NREL
Transforming ENERGY

BETO 2021 Peer Review
2.3.2.112
Enhancing Acetogen Formate Utilization to Value-Added Products

March 11, 2021
Conversion
Jonathan Lo
National Renewable Energy Laboratory

Market Trends

Product

[redacted]

Feedstock

Decreasing cost of renewable electricity

[redacted]

Closing the carbon cycle

Capital

[redacted]

Challenges and costs of biorefinery start-up

[redacted]

Social Responsibility

Carbon intensity reduction

[redacted]

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

Value Proposition

- How do we utilize CO_2 with cheaper renewable energy?
- What products can we make?

Differentiator

- **<u>Liquid C1 compounds as medium for microbial upgrading</u>**
- Diversity of potential inputs
- Long term temporal storage, easy transport

Quad Chart Overview

Timeline

- 10/01/2018 through 9/30/2021

	FY20	Active Project
DOE Funding	(10/01/2018 – 9/30/2021)	$850,000 for 3 years

Barriers addressed

Ct-H – C1 Fermentation Development

Liquid C1s are a novel and promising avenue for microbial conversion to bioproducts, but little is known about C1 liquid fermentation processes

Ct-D – Advanced Bioprocess Development

Liquid C1s can be derived from a variety of renewable feedstocks and utilized in a variety of ways, but no industrial process exists

Project Goal

- Develop acetogens as a platform for renewable liquid C1 conversion to value-added products
- Perform TEA/LCA analysis to understand CO_2 and economic considerations

End of Project Milestone

- Demonstrate production of 2 g/L of C4 compound C1 feedstocks
- TEA/LCA analysis of potential process with generated fermentation metrics

Funding Mechanism

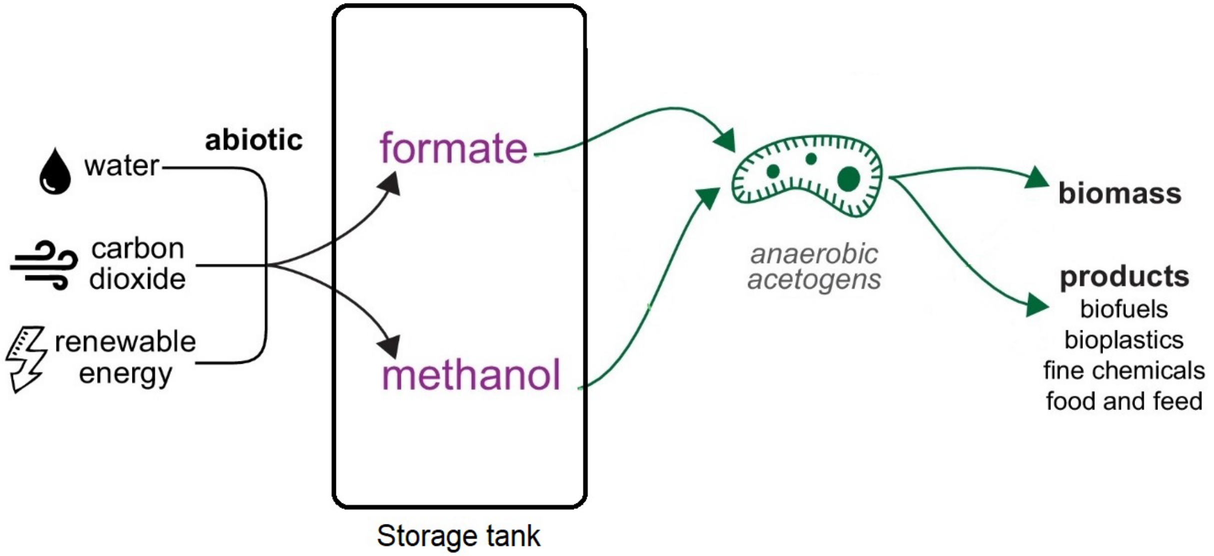
Funded through BETO Conversion 2018 Lab call

Project Overview

- **Liquid C1 compounds represent an understudied avenue for renewable energy capture and CO_2 bioconversion**
  - Electro/thermochemical approaches have focused on syngas ($H_{2,}$ CO)
  - CO_2 capture to liquid feedstock (methanol/formate)
  - High energy density –Formate = 53 g/L $H_{2,}$ methanol = 100g/L H_2
  - Easily stored/transported
  - Miscible - Avoiding mass transfer limits

- **Goal**: Develop a biological approach to convert liquid C1 into products

- Combine renewable **chemical CO_2 reduction** with **biological upgrading**



Renewable energy conversion of CO_2 combined with biological upgrading
Adapted from Cotton et al. 2020 j.copbio.2019.10.002

Project Overview

- **Relevance**: Chemicals with CO_2 and low-cost energy as feedstocks
  - Low-cost electricity to chemically reduce CO2 to formate/methanol
  - Scalable strategy as a stand-alone process or value add to existing industry

- **Outcomes:**
  - **Proof of concept:**
    - General process outline
    - Feedstocks and Organism Identification/Characterization
    - Soluble C1 Conversion to C4 compounds at **2 g/L titer**
  - **Life cycle (LCA) and technoeconomic analysis (TEA):**
    - Identify cost drivers and synergies with existing technologies

Species	Rate of Formation[a]	Selectivity[b]	Energy Efficiency[c]	Current TRL[d]
Carbon Monoxide	High	High	High	High
Ethylene	High	Medium	Low	Low
Formate	Medium	High	Medium	Low
Methane	High	High	Medium	High
Acetate	Low	High	Medium	Low
Methanol	High	High	High	High

[a] High: >200 mA/cm^2 (or commercial TC), Medium: 200 >/>100 mA/cm^2, Low: <100 mA/m^2

[b] High: >80%, Medium 80% > FE > 60%, Low: < 60%

[c] High: >60%, Medium 60% > EE > 40%, Low: < 40%

[d] High: Operated at TRL > 6, Medium: Operated TRL 4-6, Low: Operated TRL 1-3

Qualitative evaluation of product ease of formation. From "Transforming the carbon economy: challenges and opportunities in the convergence of low-cost electricity and reductive CO2 utilization"

Project Overview

Perspective | Published: 11 January 2021

An industrial perspective on catalysts for low-temperature CO_2 electrolysis

Richard I. Masel, Zengcai Liu, Hongzhou Yang, Jerry J. Kaczur, Daniel Carrillo, Shaoxuan Ren, Danielle Salvatore & Curtis P. Berlinguette

Nature Nanotechnology (2021) | Cite this article

"Units to convert CO_2 to formic acid are projected to reach pilot scale in the next year."

