




BETO 2021 Peer Review: Waste Carbon Gas Upgrading via Acetogens 2.3.2.106






Jonathan Lo
National Renewable Energy Laboratory
Conversion
March 11, 2021

Market Trends




Product

-  Anticipated decrease in gasoline/ethanol demand; diesel demand steady
-  Increasing demand for aviation and marine fuel
-  Demand for higher-performance products
-  Increasing demand for renewable/recyclable materials




Feedstock

-  Sustained low oil prices
-  Decreasing cost of renewable electricity
-  Sustainable waste management
-  Expanding availability of green H₂
-  Closing the carbon cycle

Capital

-  Risk of greenfield investments
-  Challenges and costs of biorefinery start-up
-  Availability of depreciated and underutilized capital equipment

Social Responsibility

-  Carbon intensity reduction
-  Access to clean air and water
-  Environmental equity

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- Waste carbon gas and renewable energy are substrates for microbial upgrading
- Currently microbes mainly make lower value ethanol/acetate
- What products can we make?

Differentiator

- Gaseous substrates represent a promising avenue for CO₂ conversion
- Engineering for alternative products and evaluation of engineered microbes

Quad Chart Overview

Timeline

- 10/01/2020 through 9/30/2023

	FY20	Active Project
DOE Funding	(10/01/2020 – 9/30/2023)	\$325,000 FY21

Barriers addressed

Ct-H – C1 Fermentation Development

- Unique challenges that must be overcome for gaseous feedstock such as continuous mode of operation and bioreactor configurations.

Ct-L – Advanced Bioprocess Development

- Develop robust organism via metabolic engineering to increase rate, titer, yield.

Project Goal

Evaluate Waste Carbon gas sources as a feedstock for genetically engineered microbial upgrading to novel products via non-photosynthetic mechanism.

End of Project Milestone

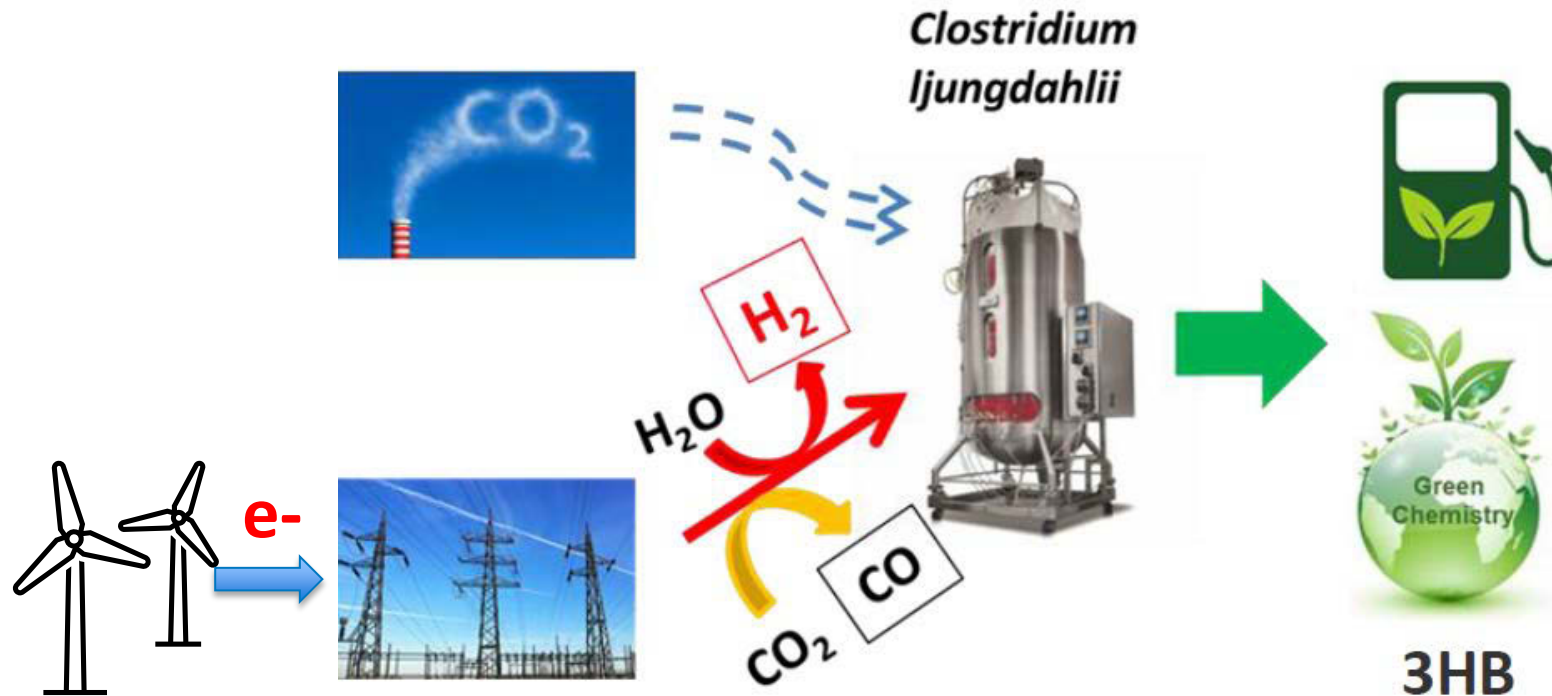
Production of high value product from CO and CO₂ containing waste gas, with a titer of 5 g/L in a ≥5L fermentation system.

Funding Mechanism

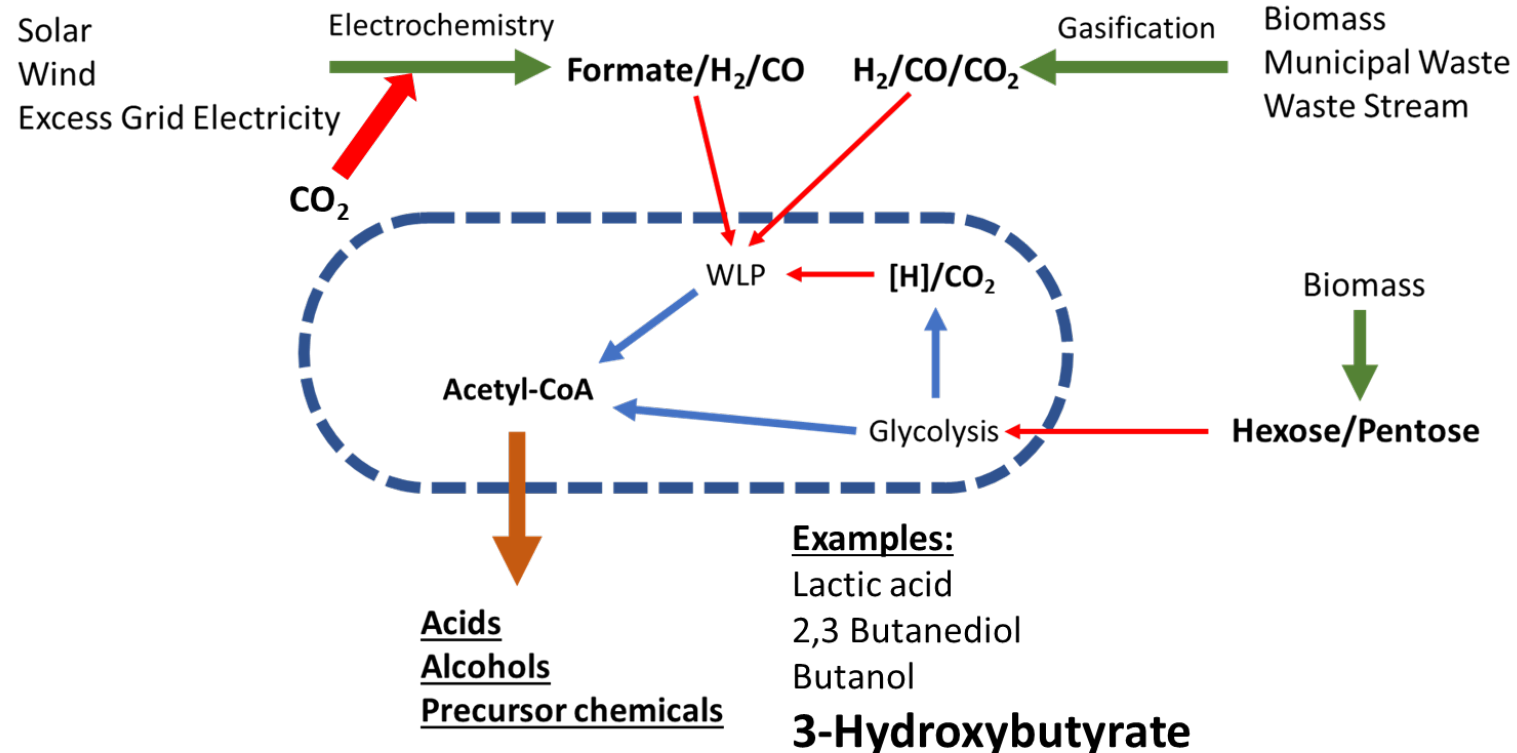
Funded through BETO Conversion 2020 Lab call renewal

