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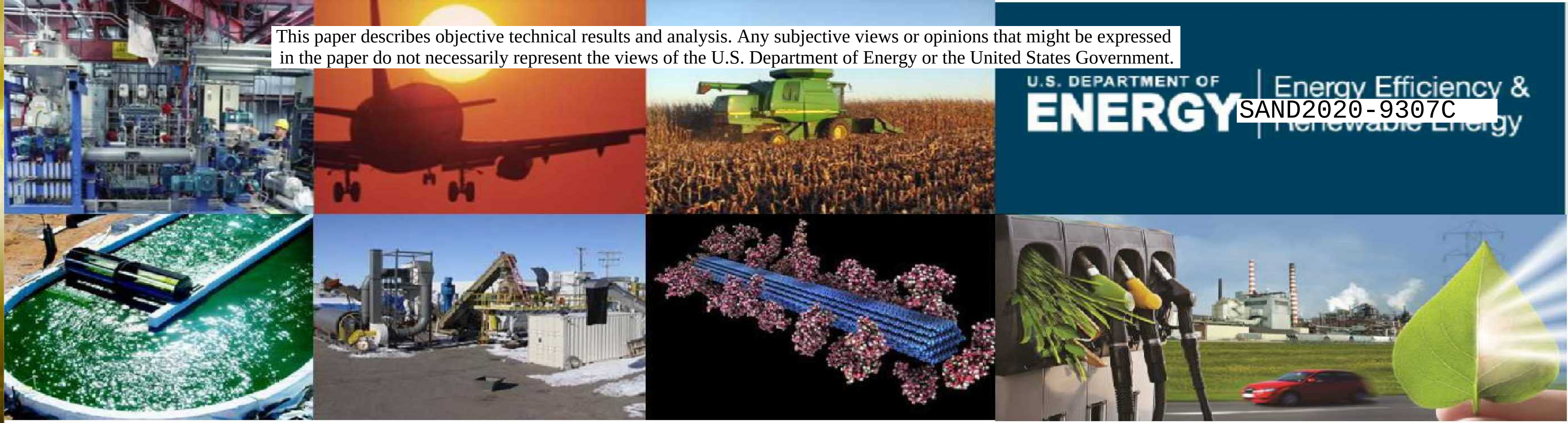

2020 Biodiesel Production Technology Summit
September 17, 2020

The Lactate and Fusel Alcohol Platform: Biofuels Production, and Biochemical Upgrading to Superior Performance Biodiesel

Somnath Shinde

Sandia National Laboratories

This presentation does not contain any confidential or otherwise restricted information

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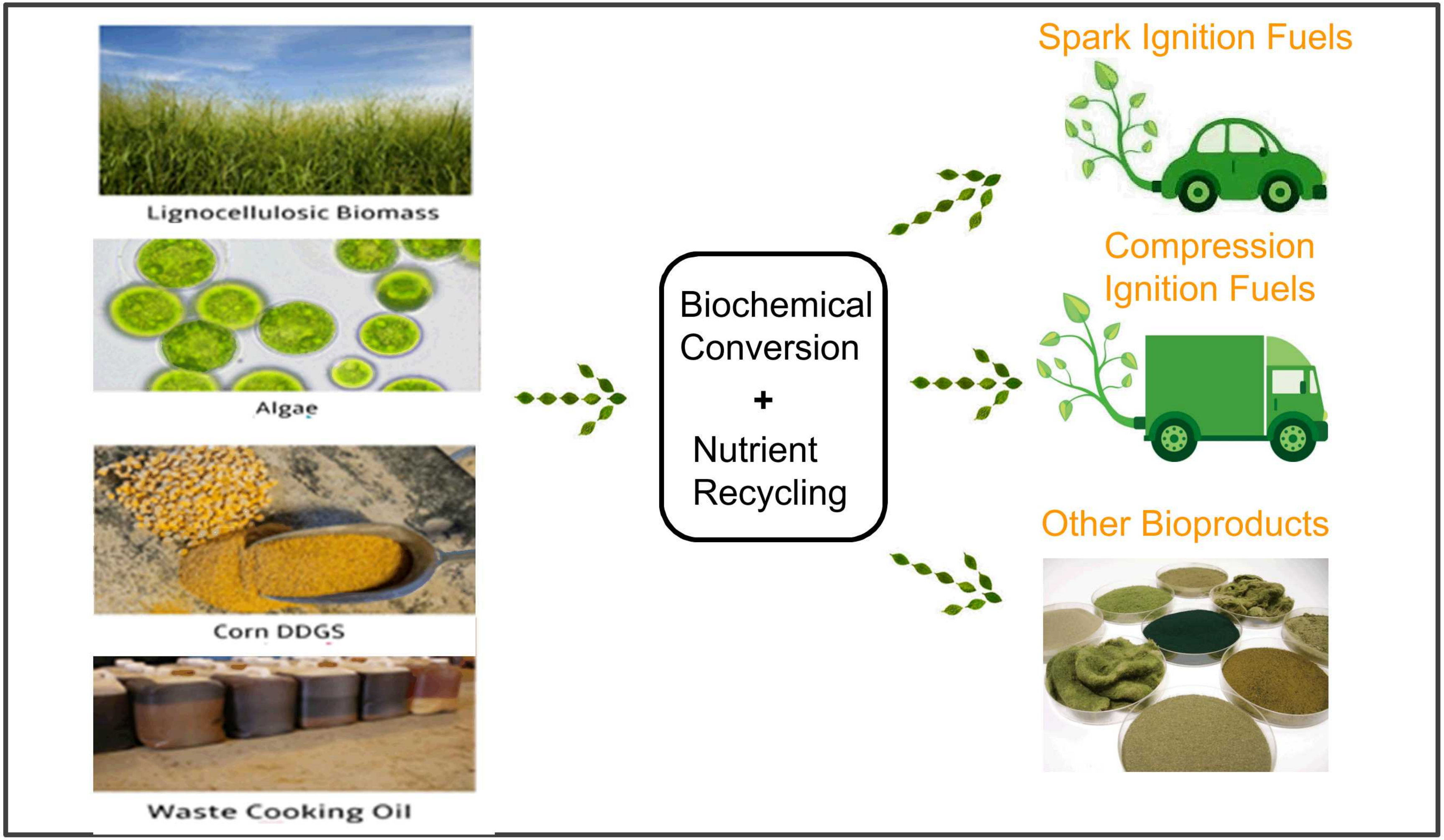
Sandia Bioscience

Sandia's perspective on biofuel production



Sandia's perspective on biofuel production

High performance fuel products can strengthen the value proposition of biofuels

Biomass Production

What can we grow?

Conversion to Fuel Products

Co-optimization of Fuels and Engines

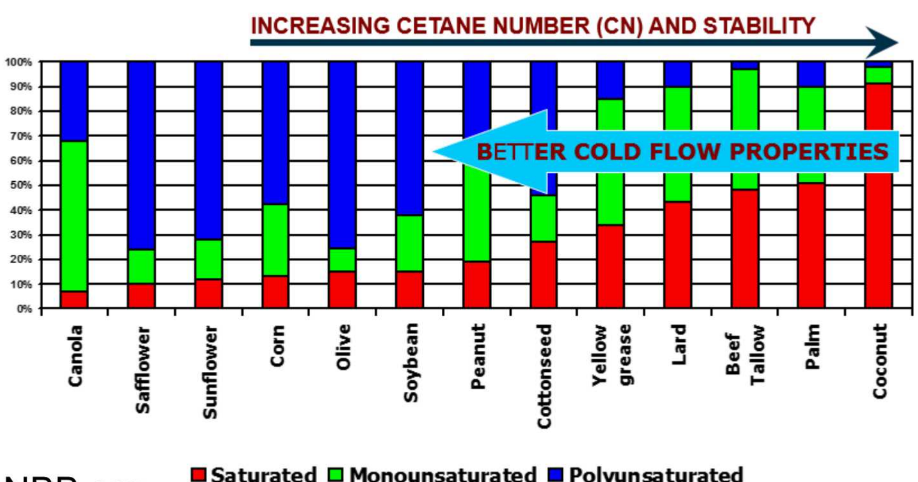
What is the best fuel?

Understanding what makes a fuel good

Evaluating a fuel is about more than just combustion

Compression Ignition Fuels

Criteria	Greatly Exceeds	Exceeds Criteria	Meets Criteria	Barriers Exist
Cetane	>50	46 to 50	40 to 45	<40
LHV (MJ/Kg)	>40	31 to 40	25 to 30	<25
Flash Point (◦C)	>70	61 to 70	52 to 50	<52
Melting Point (◦C)	<-50	-50 to -26	-25 to 0	>0
Water Solubility (mg/L)	<5	5 to 501	500 to 1000	>1000
YSI	<50	50 to 151	150 to 200	>200

