Phibro ETHANOL®

EXPERIENCE PERFORMANCE

Hot Topics in Troubleshooting

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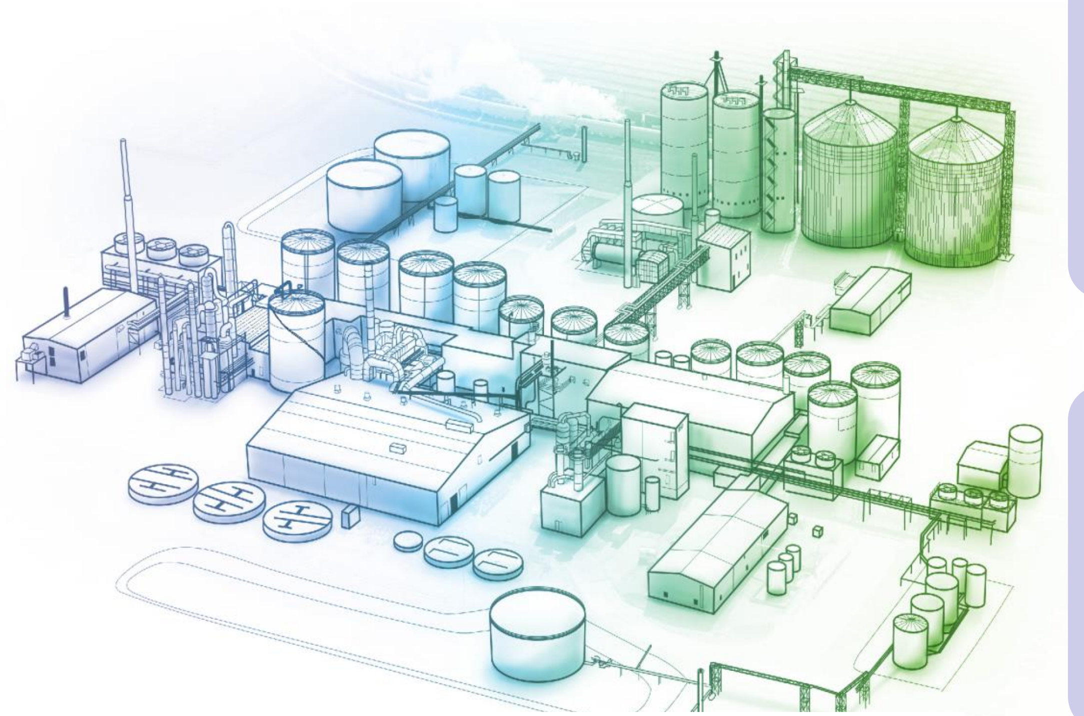
FELC 2023
FUEL ETHANOL LABORATORY CONFERENCE

Presentation Overview



FELC 2023
FUEL ETHANOL LABORATORY CONFERENCE

Fermentation Troubleshooting	*Fundamentals *Case Study Examples
Shutdowns and Startups	*Items to Consider *Plant Startup Best Practices

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Yeast Health & Fermentation

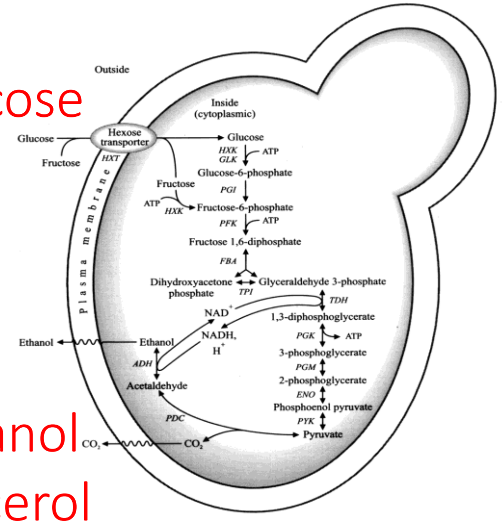
Understanding Your Baseline

Why is yeast health important?

Factors Impacting Rate and Completion

Yeast health is an essential component for successful fermentation!

Glucose



Ethanol

Glycerol

CO_2

1 Glucose --> 2 Ethanol + 2 CO2

100 kg (lbs) 51.1 kg (lbs) 48.9 kg (lbs)

Poor Yeast Health can lead to:

- ↓ **Efficiency**
- ↓ **Rates/Kinetics**
- ↑ **By-Product Formation**
- ↓ **Yield**
- ↑ **Residual Sugars**

Factors that Impact Yeast Health

Yeast Stress Factors

Operational factors

Glucose content
(10% w/v max)

Sulfite
(100 ppm)

Temperature
(35ºC = 95ºF)

Sodium
(500
ppm)

CIP
Chemicals

Nutritional factors
(media formula)

Lack of: Sterols, Nitrogen, Oxygen, UFA, Minerals/vitamins

Microbial factors

Chemical

Acetic acid
(0.05% w/v)

Ethanol
(23% v/v max)

Lactic acid
(0.8% w/v max)

pH
(<4.0 or >6.0)

Mycotoxins
(10-100 ppm)

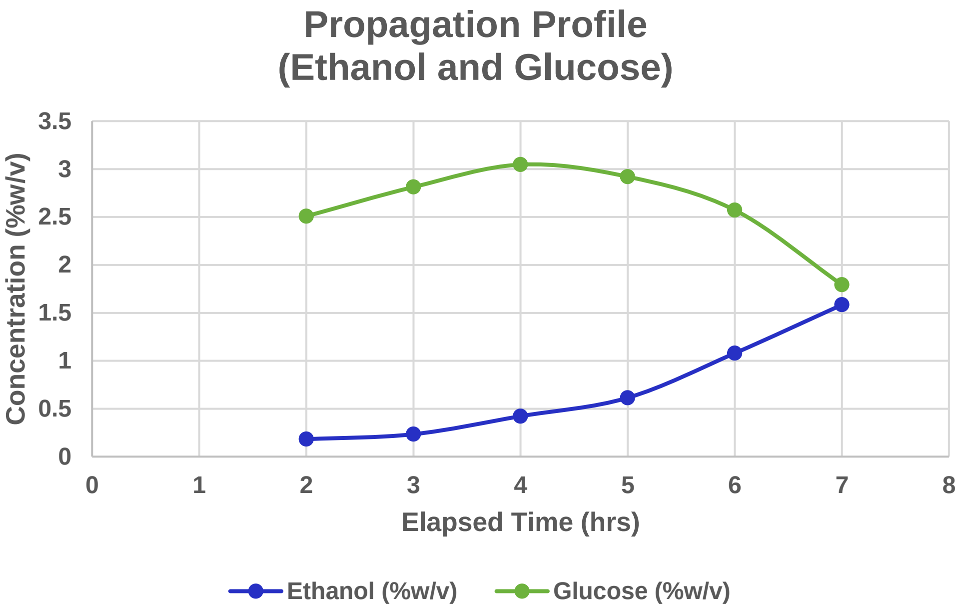
Fusel volatiles
(0.1–1 % w/v)

