Welcome
Introduction to Yeast

Kara Taylor

What we will do today

- We will dispel some myths
- We will add to your information on yeast
- We will create discussion on yeast and fermentation
- We will do some laboratory work with yeast

What we will NOT do today

- We will not tell you step by step how to treat every yeast and fermentation issue
- We will not tell you “this is the only way to do…”
- We will not stop saying ‘it depends’
- We will not give you a test

What we HOPE to do today

We hope to INSPIRE you to:

- pay attention to yeast needs
- test yeast, fermentation, and beer more
- experiment with new ideas and protocols
- create new beers

Saccharomyces cerevisiae

- One of the oldest domesticated organisms
  - Used for brewing beer in Sumeria and Babylonia around 6000 BC
- *Saccharomyces* = sugar fungus; *cerevisiae* = Roman God of crops – Ceres
- Used as a eukaryotic model organism
  - Unicellular, doesn't need a lot of room to grow, eukaryotic → can be applicable to humans
  - 1st genome to ever be sequenced in 1996

Yeast Used in Brewing

All yeast used in brewing worldwide is non-GMO

Ale Yeast

- Original brewing strain - ***Saccharomyces cerevisiae***
- Top ferment
- Warmer fermentation temps
- Wide strain variety

Other Strains

- *Saccharomyces uvarum*
- *Saccharomyces bayanus*
- *Saccharomyces eubayanus*

Lager Yeast

- Natural hybrid - ***Saccharomyces pastorianus** (Saccharomyces carlsbergensis)*
- Bottom ferment
- Colder fermentation temps
- Limited strain variety

**Saccharomyces pastorianus = Saccharomyces cerevisiae + Saccharomyces eubayanus.*

Unique Properties of Brewers Yeast

- Asexual reproduction by budding
- Little to no sporulation
- Therefore mating is rare
- Polyploid
- Phenol Flavor Negative
- Stress tolerant
- Flocculate
- Hundreds of different, stable strains currently used industrially

The ‘Real’ Significance of Yeast:

Flavor

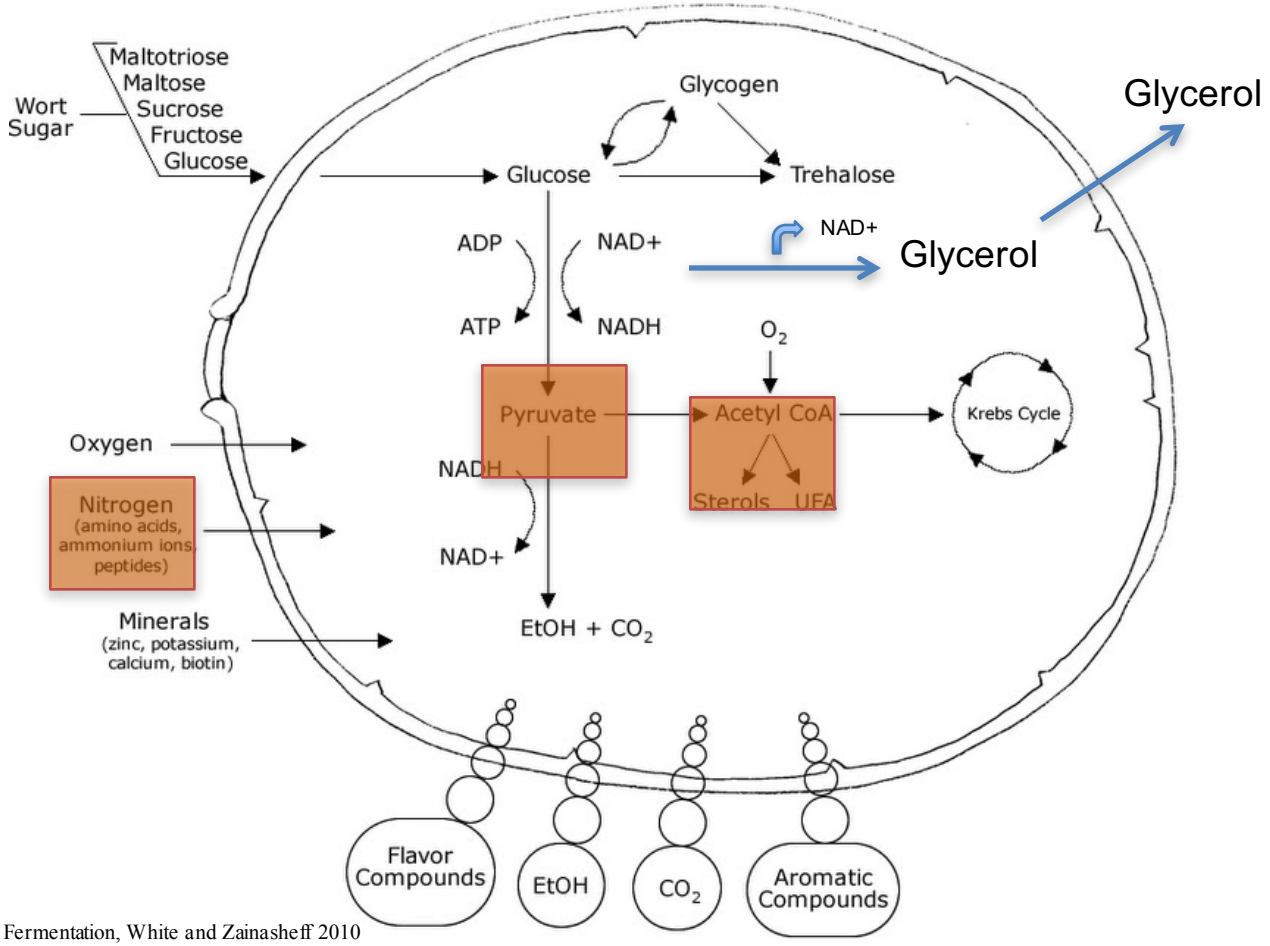
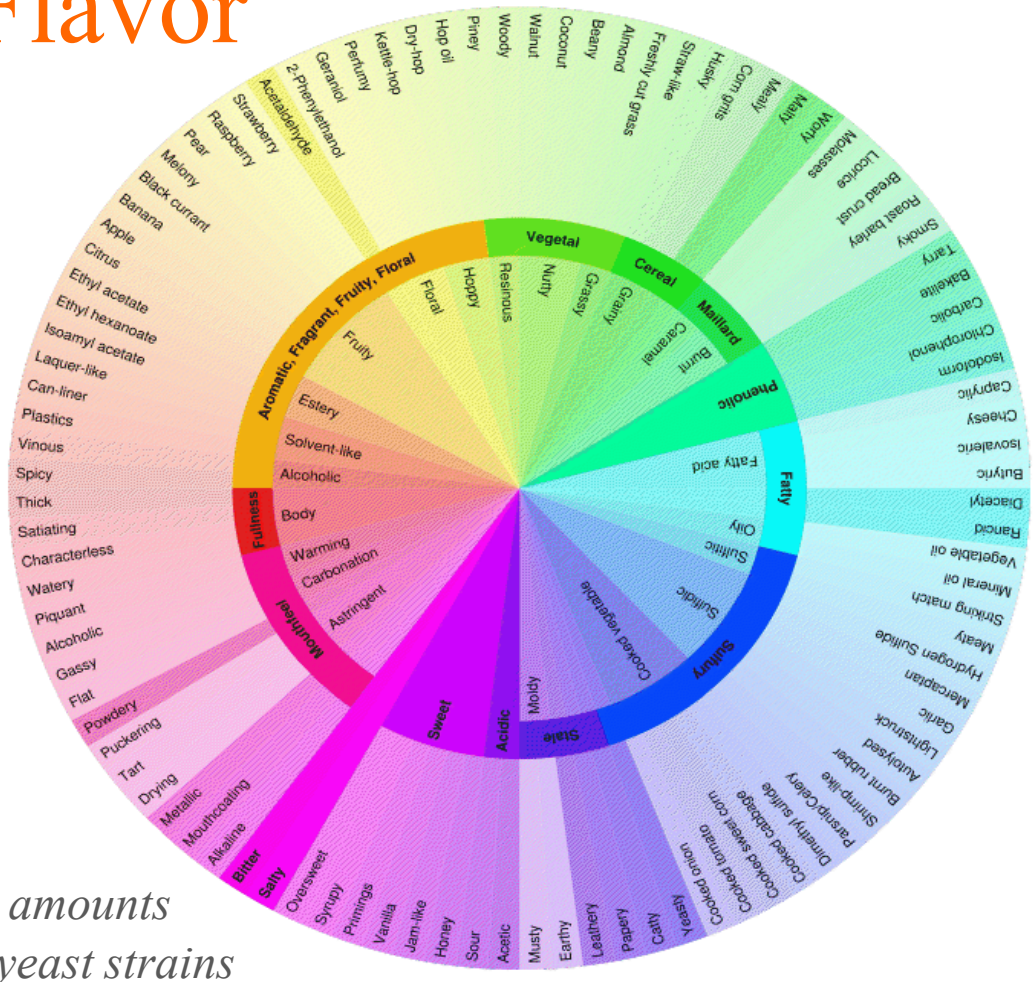


Fig 2.3 Yeast: The Practical Guide to Beer Fermentation, White and Zainasheff 2010

Why are Strains so Important:

Flavor

- Alcohol
- Higher (fusel) alcohols
- Esters
- Diacetyl
- Sulfur
- Acetaldehyde
- Phenolic compounds



**Different Yeast strains make different amounts*
Different Beers often require different yeast strains

Origins of Brewing Yeast

In the beginning, fermentation was a mystery

- Ancient beer-makers did not know what was causing fermentation

"Godisgood"

The magic froth that would appear on liquids

- Original Reinheitsgebot of 1516: water, malt, hops
  (No YEAST!)

"The Brewer," engraving by Jost Amman, 16th century

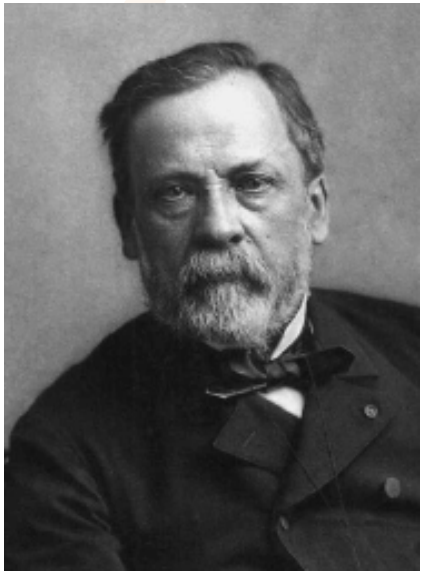
Biotechnology Before Science

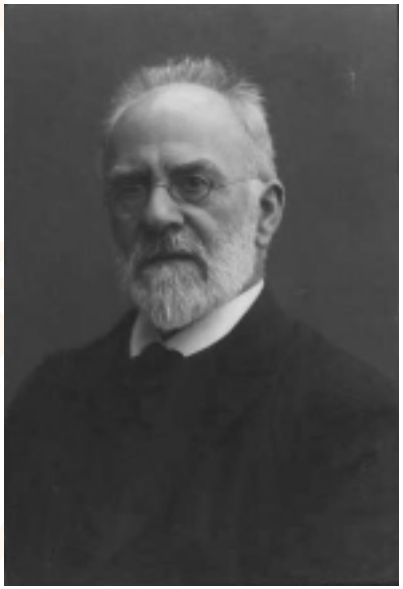
- Witch's Ring – Carlsberg Brewery
  - This wreath was retrieved from the brewing tub after fermentation, was hung on the wall to dry, ready to be thrown into the next batch of wort

In the beginning……

- Laboratories for commercial yeast did not exist
- Brewing strains were created by brewers by:
  - Continuing to use strains that performed well and tasted good
  - Passing strains to brewer to brewer

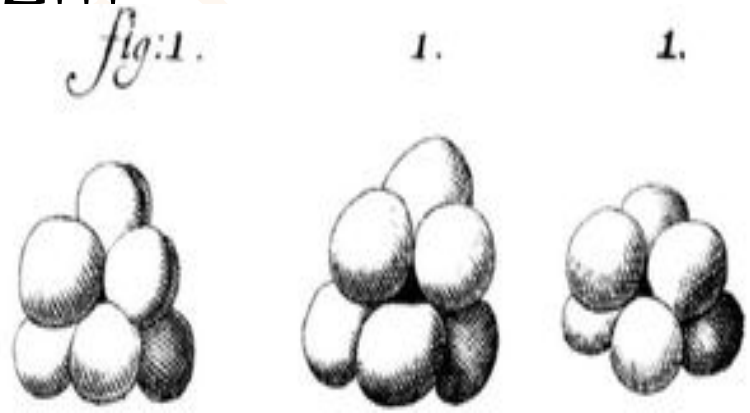
Science to the Rescue!





Origins of Brewing Yeast– Science to the Rescue!!!

- Anton van Leeuwenhoek 1680
  - "Yeast" made of small interconnected material
  - Did not think it was alive
  - Current theory of fermentation – spontaneous chemical reaction



- Antoine-Laurent Lavoisier 1789
  - Determined that sugar was being converted to EtOH and CO_2
  - Showed fermentation to be precise, quantitative, chemical analysis.

Origins of Brewing Yeast – Science to the Rescue!!!

- Theodore Schwann 1837
  - Showed that yeast was alive
  - Correctly described as a fungus
    - Zuckerpilz – German for sugar fungus
  - Findings were not immediately accepted
    - General acceptance was that yeast was a residue of the decomposition of sugar

Origins of Brewing Yeast – Science to the Rescue!!!

- 1860's
  - Current theory was that sugar liquid + air caused spontaneous generation of yeast and bacteria

Louis Pasteur

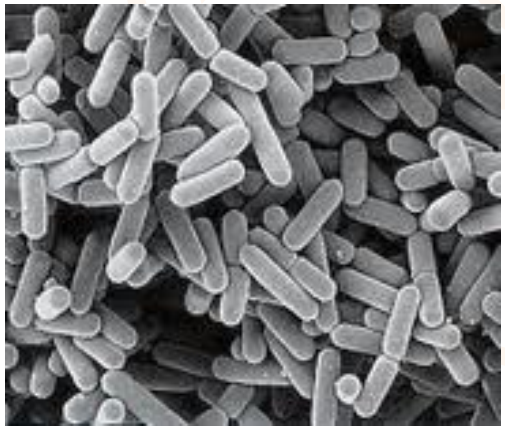
- Yeast and bacteria were found in beer and other fermented products
- Yeast were responsible for fermentation!
- Biggest discovery in fermentation science

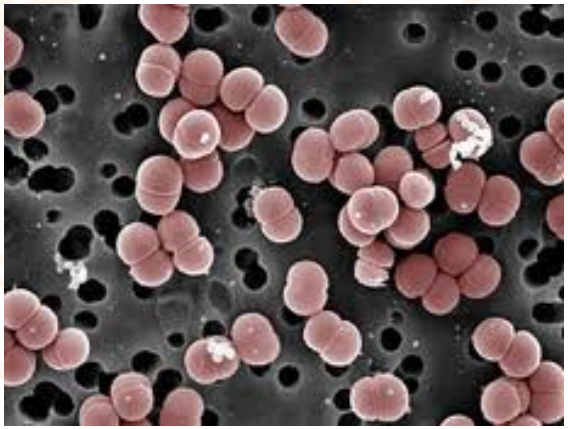
Beginnings of Fermentation Science

- Louis Pasteur

  Also determined that bacteria were causing off-flavor in beer

"The disease of beer"





The Beginning of Yeast Banking

- Emil Christian Hansen 1883
  - Developed **pure culture techniques**
- Strain selection and strain storage

The future of yeast banking

- Founded in 1995- in San Diego
- Chris was studying his Ph. D at UCSD

New location in San Diego in 2011

More locations around the world

Asheville, NC

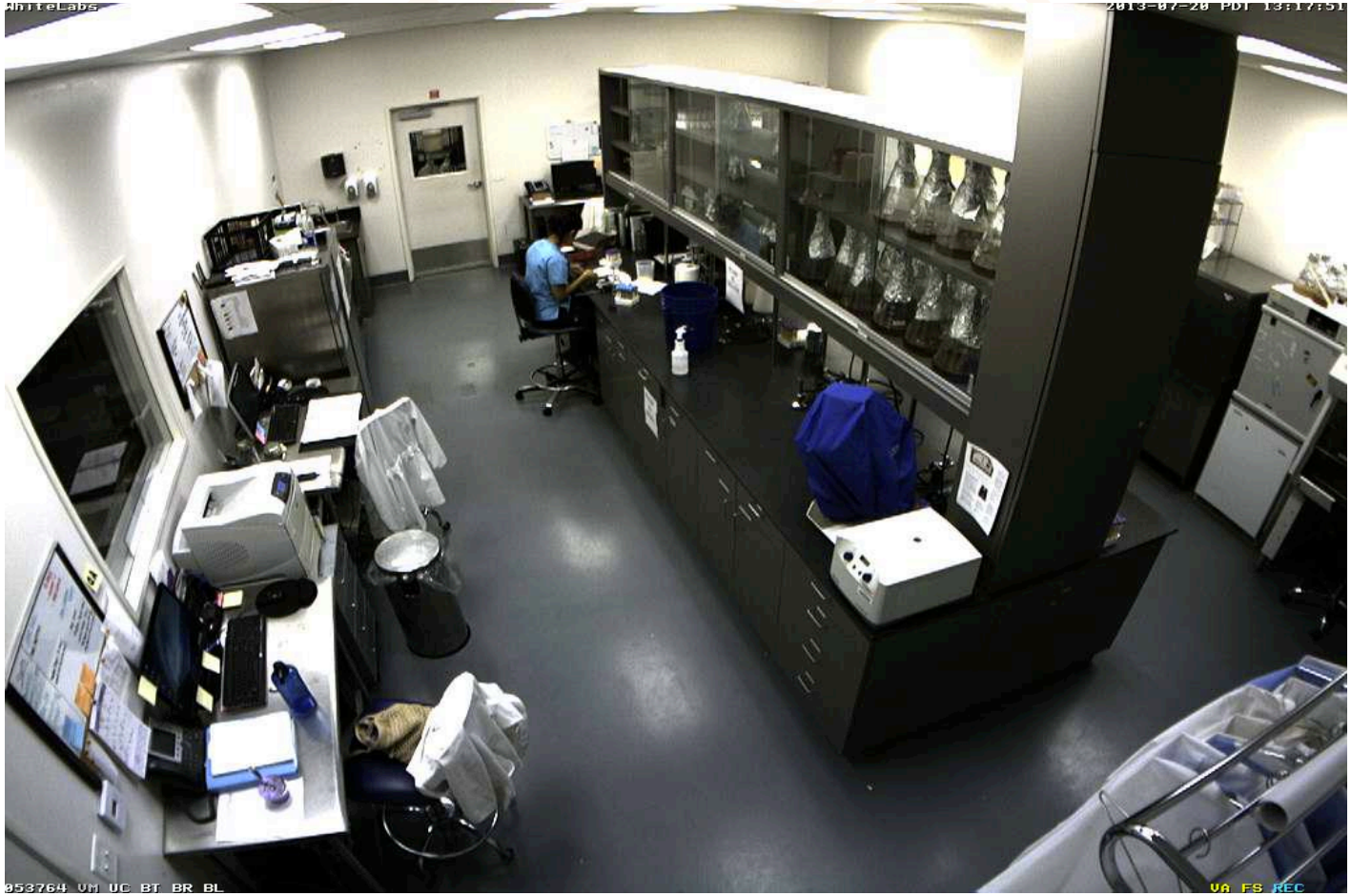
Office here since 2007
Boulder, CO

Copenhagen

Yeast is Our Main Focus

Yeast Lab

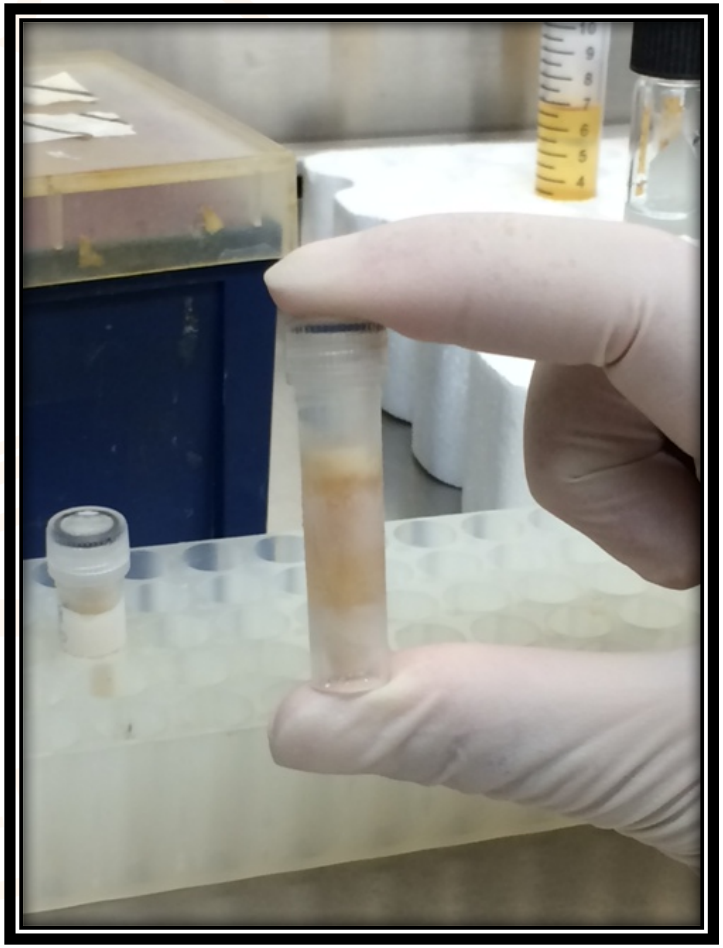
The beginning stages of our propagation



Step 1: Initial Culture Storage

- Freezes - critical
- Plates/slants
- Working plates

Yeast must be stored properly for a long, long time

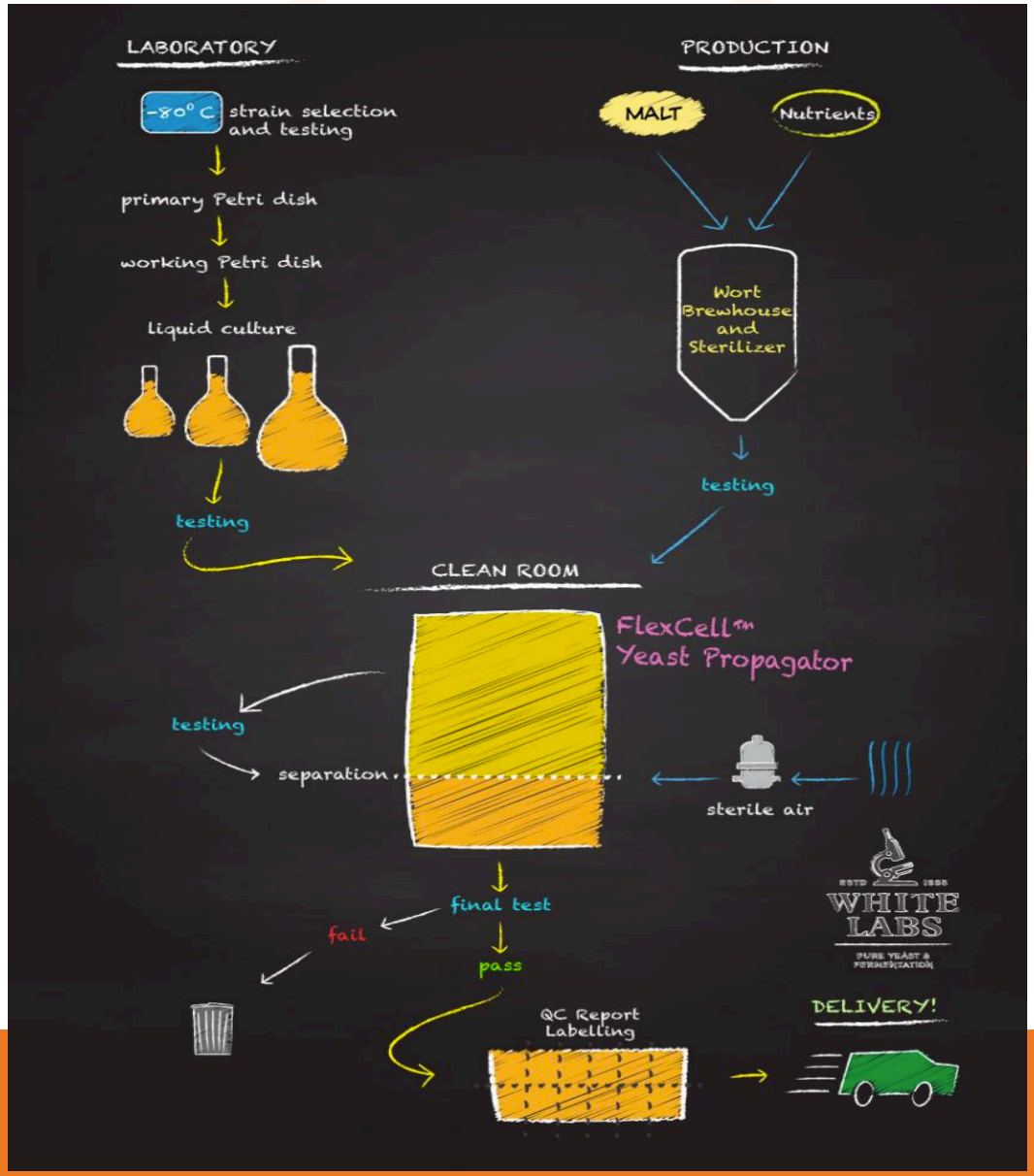


Step 2: Working agar plates/slants

Step 3: Transferring to liquid media

Step 3-5: Step-up Transfers

Our Yeast Production Process



West Exterior Elevation

East Exterior Elevation

South Exterior Elevation

Thank you

Questions?

Fermentation Control: Brewing Conditions and Strain Selection

Joe Kurowski

Outline

- Fermentation Overview
- Factors Affecting Fermentation Performance
  - Pitching Rates
  - Dissolved Oxygen
  - Temperature
- Strain Selection

Fermentation Recap

First few hours

- The yeast uses all the dissolved oxygen; there is no detectable uptake of glucose.

8-16 hours

- The first sign of active fermentation as CO_2 bubbles are formed.
- A thin head of foam can be observed.

24 hours

- Budding yeast cells observed.
- The temperature, if uncontrolled, rises due to heat generated by the fermentation.

24-48 hours

- The rate of yeast growth and carbohydrate assimilation reaches a maximum.

Post 48 hours

- The pH falls to a minimum of 3.8 - 4.4 before rising slightly towards the end of fermentation.
- The fall in pH is caused by the release of organic acids and buffering compounds (basic amino acids and phosphates) being consumed by the yeast.

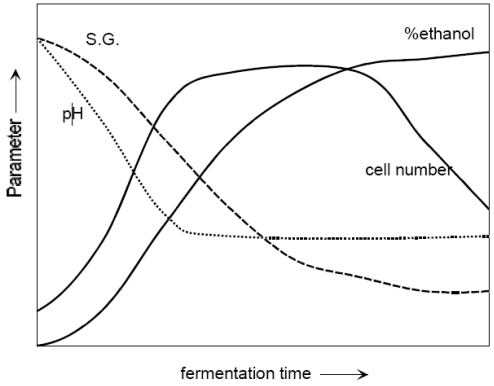


Figure 1. Fermentation profiles, showing relative changes taking place.

What Effects Fermentation?

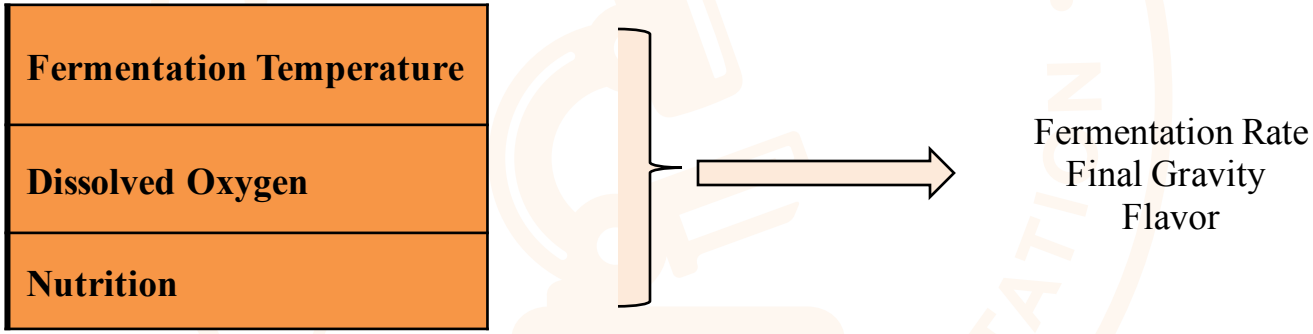
- Yeast Strain Selection
- Time
- Yeast Nutrition
- Starting Gravity
- Fermentation Temperature
- Dissolved Oxygen
- Yeast Inoculation Rates

Factors Influencing Fermentation

These mainly effect:

- Flavor production
  - Creation of metabolic byproducts from yeast
  - Influences flavor of final product
  - Can create “off-flavors”
- Rate of fermentation
  - Can affect attenuation & yield of product
  - Stuck fermentations can lead to production & scheduling issues
  - Due to promotion of YEAST GROWTH

3 factors you can control in fermentation…



This assumes great sanitation practices!

Pitching Yeast

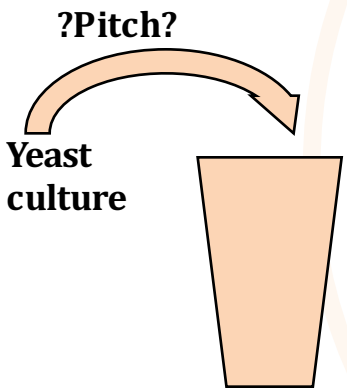
- Add a specific amount of yeast to freshly oxygenated wort, at the correct fermentation temperature
- Yeast can be new, first generation, or reused from a previous fermentation
- Yeast can be reused 5-10 times
- Pitch more yeast for high gravity beers

Pitching Yeast

Low Pitching Rates	High Cell Growth	Increased Flavor Compounds
High Pitching Rates	Low Cell Growth	Decreased Flavor Compounds

Where does "1 million cells per ml of *wort* per degree Plato" fit in?

