Pulp and Paper Chemistry and Technology Volume 1
Wood Chemistry and Wood Biotechnology

Edited by Monica Ek, Göran Gellerstedt, Gunnar Henriksson

Pulp and Paper Chemistry and Technology

Volume 1

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Wood Chemistry and Biotechnology

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Foreword

The production of pulp and paper is of major importance in Sweden and the forestry industry has a profound influence on the economy of the country. The technical development of the industry and its ability to compete globally is closely connected with the combination of high-class education, research and development that has taken place at universities, institutes and industry over many years. In many cases, Swedish companies have been regarded as the initiator of new technology which has started here and successively found a general world-wide acceptance. This leadership in knowledge and technology must continue and be developed around the globe in order for the pulp and paper industry to compete with high value-added forestry products adopted to a modern sustainable society.

The production of forestry products is based on a complex chain of knowledge in which the biological material wood with all its natural variability is converted into a variety of fibre-based products, each one with its detailed and specific quality requirements. In order to make such products, knowledge about the starting material, as well as the processes and products including the market demands must constitute an integrated base. The possibilities of satisfying the demand of knowledge requirements from the industry are intimately associated with the ability of the universities to attract students and to provide them with a modern and progressive education of high quality.

In 2000, a generous grant was awarded the Department of Fibre and Polymer Technology at KTH Royal Institute of Technology from the Ljungberg Foundation (Stiftelsen Erik Johan Ljungbergs Utbildningsfond), located at StoraEnso in Falun. A major share of the grant was devoted to the development of a series of modern books covering the whole knowledge-chain from tree to paper and converted products. This challenge has been accomplished as a national four-year project involving a total of 30 authors from universities, Innventia and industry and resulting in a four volume set covering wood chemistry and biotechnology, pulping and paper chemistry and paper physics. The target reader is a graduate level university student or researcher in chemistry / renewable resources / biotechnology with no prior knowledge in the fields of pulp and paper. For the benefit of pulp and paper engineers and other people with an interest in this fascinating industry, we hope that the availability of this material as printed books will provide an understanding of all the fundamentals involved in pulp and paper-making.

For continuous and encouraging support during the course of this project, we are much indebted to Yngve Stade, Sr Ex Vice President StoraEnso, and to Börje Steen and Jan Moritz, Stiftelsen Erik Johan Ljungbergs Utbildningsfond.

Stockholm, August 2009 Göran Gellerstedt, Monica Ek, Gunnar Henriksson

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1 The Worldwide Wood Resource

Göran Gellerstedt
KTH, Department of Fibre and Polymer Technology

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1.1 The Importance of Wood

Wood is a very old material and its importance for the development of mankind from prehistoric to present time cannot be emphasized enough. From the original use of providing heating, shelter and tools, wood, a long time ago, also became the starting material for chemicals such as charcoal, tar and pitch used for iron ore reduction and in ship construction respectively. Hand in hand with the successive societal development in different parts of the world, the use of wood and wood-based materials was further developed. Solid wood found use e.g. as construction material and for furniture production but the most important invention was done in China some 2200 years ago when it was found that wood material can be converted into paper. Thereby, the foundation was laid for the development of written communication and in the modern society of today, a day without paper is unthinkable, but now the use has been expanded to encompass not only communication but also the packaging and hygiene sectors. Even today, however, the var-

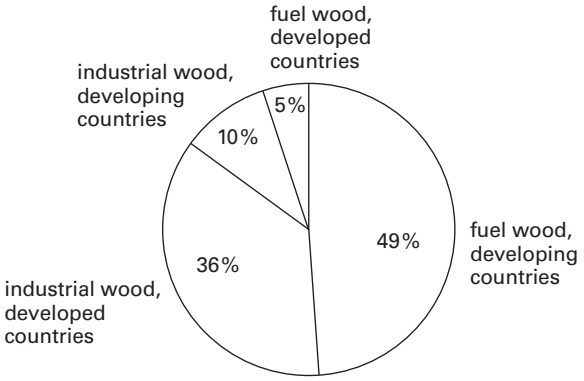

Figure 1.1. The use of roundwood in developed and developing countries in the world. Source: Skogsindustrierna, 2005.

ious uses of wood are very unevenly distributed in different parts of the world. This is shown in *Table 1.1* and in *Figure 1.1* where it can be seen that the major consumption of wood in Asia and Africa is still for fuel purposes whereas in Europe and North America, sawn wood and pulp for the production of paper and board are dominant.

Table 1.1. Total quantity of felled or otherwise removed roundwood (million m^3/year). The wood fuel is used for cooking, heating and power production. Wood for charcoal production is also included. The term pulpwood includes roundwood for pulp, particleboard and fibreboard production. Other industrial uses include e.g. wood for fences, posts and for tanning purposes.

Region	Roundwood, total	Fuel	Sawlogs	Pulpwood	Other industrial use
World	3.300	1.750	930	450	200
Africa	550	450	50	20	30
North/Central America	750	130	400	200	20
South America	300	160	80	50	10
Asia	1.150	900	150	30	70
Europe	500	100	230	140	30
Oceania	50	10	20	10	10

From ancient times until today, comprehensive deforestation in different parts of the world has been used to clear land for alternative uses such as farming. In the past, the resulting increase of atmospheric carbon dioxide that followed as a result of burning and/or microbial degradation of the wood was, however, largely compensated for by an increased production of agricultural crops. Thereby, carbon dioxide was again trapped by the photosynthetic production of biomass according to the reaction shown in *Figure 1.2*. As a consequence, only minor variations in the level of atmospheric carbon dioxide resulted. During the last ~100 years, on the other hand, the use of large and increasing amounts of petroleum for energy generation has not been compensated for by any new carbon dioxide sink and as a result, a rapid increase in the amount of atmospheric carbon dioxide can be observed (*Figure 1.3*).

$$x\,CO_2 + x\,H_2O \xrightarrow[\text{chlorophyll}]{h\nu} (CH_2O)_x + x\,O_2$$

$$\downarrow$$

$$\text{biomass}$$

Figure 1.2. The photosynthetic reaction by which carbon dioxide is converted into simple sugars and oxygen in the presence of light and chlorophyll, the pigment present in green plants. In further biosynthetic reactions, the sugars are converted to polysaccharides, lignin etc.

The rising concentration of carbon dioxide in the atmosphere constitutes a potential environmental threat since further increase may result in a global climate change with undesirable consequences. From this point of view, forest and other biomass plantation as well as an increased use of biomass in all types of applications should be encouraged since, thereby, a balance between the production and consumption of carbon dioxide can be maintained. Thus, among the various industrial sectors, the forestry industry has a great advantage since all products originate

from a renewable resource and, after use, they can be either burnt or recycled (*Figure 1.4*). The growing awareness that the use of fossil resources adds to the greenhouse effect will direct much new development towards alternative ways of producing energy, chemicals and materials using wood and other biomass as starting material.

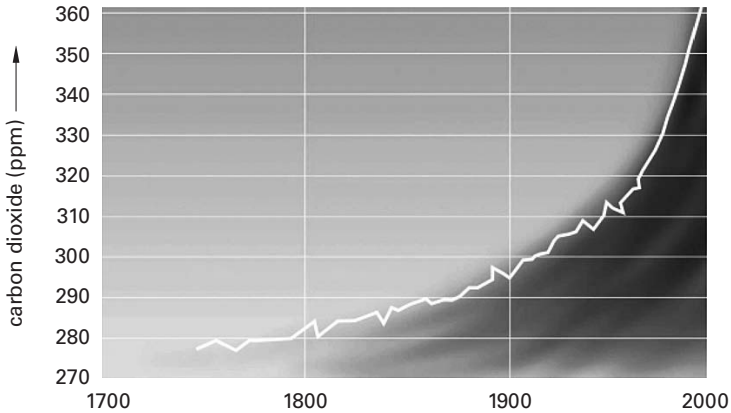

Figure 1.3. Change in the content of atmospheric carbon dioxide during 250 years. Source: Skogsindustrierna.

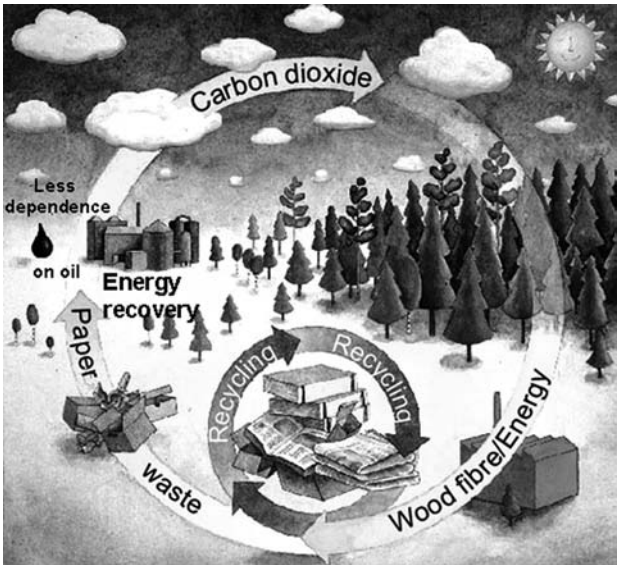

Figure 1.4. The forestry industry is in balance with nature since wood is a renewable resource and all end products, viz. carbon dioxide and minerals, can be returned to the growing forest. Source: Skogsindustrierna.

Large forest areas are present on all continents in the world. Until recently, only a few of these have been systematically used for industrial purposes, however. By tradition, the Nordic countries and Canada have been major suppliers of pulp fibers and lumber since a long time

whereas the European continent, the US and Japan have been net importers with a large domestic production of paper products. In other parts of the world, the production of pulp and paper has been comparatively small or based on annual plants. During the last decades, however, several new countries have emerged as suppliers of wood-based fibers and, in particular, the pulp producing industry has shifted focus and, today, the new capacity is found in countries such as Brazil, Chile, New Zealand and Indonesia. The major reason for this change is the establishment of large plantation forests located in close vicinity to new pulp mills. Since the climate conditions in these countries are very favourable for fast growth, the rotation time of the (softwood) tree can be reduced to very low values (10–15 years) as compared to e.g. Sweden with a rotation time of some 50–80 years. Some examples of the differences in growth rates for softwood are shown in *Figure 1.5.* Similar or even larger differences can also be seen for hardwoods.

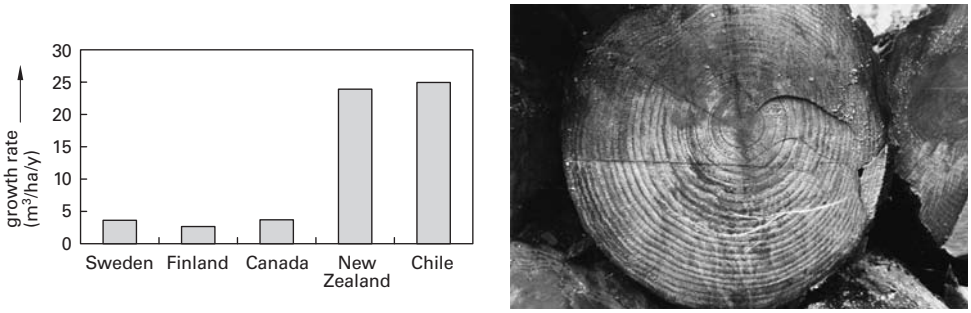

Figure 1.5. Annual growth rates for softwood plantation forests in New Zealand and Chile compared to forests in Sweden, Finland and Canada (left) and an example of a 20 year old log (*Pinus radiata*) from a plantation in New Zealand.

1.2 Pulp and Paper

1.2.1 History

Prior to the invention of paper, ancient mankind used a variety of materials for the inscription of signs and pictures such as bark, stone, silk cloth and bamboo. In ancient Egypt (~3000 BC), the pith of papyrus was made into sheets which in turn were pasted together into scrolls. On these, ink could be used to write words. Based on evidence from excavations, paper from wood-based fibers is thought to have been invented in China around the year 200 BC. It was not until much later, however, when the head of the Imperial library, Ts'ai Lung, was asked by the Emperor at that time to put the library in order that a more official birth-date of paper was obtained. Since all books in the library were made from wood boards they were heavy and never-red. Therefore, Ts'ai Lung began to study alternative ways of preserving the information while at the same time using something lighter than wood. Although the history does not give all the details, it can be assumed that previous knowledge was used to make experiments with materials that could be beaten into fibers. Finally, bark from trees belonging to the mulberry family ("kaji") was tried and, after beating in a water suspension, Ts'ai Lung was able to form paper sheets from the fi-

bers in the presence of some (unknown) mucilaginous substance. The sheets were drained of water and dried with the first sheet of paper being presented to the Emperor in the year AD 105.

After the death of Ts'ai Lung, around 125, the production of paper rapidly increased in China but it was not until the year 610 that the knowledge reached Japan through Korea (*Figure 1.6*)[1]. The westward spread of knowledge followed the silk route and arrived in Samarkand in the year 751. From there, it moved further west through the Near and Middle East and came to Europe (Spain) during the 12th century. Some 400 years later, the art of papermaking was known throughout Europe and, during the 17th century, the knowledge also reached America.

The raw material used in Europe for a long time was linen rags but with the invention of typography by Gutenberg 1445, the demand for paper increased greatly and hemp cloth and, later, also cotton rags were added. Originally, beating was done using a stamper but in the 17th century, the beating process became mechanized with the invention of the hollander. The continuous paper process, making use of an endless wire, was introduced in 1798 and a few years later (1806), the first fourdrinier paper machine was constructed.

Figure 1.6. The art of paper-making was introduced in Japan in the year 610 using the inner bark of "kozo" trees. Hand-made Japanese paper, "washi", is still made according to the original principles which involve cultivation and collecting of kozo shots, steaming, stripping off the bark, collecting "white" inner bark, boiling with wood ashes (later caustic soda), washing, manual removal of impurities ("screening"), beating, vat preparation (pulp, water, thickening agent, "neri"), paper forming, pressing, air drying (photo right), sorting and trimming.

[1] Washi, the traditional Japanese hand-made paper, is still made today mostly using bast fibers from bulberry species ("kozo") as raw material. The process is tedious and requires much labour but results in artistic paper qualities, unprecedented in the world.

The ever increasing demand for paper could not be met with the availability of cotton rags and during the 19th century, the foundation of modern pulping was laid with several important inventions based on the use of wood. Thus, in 1844, a method for making mechanical pulp was invented by Keller (Germany) and somewhat later the sulfite (1866) and the kraft (1879) processes for making chemical pulps were invented by Tilghman (GB) and Dahl (Germany) respectively.

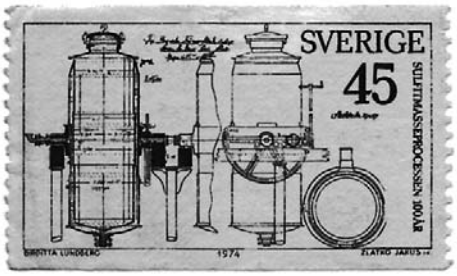

Figure 1.7. The first sulfite mill in the world was constructed by C.D. Ekman in 1874 and located in Bergvik, Sweden. 100 years later, this was commemorated by the Swedish Post Office showing the drawings from the original patent.

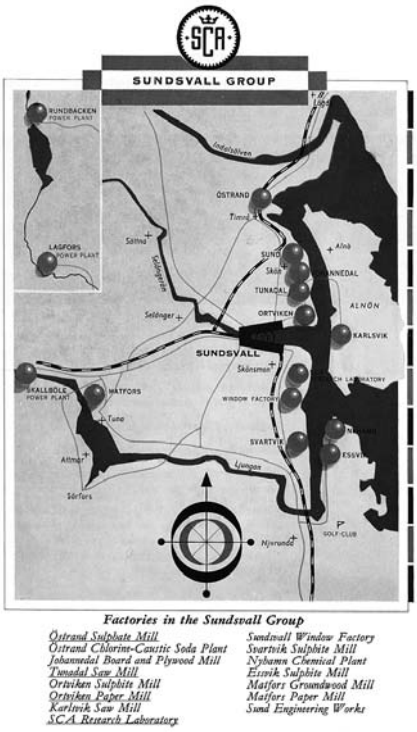

Figure 1.8. The SCA company in the Sundsvall area in 1960. Those units, still in operation, are underlined in the factory summary.

Since kraft pulps could not be bleached to give completely white fibers until some 60 years later, when bleaching with chlorine dioxide was developed, the predominant use of this type of pulps for a long time was in strong papers for packaging. Sulfite pulps, on the other hand, were more easily bleached with chlorine and hypochlorite, the dominating bleaching agents until the late 20th century, and, consequently, many sulfite mills were constructed in the late 19th and the early 20th century with the first mill in the world being built in Bergvik, Sweden, in 1874 under the leadership of C.D. Ekman *(Figure 1.7*).

With the further successive development of pulping and bleaching technologies, a shift from small sulfite mills operating only on spruce wood towards larger kraft mills with less dependency on wood species has taken place during the last decades of the 20th century. This can be illustrated by the development in the Sundsvall area, located in mid-Sweden. Thus, in 1960, the company SCA had a total of 15 industrial units in the area whereas 40 years later, the number has been reduced to 4 as shown in *Figure 1.8.* During the same time, the total production of pulp, paper and sawn wood in this area increased substantially.

1.2.2 Paper Consumption

Today, the total consumption of paper products in the world is around 300 million tons and the annual increase is forecast to accelerate due to a rapid increase of the consumption in countries like China, India and Brazil (*Figure 1.9).* This is due to the fact that several regions in the world still have a very small per capita consumption of paper as shown in *Figure 1.10.* At the same time, the economic development in many developing countries is now rapidly increasing in rate. Thus, despite being second in the world in consumption of paper products (48 million tons in 2003), China, at present, has a per capita consumption of only ~36 kg. Of this, only some 20 % is wood based whereas the rest comes from waste paper and from nonwood fibers such as straw. Although all three sources of fibers will continue to be important, a certain relative increase of wood based fibers is foreseen and, altogether, a large and rapid increase in the total consumption of fibers will take place in China. Thus, for the year 2010, the forecast is a consumption of paper and board products of around 80 million tons.

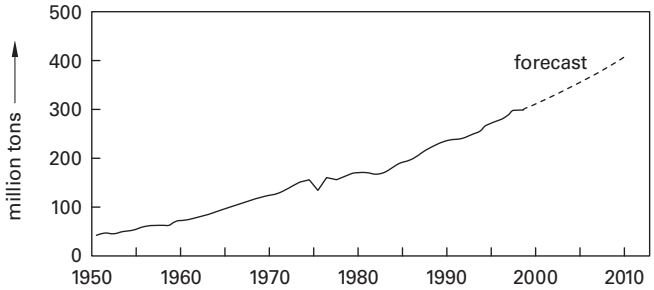

Figure 1.9. Annual production of paper products in the world and the forecast until 2010. Source: Skogsindustrierna.

In order to meet the expansion of the Chinese consumption, a further rapid increase of the import of fibers as indicated by the figures in *Table 1.2* can be foreseen. Furthermore, a large increase of the domestic production of fibers, both from plantation forests and from an increased rate of collection of waste paper (recovery rate ~29 % in 2003) must be developed. The old and

rather inefficient production apparatus, at present 3,500 mills with an average capacity of 13,000 t/y, has to be modernized.

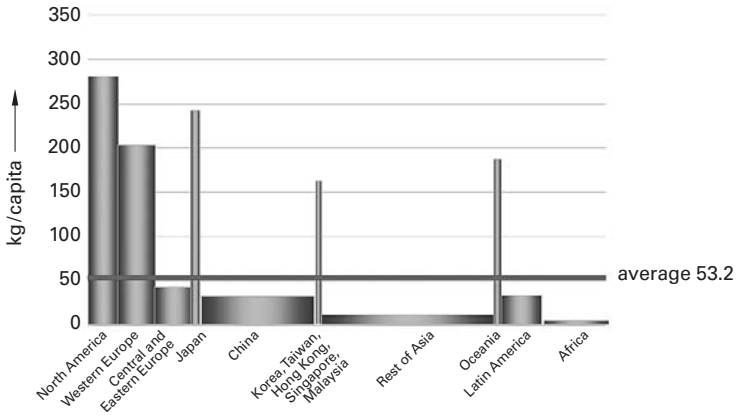

Figure 1.10. Annual per capita consumption of paper products in different regions of the world 2002. Source: Skogsindustrierna.

Table 1.2. The import of paper and board, market pulp and waste paper to China during 2001 – 2003. Source: China National Pulp and Paper Research Institute.

Import, million tons	2001	2002	2003	Increase (02→03), %
Paper and Board	5.6	6.4	6.4	–
Market Pulp	4.9	5.3	6.0	14.4
Waste paper	6.4	6.9	9.4	36.5

With China serving as an example, it can be anticipated that several other developing countries in the world will face a similar rapid change of paper consumption and, consequently, the global production of virgin fibers will continue to grow. At the same time, the rate of recovery of waste paper must be increased and rendered more effective in many countries (cf. *Figure 1.11*).

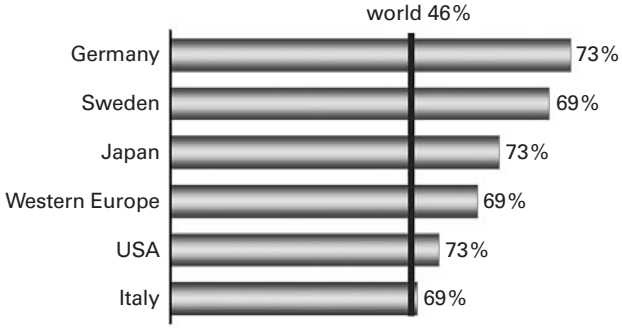

Figure 1.11. Recovery of paper products in some selected countries in the world. The average figure in the world is indicated in the figure. Source: Skogsindustrierna.

1.2.3 Pulp and Paper Trade

The great importance of the pulp and paper industry for the trade balance of the Nordic countries is indicated in *Figure 1.12* and *Figure 1.13*. Thus, a net export of some 4 million tons of pulp and 20 million tons of paper results in large export incomes, in particular for Sweden and Finland. The vast majority of customers are located in Western Europe which, at present, is the major pulp and paper importing region in the world with China being second. In the short term future, it can be assumed that a rather large increase of the paper consumption will take place in East and Central Europe (cf. *Figure 1.10*) accompanied by an increase of the pulp capacity in these countries. Since long, Canada has been the major supplier of virgin pulp in the world with the Nordic countries second. The fast development of plantation forests in Latin America will, however, result in a rapid growth of new pulp capacity based on both hardwood (eucalyptus) and softwood (pine) in this region. In addition, plantation forests in other regions in the world, such as South Africa, Southeast Asia and Oceania, will grow in importance.

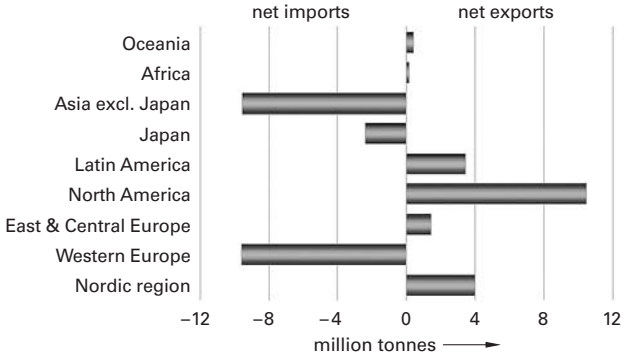

Figure 1.12. The global trade of pulp in various regions in 2002. Source: Skogsindustrierna.

The large importance of the pulp and paper industry for the economy in Sweden and Finland is further illustrated in *Figure 1.14* which shows the leading exporters of pulp and paper in the world.

In Sweden, the forest industry is the third largest export industry and from a total value for the Swedish export of 787 billion SEK (2002), forest industry products accounted for some 15 %. Since the import to this sector is relatively small, the forest industry generates a large export surplus for Sweden as shown in *Figure 1.15*.

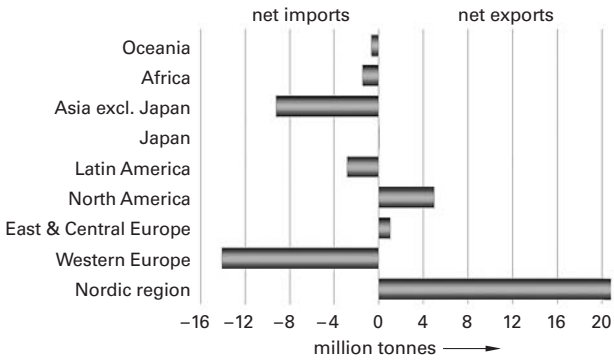

Figure 1.13. The global trade of paper in various regions in 2002. Source: Skogsindustrierna.

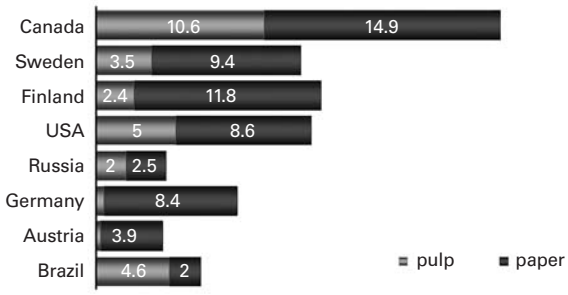

Figure 1.14. Leading export countries for pulp and paper in the world. Figures from 2003. Source: Skogsindustrierna.

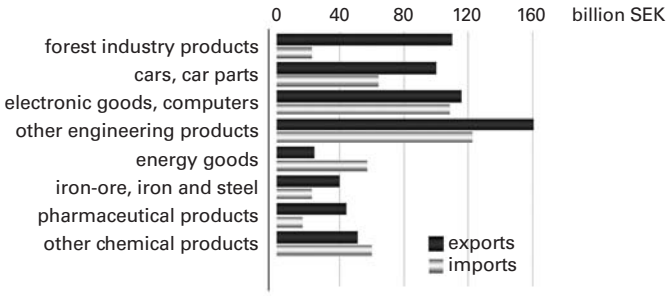

Figure 1.15. Export and import of some product groups in Sweden. Data from 2002. Source: Skogsindustrierna and Statistics, Sweden.

1.3 The European Perspective

The pulp and paper industry plays an important role in the European economy and contributes some 8 % of the EU manufacturing added value albeit with large differences in different parts

of the region. The successive changes of the industry from small local producers of forest products via national to regional or in some cases worldwide companies have taken place during a time-span of some 30–40 years. Now, several of the biggest companies in the world are European-based as exemplified in *Figure 1.16.*

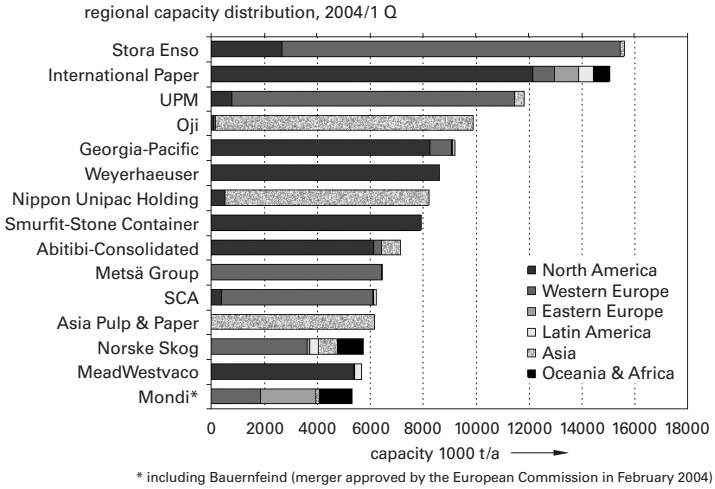

Figure 1.16. Leading global paper and paperboard producers. Data from the first quarter 2004. Source: Jaakko Pöyry.

Since about one third of the surface of Europe is covered with forests and two thirds of the annual growth is utilized for products and energy, these forests also serve as a major carbon dioxide sink (cf. page 3–4, above). Therefore, a healthy and profitable forest-based sector is of vital interest for Europe and it constitutes a corner-stone in a sustainable society since its products are recyclable and reusable for new products and energy.

The forest-based industry is multidisciplinary and, with the forest as core asset, several different value chains can be distinguished such as

- the wood products chain (including sawn lumber, house elements, furniture …)
- the paper chain (including pulp production and paper recycling)
- the energy chain (including wood, wood waste, lignin …)
- the wood-based chemicals chain (including lignin and cellulose products, ethanol...)

Along these chains, a further disintegration can be made into a wide range of products and applications as illustrated in *Figure 1.17.*

The forest-based sector in Europe has a high technological level and in many areas, a global leadership. The early recognition that chemical pulp production resulted in detrimental effects on the environment and the strong position of the machinery, the chemical and the consultancy companies has resulted in a determined and rapid change of process technologies together with a consumer-focused development of new products.

The current strength of the European forest-based sector is, however, not enough to ensure a similar position in the future and several challenges can be envisioned such as

- attracting young people and providing a qualified education
- further improvement of forest management with respect to raw material supply, environmental and recreation aspects and for mitigation of climate change
- development of less capital and energy intensive and more flexible process technology
- development of new consumer-friendly, high value added and recyclable products
- broadening the use of forest-based products to encompass “green chemicals”, biofuel and “green energy”

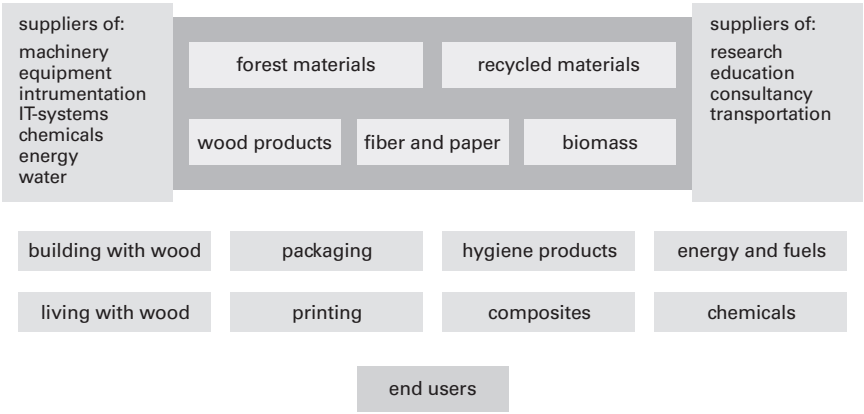

Figure 1.17. Important production areas related to the forest-based sector. Source: European Commission through CEI-Bois, CEPF, CEPI.

From an academic point of view, these challenges will require committed people, the creation of strong educational and research centres and a developed collaboration between academy and industry.

The traditional view that the forest industry only provides commodity products on a large scale must be changed.

2 The Trees

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2.1 Introduction

True plants belonging to the kingdom *Plantae* are eukaryotic[1] organisms that are photosynthetic, i.e., can utilize light for the fixation of CO_2 (some parasitic and saprophytic plants have lost this ability), use starch as nutrition storage inside cells and have cell walls rich in cellulose. Note that this definition excludes some organisms often considered as plants, such as brown- and red algae, fungi and lichens. These belong to other eukaryotic kingdoms (*Protozoa* and *Fungi*).

2.1.1 Evolution of the Plant Kingdom

The true plants are a *phylogenetic group*, i.e., they all have a common ancestor. In *Figure 2.1* an evolutionary tree of the most important phyla[2] in the plant kingdom is shown. The first plants were green algae developed in the sea, when photosynthetic bacteria start to live inside larger eukaryotic cells in symbiosis. The bacteria is the origin to the *chloroplasts*, a plant organelle[3] that is responsible for the photosynthesis in all plants and still have their own DNA encoding for some of its functional proteins. Mosses and related groups of plants, hornworts and liverworts, were developed from green algae (that probably lived in fresh water), when the dry land was colonized around 470 million years ago. In similarity to algae these plants that are called *bryophytes* with a common name, take up water by all their body and lacks thus roots and efficient systems for transport of water and nutrition within the plant, which limits their size and often their growth during dry conditions[4]. From these a more advanced group, the *vascular plants*, was developed around 440 million years ago. These contain in the opposite to the bryophytes, the hydrophobic polymer *lignin* (see Chapter 6), which allowed the creation of a new cell type, the *tracheid*. This cell type is long and has a thick hydrophobic cell wall, and it is efficient both for liquid transport and for giving strength to the plant. Simultaneously roots were developed and the plant could be much larger and grow in dryer environments. Two main *functional* types of vascular plants were developed, *herbs*, where were the plant parts over soil are non-woody and in general dies after one or a few season[5], and *woody plants* including bushes and trees, with stiff stems and branches and a permanent over-soil part[6]. The cell walls of all vascular plants contain in addition to cellulose and lignin, also other polysaccharides, *hemicelluloses* (Chapter 5), and low molecular weight compounds, *extractives* (Chapter 7).

---

[1] *Eukaryotic* organisms have the chromosomes collected in a nucleolus with membrane, in the opposite to *prokaryotic* organisms, where the DNA is free inside the cell. Plants, Fungi, Animals and other higher organized life-forms are eukaryotic, whereas Bacteria and Archebacteria are prokaryotes.

[2] A *phylum* is the systematic level directly under *Kingdom*. The next level is *class* and under that follows *order*, *family*, *genus* and *species*. Other names are used for the hierarchic levels of animals.

[3] Organelles are cell organs, i.e., separate bodies inside the cell normally surrounded by membranes that carry out special functions.

[4] There are, however, mosses growing even in desserts.

[5] Herbs are however not necessarily small. In tropical areas there are examples of herbs that are over 10 m high and thus larger than many trees. The banana "tree" is for instance actually a herb.

[6] Herbs and woody plants are off course not evolutionary groups. In general, almost all families of vascular plants contain both herbs and woody plants. Sometimes it is also difficult to say if a plant is herb or woody plant.

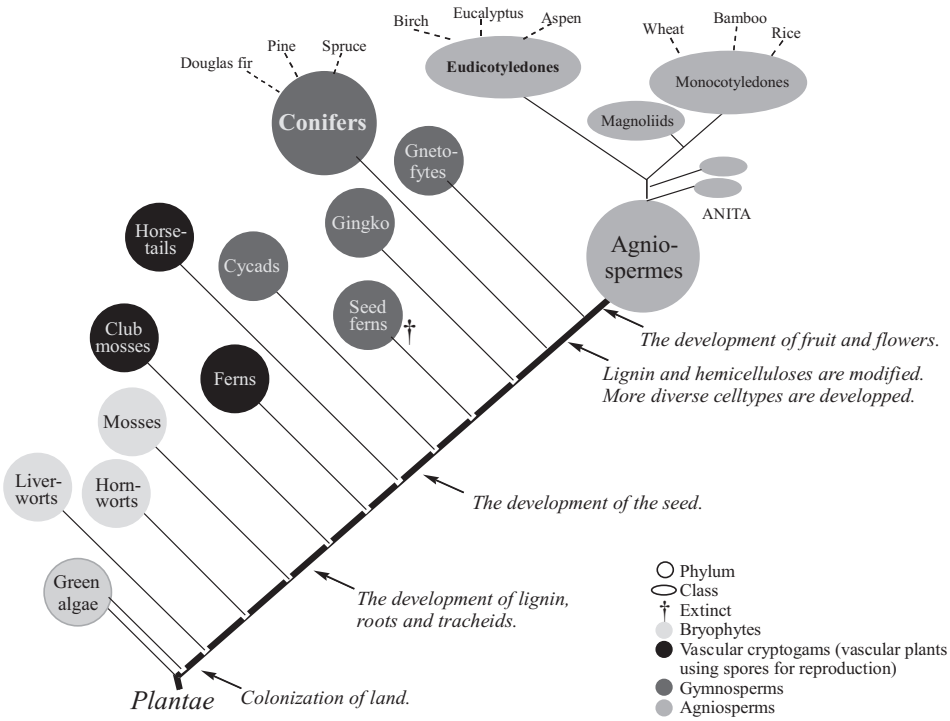

Figure 2.1. Simplified evolution tree over the plant Kingdom. All extinct phyla are not shown. The green algae consist of several separate groups, of which the stoneworts (*Chareophyta*) are closest to the land plans. Liverworts and hornworts are some times included in the moss-phylum, and in some cases the bryophytes and fern plants are divided into more phyla. The gnetophytes might be an artificial group containing several not close related groups. ”ANITA” is a group of not close related primitive angiosperms. Examples of important plants for the pulp and paper industry are shown in their representative groups.

The first vascular plants reproduced with spores, which require at least a drop of water for the fertilization. (The male spore must swim to the female spore.) Three phyla of such *vascular cryptogams* have survived the *club mosses*, the *ferns* and the *horsetails*. All of them had during the Devonian age (“the age of the amphibians” around 350–400 million years ago) species that were large trees, but today only herb forms have survived, with the exception of one family of fern-trees, *Dicksonia’cea* (*Figure 2.2*).

club moss (lycopodium clavatum)

common horsetail (assexual plant) (equisetum arvense)

fern tree

sexual horsetail plant

herbal fern

Figure 2.2. Vascular cryptogams. *Up to the left* is a *club moss* shown. This phylum once contained tree forms, but today only a limited number of herbal species have survived. They are the most primitive vascular plants still existing. *Left below and central* an example of a *horsetail* (Common horsetail, *Equisetum arvense*, maybe the oldest living species of plants). The now living horsetails are all herbs with an annual over-soil part and a permanent root and horsetails is the fern plants that is phylogentic closest to the seed plants. Yearly two plants are created, first a non-photosynthetic sexual plant, and later a asexual photosynthetic plant. The horsetails are the closest relatives to the seed plants of the vascular plants. *The ferns* (*right*) are the most advanced phylum of vascular cryptogams. Most ferns are herbs as the *lower* picture. The *fern trees* (*above*), were still important during the Triassic, and still around 650 species grew mostly in humid mountains in tropical and subtropical regions. They remain of palms and the largest species can be up to 30 m high. Fern trees are the oldest existing type of tree and may locally be rather common.

The next important jump in the evolution was the development of the seed for at least 360 million years ago. The fertilization was now carried out on the plant and a seed with stored nutrition were created. This gave advantages over the vascular cryptogams, partly since dryer environments could be colonized. The seed plants quickly expanded during the Permian age and exterminated the vascular cryptogams trees with the exception of fern-trees. (Herbal fern plants did however still dominate the undergrowth.) Dominating phyla during Triassic and Jurassic (200 million years ago, "age of the dinosaurs") were especially the *cycads* (*Figure 2.3*), but also the *gingko-plants* (*Figure 2.3*) and the now extinct *seed-ferns*. Also early *conifers* existed, but the plants in this phylum were not very abundant outside the coldest areas. These seed plants all have naked seeds that often are organised in cones, and are with a common name called *gymnosperms* (Latin for "naked seed") (*Figure 2.1*).

maidenhair tree *(ginkgo biloba)* cycad *(cycas cicanlia)*

Figure 2.3. Two phyla of primitive gymnosperms Left a *Maidenhair tree* ♂ (*Ginkgo biloba*). This is the only species left of this once so important phylum of ginkophytes. Note the characteristic leafs. Maidenhar tree has been used since ancient time as a temple tree in China and is not known with certainty as wild plant. Its discovery can be compared with finding a living dinosaur. As cycads, there are male and female trees (a primitive property). It is often used as park tree due to its decorative looking and the fact that it is easily grown in polluted air. The seeds are considered to be a delicacy in East Asian-cooking. It the most primitive tree of "oak type" (with secondary growth). Right a *cycad* (*Cycas circanlia*) ♂. This phylum of gymnosperms represents the most primitive seed plants and can be considered to be living fossils. Around 100 species of short palm like trees grow in tropical and subtropical areas. Both the male and female trees carry the characteristic cones, which are the sexual organs of the plant. Some species are used for the production of the starch rich saga. They are the most primitive seed carrying trees existing.

During the Krita (around 150 million years ago) a dramatic revolution in the plant population occurred, that is not less dramatic than the subsequent mass-extinction of the dinosaurs[7]; a new phylum, the *Angiosperms*, emerged and quickly became the totally dominating group. Today

[7] The dramatic changes in the flora of the Krita were reflected in the development of stronger teeth in the contemporary vegetarian dinosaurs. Some scientists even blame the mass-extinction of the dinosaurs on the development of angiosperms, but this is probably not correct, since dinosaurs and angiosperms coexisted for millions of years.

norway spruce *(picea abies)* | scots pine *(pinus sylvestris)* | redwood *(sequoia sempervirens)* | douglas fir *(pseudotsuga menziesii)*

Figure 2.4. Conifers. This gymnospermic phylum existed already in the Permian age (around 300 million years ago), but were for a long time in the shadow of especially the cycads. Although the trees are primitive in many aspects they are very successful, especially in colder regions where they often dominate. The needles are, in the opposite of most hardwood leafs, active for several years (The larch is an exception that lost the needles every winter.), and some pine needles can be active for 45 years. Around 600 species of trees and bushes exist. Left two examples of European softwoods belonging to the Pine family, that both are excellent raw material for papermaking and sawed wood products. Right two North American softwood species. Both are important raw material for papermaking and carpentry, and can be over 100 meters high are among the highest trees in the World. They have been introduced globally. Douglas fir belongs to the Pine family, whereas Redwood belongs to the swamp cypress family.

more than 90 % of the land plant species are angiosperms. The angiosperms had flowers, used partly insects for the fertilization and the seed are developed inside fruits. In parallel with these improvements in the fertilization system, the angiosperms developed more advanced types of leafs, the tracheids were replaced with specialized cell types for liquid/nutrition transport (vessels) and mechanical support (librioform fibres), and the lignin and the hemicelluloses were modified into different forms than in gymnosperms. The small phylum of *gnetophytes* represents an interesting transition form; they are gymnosperms (lacks fruits and flowers), but have similar cell types and lignin structures as angiosperms[8]. The angiosperms are divided into two main classes:

- The *monocotyledons*, those have one cotyledon (germinating leaf)[9]. Grasses including bamboo, banana-plants, orchids and palm trees among others belong to this group (*Figure 2.5*).

[8] The gnetophytes are today represented by around 70 species of trees, bushes, herbs and lianas. They mostly grew in South America and Western Africa. Some species are used for food and as fibre source.
[9] Gymnosperms can have two or several cotyledons.

- The *eudicotyledones* that have two cotyledons is the largest group. Ordinary leaf carrying trees as birch (*Figure 2.6*), oak, eucalyptus, etc, as well as numerous bushes and herbs belong to this class. The question is not "which plant that is an eudicotyledone," the question is "which is not". The woody plants in these groups are called "*hardwoods*", reflecting that their wood in most cases is harder than the wood of coniferous trees (softwoods)[10].

bamboo

phoenix palm
(phoenix ruebelini)

Figure 2.5. Two examples of monocotyledonic trees. Bamboo (left) is one of the most economically important monocotyledons. This fast growing woody grass is used not only as raw material for the pulp and paper industry and as construction material, but also as food, and for all kinds of ordinary things as for instance chop sticks and furniture. The palm trees (right) are another family of woody monocotyledons with over 2000 species. Palms grow mainly in tropical and subtropical areas and dominate many forests in for instance the Caribbean. The tallest can be over 50 m high and have the largest leafs and seeds among plants. The leaves are used for making special quality papers, but the stems are not regarded as a good raw material for the pulp and paper industry.

Around 97 % of the angiosperm-species belong to these two classes. The remaining species belong mainly to a third class, the *magnoliids*, which have two cotyledons, but are closer related to the monocotyledons than the eudicotyledons[11].

Over 25 000 species of hardwood trees exist. These do not form a special group within the eudicotyledonic class, but are spread out in different families (see *Table 2.1* for examples), that in many cases also include bushes and even herbs. Lime-tree is for instance closer related to jute than to beech. It is therefore not surprising that the chemical and mechanical properties of different species of hardwood trees vary considerably.

---

[10] However, there are many examples of hardwood species with rather soft wood, as lime-tree.

[11] Magnoliids include flowering trees and bushes that are often used for decoration, and also some herbs. The wood can be used for carpentry. There is also some other of primitive angiosperms, the ANITA group (*Amborella*, *Nymphaeales*, *Illiciales*, *Trimeniaceae*, *Ausrobaileyaceae*). All of these have very limited distribution with exception of *Nymphaeales*, the water-lilies.

The grasses and eudicoltyledonic herbs replaced to a large extent horsetails and club mosses from the undergrowth, whereas the gymnospermic trees were suppressed by eudicotyledonic trees (hardwoods). An exception is the coniferous trees and bushes, including all modern needle-carrying trees, or softwoods, as larch, pine and spruce (*Figure 2.4*), that rather expanded. Although these plants in many ways are more primitive than the angiosperms, they are very successful, and softwoods dominate many forests in cold and temperate regions. Before the ice age, their extension was even larger and isolated softwood forests in hardwood dominated regions are a reminiscence of this time. Two economically important families are the pine plants, *Pinaceae*, that around 30 % of the coniferous species belongs to including the genera of pines, spruces, larches, hemlocks, cedars and Douglas firs, and the cypress plants, *Cupressaceae*, with around 130 species mostly in temperate areas including the genus cypresses, junipers and thujas. Other families are swampcypresses, *Taxodiaceae*, with some old type of trees as the huge Redwood growing in western USA and living fossil tree Chinese sekivoja, and *Podiocarpaceae* and *Aroaucariaceae* growing in the southern hemisphere.

birch eucalyptus aspen maple

Figure 2.6. Four examples of hardwood trees. This group dominates normally tropical, subtropical and some temperate forests, and can be considered as the most developed trees. Birch (left) is the most used hardwood (eudicotyledonic tree) in Scandinavian pulp and paper industry, and also the most common hardwood in Scandinavian and Northern Asian forests. Eucalyptus (second from left) is a genus of Australian trees that are the most important hardwood-species internationally for the carpentry and pulp and paper industry. Several species in the genus are cultivated in tropical, subtropical and temperate regions up to Scotland (the photo is from a cultivation in Brazil). Some species can be over 100 meters high. Aspen (second from right) is a member of the poplar family of fast growing trees. They grow from subtropical to close to the tundra in both North America and the old world, and are important raw materials for the pulp and paper industry. Maple (right) is an important raw material for the pulp and paper industry in Canada and northern USA.

Table 2.1. Examples of families and trees in the eudictyledonic class.

Family	Example of trees	Example of other plants
Aceraceae	Maple	
Betulaceae	Birch	
Fagaceae	Beech, Oak	
Myrta´ceae	Eucalyptus, Clove	Myrtle
Oleaceae	Ash-tree, Rowan	Lilac
Rosaceae	Pear-tree, Cherry-tree	Roses, Cloudberry
Salica´ceae	Aspen, Poplar, Willow	Osier
Tiliaceae	Lime-tree	Jute
Ulmaceae	Elm	

2.1.2 Forests of the world

The kind of trees growing in forests in different climates and locations varies highly. Some main types can however be recognized.

- *The taiga* stretch from Scandinavia over Russian with Siberia down to northern China and Japan, towards Canada and north USA. Softwood dominates these forests, which generally are poor in species, especially the European part. Spruce and pine are the main trees in Scandinavia. In Siberia and northern Japan also larches are common. The most common hardwood in the Old World-taiga is birch, that becomes dominant in the borderline towards the tundra, whereas the North American taiga is richer in both hardwood and softwood species[12]. Except many species of pines, also hemlocks, larch and Douglas fir are common softwoods, whereas oaks and maple dominates among hardwoods. The Russian taiga represents that largest reserve of softwood timber, but is difficult to explore due to transportation problems.
- *The temperate mixed forests* are located south of the taiga. These forests are richer in species than the taiga and contain both hardwoods and soft woods, but the individual proportions vary highly, so that softwoods sometimes are absent. In Central Europe beech and oak are the common hardwood species, but in mountainous areas also spruces and larches grows. In southeast USA, mixed forest with a large portion of pines and other softwoods grow, and along the pacific coast of USA over 100 meters high softwoods (redwood and Douglas fir) are located[13]. In mountainous areas, softwoods grow in large amounts down in

[12] The richness in species in North America compared with Europe is also a fact for animals. The reason is the lack in the North American continent of barriers (as mountains and seas) in east-west direction, which prevent the spreading of the species. In Europe 10 softwood and 51 hardwood species grow naturally, whereas North America and Latin America have a much higher number of species. Some North American species as contorta pine, Douglas fir and hemlocks have been introduced in European forestry. The Monterey pine (*Pinus radiata*) is cultivated worldwide in warmer regions. East Asia is also richer that Europe in species (but not as rich as America). Many of the most important culture plats originates from America and China.

[13] These forests are reminiscences of old types of forests that before the ice ages were much more common. They are economically important for timber.

Mexico and Central America. The temperate forests in Asia are located between of the taiga and the dessert in western Asia and central and northeast China and most part of Japan. Especially the Chinese temperate forests are very rich in hardwood species[14] and have also a number of gymnospermic living fossils as maidenhair tree and the conifer Chinese sekivoja. In the south part bamboo is frequent. Also in the southern hemisphere temperate forests occur; in the Brazilian highland grows pine forests and in south Chile, the forests have a large part of softwood, but also one of the few non-tropical palm trees. The temperate forests in South Africa[15] and Australia[16] are almost exclusively hardwood forests. The latter consists mainly of Eucalyptus and Acacia, fast growing trees that have been introduced to other countries for plantation cultivation. In the South Island of New Zealand many tall softwood trees grows, some of very ancient type, together with hardwoods and fern-trees.

- Palm trees and different hardwoods dominate *the forests of the tropical circles*, but also softwoods can occur, as cedars in the Mediterarian areas. Some hardwoods are very valuable for carpentry, as teak and African black tree. In Africa and Central America also cycads occur.
- *The tropical rainforests* grow loser to the equator in Central and South America, the Congo area in Central Africa, west coast of India and Southeast Asia including Indonesia. Hardwood trees dominate them, but also gnetophytes and fern-trees occur in some forests. Rain forests are extremely rich in species. There are also drier tropical forests dominated by palm trees (for instance in Caribbean) and bamboo (in Southeast Asia).

Almost half (47%) the forests of the world are found in the tropical zone *Figure 2.7*. The forest in boreal and temperate zones together amount to about the same figure (33 % and 11 % respectively). The remaining 9 % is subtropical forest.

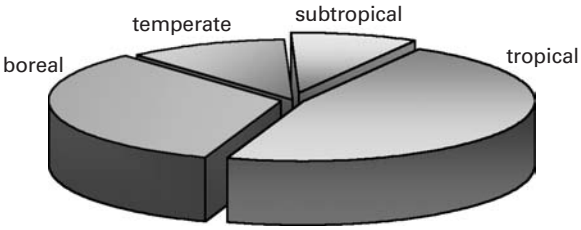

Figure 2.7. The distribution of the worlds forests in the different climate zones.

[14] Many of our common garden plants originate from these forests.
[15] Pines (*Pinus radiata*) have, however, been introduced.
[16] Australia has nevertheless some of the most ancient conifer species.

2.2 Parts of trees

2.2.1 Shape of trees

The shape of trees can be divided into three main forms[17] (*Figure 2.8*). The *palm-type* is valid, beyond palms, also for the ancient types of trees, fern-trees and cycads (Figure 2.2, 2.3), but also for some other monocotyledonic trees as the Australian grass tree and lily trees. Bamboo can be seen as a special case of this type. Palm type of trees grow in a different way and have a totally different wood, than other trees, due to their lack of secondary growth (*Figure 2.8*). Since they are not important as raw material for the pulp and paper industry[18] with the important exception of bamboo, they will not be discussed further. All other types of trees (maidenhair tree, conifers, gnetophytes, most hardwoods) are of either *spruce*- or *oak type*. Generally the spruce-type is more common among softwoods and the oak-type among hardwoods, but there are many exceptions; while spruces, Douglas firs and redwoods indeed are bottle-brush like, for instance pines and hemlocks may form crowns (but not as large as in oaks) and sometimes divided stems. Oaks and maples form mighty crowns, while birches sometimes have a shape similar to spruce. From a technical point of view, the spruce type of trees is to prefer (transportation, sawing, barking etc.). The location of the growth do off course influence the type and close growing of trees facilitates spruce type growth even among hardwoods; see the eucalyptus in *Figure 2.6*. A single hardwood tree can on the other side develop a mighty crown as the maple in *Figure 2.6*.

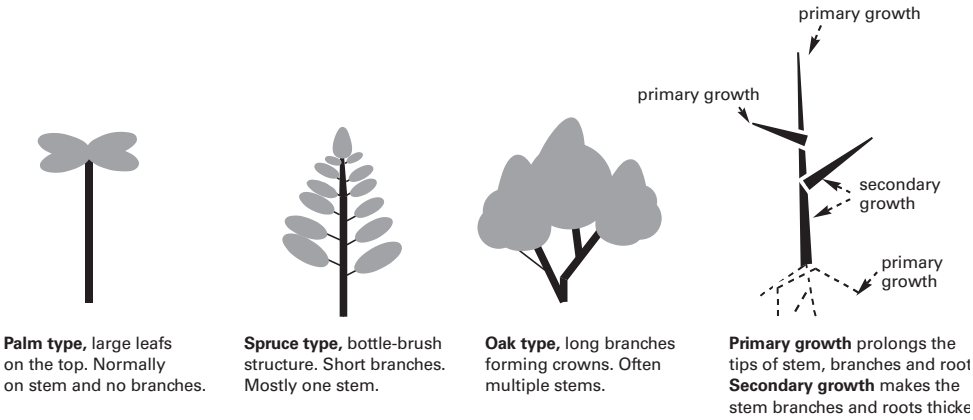

Figure 2.8. Different shapes of trees. The palm type differs from the other two main types in the aspect that they lack secondary growth.

[17] This division is *not* phylogenetic, but rather a"functional" division.
[18] Leaf fibres from palms are, however, used for some special quality papers.

2.2.2 Hierarchic Structures

The excellent properties of wood as a material is to large extent due to that it is highly organized in several hierarchic levels from parts of the tree down to molecular level. Differences in the hieractic levels are very important for the properties of the raw material for pulp and paper industry. Firstly, there are different wood species. *Table 2.2* lists the main chemical constituents in some important species. Each tree is composed of different parts: *top*, *stem*, *branches*, *leafs/needles* and *root*. Normally, only the stem and top are used for the pulping, but attempts to use root and branches for pulping have been done. The stem is further divided into bark, cambium, sapwood and heartwood, which all are composed of cells. There are different cells with different chemical composition. The cell-wall layers also vary in the different cells. Finally, the different cell-wall layers have different chemical composition and supra-molecular structure.

Table 2.2. Chemical composition of some wood species (mass %).

Species	Extractives	Lignin	Cellulose	Gluco-mannan	Xylan	Other poly-sacch.	others
Softwoods							
Norway Spruce (*Picea abies*)	1.7	27.4	41.7	16.3	8.6	3.4	0.9
Scots Pine (*Pinus sylvestris*)	3.5	27.7	40.0	16.0	8.9	3.6	0.3
Hardwoods							
Birch (*Betula verrucosa*)	3.2	22.0	41.0	2.3	27.5	2.6	1.4
Beech (*Fagus sylvatica*)	1.2	24.8	39.4	1.3	27.8	4.2	1.3
River red gum (*Eucalyptus calmaldulensis*)	2.8	31.3	45.0	3.1	14.1	2.0	1.7
Red maple (*Acer rubrum*)	3.2	25.4	42.0	3.1	22.1	3.7	0.5

The differences in chemical composition of various cells will be discussed in Chapter 3. Different parts of the tree have different chemical composition (*Figure 2.9*). The stem has the highest proportion of cellulose (> 50 %), but also bark contains 20 % cellulose. Nevertheless, the bark is removed before pulping, since bark in some cases (as birch) contain high content of extractives, which can give problems with pulp quality (Chapter 7). The needles have the highest proportion of extractives, 27 %.

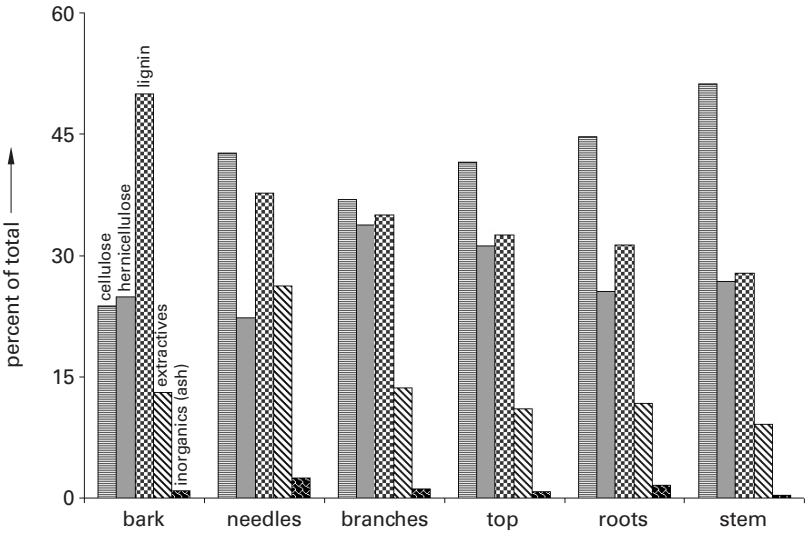

Figure 2.9. Chemical composition in different parts of slash pine (mass %). The variations on composition depends to a large extend that the different parts of the tree is build up of different tissues; the needles and the bark is constructed by other types of tissues than the bark, the top contain more of juvenile wood that the lower part of the stem, and the branches contain much reaction wood.

2.2.3 Macroscopic Structure of Wood

Wood is mainly composed of elongated cells, oriented in the longitudinal direction of the stem. They are connected by openings, pits. The cells vary in shape and function and differ in hardwoods and softwoods. The cells provide mechanical strength to the tree, perform liquid transport as well as the storage, and transport of reserve nutrients and resin. In softwoods the mechanical strength and liquid transport is performed by *tracheids*. In hardwood the *libriform fibres* (often called just "fibres") perform the mechanical strength, whereas the *vessels* transport the liquid. *Parenchyma cells* transport and store nutrients in both hardwood and softwood. These cell types will be further discussed in Chapter 3.

Figure 2.10 shows the latitudinal cut of a stem. The *phloem* is situated directly under the bark[19] and transports the nutrients from the leaves/needles or root through the branches and the stem. Some hardwoods, like lime-trees, have long strong plant cells, bast-cells, in the phloem that can be used for making ropes and similar products. In the *cambium*, under the phloem, the

[19] The bark represents for most mills a side product that that is burned for generation of energy. Due to dark colour and high content of extractives remaining bark pieces in the pulp often represents a problem at the mill. However, several drugs and spices are extracted from bark and cork from the bark of the cork oak is a product with many applications.

growth of tree takes place. The cell-dividing tissue is called *meristem*[20], and except in the cambium it exists in tips of branches, stem and roots[21]. The xylem, or wood, is organised in concentric growth rings. The outer part of the wood is called *sapwood*, whereas the inner part of the wood in many trees consists of *heartwood* (*Figure 2.8*). The heartwood does often have a darker colour than the sapwood. This is due to that the heartwood is impregnated with various extractives (these are described in Chapter 7) that work as a natural protection against microbial attack[22]. Heartwood is therefore used in constructions for special purposes as window frames etc. The *pith* represents the tissues formed during the first year of growth[23]. It can be seen as a dark stripe in the middle of the stem and branches (*Figure 2.8*). The wood close to the pitch, the *juvenile wood*, is created during the first years (10–15) of growth when the plant had more "herbal" properties. The cell walls of juvenile wood have different, and mostly less good, properties than other wood.

The outer bark and the heartwood are dead tissues, whereas the cambium and the phloem consist entirely of living cells. The sapwood has a special situation, although the majority of the volume consist of dead cells (for some tropical hardwoods, however, can the majority consist of living parenchyma cells), it is wrong to see it as a dead tissue; the water and mineral transport (that are discussed below) is carried out here, and other biological activities as defence against parasites and nutrition storage is carried out by living parenchyma cells. One can ask why the sap wood is converted to heart wood. The heartwood play an important role as mechanical support of the tree, but that role could as well be plays by sapwood. Although any definite answer to the question is not known, it seems reasonable that the conversion simply is an ageing phenomenon, where the tissue simply dies after a number of years. This idea has some support in that very old trees, as many thousands of years old redwoods, generally have a very large heartwood compared to its sapwood[24].

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[20] There are two types of meristems; the one actual in the present case (under the bark of stem, branches and roots) are called *lateral meristem* and are responsible for thickening of these plant parts, *secondary growth*. *Apical meristems* are present in the tips of stems, branches and roots and are responsible for the growth in length, the *primary growth*. Not all plants have secondary growth.

[21] All living cells of plants can, however, be reconverted to meristem cells. As a reaction to damage on a tree a growing callus can be created from for instance phloem cells, which heal the wound. Principally a complete plant can be created in laboratory from a single living cell. More about different plant tissues can be found in textbooks of plant physiology.

[22] As will be described in chapter 10 the resistance of heartwood against microbial degradation is, however, not absolute.

[23] The first year at *this level* of the stem. The top part of the tree will be a pitch when the tree grows.

[24] An interesting phenomena is that the heartwood in a living trees often are more subjected to the wood rotting fungi described in chapter 10 than the sapwood, for example are old oaks often hollowed, i.e., the heartwood is totally degraded, whereas in dead wood, sapwood is less resistant to rot than heartwood. This is explained by that the water content in the sapwood in a living tree is an obstacle for fungal attack, so is also various defence systems in the living parenchyma cells.

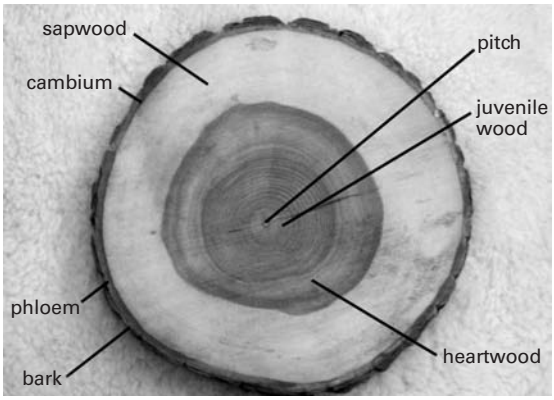

Figure 2.10. The different parts of wood in a Scots pine stem. The *pitch* is the plant from the very first year on this level and is often visible as a dark and sometimes soft spot. The *heartwood* contains more extractives than sapwood and has mostly a darker colour. All cells are dead. The central part of the heartwood consists of *juvenile wood*. The *sapwood* is mainly dead, but have a few living cells. The tree here performs water-transport. The *cambium* is the region where the growth, i.e. the cell division, takes place. The new cells inside the cambium became cells in sapwood (xylem), and outside cells in the *phloem*, living cells that are responsible for the nutrition transport. The outermost layer, *the bark*, consists of dead cells. A similar organization occur in other hardwoods and softwood, but sometimes the heartwood is less dark (spruces) or even lacking (birch). The bark can be considerably thicker (some oaks) or thinner (Eucalyptus).

Figure 2.10 exemplifies a *transverse view*, i.e., when the tree is cut across the stem. When the tree slice is seen from the bark and in, it is called a *tangential view*, and when the tree is cut across showing the cells running from the bark to the pith it is a *radial view*. This shows how ray cells extend from the outer bark either to the pith, or to an annual ring. The ray cells, that transport resin, are called *ray parenchyma cells*. They are located in 90º angle towards the longitudinal tracheids and in direction between the cambium layer and the heartwood (*Figure 2.11*). The different cell types in wood are further discussed in chapter 3.

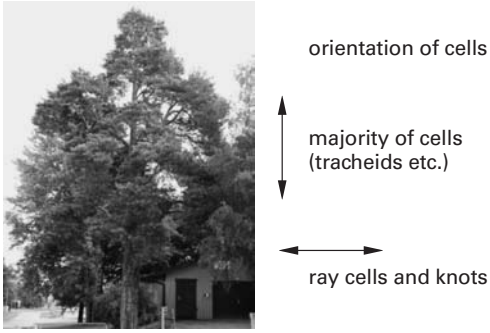

Figure 2.11. Direction of cells in wood.

2.2.4 Reaction Wood

Reaction wood is formed when the tree grows under stress (*Figure 2.12*). The purpose of the reaction wood formation is the same in softwoods and hardwoods, i.e. to enable the tree to grow in an upright position. The methods for forming reaction wood is however different in softwoods and hardwoods. Softwoods form compression wood on the lower part of the deviation to "push" the tree back in position (*Figure 2.13*). Hardwoods form tension wood on the upper side of the deviation, to "pull" the tree back in position (Figure 2.13).

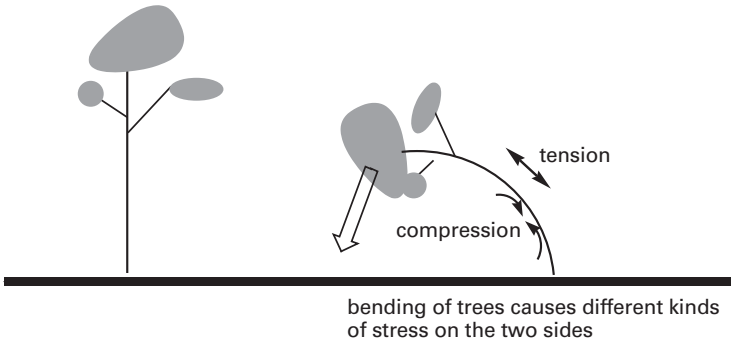

Figure 2.12. A plant under stress causes two kinds of stress.

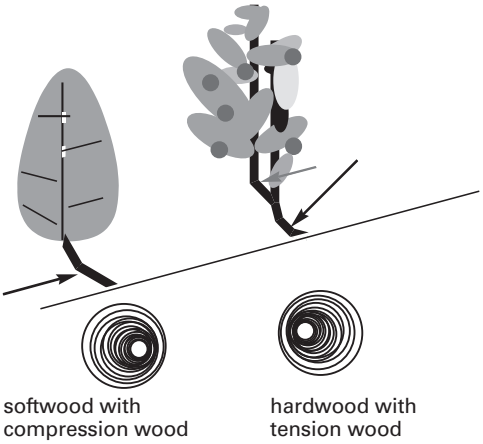

Figure 2.13. Schematic illustration showing compression wood formation in softwoods (left) and tension wood formation in hardwoods (right).

The cells formed in the reaction wood are the same as in normal wood, but the cell-wall structure and composition is different. Compression wood tracheids are more circular than normal tracheids. Intercellular spaces occur between the tracheids. The S2-wall contains helical cavities and the cells have no S3-layer. Compression wood tracheids have higher lignin content and also a different lignin structure than normal tracheids (Chapter 6), and contain a special

hemicellulose, *galactan* (Chapter 5). Tension wood fibres have a special gelatinous layer (G) instead of S3. The gelatinous layer is rich in ordered cellulose (Chapter 4) and contains a special hemicellulose, *arabinogalactan* (Chapter 5).

It shall be noted that also an apparent branch-lacking stem in general contains overgrown branches *inside* the stem. Therefore, most stocks contain some reaction-wood, although it appears to be branchless.

2.3 The Living Tree

2.3.1 Photosynthesis

All plants get all their energy from the photosynthesis, with the exception of some parasites (Pinesap, Mistletoe), saprophytes and carnivorous plants (Venus flytrap, Sundew)[25]. As mentioned above the photosynthesis takes place in chloroplasts, small organelles inside the plant cell. Chloroplasts are present in needles and leafs, but also on the surface of young branches, and in the case of herbs often al of the over-soil part of the plant. The process is complex and occurs in several steps, but the sum of the reactions is:

$$n\ CO_2 + 2n\ H_2O + \text{light} \rightarrow n\ O_2 + (CH_2O)_n + n\ H_2O$$

$(CH_2O)_n$ represents here primary a section in a carbohydrate. Here, only the general pattern of the process will be outlined. For details see a larger textbook in biochemistry. The chloroplast has a double other membrane. In the "cytosole", or *stroma*, numerous small membrane bags, *thylacoids*, are located. On these membranes a green-coloured molecule[26], chlorophyll, is located (*Figure 2.14*). A photon excites electron in this pigment and these are further transported in an electron chain leading to the generation of a pH gradient over the thylacoid membrane. This gradient gives the energy to a membrane protein that forms ATP from ADP and inorganic phosphor. Simultaneously water is oxidised to molecular oxygen (O_2), $NADP^+$ is reduced to NADPH[27]. These reactions constitute together the so-called "light reaction" in photosynthesis.

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[25] The mistletoe is a partial parasite, as well as the Venus flytraps and sundews are partial raptor. Both have also own photosynthesis. Pinesap is however complete without photosynthesis.

[26] Other photosynthetic organisms use pigments with other colours, as red or brown. Also green plants can use additional pigments to the chlorophyll.

[27] Adenosine triphosphate (ATP) to adenosine diphosphate (ADP) is in biosynthesis generally used as a thermodynamic fuel, driving unfavourable reactions. NADPH is the reductant used in biosynthesis. See a textbook in biochemistry.

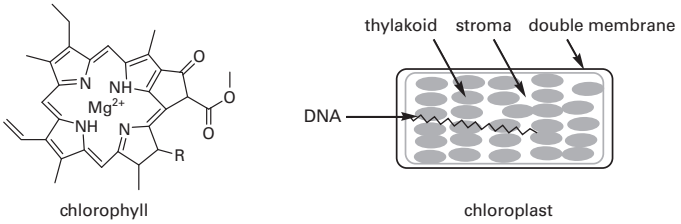

Figure 2.14. Chloroplast and chlorophyll. Chlorophyll is the green pigment that catches energy rich photons in light, and this energy is used for fixating carbon dioxide and water to simple carbohydrates. The chlorophyll is located in small membrane bags *thylacoids* that is located in a special organel, the *chloroplast*, inside the plant cell. The chloroplast carries own DNA, and originates from cyanobacteria living in synergism with plant cells.

In the "dark-reaction", or *Calvin-cycle* carbon dioxide is fixated under consumption of the ATP and NADPH created during the light-reaction. The reaction is carried out in several steps and is catalysed by several enzymes. The end product is glyceraldehydes – 3-phosphate that is further modified in different pathways inside the plant cell into higher saccharides, amino acids and precursors for lignin etc.

Light is thus the main energy source for most plants, and it is the struggle for the light that is the main force behind the evolution of originally small plants into the large contractions of trees, and thereby also behind the development of the complex composite material of wood.

2.3.2 Nutritional needs and transport

Plants are in general *photo autorophic*, i.e., they can synthesise all organic matters needed for the organism from inorganic chemicals by the help of light. The carbon needed is fixated from CO_2 mainly in leafs/needles as described above, but other minerals needed are taken up by the root system. Most of the vascular plants, including the important trees for pulp and paper industry, form mycorrhiza, a symbiotic relationship with filamentous fungi, where the plant gives sugar to the fungi, that in turn provides the plant with minerals and water. The fungal mycelium works therefore as an expansion for the root system. The most important minerals needed for the plants are nitrogen (as nitrate or ammonium), phosphorous (as phosphates), sulphur and different metal salts, as iron, manganese, potassium and cupper. Different trees vary in their need of nutrition and water and therefore diverse species dominates in different environments. Pine has for instance lower requirements than spruce. Therefore pine dominates on "poor" soils, whereas spruce is the dominating tree on soils rich in nutrition. Some plants lives in symbiosis with nitrogen fixating bacteria, that provides the plant with nitrogen fixed from the N_2 in the air[28]. Plants that trap insects (as the Venus flytrap) do probably this mainly for getting nitrogen from the chitin and protein in the animal.

The transport of nutrients in a tree is mostly that sugars and other organic molecules are transported from the leafs/needles downward and that nitrogen; phosphorous and metal salts are transported upwards from the root together with water in the sapwood. At mentioned above the

[28] The nitrogen fixating bacteria are normally located in the root. Leguminous plants and cycads are examples of such plants.

transport is mainly performed in the phloem just under the bark. This explains why large damages of the bark might be so harmful for trees. The different flows in a tree are summarized in *Figure 2.15*.

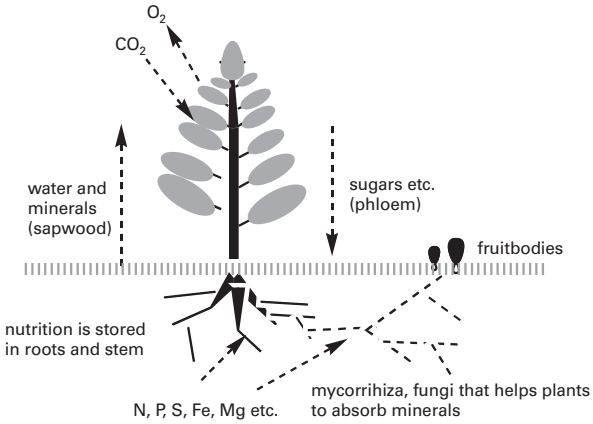

Figure 2.15. Schematic presentation of flows in a tree.

Water transport in wood cells is made possible by pits. Pits are recesses in the secondary wall between adjacent cells. Bordered pit pairs are typical of softwood tracheids and hardwood fibres and vessels. Simple pits without any border connect parenchyma cells with one another. The water-transport is of fundamental importance for plants. As shown above, water is consumed during photosynthesis – however it is only a very minor fraction of the water transported from roots to leafs that is consumed during this process; the large majority of the water is evaporated from the leafs, and this is important for the temperature control of the leafs (if the leafs was overheated the proteins performing the photosynthesis and other important reactions should be destroyed.

How is the water transported from the roots up to the leafs in the trees – a distance that can be more than 100 meters? There is no simple answer to the question and the process is still not fully understood. Several factors do probably contribute; the roots adsorb water from the soil by an *osmotic pressure* that is created by molecular pumps (consisting of special proteins), that transports ions from the soil over the cell membrane into the root cell. This generates an overpressure in the root, which presses up water (with dissolved minerals) in the vascular tissues of the wood (tracheids and vessels). Furthermore, interactions between the water and the cell walls of the vascular tissues generate *capillary forces* that further press up water in the sapwood. In the leafs there is a large evaporation of water as discussed above; this generate an osmotic overpressure, since salts will be concentrated at leafs, water will be 'sucked' from vascular tissues in the wood. Probably, living parenchyma cells in the wood play an active roll in regulating the pressure in the trachids/vessels, thereby avoiding the formation of air-bubbles blocking the flow. In summery, there are several mechanisms involved in the water transport and it is difficult to judge which is the most important.

2.3.3 The Growth of the Tree

The tree grows by cell divisions in meristem-tissues in the cambium under the bark, and in tips of stem and branches. Tracheids, fibres and vessels die when they are mature. Parenchyma cells are, however, living cells. The growth of all the cells in the tree is continuous although it is fast in the spring, slower in the summer and fall, and very slow during winter. This way the annual rings are formed; large earlywood cells in the spring when the water supply is large. In the summer the cells grow slower and become thicker, latewood. *Figure 2.16* shows the transverse view of the early- and latewood cells in softwood. The earlywood tracheids to the left are large and thin-walled, to enable a fast liquid transport.

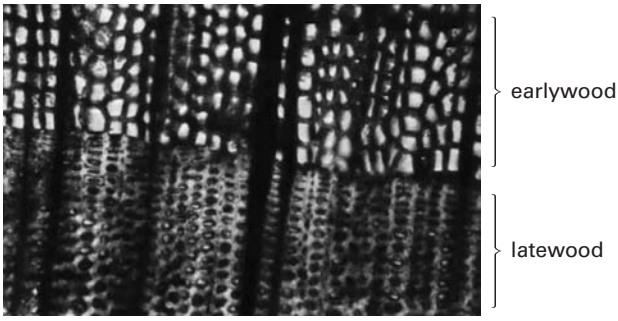

Figure 2.16. The transverse view of the early- and latewood cells in softwood (Scots pine). The difference between the thick walled latewood- and the thin walled earlywood tracheids will be visible in the wood as year rings. Year rings can be formed in other ways in hardwood as discussed in chapter 3.

At a certain age the sapwood of the stem of most trees begin to change to completely dead heartwood, and its proportion of the stem becomes successively larger as the tree grows. The dying parenchyma cells produce organic deposits such as resins, phenolic substances and pigments (cf. Chapter 7). In softwoods the bordered pits are closed when the torus is pressed towards either side of the border. In some hardwoods (such as oak) the vessels are closed by tyloses, which come from the neighbouring parenchyma cells. The anatomical and chemical differences between sapwood and heartwood have a significant effect in mechanical and chemical pulping.

2.4 Raw Material for the Pulp and Paper Industry

Availability as well as suitability determinates what raw material that is used by the pulp and paper industry. As will be described below sawmills and pulp mills often share the same raw material, i.e., side products of sawmills are used for making pulp. This means that an ideal tree for forestry both shall be as suitable for carpentry/construction purposes, as for pulp and paper. Properties of the wood that are important for pulping are *density* (for transport and packing of the boiler), *colour* (pulps made of whiter wood requires less bleaching, especially mechanical pulps), *homogeneity of wood cells*, *content of extractives* (high content of extractives gives problems especially in mechanical pulping) and *length of the wood cells* (important for the pa-

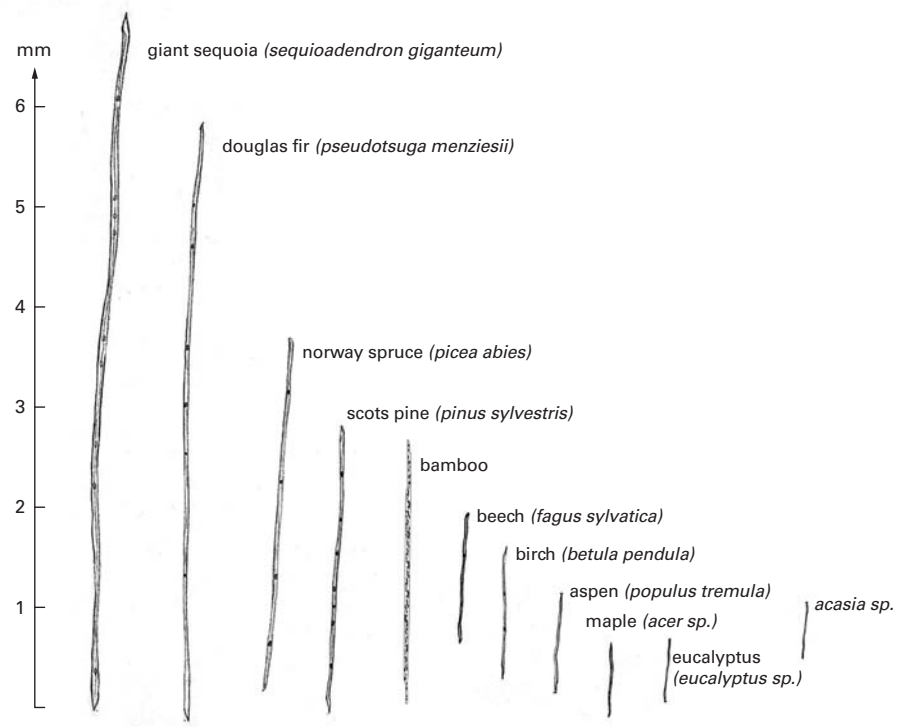

Figure 2.17. Fiber lengths of different trees. The relative length of tracheids and libriform fibers from some important wood species are shown. Fibrer length may vary considerably with different growing place etc., and the fibres above shall only be seen as representative examples. The longest fibres in wood exist in the huge North American softwoods Douglas fir and giant sequoia. However, there trees has also tracheids of similar length (~3 mm) as ordinary softwoods as Norway spruce and Scots pine. In phloem of herbs as flax and hemp there are fibres that are several cm long. Also hardwood fibres vary in length; beech and birch have relatively long fibres (1.3 mm), whereas Eucalyptus and maple may have very short fibres (0.7–0.8 mm).

per strength, *Figure 2.17*). Globally, the most important fibre-source is hardwood- (eudicotyledonic) and softwood (coniferous) trees. The advantage of trees over other plant material as straw and other agricultural waste products (that in many cases are easier to pulp) is that they are abundant (at least in nations rich in forests), they are harvested around all the year and they are easy to transport to the pulp mill. The softwoods have generally longer wood cells than hardwoods and pulp made of softwood is therefore sometimes referred to as "*long fibre pulp*", whereas hardwood pulps are called "*short fibre pulp*" (see *Table 2.3*). The softwoods are suitable for mechanical pulping and for strong papers made of chemical pulp. Hardwood is mainly chemically pulped (but for instance aspen is however also mechanically pulped), and these pulps have often good formation properties and are used for such applications as fine paper. Long- and short fibre-pulps are often mixed in order to obtain suitable properties, in boards different layers can be made of softwood and hardwood pulps respectively.

Table 2.3. Average fibre length and chemical composition in different wood types.

Wood type	Fibre length, mm	Cellulose, %	Hemicellulose, %	Lignin, %
Temperate softwood	2.5-4.5	40-45	25-30	25-30
Temperate hardwood	0.7-1.6	40-45	30-35	20-25
Eucalyptus	0.7-1.5	45	20	30

In addition to plant raw material, recycled papers is a more important fibre-source for the pulp and paper industry, while the historically significant rags, nowadays is less used. For some special papers, also synthetic fibres like glass fibres can be used. *Figure 2.18* shows the individual proportions of the fibre sources.

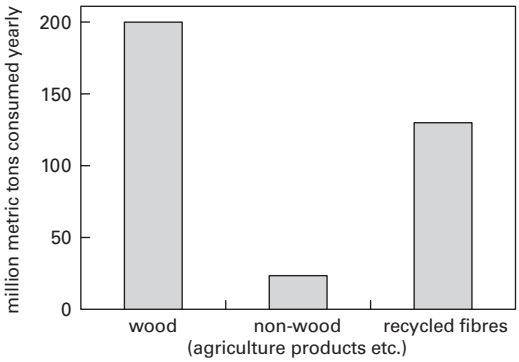

Figure 2.18. Source of raw materials for the pulp and paper industry. The majority of the pulp is made from pulpwood, but non-wood raw material (mainly agricultural products as straws from wheat, rice etc.) is locally important and for special paper qualities. The recycled fibres importance is increasing.

2.4.1 Softwoods

Most soft wood species used for pulping belongs to the pine family. Spruces (*Picea*) have long fibres and white xylem, and are very abundant, especially in Scandinavia (Norway spruce, *Picea abies*). It is therefore not surprising that this genus is the most used soft wood raw material both for mechanical and chemical pulping. Also trees from the Pine genus are widely used for especially kraft pulping, although that the content of extractives andare higher than for spruces and the tracheids are somewhat shorter. Scots pine (*Pinus sylvestris*) is the most important species in Europe. Larches are common in central Europe and in Siberia, but they are not important raw material for pulping, although that the tracheids are longer thatn for spruces, due to the very large heartwood in these trees that lead to high content of extractives. The forests in North America are richer in the number of species than European forests, and different kinds of pines are important for the pulp and paper industry. Douglas fir from western part of Canada gives pulp with very long tracheds that gives strong papers of high quality. Other important North American species are hemlock, several types of pines and spruces and also some softwood belonging to other families than the pine family, western redcedar (a cypress plant) and the huge redwood tree (a swamp cypress). Some North American softwoods have been widely spread

around the world as the contorta pine (*Pinus contorta*) in temperate areas (including Scandinavia) and the Monterey pine (*Pinus radiata*) in mediterian and subtropical climates (*Table 2.4*).

Table 2.4. Examples of wood species used for pulping and their average basic gravity range.

Species	Latin name	Basic density, kg/m^3
Softwoods		
Norway spruce	Picea abies	380–390
White spruce	Picea glauca	340–370
Black spruce	Picea mariana	380–420
Scots pine	Pinus sylvestris	390–420
Jack pine	Pinus banksiana	380–400
Western hemlock	Tsuga heterophylla	380–420
Douglas-fir	Pseudotsuga menziesii	410–450
Balsam fir	Abies balsamea	340–350
Western redcedar	Thuja plicata	290–310
Hardwoods		
Birch	Betula verrucosa	480–550
Quaking aspen	Populus tremuloides	350–400
Oaks	Quercus spp.	570–810
Maples	Acer spp.	440–550
Eucalyptus	Eucalyptus spp.	400–600
Beech	Fagus sylvatica	560–580

2.4.2 Hardwoods

A very large number of hardwood species are used in pulping, especially in tropical and subtropical contries with low access to softwoods. In some cases, rainforest is harvested and used for pulping giving mixtures of maybe hundreds of species. This quality is called *mixed tropical hardwood (MTH)*, and is often related with large problems with extractives. The trend is that MTH is replaced by plantations of Eucalyptus and Acasia. Eucalyptus is also cultivated in for instance Portugal and Great Britain. In temperate areas and within the taiga, the choose of hardwood species is more specific. In continental Europe beech is used for short fibre pulps, and also oak to some extent, although this tree give pulp of rather low quality and is not used in Sweden. In Scandianavia, birch is the preferred hardwood raw material mixed with some aspen and other hardwoods. In Russia aspen is used and in North America poplar and maple among others (*Table 2.6*).

Table 2.5. Examples of important softwoods for pulp and paper production.

Species	Distribution	Comment
Scots pine (Pinus sylvestris)	Europe, North Asia	Important for saw mills and kraft pulping in Fennoscandinavia
Black pine (Pinus nigra)	South Europe	
Aleppo pine (Pinus halepensis)	South Europe, Meditiarian region	
Contorta pine (Pinus contorta)	Rocky mountains. Introduced in Scandinavia and Scotland.	This fast growing tree is the most important introduced species in Sweden.
Weymouth pine (Pinus strobes)	North and East North America	Mostly for saw mills sensitive for a Scandinavian fungal parasite,
Slash pine (Pinus elliotti)	Southeast USA	
Caribbean pine (Pinus caribiae)	Central America. Introduced in south Asia	Common in plantations
Monterey pine (Pinus radiata)	California. Introduced in South America, South Africa Southwest Europe, Australia and New Zeeland	Maybe the most important plantation soft wood.
Norway spruce (Piciea abies)	Europe and Siberia	Longer thracheids and lower extractive content than pines. The most important raw material for Scandinavian industry.
Engelmann spruce (Picea engelmannii)	The Rocky mountains	
Black spruce (Picea mariana)	Canada	
White spruce (Picea glauca)	Canada	
Sitka spruce (Picea sitchensis)	Alaska, Western USA, introduced in United Kingdom	Important for mechanical pulp in UK
Western Hemlock (Tsuga heterotallica)	Western North America	Similar properties as pine
Douglas fir (Pseudotsuga menzisii)	North and West North America	Very long tracheids
Western redcedar (Thuja pliceta)	North and West North America	Belongs to the cypress family. One of the few important soft woods that is not a pine-plant!
Redwood (Sequoia sempervirens)	North American West coast. Introduced in Western Europe and New Zeeland	Belongs to swamp cypress family. Wood is very resistant to rot. Long tracheids. Excellent for constructions.

Table 2.6. Examples of important hardwoods for pulp and paper production.

Species	Distribution	Comment
Eucalyptus species for instance *Eucalyptus grandes* and *Eucalyptus globulus*	Australia. Widely cultivated in subtropical and temperate areas.	Very fast growing. Short fibres. Most important short fibre source.
Acacia species, for instance *Acacia melanoxylon, A. homalophylla and A. magnium*	Tropical old world and Australia.	Common in plantations in tropical areas, as Indonesia.
Birch, many species for instance *Betula pendula*	Taiga and temperate areas	Important both for carpentry and kraft pulp. Pitch problems.
Aspen (*Populus tremula*)	Taiga and temperate areas.	Very fast delignification in kraft pulping. Used for chemotermo-mechanical pulp.
Beech (*Fagus sylvatica*)	Continental Europe and south Scandinavia	Important for carpentry and chemical pulping.
Maple, many species for instance *Acer campestre*, *Acer platanoides*	temperate and subarctic areas	Very short fibres. Used in North America for kraft pulping.
Poplar, very many species for instance *Populus balsamifera, Populus fremontii* and *Populus nigra*	Globally	Fast growing and easy to deligify.

2.4.3 Pulpwood and Sawmill Chips

A distinction can be made between chips from pulpwood and sawmill chips. In the case of pulpwood, the whole wood stem, excluding bark, is chipped. The pulpwood consists to a large degree of the tips of trees and of young trees. Therefore the juvenile wood is over represented in this raw material. Sawmill-chips derive from the outer parts of the tree, and consist therefore mainly of sapwood. They constitute the waste after sawing the log into boards. Sawmill-chips have longer fibres and higher density compared to pulpwood chips of the same wood specie. Sawmill-chips give therefore often a higher quality pulp than pulpwood.

2.4.4 Density of Wood

The density (weight of oven dry wood per unit volume (kg/m^3)) is an important factor due to transportation of wood. Wood density, however, greatly depends on the moisture content of the wood, since the weight of the wood changes with variations in moisture. Additionally, higher moisture content expands the wood and a lower moisture content leads to shrinking of the wood. The true density of dry wood is not easily determined. Measuring devices using beta-rays or x-rays and electronic equipment is necessary are needed and therefore the true density of wood is not used as a routine test of wood quality. The *mass density* of wood is measured at the actual moisture content of the tree and this value varies accordingly with the moisture content. Freshly felled trees in the temperate zone have mass densities somewhere around 800 to 1000 kg/m^3, and tropical hardwoods have usually mass densities above 1000 kg/m^3.

The *basic density* is defined as the oven dry wood mass divided by volume of wood when the moisture content is above fibre saturation point. Common pulpwoods have a **S**pecific **B**asic **D**ensity in the range 350 to 650.

The parameter Basic Specific Gravity or Relative Density is defined as the oven-dry wood weight divided by the weight of water (1000 kg/m^3) displaced by the wood sample in its water-swollen state. Basic Specific Gravity is a pure number and has no unit. The relative density lies around 0.3 to 0.6 for most pulpwoods. Latewood fibres have thicker fibre wall and smaller lumen volume and thereby form wood of higher density than early-wood. Juvenile wood, with low content of latewood fibres, has lower density than mature wood.

2.4.5 Other Raw Materials

Paper is also made from non-woody raw material. In some cases the entire plant is used[29], in others specific cell types, as seed hair in cotton, and long strong bast-fibres[30] as for flax, ramie and jute. Non-wood raw material can be used for at least three reasons:

- *Lacks of sufficient amounts of forests.* Example here is China, where a large part of the paper is made of different straws (wheat, rice etc) from the agriculture. The logistic problems (i.e., collection of the straws) are solved by that the pulping to large extent is carried out in small scale on the farms. A drawback with grasses as raw material is the content of silica in the straws that obstruct recovery of the chemicals.
- *The raw material is available very cheap.* An example of this is the paper production in Cuba. In the large sugar mills of this sugar exporting country, large amounts of bagasse are produced, that are used as raw material for paper production. Another example is the paper production in Italy of algae, collected in the Adriatic Sea.
- *Special quality papers that cannot be made of wood pulps.* Currency-, tea bag-, electrolyte-, cigarette- and bible papers are examples of such qualities, as well as filter paper for laboratory use. The raw material is for instance cotton and linen (flax) fibres that have been rejected at textile manufacture. Abaca (a monocotyledon related to the banana) and hemp are examples of plant cultivated for these purposes. The material can be chemically pulped with soda pulping, but cotton can also be beaten to pulp with valley beaters. Rags (mainly cotton, but also linen) have historically been an important raw material and are still used for handmade paper used by artists and high quality book paper.

After harvesting the actual crop, the waste of some agricultural plants is used for pulping. Examples are straw from rice and cereals, stalks of corn and cotton and bagasse (the sugar cane waste after pressing out the sugar juice). Other plants are grown specifically for their fibre content. For example bamboo species are commonly used[31]. The bast fibres of jute, hemp and kenaf

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[29] In some cases, like pulp made from wheat straw, a separation of different kinds of fibres are necessary after the pulping, since some short cells lower the paper quality. This separation is mostly done by floatation.

[30] Many eudicotyledonic herbs have are long and very strong bast fibres located in the bark. The often very strong fibres are normally glued together in bundles by middle lamella rich in pectin. This is removed by a microbiological process, retting. The fibres are used mainly for textiles, but also for papers and composites.

[31] The wood of bamboo can also be used for construction and carpentry. Young plants are eaten as vegetable.

are used for paper making in addition to rope and sack production. Grasses and reeds as well as leaf fibres of sisal and abaca belong to non-wood plants used in the pulp and paper industry.

The main differences between non-wood and wood composition are the contents of lignin, extractive and inorganic material. In general, non-wood plants have lower amount of lignin, which makes them easily delignified. Some agricultural fibres have high amounts of extractives on the other hand, that is a drawback, causing problems in pulping and papermaking equipment. The inorganic content is very low in wood, less than 0.1 %, whereas it can amount up to 5 % in non-wood. The main inorganic compound is silicon dioxide, leading to scaling in the pulping equipment. This is, however, mainly a problem in monocotyledonic grasses and not in eudicotyledonic herbs.

2.5 Forestry

Trees are cultivated for timber production in two main ways; firstly an extensive form, where the timber goes to both sawmills and pulp and paper with growth time up to 100 years or more. This is the dominating form of forestry in for instance Scandinavia and Canada. Secondly, trees can be cultured in more agricultural forms in plantations. This is common in many tropical and subtropical countries as Brazil and Indonesia. The trees (mostly hardwoods as Eucalyptus) might be harvested already after 5 to 7 years and are in some cases exclusively used for pulp and paper.

The following description is mainly about forestry according to the first form. A high ambition in regeneration, clearing and thinning is a prerequisite for a sustainable high yield of good quality timber from the forestland. In general, the forest industry wants straight trees of high density and with thin, frail branches.

The fibres created in the beginning of the growth season, earlywood fibres, have a lower density compared to latewood fibres. Softwoods growing fast will form a higher proportion of earlywood fibres and thereby have a lower wood density. It is therefore desirable for softwoods to have a slow growth rate in order to have high quality wood. It is especially important for a slow growth rate during the first 20 years, in order to reduce the amount of juvenile wood with shorter fibres. By limiting the softwood seedlings access to nutrients, light and water the wood quality can be improved. Many seedlings growing close to one another and competition from bigger trees will reduce the growth rate of the seedlings. Ring-porous hardwood, such as oak, ash, and elm, the opposite is true. The faster they grow, the more latewood is formed and the higher the density of the wood becomes. The diffuse-porous hardwoods, like birch and aspen, form vessels of more or less same size in the early and late growth season. The density of these trees is not affected by the growth rate.

