

# Master Brewers Association of the Americas Annual Conference

## Fermentation, Practical Science and Techniques

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# An Introduction to Fermentation



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The International Centre for Brewing and Distilling  
Heriot-Watt University, Edinburgh, Scotland.

The University of Nottingham, Loughborough, England.

- **Ethanol and CO<sub>2</sub> are the major excretion products by yeast during wort fermentation. Ethanol has little direct impact on beer flavour.**
- **The type and concentration of many other fermentation excretion products (esters, higher alcohols, aldehydes, organo sulphur compounds, etc.) primarily determine beer flavour. The balance of these compounds is critical, and a number of factors will influence it.**

# **Brewer's Wort Fermentation has Two Objectives**

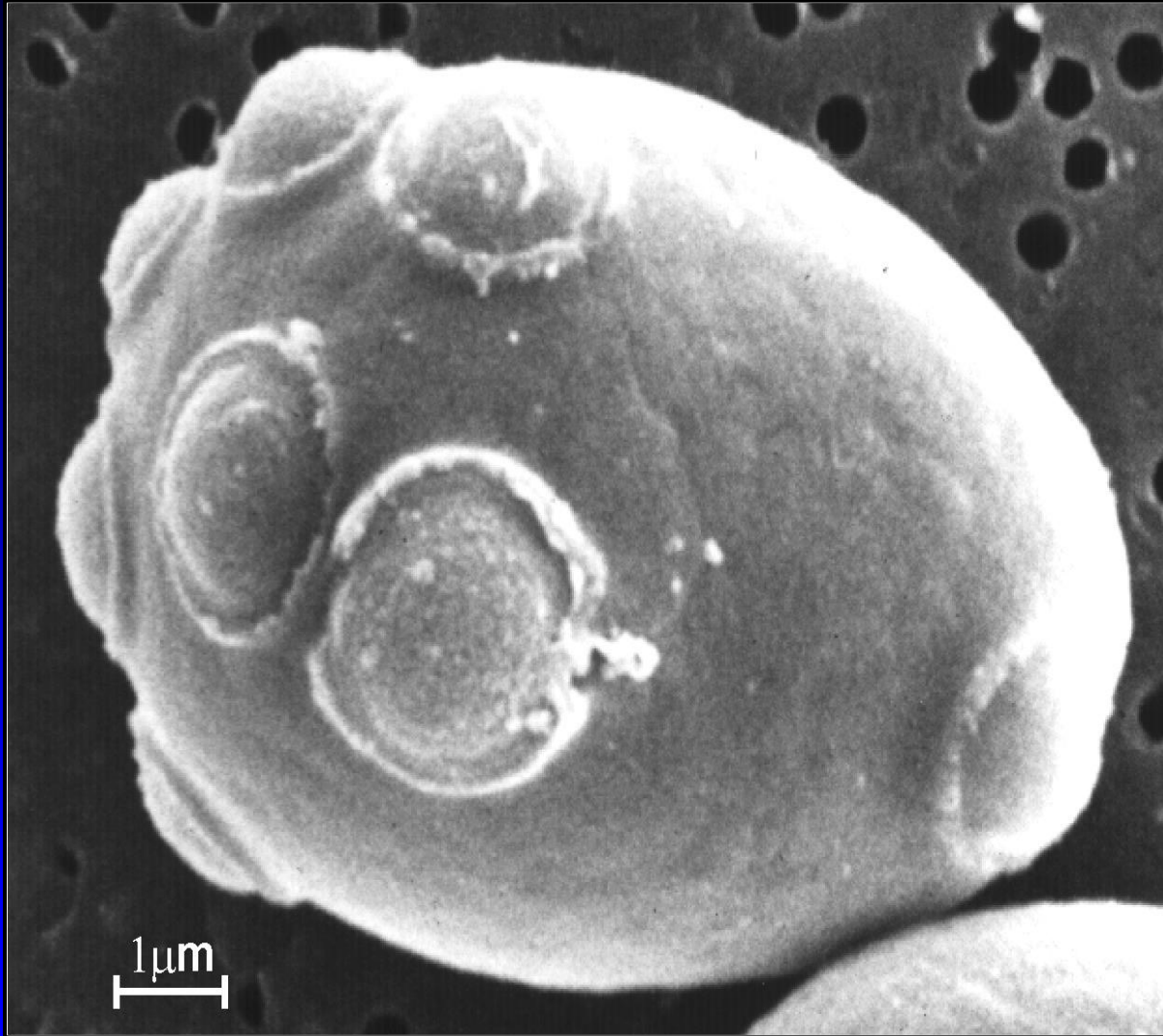
**Wort fermentation by yeast in a brewery  
can be divided into overlapping metabolic steps:**

- To consistently metabolise wort constituents into ethanol, carbon dioxide and other fermentation products in order to produce beer with satisfactory quality, drinkability and stability.**
- To produce yeast crops that can be confidently collected, stored (washed) and repitched into subsequent wort fermentations.**

# Brewer's Wort Constituents

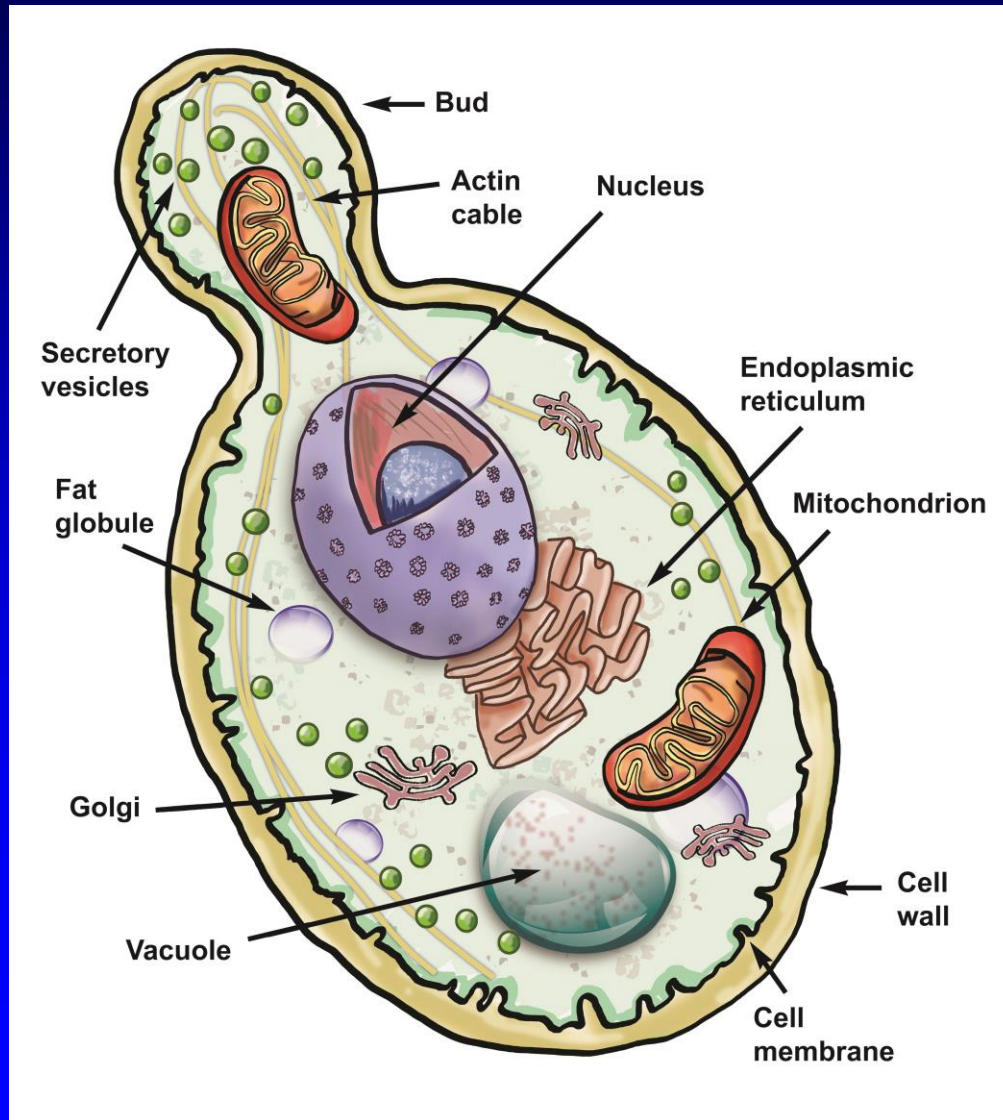
- Simple sugars and dextrans
  - Glucose.
  - Fructose.
  - Sucrose.
  - Maltose.
  - Maltotriose.
  - Maltotetraose and larger dextrans.
- Vitamins.
- Nucleic acids.
- Hop components.
- Free amino nitrogen (FAN)
  - Amino acids.
  - Ammonia.
  - Small peptides.
- Glycopeptides and proteins.
- Water.
- Ions.
- Melanoidins.
- Etc., etc.