Yeast Pitching Numbers



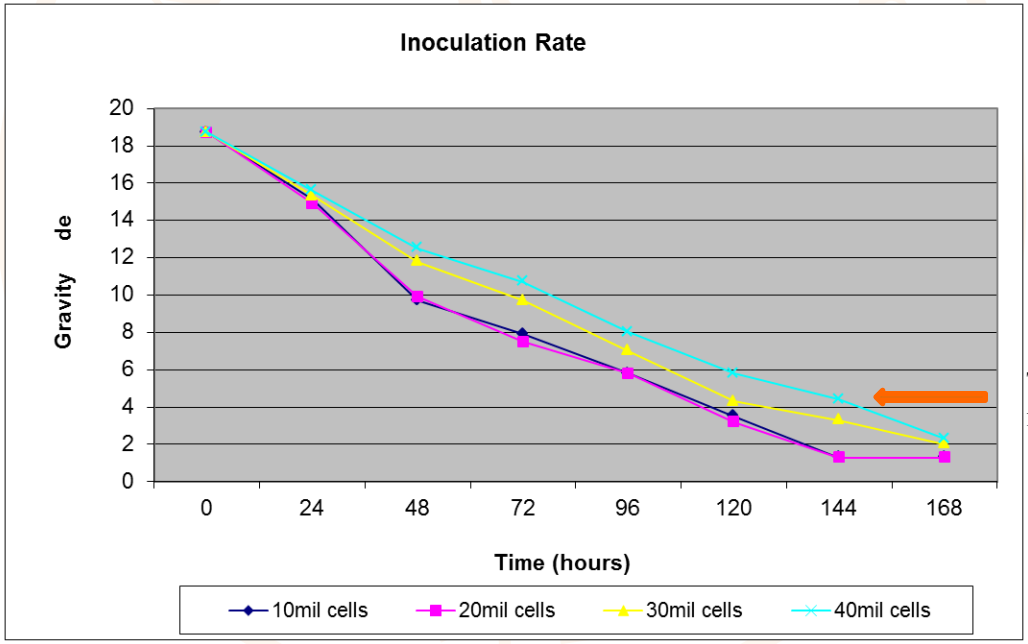
1 million cells/ml/Plato
10 Plato = 10 million cells/ml
20 Plato = 20 million cells/ml

1 lb per BBL
= 0.5 million cells/ml/Plato

Concentration of slurry?
Concentration of yeast culture/propagation?

Yeast Pitch Rate

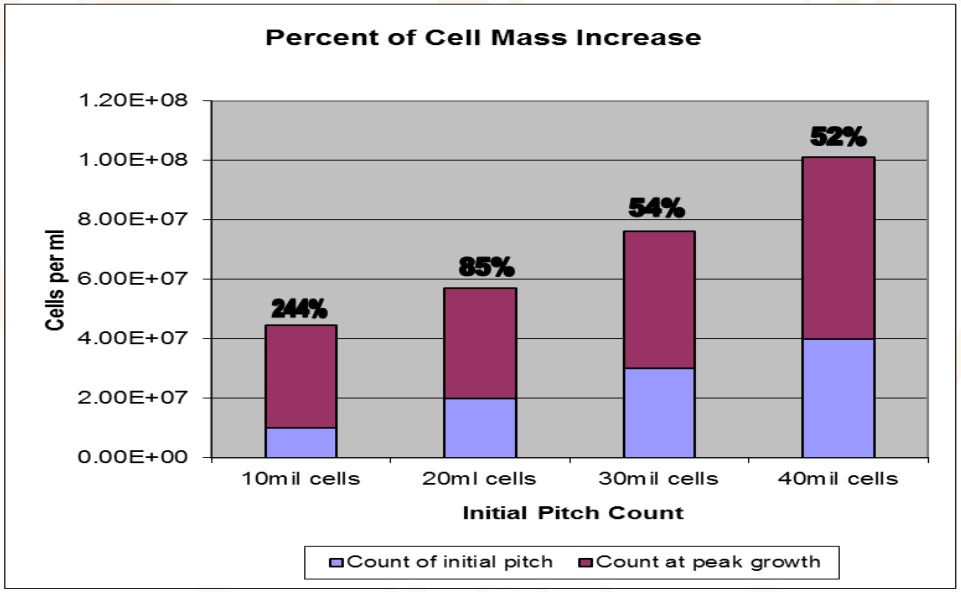
Effects on fermentation rate:



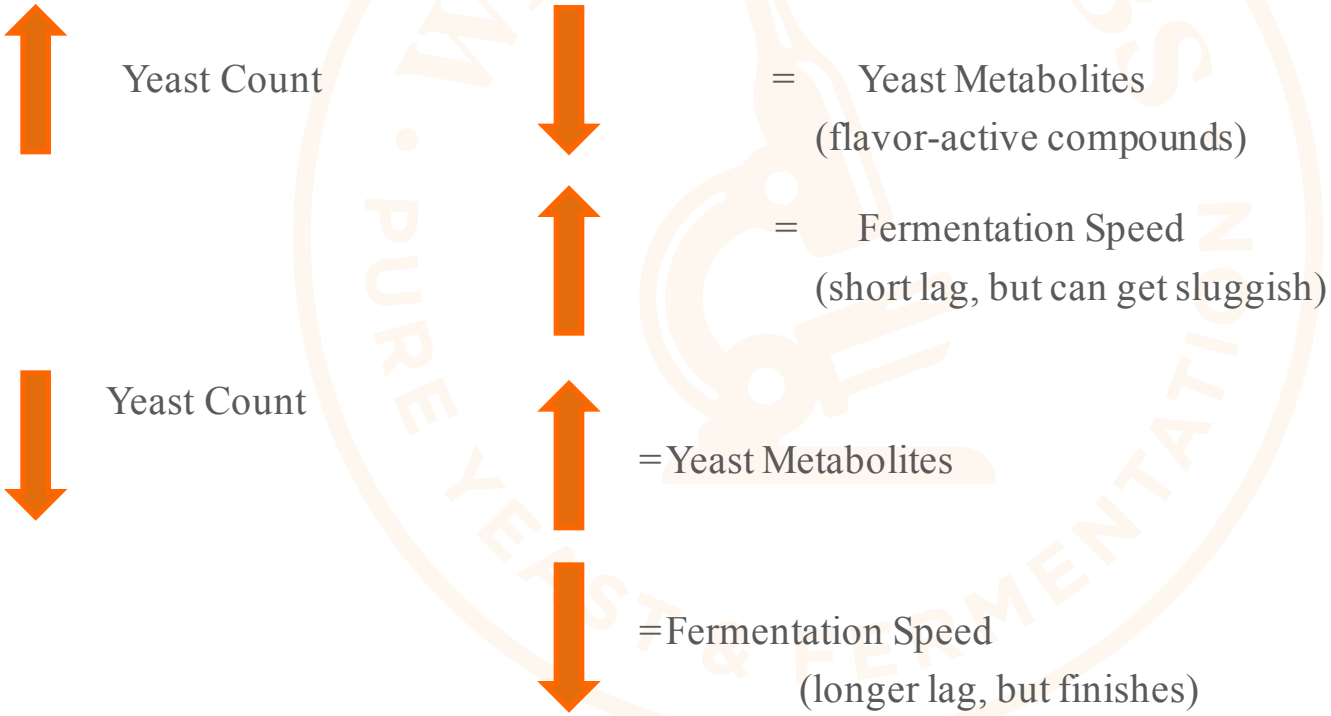
Too much yeast can result in sluggish fermentations

Yeast Pitch Rate

Effect on growth rate and flavor byproducts:



Pitch Rate and Flavor



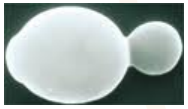
Fermentation Temperature

- Temperature affects both yeast metabolism and the speed of fermentation
- Most S. cerevisiae strains are optimal between 65-70◦F (18-21◦C), but there is a wide range
- Higher or lower temperatures can lead to varying fermentation effects

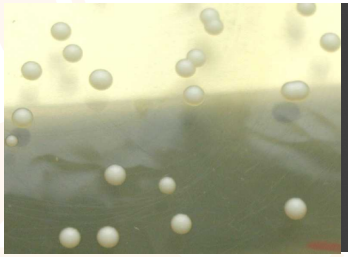
Temperature – one of the most important control factors

Fermentation Temperature

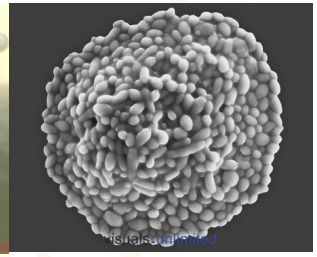
Does a few degrees really make a difference??



Single yeast cell
1000X



Yeast colonies
Naked eye



Yeast colony
1000X

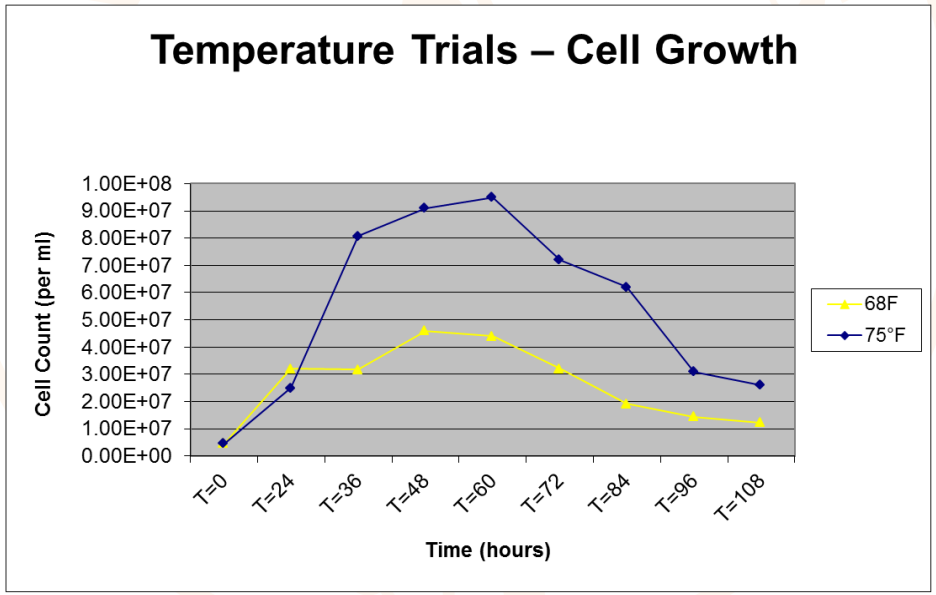
Billions of yeast cells!!!

Temperature Control Matters

	75°F	66°F	Threshold
Ethanol	5.04% abv	4.74% abv	1.4% abv
1-Propanol	22.76 ppm	23.78 ppm	600 ppm
Ethyl Acetate	33.45 ppm	22.51 ppm	30 ppm
Iso-amyl alcohol	114.92 ppm	108.43 ppm	70 ppm
Total Diacetyl	8.23 ppb	7.46 ppb	150 ppb
Total 2,3-pentanedione	3.17 ppb	5.09 ppb	900 ppb
Acetaldehyde	152.19 ppm	7.98 ppm	10 ppm

Fermentation Temperature

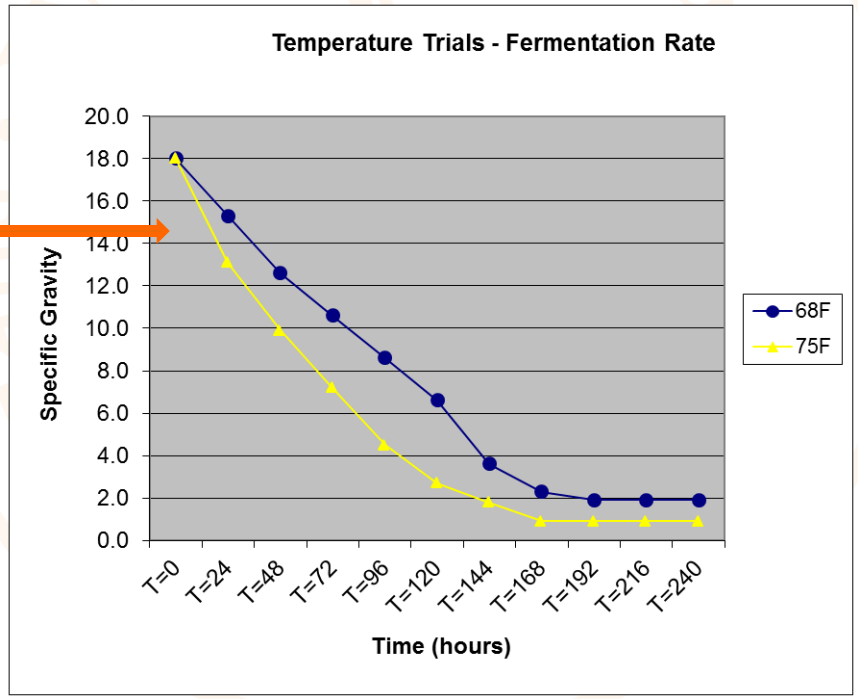
Effects on fermentation rate:



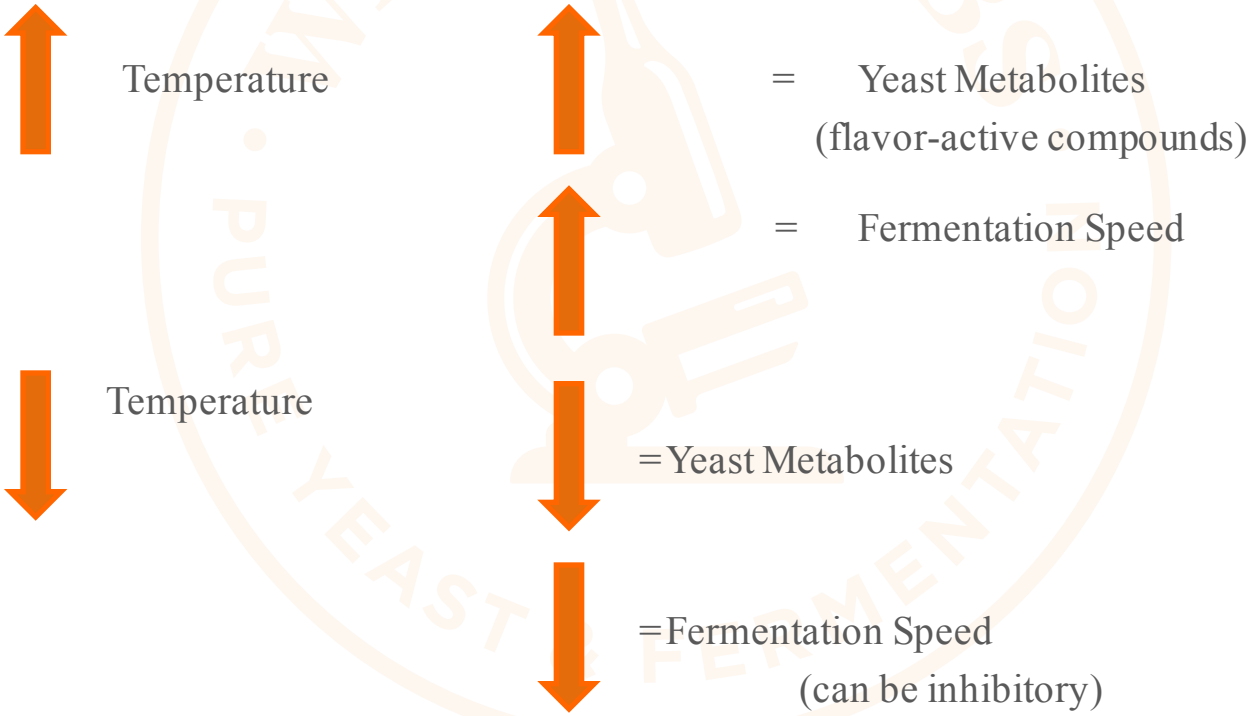
Fermentation Temperature

Effects on fermentation rate:

Faster gravity drop



Fermentation Temperature



Dissolved Oxygen

- Oxygen is necessary for production of lipids for cell wall manufacture
- Allows the yeast to be hardy and withstand environmental stresses (gravity, pH changes, temperature, alcohol)
- Optimal is 8-10ppm in wash, prior to fermentation

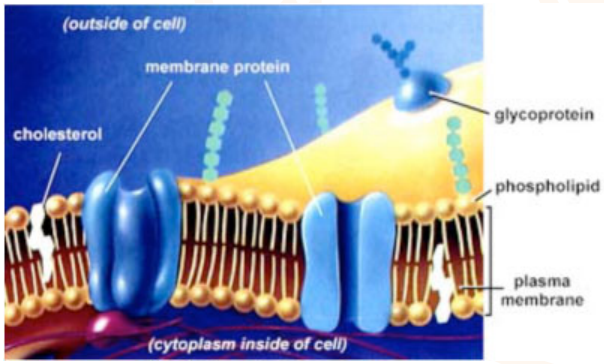
Dissolved Oxygen

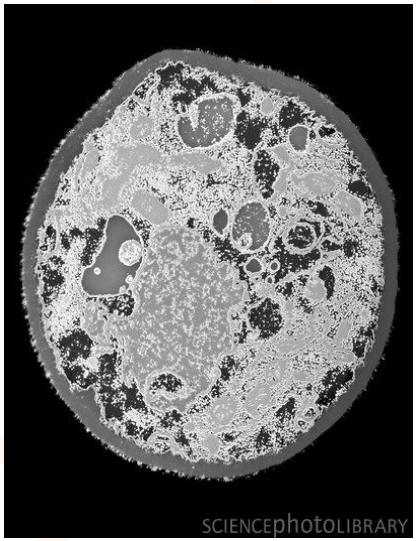
Oxygen

Unsaturated Fatty Acids

Sterols

Lipids





Dissolved Oxygen

Without it, yeast are depleted

Healthy Yeast

Unhealthy Yeast

Resulting in:

Slow fermentation

Incomplete fermentation

Poor growth

#51

Dissolved Oxygen

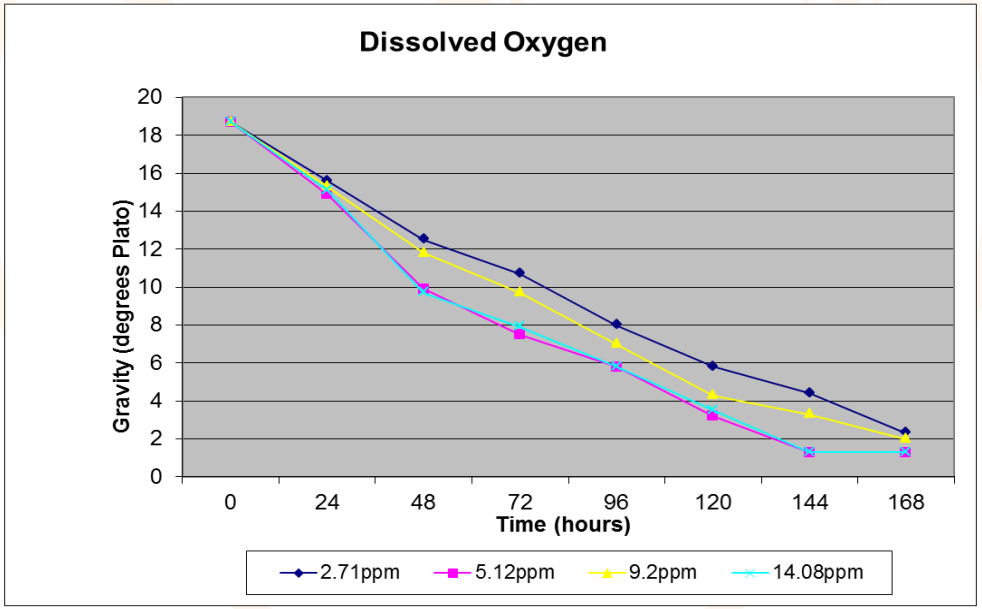
Inadequate Dissolved Oxygen		Adequate Dissolved Oxygen
Incomplete fermentations	→	Improved cell growth Improved fermentation rate Improved attenuation
Flavor issues	→	Minimize cell stress Control of flavor compounds
Low viability	→	Improved shelf life/storage

Dissolved Oxygen

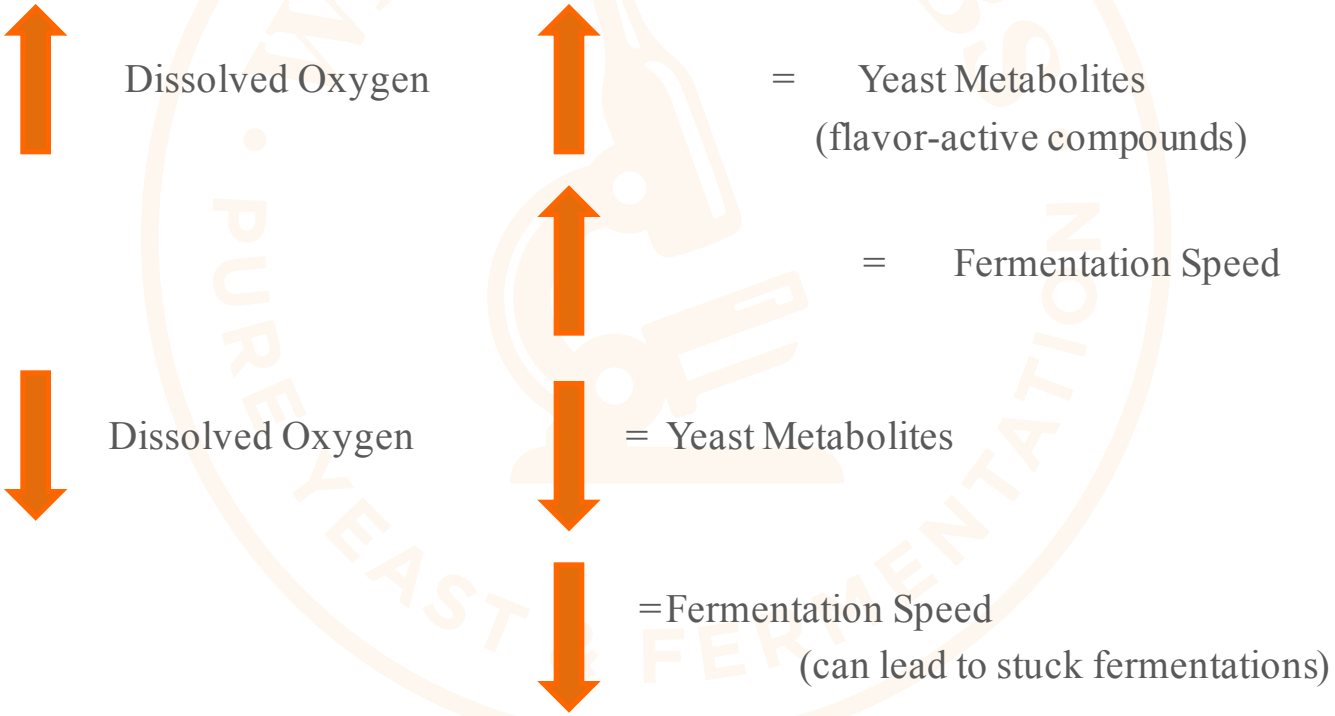
Gravity (Plato) vs. Time				
	2.71ppm	**5.12ppm**	**9.2ppm**	**14.08ppm**
Time (hours)	**shake**	**30 seconds**	**1 min**	**2 min**
0	18.7	18.7	18.7	18.7
24	17.6	17.3	17.5	16.9
48	13.5	12.8	12.7	11.9
72	11.7	10.7	9.9	9.5
96	10	9	8.8	7.8
120	7.8	7.3	6.5	6.2
144	6.4	6.3	5.5	5.2
168	5.3	5	4.3	4.3

Dissolved Oxygen

Effect on fermentation rate:

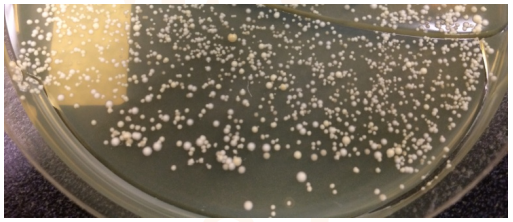


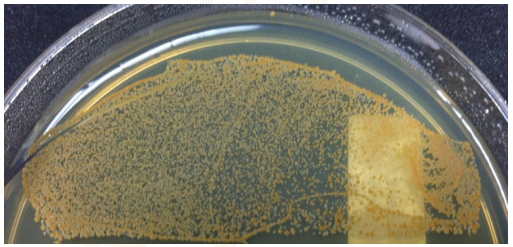
Dissolved Oxygen



Fermentation Control - Strain Selection

- Set Parameters for the beer
  - ABV, IBU, SRM
- Decide on a flavor concept
  - Malty, hoppy, other?
- Determine at least 1 or 2 key requirements
  - Temperature, sugar, and alcohol tolerance, Attenuation ranges, volatile flavor and fusel alcohol production, etc.





Monitor the Actual Values

- Gravity
- pH
- Cells in suspension
- Cell Pack
- Alcohol
- Color
- Clarity
- IBU
- Aroma

The list goes on……
Google sheets (free), excel, fancy software

Fermentation Monitoring

Do experiments!

Ferment the same wort with different yeast strains

Some things White Labs is Doing…

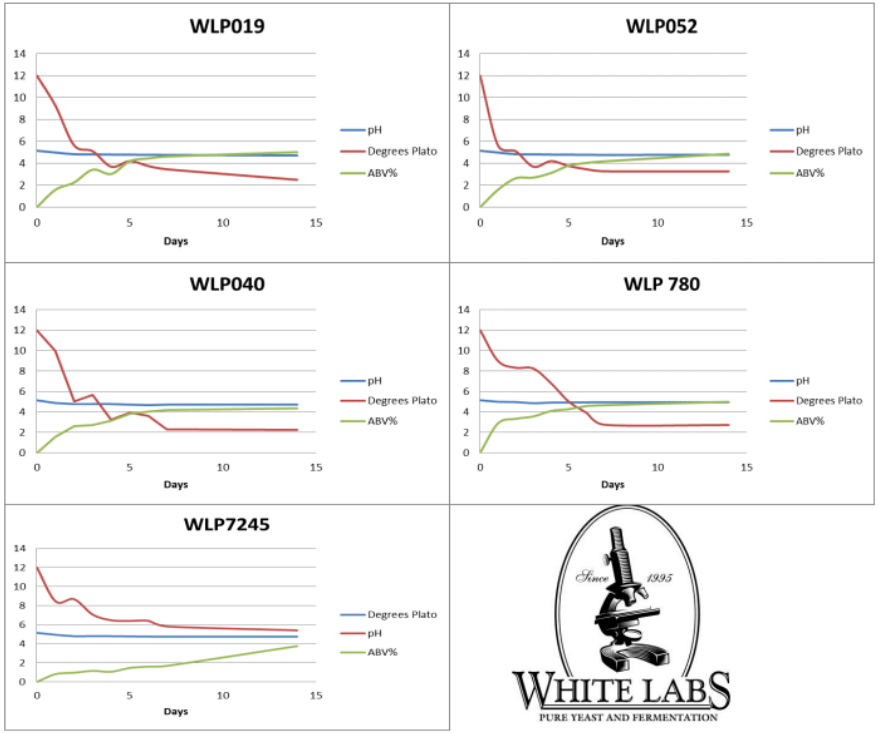
Example of analytical data on WL batch

Brewers can make more informed decisions with this type of data!

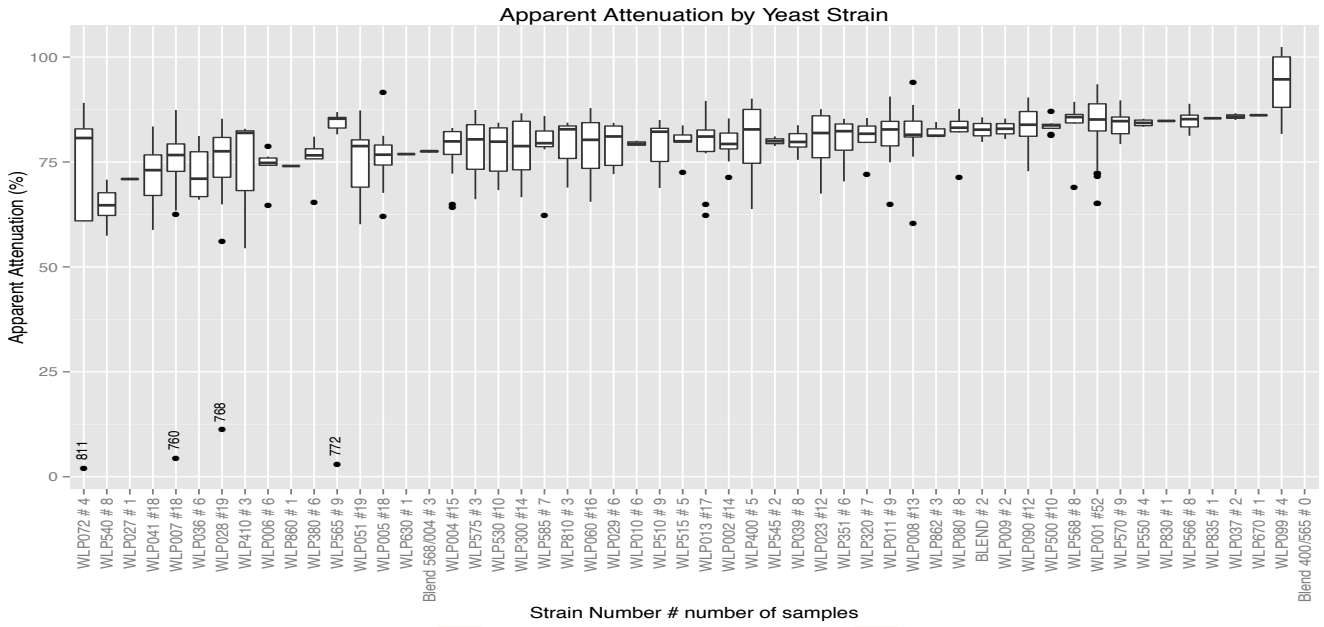
- Alcohol
- IBU
- Attenuation
- Specific Gravity
- Calories
- Diacetyl
- Wild Yeast Contaminants
- Bacteria Contaminants

Final Beer Specifications

Strain	OG	OE	pH	AE	Stain	OG	OE	pH	AE
WLP019	14	1.01000	4.72	2.5	WLP052	12.0	1.0128	4.78	3.27
WLP040	12.0	1.0091	4.7	2.26	WLP780	12.0	1.0106	4.95	2.72
WLP7245	12.0	1.02129	4.75	5.41					



Apparent Attenuation by Yeast Strain



Comparison of 96 *Saccharomyces* isolates originating from commercial brewing environments to reveal correlations between full DNA sequence and fermentation characteristics and flavor attributes in beer.

