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EXPERIENCE PERFORMANCE

Analytics of New Fermentation Products Evaluation

Stephanie Gleason Ph.D – Global Director of Technology

FELC – Tuesday, October 15th, 2024 – 10:45 am

Phibro Animal Health Corporation

A global leader in animal health

Global animal health company focused on food animals – located in Teaneck, NJ

Dedicated to supporting growing worldwide demand for animal protein

Products and solutions enhance health, nutrition and productivity of animals

>80 countries to ~4,000 customers
59% U.S.
41% International

Manufacture ~70% of our animal health products

Public company NASDAQ:PAHC

Agenda

Analytics of New Fermentation Products Evaluation

- Strain Selection and Application Evaluation
- Establishing KPIs
- Trial Execution
- Evaluating Performance

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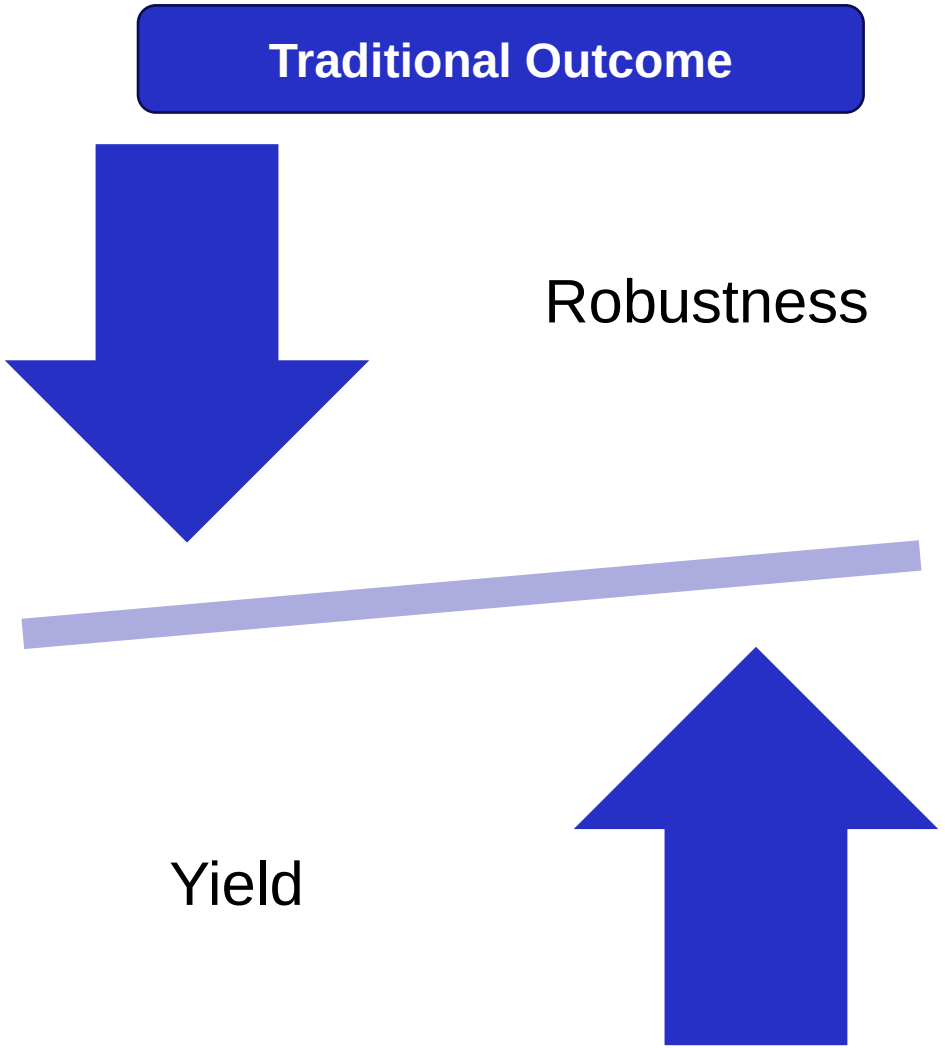
Fermentation Product Evaluation

Evaluating Options – Yeast Strain Selection

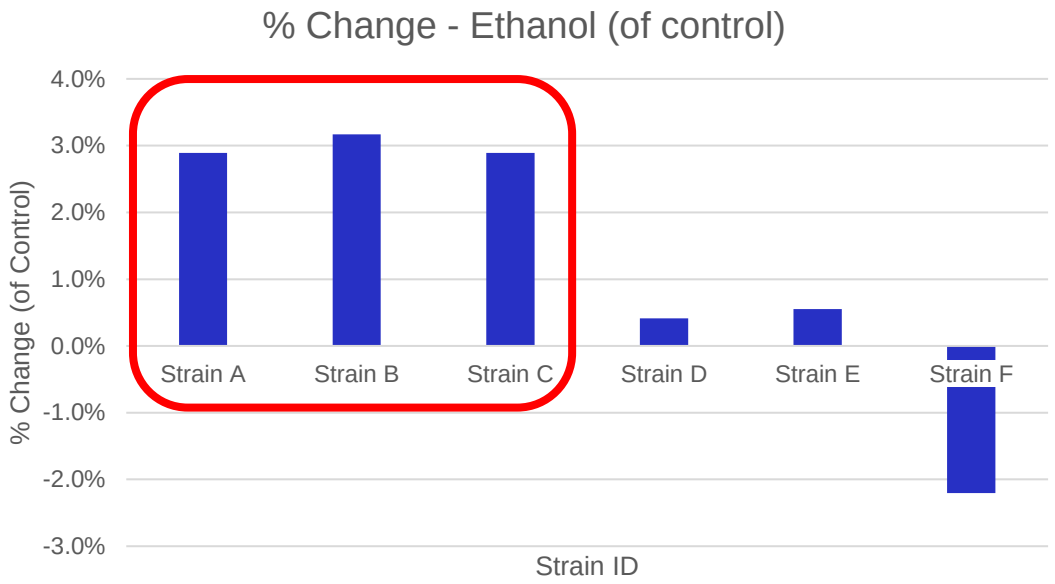
- **Traditionally, yield enhancement and pathway modification comes with trade-offs:**
  - Additional nutrient requirements
  - Reduced robustness to one or more stressor
  - Slower fermentation rates
- **Producers were often limited to picking one over the other**

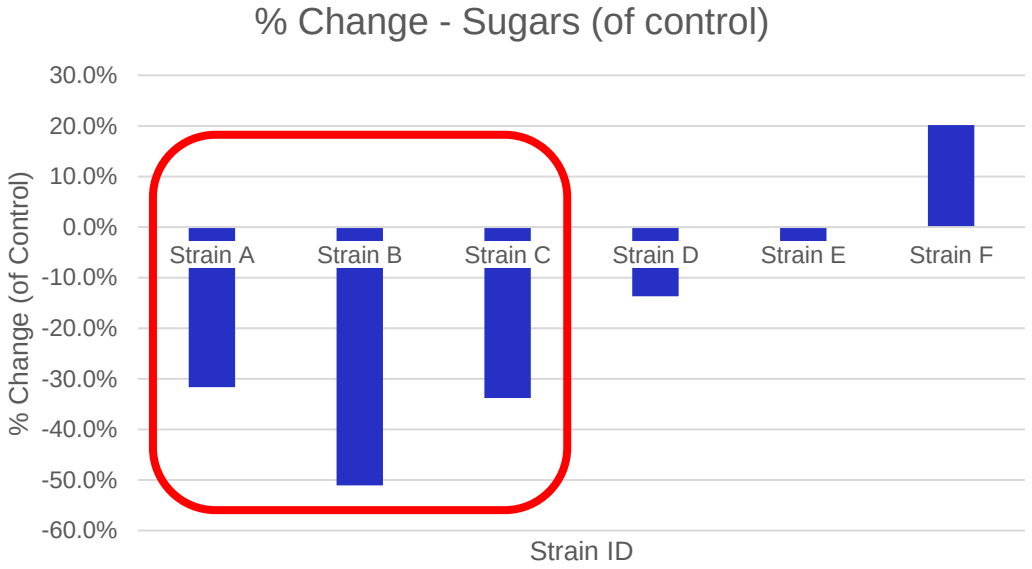


Strain Screening and Selection

Evaluating Options – Yeast Strain Selection

Yield and Performance Assessment



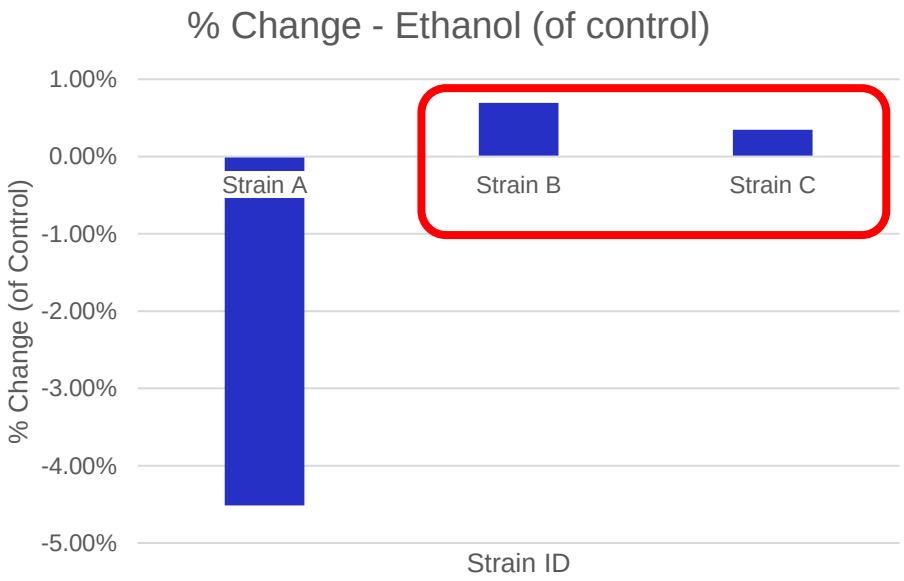


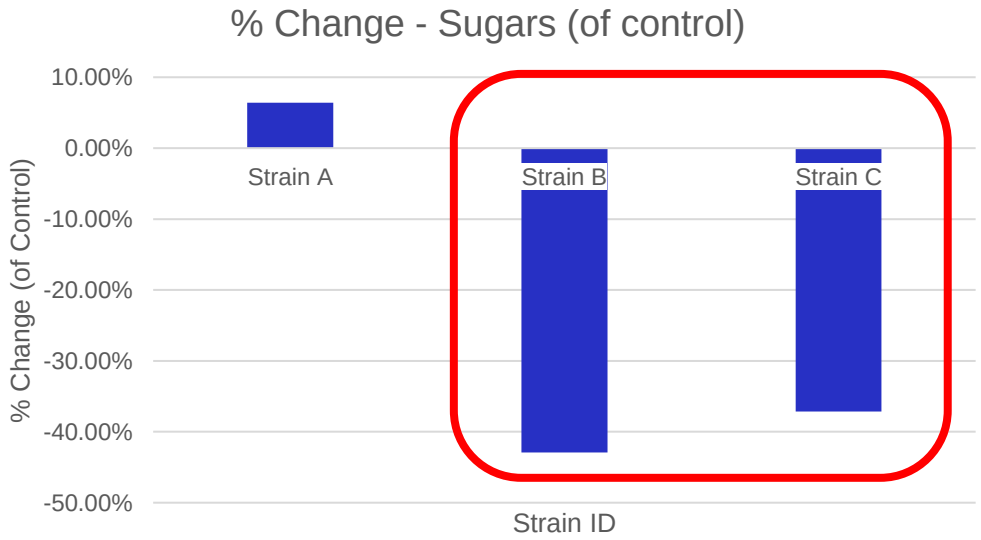
Strain engineering strategies result in multiple strains that then need to be screened/selected to narrow done options for bringing a strain to market. A robust screening program that evaluates on multiple factors/conditions is needed.

