DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Development of a Sustainable Green Chemistry Platform for Production of Acetone and downstream drop-in fuel and commodity products directly from Biomass Syngas via a Novel Energy Conserving Route in Engineered Acetogenic Bacteria

March 6, 2019

Biochemical Conversion

Sean Simpson (PI)

Michael Köpke (Technical Lead, Presenter)

LanzaTech, Inc.

Goal Statement

Project Goal:

To develop and scale up a novel technology platform for direct conversion of biomass syngas to acetone, which can be converted into a range of fuels and commodities via existing downstream technologies

Key Project Outcomes:

- Demonstration of a novel process for acetone production directly from biomass syngas at commercially relevant rates in a continuous process and scalable pilot reactor
- Optimization of natural and synthetic pathway for acetone production
- Validation of commercial potential through TEA and LCA

Relevance to Bioenergy Industry/Impact:

- Offers a new stand-alone route to advanced hydrocarbon fuels and bioproducts from biomass residues
- Provides environmentally friendly alternative to current petrochemical acetone production
- Produces a platform chemical that can be readily converted into a range of drop-in fuels (isooctane and jet fuel) or important chemical building blocks (isobutylene, propylene, bisphenol A and PMMA)
- Novel acetate-independent and energy-generating pathway that offers increased yields over traditional ABE fermentation pathway

Quad Chart Overview

Timeline

- Project start date: October 1st, 2016
- Project end date: December 31st, 2018
- Percent complete: 100%

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	N/A	$366,757	$363,637	$6,150
Project Cost Share*	N/A	$302,200	$265,863	$3,757

Partner split in total project funding:

- LanzaTech (51%)
- Oak Ridge National Lab (49%)

Sequencing input from JGI (funded separately)

Barriers addressed

- Qt-B: Cost of production
- Ct-D: Advanced bioprocess development
- Ct-H: Gas fermentation development
- Ct-L: Decreasing development time for industrially relevant microorganism
- ADO-A: Process integration

Objective

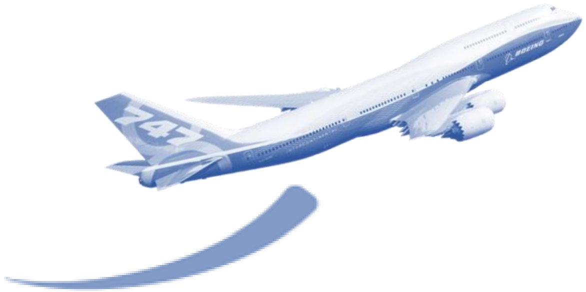
A biocatalyst capable of converting syngas from waste biomass into acetone

End of Project Goal

Demonstration of fermentation stability for acetone production for 4 days at 100% of commercial rate and 100% of commercial selectivity.

1 – Project Overview

Platform molecule for fuels and bio-products

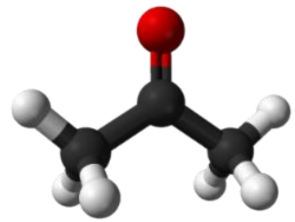


Jet-fuel
$199 billion

Propylene
$98 billion

Isobutylene
$25 billion

Isooctane
$0.6 billion (only US market)



Acetone

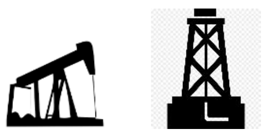
Bisphenol A (BPA)
$17.7 billion

Isopropanol
$2.5-3.5 billion

Polymethyl methacrylate (PMMA)
$7 billion

Traditional Routes vs Proposed Route

Traditional petrochemical route (Cumene process)



Reforming / Cracking / PDH

Benzene + Propylene

Cumene Process

Phenol + Acetone

Fresh Fossil

By-product

High Energy

High Water

Traditional biotechnological route (ABE fermentation)

ABE Fermentation

Butanol

Ethanol

+ Acetone

Food

By-products

High Cost

Proposed route (Gas fermentation)

