PQI 5888
Fisiologia e Biotecnologia de Leveduras

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15 de maio de 2020

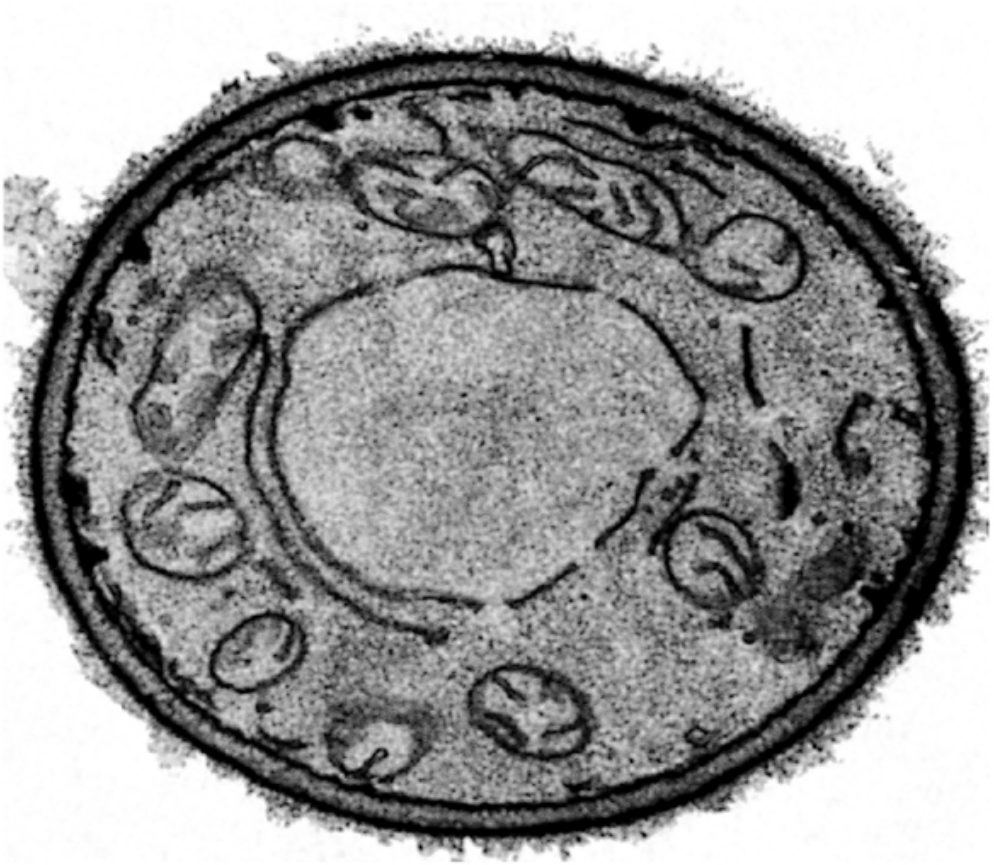
(b)

[Aula 7]

Yeast Nutrition

(aula síncrona)

Micrografia eletrônica de uma levedura



*Como as **leveduras** constroem as macromoléculas, que virarão estruturas, que virarão novas células?*

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- através de várias reações bioquímicas (METABOLISMO).

