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Bioenergy and Biorefinery: Feedstock, Biotechnological Conversion, and Products

*Jerome Amoah, Prihardi Kahar, Chiaki Ogino, and Akihiko Kondo**

Biorefinery has been suggested to provide relevant substitutes to a number of fossil products. Feedstocks and conversion technologies have, however, been the bottleneck to the realization of this concept. Herein, feedstocks and bioconversion technologies under biorefinery have been reviewed. Over the last decade, research has shown possibilities of generating tens of new products but only few industrial implementations. This is partly associated with low production yields and poor cost-competitiveness. This review addresses the technical barriers associated with the conversion of emerging feedstocks into chemicals and bioenergy platforms and summarizes the developed biotechnological approaches including advances in metabolic engineering. This summary further suggests possible future advances that would expand the portfolio of biorefinery and speed up the realization of biofuels and biochemicals.

1. Introduction

The utilization of fossils such as petroleum and coal for the development of refineries has been emphasized to take advantage of these low-cost feedstocks. The availability and the economic advantage have made the use of these feedstocks favorable for producing various chemicals such as lubricants and synthetic fibers, as well as fuels including gasoline and kerosene.[1] However, the depletion of this feedstock and the rapid increase in human population have continuously rendered its economics and sustainability doubtful.[1–3] Moreover, fossils are chief contributors to global warming due to the generation of high amounts of greenhouse gases. For these reasons, the quest for the search for sustainable and environmentally friendly alternative feedstocks and processes to meet the demands of chemicals and fuels has heightened.

Dr. J. Amoah, Dr. P. Kahar, Prof. A. Kondo
Department of Science
Graduate School of Science
Technology and Innovation
Kobe University
1-1 Rokkodai, Nada-ku
Kobe 657-8501, Japan
E-mail: akondo@kobe-u.ac.jp
Prof. C. Ogino, Prof. A. Kondo
Department of Chemical Science and Engineering
Graduate School of Engineering
Kobe University
1-1 Rokkodai, Nada-ku
Kobe 657-8501, Japan

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A new paradigm in chemicals and fuels production involving the use of biomass has led to the concept of a biomass-based refinery, referred to as biorefinery. The realization of this concept would contribute to the alleviation of global warming, as the CO_2 generated in utilizing the resources is directly consumed in the production of the biomass via photosynthesis, thus, maintaining a net zero CO_2 emission into the atmosphere. Furthermore, the production of biofuels and biochemicals could provide an alternative sustainable economy for rural communities.

The selection of biomass and microbes capable of utilizing biomass as a substrate is crucial for establishing biorefinery systems. This review summarizes the potential fuels and chemicals that can be generated under the concept of biorefinery. A comprehensive analysis of sustainable feedstocks and microbial platforms for achieving the targets of biorefinery is presented. Further, it provides insights into the biotechnological advances needed for the successful conversion of recalcitrant feedstocks into the respective forms of bioenergies and biochemicals.

2. Biomass: A Multifeedstock for Biochemicals and Biofuels

Biomass is obtained from various sources such as terrestrial and aquatic plants, including agro-forest residues, trees, and crops. Others include animal and municipal waste, and unicellular and multicellular microorganisms such as microalgae and fungi. Plants and other photosynthetic organisms have the ability to transform CO_2 and water into energy-storing compounds, as well as primary and secondary metabolites via the process of photosynthesis. Primary metabolites, often lignin and carbohydrates (starch, cellulose, hemicellulose, and sugars), form a class of biomass known as lignocellulose biomass. Lignocellulosic biomass is known to be one of the most abundant biomasses on Earth and can be converted into biofuels and biochemicals.[4] Secondary metabolites include alkaloids, tannins, and terpenoids, which are often available in small quantities in plants, but of high industrial value.[5] This class of metabolites can be exploited for the production of important chemicals including cosmeceuticals, nutraceuticals, and pharmaceuticals. Microalgae is also an emerging biomass substrate for biorefinery as most strains are capable of converting high amounts of CO_2 into useful metabolites, which

can be extracted and used directly or as intermediate for producing biofuels and biochemicals such as 5-hydroxymethylfurfural, pigments, and antioxidants.

The development of advanced biomass systems, which effectively combines an efficient production of biomass and its transformation, and the utilization of bioproducts would promote the implementation of biorefineries. Therefore, various research works geared towards the integration of biomass production and its transformation technologies have been carried out. Separation technologies employed in the biorefinery of lignocellulosic biomass have been extensively reviewed.[6] The article highlights the separation of various chemical products such as gluten and fiber from corn, as well as its prospects with the integration of ethanol production from corn and forest residue. The use of bio-based adsorbents, membranes, and extractive distillation is expected to improve the separation efficiencies of bioproducts. The role of catalysts and green chemical technologies to convert various niche biomass such as palm-based refinery have been investigated.[7] The regional challenges and opportunities of biorefinery in Australia and Canada have been independently surveyed.[8,9] Further, an investigation towards the cost-effectiveness of bioconversion of plant biomass into liquid fuels has been extensively reviewed.[10]

3. Accessory Enzymes Can Minimize the Use of Commercial Enzymes

Despite the abundance of lignocellulose biomass, the arrangement of its components presents a recalcitrant structure, which requires degradation to access the C-6 and C-5 fermentable sugars, and lignin fractions (**Figure 1**). Although this structure may vary depending on the source, it is generally composed of 40–55% cellulose and 25–50% hemicellulose covalently linked to a network of lignin via a ferulic acid ester bond, which constitutes about 10–40% of the material.[10,11]