# Yeast Cell with Multiple Bud Scars



**Yeast is a single cell fungus**

# Main Features of a Typical Yeast (*Saccharomyces*) Budding Cell



**DIFFERENCES  
BETWEEN  
ALE AND LAGER  
YEAST SPECIES  
AND  
STRAINS**

# Differences between Ale and Lager Yeast Strains

## Ale Yeast

*Saccharomyces cerevisiae* (ale type)

*Saccharomyces cerevisiae*  
(ale and distillers yeast)

Fermentation temperature (18-25°C)

Cells can grow at 37°C or higher

Cells cannot ferment the  
disaccharide melibiose

Strains with distinctive colonial  
morphology on wort-gelatin medium

“Top” fermentation.

## Lager Yeast

*Saccharomyces carlsbergensis*

*Saccharomyces uvarium*  
(*carlsbergensis*)

*Saccharomyces cerevisiae*  
(lager type)

*Saccharomyces pastorianus*  
(current taxonomic name)

Fermentation temperature (8-15°C)

Cells cannot grow above 34°C

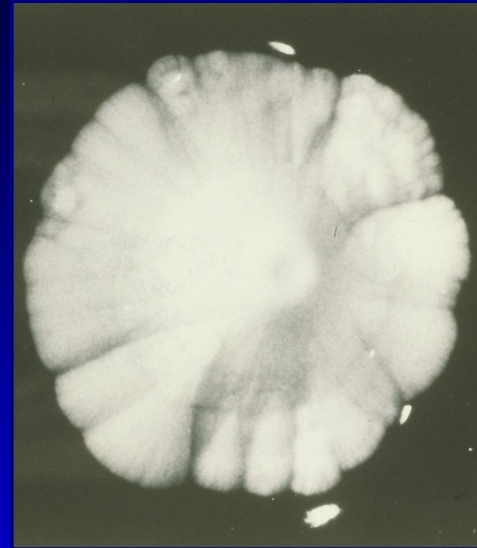
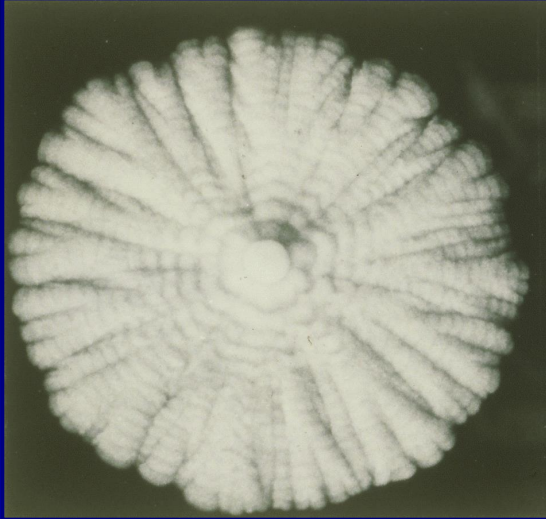
Ferments melibiose (glucose –  
galactose)

Strains do not have a distinctive  
morphology on wort-gelatin  
medium

“Bottom” fermentation.

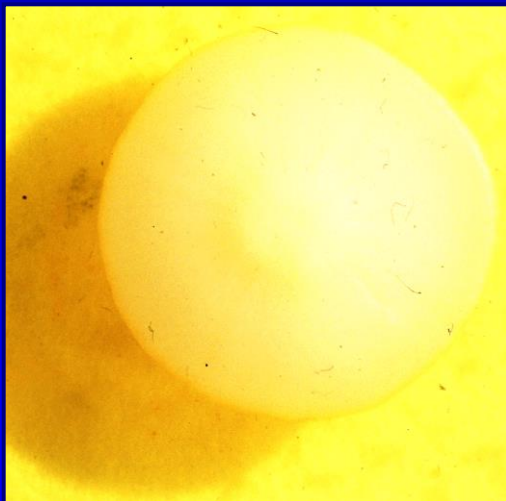


# Giant Colony Morphology of Ale Strains\*



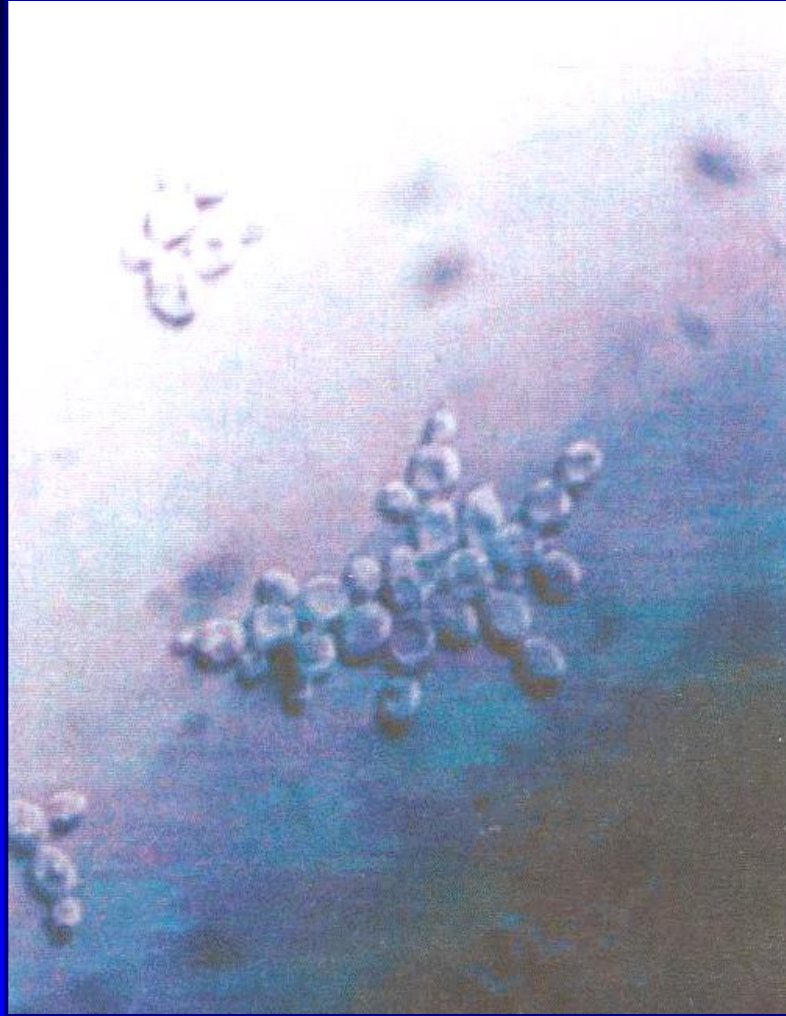
\*Cultures grown on wort-gelatin medium at 18°C for three weeks

