SIEBEL INSTITUTE OF TECHNOLOGY

The Flavor is in the Fermentation

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Control of Fermentation Flavors

- What are the primary fermentation flavor compounds?
- How does yeast make them?
- What influences their production?
- How can they be controlled?

Why do we want to control Fermentation?

- Consumer expects a consistent quality product
- Flavor matching
- Production planning, utilization
- Efficiency

General Concept

WORT
(sugars, amino acids, etc.)

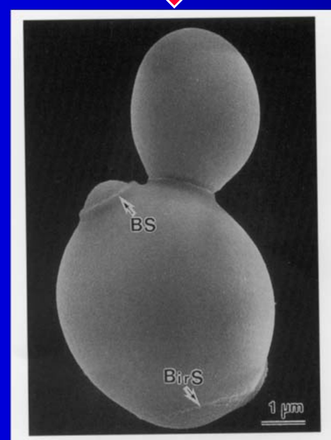
bacteria, wild yeast

Yeast autolytic compounds

Phenols

Sulfur compounds

Aldehydes & ketones



Esters

Acids (organic & fatty)

Alcohols (ethanol & fusel oils)

Glycerol

Examples of flavor-active compounds from yeast

- **Acids** **eg. Acetic acid**
- **Alcohols** **eg. Ethanol**
- **Esters** **eg. Ethyl acetate**
- **Aldehydes** **eg. Acetaldehyde**
- **Ketones** **eg. Diacetyl**
- **S-Compounds** **eg. Hydrogen sulfide**
- **Phenolics** **eg. 4-Vinyl guaiacol**

Typical flavor levels

Fermentation Product	Levels
Ethanol, CO_2 , Glycerol	g/l
Higher Alcohols, Organic acids Short chain fatty acids Aldehydes, SO_2, Keto acids Acetoin, 2,3 Butanediol	mg/l
H_2S, diacetyl, DMS	μg/l

Flavor and taste of some by-products

by-product	**flavour and taste**
acetaldehyde	unripe apple
ethyl-acetate	acetone, solvent
i-amyl-acetate	fruity, banana
n-propanol	alcohol
iso-butanol	pharmacy
i-amyl-alcohols	bitter
phenylethanol	roses
diacetyl	butter, honey

Average concentration of some by-products

compound [mg/l]	variability	average
n-propanol	5 - 17	10
i-butanol	4 - 14	8
i-amylalcohols	34 - 73	55
2-phenylethanol	5 - 50	18
ethylacetate	9 - 35	20
isoamylacetate	0,4 - 3,1	1,4
acetaldehde	2 - 19	9
diacetyl	0,01 - 0,15	0,09
2,3-pentandione	0,01 - 0,35	0,04

Significant Fermentation Related Flavors

- Effect of fermentation variables on flavor components
  - Organic Acids
  - Fatty Acids
  - **Acetaldehyde**
  - **Glycerol**
  - **Higher alcohols**
  - **Esters**
  - **Vicinal Diketones - Diacetyl**
  - Phenolics
  - Sulfur volatiles
  - **Yeast autolysis**

Fermentation Flavor Control Variables

- Yeast Strain
- Yeast Condition
- Wort Composition
- Temperature Profile
- Aeration
- Pitching

Acetaldehyde – Green Beer Flavour

- An important carbonyl is acetaldehyde; a normal intermediate product of fermentation
- Peaks during fermentation, then declines
- Formation occurs during the first three days of fermentation
- Intermediate from carbohydrate to ethanol production, 1.2 – 24.4 mg/l
- **Zinc** mediates conversion to ethanol
- Permanent reduction during post-fermentation and maturation by CO_2 washing (evaporation-volatile)
- Depends on yeast strain

