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Fisiologia e Biotecnologia de Leveduras

Prof. Thiago Basso

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(b)

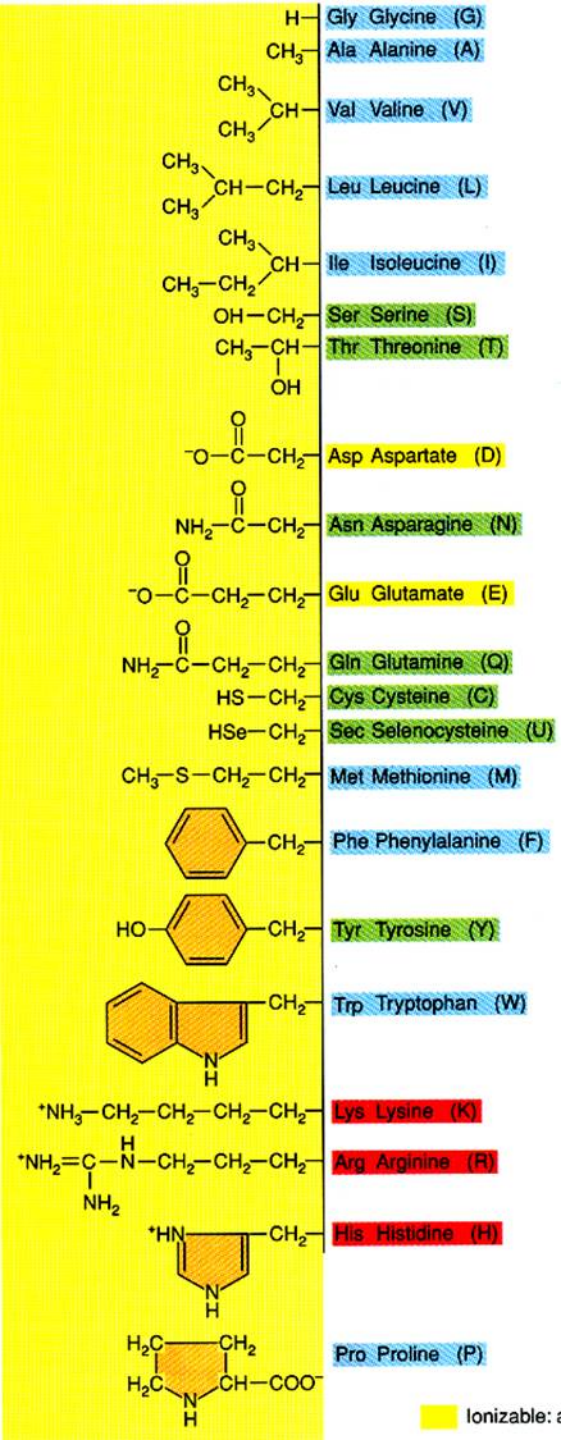
[Aula 8]

Yeast Nutrition – part 2

(aula síncrona)

Nitrogen sources for Yeasts

- Proteins - no! (*S.cerevisiae* is non-proteolytic)
- Peptides (small di, or tri-peptides)
- Amino acids (sequential uptake)
- Ammonium salts (NH_4^-)
- Urea (CH_4N_2O)
- Nitrate (NO_3^-)



Nitrogen is a basic element used by the cell to make amino acids, enzymes, structural proteins, nucleic acids

Yeast requires small molecular weight nitrogenous compounds:

- FAN = Free Amino Nitrogen
- It is found in dipeptides and amino acids

side chain
|
NH_2-CH-COOH

- YAN = Yeast Assimilable Nitrogen
- Useable forms YAN include urea, ammonia/ammonium ions
- Useable nitrogen is the most critical limiting nutrient in fermentations - below a threshold of ~150mg/L, may lead to "stuck" fermentations
- Nitrogen starvation may also lead to intracellular proteolysis (yeast death/lysis)
- ***Cereal grains & molasses are N-deficient for efficient yeast fermentation!***

Oxygen requirements for *S. cerevisiae*

- Fermenting *S. cerevisiae* cells need O_2 as a growth factor (not for respiratory metabolism)
- Yeast strains have variable requirements for O_2 (eg. brewing wort is aerated to 8-16ppm dissolved O_2)
- O_2 required for sterol and unsaturated fatty acid synthesis (e.g. ergosterol, oleic acid)
- O_2 therefore important for yeast membrane integrity (and ethanol tolerance)
- O_2 needed to mobilise glycogen (during lag-phase)