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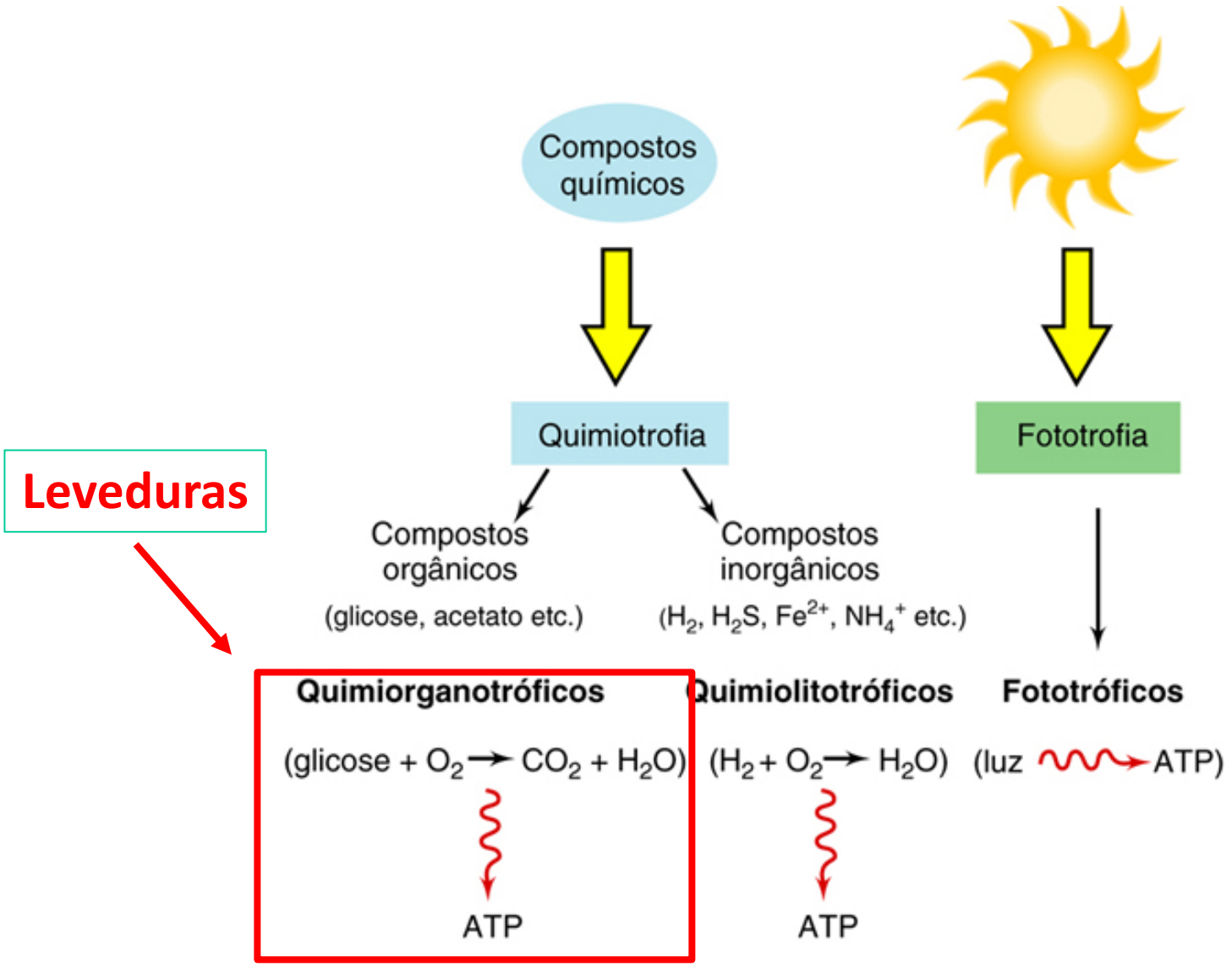
*- Vamos agora dirigir nossa atenção para o ponto de partida para estas reações bioquímicas, que são os **NUTRIENTES** (compostos orgânicos e inorgânicos) presentes no meio-ambiente (natureza) ou no meio de cultura (laboratório e indústria).*

