The Meaning of Life According to Yeast

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Overview

- General Yeast Metabolism
- Fermentation By-products
- Oxygenation
- Cell Counts and Pitch Rates

The Meaning of Life According to Yeast

- Not make Beer
- Not leaven Bread

Survive and Grow

Providing Yeast Optimum Conditions

Positive Effects;

***Fermentation temperatures warmer rather than cold.**

***Low wort pH: < 5.3 pH**

***Temperature rise midway through fermentation.**

***Pale Malts with high valine Content.**

***Adequate dissolved wort oxygen.**

Wort Stability Testing – A Pure Environment is Essential

- After incubation of sample; visible evaluation
- No CO_2
- No clouding of the wort sample.
- Sweet wort aroma and no phenolic or other off odors.
- Variations from these results indicate:
- Likely contamination of heat exchanger of process lines.
- Contamination of beer being produced and yeast in fermenter.
- Extensive cleaning required, and new yeast culture.

Wort Composition

- Fermentable Carbohydrates/ Sugars
- Amino Acids
- Dextrins
- Lipids
- Vitamins
- Inorganic Ions

Fermentable Carbohydrates/ Sugars

• Fructose	2%
• Glucose	8%
• Sucrose	6%
• Maltose	45%
• Maltotriose	10%

Phases of Metabolism

- Cell Wall Synthesis/ O_2 Uptake
- Sugar Uptake
- Nitrogen Uptake
- Fermentation (Glycolysis)
  - Energy production
  - Cell growth
  - Acidification
  - Production of By-products → BEER

Cell Wall Synthesis

- Must be permeable for nutrient uptake
- Sterol Synthesis
  - Requires
    - Internal energy reserves Glycogen
    - Oxygen
- Sterol levels

    @ pitching <0.1% cell

    @ Peak fermentation 1.0% cell
- Glycogen is depleated during storage.
- Low O2 or Glycogen leads to sluggish fermentation

Order of Sugar Uptake

Glucose

Fructose

Sucrose

Maltose

Malotriose

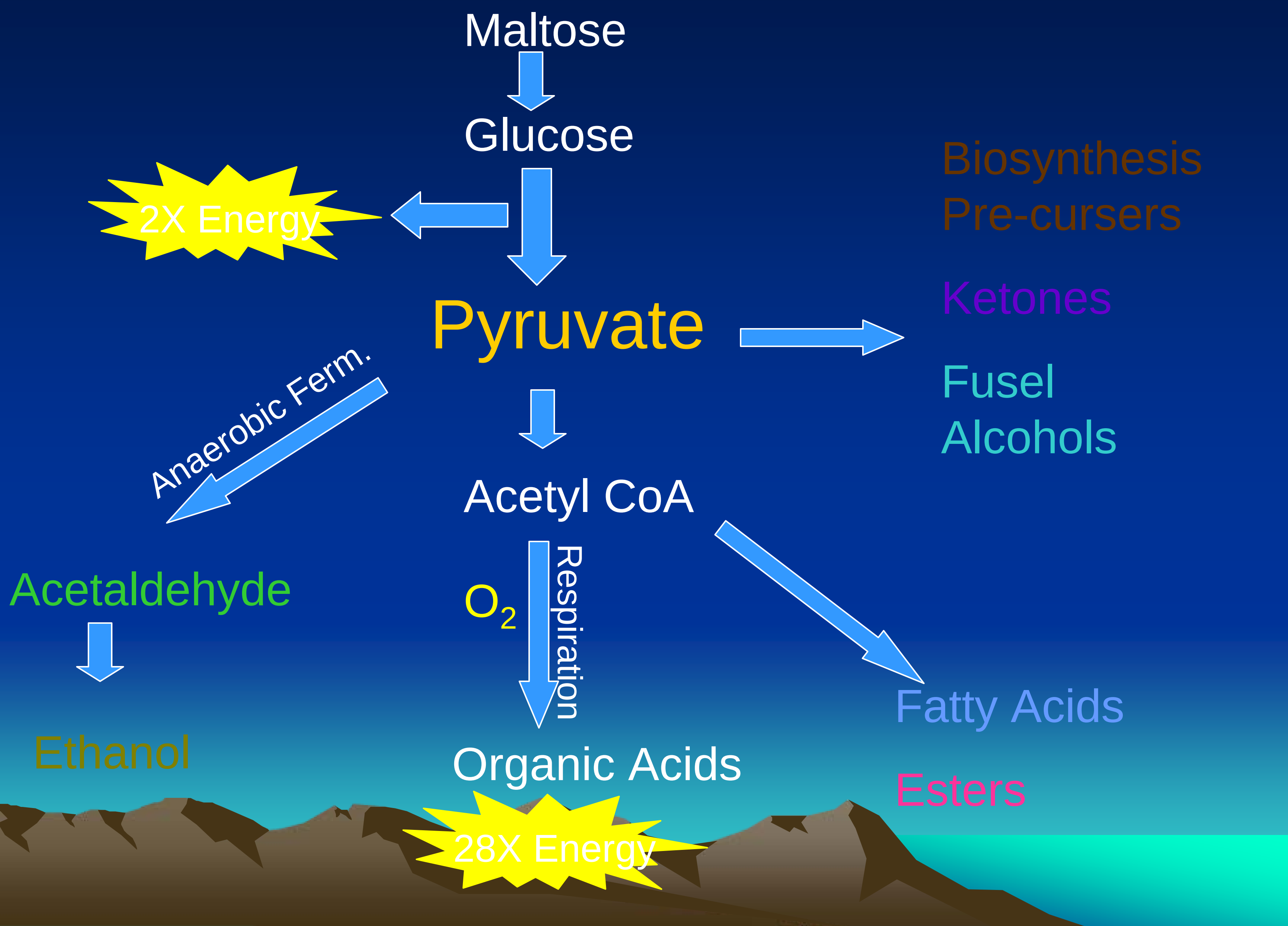
Sucrose split into Glucose & Fructose extracellularly prior to uptake, Maltose & Maltotriose are Split into Glucose Molecules by Permease and Maltase Enzymes

