XCELIS® Ethanol Solutions

FUSELS, PHYSIOLOGY, AND EARLY DETECTION AT THE PLANT

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Applied Innovation Center

IFF - APPLIED INNOVATION CENTER

Cedar Rapids, Iowa

Lab-based plant support

- Pre-trial testing
- Trial evaluation
- Optimization
- Troubleshooting

Liquefaction Services

- Cook studies
- Solubility
- Cations (Sodium, etc)

Fermentation Services

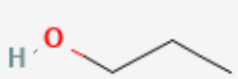
- Prop and ferm studies
- DP4+ composition
- Detailed sugar analysis
- HPLC checks
- Residual starch
- Nitrogen measurements
- Inhibitors (fusels, sodium, sulfite, organic acids, etc)

Fusels and yeast physiology

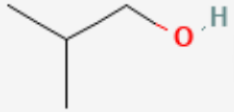
WHAT ARE FUSELS?

- Volatile, higher chain aliphatic and aromatic alcohols
- Boiling point higher than ethanol
- >40 different alcohols produced by yeast but n-propanol, isobutanol, isoamyl alcohol, and active amyl alcohol are the most abundant and relevant
- Normal byproduct of fermentation by yeast, typically measured in ppm quantities
- Fusel profile is highly yeast and process dependent
- Organoleptic properties, used in flavoring and fragrances
- Known contributor to hangovers/headaches

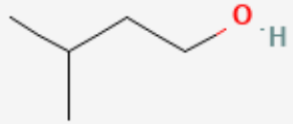
N-propanol



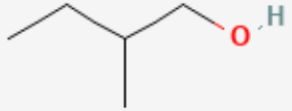
Isobutanol



Isoamyl Alcohol

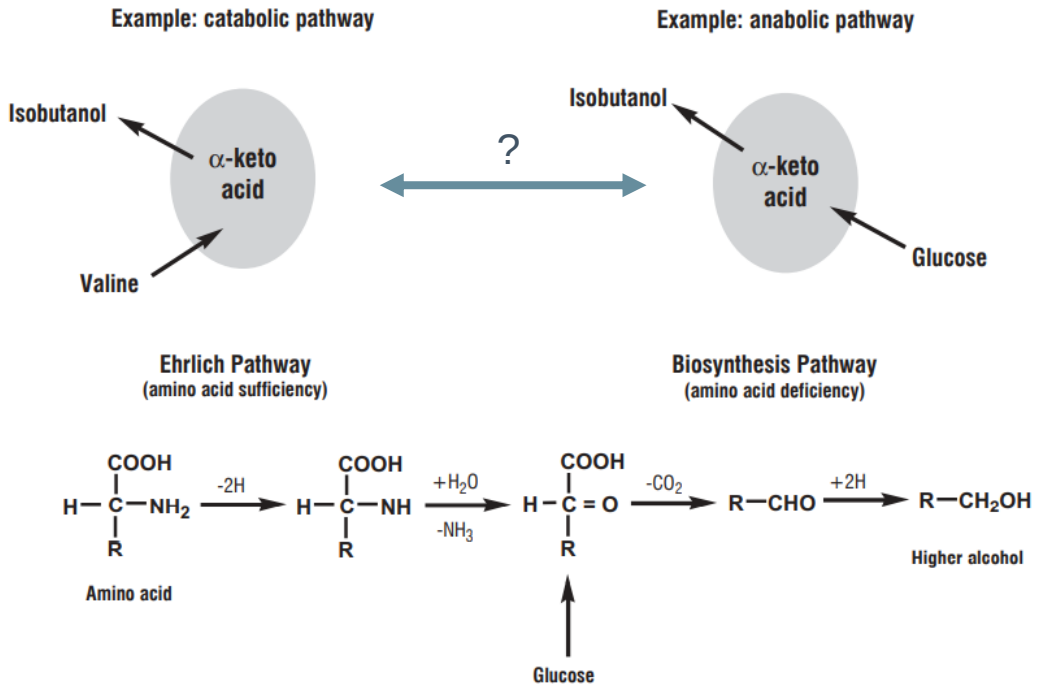


Active Amyl Alcohol



PATHWAYS FOR FUSEL FORMATION

- Can be formed from anabolic or catabolic pathways
- Which pathway is used and how much it contributes to fusel levels is nuanced



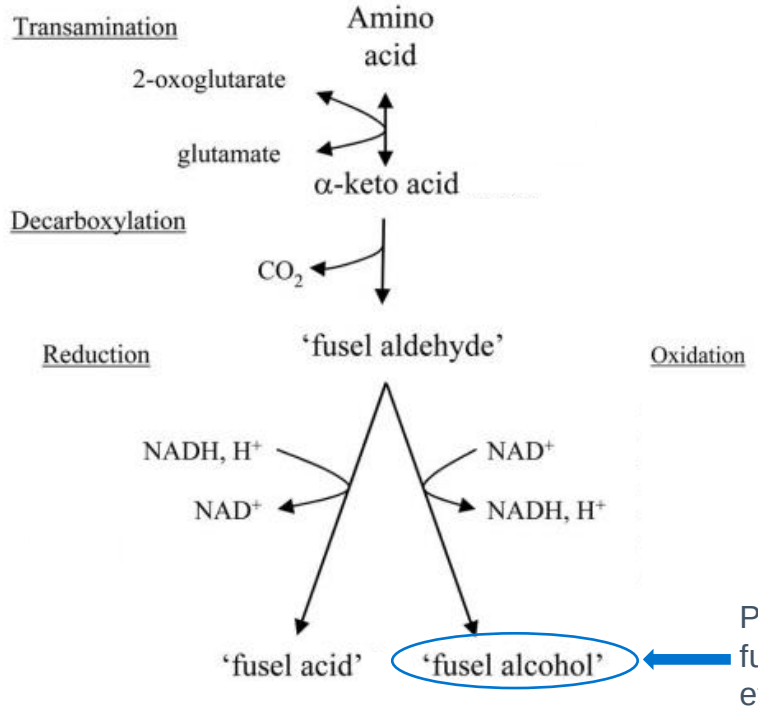
Related Amino acid pathway	Fusel formed (Systematic name)	Fusel formed (common name)
Threonine	1-propanol	N-propanol
Valine	2-Methyl-1-propanol	Isobutanol
Leucine	3-Methyl-1-butanol	Isoamyl alcohol
Isoleucine	2-Methyl-1-butanol	Active amyl alcohol

Reference: The Alcohol Textbook

AMINO ACID CATABOLISM AND YEAST NITROGEN DEMAND

- **Free amino acids can be incorporated directly into protein synthesis in the cell**
- **If the cell is low on preferred amino acids or other nitrogen sources, non-preferred amino acids can be catabolized, and the resulting nitrogen used for synthesis of preferred AA**
- **When branched chain AA are catabolized, it is via the Ehrlich pathway, resulting in fusel alcohols**
- **May also be some additional benefit to redox balancing by making fusels**

Ehrlich pathway



Predominant fusel form in ethanol plants

The Ehrlich Pathway for Fusel Alcohol Production: a Century of Research on *Saccharomyces cerevisiae* Metabolism[▽]