Clearing the forest is to remove unwanted growth from it and gives ample possibilities to influence the future stand. By clearing, suitable wood species and high quality stems are favoured. In the subsequent thinning, the trees felled have coarser stems and will give more profit. In an uncleared stand, the trees become more slender and a slender tree costs as much to fell as a coarse tree, but gives less revenue. Not to clear is a waste of capital. On the other hand, if the clearing is too severe giving too much space to individual trees, these will have the coarsest branches and the highest amount of juvenile wood.

Thinning, also called intermediate cutting, can improve the quality of the remaining stand by proper selection of the trees to be felled. The thinning material is the main wood supply to the pulp and paper industry. In other words, pulpwood is primarily thinning material.

Fertilisers, mainly nitrogen, phosphorous and potassium (see 2.3.2), can be used to increase forest production. However, the high precipitation of nitrogen from fossil fuels makes it in most cases needless to add any more nitrogen. When the air polluting nitrogen and sulphuric oxides precipitate in the forest, they leach out alkaline cations, thus decreasing the pH of the soil. The removal of big amounts of biomass, when harvesting timber, disrupts the balance of the soil productivity. Lime or wood ashes can be supplied in order to counteract the acidifying effects of air pollutants. In Scandinavia fertilizing of forests are rare nowadays.

Moose, deer, and other game can cause damages on growing forest by grazing on shoots and branches, breaking stems and gnawing off the bark of trees. Hunting, to keep the number of game on a supportable level, is an important forestry measure. Game enclosures can be necessary in certain areas to keep grazing animals out. The grazing can also be manoeuvred to areas less sensitive, by providing special grazing pastures, supplying salt stones or supportive feeding. Chopping off hardwood shrubs about one meter above ground, gives new shoots and the game access to more feed.

2.5.1 Harvesting

Harvesting can be done in two ways, either one by one when the trees are large enough, or a more or less complete harvesting. The earlier is often performed in small scaled with relatively simple equipment; horses are still used for transports (elephants in south Asia). The latter is usually performed with a harvester, which fells the tree, trims it and cuts it to desirable lengths, using a computerized cut-to-length system.

The forwarder takes the logs to the deposit where they are separated into different assortments. Trucks deliver the logs directly to the industry or to the railway for further transport to the industry. The time it takes to deliver timber from the forest to the industry is quite short. The pulping industry needs fresh raw material and the cost of storage is high. The best quality of the full-grown timber goes to the sawmills. Lower quality (damages, moderate rotted) goes to the pulp and paper industry as pulpwood as well as cleaning and thinning timber.

2.5.2 Regeneration Scandinavian Style

After cutting the timber in an area, a new forest is usually established by *planting*. In the northern part of Sweden spruce and pine are planted to the same extent, whereas in southern Sweden spruce plants dominate. Hardwood is primarily planted on abandoned arable land. Ground preparation is usually a prerequisite for a successful planting. The soil is clarified from the undergrowth so that more light reaches the plant. The soil surface temperature is thereby increased leading to a decreased risk of frost killing the juvenile plants. Soil clarification also diminishes the competition from roots of other plants and favours the sowing of other tree species.

- *Sowing* seeds is only performed on a minor portion of the regeneration area. The probability of a successful sowing is higher for the northern parts of Sweden. In the more southern

parts, the temperature can alternate between a few degrees above and below freezing point, with a risk of freezing the small plant.

- *Natural regeneration.* This can be a good way to restore pine forest, providing it is a good seed year and required preparations have been made. Before cutting for timber, the best seed trees are selected (*Figure 2.19*) and left to provide for natural sowing. Soil clarification is generally necessary and should be performed on a year when there are plenty of seeds. Some 50–150 seed trees are required per hectare. Natural regeneration is as a rule the best for *hardwoods.* Birch can be regenerated by seeds as well as by stump shoots. Root shoots regenerate aspen. Hardwood regeneration generally requires game enclosure to fence out moose, deer etc that eat plants and shoots.

Figure 2.19. Natural regeneration. Some birch trees was saved after harvesting for naturally sawing new birch plants. Note that branches are left in the wood.

- *Shelterwood* offers many advantages for regeneration and can be used for softwood as well as hardwood. The function of the shelterwood is as seed trees and protection for the plants against competing shrubbery, frost damages and harmful insects. It has been shown that regeneration under high shelterwood decreases the attacks of pine weevil (*Figure 2.20*).

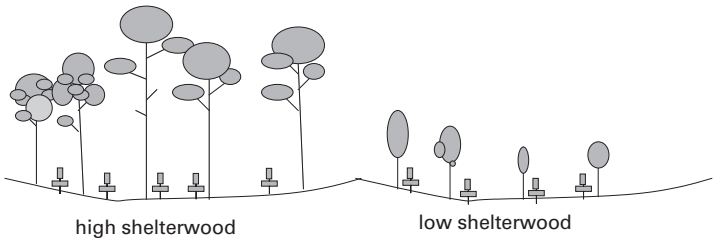

Figure 2.20. Shelterwood. Tall, storm resistant trees, mainly pine and occasionally hardwood, are chosen for high shelterwood. For low shelterwood, the hardwood appearing after harvesting timber is used to protect plants against frost.

The above-mentioned methods are based on harvesting practically all the timber in the cutting area, except for seed trees or shelterwood. Other forestry methods are gaining interest, calling for more expert knowledge and long-term planning. They include for example *edge felling* of spruce forest, in which 10–20 m wide rows are cut with 5–10 years intervals. The spruce trees along the rows will provide for the regeneration with their seeds. The age of the trees will be diverse row-wise in this kind of forest. *Group selection cutting* is a similar method, but instead of rows, circles of 20 m diameter are cut with 5–10 years interval. The openings are widened by 10 m at a time until the circles merge. The age of the trees will be diverse group-wise. In environmentally very sensitive areas, alternative forestry methods may be used, for example *selective felling* of trees with a certain diameter.

2.5.3 Plantations

As mentioned above plantations are a more agricultural type of forestry. The global trend is towards increased establishment of plantations. Some plantations are created in order to rehabilitate a certain environment or for soil and water conservation, but many plantations are an important source of raw material for the pulp and paper industry. The plantations account for some 5 % of the total forest area of the world, *Table 2.7.* The countries with major industrial plantations are China (37 million ha), the United States (16 million ha), and India (12 million ha).

Table 2.7. Forest plantation area by region. Plantations supplying raw material for industry account for half the area. (FAO report "State of the worlds forests 2001").

Region	Forest plantation area, (Million Ha.)	Plantations as % of the region's total forest
Africa	8	1
Asia	116	21
Europe	32	3
North/Central America	18	3
Oceania	3	2
South America	10	1
World	187	5

Some countries, such as for example Chile and New Zealand, have established plantations on large areas. They are able to not only supply the domestic industry with wood raw material but also export significant amounts of wood.

The tree species most commonly planted are in the genera *Pinus* (specially *Pinus radiata*), *Acasia* and *Eucalyptus.* Thus they are introduced in regions, even continents far from their origin[32]. Attacks by parasites (fungi or insects) that the trees have low resistance against are not unusual problems.

[32] Introduction of "foreign" species are not restricted to plantation forestry. *Pinus contorta* from North America have been introduced in Scandinavian forests and different American hemlock species in the continental Europe.

2.5.4 Environmental Considerations

The World Conservation Union (IUCN) has put up a classification system for protected areas shown in *Table 2.8*. Categories I and II are dominated by environmental considerations and are to be left unattended by human impact except for scientific purposes. Categories III and IV need management to preserve the special the specific natural feature or habitat. Categories V and VI are dominated by production goals.

The environmental value of a forest depends to a great deal on how it can provide for biodiversity. A variation in tree species grants a variation in species of other plants, insects, birds and mammals. However, natural monocultures of pine or spruce are biodiverse, if the age of the trees varies and many dead trees are available. Special landscape types, such as hillsides and streams, offer a habitat for a varied assortment of plants and animals. Some species are threatened by extinction, very rare or sensitive. They are denoted *red-listed species* and need special concern. In addition to environmental values, cultural values demand care. Examples are archaeological sites and old summer farm holdings. Most forests fall into categories V or VI. As an example, only 4% of Sweden's forestland is protected and excluded from wood production.

Table 2.8. The protected area management categories as defined by IUCN.

Category	Definition	Available for wood production
Category I Strict nature reserve/wilderness area	Protected area managed for science or wilderness purposes.	No
Category II National park	Protected area managed for ecosystem protection and recreation.	No
Category III Natural monument	Protected area managed mainly for preservation of specific natural features.	No
Category IV Habitat/species management area	Managed mainly for conservation through management intervention.	No
Category V Protected landscape/seascape	Area of land where the interaction of people and nature over time has produced an area of distinct character. Safeguarding this traditional interaction is vital to the protection and maintenance.	Yes
Category VI Managed resource protected area	The area is managed to provide a sustainable flow of products and to ensure long term protection and maintenance of biological diversity.	Yes

The forests are decreased by harvesting roundwood as well as a result of forest fires etc. On the other hand, forest area increases, either by plantation or by natural growth of existing forests. Historically, the deforestation of our planet has been extensive and it still continues. Globally, there is a loss of forest area by more than 9 million hectares annually, *Table 2.9*. However, as also seen from the table, the loss is of tropical forests, whereas in non-tropical areas there is an actual gain in forestland.

Table 2.9. Annual change in forest area during a ten-year period from 1990 to 2000. Source: FAO report State of the world's forests 2001.

	Loss (million ha)	**Gain (million ha)**	**Net change (million ha)**
Tropical areas	–15.2	+2.9	–12.3
Non-tropical areas	–0.9	+3.8	+2.9
World	–16.1	+6.7	–9.4

2.5.4 Certified Forestry

Certification of the forestry signifies an agreement to follow certain obligations. It is a voluntary agreement with an independent party issuing the certificate and performing regular control. The certification provides the companies with an environmental management system with routines for an efficient and structured environmental work.

- FSC, *Forest Stewardship Council*, is an environmental management system with the aim to promote an environmentally sustainable forestry. These apply mostly for bigger multinational companies. As of 2001, some 22 million ha of forestland is certified by FSC.
- PEFC, *Pan-European Forest Certification*, and FFC, *Family Forest Certification*, aim to promote family forestry that adopts a responsible approach to environment as well as production and social standards.
- The International Organization for Standardization has an Environmental Management System, ISO 14001 and the EU provides a standard EMAS*, Eco Management and Audit Scheme*. These systems present a more general environmental management and do not provide forest management certification

2.6 Suggested Readings

Raven P.H., Evert R.F., and Eichhorn S.E. (1999) *Biology of Plants, 6th edition.* New York, NY, USA: WH Freeman and company Worth Publishers, ISBN 1-57259-041-6.

Kellomäki A. (1998) *Forest resources and sustainable management.* Helsinkki, Finland: Fapet Oy, ISBN 952-5216-02-0.

3 Wood and Fibre Morphology

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3.1 Introduction

Wood (i.e. secondary xylem) is produced by the seed-bearing plants and belongs to the Spermatophytae. Wood has a complex hierarchic structure that is responsible in part for determining the mechanical and physical properties of all its products including pulp (kraft and mechanical) and sawn wood. Ultimately as outlined below, these wood properties are governed by the wood structure particularly its anatomical organization and cell wall ultrastructure. Wood is classified into soft- and hardwoods; the former produced by gymnosperms (i.e. conifers) and the latter by angiosperms (i.e. desciduous or broad-leaf trees). Wood is comprised of numerous species and altogether ca 30 000 angiosperm and ca 500 conifer tree species are currently known, the majority of the angiosperms growing in tropical regions. Gymnosperms are trees such as pine (*Pinus*), spruce (*Picea*), and fir (*Abies*) while typical angiosperms include the hardwoods birch (*Betula*), beech (*Fraxinus*), oak (*Quercus*) and poplar (*Populus*). Hardwoods lose their leaves during the autumn while the conifers are normally evergreen accept for certain species like larch (*Larix*) that lose their needles during autumn. In Sweden the most common wood species used for pulping are spruce (*Picea abies*), pine (*Pinus sylvestris*) and birch (*Betula verrucosa*).

Diagnostically each tree can be divided into several main parts commonly referred as the crown, trunk (i.e. stem) and root system (see Chapter 2); each part in turn composed of different tissues that are ultimately comprised of individual wood cells. The trunk may be divided into: a) the bark -which is dead and provides protection from physical, mechanical and biological damage; b) the phloem which is *living* and allows for the transport of nutrients and storage products; c) the vascular cambium, a thin layer of cells which by repeated division produces phloem cells to the outside and xylem (secondary) to the inside, and d) the secondary xylem which constitutes the bulk of the woody material (*Figure 3.1*). The xylem is normally divided into the sapwood and heartwood; the former composed of living and dead cells and the latter comprised of normally entirely dead cells. Finally a pith is normally present at the center of the trunk and represents tissues developed during the initial years of tree growth. These tissues are often referred to as forming the juvenile wood of xylem. The xylem is normally organized into distinct concentrically orientated rings that are referred as annual growth rings; each ring representing one years´ growth (*Figure 3.1*). Each wood tissue is comprised of a variety of different cell types having different roles in the living tree (i.e. support, transport, storage) and which can behave differently in sawn and paper products. Differences arise from variability in the physical structure of the different cell types as well as differences in individual cell wall organization (i.e. microstructure and supra-molecular organization) and chemical composition.

Wood is composed of highly ordered axial and radial cell systems. The axial system is composed of primarily elongated cells orientated in the longitudinal direction of the trunk (*Figure 3.1*). These cells vary in both size and shape (i.e. cell wall thickness and cell length, see below) features which are related to their function, with thick-walled cells providing mechanical support and strength to the tree and thin-walled cells primarily providing liquid transport and the storage of nutrients. The radial system is orientated perpendicularly to the tree and is comprised of rays that form horizontal files of cells extending from the bark to the pith (primary rays) or to specific annual rings (secondary rays). The major function of the rays is to store and redistribute storage materials (e.g. starch). Rays may contribute 5–11 % of the total softwood volume and up to 30 % in hardwoods (e.g. *Quercus sp*.).

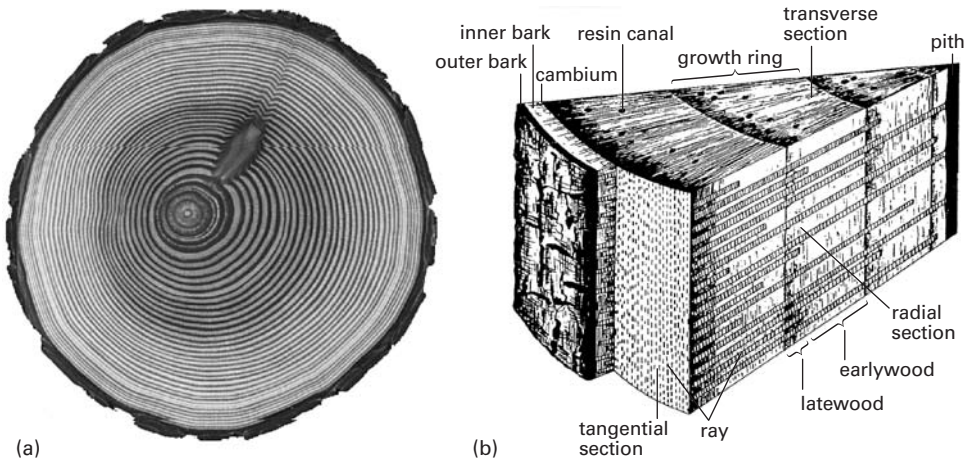


Figure 3.1. a) Transverse section of pine trunk showing the bark, sap-and heartwood regions, and the annual rings composed of early-and latewood. **b). Detailed sectional view of a young pine** stem showing its axial and radial organization and location of the major tissue types. Note that both the wood structure and its cell types can be viewed in transverse (TS), tangential longitudinal (TLS) and radial longitudinal (RLS) views.

The heartwood serves primarily as a support tissue and is present in all trees although the age at which formation begins varies according to wood species and prevailing environmental conditions. During heartwood formation chemical components known as extractives are deposited in the tissue cells making the wood less permeable and more durable (i.e. to wood decay by fungi, bacteria and animals). In certain softwoods and hardwoods like pine and oak the heartwood is more easily recognized by its darker colour while in other woods like spruce and birch the difference is not readily apparent (*Figure 3.1*).

The main difference between the sapwood and heartwood is the death of the living cells known as parenchyma cells (see below) and reduction in water conduction. In softwoods special pits (i.e. bordered pits) become closed (i.e. aspirated) and in some hardwoods the vessels become blocked by tyloses (see below). The mechanisms behind the increase in natural durability of heartwood against wood decay is a feature of considerable interest as an understanding of the process would represent a novel way for developing environmental acceptable methods of wood preservation.

3.1.1 Formation of Wood Cells

Wood cells are produced in the vascular cambium (*Figure 3.1*) from two special mother meristematic cell types known as the fusiform and ray initials; the former giving rise to all cell types of the axial and the latter the radial cell systems in both hard- and softwoods. In softwoods the axial wood cells (tracheids) are typically aligned in chains, with each line of cells derived from the same mother cell (*Figure 3.2a*). In hardwoods the situation is much more complex and distinct alignment is not apparent since a variety of different cell types (e.g. vessels, fibres, parenchyma cells) of varying sizes are produced (*Figure 3.2b*). The regulation and mechanisms

behind wood cell formation and what governs why specific cells (e.g. early- or latewood, vessels, tracheids or fibres in hardwoods) are produced is poorly understood. For more Information see Fujita and Harada (1991).

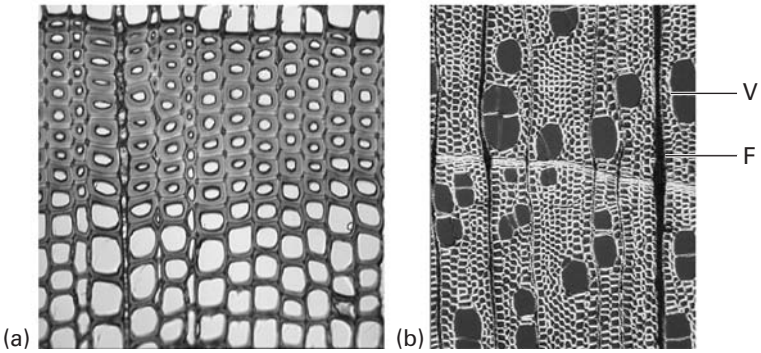


Figure 3.2. a) Transverse sections of pine and b) birch showing the microstructural organization of the annual growth rings. In pine the tracheids show alignment and appear uniform. In birch the alignment of fibres (F) is interrupted through development of vessel (V) cells. Birch shows a more complicated structure and vessels represent the most characteristic cell elements of hardwoods.

3.2 Softwood and Hardwoods

Softwoods are considered to have a much simpler structure than hardwoods and are comprised of a limited number and more uniform cell types (normally 3 axial and 2 radial) (*Table 3.1*). In contrast hardwoods are normally comprised of a greater number of axial (5–6 axial and 2 radial) cell types with much greater cell morphology; nevertheless all cell types are derived from the two meristematic cell types named above.

Table 3.1. Principle softwood cell types and their function.

Cell type	Function
1. Longitudinal tracheids:	
Earlywood	Conduction
Latewood	Support
Strand	Conduction
2. Parenchyma	
Ray parenchyma	Storage
Longitudnal parenchyma	Storage
Epithelial parenchyma	Secretion of resins
3. Ray tracheids	Conduction

3.2.1 Softwood Cell Types

Softwoods from temperate and artic regions form annual rings of tracheids that may be divided into early (i.e. forms during the spring) and latewood (forms during the summer); the former

cell types recognized by their large size and thin cell walls (s) and the latter cells recognized by their smaller radial dimensions and early-thicker cell walls (*Figure 3.3*). In softwoods from tropical regions, distinction between early- and latewood cells is more difficult due to the indistinct boundary caused by the lack of recognized seasons.

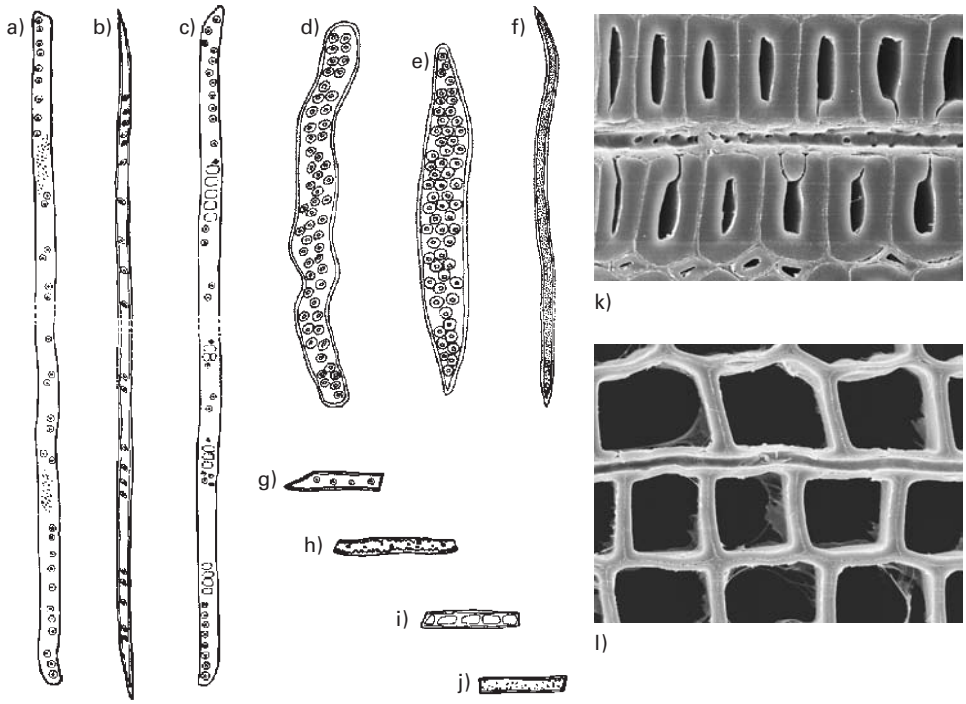


Figure 3.3. Softwood cells. a) earlywood spruce; b, c) early- and latewood pine tracheids; d) vasicentric tracheid; e, f) vascular tracheids; g, h) ray tracheids of spruce and pine; i, j) ray parenchyma of spruce and pine; k), l), Electron micrographs showing the early- and latewood tracheids of spruce.

Softwood xylem is composed of limited number of cells known as tracheids (*frequently referred incorrectly as fibres*), parenchyma and epithelial cells with tracheids normally forming between 90–95 % of the total cell volume and ray parenchyma between 5–10 % (*Table 3.3*). Because of their uniformity (homogeneity) and simplicity, the structure in softwoods tend to appear similar in appearance (*Figs. 3.2, 3.3*).

3.2.3 Softwood Tracheids

The tracheids forming the axial system are long slender cells often 100 times greater in length than in diameter and are rectangular to squarish in cross section (*Figs. 3.3, 3.4*). Tracheids have hollow centres (i.e. cell lumen, *Figure 3.4*) and closed ends which are rounded in radial or pointed in tangential orientation (*Figure 3.3*). In softwoods like pine, the earlywood tracheids are easily distinguished from latewood cells by their thin cell walls and large radial diameters

compared to the thick and smaller diameter latewood cells (*Figs. 3.2, 3.3*). The abrupt change from early to latewood is characteristic for some softwoods, like pine and larch whereas in other species like fir the transition is gradual.

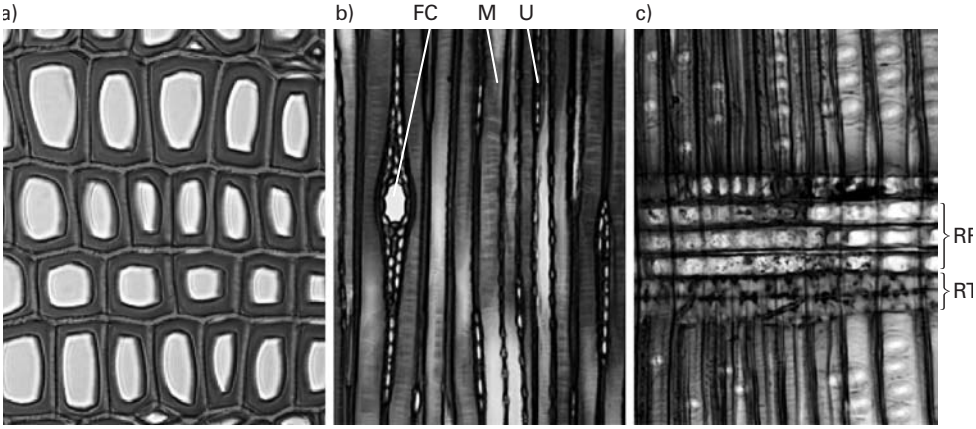


Figure 3.4. For comparison: a) Transverse (TS), tangential longitudinal (TLS) b), and radial longitudinal (RLS) c) appearance of spruce (a) and pine showing the structural organization of the axial and ray systems. In TLS, both the uniseriate (U) and a multiseriate (M) ray is shown. The multiseriate ray has a fusiform canal (FC) at its centre. Ray parenchyma (RP) and ray tracheids (RC) are shown in the TLS and RLS sections.

Tracheids of Swedish spruce and pine are normally 3.1–3.4 mm in length and 14–46 µm in diameter. Other softwoods like *Sequoia* however, produce much longer tracheids almost double that produced by spruce and pine (*Table 3.2*). The length and width of tracheids varies both within and between species and also within individual trees. The tracheids of latewood also tend to be slightly longer than those formed in earlywood. Tracheids have closed ends in contrast to that of vessels in hardwoods (see below) and pits represent therefore the major pathway for the penetration of fluids through the wood structure.

The major function of the tracheids is support (latewood) and the conduction of fluids (earlywood) (*Table 3.1*). Earlywood tracheids are thin-walled with a large cell lumina while the latewood cells are thick-walled and more rectangular in cross-section (*Figures 3.3, 3.4*), morphological attributes consistent with their function. Certain softwoods e.g. Douglas fir also possess spiral thickenings on the lumina wall of tracheids for added support.

Table 3.2. Length and width of typical softwood tracheids.

Wood Species	Tracheid length (mm)		Tracheid width (µm)	
	Mean	Range	Mean	Range
Norway spruce (*Picea abies*)	3.4	1.1–6.0	31	21–40
Scots pine (*Pinus sylvestris*)	3.1	1.8–4.5	35	14–46
Redwood (*Sequoia sempervirens*)	7.0	2.9–9.3		50–65

In certain softwood species (e.g. Larch) short, rectangular tracheids arranged in vertical series and possessing bordered pits are found. These cells are known as “strand tracheids” which are dead and function in conduction.

3.2.4 Softwood Rays

Softwood rays may be composed of entirely ray parenchyma (see below) or a combination of ray parenchyma and ray tracheids (*Figure 3.4*). Rays entirely composed of ray tracheids also exist in some species.

3.2.5 Softwood Parenchyma Cells

All softwoods possess parenchyma cells but the majority of these are normally found within the ray canals. Only a limited number of species possess longitudinal parenchyma, a feature that contrasts greatly with that of hardwoods (see below).

Ray parenchyma are living cells and are thin-walled and brick-like in appearance (*Figs. 3.3, 3.4*). They possess simple pits (see below) to allow liquid transport between the rays and the axial tracheid system.

Ray tracheids may occur at the margins of the rays (e.g. *P. sylvestris*) or within the more central regions together with the parenchyma cells (*Figure 3.4*). Morphologically ray tracheids appear similar in size to the parenchyma cells but are dead cells and possess smaller bordered pits than the axial tracheids. The function of ray tracheids is to provide liquid transport in the radial direction of the tree.

3.3.6 Longitudinal Parenchyma

In general the majority of softwoods lack this morphological cell type and when it does occur it is present in reduced amounts (*Table 3.3*). The cells are easily recognized by the presence of simple pits and frequently coloured inclusions. Swedish pine and spruce possess only traces of longitudinal parenchyma.

Table 3.3. Proportion of cells in some softwoods (Wagenfuhr & Scheiber, 1974)**.**

Wood species	**Percent of total volume**		
	(Early + late)	**Rays**	**Parenchyma**
European silver fir (*Abies alba*)	90.4	9.6	Trace
Norway spruce (*Picea abies*)	95.3	4.7	1.4
Scots pine (*Pinus sylvestris*)	93.1	5.5	
Redwood (*Sequoia sempervirens*)	91.2	7.8	1.0

3.2.7 Epithelial Parenchyma

Epithelial cells are specialized living parenchyma cells that line resin canals (*Figure 3.5*). They are present in both early and latewood. Their function is to secrete resins into the resin canals. The thickness of the epithelial cell walls varies and in pine they are thin-walled and in spruce and larch thick-walled – a feature which can be used for distinction between pines and spruces.

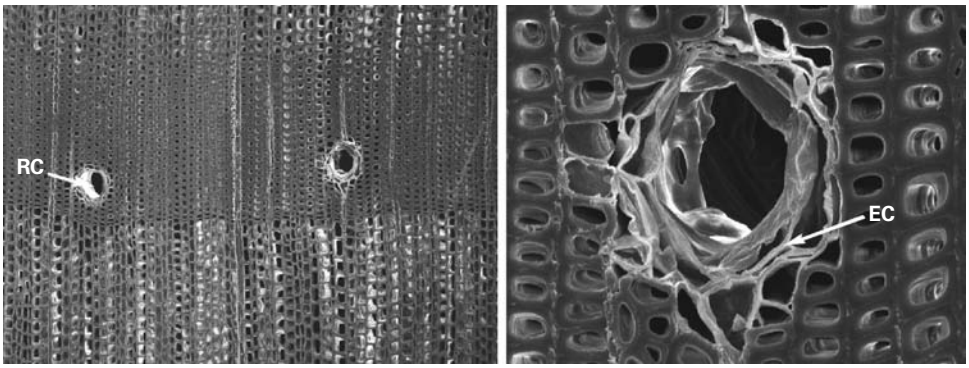


Figure 3.5. Tranverse sections of pine (*Pinus sylvestris*) showing position of ray canals (RC) in the latewood (left) and epithelial cells (EC)(right) that surround the canals. Trees under stress often develop large numbers of resin canals called "traumatic resin canals".

3.3 Resin Canals

Resin canals are frequently found in softwoods but are not present in hardwoods. Resin canals are not cells but rather represent intercellular spaces that build a network within the tree. The canals are surrounded by living epithelial cells in the sapwood and are responsible for secretion of resins into the canal lumen. Resin canals may also be found within the rays of some conifers where they are known as fusiform canals (*Figure 3.5*).

3.4 Anatomy of Hardwoods

Hardwoods are more advanced and complex in their general anatomical organization than softwoods. In addition they have a larger number of different cell types than softwoods (*Table 3.4*) including vessels, fibres (libriform fibres and fibre tracheids), and parenchyma (longitudinal and ray cells) (*Figures 3.6, 3.7*).

Vessels are comprised of single cells (sometimes called elements) which are joined end to end to form longitudinal tubes ranging from a few centimeters to several metres in length in the wood structure. Vessels represent the main conducting element in hardwoods and the cells have entirely open or perforated ends (*Figures 3.6, 3.7*), with species with open ends considered to be more evolutionary advanced. Both the size and cellular morphology of vessels varies between

Table 3.4 Major hardwood cell types and their function.

Cell type	Function
1. Vessels	Conduction
2. Fibres Libriform fibres Fibre tracheids	Support
3. Tracheids Vascular tracheids Vasicentric tracheids	Conduction
4. Parenchyma Ray parenchyma (homo and heterocellular) Longitudnal parenchyma	Storage

Table 3.5. Proportion of cells in some typical Scandinavian hardwoods (Wagenfuhr & Scheiber, 1974)

Wood species	Percent of total volume			
	Fibres	Vessels	Rays	Parenchyma (longitudinal)
Birch (*Betula verrucosa*)	64.8	24.7	8.5	2.0
Beech (*Fagus sylvestris*)	37.4	31.0	27.0	4.6
Ash (*Fraxinus exicelsior*)	62.4	12.1	14.9	10.6

hardwood species. Vessels appear in transverse sections of wood as holes, and for this reason hardwoods are known as “porous woods” in contrast to the “non-porous” softwoods.

Both the size and spatial distribution (arrangement) of vessels within the annual rings of hardwoods varies with wood species and this is used for descriptive purposes for separating hardwood types. For example, temperate zone hardwoods (*Figures 3.6* to *3.8*) exhibit three main forms described as: “diffuse-porous”, “ring-porous” and “semi-ring porous” arrangements (*Figure 3.8*). Diffuse-porous woods have vessels that are fairly uniform in size and are evenly distributed within the growth rings (e.g. Sycamore, birch, beech, willow). In ring-porous wood like Oak (*Quercus*), Ash (*Fraxinus*) and Elms (*Ulmus*), the earlywood vessels are much larger than those developed later in the growth season (*Figure 3.8*). In semi-ring porous woods, the vessels of earlywood are slightly larger or more abundant than latewood vessels (e.g. certain Poplar species, *Populus deltoides*, *P. tremuloides*). The diffuse-porous group is the most common group among papermaking hardwoods.

The cell walls of vessels are comparatively thin (*Figurs 3.6, 3.7*; *Table 3.7*) compared to that of other cell elements including the fibres and softwood tracheids and are highly ornamented with pits. The characteristic pitting of the vessel walls is used as a feature for the identification of hardwood species in pulps (Ezpeleta and Simon, 1970; Ilvessalo-Pfäffli,1995).

3.4.1 Hardwood Fibres

Hardwood fibres are divided into libriform and fibre tracheids and form together with vessels the basic tissues of hardwoods (*Table 3.4*, *Figure 3.6*). The primary function of fibres is to provide mechanical support to the tree, although fibres in some hardwoods also participate in water transport (*Table 3.4*). Fibres may contribute between 30–75 % of the basic hardwood tissue volume (*Table 3.5*) depending on wood species. Hardwood fibres are smaller than softwood tracheids, they have thicker cell walls, smaller cell lumina and the difference between early- and latewood is not so extreme as seen in softwoods.

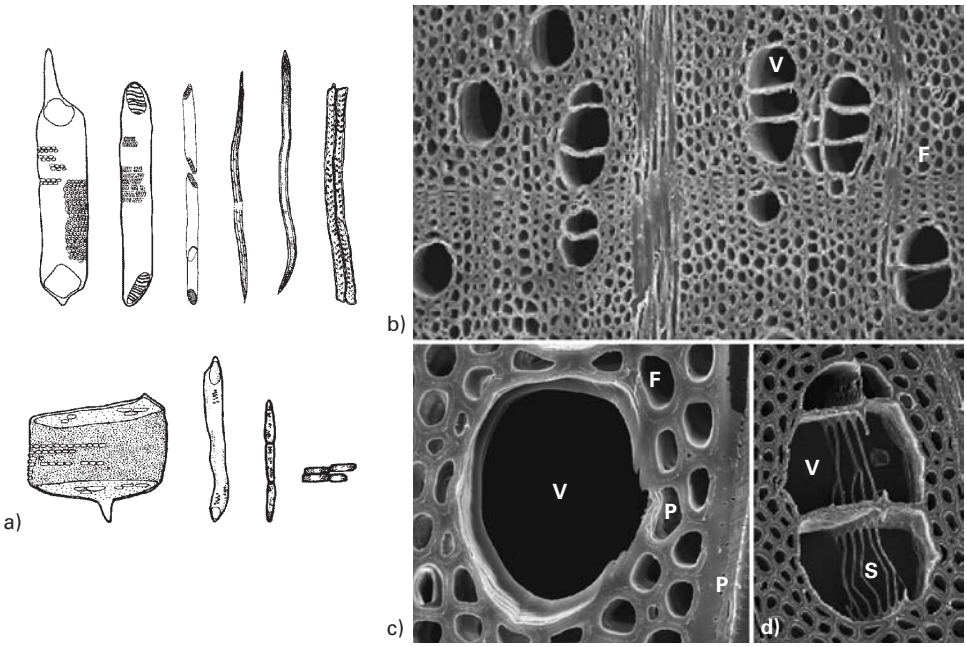


Figure 3.6. Typical hardwood cells. a) *Upper row left to right*: aspen earlywood vessel; birch earlywood vessel; birch vessels united, birch libriform fibre; birch tracheid; oak tracheid. *Lower row left to right*: oak early and latewood vessels, oak longitudinal parenchyma cells, birch ray parenchyma cell; b) Transverse sections of birch showing anatomical organization with vessels (V), fibres (F) and rays (R); c) Thin walled birch vessel surrounded by parenchyma and fibre cells; d, Birch vessel with characteristic scaliform (S) pitting found at the ends of the vessels.

Normally libriform fibres and fibre tracheids occur in the same wood species, although the proportion of fibre tracheids varies considerably. Libriform fibres are narrow cells on average 0.7–2.0 mm long with pointed ends and 10–60 µm wide. Fibres in common Swedish species lie in the range 0.4–1.8 mm long and 12–36 µm wide (*Table 3.6*). Fibre tracheids tend to be shorter than libriform fibres, have thinner walls and rounded ends (*Figure 3.6*). Libriform fibres and fibre tracheids are differentiated by the nature of their pitting with libriform fibres possessing simple pits scattered over the fibre wall and fibre tracheids bordered pits reminiscent, but smaller than those present in softwoods.

Table 3.6. Length and width of Scandinavian hardwood libriform fibres (Ezpeleta and Simon, 1970).

Wood species	Fibre length (mm)		Fibre with (µm)	
	Mean	**Range**	**Mean**	**Range**
Birch (*Betula verrucosa*)	1.3	0.8 – 1.8	25	18 – 36
Beech (*Fagus sylvatica*)	1.2	0.5 – 1.7	21	14 – 30
Ash (*Fraxinus excelsior*)	0.9	0.4 – 1.5	22	12 – 32

In some hardwoods vascular and vasicentric tracheids occur associated with vessels. Their main function is the conduction of fluids and for this purpose they are densely covered with bordered pits that provide contact with the vessels. Like softwood tracheids they have closed ends (*Figure 3.6*). Vascular tracheids occur in longitudinal series rather like vessels while the vasicentric tracheids tend to be associated with earlywood vessels and do not form longitudinal series. Vascular tracheids occur in birch and poplar species but on the whole this cell type is rather rare. Vasicentric tracheids are abundant in Oak. These types of tracheids occur in a limited number of wood species and primarily their presence is of diagnostic value. Their presence is however useful for the identification of pulps (Ezpeleta and Simon, 1970; Ilvessalo-Pfäffli,1995).

3.4.2 Parenchyma Cells

In general the proportion of parenchyma cells in hardwoods is much greater than in softwoods (*Table 3.5*) which often have large rays and often well developed longitudinal parenchyma tissues. Hardwood parenchyma cells are primarily involved in the storage of reserve materials (e.g. starch) and the cells remain living in the sapwood.

3.4.3 Longitudinal Parenchyma Cells

Longitudinal parenchyma is much more abundant in hardwoods than softwoods (*Table 3.5*, *Figure 3.6*) where it can constitute over 50 % of the total wood volume. It is particularly abundant in trees from tropical and subtropical zones. Longitudinal parenchyma associated with vessels is known as *paratracheal* while that not in contact is known as *apotracheal* parenchyma.

3.4.4 Ray Parenchyma

Rays in hardwoods consist entirely of parenchyma cells and ray tissues can comprise *ca* 7–30 % of the total wood volume. Rays in hardwoods vary greatly in width and height in contrast to that of softwoods. Both uniseriate (i.e. one cell width, *Figure 3.7*) and multiseriate (e.g. 1–3, birch) or abundant multiseriate (*Figure 3.7*) (e.g. 1–36, Oak) forms exist. Ray cells like longitudinal parenchyma cells vary greatly in size and shape, but morphologically tend to be short, isodia-

metric cells. Two types of ray parenchyma cells are known *viz: homocellular* – where the parenchyma cells are of one morphological form; and *heterocellular* – where the parenchyma is composed of 2 or 3 morphological cell types.

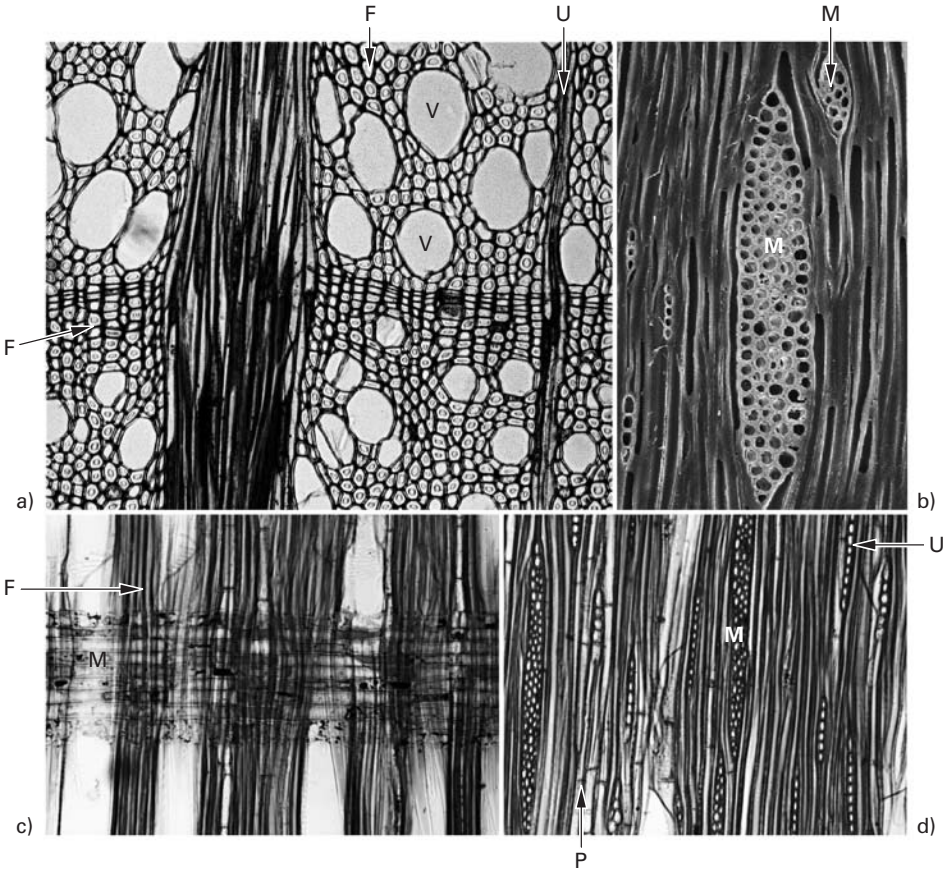


Figure 3.7. Anatomical organization of beech. a) Transverse (TS), tangential (TLS) (b, d) and radial longitudinal sections (RLS) (c) of beech showing the anatomical organization. Uniseriate (U) and multiseriate (M) rays are present in the TLS sections while the presence of vessels (V) and fibres (F) are shown in all sections.

3.5 Hardwood Tyloses

The vessels of some hardwoods in heartwood may be partly or entirely filled with characteristic inclusions known as tyloses. Tyloses represent outgrowths from adjacent living parenchyma cells (ray or longitudinal parenchyma) into the vessel through pits (half-bordered pit pairs). Following partial dissolution of the pit membrane, tyloses extend like balloons into the vessels from adjacent parenchyma cells. Structurally, tyloses consist of two walls and are chemically composed of cellulose, hemicelluloses and lignin. Studies indicate that tylose formation is initi-

ated due to the absence of water in the vessels which stimulates the adjacent parenchyma to develop tyloses into the vessel. Tyloses are frequently formed in the ring porous vessels of certain hardwoods (e.g. Oak, *Figure 3.9*).

Development of tyloses is a natural physiological event normally associated with heartwood formation or death of the sapwood through example tree felling. Tyloses may also be initiated through mechanical damage or through viral or fungal infection of the parenchyma cells. Wood exhibiting tyloses is impermeable to liquids (e.g. Oak) and represents excellent material for water holding materials (e.g. whisky barrels).

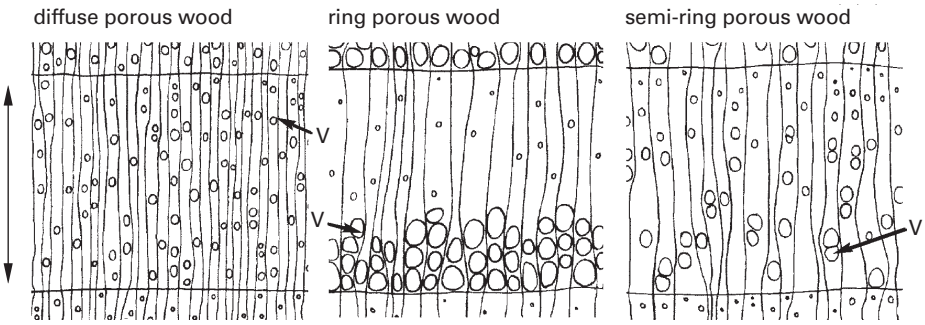


Figure 3.8. Different types of hardwood. Spatial arrangement of hardwood vessels (V) used to distinguish between different wood species. The vessels are either associated with the ends of the annual rings, or randomly distributed throughout the rings.

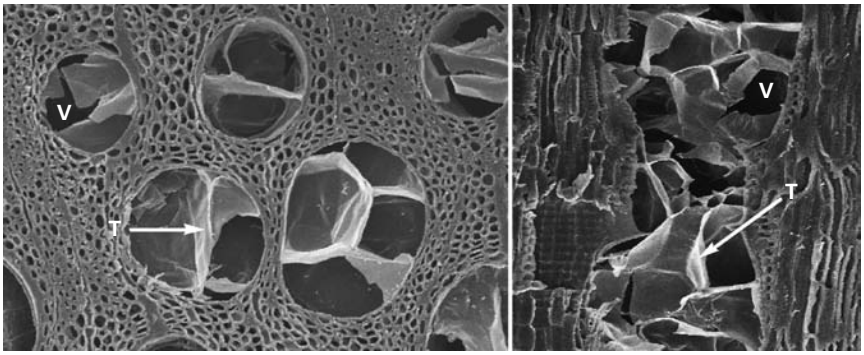


Figure 3.9. Morphology of tyloses. Ring porous oak wood (*Quercus spp)* showing tyloses (T) occluding vessels (V) in transverse (left) and longitudinal (right) sections. The tyloses are produced into the vessels through pits by the living parenchyma cells adjacent to the vessels.

3.6 Wood Pits: Simple, Bordered and Cross-field Pitting

Pits are one of the most characteristic microstructures that occur in cell walls of both soft- and hardwoods. They represent canals that allow the flow of liquids both laterally and vertically through the cell walls. Normally in living and frequently even after death a membrane of vari-

able permeability divides the cells. Pits have a variety of shapes and sizes that together with their location on wood cells can be used as a diagnostic feature for wood classification. Pits of adjacent cells are normally always "paired" thereby forming "pit pairs" – "simple, bordered and half-bordered pit pairs". All pits have essentially two main components: the pit cavity and pit membrane (*Figures 3.10* to *3.12*). The pit membrane consists of a primary wall and middle lamella and since the pits occur in pairs the membrane is composed of two primary walls and a middle lamella.

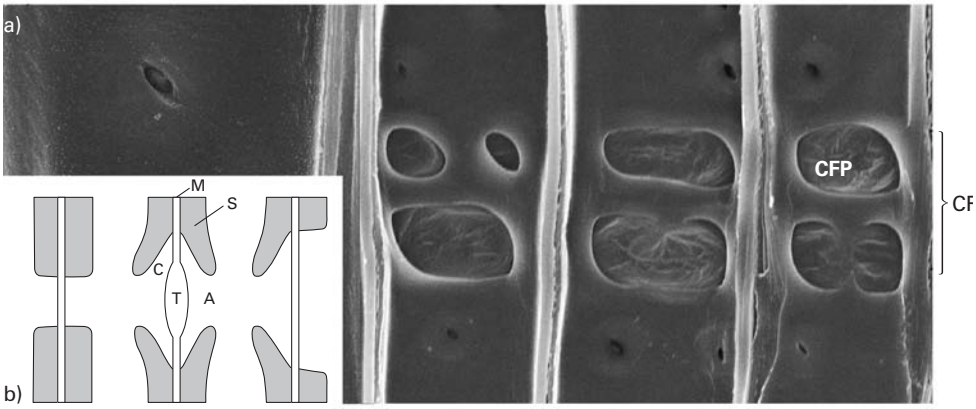


Figure 3.10. Structure of softwood pits. Electron micrographs showing cross-field (CF) pitting in *P. sylvestris* (a) and a diagram of the three pit types (b). Cross-field pits (CFP) connect the rays (ray parenchmya cells) with the axial tracheid system. The morphology of the cross-fields can be used as a taxonomic tool for species identification. The diagram shows (left) sections through a simple pit, bordered pit and half-bordered pit. C, pit chamber; T, torus, S, secondary cell wall; M, middle lamella; A, pit aperture.

3.6.1 Softwood Pits

Simple pits have a straight channel through the cell wall (*Figure 3.10*) while the bordered pit is formed by the secondary wall arching over the pit to form a characteristic dome-shaped chamber (*Figure 3.11*). In softwoods, simple pits are found uniting parenchyma cells. Bordered pits are found uniting axial tracheids and ray- and axial tracheids. Half bordered pits are also found uniting parenchyma and tracheids whereby the wall of the parenchyma cell has a straight opening and the wall of the tracheid an arched dome-shaped opening.

The area of pitting in softwoods that unites the radial parenchyma cells in rays with the axial tracheids is known as "cross-field pitting". Cross-fields occur on the radial walls of tracheids (*Figures 3, 13*) and is an important feature for identifying softwoods. It is best observed in earlywood tracheids where the pits are larger than in the latewoods (*Figure 3.10*). The large window-like openings seen in pine (*Figure 3.10*) are known as "pinoid" and the smaller pitting in spruce "piceoid".

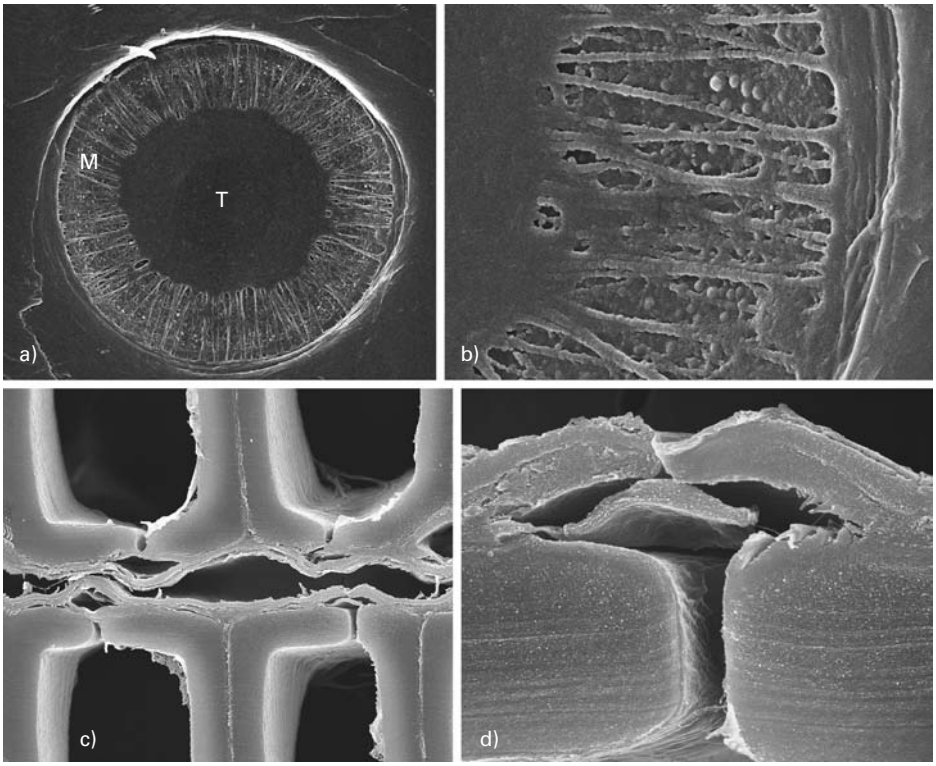


Figure 3.11. Softwood pits. Bordered (a, b, c, d) pits (BP) of Scots pine (*P. sylvestris*). A central torus (T) is shown surrounding margo (M) network of cellulose strands. In cells subjected to stress the torus may be pressed against either side of the pit chamber thereby closing the cell (i.e. the cell is aspirated). This process also occurs during heartwood formation and is partly why heartwood is difficult to impregnate with fluids. The lower micrographs show the smaller bordered pits of ray tracheids.

3.6.2 Hardwood Pits

In hardwoods, simple pits are found connecting parenchyma cells as well as vessels with parenchyma cells. Bordered pits connect vessel elements. They are often closely packed and assume a variety of patterns that can be used as a diagnostic feature of classification (*Figure 3.12*). Connections between fibre tracheids are by bordered pits and between fibres and parenchyma cells by half-bordered pits. Libriform fibres have simple pits like the parenchyma cells.

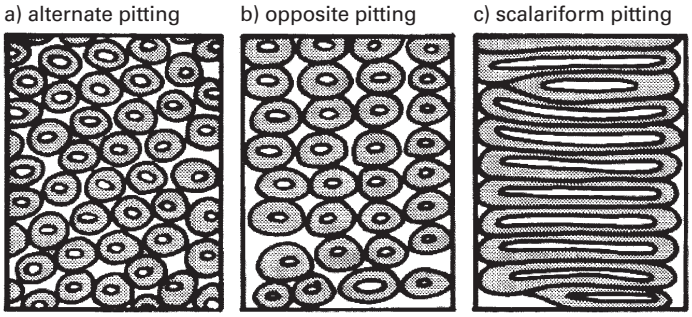


Figure 3.12. Characteristic vessel to vessel pitting in hardwoods. a) alternate pitting; b) opposite pitting; c) scalariform pitting. (Haygreen and Bowyer, 1989).

3.7 Wood Cell Wall Structure and Ultrastructure

Wood cell walls are comprised of three major chemical components namely cellulose, lignin and hemicelluloses (see later chapters in this book). In simplified terms the cellulose forms a skeletal matrix that is surrounded and encrusted by the hemicelluloses and lignin. Cellulose is composed of glucose units that are organized into chains with the smallest building element considered as represented by the elementary fibril bundles of 36 parallel-aligned cellulose molecules (see chapter 4). Cellulose microfibrils (aggregated fibrils 10–20 nm in diameter) are visible using electron microscopy and may be aggregated further into macrofibrils and lamellae, the latter organized into a concentric arrangement around the wood cell wall layers (see below). The hemicelluloses are amorphous and are associated and orientated along the cellulose while lignin is amorphous and isotropic and encrusts both the hemicelluloses and cellulose. The cellulose major function is skeletal and the provision of support to the individual wood cell and tree and ultimately the final wood or paper product.

3.7.1 Models of Wood Cell Wall Organization

Wood cells are composed of a number of cell wall layers forming the primary (one layer) and secondary cell walls (2–3 layers) sometimes referred as the "*3-ply structure*" (*Figure 3.13*). Individual cells are connected together by the intercellular middle lamella region (*Figure 3.13*). Controversy still exists as to the "true structure" of the individual layers as reflected by the numerous different models shown in textbooks. *Figure 3.13* shows a typical model for the cell wall organization of a tracheid.

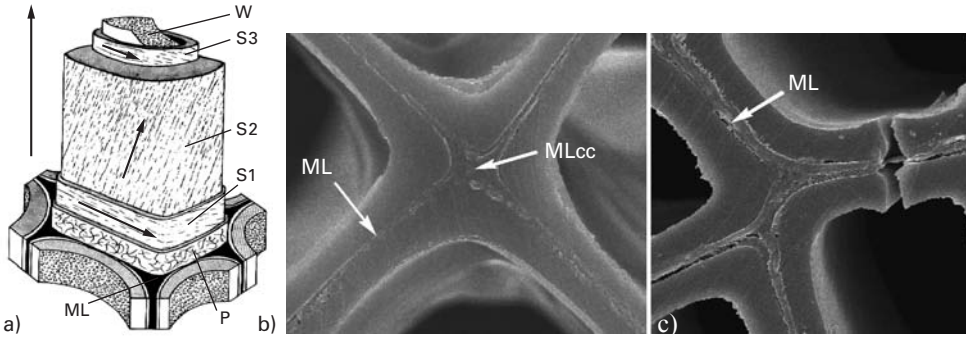


Figure 3.13. Different layers in a tracheid. a) Simplified structure of a typical tracheid cell wall (Cote, 1967) showing the middle lamella (ML), primary wall (P), secondary cell wall layers (S1, S2, S3) and warty (W) layer lining the cell lumen. Arrows indicate the orientation of the cellulose microfibrils (MFA) in the individual secondary cell wall layers. The micrographs on the right show the location of the intercellular lignin rich middle lamella (ML) and middle lamella cell corner (MLcc) between tracheids of pine b) and fibres of birch c). The S1 and S3 layers are not very apparent in the micrographs.

3.7.2 Structure of Cell Wall Layers

Wood cells are "glued" to one another by the lignin rich middle lamella (ML) region (*Figure 3.13*). The lignin content of the middle lamella region is high but because the layer is thin only 29–25 % of the total lignin is present in this layer. The primary wall (P) forms the outer layer of the cell and is comprised of randomly orientated cellulose microfibrils (*Figures 3.13, 3.14*). The middle lamella and primary walls of adjacent cells together form the compound middle lamella.

Figure 3.14. Example of multilayer arrangement in the S3 layer in a tropical hardwood. a) Cellulose microfibrils (MFA) in S or Z orientation have a helical arrangement in the S2 along the cell axis. b) MFA in S2 as shown with soft rot cavities (holes) following a helical orientation (arrows). Multilaminate structure of fibre S2 and S3 layers in *Homalium foetium*. The fibre has been partially degraded by a soft rot fungus producing the same types of cavities shown in b).

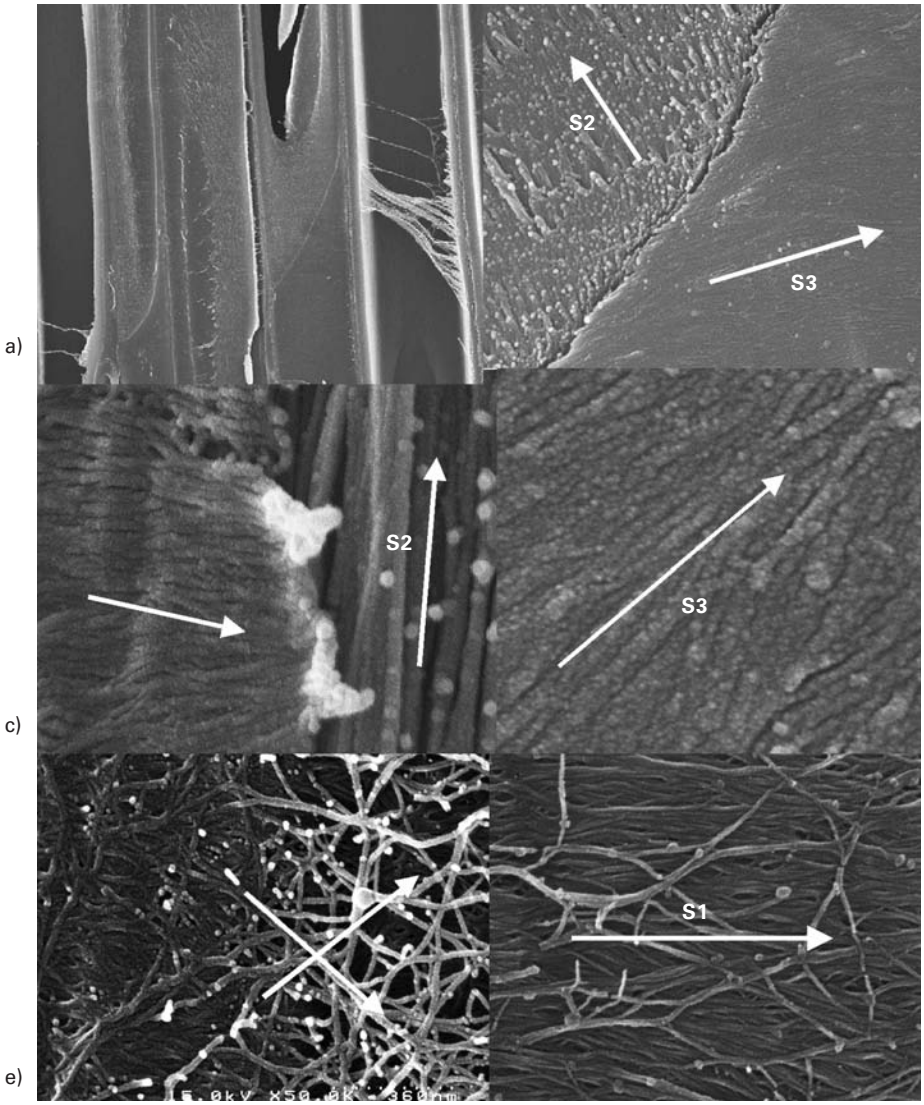


Figure 3.15. Structural organization of cell wall layers. Electron micrographs showing the structural organization of the primary and secondary cell wall layers from delignified spruce tracheids and the presence of cellulose macrofibrils. a) RLS view of axially orientated tracheids; b, c) S3 and S2 layers showing differential orientation of cellulose macrofibrils. In c, the S2 macrofibrils show an axial orientation and the S3 macrofibrils are typically perpendicular to the fibre axis; d) Surface view of S3 macrofibrils; e) primary cell wall with random orientation of cellulose macrofibrils; f) S1 layer with low MFA. (arrows indicate the MFA of cell wall layers).

The secondary cell wall is composed of 2–3 layers known as the S1, S2, and S3 layers, the S1 and S3 layers being comparatively thin (*Table 3.7*) and the central S2 middle layer forming the major part of the cell wall in both soft- and hardwoods. Variations in thickness of the cell wall

Table 3.7. Average thickness (μm) and percentage (%) of the various wall layers in a soft- and hardwoods. P + ML = primary wall + middle lamella; S1, S2, S3 = secondary cell walls; T = tertiary cell wall. Long. = longitudinal parenchyma cell. * Measurements where P + ML were not distinguishable from the S1 layer. (Data from: Fengel & Stoll, 1973; Harada, 1962)

Species	**P + ML**	**S1**	**S2**	**S3**	**T**
Norway spruce *Picea abies*					
(EW)	0.09 (4.2)	0.26 (12.5)	1.66 (78.7)	0.09 (4.5)	
(LW)	0.09 (2.1)	0.38 (9.0)	3.69 (85.4)	0.14 (3.3)	
Japanese beech *Fagus crenata*					
Vessel	0.25 (25.0)*		0.50 (50.0)		0.25 (25.0)
Libriform fibre	0.07 (1.0)	0.51 (10.0)	4.32 (87.0)		0.10 (2.0)
Fibre tracheid	0.07 (5.0)	0.24 (16.0)	0.99 (67.0)		0.17 (12.0)
Long. parenchyma	0.06 (4.0)	0.35 (21.0)	0.37 (22.0)		0.09 (5.0)
Ray parenchyma	0.50 (27.0)*		0.92 (50.0)	0.37 (20.0)	0.07 (3.0)

layers occur (e.g. between early- and latewood cells) and it also varies with cell type (see below). Some cells also have a warty layer lining the inner S3 adjacent to the cell lumen (*Figure 3.13*). The individual layers of the primary and secondary cell wall can be distinguished from one another by the orientation of the cellulose microfibrils which wind around the cell axis in different directions either to the right (i.e. Z-helix) or to the left (i.e. S helix) within the individual layers (*Figures 3.14, 3.15*). The orientation of the cellulose microfibrils is of major importance for governing the physical properties of the wood cell and in-turn the wood structure. The S1 layer possesses almost horizontal cellulose microfibrils while in the S2 they are almost vertical and in the S3 they are once again almost horizontal with regard to the fibre axis (*Figures 3.13* and *3.14*). Of the three secondary wall layers the S2 layer is of overriding importance because of its thickness and almost vertical orientation of cellulose microfibrils giving wood cells strength and ultimately imparting the major mechanical and physical properties to wood products. This cellulose orientation is known as the microfibril angle (MFA) and can vary considerably between early- and latewood in juvenile, mature and compression wood as well as different clones of the same species. The MFA is also known to vary in tracheids across individual growth rings as well as from pith to the outer sapwood and from the base of the tree to its crown. The orientation (i.e. angle) of the S2 layer microfibrils is frequently used as a major feature for characterizing (distinguishing) the strength properties of wood.

In all wood cell types from both soft- and hardwoods, the S2 layer accounts for the major part of the cell wall (*Table 3.7*). In the latewood tracheids of softwoods and libriform fibres of hardwoods the S2 layer may contribute as much as 80–90 % of the total cell wall (*Table 3.7*). The change from early- to latewood during seasonal growth is primarily determined by the increase in thickness of the S2 layer with the S1 and S3/tertiary wall layers only contributing marginally to the change in thickness (*Table 3.7*). In contrast the S2 layer in vessels tends to be thin in comparison with other cell types (*Figure 3.6*, *Table 3.7*).

3.7.3 Molecular Models of Cell Wall Ultrastructure

Several models depicting the molecular arrangement of cellulose, hemicellulose and lignin in wood cell walls have been put forward although there is no accepted model (*Figure 3.16*). Just like a single “3-ply model” is not truly accurate for all cell walls from all wood cell types, no one molecular model will reflect all wood cells. This is because wood cells vary considerably in their chemical composition. Wood fibres can vary greatly in both degree and type of lignification as well as hemicellulose and pectin content. For example fibres in hardwoods are syringyl lignified while tracheids in softwood and vessels in hardwoods are guaiacyl lignified. Ray parenchyma cells in softwoods tend to be non-lignified and high in pectic substances.

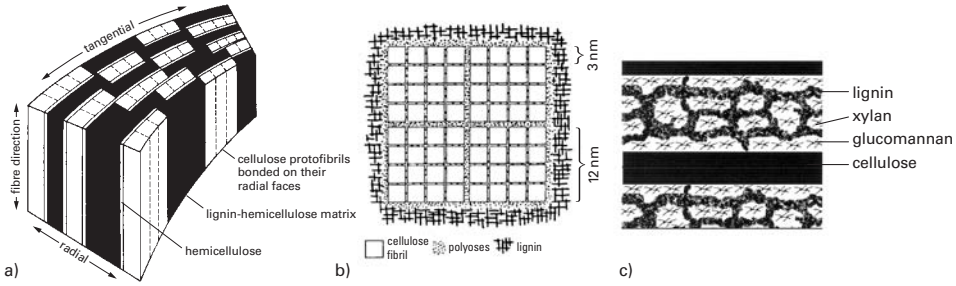


Figure 3.16. Proposed molecular arrangement of wood polymers in wood fibre cell walls. a) Kerr and Goring (1975), with cellulose microfibrils forming interrupted lamellae embedded in a matrix of lignin and hemicellulose; b) Fengel and Wegener (1984) with elementary fibrils surrounded by monolayers of hemicellulose and larger units (macrofibrils) enclosed with hemicellulose and lignin; c) Salmén and Olsson (1998) where glucomannan is closely associated with cellulose and xylan to lignin.

In recent years, greater attention has been applied to understanding the ultrastructural architecture of wood cell walls particularly the configuration and arrangement of the cellulose microfibrils. While it is generally agreed that cellulose microfibrils (*ca* 2–4 nm) or sub-elementary fibrils (*ca* 1.5–2.0 nm) comprise the basic armature, there is increasing evidence that there may be a greater order in which the cellulose microfibrils are complexed together to form what has been termed cellulose "aggregates" or "macrofibrils" (i.e aggregation of microfibrils) (*Figure 3.15*). Evidence for these aggregates has been documented in delignified softwood cells (e.g. spruce kraft pulp tracheids), for all secondary cell wall layers using advanced microscopical (*Figure 3.15*) and spectroscopic techniques. At the ultrastructural and molecular levels wood

Table 3.8. Distribution of cellulose in different cell wall layers (Meier, 1964).

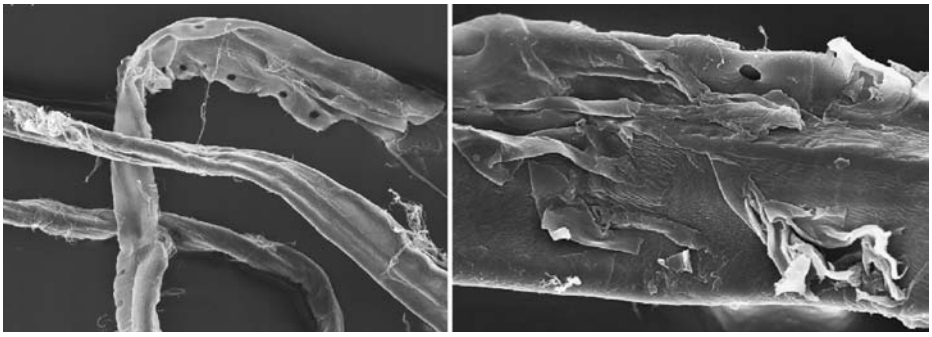
Wood species	ML + P	S1	S2(outer)	S2(inner)+ S3
	% of total polysaccharide			
Scots pine *Pinus sylvestris*	33.4	55.2	64.3	63.6
Norway spruce *Picea abies*	35.5	61.5	66.5	47.5
Birch *Betula verrucosa*	41.4	49.8	48.0	60.0

cell walls consist of numerous concentrically orientated lamellae composed primarily of cellulose macrofibril aggregates with the lamellae showing similar or variable S or Z orientation in respect of the fibre axis. Each lamellae is composed of cellulose aggregates (i.e. macrofibrils) which are in turn built up of several microfibrils, each of which is composed of elementary fibrils. Data for lignified woods cells is also mounting to indicate a similar wall construction with the cellulose aggregates embedded in lignin.

3.7.4 Chemical Composition of Different Cell Wall Layers

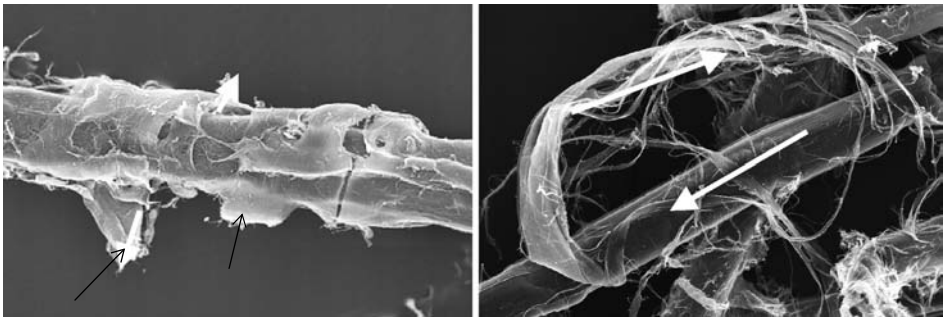
The chemical composition of wood cell wall layers varies between different cell types and between soft- and hardwoods. The compound middle lamella region is rich in lignin and contains the highest lignin content (g/g) in wood cell layers but also contains pectin and cellulose. The primary cell wall contains high levels of pectin as well as the glycoprotein extensin – thought to hold the cellulose microfibrils in their criss-cross network- and also the hemicellulose xyloglucan. The secondary cell wall layers (S1, S2, S3) also vary in their chemical composition with the concentration of lignin being greater in the S1 layers than S2 and S3 and the total amount of cellulose and hemicelluloses greater in the S2 than either S1 or S3 layers (*Table 3.8*). This can only be recognized as a general trend and exact chemical analyses of the individual wall layers is not available since adequate methods to separate sufficient quantities of the layers in a truly purify form have not so far been developed. As outlined earlier, considerable chemical differences exists in type of lignification (see later chapters) between soft- and hardwoods cells. Chemical differences also exist between reaction wood and normal wood cells as well as between juvenile and mature cells. Some reports even indicate minor differences in lignin content between early- and latewood cells.

The organization of the wood cell wall – particularly the tracheids and fibres that represent the greatest proportion of soft- and hardwood tissues is of considerable importance during processing for pulp and paper production. During chemical pulping the majority of the lignin is removed (i.e. the middle lamella is removed), the wood is defibrated and the wood cells are separated (*Figure 3.17*). Thus the exposed wood cell wall surface which will be in contact during paper-making – assuming no damage will be represented by the thin primary (P) (which is often removed) or S1 wall layer. During beating of the fibre the S1, and sometimes the S2 (in severe cases) (*Figure 3.18*) layers fibrillate and are responsible for the fibre-fibre bonding in paper. In contrast, during mechanical pulping, the surfaces that come into contact during paper or board production may be partly composed of the middle lamella, primary wall, S1 or S2 layers and is dependent on where the fractures occur in the wood cell walls during initial refining (*Figure 3.17*). Naturally, the chemistry of the different cell wall layers is also of considerable importance, nevertheless the nature of cell wall construction plays an overriding role.



a) b)

Figure 3.17. Structure of pulped wood fibres. a) When wood fibres are chemically processed the lignin is removed, the fibres are separated, and appear as long threads and the outer part of the secondary cell wall – the primary and S1 layers are exposed. b) Wood fibres processed for mechanical pulp may show an outer surface structure composed of remaining middle lamella materials, primary wall, S1 or even S2 wall materials, depending on where the fractures have occurred in the wood cell wall structure.



a) b)

Figure 3.18. Importance of the wood cell wall layers in pulping. a) When wood fibres are refined the primary wall is often removed and the S1 layer becomes fibrillated (arrows); b) If refining is severe, fibrillation may also occur within the S2 layer. The orientation of fibrillation (arrows) reflects the MFA of the original wall layer.

3.8 Reaction Wood

Reaction wood is developed in both soft- and hardwoods when trees are subject to stress (e.g. mechanical forces such as wind, gravity etc) and its purpose is to retain the tree in an upright position. The mechanisms behind reaction wood formation and its development however differ between soft- and hardwoods. Softwoods form compression wood (normally dark in colour due to higher lignin content – compared to normal wood) on the lower part of leaning stems in order to revert the tree into an upright position while hardwoods form tension wood on the upper side for the same reason (see Chapter 2). Anatomically the types of cells formed in reaction wood are similar to those in normal wood but differ considerably in cell wall structure (e.g. MFA) and chemical composition. The development of reaction wood in trees represents a problem for

wood utilization although its importance in pulp manufacture is considered of less importance. The problem arises from the very different physical and chemical characteristics of reaction wood compared to normal wood.

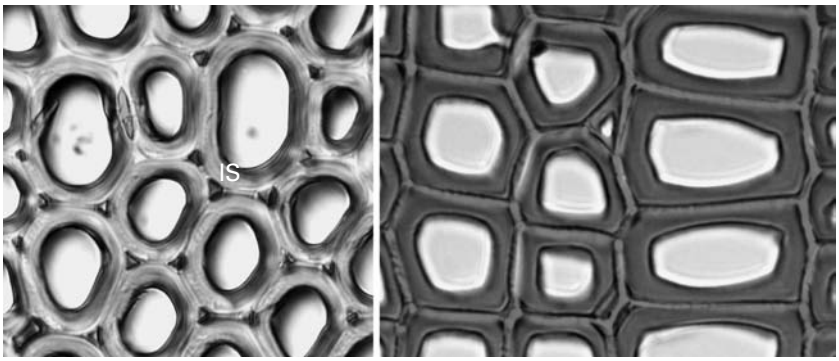


Figure 3.19. Difference between compression wood and normal wood. Compression latewood (left) and normal tracheids (right) from spruce in transverse section. Compression fibres are round and there are intercellular spaces (IS) at the corners between the cells. Normal spruce tracheids have a more rectangular-squarish appearance and lack intercellular spaces.

3.8.1 Morphology of Compression Wood Tracheids and Tension Wood Fibres

Compression wood tracheids normally have a more circular outline than normal tracheids and usually have intercellular spaces located between the cells at the corners of the middle lamella regions (*Figure 3.19*). In addition, the tracheids lack an S3 layer and the S2 cell wall normally contains helical cavities or checks that follow the microfibril angle (*Figure 3.21*). These checks

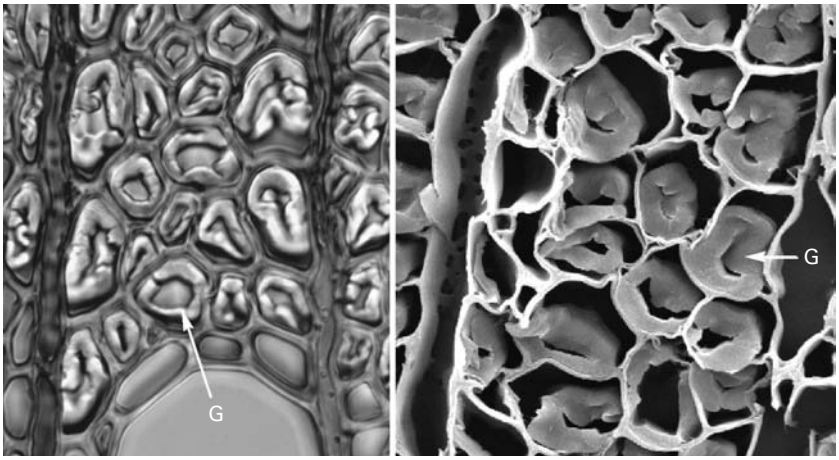


Figure 3.20. Tension wood fibres from *Poplar tremeloides*. The G-layer (G) is developed on the inside of the S2 layer.

are normally about 45–60° in relation to the fibre axis. Chemically, compression wood tracheids also have a marginally higher lignin content than normal tracheids and also contain higher amounts of galactose.

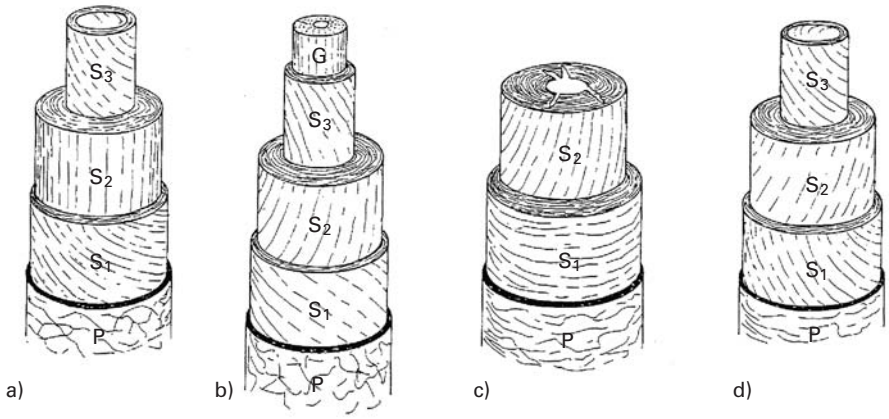


Figure 3.21. Diagrams comparing the cell wall structure of a) normal- and b) tension wood from a hardwood with that of c) compression- and d) normal wood of a softwood. P, primary cell wall; S1, S2, S3, secondary cell wall layers; G, gelatinous layer. Note the presence of the G-layer in the tension wood and absence of S3 in compression wood. The lines show the approximate MFA in the different layers. (Data from Barefoot and Hankins, 1982).

Anatomically, tension wood normally contains fewer and smaller vessels than normal hardwood. Tension wood fibres are also characterized by the development of an extra cell wall layer, the gelatinous layer (termed G-layer) produced around the cell lumen (*Figures 3.20, 3.21*). Depending on wood species, the G-layer may be present instead of the S2 or tertiary wall layers or as an additional layer (*Figure 3.21*). Chemically, the gelatinous layer is comprised of almost pure cellulose. The G-layer cellulose is highly crystalline in nature, is arranged in concentric lamellae and has a microfibril angle aligned with the fibre axis.

Studies in recent years have also shown the development of mild reaction wood in plantation timber (e.g. in Monterey pine, *Pinus radiata,* in New Zealand) and furthermore that reaction wood may be present even in the trunks of apparently upright stems. Here reaction wood is thought to be periodically produced during tree development to maintain the tree upright.

Despite the negative aspects of reaction wood for wood utilization, such wood is of considerable interest for examination of wood structure in respect to its biosynthesis, particularly the molecular events and enzymes involved in abnormal wood development. For example, understanding the molecular events signaling gelatinous layer synthesis may provide novel information on cellulose synthesis and cell wall deposition.

3.9 Methods for Studying Wood and Cell Wall Structure

Wood is a matrix material and its preparation (e.g. sectioning) for routine microscopical methods is rather simple since it retains its structure during processing. Observations on wood mac-

ro- and microstructure as well as fibre morphology are primarly conducted using a variety of microscopic methods. Macrostructure is studied using stereomicroscopy (up to 100x) while light microscopy (up to 1000x) is the most frequently used technique for observations on tissue arrangement and spatial distribution of wood cells. It is also used for studies on fibre morphology for taxonomic purposes. More specialized techniques such as polarized light microscopy are used for studying cellulose microfibril angles (MFA) in wood cells and fibres and ultraviolet light (UV) is used for studies on lignin distribution across wood cell walls. For studies at higher magnification (e.g. 500–100 000x) scanning (gives a 3 dimensional view)(SEM) and transmission electron microscopy (TEM) (e.g. 1000–300 000x) are routinely used to give details of the ultrastructural architecture of individual wood cells and wall layers as well as cellulose structure. In recent years Atomic Force Microscopy (AFM) has also been used to study wood cell wall ultrastructure and for studies on the molecular structure of cellulose.

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4 Cellulose and Carbohydrate Chemistry

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4.1 Introduction

In this and the following chapters, the main components of the woody cell walls, *cellulose*, *hemicellulose*, *lignin* and *extractives*, will be described from a chemical perspective. First out is cellulose[1], which is normally the main component in the cell walls of all true plants. This and the abundance of plant material make cellulose the outstandingly most common bio-compound on earth. Although its chemical structure is very simple, very long[2] unbranched chains of glucose, it displays various special properties that are of great scientific and technical interest. However, cellulose, as well as different types of hemicelluloses, belongs to the large group of biomolecules, *the carbohydrates*, which play central roles in all forms of life. Before the structure, biosynthesis and properties of cellulose are discussed, an introduction to carbohydrate chemistry is given.

4.2 Carbohydrate Chemistry

The term *'carbohydrate'* (French 'hydrate de carbone') was originally applied to the large group of biomolecules which formula can be expressed as $C_n(H_2O)_n$. However, this definition is not ideal, since many biomolecules with similar functions and properties as carbohydrates do not fulfill this formula due to substitution with for instance amines or phosphate, or a reduction or oxidation. Furthermore, the simplest chemical fulfilling the formula ($n = 1$) is formaldehyde ($H_2C{=}O$) – a gas that has neither the physical or biological properties of other carbohydrates. A more useful definition is:

Carbohydrates are polyhydroxic carbon chains with at least one aldehyde- or keto group.

It follows the definition that a simple carbohydrate (i.e., with one single carbon chain), a *monosaccharide*, must have at least three carbons ($n \geq 3$). Monosaccharides with up to six carbons are common in nature and there exist also larger molecules, although they are rare. The monosaccharides have excellent possibilities to couple to each other by the hydroxyl groups. If two monosaccharide residues are covalently connected a *disaccharide* is created. Three connected monosaccharide residues give a *trisaccharide* etc. *Oligosaccharide* has a more vague definition and can contain from 3 up to approximately 10 monosaccharide units, and if it is even more monosaccharide residues it is a *polysaccharide*. The term *'sugar'* is frequently applied to monosaccharides, disaccharides, and sometimes to short oligosaccharides. Carbohydrates differentiate from each other by rather small structural differences that however can be very important from a biological and mechanical perspective; even small differences as the direction of a hydroxyl group can give important biological differences. However, especially in the case of the heavier monosaccharides with several carbons, it can be difficult to show the structural differences in a clear way by normal chemical presentation. In this chapter we will use a way of drawing structures called *Fischer projection*. It is explained in figure 4.1.

---

[1] Cellulose was named by the French botanist Anselm Payen, who 1842 isolated it from plant material as a solid residue after extensive alkali extractions. The word comes from *cellulosus* – Latin for “rich in small cells”. This name is indeed appropriate for the most important constituent in plant cell walls!

[2] Cellulose is one of the largest biopolymers known. Of other cell wall components lignin might have an even higher molecular weight in the wood, but it cannot be isolated in pure form without fragmentation.

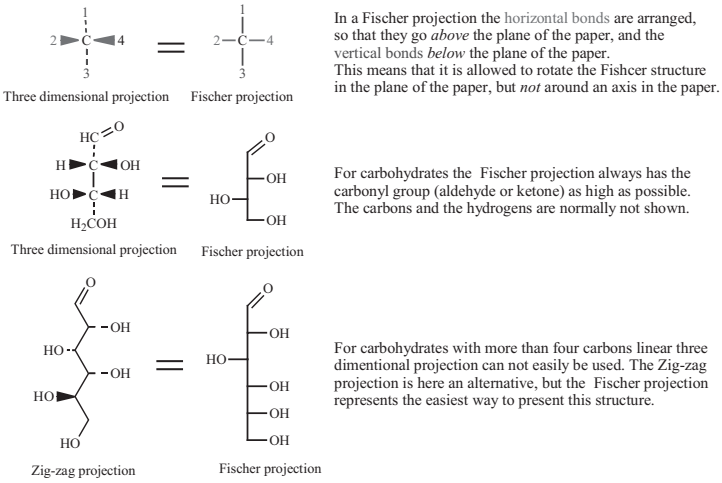


Figure 4.1. Fischer projections of carbohydrates.

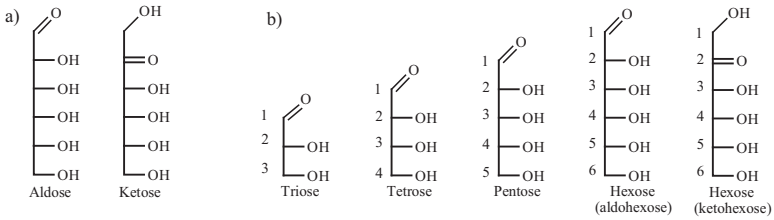


Figure 4.2. Nomenclature of monosaccharides. a) A monosaccharide with an aldehyde is called *aldose* and a monosaccharide with a keton is called *ketose*. b) Mono-saccharides with 3 carbons are called trioses, with 4 tetroses, with 5 pentoses and with 6 hexoses. The numbering of the carbons are made so that the carbonyl, i.e., that keton- or aldehyde group got as low number as possible.

4.2.1 Common Monosaccharides

The general term ”monosaccharide” (as opposed to oligosaccharide or polysaccharide) denotes a single unit, without connections to other such units. When a monosaccharide is a part of a larger molecule, disaccharide, oligosaccharide polysaccharide etc., it is called a *monosaccharide residue*[3]. Monosaccharides are either *aldoses*[4], i.e., polyhydroxy aldehydes H–[CHOH]$_n$–CHO, or *ketoses*, i.e., polyhydroxy ketones H– [CHOH]$_n$–CO– [CHOH]$_m$–H called *(Figure 4.2)*. A monosaccharide with 3 carbon atoms is called *triose*, with 4 carbon atoms *tetrose*, with 5 *pentose*, 6 *hexose*, 7 *heptose*, 8 *octose*, etc., i.e., the names are based on the

[3] This nomenclature is explained by the fact that water is lost from the monosaccharides when they form covalent bond to each other, as will be discussed below.
[4] The suffix “ose” is used for all carbohydrates with the exception of polysaccharides.

Greek numbers (*Figure 4.2*). In plant cell walls almost all monosaccharides are pentoses or hexoses. Trioses and tetroses are important metabolites in plants, whereas heptoses and octoses are, as mentored above, rare and will not be discussed further[5]. The chain is numbered so that the carbonyl carbon (C=O) get as low number as possible (*Figure 4.2*).

In addition to the difference in number of carbons and if they are aldoses or ketoses, the orientation of the hydroxyl groups defines the diversity between different monosaccharides. We start with the aldoses; the simplest is aldotriose or *glyceraldehyde* (*Figure 4.3*). Since all the four groups bound to the carbon at C2 are different, this carbon is asymmetric, i.e., it has a *chiral center* at C2. In other words the glyceraldehyde can exist in two mirror images, *enantiomers*, called D-glyceraldehyde and L-glyceraldehyde[6]. As shown in figure 4.3 the D-sugars have the hydroxyl to the right and the L-sugars the hydroxyl to the left in Ficher projection. Although the physical and chemical properties of enantiomers normally are identical, there are normally large biological differences between them, making the stereochemistry to an important aspect in biochemistry.

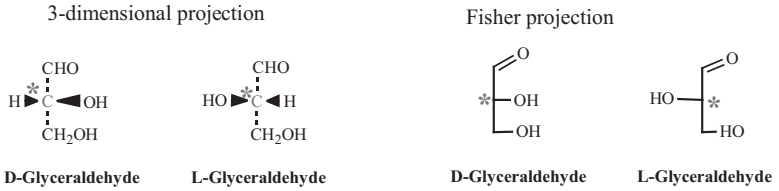


Figure 4.3. D- and L- forms of glyceraldehyde. The asymmetric carbons responsible for the D/L-system are marked with *. In D-glyceraldehyde the hydroxyl group at the asymmetric carbon points to the right, whereas the hydroxyl group at the asymmetric carbon points to the left in L-glyceraldehyde.

The names of the aldoses are given starting from D- and L-glyceraldehyde. The *D-aldoses* are formed by a theoretical "expansion" of D-glyceraldehyde, and the *L-aldoses* are formed by an "expansion" of L-glyceraldehyde. D-Aldotetroses can be regarded to be constructed by adding a new carbon with hydroxyl group (HCOH) between the C1 and C2 of D-glyceraldehyde. Since there exist two possible orientations of the hydroxyl group, there exist two D-aldotetroses (*Figure 4.4*). D-Pentoses are formed by an addition of another HCOH-group, and due to the two possible orientations of the hydroxyl it exist $2 \times 2 = 4$ pentoses. The D-hexoses are formed a third addition of a hydroxylated carbon and there are consequently $2^3 = 8$ different D-hexoses (*Figure 4.4*). A corresponding tree excist for L-aldoses with monosaccharides that are mirror images, *enatiomers*, of the D-monosaccharides, but most of the important aldoses in plant cell walls are D-aldoses, althogh L-aldoses also occur. Aldotrioses and aldotetraoses are important in the plant cell metabolism, but are insignificant constituents in the plant cell wall. The aldopentoses *xylose* and *arabinose* and the aldohexoses *glucose*, *mannose* and *galactose* are important building stones in the cell wall. Ribose is also a very biologically important pentose with central roles in

[5] A heptose has however an important function in the Calvin cycle of the photosynthesis (2.3.1). Octoses with unknown function are synthesized by avocado.

[6] The enantiomers differ in the way they rotate polarized light; the D-glyceraldehyde ((+)-glyceraldehyde according to another nomenclature) rotate clockwise, *dextro-rotating*, and the L-glycerladehyde ((–)-glyceraldehyde according to another nomenclature) rotate counter-clockwise, *levo-rotating*.

the gene expression system and cell metabolism. The other pentoses and hexoses are uncommon. D-Glucose is by far the most abundant monosaccharide and it is a good idea to learn the structures of the most important monosaccharides as related to this hexose. D-Mannose differs from glucose only in the orientation of hydroxyl on the C2. D-Galactose differs from glucose in the orientation of the C4 hydroxyl. D-xylose is identical to glucose except that is has no C6. L-arabinose differs from xylose only in the orientation of the C3 hydroxyl, i.e., the C1–C3 have identical conformation as in D-glucose.

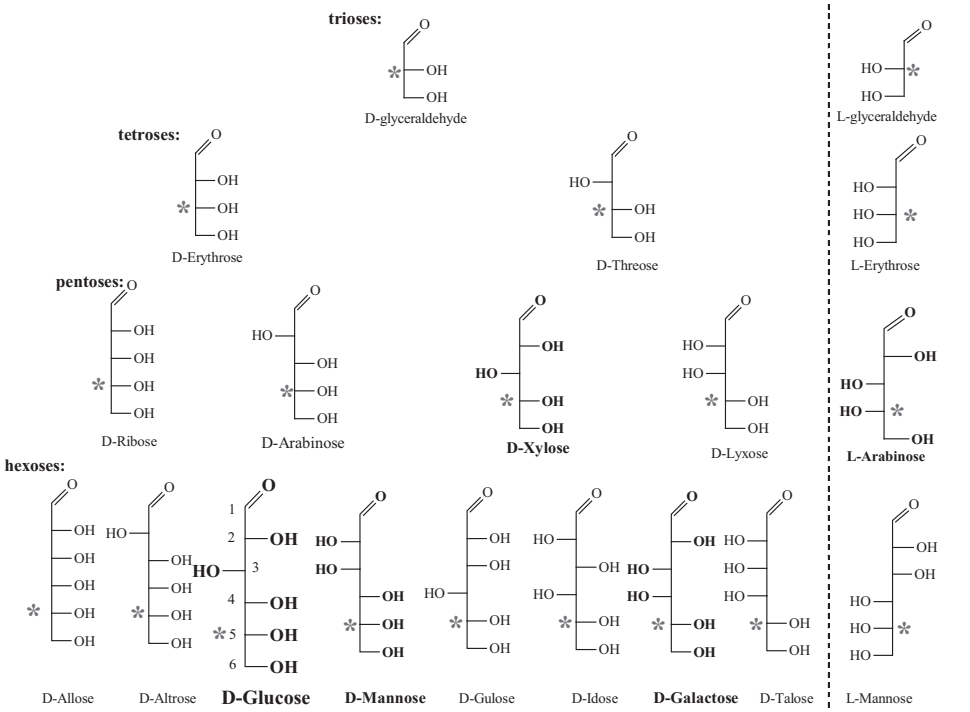


Figure 4.4. The structures of the aldoses. The most relevant monosaccharides in wood chemistry is shown in bold letters. The D-aldoses can be seen as derivated from the D-glyceraldehyde, where novel HCOH groups are added between the chiral carbon in glyceraldehydes (*) and the C1, i.e., the aldehyde carbon. For al D-aldoses there is an L-aldose that is a mirror image of the D-sugar. Some examples are shown right of the line. L-aldoses are however, more rare in nature than D-aldoses. In wood some are however present, as L-arabinose.

