Physiology of Ethanol-Producing Yeasts

The Alcohol School

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& Abertay University

WIRSPA

West Indies Rum & Spirits Producers' Association Inc.

Ethanol Technology Institute

Place: Caribbean Distilling Seminar, St Lucia

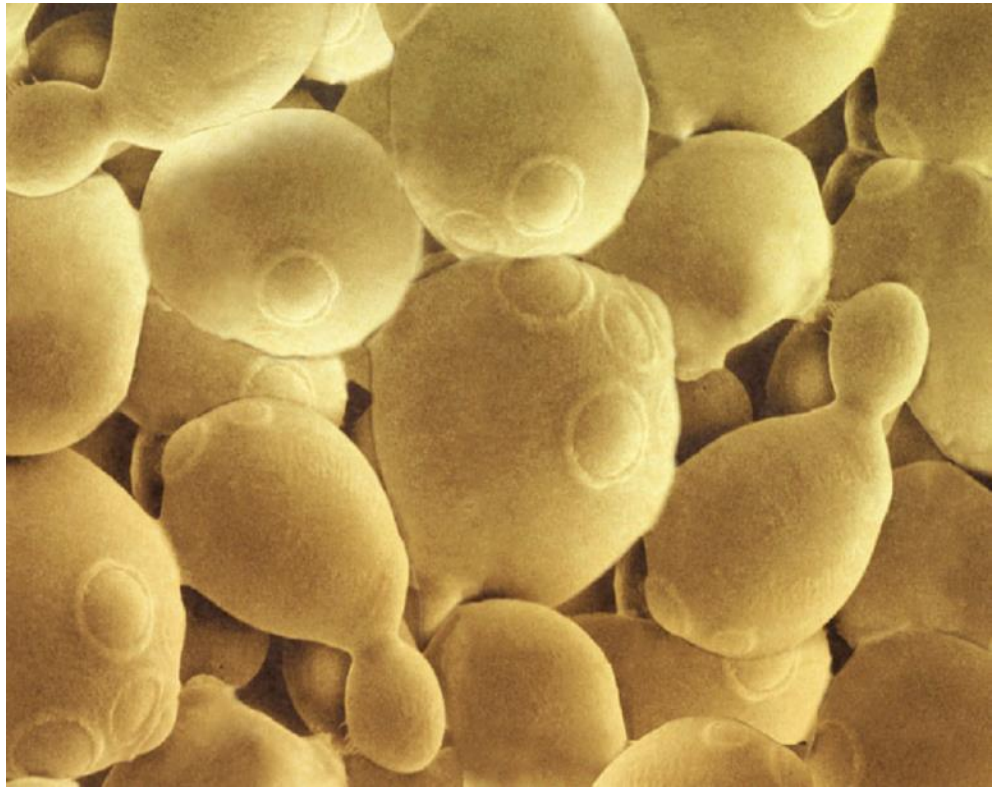
Date: April, 2024

Outline

- **Introduction:** *Saccharomyces cerevisiae*

- **Yeast – some basic physiology**
  - Cytology: structure/function relationships
  - Nutrition & Growth
  - Metabolism during fermentation
  - Flavour metabolites from yeast

- **Conclusions**



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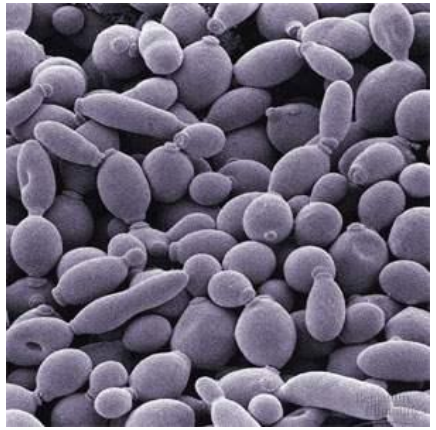
Yeast is the Key to Successful Alcohol Production

Raw materials



FERMENTATION

Distillation

Distilled Spirits

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Yeast & Fermentation topics discussed in The Alcohol School

- **Yeast physiology**

– how do distiller's yeasts grow and conduct fermentation?

- **Yeast nutrition**

– does your fermentation medium have everything yeast needs?

- **Yeast production & propagation**

– how do we provide enough active yeast for distillery fermentations?

- **Yeast improvements**

– what new and exciting strains are available, what can they do?

- **Problem fermentations!**

– how to tell when things go wrong, and how to fix them?

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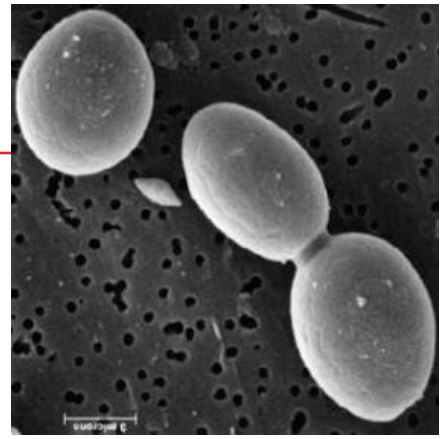
YEASTS – *What are They*?



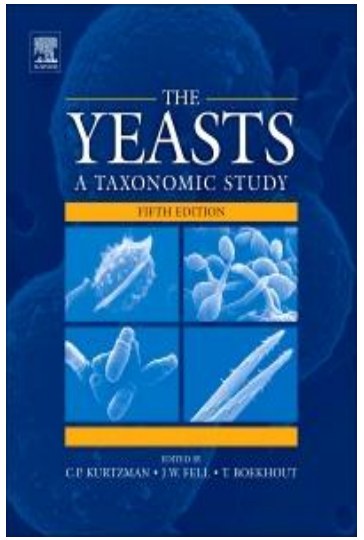
- Yeasts are UNICELLULAR FUNGI
- Yeasts are EUKARYOTIC MICROORGANISMS

  [Bacteria are PROKARYOTES]
- Most industrial yeasts (brewing, baking, winemaking, distilling, bioethanol etc.) are

Saccharomyces cerevisiae

~2400 Yeast species known

Other yeasts:
Kluyveromyces marxianus,
Schizosaccharomyces pombe,
Dekkera bruxellensis,
Candida utilis,
Saccharomyces bayanus
etc. etc….



Which strain of *Saccharomyces cerevisiae* Do I Choose?

Beer

Wine

Spirits

Bread

Ethanol

Specific characteristics of fermentation products are yeast strain dependent!

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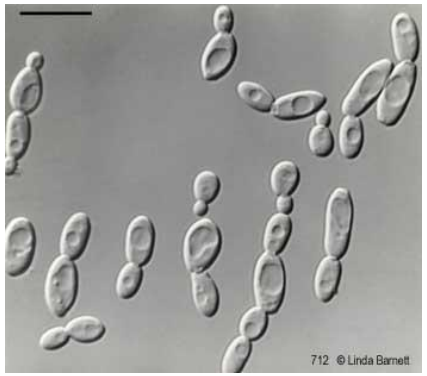
Other Distiller's *Yeasts*?

- ***Schizosaccharomyces pombe***

  (Rum fermentations, some whisky)

- ***Kluyveromyces marxianus***

  (Cheese whey lactose fermentations)

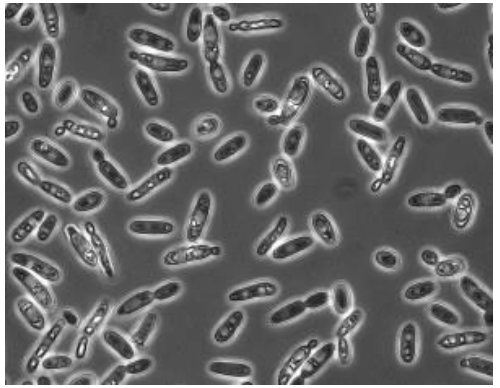


- Wild (contaminant) yeasts:

  Brettanomyces, Pichia,

  Candida, Torulaspora etc.

  & **WILD *S. cerevisiae***



What Makes a Good Distilling Yeast?

- Rapid & complete fermentation of available sugars
- Good congener profile (if desired)
- General stress tolerance (ethanol, pH, temp, osmotic, microbial)
- High viability (during propagation, storage, rehydration, fermentation)
- Non-flocculent
- GM?

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- **Introduction:** *Saccharomyces cerevisiae*

- **Yeast – some basic physiology**
  - **Cytology: structure/function relationships**
  - Nutrition
  - Growth during fermentation
  - Metabolism during fermentation
  - Flavour metabolites

- **Conclusions**

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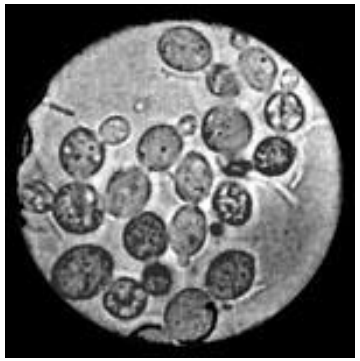
What Do Yeasts Look Like?