To efficiently hydrolyze this structure into fermentable sugars, high amounts of commercial enzymes are needed.[12] As it stands, a kilogram of cellulose requires 20 g of cellulase to attain approximately 70% hydrolysis in five days.[13,14] It is necessary to lower the amount of commercial cellulase to minimize the cost associated with the use of enzymes. The prohibitively expensive enzyme in enzymatic hydrolysis is one of the largest contributors to the overall cost of biofuel and biochemical production from lignocellulose biomass. Reducing the cost associated with the use of enzymes is prudent in making enzymatic hydrolysis more economically feasible. To achieve this, several approaches including substrate and enzyme-related approaches have been evaluated. In the enzyme-related approach, the achievement of optimized amounts of fermentable sugars via the cohydrolysis of both the cellulosic and hemicellulosic portions of the lignocellulosic biomass is crucial. By supplementing cellulase with accessory enzymes, this approach has shown significant improvements in reducing the total enzyme load.[15–17] The degradability of cellulolytic material is drastically improved through the hydrolytic effect of accessory enzymes like acetyl xylan esterases, feruloyl esterases, and xylanase. These enzymes hydrolyze the intermolecular bonds within hemicellulose, as well as interconnecting linkages of lignin and hemicellulose.[18,19] Improved hemicellulose breakdown is a key factor in maximizing biomass utilization, because the effective hydrolysis of hemicelluloses would both rupture this polysaccharide and increase the enzymatic access to the other component of lignocelluloses.

The amorphous nature of hemicellulose provides a relatively easier enzymatic hydrolyzability than cellulose. Further, rupturing the hemicellulose improves the accessibility to cellulose by enhancing the porosity and surface area of the lignocellulosic material.[20] Due to the high proportion of xylan in the plant cell wall, xylanase is often critical for the decomposition of hemicellulose. Xylanase primarily hydrolyzes the linear xylan polysaccharide and provides a synergistic effect with feruloyl esterase in cleaving the diferulic bridges between xylan chains to release lignin.[21] The cross linkages within hemicellulose and between hemicellulose and lignin are hydrolyzed through the catalytic effect of feruloyl esterases.[21,22] The β-1,4-glycosidic linkages connecting the backbone of xylans and xylose residues are hydrolyzed by endo-1,4-β xylanase.[18]

4. Culture Collection as Microbial Sources for Biorefinery

Another bottleneck in microbial fermentation of lignocellulose biomass is the presence of inhibitory chemical compounds

Jerome Amoah received his B.Sc. Chemistry from Kwame Nkrumah University of Science and Technology, Ghana, and his Ph.D. in Chemical Science and Engineering from Kobe University, Japan. He is currently a postdoctoral research engineer at the Bioproduction Engineering Lab, Kobe University. He was a visiting scientist at the Laboratory of Biomass and Green Technologies at the University of Liege, Belgium. His research interests include bioprocess engineering for biofuel and biochemical production, elucidation and valorization of microalgal biomass, and benzylisoquinoline alkaloid production in microbial platforms.

Akihiko Kondo is the Dean and a Professor of School of Science, Technology and Innovation, Kobe University. He received his Ph.D. in Chemical Engineering from Kyoto University, Japan. His research interests cover production of biofuels and chemicals from biomass, development and application of novel cell surface display systems, and combinatorial bioengineering. He is the team leader of Cell Factory Research at RIKEN Center for Sustainable Resource Science and also launched the "Innovative Bioproduction Kobe" project at Kobe University.