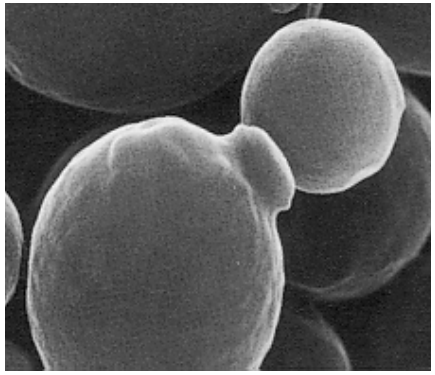
Diversity of yeast O_2 requirements

Obligate Aerobic — Obligate Anaerobic

RESPIRERS----------Facultative----------**FERMENTERS**

Rhodotorula, Cryptococcus

S. cerevisiae



Candida pintolopesii

- can grow fermentatively without O_2 or oxidatively with O_2
- for truly respiratory growth, O_2 and low sugar is required (Crabtree effect)
- cannot grow **strictly** anaerobically (O_2 needed for membrane sterols & fatty acids)

The *Crabtree effect* (absent in some yeasts like *Kluyveromyces marxianus*)

- *S. cerevisiae* metabolizes fermentatively even in presence of oxygen - mainly due to the high level of carbohydrates (glucose)

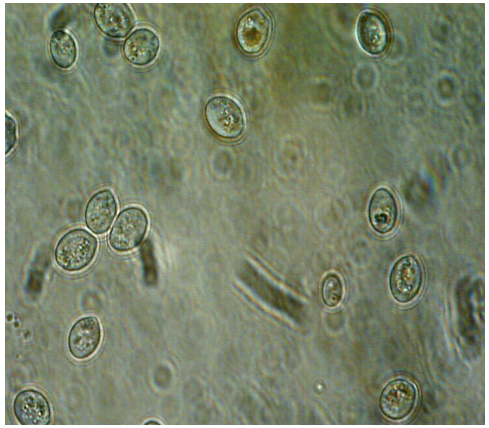
- In the presence of large amounts of oxygen (>1 vvm) yeasts can obtain energy by respiration

BUT: sugar must be very low - eg. <0.2% glucose

- Aerobic respiratory growth occurs in a yeast production plant, using fed-batch propagation (not in an ethanol distillery)

Lipid supplements instead of oxygen?

- Lipid-phospholipid complex
- Hosphatidylcholine, palmitic acid and cholesterol
- Phosphatidyl serine
- Ergosterol or camposterol and linoleic acid
- Oleic, linolelic or linolenic acid
- Linseed/cotton seed or soyabean oil
- Yeast foods
- Yeast hulls



Phosphorus & Sulphur

- **P** & **S** are essential for yeast metabolism and growth (molasses is usually deficient)
- **S**: sulphate, sulphite, thiosulphate, methionine and glutathione
- **P**: Orthophosphate ($H_2PO_4^-$) and condensed inorganic phosphate
- Available phosphorus is required at ~1-2% of the dry weight of expected yeast mass per unit volume of medium
- Available sulphur is required at ~ 0.3-0.5% of the dry weight of expected yeast mass per unit volume of medium

Inorganic nutrients for yeast

- Essential bulk minerals: **P, S, K, Mg**
- Essential trace ions: Zn, Ca, Fe, Mn, Mo, Ni
- **Toxic** trace ions: Pb, Cd, Cr, Hg, Cu, Al etc.

NOTE

Phosphorus, sulphur and potassium generally in sufficient supply

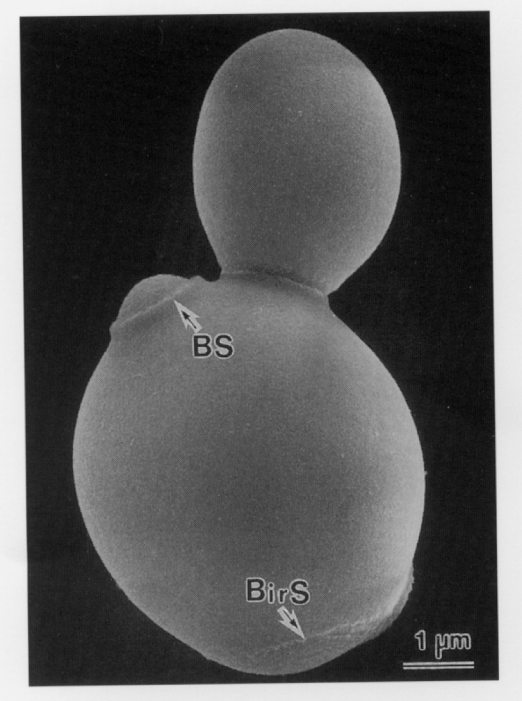
Metal cations may occasionally be deficient (and ratios of metal ions may be sub-optimal)