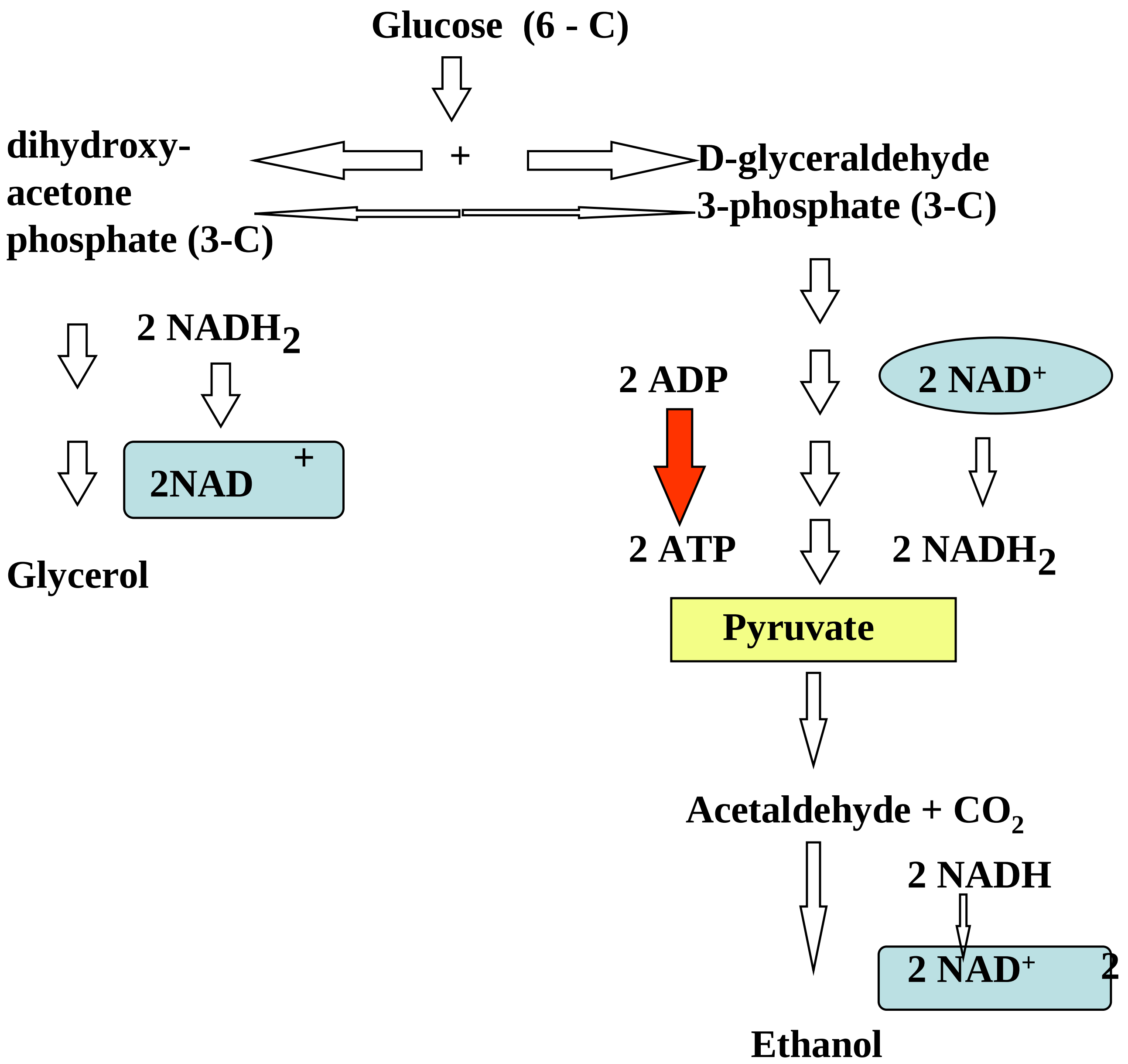
Acetaldehyde – Green Beer Flavour

- Concentration **increases** by:
  - intensive fermentation
    - high fermentation temperature
    - excessive aeration
    - stirred fermentations
  - high pitching rates
  - high pH
  - pressure during fermentation
  - can be complexed by sulfite
  - infection
- Concentration **reduces** by:
  - intensive post-fermentation and maturation
  - high yeast concentration during maturation

Glycerol Production by Yeast

Glycerol is quantitatively one of the most important products of yeast fermentation and contributes to the viscosity and "body" of beer (and wine).

Glycolysis


Glucose (6 - C)
+
dihydroxy-
acetone
phosphate (3-C)
D-glyceraldehyde
3-phosphate (3-C)
2 NADH$_2$
2NAD$^+$
Glycerol
2 ADP
2 NAD$^+$
2 ATP
2 NADH$_2$
Pyruvate
Acetaldehyde + CO_2
2 NADH
2 NAD$^+$
2
Ethanol

Higher Alcohol (fusel oil)