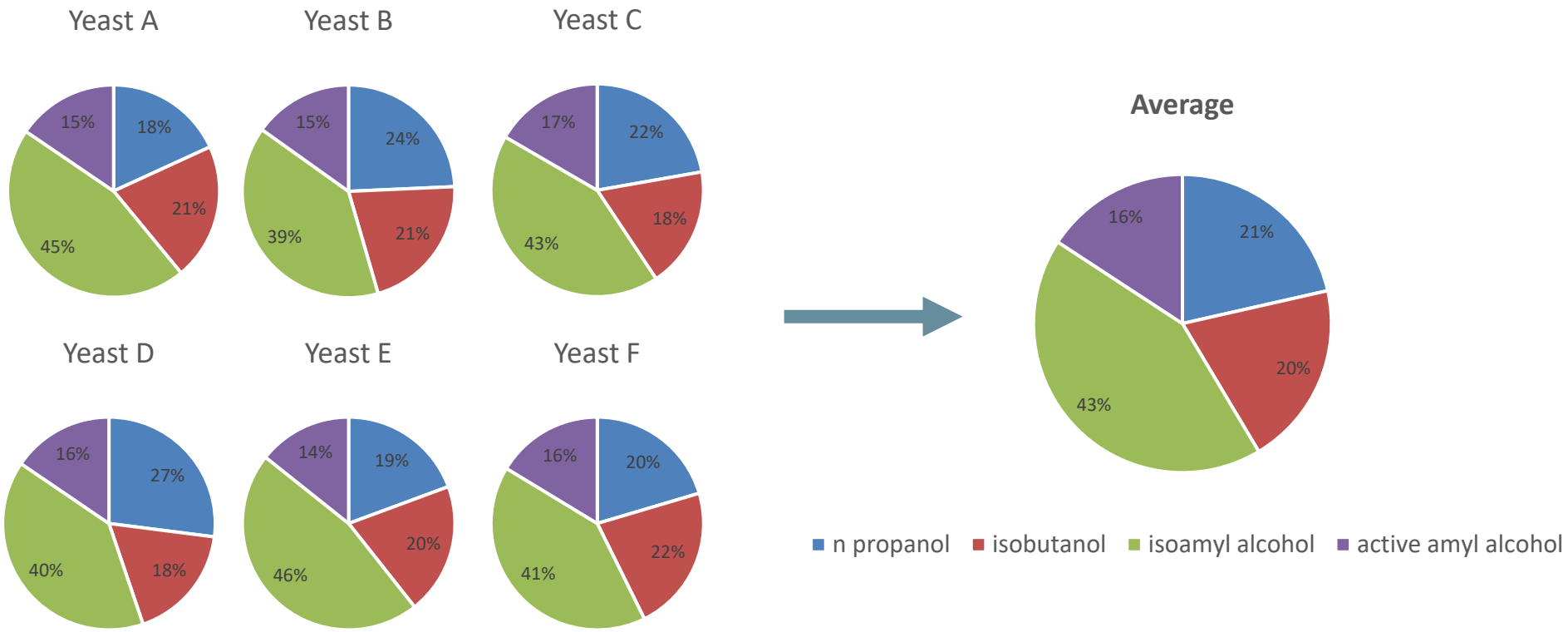
Lucie A. Hazelwood,[1,2] Jean-Marc Daran,[1,2] Antonius J. A. van Maris,[1,2] Jack T. Pronk,[1,2] and J. Richard Dickinson[3]*

Department of Biotechnology, Delft University of Technology,[1] and Kluyver Centre for Genomics of Industrial Fermentation,[2] 2628 BC Delft, The Netherlands, and Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3TL, United Kingdom[3]

Modified from:

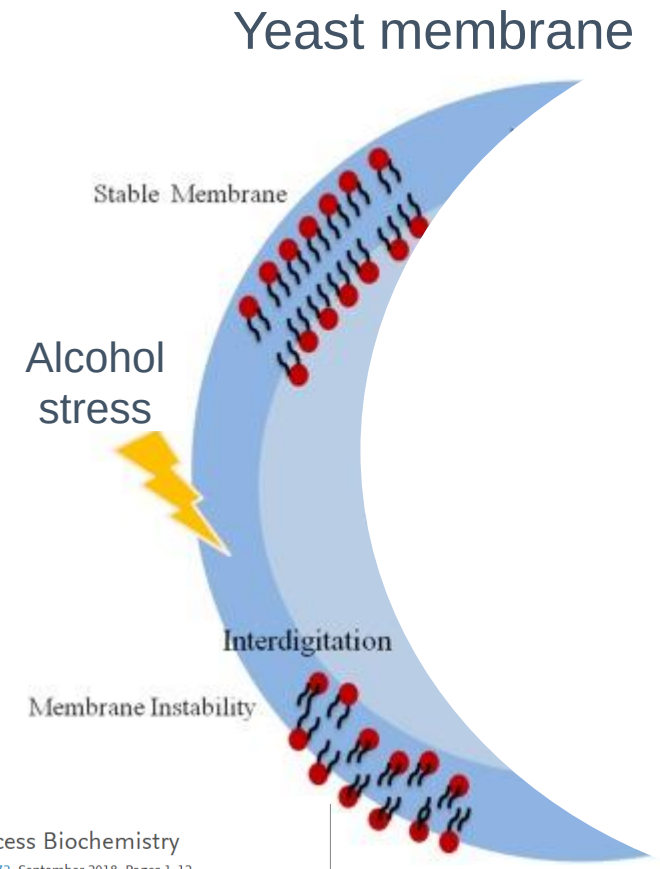
FUSEL PRODUCTION DIFFERENCES BY YEAST

Ratios shift slightly depending on yeast but isoamyl alcohol is always the highest fusel produced and active amyl alcohol is the lowest.



MECHANISM OF FUSEL INHIBITION

- **Fusels and other alcohols accumulate in the yeast membrane, increasing it's fluidity, disrupting structure and stability**
- **Compromised membrane results in:**
  - Decreased proton motive force
  - Inability to transport solutes across the membrane
  - Reduced ability for cellular maintenance, growth inhibition, and cell death



Modified from:

ELSEVIER

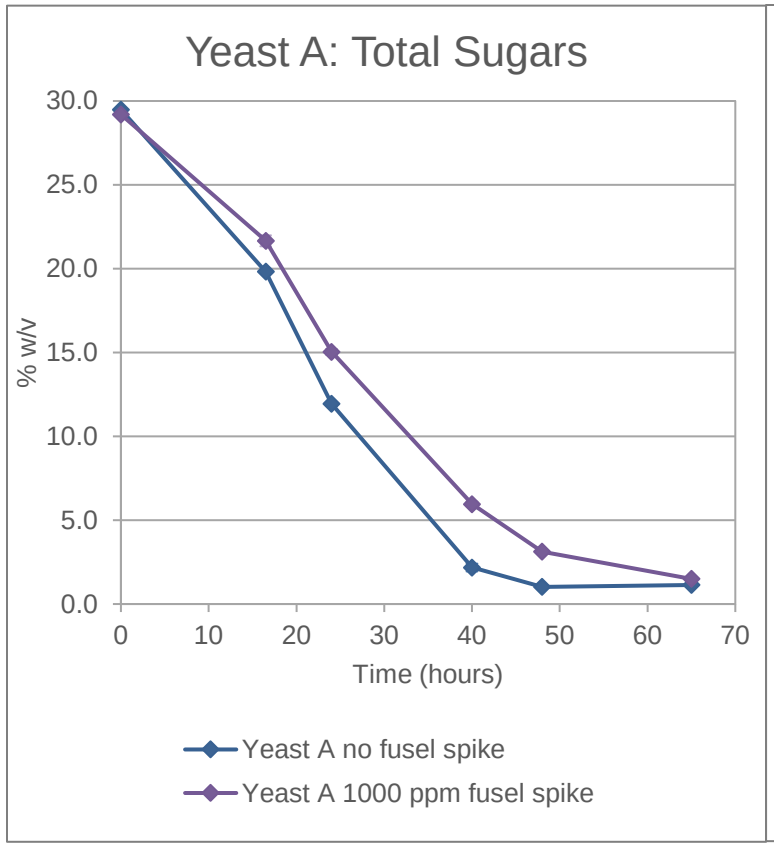
Process Biochemistry
Volume 72, September 2018, Pages 1-12