Monosaccharides with a carbonyl are called ketoses as mentioned above. In practice, the carbonyl is always located at C2 in all important ketoses[7]. The simplest ketose, dihydroxyaceton, figure 4.5, has no chiral centers, and thus the ketose "tree" characterizing the compounds as D-

[7] This has to do with the fact that the double bond oxygen is involved in the ring formation that is discussed in section 4.1.3. If the carbonyl had been located on the C3, ring formation had been difficult and the properties of the monosaccharide had been different.

or L-forms starts with D- and L-erytrulose, figure 4.5. The by far most important ketose is *fructose*, but ketoses play generally a minor role in plant cell walls.

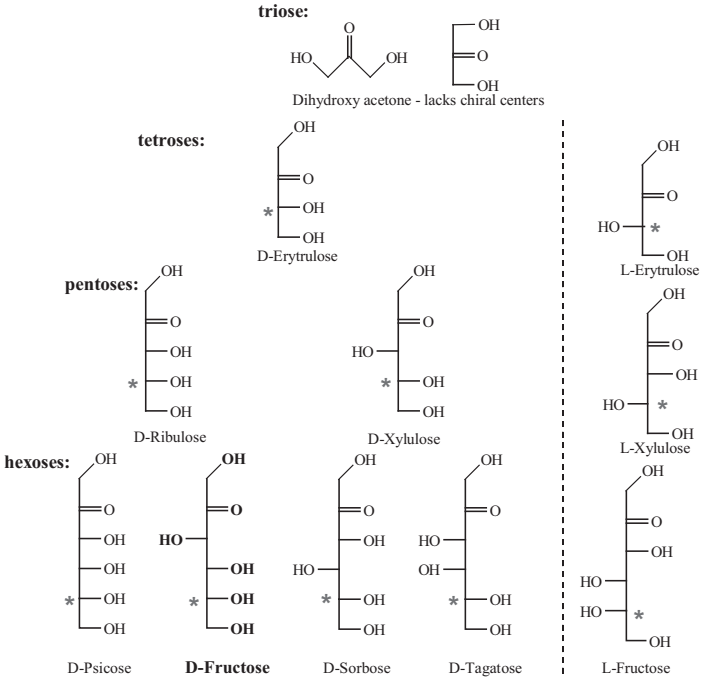


Figure 4.5. Ketoses. The asymmetric carbons furthest from the most oxidized carbon (*) have hydroxyl groups that point to the right for D-ketoses, and to the left for the unusual L-ketoses of which some examples are shown right of the line. As with aldoses, the D- and L-ketoses with the same name are mirror images, enatiomers. The names of the ketotetroses and pentoses are formed from the names of the corresponding aldoses by the addition of "-ul-" to the suffix.

4.2.2 Modified and Branched Monosaccharides

Different types of modifications of the common monosaccharides are very widespread; if the carbonyl in a ketose or an aldose is reduced to an alcohol an *alditol* is created. Thus alditols are polyols (*Figure 4.6*). Oxidation of the aldehyde group leads to an *aldonic acid*. If a primary alcohol, i.e., on the end of the chain is oxidized to an aldehyde a *dialdose* is formed and if it is oxidized to a carboxylic acid, it is an *uronic acid.* Oxidation of a secondary alcohol form an *aldoketose*, and also *diketoses* exists. If an amino group replaces one or more of the hydroxyl groups, the residue is called *amino sugar*, and if it is reduced to a $–CH_2–$ or $–CH_3$ group it is called a *deoxysugar* (*Figure 4.6*). Hydroxyls groups are often derivated; it can be phosphorylated, acetylated or metylated (eterized by methanol) (*Figure 4.6*).

Some monosaccharides with modified structure are important building stones in the plant cell wall, and some of them have trivial names[8], the deoxy sugars *rhamnose* and *fucose*, and the oxidized sugars, *O-methyl-glucuronic* acid and *galacturonic* acid (*Figure 4.7*).

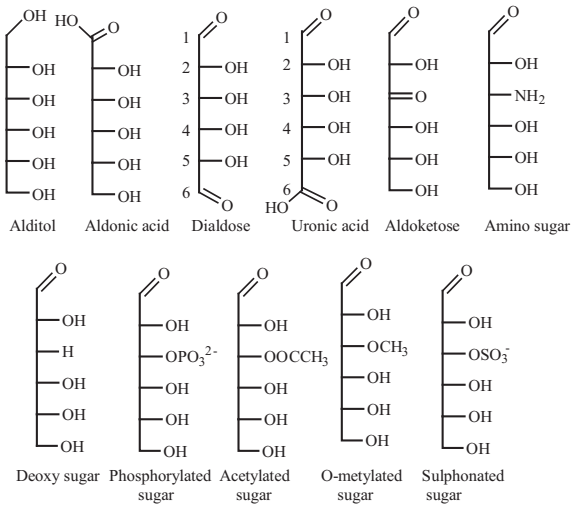


Figure 4.6. Examples of monosaccharides with modified structure. The examples here are based on an aldohexose, but similar structures exist also in other monosaccharides. In the aldotol, the aldehyde has been reduced to an alcohol. In the aldonic acid the aldehyde has been oxidized to a carboxylic acid. In the dialdos, the primary alcohol (6) have been oxidized to an aldehyde, while in the uronic acid, the same carbon have been oxidized to a carboxylic acid. An aldoketose has a keto group instead of a secondary alcohol and the amino sugar has an alcohol replayced by a aminogroup. In the deoxy sugar an alcohol is replaced by a hydrogen. Sugars can be phosphorylated, acetylated metylated and sulphonated. Note that none of this sugars fulfil the $C_n(H_2O)_n$ formula. The aldotol and aldonic acid do not fulfil definition of carbohydrate used in this textbook.

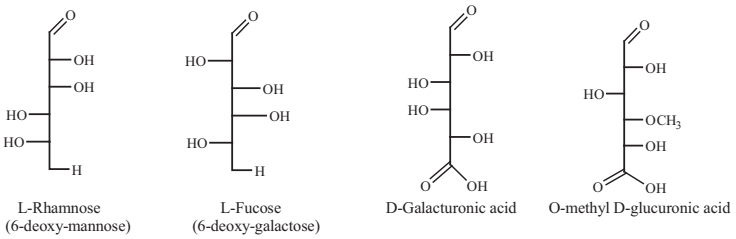


Figure 4.7. Sugars with modified structures that are important in plant cell walls.

The monosaccharides discussed so far have unbranced carbon chain, but there are also examples of monosachcarides with branched chains (*Figure 4.8*). Generally, they are rather uncommon in nature, but one of them, *apiose* is present in the plant wall polysaccharide pectin (rhamnogalactouran II, 5.7). With this the structures of the ten monosaccharides of which most of the plant cell wall consist have been presented. In *Table 4.1* abbreviation systems for them

[8] Trivial names are often given beside the functional name for biologically or technically important components. They are generally fully accepted in technical/scientific literature. For example is "acetic acid" a trivial name for etanoic acid.

are shown. From now we will not use D- and L- nomenclature, unless it need to be specified which enantiomer that is discussed.

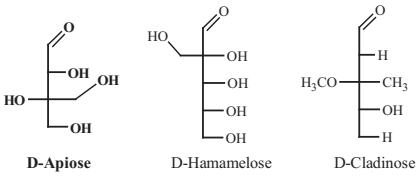


Figure 4.8. Examples of monosaccharide with branched carbon chain. Apiose is present in pectin. Hamamelose is a product in carbon fixation of the photosynthesis of many plants, and cladinose is present in some bacterial antibiotics.

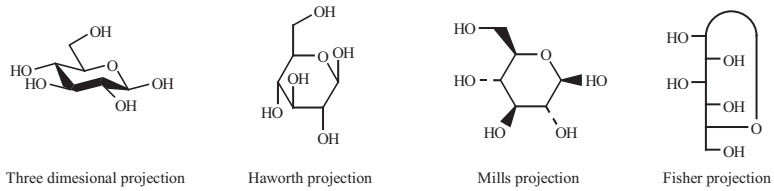


Figure 4.9. Different ways of presenting the cyclic form of monosaccharides. In this textbook we will mainly use the more realistic three-dimensional projection, but especially the Haworth projection is very commonly used. As with Fisher projection, carbons and hydrogens directly bound to carbon is normally not shown.

4.2.3 Cyclic Structures

Monosaccharides from pentoses and up have a strong tendency for forming cyclic structures. There can several ways that the structure can be presented (*Figure 4.9*); in this textbook we will use tree dimensional drawing or fisher projections. The structure is formed through an internal reaction between a nucleophilic hydroxyl group and the electrophilic carbonyl (*Figure 4.10*) creating a *hemiacetal* bond[9]. This creates *a novel chiral center* called the *anomeric carbon*. The two newly formed isomers, or *anomers*, are called α and β. As shown in *Figure 4.10*, an *α-anomer has the hydroxyl group on the same side of the ring as the oxygen forming the ring* (in the case of glucose upwards), and the *β-anomer has the hydroxyl directed to the opposite side* (in the case of glucose downwards). In the same manner, ketoses also form cyclic hemiketals, *Figure 4.13*. The ring can contain 6 atomes, called a *pyranoside* (due to the similarity to the cyclic hydrocarbon pyran), or 5 atoms, called *furanoside* (due to the similarity to the cyclic hydrocarbon furan) (*Figure 4.10*).

[9] The chemical definition of a hemiacetal is a bond formed by an alcohol and an aldehyde. If it is an alcohol and a keton it is hemiketal. Note the difference to ether and ester bonds.

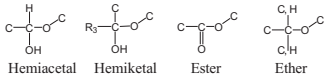
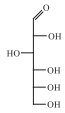
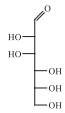
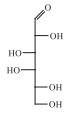
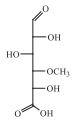
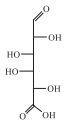
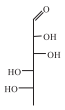
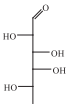


Table 4.1. The short form of the most common monosacharides in plant cell walls. The letter code will be used in this book, but the graphical symbols are rather common, especially in biochemical literature. The monosaccharides are ordered according to their abundance in normal conifer wood. The wood polysaccharides are described later in this chapter (Table 4.4, section 4.4–4.6), and in chapter 5.

Monosaccharide	Letter code	Structure	Occurrence in wood polysaccharides
D-Glucose	Glc		Cellulose, glucomannan, xyloglucan.
D-Mannose	Man		Glucomannan
D-Xylose	Xyl		Xylan, xyloglucan
D-Galactose	Gal		Glucomannan, Pectin, Larch galactans
L-Arabinose	Ara		Xylan, Pectin
O-metyl D-glucuronic acid	4-O-Me-GlcUA		Xylan
D-Galacturonic acid	GalUA		Pectin
L-Rhamnose	Rha		Pectin, traces in xylan
L-Fucose	Fuc		Xyloglucan, traces in pectin
D-Apiose	Api		Small amounts in some pectins

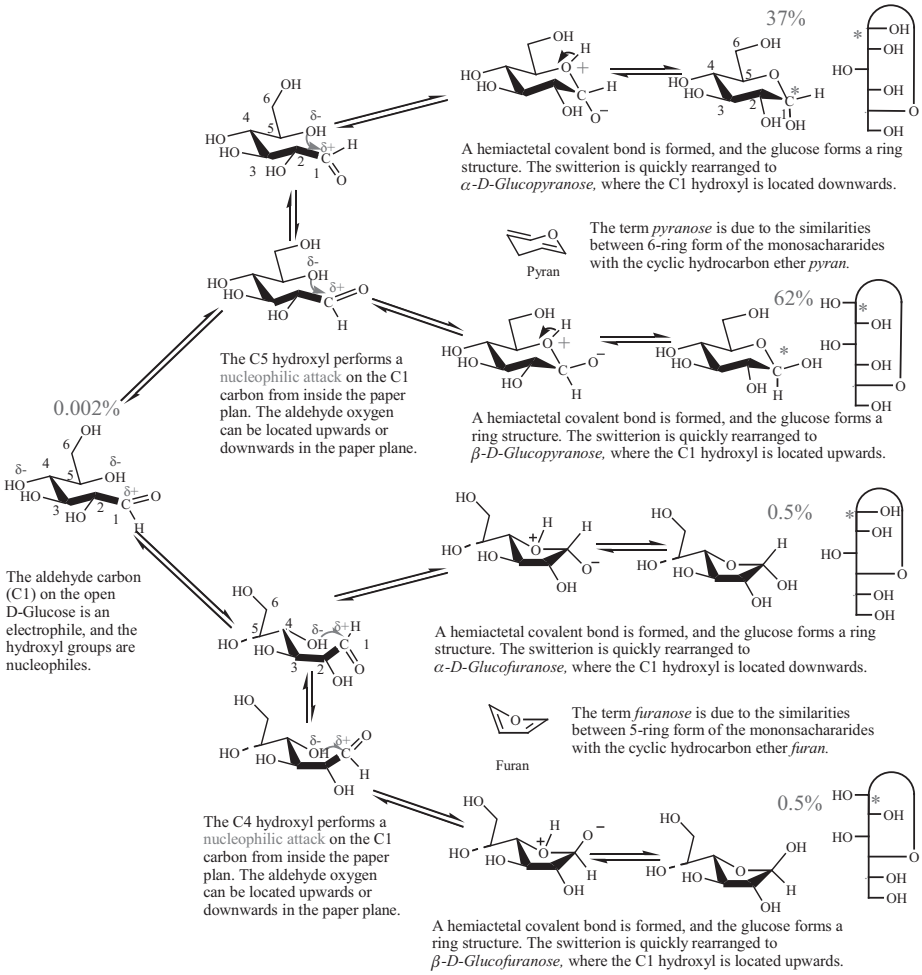


Figure 4.10. Ring closure of monosaccharides. Here the mechanism is shown for the glucose, but similar reactions occur also with other pentoses, hexoses and heavier monosaccharides. If the nucleophilic attack is performed with the C5 hydroxyl it forms a pyranoside (6-ring), and if it is performed with the C4 it forms a furanoside (5-ring). If the novel hydroxyl group formed from the aldehyde oxygen is located downwards it is an α-anomer , and if it is located upwards it is a β-anomer. All forms including the open forms are in equilibrium with each other and the fraction of forms in each form is given in percent. As seen the pyranose and especially the β-anomer is the most common.

For glucose and other aldohexoses, the equilibrium is strongly towards the pyranosides; the furanosides and open forms are normally minor components when in water solution (*Table 4.2*). All forms are however in equilibrium with each other (by opening and closing of the cyclic structure), this means that if a pure anomer, as α-pyranoside is dissolved in water, the other forms will be formed spontaneous. This phenomenon is called *mutarotation* and is catalyzed by acid or base. At neutral pH and room temperature, the reaction is relatively slowly, and it can take up to 30 minutes before equilibrium is reached.

Table 4.2. Distribution of ring- and open-chain forms of monosaccharides of some monosaccharides at equilibrium in water. The pyranoses (6-rings) dominates strongly over the furanoses with the rare sugar idose somewhat of an exception. The β-pyranose is generally more common than the α-pyranose with mannose and idose as exceptions. These differences are due to the orientation of the hydroxyl groups of the sugars.

	Temp. (°C)	**α-pyranose (%)**	**β-pyranose (%)**	**α-furanose (%)**	**β-furanose (%)**	**Open-chain (%)**
D-glucose	31	38.0	62.0	0.5	0.5	0.002
D-mannose	44	65.5	34.5	0.6	0.3	0.005
D-galactose	31	30.0	64.0	2.5	3.5	0.02
D-idose	31	38.5	36.0	11.5	14.0	0.20
D-fructose	31	2.5	65.0	6.5	25.0	0.8
D-xylose	31	36.5	63.0	0.3	0.3	0.002
D-ribose	31	21.5	58.5	6.4	13.5	0.05

If one shall underline that a sugar is in the pyranose form, the suffix '*pyranoside*" is added to a short form of the monosaccaride name and α or β is added before depending on which anomer it is, i.e., α glucopyranoside, β mannopyranoside and α xylopyranoside. Similarly, furanose forms are named *furanosides*, i.e., β-arabinofuranoside and α-galactofuranoside. Pyranosides are in short form "*p*" (Glu*p*), and furanosides "*f*" (Ara*f*).

Since the covalent bonds around the carbon are tetrahedral distributed, the pyranose ring is not flat. This means that the ring can take several conformations, *chairs*, *boats* and screw boat[10]. Of these the chair form is the most stable and dominates, but other forms can occur for instance during enzyme catalysis of carbohydrates (*Figure 4.11*).

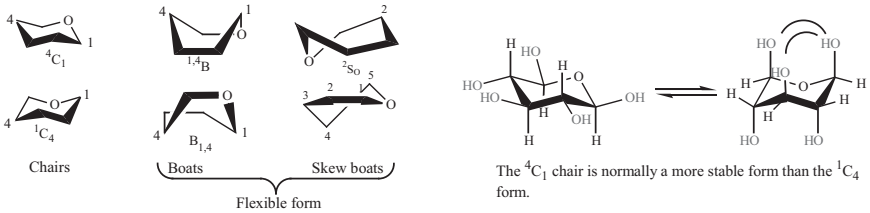


Figure 4.11. Conformations of pyranoses. The conformations of the six-membered ring systems are better characterised than those of the less stable five-member rings. The cyclohexane molecule can occur in two relaxed forms, the *chair* and the flexible form. The latter can exist in a variety of forms, of which only the *boat* and *skew-boat* are easily described in two dimensions on paper. The nomenclature of the conformations: the letter means "C" as in chair, "B" as in boat, and "S" as in Skewboat. Figures or "O" in ^{super}- or $_{subscript}$ notifies the atoms (carbon number or oxygen) most over or under the plane of the molecule.

[10] The boat and the skew boat can be seen as variants of a more flexible form of ring than the chair form. In the latter the covalent bonds in the ring can be regarded as forming a zigzag pattern.

A closer look at the for chair form of common cyclic form of glucose, β-glucopyranoside, give a clue to why glucose is the most important monosaccharide; the cyclic structure forms a flat disc with hydroxyl groups located outwards and a relatively hydrophobic up- and downside. This conformation allows β-glucopyranosides to pack on the top of each other exposing the hydroxyls for hydrogen bonding and chemical substitution. As shown in *Figure 4.12*, the rare aldohexose idose has not such regular orientation, when in β-pyranose form. These differences are probably the reason why glucose and structurally similar sugars play central roles in biochemistry, whereas idose does not.

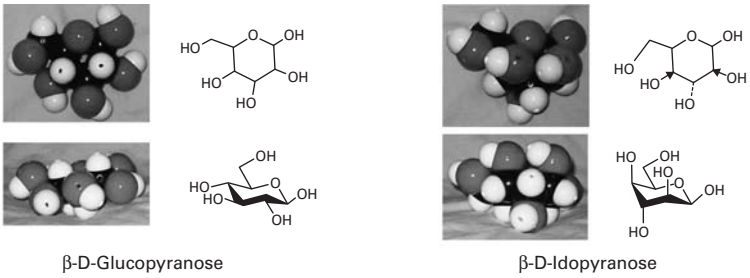


Figure 4.12. The reason why glucose is the most important monosaccharide? The D-glucopyranose forms a "discus" with the hydroxyl groups directed outwards and a relatively hydrophobic centre. The β-D-idopyranose, on the other side, has the hydroxyls more orientated up and down. Has nature chosen glucose instead of idose, due to that it has better possibilities for horizontal interaction with other groups and stacking in top of each other?

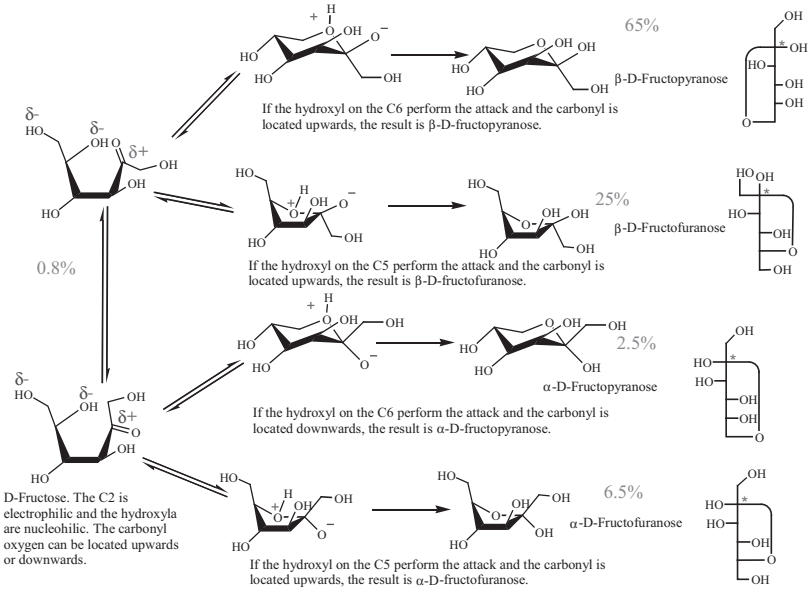


Figure 4.13. Formation of cyclic hemiketal forms of the ketose fructose. Compare with the reaction schedule in figure 4.10. The fractions of the forms at equilibrium are given in percent.

Also aldonic acids can form ring structures through a condensation forming an internal ester, called lactone (*Figure 4.14*). The equilibrium is slightly more to the open acid form in water solution, but the cyclic form constitutes 35–45 %. Lactones are a common product of enzymatic oxidation of aldoses (chapter 11).

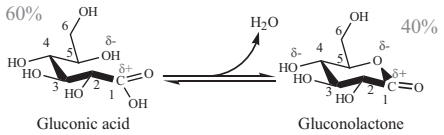


Figure 4.14. Formation of gluconolactone from gluconic acid. The cyclic lactone is formed by a condensation. The fraction of the forms at equilibrium are given in percent.

4.2.4 The Glycosidic Bond

Monosaccharides have excellent possibilities for covalently couples other molecules. Principally all hydroxyl groups can bind other groups and thus a glucose pyranoside have five possible interaction points, while for instance an amino acid has just two or three. Above (4.2.2, *Figure 4.6*) is shown that hydroxyls of monosaccharides can be acetylated, metylated, phosphorylated etc., but the most important bond is the one to other monosaccharide. In practice such bonds does always involve the hydroxyl on the anomeric carbon. The link between this and a hydroxyl on another monosaccharide is formed by a condensation (*Figure 4.15*), and is called a *glycosidic bond*. As discussed above pentoses and hexoses in water solution is in equilibrium between different cyclic and open forms (α-pyranoside, β-furanoside etc.); however, when a glycosidic bond have been formed to the anomeric carbon, the monosaccharide conformation is locked (*Figure 4.15*). Therefore, conformations that are less favorable in solution, furanosides and α-pyranosides (*Table 4.2*), can occur in di- and polysaccharides.

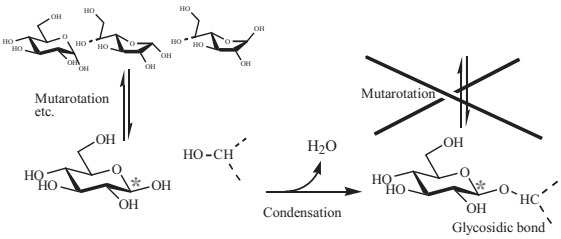
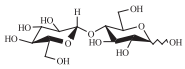
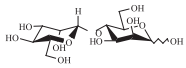
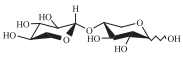
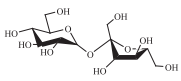
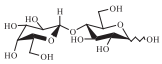
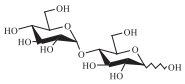
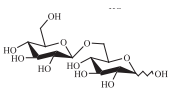
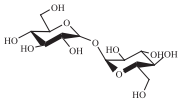


Figure 4.15. Formation of a glycosidic bond. Glycosidic bonds can be formed between the anomeric carbon (*) on a monosacharide and a hydroxyl group on another monosaccharide, but also to much simpler molecules as methanol. A monosaccharide in solutions undergo mutarotation and ecist thus in several different conformations. When a glycosidic bond has been formed to the anomeric carbon, the structure is however “locked” and cannot undergo mutarotation.

Table 4.3. Examples of important disaccharides.

Trivial/short name	Formal name	Structure	Comment
Cellobiose	4-O-(β-D-Glucopyranosyl)-D-Glucopyranose		The main product of enzymatic degradation of cellulose
Mannobiose	4-O-(β-D-Mannopyranosyl)-D-mannopyranose		A product of enzymatic degradation of hemicellulose
Xylobiose	4-O-(β-D-Xylopyranosyl)-D-xylopyranose		A product of enzymatic degradation of hemicellulose
Sucrose	4-O-(α-D-Glucopyranosyl)-β-D-Fructofuranose		The commonly used table sugar. Occurs in fruits, honey, seeds, roots and pitch of some plants. Raw material in cellulose biopolymerizarion. A non-reducing disaccharide.
Lactose	4-O-(β-D-Galactopyranosyl)-D-Glucopyranose		Occurs in milk of mammalians.
Maltose	4-O-(β-D-Glucopyranosyl)-D-Glucopyranose		A main degradation product of amylose (starch)
Gentobiose	6-O-(β-D-Glucopyranosyl)-D-Glucopyranose		Degradation product of some plant polysaccharides
Trehalose	1-O-(β-D-Glucopyranosyl)-α-D-Glucopyranose		Used for energy storage in insects. A non-reducing disaccharide.

4.2.5 Di-, Oligo- and Polysaccharides

A disaccharide is two monosaccharides joint by a glycosidic bond. They occur as intermediates in enzymatic degradation of polysaccharides (see chapter 11 for examples), or are used as energy storage in for instance milk, fruits and roots[11]. Formally disaccharides are named according to similar rules as used in organic chemistry; one of the residues is regarded to be the "main" group and the other as a substitution. The substitution groups are named by adding the suffix "*yl*" to a short form of the monosaccharide, and this is added as a suffix to the name of the "main" residue. As a prefix the number of the compelling oxygen is added (see *Table 4.3* for ex-

[11] Except disaccharides also polysaccharides are used for energy storage. Why do not living cells store energy as glucose? The explanation is probably that the osmotic pressure of the corresponding glucose concentration is much higher.

amples) but in practice short forms and trivial names are used for the most common disaccharides. In *Table 4.3* data for some important disaccharides are shown.

Disaccharides are divided into *reducing*- and *non-reducing* sugars, where the later lack a free anomeric carbon of aldose type (*Table 4.3*). The term "reducing" is related to the fact that free anomeric aldose carbons can perform reductions as will be discussed below (4.2.5). Non-reducing sugars can undergo mutarotation in the monosaccharide residue carrying the free anomeric carbon, similar as monosaccharides.

Oligosaccharide is a rather vague term for saccharides containing from three to approximately ten monosaccharide residues. If the number shall be specified terms based on the Greek number is used, *trisaccharide*, *tetrasaccharide*, *pentasaccharide* etc. Oligosaccharides are often chemically coupled to other biomolecules, as membrane phospholipids, proteins and polysaccharides. Their role is sometimes in recognition, where the rich possibility for combination is explored.

Polysaccharides are used by nature as energy storage or as construction materials. The number of monosaccharide residues (degree of polymerization, DP) can vary from around ten (there is no sharp borderline towards oligosaccharides) up to ten thousands. A *homopolysaccharide* consists exclusively of one kind of monosaccharide residue, whereas a *heteropolysaccharide* consists of two or more kinds of monosaccharide residues. Polysaccharides can be *linear*, i.e., consist of one single unbranched chain, or *branched*, i.e., have side groups or side chains. There are even examples of *cross-linked polysaccharides*, where two main chains are covalently connected by oligosaccharides.

A homopolysaccharide is named by adding the suffix "*an*" to a short form of the monosaccharide, i.e., an *arabinan* consist of arabinose, a *glucan* of glucose and a *galactouran* of galacturonic acid[12]. Heteropolysaccharides are named by combinations of short form of the monosaccharide with the most common last and the least common first, i.e., xyloglucan consist of more glucose- than xylose residues, and galactoglucomannan has most mannose and least galactose residues with glucose residues in between. If a polysaccharide have a very complex structure with many kinds of monosaccharide residues or if some mononosaccharide residues are present in vary low amount, only the most important monosaccharide residues are used in the name. The most important polysaccharides have also trivial names. As with reducing disaccharides a free end with anomeric carbon can undergo mutarotation. Examples of some common polysaccharides are shown in *Table 4.4*.

If one compares the main polysaccharides with structural function, one can seen that many of them have structural similarities with cellulose, chitin, peptidoglucan, most hemicelluloses, the galactouran part of pectin etc. Many of them are connected with β-1→4 glycosidic bonds – with the exception of galactans that are connected with α-1→4 glycosidic bonds, but this is explained by the fact that the C4 hydroxyl group has the opposite direction to the one in glucose and mannose (*Table 4.1*). This means that there are structurally similarities between cellulose, which will be discussed more deeply later in this chapter, and many other polysaccharides. There are however also differences; pectin and hemicelluloses (discussed in detain in the chapter 5) are branched polysaccharides while cellulose is unbranched. The polysaccharide with the largest similarities to cellulose is probably chitin. The nomenclature rules for monosaccharide are summarized in *Figure 4.16*.

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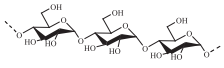
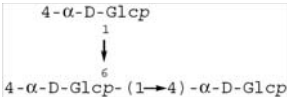
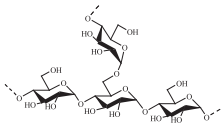
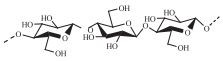
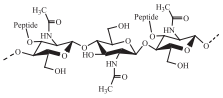
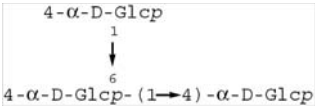
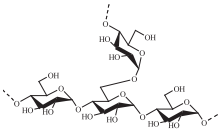
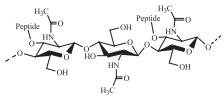
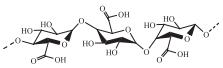
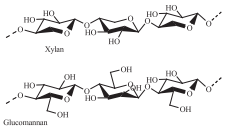
[12] For polymers of uronic acid an alternative nomenclature does exist that is used in parallel with the one describide here. According to this a polymer of galacturonic acid is called *polygalacturonic acid*. This terminology shall be avoided since it is inconsistent.

Table 4.4. Examples of important polysaccharides.

Trivial name	Composition	Structure	Occurrence and function
Amylose	4-α-D-Glc*p*-(1→4)-α-D-Glc*p*		One of the main components in starch. Energy storage in plants.
Amylopectin			One of the main components in starch. Energy storage in plants.
Cellulose	4-β-D-Glc*p*-(1→4)-β-D-Glc*p*		Construction material in cell wall of plants and some other organisms.
Chitin	4-β-D-GlcN*p*-(1→4)-β-D-GlcN*p*		Construction material in the cell walls of insects, shellfish and fungi.
Glycogen			Energy storage in animals.
Peptidoglycan	β-D-GlcNAc*p*-(1→4)-D-MurNAc*p*		Construction materials in cell walls of bacteria.
Galactouran	4-α-D-GalU*p*-1→4-α-D-GalU*p*		Main part in pectin, a construction material in fruits and plant cell wall.
Hemicelluloses (xylan glucomannan etc.)	For example 4-β-D-Xyl*p*-(1→4)-β-D-Xyl*p* 4-β-D-Man*p*-(1→4)-β-D-Glc*p* See chapter 5		Group of hetero-polysaccharides that are construction material in plant cell walls.

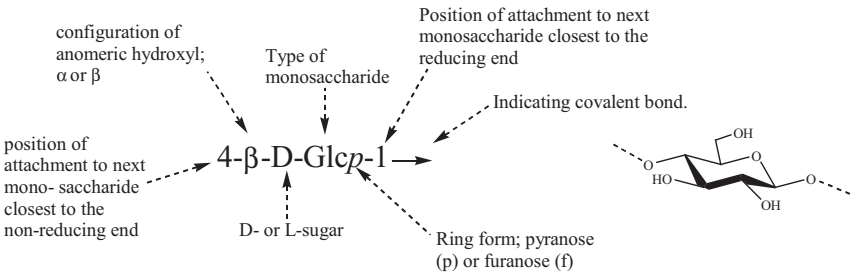


Figure 4.16. Nomenclature for monosaccharide residues in polysaccharide chains.

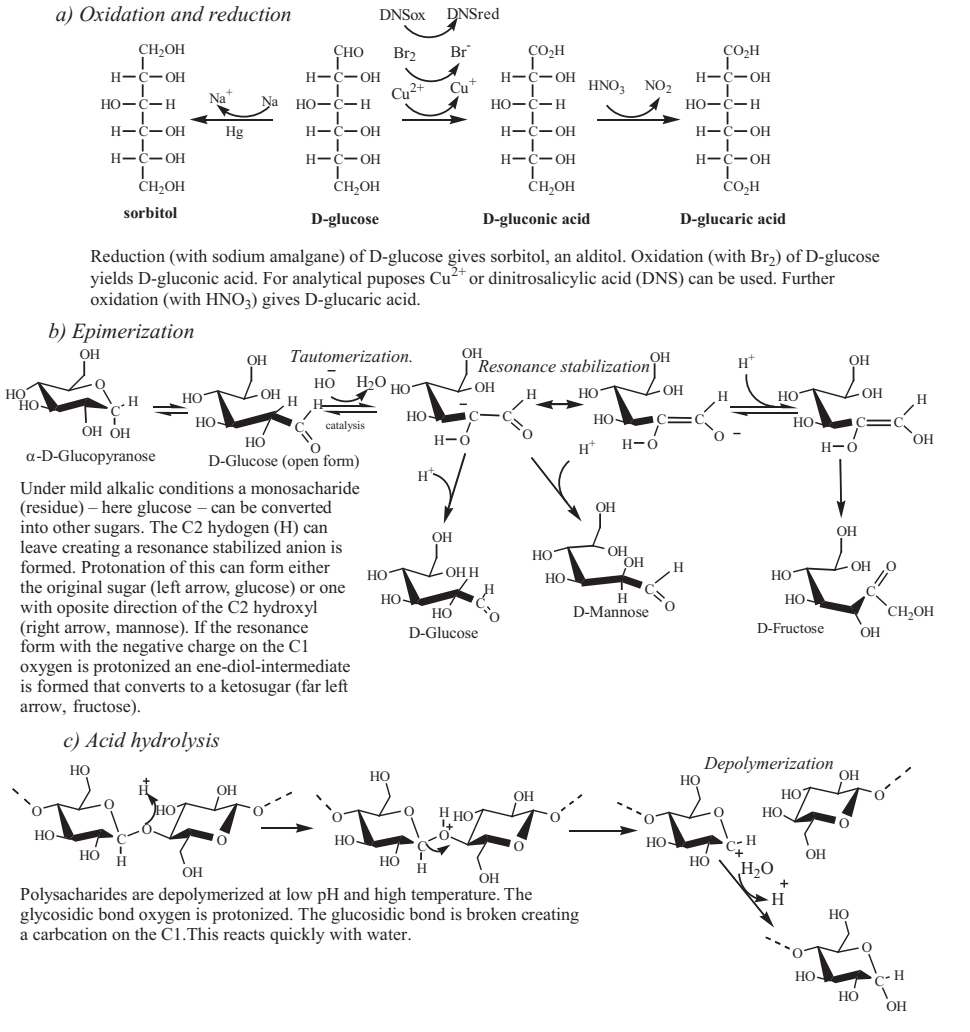


Figure 4.17. Examples of important chemical reactions of carbohydrates.

4.2.6 Carbohydrate Reactions

Carbohydrates react chemically in a number of characteristic ways. These reactions are important in chemical analysis, and some are also occurring during pulping and bleaching where they have central roles.

- *Reductions* and *oxidations* of carbohydrates are often performed in chemical carbohydrate analysis (chapter 9), and can also happened during biological and technical reactions. Reduction of ketons and aldehydes (in aldoses and ketoses) to an alcohol, produce an aldi-

tole (4.2.2, *Figure 4.6*). Sodium borohydride is often used for such a reduction *(Figure 4.17)*. Oxidation of aldoses yields first aldonic acids. This reaction is important for concentration determination of reducing sugars and uses for instance Cu^{2+} or dinitrosalicylic acid as oxidants. Oxidation of the primary alcohol (CH_2OH-group) in aldoses yields uronic acids. If it is both an aldonic acid and an uronic acid it is called an *aldaric acid (Figure 4.17)*

- *Epimerisation*. In weakly alkaline solutions aldoses and ketoses may undergo rearrangements. The rearrangements have importance in reactions during chemical pulping (peeling and stopping). One type of rearrangement is epimerisation. It starts with a rearrangement of the aldehyde to an ene-diol group, (*tautomerisation*), and this reacts further in forming other sugars *(Figure 4.17)*.
- *Hydrolysis* of glycosidic bonds can be catalysed by acid, base or enzymes and is of importance in many technical processes with wood as raw material (*Figure 4.17*). They are also important in analysis of the sugar content of wood and other materials containing polysaccharides. This type of reactions is further discussed in chapter 11.
- *Radical degradation.* Radicals as hydroxyl radical ($OH^{\cdot}$) and chloromonoxid radical ($ClO^{\cdot}$) can degrade carbohydrates among others by breaking glycosidic bonds. Such reactions are further discussed in chapter 26.
- *Caramelization*. When carbohydrates are heated to 110–180C (varies between different carbohydrates) under water free conditions, it undergo a series of complex reactions that includes dehydrations and condensations, which produced modified carbohydrate structures that include double bonds. The reaction products are often coloured, and may have pleasurable taste and smell.

4.3 Occurrence and Function of Cellulose

As discussed in chapter 2, cellulose is a main component in the cell walls of all true plants (Kingdom *Plantae*), but there are also several other types of organisms that produce cellulose – among eucaryotes, sea squirt (also called tunicates, a sea animal), the Oomycetes ("water molds" fungal like protist[13] eucaryotes) and some other protists, and some algae (for instance the bubble algae *Valonia*) synthesize cellulose, and there are also procaryoic bacteria, as *Acetobacter*, that makes cellulose (*Figure 4.18*). Most likely, cellulose has been "invented" several times during the evolution. In spite of this, the covalent pattern is always identical in cellulose, although the aggregation pattern might be very different between different organisms and even tissues within the same organism. The function of cellulose is always mechanical, and it occurs either in pure form as in the seed hair of cotton, or mixed with other polysaccharides and lignin, as in wood. The role of cellulose in this "composite" is to work as an enforcing fiber.

[13] Protists is a Kingdom of primitive eukaryotes that are either plants, animals or fungi. In recent classifications the protests are divided into numerous Kingdoms. The Oomycetes seems to be closer related to certain algaes than to fungi.

The function of the cellulose is somewhat different in various types of organisms; in plants, Oomycetes and probably also some of the bacteria, the cellulose is located in the cell wall; in sea squids, the cellulose forms a tunic surrounding the complete animal, and in the cellulose producing bacterium *Acetobacter*, the cellulose is produced as extracellular fibrils, forming sheets that the bacteria float on.

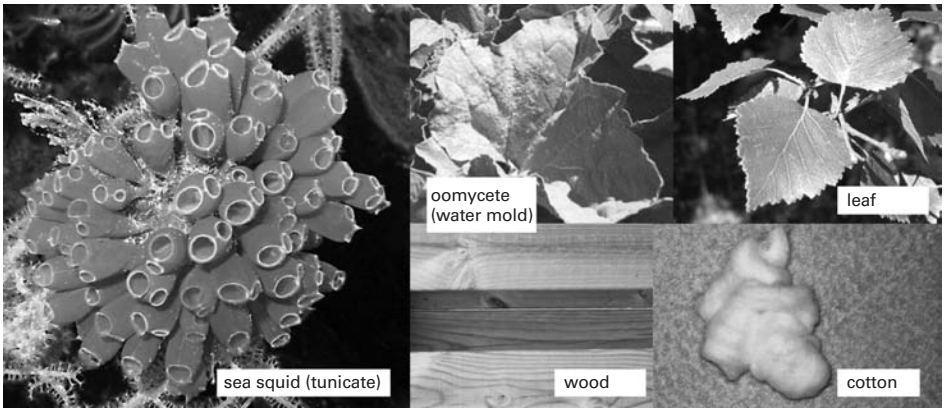


Figure 4.18. Examples of natural sources of cellulose. Several not close related organisms produce cellulose. Thus cellulose has probably not a common origin. Sea squids are sea animals that have a tunic of cellulose surrounding their body. Oomycetes are filamentous eukaryotes with some similarities to fungi. The species shown here is a parasite on plants. They have cellulose in their cell walls. In plants cellulose can occur together with lignin and hemicelluloses as in wood, or in lignin free, but hemicellulose rich tissues as in leafs. In some cases the cellulose occurs in almost pure forms as in the seed hairs of cotton. The photo of the sea squid is by Adam Laverty and the photo of the Oomycete is by Mike Matheron.

4.4 Primary Structure of Cellulose

The primary structure, i.e., its covalent bond pattern, of cellulose is very simple - a linear unbranced polymer of ß-glucopyranoside residues, connected with 1→4 β-glycosidic bonds. The degree of polymerization is often very high; values of 15 000 residues in one chain are reported making cellulose to one of the longest of known polysaccharides. The fact that the glucopyranose units are in the form of ß-anomers, makes the polysaccharide strait and extended, in the opposite to the 1→4 glucan of α-anomers, amylose, which is hexicalled shaped. The cellulose chain is not *totally* strait; theoretical calculations indicate that a cellulose chain form a very extended helix. If this has some biological significance is not known. As shown in *Figure 4.19* every second glucose residue is “turned upside down” compared to the previous, i.e., the residues are rotated 180º towards each other. Thus, the repeated unit in cellulose is a cellobiose residue rather than a glucose residue.

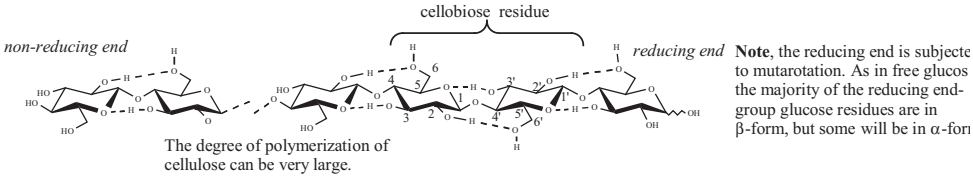


Figure 4.19. Primary structure of cellulose. The structure is a linear 1→4 polysaccharide of β-D- glucose residues. The degree of polymerization may be far over 10 000. The glucose residues are 180° towards each other, making the repeated unit to a cellobiose residue rather than a glucose residue.

4.5 Secondary Structure of Cellulose

The primary structure of cellulose is as discussed above very simple, and the properties of cellulose that have made it to such a biological and technical interesting polysaccharide, are dependent on the secondary structure.

4.5.1 Hydrogen Bond Pattern

Two hydrogen bonds – between the C6 hydroxyl and the C2 hydroxyl and between the C5 oxygen and C3 hydroxyl (*Figure 4.19*) – stabilize the glycosidic bond and make the structure stiff. There are also hydrogen bonds between cellulose chains forming *sheets*. The hydrogen bond is located between the hydroxyls at C6 and C3 (*Figure 4.20*).

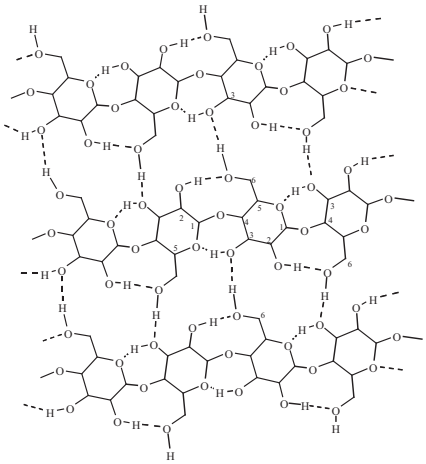


Figure 4.20. Hydrogen bonds between cellulose chains. The glucose residues in the cellulose chains are in this figure seen from "above". One hydrogen bond between C6 and C3 hydroxyls per glucose residue is formed between parallel chains. Parallel cellulose chains side by side like this form a *cellulose sheet*.

4.5.2 Interaction of Cellulose Sheets

Cellulose sheets are stocked over each other and interact with van der Waals bonds[14] and χ interaction[15], but no hydrogen bonds. As discussed above the top and bottom of the β-glucosepyranose is relatively apolar.

4.5.3 Cellulose I_α and I_β

When the cellulose sheets bind to each other, they can for two different crystal forms, cellulose I_α and I_β. This is due to that the glucose residues of the different sheets do not stack directly over each other, but there is a displacement in the position of the chains in the adjutant cellulose sheets. The third layer can be displaced in the same direction as the second, forming cellulose I_α, or in the opposed direction, forming cellulose $I\beta$ (*Figure 4.21*). There are also differences in the hydrogen-bonding pattern of cellulose I_α and I_β. As a result of this cellulose I_α and $I\beta$ have different unit cells. Cellulose I_α has a one-chain triclinic unit cell and cellulose I_β a two-chain monoclinic unit cell[16]. Both crystal forms are thought to co-exist in the cellulose. Cellulose Iα is meta-stable and can be transformed to the more stable cellulose I_β at high temperature and pressure in alkaline or acidic solution.

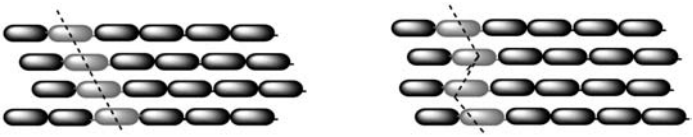


Figure 4.21. Cellulose I_α and I_β. The figure shows cellulose chains, or rather layers, stacked on the top of each other seen from aside. The left structure is cellulose Iα, and the right is cellulose I_β.

4.5.4 Shape of Fibrils

Based on the description above, one could believe that cellulose should be a continuous material consisting of large sheets stacked over each other. This is however not the case; long, relatively

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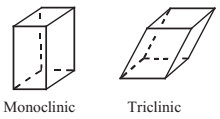
[14] Van der Waals interactions are weak interactions between apolar structures on molecules. They are regarded to be electrostatic to the nature being interaction between temporary polarities within the structures.

[15] The χ-interaction is often called "hydrophobic interaction" in biochemical literature.-It might be surprising that hydrophobic forces are important in the cellulose structure, since both glucose and cellulose is considered to be very hydrophilic. However, the chair conformation of glucose can be described as a discus with the hydroxyl groups pointing outwards. Thus the top and the bottom of the anhydrous glucose is actually rather hydrophobic. Furthermore, the hydroxyl groups are locked in hydrogen bonds in the structure. Interestingly cellulose-binding proteins often use χ-interaction to bind cellulose.

[16] Monoclinic ia a crystal system, where the repeated unit cell dimension are of different length. Two of the axes are at 90° to each other, whilst the third is not. Triclinic is a crystal system, where the repeated unit cell dimensions are of unequal length and none of the corresponding axes are at 90°.



narrow sheets, forms highly organized bundles called *fibrils*. The size of them varies between different organisms, and between different tissues, as leaf and wood; the size can even vary between cell wall layers. How many chains contain a fibril? A common suggestion is 36 for plant secondary walls, but this figure can probably be both higher and lower. In plants, the fibrils are quadratic (possibly with rounded edges), but fibrils from other sources, as *Acetobacter*, can be much flatter, so that the fibril gets the shape of a long band. In *Figure 4.22* shows some suggestion for the dimensions of fibrils. Notable are the very huge fibril of the green algae *Vallonia* that contains over 1000 cellulose chains.

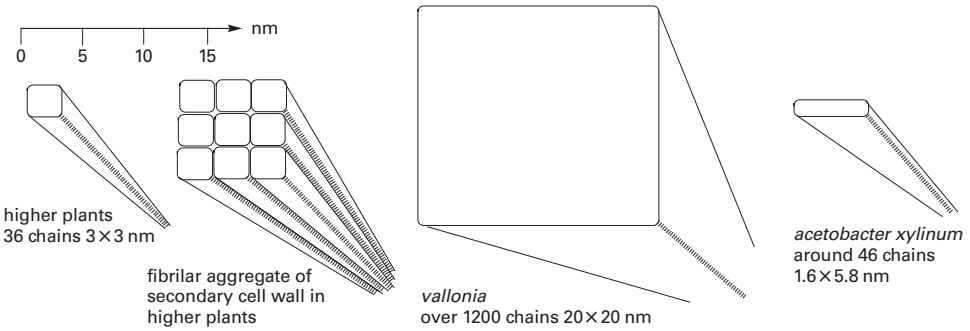


Figure 4.22. Suggested shapes of cellulose fibrils from various sources. To determine shape and size of cellulose fibrils is difficult, since they have a strong tendency to form fibrilar aggregates, and it can be difficult to differentiate between these and true fibrils. Aggregation seems to be more frequent in the secondary cell wall.

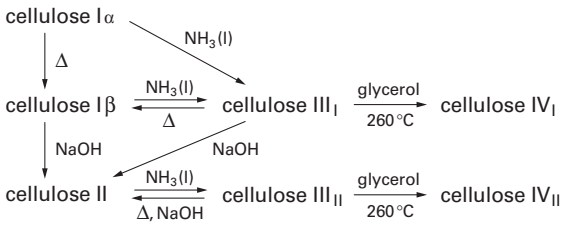


Figure 4.23. Conversion of various crystalline forms of cellulose. Cellulose I is converted to cellulose II by treatment in strong alkali (mercerication). Treatment of both cellulose I and II with liquid ammonia form cellulose III, which in turn is converted to cellulose IV by treatment of cellulose III with glycerol at high temperature.

A cellulose chain can maybe be 5–7 μm long, but a fibril can be much longer, probably at least 40 μm due to that several chains overlap each other. *In summary*, the cellulose fibrils are thus ordered 3-dimensional crystals. Thus, it may be interpreted as the crystals have different bonds in each dimension. *The first dimension* is given by the covalent bonds (enforced by some hydrogen bonds) along the cellulose chains, giving the final length of the fibril. Since cellulose often has a high degree of polymerization (DP), the fibrils are quite long. *The second dimension* constitutes the hydrogen bonds, holding the cellulose chains together in sheets (*Figure 4.20*). The Van der Waals bonds and χ-interaction bridging the cellulose sheets in the fibril form the third dimension.

4.6 Other Crystalline Forms of Cellulose

The secondary structure of cellulose with parallel chains is as mentioned above called cellulose I. However, there exist several other secondary structures that can be formed by various chemical treatments, cellulose II, III and IV (*Figure 4.23*). Of these, cellulose II is the most important. It is technically used as fibers of regenerated cellulose (the chapter "Cellulose products and chemicals from wood."). Cellulose II differs from cellulose I by that the chains are anti parallel, i.e., that every second chain has opposite polarity to the next. Thus the hydrogen bond pattern is different, and there is one more hydrogen bond per glucose residue in cellulose II compared with cellulose I. This might be the explanation for the fact that cellulose II is the thermodynamically stable form of cellulose, while cellulose I just represent a local thermodynamic minimum. The unit cell of cellulose II is monoclinic.

Cellulose II can be formed from cellulose I principally by two methods:

1. *Mercerization*[17]. The first step in a mercerization is called alkalization. Here the cellulose is immersed in strong (18%) NaOH solution, and so-called alkali-cellulose, or Na-cellulose, is formed. The material is washed to remove the alkali and then cellulose II is formed.
2. *Regeneration.* Cellulose II can also be formed by precipitation of dissolved cellulose.

That a cellulose crystal change polarity from parallel cellulose I to anti parallel cellulose II during regeneration, where the cellulose I is first dissolved and then regenerated is perhaps not so strange. But how can this happen during a solid-phase reaction such as mercerization, where the cellulose I material is swelled in alkali? There are two main ideas for how this is carried out:

1. This has been explained by imaging that cellulose I fibrils of different polarity lie close to each other (*Figure 4.24*). This can happen for example in different cell-wall layers, where it can be thought that the cellulose fibrils have been biosynthesisied in different "directions". During the mercerization the cellulose is treated with alkali to form alkali cellulose, a highly swelled cellulose form. Two fibrils with different polarity can then swell into each other. When the alkali is removed the material gets less swelled, but the chains have adopted the anti parallel mode,
2. The anti parallel mode of the chains in cellulose II has been suggested to result from chain folding (*Figure 4.24*). As shown by conformation analysis, folds in the cellulose chains are theoretically possible[18].

Cellulose III and IV are less important structural forms of cellulose. As cellulose II they are with few exceptions only made by man and does not occur in nature to large extent[19]. Cellulose III has some similarities with cellulose II although it is believed to have parallel chains. Cellulose IV is rather similar to cellulose Iβ.

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[17] Invented by John Mercer 1844, as a method for improving the strength and color-absorption of cotton cloth.
[18] During the recent years some scientist have questioned if cellulose II from mercerized cellulose really is anti parallel; alternatively this structure should have a different hydrogen bonding pattern than cellulose I.
[19] There are a few bacteria that are believed to synthesize cellulose II. Cellulose IV has been suggested to occur in small amounts in primary cell walls of cotton.

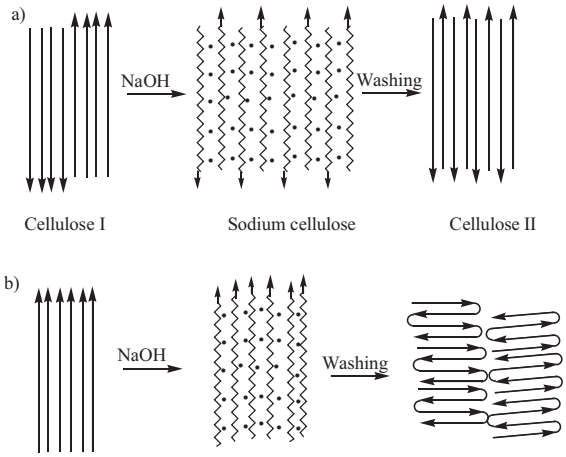

Figure 4.24. Suggested conversation of parallell cellulose I to antiparallell cellulose II (Two theories.) a) Fibrils with different polarities are swelled as sodium cellulose, and diffuse into each other. When the alkali is washed away, the aniparallell cellulose II is formed. b) Parallell cellulose chains swells in NaOH forming sodium cellulose. When the alkali is washed away a zigzak antiparallell cellulose structure is formed.

4.7 Biosynthesis

Cellulose biosynthesis is the formation of cellulose chains from glucose monomers, the formation of fibrils from the cellulose chains, and the orientation of the fibrils in the cell wall. In the two latter aspects, the knowledge is incomplete, and therefore the description here is in part based on speculation, that might be in part revised in the future.

The formation of the ß-(1→4)-glycosidic bonds in cellulose is a condensation reaction, that is thermodynamically unfavorable, i.e., the equilibrium is highly towards the monosacchaides (*Figure 4.25*). How can then the glycosidic bond be formed? The plant solve problem by couple the thermodynamically unfavorable reaction to a highly favorable process – the hydrolysis of a phosphate bond in UTP (uracyl triphosphate a nuclotide) (*Figure 4.25*). The hydrolysis of this bond thus works as a thermodynamically "fuel" for the overall reaction. In practice this means that firstly an *activated glucose residue* is formed from sucrose and UTP in an enzymatic catalyzed reaction. This reaction is thermodynamically favorable due to the release of a phosphate ion. Thereafter the activated glucose residue (UDP-glucosyl) couples to the non-reducing end of the growing cellulose chain catalyzed by a second enzyme, *cellulose synthase*. This reaction became thermodynamically favorable by the release of the UDP. After the addition of the novel glucose residue to the cellulose chain, it moves within the cellulose synthetase that hereby becomes ready for accepting a novel glycosyl-UDP.

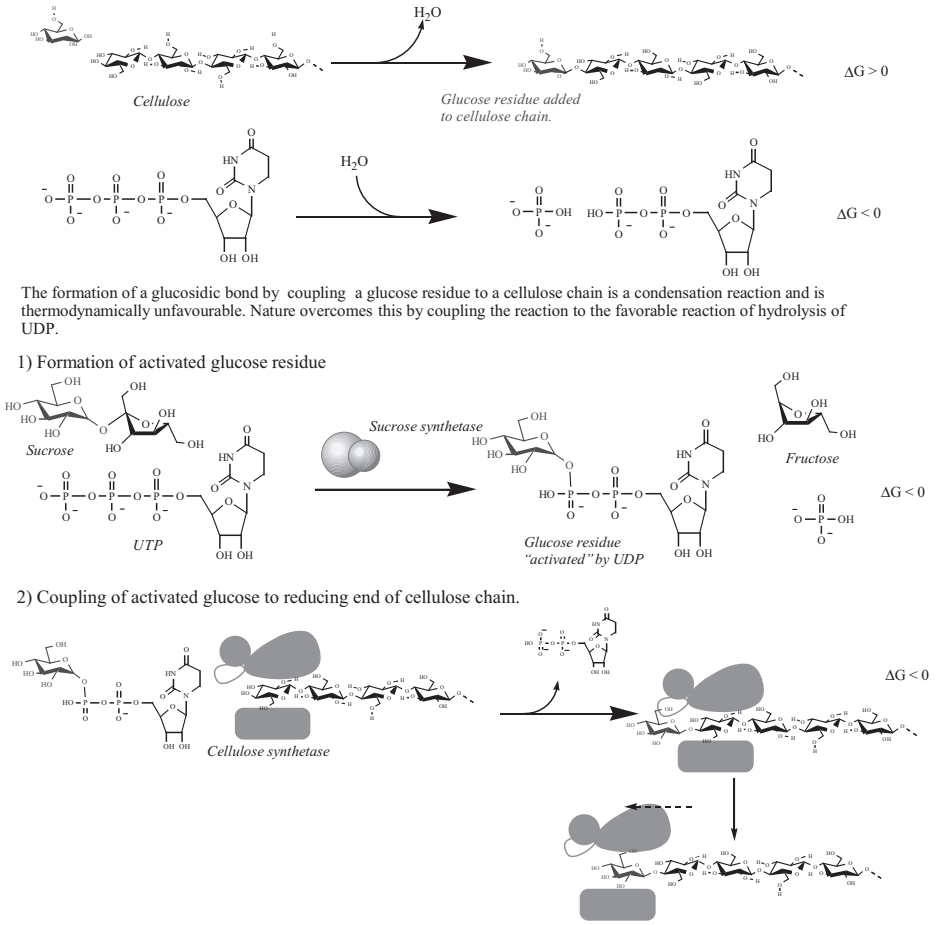

Figure 4.25. Formation of the glycosidic bonds in cellulose The formation of the glycosidic bond is a condensation that is theromdynamicallt unfavourable. The nature has overcome this problem by coupling the raction to the favourable hydrolysis of an energy rich phosphate bound. In practive this means that an activated intermediate, UDP-glucose is created first,and coupleded to a growing cellulose chain.

4.7.1 Terminal Complexes Synthesize Cellulose

This description for how the covalent bonds are formed, are in its main lines also valid for other polysaccharides, such as hemicelluloses. However, cellulose is due to its high degree of polymerization and crystallinity totally insoluble in water at physiological conditions, in the opposite of most other polysaccharides. Therefore, cellulose cannot be synthesized intracellular and excreted to the cell wall were deposited (as hemicelluloses and pectin, 5.3). Instead the cellulose synthase is located inside the outer cell membrane[20] and the cellulose chains are thus directly deposited in the cell wall (*Figure 4.26*).

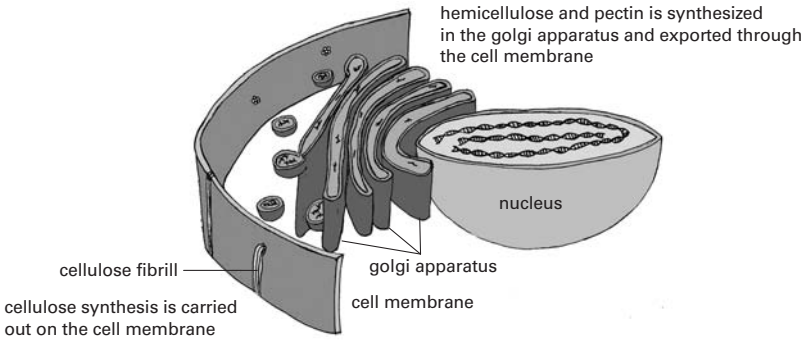

Figure 4.26. Localization of cellulose biosynthesis in the cell.

It is believed that several cellulose synthases form aggregates in the cell wall, *terminal complexes*, and that several cellulose chains thus are synthesized in parallel. Outside the terminal complex, the cellulose chains crystallize spontaneously in a parallel cellulose I-structure. By being synthesized in close vicinity to other cellulose chains, it avoids to form the thermodynamically more stable antiparallel cellulose II (see above, 4.6)[21].

Although terminal complexes synthesizing cellulose have been visualized in electron microscopy, the details of the process are not known with certainty. However, a possibility is that a cellulose fibril is synthesized by one single terminal complex. If this is the case, the size and shape of the cellulose fibrils, may be determined already at the biosynthesis. Thus, the variation of the fibrils between species, plant tissues and cell wall layers (4.5.4), is possibly explained by that the terminal complexes are constructed in different ways. In higher plants, the terminal complex seems to be formed as a circle of 6 units (believed to synthesize 6 chains each); the structure is called *rosette* ("small rose"). In other cellulose-synthesizing organisms as algae and bacteria, the terminal complexes seem to be linear, and not seldom much larger than in higher plants (*Figure 4.27*). Cellulose Iα is thought to be biosynthesised by linear terminal complexes to large extent, while by rosette-like terminal complexes in the cell wall make more cellulose Iβ.

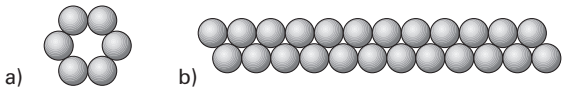

Figure 4.27. Terminal complexes seem from above. a) Rosette type in higher plants. b) Linear type present in bacteria and some algae. The size might vary highly. Compare the terminal complex dimensions with the form of the cellulose fibrils in *Figure 4.22*.

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[20] Biological membranes consist of amphophilic phospholipids and similar compounds and can be described as a "two-dimensional liquids" in which different proteins is "floating". The structure and properties of biological membranes can be studied in any larger textbook of biochemistry.

[21] There are actually a small number of bacteria that naturally synthesize cellulose II. There are most likely fundamental differences between these organisms and other cellulose-synthesizing organisms. One possibility is that the cellulose chains are made one by one and therefore form the thermodynamically most stable form of cellulose – cellulose II.

Except for cellulose synthesizes, the rosettes might contain several other proteins involved in the construction of the rosettes. There seems also to be a cellulase, i.e., an enzyme hydrolyzing glycosidic bonds in cellulose present (see chapter 11).

4.7.2 Control over Fibrilar Angle

As discussed in chapter 3, the cellulose fibrils have a specific orientation that is different in the various cell wall layers. How does the plant control this? Inside the cell membrane, *the cytoskeleton*, a network of protein fibers (microtubule) is located, and it is believed that these direct the terminal complex in some way. By altering the direction of the microtubule, the cellulose fibril direction will be controlled (*Figure 4.28*).

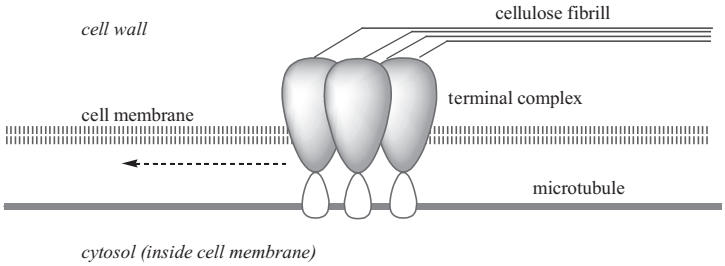

Figure 4.28. A model for the control of fibrilar angle of cellulose. The terminal complex "flutes" in the cell membrane and synthesizes a cellulose fibril that is deposited in the cell wall. During this process the terminal complex is moving in the cell membrane, and the microtubule, a network of protein fibers inside the cell membrane directs the movements of these - maybe similar as a railway directs the movements of a train.

4.8 Super Fibrilar Organization of Cellulose

The knowledge for how cellulose is arranged on the superfibrilar level is very limited, due to absence of suitable analysis methods, and that this aspect of the cellulose structure is highly affected on cellulose preparation and such processes as pulping and bleaching. However, there are two facts that are uncontroversial:

1. Cellulose contains both highly (crystalline) ordered and less ordered (semi-crystalline or even amorphous) structures. The less ordered cellulose has been suggested to be located either on the fibrilar surface, or in "amorphous" segments of fibrils (*Figure 4.29*). In favor of the last hypothesis is the fact that cellulose subjected to strong acid hydrolysis produce crystalline cellulose with a low degree of polymerization – about 200.
2. Cellulose fibrils tend to aggregate into larger units,*fibril aggregates* (*Figure 4.22*). How the aggregation is controlled is not known, but both microtubuli (*Figure 4.25*) and presence of hemicellulose have been suggested to play roles. It is believed that the aggregation if more propounded in the secondary cell wall.

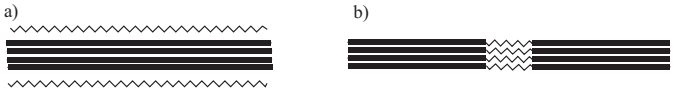

Figure 4.29. Suggested distribution of crystalline and less ordered cellulose. a) Less ordered cellulose ( ) occurs on the surface of the cellulose fibrils, while the highly crystalline polysaccharide ( ) is centrally located in the fibril. b) Segments with less ordered cellulose occur within the fibril.

4.9 Reactivity and Properties of Cellulose

The *high degree of polymerization*, the strong and *regular interactions between the chains*, and the *organization of fibrils*, give cellulose it's among polysaccharides unusual properties:

1. Cellulose is a *very strong material*; it has been said that a cellulose fibrils stronger than steel of the corresponding dimensions.
2. Cellulose is in spite of all hydroxyl groups *totally insoluble in water under physiological conditions*[22]. Even such a short oligosaccharide as celloheptose is virtually insoluble. This does not mean that cellulose interacts poorly with water - a cellulose surface is very hydrophilic, and defatted cotton can absorb 10 times its on weight of water. During drying of wet celluloses, as for instance chemical pulp, the fibers often got hart and inflexible - this is normally negative for the technical properties in for instance papermaking and chemical derivatization. The phenomenon is called *hornification*, and its molecular basis is not fully understood. Cellulose II has a very high tendency for hornification.
3. Cellulose is *relatively resistant to chemical derivatization*, and added groups are not seldom unevenly distributed. Nevertheless, there is a large industry based on derivatization of cellulose, as described in the chapter "Cellulose products and chemicals from wood". The chemical reactivity differs, however, between celluloses prepared with different methods
4. Cellulose interacts well *with aromatic compounds*. This might be somewhat surprising when the hydrophilic surface of cellulose is taken into account, but the top and bottom of glucose units are actually rather hydrophobic (*Figure 4.12*) and have a similar size as aromatic rings. This property might be important for the interaction of the aromatic polymer lignin (chapter 6) with cellulose. Cellulose-binding proteins (chapter 11) often use aromatic groups for their binding to the polysaccharide.

[22] There are however a number of suitable solvents for cellulose. These are mineral acids as phosphourous acid and triflouroacetic acid. They might however cause hydrolysis and other chemical changes in the cellulose. There are metal based solvents as LiCl-dimethylacetamid, Copper -ethylenediamine (CED, Cuen) and Cadmium -ethylenediamine (Cadoxen). Strong alkali (NaOH) can also dissolve short chained cellulose.

4.10 Further Reading

4.10.1 General literature

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4.10.2 Ph.D. Theses

Hult, E-L. (2001) *CP/MAS 13C-NMR spectroscopy applied to structure and interaction studies on wood and pulp fibers.* Stockholm.

Wickholm, K. (2001) *Structural elements in native cellulose.* Stockholm.

5 Hemicelluloses and Pectins

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5.1 Introduction

For historical reasons, the plant cell wall carbohydrates are grouped into three principal groups: the pectins, the hemicelluloses and cellulose. One reason for the grouping is the extractability. The pectins are extracted with neutral aqueous solutions of divalent metal chelators. The hemicelluloses require fairly concentrated solutions of sodium or potassium hydroxide. It should though be pointed out that some hemicelluloses, especially from larches, are partly extractable with water. The residue after extraction of the pectins and the hemicelluloses is rich in cellulose.

The grouping of polysaccharides into hemicelluloses is ambiguous. E. Schulze (1891) proposed the name hemicellulose for polysaccharides extracted from plants with dilute alkaline solution. The name is based on the assumption that these polysaccharides are hydrolysed more easily than cellulose and are related chemically and structurally to cellulose. They were thought to be intermediates in cellulose biosynthesis. However, it turned out that these polysaccharides represent a distinct and separate group of plant polysaccharides. A common way to define hemicellulose is cell wall polysaccharide of land plants, except cellulose and pectin. Hemicelluloses and pectins are sometimes called matrix polysaccharides. In this chapter non-cellulosic polysaccharides including hemicellulose, pectin and starch will be discussed.

5.1.1 Properties

Hemicelluloses are one of the main constituents of wood, usually between 20 and 35 % of dry mass (*Table 5.1*). Cellulose and most of the hemicelluloses are structural carbohydrates as they form the bulk of the plant cell's supporting structure – the *cell wall*. Hemicelluloses are found in the matrix between cellulose fibrils in the cell wall. The components in lignocellulose are tightly associated and in several processes it has been proved to be difficult to separate hemicelluloses from lignin and cellulose without modifying the hemicellulose. Besides wood, hemicelluloses are also found in grasses, cereals and some primitive plants. The type and amount of hemicellulose varies widely, depending on plant materials, type of tissues, growth stage, growth conditions, storage and method of extraction.

Hemicelluloses generally occur as heteropolysaccharides. Some hemicelluloses that are mainly homopolysaccharides have been characterised (Sections 5.6.3 and 5.8.3). The major wood hemicelluloses were extensively studied in the 1960s and have a degree of polymerisation up to 200. They are clearly less well defined than cellulose. Their main building units are hexoses (D-glucose, D-mannose and D-galactose) and/or pentoses (D-xylose and L-arabinose). Small amounts of deoxyhexoses (L-rhamnose and L-fucose) and certain uronic acids (4-*O*-methyl-D-glucuronic acid, D-galacturonic acid and D-glucuronic acid) are also present. These units exist mainly as six-membered (pyranose) structures either in the α- or β-forms. Acetyl groups are commonly a part of hemicelluloses. The chemical and thermal stability of hemicelluloses is generally lower than that of cellulose. The sugar residues of the poly- or oligosaccharides of plant cell walls contain no nitrogen derivatives, while many of the animal extracellular sugars have them, mostly as amides.

Table 5.1. Major hemicelluloses in softwoods and hardwoods.

Occurrence	Hemicellulose	Amount, %[1)]	Units	Molar Ratio[2)]	Linkage
Softwood	Galactoglucomannan	5–8	β-D-Man*p*	3–4	1→4
			β-D-Glc*p*	1	1→4
			α-D-Gal*p*	1	1→6
			O-Acetyl	1	
Softwood	Glucomannan	10–15	β-D-Man*p*	3–4	1→4
			β-D-Glc*p*	1	1→4
			α-D-Gal*p*	0.1	1→6
			O-Acetyl	1	
Softwood	Arabinoglucuronoxylan	7–15	β-D-Xyl*p*	10	1→4
			4-*O*Me-α-D-Glc*p*A	2	1→2
			α-L-Ara*f*	1.3	1→3
Larch wood	Arabinogalactan	3–35	β-D-Gal*p*	6	1→3, 1→6
			L-Ara*f*	2/3	1→6
			β-D-Ara*p*	1/3	1→3
Hardwood	Glucuronoxylan	15–35	β-D-Xyl*p*	10	1→4
			4-*O*Me-α-D-Glc*p*A	1	1→2
			O-Acetyl	7	
Hardwood	Glucomannan	2–5	β-D-Man*p*	1–2	1→4
			β-D-Glc*p*	1	1→4
			O-Acetyl	1	

[1)] By dry weight;
[2)] Approximate values

5.1.2 Biological Function

The hemicelluloses almost certainly contribute in an important, although by no means fully understood, way to the mechanical properties of the cell wall. The predominant wood hemicelluloses have a common structural feature of a linear β-(1→4)-linked backbone of conformationally related sugar residues in the pyranose ring form. The backbone is laterally substituted by short branches. This structural pattern may be of significance for the supermolecular interactions, which almost certainly occur within the secondary cell wall. It is possible that hemicelluloses serve as an interface between the cellulose and lignin, perhaps facilitating the encrustation of the fibrils. They may maintain the ordered spacing of cellulose fibrils and perhaps regulate the wall porosity and strength. It has been shown that for cellulose synthesised by *Acetobacter xylinum*, the presence of acetyl glucomannan in the medium prevents the assembly of cellulose microfibrils and changes the crystal structure of cellulose. In the primary cell wall xyloglucan may have a substantial role in maintaining the three-dimensional conformation via interfibrillar polysaccharide linkages in the cell wall.

The exact physical state of the hemicelluloses in wood is not known. Most probably hemicelluloses influence the moisture equilibrium of the living tree. The macromolecules in the cell wall have different ability to store water and thereby increase their volume. They can be grouped according to their capacity to bind or store water as follows: pectin > hemicellulose > cellulose > lignin. The effect of locally unequal changes of the state of hydration can be directly observed when timber warps at high humidity.

For a long time it has been known that polysaccharides in the plant have a biological purpose to serve as structural material and as energy source. However, during the last two decades it has been discovered that fragments of cell wall polysaccharides perform many other important biochemical functions as well. Certain complex biologically active oligosaccharides, called oligosaccharins, act as signal molecules in plants. They are usually low molecular weight breakdown products of the cell wall. Hormonal concentrations of oligosaccharins influence growth and differentiation of other cells and tissues and they participate in defensive reactions against fungi and other micro-organisms. Oligosaccharines act by regulating gene expression. It appears as if the oligosaccharines have an importance for the plants that can be compared to that of peptide hormones in animals.

Generally polysaccharides are classified by molecular structure. However, structures are most interesting when they can be interpreted in terms of the biological functions that they subserve. The three-dimensional structures of carbohydrates provide the driving force for all intermolecular interactions and therefore predetermine their function. Furthermore, the flexibility of certain glycosidic linkages produces multiple conformations, which co-exist in equilibrium. Correlation of carbohydrate structure and function is a relatively new research field in which methods are being developed and tested.

5.2 Localisation in Cell Elements

The woods have a high proportion of lignified cells with thick secondary cell walls. Consequently, any polymers extracted with alkali from such tissues will inevitably reflect the composition of the secondary cell wall. Hemicelluloses from gymnosperms (softwoods) and angiosperms (hardwoods) are not the same (*Table 5.1*). In hardwoods, the predominant hemicellulose is a partially acetylated (4-*O*-methylglucurono)xylan (Section 5.4.1) with a small proportion of glucomannan (Section 5.5.2). In softwoods, the major hemicellulose is partially acetylated galactoglucomannan (Section 5.5.1). A smaller amount of an arabino-(4-*O*-methylglucurono)xylan (Section 5.4.2) is also present. This is the chief chemical difference between softwoods and hardwoods. Among the softwoods, the larches are unique in that their major hemicellulose is an arabinogalactan (Section 5.6). Other polysaccharides might be important components of the living tree, although of little interest when considering technical applications.

The composition of other cell wall layers and other cell types in the same tree might be very different. For the different xylemic cell elements, the hemicellulose content in parenchyma cells is higher than in the other cell types. In pine there is more glucomannan and less xylan in latewood than in earlywood. Reaction wood (Section 2.2) differs from normal wood anatomically, physically and chemically. Compression wood in gymnosperms contains 10-15% of a galactan, which is present in only minute amounts in normal wood. Tension wood in hardwood species contains less glucomannan and two to five times as much galactan in comparison to normal wood.

The primary cell wall of wood contains cellulose, pectin and hemicellulose polymers, some of which are quite distinct in structure from the hemicelluloses of the secondary wall. The hemicellulose fractions from wood primary cell walls normally contain xyloglucan (Section 5.8.1) as the principal component.

Pectic (Section 5.7) fractions form a separate coextensive network. Pectin levels are high in middle lamella and very low in the secondary cell wall. Pectins with different amounts of methylesters have different distributions within the cell wall.

5.3 Biosynthesis

Cellulose (Section 4) and β-(1→3)-glucans (Section 5.8.3) are synthesised on the plasma membrane by enzymes bound to the membrane. The polysaccharides are directly deposited onto the cell walls. However, the majority of hemicellulose and pectin biosynthesis occurs in the Golgi apparatus. There is evidence that some early stages, perhaps including any priming reactions, may take place in the endoplasmatic reticulum. The hemicelluloses and pectins are transported to the cell wall via Golgi vesicles (*Figure 5.1*).

Nucleoside diphosphate sugars are the activated intermediates in nearly all synthesis of glycosidic linkages. The sugar nucleotides are formed by enzymes, which are located in the cytosol. Formation of polysaccharide chains is carried out by enzymes tightly bound to Golgi membranes (*Figure 5.1*). Glycosyl units are added, usually at the non-reducing end, to a pre-existing polysaccharide molecule, to lengthen it by one unit, with liberation of the free nucleoside diphosphate (*Figure 5.2*). The equilibrium of this type of reaction is strongly to the right, due to the breakage of a high-energy bond in the sugar nucleotide.

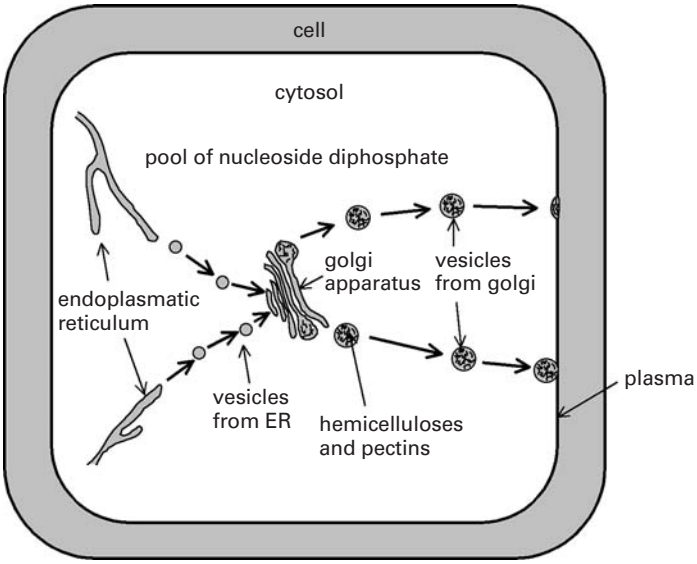


Figure 5.1. Site of synthesis of the majority of hemicelluloses and pectins in plant cells.

The biosynthetic nucleoside diphosphate sugar pathway is part of the overall carbohydrate metabolism of the cell. Other relevant processes are the photosynthetic process, the processes of breakdown of storage carbohydrates and the pathways of sucrose catabolism. The phosphorylated sugar intermediates, which may participate in the regulation of cell wall polysaccharide bio-

synthesis, are generated by the pathways of sucrose catabolism. The pathways of biosynthesis of individual cell wall components are not so well understood. The regulation of the biosynthesis is reflected in the polysaccharide; when the enzymes interact in a precisely controlled manner, they produce a precise structure in the product, and when they are only loosely controlled, they introduce a degree of randomness into the polysaccharides.

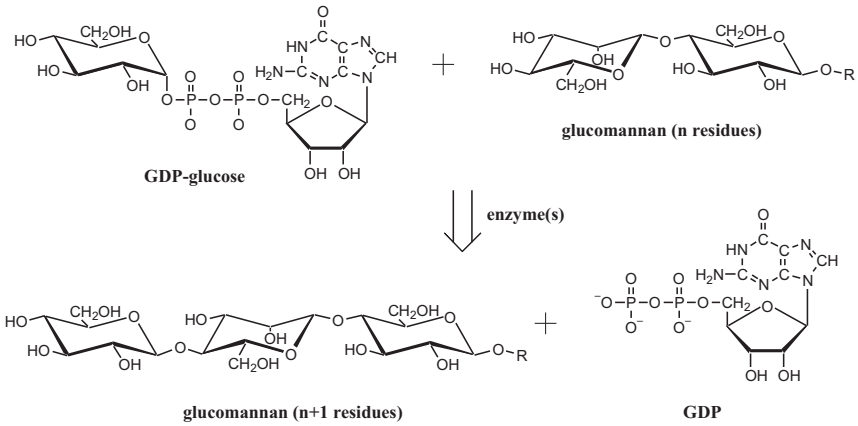


Figure 5.2. Transfer of glucose from GDP-glucose to a growing glucomannan chain. GDP = guanosine diphosphate.

Xyloglucan (Section 5.8.1) biosynthesis is partly understood. The addition of xylose and glucose to an existing xyloglucan chain is probably tightly coupled, which explains the regular structure of a series of Glc_4Xyl_3 repeating units. Galactose and fucose appear to be added later, and can be added to the preformed polymer.

In glucomannan synthesis, the backbone is formed by addition of mannose and glucose. A single enzyme complex adds both types of monosaccharide. There is no precise pattern in the sequence of glucose and mannose residues, however, precise statistical rules may govern the distribution. Little is known about the control of the ratio mannose/glucose.

Some modifications are made subsequent to the initial polymerisation. Methyl groups are added to galacturonans (esters are formed) and glucuronoxylans (ethers are formed) after polymerisation but while the polymers are still in the endomembrane system, prior to deposition in the wall. Addition of acetyl groups and ferulic acid is also thought to occur prior to deposition; neither the substrates nor the enzymes have been identified.

The activity of enzymes, which participate in conversion of monosaccharides and in polymerisation, is controlled depending on the stage of cell wall differentiation. Living cells might be expected to possess the metabolic machinery to rearrange the molecular structures of their (mainly primary) cell walls. During growth the primary cell wall extends, whereas the much stronger secondary cell walls show only a limited ability to extend. It has been suggested that xyloglucan (section 5.8.1), which binds with high specificity and affinity to cellulose fibrils, play an important role in the growing cell. The xyloglucan metabolism is closely associated with cell expansion. An enzyme, xyloglucan endotransferase (XET), can cleave and rejoin xyloglucan chains. Thereby may two adjacent fibrils move against each other without loosing the cell wall structure (*Figure 5.3*). Xyloglucan adsorbs strongly but non-covalently to cellulose

and forms cross-links between different fibrils. The enzyme XET cleaves the bridges and remains covalently attached to one of the xyloglucan ends. The fibrils can now move longitudinally allowing the cell wall to expand. Finally the enzyme connects the xyloglucan end with another xyloglucan chain, and is released.

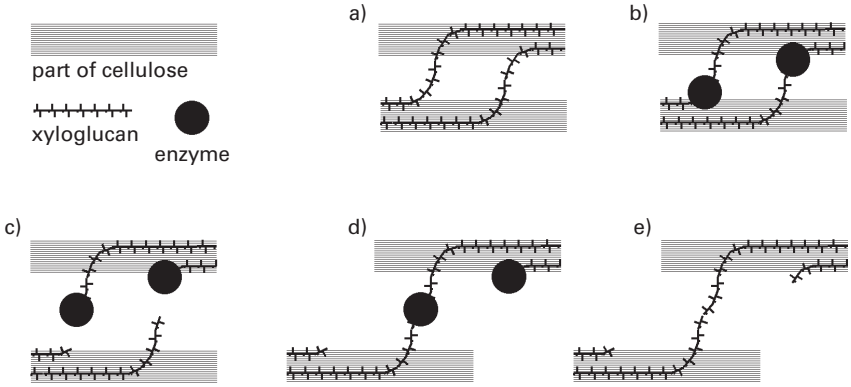


Figure 5.3. A suggested role of xyloglucan in the expansion of primary cell walls.

A change in the biosynthesis according to external forces can occur. Reaction wood is synthesised in branches and in stems, which are growing at an angle to the vertical, to resist the force of gravity (Section 2.2). The hemicellulose content in reaction wood differs from normal wood. Tension wood, which is formed on the upper side of an inclined hardwood stem, contains less glucomannan and xylan, and more galactan than normal fibres. Compression wood is formed at the lower side of the branch or stem when the softwood grows under stress. It contains less glucomannan and more galactan than normal tracheids.

Another example of a response to external forces is the formation of callose (Section 5.8.3) as an additional barrier to penetration of fungal hyphae. The deposition of callose is probably triggered by an influx of Ca^{2+} into the cell, since callose synthase is calcium-dependent.

5.4 Xylans

The xylans that are ubiquitous in plants have a backbone of β-(1→4)-D-xylopyranosyl residues. Most plant xylans bear some short side chains consisting of single sugar residues. Xylans are essentially linear polysaccharides. In wood xylans some of the xylopyranosyl residues carry a side-group consisting of 4-*O*-methyl-D-glucuronic acid (MeGlcA), α-(1→2)-linked to the xylan chain (*Figure 5.4*). Softwood xylans are more acidic than hardwood xylans, due to a higher content of MeGlcA. The distribution of uronic acid side groups is different for hardwood and softwood xylans. A regular distribution is found in softwood xylans, whereas the MeGlcA units appear to be irregularly distributed in hardwood xylans. Hardwood xylans contain acetyl groups (*Figure 5.4*) whereas softwood xylans contain L-arabinose side groups (*Figure 5.5*). The xylose based hemicelluloses in both softwoods and hardwoods are often simply called xylan. It has been reported that the reducing end group in xylan has an irregular shape due to the insertion of specific sugar units as shown in (*Figure 5.6*).

The composition of xylans from various plants appears to be related to their belonging to evolutionary families. *O*-Acetyl-(4-*O*-methylglucurono)xylan from flax, a non-woody dicot, has a similar structure as hardwood xylan. The grass (including bamboo) xylans have in addition to the arabinose and methylglucuronic acid sidegroups in the softwood xylan, a great variety of short side chains containing arabinose, galactose, xylose as well as esterified ferulic or *p*-coumaric acid. The ferulic acid components may cross-link to other wall polymers.

5.4.1 Hardwood Xylan

The most abundant hemicellulose in hardwoods is *O*-acetyl-(4-*O*-methylglucurono)xylan, sometimes called glucuronoxylan (*Table 5.1*). There is on the average one MeGlcA side group per 8–20 xylopyranosyl residues. The glucuronoxylan is partially acetylated in its native state. Average molar masses of 5,600–40,000 and average degree of polymerisation of 100–220 have been reported for hardwood xylans. These values probably depend on wood species, mode of isolation and analysis method. Recent values of molar mass parameters for birch and aspen glucuronoxylans (*Table 5.2*) indicate that the average number of xylose units per xylan molecule, DP_n, is 84–108 (DP_w = 101–122). The range of molar masses is rather narrow, i.e. the polydispersity index M_w/M_n is approximately 1.1.

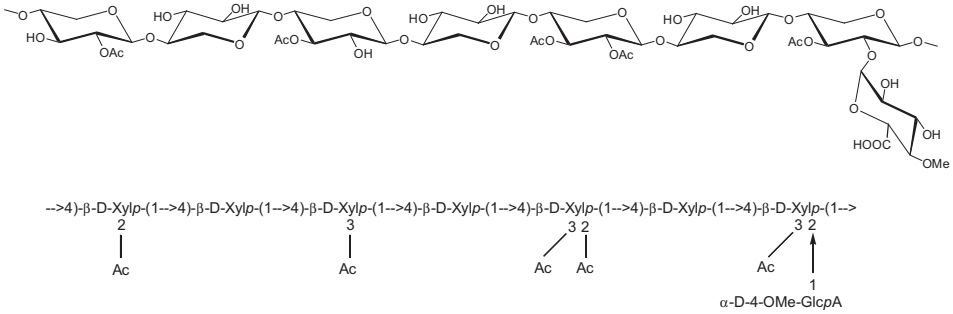


Figure 5.4. Representative structural formula for hardwood glucuronoxylan.

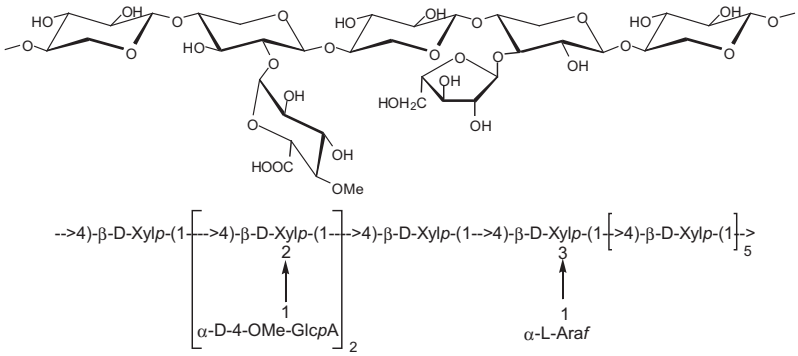
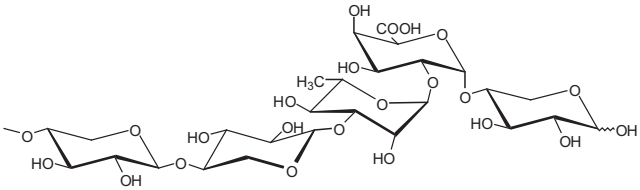


Figure 5.5. Representative structural formula for softwood arabino-4-O-methylglucuronoxylan.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COOH
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H3C
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Table 5.2. Molar mass parameters[1)] for the major hemicelluloses in softwoods and hardwoods.

Hemicellulose	Species	M_n [2)]	M_w [3)]	M_w/M_n [4)]
Glucuronoxylan	birch	14000	16500	1.13
	aspen	15600	17100	1.09
Arabinoglucuronoxylan	spruce	16100	19200	1.16
	pine	17200	20800	1.18
	larch	14400	17300	1.18
(Galacto)glucomannan	spruce	14700	20200	1.33
	pine	16600	21400	1.26
	larch	15500	19100	1.22

[1)] In deacetylated form.; [2)] M_n = number-average molar mass; [3)] M_w = weight-average molar mass
[4)] M_w/M_n = polydispersity index

two hydrogen atoms at position 5. The presence of water is apparently necessary for xylan to assume a stable, ordered structure. The water molecules are incorporated between neighbouring xylan chains, where they are believed to form a column along the xylan chains.

The supermolecular structure of xylan is highly dependent on the nature of the immediate environment. In an aqueous environment, cellulose fibrils interact with xylan, thereby causing xylan to adopt a conformation not observed with the isolated hydrated polymer.

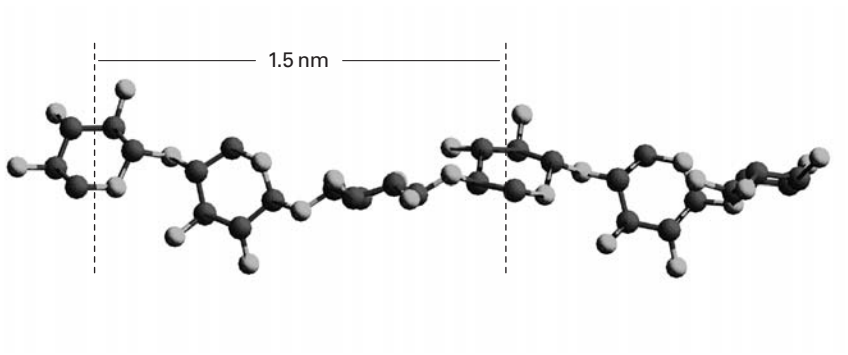


Figure 5.7. The repeat of the three-fold helix of xylan. One of several possible conformations for xylohexaose. The grey and red (dark-grey) colour represents carbon and oxygen atoms respectively. Hydrogen atoms are omitted. Courtesy: Tomas Larsson, STFI-Packforsk AB.

5.4.4 Reactivity

The uronic carboxylic acid groups have a pK_a about 3, implying that the uronic acid groups are negatively charged in neutral and alkaline water solutions. The metal carboxylate salt increases the water solubility of glucuronoxylan because of its ionic structure. The uronic acids might bind heavy metal ions.

The acetyl groups are easily cleaved by alkali. The acetate formed in kraft pulping mainly originates from these groups. The glycosidic linkages might be hydrolysed at the drastic conditions prevailing during alkaline kraft pulping. The peeling reaction starting from the reducing end group, usually, is the most significant carbohydrate reaction under alkaline conditions. The endgroup of xylan (*Figure 5.6*), due to the presence of arabinose and uronic acid substituents, is, however, stabilized against peeling.

The MeGlcA side group is degraded under kraft pulping conditions. The H-5 of MeGlcA is removable at the alkaline conditions and high temperature in kraft pulping. An inversion of the configuration at C-5 might occur if a proton is added to form the epimer 4-*O*-methyl-L-iduronic acid (MeIdoA) (*Figure 5.8*). The conformation of 4-*O*-methyl-α-D-glucuronic acid and 4-*O*-methyl-β–L-iduronic acid is $^4C_1$ and $^1C_4$, respectively. Both the MeGlcA and the MeIdoA side-group are partly converted to 4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid (hexenuronic acid, HexA) via β-elimination of methanol during kraft pulping (*Figure 5.8*). The HexA groups consume bleaching chemicals, bind heavy metal ions and cause colour reversion of pulps. The HexA linkage may be selectively hydrolysed under mild acid conditions without significant hydrolysis of xylosidic linkages.

In the degradation of xylans by the action of acids, the rates of hydrolysis of the various glycosidic linkages differ significantly. The hydrolysis rate of the glycosidic linkages decreases in the order HexA > Ara > Xyl > MeGlcA. In acid hydrolysis the HexA group is mainly converted to 2-furoic acid and formic acid. A minor amount of 5-carboxy-2-furaldehyde is also obtained. The furanosidic form of the arabinose unit contributes to a relatively weak glycosidic linkage. Pentoses are degraded to furfural upon prolonged heating in the presence of concentrated mineral acids. The MeGlcA linkage is a relatively acid resistant glycosidic linkage.