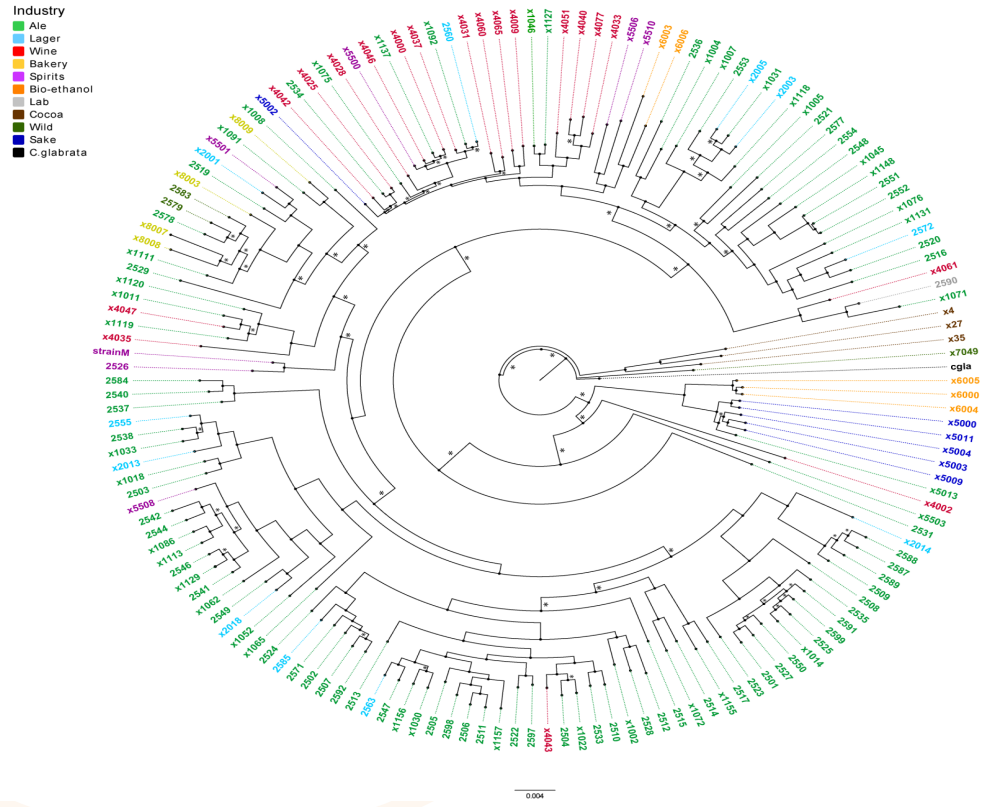
Illumina in collaboration with White Labs Inc. have sequenced 96 closely related *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* strains used in brewing, in order to capture the biological diversity and gain insight into the difference between the strains.

illumina

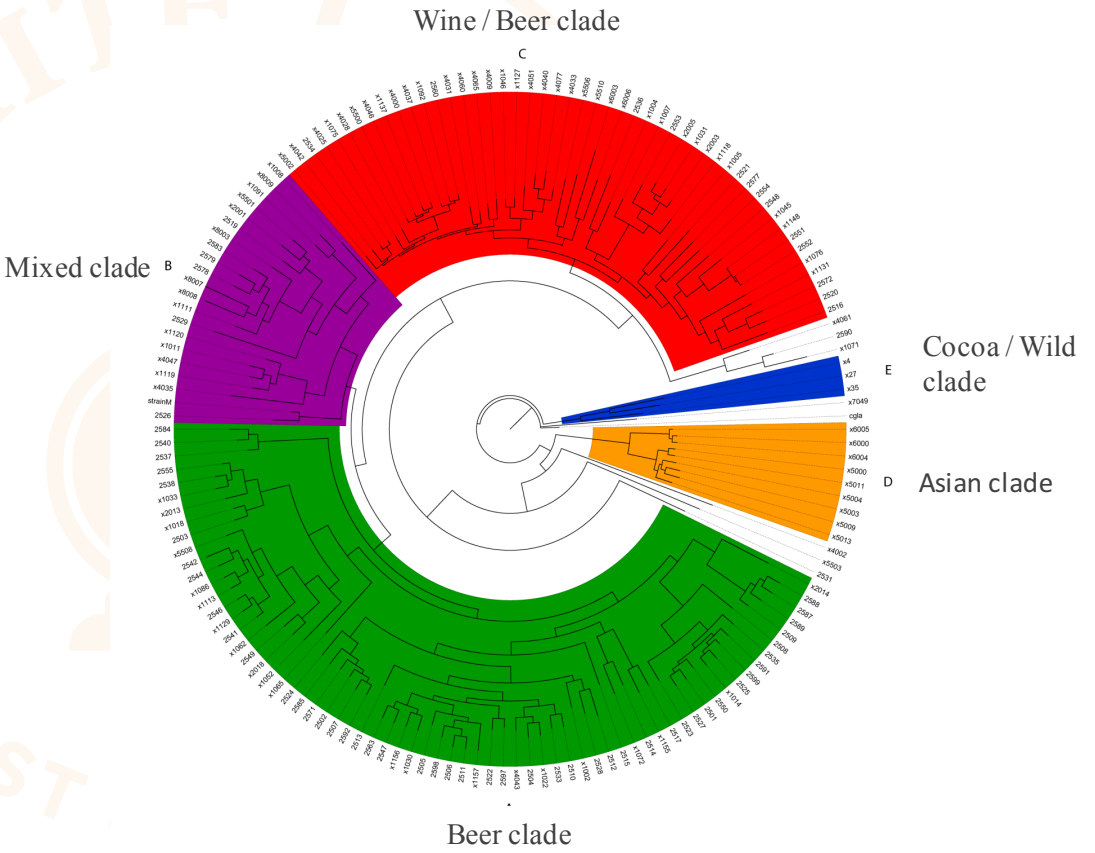
Comparative genomics is the study of the relationship between genome structure and function across different species or strains. The purpose of this study is to determine the phylogenetic relatedness among different *Saccharomyces* samples and compare the data to fermentation performance and flavor characteristics of the isolates in beer production.

Sequencing of the isolates was done by Illumina using the HiSeq 2500 and the MiSeq with different data handling tool applied. Fermentation characteristics were described on the basis of 20--80 L fermentations of brewer's wort covering a variety of beer styles true to the individual strain

Comparative Genomics

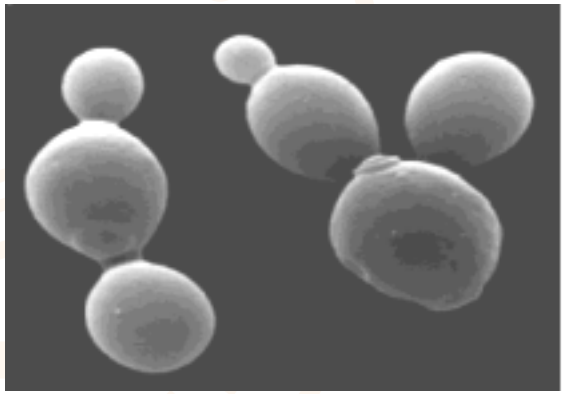


Comparative Genomics



Summary

1. Pitch Rate
2. Temperature
3. Aeration
4. Yeast Strain Selection
   - Classic and the FUTURE!
5. And…Sanitation



Thank you

Questions?

Yeast Metabolism Basics

Karen Fortmann, Ph.D.

Outline

- Aerobic vs. Anaerobic metabolism
  - Propagation vs. fermentation
- Yeast nutrition
- Critical metabolic pathways in brewing
- Yeast flavor and aroma contribution

Glucose Catabolism of *Saccharomyces*

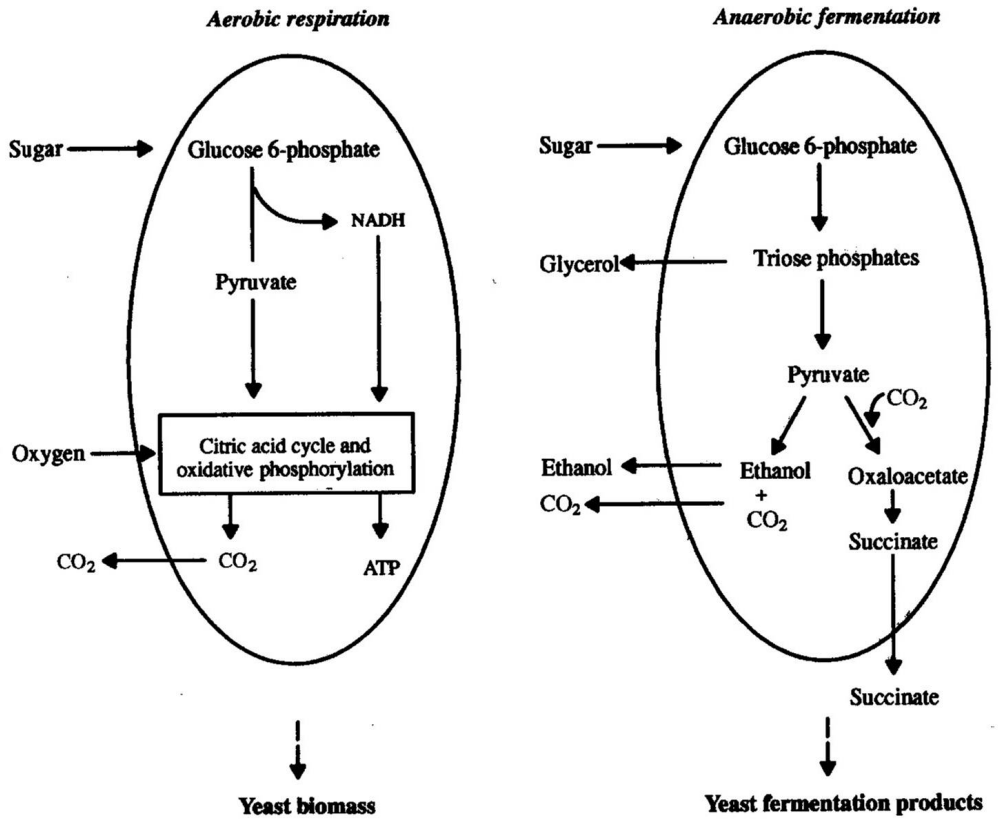
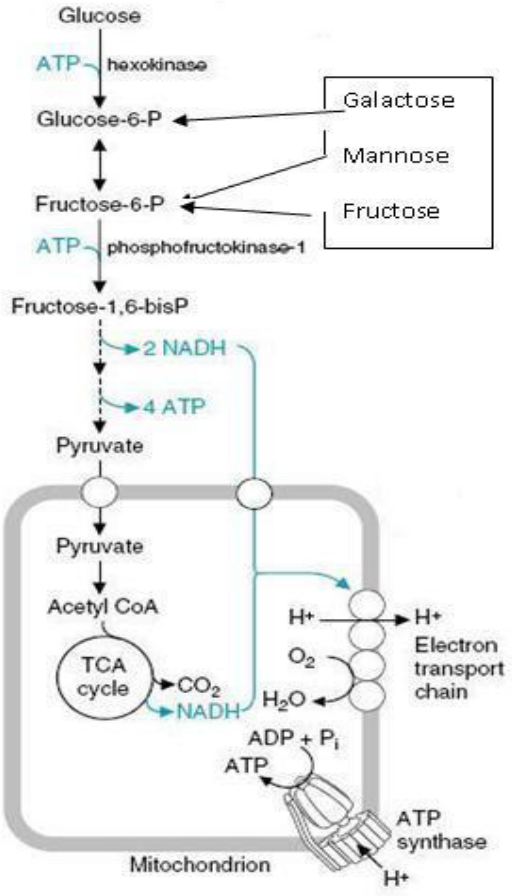


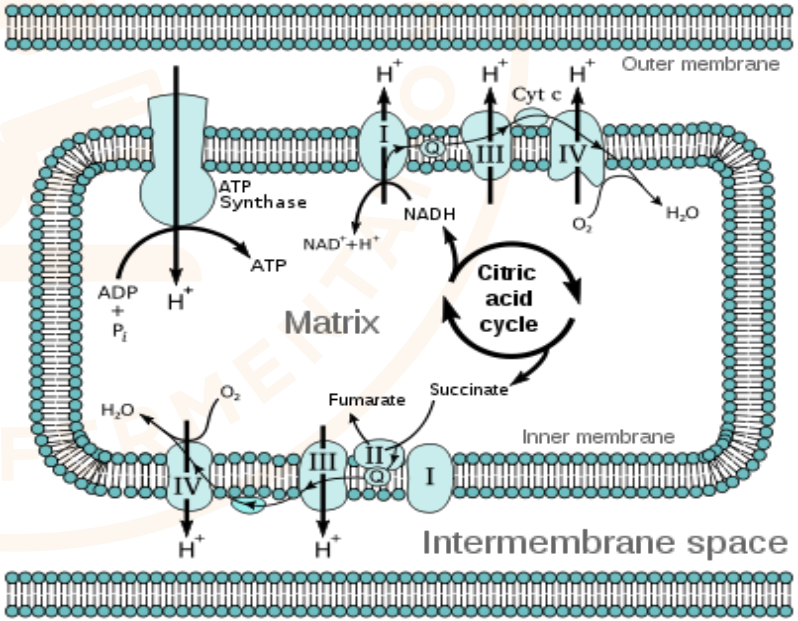
Figure 5.9. Summary of major sugar catabolic pathways in yeast cells.

Yeast Physiology and Biotechnology, Graeme Walker, 1998

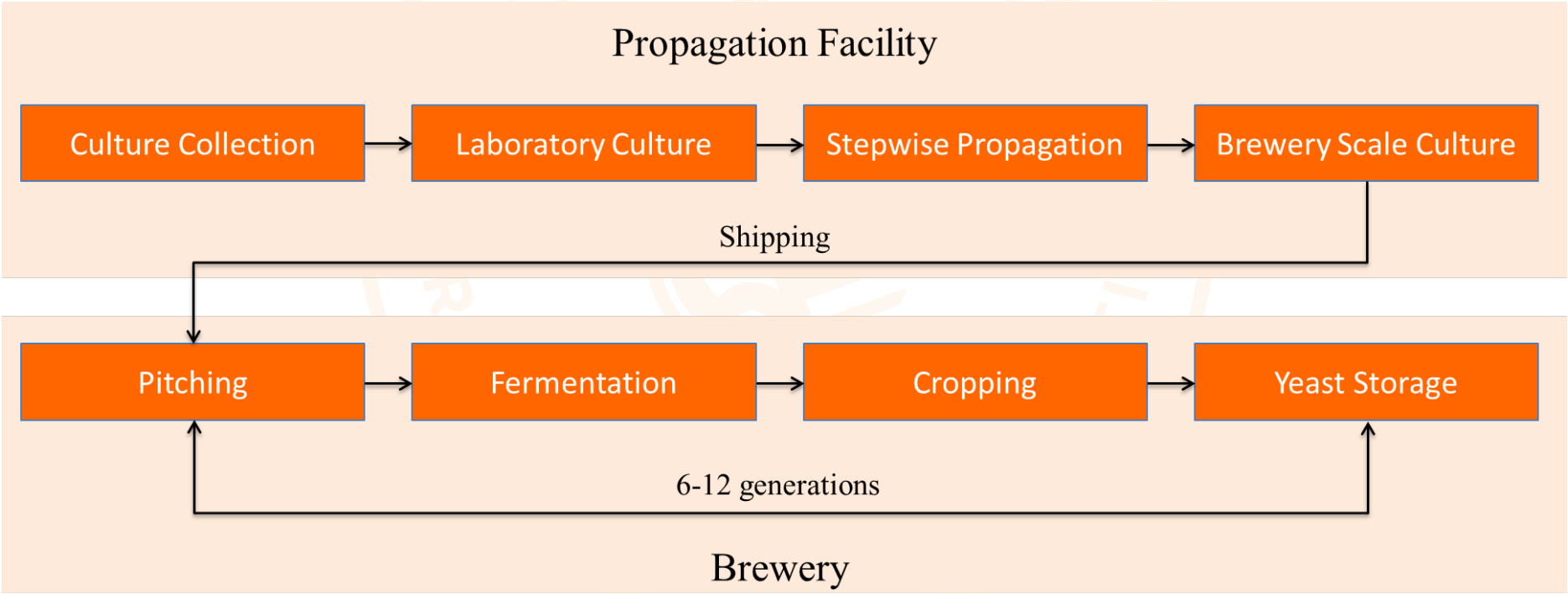
Respiration – The Power Plant



The goal of a yeast cell is not to produce alcohol but to survive and reproduce



Flow Chart of Yeast Culture



Propagation

- Purpose of propagation:
  - To produce yeast for beer production with high viability and vitality
    - Viability: Percentage of living & dead yeast cells
      - Example: 90% viability equals 10% dead cells
    - Vitality: Physiological fitness of living cells
  - Avoid mutation
    - Start with the best possible culture (pure, high viability high vitality)

Making Yeast or Making Beer?

Propagation

- Yeast **respiration** is preferred over fermentation
- Molecular oxygen (O_2) is necessary for yeast growth
- Nutrients are even more important than during alcoholic fermentation (growth)
- Optimal temperature for propagation is higher than for fermentation (+5-10 degrees)

Propagation Medium

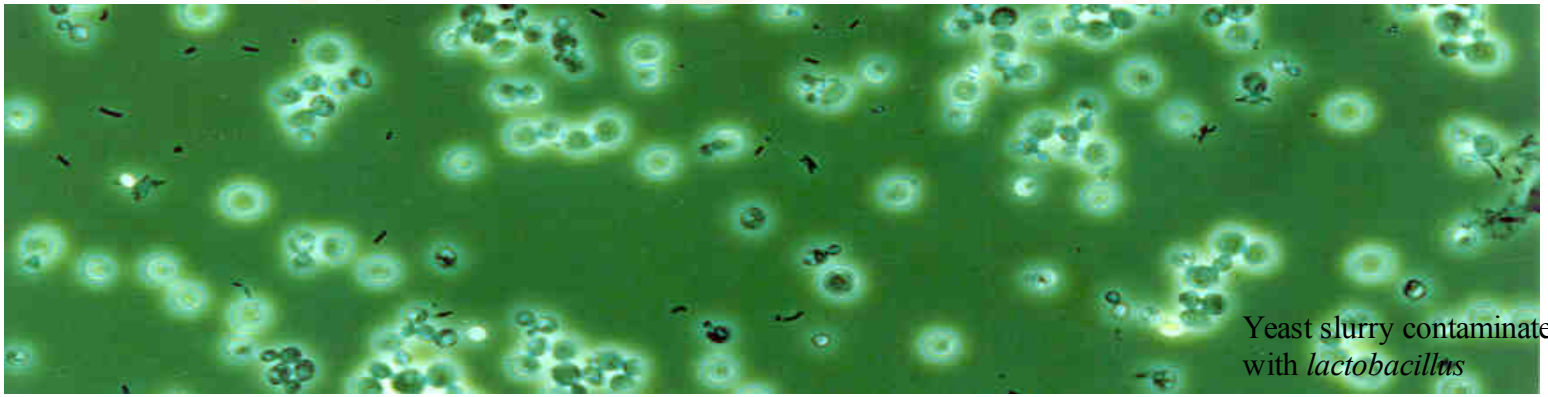
- Normal to slightly lower gravity (8-12 Plato)
- Wort sugars rather than simple sugars
- Light wort rather than dark
- Hops? (as antimicrobial factor: yes!)

Keep it Clean!

Propagation process is much more susceptible to contamination than alcoholic fermentation

Why?

- Low alcohol
- Aerobic environment
- Possibly no hops
- Constant introduction of new material (wort?, air)
- More transfers

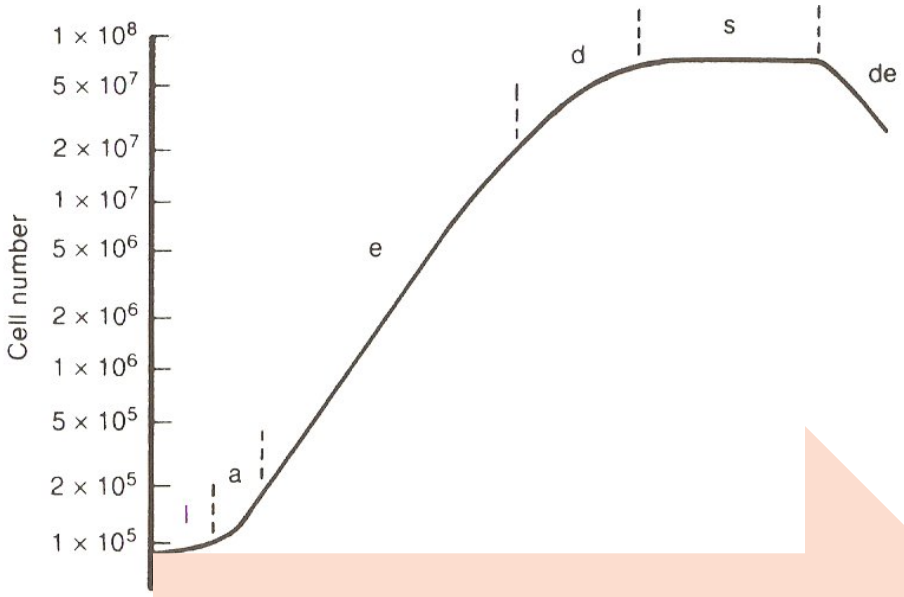


Yeast slurry contaminated with *lactobacillus*

Aerobic vs. Anaerobic

	Aerobic	Anaerobic
Reactants	Glucose & Oxygen	Glucose
Energy Yield	High (36-38 ATP)	Low (2 ATP)
Products	CO_2 & H_2O	Yeast: Ethanol and CO_2 Animals: Lactic Acid
Location	Cytoplasm & Mitochondrion	Cytoplasm
Stages	Glycolysis; TCA; ETC	Glycolysis; Fermentation

Fermentation



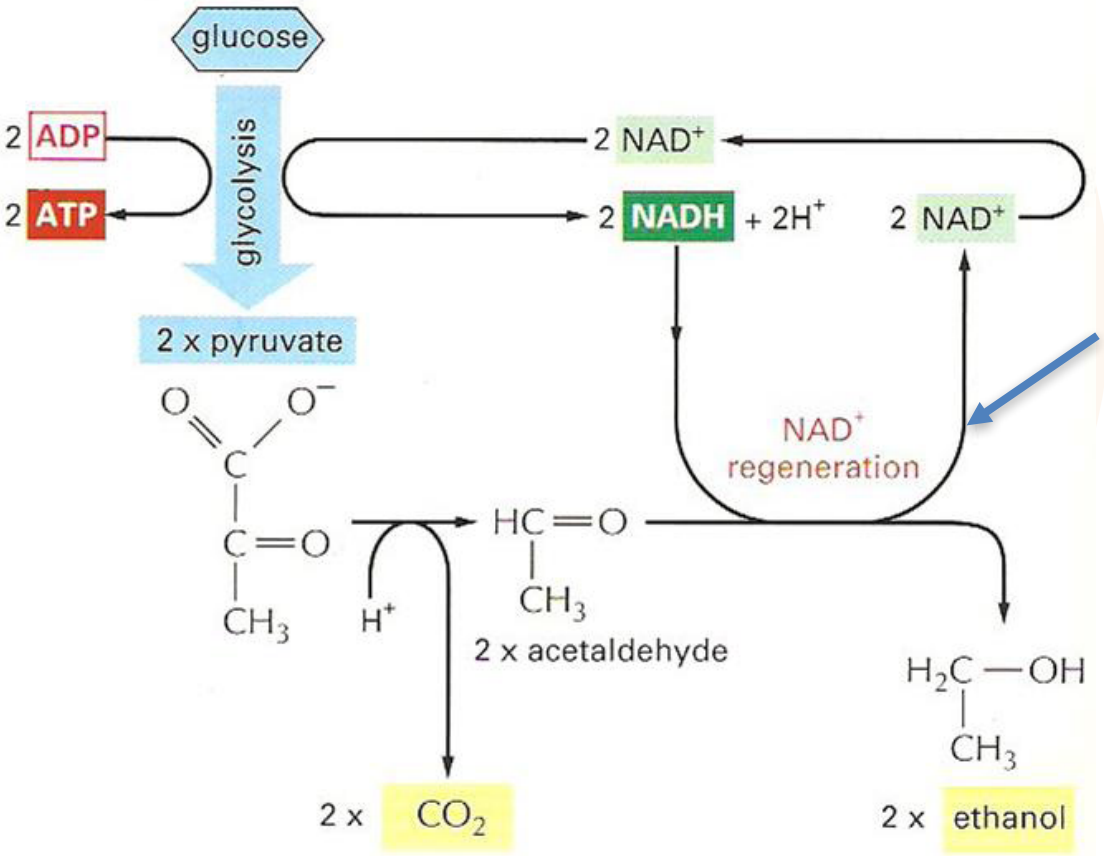
Lag Phase

Exponential Phase

Stationary Phase

Death Phase

Fermentation



Keeps glycolysis going

Glucose Catabolism of *Saccharomyces*

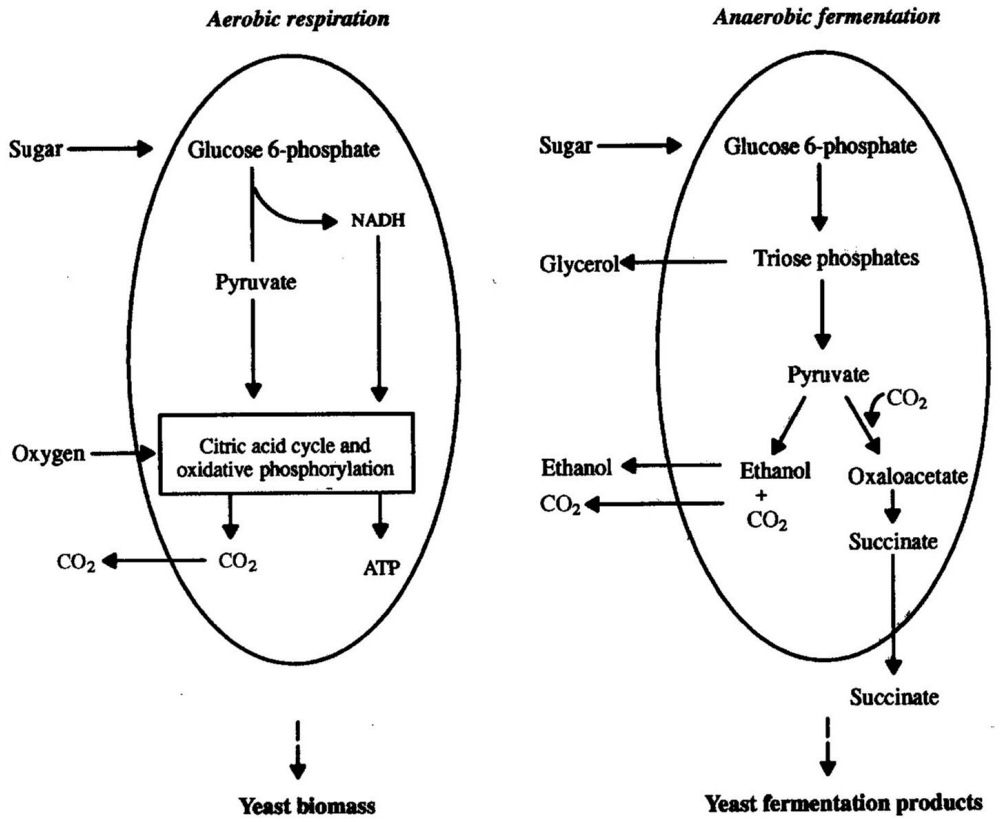


Figure 5.9. Summary of major sugar catabolic pathways in yeast cells.

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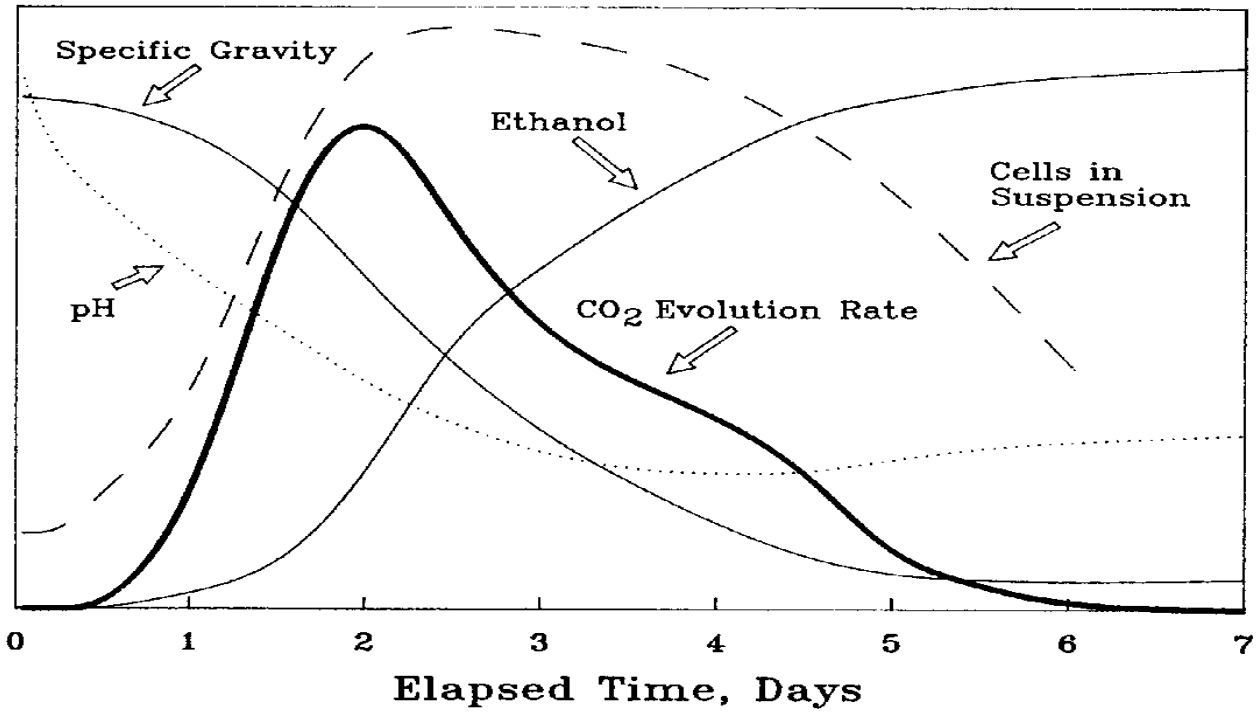
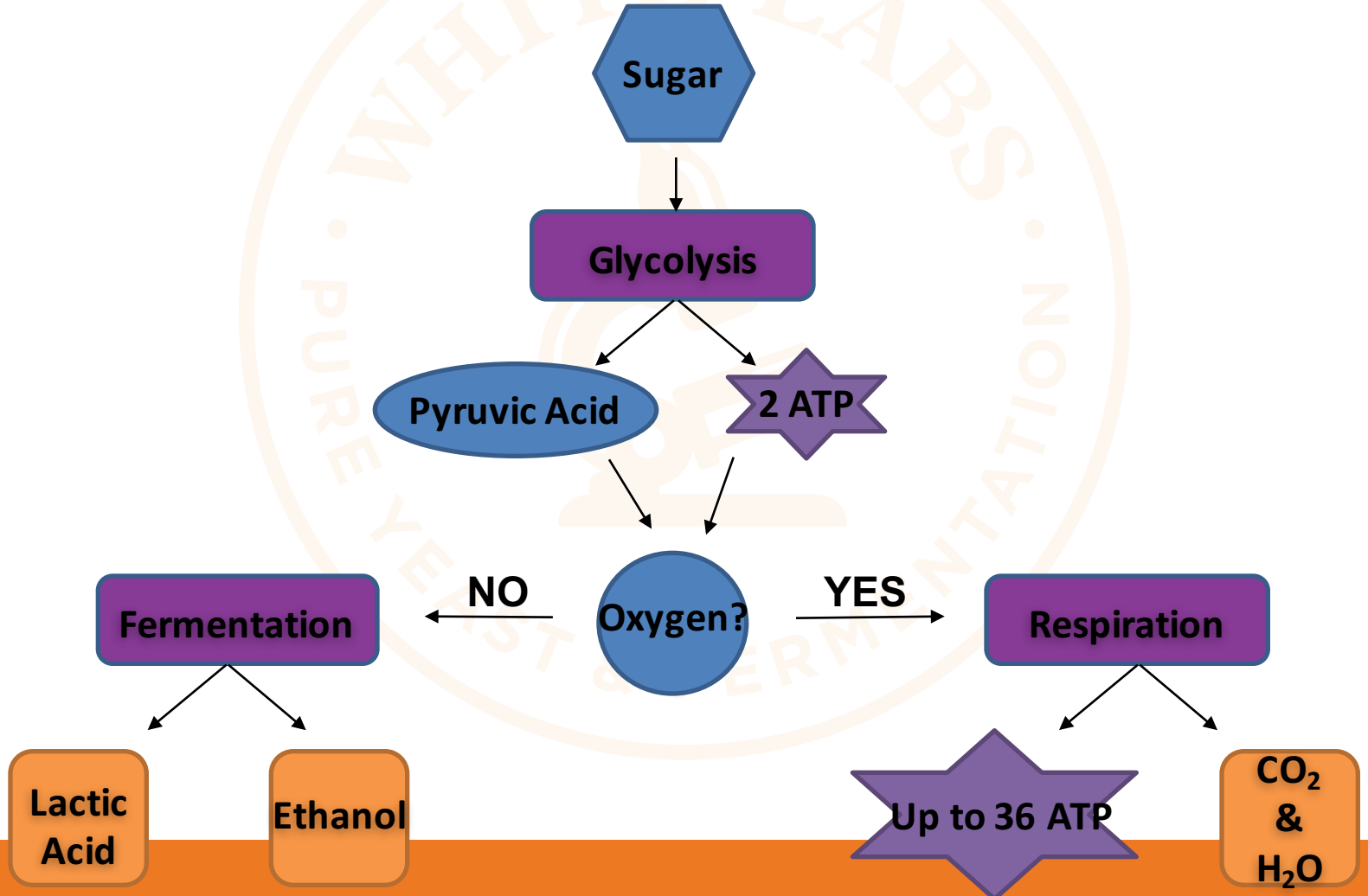
Fermentation Recap



