DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Bioproduction and Evaluation of Renewable Butyl Acetate as a Desirable Bioblendstock for Diesel Fuel

March 16, 2021

Co-Optimization of Fuels and Engines (Co-Optima)

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Co-Optimization of Fuels & Engines

Project Overview

Why Butyl Acetate (BA)? (Motivation)

Butyl acetate

❖ ***n*-butyl acetate (BA) has great potentials as a fuel additive:**

- BA's high flash point (295 K) makes it safe for operation as fuel additive
- BA's low freezing point (200 K) can help improve cold flow properties of the fuel
- As an oxygenated fuel, BA is anticipated to lower the sooting propensity of a BA/diesel-based mixture
- BA as a bioblendstock for diesel could decrease combustion pollutants and thus reduce the energy penalty of the aftertreatment
- **However, still, there is a lack of direct evidence concerning the combustion performance of BA as a bioblendstock for diesel**

→ Droplet and spray combustion experiments followed by heavy-duty engine testing

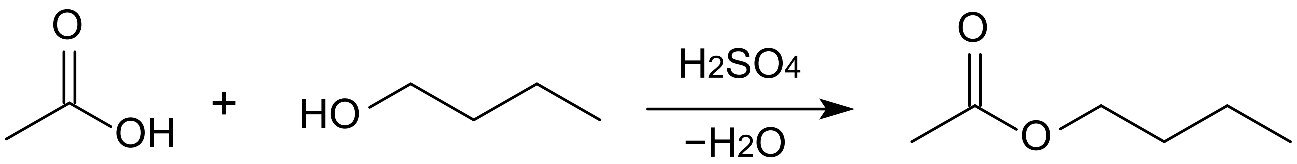
***DE-FOA-0001919 (Advanced Vehicle Technologies Research)**

Production of Butyl Acetate (BA)

❖ **Can enough BA be produced to meet needs of diesel transportation sector?**

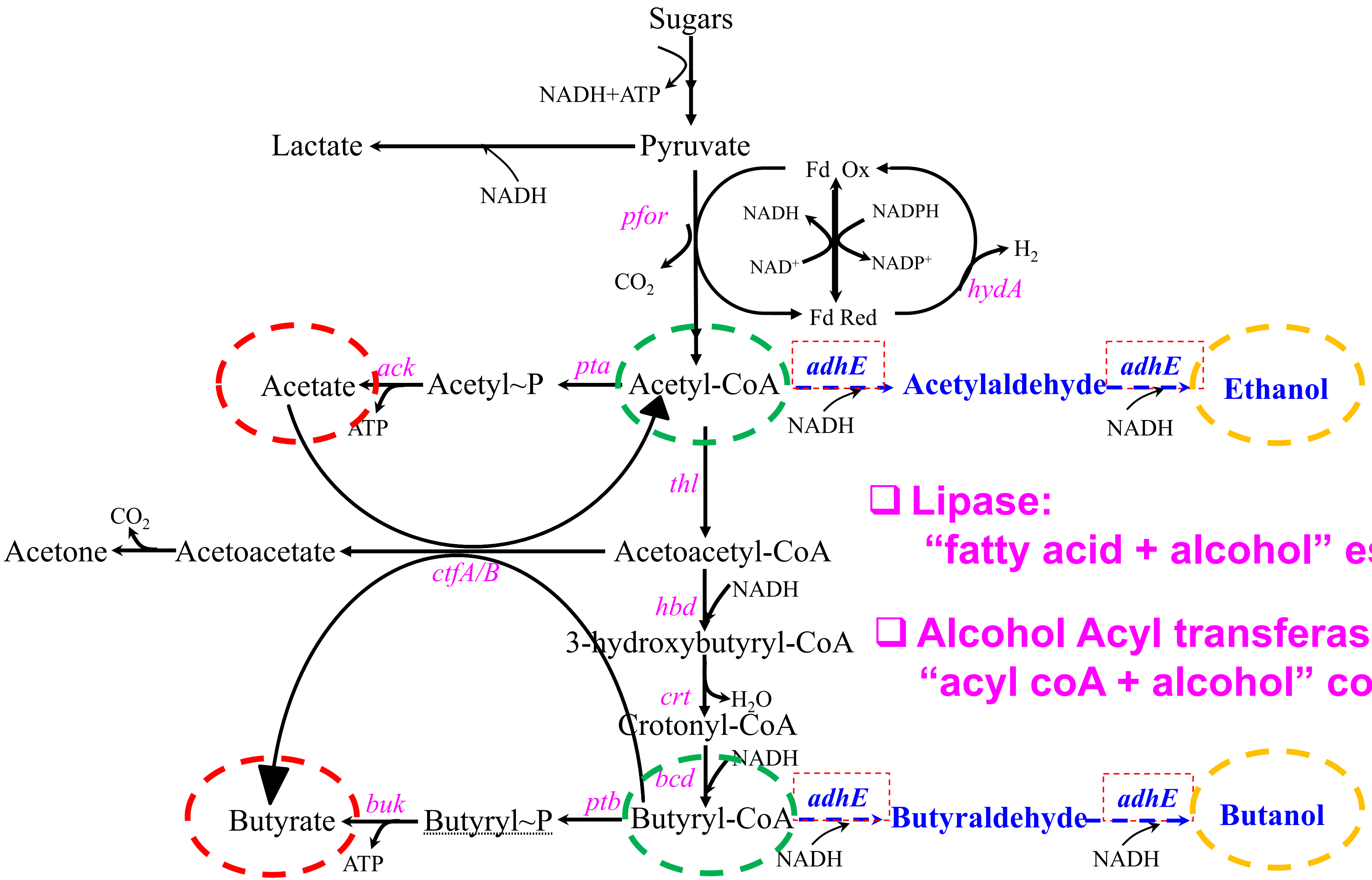
- Based on US-EIA: in 2019, 47B gallons diesel was consumed; assume 10% blending of BA → **~5B gallons of BA is needed each year!**

- Traditional BA production through Fischer esterification is highly energy consuming and non-environmentally friendly.



How can we produce BA in an environmentally sustainable and economically viable manner?

Clostridium for Fatty Acid Ester Production



- **Lipase:**
  "fatty acid + alcohol" esterification
- **Alcohol Acyl transferase (AAT):**
  "acyl coA + alcohol" condensation

*Solventogenic clostridia: non-pathogenic *Clostridium* strains for solvent (acetone, *n*-butanol, ethanol) production

- Solventogenic clostridia* are excellent platforms for fatty acid ester (particularly, BA) production.

→ **Systematic genome engineering**

*Solventogenic clostridia: non-pathogenic *Clostridium* strains for solvent (acetone, *n*-butanol, ethanol) production

Development of Integrated Bioprocess

- BA is a highly toxic endproduct (>1-2 g/L strongly inhibits cell viability)
- A materials science platform (called MicroNiche Engineering, or MNE) develops panels of polymer-microorganism composites ("MNE biocatalysts, or MNE BCs"). The candidate MNE BCs are downselected based on performance indicators, such as increasing tolerance to BA toxicity.
- MNE works in tandem with genome engineering and enables the use of microenvironmental stimuli to improve the performance of fermentation processes.

→ **Materials science-based Integrated bioprocess**

Project Goal (Statement)

- Develop integrated bioprocess for efficient BA production through strain development by systematic genome engineering and process development using biocatalyst composites (MicroNiche Engineering technology, or "MNE biocatalysts").

- BA will be evaluated for its potential as a bioblendstock for diesel through fundamental combustion experiments using configurations ranging from the sub-grid element of a fuel spray (i.e., droplet combustion dynamics) to lab scale testing in a CPFR and full-scale engine testing.

- Life-Cycle Analysis (LCA) and Techno-Economic Analysis (TEA) will be performed throughout the project to provide reference for sustainable and economically viable production of BA from lignocellulosic biomass.

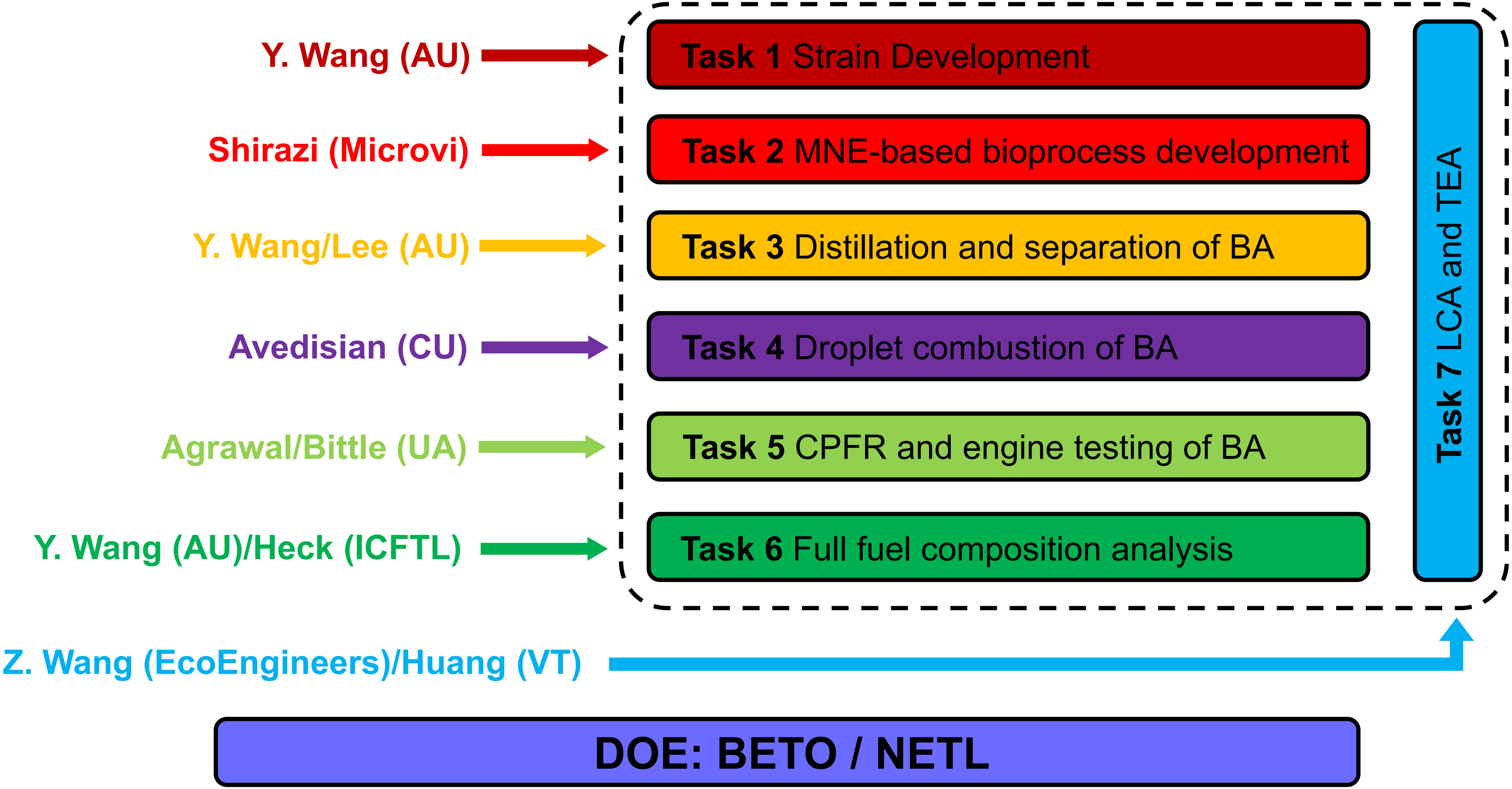
Project Goal (End-of-Project Milestones)

- BA production (end-of-project): titer of 30 g/L, yield of 0.4 g/g, and productivity of 0.6 g/L/h in the extractive batch fermentation from the NREL hydrolysates.

- Combustion & Engine testing: will provide evidence supporting BA as a desirable bioblendstock for diesel by improving the sooting propensity and cold weather behavior of the finished fuel, decreasing combustion pollutants and reducing the energy penalty of the aftertreatment for diesel fuel.

1 – Management

Management Structure (Overview)

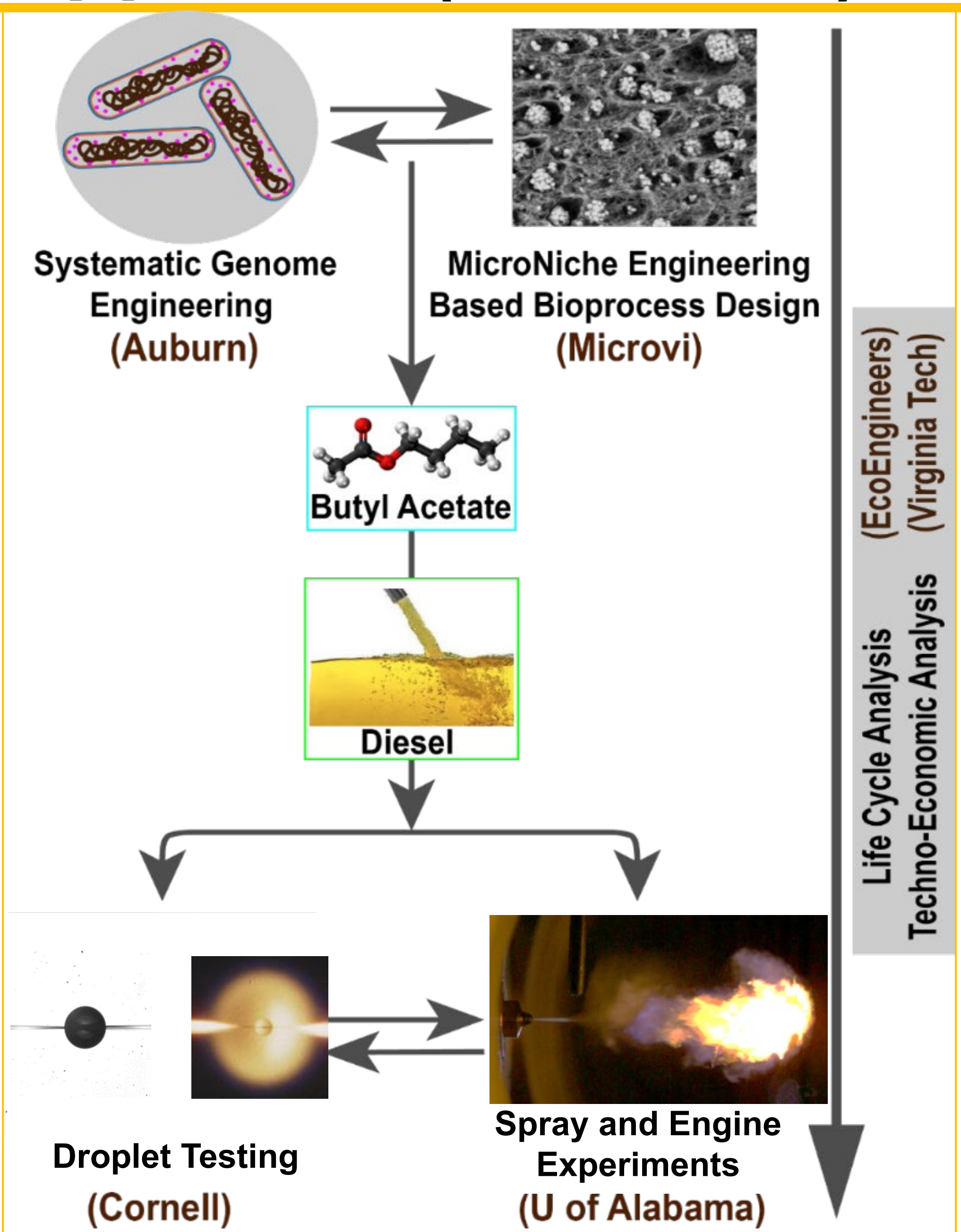


Management (Details)