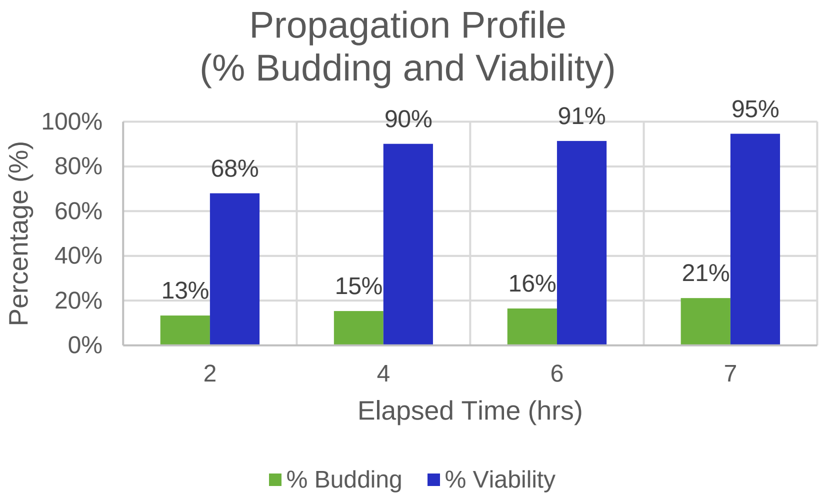
Competition

Establishing a Baseline – Propagation

Establishing a Baseline and Identifying Deviations

- **Goal of Propagation**
  - Produce Biomass (good cell counts and viability)
    - Build a healthy yeast population to set fermentation up for success
- **Review of Past and Current Data (KPIs)**
  - Cell Count and Viability
  - % Budding
  - Environmental Conditions (i.e. temperature and pH)
  - Process Variables (%DS, mash:water ratio, nitrogen additions, DO)
  - Propagation sugar and ethanol profiles
- **Identify what a "normal" propagation would be for your plant**

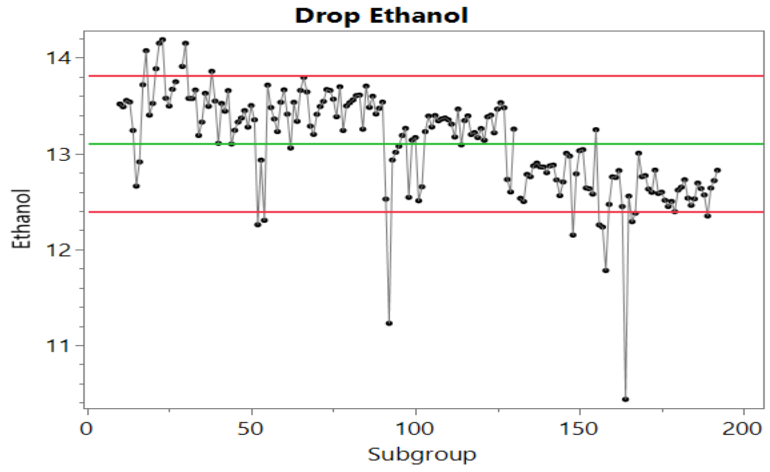


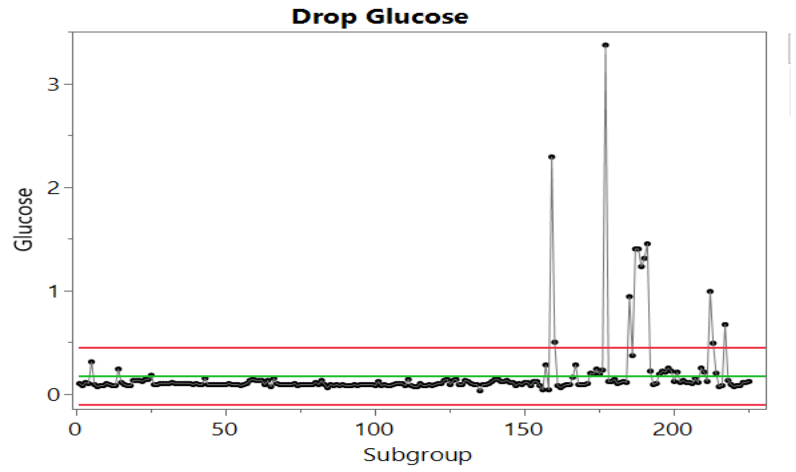


Establishing a Baseline – Fermentation

Establishing a Baseline and Identifying Deviations

- **Goal of Fermentation**
  - High product titers and substrate utilization
    - Maximize performance for yield and efficient and complete substrate utilization
- **Review of Past and Current Data (KPIs)**
  - Environmental Conditions (i.e. temperature and pH)
  - Process variables (%DS and nitrogen)
  - Fermentation profiles (sugar, ethanol., by-products, etc…)
- **Identify what a "normal" fermentation would be for your plant**





Identifying “Stressed” Propagations and Fermentations

Troubleshooting Tips

- **Establish a baseline for propagation and fermentation “normal” operating conditions**
- **Continuously monitor both for deviations from the established baseline**
- **Use the tools available to look for key indicators/signs of stress**