Methanol category	Commercial	Feasibility and R&D
Bio-methanol	BASF (GER) BioMCN (NL) Enerkem (CAN) New Fuel (DEN)	Biogo (GER) Enerkem (NL) LowLands Methanol Heveskes Energy (NL) NREL (USA) Origin Materials (USA) Södra (SE)
Renewable methanol	CRI (IC) Innogy (GER)	Advanced Chemical Technologies (CAN) Asahi Kasei (JPN) Blue Fuel Energy (CAN) bse Engineering (GER) Catalytic Innovations (USA) CRI (CN/GER) Gensoric (GER) Infraserv (GER) Liquid Wind (SE) MefCO2 (GER) Neo-H2 (USA) Port of Antwerp (BE) Quantiam Technologies (CAN) STEAG (GER) Swiss Liquid Future (CH) thyssenkrupp (GER) USC (USA) ZASt (GER)
Low carbon methanol	GPIC (BAH) Methanex (CAN) QAFAC (QAT) SABIC (KSA)	Carbon2Chem (GER) FRESME (SE) GasTechno (USA) Haldor Topsoe (DEN) Maverick Synfuels (USA) NCF (CN) OPTIMeoH (GER)

From Methanol.org

Management

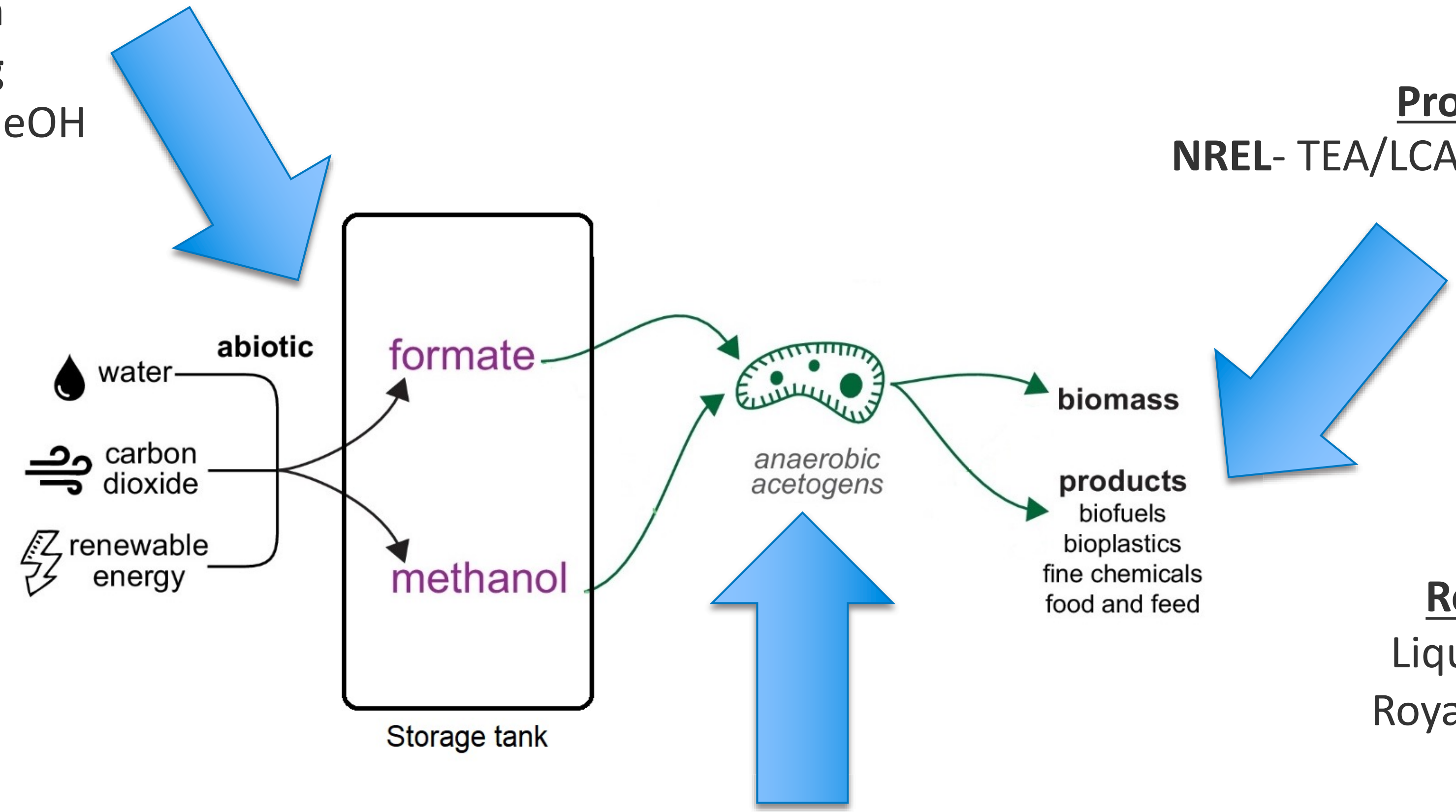
Feedstocks

NREL- C1 Electrochemist Kenneth Neyerlin

Carbon Recycling International - MeOH producer

Products

NREL- TEA/LCA analysis Ling Tao



Related Industry Contacts

Liquid C1 Conversion Startups

Royal Dutch Shell C1 conversion

C1 Organisms

NREL- Microbiologist Jonathan Lo

Management

Methanol and Formate Feedstocks

How are they generated?

H_2/CO_2 synergy?

At what prices?