Gas Fermentation

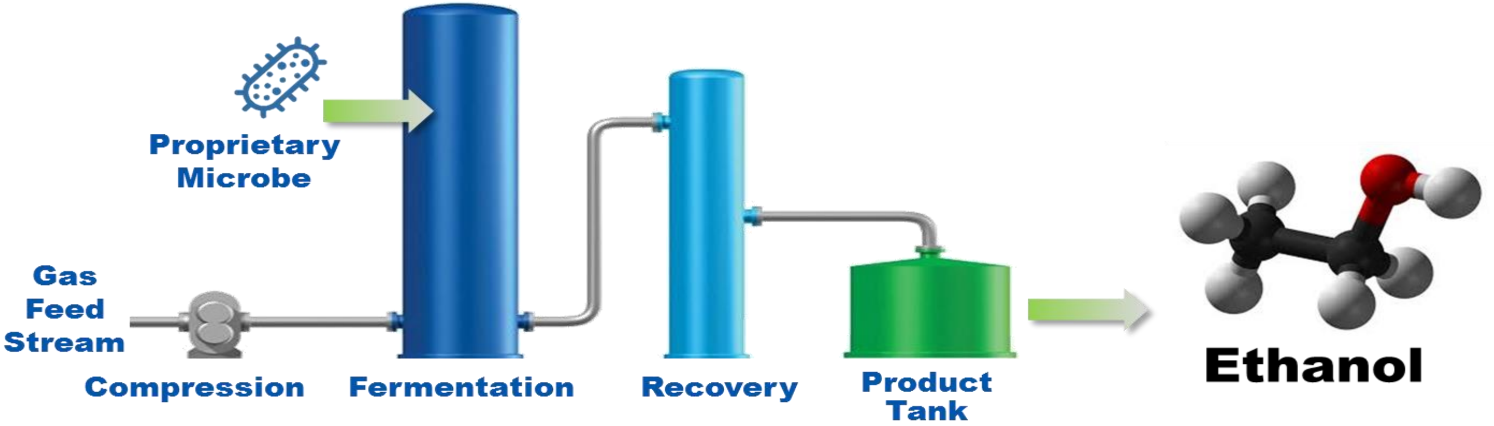
(Ethanol) + Acetone

Waste and Residues

Reduced GHGs

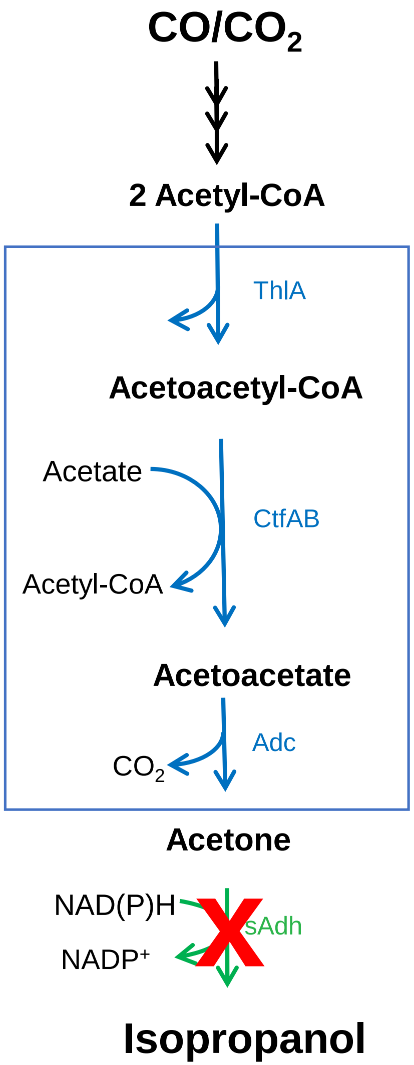
Reduced Water

The LanzaTech Process - Commercial Ready



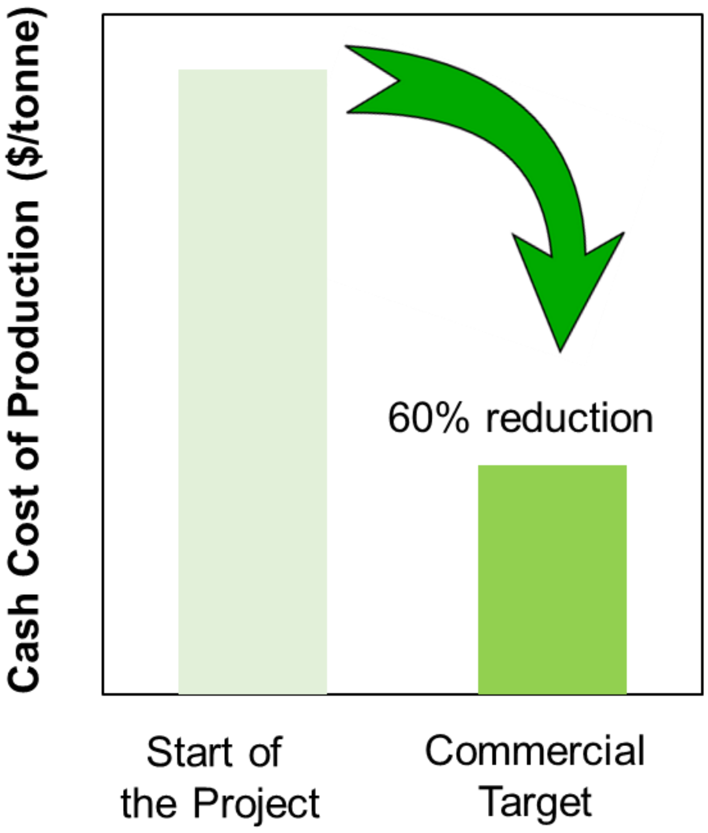
Started up May 3rd, 2018

The LanzaTech Acetone Process at the Start of the Project



- **Acetone production demonstrated by implementing pathway from ABE model organisms**
- **Identified a native secondary alcohol dehydrogenase that is converting acetone away to isopropanol**
- **Lab-scale fermentation established, but acetate as major product and not stable over time**
- **Process requires optimization to be cost competitive**

Cash Cost of Production (CCOP)



Gas feed basis: 2000 MT/day biomass feedstock converted to syngas through gasification

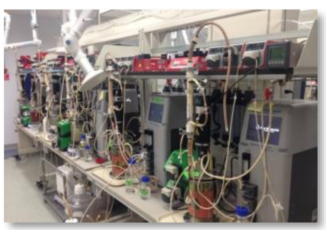
2 – Approach (Management)

Project Team

LanzaTech

(Primary)

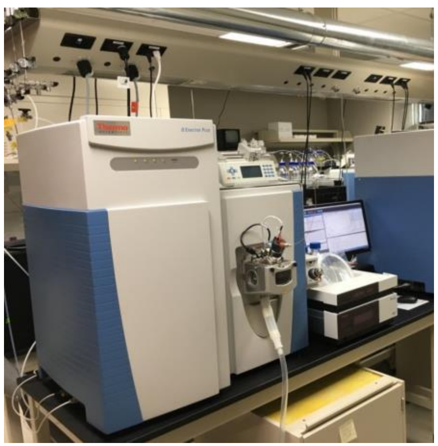
- **Strain Engineering & Modelling**
- **Fermentation & Scale Up**
- **TEA & LCA**

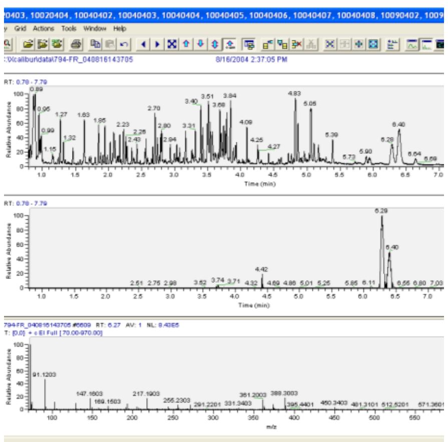


Oak Ridge National Laboratory

(Subawardee)

- **Omics**
- **Data Analysis**

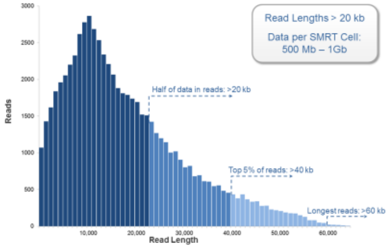


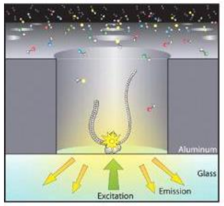


JGI Joint Genome Institute Department of Energy

(DOE User Facility)

- **Genome sequencing**





Project Team

Principal Investigator
Dr. S. Simpson

Technical Lead
Dr. M. Köpke

Task 1 – Strain Engineering
- **Strain Engineering**: Dr. C. Leang, R. Nogle
- **Genome Scale Modeling**: Dr. J. Daniell

Task 2 – System Biology & Omics
- **Sample Prep**: Dr. R. Jensen, Dawn Klingeman, Nancy Engle, Zamin Yang
- **Proteomics**: Dr. R. Hettich, Dr. R. Giannone, Suresh Poudel
- **Genomics/ Transcriptomics**: Dr. R. Jensen, A. Dassanayake, Dr. S. Nagaraju
- **Metabolomics**: Dr. T. Tschaplinski, Payal Charania

Task 3 – Pathway Engineering
- **Native Pathway Optimization**: Dr. E. Liew
- **Synthetic Pathway Optimization**: Dr. R. Tappel

Task 4 – Process Development
- Dr. S. Brown, T. Abdalla, L. Tran

Task 5 – TEA & LCA
- **TEA**: Dr. A. Gao
- **LCA**: Dr. C. Canter

- LanzaTech and ORNL have collaborated on numerous projects since 2011 and have developed successful mechanisms for technical coordination, data sharing and integration.
- Project meetings:
  - Monthly teleconference meetings
  - Three in-person meetings alternating between LanzaTech and ORNL offices.

3 – Approach (Technical)