# Giant Colony Morphology of Lager Strains\*



\*Cultures grown on wort-gelatin medium  
at 18°C for three weeks

# Chain Formation in Ale Yeast Strains



Lager strains do NOT form chains



# Lager Yeast from Patagonia?

- *Saccharomyces pastorianus* is a yeast species created by the fusion of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*.
- *Saccharomyces eubayanus* exists in the forests of Patagonia and has not been found in Europe – Tibet?
- The genome sequence of *Saccharomyces eubayanus* is 99.5% identical to the non- *Saccharomyces cerevisiae* portion of the *Saccharomyces pastorianus* genome sequence.
- The unique location of *Saccharomyces eubayanus* in the Patagonian forests is questionable!

# Some Brewing Differences between Ale and Lager Yeast Strains

- **Giant colony morphology – chain formation.**
- **Co-flocculation.**
- **Wort sugar uptake**
  - **Effect of glucose on maltose uptake.**
  - **Derepressed variants.**
  - **Wort maltotriose uptake rates and extent greater with lager strains.**
- **Wort assimilable nitrogen uptake.**
- **Dried yeast - viability characteristics.**

(Cont'd)

# **Some Brewing Differences between Ale and Lager Yeast Strains (Cont'd)**

- **Sulphur dioxide production greater under lager fermentation conditions.**
- **Diacetyl metabolism – formation and subsequent re-uptake.**
- **Temperature tolerance – lager (lower) and ale (higher).**
- **Stress tolerance – use of concentrated worts.**
- **Sporulation.**



**WORT**

**FERMENTATION**

**PROFILE**



**SIMPLE WORT SUGARS**

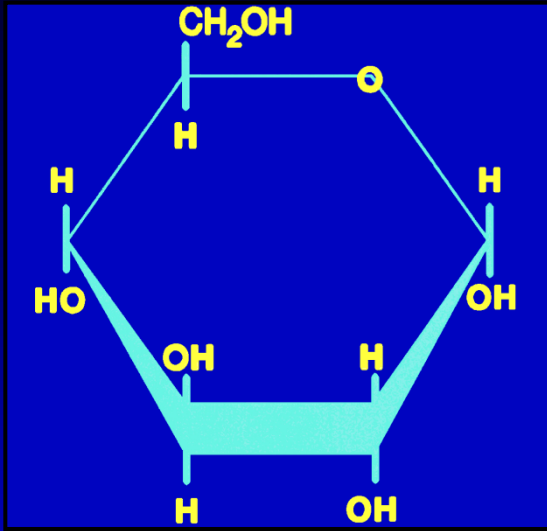
**AND**

**DEXTRINS**

# Typical Sugar Spectrum of Wort

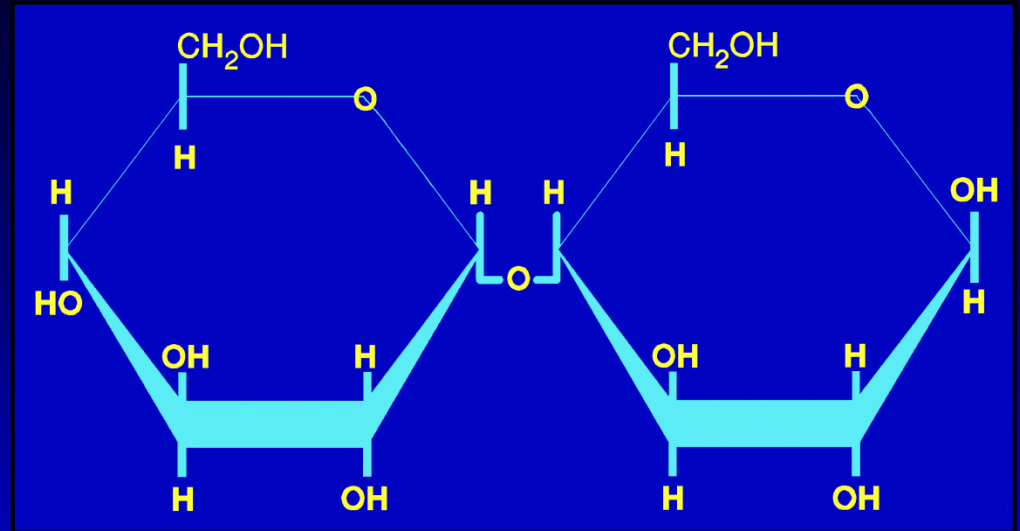
|             | Percent<br>Composition |
|-------------|------------------------|
| Glucose     | 10 - 15                |
| Fructose    | 1 - 2                  |
| Sucrose     | 1 - 2                  |
| Maltose     | 50 - 60                |
| Maltotriose | 15 - 20                |
| Dextrins    | 20 - 30                |