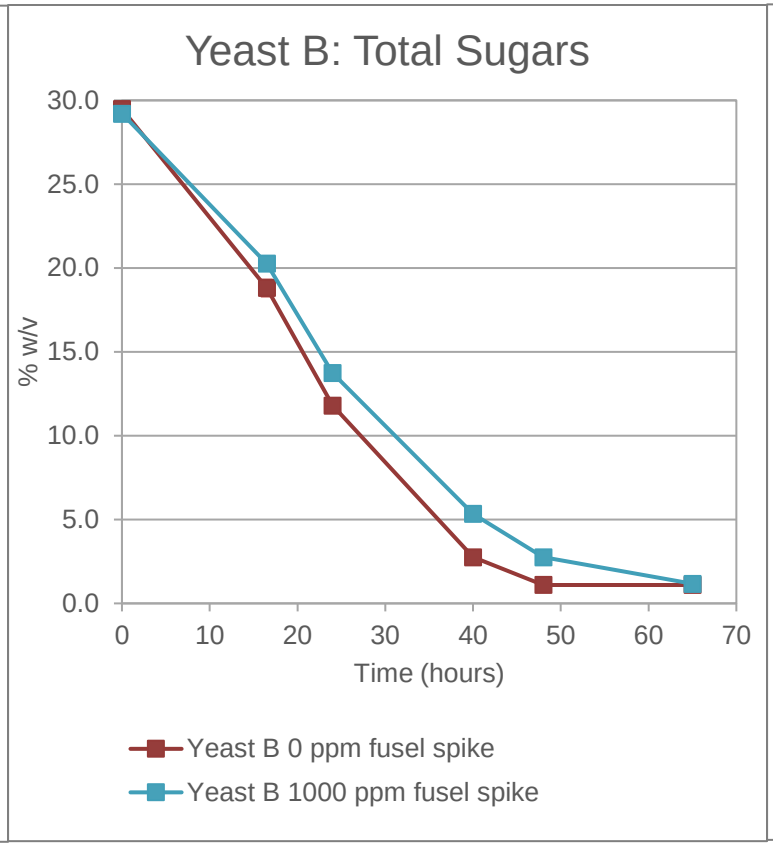
Response and tolerance of yeast to changing environmental stress during ethanol fermentation

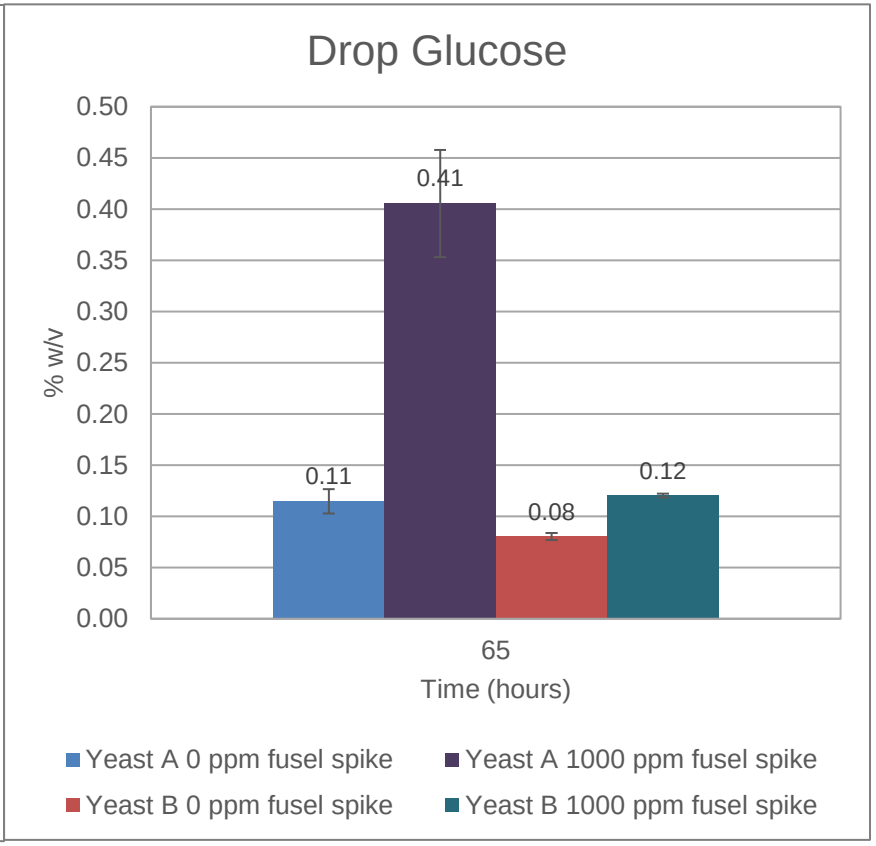
Priyanka Saini, Arun Beniwal, Anusha Kokkiligadda, Shilpa Vij

IMPACT OF FUSEL RECYCLE ON YEASTS

All yeasts are impacted by fusel recycle, which could slow finishing time by up to 10 hours or in low robustness yeasts cause fermentation to not finish