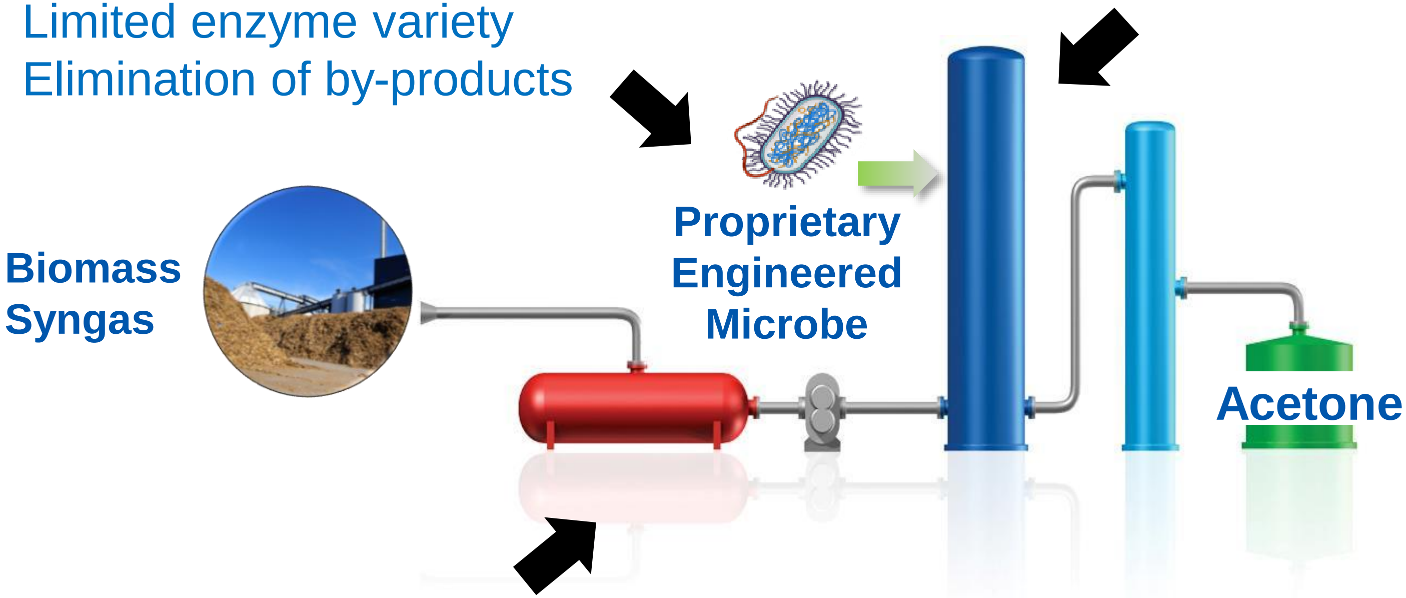
Approach Overview

Strain engineering
- Genome Scale Modelling
- Chassis strain construction
- Pathway engineering

Challenges:
- Limited enzyme variety
- Elimination of by-products

Process development
- Fermentation
- Omics

Challenge: Continuous process



Scale up & Process Integration
- Scalability
- Separations

Challenge: Novel process

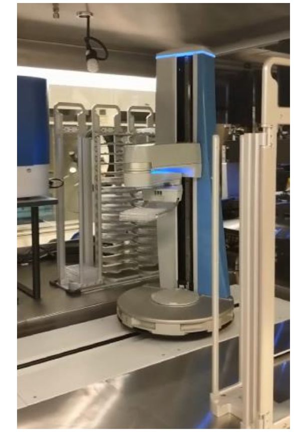
Economics
- TEA
- LCA

Critical success factor: Cost competitive

Strain Engineering & Modelling

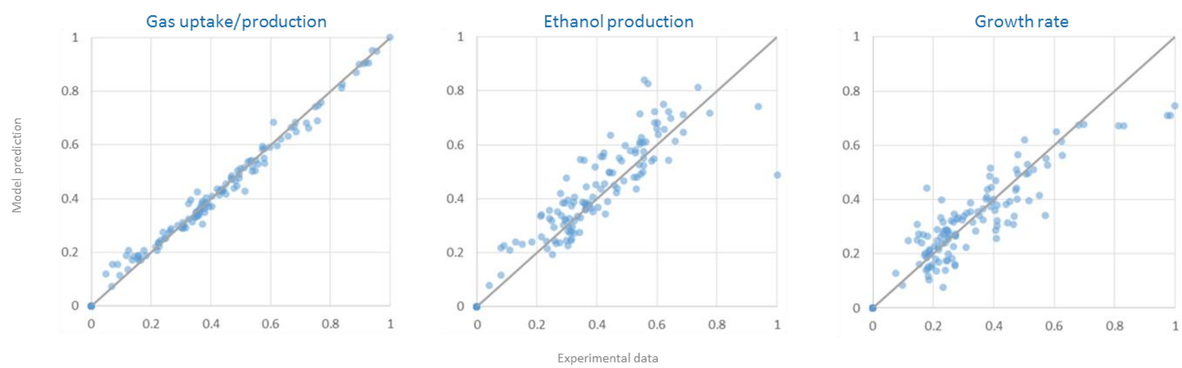
State of Art Strain Engineering Platform

- Robust genetic toolbox including extensive part library and proprietary genome engineering tools
- Fully-automated, anaerobic biofoundry



State of Art Genome Scale Model

- Validated against 1000s of steady-state fermentation runs and KO strains, enriched with Omics data
- Informs strain designs; Accurately predicts gas profile, growth, and product formation across the full spectrum of gas mixes.

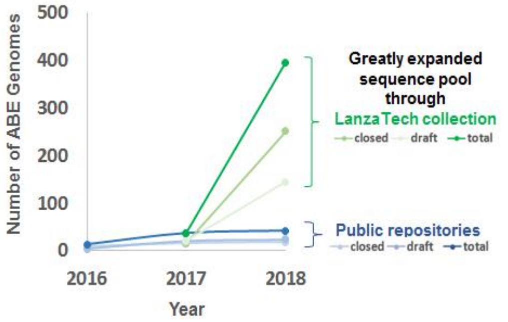


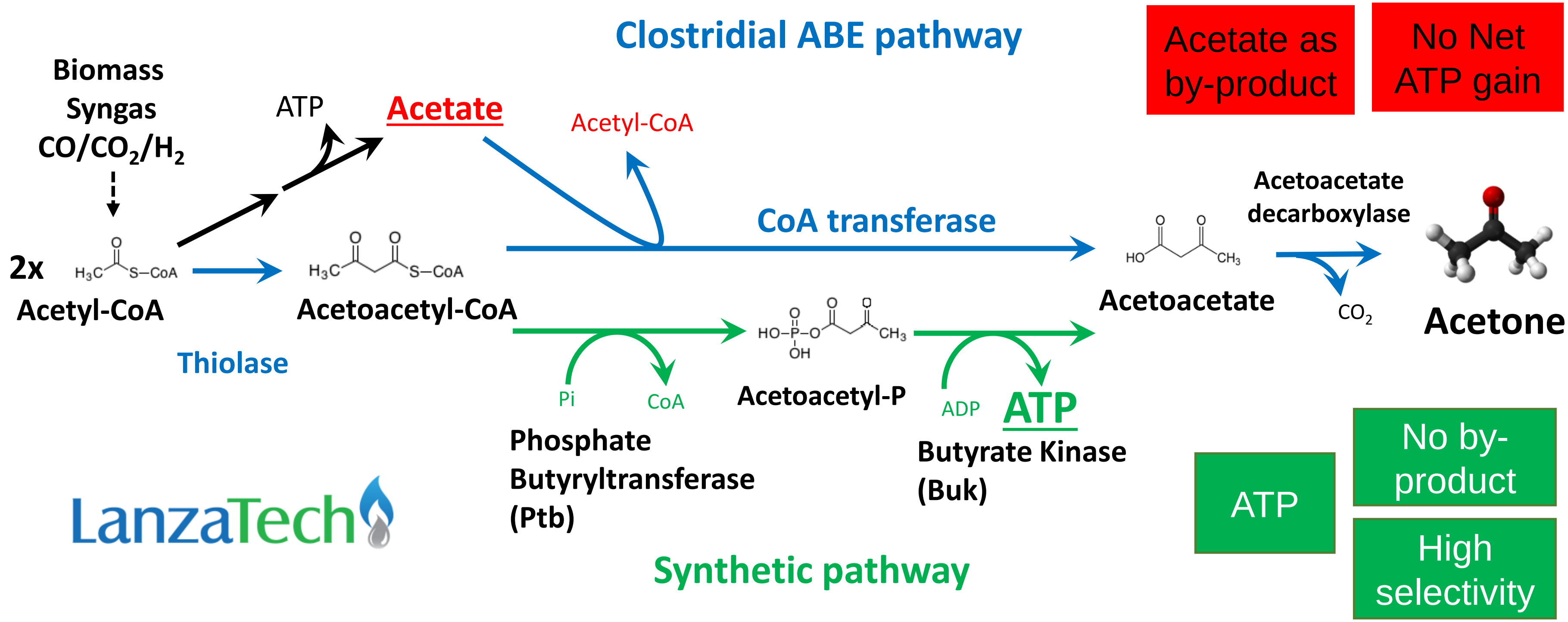
Pathway Engineering

- LanzaTech owns collections of >400 industrial ABE strains spanning four decades of commercial development.
- Ptb-Buk are promiscuous enzymes, shown to also use acetoacetyl-CoA as a substrate, but with low activity.