Opções metabólicas para a obtenção de energia



Composição elementar de uma célula microbiana

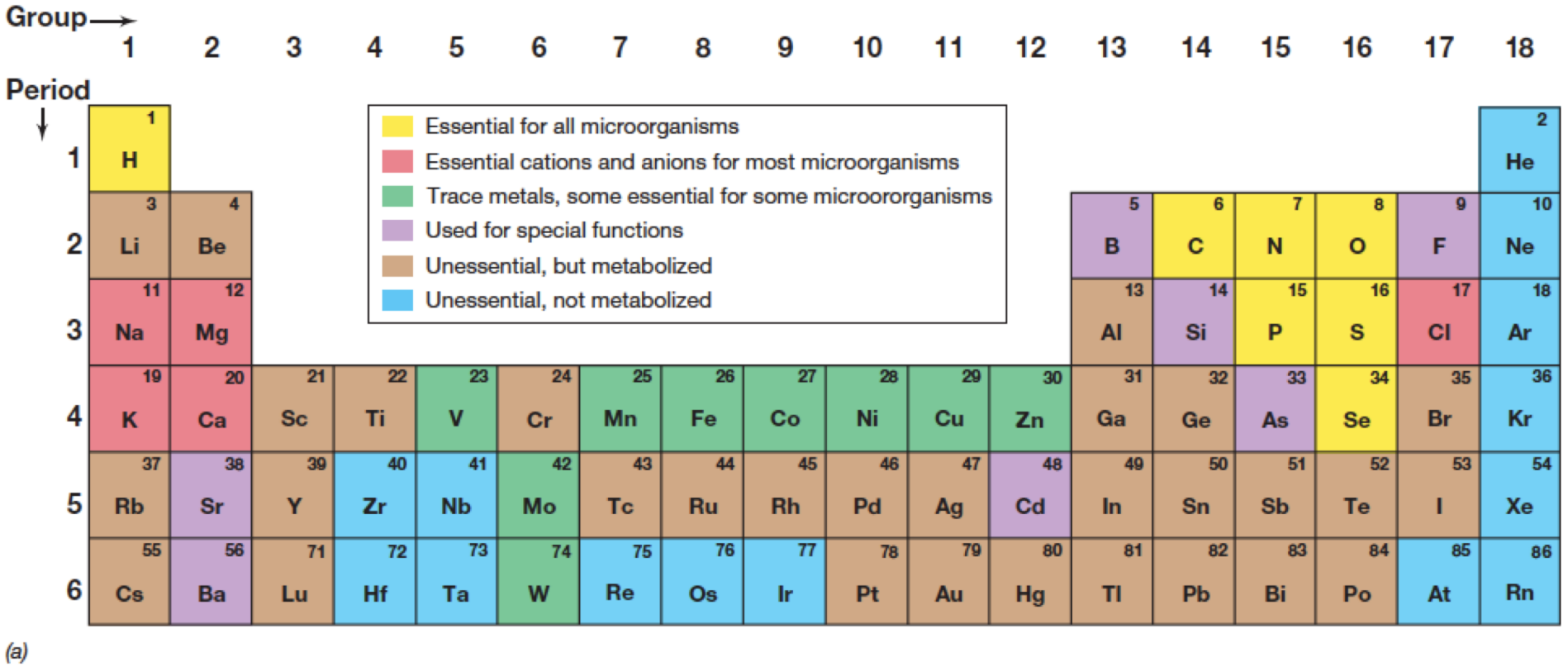
TABLE 4.2. *Element composition of bacteria, yeasts and fungi (% by dry weight)*

Element	Bacteria (Luria, 1960; Herbert, 1976; Aiba *et al.*, 1973	Yeasts (Aiba *et al.*, 1973; Herbert, 1976)	Fungi (Lilly, 1965; Aiba *et al.*, 1973)
Carbon	50–53	45–50	40–63
Hydrogen	7	7	
Nitrogen	12–15	7.5–11	7–10
Phosphorus	2.0–3.0	0.8–2.6	0.4–4.5
Sulphur	0.2–1.0	0.01–0.24	0.1–0.5
Potassium	1.0–4.5	1.0–4.0	0.2–2.5
Sodium	0.5–1.0	0.01–0.1	0.02–0.5
Calcium	0.01–1.1	0.1–0.3	0.1–1.4
Magnesium	0.1–0.5	0.1–0.5	0.1–0.5
Chloride	0.5	—	—
Iron	0.02–0.2	0.01–0.5	0.1–0.2

Composição macromolecular

Macromolecule	Percent of dry weight
Protein	55
Lipid	9.1
Polysaccharide	5.0
Lipopolysaccharide	3.4
DNA	3.1
RNA	20.5

Macro- & **Micro**nutrientes



(a)