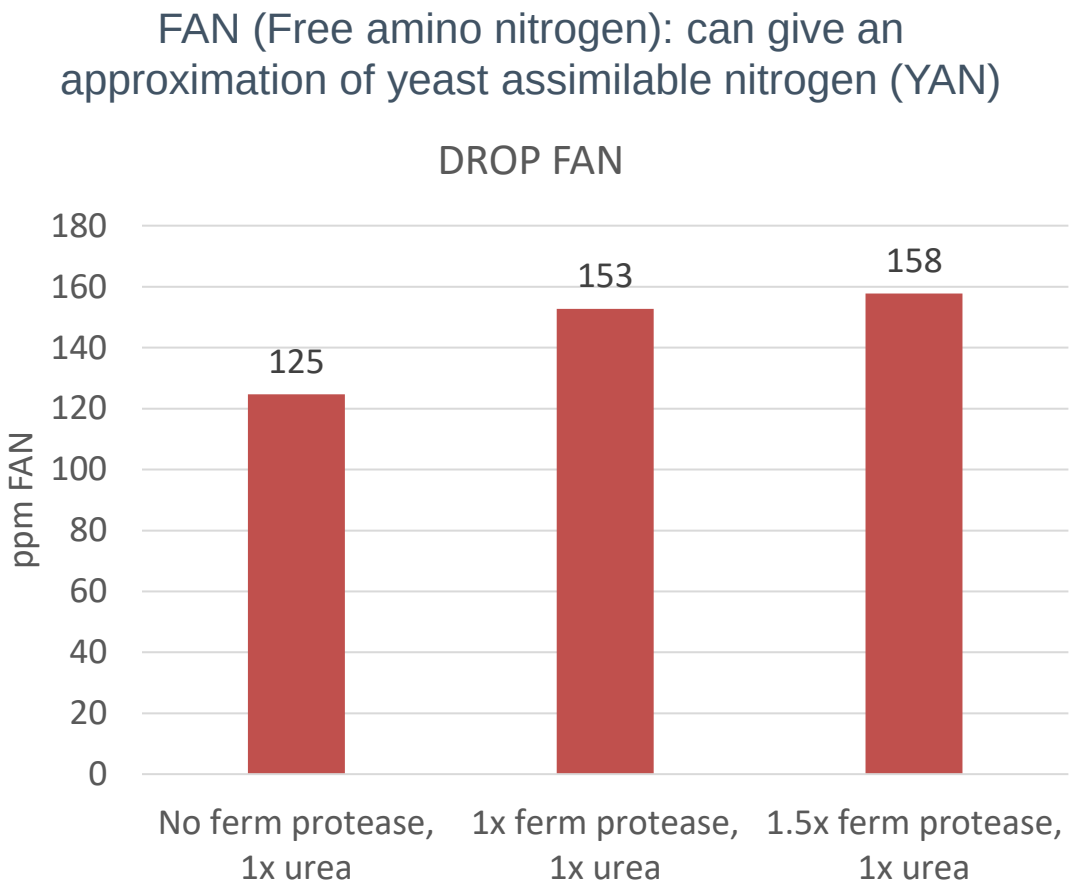


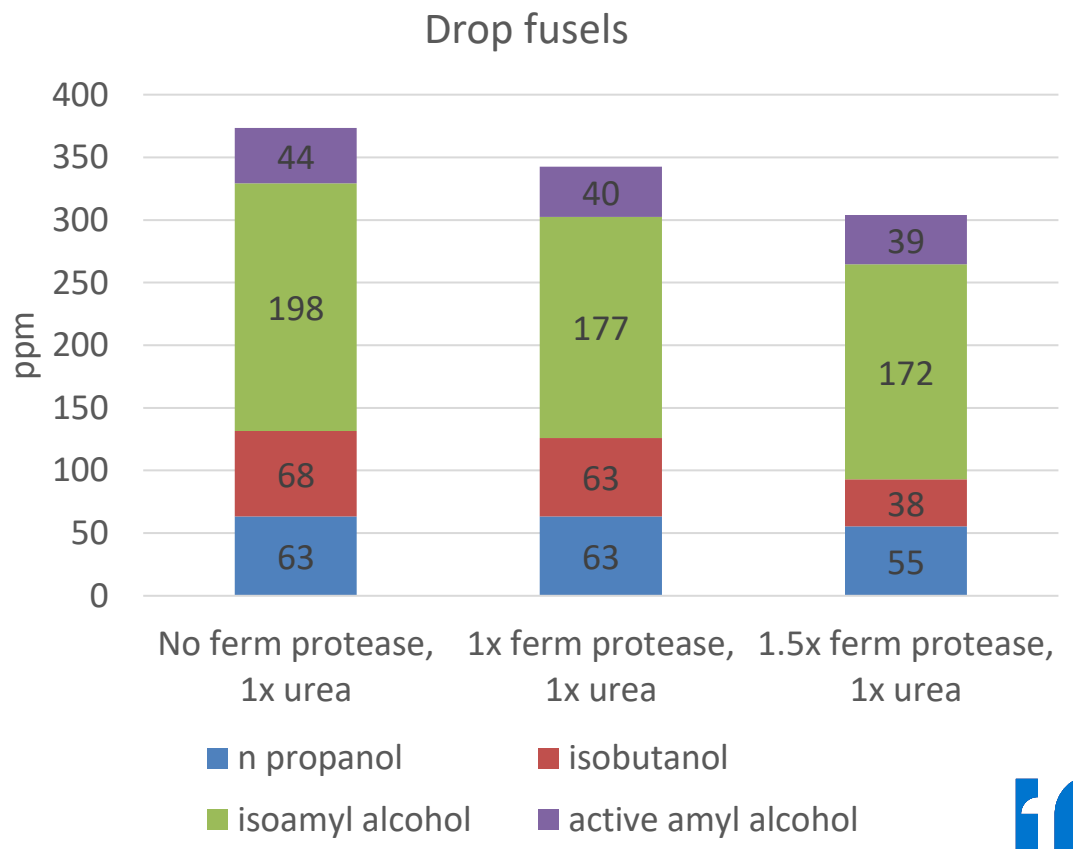


Manipulating yeast fusel production

CAN YOU REDUCE FUSELS BY INCREASING FERM PROTEASE?

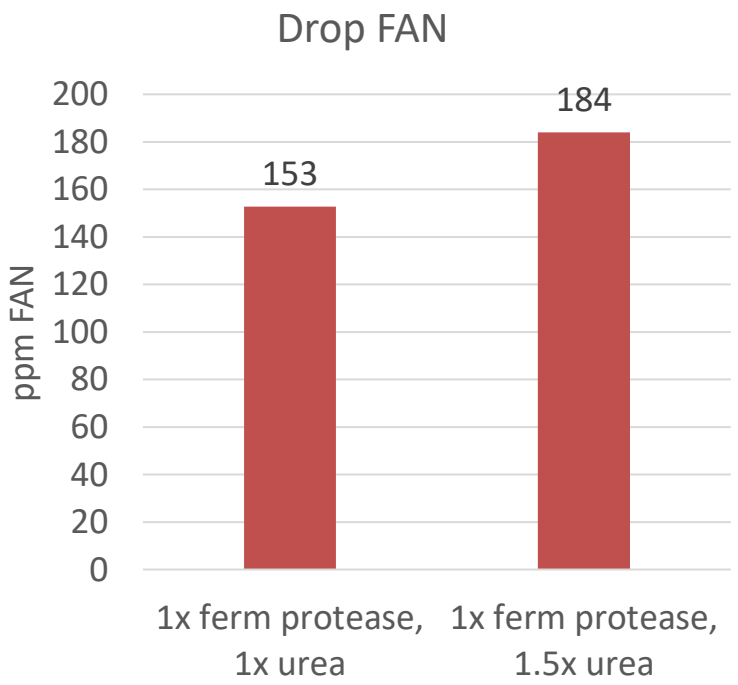
Increasing ferm protease increases FAN in ferm and reduces fusel production by up to 18%.

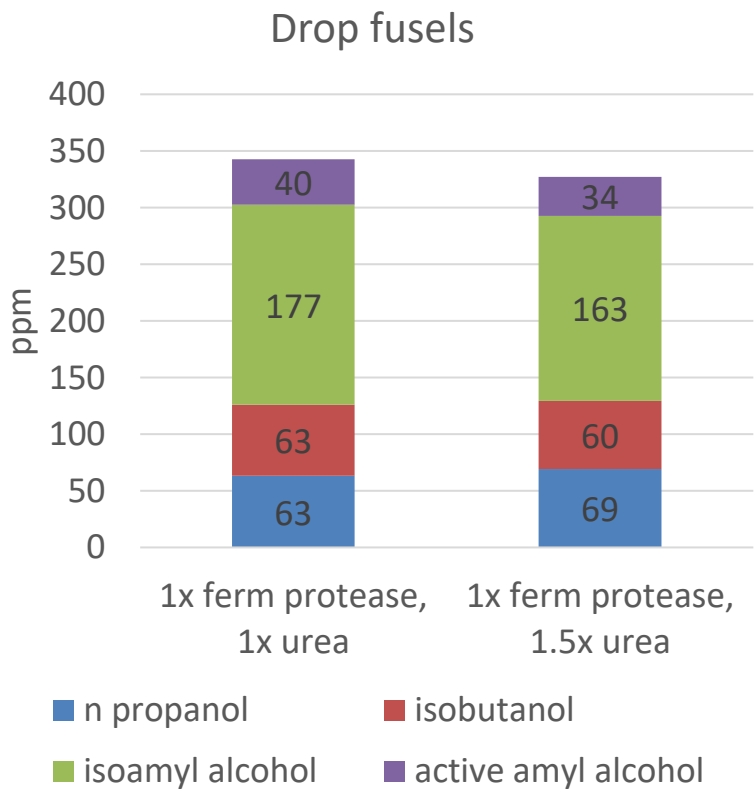




CAN YOU REDUCE FUSELS BY INCREASING FERM UREA?

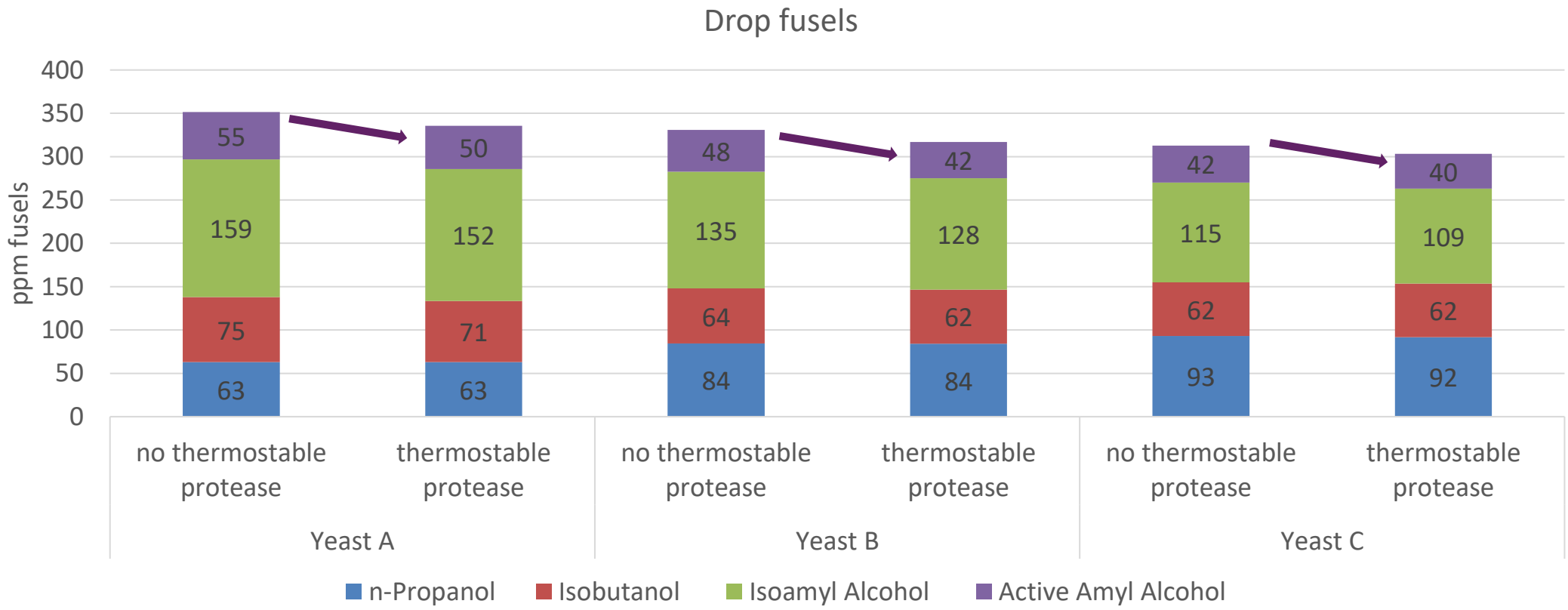
Increasing ferm urea increases FAN in ferm and reduced fusel production by 4% in this case





