BETO 2021 Peer Review
2.3.2.106
Waste Carbon Gas Upgrading via Acetogens

March 11, 2021

Conversion

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Market Trends

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 CO_2 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

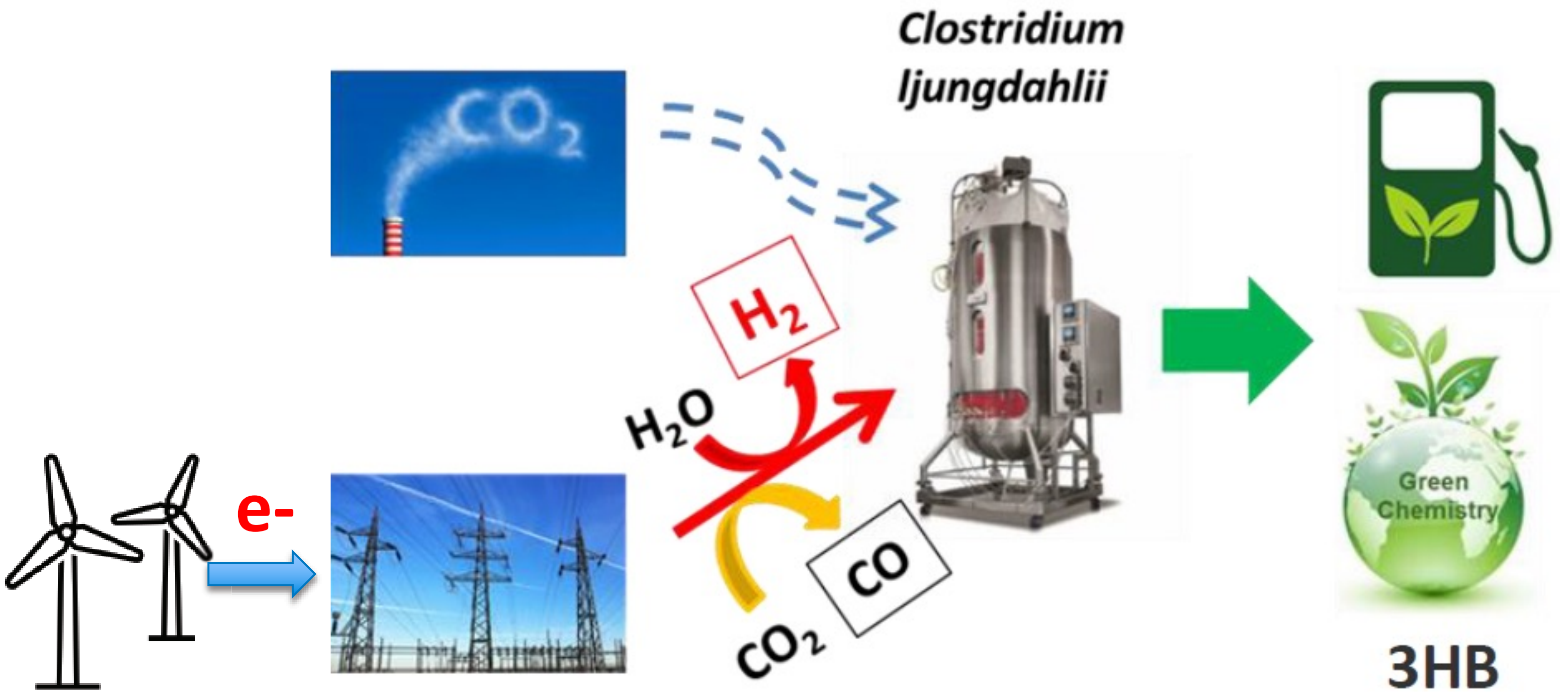
Production of high value product from CO and CO2 containing waste gas, with a titer of 5 g/L in a ≥5L fermentation system.

Funding Mechanism

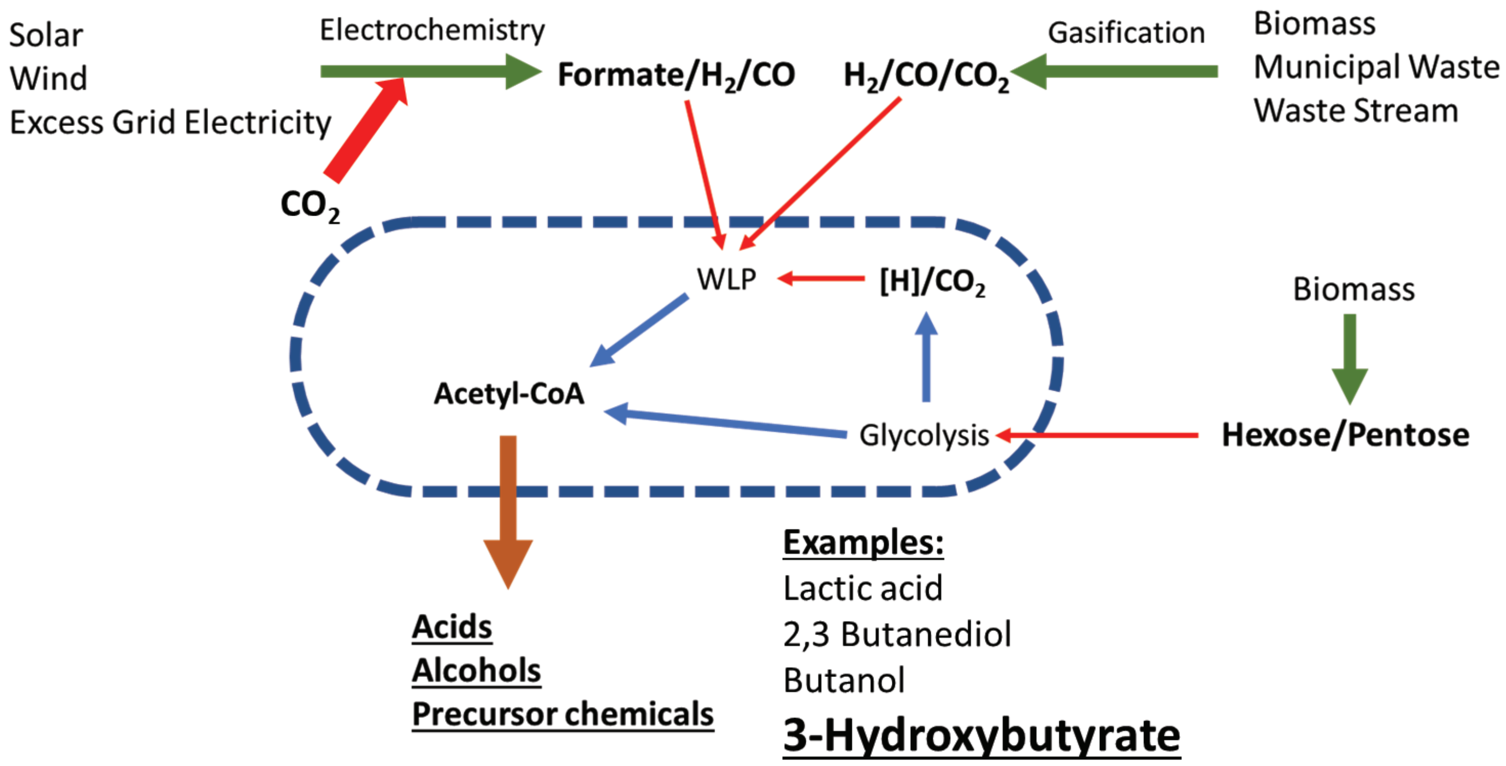
Funded through BETO Conversion 2020 Lab call renewal