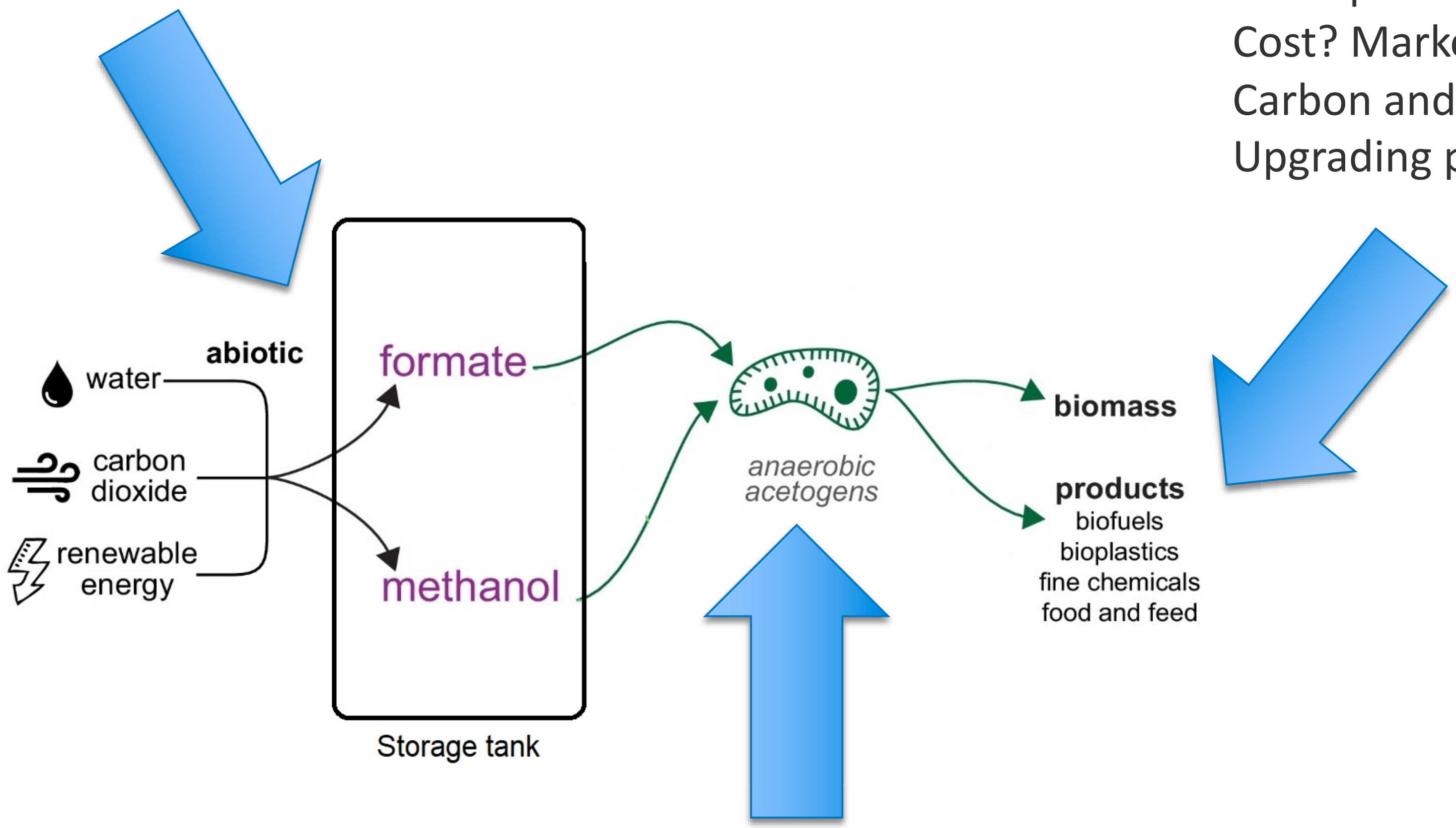
Products

What products can be made?

Cost? Market price?

Carbon and electron efficiency?

Upgrading paths and market size?



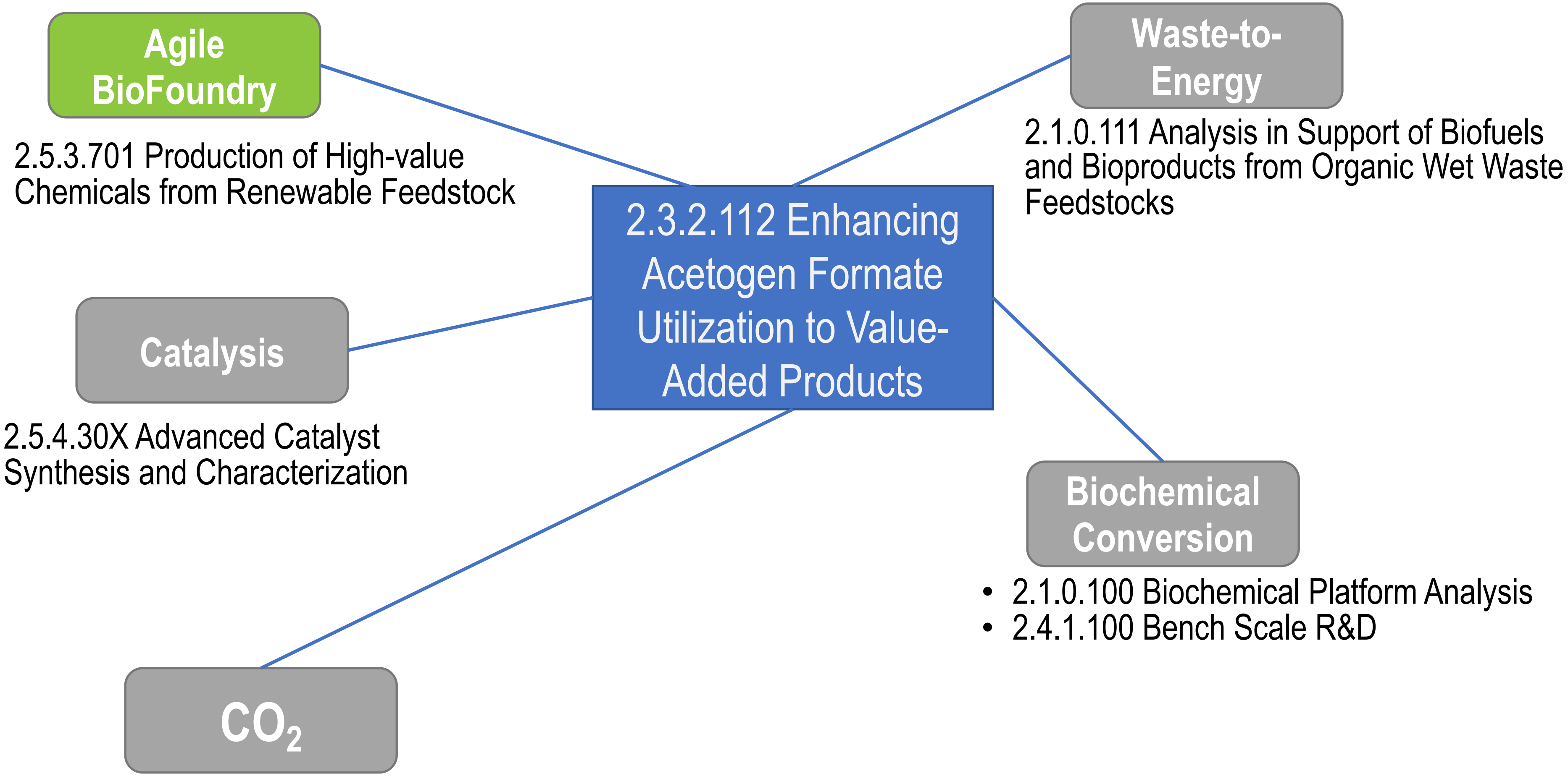
C1 Microbes

Who can use them? How well?