Macronutrientes

TABLE 4.2 Macronutrients in nature and in culture media

Element	Usual form of nutrient found in the environment	Chemical form supplied in culture media
Carbon (C)	CO_2, organic compounds	Glucose, malate, acetate, pyruvate, hundreds of other compounds, or complex mixtures (yeast extract, peptone, and so on)
Hydrogen (H)	H_2O, organic compounds	H_2O, organic compounds
Oxygen (O)	H_2O, O_2, organic compounds	H_2O, O_2, organic compounds
Nitrogen (N)	NH_3, NO_3^-, N_2, organic nitrogen compounds	*Inorganic:* NH_4Cl, $(NH_4)_2\,SO_4$, KNO_3, N_2 *Organic:* Amino acids, nitrogen bases of nucleotides, many other N-containing organic compounds
Phosphorus (P)	PO_4^{3-}	KH_2PO_4, Na_2HPO_4
Sulfur (S)	H_2S, SO_4^{2-}, organic S compounds, metal sulfides (FeS, CuS, ZnS, NiS, and so on)	Na_2SO_4, $Na_2S_2O_3$, Na_2S, cysteine, or other organic sulfur compounds
Potassium (K)	K^+ in solution or as various K salts	KCl, KH_2PO_4
Magnesium (Mg)	Mg^{2+} in solution or as various Mg salts	$MgCl_2$, $MgSO_4$
Sodium (Na)	Na^+ in solution or as NaCl or other Na salts	NaCl
Calcium (Ca)	Ca^{2+} in solution or as $CaSO_4$ or other Ca salts	$CaCl_2$
Iron (Fe)	Fe^{2+} or Fe^{3+} in solution or as FeS, $Fe(OH)_3$, or many other Fe salts	$FeCl_3$, $FeSO_4$, various chelated iron solutions (Fe^{3+} EDTA, Fe^{3+} citrate, and so on)

Micronutrientes

TABLE 4.3 Micronutrients (trace elements) needed by living organisms[a]

Element	Cellular function
Chromium (Cr)	Required by mammals for glucose metabolism; no known microbial requirement
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (propionic acid bacteria)
Copper (Cu)	Certain proteins, notably those involved in respiration, for example, cytochrome *c* oxidase; or in photosynthesis, for example, plastocyanin; some superoxide dismutases
Manganese (Mn)	Activator of many enzymes; present in certain superoxide dismutases and in the water-splitting enzyme of photosystem II in oxygenic phototrophs
Molybdenum (Mo)	Present in various flavin-containing enzymes; also in molybdenum nitrogenase, nitrate reductase, sulfite oxidase, DMSO-TMAO reductases, some formate dehydrogenases, oxotransferases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles (for example, aldehyde: ferredoxin oxidoreductase of *Pyrococcus furiosus*)
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Present in the enzymes carbonic anhydrase, alcohol dehydrogenase, RNA and DNA polymerases, and many DNA-binding proteins
Iron (Fe)[b]	Cytochromes, catalases, peroxidases, iron–sulfur proteins (for example, ferredoxin), oxygenases, all nitrogenases

[a]Not every micronutrient listed is required by all cells; some metals listed are found in enzymes present in only specific microorganisms.

[b]Needed in greater amounts than other metals—not generally considered a trace element.

Vitaminas e suas funções

TABLE 4.4 Vitamins and their functions

Vitamin	Function
p-Aminobenzoic acid	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl group transfer
Biotin	Fatty acid biosynthesis; β-decarboxylations; CO_2 fixation
Cobala	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α-ketoglutarate
Nicotinic acid (niacin)	Precursor of NAD^+; electron transfer in oxidation–reduction reactions
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives
Riboflavin	Precursor of FMN, FAD in flavoproteins involved in electron transport
Thiamine (B_1)	α-Decarboxylations; transketolase
Vitamins B_6 (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations
Vitamin K group; quinones	Electron transport; synthesis of sphingolipids
Hydroxamates	Iron-binding compounds; solubilization of iron and transport into cell
Coenzyme M (CoM)	Required by certain methanogens; plays a role in methanogenesis

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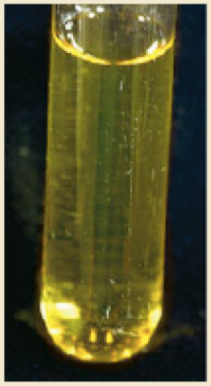
Meio DEFINIDO e Meio COMPLEXO

Table 4.2 *Examples of culture media for microorganisms with simple and demanding nutritional requirements*[a]