- PI Wang (AU) –Overall project management (administration, organization, and reporting).
- The Project Team maintain effective communications and share progress through monthly conference calls.
- PI Wang meets specific Co-PI(s) virtually when needed to evaluate Subtask progress.
- When project risks arise, PI Wang first communicates with particular Co-PI to solve the problem. When necessary, PI Wang communicates with the DOE program manager to find alternative solutions or revise the proposed framework if needed.
- **Eric Sundstrom, ABPDU (National Lab Liaison)**
- ***Interactions with other DOE Projects:***
  - ✓ DOE Co-Optima Task #: A.5.18 (Lelia Cosimbescu, PNNL): *Lubricant-oxygenate compatibility*
  - ✓ DOE BETO WBS#: 3.4.2.201 (Dan Schell, NREL): *Biochemical Pilot Scale Support and Process Integrations (Generating hydrolysates using the 'deacetylation and mechanical refining in a disc refiner (DDR)' approach)*

2 – Approach

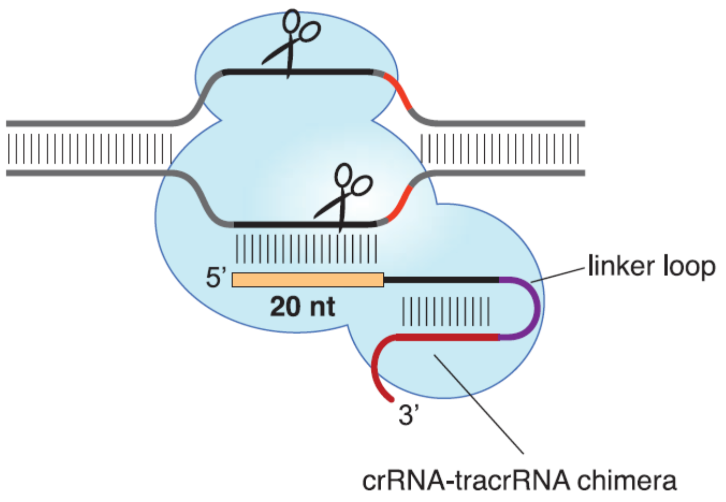
Approach (Overview)



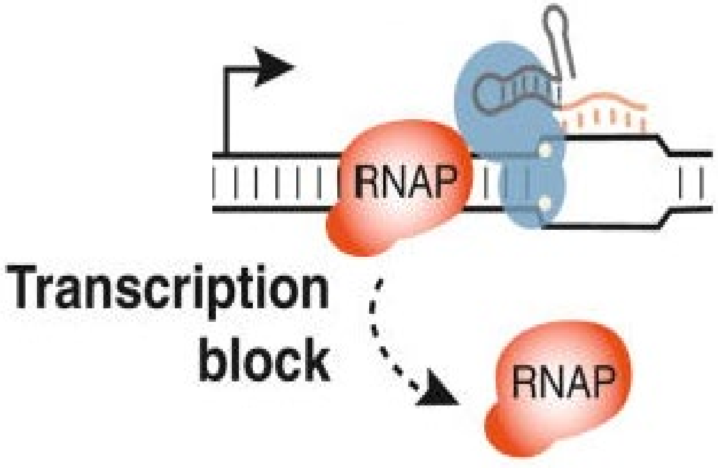
Project efforts breakdown:

- Auburn = 42%
- Microvi = 15%
- Cornell = 15%
- U of Alabama = 17%
- Virginia Tech = 5%
- EcoEngineers = 5%

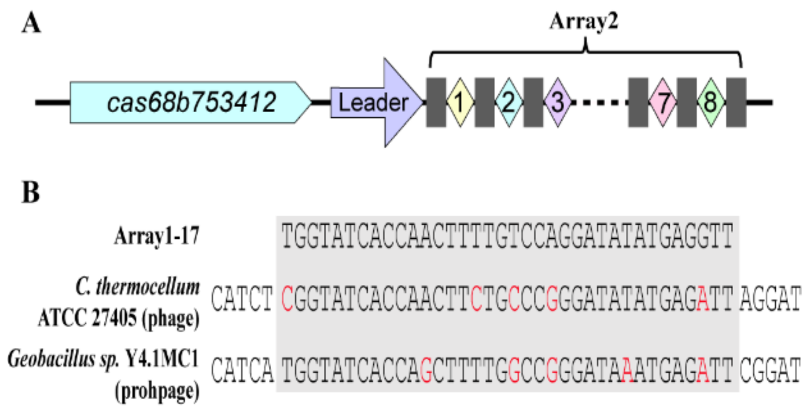
Approach (contd.): Strain Engineering



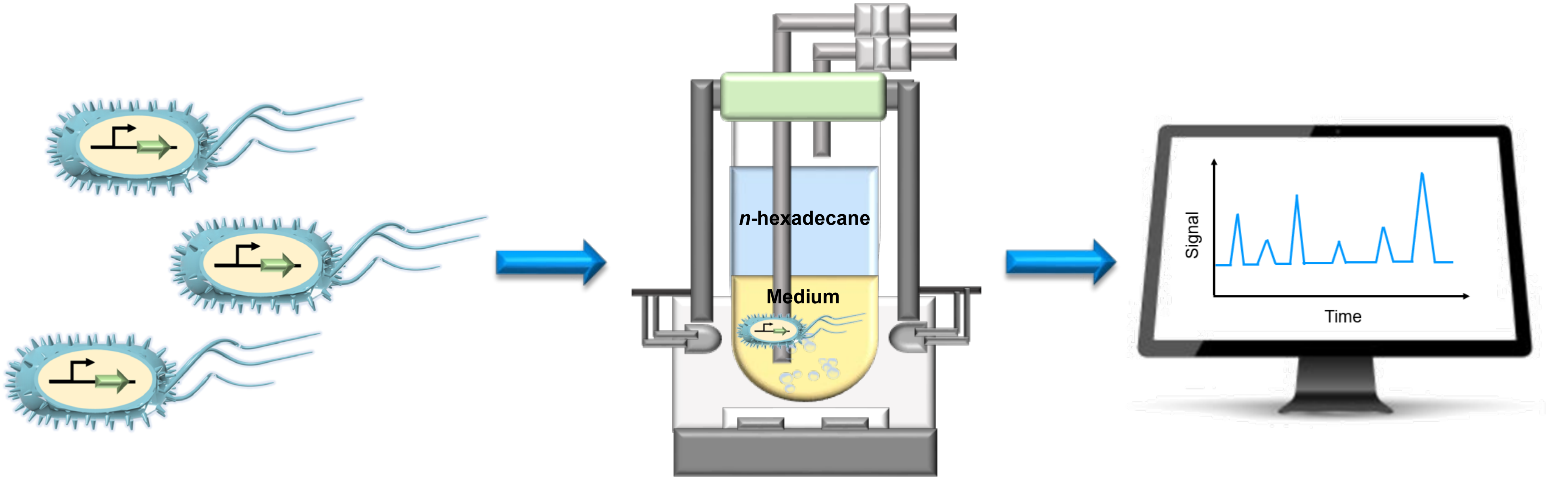
CRISPR-Cas9
(Wang et al., 2016a; Wang et al., 2017)



CRISPR-dCas9 (CRISPRi)
(Wang et al., 2016b)



Native CRISPR
(Zhang et al., 2018)

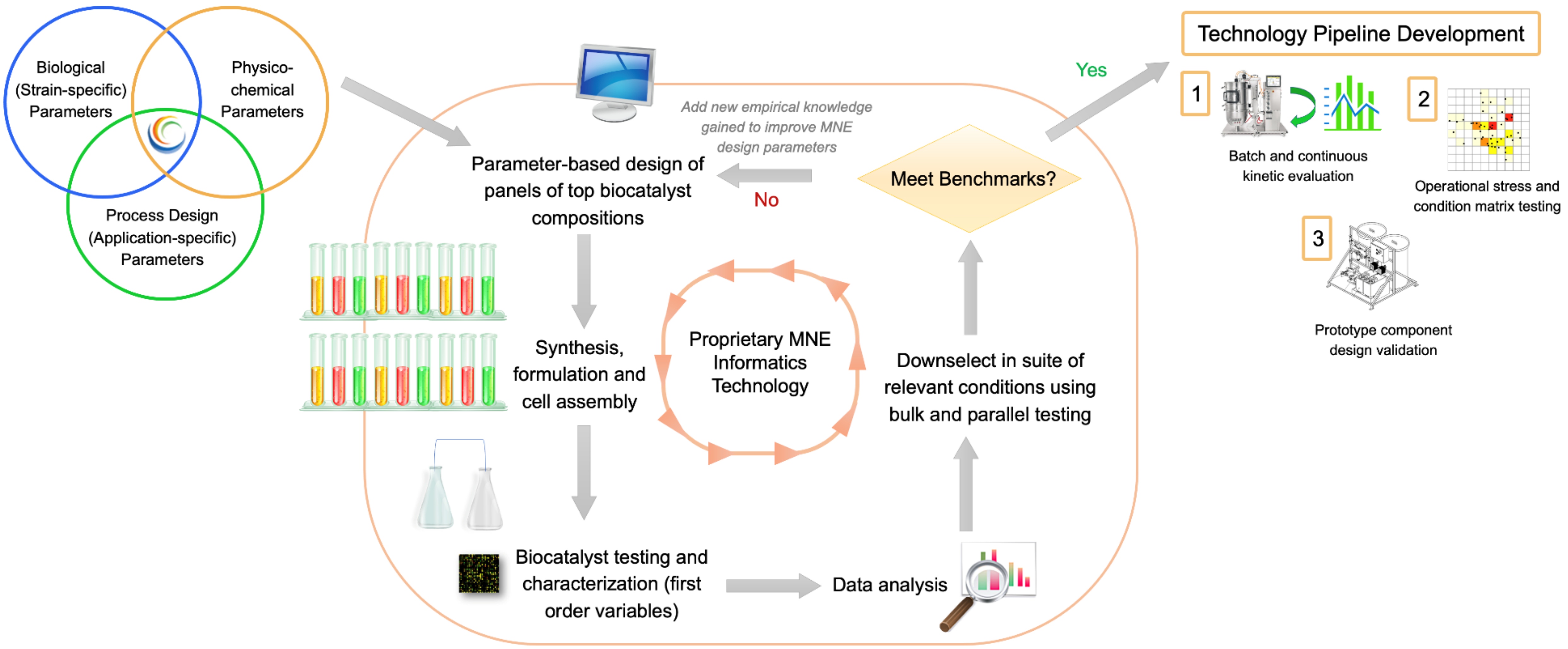


Engineered *Clostridium* for ester production

Extractive fermentation

HPLC/GC-MS analysis

Approach (contd.): MNE-based Bioprocess



Approach (contd): Butyl Acetate Combustion Dynamics

Combustion testing is necessary to...

-determine BA ignition and burning characteristics (largely unknown)
-answer question: does genome-produced BA = commercial BA?

chemical →	BA	n-butanol	n-hexadecane	iso-propanol	ethyl acetate	? unknown
formula →	$C_6H_{12}O_2$	$C_4H_{10}O$	$C_{16}H_{34}$	C_3H_8O	$C_4H_8O_2$	?
mass fraction in synthesized BA (%) →	94.3	3.8	0.4	0.134	0.118	1.248

(role of dissolved by-products)

-determine effect of blending BA with diesel

Combustion configurations being used in the project:

*droplets
*constant pressure flow rig (sprays)
*engine

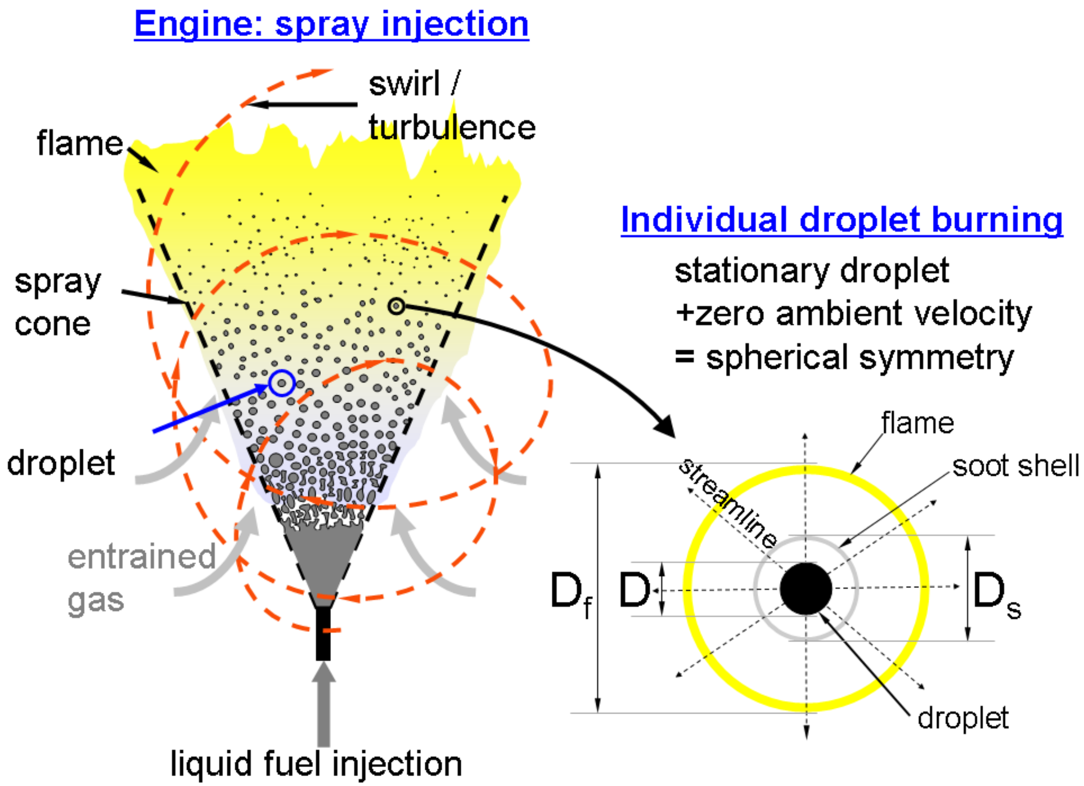
Approach (contd): Droplet Combustion

Why droplet burning in a study of BA?

-engine testing requires several **<u>liters/exp</u>**
-genome-engineered BA initially produces **<u>ml</u>**
-droplet burning experiments require only ***nano* L/exp**
-key question of our work, does 'neat' BA = synthesized BA, can be cheaply and easily answered.

also

-cost of commercial 'ultrapure' (>99.9%) BA is high: $2,100/L



Are droplets relevant in practice (i.e., to a spray)?

-Yes, BA droplet burning is governed by similar combustion physics of heat/mass transfer and reactions as found in spray flames

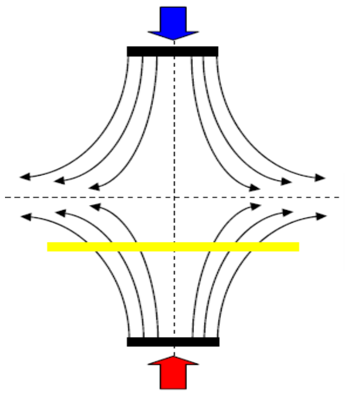
Additional consideration: droplet burning can enable combustion kinetics development

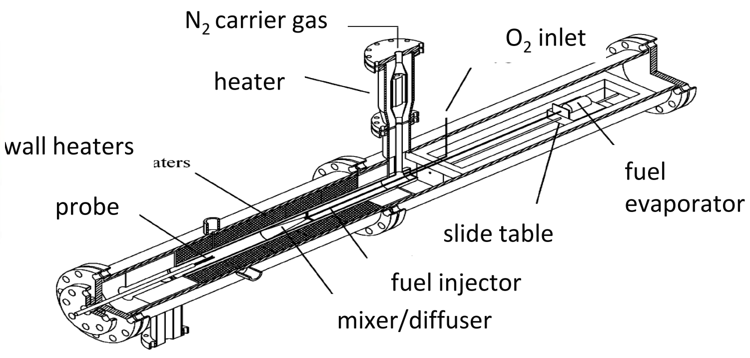
-combustion kinetic mechanisms are foundational to enable simulating engine performance (e.g, Converge code), including predicting engine efficiency and fuel economy (i.e., mpg)
-kinetics determine heat release and effect fuel evaporation
-BA droplet burning data can bring a new dimension to mechanism validation (see Cuoci, Avedisian, et al.*Fuel*, 288, 119451 (2021)) by incorporating gas/liquid coupling with fuel and soot chemistry.