Respiration vs. Fermentation



Yeast Nutrition

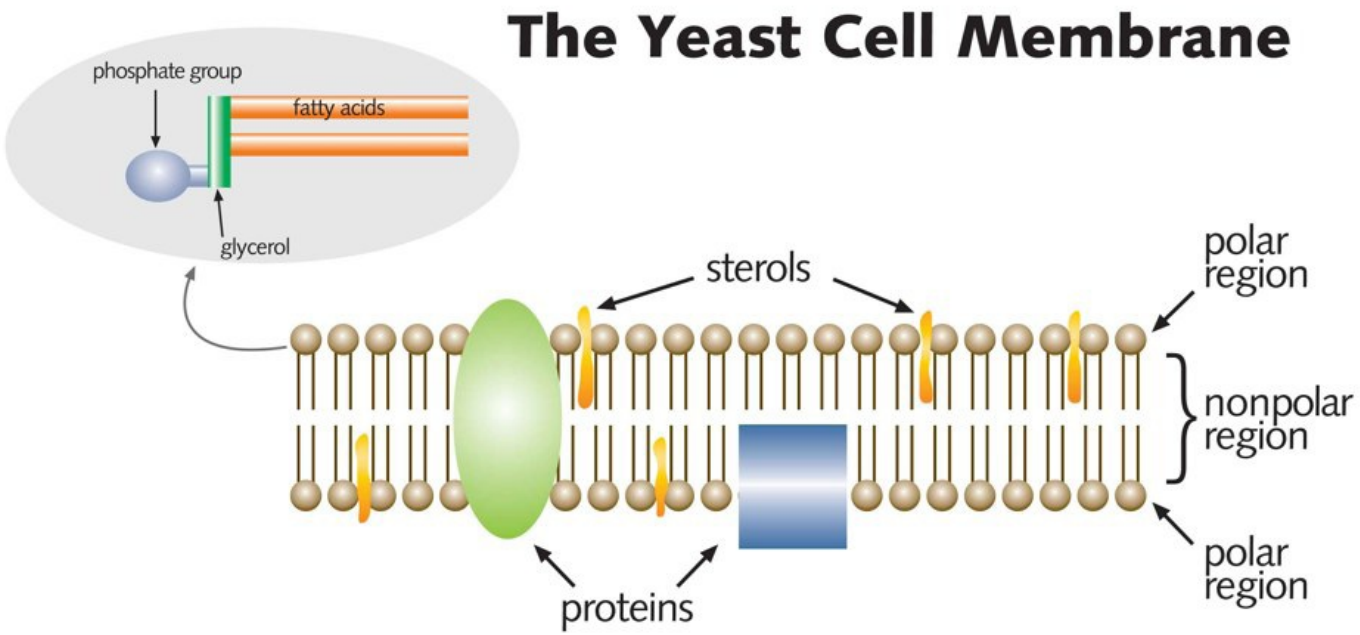
- Carbohydrates (carbon source: malt sugars)
- Oxygen (from aeration or agitation)
- Amino acids (nitrogen from malt)
- Minerals (from malt and brewing water)
- Vitamins (from malt)

Fermentation monitoring is an important indicator of possible nutrient deficiency in wort

Yeast Nutrition – Oxygen

- Oxygen is needed to synthesize sterols & fatty acids
- Essential components of yeast cell membrane
- Yeast is only capable of growth under anaerobic conditions if a surplus of sterols is available
- Yeast growth is sterol-limited

Yeast Nutrition – Oxygen



Yeast Nutrition – Oxygen

- Requirements are strain-dependent
- Generally 8-10ppm for moderate gravity wort (higher with increasing gravity)
- Without adequate supply → low vitality → poor fermentation performance
- Especially important in later generations when yeast are in an anaerobic physiological state

Yeast Nutrition – Nitrogen

- Used in production of proteins – protein complexes for cell wall components, enzymes
- In the form of FAN (free amino nitrogen) or amino acids
- Ideally, 100-150ppm FAN
- All-malt worts are usually sufficient in amino acid content, unless high gravity
- High adjunct brewing will require additional

Yeast Nutrition – Nitrogen

- Preferential uptake of amino acids, based on specificity of permeases utilized in their transport

Taken up first

Class A	Class B	Class C	Class D
Arginine	Histidine	Alanine	Proline
Asparagine	Isoleucine	Ammonia	
Apartate	Leucine	Glycine	
Glutamate	Methionine	Phenylalanine	
Glutamine	Valine	Tyrosine	
Lysine		Tyrptophan	
Serine			
Threonine			

The Role of Nitrogen in Brewing. (Pierce, 1987)

Yeast Nutrition – Nitrogen

- Some amino acid concentrations also affect production of flavor compounds

Has least impact

Class 1	Class 2	Class 3
Asparagine	Isoleucine	Lysine
Apartate	Valine	Histidine
Glutamate	Phenylalanine	Arginine
Threonine	Glycine	Leucine
Methionine	Tyrosine	
Proline		
Serine		

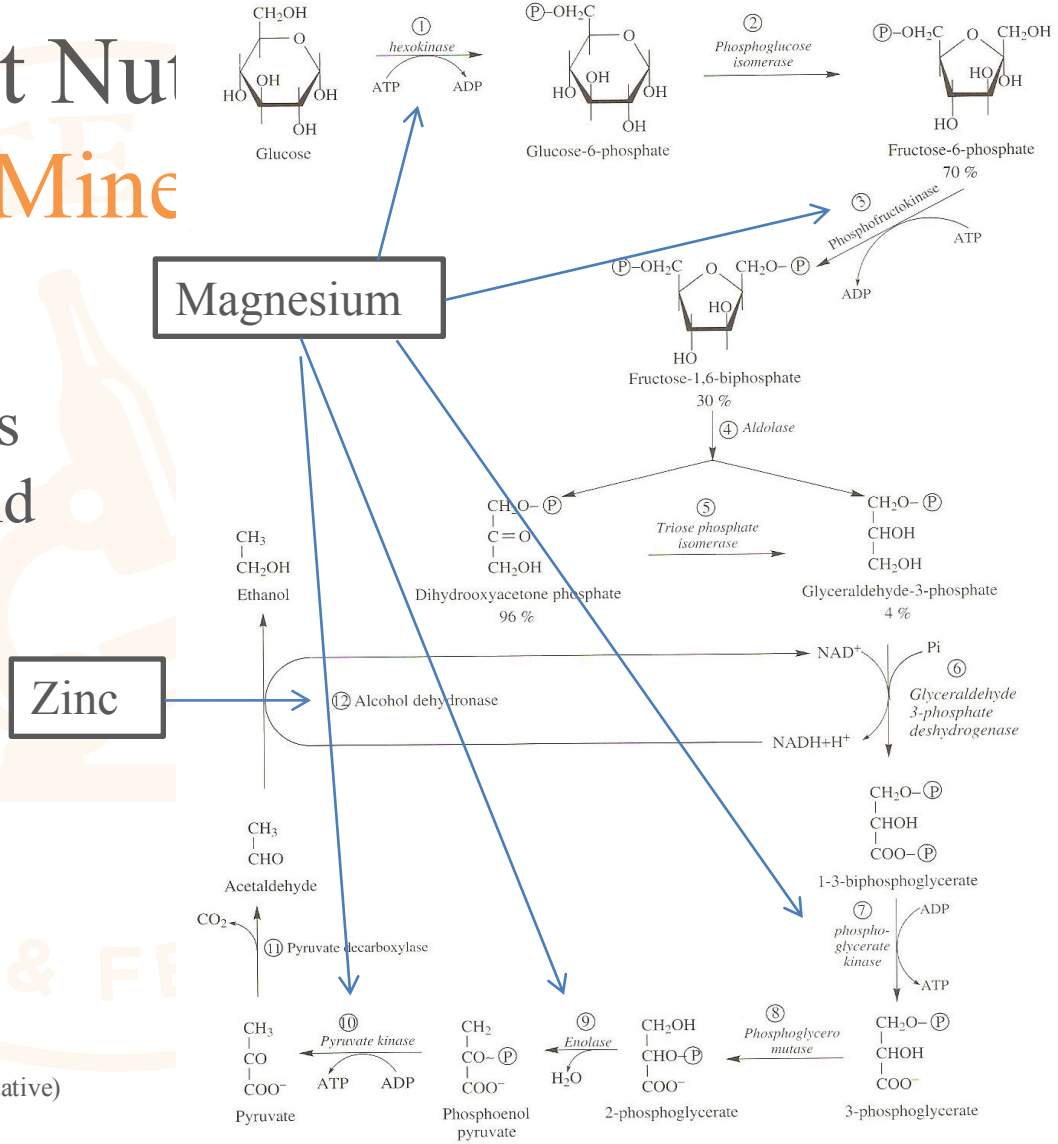
The Role of Nitrogen in Brewing. (Pierce, 1987)

Yeast Nutrition – Minerals

- Magnesium → cofactor for yeast metabolic enzymes
- Zinc → specific cofactor for alcohol dehydrogenase
- Calcium → essential in yeast flocculation pathway
- Manganese & Potassium (trace)

Yeast Nu
Mine

- Enzymes in glucose pathway require metal ions to work, primarily Zinc and Magnesium



Utilization of glucose by Saccharomyces cerevisiae under anaerobic (fermentative) conditions. (Ribéreau-Gayon 2000)

Flavor Active Compounds

Apart from ethanol and CO_2, yeast contribute significantly to the flavor & aroma of beer

- Esters
- Alcohols (fusel)
- Vicinal diketones (diacetyl, 2,3-pentandione)
- Aldehydes (primarily acetaldehyde)
- Phenols
- Organic acids
- Sulfur compounds
- Fatty acids

Alcoholic
Solventy

Estery
Fruity

Grassy
Butterscotch

Sulfury
Yeasty

Autolysis

Yeast

Metallic
Salty

Water

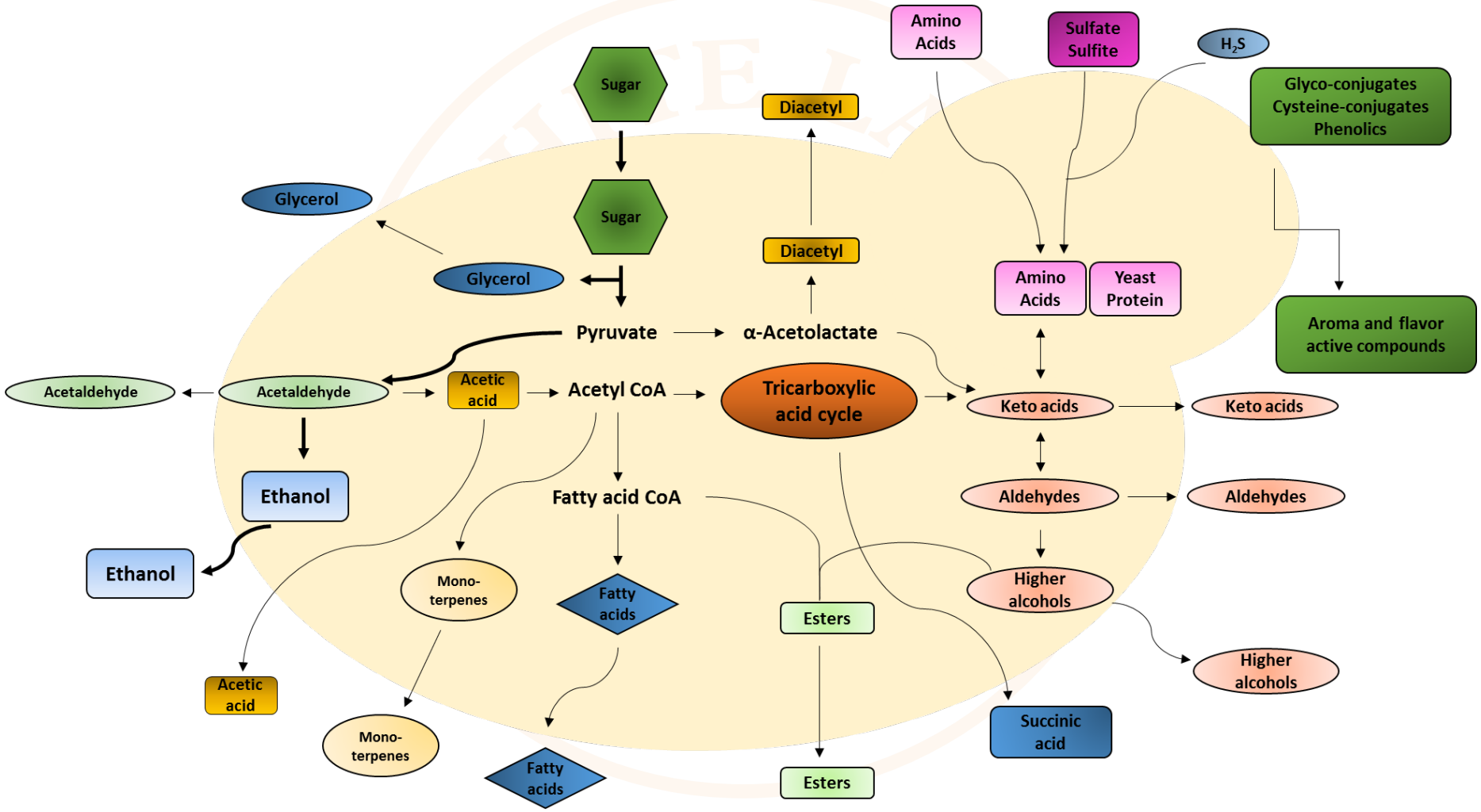
Hops

Floral
Citrus
Bitter

Malt

Grainy
Bready

Worty
Caramel


Amino Acids
Sulfate Sulfite
H_2S
Glyco-conjugates
Cysteine-conjugates
Phenolics
Sugar
Diacetyl
Glycerol
Sugar
Diacetyl
Glycerol
Amino Acids
Yeast Protein
Aroma and flavor active compounds
Pyruvate
α-Acetolactate
Acetaldehyde
Acetaldehyde
Acetic acid
Acetyl CoA
Tricarboxylic acid cycle
Keto acids
Keto acids
Ethanol
Fatty acid CoA
Aldehydes
Aldehydes
Ethanol
Mono-terpenes
Fatty acids
Higher alcohols
Esters
Higher alcohols
Acetic acid
Mono-terpenes
Succinic acid
Fatty acids
Esters

Yeast Flavor & Aroma

Remain at Levels Produced After Primary Fermentation

- Esters
- Higher alcohols
- Sulfur dioxide
- Phenols

Decline During Beer Maturation

- Acetaldehyde
- Diacetyl

Yeast Flavor & Development

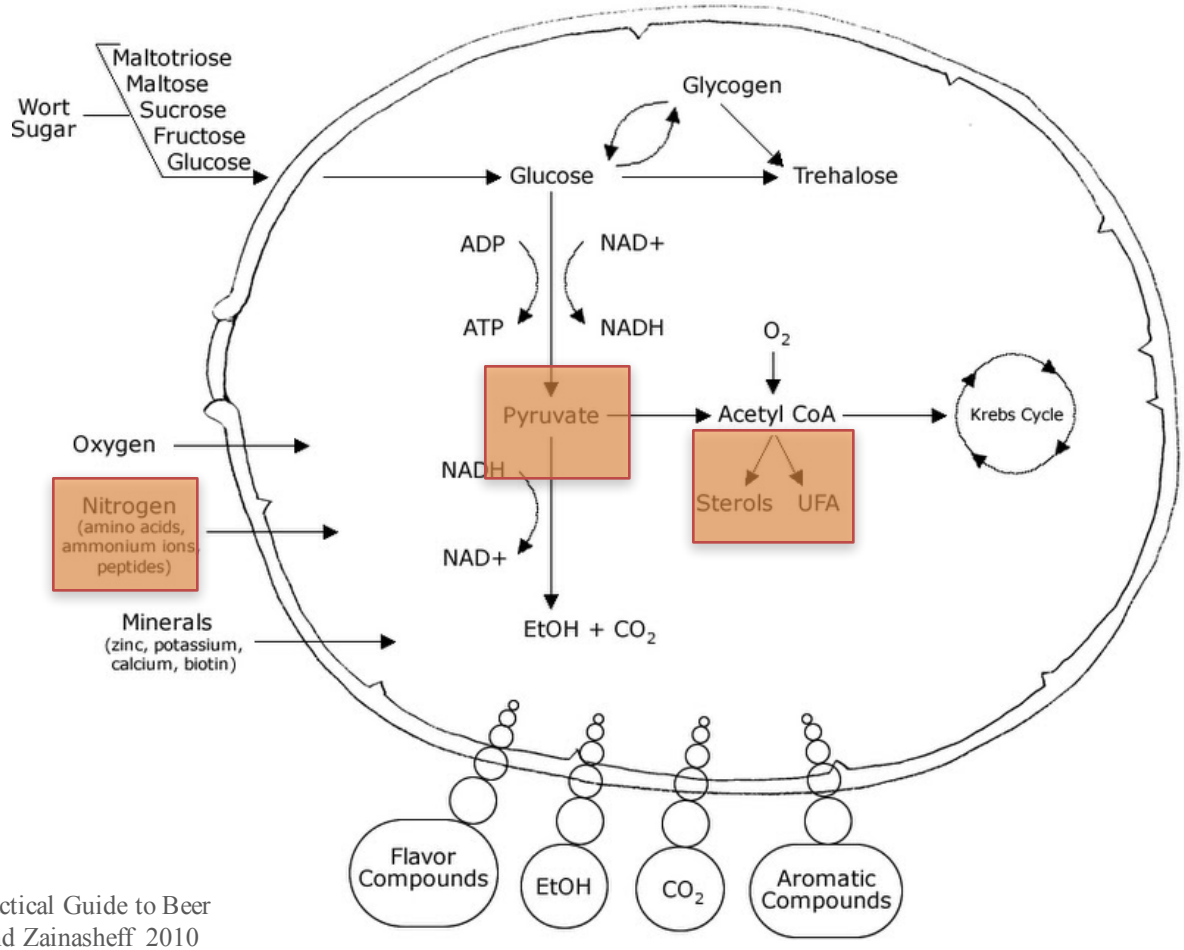


Fig 2.3 Yeast: The Practical Guide to Beer Fermentation, White and Zainasheff 2010

Esters

Flavors - fruity, banana, apples, perfume, solvent, nail polish remover

Formation

- Reaction of alcohol group and acid group in the yeast cell
- Alcohol part comes from ethanol and fusel alcohols
- Acid part comes from various acids that are inside the yeast (acetyl-CoA compounds)
- Reaction is catalysed by an enzyme (alcohol acetyltransferase)

Esters

Control

- Ester synthesis not that simple.
- No direct relationship between yeast growth and ester synthesis.
- Strain dependent

Formation depends on:

- The amount of the acid (acetyl-CoA compounds)
- The amount and activity of the enzyme (Alcohol acetyltransferase)
- The amount of the higher alcohol
- Low temperature = low esters
- More Oxygen = low esters
- Highly yeast strain dependent
- More problematic in very strong beers
- May be symptom of Acetobacter

Higher (Fusel) Alcohols

Flavor- alcoholic, spicy, vinous, warm

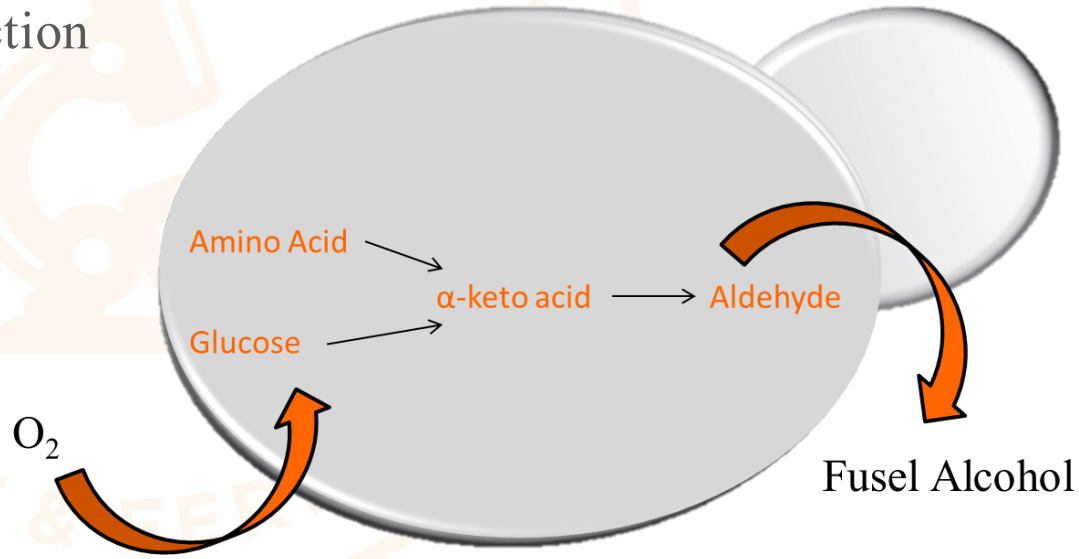
Formation

- Intermediates in amino acid metabolism
- Produced during uptake of amino acids
- Produced from glucose when yeast needs to make amino acids
- Directly related to yeast growth

Higher (Fusel) Alcohols

Control

- Any conditions that stimulate yeast growth will stimulate fusel alcohol production
  - Aeration
  - Lipid (fat) content of the wort
  - Trub
  - Agitation
  - Temperature



Sulfur Compounds

Flavor – sulfury, rotten eggs, burnt rubber, striking a match

Formation

- Intermediates in amino acid metabolism
- When yeast needs to make sulfur containing amino acids

Control

- Wort oxygen content (more is better)
- Fermentation temperature
- Yeast "health"

Phenols

Some yeasts are able to convert phenol carbon acids into phenols in the beer

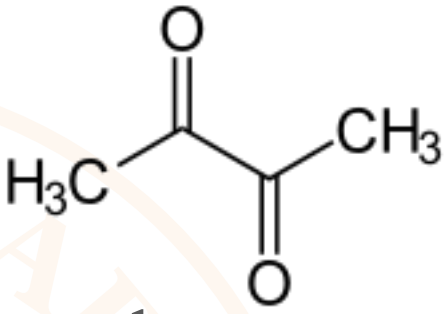
- **Phenolic Off Flavor (POF)** – POF positive yeasts are generally unwanted in brewing (wild yeast characteristics)
  - **Exception -** Bavarian Hefeweizen style where the phonol 4-Vinyl Guiacol is a desired compound due to its clove character as well as some Belgian beers

Flavor – Clove, solvent, plastic, bandaid, smoke (Wild/Belgian!)

Formation:

- During primary fermentation
- POF positive yeasts decarboxylate cinnamic acid derivates in wort to produce vinylphenols

Diacetyl

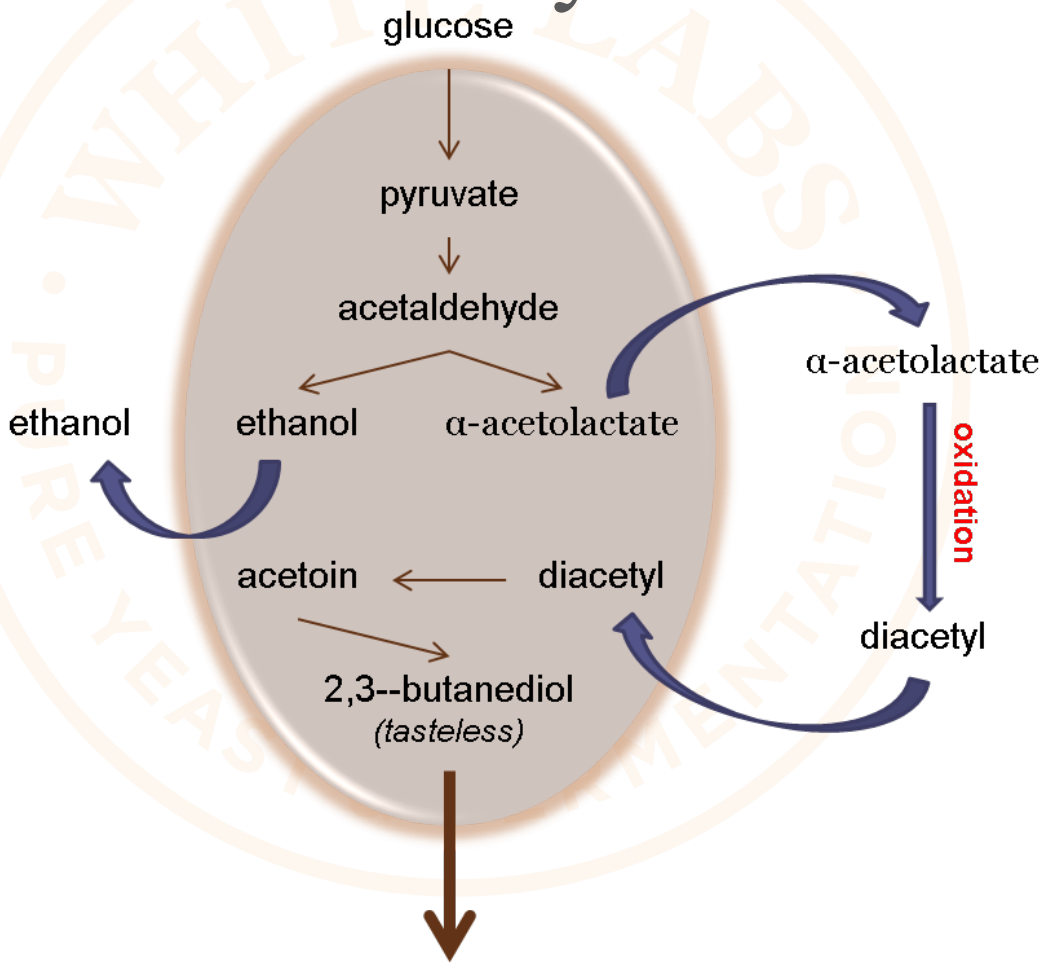


Flavor – Buttered popcorn, butterscotch, sweet yogurt, slick mouthfeel

Formation:

- Precurser (α-AL) produced during primary fermentation
- α-AL is converted to diacetyl outside cell
- Diacetyl is again taken up and metabolized by yeast during maturation
- Reaction related to amino acid synthesis
- pH and temperature dependent

Diacetyl


glucose
pyruvate
acetaldehyde
ethanol
ethanol
α-acetolactate
α-acetolactate
oxidation
acetoin
diacetyl
diacetyl
2,3--butanediol
(tasteless)

Acetaldehyde

Flavor – Grassy, green apples

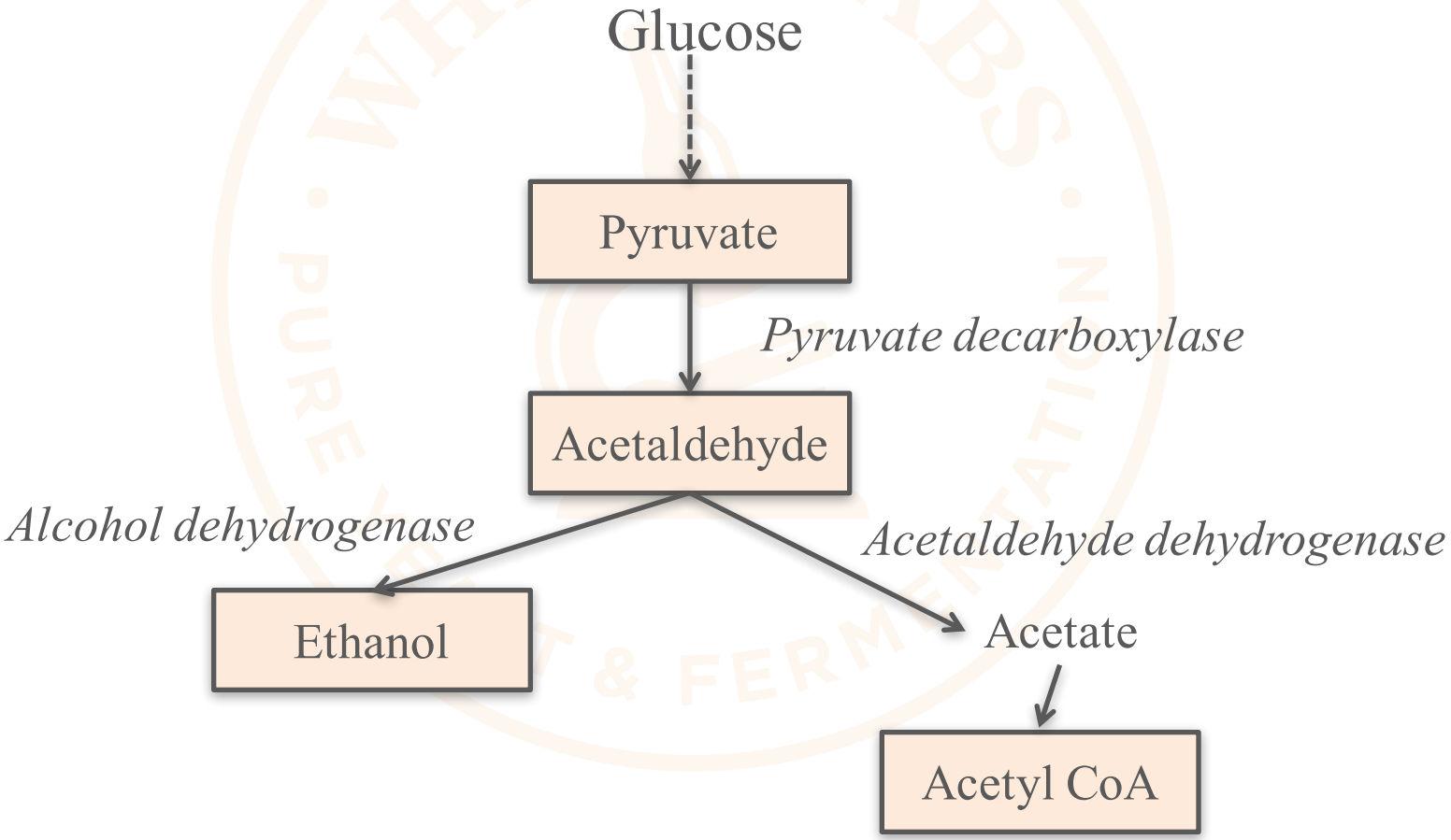
Formation:

- During primary fermentation, then reduced during maturation
- Intermediate of alcoholic fermentation pathway
- Metabolized to ethanol during maturation

Control:

- Healthy yeast
- Adequate conditioning time
- Temperature

Importance of Conditioning Time



Conclusions & Take Away

- Yeast metabolism is a complex biological process
  - Don’t worry about understanding the complex details!
  - The more you know the better you can control your fermentations

Thank you for listening!