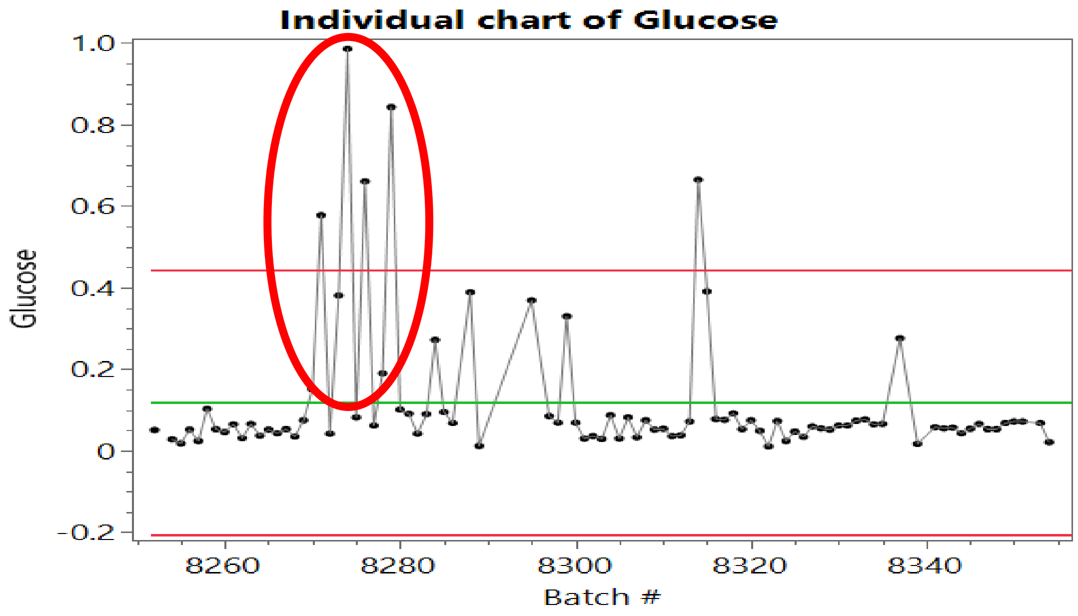
Troubleshooting and Mitigation

Stressed Fermentations

Troubleshooting Fermentation

Troubleshooting and Mitigation

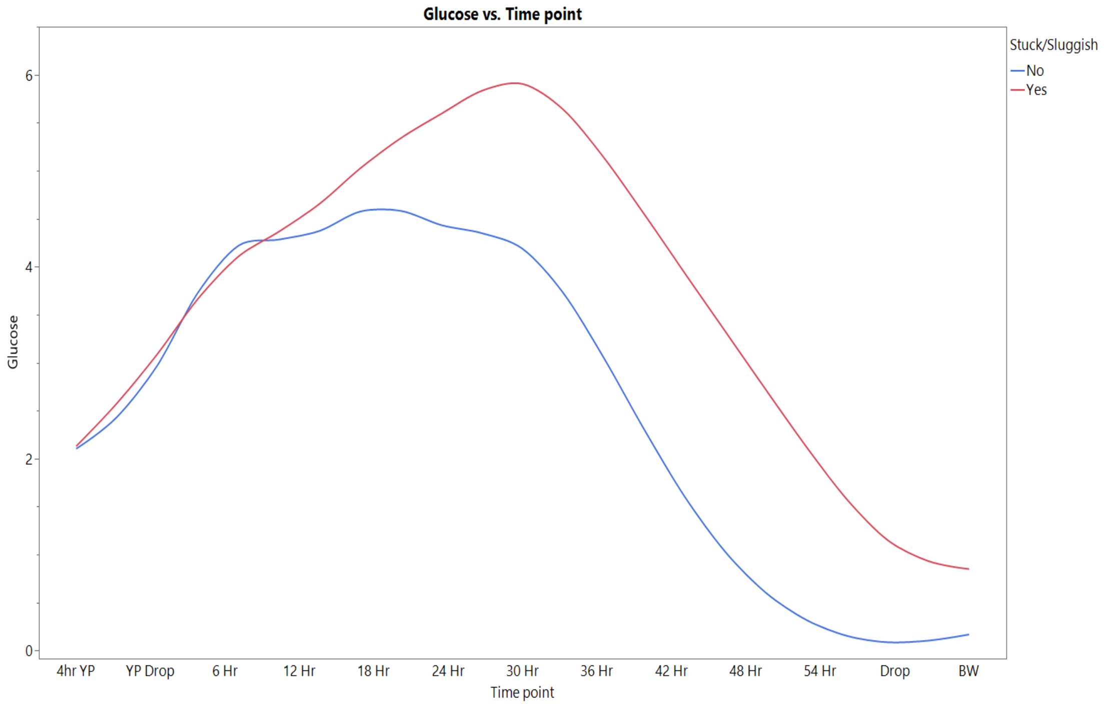
- **Data Review**
  - Compare to established baseline
  - Identify issues as they arise
- **Review procedures, recipes, and data to determine the potential causes of stuck/sluggish fermentations**
  - Contamination
  - Temperature stress
  - Missed or changed inputs (such as enzymes additions, nitrogen additions, other inputs to the process).



Troubleshooting Fermentation - Continued

Troubleshooting and Mitigation

- **Rule in/out potential causes by leveraging information available**
  - HPLC and other laboratory metrics
  - DCS data/trends
  - Process logs
- **Onsite and Laboratory Testing**
  - Nutritional/Ion Analysis
  - Nitrogen Availability
  - Ethanol and Acids
  - Other Inhibitor Testing



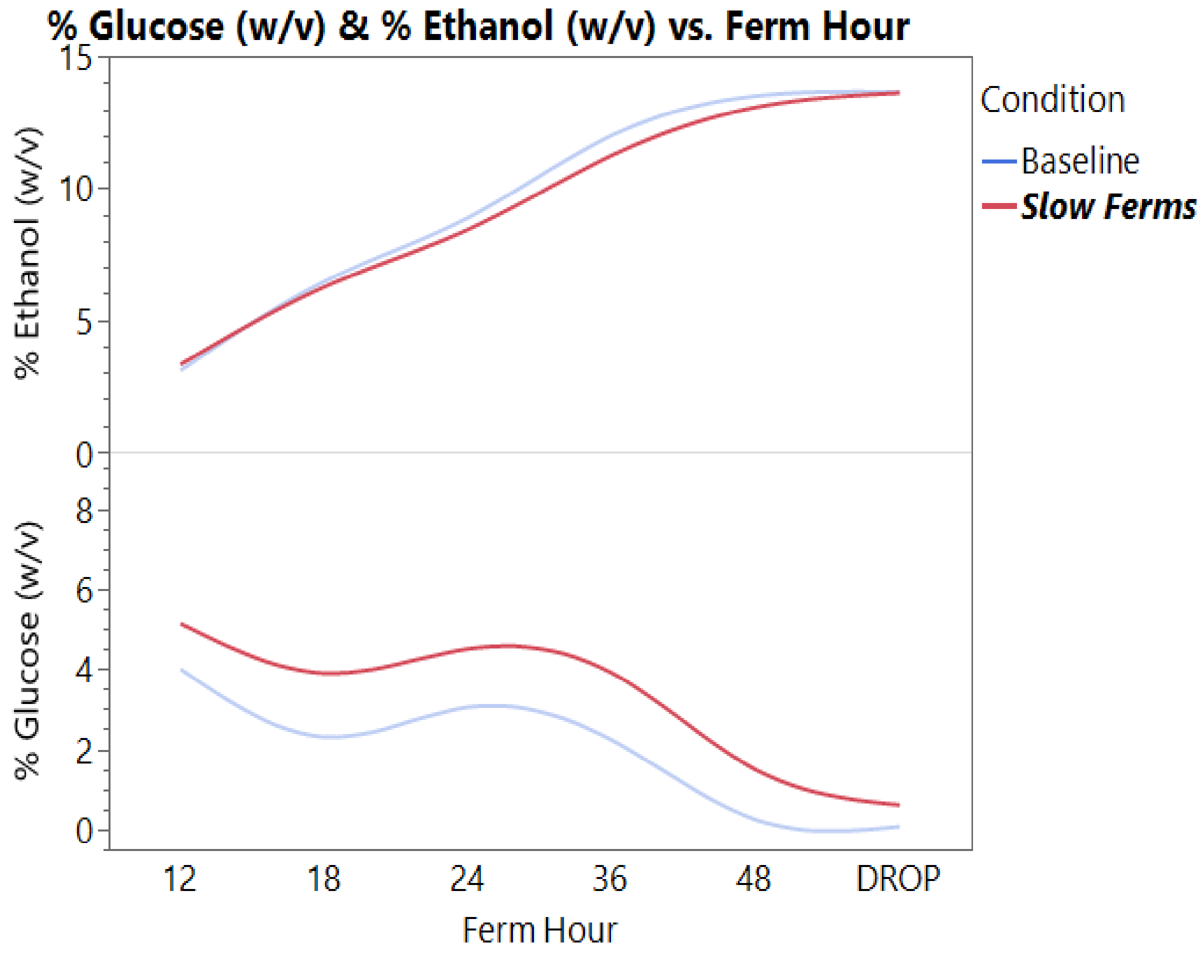
- **Identifying the cause can help with mitigation steps to reduce the impact and potentially recover the fermentation**

