
SAND2017-7386C

## 2nd International Solar Fuels Conference
## Biomass Conversion

# Production and Upgrading of Fusel Alcohols from Biomass Hydrolysates

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# A holistic approach to biofuel production

**Biomass Production**

What can we grow?

**Conversion to Fuel Products**

**Co-optimization of Fuels and Engines**

What is the best fuel?

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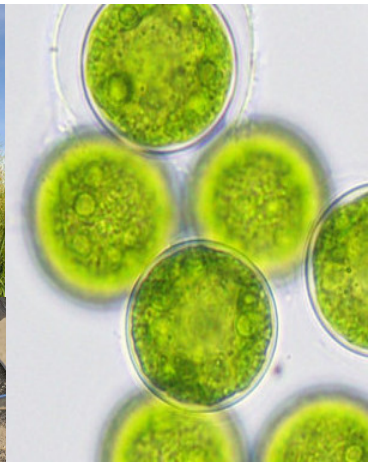
# Biofuel production overview: sustainable, low cost, and high yield

- Focus on renewable feedstocks:
  - Non-edible crops
  - Waste streams (residual from corn-ethanol process, others)
  - Agricultural lignocellulosic residues
  - Algae (micro and macro)

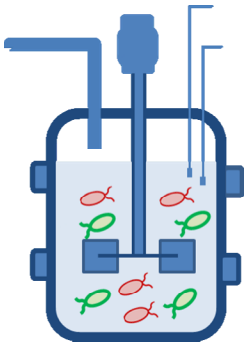
Lignocellulosic Biomass

Corn Waste

Algae

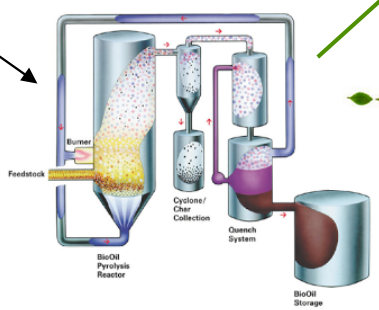


Biochemical Conversion



(Liu, Fang 2017)

Chemical Conversion




(http://4.bp.blogspot.com/fast_pyrolysis

Spark Ignition

Compression Ignition

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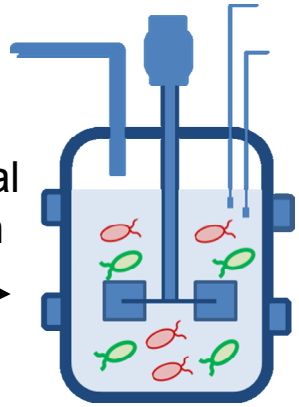
# Sustainable and high productivity biomass correlates to high protein content

**Low-Quality Biomass**

Benthic Algal Polycultures

Biochemical Conversion

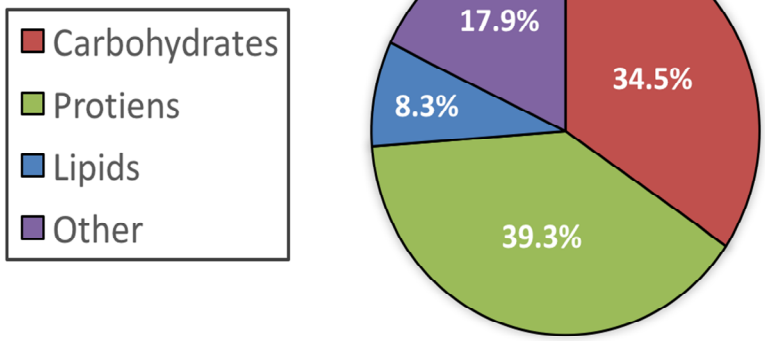


(Liu, Fang 2017)