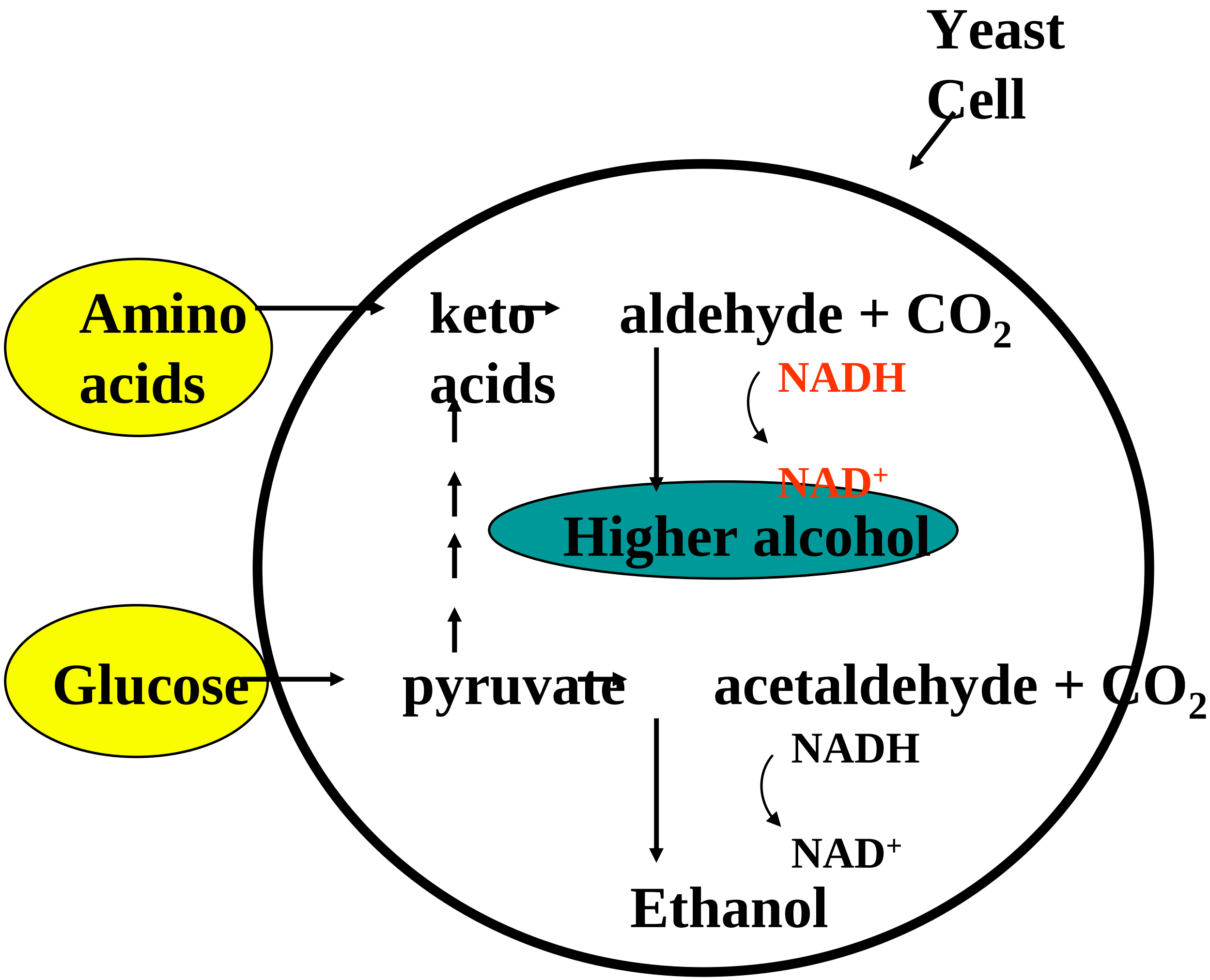
More than 40 alcohols identified in beer!

Compound	Threshold (mg/l)	Aroma or Taste	Bottom Fermentation	Top Fermentation
n-propanol	600 – 800	alcohol	7 – 9	20 - 45
iso-butanol	100 – 200	alcohol	4 – 20	10 - 24
2-methylbutanol	50 - 70	alcohol	9 - 25	80 - 140
3-methylbutanol	50 - 65	fusel, pungent	25 - 75	80 - 140
2-phenylethanol	5 - 75	rose, perfume	11 – 51	8 - 50
Tyrosol	10 - 20	bitter	6 – 15	8 - 22
Tryptophol	10 – 20	almonds	0.5 – 14	2 - 12

Higher Alcohol (fusel oil) Metabolism by Yeast

- Formed as a by-product of protein synthesis from keto-acids

HIGHER ALCOHOLS



Higher Alcohol (fusel oil) Metabolism by Yeast

- When amino acids are sufficient (early in fermentation) fusel oils originate from the CATABOLIC PATHWAY (Ehrlich pathway)

amino acid $\longrightarrow$ α-ketoacid $\longrightarrow$ higher alcohol

- When amino acids are deficient (later in fermentation) they originate from the ANABOLIC PATHWAY from pyruvate

carbohydrate $\longrightarrow$ pyruvate $\longrightarrow$ α-ketoacid $\longrightarrow$ amino acid

