PQI 5888
Fisiologia e Biotecnologia de Leveduras

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[Aula 9]

Yeast Metabolism

(aula síncrona)

(b)

Yeast Metabolism

Based of Graeme Walker´s class

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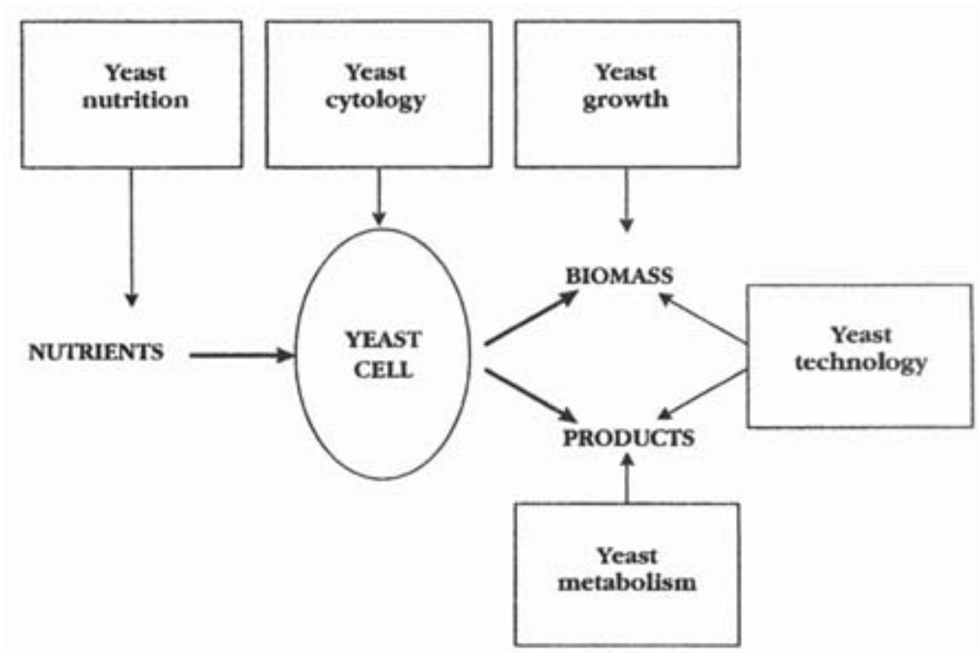
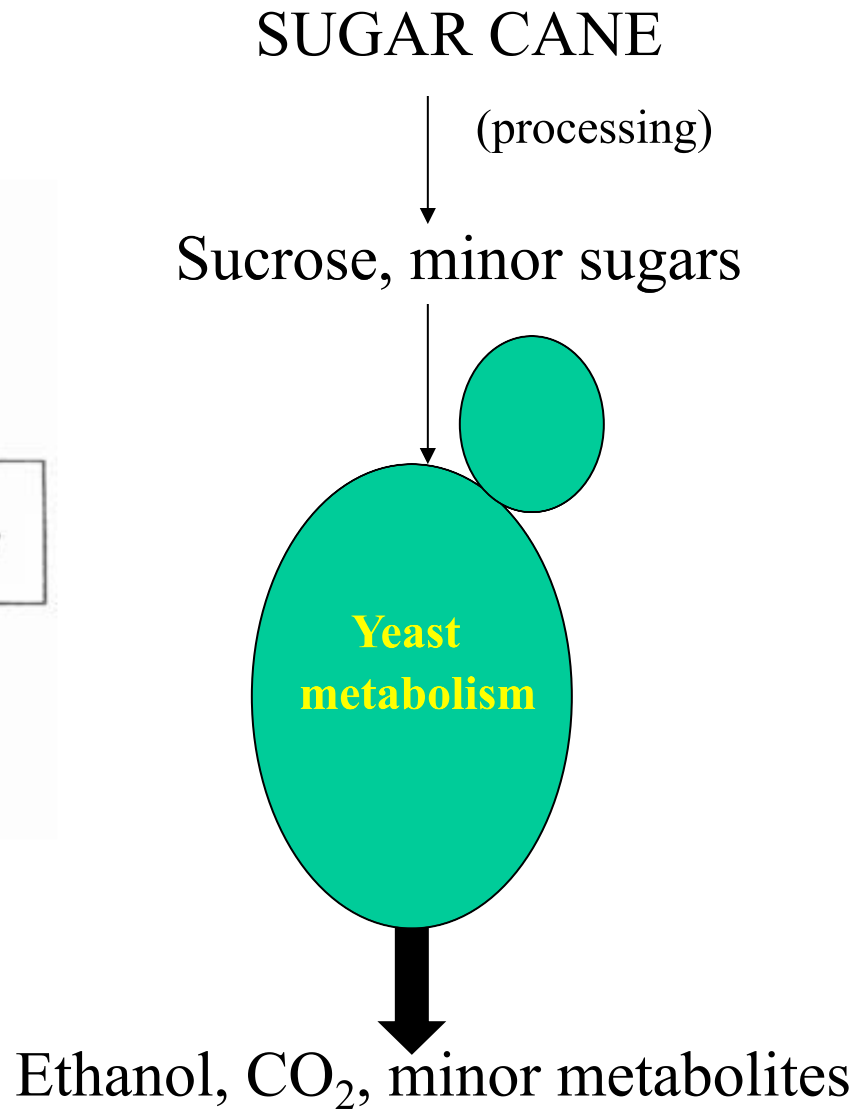
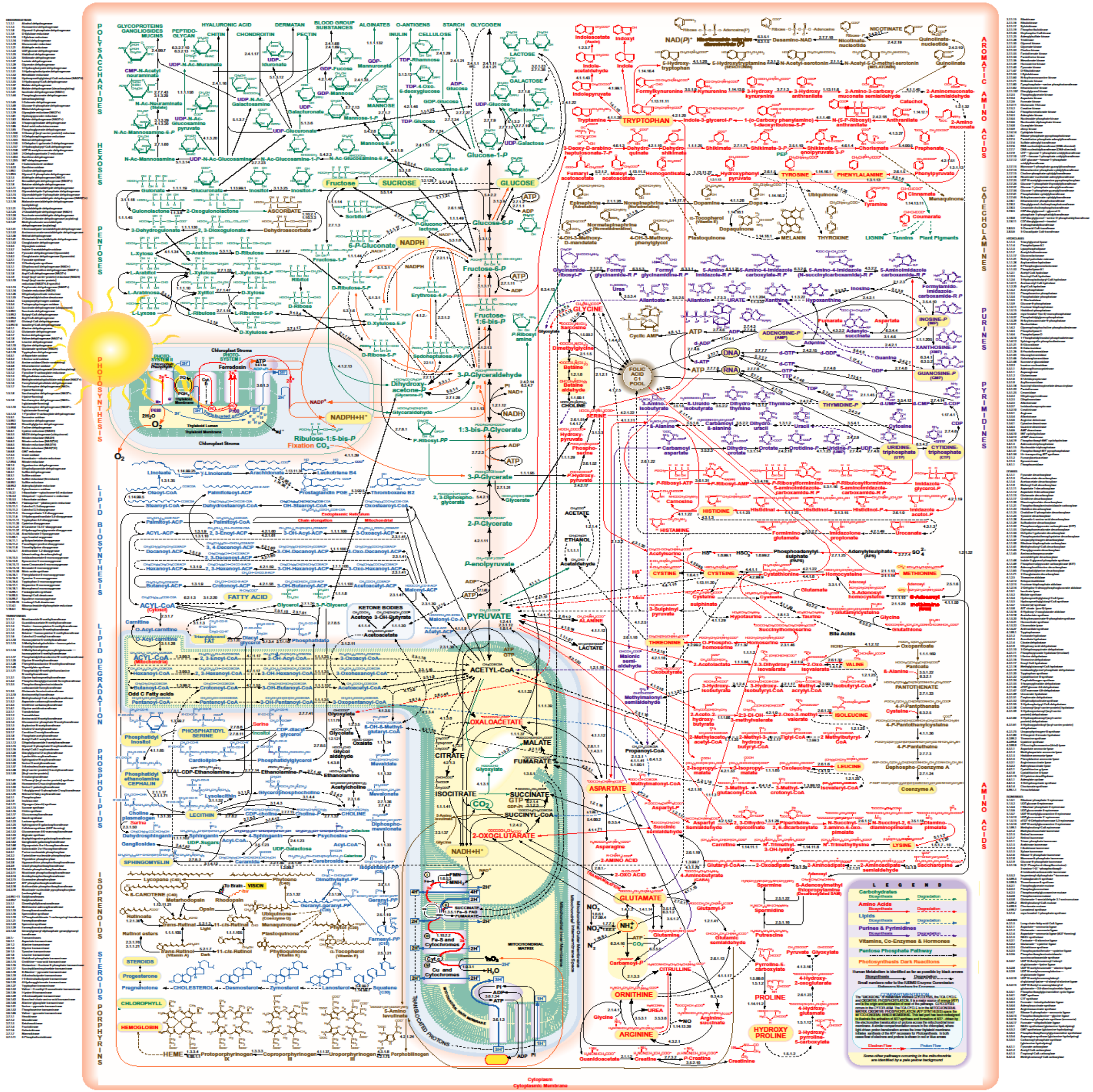
Yeast Physiology and Biotechnology