Project Overview

- Valorize waste CO_2 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 CO_2 upgrade



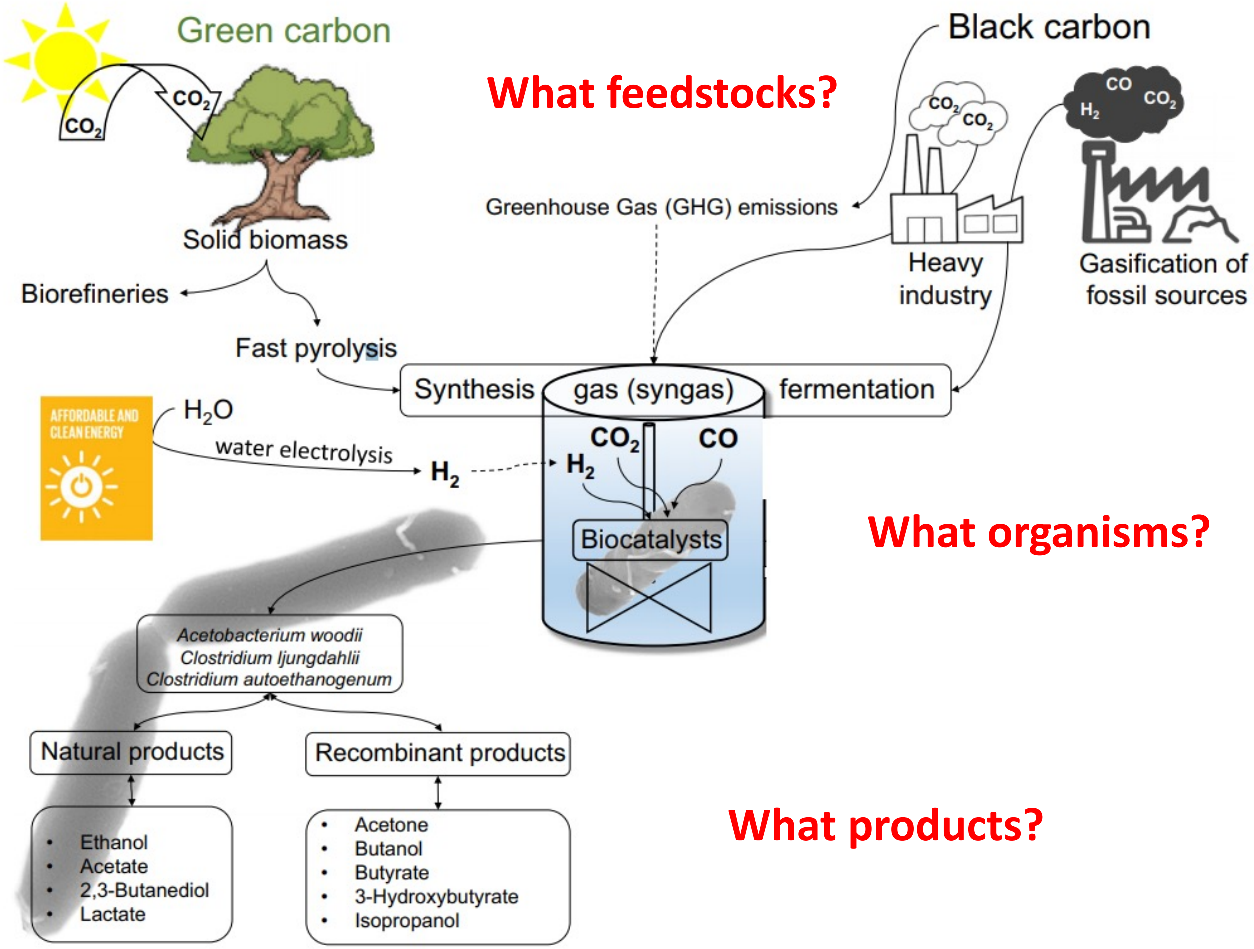
Project Overview



- Waste carbon from gas represents a diverse and plentiful set of feedstocks
- This waste gas can be converted to syngas, a mixture of CO/H_2/CO_2
- Syngas can be microbially upgraded using anaerobic microbes called acetogens
- Acetogens use the Wood-Ljungdahl Pathway (WLP) to fix CO_2 to acetyl-CoA
- Acetyl-CoA can be biologically converted to 3-hydroxybutyrate, a C4 fuel & plastic precursor

Management

Process idea



What feedstocks?

What organisms?

What products?

Adapted from: Gas fermentation for commodity chemicals and fuels
doi: 10.1111/1751-7915.12763

Process Development

Princeton – Electrochemical CO_2 reduction
NREL- Gas fermentation Lauren Magnusson

