Yeast Nutrition to Improve Fermentations

The Alcohol School Concise

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Ethanol Technology Institute

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Date: Caribbean Distilling Seminar, St Lucia

Outline

- Yeast nutritional requirements
- Nitrogen sources and assimilation by yeast
- Feedstock nutrient availability
- Effect of nutrition on fermentation (kinetics and yield)

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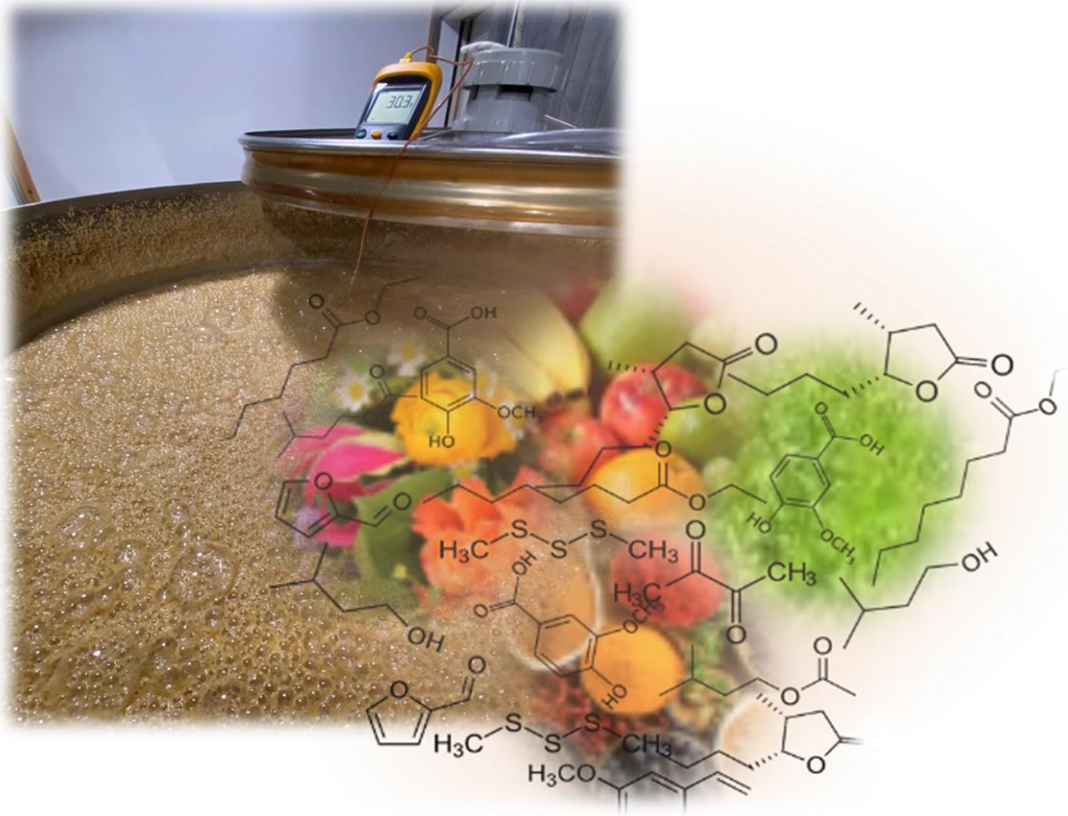
Importance of Nutrition in Fermentation

To sustain yeast growth and health:
Viability and vitality

To sustain fermentation:
Synthesis of ethanol and congeners



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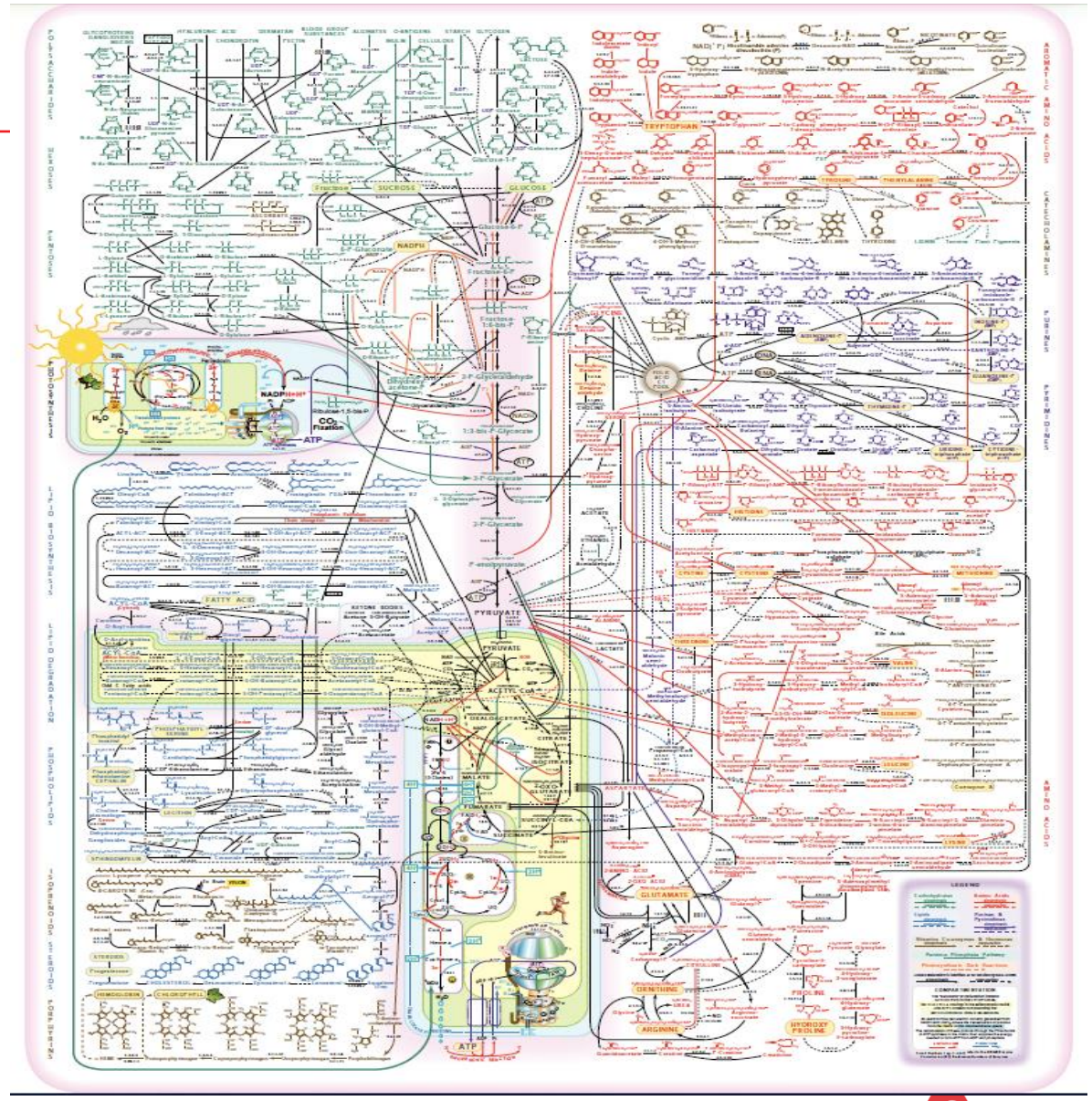
How Do Yeast Work?

- Incredibly complex pathway
- Takes lots of inputs
- Uses lots of enzymes!!!
- Enzymes are proteins, high turn over
- Building requires N
- Maintaining/Usage requires Mg, Zn, Ca, etc…



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Yeast Nutritional Requirements

Carbohydrates

- Source of carbon and energy to generate biomass and metabolites

Nitrogen

- Biosynthesis of protein, enzymes, nucleic acids (anabolic role)
- Synthesis of higher alcohols and esters (catabolic role)

Vitamins: Coenzymes

Sterols and Fatty Acids: Membrane and ethyl esters synthesis

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

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Yeast Nutritional Requirements

Sugar feedstocks

Starch feedstocks

Different feedstocks have different composition and require different nutritional supplementations

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Where Do We Add Nutrients?

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Nitrogen and Inorganic Ions

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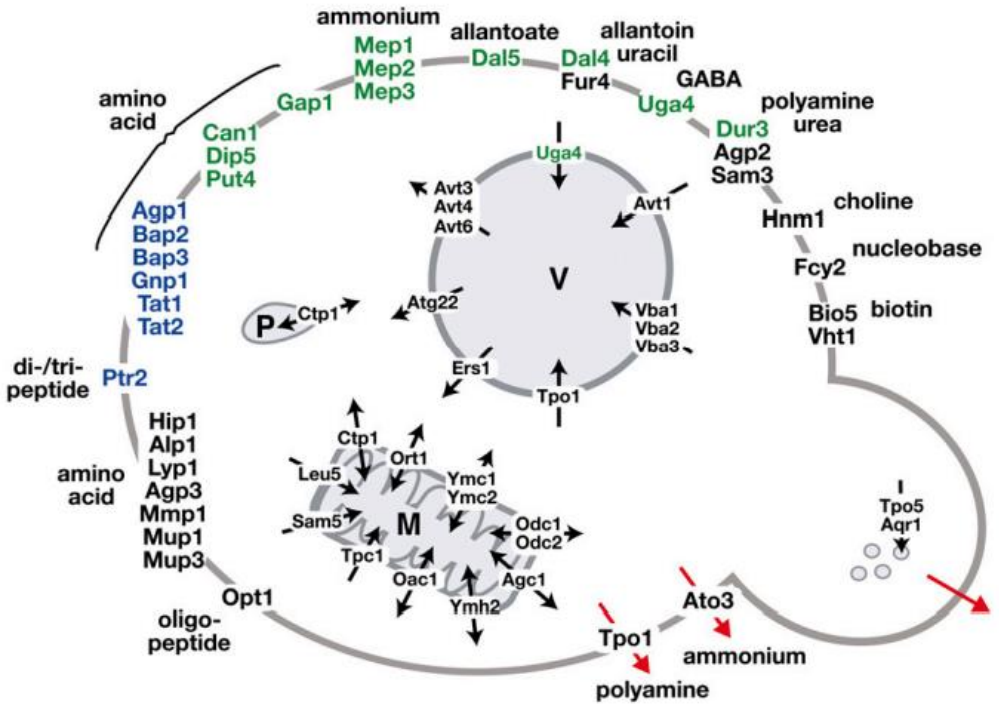
Sources of Assimilable Nitrogen

YAN: Yeast **A**ssimilable **N**itrogen:

- **Inorganic Nitrogen**
  - Ammonium ions (NH_4^+): MAP, DAP
- **Organic Nitrogen**: **F**ree **A**mino **N**itrogen (**FAN**)
  - Amino acids
  - Small peptides (up to pentapeptides)
- Proteins and longer oligopeptides cannot be assimilated by yeast (but there is an enzymatic solution for that – see next slides!).
- Urea should not be used in beverage fermentations being a precursor of ethyl carbamate (carcinogenic). `