Robustness Assessment

Evaluating Options – Yeast Strain Selection

Temperature Tolerance



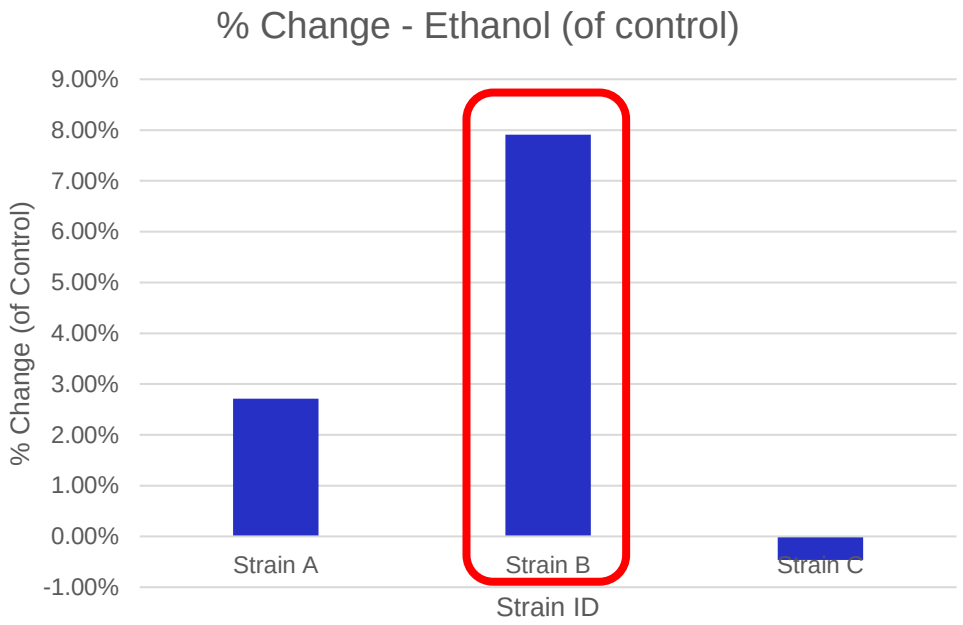


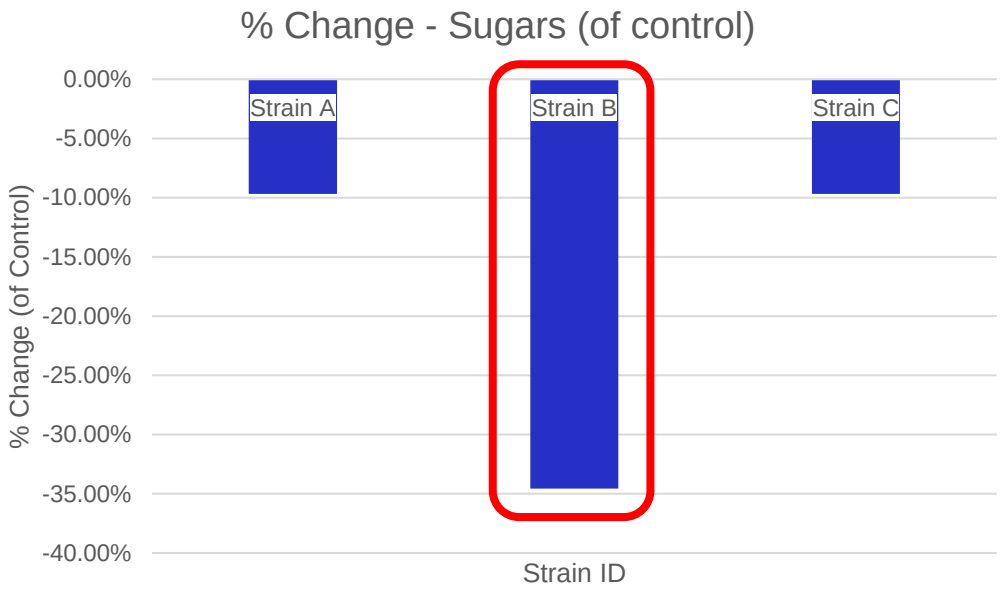
Strains were further screened for tolerance to high temperatures and contamination.

Robustness Assessment

Evaluating Options – Yeast Strain Selection

Organic Acid Tolerance



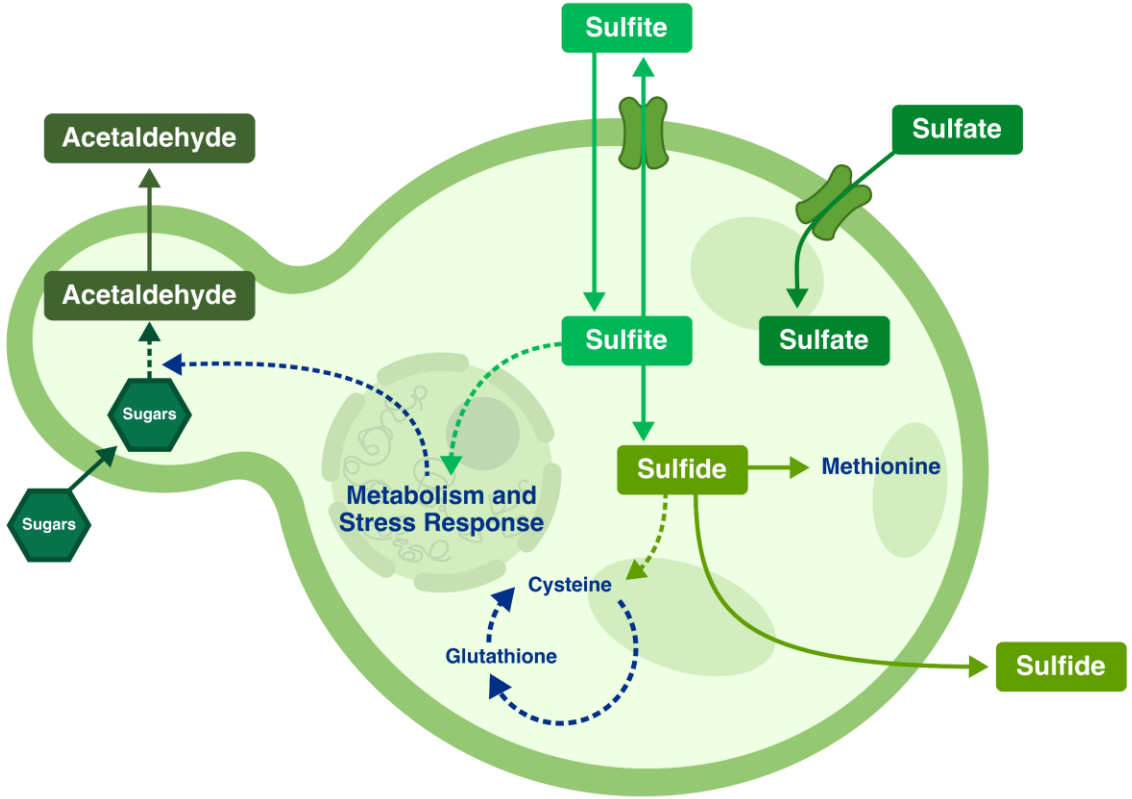


Strains were further screened for tolerance to high temperatures and contamination.

Other Considerations for Selection

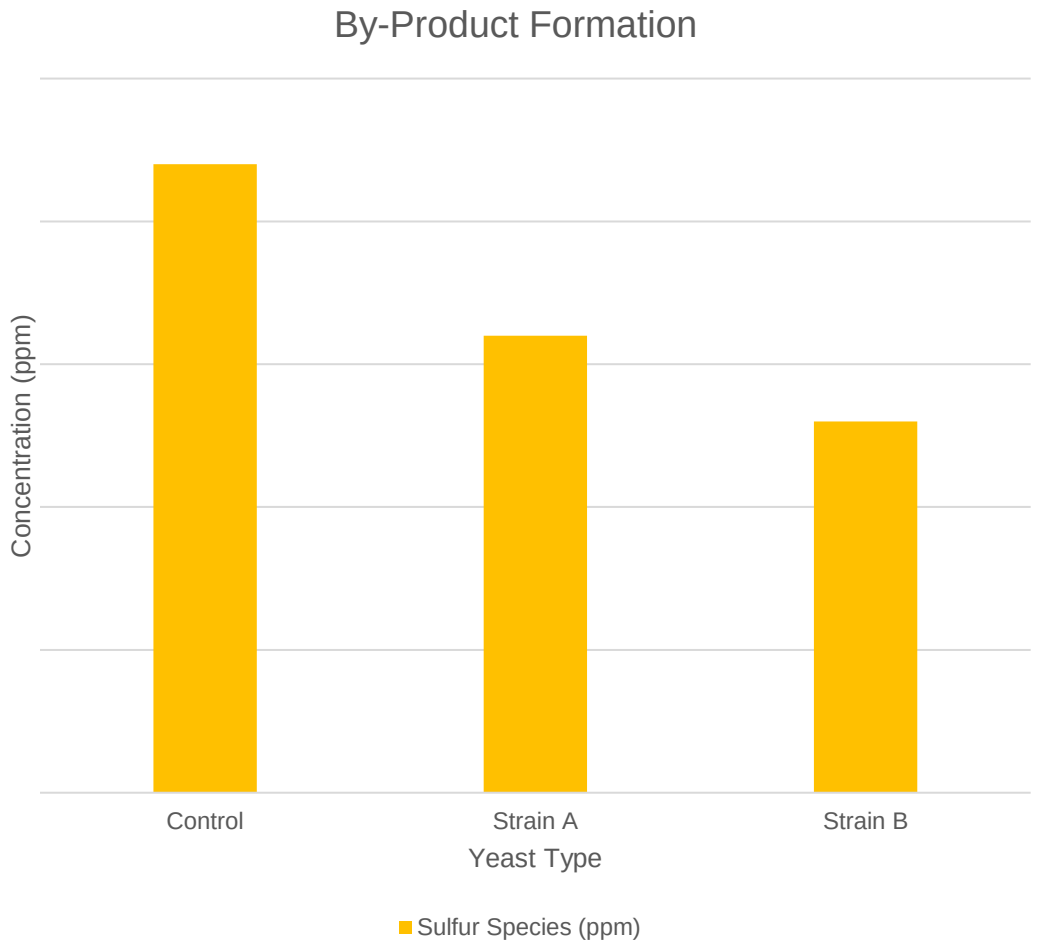
Evaluating Options – Yeast Strain Selection