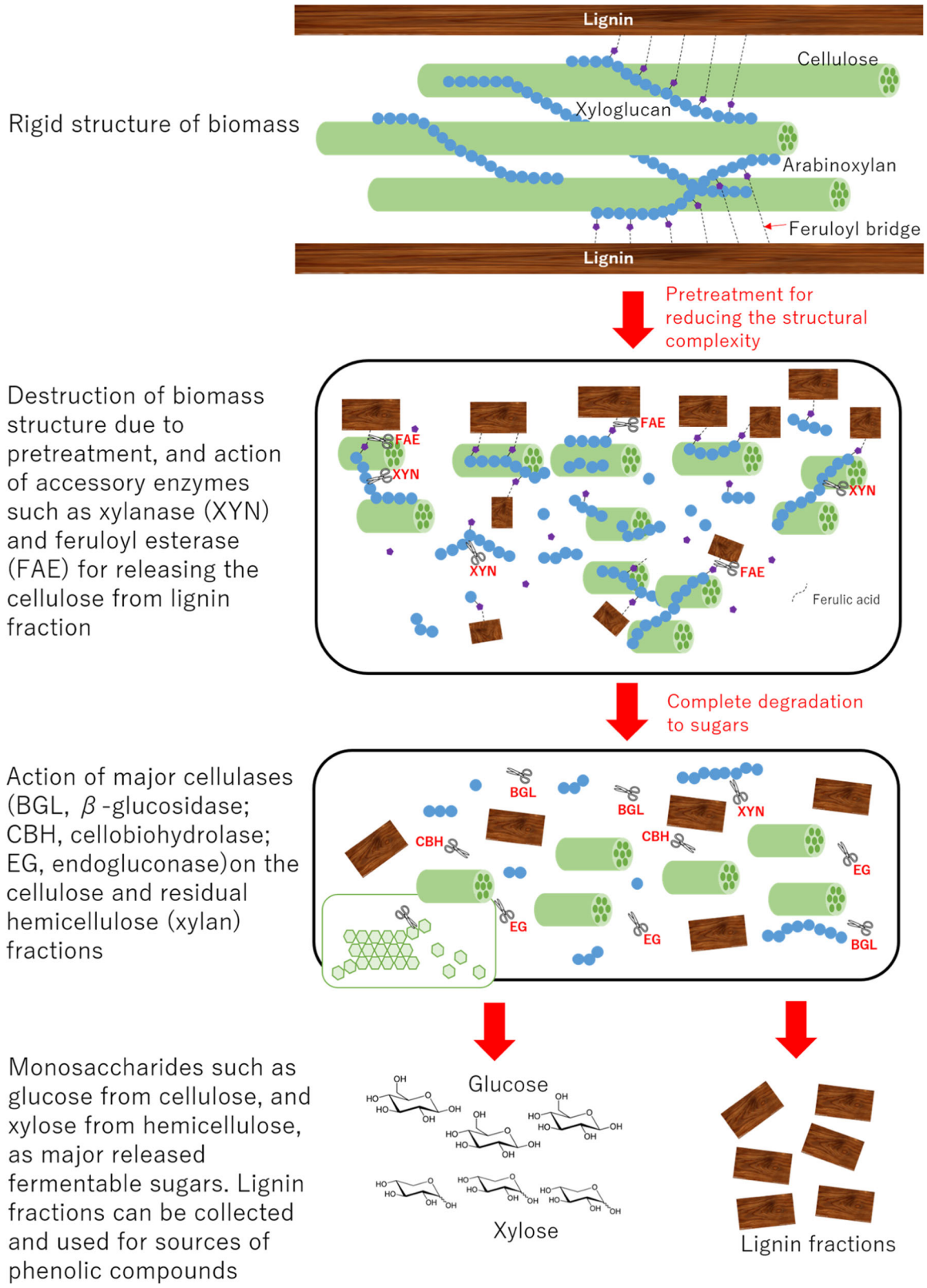

Figure 1. Degradation of lignocellulose biomass to valuable lignin fractions and fermentable sugars.

(ICC), which are released upon the pretreatment and the hydrolysis of the biomass.[23] The selection of microbes is critical to the production of biofuels and biochemicals from lignocellulose biomass hydrolysate.

Microbial platforms such as *Escherichia coli* (*E. coli*), actinomycetes, corynebacterium, yeast, fungi, lactic acid bacteria are well known to produce some important biochemicals (**Figure 2**). Typically, *E. coli* has the potential to produce some alcoholic compounds such as isopropanol and long-chain fatty acid alcohols. Due to its ease to be used in genetic modifications, many approaches regarding the production of various biochemicals in *E. coli* have been attempted. Actinomycetes, particularly *Streptomyces,* are commonly known to produce nonribosomal peptides and polyketides among other secondary metabolites through intrinsic metabolic gene clusters.[24] Aside these metabolites, Streptomyces are good producers of industrially important enzymes including lipases, amylases, cellulases, and proteases, which can be used in the decomposition of various polymers, and carrying out other green processes.[25] *Corynebacterium* also has a great potential to produce amino acids required for food and pharmaceutical applications. The current stage of metabolic engineering technology enables this microbe to produce a variety of biochemicals beyond classical amino acids from renewable substrates.[26] *Saccharomyces cerevisiae* (*S. cerevisiae*) is the most widely used yeast for biotechnological applications and it is regularly employed at an industrial scale to produce biochemicals and biofuels. Current metabolic engineering technologies enable the directed evolution of some yeast to nonconventional forms. These present biocatalytic alternatives to be used as economically feasible whole cell production platforms. Examples include the use of the oleaginous yeast *Yarrowia lipolytica* for the production of lipids, *Candida guilliermondii* for xylitol production, and *Ashbya gossypii* for the production of riboflavin (vitamin

