Cornell University

How to Feed Your Yeast

Nutrient Addition Strategies for Effective Fermentations

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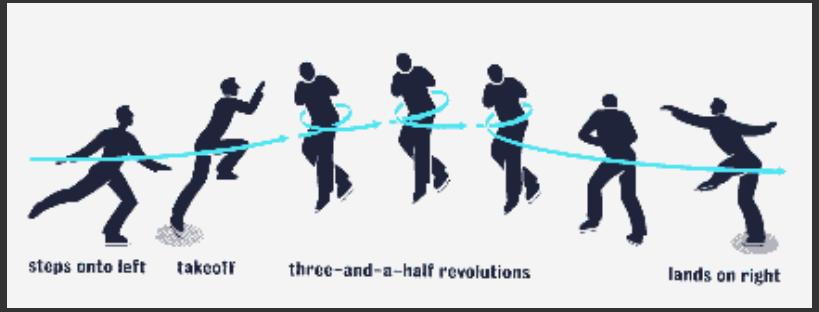
August 14th, 2010

Difficult vs. Onerous

- Some things are legitimately hard to do:
  - Neurosurgery
  - Triple Axel
  - Health Care Reform
- Some are just a pain:
  - Measure Carefully
  - Keep Glassware Clean
  - Wait Specified Times



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Objectives:

- What?
  - Yeast Nutrient Requirements
- Why?
  - Metabolic Pathways for Yeast Nutrients
- Where?
  - Yeast Nutrition Products
- When?
  - Effective Strategies for Feeding Your Yeast
- Resources for More Information

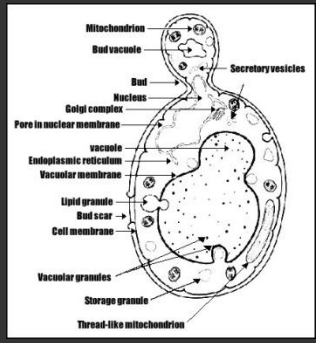
Surviving in a Hostile Environment

- Like any living organism, microbes need certain conditions to survive.
- Yeast & bacteria have temp., pH ranges that affect their growth and survival.
- They also have dietary needs and restrictions.

Yeast

- Yeast are present on grapes, in winery and in the air, so why do we need to add them?
- Control, behavior, performance- Added strains are much more likely to finish the fermentation quickly without creating off-aromas or other problems.

– Strains we inoculate with are selected from vineyards and wineries around the world as performing well. Almost all commercial strains are ***Saccharomyces cerevisiae.***

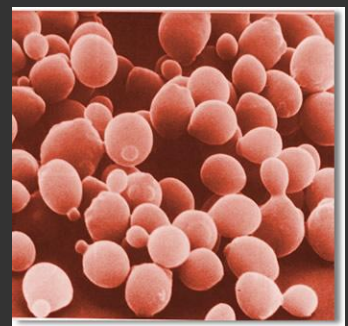


Yeast: Inhibitory Factors

- High or low pH
- Lack of nutrients 
- High or low temperature
- High alcohol
- Rapid temperature changes (temperature shock)

Yeast Hydration

- Weigh out 1 gram yeast per gallon of juice
  - 10 ml of water for each gram of yeast
  - Water should ideally be chlorine-free tap water, and *must* be at **40ºC/104ºF**
  - Let the yeast rehydrate (no stirring) for 20 minutes
  - Stir for 10 minutes



Yeast Inoculation

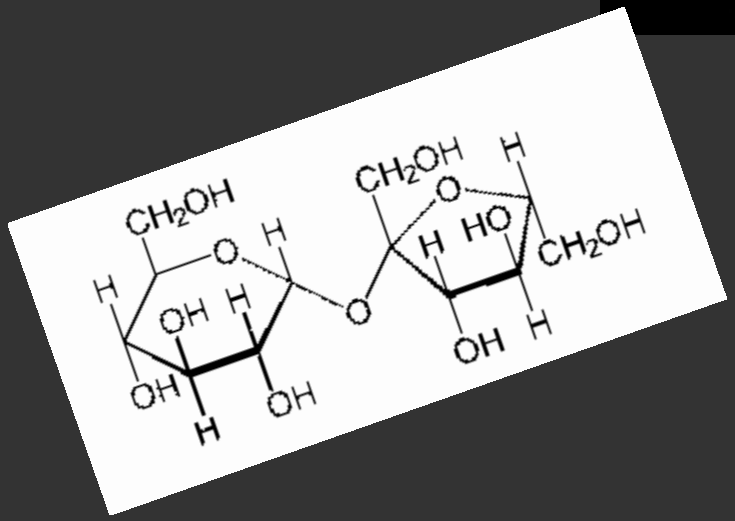
- Yeast should be within 10º of fermenter temp before it is added. Big temperature changes can cause shock.
  - Add juice from fermenter to yeast slurry in steps so as not to reduce temp of slurry more than 10º in one step, waiting 15 minutes between each step.
  - Example: Juice is 20º cooler than yeast, so I'll need 2 juice addition steps to cool the yeast.
  - Stir the tank after addition.

Nutrient Needs

- Carbon
  - From Sugars
- Nitrogen
  - Ammonium
  - Amino Acids
- Oxygen
- Vitamins
- Minerals

CARBON

$C_6H_{12}O_6$



Sugars

- Glycolysis Pathway
- Precursors for everything
  - ATP – Energy
  - Amino Acids
  - Ethanol
  - Acetic Acid