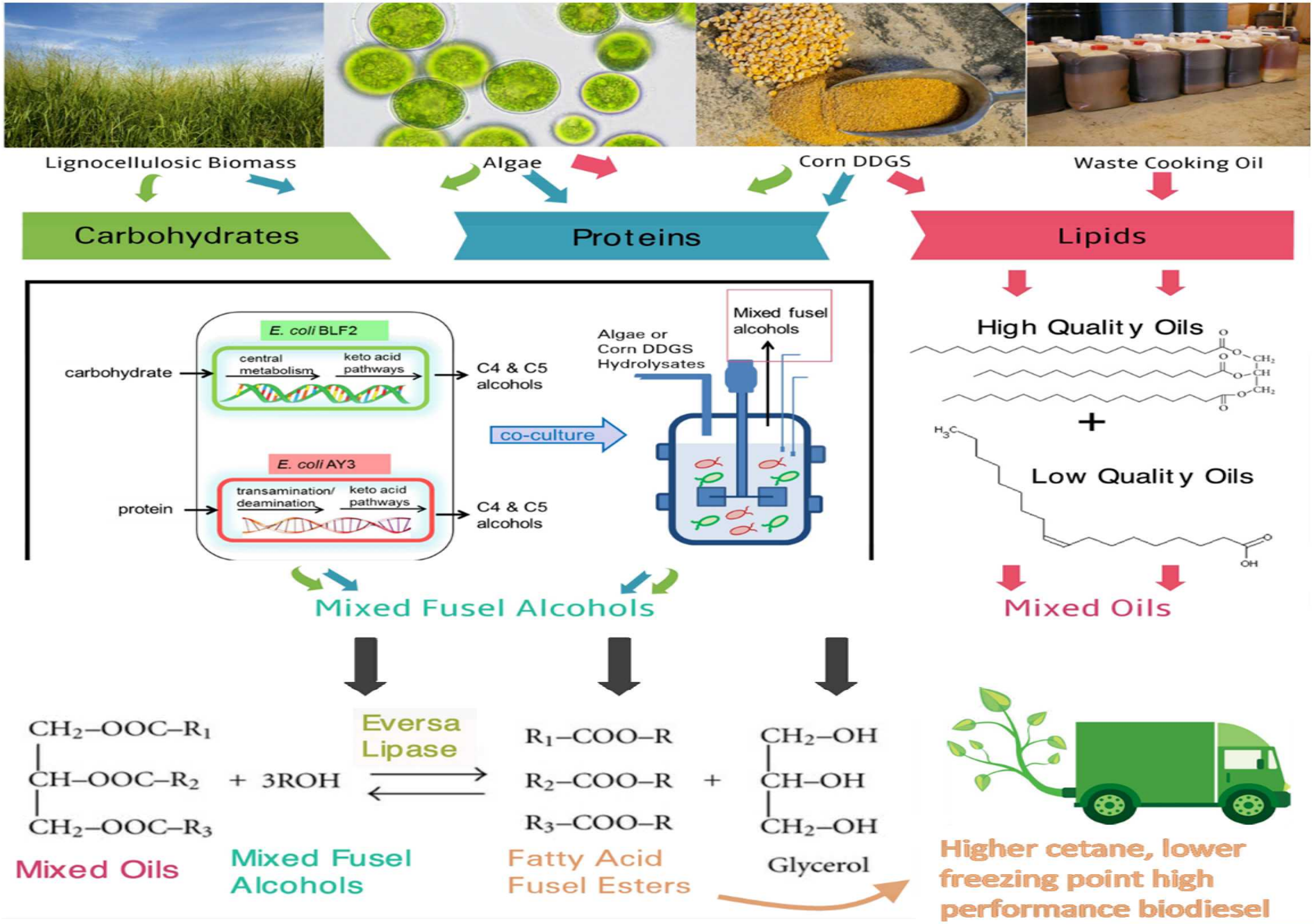


NBB.org

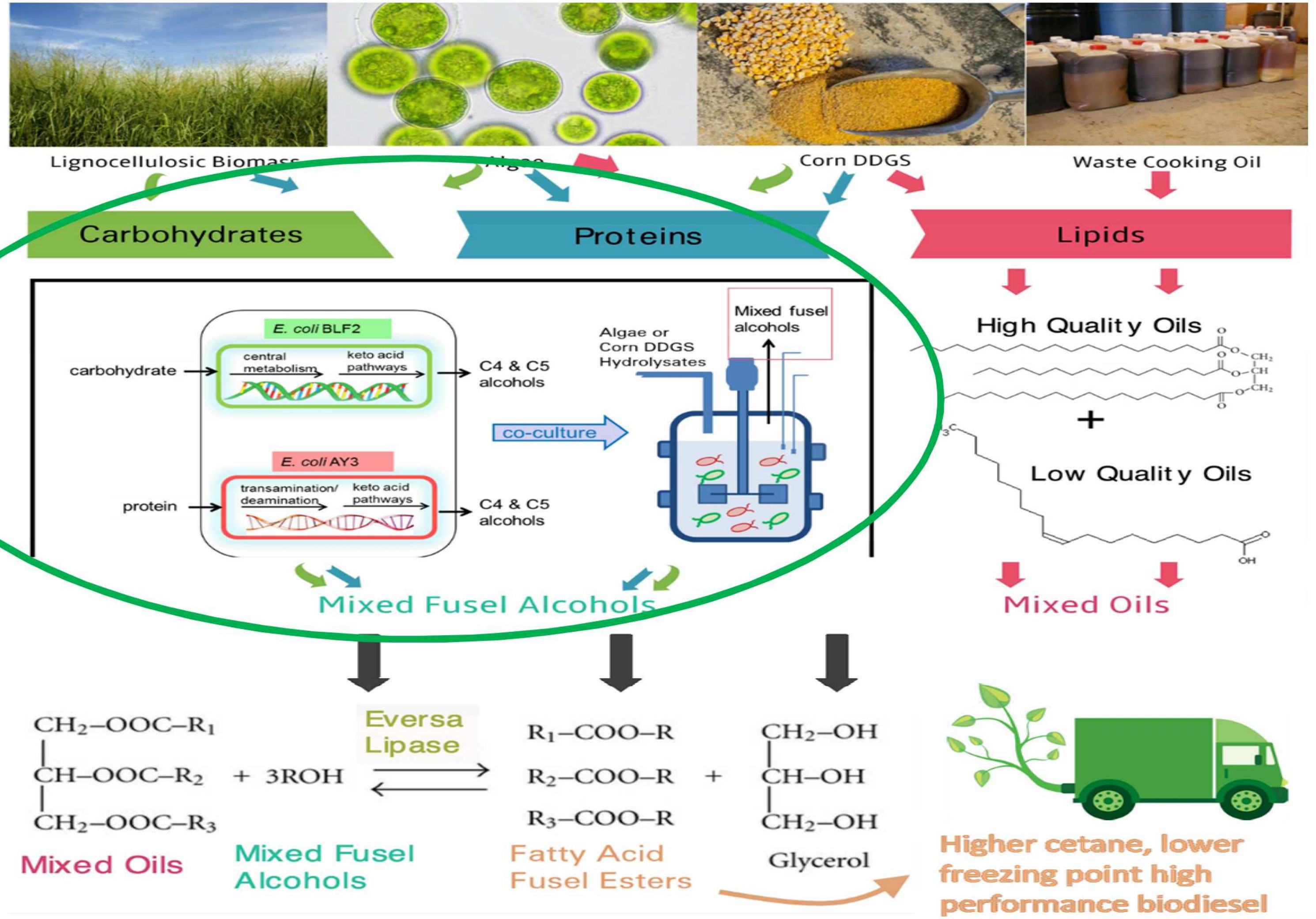
High Performance Fuels (HPF)

- Fusel Alcohols- Biofuels production and upgrading to **Fatty Acid Fusel Esters (FAFE)**
- **Biodiesel Hydroxyalkanoates**- Biochemical production of lactic acid and upgrading to HPF
- **Biodiesel Ethers**- Fatty Acid Derived Alkyl Ether Fuels

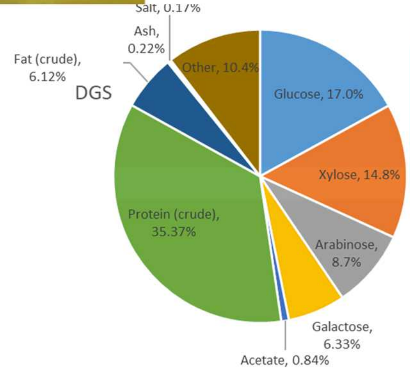
Our goal: a robust, feedstock agnostic bioconversion process to utilize this biomass

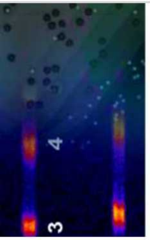


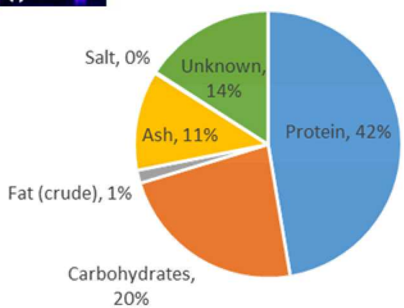
Our goal: a robust, feedstock agnostic bioconversion process to utilize this biomass



