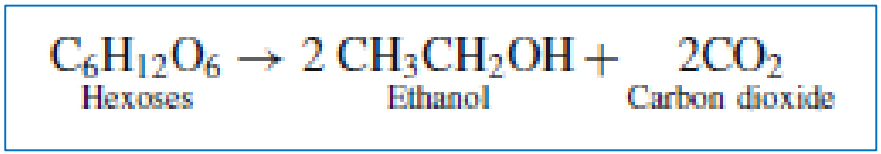
FDE 328
INDUSTRIAL MICROBIOLOGY

Biochemistry of Alcoholic Fermentation

- Alcoholic fermentation is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide.
- This process is carried out by yeast (generally by *Saccharomyces cerevisiae*) and also by some bacteria such as *Zymomonas mobilis*.
- The alcoholic fermentation process can be summarised by this overall reaction.

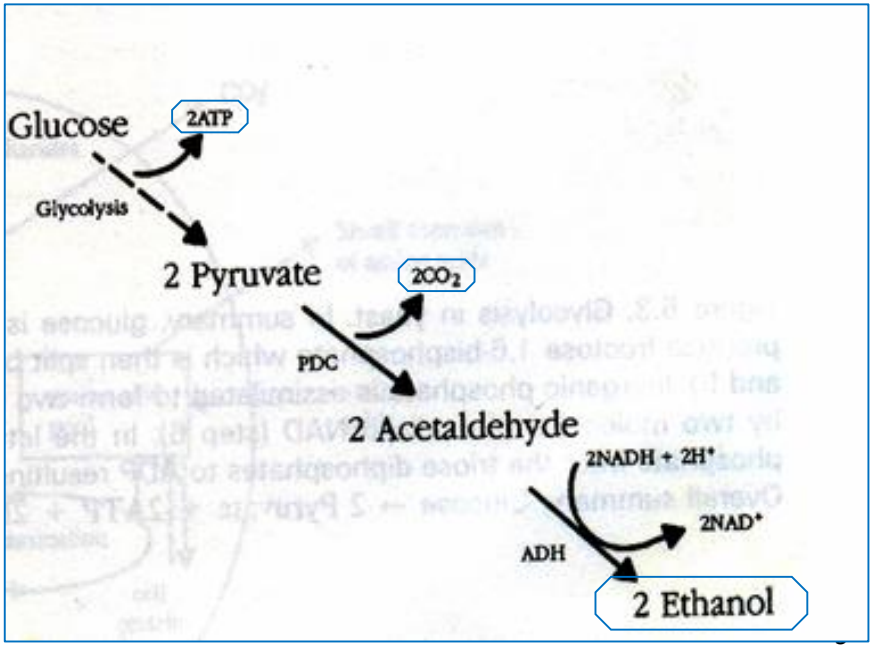


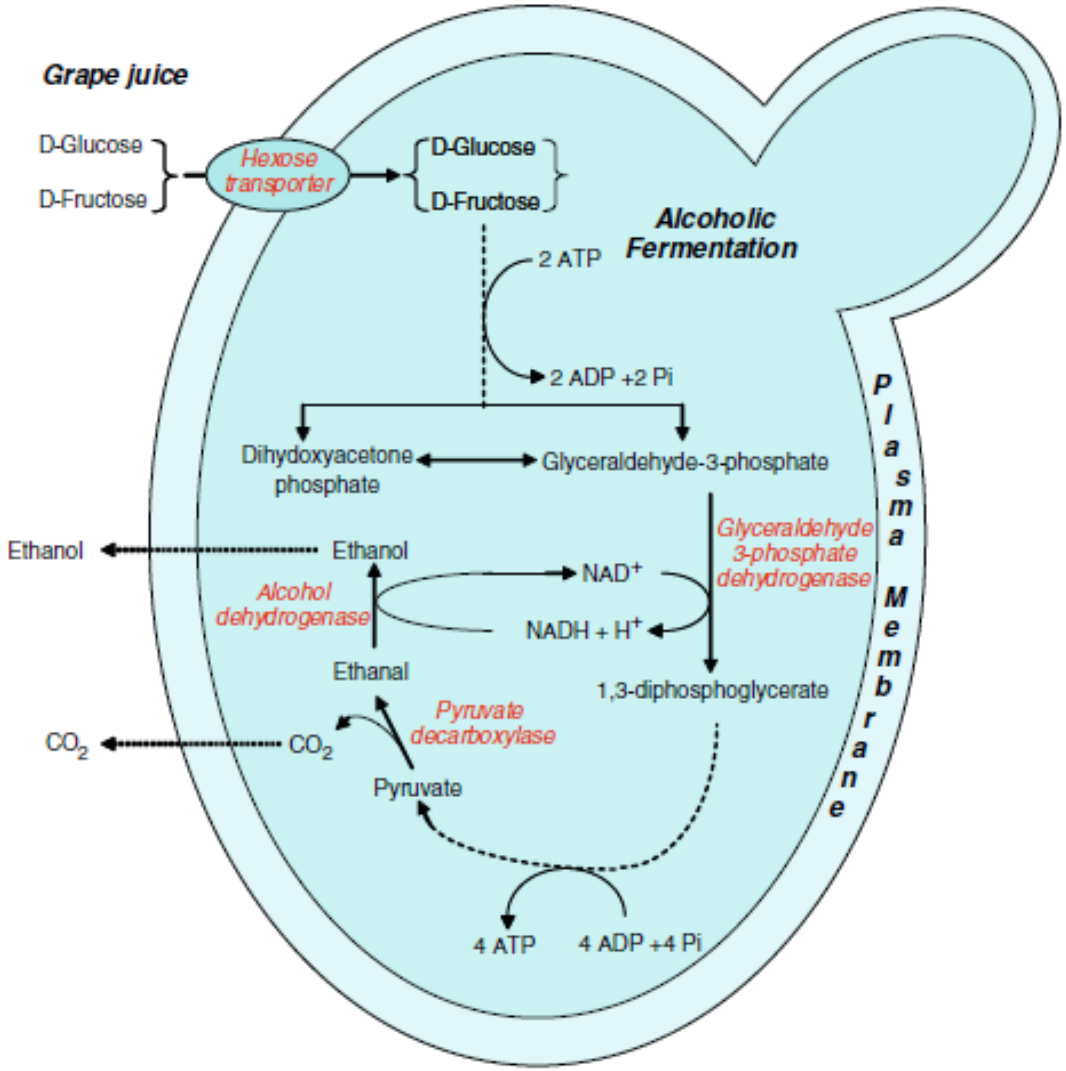
- However, alcoholic fermentation is a much more complex process.
- At the same time as this overall reaction proceeds, a lot of other biochemical, chemical and physicochemical processes take place, making it possible to turn the grape juice into wine.
- Besides ethanol, several other compounds are produced throughout alcoholic fermentation such as higher alcohols, esters, glycerol, succinic acid, diacetyl, acetoin and 2,3-butanediol.
- Simultaneously, some compounds of grape juice are also transformed by yeast metabolism. Without the production of these other substances, wine would have little organoleptic interest.

Biochemistry of Alcoholic Fermentation