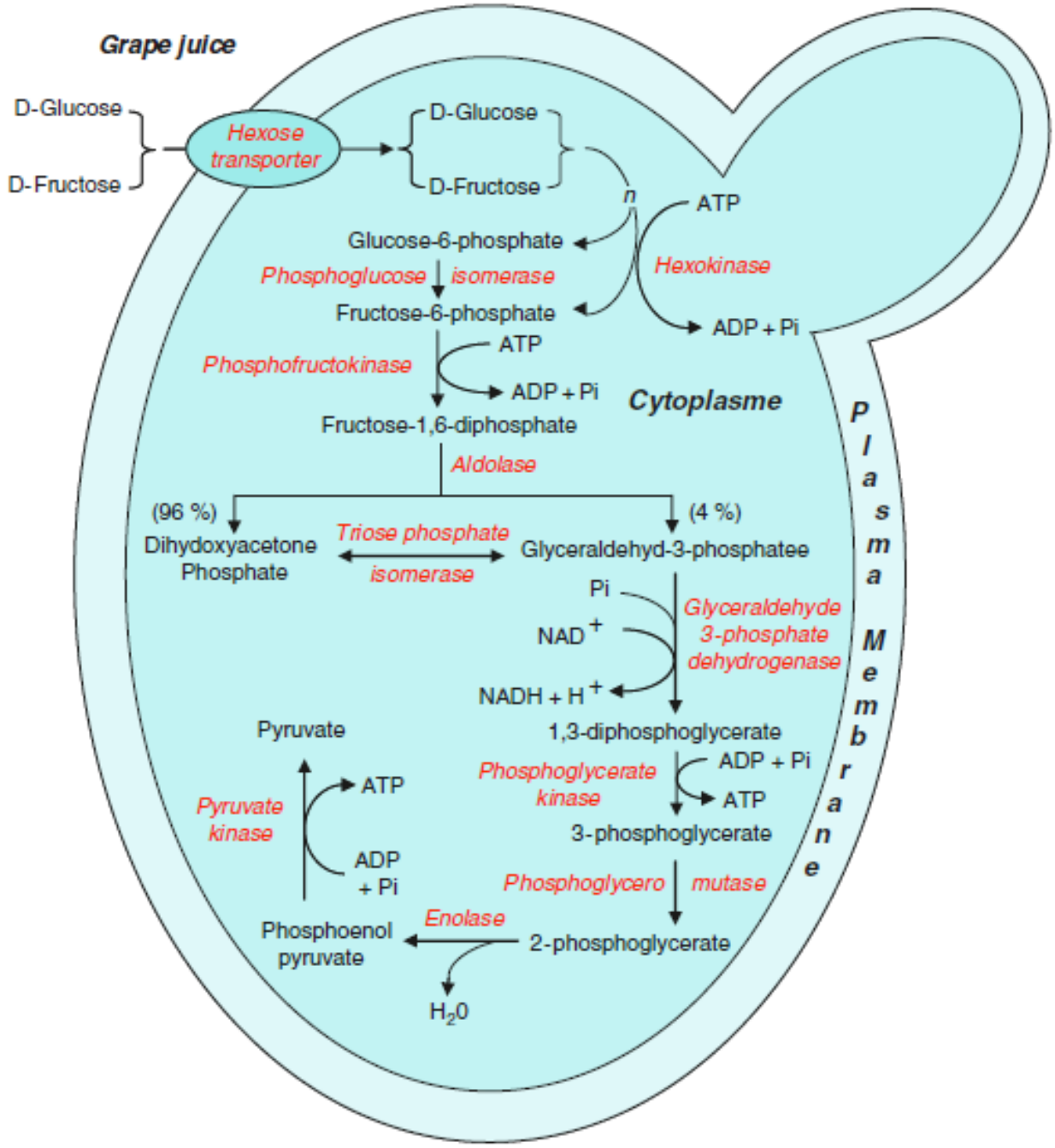


Fig. 1.2 Biochemical mechanism of glycolysis

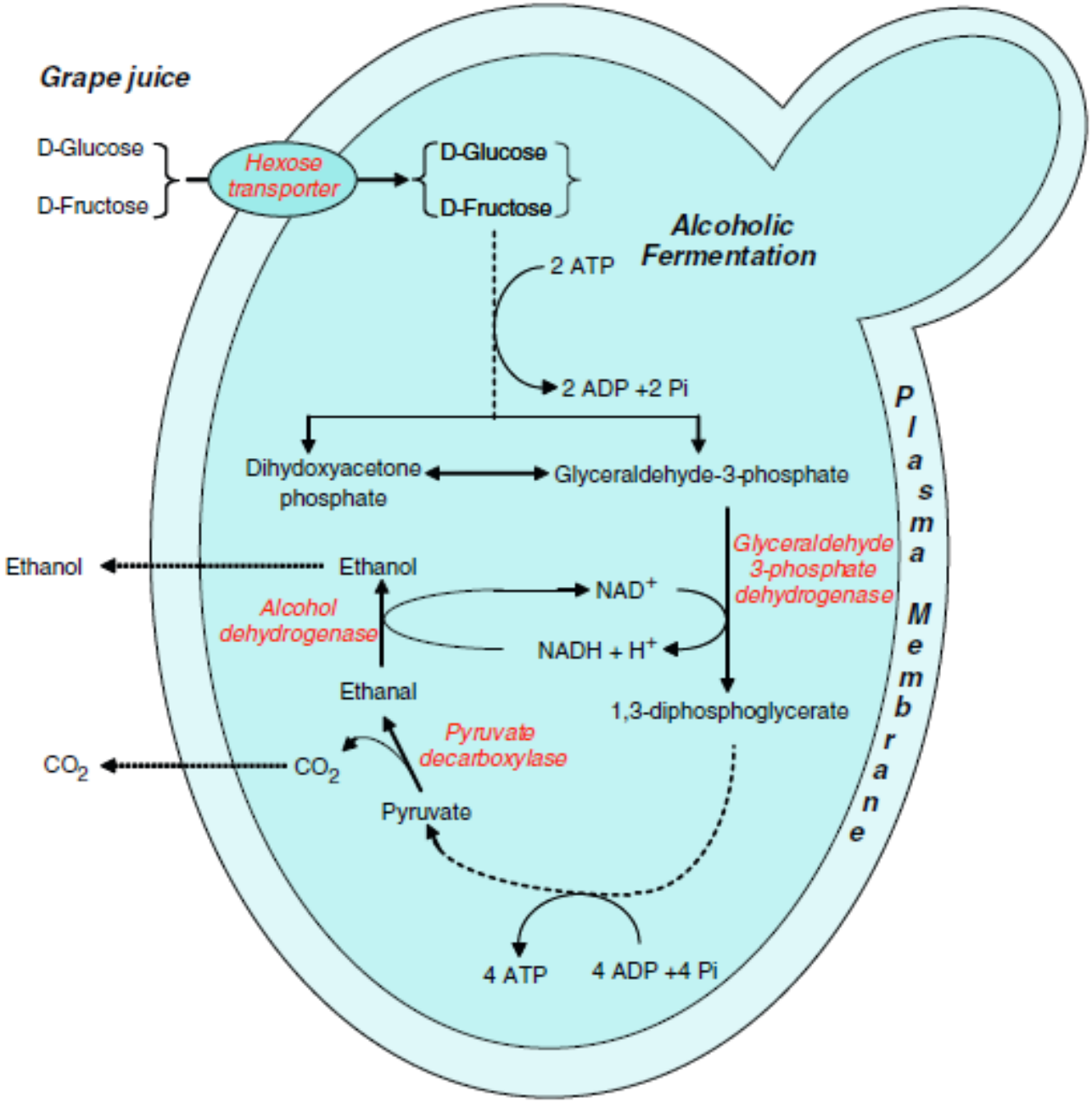
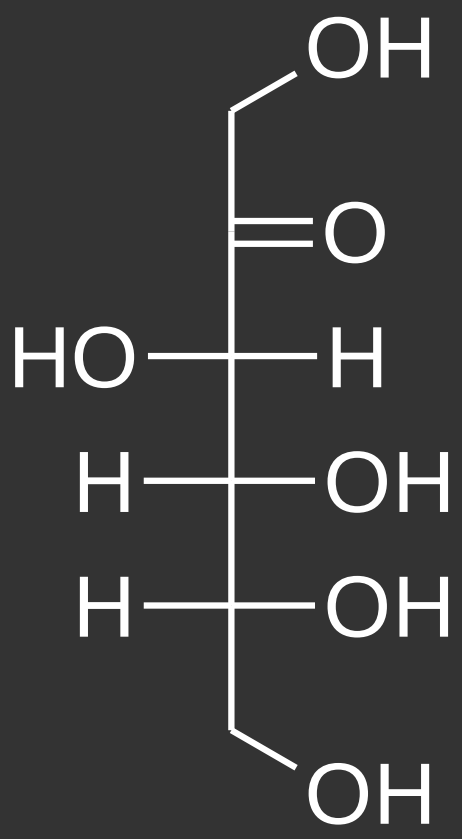