Project Overview

- Valorize waste CO₂ to C-C bonds for seasonable or long-term storage
- Leverage low-cost renewable electrons from wind and/or solar PV
- Engineer *Clostridium ljungdahlii* to produce 3-hydroxybutyrate (3HB), a building block in the carboxylate platform chemicals and bioplastic monomer.
- Synergistic with the BETO Waste-to-Energy (WTE) platform for CO₂ upgrade



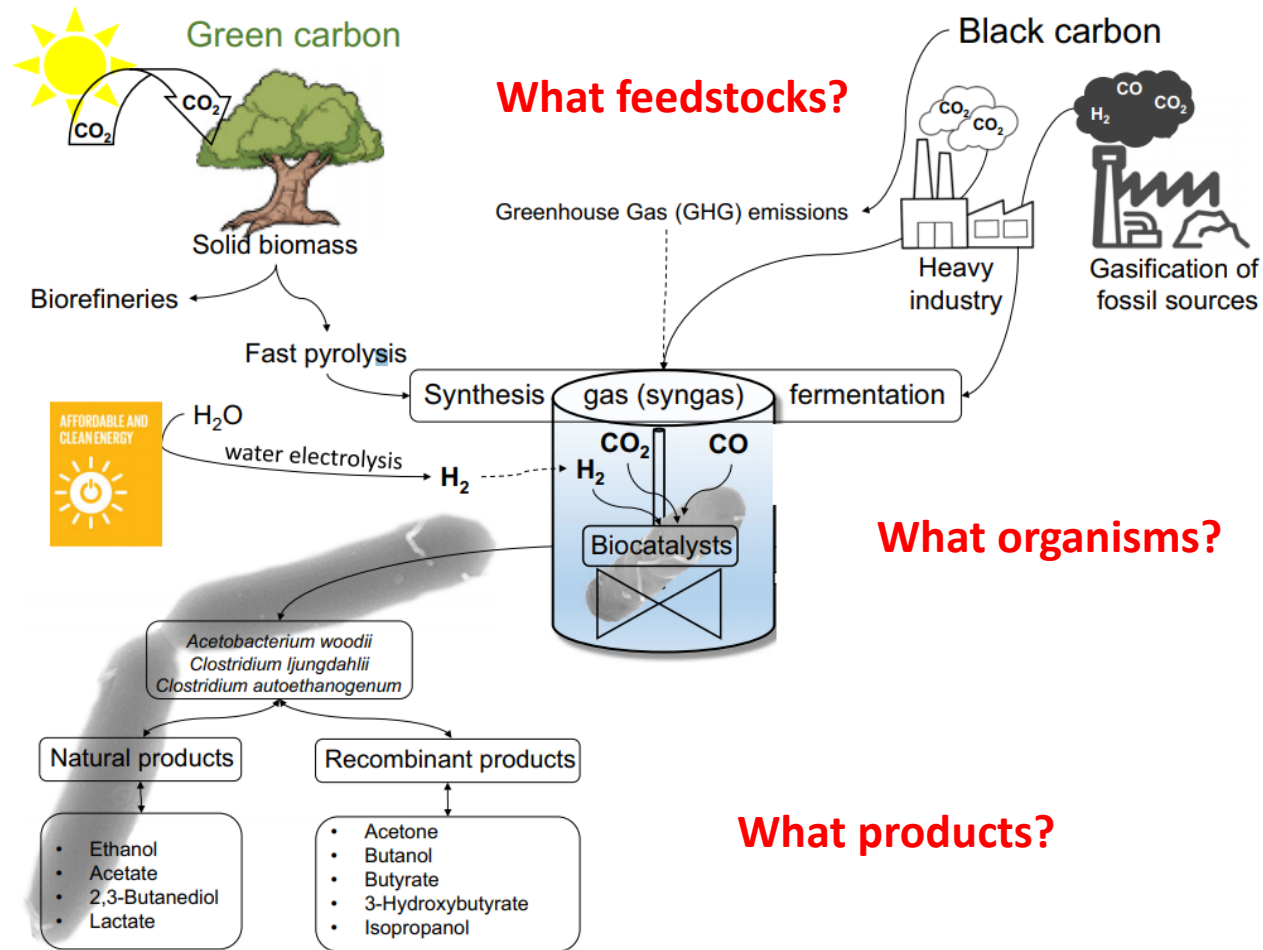
Project Overview



- Waste carbon from gas represents a diverse and plentiful set of feedstocks
- This waste gas can be converted to syngas, a mixture of CO/H₂/CO₂
- Syngas can be microbially upgraded using anaerobic microbes called acetogens
- Acetogens use the Wood-Ljungdahl Pathway (WLP) to fix CO₂ to acetyl-CoA
- Acetyl-CoA can be biologically converted to 3-hydroxybutyrate, a C4 fuel & plastic precursor

Management

Process idea



Process Development

Princeton – Electrochemical CO₂ reduction
NREL- Gas fermentation Lauren Magnusson