Case Studies

Problem Specific Troubleshooting

Case Study #1 – Reduced Fermentation Rate

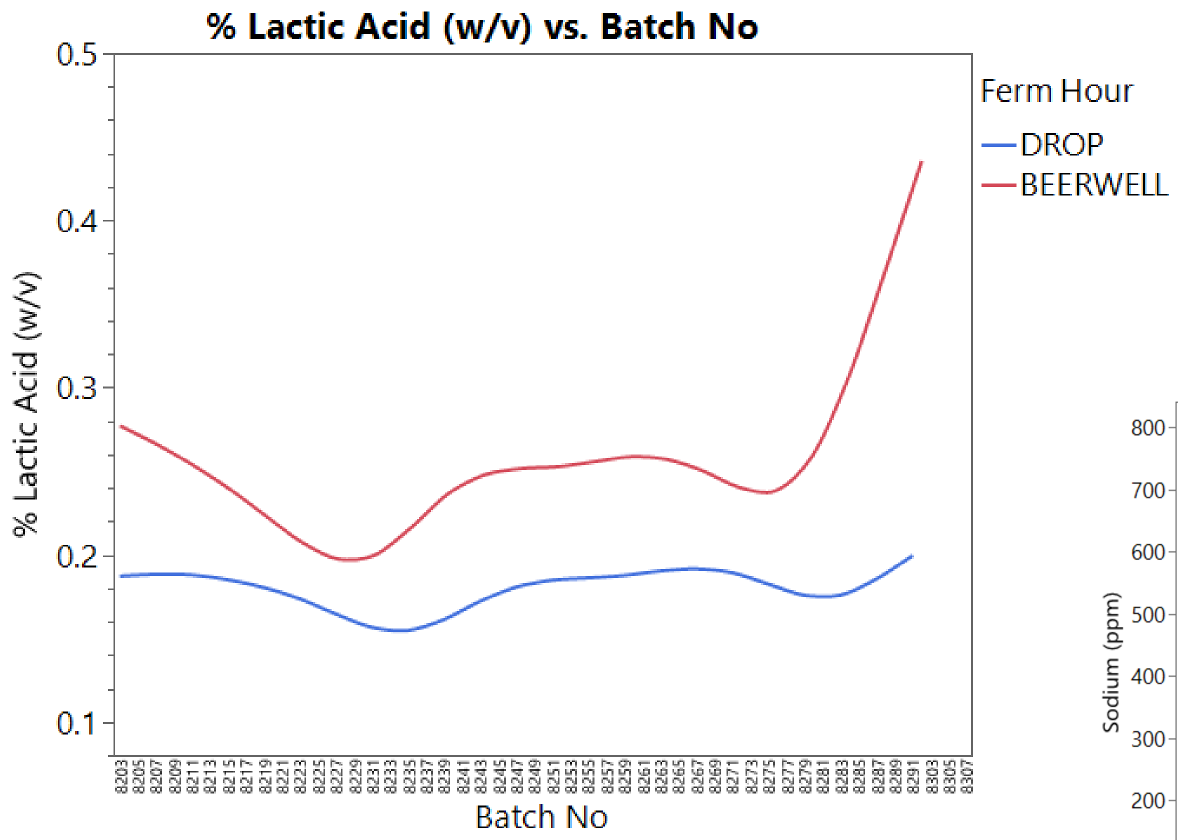
Problem Specific Troubleshooting



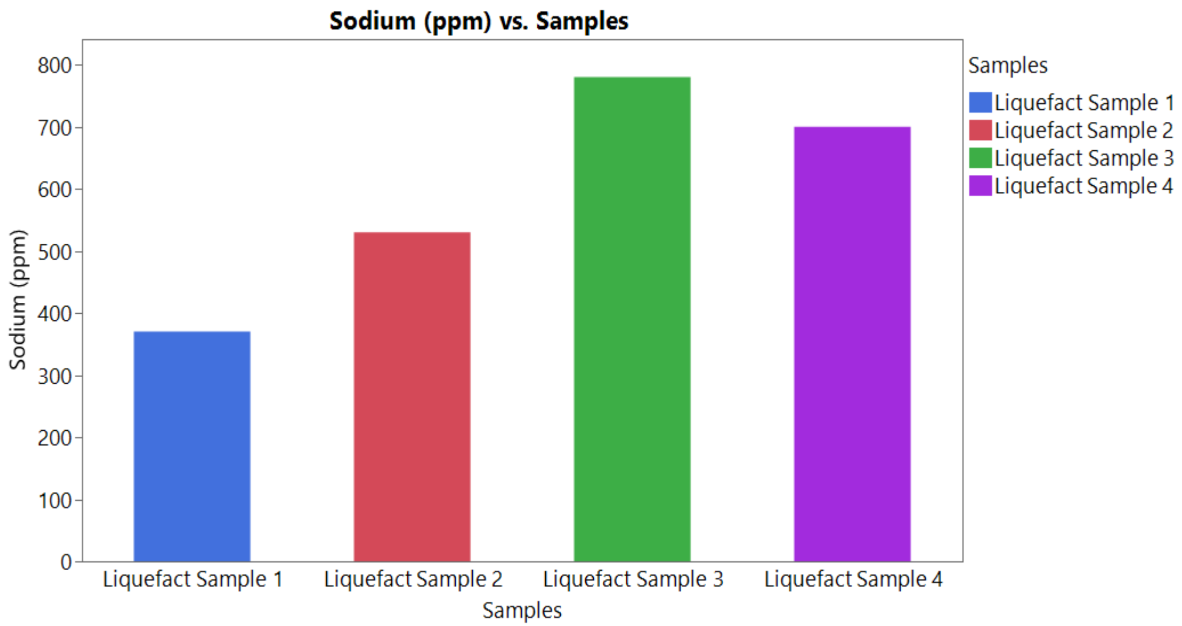
- **Slower fermentation kinetics were observed after a plant slowdown event; leading to:**
  - Reduced substrate utilization (i.e. high drop glucose)
  - Reduced productivity and yield

Case Study #1 – Reduced Fermentation Rate

Problem Specific Troubleshooting

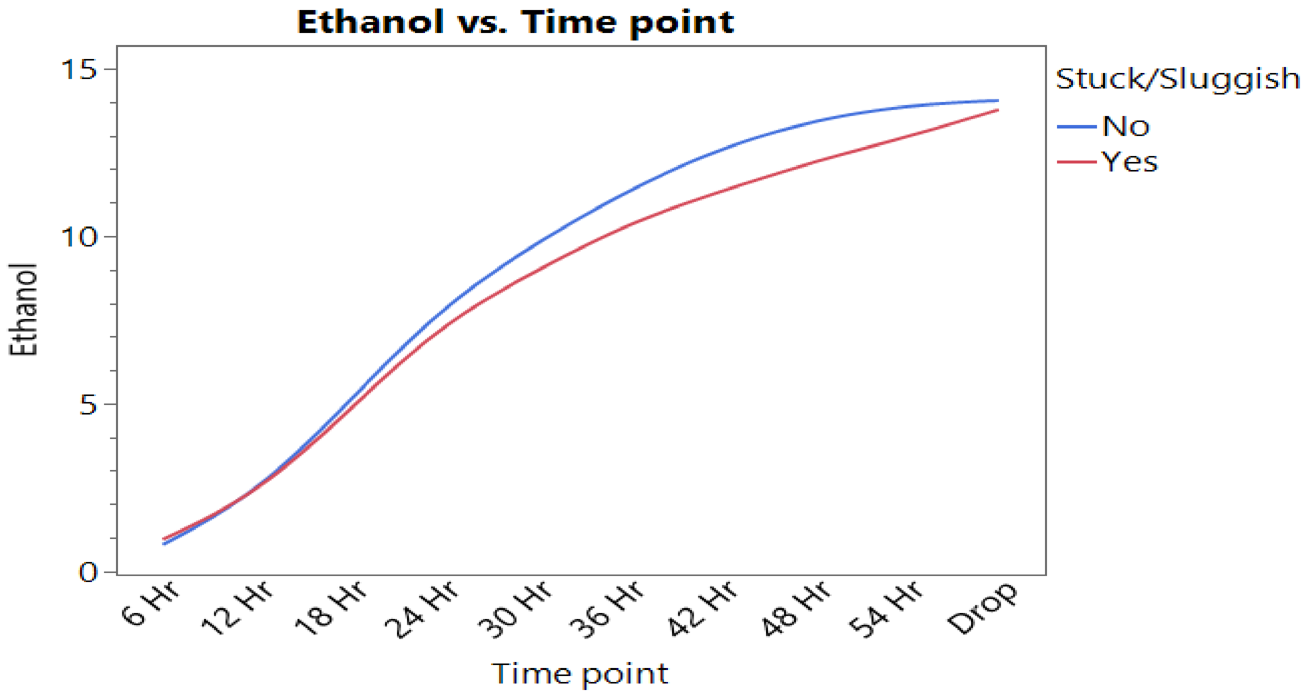


- **Troubleshooting was done by combining data analysis and laboratory testing:**
  - Increased organic acid recycle – beerwell infection
  - Elevated sodium concentrations in liquefaction