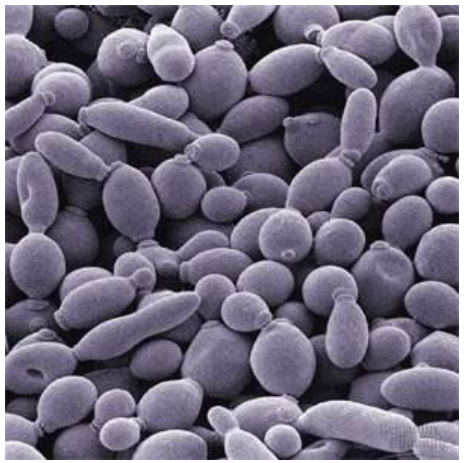
Cake, dried & cream Yeast)

Yeast Colonies
(agar Petri dish)

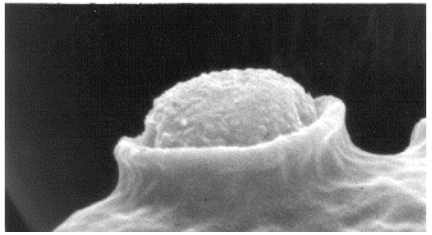
Top-fermenting Yeast
(ale brewery)



Yeast cells
(light microscope)



Yeast Budding
(scanning electron microscopy)



Yeast Bud Scar

(Rich in a material called **chitin**)

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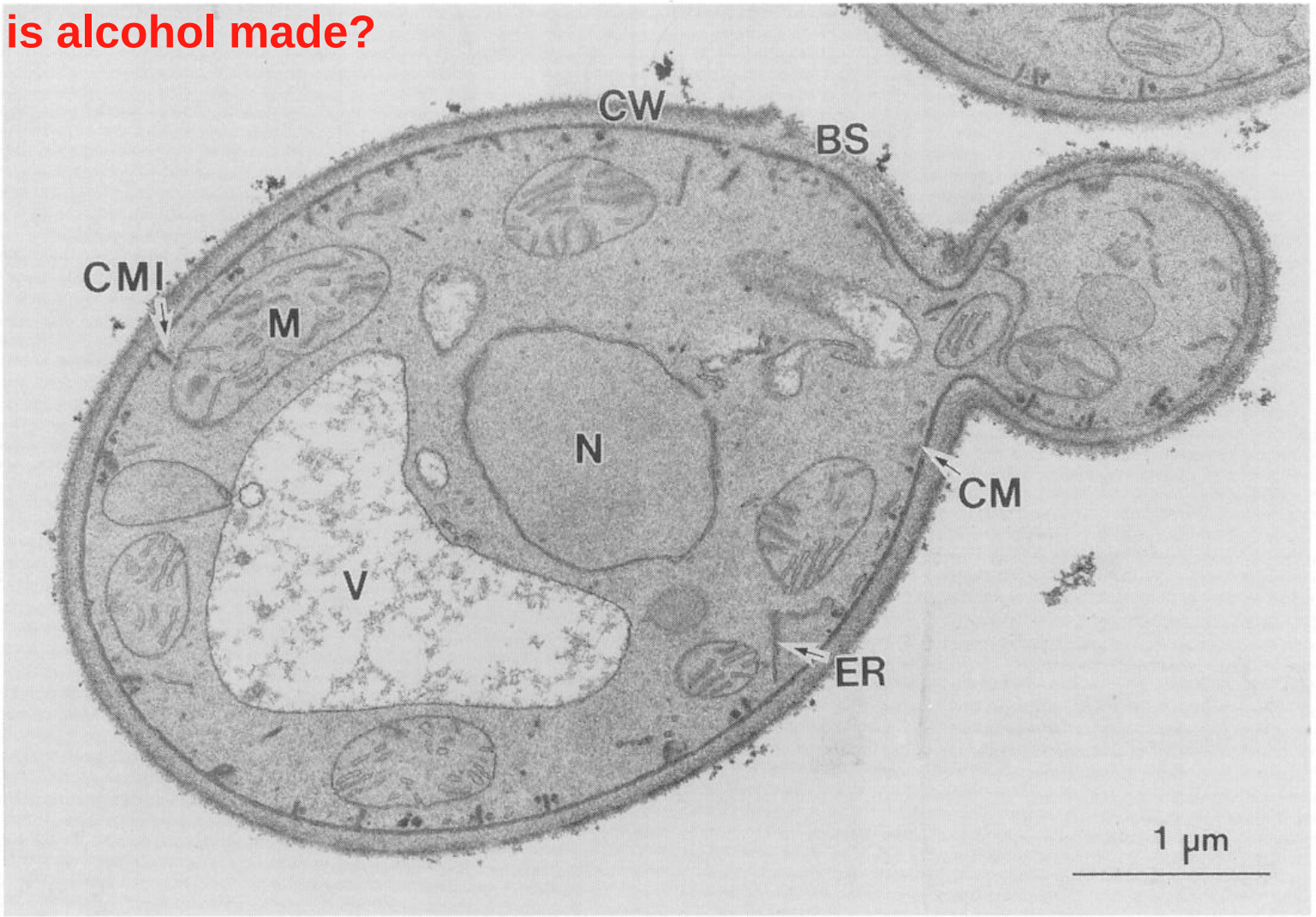
Sub-cellular ultrastructure of *S. cerevisiae*

(electron microscope picture)

Q. Where is alcohol made?



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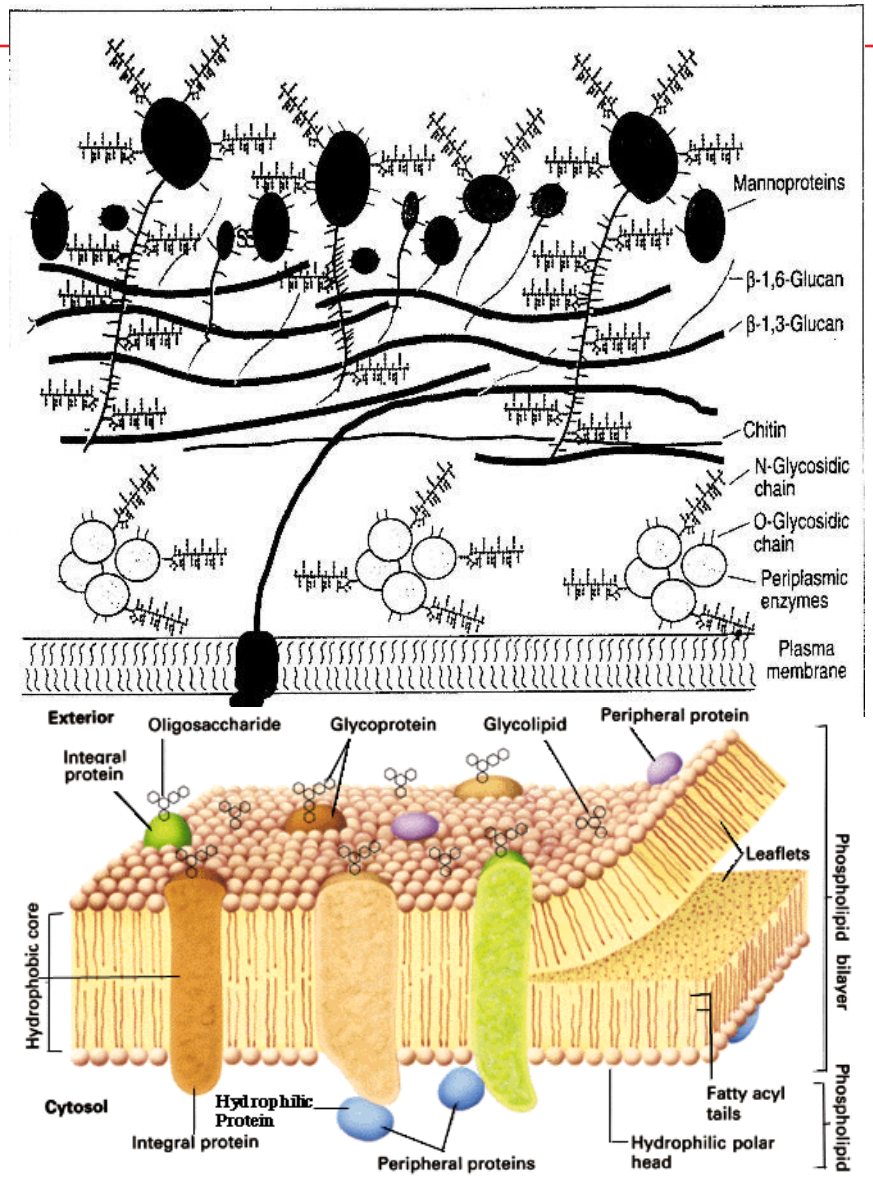
Yeast Cell Envelope



Role of Cell Wall

- Physical protection
- Shape maintenance
- Some enzymes (e.g. invertase)
- Cation binding
- Flocculation

