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2017 Algae Biomass Summit
Salt Lake City, Utah

Production, upgrading, and testing of fusel alcohols as high performance fuels from whole algae bioconversion

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A holistic approach to biofuel production

High performance fuels can strengthen the value proposition of biofuels

Biomass Production

What can we grow?

Conversion to Fuel Products

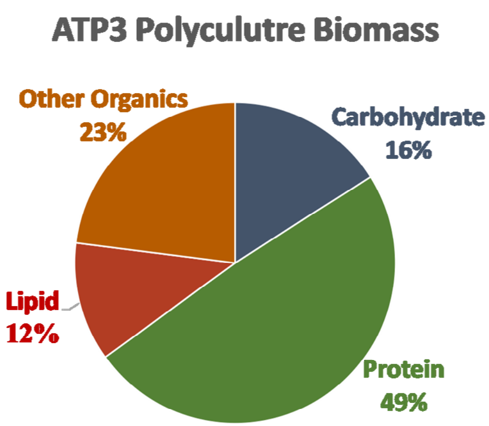
Co-optimization of Fuels and Engines

What is the best fuel?

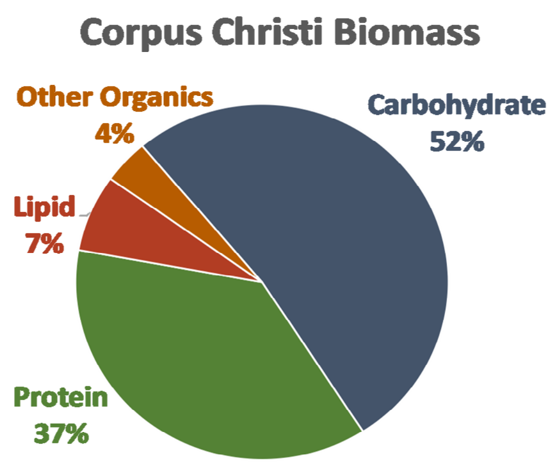
Low-cost, high productivity is priority number one

What can we grow cheap?

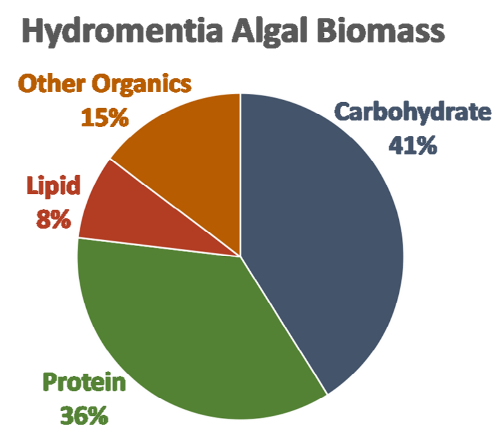
ATP3 Pond Microalgae Polyculture
-ASU, Arizona



Sandia/Hydromentia Benthic Algae Turf Scrubber
-Corpus Christi, Texas



Hydromentia Benthic Algae Turf Scrubber
-Vero Beach, Florida



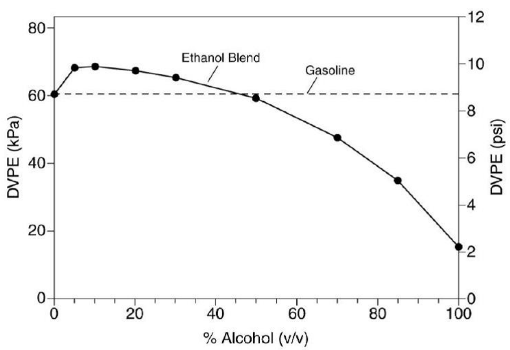
What Makes a Fuel Good?