Case Study #2 – Reduced Fermentation Rate

Problem Specific Troubleshooting



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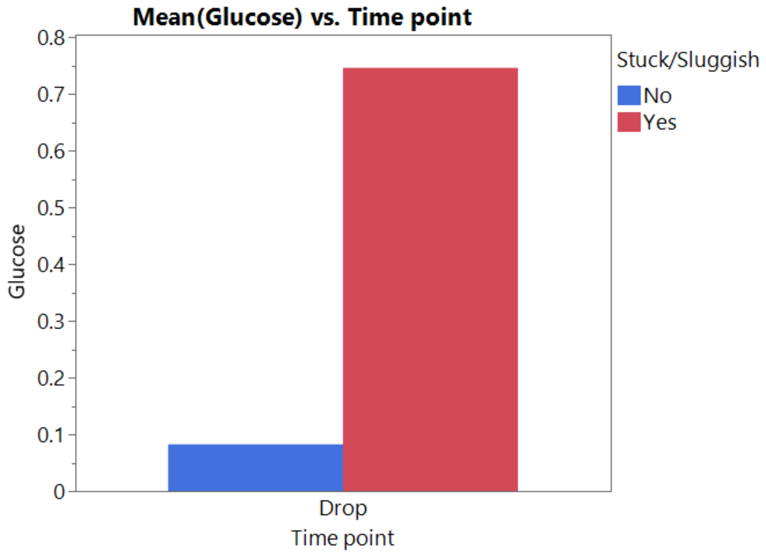
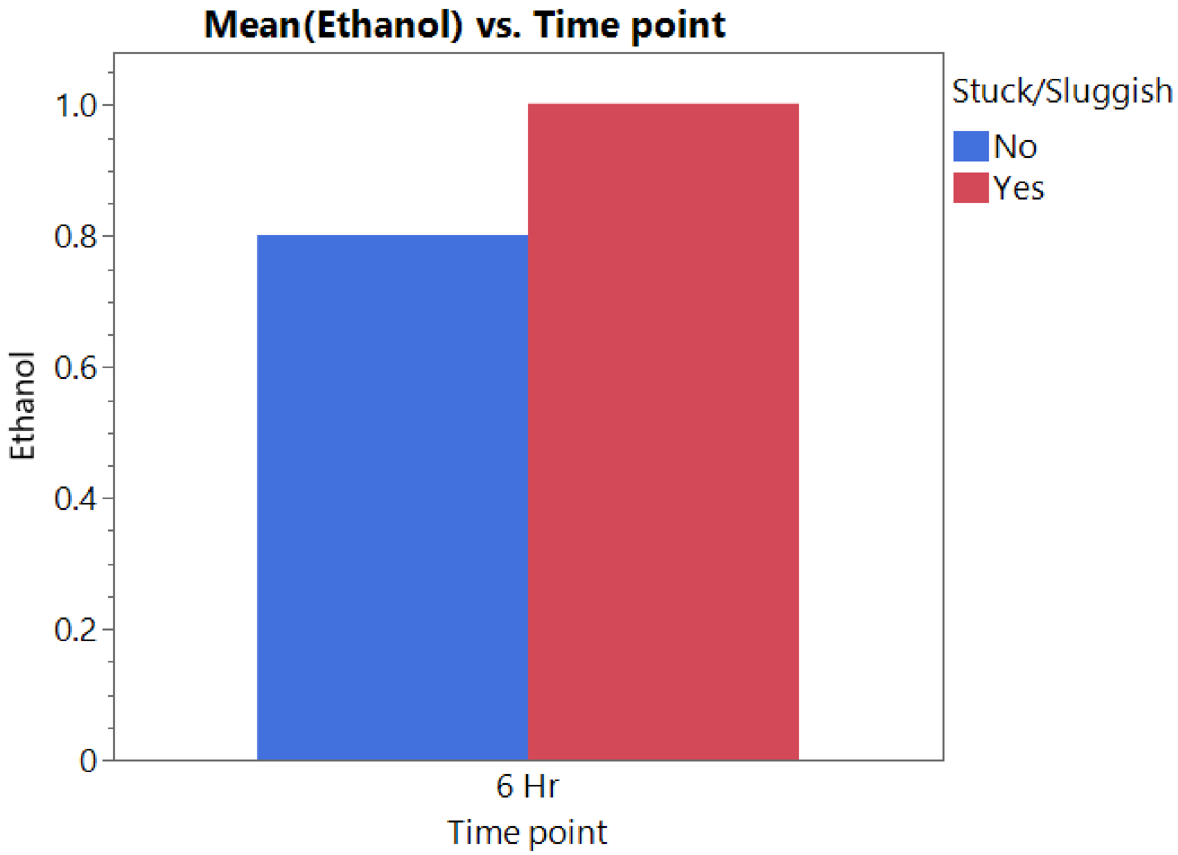
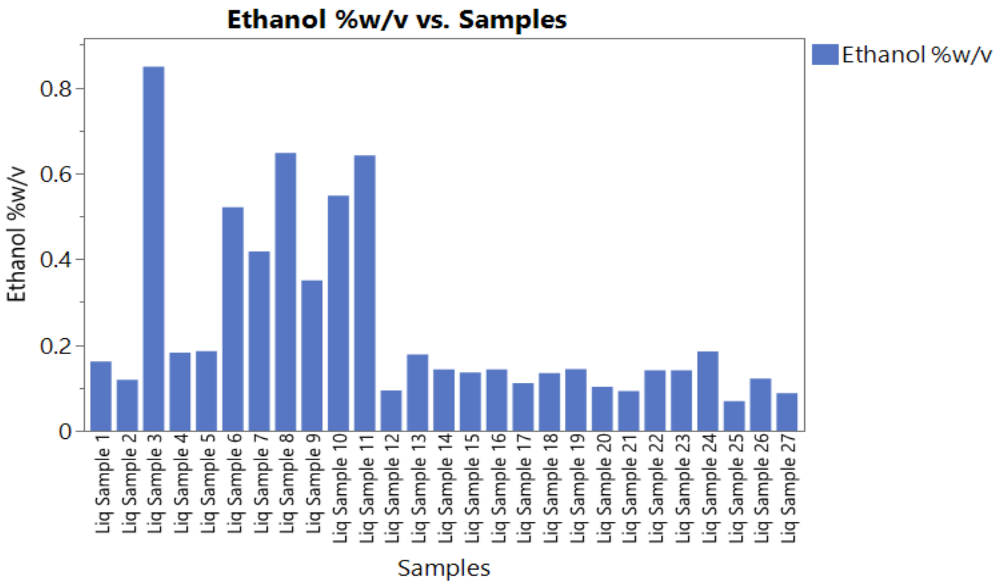


Case Study #2 – Reduced Fermentation Rate

Problem Specific Troubleshooting



- **Troubleshooting was done by combining data analysis and laboratory testing:**
  - Increased early fermentation ethanol
  - Elevated ethanol concentrations in cookwater and liquefact