Role of Cell Membrane

- Nutrient uptake
- Metabolite secretion
- Stress resistance

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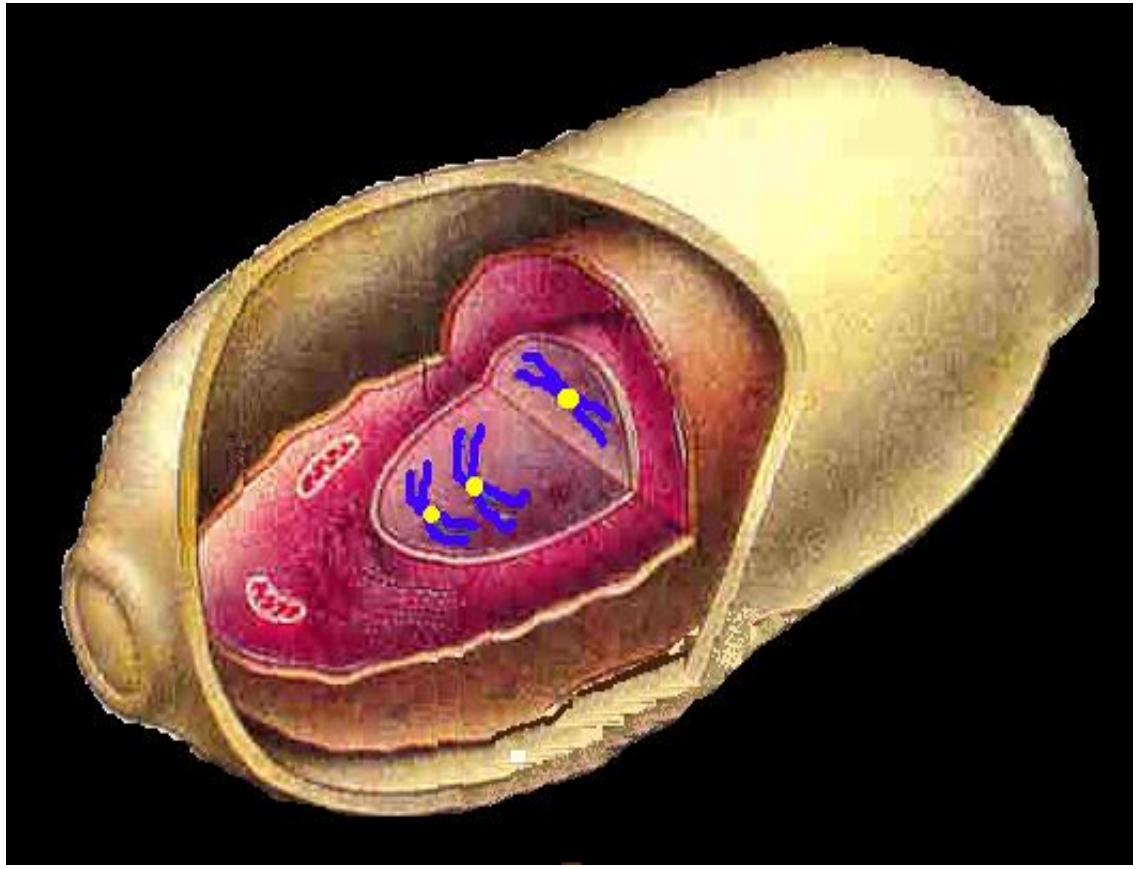
The Yeast Nucleus (containing genes within chromosomes)

It divides when the cell divides to ensure transfer of genetic material to daughter cells (when chromosomes segregate at mitosis)



- *Haploid* cells of *S. cerevisiae* contain 1**6 chromosomes**
- *Diploid* strains have 2 copies of these chromosomes
- Many industrial strains are *Polyploid* – multiple chromosomes

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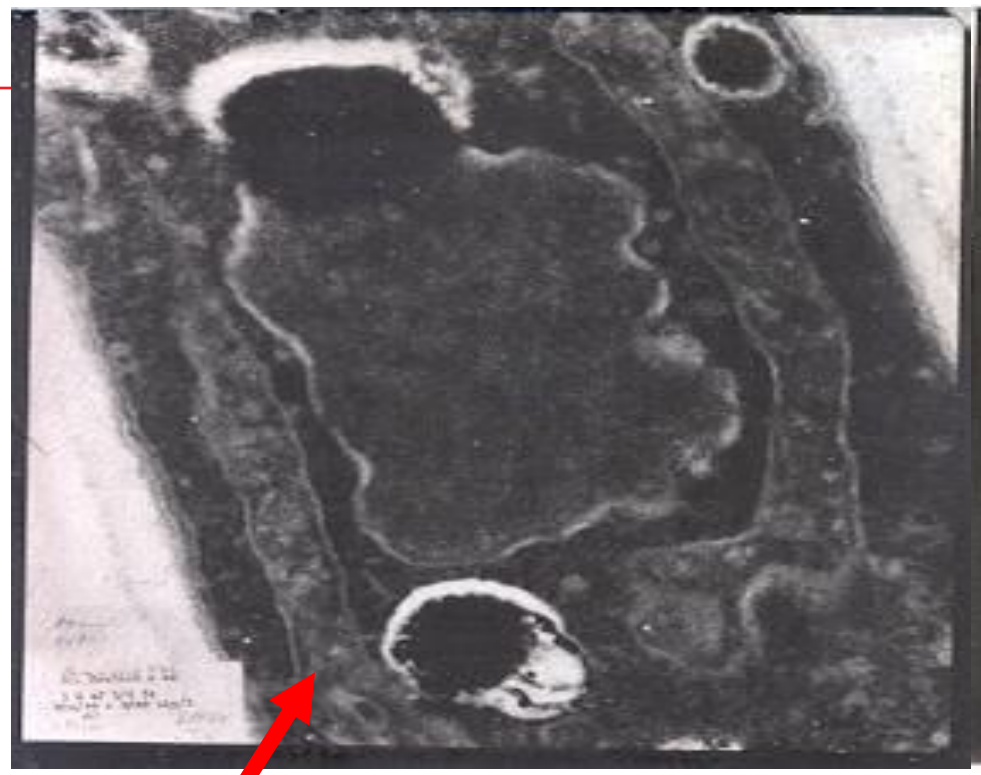
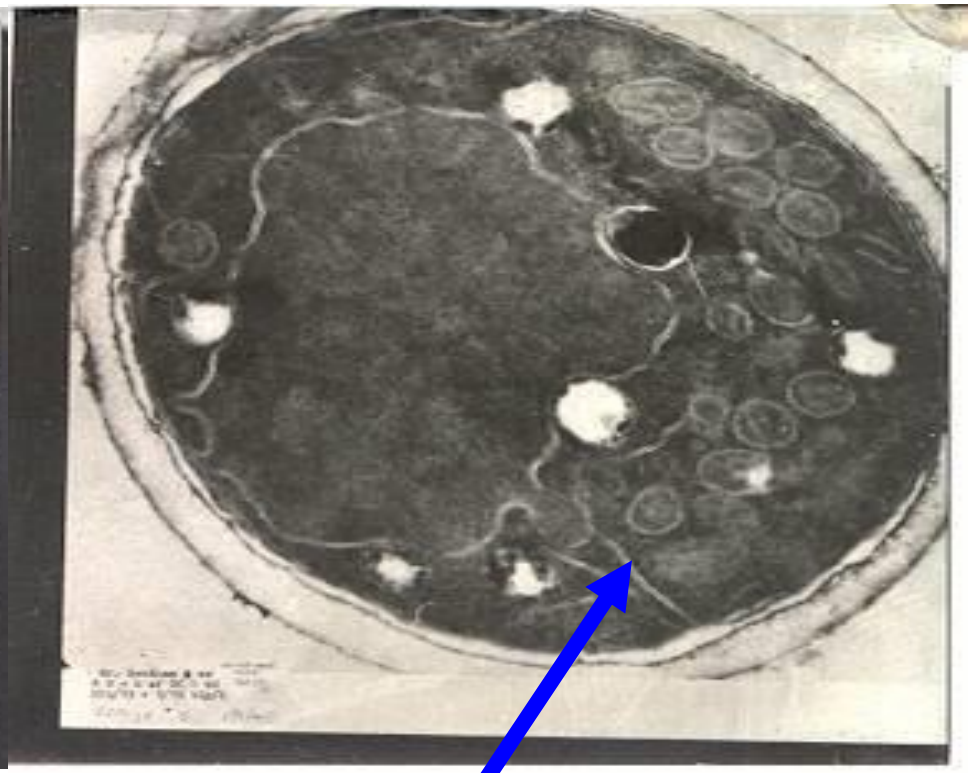
Yeast MITOCHONDRIA





Mitochondria **OFF** (no ATP)

FERMENTING CELLS

Mitochondria **ON** (lots of ATP)

RESPIRING CELLS

NOTE: Mode of metabolism depends on availability of OXYGEN & GLUCOSE
(basis of ***Crabtree Effect***)

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The *Crabtree Effect*

- *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 yeasts can obtain energy by ***respiration***

  But sugar must be very low eg. <0.2g/l glucose

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

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- **Introduction:** *Saccharomyces cerevisiae*

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  - Growth during fermentation
  - Metabolism during fermentation