The three main reactions that take place during the conversion of sugar in grape must to ethyl alcohol by wine yeasts are;

1. **Glycolysis, or breakdown of sugar**,
2. **Decarboxylation of pyruvic acid**, and
3. **Reduction of acetaldehyde**




Grape juice
D-Glucose
D-Fructose
Hexose transporter
D-Glucose
D-Fructose
Alcoholic Fermentation
2 ATP
2 ADP +2 Pi
Dihydoxyacetone phosphate
Glyceraldehyde-3-phosphate
Ethanol
Ethanol
NAD+
Glyceraldehyde 3-phosphate dehydrogenase
Alcohol dehydrogenase
NADH + H+
Ethanal
1,3-diphosphoglycerate
Pyruvate decarboxylase
CO2
CO2
Pyruvate
4 ATP
4 ADP +4 Pi
Plasma Membrane

Alcoholic Fermentation

- When fermenting grape juice, *Saccharomyces cerevisiae* mainly directs the pyruvate to produce of ethanol in order to regenerate the NAD+ consumed by glycolysis. This process, called alcoholic fermentation.

- Pyruvate is initially decarboxylated into ethanal by pyruvate decarboxylase. This enzyme needs magnesium and thiamine pyrophosphate as cofactors.

- Thereafter, alcohol dehydrogenase reduces ethanal to ethanol, recycling the NADH to NAD+. Alcohol dehydrogenase uses zinc as cofactor.