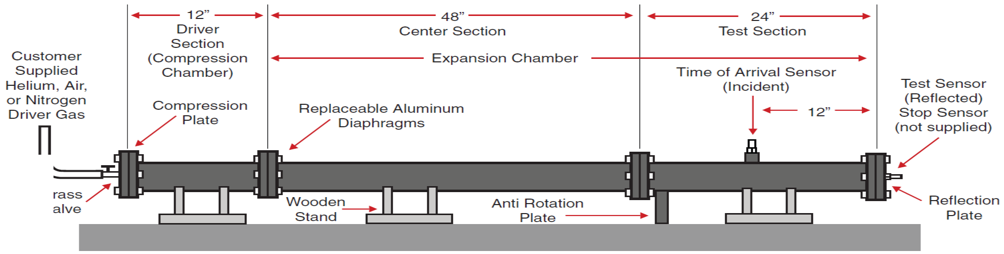
Approach (contd): Droplet Combustion

Prior art for kinetic mechanism validation: data from these configurations

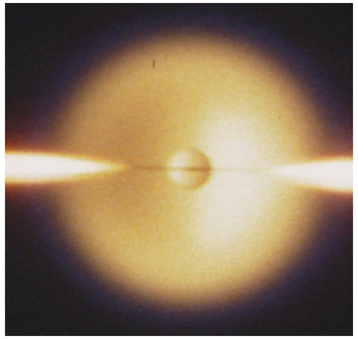
CF Flame **jet-stirred reactor** **Flow Reactor** **Shock Tube**



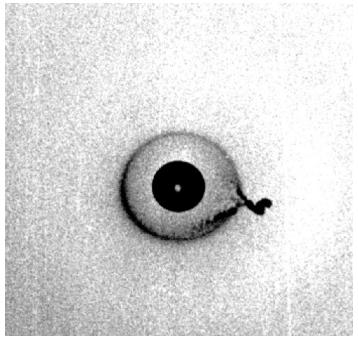




1-D droplet burning (no convection)



flame image



droplet image (black ring is soot)

Shared features;

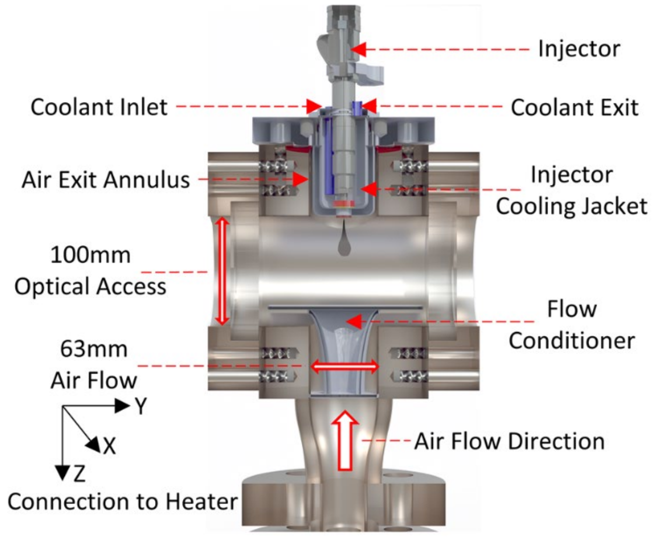
-fuel is pre-vaporized: why? with liquid present simulation is too complex and codes do not exist (e.g., in a shock tube, kinetic mechanisms are strongly connected to evaporation and flame droplet dynamics)

-only gas is present: without liquid, detailed modeling is possible and for 0D or 1D transport commercial codes exist (e.g,.Chemkin, etc.)

New approach: droplet database for 1-D burning of BA may be simulated in detail (no convection case - see photograph) to validate BA combustion chemistry; this work is on-going

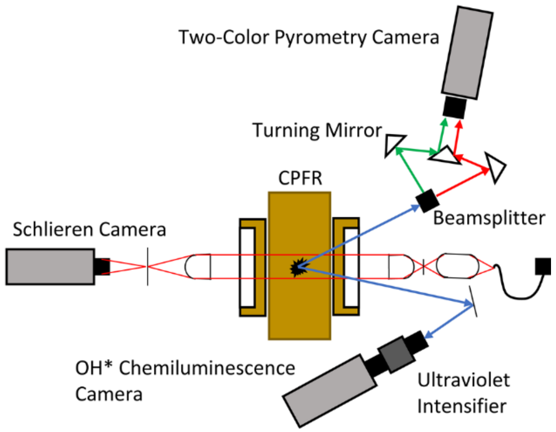
Approach (contd): Spray Combustion Testing

Constant Pressure Flow Rig (CPFR)

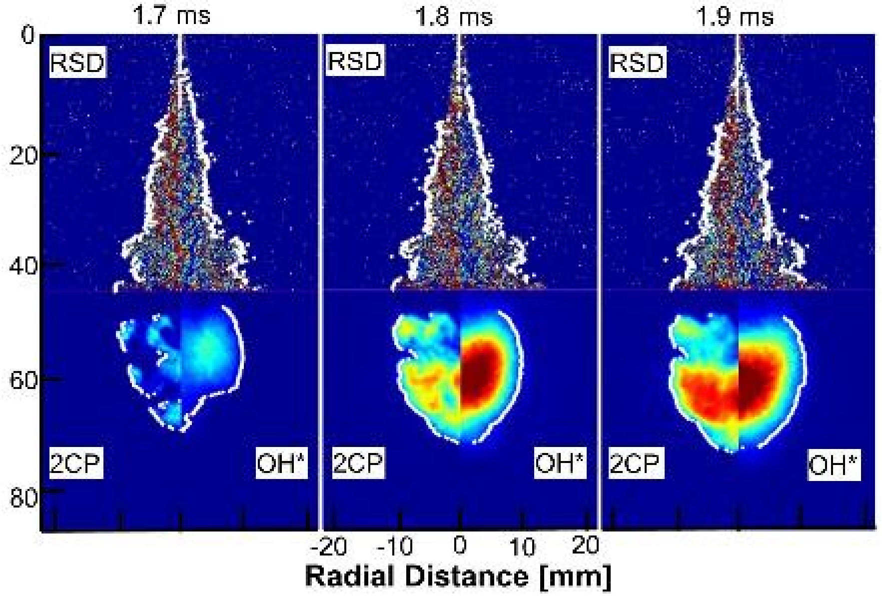


Natural Luminosity Image

Quantitative Optical Diagnostics



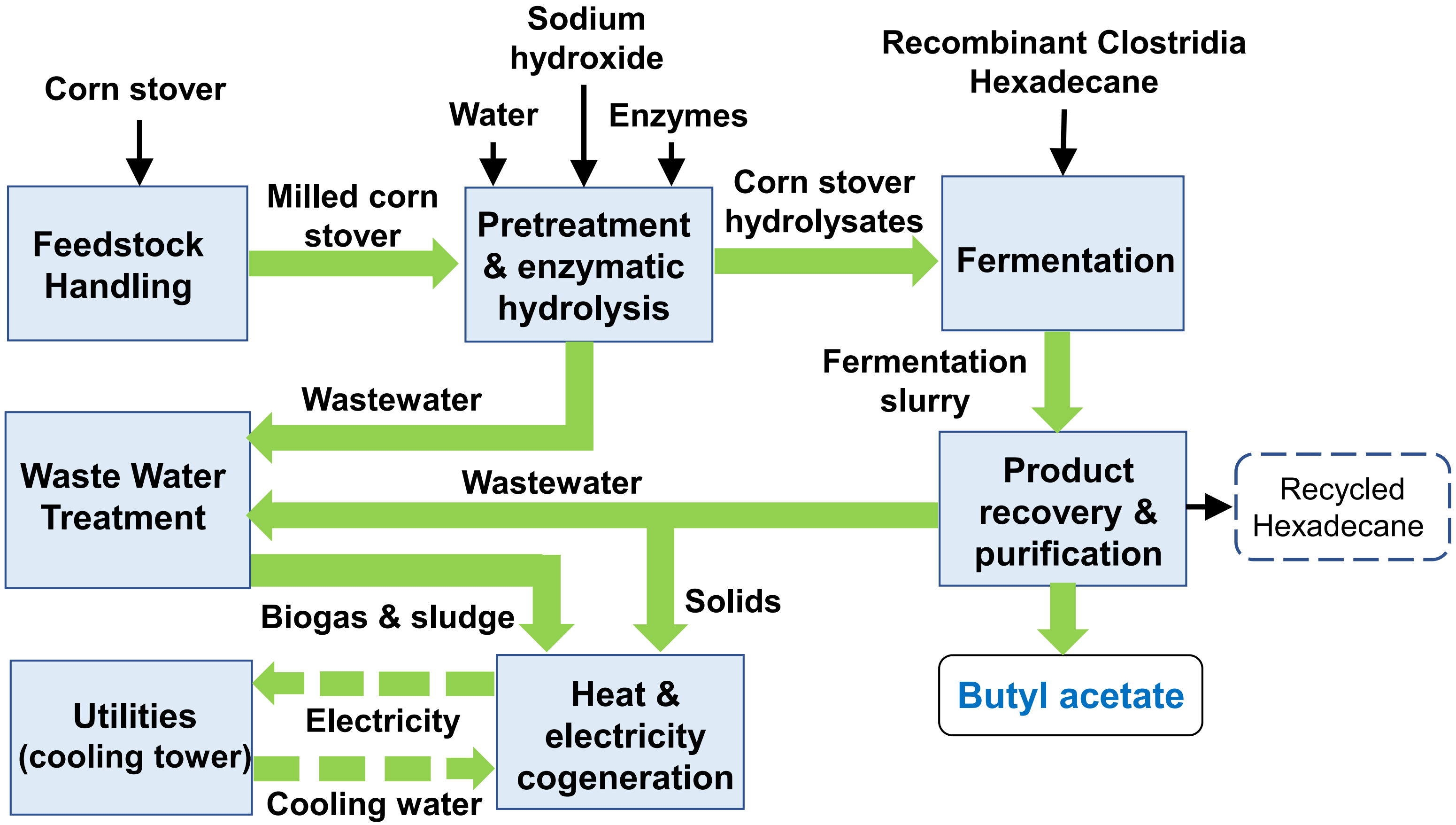
Sample Raw Results



- Commercial BA tested at blend ratio of 0, 10, and 20% in n-heptane
- Injected with modified diesel injector into 30 bar, 800 K ambient air
- Simultaneous high-speed diagnostics capture mixing (RSD), ignition (OH*), and soot formation (2CP).
- Hundreds of injections captured to evaluate impact of BA blending.

Approach (contd): Techno-Economic Analysis (TEA)

- TEA was conducted to evaluate the economic feasibility of BA production from corn stover.
- Corn stover hydrolysate was produced using the deacetylation and disk refining (DDR) pretreatment hydrolysis process developed by NREL.
- Process capacity: 2,500 MT wet corn stover (20%) per day.



Approach (contd): Life-Cycle Analysis (LCA)

- LCA was conducted to evaluate the life-cycle greenhouse gas (GHG) emissions of the BA produced from corn stover.
- Greenhouse gases, Regulated Emissions, and Energy use for Transportation (GREET®) model was the model selected for this project as it has been widely used for LCA of transportation fuels by stakeholders from academia, industry, and government agencies.
- The corn stover to BA pathway was established in the “Bioproducts” section based on existing corn stover to ethyl acetate pathway.
- Inventory data was obtained from the TEA results, and constantly updated with the updated results from all parties in this project.

3 – Impact

Impact

- The general principle for producing a valuable bioproduct that is easily recoverable is highly applicable to other bioprocesses for biofuels and biochemical production.
- The implementation of CRISPR-Cas9 for systematic genome engineering in anaerobic non-model microorganisms provides essential references for general metabolic engineering and synthetic biology research areas.
- The developed technology could lead to an enabling industrial bioprocess in support of the bioeconomy.
- Rigorous fundamental droplet, spray, and engine testing results will add significant data to broader body of knowledge regarding BA combustion and give confidence in ultimate impact BA will have if used as blendstock.

Impact

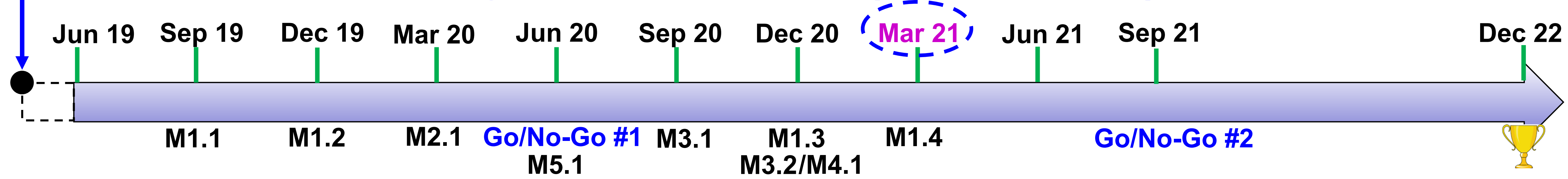
- ✓ **Publications:** *Nature Communications (IF=12.121)*; Current Opinion in Biotechnology (IF=8.288); Biotechnology for Biofuels (IF=4.815); Proceedings of the Combustion Institute (IF = 5.627)*
- ✓ **Patents:** 1 PCT application (PCT/US20/66452)
- ✓ **License:** Potential Option Agreement/Licensing (with a Canadian Company)
- ✓ **Other disseminations:**

  AIChE Annual Meeting (2020), ASABE Annual Meeting (2019, 2020), SIMB-SBFC (2019), ASGSR Annual Meeting (2020), DoE VTO AEC Meeting (2020)

*Currently under revision (*close to acceptance*)

4 – Progress and Outcomes

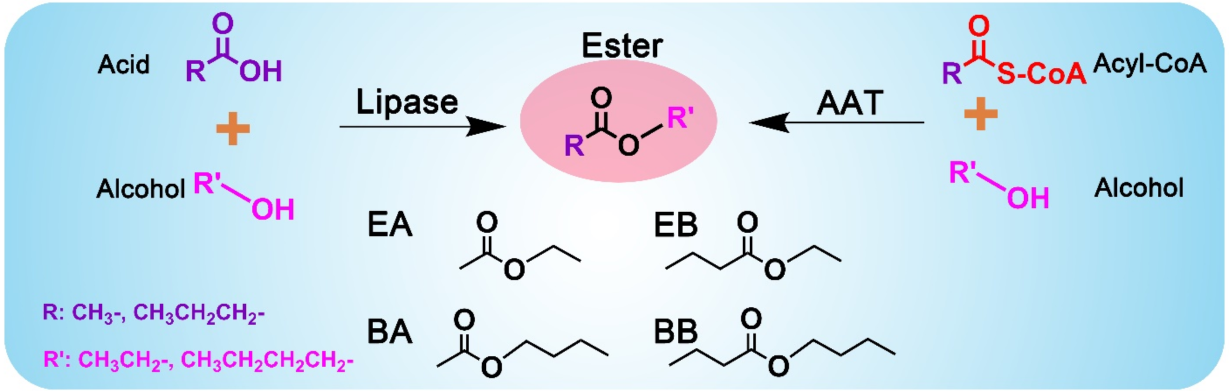
Progress Overview (Milestones)