Mineral nutrition: ***Why do yeasts need metals?***

Cell growth

Enzyme Activity (fermentation)

Cell structure



Stress tolerance

Cell -cell Interactions

Cell division

Vitamins for yeasts?

- Biotin, pantothenic acid & inositol are commonly required

  (Biotin deficiency in some molasses)
- Also thiamin, nicotinic acid and pyridoxine
- Vitamin deficiencies may be problematic

  (can supplement with "yeast foods")

Vitamin content of a typical molasses

Biotin	1-3ppm
Inositol	6000ppm
Pantothenic acid	50-60ppm
Thiamin	~2ppm
Nicotinic acid	30-80ppm

Typical Vitamin Levels in Cereal Wort

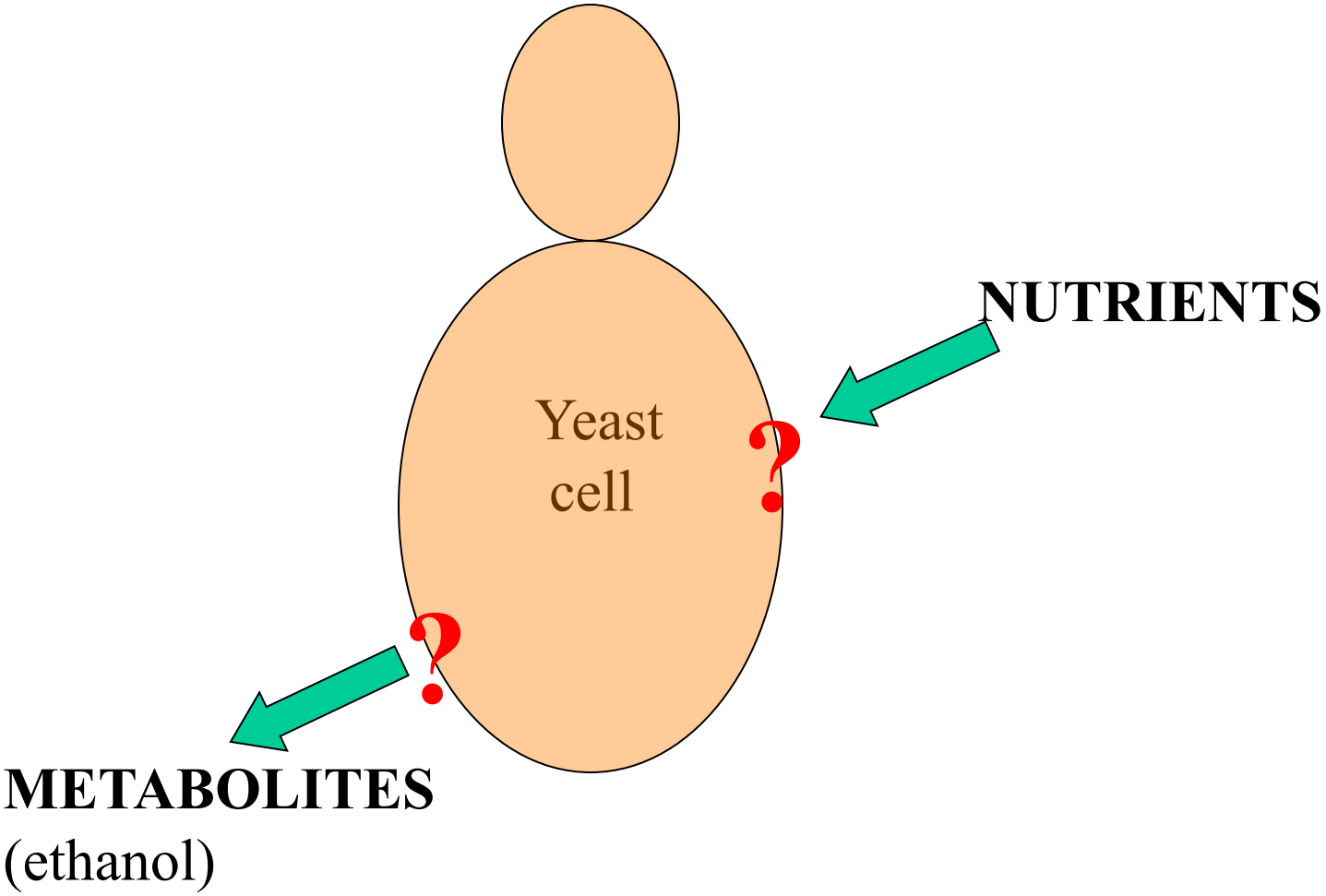
VITAMIN	LEVEL IN WORT /100 ml	METABOLIC ROLES
Biotin	0.5 μg	Carboxylations
Thiamin (B1)	60 μg	Decarboxylations
Pantothenate	~50 μg	Acetylations, CoA
Nicotinic acid	1000 μg	Coenzymes
Riboflavin	50 μg	Coenzymes
Pyridoxine	85 μg	Amino acid metabolism
Inositol	10 mg	Membranes