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







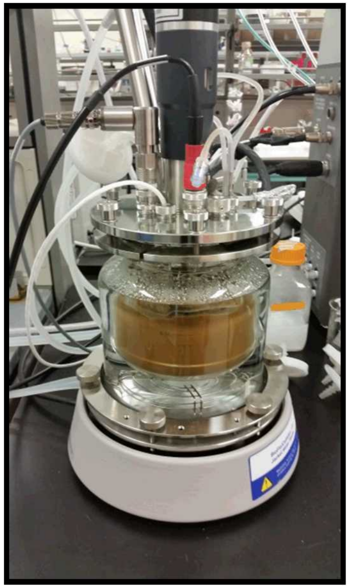
Biomass

Carbohydrates

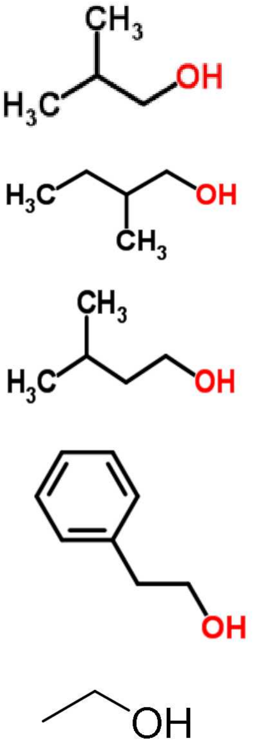
Proteins

Pretreatment

Fermentation



Fusel Alcohol Product Mix



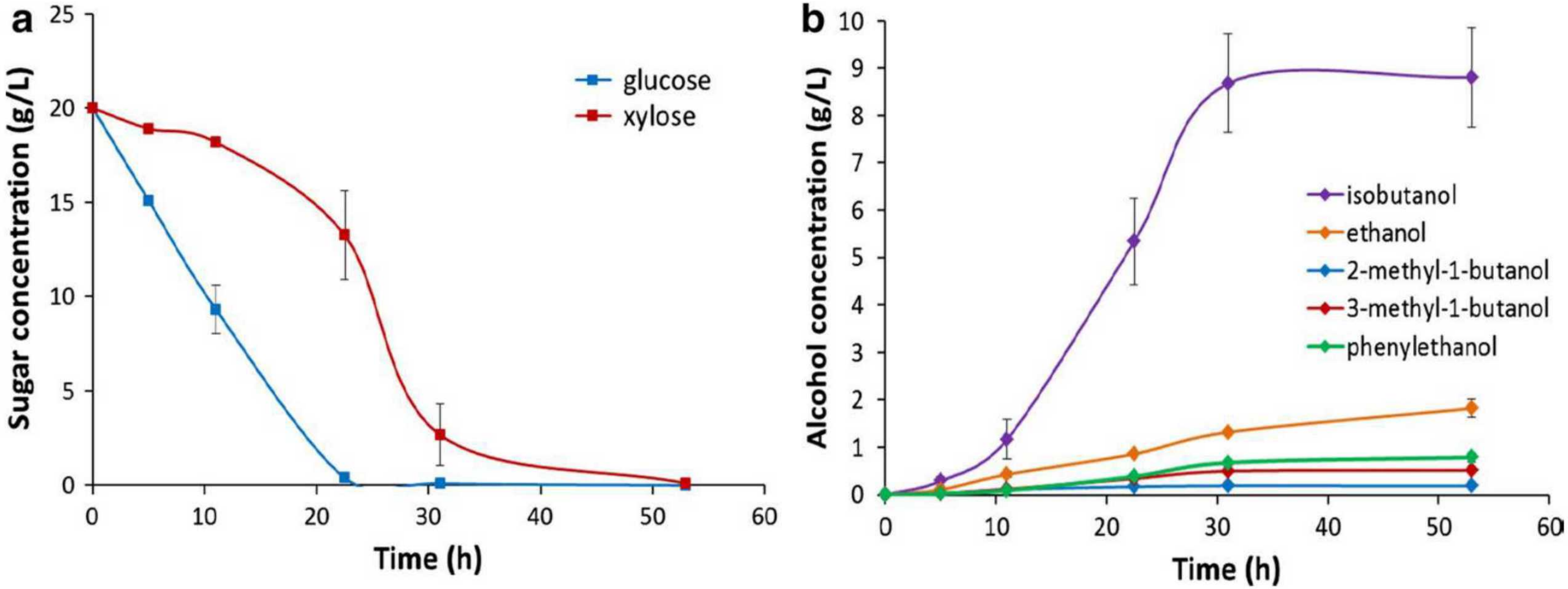
Huo et al Nature Biot. 2011

Wu et al Algal Res 2016

Wu and Davis *Algal Res* 2016

Liu et al Microbial Cell Factory 2017

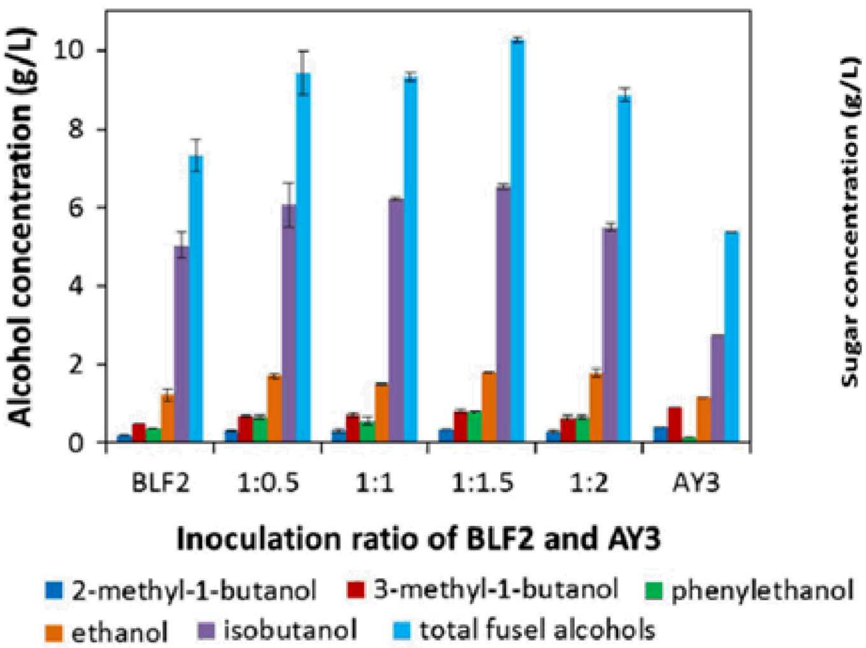
Fermentation of a glucose and xylose mixture by E. coli BLF2.

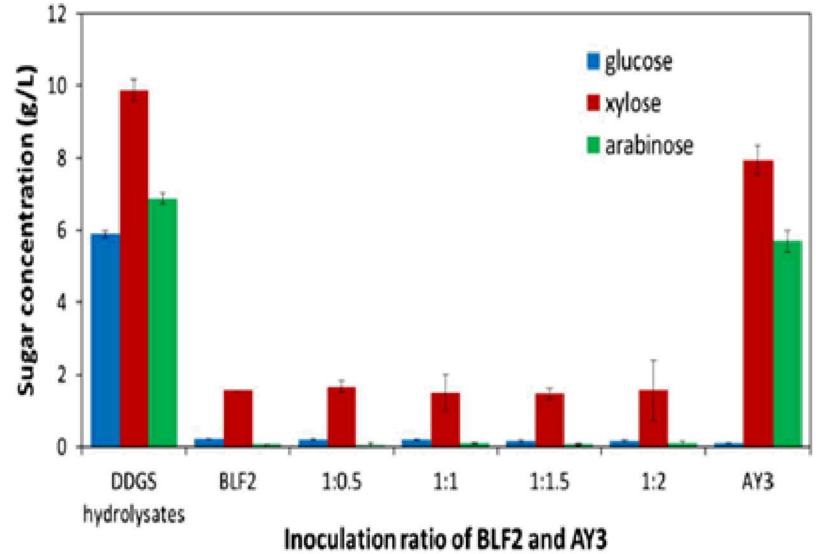


- The volumetric productivity for the total fuel alcohols from the sugar mixture was about 0.37 g/L h which was lower than that from glucose but higher than when xylose was used as a sole carbon source

Liu et al Microbial Cell Factories (2017) 16 (1),192

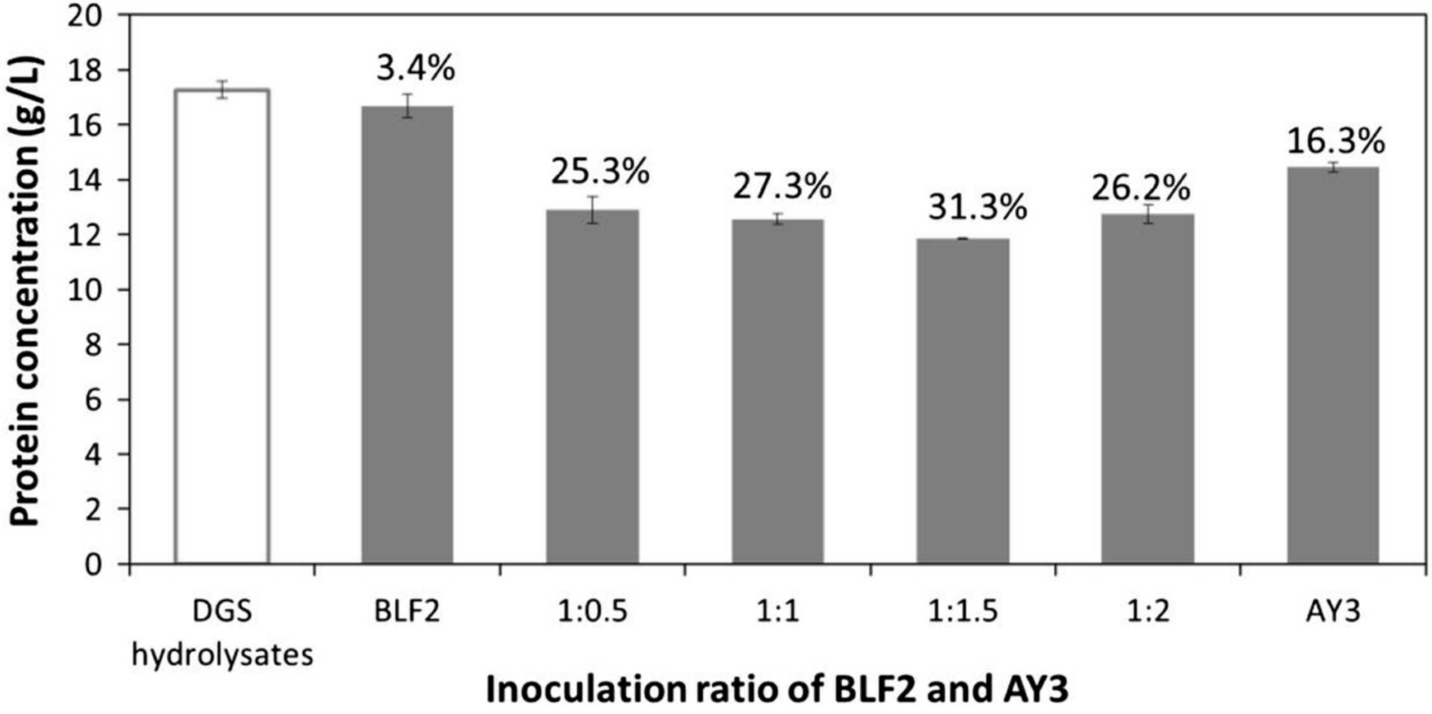
Fusel alcohols production from DGS





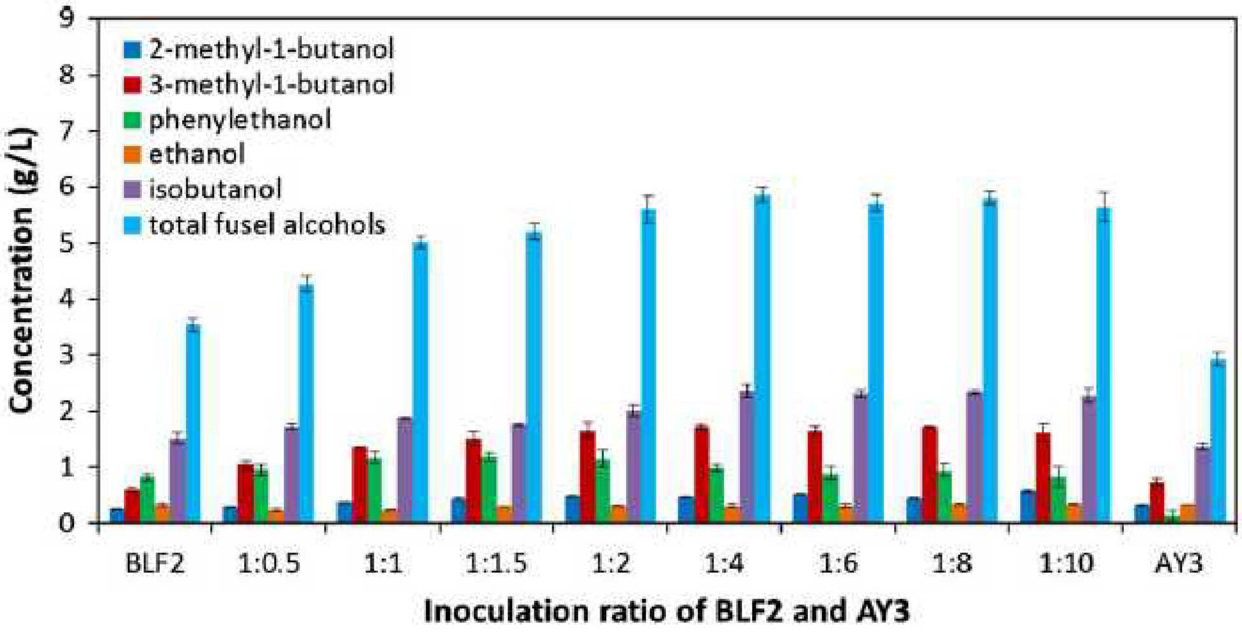
- The co-culture with an inoculation ratio of 1:1.5 of *E. coli* BLF2 and AY3 achieved the highest total fuel titer of up to 10.3 g/L from DGS hydrolysates.