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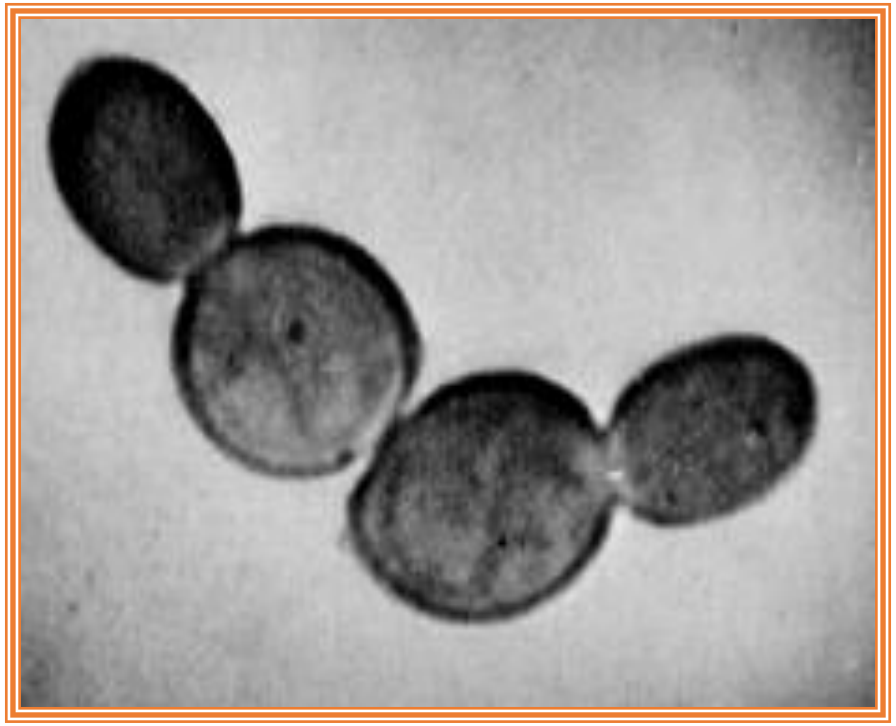
Nutrients for Yeast Growth & Metabolism

- **Carbohydrates**
- **Nitrogen sources**
- **Vitamins**
  Minerals
- **Oxygen** (special roles for alcohol fermentation)



NOTE: ***poor yeast nutrition = poor fermentation***

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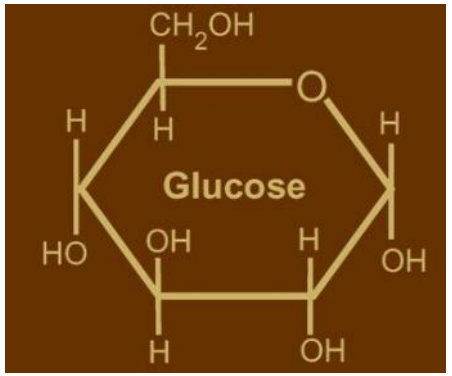
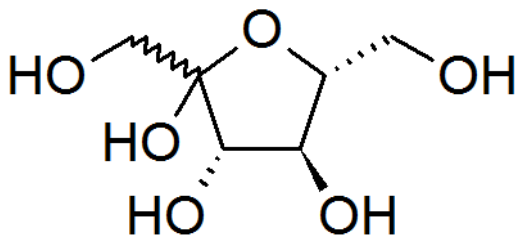
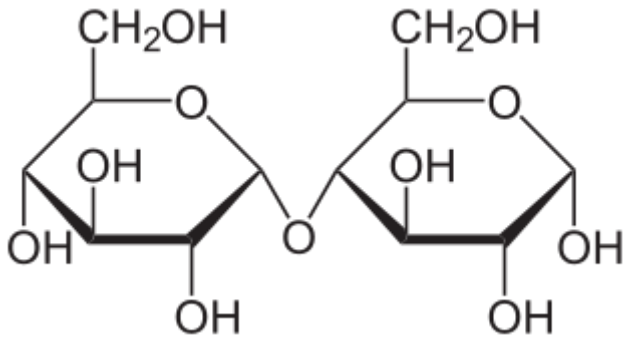
Main Sugars Fermented by *S. cerevisiae*



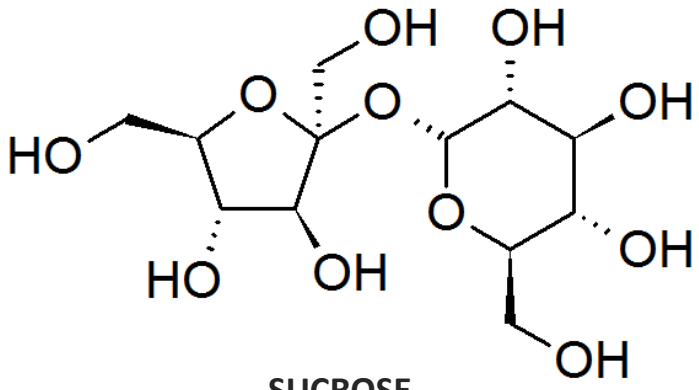
Glucose is the primary, and preferential, source of energy and basic carbon backbone for yeast growth



FRUCTOSE



MALTOSE



SUCROSE

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Yeast Carbohydrate Sources for Ethanol Fermentations







Ethanol

Ideally with >90% conversion efficiency

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Question:

Do industrial fermentation feedstocks always provide the correct balance of nutrients for yeast to conduct efficient fermentation?

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Problem Fermentations!

- **Spectrum/availability of sugars**
- **Low Yeast Available Nitrogen, YAN** (>150ppm)
- **Metal ion bioavailability** e.g. Low Zn, excess Ca, insufficient Mg
- **Insufficient O_2**
- **Vitamin deficiency**
- **Inhibitory components** e.g. acids, mycotoxins, cleaning agents
- **Stressed yeast** - impaired nutrient uptake

Poor nutrition, contamination, & stress may result in stuck/sluggish fermentations

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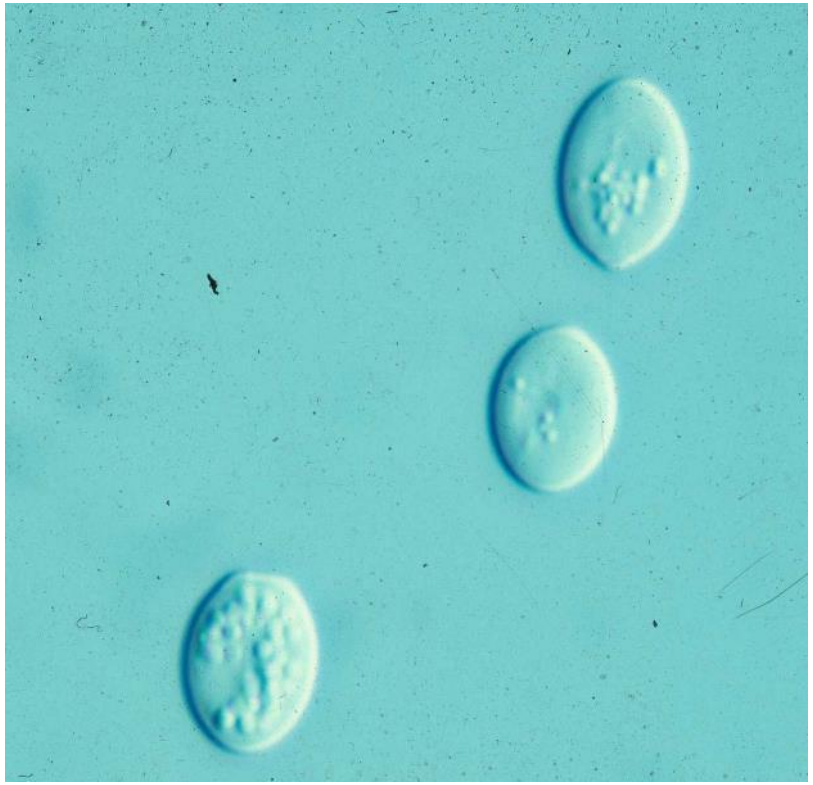
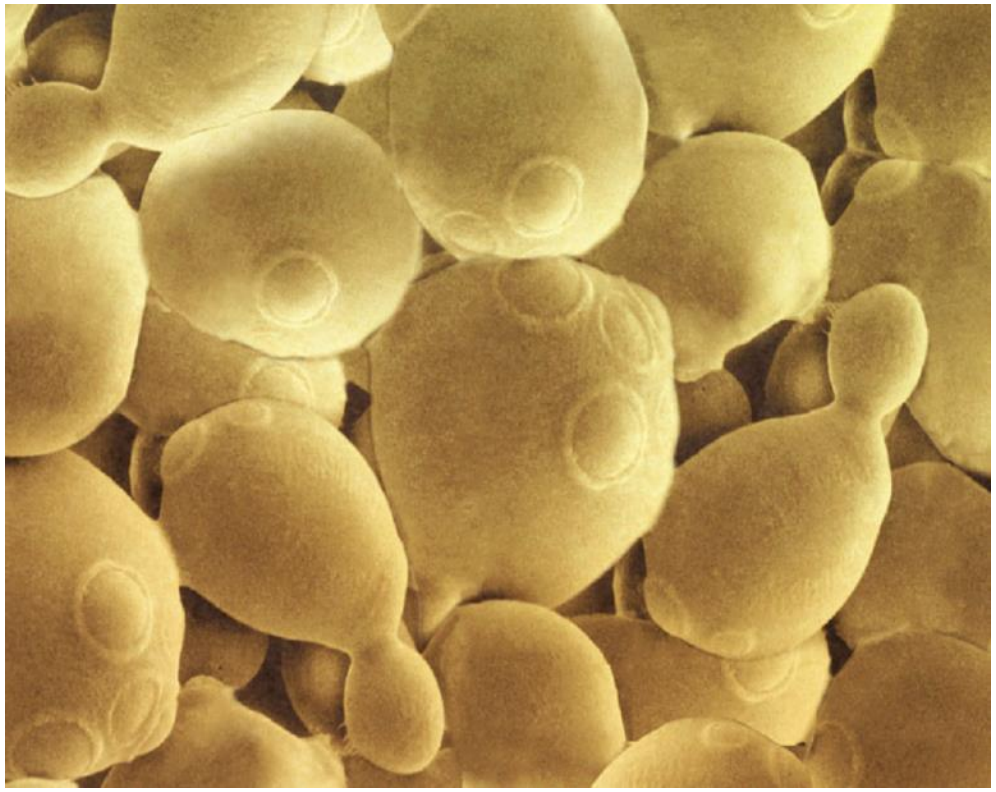
Budding in *Saccharomyces cerevisiae*