Questions?

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Yeast Handling, Storage, & Maintenance

Joe Kurowski

Yeast Handling – What Do We Mean?

- Best practices for working with yeast
  - Maintaining a pure culture
    - Avoiding contamination by bacteria, wild yeast, or cross-contamination of brewing strains
  - Maintaining a healthy culture
  - Minimizing stress to yeast

Yeast Handling – What Do We Mean?

- This includes:
  - Yeast collection and harvesting
  - Yeast storage
  - Yeast propagation
  - Yeast maintenance

Yeast Collection & Harvesting

Yeast Collection & Harvesting

When is the best time to harvest?

- End of fermentation
- When early flocculating yeasts begin to drop to the bottom of the cone – discard
- Within 3 days of start of fermentation

Yeast Collection & Harvesting

How should yeast be collected?

Top Cropping

- Benefits
  - Yeast rises at a time of high vitality and viability
  - Free from trub – better shelf life
  - Faster turnaround time for yeast collection
- Disadvantages
  - Beer & yeast are exposed to environment

Yeast Collection & Harvesting

How should yeast be collected?

- Top Cropping – Best practices

  More flocculent yeast = better top croppers

  - Timing – 48-72 hours
  - Location – past first layer (protein)
  - Skim yeast with a paddle, shovel, or bucket which can be sterilized (stainless steel)

Yeast Collection & Harvesting

How should yeast be collected?

Bottom Cropping

- Benefits
  - Equipment design lends well to bottom cropping
  - Some strains can't be cropped from top
- Disadvantages
  - Breakdown of yeast happens faster – stress from hydrostatics, alcohol, temperature
  - High percentage of trub
  - Turnaround time to collect yeast is longer

Yeast Collection & Harvesting

How should yeast be collected?

- Bottom Cropping – Best practices
  - Timing – end of fermentation, depending on strain
    - Remove as soon as possible without risking integrity of beer
  - Discard the first runnings
  - Use only the middle pack

Yeast Collection & Harvesting

Stratification of yeast during collection

Yeast Collection & Harvesting

How should yeast be collected?

Cone to cone?

- Need to visually verify yeast
  - Color
  - Trub
  - Concentration
  - Contamination analysis

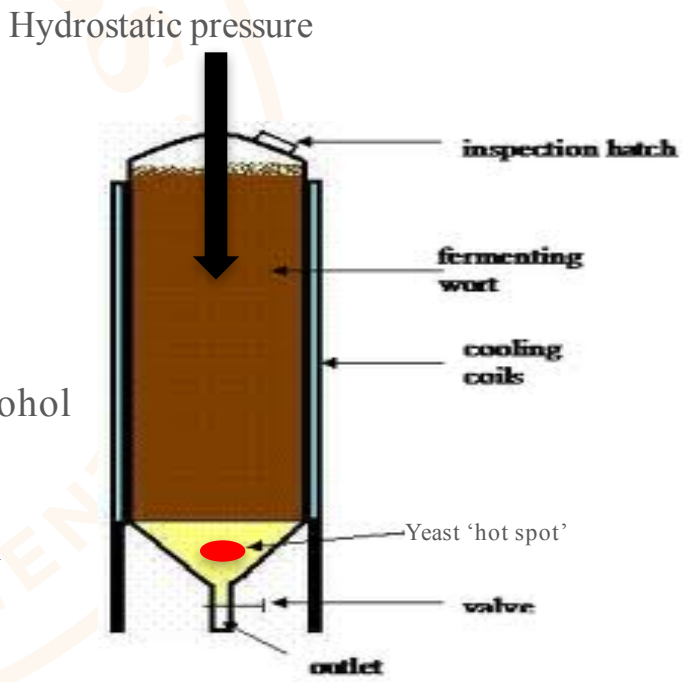
Collection

Collection vessels:

- Ferm-Flask or yeast brink
- Converted kegs
- Stainless steel bucket with lid
- Food-grade plastic bucket with lid
  - Polyethylene
  - Polypropylene

Storage

- Cone storage can be stressful
  - Hydrostatic pressure
  - Inhospitable environment – alcohol
  - Temperature in the cone
- Storage Medium:
  - On beer, wort, or water?
    - Beer – no transfer; great short term if under 6% alcohol
    - Wort – short term; carbohydrates present can be harmful
    - Water – best long term solution because it's neutral



Storage

Considerations for yeast storage:

Objective:

Keep metabolic activity to an absolute minimum in order to preserve viability and vitality

1. **Chilling the yeast**

- Chill yeast to between 2 - 4 ^{0} C
  - Keep metabolic activity to an absolute minimum
- If colder than 2^{0}C
  - Risk of freezing the yeast
  - Irreparable cell damage and subsequent death

Storage

Considerations for yeast storage:

1. **Chilling the yeast (cont'd)**

- If warmer than 4^0C
  - Alcohol toxicity
  - Limited nutrients
  - Depletion of glycogen
  - Loss of viability / vitality

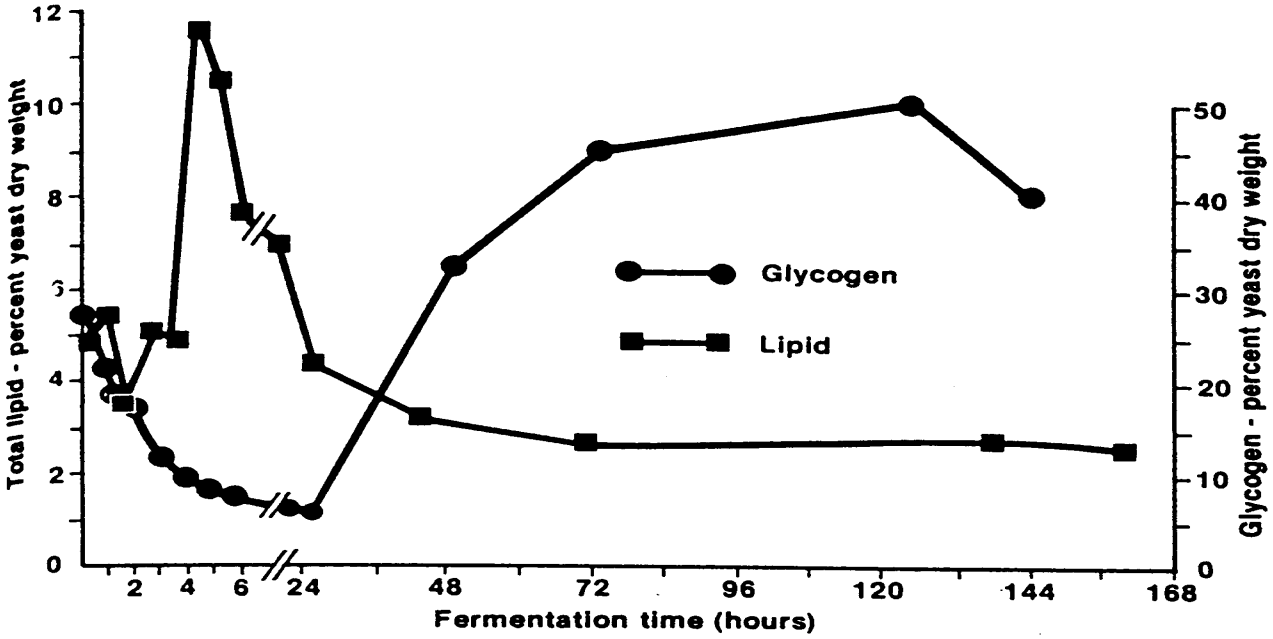
Storage

Considerations for yeast storage:

2. Glycogen and lipids

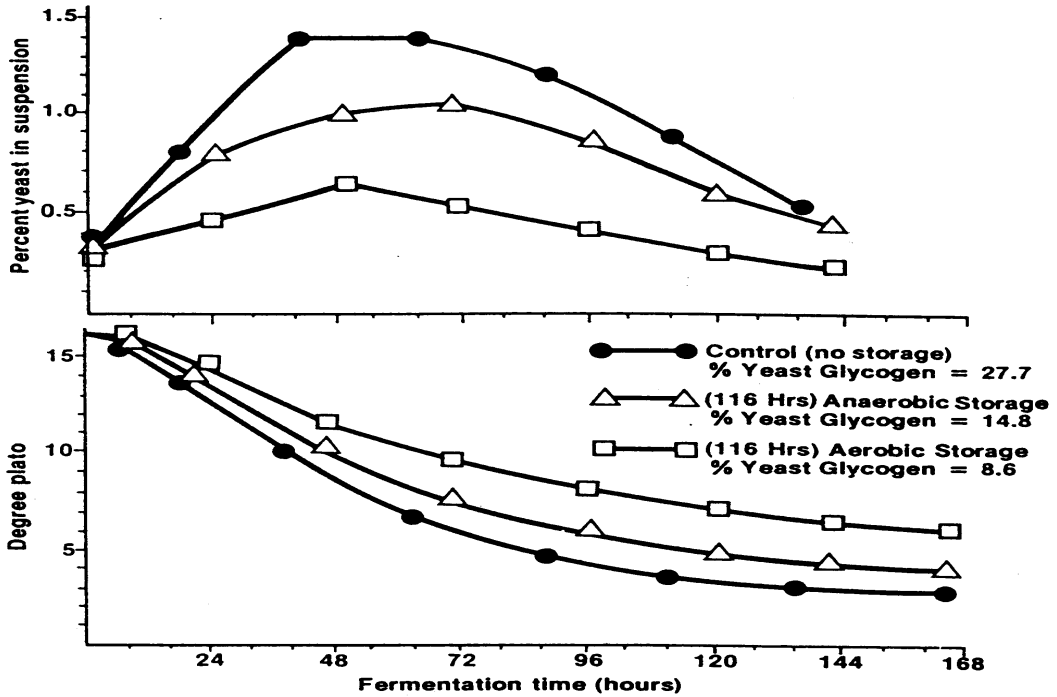
- Glycogen is the major reserve carbohydrate stored within the yeast cell.
- "Store" of to sustain the cell during periods of starvation
- In the presence of oxygen, glycogen is rapidly mobilized to fuel lipid (sterol and unsaturated fatty acids) synthesis.

Yeast Glycogen and Lipid during a 16 ^{0}P Lager Fermentation



C.R. Murray, T. Barich and D. Taylor
MBAA Technical Quarterly, 21 (4) 1984

The Effect of Yeast Glycogen Concentration at Pitching on a 16 ^{0} P Lager Fermentation



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MBAA Technical Quarterly, 21 (4) 1984

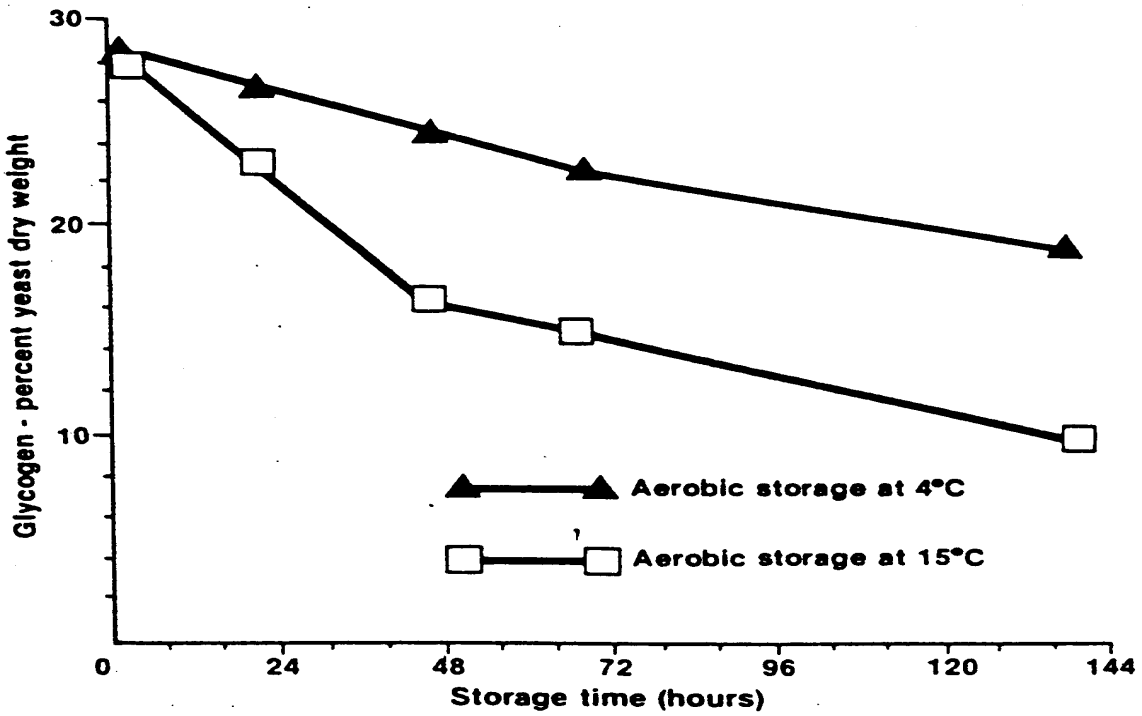
Storage

Considerations for yeast storage:

3. Temperature of storage

- Temperature must be maintained uniformly at ~ 4^0C
  - Yeast mixers - no "hot spots"
- Temperature affects glycogen storage

The Effect of Yeast Storage Time and Temperature on the Concentration of Intracellular Glycogen



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Storage

Considerations for yeast storage:

4. Oxygen/air during storage

- Oxygen is the trigger which causes the yeast to rapidly deplete it's glycogen stores.
- Under nutrient limitation (during storage) this glycogen is used for maintenance metabolism and not to fuel lipid synthesis
  - This depletion is deleterious to the yeast as the energy stores will not be available when they are required

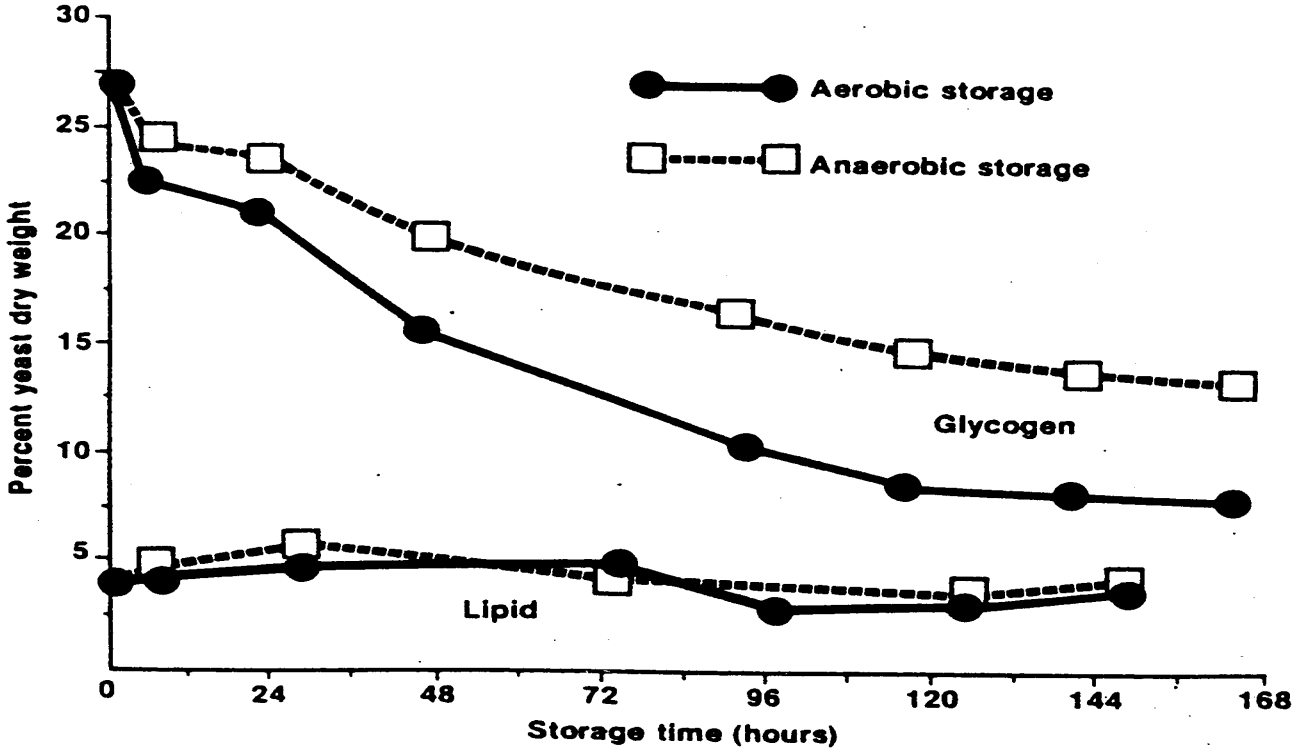
Storage

Considerations for yeast storage:

4. Oxygen / air during storage (cont'd)

- Metabolic heat generated compounds alcohol toxicity
  - Accelerated cell death
- Avoid oxygen/air pick-up during yeast storage

The Effect of Oxygen and Storage time on the Concentration of Intracellular Glycogen of Storage Yeast at 6 ^{0} C



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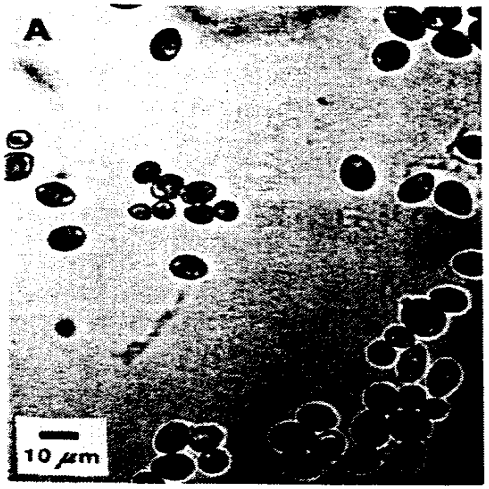
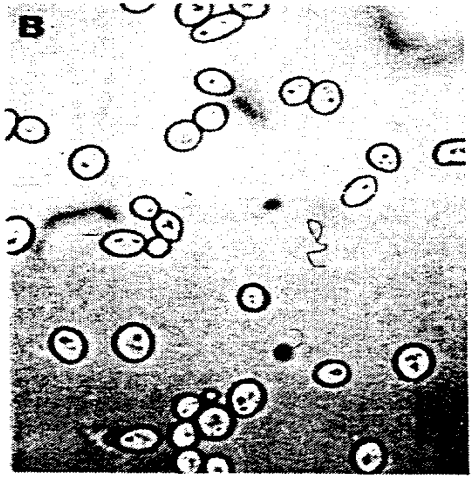
Photomicrograph of *Saccharomyces pastorianus* stained with Lugol's iodine.



Fermentation Vessel
(48 hrs)

(A)Yeast removed from a 16 ^{0}P Lager fermentation 48 hrs after pitching.



Storage Tank
(5 days)

(B) Yeast which has been stored aerobically at 6 ^{0}C for five days

Storage

Considerations for yeast storage:

5. Time

- Store yeast for as short a time as is possible
  - Recommended 1-3 days, ideally
  - Up to 2 weeks, with exceptions
- Petite mutants increase with increasing storage time
- Glycogen reserves will be slowly but surely reduced
- Ethanol stress

Storage

Considerations for yeast storage:

5. Time (cont'd)

The actual time that yeast can be stored without significant deterioration is influenced by:

- Yeast strain
- Process conditions (O.G., alcohol)
- Viability / vitality of the yeast
- Storage conditions

Storage

What can I do if I need to store it longer than recommended?

- Revitalizing, in some cases
- Best practices:
  - Feed the yeast some fresh wort to activate the cells
  - Add concentrated wort (~20P) to make up 5% of total volume of yeast/wort
  - Hold at room temp for 12 hours
  - Allow dead cells to drop to the bottom and decant the active yeast into fermentation

Yeast Maintenance

Re-pitching yeast – what to expect

- How many generations? – conditions & strain
  - Ales: 8-10
  - Lagers: 3-5
  - Wheat & Belgian: 3 or less
- First generation vs. later generations – why the differences?

Yeast Maintenance

Consistent pitch rate

- Fermentation speed
- Flavor profile
- Identification of problems early

Pitch the right amount of yeast for your beer!

Weight, volume, % yeast solids

Yeast Maintenance

Yeast Differentiation

- Simple microbiological methods:
  - Cell morphology
  - Giant colony (dye uptake on agar media – WLN)
  - Ale vs. lager tests:
    - Incubation at 37° C
  - Mellibiose
- Genetic testing
  - PCR

Yeast Maintenance

- Fermentation problems in second or third gen?
  - Related to poor conditions for yeast
  - Improper collection or storage
  - Inadequate oxygen
  - Inadequate nutrients

Summary

- Harvest yeast as soon as the bulk of the yeast has separated from the beer
- Chill rapidly to ~ 4°C and maintain that temp
- De-carbonate
- Exclude air
- Store for as short a period as possible
- Pitch accurately
- Evaluate the culture before using/reusing
- Keep it clean

Thank you!

Questions?

Brettanomyces & Bacteria Handling Guide

Karen Fortmann, Ph.D.

Outline

- Fermentation Techniques
  - Wort Inoculation
  - Inoculation Rates
  - Environmental conditions – temperature, pH, alcohol
- Brewery propagation & culture maintenance –
  - Propagation procedures
  - Harvest, reuse, storage

Fermentation Techniques – Wort Inoculation

- Primary vs. Secondary
  - Affects flavor characteristics of beer
  - Inoculation rates for organisms differ
  - Some strains of *Brettanomyces* do not perform well as primary fermenters
  - Two most commonly practiced methods currently

Fermentation Techniques – Wort Inoculation

- Primary vs. Secondary

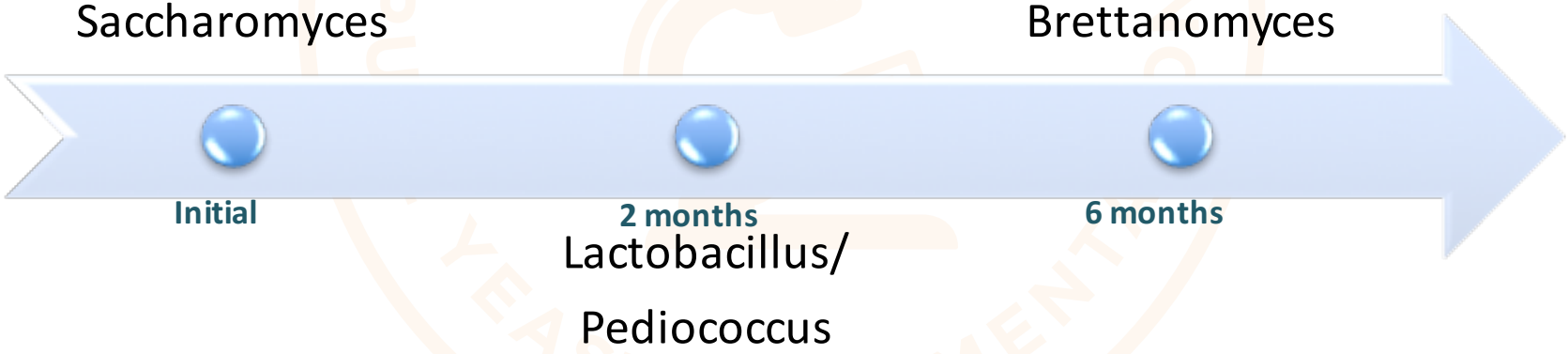
Strain	Tasting Notes – Primary	Tasting Notes - Secondary
WLP644 – S. "brux" trois	Tart, bright cherry, complex	Slightly phenolic, acidic
WLP645 – B. claussenii	Vinegary , Fruity	Esters, pineapple, tart
WLP650 – B. bruxellensis	Tart/sour, slightly horsey, fruity	Acidic, tart, malty
WLP653 – B. lambicus	Grassy, barnyard, phenolic	Acidic, dark fruit

**Using Flanders Red recipe*

Fermentation Techniques – Wort Inoculation

- Inoculation schedule
  - Succession of growth of various organisms
  - Attempt to represent timeline of spontaneous fermentation
  - Gives brewer control
  - Takes more planning
  - Can lose some of the organism symbiosis

Fermentation Techniques – Wort Inoculation



Fermentation Techniques – Wort Inoculation

- Mixed culture inoculation
  - All cultures added simultaneously
  - Closer to spontaneous fermentation
  - Concentration of individual organisms is critical
  - Potential for competition if environment isn't right

Fermentation Techniques – Inoculation Rates

Ale & Lager Brewing

Pitch Rate?

1 million cells/ml/degree Plato

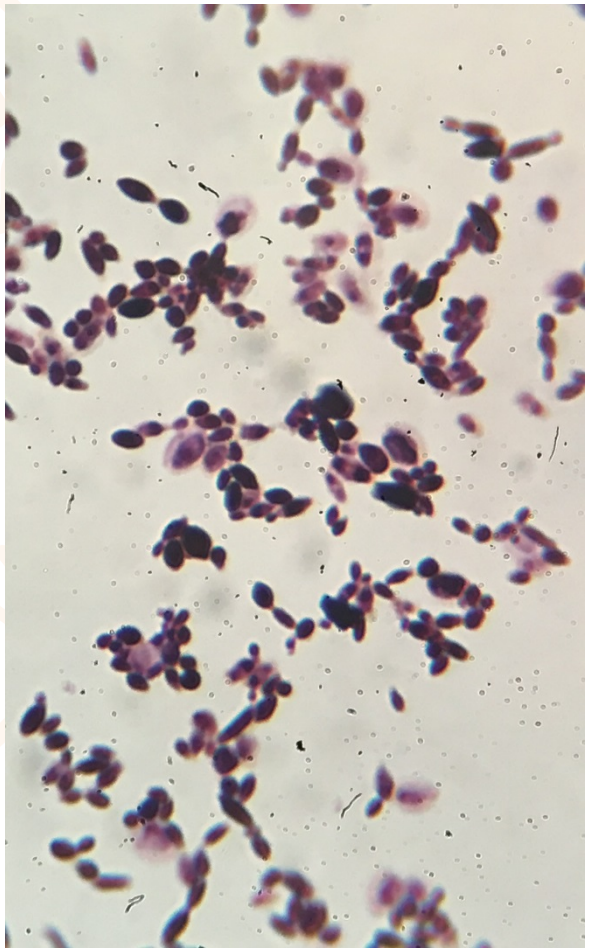
Fermentation Techniques – Inoculation Rates

Sour Beers

- *Saccharomyces*
  1 million cells/ml

- *Brettanomyces*
  100,000 - 200,000 cells/ml for secondary or mixed culture
  500,000 -700,000 cells/ml for primary

- *Lactobacillus/Pediococcus*
  200,000 cells/ml



Fermentation Techniques – Inoculation Rates

UNDERPITCH

WHY?