Nitrogen Uptake

Amino Acids → NH_3 + Carbon Skeleton

- NH_3 utilized for protein synthesis
- Carbon Skeleton pre-curser Fusel Alcohols
- Shock Excretion
  - Osmotic pressure from high wort gravity can cause cell to excrete essential Nitrogen back into wort.

Fermentation



Fermentation By-products

- Alcohol
- Acetaldehyde
- Fusel High Alcohols
- Esters
- Ketones

Alcohol

- Major excretion product
  - Anaerobic Fermentation
  - Cell detoxification mechanism from Pyruvate and Acetaldehyde build up

- Little impact on overall flavor

Acetaldehyde

- Flavor
  - Green beer flavors, grassy, green apple
- Formed as an intermediate product of fermentation
- Conditions that favor formation include:
  - Increase Temp
  - Increase Oxygen
  - High pitching rates
  - Pressure during fermentation

Higher/Fusel Alcohols

- Flavors
  - Alcohol solvent character, rough, possible roses, banana, chemical, medicinal
- Formed
  - carbon skeletons from amino acids reduced to corresponding alcohol
- Conditions that favor formation include;
  - Increase Oxygen
  - Decrease Pitch Rate
  - Increase Temp
  - Increase Gravity
  - Amino Acid Supplementation or Deficiency
- Examples include
  - Isoamyl alcohol — banana, solvent
  - Isobutanol — alcohol, rough
  - n-Propanol — alcohol, rough

Esters

- Flavors
  - Fruity, banana, solvent, apples

- Formed
  - Fusel Alcohol combines with a Acetyl CoA Fatty Acid

- Conditions that favor formation include;
  - Decrease Oxygen
  - Increase Temperature
  - Fusel Alcohol pre-cursers
  - Increase gravity
  - Increase trub
  - Contradictory information on cell growth effects

- Examples include

– Isoamyl acetate	Banana
– Ethyl acetate	Light fruity solvent
– Phelylthyl acetate	Roses, honey, Apple
– Ethyl Caprylate	Apple-like

Ketones/ VDK(vincinal di-ketone)

- Flavors
  - buttery, butterscotch, fruity, musty, honey, rubber

- Formed
  - Oxidation of Amino acid synthesis intermediates (Valine, Isoleucine).
  - Can be reduced later in fermentation

- Conditions that favor formation include;
  - Increase O2 post fermentation
  - Decrease pitch rate
  - Increase Temperature during fermentation
  - Yeast deficiency of Amino acid uptake
  - Wort deficiency of Amino acid