# Structure of Glucose, Maltose and Maltotriose



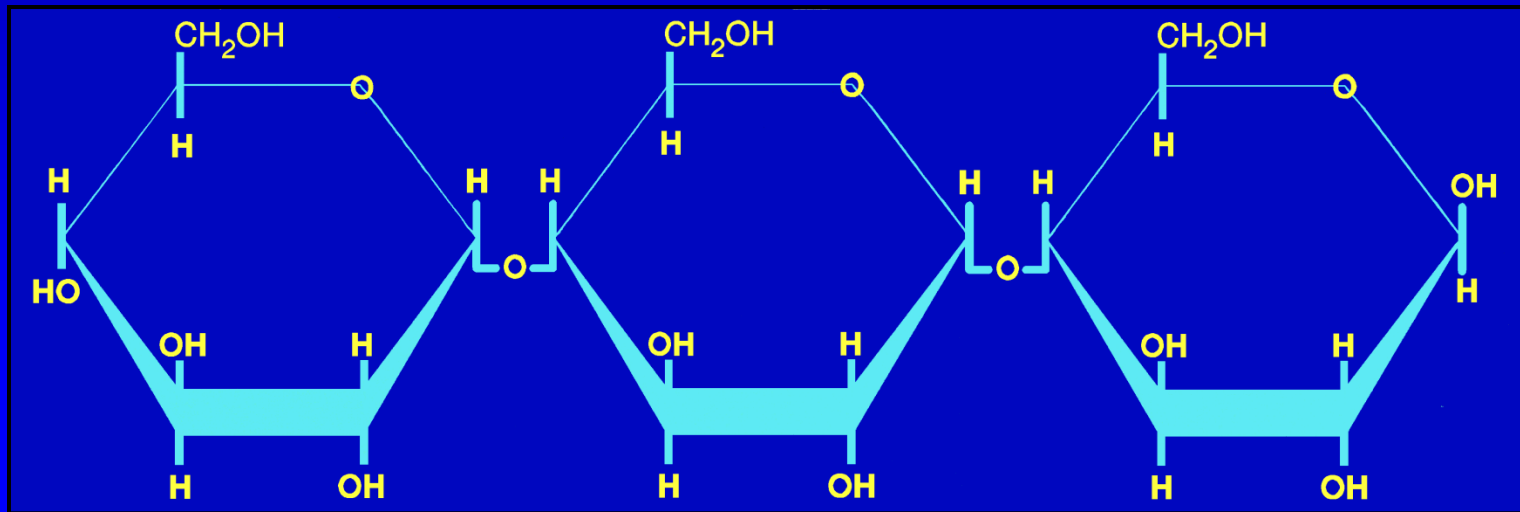
**Glucose**

Molecular weight 180



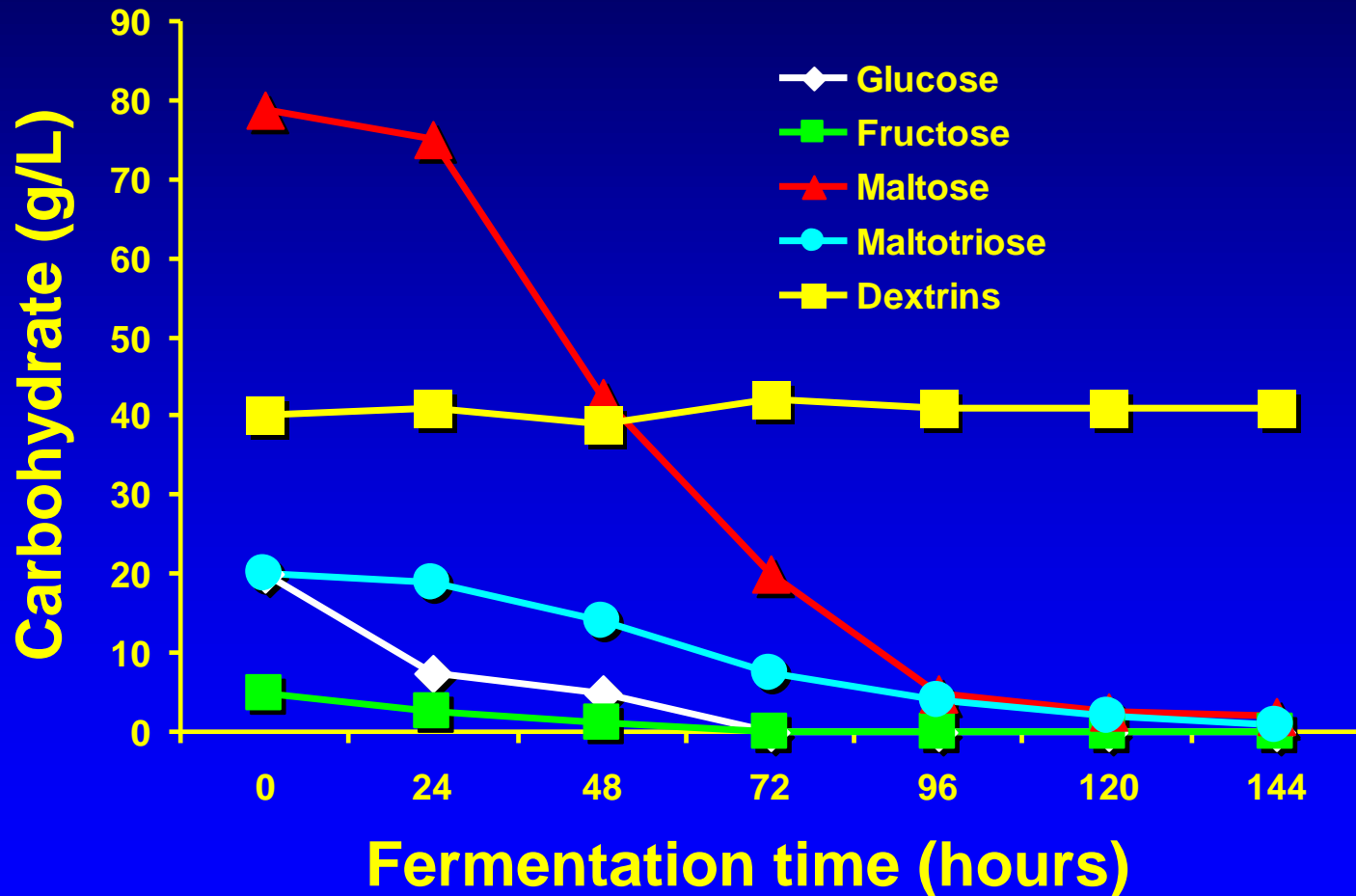
**Maltose**

Molecular weight 342



**Maltotriose** Molecular weight 504

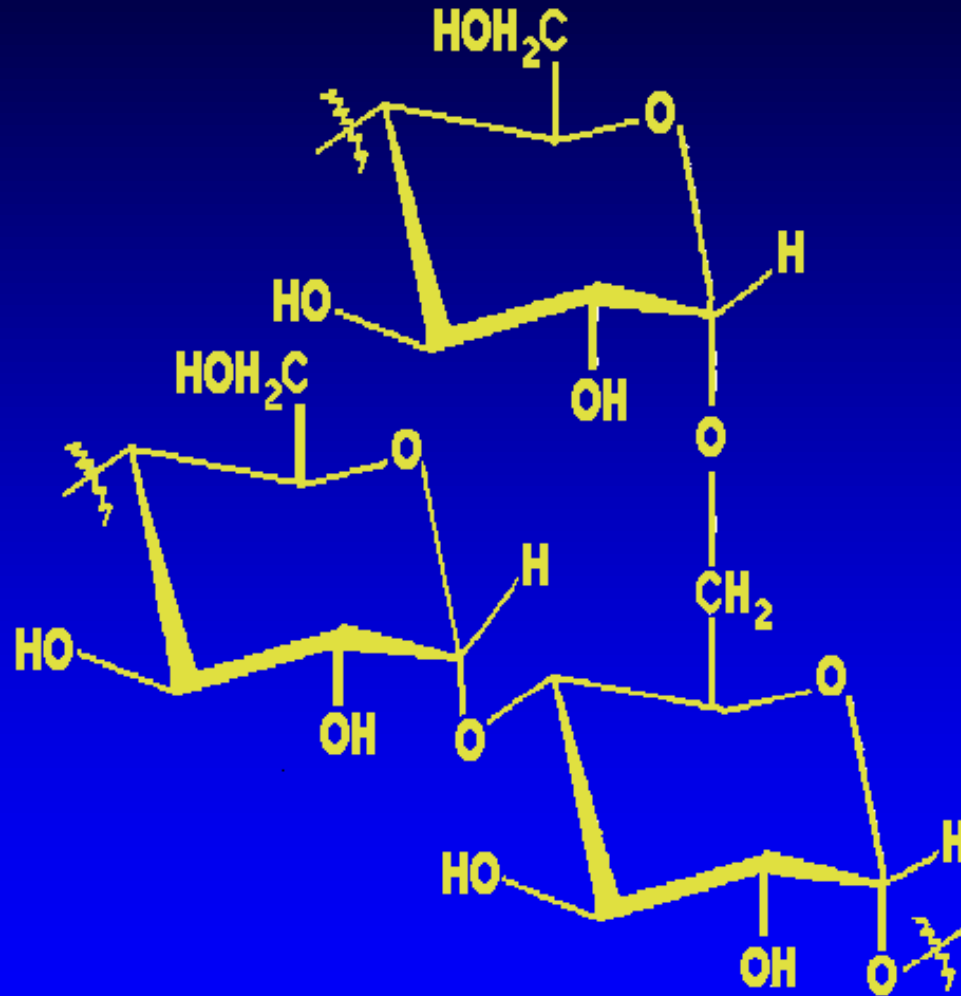
# Order of Uptake of Wort Sugars by Brewer's Yeast