Compression Ignition Fuels

Spark Ignition Fuels

Evaluating a fuel is about more than just cetane and octane

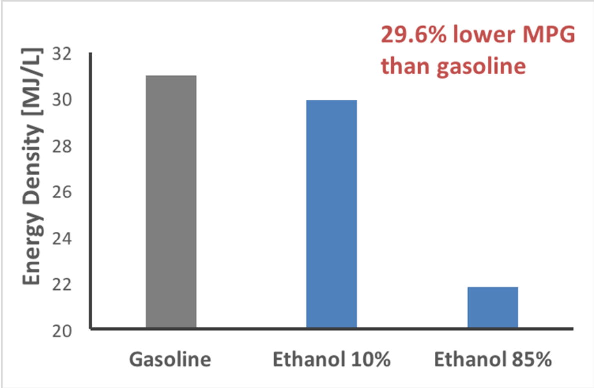
Blending Vapor Pressure



McCormick et al *NREL 2012*

Corrosivity/Infrastructure Compatibility

Energy Density



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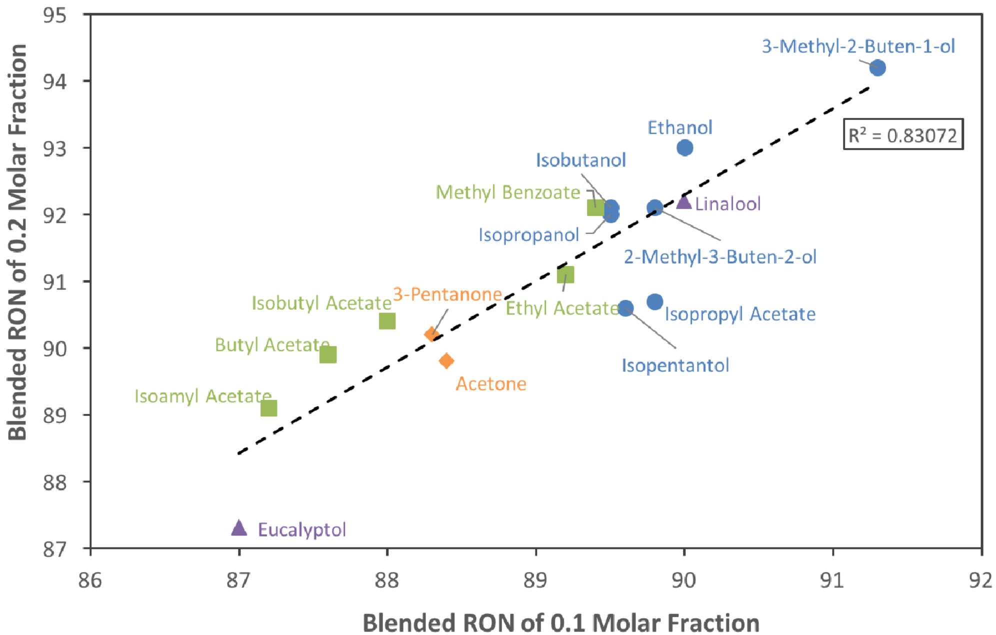
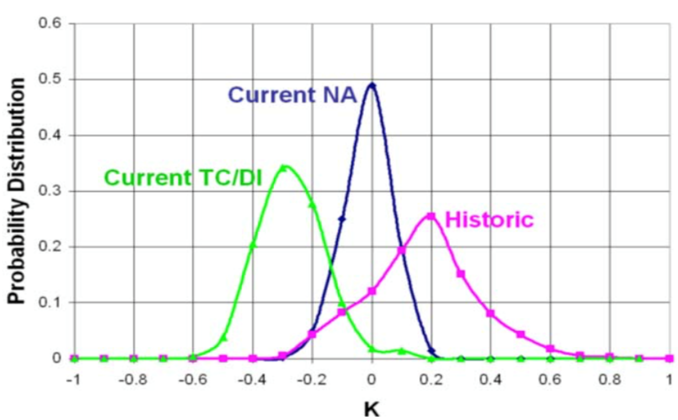
Octane blending behavior is complicated unpredictable



- Octane blending behavior is extremely unpredictable and poorly understood

"All we know is that we know nothing"
- Socrates and Co-Optima Team



Fuel performance metrics for diesel fuels not well defined. Infrastructure compatability is most important

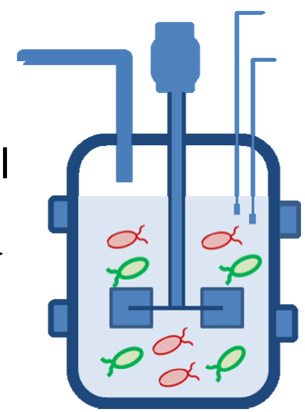
Mittal & Heywood *SAE International* 2009

In the meantime a robust, feedstock agnostic conversion process is necessary

Low-Quality Algae Biomass

-High Protein
-High Carbohydrate

Biochemical Conversion



(Liu, Fang 2017)

Spark Ignition Fuels

Compression Ignition Fuels

Other Bioproducts

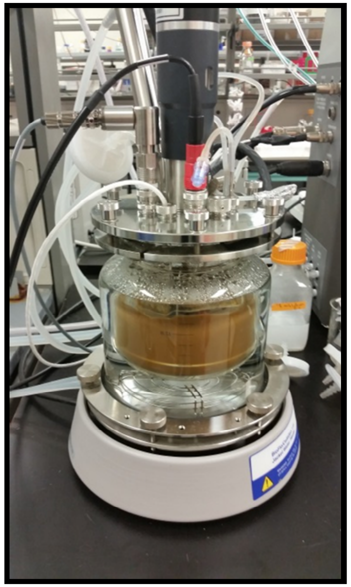
Development of *E. coli* strains for protein conversion and carbohydrate conversion to fusel alcohols in co-culture system