- Avoid one organism outcompeting others
- Allows all organisms (non-fermenting and fermenting) to create complex flavor compounds
- Plenty of time for growth over the long haul
- Encouragement of growth for development of flavor compounds

Fermentation Techniques – Environmental Considerations

- Temperature: Range 60-90°F (15-32°C)
  Optimal 65-77°F (18-25°C)
  - Too high – overgrowth and potential off-flavors
  - Too low – slowed growth & fermentation
  - Typically fluctuations between high & low

- pH Tolerance: low of 2.5

- Alcohol Tolerance: < 10%

Fermentation Techniques – Environmental Considerations

- Oxygen requirement:
  - *Brettanomyces*:
    - Fermentation/ethanol production stimulated by oxygen – Custers effect
    - Acetic acid (vinegar like) produced in presence of oxygen
  - *Lactobacillus & Pediococcus*:
    - Anaerobic fermentation

Fermentation Techniques – Environmental Considerations

- Carbohydrate requirement
  - Dextrins – for *Brettanomyces* later

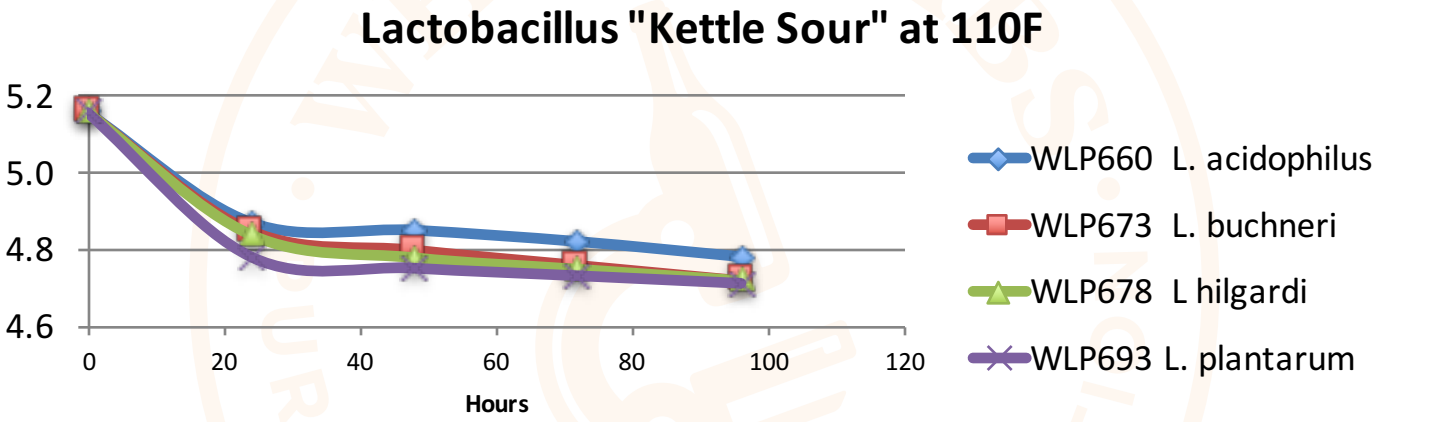
- Nutrient Requirement
  - Specific but fewer requirements than *Saccharomyces*
  - Yeast trub (from autolyzed primary yeast)

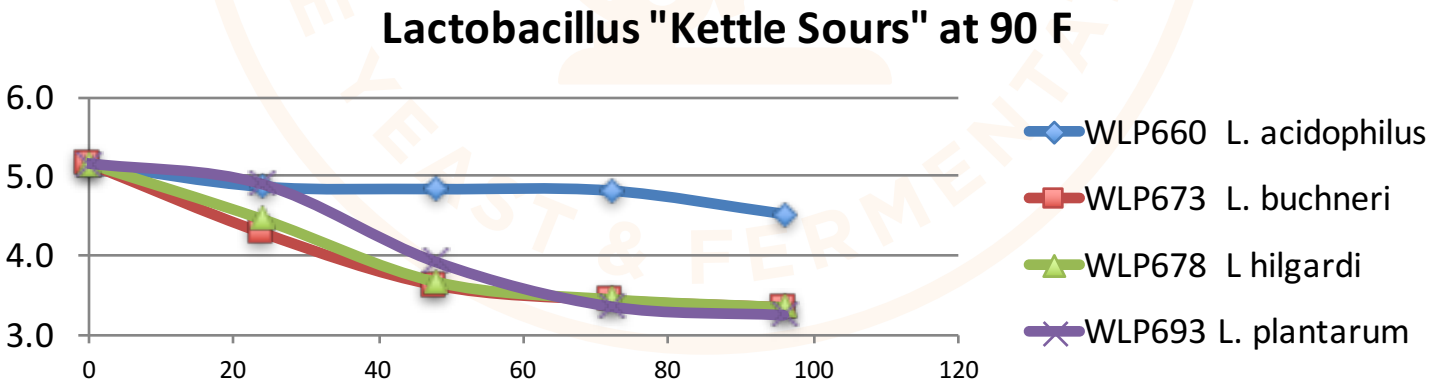
Fermentation Techniques

- Characteristics of isolated Lactic Acid Bacteria fermentations
  - Long lag phase – 3-7 days before active fermentation begins
  - Long fermentation time – actively fermenting over 5-6 months
  - Two types of sub-species

Homofermentative	Heterofermentative
Pediococcus, L. delbrueckii	L. brevis, L. plantarum
Metabolizes glucose to lactic acid	Metabolizes glucose to lactic acid, ethanol & CO_2

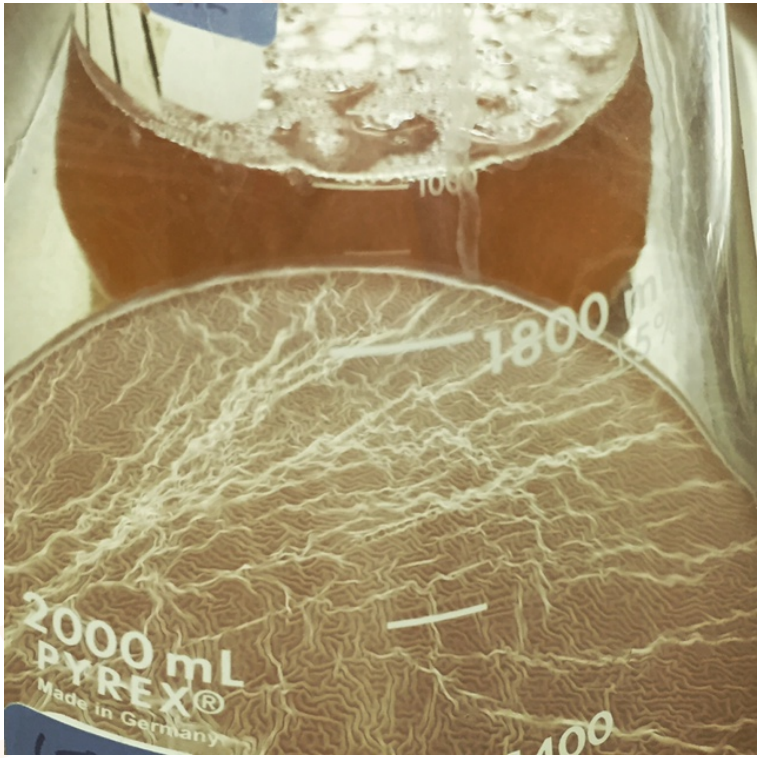
Kettle Sours



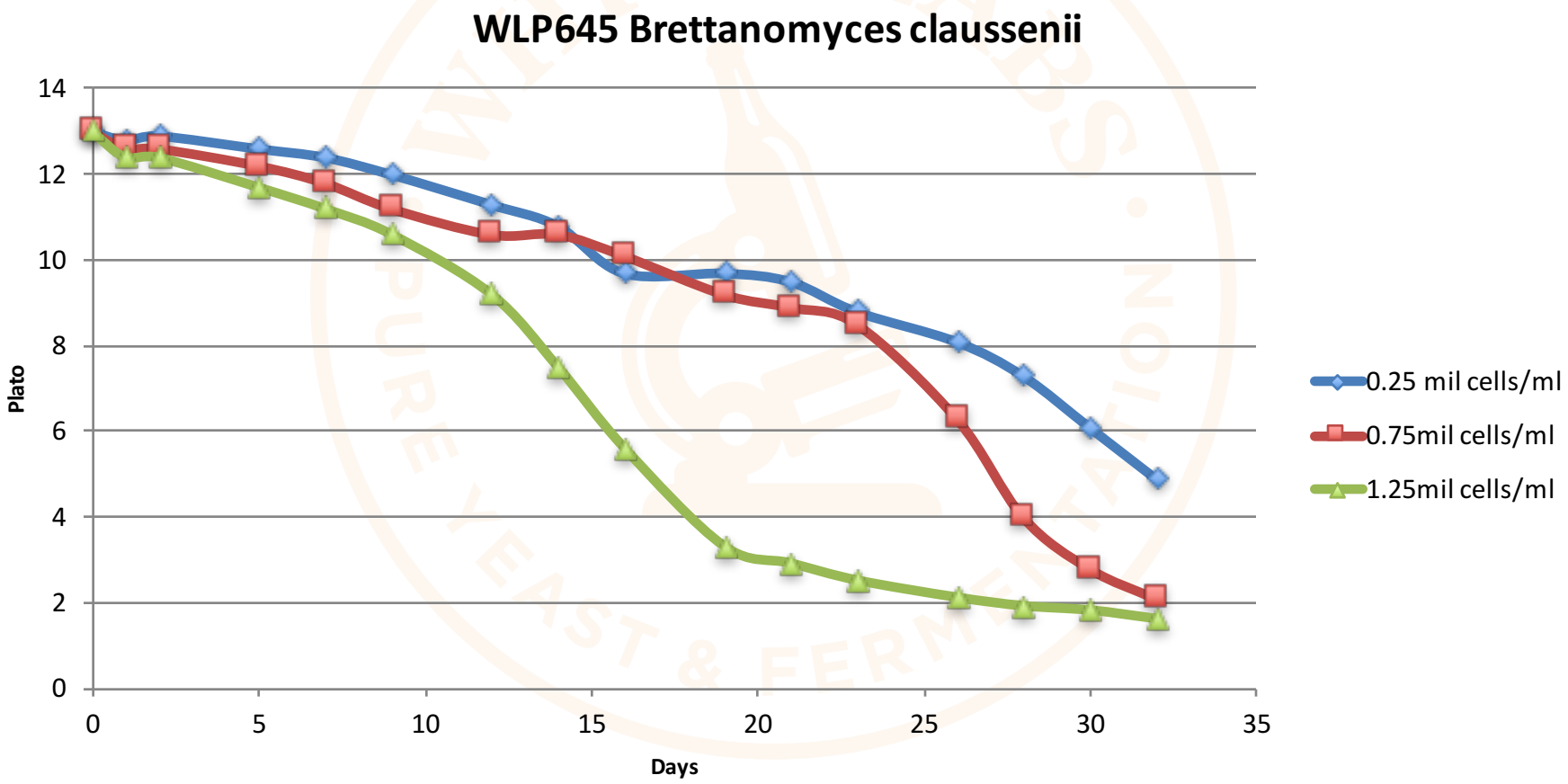


Fermentation Techniques

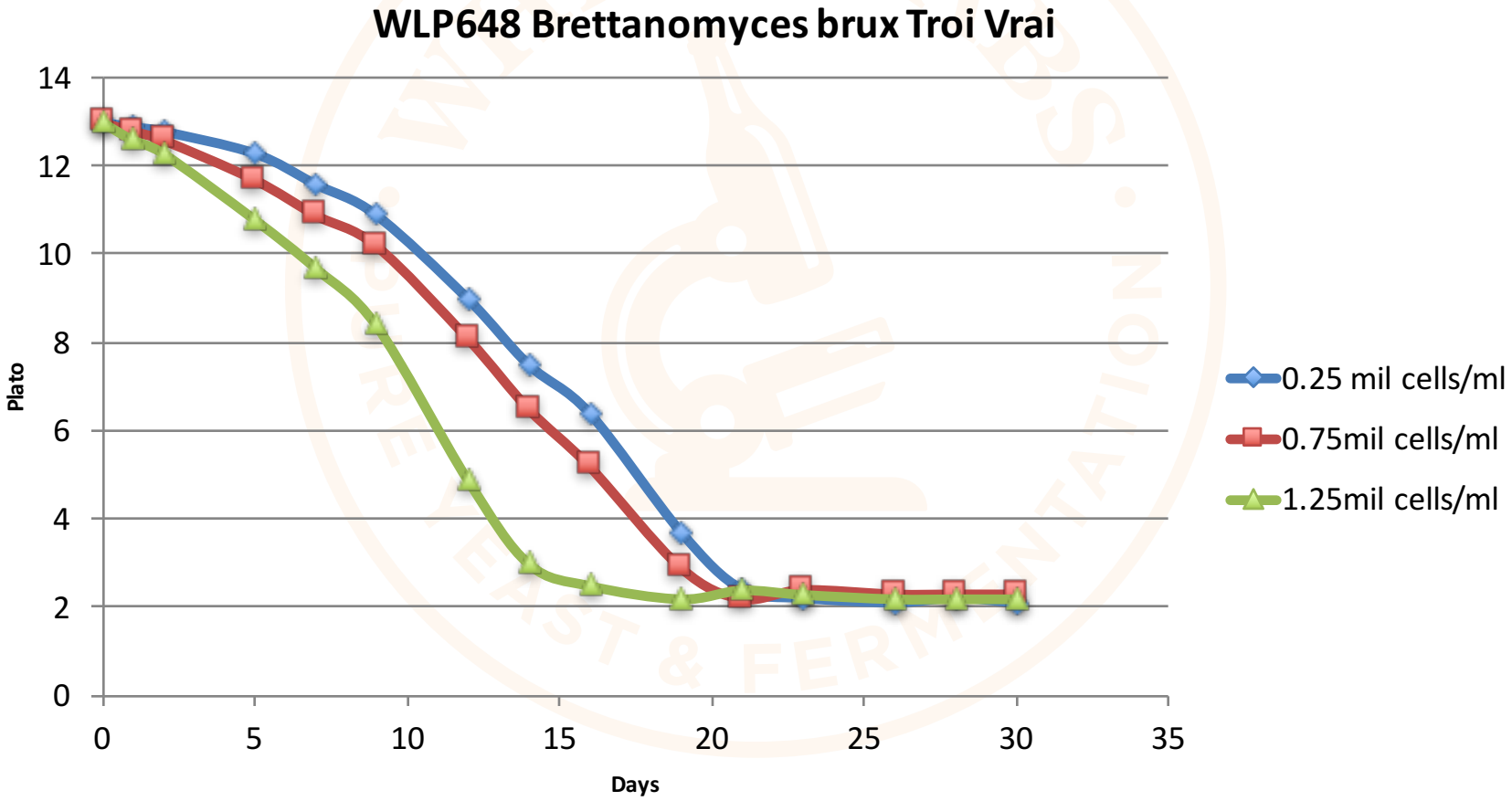
- Characteristics of *Brettanomyces* primary fermentations
  - Longer lag phase
  - Long fermentation time
  - Super-attenuating
  - Biofilm or pellicle forms at top of wort



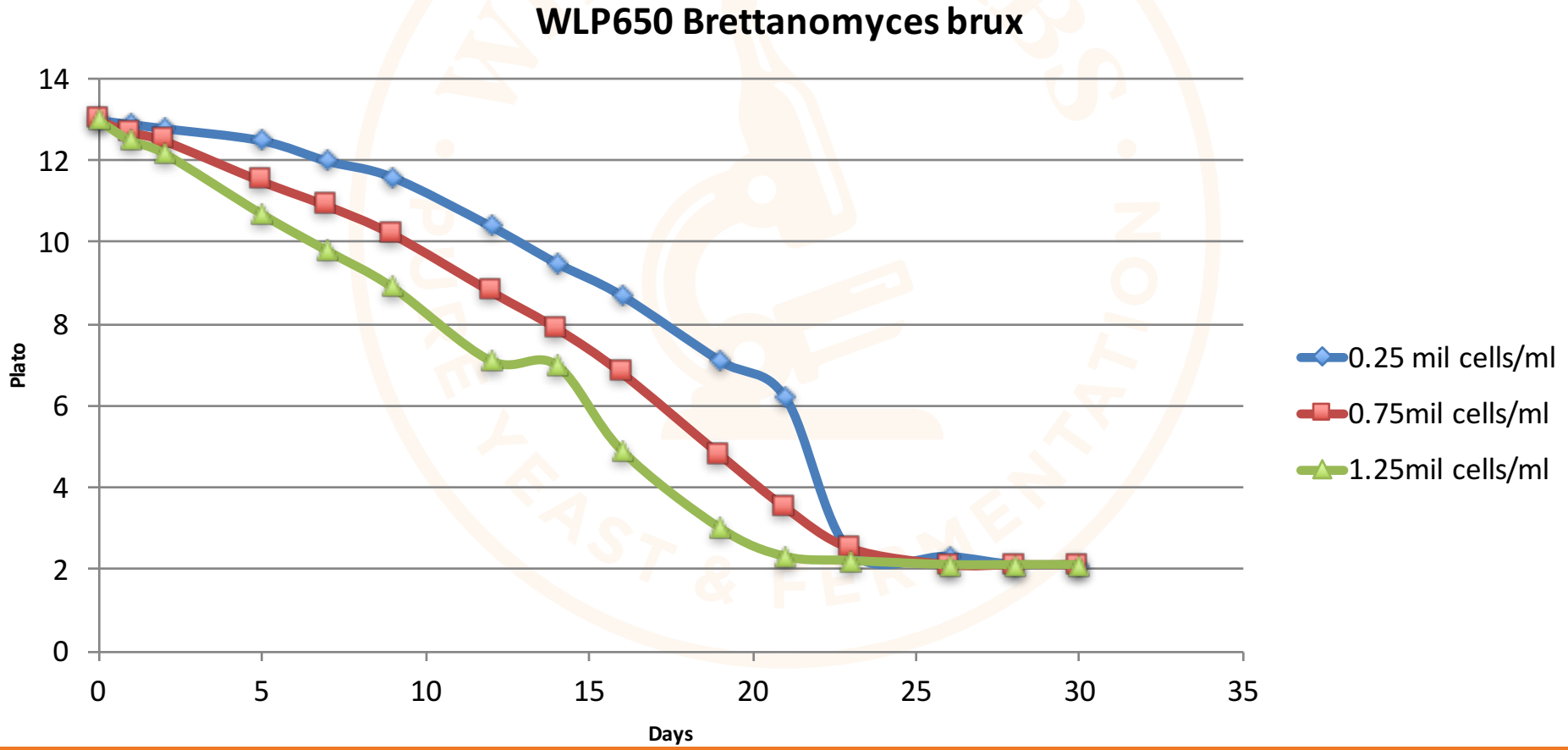
Pitching Rate vs. Attenuation



Pitching Rate vs. Attenuation



Pitching Rate vs. Attenuation



Fermentation Techniques

- Other considerations
  - Nutrient additions?
  - Fermentation vessel
    - Stainless tank
    - Plastic fermenter
    - Wooden barrels
  - Racking beer
    - To rack or not to rack?
  - Oxygen ingress during fermentation as beer evaporates (barrels)
  - Temperature regulation of the room? Humidity control?

Brewery Propagation & Maintenance

- Challenges to lab-scale propagations:
  - Organisms have strict & specialized nutrient requirements
  - Defined media can be expensive
  - Organisms cannot survive long periods without this media
  - Some expensive equipment necessary (i.e. 1000x microscope)
  - Difficult to maintain viable cultures over long periods of time
    - *Brettanomyces* need to be transferred to new slants approx. every 2 months

Brewery Propagation & Maintenance

Brewery Propagations – Simple way to scale up

- Wort
  - 8-10 Plato (ideally from malted barley rather than extract)
  - Buffer with 2% Calcium Carbonate (CaCO3)
  - Hops – 15-20IBU range
  - Additional nutrients: *Lactobacillus* & *Pediococcus*
    - Tomato juice – 10% OR
    - Dried brewers yeast – 0.5% OR
    - Yeast extract – 0.5%

Brewery Propagation & Maintenance

Brewery Propagations – Simple way to scale up

- Propagation

Temperature	**80-90°F (27-32°C)**
Time	5-7 days
Aeration	Yes – Brettanomyces No – Lacto & Pedio
Krausen	No
Scale-up size	10-20 fold

Brewery Propagation & Maintenance

- Harvesting mixed cultures
  - Skimmed off an actively fermenting culture, usually 5-6 months into fermentation
  - Timing when key organisms are at their peak
    - Saccharomyces present but declining
    - First round of *Pediococcous*/*Lactobacillus* present
    - *Brettanomyces* beginning to upsurge

Brewery Propagation & Maintenance

- Storing mixed cultures
  - Various containers
  - Store at 40°F (4°C)
  - Should be fed with fresh wort or used within 6-8 weeks to maintain viability of organisms

Brewery Propagation & Maintenance

- Reusing mixed cultures
  - Directly inoculate into next beer
  - Population densities for each organism will change over generations
  - Bacterial populations generally increase steadily
    - Higher concentrations of acids in beer
  - Acid wash cultures when bacteria too high?

Take Away Message

- Fermentation techniques vary from *Saccharomyces*
  - Will also vary for primary vs. secondary
- Bacteria and Wild Yeast are not as predictable as Brewer's yeast
  - Wild and sour beer is as much of an art as it is science

Thank you for listening!
Questions?

kfortmann@whitelabs.com

Setting up a Brewery Lab

Kara Taylor

Outline

- Why a QC Lab?
- Planning considerations
- Quality control measures and equipment
  - Yeast management
  - Microbiological control & identification
  - Fermentation analysis
  - Beer evaluation
  - Record keeping
- Size Considerations

Why a Quality Control Program?

- Consistent beer from batch to batch
- Consistent flavor profile
- Predictable fermentation rates
- Know your enemy!
  - Detection, identification, and control of brewery contaminants:
- Proactive vs. reactive
- Save money

Why Do I Need a Brewery Lab?

"I now believe there are only two types of breweries; those that have had a contamination and those that will"

– Fal Allen (Anderson Valley Brewing)

Planning a Brewery Lab

1. Budget
2. Create a timeline
3. Goals
   - Consider space requirements
   - Imagine expansion
   - Avoid unnecessary construction costs
   - Gas Lines
   - Electricity
   - Filters and air handling

Planning a Brewery Lab

- Goals
  - Yeast health & management
  - Clean brewing process
  - Predictable fermentations
  - No undesirable off flavors
  - Identify contaminants
- What you need to know
  - Cell counts, viability, morphology
  - Meaning of clean & forced wort testing
  - Forced fermentations
  - Forced diacetyl testing
  - Media plates & Gram staining

All of procedures and analyses should include **good record keeping!**

Cell Counts/Yeast Morphology

- Know your slurry concentration!
- Manipulation of yeast pitching rate
  - Performance
  - Yeast derived flavor compounds
  - Longevity of a batch of yeast (number of possible re-pitches)
- ***Bonus effect*:** Visual evaluation of the yeast culture helps the brewer understand the state of the culture

Yeast Viability vs. Vitality

- Definition of "viability"

"Capacity of a Cell to Exhibit Life Functions"

DEAD OR ALIVE

- Definition of "vitality"

"Yeast Activity or Physiological Health" Or
"Potential To Endure Stress and Still Perform"

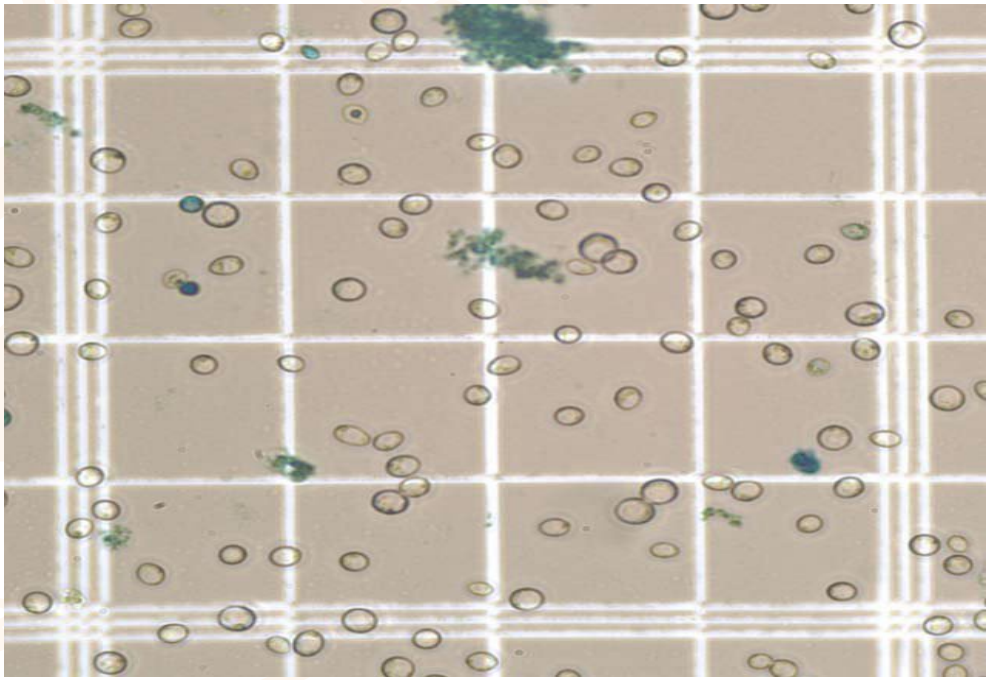
METABOLIC FITNESS

Yeast Viability Assay

Methylene Blue staining method

Pros: Quick, easy, inexpensive

Cons: Can be inaccurate, great risk of human error in dilutions



Yeast Management

What does it involve?

- Cell counts
  - Pitching rates
  - Viability
  - Cell Morphology

What equipment is needed?

- Microscope
- Hemocytometer + glass coverslip
- Handheld counter
- Methylene blue viability stain (ready-to-use liquid or powder concentrate)

Optional Equipment:

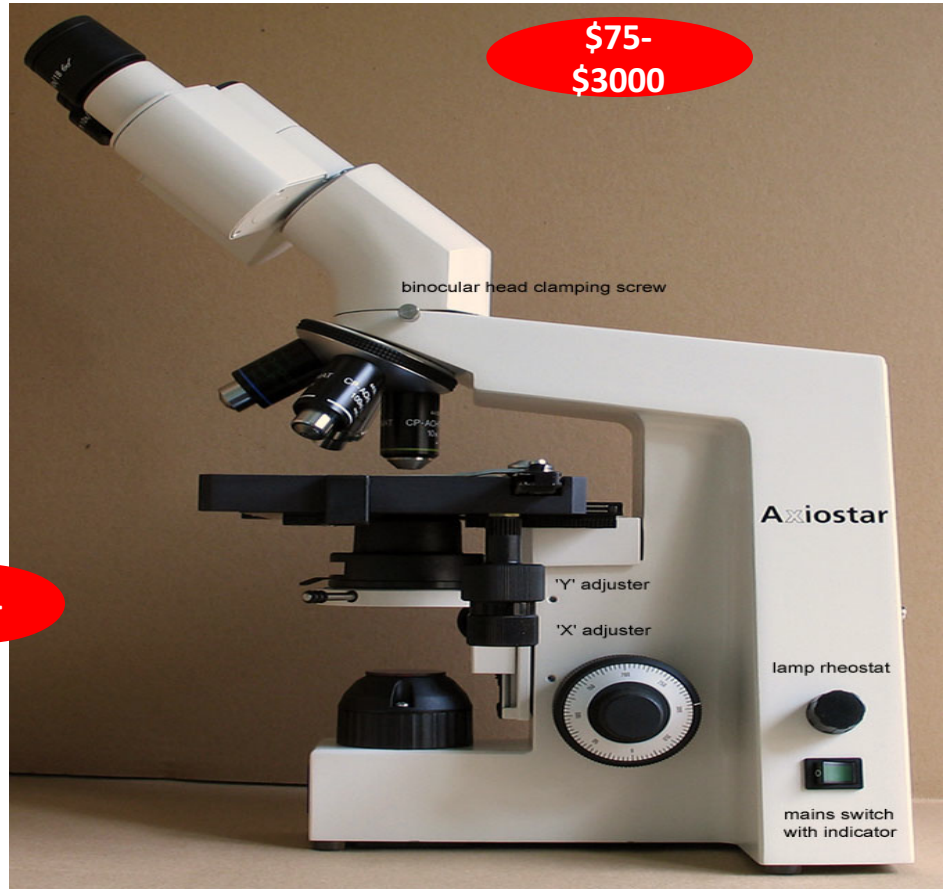
- Advanced cell counter
- Digital microscope camera

LIGHT MICROSCOPE

Specifications to consider when purchasing a microscope:

Require 1000X total magnification for bacteria visualization

•Having an adjustable stage is helpful



Cellometer

PROS:

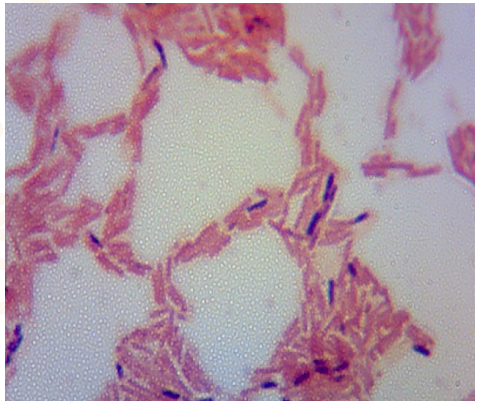
- Vitality + viability
- Less human error
- Easy to read

CONS:

- Not portable
- Counts trub as cells
- Difficulty counting budding or clumped cells
- No morphology observation

Digital Microscope Camera

This image was taken with a 2MP digital microscope camera!



Microbiological Control: The Meaning of Clean

Definitions:

- Clean – soil reduced to an acceptable level. Usually done with a combination of water and detergent
- Sanitized – viable organisms reduced to an acceptable level on a clean surface.
- Sterile – all organisms including spores and viruses are completely destroyed.

Microbiological Control: Cleaning & Sanitizing

"If you don't get it clean the first time, try, try again"

Frequency of cleaning
Length of exposure time to cleaning/sanitizing

- Importance of SOPs and audits
- Consider your brewery's needs
- Follow manufacturer's recommendations
- Validate with ATP meters

ATP Meter

PROS:

- Offers real time data
- Most machines allow testing of liquids and surfaces
- Quick, simple way to test surfaces

$1,600 - $2,100

CONS:

- Numbers are arbitrary without a baseline
- Need to test a "positive" surface and "negative" surface for a baseline.

