

Tools for Determining Yeast Health

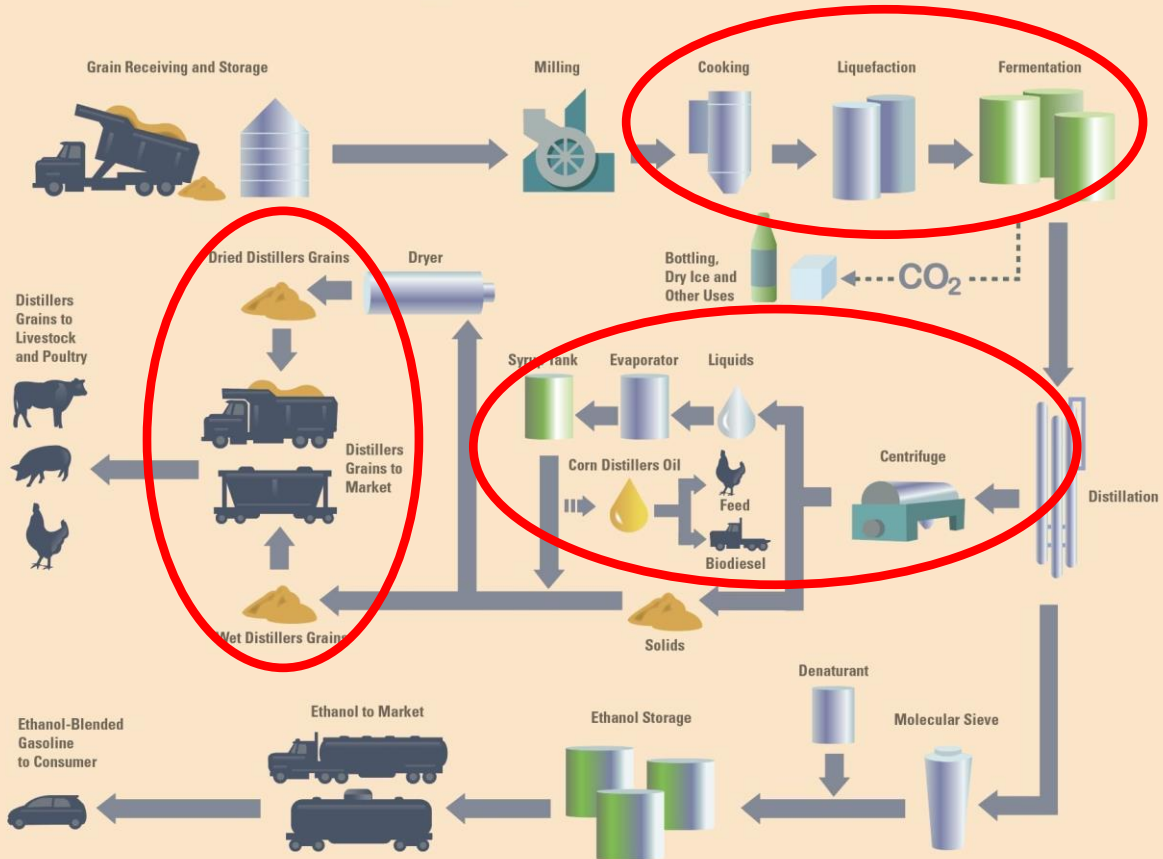
James (Jim) Miers

2024 Fuel Ethanol Laboratory Conference

Omaha, Nebraska

We Have Multiple Levers for Fermentation Success

Dry Mill Ethanol Process



Total Potential Value is \$6.31/bu

*** Based on USDA weekly grain report (5/15/2024)

Ethanol

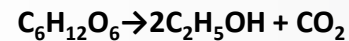
1 bushel of corn

56 lbs/bu x 73% starch x 15% moisture = 34.7 lbs of starch/bu

34.7 lbs starch x 1.11 lbs glucose/lb starch = 38.5 lbs glucose/bu

38.5 lbs glucose/bu x 5% utilized for yeast growth = 36.6 lbs glucose/bu

The reaction of glucose to ethanol:



180g/mol 2*46 g/mol

36.6 lbs glucose x 92 lbs EtOH/180 lbs glucose = 18.7 lbs EtOH/bu

18.7 lbs EtOH x 1 gal EtOH/6.6 lbs = 2.83 gal EtOH/bu theoretical

Oil

1 bushel of corn

56 lbs/bu

3.5% corn crude fat (80% germ, 20% endosperm)

15% moisture = 1.7 lbs oil /bu theoretical basis

Dry Distillers Grain

1 bushel of corn

56 lbs/bu = 23.8 lbs/bu based on 15%

moisture based on fermentation efficiency



How Can We Meet Our Goal?