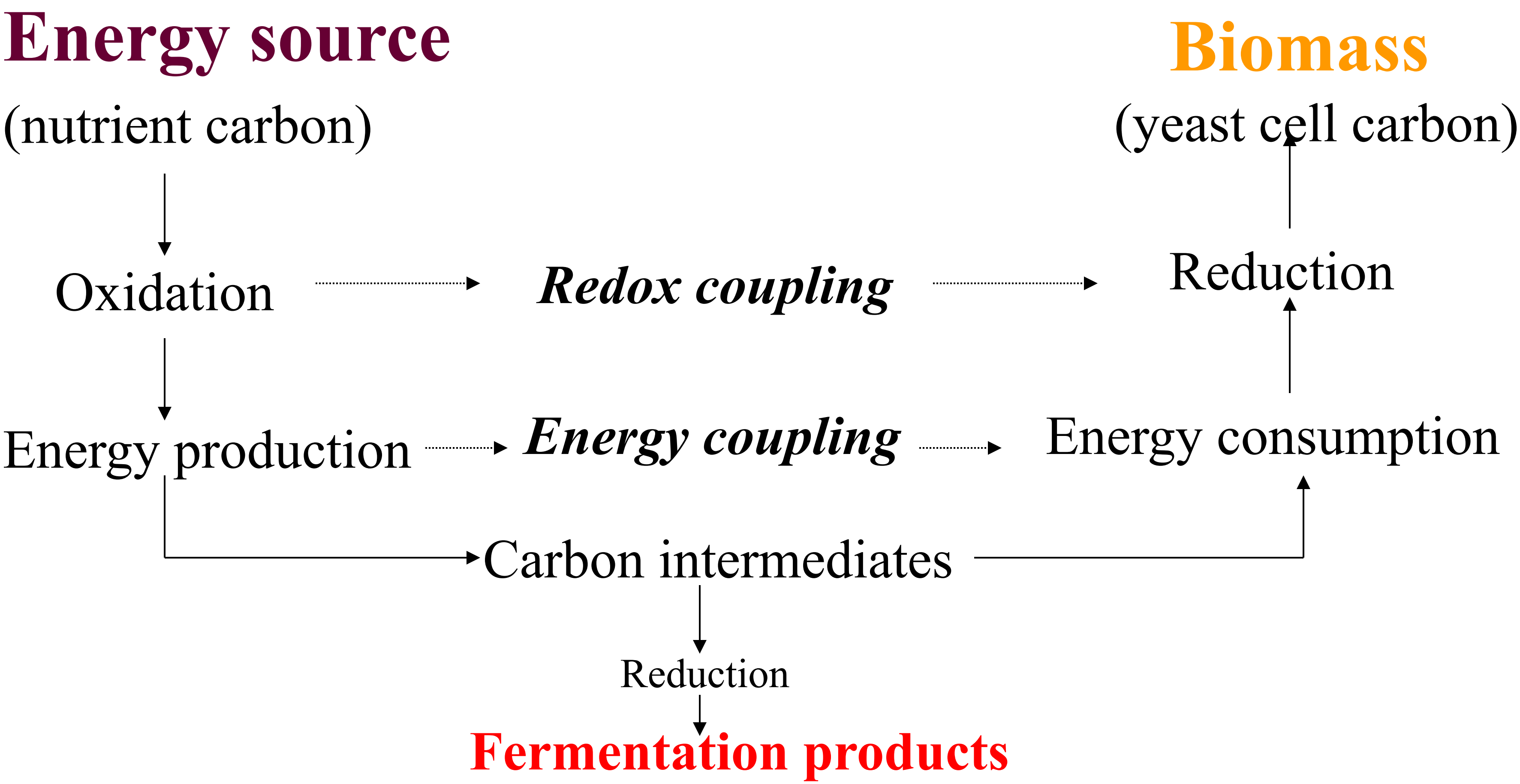


Yeast metabolism!



So how does yeast make alcohol?