Fusel alcohols production from mixed C5 and C6 sugars by BLF2 strain

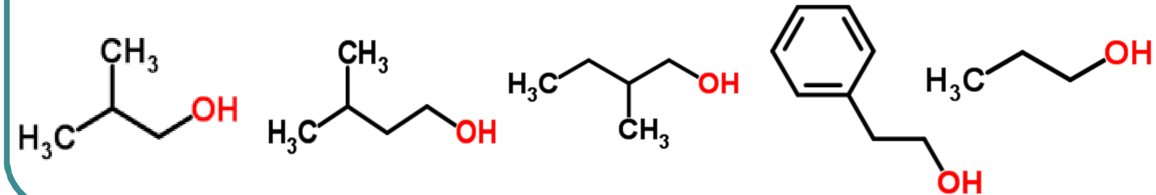
Mutant development for protein fermentation strain AY3 based

- **Two breakthroughs:**
  1) *redox engineering* of key proteins provides bioenergetic efficiency gains in the fusel alcohol pathway.

  2) *consortium conversion* for simultaneous utilization of sugar and peptide substrates to minimize unit operations.



Fusel Alcohol Product Mix



Process Byproducts

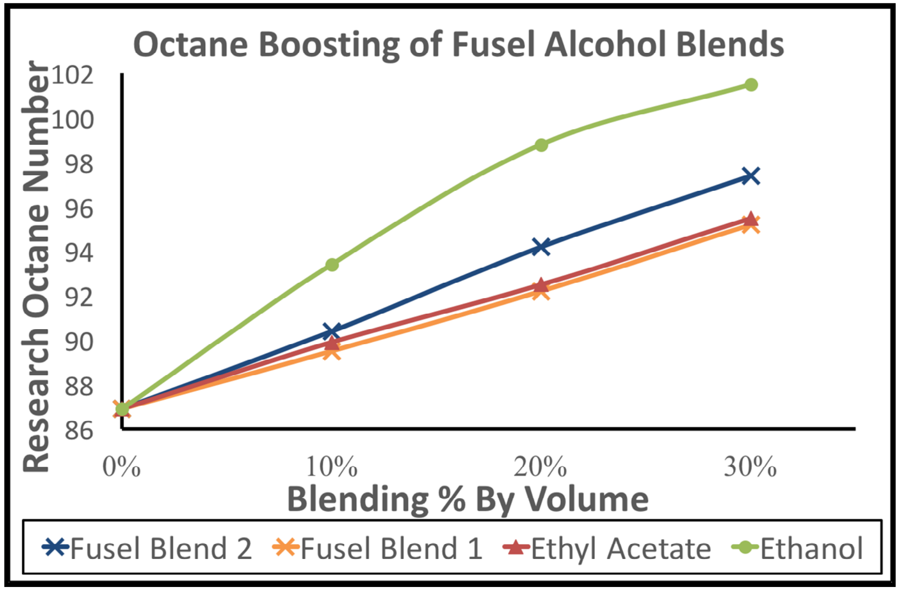
- Residual Lipids
- Acetate

Wu and Davis *Algal Res* 2016

Huo et al Nature Biot. 2011

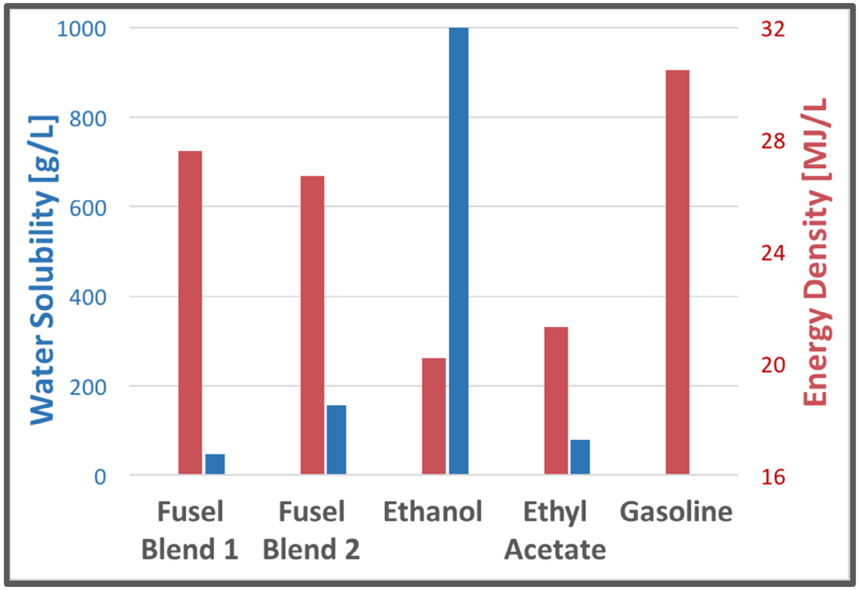
Wu et al Algal Res 2016

Investigation of fusel alcohol products as spark ignition fuels.