NCP commercial ABE plant 1935-1982

Jones DT and Woods DR, Microbiol Rev (1986).



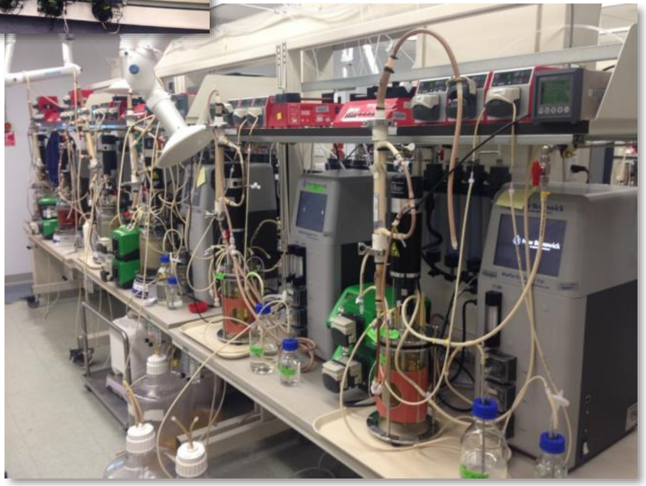


Process Development

State of Art
Gas Fermentation Facilities

- Total gas composition flexibility
- On-line analytics (gas, biomass, metabolites)
- Multiple reactors configurations
- Over 40 dedicated gas fermentation reactors

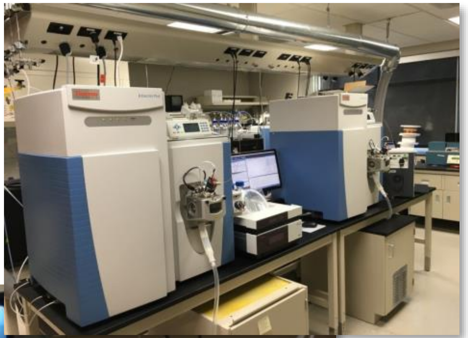
LanzaTech



State of Art
Multi-Omics Facilities

- Metabolomics (including energy related metabolites)
- Proteomics
- Transcriptomics

Oak Ridge National Laboratory



Scale Up

LanzaTech

- In-house pilot reactors
  - >80L
- Freedom Pines Biorefinery
  - >150L

Freedom Pines Biorefinery

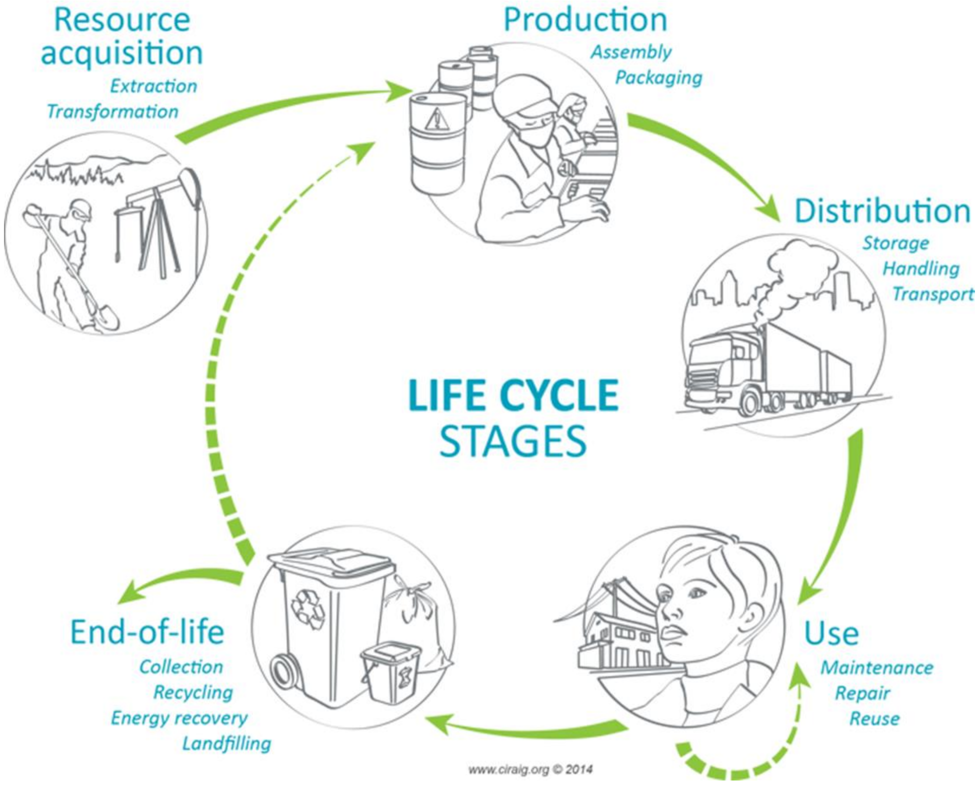
Economics Assessment

TEA

• TEA sensitivity analysis guided strain and process development

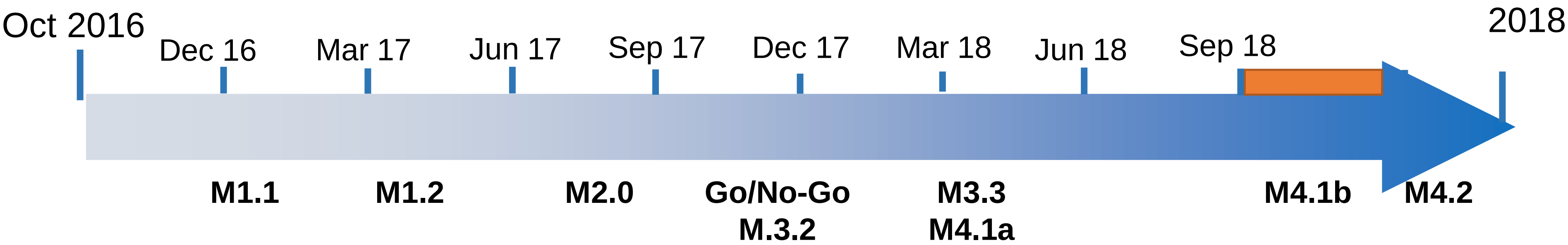
LCA

• Life cycle analysis from crate to gate to determine LanzaTech process GHG emissions reductions compared to other conventional processes.



