



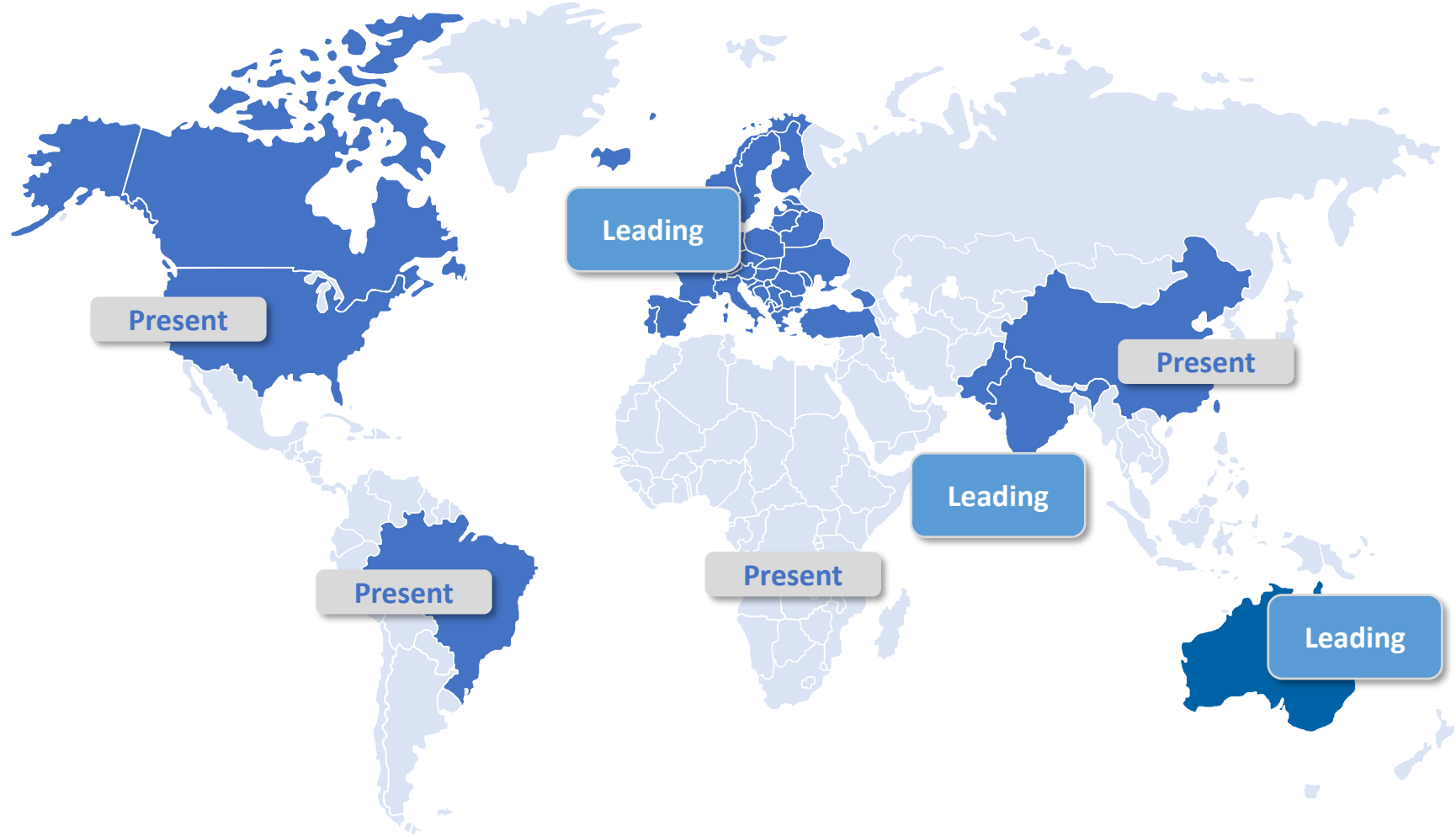
Role of Fermentation Yeast in Efficiency & Yield

Grain Ethanol Manufacturers Association

AB Biotek

19th July 2025

ABB Present in All Major Markets



Manufacturing and R&D Footprint



Spring 2026 our new plant in Pilibhit, Uttar Pradesh will start



ABB Yeast Formats

- **Dry:** efficient transport and storage, long shelf life
- **Cream:** easy handling, high viability
- **Compressed:** for distilled
- **Crumbled:** for distilled

Local yeast production gives many advantages like optimal pricing, easier logistics



What ethanol plants want from yeast

Titer, Rate, Yield

- Titer (final ethanol concentration)
 - High Ethanol Resistance
 - High-gravity fermentation yeast >15 %w/v
 - Increases volumetric productivity
- Rate (how fast yeast produces ethanol)
 - High fermentation rate
 - Increases
- Yield (how much ethanol for consumed sugar)
 - Low glycerol and other by-product production
 - Low residual sugar

Robustness

- Organic Acid Tolerance
 - Ability to ferment at high lactic/acetic acids at low pH
- Temperature Tolerance
 - Ability to ferment at high temperature
 - Temperature excursions in ethanol plants can stall fermentation and kill yeast
- Osmotolerance
 - Low glycerol production in response to high sugar/solids conditions



Agenda

- **Part 1: Typical Grain-to-Ethanol Process**
- **Part 2: Yeast Fermentation Basics**
- **Part 3: Yeast Deployment in Ethanol Plants**

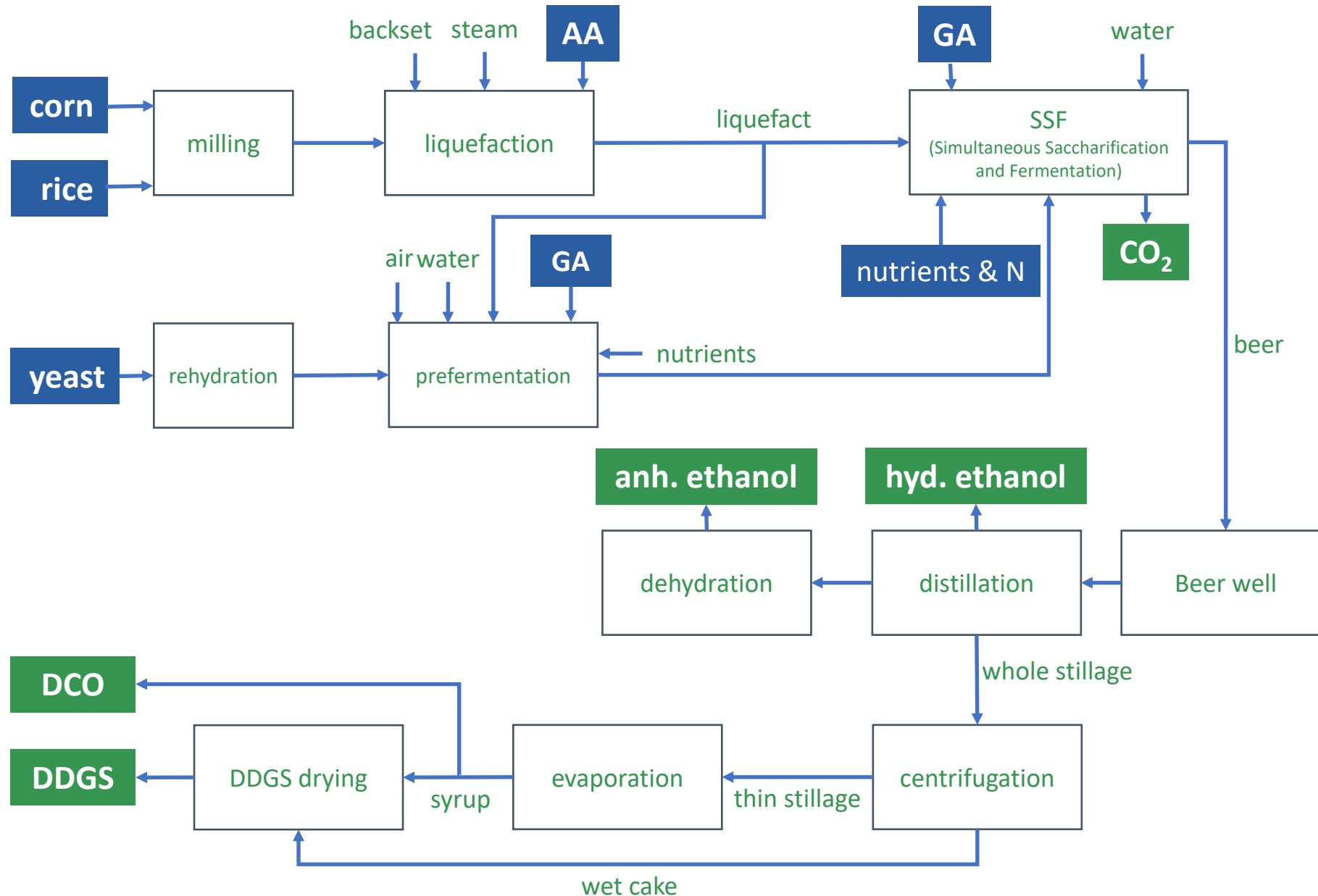


Part 1: Typical Grain-to-Ethanol Process

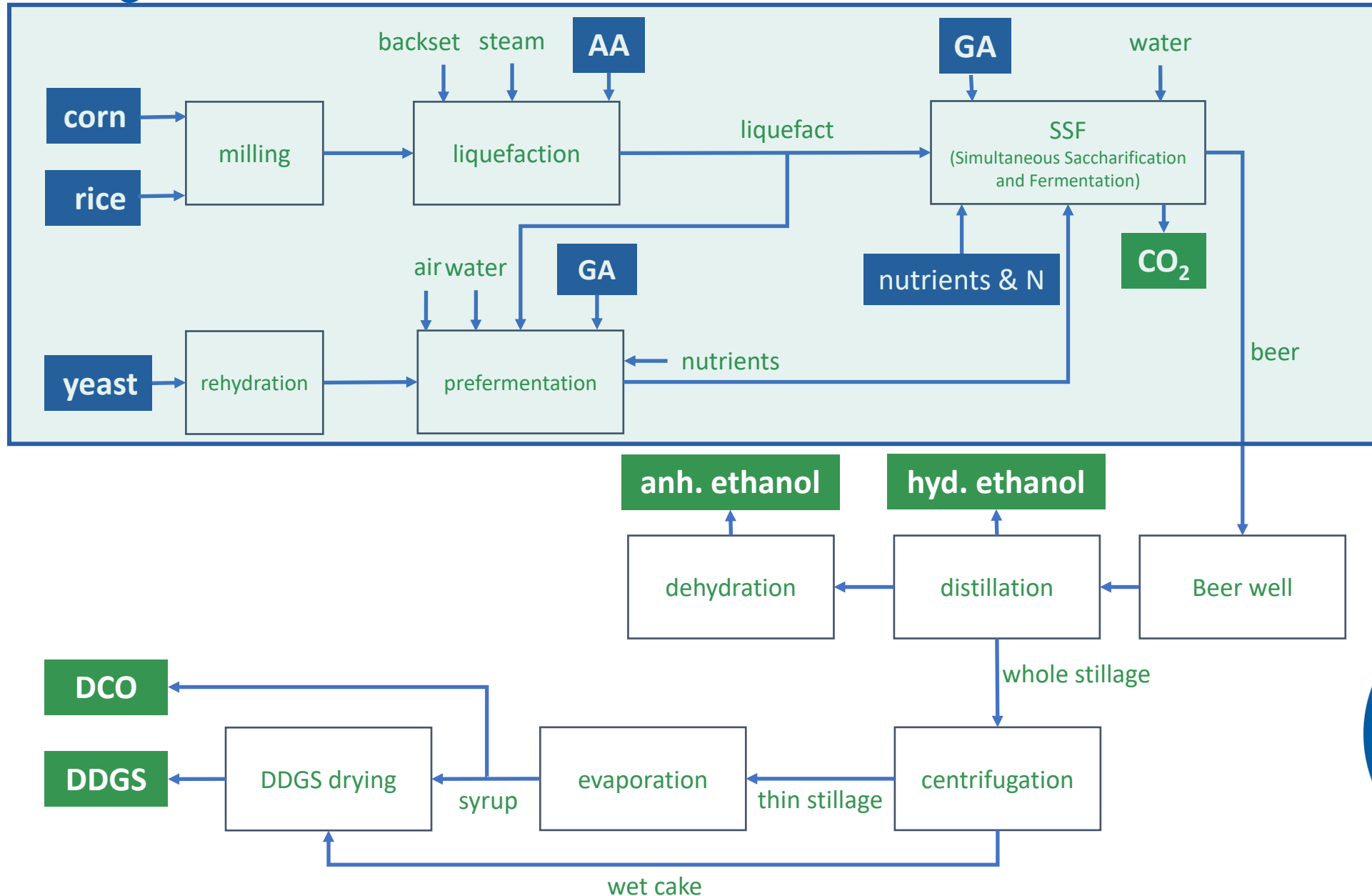
- Dry Grind Process Flow
- Starch Conversion to Glucose
- Simultaneous Saccharification and Fermentation (SSF)
- Problem Areas in Fermentation