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Shutdowns and Startups

Planning and Items to Consider

Shutdowns and Startups

Planning

Proactive Work

- Items to Verify
- Scheduled Activities

Plant Startup Best Practices

- Considerations to Help Effectively Navigate a Restart
- Case Study Examples

Proactive Maintenance

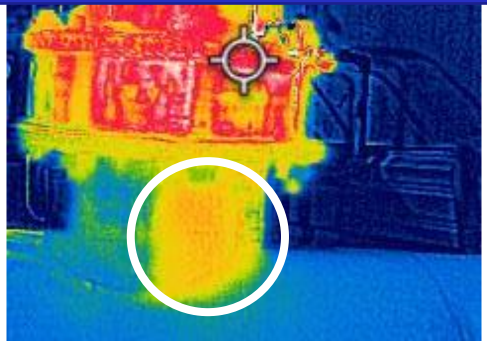
Items to Verify and/or Fix

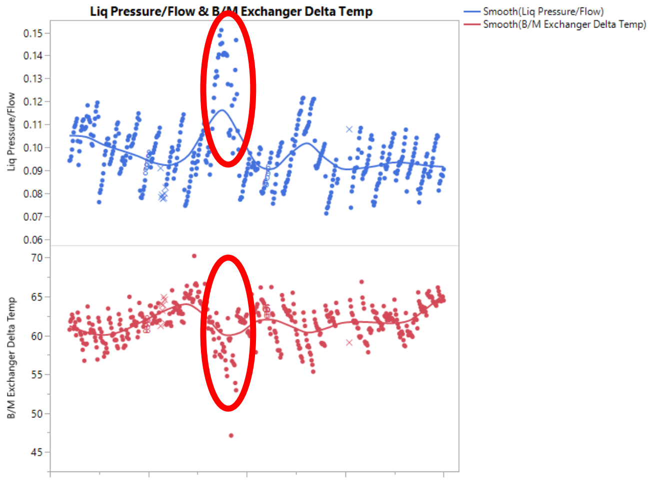
Leaking Valves
- Leaking caustic or acid into mash can increase yeast inhibition/stress factors (i.e. sodium, acids, etc).
- A leaky valve can also slowly feed contaminated mash into the process and cause infections

Plugged/Blocked Heat Exchangers
- Reduced heat transfer across exchangers.
- Biofilm development - leading to risk of periodic bacterial contamination.

Worn Pump Impellors
- Reduced flow rate can impact solids removal during flushes.
- Reduced velocity impacts effectiveness of recirculation loops.

Ferm fill valve during Ferm Fill Header CIP





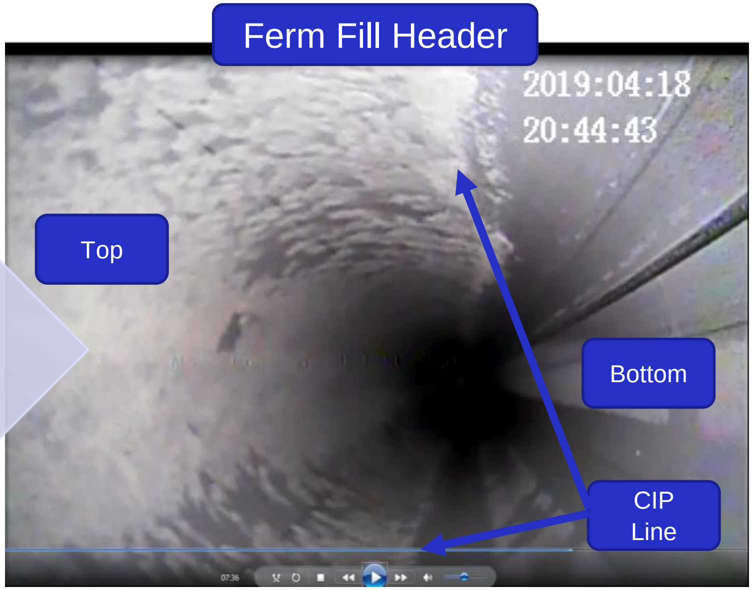
Proactive Maintenance

Scheduled Activities

Borescoping
- Helpful for monitoring before and after cleaning/hydroblasting.
- Can identify problem areas or deficiencies in normal cleaning loops.

Temperature and Pressure Transmitters
- If out of calibration, cleaning tank actual temperature could be far from target setpoint.
- Pressure transmitters can be essential for identifying poor or plugged flow conditions.

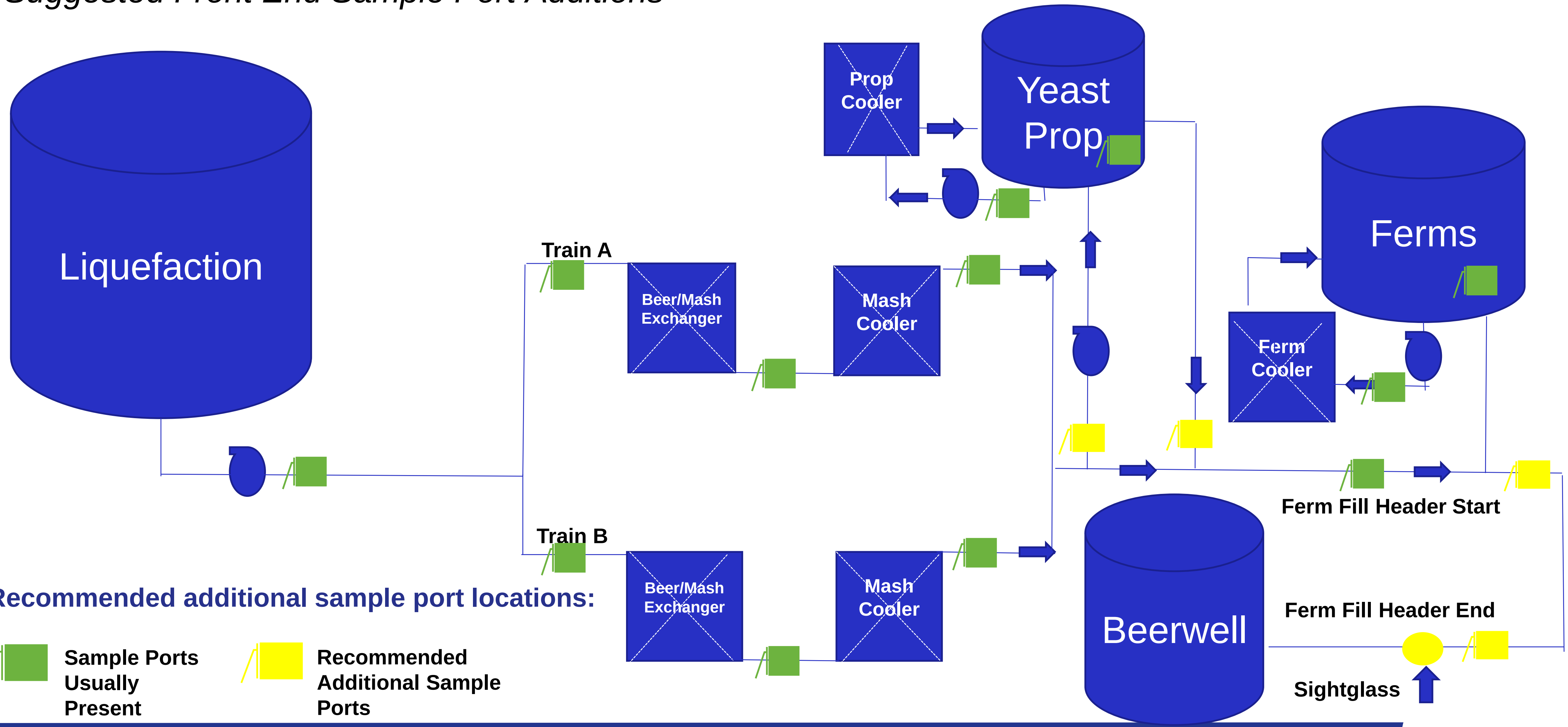
Additional Sample Ports
- Increases the number of locations where mash samples can be collected.
- Improves infection troubleshooting efforts.



