DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

WBS 2.3.2.106
CO_2 Valorization via Rewiring Carbon Metabolic Network

CO_2 Utilization Technology Session

March 7, 2019

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National Renewable Energy Laboratory

Goal Statement

Project Goal.

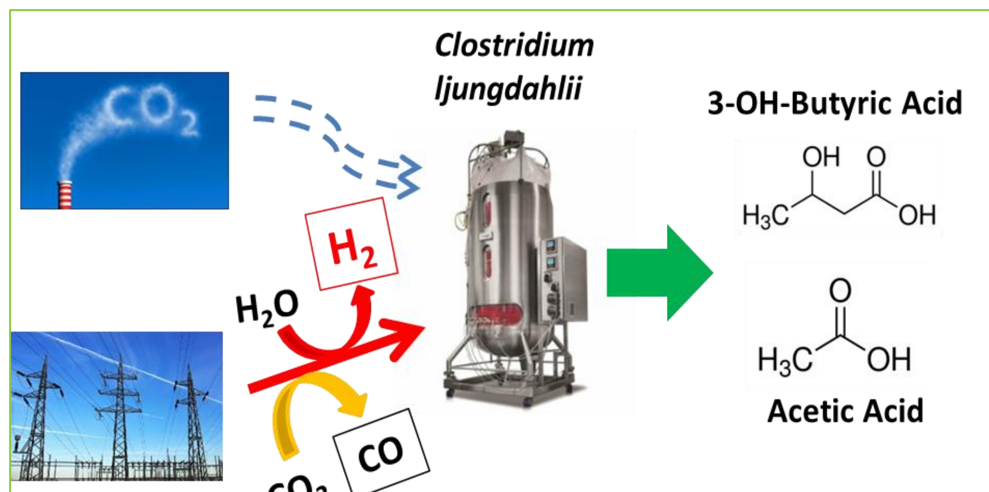
- Develop an efficient biological approach to convert waste CO_2 to hydrocarbon products in a CO_2-fixing microbe without photosynthesis, by leveraging low-cost electricity leading to rewiring a carbon economy based on CO_2 valorization.
  - Develop enabling tools to build more durable *Clostridium ljungdahlii* model microbe via metabolic engineering

Project Outcome.

- Produce **3-hydroxybutyrate (3HB) at a titer of 2 g/L** (proof of concept) as well as acetate co-product from **waste CO_2**, with high carbon-conversion efficiency.
- Valorize CO_2 to 3HB, a high-value chiral building block in the carboxylate platform with a global carboxylic acid market value of $14.2 billion in 2017.

<u>Relevance</u>

- Develop cross-cutting technology to rewire CO_2 - a BETO mission.
- Help fossil power plants, biofuels, and manufacturing industries in reusing their waste CO_2
- Monetize CO_2 in a new economy.



Quad Chart Overview

Timeline

- Project start date: 10/1/2017
- Project end date: 9/30/2020
- Percent complete: 45%

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$0	$0	$24 4K	$731K
Project Cost Share*	$0	$0	$0	$0

•**Partners:** **If multiple DOE recipients are involved in the project, please list level of involvement, expressed as percentages of project funding from FY 17-18. [(i.e. NREL (70%); INL (30%)]-**

*Only fill out if applicable. If there are multiple cost-share partners, separate rows should be used.

**Only fill out if applicable.

Barriers addressed

Ct-H. Gas Fermentation Development.

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

Ct-L. Decreasing development time for industrially relevant microbe

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

Objectives

- Develop a biological approach to valorize waste CO_2 to high-value products in a CO_2-fixing non-photosynthetic microbe by rewiring its carbon metabolic network,

End of Project Goal

- Develop a robust microbe to produce 3HB and acetate from CO_2 with **a 3HB titer of 2 g/L.**
- The robust CO_2-fixing process will help biofuels industry and industrial processes to reuse CO_2 and hence mitigate CO_2 emission.

1. Project Overview

History: A BETO Lab Call proposal award started in FY18, using a CO_2-fixing ***non-photosynthetic microbe*** to convert CO_2 to high-value hydrocarbon products.

Goal: Increase the productivity of 3HB from CO_2 via *C. ljungdahlii* organism development.

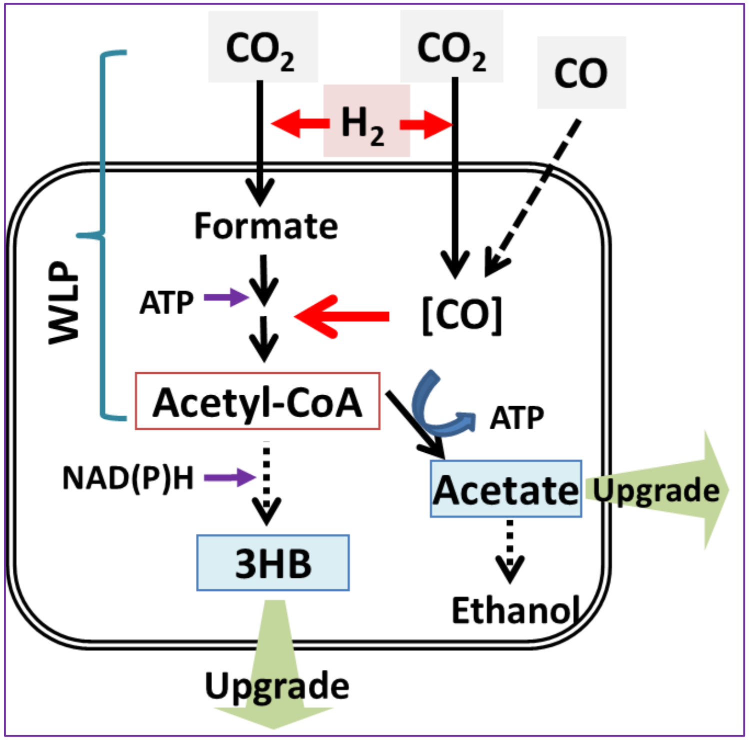
— Develop enabling tools to afford 3HB production with high titer/yield