Overview of yeast carbon metabolism

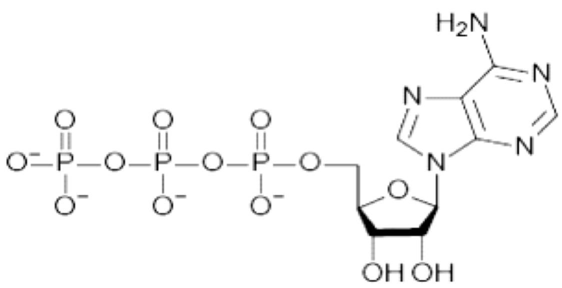


2 Key players in metabolism

ATP

adenosine **t**ri-**p**hosphate

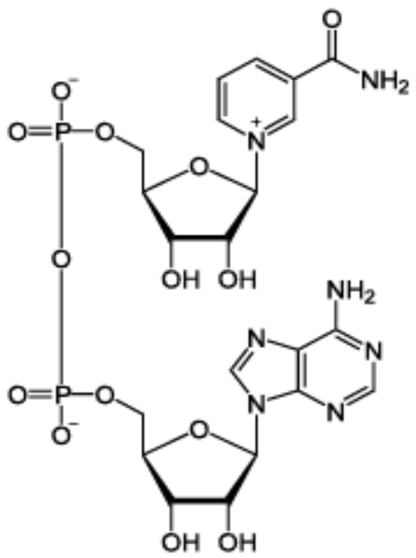
(Biologically useful energy)



NAD

nicotinamide **a**denine **d**inucleotide

(Co-enzyme transferring electrons – cellular oxidation-reduction reactions)



ENERGY COUPLING: facilitated by high-energy phosphate compounds (ATP)

$$ADP + Pi \rightleftharpoons ATP$$

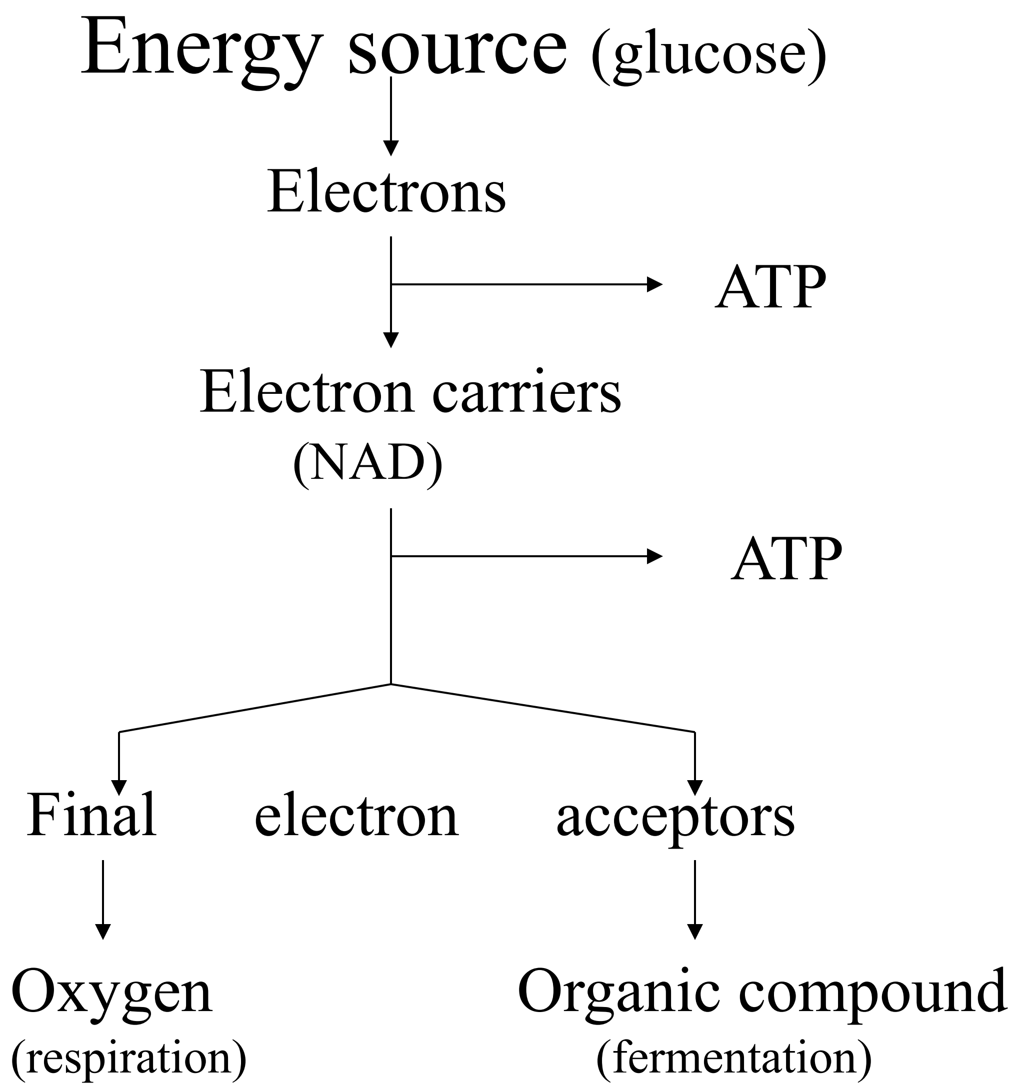
REDOX COUPLING: facilitated by co-enzymes (NAD)

REDUCTION = gain of electrons (H- ions) **OXIDATION** = loss of electrons

Yeast cells need to maintain a balance between the two, called the REDOX balance, and NAD participates in achieving this