- Examples include
  - Diacetyl Butter
  - 2,3 pentadione honey

Main factors in By-product formation

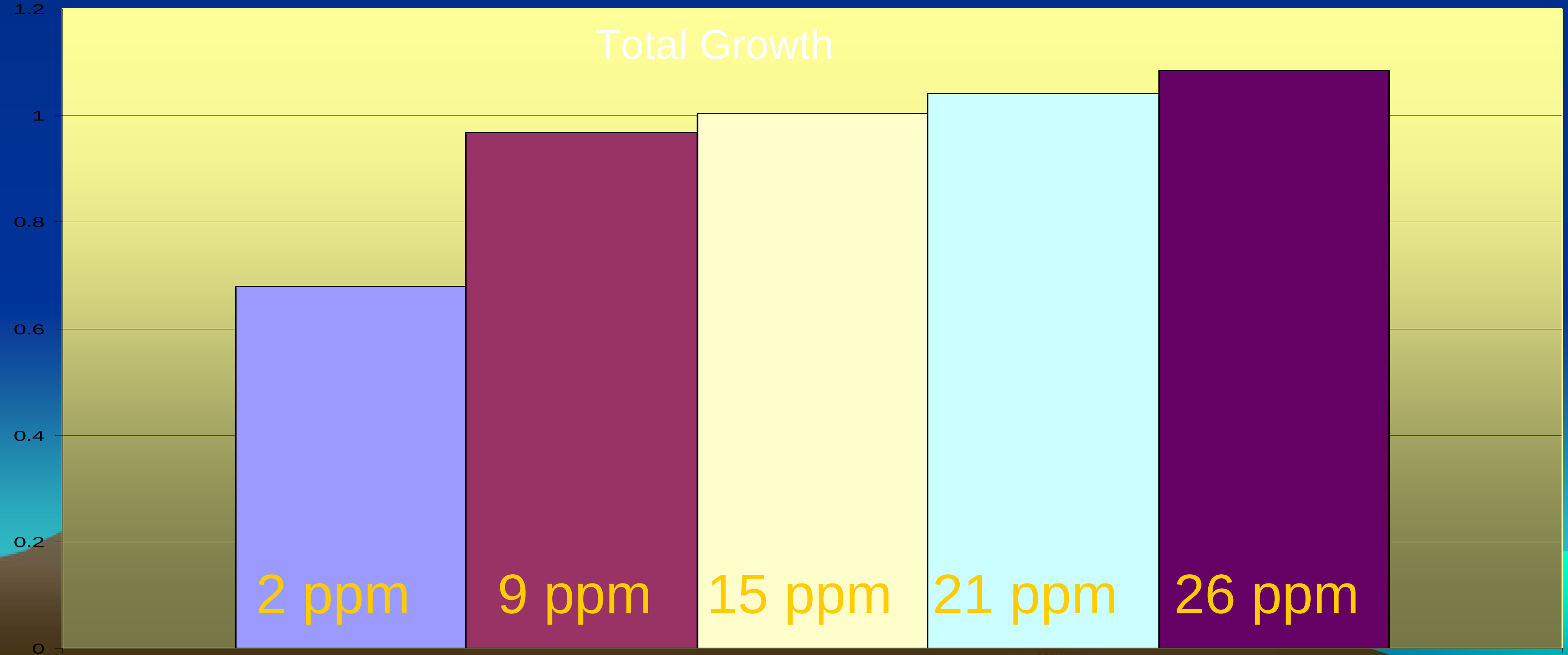
- Yeast Strain
- Yeast Condition
- Wort composition
- Temperature
- Level of Aeration
- Pitch Rates

By-product formation influenced by conditions that favor cell growth

- Increase Dissolved Oxygen
- Decrease Pitch Rate
- Increase Temperature
- Increase Gravity

Aeration
DO Dissolved OxygenExperiment

- 5 flask fermentations with increasing levels of DO
- Optical density measured every 12 hours
- Results
    - Increases in DO = increased cell growth



Aeration
Dissolved Oxygen Experiment

Doublings vs. DO

DO ppm	# Doublings
2ppm	1.2
9ppm	1.5
15ppm	1.62
21 ppm	1.66
26 ppm	1.70

Sensory Experiment
DO levels affect on Beer Flavors

- Flask fermentations with increasing levels of DO
  - DO levels 6, 14, 28 ppm
  - Wyeast # 1007 Alt- Pale Wort
  - Wyeast # 3068 Wheat- Amber Wort

DO levels affect on Beer Flavors Results

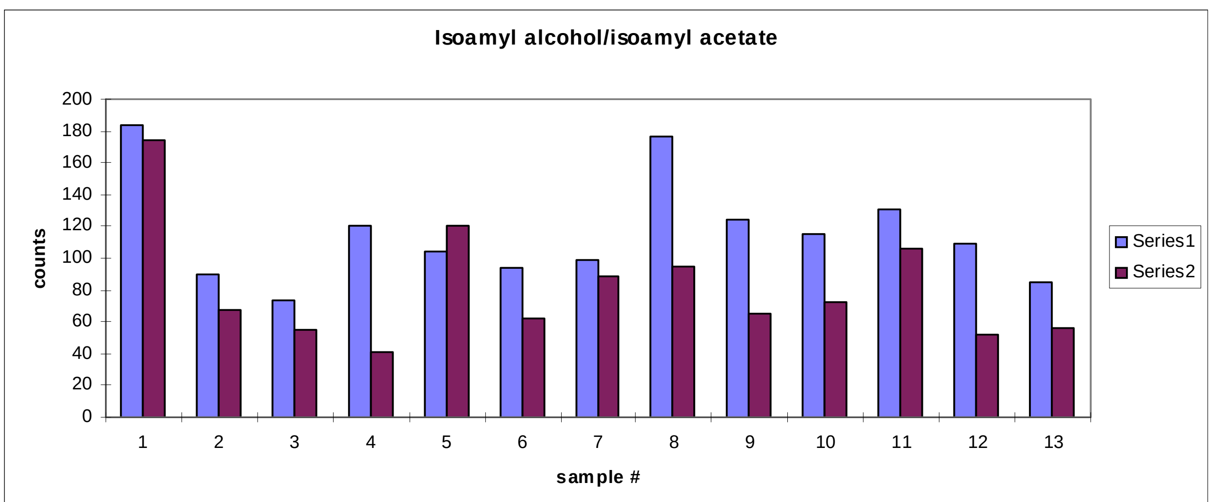
- 1007- Pale
  - Prefered sample 28 ppm
  - Coments were mixed