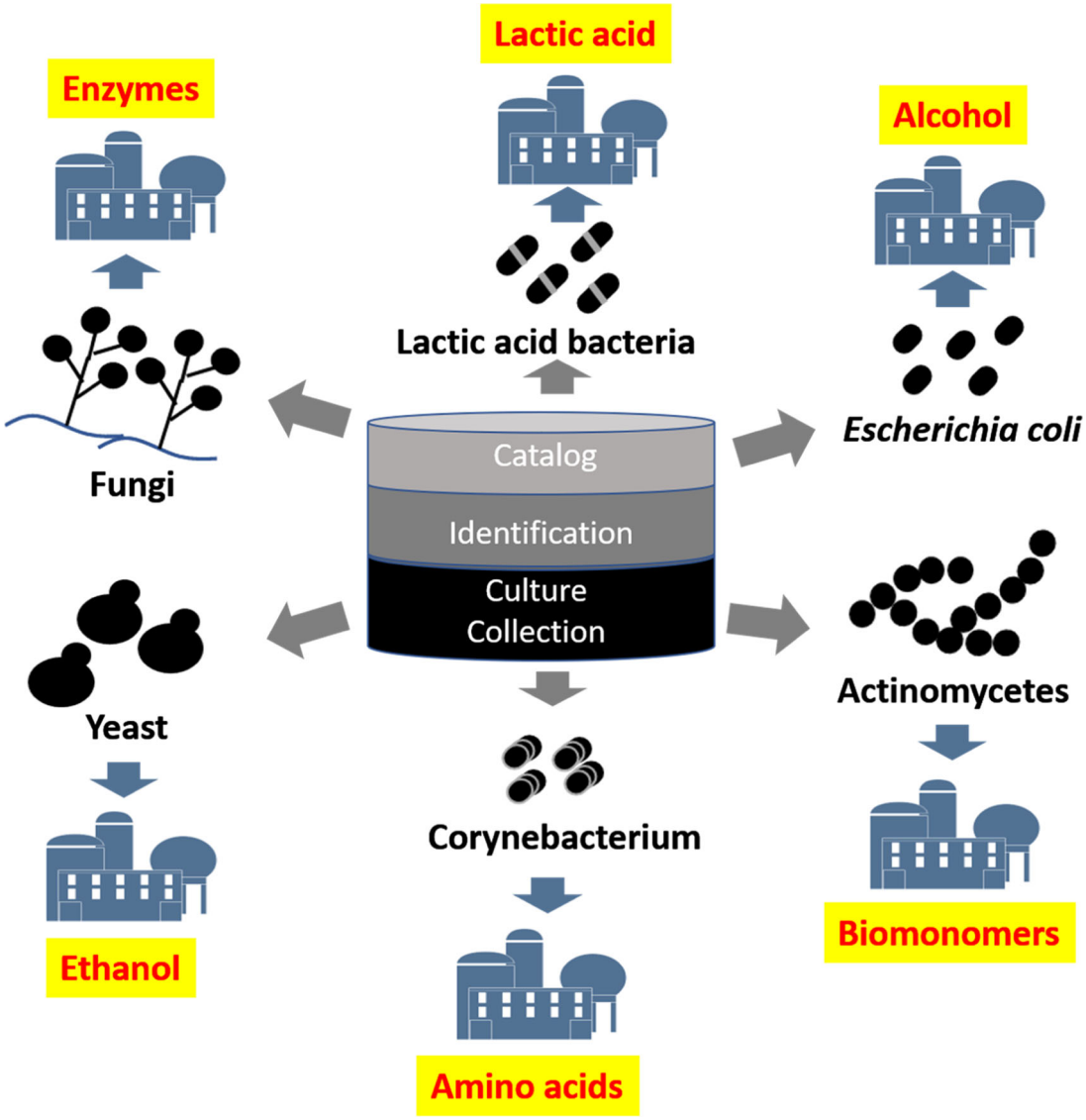

Figure 2. Industrial microbes for biorefinery.

B2).[27–30] Recent advances in genetic engineering technologies, such as the discovery of the CRISPR–Cas9 system and approaches, offer a great opportunity to explore the biotechnological potential of these nonconventional yeasts. Fungi is extensively used in the production of industrial enzymes, secondary metabolites, and fermented foods.[31] Lactic acid bacteria have been proposed and proven for their production of lactic acid, which is a potential building block for biodegradable plastics.[32,33]

Despite these developments, little is known about the capability of these microbes for the fermentation of the sugars generated from lignocellulosic biomass into useful biochemicals. Microbial screening processes are required for the selection of microbes for targeted products from lignocellulose hydrolysate. It is comparatively easy to screen from a group of microbial sources that have been already systematically identified. The role of culture collection is therefore crucial for utilizing microbes in the fermentation of lignocellulose biomass.

5. Bioenergy Platforms

Bioenergy is an integral part of the biorefinery concept as sustainable and renewable alternatives of fuels are being sort for. Over the past decade, bioenergy continues to be the single most produced and consumed form of renewable energy in the United States accounting for approximately 50% of the total renewable energy forms.[34] Globally, the production of biofuels continues to see sturdy growth over the past decade. Among the top producers, national energy polices, feedstock availability, and technology readiness are the decisive conditions affecting the production and consumption of bioenergy. Biofuel synthesis generally involves the transformation of the chemical components stored up in biomass into forms that can easily be used in engines to generate energy (**Figure 3**). Traditional conversion methods involve the use of thermal or chemical methods, which are rather energy-intensive and further contribute to environmental pollution.[34,35] Newer strategies involving the use of biotechnological tools show great potential in addressing these challenges.

5.1. Bioethanol

Bioethanol continues to be the largest contributor to biofuels with the United States and Brazil having 85–90% of the total global shares.[35] To avoid the worsening case of an already looming food crises, the use of nonedible feedstock such as lignocellulosic biomass has been suggested to be a sustainable substitute to their edible counterpart such as corn and sugar cane.[35,36]

Bioethanol is a product of the fermentation of glucose and xylose by specialized microbes harboring glucose and xylose assimilation pathways, respectively. As discussed in Section 3, the structure of lignocellulose biomass requires pretreatment and subsequent hydrolysis to obtain fermentable sugars. These processes have a direct consequence on subsequent fermentation as some are known to generate compounds that inhibit fermentation microbes. Recent discoveries of using ionic liquids for pretreatment result in fewer inhibitors and offer better efficiencies in terms of yield and energy requirements.[37,38] Ionic liquids tend to transform the structure of the cell walls, increase cellulose surface accessibility, and decrease cellulose crystallinity.[39] Although ionic liquids seem to be a preferred choice of biomass pretreatment, some are known to have inhibitory effects on saccharification enzymes, and acetate containing ionic liquids tend to have inhibiting effects on several naturally occurring yeast strains.[40] The

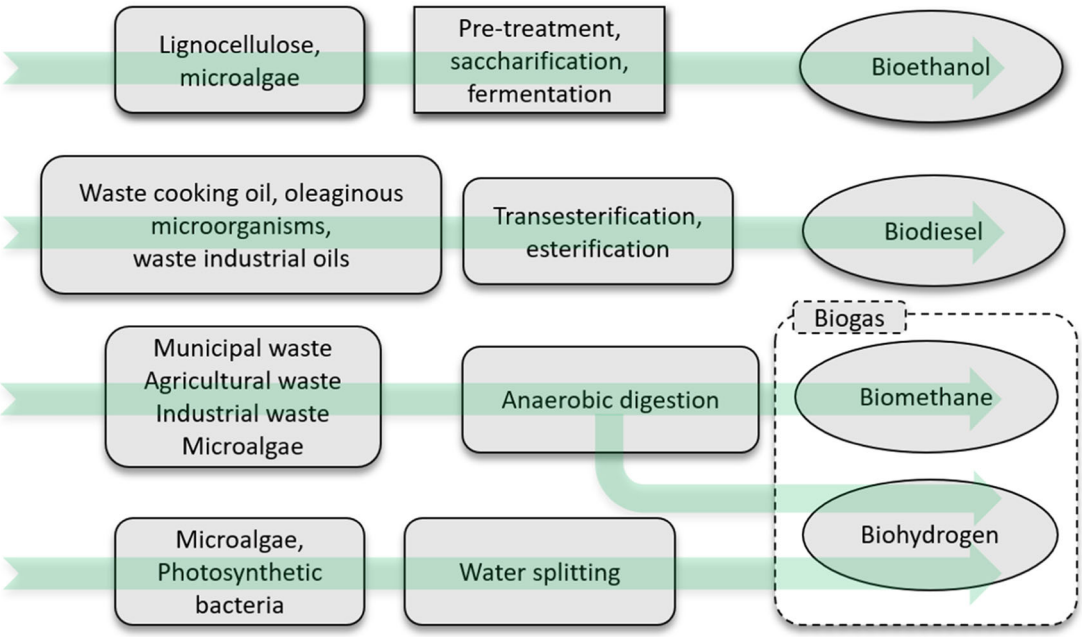

Figure 3. Technological transformation of biomass into various major bioenergy platforms.