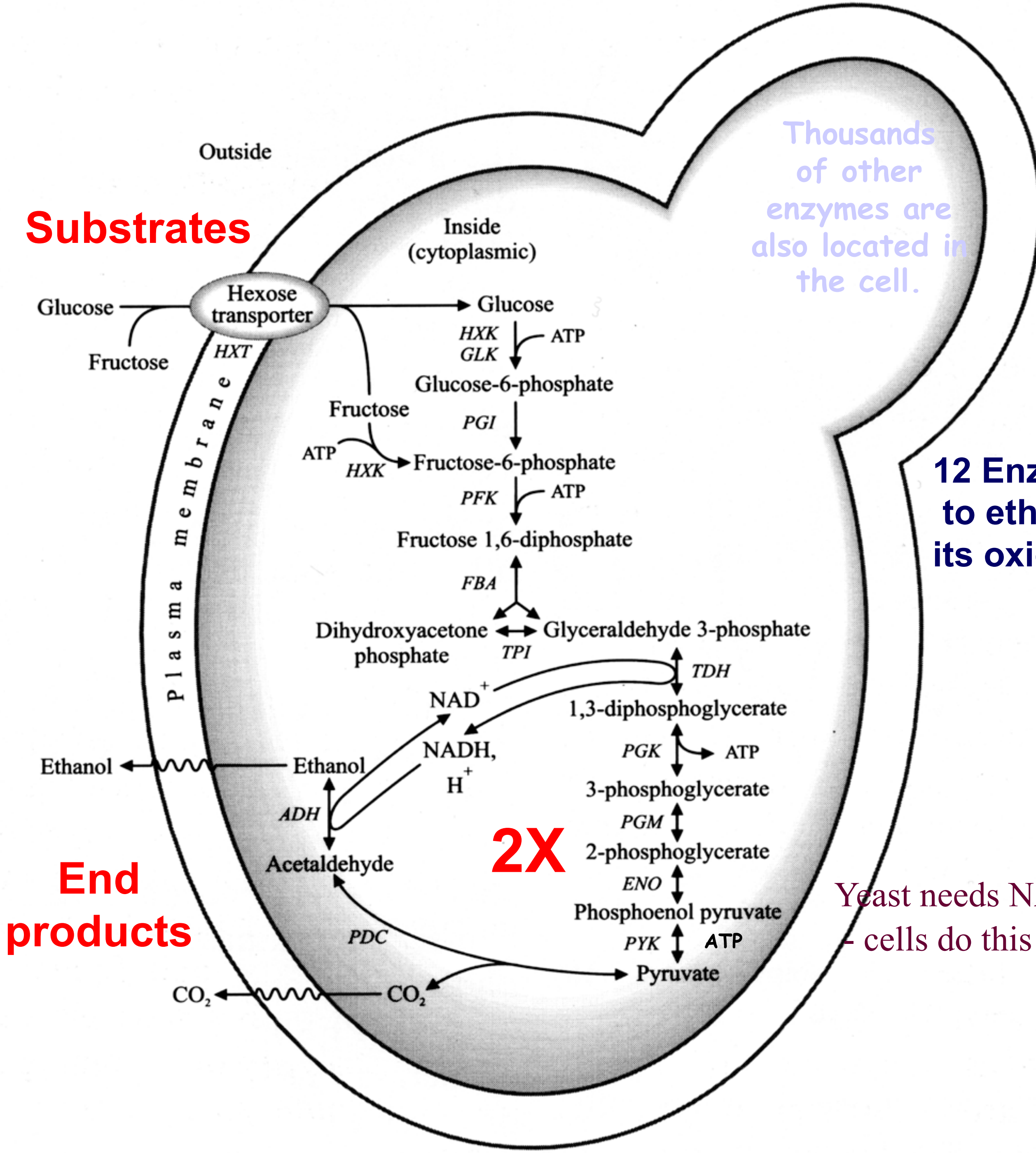
$$NAD^{+} + 2H \rightleftharpoons NADH + H^{+}$$

Summary of electron transfer by yeast



Glycolysis in yeast:

the degradation of sugar with production of energy (ATP) for cell growth



12 Enzymes convert ~90% of glucose to ethanol and CO_2 as yeast controls its oxidation/reduction balance.

Yeast needs NAD to keep glycolysis going
- cells do this by producing alcohol in fermentation

Glycolysis *(EMP Pathway):*

overall equation

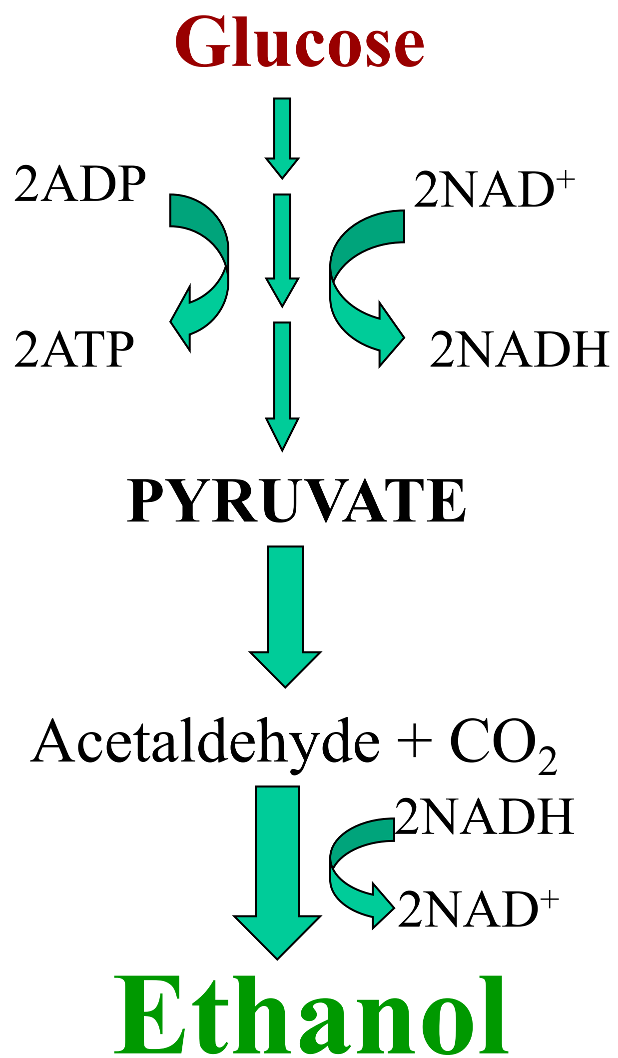
Glucose + 2ADP + 2Pi + $2NAD^+$

↓

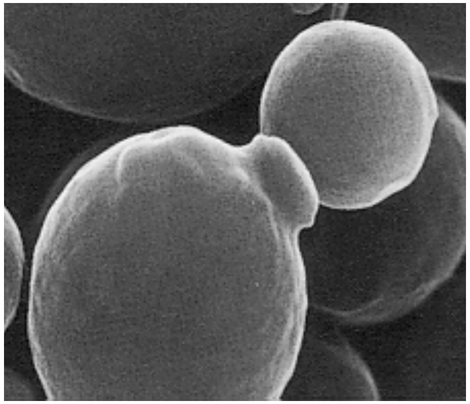
2Pyruvate + 2ATP + $2NADH^+$ + $2H^+$

GLYCOLYSIS & FERMENTATION

2 Key players: NAD (electron-accepting coenzyme) + ATP (biological energy)



YEAST is the (Bio)CATALYST!



Ethanol CO_2, energy production, yeast growth are all COUPLED - proportional to metabolic rate

- As sugar is used, energy (ATP) and pyruvic acid are made (1 glucose->2 pyruvate)
- ATP is used for yeast growth, but is limiting in anaerobic fermentors
- Pyruvic acid is used as a sink for the reduced enzyme cofactor made in glycolysis (NADH)
- Pyruvic acid is converted through acetaldehyde to ethanol using reduced NADH + H^+ and regenerating cofactor NAD^+
- Basically, yeast makes alcohol as it tries to balance its electrons!