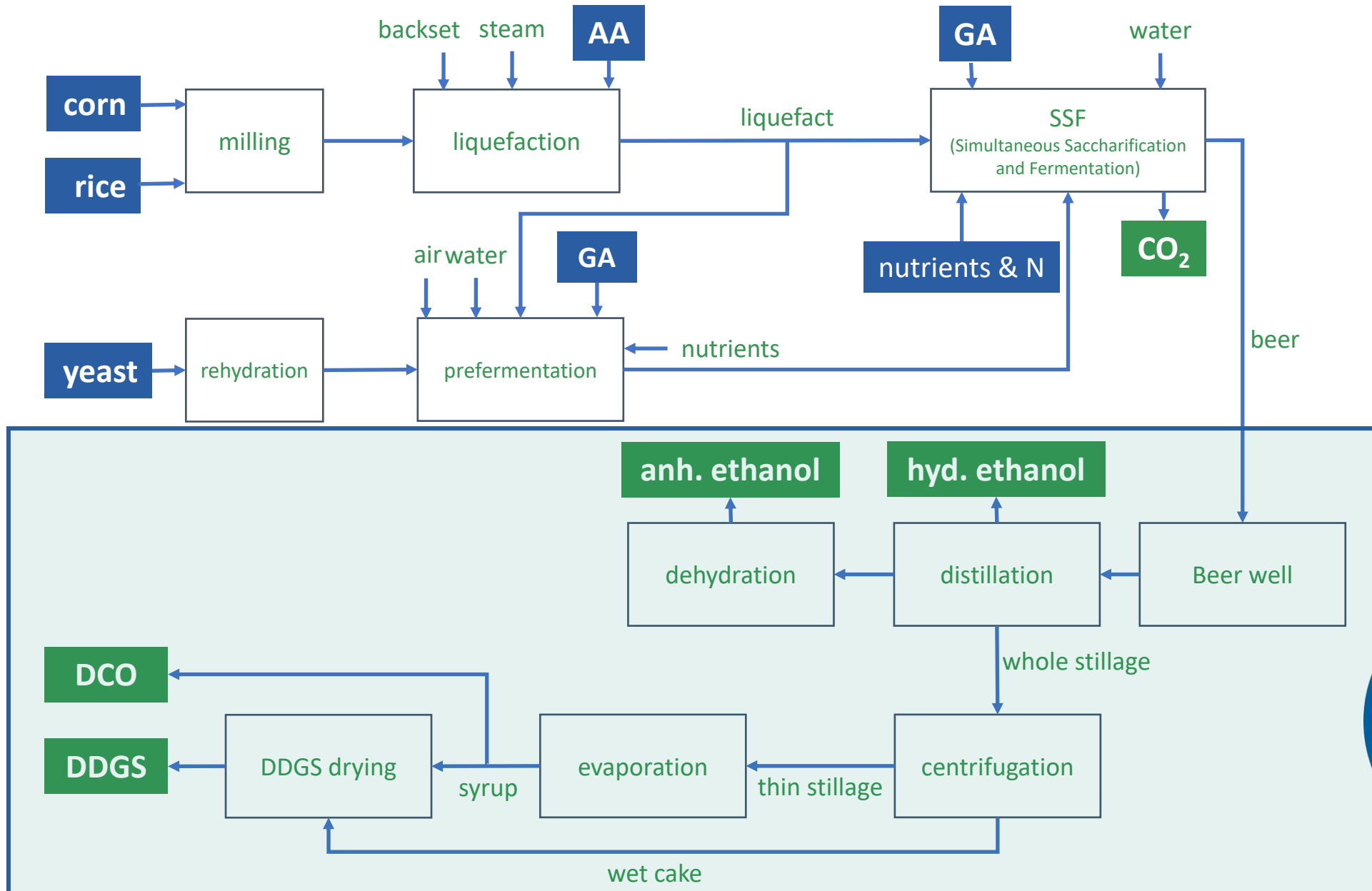
Dry Grind Process Flow – Corn ethanol



Dry Grind Process Flow – Corn ethanol



Dry Grind Process Flow – Corn ethanol



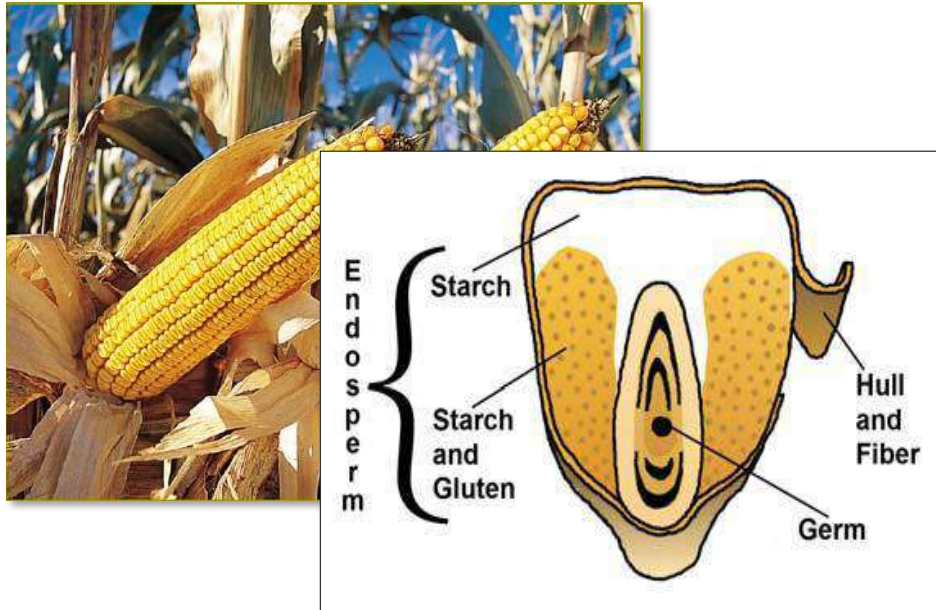
Starch Conversion to Glucose

- All grains have complex structure



Starch Conversion to Glucose

- All grains have complex structure



Corn kernel components

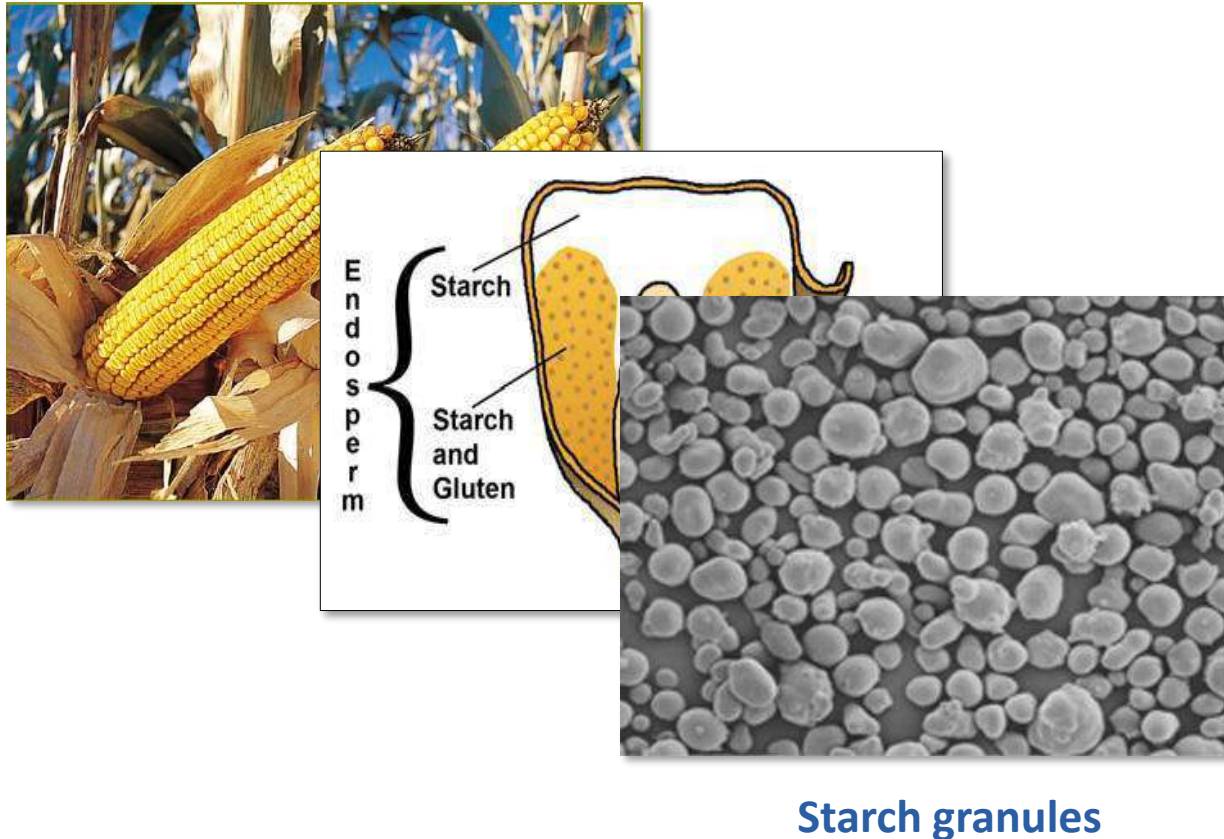
Corn grain

- 11 – 13 % moisture
- 87 - 89% DM
 - **69 - 72 % starch**
 - 8 - 9 % protein
 - 3 - 5 % oil
 - 2.5 % fiber
 - 1.5 % ash