# Yeast Cells Contain Four Major Carbohydrates

- **Glucan - structural material in the cell wall.**
- **Mannan - cell wall material important in flocculation and haze formation.**
- **Glycogen - intracellular storage material.**
- **Trehalose - disaccharide, protection against cell stress (eg, drying, osmotic pressure and ethanol).**

# Structure of Glycogen

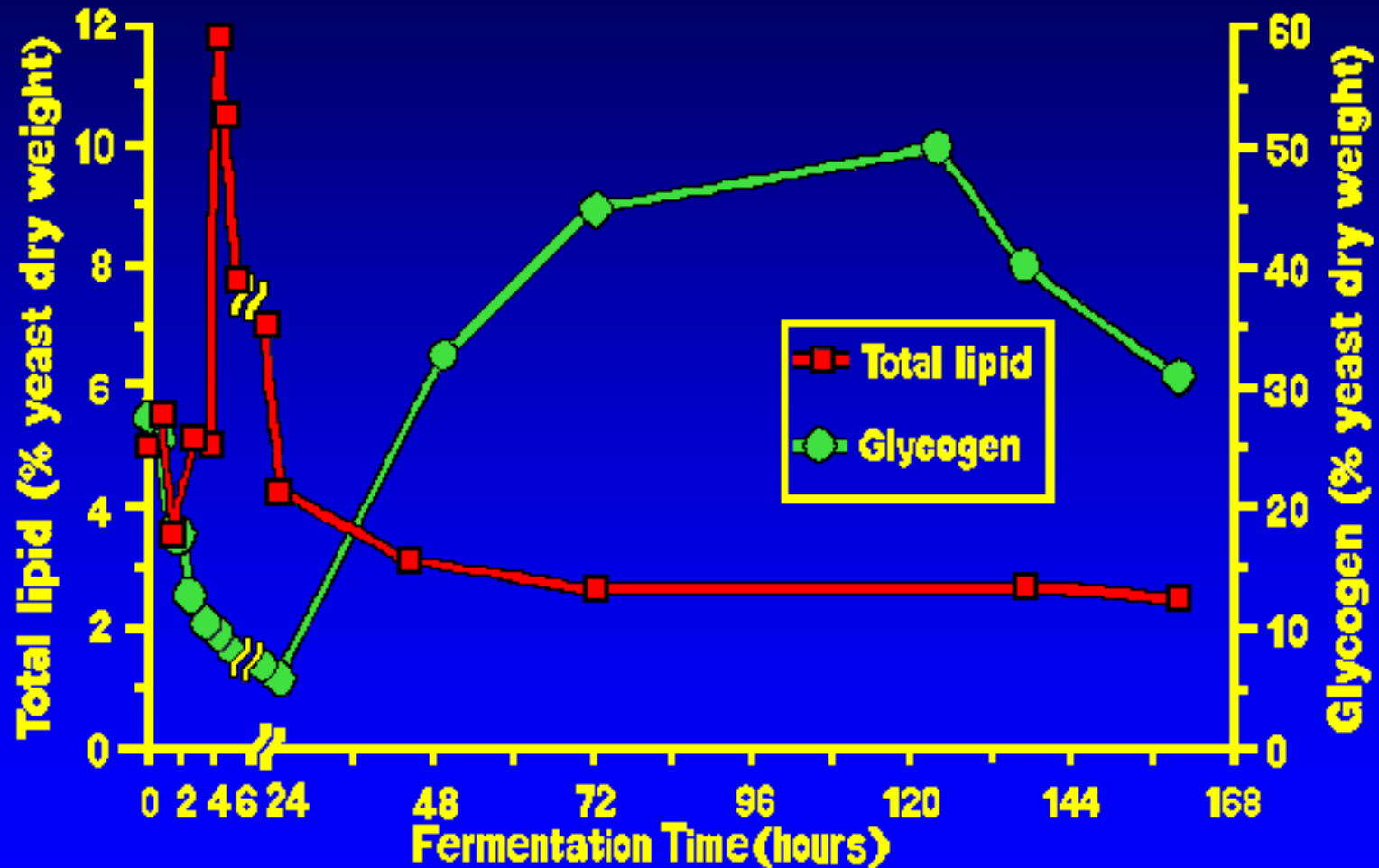


Provides yeast with an immediate source of carbon and energy

# Functions of Yeast Glycogen

- **Glycogen is the major reserve carbohydrate stored within the yeast cell.**
- **It serves as a store of biochemical energy during the lag phase of fermentation when energy demand is intense for the synthesis of such compounds as sterols and fatty acids (lipids).**
- **An intracellular source of glucose (from the glycogen) is required to fuel lipid synthesis at the same time that oxygen is available to the cell.**
- **There is no appreciable uptake of wort glucose during the first six (6) hours (or later) after yeast pitching.**