What do they make?

Can they be genetically engineered?

Approach and Management

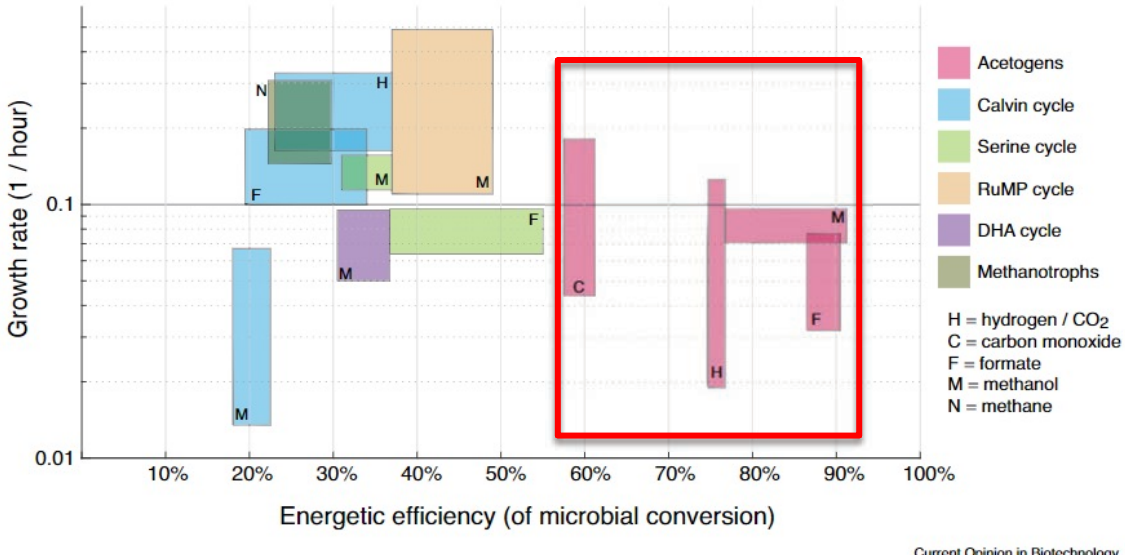


- 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO_2 and Biomass
- 2.3.2.111 Improving formate upgrading by *Cupriavidus necator*
- 2.3.2.113 Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis
- 5.1.3.101 Integration of CO_2 Electrolysis with Microbial Syngas; Upgrading to Rewire the Carbon Economy
- 2.3.2.106 CO2 Valorization via Rewiring Metabolic Network
- Royal Dutch Shell/Princeton CO2 Conversion ACT Project

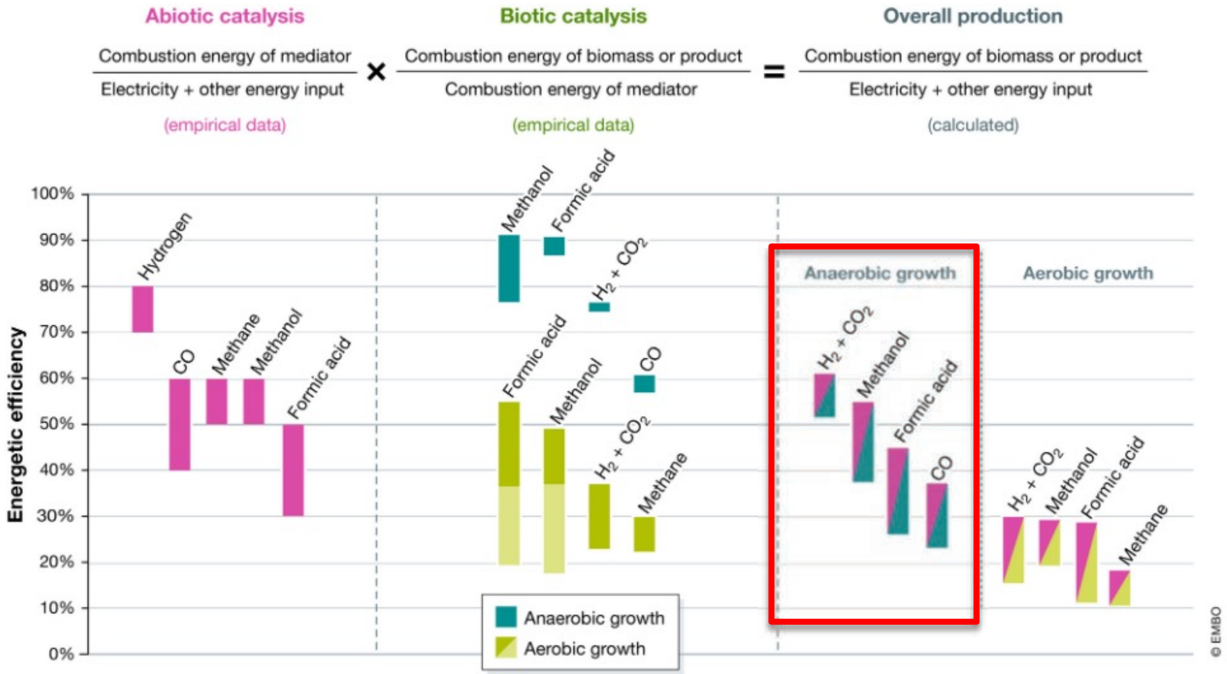
Approach: Highest biological efficiency for CO_2 fixation