Defined culture medium for **Escherichia coli**	*Defined culture medium for* **Leuconostoc mesenteroides**	*Complex culture medium for either* **E. coli** *or* **L. mesenteroides**
K_2HPO_4 7 g KH_2PO_4 2 g $(NH_4)_2SO_4$ 1 g $MgSO_4$ 0.1 g $CaCl_2$ 0.02 g Glucose 4–10 g Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo) 2–10 μg each Distilled water 1000 ml pH 7	K_2HPO_4 0.6 g KH_2PO_4 0.6 g NH_4Cl 3 g $MgSO_4$ 0.1 g Glucose 25 g Sodium acetate 25 g Amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) 100–200 μg of each Purines and pyrimidines (adenine, guanine, uracil, xanthine) 10 mg of each Vitamins (biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid) 0.01–1 mg of each Trace elements (as in first column) 2–10 μg each Distilled water 1000 ml pH 7	Glucose 15 g Yeast extract 5 g Peptone 5 g KH_2PO_4 2 g Distilled water 1000 ml pH 7

(a)



(b)

[a]The photos are tubes of *(a)* the defined medium described, and *(b)* the complex medium described. Note how the complex medium is colored from the various organic extracts and digests that it contains. Photo credits: Cheryl L. Broadie and John Vercillo, Southern Illinois University at Carbondale.

Table 3.6. Principal ingredients of selected industrial media for yeasts.

Components	Molasses	Malt wort	Wine must	Cheese whey
Carbon Sources	Sucrose Fructose Glucose Raffinose	Maltose Sucrose Fructose Glucose Maltotriose	Glucose Fructose Sucrose (trace)	Lactose
Nitrogen Sources	Nitrogen compounds as unassimilable proteins. Nitrogen sources need to be supplemented	Low molecular α-amino nitrogen compounds, ammonium ions and a range of amino acids	Variable levels of ammonia nitrogen, which may be limiting. Range of amino acids	Unassimilable globulin and albumin proteins. Low levels of ammonium and urea nitrogen
Minerals	Supply of P, K, and S available. High K^+ levels may be inhibitory	Supply of P, K, Mg and S available	Supply of P, K, Mg and S available. High levels of sulphite often present	Supply of P, K, Mg, S.
Vitamins	Small, but generally adequate supplies. Biotin is deficient in beet molasses	Supply of vitamins is usually adequate. High adjunct sugar wort may be deficient in biotin	Vitamin supply generally sufficient	Wide range of vitamins present.
Trace Elements	Range of trace metals present, although Mn^{2+} may be limiting	All supplied, although Zn^{2+} may be limiting	Sufficient quantities available	Fe, Zn, Mn, Ca, Cu present
Other Components	Unfermentable sugars (2-4%), organic acids, waxes, pigments, silica, pesticide residues, caramelized compounds, betaine	Unfermentable maltodextrins, pyrazines, hop compounds	Unfermentable pentoses. Tartaric and malic acids. Decanoic and octanoic acids may be inhibitory. May be deficient in sterols and unsaturated fatty acids	Lipids, NaCl. Lactic and citric acids

AIMS of this presentation

- Introduce yeast nutritional physiology
- Describe the nutritional requirements of yeast
- Discuss the concept of nutrient bio-availability
- Explain the uptake and utilisation of nutrients
- Highlight the industrial relevance of yeast nutrition

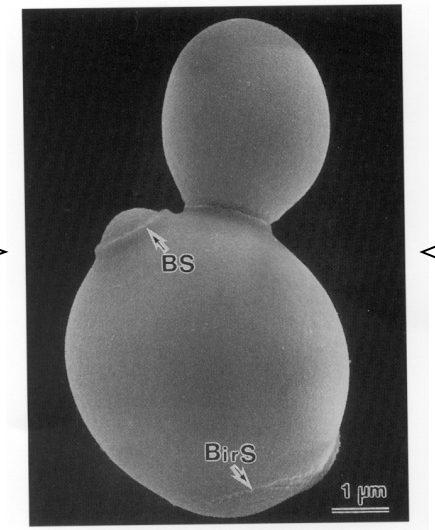
Yeast physiology and biotechnology - important note

A pre-requisite for the success of any yeast-based industry is a thorough understanding of the factors that regulate nutrition, growth, stress responses and metabolism in yeast cells

Factors influencing yeast behaviour

GENOTYPE

Physical environment



Nutrient availability

PHENOTYPE

Nutrients influence:

- Fermentation performance (eg. ethanol yield)
- Yeast growth rate/yield
- Yeast viability/vitality/stress tolerance
- Extent of foaming, yeast flocculation
- Minor metabolites (glycerol, acetaldehyde, fusel oils, etc.)