Spark Ignition

Compression Ignition

**Hydromentia ATS Biomass Composition**




Corpus Christi, TX

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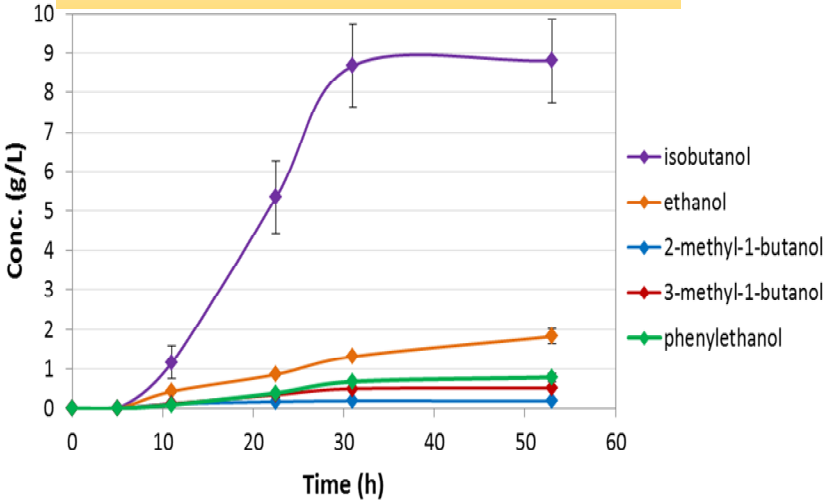
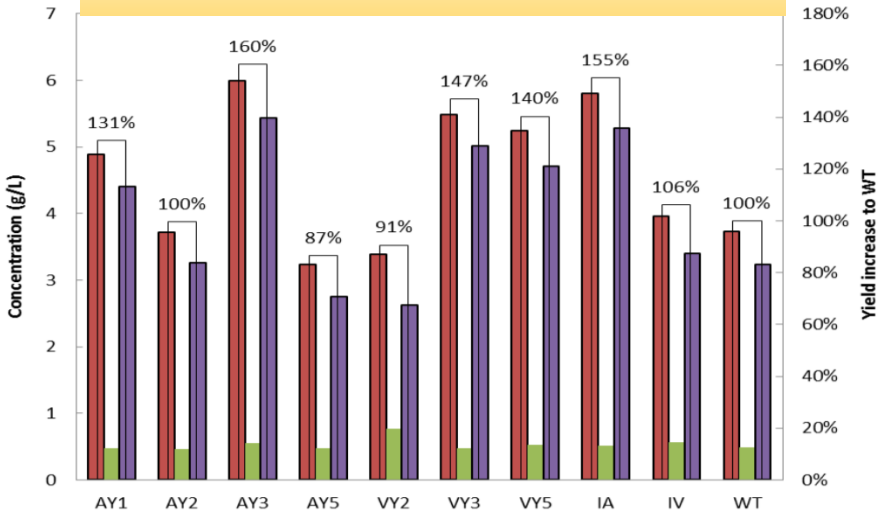
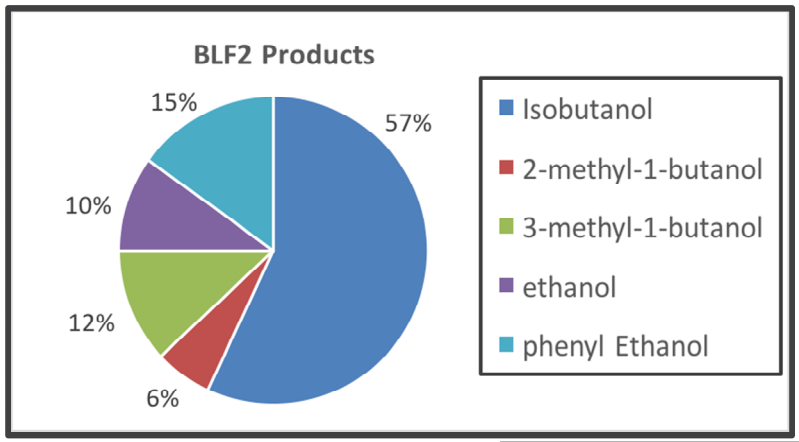
# Development of *E. coli* strains for protein conversion and carbohydrate conversion

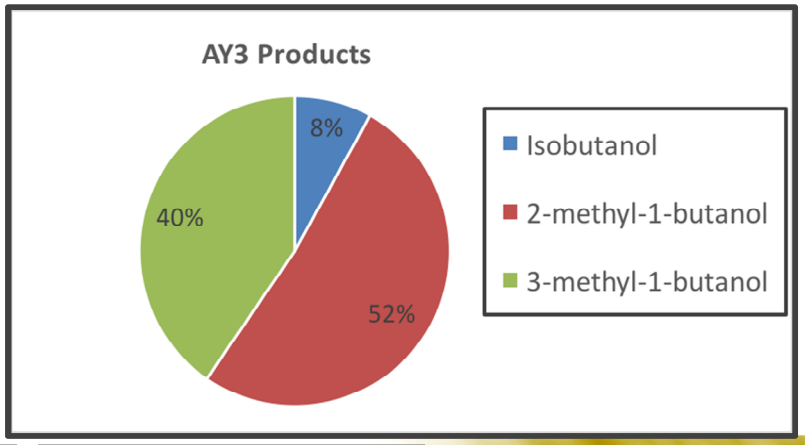
Fusel alcohols production from mixed sugars by BLF2 strain