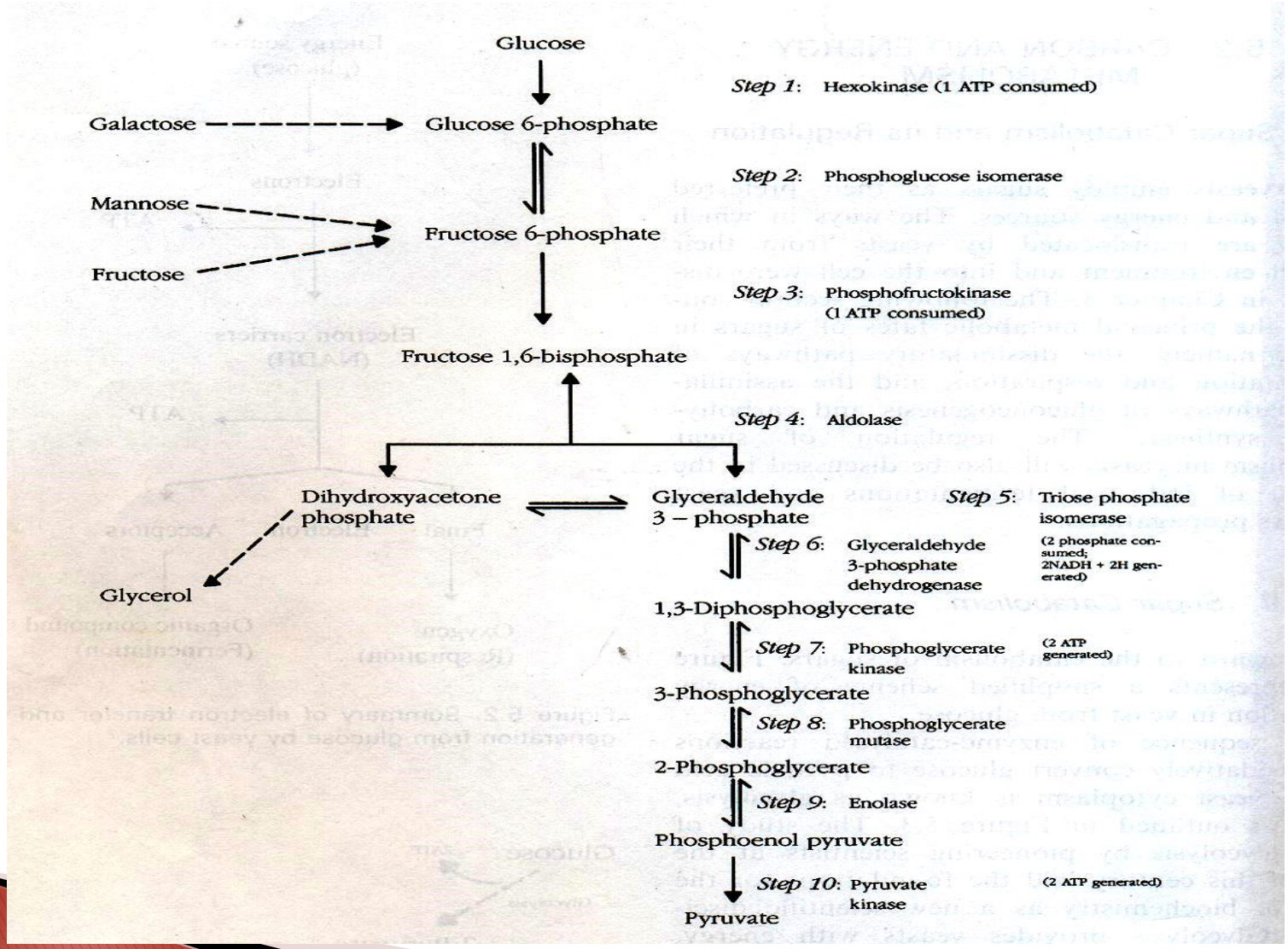
- Both final products of alcoholic fermentation, ethanol and carbon dioxide, are transported outside the cell by simple diffusion.

Yeast Metabolism

- Sugar Transport
  - Glucose is transported into the cells via facilitated diffusion with a non-energy requiring process.
- (Regardless of the metabolic route (TCA or EMP pathways), glucose and fructose metabolism begins with transport into the cell via specific transport systems. For both, transport is mediated via facilitated diffusion, driven simply by the concentration gradient.

- Glycolysis
  - Glucose is converted to pyruvic acid by enzyme-catalyzed reactions and energy is generated via process called substrate level phosphorylation.