# Intracellular Concentration of Glycogen and Lipids During Wort Fermentation of a 15° Plato Wort





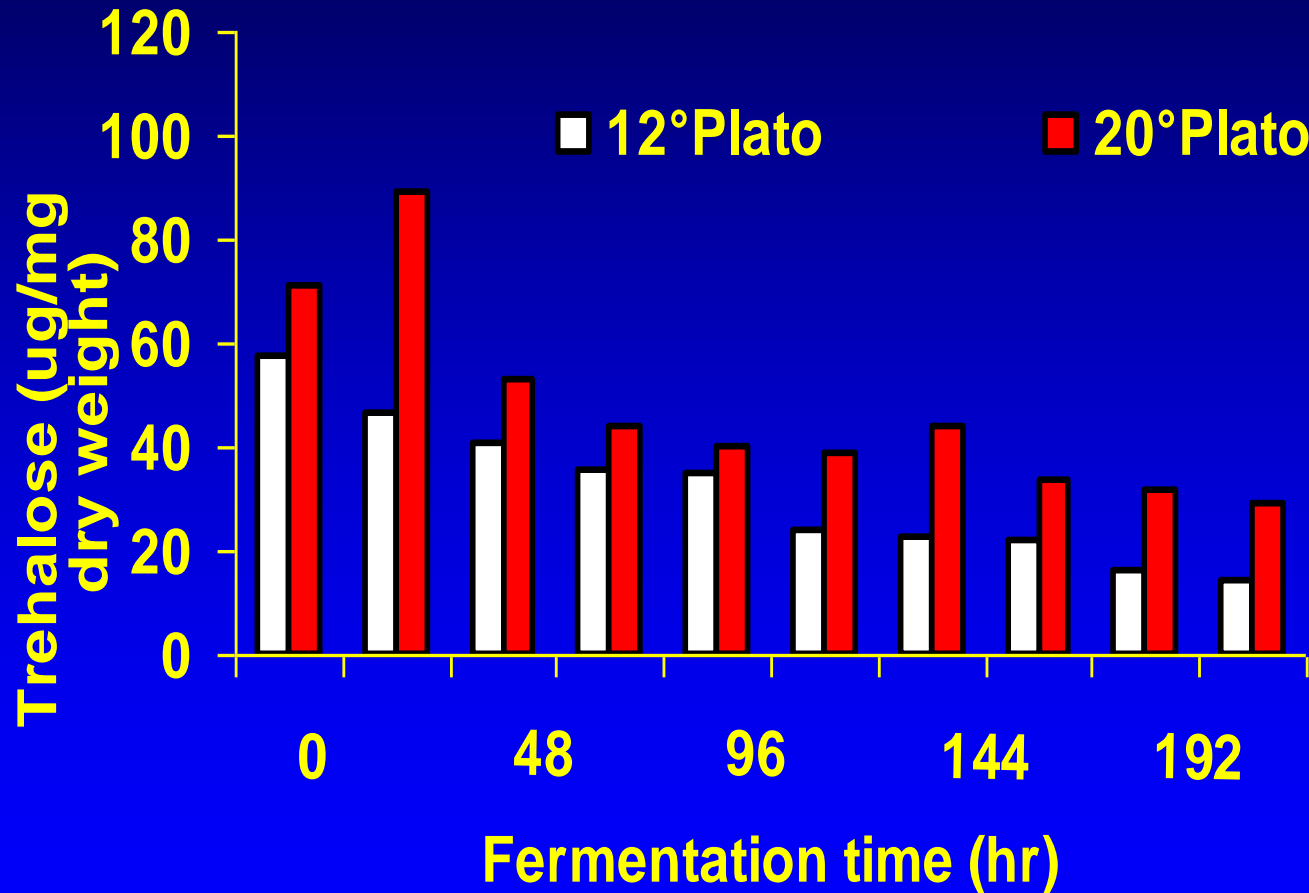
**STRESS EFFECTS**

**ON**

**BREWER'S YEAST STRAINS**



# Effect of Wort Gravity on Trehalose Metabolism in a Lager Yeast Strain



# **ESTER FORMATION**

# Important Esters in Beer

- Ethyl acetate (fruity/solvent).
- Isoamyl acetate (banana/apple).
- Isobutyl acetate (banana/fruity).
- Ethyl caproate (apple/aniseed).
- $\beta$ -phenylethyl acetate (roses/honey).

# Factors that Influence the Level of Ester Production During Wort Fermentation

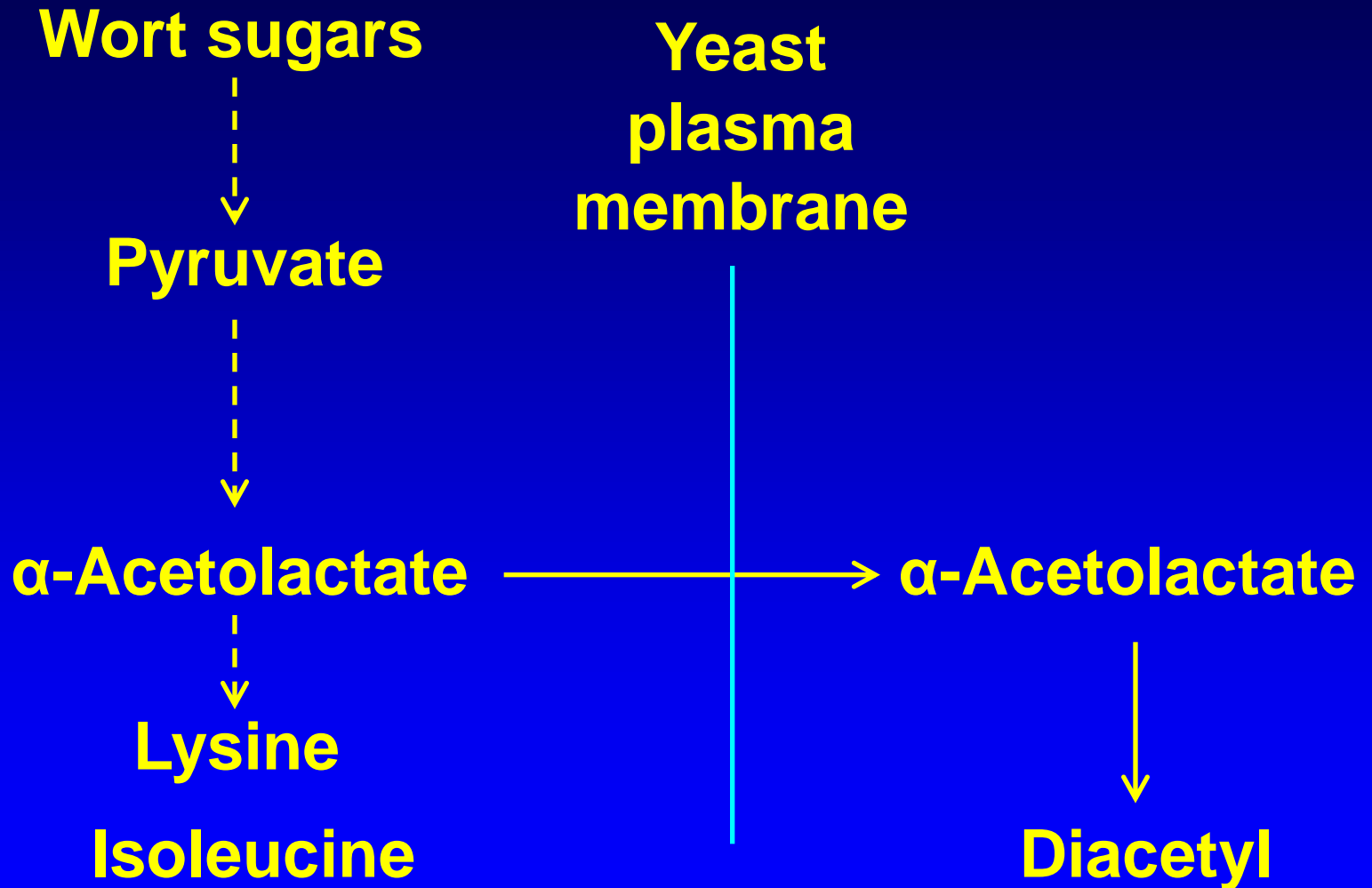
- **Yeast characteristics – yeast strain, physiological state of the culture.**
- **Wort composition – sugar and amino acid spectrum, lipids, vitamins, inorganic nutrients, dissolved oxygen, clarity (trub), original gravity.**
- **Fermentation conditions – temperature, agitation, CO<sub>2</sub> tension, wort pH, fermenter design, pitching rate.**