α-ketoacid $\longrightarrow$ higher alcohols

Example: isobutanol production

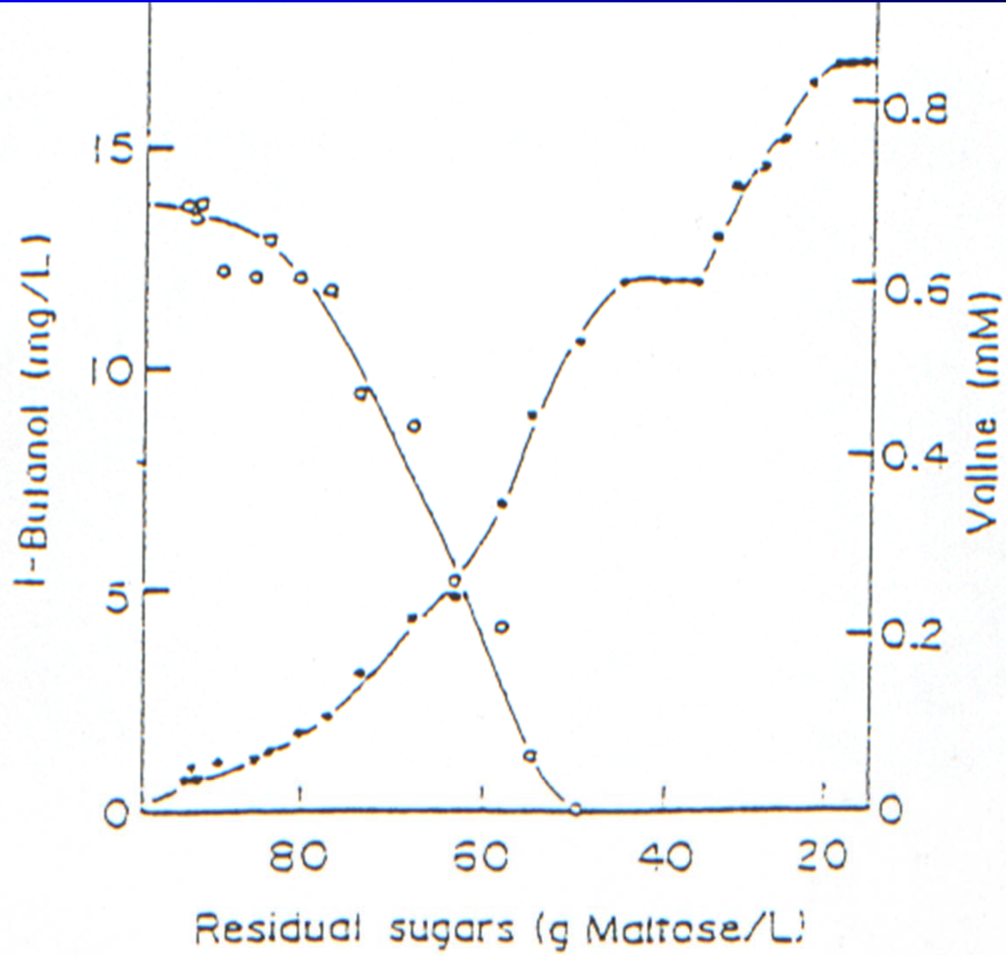


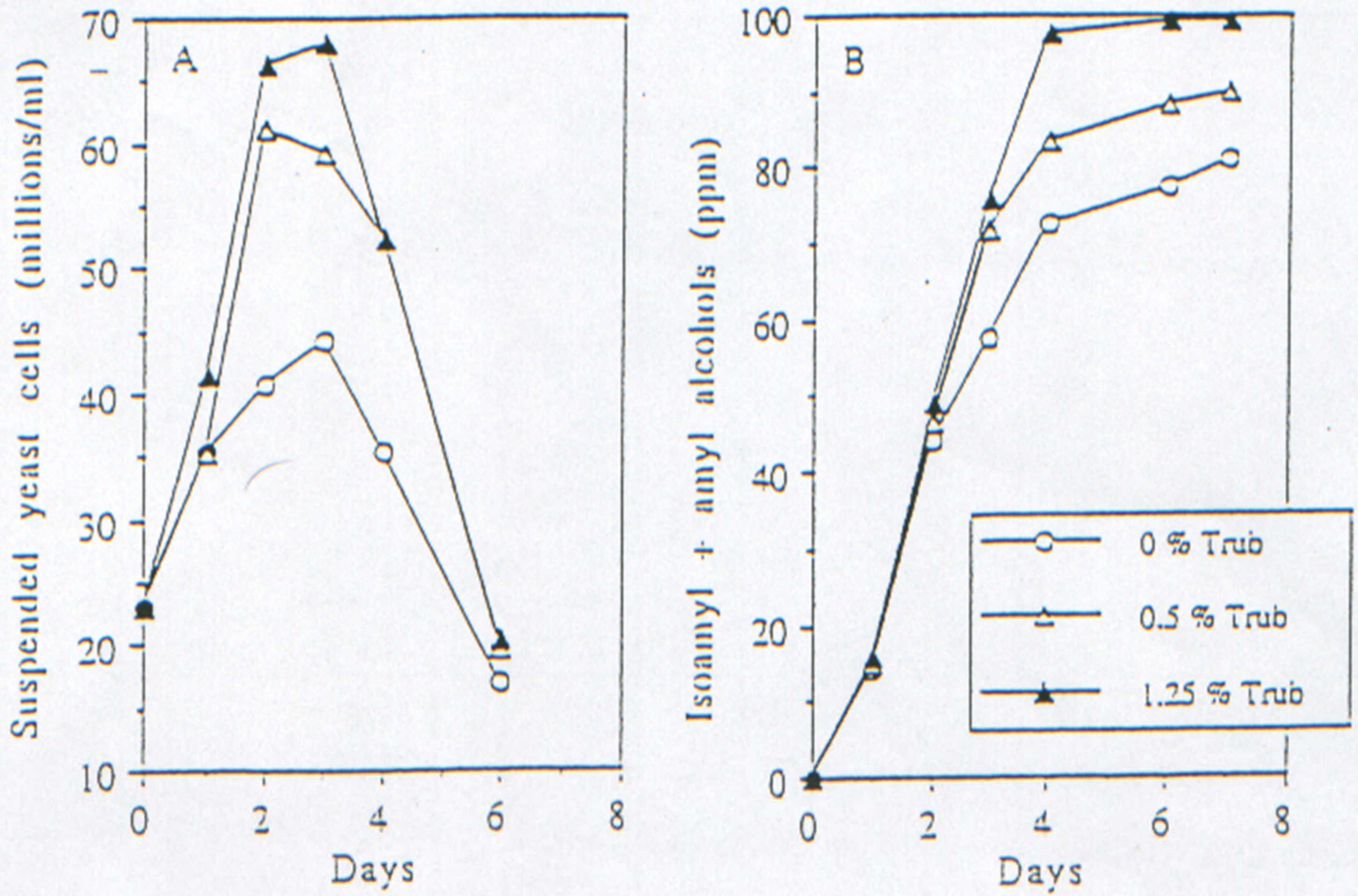
Fig. 5. Valine consumption and isobutanol formation during stirred wort fermentation at 20°C. ○: valine; ●: isobutanol.

Factors effecting Fusel Alcohol production by yeast

- **Extent of yeast growth**
  - Conditions **promoting yeast growth** (increased O_2) result in increased fusel alcohol production

Higher alcohols

- increased by
  - ↑ **Yeast Growth**
    - high fermentation temp
    - stirring and pumping
    - high concentration of amino-acids in wort, higher FAN utilization
    - intensive aeration
- reduced by
  - ↓ **Yeast Growth**
    - high pitching rate
    - cold pitching temperature and fermentation
    - pressure during fermentation
    - avoid oxygen after pitching



'igure 7. Relationship between yeast growth (A) and isoamyl (+ amyl) alcohol synthesis (B) (from Sa Almeida et al., 1989).

Ester production by yeast

- Over 90 distinct esters in beer - flowery and fruity flavours and aromas
- Desirable at low concentrations, but undesirable at high concentrations
- Important esters: ethyl acetate, isoamyl acetate,
- Produced by reaction of fatty acids with alcohols

Ethanol + acetyl CoA----->Ethyl acetate + CoA

Control

- Ester synthesis not that simple.
- No direct relationship between yeast growth and ester synthesis.
- The amount of ester formed will depend on :

- The amount of the acid (Acyl CoA compounds)
- The amount and activity of the enzyme (Acyl-alcohol transferase)
- The amount of the higher alcohol

Factors effecting Ester Production

- Yeast strain dependent
- Wort gravity - higher °P, higher esters
- Fermentation temperature – slightly increases fruity esters, high temperature increases floral esters
- Pitching rate - low rates decrease esters (Quantitatively)
- Oxygen - low wort O_2 enhances esters
- Zinc - promotes esters
- Fermenter pressure - reduces yeast growth and esters

Esters

- enhanced by
  - yeast strain
  - high gravity
  - high fermentation degree
  - low wort aeration
- reduced by
  - low wort concentration
  - pressure during fermentation
  - deep fermenters
  - higher lipid content