- **In addition to evaluating strains for performance enhancement, an understanding of secondary characteristics is also needed**
  - Fermentation Kinetics
  - Nutritional Requirements
  - By-Product Formation
- **This factors can impact how the strain performs at scale and/or its suitability for certain applications**



Considerations for Selection – By-Product Formation

Evaluating Options – Yeast Strain Selection

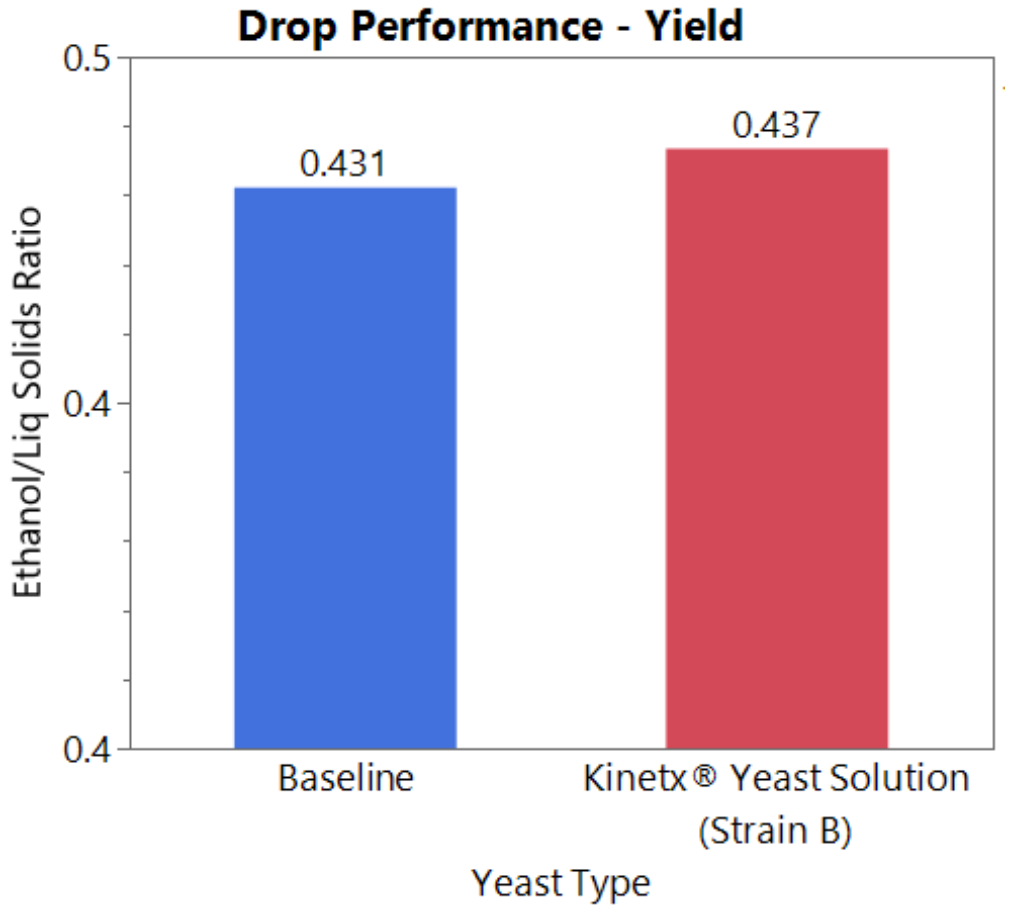


Yeast Strains	Acetaldehyde (ppm)	Acetal (ppm)	Total Fusels (ppm)
Strain A	Med	Low	Neutral
Strain B	Low	Low	Low

Applications and Evaluation

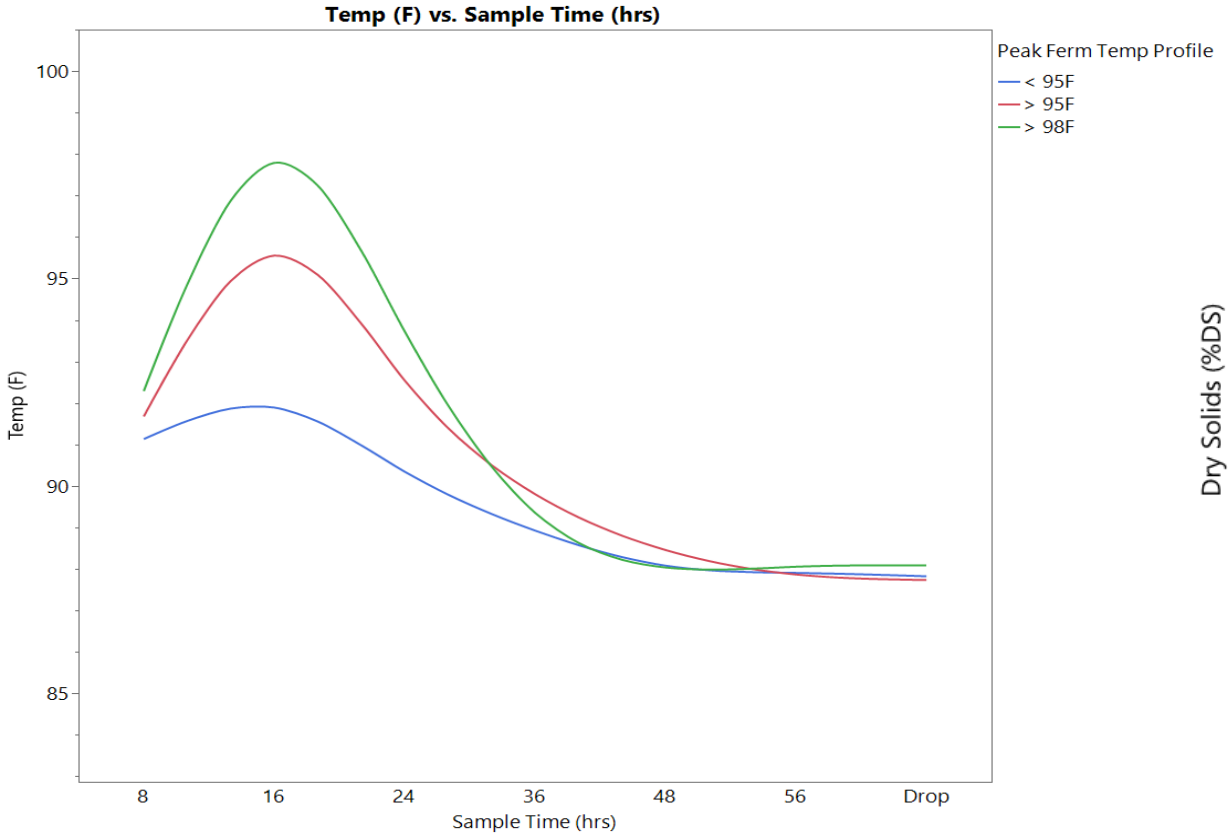
Evaluating Options – Yeast Strain Selection

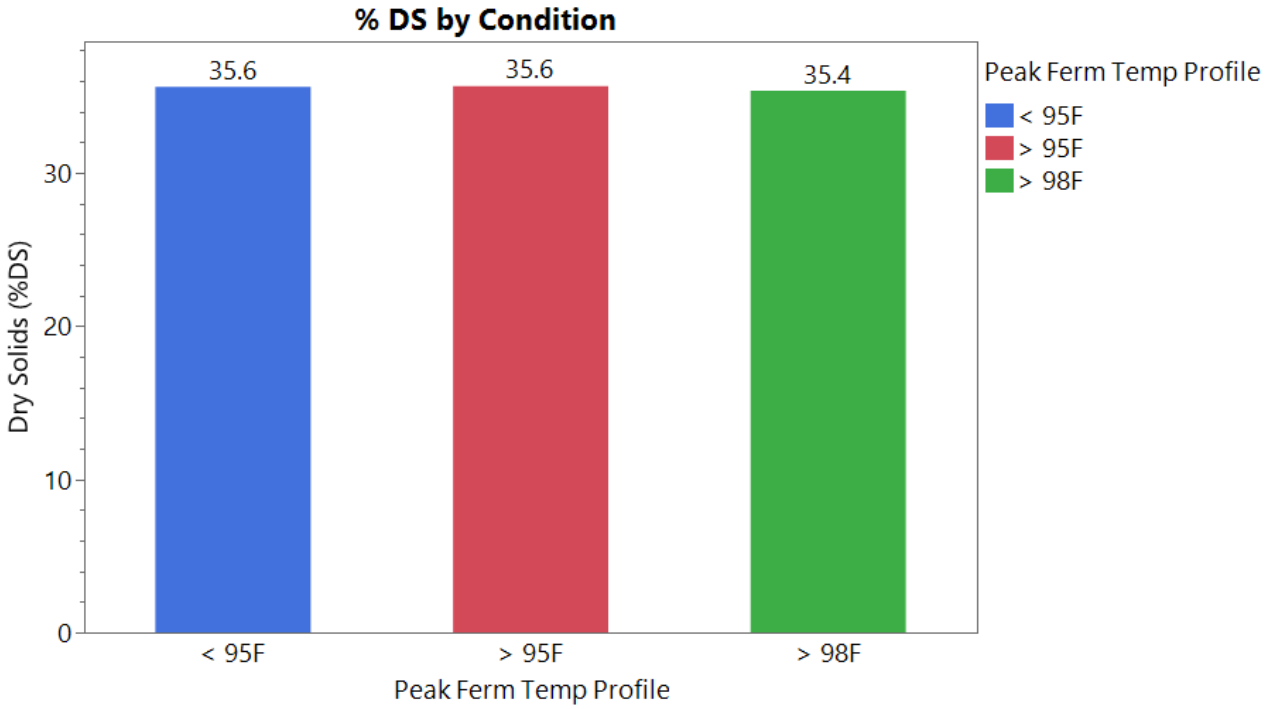
- **Once strain is selected and manufactured, testing at full scale is required**
- **Does the selected strain perform at scale?**
  - Validation that the development process produces a strain that performs at scale
  - Confirmation that the selected strain confers the desired benefits and characteristics