Mutant development for protein fermentation strain




Wu and Davis *Algal Res* 2016

Huo et al Nature Biot. 2011

Wu et al Algal Res 2016

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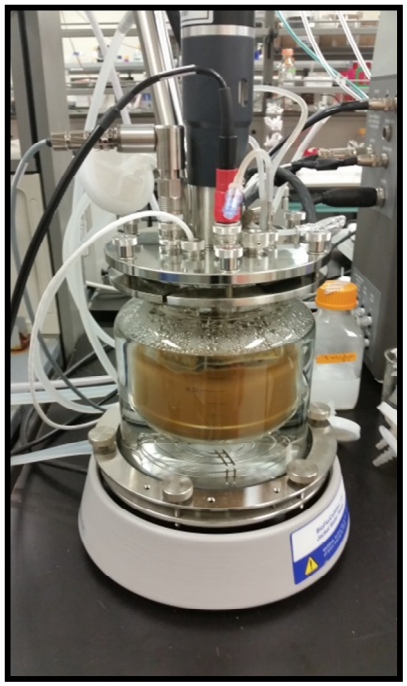
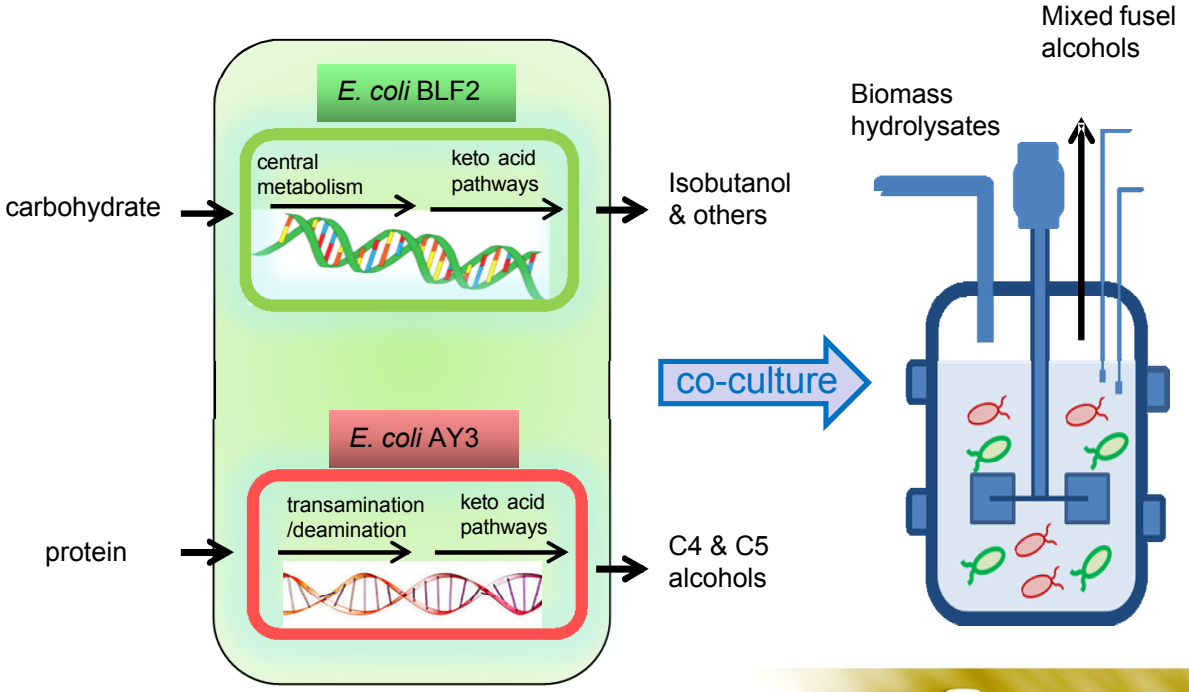
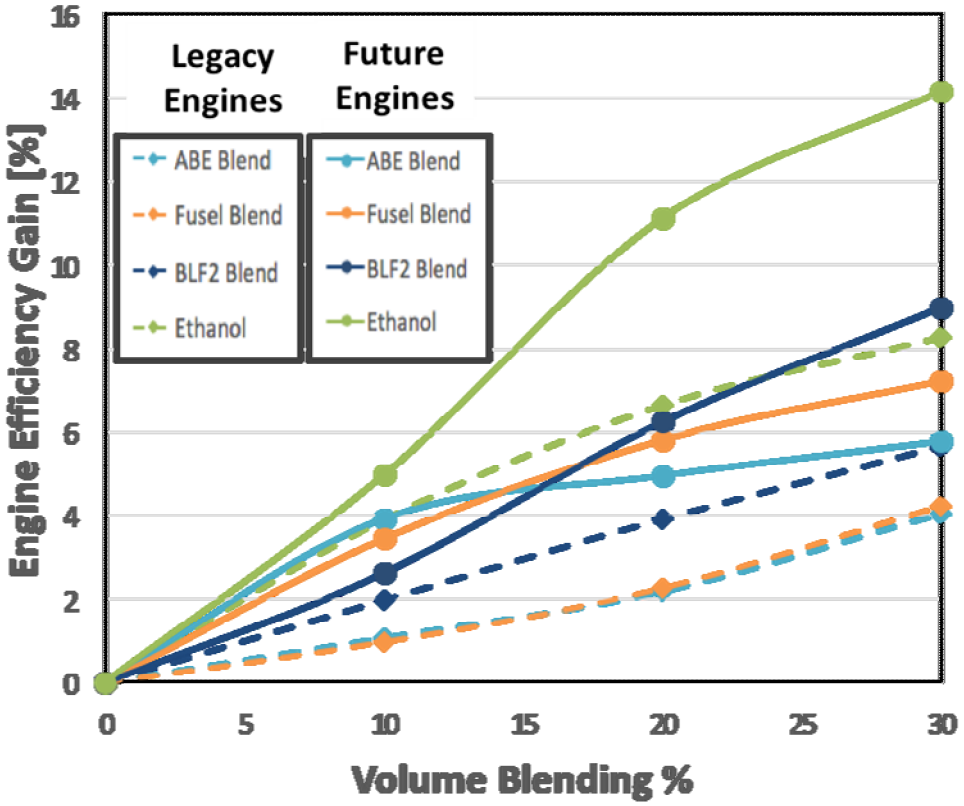
# Engineering the *E.coli-E.coli* co-culture system for fusel alcohol production

- Consortium improves culture stability and has titers >10 g/L
- "One-pot" bioconversion reduces unit operations and cost
- Significant room for optimization of fermenters or exploration of other organisms

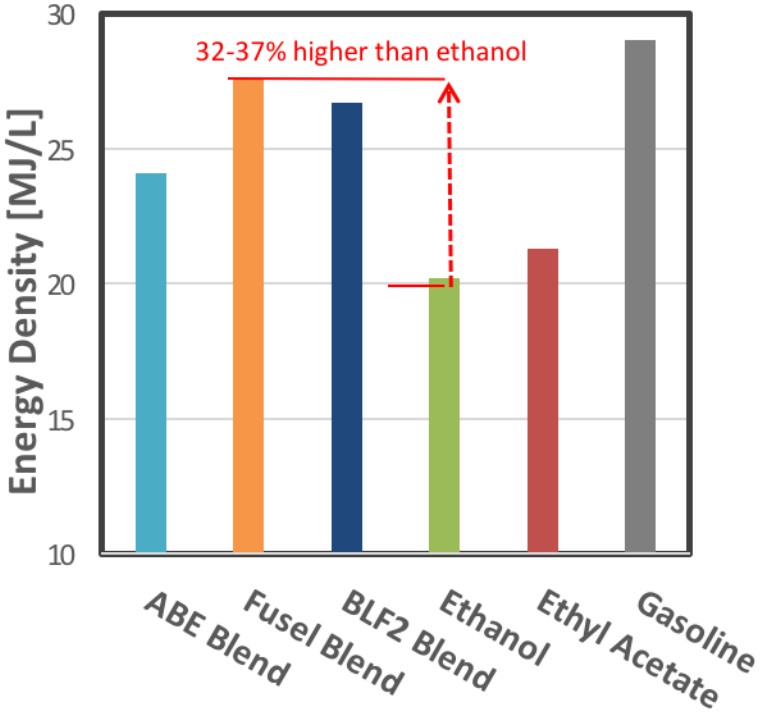




# Fusel alcohols as a high performance spark ignition fuel








Doesn't factor in other important considerations
- Hygroscopicity/Corrosivity
- Vapor pressure of gasoline blends