C1 Organisms

NREL- Microbiologist Jonathan Lo

Products

NREL- TEA/LCA analysis Ling Tao

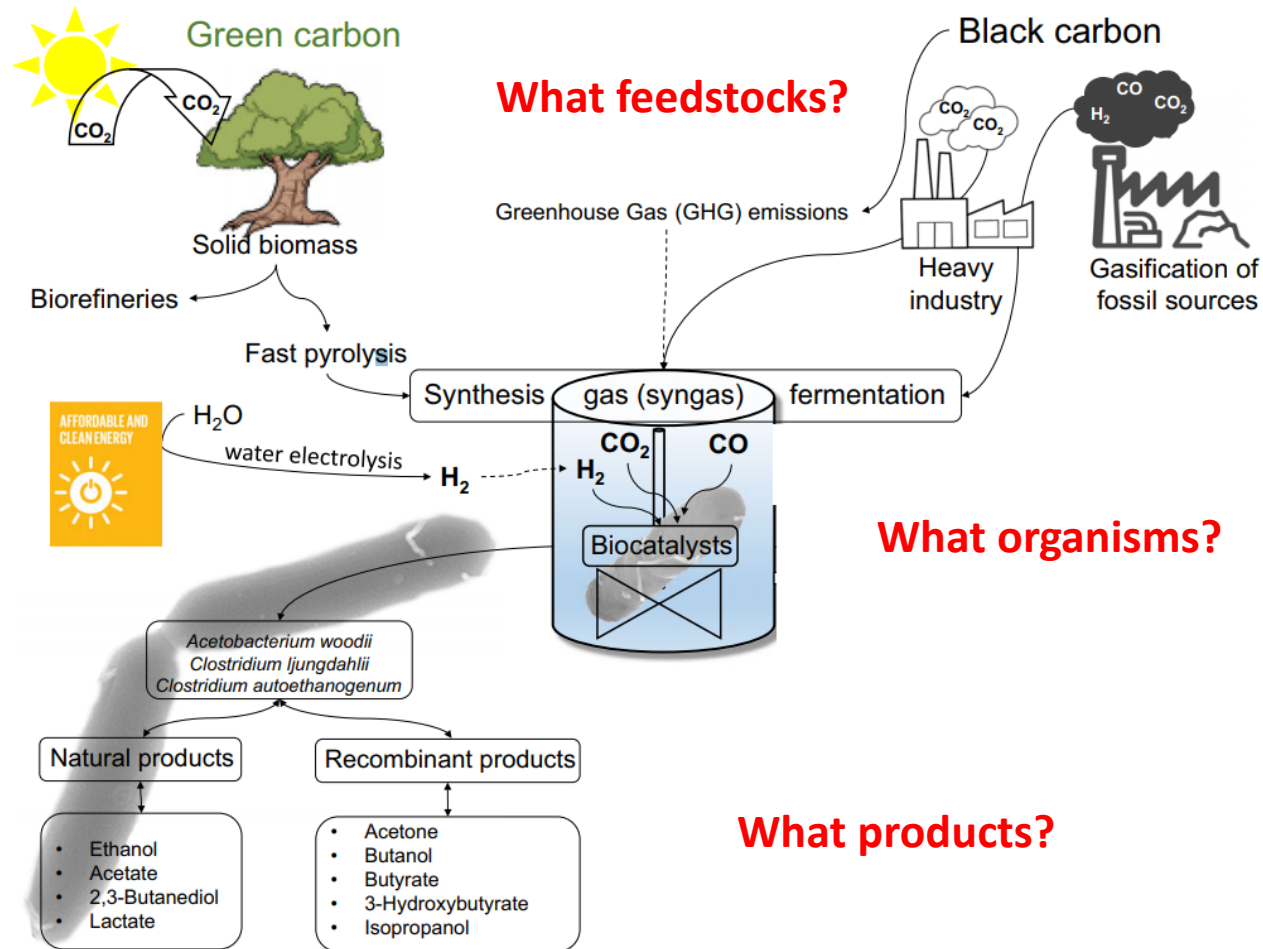
Related Industry Contacts

Royal Dutch Shell C1 gas conversion

Adapted from: Gas fermentation for commodity chemicals and fuels
doi: [10.1111/1751-7915.12763](https://doi.org/10.1111/1751-7915.12763)

Management

Process idea



Adapted from: Gas fermentation for commodity chemicals and fuels
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Gas Feedstocks

How are they generated?
What is their composition? (H₂/CO/CO₂)
At what prices?
How does that affect carbon efficiency?

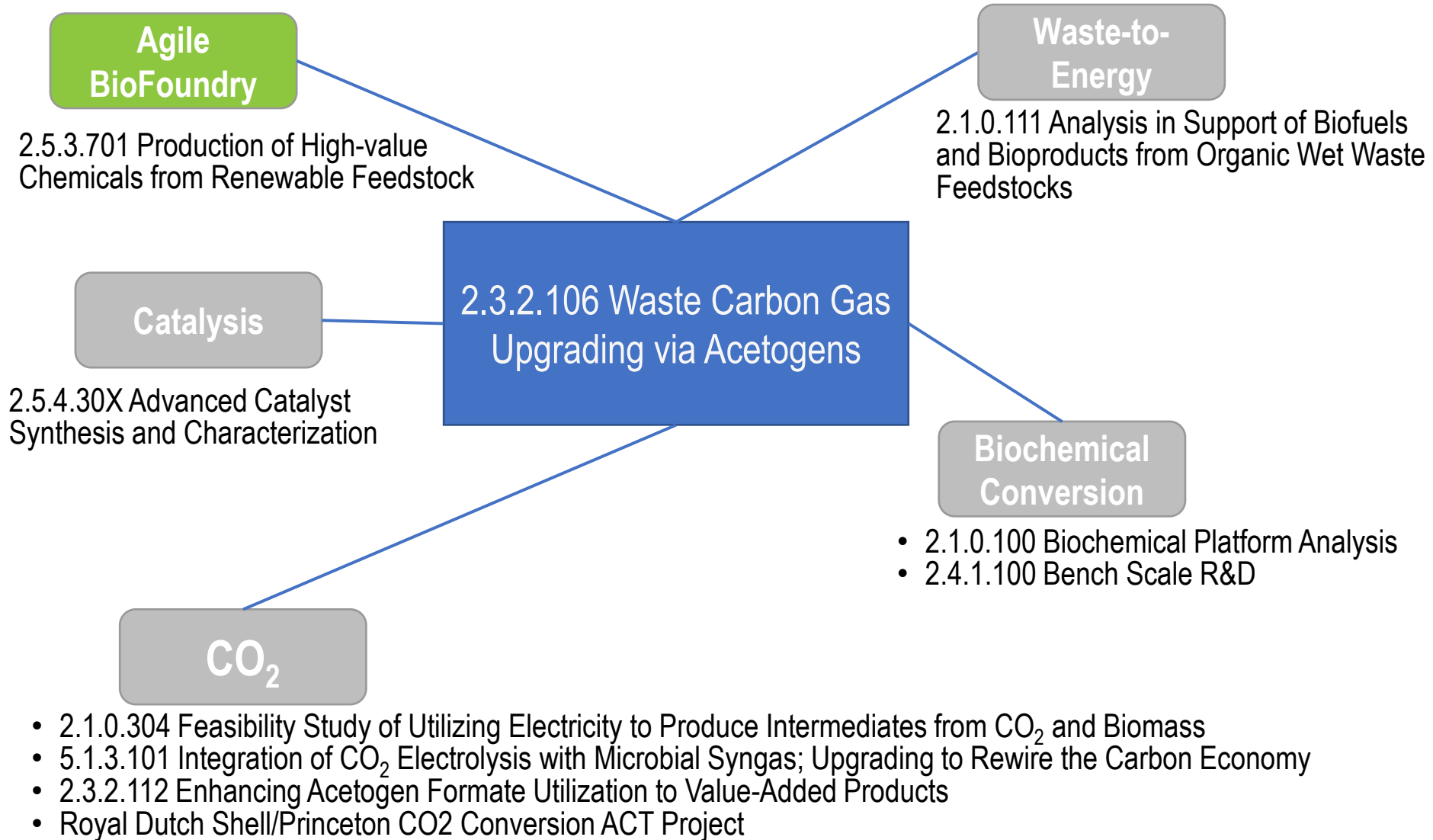
Microbial Syngas Conversion

Does syngas composition effect growth?
Products?
How much is known about them?
Can they be genetically engineered towards our products of interest?

Scaling Gas Fermentations

Strain stability?
Performance at larger scale?
Mass transfer of syngas?
Separations?
Carbon and electron efficiency?
Yield/rate/titer?

Management



Approach – Feedstocks and products

- Direct CO₂ conversion to C1 chemicals has a high technology readiness level, but ethanol/acetate have a low market price and limited market size.
- C1 sources have a diversity of potential feedstocks with different characteristics
 - CO₂ from ethanol plants (50 million tons) Colocalized to Wind Energy
 - Methane from livestock (4 millions tons) Methane → syngas
 - Iron and Steel (75 million tons) Potential CO/H₂ in stream
- Developing CO₂ microbial conversion to valuable products
 - 3 Hydroxybutyrate as a biodegradable plastic
 - Carboxylates/alcohols as transportation fuel replacements

Approach

- What is the source of gas? Cost and yield?

TEA/LCA analysis

Feedstock size

- How well can microbes be engineered for conversion?

Growth Characterization

Metabolic Engineering

- How can gas fermentations be scaled?

Bioreactor studies

Improve syngas fermentation

Gas Feedstocks

How are they generated?

What is their composition? ($H_2/CO/CO_2$)

At what prices?

How does that affect carbon efficiency?

Microbial Syngas Conversion

Does syngas composition effect growth?

Products?

How much is known about them?

Can they be genetically engineered towards our products of interest?

Scaling Gas Fermentations

Strain stability?

Performance at larger scale?

Mass transfer of syngas?

Separations?

Carbon and electron efficiency?

Yield/rate/titer?