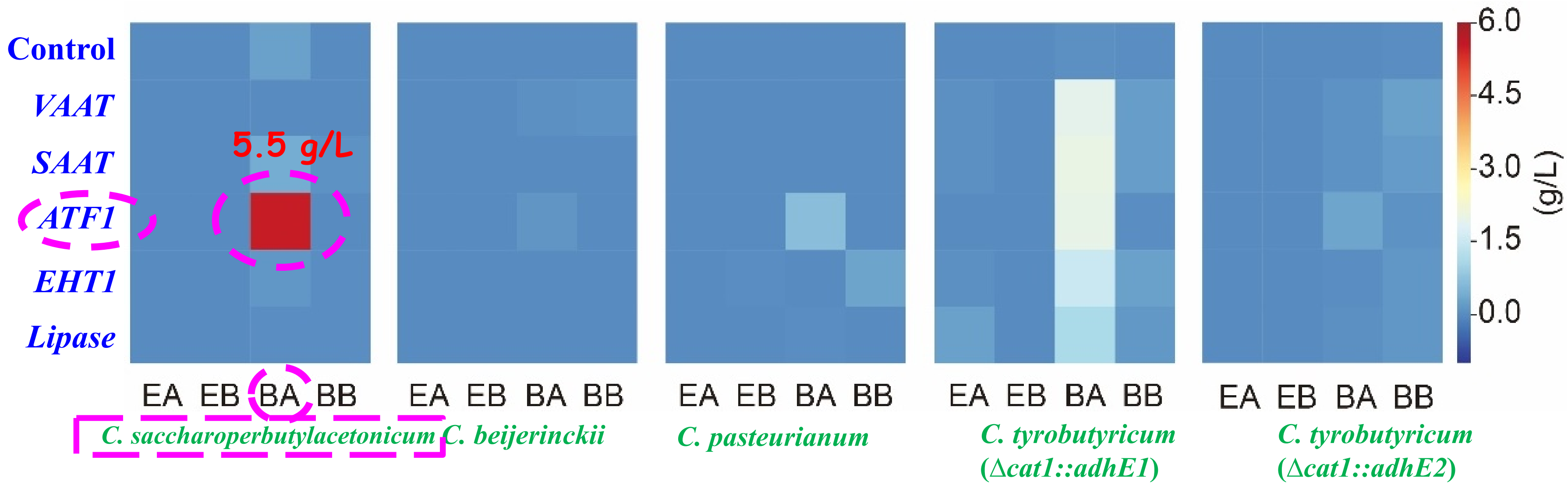


Progress Summary		
Tasks		**Status**
1.0 Enhance BA production in Clostridium through systematic genome engineering		
M1.1	Engineer the strain for mixed sugars co-fermentation	***Close to completion**
M1.2	Delete BA production competing pathways	**Completed**
M1.3	Improve the pool of acetyl-CoA for enhanced BA production	**Completed**
M1.4	Evaluate various promoters for *AAT* expression	**Completed**
2.0 MicroNiche Engineering Technology (MNE) based bioprocess design for enhanced BA production		
M2.1	Develop a panel of composites for the desired process application	**Completed**
3.0 Distillation and separation of BA from extractant		
M3.1	Evaluate/optimize parameters for vacuum distillation in small scale	**Completed**
M3.2	Scale up the vacuum distillation to 20 L scale	**Completed**
4.0 BA/diesel and BA/heptane droplet experiments-using BA/heptane mixture		
M4.1	Recording droplet burning histories--using BA/heptane mixtures	**Completed**
5.0 Constant Pressure Flow Rig (CPFR) and Engine Testing		
M5.1	CPFR Experiments using commercially available BA	**Completed**
6.0 Full fuel composition analysis		
7.0 Life-cycle analysis (LCA) and techno-economic analysis (TEA)		

Strain Engineering

- Screen host strains and genes for fatty ester production





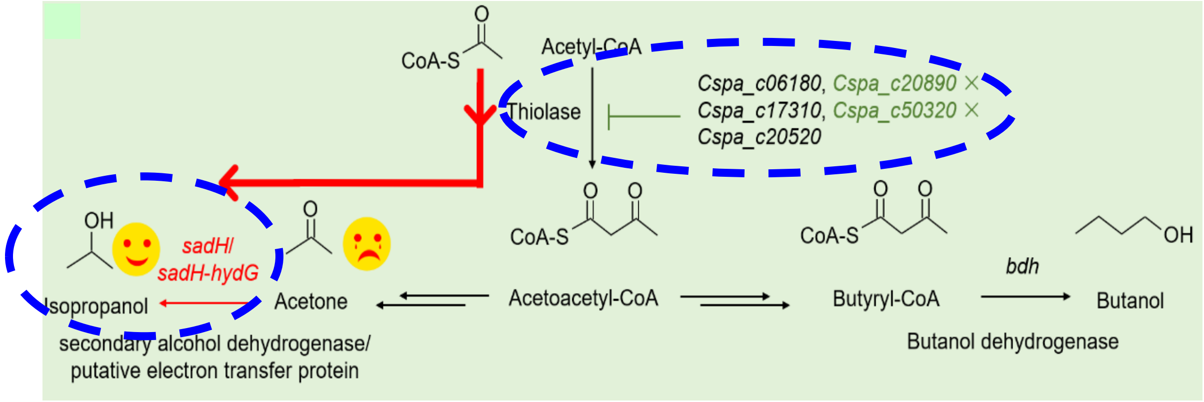
- VAAT, SAAT, ATF1, EHT1: Alcohol Acyl transferase (AAT) from various sources
- EA: ethyl acetate; EB: ethyl butyrate; BA: butyl acetate; BB: butyl butyrate

Strain Engineering

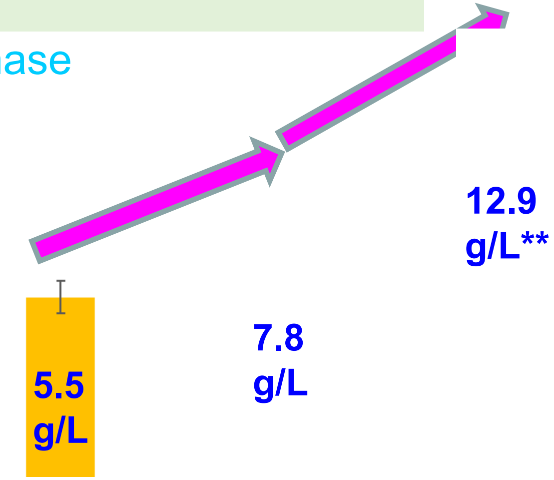
➢ **Improve NADH pool & Acetyl-CoA metabolic flux**

1. NADH *nuoG* **X→ NADPH**

2. Enhance Acetyl-CoA metabolic flux



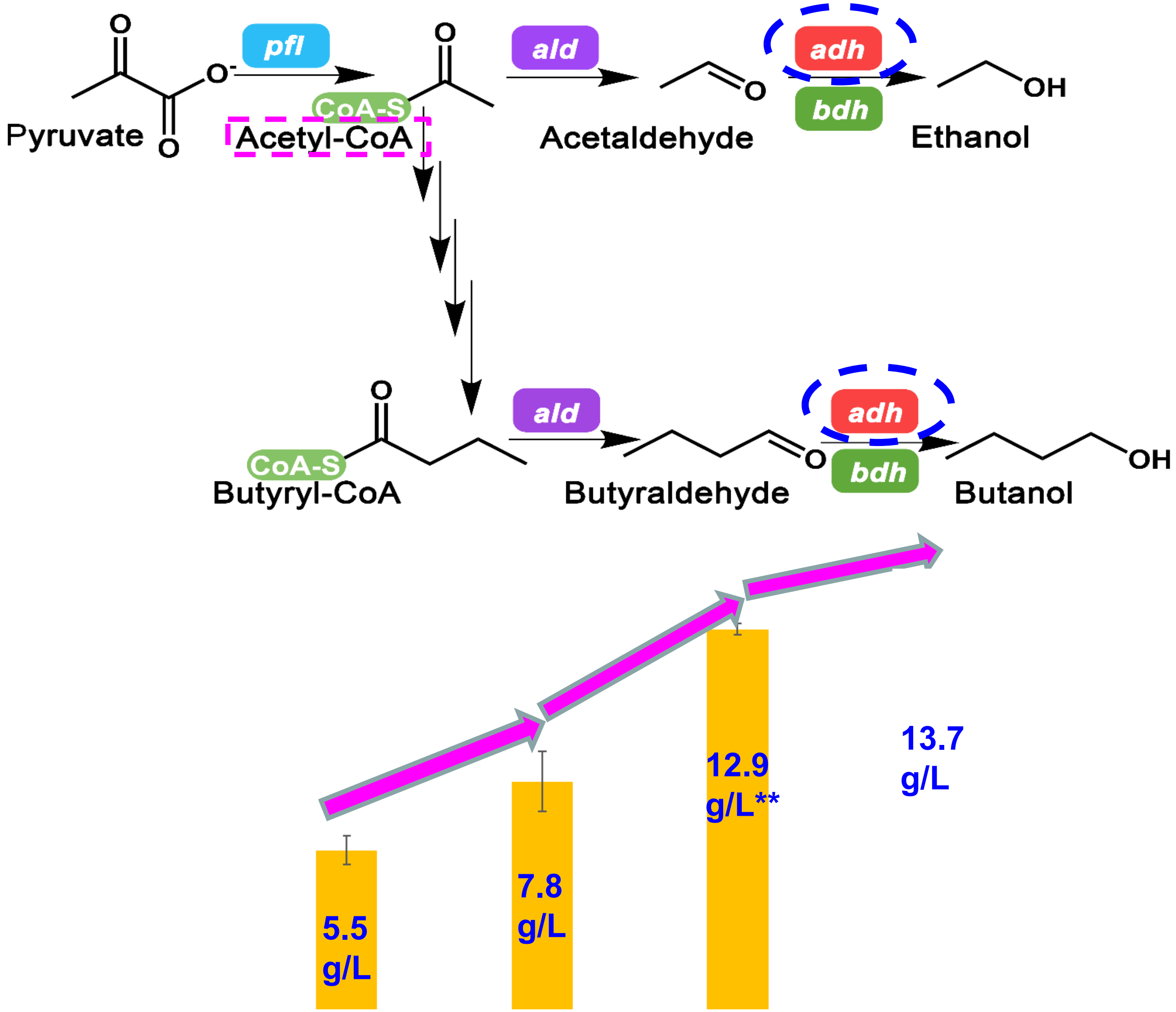
**sadH*: secondary alcohol dehydrogenase



**DOE project starting point: yield of 0.2 g/g, productivity of 0.22 g/L/h.

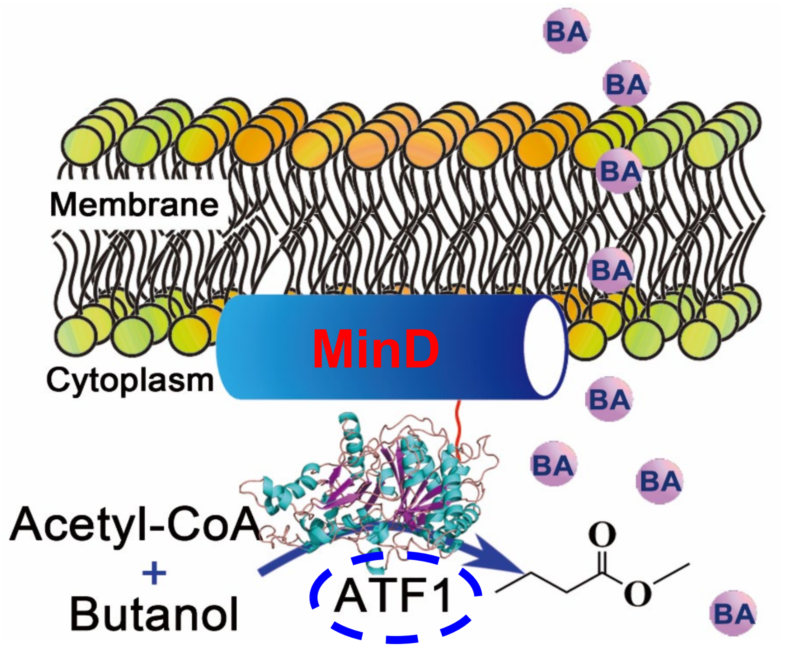
Strain Engineering

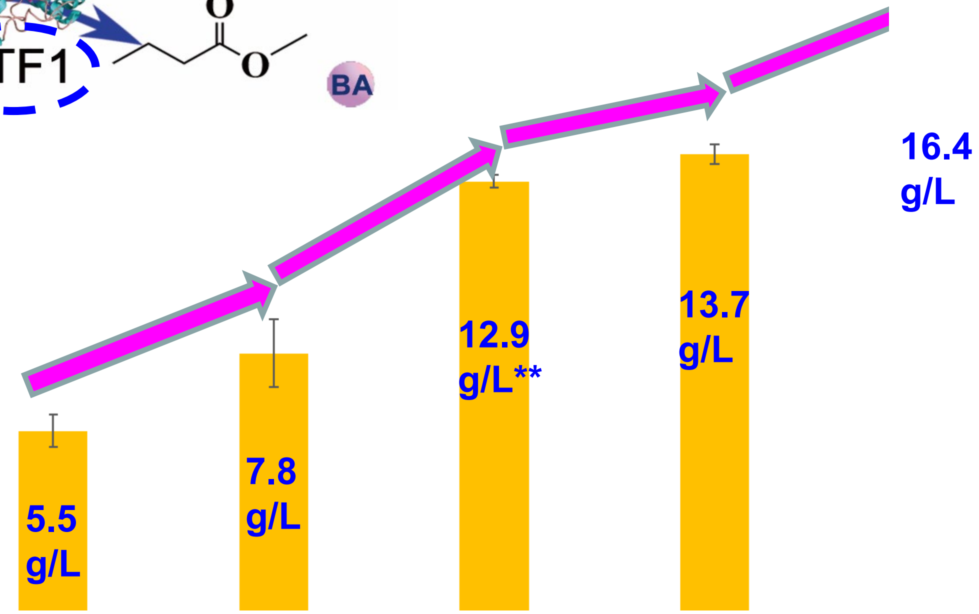
➢ **Evaluate various promoters for *AAT* expression**



Strain Engineering

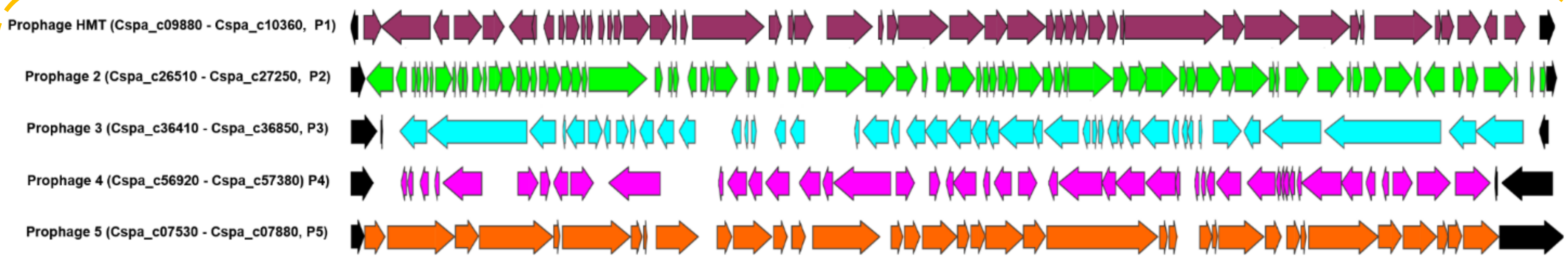
- **Targeting *AAT* enzyme (*ATF1*) into the cell membrane to facilitate BA secretion**