Fusel Blends expected to significantly decrease blended vapor pressure and to fit into existing fuel distribution infrastructure. Testing coming in the near future.

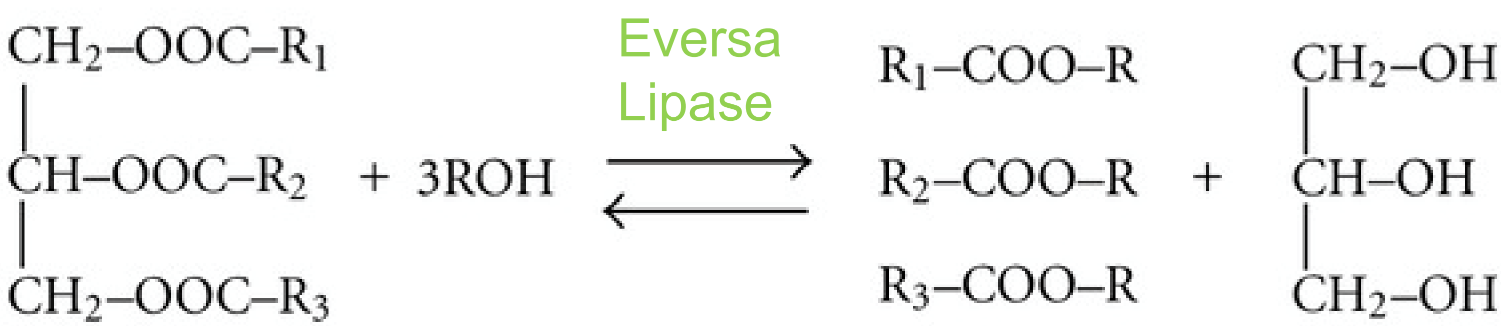
- Similar octane boosting as ethanol
- Energy Densities ~35% better than ethanol
- Corrosivity much less of an issue
- Vapor pressure of blends expected to be much lower than ethanol



Closing the biomass loops: use of residual lipids

In collaboration with Novozymes, we developed a method to react fusel alcohol mixtures with high FFA lipid fraction to make Fatty Acid Fusel Esters

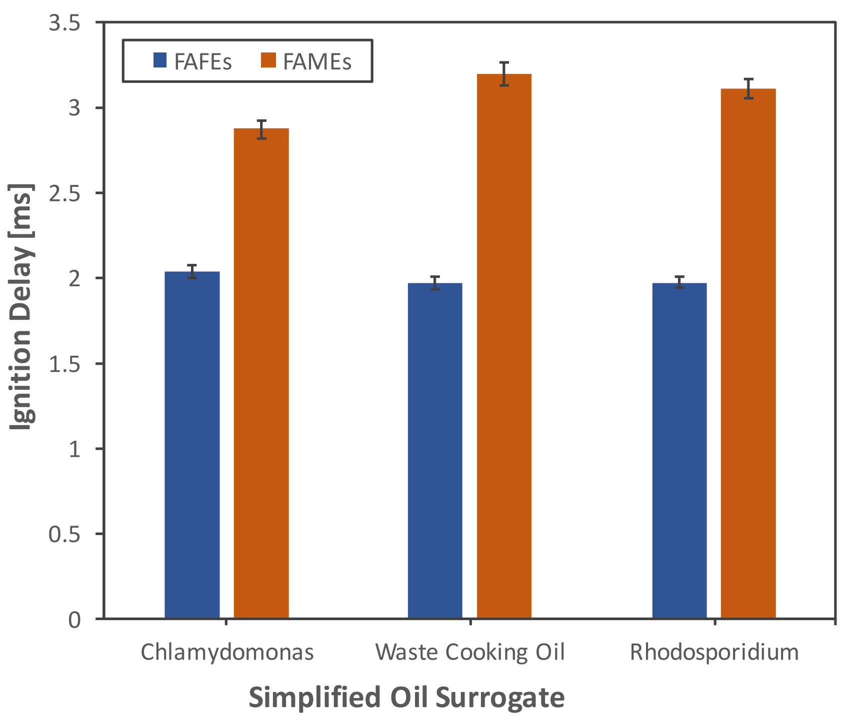
- >97% Yield after 24 hrs using 5:1 molar ratio of alcohol:oil and mixed alcohol samples.
- Significant room for reaction optimization to improve economics (lower enzyme loading, shorter reaction times, etc.)