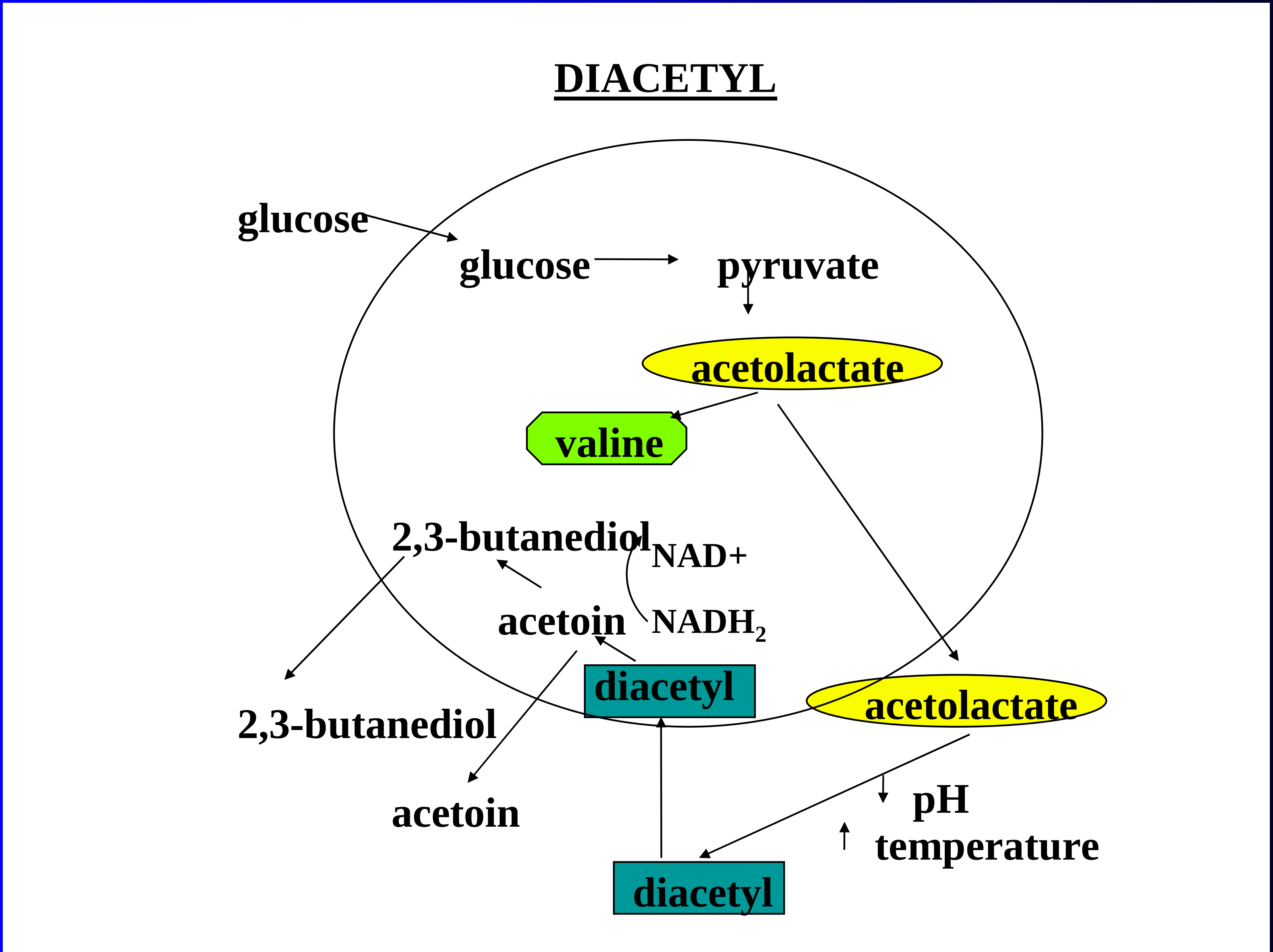
Production of Carbonyls by Yeast

- Several carbonyls have important flavour effects on beer: eg. acetaldehyde (unripe apples) and diacetyl (rancid butter)
- Diacetyl ($CH_3COCOCH_3$) has a very low flavour threshold, 0.1ppm
- A critical aspect of fermentation management and beer maturation is the control of diacetyl