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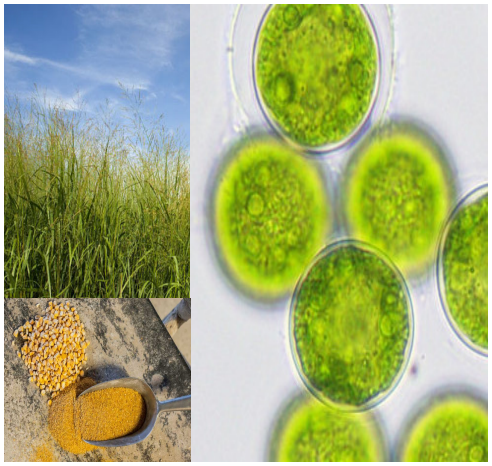
# Closing the loop: usage of residual lipids

Grease, Waste cooking oil, other lipid sources

- Allows for use of all components of a diverse suite of high productivity biomass sources
- Allows for significant fuel tunability based on evolving transportation fleet
- Use of new biocatalyst to lower biodiesel operational costs

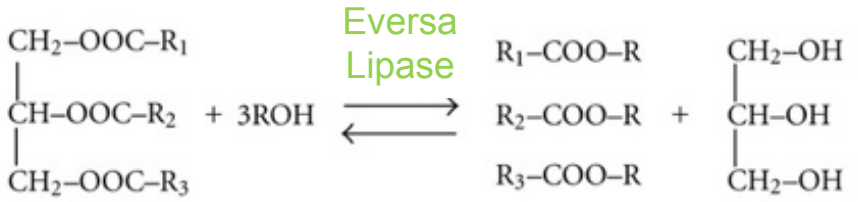
Lignocellulosic Biomass



Corn Waste

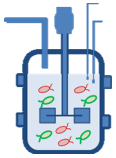
Algae

Residual Lipids




Low Quality Lipids + Fusel Alcohols → Fatty Acid Fusel Esters (Biodiesel) + Glycerol

Carbs + Proteins



Mixed Fusel Alcohols

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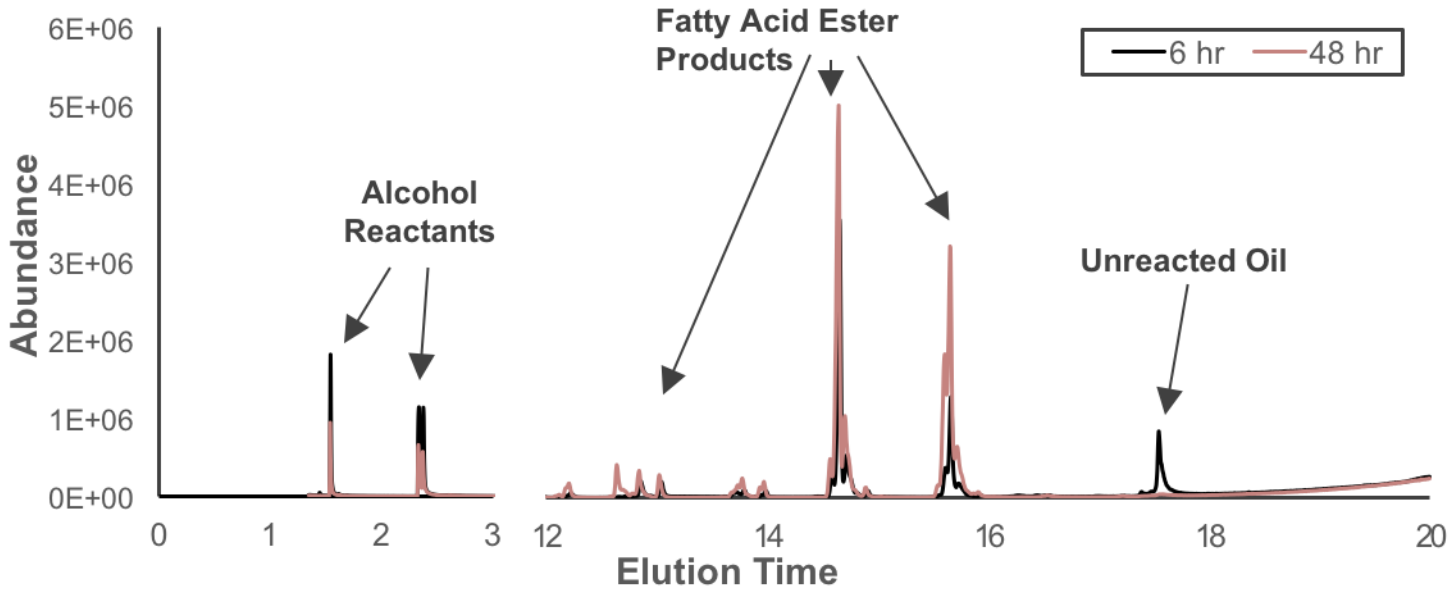
# Upgrading to CI fuels: fatty acid fusel esters (FAFEs)

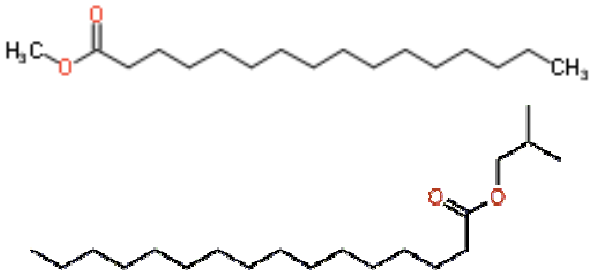
- >97% Yield after 24 hrs using 5:1 molar ratio of alcohol:oil and mixed alcohol samples.
- Demonstrated on model replicates of fermentation products (only isobutanol and isopentanol isomers).
- Significant room for reaction optimization to improve economics (lower enzyme loading, shorter reaction times, etc.)