3 – Technical Accomplishments/ Progress/ Results/ Milestones

Milestones



Progress summary		
Task		**Status**
1.0 Genome Scale Model (GEM) guided chassis strain engineering		
1.1	Strain selection	Completed
1.2	Chassis strain construction	Completed
1.3	Fermentation and data feedback to GEM	Completed
2.0	Data analysis and genotype-phenotype modeling	Completed
3.2	Platform established to screen and select synthetic pathway variants *in vivo* and *in vitro*	Completed
3.3	Combinatorial assembly of acetone pathway	Completed
4.1	Fermentation and evaluation of strains	Completed
4.1	Fermentation and evaluation of strains	Completed
4.2	Piloting of process 80L pilot reactor	Milestone adjusted in agreement with DOE

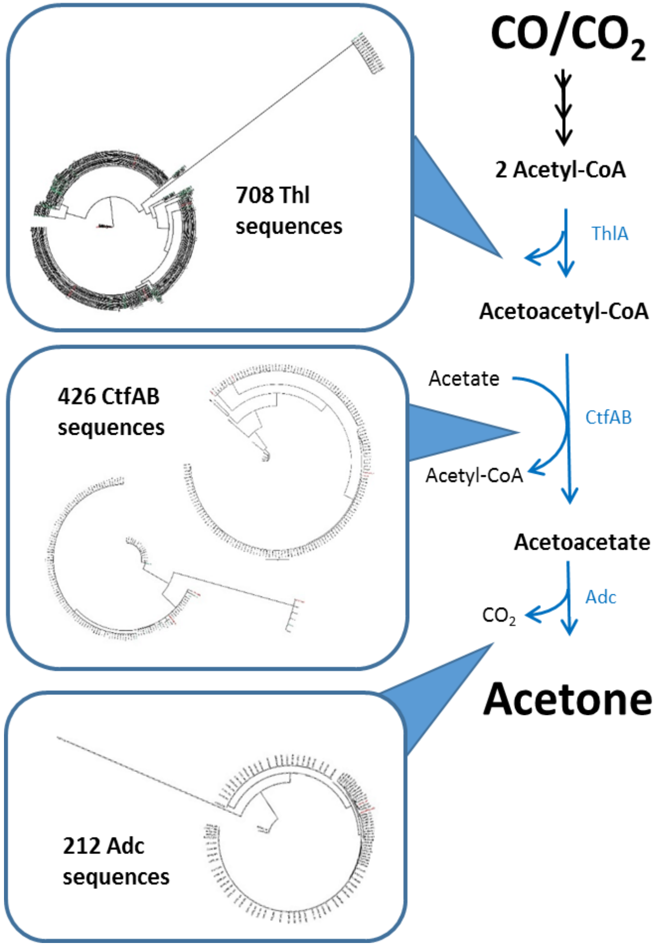
Strain Engineering

- Once improving flux to pathway, production of unwanted byproducts (e.g. 3-HB) was observed
- Guided by genome scale simulations, a series of gene KOs was applied to increase flux into acetone pathway and avoid unwanted byproducts
- Best pathway was introduced into the genome

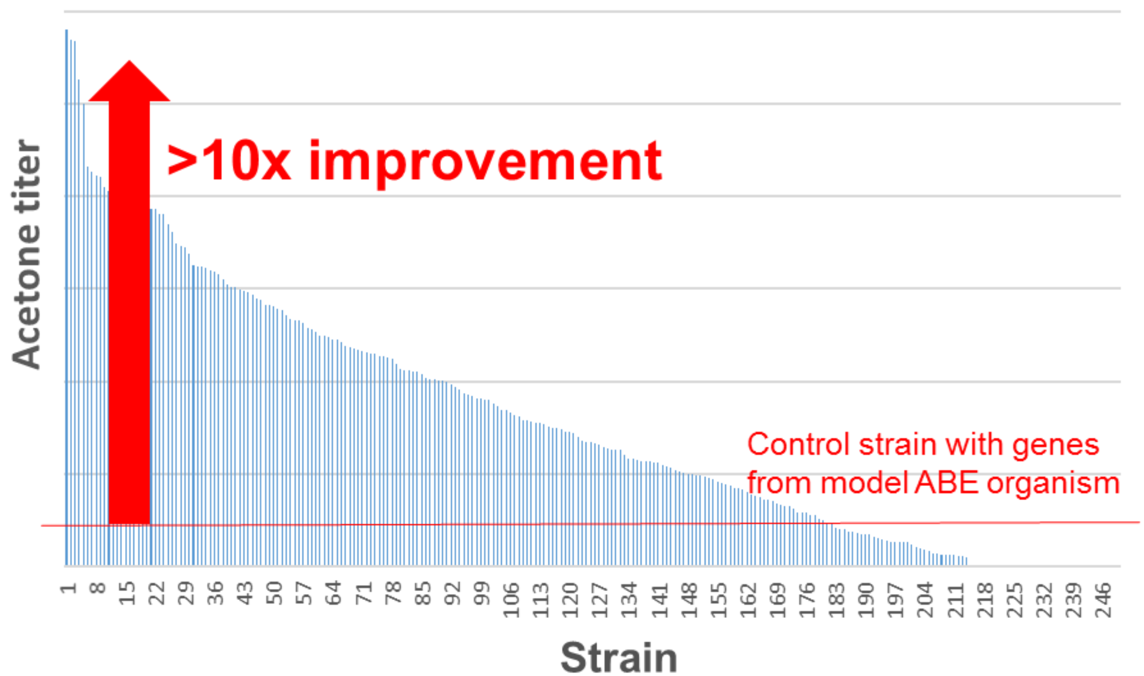
Acetone pathway	Gene knock-outs	Productivity (g/L/h)	Byproducts	Stability	Inducer	Antibiotic
Plasmid	-		2,3-BDO 3-HB IPA	3 days	Required	Required
Plasmid	Adh KO		2,3-BDO 3-HB no H_2 uptake	3 days	Required	Required
Plasmid	Adh KO*		2,3-BDO 3-HB	1 week	Required	Required
Plasmid	Adh KO* + 1		2,3-BDO	1 week	Required	Required
Plasmid	Adh KO* + 2		2,3-BDO	1 week	Required	Required
Integrated	Adh KO* + 2			>3 weeks	Not required	Not required
Integrated	Adh KO* + 3			>3 weeks	Not required	Not required

- **Challenge overcome:**
  - ✓ **Elimination of by-products**

Pathway Engineering



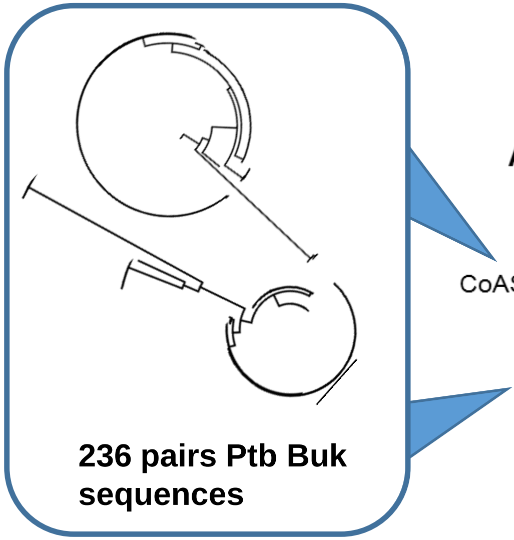
- Mined sequenced genomes of Clostridium collection for acetone pathway genes
- Combinatorial library of identified genes increased acetone production by >10x