Starch Conversion to Glucose

- All grains have complex structure



Starch granules

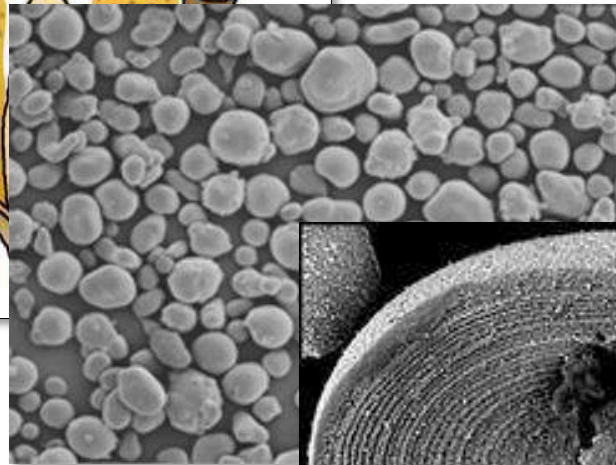
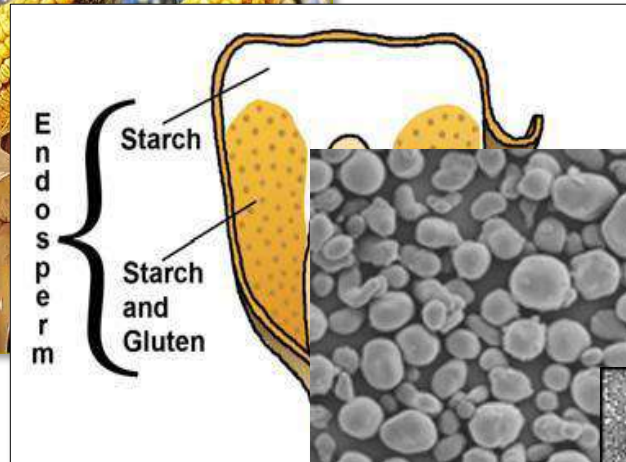
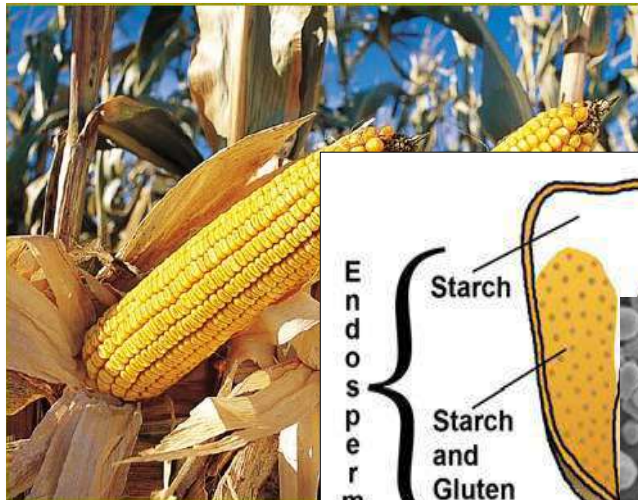
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Starch Conversion to Glucose

- All grains have complex structure



Corn grain

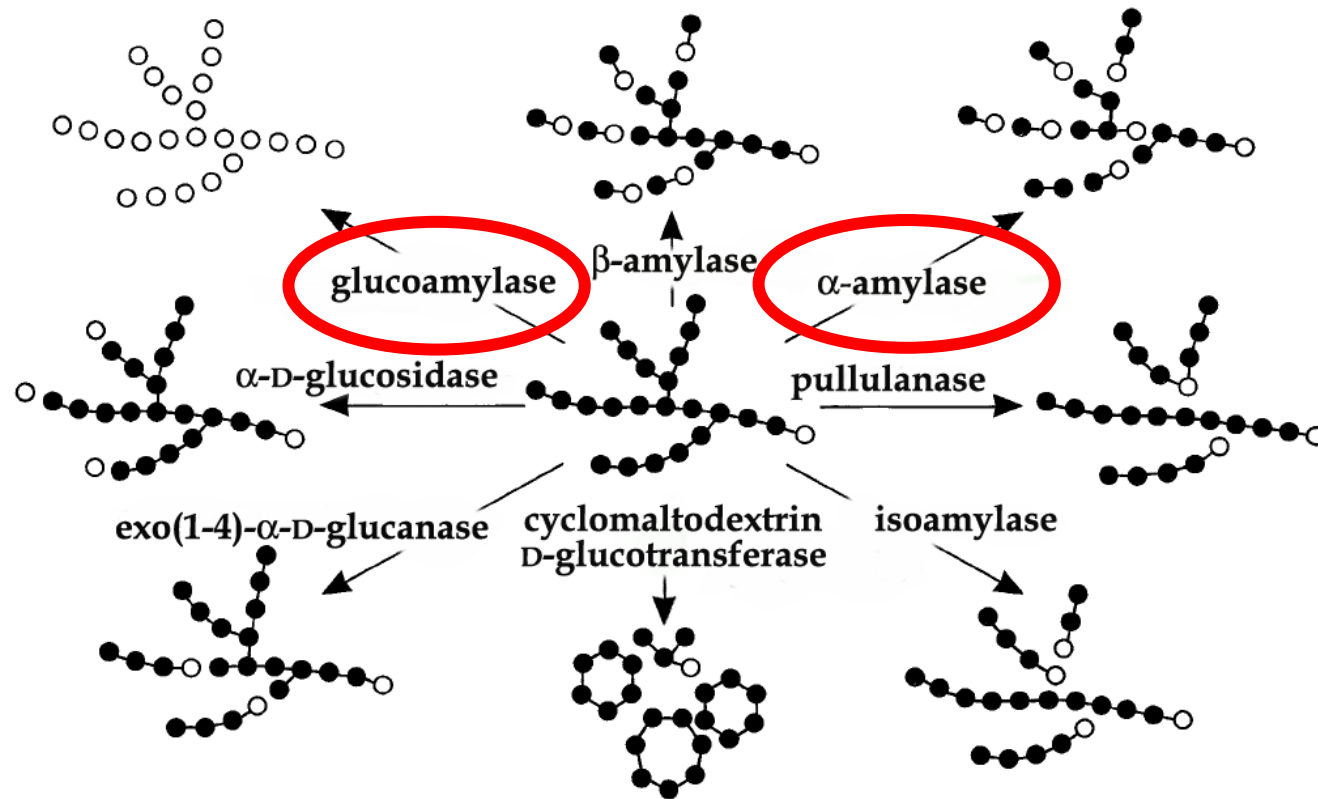
- 11 – 13 % moisture
- 87 - 89% DM
 - **69 - 72 % starch**
 - 8 - 9 % protein
 - 3 - 5 % oil
 - 2.5 % fiber
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Starch granule
cross section
showing growth
rings

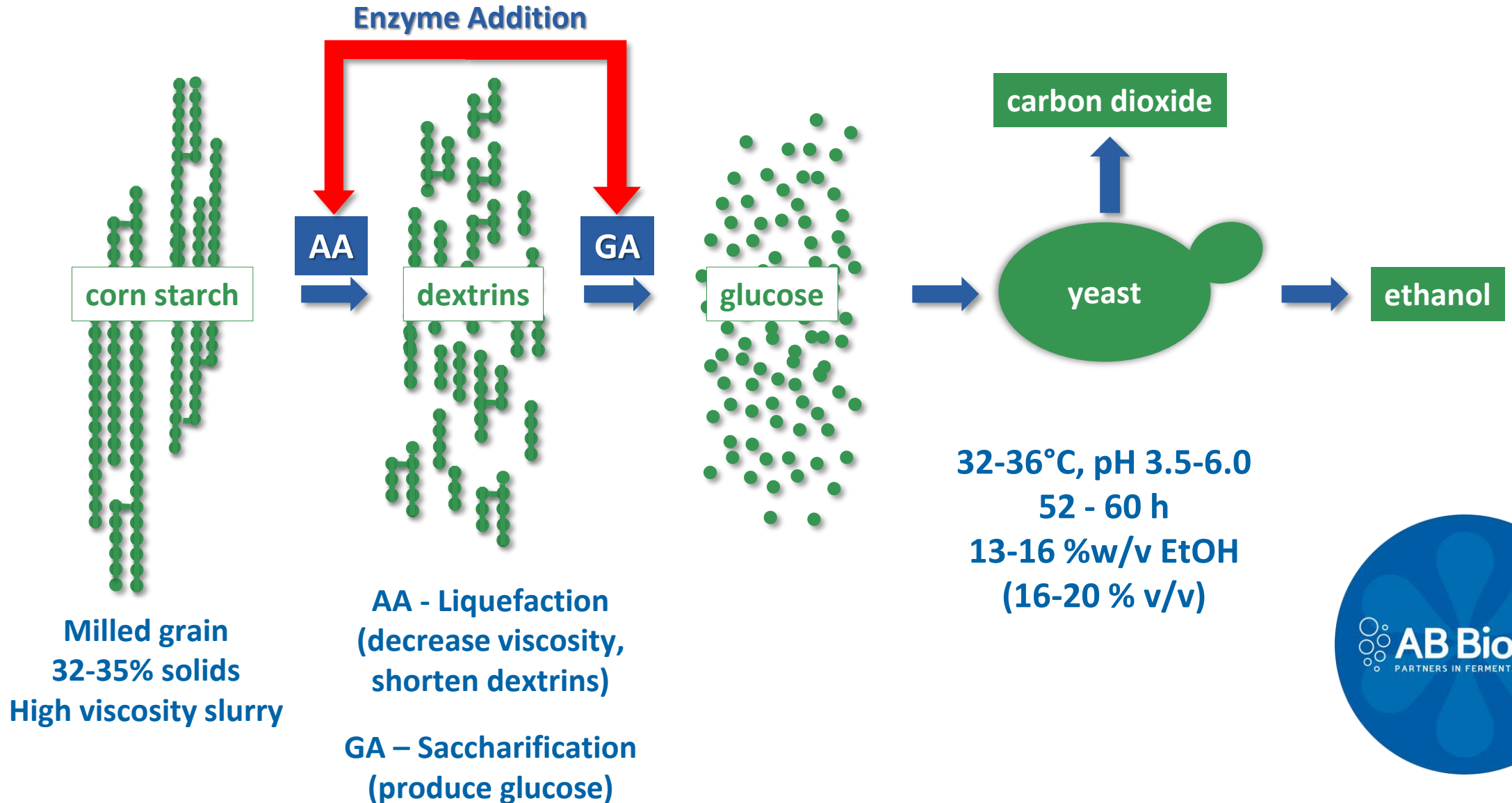


Starch Conversion

- Different enzymes convert starch to short chains or glucose
- Important enzymes are α -amylase and glucoamylase