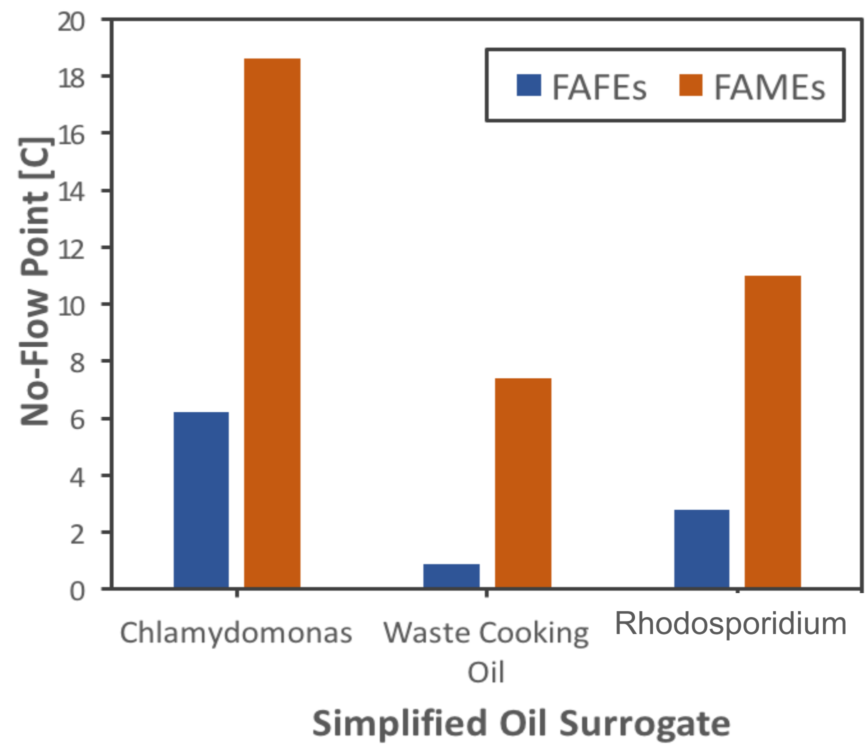


Lipids | Mixed Fusel Alcohols | Fatty Acid Fusel Esters | Glycerol

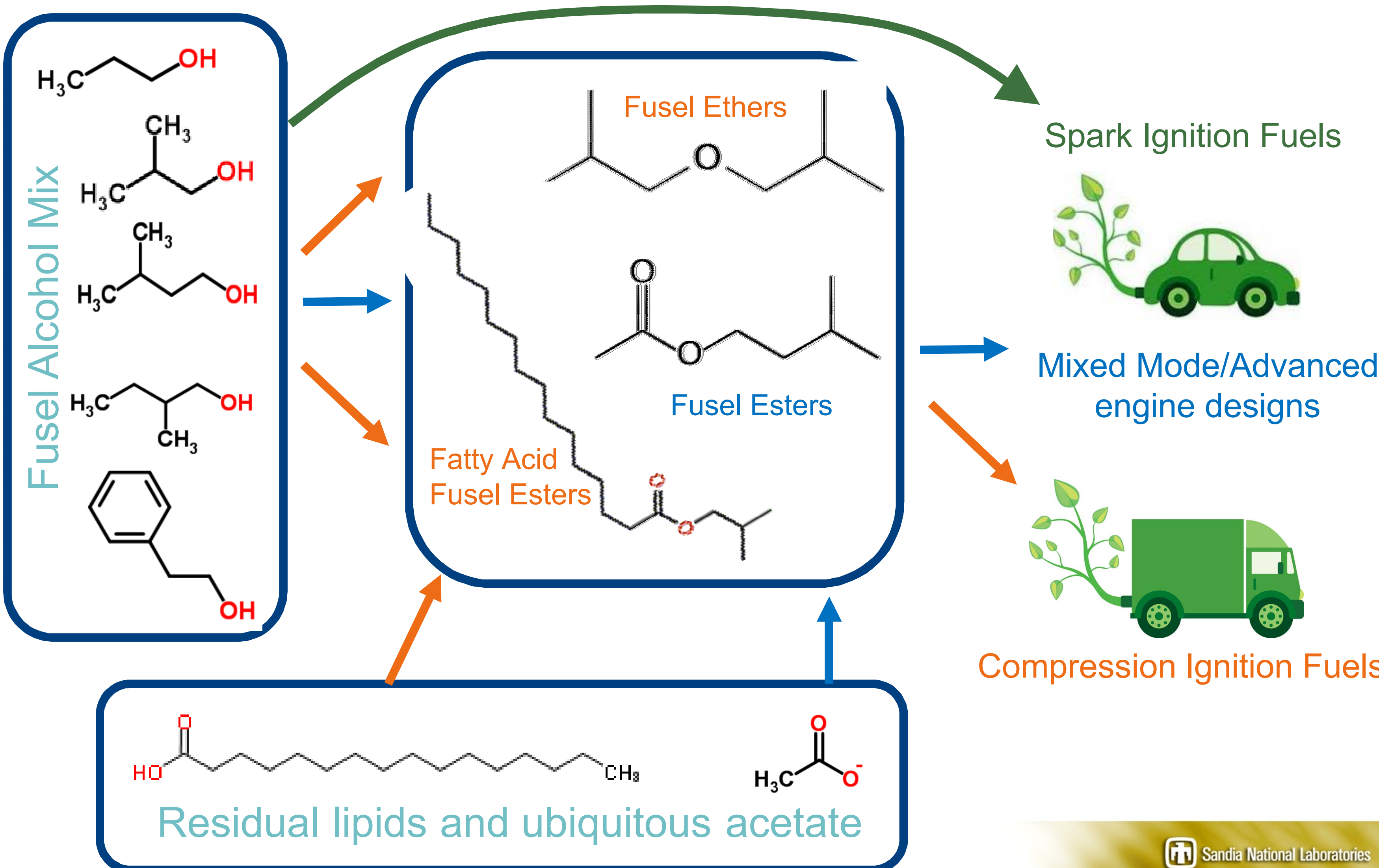
FAFEs as high performance compression ignition fuels