Carbohydrates

- Source of carbon and energy to generate biomass

Nitrogen

- Biosynthesis of protein, enzymes, nucleic acids
- Synthesis of higher alcohols and esters

Vitamins: Coenzymes

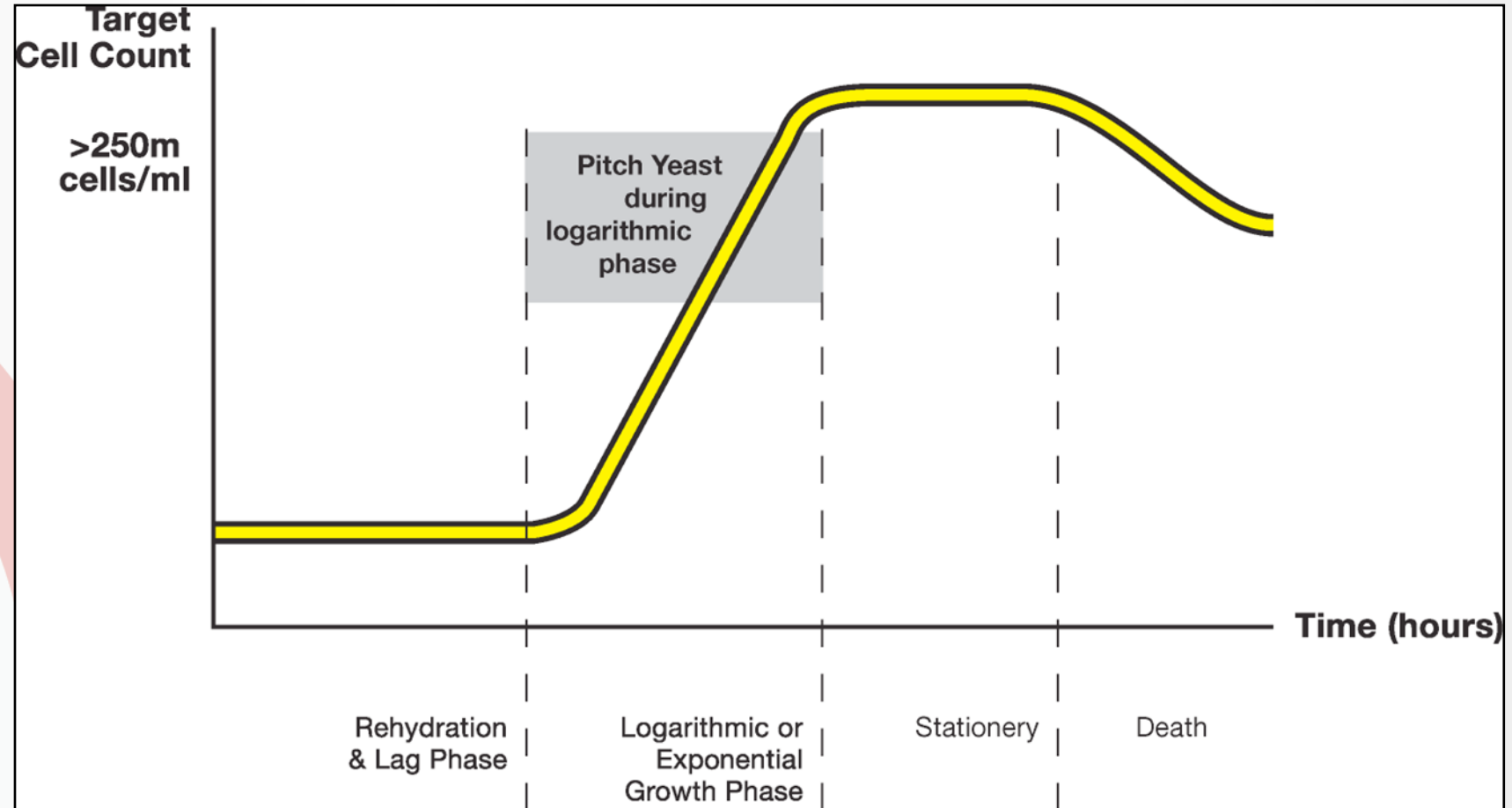
Sterols and Fatty Acids:
Membrane to handle stress

Inorganic Ions (Minerals and Metals)

- Sulfur: Synthesis of sulfur amino acids and coenzymes
- Phosphorous: Synthesis of nucleic acid, phospholipids and ATP
- Potassium: Osmoregulator, enzyme cofactor
- Magnesium: Enzyme cofactor, associated with yeast robustness to stresses
- Manganese and Zinc: Enzyme cofactors