Table 3.4. Growth factor requirements of some yeasts.

Yeast	Requirements	Comments
Saccharomyces cerevisiae	Biotin, pantothenic acid,	Required by practically all strains
	Inositol, thiamine	Required by some strains
Saccharomyces carlsbergensis	Biotin, pantothenic acid	Required by all strains
	Inositol, uracil, guanine	Required by some strains
	Thiamine, pantothenic acid, nicotinic acid	Stimulatory for growth of some strains
Schizosaccharomyces pombe	Inositol, biotin, pantothenic acid	Required by all strains
	Nicotinic acid	Required by some strains
	Pyridoxine, thiamine	Stimulatory for growth of some strains
Pichia spp.	Variable	Some strains (e.g. *P. membranefaciens*) have no requirements whereas others require one (e.g. thiamine) or more (e.g. biotin plus pyridoxine)
Hansenula spp.	Variable	Vitamin requirements of this genus have been used in differentiating species. *H. anomala* has no requirements for pre-formed vitamins
Schwanniomyces spp.	Biotin	Required by *S. occidentalis*
Candida spp.	Biotin, nicotinic acid	Required by most species
	Thiamine	Required by *C. lipolytica* and stimulatory to *C. albicans*
	Cyanocobalamin	Required by *C. albicans*. *C. utilis* has no requirements for preformed vitamins
Kluyveromyces spp.	Nicotinic acid	All lactose-fermenting yeasts need this vitamin
Rhodotorula spp.	Thiamine, *p*-aminobenzoic acid	Generally required by this genus

Summarized from information provided in Koser (1968).

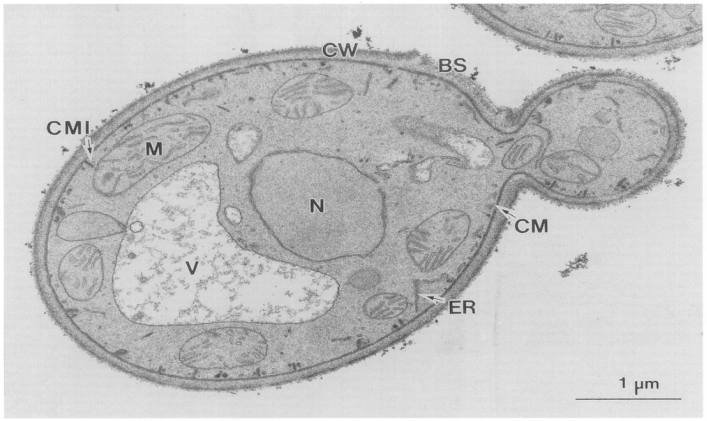
Yeast transport strategies for nutrients