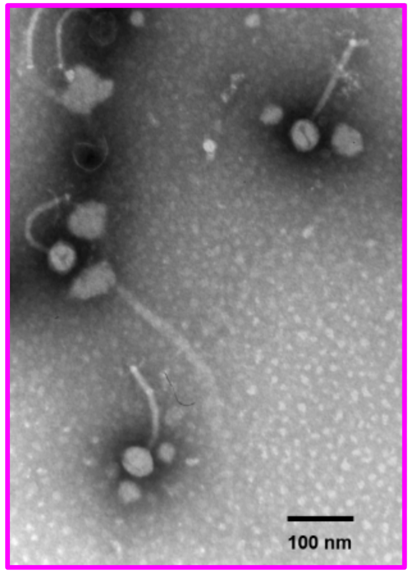


Strain Engineering

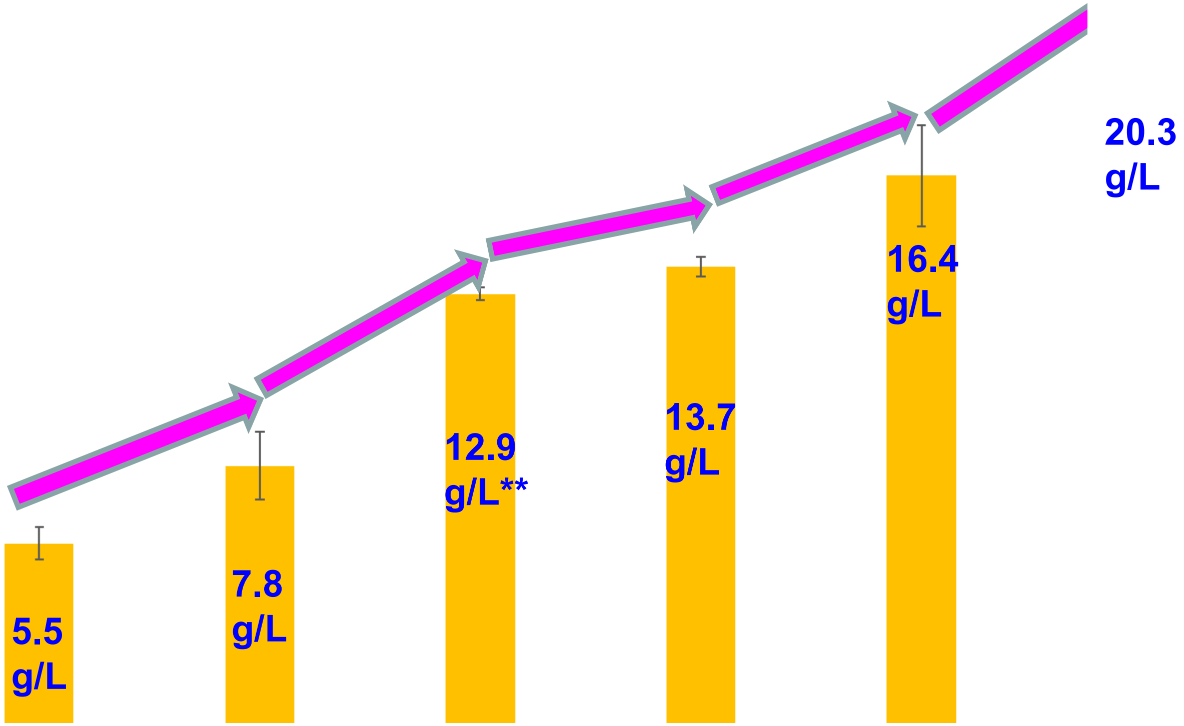
➢ Eliminating prophages from the chromosome



Putative prophage gene clusters within the Chromosome

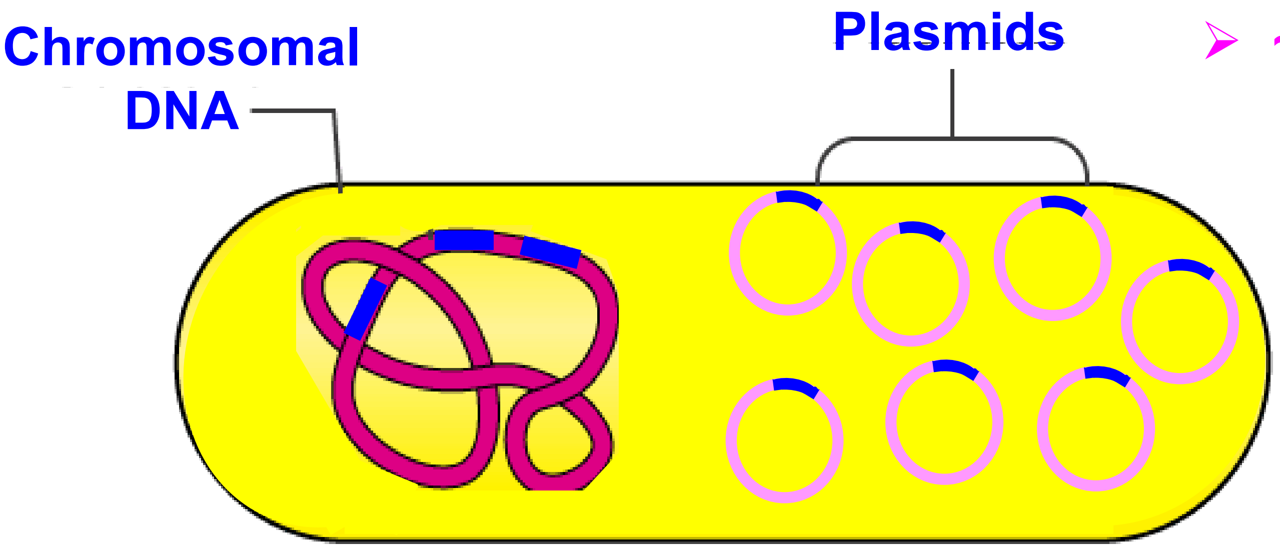


The HM T prophage

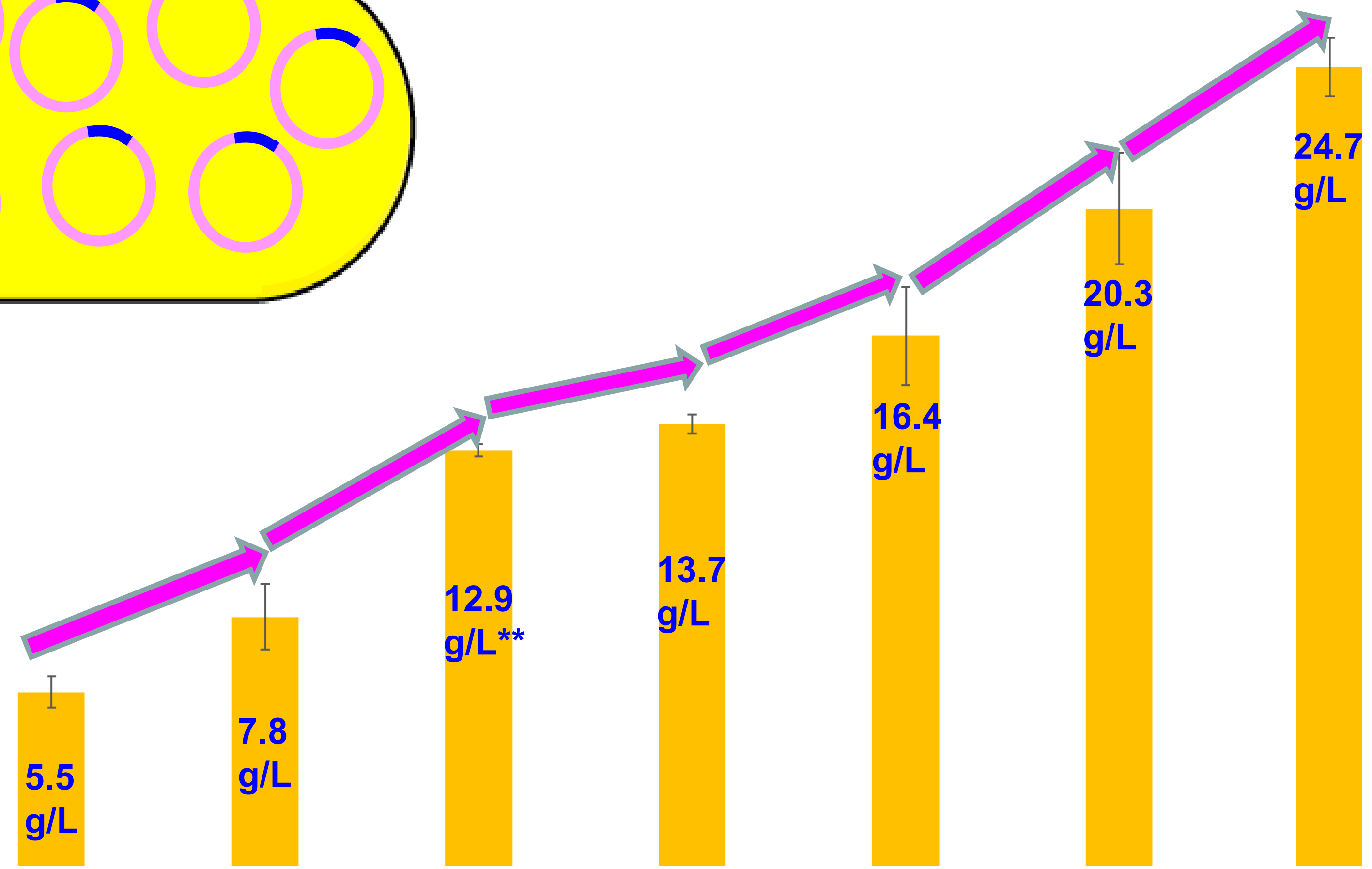


Strain Engineering

- Chromosomal integration (in addition to plasmid-based expression)



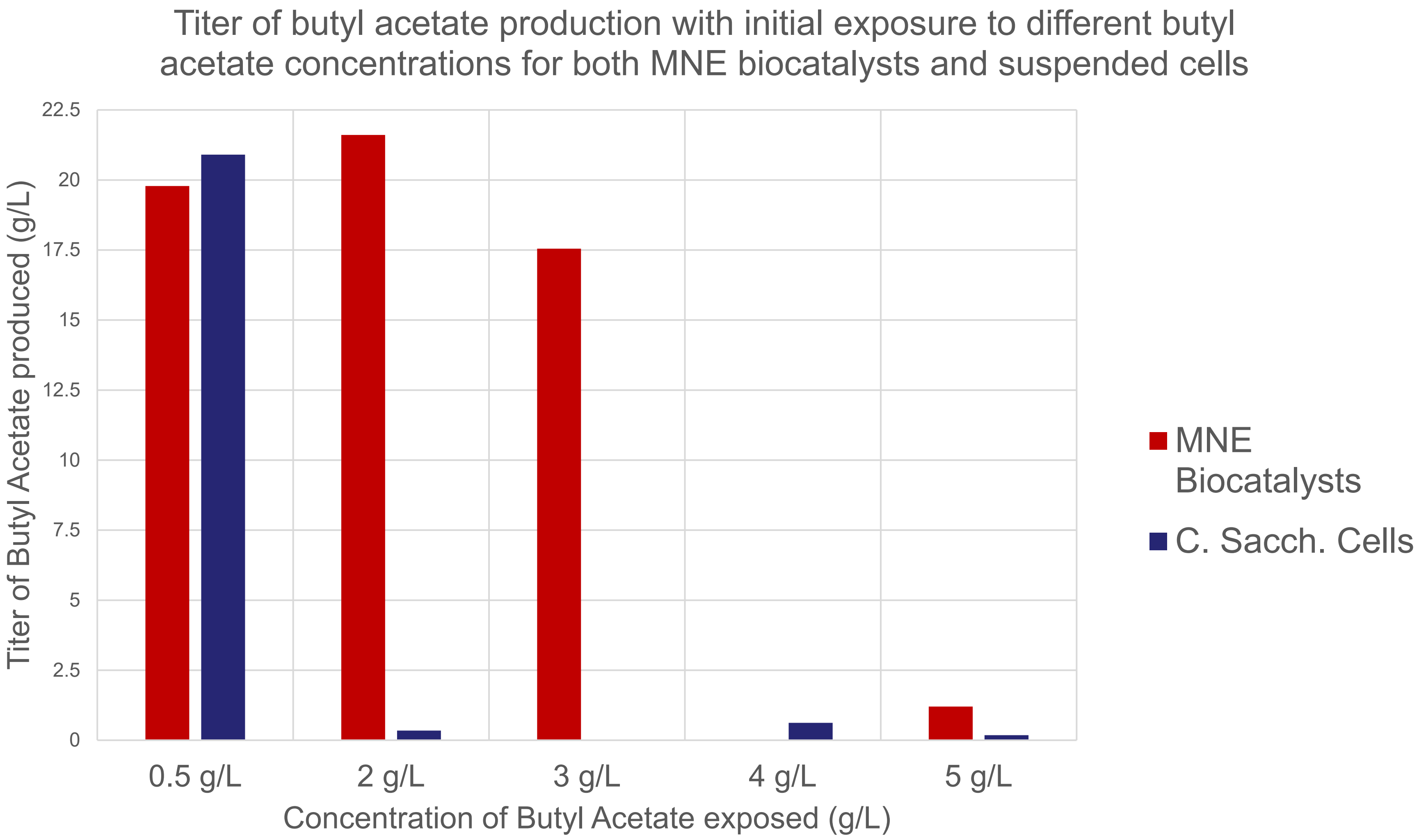
- ~2950-fold higher than the previous reported* (24.7 g/L, 0.31 g/g)



*Noh, et al. *Appl. Microbiol. Biotechnol.* 2018, 102(19):8319-8327.

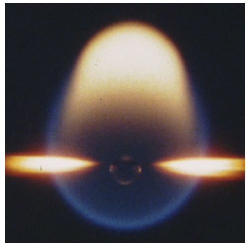
MNE-based Bioprocess

- ❑ Five MNE panels have undergone downselection with additional manufacturing and testing of promising candidates.
- ❑ Demonstrated ability to tolerate higher concentrations of BA than suspended cells
- ❑ Increased titer by up to 20% over suspended cells in batch fermentation
- ❑ Yield of up to 0.39 g/g
- ❑ Long-term storage (7+ days) without special conditions
- ➢ Continued iterative development of biocatalyst composites with strain engineering