Non-budding cells



Budding cells



Q. Which form do we want in a fermentor?

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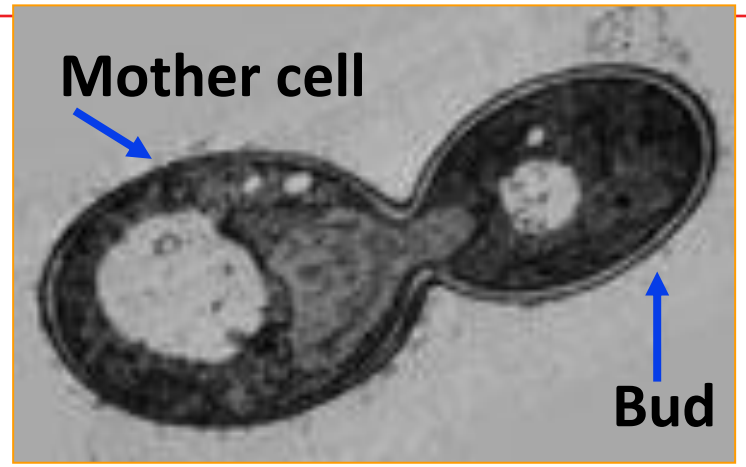
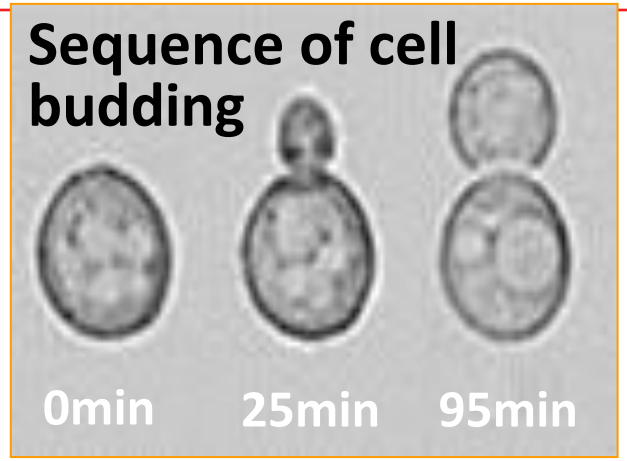
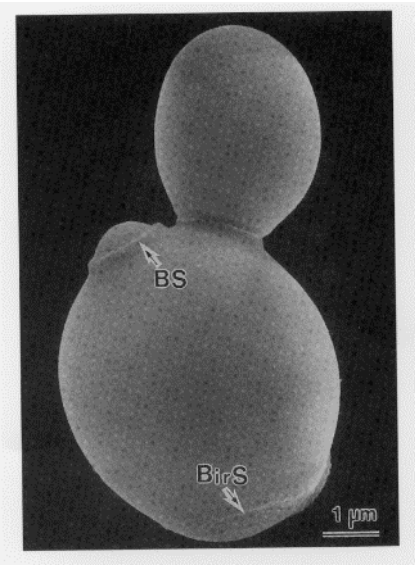
Budding Sequence





Single-budded cell



- **In the lab, *S. cerevisiae* can divide every ~2hours, but longer in a fermentor**
- **Yeasts divide ~4 times during a typical fermentation**
  - Example: Corn mash (batch) fermentation
  - $10x10^6$ cells/mL inoculated will grow to ~250 x 10^6 cells/mL
- **Multi-budded cells are old, and poor fermenters**

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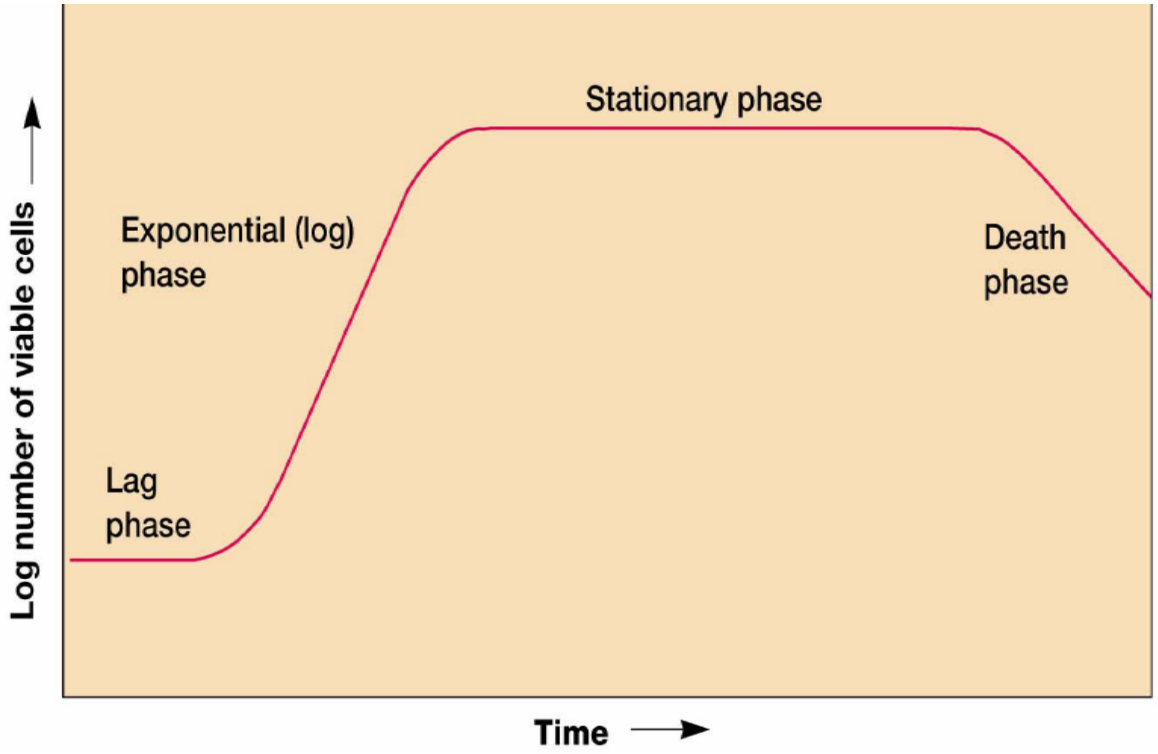
Yeast Growth

After pitching, yeast cells go through different growth phases:

LAG, LOGARITHMIC, & STATIONARY

(also death phase)

- Yeast growth & fermentation are coupled.
- Growing cells produce alcohol 33 times faster than non-growing cells!
- Growth is limited by lack of essential nutrients and ethanol toxicity.
- If substrate remains, loss of product occurs.



✓***Keep cells viable and actively growing for consistent alcohol fermentations***

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Yeast Growth in the Distillery

Important factors must be taken into consideration:

- ***Pitching rate*** (initial yeast concentration) influences fermentation
- ***Dissolved oxygen*** needed by yeast to synthesize the building blocks necessary for new cellular membranes
- ***Temperature*** affects the yeast growth and fermentation rate
- ***Flocculation pattern*** of the yeast strain being used may affect fermentation rate (generally – flocculation undesired by distillers)
- ***Nutrients*** *(in the wort, must, juice)* affect yeast growth & fermentation

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Yeast Sugar Metabolism - Overview

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)

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

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

Alcohol, CO_2

Yeast biomass

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Aerobic & Anaerobic Growth of *S. cerevisiae*

Fermentation: BATCH, ANAEROBIC

↓

Alcohol

Propagation: FED-BATCH, AEROBIC

↓

Yeast

Easy only in a yeast plant!