Applications and Evaluation – Temperature Stress

Evaluating Options – Yeast Strain Selection

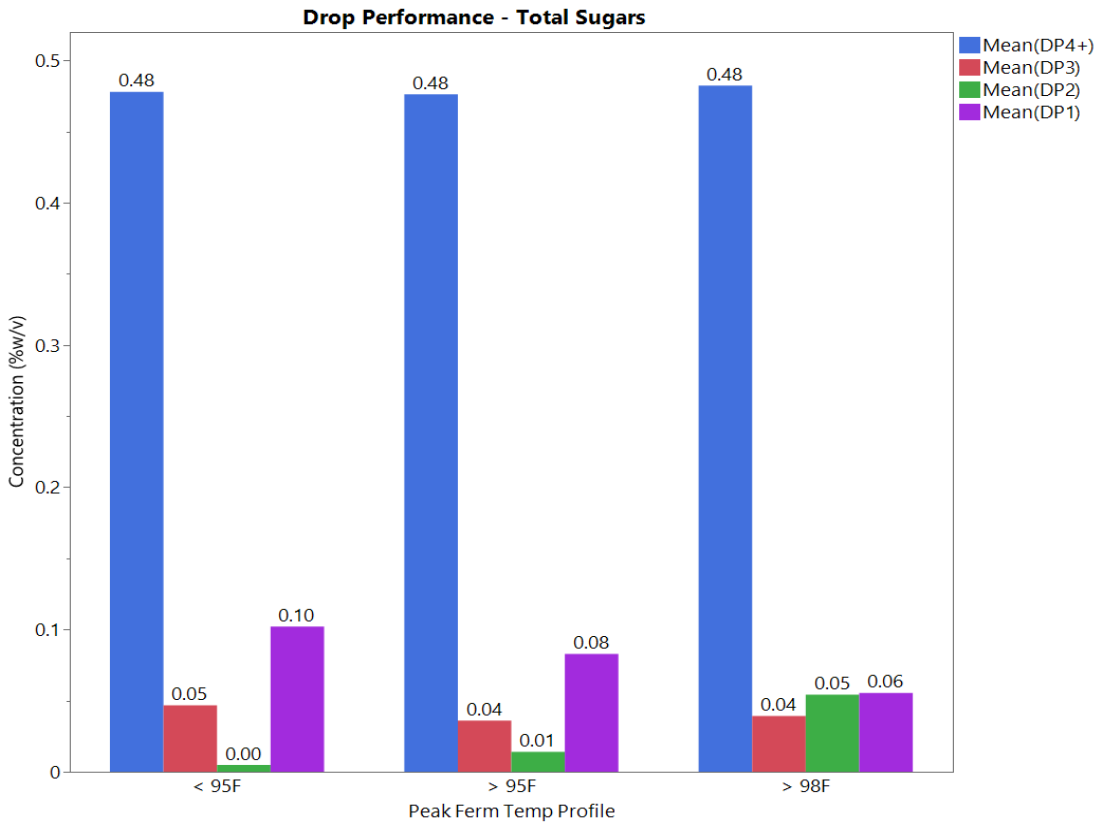


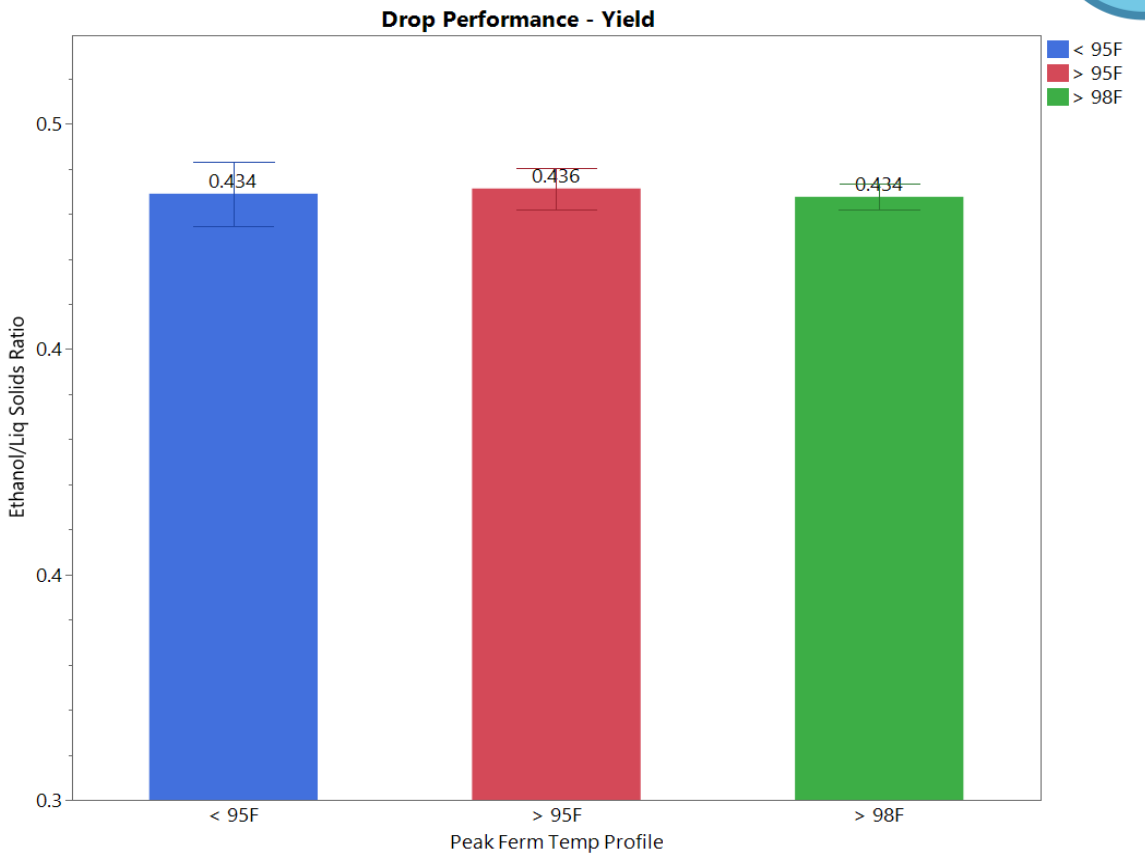


Kinetx® Yeast Solution (Strain B) experienced some high temperature excursion during evaluation at full scale.