Fusel alcohols production from DGS



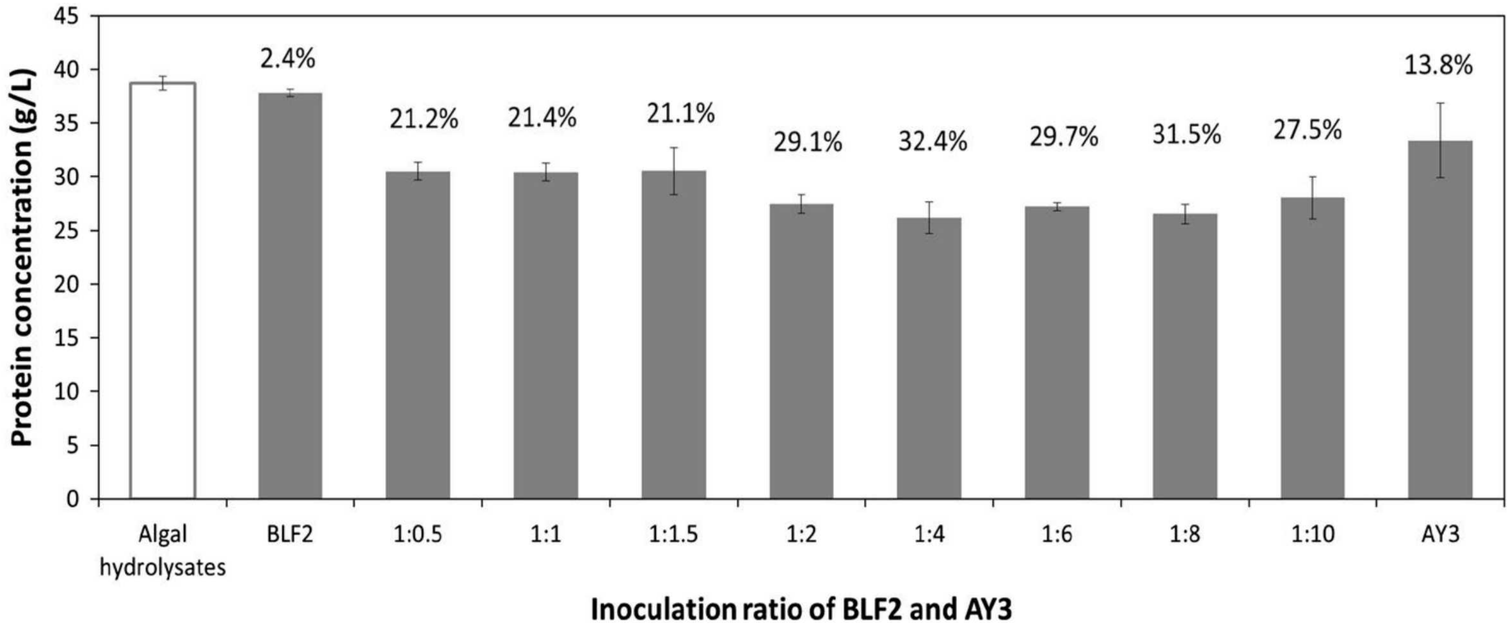
- Up to 31.3% of the initial 17.5 g/L proteins in the DGS hydrolysates were converted by the co-culture with an inoculation ratio of 1:1.5

Fusel alcohols production from *Nannochloropsis sp.* algae hydrolysates



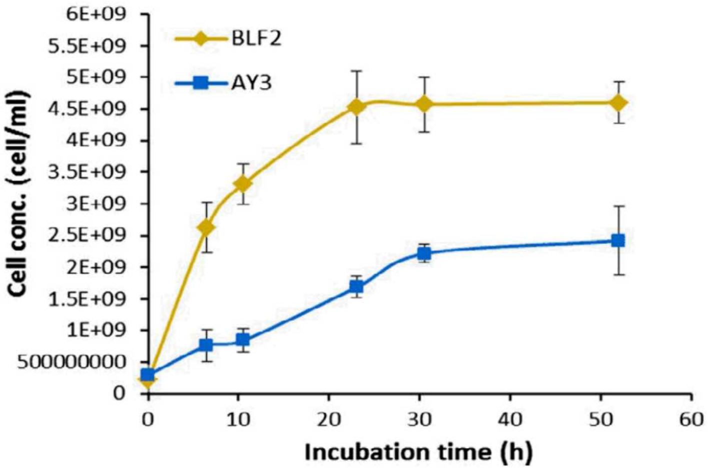
- The composition of the fusel alcohols products from algae hydrolysates included isobutanol (40.3% (w/w)) and mixed isopentanols (2-methyl-1-butanol and 3-methyl-1-butanol (37.3% (w/w)), indicating significant enrichment of the C5 alcohols compared to the product spectrum produced from DGS, where isobutanol was the major product (63.1% (w/w))

Fusel alcohols production from *Nannochloropsis sp.* algae hydrolysates

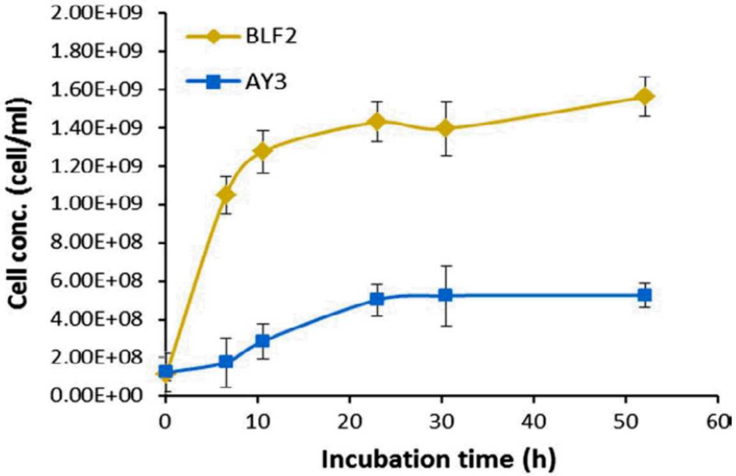


- Up to 32.4% of the initial 38.7 g/L proteins in the algae hydrolysates were converted by the co-culture with an inoculation ratio of 1:4

Growth dynamics of individual populations in the co-culture



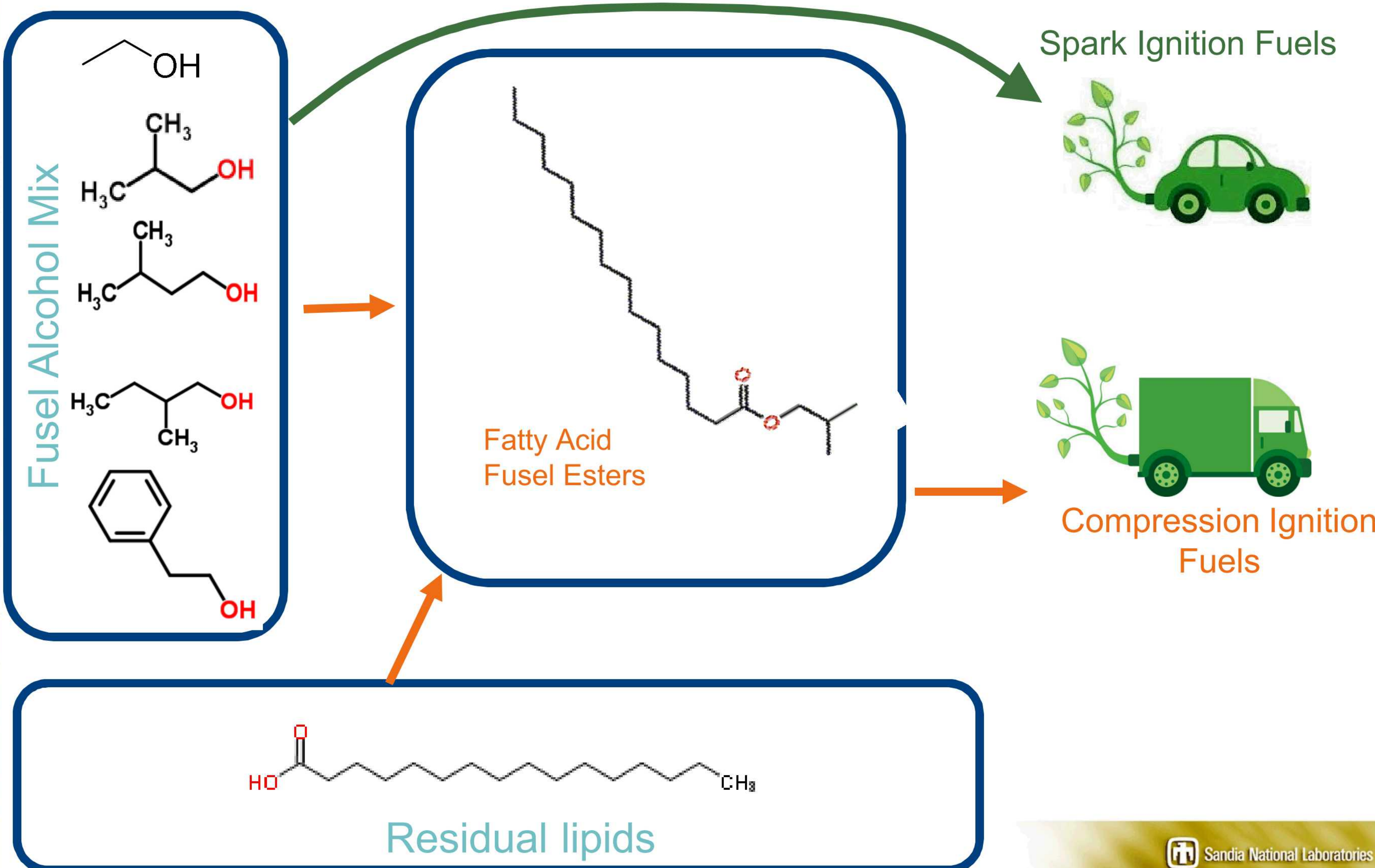
DGS hydrolysate with a BLF2/AY3 inoculation ratio of 1:1.5