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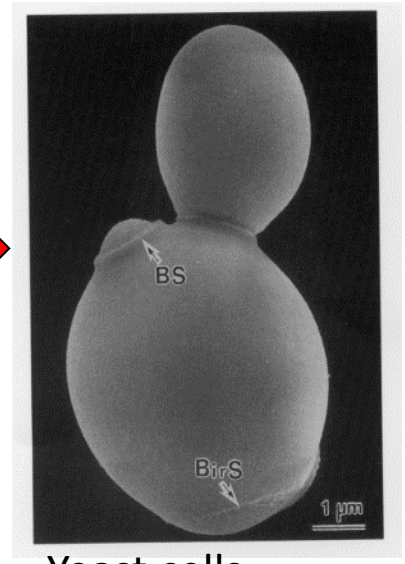
How Does Yeast Make Alcohol?

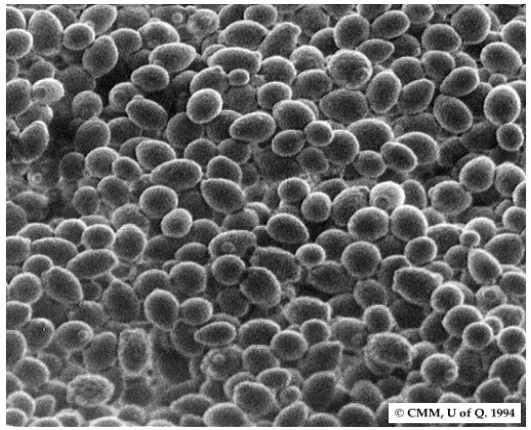
Sugary nutrients



Yeast cells

Growth

Yeast biomass



Fermentation

Alcohol + CO_2

$$C_6H_{12}O_6 \Rightarrow 2CO_2 + 2C_2H_5OH$$

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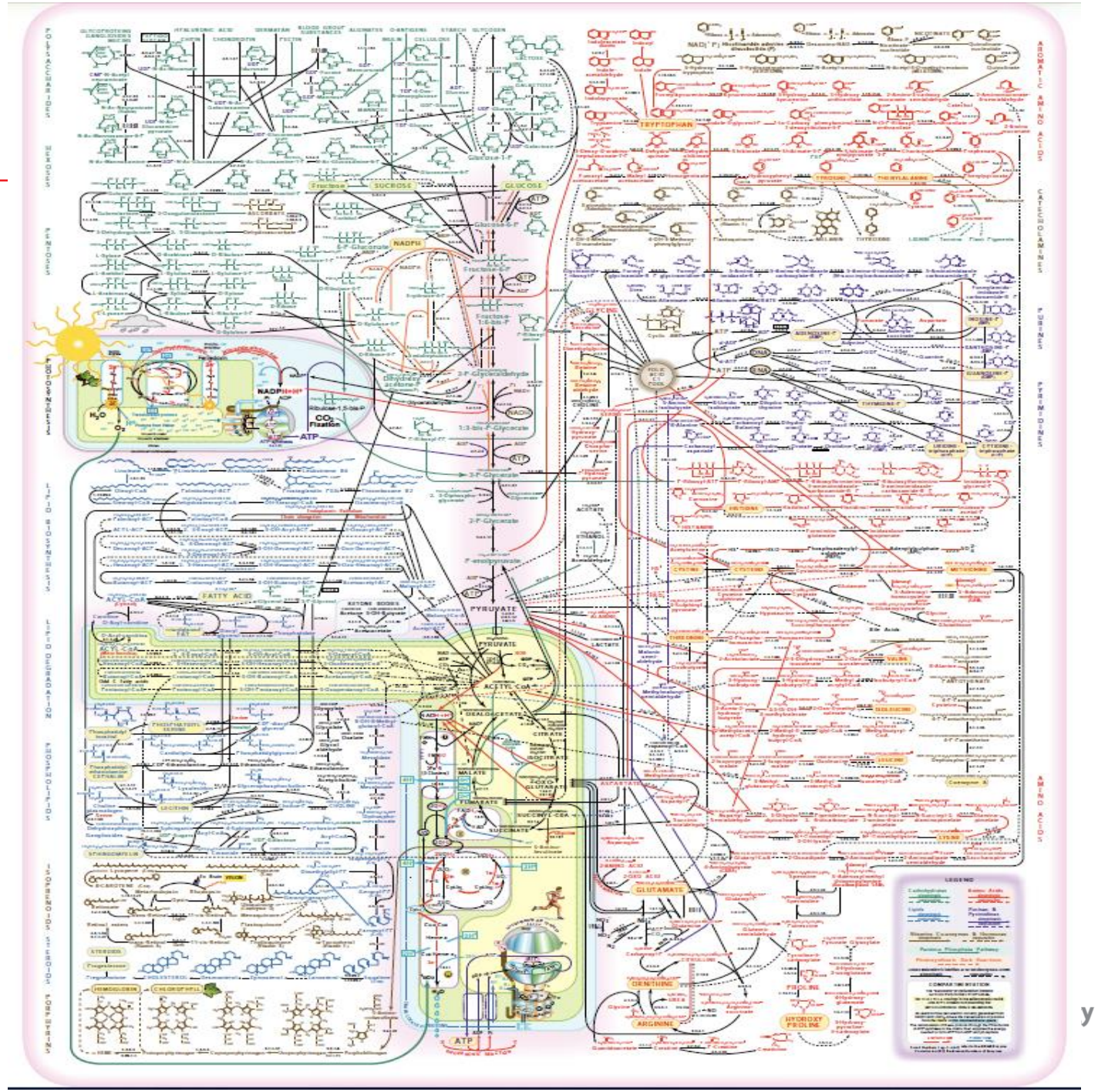
Yeast metabolism – it is complex!

So how does yeast make alcohol?



Ethanol from sucrose?

Sucrose

H_2O + Yeast Invertase

Glucose + Fructose

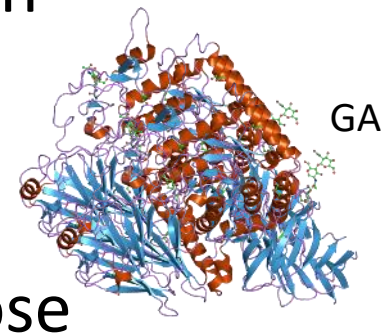
Yeast Enzymes

Ethanol & CO_2

Ethanol from starch?

Starch

H_2O + Enzymes



GA

Glucose

Yeast Enzymes

Ethanol & CO_2

Ethanol from cellulose?

Cellulose

Acids + heat

H_2O + Enzymes

Glucose, Xylose etc.

Yeast Enzymes, GM Yeast

Ethanol & CO_2

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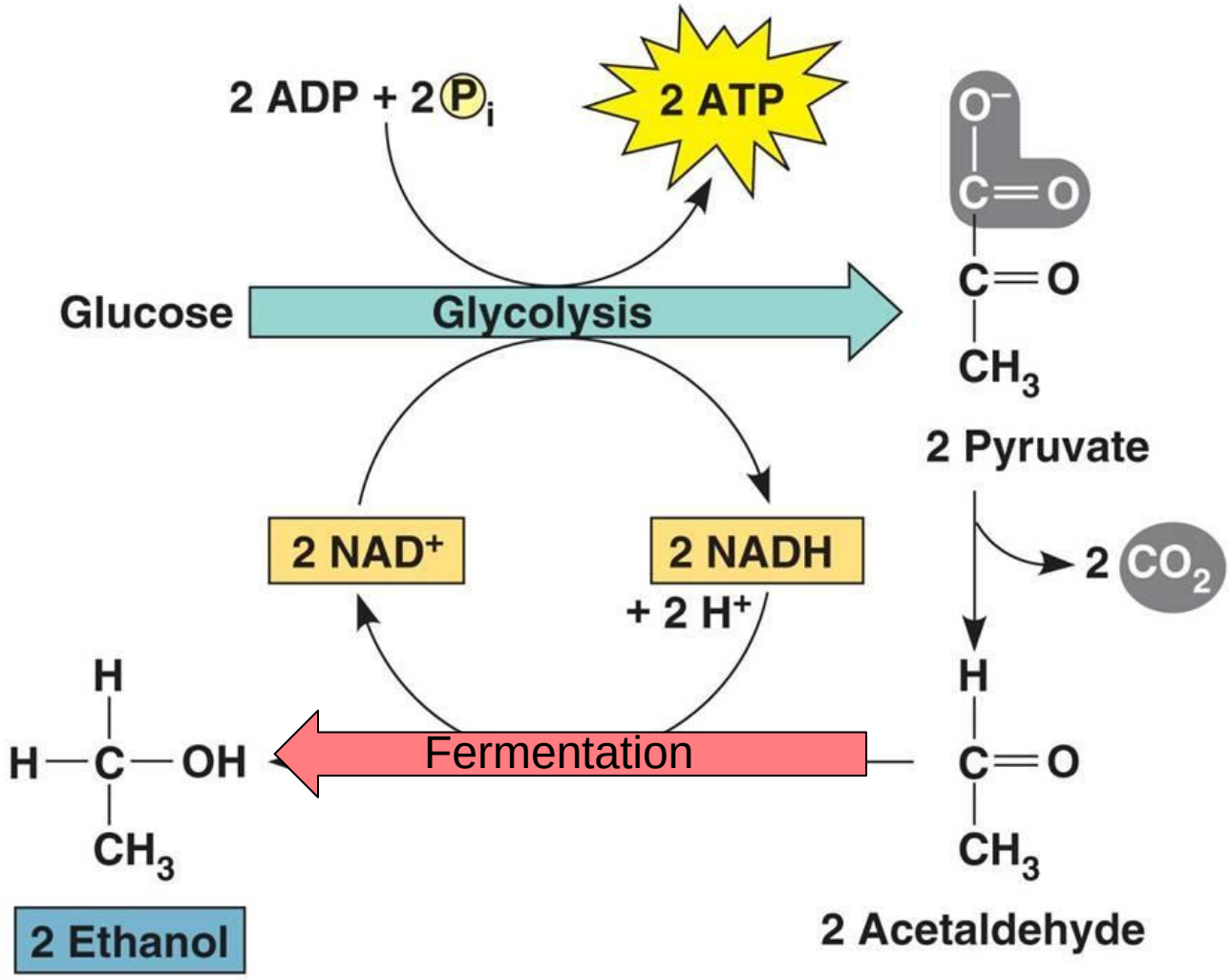
But, why does yeast do this?