Yeast nutrition

This relates to how yeast cells feed.

That is:

- **How yeasts transport nutrients that are *bioavailable***
- **How yeasts utilise these nutrients in growth & metabolism**
- **How yeasts store excess nutrients**

Elemental requirements for yeast growth

Macroelements

(**mM** requirements)

H - cell water, organic material

O - respiration, cell water, organics

C - organic material

N - proteins, nucleic acids, coenzymes

S - proteins, coenzymes

P - nucleic acids, phosphoslipids

K - cell electrolyte, enzyme cofactor

Mg - enzyme cofactor, cell division

Microelements

(**µM requirements**)

Ca - flocculation, signalling

Mn - enzyme cofactor

Fe - cytochromes

Zn - alcohol dehydrogenase

Co - vitamin B12, coenzymes

Cu, Ni, Mo - specialised enzymes

Nutrient sources for yeast growth

-Water (yeast cells are 75% water)

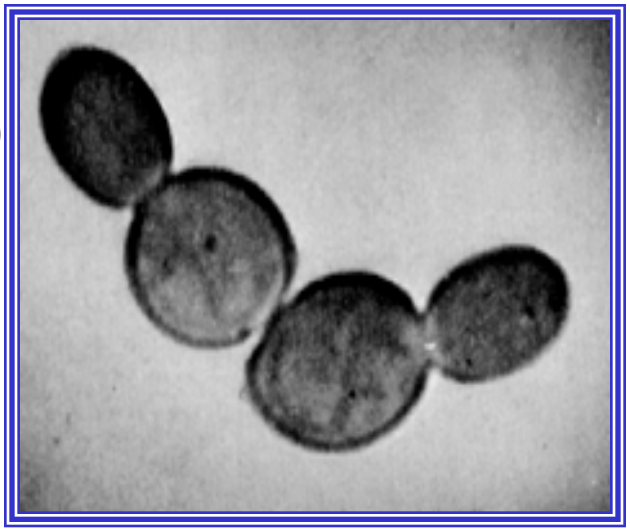
-Carbohydrates (hexoses, pentoses, disaccharides)

-Nitrogen sources (NH_3, urea, amino acids)

-Vitamins (biotin, niacin, pantothenate etc)

-Minerals (Mg, Zn, K, P, Ca etc.)

-Oxygen (special roles)



NOTE: poor yeast nutrition = poor fermentation

Yeast nutrition: **WATER**

- Yeast needs very high amounts of water for growth
  (eg. molasses is appropriately diluted)

- Water should not contain impurities (NaOH wash water,
  ions, solvents, acids, anti-scalants etc. that are harmful to yeast viability)

- ***A fermentor is not a rubbish bin!***

It is a delicate balance of yeast in its environment

Carbon sources for *S. cerevisiae*

Carbohydrate is the source of energy and basic carbon 'backbones' for yeast growth AND is directly proportional to ethanol made by fermentation

Hexose sugars: glucose, fructose

Pentose sugars: no (but possibly xylulose)

Disaccharides: maltose, sucrose (lactose unfermented)

Trisaccahrides: maltotriose (slowly)

Tetrasaccharides: maltotetraose (some strains)

Oligosaccharides: maltodextrins (unfermented)

Polysaccharides: starch (unfermented)

Others: ethanol, acetate, glycerol (only respired)

Destination of sugars in yeast?