Translocation of nutrients into yeast

CELLULAR BARRIERS

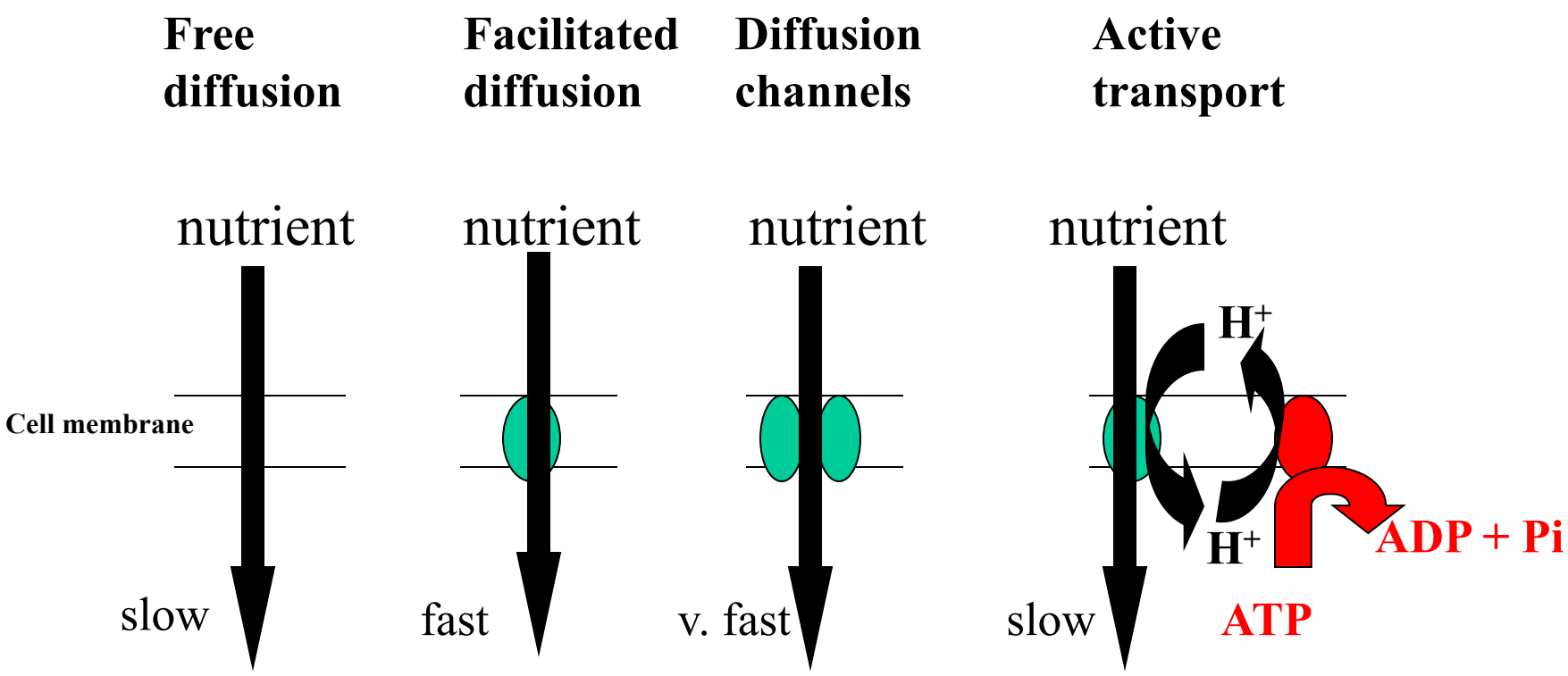
capsule, cell wall, periplasm, plasma membrane, organelles



PHYSICO-CHEMICAL BARRIERS (nutrient bioavailability)

chelation, adsorption, molecular size, binding

Yeast cell membrane nutrient transport mechanisms



- undissociated organic acids
- export of ethanol and gaseous compounds

Inside yeast cell

- permease, or carrier, or facilitator

- voltage-dependent 'gates' to move ions (K^+)

- driving force is the membrane potential and the proton gradient

Proton (H^+) - pumping ATPase

- Very important enzyme for nutrient uptake in brewing yeast (regulates growth and fermentation)
- Enzyme excretes acidity and regulates cell pH
- Yeasts can lower external pH to ~1.5
- In fermentation, ~30% of acidity due to ATPase

  (Rest is due to organic acids, CO_2 etc.)