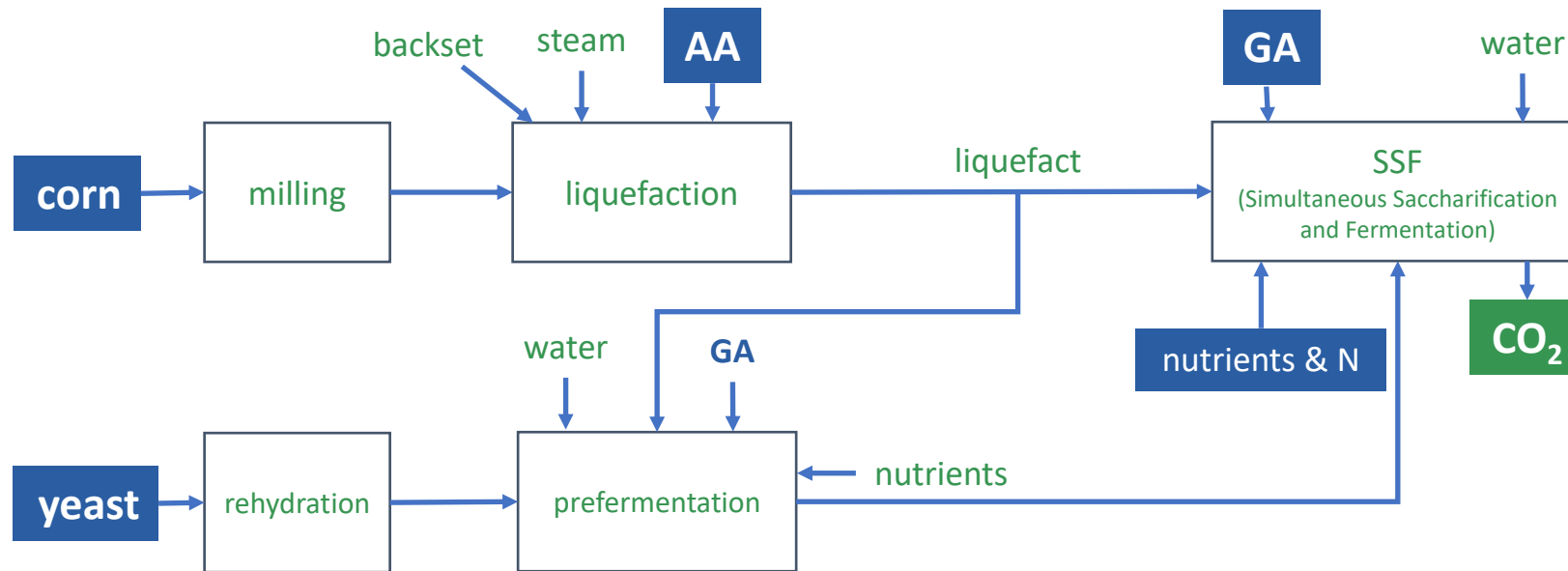


Starch Liquefaction & Hydrolysis



Upstream Problem Areas

- All upstream process areas are prone to process upset



Upstream Problem Areas

- Yeast handling and preparation

Problems

Yeast storage

- Heat damage
- Air and moisture in yeast

Rehydration

- Bacterial contamination
- Incorrect yeast dosing

Prefermentation

- Bacterial contamination
- Poor yeast growth/doubling

Solutions

Yeast storage

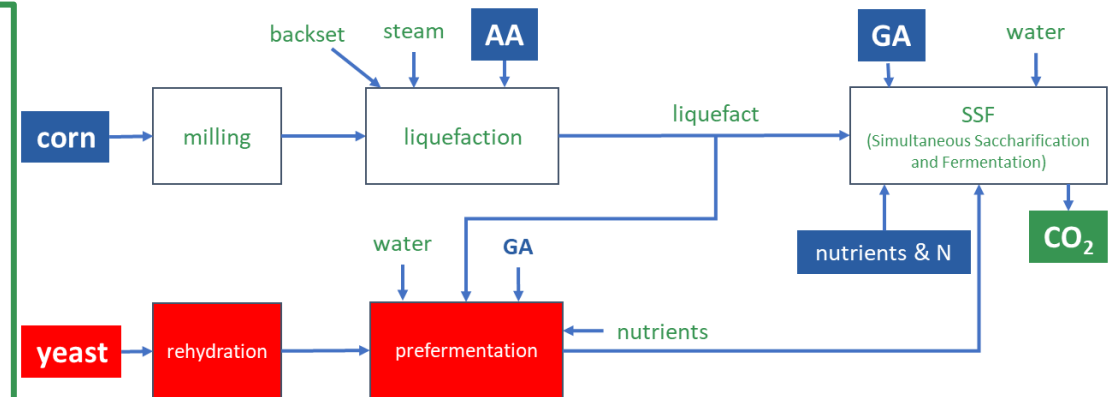
- Store in cooler areas of the plant out of direct sunlight
- If partial package is used, package needs to be well sealed

Rehydration

- CIP vessel and transfer lines
- Keep good records on yeast dosing and monitor yeast counts at transfer

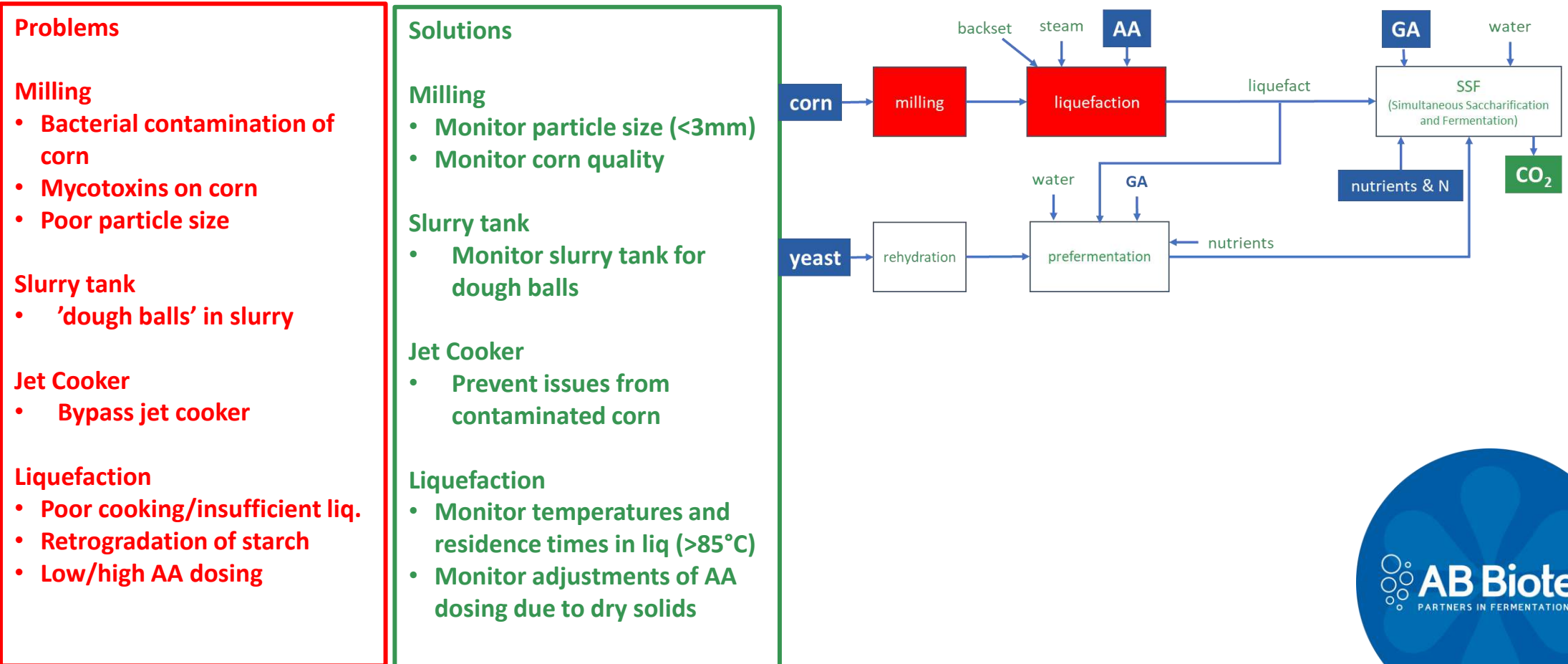
Prefermentation

- CIP vessel and transfer lines
- Keep good records on yeast dosing and monitor yeast counts at transfer



Upstream Problem Areas

- Corn, milling and liquefaction



Upstream Problem Areas

- Fermentation

Problems

SSF (Fermentation)

- Bacterial contamination; high lactic and/or acetic acids
- Temperature excursions during fermentation (high deviation from set point)

GA

- Incorrect GA dosing (low or high saccharification)

Nutrients & N

- Stuck fermentation (insufficient FAN, fusel alcohols)

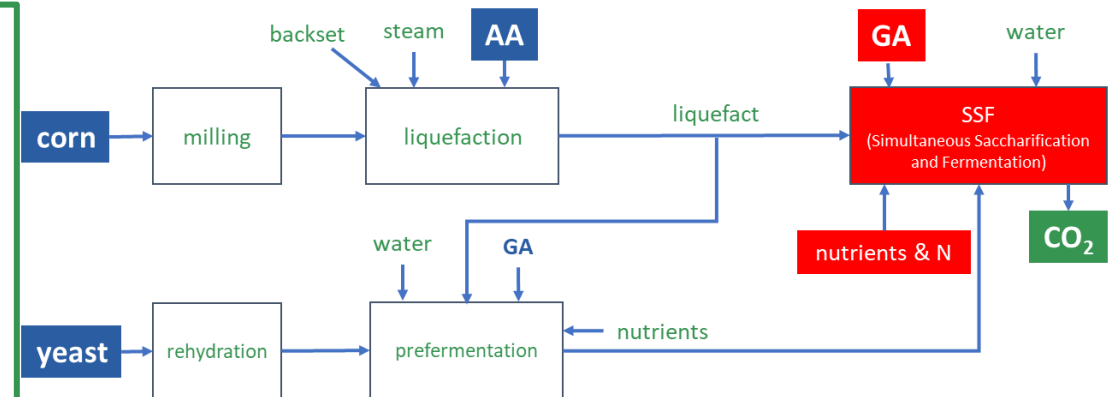
Solutions

SSF/Fermentation

- Monitor lactic/acetic in preferm & ferm
- CIP vessels and lines (check caustic)
- Monitor temperatures

GA

- Modify GA dosing to mitigate temp excursions
- Track yield versus GA adjustments on liq solids
- Monitor FAN at start and ferm drop
- Monitor fusels occasionally