choice of microbes for saccharification and fermentation should therefore be factored in the selection of ionic liquids for pretreatment. Pretreated lignocellulose biomass usually contains cellulose with both amorphous and crystalline regions as well as some amounts of cellobiose. The high specificity of saccharifying enzymes calls for the introduction of multiple kinds of enzymes capable of hydrolyzing the respective parts of the cellulosic material. Endoglucanase (EG) and cellobiohydrolase (CBH) are responsible for hydrolyzing the amorphous and crystalline components, respectively, whereas β-glucosidase (BGL) is specialized for hydrolyzing cellobiose. A typical form of commercially available enzyme formulation for cellulose degradation is the Cellic CTec from Novozyme Co. Ltd., which consists of blend cellulases, BGL, and hemicellulase.

Despite its notoriously slow reaction rate, enzymatic saccharification occurs at low temperatures, produces high quantities of fermentable sugars with no inhibitors and allows for simultaneous saccharification and fermentation (SSF), as suggested by Takagi et al.[41] SSF involves the breakdown of cellulose into sugars and direct conversion of the sugars into ethanol in a single unit process. This reduces the overall production time and prevents potential contamination of the sugars by other microorganisms.[42,43] A novel technology of expressing proteins on the surface of cells via glycosylphosphatidylinositol anchor proteins has been developed and this technology offers a major boost in the design of SSF processes.[44] The application of cell surface engineering has paved way for the construction of the arming yeast, which allows for the expression of various cellulolytic enzymes on the surface of yeast cells. *S. cerevisiae* has been one of the best candidates for this technique as it allows the folding and glycosylation of expressed heterologous eukaryotic proteins and has a superiority in the natural ability to convert glucose into ethanol.[45] Matano et al.[46] successfully designed a yeast strain expressing fungal EG, CBH, and BGL on the cell surface. This strain improved the ethanol production from high-solid rice straw biomass by 1.4-fold when used in combination with the commercial cellulase, cellulase SS. In order to improve the enzymatic hydrolysis of cellulose in rice straw, a diploid strain of *S. cerevisiase* with an optimized expression ratio of EG, CBH, and BGL was constructed.[47] This strain successfully produced 7.5 g L^{-1} ethanol from rice straw in an SSF system without the addition of exogenous enzymes. Unlike some naturally cellulolytic enzyme expressing microorganisms such as *Trichoderma reesei*, the simultaneous controlling of the expression levels of cellulases in recombinant *S. cerevisiae* is limited. Rectifying this would enhance the optimization of the expression ratio of the multiple cellulases expressed by the microorganism, as the optimum expression ratio differs depending on the content of the cellulosic material. Building on a previous development, a simple one-step method of inserting several kinds of different cellulase expression cassettes into yeast chromosomes was designed.[48] This method, known as the cocktail δ-integration, resulted in yeast strains of higher activity towards the degradation of phosphoric acid swollen cellulose. In order to match yeast strains of optimized cellulase expression ratio with various lignocellulosic biomass, Amoah et al.[49] designed a screening method, which allowed for the high production of ethanol from ionic liquid-pretreated bagasse and Laubholz unbleached Kraft pulp (LUKP), respectively. A detailed investigation revealed that cell surface displaying yeast cells binds tightly to cellulosic materials and further exerts a more extensive tearing effect on the material enhancing ethanol production.[50]

5.2. Biodiesel

Biodiesel, which is a mixture of fatty acid alkyl esters (FAAE) with varying chain length, is generally produced via the transesterification of oils and fats from plants, animals, and oleaginous microorganisms.[34,51] It can be used wholly as B100 or as blends with petrodiesel.

Similar to bioethanol production, the feedstock for biodiesel varies according to geographical areas and depends on the availability and viability for production. Although it seems to be in abundance, one of the major setbacks in biodiesel production is the feedstock. Theoretically, to replace 50% of the US transport fuel needs with biodiesel produced from soybean, which is the main feedstock in the United States, about 326% of the existing U.S. cropping area is required.[52] In addition, the use of refined oils that are considered as first-generation feedstock contributes to about 60–80% of the cost of biodiesel production resulting in high production cost and less economic attractiveness.[53] Biodiesel feedstocks greatly influence the properties of the product since a great portion of the molecular structure of these feedstocks are retained in the final product. Recent research has therefore focused on the use of crude nonedible feedstock for biodiesel production. Due to the unconventional nature of this class of feedstock, they present various technological barriers in their conversion to biodiesel.

Enzymatic biodiesel production presents the advantages of low-energy requirements, efficient conversion of oils containing high contents of free fatty acids (FFA), high purity of glycerol by-product, and enzymes in their immobilized forms offer an easy product separation and reuse.[34,54] The class of enzymes used for biodiesel production is EC 3.1.1.3 lipases. These are carboxylic ester hydrolases that catalyze the breaking and formation of ester bonds in carboxylic esters. Although this may vary slightly from lipase to lipase, the active site is generally characterized by the presence of Ser, His, and Asp amino acid residues.[55] This catalytic triad is activated by an aqueous–nonaqueous interface, thus generally requiring some droplets of water for effective conversion of triglycerides into biodiesel.