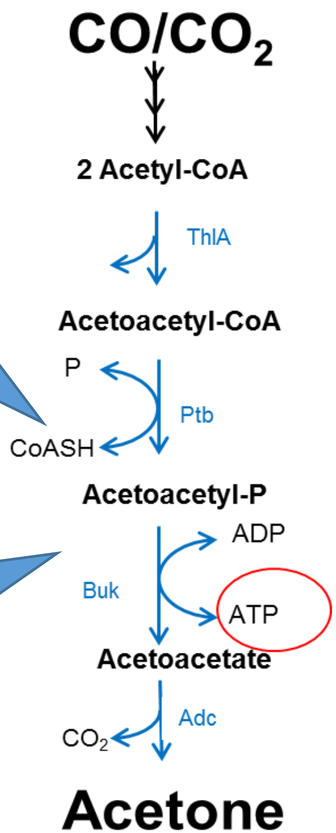


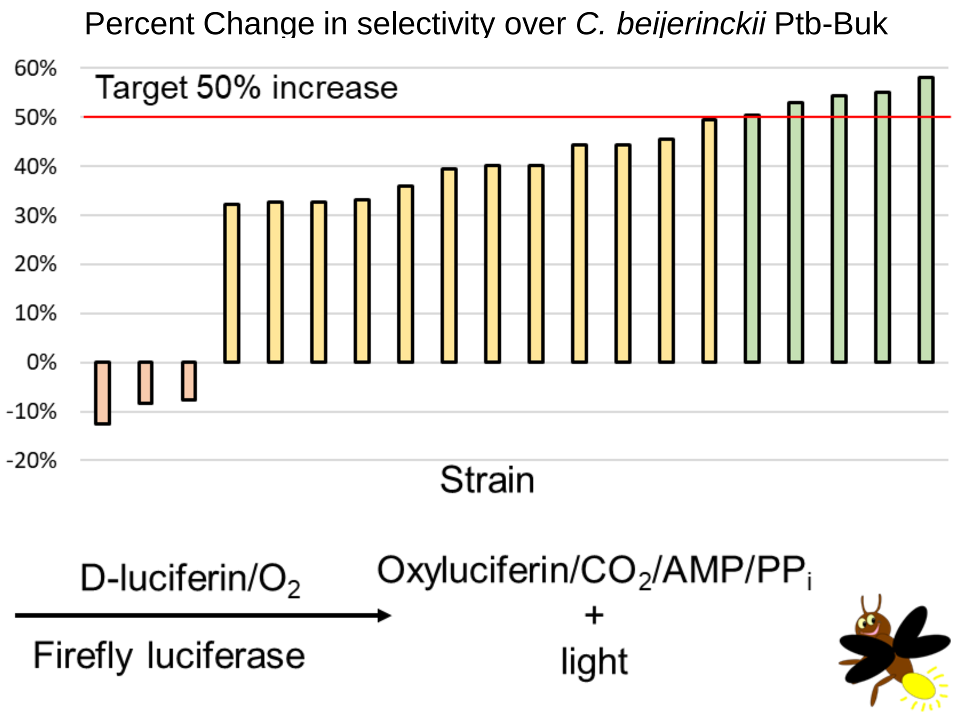
- **Challenge overcome:**
  - ✓ **Limited enzyme variety**

Synthetic Pathway

- Successfully identified Ptb-Buk pairs with increased specificity towards acetoacetyl-CoA



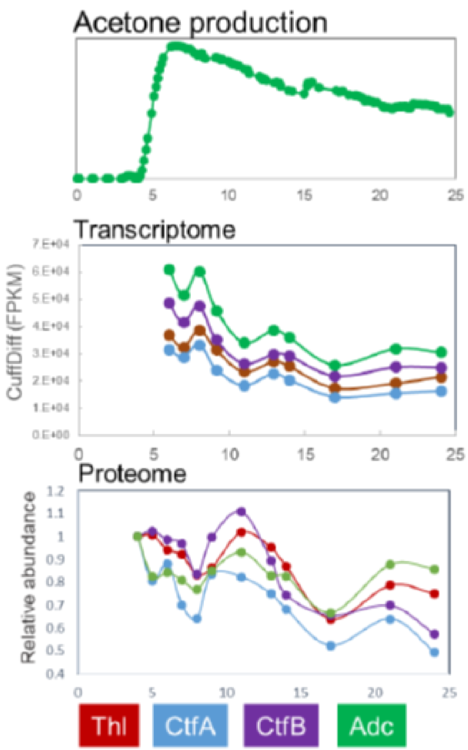


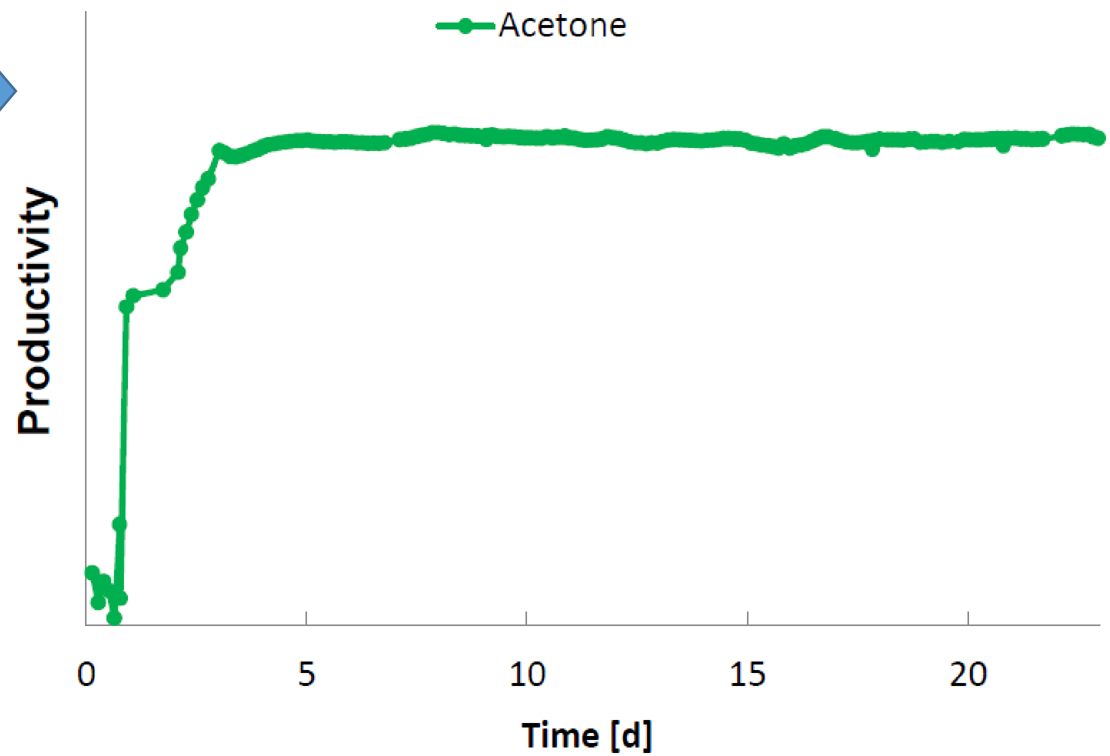


- **Challenge overcome:**
  - ✓ **Limited enzyme variety**

Process Development

- Acetone pathway gene/protein expression was found decreasing over time in continuous culture
- Stability established through multi-omics guided process changes