- 3068- Amber
  - Prefered sample 6 ppm
  - Most banana/ fruit character

Fruity

banana & fusel oil

#	yeast type	isoamyl acetate normalized	GC-O value	Isoamyl alcohol normalized	GC-O value	flavor panel
1	Belgian Ale Yeast	87.4	1537	184	712	strong banana
2	Trappist High Gravity	33.5	1413	90	682	
3	Belgian Ardennes Yeast	27.3	1102	73	1054	banana
4	German Wheat Yeast	20.4	846	120	1515	banana
5	Weihenstephen Weizen Yeast	60.3	1946	104	1293	strong banana
6	American Wheat	31.1	1584	94	1896	
7	Belgian Abbey Yeast II	44.3	2197	99	1856	
8	Forbidden Fruit Yeast	47.2	694	177	1153	
9	Belgian Wheat Yeast	32.9	527	124	664	banana
10	Belgian Whitbier Yeast	36.2	1062	115	591	
11	Bavarian Wheat Yeast	52.9	1213	131	1563	strong banana
12	Canadian/Belgian Style Yeast	26.2	998	109	1198	
13	Leuven Pale Ale Yeast	27.9	835	85	449	banana



1) Isoamyl acetate is the yeast metabolite responsible for the banana flavor in beer.

2) Sample 1, 5 and 11 have the highest amounts of isoamyl acetate and these where found to have the strongest banana flavor by the panel.

3) The samples with lower levels of isoamyl acetate have their banana flavor blended with the other fruity notes so that no distinct banana is perceived.

4) Isoamyl alcohol is a yeast metabolite which is responsible for the fusel oil note in beer.

5) Isoamyl alcohol is the only other aliphatic alcohol in beer besides ethanol present at levels that make a flavor contribution.

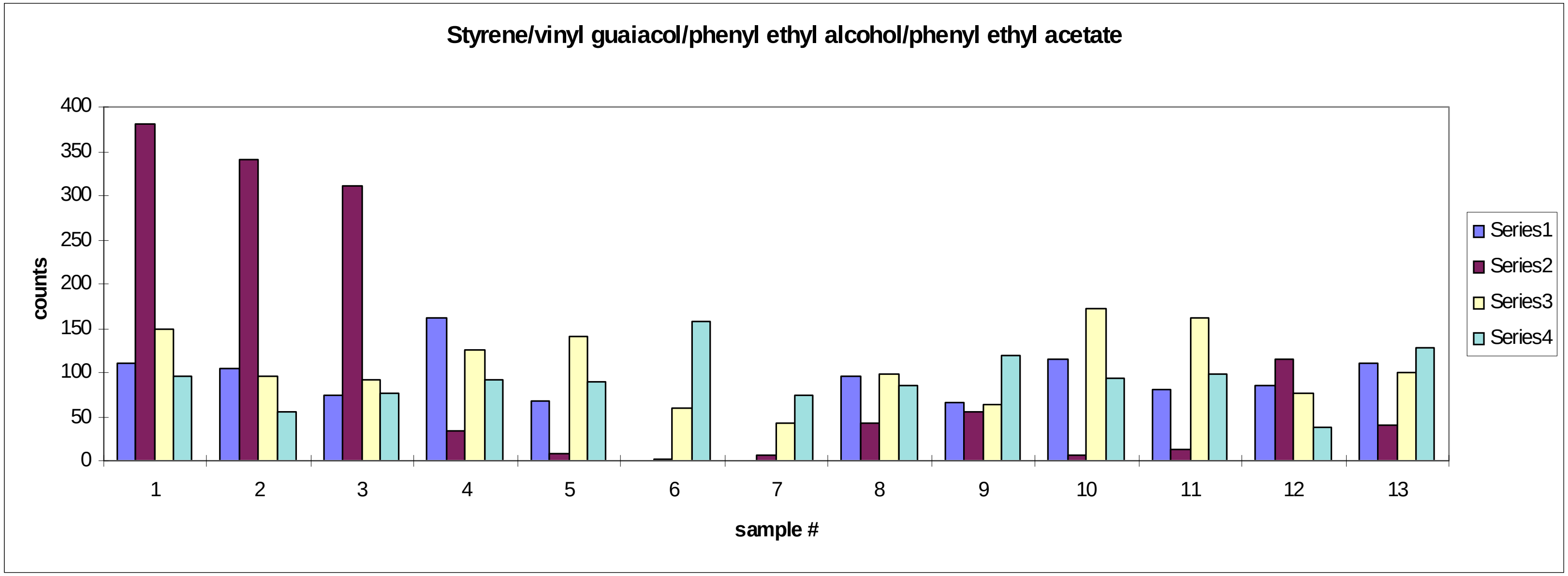
series 1 is isoamyl alcohol	
series 2 is isoamyl acetate * 2	

Aromatic-based Flavors:

styrene/vinyl-guaiacol/phenyl ethyl alcohol/phenyl ethyl acetate

1) The same two beers that have no styrene, samples 6 and 7, also have very low levels of 4-vinyl-guaiacol. This suggests a metabolic link between styrene and 4-vinyl guaiacol.