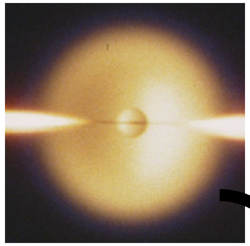
Droplet Combustion

with convection



vs

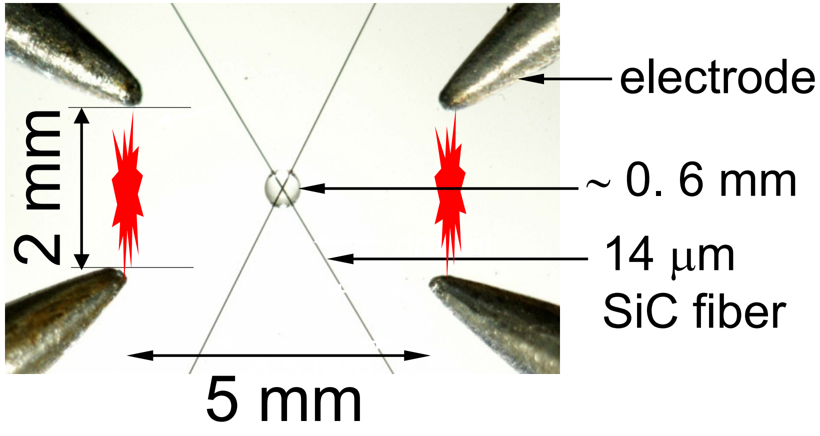
without convection



Fuels being investigated

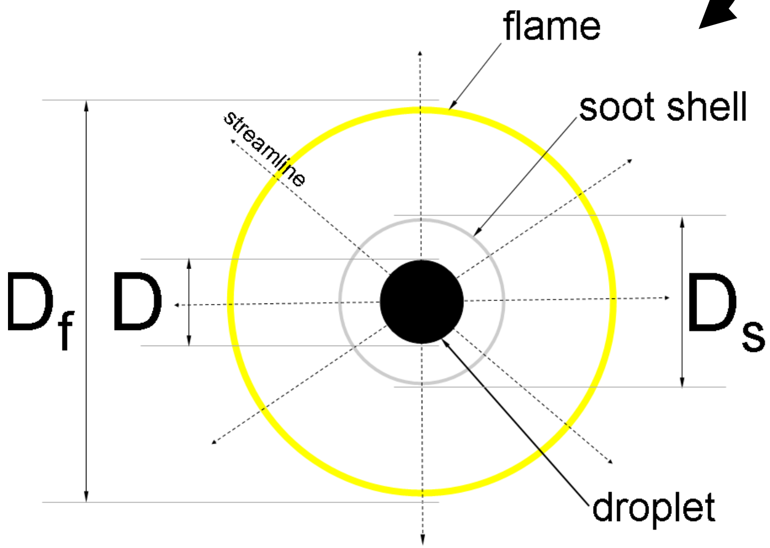
*commercial (neat) BA (>99.9%)
*genome engineered BA (94.3%)
*BA+heptane
*BA+diesel

parameters:
initial droplet diameter

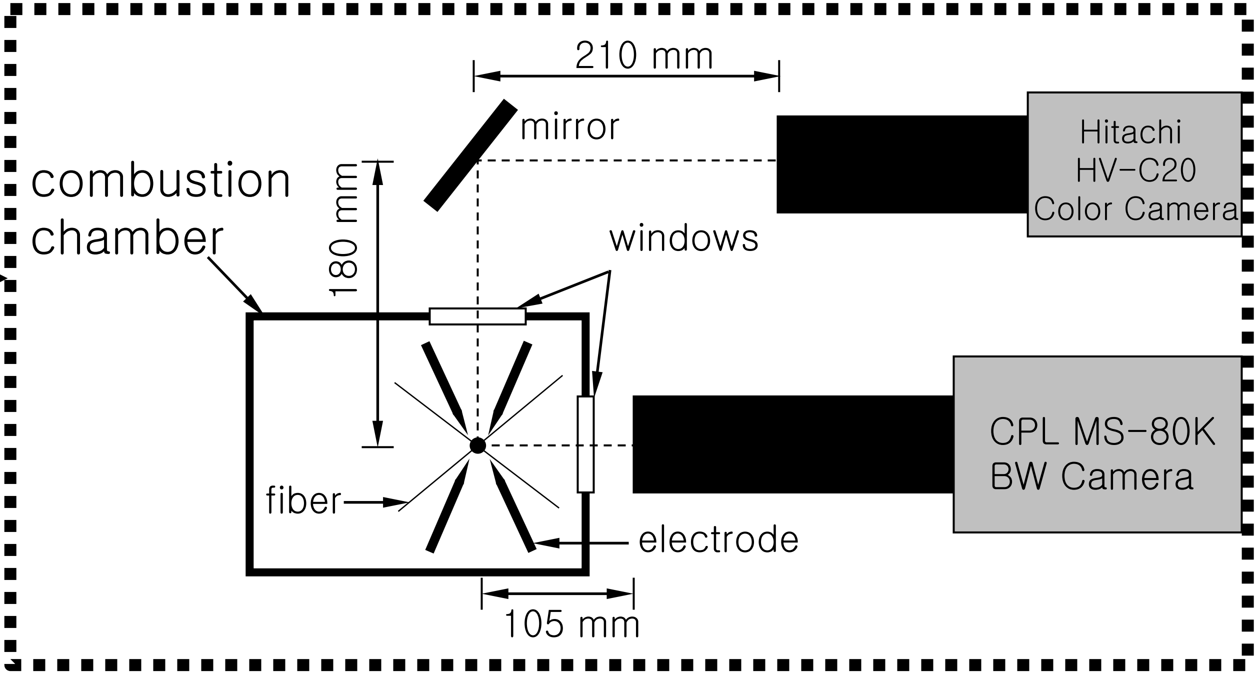


[10^{-9} L /experiment]

Data from experiments:



instrumentation package
(for burning without convection; in free-fall)

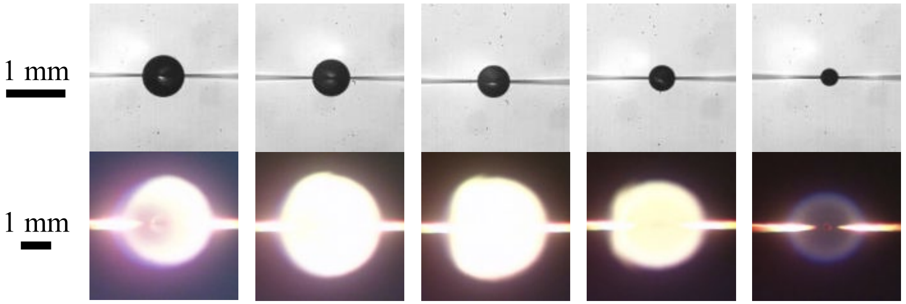


Droplet Combustion

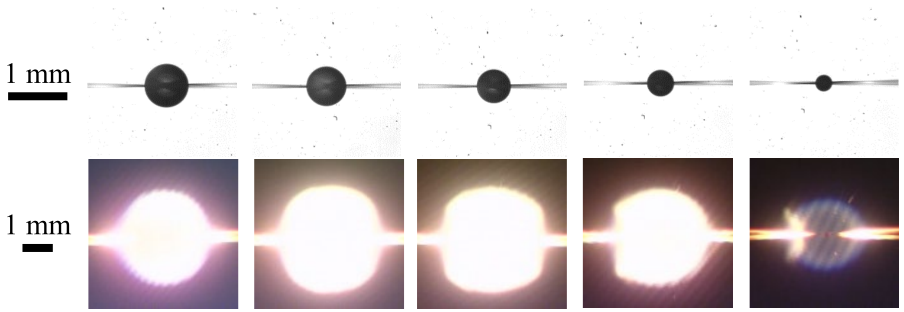
synthesized BA and by-products

chemical →	BA	n-butanol	n-hexadecane	iso-propanol	ethyl acetate	? unknown
formula →	$C_6H_{12}O_2$	$C_4H_{10}O$	$C_{16}H_{34}$	C_3H_8O	$C_4H_8O_2$	?
mass fraction in synthesized BA (%) →	94.3	3.8	0.4	0.134	0.118	1.248

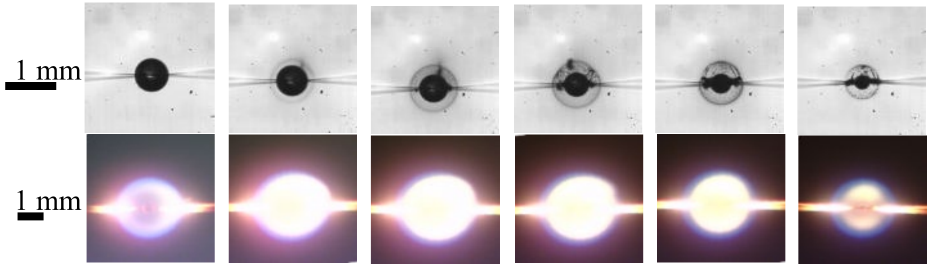
raw data: digital video images
quantitative data: image extraction



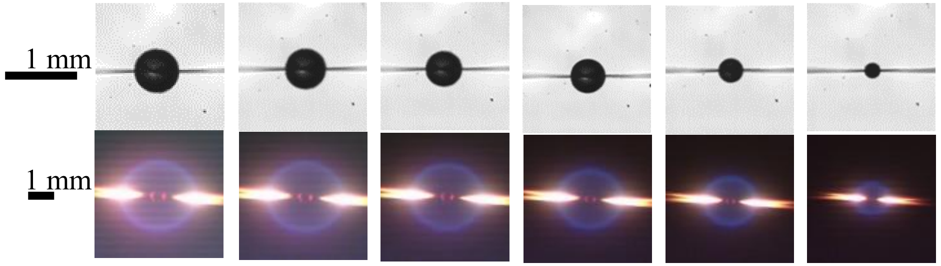
synthesized BA (94.3%)



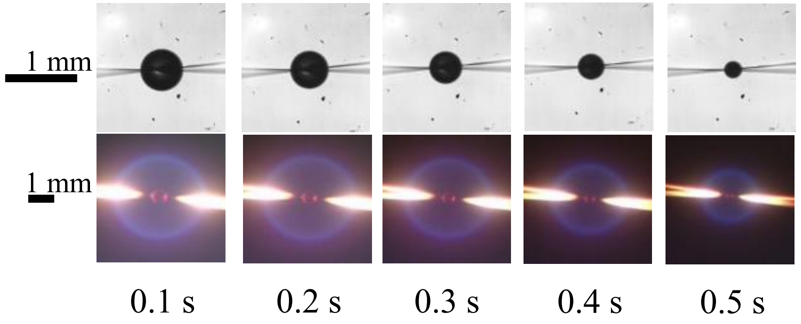
commercial BA (>99.9%)



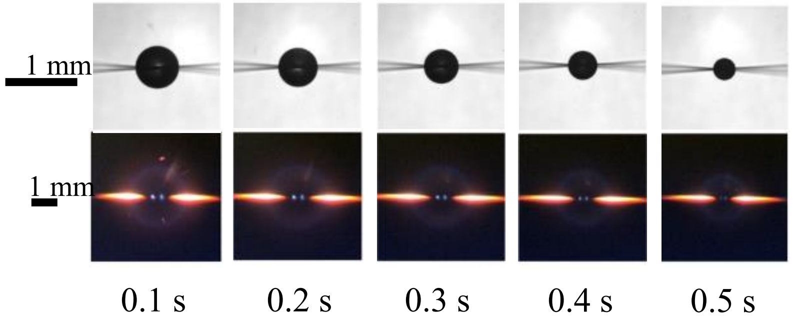
hexa-decane (0.4%)



iso-propanol (0.134%)

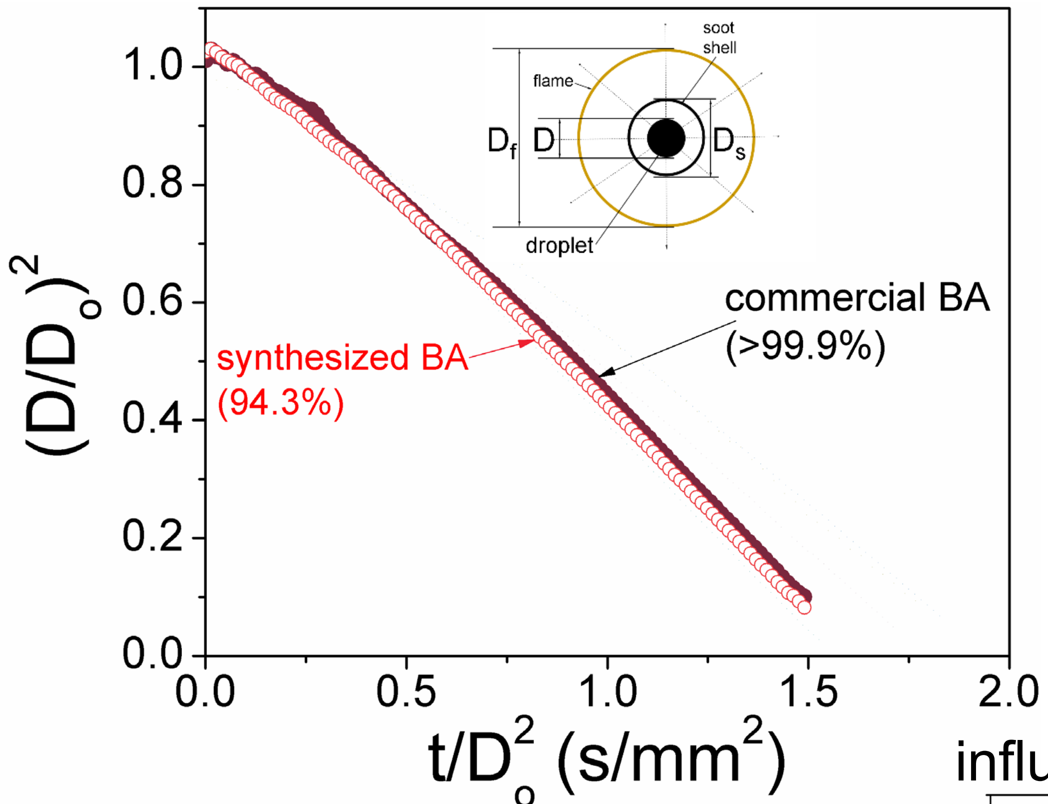


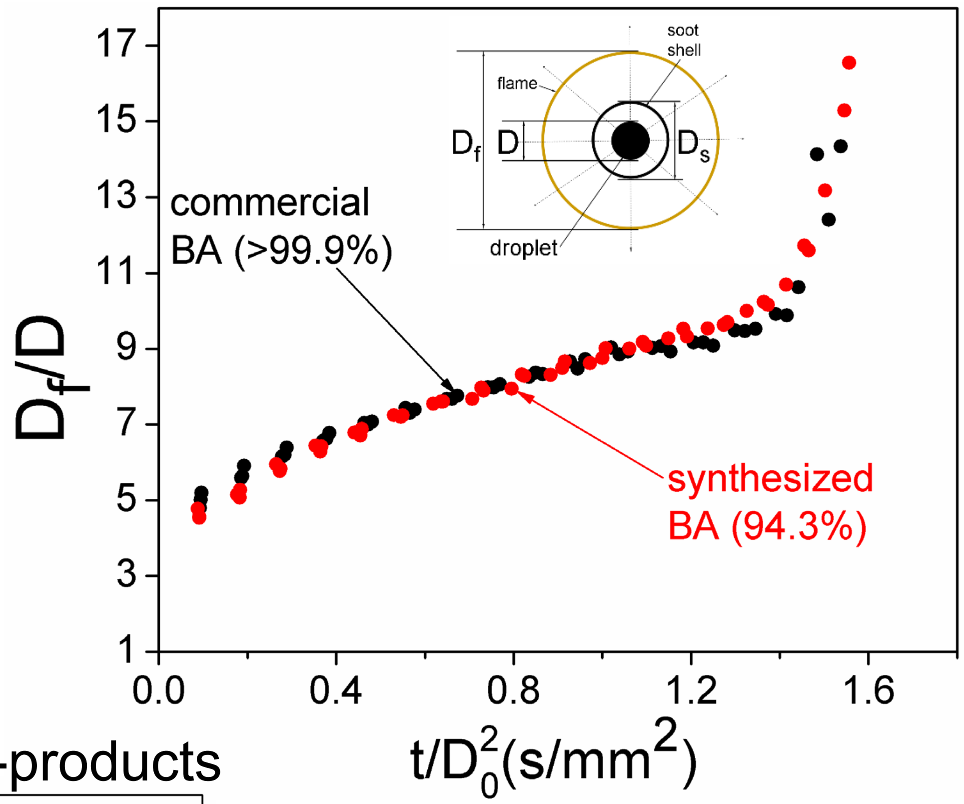
ethyl acetate (0.118%)



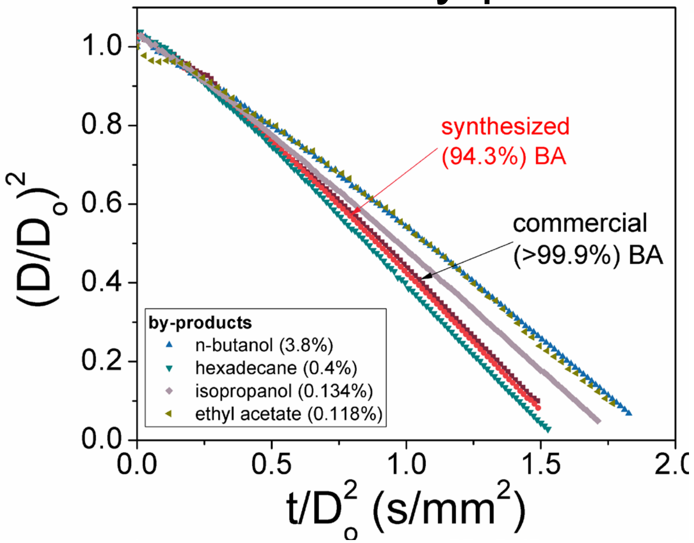
n-butanol (3.8%)