Applications and Evaluation – Temperature Stress

Evaluating Options – Yeast Strain Selection

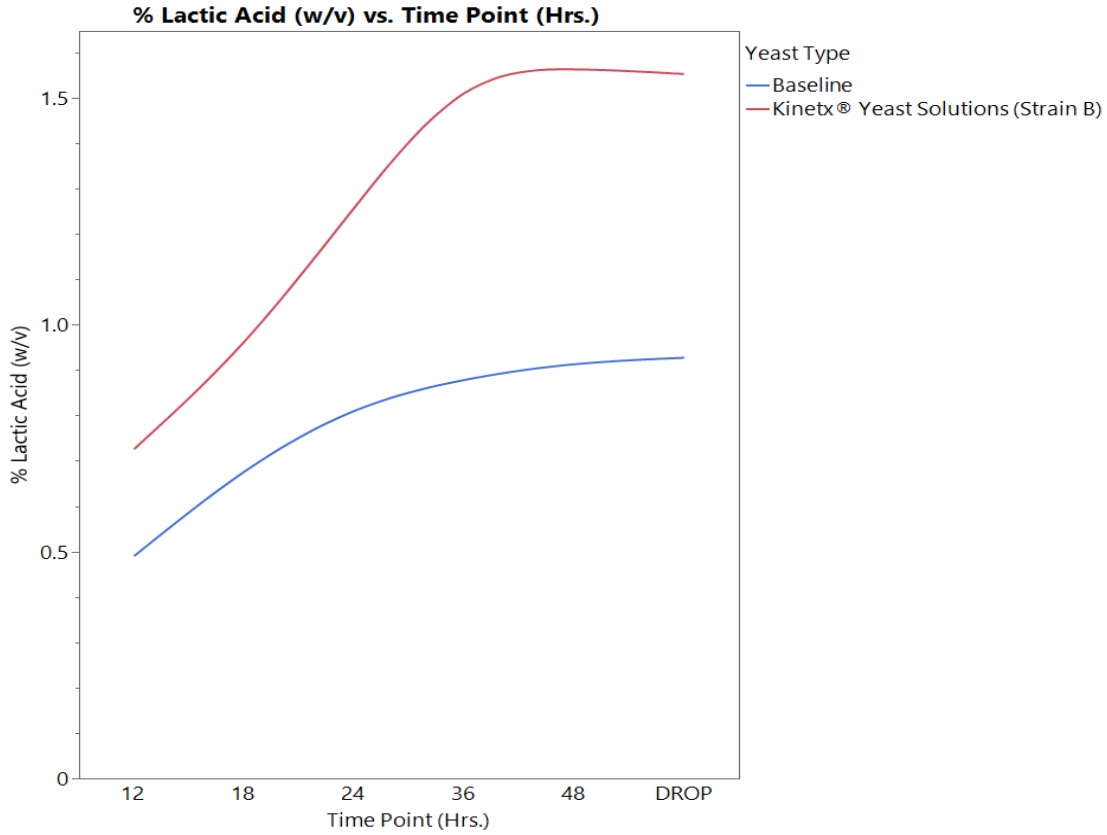


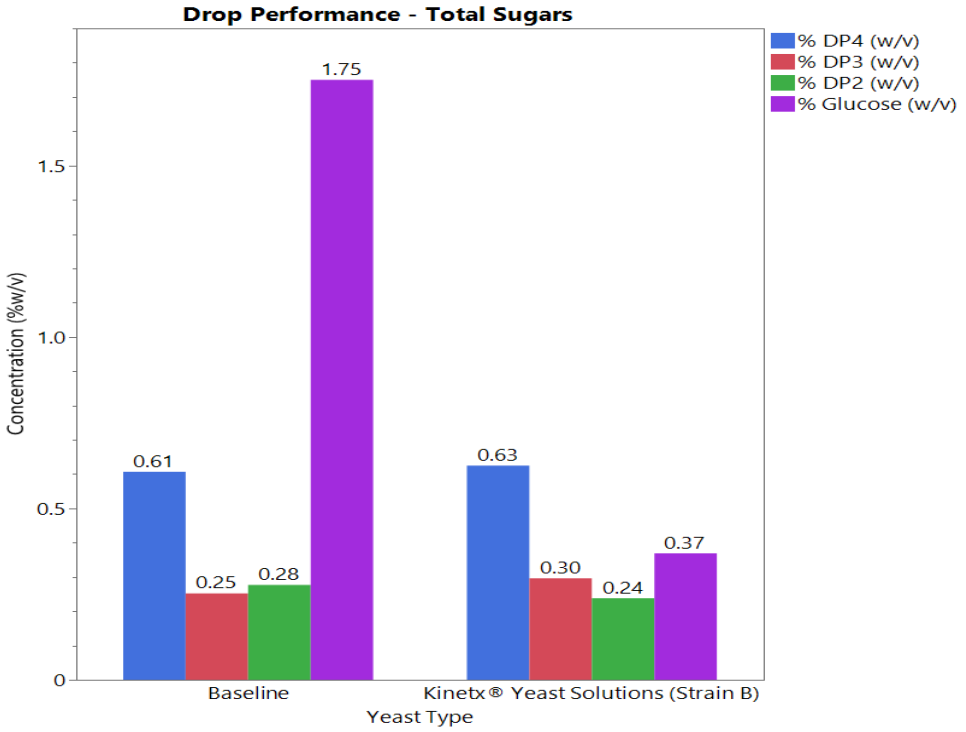


Kinetx® Yeast Solution (Strain B) experienced some high temperature excursion during evaluation at full scale.

Applications and Evaluation – Contamination

Evaluating Options – Yeast Strain Selection

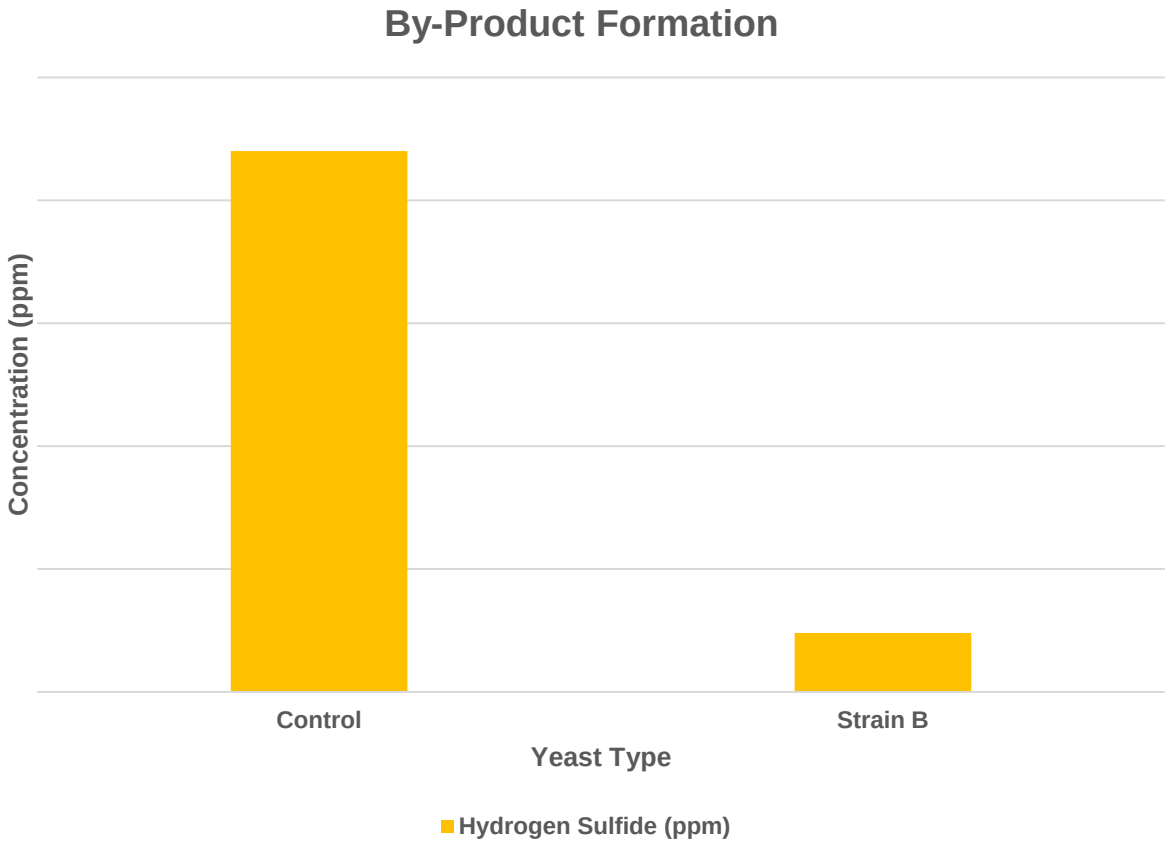


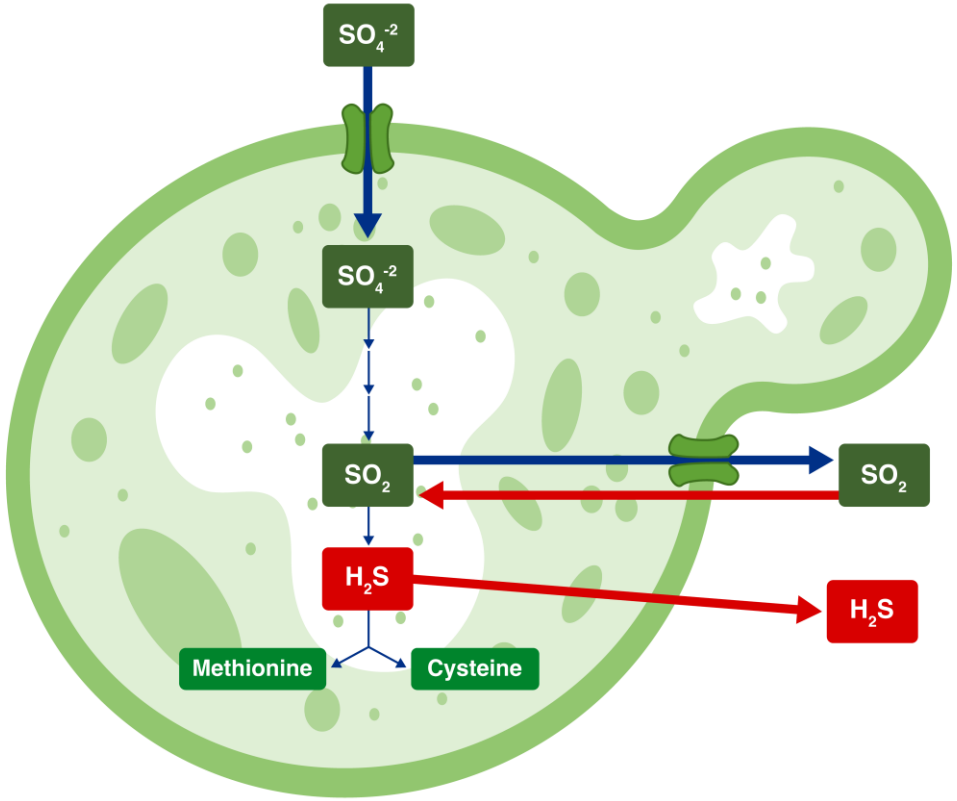


In contaminated batches (lactic >1.0 % w/v), Kinetx® Yeast Solutions (Strain B) outperformed the baseline yeast with significant glucoamylase reduction.

Applications and Evaluation – By-Product Formation

Evaluating Options – Yeast Strain Selection





Summary and Conclusions

Evaluating Options – Yeast Strain Selection

Rigorous screening, allows for selection of engineered strains that are extremely robust and have low by-product formation

Screening can also allow for the selection of strains that have yield benefits plus other performance improvements

Understanding how potential modifications may impact performance at scale is extremely

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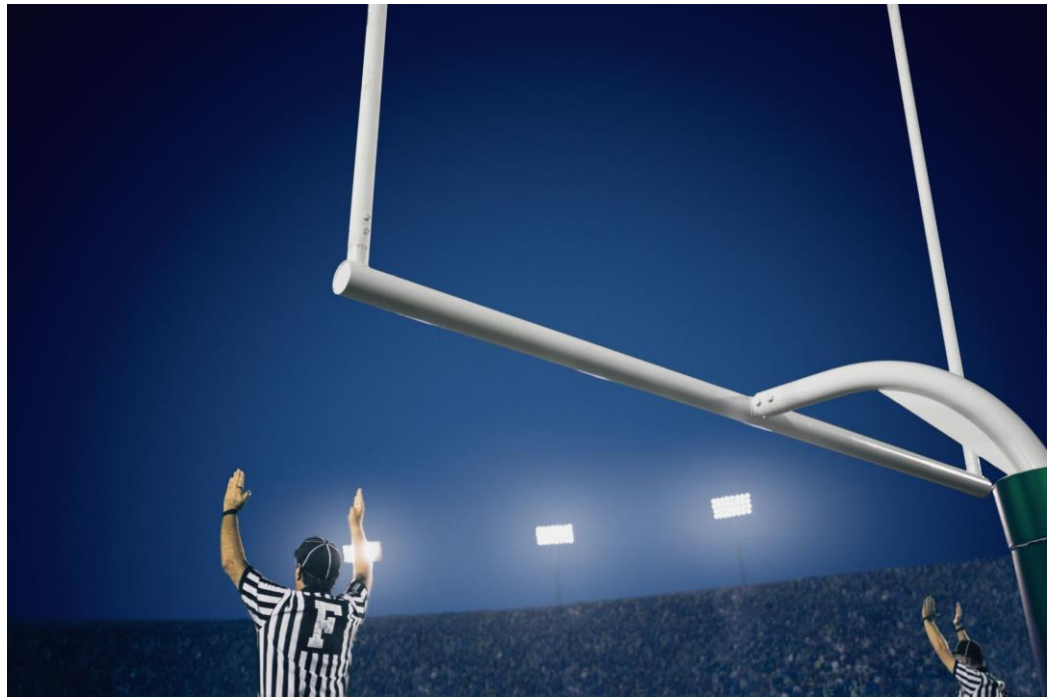
Evaluating Performance – Producer Prospective