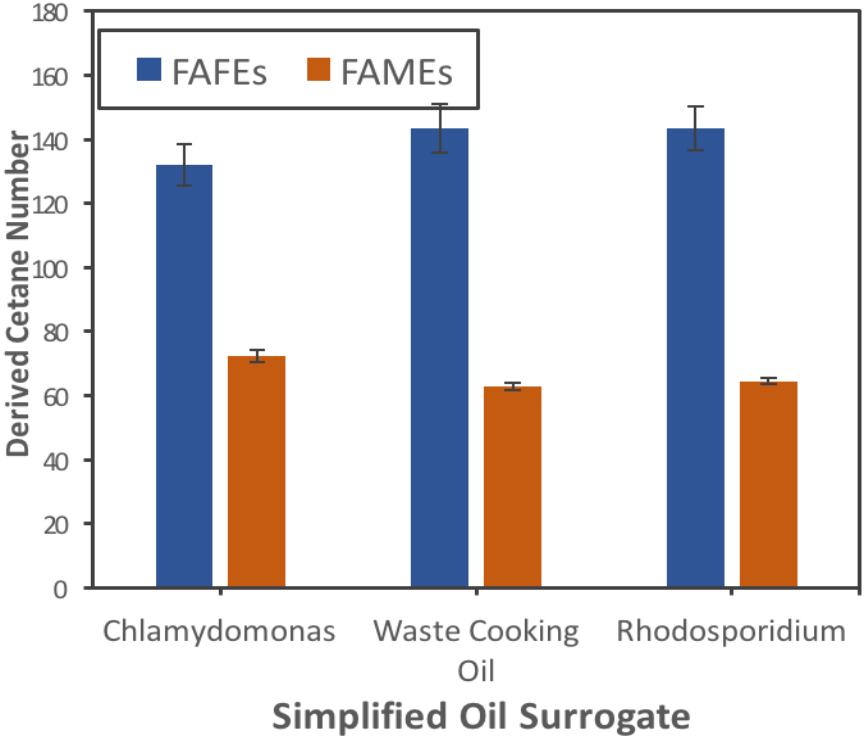
GC-MS Chromatogram

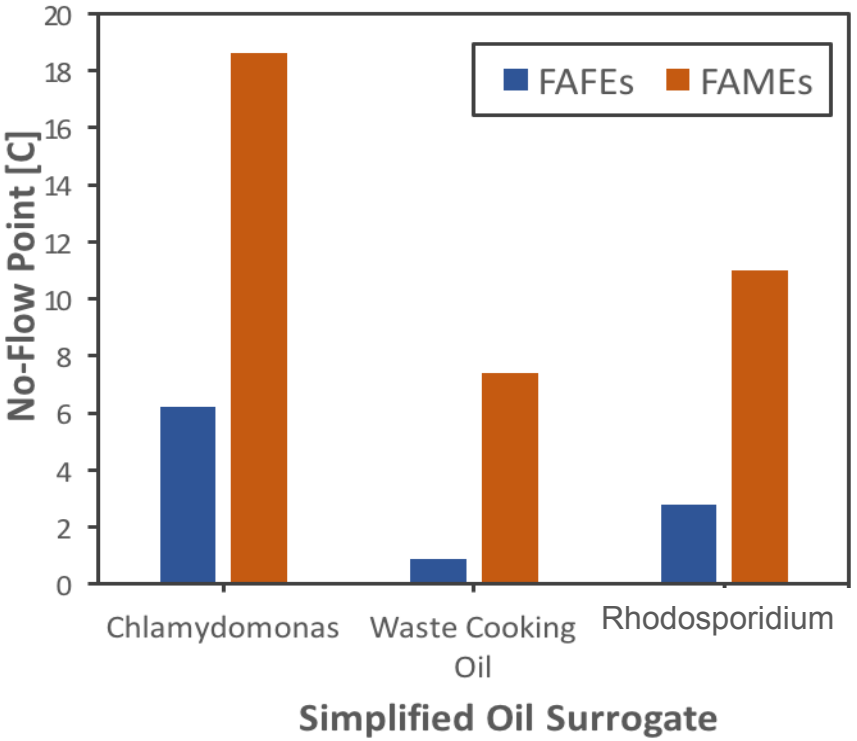


# FAFEs as high performance compression ignition fuels

- Results shown are based on simplified surrogates of expected FAFE reaction mixtures
- "Off the charts" Cetane Numbers are outside accurate range for the calculation
- No-Flow point improved by an average of 9 C which addresses biodiesel's significant cold-flow performance issues.