The high specificity of lipases and the broad range of oil substrates require creative genetic engineering and process engineering approaches to ensure efficient reactions. The immobilization strategy and immobilization matrix seem to affect the specificity and the performance of lipases. *Candida antarctica* lipase B (CALB), one of the most used lipases, is known to be nonspecific in its free state. CALB immobilized on polyurethane matrix, however, shows no activity towards triglycerides.[54,56] Moreover, CALB immobilized on the hydrophobic acrylic resin (Novozym 435), one of the most extensively studied commercial lipase for biodiesel production, loses its transesterification activity in the presence of high amount of water.[56,57] **Table 1** outlines some potential nonconventional feedstocks and techniques to improve their enzymatic conversion to biodiesel.

Another potential feedstock that has gained attention is lipid from oleaginous microorganisms. These are microorganisms

Table 1. Overcoming the biotechnological challenges associated with nonconventional biodiesel feedstock.

Raw material	Biocatalyst	Technological barrier	Conversion technique	Yield [%]	References
Used palm oil	Lipases from *Pseudomonas fluorescens* and *Candida rugosa*	Broad range of substrates compared to the specificity of lipases	Combination of lipase AY and lipase AK	91	[58]
Chlorella vulgaris ESP-31 lipids	*Burkholderia* sp. lipase immobilized on nano Fe_3O_4–SiO_2	Diffusion limitation of microalgal oil	Use of microalgal cells with higher lipid content	>90	[59]
Acid oil from vegetable oil refinery	Novozym 435	High FFA content (>78%)	Methyl esterification followed by methanolysis of TAGs with 10 wt% glycerol	91.1	[60]
Soybean oil	Recombinant *Aspergillus oryzae*	High amounts of unconverted mono- and di-glycerides	Coexpression of triglycerides and partial glyceride lipase gene in *A. oryzae*	98	[61]
Waste oil	Immobilized lipase from *Penicillium expansum*	27% FFA resulting in excess water hindered high FAME yield	Conversion in cosolvent tert-amyl alcohol using blue silica gel as adsorbent	92.8	[62]
Waste activated bleaching earth (WABE)	Powdered lipase *C. cylindracea*	Accessibility of oil in wABE is limited	Use of petrodiesel as cosolvent	97	[63]
Jatropha oil	*Enterobacter aerogenes* immobilized on silica	Deactivation by methanol resulting in 0% yield	Conversion in cosolvent (tert-butanol)	68	[64]
Crude palm oil	Lipozyme TL IM	High acid value	Using ethanol as acyl acceptor and an alcohol-tolerant lipase	25	[65]
Chlamydomonas sp. JSC4 lipid	Immobilized recombinant *Fusarium heterosporum* lipase	High FFA (>9%) High phospholipid (>16%)	Low agitation to reduce the risk of reverse micelle formation, coupled with high water content	98.1	[66]
Crude soybean oil	Callera Trans L	High phosphorus content (900 ppm) representing high phospholipids	Combining Callera Trans L with phospholipase	95	[67]
Rapeseed oil	Callera Trans L	High FFA (5%) resulting in high amount of remaining FFA	Substituting a portion of Callera Trans L with a lipase of different specificity, CalA	85	[68]
Partial soybean oil hydrolysate	Immobilized recombinant *Fusarium heterosporum* lipase	High FFA (>70%)	Using lipase with broad-substrate specificity and high water tolerance	93	[56]

including yeast and microalgae, which produce lipid content in excess of 20% of their cell weight as a consequence of their metabolic activities. Due to the single-celled nature of these microorganisms, the characteristics of the lipids are directly affected by the metabolic activities within the cells. Typically, under favorable conditions, the triglycerides in some microalgal cells are hydrolyzed to FFA by endogenous lipases.[69] The membrane lipids of these cells also tend to contribute a large portion of polar lipids in the form of phospholipids and glycolipids. These polar lipids are known to hinder enzymatic biodiesel conversion.[70] Although degumming can be used to remove these polar lipids, it adds extra cost to production and also causes significant loss of feedstock. As surfactants, phospholipids in oils form water-in-oil reverse micelles with the added water required for lipase activation. This alters the nature of the reaction medium and increases the concentration of alcohol within the locality of the lipase. Phospholipid itself may not exert an inhibiting effect on lipases; however, methanol, which is the most widely used acyl acceptor for biodiesel synthesis, tends to alter the conformation of lipases leading to deactivation. The excessive amounts of methanol retained in the reaction mixture thus deactivate the immobilized lipases.[71,72]

Although microalgal lipid is one of the highly potential feedstocks for biodiesel, it is characterized by the presence of both FFA and phospholipids. Low-conversion efficiencies of microalgal lipids and poor lipase reusability resulting from high amounts of phospholipids have been observed.[73] Successful enzymatic conversion of these lipids to high fatty acid methyl esters (FAME) content has been achieved with robust biocatalyst and effective process engineering.[66,74] Improving the robustness, processing time, and overcoming other technological barriers could further promote the enzymatic conversion of lipids from oleaginous microorganisms.

5.3. Biogas

Aside from liquid bioenergy platforms, biogas in the form of hydrogen and methane can be obtained from bioresources. Biohydrogen is produced through various biological routes and the choice of production route has a great impact on the yield from a given substrate. It has been widely produced by the use of fermentative microorganisms such as *Clostridium*, *Enterobacter*, and *Bacillus*.[75,76] Under anaerobic conditions, H_2 is produced as a by-product of oxidation of organic substrates via the sequential neutralization of excess electrons catalyzed by endogenous hydrogenase of the microbes. This natural phenomenon has been adapted for the systematic production of biohydrogen. The actual yield of H_2 is usually lower than the theoretical yield because a significant amount of the substrate is consumed in biomass growth. Moreover, the process catalyzed by hydrogenase is known to be reversible where the discharge and uptake of hydrogen are catalyzed by hydrogenases in the cytoplasm and the periplasm, respectively.[77] Methane, on the other hand, is produced by a group of microbes referred to as methanogens. Although it can be produced from $H_2 + CO_2$, some limited number of the methanogenic genera are known to utilize organic acids such as acetic acid as a substrate for methane production.[78] An additional interest in the controlled production of these biogases is the vital role it can play in waste treatment. A popular route for hydrogen production is via dark fermentation, which uses organic carbon such as glucose as its substrate. The acetate intermediate of this process can then be used as a substrate for methane production. The bioavailability and biodegradation rate of the substrates are crucial for H_2 yields.[79,80] Three main classes of organic wastes have been systematically studied for their potential conversion to biogas. These include municipal waste, agricultural waste, and industrial waste.