ATPase and toxic acids

- Organic acids are more harmful than mineral acids (e.g. 0.8% lactic acid or 0.05% acetic acid are generally toxic to yeast)

- These acids dissociate inside the cell, releasing protons that lower cell pH

- ATPase expels these protons, but energy (ATP) needed for growth is shifted to pump H^+

Yeast transport mechanisms for nutrients

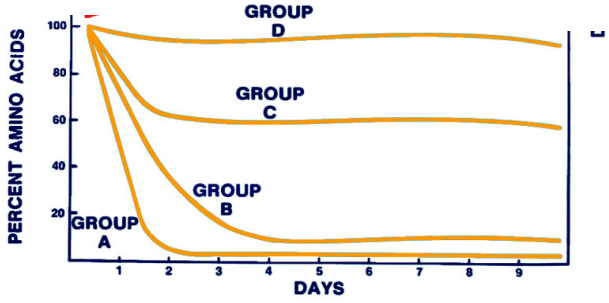
- **Water?** Turgor potential, and maybe water "channels"
- **Sugars?** Various mechanisms
- **Amino acids?** General and specific permeases
- **Ions?** Diffusion channels and specific carriers
- **Ethanol?** Free diffusion
- **Others?** Various active mechanisms for sterols, vitamins, fatty acids etc.

Amino acid uptake by brewing yeast

- **GROUP A (fast)**
  Glu, Asp, Asn, Gln,
  Ser, Thre, Lys, Arg
- **GROUP B (intermediate)**
  Val, Met, Leu, Isoleu, His
- **GROUP C (slow)**
  Gly, Phe, Tyr, Try,
  Ala, NH_3
- **GROUP D (little or no)**
  Pro

NOTE: A general amino acid permease (GAP) plus a variety of specific transport permeases exist for (active) amino acid uptake by brewing yeast

Brewing yeast amino acid uptake



Sugar transport by *S. cerevisiae*

- **Glucose & Fructose** - facilitated diffusion
- **Maltose** (4-O-α-D-glucopyranosyl-D-glucopyranose) - maltase, active transport by H^+ symport
- **Sucrose** (β-D-fructofuranosyl α-D-glucopyranoside) - invertase (then glucose/fructose by facilitated diffusion) Note that a constitutive proton symporter for sucrose has been identified in some strains of *S. cerevisiae*
- *In Kluyveromyces marxiunus, sucrose hydrolysed by extracellular inulinase*
- Maltotriose, etc. - specific permeases

Glucose uptake by yeast?

- Facilitated diffusion (energy-independent)