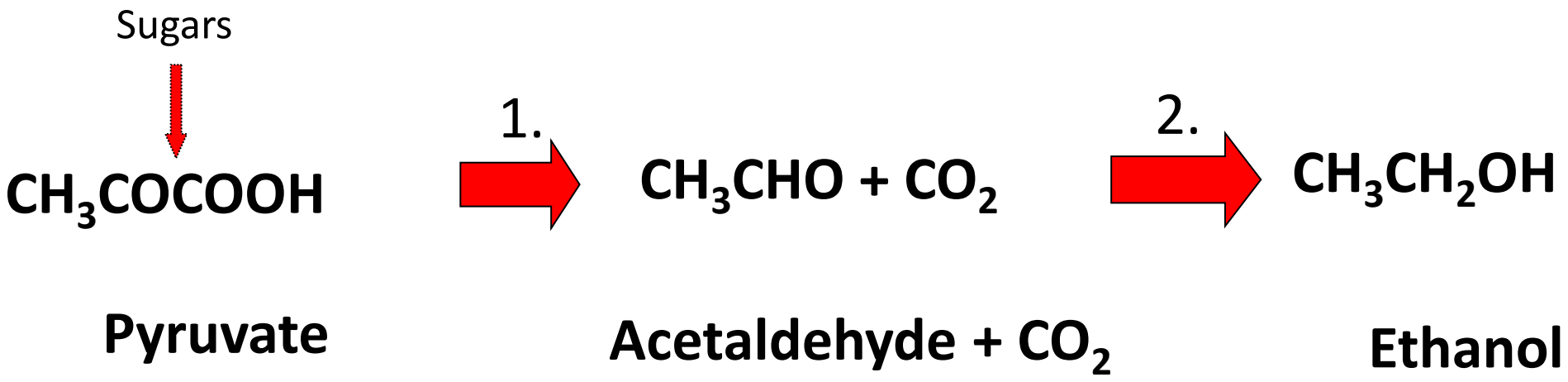


Glycolysis (10 enzymes)
degrades glucose to pyruvate with production of energy
(2ATP) for yeast cell growth

Fermentation (2 enzymes)
Convert pyruvate to ethanol & CO_2 as yeast controls its redox balance

Basically, yeast makes alcohol as it tries to produce energy and balance its electrons

Yeast Alcoholic Fermentation



Enzyme 1. Pyruvate decarboxylase

Enzyme 2. Alcohol dehydrogenase - regenerates NAD^+

In **FERMENTATION**, ethanol CO_2, energy production, yeast growth are all COUPLED - proportional to yeast metabolic rate

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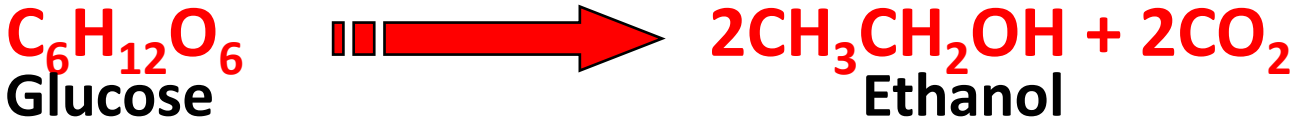
Ethanol Yields from Glucose?



Taking glucose as 100 parts by weight, reactant weights would be

100 **51.1** **48.9**

Theoretical maximum yield of ethanol is therefore **51.1%** from 100 parts of 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 etc. 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

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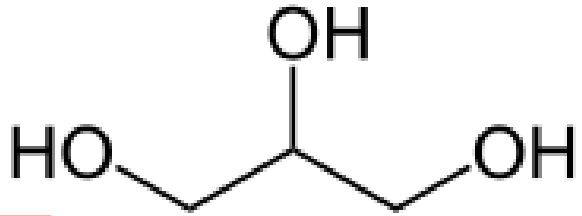
Yeast Also Makes Glycerol



- Glycerol is made due to the accumulation of excess NADH + H^+
  - used to convert dihydroxyacetone phosphate to glycerol

- Glycerol over-production means less ethanol
  - X bad news for alcohol producers: glycerol can be as high as 10% of ethanol
  - √ good for wine producers: to improve mouthfeel/viscosity of beverage

- Stressed cells also accumulate glycerol (membrane protectant)
  - Caused by osmostress & acids
  - Can reduce glycerol by minimising yeast stress!

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Can We Increase Ethanol Productivity?

Q. Would a 2 or 3% increase (eg. 90 to 93%) in yield help your plant?

- ❑ **Minimize microbial contaminants**
- ❑ **Optimize yeast nutrition**
- ❑ **Minimize yeast stress**
- ❑ **Choose correct yeast strain (maybe GM)**
- ❑ **Control glycerol & other secondary yeast metabolites**
- ❑ **Use correct bioprocess and downstream technologies**

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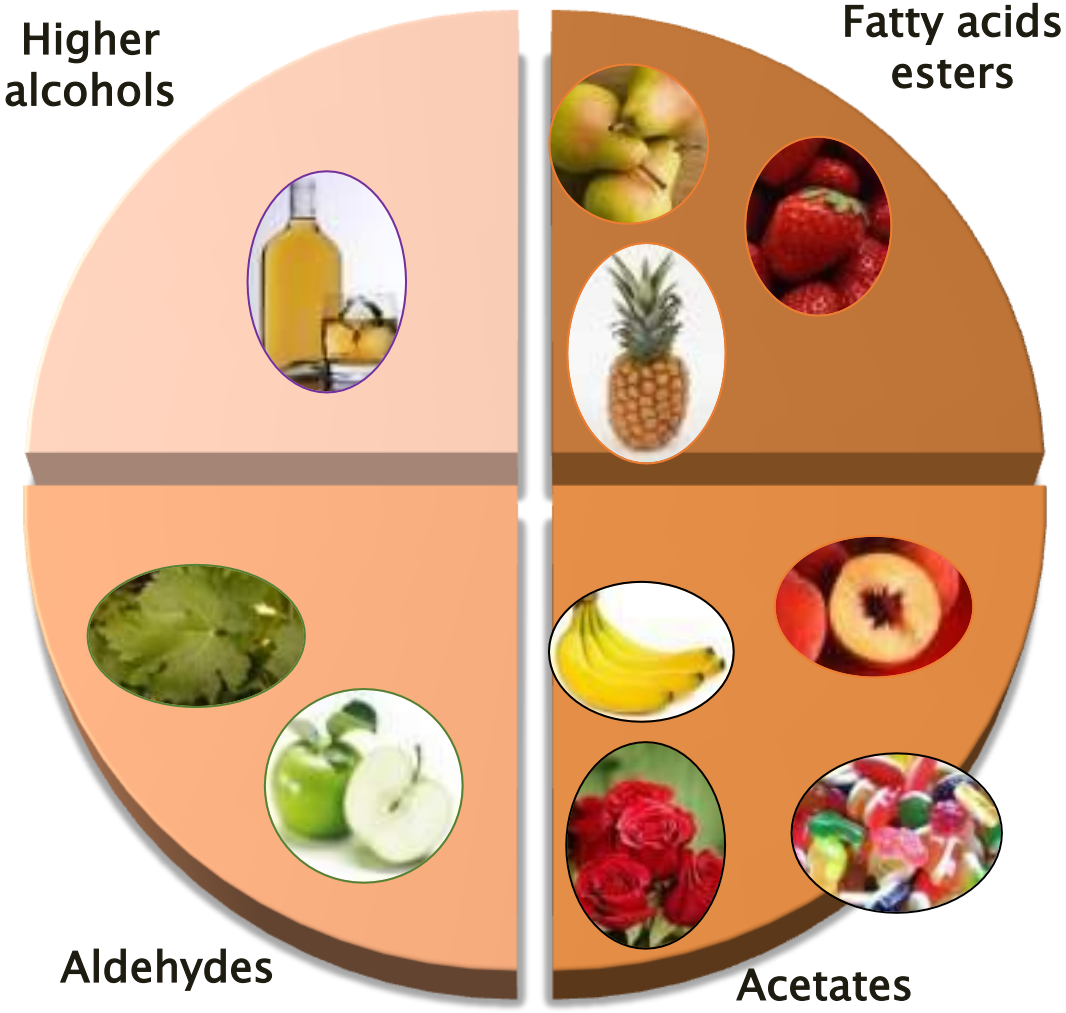
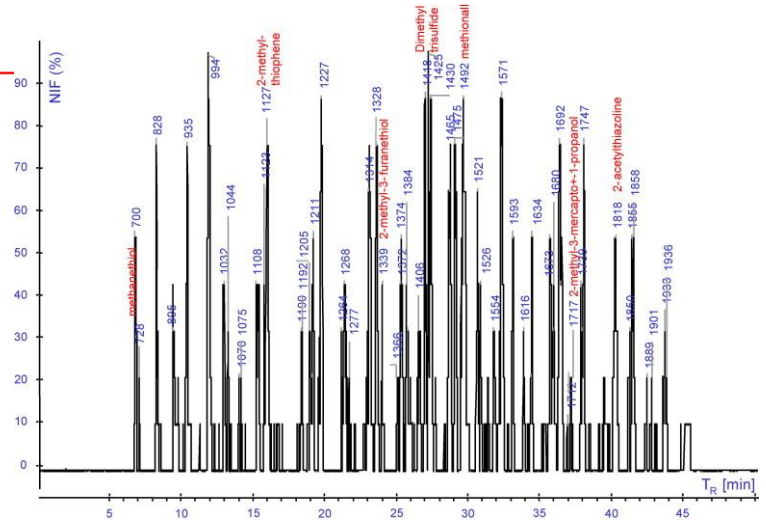
What about Fermentation Metabolites -> CONGENERS