Fig. 1.4 Alcoholic fermentation

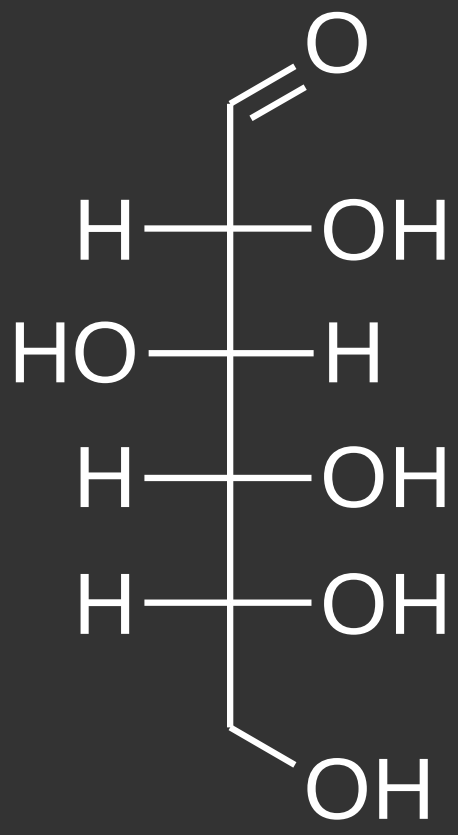
Sugars

- 180 – 250 g/kg (18-25 °Brix) in Must
- Glucose/Fructose Ratio
  - Must 0.75 – 1.1
  - Dry Wine 0.25
  - S. cerevisiae is glucophylic
    - Preferentially consumes glucose

°Brix of must	Alcohol % by vol
18	9.90
19	10.45
20	11.00
21	11.55
22	12.10
23	12.65
24	13.20

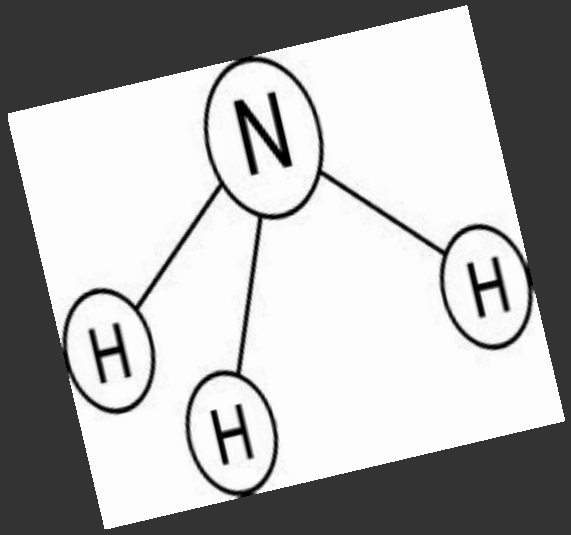


Fructose



Glucose

Nitrogen


N
H
H
H


www.haivchem.com
DAP
DIAMMONIUM PHOSPHATE
磷酸氢二铵
(NH4)2HPO4
什邡市海星化工有限公司

Nitrogen Requirements

- Amino Acids
  - Often yeast amino acid requirements are different from the available amino acids in grape must
  - Required for protein synthesis
- Cell Wall constituents
  - Chitin
- Nucleic Acids
  - Reproduction
  - Protein synthesis

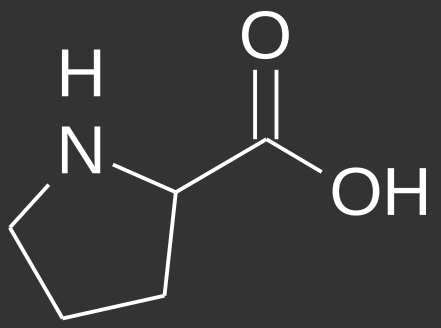
Effects of Nitrogen Deficiency

- Slow/Stuck Fermentations
- Hydrogen Sulfide (H_2S) Produced

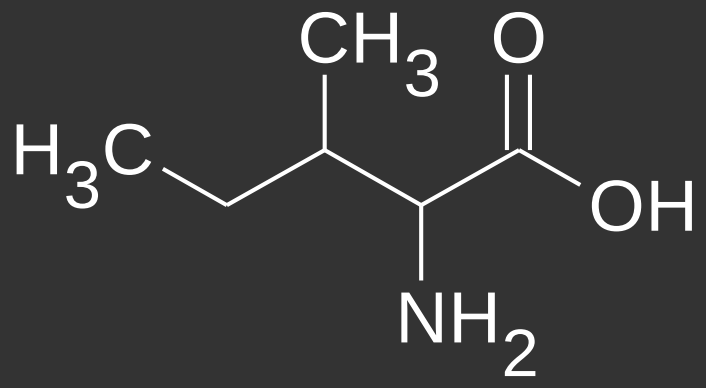
- Nitrogen Supplementation Common
  - Diammonium phosphate (DAP)
    - Can lead to a cleaner, but less complex wine

Available Nitrogen

- Yeast Assimilable Nitrogen (YAN) =
  $[NH_4^+]$ + [Primary Amino Nitrogen]



Proline –
Not Assimiable



Isoleucine –
Primary amino acid

YAN in wine

- Estimated that 14-25% of must is deficient (less than 150 mg N/l)
- Number could be higher in Finger Lakes