Approach - Milestones

- Fermentation performance of WT under three different syngas concentrations. (Q1)
- Compare fermentation metrics with engineered strains and understand bottlenecks (Q2). Work with industrial partners and NREL SEAC Teams to determine near term available feedstock streams.
- Omic analysis of the WT and engineered strains to determine gene candidates for strain improvements (Q3)

End of project goal/milestones (9/2023)

- Production of high value product from CO and CO₂ containing waste gas, with a titer of 5 g/L in a ≥5L fermentation system.

Go/No-Go (3/2022)

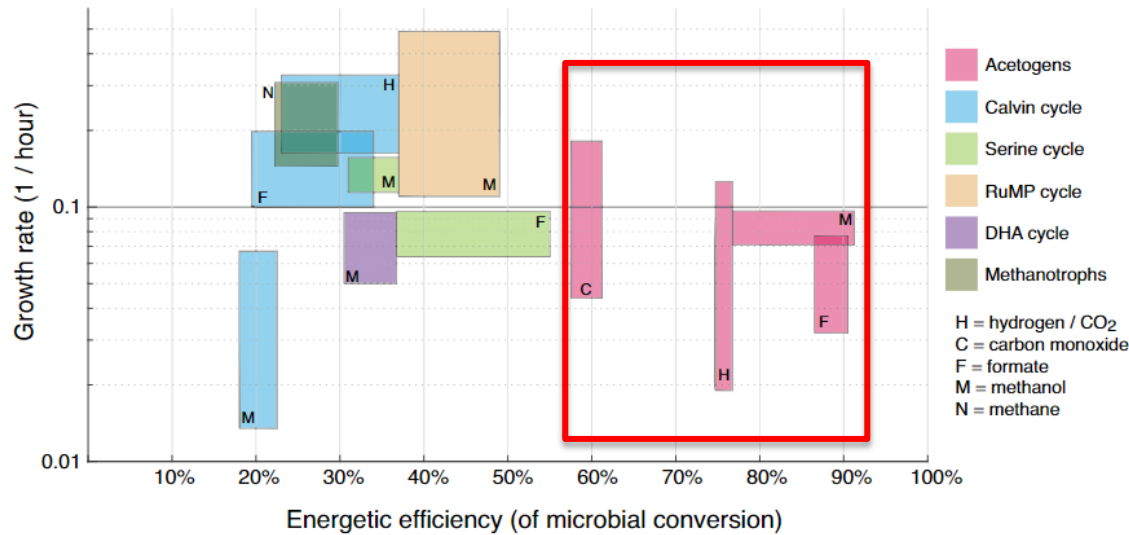
- Obtain a productivity of at least 0.1 g/L/hr from H₂/CO/CO₂ with a genetically engineered acetogen expressing butanol/3-Hydroxybutyrate pathway

Approach: Acetogens have highest biological efficiency for CO₂ fixation

Renewable methanol and formate as microbial feedstocks

Charles AR Cotton¹, Nico J Claassens¹, Sara Benito-Vaquerizo¹ and Arren Bar-Even

Current Opinion in Biotechnology 2020, 62:168–180

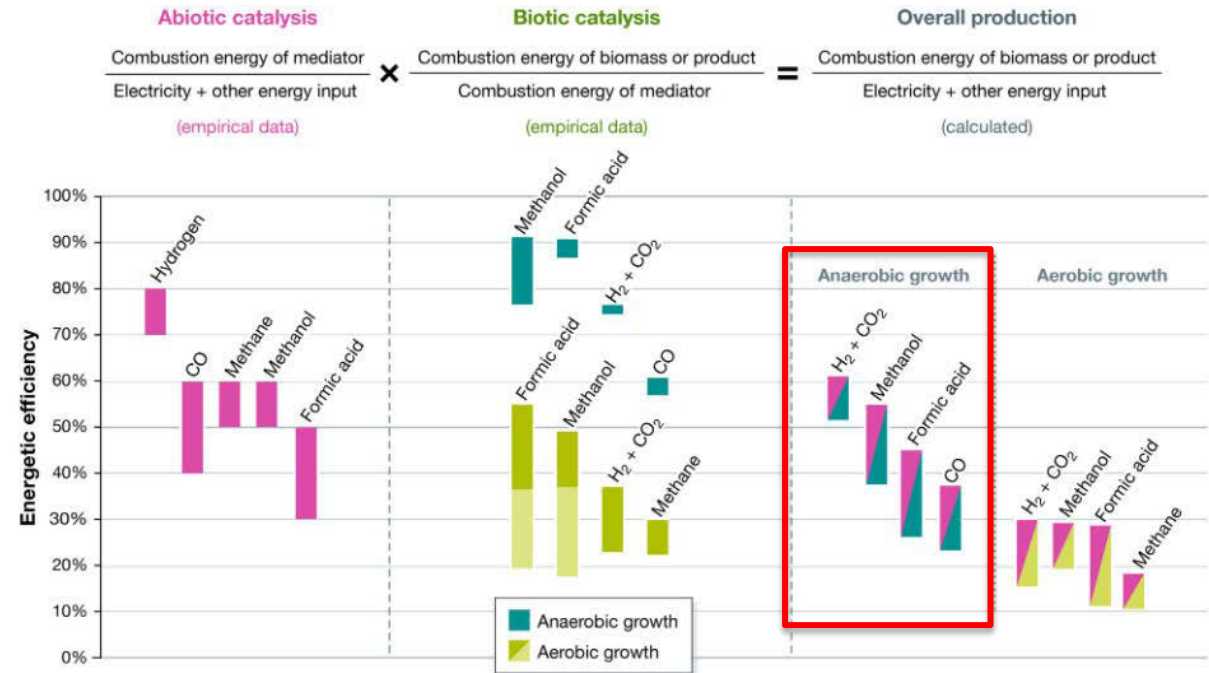


Current Opinion in Biotechnology

A one-carbon path for fixing CO₂

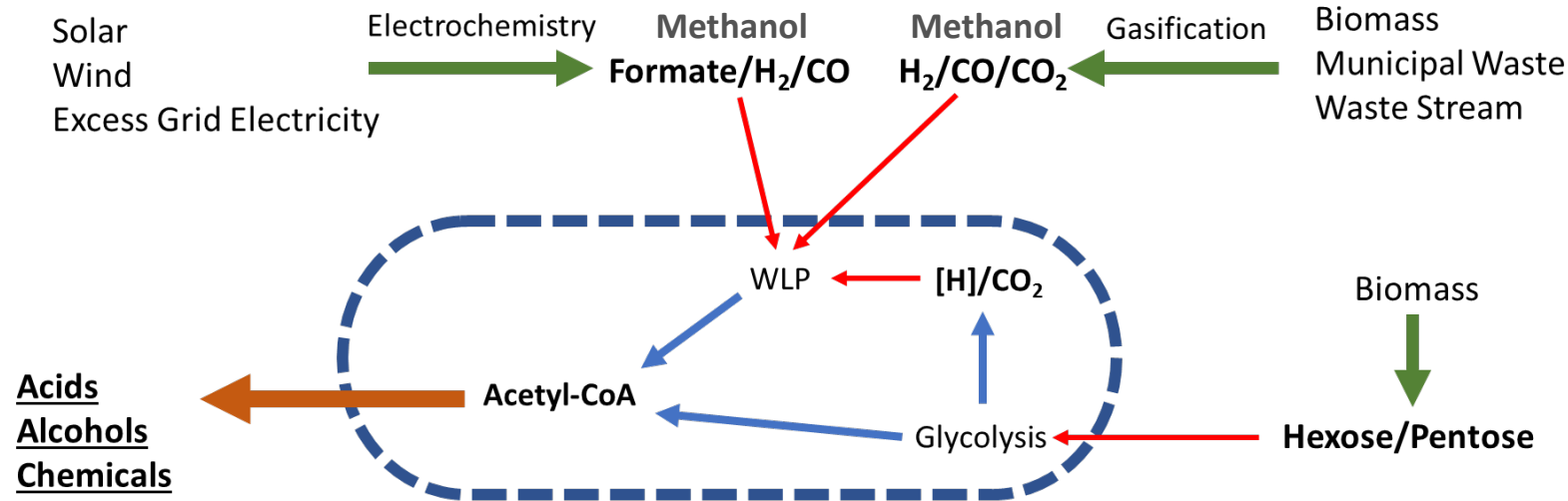
Ari Satanowski, Arren Bar-Even  

EMBO Rep (2020)21:e50273



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Approach: Acetogens for CO₂ fixation