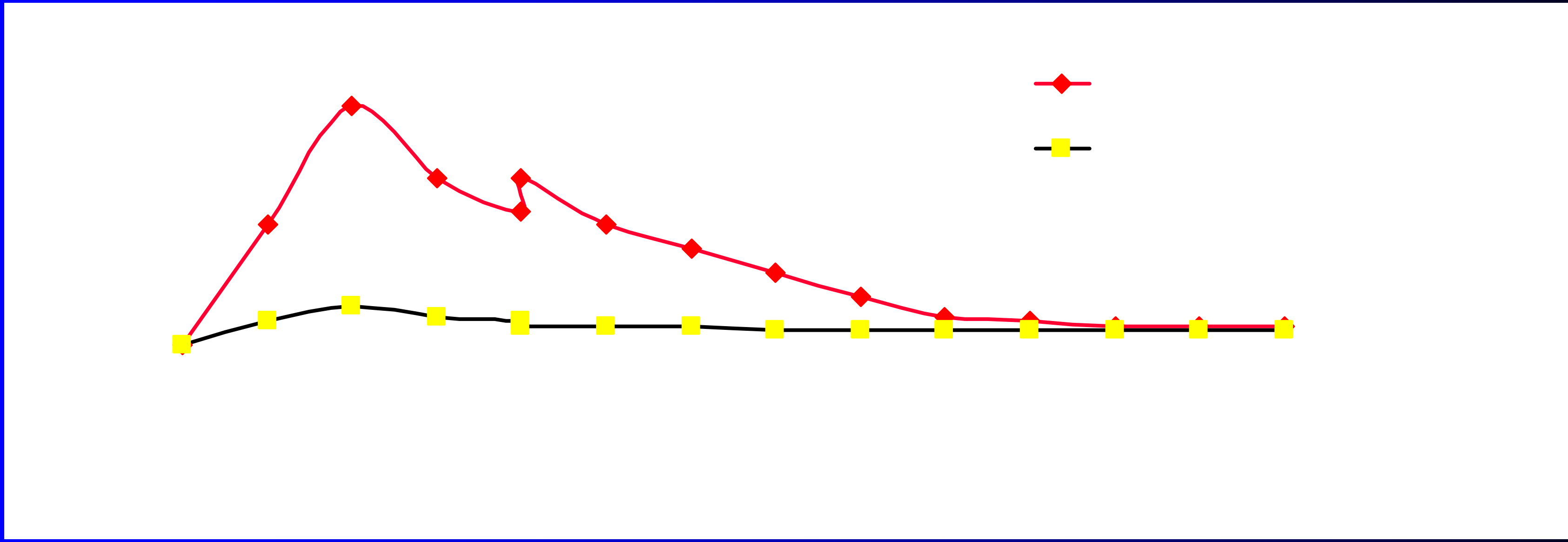
Diacetyl

- VDK (butter) flavour
- Diacetyl accounts for 80-90% of VDK flavor while remainder is from 2,3-pentanedione

Diacetyl


DIACETYL
glucose
glucose
pyruvate
acetolactate
valine
2,3-butanediol
NAD+
acetoin
NADH$_2$
diacetyl
acetolactate
2,3-butanediol
acetoin
pH
temperature
diacetyl

Development of Diacetyl



- during the first days of main fermentation the aceto-hydroxy-acids increase drastically
- uptake of oxygen increases content again
- during secondary fermentation, diacetyl steadily reduced

Reduction of Diacetyl Concentration

- Reduced by
  - low pH
  - active yeast
  - high yeast cell count
  - low pressure
  - low fermentation temperature and higher end fermentation temperatures
  - long storage on yeast (i.e., diacetyl rest)

Some Strategies for Diacetyl Reduction

- Fermentation temperature control
- Increased temperature half way through fermentation (speeds up diacetyl reduction)
- Traditional “lagering”

Diacetyl Diagnostic Test

- **Method :**
  - Sample beer from the fermenter
  - Remove the yeast (filter through filter paper)