Brix of Must	Target YAN (mg N/L)
21	200
23	250
25	300
27	350

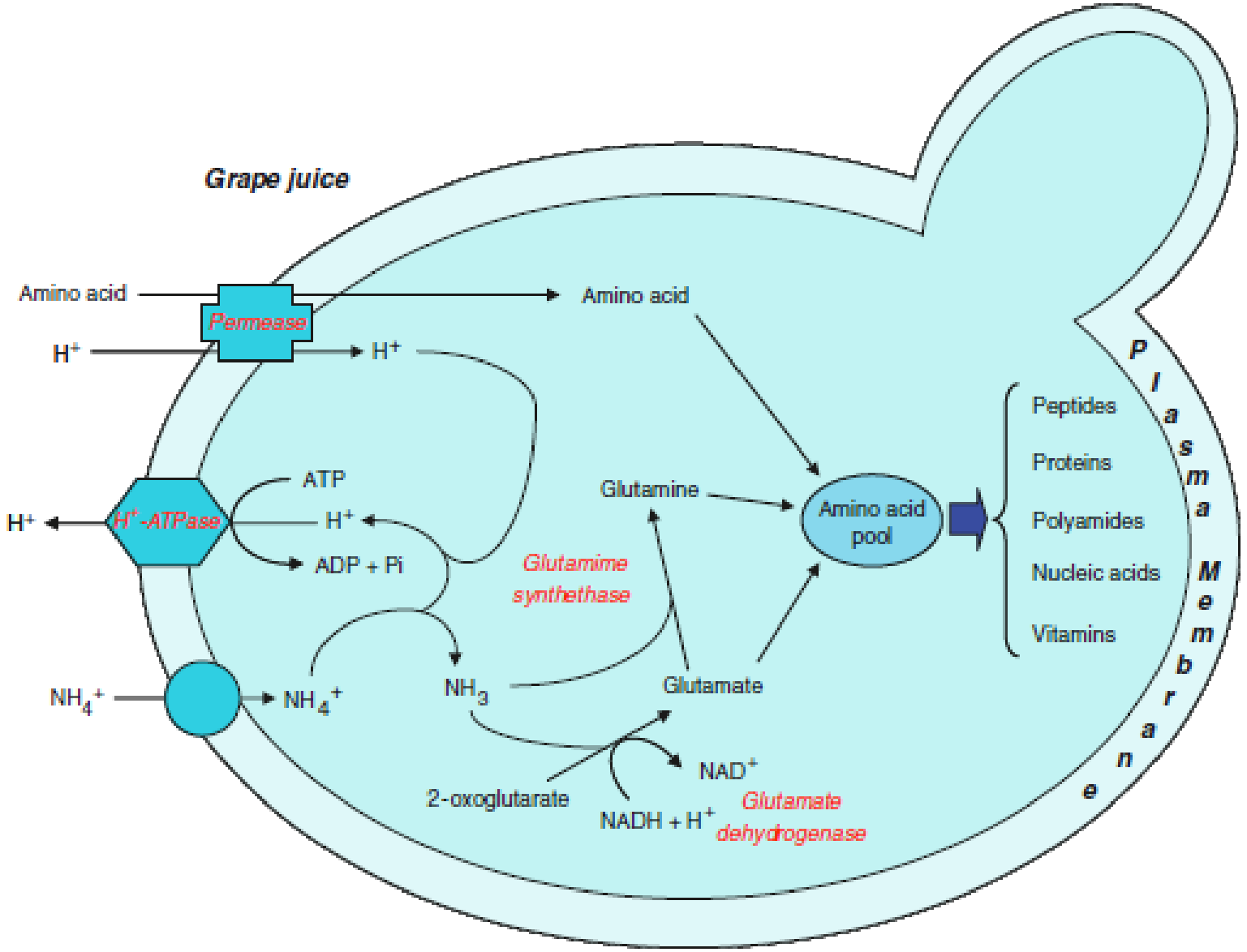
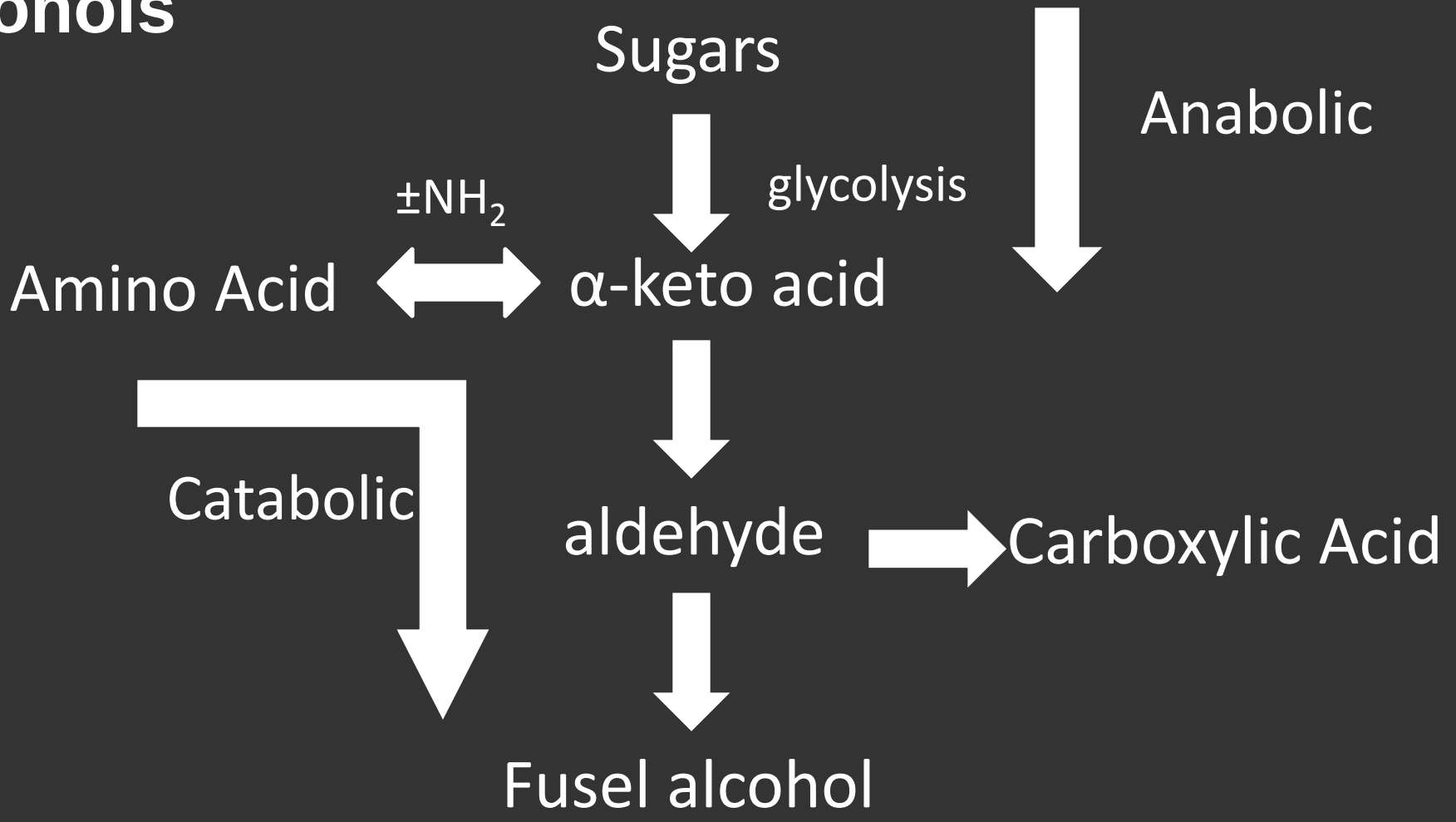