Synthesis (new yeast cells)

Secretion (fermentation products)

Storage (glycogen, trehalose)

Survival (trehalose, glycerol)

Sugar sources for yeast bioethanol fermentations





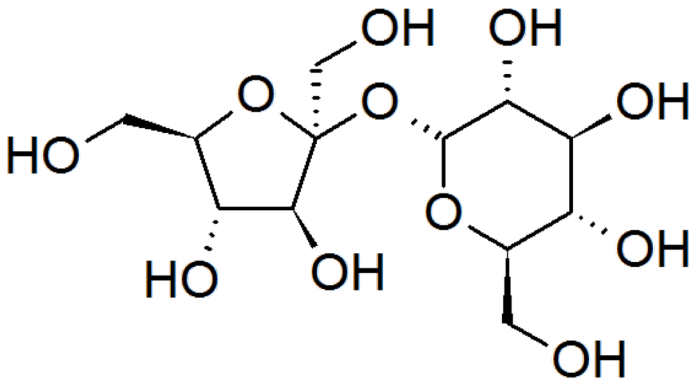




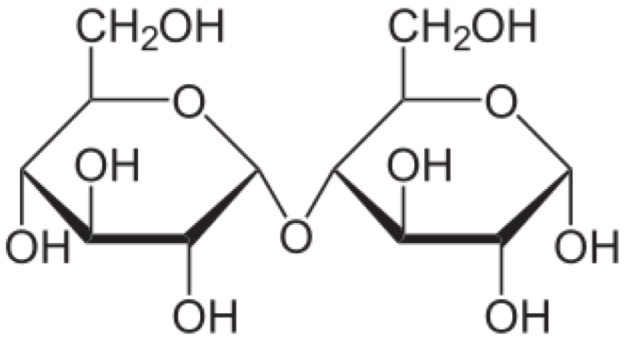
Ethanol

Ideally with >90% conversion efficiency

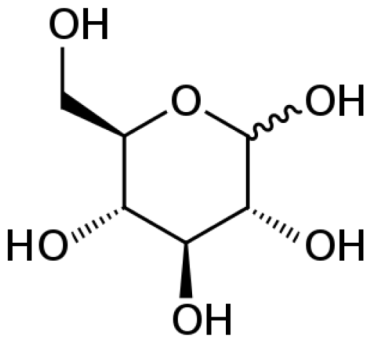
Main sugars fermented by *S. cerevisiae*