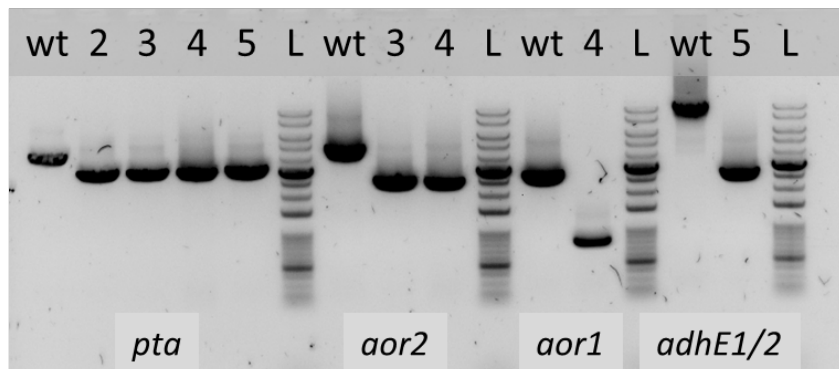
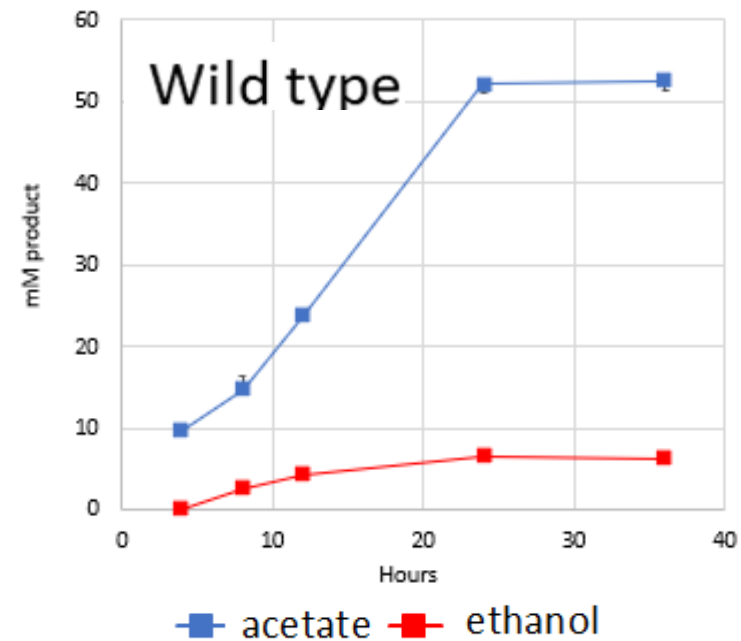
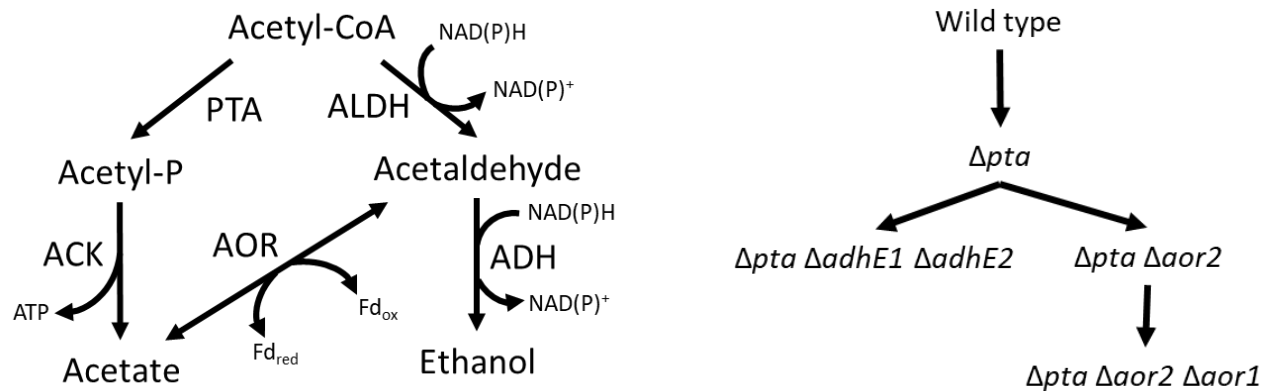


Acetogens **non-photosynthetically, anaerobically** fix CO₂

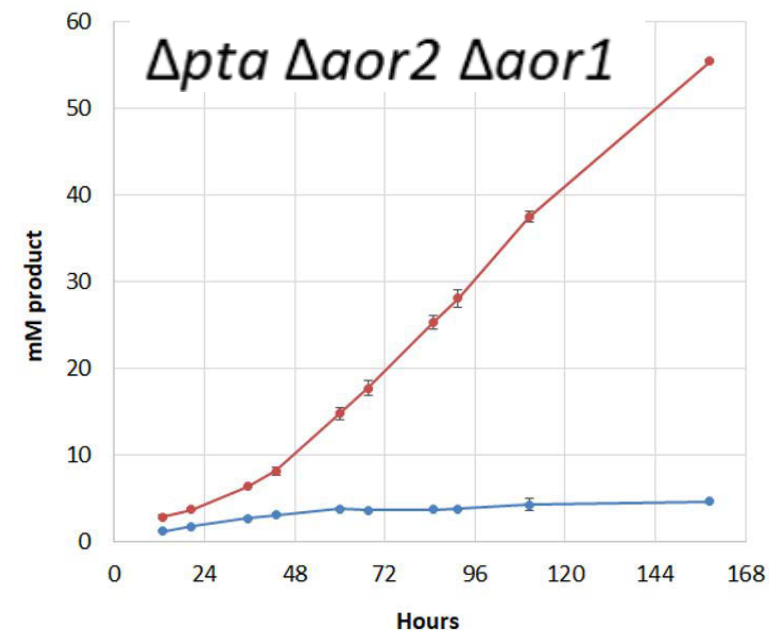
- Use Wood Ljungdahl Pathway (WLP), most efficient for CO₂ fixation
- Investigated for syngas conversion, but can use liquid C1 formate and methanol
- Can simultaneously use gases, liquids, and biomass related sugars
- Produce interesting products at high carbon and electron efficiency
- Focus on C4 product 3-hydroxybutyrate due to potential as bioplastic PHB and fuel precursor