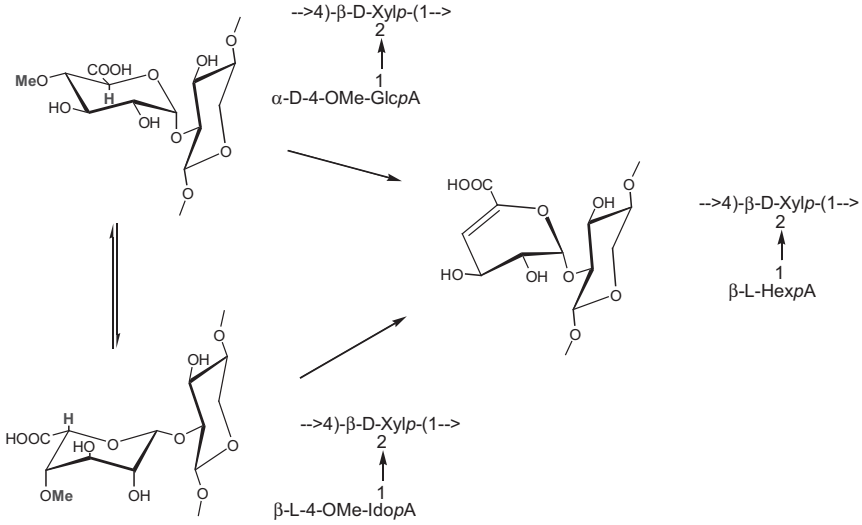


Figure 5.8. Reactions of the MeGlcA side-group in xylan under alkaline conditions and high temperature (kraft pulping). An epimerisation of the MeGlcA side-group to a MeIdoA group occurs. Both MeGlcA and MeIdoA are degraded to the HexA side-group via β-elimination of methanol (blue colour). Notice the change from D to L and from α to β.

5.5 Glucomannans

Mannans are widespread in the vegetable kingdom. In general, three kinds of (galacto)glucomannans have been isolated from softwoods and hardwoods (*Table 5.1*). The (galacto)glucomannans consist of linear chains of (1→4)-linked β-D-mannopyranosyl and β-D-glucopyranosyl residues. The ratio of glucosyl to mannosyl residues varies between 1:1 and 1:4. The distribution of the two residues may be statistically random. In softwood (galacto)glucomannans some of the mannosyl residues carry a side-group consisting of single D-galactopyranosyl residues attached by α-(1→6)-linkages. In its native state, the (galacto)glucomannans are partially acetylated on mannosyl residues. The *O*-acetyl groups are irregularly distributed.

5.5.1 Softwood (galacto)glucomannans

In softwoods, galactoglucomannans can be roughly divided into two fractions having different contents of α-D-galactopyranosyl units (1→6)-linked to mannose (*Table 5.1*). The fraction that has a low content of galactosyl units has a ratio of galactose to glucose to mannose units of 0.1:1:3-4, whereas in the galactose-rich fraction, the corresponding ratio is 1:1:3-4. The lower degree of substitution with galactose units makes glucomannan less water soluble than galactoglucomannan. Often, the two forms are simply called glucomannan. Native (galacto)glucomannans from softwoods have *O*-acetyl groups in the 2- or 3-positions of the mannose residues (*Figure 5.9*). The amount of acetyl groups ranges from DS 0.17 to 0.36, which is approximately one acetyl group per 3-6 backbone hexose units. *O*-Acetyl-glucomannans are found primarily in the lignified secondary cell wall of softwoods. Recent molar mass parameters for galactoglucomannans (*Table 5.2*) indicate that the number of mannose and glucose units per galactoglucomannan molecule, DP_n, is 90-102 (DP_w = 118-132). The galactoglucomannans appear to have a slightly higher polydispersity than the xylans.

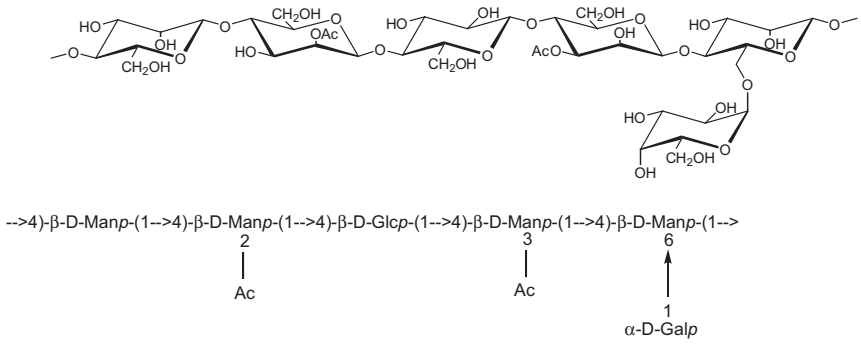


Figure 5.9. Representative structural formula for softwood galactoglucomannan.

5.5.2 Hardwood Glucomannan

Hardwoods usually contain 3 to 5 % (w/dry wood weight) of glucomannan (*Figure 5.10*). The glucose:mannose ratio varies between 1:2 and 1:1, depending on wood species. A galactoglucomannan with α-(1→6)-linked galactose residues has been isolated from bark of trembling aspen. Otherwise, galactose substituents are either infrequent or absent altogether. Recent findings show that the native glucomannan is partially *O*-acetylated to the 2- or 3-positions of the mannopyranosyl residues. An average degree of polymerisation of approximately 60–70 has been reported for glucomannan.

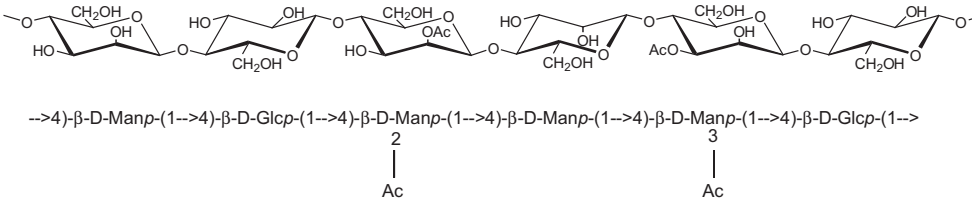


Figure 5.10. Representative structural formula for hardwood glucomannan.

5.5.3 Supermolecular Structure

The irregularly arranged *O*-acetyl and galactosyl groups of native glucomannans seem to prevent a strict order over longer distances, as in crystalline structures. Glucomannans can be crystallised after removal of a portion of the side chains and/or some reduction in chain length. Deacetylated softwood glucomannans crystallise in mannan I- or mannan II-type crystals depending on the experimental conditions. Mannan I and mannan II are the two polymorphs of pure mannan, poly β-(1→4)-mannose. The former is found in the native state. The glucomannans crystals are less perfect than those of mannan. Glucomannans are structurally similar to cellulose and might occur in association with cellulose.

5.5.4 Reactivity

The acetyl groups are easily cleaved by alkali. The acetate formed in kraft pulping mainly originates from these groups. The α-glycosidic linkage of the galactose units is a sensitive linkage, which may be cleaved during alkaline extraction and kraft pulping. Glucomannans undergo extensive peeling reactions at the alkaline conditions and high temperature during kraft pulping.

The α-(1→6)-galactosidic linkages are acid-labile and can be selectively hydrolysed by mild acidic treatment. The β-D-mannosidic linkage is more easily hydrolysed by acid compared to the β-D-glucopyranose linkage. Glucomannan is easily depolymerised at acidic conditions.

5.6 Galactans

Galactans and arabinogalactans have a backbone of β-D-galactopyranosyl residues. They are distributed throughout the plant kingdom as constituents of pectic substances in middle lamella and primary cell walls (Section 5.7) or hemicelluloses in plant cells.

5.6.1 Larch Arabinogalactan

A neutral water-soluble arabinogalactan occurs in large amounts, 10–25 % by dry weight, in the heartwood of larches. In other softwoods the amount is generally less than 1 %. Larch arabinogalactan is probably synthesised in the living ray cells in the transition of sapwood to heartwood and occurs in the lumen of tracheids, ray cells and epithelial cells. It might be extracted quantitatively from the untreated heartwood with water. The backbone consists of (1→3)-linked β-D-galactopyranosyl residues (*Figure 5.11*) and has a high degree of branching. The side chains consist of (1→6)-linked β-D-galactopyranose chains of variable length and arabinose substituents (α-L-Ara*f* and β-L-Ara*p*). Approximately one third of the arabinose units are in the pyranose and two thirds in the furanose form. Small amounts of D-glucuronic acid side groups might also be present. The ratio of galactose and arabinose units varies from 3 to 10. Arabinogalactans are separated into two components, arabinogalactan A and B, according to their molecular weight. Arabinogalactan B with a molar mass around 11000 appears to be a degradation product of arabinogalactan A with a molar mass around 70000. Arabinogalactans from other softwood species have a similar basic structure.

Larch arabinogalactan has a commercial use as medium in density gradient separations of cells, viruses and organelles. It might be used instead of Arabia gum in the food industry. Larch arabinogalactan has a very low viscosity in water.

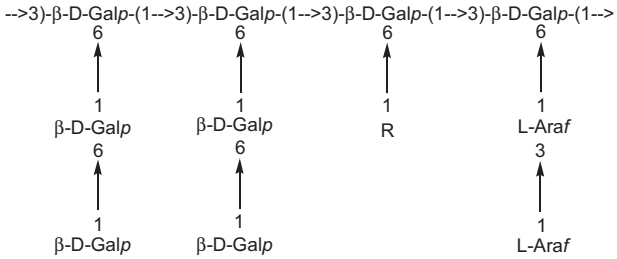


Figure 5.11. Representative structural formula for larch arabinogalactan. R = β-D-galactose or, less frequently, L-arabinose or D-glucuronic acid.

5.6.2 Tension Wood Galactan

Tension wood is formed by some hardwoods in attempts to maintain or resume a proper orientation of stem and branches. It contains 3–11 % by dry weight of a complex galactan localised in the S1 and S2 layers of the secondary wall. The galactan has a low intrinsic viscosity indicating that it must be extensively branched. A possible structure has been proposed based on proven

structural elements (*Figure 5.12*). The galactan consists of a backbone of (1→4)-linked β-D-galactopyranosyl residues, approximately half of which are substituted at C-6 with complex side-chains. Most of the side-chains contain (1→6)-linked β-D-galactopyranosyl residues, some of which are substituted at C-6 of their end units with terminal 4-*O*-methyl-β-D-glucuronic acid or β-D-glucuronic acid. Other side-chains contain L-rhamnopyranosyl residues with an α-D-galacturonic acid residue at C-2. Most of the L-arabinofuranose residues are terminal, while some are substituted at C-5.

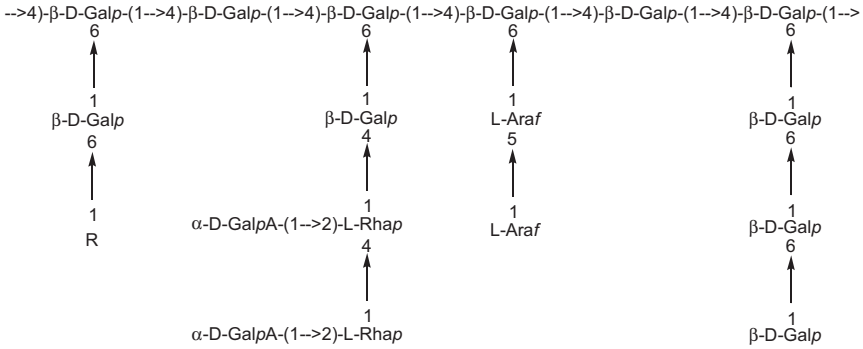


Figure 5.12. Proposed structural formula for galactan from tension wood. R = β-D-glucuronic acid or 4-*O*-methyl-β-D-glucuronic acid.

5.6.3 Compression Wood Galactan

Compression wood is formed by all gymnosperms and is also found in ginkgo, when the tree attempts to revert to or retain its normal growth pattern. It contains 7–12 % by dry weight of an acidic galactan. The galactan is a major hemicellulose in compression wood and occurs in the outer portions of the cell wall of the tracheids. The galactan consists of a main chain of 100–300 (1→4)-linked β-D-galactopyranosyl residues (*Figure 5.13*). One galactose residue out of 20 carries a side-group consisting of β-D-galacturonic acid, (1→2)-linked to the galactan chain. A few glucuronic acid residues might also be present. The galactan is freely soluble in water.

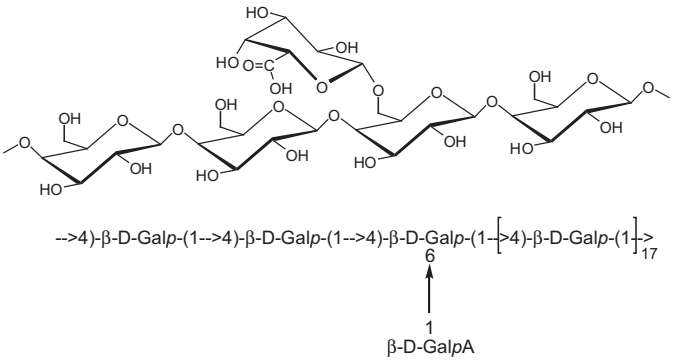


Figure 5.13. Representative structural formula for an acidic galactan from compression wood.

5.7 Pectins

Pectins are usually not classified as hemicelluloses. The amount of pectin present in wood is low, only a few percent. To the food manufacturer (or consumer) pectin is a natural fruit polysaccharide which is used, in jams for example, because of its ability to gel in the presence of high concentrations of sugar. Pectins are present in the middle lamella between cells of all types. These polysaccharides appear to be present universally in primary cell walls, and are a major constituent of primary cell walls in wood. They thus contribute to the mechanical properties of the cell wall and influence cell adhesion.

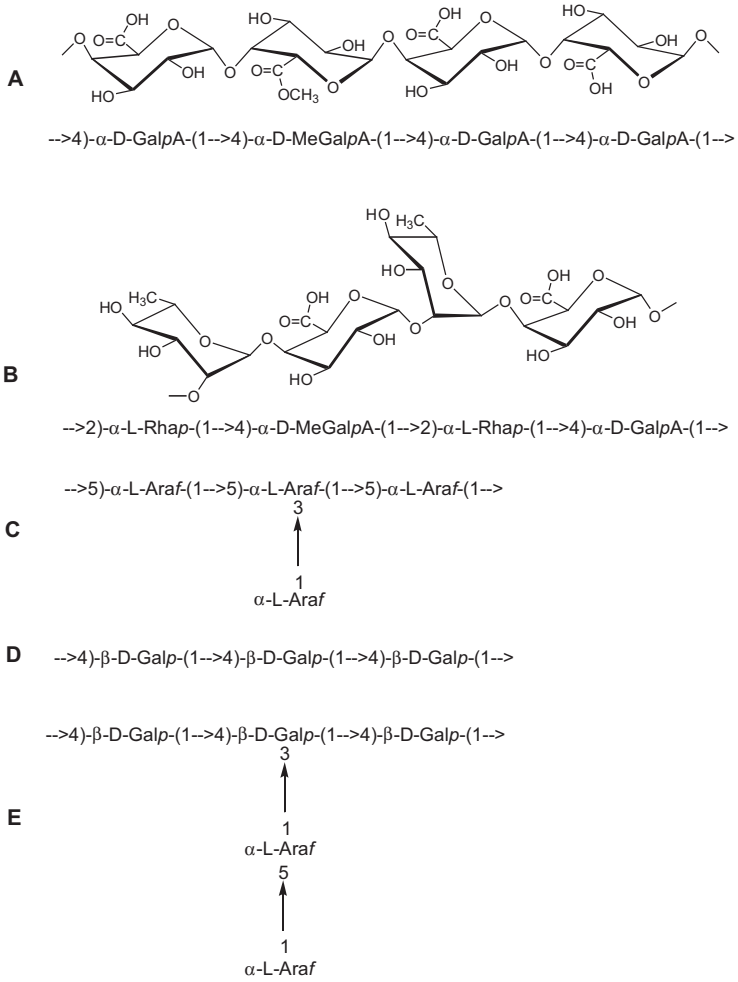


Figure 5.14. Structural features of components in pectins. A = Polygalacturonan; B = Backbone of rhamnogalacturonan-I; C = Arabinan; D = Galactan; E = Arabinogalactan.

Pectins are acidic, irregular and susceptible polysaccharides. They are very easy to dissolve in and easily degraded by alkali, and are thus difficult to isolate and study. Therefore, the native structure of pectins is not fully understood. They appear to have a block copolymer structure, that is a heterogeneous structure, with regular parts. Galacturonan and rhamnogalacturonan form backbones in pectin polymers. The galacturonan part consists of α-(1→4)-linked D-galactopyranosyl uronic acid residues (*Figure 5.14*) that adopt a helical configuration with three monosaccharide units per turn. The uronic acid groups are partly methylated. Lignified tissue contains mostly methylesterified galacturonan, whereas unlignified tissue also contains much unesterified galacturonan. Some L-rhamnose (6-deoxy-L-mannose) residues are interspersed within the backbone. Rhamnose introduces a kink in the otherwise straight chain. In rhamnogalacturonan-I, every second residue in the main-chain is a rhamnose and the repeating sequence is →2)-α-L-Rha*p*-(1→4)-α-D-GalA-(1→ (*Figure 5.14*). The rhamnose residues can serve as anchorage points for side chains. Rhamnogalacturonan-I carries relatively short side chains consisting mainly of neutral sugar residues, like galactose and arabinose. Rhamnogalacturonan-II is a small, but highly complex polysaccharide, which appears to be present in an individual network.

Pectins contain variable proportions of neutral polysaccharides. The neutral components are probably attached to the acidic pectic backbone as large side chains. Three main structural types are known; arabinans with a core of α-(1→5)-linked arabinofuranosyl residues, galactans with a backbone chain of β-(1→4)-linked D-galactopyranosyl residues and arabinogalactans that are branched polysaccharides (*Figure 5.14*). The regions with rhamnose residues and side chains are called "hairy regions", or rhamnogalacturonans, and the regions with galacturonan residues are called "smooth". Smooth regions with low degree of methylation, i.e. with many carboxylic acids, have a strong tendency to form complex with Ca^{2+}. The calcium ion can bind to two carboxylic acids, so called "egg boxes". In pectin/calcium gels these often come from two pectin chains and thereby the metal ion forms a crosslink between two polysaccharide molecules. This structure is important in non-lignified middle lamellas in dicot plants. This self-interaction may itself form the basis of a network within the primary cell wall and the middle lamella.

Pectins work as an ion exchanger and can effectively bind metal ions. It is probable that pectins are responsible for binding a part of the metal ions in wood. It has been suggested that calcium ions bonded to acidic pectins may be important in lignification.

5.8 Glucans

Glucans have a main-chain of glucopyranosyl residues. The most abundant glucan in plants is cellulose, which is a β-(1→4)-glucan (Chapter 4). Starch is an α-glucan. Both cellulose and starch are homopolysaccharides and are the two most widely occurring polysaccharides in nature. Callose, which is found in a number of special situations in plant cell walls, and laricinan in compression wood are both β-(1→3)-glucans with a few uronic acid side-groups. Xyloglucan is a heteropolysaccharide.

5.8.1 Xyloglucan

Xyloglucan is a major hemicellulose in primary cell walls of higher plants. It might account for about 20 % of the dry weight of the primary cell wall. The main chain consists of β-(1→4)-D-

glucopyranosyl residues (like in cellulose), where some of the residues are substituted on C-6 with α-D-xylopyranosyl side-groups (*Figure 5.15*). Three consecutive glucosyl residues carry substituents while the fourth does not. It appears that xyloglucans are composed of this repeating heptasaccharide unit to which variable amounts of certain monosaccharides are attached. The two xylosyl residues farthest from the non-reducing end of the heptasaccharide might be substituted with β-(1→2)-D-galactosyl residues. A further substituent might occur, namely an L-fuco-pyranosyl group α-(1→2)-linked to galactose. *O*-acetyl substituents have also been detected on xyloglucans, predominantly on the galactosyl residues.

The glucan backbone of xyloglucans has an extended, two-fold helix conformation similar to cellulose. In the primary cell wall, xyloglucans are highly associated to the cellulose fibrils and isolated xyloglucans have been observed to bind to purified cellulose by hydrogen bonds. Xyloglucans can be as long as 700 nm. Cellulose fibrils in the primary cell wall are typically 20–40 nm apart, implying that a single xyloglucan chain has the potential to cross-link several adjacent fibrils. Oligosaccharides derived from xyloglucans can have a range of growth promot-

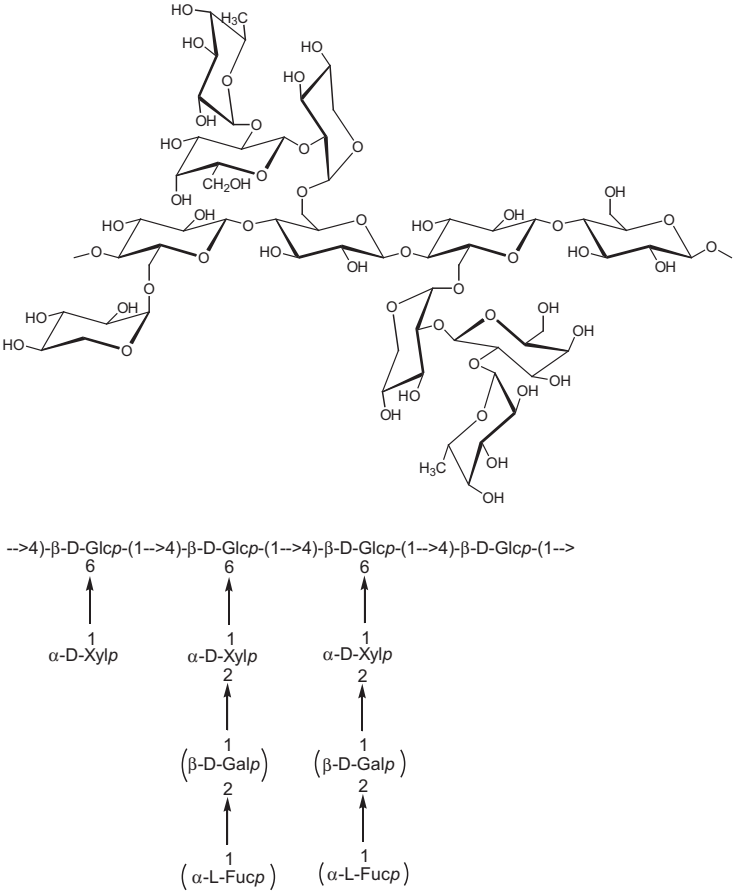


Figure 5.15. Representative structural formula for xyloglucan. [β-D-Gal*p*-1] = possible galactose substituent; [α-L-Fuc*p*-1] = possible fucose substituent.

ing or inhibiting effects depending on concentration and side-chain composition. Xyloglucan can be relatively easily extracted in considerable amounts from Tamarind seeds. The capacity of the polysaccharide to form gels is used in food preparations.

5.8.2 Starch

Starch is the principal reserve polysaccharide in plants and is no hemicellulose. It is present in parenchyma cells of wood tissue. Starch normally occurs as starch granules which can be separated into two fractions; a fraction soluble in water, called amylopectin, and a fraction insoluble in cold water, called amylose. Amylose, which accounts for about 20 % by weight of starch, is mainly a linear polysaccharide formed by up to several thousand α-(1→4)-linked D-glucopyranosyl residues (*Figure 5.16*). Amylopectin, which accounts for the remaining 80 % of starch, has a backbone of α-(1→4)-linked D-glucopyranosyl residues but also contains α-(1→6)-linked branching points at approximately every 25 glucopyranosyl units (*Figure 5.16*). It may contain up to 10^6 glucopyranosyl residues. Modified starches are used in the paper industry for coating and surface sizing of paper. Oxidised starches are added as stabilisers in internal sizing emulsions and are used in surface sizing as well as cobinders for coating colours. The oxidation results in a low-viscosity product with reduced tendency to retrogradation and gelling in solution.

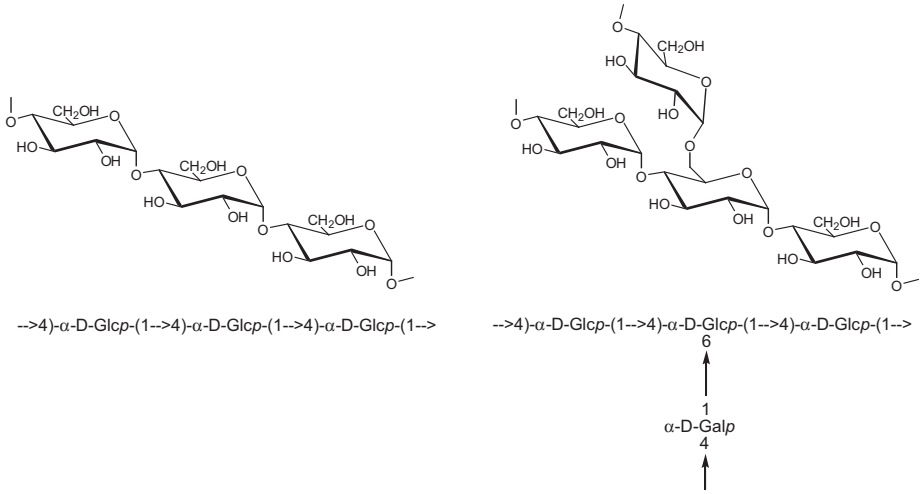
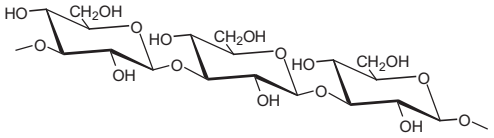


Figure 5.16. Chain structure of amylose (left) and a segment of amylopectin at the branching point (right).

5.8.3 Callose and Laricinan

Callose is common in higher plants, but not abundant. It is readily formed in response to wounding of cell walls and cell membranes or chemical and physical stress. This polysaccharide is found in a number of specialised tissues, such as the phloem sieve tubes, the pollen tube and the walls of some pollen. Laricinan has so far been isolated from only a few softwood spe-

cies, but it can be assumed that compression wood contains about 3 % laricinan, whereas only trace amounts occur in normal softwood. Both callose and laricinan have a main chain largely composed of β-(1→3)-D-glucopyranosyl residues (*Figure 5.17*). Some of the linkages might be β-(1→4). They contain a small amount of uronic acid side-chains. A small amount of glucuronic acid and an even lesser quantity of galacturonic acid have been reported for laricinan. The number-average degree of polymerisation of laricinan is approximately 200. Callose adopts a helical conformation and can form gels under some circumstances, but can also form fibrils.



-->3)-β-D-Glc*p*-(1-->3)-β-D-Glc*p*-(1-->3)-β-D-Glc*p*-(1-->

Figure 5.17. Main chain of callose and laricinan.

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6 Lignin

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6.1 Introduction

Cotton and wood are both fibrous plant-materials that have high tensile strengths. Cellulose is a main functional compound of both, but in spite of this, the properties of the two materials are very different; cotton is soft, flexible and absorbs water up to 10 times its weight, whereas wood is a stiff material with low water adsorption. What is the chemical background to these differences? The answer is that wood in contrast to cotton contains large amounts of *lignin* (15–35 % for softwoods and around 20 % for hardwoods), a hydrophobic polymer that fills up between the cellulose microfibrils and hemicellulose fixating them towards each other and thus giving the cell wall its “woody” properties. Thus, wood can be considered as a micro-composite type material, with cellulose as enforcing fibers and lignin playing the role of a phenolic plastic. Since wood is so common, lignin is one of the most abundant biopolymers. In spite of this, it is in many ways an oddity among biomolecules; lignin is neither a polysaccharide, a lipid, a protein, nor a nucleotide. It has the most complex structure among naturally occurring polymers with a mixture of aromatic and aliphatic moieties. It is not a linear polymer as cellulose, or a branched polymer as the hemicelluloses or pectin but rather a three-dimensional web, with the

monomers (i.e., the building blocks) connected with a number of different ether (C-O-C)- and carbon-carbon (C-C) bonds that are randomly distributed, and the first impression of the covalent structure may appear chaotic (*Figure 6.1*). Most surprisingly, *the lignins are optically inactive*! i.e., in contrast to almost all other biomolecules, the asymmetrical carbons are racemic[1]. The structure and properties of lignin are of great interest for the pulp and paper industry, since the chemical pulping and bleaching of pulp are mainly based on chemical reactions on lignin and lignin released during chemical pulping represents an incompletely unexplored natural resource. Furthermore, lignin biodegradation is a large research field with importance for wood preservation and various biotechnological processes. We will in this chapter discuss the structure, formation and properties of various lignins.

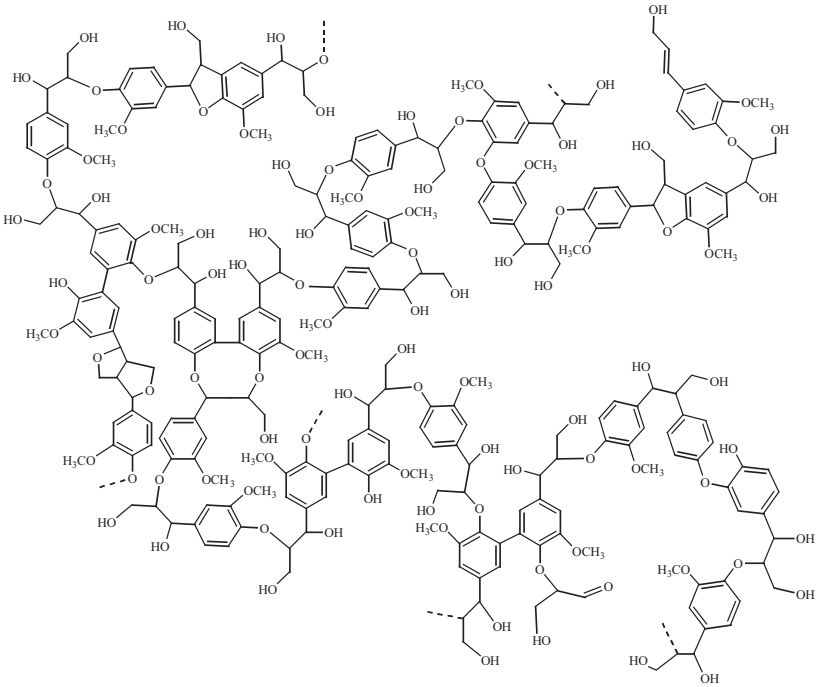


Figure 6.1. A suggested structure of soft wood lignin. The lignins in hardwoods and monocotyledons differs mainly in the content of metoxy groups ($-OCH_3$).

Lignins are polymerized from mainly three monomers called *monolignols*, namely, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. These are propylphenol derivatives, with the differences in the number of methoxy groups attached to the ring (*Figure 6.2*). Three main types of lignin are recognized according to their content of monolignols:

[1] In secion 4.3.1 chirality is discussed.

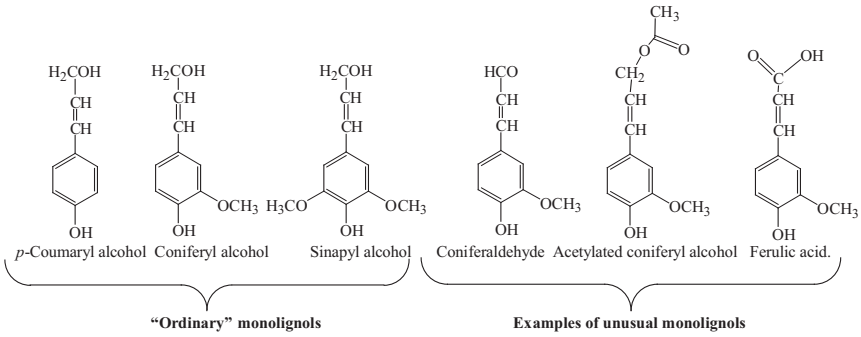


Figure 6.2. The monomers forming the lignin polymer. *p*-Coumaryl alcohol, conferyl alcohol and sinapyl alcohol are present in large amounts in lignin, although the individual proportions vary as described in the text. There are also a number of other monolignols (lignin monomers), that might be present in small amounts (as conifer aldehyde) or special plant species.

- *Softwood lignin* or *guaiacyl lignin* consist almost exclusively of coniferyl alcohol, and may contain small amounts of *p*-coumaryl alcohol (mainly in the compression wood), but no or only traces of sinapyl alcohol. This type of lignin is present in the gymnosperms coniferous trees, i.e., softwoods, and the more primitive gymnosperms, gingkophytes (maidenhair tree) and cycads[2]. Some tropical hardwoods (eudicotyledones) have also lignin of this type and, as will be discussed below (6.3), vessels and middle lamellas in ordinary hardwoods contain similar lignin.
- *Hardwood lignin* or *syringyl-guaiacyl lignin*, contain both coniferyl and sinapyl alcohols with proportions from approximately equal amounts, to three times higher levels of sinapyl alcohol. Some hardwood lignin may also contain small amounts of *p*-coumaryl alcohol. This type of lignin is present in eudicotyledonic angiosperms, including broad leaf-trees, i.e., hardwoods. Also gnetophytes, the group of gymnosperms standing evolutionary closest to angiosperms, are reported to contain this type of lignin.
- *Grass lignin*, or *HGS-lignin* (**H**ydroxy phenol, **G**uaiacyl, **S**yringyl), contains all three monolignols and has a higher content of *p*-coumaryl alcohol than other types of lignin. This type of lignin is present in grass and also other types of monocotyledonous angiosperms as palm trees and banana-plants. In grasses, coniferyl alcohol seems to be the most common monomer, whereas the lignin in palm trees has probably a higher content of syringyl alcohol. The lignin in fern plants seems to be similar to this type, but has often very high content (up to over 90 %) of coniferyl alcohol residues.

Monolignol-ratios for different lignins are shown in *Table 6.1*. A glance at the evolution schedule of different plants in chapter 2 (*Figure* 2.1) shows that these differences make sense from an evolutionistic point of view; the softwood lignins may represent an older type, when the grass lignins and hardwood lignins are later developed versions. More confusing is the similarities between the lignin in fern plants and monocotyledons as grasses.

There are also some additional, more unusual, monolignols (examples are shown in *Figure 6.2*); coniferaldehyde is present in a few percent in coniferous wood; the monolignols are acety-

[2] See chapter 2 for an overlook of different groups of plants.

lated to large degree in the eudicolyledonous bast-fiber plant kenaf, and in grass, γ-carboxylic acid derivatives of the monolignols are present (*Figure* 6.2). It shall be underlined that many questions still remain unanswered in lignin research; some of the facts presented within this chapter may thus be reconsidered by new discoveries.

Table 6.1. Composition of monolignols (lignin monomers) in different plants.

Plant	p-Coumaryl alcohol (%)	Coniferyl alcohol (%)	Sinapyl alcohol (%)
Coniferous¨ softwood	<5	>95	None or Trace
Eudocotyledonous hard-wood	0–8	25–50	46–75
Monocotyledonous grass	5–33	33–80	20–54

6.2 Occurrence and Function of Lignin

The word lignin is derived from the Latin word for wood, ”*lignum*”, and the polymer is indeed the most essential compound in the formation of woody tissues in plants. However, lignin is not restricted to woody plants, but occurs in all vascular plants, including herbs in various systematic groups and the more primitive vascular plant, ferns, horsetails and club mosses. The lignin contents in non-woody plants are, however, much lower (1–20 %) than in woody plant tissues. Green algae and bryophytes (mosses and related groups) do not synthesize lignin[3], but make in many cases smaller molecules, *lignans*, that are dimers or oligomers of monolignols and closely related structures. In some cases lignans have been wrongly reported as “primitive” or “abnormal” lignin. This group is also present in wood, and was earlier believed to be incompletely polymerized lignin. However, unlike lignin, *lignans are optically active*, are thus a different group of molecules chemically divergent from lignin, and are synthesized in a different way. Suberin, a polymer present in the bark of plants has some similarities with lignin, but is less studied. Lignans and suberin are further discussed in chapter 7. Lignin was apparently developed together with the vascular plants and this make it to the probably latest developed group of biopolymers.

What is the biological function of lignin? The answer is complex since lignin has at least four important roles in plants:

- *Lignin gives stiffness to the cell walls.* As discussed above, lignin works as a cementing and fixating polymer in the woody plant cell wall, in close association with polysaccharides. The composite nature of wood with its mixture of cellulose, hemicellulose and lignin, makes the fibers relatively stiff and rigid, making it able to serve as a mechanical support to build up the stem and branches and thereby giving the plant better chances to compete for the sunlight.
- *Lignin glues different cells together in woody tissues*. In wood, the middle lamella consists mainly of lignin, which works as an efficient and resistant glue keeping the different cells

[3] Outside the plant kingdom, aromatic polymers with some similarities with lignin, are synthesized by some fungi (melanin) and some insects (sclerotizine). The knowledge of the structure of these polymers is very limited.

together. This function is displayed by pectin in many herbs and in the non-woody tissues of trees.

- *Lignin makes the cell wall hydrophobic*. The polymer inhibits swelling of the cell walls in water, and thereby that water leaks from a woody cell wall, i.e., it makes the cell wall waterproof. This is a prerequisite for the development of cells for efficient water- and nutrition transport, and the introduction of lignin can thus be considered to be the key to the evolution of the vascular plants. In non-woody plants this is the main function of lignin.
- *Lignin is a protection against microbial degradation of wood*. A lignified woody tissue is simply so compact that the polysaccharide degrading proteins excreted by microorganisms cannot penetrate into the cell wall. Thus it serves as a barrier against microorganisms, and this together with complexity and heterogeneity of the lignin, make sound wood resistant against most molds. Some specialized fungi, bacteria can, however, degrade lignin efficiently, and they have potential applications in pulp and paper industry as further discussed in the chapter 12. Nevertheless, wood is degraded much slower than non-lignified plant materials and heavily lignified wood is generally more resistant to biological degradation than lightly lignified[4]. A second lignification can also occur as a defense of a plant against an attack by a fungal parasite.

The structure of lignin (*Figure* 6.1) makes it very suitable for fulfilling these functions; the aromatic rings and the hydroxyl groups give lignin good possibilities for formation of non-covalent dipole aromatic interaction and of hydrogen bonds with cellulose and hemicellulose, and its branched network structure makes lignified materials stiff. As will be discussed later, covalent linkages are also formed between lignin and polysaccharides in wood.

6.3 Lignin Polymerization and Covalent Structure

Lignin always appears in close association with polysaccharides (cellulose, hemicellulose, pectin), and it is thus difficult to prepare the polymer in native form (chapter 9). Therefore it is impossible to determine the molecular weight of intact lignin in woody plants[5]. The sparse data suggest however that lignin in woody tissues at least has a degree of polymerization of several thousands, but the true value might be much higher. At least in theory, it is not impossible that the lignin in the top of a tree and the lignin in the root belong to the same “molecule”, i.e., that there exist covalent bonds in an unbroken chain.

In lignin, mainly three types of ether linkages and four kinds of carbon-carbon bonds, or *condensed bonds*[6] connect the monolignols. Monolignols can be split into two parts and the phenolic rings can in some cases be converted to an aliphatic structure. No strong evidence that there is any repeated structure of the bond patterns has been presented, and the distribution of the

[4] The above-mentioned lignans also serve as toxic defense substances in plants. There are reasons to believe that these substances were the starting point to the ”innovation” of lignin and the evolution of vascular plants.

[5] Residual lignins from chemical pulp, on the other side, can principally be measured, since the original lignin has been pertly depolymerized during pulping. Reported values are up to 10 000 Da.

[6] The term is inappropriate, since the carbon-carbon linkages are not at all formed by condensations. “Condensed bonds” is however an established term and will be used herein. Often ether bonds directly connecting aromatic rings (4–O-5’ bonds) are regarded as condensed.

linkages appears to be random with few exceptions. Before we discuss the covalent pattern of lignin an introduction to nomenclature of the chemical bonds is given.

6.3.1 Nomenclature of Covalent Bonds in Lignin

As shown in *Figure 6.3* the carbons in the aromatic ring are numbered from 1 to 6 starting with the carbon attached to the propyl chain. The aliphatic carbons of the propyl group are named with the Greek letters α, β and γ starting from the carbon next to the aromatic ring; an alternative nomenclature used by some scientists is to name the aliphatic side chain carbons 7, 8 and 9. Atoms on different monolignols are separated by marking the carbon atoms on the second monolignol with prim (´), and on the third with even bis (´´). A β–O–4´ bond is thus an ether linkage between a para-position in the aromatic ring and the central carbon in a propyl group, and a 5-5´ linkage represents a covalent linkage directly between the 5-carbons in two aromatic rings. The Greek letters in the lignin nomenclature shall not be confused with α- and β-anomers of carbohydrates.

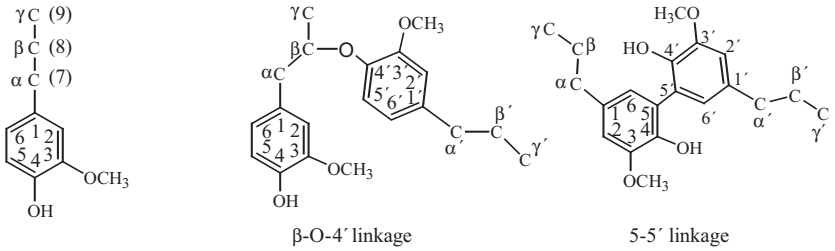


Figure 6.3. Nomenclature of lignin carbons.

6.3.2 Covalent Pattern in Lignin

During the last decades, new data about lignin structures have been accumulated due to progress in among others NMR-analysis (see chapter 9). This has led to a reconsideration of the covalent pattern of lignin, and some structures earlier believed to be common have been ruled out. On the other side, some new structures have been discovered, and the frequencies of some of the bonds have been reevaluated. In *Table 6.2* the structures, names and frequencies of hardwood and softwood lignin based on the latest data are shown. Grass lignin is less studied, and the frequencies of the different structures are more uncertain

Table 6.2. Bonds between monolignols and lignin functional groups.

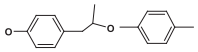
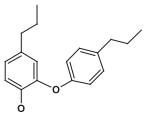
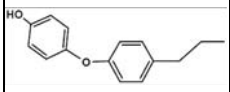
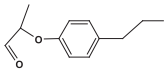
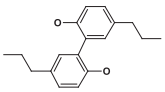
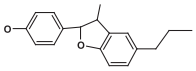
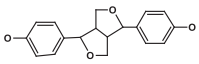
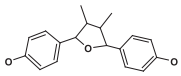
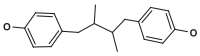
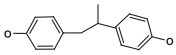
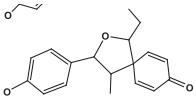
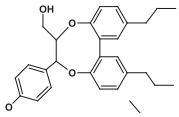
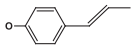
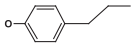
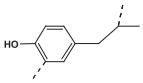
Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)
Ether bonds				
β-aryl ether	β-O-4´		35–60	50–70
Diaryl ether	4-O-5´		<4	7?
	1-O-4´		low	low
Glyceraldehyde aryl ether	β-O-4´		<1	<1
Carbon-carbon bonds (condensed bonds)				
Dihydroxy biphenyl	5-5´		10	~5
Phenyl coumarane	β-5´		11–12	4–9
Pinoresinol	ββ´		2–3	3–4
	ββ´		<1	none
Secoisolariciresinol			1–2	none
	β-1´		1–2	1
Spirodienon	β-1´		1–3	2–3

Table 6.2. Bonds between monolignols and lignin functional groups. (cont.)

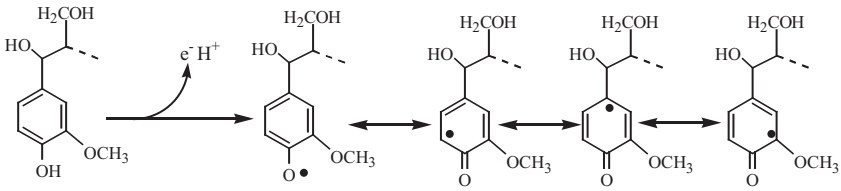
Name	Bonds	Structure*	Frequency softwood (%)	Frequency hardwood (%)
Dibenzo-dioxocin			4–5	trace
End groups				
Coniferyl alcohols			1–6	Trace–6
Dihydroco-niferyl alcohol			2	none
Free phenol			11	9?

* Note that only the "carbon skeletons" of the structures are shown. In complete structures hydroxyls and metoxyl groups shall be added and off course also ethers and carbon-carbon bonds to other monolignol residues.

As shown in *Table 6.2*, the by far most important bond between monolignols is the β-O-4′ linkage. The most important chemical reactions in pulping, bleaching and biological lignin degradation, involve this bond. All inter-monolignol bonds are relatively stable (with the α-O-4′ bonds as somewhat of an exception), but the carbon-carbon bonds (condensed bonds) are the most resistant, and these structures survive often chemical pulping. Only 10–13 % of the aromatic rings in native lignin are free phenols, i.e., the oxygen in 4-position does not form an ether-bond. Non-phenolic structures require much higher redox-potential for being oxidized to resonance stabilized radicals than free phenols, due to that more resonance forms are possible for the phenolic radical than for the non-phenolic (*Figure 6.4*). Since both lignin biodegradation and many pulp bleaching methods are based on oxidation of aromatic rings, the phenolic structures represent the "weak points" and content of phenols is important for the reactivity of the lignin. Also for chemical pulping the phenolic groups play a central role[7]. Physical and chemical properties of lignin will be further described in Volume 2.

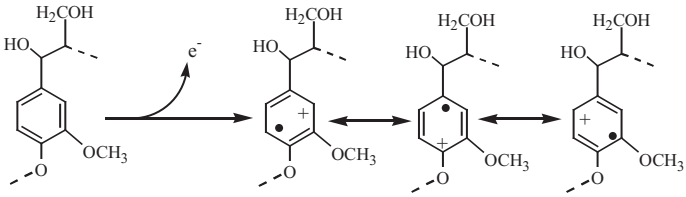
[7] In this case it is however not the redox potential that is the interesting, but rather the possibility to deprotonization of the phenolic hydroxyl group.

Oxidation of a phenolic aromate requires a moderately strong oxidant.



The generated phenoxy radical is a strong acid (pK_a<0) and thereby can the radical be delocalized to the oxygen.(All possible forms are not shown.)

Oxidation of a non-phenolic aromate requires a very strong oxidant.



The generated cation radical has the unpaired electron delocalized over the aromatic ring, but the number of possible resonance forms are smaller than for the phenolic radical.(All possible forms are not shown.)

Figure 6.4. Oxidation of phenolic and non-phenolic lignin structures.

In older literature some additional lignin-structures are often described: α-carbons oxidized to carbonyls (ketons); γ-O-4´-bonds; α-O-4´ bonds other than the one in the β-5´ and dibenzo-dioxocin- structures (*Table 6.2*), i.e., as branch points, condensed bonds to the 6-carbon, and vanillin type end groups. However, some of these structures exist only in synthetic lignin (see 6.2.5) and natural lignin structures might be altered during the preparation of the samples. Recent data indicate that branch point α-O-4´ bonds, and γ-O-4´-bonds does not exist in natural lignin. α-carbonyls and vanillin structures do not seem to exist in newly synthesized lignin, but might be created during ageing[8], maybe involving microbiological processes (chapter 11, *Figure* 11.7). The existence of covalent bonds involving the 6-carbons, as the β-6´-bond, has some experimental support, although the results are contradictory. This structure is under all circumstances infrequent.

At least in softwood-lignin another type of β-β´ bond exist in addition to the pinoresinol structure (*Table 6.2*), where the α- carbons are in a reduced stage, CH_2. Thus, the ether bonds do not occur in this structure and the monolignols are purely connected by the β-β´ bond. Around 25 % to 50 % of the β-β´ bonds are in this reduced form. The mechanism behind the formation of this reduced form is unknown.

[8] The lignin in a harvested timber can be several hundred years old

6.3.3 Polymerization of Monolignols

How can the complex and racemic pattern of lignin be formed from the monolignols? As described in chapters 4 and 5 cellulose and hemicellulose are polymerized inside protein complex in a nucleotide triphosphate-driven reaction giving well-defined and non-racemic structure. Lignin is not polymerized according to this concept. According to a theory by the Swedish scientist Ertman, the chemical bonds between the monolignols are the result of radical-radical couplings, and this got experimental support when the German scientist Karl Freudenberg 1959 succeded to make a lignin type polymer in the laboratory. The monolignols[9] are according to this theory oxidized either by a peroxidase (using H_2O_2 as oxidant) or a laccase[10] (using O_2 as oxidant) to radicals with high resonance stabilization (*Figure 6.5*). As shown the unpaired electron can be localized either at 4-O-, 3-, 5-, 1- or β-position. When two radicals meet in an uncatalyzed reaction (i.e., without any enzymes present), the unpaired electrons can form a covalent bond. However, all theoretically possible covalent bonds are not present in lignin; linkages at 3-position is prevented by sterical reasons (due to the methoxy group), and 4-O-O´-4´-bonds are chemically unstable.

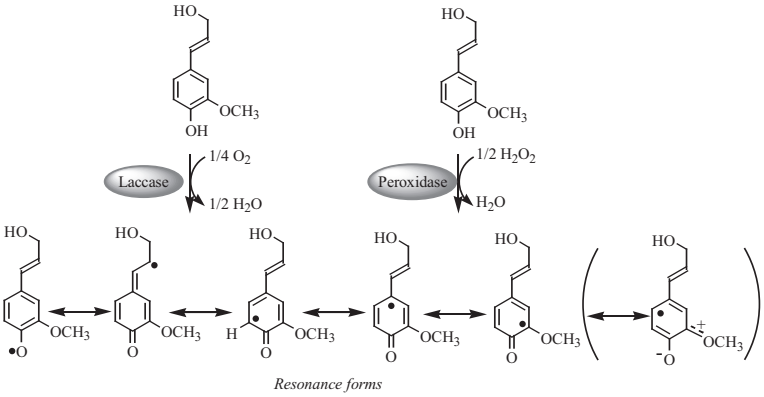


Figure 6.5. Enzymatic generation of resonance stabilized monolignol radicals.

This explains how dimers of monolignols are formed, but how does the polymerization continue? As shown in *Figure 6.5*, radicals can also be present at phenolic groups on the lignin polymer. These unpaired electrons can be located on either the 4-O-, the 3-, the 5-, or the 1- positions, and they can form covalent linkages by coupling to monomer radicals and to each other with the same limitations as for the monolignols[11], thereby creating a growing polymer. This model is called end-wise polymerization. Non-phenolic radicals are not created during the

[9] Coniferyl alcohol is used in all examples. The differences in behavior of the other main monolignols will be discussed below.

[10] These types of enzymes are further discussed in chapter 11. Interestingly there are similarities between the enzymes polymerizing lignin and the proteins degrading the polymer.

[11] Thus, covalent bond between monolignols ban be created in principally three ways – between monolignol radicals, between one monolignol radical and an end group radical, and between two end group radials. The 5-5´- and 4-O-5´bonds seems almost exclusively be created from two end-groups.

lignin bio-polymerization. Thus, the polymerization of lignin chain always starts from phenolic residues. An alternative way is that monolignol dimers became oxidized to radicals that couples to tetramers, that in turn became oxidized and so on until a polymer is created, batch-wise polymerizarion. At least in softwood it appears like the end-wise polymerization dominates[12].

How is the radical introduced to the phenolic group on the polymer (or oligomer)? There are two possible ways: either directly by the enzymes, or by an oxidation from a monolignol radical; the radical-radical coupling will thereafter be carried out with a second monolignol radical (*Figure 6.6*).

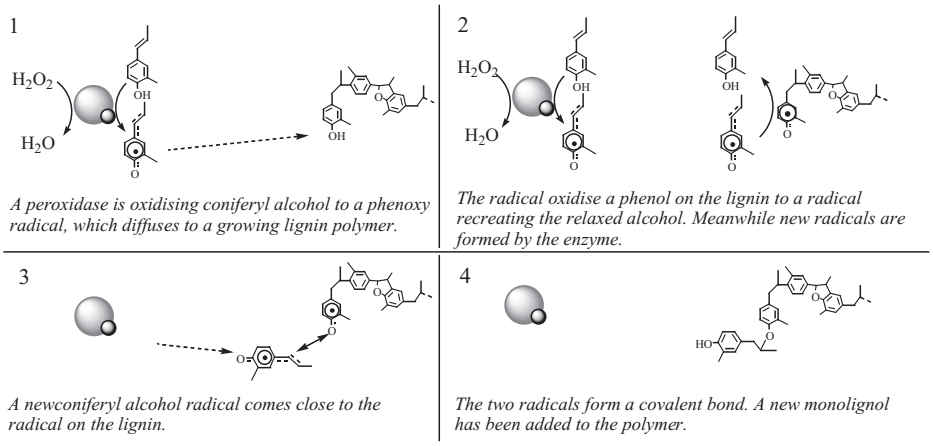


Figure 6.6. Principe for indirect enzymatic driven polymerization.

Lignin biosynthesis can be compared with the formation of thermosetting plastics. Radical polymerization of this kind is not totally unique in nature; other examples of biopolymers created this way are suberine, tannins and various pigments. We will now discuss the mechanisms of formation of covalent bonds in lignin in detail. The simplest mechanisms are the formation of 5-5´ and 4-O-5´ bonds that consists of a simple radical coupling followed by a rearrangement of protons that reform the aromatic structure as shown in *Figure 6.7*.

[12] The argument for this is an exception for the rule that the covalent bonds in lignin is randomly distributed; the ββ' -bonds seems always to occur together with another condensed bond as 5-5´, or 4-O-5', and this is difficult to explain according to the batch wise mode of polymerization. The method for studying this is thioacedolysis that is described in chapter 9.

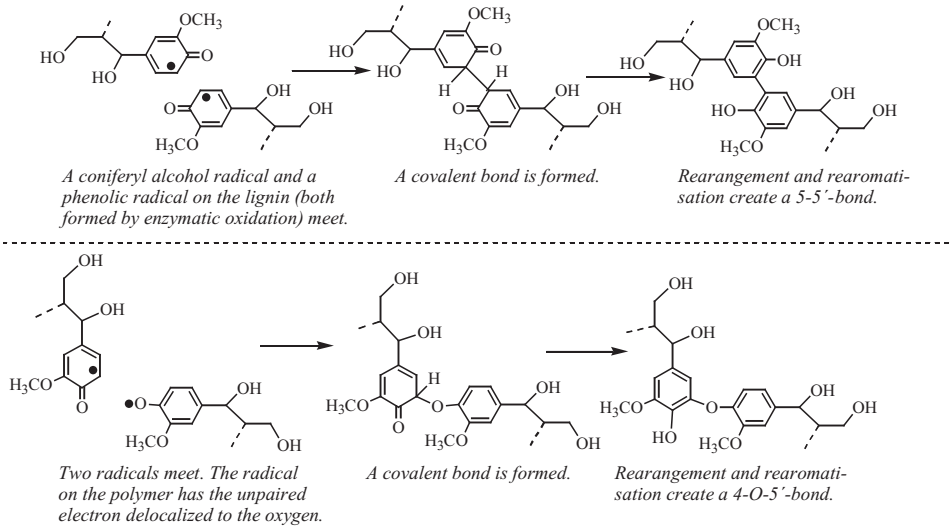


Figure 6.7. Formation of 5-5´- and 4-O-5´-bonds.

The reaction mechanisms become somewhat more complicated when radicals on the β-carbons are involved in the coupling. In these cases the reactions occur in two steps: first a radical coupling and thereafter a nucleophilic attack by oxygen atoms (in water or sugar alcohols etc) on quinone methide intermediates. In *Figure 6.8* the formations of β- β´, β-5´and β-O-4´ bonds are shown. The δ+ on the α–carbon on the quinone methide intermediates might appear surprising, but they are explained by the resonance structures shown in *Figure 6.9*.

As shown in *Figure 6.8* the nucleophilic attacks are carried out by γ-hydroxyl-groups in the formation of the pinoresinol structure, by the 4-O in the formation of the β-1´ structure and by water in the case of the β-O-4´ bond. In the latter case, however, also alcohol- or carboxylic acid groups on polysaccharides can perform the nucleophilic attack. The result will then be a covalent bond between lignin and polysaccharides in wood. Such structures are called *lignin-carbohydrate complexes*, LCC[13]. These may occur between lignin and the polysaccharides of wood, hemicellulose, cellulose and pectin. In *Figure 6.10* the structure of some LCC are shown.

[13] Again an inappropriate name; LCC are covalent bonds and *not* simple complexes. The term is however very established.

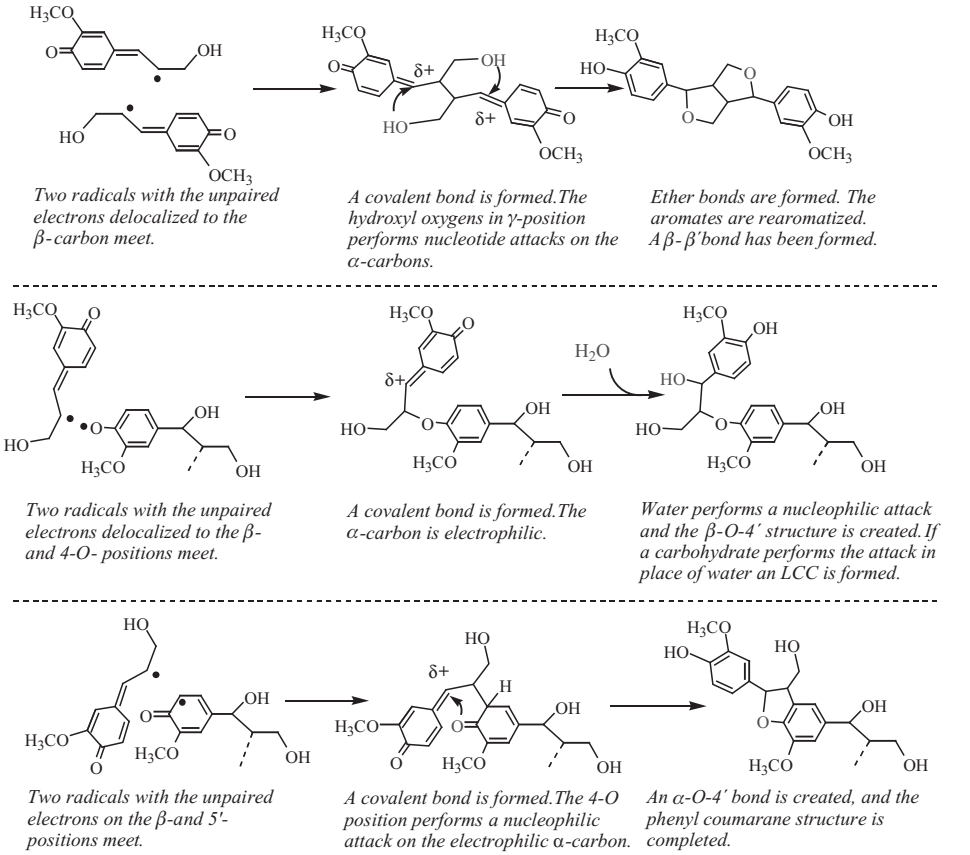


Figure 6.8. Mechanism of formation of β- β′, β-O-4′ and β-5′ bonds in lignin.

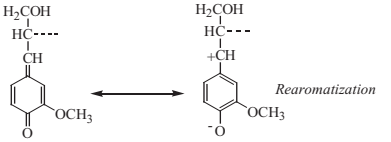
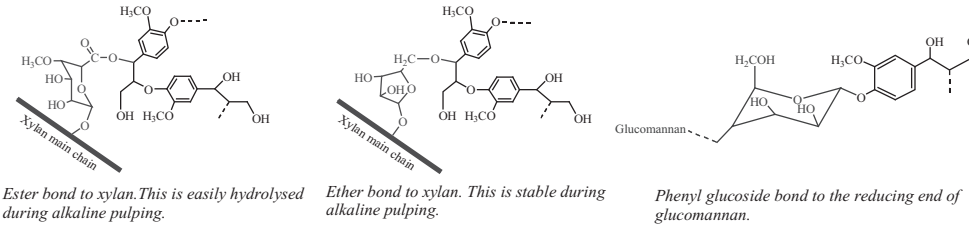


Figure 6.9. Resonance structures make the α–carbon electrophilic. As shown in the figure, the quinomethide has a switterion isoform, where a positive charge is located on the α-carbon; in practice this means that it will be electrophilic.



Ester bond to xylan.This is easily hydrolysed during alkaline pulping.

Ether bond to xylan. This is stable during alkaline pulping.

Phenyl glucoside bond to the reducing end of glucomannan.

Figure 6.10. Examples of covalent bonds between lignin and carbohydrates – LCC. Here the ester and ether bonds are shown to xylan and the phenyl glucosidic bond to glucomannan, but these types of bonds may probably occur to all kinds of wood polysaccharides. While the esters and ethers probably re formed during the lignin bio-polymerization though nucleophilic attacks to quinone methods, the mechanism for the formation of phenyl glucosides are unclear. In wood as much as over 5 % of the lignin aromates form phenyl glucosides.

When radical coupling occurs to the 1-carbon, the result will either be that a monolignol splits in two parts, one aliphatic and one aromatic, or that the final structure is totally aliphatic (spiro-dienon). The reaction mechanisms are shown in *Figure 6.11*.

As discussed in the introduction to this chapter, lignin has a branched network structure. How are the branch points created? In principle in two different ways, firstly if a monolignol is coupled to a polymeric phenolic group, so that two free phenol structures are preserved, i.e., 5-5´ bonds (*Figure 6.7*), both phenols can couple new monolignol and one chain is split into two. Secondly, if two polymeric phenols form a covalent bond that preserve at least one phenol, i.e., 4-O-5´ and 5-5´ bonds (*Figure 6.7*), that can couple to new monolignol residues, i.e., two chains are united into one. The formation of the maybe most common branch point, dibenzodoxocin, is shown in *Figure 6.12*.

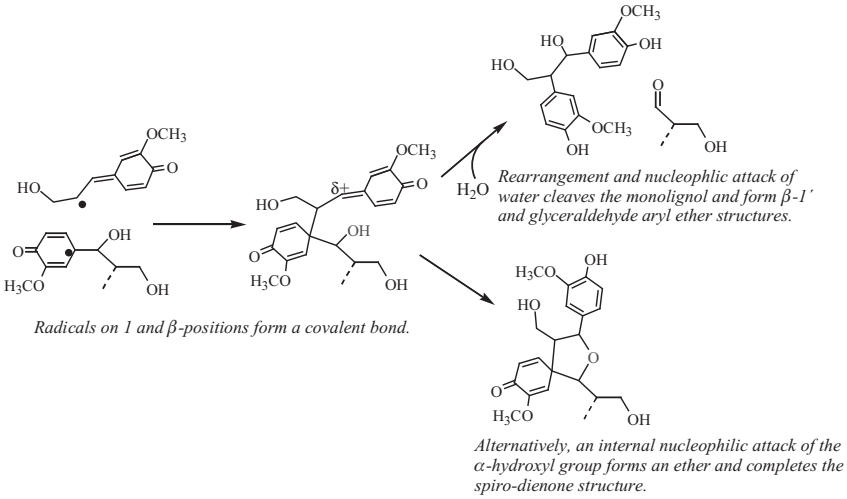


Figure 6.11. Formation of β-1´ and 4-=-1´structures.

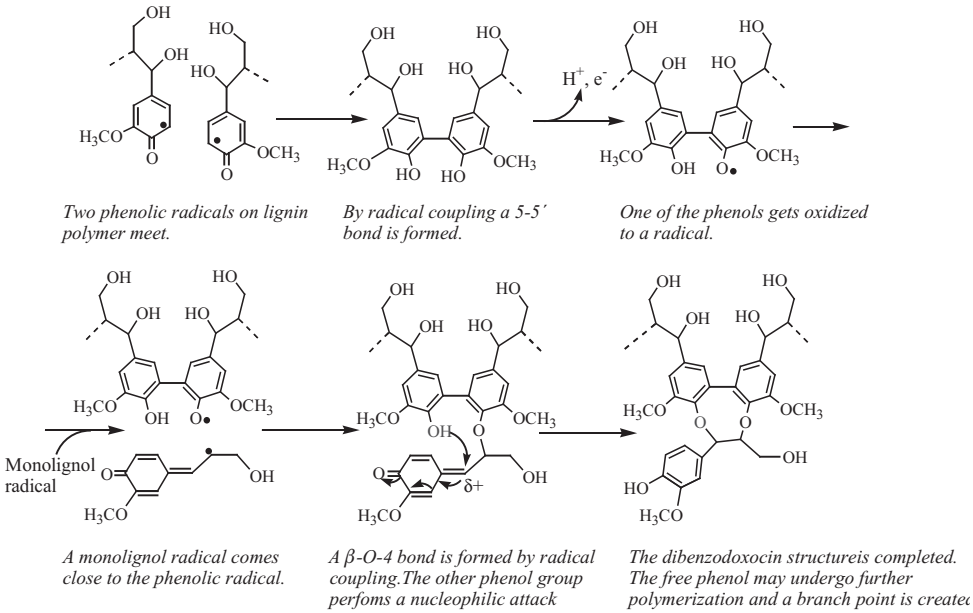


Figure 6.12. Formation of dibenzodoxocin, one of the most important branch points in lignin.

The fully polymerized lignin will contain a fraction of free phenolic rings that have not undertaken oxidations during the polymerization. It will also contain end-groups, where the double bond between Cα and Cβ has remained from the original monolignol-structure (*Table 6.2*). For a mechanism, replace for instance the polymeric phenol with a coniferyl alcohol in the formation of the β-O-4´ bond in *Figure 6.8*. However, there are also reduced end-groups α, β and γ carbons reduced to CH_2 and CH_3 groups. The β-6´ bonds, that according to many scientist occur in small amounts in lignin, have been suggested to be a result of an acidic condensation reaction, but might also have been created by a radical coupling, since the unpaired electron can be delocalized to some extent also to the 6.carbon (*Figure 6.5*), and that this electron form a covalent bond with another radical in similar was as described in *Figures 6.7* to *6.9*.

6.3.4 Differences Between Various Types of Lignin

The polymerization mechanisms in section 6.2.3 are all shown with coniferyl alcohol, which is the totally dominating monolignol in softwood lignin (*Table 6.1*). Hardwood lignin contains both sinapyl alcohol- and coniferyl alcohol units. Sinapyl alcohol differs from coniferyl alcohol by that it has an extra methoxy group on the 5-position (*Figure 6.2*). How will this interfere with the coupling pattern? For sterical reasons sinapyl alcohol radicals cannot couple in 5-position, but are restricted to covalent bonds in the 4-O-, the β-, and the 1-positions. Thus, hardwood lignin contains more β-O-4´-, β- β´-bonds and fewer bonds on the 5-position than softwood lignin, whereas the β-1´ bonds are approximately the same (*Table 6.2*). As a consequence of this, hardwood-lignin is believed to be more linear and less branched than softwood

lignin. These differences explain why it is easier to make kraft pulp from hardwoods than from softwoods. Another consequence of the presence of sinapyl alcohol units in hardwood lignin is that the content of methoxy groups is higher in hardwood lignin (140 to 160 per 100 aromatic ring) than in soft wood lignin (92 to 96 per 100 aromatic rings). Softwood lignin contains also *reduced structures* as shown in *Table 6.2* (dihydroconiferyl alcohol and secoisolariciresinol). These structures are rare in lignin, but are theoretical interesting, since they cannot be directly created by the radical coupling mechanisms.

Grass lignin contains much higher amounts of *p*-coumaryl alcohol that lack methoxy-groups, than the other two main lignin types. Expected consequences of this should be lower content of the β-O-4´-bond and higher content of carbon-carbon bonds and thereby a more branched polymer structure, since this monolignol can perform radical couplings also in the 3-position. Some investigations support this, but any final conclusion cannot be drawn since lignins from monocotyledons are less investigated. Furthermore, grass lignin contains ester-bonds formed by the hydroxyl group on the γ-carbon, and the carboxylic acid on the γ-carbon of oxidized monolignols such as *p*-coumaric acid or ferulic acid (*Figure 6.2*), or to hemicelluloses forming LCC. Also some eudicotyledonous lignins contain ester-bonds; in aspen and other trees in the Poplar family (chapter 2), LCC is formed by esters between carbohydrate alcohols and *p*-coumaric acid.

6.3.5 Alternative Theories of Lignin Polymerization

The above description of lignin-polymerization with an uncatalyzed polymerization of enzymatic-generated monolignol radical generating a polymer of partly random structure, follows with some minor modifications the original ideas of Freudenberg. However, there are some problems coming with this model; when monolignols are polymerized in a laboratory by a peroxidase, the polymer produced will have covalent bonds of lignin type, but the ratios of different bond types differs from that in natural lignin; the synthetic "lignin" (or *dehydrogenated polymer*, *DHP*) contains less of the β-O-4´ bond, and more of the carbon-carbon bonds than natural lignin. Furthermore, DHPs often contain considerable amounts of branch points formed by α-O-4´ bonds, a structure not observed in natural lignin. In addition to this, natural lignin contains structures that cannot be directly explained by the classical Freudenberg chemistry (reduced structures and phenol glucosides to polysaccharides), as discussed above. These observations have lead to the formulation of two alternative models for lignin biopolymerization[14].

According to one school, the lignin structure is not randomly polymerized, but rather under very strict biological control. This is hypothesized to carry out by that special "*dirigent sites*" in the cell wall catalyze the coupling of the monolignol radical, so that certain types of bonds and sequences of bonds are formed. In support of this idea, there are reports that lignin indeed displays a repeated structure, but these findings are controversial. What does the dirigent sites consist of? Dirigent proteins (DiP) is a group of enzymes[15] present in plant cells during lignification that are able to efficiently catalyze the coupeling of monolignol radicals to structure as ββ' or β-O-4' (*Figure 6.13*). However, since the product of this coupling is optically active (in the oppo-

[14] Both of these models are actually expansions and modifications of Freudenberg´s classical model, rather than totally novel theories.

[15] In the literature it is sometimes claimed that the DiP is a "non-catalytic protein". In fact, DiP has the typical characteristic of an enzyme, i.e., catalyzing the radical coupling of two phenolic radicals.

site to the racemic lignin), it is unlikely that DiP:s play a fundamental role in lignin formation[16]. The American researcher Simo Sarkkanen, has suggested that a lignin chain by itself may work as dirigent site catalyzing radical couplings. The original "first" lignin chain might be formed with the help of protein catalysis (*Figure 6.14*). This might theoretical give an optically inactive product, but recent data talk against that lignin can have such template activity.

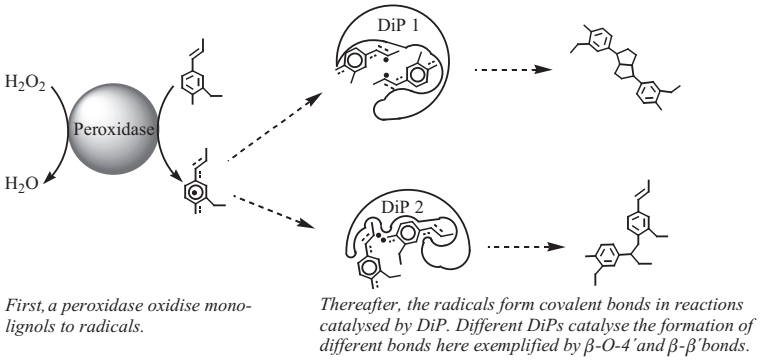


Figure 6.13. Dirigent proteins (DiP) catalyze radical couplings. Several types of DiP:s are known that catalyze radical couplings of monolignol radicals specifically to various types of bonds. The products are however optically active in the opposite to lignin, and DiP:s are more likely involved in the formation of the extractive *lignans* (optically active dimmers and oligomers of lignans) than in lignin biopolymerization.

According to one school, the lignin structure is not randomly polymerized, but rather under very strict biological control. This is hypothesized to carry out by that special "*dirigent sites*" in the cell wall catalyze the coupling of the monolignol radical, so that certain types of bonds and sequences of bonds are formed. In support of this idea, there are reports that lignin indeed displays a repeated structure, but these findings are controversial. What does the dirigent sites consist of? Dirigent proteins (DiP) is a group of enzymes[17] present in plant cells during lignification that are able to efficiently catalyze the coupeling of monolignol radicals to structure as ββ' or β-O-4' (*Figure 6.13*). However, since the product of this coupling is optically active (in the opposite to the racemic lignin), it is unlikely that DiP:s play a fundamental role in lignin formation[18]. The American researcher Simo Sarkkanen, has suggested that a lignin chain by itself may work as dirigent site catalyzing radical couplings. The original "first" lignin chain might be formed with the help of protein catalysis (*Figure 6.14*). This might theoretical give an optically inactive product, but recent data talk against that lignin can have such template activity.

A totally other model was suggested by the Swedish scientist Ulla Westermark; here the peroxidases[19] do no not directly oxidize the monolignols. Instead the enzyme oxidizes a low-molecular weight compound, *the redox shuttle*, which in turn oxidizes monolignols and phenolic groups on a lignin polymer. The advantage with this is that oxidations are performed at approx-

[16] The DiP:s are probably involved in the biosynthesis of lignans, that in the opposite to lignin is optically active.
[17] In the literature it is sometimes claimed that the DiP is a "non-catalytic protein". In fact, DiP has the typical characteristic of an enzyme, i.e., catalyzing the radical coupling of two phenolic radicals.
[18] The DiP:s are probably involved in the biosynthesis of lignans, that in the opposite to lignin is optically active.
[19] Westermark did actually use an oxidase, xhantine oxidase, instead of a peroxidase in her experiment. Other scientists have later obtained similar results with peroxidases.

imately the same rate on both the monomer and the polymer, which should favor polymerization over dimerization. Furthermore, according to this model, lignification could easily be performed also in very compact structures. Westermark proposed a complex of Ca^{2+} and superoxide anion ($O_2^{-}\cdot$) as redox shuttle and other scientist have suggested Mn(II)/Mn(III). In support of the model, DHP with high similarity to natural lignin have been synthesized. However, no redox shuttle system has been isolated from plant cells and thus the model remains speculative. See *Figure 6.14* for a schematic presentation of the Sarkanen- and Westermark models.

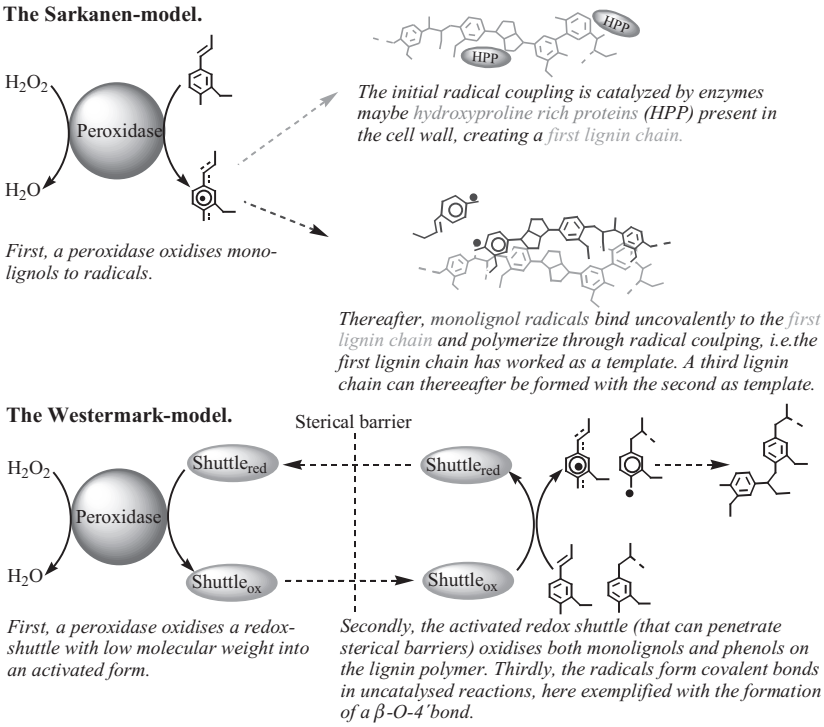


Figure 6.14. Two alternative models of lignin polymerization. It shall be emphasized that none of these models are proven or generally accepted.

6.4 Morphological Aspects of Lignification

The concentrations of lignin vary in different *hierarchic levels* of the wood, as different species (*Table 2.2*), different parts of the tree (*Figure 2.6*), but also within the stem wood as late wood compared with early wood (*Table 6.3*). The structure of lignin does not only differ between different kinds of plants; it can also vary between different plant tissues, different types of cells and even between different cell wall layers. In compression softwood, the lignin has a higher content of *p*-coumaryl alcohol units[20] (around 30 %), and, as expected, compression lignin contains more carbon-carbon bonds than normal soft wood lignin. This gives a more cross-linked lignin in the compression wood, but the lower amount of methoxy-groups might also facilitate

mobility within the lignin chain (ring rotations etc.). The lignin content in the compression tracheids is somewhat higher than the normal tracheids.

In hardwood the monomer composition differs between different cell types; the ray parenchyma cells and especially the vessels have a higher content of coniferyl alcohol than the fibers, and their lignin can thus be considered to be more similar to the lignin in soft wood. The lignin concentrations in these cells are also higher (24–28 % in vessels and 27 % in ray cells) than in libriform fibers (19–22 %)[21].

Table 6.3. Distribution of lignin in cell wall layers of softwood tracheids and hardwood fibers.

Wood cells	Cell wall layer	Tissue volume (%)	Part of total lignin (%)	Lignin-conc. (%)
Loblolly pine tracheids (Softwood)				
Early wood	S1	13	12	25
	S2	60	44	20
	S3	9	9	28
Middle lamella +	primary wall	12	21	49
	Cell corner	6	14	64
Late wood	S1	6	6	23
	S2	80	63	18
	S3	5	6	25
Middle lamella +	primary wall	6	14	51
	Cell corner	3	11	78
White birch (Hardwood)				
Fiber	Secondary cell wall	73	60	19
Middle lamella +	primary wall	5	9	40
	Cell corner	2	9	85

Also within the different cell wall layers differences in lignin concentration and structure occur. The lignin deposition proceeds in three steps in tracheids and fibers, always preceded by deposition of carbohydrates. The first step begins in the corners of the primary cell wall and continues in the middle lamella[22]. The second lignification stage occurs after the deposition of

[20] According to some researchers almost all *p*-coumaryl in soft wood lignin originates from compression wood. The "normal" soft wood lignin should then be built up exclusively by coniferyl alcohol.

[21] The data come from a UV-microscopy study of white birch (*Betula papyrifera*) by Fergus and Goring. See suggested reading.

[22] It is suggested that special *lignin initiation sites* located in the cell corners initiate the lignification. These could maybe be proteins rich in tyrosine residues acting like starting points for the polymerization. The hypothesis is however not confirmed.

cellulose and hemicellulose in the S2 layer. The main lignification takes place in the third stage, when cellulose microfibrils have been deposited in the S3 layer. The different stages of lignification are reflected in variations in the monolignol composition, in the structure of the lignin and in different lignin contents (*Table 6.3*). The S2-layer lignin is the most abundant in wood, but the middle lamella lignin has a special interest, since the functional reactions in chemical pulping leading to separation of fibers and tracheids to a large extent take place in this lignin. Both in hardwood and softwood the middle lamella and the S3-layer[23] have higher lignin contents than the S2 layer, but the larger volume of the latter makes the S2 lignin the most abundant in the plant. In hardwood (birch) the coniferyl alcohol residues dominates over the syringyl alcohol residues in the cell corners, whereas the opposite is the case in the S2-layer. Thus, the middle lamella lignin in hardwood has a more branched structure with a higher content of carbon-carbon bonds than secondary wall lignin. Also in soft wood, the middle lamella lignin is believed to contain more of carbon-carbon bonds and has a more cross-linked structure than the secondary cell wall-lignin, but nevertheless coniferyl alcohol is the dominating monolignol in both lignins[24].

On a *close to molecular level*, the lignin seem to be closer associated with xylan than with mannan and cellulose. LCC in wood are very common, especially to the hemicelluloses, but bonds to cellulose and pectin does also occur, so that complex *networks* are created. Such networks probably play an important role for the mechanical properties for wood and are also an obstacle for delignification during pulping and bleaching (see Volume 2). In *Figure 6.15*, a model for the organization of lignin, hemicellulose and cellulose in S2 layers is shown.

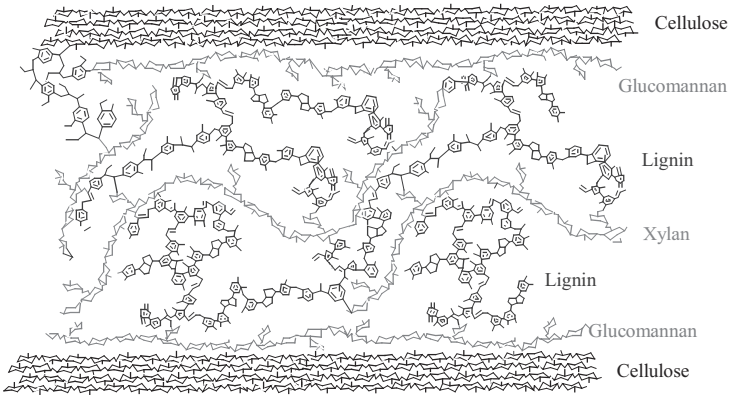


Figure 6.15. Distribution of lignin and polysaccharides on molecular level. This model for the S2 layers is based on studies by Sahlmén and coworkers and Lawoko and coworkers. Most of the lignin is believed to be located between glucomannan and xylan, but there are also some direct contacts between lignin and cellulose. In softwood covalent bonds between lignin and both hemicelluloses (glucomannan and xylan) are frequent. In hardwood the glucomannan content is very low and thus the LCC between lignin and xylans dominates. For both types of wood, the lignin covalently cross-links the different polysaccharides forming networks.

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[23] The high content of lignin in the S3 layer may be a defense against attack of microorganisms, since the degradation in many cases starts from the lumen. Some wood degrading microorganisms, i.e., brown rot fungi, have also difficulties in degrading this layer.

[24] Some researchers claim that the middle lamella lignin in softwood contains more of *p*-coumaryl alcohol, but this might be due to contamination of the samples with compression wood.

6.5 Biosynthesis and Genetic Modification

Lignin is special among biopolymers due to its *anabolic plasticity*, i.e., it is in many cases possible to at least partly replace the monomers to other structures or at least modify the monolignols, and still getting a lignin with functional properties for the plant[25]. Since it is of large economical interest to get trees with lower lignin content or a modified lignin that is easier to remove by pulping and bleaching, or has other interesting properties, the biosynthesis of the monolignols is interesting not only from a basic-research perspective, but also for potential applications.

The biosynthesis of monolignols takes place intracellular (inside the cell membrane) in the opposite to the lignin polymerization that is performed in the cell wall. A biochemical pathway for synthesizing a specific molecule (as the monolignols) in a living cell can be compared with an assembly line in an engineering industry, where the different enzymes catalyzing a specific modification of a molecule, play the role of machines performing a special step in the manufacturing of the industrial product. Steps that require reduction are driven by oxidation of nicotine amide dinucleotide, NAD(P)H:

$$\mathrm{NAD(P)H} \rightarrow \mathrm{NAD(P)^{+}} + \mathrm{H^{+}} + 2\mathrm{e^{-}}$$

In similar way, thermodynamic unfavorable steps, are made thermodynamically possible by the hydrolysis on nucleotide triphosphates (ATP, GTP, UTP, CTP, TTP) as “fuel”:

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{PO_4^{2-}} \quad \Delta G < 0$$

Furthermore, one or several enzymes in a pathway are generally possible to regulate (by phosphorolation, product inhibition etc.); in the parallel to the engineering industry, this corresponds to the regulation technology. The general principles for biochemical synthesis can be studied further in biochemical textbooks.

6.5.1 Biosynthesis of Monolignols

A considerably fraction of the carbon fixated in the photosynthesis is consumed in lignin biosynthesis. As described in chapter 2 the product of the photosynthesis is erythrose 4-phosphate. This tetraose is used for glucose synthesis, but together with phosphoenolpuruvate, it forms also the starting material for the *shikimic acid pathway* to the aromatic amino acids phenylalanine and tyrosine. These amino acids belong to the set of twenty amino acids that are the monomers to proteins[26]. This pathway, which has gained some interest as target for some attempts of down-regulation of lignin (manipulation of enzyme levels that lead to lower production of lignin), can be studied in any larger textbook in biochemistry, and will not be discussed further here. See *Figure 6.16*.

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[25] This is generally not possible for other biopolymers, although modified monosaccharides might be included in some polysaccharides in rare cases.

[26] This pathway exist in fungi, archebacteria and bacteria, but not by animals, for which phenyl alanin and tyrosine are essential nutrient.

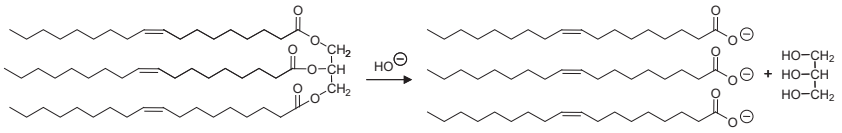

Figure 6.16. Structures of some important intermediates in the pathway from photosynthesis to monolignols.

The pathway (*general phenylpropanoid* (C6.C3) *pathway* or *monolignol pathway*) for the conversion of these amino acids (*Figure 6.16*) to monolignols, is specific for vascular plants,[27] and is shown in a simplified form in *Figure 6.17*. Initially on the pathway, the amino group is removed with introduction of a double bond between Cα and Cβ in a lyase[28] reaction (PAL). In the subsequent steps, the carboxylic acid on the Cγ is reduced to an alcohol (in two steps (4CL, CCR and CAD); the reduction from carboxylic acid to aldehyde involves intermediates with the coenzyme A[29]), the aromatic ring is hydroxylated, and thereafter methylated on proper positions (C4H, C3H, COMT and F5H). Since some of the enzymes involved in the catalysis are relatively unspecific, and, as seen in the figure, the same enzyme is believed to catalyze similar reaction on several various substrates, the exact order of the different reactions is thus uncertain, and the pathway has in some cases the form of a web of alternative pathways. In addition to lignin, the pathway produce monomers for other important plant products as lignans, flavanoids and the hydrophobic polymer suberine (see chapter 7).

The lignin monomers are toxic and unstable and cannot thus accumulate to high levels in plant cells. Monolignols seem, however, to be stored in the living plants as glucose ethers on the phenol group. These are stable and non-toxic species.

6.5.2 Genetically Modified Lignin

In order to get a wood having better properties for chemical pulping by manipulation with the monolignol biosynthesis, two main strategies have been followed: i) by lowering the total content by down-regulating the genes encoding for one or several of the enzymes in the monolignol pathways, and thereby facilitate pulping, or ii) by down-regulating or knockout the gene encoding for any of the enzymes catalyzing the different steps in the pathway (*Figure 6.17*), or even introduce a gene encoding for a new enzyme in the plant[30], a lignin made of other monolignols than the normal, i.e., a lignin with a different covalent structure can be obtained. According to strategy i), the genes encoding CCR, COMT, CCoAOMT successfully have been down-regulated and in some cases "functional" plants with lower lignin content have been obtained. There is, however, a limit how much the lignin content a fully functional plant can be reduced.

For strategy ii) several possibilities have been tested:

- By down-regulating COMT an interesting possibility should be to obtain a lignin with hydroxyl groups instead of methoxy groups on 3-position. Such a lignin should be easier to

[27] A few fungi (Basidiomycetes) seem to have at least part of the pathway, however.
[28] See a textbook in enzymology for the definition of different types of enzymes.
[29] Coenzyme A is a cofactor used for many biochemical processes inside the cell.
[30] "Down-regulate" a gene means that the plant produces less of this enzyme. "Knockout" means that the gene and thereby the protein is totally eliminated.

oxidize (*Figure 6.4*) and thus easier to remove in bleaching and pulping. However, in some cases a lignin that is more *difficult* to remove by chemical pulping has been obtained. Investigations of this lignin indicate that a new type of chemically stable bond has been created (*Figure 6.18*). Interestingly, this structure is very similar to the main bond type in the insect polymer sclerotizine.

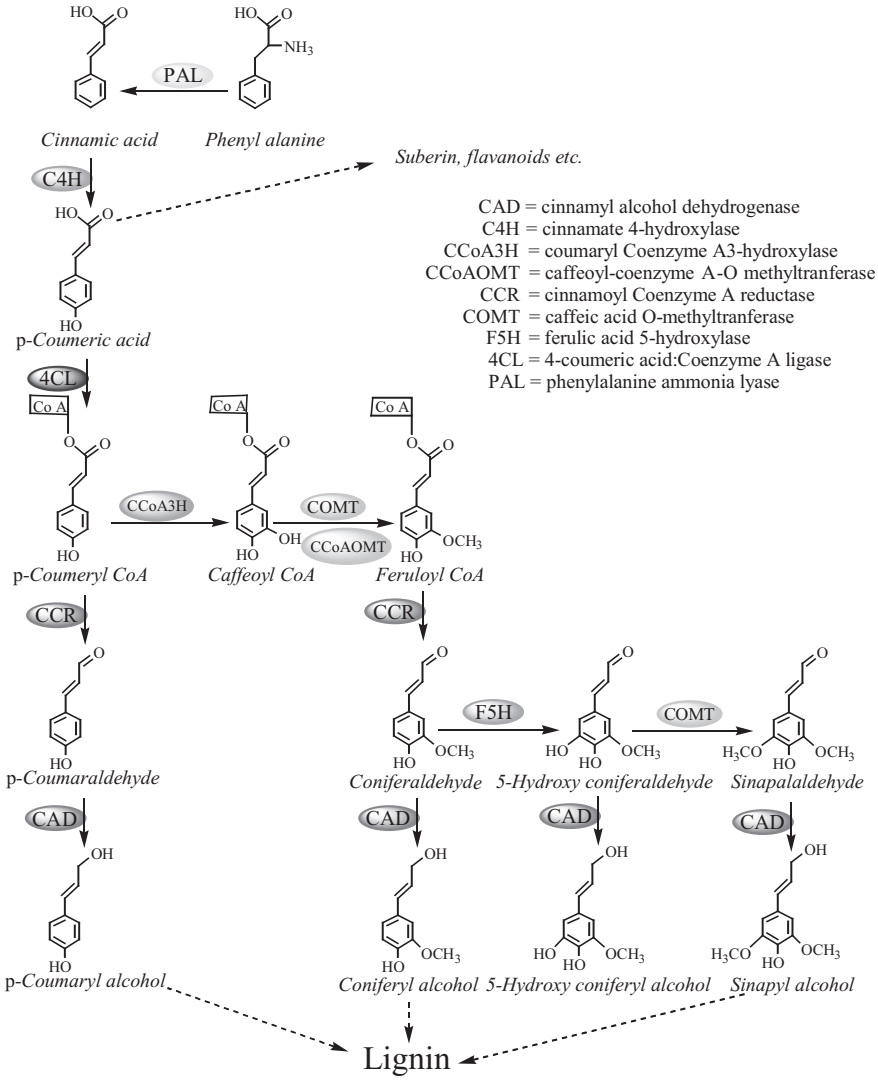

Figure 6.17. Pathways for the biosynthesis from phenylalanine to monolignols. In the conversion from the amino acid phenyl alanin to monolignols, the reducing power of 2 NADPHs and the free energy of one ATP are consumed.

- By down-regulating CAD in poplar[31] a lignin partly based on aldehydes were obtained. The wood had a red colour (but this might be due to modified lignans as well as new lignin structures), and was easier pulped than normal poplar. The chemical background for this is still unclear, but also here a novel type of bond is created (*Figure 6.18*).
- By over-expression or introduction of F5H (in softwoods) higher syringyl content in the lignin can be obtained. This is interesting; since it could lead to a higher content of β-O-4´-bonds and thereby an easier chemical pulping. A spruce with lignin of hardwood type should indeed be interesting for the pulp and paper industry, but this approach is more difficult to realize, since it generally is more difficult to over express a gene, than to suppress it.

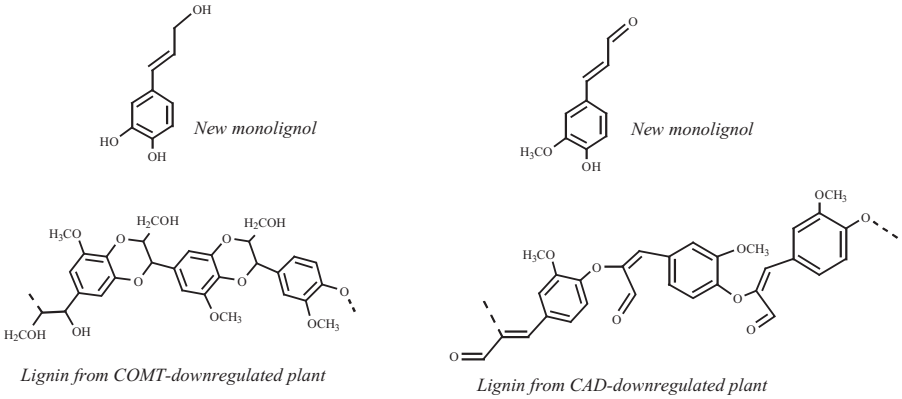

Figure 6.18. Lignin segments with structures unique for COMT and CAD down-regulated plants. These "unnormal" structures can be be explained from the different chemical properties of the monolignols.

Both CAD and COMT down-regulated poplars have been cultivated in field tests and appears to be growing well. The attempts to make plants with altered polymer structure are further discussed in chapter 12.

6.6 Folding of Lignin

Many biopolymers, such as proteins and DNA, are organized also on a higher level than the pattern of covalent bonds. Stabilized by non-covalent forces as hydrogen bonds, aromatic interaction, salt-bridges and hydrophobic interaction, they fold into complex, but well-defined structures. As we have seen in chapter 4, this is the case also for cellulose, the microfibrils. Any corresponding structure is not known for lignin, and it is possible that the three-dimensional structure of lignin is totally amorphous. Since lignin naturally always occurs in close association with cellulose and other polysaccharides (*Figure 6.15*), it is also very difficult to study the subject. However experimental data indicate that the aromatic rings tend to orient themselves in parallel to cellulose surfaces; this is in concert with the ideas that the aromatic rings in lignin are

[31] Aspens with both COMT and CAD down-regulation have been cultivated in field, and were found to grow normally. A tendency for the CAD down-regulated wood, to be more sensitive to rot was, however, observed.

linked not covalently to polysaccharides In this case, the lignin folding should not be completely random.