Data and Tools for Benchmarking Strains at Scale

Preparation and Planning

Establishing Key Performance Indicators (KPIs)

- **Knowing the goals will establish the KPIs**



- **Yeast trial goals may include:**

Ethanol yield

Robustness

Fermentation efficiency

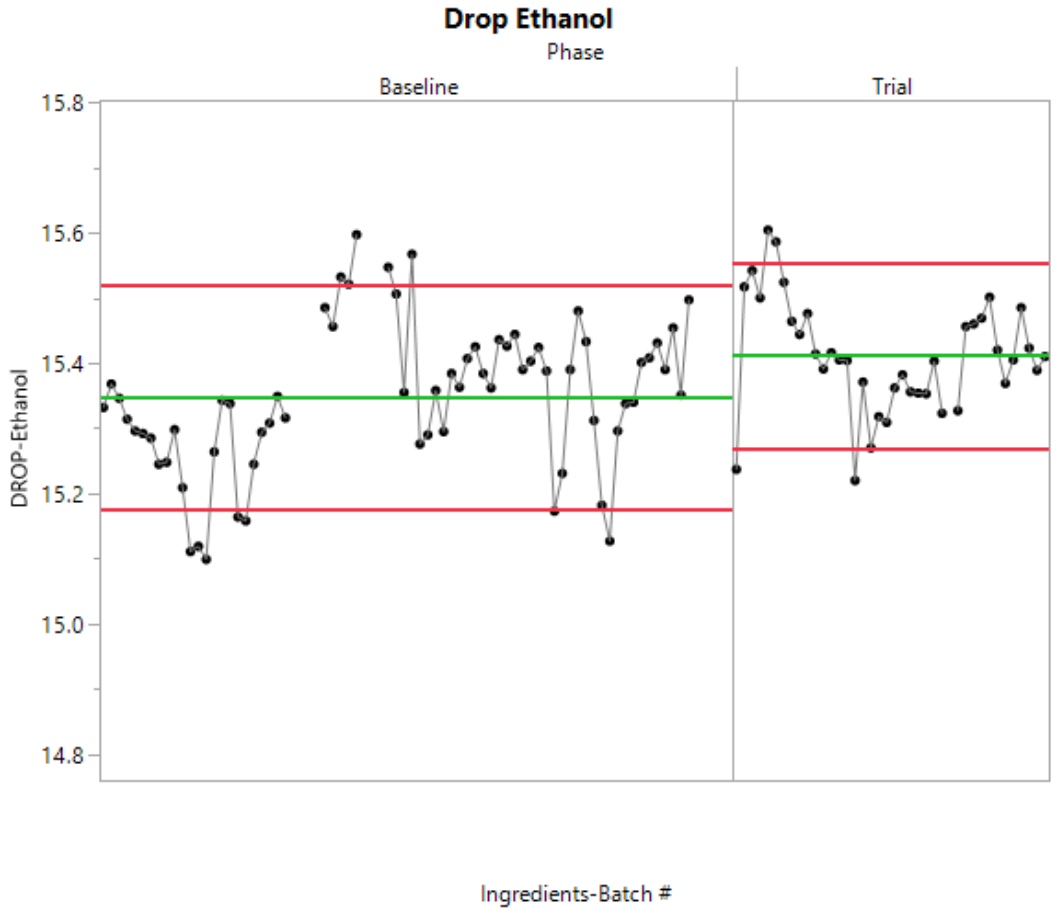
Cost

Reduced by-product formation

Preparation and Planning

Selecting a Baseline

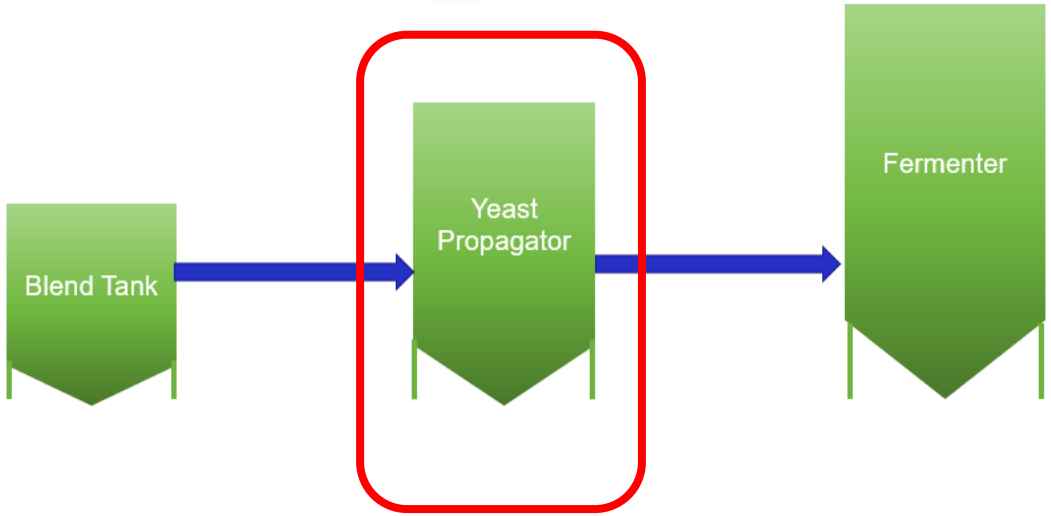
- **What is the “baseline”?**
- **Factors to consider when establishing the baseline**
  - Number of batches
    - Similar samples size
  - Significant recipe or operational changes
  - Process changes/upsets
    - Time since last shutdown or process upset
  - Capital commissioning and additions
- **Does it represent current “normal” operations?**
- **Is it really what your plant wants to measure performance against?**



Preparation and Planning

KPI Examples by Unit of Operation

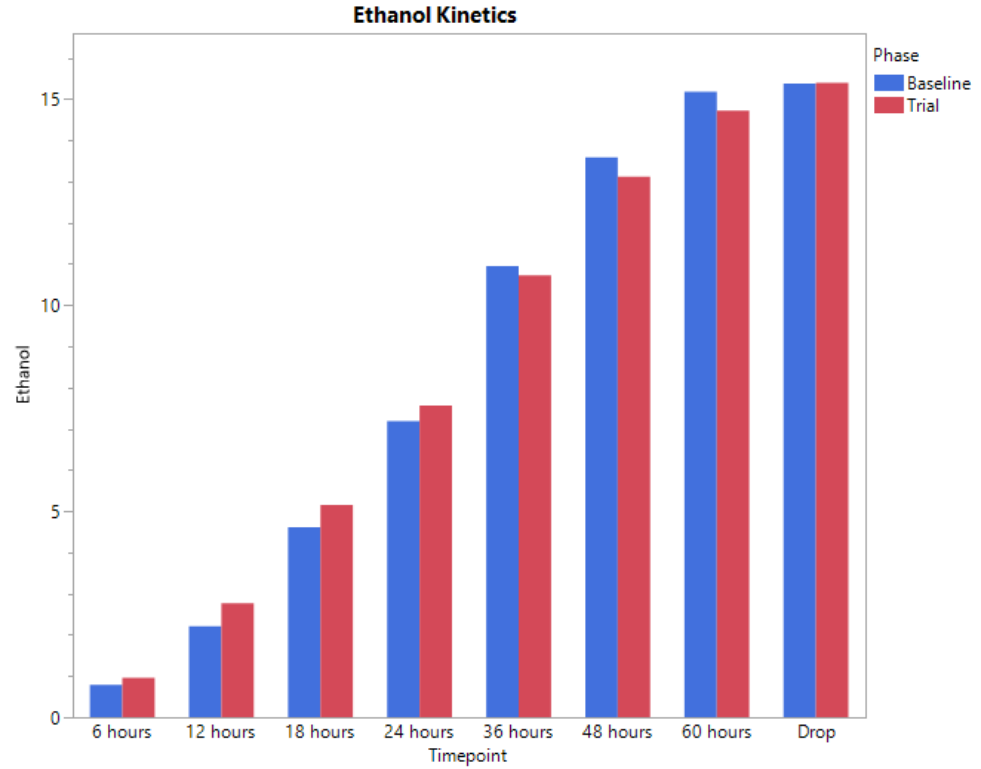
Propagation	
GOAL	**KPIs**
Increased biomass	Cell counts, %budding
Improved yeast robustness	%Viability
Faster prop time	Prop profiling
Lower cost	Input reductions

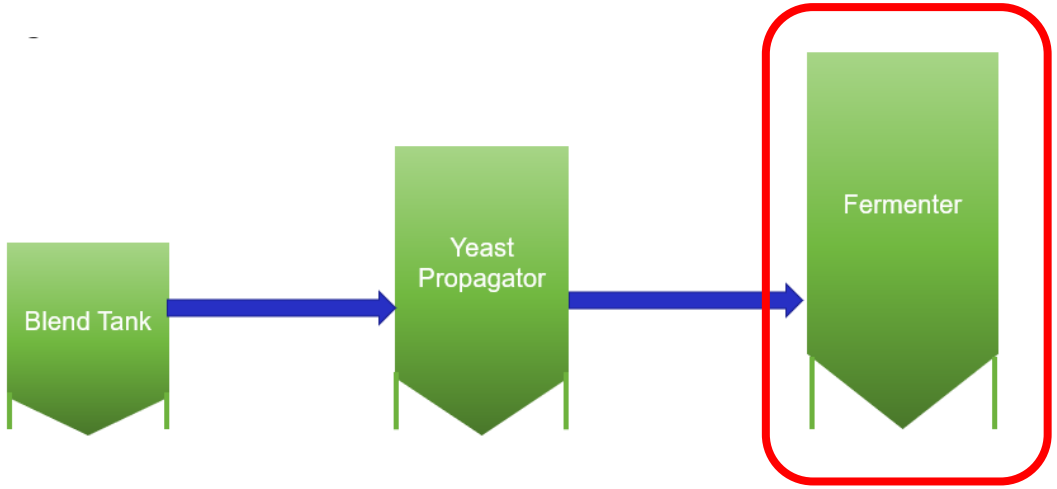


Preparation and Planning

KPI Examples by Unit of Operation

Fermentation	
GOAL	**KPIs**
Improve fermentation kinetics	HPLC profiles
Increase ethanol yield	Ethanol/liq solids
Increase ferm efficiency, less by-product generation	Ethanol/glycerol ratio Ferm efficiency Delta glycerol
Improve yeast health, robustness	Cell counts, %Budding, %Viability
Lower input costs	Input reductions (enzyme, nitrogen, yeast)