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

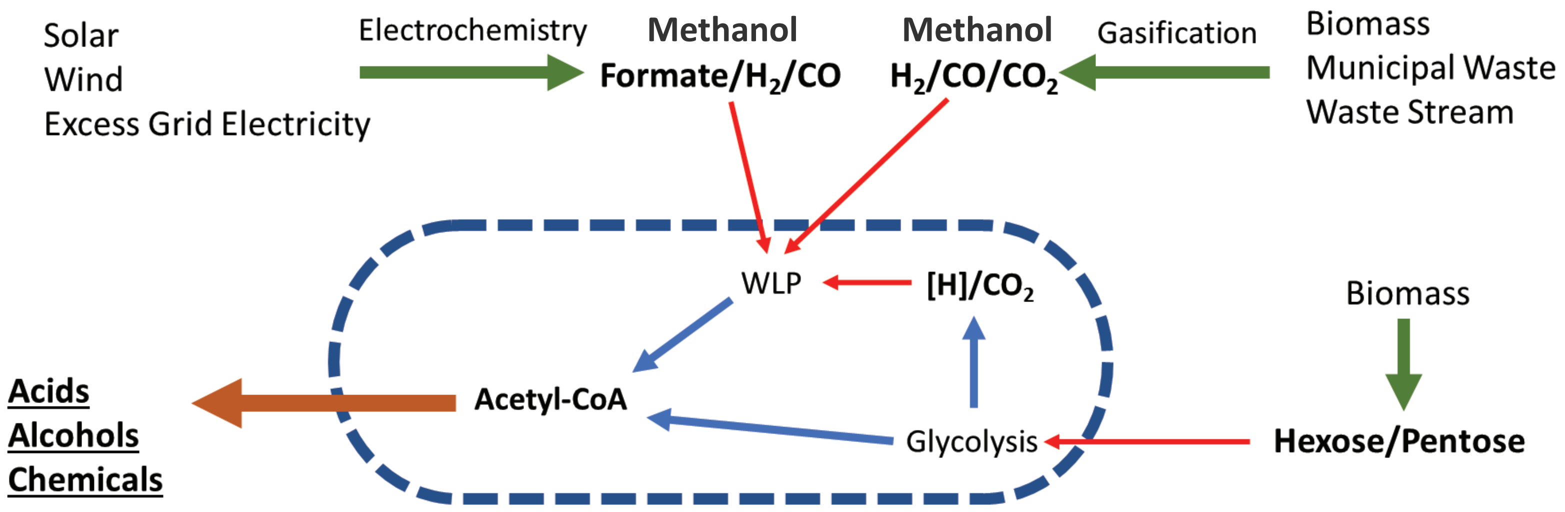


A one-carbon path for fixing CO_2
Ari Satanowski, Arren Bar-Even
EMBO Rep (2020)21:e50273



"Bioproduction with acetogens is thoroughly researched and commercially exploited using gaseous C_1 feedstocks...**only a small number of acetogens have been tested for growth on methanol and formate...miscible carbon sources support higher energetic efficiencies of bioproduction**"

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
- Avoids gas mass transfer issue, easier to store and transport
- Can simultaneously use gases, liquids, and biomass related sugars
- Produce interesting products at high carbon and electron efficiency
- Focus on C4 products (butanol/butyrate) due to their ease of upgrading to fuels

Approach - Milestones

- Transform C4 overexpression pathway into acetogen to boost yield of C4 products (Q1)
- Growth ≥1L reactor for TEA/LCA metrics analysis (Q2)
- TEA/LCA analysis of butanol/butyrate production with different feedstock mixes to determine cost drivers, carbon efficiencies, product separation, purification, and upgrading (Q3)

<u>End goal</u>

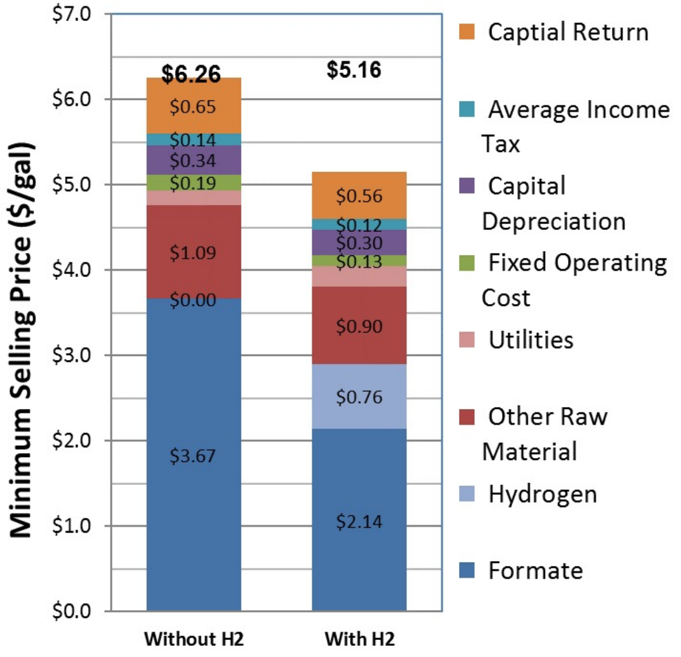
- <u>Proof of concept:</u> Demonstrate production of 2 g/L of a C4 compound in an engineered acetogen using 1-carbon feedstocks (Q4)

Progress and Outcomes
TechnoEconomic Analysis

Assumptions for CO_2 Reduction to Formate

Parameters	Value
Cell Voltage (V)	2.08
Current Density (mA/cm^2)	300
Faradaic Efficiency (%)	95
CO_2 Single pass Conversion (%)	90
CO Faradaic Efficiency (%)	0
H_2 Faradaic Efficiency (%)	5
Electrolyzer Capital Cost ($\$/m^2$)	10,000
Electricity Price ($kWh)	0.03
CO Market Price ($/kg)	0.23
H_2 Price ($/kg)	1.57
Water Price ($/kg)	0.00022
CO_2 Capture Price ($/ton)	20

Preliminary Minimum Butanol Selling Price ($/gal)



- Formate is a poor electron source
- H_2 improves Carbon yield
- Butyrate versus butanol?