Progress and Outcomes

Organism development



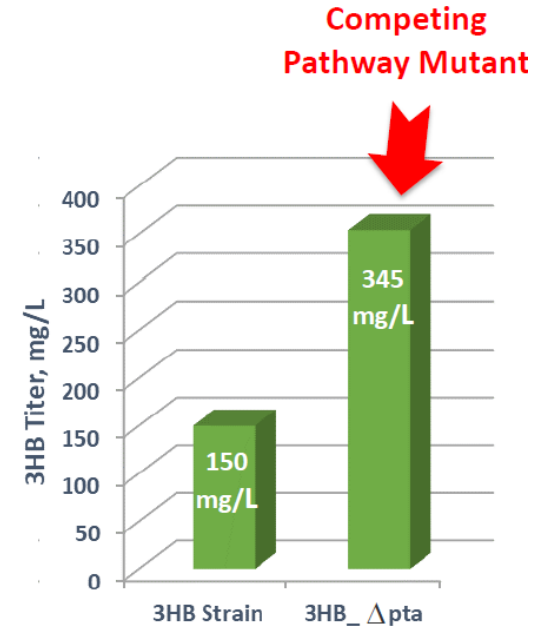
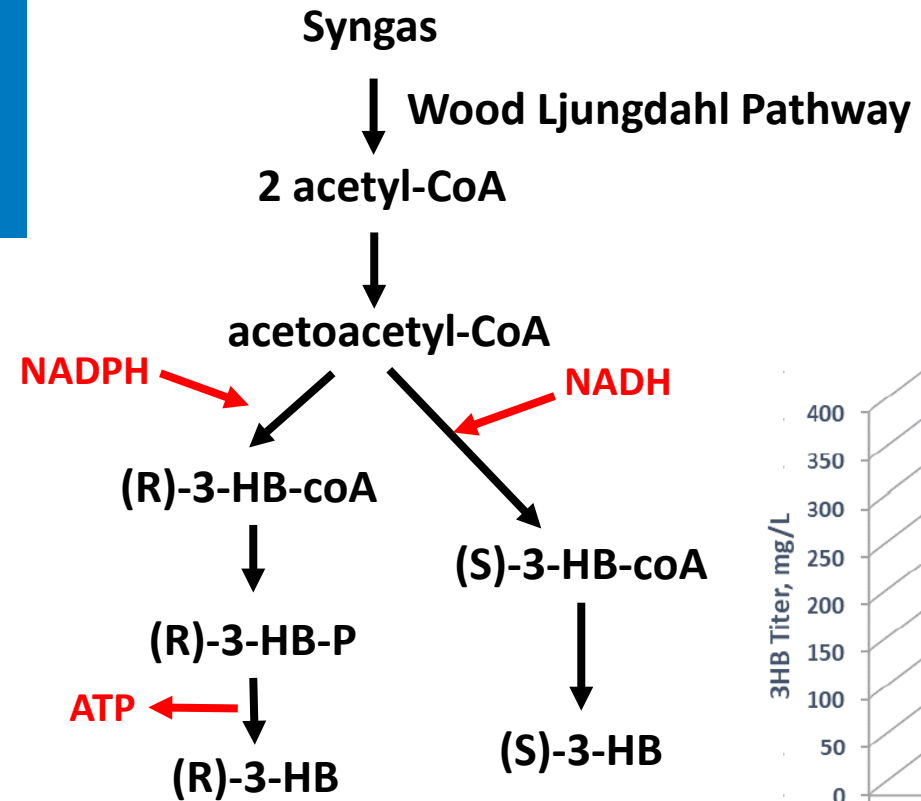
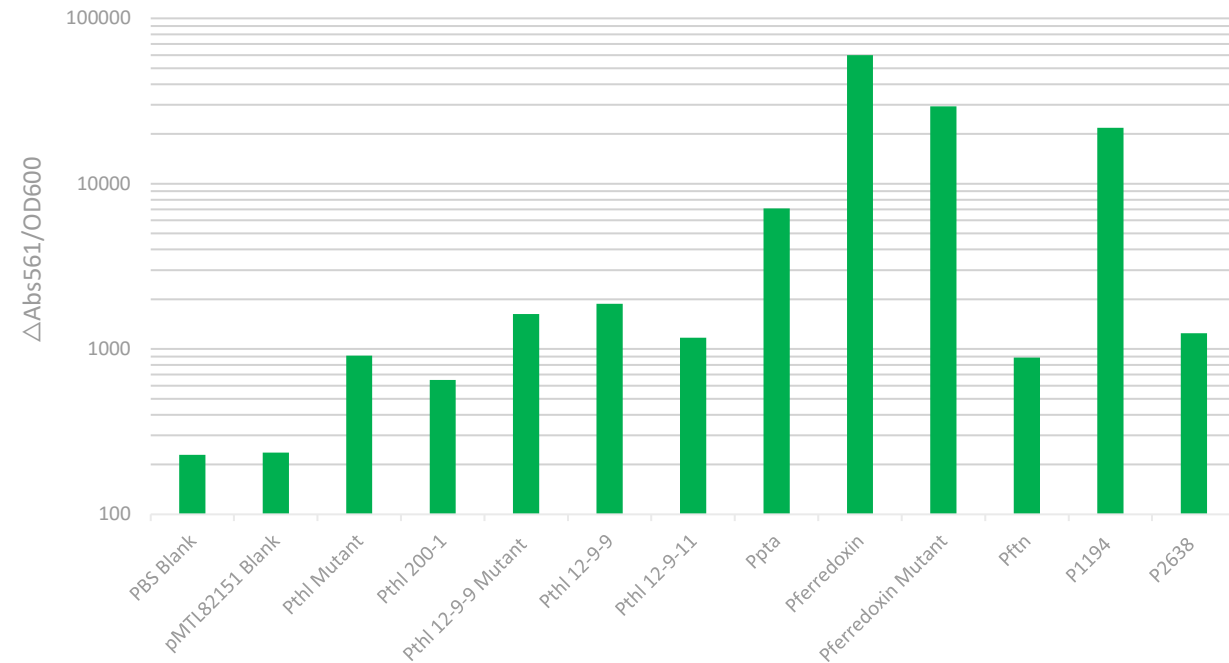
C. ljungdahlii metabolism and CRISPR/Cas9 engineering



Progress and Outcomes

Organism development

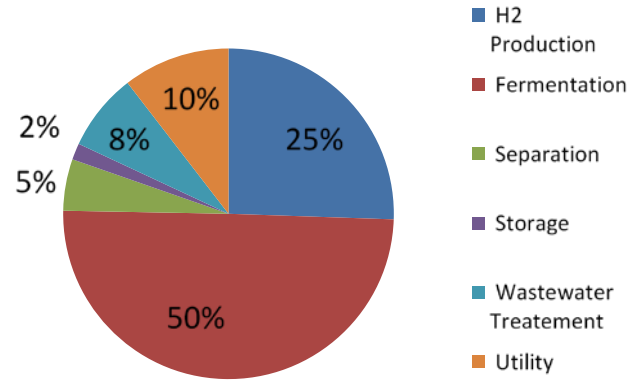
Adam Guss, ORNL



- Characterizing promoters to drive 3HB expression
- Testing different metabolic pathways to make 3HB
- Combining 3HB pathways with *C. ljungdahlii* deletion variants yields more 3HB

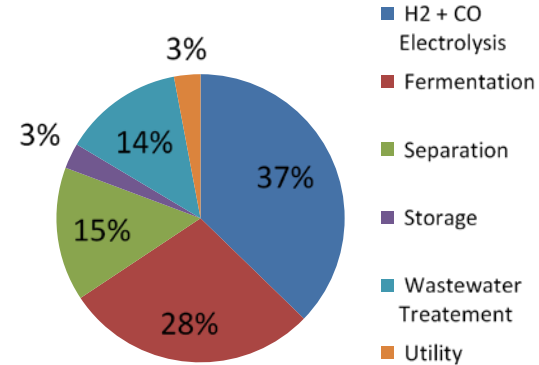
Progress and Outcomes

TechnoEconomic Analysis



* MSP=\$1.63/kg 3HB or \$8.92/GGE

~\$2.50/kg market 3HB price



* MSP= \$1.66/kg 3HB or \$9.09/GGE

Cost Distributions of CO₂-to-3HB (left) and CO-to-3HB (right).