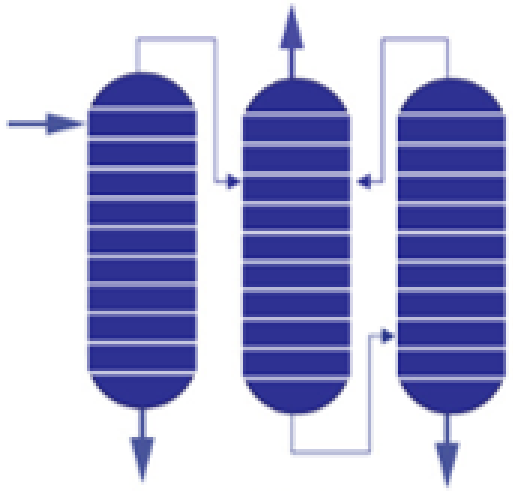




Preparation and Planning

KPI Examples by Unit of Operation

Distillation/Co-products	
GOAL	**KPIs**
Increase ethanol production	Daily gallons
Improve production yield	Daily gallons, corn grind
Reduce by-products	Acetaldehyde, Hydrogen Sulfide
Improve DDGS feed composition	Protein content Amino acid composition

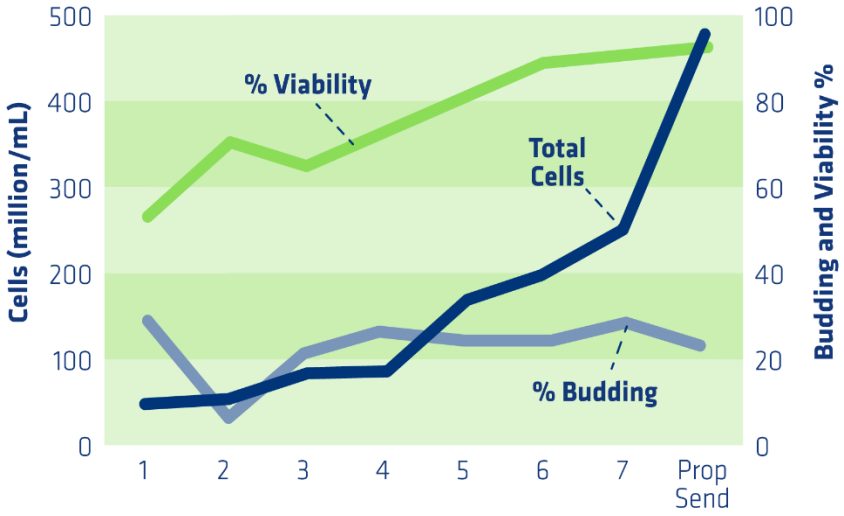


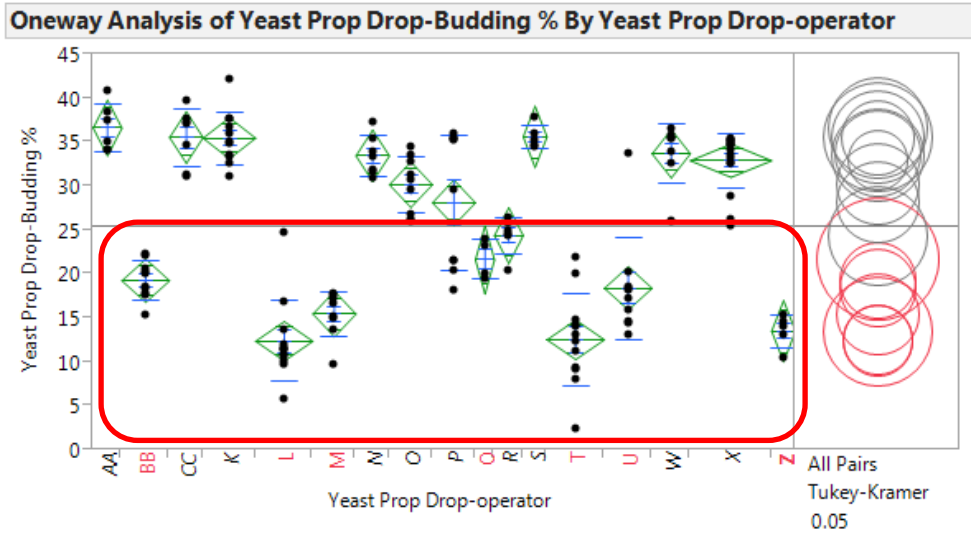
Trial Execution

Propagation

- **Keep those KPIs in mind!**
- **Prop profiling recommended**
  - Cell count considerations
    - Variability?
  - Baseline for comparison
- **Operator training and audit opportunity**
  - Rehydration steps
  - Timing of additions

Yeast Count: Total Cells Profile

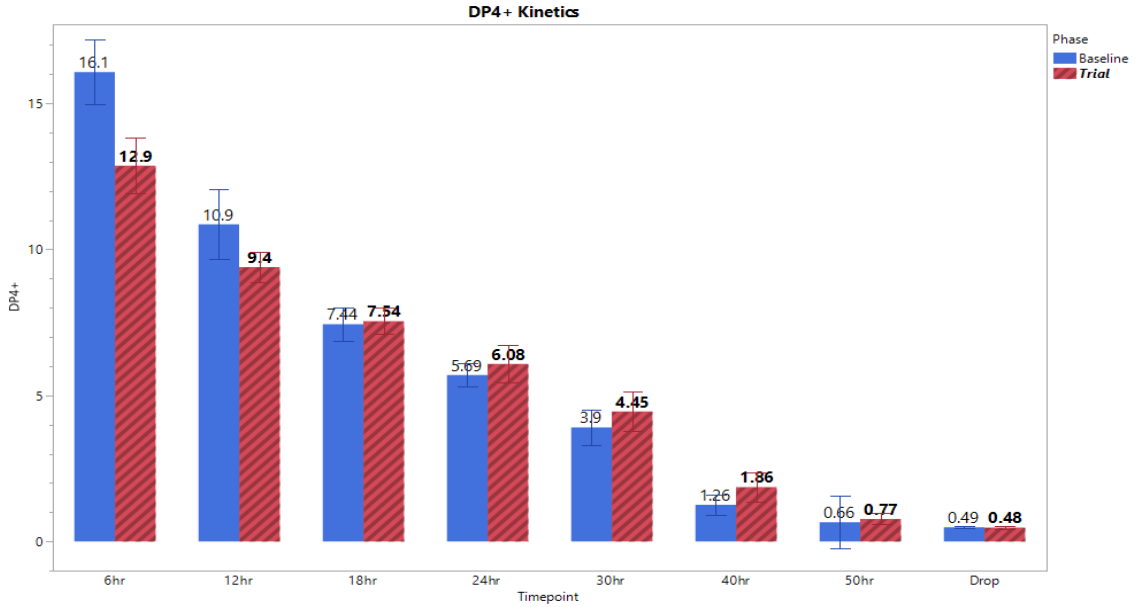


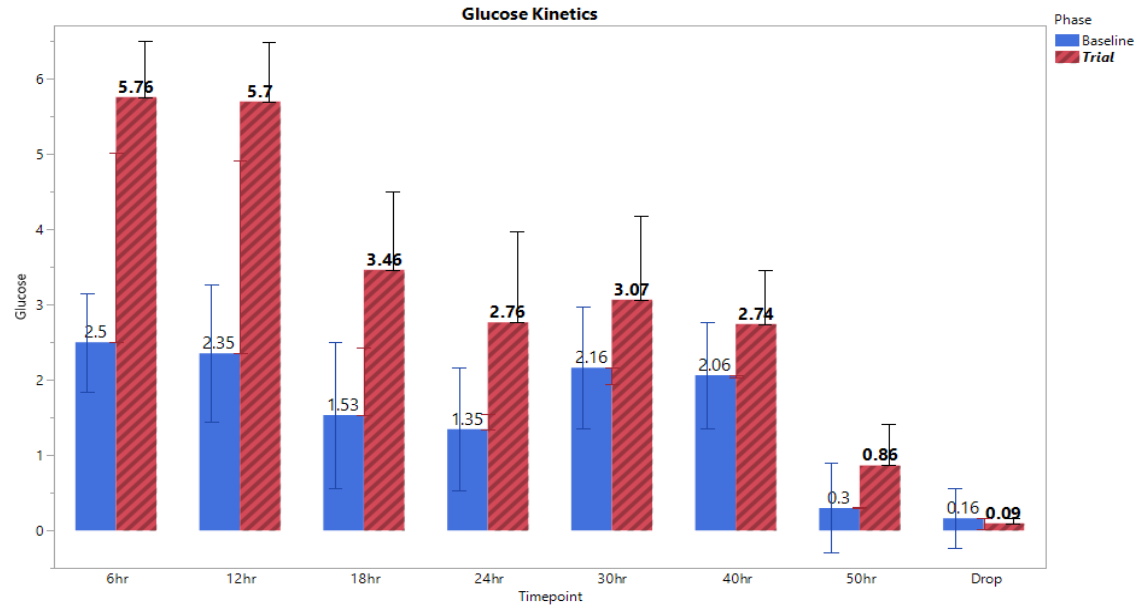


Trial Execution

Fermentation

- **Closely monitor fermentation kinetics**
  - Enzyme considerations
    - Dosing scheme
    - Side activities
- **Consider extra sample timepoints**
  - Early fermentation
    - Cell counts?
  - Late fermentation
- **Save samples for further analysis**
  - FAN, residual starch, etc.





Trial Execution

Distillation and Co-Products

- **Track daily production gallons against trial conditions**
  - Considerations: beer feed rate, base loss
  - A way to verify HPLC results
- **Yield monitoring**
  - Determine duration
    - Daily, weekly, monthly?

Evaluation of Performance

Back to the KPIs

- **Good preparation makes this part easy!**
- **Go back to the trial KPIs and the agreed upon baseline**
  - How do the results compare?
  - Are they statistically significant?