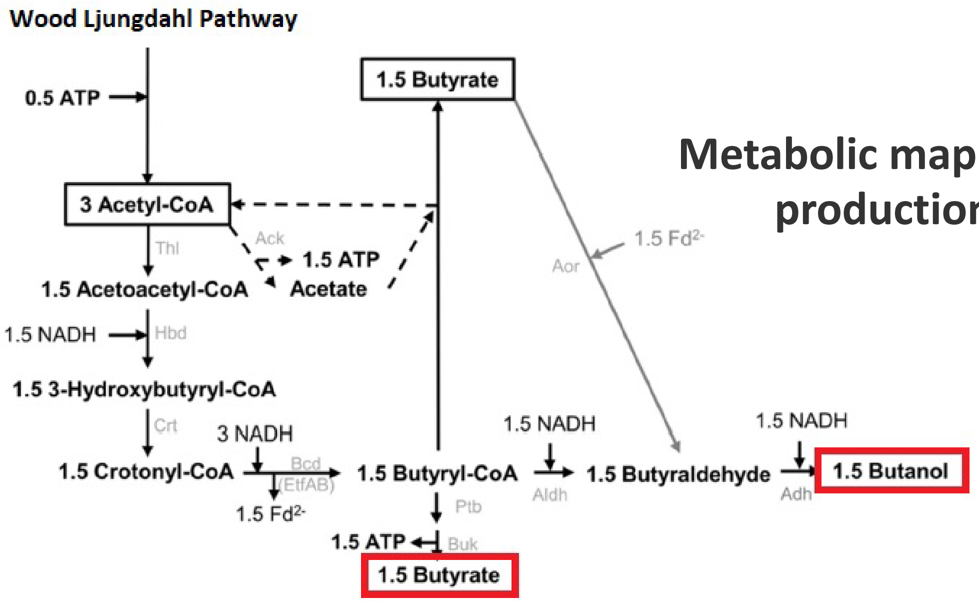
Equation	[C] Yield	Mass Yield
$C_6H_{12}O_6$ (sugar) → $C_4H_{10}O_2$ (butanol) + $2CO_2$ + H_2	0.67	0.41
12 CH_2O_2 (formate) → $C_4H_{10}O$ (butanol) + $8CO_2$+ $7H_2O$	0.33	0.13
7 CH_2O_2 (formate) +5 H_2→ $C_4H_{10}O$ (butanol) + $3CO_2$+ $7H_2O$	0.57	0.23
4 CH_2O_2 (formate) +8 H_2→ $C_4H_{10}O$ (butanol) + $7H_2O$	1.00	0.40

- Methanol is cheap, electron rich, soluble
- From methane or electrochemically
- Potential cosubstrate
- C1 miscible

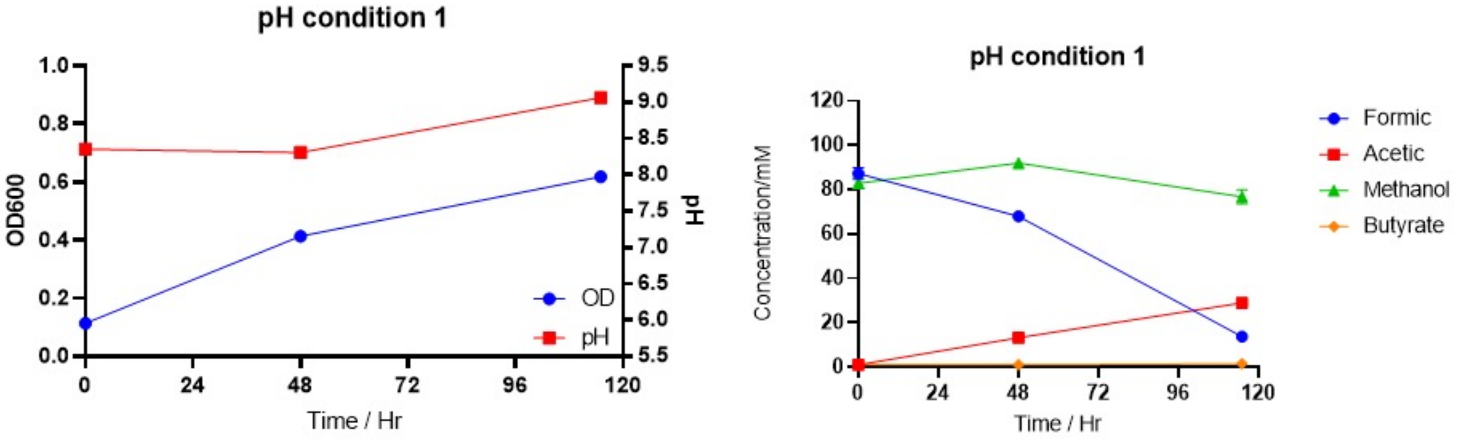
	USD/kg $	MW	Mass yield g/g	Input cost $ per kg	% Cost reduction
Methanol	$0.29	32.04	0.58	$0.49	
Glucose	$0.29	180.16	0.41	$0.70	30%
Butanol	$1.20	74.12			

Progress and Outcomes
C1 conversion

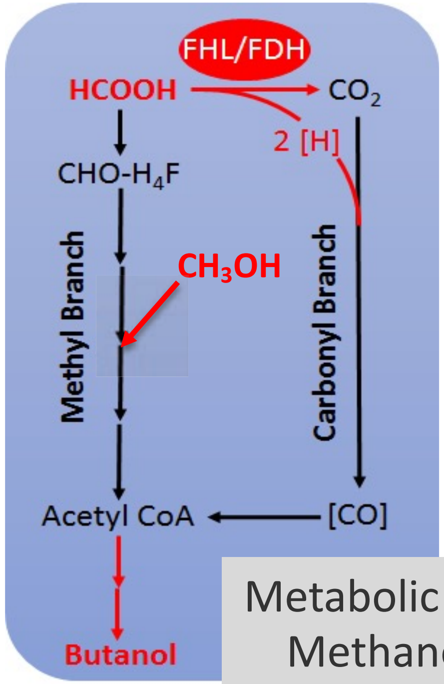
- ***Butyribacterium methylotrophicum* (Bm)**
  - Can use formate/methanol alone
  - No genetic tools
  - No metabolic models
  - Acetate/ethanol/butyrate/butanol