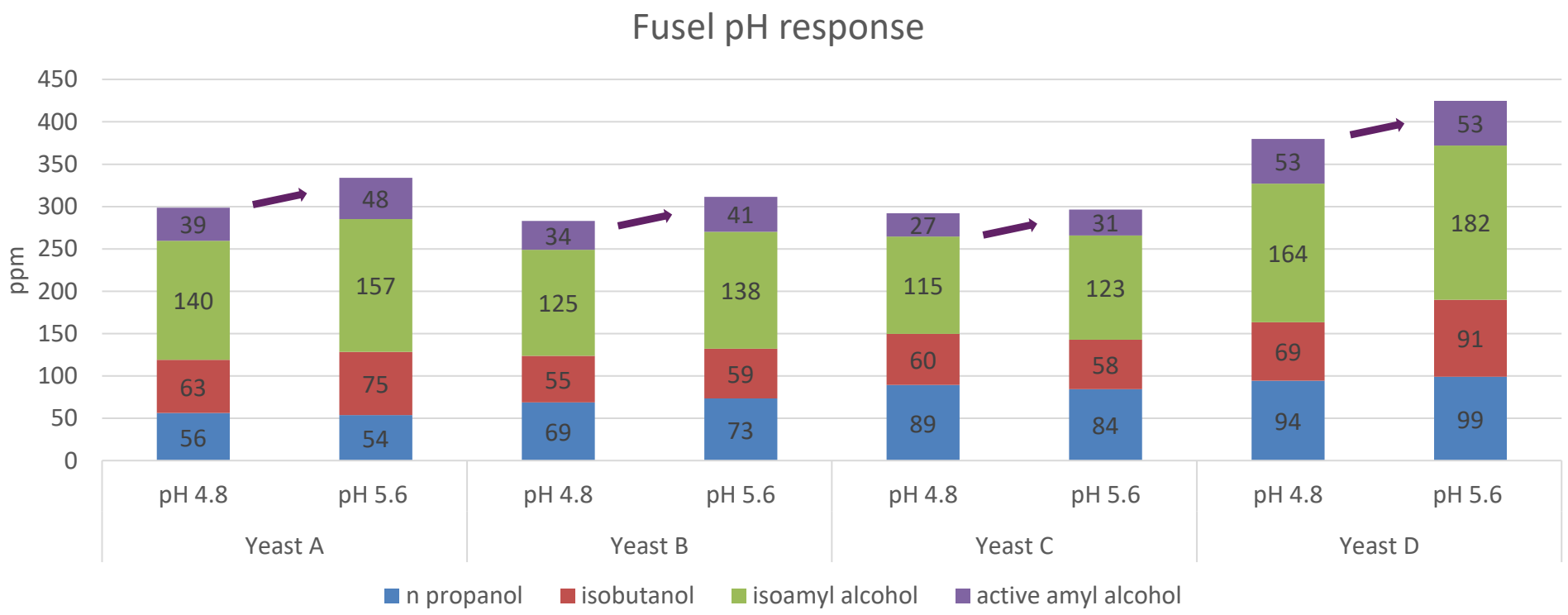
CAN YOU REDUCE FUSELS BY USING A THERMOSTABLE PROTEASE IN LIQUEFACTION?

Inclusion of a thermostable protease decreases fusel production by 3-4% for the three yeasts evaluated.



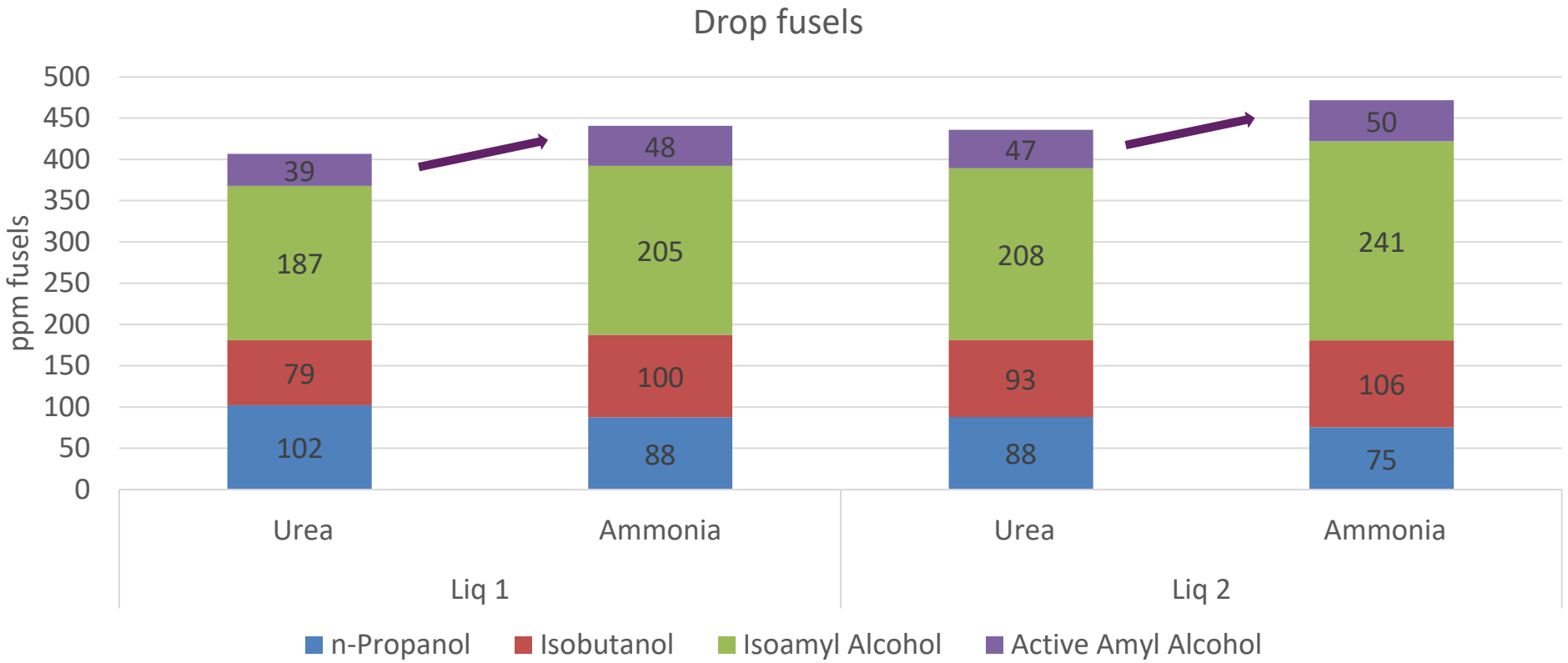
CAN YOU REDUCE FUSELS BY ADJUSTING PH?