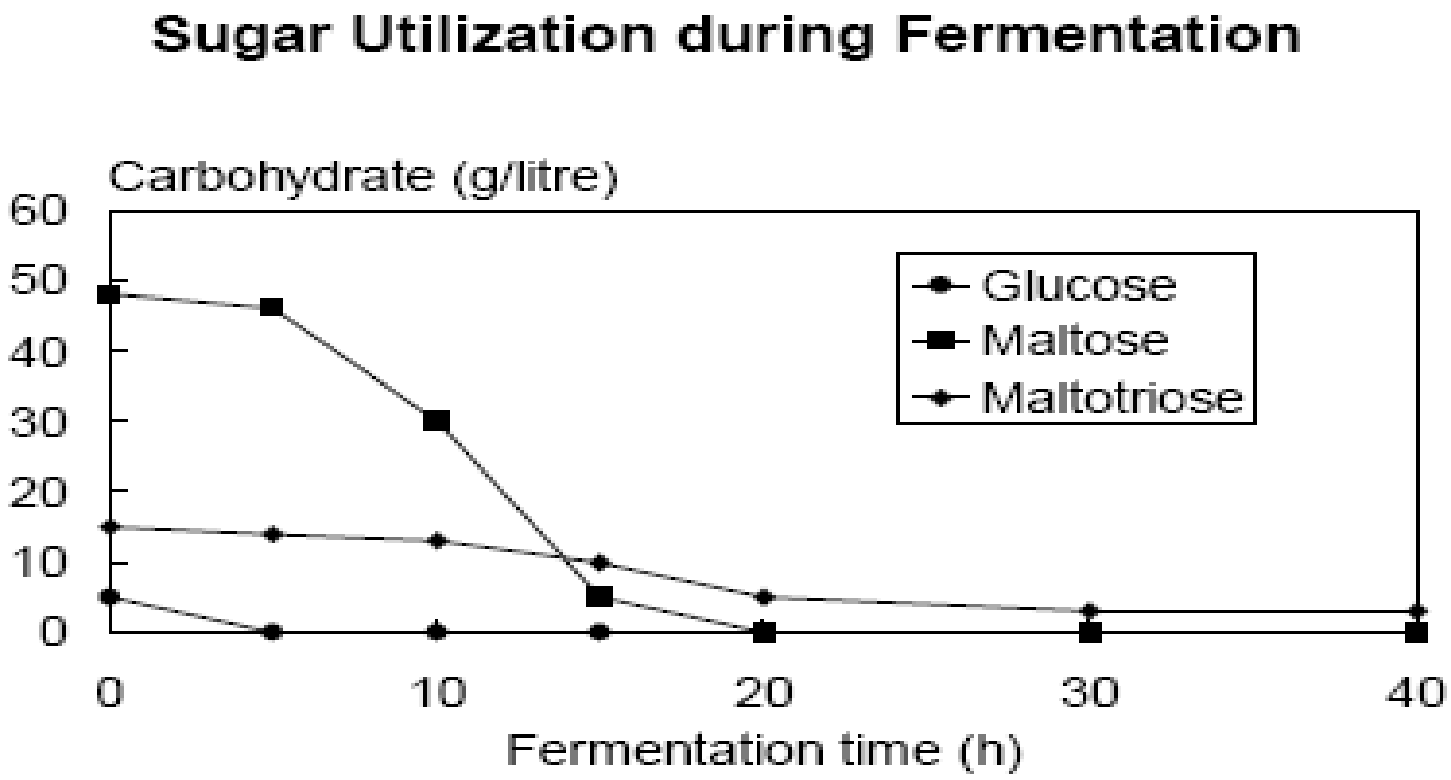
  [Some glucose (~1 in a million) may enter by passive diffusion]
- Driving force=glucose concentration gradient across yeast membrane
- Translocation via specific permeases (carrier proteins)
- Transport coupled to glucose phosphorylation
- Glucose uptake is preferential (over other sugars)

Maltose uptake by yeast?

- Active transport (energy-dependent)
- Driven by proton gradient (H^+ symport)

  [ATPase expels protons which then re-enter cell with maltose]
- Glucose represses maltose uptake (and its utilisation)

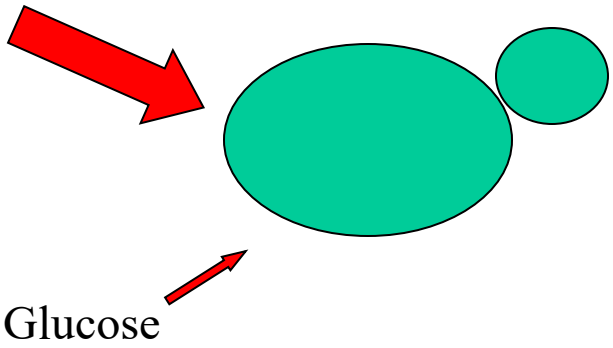
Malt distillery & brewing fermentations



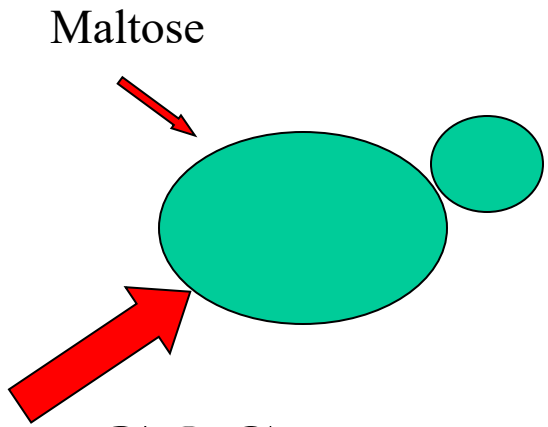
- Maltose (and other sugars) not used until glucose is taken up by yeast
- Sequential sugar uptake can slow fermentation (especially if glucose syrups are used as adjuncts)

Brewer's view

MALTOSE



Yeast's view



GLUCOSE

Biotechnological significance?

(Of sugar uptake in yeast)

1. Competition for sugars between different yeasts:

   Saccharomyces cerevisiae (low affinity glucose transporter)

   versus.