Fig. 1.6 Nitrogen metabolism

Relationship of Amino Acids and Higher Alcohols



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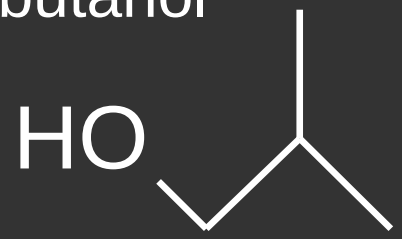
Amino Acids and Fusel Alcohols

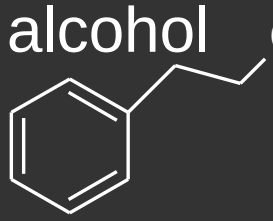
Alcohol

- Isobutanol



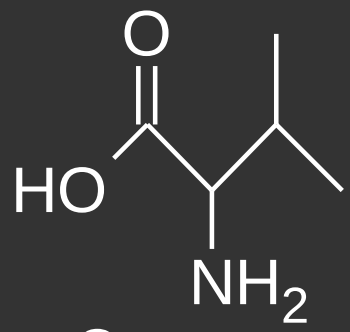
- Isoamyl Alcohol

- 2-Phenylethyl alcohol

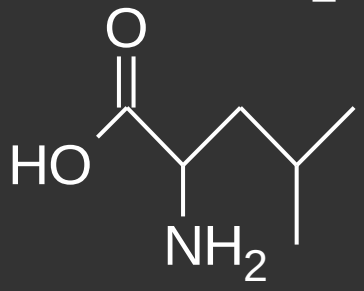


Related Amino Acid

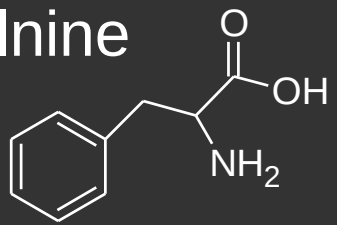
- Valine



- Leucine

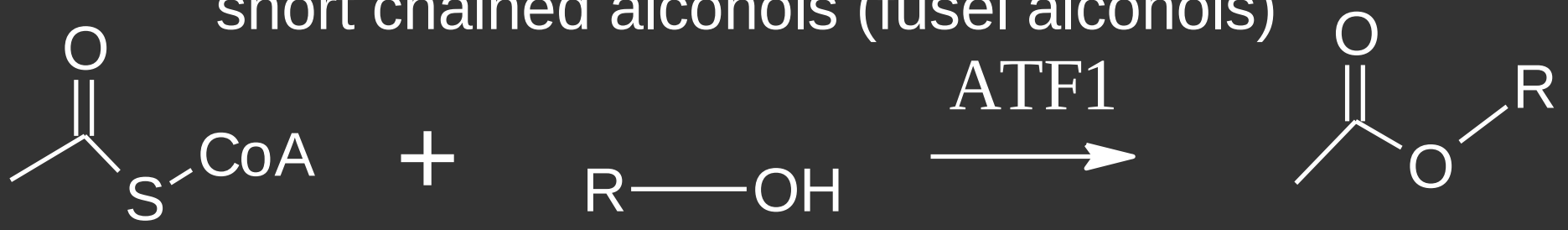


- Phenylalnine



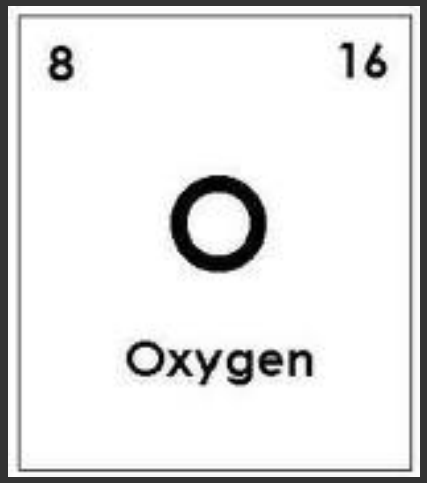
Formation of Acetate Esters

- Enzymatically formed from acetyl-CoA and short chained alcohols (fusel alcohols)



Acetyl-CoA Alcohol Acetate Ester

Oxygen

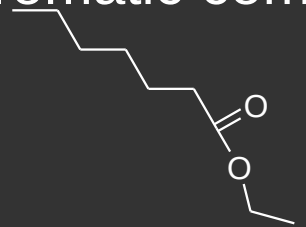


Oxygen in Yeast Metabolism

- Yeast are Facultative Anaerobe
  - Can metabolize carbohydrates aerobically and anaerobically
    - Aerobic respiration (TCA cycle) much more efficient than anaerobic 38 ATP vs 2 ATP
    - Byproduct of anaerobic metabolism is ethanol

Oxygen Requirements

- Sterols
- Unsaturated fatty acids
  - Provide fluidity to cell membrane especially during cold fermentations
  - In absense of O_2 production of short chain fatty acids can be produced
    - Esters of medium and short chain fatty acids can be esterified to form fruity aromatic compounds
    - Ethyl octanoate