Biogas production from municipal waste is greatly influenced by the environment of the microbial communities. In a mesophilic fermentation regime of food waste, the dominant microorganisms found were *Thermotogales* and *Bacillus* species resulting in the production of both H_2 and CH_4.[81] Contrarily, the authors found that *Thermoanaerobacterium thermosaccharolytium* and *Desulfotomaculum geothermicum* were the dominant species in the thermophilic regime and resulted in the evolution of only H_2. Mixing of municipal waste from several sources provides a synergistic effect where the nutrition supply for the microbes is supplemented by auxiliary substrates.[82] Agricultural waste is projected to increase with increasing human population.[83] This waste, mainly made up of bagasse and peelings from crops as well as manure from animals, contain high amounts of carbohydrates, which could be converted into fermentable sugars and subsequently for biogas production. Blending high carbon-content bagasse with manure from animals provides a good C/N ratio, typically 20–30, which is essential for efficient biogas production. Most of these waste materials, however, are made up of lignocellulosic biomass, and similar to other bioproducts, pretreatment methods are required for efficient accessibility of the soluble sugars.[84,85] Nonetheless, a 24.8 mL g^{-1} TS hydrogen production was obtained from untreated rice straw.[86] The authors claimed that, by mixing the lignocellulosic substrate with heat-treated municipal waste, the presence of both hydrolytic and fermentative bacteria aided in the hydrolysis of cellulose and subsequent fermentation. Ionic liquid-pretreated pure cellulose was found to show superior yields of hydrogen compared to acid- or alkaline-pretreated counterparts.[87] Exploitation of ionic liquid pretreatment of lignocellulose biomass for biohydrogen and biomethane production, however, still remains low. Resources from some industrial waste are known to contain fermentable carbohydrates that can be used as a substrate for hydrogen fermentation. Cheese whey from the cheese industry has been explored extensively for hydrogen production due to its abundance and high lactose content.[88–90] High biohydrogen production yield comparable to those from other biomass sources have been successfully achieved. Other industrial waste biomass sources such as cassava waste from mills, rice slurry, and oil seed mill effluent have been suggested to be potential sources for biogas production.[91–93]

The process of hydrogenesis is usually in competition with methanogenesis as most methanogens utilize hydrogen for methane production. This usually leads to lower yields of both gases. Efforts to optimize the yields of both gases focus on the separate production of the individual gases starting from the same substrate (**Figure 4**). A swift approach is by playing with

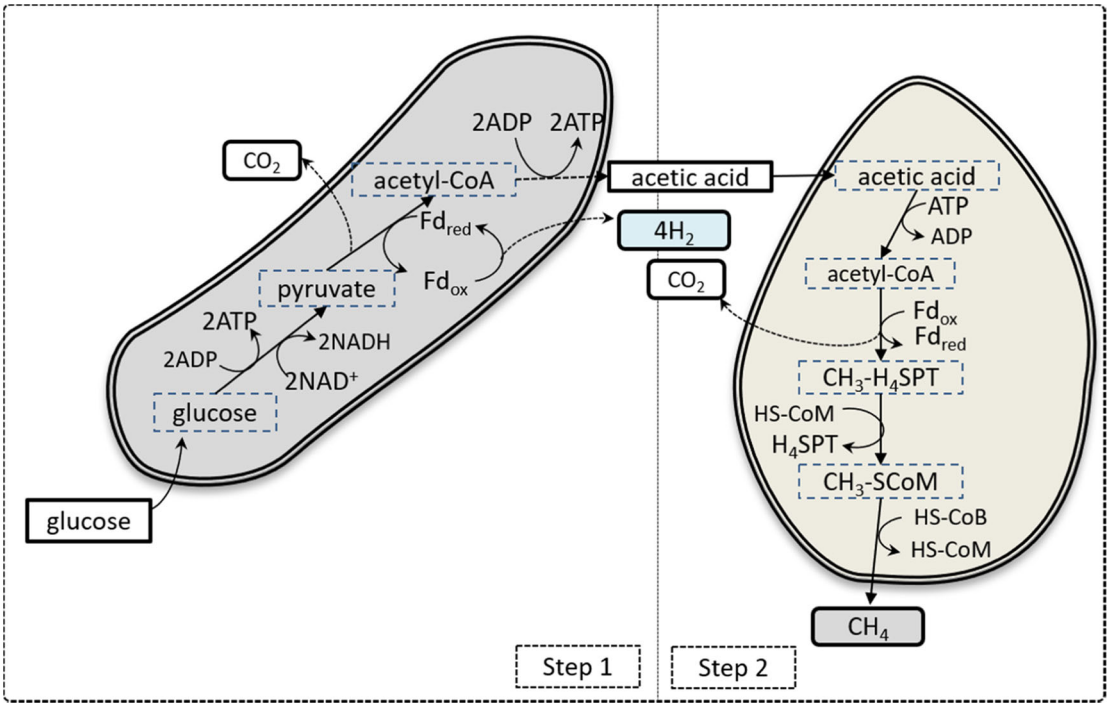

Figure 4. Simplified metabolic pathways for H_2 synthesis and CH_4 synthesis in a two-step conversion of biomass. The organic acid produced from the oxidation of glucose in the first stage is utilized as a substrate for the production of methane in the second stage. HS-CoB, coenzyme B; HS-CoM, coenzyme A; H4SPT, tetrahydrosarcinapterin.