Computer simulations on β-O-4′ lignin oligomers have suggested an energy-minimum where the aromatic rings were located in approximately 70° towards each other, i.e., a zigzag appearance. The moveability of the Cα-Cβ bond is lower for dimers with sinapyl alcohol residues than for coniferyl alcohol residues, which in turn have lower moveability than *p*-coumaryl alcohol dimers. This suggests that the methoxy-groups have a function in adjusting the elasticity of the lignin, and therefore the monolignol composition is different types of lignin may thus not only influence the covalent pattern, but also the physical properties of the wood. It shall, however, be underlined that these studies are only theoretical, and the conclusions must therefore be regarded as preliminary.

6.7 Further Reading

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7 Wood Extractives

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7.1 Background

Wood extractives are compounds with low molecular mass that are extractable from wood. In contrast to the structural polymers of wood, i.e. cellulose, hemicelluloses and lignin, their composition varies considerably between tree families and genera. Some extractives play a role in the metabolism of the living cells (the parenchyma cells) in the tree. Other extractives are produced to protect the tree against fungi and insects. The total amount of extractives is normally only a few percent of the wood, but it can be considerably higher in parts like bark and branches and is normally also increased in wounded wood.

Wood extractives have mainly been studied by pulp and papermakers because they cause problems in the production process and also influence several paper properties. Some extractives have also been identified as main contributors to the toxicity of untreated effluents. Lipophilic extractives are difficult to remove in the pulp washing line and they often form sticky deposits on process equipment, e.g. screens and wires, and may give rise to spots in paper. Many extractives are surface-active compounds and in paper they affect the surface properties, such as the binding between fibres, the water adsorption and friction. Further, the smell of the paper, especially important in food contact applications, is affected by the extractives. Also foaming in process liquors is a problem often connected with extractives.

In the kraft process the main part of the wood extractives are dissolved in the black liquor and either burnt to give energy or separated, and used as a source for production of speciality chemicals. Especially during kraft pulping of pines the gases released from the digester are condensed and a turpentine fraction can be separated from the aqueous distillate. Further, the removal of the sodium salts of fatty and resin acids from the black liquor followed by acidification gives tall oil, which is refined into a number of commercially important products, like paper sizes, adhesives, and ink and paint ingredients.

7.2 Definitions

Wood extractives can be defined as the chemical compounds that are extractable from wood with various neutral solvents. That means that the solvent and the extraction procedure used must be specified, since these variables will affect the yield and the composition of the obtained extract. Naturally, the composition of a water extract will be very different from that of a hexane extract. However, since the water soluble extractives, such as sugars, lignans and other phenolic compounds, normally are of less importance during pulp production, these compounds will only be mentioned briefly in this chapter. The main focus will be on the lipophilic part of the extractives, often referred to as wood resin.

Wood resin can be defined as components that are soluble in liquids of low polarity, e.g. hexane or diethylether. Such an extract will contain the following four main classes of lipophilic components:

1. Fats and fatty acids
2. Steryl esters and sterols
3. Terpenoids including terpenes and polyisoprenes
4. Waxes, i.e. fatty alcohols and their esters with fatty acids

At present the standard method for determination of extractives in wood and pulp is based on acetone extraction. Acetone is a good solvent for wood resin, but it also dissolves lignans and monosacharides to some extent. Previously dichloromethane (DCM) was used for the determination of wood extractives. The gravimetrically determined amounts of extractives will be higher with acetone compared to DCM.

Another aspect that may need to be considered is the volatile part of the wood resin, such as the monoterpenes. Volatile compounds will evaporate together with the solvent during the drying of the extract and will not be included in the extract weight. The volatile wood resin components are also mainly lost during chip storage and the pulping procedure. Deposits in the mills and specks in pulp and paper that contain wood resin components are often called pitch. Measures to minimize such problems are referred to as pitch control.

7.3 Amounts and Variations in Different Species

The content of wood resin and its composition varies significantly between different wood species, *Table 7.1*. There is also a variation between individual trees of the same species, depending on the age of the tree, genetic factors and growth conditions e.g. climatologic and geographic factors. Within a single tree there is also a variation between different parts, higher in stump and often also in top and branches compared to the stem. There is also a difference between the sapwood and heartwood. Generally a more slowly growing individual has a higher resin content.

Table 7.1. Examples of diethyl ether extract in some wood species [1].

Species	%
Pine (Pinus sylvestris)	2.5–4.8
Spruce (Picea abies)	1.0–2.0
Birch (Betula pendula)	1.1–3.6
Aspen (Populus tremula)	1.0–2.7
Beech (Fagus grandiflora)	0.3–0.9

One example of how the extractive content can vary within a single tree is given in *Figure 7.1*. The figure shows the extractive content at three different heights of a birch tree. At each height, samples were taken from the inner, middle at outer parts of the trunk. As seen the extractive content in the top of the tree is the highest. Further, at all levels the extractive content increases from the cambium to the pith.

Northern hardwood species such as birch and aspen usually have a larger resin content than southern hardwoods, which to some extent, store starch instead of fatty materials in their parenchyma cells. Both the fatty material (the glycerides) and the starch are partly considered as a reserve food supply for the living tree, i.e. the sapwood. Consequently the amount of glycerides in the heartwood is very low.

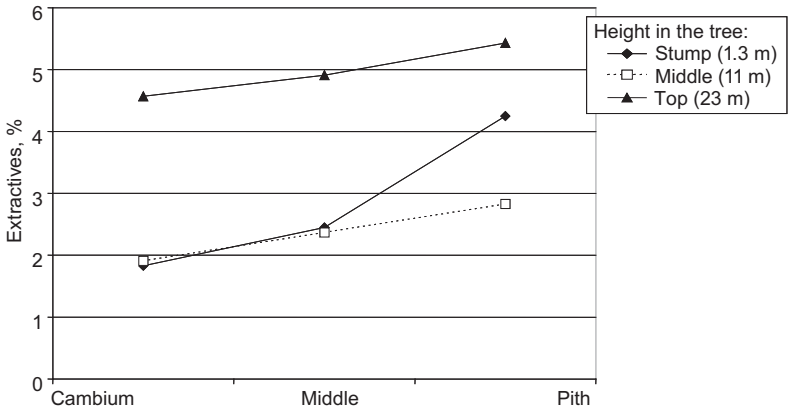

Figure 7.1. Acetone extractives across the stem wood of a 73-years old birch tree (*Betula pubescens*) determined at tree different heights [2].

7.4 The Chemistry of Wood Resin

Wood extractives can chemically be classified into several different groups of compounds. The main constituents can be divided in aliphatic compounds, terpenes and phenolic compounds. Of these the phenolic compounds are more soluble in water and are normally not included in wood resin.

7.4.1 Aliphatic Compounds

The main aliphatic compounds are fatty acids and fatty alcohols, but also hydrocarbons, such as n-alkanes, are present. The fatty acids are in the growing tree mainly present in the form of esters, either esterified with glycerol in the form of mono-, di-, and triglycerides (fats) (cf. *Figure 7.16*) or as esters with fatty alcohols (waxes) or sterols (steryl esters). The fatty alcohols are also present as free alcohols. The dominating fatty alcohols have an even number of carbon atoms, usually 18 to 24.

The dominating fatty acids in most wood species also have an even chain length of 16 to 24 carbon atoms, (denoted C16 to C24), but acids from C10 to C28 can be found. Also some branched chain acids, such as anteisoheptadecanoic acid, are often present. Normally the unsaturated C18 acids, such as oleic, linoleic, and linolenic acid are the main constituents. In pine and spruce an isomer to linolenic acid, called pinolenic acid is one of the major fatty acids. The names and structures of the most common fatty acids are given in *Table 7.2* and *Figure 7.2*. *Table 7.3* gives an example of the fatty acid composition in spruce, pine and birch wood. The unsaturated C18 acids linoleic, oleic and linolenic (in pine and spruce pinolenic) clearly dominates and make up between 75 and 85 % of the fatty acids. The saturated acids in this example only make up about 3 % in pine and 10 % in spruce. Birch and other hardwoods often contain more saturated acids, in this example 27 %.

Table 7.2. The most common saturated fatty acids in wood.

Systematic name	Name	Formula
Dodecanoic acid	Lauric acid, C12:0	$C_{11}H_{23}COOH$
Tetradecanoic	Myristic, C14:0	$C_{13}H_{27}COOH$
Hexadecanoic	Palmitic, C16:0	$C_{15}H_{31}COOH$
Oktadecanoic	Stearic, C18:0	$C_{17}H_{35}COOH$
14-methylhexadecanoic	Anteisoheptadecanoic, C17:0 a.i.	$C_{16}H_{33}COOH$
Eicosanoic	Arachinic, C20:0	$C_{19}H_{39}COOH$
Docosanoic	Behenic, C22:0	$C_{21}H_{43}COOH$
Tetracosanoic	Lignoceric, C24:0	$C_{23}H_{47}COOH$
Hexacosanoic	Cerotic, C26:0	$C_{25}H_{51}COOH$

Table 7.3. Example of fatty and resin acid composition in pine (*Pinus sylvestris*), spruce (*Picea abies*) and birch (*Betula pendula*).

Fatty acid	Pine wood [1] (% of total fatty acids)	Spruce wood [1] (% of total fatty acids)	Birch wood [2] (% of total fatty acids)
Saturated:			
Palmitic (C16:0)	1.0	3.6	9.2
17:0ai*	0.8	3.0	n.d.
Stearic (C18:0)	<0.2	0.6	4.9
Lignoceric (C24:0)	<0.2	<0.2	8.1
Other saturated*	0.9	2.8	5.3
Unsaturated:			
11-18:1	0.5	2.0	n.a.
Oleic	35.3	25.0	5.6
Linoleic	40.5	36.4	59.0
Linolenic	0.8	0.9	1.3
Pinolenic	10.6	14.9	0
20:1	<0.2	<0.2	4.7
5,11,14-20:3	4.6	3.4	n.d.
Other unsaturated**	3.5	4.6	n.d.
Other minor < 0.2 % each	1.5	2.8	1.9
Sum	100	100	100
Resin acid	Pine wood (% of total resin acids)	Spruce wood (% of total resin acids)	Not present
Pimaric	8.1	6.2	–
Sandaracopimaric	1.6	6.4	–
Isopimaric	3.5	13.3	–
Levopimaric	30.0	16.2	–
Palustric	15.1	13.5	–
Abietic	15.8	11.2	–
Neoabietic	11.1	10.2	–
Dehydroabietic	14.4	22.6	–
Other minor	0.4	0.4	–
Sum	100	100	–

* a.i. (anteiso) = branched (methyl group) at the carbon atom n-2. ** < 1.0%
[1] Reference 3. [2] Compilation of data from different sources

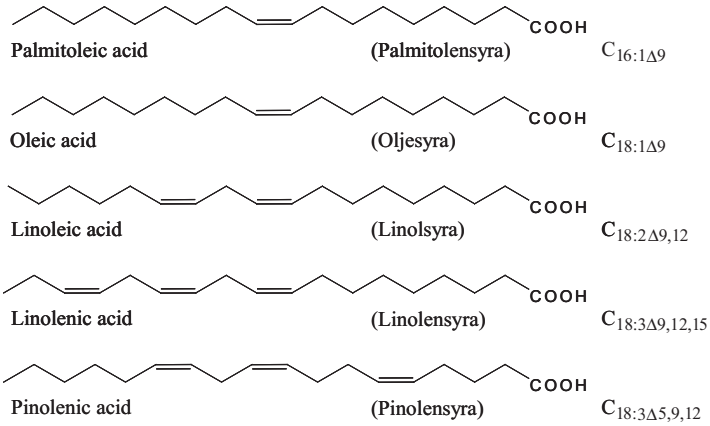

Figure 7.2. Common unsaturated fatty acids in wood. Δn means that the acid has a double bond between the nth and the $(n+1)^{th}$ carbon atom.

7.4.2 Terpenes

The terpenes constitute a variety of mainly cyclic compounds, which are present in most plants and animals. They are all built up from isoprene units (5 carbons) and the main groups are monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20) and triterpenes (C30), as illustrated in *Figure 7.3*. Oxygen-containing terpenes are often called terpenoids. Sterols comprise a special group with a tetracyclic ring system, same as sitosterol (see examples in *Figure 7.6*). In most wood sterols the number of carbon atoms are between 28 and 32.

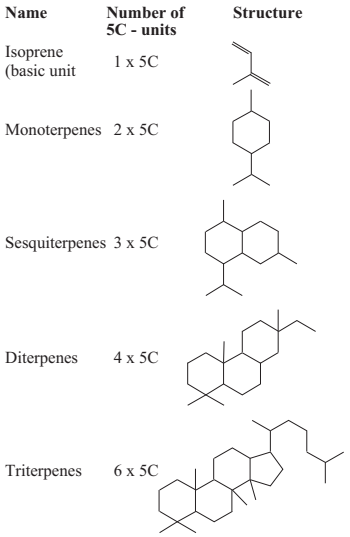

Figure 7.3. Basic structure of the various terpenes.

In wood there is a clear difference in the terpene composition between hardwoods and softwoods. Whereas the softwood terpenes include both mono-, sesqui- and diterpenes together with sterols, the hardwoods mainly contain sterols, triterpenoids and higher terpenes, such as polyprenoles. The mono- and sesquiterpenes are very characteristic compounds in the softwood resin, giving the typical aroma of "pine forest". Chemically they are most often hydrocarbons or alcohols. Especially the monoterpenes are volatile and neither mono- or sesquiterpenes are, due to their volatility, not present in kraft pulps. Also in extracts from wood they are most often evaporated during the drying of the extract and therefore not included in the extract weight. In the kraft process, the mono- and sesquiterpenes are often collected as a turpentine fraction from the digester. Examples of typical mono- sesqui- and diterpenes are shown in *Figure 7.4*.

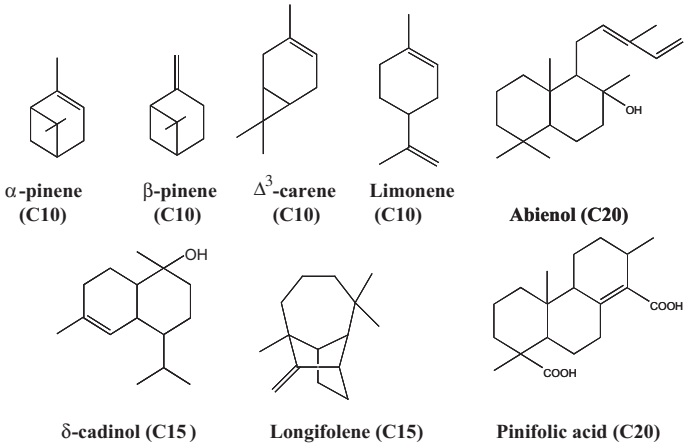

Figure 7.4. Examples of mono-, sesqui- and diterpenes/terpenoids.

A dominating group in softwood resin are the resin acids, which constitute a group of tricyclic diterpenoic acids with similar structures, *Figure 7.5*. An example of the resin acid composition in pine and spruce wood is given in *Table 7.3* above. There are also other diterpenoids such as alcohols and aldehydes present, but in lower quantities. The resin acids are the compounds causing the main part of the toxicity in effluents from mechanical pulping. Oxidised resin acids have also been shown to be allergenic.

In kraft pulping the resin acids are dissolved in the alkaline black liquor in the form of soluble sodium soaps and together with the fatty acids they are often separated as a soap phase, called tall oil soap.

Triterpenoids are most often found in hardwood resin. For producers of birch pulp especially betulinol from the birch bark is often seen as a major problem due to its tendency to form deposits in the mill. Betulinol is a white, crystalline compound present in up to 30 % in the outer layers of birch bark.

The sterols are closely related to the triterpenes, but they are found in both softwoods and hardwoods. The main wood sterol is sitosterol (in older literature often referred to as β-sitosterol). Some common wood sterols are presented in *Figure 7.6*. Sitosterol and campesterol are structurally very close to cholesterol, one of the main sterols in humans and animals, which is also present in wood in low quantities.

Abietic acid type

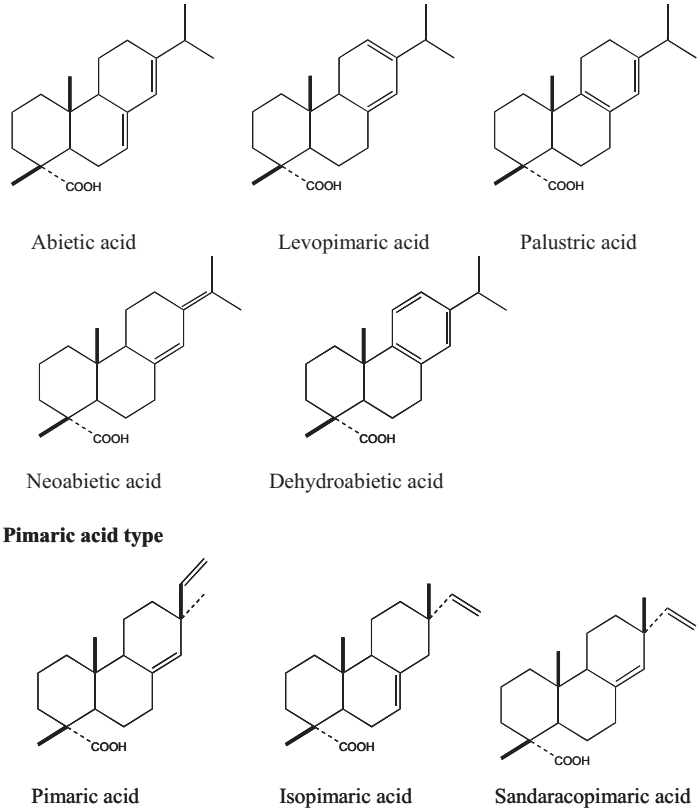

Figure 7.5. The most important resin acids, devided in abietic and pimaric acid type, found in pine and spruce.

In some wood species also terpenes of higher molecular mass are present. The most well-known is rubber (cis-polypren) and gutta (trans-polypren). Betulaprenols, present in birch wood, is another example of polyterpenes (*Figure 7.7*).

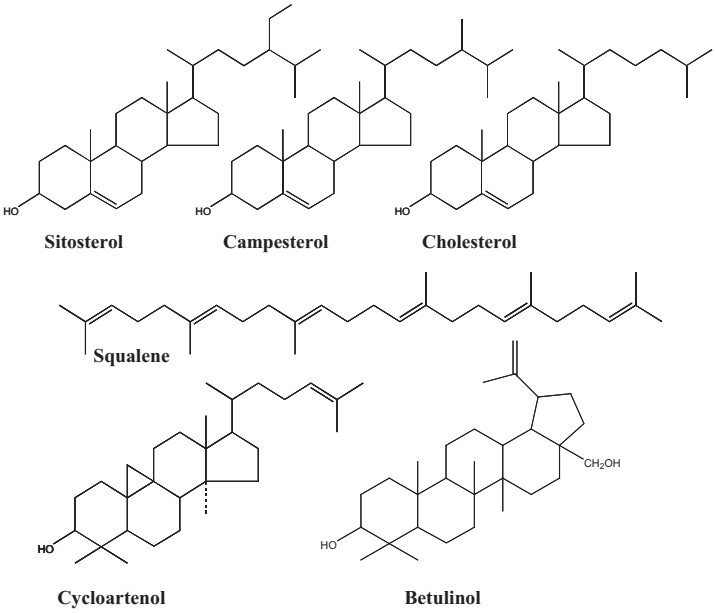

Figure 7.6. Examples of sterols and triterpenes.

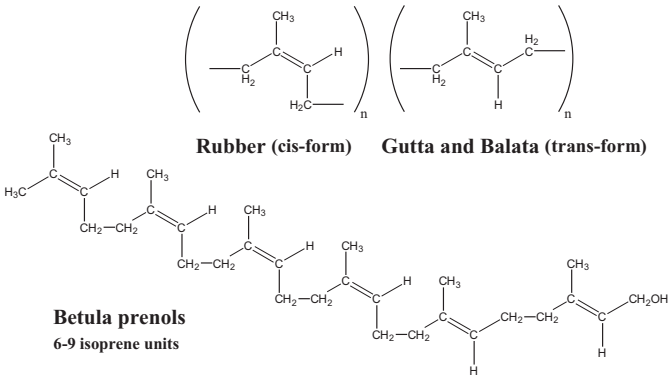

Figure 7.7. Examples of polyterpenes in wood.

7.4.3 Phenolic Extractives

Some groups of phenolic extractives are illustrated in *Figure 7.8*. The phenolic compounds are usually only found in the heartwood and in the bark, where they act as fungicides. (See also Part 7.6.) Since most of these compounds are water soluble, they are removed in the cooking stage and thus not present in chemical pulps. However, they can be found in acetone extracts from

wood and mechanical pulps. Together with the resin acids the phenols are responsible for the main toxicity in effluents from mechanical pulping.

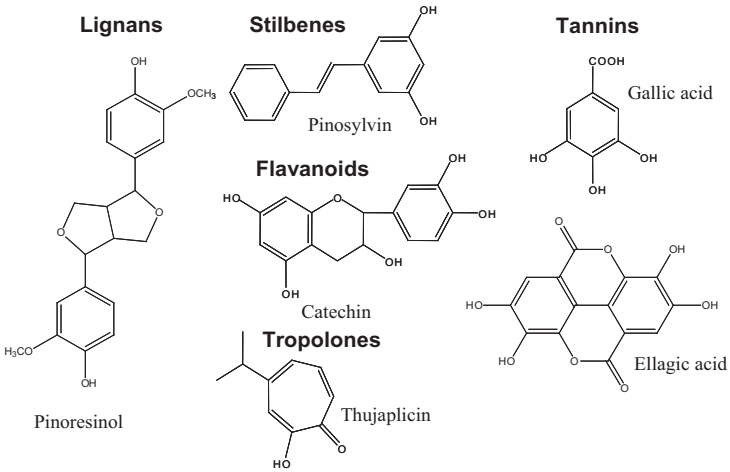

Figure 7.8. Representatives of different groups of phenolic extractives in wood.

7.5 Location in Wood

7.5.1 Canal Resin and Parenchyma Resin in Sapwood

In wood the resin is mainly located in the parenchyma cells and, in softwoods, also in the so called resin canals. There are fundamental differences between canal resin and parenchyma resin and also between the resin in sapwood (the living wood) and in heartwood, both with regard to physical accessibility and chemical composition. Resin canals are formed as interspaces between the fibres both vertically and horizontally in most pulpwood conifers, *Figure 7.9*. This means that the resin inside these canals is physically freely exposed when the fibres are separated during pulping. Resin canals are most frequent in pine (*Pinus*) but are also present in e.g. spruce (*Picea*) and larch (*Larix*) and some other softwood genera. Similar canals exist also in some tropical hardwoods.

The resin in the resin canals serves mainly as wound secretion material to protect the tree. The resin in the parenchyma cells on the other hand, is believed to take part in the metabolism of the cell. Thus the chemical composition of the canal resin in the conifers differ from that of parenchyma resin. Canal resin generally consists of an amorphous mixture of terpenes and terpenoids. The canal resin in pine and spruce typically consists of free resin acids (90 %) and other diterpenoids (10 %) dissolved in a monoterpene fraction containing also a small amount of sesquiterpenes to give the mixture the right viscosity. When the tree is wounded the resin flows out and covers the wound and the monoterpenes evaporate. Especially pines have been used for tapping of canal resin (in this context also called rosin), which is then used as a base for production of special chemicals.

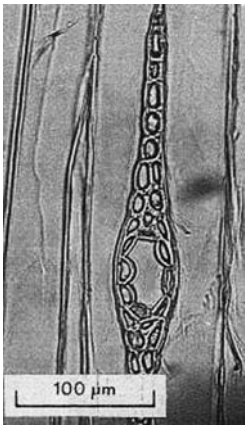

Figure 7.9. Resin canal with surrounding parenchyma cells [4].

In contrast the parenchyma resin in sapwood contains mainly fats and steryl esters. The fats are mainly triglycerides, but also diglycerides are present. Other components, such as the betulaprenoles in birch, may also be present. The fat is believed to constitute reserve energy for the cells, whereas the steryl esters play a role in the function of the living cell.

There are obvious differences between the parenchyma resin and the canal resin with respect to their behaviour in pulping processes. After acid (sulphite) or alkaline (kraft) pulping, or after a mechanical defibration process, the canal resin is physically exposed. Even if the resin is still undissolved, its surface is easily accessible for chemical reactions. This may lead to pitch problems with deposition but may also facilitate deresination. The parenchyma resin, on the other hand, is still protected within the parenchyma cells, unless these have been broken. The dissolution of resin from parenchyma cells thus requires the diffusion of dissolved resin material out of these cells.

In the sapwood of northern hardwoods, e.g. birch and aspen, all the resin occurs in the parenchyma cells. These parenchyma cells make up a considerable part of the hardwood volume. For example rays, consisting mainly of parenchyma cells, constitute from 10 % of the wood volume in Betula species (birch) up to about 40 % for certain Quercus species (oak).

The parenchyma cells both in softwood and hardwood also have a higher lignin content than the tracheids, which in combination with their resinous material, makes them more resistant to pulping conditions. As an example, the wood of *Picea abies* holds 3 % by weight of ray parenchyma, with a lignin content of 43 %, compared with a lignin content of 26 % in the tracheids.

Of importance for deresination is also the structure of the ray parenchyma pits. The pronounced difference in this respect, between Pinus with a pit opening of about 20 µm and Picea with 2–3 µm is thought to be one of the reasons for the more difficult deresination of spruce wood pulp compared to pine. In production of mechanical pulps most of the parenchyma cells are broken.

7.5.2 Heartwood

In heartwood, not only the tracheids (fibres), but also the parenchyma cells are dead. The moisture content of the heartwood is much lower than in sapwood, making the wood more vulnerable to fungi attacks. Therefore, in some species, during heartwood formation (before dying), the parenchyma cells greatly increase their production of resin and/or phenolic compounds, and in many species the heartwood can be visibly identified by a change in colour, originating from polyphenols formed. The parenchyma resin is also chemically changed during the heartwood formation. The reactions taking place are similar to those that occur during wood seasoning. The main reaction is that fats and other esters are hydrolysed, forming free fatty acids and sterols.

7.5.3 Heartwood in Softwoods

During heartwood formation, the extractives partly become distributed throughout the wood structure. Thus, in the heartwood of softwoods, wood extractives also exist in normal tracheids and contribute to the clogging of the bordered pits. The increase in resin content in heartwood compared with sapwood is about 100 % or more for several Pinus species and thereby the microbiological resistance of the wood increases. The heartwood extractives of pines also include special fungicide phenolic substances such as pinosylvin (see *Figure 7.8*). *Figure 7.10* shows the radial variation of wood extractives in *Pinus sylvestris*. On the other hand for some genera, e.g. spruce, the formation of heartwood is not accompanied by an increase in wood resin. As an example the composition of heartwood and sapwood resin in *Picea abies* is given in *Table 7.4*.

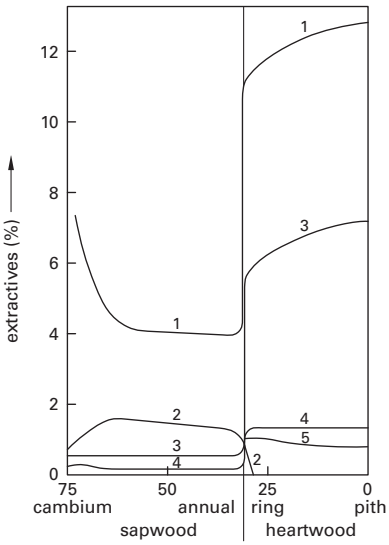

Figure 7.10. Distribution of wood resin components groups across the stem wood of a 75-years old Scots pine tree. 1 = Total, 2 = fats, 3 = resin acids, 4 = fatty acids, 5 = pinosylvin [1].

Table 7.4. Petroleum ether extractives from *Picea abies* in mg/g dry wood [5]. Analysis of one tree.

Component	Sapwood mg/g wood	Heartwood mg/g wood
Fatty acids	6.77	3.29
– triglycerides	5.54	1.67
– mono- and diglycerides	0.57	0.80
– free	0.66	0.53
Resin acids	1.21	0.95
Sterols	1.00	0.94
free	0.20	0.29
esterified	0.80	0.65
Triterpene alcohols	0.13	0.13
free	< 0.01	0.02
esterified	0.13	0.11
Diterpene alcohols + aldehydes	0.40	0.29
Alkyl ferulates	0.12	0.19
Glyceryl residues	0.36	0.22
Total	10.0 (1.0 %)	6.0 (0.6 %)

7.5.4 Heartwood in Hardwoods

Hardwoods produce heartwood in two different ways. Either by clogging of the vessels with tyloses or by resin secretion into the vessels. Tyloses are formed by the walls of the parenchyma cells, which are expanding balloon-like. *Figure 11* illustrates the heartwood formation in hardwoods. The aim in both cases is to protect the wood against fungi attacks through the vessels, which would otherwise be easily accessed when the moisture content in the wood is reduced. There seems to be a connection between the pit size in the parenchyma cells and the way the heartwood is formed. Thus, tyloses form when the size of the pits exceeds 2–5 μm. Genera forming tyloses are e.g. aspen (Populus), beech (Fagus) and most eucalypts (Eucalyptus). Among the pulpwood genera that forms heartwood by secreting resin are birch (Betula) and Acacia.

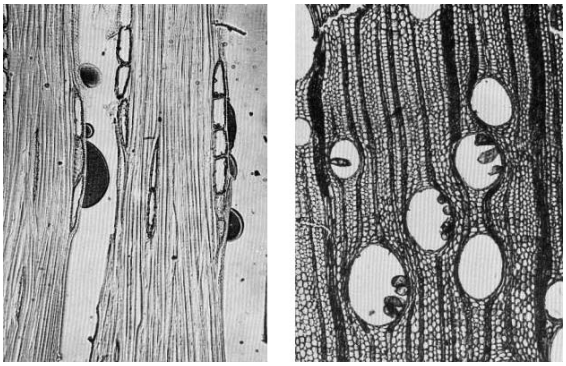

Figure 7.11. Heartwood formation by resin secretion (left) and by formation of tyloses (right) [4].

7.6 Extractives in Bark

Bark can morphologically be divided in inner and outer bark. The composition of the extractives is often very different. Generally the resin content in bark is higher than in wood, especially for species forming a smooth bark, such as birch and aspen. A clean debarking is thus important for deresination. Below some typical bark extractive components are described.

7.6.1 Suberin

A typical component in bark is suberin. It is found in most types of bark, and especially the outer bark of birch. It is a polymeric substance and its structure is still not fully identified. A proposed structure is shown in *Figure 7.12*. Suberin is built up by an aromatic matrix, similar to lignin, which is cross-linked with waxy, aliphatic components. The dominating aliphatic monomers are ω-hydroxy acids and dicarboxylic acids (C16-C22) and long chain fatty acids and alcohols (C20-32). The phenol composition of the polymer is still not fully characterized, but one component identified in several species is ferulic acid.

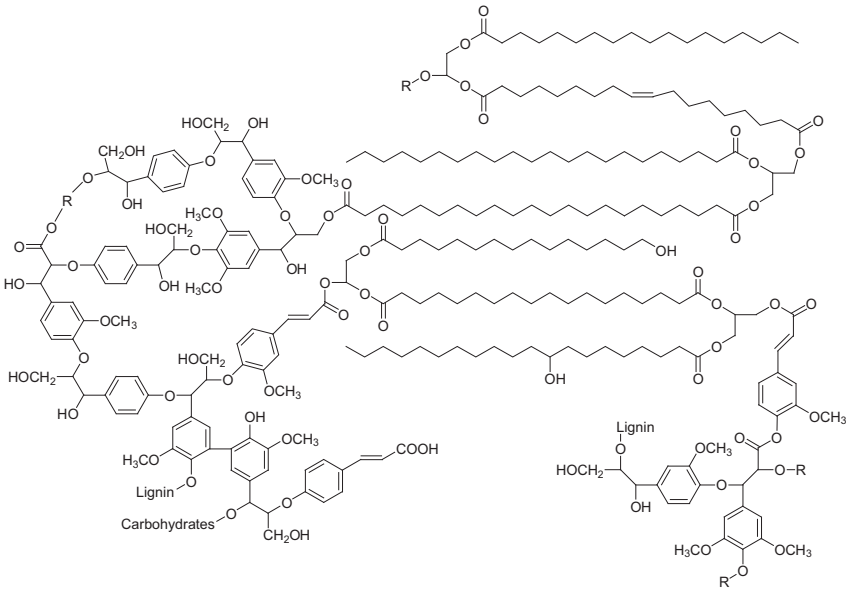

Figure 7.12. Proposed structure for suberin.

7.6.2 Tannins

One important group of phenolic extractives in bark and also in the heartwood of some species is the tannins. These are of two types, the hydrolysable tannins and the condensed tannins. One characteristic of the tannins, that has been known for centuries, is their ability to interact with

proteins. Still, one of the most important commercial applications for wood extractives is the use of tannins in leather manufacture, even though the use of natural vegetable tannins today is decreasing. The hydrolysable tannins are polymers of gallic or ellagic acid esterified to a core molecule commonly glucose or a polyphenol such as catechin (cf. *Figure 7.8*). This type of tannins is also found in Eucalypt heartwood, and is reported to give rise to manufacturing problems, e.g. dark colour of the pulp and deposits on metal surfaces. The condensed tannins were not chemically characterized until the beginning of the 1980s. They are so called proanthocyanidines, which comprise a group of oligomeric flavanoids found in many plants but also in vegetables (*Figure 7.13*).

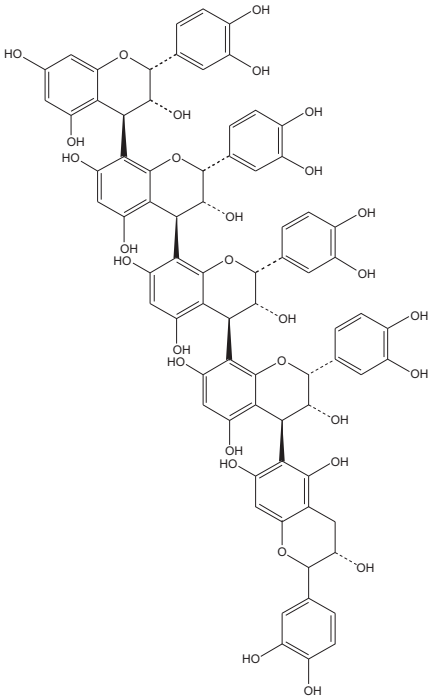

Figure 7.13. Example of structure of condensed tannin. In this case built up by five catechin units.

7.6.3 Betulinol

Of special importance in Scandinavia is the very high triterpene content of birch bark. In the outer bark of *Betula pendula*[1] and several other Betula species, 25–35 % of betulinol has been found. The average resin content in birch bark is 10–12 %. Betulinol (cf figure 6) is crystalline, with a melting point of 242 °C. It is betulinol that gives birch bark the white colour. It is alkali insoluble and therefore very difficult to remove by alkaline pulping. Since the wood never is

[1] Swe. = vårtbjörk; earlier often called *Betula verrucosa*

completely debarked, substantial amounts of betulinol enter the pulp mill. Betulinol is normally the most dominant component of birch pitch deposits, although it is normally not found in birch wood. In other species such as pines the resin in bark is mainly made up of the same compounds as in the wood. That is, in pine bark diterpenoids and monoterpenes dominate.

7.7 Wood Seasoning

It is well established that wood seasoning reduces pitch problems, especially in sulphite and mechanical pulps, and 30 year ago it was still a normal procedure to store the wood as logs or chips before using them in the process. However, in kraft pulping, the effect is small and since storage is economically unfavourable, most mills now operate with only a small but sufficient wood buffer. Seasoning binds capital and also the general loss in total wood weight due to microbial activity must be considered.
The main reactions during wood storage are

1. Oxidation of wood resin, partly due to autoxidation and partly due to metabolic reactions, i.e. a continuation of the life functions in the still living parenchyma cells.
2. Glycerides and other esters are hydrolysed
3. Volatile components are lost
4. Microbial degradation

Alltogether these reactions lead to a decrease in the total amount of wood resin.

The oxidation of especially unsaturated compounds introduces more hydrophilic groups, thereby making the wood resin more hydrophilic. The oxidation processes can be followed by measuring the production of CO_2. The hydrolysis of the glycerides and other esters is thought to be due to the activity of enzymes, such as lipases. During the first month of chip seasoning a decrease in the bound fatty acids of 70 % has been reported. When wood is stored in the form of logs and especially if these are stored in water, the above-mentioned reactions are strongly suppressed by the lack of oxygen and by low temperature. In a chip pile the temperature is substantially raised by the heat from oxidation processes.

The decrease in total wood resin content and the hydrolysis of fats and especially steryl esters and waxes will facilitate the deresination, especially for sulphite pulp but also for spruce kraft pulp. For pine kraft pulp no such positive effect by chip storage is seen, probably since pine resin is initially low in waxes. Both the turpentine and the tall oil yields are substantially decreased when wood is stored as chips. The turpentine decrease is mainly due to a loss of monoterpenes to the atmosphere.

7.8 Wood Resin in Pulping and Bleaching

7.8.1 Solution Properties of Resin and Fatty Acids

Resin and fatty acids are examples of amphiphilic compounds, often called surfactants. They are built up by a hydrophilic, polar carboxyl group and a hydrophobic, nonpolar hydrocarbon moiety. The undissociated fatty acids have a very low solubility in water. However, at higher

pH, they dissociate, and in kraft cooking they are present mainly in the form of soluble sodium soaps. The solubility of the sodium soaps of fatty and resin acid in water is characterized by a sharp increase in solubility at a certain temperature, called the Krafft temperature or the Krafft point. At temperatures below the Krafft point, the fatty acids are mainly in the form of monomers, but at this point they start to aggregate with formation of micelles and the solubility increases dramatically. The concentration at the Krafft point is often referred to as the critical micelle concentration (cmc). Cmc decreases with an increased length of the fatty acid chain (decreased water solubility) and increases with the number of double bonds in the chain (increased water solubility). The micelles can be described as spherical aggregates built up by a large number of soap anions, arranged so that the hydrophobic carbon chains are located inside the sphere, while the carboxyl groups form the surface of the sphere in contact with the water (*Figure 7.14*).

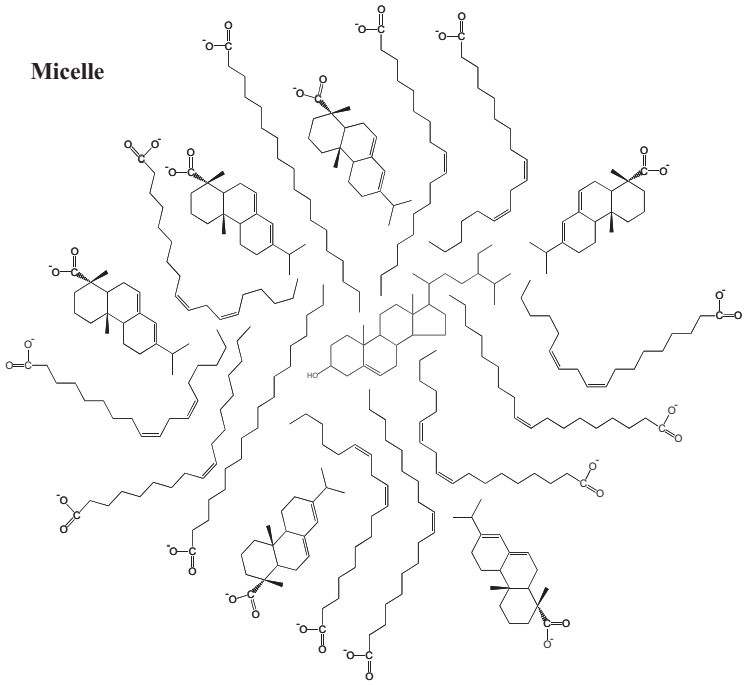

Figure 7.14. Illustration of the function of a mixed micelle with resin and fatty acids, having a solubilized sterol in the interior.

In a micellar solution there is a dynamic equilibrium between compounds dissolved as monomers and the different aggregates present. Mixtures of different fatty acids and resin acids form mixed micelles, and the cmc will be influenced by the different compounds in the mixture. The solubility is also strongly influenced by the ionic strength of the solution. As shown in *Figure 7.15*, the solubility in pure water is about 18 % for both sodium abietate and sodium oleate. For a mixture of abietic acid:oleic acid (1:1) the solubility increases to 40 %. However, when salt is added to the solution, the solubility of the mixture falls rapidly at a sodium chloride con-

centration of about 0.8 M. At this ionic strength the solubility of the micelles is dramatically reduced and the micelles separates from the liquor, as a so-called liquid crystalline phase, a soap phase is “salted out”.

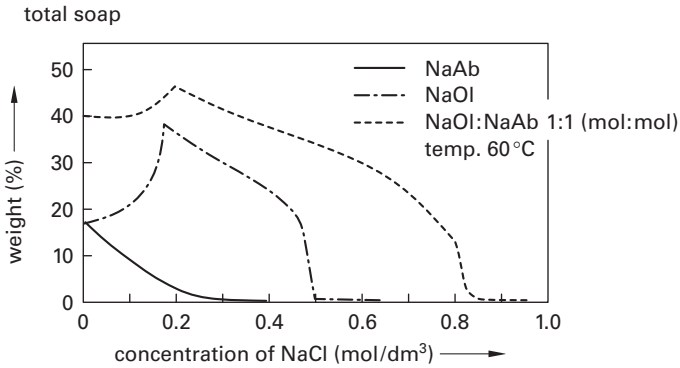

Figure 7.15. Solubility of oleic and abietic acids alone and in mixture at 60 °C as a function of NaCl concentration [6].

Of importance for the deresination of kraft pulps is also the capability of the soap micelles to solubilize otherwise water-insoluble hydrophobic substances inside the micelles. This is the principle of most washing, and this makes it possible to dissolve and remove also water-insoluble extractives. In contrast to the sodium soaps, calcium soaps are very insoluble in water. As an example the solubility product, pKs, in pure water, for calcium palmitate is 17.4 and for calcium oleate 15.4.

7.8.2 Resin in Kraft Pulping and Washing

During kraft pulping, the free fatty and resin acids are dissolved as soaps in the alkaline cooking liquor. Furthermore, the triglycerides are hydrolysed in the alkaline liquor and the formed fatty acids dissolved. In *Figure 7.16*, the hydrolysis of a triglyceride is illustrated.

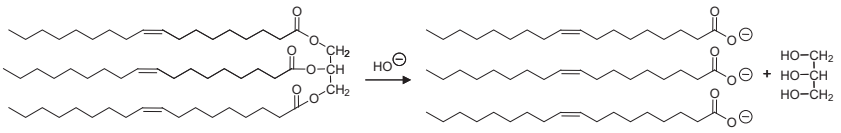

Figure 7.16. Hydrolysis of a triglyceride, triolein, to free oleic acids and glycerol.

Also the main part of the steryl esters and the waxes are hydrolysed, but the hydrolysis rate is lower than for the triglycerides and some esters may remain intact after the cook. The free sterols and alcohols formed from these esters have in contrast to the fatty acids a low solubility in the cooking liquor, but they may be solubilized within the micelles formed by the dissolved resin and fatty acids. It has been shown that mixed micelles from fatty and resin acids have a higher capacity to solubilize the neutral compounds than micelles of fatty or resin acids alone.

Furthermore, at cooking conditions, some of the unsaturated fatty acids will become isomerised to acids with conjugated double bonds, mainly with cis-trans configuration. The main change in the resin acid composition is a partial isomerization of levopimaric acid to abietic acid.

After the cook, most of the black liquor, containing the dissolved lignin and the cooking chemicals, is separated from the pulp. The hot black liquor also contains most of the wood resin and later, in the evaporation stages, when the concentration and thereby the ionic strength increases, a soap layer (= liquid crystalline phase) is formed, that can be separated. After acidification, the soap is called tall oil. Today, some mills do not separate the tall oil soap but burn it in the recovery boiler.

In the next step, the pulp is washed in order to separate the remaining black liquor. Important for a good deresination is the combination of temperature, ionic strength and pH in the washing line. The solubility in the black liquor decreases when the liquor is cooled down and therefore washing at higher temperatures, such as in a pressurized diffuser at about 110°C, is regarded as more favourable for deresination.

Most mills also have an oxygen delignification stage after the cook and this stage is normally very efficient in decreasing the extractives content. Two examples for TCF (totally chlorine free) bleaching sequences are given in *Figure 7.17*. As can be seen, very little happens to the extractives content in the following bleaching stages. When chlorine is used for pulp bleaching (not used in Scandinavia today) several of the unsaturated wood resin components will become chlorinated.

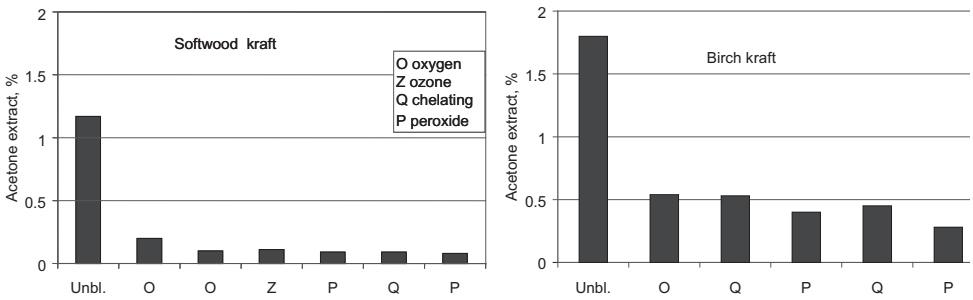

Figure 7.17. Example of extractive content in unbleached pulp and pulp from different bleaching stages for a TCF bleached softwood kraft (OOZPQP) and a birch kraft pulp (OQPQP).

For kraft pine pulps the relation between fatty and resin acids and neutral substances is such that the formed micelles are capable of solubilizing the main part of the neutrals, giving a very efficient washing. For birch kraft pulps, especially for fresh wood, the relation between acids and neutrals is much less favourable. Furthermore, no resin acids are present in the birch extract (*Figure 7.18*). Spruce resin has a composition between pine and birch. In practice, when comparing pine and spruce pulps, this means that even though the total extractives content in pine wood is much higher than in spruce wood, normally the resin content in the pulp after washing is lower for pine kraft pulp than for spruce pulp. Another factor affecting the much easier deresination of pine pulp compared to spruce pulp is the parenchyma larger pit size and the larger relative pit area in the walls of the parenchyma cells.

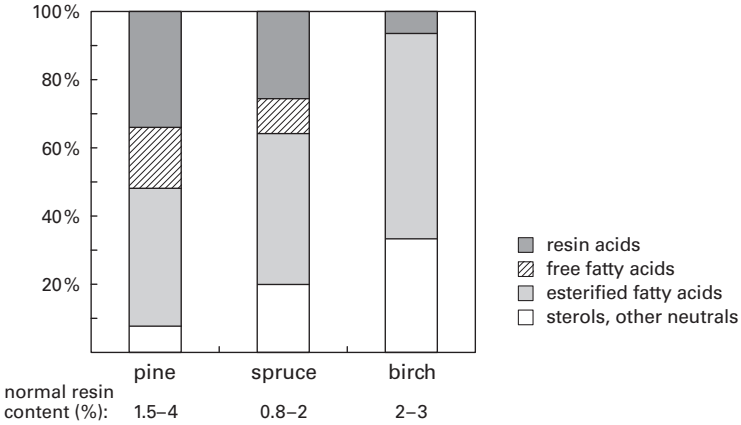

Figure 7.18. Composition of the diethyl ether extract in pine, spruce and birch wood.

The resin composition in birch pulp has an even more unfavourable composition than in spruce. Thus, in order to improve the washing of birch pulp it is a normal procedure to add tall oil soap to the cook. Additions of 1–3 % on dry wood are used. The soap contains mainly fatty and resin acids that will form micelles capable of solubilizing the high amount of neutrals in the birch extract. Even with the addition of tall oil, the resin content in birch pulps will be higher than in spruce and especially pine pulps. Bleached softwood kraft pulp normally has an extractives content between 0.05 and 0.15 %, whereas bleached birch kraft pulp varies from 0.2 to 0.8 %. The higher value is typical for a birch pulp produced without addition of tall oil. For birch pulps, the debarking is also very important since betulinol from the bark is difficult to remove in the pulp washing.

Calcium ions, mainly from the wood, play an important role for the behaviour of wood resin during washing. Calcium is, after potassium, the most frequent metal ion in wood, and pulpwood normally contains about 600 mg of calcium per kg. Insoluble calcium soaps often constitute a major part of the pitch deposits in the washing and screening departments.

During kraft pulping, the calcium ions take part in several equilibria:

- with carbonate
- with ionic groups on the fibre
- with fatty acids
- with lignin

Most of the carbonate ions enter with the white liquor, but some carbonate is also formed during cooking, and it has been shown that calcium carbonate precipitates out during a kraft cook. After the cook, most of the calcium follows the pulp, and not the black liquor. The main part of the calcium is bound to the ion exchanging groups of the fibres or adsorbed as calcium carbonate. However, a portion of the calcium is bound as calcium salts of fatty acids. The solubility of the calcium soaps is depending on the length and the number of double bonds in the carbon chain Long, unsaturated fatty acids form the most insoluble calcium soaps, and these fatty acids are washed out to a less degree, than acids of shorter chain lengths. The resin acids, which do not form calcium soaps during pulp washing conditions, are washed out very effi-

ciently. The dissolved kraft lignin also affects the deresination, possibly by acting as a dispersion agent. In laboratory experiments it has been shown that addition of kraft lignin inhibits the precipitation of calcium soaps.

7.8.3 Resin in Sulphite and Mechanical Pulping

The conditions during the classic acid sulphite and also during the bisulphite process are much less favourable for deresination compared to the kraft cook. Only a minor amount of the esters will get hydrolysed and neither the fatty nor the resin acids are soluble in the acid cooking liquor. Therefore, the deresination during sulphite pulping is mainly achieved by the dispersion of resin components by the lignosulphonates formed from the lignin. Also wood seasoning, leading to increased ester hydrolysis, is very beneficial for the deresination of sulphite pulps. The deresination is usually better when the pH is increased as in neutral sulphite (NSSC). Most of the deresination in sulphite pulping will be done during the washing and bleaching stages. Normally, bleached sulphite pulps have a slightly higher extractives content compared to kraft pulps, ~0.2 %, also after optimal deresination. Pine wood is normally not used for sulphite pulping, mainly due to the high extractives content and also due to the high content of pinosylvin in the heartwood. The pinosylvin will condense with the lignin during acid sulphite pulping and thus inhibit pulping of the heartwood.

During mechanical pulping most or all of the parenchyma cells will be broken thereby exposing the resin. However, since the pH is not alkaline enough for hydrolysis of the esters, the water solubility of the resin is low. The extractives content of spruce groundwood pulp or thermo mechanical pulp (TMP) normally is about 0.6–0.7 %. After peroxide bleaching this amount will decrease somewhat due to the alkaline washing. In contrary, the resin content of CTMP can be as low as in kraft pulps.

7.9 Effects on Paper Properties

Wood resin can affect paper properties in many different ways. Visible defects, such as spots and/or holes in the paper are often the main problem caused by extractives. But also properties that are related to the fibre surface energy will be affected. Thus, affects can be seen in widely separate areas such as e.g. binding between the fibres (paper strength), linting, hydrophobation and wetting, behaviour in printing and lamination processes, and friction. Other areas that are affected are pulp and paper odour, and in some cases optical properties.

7.9.1 Visible Defects

The cleanliness of pulp and paper is an important quality parameter. Pitch deposits in the process line may cause problems during the production process. The deposits may also come loose and cause spots or specks in the paper. Deposits can also cause streaks in paper, for instance if they occur on the wall of a head box. Another well recognized problem is holes or thin areas in the paper, which can be formed when resin is clogging a felt or a wire cloth, thereby affecting the dewatering of the web. The solution of the problem of specks in pulp and paper often in-

volves a chemical identification, and the deduction of its origin. Other sources, besides wood resin causing deposits in a mill may be present. Both process chemicals, e.g. defoamers, and paper additives, e.g. hydrophobation agents or latex may give rise to deposits. Today, techniques based on infrared spectroscopy, or on pyrolysis in combination with GC-MS, are the most efficient methods for identification of extractives in specks and deposits.

7.9.2 Surface Properties

In the dry sheet, the surface energy of the cellulose is higher than for the lipophilic extractives. This will lead to a redistribution of the extractives to cover the fibre surfaces, the driving force being to lower the surface energy. This autohydrophobation process is often referred to as "self-sizing". The effect can be noticed already a few hours after production and is naturally greater for paper made from pulps with a high extractives content, such as mechanical pulps. The process leads to a decreased wettability, which for paper qualities such as tissue has a large negative impact.

The layer of extractives on the paper surface may also affect the bonding of toner particles in printing papers made from mechanical pulps. Furthermore, in lamination of paper, e.g. with polyethylene (PE), a high surface energy is needed for good bonding. Therefore the paper surface is normally activated by oxidation, e.g. a flame treatment, immediately before the lamination. This treatment will destroy the surface layer of extractives. However, after the treatment the surface will again become covered by extractives if the paper is stored.

A high extractives content in the pulp also decreases the active bonding area between the fibres in the sheet and thereby the paper strength. An example is given in *Figure 7.19*, showing the tensile index and Scott Bond (z-strength) for a peroxide bleached groundwood pulp, as a function of the resin content before and after washing. The alkaline pH in the peroxide stage, about pH 10, is advantageous for the washing of extractives. A decreased degree of fibre bonding will also be seen as an increased tendency for linting, especially of the fines material. This is especially a problem in printing of papers made from mechanical pulp.

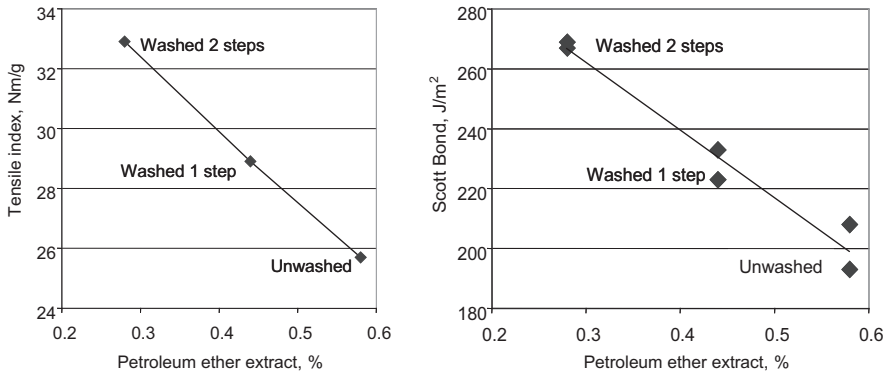

Figure 7.19. Tensile index and Scott bond as a function of the resin content in a peroxide bleached groundwood pulp before washing and after one and two washing steps [7].

Another property that is affected by extractives on the paper surface is the friction. Friction is important e.g. for packaging papers, such as sack paper and liner, where a low friction will increase the risk for sliding of sacks or boxes during storage or transport. Friction is also important for paper behaviour in copy machines, where a constant friction is desirable. One example of the effects exerted by fatty acids on friction is shown in *Figure 7.20*. It can be seen that saturated fatty acids with a chain length of more than 14 carbon atoms have a substantial effect in lowering the friction coefficients.

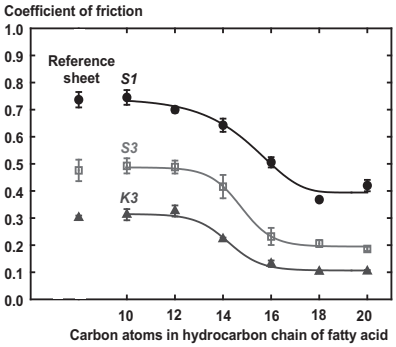

Figure 7.20. Coefficient of friction for paper impregnated with fatty acids of different chain lengths [8]. For paper the coefficient of friction will change with the number of slides. S and K stands for the static and kinetic coefficients of friction, respectively, and the number is the number of slides in the test.

7.9.3 Colour and Brightness Reversion due to Extractives

During ageing, pulp and paper may suffer from brightness reversion, i.e. a decrease in brightness or absolute colour, due to an increased light absorption. This has been blamed on almost every constituent present in the pulp or paper, including the extractives. However, the contribution from native wood extractives to the specific light absorption coefficient for wood and different unbleached pulps is usually insignificant. In general, wood extractives are non-coloured, but exceptions exist. Particularly in tropical species, extractives containing phenolic groups may contribute to the colour, especially in complexes with metal ions or after oxidation to quinone structures. Furthermore, lignans can be oxidised to coloured structures; however, phenolic extractives are normally easily removed during pulp washing. Metal soaps of fatty acids exhibit different colours depending on their counter ion.

Bleaching with chlorine leads to chlorination of aliphatic unsaturated extractives and it is well documented that chlorinated extractives contribute to brightness reversion of chlorine bleached pulp. Chlorinated extractives have been shown to have an indirect adverse effect on pulp brightness stability, by liberation of hydrogen chloride. The increased acidity promotes degradation of polysaccharides to coloured structures, which also constitute the main portion of the extractable material formed during ageing of chlorinated pulps.

7.9.4 Pulp and Paper Odour

Paper and board are used extensively as food packaging material, solely or in combination with other materials. In food packaging applications it is vital that the packaging material does not affect the odour or taste of the packed food. One well-recognized source of off-flavor in packaging papers is wood extractives or their degradation products. Among the compounds identified in the gas phase over a pulp sample are hydrocarbons, alcohols, aldehydes, esters and terpenes. Especially in the headspace of fresh TMP samples, mono- and sesquiterpenes can be found.

The concentration of terpenes in TMP-based board samples normally decreases during aging and, usually, the n-aldehydes (C5-C10) are dominating substances. Some pulp and paper producers measure the content of hexanal continuously as one parameter in their quality control. The odour threshold for these aldehydes is very low and, contrary to the terpenes, the smell of these aldehydes is highly unpleasant. The aldehydes are not a part of the original wood extractives, however, but are formed through autoxidation of the double bonds in the unsaturated fatty acids. The reaction mechanism is complex and involves a first step with formation of a hydroperoxide and a second step with cleavage of the peroxide to form aldehydes, unsaturated hydrocarbons and shorter acids. The autoxidation takes place over a long period of time (several months) and is often considered a reason for the increase in odour with time that can be found for some types of paper. The reaction is enhanced by the presence of heavy metal ions and it can be retarded by addition of complexing agents such as EDTA.

The type of pulp may also have an influence on the odour level. Bleached board samples often display a lower odour level than boards containing unbleached pulp or TMP. The higher odour level found in TMP is most probably due to the higher content of extractives normally found in such pulps compared to e.g. kraft pulps.

7.10 Further Reading

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8 Cellulose Products and Chemicals from Wood

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8.1 Introduction

Wood is a versatile raw material and it has an immense importance to mankind as energy source, construction material and as pulp and paper products. The use of wood (and other biomass) for the production of chemicals is, however, of a rather moderate size since petroleum constitutes an excellent and versatile starting material for the chemical and polymer industry. Only in a few niche areas, wood-based chemical products have been able to compete successfully with the petroleum-based. The most important of these is based on cellulose which can be purified and further transformed into regenerated cellulose, i.e. Rayon or Lyocell, or derivatized into a large variety of cellulose esters and ethers. In some sulfite and kraft mills in the world, the dissolved lignin is recovered by evaporation or precipitation and further processed into products such as dispersing agents. From softwood kraft mills, the volatile extractives are recovered as turpentine whereas the remaining portion of the extractives form the so-called tall oil. Both these fractions can be further refined. The flavoring agent vanillin is made from sulfite liquor by oxidation with air.

Several other uses of wood for the production of chemicals have, however, also been tried, some of which are old technologies (*Figure 8.1*). Except for the direct combustion for energy

purposes, wood can also be degraded by either pyrolysis or hydrolysis. In the former case, charcoal together with wood pitch and low molecular weight liquid and gaseous compounds can be produced provided that the temperature is not too high. In industrial scale, a final temperature of around 500 °C is used. At pyrolysis temperatures of around 1000 °C and in the presence of oxygen or air, further degradation of the wood occurs and the predominant products are gases such as hydrogen, carbon monoxide and carbon dioxide (water gas). A further cleaning of this gas and addition of more hydrogen converts it into synthesis gas which, with a CO/H_2 ratio of 1:2, in turn can be catalytically converted into methanol. This is one of the currently discussed options for a future liquid fuel system.

Figure 8.1. Wood stack from the early 1900s for making charcoal.

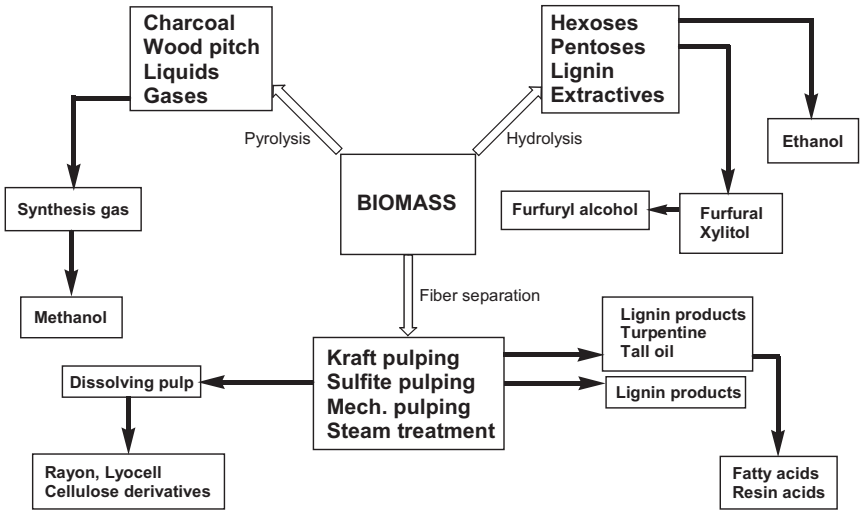


Figure 8.2. Thermal and chemical techniques for the conversion of wood (or other biomass) into chemical products.

The direct hydrolysis of wood (or other biomass) aims at degrading the polysaccharides into monosugars which can be further processed into a variety of end products. From the hexoses, ethanol can be obtained by fermentation but, at present, a similar facile conversion to ethanol of the pentoses is not possible and, consequently, much research work is currently done in order to develop such a technology. In principle, an alternative use of the pentoses (xylose) could be as starting material for the production of furfural by acid treatment. The production of furfural (and furfuryl alcohol) is, however, based entirely on agricultural residues which affords much higher yields. In Finland, catalytic hydrogenation of pure xylose is a commercial process for making the sweetening agent xylitol. Some process streams, currently used or suggested, are shown in *Figure 8.2*.

8.2 Cellulose Chemistry

8.2.1 General Aspects

The processing of cellulose for production of regenerated cellulose and cellulose derivatives requires a cellulose of high purity, i.e. cotton linters or dissolving pulp. The latter can be produced either by prehydrolysis-kraft or acid sulfite pulping. In both processes, the conditions must be chosen such that the remaining amount of hemicellulose in the fibers is reduced to a minimum. Furthermore, bleaching to high brightness is required in order to remove all lignin. The pulp yield in these processes is low and in the order of 35 % making it a rather expensive product.

An alternative and more energy-efficient way of separating the wood components from each other has been suggested and is usually referred to as the "wood explosion" process (*Figure 8.3*). Here, the wood material (or other biomass) is treated with steam at temperatures in the range of 190–240 °C for a few minutes followed by a rapid release of the pressure. This forces the material to "explode" with formation of individual fibers and fiber bundles whereas volatile extractives can be collected separately. Under the conditions of the steam treatment, wood acids are liberated and acid hydrolysis of the polysaccharides takes place together with simultaneous hydrolysis and condensation reactions of the lignin. Most of the hemicelluloses are degraded into low molecular weight sugars and oligosaccharides and can be removed by washing with water. A redistribution of lignin in the fiber walls also occurs and results in a rather facile elimination of a large portion of the lignin by extraction of the fibrous material with either aqueous alkali, sodium sulfite or an organic solvent. The remaining material consisting of cellulose with a low to medium degree of polymerization together with the remainder of the lignin and some degraded carbohydrates like furfural-derived polymers can be easily bleached giving a rather pure cellulose. Unfortunately, the process suffers from the difficulty in obtaining a homogeneous reaction in the biomass material and this results in high amounts of shives. Therefore, the process has not yet reached the commercial scale.

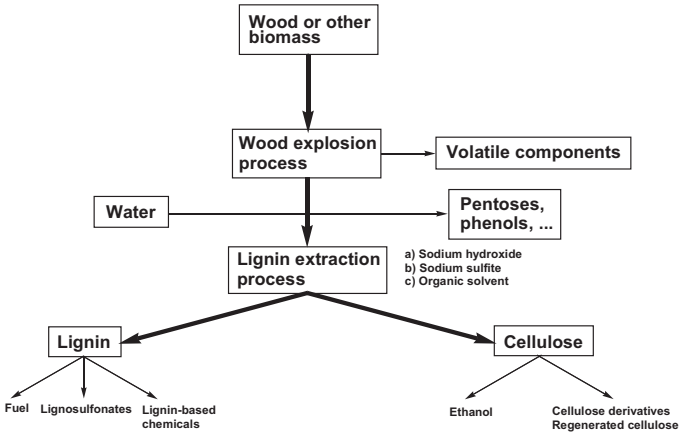


Figure 8.3. Principal scheme for the "wood explosion" process and the types of products that can be obtained. Typical reaction conditions are 190–240 °C, 1–5 min.

From cellulose, a large variety of derivatives, esters and ethers, can be manufactured. The largest volume is, however, regenerated cellulose which is produced as Rayon fibers, Cellophane and Lyocell fibers (*Figure 8.4*). Several major difficulties are encountered in the production of cellulose-based products such as the heterogeneity of the starting material, the reproducibility of the experimental conditions, the heterogeneous phase of the reaction, the purification difficulties, the effluent disposal and the product quality control. In addition, there has been no real driving force to further develop the technologies used because of the strong competition from the petroleum-based industry.

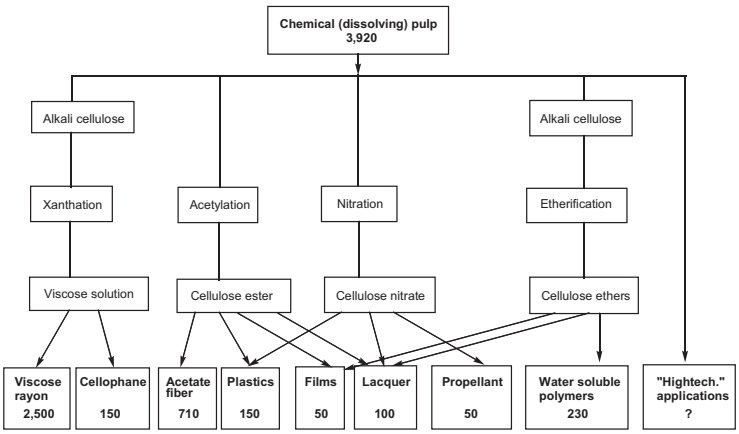


Figure 8.4. Major uses of cellulose derived products in the world with production volumes in tonnes $\times 10^3$. Data from 1990.

Thus, a successive decline in the production of cellulose-based fibers has been encountered during the last 20 years. At present (2002), the global production of manufactured fibers is in

the order of 36 million tons/y, an increase of 155 % from the production in 1982. Of this production, only some 6 % (~2.2 million tons) is based on cellulose, however (*Figure 8.5*).

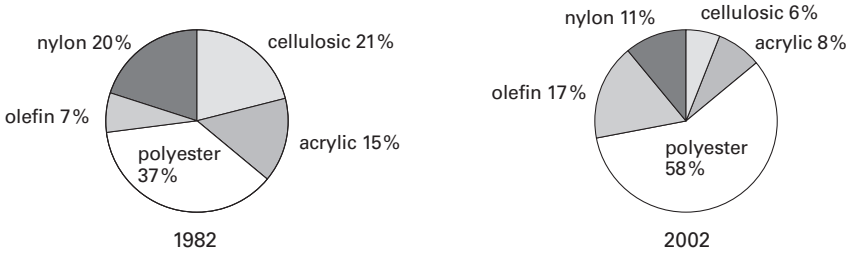


Figure 8.5. Worldwide production of manufactured fibers shared by fiber types in 1982 and 2002.

8.2.2 Regenerated Cellulose

The traditional way of making regenerated cellulose fibers and films is by treating the cellulose with strong alkali (mercerisation) in order to adjust (decrease) the degree of polymerization (DP) to a suitable value followed by reaction with carbon disulfide. The solution that is formed is termed viscose and this is also the name of the process. Chemically, the mercerisation converts the cellulose I to cellulose II which subsequently is converted to cellulose xanthate by reaction with carbon disulfide. The xanthate is dissolved in aqueous sodium hydroxide and allowed to equilibrate in order to get the substitution as evenly distributed as possible. Finally, the xanthate is pressed through a spinnerette into a solution of sulfuric acid where the acid re-

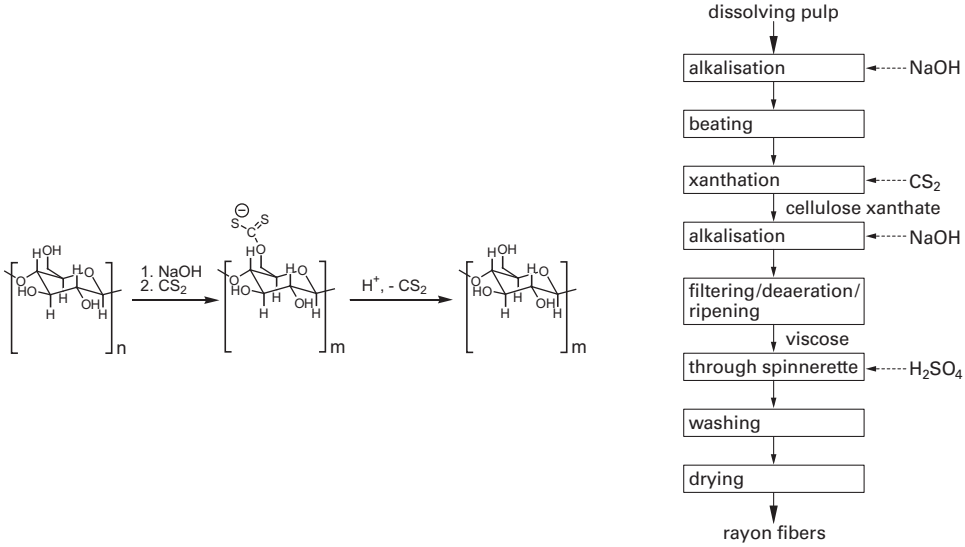
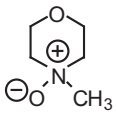


Figure 8.6. Process steps and chemical reactions encountered in the manufacturing of rayon fibers.

generates the cellulose as fine filaments resulting in rayon fibers. The process and the chemical reactions are schematically shown in *Figure 8.6*. In a similar way, cellophane can be made by pressing the viscose solution through a fine slit.

A newly developed alternative to the viscose process is a direct dissolution of the cellulose in NMMO (N-methyl-morpholine-N-oxide, *Figure 8.7*) and subsequent precipitation of the cellulose filaments in an NMMO-water mixture. These fibers are termed Lyocell fibers and like the rayon fibers, their major use are in textiles.



N-methyl-morpholine-N-oxide

Figure 8.7. N-methyl-morpholine-N-oxide, NMMO, a cellulose solvent used to manufacture Lyocell fibers

8.2.3 Cellulose Derivatives

Carboxymethylcellulose (CMC) is one of the most important cellulose derivatives. In Sweden, it is manufactured by Metsä-Serla Chemicals AB in Skoghall which has an annual production capacity of around 20,000 tonnes (1995). The process involves mercerisation of the starting cellulose, usually dissolving pulp, followed by reaction with sodium monochloroacetate to form an ether linkage. After neutralisation, washing and beating the product is dried as its sodium salt. Normally, the degree of substitution (DS) is around 0.60–0.95. The process is outlined in *Figure 8.8*.

CMC has a wide area of use. Highly purified CMC is used in the food, pharmaceutical and cosmetic industry when a taste- and smell-free non-toxic thickening agent, stabilizer or dispersing agent is needed. Typical examples are ice cream, tooth-paste, deodorants and schampoos. A somewhat less pure, highly viscous and water-soluble form of CMC is used in various industrial applications such as dispersing agent, flow property regulator and for the development of thin films in e.g. paper coating colors.

In Sweden, the non-ionic cellulose derivative ethylhydroxyethylcellulose (EHEC) is manufactured by Akzo Nobel Surface Chemistry AB in Örnsköldsvik with an annual production of around 20 000 tons (1995). In the manufacturing of EHEC, the cellulose is first mercerized and subsequently reacted with ethyleneoxide to form hydroxy-polyethoxy ether groups along the cellulose chain. Typically, a DS ~1.2 is obtained. In a second reaction step, ethylchloride is used to introduce ethyl ether groups with a DS of around 0.8–1.0. The process is outlined in *Figure 8.9*. In water, EHEC forms colloidal solutions that are used for water retention in cement and other applications in the construction industry. Other important uses are as thickening and dispersing agents and as stabilizer in water-based latex paints.

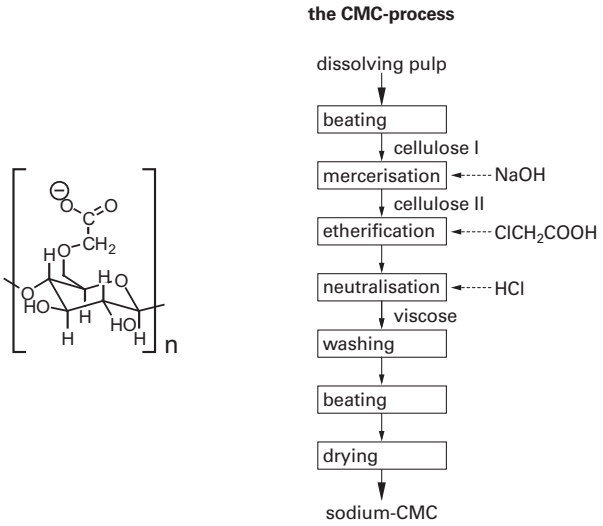


Figure 8.8. Process steps in the manufacturing of carboxymethylcellulose (CMC) together with a simplified chemical structure of CMC.

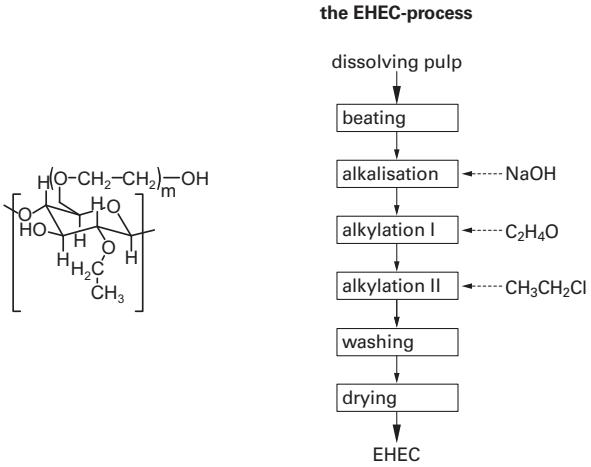


Figure 8.9. Process steps in the manufacturing of ethylhydroxyethylcellulose (EHEC) together with a simplified chemical structure of EHEC.

Several products based on cellulose acetate are produced commercially having different degrees of substitution along the cellulose chain. Major uses are as lacquers, fibers, photographic films and fabrics (*Table 8.1*). Normally, the reaction is carried out in acetic acid with acetic anhydride as the acetylation reagent and sulfuric acid as the catalyst. The reaction conditions determine the DS but also the solvent and the catalyst.

Table 8.1. Different types of cellulose acetates with degree of substitution (DS), suitable solvent and typical application areas.

Acetyl content, %	DS	Solvent	Applications
22–32	1.2–1.8	2-methoxyethanol	Plastics, Lacquers
36–42	2.2–2.7	Acetone	Fibers, Photographic films
43–45	2.8–3.0	Chloroform	Fabrics, Fibers, Foils

A further cellulose ester of commercial interest is cellulose nitrate which also can be produced with a variety of DS as shown in *Table 8.2*. The process involves treatment of the cellulose (dissolving pulp) with a mixture of nitric acid and sulfuric acid in which the treatment conditions determine the DS of the product. The crude product is washed with water and subsequently treated with boiling sodium carbonate solution in order to adjust the degree of polymerization ("stabilization") before beating and dewatering to form the final product. The process is outlined in *Figure 8.10*.

Table 8.2. Different types of cellulose nitrates with degree of substitution (DS), suitable solvent and typical application areas.

Nitrogen content, %	DS	Solvent	Applications
10.5–11.1	1.8–2.0	Ethanol	Plastics, Lacquers
11.2–12.2	2.0–2.3	Methanol, Acetone	Lacquers, Adhesives
12.0–13.7	2.2–2.8	Acetone	Explosives

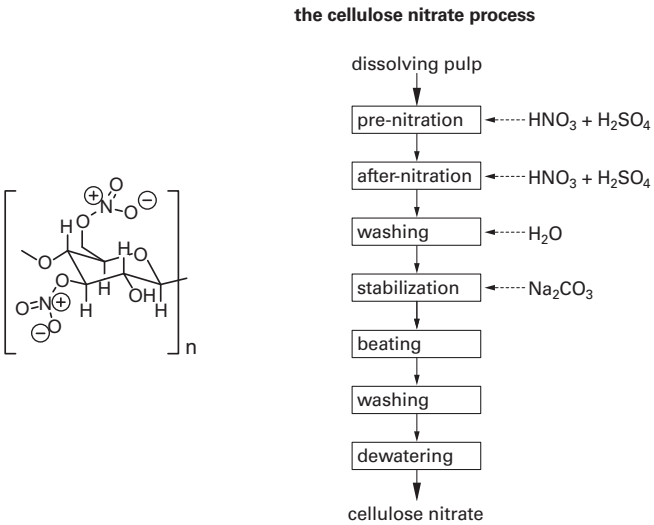


Figure 8.10. Process steps in the manufacturing of cellulose nitrate together with a simplified chemical structure.

In Sweden, cellulose nitrate is manufactured by Bofors Explosives AB in Karlskoga which has an annual capacity of around 1000 tons (1995). The DS is around 2.2–2.5 with more than 50 % of the substitution in the positions 2 and 3. The products are gunpowder and explosives, made by mixing of low and high nitrated cellulose together with a solvent like ethanol.

A different way of substituting the cellulose is by graft polymerization. Although several modes of adding reactive compounds to cellulose have been tried, these attempts have so far been without much success. One major difficulty encountered in these experiments is the relative ease by which homopolymers are formed together with the desirable copolymerization. Therefore, comparatively large losses of the grafting compound can be obtained. One example is shown in *Figure 8.11* where radical polymerization between cellulose and vinyl chloride has been tried with the major product being polyvinyl chloride.

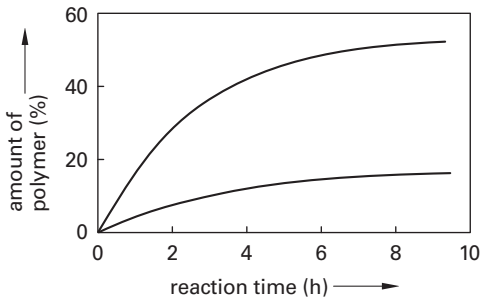


Figure 8.11. Radical initiated polymerization of vinyl chloride on bleached softwood kraft pulp. Amount of copolymerization (C) and homopolymerization (H) respectively.

8.3 Lignin Chemistry

8.3.1 General Aspects

In the pulp industry, hugh quantities of organic material, dissolved in the pulping process, are burnt in order to regenerate the cooking chemicals and to provide the necessary energy for the mill operations. Additional uses are limited but in a few mills in the world, the evaporation of sulfite cooking liquor has been developed for the production of lignosulfonates. Here, a strong driving force has been the fact that the calcium-based sulfite process lacks a suitable recovery system for the cooking chemicals. Thus, in order to avoid severe environmental problems, a complete evaporation of the spent liquor can be done, sometimes followed by further purification and chemical modification steps. In kraft mills, a partial precipitation of kraft lignin from the black liquor can be made by addition of carbon dioxide or mineral acid to a pH of around 9–10 (*Table 8.3*). Thereby, approximately 80 % of the lignin can be recovered and further processed into sulfonated or oxidized lignin products.

Other uses of lignin except energy have been suggested frequently in the literature since the availablity of lignin is high and, potentially, large volumes of organic material can be produced. Despite many efforts, however, the market for lignin-based products has only developed slowly and, still, the major use is as a macromolecule in solution. A major weakness of lignin is the large heterogeneity since the various technical pulping processes give rise to molecules ranging from virtually monomeric phenols to high molecular weight polymers. As a result, the physical properties of a certain lignin product cannot be well defined and, accordingly, the technical performance is such that only rather low value added uses can be found. The heterogeneity of

lignins can be expressed as the polydispersity, calculated as M_w/M_n with typical values for lignosulfonates of 5–7 and for kraft lignins of 2–3.

Table 8.3. Fractional precipitation of kraft black liquor lignin from spruce wood by successive acidification with sulfuric acid.

Precipitation (pH)	Amount (% of total)	Sulfur content (%)
10.75	27	1.2
10.5	32	2.1
10.2	10	–
10.0	7	1.4
9.9	4	–
9.5	4	–
9.4	3	1.5
8.2	2	3.9
7.8	1	–
1.5	10	6.5

8.3.2 Lignosulfonates

At present, approximately 1 000 000 tons/y of lignosulfonates are produced in the world with the major producer being Borregaard Lignotech (Norway) with production facilities in 6 different countries. Major uses are found in the areas of industrial binders and as dispersing agents. In the production process (for making sulfite pulp), the water soluble lignosulfonate is formed by an extensive sulfonation and partial hydrolysis of the native lignin macromolecule. Simultaneously, some condensation reactions will occur within the lignin. In addition, a certain degradation of the wood polysaccharides takes place giving rise to monomeric sugars and to the formation of small amounts of furfural and hydroxymethyl furfural (*Table 8.4*). Normally, the counter-ion is calcium. From this mixture, a variety of products can be manufactured with the major changes being the amount of sugars (which can be reduced) and the counter-ion used (Ca, Mg, Na, NH_4).

Table 8.4. Main components present in spent pulping liquor from acid sulfite pulping (kg/ton of pulp).

Component	Spruce	Birch
Lignosulfonate, tot.	480[1)]	370[1)]
M_w>5,000	245	55
Carbohydrates	280	375
– Arabinose	10	10
– Xylose	60	340
– Mannose	120	10
– Galactose	50	10
– Glucose	40	5
Aldonic acids	50	95
Acetic acid	40	100
Extractives	40	40
Misc. compounds	40	60

[1)] Amount of lignosulfonate calculated as lignin

The structure of the lignosulfonate molecule is that of a spherical microgel rather than a linear molecular chain. Thus, the charge is predominantly located on the surface of the molecule whereas sulfonate groups located at the interior are neutralized through ion pairing with adjacent cations. Condensation reactions withing the microgel results in a certain degree of cross-linking which further favours the spherical structure. A schematic picture is shown in *Figure 8.12*.

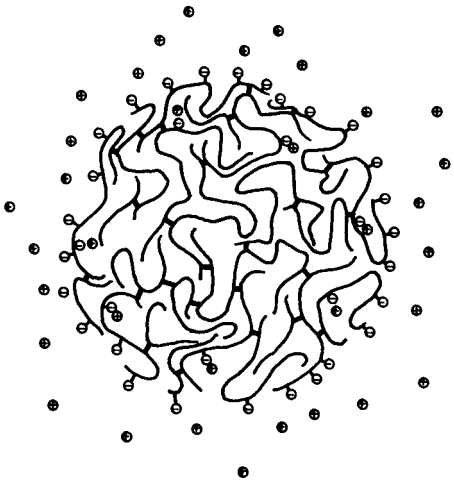


Figure 8.12. Schematic picture of a lignosulfonate molecule.

Below a certain (undetermined) molecular weight, the spherical structure is, however, no longer applicable since the number of phenylpropane units and cross-links within the molecule becomes too small. On the other hand, at the high end of the molecular weight range, the lignosulfonate molecules may again deviate from a spherical shape thus giving more interactions with each other. Therefore, the efficiency of a lignosulfonate to act as a dispersing agent is highly dependent on the molecular weight range since the molecules must be able to efficiently cover the surface of the particle as shown in *Figure 8.13*. It has been demonstrated that for different types of particles (having different particle sizes and shapes), the maximum dispersing power that can be reached requires lignosulfonates of different molecular ranges. Therefore, in commercial operation, different customers will require different formulations making the business highly demanding.

Simple evaporation of the crude lignosulfonate solution results in products used as binders in various applications such as pellet binder in animal feeds and as dust binder for unpaved roads. By fermentation to ethanol of the hexoses present in the original solution and by chemical degradation of the pentoses, purified lignosulfonate products can be obtained. These can be further modified by cation exchange and by chemical modification reactions. The major application areas are as dispersing agents for mineral and dye pigments like in the manufacturing of bricks and concrete, as additive in oil well drilling and as dispersing agent for textile dyestoffs.

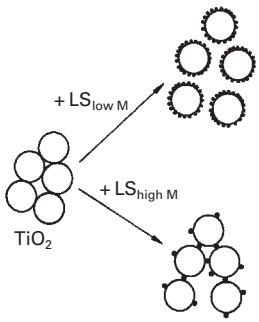


Figure 8.13. Schematic picture of the dispersing action on titanium dioxide pigment of lignosulfonates having an optimal (LS_{lowM}) and a non-optimal (LS_{highM}) molecular weight range. In the former case, a molecular weight range of 10000–40000 Dalton was found.

8.3.3 Kraft Lignins

The black liquor from kraft pulping contains a large amount of degraded lignin together with low molecular weight acids formed on degradation of the polysaccharide components of the wood. Furthermore, extractives are present and can be recovered as turpentine and talloil (*Table 8.5*).

Table 8.5. Main components present in the spent liquor from kraft pulping (kg/ton of pulp).

Component	Pine	Birch
Lignin	490	330
Carbohydrate derived		
– Hydroxy acids	320	230
– Acetic acid	50	120
– Formic acid	80	50
Turpentine	10	–
Resin and/or fatty acids	50	40
Misc. products	60	80

Precipitation of lignin from the spent cooking liquor is done in only a few mills in the world. At present, the total production is in the order of 100,000 tonnes/y with Westvaco (USA) and Borregaard Lignotech (Norway) as the producers. The acidification of the liquor to a pH = 9–10 gives a lignin precipitate which on evaporation still contains a large amount of sodium ions. The major use of kraft lignins is, however, as a dispersant in aqueous solution and, consequently, the lignin is modified by sulfonation or carboxylation thereby rendering water solubility to the product. Several different ways of introducing hydrophilic groups into the lignin molecule have been tried such as direct treatment with sodium sulfite at high temperature, sulfomethylation, oxidative sulfonation with sodium sulfite and oxygen and ozone treatment. Of these, the former two methods are commercial. The reactions involved in these treatments are summarized in *Figure 8.14*.

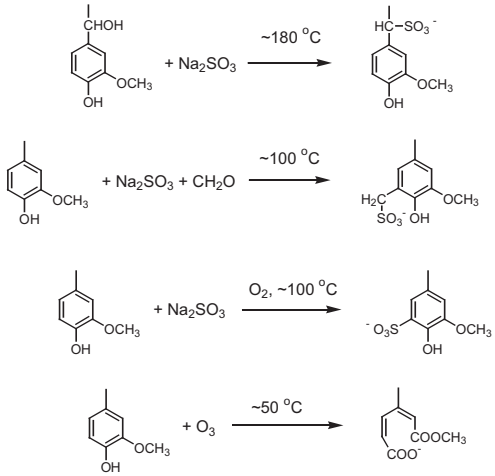


Figure 8.14. Different ways of introducing hydrophilic groups in kraft lignin.

Large differences in dispersing efficiency are found when comparisons between sulfite-based lignosulfonate and sulfonated kraft lignin are done. Both the polydispersity and the chemical structure seem to play a role. The effect has been illustrated through molecular weight fractionation of lignins and comparison of the efficiency of the various fractions. In *Table 8.6*, the results from such fractionations are shown for commercial lignosulfonate and sulfonated kraft lignin respectively.

Table 8.6. Fractionation of a lignosulfonate (average $M_w = 6{,}940$) and a sulfonated kraft lignin (average $M_w = 4{,}430$) by ultrafiltration. The kraft lignin was sulfonated by sulfite treatment at 170 °C.

Fraction No	Yield (%)	Molecular weight (M_w)
Sulfite		
1	20	590
2	29	1,440
3	18	3,690
4	4	6,500
5	6	10,880
6	22	21,950
Sulfonated kraft		
1	0.4	670
2	8.5	920
3	62.8	2,560
4	16.9	7,200
5	11.8	>13,000

It can be seen that the sulfonated kraft lignin contains a predominant fraction of material centered around a molecular weight of around 2500. For the lignosulfonate, on the other hand, a corresponding major fraction does not exist and most of the fractions contain similar amounts of material. When the ability of each one of these lignin fractions to act as a dispersant for a dyestuff are compared, large differences were found as shown in *Figure 8.15*. Here, the efficiency

on a weight basis of a sulfonated kraft lignin to act as a dispersant was found to be much higher as compared to a lignosulfonate.

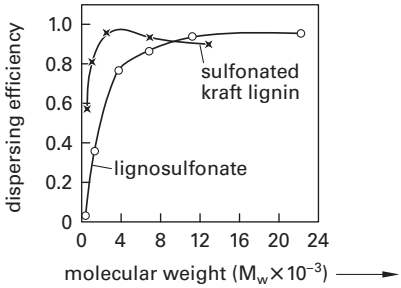


Figure 8.15. Relationship between the molecular weight of lignin fractions and dispersing efficiency.

At present, the sulfonation of a kraft lignin is done on the whole of the precipitated and isolated material obtained from the black liquor after the cook. It has been demonstrated, however, that the lignin that goes into solution early in the kraft cook has a lower molecular weight distribution than that which is dissolved later. Futhermore, by using a solvent fractionation technique, kraft lignin can be divided into fractions of largely different structure. Some of these features are shown in *Table 8.7*.

Table 8.7. Analytical data for softwood kraft lignin, fractionated by solvent fractionation (P OH denotes phenolic and A OH alifatic hydroxyl groups).

Fraction, soluble in	Yield (%)	M_n	M_w	M_w/M_n	P OH (Mmol/g)	A OH (Mmol/g)	COOH (Mmol/g)	T_g (°C)
CH_2Cl_2	9	$4.5 \cdot 10^2$	$6.2 \cdot 10^2$	1.4	5.1	1.0	2.3	32
$CH_3CH_2CH_2OH$	22	$9.0 \cdot 10^2$	$1.3 \cdot 10^3$	1.4	5.0	2.3	1.1	62
CH_3OH	26	$1.7 \cdot 10^3$	$2.9 \cdot 10^3$	1.7	4.3	2.1	0.8	164
CH_3OH-CH_2Cl_2	28	$3.8 \cdot 10^3$	$8.2 \cdot 10^4$	22	3.9	2.4	0.4	173
undissolved	14	$5.8 \cdot 10^3$	$1.8 \cdot 10^5$	31	3.0	2.4	0.3	–
unfractionated	100	$1.4 \cdot 10^3$	$3.9 \cdot 10^4$	28	4.3	2.2	0.8	~175

Thus, the prospect of further improving the properties of kraft lignins by fractionation techniques seems possible. Such development is currently being explored by the use of ceramic membranes able to operate in-line at pulping temperatures. Thereby, lignin fractions with more well defined molecular weights and functional groups may be a future possibility.

8.4 Low Molecular Weight Products

8.4.1 Turpentine

In kraft pulping of softwood, the volatile extractives, predominantly monoterpenes, can be isolated in a yield of approximately 10 kg/ton of pulp. After distillation to remove impurities such as methyl mercaptan and dimethyl sulfide together with higher terpenoid compounds, turpen-

tine is obtained. The major constituent in turpentine is α-pinene but the composition can be different depending on the origin as shown in *Table 8.8*.

Table 8.8. Main components in turpentine from A: Nordic countries, B: southeastern US.

Component	Turpentine A	Turpentine B
α-Pinene	50–80	60–70
β-Pinene	2–7	25–35
3-Carene	10–30	–
Other terpenes	5–10	6–12

In many countries, turpentine is produced by direct solvent extraction of wood whereas in pulp producing countries, sulfate turpentine is predominant. The global production of turpentine is in the order of 240,000 tons/year and major uses are as raw material for the manufacturing of chemicals and resins and as fragrances and flavors. The single largest use of turpentine is for the production of pine oil which chemically involves a hydration of α-pinene to α-terpineol. Another major use is for the production of resins, which when starting with β-pinene and employing a Lewis acid as catalyst gives rise to the adhesive in tape (*Figure 8.16*).

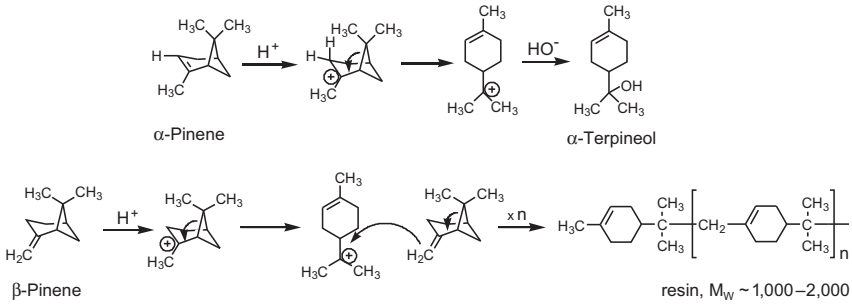


Figure 8.16. The hydration of α-pinene with mineral acid for the production of pine oil, α-terpineol, (upper line) and the polymerization of β-pinene with Lewis acid to form tape adhesive (lower line).