Ljungdahl P.O. *et al.* 2012 Genetics

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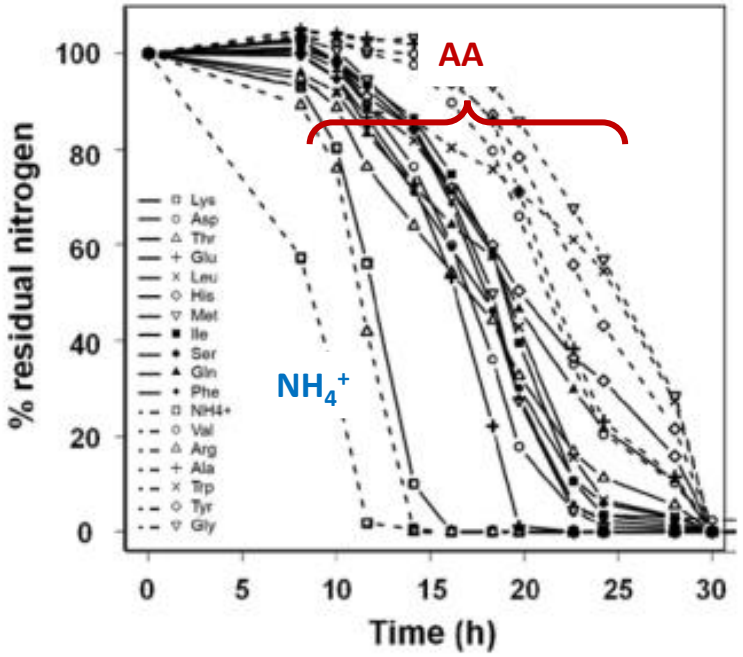
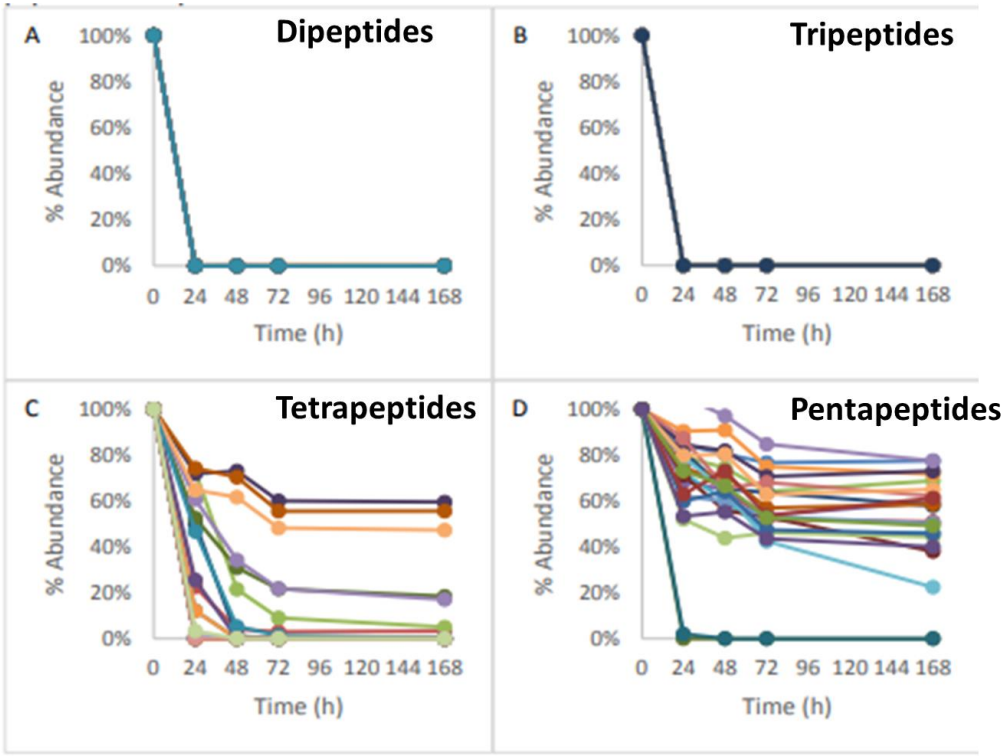
Nitrogen Assimilation Profile

Ammonium and AA consumption during fermentation in synthetic medium



Crépin L. *et al.* 2012 AEM

Peptide consumption during fermentation in synthetic medium



Lallemand Study

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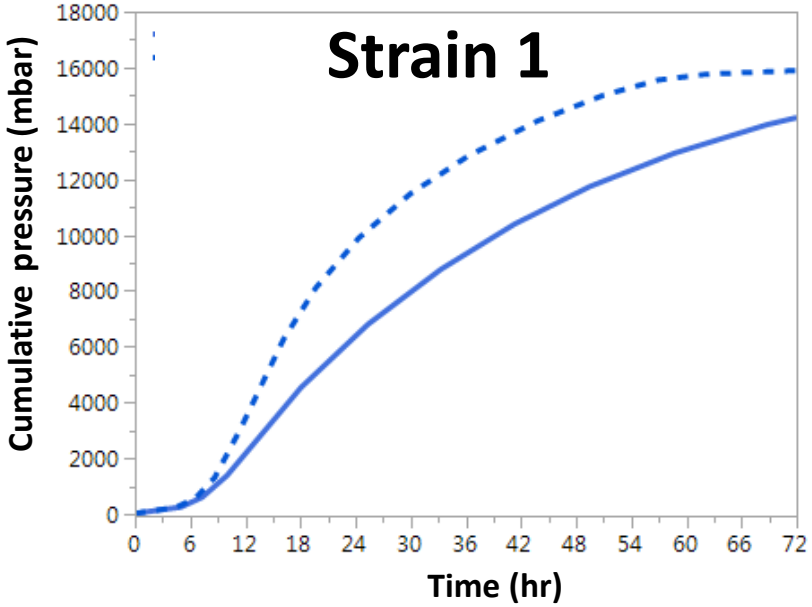
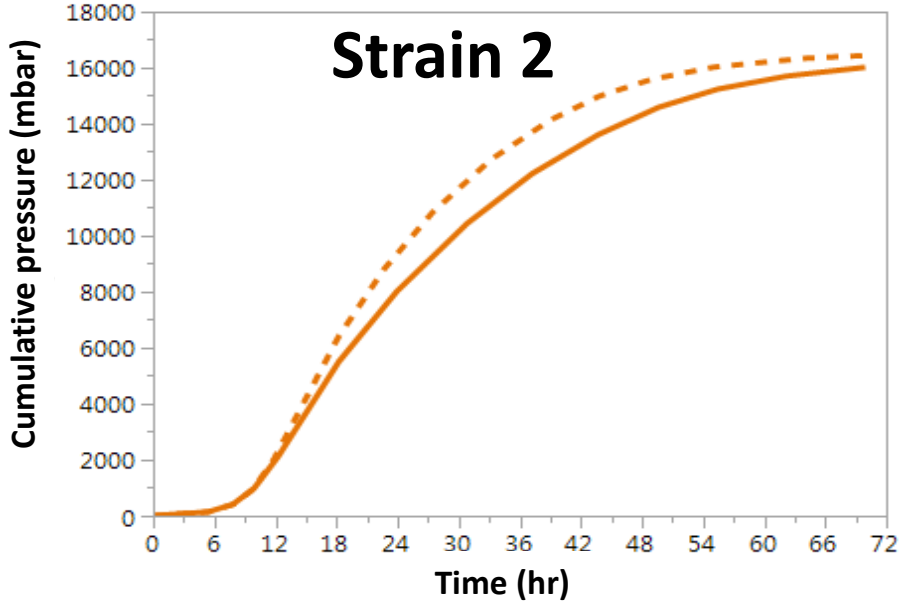
Nitrogen Requirements are Strain Specific

As a rule of thumb: **200-250 ppm (mg/L) of YAN** is required by most strains for growth and efficient fermentations.

100 % corn whiskey fermentation





No nitrogen addition (70 ppm YAN from the mash)

Addition of a nitrogen source (300 ppm YAN)

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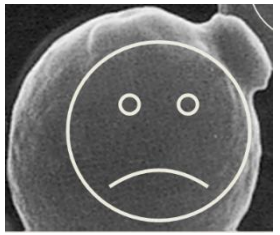
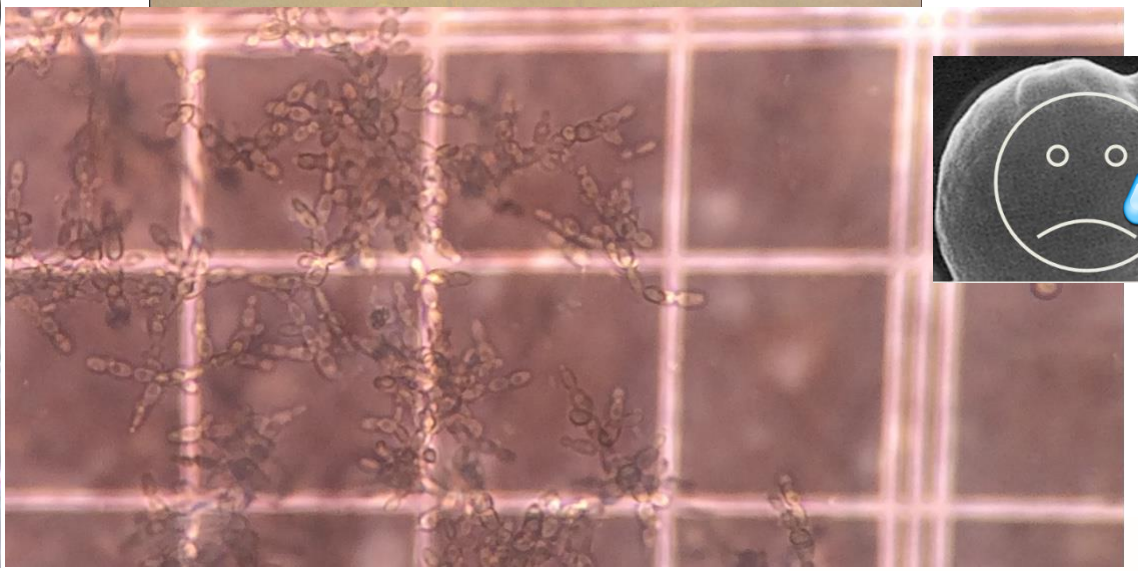
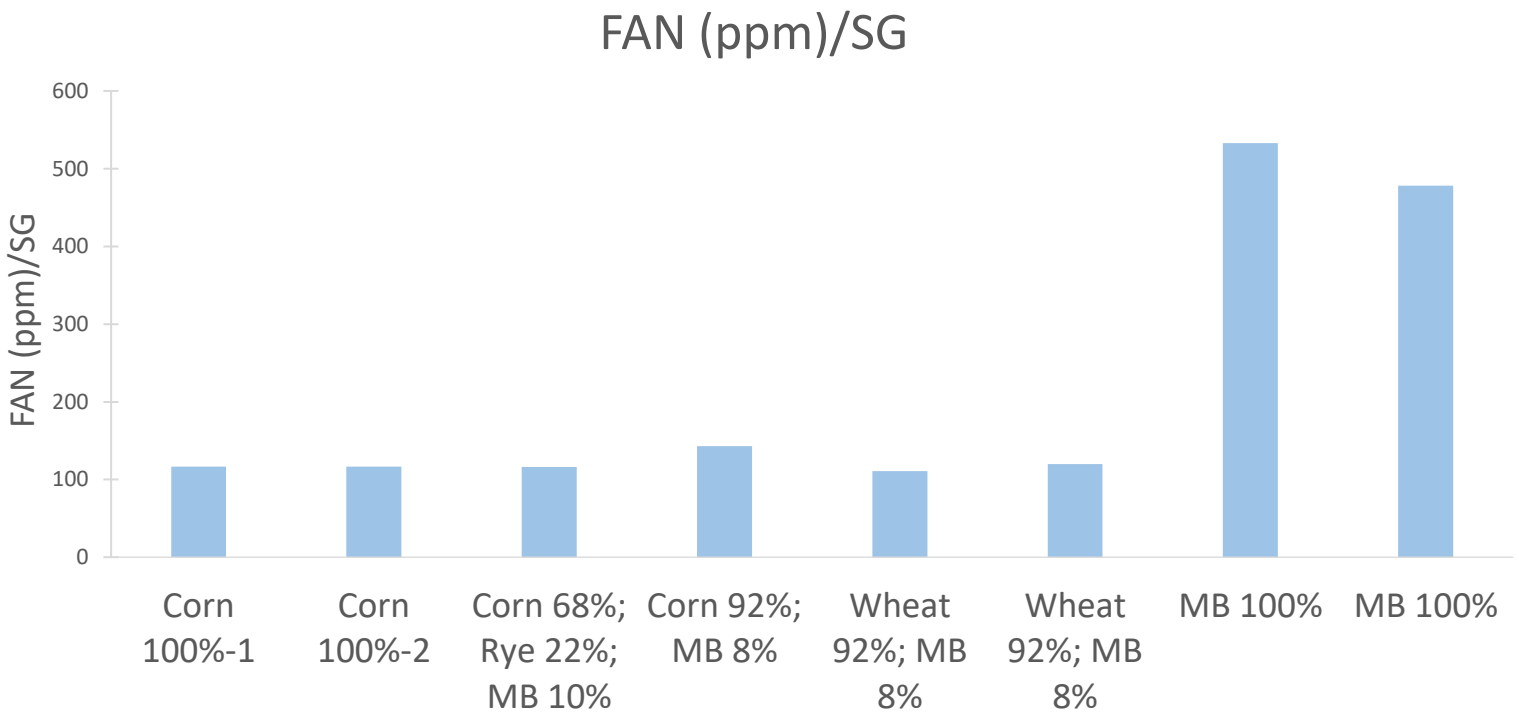
How Can Lack of Nutrition Impact the Yeast?





LALLEMAND BIOFUELS & DISTILLED SPIRITS

FAN in Starch Feedstocks



MB = Malted Barley
SG of mash bills shown in the graphs: 1.064 -1.090

- Nitrogen is naturally present in starch-based feedstocks, but its content is often insufficient or not readily available to yeast
- Malted grains are a source of active proteases.