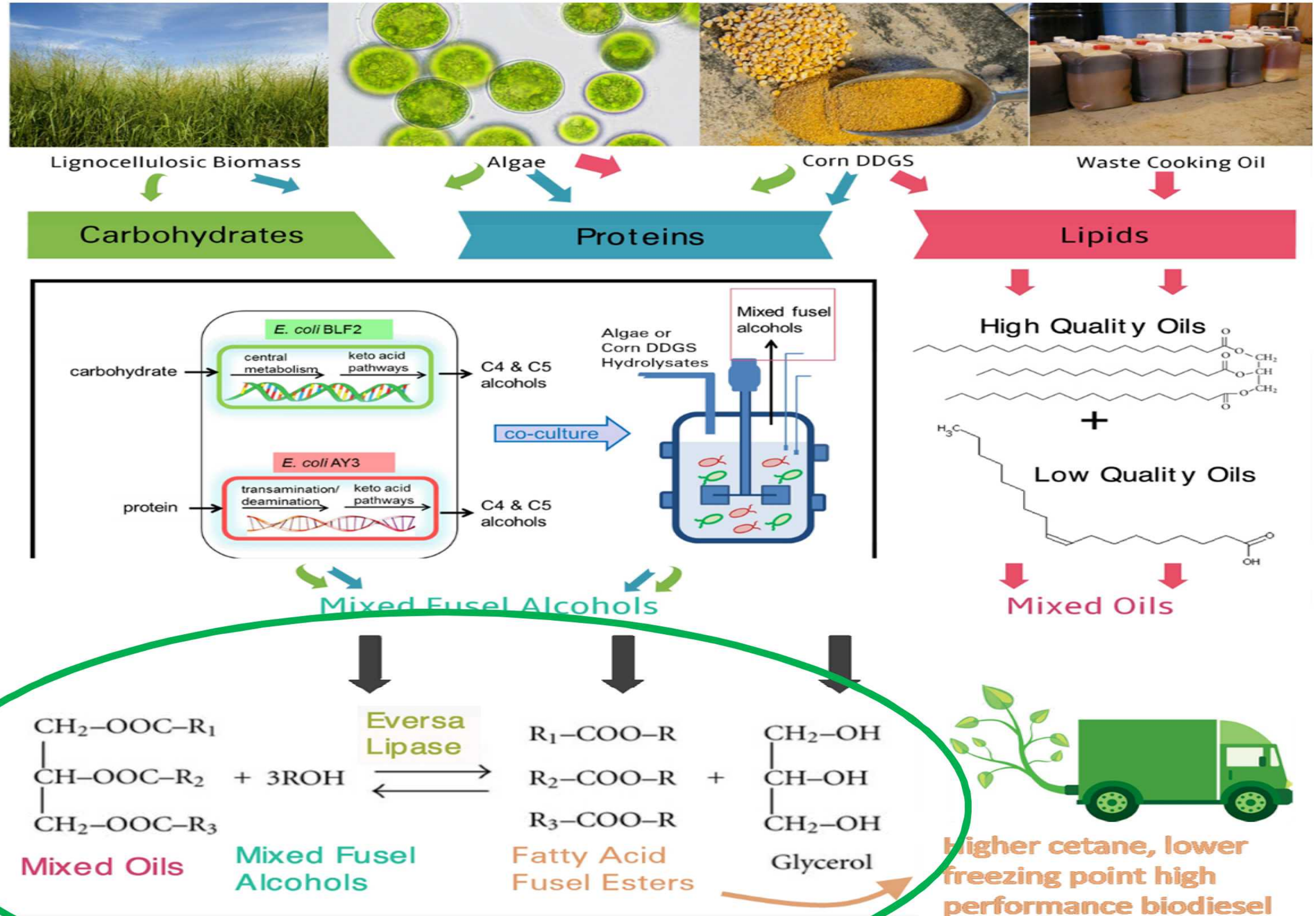
Algae hydrolysate with a BLF2/AY3 inoculation ratio of 1:4

- In both of the hydrolysates, the cell number of the two strains continuously increased until reaching plateau, which indicated that despite the growth rate difference between the two strains, the co-culturing didn't adversely affect the growth of each strain.
- The final cell numbers of AY3 in the co-cultures at proper inoculation ratios of BLF2/AY3 were no less than the cell number of AY3 monoculture in the hydrolysates.

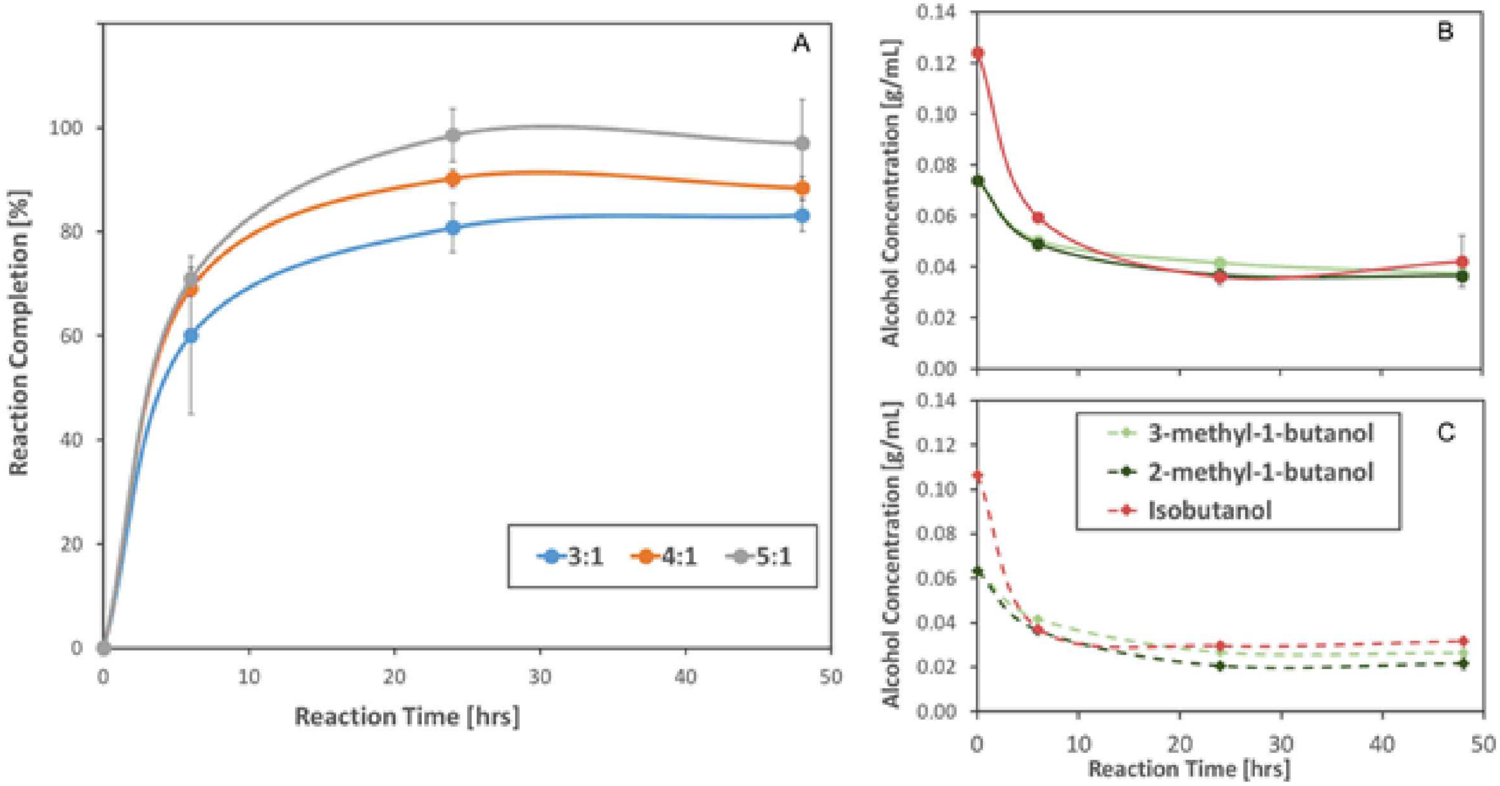
Fusel Alcohols as a platform for a diverse suite of high performance fuels



Our goal: a robust, feedstock agnostic bioconversion process to utilize this biomass



Fatty Acid Fusel Esters (FAFE)

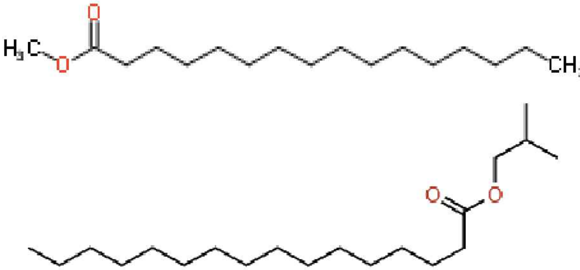


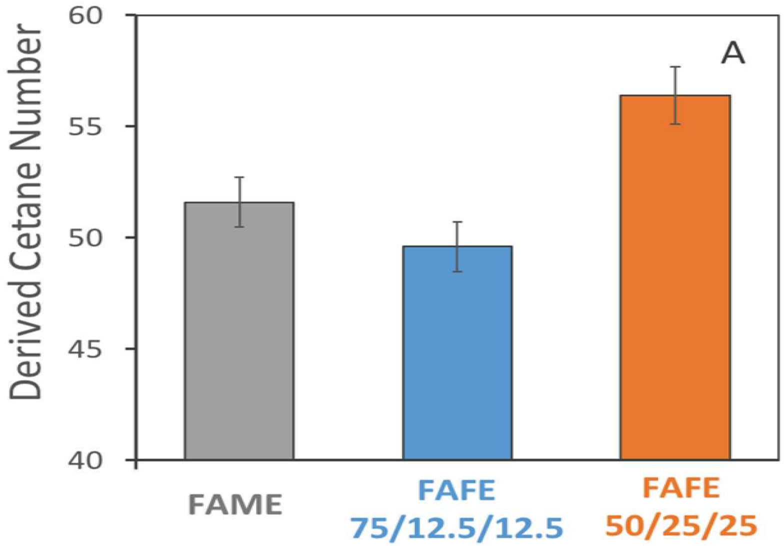
- >97% Yield after 24 hrs using 5:1 molar ratio of alcohol:oil and fusel alcohol mixture.

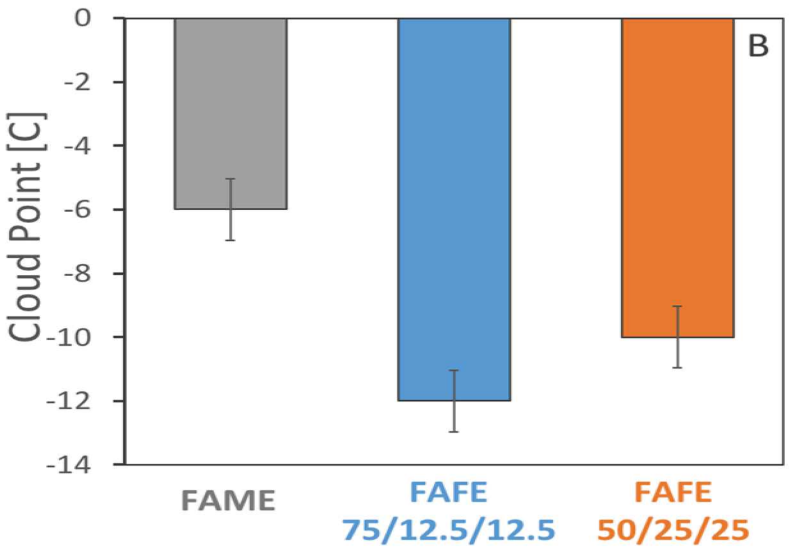
Monroe et al Fuel 268,117408

FAFEs as high performance compression ignition fuels

- FAFE 50/25/25 having a 4.8 higher DCN than FAME, while FAFE 75/12.5/12.5 was not significantly different than FAME.
- FAFE 50/25/25 and FAFE 75/12.5/12.5 demonstrating a 4 ◦C and 6 ◦C lower no-flow point respectively when compared to FAME.