Yeast Alcoholic Fermentation

SUGARS

$CH_3COCOOH$ → (1.) $CH_3CHO + CO_2$ → (2.) CH_3CH_2OH

Pyruvate **Acetaldehyde + CO_2** **Ethanol**

Enzyme 1. Pyruvate decarboxylase

Enzyme 2. Alcohol dehydrogenase - regenerates NAD^+

Metabolic biodiversity in yeast

Vast range of substrates, metabolic pathways and products

- **Saccharides** hexoses, pentoses, oligosaccharides
- **Polysaccharides** starch, pectin, inulin, (cellulose?)
- **Hydrocarbons** n-alkanes
- **Alcohols** ethanol, methanol, glycerol, glucitol
- **Organic acids** acetate, citrate, lactate, malate
- **Fatty acids** oleate, palmitate
- **Aromatics** phenol, cresol, quinol, catechol, benzoate

Ethanol from starch

Industrial enzymes | Yeast enzymes (12)

$$(C_6H_{10}O_5)n + (n\text{-}1)H_2O \rightarrow n\ C_6H_{12}O_6 \rightarrow 2n\ CH_3CH_2OH + 2n\ CO_2 \uparrow$$

Starch	Water	Glucose	Ethanol	CO_2
Taking glucose as 100 parts by weight, reactant weights would be				
90	**10**	**100**	**51.1**	**48.9**

The theoretical maximum yield of ethanol from 100 parts of substrate is therefore 56.7% from starch and 51.1% from glucose

Industrial alcohol producers should aim for >90% of this theoretical yield

Can never get 100% because:

- new cells are made (the yeast's objective)
- glycerol, organic acids, higher alcohols, esters and other end products are made
- some losses occur in factory operation
- some sugar is retrograded or reacts in the Maillard reaction
- contamination by bacteria, wild yeasts
- stuck and sluggish fermentations/stress on the yeast

Note!

In addition to the **PRIMARY** fermentation metabolites (ethanol & carbon dioxide),

S. cerevisiae also produces **SECONDARY** fermentation metabolites which are very important in governing flavour characteristics of alcoholic beverages (beer, whisky, cachaça, wine etc).

Many secondary metabolites are produced by yeast NAD-regeneration reactions:

- Fusel alcohols (isopentanol, phenyl ethanol, n-propanol, isobutanol, 2-methyl butanol)
- Vicinal diketones (diacetyl, pentane 2,3-dione)
- Esters (ethyl acetate, isoamylacetate)
- Sulphur compounds (H_2S, dimethyl sulphide)
- Phenolics (4-vinyl guaiacol) etc…

Fermentation nutrients

(Sugars, amino acids, etc.)

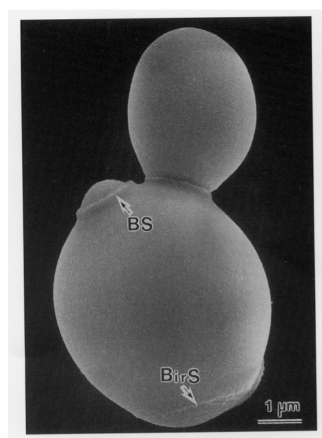
More yeast!

Yeast autolytic compounds

Phenols

Sulphur compounds

Aldehydes & ketones



Esters

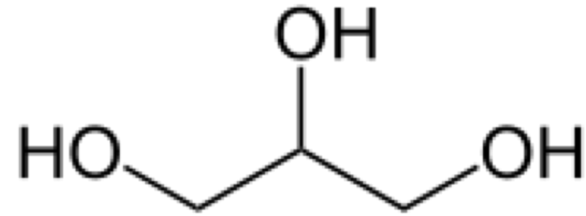
Acids (organic & fatty)

Alcohols (ethanol & fusel oils)

CO_2

Glycerol

Yeast also makes Glycerol



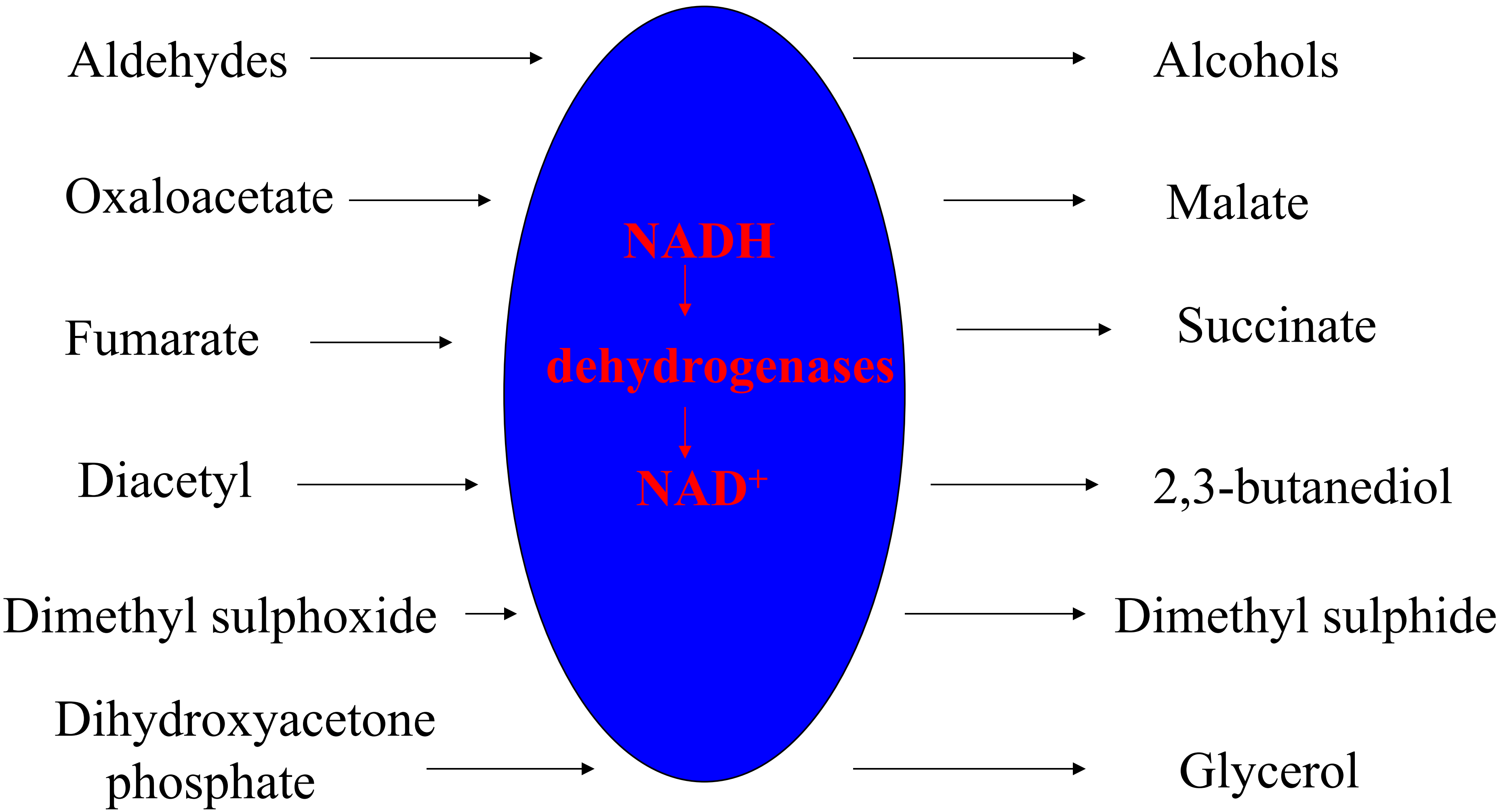
- Glycerol is made due to the accumulation of excess NADH + H^+
  - used to convert dihydroxyacetone phosphate to glycerol
- Stressed cells also accumulate glycerol (protectant)

  (eg. Caused by osmostress, acids – increased pH inside yeast leads to excess NADH which diverts glycolysis to glycerol)
- Glycerol over-production means less ethanol

  X bad news for fuel alcohol companies: glycerol can be as high as 10% of ethanol
- Reduce glycerol!