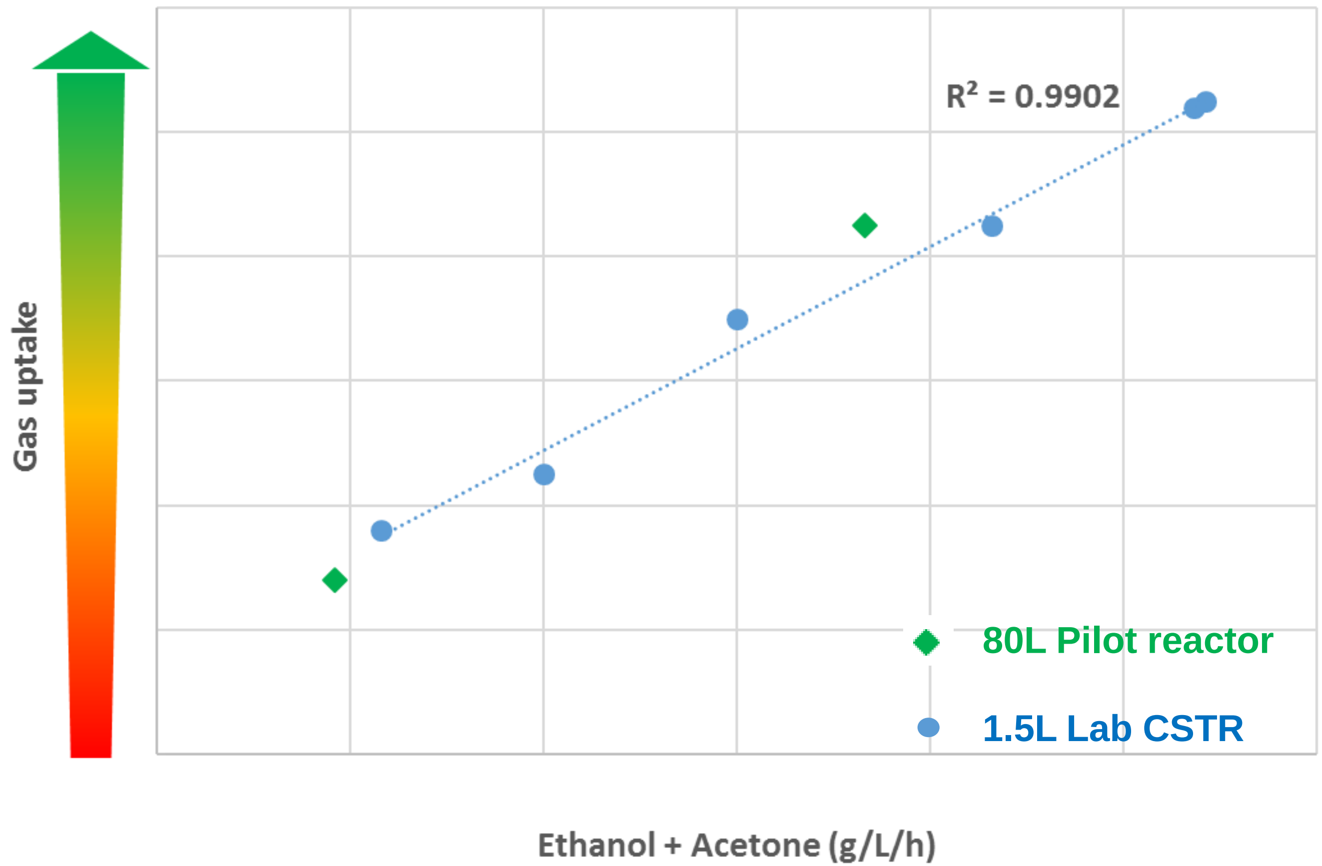




- **Challenge overcome:**
  - ✓ **Continuous process (Instability of acetone production over time)**

Scale Up

- Process scaled up from bench-scale CSTR to 80L reactor with good correlation of performance