Microbiological Control: Forced Wort Testing

Method

- Used to validate cleanliness of brewing hot side
  - Simple
  - Affordable
  - Effective
- After cooling, oxygenation, and wort transfer, a small sample is collected prior to pitching the yeast
- Incubated for evidence of contamination

Microbiological Control: Forced Wort Testing

Results

- Clear wort = Beer is clean
- Cloudy wort or wort with bubbles = contamination

Duration	Result
1 day	Very dirty, clean heat exchanger and hoses. Beer will need to be dumped.
2–3 days	Major contamination. Need to clean problem, beer most likely will be affected. Do not collect yeast for re-use from this batch.
3–6 days	Mild contamination build up, clean problem. Beer may or may not be affected.
7 or more	Very clean, keep up the good work

Microbiological Control: Forced Wort Testing

– Erlenmeyer flask or sterile single use containers (sampling bags, centrifuge tubes)

Microbiological Control: Water Testing

- Vacuum Pump

$600 +

- Sterile filters

$15+/ea

Contamination Detection

Use your senses: sensory evaluation

- Smell
- Taste
- Sight

Implement media into your QC program:

- Selective media plating
- Environmental plates in the brewery

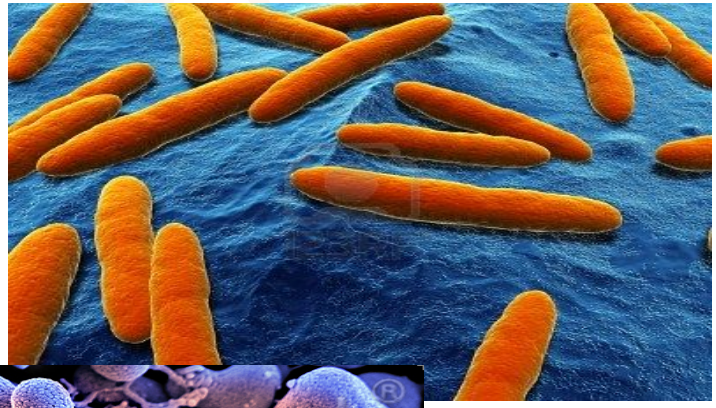
The Contaminants

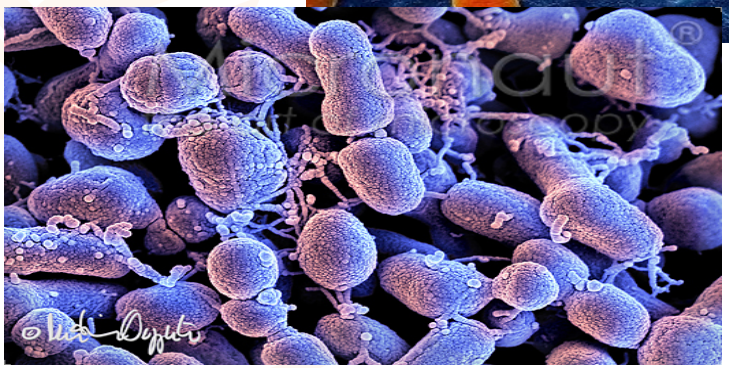
Acetic Acid Bacteria

- Gram-negative rods "beer spoilers"

Aerobic (don't survive in the absence of oxygen)

Acetobacter
Gluconobacter





The Contaminants

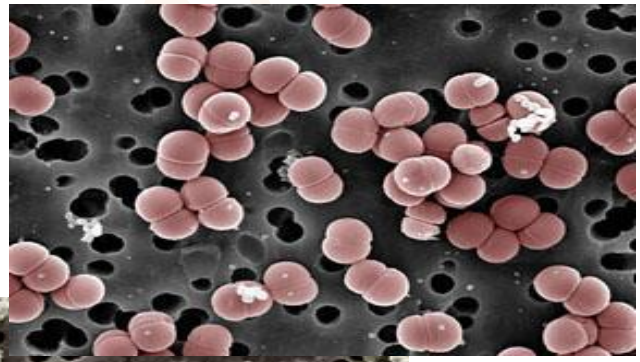
Lactic Acid Bacteria

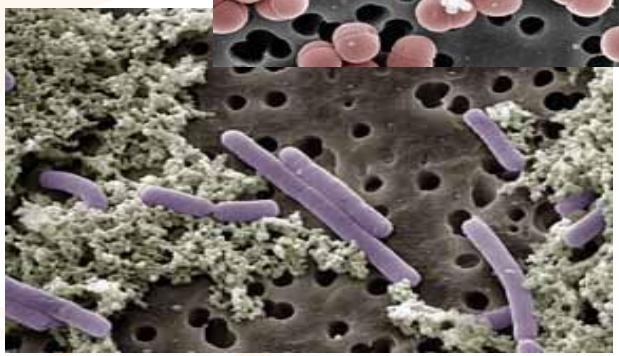
- Gram-positive rods or cocci

Aerotolerant anaerobes

Temperature tolerant (2-53°C)

Optimum temperature 30-40°C





The Contaminants

Wild Yeast

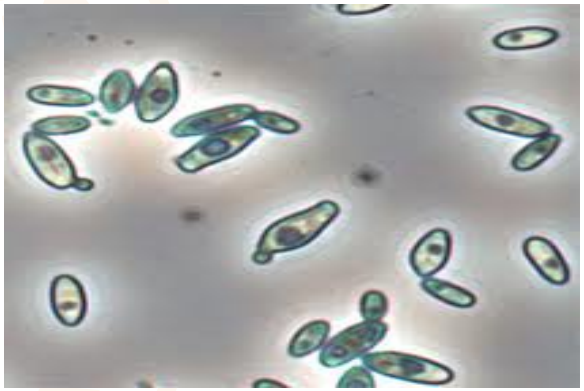
- Gram-positive cocci, lemon, football, or elongated

Temperature tolerant (2-53°C)

Optimum temperature 30-40°C

Alcohol tolerant

pH tolerant (down to 3.0)



Microbiological Control

- Selective/differential media
  - Pre-poured (easy but higher price)
  - [illegible] but need autoclave)

$1/plate

$1.50/ea

Microbiological Control

Optional Equipment

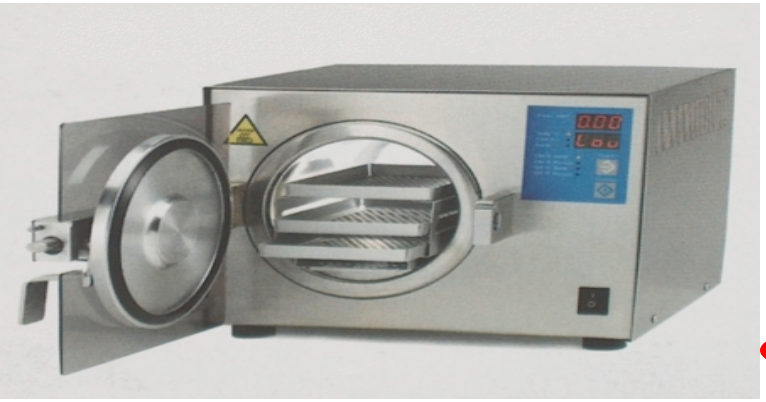
- Pressure cooker
  - "Starter autoclave"
    - Pros: cheap
    - Cons: not as effective or versatile
- Autoclave
  - Pros: versatile, helps save money in the long run
  - Cons: expensive
- Hot water bath
- Incubator
- Scale
- ATP Meter

$100+

$250+

$350+

$300+



AUTOCLAVE

$2,000 - $$

- Expensive!
- Small bench top autoclave:
  - Media
  - Small flasks
  - Some tubing
- Larger floor autoclave:
  - Run other equipment during a COP (ex: bottling line parts, butterfly valves)
  - Run less frequently and fit larger items
  - Yeast propagations will require an autoclave!

Selective or Differential Growth Media

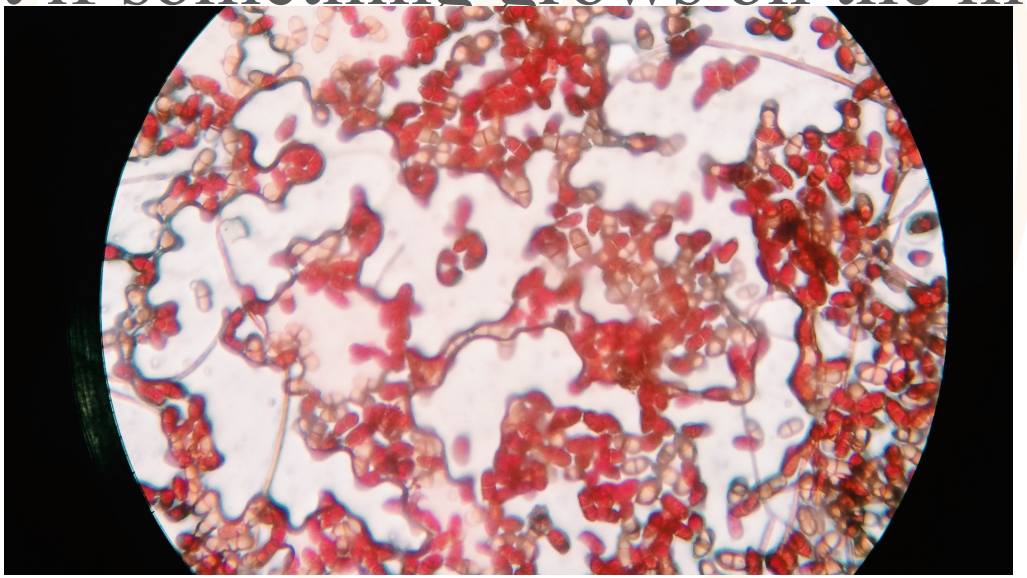
- Selective Media – only permits growth of certain organisms
- Differential Media – distinguishes between individual microorganisms
- Growth of organisms based on environmental and metabolic conditions
- Involves specific substrates and inhibitory compounds

Types of media

- Wallerstein Differential Media (WLD)
- Wallerstein Nutrient Media (WLN)
- Lin's Cupric Sulfate Media (LCSM)
- Lin's Wild Yeast Media (LWYM)
- Hsu's Lactobacillus and Pediococcus Media (HLP)
- Schwartz Differential Media (SDA/ LMDA)

Selective or Differential Growth Media

- So what if something grows on the media?



Identification from Plates

This

Not this

Identification from Plates

- Microscopy & Gram Staining (least expensive)
  - Simple, rapid, and requires minimal equipment and training
- Differentiate organisms by:
  - Cell morphology (shape & grouping)
  - Gram-positive or negative (purple or red)

Pros: Easy, cheap, relatively quick

Cons: Requires some microbial knowledge and skills.

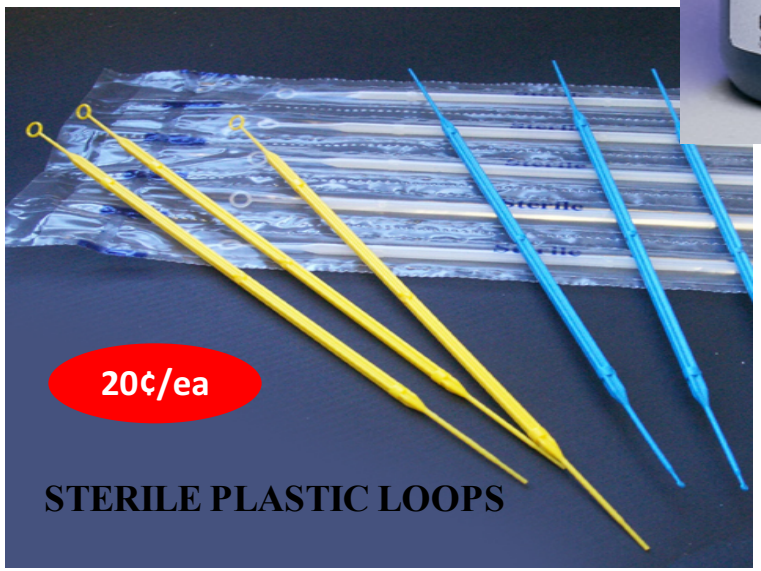
Microbiological Control

What is needed?

- Gram staining
  - Gram stain kit: slide + stains
  - Plastic sterile loops or metal loops
  - Flame – lighter or Bunsen burner
- Tolerance tests
  - Oxygen tolerance – anaerobic pouches
  - Catalase test – hydrogen peroxide
  - Oxidase test – oxidase slides
- Genetic analysis
  - Send out microbial samples for genetic ID


GRAM STAIN SLIDES
$4+/ea
AFB SLIDE
POSITIVE
NEGATIVE
BBL


$30+
BD
Gram Crystal Violet
Primary Stain
Stabilized Gram Iodine
Mordant
Gram Decolorizer
Primary Stain
Gram Safranin
Counterstain
GRAM STAIN KIT


20¢/ea
STERILE PLASTIC LOOPS

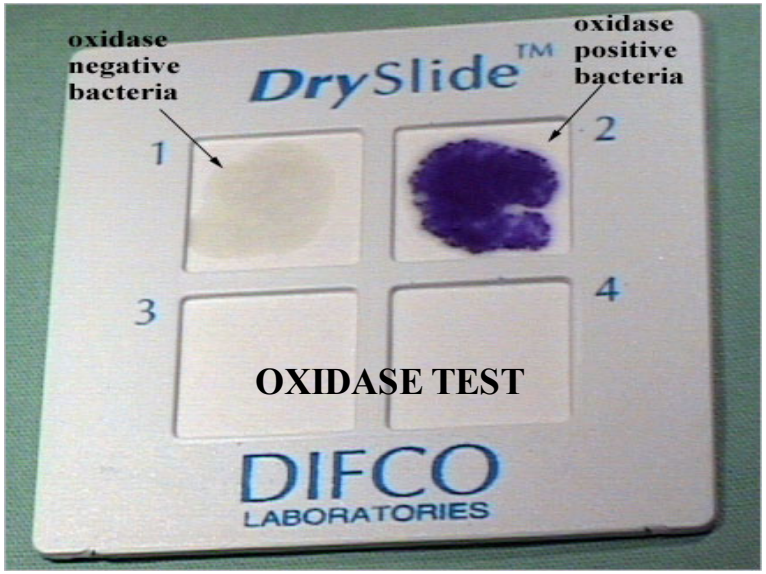

LIGHTER
$1+


BUNSEN
BURNER
$25+

Identification

Tolerance Tests

- Oxygen tolerance (aerobic vs. anaerobic)
- Catalase positive or negative
- Oxidase positive or negative



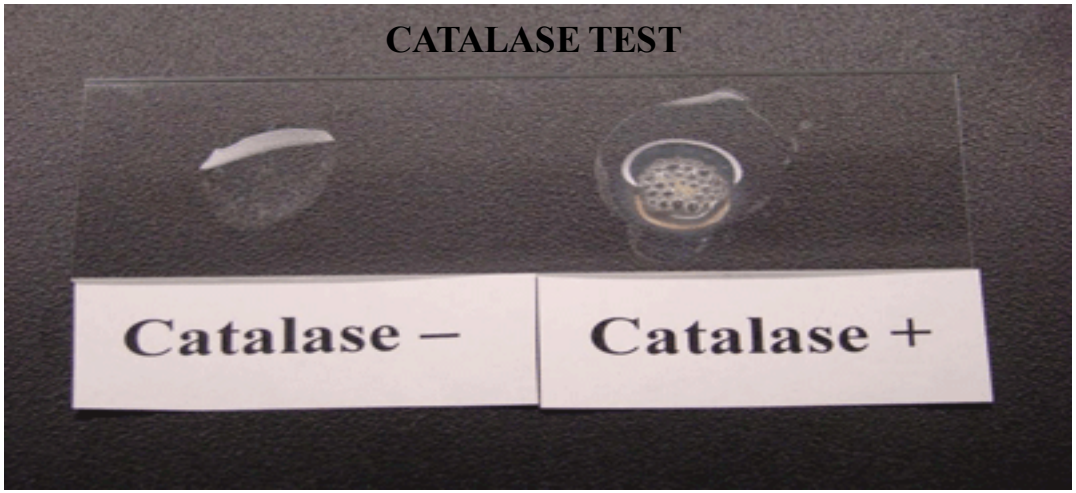
OXIDASE TEST

OXYGEN TOLERANCE

TOLERANCE TESTS

$2+ depending on test

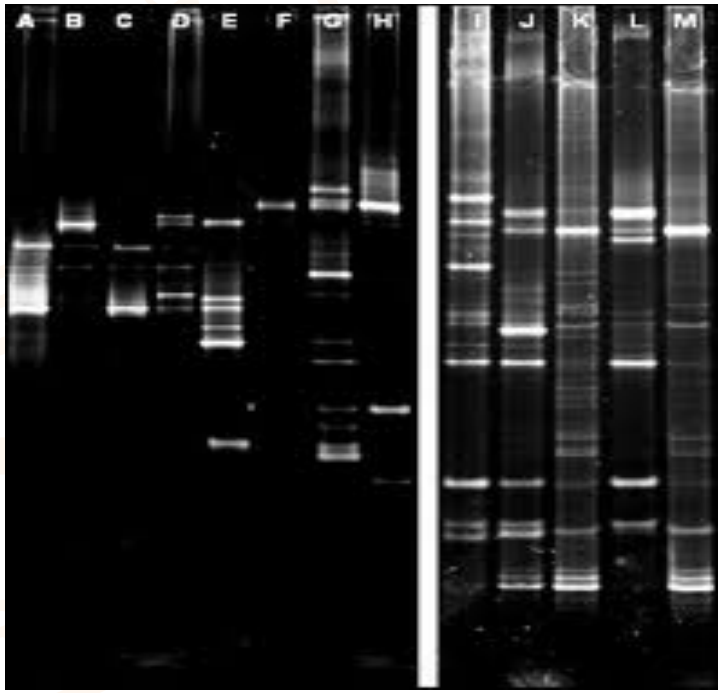
CATALASE TEST



Identification

Advanced Genetic Technology

- PCR (polymerase chain reaction), Genetic sequencing
  - Pros: accurate
  - Cons: expensive, results can take weeks



Identification

When is the best time to use genetic analysis?

- Most effective when you continue to see the same types of issues and growth
- Conduct research to see how people in the industry are dealing with this problem

Fermentation Analysis

- What should you be recording?
  - Dissolved oxygen
  - Cell count, viability, and pitch rate
  - Morphology of cells seen under microscope (i.e. round, oval, etc)
  - Real degree of fermentation (RDF) or Apparent attenuation (ADF)
  - Lag time – especially in terms of yeast generation
  - pH – starting pH and the drop in pH over the course of fermentation
  - Attenuation – time it takes yeast to attenuate the beer

Record all this data for each fermentation.

You can use it to determine when it's time to use a new pitch of yeast.

Fermentation Analysis

- Fermentation documentation and strain performance
  - Dissolved oxygen meter: portable or b
  - Written records or computer program
- Commonly used D.O. meter brands
  - Hach/Orbispheres
  - Mettler Toledo
  - Haffmans

Forced Fermentations

- Force fermentations to max attenuation
  - High temp
  - Constant stirring
- Once the activity stops:
  - Measure specific gravity
  - Result is lowest potential gravity with this *wort & yeast combination*.

Forced Diacetyl Testing

Method

- Forcing conversion of precursor to diacetyl with heat and oxygen
  - 2 samples
    - Heated (water bath at 140-160°F)
    - Room temperature

Procedure:

- 10-20 minutes
- Cool
- Smell

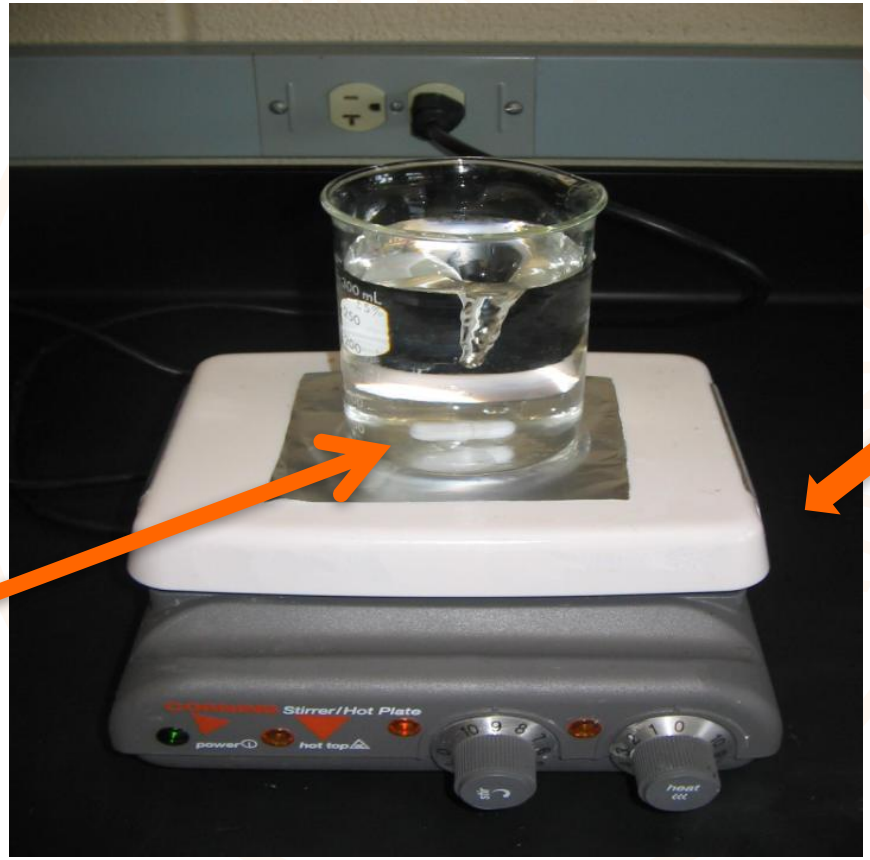
Forced Diacetyl Testing

Results

Room Temp Beer	Heated Beer	Conclusion
Negative	Negative	Little to no precursor present, beer is ready to go
Negative	Positive	Precursor present, beer needs more time on yeast
Positive	Positive	Beer is loaded with precursor or possibly contaminated

Equipment Requirements

- Forced diacetyl
  - Flasks
  - Hot water bath
- Forced fermentation
  - Flask
  - Heating element (typically hot plate with magnetic stirring bar)



STIRRER HOT PLATE

$250+

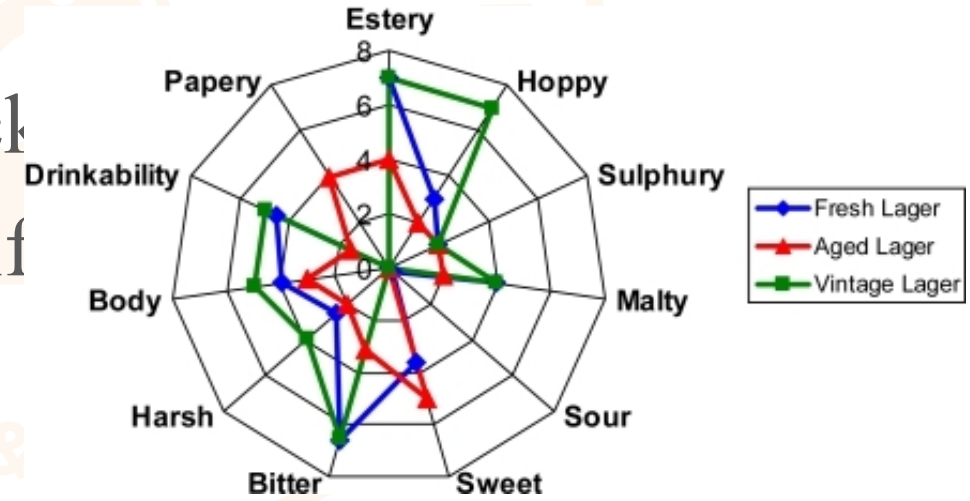
MAGNETIC STIR BAR

$2 - $15

Sensory Program

- Creating a panel of trained tasters can help identify contamination and other changes in your beers.
- Don't just taste, track
- How can you quantif

subjective?



Beer Evaluation

- Forced Diacetyl
- Sensory analysis
- Quality control - packaging

EQUIPMENT NEEDED

- Sensory analysis
  - Off flavor kit ($100 - $250)
  - Light beer
  - Notes on different batches
- Quality control – packaging (beer punishing)
  - Use hot/cold cycling to determine each beer's 'best by' date.
  - EBC Method – Hot/cold cycling to determine colloidal stability

• Place the bottles in a refrigerated bath at 0 °C, for chilling overnight • Read initial haze • Place the bottles in a bath at 60°C for 48 hours • Place the bottles in a refrigerated bath at 0 °C, for chilling overnight • Read final haze

Record Keeping

- Track as many parameters as possible
- Hot side parameters:
  - Oxygen
  - pH – mash and knockout
  - Original Gravity – first runnings, pre-boil, knockout
  - FAN analysis
- Cold side parameters will relate back to fermentation analysis
  - Alcohol
  - Color
  - Diacetyl
- Fermentation, CIP treatments, etc.

Record Keeping

Google Drive offers a free and convenient option for any brewery.

Date	Beer	FV#	Batch	Target gravity	Batch size (BBL)	Silo # for 2 row	Mash H2O Amount (bbl's)	Mash PH	1st Runnings Gravity	Last Runnings Gravity	Sparge H2O Amount (bbl's)	Vorlauf/ Lautering Issues?	Post Boil Gravity for Calculation	Required Water Addition (BBL)	*Actual Water Added (BBL)*	Knock-out Gravity	Wort DO (ppm)	O2 LPM	Knock-out PH	Knock-Out Volume (BBL)
9/9/2013		11	336.2	16	40	1	25.6	5.25	20.8	8.1	16.8	no	18.2	4.4	**4.4**	15.9	46.9	8	5.16	42.16
9/10/2013		4	11	17.5	50	1&2	33.2	n/a	21.5	8	24.8	no	17.6	0.3	**0.0**	17.5	n/a	8	4.80	49.87
9/10/2013		16	337.1	16	50	2	31	5.18	21.7	N/A	26	no	18.1	5.2	**5.2**	16.0	50+	8	5.06	55.45
9/10/2013		16	337.2	16	50	2	30.4	5.17	23	7.6	26.6	no	18.1	5.2	**5.2**	16.2	48.8	8	5.08	55.96
9/10/2013		16	337.3	16	50	2	32	5.2	21.6	8	25.2	no	18.3	5.7	**5.7**	16.0	50+	8	5.08	58.52
9/10/2013		16	337.4	16	50	2	32	5.23	21.6	8.1	26	no	18.0	5.0	**5.0**	16.0	?	8	?	57.10
9/11/2013		1	389.1	13.5	50	2	25.8	5.14	20.8	4.8	30.2	no	13.6	0.3	**0.0**	13.7	?	7	4.95	52.65
9/11/2013		1	389.2	13.5	50	2	24.3	5.15	21.4	4.8	31.7	no	14.0	1.6	**1.6**	13.5	48.8	7	4.96	51.90
9/11/2013		18	338.1	16	50	2	31.1	5.36	21	8	?	no	17.7	4.3	**4.3**	15.8	50+	8	5.11	56.71
9/11/2013		18	338.2	16	50	2	32.5	5.35	21	8.1	24.5	no	17.8	4.6	**4.6**	15.8	50+	8	5.12	56.23
9/12/2013		18	338.3	16	50	2	30.9	5.32	21.2	8	25.8	no	18.0	5.0	**5.5**	15.9	50+	8	5.13	57.16
9/12/2013		18	338.4	16	50	2	32	?	21.5	8.8	25	no	17.2	3.1	**3.1**	16.0	50+	8	5.12	54.45
9/12/2013		9	60	10.5	40	2	16.2	5.48	18.6	3.6	26.3	no	10.6	0.3	**0.0**	11.5	?	6	5.65	38.68
9/12/2013		32	756.1	12.5	50	2	21.4	5.21	20.5	4	31.6	no	13.0	1.7	**0.0**	12.6	N/A	6	5.18	53.86
9/13/2013		32	756.2	12.5	50	2	22.3	5.19	20.6	4	33.2	no	13.0	1.7	**1.0**	12.6	N/A	6	5.18	52.50
9/13/2013		32	756.3	12.5	50	2	21.7	5.22	20	3.1	33.8	no	13.2	2.4	**2.4**	12.5	48.8	6	5.18	56.09
9/13/2013		32	756.4	12.5	50	2	22	5.35	19.8	3.5	33.8	no	13.2	2.4	**2.5**	12.4	50+	6	5.22	54.67
9/13/2013		36	339.1	16	50	1	34.2	5.26	21.4	10	22.8	yes	17.9	4.8	**4.8**	16.0	50+	8	5.07	55.50
9/13/2013		36	339.2	16	50	1	31.8	5.34	21.5	8	25.2	yes	17.6	4.1	**4.1**	16.2	50+	8	5.11	54.39
9/13/2013		36	339.3	16	50	1	31	5.32	21.3	8.6	25.3	no	17.8	4.6	**4.6**	16.2	50+	8	5.08	55.48
9/14/2013		36	339.4	16	50	1	30.8	5.32	21.4	8.4	25.8	no	17.9	4.8	**4.8**	15.8	47.9	8	5.08	56.41
9/14/2013		15	340.1	16	50	1	30.9	5.25	21.2	8.8	25.4	?	17.8	4.6	**4.6**	15.8	50+	8	5.07	55.35
9/14/2013		15	340.2	16	50	1	30.2	5.24	21.8	?	25.2	?	18.5	6.1	**?**	16.0	?	8	5.06	59.30
9/14/2013		5	10-Jan	15	50	bag	29.6	5.1	20.3	8.8	26.9	?	15.9	2.5	**?**	14..9	?	8	4.97	55.45
9/14/2013		7	26.1	16	50	1	31.4	5.29	22.4	na	25.1	no	17.5	3.9	**3.9**	16.0	47.2	8	5.12	54.35

Lab Breakdown

1. “Breakroom” Lab
2. Small Lab
3. Medium Lab
4. Large Lab

- Associated costs
- Space requirements
- Outputs and functionality

*Note: costs included cover equipment only, not construction cost

“Break Room” Lab

- Approximate cost: $800+
- Space needed: Small area only!
- An extra desk in an office area may be enough.
- What does it have?
  - Microscope
  - Hemacytometer + glass coverslip + counter
  - Methylene blue/crystal violet
  - Hydrometer, refractometers
  - pH strips

“Break Room” Lab

Small Lab

- Approximate cost: $3000+
- Space needed: 50 square feet +
- What does it have?
  - Everything in “break room” lab
  - Forced diacetyl testing (flasks, heating supply)
  - Forced fermentation (sanitized flasks, heated stir plate, magnetic stir bar)
  - Some plating (possibly just HLP)
  - CO2 testing (Zahm & Nagel)

Medium Lab

- Approximate cost: $9,000+
- Space needed: 175 square feet +
- What does it have?
  - Everything in small lab
  - Laminar flow hood ($3,000+)
  - More extensive microbial plating/identification
  - CO2 testing fill levels
  - Pressure cooker
  - IBU/Color – spectrophotometer ($700+)
  - Turbidity meter ($350+)
  - pH meter ($50+)
  - ASBC methods of analysis subscription ($995/year)

Large Lab

- Approximate cost: $100,000
- Space needed: 350 square feet +
- What does it have?
  - Everything in medium lab
  - Autoclave
  - Alcolyzer ($60,000+)
  - Gas chromatograph ($8,000+)
  - Inline monitoring – gravity, pH, temperature
  - Propagation materials – ferm flask, slants, plates, shaker

Save Money!

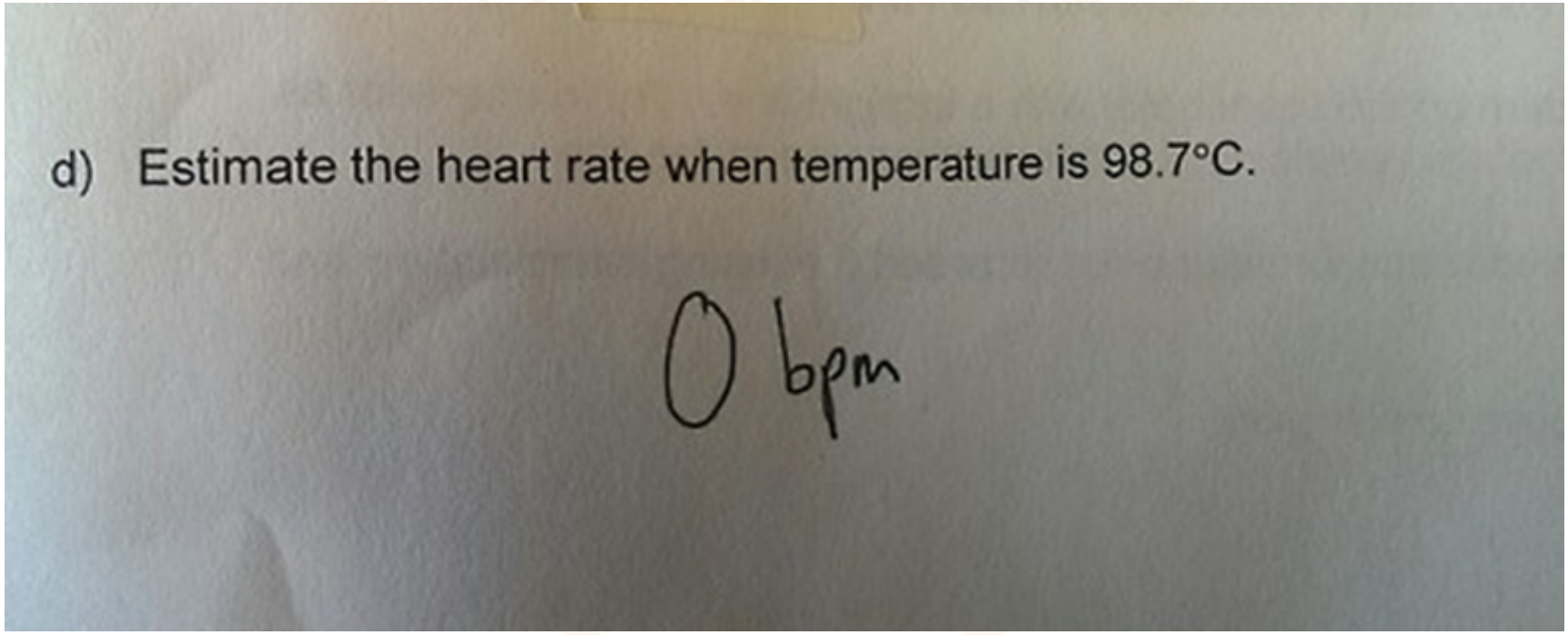
- Buy used
  - Many websites that sell used lab equipment
  - Check local universities
- Invest
  - More expensive items that will be used frequently
  - Multipurpose items (Ex: autoclave – use to sterilize media, tubing, etc.)
- Buying new?
  - Buy direct from manufacturer to get best price and advice
- Consult your peers
- **probrewer.com**

Putting it all Together

- Record keeping is key!
  - Gravity
  - Fermentation temperatures
  - Pitch rates
  - pH

- Develop your testing program
  - Set your limits
  - Set your timelines
  - Protocols – best way to ensure consistency

Being a Real Scientist



Summary

- Even small lab programs can benefit your end product
- Start with a microscope and build from there
- Have good record keeping
- Be proactive, not reactive!