Their level depends on

- fermentation media
- yeast strain & physiology
- fermentation conditions

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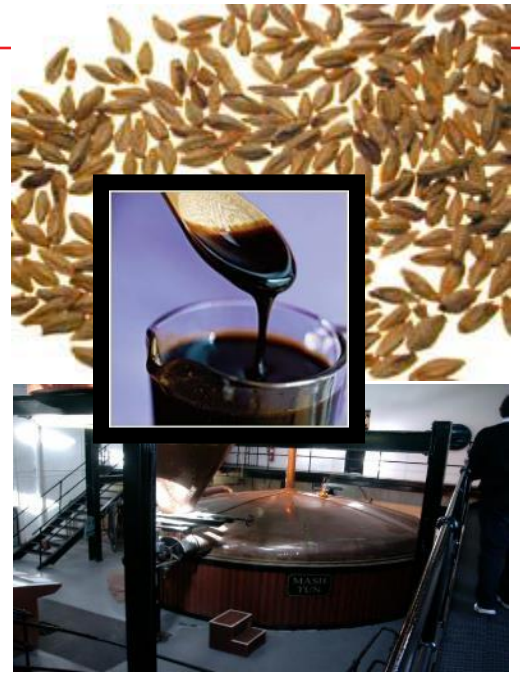
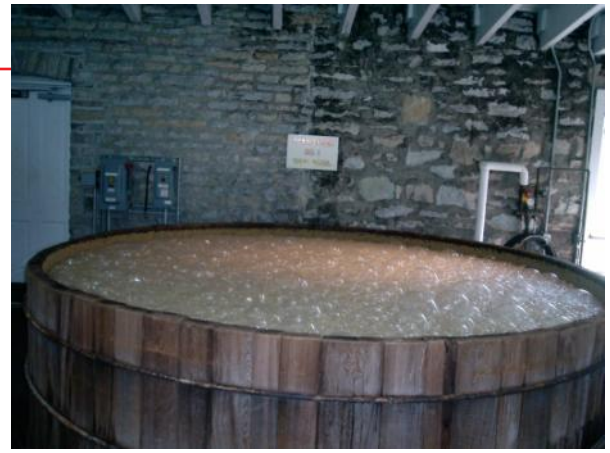
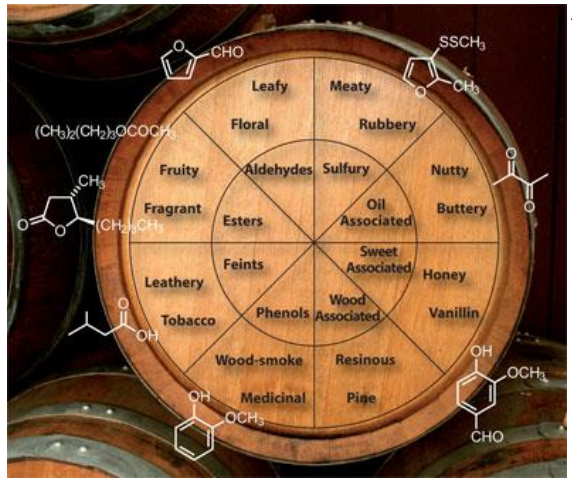
Where Does Spirit Flavour Come From?







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Fermentation Control of Congeners?

- **Yeast strain & yeast condition**
- **Yeast growth** (with increased O_2 results in increased higher alcohol production)
- **Fermentation media** (eg. more YAN stimulates fusel oils)
- **Temperature** (eg higher alcohols & esters increase with temperature)
- **Pressure** (decreases higher alcohols & esters, increases acetaldehyde)
- **Mixing** (increases fermentation & higher alcohols)
- **CO_2** (reduces yeast growth & lowers higher alcohols)
- **Contamination**

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Could we control yeast-derived congeners?

eg. What would happen if you increased the following?

Aeration	Yeast Pitch	Temperature	Pressure
Higher alcohols ↑	Higher alcohols ↓	Higher alcohols ↑	Higher alcohols ↓
Esters ↓	Esters ↓ (iso amyl acetate - banana)	Esters ↑	Esters ↓
Acetaldehyde ↓		Acetaldehyde ↑	Acetaldehyde ↑
	Yeast growth ↓		Yeast growth ↓

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Outline

- **Introduction:** *Saccharomyces cerevisiae*

- **Yeast – some basic physiology**
  - Cytology: structure/function relationships
  - Nutrition
  - Growth during fermentation
  - Metabolism during fermentation

- **Conclusions**

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How to ensure consistently good fermentations?

- Choose the *correct yeast strain*
- Remember that *yeast growth is coupled to fermentation*
- We need to ensure *correct yeast nutrition*
- We need to *minimize yeast stress*
- We need to optimize *fermentation conditions*
- We need to minimize *contamination*

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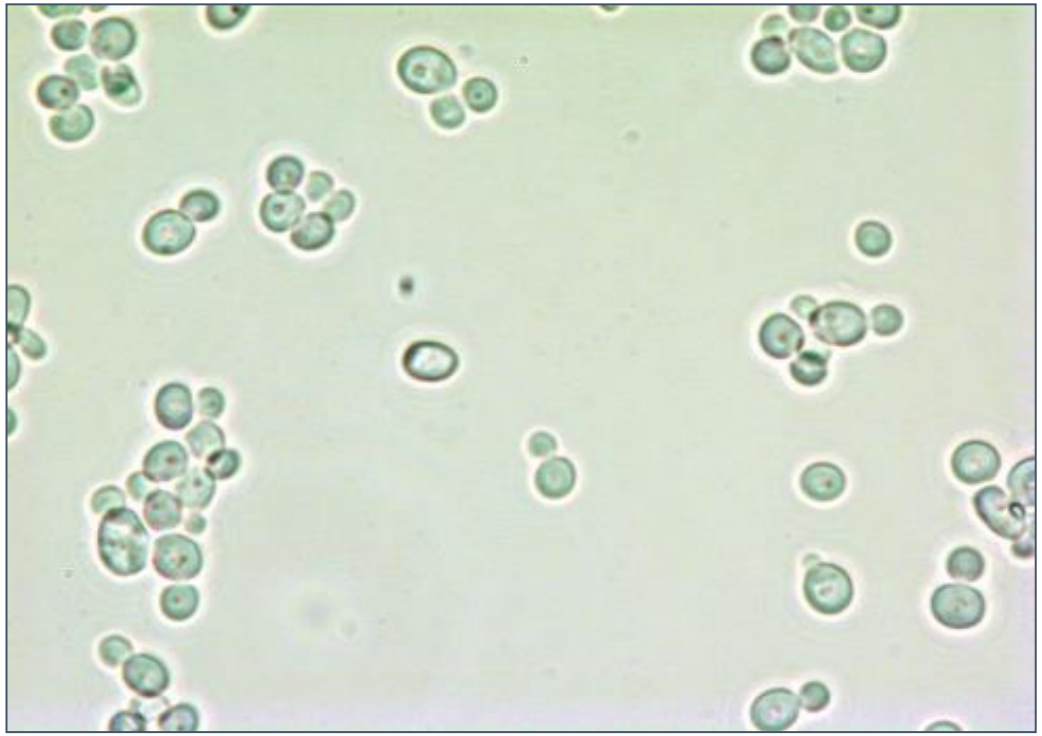
CONCLUSION:

Yeast is the beating heart of the alcohol distillery!

- **Yeast is the most important input in ethanol processes**
  - but least understood!
- **Maintain viable & healthy yeast**
  - avoid stress and contaminants!
- **Ensure proper yeast nutrition**
  - avoid stuck & sluggish fermentations
- **Essential to understand yeast physiology!**



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Thank you!

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