8.4.2 Tall Oil Products

In kraft pulping of softwood, the non-volatile extractives, predominantly soaps of resin acids and fatty acids, are separated from the black liquor after the cook. Addition of sulfuric acid liberates the free acids which form crude tall oil. In areas with high density of pine, the yield of tall oil might reach ~50 kg/ton of pulp. In Sweden, tall oil fractionation is done in one production unit located in Sandarne which has an annual capacity of ~140,000 tons. After distillation, approximately 25 % of that amount is obtained as rosin (abietic acid as predominant component), another 30 % as fatty acids (oleic acid and linoleic acid as predominant components) whereas the remainder form tall oil pitch and fractions of mixed composition. A general production and product scheme is shown in *Figure 8.17*. Much of the rosin products are used in the sizing of paper, as adhesives and in printing inks whereas a major use of the fatty acids are in alkyd resins.

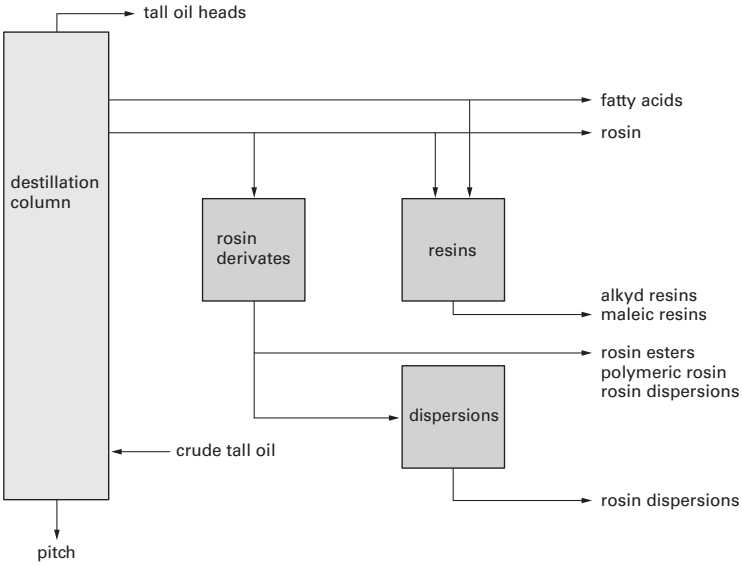


Figure 8.17. Schematic picture of a distillation unit for tall oil together with major product types.

8.4.3 Ethanol

In the traditional acid sulfite pulping process, a portion of the wood polysaccharides are hydrolysed into hexoses and pentoses which can be found in the spent pulping liquor (*Table 8.4*). Since long, fermentation of the hexoses to ethanol has been applied in some sulfite mills. In Sweden, the Domsjö mill outside Örnsköldsvik produces around 10,000 tons/year. As part of a major international development project, great efforts are being done in Sweden to find suitable ways of converting wood waste like branches and other non-pulpable parts into ethanol by direct acid hydrolysis. Three alternative methods have been tried, hydrolysis with concentrated sulfuric acid or hydrochloric acid at 20–40 °C (the CHAP method), hydrolysis with diluted acid at high temperature (the CASH method) or enzymatic hydrolysis. Of these, the CASH (Canada-America-Sweden-Hydrolysis) method looks the most promising. Such an ethanol plant can easily be incorporated in a normal pulp mill thus making efficient use of the raw material, energy, water and effluent treatment facilities. This is schematically shown in *Figure 8.18*. At present (2004), a pilot plant unit has been installed in Domsjö for the further evaluation of the process.

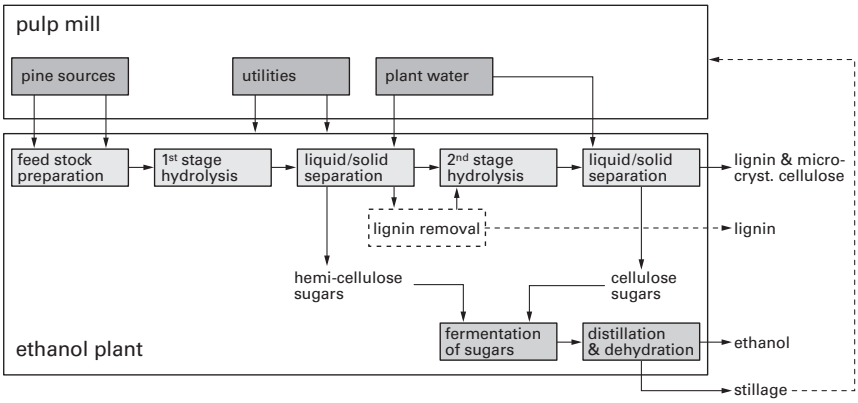


Figure 8.18. Suggested production unit for conversion of wood waste to ethanol using the CASH process employing sulfur dioxide and sulfuric acid in two stages. The integration with a pulp mill is indicated in the figure.

8.4.4 Vanillin

The World production of vanillin is around 3500 tons/year with the predominant producer being Borregaard, Norway. It is used as a food and beverage flavouring agent. Most of the vanillin is obtained by treatment of lignosulfonate with alkali at an elevated temperature under oxidative conditions although synthetic routes from phenol also exist. In addition, vanillin can be obtained from natural sources such as vanilla beans. The formation of vanillin from lignosulfonate has been investigated using a phenolic β-O-4 structure with a sulfonic acid group in the α-position as a model compound. On treatment with alkali, the structure was degraded into vanillin and acetaldehyde whereas the β-substituent was released as a phenol. Thus, it can be assumed that phenolic end groups in the lignosulfonate give rise to vanillin according to the reaction scheme shown in *Figure 8.19*. The modified lignosulfonate that is obtained after treatment can be further processed into commercial lignosulfonate products.

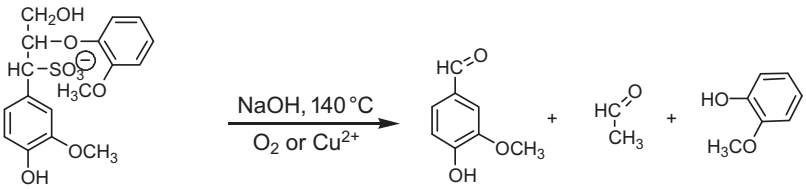


Figure 8.19. Products formed on treatment of a lignosulfonate model compound with alkali under oxidative conditions.

8.5 Future Development

The large societal consumption of non-renewable recources such as petroleum will undoubtedly result in successively higher energy costs and a large net increase of the concentration of carbon dioxide in the atmosphere can be observed (*Figure 8.20*). The only alternative to petroleum, available worldwide, is biomass present in wood and annual plants which can be converted e.g. into ethanol or methanol as described above. A commercial production of fuel ethanol, based on sugarcane bagasse, corn or wheat, has already started in a few countries. This development will continue and, successively other biomass will also be included, but the rate will be much dependent on political decisions such as tax rates.

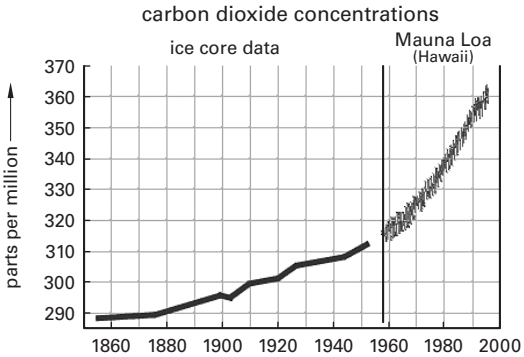


Figure 8.20. Atmospheric concentration of carbon dioxide from 1855 to 1996.

Based on the apparent simple photosynthesis reaction, the production of carbohydrates and oxygen from carbon dioxide and water shown in *Figure 8.21*, the annual production of biomass in the world has been estimated to around 12,000 million m^3. Of this amount, some 7000–9000 million m^3 is wood-based.

$$x\ CO_2 + x\ H_2O \xrightarrow[\text{green plants}]{h\nu} (CH_2O)_x + x\ O_2$$

Figure 8.21. Formation of carbohydrates and oxygen from carbon dioxide and water in the presence of day-light and chlorophyll in green plants.

At present, approximately 40–45 % of this wood quantity is used for various purposes of which fuel wood is the single most common (*Figure 8.22*). The uncontrolled elimination of large areas of tropical rainforests together with the high and increasing rate of combustion of non-renewable resources such as petroleum and coal will, however, result in a growing imbalance in the global carbon cycle as shown in *Figure 8.23*. Here, it can be seen that hugh quantities of carbon dioxide are stored on earth and present, organically bound, as wood and other types of plant material, i.e. biomass. Inorganic carbonate-containing minerals as well as free carbon dioxide present in the atmosphere and physically dissolved in the oceans constitute further important sources. As long as the continuous consumption of carbon dioxide by photosynthesis is counterbalanced by the release caused by decay of biomass material, no net production

of carbon dioxide is obtained. The increased level of atmospheric carbon dioxide that in fact is observed (*Figure 8.20*) may, however, be counterbalanced by an increase of new forest plantations and by the development of alternatives to the large scale consumption of petroleum and coal. Therefore, a strongly increased use of biomass for the production of fuel as well as of chemicals can be foreseen in the future. This field of "green chemicals" is presently under strong development although it will still take a long time until a noticable penetration of the areas traditionally using petroleum-derived products will be achieved.

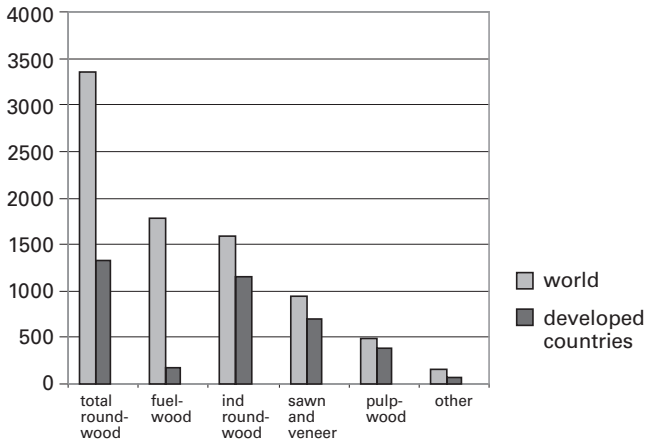


Figure 8.22. Annual consumption of wood for various end uses with values in Million m^3. Data from 2003, FAO statistics. Total roundwood = Fuelwood + Industrial roundwood; Industrial roundwood = Sawn and Veneer + Pulpwood + Others.

During the years 1996–2002, a major Swedish project, directed by the Swedish Pulp and Paper Research Institute, on the potential for future development of kraft mills was carried out; the Ecocyclic Pulp Mill. Within that project, a theoretical pulp mill (Reference mill 2000) was constructed based on "best available techniques" and its energy efficiency calculated and compared to that of the average Swedish pulp mill. As shown in *Figure 8.24*, the Reference mill is a net producer of energy. Thus, a withdrawal of a portion of the lignin from the pulping process could be advantageous since that would permit a higher output of pulp without a corresponding increase in recovery boiler capacity. At the same time, the lignin could be isolated and used elsewhere as a biofuel or, alternatively, as a chemical feedstock. The vision that a kraft pulp mill may become an essential part of a biorefinery system in the future is attractive. The experience of handling and processing large volumes of wood, water, chemicals and energy together with the use of sofisticated process control systems will facilitate an expansion to encompass new uses of wood.

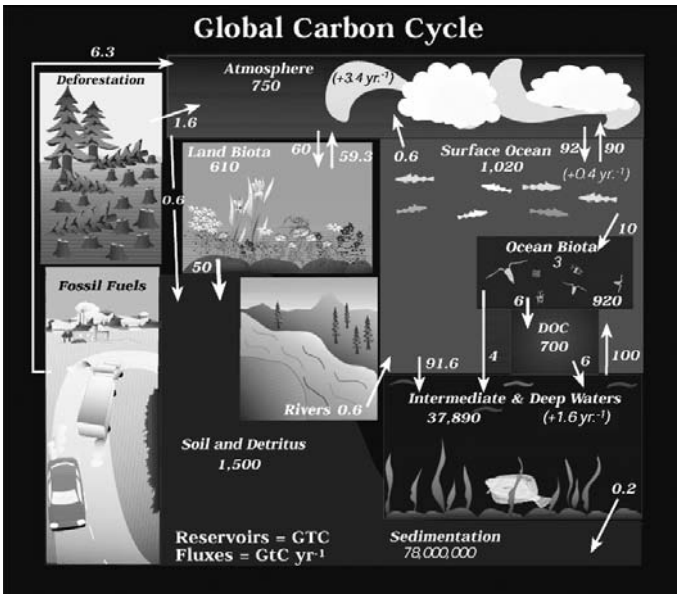


Figure 8.23. The distribution and flux of carbon in nature. GTC = gigatonnes of carbon.

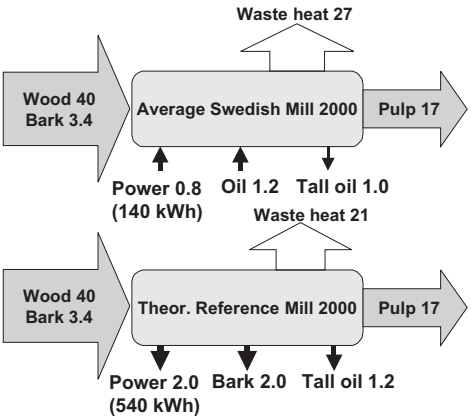


Figure 8.24. Energy balance (GJ/ton of pulp) in the average Swedish pulp mill in the year 2000. Comparison with a theoretical Reference Mill. Data from the Final Report of "The Ecocyclic Pulp Mill" (STFI 2003).

Several other research institutions in the world are also currently involved in projects directed towards the development of biorefineries. The efficient and cost-effective separation of individual polymers, i.e. cellulose, hemicellulose and lignin, is, however, a difficult task. In addition, a large scale production of value-added products based on each one of the biomass polymers must be developed. The steam explosion process described above (*Figure 8.3*) is one example of an unconventional way of achieving a separation of the biomass polymers, another is shown in *Figure 8.25*. Here, biomass of various origin is pulped with a mixture of nitric acid

and acetic acid thus providing degraded hemicelluloses, microcrystalline cellulose and nitrolignin. This approach forms a major research area at the "Petru Poni" Institute of Macromolecular Chemistry in Iasi, Romania, where the nitrolignin as well as technical lignins from other more conventional sources constitute the starting materials for a variety of products.

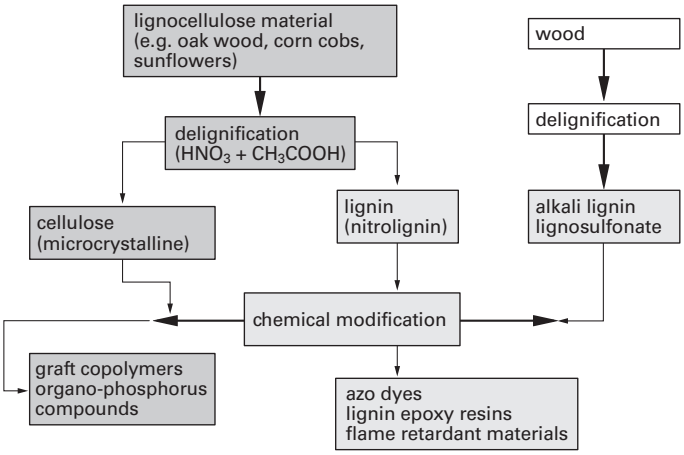


Figure 8.25. Schematic view of the research area on biomass utilization advocated at the "Petru Poni" Institute of Macromolecular Chemistry in Iasi, Romania.

9 Analytical Methods

Göran Gellerstedt
KTH, Department of Fibre and Polymer Technology

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9.1 Introduction

The chemical characterization and analysis of wood and of wood and fiber components constitute a formidable challenge due to the complexity of the material and to the large number of chemical changes that may occur in various process stages. During the years, many analytical methods, specially designed to give information about the composition of wood and fibers as well as the structure of the individual wood polymers and the identity of the extractive components have been developed. Traditionally, these methods have been based on wet chemistry, often in combination with gas chromatography and mass spectrometry. The possibilities of analysing the polymers as such have been limited, however. With the continous development of the separation techniques and the introduction of advanced spectroscopic methods such as FTIR and NMR spectroscopy, in combination with new data handling techniques, the analyst, today, has access to a much larger number of methods. Often, such a method can replace the older wet chemical analysis but, due to the analytical complexity, many wet chemical analyses are still in use and new methods are being developed.

In different situations, different types of analyses are required. In industry, the detailed chemical structure is usually not of interest and, often, the methodology is based on rapid and relevant information, e.g. degree of delignification in pulping, amount of organically bound chlorine in the bleaching effluent or gross composition of a deposit in the paper machine. In the R&D laboratory, on the other hand, much more detailed information is usually necessary since the objective is to understand a certain phenomenon. In the following, a survey of different analytical methods are presented with a focus on the composition of wood and methods for the analytical elucidation of pulp fibers and of wood and pulp polymers.

9.2 The Composition of Wood

The amount of the various wood components, viz cellulose, hemicelluloses, lignin, extractives and inorganics can vary widely depending on wood species. In addition, structural differences exist between the various types of wood tissue as well as between the individual cell wall layers. Irrespective of morphological differences, the gross composition of wood (or pulp) can be determined by first applying a homogenization of the material, followed by solvent extraction to remove low molecular weight organics. Acid hydrolysis of the residue results in a precipitate consisting of highly condensed lignin (Klason lignin) and a solution of monomeric sugars. The latter are separated (by GC, HPLC or CE analysis) and quantified whereas the Klason lignin is determined gravimetrically (*Figure 9.1*). For the analysis of inorganics, present in the wood as carbonates, phosphates etc, combustion of the wood sample and gravimetric determination of the ash can be used. The individual components of the ash can be further analysed using e.g. inductively coupled plasma mass spectrometry (ICP-MS). Quantification of uronic acid residues is also done directly on the wood sample by decarboxylation with hydriodic acid and determination of the carbon dioxide that is formed.

The analytical protocol described above is generally applicable and provided that each step is optimized, accurate and reproducible results can be obtained. For some common softwood and hardwood species used for pulping, average values for the component composition (except inorganics) are given in *Table 9.1* and *Table 9.2*. The large deviations from the normal wood com-

position, present in compression wood and tension wood respectively, illustrate the difficulties in getting reproducible data and show that great care must be exercised in the sampling of material for analysis.

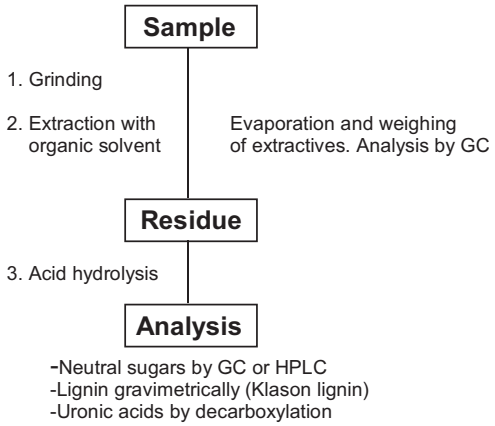

Figure 9.1. Analytical protocol for the analysis of wood and pulp samples.

Table 9.1. Average chemical composition in softwood (% of dry matter).

Component	Normal wood	Compression wood
Cellulose	37–43	29–31
Galactoglucomannan	15–20	9–12
Arabinoglucuronoxylan	5–10	6–8
Galactan	–	9–11
Laricinan	–	3–5
Lignin	25–33	37–40
Extractives	2–5	2–5

Table 9.2. Average chemical composition in hardwood (% of dry matter).

Component	Normal wood	Tension wood
Cellulose	39–45	50–65
Glucuronoxylan	15–30	16–23
Glucomannan	2–5	2–4
Galactan	–	0–10
Lignin	20–25	16–20
Extractives	2–4	2–4

9.3 Carbohydrates

9.3.1 Periodate Oxidation

Wet chemical analyses of carbohydrates and polysaccharides were developed a long time ago and are still in use frequently although NMR methods can also be used. For the general detec-

tion of carbohydrate structures, an oxidation of the sample with periodate is convenient since periodate reacts with vicinal dihydroxy compounds. In the reaction, each hydroxyl group is converted to an aldehyde or, for glycerol structures, an additional formation of formic acid (*Figure 9.2*). The reaction can be monitored by analysis of the periodate that is consumed and, consequently, information about the total amount of carbohydrates can be obtained.

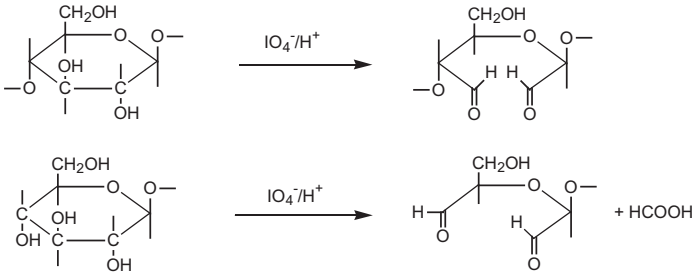

Figure 9.2. Periodate oxidation of an inner and a terminal sugar unit in a polysaccharide structure.

9.3.2 Separation of Polysaccharides

The separation of the individual polysaccharides present in wood and other lignocellulosic material is not trivial due to the complex arrangement of polymers making up the cell wall with lignin acting as an incrusting material. The necessary increase in accessibility of the polysaccharides can be obtained by a selective oxidative degradation of the lignin with sodium chlorite under mild acid conditions (*Figure 9.3*). Thereby, chlorine dioxide is formed in situ and further reactive chlorine-containing species such as hypochlorous acid and chlorine are formed during the course of reaction. The resulting product, holocellulose, contains virtually all the original polysaccharide components together with minor amounts of a lignin residue. As an alternative to chlorite, gasous chlorine can be used.

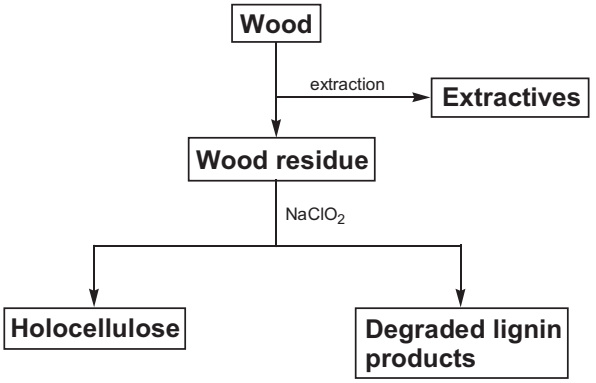

Figure 9.3. Reaction scheme for the preparation of holocellulose.

The further separation of the individual polysaccharide components present in wood can be done with holocellulose as the starting material. In the complicated separation scheme (*Figure 9.4*), strong alkali is used to dissolve the predominant portion of the xylan and galactoglucomannan leaving cellulose and glucomannan as a residue. The fact that sugar units like mannose having vicinal hydroxyl groups in a cis-configuration can form soluble borate complexes and insoluble barium salts (*Figure 9.5*) can be used to further separate the individual hemicelluloses.

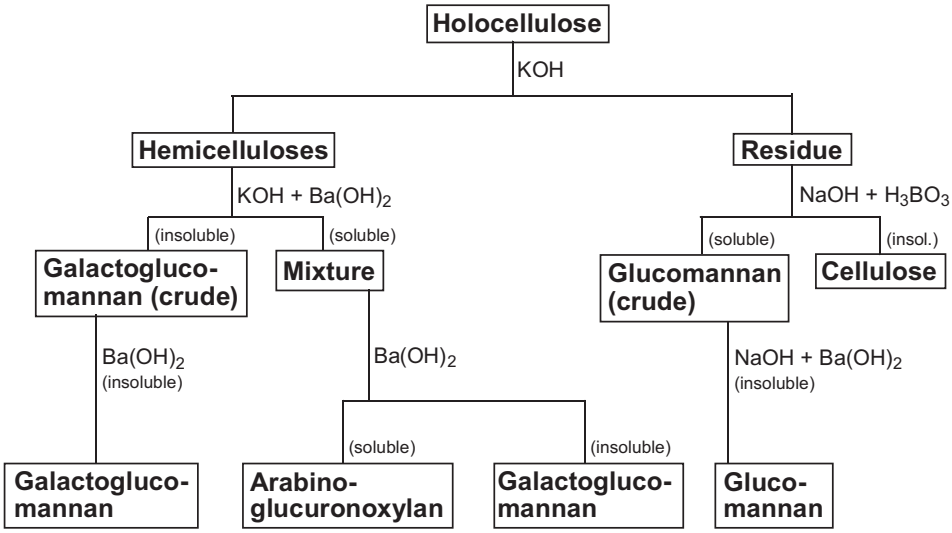

Figure 9.4. Isolation of individual hemicelluloses from softwood holocellulose by alkaline extraction combined with soluble borate complex and insoluble barium salt formation.

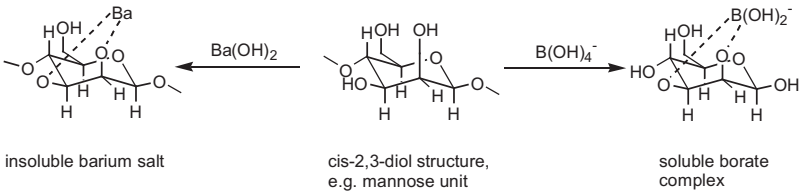

Figure 9.5. Reactions of a mannose unit with barium hydroxide and borate ions respectively.

9.3.3 Sugar Composition

The composition, structure and morphology of the polysaccharides present in wood and pulps are of great importance since these to a major extent determine the properties of the resulting fibers. A simple quantification of the monomeric sugar units present in a wood or pulp sample can be done as outlined in *Figure 9.1*. After the acid hydrolysis, the mixture of sugars can be reduced with sodium borohydride to convert all reducing end-groups to the corresponding alcohol

groups. Thereby, only one product from each pair of anomeric sugar units is obtained. After a subsequent acetylation, the mixture is analysed by gas chromatography (*Figure 9.6*). Other separation techniques are also frequently used such as HPLC or CE (capillary electrophoresis) with or without derivatization.

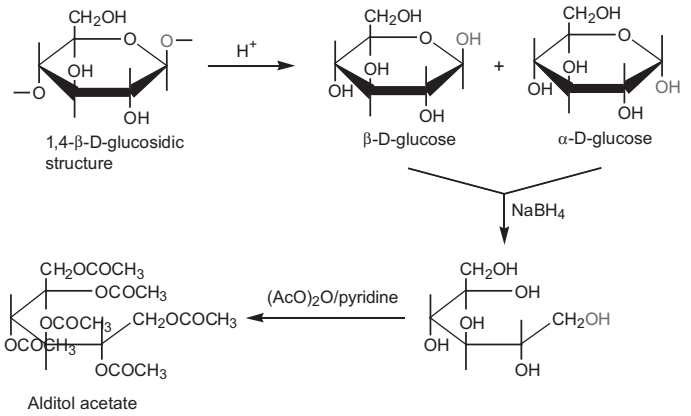

Figure 9.6. Reaction sequence for the formation of alditol acetates from a polysaccharide structure via acid hydrolysis, reduction with sodium borohydride and acetylation.

Acid hydrolysis of kraft pulp will result in the formation of 2-furoic acid and 5-carboxy-2-furaldehyde in addition to the normal sugars. The former are generated by acid catalysed conversion of hexenuronic acid groups present in the xylan and formed during the pulping procedure from the corresponding 4-O-methyl-glucuronic acid groups (*Figure 9.7*). By using mild acid conditions, the two degradation acids can be formed selectively and used for quantification of the hexenuronic acid groups present in a pulp sample.

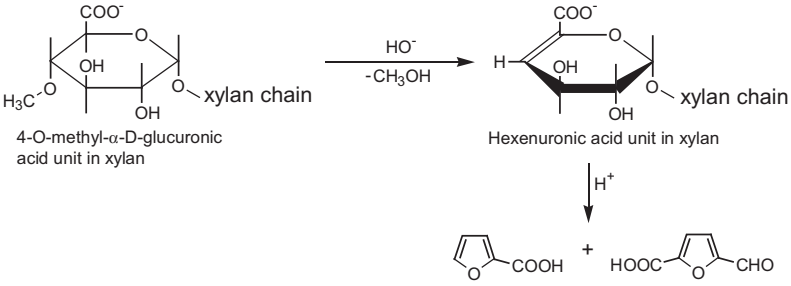

Figure 9.7. Formation of hexenuronic acid in alkaline pulping and its further conversion into 2-furoic acid and 5-carboxy-2-furaldehyde on treatment with acid.

9.3.4 Methanolysis

An alternative method for the analysis of monomeric sugar units is based on acid hydrolysis in methanol (methanolysis). The reaction results in a conversion of the polysaccharides to the corresponding methylglucosides with a major advantage being a much better preservation of the uronic acid groups. In the reaction, the latter are esterified and this promotes a more complete hydrolysis of otherwise resistant glucosidic linkages such as those between xylose and 4-O-methyl-glucuronic acid in xylans.

9.3.5 Permethylation

Information about the linkage pattern in polysaccharides can be obtained through permethylation analysis. As an alternative, the linkage pattern in polysaccharides can also be analysed by two-dimensional (2D) NMR techniques. The reaction sequence in the permethylation analysis involves a complete methylation of all hydroxyl groups in the sample with e.g. methyl iodide in

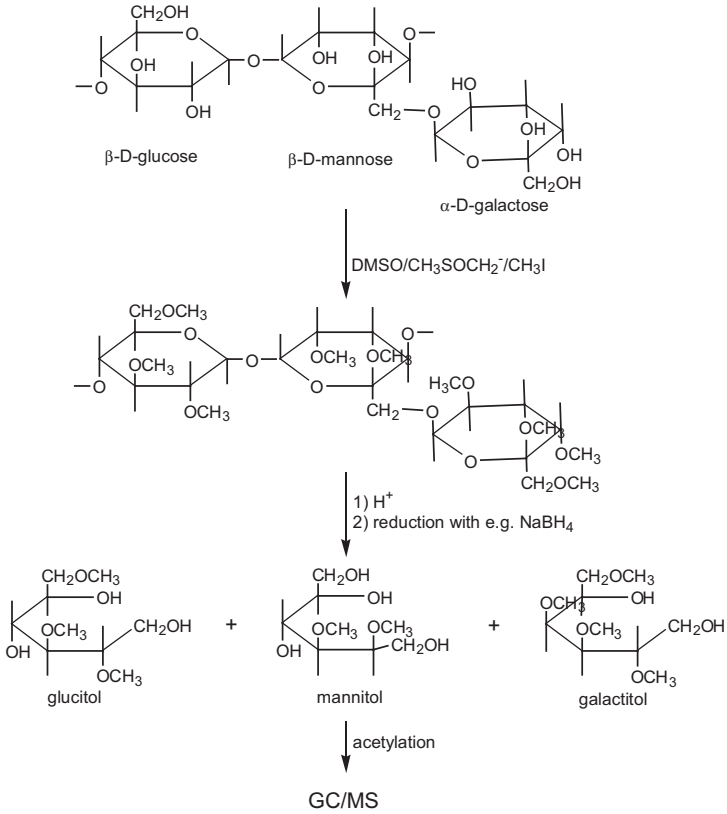

Figure 9.8. Scheme for the determination of linkage patterns in polysacharides using permethylation analysis on a segment of galactoglucomannan.

DMSO with dimethyl sulfinyl anion as the base followed by acid hydrolysis. Reduction of the anomeric carbon atoms with sodium borohydride to form hydroxymethyl groups and finally acetylation of all hydroxyl groups results in a mixture of methylated alditol acetates which can be separated by gas chromatography and identified with mass spectrometry (*Figure 9.8*). If carboxyl groups are present in the sample, these must be eliminated prior to the methylation step.

9.4 Lignin

The chemical structure of lignin is completely different from that of the polysaccharides with the major feature being the presence of aromatic rings. As discussed in Chapter 6, these rings are connected to each other through ether and carbon-carbon linkages both in side-chains and directly between the aromatic rings. The presence of aromatic rings makes it possible to utilize UV-light spectroscopy to detect lignin and this feature is commonly used both in the industry and in the laboratory. Thus, the degree of delignification in kraft pulping can be followed by the degree of UV-absorbancy of the pulp or in the cooking liquor. For soluble lignins, further use of UV-spectrometry is in the quantification of phenols and other specific groups such as conjugated carbonyl groups and double bonds. Absorption spectra of spruce and beech MWL are shown in *Figure 9.9*.

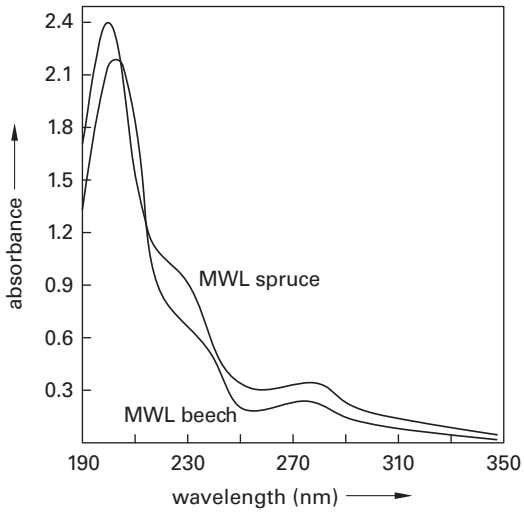

Figure 9.9. UV-light spectra of spruce and beech MWL.

The complex structure of lignin has been a challenge for wood chemists for long times and different chemical methods for the elucidation of the structure have been developed and optimized. All these are based on chemical degradation of the lignin polymer and identification of the fragments that are formed. Most of these methods suffer, however, from the fact that they do not result in a quantitative yield of degradation products and, usually, correction factors have been used in attempts to reconstruct the lignin structure. In addition to the structural analysis,

specific methods for the analysis of various functional groups have been developed. With the introduction of NMR spectroscopy, great advances in the structural elucidation of the polymeric lignin has been obtained albeit only on lignin samples that can be isolated and subsequently dissolved in a suitable NMR solvent.

9.4.1 Isolation of Lignin

The isolation of lignin from wood or other biomass, can be achieved by extensive milling of the extractives-free material in a vibratory mill or a ball mill followed by extraction with a good lignin solvent such as dioxane (with some water to increase swelling). The Milled Wood Lignin, MWL, that is obtained has been extensively used for structural studies of a variety of wood species although the polymer is degraded to some extent as a result of the milling procedure. Thus, the content of phenolic hydroxyl groups is much higher in MWL as compared to the value obtained on direct analysis of the corresponding wood sample, viz. around 20 instead of 10–12. This increase, most probably, originates from a homolytical cleavage of (predominantly) β-O-4 structures in the native lignin polymer. Thereby, new phenolic hydroxyl groups are formed together with lignin end groups of the Hibbert ketone type (*Figure 9.10*). For the isolation of lignin from kraft pulp, methods based on acid and/or enzymatic hydrolysis of carbohydrate-carbohydrate and lignin-carbohydrate linkages are frequently employed whereas lignin from alkaline pulping liquors can be isolated by precipitation with acid.

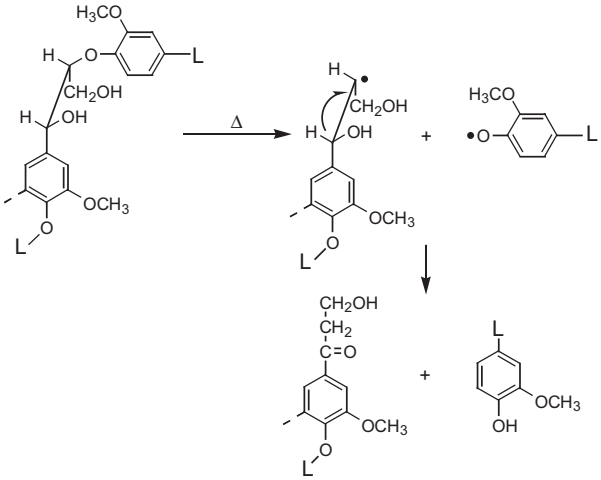

Figure 9.10. Homolytic cleavage of a β-O-4 structure in lignin and formation of a new phenolic end-group together with an α-keto-structure (a Hibbert ketone). L denotes a lignin residue.

9.4.2 Methoxyl Groups

Lignin is a multi-functional macromolecule with high reactivity towards a variety of chemical reagents under acidic as well as alkaline conditions. The characteristic feature of a lignin (or lig-

nan) is the presence of aromatic methoxyl groups. Except for the p-hydroxyphenyl units constituting part of compression wood and grass lignins, virtually all aromatic units contain at least one methoxyl group. This can be analysed by reacting the sample with hydriodic acid followed by gas chromatographic analysis of the methyl iodide that is formed (*Figure 9.11*).

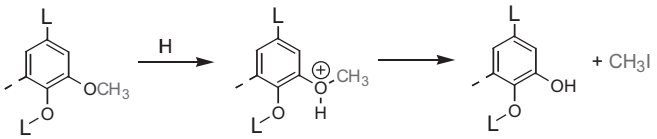

Figure 9.11. Cleavage of methyl aryl ether groups in lignin by hydriodic acid (Zeisel procedure).

9.4.3 Oxidation with Permanganate – Hydrogen Peroxide

For the direct chemical analysis of isolated lignin or of lignin present in wood or pulps, degradation methods employing oxidation with potassium permanganate – hydrogen peroxide or ozone can be used. Among other methods, acid hydrolysis in the presence of ethanethiol, thioacidolysis, is the most important. The first of these reactions is outlined in *Figure 9.12*. In a four-step procedure, the lignin-containing sample is first alkylated to protect the phenolic hydroxyl groups. Oxidation in two steps with potassium permanganate and hydrogen peroxide converts all side-chains to aromatic carboxyl groups and these are finally esterified to provide derivatives suitable for gas chromatographic analysis. The overall reaction results in a mixture of mono-aromatic and di-aromatic carboxylic acids (*Figure 9.13*). The product pattern acts as a sensitive fingerprint for the lignin structure although the fact that only originally phenolic structures can be analysed sometimes is a serious drawback. Furthermore, the overall yield of products is moderate. Although the method has been used extensively for the analysis of native and technical lignins, its major advantage is the possibility of detecting minor structural units of technical importance such as catechols and hydroquinones (can form quinones), condensed structures (sulfite-based pulping) and chlorinated aromatic structures (bleached pulp and bleaching effluents).

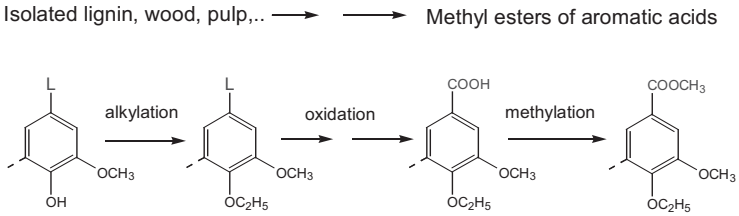

Figure 9.12. Principle for oxidative degradation of lignin with potassium permanganate – hydrogen peroxide. L denotes a lignin residue.

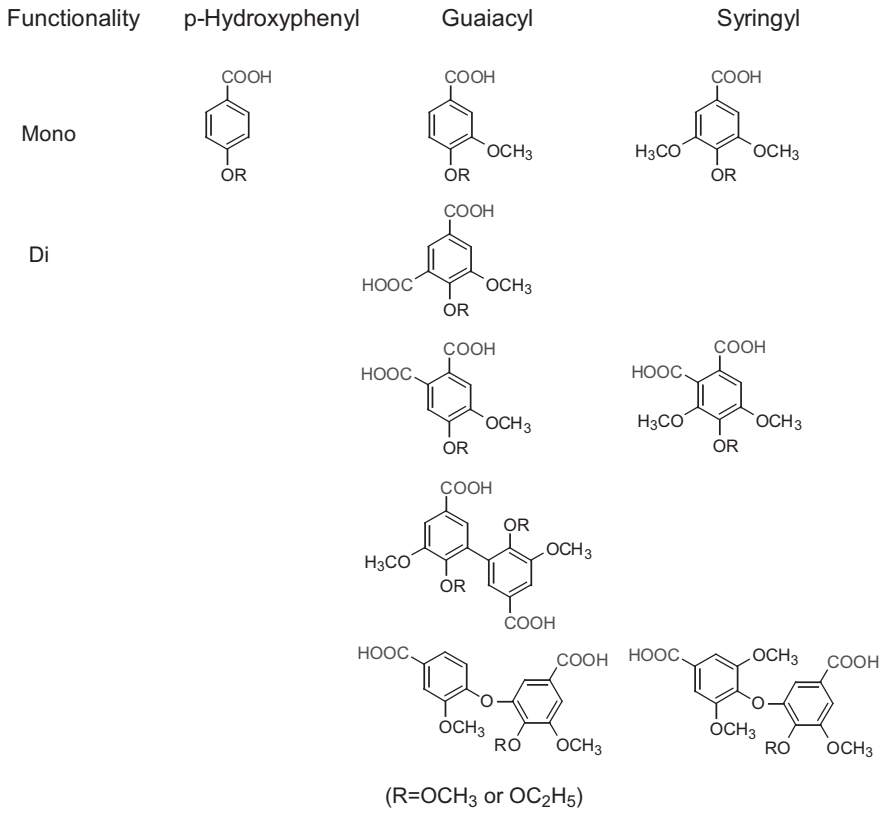

Figure 9.13. Major acids from oxidative degradation of lignin with potassium permanganate – hydrogen peroxide.

9.4.4 Ozone Oxidation

Oxidative degradation of lignin with ozone results in a degradation of the aromatic rings by ozonolysis of the double bonds to form carboxylic acid residues attached to the original side chain. Thus, this method supplements the permanganate – hydrogen peroxide method described above since, here, the side chains are analysed. For certain sub-structures in lignin such as the β-O-4 structure, the method provides quantitative information of the frequency as well as of the ratio of erythro to threo forms since the stereochemistry of the side chain is retained (*Figure 9.14*).

On ozonolysis, lignin sub-structures of the β-5 (phenylcoumaran) and β-1 (1,2-diaryl-1,3-propanediol) types both give rise to 2-hydroxy-3-hydroxymethylbutanedioic acid. The latter lignin structure is present in both the erythro and threo form and, consequently, both forms of the acid are formed. The β-5 structure, on the other hand, is only present in the trans form giving the erythro acid on ozonolysis (*Figure 9.15*). Thus, by quantification of these acids, a good estimate of the proportions of these two sub-structures in lignin can be obtained.

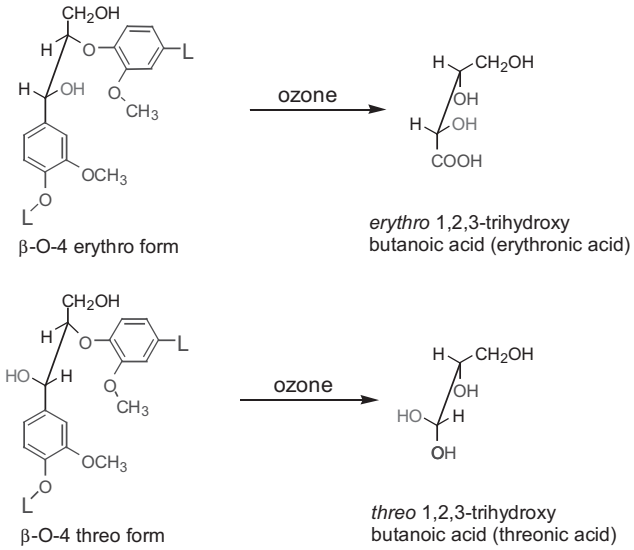

Figure 9.14. Ozonolysis of β-O-4 structures in lignin present as a mixture of erythro and threo forms. L denotes a lignin residue.

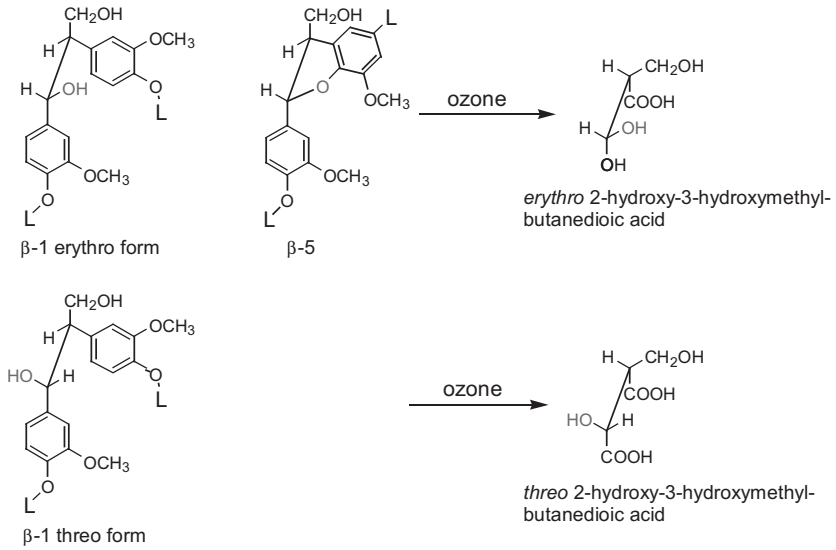

Figure 9.15. Ozonolysis of β-1 and β-5 structures in lignin. L denotes a lignin residue.

9.4.5 Thioacidolysis

To date, the most powerful wet chemical degradation method of lignin is thioacidolysis with ethanethiol which can be used to quantify the β-O-4 structures present in a sample of wood, pulp or isolated lignin. The method relies on a selective hydrolysis of the β-aryl ether linkage with formation of a mixture of the erythro and threo forms of an ethylthio derivative which can be quantified by gas chromatography. For lignins from different types of origin, the method can be used to distinguish between guaiacyl, syringyl and p-hydroxyphenyl based units and, since the method gives high yield of the product, an accurate estimate of the amount of β-O-4 structures can be obtained. The reaction sequence is shown in *Figure 9.16* and involves a successive replacement of the side chain oxygen functions with thioethyl groups using a Lewis acid, boron trifluoride, as the catalyst.

When the thioacidolysis reaction is applied on a wood sample, a rather complete degradation of lignin to low molecular weight products, predominantly containing monomeric – trimeric phenylpropane units, takes place. Except the major product, shown in *Figure 9.16*, a variety of dimers and trimers containing stable carbon-carbon and carbon-oxygen linkages such as in 5-5, 4-O-5, β–β and β-1 structures can be identified. Thus, the thioacidolysis reaction constitutes an almost ideal delignification reaction. Unfortunately, the commonly used technical delignification processes are far less efficient.

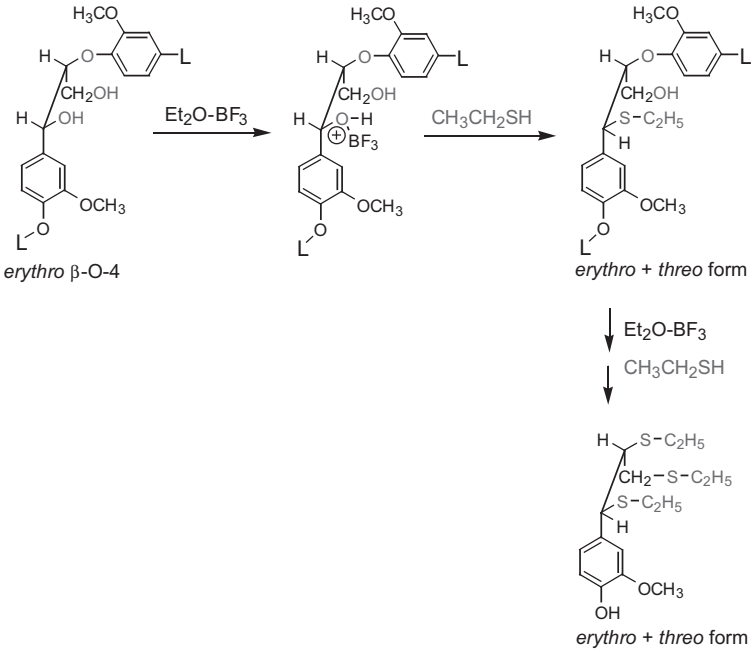

Figure 9.16. Mechanism for the degradation of a β-O-4 structure by thioacidolysis. L denotes a lignin residue.

9.4.6 Phenolic Hydroxyl Groups

The content of phenolic hydroxyl groups in lignin is of great importance in several different technical processes such as in kraft pulping, in bleaching and in reactions resulting in oxidative discoloration of pulps. For wood and pulp samples, two methods have been developed which give similar results. In the first of these, the lignin-containing sample is oxidized with sodium periodate and provided the phenolic group has at least one o-methoxyl substituent, the methyl group is split off as methanol which can be quantified by gas chromatography. The reaction is shown in *Figure 9.17*.

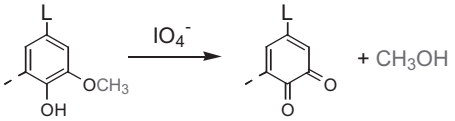

Figure 9.17. Determination of phenolic hydroxyl groups in lignin by periodate oxidation.

In a somewhat more complicated procedure, aminolysis, a sample is first subjected to a complete acetylation. For lignocellulosic samples, however, the acetylation must be preceded by a reductive step with sodium borohydride in order to convert all reducing sugar end-groups to the corresponding alditoles. In a second step, the aromatic acetyl groups are selectively eliminated by treatment of the sample with pyrrolidine under mild conditions and quantification of the 1-acetylpyrrolidine that is formed (*Figure 9.18*). The advantage of the method rests on the fact that also p-hydroxyphenyl structures can be included in the analysis. Both these methods for phenolic group analysis are time-consuming and require skilled operators. For the analysis of isolated lignin samples, further methods exist based on e.g. UV-light absorption or titration. Aminolysis can also be used for analysis of the total content of hydroxyl groups in a sample by adjusting the reaction conditions in the pyrrolidine treatment step such that all acetyl groups are converted into 1-acetylpyrrolidine.

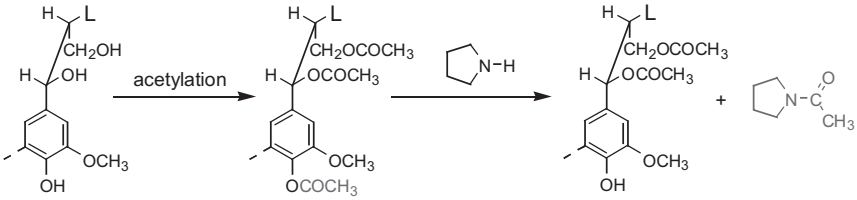

Figure 9.18. Determination of phenolic hydroxyl groups in lignin by aminolysis.

9.4.7 NMR Analysis

For isolated lignin samples from wood or pulp, one-dimensional (1D) ^{1}H and ^{13}C NMR as well as combinations of these in various two-dimensional (2D) pulse sequences constitute, by far, the most informative type of analysis. To date, a large number of individual signals has been assigned in the NMR spectra from native and technical lignins, in most cases by comparison with model compounds. With few exceptions, the possibility of getting quantitative information

from 1D-spectra is, however, low due to the extensive overlap of the various signals. One example is shown in *Figure 9.19* and illustrates that a good separation of aromatic and aliphatic carbon atoms is possible in a ^{13}C NMR spectrum. The signals are broad, however, and do not permit accurate integration of individual peaks. With the continous development of both the 2D techniques and the instrument performance, lignins can now be analysed with greater accurracy and, for certain sub-structures and functional groups, quantification is possible.

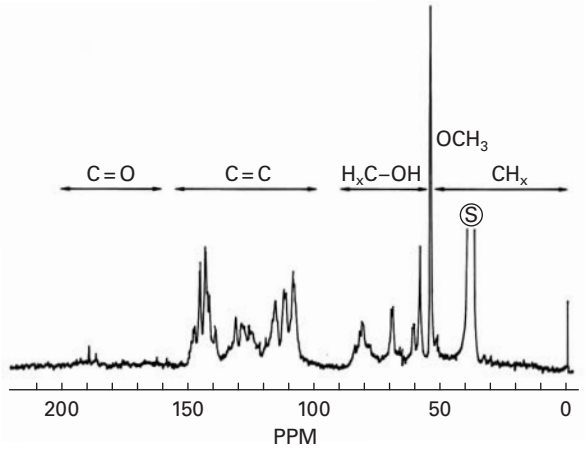

Figure 9.19. ^{13}C NMR of milled wood lignin (MWL) from spruce. The regions of different types of carbon atom signals are denoted in the spectrum. (CH_x = CH or CH_2. S = solvent.)

In correlation spectra of the HMQC type, the various types of CH-groups present in a lignin sample can be analysed. By a careful choice of operating parameters in the NMR experiment, the signals can be integrated in the z-direction, and, since the separation of the individual signals is much higher in a 2D spectrum, pure signals are often possible. In *Figure 9.20*, a portion of an HMQC spectrum of a native lignin sample from spruce is shown together with the proper assignments of individual signals. The corresponding ^{1}H and ^{13}C NMR spectrum are also shown on the x-axis and y-axis respectively.

9.5 Extractives

The extraction of wood, pulp or paper with an organic solvent (cf *Figure 9.1*) like acetone (with 10 % water to increase the swelling) is used to isolate the low molecular weight organic material present in the sample. The further analytical protocol is much dependent on the problem to be solved. In many cases, only a group separation is of interest and for such a purpose, derivatization followed by short column gas chromatography or, alternatively, thin layer chromatography can be sufficient. As a result, quantification of the various groups of extractives like fatty acids, resin acids, lignans, sterols, steryl esters and triglycerides is possible (*Figure 9.21*).

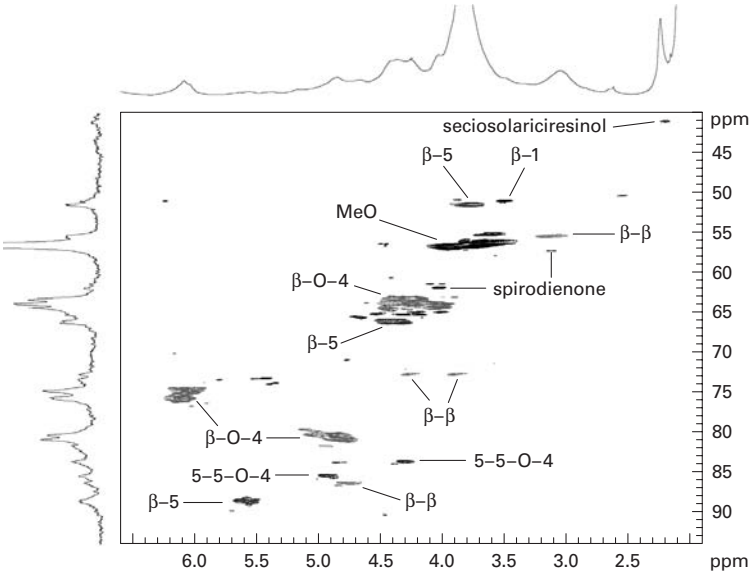

Figure 9.20. Portion of a HMQC spectrum of MWL demonstrating the resolution of individual signals in the region of 40–90 ppm for carbons and 2.0–7.0 ppm for protons.

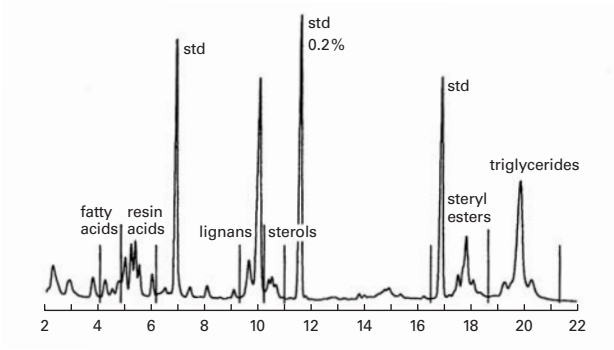

Figure 9.21. Gas chromatographic group separation on a short column of an acetone extract from spruce TMP (Holmbom 1999. With kind permission of Springer Science and Business Media).

For a more detailed analysis of individual components in an extract, gas chromatography, sometimes in combination with mass spectrometry, is the method of choice and performed after suitable derivatization e.g. through silylation. A pre-separation into substance groups is advantageous since, otherwise, the complexity of the chromatogram may prevent a meaningful interpretation (*Figure 9.22*).

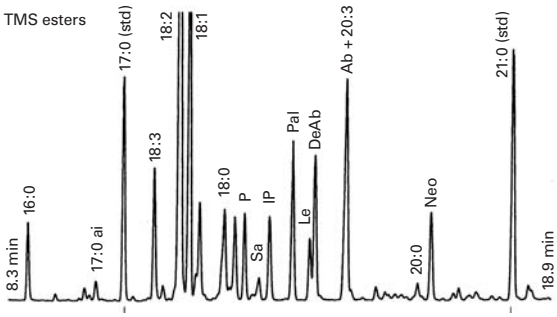

Figure 9.22. Gas chromatographic analysis of fatty acids and resin acids from a softwood kraft soap mixture (Holmbom 1999. With kind permission of Springer Science and Business Media).

9.6 Chemical Analysis of Fibers

9.6.1 Kappa Number

In chemical pulping, determination of the kappa number of the pulp constitutes one of the most common analytical methods and it is used frequently both by industry and research laboratories to evaluate the performance of the kraft cook and the subsequent bleaching operations. In the method, the consumption of acidic potassium permanganate by a pulp sample under standardized conditions is measured by adding an excess of permanganate and determination of the remaining amount after a given time. The reaction steps are shown in *Figure 9.23*. The content of oxidizable groups in the pulp, predominantly lignin, is calculated as the kappa number and used e.g. for process control. The kappa number not only reflects the lignin content of the pulp, however, but also includes hexenuronic acid from xylan as well as different types of functional groups probably formed during the cook and present in the polysaccharides. By the use of model compounds, the consumption of permanganate by various structures assumed to be present in pulp fibers can be calculated. Such values are shown in *Table 9.3* and demonstrate that the aromatic rings present in lignin together with hexenuronic acid from xylan contribute to a major portion of the permanganate consumption. Other functional groups which can react during the conditions of the kappa number measurement, include carbonyl and conjugated carbonyl groups.

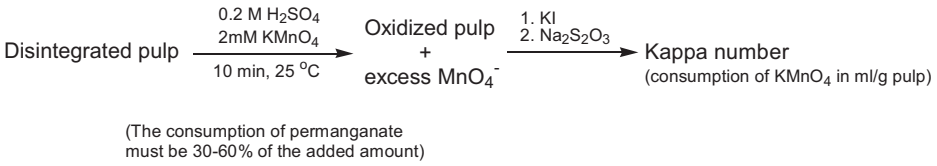

Figure 9.23. Procedure for the determination of Kappa Number according to SCAN-C1:77.

Table 9.3. Consumption of permanganate by lignin and by different functional groups under the conditions of kappa number determination.

Functional group	Consumption of permanganate, Equivalents/mole
Aromatic ring in pulp lignin	11.6
Hexenuronic acid group in pulp	8.5
Aromatic ring in model compounds	~15
Double bond	5.7
Aldehyde	2.4
α,β-unsaturated aldehyde	7.7
α-keto-carboxyl group	2.0
Glucose	0.1

9.6.2 Ionizable Groups

Pulp fibers are negatively charged as a result of the pulping procedure. For mechanical pulps, the major source of charge is from the native carboxyl groups that are present in e.g. xylan and pectin. After peroxide bleaching, further carboxyl groups are formed as a result of hydrolysis of ester groups in the pectin and oxidation of certain lignin structures although, at the same time, some hemicellulose can be lost due to the alkaline conditions. In the production of CTMP, sulfonic acid groups are introduced in lignin resulting in a further increase in the total amount of acidic groups (*Table 9.4*).

Table 9.4. Concentration per gram of fibers of ionizable groups in mechanical pulps. 4-OMe-GlcA = 4-O-methylglucuronic acid in xylan, GalA = galacturonic acid in pectin.

Pulp sample	4-OMe-GlcA, μmol/g	GalA, μmol/g	Total charge, μmol/g
TMP	63	19	85
TMP + alkali	63	85	196
TMP + H_2O_2/HO	58	80	220

In unbleached kraft pulp fibers, the negative charge can originate both from the polysaccharides, with a major contribution from the uronic acid groups in xylan and from lignin. The presence of acidic extractives such as fatty acids can also play a role. In bleaching, some of the acidic groups can be eliminated although large differences exist between different bleaching sequences. Thus, in pulps bleached with only oxygen and hydrogen peroxide, the remaining amount of hexenuronic acid after bleaching can be substantial and contribute to a considerable concentration of charged groups (*Table 9.5*).

Table 9.5. Concentration of ionizable groups per gram of pulp in some kraft pulps. SW and HW = softwood and hardwood respectively.

Pulp sample	Total charge, μmol/g	Uronic acids, μmol/g	Lignin-bound, μmol/g	Surface charge, μmol/g
Unbleached SW, kappa 24	77	40	25	
Bleached SW, OO QQ PO	68			11
Bleached HW, OO Q PO	119			16
Bleached HW, D EO PDD	55			7

Several fiber properties such as swelling and tensile strength are affected by the amount of charged groups present. These can be determined by methods such as ion exchange, conductometric or potentiometric titration. If further details of the location of the charged groups, i.e. surface charge versus total charge, are desired, polyelectrolyte adsorption can be employed (*Table 9.5*). By selecting a cationic polymer with very high molecular mass, only the surface charges are neutralised whereas a low molecular mass polymer can give a complete penetration of the fiber wall. In the latter case, values in close agreement with those from conductometric or potentiometric titration are obtained.

9.6.3 Pyrolysis – Gas Chromatography

Rapid heating (< 0.5 sec) of a small wood or pulp sample (~100 μg) to temperatures in the range of ~600 °C results in a fragmentation of the polymeric material to low molecular weight compounds. These can be separated by gas chromatography and further analysed by mass spectrometry. Both lignin and polysaccharides can be analysed and provide information about the structure of the original polymers. From the polysaccharides, a series of 1,6-anhydrohexoses and 1,4-anhydropentoses are obtained as exemplified in *Figure 9.24*. A relative quantification of these can give information about the monosaccharide composition in the sample.

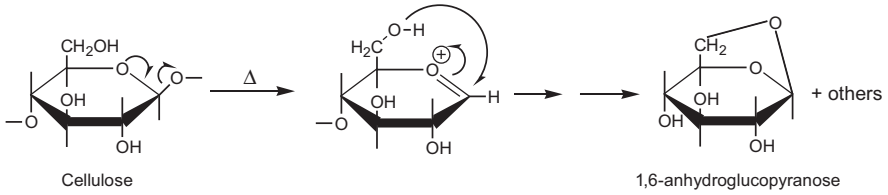

Figure 9.24. Analytical pyrolysis of cellulose and formation of an anhydrosugar.

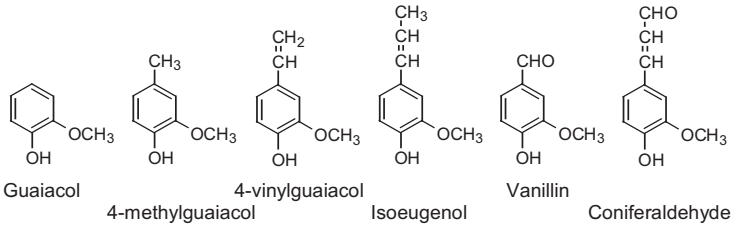

Figure 9.25. Some typical pyrolysis products from softwood lignin in wood or pulps.

From the lignin portion of a sample, a large number of phenols with and without side-chain residues are obtained in which the oxygen substituents attached to the aromatic ring are still present. Thus, the pyrolysis-GC technique is a convenient way of distinguishing between different types of lignin units, viz. p-hydroxyphenyl, guaiacyl and syringyl. Both native and technical lignins give similar degradation products (*Figure 9.25*) albeit in different proportions but the origin of each one of these is not well understood. Consequently, pyrolysis-GC analysis of lignin can only be used to provide a "finger-print" but without much structural information.

9.7 Gel Permeation Chromatography, GPC

The macromolecular properties of both lignin and polysaccharides can be analysed by GPC (also denoted SEC, size exclusion chromatography), thus providing information about the molecular size and the size distribution of the individual polymers. In GPC, a solution of the sample is eluted through a column of a molecular sieving material such as cross-linked polydextran (e.g. Sephadex^R) or semi-rigid and cross-linked polystyrene. The latter can be used in high pressure systems resulting in small column dimensions, short elution times and high resolution power.

For lignin samples, a derivatization is usually necessary in order to assure a complete solubility in a solvent such as tetrahydrofurane. In many cases, this can be achieved by acetylation thereby blocking all types of hydroxyl groups. Oxidized lignins contain carboxyl groups and in such a case, silylation or methylation with e.g. diazomethane must be used. For all types of lignins, a broad molecular mass (size) distribution is obtained as shown in *Figure 9.26*. Although no exact mass can be obtained by GPC, a calibration of the column with polymers of known molecular mass can be done to provide a comparison between the mass and the elution volume. For lignin analysis, polystyrene standards are commonly used whereas for polysaccharides, pullulan mixtures serve the same purpose.

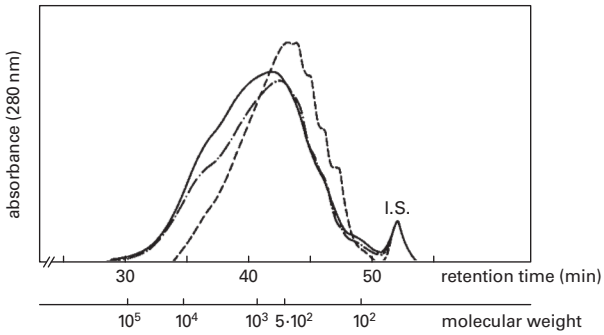

Figure 9.26. GPC of acetylated black liquor lignins from initial (--), bulk (-.-) and residual (—) delignification respectively. I.S. = acetone (internal standard). Calibration with polystyrene standards.

The finding that a mixture of dimethylacetamide and lithium chloride (DMAC-LiCl) is able to dissolve cellulose as well as unbleached and bleached kraft pulp fibers (partially in the case of softwood pulps) has resulted in a rapid development of the GPC-techniques for analysis of

various pulps. Thus, by the use of dual detectors for the simultaneous detection of lignin (UV-absorbance) and carbohydrates (refractive index), it is possible to get information about the composition and molecular mass distribution of the different types of polymers constituting the fiber. One example is shown in *Figure 9.27* and illustrates that in an unbleached birch kraft pulp, both the hemicelluloses and lignin are eluted together indicating that these polymers are linked whereas the (high molecular weight) cellulose only contains very small amounts of UV-absorbing material.

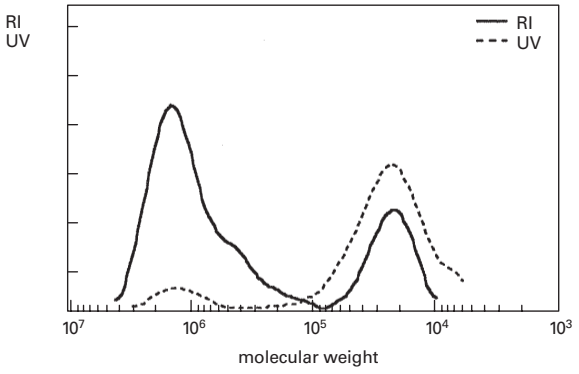

Figure 9.27. GPC of unbleached birch kraft pulp, dissolved in DMAC/LiCl. A series of pullulan standards were used for calibration and detection was done with UV-light at 295 nm and with refractive index (RI) respectively.

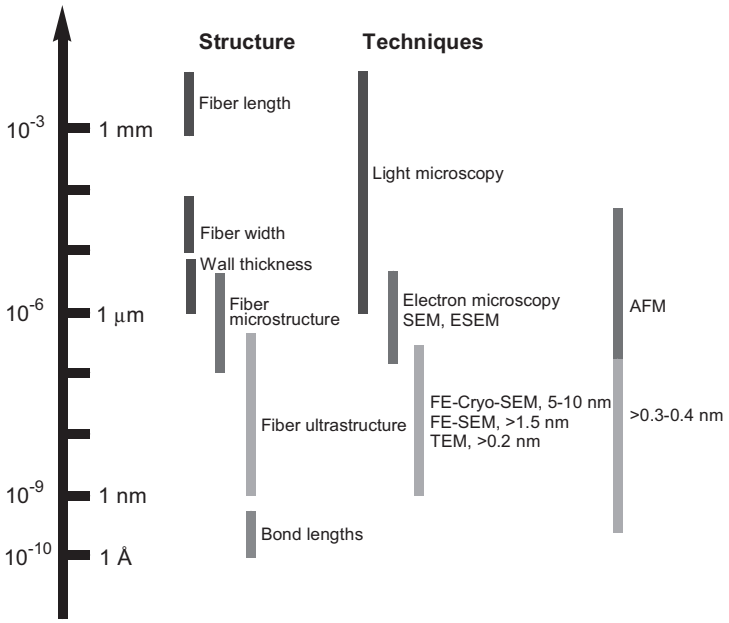

Figure 9.28. Morphological structure levels of fibres and available microscopic techniques.

9.8 Microscopic Analysis of Fibers

Information about the morphological structure of a fiber can be obtained on different levels depending on the type of information that is wanted. This is illustrated in *Figure 9.28* where it is shown that several microscopic techniques are available each one with its own characteristics in terms of information accuracy.

Light microscopy can be used to obtain information about fiber dimensions such as length and width. By applying polarized light, the crystalline cellulose can be readily seen in kraft pulp fibers and features like e.g. dislocations in the fiber wall can be visualized. For the more detailed information about the fiber wall structure, electron microscopic techniques like Scanning Electron Microscopy (SEM), Environmental SEM (ESEM) and Transmission Electron Microscopy (TEM) are available. As a complementary and powerful analysis, the development of atomic force microscopy (AFM) has made it possible to analyse the surface topography down to a few Ångström in resolution power. In *Figure 9.29*, examples of the various techniques are shown.

9.9 Further Reading

9.9.1 General literature

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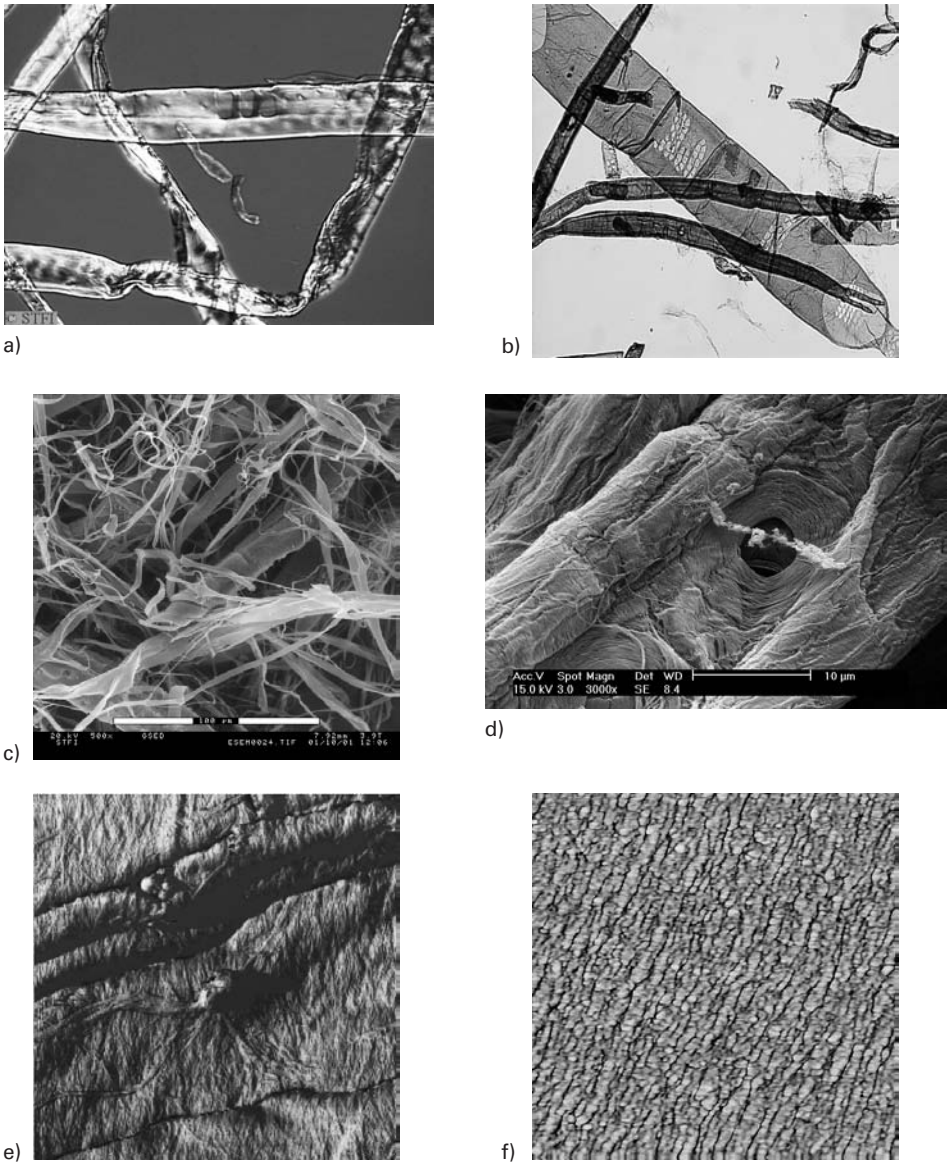

Figure 9.29. Light microscopy of a) pine kraft pulp (100×) and b) aspen kraft pulp (200×). ESEM of c) spruce TMP, 50–200 mesh (500×) and d) pine kraft pulp (3000×). AFM of e) fiber surface (5 × 5 µm) and fibrillar aggregation (1 × 1 mm, aggregate dimensions 17 nm) (Courtesy: Joanna Hornatowska, STFI-Packforsk AB).

10 Biological Wood Degradation

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10.1 Introduction

Wood is a biological material and is, like most such materials, degraded by a variety of organisms. Wood degradation constitutes an important part of carbon cycling, a process that is required for a continued biological life on earth. Various organisms have become adapted to wood degradation in very diverse environments, terrestrial and aquatic. Biological degradation appears to be prevented only by complete absence of oxygen, extreme temperatures or enclosures that prevent the access of organisms. Environments lacking oxygen can be found in very deep waterlogged sediments. Wood is known to have survived in such environments for ca. 10 000 years. Enclosures can be found in the form of deep burial under sediments, where high temperatures and pressures deny access and life of organisms. Such wood is slowly undergoing coalification leading to formation of brown coal. Here, the non-coalified parts of wood may look and smell like fresh wood even after a million years.

The wood-degrading organisms are found in quite diverse groups; insects, molluscs, fungi and bacteria. In addition, mechanical damage to wood is caused by higher animals, such as beavers, rats and woodpeckers. Termites represent the most important group of insects that are specialised in living on wood. The larval stages of other insects represent another form of insect

attack. Shipworm (*Teredo*) is a mollusc which causes very rapid destruction of wood in marine environments. This review will only deal with microbial degradation of wood, i.e. caused by microorganisms, fungi and bacteria. Degradation or decay is used here to imply attack on the wood structural components.

All wood-degrading fungi are filamentous. This means that the organisms form thin microscopic threads, called hyphae (*Figure 10.1*). The hyphae exude enzymes and absorb nutrients from the immediate surroundings. The hyphal system also allows translocation of elements over long distances. The hyphae grow in length by extension of the hyphal tip (apical growth). A mass of hyphae form a mycelium, often visible to a naked eye (*Figures 10.2* and *10.3*). Substrates, like wood, are invaded and colonised through hyphal growth. Some fungi can form aggregates of hyphae, rhizomorphs, which makes it possible to reach substrates considerable distances away. Yeasts, which are unicellular fungi, frequently occur in wood, but they are unable to degrade it. Fungi form spores by a sexual process, but may also form spores asexually. The sexual spores are produced in fruit bodies, whose structure is typical for each species (*Figure 10.4*). Fungi are heterotrophic organisms that depend on organic carbon; they derive their energy from a saprophytic or parasitic life.

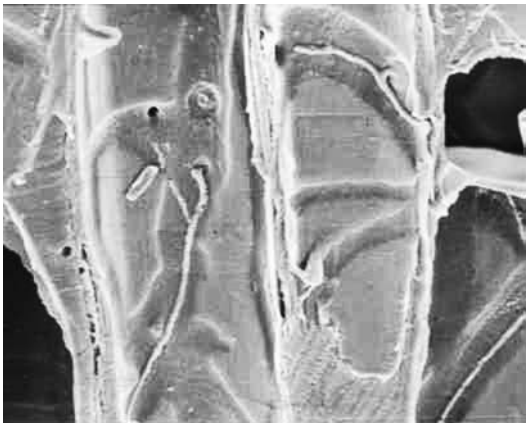

Figure 10.1. Hyphae of the white-rot fungus *Phlebiopsis radiata* growing in pine tracheids. Note erosion of the tracheid walls along the hyphae. SEM.

Figure 10.2. Mycelium of a white rot. fungus growing over discoloured birch wood chips. Note the bleaching effect.

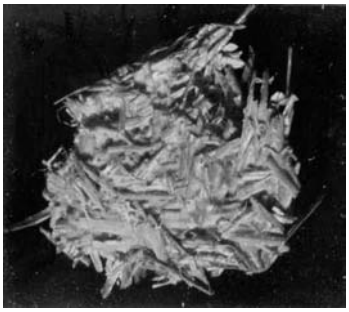

Figure 10.3. Mycelium of a *Pycnoporus* embedding wheat straw.

The main wood-inhabiting organisms of the Kingdom of Fungi are found in the classes *Zygomycota*, *Ascomycota* and *Basidiomycota* and the wood-degrading bacteria belong to the Eubacteria. *Figure 10.5* illustrates their phylogenetic relations. The classification is based on the form the sexual phase of the fungal life cycle. Some fungi, the *Deuteromycetes* or *Fungi Imperfecti*, only produce asexual spores. Other characteristics suggest that most Deuteromycetes in fact belong to the *Ascomycota*. Members of *Zygomycota* are not able to degrade wood, but some species within genera like *Mucor* and *Rhizopus* occur as moulds on timber. Degradation of wood is caused by fungi from the classes of *Basidiomycota*, *Ascomycota* and the *Deuteromycetes*. *Basidiomycetes* are usually referred to as higher fungi and the wood-degrading species are sometimes called "*true wood-decay fungi*". They often form macroscopic fruit bodies such as brackets and mushrooms. *Ascomycetes* and *Deuteromycetes* are usually referred to as "microfungi" due to their generally microscopic appearance. Some are capable of degrading wood, by causing soft rot, other species may cause sapstain or grow as moulds on wooden surfaces. Most wood-degrading organisms are also able to degrade other types of lignocelluloses, such as straw, bagasse and various plant components such as bark, needles, cones etc. The dominant degraders of such substrates in nature are, however, organisms that are specifically adapted to a particular substrate.

Figure 10.4. Fruit bodies of the brown rot fungus *Laetiporus sulphureus* on living ash tree (*Fraxinus excelsior*).

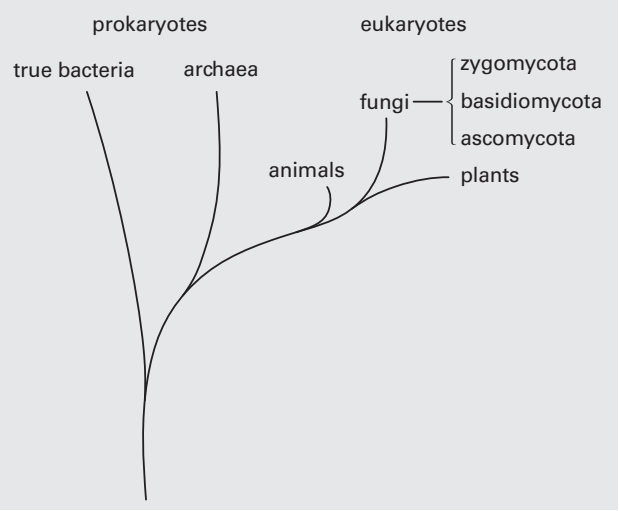

Figure 10.5. Phylogenetic tree illustrating the position of the wood-degrading microorganisms; true bacteria and fungi.

Kingdom Fungi

Classes		
Zygomycota	*Ascomycota*	*Basidiomycota*
Moulds	Moulds, sapstain	White rot
	Soft rot	Brown rot

Wood degradation may start already in the heartwood in the living trees (*Figure 10.6*). The most well known attack of this type is the root rot in spruce, caused by the root rot fungus (*Heterobasidion annosum*.). Decay is known to occur during storage of wood, in the form of logs or pulpwood chips. Longterm use of wood for constructional purposes often runs a risk of decay if measures not are taken to prevent the attack.

Figure 10.6. Brown rot in the heartwood of a pine tree. The rot started in the living tree.

Bacteria are taxonomically very distant from fungi (*Figure 10.5*). Fungi are eukaryotes, having a true cell nucleus, whereas bacteria are prokaryotes lacking a nucleus. Most bacteria are single celled organisms, but some form thin hyphae from which spores are produced. Hyphal forms are called actinomycetes. So far, active wood degradation has only been observed for single celled bacteria.

10.1.1 Wood as a Substrate for Microorganisms

Decay of wood results in a number of physical and chemical changes in the wood structure. The most obvious result is the loss of wood substance, through conversion to carbon dioxide and water, which leads to a decreased density of the material. The strength of wood and of individual fibres is greatly reduced, often already at rather small losses of wood substance. Brown rot decay also leads to increased solubility in hot water and in alkaline solutions (1 % sodium hydroxide). Viscosity of holocellulose and degree of polymerisation is also reduced, the extent depends on the type of decay.

10.1.2 Morphological Aspects of Wood

The structure of wood is described in Chapters 2 and 3. From a microbial perspective wood is a rather porous material, thus easily accessible even for microorganisms, such as yeasts, staining fungi and mould fungi and many bacteria, that are unable to degrade the lignified wood cell walls. Colonisation, often occurs along the natural routes provided by the structure, such as rays, resin canals, vessels and pits. Most wood-inhabiting fungi are also capable of growing through the wood cell walls, thereby causing bore holes of varying sizes.

The wood-degrading microorganisms may grow and affect degradation of the wood cell walls either in the cell lumena or within the cell walls. This suggests that the cell wall thickness has some influence on the degradation process. However, it is important that we do not apply human perspectives when trying to consider the influence of factors such as high density and narrow annual rings. We may feel that such wood would be difficult to degrade, but the microorganisms seem to be less bothered. Most experiments suggest that high density and narrow annual rings have very little influence on the rate of degradation.

It seems that most wood-degrading microorganisms have adapted their strategies to the natural structure of wood. This makes it possible for the organisms to separate different stages of the degradation process both in time and in space in a way that is not possible when the organisms are grown in liquid cultures or on milled wood. At a microscopic level morphological differences can clearly be seen between the different decay forms. The highly lignified middle lamella and S3 layer in soft-woods are more resistant to microbial degradation. Some resistance of the S1 layer is often observed. Spatially there are two distinct possibilities for growth at the fibre level, either in the cell lumen or within the fibre cell wall. Some species make use of both ways.

10.1.3 Chemistry of Wood and Microbial Degradation

The chemistry of wood is described in Chapters 4 to 6. The main components are cellulose, hemicelluloses and lignin. The carbohydrate polymers are embedded in a lignin matrix which protects them from being degraded at the same rate as the non-associated carbohydrates. The high durability of wood when compared with paper or cotton is an effect of the lignin. There is also a general trend that durability increases with increasing lignin content. The higher susceptibility of hardwoods is due to the lower content of lignin, rather than to any anatomical differences. This is supported by the observations, that the susceptibility of certain highly lignified tropical hardwoods, is comparable to that of the softwoods.

Ordinary cellulolytic moulds and bacteria are mostly incapable of degrading wood. These organisms are generally only capable of degrading the non-lignified tissues, such as pit membranes and some ray parenchyma cells. Slight attack can, however, be induced over long time in low lignin hardwoods, *e.g.* aspen and birch wood, after extended exposure and particularly if extra nitrogen is provided.

All groups of wood-decaying microorganisms have some means of overcoming the hindering effects of lignin. White rot fungi and the wood-degrading bacteria degrade the lignin, soft rot fungi appears somehow by growing within the wood cell walls at least to some extent reduce the hindering effects of lignin. Brown rot fungi, finally, has a way of simply "extracting" the carbohydrates from the wood cell walls, with only minor modifications of the remaining lignin. Wood decay will often lead to changes in the proportions of the main components in the remaining wood material.

Differences in chemical composition between different wood cell types will influence the degradation. This is not so evident in softwoods where 95 % of the wood volume is occupied by tracheids. It has been observed, however, that ray tracheids generally are less degraded and that ray parenchyma and epithelial cells associated with resin canals in spruce are more resistant than the tracheids. Chemical differences in cell wall composition between the many different cell elements in hardwoods effects the degradation. It is a general observation that the vessel elements, which are highly lignified and contain a greater proportion of guaiacyl lignin, are more resistant than the fibre cell walls. Several decay organisms are unable to degrade the middle lamella, probably due to the high lignin content and a more condensed lignin structure.

Extractives occur in varying amounts depending on the type of timber. Heartwood generally contain higher amounts of extractives. In certain timbers, like pine, this results in a considerable increase in durability. The durability varies depending on the type of degrading organism and also on the location of the extractives at a microstructural level. Extractives are usually found in parenchyma cells and resin canals, where they are being utilised by many sapstain fungi as a nutrient source. Extractives are often present also in fibre cell lumena and even within the fibre cell walls. Extractives may also protect dried wood from attack by retarding uptake of moisture.

Extractives can be modified or degraded. Sapstain and mould fungi are often able to utilise extractives as a source of nutrients. It was observed during early studies on pulpwood chip storage that some of the more common moulds could live on extractives as a sole source of carbon.

10.2 Overview of Wood-attacking Microorganisms

The definitions of the fungal decay types are arbitrary and based on a mixture of taxonomic, chemical and micromorphological criteria. This has lead to some confusion with respect to certain fungi. The definitions used here follows Nilsson (1988). He suggested that all white- and brown-rotting fungi belong to the basidiomycetes. The remaining wood-degrading fungi, *i.e.* ascomycetes and deuteromycetes are regarded as soft rot fungi. The high variability within the groups causing white rot and soft rot makes a clear differentiation difficult. The fact that many fungal species have many different ways of degrading the wood and that these may be influenced by environmental conditions causes more confusion. The biochemistry of microbial degradation of wood is described in chapter 11.

10.2.1 White Rot Fungi

White rot is defined as decay caused by basidiomycete fungi that are capable of an extensive degradation of lignin, including the more heavily lignified middle lamella. In the literature, higher ascomycetes such as *Daldinia*, *Hypoxylon* and *Xylaria*, are often referred to as white rot fungi. This is based on their rather high rate of wood decay, especially in low lignin hardwoods and a quite strong attack on the lignin, plus the fact that several species bleach the substrate. They cannot, however, degrade the middle lamella, not even in the hardwoods.

White rot is caused by a very large number of basidiomycetes. In addition to those living on wood, most of the litter-degrading basidiomycetes also cause white rot. White rot attack is highly variable in its chemical effects on the substrate and also with regard to the micromorphological effects. This may even apply to a single species and it has been observed that environmental conditions often influence the decay patterns.

Figure 10.7. Typical white rot attack in wood. Note the bleaching and the fibrous appearance.

Figure 10.8. White rot erosion of tracheid walls in pine wood. (light microscopy).

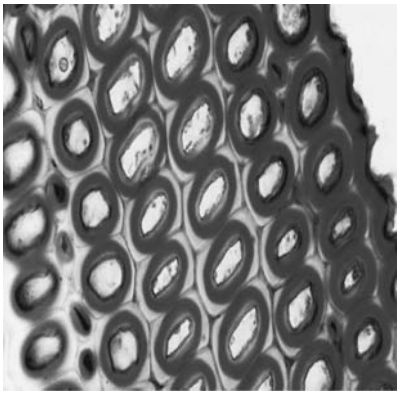

Figure 10.9. Preferential degradation of lignin in pine wood by the white rot fungus *Ceriporiopsis subvermispora*. The red zone next to the lumen indicates area of lignin degradation.

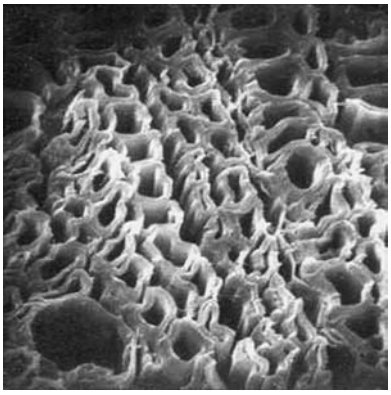

Figure 10.10. Birch wood selectively delignified by a white rot fungus. Note the seemingly intact fibre separating from each other.

One common form of white rot results in a rather uniform depletion of cellulose, hemicelluloses and lignin, *i.e.* the proportions between these components remain fairly constant. White rot of this kind has been referred to as *simultaneous* white rot. Macroscopically the wood appears bleached and somewhat fibrous (*Figure 10.7*). Microscopically a gradual erosion, caused by hyphae in cell lumena, can be seen leading to increasingly thinner cell walls (*Figures 10.1* and *10.8*). After removal of the cell wall, the middle lamella will finally be degraded. Thus, nothing remains of the wood structure. These white rot fungi are the only microorganisms that can cause a complete degradation of the wood structure.

Another type of white rot is characterised by preferential removal of lignin and hemicelluloses, leaving defibrated cellulose fibres behind. These fibres may eventually be degraded in the final stages of decay. Macroscopically the wood appears bleached and distinctively fibrous. The degradation is caused by hyphae situated in the cell lumena. The first effect on the wood cell walls can be seen, using light microscopy, as an increased uptake of safranin stain in a narrow band next to the lumen (*Figure 10.9*). This zone later becomes delignified, and the zone with increased safranin uptake has now moved further into the cell wall towards the middle lamella. This process continues, leading to a complete delignification of the wood cell wall. Finally, the middle lamella is degraded, leading to a separation of the individual fibres (*Figure 10.10*). Chemically, this type of white rot leads to a decrease in lignin and hemicelluloses, whereas the cellulose content remains constant. Several studies have suggested that the biologically delignified cellulose remains unchanged. One study on heavily delignified birch wood fibres suggested, however, that the remaining cellulose was extensively fragmented. This could have been a result of radical reactions during the degradation of lignin. The idea to use white rot fungi for "biopulping" is quite old, but it is only in recent decades that more serious studies have been carried out. The research has focused on selection of very active species, such as *Ceriporiopsis subvermispora*, and production of mutants that do not degrade cellulose. Most studies have concerned mechanical pulping operations, where fungal pretreatment has been reported to result in considerable energy savings. So far no largescale industrial operations seem to exist. Biological delignification has also been found to considerably increase the digestibility of straw intended to be used as cattle feed. The unique ability of white rot fungi to degrade complex organic molecules have been explored for the remediation of sites contaminated by environmental pollutants.

As stated above, white rot fungi are highly variable; this is exemplified by the commonly observed occurrence of simultaneous and preferential rot within a wood block inoculated with only one white rot fungus. The type of attack may also be influenced by addition of sugars or nitrogen.

White rot fungi also frequently grow through neighbouring fibre walls. This leads to the formation of a small bore hole, which sometimes may enlarge dramatically (*Figure 10.11*). Such large bore holes should not be confused with the preferential attack on bordered pits in softwood tracheids, which gives the impression of bore holes when the pit borders have been removed. Growth and irregular branching of hyphae within the fibre cell walls and degradation of the adjacent wood substance, may also lead to a near complete degradation. Cavities, more or less similar to those produced by soft rot fungi, have been observed for a number of white rot species.

The fact that white rot fungi gain access to the cellulose and hemicelluloses in wood through degradation of the lignin component explains why more lignified timbers are less susceptible to white rot. White rot attack may be quite uniform throughout the wood, but some species cause

distinct pocket rots. Wood attacked by such species has a mottled appearance and is characterised by localised areas, pockets, of heavily degraded wood surrounded by sounder wood. The decay observed in the pockets is often characterised by a delignified fibrous tissue. Differences in susceptibility have also been observed between early- and latewood tracheids in softwoods. Some species of white rot seem to preferentially degrade earlywood, whereas other species prefer the latewood.

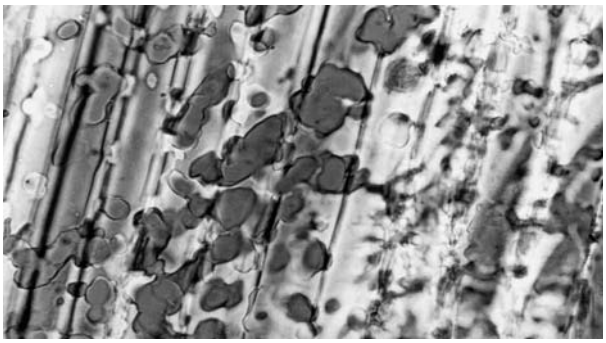

Figure 10.11. Large bore holes in birch wood fibres as a result of white rot attack (light microscopy).

Wood-degrading fungi generally have an ability to translocate and redistribute elements from one place to another. Some white rot fungi have been observed to redistribute manganese or translocate this element from sources external to the wood. Manganese can often be seen as blackish specks in the wood. This is a typical feature of wood degraded by the root rot fungus (*Heterobasidion annosum*). Increased levels of potassium, calcium and iron have also been observed.

Chipping of white-rotted wood yields more pin chips and fines. Studies on kraft pulping of white rotted wood have shown decreases in pulp yields. The losses are not so significant when the losses are calculated on weight basis. The yields may even be slightly higher compared with sound wood, due to a selective removal of the lignin. The losses become more evident when calculations are done on a volume basis, due to the decreased density of the wood. Alkali requirements have been found to increase for rotted wood. White rot results only in small changes in the DP of cellulose, but the strength is usually reduced in relation to the level of attack. However, published results from pulping experiments suggest great variations in the effect on strength parameters. A conclusion is that white rot decay decrease pulp yield and quality, but not disastrously. Strength properties may actually increase for mechanical pulps after incipient decay. This has been demonstrated in biopulping experiments. The discovery that white rotted wood may contain increased levels of manganese means that it is no longer acceptable.