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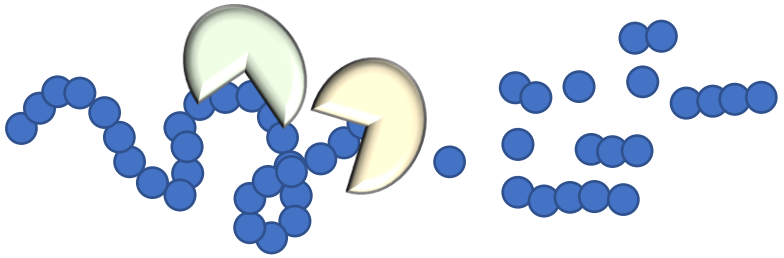
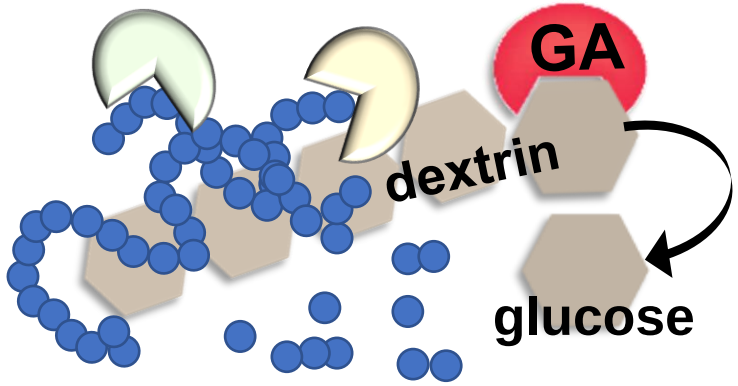
How Do Proteases Work?



The proteases are enzymes that cleave proteins and polypeptides present in the mash.

→ **Gradual release of FAN during the fermentation resulting in faster fermentation kinetics**



The proteases can also cleave starch-bound proteins liberating additional fermentable sugars.

→ **Higher yields**

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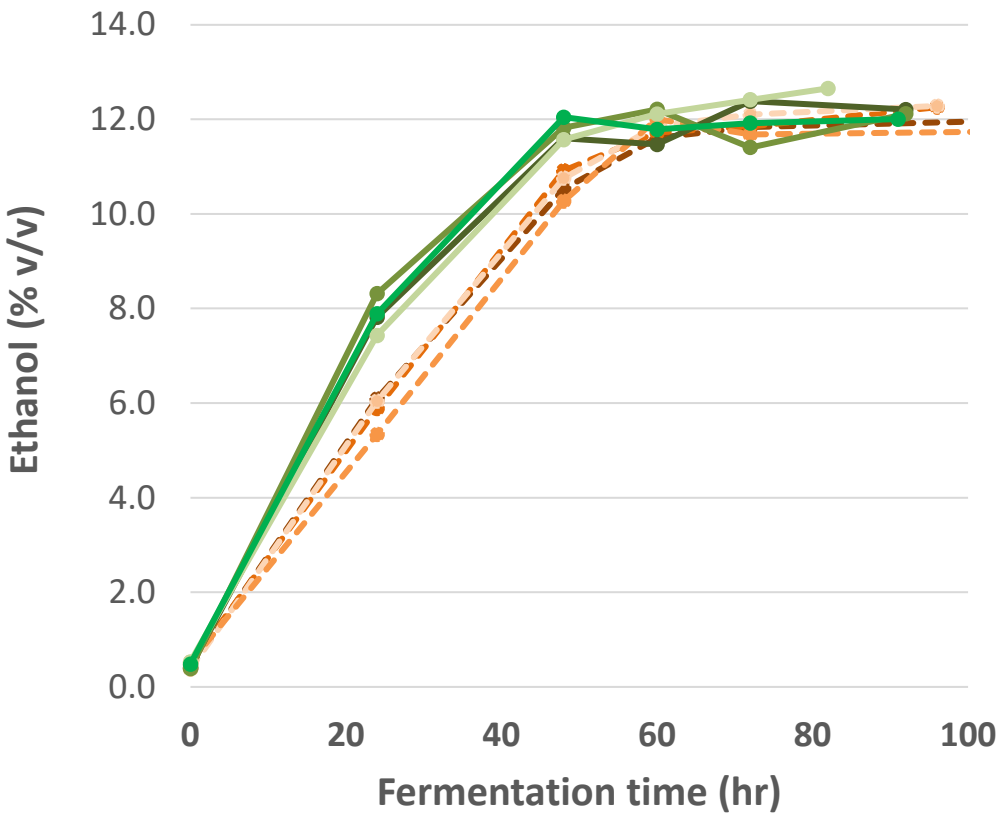
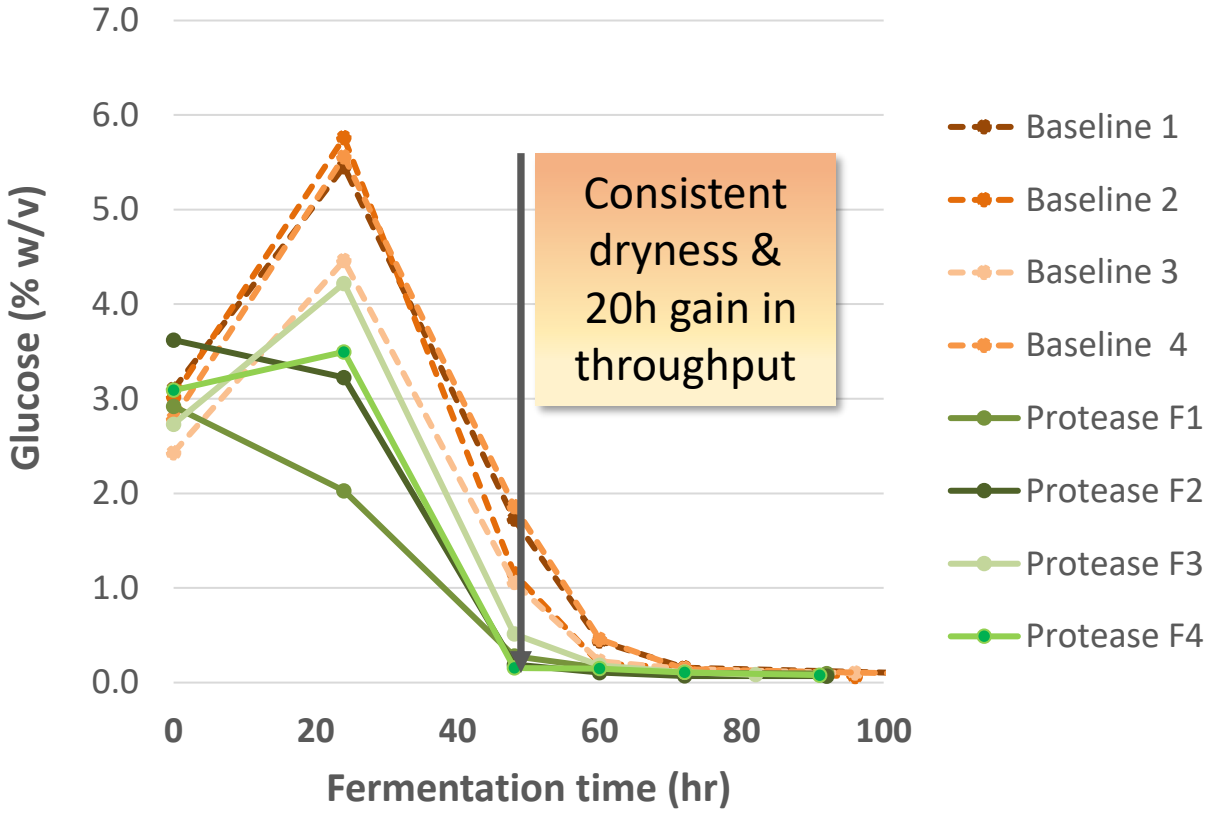
Proteases: Rye Whisky Plant Trial





Plant Data, Rye SSF

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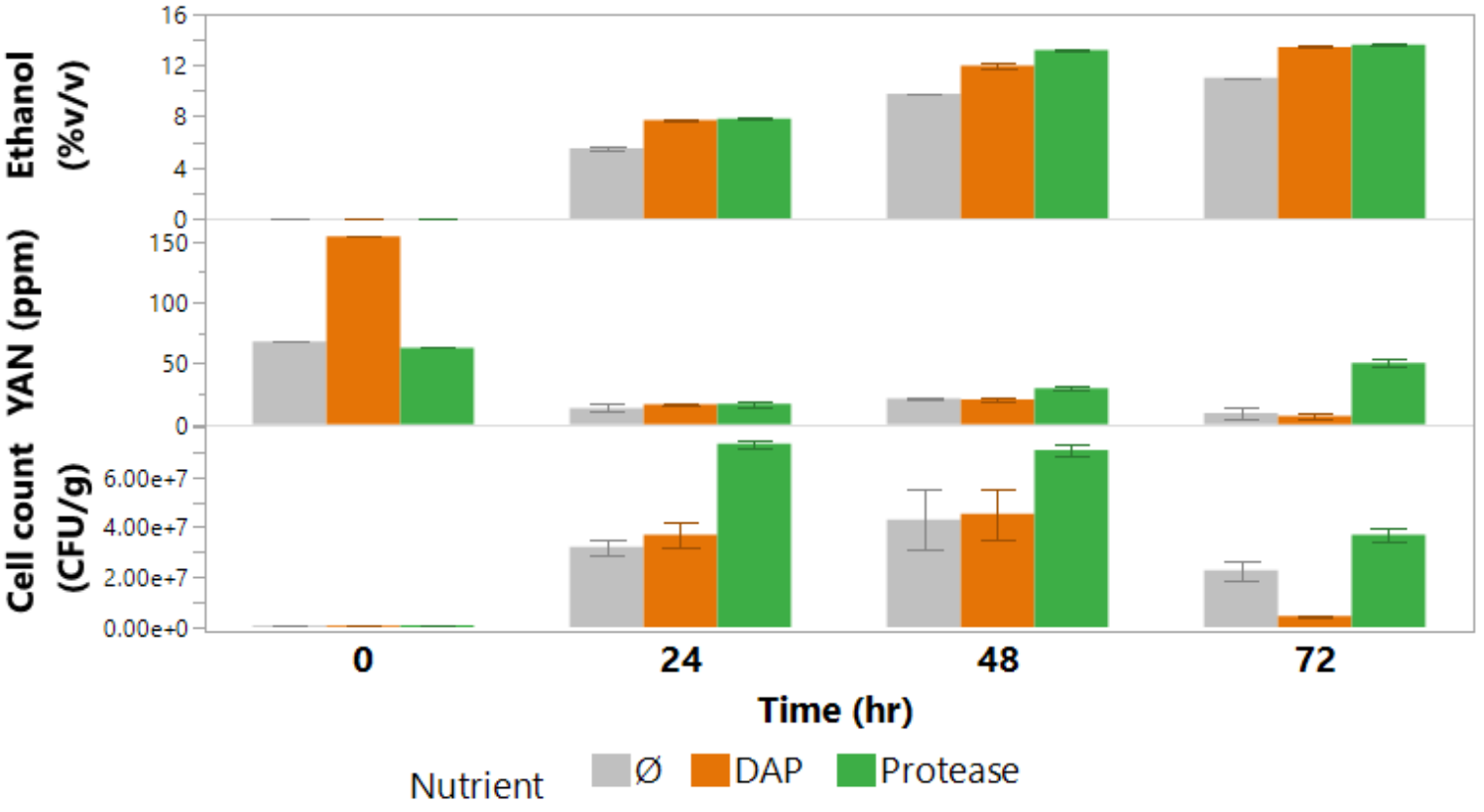
Impact of Nitrogen Sources on Yeast Viability



- Inorganic nitrogen is assimilated rapidly at the beginning of fermentation
- Organic nitrogen is released throughout the entire fermentation time sustaining higher cell viability