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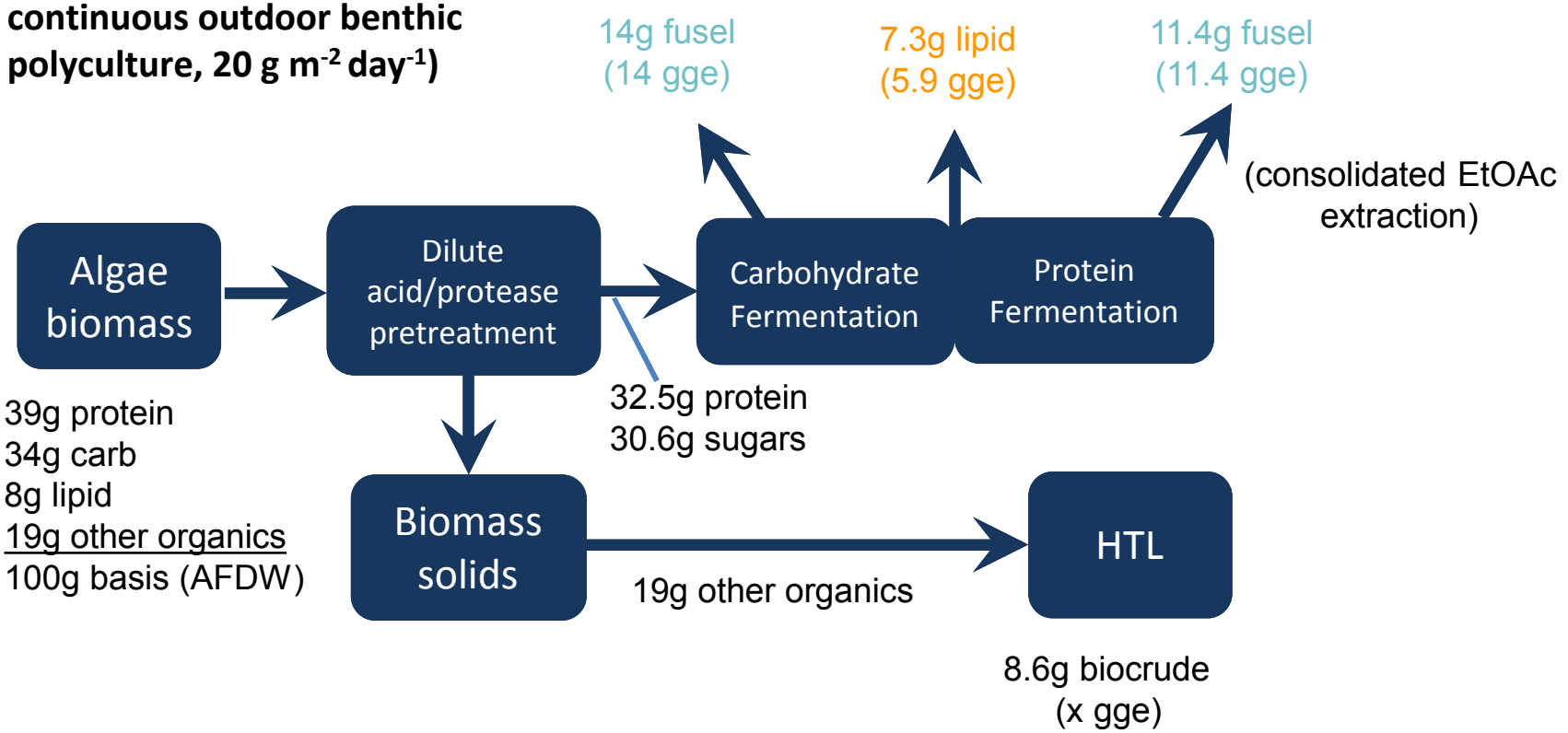
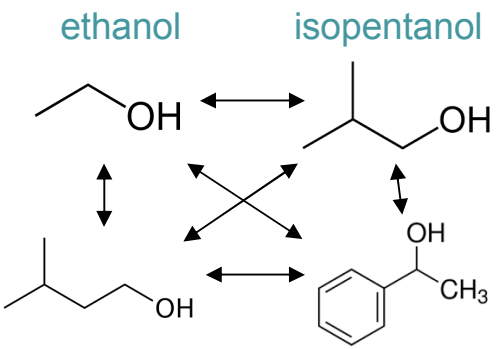
# Is the loop closed and what biomass is going where?

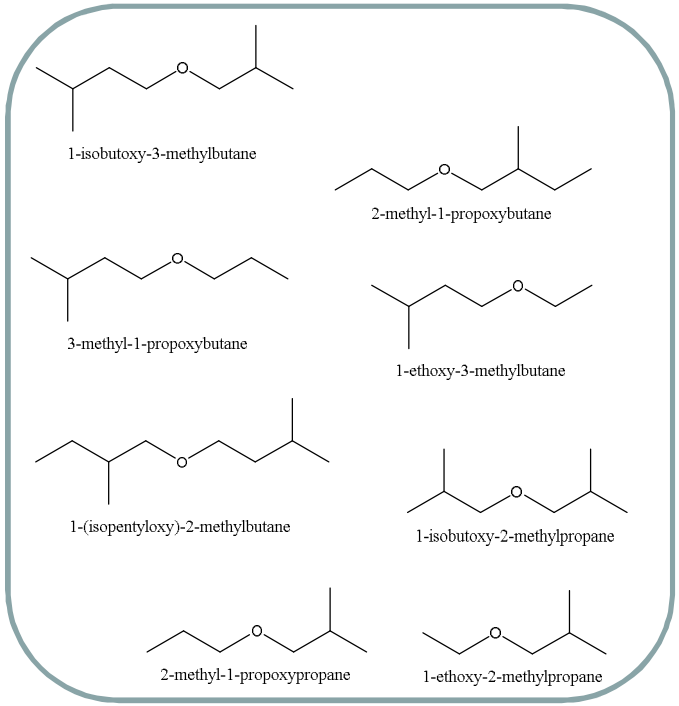
**HydroMentia Biomass (semi-continuous outdoor benthic polyculture, 20 g $m^{-2}$ $day^{-1}$)**