2) It is also seen that there is generally a correlation between styrene and phenyl ethyl alcohol concentrations.



Note: The counts scales are different for each compound

Series 1: styrene

Series 2: 4-vinyl guaiacol

Series 3: phenyl ethyl alcohol

Series 4: phenyl ethyl acetate

Methods of Aeration Test

- Syphon Spray
- Splashing and Shaking
- Aquarium pump though stone
- “Oxynater” Pure oxygen through stone

OR?

OK

BEST

Aeration Test Results

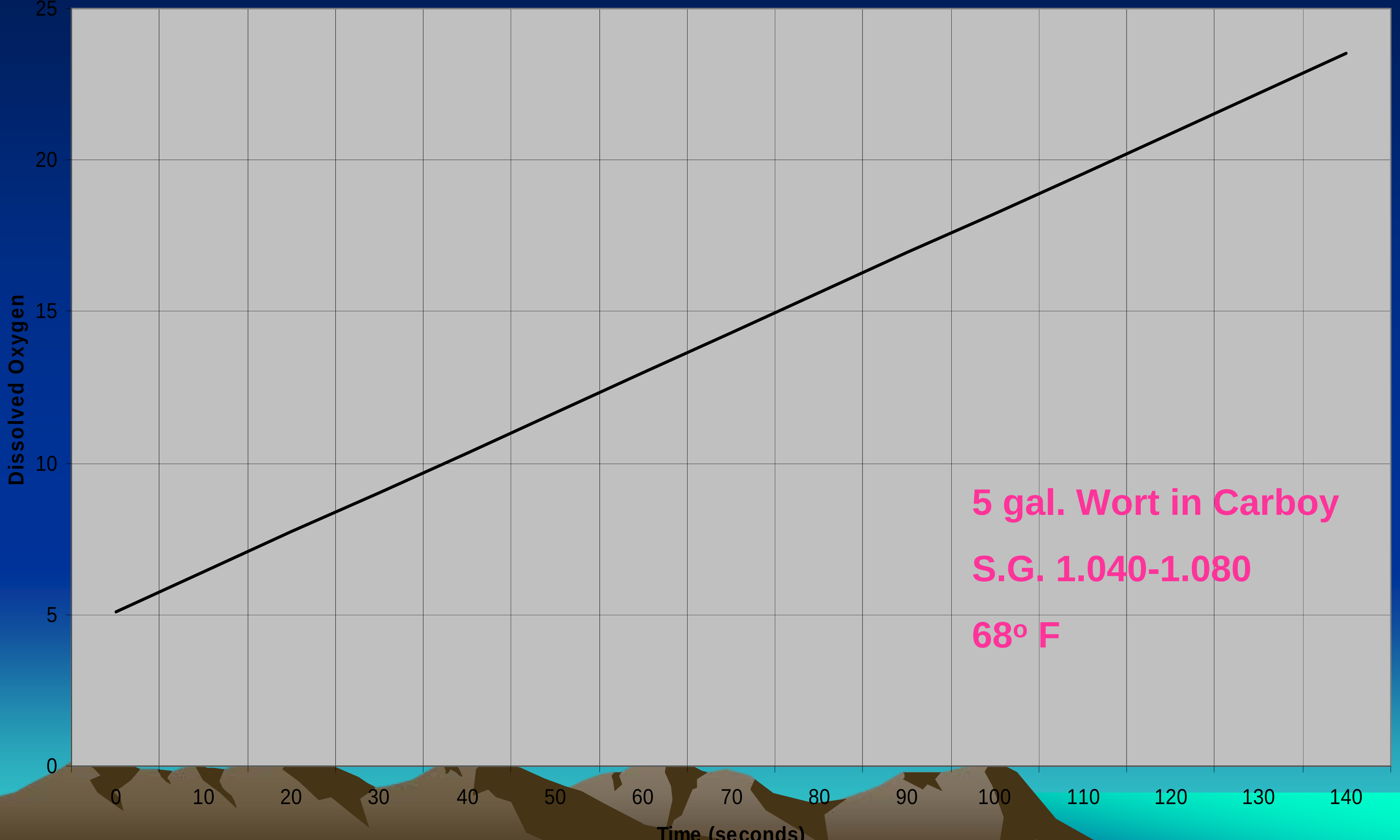
Method	DO ppm	Time
Syphon Spray	4 ppm	0 sec.
Splashing & Shaking	8 ppm	40 sec.
Aquarium Pump w/ stone	8 ppm	5 min
Pure Oxygen w/ stone	0-26ppm	60 sec

Conclusions

- Pumping air though a stone is not efficient
- Splashing/ Shaking is effective up to 8 ppm
- Pure Oxygen is easiest and most effective with most control.