Laboratory Data, Corn SSF

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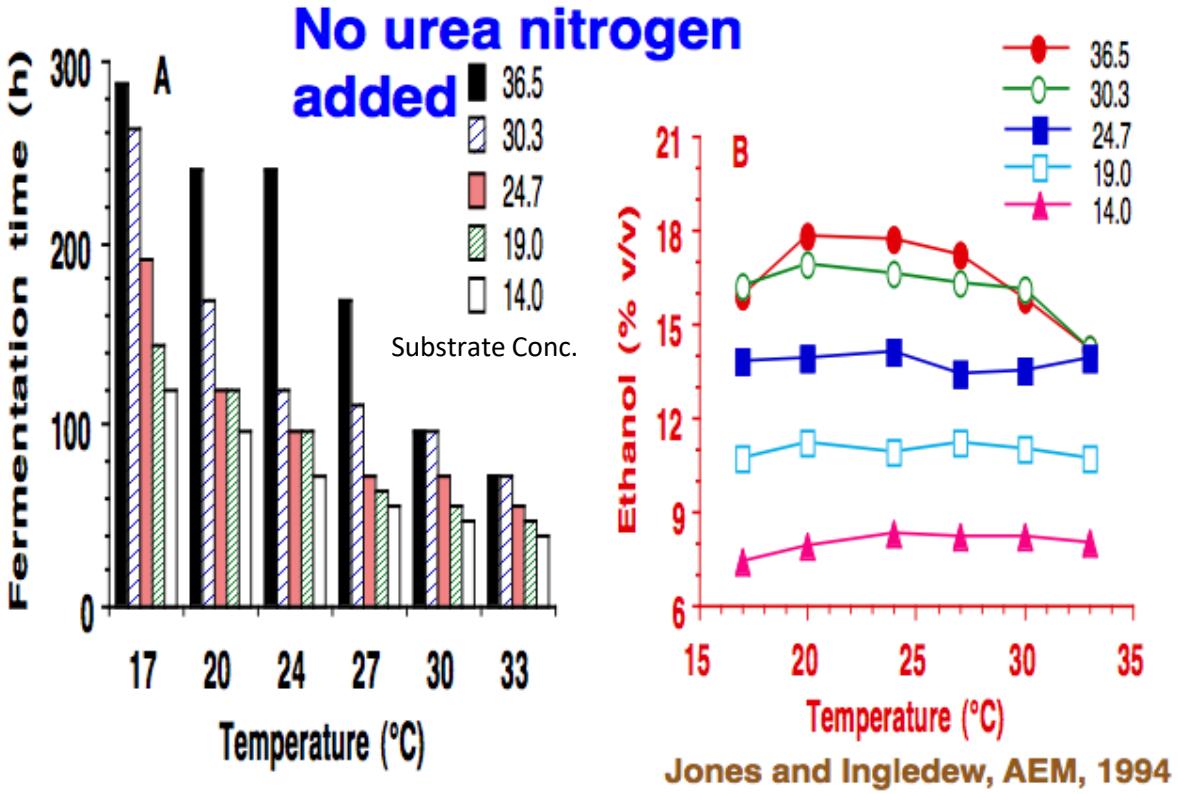
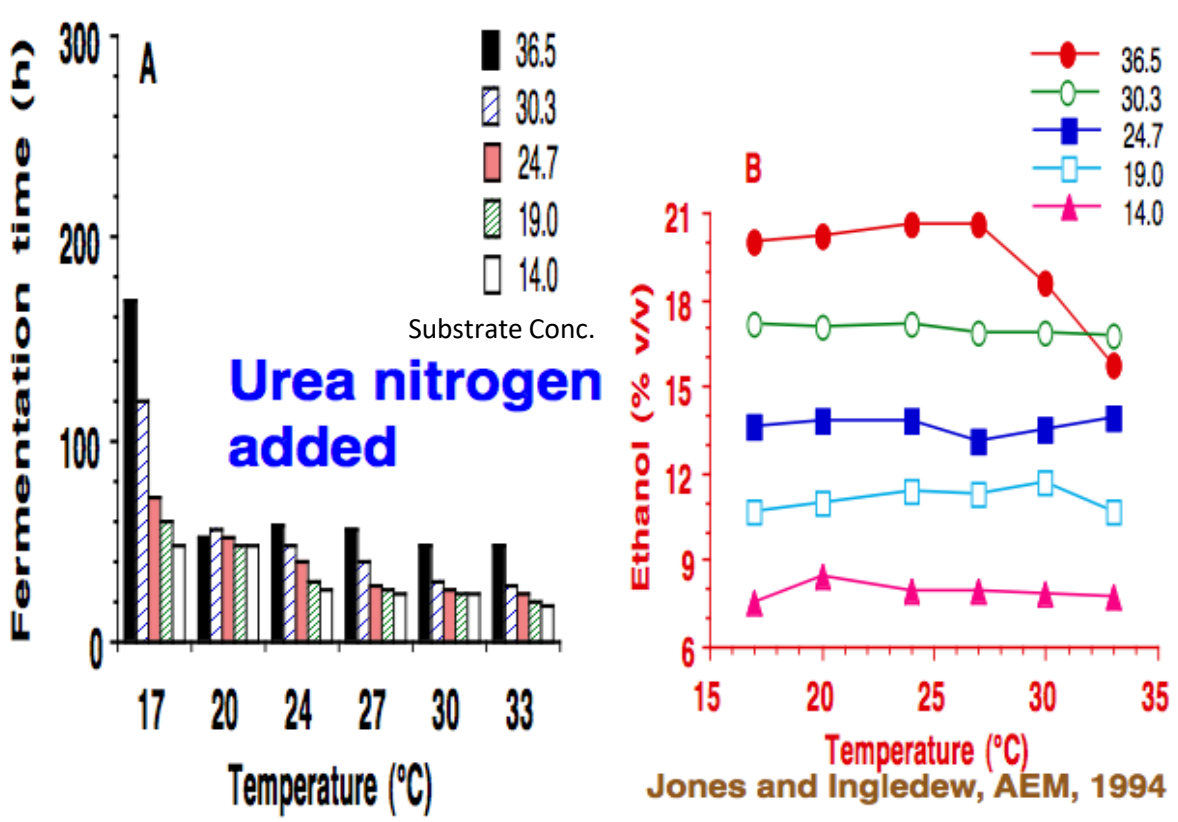
Impact of Nitrogen on Kinetics and Yield





82 mg/L FAN present in wheat mash

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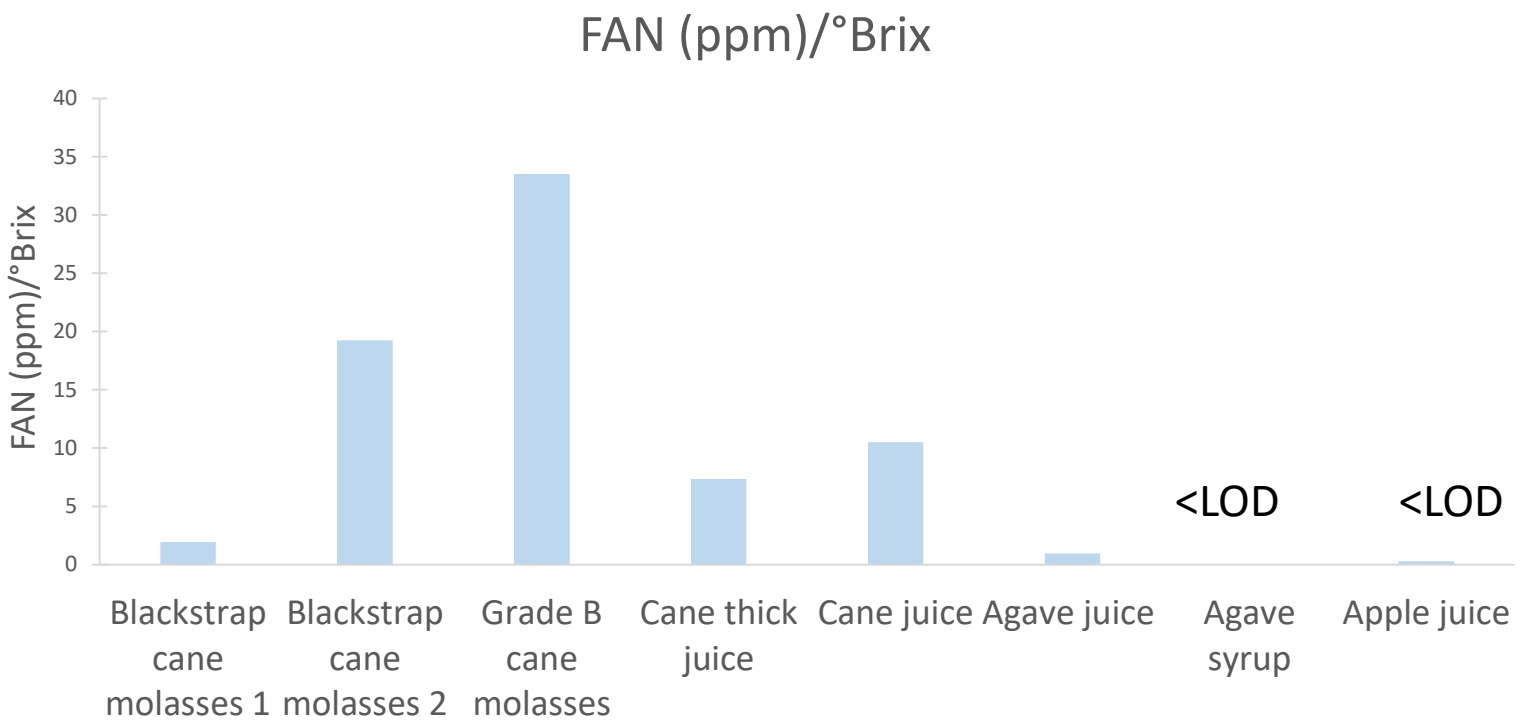
FAN in Sugar Feedstocks



- Sugar-based feedstocks generally benefit from nitrogen supplementation
- 200-250 ppm (mg/L) of YAN is required by most strains for growth and efficient fermentation

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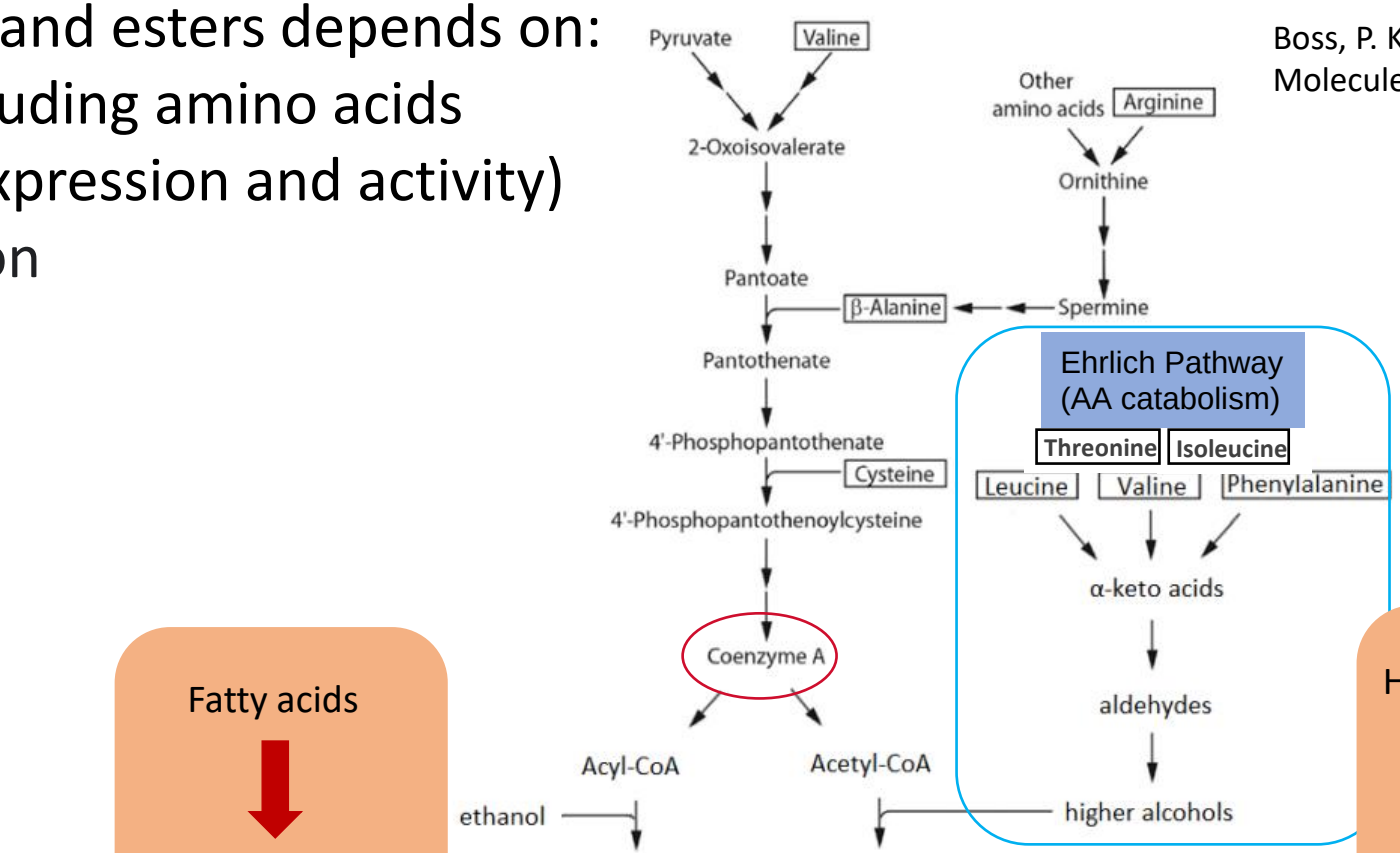
Role of Organic Nitrogen in the Biosynthesis of Higher Alcohols and Esters

Synthesis of higher alcohols and esters depends on:

— Precursor availability, including amino acids

— Yeast genetics (enzyme expression and activity)