- 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.



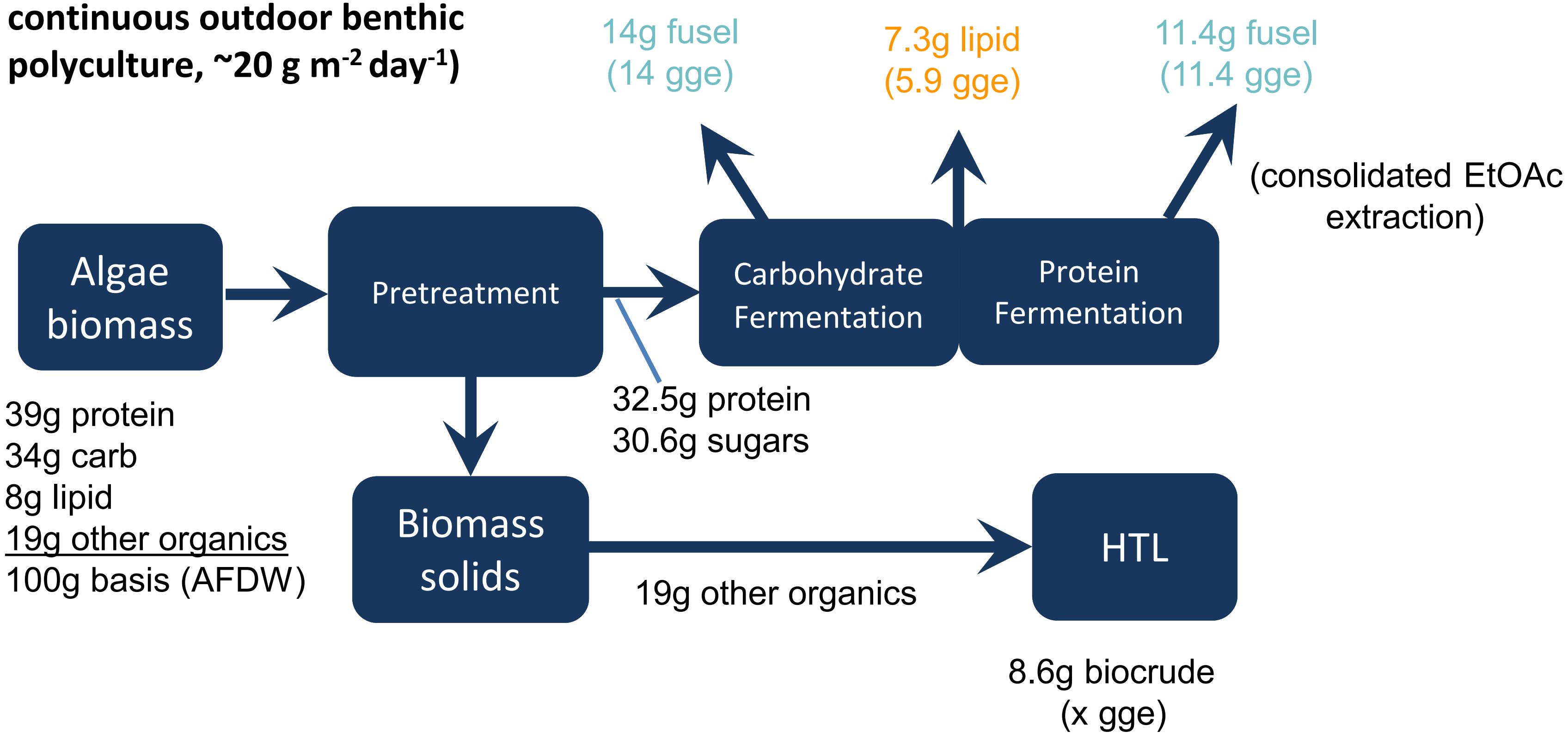


Fusel Alcohols as a platform for a diverse suite of high performance fuels



Is the loop closed and what biomass is going where?