2 Cases modeled:

CO₂ with electrolyzed H₂ to 3-HB

Electrochemical CO₂ reduction to CO to 3-HB

- Costs are driven by 2 factors:
 - Electrochemistry: electricity price
 - Gas fermentation: productivity and setup
- Improving productivity is paramount to reducing costs
 - CO fermentations are much faster

NREL - Ling Tao

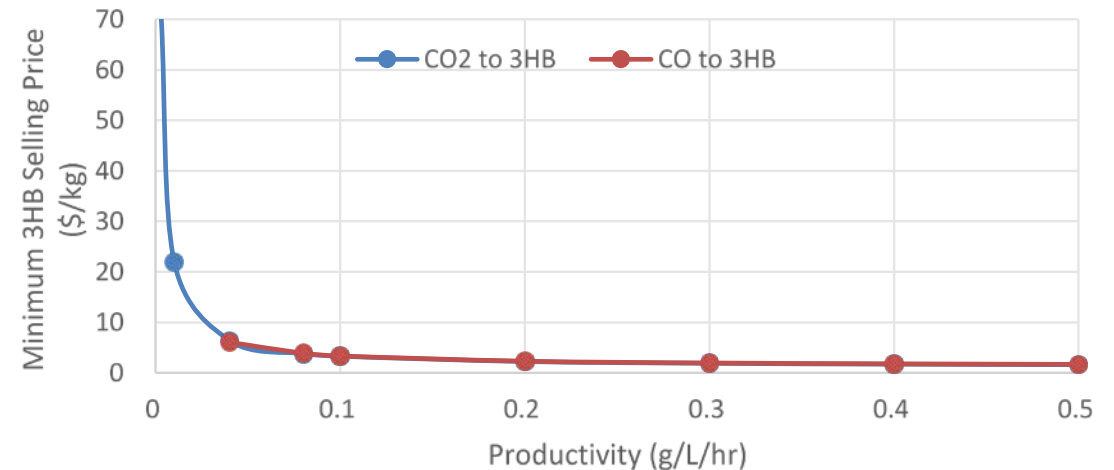


Figure 1. Minimum 0.1 g/L/hr is needed for both CO₂-to-3HB and CO-to-3HB cases.

Progress and Outcomes

TechnoEconomic Analysis

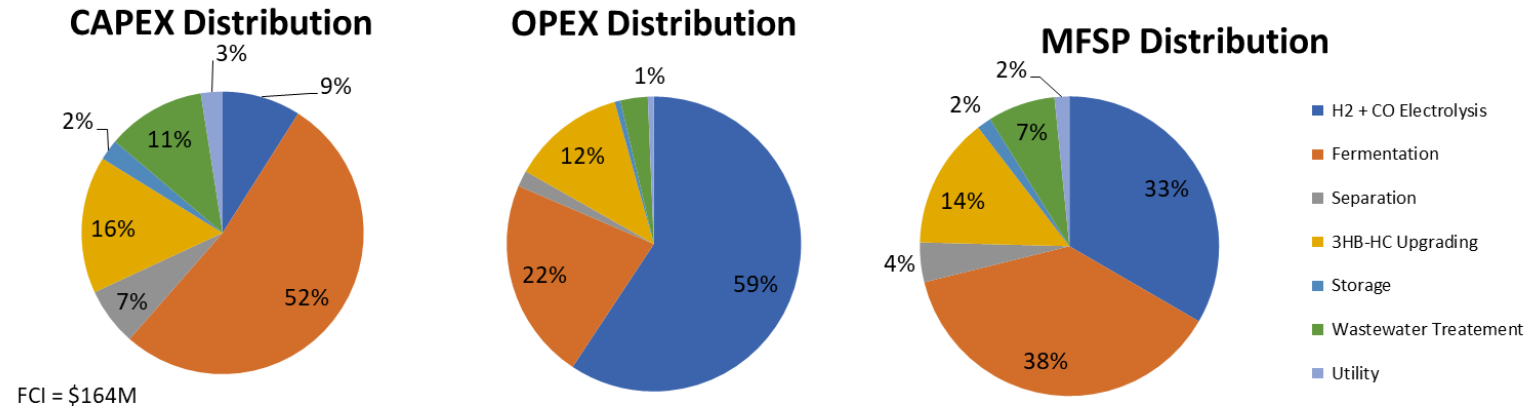


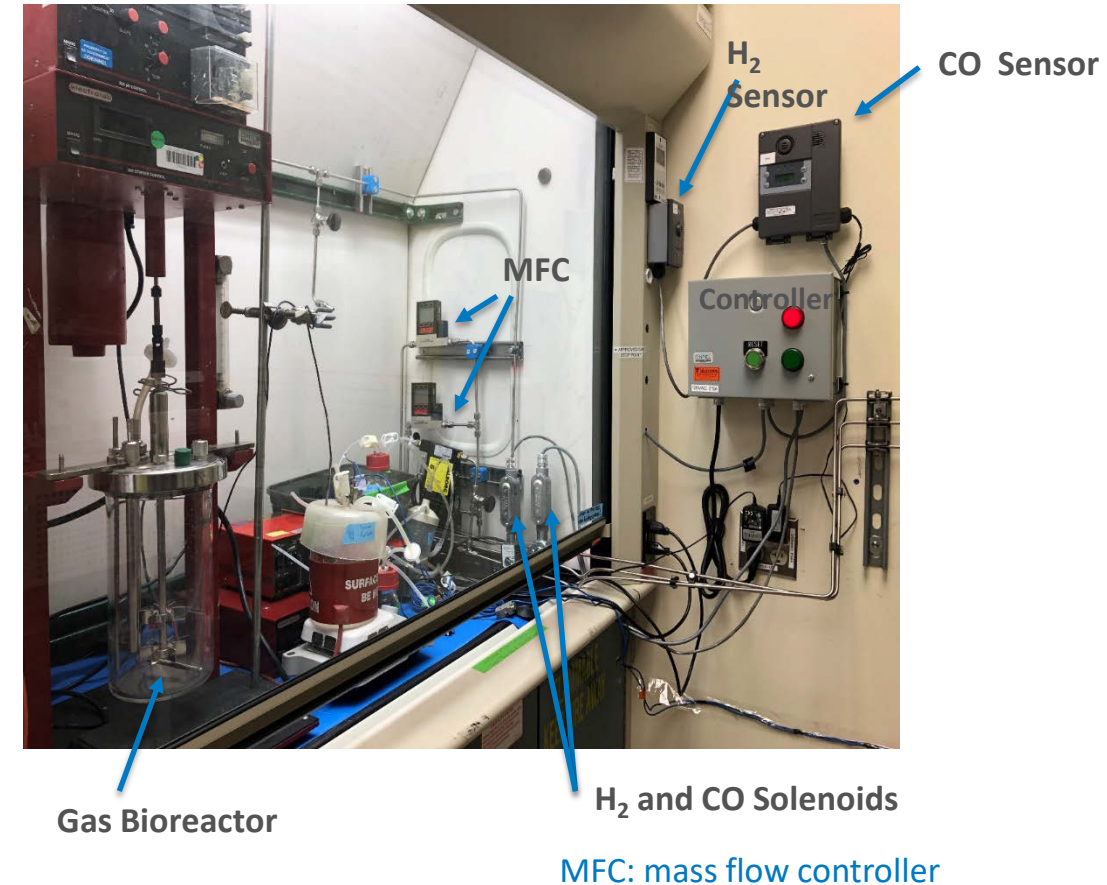
Figure 3. Preliminary MFSP distribution of processing areas including hydrogen, biological conversion, 3HB purification, upgrading and outside battery limit facilities.

- CAPEX is driven by gas fermentation investment
- OPEX is driven by electrochemistry (electricity price)
- Upgrading 3-HB to 3 carbon HC fuel drops efficiency, loses CO₂, making less viable fuel
- 3-HB is a monomer for biodegradable plastic, ~\$2.50/kg market

Progress and Outcomes

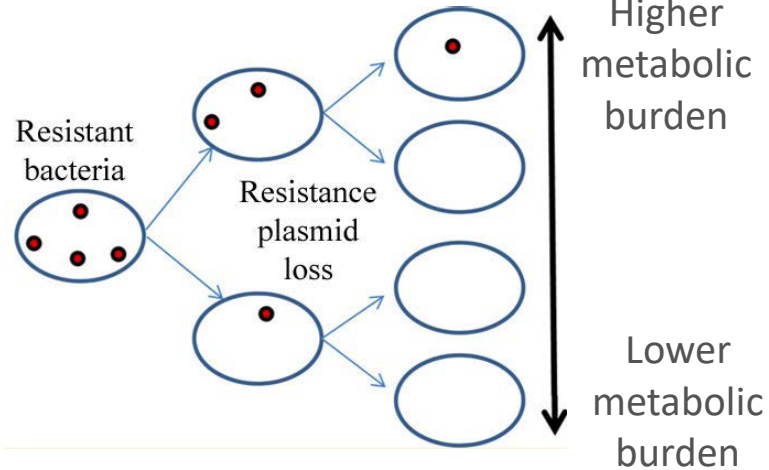
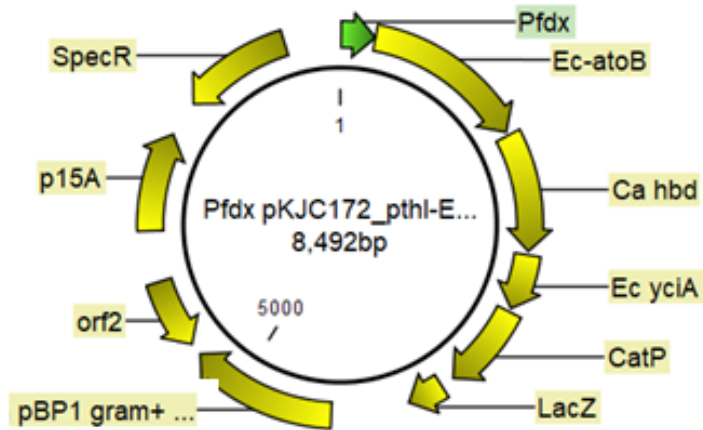
Process development