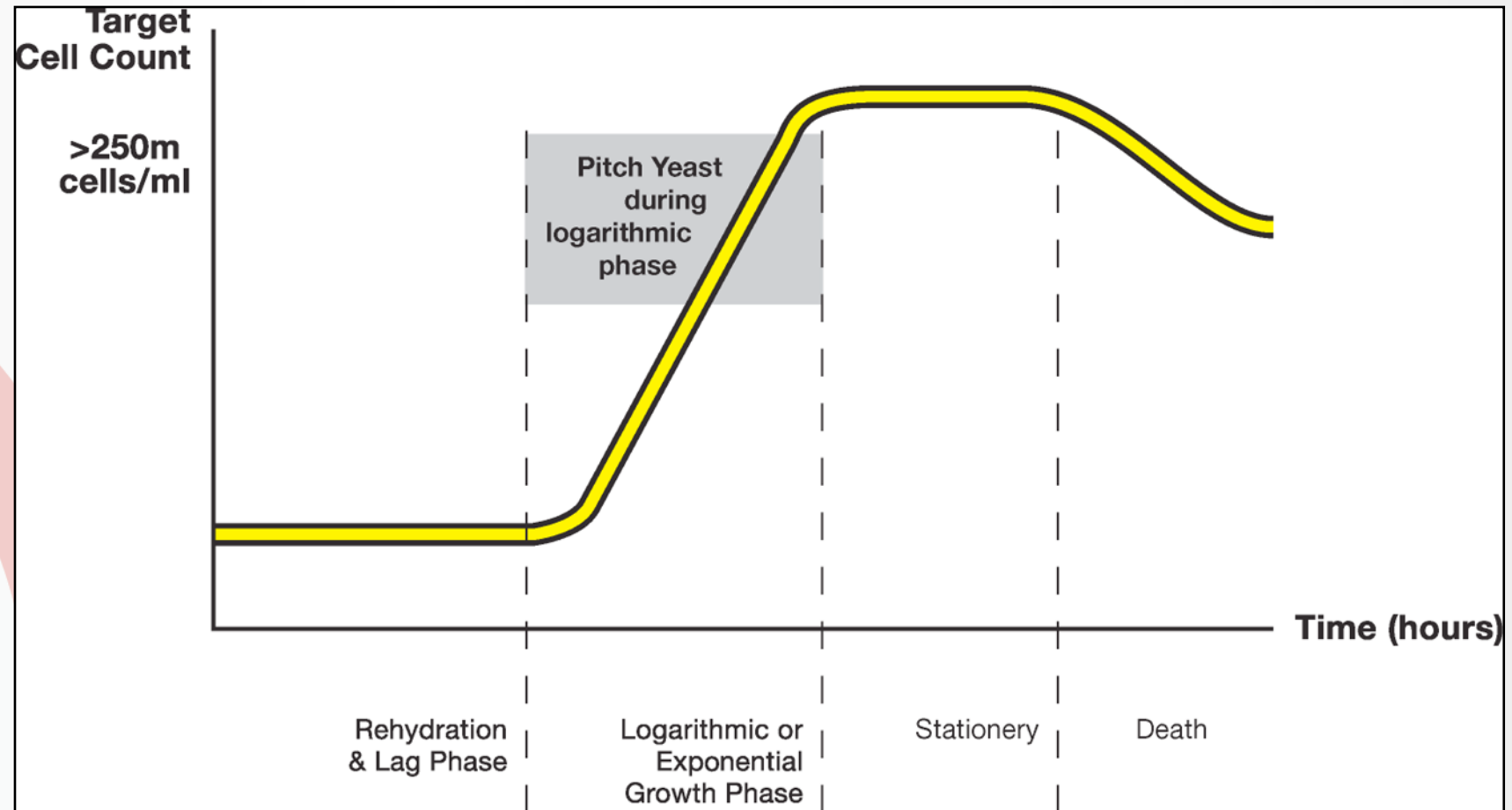
Yeast Health - Goals

- We need to keep growth anaerobically as long as possible – highest cell mass as possible.



Yeast Health - Goals

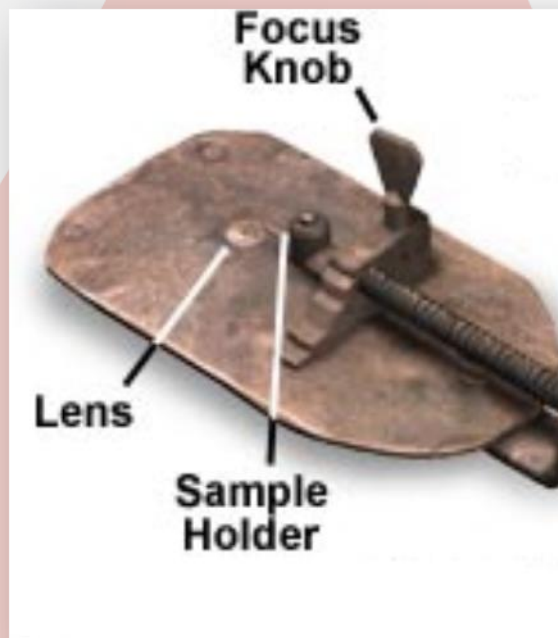
- We need to keep growth anaerobically as long as possible – highest cell mass as possible.
- Growth is limited by lack of substrates, inhibition by products, or limiting essential nutrient(s).