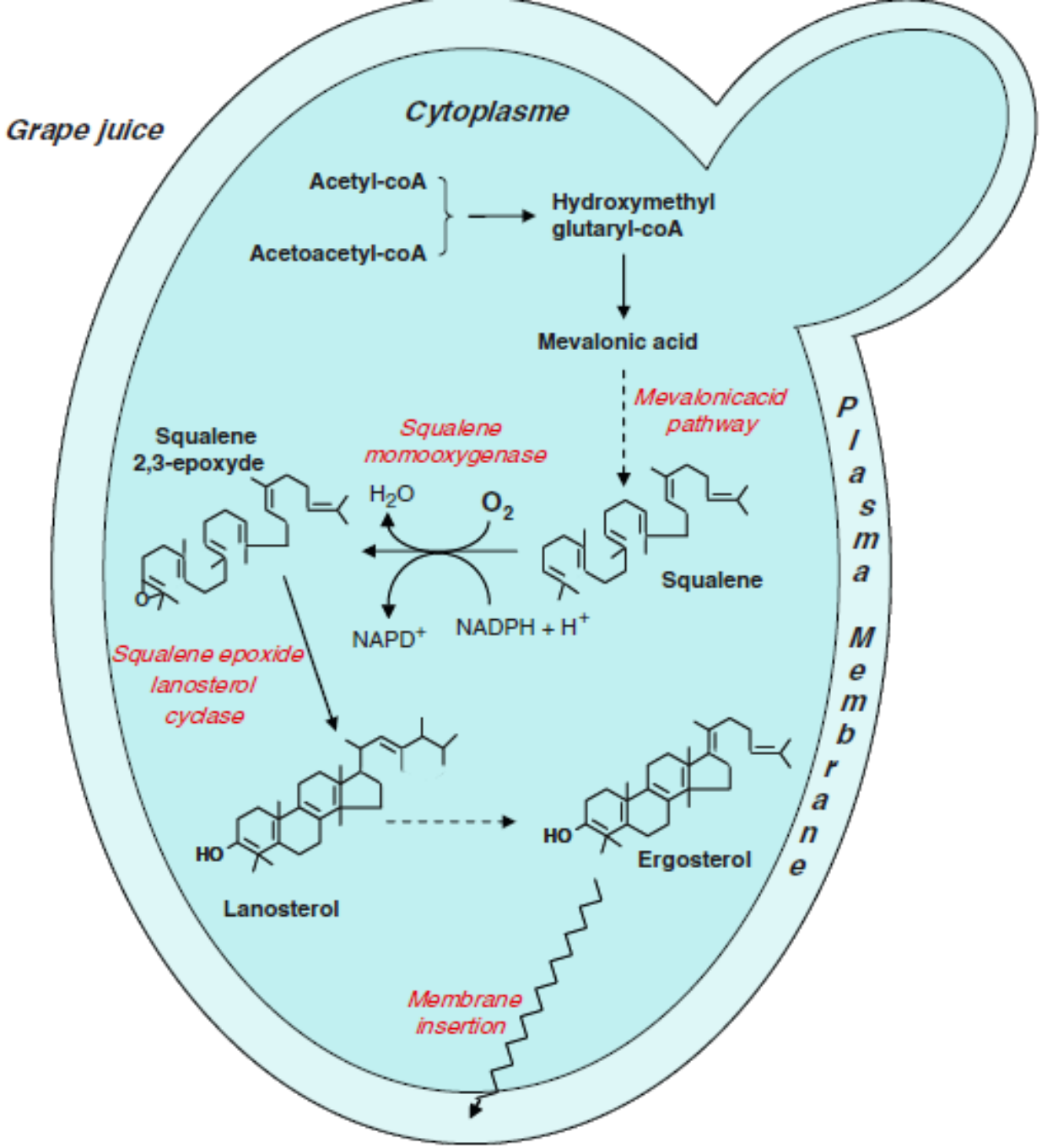


Fig. 1.7 Synthesis of ergosterol in yeasts

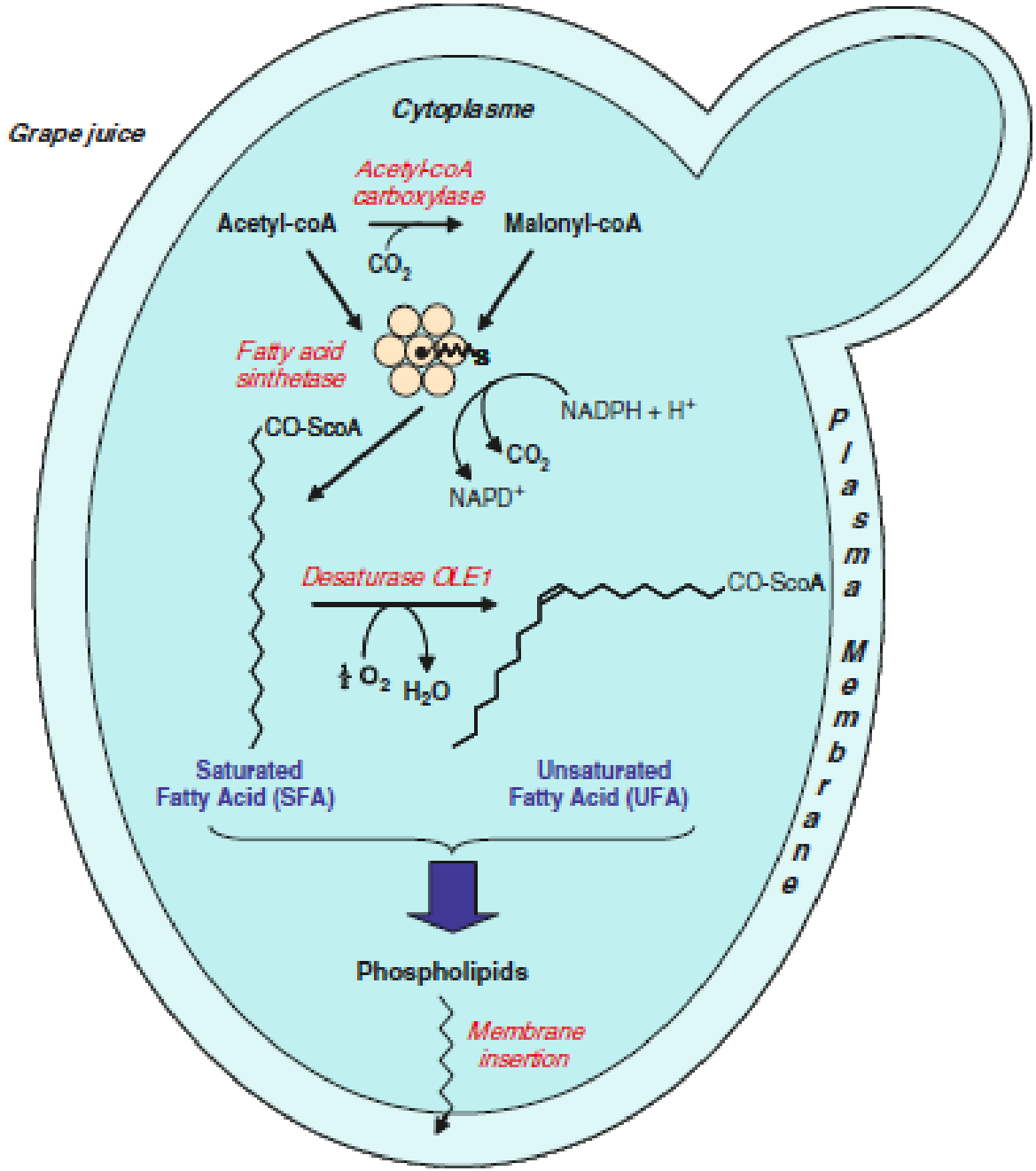
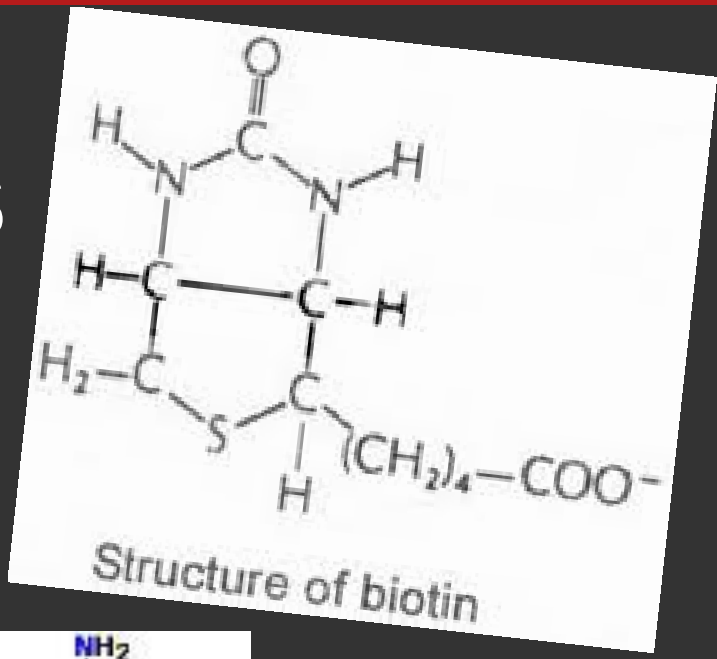
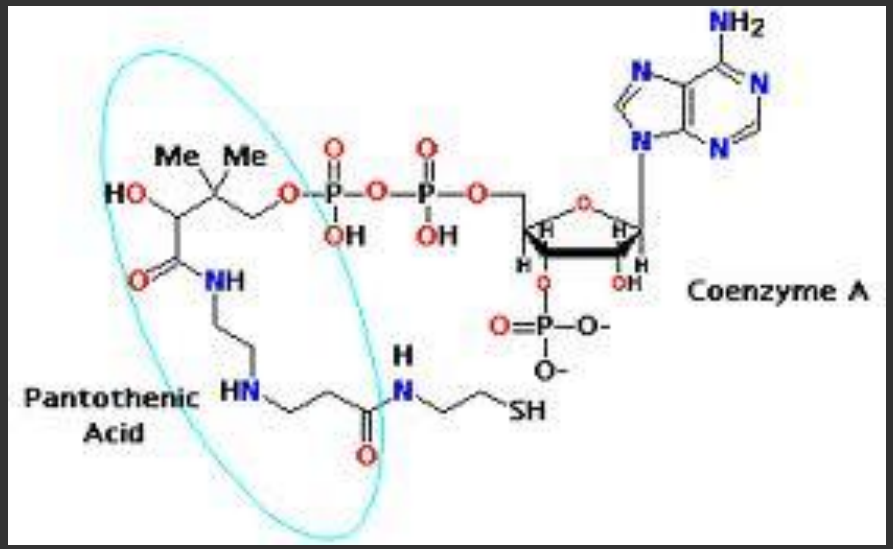