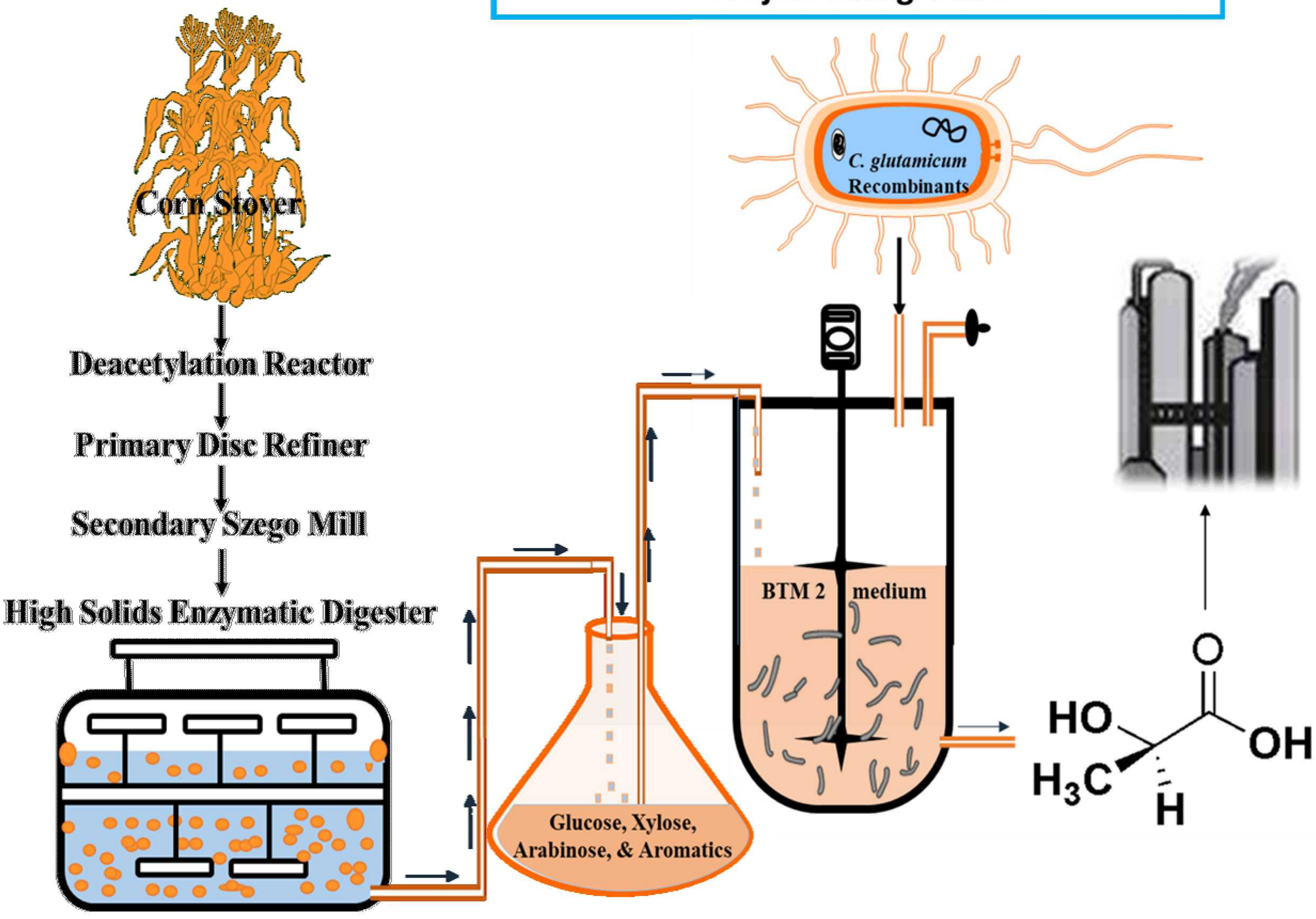




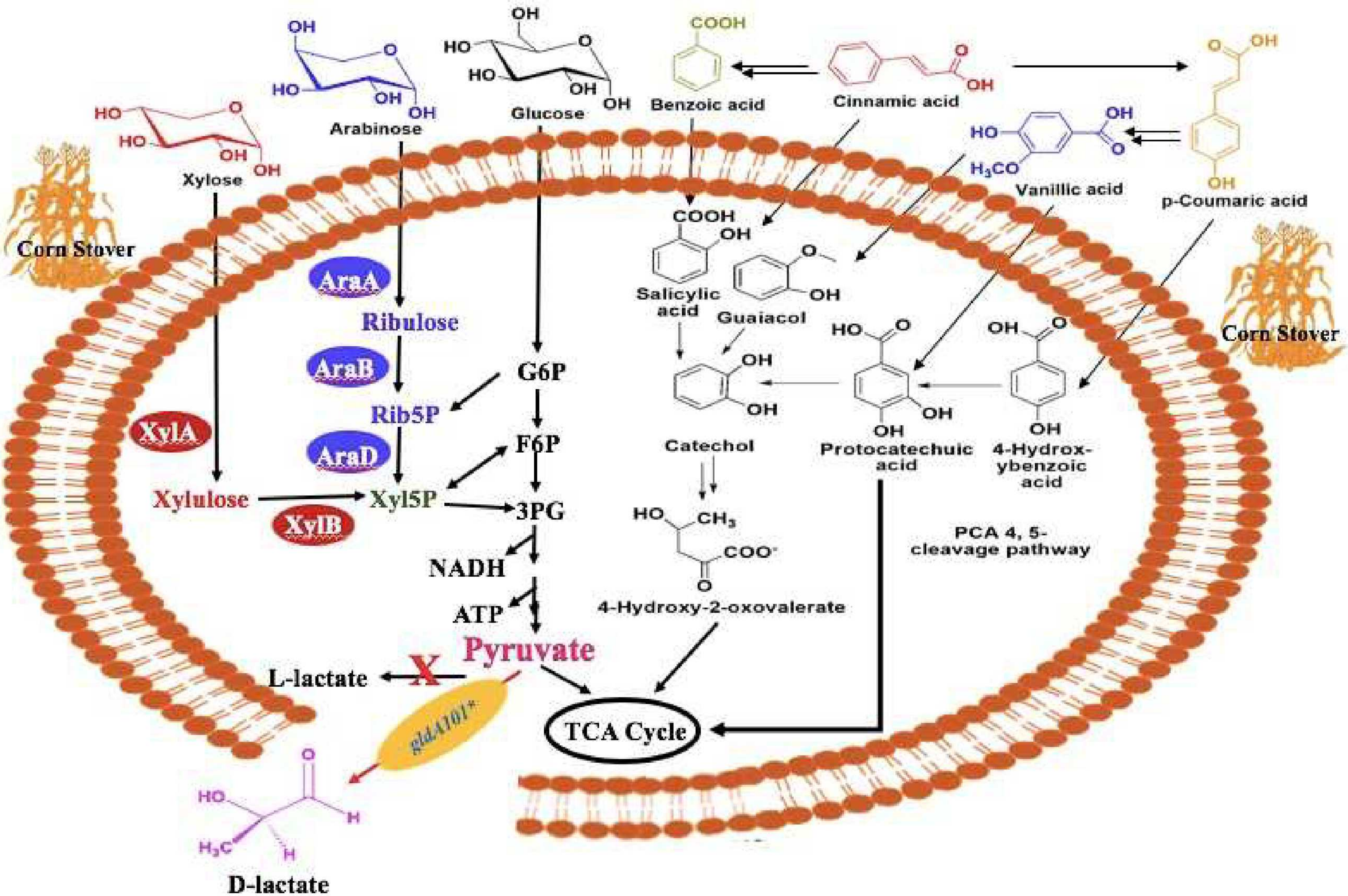
Monroe et al., Fuel, Volume 268 (2020) 117408

Hydroxyalkanoates: Biological production and upgrading to high performance fuels

Project background

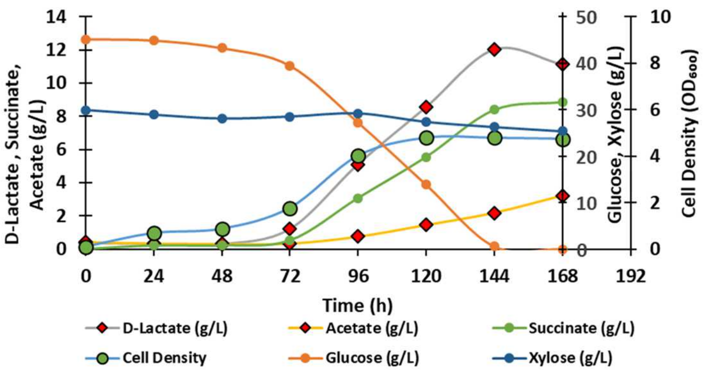


Metabolic Engineering strategy



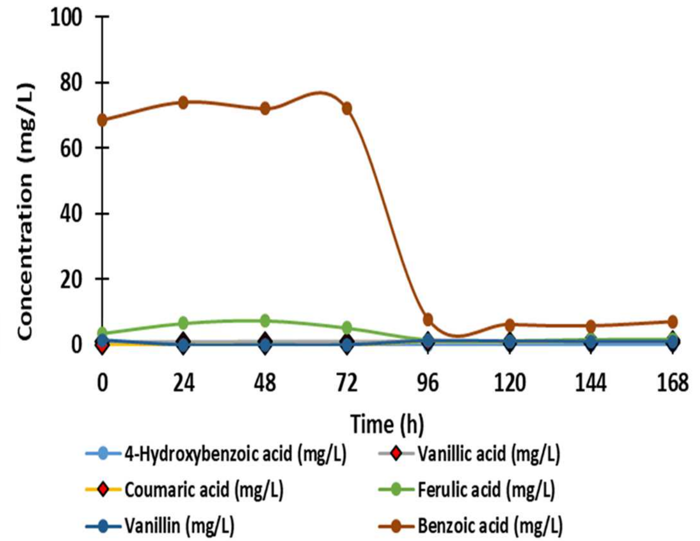
Batch fermentation studies of D- lactate production on DMR corn stover hydrolysate