  Minimise yeast (osmo)stress. Use GM yeasts (eg. Pronk et al)

Regeneration of NAD^+ by fermenting yeast



Respiration & Fermentation

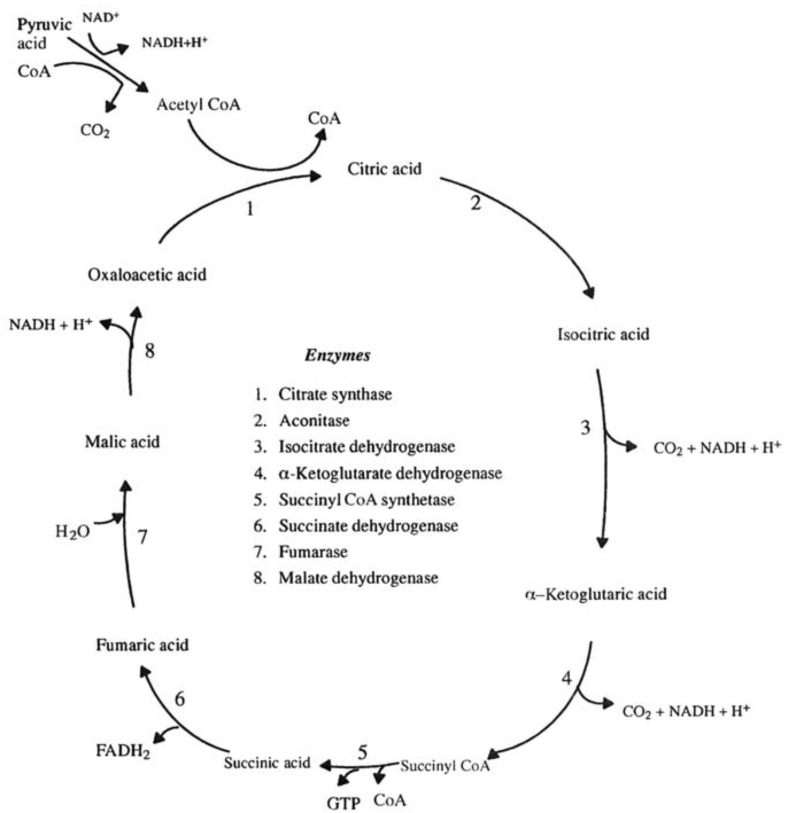
→ ***RESPIRATION*** (uptake of oxygen to generate energy)

Under aerobic conditions, pyruvate is oxidized to CO_2 and water by specific metabolic routes (TCA cycle). This uptake of O_2 generates large amount of energy (38ATP).

→ ***FERMENTATION*** (conversion of sugar to alcohol in absence of oxygen)

Under anaerobic conditions, pyruvate is converted to CO_2 and acetaldehyde which in turn will form ethanol. Only 2 ATP generated.

The **citric acid cycle** is referred to as amphibolic since the pathway performs both catabolic and anabolic functions.



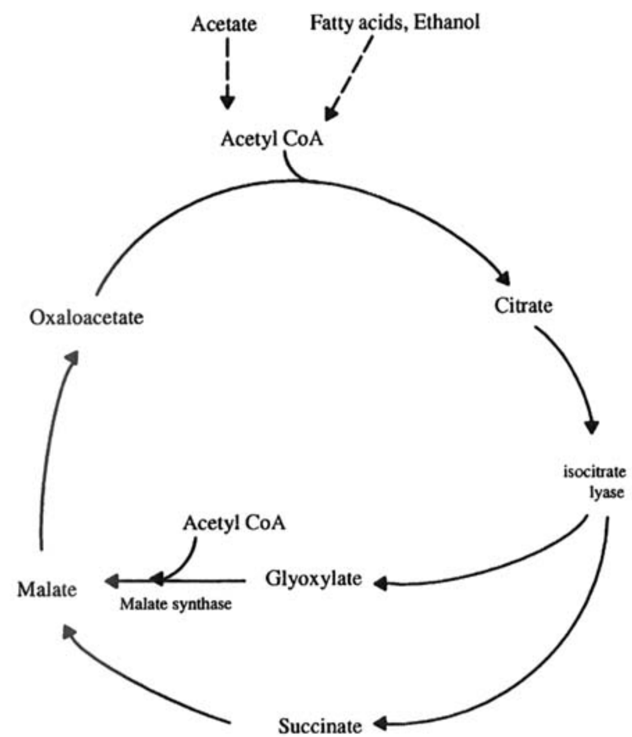


Figure 5.7. The glyoxylate cycle.

Notes

Not only glucose can be respired by yeasts; in fact, a greater array of carbon sources can be respired than fermented.

Substrates which are **respired** by yeast cells include:
pentoses (e.g. xylose),
sugar alcohols (e.g. glycerol),
organic acids (e.g. acetic acid),
aliphatic alcohols (e.g. methanol, ethanol),
hydrocarbons (e.g. n-alkanes) and
aromatic compounds (e.g. phenol)

Overview of yeast sugar metabolism

Sugars

glycolysis

PYRUVATE

$-O_2$

$+O_2$, low sugar levels

Fermentation

Respiration

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 2ATP$$

(glucose) (ethanol) (carbon dioxide) (energy)

Alcohol, CO_2

Yeast biomass

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 38ATP$$

(glucose) (oxygen) (carbon dioxide) (water) (energy)