Depending on the location of the temperature transmitter, tank may need to be emptied or level lowered

Additional Sample Ports

Suggested Front End Sample Port Additions



- **Recommended additional sample port locations:**

Sample Ports Usually Present

Recommended Additional Sample Ports

Considerations to Help Effectively Navigate a Restart

Plant Startup Best Practices

Plant Startup Best Practices

Impact of Stored Water/Stillage Sources – General Information

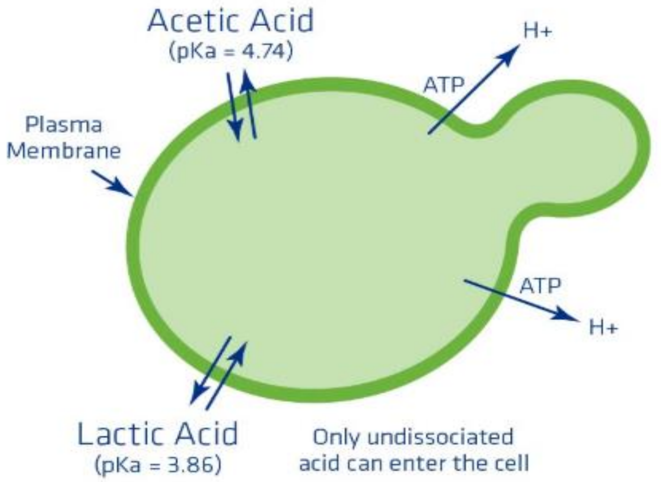
- **When preparing to startup after a shutdown or speeding up after a slowdown, understanding the make-up of process waters is critical for developing the right startup strategy. Key metabolites to monitor include:**

Metabolite	Details
Lactic and Acetic Acid	• Mainly produced by Lactic Acid Bacteria (LAB) but also potentially acetogenic and methanogenic bacteria (similar to what occurs in an anaerobic digestor) if liquids sit too long during shutdown.
Propionic and Butyric Acid	• Propionic and butyric acids are found in all plant and animals as a part of normal fatty acid metabolism. They both have a pungent odor. • In fermentations, propionic acid is mainly produced by bacteria of the genus *Propionibactera* and while butyric acid is most commonly produced by bacterial of the genus *Clostridia*.
Fusel Compounds	• Often enter process waters as part of base loss during the process of shutting the plant down.
Hydrogen Sulfide	• Produced by Sulfur Reducing Bacteria (SRB). This has more of a health/safety impact rather than fermentation inhibition. See Phibro's guidance document titled "Phibro EPG Sulfur Reducing Bacteria Monitoring Guide" for more details.

Impact of Stored Water/Stillage

pH Impact on Yeast

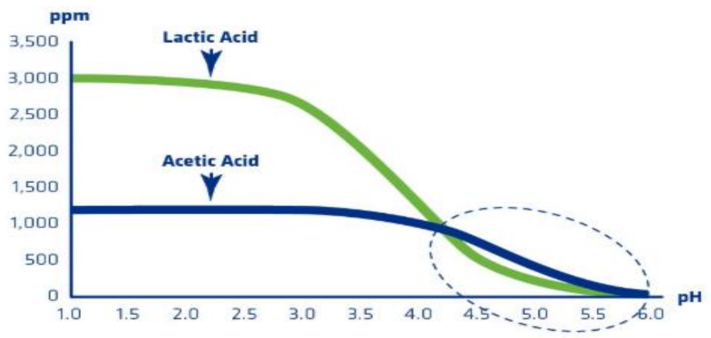
- **Organic acids can become inhibitory to yeast at startup in the following ways:**



At a pH equal to the acid dissociation constant (pKa), half of the acid in solution is dissociated and half is undissociated.

- Lactic = 3.86
- Acetic = 4.74
- Propionic = 4.87
- Butyric = 4.82

Concentration of Undissociated Lactic & Acetic Acid at Different pH Values



As the pH drops below the pKa, the more toxic/inhibitory it is for yeast because more acid can enter the cell.

- Undissociated acids diffuse passively into the cell

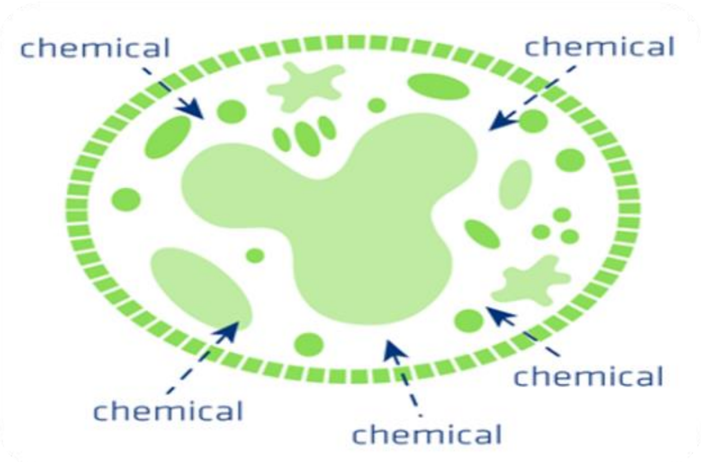
*NOTE: With all organic acids and fusels, the presence of other inhibitory compounds and/or stressors can reduce the levels of inhibition.

Impact of Stored Water/Stillage

Fusels Impact on Yeast

Fusels can become inhibitory to yeast at startup in the following ways:

- Inhibit internal yeast cell enzymes
- Change membrane composition and destroy integrity
- Increase membrane fluidity
- Glucose transport disruption