Metabolic map of C4 production



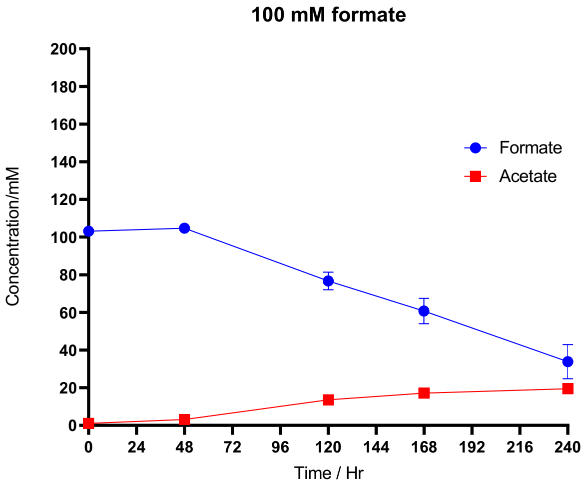
Growth and production on formate/methanol

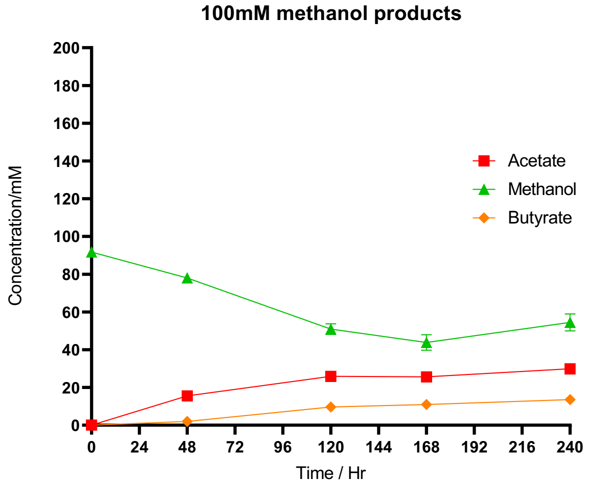


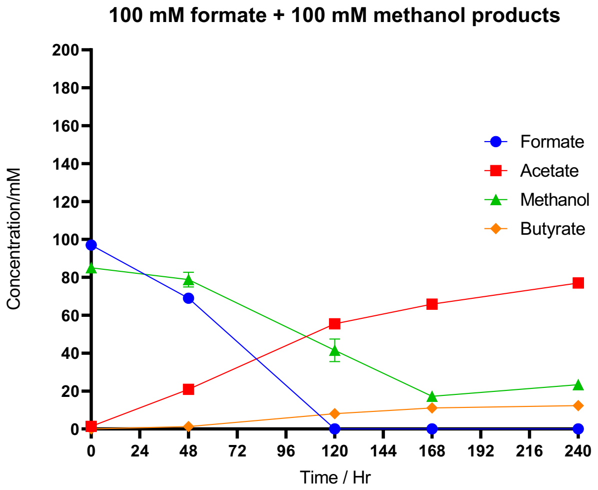
Metabolic conversion of Methanol/Formate

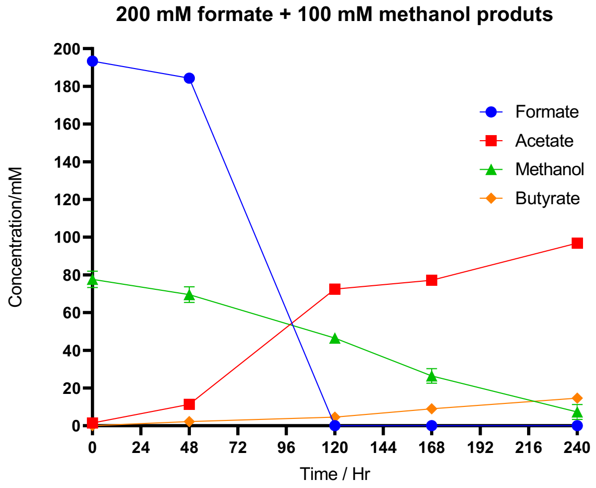
Progress and Outcomes
C1 conversion specifics

- **Bm** can naturally make C4 (butyrate/butanol)
  - 100 mM formate 200 mM MeOH
  - 41 mM (3.6 g/L) butyrate
- Methanol seems to drive formation for butyrate, but does not make butanol
- Different C1 mixtures and conditions for different product formation
- Single substrate C1 fermentation is slow
- C1 mix is synergistic
  - Tolerance (~500mM formate, >1M methanol)
  - Consumption kinetics (yields, rates, titers)