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



TEA Modeling underway with Colorado State with promising preliminary results

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.
- The high performance of fusel alcohols and their downstream products can change the value proposition of algal biofuels in order for faster market impact.

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Co-Optimization of Fuels & Engines

novozymes

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

HydroMentia

IID A century of service.

Old Dominion University 1930

Texas A&M AgriLife Research

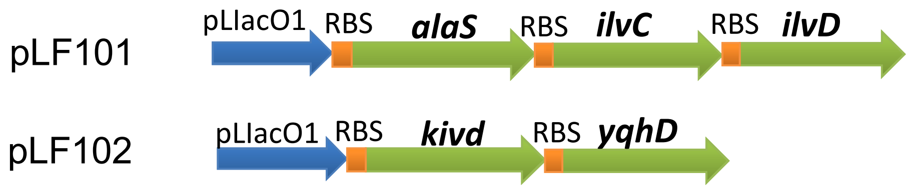
California Polytechnic State University 1901 Discere Faciendo

Colorado State University

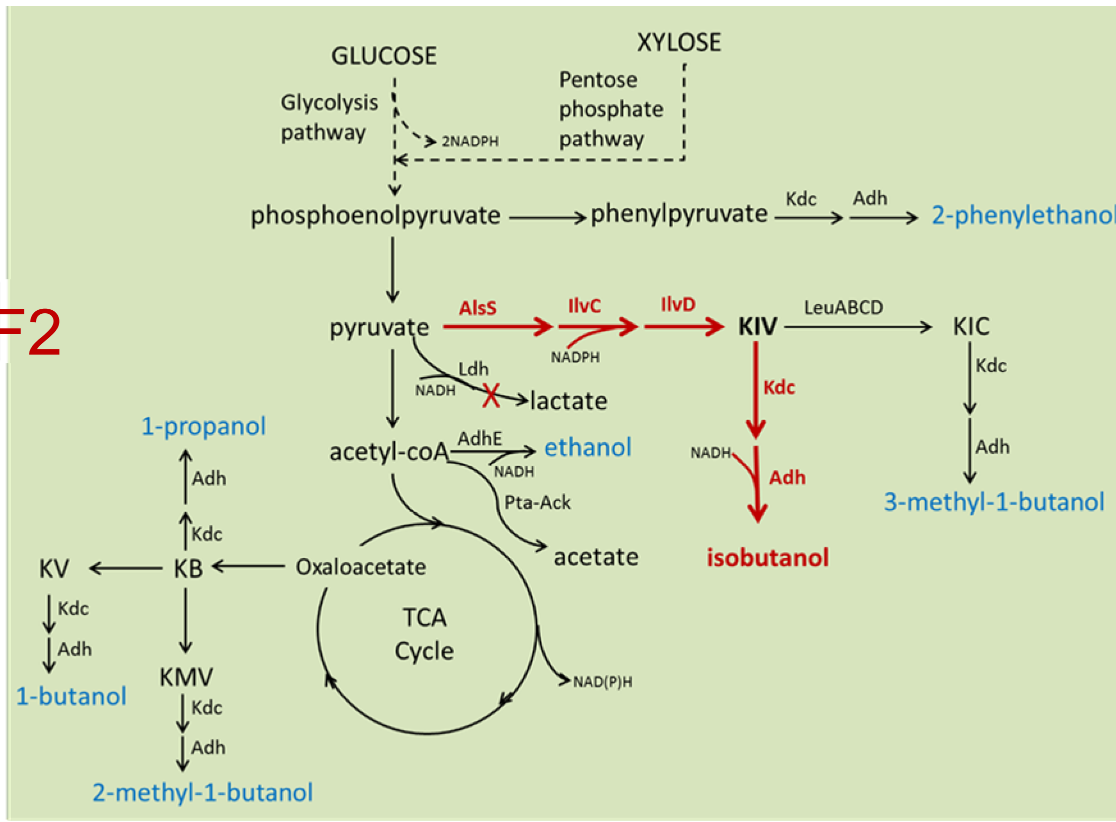
ABPDU Advanced Biofuels Process Demonstration Unit

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.