Lower pH decreased fusels slightly for all yeasts, ranging from 1-10% depending on the yeast.



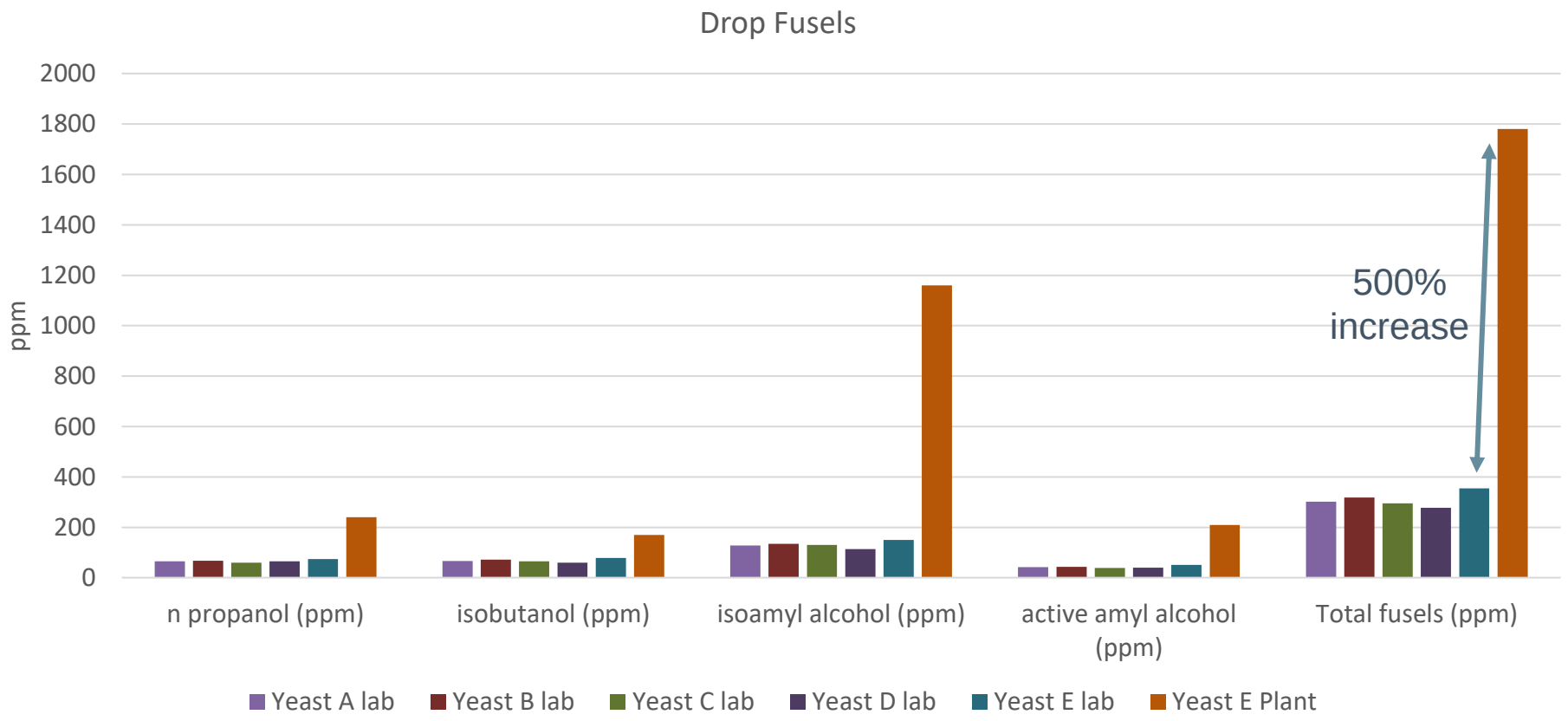
DOES TYPE OF CHEMICAL NITROGEN TYPE IMPACT FUSEL PRODUCTION?

When dosed at equal ppm N, 5-10% increase in fusel production when ammonia is used relative to urea.



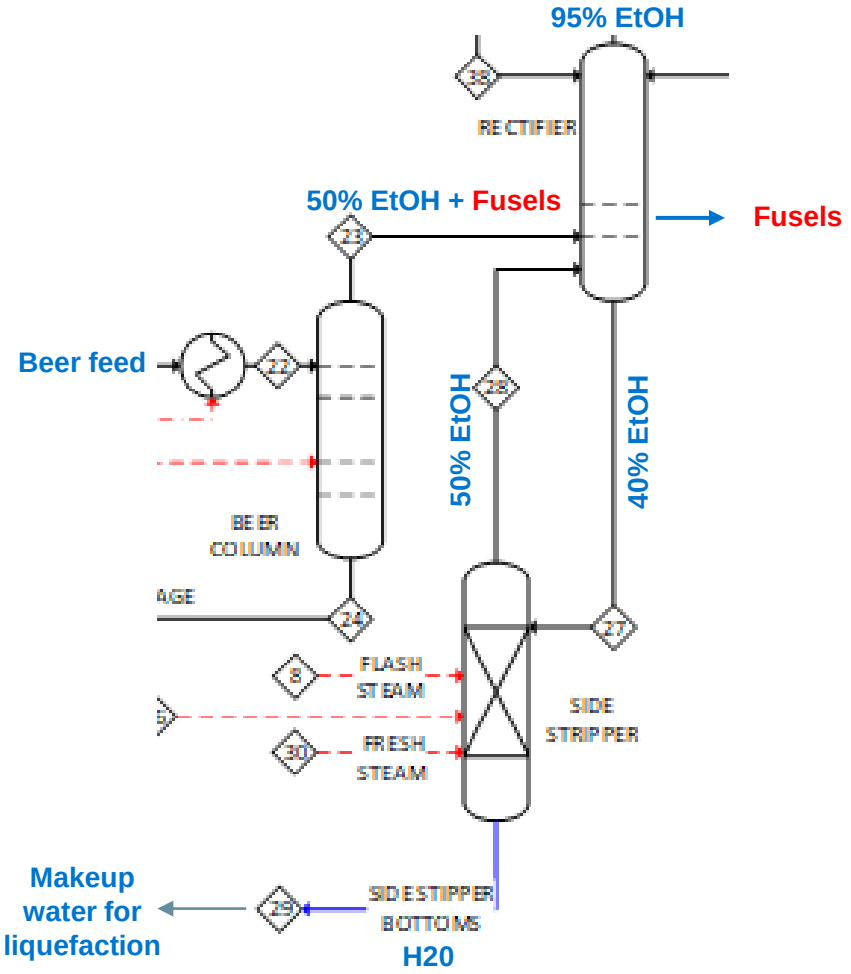
DOES THE AMOUNT OF FUSELS PRODUCED BY YEAST MATTER?