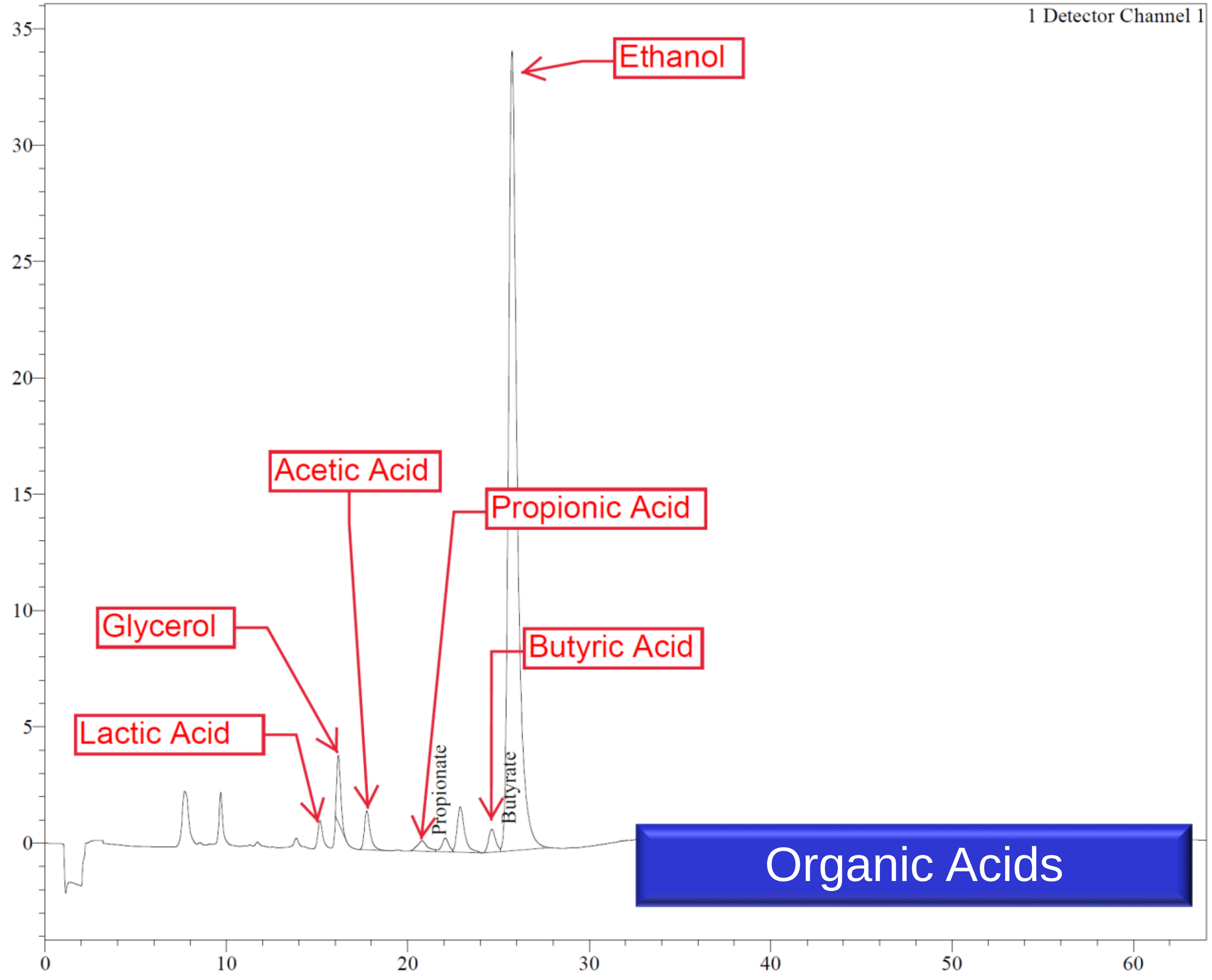
Case Studies

Shutdowns and Startups

Case Study #1 - Impact of Stored Water/Stillage

Measuring/Identifying Organic Acids

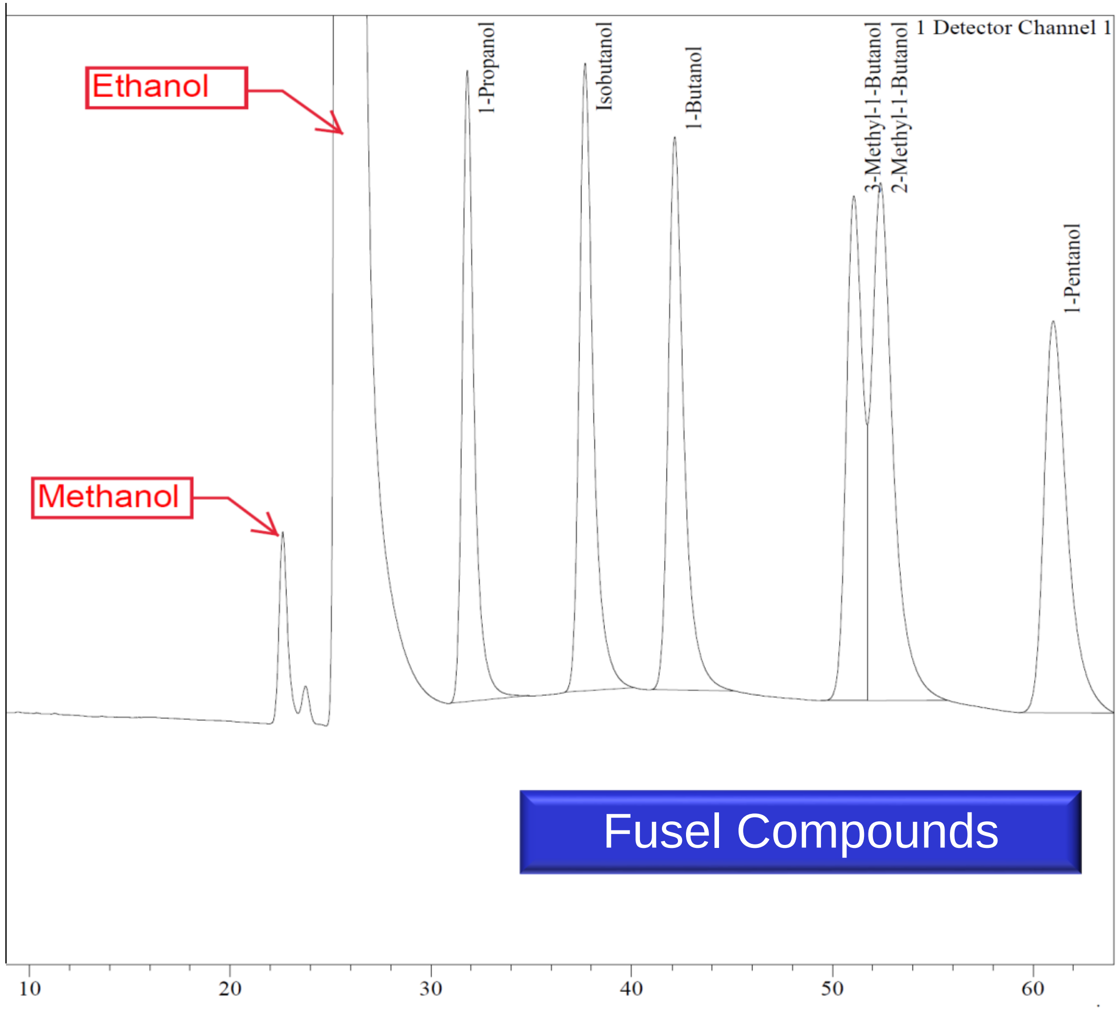
- **Plant came out of a slowdown where stored water/stillage tank levels had increased (increased residence time)**
- **Slowed fermentation kinetics were observed**
- **Process water samples were collected and analyzed on the HPLC**
  - Propionic and butyric acids were found (quick presence/absence analysis)
  - Plant processed waters more effectively to remove inhibitory compounds



Case Study #2 - Impact of Stored Water/Stillage

Measuring/Identifying Organic Acids

- **Plant startup following shutdown**
- **Distillation/plant rate upsets occurred going into and out of shutdown**
- **Problem specific troubleshooting including mash analysis identified presence of these compounds via HPLC**



Plant Startup Best Practices

Startup Steps to Minimize Impact of Inhibitory Products

FortiPhi®
NUTRITIONAL SOLUTIONS

Increase:

- CIP Strength
- Flush Duration
- Antibiotic Additions
- pH
- Nitrogen (20-50%)

Other products that can help mitigate the impact of inhibitory compounds present in the system:

- FortiPhi® Nutritional Solutions
- Kinetx™ Supplementation

Decrease:

- Target Mash Solids
- Backset and Process Water Addition Rate

Presentation Summary

Hot Topics in Troubleshooting

Establish a baseline for propagation and fermentation “normal” operating conditions

- Continuously monitor both for deviations from the established baseline
- Use the tools available to look for key indicators/signs of stress

Troubleshooting

- Analyze data and rule in/out potential causes
- Identifying the cause can help with mitigation steps to reduce the impact and potentially recover the fermentation

Plan ahead for startups and shutdowns

- Schedule maintenance items that are easiest to get when the plant is shutdown
- Look for opportunities to add sample ports to improve plant troubleshooting

Startup best practices

- Monitor process waters/stillage at startup and follow recommended startup measures to minimize unnecessary yeast stress

QUESTIONS?

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