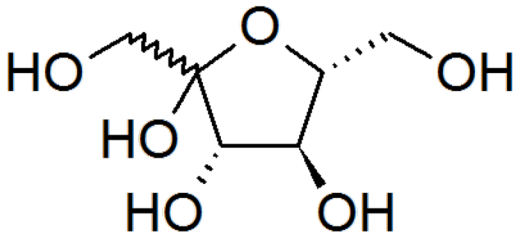
SUCROSE



MALTOSE



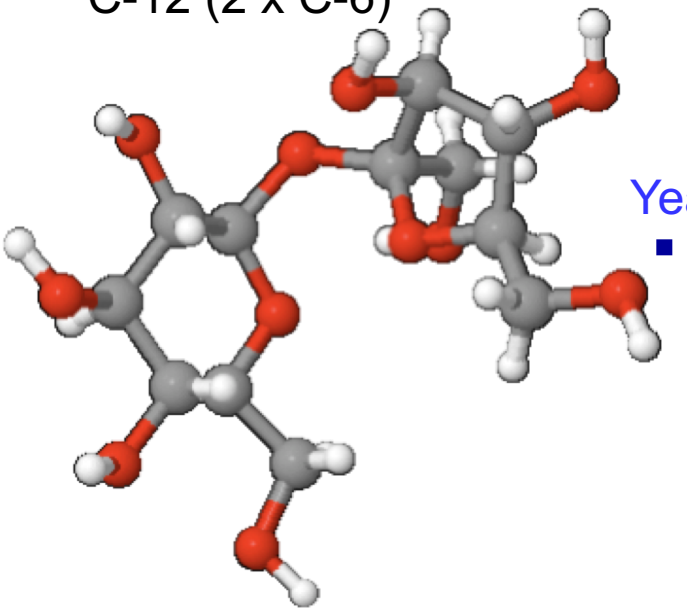
GLUCOSE



FRUCTOSE

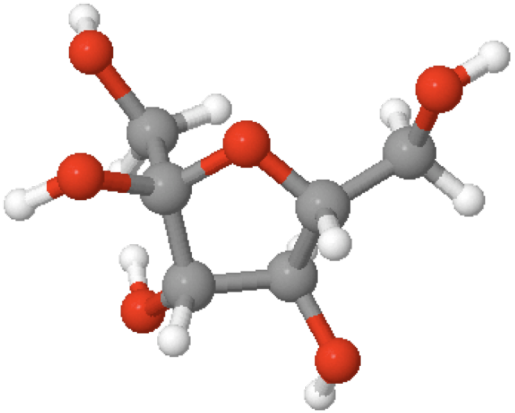
Sucrose cleavage by yeast

Sucrose
C-12 (2 x C-6)



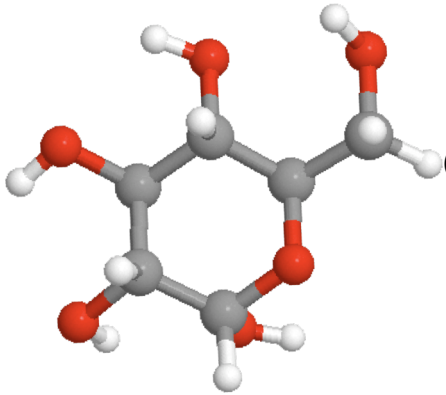
Yeast invertase

+ H_2O

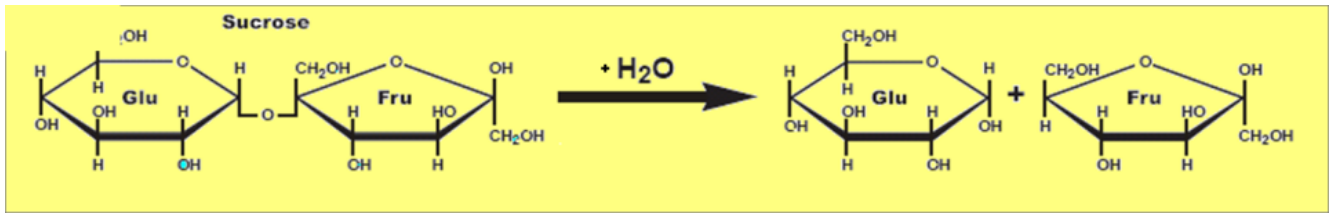


Fructose
C-6

+



Glucose
C-6



Molasses (for yeast & ethanol production)

Sugar cane

Sugar beet

Molasses

Cane
~35%sucrose

Beet
~50%sucrose

A Type one crystallization step (rare)
B Type two crystallization steps
C Type three crystallization steps. Most common form of ***blackstrap****
Refiners From the cleanup of raw sugar (lower minerals, higher sugar)

* *No one makes Blackstrap Molasses as a product - it is a waste product of sugar production!*

	Cane	*Beet*
	Range or typical value (%)	
Total sugars	45-55	48-52
Sucrose	25 to 35	46-52
Reducing sugars	20 to 35	0.5-4.0
pH	5-5.5	~7.0
Ash	10-16	10-12
Non-sugar organic matter	10-12	12-17
Starch/polysaccharides	0.5%	
Calcium	0.4-0.8	0.1-0.5
Sodium	0.1-0.4	0.5-1.0
Potassium	1.5-5.0	2.0-7.0
Magnesium	0.05-0.98	0.1-0.15
Phosphorous	0.03-0.1	0.02-0.07
Sulfur	0.3-0.8	0.15-0.5
	Range or typical value (ppm)	
Biotin	1.2-3.2	0.04-0.13
Folic acid	0.04	0.2
Riboflavin	2.5	0.4
Copper	2.2-38	2.5
Manganese	4-300	3.0
Zinc	4-48	0.15

United Molasses

Molasses is highly variable!

	#1	**#2**
Brix	84.90	88.32
Sucrose	32.36	33.31
Total Sugar	51.30	58.39
TSAI	53.30	60.30
Reducing Sugars	18.97	23.58
Non fermentable Sugars	2.96	3.50
Ash	13.40	11.08
Calcium	0.41	0.35