C1 Organisms

NREL- Microbiologist Jonathan Lo

Products

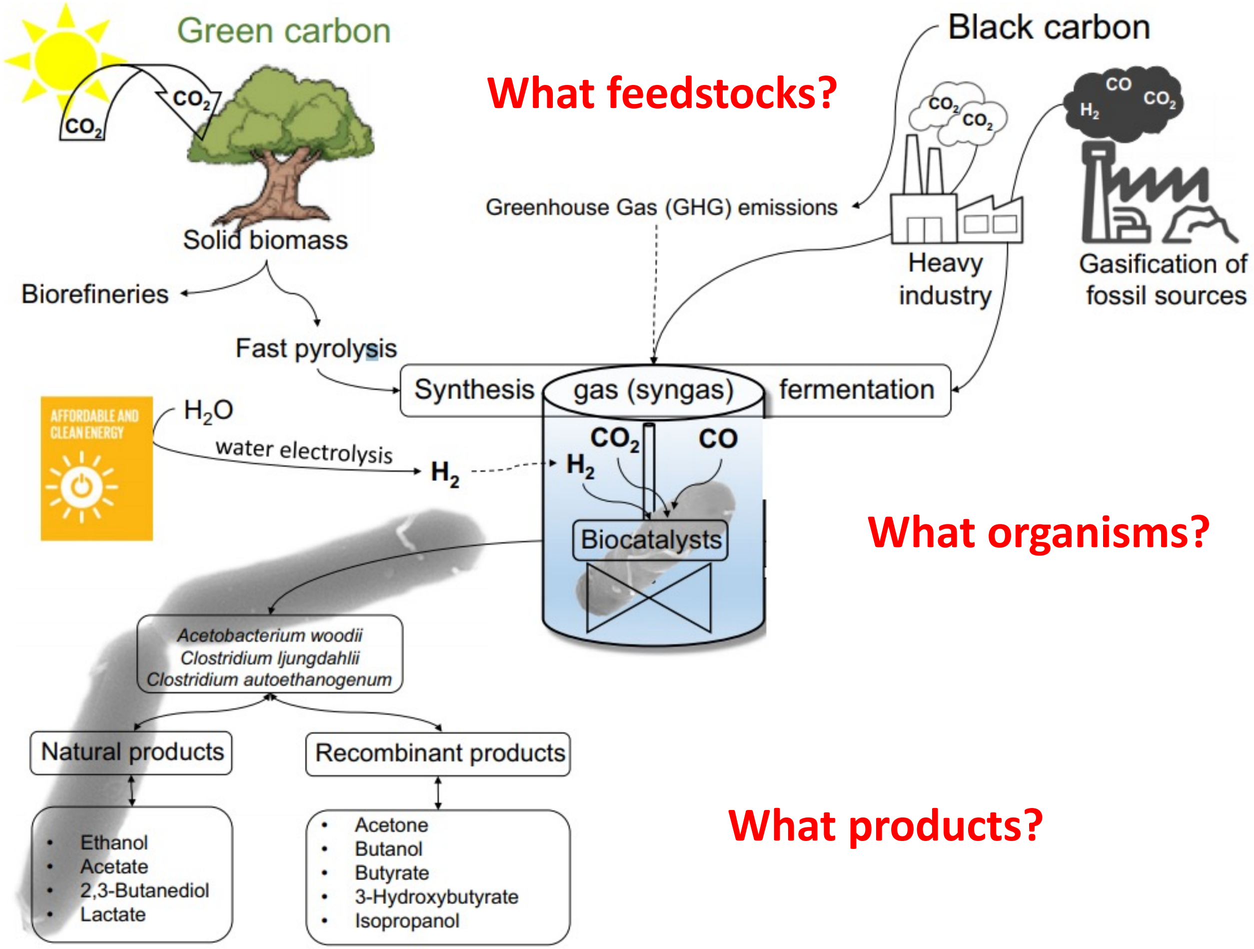
NREL- TEA/LCA analysis Ling Tao

Related Industry Contacts

Royal Dutch Shell C1 gas conversion

Management

Process idea



Adapted from: Gas fermentation for commodity chemicals and fuels
doi: 10.1111/1751-7915.12763

Gas Feedstocks

How are they generated?
What is their composition? (H_2/CO/CO_2)
At what prices?
How does that affect carbon efficiency?

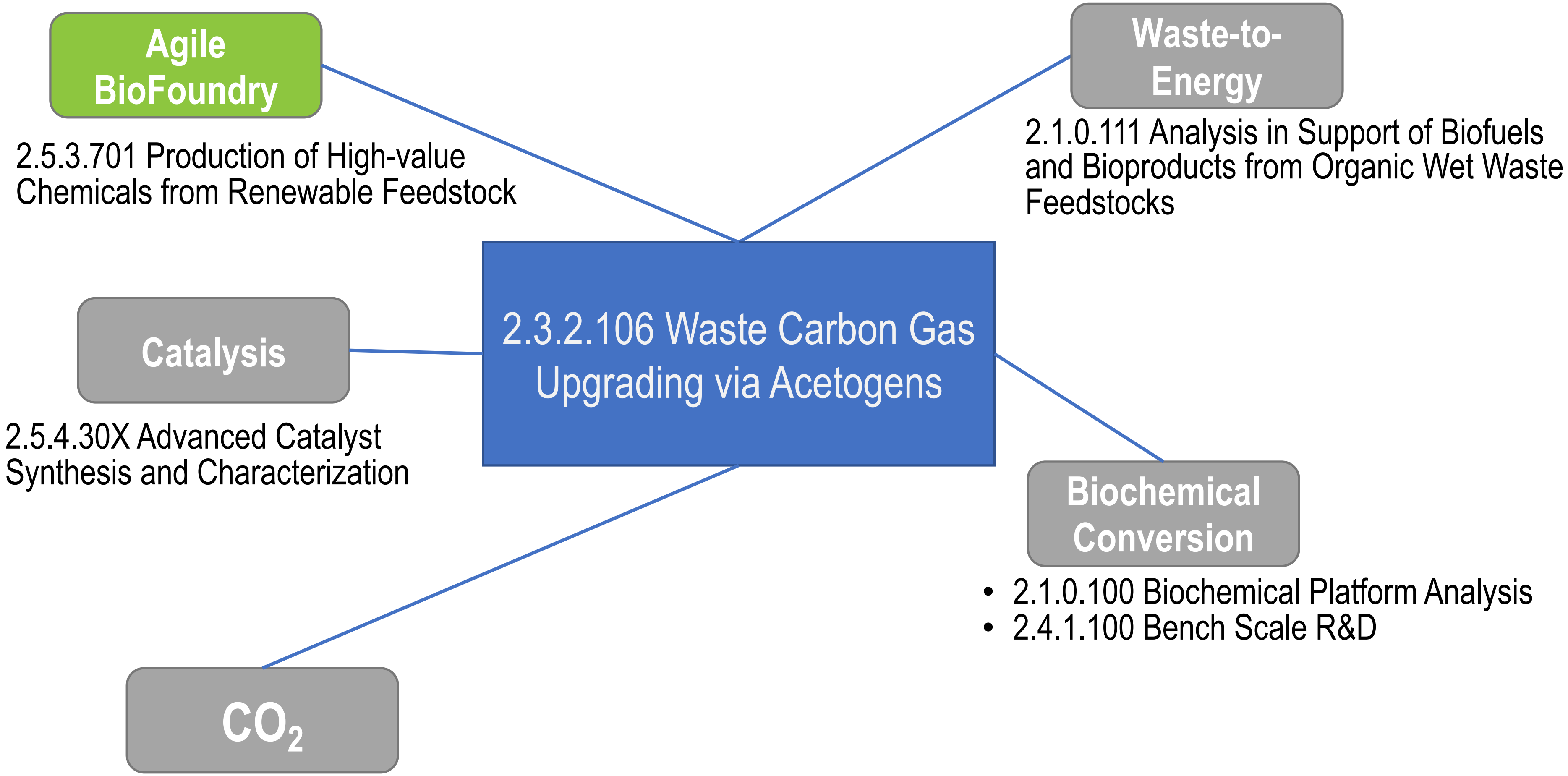
Microbial Syngas Conversion

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?