Aeration Pure O_2 and Stone

DO levels vs. Time



Pitch Rates

- Pitch Rate Influences Cell Growth
  - Low Pitch Rate ⟶ High cell growth
  - High Pitch Rate ⟶ Low cell growth

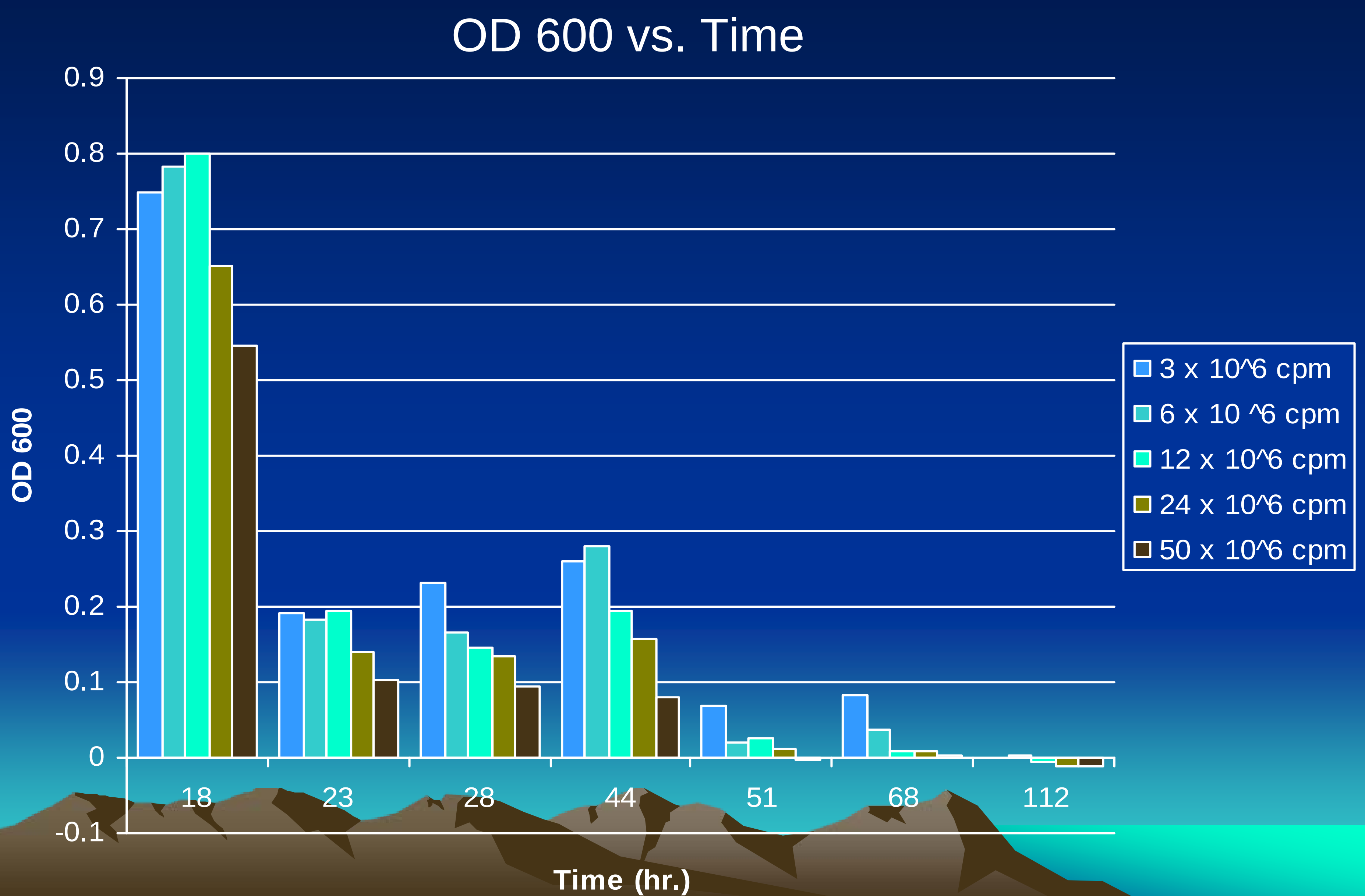
- Rule of thumb $1x10^6$ cells/ml/ P^o

Cell Growth vs. Pitch Rate Experiments

- Flask fermentations with varying pitch rates
  - Monitored OD, pH, Gravity Drop