— Conditions of fermentation



Boss, P. K. *et al.* 2015. Molecules

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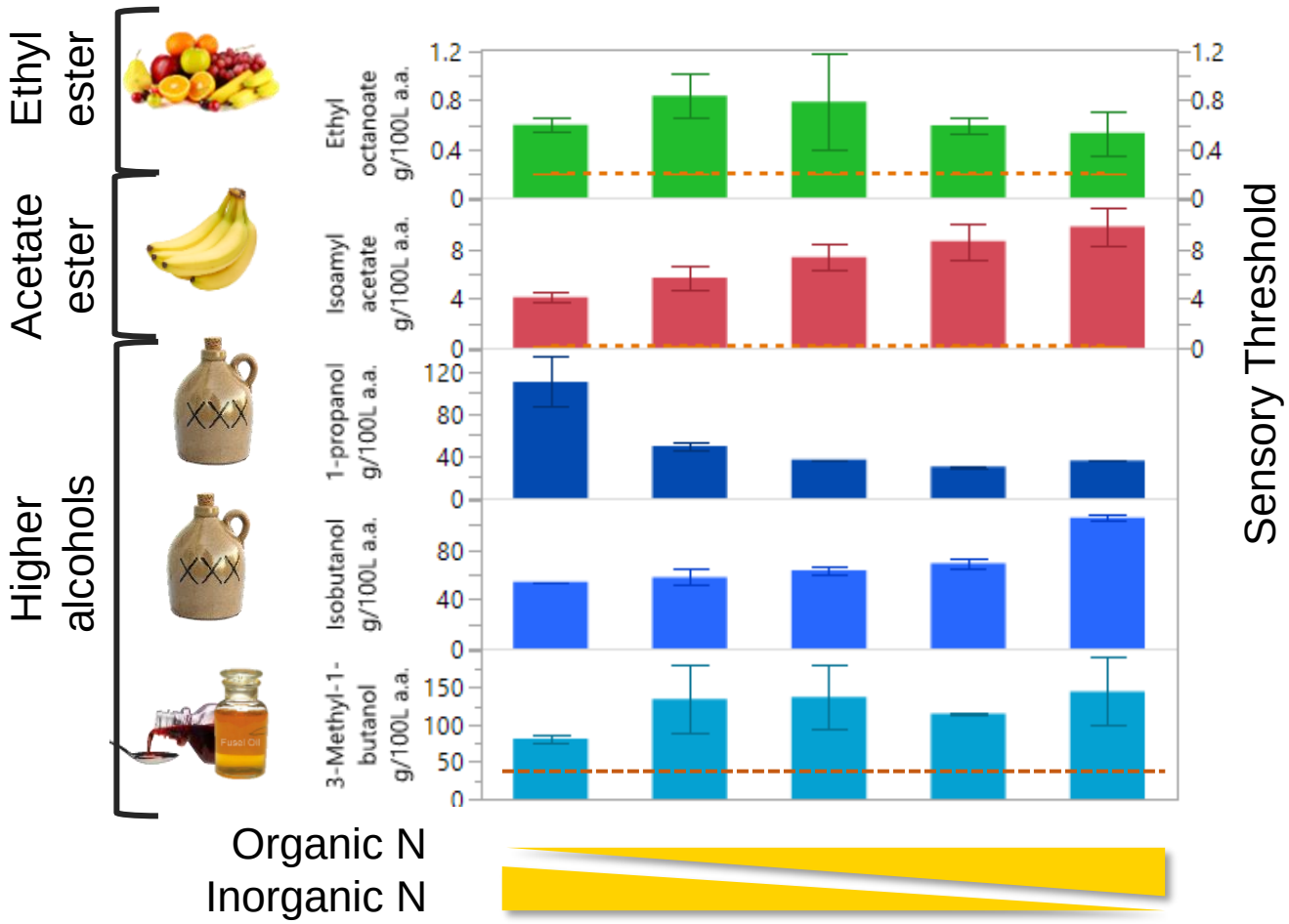
Impact of the Nitrogen Source on Congeners

- Lab-scale study in industrial whisky corn mash
- Different ratios of diammonium phosphate and amino acids tested but same total YAN = 300 ppm
- Distillation in small copper alembics

→ **The balance** between organic and inorganic nitrogen impacts flavour and aroma congener production.



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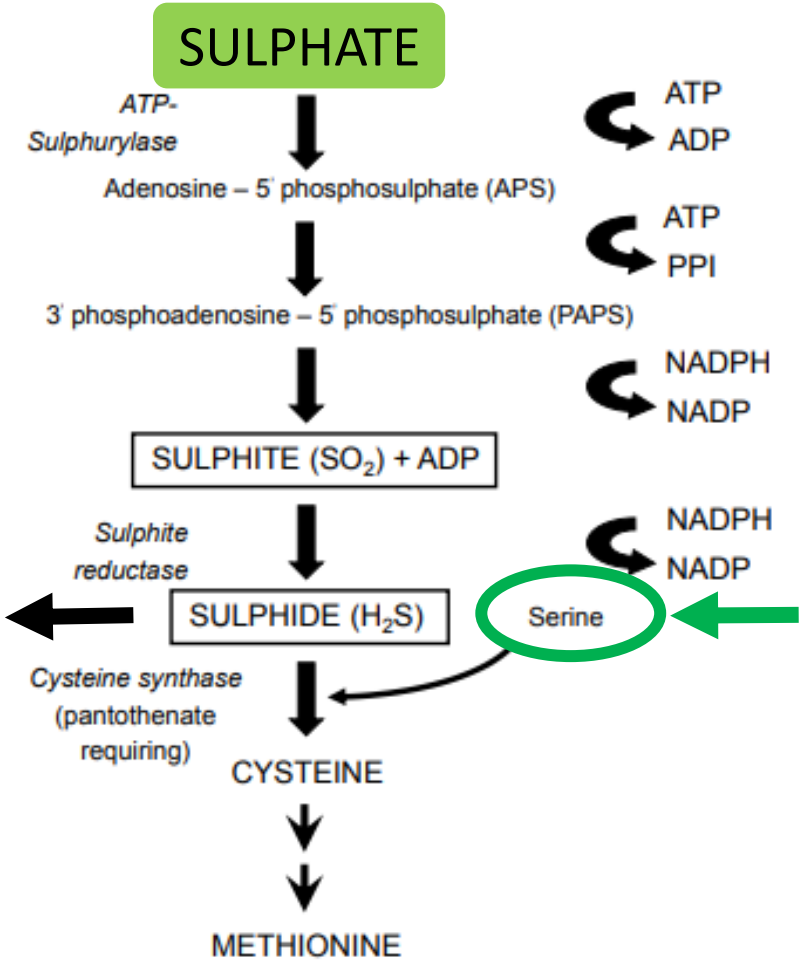
Role of Nitrogen in Sulfur Formation in Fermentation

- Accumulation of sulfur compounds in fermentation is **substrate and nutrition** dependent



DMS, DMSO from the substrate

NITROGEN (NH_4, amino acids)

Thiols

ALKIL SULFIDES (DMS, DMDS, DMTS, MMFDS)

CHEMICAL REACTION

- Major sulfur off-flavors in NMS
- Distillation control
- Role of copper

Stewart, G.G. 2017. Fermentation

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Nitrogen: Conclusions

- Nitrogen is limiting in most fermentations.
- Timely and adequate delivery of Nitrogen is one of the fundamentals in efficient fermentation.
- The majority of stuck or sluggish fermentations can be attributed to deficiencies in nitrogen.
- Ethanol/ beverage plants should target 200-250 ppm YAN at the start of fermentation to provide adequate nitrogen.
- Fermentation rate is directly related to available nitrogen. Above 700-800 ppm does not appear to improve fermentation.

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Oxygen

- We do not need for yeast to ferment
- We need some O_2 if we want a good synthesis of sterols and unsaturated fatty acids => better resistance of the yeast membrane during the last third of fermentation
- Fermentors and propagation remain anaerobic due to sugar levels. And the yeast savaging the oxygen.
- Our recommended rate is 5 -10 mg O_2/L per hour ONLY when yeast is growing.
- For 100,000 Litre PROP that is about 36 -72 litres per minute

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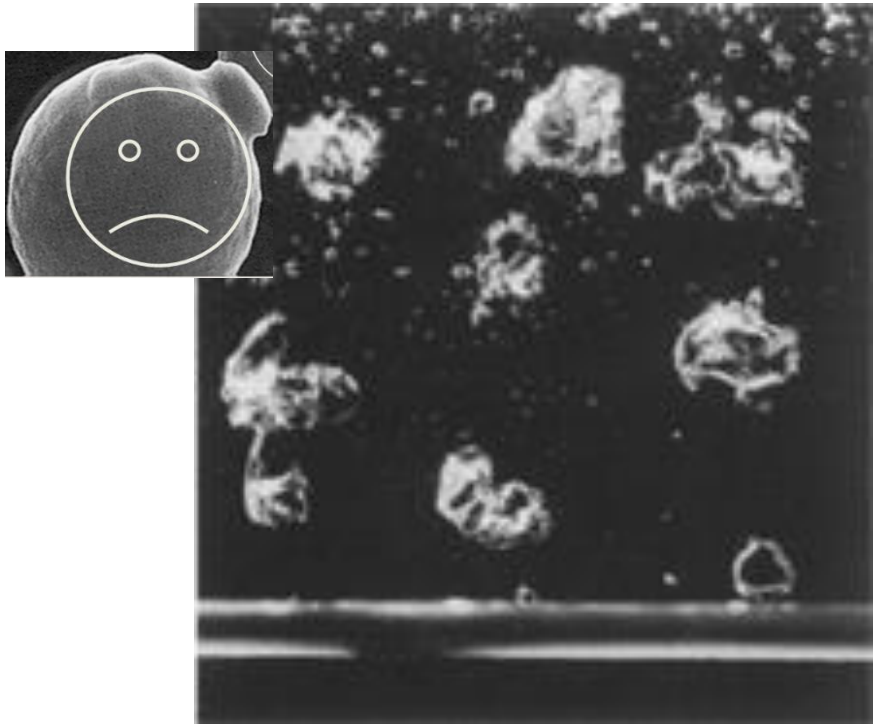
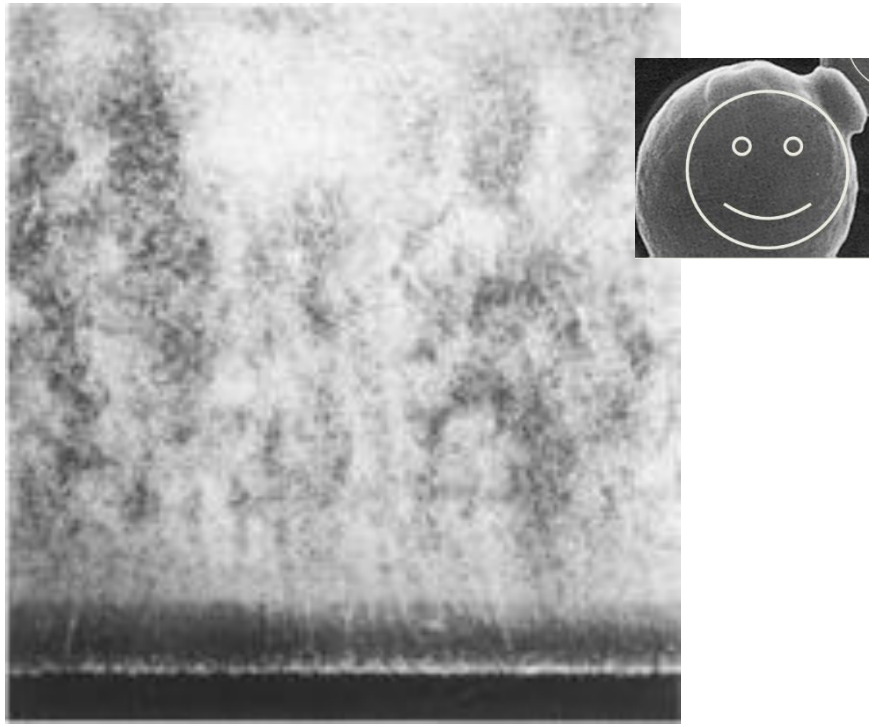
Oxygen

Add O_2 correctly at the right moment





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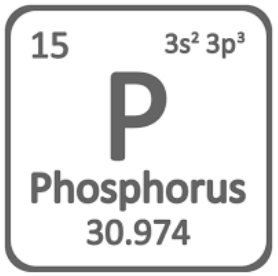
Phosphorus



- Yeast cell is about 1,8 % (from 1,4% to 2%) P
- Phosphates are essential for biosynthesis of nucleic acids, phospholipids and ATP
- Important for storage of cell energy reserves
- Used during the glycolysis
- Can be added in the form of DAP
- Phytases may help for liberation of useable phosphorous in grain mashes but too much liberation may make divalent cations unavailable