D-lactic acid production from DMR hydrolysate



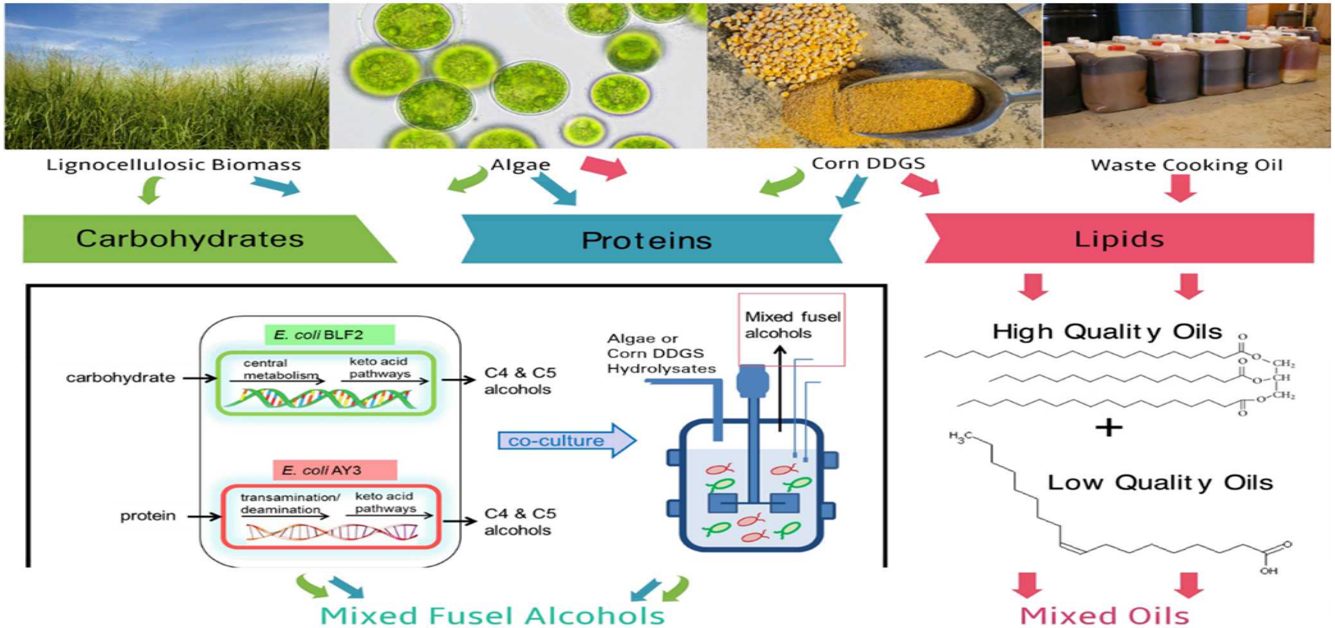
Conditions: Anaerobic, batch, pH 7, SSL09, DMR hydrolysate

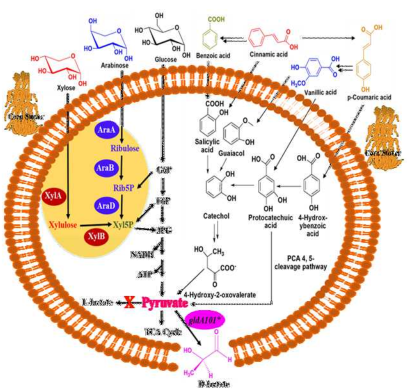
Aromatics utilization

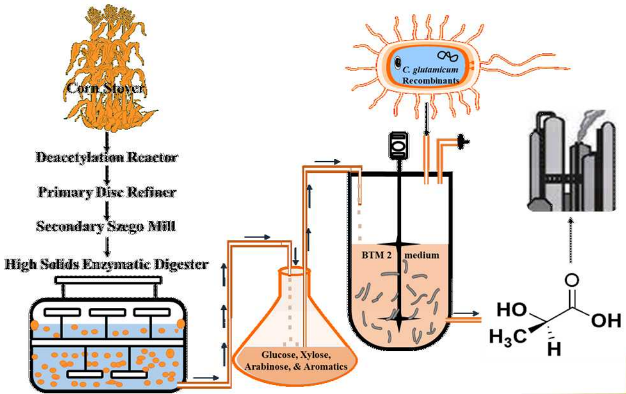


Yp/s (g D-lactate/g sugar)	0.148
D-lactate (g/L)	11.1
D-lactate productivity (g/L/h)	0.066