Evaluation of Performance

Available Statistical Tools

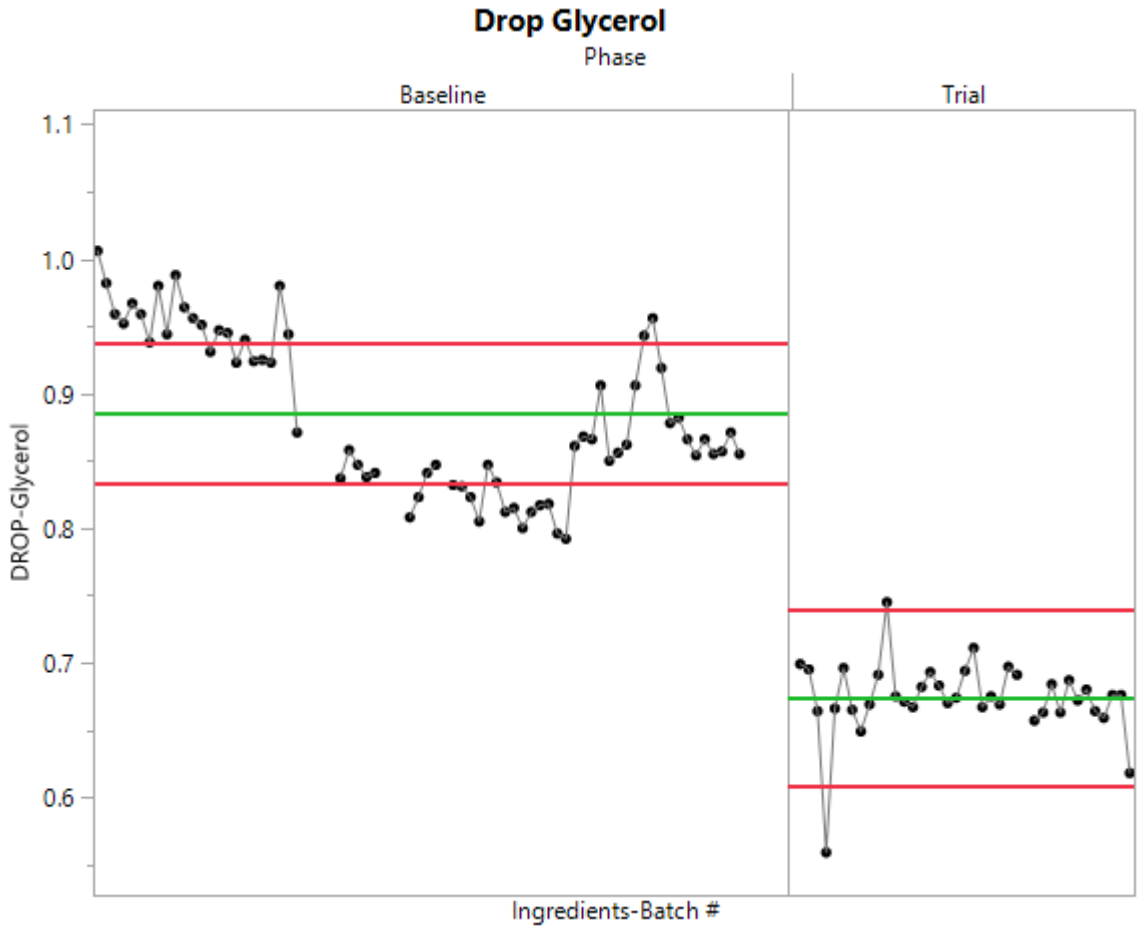
- Linear Trends
- Bar Graphs ★
- Control Charting ★
- Means Comparison Testing ★
- Regression Analysis
- Multivariate Analysis

Evaluation of Performance

Commonly Used Graphs

Control charts are most commonly used for:

- Tracking the difference in performance after a recent process change
- Comparing different products against a specific metric
- Identifying phase shifts in the data

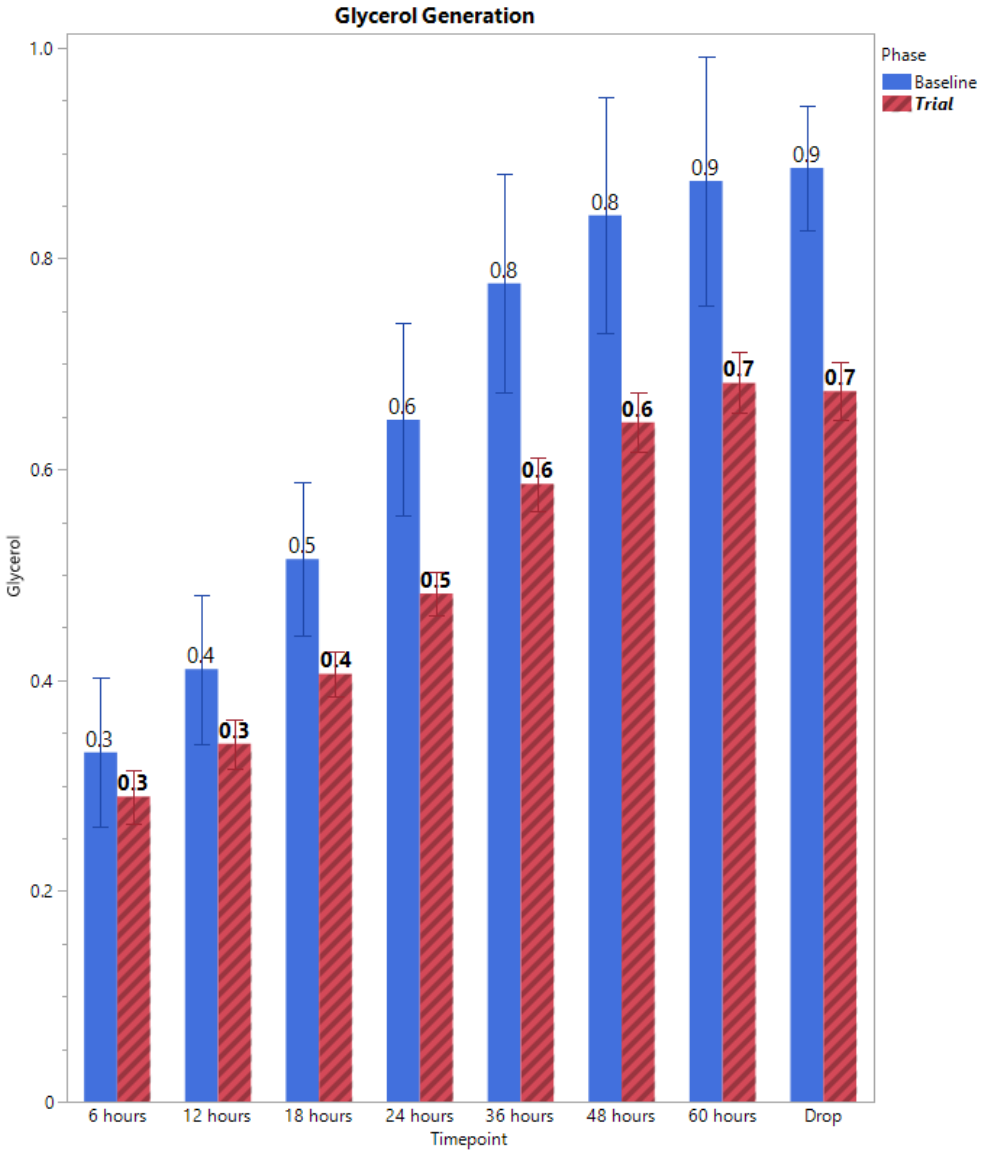


Evaluation of Performance

Commonly Used Graphs

Linear graphs/bar charts are most commonly used for:

- Fermentation kinetics
  - When monitoring several parameters or several sample times in batch order
- An initial screening of parameters that may or may not correlate with each other

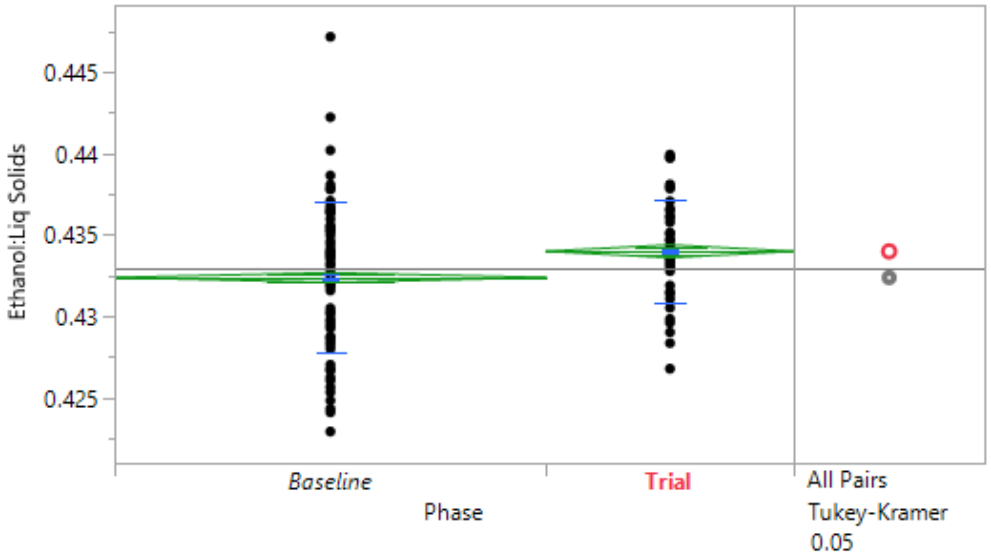


Evaluation of Performance

Commonly Used Graphs

Analysis of Variance (ANOVA) visualizations are most commonly used for:

- Looking for differences within the same parameter by trial condition, fermenter, mash train, etc



Connecting Letters Report

Level			Mean
Trial	A		0.434
Baseline		B	0.432

Levels not connected by same letter are significantly different.

Means comparisons are most commonly used for:

- When wanting to test for statistical significance
- Primarily used for operator, product, and change analysis stats testing

Summary and Conclusions

Summary

Numerous yeast options are available to producers and through a targeted approach they can evaluate them with confidence

Establishing a baseline for comparison and the key performance indicators give producers and suppliers alignment on selection criteria and goals

Use all available data, internal and external, to evaluate your KPIs and the overall performance of the solution

Through a robust understanding of how these strains can be compared, producers can make informed decisions regarding yeast selection for their facility.

Thanks

Contributors & References

- Anne Chronic
- Lindsay Kuhn
- Phibro Ethanol Laboratory
- Phibro Technical Services

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