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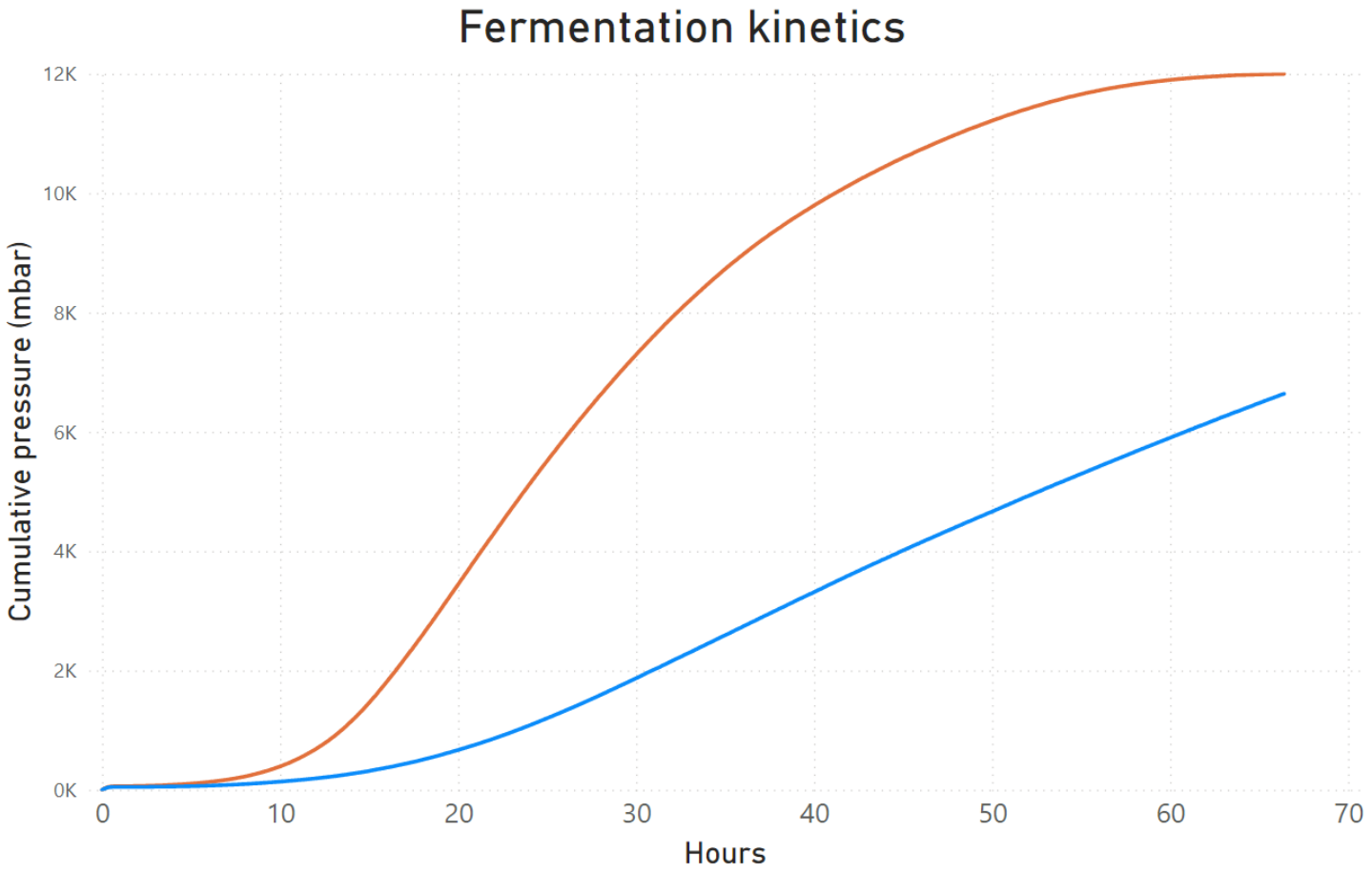
Impact of Phosphorus



Control

Uzbekistan molasses

Laboratory data

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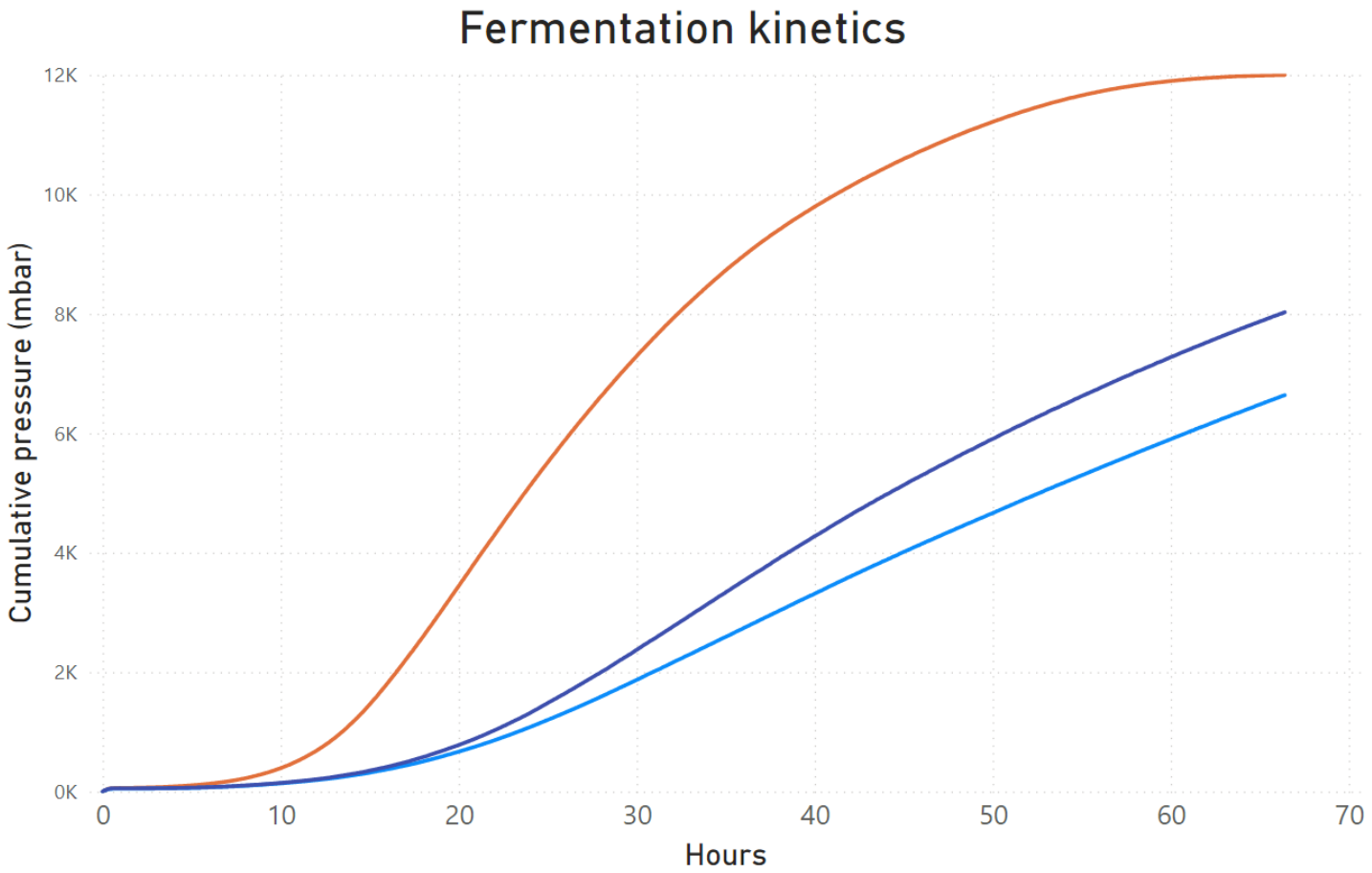
Impact of Phosphorus



Control

Uzbekistan molasses + urea

Uzbekistan molasses

Laboratory data
Urea: 300 ppm FAN

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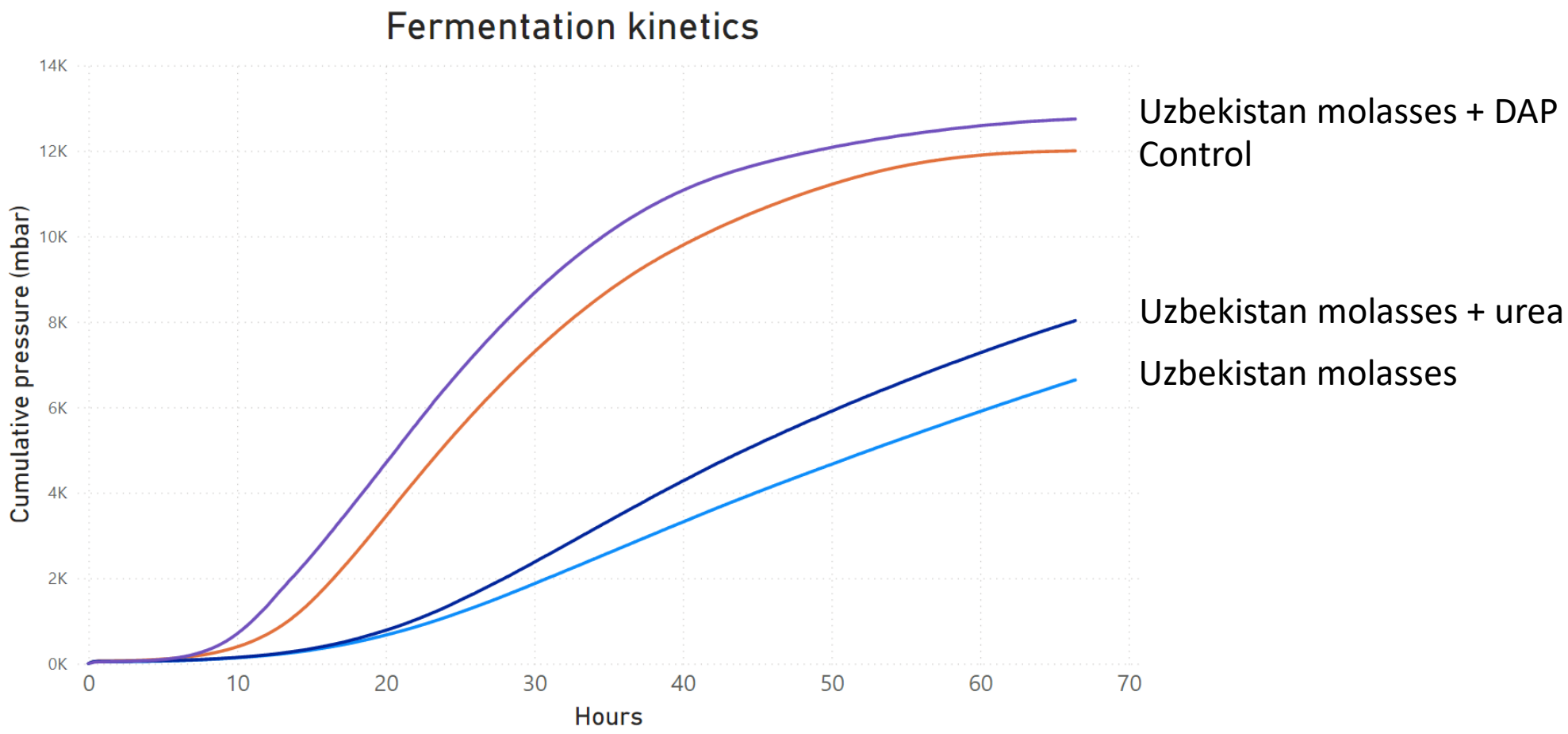
Impact of Phosphorus



Laboratory data

*targeting 300mg/L FAN in both DAP and Urea treatments

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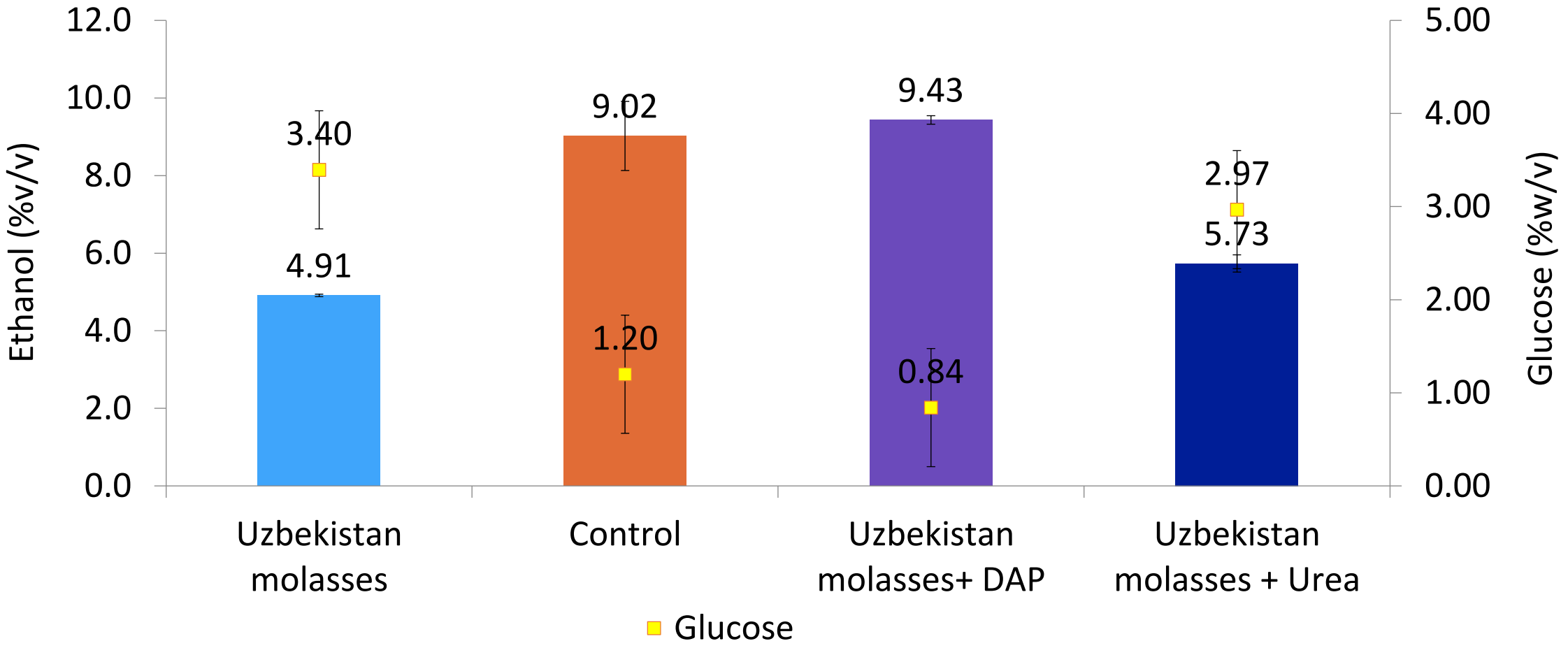
Impact of Phosphorus