E. coli BLF2



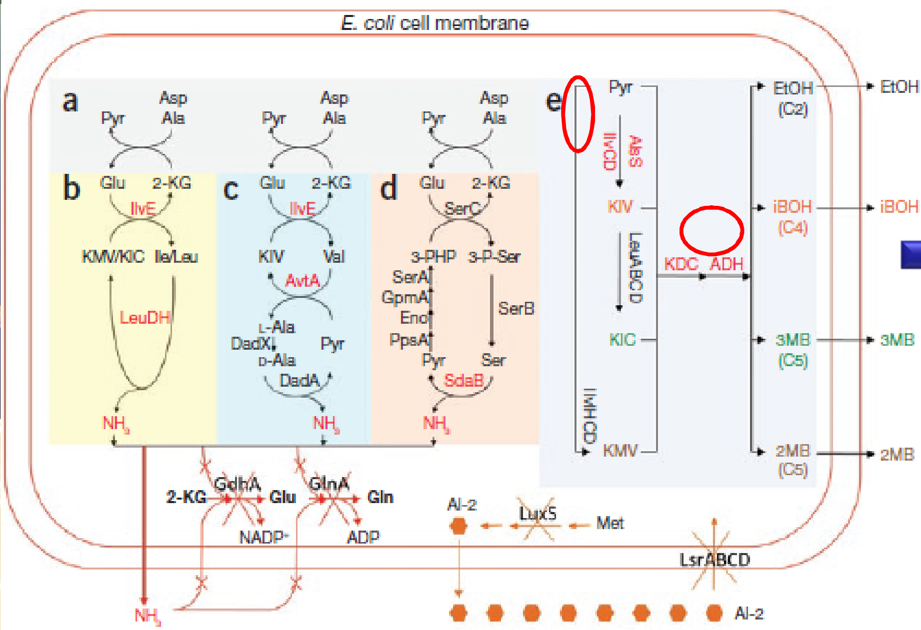
Protein fermentation strain

- Protein utilization strain

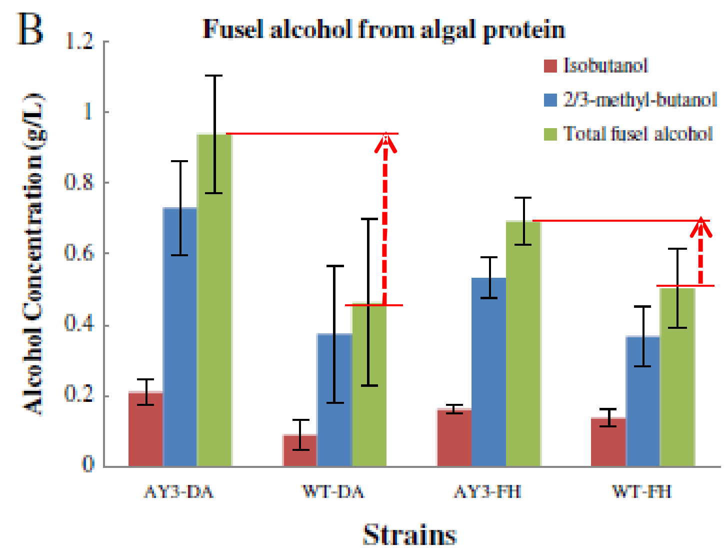
  - deaminate protein hydrolysates and convert proteins to C4 and C5 alcohols

- Improved strain: *E. coli* AY3

  - by modifying the cofactor specificity of two key enzymes (IlvC and YqhD) from NADPH to NADH



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

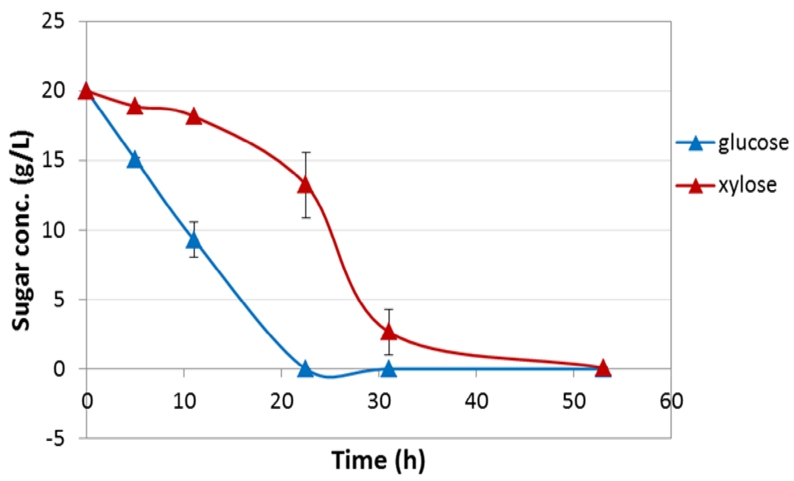


(Wu et al, *Algal Research,* 2016,19: 162-167)

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