Things To Consider For Success (Agenda)

- What type of instrument are we using?
 - Manual vs. Automated
 - Advantages and Disadvantages
 - Measurement of Interest
 - Consistency
- Fermentation
 - Propagation
 - Cell Viability
 - Budding
 - Cell Mass
 - Fermentation Efficiency
 - Yield!

Instrumentation



Van Leeuwenhoek 1680



Microscope Analysis

Oculyze Analysis
Digitalization allows
laboratory microscope
counting functional

Requirements

- A laboratory microscope with a 10x objective
- A Neubauer / Thoma chamber for cell counting
- A compatible external camera which can be bought directly from the manufacturer or alternatively from [Oculyze](#)
- A compatible Android mobile device
- An active Oculyze user account
- A cable to connect the compatible mobile phone with the camera
- If you have a device with USB-C: USB-C (male) to USB-B (male) cable OR USB-A (female) to USB-C (male) adapter
- If you have a device with Micro USB: USB-A (female) to Micro-USB (male) adapter

External camera is compatible with generic microscopes, uses Oculyze BB 2.0 mobile app and cloud server. The camera can be connected via the C-Mount or the eyepiece (eyepiece adapters included for the following diameters: 30, 30.5 and 31.75 mm).





Pipette 20µl

Cellometer® K2

Cellometer®

Cell Count: 0 F1 Count: 1780
0 F2 Count: 84

Tint F

View Image: A, 1, 2, 3, 4, Zoom In

● B1 ● B2
● F1 Image
● F2 Image

Combined
 Counted

Count In View: 118
Manual Adjust: 0

Count

?

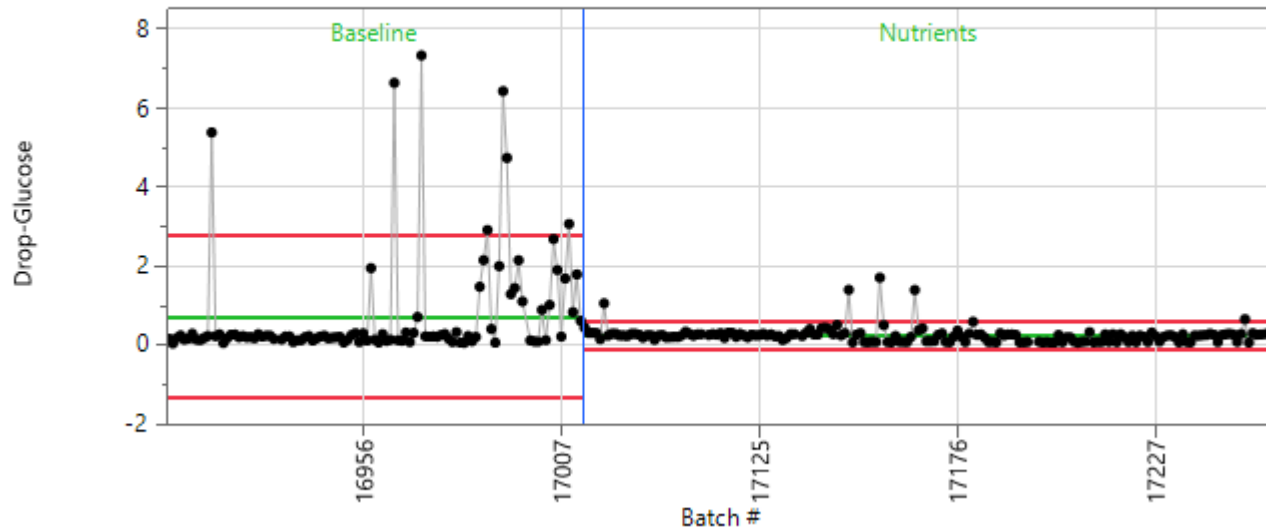
Results:

Count	Concentration	Mean Diameter
Total: 1864	6.44x10 ⁶ cells/mL	6.4 micron
Live: 1780	6.14x10 ⁶ cells/mL	6.2 microns
Dead: 84	2.93x10 ⁵ cells/mL	9.5 micron