Droplet Combustion: Synthesized vs. Commercial





influence of by-products

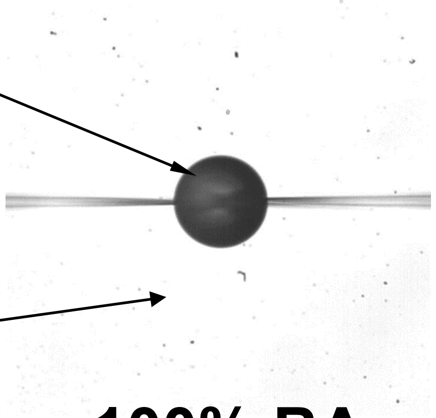


Droplet Combustion: BA+heptane mixtures (preliminary)

droplet
~0.5 mm

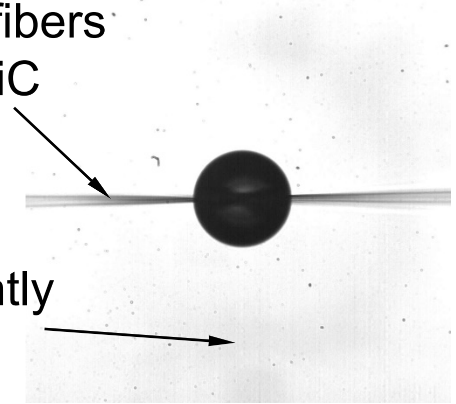
support fibers
14 μm SiC

no soot visible
at 1 atm



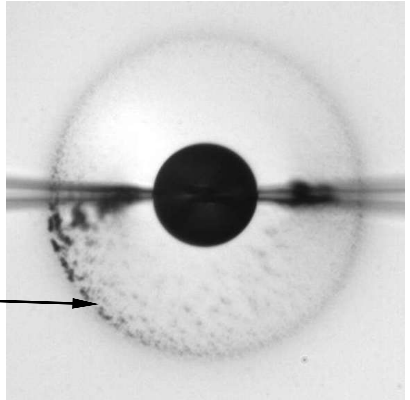
100% BA

soot faintly
visible

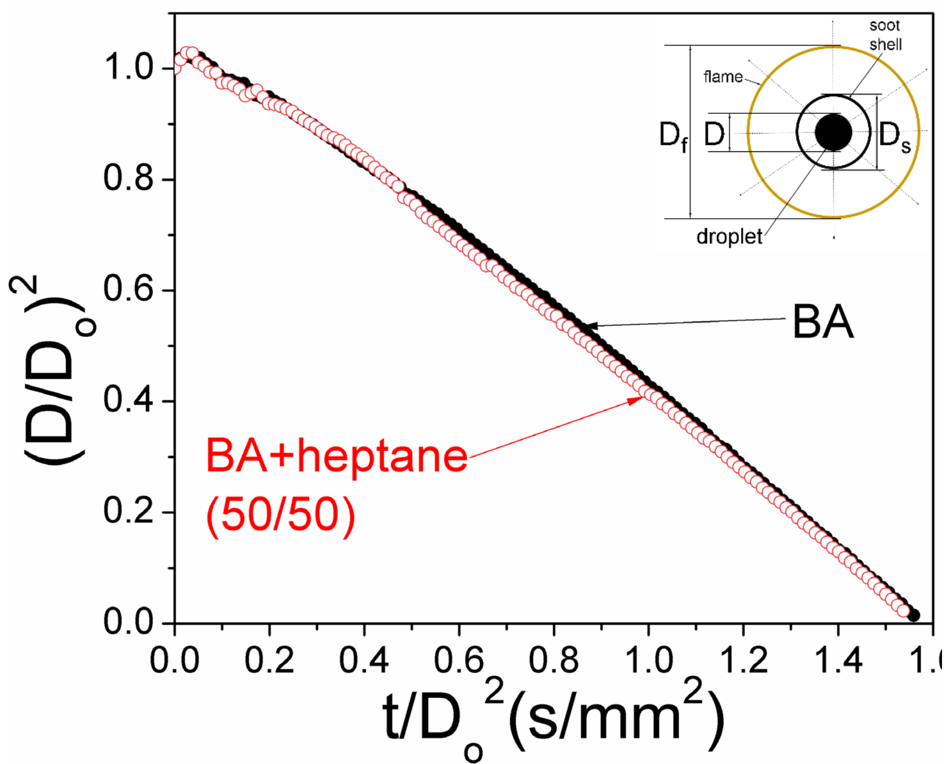


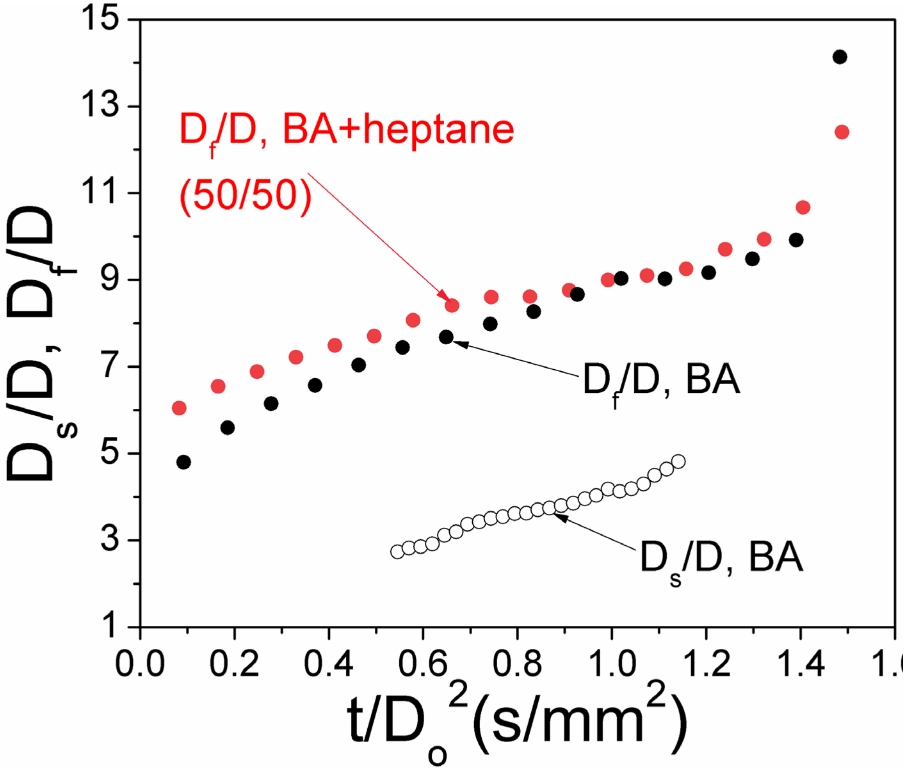
50/50 BA/heptane

soot 'shell'



100% heptane





Spray Combustion Testing

Experimental Conditions

Chamber Conditions:

- n-heptane and n-heptane/butyl-acetate (0, 10, 20% blend)
- 30 bar, 800 K nominal ambient conditions
- 1000 bar injection pressure, 100 μm nozzle
- 100-200 injections captured for each blend

Diagnostic Details:

- RSD, OH* CHE, and 2CP used simultaneously
- RSD acquired at 20 kHz and 90 μm/px,
- OH* CHE acquired at 10 kHz and 160 μm/px
- 2CP acquired at 10 kHz 240 μm/px

	n-Heptane	Butyl Acetate
Molecular Formula	C_7H_{16}	$C_6H_{12}O_2$
Density	0.6838 g/cm^3	0.8760 g/cm^3
Lower Heating Value	44.93 MJ/kg	27.43 MJ/kg
Cetane Number	53.8	-
Boiling point (1 atm)	98.0 ^{o}C	126.1 ^{o}C
Vapor Pressure	4.60 kPa	1.20 kPa
Critical Pressure	2.7 MPa	3.1 Mpa
Critical Temperature	540.1 K	575.0 K

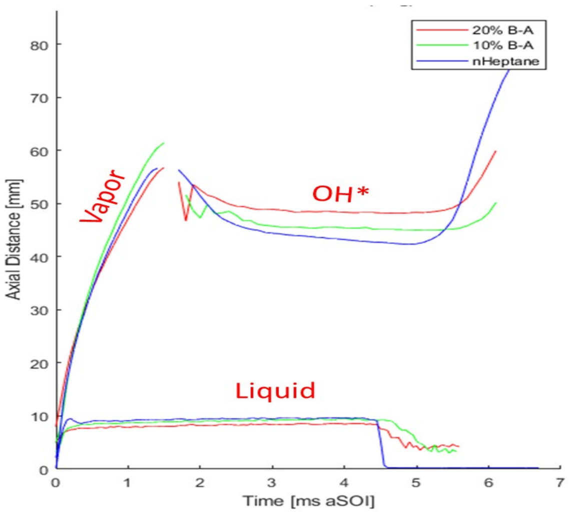
Based on properties, expect

- slower evaporation due to higher boiling point/lower vapor pressure and thus longer physical ignition delay
- Oxygenation should promote lower sooting tendency

Sample Two-color pyrometry results

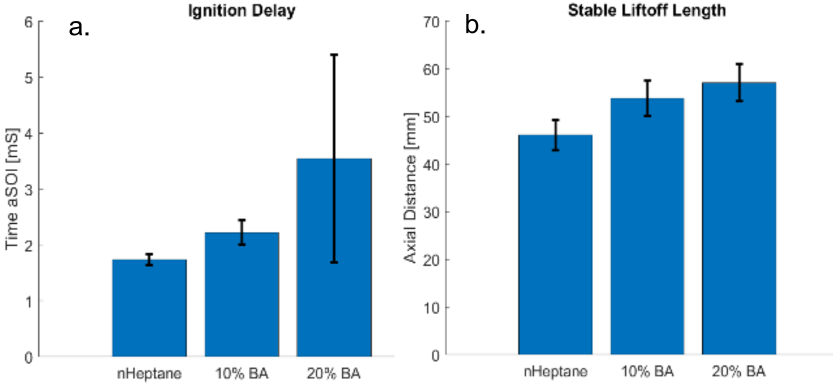
Sample data showing temperature (left) and soot concentration, KL (right) for a pure n-heptane spray combustion event.

Mixing and Ignition Evolution



Liquid and vapor penetration, and OH* (reaction front) lift-off length for BA blends.

BA Blend Ignition and Lift-off Length



Average Ignition Delay (a) and Average Stable Lift-off Length (b) for n-Heptane, 10% n-Butyl Acetate, and 20% n-Butyl Acetate fuel blends.

Summary of Current Status

- Significant test data has been acquired for 0, 10, and 20% BA blended into n-heptane.
- Uncertainty analysis is being incorporated into two-color pyrometry analysis prior evaluating impact of BA on sooting tendency.
- Ignition and lift off-length results are as expected based on expected impact macroscopic fuel properties have on physical ignition delay.
- Testing of biosynthesized BA is still outstanding with comparisons of spray combustion behavior to commercial BA.
- Engine testing of commercial BA in heavy-duty engine will be completed in final BP.

Techno-Economic Analysis (TEA)

➢ **The TEA results showed an economic feasibility of biological production of BA from corn stover using our engineered strain.**