Fig. 1.8 Synthesis of fatty acids in yeasts

Vitamins


Structure of biotin


Me Me
HO
NH
HN
H
N
SH
Pantothenic Acid
NH2
Coenzyme A

Essential Vitamins

- Pantothenic Acid
- Biotin
- Thiamin
- Inostitol
- Nicotinic acid

Pantothenic Acid

- Required for synthesis of Coenzyme A
  - Utilized in sugar and lipid synthesis
- Deficiency associated with hydrogen sulfide production (H_2S)

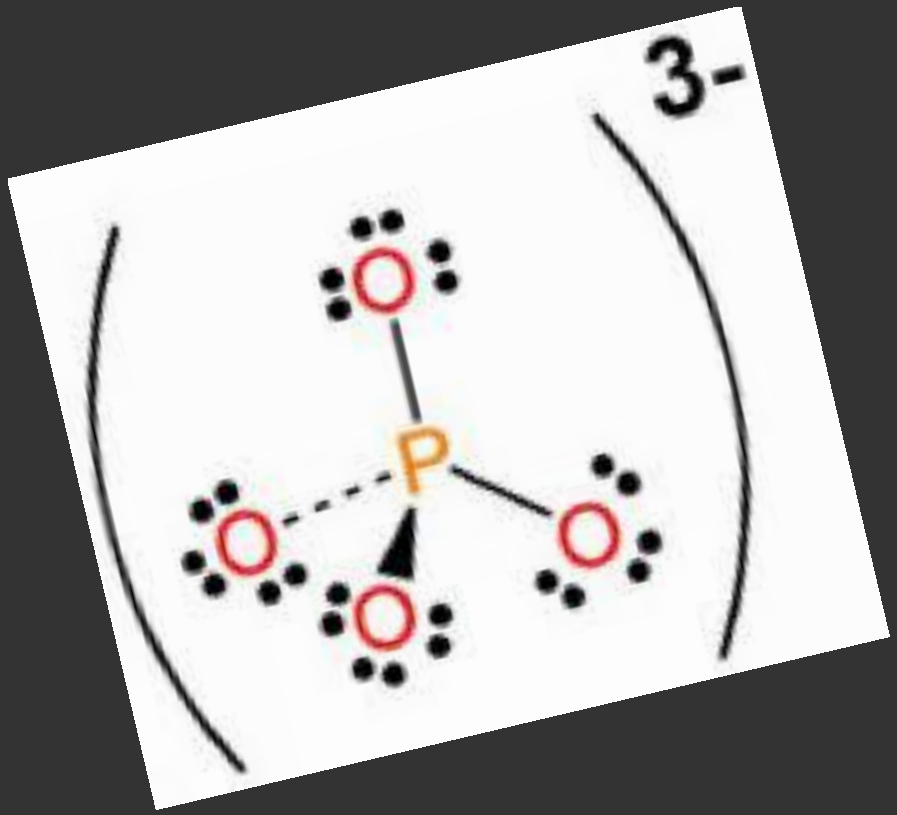
Biotin

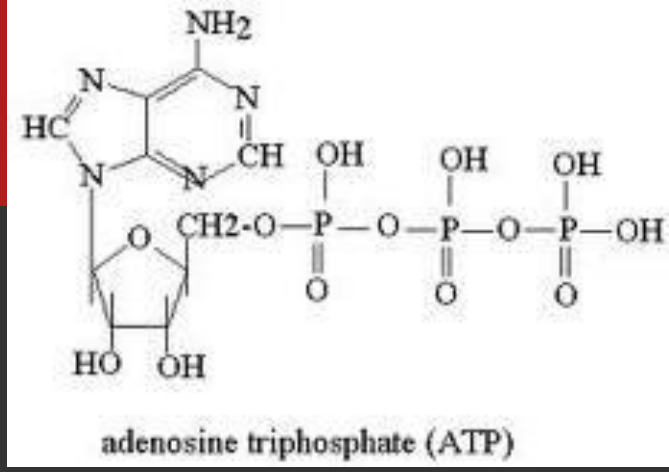
- Required for carboxylation of pyruvate
  - Synthesis of:
    - Nucleic acid
    - Protein
    - Fatty acid