Viability: 95.4%

110 mmgy plant - No Nutrient vs. Combination Nutrient

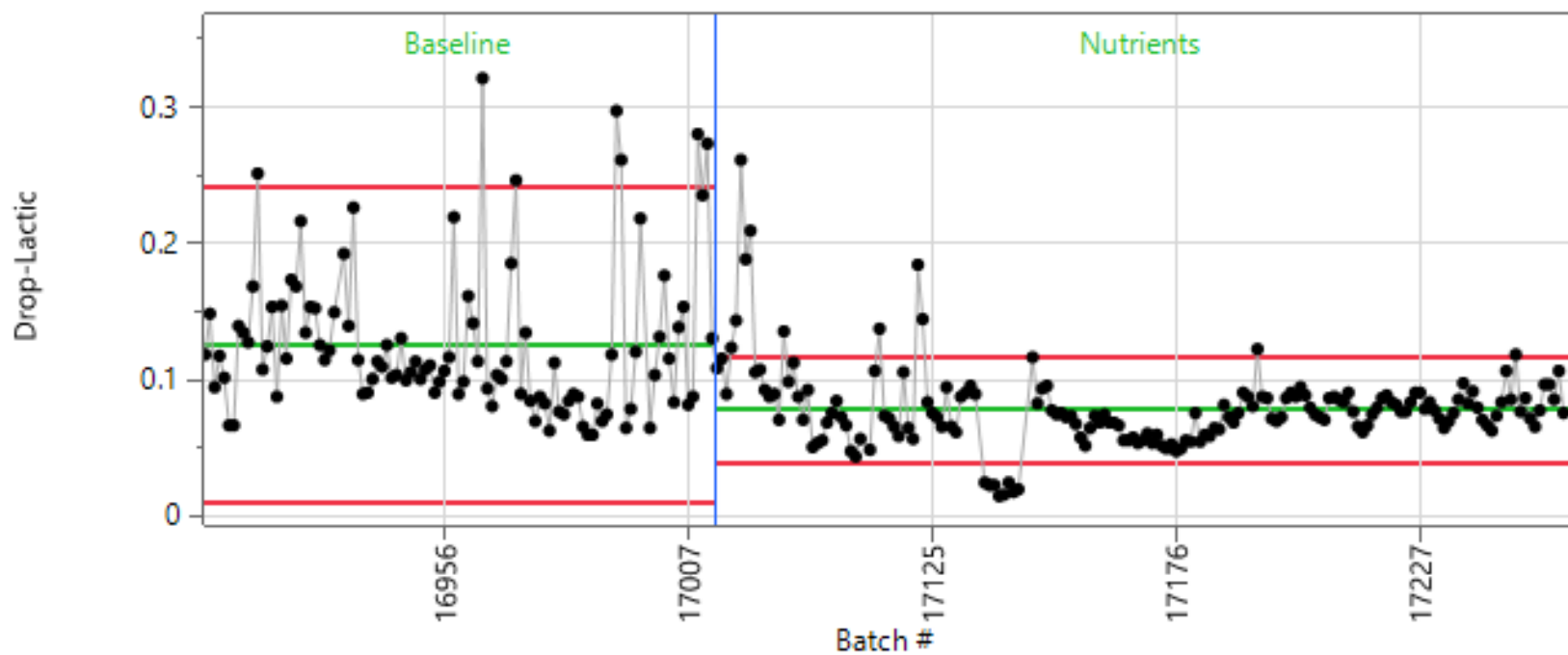
Individual Measurement of Drop-Glucose



		Baseline NH3	10 gal L, 1 bag Pro
Prop Send-Cell Count	Mean	341.2	425.5
Prop Send-% Viab	Mean	90.9	91.6
10 Hrs-Cell Count	Mean	253.9	357.2
10 Hrs-% Viab	Mean	79.0	85.3
15 Hrs-Cell Count	Mean	286.7	409.3
15 Hrs-% Viab	Mean	83.7	87.5
20 Hrs-Cell Count	Mean	282.6	404.0
20 Hrs-% Viab	Mean	83.7	86.3
30 Hrs-Cell Count	Mean	290.1	404.2
30 Hrs-% Viab	Mean	83.5	86.4