Management



- 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO_2 and Biomass
- 5.1.3.101 Integration of CO_2 Electrolysis with Microbial Syngas; Upgrading to Rewire the Carbon Economy
- 2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products
- Royal Dutch Shell/Princeton CO2 Conversion ACT Project

Approach –Feedstocks and products

- Direct CO_2 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
  - CO_2 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 CO_2 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

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Scaling Gas Fermentations

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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)

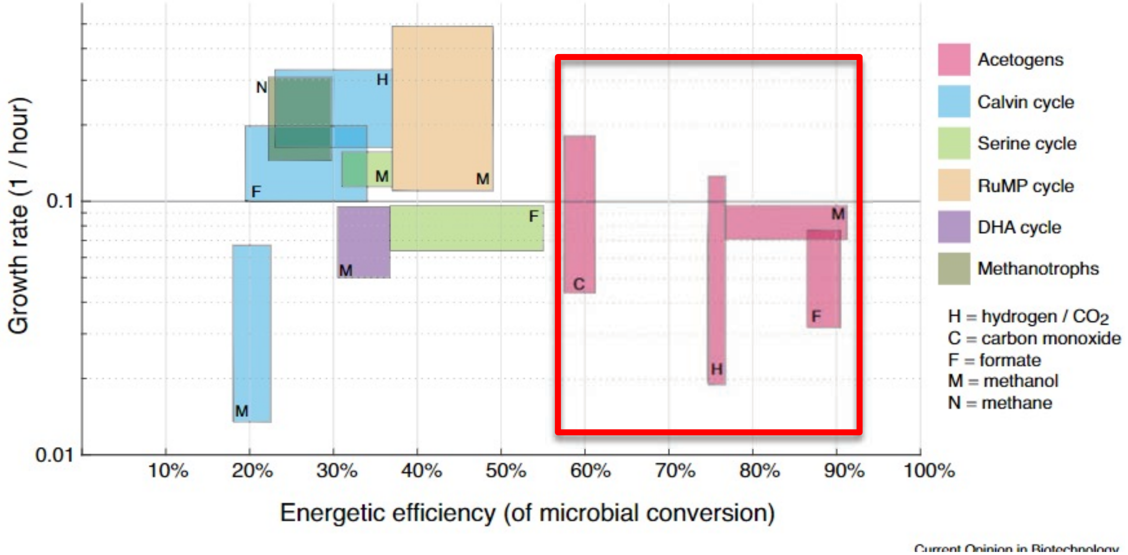
- Obtain a productivity of at least 0.1 g/L/hr from H2/CO/CO2 with a genetically engineered acetogen expressing butanol/3-Hydroxybutyrate pathway

Approach:
Acetogens have highest biological efficiency for CO_2 fixation

Renewable methanol and formate as microbial feedstocks

Charles AR Cotton[1], Nico J Claassens[1], Sara Benito-Vaquerizo[1] and Arren Bar-Even

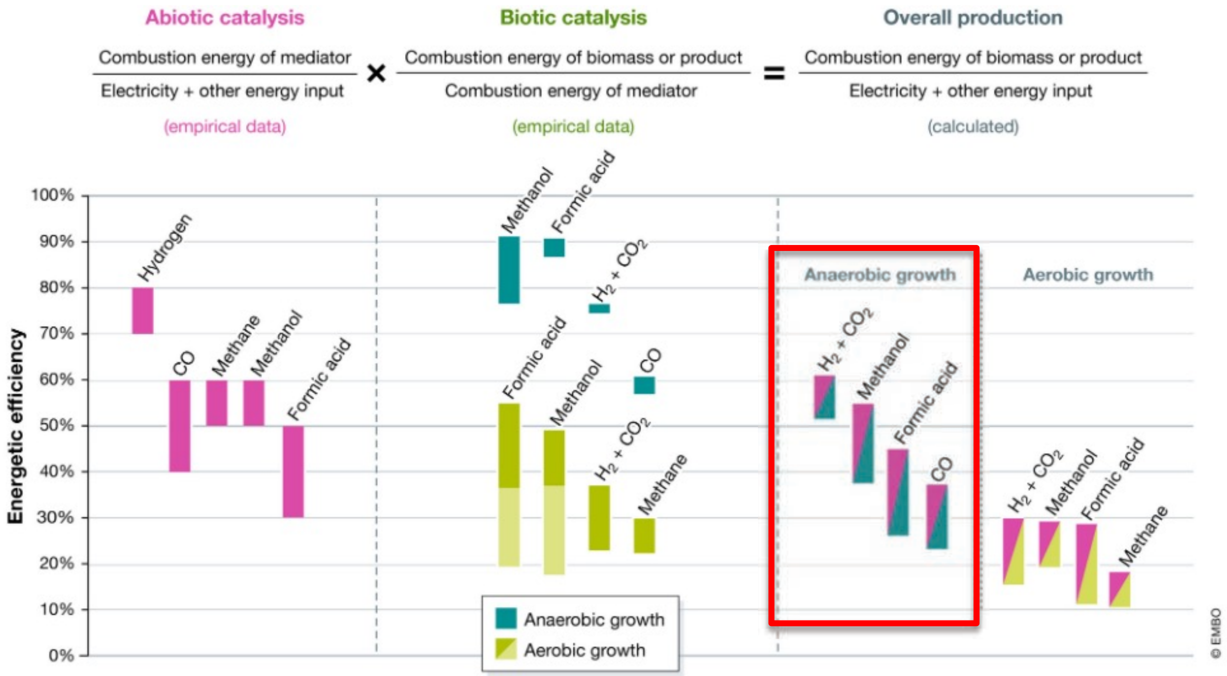
Current Opinion in Biotechnology 2020, 62:168–180