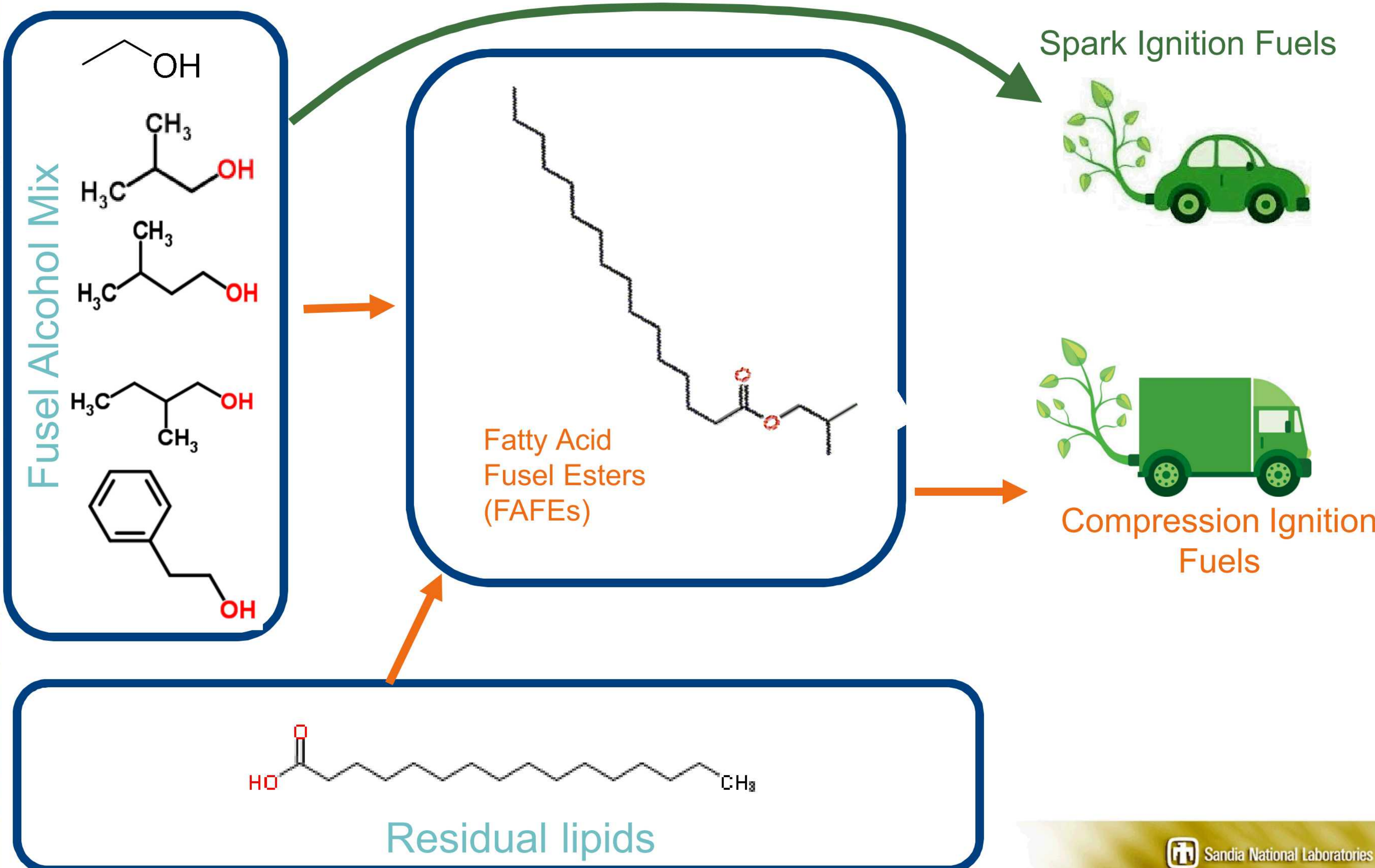
The Lactate and Fusel Alcohol Platform



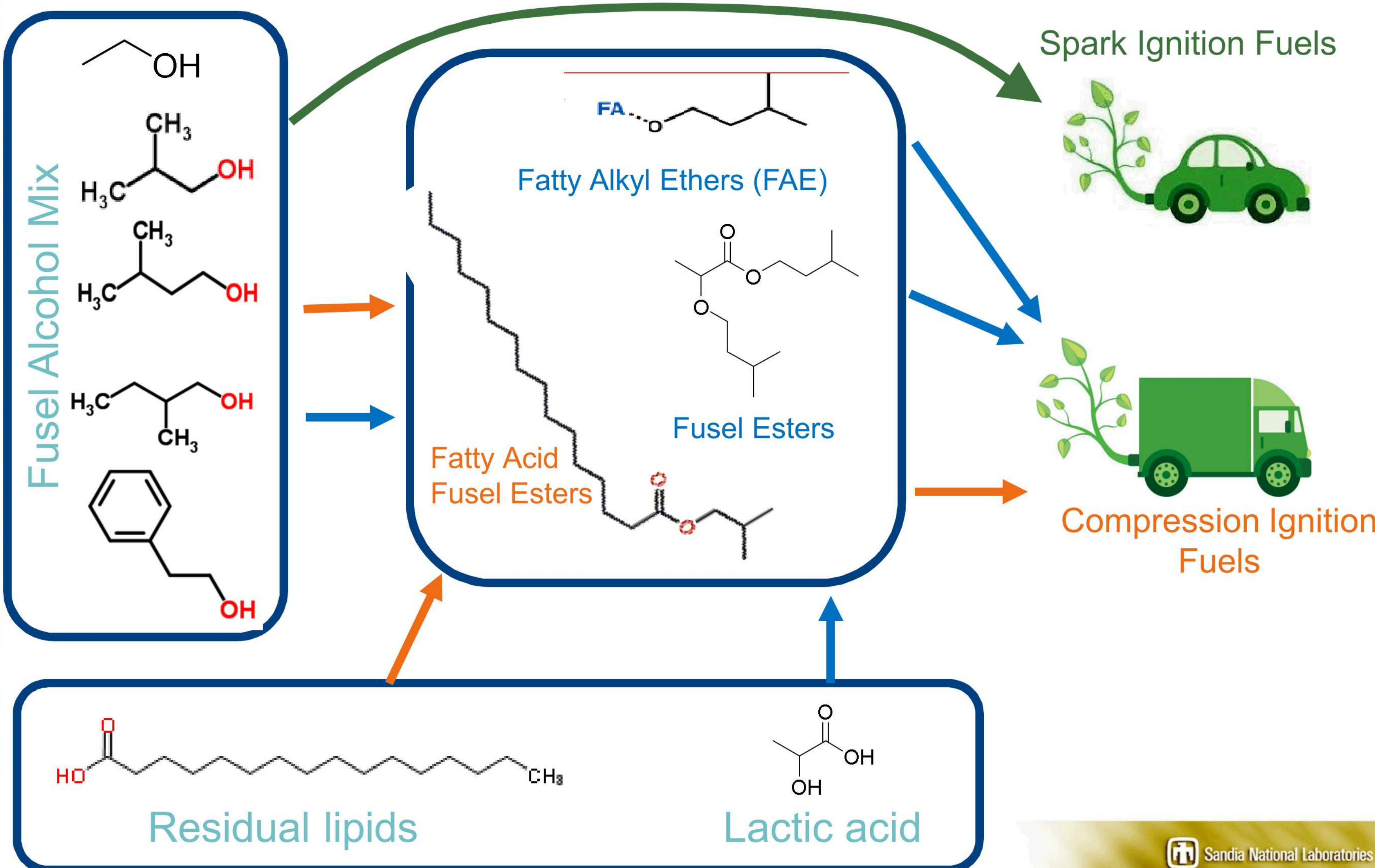




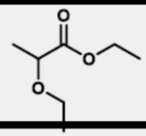
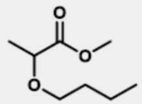
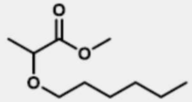
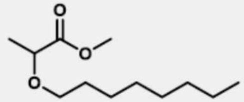
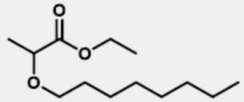
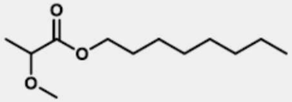
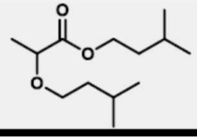
Fusel Alcohols as a platform for a diverse suite of high performance fuels



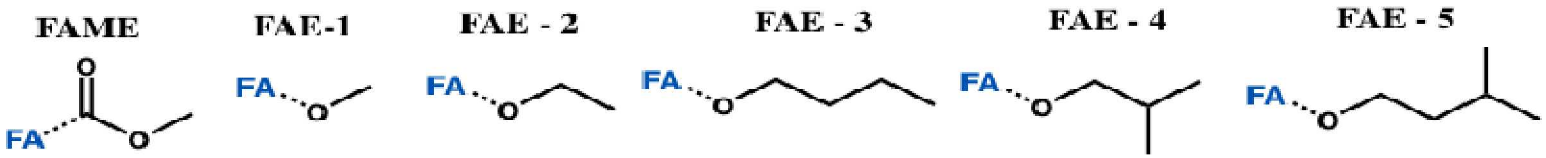
The Lactate and Fusel Alcohols platform for a diverse suite of high performance fuels



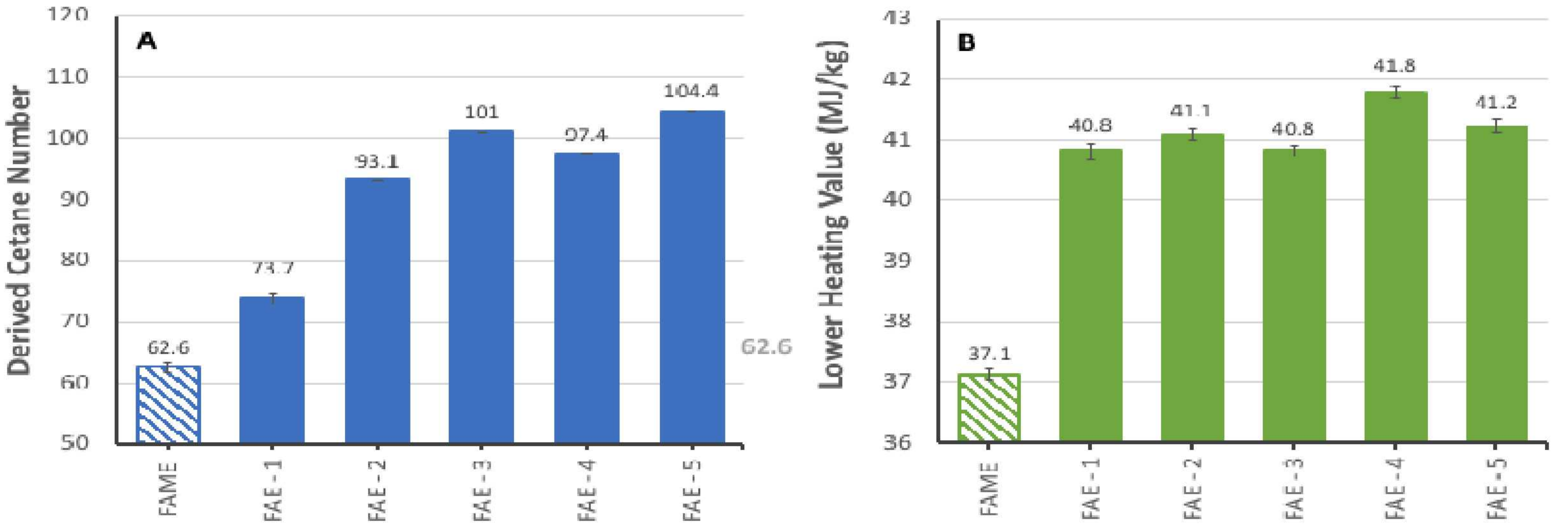
Hydroxyalkanoates as high performance fuels

Trend	Chemical Structure	C:O ratio	DCN	LHV (MJ/kg)	Flash Pt (° C)	YSI	M.P. (° C)	B.P. (° C)
C2, C2		2.33	23.1	25.48	TBD	21.7	<-50	167
		2.67	46.3	TBD	89	29.9	<-60 (CP)	189.8
		3.33	35.4	TBD	64.5	42.9	<-60 (CP)	235.6
Increasing **Ether** Carbon Length		4.00	59.4	30.79	TBD	56.0	TBD	281
		4.33	62.2	TBD	117.5	60.8	<-60 (CP)	281.4
Increasing **Ester** Carbon Length		4.00	57.5	31.71	TBD	56.0	TBD	281
Increased Branching		4.33	43.6	34.5	TBD	85.8	<-50	N/A

Fatty Alkyl Ether (FAE) biodiesel

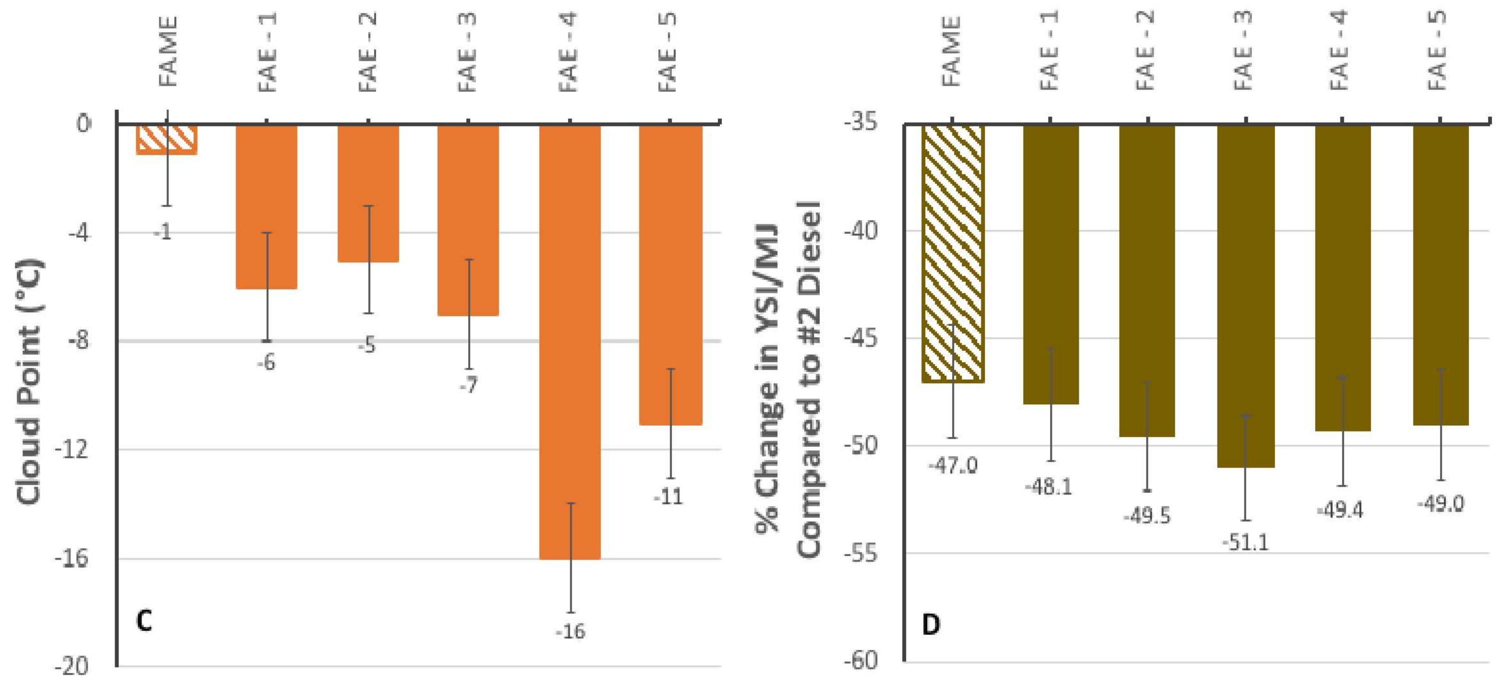


FA = Fatty Acid Chain



- All FAEs show a significant improvement as compared to a FAME control
- Increasing carbon chain length improved DCN and LHV while increased branching lowered DCN

Fatty Alkyl Ether (FAE) biodiesel



- **Increasing carbon chain length improved DCN, LHV, and CP, while increased branching lowered DCN, but improved LHV and CP.**

Conclusions

- We have developed a proof of concept “one-pot bioconversion” with engineered *E. coli* and *C. glutamicum* for efficient production of mixed fusel alcohols and D-lactate from a wide variety of biomass sources, respectively.
- 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, lactic acid and fatty acids 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.
- **Fusel alcohols, lactic acid and fatty alkyl ethers represent a few example of this, but the co-optima effort has identified a variety of biofuel molecules that each have slightly unique value propositions as industrial fuels.**

Open for Collaboration:
Please reach me at sdshind@sandia.gov or Ryan Davis at rwdavis@sandia.gov

Acknowledgments

- **Ryan W. Davis** (Sandia)
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- Amit Jha (Sandia)
- Mysha Sarwar (Sandia)
- John Gladden (Sandia, JBEI)
- Mary Tran (Sandia)
- Apurv Mhatre (ASU)
- Arul Varman (ASU)

Co-Optimization of Fuels & Engines

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ABPDU ADVANCED BIOFUELS PROCESS DEMONSTRATION UNIT

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

ASU Arizona State University

Thank you

Exceptional service in the national interest