Lactic Acid Control

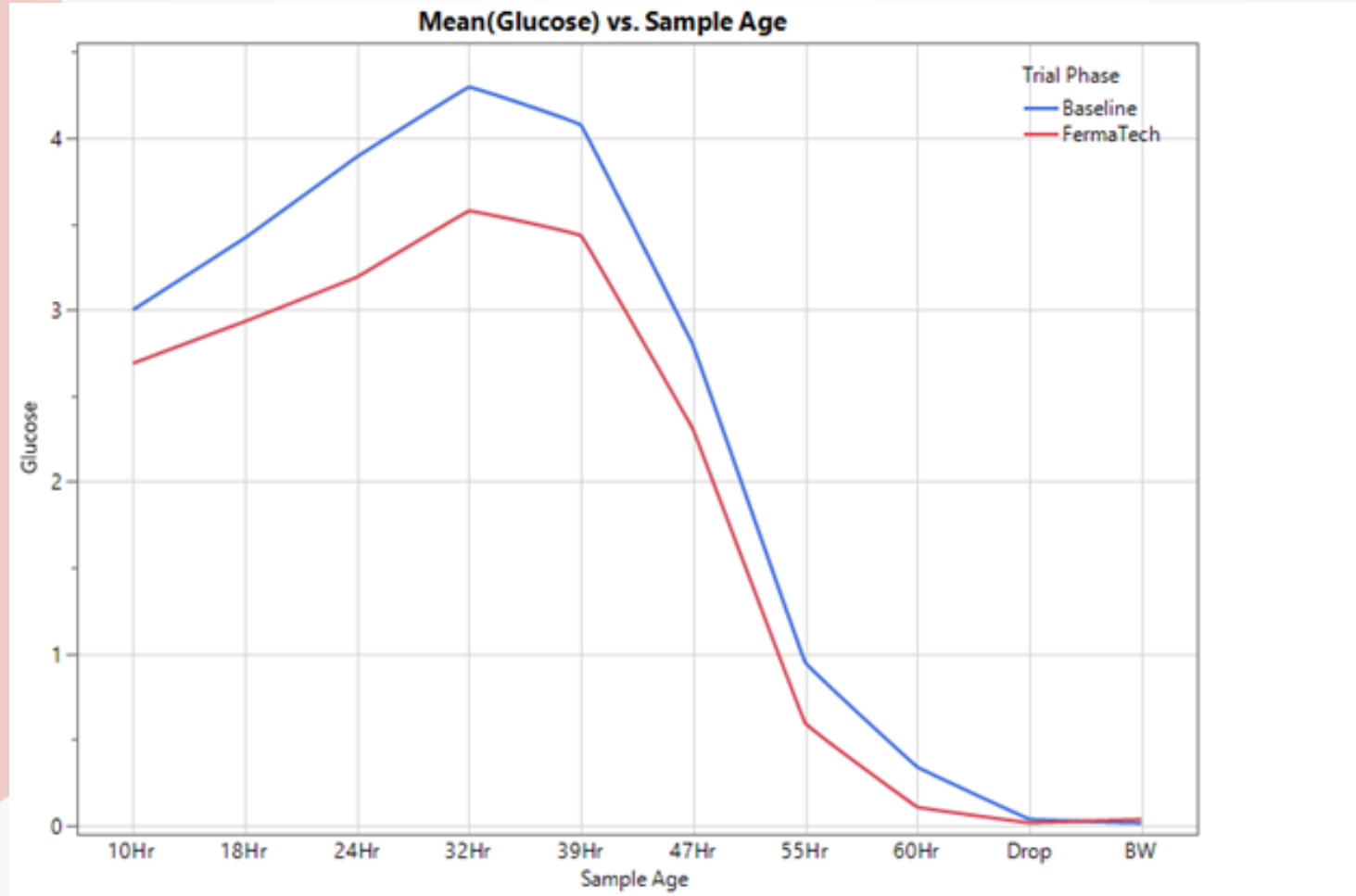
Individual Measurement of Drop-Lactic



Phase Limits

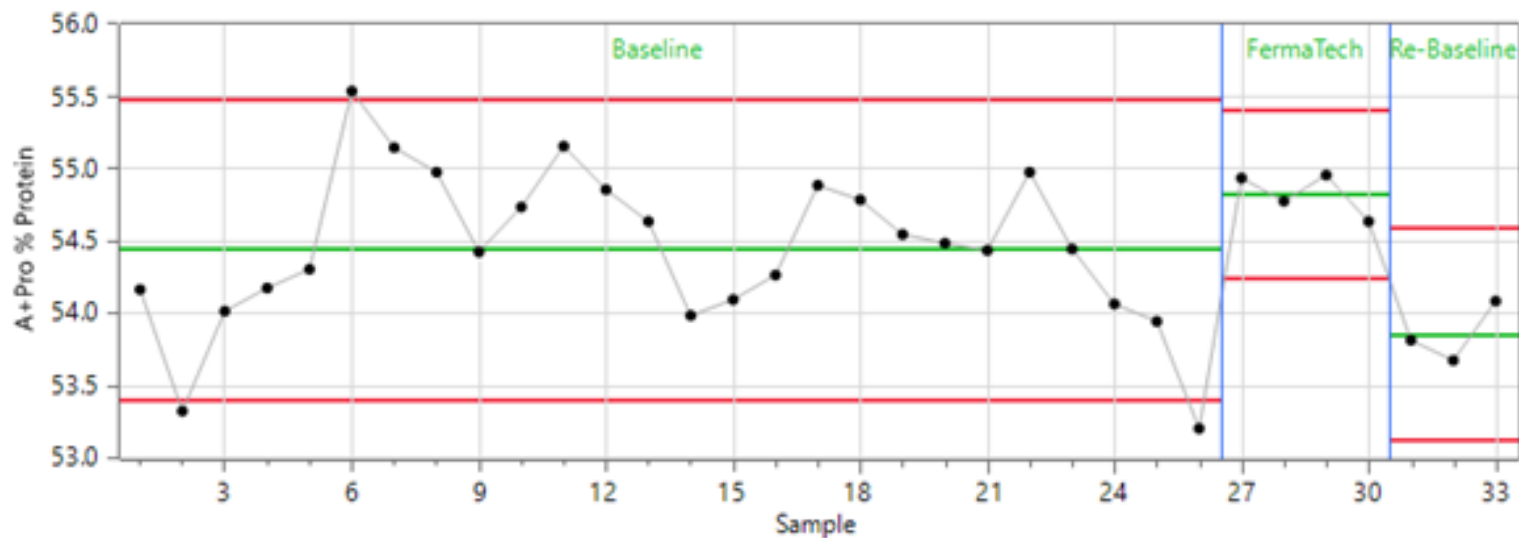
Phase	LCL	Avg	UCL
Baseline	0.010	0.126	0.242
Nutrients	0.039	0.078	0.117