**DIACETYL**

**(BUTTERSCOTCH/STALE MILK)**

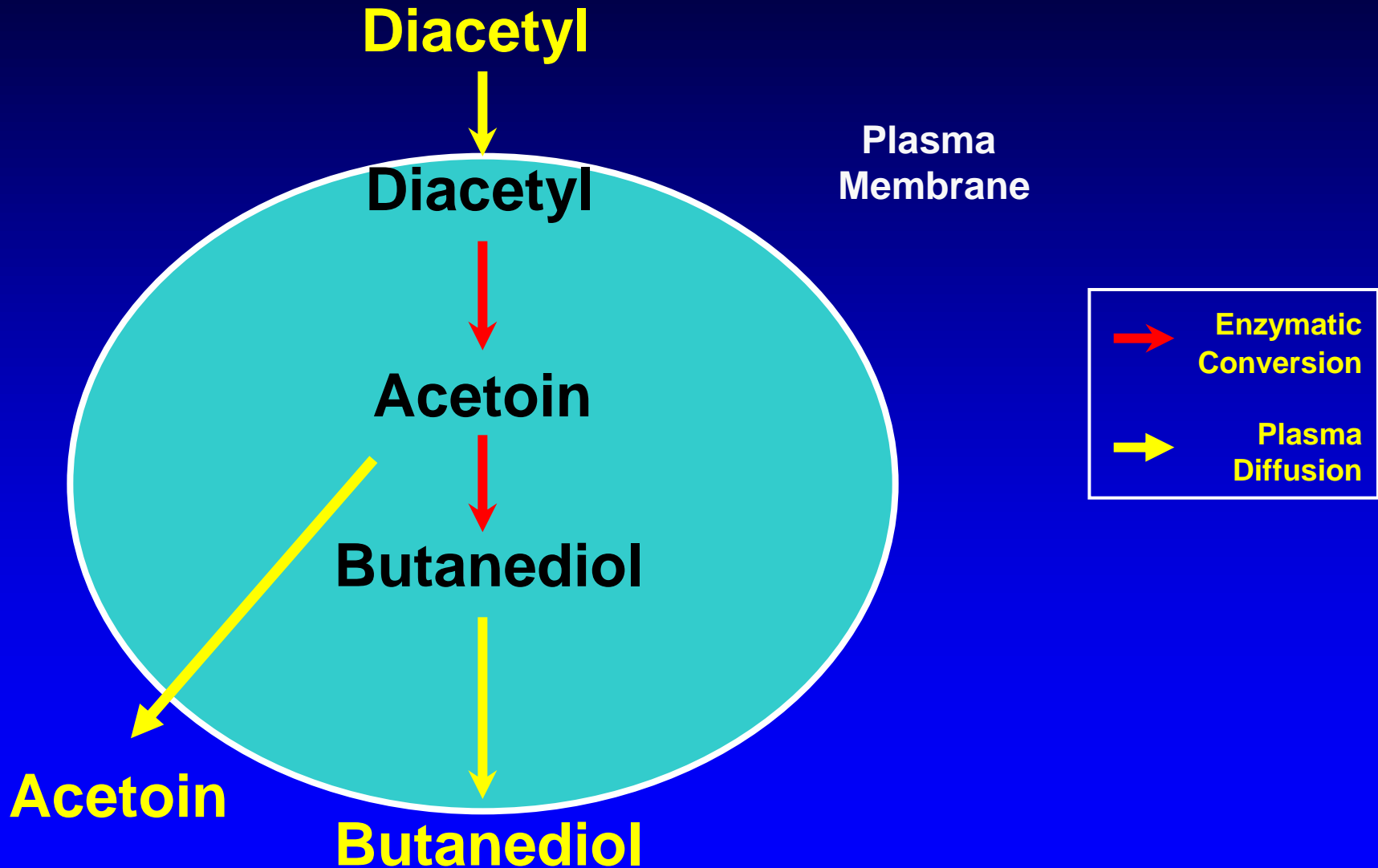
**MANAGEMENT**

# Formation of Diacetyl

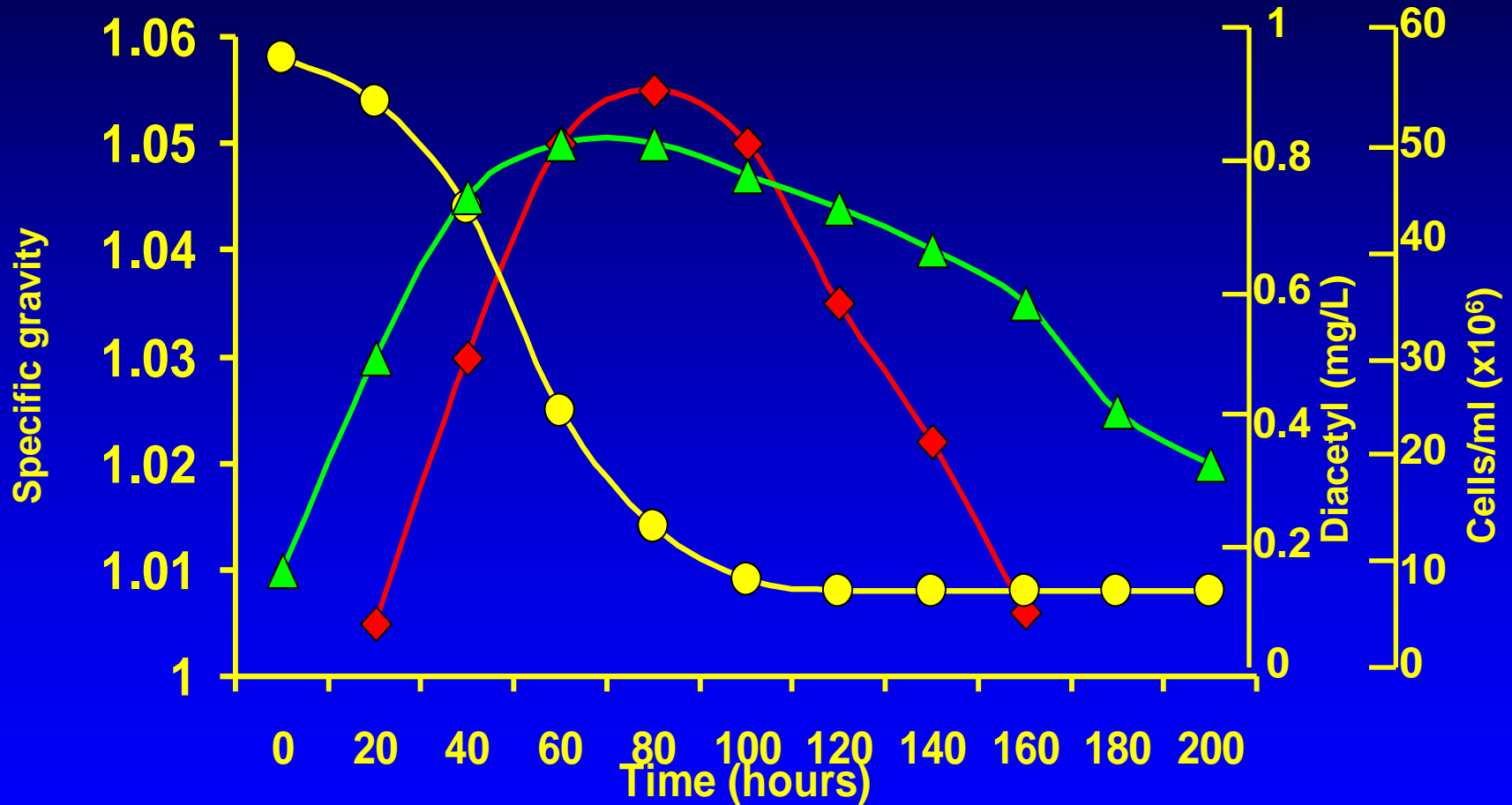




# Reduction of Diacetyl to Acetoin and 2,3-Butanediol



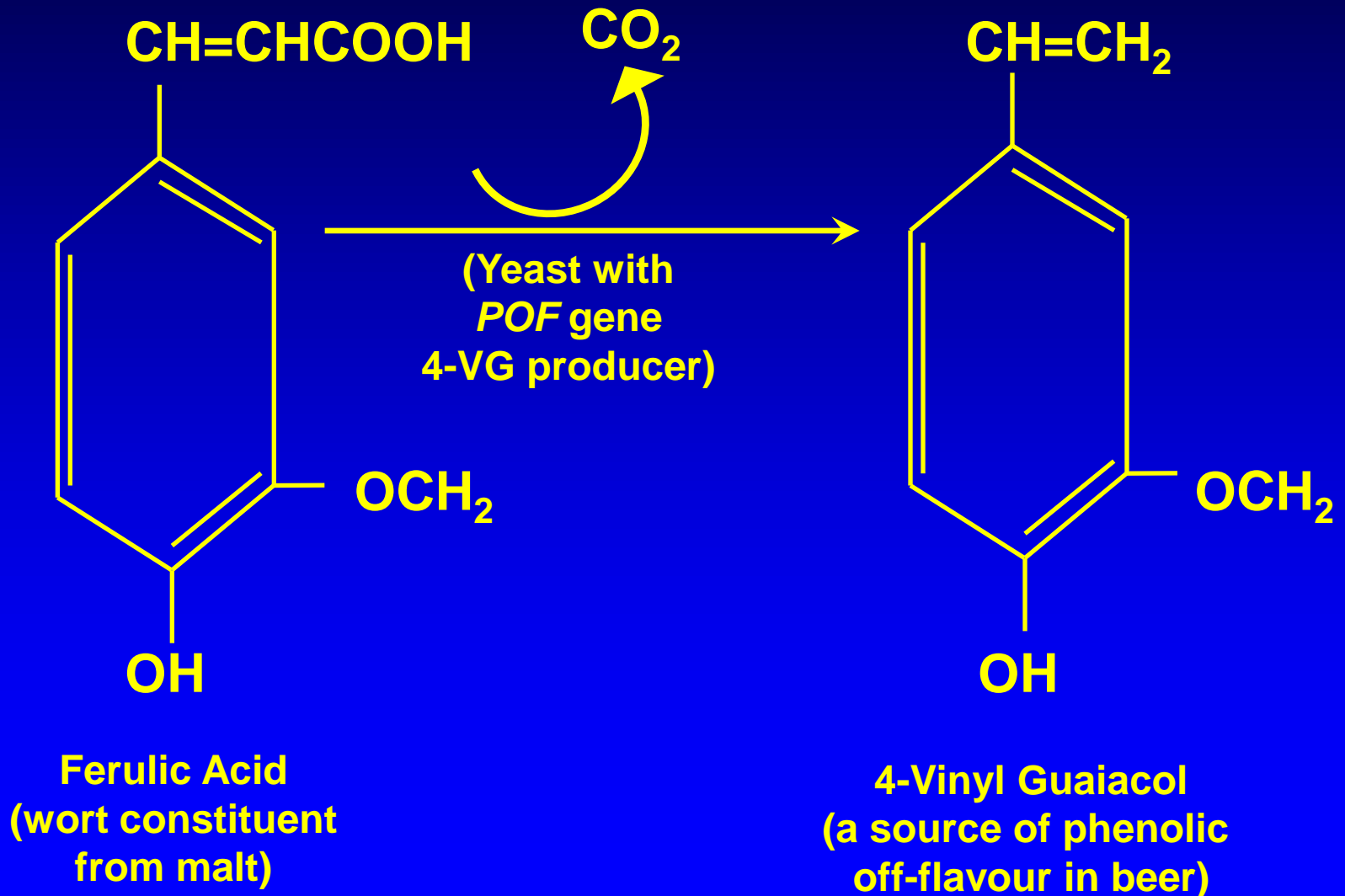
# Diacetyl Metabolism During Wort Fermentation



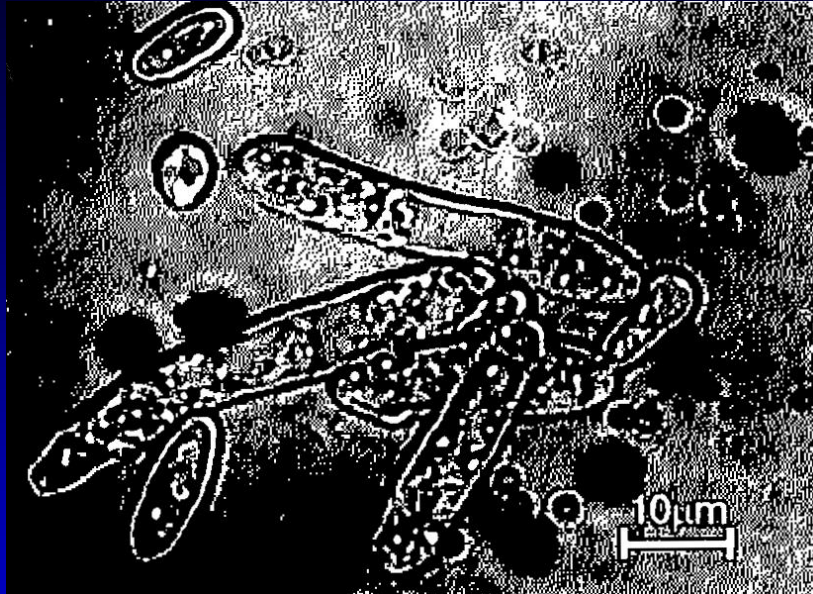
◆ Total diacetyl ● Specific gravity ▲ Suspended yeast count

**PHENOLIC  
OFF-FLAVOURS (POF)  
(4-VINYL GUAIACOL – CLOVES)  
IN  
BEER**

# Enzymatic Decarboxylation of Ferulic Acid to 4-Vinyl Guaiacol (4-VG) by *Saccharomyces sp.*



# Brettanomyces (Dekkera)



- *Brettanomyces* is a yeast and considered to be a contaminant by many brewers.
- Lambic and Gueuze Belgian beers owe their unique flavours and character to *Brettanomyces*. It used to occur in some British ales.
- Currently, it is not uncommon for craft brewers in North America to employ these yeasts as a part of the pitching yeast culture (sometimes with *Lactobacillus* and *Pediococcus*).

# Summary

- Yeast is a unicellular fungus.
- Ale and lager yeast strains exhibit a number of unique features and consequently produce characteristic beers.
- The uptake of wort sugars is an important property of brewer's yeast strains.
- The formation of esters and diacetyl by yeast significantly contributes to beer flavour.
- Yeast species such as *Brettanomyces* and yeast strains producing 4-vinyl guaiacol are important cultures in the production of unique beer types.

# Acknowledgements

Thanks are due to Anne Anstruther for her assistance and encouragement during the development of this presentation.