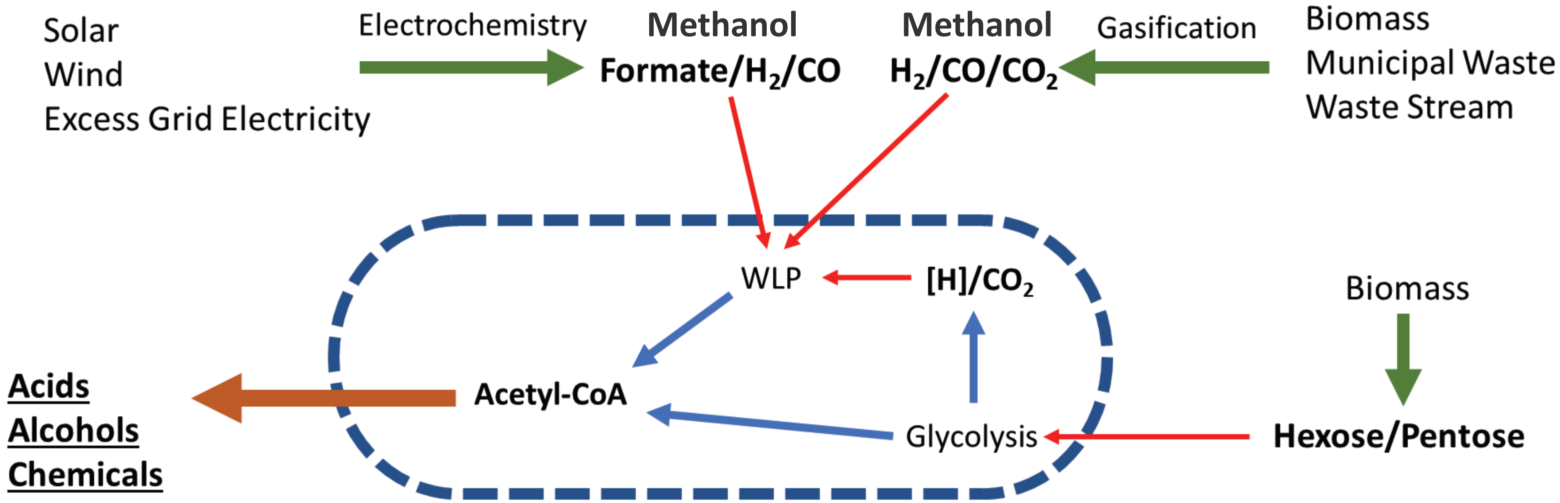
A one-carbon path for fixing CO_2

Ari Satanowski, Arren Bar-Even

EMBO Rep (2020)21:e50273



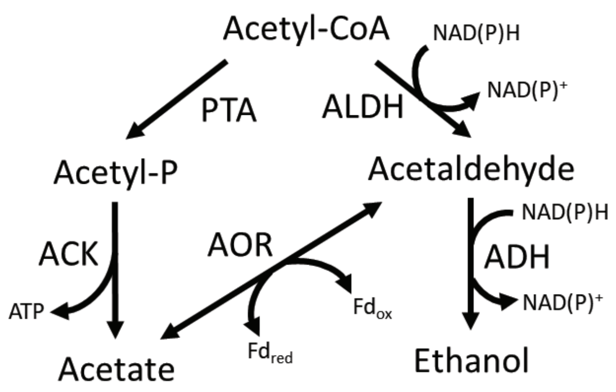
Approach: Acetogens for CO_2 fixation

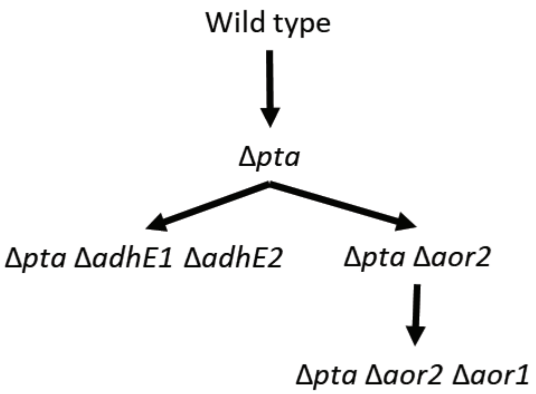


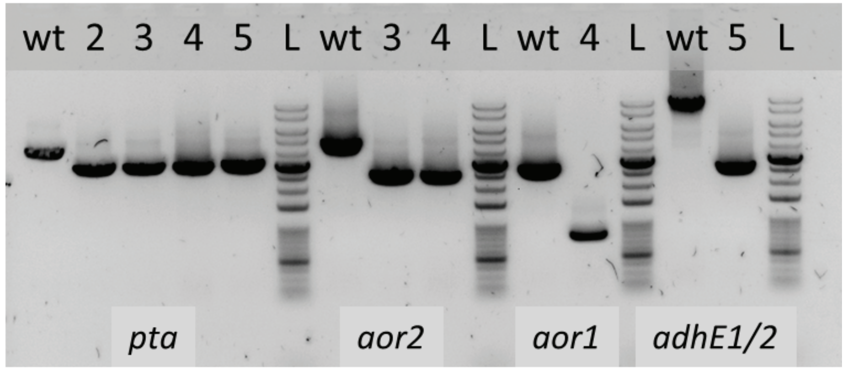
Acetogens **non-photosynthetically, anaerobically** fix CO_2

- Use Wood Ljungdahl Pathway (WLP), **most efficient for CO_2 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

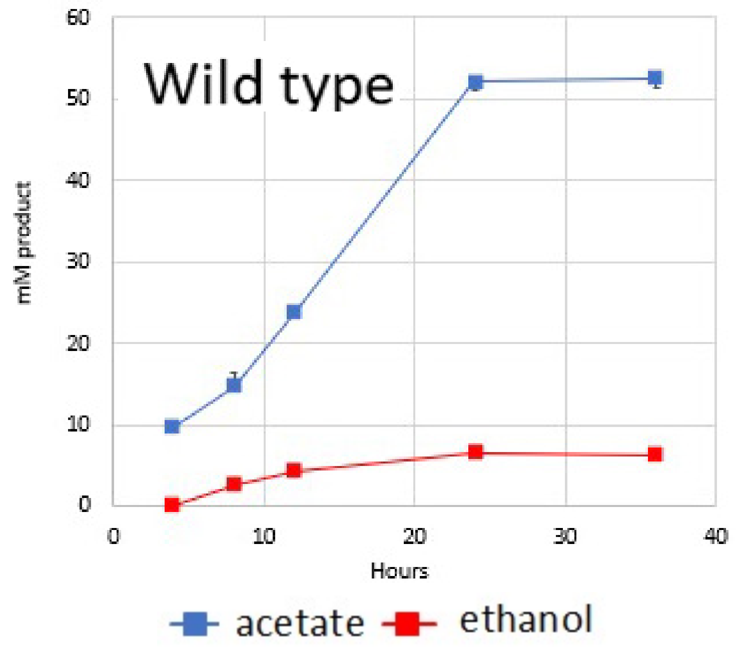
Progress and Outcomes
Organism development