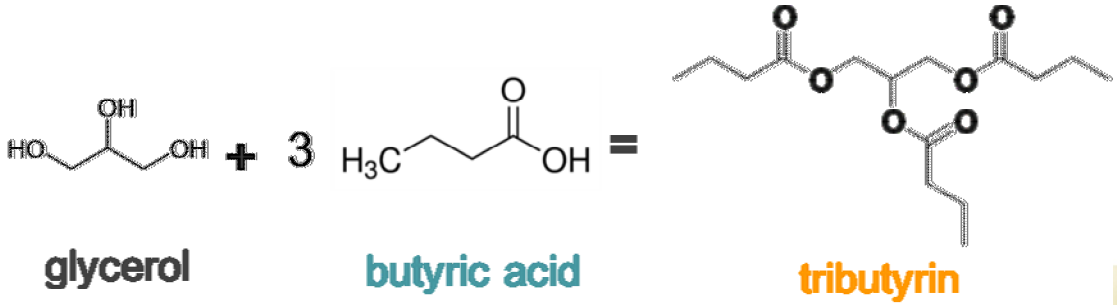
# Future work: other high performance biofuels of interest that further close the loop

- Ethers as CI Fuels




- Upgrading glycerol to viable fuel products/blending agents

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# Conclusions

- A key bottleneck for large-scale biofuel feasibility is sustainable growth of high productivity biomass, which is often comprised of mostly proteins and complex carbohydrates.
- We have developed a proof of concept “one-pot bioconversion” with engineered *E. coli* for efficient production of mixed fusel alcohols from a wide variety of biomass sources.
- These fusel alcohols show promise as drop in fuels, or as blending agents with gasoline for SI engines, with properties comparable or better than ethanol.
- Fusel alcohols can further be upgraded to other high performance fuel compounds or reacted with residual lipids to utilize all major biochemical components of the biomass and “close the loop” allow for tunablity to different engine architectures.

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# Partnerships & Acknowledgments


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- Robinson Barzuto (HyrdoMentia)
- Mary Tran (Sandia)
- Leanne Whitmore (Sandia)


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HydroMentia

Old Dominion University 1930

Texas A&M AgriLife Research

California Polytechnic State University 1901 Discere Faciendo

IID Irrigation District Water Power — A century of service.

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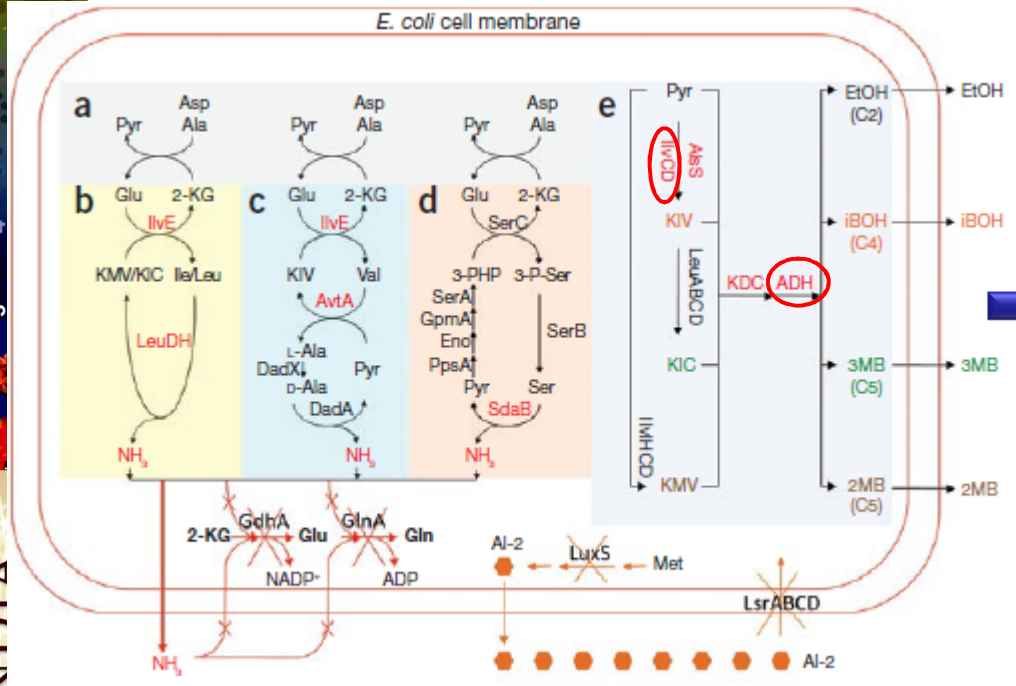
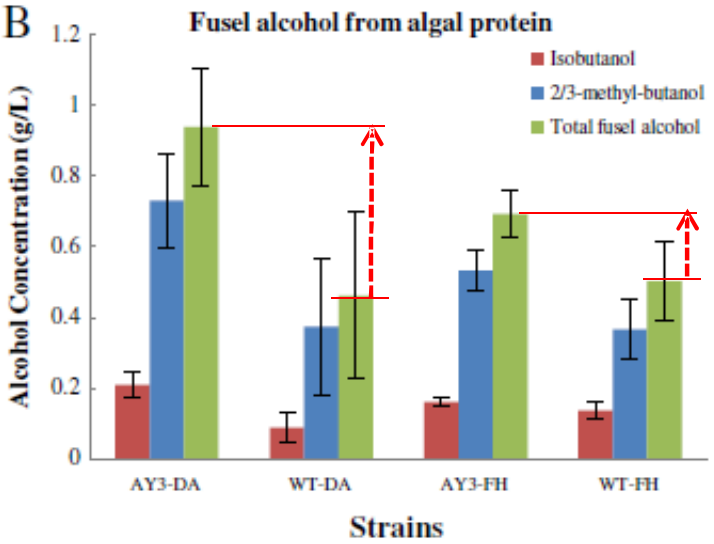
# Protein fermentation strain