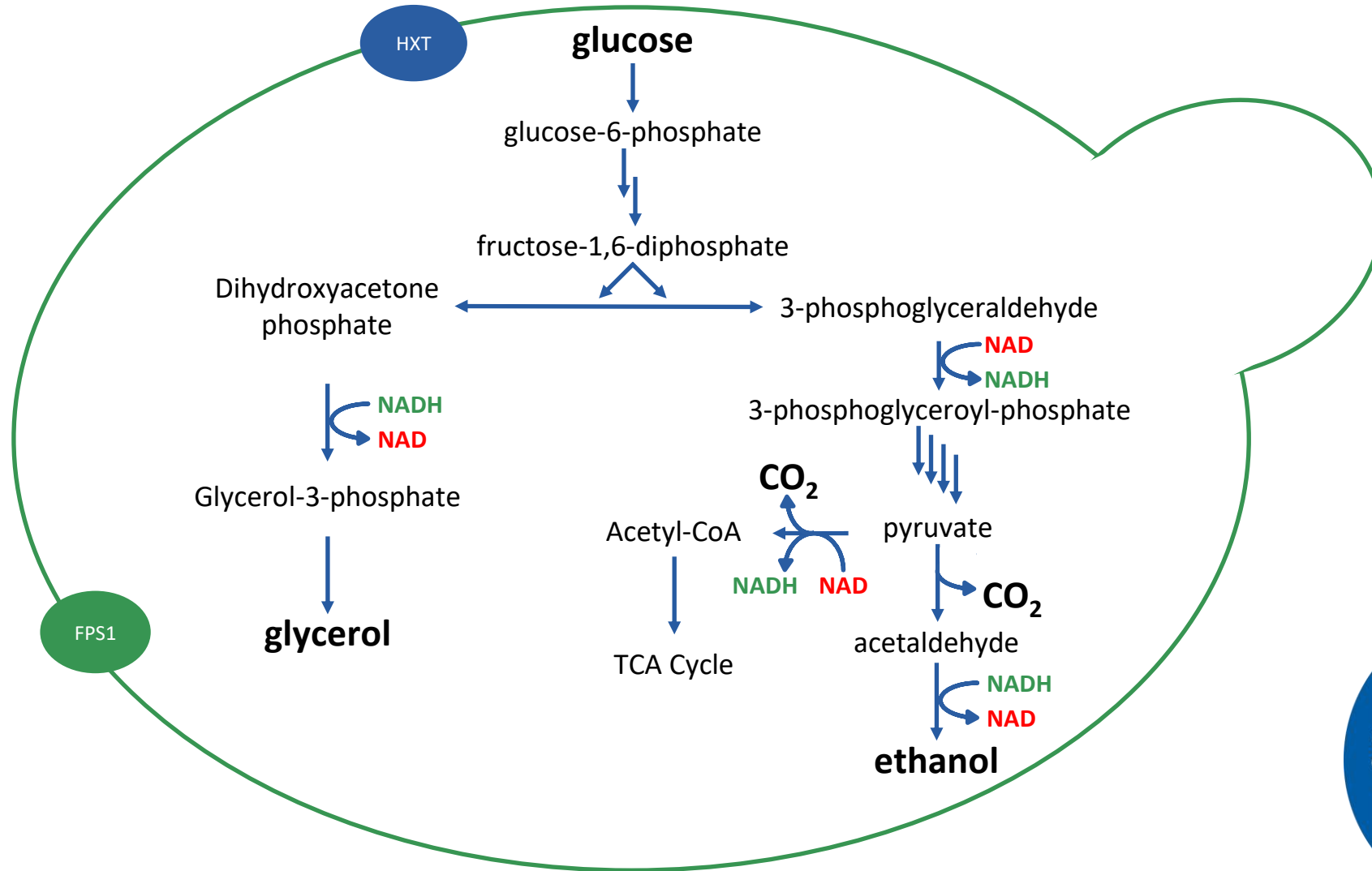


Part 2: Yeast Fermentation Basics

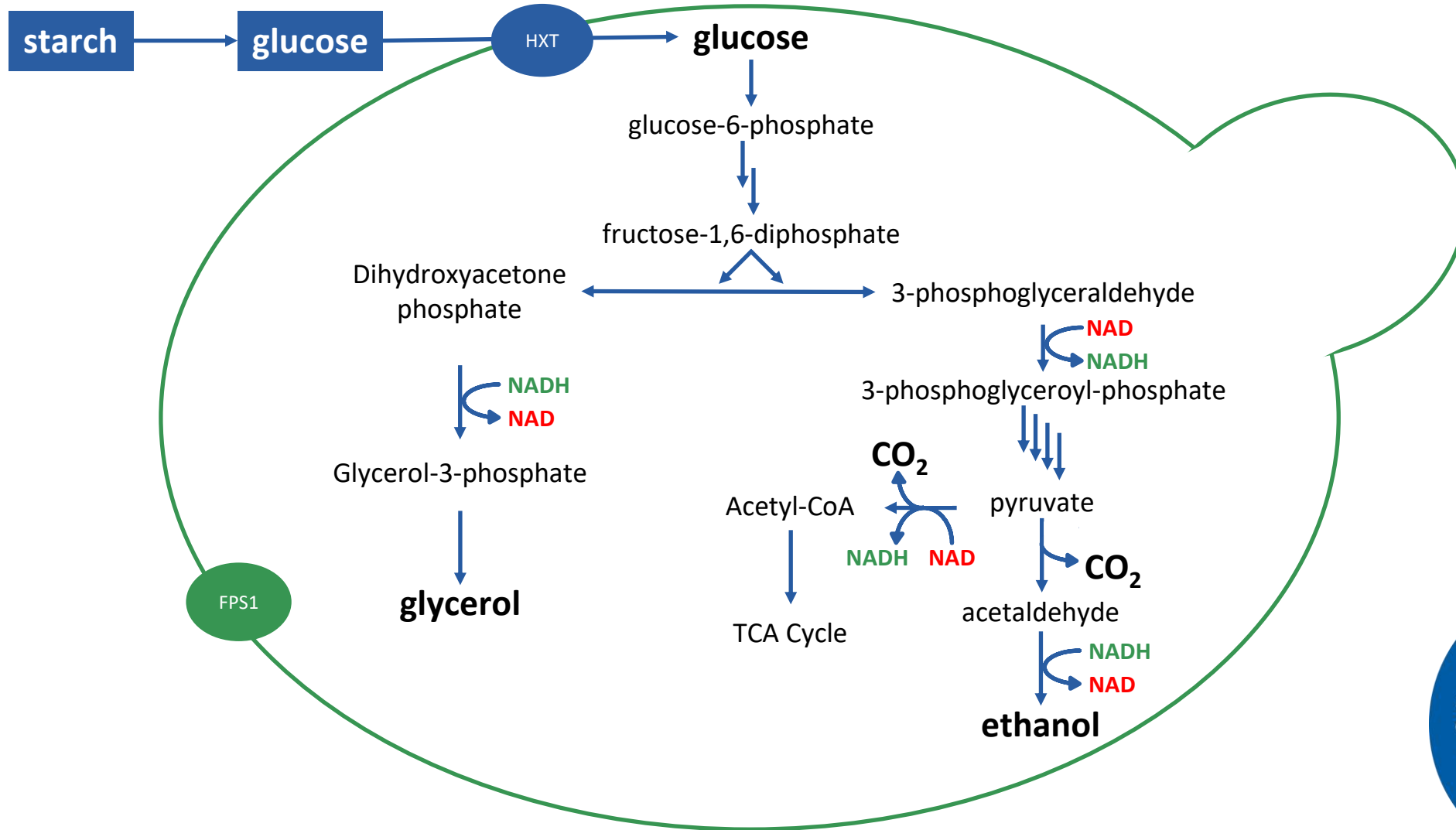
- Metabolism
- Nutrition
- Stressors
- Fermentation Realities



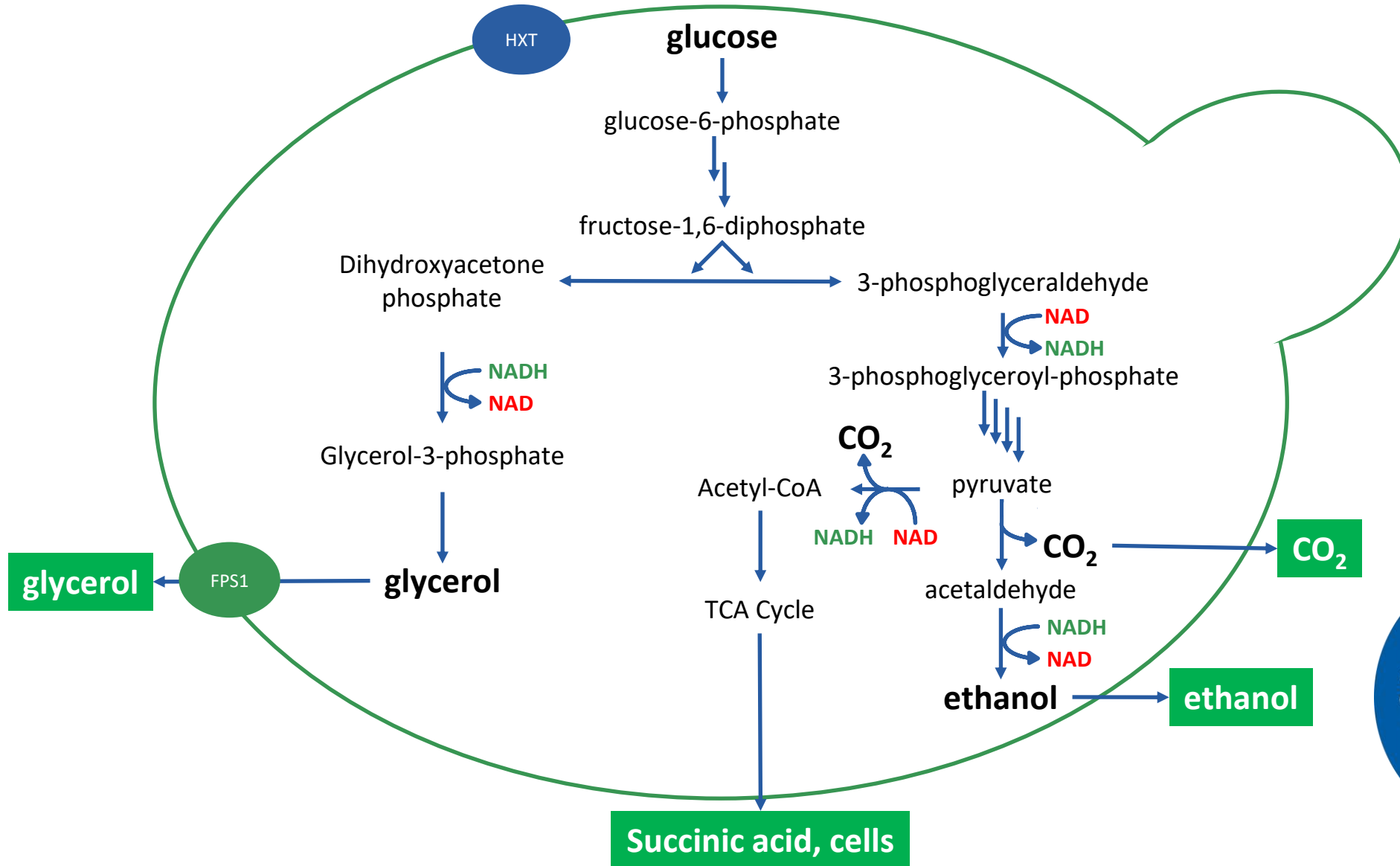
Yeast in Ethanol Production



Yeast in Ethanol Production



Yeast in Ethanol Production



Yeast Nutrition

- **B vitamins**

- thiamin (B1) – catabolism of sugars and amino acids
- riboflavin (B2) – required for coenzymes
- niacin (B3) – co-enzymes for NAD/NADP – important in fermentation
- pantothenic acid (B5) – required for coenzyme A , important in many cellular processes
- pyridoxine (B6) – metabolic coenzyme
- biotin (B7) – for fatty acids
- folic acid (B9) – important for DNA in cell

- **Inositol**

- Required for important membrane lipids (PI)
- PI required for ethanol tolerance

- **Quality fermentation supplements will have all vitamins and inositol**



Yeast Nutrition

- **Bulk and Trace elements**
 - Bulk
 - Phosphate
 - Magnesium
 - Zinc – required for ethanol production
 - Trace
 - Potassium
 - Manganese
 - Copper



Quality fermentation supplements will have all trace elements

Bulk elements usually added by plant



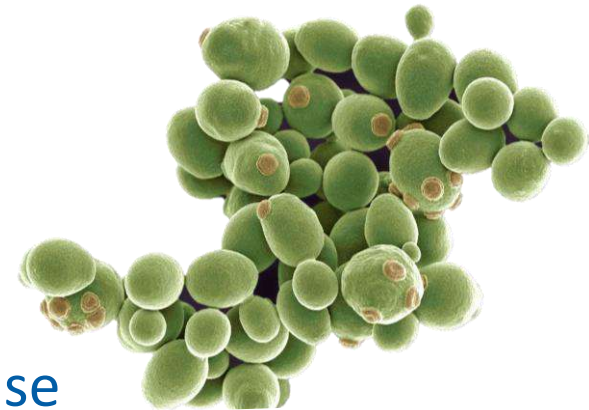
Yeast Nutrition

• Nitrogen

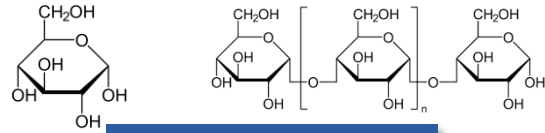
- Yeast need nitrogen to reproduce
 - Reproducing (“budding”) yeast is a good sign of active fermentation
- Nitrogen usually supplied as urea and/or ammonia
 - The enzyme Urease converts urea into ammonia in yeast
- Lack of Nitrogen results in “stuck” fermentation

• Nitrogen in Ethanol Production

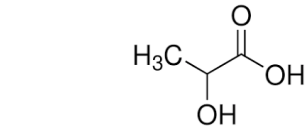
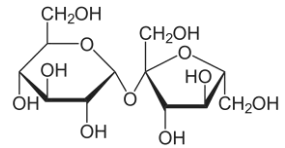
- YAN (Yeast assimilable nitrogen) – Nitrogen for yeast use
 - Ammonium ions (e.g. from MAP or DAP)
 - FAN (Free Amino Nitrogen) – from amino acids
 - Target 400-600 ppm in the prefermenter and 300-400 ppm in the fermenters
 - Protease added to corn fermentations can hydrolyze proteins and add to FAN
 - More cost effective than adding NH₄, DAP/MAP, or urea



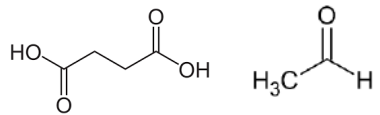
Yeast Stressors



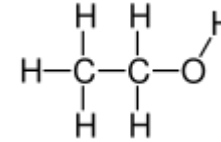
Osmotic stress



Organic Acids



ethanol



High Temperature

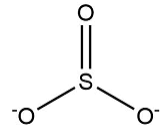


Poor Fermentation

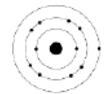
Ethanol Yield Loss

High Residual Sugars

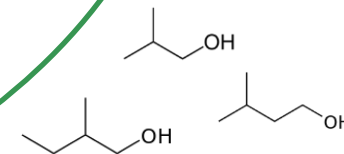
Sulfite



Sodium

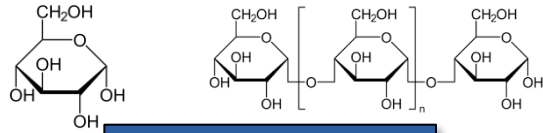


Fusels (higher alcohols)