Fermentation Kinetics Trial Comparison



Protein – High Value

Individual Measurement of A+Pro % Protein

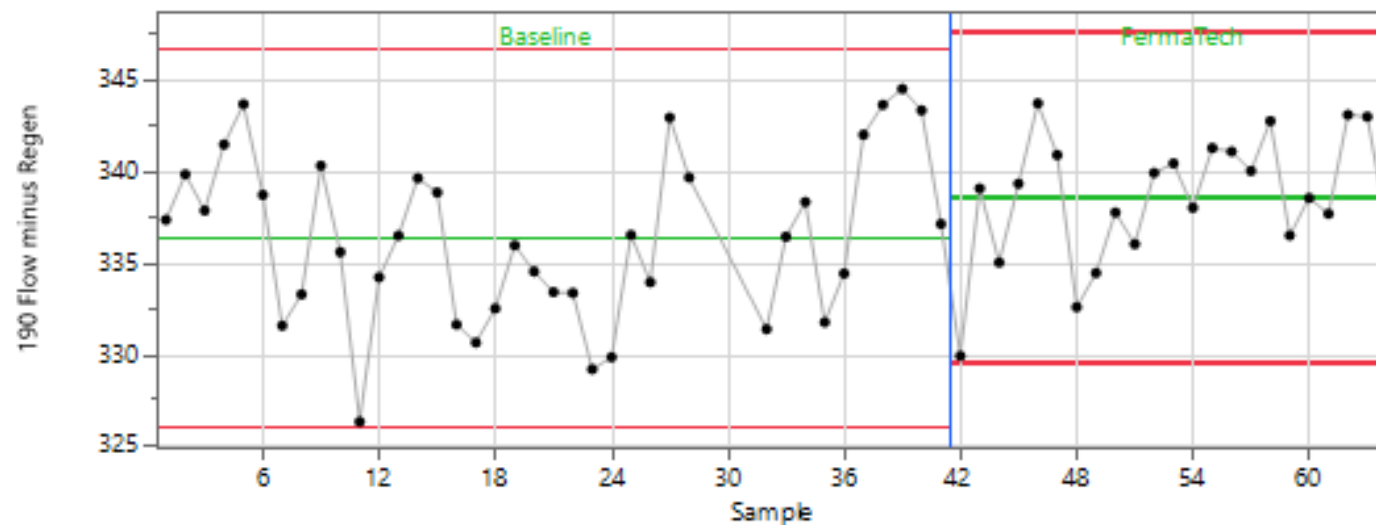


		Trial Phase	
		Baseline	FermaTech
Slurry Solids	Mean	31.61	31.56
DDGS % Protein	Mean	25.06	25.68
A+Pro % Protein	Mean	54.38	54.82
A+Pro Protein % Change	Mean	-0.00%	0.81%
Protein Line Mass Flow Rate	Mean	4.66	5.26
Protein Mass Flow Rate % Change	Mean	0.00%	12.86%
N		29	4

		Trial Phase	
		Baseline	FermaTech
Send Prop-% Bud	Mean	20.4	14.5
Send Prop-Cell Count	Mean	174.5	212.2