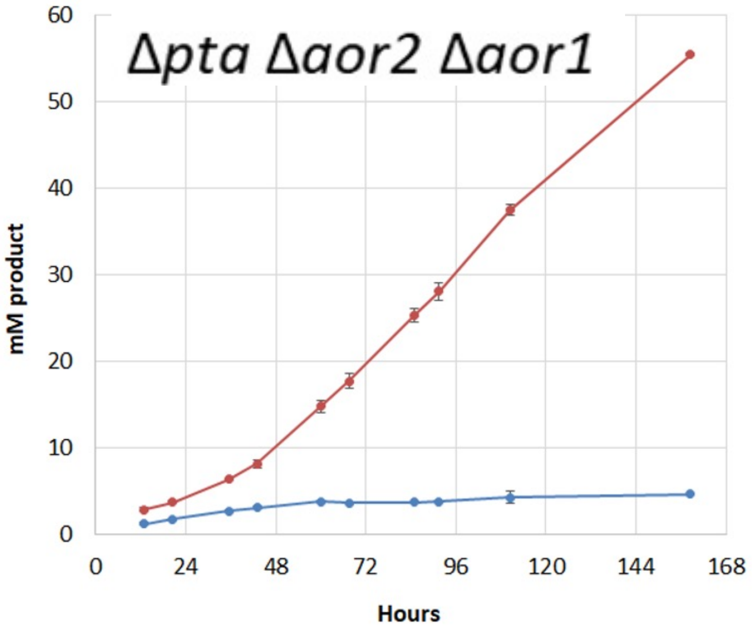






C. ljungdahlii metabolism and CRISPR/Cas9 engineering

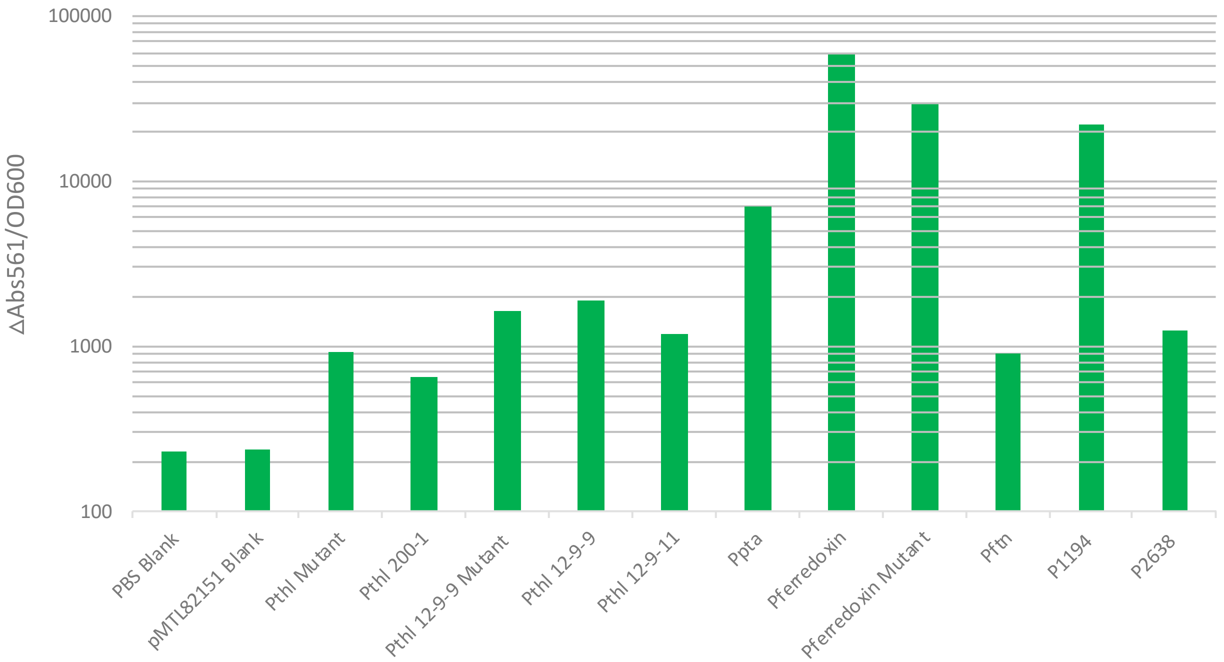


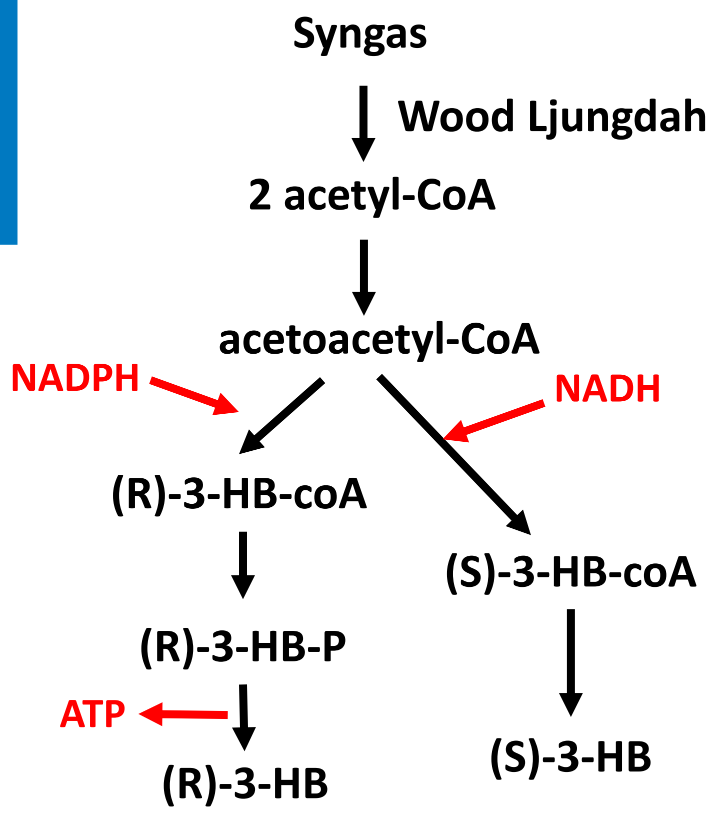


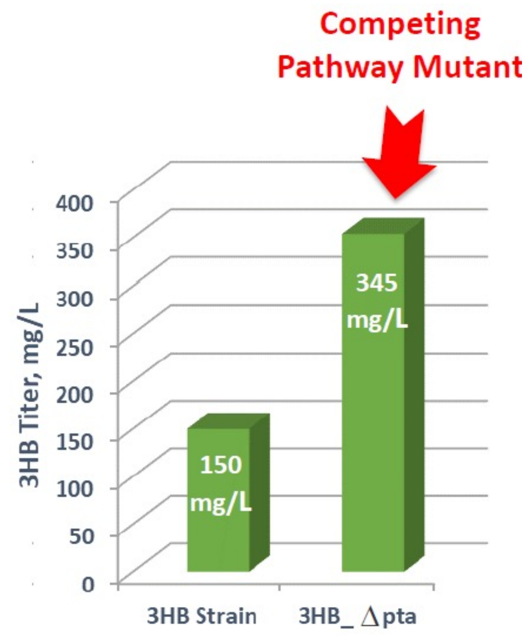
Progress and Outcomes

Organism development

Adam Guss, ORNL



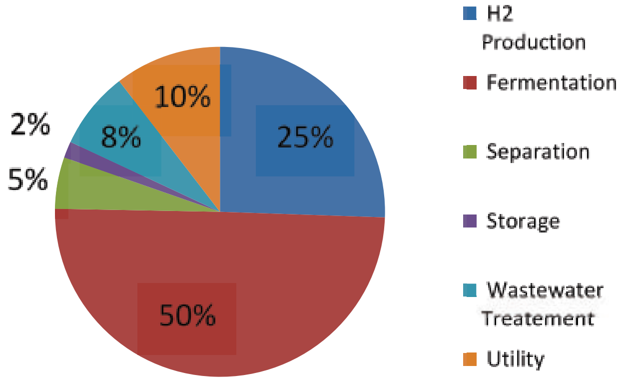




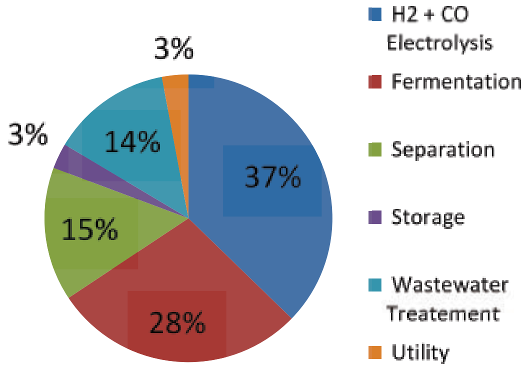
- Characterizing promoters to drive 3HB expression
- Testing different metabolic pathways to make 3HB
- Combining 3HB pathways with *C. ljungdahlii* deletion variants yields more 3HB

Progress and Outcomes
TechnoEconomic Analysis