Laboratory data

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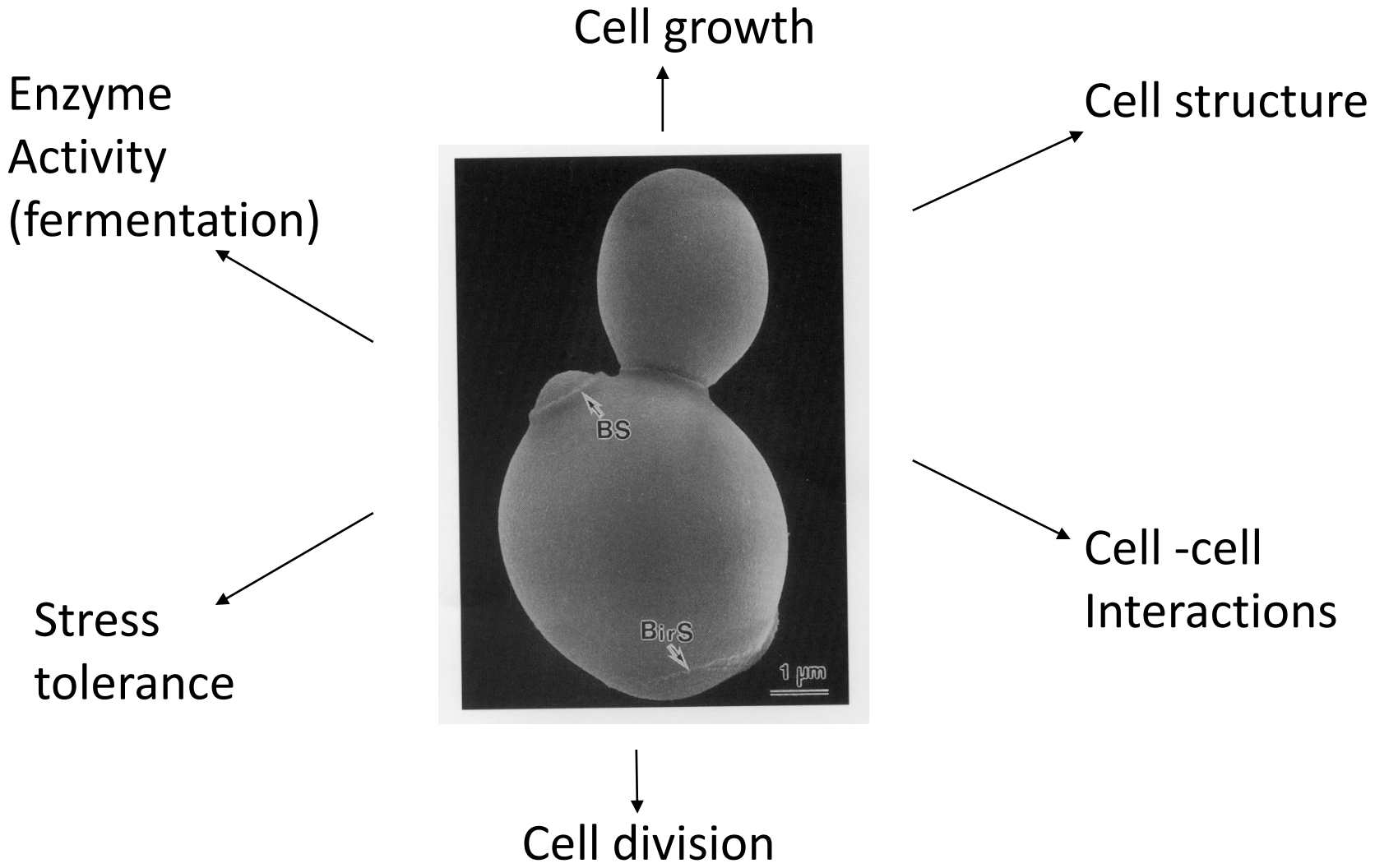
Yeast Nutrition: *Why Do Yeasts Need Metals and Vitamins?*



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Yeast Nutrition in Fermentation: Minerals & Vitamins

Macroelements

- Phosphorus, sulfur, magnesium, potassium

Microelements

- Zinc, manganese, iron, calcium, cobalt, boron, cadmium, chromium, iodine, molybdenum, nickel, and vanadium

Key vitamins

- Biotin, pantothenic acid
- Inositol, thiamin, nicotinic acid and pyridoxine

Most of these are supplied by mash

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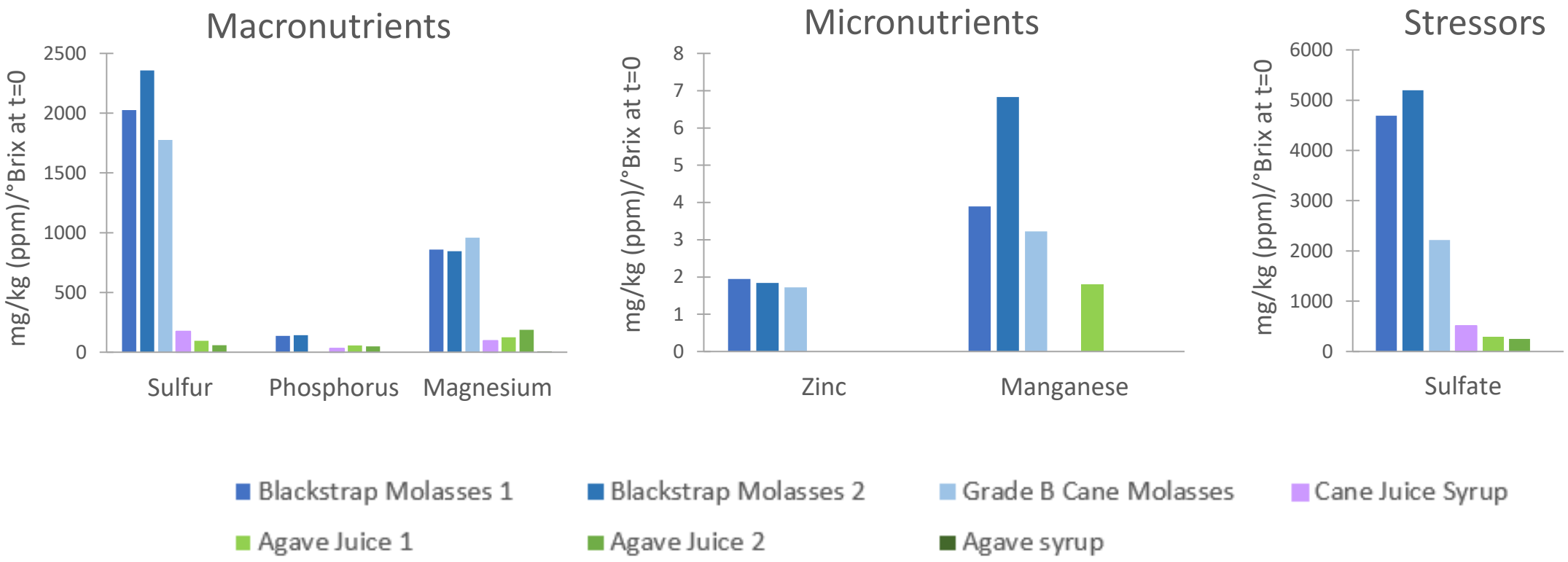
Inorganic Ions in Sugar Feedstocks



°Brix used for the graphs:
Molasses 25 °Brix
Agave juice: 9-10 °Brix
Agave syrup: 15 °Brix

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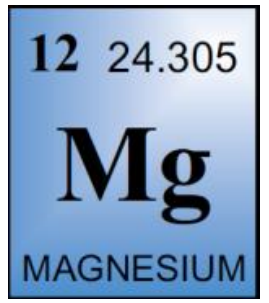
Nutrition: Magnesium



- Absolutely essential (growth, fermentation and to resist stress factors)
- Cell Mg correlates with viability/vitality
- Maintains structural integrity of yeast in correlation with the phospholipides => Helps to insulate the cell against stress factors such as temperature, osmotic pressure and alcohol
- Required for glycolysis : for each phosphorylation reaction
- Stored in the yeast vacuole for a part.

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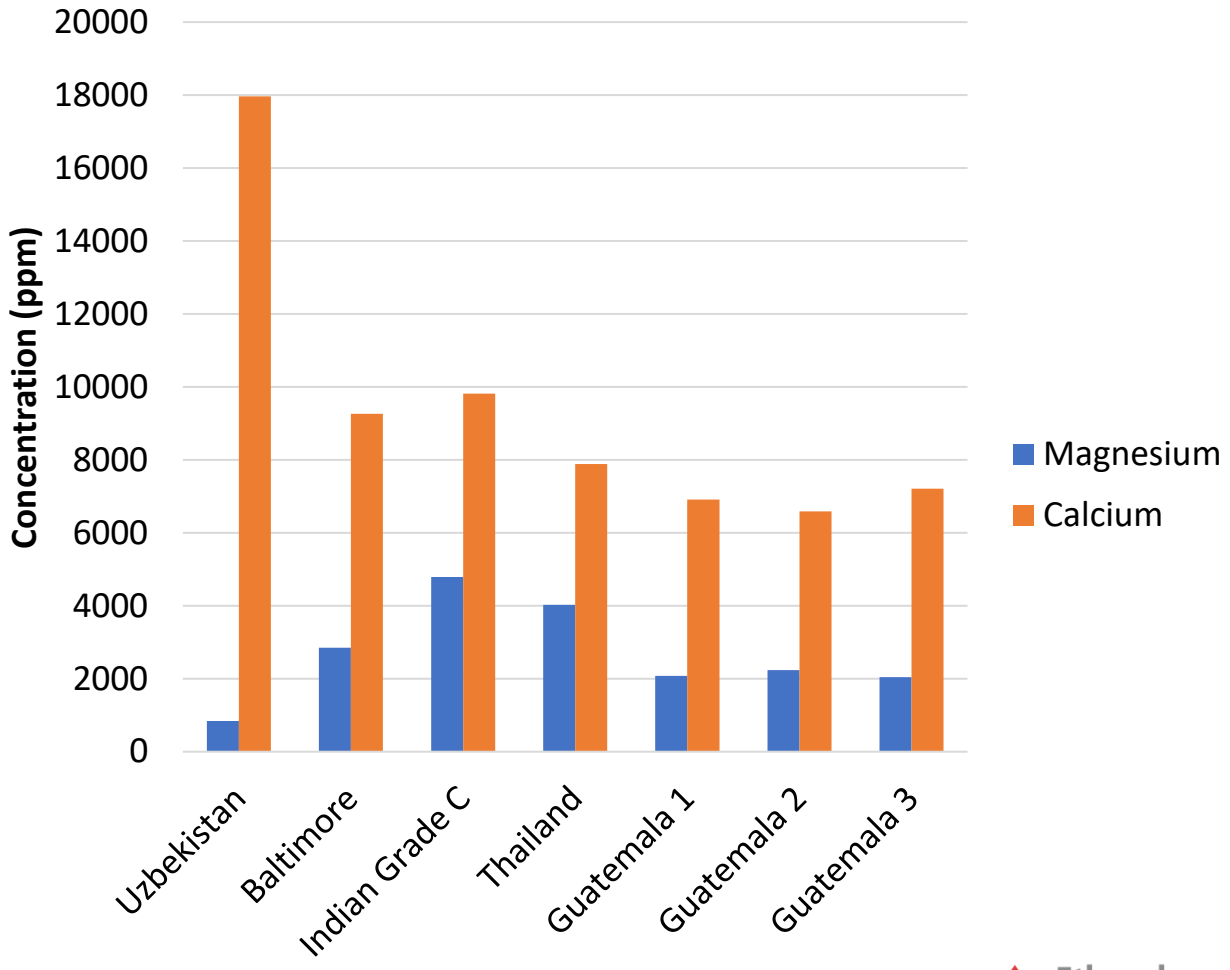
Molasses Fermentation Challenges