No, each yeast has a different fusel profile and amount, but pales in comparison to a plant suffering from a fusel recycle issue

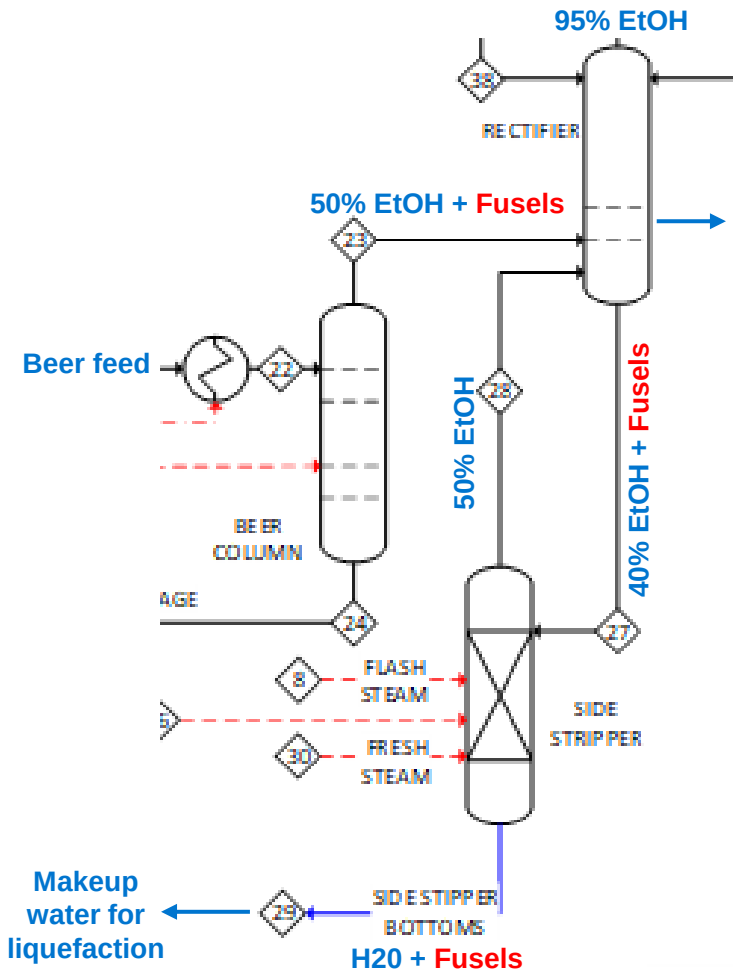


HOW DO FUSELS RECYCLE TO THE FRONT OF THE PLANT?

Normal distillation



Distillation with rectifier issues

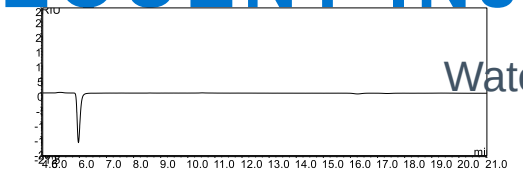


Fusel detection and “XCELIS quick detect”

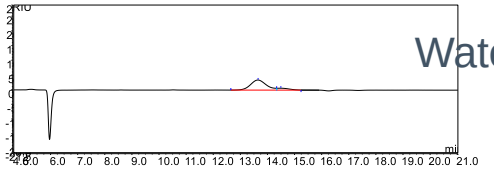
DETECTING A FUSEL PROBLEM

Method	Indication	Advantages	Disadvantages
Smell	Varnish-like odor	Quick and simple	Not always obvious, qualitative, not easily traceable
Saturated salt test	Fusels will phase separate in saturated salt solutions	Simple, non-complex materials to carry out test	Not effective for detecting fusels in fermentation samples
HPLC or GC	Chromatographic separation	Accurate, quantitative	Not common at most plants, can take a long time to get results from 3rd party lab
XCELIS quick detect	Chromatographic separation	Quick, semi-quantitative, uses available HPLC instrumentation	Not fully quantitative

FUSELS IN SAMPLES CARRYOVER INTO THE SUBSEQUENT INJECTION



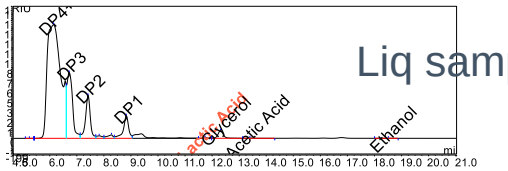
Water injection after a normal ferm sample injection



Water injection after a fusel ferm sample injection

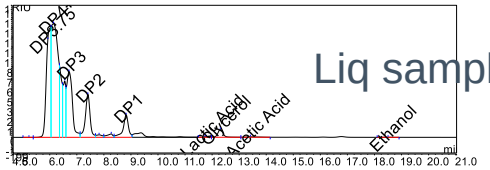
Fusels carry over from previous injection

FUSEL CO-ELUTION IMPACTS HPLC PROFILES



Liq sample injected after normal ferm sample injection

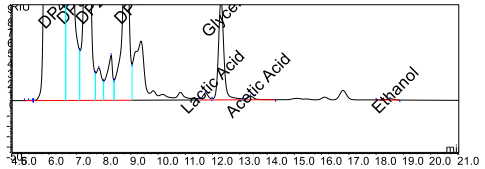
Chromatograms look the same?



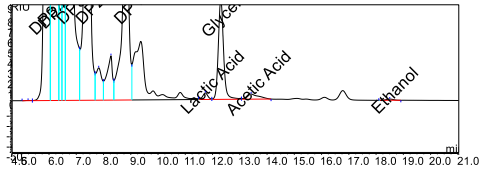
Liq sample injected after fusel recycle ferm sample injection

ZOOMED IN CHROMATOGRAPHY

Acetate peak is higher and broader when the previous injection contained high levels of fusels



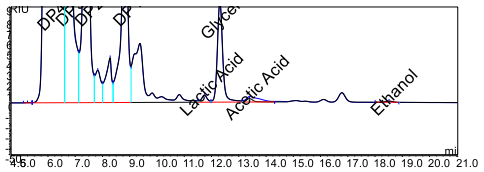
Liq sample ran after normal ferm sample



Liq sample ran after sample with fusel recycle

CHROMATOGRAM OVERLAY TO MAKE IT CLEARER

Fusels not present in black chromatogram but are in the blue as indicated by the change in acetate peak

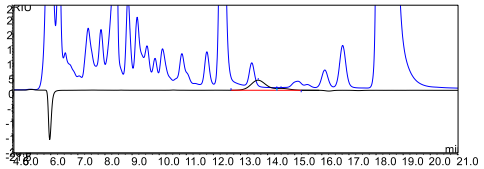


Larger and broader acetate peak is not typical, acetate integration difference of 0.04 vs. 0.1% w/v

FUSEL CARRYOVER ON HPLC

Fusel carryover elutes near the acetate peak

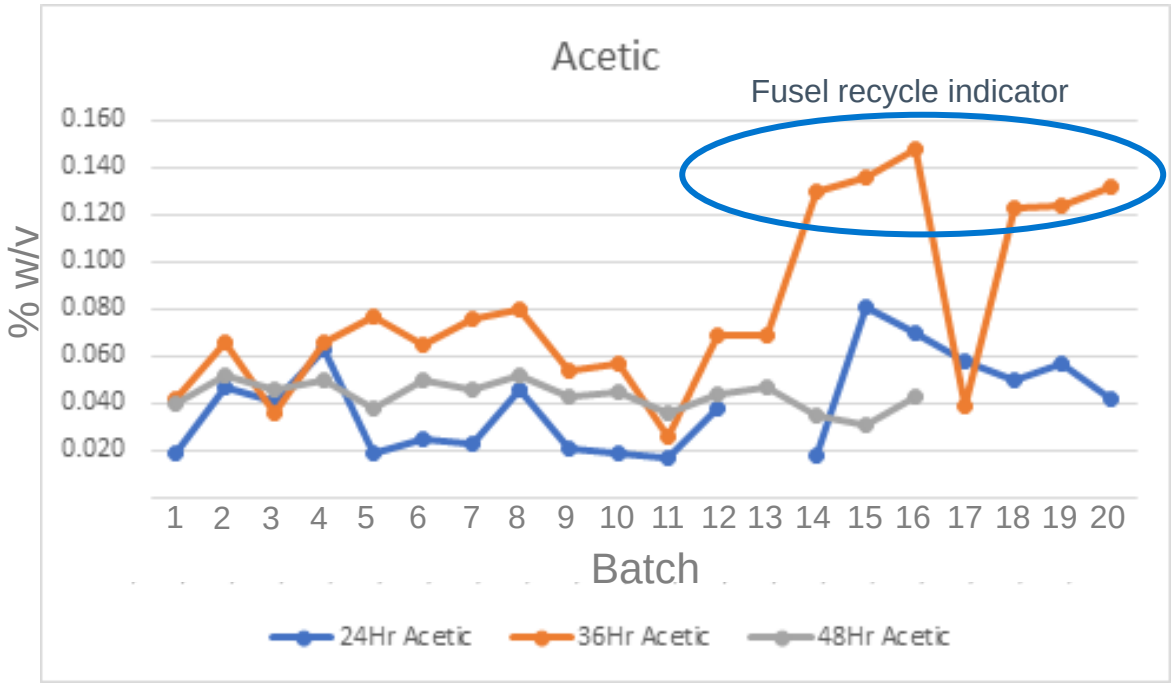
Fusel recycle drop sample overlayed with a subsequent water injection