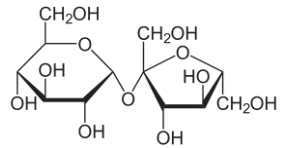


AB Biotek
PARTNERS IN FERMENTATION™

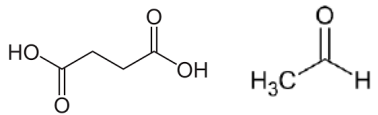
Yeast Stressors



Osmotic stress



Organic Acids



High Temperature



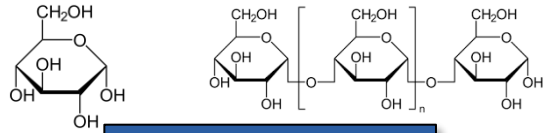
Osmotic stress in Fermentation:

- Caused by high sugars and high dextrin (grain)

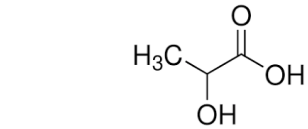
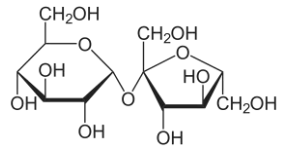
Results in:

- High glycerol / low ethanol yield
- Increased glycogen synthesis
- Lower cell reproduction
- Loss of membrane integrity

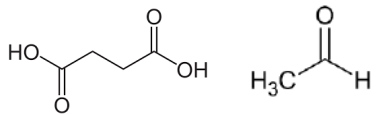
Yeast Stressors



Osmotic stress



Organic Acids



High Temperature



Organic acid stress:

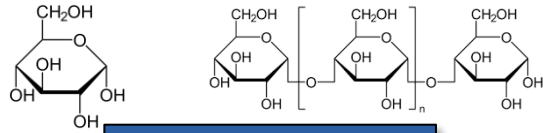
- Caused by lactic and acetic acids, usually from bacterial contamination

Results in:

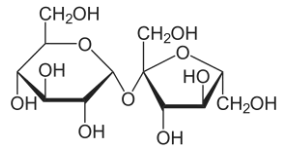
- acidic conditions inside yeast – stops metabolism
- Oxidative damage and cell death
- Low ethanol and high residual sugar



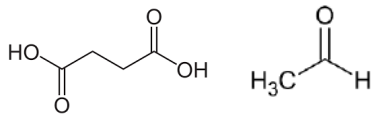
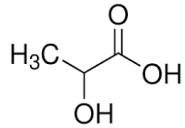
Yeast Stressors



Osmotic stress



Organic Acids



High Temperature



High Temperature:

- Caused by loss of cooling control; temperature excursion

Results in:

- Loss of membrane integrity & cell death
- Slow/no growth

Fermentation Chemistry

MAIN CONCEPTS:

- Ethanol is made by yeast from glucose in the fermentation process
- Glucose can be made from starch from grain

1 glucose molecule → 2 ethanol molecules + 2 carbon dioxide molecules (gas)

1 kg glucose → 511 g ethanol (0.65L) + 489 g carbon dioxide



In grain, starch is broken down to glucose

starch + water enzymes glucose
1 Kg Starch → 1.11 kg Glucose



Fermentation Realities

MAIN CONCEPTS:

- By-products are *always* made in fermentation
- Cells are *always* made in fermentation
- *Never* achieve stoichiometric ethanol production from glucose

1 glucose molecule → <2 ethanol molecules + <2 carbon dioxide molecules (gas)

1 kg glucose → <511 g ethanol (0.65L) + <489 g carbon dioxide

→ glycerol

→ acetic and succinic acids

→ acetaldehyde

→ yeast cells

Fermentation
by-products

By-product formation depends on the yeast and the fermentation conditions



Mass Balance in Fermentation

MAIN CONCEPTS:

- $\text{Mass}_{\text{in}} = \text{Mass}_{\text{out}}$
- There can be no exception to the above rule – Law of Conservation of Mass

The Case of Bioethanol Fermentation

- All mass inputs must be accounted by product output
- Balancing input and output masses validate analytical values
 - There is otherwise no way to check on the input/output analytics



Balancing on Glucose (grain)

INPUTS

If starting from starch/dextrin (dry basis)

1 g starch = 1.11 g glucose
(due to water addition in hydrolysis)

If starting from typical corn flour (~72% starch)

1 g flour = 0.72×1.11 g glucose

If starting from corn mash of known composition

1 g DP4+ = 1.111 g glucose
1 g DP3 = 1.071 g glucose
1 g DP2 = 1.053 g glucose
1 g glucose = 1.000 g glucose

Do not count input glycerol, acetic or lactic acids, or cell mass

OUTPUTS

HPLC and/or GC analytes

| | |
|------------------------|-------------------|
| 1 g DP4+ | = 1.111 g glucose |
| 1 g DP3 (maltotriose) | = 1.071 g glucose |
| 1 g DP2 (maltose) | = 1.053 g glucose |
| 1 g glucose (glu) | = 1.000 g glucose |
| 1 g glycerol (gly) | = 0.978 g glucose |
| 1 g ethanol | = 1.957 g glucose |
| 1 g acetic acid (a.a.) | = 1.500 g glucose |
| 1 g lactic acid (l.a.) | = 1.000 g glucose |
| 1 g dry cell mass | = 1.923 g glucose |

Subtract input glycerol, acetic and lactic acids, and cell mass.

Using glucose equivalents corrects for CO₂ loss in ethanol and acetic acid production by the cell

Assume cell yield on sugar is 0.52 g/g.
Assume 2×10^{10} cells/g



Example - Grain

Assumptions

- *Starting from starch/dextrin (dry basis)
- *Analytical values in % mass/volume (%m/v)
- *No dilution or water addition to fermentations (amount of water remains constant)

INPUTS

$$(DP4 \times 1.111) + (DP3 \times 1.071) + (DP2 \times 1.053) + (glu) = g \text{ glucose equivalent input}$$

OUTPUTS

From HPLC and or GC data in %w/v:

$$(DP4 \times 1.111) + (DP3 \times 1.071) + (DP2 \times 1.053) + (glu) + (ethanol \times 1.957) + [(gly_f gly_i) \times 0.978] + [(a.a._f a.a._i) \times 1.500] + [(l.a._f l.a._i) \times 1.000] = g \text{ glucose equivalent output}_{analytes}$$

If acetic and lactic acids are <0.05 %w/v, both can be ignored (typically account for ~0.5 % of input glucose)

Cell mass calculation assumes 2×10^{10} dry cell/g and 0.52 g cells per g glucose. Cell count at end of fermentation required.

$$\left(\frac{((cells/mL_f - cells/mL_i) \times \text{fermentation volume in mL})}{2 \times 10^{10}} \right) \times 1.923 = g \text{ glucose equivalent output}_{cells}$$

$$\text{Total OUTPUT } g \text{ glucose equivalent} = g \text{ glucose equivalent output}_{analytes} + g \text{ glucose equivalent output}_{cells}$$



Mass Closure

Importance

- If the closure is not within $100\pm 5\%$, there are problems with the analytical results and they are not reliable
 - Check HPLC chromatograms, standards, etc.
 - Check inputs (moisture, etc.)
- For applications testing, it is important to compare the flask results with plant results for relevance
 - For confidence, the mass balances and EOF glucose equivalents should be close



Why Bother with Mass Balance & Closure

Importance

Determine ethanol yield

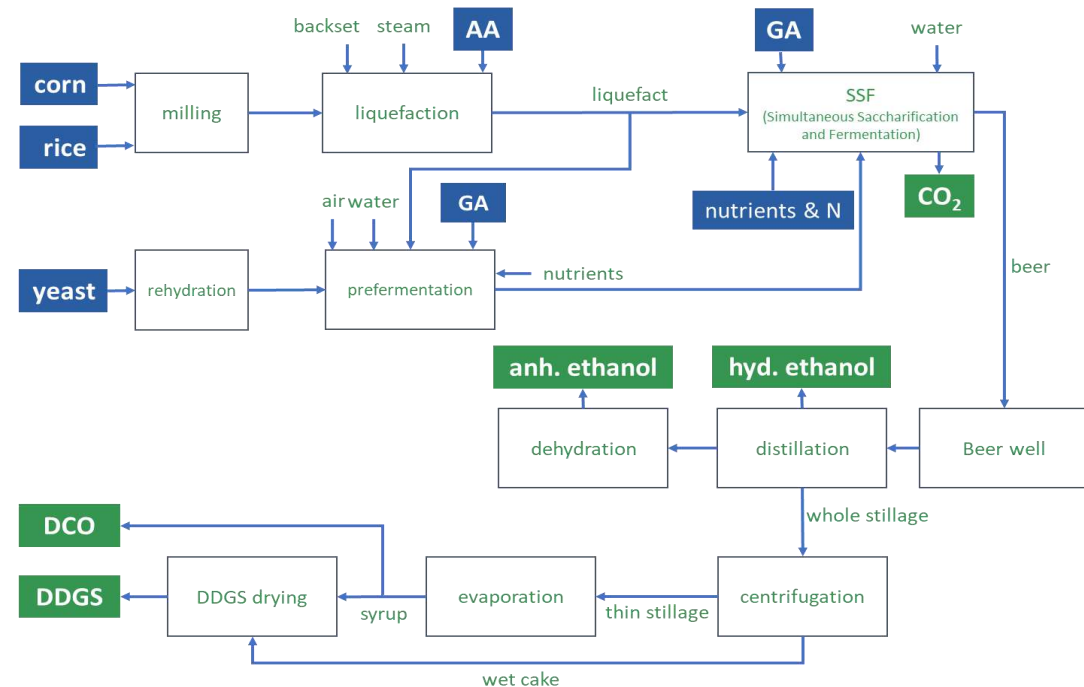
- One of the most important plant metrics
- Important to have certainty of yield when conducting trials
- Determine variance of yield in plants
- Back-calculate to corn starch and corn flour input (compare to plant targets)

Have confidence in analytical results

- Confidence in trial results
- Need to determine fate of glucose in fermentations

Diagnose problems with the plant

- Plants differ widely in their ability to diagnose problems based on analytical results
- Outputs known to vary when inputs are unknown/unreliable



Corn ethanol plant block flow diagram



Part 3: Yeast Deployment in Ethanol Plants

- Yeast Selection
- 'Big Data' in industrial fermentation
- Technical Services



Importance of Advanced Yeast - Yield

Ethanol Yield

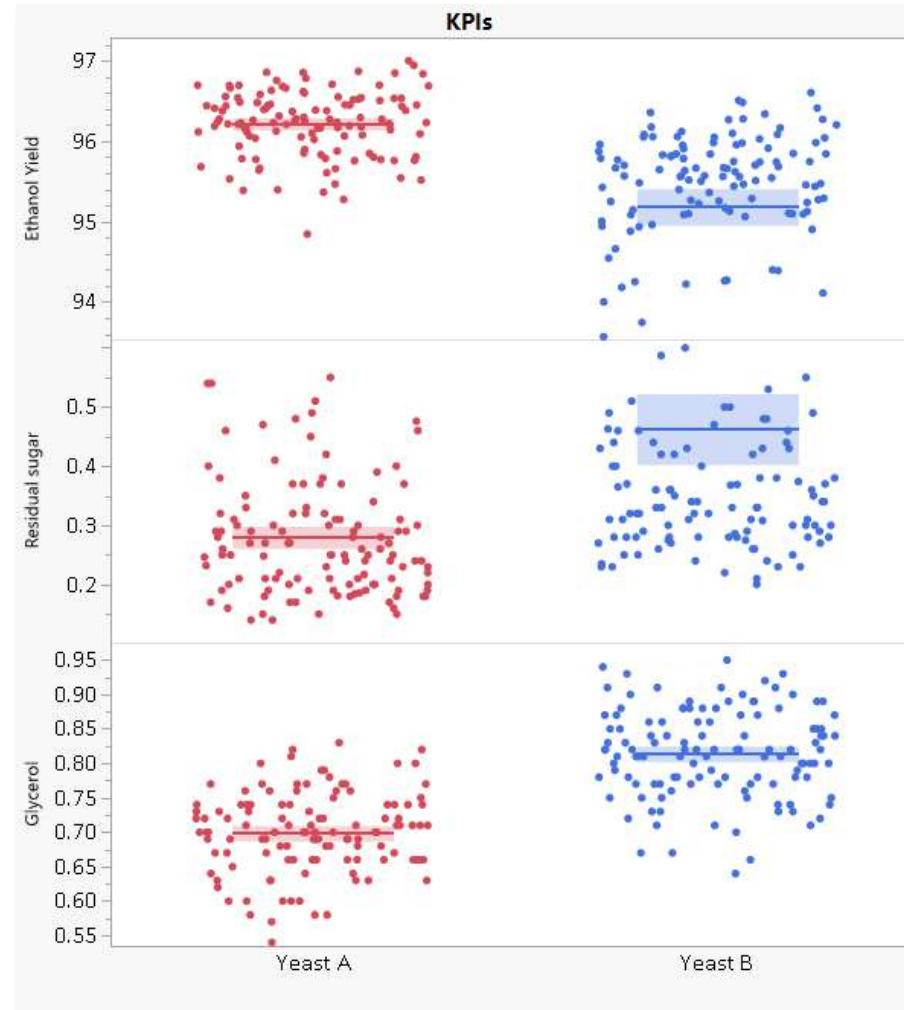
- Yeast A - 96.2%
- Yeast B - 95.2%
- Yeast A exhibits 1.0% increase in Ethanol Yield
- **More ethanol produced from corn**