10.2.2 Brown Rot

Brown rot is caused exclusively by basidiomycete fungi. This form of decay has unique features that do not show little resemblance to other types of decay. Brown rot fungi degrade cellulose and hemicelluloses and leave the lignin as a slightly modified residue. Strongly degraded wood

is brown in colour (*Figure 10.12*), hence the name of this rot. Heavily degraded wood has very little residual strength and can easily be powdered between the fingers. At drying, the wood cracks in a cubical manner (*Figure 10.12*).

Figure 10.12. Brown rot in pine wood. Note the cubical cracking.

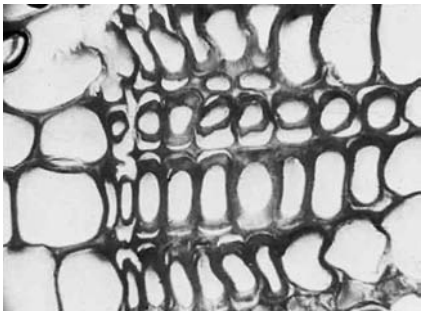

Figure 10.13. Brown rot in pine wood. Most carbohydrates have been degraded, leaving a lignin skeleton (light microscopy).

Figure 10.14. Brown rot in pine wood. Only the crossfield pit areas exhibit birefringence (light microscopy, polarized light).

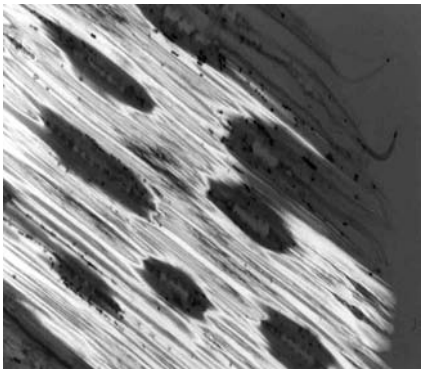

Figure 10.15. Brown rot in pine wood. Ray cell areas appear dark due to loss of birefringence (light microscopy, polarized light).

The hyphae grow in the cell lumena where they effect degradation of the carbohydrates. Hemicelluloses are degraded in the initial stages, followed by extensive degradation of the cellulose, finally leaving a coherent lignin skeleton (*Figure 10.13*). A unique feature is that the cellulose becomes extensively depolymerised already at low losses of wood substance. At a weight loss of 10 % the cellulose is already strongly fragmented. This explains why the remaining wood is quite soluble in 1 % NaOH. The initial attack has been observed to start in the S2 layer; later S1 and S3 are also degraded. Attack on the middle lamella is only occasionally observed. Early attack by brown rot is very difficult to detect using light microscopy. It is only when a substantial amount of the cellulose has been lost, that brown rot can be detected, by a loss of birefringence seen when polarised light is employed (*Figures 10.14* and *10.15*).

Chemically brown rot leads to losses of cellulose and hemicelluloses, where the remaining cellulose becomes fragmented already in the early stages of attack. The lignin content remains relatively constant, but the lignin has undergone modifications in the form of loss of methoxyl groups and an increased alkali solubility. Partial depolymerisation, and increase in phenolic and aliphatic hydroxyl and carboxyl groups has also been observed. The chemical changes result in very low yields during pulping, since a large fraction of the rotted wood will dissolve in the cooking liquors.

The decay mechanisms of brown rot fungi have for a long time remained obscure. A fascinating characteristic of several brown rot fungi has been their inability to degrade pure cellulose in the form of cotton or pulp fibres. This is particularly evident when the fungi are grown in liquid cultures. Today, there is strong evidence that the brown rot fungi use a Fenton-type system, involving hydroxyl radicals, to achieve the fast degradation of the cellulose in wood.

Lignin content or type does not, in contrast to all other decay forms, appear to influence the rate of degradation by brown rot fungi. Thus, hardwoods are degraded at the same speed as softwoods, and even the more lignified compression wood in conifers is degraded with the same speed as normal wood.

Chipping of brown-rotted wood leads to an increase in pin chips and fines. Pulping of brown-rotted wood results in significantly decreased yields and inferior strength.

10.2.3 Soft Rot

Soft rot is by definition caused by ascomycetes and deuteromycetes. The inclusion of a very large number of very diverse fungi has resulted in very wide variations in the types of decay that can be found among soft rot fungi. The fact that many cellulolytic fungi, usually seen as moulds, are capable to degrade low lignin hardwoods after prolonged exposure and stimulus in the form of nitrogen, complicates the picture even further.

Two distinct forms of attack are observed, usually referred to as Type 1 (cavity formation within wood cell walls) and Type 2 attack (erosion of wood cell walls). A large number of soft rot fungi cause attack of Type 1 and Type 2 more or less simultaneously. Others produce only Type 2 attack. Like most wood-inhabiting fungi, soft-rotting species are capable of invading and colonising wood. The open avenues, rays, vessels and pits are often used for rapid access to the wood substrate. Boring directly through the cell walls is, however, also frequently observed. In contrast to white- and brown rot fungi, the bore holes of soft rot fungi always remain small. Another feature that differentiates soft rot from white rot is that the middle lamella escapes degradation even in the final stages of soft rot attack. This suggests that soft rot fungi lack the ability to degrade the middle lamella lignin, which may have a more condensed structure compared with the lignin in the secondary cell walls.

Macroscopically soft-rotted wood becomes dark, often dark brown or nearly black. This is probably due to melanin synthesised by the soft rot species. Many of the typical soft rot hyphae have a dark colour due to melanin. Other species have hyaline hyphae, but they produce melanin when attacking wood. Thus in soft-rotted wood, melanin can be found not only within the fungal hyphae but is also secreted into the surrounding tissue. The term *soft rot* relates to the fact that it was described and named from very wet waterlogged wood that appeared soft. Softness is, however, not a typical characteristic of soft rotted wood. Drier wood from terrestrial exposure, may be extensively degraded, but still appear quite hard. The decay is then only revealed by poking the wood with a sharp pointed tool. This results in a typical brash fracture (*Figure 10.16*). The surface of heavily degraded wood is dark and crackled.

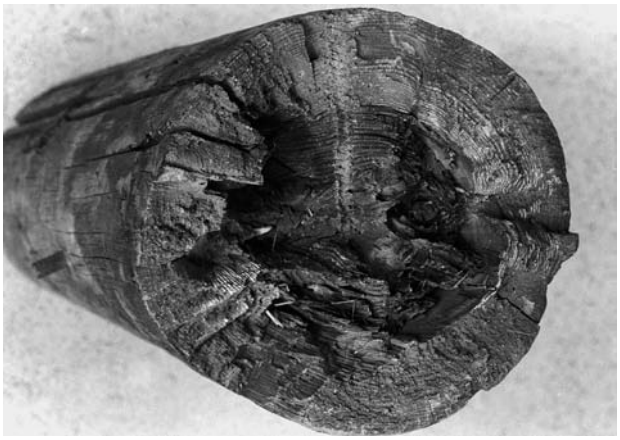

Figure 10.16. Severe soft rot attack in a preservative treated transmission pole has resulted in a brash fracture.

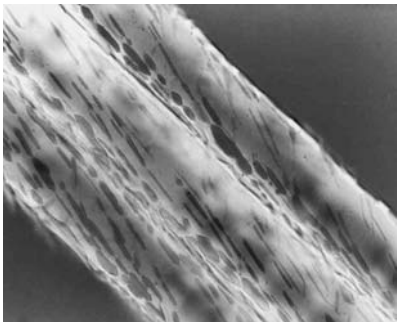

Figure 10.17. Chain-like arrangement of soft rot cavities in tracheid walls of pine wood. The longitudinal axis of the cavities indicate orientation of cellulose fibrils (light microscopy, polarized light).

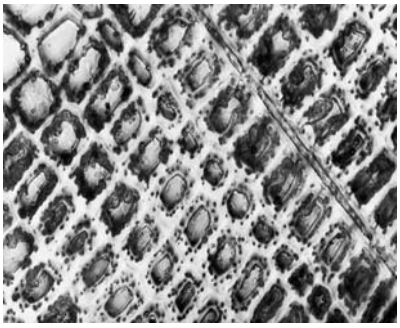

Figure 10.18. Soft rot attack seen in a transverse section of pine wood The numerous holes in the tracheid cell walls represent individual cavities (light microscopy).

Type 1 attack is characterised by formation of discrete cavities within the secondary layer of wood cell walls appearing as rounded holes in transverse sections (*fig. 10.18*). The cavities, best observed in longitudinal sections, may occur single or in chain-like sequences (*Figure 10.17*). The longitudinal axis of the cavities is always oriented along the cellulose microfibrils. This property has been used in microscopic studies on microfibril orientation in fibre cell walls. Type 1 attack is initiated by hyphae that penetrate the cell walls by means of very narrow hyphae. This hypha may penetrate directly through two adjacent cell walls without forming a cavity. Attack on the cell wall is only accomplished when the penetrating hypha orients itself to grow parallel to the cellulose microfibrils. This can be accomplished by forming a T-shaped branch (T-branching) or through a simple bend in direction (L-bend). When oriented inside the cell wall, the hypha ceases its growth and starts to produce a cavity. At some stage, when the cavity has enlarged, growth is resumed, either from one end of the cavity or from the two ends. The hypha only grows for a short length, than growth stops again and a new cavity is produced. This stop- and start process will continue repeatedly, resulting in chains of cavities.

Cavities are most frequently observed in the S2 layer of wood cell walls. Cavities are much less frequent in the much thinner S1 layer in normal wood cells (*Figure 10.19*), but are regularly observed in the comparatively thicker layer of compression wood tracheids. Cavities have never been observed in the S3 layer. Extensive cavity formation eventually leads to a complete degra-

dation of the S2 layer. S1 and S3 seem to persist rather long, but are eventually degraded through cavity formation (S1) or through expansion of cavities initiated within the S2 layer. The remaining wood structure then consists of a very fragile network of middle lamellae. There are early reports that state that the S3 layer in hardwoods and softwoods is very resistant to decay and can be seen as a discrete loosened layer next to the cell lumen. Recent studies indicate, however, that this layer in the final stages lacks cellulose. What is seen in the microscope is probably a layer of melanin synthesised by the soft rot fungus.

In softwoods, cavity formation usually starts in the thick walled latewood tracheids. Earlywood tracheids are attacked during later stages of attack. In hardwoods, the more lignified and guaiacyl rich vessel walls are more resistant than the fibres to soft rot attack.

Type 2 attack is somewhat similar to simultaneous white rot. Erosion of the cell walls accomplished by hyphae growing in the cell lumen (*Figure 10.20*) resulting in a complete removal of the secondary cell walls is the typical feature, but in contrast to white rot fungi, middle lamellae remain. Type 2 attack is mainly found in hardwoods, the attack in softwoods is usually quite limited. This may be explained by the higher lignin content, a more guaiacyl rich lignin and a more resistant S3 layer in softwoods, which prevents access to the S2 layer.

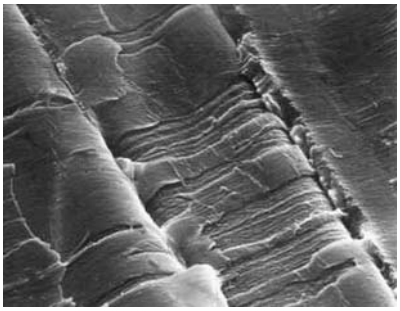

Figure 10.19. Soft rot cavities formed in the S1-layer of pine wood.tracheids (SEM).

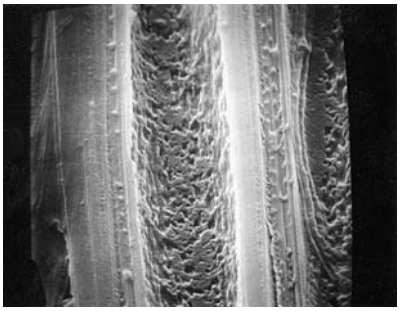

Figure 10.20. Soft rot erosion of birch wood fibre walls (SEM).

A third form of attack by soft rot has also been described, referred to as *diffuse cavity formation.* The attack is caused by hyphae growing within the wood cell walls just like for Type 1 attack, but the cavities are diffuse and irregular. This form is rarely seen in field material.

Chemical analyses of soft-rotted wood show depletion of cellulose and hemi-celluloses. There is no evidence for depolymerisation of the cellulose remaining in the wood. Lignin degradation appears highly variable and is dependant on fungal species and wood species. Klason lignin analyses may underestimate lignin losses since the fungally synthesised melanin may remain in the residue together with lignin. Lignin methoxyl content decreases with proceeding decay. Soft rot attack leads to significant reduction in wood strength even at small losses of wood substance. This effect is most likely evident also at a microscopic fibre level.

Lignin content and lignin type have great influence on the rate of attack by soft rot. Laboratory experiments have clearly demonstrated that low lignin hardwoods are considerably more susceptible than softwoods to soft rot attack. In an extensive Swedish study it was found that the timbers could be ranked in the following order of decreasing susceptibility: aspen, birch, beech, pine and spruce. Aspen wood was significantly more susceptible than birch wood. This is surprising since the chemical differences between aspen and birch are small. It has also been demonstrated that a slight chemical delignification greatly increases the susceptibility towards soft rot.

Soft rot is usually of little importance during storage of pulpwood, but may occur after prolonged chip storage in outdoor piles. Thus, there are no reports on pulping experiments of soft rotted wood. It may be assumed, however, that the cavities formed within the fibre cell walls, result in considerable strength losses of the individual fibres.

10.2.4 Sapstain and Mould Fungi

Sapstain fungi belong to the ascomyctes and deuteromycetes. They have dark coloured hyphae that invade the sapwood, often through the rays. They live on extractives and the small amounts of sugars present in wood. Pit membranes are also degraded, increasing the porosity of wood. All species are also capable of direct penetration of the wood fibre walls, but most of them cannot degrade lignified wood cells. A few species, however, are capable of causing soft rot. Some of these species may function as typical staining fungi in logs stored above the ground, but behave as typical soft rot fungi if the wood is in contact with the ground. The colours of the discoloration vary from light brown, blue or nearly black and become more intense with time. Sapstain fungi may under certain conditions, low temperatures or very wet wood, colonise the wood with hyaline hyphae. At higher temperatures or during drying out such hyphae turn rapidly dark. This explains why apparently sound wood may become discoloured after only a few days.

Sapstain is spread via airborne spores, but may also be inoculated into the wood by insects. The insect borne stain often penetrates deeper into the wood. Staining fungi are often observed in living trees of hardwoods, e.g. birch and aspen.

Moulds are classified within the *ascomycetes* and *deuteromycetes*. Growth of a few basidiomycetes, e.g. *Phlebiopsis gigantea*, may on certain substrates resemble mould growth. Like sapstain fungi, moulds live on simple sugars and extractives, but do not generally penetrate very deep into the wood. They are able to grow through wood fibre walls and some species are cellulolytic. Only a few species may after long time cause any degradation of lignified wood elements.

Moulds are mostly spread by airborne spores and rapidly colonise freshly sawn or debarked wood. This is the reason for the prolific mould growth observed on pulpwood chips during out-

door storage. There is also evidence that some may be carried by insects visiting wood. Many of the moulds produce large numbers of spores that can be seen on the wood surface. The spores are often coloured in shades of green, brown and black (*Figures 10.21 – 10.22*).

Figure 10.21. Heavy mould growth on untreated oak wood panels.

Figure 10.22. Mould growth on untreated larch wood panels and battens.

10.2.5 Bacteria

Bacterial degradation of wood has been discussed over a long time. Bacterial degradation of pit membranes in water-logged or water-sprinkled wood has been known since long, but it was only recently, around 1980, confirmed that single celled bacteria are capable of degrading even highly lignified wood cell walls. The evidence comes from electron microscopy studies, where bacteria can be seen to actively degrade the cell walls. So far none of the wood-degrading bacteria have been isolated in pure culture and identified. One reason may be that the bacteria not are culturable using standard procedures. This is a very common feature of the majority of bacteria observed in natural environments, such as soil and water. The distinction made between different types of bacterial attack is solely based on the micromorphology of the attack as seen when using microscopy and not on chemical changes occurring in wood or any taxonomic affiliations. Bacteria are capable of very rapid invasion of wood. It is a common misconception that fungi are better suited, due to their hyphal growth, but several studies have demonstrated that bacteria colonise wood well before the fungi. This is probably due to the fact that bacteria can swim or glide in liquid water present in cell lumena. Thus, their invasion is not determined by a growth rate. Bacteria cannot, however, compete successfully with the more rapid fungal decay forms.

Thus, bacterial attack is usually observed in substrates or under conditions that restrict fungal degradation.

Bacteria that degrade the pit membranes in softwoods are the main cause of the increased porosity observed during waterlogging or sprinkling with water. It may be possible that such bacterial attack may occur even under anoxic conditions. Pit membranes of hardwoods appear to be more resistant to bacterial attack. Ponding of refractory timbers has been used as a way to increase the penetrability of wood preservatives. The attack often also leads to a patchwise excessive absorption of liquids into the wood. This is easily observed when board surfaces are painted with a watersoluble stain. Bacteria have also been observed to cause a brown stain in certain timbers after felling. Mycelia-forming bacteria, i.e. actinomycetes are often present in decaying wood. There is, however, no conclusive evidence for wood degradation. We have noticed slight erosion of birchwood fibre walls, but no attack in pine. Many actinomycetes produce a strong smell similar to that of soil, a fact that has lead to the suggestion that the smell of soil is derived from metabolites of actinomycetes. Such smells may be found in buildings having problems with mould growth.

10.2.5.1 Tunnelling Bacteria

Tunnelling bacteria are quite peculiar in their mode of attack. The attack is initiated by single bacteria that bore their way into the wood cell walls. At later stages the bacteria can be seen at the front of tunnels (*Figure 10.23*) within the cell walls. The bacteria divide and the new individuals bore new tunnels. The result is a highly branched network of tunnels (*Figure 10.24*) that leads to complete destruction of the wood cell walls (*Figure 10.25*). The ubiquitous occurrence of tunnelling bacteria suggests that they have an important role in the carbon cycle. They are always present in fertile soils and in the marine environment. Their role seems to be to degrade recalcitrant lignocellulosic substrates resistant to fungal decay. The resistance may be due to high amounts of toxic extractives within the heartwood of certain timbers or to chemical treatments with wood preservatives or chemicals, which modify the wood structure. Tunnelling bacteria cause serious destruction of preservative treated timbers used in ground contact or in cooling towers. They are of no importance during storage of pulpwood, except that they may cause some degradation of waterlogged timbers after extended storage time.

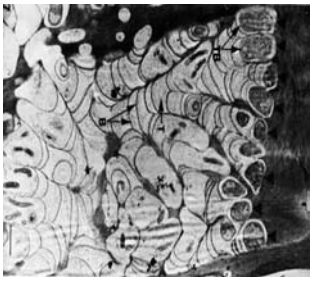

Figure 10.23. Branching tunnels caused by tunnelling bacteria. Note the bacteria at the front of each tunnel (TEM, Courtesy Adya Singh).

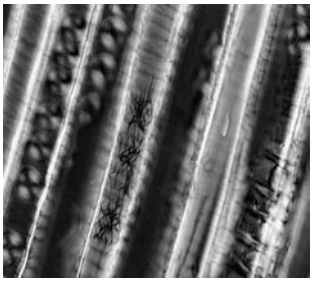

Figure 10.24. Branching tunnels caused by tunnelling bacteria in Douglas fir tracheids (light microscopy).

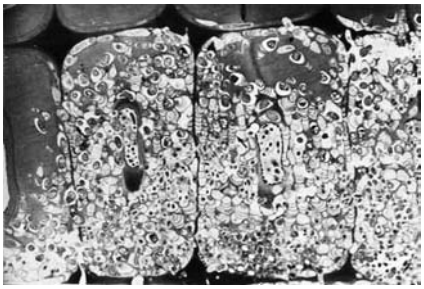

Figure 10.25. Almost complete destruction of wood fibres of *Homalium foetidum* caused by tunnelling bacteria (TEM, Courtesy Adya Singh).

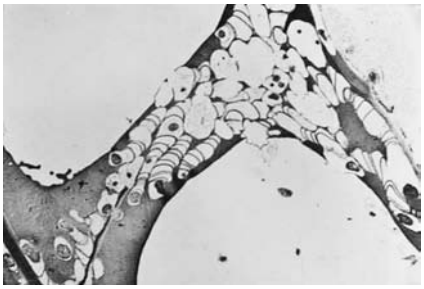

Figure 10.26. Extensive attack in pine wood by tunnelling bacteria. Note that the middle lamella is degraded indicating ligninolytic activity (TEM, Courtesy Adya Singh).

Tunnelling bacteria have a rodlike shape and are gram negative. Nothing is known about the enzymes used by tunnelling bacteria. The fact that they are capable of degrading woody substrates that are completely resistant to fungal decay, suggests that their enzyme system is rather unique. Chemical analyses of degraded wood and the observation that they can tunnel through the highly lignified middle lamella prove that these bacteria can degrade lignin (*Figure 10.26*).

10.2.5.2 Erosion Bacteria

Erosion bacteria are rod shaped and gram negative. They erode the cell walls starting at the S3 layer and progress towards the middle lamella (*Figure 10.28*). The rays appear to be the main

pathway into the wood (*Figure 10.27*). The S1 layer is rather resistant to their attack and the middle lamella seems not to be degraded. Thus, in the final stages, the remaining wood is a fragile skeleton of middle lamellae (*Figure 10.29*). During degradation erosion bacteria have been observed to align themselves along the cellulose fibrils.

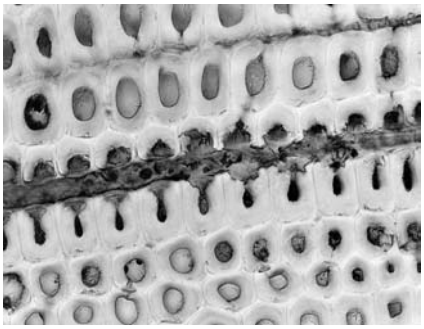

Figure 10.27. Attack spreading from a pine wood ray into adjacent tracheids (light microscopy).

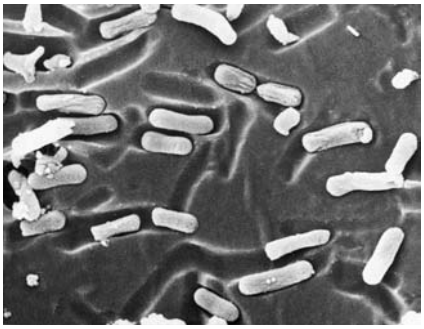

Figure 10.28. Erosion bacteria eroding pine wood tracheid wall. Note the typical rod shape (SEM).

Chemical analyses of wood degraded by erosion bacteria show that cellulose and hemicelluloses are degraded, but the lignin content remains unchanged, suggesting that lignin is not degraded to a large extent. The rate of degradation is clearly dependent on the lignin content of the wood, hardwoods are more susceptible than softwoods. Erosion bacteria have little importance during storage of pulpwood, except for prolonged storage under waterlogged conditions. Severe attack has been observed in birch logs that have been recovered after several years from the bottom of a river.

Erosion bacteria is the main form of degradation in waterlogged archaeological wood. Most archaeological wood has been lost due to decay, but the near anaerobic conditions during waterlogging have prevented attack by other decay forms. In the final stages wood is a very fragile skeleton of remaining middle lamellae. During drying such wood will collapse irreversibly, but this can be prevented by impregnation of the fragile structure with wax (polyethylene glycol, PEG). PEG was used to stabilise the timbers of the warship Vasa which was recovered from its aquatic environment 1961. Recent findings have shown that not only was the Vasa timbers attacked by erosion bacteria, the activity of other bacteria had led to increased levels of sulphur in

the wood. The sulphur is now being oxidised to sulphuric acid by atmospheric oxygen. The reaction is probably catalysed by iron resulting from corroding iron bolts.

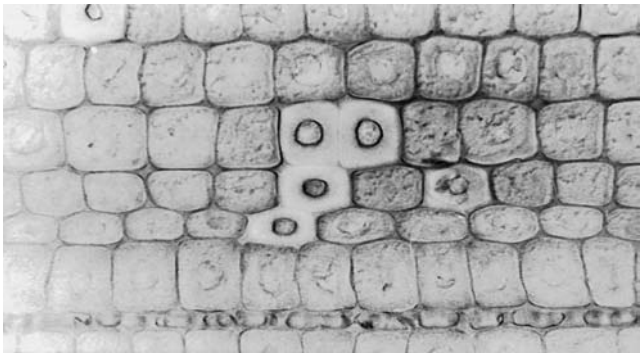

Figure 10.29. Transverse section of heavily degraded pine wood. Note that the tracheid walls have been transformed to an amorphous structure, representingslime and possibly residual lignin. Note that the middle lamellae are intact. The four tracheid sections in the centre are still sound (light microscopy).

10.3 Ecology

It should be remembered that the microorganisms causing decay are microscopic and thus usually not visible to a naked eye. The hyphae (*Figure 10.1*) and bacteria growing on wood or within cell lumena where they cause decay are only seen with the aid of microscopy. A large mass of hyphae forming a mycelium (*Figures 10.2* and *10.3*) and many fruiting bodies of many basidiomycetes, such as brackets (*Figure 10.4*) and toadstools, are clearly visible. The occurrence of higher fungi is often estimated from the number of fruiting bodies observed, disregarding viable mycelia growing in wood or other substrates. This clearly underestimates the number of individuals. Furthermore, fungi may be quite common without ever producing a fruiting body. One example is *Phanerochaete chrysosporium*, a fungus that seems to be omnipresent in pulpwood chip piles, but so far no fruit bodies have been found in Sweden.

Most fungi produce very large number of spores, sexually in fruit bodies or asexually directly on the mycelium. The spores become dispersed in the air and may be carried over vast distances by the winds. This explains why many fungi are cosmopolitan, occurring almost anywhere on planet Earth where the conditions are suitable. One example, is the white rot fungus *Phanerochaete chrysosporium*, which globally has found to be an important rot fungus in pulpwood chips during outdoor storage. Spores may also be actively spread by insects or unintentionally spread by insects and other mobile organisms. Water dispersal is typical for marine organisms. A successful colonisation depends on landing on the right substrate and suitable environmental conditions. Most substrates will already be occupied by other microorganisms; this will considerably restrict the success of late arrivals.

Knowledge of the distribution of wood-inhabiting microorganisms is clearly inadequate. Our experience seems to indicate that sapstain fungi, moulds and wood-degrading bacteria and soft rot fungi are more or less omnipresent. The knowledge of distribution of individual species is, however, meagre. Sapstain and moulds will always occur wherever the conditions are suitable.

Certain common moulds, of genera like *Cladosporium*, *Aspergillus* and *Penicillium* dominate among the air borne spores. We have observed that erosion bacteria appear to be present and active mainly in waterlogged wood where the low levels of oxygen excludes other forms of decay. Erosion bacteria are found in fresh as well as saline water and in soil. Tunnelling bacteria are omnipresent in fertile soils and saline water. They appear to be common also in fresh water, but absent from acid soils (pH below 4). Their distribution indicates that they have higher oxygen demands than erosion bacteria. Wood bacteria that degrade pit membranes, thereby increasing the permeability of wood appear also to be present everywhere.

Soft rot fungi are omnipresent in soil and aquatic environments. Like the tunnelling bacteria, soft rot fungi are more oxygen demanding than erosion bacteria. The decay activity differs widely depending on the environment. It has been clearly been demonstrated that soft rot activity is higher in fertile soils than in less fertile soils. Thus, the activity is considerably higher in a good garden soil compared with a poor forest soil. This is most likely an effect of the higher nitrogen levels in the fertile soils. Soft rot activity in timber exposed above ground is low and becomes important only after long time (extended storage in pulpwood chip piles) or under conditions that impede the activity of wood-rotting *Basidiomycetes*.

The distribution of white- and brown-rotting *Basidiomycetes* is not well known. The occurrence is usually estimated from visual observations of fruiting bodies appearing on woody substrates. Isolation and identification of mycelia from these fungi is rarely carried out. The knowledge gathered so far derives from specific research projects on a quite limited material. Thus, the information gained from observations on fruiting bodies still provides some information on frequency of a number of species, but there is a large uncertainty. Wood in ground contact is often attacked by basidiomycetes and it is obvious in most cases that this decay is caused by fungi residing in the soil, but the distribution of such fungi in soils is not known.

Figure 10.30. Heart rot caused by *Phellinus pini* in a pine wood tree used to construct. the bulwark in lake Tingstäde ca 1100 AD. The log has been laying at the bottom of the lake for nearly 900 years. Soft rot attack can be seen as a thin outer blackish layer. Attack by erosion bacteria has occurred to a depth of ca 30–40 mm. Soft rot and bacterial attack occurred while the log was submersed in the lake water.

Figure 10.31. Heart rot in the pine wood foremast of the ship Vasa. The tree felled to be used as a mast was already rotten!

The wood-degrading microorganisms have become adapted in various ways to the woody substrate and the environment. Many fungi are parasitic and decay the wood already in living trees. The most well known is the root rot fungus, *Heterobasidion annosum*, causing white rot in spruce trees. *Phellinus pini* is also causing white rot, but is found in the heartwood of old pine trees (*Figure 10.30* and *10.31*). *Fistulina hepatica* and *Laetiporus sulphureus* cause brown rot in the heartwood of oak trees. *Piptoporus betulinus* causes brown rot in birch trees. The heartwood of aspen trees is often attacked by the white rot fungus *Phellinus igniarius*. *Fomes fomentarius*, a white rot fungus, is often found in aspen and birch trees. Certain heartrotting fungi may continue their attack even after felling of the tree, whereas other species cease their activity and are replaced by other types of fungi. A large number of wood-decaying ascomycetes are known to attack living hardwoods where they cause cankers and decay. One hypothesis suggest that many trees contain latent decay fungi, i.e. present but not active. They only become active when conditions change, e.g after felling. Bacteria that do not attack the lignified wood cell walls are usually abundant in woody substrates. Their role here is not known. They even occur in living trees, where certain species may cause wetwood in the outer parts of the heartwood. Here the wood is wetter and less acid than the surrounding wood and has often an unpleasant smell.

The occurrence of microorganisms in woody substrates is determined by a large number of factors. The most important are: wood quality, or more precisely wood chemistry, water, oxygen and competing organisms. These factors interact in ways that are not very well understood. Wood quality could be seen to be reflect the chemical composition of wood;

Water is a key factor; dry wood does not rot! Water is present in wood as bound water in the cell walls and as free water in cell lumena. At the fibre saturation point, ca 30 % water calculated on the oven dry weight, the cell walls are saturated with water, but there is no water in the cell lumena. Wood decay activity starts at moisture contents above the fibre saturation point. It should be noted that the moisture content of a woody substrate may be highly variable, with parts below the fibre saturation point and with other parts well above it. Wood-decaying basidiomycetes are most active at moisture contents within a wide range of 35–160 %. A moisture content of ca 80 % has been found to be optimal. Basidiomycetes are usually absent from waterlogged wood, where only soft rot fungi and bacteria are active. Some basidiomycetes, like the dry rot fungus, *Serpula lacrymans*, are capable of actively transporting water to wood if a

source of moisture is available. This explains why the fungus, starting from scrap wood on a wet soil under a house, is able to attack the dry timbers in the house.

Temperature has a great influence on growth, rate of decay and the occurrence of microorganisms in nature. Fungi are able to grow within a temperature spanning from a few degrees below zero to ca 60 ºC. Bacteria are considerably more heat tolerant, some species can even grow in boiling water. Bacterial wood degradation has been observed at 70 ºC in laboratory experiments. Most basidiomycetes causing white- and brown rot cease to grow at temperatures around 35–40 ºC. Their optima lie at 20–30 ºC. One white rot fungus, *Phanerochate chrysosporium*, is exceptional, by being able to grow up to a temperature of ca 50 °C. It has its optimum at ca 40 °C. The high heat tolearance allows the fungus to become established in the interior mid-warm areas of piles of pulpwood chips, where it may cause quite fast degradation. Some soft rot fungi are able to grow at temperatures close to 60 °C. They can also be found in the warmer parts of piles of pulpwood chips. One thermophilic soft rot fungus, *Allescheria terrestris*, has in a laboratory experiment been found to cause a weight loss of almost 60 % after 4 months at 45 °C.

Oxygen seems crucial for degradation of lignified wood elements. Non-lignified tissues may be degraded under anoxic conditions, but there is no conclusive evidence for degradation of other wood cells under such conditions. Waterlogging or sprinkling is often used to protect timber from decay. The protection is partly due to reduction in oxygen availability. Recent experiments where stormfelled timbers have been enclosed in sealed plastic envelopes have been found to provide excellent protection due to the anoxic conditions resulting from consumption of the oxygen and production of carbon dioxide by the still living parenchyma cells.

The wood-degrading microorganisms need like all living organisms essential nutrient elements like nitrogen, phosphorus, sulphur, iron, manganese etc. The requirements of the basidiomycetes are fulfilled by low concentrations of these elements in wood. However, addition of extra nitrogen often results in some increase in decay. In contrast, the decay activity of soft rot fungi is greatly stimulated by addition of nitrogen. This explains why soft rot attack is more severe in fertile soils. Little is known about the requirements of the wood-degrading bacteria. It is known, however, that the attack by tunnelling bacteria is more severe in fertile soils, suggesting that they also benefit from an external source of nitrogen. Field studies suggest that nitrogen also may stimulate the activity of erosion bacteria.

Antagonistic activities between microorganisms probably play a significant role during the decomposition of wood. It has known since long that bacteria and moulds may have a very strong antagonistic effect on wood-degrading basidiomycetes, but this never prevents degradation to take place. The basidiomycetes also compete for the wood substrate. The great variation in susceptibility towards antagonistic organisms, selects for more resistant species. The basidiomycetes also have the great advantage of being able to more efficiently use the wood as a nutrient. It is likely that the microflora that becomes established in a woody substrate, at least in part, is determined by the outcome of a competition between competing organisms. Studies on pile storage of pulpwood chips suggest that the heavy mould infestation retard basidiomycete colonisation in the more temperate regions of the piles. The most dominating basidiomycete in chip piles, *Phanerochaete chrysosporium*, is successful not only because it can tolerate higher temperatures, but also because it is quite resistant to antagonistic moulds. Attempts to make use of antagonistic moulds and bacteria for protection of wood against decay and sapstain have so far not been successful.

10.4 Storage of Wood

The ambition today is to avoid storage of wood as much as possible. Advancement in technology has also made it possible to shorten the time from felling to conversion. Storage of roundwood timber was earlier a common practice and several studies have been carried out to study the deterioration during storage.

The moisture content of wood at the time of felling is usually too high to suit the wood-decaying basidiomyctes, but they will soon attack when the wood has dried out somewhat. Peeling increases drying out of the roundwood. Unpeeled roundwood, particularly birch and aspen, stay wet for very long. Storage experiments on roundwood have clearly demonstrated that there are great differences in rate of decomposition. Birch roundwood is decomposed much more rapidly than aspen. This cannot be explained solely by inherent differences in susceptibility of the two timbers, since laboratory experiments with pure cultures of decay fungi show only minor differences. Results from different regions in Sweden have shown that the timbers can be ranked in the following order with respect to decreasing losses: birch, pine and spruce. Temperature appears to be the main rate-limiting factor. White rot fungi dominate throughout the storage and chemical analyses have shown that the relations between the cellulose and lignin do not change. Losses in kraft pulp yield from birch roundwood stored for more than one year in southern and middle Sweden have been reported to be 10 % or more. Similar losses were reported for aspen roundwood stored for longer than two years. Much lower wood substance losses have been reported for peeled roundwood of pine; ca 1 % after one season and 1.7 % after two seasons.

The dominating white rot gives the wood a bleached appearance. In old hardwood logs numerous very dark lines or zones are regularly observed. They often mark where two different mycelia meet, either two different species or two different strains of the same species. The dark areas represent a reaction or battle zone, where the mycelia produce the darkly coloured melanin. *Ascomycetes* and *Deuteromycetes* may also grow in the “no mans land” and sometimes produce typical soft rot cavities.

Ponding or water sprinkling of timber carried out to protect against decay and sapstain results after some time to an increased permeability of the wood. This is due to the bacterial degradation of pit membranes. This has some negative effects. One is the overabsorption of liquids, often seen to occur in a patchwise manner. Extended waterlogging results in attack by soft rot and erosion bacteria. Such attack has been observed in logs sunken for many years in lakes or rivers.

Outdoor storage of pulpwood chips become common practice in the 1960´s. The chips were store in large piles that tended to heat up due to the respiration of the living wood parenchyma cells and microbial activity. Selfignition of piles have been observed as well as chemical degradation caused by acetic acid produced in the chips at temperatures above 60 °C. This type of storage create conditions which differ widely from storage of roundwood. Early stages of storage are characterised by a rise in carbon dioxide and a decrease in oxygen. The temperatures are for the most parts of the piles much higher and the chipping of the wood has increased the exposed wooden surface. This results in a quite heavy growth of mould fungi, including some truly thermophilic species. The temperatures in the outermost parts of a chip pile are quite similar to the fluctuating outdoor temperature, but the temperatures rise to about 60 °C in the central parts. A thermotolerant white rot fungus, *Phanerochaete chrysosporium*, is found in areas with temperatures around 30–45 °C. The highest wood substance losses are found in such areas. The outer, cooler parts with temperatures suitable for typical wood-decaying basidiomycetes are not

suffering from high losses. This is explained by the antagonistic effects of the moulds, which restricts the colonisation by the basidiomycetes. Technical aspects of wood storage and microorganisms are further discussed in Volume 2.

The average wood substance losses were found to be about 1 % per month. The pulp yields based on wood fed to the digester was little effected, except for chips from overheated parts of the pile. Chip storage was found to considerably reduce the extractive content of the wood.

Decay of wood during storage leads to losses in wood substance and also to a loss in strength. The latter effect may lead to increased losses during further processing, e.g. debarking. Capacity losses occur when the wood is fed to the digester, since the decayed wood occupies the same volume as sound wood. Furthermore, the pulp yield will decrease, depending on type of rot. The wood consumes more alkali and bleaching may consume more chemicals and the strength of the paper may be reduced. Energy savings may be seen during mechanical pulping, due to the softer wood, but bleaching of the pulp may be more difficult. The effect on strength will depend on the extent of decay and the type of decay. Tear strength of kraft pulps from rotted birch wood has been reported to decrease ca 10 % for every 5 % increase in wood loss due to decay. Studies on biomechanical pulping suggest that mild white rot may even result in increased strength properties, as is further discussed in chapter 12. Microbial activity can also have positive effects in the aspect that it degrades pitch, and especially triglycerides during seasoning. This is further discussed in chapters 7 and 12.

10.5 Microorganisms During Processing

Extensive diminution of wood during mechanical pulping or delignification during chemical pulping will make the material more accessible to a variety of microorganisms. Furthermore, soluble sugars and extractives will contribute to render the pulps susceptible to attack. Cellulolytic organisms may also increase the sugar levels through degradation of cellulose and hemicelluloses. The attack will result in loss of cellulose, staining and production of slime. Slime consists of polysaccharides produced by fungi and bacteria. Earlier, strong biocides like mercury compounds and others were used to prevent microbial growth.

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11 Enzymes Degrading Wood Components

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11.1 Introduction

Pulp and paper products are made from wood, which is a natural raw material and thus fully biodegradable. Biodegradation of natural compounds is catalyzed by *enzymes*, which have evolved for the needs of their host and which therefore operate best in the physiological conditions of the production organism. The biological degradation of wood in nature is based on enzymes produced by various wood degrading microorganisms. Owing to its high energy content, wood is indeed an attractive energy source for many microbes. However, the extensive lignification of the woody cell walls is a significant obstacle for efficient biodegradation (Chapter 6) and only a few groups of microbes are able to attack sound wood. Instead, the degradation of

the different wood components takes place by synergistic action of microbes in a wide variety of taxonomic groups (Chapter 10). Some wood degrading organisms can produce all of the enzymes required for a complete degradation while others are specialized to one or few wood components. Owing to the insoluble nature of the substrate, which cannot enter the microbial cells, wood degrading enzymes are commonly secreted to the culture medium of the production organism. These enzymes are typically inducible, i.e. only produced upon contact with available substrate. In some organisms, the entire repertoire of e.g. cellulolytic enzymes is induced simultaneously while in others, contact with different substrates give rise to different enzyme compositions. In addition to the enzymes, different low molecular weight compounds essential for the degradation of some wood components are also secreted.

11.1.1 Enzymes are the Biological Catalysts

Enzymes are protein molecules built of linear heteropolymers consisting of 20 different amino acids. A typical hydrolytic enzyme, such as a cellulase, consists of approximately 200 to 800 amino acid residues. Ribosomes are the machinery that join the amino acids into long linear polypeptide in the cells. During or after its synthesis, the polypeptide chain folds to adopt a three dimensional structure governed by its amino acid sequence and held together by non-covalent interactions and covalent bonds such as disulphide bridges. The three-dimensional structure is required for full activity of the enzyme protein. Some proteins, such as the wood degrading enzymes, contain export signals that direct them to the secretory pathway, resulting in their secretion to the surrounding medium of the cell. Besides amino acids, mature proteins can contain carbohydrates (common in cellulose and hemicellulose degrading enzymes), metal ions and smaller organic molecules, called prosthetic groups, which are often necessary for the enzyme function of for example ligninases. The carbohydrate moieties fall in two different types: O-glycosylation occurs when sugars form an ether to an amino acid oxygen, and N-glycosylation when the bond is on an amino acid nitrogen.

Enzymes are efficient and very specific catalysts. One enzyme normally only catalyses a single reaction, which is determined by the type of substrate recognized and the type of chemical bond cleaved or synthesized. Enzymatic reactions proceed via high-energy transition states of the enzyme substrate-complexes. The catalytic efficiency of enzymes relies on their ability to bind the transition state of the substrate tighter than its ground state. The essential features of enzymatic catalysis are thus 1) recognition and binding of the substrate, 2) catalysis, and 3) dissociation of the enzyme substrate-complex to release the products.

Wood is a very challenging material for enzymatic attack due to its complex and compact structure composed of many different polymers, each with a different chemical composition and physical structure. Therefore, complete degradation of wood almost always requires multi-enzyme-systems; no single enzyme can efficiently degrade any of the major wood polymers to its components. It is thus important to realize that descriptions such as ‘cellulase’, ‘hemicellulase’ and ‘ligninase’ often refer to groups of enzymes rather than single enzymes. Each of these enzymes has established a different approach to overcome the problems presented by the poor accessibility and/or the chemical heterogeneity of their substrates as will be described below.

11.2 Cellulolytic Enzyme Systems

Crystalline cellulose is the major structural component of plant cell walls and it has evolved towards resistance to enzymatic degradation. The long polymeric chains of glucose attach to one another by networks of hydrogen bonds and van der Waals interactions leading to the formation of crystalline bundles, *microfibrils*. In plant cell walls, cellulose is closely associated with other cell wall components. In nature cellulose is rarely perfectly crystalline: the structure of the surface is commonly less ordered, para-crystalline, and some regions even along the crystals exhibit poorly ordered structures. These disturbed regions are often the starting points of enzymatic degradation of various cellulosic substrates. Complete solubilization of crystalline cellulose almost always requires coordinated actions of many enzymes. Microbes producing efficient cellulolytic enzyme systems are generally found among aerobic fungi and aerobic as well as anaerobic bacteria. Many celluloytic fungi belong to *Ascomycetes* among which the best-studied cellulolytic genera are *Trichoderma*, *Fusarium, Penicillium, Humicola* and some *Aspergilli. Trichoderma reesei,* in particular, secretes very efficient cellulolytic and hemicellulolytic enzymes and is therefore widely used for commercial enzyme production. The cellulose biodegradation by the brown rot fungi differs considerably from other aerobic fungi. Although at least one fungus, *Coneophora puteana* produce „normal“ cellulases, the degradation seems to be dependent on small redox-mediators or hydroxyl radical as will be discussed below for lignin biodegradation.

Besides fungi, many different bacteria can degrade cellulose and/or hemicellulose. Aerobic cellulolytic soil bacteria produce secreted enzymes similar to filamentous fungi and include well-studied species such as *Thermobifida fusca* (previously *Thermononospora fusca*), *Cellulomonas fimi* and alkalophilic *Bacilli*. *Erwinia* species are plant pathogens producing a variety of pectinases, proteases and cellulases. Cellulolytic anaerobic bacteria and anaerobic rumen fungi, such as *Piromyces equi*, use large multienzyme complexes called *cellulosomes* for cellulose degradation.

11.2.1 Cellulases Produced by Aerobic Microorganisms

All cellulases catalyze the cleavage of the $\beta(1 \rightarrow 4)$–glucosidic bonds, but they must be able to retrieve the bonds from a heterogeneous and mostly insoluble substrate. Different cellulases have established different strategies to approach their solid substrate. Since the early days of cellulase research, the enzymes have been broadly divided into two main classes: Cellobiohydrolases (EC[1] 3.2.1.91) remove cellobiose, the repeating unit of a cellulose polymer (see Chapter 4), from either the non-reducing or the reducing ends of the chain. The action of cellobiohydrolases results in a rapid production of soluble sugars but slow decrease in the degree of polymerization (DP). Endoglucanases (EC 3.2.1.4) cleave bonds randomly in the middle of the chains. Endoglucanase action is characterized by a rapid reduction in the DP (degree of polymerization), but slow release of soluble sugars from crystalline cellulose. They usually produce a range of oligosaccharides of different size, and glucose. Cellobiohydrolases are the en-

[1] EC, enzyme classification code – a classification system for different types of reactions catalysed by enzymes.

zymes with highest apparent activities on crystalline cellulose while endoglucanases typically act on more irregular physical structures of the substrate (Fig 11.1).

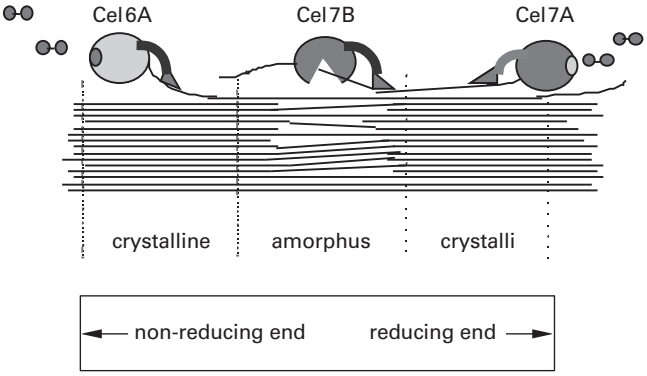


Figure 11.1. A schematic representation of cellulose degradation by enzyme produced by the filamentous fungus *Trichoderma reesei*. The cellobiohydrolases, Cel6A and Cel7A cleave cellobiose form the cellulose chain ends, while the endoglucanase Cel7B makes internal cuts in the chains.

The complementary action of the 'free' cellobiohydrolases and endoglucanases produced by the aerobic micro-organisms leads to synergism, which means that the combined activity of the enzymes working in a mixture is higher that the added activities of the same enzymes acting individually (*Table*11.1).

Table 11.1. Synergistic action of *T. reesei* Cel7A and Cel7B in the degradation of cotton (Srisodsuk *et al*, 1998).

Time h	Cel7B[a] (endoglu-canase)	Mass Loss Cel7A[b] (cellobiohydrolase)	Cel7B+Cel7A	Degree of synergy[d]
0	0	0	0	–
12	11	1	19	1.52
24	15	1	34	2.06
48	21	1	39	1.73
96	31	3	51	1.50
198	34	3	58	1.57

[a,b]Each enzyme was incubated with the substrate individually; [c] Both enzymes were incubated with the substrate simultaneously; [d] $c/(a + b)$

Synergism is often explained by assuming that the endoglucanases generate free chain ends, which in turn are substrate for the cellobiohydrolases. The synergism sometimes observed between two cellobiohydrolases may occur since the cellobiohydrolases peeling individual chains off the cellulose surface expose new starting points for each other. The oligosaccharides and cellobiose produced by the cellobiohydrolases and endoglucanases are degraded to glucose by β-glucosidases (EC 4.2.1.21) (Clarke, 1997). Although the β-glucosidases no not operate on cellulose and can thus not be described as cellulases, they are nevertheless an essential compo-

nent of complete cellulolytic enzyme systems. Typical aerobic cellulolytic fungi and bacteria produce mixtures of two different cellobiohydrolases, one for each of the ends of a cellulose chain, a series of different endoglucanases and one or two β-glucosidases.

Cellulolytic enzymes are glycoside hydrolases, which cleave the β(1→4)–glucosidic bonds by either inversion or retention of the configuration of the anomeric carbon (*Figures 11.2* and *11.3*).

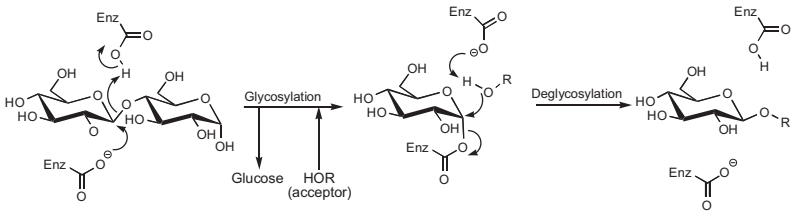


Figure 11.2. Two-step retaining glycosyl transfer mechanism. R = H for hydrolytic enzymes, R = sugar moiety for transglycosylating enzymes.

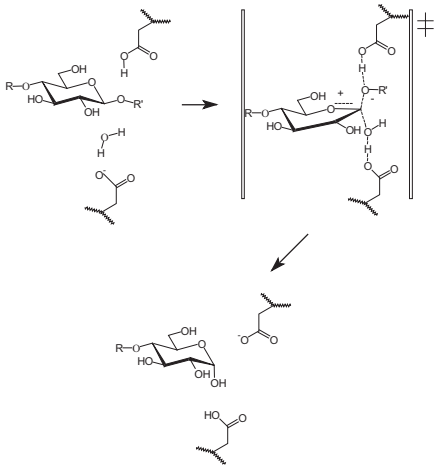


Figure 11.3. General Mechanism for an inverting β–glucanase.

Based on amino acid similarities, different cellulases have been divided in families sharing the same catalytic reaction mechanism and the same overall three-dimensional structure (http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html). Experimental work has shown that the enzymes within a given structural family also share the same chemical reaction mechanism. As the family classification was developed it was noticed that enzymes with both endoglucanase and cellobiohydrolase activity can be found within a single family. Structural comparison of the two types of enzymes in a given family revealed that the enzymes do indeed share similar overall structures but differ in the topology of their active sites. Endoglucanases have more open active site clefts, which allow them to bind to and cleave bonds in the middle of the cellulose chains (*Figure 11.1*). After a single endolytic cleavage the affected cellulose chain remains associated with the rest of the crystal without any detectable release of soluble reducing sugar. In the cello-

biohydrolases, which act at the chain ends, the active site is covered by several long surface loops, which create tunnel-shaped active site extending deep inside the enzyme molecule (*Figure 11.4*). The individual cellulose chains will thus have to be transported into the tunnel from one of the openings („the entrance“) for the hydrolysis to take place. After the bond cleavage, the product – cellobiose – is released from the opposite end of the tunnel („the exit“) (*Figure 11.4*).

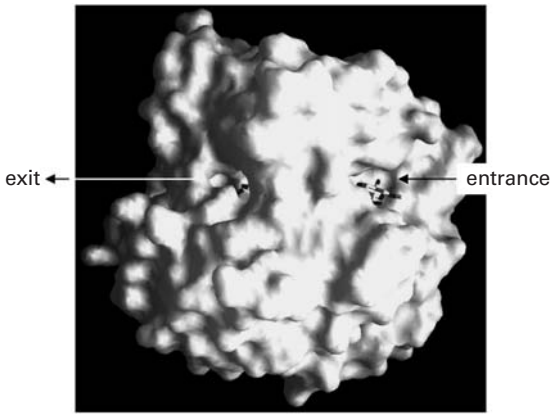


Figure 11.4. Structure of a cellobiohydrolase with characteristic tunnel. Solvent accessible surface of the X-ray structure of the *T. reesei* cellobiohydrolase Cel6A demonstrating the shape of the active site with a substrate molecule bound inside the enzyme.

Based on amino acid similarities, different cellulases have been divided in families sharing the same catalytic reaction mechanism and the same overall three-dimensional structure (http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html). Experimental work has shown that the enzymes within a given structural family also share the same chemical reaction mechanism. As the family classification was developed it was noticed that enzymes with both endoglucanase and cellobiohydrolase activity can be found within a single family. Structural comparison of the two types of enzymes in a given family revealed that the enzymes do indeed share similar overall structures but differ in the topology of their active sites. Endoglucanases have more open active site clefts, which allow them to bind to and cleave bonds in the middle of the cellulose chains (*Figure 11.1*). After a single endolytic cleavage the affected cellulose chain remains associated with the rest of the crystal without any detectable release of soluble reducing sugar. In the cellobiohydrolases, which act at the chain ends, the active site is covered by several long surface loops, which create tunnel-shaped active site extending deep inside the enzyme molecule (*Figure 11.4*). The individual cellulose chains will thus have to be transported into the tunnel from one of the openings („the entrance“) for the hydrolysis to take place. After the bond cleavage, the product – cellobiose – is released from the opposite end of the tunnel („the exit“) (*Figure 11.4*).

Sugar binding in the tunnel shaped active sites is mediated by hydrogen bonding and by hydrophobic stacking/charge transfer interactions between aromatic side chains and the glucose rings. Owing multiple protein-carbohydrate interactions, cellobiohydrolases act processively, catalyzing several bond cleavages before the dissociation of the enzyme-substrate complex. The

processivity brought about by the tunnel shaped active site is clearly the most important feature in enzymes that are able to degrade highly crystalline cellulose. Endoglucanases are generally relative inefficient on highly crystalline substrates when acting alone, without the help of cellobiohydrolases.

Another characteristic feature of most cellulases is a modular structure consisting of a catalytic module accommodating the active site and one or several carbohydrate-binding modules (CBM) or other auxiliary modules (*Figure 11.5*). The catalytic and carbohydrate-binding modules are generally joined by O-glycosylated linker sequences, which are assumed to adopt an extended, flexible conformation. Removal of the CBM has no effect on the catalytic efficiency of the enzyme on small soluble substrates but reduces its activity dramatically on solid substrates. It has been speculated that the CBM is needed to keep the catalytic module in contact with the substrate as long as it takes the catalytic module to access the substrate. Since cellulases are secreted enzymes, the CBM may also help to keep them close to the host, thus helping to avoid competing organisms to utilize the soluble sugar produced. A structure with two independent domains bonding to cellulose (CBM and catalytic module) that are connected with a linker, will furthermore allow the enzyme to diffuse on the cellulose surface similar to a caterpillar. Analogous two-domain structures are also common in many other enzymes active on other solid substrates and apparently form the basis of their efficient attack on such material. Some, particularly bacterial cellulases may carry many different carbohydrate modules and even several catalytic modules in a single polypeptide chain.

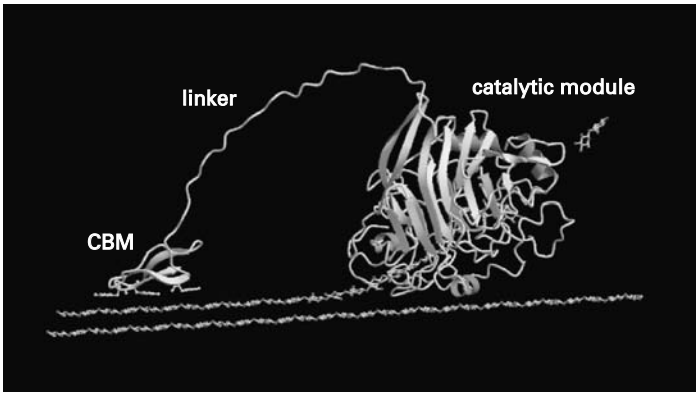


Figure 11.5. The modular structure of the cellobiohydrolase Cel7A of *T. reesei*.

Most cellulases have identical sugar and bond specificities. There are a few exceptions and for example some endoglucanases can work on both cellulose and xylan or on both cellulose and glucomannan. Nevertheless, as a rule, the differences in the cellulase specificity are a matter of 'where' in the substrate surface the attack is localized rather „which" sugar or bond is recognized.

Since the substrate presents many, quite different structures to the enzymes, evolution has responded by producing a number of different enzyme structures. In addition to the 'strict' cellobiohydrolases and endoglucanases, a whole series of different intermediate structures have been found. For example, some cellobiohydrolases have mobile active site loops, which allow a certain opening and closing of the tunnel during the degradation and may lead to residual endoglu-

canase activity on soluble and amorphous substrates. Similarly, some endoglucanases have loops, which enclose the cellulose chain into a mini-tunnel after the first, endo-type cut, resulting in processive action.

Even the modular structures influence the type of activity exhibited by a given enzyme. The classical cellulases, typically produced by aerobic fungi, contain only one catalytic and one cellulose-binding module. However, some cellulases in e.g. families 12 and 7 have only a single catalytic module and no CBM. The modular structures of bacterial cellulases are often more complicated and may consist of more than one catalytic as well several different substrate-binding modules and even other modules of unknown function. Both *C. fimi* and *T. fusca* produce family 9 cellulases, which contain a single catalytic domain and two different cellulose-binding modules. In this case, however, one of the cellulose-binding modules is linked directly to the catalytic domain and thus helps to form an extended active site resulting in a processive mode of action. Only the second cellulose-binding module binds to crystalline cellulase. In some cases, two catalytic modules with different substrate specificities can be linked with each other and one or more CBMs. This diversity in the 'topological' specificities of the enzymes reflects the heterogeneity of the substrate; each different variation that has been maintained during the evolution of cellulases apparently contributes to the general efficiency of the cellulose degrading ability of the organism.

Cellulases from aerobic fungi are among the most commonly exploited industrial enzymes, used e.g. for food processing, extraction of fruit juices, surface treatment of textiles, an additive in laundry detergents, and even as a tool in chiral analysis of chemically synthesized molecules. There are also many applications for cellulases in the pulp and paper industry as discussed in Chapter 12.

11.2.2 Cellulases Produced by Anaerobic Microorganisms

Anaerobic bacteria have developed a different strategy for crystalline cellulose degradation and extend on the theme of multimodular enzymes. They produce a large number of different catalytic modules, which – with a number of accessory proteins – form large enzyme complexes called *cellulosomes* (Shoham *et al*, 1997) (*Figure 11.6*). En integral part of a cellulosome is a scaffolding protein carrying a bacterial cell wall anchoring module, a cellulose-binding module and a series of „*cohesin*" modules In turn, each of the catalytic modules carries a „*dockerin*" domain, which binds to a cohesin domain thereby making the catalytic domain a part of the enzyme complex.

The number of catalytic domains with a dockerin is about double the number of cohesin domains on the scaffolding protein. It has thus been speculated that the exact composition of a cellulosome may be dictated by the relative concentrations of the different catalytic domains at a given time. This would allow the cellulosomes to act dynamically, with different ratios of cellulolytic and hemicellulolytic enzymes depending on the type of substrate and the phase of degradation. The component enzymes of cellulosomes all contain typical secretion signals and the assembly of the complex thus probably takes place extracellularly although in close contact with the bacterial cell wall. The contact with the cell wall is maintained by fibrous material, which is sometimes referred to as protuberances. Upon contact with the substrate, these organelles elongate into fibers, which may have a role in transferring the soluble sugars produced to the cell wall premises.

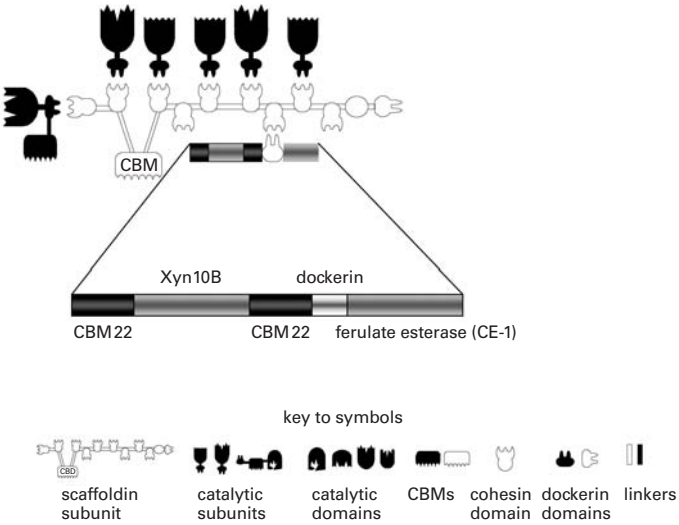


Figure 11.6. Typical structure of a cellulosome. The insert shows an example of a multimodular enzyme with two different catalytic modules and two CBMs.

11.3 Hemicellulases

Hemicelluloses represent about 20–25 % of the lignocellulosic biomass. Hemicelluloses are chemically more heterogeneous that cellulose and usually consist of a backbone of one or two sugars with side chains and other substitutions in different branched configurations. The main hemicellulose in the wood primary cell walls is xyloglucan, which has a backbone of (1,4)-β-D-glucose. Xylan residues are attached to the backbone at regular intervals, and these are in turn glycosylated with D-galactose and D-fucose residues. Xylans and glucomannans are the main hemicelluloses in the secondary cell walls (wood). The xylan backbone consists of 1,4-linked β-D-xylopyranose units, which can be acetylated or linked to a variety of side chains such as galacturonic acids or arabinose. Galactomannans have a backbone of (1,4)- β-linked D-glucose and D-mannose units in ratios depending on the species. Some glucomannans carry galactose side chains on their glucose residues and in some cases acetyl esters may be present on the mannose residues. In addition, both hardwood and softwood contain small amounts of other hemicelluloses such as galactan and arabinan as well as pectin (Chapter 5). Degradation of hemicelluloses also requires the combined actions of many enzymes with specificities for the different sugars and the different types of chemical linkages involved (*Figure 11.7*).

Xylans, and other hemicelluloses with side chains, do not generally form tight crystalline structures such as cellulose. Instead, the need for many different enzymes in hemicellulose hydrolysis springs from the variety of the different sugar-bond combinations in these polymers. For example, the total degradation of xylan requires endo-xylanases (EC 3.2.1.8) attacking the main chain and β-xylosidases (EC 3.2.1.37), which hydrolyze xylo-oligosaccharides to xylose. Debranching enzymes, e.g. 1,2-α-D-glucuronidases (EC 3.2.1.139) and 1,3-α-L-arabinosidases

(EC 3.2.1.55) are needed for the removal of the side chains and esterases (EC 3.1.1.72) to hydrolyze the ester linkages between xylan and the acetic or phenolic acids (*Figure 11.7*). The order of the events may also play a role in hemicellulose degradation: Some xylanases do not cleave the xylan main chain in the presence of substituents and the side chains must thus be removed prior to the degradation of the backbone. On the other hand, some of the side chain active enzymes can only act on xylo-oligosaccharides, which must be first produced by endoxylanases. The hydrolysis of the other hemicelluloses is analogous to that of xylans, but relies on enzymes specific for the resident structures in these polymers (*Figure 11.7*).

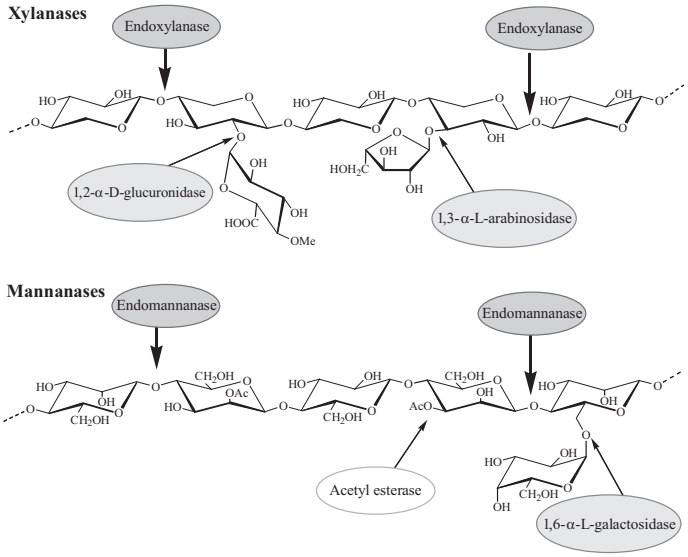


Figure 11.7. Substrate specificity of some hemicellulases. Most hemicellulases can be divided into two main groups; endo-enzymes and debranching enzymes, i.e., enzymes removing side-groups. Except for the shown enzymes, there are some other reported as exo-xylanase. All shown enzymes are hydrolases, i.e., they cleave glucosidic and ester bonds with addition of water, but hemicellulose can also be depolymerized by cellobiose dehydrogenase using a radical mechanism (see 11.4.4).

Similar to cellulases, some of the hemicellulases also carry cellulose-binding modules, presumably in order to come close to the substrate associated with cellulose. In some cases, hemicellulases may also carry different hemicellulose-binding modules, such as xylan- or mannan-binding modules.

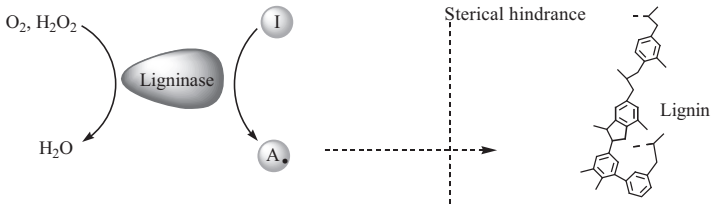
Xylanases and mannanases are used in the pulp and paper industry for bleaching of chemical pulps as well as tools in analytical wood chemistry as discussed in Chapter 12.

11.4 Ligninases

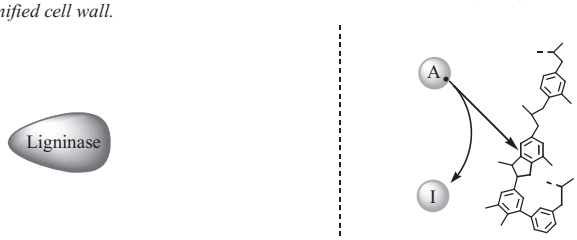
Lignin is a hydroxylated phenolic polymer intimately associated with the cellulose and hemicellulose in the wood fiber cell walls (see Chapter 6). Even though lignin contains somewhat more energy than cellulose or hemicellulose, it is not generally used as an energy source by microbes.

Instead, the role of microbial lignin degrading enzymes, *ligninases*, seems to be to make wood polysaccharides more accessible for the carbohydrate active enzymes. All true wood degrading organisms seem to be able to degrade and modify lignin to some extent, but it is only the white rot fungi and the tunneling bacteria that can degrade lignin completely (Chapter 10). The lignin degrading system from white rot fungi is the best known and most of the discussion below will focus on these enzymes.

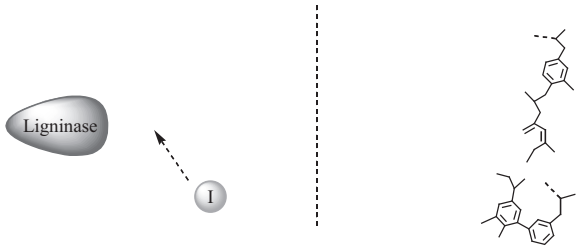
Lignin is a complex substrate for enzymatic degradation for two main aspects: Firstly, the covalent pattern of the lignin polymer is very complex and contains many different chemical bonds. Lignin is also one of the few biopolymers, which is racemic. This adds to the structural complexity since the racemic carbons of lignin consist of a mixture of both stereo-forms. Secondly, impregnation of the woody cell walls by lignin makes the wall structure so compact that large molecules, such as enzymes, cannot penetrate into the cell wall. Direct contact between lignin and the enzymes is thus only possible on the cell surfaces during the initial stages of the microbial attack on the wood. From nature's point of view this is an excellent asset as it forms an effective obstacle for microbial attack. Lignin-degrading microorganisms overcome these



The enzymes use oxygen or hydrogen peroxide to oxidize a redox mediator (I) into an active form (A), that might be a radical. The redox mediator is small enough to penetrate into the lignified cell wall.



The activated redox mediator diffuses to the lignin and performs an oxidation that introduces a radical on the lignin. The redox mediator is simultaneously transformed into its inactive form.



The lignin gets fragmentated by uncatalyzed reactions following the oxidation and the inactive redox mediator can diffuse back to the ligninase for a new reaction cycle.

Figure 11.8. The principle for enzymatic lignin degradation with redox mediators.

difficulties by a sophisticated strategy: instead of direct contact of the enzyme molecules with the substrate, diffusible low-molecular-weight reagents, *redox mediators*, which are activated by the ligninases, mediate the reaction. The activated redox-mediators are small enough to penetrate though narrow pores in the lignified cell walls and can eventually perform an oxidation of the aromatic rings to resonance- stabilized radicals. The inactivated redox-mediator can then return to the ligninases for a new oxidation (*Figure 11.8*) (see also 6.2.5).

The activated redox-mediator is relatively unspecific and can oxidize various phenolic, and in some cases also non-phenolic, structures in lignin. Lignin will thereafter be fragmented or modified, when the radicals react in uncatalyzed reactions. Some examples of such reactions are shown in *Figure 11.9*. In addition, many other types of reactions have been suggested, such as openings of the aromatic rings of lignin forming carboxylic acids. Such reactions are expected to cause both swelling of the lignified cell wall and opening of pores. Thus, in later stages of

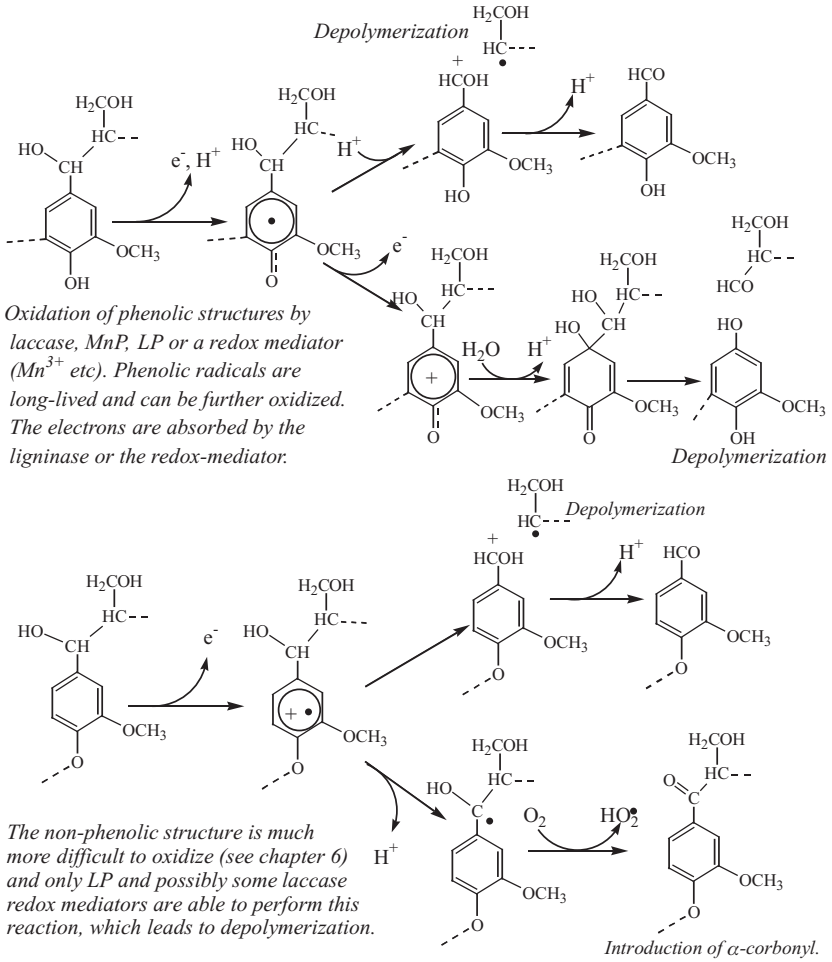


Figure 11.9. Reactions on lignin caused by oxidation of aromates by ligninases as MnP, LP and laccase.

wood degradation, direct contact between ligninases and fragmented lignin is possible, and this may be important for the degradation of lignin. Ligninases have generally properties that make them suitable to perform oxidations of large hydrophobic surfaces.

Enzymatic lignin degradation is based on oxidations and the chemistry of enzymatic lignin degradation has many similarities with the chemistry of oxygen lignification. Total degradation of wood is known to be a strictly aerobic process, and it may be that it is the ligninases, which are responsible for the dependence of oxygen. Cellulases and hemicellulases catalyze hydrolysis reactions, which operate even in anaerobic conditions.

The feature common to all ligninolytic enzymes so far characterized is that they are redox-enzymes, i.e. they catalyze oxidation or reduction reactions. A number of different enzymes described as ligninases have been purified from different white rot fungi. The word „ligninase“ is sometimes used as a synonym to lignin peroxidase. In this text the term „ligninases“ is, however, used to describe the group of enzymes that can depolymerize lignin in various ways. These include laccase (EC 1.11.3.2), lignin peroxidase (EC 1.11.1.71, LP), manganese peroxidase (EC 1.11.1.13, MnP), and cellobiose dehydrogenase (EC 1.1.99.18, CDH). It should be emphasized that all of these enzymes are not necessary produced by all white rot fungi. Ligninases carry normally metal ions or prosthetic groups that are necessary for their function. In addition to true ligninases, wood degrading fungi also produce various accessory enzymes, e.g. producing hydrogen peroxide. However, it should be emphasized that detailed mechanism of enzymatic lignin degradation is still not fully understood, and that new findings may change the present hypotheses. In particular, the recent publication of the genome sequence of *P. chrysosporium* will pave the way for a detailed understanding of its biochemical pathways leading to efficient lignin degradation. The nonspecific nature and extraordinary oxidation potential of the lignin-modifying enzymes enables these organisms to also degrade toxins and contaminants, including many pesticides, polyaromatic hydrocarbons, polychlorinated biphenyls and other halogenated aromatics (including dioxins), tri-nitro toluene (TNT) and other nitroaromatic explosives, and a range of other toxic pollutants such as cyanides, azide, carbon tetrachloride and pentachlorophenol. These organisms have therefore great potential for „cleaning“ soil contaminated with environmental toxins (bioremediation).

11.4.1 Laccases

Laccases are phenol-oxidases, which are wide spread among different organisms and carry our many different functions. In white rot fungi, laccases are involved in lignin degradation and in trees in lignin synthesis. The substrate specificity of laccases is very broad, but the preferred substrates are phenols that are oxidized to quinones or phenolic radicals. Oxygen is used as electron acceptor and is reduced all the way to water. Thus, the balance is: one molecular oxygen to four phenol molecules if phenolic radicals are the product, and one molecular oxygen to two phenols if quinones are the product (*Figure 11.10*).

A laccase contains four copper ions that are active in the catalytic cycle. Some laccases carry two copper ions and one PQQ, a prosthetic group that can carry two electrons. The enzyme can oxidize phenolic lignin structures, but not non-phenolic structures, directly, and has been suggested to work together with a redox mediator *in vivo*. In *Figure 11.11* some low molecular compound produced by white rot fungi that have been suggested to work as redox mediators are shown. The activated form should then be a resonance stabilized radical on the structures. In

laboratory experiments, the presence of some redox-mediators has allowed laccase also to attack non-phenolic lignin structures. This will be further discussed in Chapter 12. Most laccases have pH optima around 5.

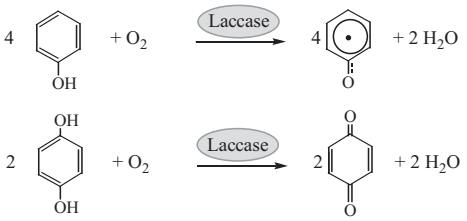
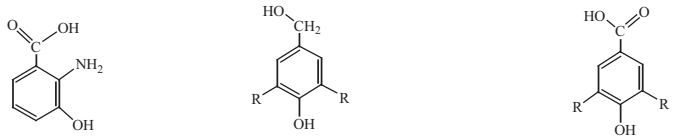


Figure 11.10. Reactions catalyzed by laccase. Laccase is a phenol oxidase and the reaction products can be phenol radicals or quinones. The oxygen is reduced al the way to water.



3-hydroxy anthranilate *p*-hydroxy benzaldehyde deriavates *p*-hydroxybenzoic acid derivate

Figure 11.11. Some suggested natural redox-mediators for laccase. 3-hydroxy anthranilate was the first redox-mediator reported to be involved in lignin degradation. Later experiments suggest, however, that it actually is involved in formation of a colored polymer.

11.4.2 Manganese Peroxidases

Manganese peroxidases (MnP) are a group of isoenzymes, i.e., enzymes from close related genes with similar activity, produced by many white rot fungi. They catalyze the oxidation of Mn^{2+} to Mn^{3+} with hydrogen peroxide as oxidant. In turn, Mn^{3+} is the reactive redox mediator, which can perform a one-electron oxidation on phenolic structures in lignin. Mn^{2+} is recreated and the lignin modified in uncatalyzed reactions (*Figure 11.12)*. The importance of the MnP-system is supported by mutant studies, where MnP-lacking mutants had low ligninolytic activity, and by the fact that manganese salts are transferred into the attacked wood from the soil by the fungal hyphen, so that the Mn-concentration in wood can be increased with a factor 100.

For proper function, the manganese ion must in complex with a suitable chelator, such as carboxylic acids (e.g. malonate or oxalate) or α-hydroxyl acids (e.g. lactate or tartate). The activated MnP system is able to oxidize phenolic structures in lignin, but cannot directly attack non-phenolic structures. However, in the presence of unsaturated fatty acids, it has been shown to oxidize even non-phenolic lignins. This is probably due to a secondary reaction between the Mn^{3+}-ion and double bonds in unsaturated fatty acid that generates carbon centered radicals. It is, however, unclear if this reaction is biologically important. Similar reactions have been reported if thiols, such as gluthatione[2] are present.

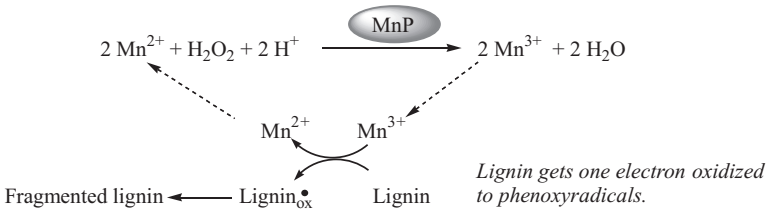


Figure 11.12. The reactions catalyzed by manganese peroxidase.

MnP is a relatively small enzyme consisting of around 350 amino acid residues, and carries a haem as prosthetic group (an iron ion chelated by a porphyrine ring, the same as in the blood protein haemoglobin). The pH optimum is around 4. Hydrogen peroxide concentrations higher than 1 mM inactivate the enzyme due to irreversible oxidation of the haem group. MnP can furthermore catalyze the reaction between oxalic acid (that is produced by many white rot fungi and a product of lignin biodegradation) and oxygen that produces hydrogen peroxide (*Figure 11.13*)[3]. Thus, the MnP-system is independent of hydrogen peroxide producing enzymes, if oxalic acid is present.

Figure 11.13. Manganese peroxidase can create hydrogen peroxide by the oxidation of oxalic acid by oxygen.

11.4.3 Lignin Peroxidases

Lignin peroxidase (LP) is another hydrogen peroxide dependent, haem-carrying enzyme produced by many white rot fungi. Similar to many other cell-wall-degrading enzymes, also lignin peroxidases are often produced as several isoenzymes. They belong to the same protein family as MnP and some enzymes in this family can even display both activities. LP is interesting since, in contrast to MnP and laccase, it can directly oxidize non-phenolic lignin structures to aromatic cation radicals.

The activity of LP has been suggested to depend on veratryl alcohol (*Figure 11.14*), a metabolite excreted by white rot fungi, but the role of veratryl alcohol is not clear. LP might either oxidize it to a radical, thus working as a redox mediator, it may work as a kind of coenzyme working close to the active site of the enzyme. Veratryl alcohol may even protect the enzyme from inactivation as MnP and LP are inactivated by excess of hydrogen peroxide. However, using a second active site, LP can also directly oxidize lignin polymer into radicals also in the ab-

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[2] Gluthatione is a radical-scavenger present inside eukaryotic cells with the function to protect DNA from damage created by radicals.

[3] Principally, MnP is therefore also an oxalic acid oxidase. There are also specialized oxalic acid oxidases from both plants and fungi. These enzymes have large potential technical application in the pulp and paper industry and are discussed further in Chapter 12.

sence of veratryl alcohol. The catalytic cycle of LP is shown in *Figure 11.14*. Some white rot fungi seem to produce peroxidases that display both MnP and LP activities.

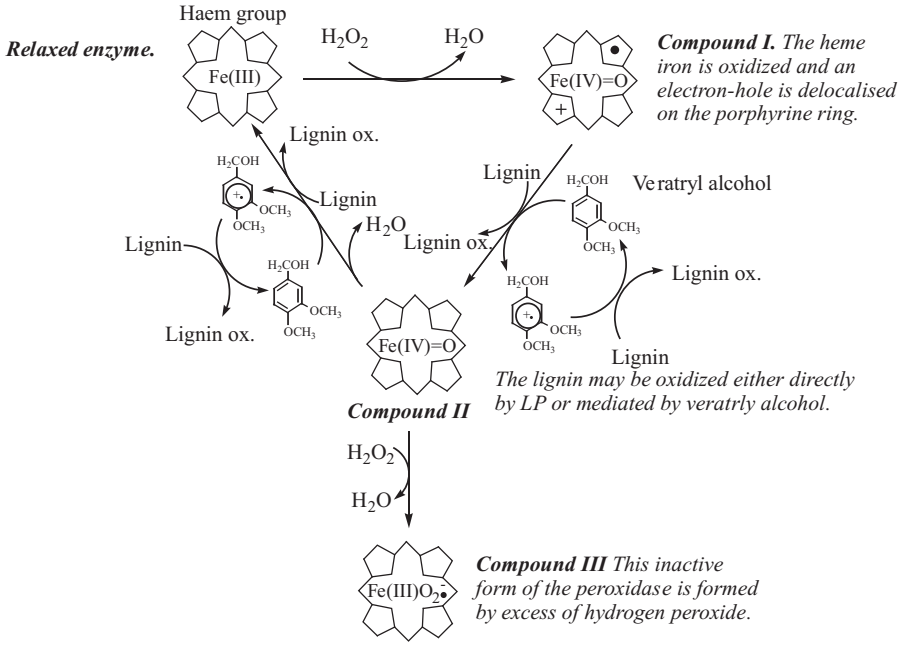

Figure 11.14. Catalytic cycle of lignin peroxidase. Note that LP can oxidize veratryl alcohol *or* lignin directly.

11.4.4 OH·-generating Enzyme Systems

There are several enzyme systems produced by wood degrading fungi capable to generate hydroxyl radicals that may play an important role in lignin biodegradation. Cellobiose dehydrogenase (CDH) is produced by white rot fungi, but also by many soft rot and brown rot fungi. It oxidizes cellobiose, the main product of cellulose biodegradation (see 11.2), and reduces a large number of different electron acceptors, including quinones and phenolic radicals, which are reaction products of other ligninases. Furthermore, CDH can create hydroxyl radicals by reducing O_2 to hydrogen peroxide and Fe^{3+} to Fe^{2+}. In this case, the latter must be chelated with some suitable acid as for instance acetic acid. Hydrogen peroxide and Fe^{2+} are small relatively stable molecules that easily can penetrate pores in the lignified plant cell wall to small for enzymes and thereby serving as redox mediators (*Figure 11.8*). When they meet they will form the highly reactive hydroxyl radical in a *Fenton-type reaction* (*Figure 11.15*). It is this reactive species that is believed to attack lignin.

In *Figure 11.16* the reaction between a non-phenolic lignin structure and a CDH-generated hydroxyl radical is shown. Note that the common β-O-4´ linkage[4] is broken and that the reaction product is a phenolic lignin structure. This means that the created structure can be oxidized by the MnP- and laccase systems, suggesting a pathway in lignin biodegradation by white rot fun-

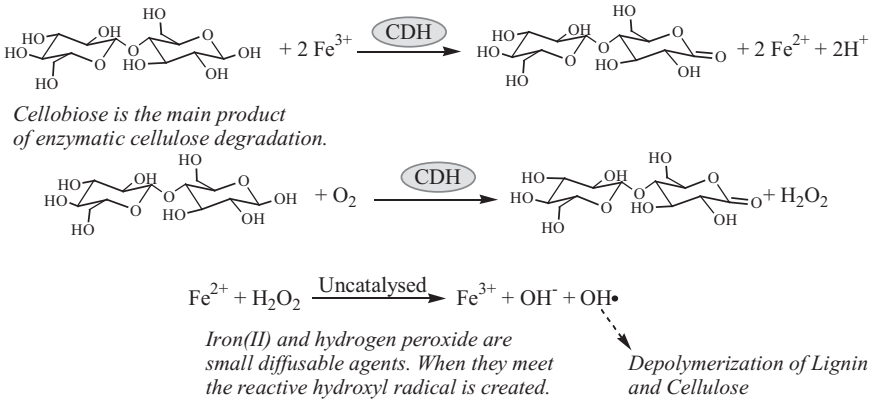

Figure 11.15. Generation of hydroxyl radicals by CDH.

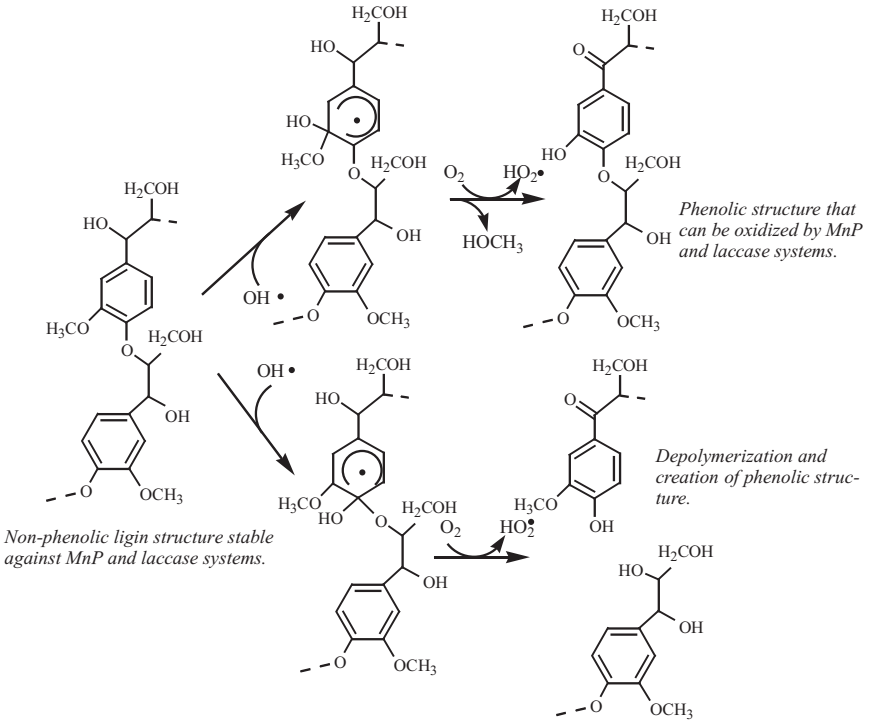

Figure 11.16. Reactions on non-phenolic lignin structure by CDH- generated hydroxyl radicals.

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[4] The nomenclature for different types of bonds in lignin is explained in Chapter 6. The β-O-4' bond is the most common linkage in lignin.

gi, where the CDH system first attack non-phenolic lignin causing depolymerization and conversion to phenolic structures, that are oxidized by MnP and laccase for further depolymerization. In brown rot and soft rot fungi, the initial attack with hydroxyl radicals should than not been followed up by other enzyme systems and therefore a modified residual lignin is left. CDH can thus be regarded to be both a depolymerising and activating enzyme and may be important in the initial attack of lignin.
A CDH-knockout mutant of the white rot fungus *Trametes versicolor* lacked the ability to degrade wood suggesting a key role for CDH in the attack of wood. CDH seems also to support cellulose biodegradation by the generation of hydroxyl radicals, which can depolymerise cellulose. Cellulose that was treated with CDH was faster degraded by both endoglucanases and cellobiohydrolases.

CDH carries two prosthetic groups that are active during the catalytic cycle, one flavin (a B-vitamin that can take up and deliver two electrons) and one haem. The latter is, however, of a different type than in the peroxidases described above and its function is probably to store one electron during the catalysis[5]. The arrangement with two prosthetic groups is probably an adaptation to the problem of reducing a one-electron acceptor (Fe^{3+}) with a two-electron donor (cellobiose), and is unique for extra-cellular enzymes. CDH binds strongly to cellulose. In some fungi the binding is mediated by a CBM, whereas other enzymes bind with a special surface, which is spatially separated from the active site but located on the catalytic module.

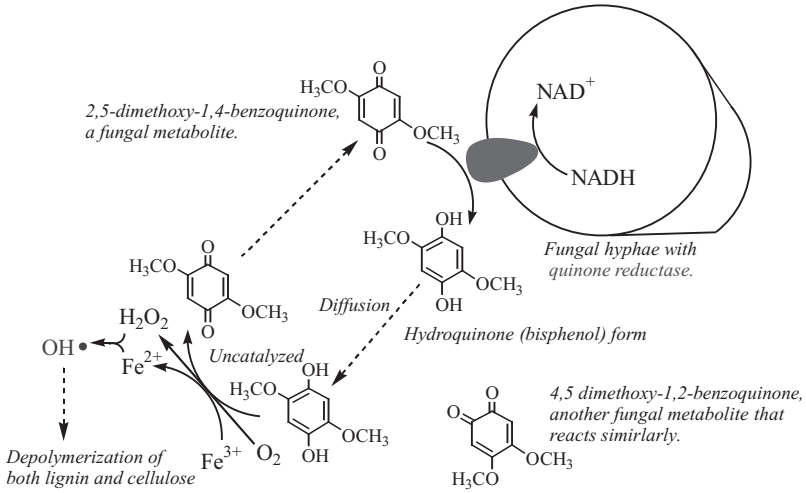

Figure 11.17. Generated hydroxyl radicals by brown rot fungi by cooperating metabolites and quinone reductase.

Other enzyme-systems have also been suggested to function in the generation of hydroxyl radicals by wood degrading fungi. The brown rot fungi *Gloeophyllum trabeum* and *Posita placenta* produces two extra-cellular metabolites, 2,5-dimethoxy-1,4-benzoquinone and 4,5 dimethoxy-1,2-benzoquinone, which are reduced to hydroquinones (bisphenols) by a mycelial reductase[6]. The created hydroquinone can reduce Fe^{3+} to Fe^{2+}, and the resulting semiquinone

[5] Some traces of peroxidase activity are actually reported for CDH, but this has probably no biological significance.

can reduce molecular oxygen to hydrogen peroxide. Hydroxyl radicals are thereafter formed in a Fenton-type chemistry, and can react with lignin as in *Figure 11.16*. The quinones are simultaneously recreated for a new catalytic cycle. See *Figure 11.17*.

11.4.5 Hydrogen peroxide Producing Enzymes

A number of hydrogen peroxide producing redox enzymes have been isolated and are hypothesized to be producers of H_2O_2 for the lignolysis by MnP, LP and CDH. Some of these enzymes are, however, intracellular and therefore not likely to be involved in this process. Of the extracellular enzymes, at least three enzymes appear to be plausible candidates: glucose oxidase[7] (EC 1.1.3.4), glyoxal oxidase (EC 1.2.3.-) and veratryl alcohol oxidase (EC 1.1.3.7). Although glucose oxidase and CDH display rather different catalytic properties, they are genetically related to and belong to the GMC – oxidoreductase family. Veratryl alcohol oxidase belongs also to this family. *Figure 11.18* shows the reactions catalyzed. Veratryl alcohol oxidase and glyoxal oxidase have relatively broad substrate specificities and oxidize thus several aldehydes respectively alcohols. As described above both CDH and MnP can synthesize hydrogen peroxide by themselves.

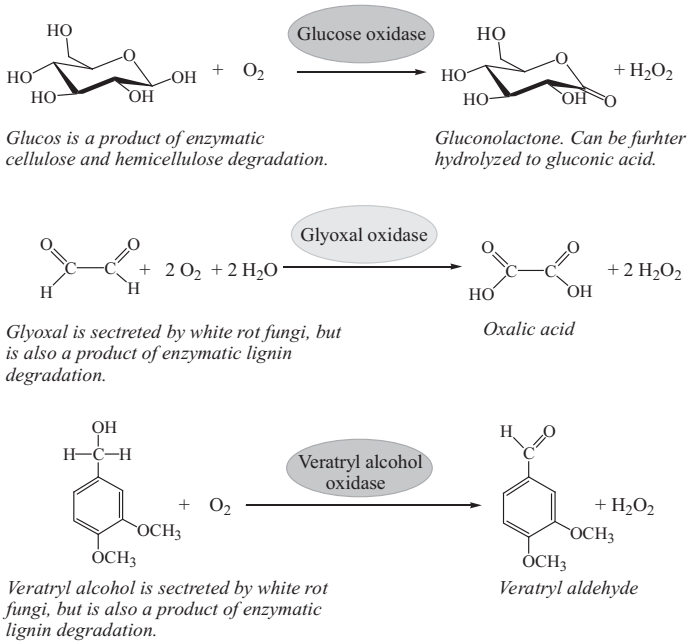

Figure 11.18. Reactions catalyzed by hydrogen peroxide producing enzymes.

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[6] NADH, the general reducing agent in biochemistry, may drive this reaction.

[7] An oxidase in an enzyme that use oxygen as electron acceptor, normally producing water (H_2O), hydrogen peroxide (H_2O_2) or superoxide anion ($O_2^{-}\cdot$).