the optimum production conditions of the individual gases, predominantly the pH. The optimum pH for hydrogen production is generally between 5–6, whereas that of methane is 7–8. This has led to the development of a two-step process of producing hydrogen in the first stage followed by the methanogenesis of the organic acid by-products.[94,95] The first stage, which is optimized for the activity of hydrogenase in the acidogenic microbes, produces organic acids, usually acetate, propionate, and butyrate as by-products. These organic acids are then used as a substrate for the methanogenic microbes. Superior yields of total biogas produced in the two-stage process over the one-stage process have been reported.[95]

A more recent approach of producing biohydrogen, which uses the water-splitting abilities of some photosynthetic microbes such as photosynthetic bacteria and microalgae, is on the rise. These microorganisms containing light-harvesting pigments are functional in CO_2 fixation by using absorbed light energy. A deviation from the normal physiological processes can lead to hydrogen production through water splitting.[96]

6. Metabolic Engineering and Its Role in the Development of Cell Factories for Biorefinery

Microorganisms offer a platform for easy manipulation of metabolic activities. This has allowed for the accumulation of sufficient information for the successful engineering of metabolic processes in microbes such as *E. coli*, *S. cerevisiae*, and some microalgal strains. This process, referred to as metabolic engineering, seeks to optimize genetic and regulatory processes of cell factories for the production of increased amounts and novel compounds by microbes.

Advances in metabolic engineering have been a major pillar in the progress of biorefineries. Blocking of competitive pathways and the overexpression of relevant genes in the biosynthesis of fatty acids and carotenoids in some microalgal strains have been used to improve the yields of lipids and pigments, which can be used as precursors for biodiesel and other pharmaceutical products.[27] Metabolic engineering has aided in expanding the range of feedstock that can be used in biorefinery. A typical scenario is the assimilation of xylose by *S. cerevisiae* through the heterologous expression of xylose isomerase, or xylose reductase and xylitol dehydrogenase, or the overexpression of endogenous GRE3.[97] This promotes the efficient utilization of biomass, as xylose is the second most abundant sugar in biomass. Moreover, recent trends in metabolic engineering provide tools for mimicking pathways in cell factories to enhance the biosynthesis of novel compounds in cell factories. Plant secondary metabolites, such as benzylisoquinoline alkaloids, which are used in pharmaceuticals and nutraceuticals have been successfully produced in high titers via a biosynthetic pathway in *E. coli*.[98] The successful introduction of these metabolic pathways in microorganism offers a more rapid and more flexible approach of synthesizing a variety of these secondary metabolites.

Recent advances in analytical procedures under metabolic engineering including metabolic flux analysis and various omics have made it possible for characterizing not only metabolic fluxes, but also obtaining information as fundamental as gene expression levels. The data set (multiomics)

obtained under these analyses can be used to generate mathematical models. These models, which can be computerized, are then applied to the Design–Build–Test–Learn (DBLT) approach to study genetic perturbation and metabolic shifts. This is expected to assist and reduce cost and time in strain design and process engineering development.

7. Rethinking the Concept of Biorefineries

The concept of biorefinery is based on the use of biomass as feedstocks for the production of multiple bio-based products including fuels and chemicals, relieving the dependency on fossils. Earlier documented works differentiated biorefineries according to feedstock, process flexibility, and products. The first approach produces dried distillers' grain, ethanol, and CO_2 from dry grains using a fixed processing capability. Another approach, which offers more processing flexibility, produces corn oil, starch, high-fructose syrup, ethanol, dried distillers' grain, and CO_2 from grains. A more advanced approach to biorefinery employs the concept of high value–low volume and low value–high volume in its targeted products, using an integration of processing technologies and a variety of feedstock. This advanced approach is still under intensive research and developmental stages.

Biorefinery is geared towards the production of both traditional and novel fuels and chemicals by combining specially designed technologies and processes for the transformation of biomass. The goal is not solely to imitate the petroleum refinery but rather generate novel products, which are otherwise not obtainable from fossils, in addition to the possible substitutes of petroleum products. Arriving at this goal requires an in-depth understanding of the fundamental chemistries of biomass to assist in designing effective conversion technologies for optimized product generation. Coupling this with comprehensive knowledge in production technology and economics and environmental impacts is essential to achieving the goals of biorefinery.

8. Conclusion

The current approach of biorefinery encompasses various feedstocks and diverse conversion technologies to achieve multiple products in the form of biochemicals and biofuels. Lignocellulose, which is the most abundant biomass, often requires pretreatment processes to liberate value-added products and fermentable sugars, which could be used as feedstock for microbes to generate an array of biochemicals and biofuels. Other emerging forms of biomass such as nonedible oils and microalgae can be used as feedstock in biorefinery. Development of robust microbial culture collection will facilitate the efficient biotechnological transformation of these feedstock, which possesses various technological barriers. Combining this with the advances in metabolic engineering could leverage the rapid development of cell factories for the efficient utilization of biomass feedstock for the production of a variety of products under biorefinery. Moreover, an integrative approach towards biorefinery where the use of the waste from one production stream can be employed as a feedstock for another should be investigated. This symbiotic effect should not be limited to industrial levels but also extended to microbial levels. As biomass varies greatly from one geographical region to the other, the development of global-scale technologies should be supported by local-scale developments such as developing culture collection for microbes that are adapted to the local environment and feedstock.

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Conflict of Interest

The authors declare no conflict of interest.

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biochemicals, bioenergy, biofuels, biorefinery, industrial microbes, lignocellulose biomass

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