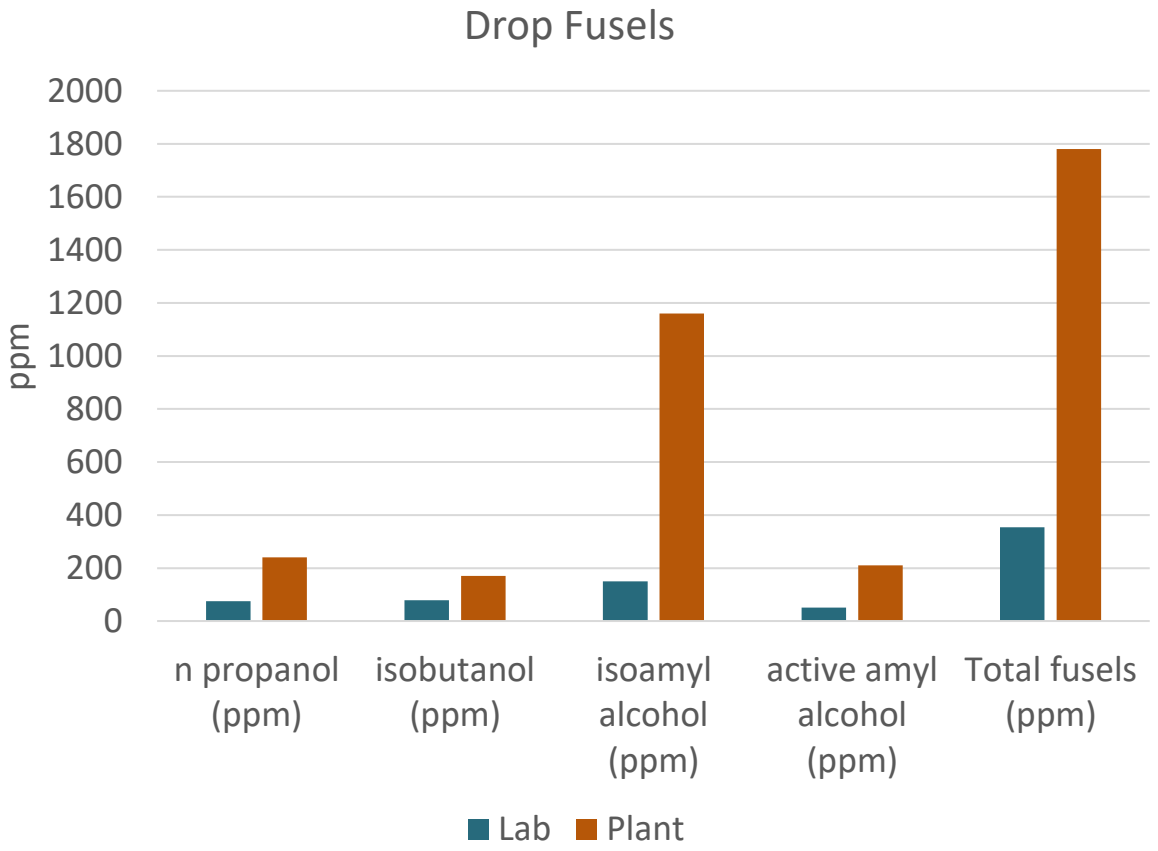


Fusel carry over elutes under acetate

FIELD EXAMPLE OF ACETIC AS A FUSEL INDICATOR

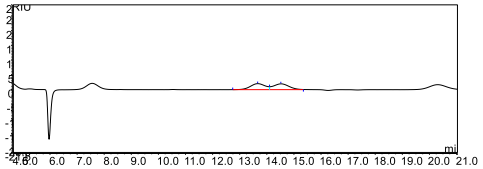
Plant was experiencing sluggish fermentations and seeing unusually high acetate in ferm samples. Lab fusel analysis confirmed fusel recycle issue.





WHAT FUSEL IS INTERFERING WITH ACETATE?

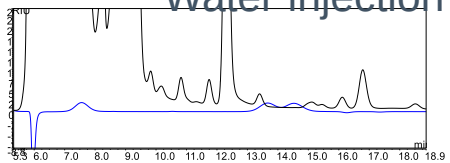
Water injection after injecting a 350 ppm fusel standard



Isoamyl alcohol

Active amyl alcohol

Water injection after 350 ppm fusel standard overlaid with liq sample



Acetate

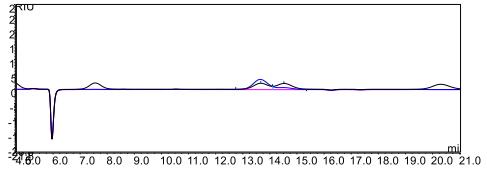
Isoamyl alcohol ★

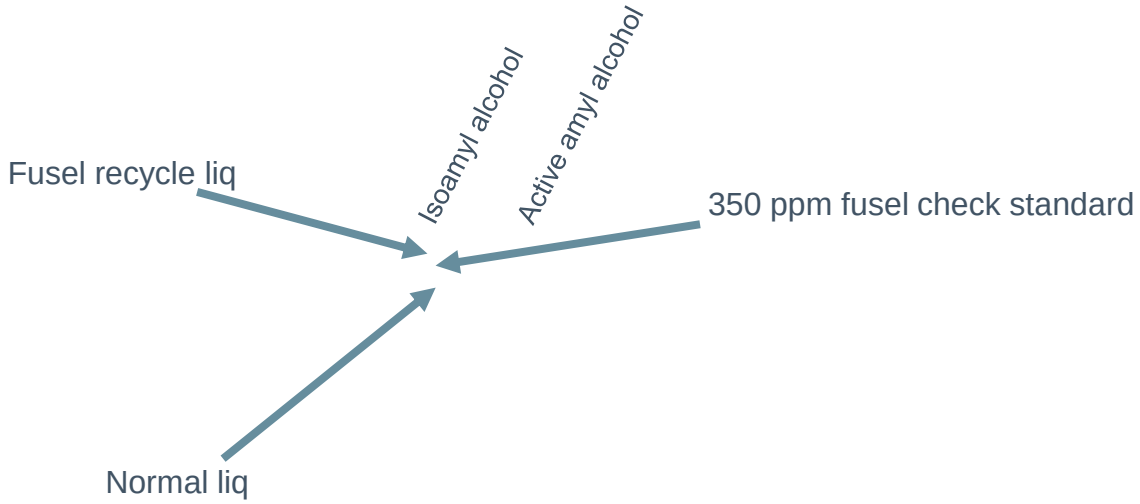
Active amyl alcohol

QUANTIFYING FUSEL RECYCLE

By comparing the water injections from after fusel check standard and the fusel recycle liq sample we can determine it has >350 ppm isoamyl alcohol recycling, a sign of significant distillation issues

Water injections after fusel recycle liq, 350 ppm fusel standard, and no fusel recycle liq





APPLYING THE METHOD TO YOUR PLANT

Is it perfect? No. But it can give you a quick answer to help you navigate process upsets at your plant

If you suspect fusel recycle issues or see unexplained shifts in acetate:

1) Inject the suspected sample (liquefact is best since it typically has zero- or single-digit ppm fusels)
2) Inject a water sample immediately after
3) If a notable peak appears in the water sample near where acetate normally elutes, it is likely a fusel recycle issue
4) To semi-quantify the fusels, inject a fusel check standard
5) Inject a water sample immediately after
6) Compare chromatograms for both water injections, if the fusel sample peak is higher than the fusel check standard, you have an idea of the severity of the fusel recycle

This should work for HPLC systems that use standard columns with 0.01N or 0.005N sulfuric acid

Special thanks to Keaton Albers, Senior Application Associate, AIC

SUMMARY

Fusels and yeast physiology

Manipulating yeast fusel production

Fusel detection and “XCELIS quick detect”

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