Thank you

Questions?

Troubleshooting Problem Fermentations

Kara Taylor

Goals for Today

But remember – this is a guide, not the Bible

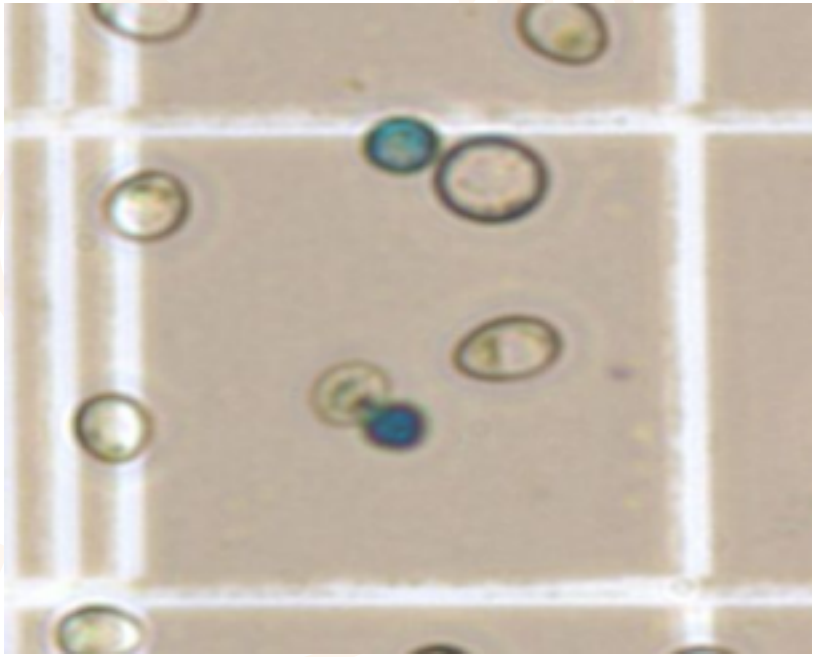
Outline

- 4 common fermentation problems
  - Slow, sluggish or stalled fermentations
  - Decline in yeast viability
  - Change in flocculation
  - Fermentation off-flavors
- Possible causes for the problems
- Remedies the problems

Problem 1

Slow, Sluggish, or Stalled Fermentation

Low Viability



Problem 1

Slow, Sluggish, or Stalled Fermentation

Low vitality (poor yeast health)

"My yeast has 97% viability, why isn't it fermenting well?"

Problem 1

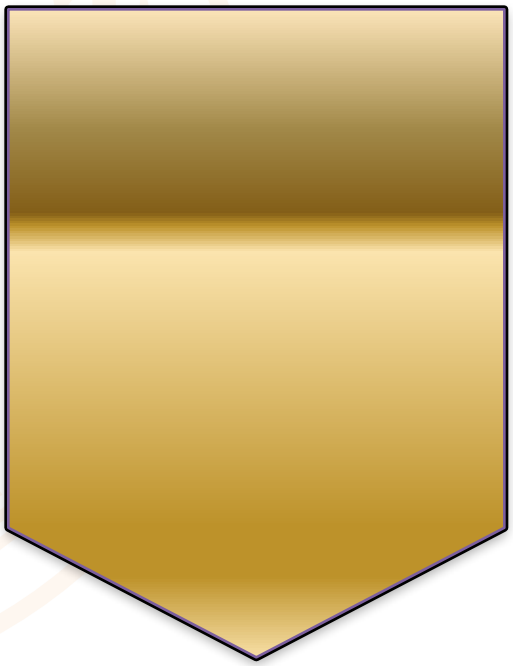
Slow, Sluggish, or Stalled Fermentation

- Under-pitching
  - Not enough yeast to do the job
    - "Too few hands…"
- Over-pitching
  - Competition for food & nutrients
  - Low growth rate
  - Older yeast populations when harvested & re-used
  - High % of bud scars

Problem 1

Slow, Sluggish, or Stalled Fermentation

- Poor mixing or agitation in fermentor
  - Multiple fills
  - Premature yeast flocculation



Problem 1

Slow, Sluggish, or Stalled Fermentation

- Inadequate dissolved oxygen levels
  - Ideally 8-10ppm

…but how do you achieve that?

Brewery	Brewery Flow Rate	Length of Time In-line	Original Gravity (P°)	Final Gravity (P°)	Actual DO (ppm)
1	6L/min	40 min for 40bbl	12.5	2.3	**5**
2	7L/min	30-40min for 10bbl	12	1.8	**8.25**
3	7L/min	20min for 8bbl	12.8	3.3	**9**
4	12L/min	75-80 min for 15bbl	25.5	4.1	**5.5**
5	6L/min	25-30min for 10bbl	12.8	3.2	**35.8**
6	5L/min	90min for 15bbl	12.7	3.2	**24.4**
7	7L/min	40min for 40bbl	12.3	2.2	**6.2**
8	6L/min	30min for 10bbl	14.4	2.3	**8.1**
9	6L/min	45min for 15bbl	13.2	3.3	**5.42**
10	7L/min	35min for 10bbl	12.5	2.4	**7.2**
11	7L/min	40min for 15bbl	12.7	2.2	**6.54**
12	6L/min	35min for 10bbl	12.3	2.3	**5.85**

Problem 1

Slow, Sluggish, or Stalled Fermentation

- Inadequate dissolved oxygen levels
  - Effect of multiple generations

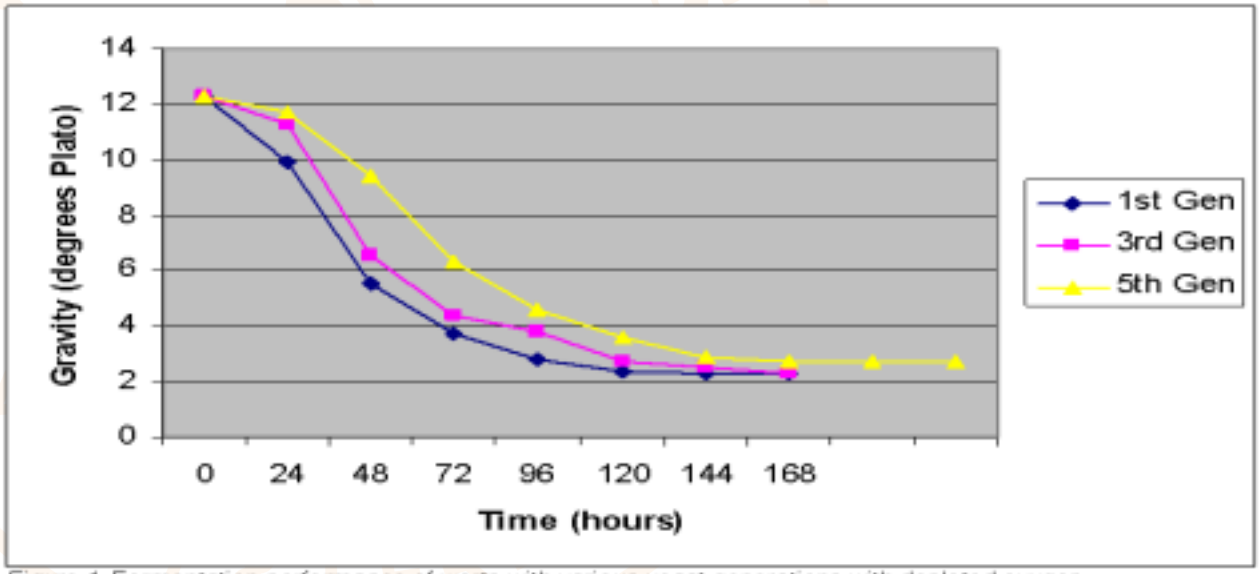


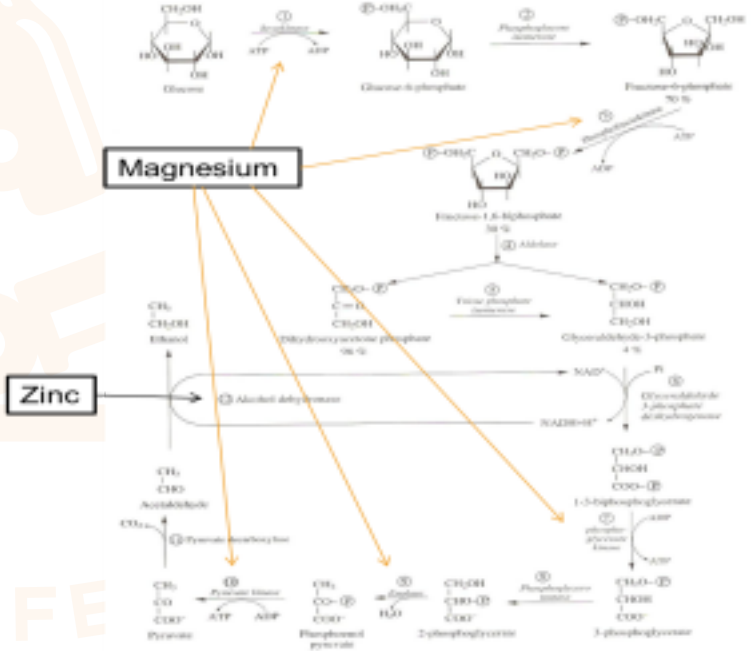
Figure 4. Fermentation performance of worts with various yeast generations with depleted oxygen resources.

Problem 1

Slow, Sluggish, or Stalled Fermentation

Nutrition

- Minerals, specifically
  - Magnesium
  - Zinc



Problem 1

Slow, Sluggish, or Stalled Fermentation

- Can more oxygen be added?
- What about adding more yeast?
- More nutrients?

Problem 2

Decline in viability

- Poor yeast collection or yeast storage practices

When is the best time to harvest?

- End of fermentation
- When early flocculating yeasts begin to drop to the bottom of the cone – discard
- Within 3 days of start of fermentation

Bottom-Cropping

Top-Cropping

Problem 2

Decline in viability

- Poor yeast collection or yeast storage practices

How should yeast be collected?

- Best practices
  - Remove as soon as possible without risking integrity of beer
  - Discard the first runnings
  - Use only the middle pack

Problem 2

Decline in viability

- Poor yeast collection or yeast storage practices

How should yeast be stored?

- Ideally, only 1-3 days after collection
- Up to two weeks, with exceptions
- On beer, wort, or water?
- Temperature – 33-36◦F

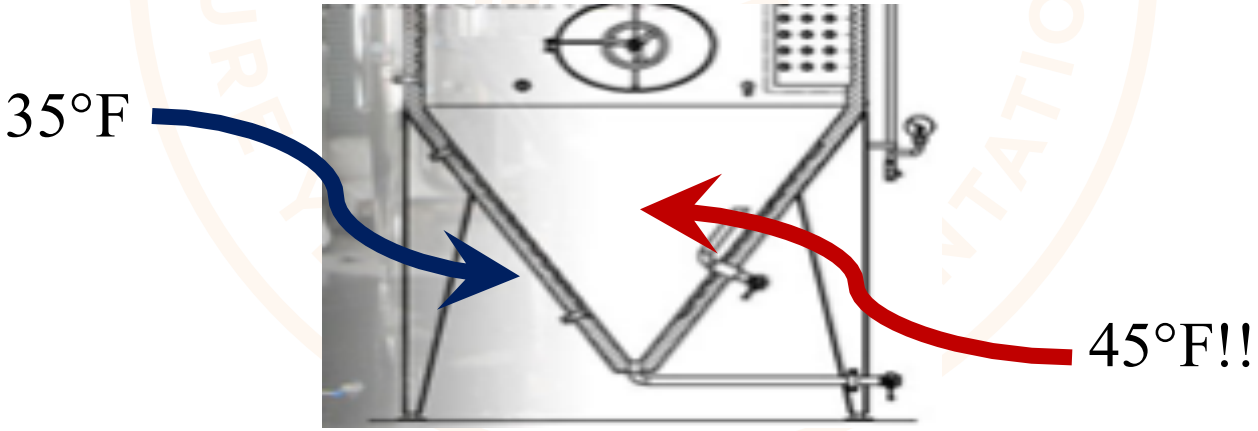
Problem 2

Decline in viability

Should yeast be stored in the cone?

No – why?

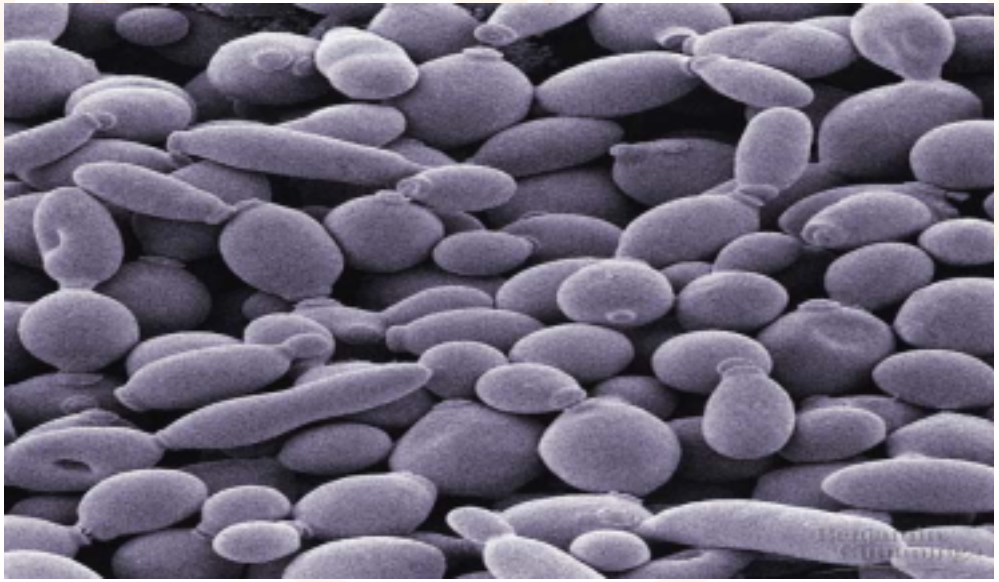
Insufficient cooling in the fermentor cone



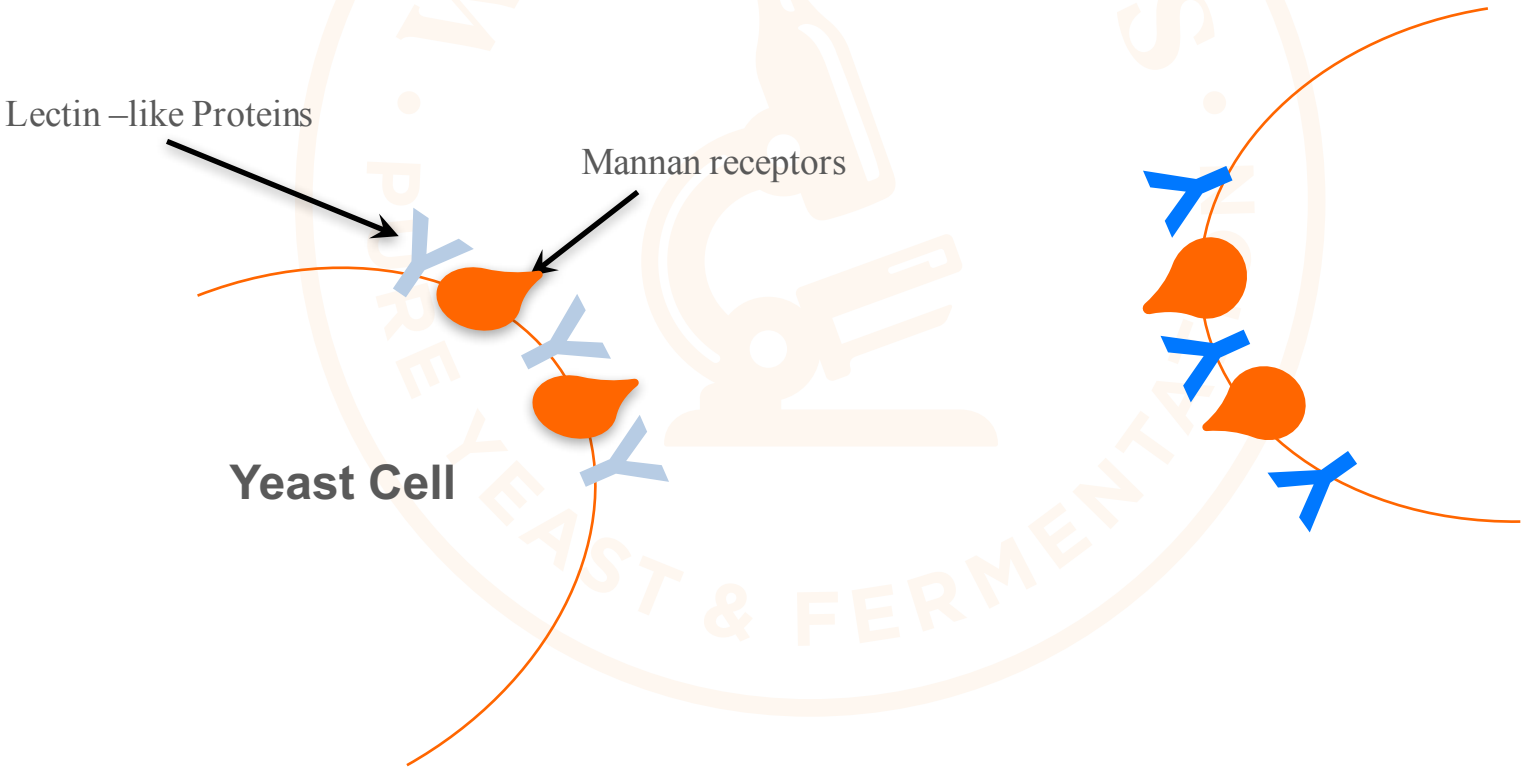
Problem 3

Flocculation

- Yeast sticking together and forming aggregates



Problem 3
Flocculation



Problem 3
Flocculation

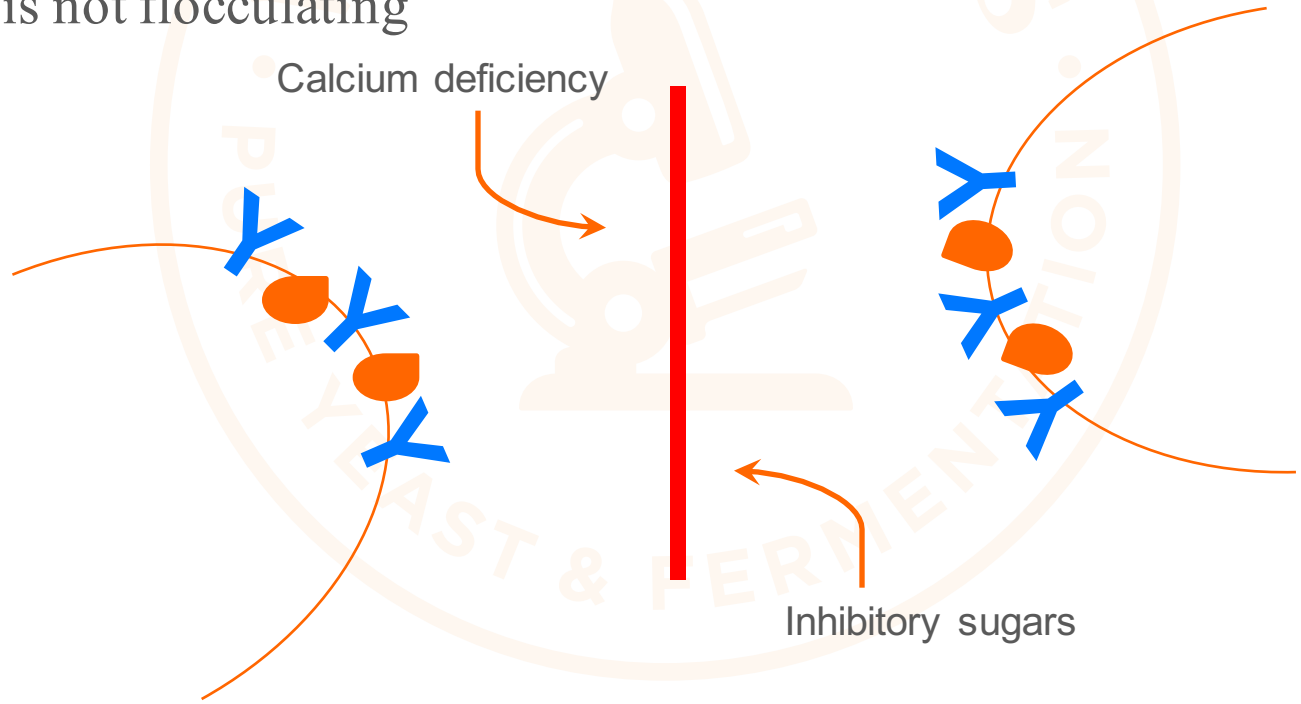
Yeast is not flocculating

- Problems with pitching yeast
  - Old yeast population
  - Mutation of yeast – petite mutants

Problem 3

Flocculation

- Yeast is not flocculating



Problem 3

Flocculation

Yeast is flocculating too quickly

- Insufficient turbulence in fermentor
- Premature yeast flocculation

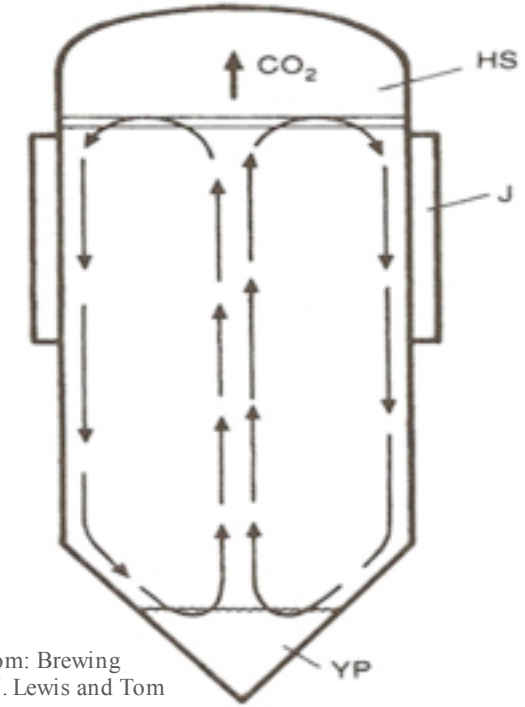


Figure from: Brewing
Michael J. Lewis and Tom
Young

Problem 3
Flocculation

Yeast is flocculating too quickly

- Slow or incomplete fermentation
  - Could be due to low viability/vitality yeast
- Mutation of yeast
  - Wheat beer strains, Belgian strains

Problem 4

Off Flavors

Phenolic Off-Flavors

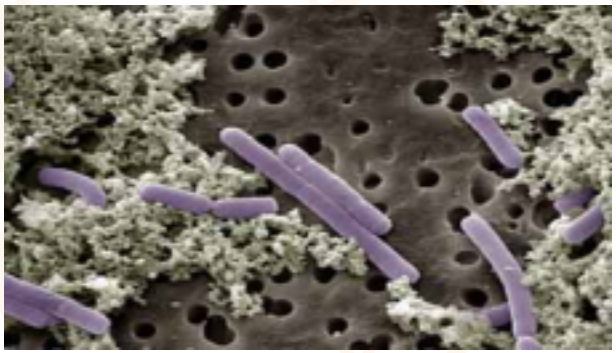
- Mutation of yeast
- Cross-contamination
- Stressed yeast
- Contamination by wild yeast

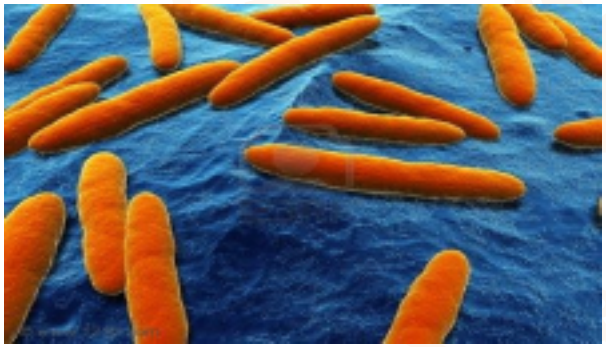
Problem 4

Off Flavors

Acidic flavors and aromas

- Lactic acid bacteria
- Acetic acid bacteria



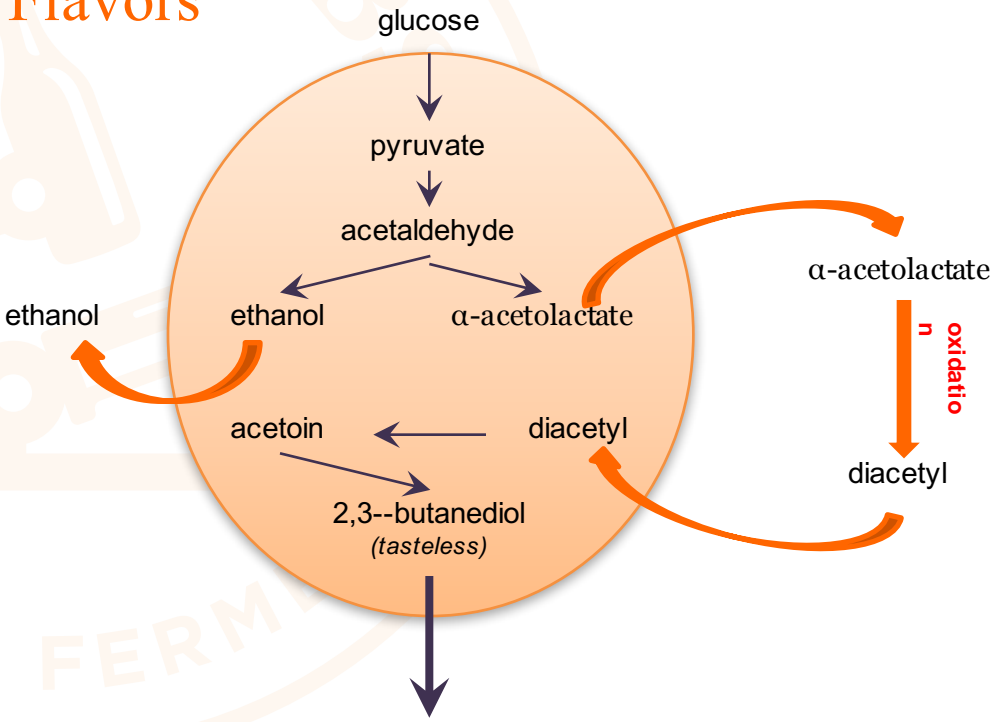


Problem 4

Off Flavors

Diacetyl

- Slow or incomplete fermentation
- Premature flocculation
- Premature removal of yeast from beer



Problem 4

Off Flavors

- Diacetyl
  - Contamination by anaerobic bacteria
  - Poor re-uptake of diacetyl at end of fermentation

Problem 4

Off Flavors

Esters and/or fusels

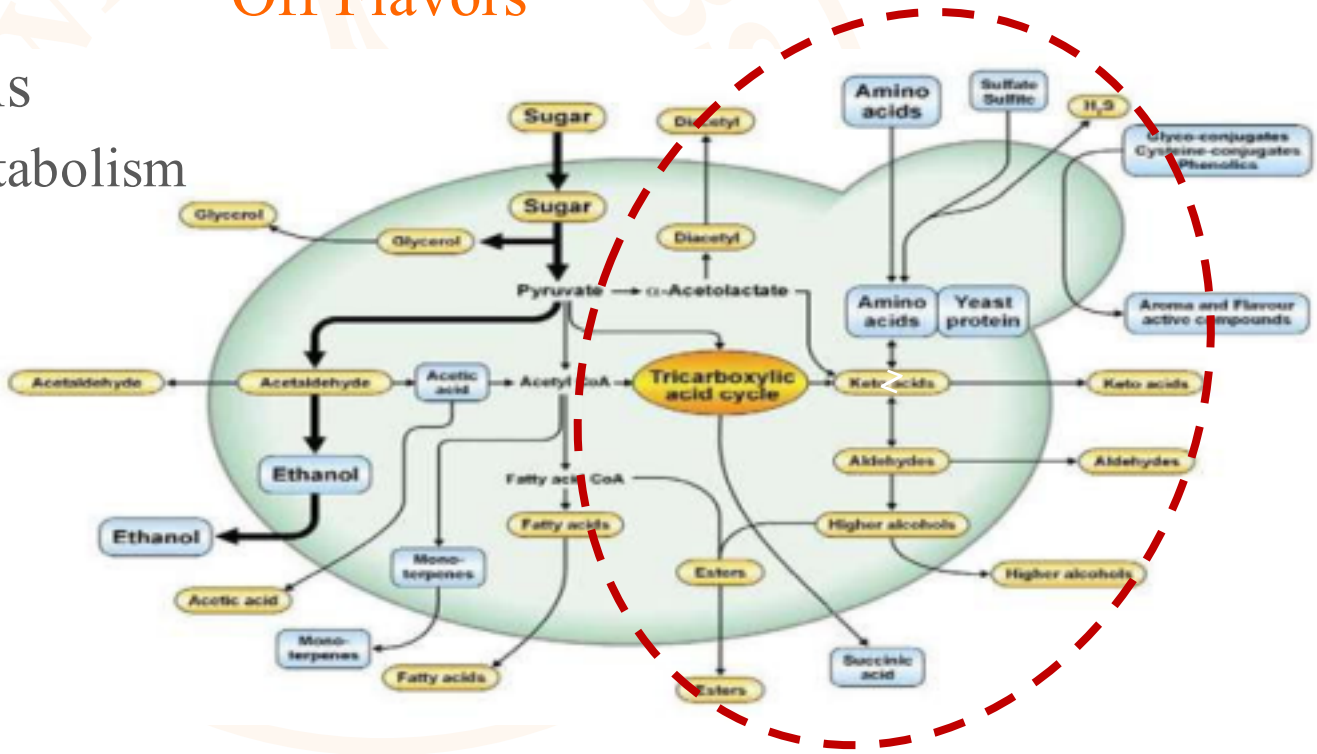
- Insufficient temperature control
- Increased oxygen
- Over-pitching yeast

Problem 4

Off Flavors

Esters and/or fusels

– Amino acid metabolism



Summary

- Fermentation is a metabolic process, and yeast are living organisms
- Fermentation and yeast handling affect yeast condition
- Yeast condition affect subsequent fermentations

Knowing your fermentations is your best arsenal!

Summary

- Knowing your fermentation – what you need to know:

Fermentation Data	
Gravity (degrees Plato)	**Sensory**
Time (hours)	
0	
24	
48	
72	
96	
Final	
Flocculation	
Beer Phase (c=clear, m=medium, b=blurry)	
Phase Separation (s=sharp, d=diffuse)	
Film of sediment in the cone/over yeast pack (+/-)	
Attenuation	
AT 50% (Time to 50% attenuation)	
% Attenuation	

Summary

The take home message…

"All yeast are female by definition, mother cells and daughter cells. If you don't treat women with respect they *will* eventually bring you to your knees…"

Thank you

Questions?