Ethanol Production - Yield

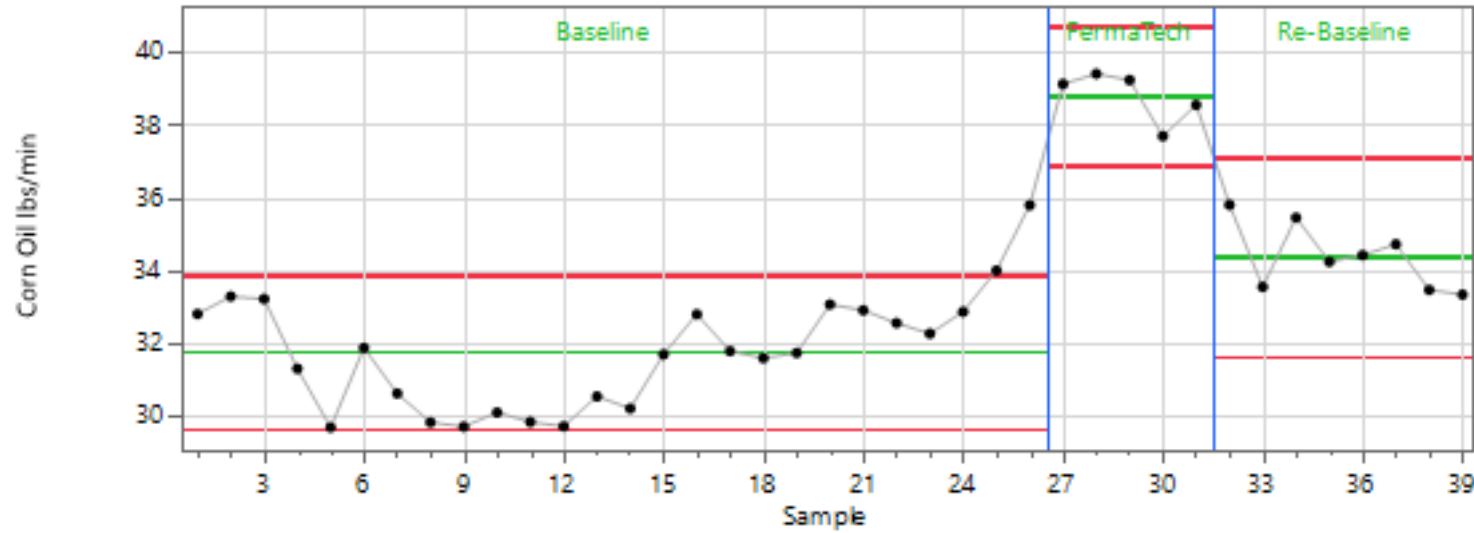
Individual Measurement of 190 Flow minus Regen



		Phase	
		Baseline	FermaTech
Liq Solids (Data)	Mean	34.82	34.32
190 Proof Product Rate Flow	Mean	381.02	389.01
Regen to Rectifier Flow	Mean	44.70	50.46
190 Flow minus Regen	Mean	336.32	338.56
190 Net Flow % Change	Mean	-0.00%	0.67%
190 gal/Liq Solids	Mean	4436.2	4580.8
190 gal/Liq Solids % Change	Mean	-0.00%	3.26%
N		38	24

Oil Production

Individual Measurement of Corn Oil lbs/min



		Trial Phase	
		Baseline	FermaTech
Slurry Solids	Mean	31.61	31.56
Corn Oil lbs/min	Mean	32.37	38.78
Corn Oil lbs/ferm	Mean	35,448	42,462
Corn Oil Ferm Yield lbs/solids	Mean	1,121	1,345
Corn Oil Ferm Yield % Change	Mean	-0.00%	19.96%
N		34	5

It's All About Value!

DAILY PRODUCTION CALCULATION			
Fill Time	7.75	Baseline	Trial
Actual Oil Production per Day (lbs)		112085	142053
Oil Price per lb (¢/lb)			\$ 0.46
Ethanol Price per gal (\$/gal)			\$ 1.46
DDG Price per Ton (\$/Ton) (at ~10% Moisture)			\$ 183.00
Gallons of Ethanol Production per Day (200 proof)		478,558.0	486,500.0
Gross Increase in Oil Revenue (\$) per Day			\$ 13,785.28
Lbs of Ethanol Increase			52,293.75
Ton decrease DDGS, DWB			39.22
Ton decrease DDGS, 90% solids			43.58
\$ lost from DDGS per Ton (Day)			\$ 7,974.80
Gross Ethanol Revenue (\$) per Day			\$ 11,595.32
Net Revenue (\$) per Day			\$ 13,874.84
Net Revenue (\$) per Year			\$ 4,856,193.79

This financial sheet does not account for cellulosic ethanol sold



Conclusions

- Proper training
 - counting cells, viability and budding
- Equipment – take a good look at what you want from them
 - Evaluate the advantages and disadvantages
- Remember the goal !
 - Most yeast cells
 - Peak budding
 - Highest viability
- Make money through fermentation

Thank You