- Sensory Analysis with tasting panel 3 strains
  - Wy 1007 German Alt- Pale Wort
  - Wy 3068 Weihenstephan Wheat- Amber Wort
  - Wy 2124 Bohemian Lager- Amber Wort

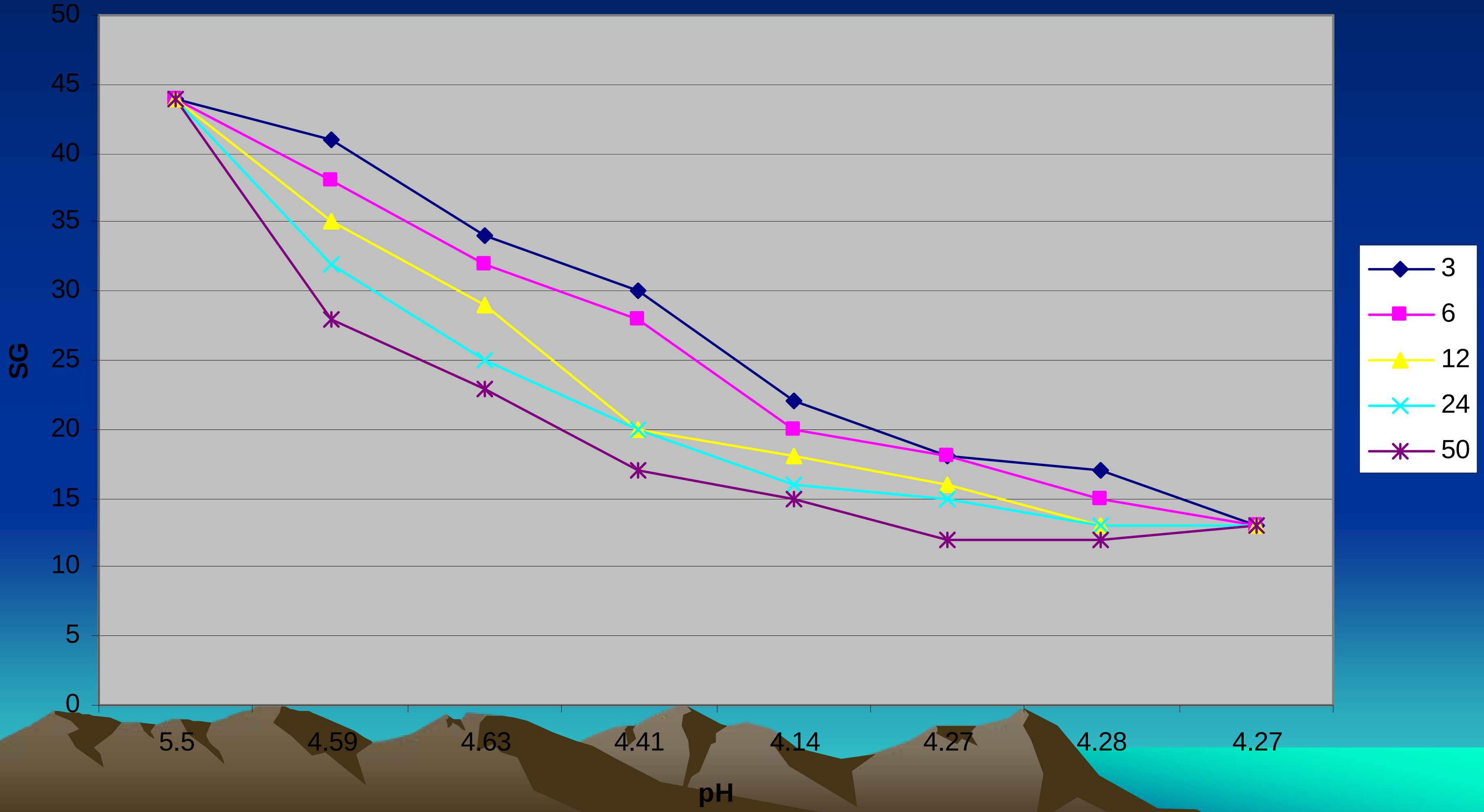
Cell Growth vs. Pitch Rate



Cell Growth vs. Pitch Rate

Pitch Rate	Doublings	Increase OD
$3x10^6$ cells/ml	2.22	1.58
$6x10^6$ cells/ml	1.81	1.47
$12x10^6$ cells/ml	1.56	1.37
$24x10^6$ cells/ml	1.02	1.09
$50x10^6$ cells/ml	0.63	0.81