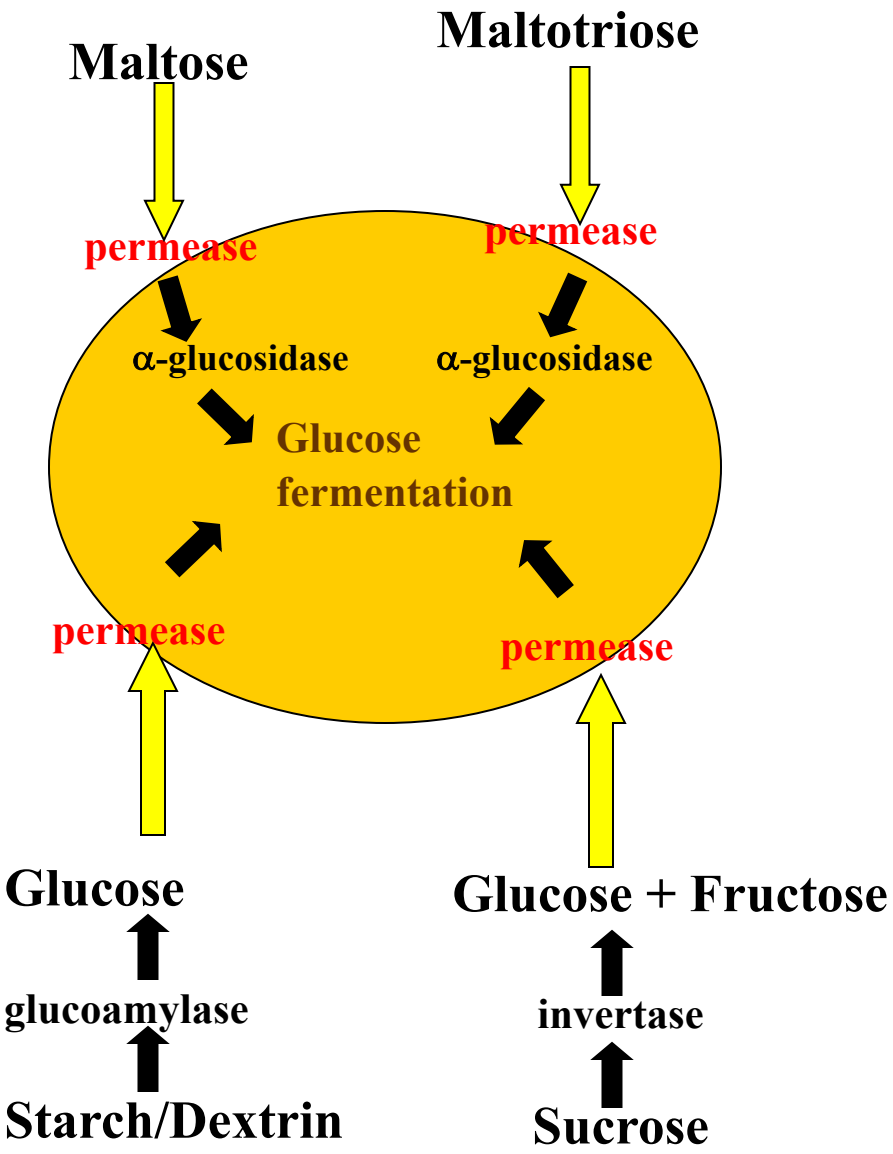
   Candida utilis (high affinity glucose transporter) in molasses fermentations

2. Preferential (sequential) glucose uptake by *S.cerevisiae* can slow fermentations of mixed sugars

3. Glucose-derepressed GM baker's & brewer's yeasts

   (lead to faster baking & brewing processes)

Sugar uptake by *S. cerevisiae* - summary



Yeast nutrition - potential problems for fermentation

- Spectrum/availability of sugars
- Metal ion bioavailabitiy
- Inhibitory components (e.g. pesticides, cleaning agents)
- Insufficient O_2 (slows growth)
- Vitamin deficiency
- Stressed or starved yeast - impaired nutrient uptake

Yeast nutrition - **SUMMARY**

- Yeasts have relatively simple nutritional requirements
- Attention to yeast nutrition will prevent slow and premature fermentations
- Different strategies adopted for nutrient uptake
- Control of sugar uptake and metabolism in *S. cerevisiae* is very important for industrial fermentation processes
- Stressed yeast has impaired nutrient uptake and fermentation performance