$\swarrow$ $^1/_2$ $\quad$ $^1/_2$ $\searrow$

A	**B**
Refrigerate	Heat to 60°C for 1/2 hr
Taste	Cool and taste

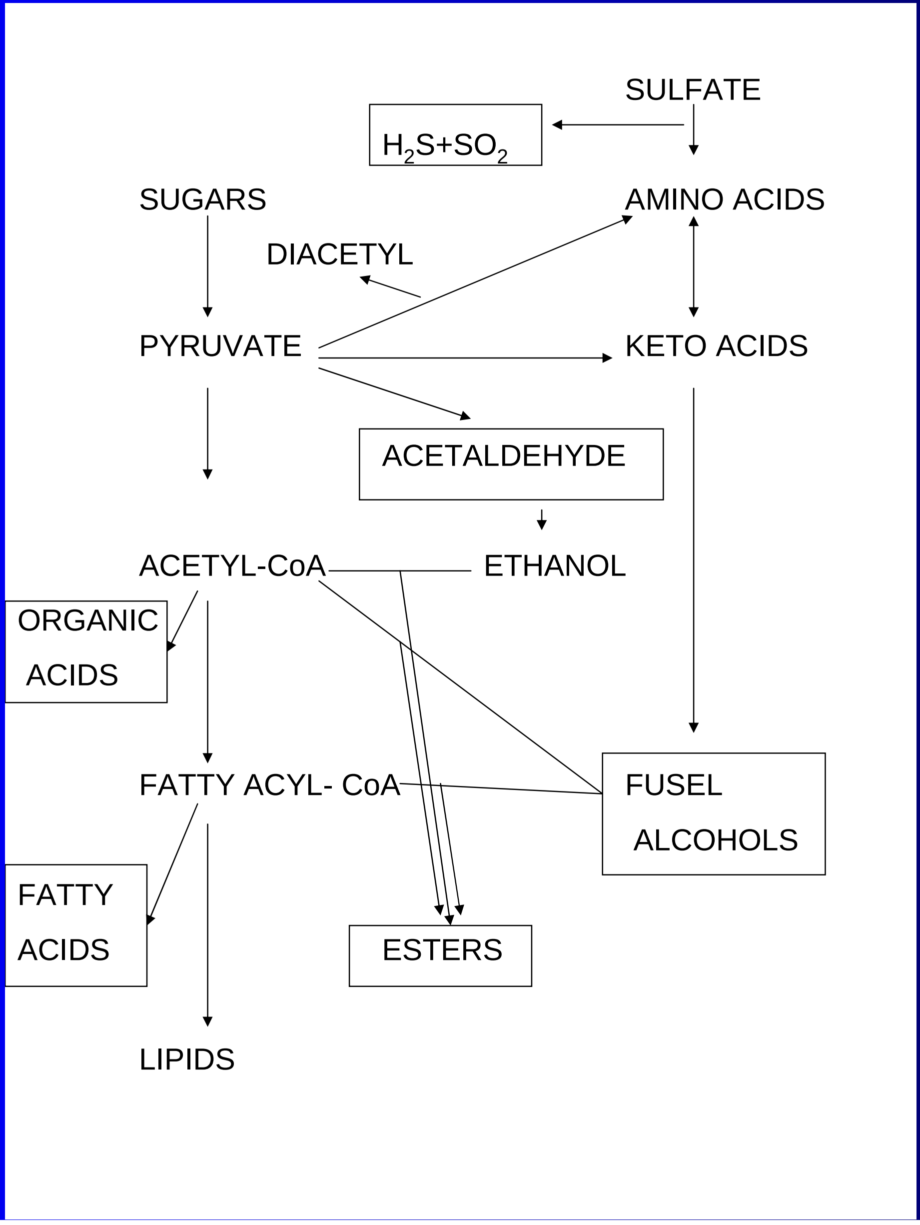
Diacetyl Diagnostics

- **Interpretation I**
  - **A** (no heat) tastes fine **B** (heated) tastes of diacetyl
  - Precursor left in beer that will go to diacetyl over time (accelerated with heat)
- **Interpretation II**
  - **A** (no heat) tastes of diacetyl **B** (heated) tastes of diacetyl
  - (Same intensity)
  - Diacetyl left at end of fermentation
- **Interpretation III**
  - **A** (no heat) tastes slight diacetyl **B** (heated) tastes strong diacetyl
  - **(B** more intense than **A)**
  - Diacetyl and precursor left at end of fermentation

Yeast Autolysis
Flavors and Aromas

- Gives meat-like flavors and aromas, harsh bitterness
  - Yeast viability significantly decreases
  - Yeast releases proteases
  - pH increases
- Increased by:
  - Length of time beer sits on yeast
  - Temperature of yeast
- Timely remove settled yeast

Inter-relationships between yeast metabolism and formation of flavor-active compounds



Summary

Control of Fermentation Flavors

Important Factors

- YEAST
  - Strain
  - Pitching rate and consistency
  - Viability (autolytic “yeasty” flavours)
  - Contaminants
- WORT
  - Gravity, pH, dissolved oxygen, contaminants, yeast foods
- FERMENTATION
  - Design/geometry, temperature, pressure

Summary

- Wort Composition

High Gravity:	↑ Esters	
High FAN:	↑ Yeast Growth	↑ Fusel Alcohols
Increased Zinc:	↑ Fusel Alcohols	↑ Esters
All Malt:	↑ Diacetyl	
Glucose Adjunct:	↑ Esters	↑ SO_2

Summary

- Increasing Oxygen (Aeration)

  $\uparrow$ Higher Alcohols

  $\downarrow$ Esters

  $\downarrow$ SO_2

  $\downarrow$ Acetaldehyde

  $\downarrow$ Diacetyl

Summary

- Increasing Yeast Pitch Rate
  - ↓ Yeast Growth
  - ↑ Ethyl Acetate (solvent)
  - ↓ Iso-amyl Acetate (banana)
  - ↓ Higher Alcohols

Summary

- Increasing Temperature
  - ↑ SO_2
  - ↑ Acetaldehyde
  - ↑ Higher Alcohols
  - ↑ Floral, solvent Esters
  - ↑ Diacetyl (depending upon flocculation), but speeds reduction