~$2.50/kg market 3HB price



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



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

Cost Distributions of CO_2-to-3HB (left) and CO-to-3HB (right).

NREL - Ling Tao

2 Cases modeled:

CO_2 with electrolyzed H_2 to 3-HB

Electrochemical CO_2 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

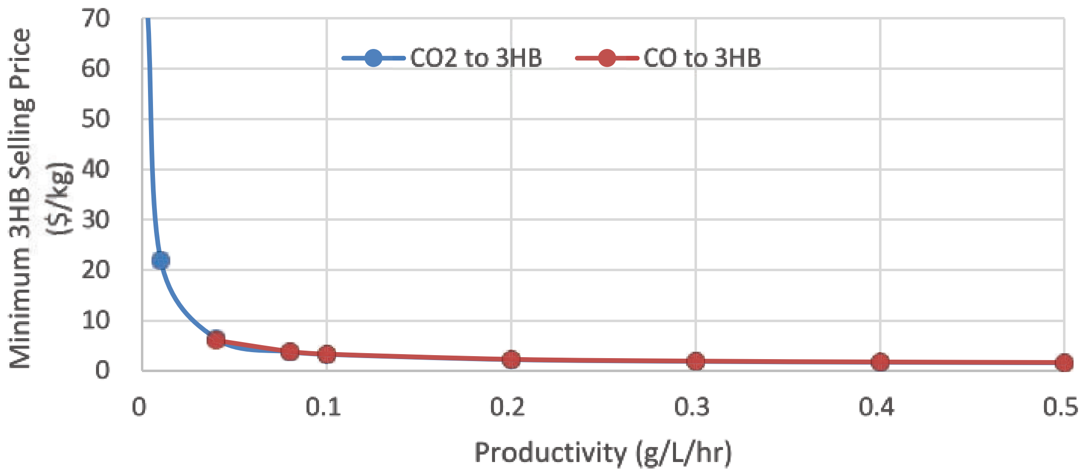


Figure 1. Minimum 0.1 g/L/hr is needed for both CO_2-to-3HB and CO-to-3HB cases.

Progress and Outcomes
TechnoEconomic Analysis

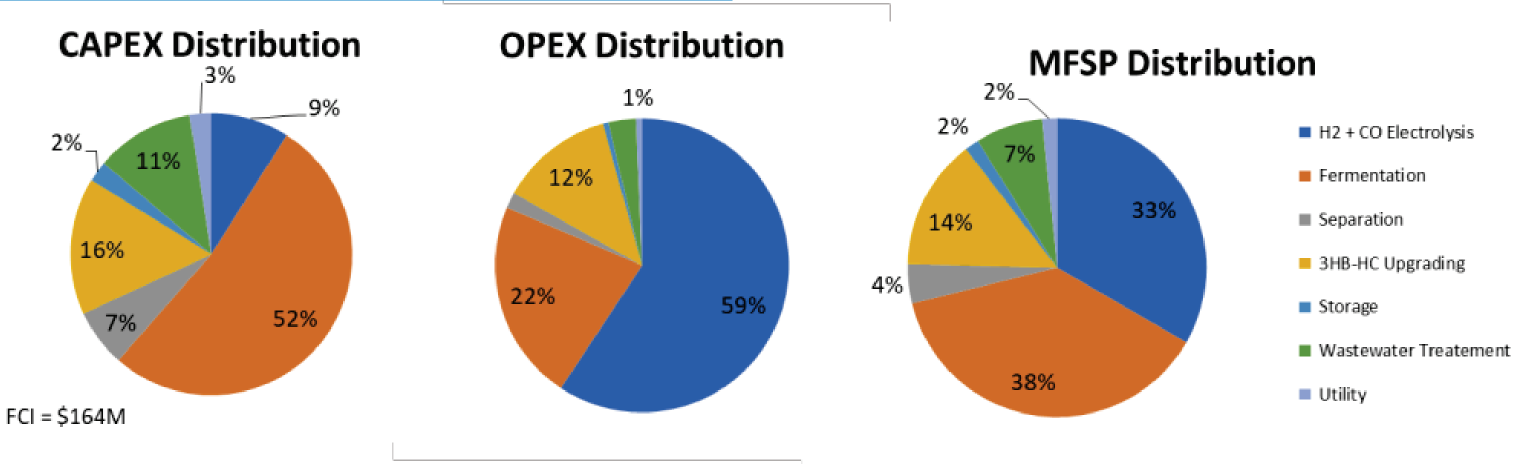
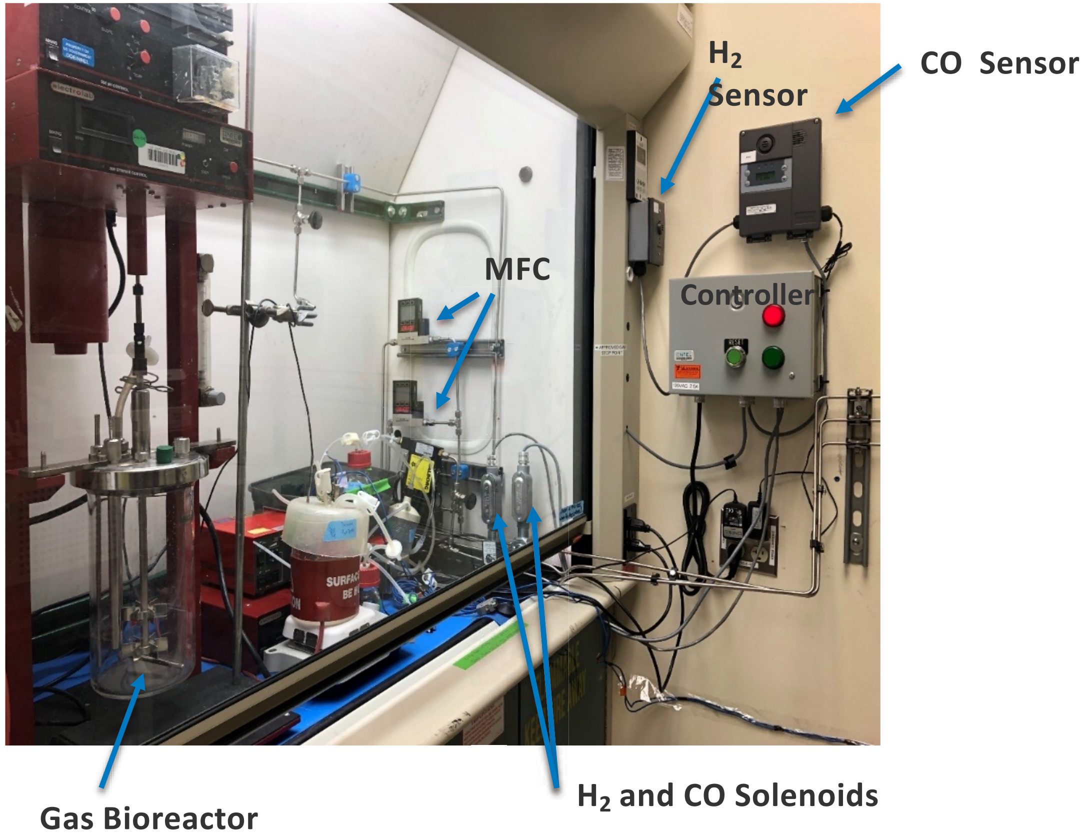