- Protein utilization strain
  - deaminate protein hydrolysates and convert proteins to C4 and C5 alcohols




( Huo et al, *Nat. Biotech,* 2011, 29(4): 346-352 )

- Improved strain: *E. coli* AY3
  - by modifying the cofactor specificity of two key enzymes (IlvC and YqhD) from NADPH to NADH




( Wu et al, *Algal Research,* 2016,19: 162-167 )

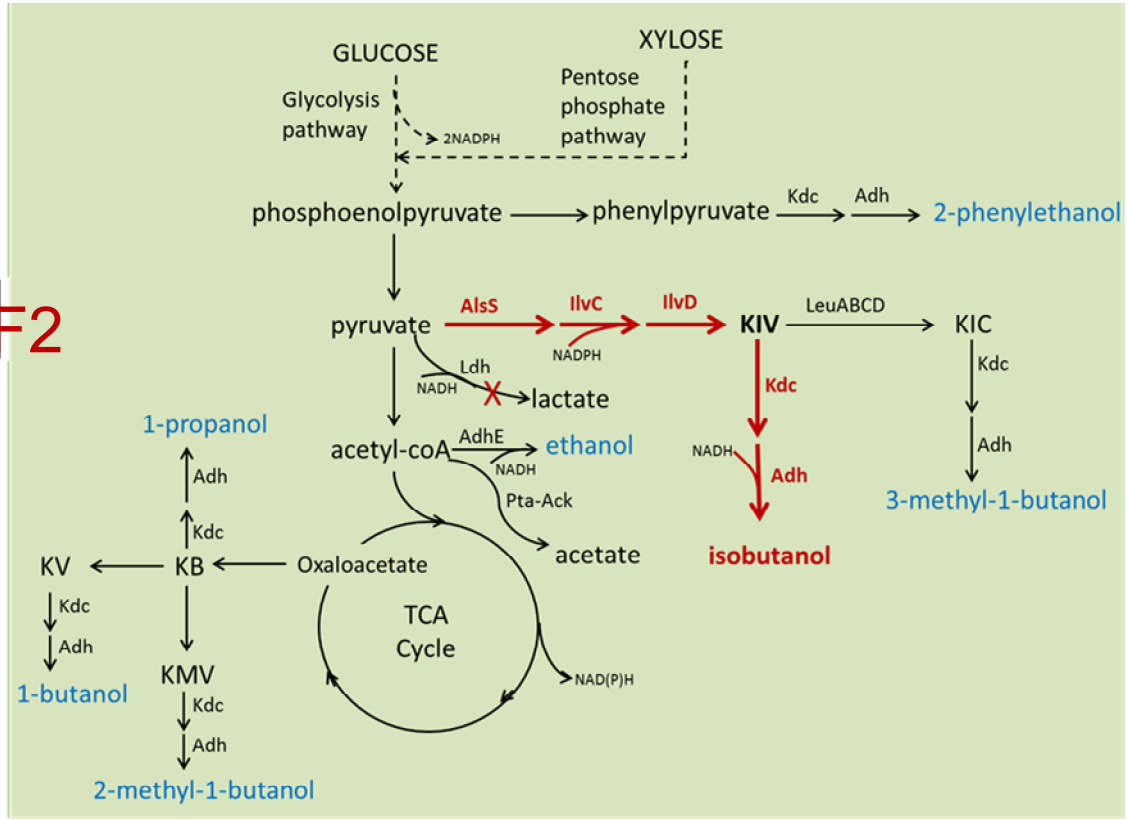
# Engineering *E. coli* strain B for fusel alcohol production using carbohydrates

- Deleted *ldh* gene from the chromosome of strain B ATCC 11303 and replaced with chloramphenicol resistance gene ($Cam^R$).
- Cloned the 2-keto acid pathway to strain B ATCC11303.

pLF101


pLF102

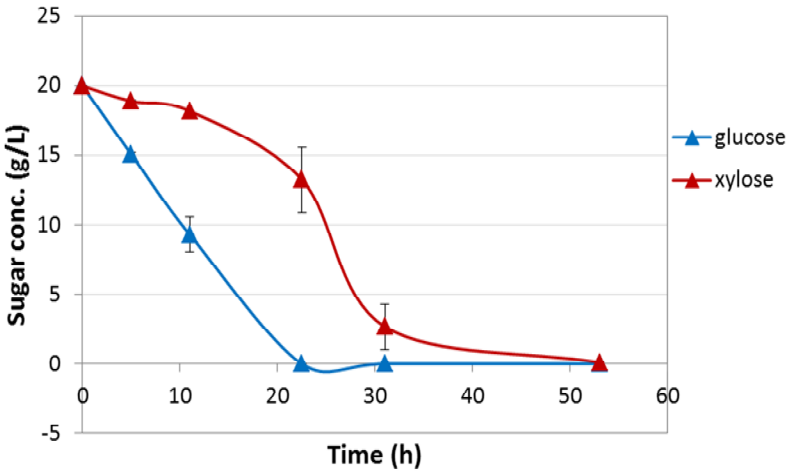

*E. coli* BLF2



# Alcohols production from glucose and xylose by *E. coli* BLF2

- The uptake of xylose was slow until glucose was completely metabolized, which is the result of carbon catabolite repression.

Glucose and xylose consumption in sugar mixture




Alcohols production from sugar mixture