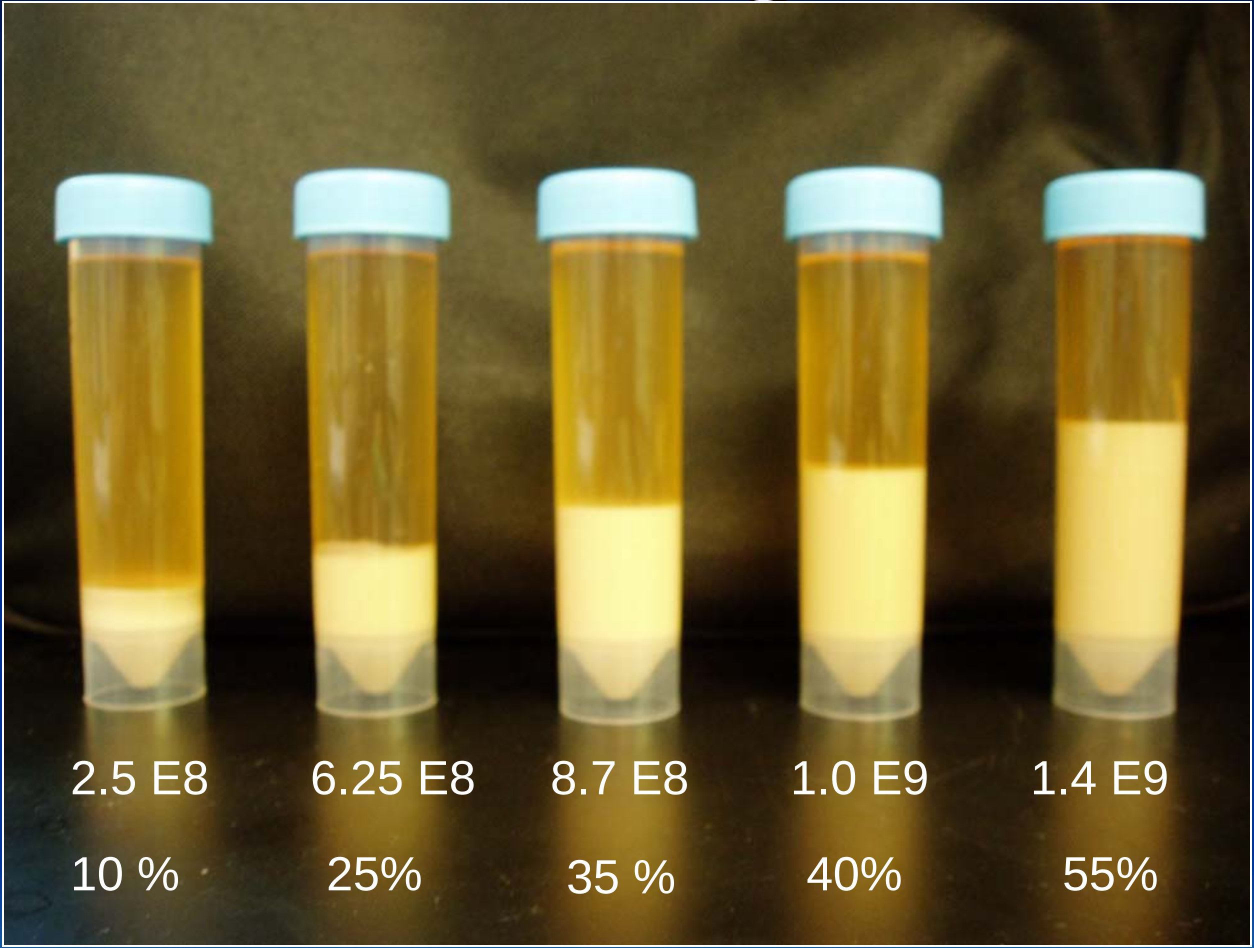
Gravity Drop vs. pH



Sensory Results

- 1007 German Alt- Pale Wort
  - Prefered sample 24 X 10^6 cpm
  - Nice Hop character, Dry, Clean and balanced
- 3068 Weihenstephan Wheat- Amber Wort
  - Prefered sample 6 X 10^6 cpm
  - Balanced, complex banana and fruit
- 2124 Bohemian Lager- Amber Wort
  - Prefered sample 24 X 10^6 cpm
  - Balanced with nice hops, mild fruit

Cell Estimating/Count



Allow Yeast to settle under refrigeration

Estimate Yeast Pack

Take into consideration Trub and Flocculation (Powdery strains don't pack)

Calculating Pitch Rates

- Decide how many cells/ ml wanted in fermenter
  - eg. $6x10^6$ cells/ ml
- Calculate Total Cells Needed
  - (Cells/ ml) x (total mls In fermenter)
  - (19 liters =$1.9x\ 10^4$ mls)
  - eg. ($6x10^6$ cells/ml) x ($1.9x10^4$ mls) = $1.14x10^{11}$ cells
- (Divide Total cells)/(Cell count of slurry)= mls slurry to pitch
  - eg. ($1.14x10^{11}$ total cells)/($1.0x10^9$ cells/ ml)

Answer 114 mls slurry

Science vs. Reality

What the book says isn't always how it works out. Trust the results that you achieve in you brewery first. There are a lot of variables.

Questions?

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