Figure 3. Preliminary MFSP distribution of processing areas including hydrogen, biological conversion, 3HB purification, upgrading and outside battery limit facilities.

- 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 CO_2, making less viable fuel
- 3-HB is a monomer for biodegradable plastic, ~$2.50/kg market

Progress and Outcomes
Process development

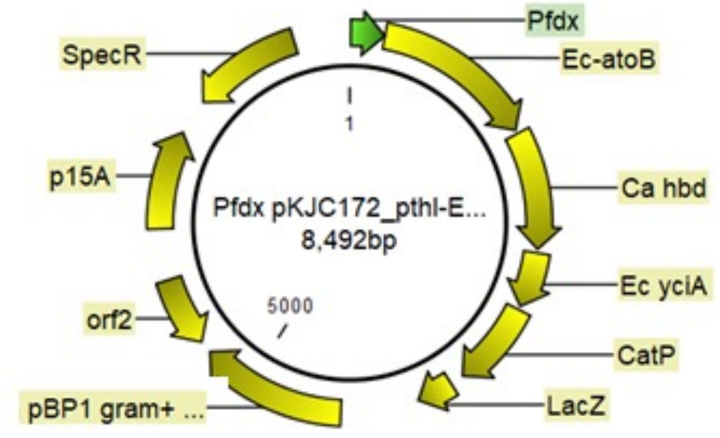
- Bioreactor gas scale up
  - Dynamic $H_2/CO_2/CO$ control
  - Can run gas fermentations at ≤2L
  - Different gas diffusion strategies
  - Numerous safety controls
  - Improving yield/rate/titer
  - Capturing metrics for further TEA/LCA analysis

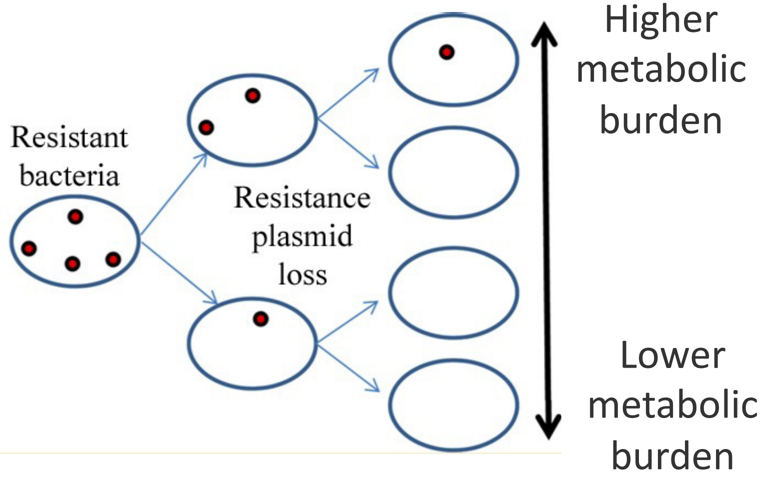


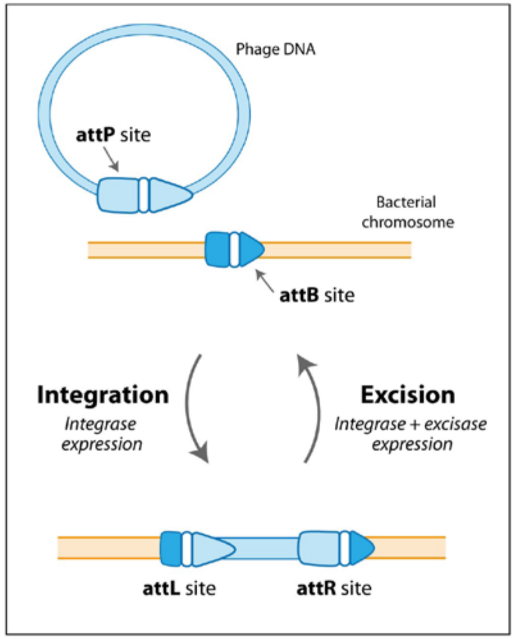
MFC: mass flow controller

Progress and Outcomes
Organism scaling

Pathway insertion into genome







100% CO, 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

Two strategies:

Phage attB integrase multiple sites

Homologous recombination

Impact –Data and Dissemination

- Project with Royal Dutch Shell/Princeton for C1 CO_2 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. Humphreys[1], Joshua Jack[2], Chris Urban[1], Lauren Magnusson[1], Wei Xiong[1], Yang Gu[3], Zhiyong Jason Ren[2] and Pin-Ching Maness[1]*

[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 CO_2 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]

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

Joshua Jack [a, b], Jonathan Lo [b], Pin-Ching Maness [b], Zhiyong Jason Ren [a, c]

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 CO_2 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 H_2
- 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

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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 H_2 and CO_2 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.