Thiamin, Inositol, and Nicotinic Acid

- All needed for cell devision
- Synthesis of NAD^+

Minerals





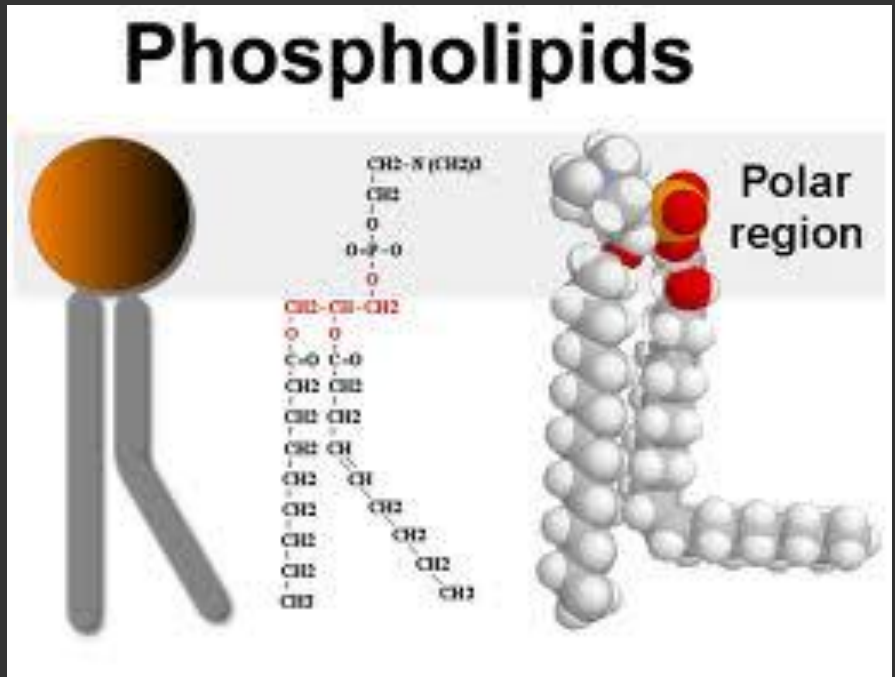
adenosine triphosphate (ATP)

Potassium

- Required for Phosphate uptake
- Cell osmotic balance

Phosphate

- Nucleic Acids
- ATP
- Phospholipids



Nutrient Supplements

Nitrogen Inorganic (Ammonium Salts)

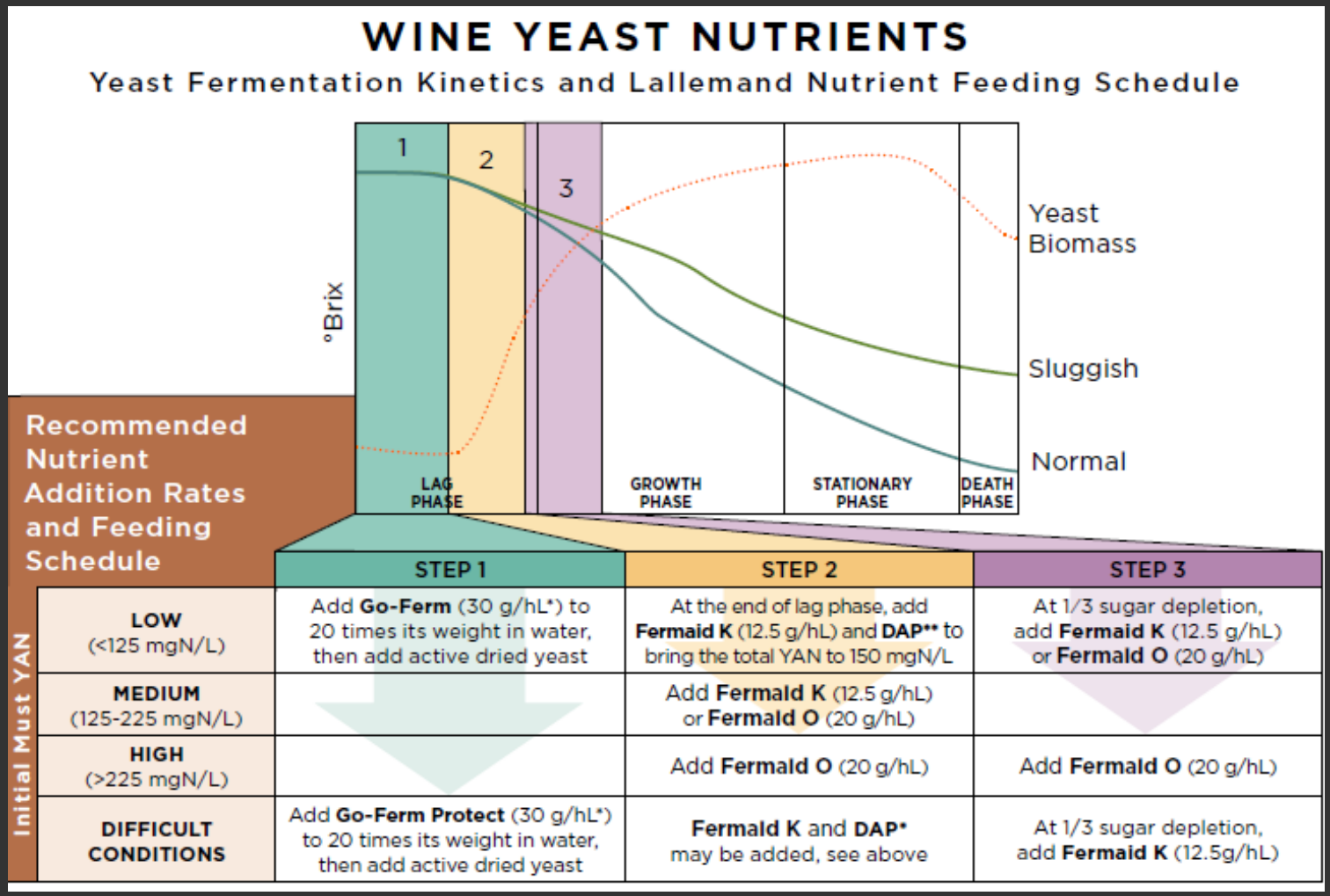
- Diammonium Phosphate (DAP)
  - 21% N
  - 100 mg N = 0.47 g DAP
  - About 9 g per 5 gal
  - 100 mg N/l is a good prophylactic dose

Nitrogen Organic (Autolysed Yeast)

- Bioactiv
- Dynastart
- Fermaid K (both organic and inorganic N)
- Fermaid O
- Fortiferm
- Go-Ferm

Many of these products contain vitamins and minerals as well

Lallemand Wine Yeast Nutrient Addition



Where to Go for More Information

- Scott Labs Fermentation Handbook
  - Electronic copy at www.scottlab.com
  - Many different types of yeast, nutrients, fining agents, ect.
  - Practical resource, updated yearly
- Wine Chemistry and Biochemistry

  M. Victoria Moreno-Arribas & M. Carmen Polo
- Wine Microbiology

  Kenneth C. Fugelsang & Charles G. Edwards