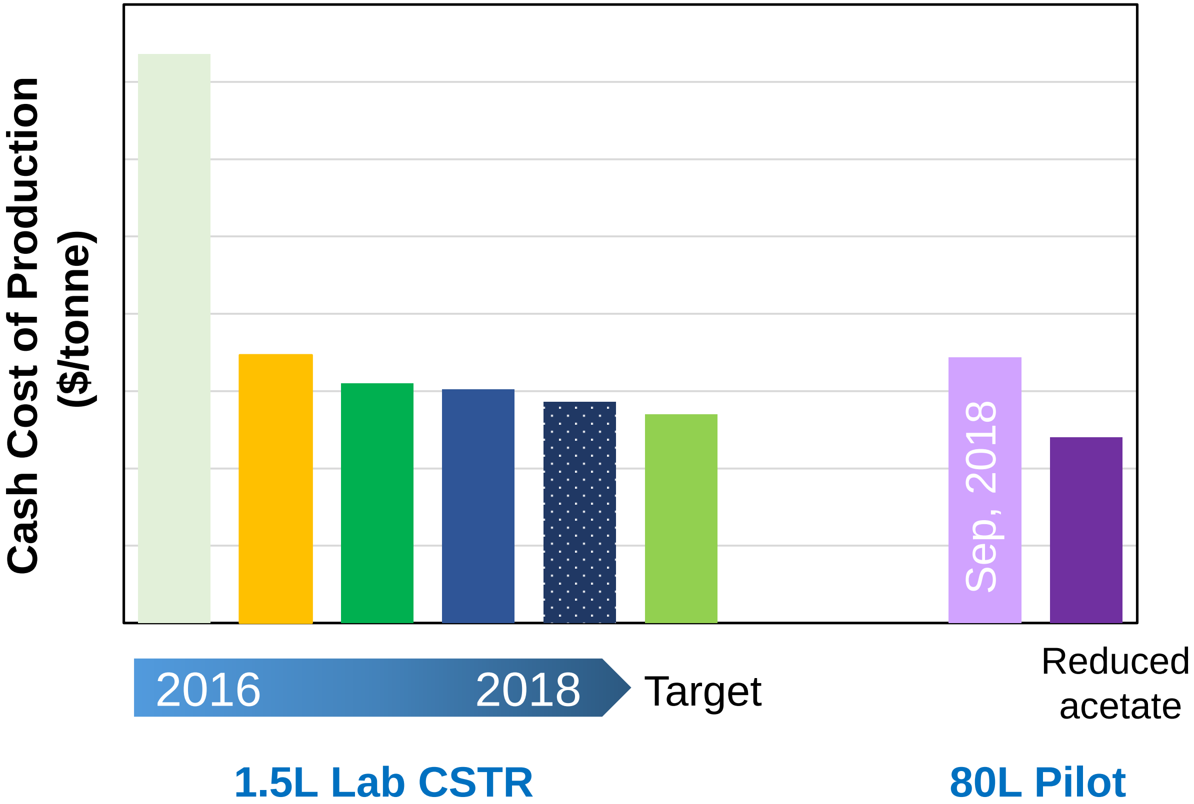


- **Challenges overcome:**
  - ✓ **Novel process (Scalability)**

Economics

Reduction in Cash Cost of Production (CCOP) over time

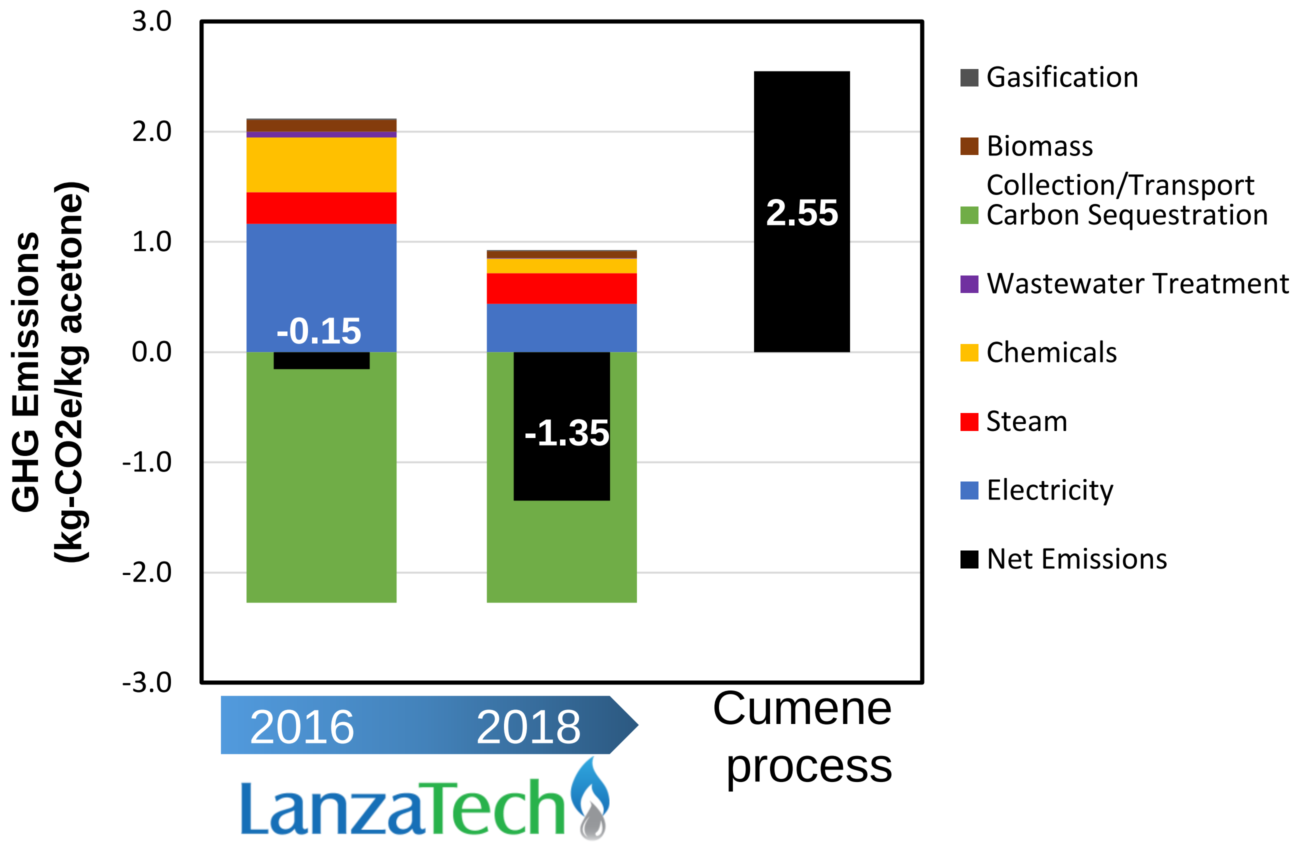
- Gas feed basis is 2000 MT/day biomass feedstock converted to syngas through gasification
- Improvements in productivity and selectivity have driven down cash cost of production.



- **Challenges overcome:**
  - ✓ **Cost competitive**

GHG Emission Reduction

- Results are compared to conventional acetone, considering cradle-to-gate only (acetone production).



LanzaTech acetone process at the end of the project projected to show GHG emissions savings of ~150%.

4 - Relevance

Project Goal:

Develop and scale up a sustainable alternative to current acetone production routes, with an improved safety and environmental footprint, enabling cost-competitive production of acetone as a fuel intermediate or co-product.

Relevance to BETO Mission:

- The process contributes to the Conversion R&D goal of commercially viable technology to convert biomass to finished liquid transportation fuels via a biological route
- Coproduction of acetone and hydrocarbon fuels will enable production of biofuels at or below BETO's target of $3/gge target with 150% GHG reductions over cumene process for acetone.
- Demonstration of continuous acetone gas fermentation processes.

Industry Relevance:

- The acetone market value is heavily dependent on crude oil prices (~$3B in 2015, ~$6B in 2014). Production of acetone from biomass and residues will reduce price volatility and environmental impact.
- This project offers a sustainable, cost-effective alternative to meet the needs of end users in the chemical and consumer products industries seeking to "green" their supply chains.

5 – Future Work

- **Project completed**
- **Scale up and build commercial plant for acetone production**

Summary

Overview:

- Project to develop a novel microbial process for the production of acetone directly from biomass syngas at high yields and efficiencies.

Approach:

- Interdisciplinary approach of strain engineering, process development, scale up and economic modelling. State-of-the-art Clostridium Synthetic Biology, gas fermentation process development and Multi-Omics combined with integrated models.

Technical Accomplishments/Progress/Results:

  - Demonstrated production of acetone in continuous process and at commercially relevant rates and yields from biomass syngas in a scalable system.
  - 150% GHG reduction
  - Largest number of modifications ever made in single Clostridium strain
  - Largest number of library in Clostridium
  - Largest sequences pool of acetone pathway genes derived from

Relevance:

- Acetone is a high value chemical with a growing market demand; the fully developed process will provide a cost competitive route to this product from sustainable resources and enable low-cost biofuels at or below DOE's $3/gge target.

Future work:

  - *Project completed.*

Thank you!

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

BIOENERGY TECHNOLOGIES OFFICE

Technology Manager: David Babson
Project Monitor: Clayton Rohman

Response from Previous Reviewers' Comments

- The demand for acetone in the U.S., European, and Asian markets is estimated at 6.4 million tons per year and is valued at $7 billion per annum. Acetone is also a direct precursor of valuable downstream products, such as direct drop-in fuels, fuel additives, polymers, and important chemical building blocks. In addition to its direct use, acetone can serve as a platform intermediate for conversion to a number of downstream products, including propylene ($125 billion), isobutylene ($25 billion), bisphenol A ($10 billion), poly(methyl methacrylate) (a fast-growing $7 billion market), and drop-in fuel isooctane, further diversifying the utility of renewable acetone as a co-product.
- The wide range of uses for acetone, in conjunction with the increasing average market price (the 2016 price was roughly 50% higher than 2015), will help offset the effective cost of the fuel product, thus enabling us to meet the $3/gge target set forth.
- LanzaTech has developed detailed techno-economic models that we use to consistently evaluate the economics of our process. Acetone as co-product can enable ethanol fuel production with a target price of $3/gge. Per this TEA analysis, a 1:1 acetone to ethanol ratio enables meeting this $3/gge fuel target.

Highlights from Go/No-Go Reviews

- We not only met our Go/No-Go milestones but exceeded the Go/No-Go criteria (50% of the commercial productivity and 85% of commercial selectivity).

Presentations

Conferences	Dates	Location	Presenter
Molecular Basis of Microbial One-Carbon metabolism (GRC)	July 29-Aug 3, 2018	Newry, ME USA	Dr. C. Leang
Foundations of Systems Biology in Engineering (FOSBE)	Aug 5-8, 2018	Chicago, IL USA	Dr. S. Brown
SIMB Annual Meeting	Aug 12-16, 2018	Chicago, IL USA	Dr. M. Koepke
Metabolic Engineering	June 24-28, 2018	Munich Germany	Dr. M. Koepke
Symposium on Biotechnology for fuels and chemicals (SBFC)	Apr 29th-May 2nd, 2018	Clearwater, FL USA	Dr. R. Jensen
Eco-Bio	March 4-7, 2018	Dublin Ireland	Dr. M. Koepke

Commercialization

- In addition to in-house piloting, we carried out a proof of concept demonstration of acetone production in our FP reactor in Soperton, Georgia.