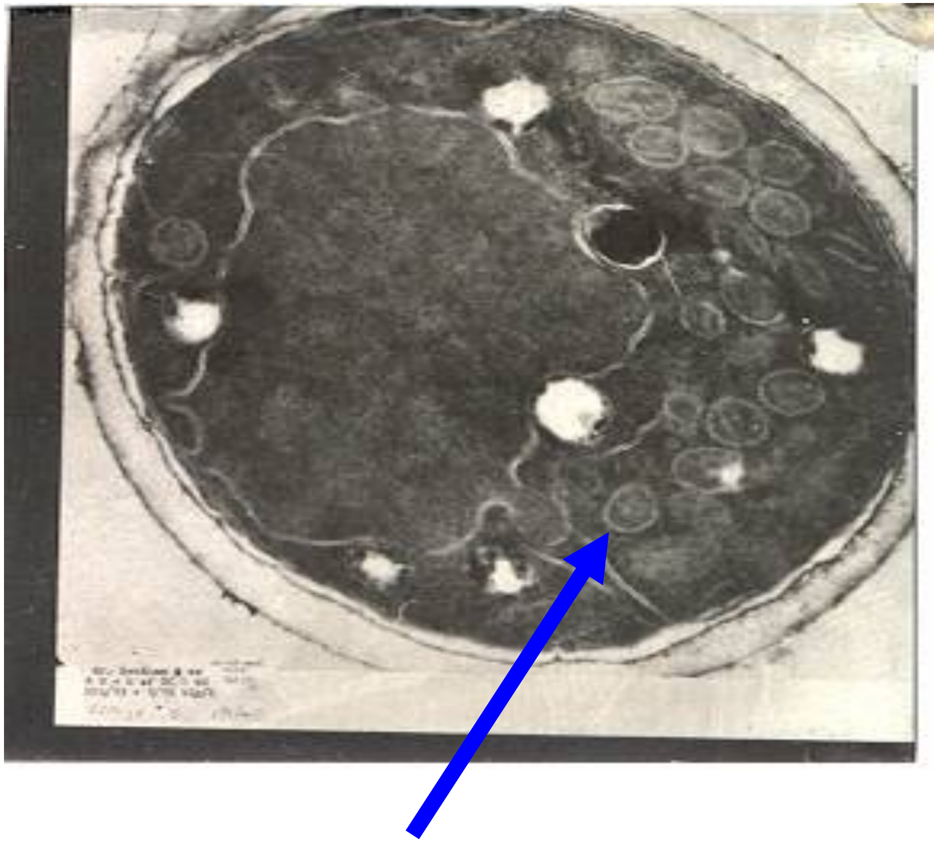
Regulation of Sugar Catabolism

Fermentation or Respiration?

S. cerevisiae can ferment or respire depending on availability of:
OXYGEN and GLUCOSE

Reticular mitochondria

FERMENTING CELLS



Vesicular mitochondria

RESPIRING CELLS

Biochemical pathways in yeasts may be regulated at various levels:

- **enzyme synthesis** (e.g. induction, repression and derepression of gene expression)
- **enzyme activity** (e.g. allosteric activation, inhibition or interconversion of isoenzymes)
- **cellular compartmentalization** (e.g. mitochondria1 localization of respiratory enzymes)

The following discussion will focus primarily on **external factors** (carbon source and oxygen) which influence respiratory and fermentative metabolism in yeasts of biotechnological significance

The *Crabtree effect*

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

- The Crabtree effect appears to be correlated with suppression of respiration by high 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

Explanation of the Crabtree Effect?

Glucose acting as repressor/inactivator of yeast respiration

SHORT **TERM**
Crabtree effect
(seconds)

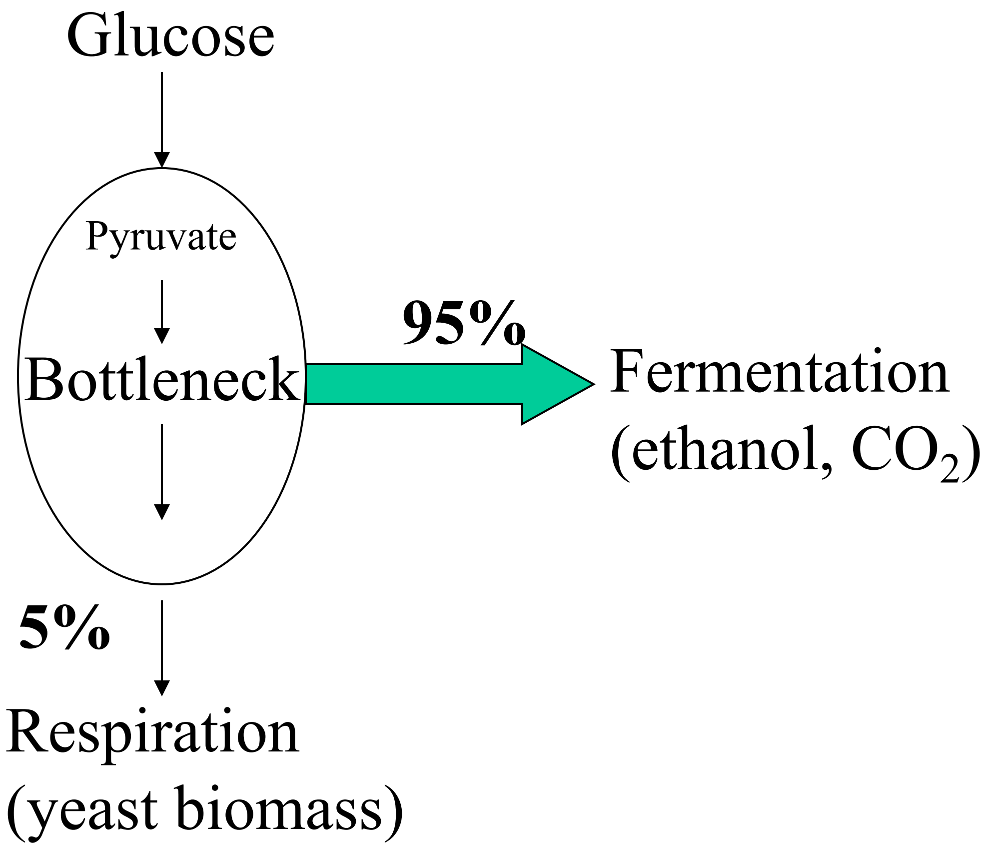
→ Glucose-induced INACTIVATION (degradation of respiratory enzymes)

LONG **TERM**
Crabtree effect
(hours)

→ Glucose-induced REPRESSION (synthesis of respiratory enzymes repressed)

Limited respiratory capacity of *S. cerevisiae*?

Aerobic metabolism of glucose by yeasts showing the Crabtree effect is termed
Respiro-fermentative



Industrial significance of the ***Crabtree effect***?

- If you want to produce as much yeast as possible, respiration is desired (e.g. during propagation), and alcoholic fermentation is undesired
- Therefore, keep O_2 levels high, but keep sugar levels low (to avoid Crabtree effect)
- This can be achieved by ***fed-batch*** systems to control sugar feed to growing yeast

Industrial growth of *S. cerevisiae*

Fermentation: BATCH, ANAEROBIC

↓

Alcohol

2ATP/molecule glucose - High ethanol yield – No sterol & unsaturated fatty acid synthesis

Cell yield = 0.05-0.1 g/g glucose *[Biomass yield of ~50 million cells/ml]*

Propagation: FED-BATCH, AEROBIC

↓

Yeast

38ATP/molecule glucose - Low (zero) ethanol yield - Sterol & unsaturated fatty acid synthesis