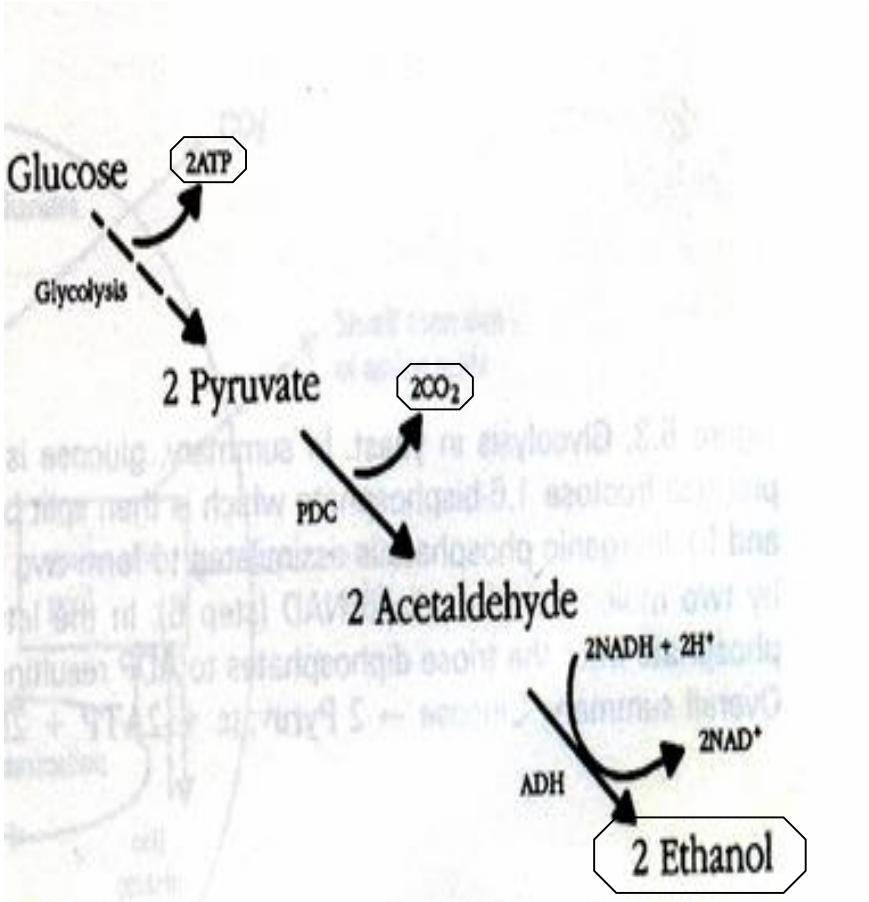
Glycolysis



(Walker 1998)

Alcoholic Fermentation

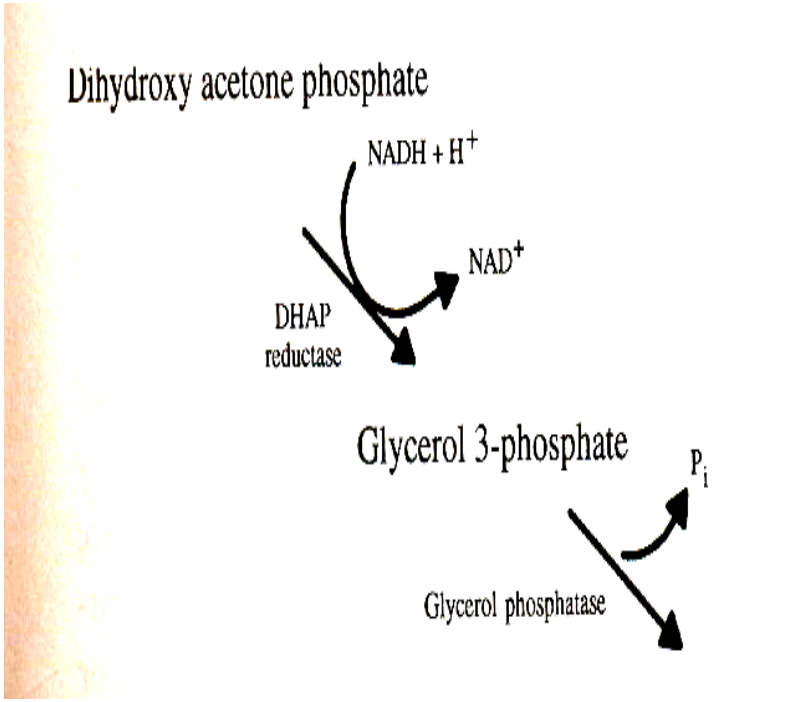
- Pyruvate is decarboxylated to acetaldehyde by pyruvate decarboxylase generating CO_2.
- Acetaldehyde is then reduced to ethanol by alcohol dehydrogenase.
- NADH is reoxidized to NAD^+ by transferring the hydrogen to acetaldehyde.
- End products of alcoholic fermentation:
  - 2 mol ethanol
  - 2 mol CO_2
  - 2 mol ATP per mole of glucose.



(Walker 1998)

Glycerol is quantitatively one of the most important products of yeast alcoholic fermentation, besides ethanol and carbon dioxide. This production can be enhanced by trapping acetaldehyde with sodium sulphite which prevents ethanol synthesis via alcohol dehydrogenase:

- **Glycerol formation**
  - Dihydroxy acetone phosphate is reduced to glycerol 3-phosphate by dihydroxy acetone phosphate reductase generating NAD^+ from NADH.
  - Glycerol 3-phosphate is then dephosphorylated yielding glycerol.
  - 2 moles of ATP is consumed and no ATP is generated by the conversion of one mole of glucose to two moles of glycerol.



(Walker 1998)