Residual Sugar (DP1+DP2+DP3+DP4+)

- Yeast A – 0.28 %w/v
- Yeast B – 0.46 %w/v
- 0.18 %w/v decrease in Residual Sugar
- **More sugar used in fermentation**

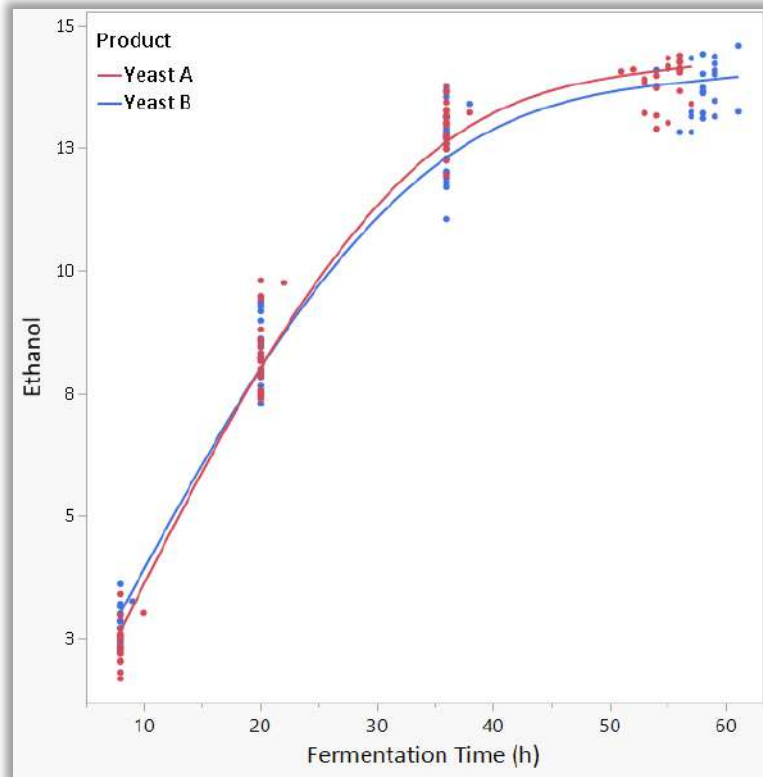
Glycerol

- Yeast A – 0.70 %w/v
- Yeast B – 0.81 %w/v
- 0.11 %w/v decrease in glycerol
- **Less glycerol produced in fermentation**

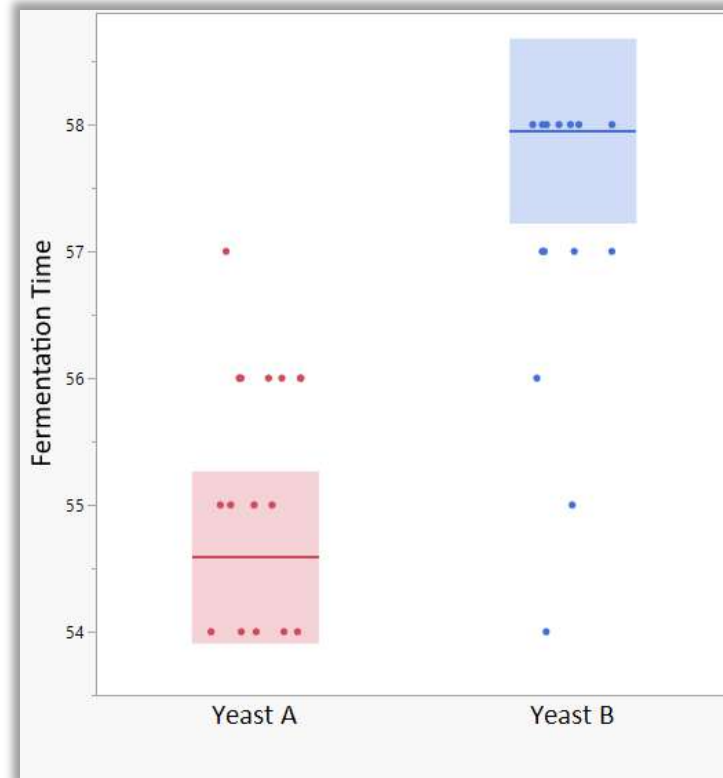


Importance of Advanced Yeast – Rate

- Increased Volumetric Productivity – More ferms/year



Time course shows increased rate and yield and decreased ferm time of Yeast A versus Yeast B.



Trial data shows a 3.4 h decrease in ferm time for Yeast A versus Yeast B (5.8% reduction in ferm time).



Importance of Advanced Yeast - OA Resistance

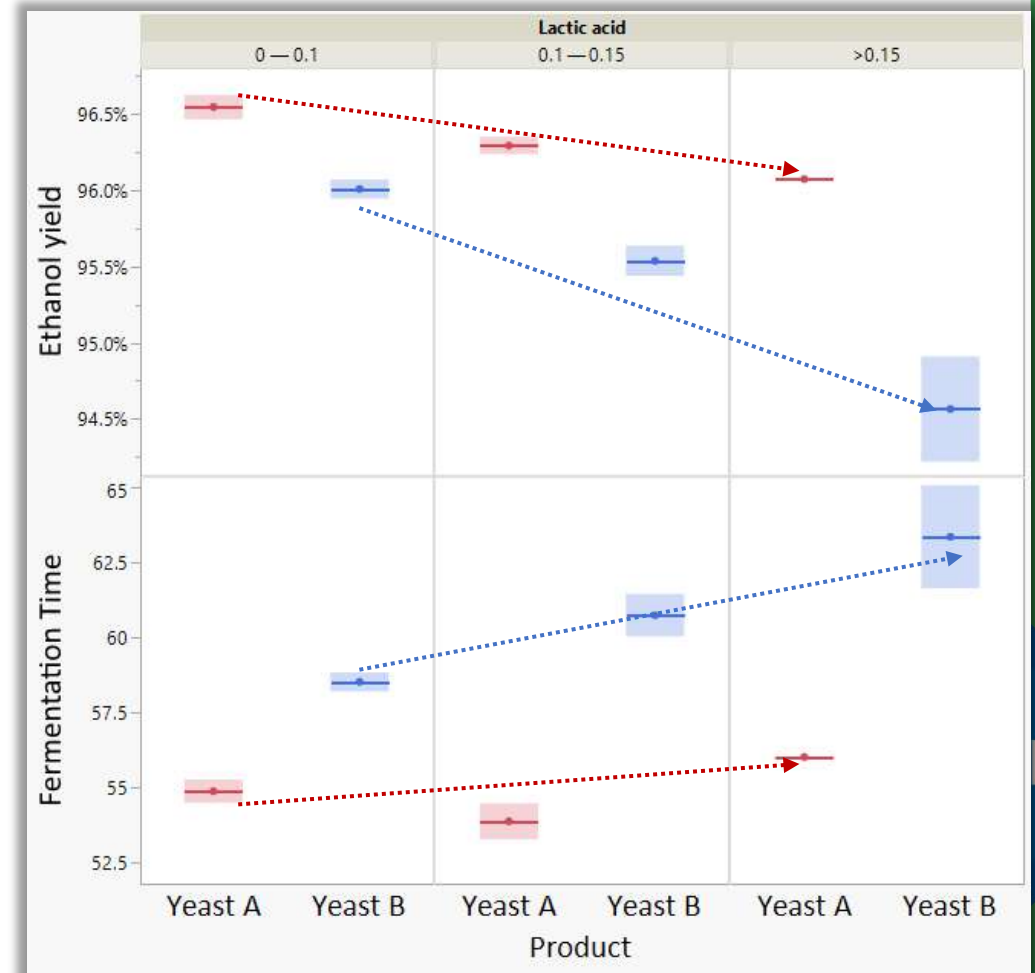
- Increased Yield and Shorter Ferm Times in high OA conditions

Ethanol Yield

- Yeast A – 0.4% decrease with high lactic acid
- Yeast B – 1.5% yield drop with high lactic acid

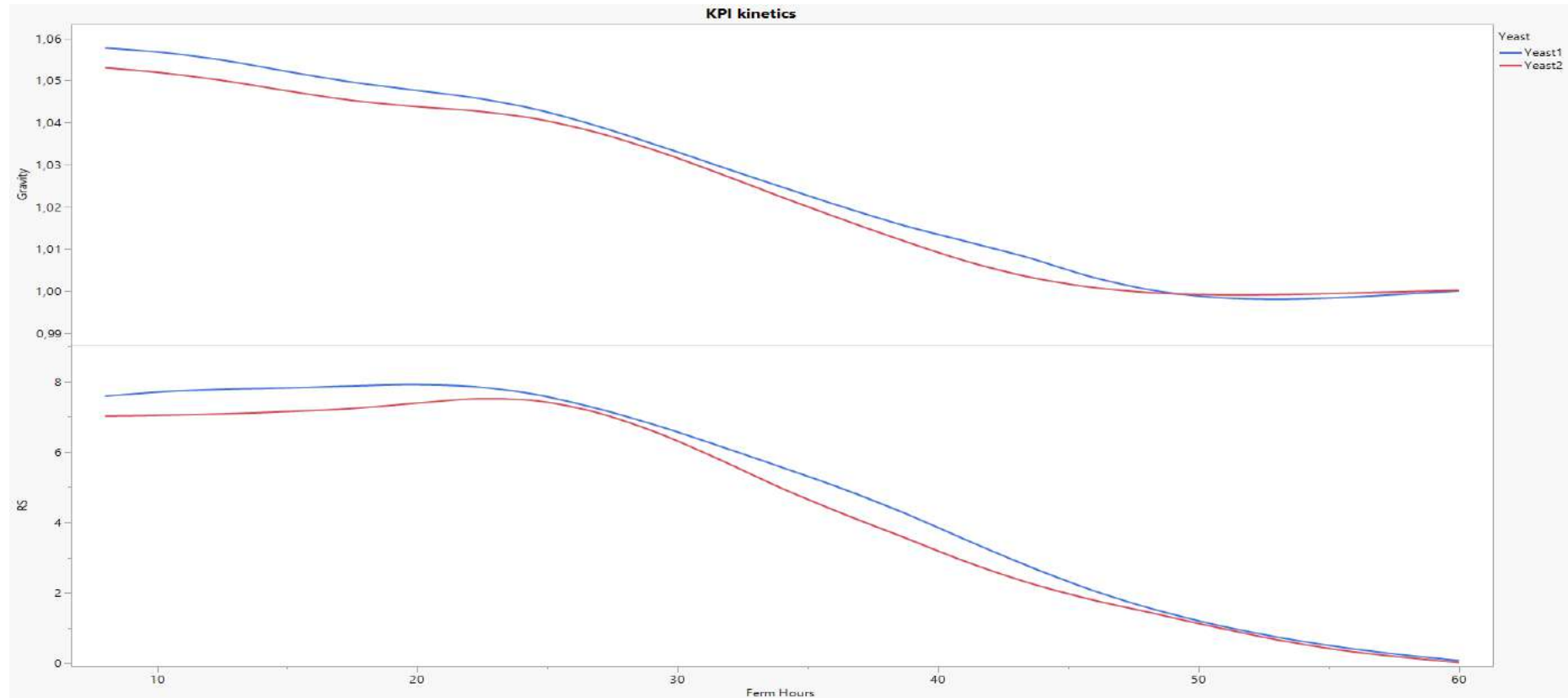
Fermentation Time

- Yeast A – 53-55h. Fermentation unaffected by lactic acid
- Yeast B – 58-63h. Fermentation increased by ~5h by lactic acid