Cell yield = 0.5 g/g glucose *[Biomass yield of >200 million cells/ml]*

Fed-batch yeast propagations

-Nutrient fed incrementally

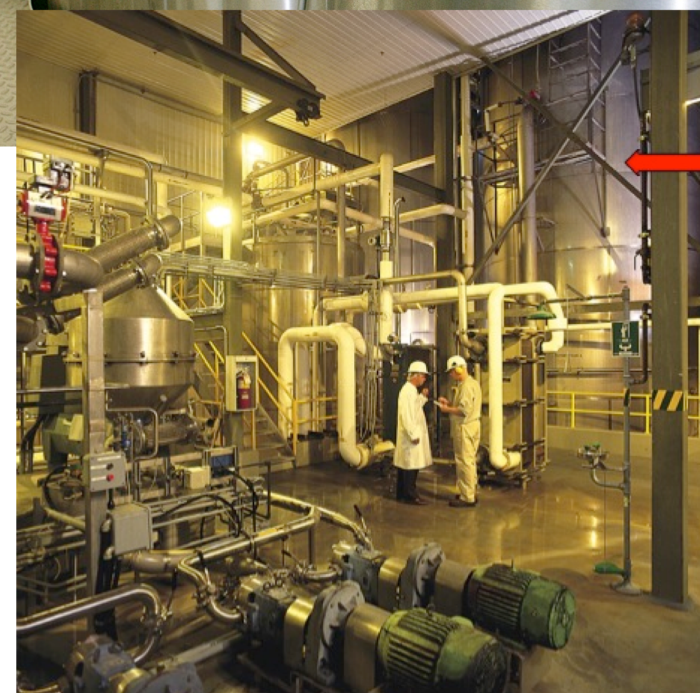
(exponentially increasing rate which parallels yeast growth)

-Extends exponential phase

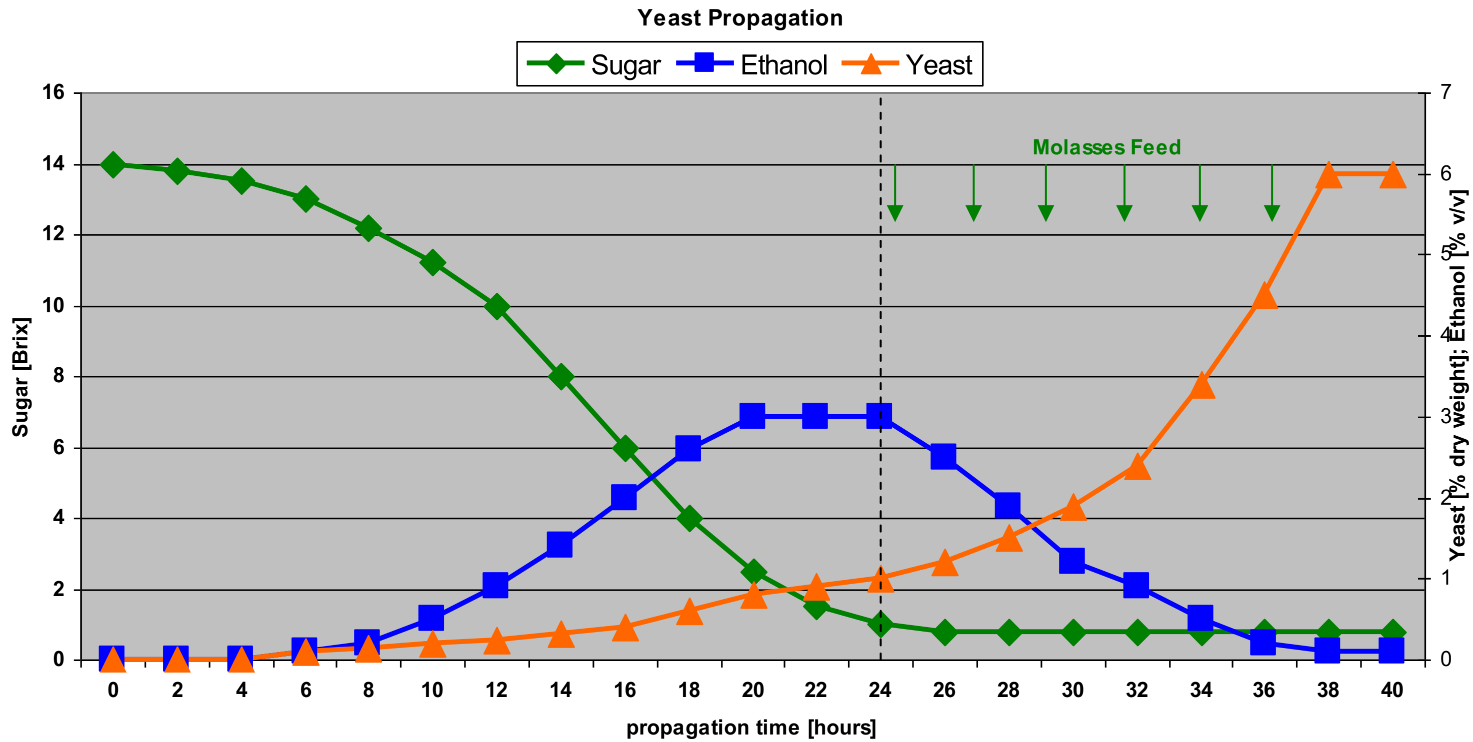
(low μ, but high cell densities)

-Highly aerobic

(oxygen transfer coefficients, K_La of 0.07-0.1sec^{-1})



Batch v. Fed-Batch



- ***Batch propagation***
  - All nutrients are in fermenter
  - Alcohol and little yeast is produced
- ***Fed Batch***
  - Carbohydrates and nitrogen are added at a certain flow rate
  - Aerobic fermentation => Alcohol decreases, yeast production

Effect of yeast growth rate

Slow Growth: More mature cells
Higher carbohydrate
Higher yield
Lower initial activity
Better stability

Rapid Growth: Less mature cells
Higher protein and enzymes
Lower yield
Higher initial activity
Lower stability

The Pasteur effect

The Pasteur effect relates **oxygen** with the **kinetics of yeast sugar catabolism** and states that under anaerobic conditions, glycolysis proceeds faster than it does under aerobic conditions

Only observable when glucose **concentrations are low** (yeast-dependent)

The Custers effect

This may be defined as the transient **inhibition** of fermentation by **anaerobiosis** and is observed when small levels of oxygen abolish this anaerobic inhibition of fermentation (*Brettanomyces* and *Dekkera* spp.)

The Custers effect is therefore explained on the basis of a disturbed redox balance in the absence of oxygen

The Kluyver effect

Several yeasts which can ferment glucose anaerobically are able aerobically to assimilate, but not ferment, other sugars such as galactose and certain disaccharides.

The biotechnological relevance of the Kluyver effect lies in the production of yeast biomass or heterologous proteins on inexpensive disaccharide-based growth media (cheese whey or molasses)

SUMMARY

- 2 key players in yeast carbon metabolism: **ATP & NAD**
- Alcohol is produced in fermentation as yeast cells maintain Redox balance
- *S. cerevisiae* can respire and ferment sugars, depending on prevailing conditions **(O_2 & Glucose)**
- The expression of the *Crabtree Effect* necessitates fed-batch propagation when producing yeast in industry