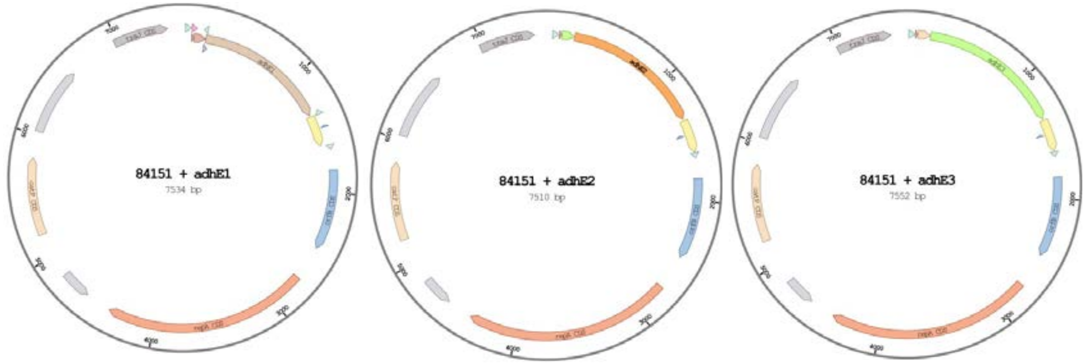




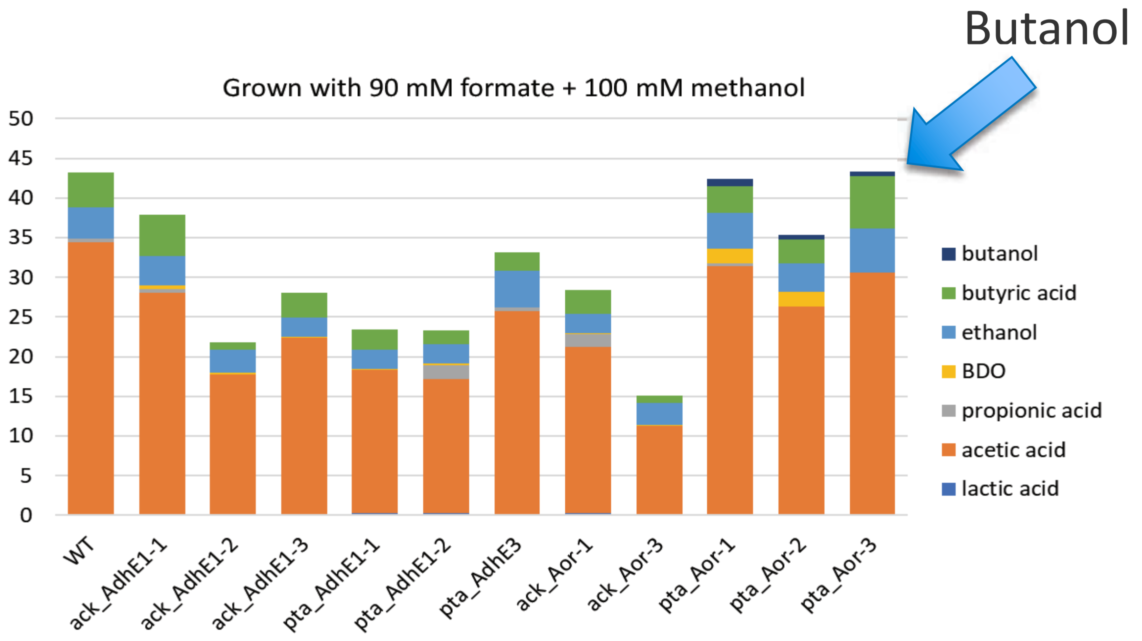
Progress and Outcomes
Organism development

- Genetics tools development
  - Protocol established
  - Building CRISPR/Cas9 plasmids
  - Butanol production strategies
  - Promoter testing

- Metabolic analysis
  - RNAseq analysis
  - Substrate specificity
  - Product profile
  - Linking genes to products



Plasmids expressing different alcohol dehydrogenases (AdhE). AdhE is known to convert aldehydes into alcohols and is needed to create ethanol and butanol.



Growth of *B. methyltrophicum* engineered strains on 90 mM formate and 100 mM methanol.

Progress and Outcomes
Organism development

- Test tube scale
  - 3.6 g/L butyrate
  - 50% Carbon efficiency
- Bioreactor scale up
  - Fermentation control
  - Experimenting with conditions
  - Feeding strategies
  - Improving yield/rate/titer
  - Capturing metrics for second TEA/LCA analysis

Impact –Data and Dissemination

- Direct CO_2 conversion to C1 chemicals has a high technology readiness level, but C1 chemicals have a low market price.
- Larger compounds C2-C4 have a higher value
- Direct contact with Royal Dutch Shell, and renewable Methanol Carbon Recycling International (CRI)
- Several forthcoming publications around microbial C1 conversion.
  - Genetic engineering and describing metabolism
    - Describing techniques, new tools, analysis
  - Metrics regarding C1 fermentation
    - Yield/rate/titer, fermentation strategies to change products
  - Fermentation process using real liquid C1s from CO_2

Feedstocks		USD/kg	$/mole
C1	Methanol	$0.29	$0.009
C1	CO2	$0.00	$0.000
C1	Formate	$1.00	$0.046
Products		USD/kg	$/mole
C2	Acetate	$0.80	$0.048
C4	Butyrate	$1.50	$0.132

Feedstock and product market costs.

Impact – Future Work

- Liquid C1 fermentation strategies
  - Implementing real CO_2 reduction streams
  - Fed batch, continuous, in situ extraction
  - Supplement CO/H_2 for better growth and carbon efficiency?

- Direct production of methanol conversion to C4?
  - Methanol is cheap ($276/MT), readily available, derived from methane, or electrochemically renewable from CO2 (Vulcanol).

- Engineering *B. methyltrophicum* to make C4 (butyrate /butanol) at higher yield
  - Target native pathways, adding C4 pathways, planning RNAseq experiment
  - CRISPR/Cas9 gene deletion, genomic integration

- **Proposals to further develop process, explore variations, outside partner collaboration**

Summary

Product
- [redacted]
- [redacted]
- [redacted]
- [redacted]

Feedstock
- [redacted]
- Decreasing cost of renewable electricity
- [redacted]
- [redacted]
- Closing the carbon cycle

Capital
- [redacted]
- Challenges and costs of biorefinery start-up
- [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

- CO_2 conversion to liquid C1 for microbial upgrading represents an interesting proposition for renewable energy integration with CO_2 as a feedstock

Key Accomplishments

- Identified and developed microbial C1 conversion to C2 and C4 products
- Developed a TEA/LCA analysis for understanding the process
- Filling in knowledge gaps and disseminating knowledge among academic and industry institutions

NREL

- Jonathan Humphreys
- Lauren Magnusson
- Holly Rohrer
- Yi Pei Chen
- Wei Xiong
- Ling Tao
- KC Neyerlin
- Pin Ching Maness

Thank You

www.nrel.gov

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Program 2.3.2.112

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NREL Transforming ENERGY

Responses to Previous Reviewers' Comments

This project aims to convert formate to butanol using Clostridia ljungdahlii. Formate is one of the target intermediates that can be produced via chemical synthesis. It is not clear whether butanol is the best target product considering their value and subsequent separation issues etc. Thus, a better system engineering should be incorporated to fully evaluate the proposed technology.

Response: The TEA analysis is utilized to evaluate which products could best be made from formate. In our system, formate is first converted into acetyl-CoA, which is a precursor to many other products that could be made instead of formate, including ethanol, buytrate, and mevalonate, which could be made instead of butanol.

As a benchmark, assuming 100% formate conversion and 3 V for the electrochemical cell producing formate, the electricity demand will be ~26 kWh per kg butanol. The energy demand for state-of-the-art formate producing cells is substantially more than the thermodynamic minimum. Even if electricity is cheap, the extra cost of an inefficient energy conversion may make the process uncompetitive.

Response: Having high conversion to product is an important consideration for reaching economically feasible. It may be that we need higher efficiency of formate conversion to products, and that may be through co feeding other substrates like H2/CO to better efficiencies so that the formate carbon and electrons are better matched towards products.

Publications, Patents, Presentations, Awards, and Commercialization

We anticipate at least 2 publications from this project in progress:

Butyribacterium methylotrophicum C1 liquid conversion characterization via RNAseq analysis and bioreactor data

Development and Genetic Engineering of *Butyribacterium methylotrophicum* as a chassis organism for conversion of C1 compounds to Value Added products