- Bioreactor gas scale up
 - Dynamic H₂/CO₂/CO control
 - Can run gas fermentations at ≤2L
 - Different gas diffusion strategies
 - Numerous safety controls
 - Improving yield/rate/titer
 - Capturing metrics for further TEA/LCA analysis



Progress and Outcomes

Organism scaling



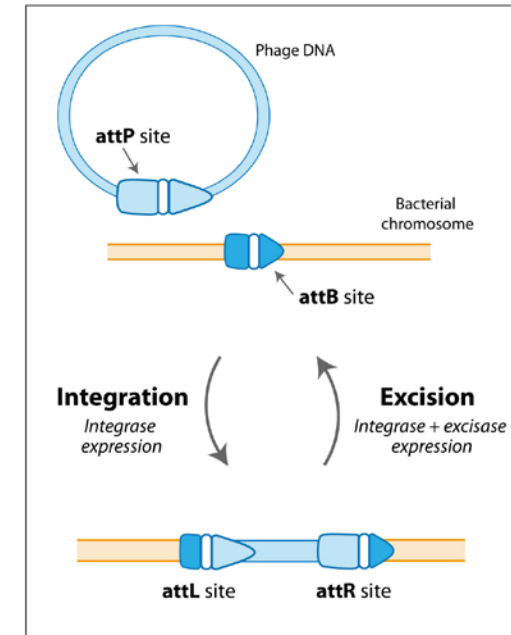
100% CO₂, 3HB (mg/L)

Volume	1 mL	10 mL	100mL
Titer	220	52	18

Plasmid loss due to metabolic burden

Reduces 3HB titer as volume increases

Pathway insertion into genome



Two strategies:

Phage attB integrase multiple sites

Homologous recombination

Impact –Data and Dissemination

- Project with Royal Dutch Shell/Princeton for C1 CO₂ acetogenic conversion
- Publications around microbial C1 conversion
 - Genetic engineering, metabolism, and novel strains
 - Describing new techniques and new tools
 - Insights into engineering/metabolism
 - Distributed new strains to interested parties
 - Metrics regarding C1 fermentation
 - Yield/rate/titer, scaling gas fermentation, gas mixes
 - TechnoEconomic and Life Cycle Analysis

The Metabolism of *Clostridium ljungdahlii* in Phosphotransacetylase Negative Strains and Development of an Ethanologenic Strain

Jonathan Lo^{1*}, Jonathan R. Humphreys¹, Joshua Jack², Chris Urban¹, Lauren Magnusson¹, Wei Xiong¹, Yang Gu³, Zhiyong Jason Ren² and Pin-Ching Maness¹

¹ National Renewable Energy Laboratory, Golden, CO, United States, ² Andlinger Center for Energy and Environment, Princeton University, Princeton, NJ, United States, ³ Key Laboratory of Synthetic Biology, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China

High rate CO₂ valorization to organics via CO mediated silica nanoparticle enhanced fermentation

Joshua Jack ^{a, b}, Jonathan Lo ^b, Bryon Donohue ^b, Pin-Ching Maness ^b, Zhiyong Jason Ren ^{a, c, d}

Directing *Clostridium ljungdahlii* fermentation products via hydrogen to carbon monoxide ratio in syngas





Joshua Jack ^{a, b, d}, Jonathan Lo ^{b, d}, Pin-Ching Maness ^{b, d}, Zhiyong Jason Ren ^{a, c, d}

Impact – Future Work






- **Proposals to further develop process, explore variations, outside partner collaboration**
- Acetogenic metabolism engineering and fundamental understanding
- Increasing knowledge of scaling gas fermentation
 - Strain stability
 - Improving mass transfer
 - Studying gas mixes
- Other implementations of acetogenic microorganisms for CO₂ efficiency
 - C1 liquids and gases, co-utilization
 - Mixotrophy (sugar + C1 gases)
 - Co-cultures
 - Expanded products and increased carbon efficiency

Summary




Product

-  [Redacted]
-  [Redacted]
-  [Redacted]
-  [Redacted]




Feedstock

-  [Redacted]
-  [Redacted]
-  Sustainable waste management
-  Expanding availability of green H₂
-  Closing the carbon cycle

Capital

-  [Redacted]
-  [Redacted]
-  [Redacted]

Social Responsibility

-  Carbon intensity reduction
-  [Redacted]
-  [Redacted]

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

Value Proposition

- Waste Carbon Gas is a diverse and plentiful feedstock for microbial upgrading
- Syngas microbial upgrading is still a new process with limited product diversity and value

Key Accomplishments

- Microbial understanding and tool development
- Engineered production of 3HB from CO
- TEA analysis for understanding process
- Filling in knowledge gaps and disseminating knowledge among academic and industry institutions

NREL

- Lauren Magnusson
- Jonathan Humphreys
- Yi Pei Chen
- Wei Xiong
- Ling Tao
- Pin Ching Maness

Thank You

www.nrel.gov

NREL/PR-2700-79320

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Program 2.3.2.106

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Responses to Previous Reviewers' Comments

TEA should be very valuable to direct the performers to the market size of 3HB, and what the necessary titer/productivities should be in order to commercialize.

Response: The preliminary TEA has revealed a 3HB minimum selling price of \$1.90/kg, provided a titer of 10 g/L and a productivity of 0.2 g/L/h can be achieved. This can set research targets. A minimum selling price for coproducts like acetate and 2,3- butanediol are \$1.80 and \$2.20/kg, respectively. The major cost drivers are H₂ and CO₂ feedstock cost and capital expenditure of biological conversion. The former can be addressed through improving the production yield to near theoretical maximums and the latter through increasing productivity and product titers. These findings guide R&D efforts carried out by the project team.

Publications, Patents, Presentations, Awards, and Commercialization

Lo, Jonathan, Jonathan R. Humphreys, Joshua Jack, Chris Urban, Lauren Magnusson, Wei Xiong, Yang Gu, Zhiyong Jason Ren, and Pin-Ching Maness. “The Metabolism of *Clostridium Ljungdahlii* in Phosphotransacetylase Negative Strains and Development of an Ethanologenic Strain.” *Frontiers in Bioengineering and Biotechnology* 8 (2020).

<https://doi.org/10.3389/fbioe.2020.560726>.

Jack, Joshua, Jonathan Lo, Bryon Donohue, Pin-Ching Maness, and Zhiyong Jason Ren. “High Rate CO₂ Valorization to Organics via CO Mediated Silica Nanoparticle Enhanced Fermentation.” *Applied Energy* 279 (December 1, 2020): 115725.

<https://doi.org/10.1016/j.apenergy.2020.115725>.

Jack, Joshua, Jonathan Lo, Pin-Ching Maness, and Zhiyong Jason Ren. “Directing *Clostridium Ljungdahlii* Fermentation Products via Hydrogen to Carbon Monoxide Ratio in Syngas.” *Biomass and Bioenergy* 124 (May 1, 2019): 95–101.

<https://doi.org/10.1016/j.biombioe.2019.03.011>.