Example limited parameters data set

- Plant trial using different yeasts

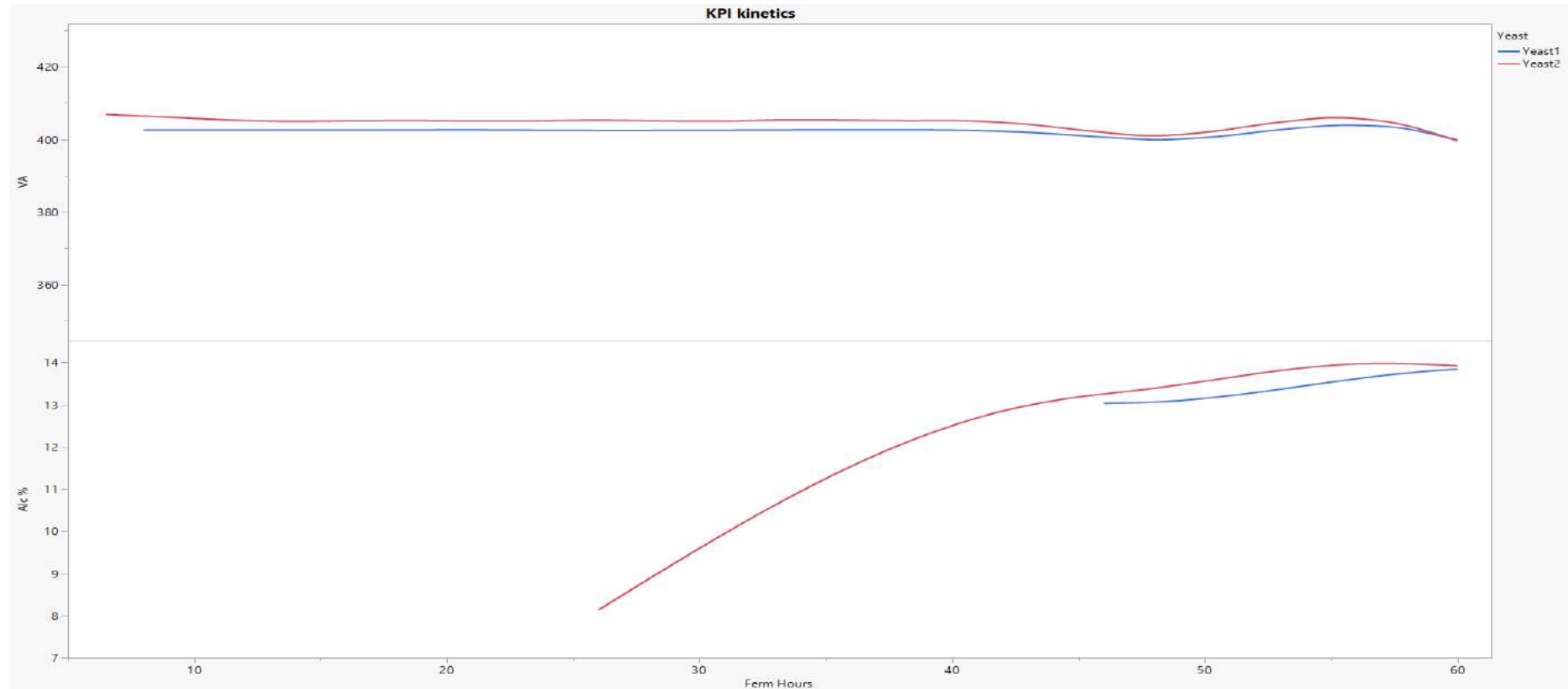


With data collected during a plant trial comparing 2 yeast samples, Yeast B shows better performance than Yeast A, faster kinetics, higher ethanol titer.



Example limited parameters data set

- Plant trial using different yeasts



With data collected during a plant trial comparing 2 yeast samples, Yeast B shows better performance than Yeast A, faster kinetics, higher ethanol titer.



Economic Importance of Advanced Yeast

High-Level Economic Benefit for Advanced Yeast*

| Biorefinery capacity | 66.00 m ltr | | Assumptions | |
|--|-------------|--|-----------------------|------|
| 1% Ethanol Yield increase | 0.66 m ltr | | Ethanol price (Rs./l) | 58.5 |
| 5% Volumetric Productivity increase | 3.33 m ltr | | | |
| 1.5% Yield & 10% Productivity increase in 10% infected farms | 0.81 m ltr | | | |
| TOTAL Ethanol Increase | 4.80 m ltr | | | |
| Increased ethanol revenue (pa)** | 280 m INR | | | |
| Increased yeast cost (pa) | 1.20 m INR | | | |

*Value of lower evaporation fouling and natural gas savings in DDGS drying not included

**Increased distillation cost not included

'Big Data' Approach

Use of 'Big Data'

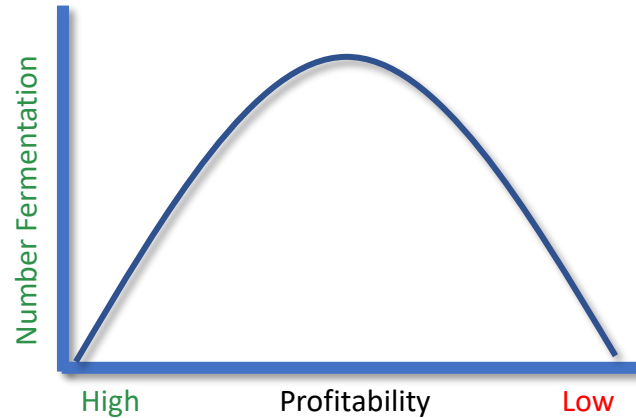
- Uncovers efficiencies in plants
 - Increases profitability
 - Reduces CI of ethanol and co-products

Big Data collection

- Ideally, all DCS, PLC, lab, and other data uploaded in statistical software
 - Data curation important
- True 'Big Data' handling of customer data, 10^4 rows with 10^3 columns, $> 10^7$ data points per plant/fermentation

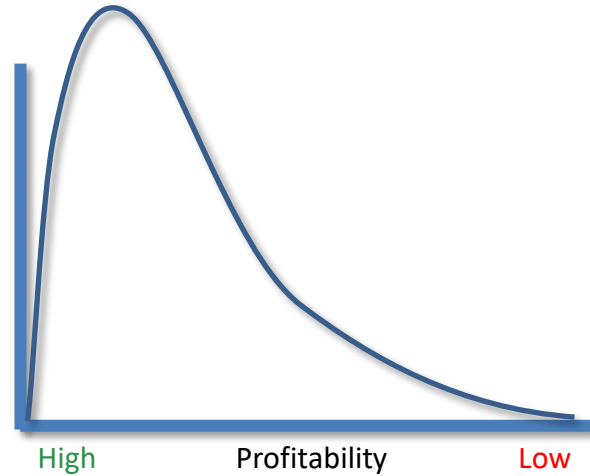
A screenshot of the JMP software interface. It shows a data table with many columns and rows. The columns are labeled with various variables, and the rows contain numerical data. The interface includes a sidebar with a tree view of the data structure and a main window displaying the data table.

Economic Benefit to Optimization



Many plant operators wrongly think ethanol yield should generate a bell shaped curve

- Data from DCS, PLC, lab, and notes are “looked at” and decisions made by “gut feel”
- “This is how we have always done it”

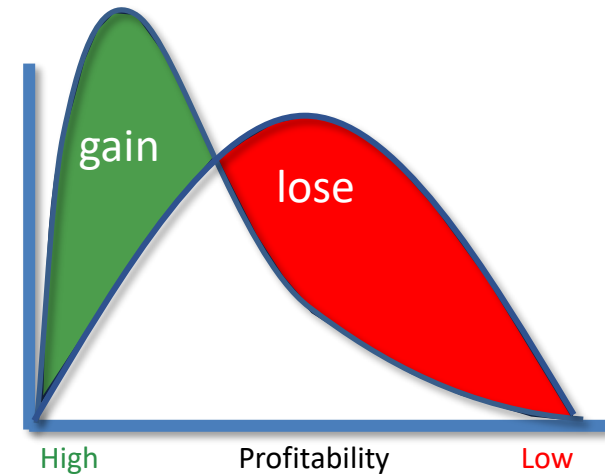


Using statistical methods allow more high profit fermentations

- Data-driven decisions
- Statistical analysis using advanced software (JMP)
- All plant data (data from data historians, PLC, DCS, laboratory)

JMP Dashboards & KPIs replace Excel charting

- data-driven optimization
- Reduce variance, increase profitability



End game is higher profits with statistical optimization

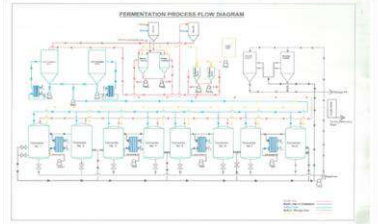


Technical Service – What to Expect?

Plant Understanding

Indian Sugar Mills Distillery -1

Site Visit Report



- Batch/ Cont. process
- Propagation
- Yeast dosage
- Substrates
- ...

Data Collection



| ITEM | Unit | Plant | Distillery | Substrate | Yeast | Temp | Pressure | Flow | Other | Distillery | Distillation |
|------|------|-------|------------|-----------|-------|------|----------|------|-------|------------|--------------|
| ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

- Baselining
- Establish visibility to customer plant operation via data sharing

Trial Design & Execution

Trial KPIs:

- Increase ethanol yield on sugar from 80.15% to 83.84 15.4%
- Increase ethanol flow average from 8.25 to 1.56% to 9.40 %w/v
- Reduce end TS from average 2.25 59.02 %w/v to 1.65 %w/v (D4 %w/v decrease with 26% yield)
- Increase CO2 recovery 3-4%

The targets may be modified based on additional analysis (see below). Based on AB Biotek's experience in similar ethanol distilleries, the above targets seem reasonable. Please confirm if the plant captures CO2 as the PFD shows.

Trial outline:

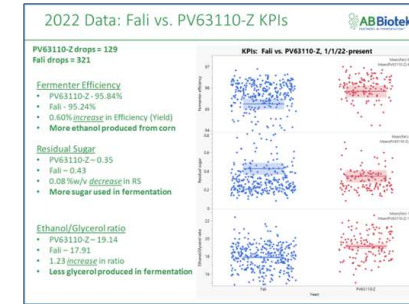
- 30 d SAF Instant baseline: 10 d fall MC-20, 10 d SAF, 10 d fall MC (so called offgas)
- Only yeast product changed during this trial
- Important to evaluate yeast cell counts (start and end) after a few days of Fall MC to ensure adequate dosing
- Continue with data collection as before, with these modifications
 - adding the time of day (morning/noon/evening) for the data points (not just date, noon date & time)
 - temperature and other data cannot be reached well to determine kinetics without flow of dry captured
 - Will make it easy to determine the cross representing chain
- Baseline measurements of feed including sugar (S) & feed (TS) %?
 - Currently, these are calculated values from the single determination of residual RS/TS
 - Additional feed determinations are needed to determine yield with less error
 - Determine DM gravity (with a range is provided here)

Yeast & Yeast Prep:

- 15 kg compressed (assuming 20% solids)
- Important to evaluate yeast cell counts (start and end) after a few days of Fall MC to ensure adequate dosing (same counts as offgas)

- Based on customer operation priorities to design the trial

Evaluation & Benefits



- Understand plant operation..
- Identify technical benefits
- Value pricing

BAU Tech Services



- Regular data analysis
- Regular sampling and lab analysis



Questions?