Both glucose oxidase and veratryl alcohol oxidase carry flavins as prosthetic groups (the same as CDH). Glyoxal oxidase has an unusual redox active centre consisting of a radical delocalized on covalently bonded tyrosine- and cystein- residues in complex with a copper ion.

11.4.6 Lignin Degrading Enzymes from Soil Organisms

As discussed in Chapter 10 some wood degrading organisms, i.e., brown rot fungi and soft rot fungi, do not degrade lignin totally, but leaves a modified remaining lignin. Eventually this material ends up in soil, and is one of the main sources for the organic compound in soil, *humus*. This modified lignin is there slowly degraded under aerobic condition to carbon dioxide by both fungi and bacteria. Some of these organisms have an enzymology that is very similar to white rot fungi, but there are also totally other types of lignin degrading enzymes. Some of these remain more of „classical“ enzymes, than of the above-described ligninases, since they have direct contact with the polymer they degrade in „normal“ enzyme-substrate complex. The reason for this is off course that the compact structure of wood does not exist in soil. The humus lignin is an amorphous polymer, where enzyme can get in direct contact with reactive groups. A bacterium (*Spingomonas paucimobilis*) has an intracellular enzyme system for lignin degradation, where, a phenolic or non-phenolic b-O-4´ bond is cleaved by an oxidation of the α-carbon to a carbonyl followed by a reductive cleavage of the ether bond (*Figure 11.19*). The biological niche of this type of enzymatic lignin degradation is more likely degradation of fragmented lignin in soil rather than attack of lignin in sound wood, and it seems like at least some soil organisms utilize the lignin as carbon and energy source; for instance can *S. paucimobilis* grow on lignin fragment as sole carbon source.

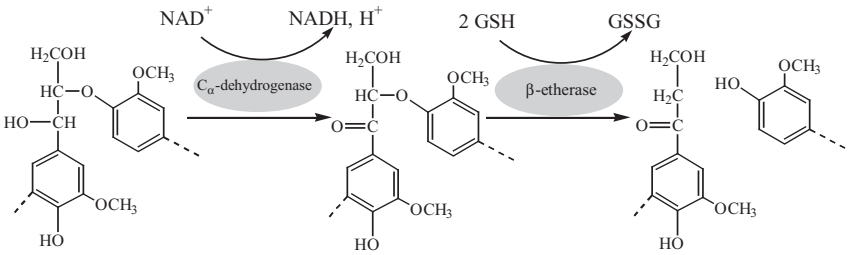

Figure 11.19. Enzymatic mechanism for breaking of β–O-4' esters in bacteria. This is an intracellular mechanism involving two redox reactions. First, the α–carbon is oxidized by Cα-dehydrogenase leading to a carbonyl (O=C) at the α-position. Nicotinamide adenine dinucleotide ($NAD^{+)}$ is used as oxidant. Thereafter, a second enzyme, β-etherase (that *not* is a hydrolase!) breaks the β-O-4´ bond by a reductive cleavage, where gluthathione (GSH) is oxidized to glutathione disulphide (GSSG). Both nicotinamide adenine dinucleotide and gluthathione are common intracellular substrate for redox reactions.

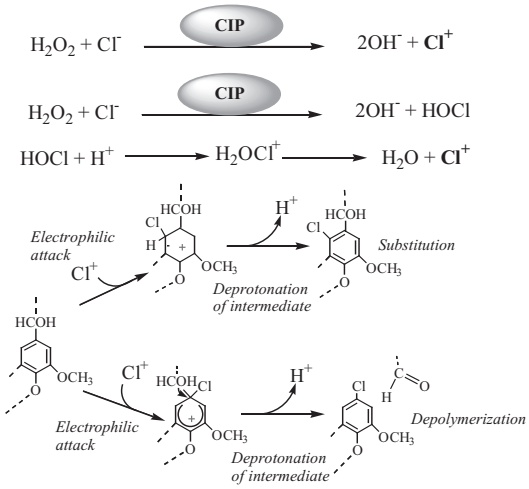

Figure 11.20. Lignin degradation with chloroperoxidase (ClP). ClP catalyze the oxidation of chloride ions to chloronium ions either directly, or via hyperchloric acid. The chloronium ions (Cl^+) is a very strong electrophile that can chlorinate aromatic rings in lignin, which may lead to depolymerization.

11.4.7 Other Ligninolytic Enzymes

There are also some other fungal enzymes that have been suggested to be involved in lignin biodegradation, among them a *dioxygenase*, an enzyme able to include two oxygen atoms in a lignin structure. This enzyme requires, however, direct contact between enzyme and lignin polymer. It appears also as some organisms attack lignin with *chloroperoxidases*. These enzymes generate hypochlorite, HOCl, or even *chlorionium ion*, Cl^+, from chloride ions, Cl^-. These species can chlorinate and depolymerise lignin (*Figure* 11.20) in reactions similar to the one described for chlorine bleaching chemistry.

11.5 Enzymes Degrading Extractives

Extractives (see Chapter 7) constitute a minor component in wood. Nevertheless, enzymatic systems degrading this group of molecules are important, since extractives are associated with many technical problems in mechanical and chemical pulping of wood. Furthermore, extractives are important carbon and energy sources for certain wood-invading microorganisms. For some, such as the sapstain fungi (see 9.2.4) and many bacteria, it is even the dominating carbon source. Nevertheless, the knowledge of the enzyme systems for degrading extractives is very limited. Since extractives are generally small molecules, and thus easy for the microorganisms to import through their cell membrane, many of the enzymes are probably intracellular. However, there are also extracellular enzymes that attack extractives, maybe due to that many of them are very hydrophobic and have poor solubility in water.

The best understood and maybe also the most important of the extracellular extractive-degrading enzymes are *lipases* (EC 3.1.1.3), and *tannases* (EC 3.1.1.20).

11.5.1 Lipases

Lipases are hydrolytic enzymes that cleave the ester bonds in triglycerides to release fatty acids. These hydrolases commonly have broad substrate specificities. They can degrade diglycerides and monoglycerides, and in some cases even steryl-esters, all of which are important extractives in wood (*Figure 11.15*). A wide range of organisms, ranging from plants and animals to filamentous fungi, yeasts and bacteria, produce lipases. The technically most used lipases are produced by yeasts as *Candida anthartica* and eubacteria such as *Pseudomonas*. Lipases have a number of properties that are unusual among enzymes: they are often relatively stable in organic solvents that denature and inactivate most other enzymes. Proteins are generally folded to hide their hydrophobic core inside the molecule, thereby exposing a largely hydrophilic surface. Exposure to non-polar solvents therefore tends to destabilize proteins by unfolding them, which destroys their function. However, lipases can also tolerate extreme pH, high temperatures and can often act in the presence of organic solvents. Lipases thus resemble enzymes from extremophilic organisms that grow under extreme conditions, although they are produced by ordinary microbes from moderate conditions. This is probably since lipases have adapted to the special problems associated with hydrolyzing very hydrophobic substrates. The low solubility of triglycerides and other fatty components makes them form a special phase, „oil drops“ in water, which is needed as co-substrate for the hydrolysis of the ester bonds (*Figure 11.21*).

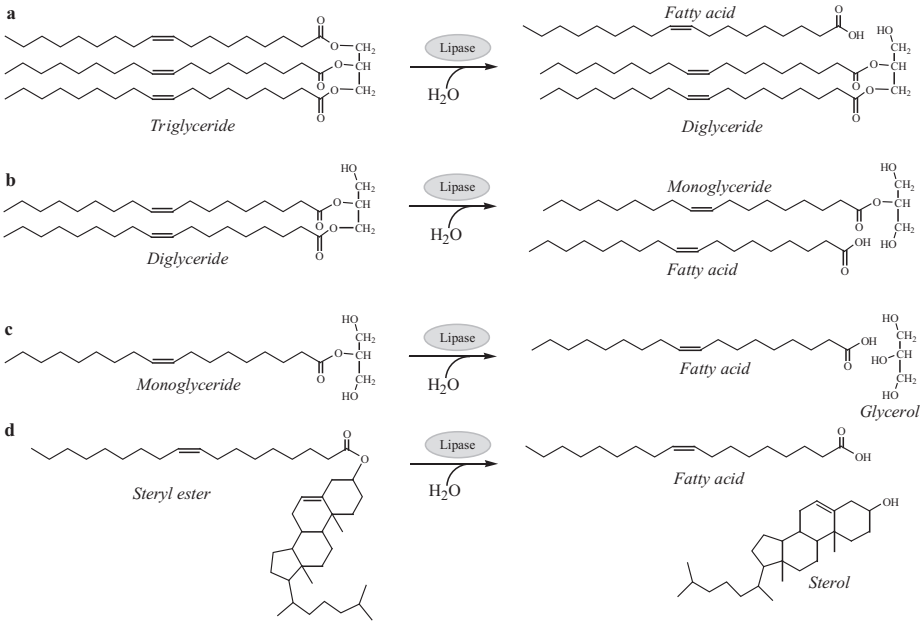

Figure 11.21. The substrate specificity of lipases. The specificity of different lipases varies; some lipases hydrolyze triglycerides and diglycerides (reactions a and b), but cannot hydrolyze the central ester bond (reaction c), whereas other lipases also can hydrolyze the ester bond in steryl esters (reaction d), in addition to triglycerides.

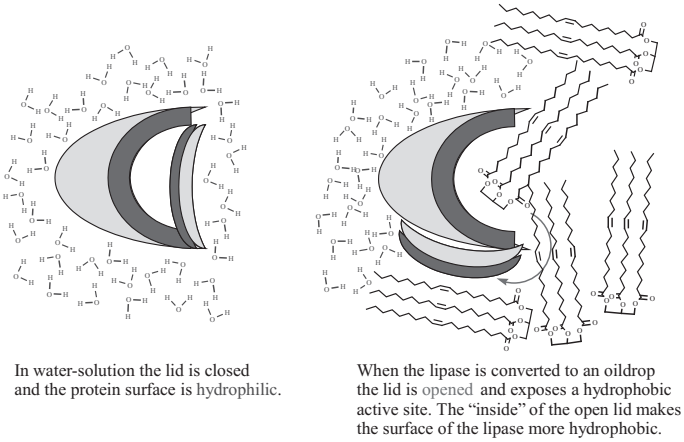

Figure 11.22. Structural conformation-change in lipases during catalysis.

Lipases act in the borderline between a lipophilic phase and water phase, which imposes special demands on the protein structure in terms of stability in both polar and non-polar solvents. Many lipases have solved this problem by performing a conformational change that makes the surface more hydrophobic; the hydrophobic catalytic site is covered by a „lid" with a hydrophilic outside and a hydrophobic inside. When the lipase is transferred into a non-polar phase, the lid is opened and a large exposed hydrophobic surface is created by the catalytic site and inside of the lid (*Figure 11.22*).

Hydrophobic molecules as triglycerides etc. will then direct themselves to the active site, where they are hydrolyzed by a catalytic triade of amino acids similar to many other hydrolases (*Figure 11.23*). These structural characteristics explain the unique properties of lipases in terms of their extraordinary stability in organic solvents. They may also be important for another unusual property of lipases: some lipases have no activity towards triglycerides in the presence of mild detergents solubilizing the triglycerides into the water phase – the lid to the lipase is simply closed in the water phase, and thus can the enzyme not hydrolyze the ester bonds. However, even though all known lipases belong to the same gene family, i.e., they are evolutionary and structurally related, all of them are not inactive in water phases, and there are also examples of lipases lacking the lid over their active sites.

Lipases are used in soap making, food industry, laundry-detergents and for the reduction of pitch problems in the pulp and paper industry (see Chapter 12). Their extraordinary stability in organic solvents and broad substrate specificities have also made them to a useful tool in organic synthesis, where they are used in water free, or close to water free organic solvents to carry out synthetic reactions. The water in the hydrolysis (*Figure 11.23*) is then replaced with an alcohol.

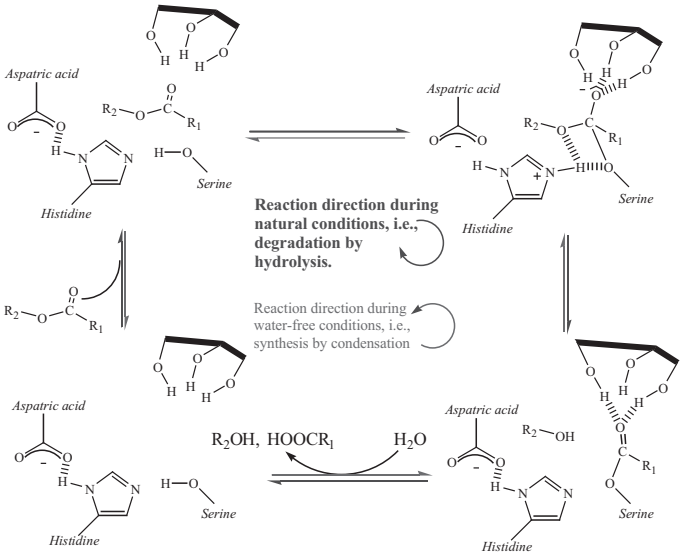

Figure 11.23. Catalytic mechanism of lipases. Compare with the mechanism of glucosylases in scheme 11.2. In water-free systems lipases can catalyze the opposite reaction, i.e., the *formation* of an ester bond by catalyzed condensation, and can therefore be used in organic synthesis.

11.5.2 Tannases

Tannins are polymers that represent one of the main components of wood bark, and they are also found in the heartwood of certain plants, such as Eucalyptus. There are two main types of tannins, *condensed*[8] *tannin*, where the monomers are connected with mainly with carbon –carbon bonds, and *hydrolyzable tannin*, dominated by the presence of ester-bonds and covalent linkages to carbohydrates (as glucose) (*Figure 11.24*, Chapter 7). In general, the presence of tannin in plant material makes it more resistant to microbial degradation (Chapter 10), but several microorganisms, as well as plants and some animals, have tannin degrading enzyme systems. The enzyme system degrading condensed tannin are practically unknown, but have been suggested to consist of both hydrolases and dioxygenases[9]. In contrast, enzymes depolymerising hydrolyzable tannin are hydrolytic esterases similar to lipases. These enzymes are called *tannases* or *tannin acyl hydrolases*.

The best-known tannases are produced extracellularly by various types of moulds such as *Aspergillus*. They are rather large enzymes of 186–300 kDa and typical pH optimum of 5.5–6. Tannases, that have stability comparable with extracellular fugal cellulases, are commonly used in e.g. the food industries, but have so far not found any application in the pulp and paper industries.

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[8] The term „condensed" is established, but actually the „condensed tannin" is not necessarily formed by condensation reactions. Compare the discussion with „condensed bonds" in lignin (Chapter 6).

[9] A dioxygenase is an enzyme that include two oxygen from a dioxygen molecule into a substrate, i.e., the reaction: $O_2 + A \rightarrow AO_2$. See a textbook in biochemistry for details.

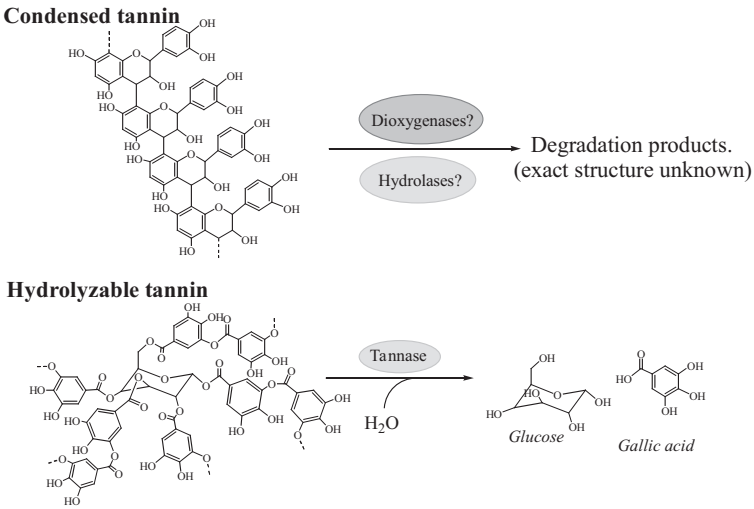

Figure 11.24. Enzymes degrading tannins. The knowledge of the enzyme degrading condensed tannin, and the structure of the degradation products, is very small, but it has been suggested that hydrolases and dioxygenenases are involved. Hydrolysable tannins are degraded by *tannases*, esterases that hydrolyzes ester bonds between gallic acid- and glucose residues.

11.6 Other Enzymes involved in Wood Degradation

In addition to enzymes degrading the main wood polymers, the wood degrading organisms also produce other types of enzymes, which - although they attack minor wood components - can be physiologically very important for the organisms. Some of these enzymes are also technically very important. For example, *pectinases* and *α-amylases* are hydrolytic enzymes degrading the polysaccharides pectin and starch (respectively) that occur in wood in small amounts. Both these groups of enzymes are widely used technically (Chapter 12). Pectinases include both *main-chain cleaving enzymes* (mainly endo-polygalacturonases, but also exo-polygalacturonases[10]) and *debranching enzymes* that attack the arabinose and galactose rich side chains of the „hairy regions“ of pectin. There are also enzymes specialized on methylesterized and non-esterized polygalacturonic acid respectively (Chapter 5). The enzymatic degradation of insoluble pectin is strongly stimulated by oxalic acid or other chelating agents that remove calcium ions cross-linking pectin chains. In addition to the hydrolytic pectinases there are also *pectin lyases*[11] (*Figure 11.19*). Even *proteases* are needed in order to degrade some of the protein components of the cell walls.

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[10] As with cellulases, the exo enzymes attack the chain from free ends, and the endo-enzyme cleave within the chain.

[11] Lyases are enzymes that breaks bonds udner introduction of double bonds. It seems as this type of enzymes can compete with hydrolases for the cleavage of electrically charged polysaccharides as pectin, but not with uncharged polysaccharides as cellulose.

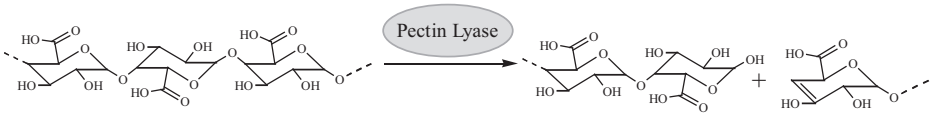

Figure 11.25. Reaction catalyzed by pectin lyase. Note that no water is consumed during the cleavage and that a double bond is formed. This is the difference towards a hydrolysis – compare scheme 11.1 and 11.2.

11.7 Further Reading

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12 Biotechnology in the Forest Industry

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12.1 Introduction

Biotechnology is often defined as *the technical use of living organisms or parts thereof*. However, using this definition the entire pulp and paper industry could be regarded as biotechnology. Therefore, we will here use a narrower definition of biotechnology: *The technical use of microorganisms, genetically modified organisms or enzymes.* Biotechnology is not even by this definition very recent invention; microbial techniques have been used by man for thousands of years to brew beer, to bake bread and to pretreat flax fibers for use in textiles. In the pulp and paper industry, seasoning of wood is a traditional technique that is in part based on microbial action (Chapter 7.7). The advent of molecular biotechnology over the past few decades has opened the way for the development of powerful expression systems for industrial enzymes, and their engineering for improved performance. Owing to this rapid development, enzyme

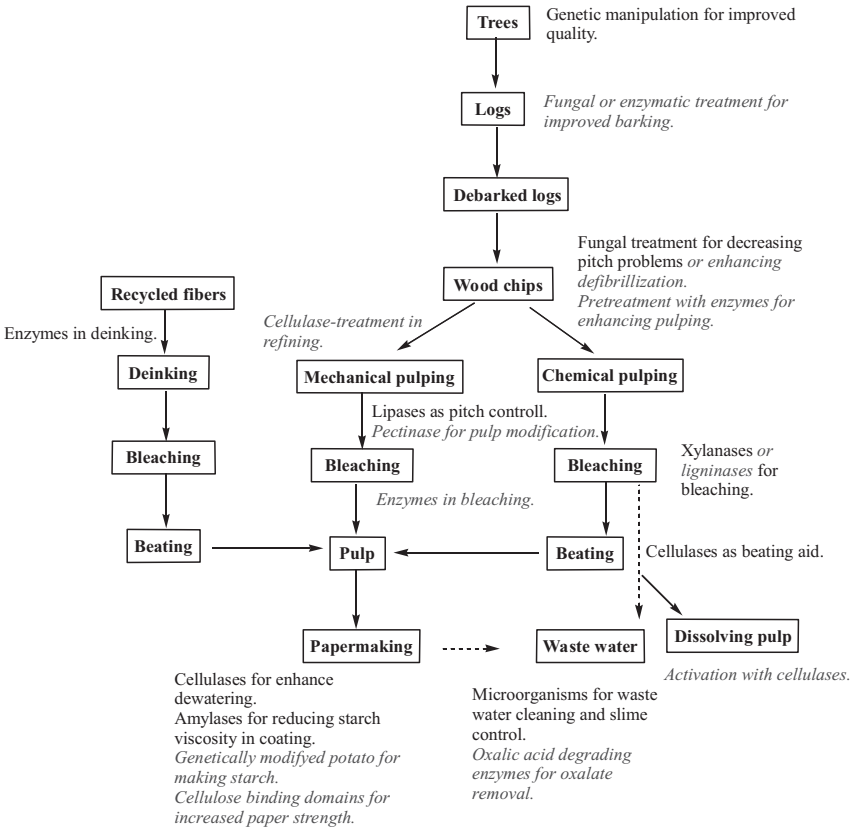


Figure 12.1. Examples of biotechnological applications in the pulp and paper industry. See Volume 2 for a summary of the different pulp and paper industrial processes. The biotechnological methods most commonly exploited include xylanase assisted bleaching of pulp, lipase-mediated depitching of mechanical pulp, viscosity reduction of coating mixtures with amylases and microbiological effluent treatment. Techniques already implemented in the industry are in normal typeface, while *experimental methods are in italics*.

technology is used today for a number of applications in the pulp and paper industry. However, the full potential of the technology has by no means been reached yet.

Pulp and paper industries exploit biological raw materials, which are synthesized, modified and degraded in nature by a number of microbes using a vast array of specific enzymes (see Chapters 10 and 11). Even though the pulp and paper industries have traditionally relied on mechanical and chemical processes, the potential for biotechnology is significant. Microbiological processes used e.g. for wastewater treatment led the way to pulp and paper biotechnology, followed by the first commercial enzyme processes for pulp bleaching in the 1990's. Today there is an increasing interest in biotechnology in order to develop environmentally compatible processes, to lower the energy consumption in mechanical pulping procedures and to develop new tools for improving the quality and the performance of the products (*Figure 12.1*).

The application of enzymes in the enormous scale of pulp and paper industries presents significant challenges at many levels. How can we identify the best organisms that produce enzymes with the most useful properties for wood and pulp processing? How can we produce the enormous quantities of enzymes needed? How can the enzyme stability and activity be guaranteed in the high temperature or extreme pH of some processes? So far, it is the wood degrading fungi that have been most commonly used as the sources of enzymes. They produce complex mixtures of enzymes attacking different parts of wood structure. When used as a mixture, these enzymes lead to practically complete degradation of milled or steamed wood. In contrast, sound wood cannot be degraded by cell free culture filtrates, since lignification makes the structure impermeable. Pure enzymes or partially purified fractions can be used to achieve better controlled modifications. Most of the present day industrial enzymes have been obtained by activity-based screening of microbial enzymes secreted to the culture media, followed by chemical or irradiation-based mutagenesis programs to optimize the protein production and properties for industrial use. Once the desired enzyme has been identified and characterized, the corresponding gene can be transferred into a suitable production organism. Wood degrading fungi, including both soft rots and white rots (see Chapter 10), can be cultivated in large fermentors using cheap carbon and energy sources, and the culture conditions can be optimized for the production of the desired spectrum of enzymes in high yield. Also non-true wood degrading organisms such as moulds including *Aspergillus* or *Trichoderma* species, can produce interesting enzymes as cellulases, and have high capacity to secrete enzymes in their culture media are typically used as hosts for industrial enzyme production.

The first enzyme preparations used in the pulp and paper industries typically consisted of the culture filtrate with up to several hundred different enzymes with diverse activities. Uncontrollable side reactions are a drawback of such crude enzyme preparations. The development of gene technology in the early 1980's made it technically possible to modify the enzyme composition of the production organism, thus paving the way for new large-scale production processes of essentially pure enzymes. Genes encoding enzymes catalyzing side reactions can be inactivated, the level of expression of desired enzymes can be increased, and genes encoding new activates can be added. *Figure 12.2* summarizes the strategy for recombinant protein production.

Due to the high specificity of *monocomponent* enzyme products produced in this way, it became possible to design processes for well-controlled, limited hydrolysis of substances on the pulp fiber or paper surfaces. For instance, in the case of cellulases, the use of a culture filtrate product containing several different endoglucanases and cellobiohydrolases commonly decreases the fiber quality. In contrast, a monocomponent endoglucanase (Chapter 11) can be used for smoothening the fiber surfaces with little or no loss of fiber strength.

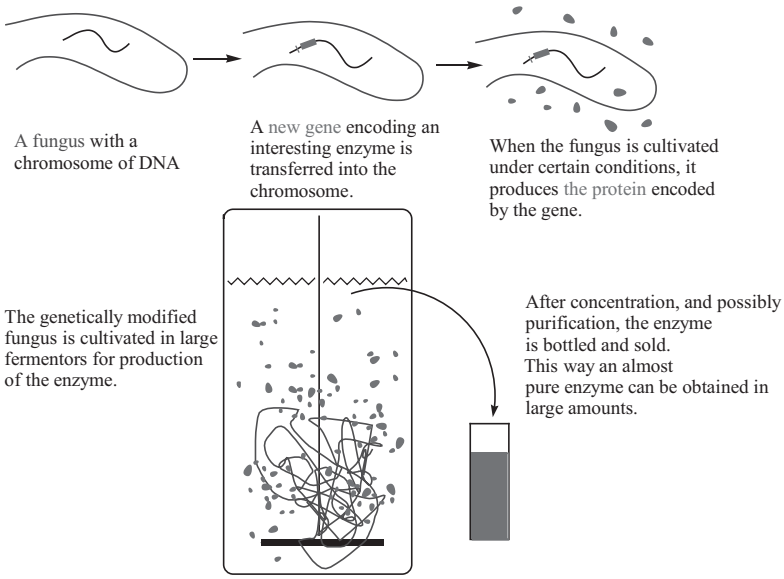


Figure 12.2. Principles of genetic modification for obtaining monocomponent products. There are several organisms used for enzyme production including the moulds *Aspergillus oryzae* and *Trichoderma reesei* the yeast *Pichia pastoris*.

The processes developed for use in pulp and paper applications have been largely developed by empirical means, by treating the pulp with different enzyme preparations followed by measuring their influence on the pulp performance. Due to the high heterogeneity of the natural raw material, lack of pure enzyme preparations and sometimes poor understanding of the reaction mechanisms of the enzymes involved, the exact basis of the effects obtained is rarely understood in detail. Enzymes are also relatively expensive reagents compared to most process chemicals used in the enormous scale of pulp and paper industries. Intensive efforts have therefore been engaged in developing efficient, robust expression systems allowing efficient enzyme production at low cost. However, continued basic research on the properties and production of enzymes involved in the degradation of woody raw material is essential in order to be able to develop better processes for the forest industrial sector.

In addition to microbes, *plants produce enzymes* that could be used to modify the structure and properties of lignocellulosic materials for industrial purposes. In contrast to microbial enzymes, most fiber-active enzymes in plants are engaged in the synthesis rather than degradation of the cell wall components. The advantage of synthetic enzymes is that they can be used to alter the raw material for both better yield and improved fiber performance. The disadvantage of plant enzymes is that they have evolved to operate in moderate temperatures, protected by the plant cell walls. Many relevant plant enzymes are also expressed in plant in membrane found form. Enzymes from plants are therefore more difficult to adapt for industrial use than the more robust microbial enzymes.

The properties of any proteins, including those from plants, can be improved by using *protein engineering*. By this way the amino acid sequence of a protein can be changed by altering the corresponding gene, e.g. to increase protein stability. The modified protein is then expressed

in efficient microbial production systems. Furthermore, the dawning possibilities for fiber engineering *in vivo* may help to overcome the problems of both enzyme stability and the enzyme cost. This is since either native or modified genes encoding proteins capable of fiber modification are expressed in the plant during its growth and development. An alternative to protein engineering for stability is to use enzymes from extremeophiles living in environment with high temperature and extreme pH.

12.2 Microbiological Techniques

The simplest application of biotechnology is to use an entire organism to facilitate pulp and paper processing. Many different organisms are able to attack wood components in nature, using widely different strategies. While some microbes can digest all wood components (see Chapter 10), others are more selective in their action. Thus, by choosing the microbial species carefully, it is possible to achieve selective modification instead of total hydrolysis of the wood components. For instance, white rot fungi can be applied to pre-treat wood chips for enhancing delignification (*biopulping*), and albino strains of sap stain fungi can be applied in reduction of extractive content (*controlled seasoning*). Since microbial processes exploit living organisms, they cannot be carried out in the severe conditions of e.g. the bleaching towers. Microbial activity is also generally insignificant in frozen wood, which is a problem for microbial treatment of logs and chips for mills located in the cold climates of e.g. Canada and Scandinavia. Most microbial processes are also relatively slow, and therefore best suited for treating the raw materials or waste, not for the pulping processes themselves. A concern is also that microbes may cause allergies and other health problems. However, also "normally" stored wood contains a large number of microorganisms growing on the wood.

12.2.1 Biological Waste Treatment

Both pulping and papermaking processes result in large volumes of wastewaters, which contain dissolved wood-derived substances and residual process chemicals. Owing to the increased environmental awareness, these potentially highly polluting raw wastewaters from the mills cannot be directly released in nature. Instead, recycling of the wastewaters is becoming an attractive alternative for many pulp and paper mills as it also offers potential savings in the cost of fresh water. Recycling of the wastewaters can be done either by closing up the systems in the mill or by treating the wastewaters such that they can be reused.

Wastewater purification is usual carried out by a sequential approach. The first step involves a primary clarification by sedimentation or flotation in order to remove solid materials. The secondary treatment involves either an aerobic microbial process called the activated sludge operation, or an anaerobic digestion. The activated sludge process, which is the most commonly used approach for treating the waste water in pulp and paper mills, operates through successive action of many different microbes active in the sludge. The process is often carried on site in large aerated tanks. The success of the process is dependent on successful maintenance of the dissolved oxygen as well as good settling of the sludge. The settlement, in turn, depends on the type of microbial flora involved; the presence of excessive amounts of filamentous bacteria forming a matrix for flock forming bacteria can cause significant settlement problems. Howev-

er, at the growth requirements are different for different microbial species, these problems can often be controlled by varying the rate of aeration, temperature or the nutrient composition of the process (Thompson et al, 2001). In biological waste treatment a very large number of different microbes can be used. Sometimes additional nutrition, as nitrogen in the form of urea, is added to enhance the biological waste treatment.

12.2.2 Biopulping

Pulping processes rely on mechanical or chemical disintegration of the wood structure in order to liberate the fibers. Mechanical pulping processes consume very much energy and result in paper, which is weaker than paper produced by chemical means. Biopulping is a microbial process in which wood decaying fungi are used to pretreat wood chips in order to facilitate subsequent mechanical, thermomechanical or chemical pulping. The idea of fungal pre-treatment of wood arose already in the 1950's, upon the realization that certain white rot fungi that grows mainly to the delignifying strategy – see Chapter 10) can remove lignin (and hemicellulose) from the wood cell walls leaving the cellulose. However, the cellulose from wood delignified in this way has normally a low degree of polymerization and thus produce a very weak paper. A shorter treatment with white rot fungi, that not remove all of the lignin, have however been shown to enhance both mechanical and chemical pulping. However, the need to solve the secrets of enzymatic delignification and to identify a microbe suitable for the technical requirement of biopulping have led to a long development time towards a functional industrial process.

There are several requirements for a fungus to be able to work commercially in biopulping. These include:

1. Relatively fast growth rate,
2. Ability to grow on both hardwood and soft wood,
3. Preferred activity against hemicellulose and lignin combined with low activity on cellulose,
4. Ability to degrade extractives,
5. Inability to elicit allergies, since the growth of moulds on chip pales causes health problems for the workers in the pulp and paper industry,
6. Aggressive competition against other microorganisms that could damage the cellulose and give other problems,
7. Low pigmentation that might decrease pulp brightness,
8. Good ability to sporulate in order to facilitate the inoculation of the wood chips.

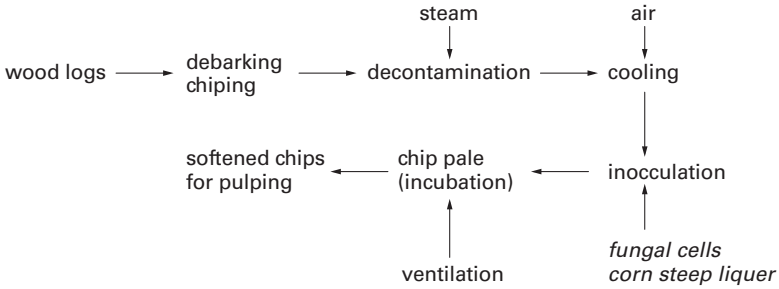


Figure 12.3. Overview of the basic components of a biopulping operation. Details of the process may vary depending on the conditions and facilities in particular mills.

Several fungi have been tested with promising results among them the white rot fungi *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora*. While the first grows according to the “simultaneous rot” strategy (see Chapter 10), and can degrade also cellulose efficiently, most focus has been on *C. subvermospora*, a "delignifying" fungus that fulfils the first five criteria. At the university of Wisconsin in Madison, USA, research has been carried out on mainly biomechanical pulping with this organism, and several full-scale tests have been performed. The biopulping processes so far developed (*Figure 12.3*) are compatible with the operations at a mechanical pulping mill, although positive effects have been also observed in connection with chemical pulping.

The wood chips are first decontaminated on site by steaming to remove competing microbes, which can attack cellulose and thus destroy the fiber quality. After a cooling phase, the chips are inoculated with the biopulping fungus. Supply of additional nutrients such as a cheap sugar solution (corn steep liquor) facilitates the fungal colonization process, but is not absolutely necessary. The inoculated chips are then piled and the treatment is allowed to proceed for 1–4 weeks in the optimal temperature and moisture content for fungal growth. Energy savings of 30–40 % in the refining step (see Volume 2 for a description of mechanical pulping) have been reported for the biopulping compared with untreated control, and the yield loss were insignificant (99 %). The paper produced is often also stronger than the control, but the pulp brightness often decrease (in one case from 55 % ISO to 40 % ISO). It was however easy to adjust this brightness loss with hydrogen peroxide bleaching. Although, the energy saving thus is considerably, the biopulping with *C. subvermospora* have weaknesses – this fungus is not particularly aggressive against other organisms, and the inoculum is relatively costly, and therefore "sterilized" by using a quick steam treatment and nutrition addition was necessary (*Figure 12.3*). Presently (2005), there is no commercial use of biopulping, and the search for other suitable fungi and technical solutions have been activated. One candidate is the white rot fungus *Phlebiopsis gigantea* that is very aggressive towards other microorganisms. This organism may also stimulate debarking of logs as described below. The non-selective white rot fungi *Coriolus hirsutus* have been modified, so that cellulose degrading enzymes are partly suppressed, and have been shown to enhance Kraft pulping of hardwoods (Eucalyptus). This fungi is interesting since it grows at higher temperatures that most other organisms.

In spite of intensive research efforts, the exact mechanism of biopulping has remained obscure. Microscopic studies have shown that, in some cases, the cell wall morphology is not visibly altered but the material no longer reacts with lignin stains. In other cases, improvements in pulp properties have been observed with samples with a mass loss of less than 10 % during the fungal treatment. It seems thus that other factors than delignification also contribute to the biopulping process. Gradual penetration of marker proteins has been observed as a function of biopulping time, indicating a generation of pores in the cell wall structure. Since the pores do not seem to be large enough to allow diffusion of ligninolytic enzymes, these effects may be result of action of the lignin-oxidizing intermediates generated by the fungus. The opening of the pores in the cell wall may actually also contribute to easier penetration of pulping chemicals, providing a plausible explanation for the positive effects also noted during chemical pulping. The lignin may off course also be structurally modified, although the fungus not does remove it.

12.2.3 Controlled Seasoning of Wood

Seasoning of wood, i.e. storage of the wood logs or chips for a time prior to pulping decrease the pitch content in wood and is a traditional method used to diminish pitch problems during the paper-making processes and in the equipment (7.7). Pitch consists of waxy, hydrophobic extractives, such as triglycerides and steryl esters, and cause a lot of technical problems in especially mechanical pulping; it deposits on equipments, disturb the fiber-fiber bonding (due to deposits on the fiber surface that disturb hydrogen bonding) thus leading to weaker paper, and larger pitch deposits (that have get lost from equipment) can also cause tearing of the paper sheets in the paper machine, leading to very costly web brakes. However, traditional seasoning offers no control over the microbial species growing on the chips, and the results are therefore highly unpredictable; the formation of color is common (loss of brightness), and strength loss due to attack of soft rot and white rot fungi is not uncommon. Methods for *controlled seasoning* have thus been developed using microbes that can both effectively remove extractives without causing discolorization or strength loss, and suppress the growth of other microbes. An albino strain of the sap stain fungus (9.2.4), *Ophiostoma piliferum*, is commercially available for controlled seasoning under the name "Cartapip™". It degrades extractives and prevents growth of other microorganisms on the chips if it is applied on fresh chips. This treatment reduces the risks of pigment formation, loss of pulp yield and fiber weakening, and decreases the pitch content in both high- and low-extractive containing wood, although it is mainly used for pines, that often have high pitch content. As shown in *Figure 12.4*, the main targets are the triglycerides, which are the likely key component in pitch deposits (see also 12.2.2). In the case of e.g. pine wood, with high content of extractives, the fungal treatment also improves the fiber properties in mechanical pulp and even seems to lower the energy consumption in refining. This might be because the fungal treatment open pits in the wood that might improve the steaming pre-treatment in mechanical pulping and by an enhanced fiber-fiber interaction (*Figure 12.5*).

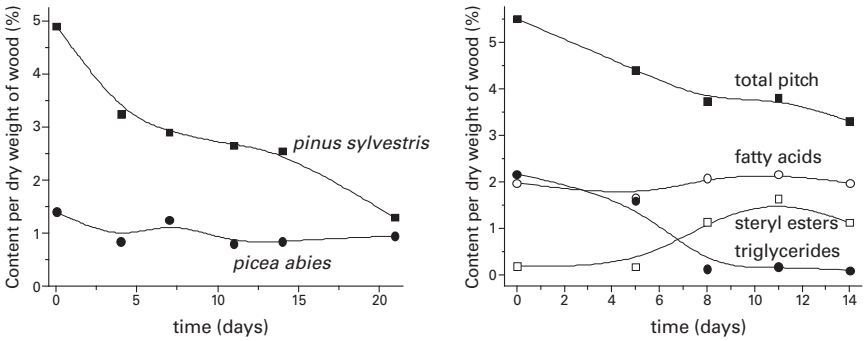
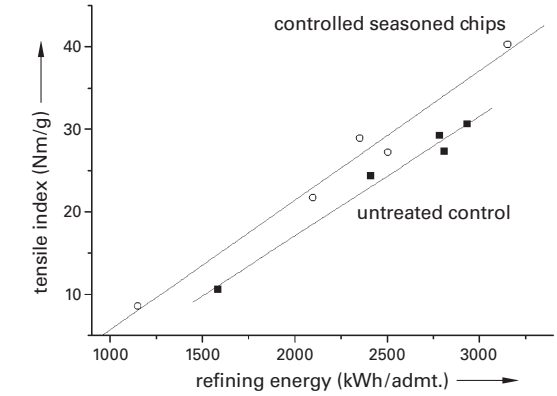
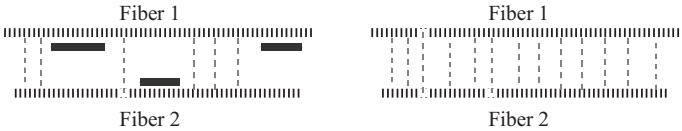


Figure 12.4. Pitch decrease by controlled seasoning using Cartapip™. Left, effects of controlled seasoning of Norway spruce (*Piciea abies)* with low content of extractives, and Scott's pine (*Pinus sylvestris*) with a relatively high content of extractives. Right, the rate of degradation of different classes of extractives in Scots pine. As shown it is mainly the triglycerides that are degraded.

Apart from *Ophiostoma piliferum* (Cartapip™), many moulds and also *C. subvermospora* (see above) are similarly able to degrade extractives. However, by using Cartapip™ instead of the traditional uncontrolled seasoning, a better control and reproducibility of the seasoning pro-

cess can be obtained, and the negative side effects of discolorization and strength loss can be avoided. Technically, controlled seasoning is much easier to use than the biopulping process described above, since no sterilization, ventilation or nutrition addition are necessary. In spite of these apparent advantages, practical use of controlled seasoning is limited to a few mills making mechanical pulp from pines in North America.





In the pulp made of untreated chips the extractive content is high and deposits with a core of triglycerides lay on the fiber surface and partly block the possibility to form hydrogen bonds giving a weak paper.

In the pulp made of controlled seasoned chips the extractive content is low. Few deposits lay on the fiber surface and many hydrogen bonds are created between the fibers giving a strong paper.

Figure 12.5. Effect of controlled seasoning on paper strength of thermomechanical Scots pine pulp. The graph above shows that the tensile strength is higher at similar refining energy for controlled seasoned chips than the untreated control. Thus a fungal pretreatment may save energy, or alternatively produce a stronger paper. The cartoon below shows a hypothesis for the mechanism behind the effect. For a discussion of the complex strength developments in mechanical pulp, see Volume 2.

Microbial destruction of wood is a large problem in forestry; in Scandinavia around 15 % of the trees are infected with rooting fungi and in tropical countries the situation is even worse. In a process similar to controlled seasoning, the white rot fungi *Phlebiopsis gigantea* is applied to tree stumps directly after harvest This is carried out automatically by the harvesting machines. The purpose of the treatment is to prevent the growth of another fungus, *Heterobasidion annosum* that can infect the stand through the fresh wood surface and subsequently spread via root contacts to nearby trees to cause damage and discolorization of the wood. The method is in practical use in Swedish forestry.

12.2.4 Microbial Aided Debarking

Birch bark contains large amounts of the extractive betulinole (see Chapter 7) that causes depositions in Kraft pulp made from chips contaminated bark. The depositions may form spots in papers, web brakes and sets off on equipments (see Chapter 7). The colored bark of pines and spruces may give dark spots in mechanical paper. Efficient debarking is therefore needed. Debarking of logs is, however, not a trivial task and intensive debarking leads to significant losses of wood yield. Thus, techniques for enhancing debarking are interesting. The innermost layer of the bark, i.e., the phloem and the cambium has a different chemical composition than the wood log itself (Chapter 2, *Figure 12.6*); The middle lamellas in this region consist mainly of pectin, which is more susceptible to microbial degradation that the lignified fibers. Microbial or, potentially, enzymatic removal of pectin could thus be used to weaken the binding of the bark to the log, thus facilitating debarking. There are different types of fungi that are able to degrade attack this tissues, both white rot fungi, and specialized moulds. A problem is that the cambium/phloem are living tissues and have defense systems against microbial attacks. A few studies with treatment of logs (mainly softwoods) with different types of fungi – the inoculum is added to the stumps of the logs – have shown that barking can be enhanced, but the technique has not been commercialized.

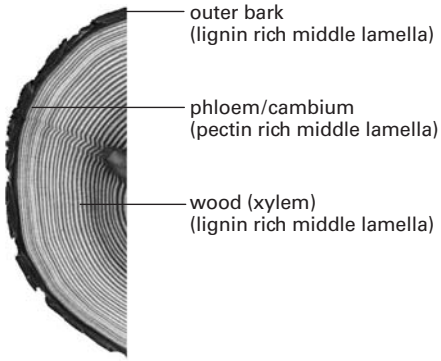


Figure 12.6. Principle for microbial aided barking. The Phloem and cambium layers (inner bark) located between the wood (xylem) and the other bark in wood are only lignified to limited extend and can therefore be degraded by moulds.

However, a similar microbial technique called *retting*, has been used for thousands of years for the processing of fibers from flax and hemp. The valuable fibers in these plants are located in the phloem, whereas the xylem (wood) contains short fibers of low value. The retting helps the "debarking" but, in contrast to wood, in this case it is the bark that represents the valuable product.

12.2.5 Microbial Treatment of Sawed Timber Products

The possibilities to use biotechnology to improve the properties of sawed timber products are less explored than those improving pulping processes. However, the possibilities to exploit the

natural process of sap stained wood (blue wood) have been explored to some extent in carpentry. Recently there has also been some interest in utilizing the Cartapip™ (see above 12.2.4) treatment for enhancing the effectiveness of impregnation of wood. The mechanism behind this is probably that the sap-stain fungus opens pores in wood by degrading boarded pits and extractive deposits.

12.3 Enzymatic Techniques

The main advantage of using enzymes in industrial processes is their high specificity. Normally an enzyme catalyzes only one single reaction on one single substrate, and such high specificity is rare among industrial chemicals. Furthermore, enzymatic reactions are carried out at mild conditions, whereas many other chemicals require high temperature, or high or low pH, that might affect the pup structure. Therefore it is possible to do things with a pulp with enzymes that cannot be done by ordinary chemicals. As discussed above, there are two main types of commercial enzyme products, *culture filtrates*, which are mixtures of many different proteins with various specificity, and *monocomponent enzyme preparations*, which consist of a single type of enzyme. However, there are also limitations in using enzymes in a large-scale industry employing harsh processing conditions. Enzymes are polymers of amino acids. Their activity and other properties depend on folding of the amino acid chain into a specific three-dimensional structure held together by non-covalent interactions and covalent bonds such as disulphide bridges (10.1). Most protein structures, and thus their activity, are relatively sensitive to high temperatures, extreme pH and organic solvents. These are conditions typically used in many pulping processes such as Kraft cooking or oxygen delignification (see Volume 2). There are, however, other modern pulping processes more compatible with enzymatic treatments. For instance, chlorine dioxide bleaching, which is one of the most important bleaching methods, is carried at pH around 4.5, and at 60 °C. Nevertheless, enzyme stability is an issue of high relevance for their use in the pulp and paper industry. Most industrial enzymes used today are extracellular enzymes from microbes (Chapter 10 and 11). Since these enzymes are secreted in the culture medium of their host, they have evolved to act in the challenging extracellular environments requiring high stability. Furthermore, even more stable enzymes can be isolated from organisms living in extreme environments such as volcanic hot springs.

The cost of the enzymatic treatments is another issue limiting their use in pulp and paper processing. Enzymes are catalysts and, unlike chemical bleaching agents such as hydrogen peroxide or chlorine dioxide, they are not consumed in the reactions. However, due to the relative instability of most enzymes, they have limited lifetimes, and in practice it is seldom possible to reuse them. It is also for the enormous scale of the operations that enzymes are needed in vary large quantities. As mentioned above, it is mainly only filamentous fungi that are have high enough production capacity to meet the needs of the pulp and paper industries. *Mixing the enzymes with the pulp* is yet another critical step for enzymatic treatments, since heavy mixing often leads to foaming and inactivation of the enzymes. Most cellulases and also some other enzymes have a strong affinity to cellulose (Chapter 11), which may lead to uneven treatment of the pulp. It is therefore often necessary to add the enzymes in a diluted form.

12.3.1 Enzymatic Pre-treatment of Chips and Logs

Enzymatic treatment of native wood does not, at the first glance, seem like a good idea, since lignification makes the wood so compact that the large enzyme molecules cannot penetrate into the cell wall (6.1). Nevertheless, enzymatic treatment of wood chips with different enzymes has given positive effects on both mechanical and chemical pulping.

Pre-treatment of pine chips with the ligninolytic enzyme, laccase (10.4.1), has been shown to lower the energy consumption of mechanical pulping by 6–8 %. Also pre-treatment with manganese peroxidase (10.4.2) seems to have similar effects. The mechanism behind this effect is probably depolymerization or modification of lignin structure, although no mass loss of the treatment has been detected. However, manganese peroxidase is technically more difficult to apply in the process, as discussed below (12.2.6).

Pre-treatment of wood chips with polysaccharide degrading enzymes (cellulases, xylanases and pectinases) give some improvements of Kraft pulping (lower reject and increased delignification). The mechanism behind these effects may be that non-lignified parts in the wood, such as boarded pits, are degraded, and that this enhances the penetration of the white liquor in the wood chips during pulping.

Pulping of non-wood raw material with assistance of enzymes has better possibilities. High quality pulps of cotton and bast fibers of flax and hemp, is made by an intense beating of the raw fibers that make them more flexible and shorten them. This process is energy consuming, but addition of cellulases and – for hemp and flax – pectinases shorten the beating time considerably. The technique has been tested in full scale, but is presently not used.

12.3.2 Lipases for Pitch Control

Pitch is a common name for heterogeneous wood extractives mainly composed of non-ionic triglyceride esters of fatty acids. Pitch deposits accumulating on paper represent a problem especially in mills focusing on mechanical and sulphite pulps. As discussed above, pitch depositions lower the graphical quality of the paper by giving dark spots, and can also cause breaking of the paper on the paper machine, as discussed above (12.2.3). The extractives often aggregate around a “core“ of triglycerides. It has been shown that addition of lipases, enzymes hydrolyze the triglyceride to glycerol and fatty acids, reduces the problems with pitch deposits (*Figure 12.7*).

Lipases (Chapter 11) have been available for commercial use for decades e.g. as components in laundry detergents, and are also used to control pitch problems in mechanical pulp mills especially in Japan and China, relying on oriental tree species with high content of extractives (See Chapters 2 and 7). By using lipases, the pitch deposits in the paper may be decreased up to 30 %, and improved brightness and paper strength are obtained. The effect on paper strength may be due to improved fiber-fiber interaction when the triglycerides deposited on the fiber surfaces are removed (see *Figure 12.5*). The lipases are normally added directly after the refining and the bleaching.

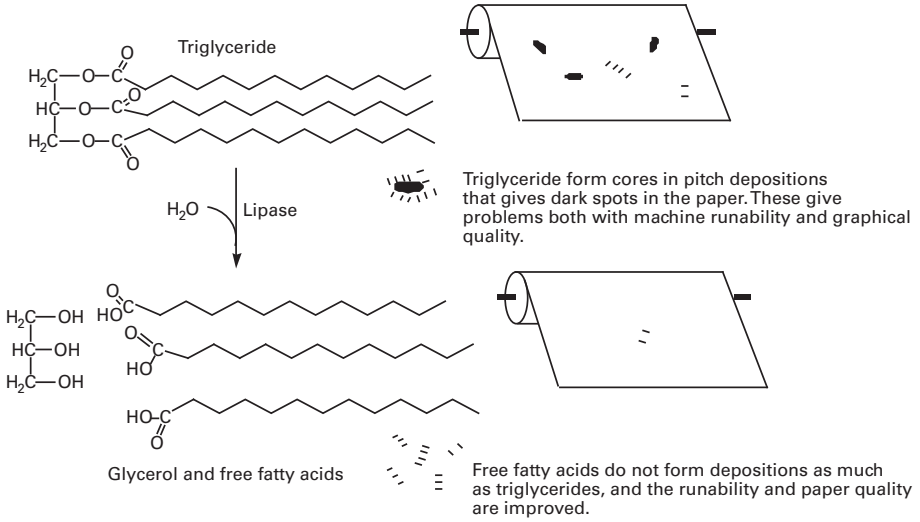


Figure 12.7. A hypothesis for the effect behind the effect of lipases on pitch problems.

12.3.3 Pectinase Treatment of Mechanical Pulp

Pectins constitute a minor component in wood in general, except in the primary cell wall, which has relatively high pectin content (Chapter 5). In mechanical pulps, large proportions of the primary wall is preserved on the fiber surfaces, which thus have high pectin content. Since pectin is a highly a charged polymer, it contributes to the electrical charge of the fiber surface. This gives sometimes problems with the interactions of positively charged coating polymers, and treatment with the pectinase, endopolygalacturonase (10.6), is way to adjust the charge. The method has been commercial used.

12.3.4 Enzymatic Deinking

Recycled paper is an important source of raw material for the pulp and paper industry. In this case, one of the major problems is that the ink needs to be removed from the paper if it shall be re-used for making white paper. Therefore, recycled paper is treated with different deinking processes based on detergents and separation of ink particles by floating, i.e., that the particles are concentrated at the surface and removed.

The deinking process can be substantially improved by the addition of various enzymes to the detergent mixture. This approach is in commercial use in many countries, in fact, enzymatic deinking is one of the most common enzymatic technique in the pulp and paper industry. The detergents and the enzymes must be carefully chosen in order to avoid enzyme inactivation. The exact composition of the industrial enzyme preparations used for deinking are industrial secrets, but robust fungal amylases (starch degrading enzymes), cellulases and to some extent, lipases and hemicellulases are generally included. Lipases probably attack fatty structures in the ink it-

self, whereas the other enzymes are believed to attack either the starch used in paper coating or the cellulose around the printed area. *Figure 12.8* shows a suggested hypothesis for how cellulases facilitate deinking.

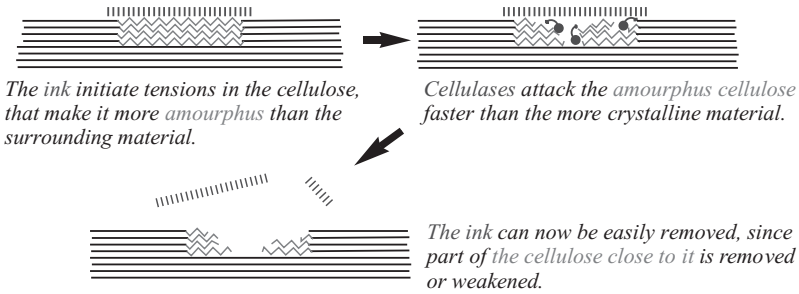


Figure 12.8. A hypothesis for how enzymatic deinking works.

12.3.5 Xylanase Supported Bleaching

The discovery that xylanases can be used to facilitate pulp bleaching was made in late 1980's, and xylanase-assisted bleaching processes have since become established technology in many countries, including Finland and Canada. The symbol used for this step in describing the various bleaching sequences is normally "X" (see Volume 2). By using xylanases, the bleaching chemical consumption can be reduced by 10–20 % with concomitant improvements in product quality.

The xylanase treatment is normally carried out as an early step in the bleaching sequence, and it usually leads to the degradation of less than 10 % of the xylan in a pulp. Xylanases cannot degrade or modify lignin directly. The mechanism for how xylanase bleaching works is not clear, but three main hypotheses are shown in *Figure 12.9*. On one hand, it has been observed that the kappa number, which reflects the lignin content of pulp, is decreased during the xylanase treatments. This suggests that xylanases contribute to delignification of the pulps On the other hand, it has been suggested that xylanases might act by removing hexenuronic acid, which is a component of some xylan polymers, and which consumes bleaching chemicals (see Volume 2). Depending on the type of pulp, either one or both of these mechanisms may contribute to the bleaching effects observed. A third explanation is offered by the observation that some of the xylan dissolved during Kraft pulping of birch-wood under classical conditions reprecipitates on the fiber surfaces. These precipitates may trap some residual lignin, the removal of which could explain the bleaching effect of the xylanases. However, xylanases also improve the bleaching of Kraft pulps made of softwood and pulps prepared by prolonged cooking. In these cases, there should be much less xylan deposited on the fiber surfaces, and the bleaching effect is much more difficult to explain according to this hypothesis.

In addition to xylanases, other hemicellulases, such as mannanases, can be used to facilitate pulp bleaching. Both xylanases and mannanases have been divided into several enzyme families with different specificities as discussed in Chapter 11. Although bleaching with hemicellulases in most cases is performed with a crude mixture of different enzymes, experiments with pure enzymes have shown that most of the effect is dependent of a single enzyme, the endoxylanase.

This can be partly explained by pulping chemistry; the side-groups are removed to a large extend during the Kraft cook and debranching enzymes are thus generally unnecessary. Addition of endomannanases may support bleaching of soft wood pulps, but they are generally relatively inefficient when acting alone. Endomannanases may also act to release physically entrapped lignin from the pulp. However, solubilized mannan is normally more extensively degraded during cooking than xylan, thus explaining the weaker bleaching effect of mannanases.

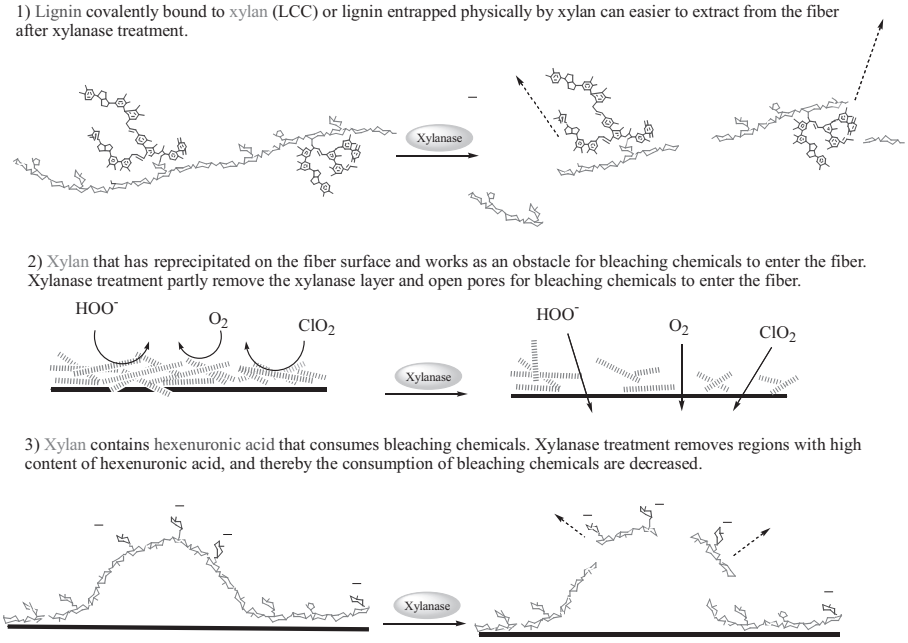


Figure 12.9. The three main theories on the mechanism of xylanase bleaching.

During the early years of xylanase bleaching, decreased viscosity of the xylanase-treated pulps was commonly observed. This was probably due to contamination of the enzyme preparations by cellulases. Once totally cellulase free xylanases became available, increases in the viscosity of the pulps were observed instead. This may be due to preferential degradation of xylan polymers that has with low degree of polymerization compared to cellulose, and therefore the average degree of polymerization increase.

In Canada the following sequence is used frequently for xylanase assisted bleaching:

OXDEDED

The pH of the pulp is first adjusted to a suitable value for the enzyme. Most enzymes form soft or white rot fungi work best between pH 4 and 6. However, screening of enzymes in organisms living in extreme conditions has led to the identification of xylanases that work at higher pH. The enzyme is added to the bleaching tower as a water solution, and the incubation is carried out for a couple of hours. The temperature should in most case be 40 to 50 ºC, but some enzyme works even at 100 ºC.

Xylanase bleaching has also been used in total chlorine free (TCF) bleaching sequences (see Volume 2), and in chlorine bleaching still used in many countries. Also in these cases the X step is early in the bleaching sequence, normally after oxygen-mediated delignification. Xylanase bleaching has also been tested as a final bleaching step in TCF bleached hardwood pulps. The purpose is to remove hexenuronic acid from these (*Figure 12.9*). Hexenuronic acid is known to cause post yellowing, and this effect is thereby partly prevented.

12.3.6 Ligninases in Bleaching

One of the first concrete ideas in forest industrial biotechnology was to use ligninolytic enzymes to facilitate pulp bleaching. In contrast to xylanases that act indirectly to remove lignin, bleaching with ligninases would constitute a real delignifying step. However, due to the very complex chemistry of ligninolytic enzyme systems, it was not until the end of 1990's that the first promising results began to emerge. Bleaching with ligninolytic enzymes from white rot fungi appears most promising, since this class of fungi can perform relatively selective lignin degradation. However, the cellulose always appears to be damaged somewhat, even by "delignifying" white rot fungi (Chapter 10). Lignin degradation by white rot fungi is based on oxidations of non-phenolic and phenolic aromates in lignin, and by radical attacks of hydroxyl radicals, as described in Chapter 11. This chemistry is very similar to the reactions in oxygen delignification described in Volume 2. Some of the ligninolytic systems have, however, a much higher selectivity for lignin than oxygen delignification (that also damages cellulose), and therefore the concept of bleaching with ligninases has gained much interest. However, significant technical problems remain with ligninolytic bleaching techniques, and highly selective bleaching can also be obtained by using chemicals such as for instance chlorine dioxide and peracetic acid. For these reasons, methods based on ligninase based bleaching have not been commercialized, and the interest for the technique is gradually declining.

As described in Chapter 11, ligninases generally cooperate with low molecular weight cofactors, redox mediators, and need an oxidant, such as hydrogen peroxide or oxygen. This makes these techniques more complicated than xylanase bleaching, and reasonably realistic bleaching stages have only been presented for two ligninases, manganese peroxidase (MnP) and laccase.

Manganese peroxides (MnP) depend on hydrogen peroxide and manganese (II) (Mn^{2+}) for the bleaching reaction. In the presence of hydrogen peroxide, Mn^{2+} is oxidized to Mn^{2+}. This is the reactive species performing a one-electron oxidation on the lignin to recreate Mn2+ (see Chapter 11). Furthermore the manganese ion needs a chelator, such as malonate or oxalate. Over-dosing of hydrogen peroxide will inactivate the enzyme as described in Chapter 11. Although these difficulties a bleaching stage for laboratory use have been developed based on MnP. *Table 12.1* shows the results of a representative experiment.

Table 12.1. Results of manganese peroxidase bleaching[1].

Step	MnP bleaching	Control without enzyme
	Kappa number	
O(MnP+Q)/OQ	25.8	28.7
EP	21.1	24.3
P	15.7	21.5

As shown above, the kappa number is lower for the MnP bleached pulp than for the control in all steps but the largest difference is seen in the final hydrogen peroxide bleaching step. Thus, it appears that the MnP treatment in some way enhances the hydrogen peroxide bleaching. The most probable explanation is that reactive groups are introduced. Carbonyl groups in α-position on the lignin, that are very easily attacked by hydrogen peroxide bleaching might be a possibility, something that is supported by that the MnP bleached pulp actually had a lower brightness than the control directly after the MnP + Q/Q stages. The opposite was the case after the P-stage.

Laccases are in many ways easier than manganese peroxidases to apply technically, since they use molecular oxygen as the oxidant and cannot be inactivated by over-dosage (See Chapter 11). Laccases based bleaching ban be performed in bleaching in equipment similar to that in oxygen delignification, and the enzyme can also be produced at relatively moderate cost. However, efficient bleaching by laccases is dependent on the addition of a low molecular weight redox mediator (see Chapter 11). Although some putative natural redox mediators have been identified (Chapter 11), most research has been focused on finding a synthetic mediator more efficient than the natural mediators. The structures of some of them are presented in *Figure 12.10*. One has also tried to find lignin fragments that work as redox mediators.

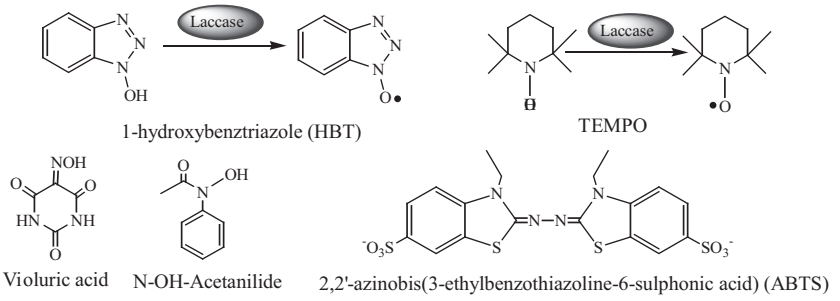


Figure 12.10. Synthetic redox mediators for laccase used in pulp bleaching. For HBT (the most used mediator) and TEMPO, the oxidation to the active radical form is shown. In the case of ABTS the active form might be a two electron oxidized species. All these mediators are relative specific for lignin, with TEMPO as an exception; this chemical can also oxidize cellulose.

The role of the redox mediator in the laccase system is not only to overcome sterical barriers, as discussed in chapter 11. The activated mediators also perform different types of reactions on the lignin than the laccase itself; activated mediators can abstract hydrogens from the α-carbon leading to depolymerization reactions, whereas laccase mainly oxidize the phenol, to a stabilized radical, that can decompose, but also lead to coupling reactions (see 12.4.1.). In *Figure 12.11*, some suggested lignin reactions in laccase mediator systems are shown.

Most efficient redox mediators contain an NO group that is believed to be oxidized by the laccase to stabilized NO· radicals, which are believed to selectively attack the lignin. In the case of ABTS there are, however, some indications that the active component may be a two-electron oxidized form. It has been shown in numerous lab scale experiments that efficient delignification of pulp can be achieved by using a combination of a laccase and a mediator. Experiments with lignin model compounds and HBT have shown that the system is potent to break αβ-link-

[1] *P*, alkaline hydrogen peroxide bleaching. *Q*, treatment of pulp with a chelator, *E*, alkalic extraction.

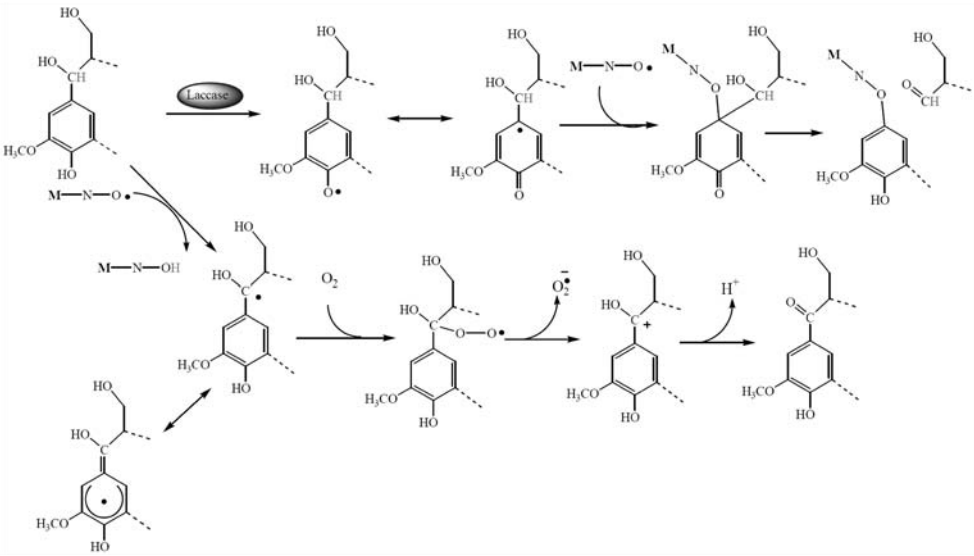


Figure 12.11. Some suggested lignin reactions in laccase mediator systems. M–N–O is a redox mediator as TEMPO or HBT. Laccase oxidizes the phenols to stabilized radicals. Activated mediators can in opposite to the enzyme abstract hydrogen from the α-carbon, forming a resonance stabilized radical, that can be oxidized by O_2, eventually forming a carbonyl, that can be attacked during hydrogen peroxide bleaching. Activated mediators may possible also couple to aromatic radicals, wich may lead to depolymerization of the lignin

ages in lignin. The result of a full-scale experiment has also been done with HBT as mediator is shown in *Table 12.2*.

The data shows that the laccase step decreases the kappa number without any decrease in pulp viscosity. On the other hand, the brightness decreases, which may be explained by the formation of quinones in the pulp. The brightness increases however quickly in the following extraction and hydrogen peroxide bleaching steps.

Table 12.2. Mill scale bleaching with laccase and HBT. A softwood Kraft pulp was uses. The conditions for the laccase stage were 45 °C, pH 4.5 and a pressure of 2 bars. Two kilograms of laccase and 13 kg of mediator were applied per ton of pulp.

Step	Kappa number	Viscosity* (ml/g)	Brightness (ISO%)
Oxygen delignific.(O)	10.7	830	36.5
Laccase bleaching (L)	8.2	856	34.2
Alkalic extraction (E)	6.0	755	49.7
Chelator extraction and peroxide bleaching (QP)	Not measured	604	84.5

* The viscosity measured this way is dependent of the DP of cellulose in the pulp.

Although the laccase bleaching stage is easier to upscale than MnP bleaching, it cannot presently compete with traditional techniques, partly due to the cost of HBT, and environmental concerns (many redox mediators are toxic).

12.3.7 Cellulases in Mechanical Pulping

Mechanical pulping of softwood, and to some degree also hardwood, is an important process for providing pulp for cheaper qualities of paper, such as newsprint and journal paper. The energy consumption of mechanical pulping is, however, very high and techniques for decreasing this are thus very interesting. Biopulping is a microbiological approach that is clearly effective in decreasing the energy consumption of different pulping processes as described above. Enzymatic techniques may offer alternative approaches even though enzymes cannot directly attack the lignified cell walls in wood (Chapters 6, 10 and 11). However, if the enzyme is added after the primary refining step, which has softened the wood structures, energy can be saved in the secondary refining steps (*Figure 12.12*). Among the different cellulases so far tested, purified cellobiohydrolases, which can efficiently degrade even the most crystalline parts of cellulose (Chapter 11) gave the best effects.

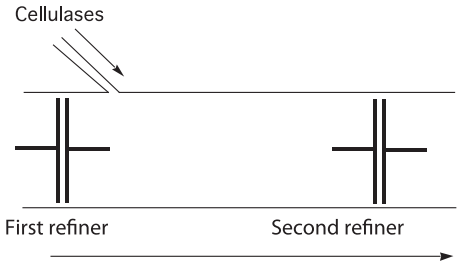


Figure 12.12. Cellulase treatment for decreased energy consumption in mechanical pulping.

In spite of the clear technical benefits, this method has so far not been commercialized. One problem is that the conditions between primary and secondary refiners in most mills are not suitable for enzymatic treatments, in aspects as high temperature and low swell time.

12.3.8 Cellulase Supported Beating

Cellulase treatment has also been applied to facilitate the beating of chemical pulps. Especially endoglucanase treatment has been demonstrated to decrease the need of beating, leading to improved pulp properties. Various combinations of the beating procedures and enzymatic treatments have been tested. For example, the enzymes may have been added before the beating, or the enzyme step has been added in between two cycles of beating. In this way effects such as improved formation of the paper sheets have been observed, but the exact mechanism remains unclear. Under some conditions a long fiber pulp (softwood) can be converted to a more short fiber type pulp with improved formation as a consequence. The method has been tested in industrial scale, but it is presently not in commercial use. Exactly how the enzymes improve the beating is unclear, but that they increase fibrillation in some way, appears to be a plausible hypothesis.

12.3.9 Cellulase Treatment for Enhancing Runability

Cellulases have also been applied for treatment of chemical pulp after beating in order to facilitate the dewatering rate, thereby increasing the maximum speed of the paper machine. A possible explanation for this phenomenon is that the enzymes reduce the content of fines and reduce fibrillation of the cellulose[2] (*Figure 12.13*).

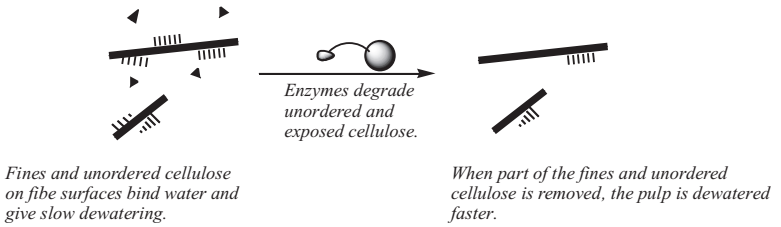


Figure 12.13. Hypothesis for how cellulose treatment increases the dewatering rate of chemical pulps.

12.3.10 Amylase Treatment of Coating Mixtures

Amylases are commonly used for adjusting the viscosity of starches used for coating of papers. This is one of the most common biotechnological method presently in use in the pulp and paper industry.

12.3.11 Enzymatic Activation of Dissolving Pulps

Dissolving pulps are either acidic sulphite pulps or pre-hydrolyzed Kraft pulps[3], and are used for production of regenerated cellulose, as rayon and cellulosolv, used for among other textiles and non-woven, and cellulose derivatives as cellulose acetate, carboxymethyl cellulose and cellulose nitrate. In these applications it is not the strong structure of the fiber that is exploited but the cellulose rather used as a chemical. *The reactivity of cellulose* is very important for many applications of dissolving pulps. High reactivity saves chemicals and, even more importantly, can give a *more even product*; residues of un-reacted cellulose in cellulose derivatives are often regarded as a factor lowering the value.

It has been demonstrated that even a short treatment of low amounts of monocomponent endoglucanases increase the reactivity of dissolving pulps. The endoglucanase lowers the viscosity of the pulp, but the increased reactivity seems not to be directly dependent of this as indicated in *Figure 12.14*. Although the technique mechanism behind the effect is unknown, a hypothesis is presented in *Figure 12.15*.

The technique is yet not commercialized.

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[2] Interestingly the effects of cellulose treatment seem to be the opposite in some aspects, whether it is preformed before or after beating.

[3] Dissolving pulps, regenerated cellulose and cellulose derivatives are further discussed in chapter 9.

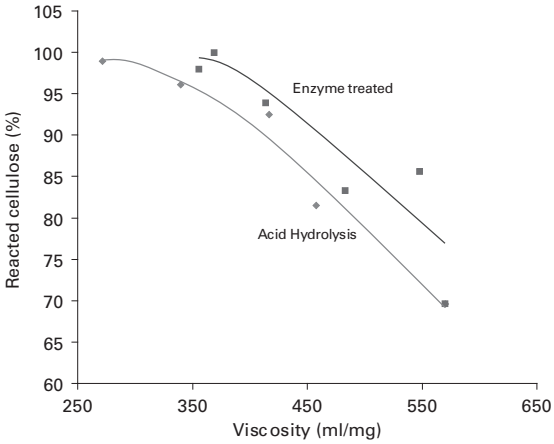

Figure 12.14. Reactivity of enzymatic pretreated pulp is higher at a given viscosity. The reactivity is measured according to a simulation of the viscose process (see chapter 9).

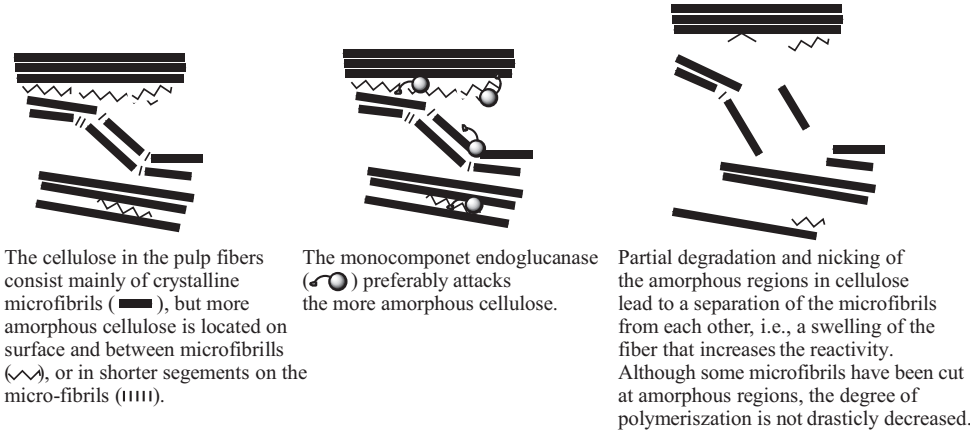

Figure 12.15. A model for how endoglucanases increase the reactivity of dissolving pulps.

12.3.12 Enzymes in Wastewater Treatment

As been described above (12.2.1) microorganisms have since long been applied in wastewater cleaning. However, there is also a potential for applying enzymes here. A serious problem in the pulp and paper industry is the deposition of calcium oxalate in especially the bleaching effluents, as further discussed in Volume 2 (*Figure 12.16*). Enzymatic reduction of the content of oxalic acid in the effluents could be an interesting alternative – possible with the enzyme immobilized to a solid support. Two types of enzymes are able to degrade oxalic acid, oxalate oxidase[4] and oxalate decarboxylase, the latter of the enzymes is independent of oxygen (*Figure 12.16*). The problem with the idea has so far been that bleaching effluents contains many com-

pounds including various metal salts that act as inhibitor to the enzymes. The search for a suitable oxalate-degrading enzyme is ongoing.

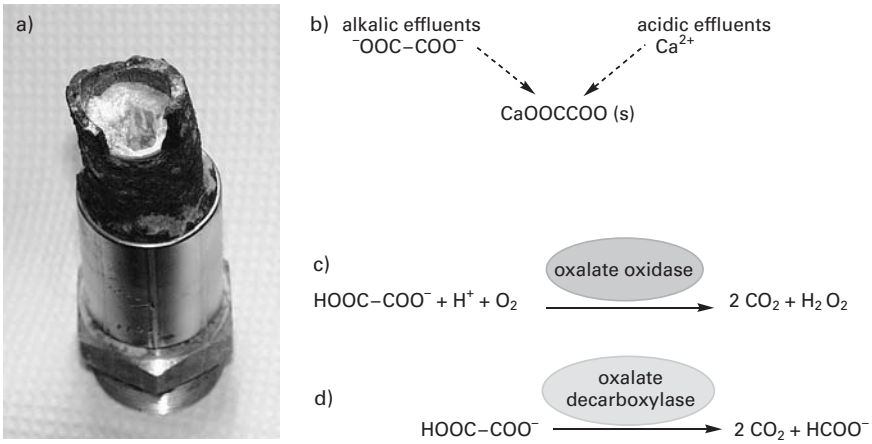

Figure 12.16. Enzymes degrading oxalic acid a) spray pipe with calciumoxalate precipitation. b) Principle behind precipitation. c) degradation of oxalic acid with oxalate oxidase d) Degradation of oxalic acid with oxalate decarboxylase.

12.4 Enzymatic Processes for Wood Based Materials

Enzymatic methods for processing of other wood based materials such as fiberboard and sawed wood products are not investigated to the same extend as processes for pulp and paper industry. Partly this is due to that this type of material generally is based on intact wood, wood pieces of mechanically released fibers. Since enzymes cannot penetrate into lignified wood fibers, enzymatic treatment can mainly give surface effects.

12.4.1 Laccases as Glue in Fiberboard Materials

Medium density fireboards (MDF) is a widely used material that is used for indoor construction materials and various house hold equipments. It is made by a mechanical pulp (often a simple refiner pulps that can be made of both hardwood and softwood) that is glued together by glue based on formaldehyde and urea. The fact that formaldehyde is toxic is a problem in the manufacture, and the bad resistance to humidity excludes its use outdoors. Laccase can be used to replace the glue, in a process where phenolic groups on the lignin is oxidized to long-lived resonance stabilized radicals. If treated fibers, then are pressed together, covalent bonds are formed between the fibers (*Figure 12.17*). The process is similar to the biosynthesis of lignin that is described in Chapter 6.

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[4] It shall be noted that the ligninolytic manganese peroxidase also have oxalate oxidase activity.

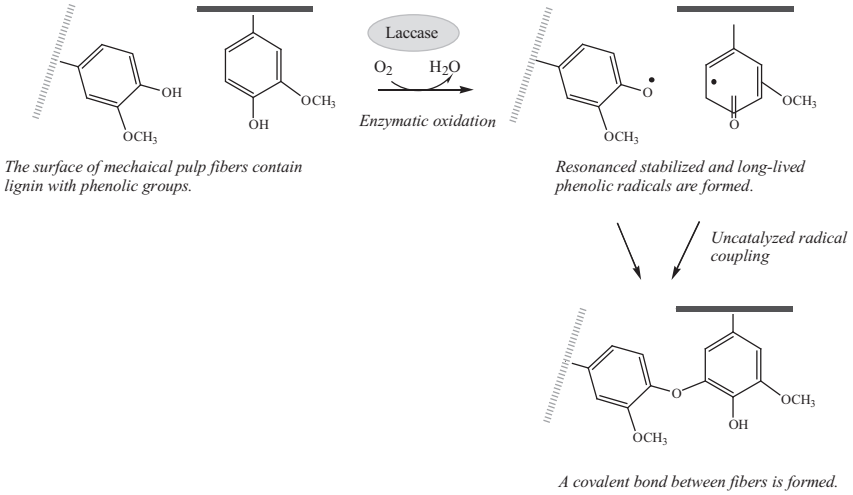

Figure 12.17. Model for how laccase can introduce covalent bonds between the lignin in different fibers.

12.5 Genetic Modification of Forest Trees and other Plants

Prior to agriculture, humans lived as nomads feeding on wild plants and animals. The foundations of modern agriculture were laid over 10 000 years ago with domestication of the wild species by selecting seed of plants with favorable properties. Plant breeding has led to significant improvements in e.g. growth, resistance to pathogens and pests, and grain yield of important agricultural crops. Economically important woody species such as fruit and forest trees have undergone little domestication and breeding compared with food crops such as e.g. maize and cereals. The main reason for this delay is the long generation times of trees that lead to breeding cycles of decades.

Traditional plant breeding involves the crossing of hundreds or thousands of genes. Plant biotechnology extends the repertoire of traditional breeding by permitting the transfer of only one or a few genes in a precise and controlled manner. With enough knowledge of the relevant biochemical processes, genes encoding selected enzymes can be silenced or new genes can be inserted to achieve specific modifications in e.g. the structures and composition of plant biopolymers. Unlike traditional breeding, genetic transformation is not limited to the genes within a given species but genes with desirable properties from other species can also be used. In addition to the transgene technology, modern genetics can accelerate domestication of species by intelligent exploitation of genetic diversity in breeding programs. Both strategies depend on a profound understanding of gene–function relationships. Recent developments in plant molecular biology and genomics are gradually leading to deeper understanding of the genes, regulatory networks and molecular mechanisms underlying plant physiology and development. A small flowering weed, *Arabidopsis thaliana*, characterized by a small, completely sequenced genome, rapid life cycle, efficient transformation, and many available mutant lines, has emerged as the main model for studies of plant biology. However, in spite of its many advantages, *Arabidopsis* is not an ideal model for studies of many essential processes characteristic to commercially rel-

evant forest trees. In particular, wood development including the formation of the thick secondary cell walls is poorly represented in *Arabidopsis*.

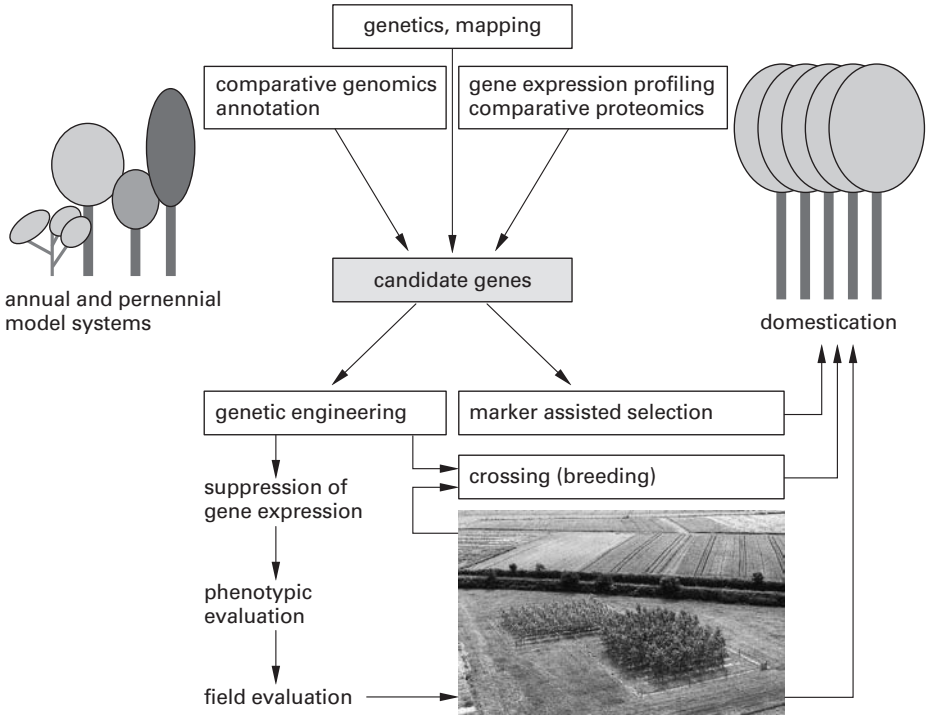

Figure 12.18. Strategies for candidate gene identification and domestication of forest trees. Genes determining the yield and quality traits of trees can be identified by different approaches. Gene expression and protein profiling identify genes that are involved in a given process, suggesting a role in that process. Gene mapping and genetic studies provide support for the involvement of a gene in a given trait. Comparative genomics and genome annotation allow comparison of different model systems to identify candidate genes. Genetic engineering by suppression of the candidate gene expression is used to demonstrate the role of a candidate gene. If the resulting phenotype is favorable, two avenues can be followed: either elite clones previously selected by other means are genetically modified with the gene, or alleles of this gene that are associated with beneficial phenotypes are identified and the genotypes harboring these alleles introduced in the breeding program. Adapted from Boerjan 2005.

Among forest trees, members of the angiosperm trees, poplars, feature easy transformation and regeneration, vegetative propagation, rapid growth, and modest genome size. Extensive genomic resources, including a fully sequenced genome, are currently available for poplars thus making them ideal model organisms for tree molecular biology and biotechnology. Genetic modification of conifers, such as pine and spruce, is also important for forest biotechnology. Genome sequencing of conifers is a considerable challenge owing to their huge genome size, but methods for genetic transformation are available. The main focus of the new biotechnology of forest trees has been on improving growth rate, wood properties and quality, pest resistance, stress tolerance, and herbicide resistance. Recent examples of successful genetic modification of industrially important traits of forest trees are described below. See *Figure 12.18*.

12.5.1 Genetic Engineering of Wood Quality

Lignin is the main wood component that must be effectively removed from pulps in order to guarantee high brightness of the subsequent paper products. Owing to its importance for the pulp and paper industries, the biochemistry and molecular biology of lignin biosynthesis are currently well understood. Since the key enzymes in the relevant biochemical pathways have been identified and characterized, it has been possible to use genetic engineering to modify lignin content and/or composition in poplars (see Chapter 6). For example, suppression of cinnamyl alcohol dehydrogenase (CAD), the final enzyme in the biosynthesis of lignin monomers, results in lignin with altered structure, and suppression of caffeate/5-hydroxyferulate *O*-methyltransferase (COMT), an enzyme involved in syringyl (S) lignin synthesis, results in dramatic reduction in S lignin content. The pulping performance of transgenic trees with altered lignin has also been evaluated in long-term field trials carried out in France and England. Kraft pulping of the transgenic tree trunks showed that the reduced-CAD lines had improved characteristics, allowing easier delignification, using smaller amounts of chemicals, while yielding more high-quality pulp. These experiments demonstrate for the first time the potential of genetic engineering in producing wood that is more easily processed by Kraft pulping, and producing pulp with improved properties. Owing to the genetic modification savings in energy and pollutant chemicals were also achieved, thus leading to an environmentally more sustainable process.

12.5.2 Increased Growth Rate

Wood yield is one of the most important traits of industrially important forest trees. Intensive research efforts have therefore been focused on improving the growth rates of tress. Consequently several genes have been identified that improve the growth of transgenic poplars. Among these is the cytosolic pine glutamine synthase (GS), a key enzyme involved in nitrogen assimilation. Overexpression of this gene in poplar increases height by 41 % and stem diameter by 36 %. Enhanced growth and cellulose production have also been obtained by overexpression of a fungal xyloglucanase gene and an *Arabidopsis* endoglucanase gene in poplar. Remarkably, overexpression of a horseradish peroxidase in poplar enhances plant height by 25 % and stem volume by 30 %, and increases oxidative stress resistance. It has been proposed that the mechanism behind the enhanced growth rate is related to altered ascorbate/dehydroascorbate levels that are thought to play an important role in cell division and elongation.

12.5.3 Earlier Flowering

Unlike many other plants, trees have very long juvenile phases during which they are unable to initiate flowering and subsequent seed development. This is the main factor contributing to the long breeding cycles in trees. Investigations of herbaceous and woody species suggest that the juvenile to adult transition is regulated by genetic and environmental controls. Therefore, the juvenile to adult transition allowing the seasonal induction of flowering could be controlled through genetic engineering of the regulatory pathways. Current understanding of the control of flowering transition is mostly based on studies in *Arabidopsis*, leading to the identification of some of the key genes. Among these is a unique transcription factor denoted *LFY* (*leafy*). It has

been demonstrated that constitutive expression of the *Arabidopsis LFY* gene in a male hybrid aspen clone (*Populus tremula×Populus tremuloides*) induced the development of flowers in transgenic juvenile trees, which would normally take 15 years to develop flowering competence. This is the first demonstration that genetic engineering can indeed be used to influence complex physiological processes such as the generation time of trees.

12.5.4 Modified Starch Structure in Potato

Cationic starch derivates are widely used as a strength-increasing additive to papers. The origin of the polysaccharide is often special potato strains cultivated entirely for production of technical starches. Starch consist of two main structures, *amylopectin*, that is a branched polysaccharide with high molecular weight, and amylose, that is a linear glucan (see Chapter 5 for further details). By genetically manipulation of the enzymes in the biosynthesis of starch, potato strains with altered amylase/amylopectin balance have been obtained, as well as with higher total starch content. Higher amylopectin content in the starch used as wet end additive to pulp giving improved strength properties of the paper.

12.6 Biotechnology in Fiber Analysis

Methods based on microorganisms and especially enzymes are today very important in modern wood and pulp analysis. Soft rot fungi can be used to determine the microfibrillar angel in fibers as described in Chapter 10. Specific hydrolases and sugar oxidases are used for analysis of wood components – for instance hexenuronic acid (see Volume 2) was first identified pulps with the help of enzymatic methods. Monocomponent endoglucanases are very suitable for isolation of lignin with intact lignin-polysaccharide networks (Chapter 6) from pulp and milled wood.

Since enzymes have the ability recognize and degrade different components of the plant fiber walls with high specificity, they can also be used to characterize pulps. Enzymatic solubilization can be applied for qualitative and quantitative analysis of pulp components, without attacking other components in pulps. Enzymes can for example to use for peeling off hemicelluloses and the chemical composition of the peeled surfaces can then be analyzed by ESCA. ESCA (Electron spectroscopy for chemical analysis), also known as XPS (X-ray photoelectron spectroscopy) is a powerful method to characterize different surfaces and provides information on e.g. the chemical bonding between different functional groups. In this way, Finnish researchers have used specific xylanases and mannanases able to analyze the location of xylan, glucomannan and lignin on the fiber surfaces. The removal of the accessible portion of pine Kraft xylan was shown increase the amount of lignin exposed at the fiber surfaces, whereas mannan removal had no effect on the amount of lignin on the surface. In the case of birch Kraft pulp, the removal of accessible xylan did not enhance the amount of lignin on the surface. Instead, the removal of xylan decreased the amount of extractives covering the birch Kraft pulp surfaces. This indicates differences in the surface composition of pulps form different species. Thus, by enzymatic peeling, information can also be obtained on the role of these components on the pulp surface on the technical properties of pulps.

Enzymatic methods have also been used to investigate the occurrence of covalent bonds between residual lignin and polysaccharides in birch and pine Kraft pulps. Pure xylanases and mannanases were used both separately and in combination to peel of carbohydrates from the fiber surfaces. Comparison of the molar masses of polysaccharides and lignin in the original pulps and in the pulps treated with enzymes showed that residual lignin in birch Kraft pulp is linked at least to xylan, and perhaps also to cellulose. In pine Kraft pulp some of the residual lignin appears to be linked to cellulose, glucomannan and xylan. In another method developed by Swedish researchers, chemical pulp or slightly milled wood is subjected to treatment with a monocomponent endoglucanase that shortens the cellulose chains without significant damage of the hemicelluloses. This treatment allows quantitative preparation of intact lignin-polysaccharide networks based on covalent bonds between lignin and polysaccharides, as further discussed in Chapter 6. A suggested model for the method is shown in *Figure 12.19*.

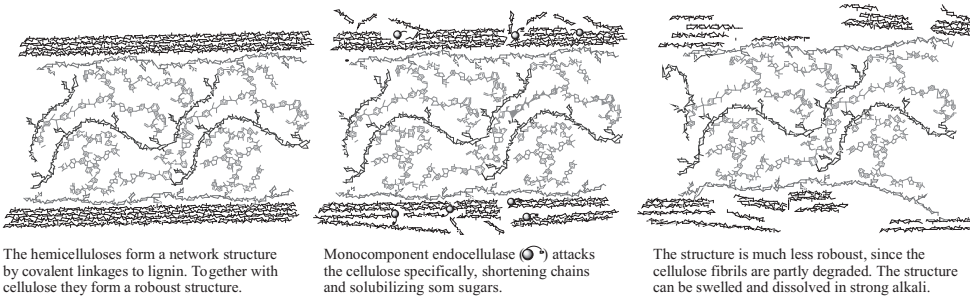

Figure 12.19. Role of endoglucanase in quantitative preparation of lignin-polysaccharide networks.

Yet another interesting application of enzymatic peeling is determination of the carbohydrate composition of extractive-free delignified wood and pulp has been developed in Sweden. The polysaccharides in the sample are first hydrolyzed using a mixture of cellulases and hemicellulases, and the solubilized sugars in the hydrolysate are then chemically derivatised and quantified by capillary zone electrophoresis (CZE). Remarkably, all neutral monosaccharides and uronic acids, which occur as structural elements in the polysaccharides of wood and pulp, could be quantified in a single analytical run. The method was also very sensitive, permitting the detection of carbohydrate constituents constituting as little as 0.1 % of the dry mass of the sample. The total yield of carbohydrates was consistently around 93–97 % when the enzymatic method was used. This is clearly better that the yield of about 85–93 % achieved by using the traditional procedure for carbohydrate analysis, involving acid hydrolysis and gas chromatographic analysis.

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