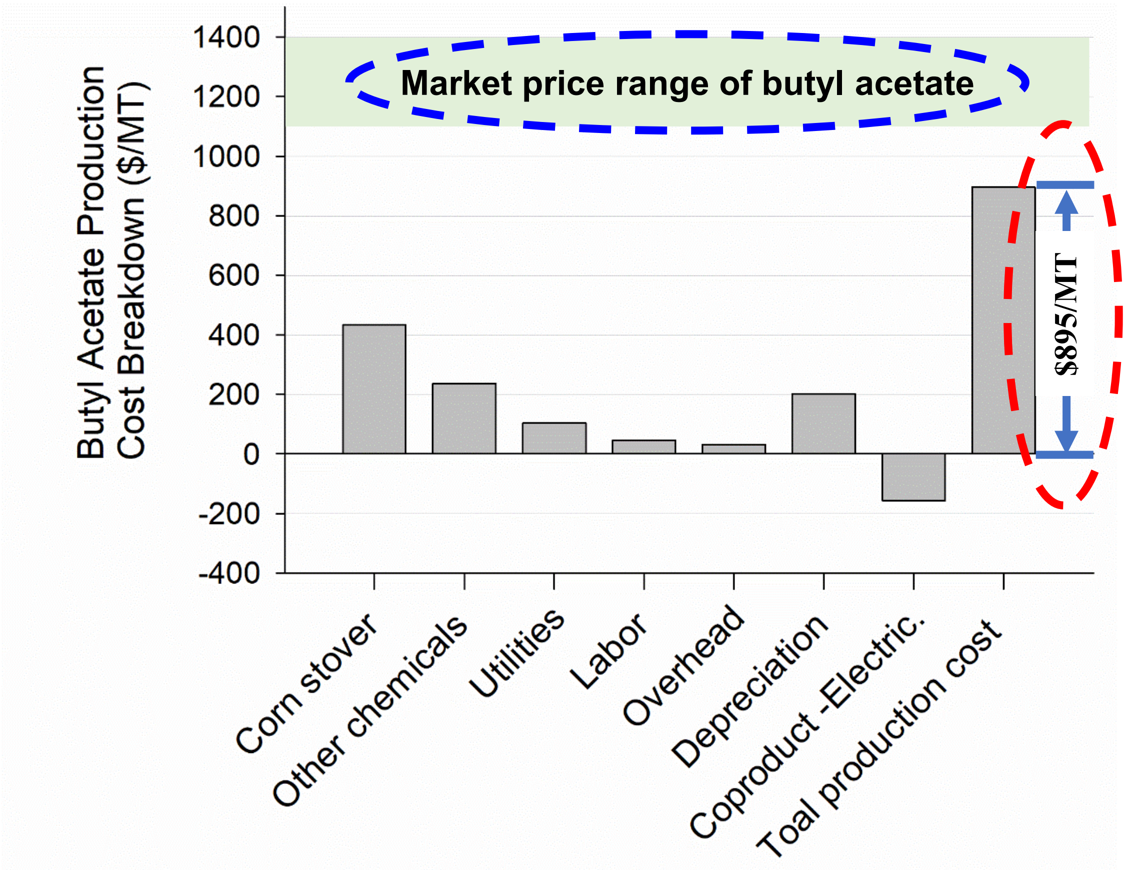


Fig a: BA production cost with the ***current*** strain

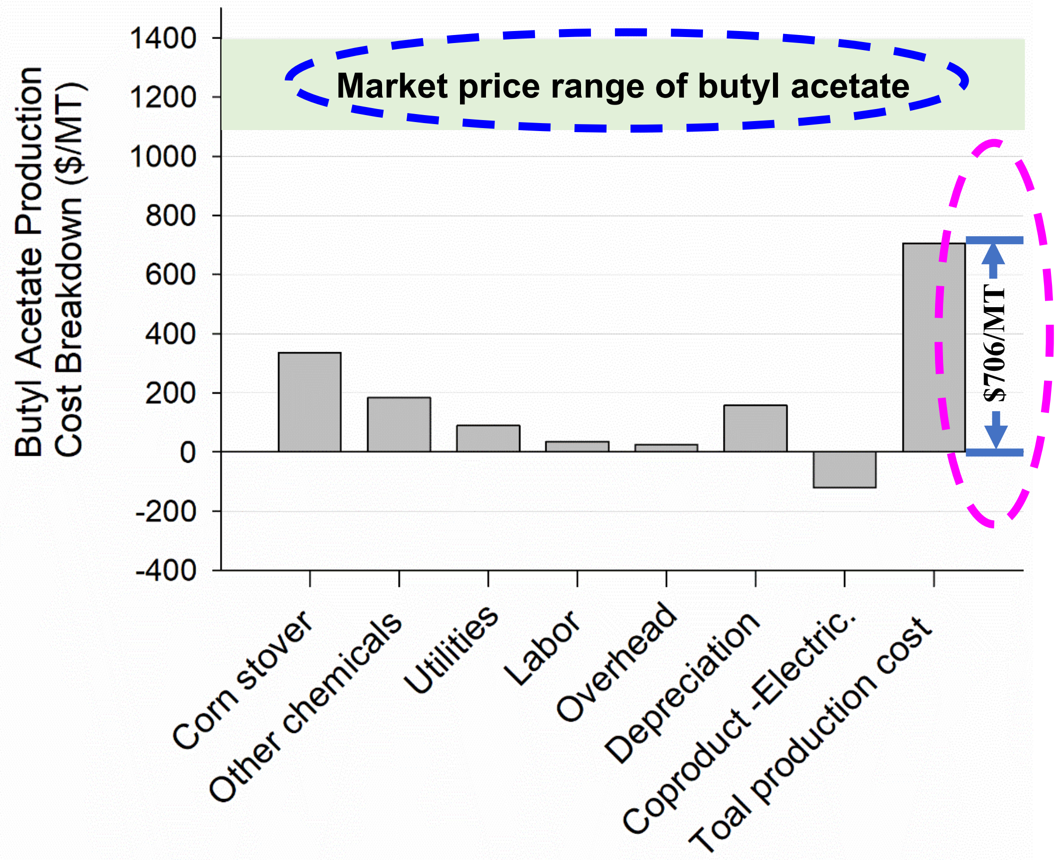
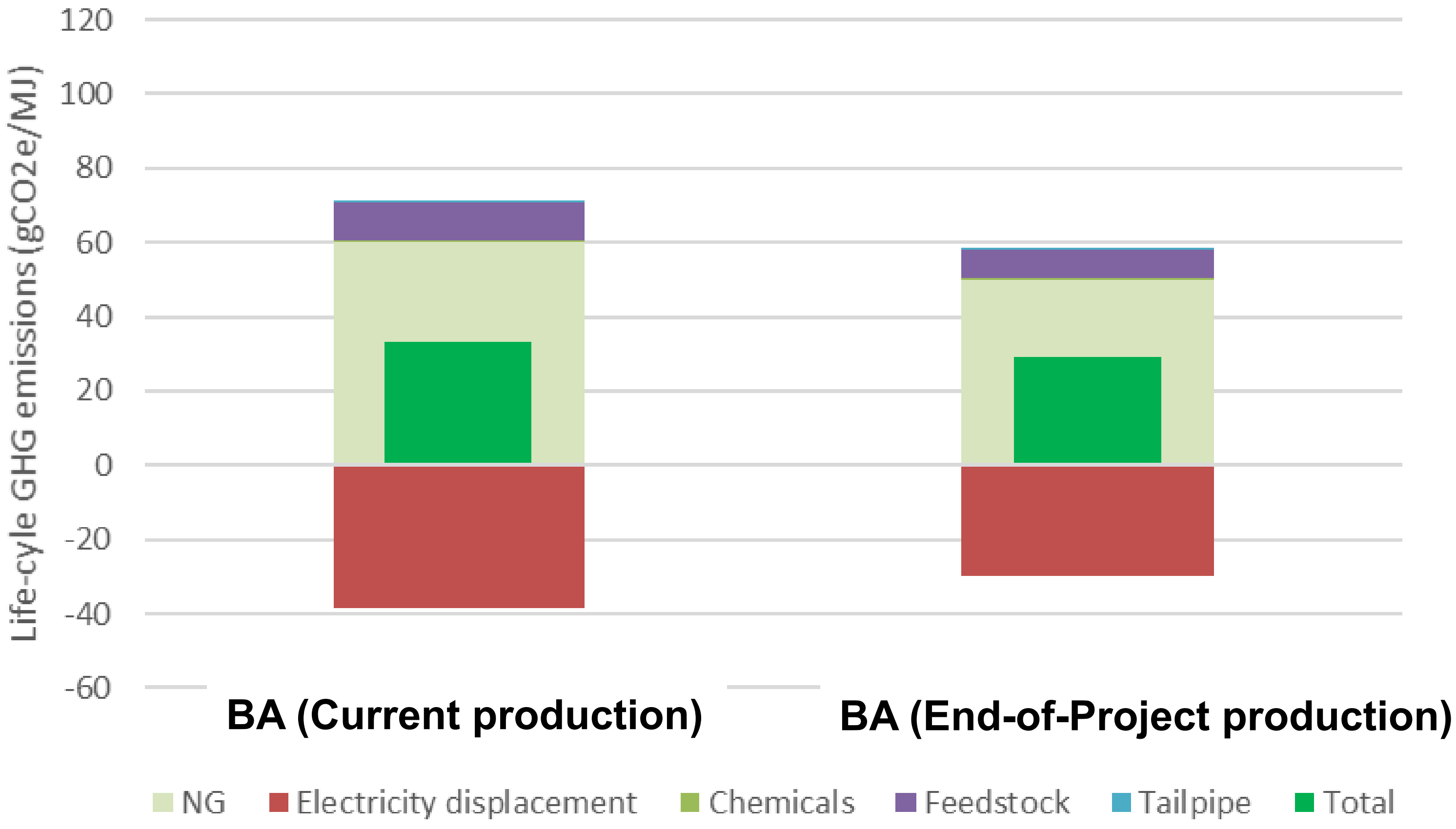


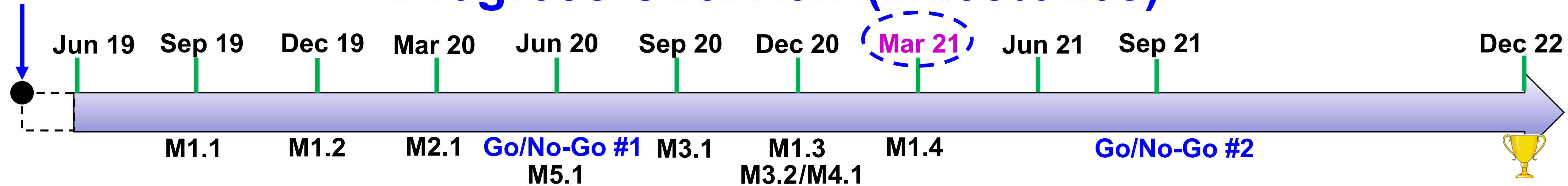
Fig b: ***Projected*** BA production cost with achieving the end-of-project goal: titer of 30 g/L titer, yield of 0.4 g/g, and productivity of 0.6 g/L/h

Life-Cycle Analysis (LCA)



- The LCA results showed a significant GHG reduction for the BA production from corn stover in this project, compared to its petroleum counterparts;
- The BA from this project meets the 60% GHG reduction threshold that US EPA defined for D3 RIN generation.

Progress Overview (Milestones)



- Go/No-Go #1: BA titer of 20 g/L with a yield of 0.3 g/g

- Go/No-Go #2: 1) BA titer of 25 g/L with a yield of 0.4 g/g;
  2) 10.5 L BA of 95% purity will be generated and provided for testing;
  3) Ignitability of BA/heptane blend and surrogate blend will be assessed in droplet experiments

Summary

- In this project, we target to develop an integrated bioprocess for efficient BA production through systematic genome engineering of *Clostridium* and design of materials science-based cell immobilization process. Meanwhile, we evaluate BA as a desirable bioblendstock for diesel through fundamental combustion and full-scale engine testing.
- By now, our engineered strain can produce 24.7 g/L BA with a yield of 0.31 g/g in a batch fermentation.
- We have developed MNE biocatalyst composites that can provide consistently high BA yield, enhance toxicity resistance and offer storage capabilities.
- Droplet and spray combustion studies show impact on ignition and sooting behaviors, and still more exploration needed.
- TEA and LCA results demonstrated economic feasibility and environmental sustainability of our bioprocess even based on the current BA production level.
- We have met the requirement for Go/No-Go #1, and close to meet the requirement for Go/No-Go #2.
- By far, our project has brought broader impacts through publications, patents, presentations, interactions with industries, and other dissemination activities.

Quad Chart Overview

Timeline

- 10/1/2018
- 12/31/2022

	FY20 Costed	Total Award
DOE Funding	$747,340	$1,999,990
Project Cost Share	$167,736	$525,898

Project Partners:

- Auburn University: Y. Wang, Y.Y. Lee
- University of Alabama: A. Agrawal, J. Bittle
- Cornell University: C. T. Avedisian
- Virginia Tech: H. Huang
- Microvi Biotech: F. Shirazi
- EcoEngineers: Z. Wang

Project Goal

1) Develop an integrated bioprocess for efficient butyl acetate (BA) production through strain development by systematic genome engineering and process development using biocatalyst composites (MicroNiche Engineering technology, or "MNE biocatalysts");

2) further, BA will be evaluated for its potential as a bioblendstock for diesel through fundamental droplet combustion, lab scale CPFR testing, and full-scale engine testing.

End of Project Milestone

1) BA production of 30 g/L with yield of 0.4 g/g and productivity of 0.6 g/L/h with MNE-biocatalysts from NREL hydrolysates;

2) Droplet experiments and full engine testing determine the range of BA that can be used as diesel blendstock;

3) Documentation of the impact of BA on finished fuel energy density, sooting propensity, cetane number, and cold weather behavior (pourpoint, cloudpoint);

4) LCA and TEA confirming sustainable and cost-competitive BA production with our bioprocess.

Funding Mechanism

- **Year:** 2018;
- **FOA:** DE-FOA-0001919 (Advanced Vehicle Technologies Research);
- **Topic Area:** 5b-Bioblendstocks to Optimize Mixing Controlled Compression Ignition Engines.

Acknowledgements

- Funding from DOE Energy Efficiency and Renewable Energy (EERE) under the Co-Optima Initiative Award Number DE-EE0008483;
- Program Managers:

  BETO: Alicia Lindauer, Robert Natelson

  NETL: Michael Ursic, Ralph Nine
- National Lab Liaison:

  ABPDU: Eric Sundstrom, Todd Pray
- Dan Schell (NREL)
- Lelia Cosimbescu (PNNL)
- Many others…

Additional Slides

Publications, Patents, Presentations, Awards, and Commercialization

Publications:

- J. Feng, J. Zhang, Y. Feng, P. Wang, P. Jiménez-Bonilla, Y. Gu, J. Zhou, Z.T. Zhang, M. Cao, Z. Shao, I. Borovok, H. Huang, **Y. Wang***. 2020. Renewable fatty acid ester production in *Clostridium*. *Nature Communications*. ***In revision (close to acceptance)***.
- Z. Chen, J. Zhou, Y.F. Wang, **Y. Wang***. 2020. Nano on micro: tuning microbial metabolisms by nano-based artificial mediators to enhance and expand production of biochemicals. *Current Opinion in Biotechnology.* 64:161-168.
- J. Feng, W. Zong, P. Wang, Z.T. Zhang, Y. Gu, M. Dougherty, I. Borovok, **Y. Wang***. 2020. RRNPP-type quorum-sensing systems regulate solvent formation, sporulation and cell motility in *Clostridium saccharoperbutylacetonicum*. *Biotechnology for Biofuels*. 13:84.
- Allen Parker, C. Taber Wanstall, Shawn A. Reggeti, **Joshua A. Bittle, Ajay K. Agrawal***, 2020. Simultaneous rainbow schlieren deflectometry and OH* chemiluminescence imaging of a diesel spray flame in constant pressure flow rig. Proceedings of the Combustion Institute.

Patents:

- **Y. Wang**, J. Feng, Y. Ma. 2020. Microbial ester production. Auburn University. PCT Patent Application number: PCT/US20/66452.
- **Y. Wang**, J. Feng. 2019. Engineered *Clostridium* for ester production. Auburn University. Application number: 62/950,564.

Publications, Patents, Presentations, Awards, and Commercialization

Presentations:

- J. Feng, J. Zhang, M. Cao, Z. Shao, I. Borovok, **Y. Wang**. Rational engineering of *Clostridium* for efficient production of renewable fatty acid esters. 2020 Virtual AIChE Annual Meeting. November 16-20, 2020.
- P. Jiménez-Bonilla, David Blersch, Yifen Wang, Luz-Estela Gonzalez-de-Bashan, **Y. Wang**. Autolysin gene deletion in *Clostridium saccharoperbutylacetonicum* N1-4 increased strain stability and production for biobutanol fermentation. 2019 Bioenergy Sustainability Conference. Nashville, TN, October 21-22, 2019.
- P. Jiménez-Bonilla, Jie Zhang, David Blersch, Yifen Wang, Luz-Estela Gonzalez-de-Bashan, **Y. Wang**. *srpB* efflux pump from *Pseudomonas putida* increases robustness of *Clostridium saccharoperbutylacetonicum* N1-4 for biobutanol production. 2019 ASABE Annual International Meeting. Boston, MA, July 07-July 10, 2019.
- Jie Zhang, Jun Feng, Pixiang Wang, **Y. Wang**. Systematic genome engineering of solventogenic clostridia for biofuel and biochemical production. 41st Symposium on Biotechnology for Fuels and Chemicals, Society of Industrial Microbiology and Biotechnology. Seattle, WA, April 28-May 1, 2019.
- P. Jiménez-Bonilla, **Y. Wang**. Exogenous efflux pump expression increase robustness against biomass hydrolysates inhibitors on fermentations of the hyperbutanol producer *C. saccharoperbutylacetonicum* N1-4. 41st Symposium on Biotechnology for Fuels and Chemicals, Society of Industrial Microbiology and Biotechnology. Seattle, WA, April 28-May 1, 2019.

Publications, Patents, Presentations, Awards, and Commercialization

Presentations (contd.):

- Bittle, J.. Agrawal, A. Simultaneous Mixing, Ignition and Soot Measurements in a Constant Pressure Facility. Department of Energy, Vehicle Technology Office's Advanced Engine Combustion Program Meeting. Sandia National Laboratory. Livermore, California. February 2020.
- S. Guo, Y. Wang, M. Haefner, C. T. Avedisian, "Combustion of petroleum-based transportation fuels and their blends with oxygenates," NSF Workshop: New Frontiers of Thermal Transport, University of Central Florida, December 14-16, 2020.
- Y. Wang, J. Feng, S. Guo, M. Haefner, J. Zhang, Yi Wang, C.T. Avedisian, "Combustion of Butyl acetate as an alternative additive to petroleum fuels," 36th Annual Meeting, American Society of Gravitational and Space Research, November 5-6, 2020.

Commercialization:

- A Canadian company is negotiating with Auburn University for an option agreement or licensing of the technology.

Combustion testing strategy:

-DROPLETS: small-scale configuration, need 10^{-8} gal/exp

-ENGINE: large scale and end-use configuration: need liters/exp

Purpose

COMPARE synthesized (94.3% pure) BA with 'commercial' (>99.9%) BA

EVALUATE blending effects using droplet and engine testing:

- **-1st, BA; 2nd, BA+n-heptane; 3rd, BA + diesel; 4th, BA+diesel surrogate**
- **-why heptane to start?**
  - **--heptane is 'simple', results 'easy' to interpret**
  - **--heptane widely used as diesel surrogate**
    - ***heptane oxidation chemistry ≈ diesel oxidation chemistry**
- **-results useful for detailed numerical modeling**
- **-diesel fuel is very complex so start 'simple' then progress to complex**

Summary (contd.)

- BA produced by genome engineering burns in an almost identical manner as commercial BA in terms of the fuel burn rate and relative position of the droplet to its flame.

- By-products of production do not significantly influence the burning process.

- Initial experiments with BA+heptane mixtures show remarkably similar burning of BA and BA/heptane mixtures; these results are preliminary and additional experiments are in progress to expand the range of heptane concentrations.

- Continuing work will be carried out on BA/diesel mixtures and mixtures of BA with a diesel surrogate in anticipation of validating oxidation kinetics important for simulating in-cylinder processes in combustion engines.

Responses to Previous Reviewers' Comments

- If your project has been peer reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers' comments which you have since addressed
- Also provide highlights from any Go/No-Go Reviews

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.