- Mg^{2+}:Ca^{2+} (Particularly with cane molasses)
  - Magnesium benefits glucose uptake during fermentation however, high calcium levels prevent sugar assimilation resulting in poor yeast health and reduced ethanol tolerance
  - Managing this ratio is important for fermentation



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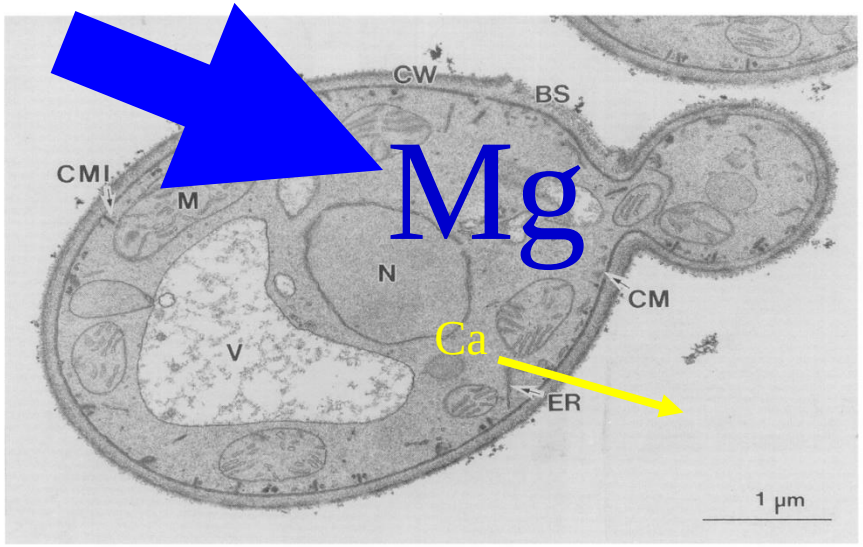
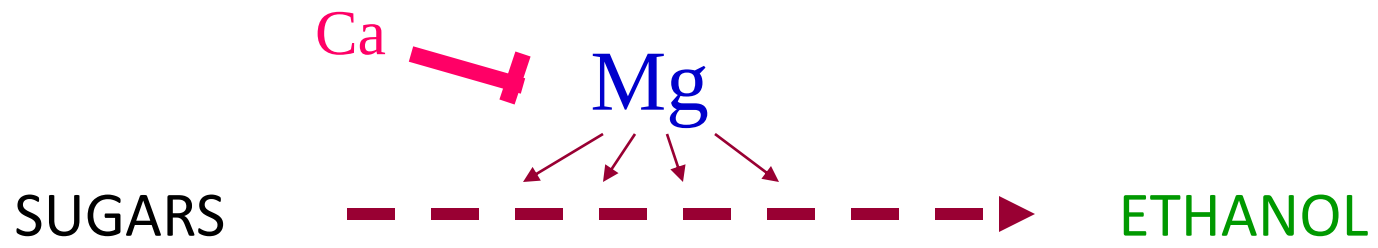
Nutrition: Magnesium vs Calcium

- Cells actively include Mg, but exclude Ca
- High growth demand for Mg, but not for Ca
- Mg required for many enzymes, Ca very few





Ca (which is not needed in high amounts by yeast) acts antagonistically toward Mg

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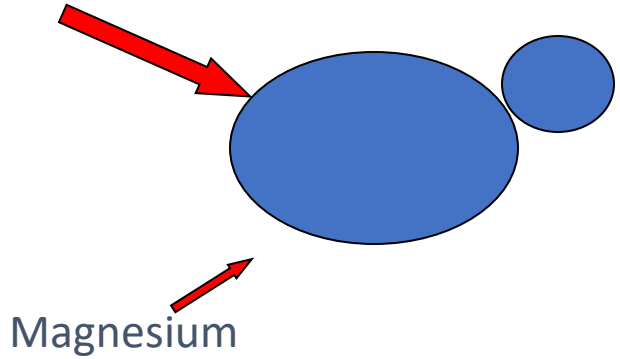
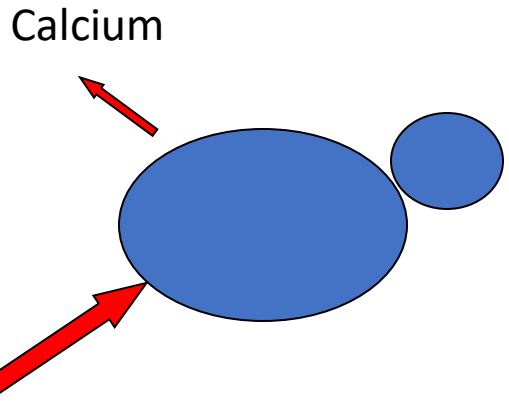
Nutrition: Magnesium vs Calcium in Molasses

Industry's View

CALCIUM



Yeast's View



MAGNESIUM

Therefore: ***Maintain a high Mg:Ca ratio in fermentation media***

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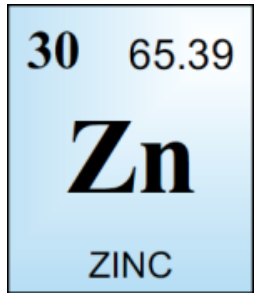
Nutrition: Zinc

The most common trace element deficiency in yeast is ZINC



- Rapidly taken up by yeast
- Vacuole is main sink for zinc
- It should be around 0,4 ppm – 1 ppm
- Zinc-limitation (<0,1 ppm) may cause slow or sluggish fermentations and poor yields
- Stabilizes cell membranes - possible role in stress
- Result in consistent flavour profiles for beverage alcohol
- Zinc is a requirement for activity of alcohol dehydrogenase (ADH), the enzyme converting acetaldehyde to ethanol (and other enzymatic reactions)
- An excess can be problematic if similar amounts of Manganese are not present

(Walker *et al.*)

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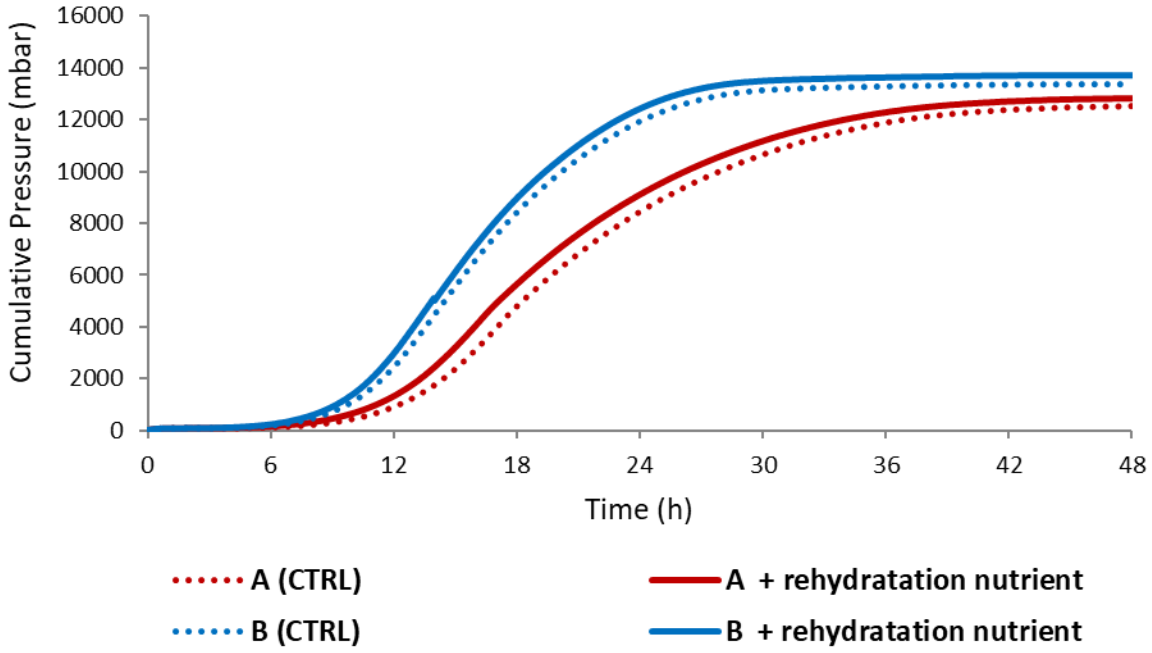
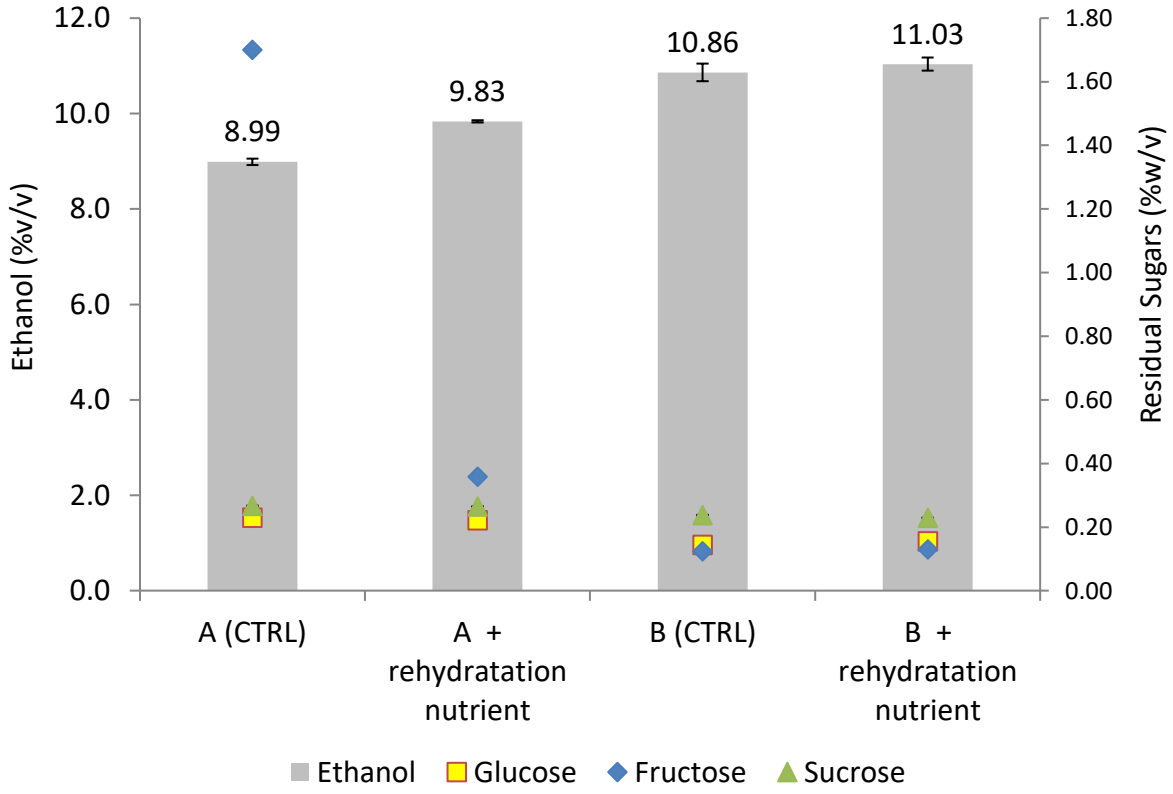
Effect of Complex Rehydration Nutrient on Molasses Fermentations

Ferm °Brix: 29.5; pH = 5.1; FAN = 57 ppm
576 ppm urea added in fermentation
Yeast pitch: 0.35 g/L; 35 °C, 48 hr fermentation
Batch laboratory scale fermentations





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Vitamins

- Vitamins are important regulators and cofactors of numerous metabolic processes
- Most strain have an absolute requirement for biotin, and many require pantothenate and inosiltol
- Grain mash should contain sufficient amount of vitamins
- Molasses: vitamins concentrations vary for cane and beet molasses
  - Biotin is normally present in great excess in cane molasses, but is normally deficient in beet molasses.
  - Pantothenate is normally borderline-to adequate in cane molasses, but is generally in excess in beet molasses

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Nutrition for Consistency in Fermentation

- Nutrition allows for consistency among batches
- It is a good idea doing small scale tests at the reception of new batches of substrates!

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Conclusions

- Nutrition is essential for yeast metabolism and therefore for efficient and consistent fermentation and congener biosynthesis.
- Different substrates have different composition and required different nutrition solutions.
- Organic and inorganic nitrogen sources are assimilated differently from yeast and affect yeast viability, metabolism and congener accumulation differently.
- Nitrogen supplementation must be balanced to maximize flavour congener synthesis and contribute in controlling off-flavour congener synthesis.

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Thank you for your attention!
Any Questions?

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