Rationale: 3HB is derived from acetyl-CoA, the latter a direct product of CO_2-fixation hence with higher electron efficiency.

Merits: High carbon-conversion efficiency

— **82% carbon yield** from sugar alone.

— **No CO_2 emission** in mixotrophic mode (sugar + H_2).

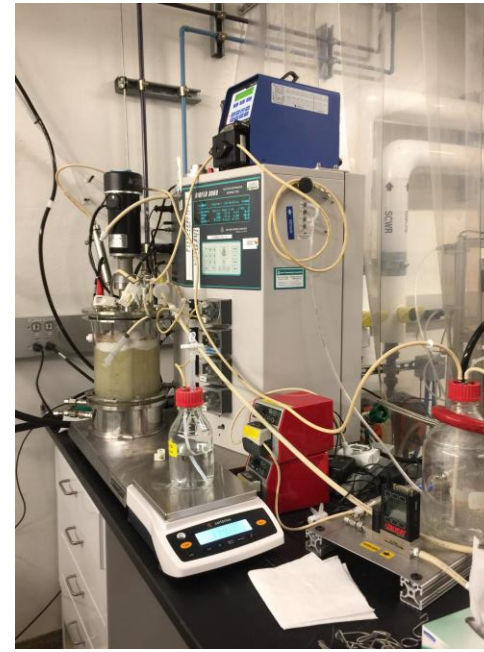


WLP: Wood-Ljungdahl Pathway, an energy efficient CO_2-fixation pathway.

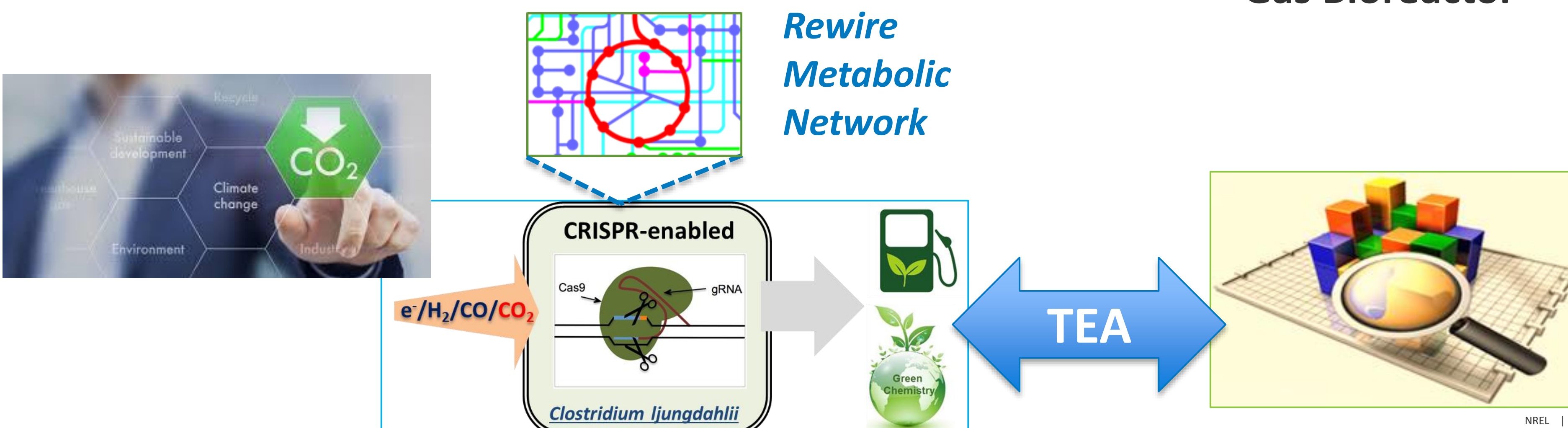
Advantage: Developing *C. ljungdahlii* as the model catalyst for CO_2-derived products will **"*rewire*"** and enable a new renewable carbon economy with high carbon- and energy-conversion efficiency.

2 – Approach (Management)

- Multi-disciplinary team approach recruiting molecular biologist, microbiologist, computational modeler, chemical engineer, and process engineer.
- Research guided by TEA and monitored by Go/No-Go.
- **Task 1. Strain Development**
  - CRISPR genetic tools for metabolic pathway engineering (Jonathan Lo and Katherine Chou)
  - ^{13}C-metabolic flux analysis (Wei Xiong)
  - Gas bioreactor fermentation (Lauren Magnusson)
- **Task 2. Technoeconomic Analysis (TEA)** (Ling Tao)

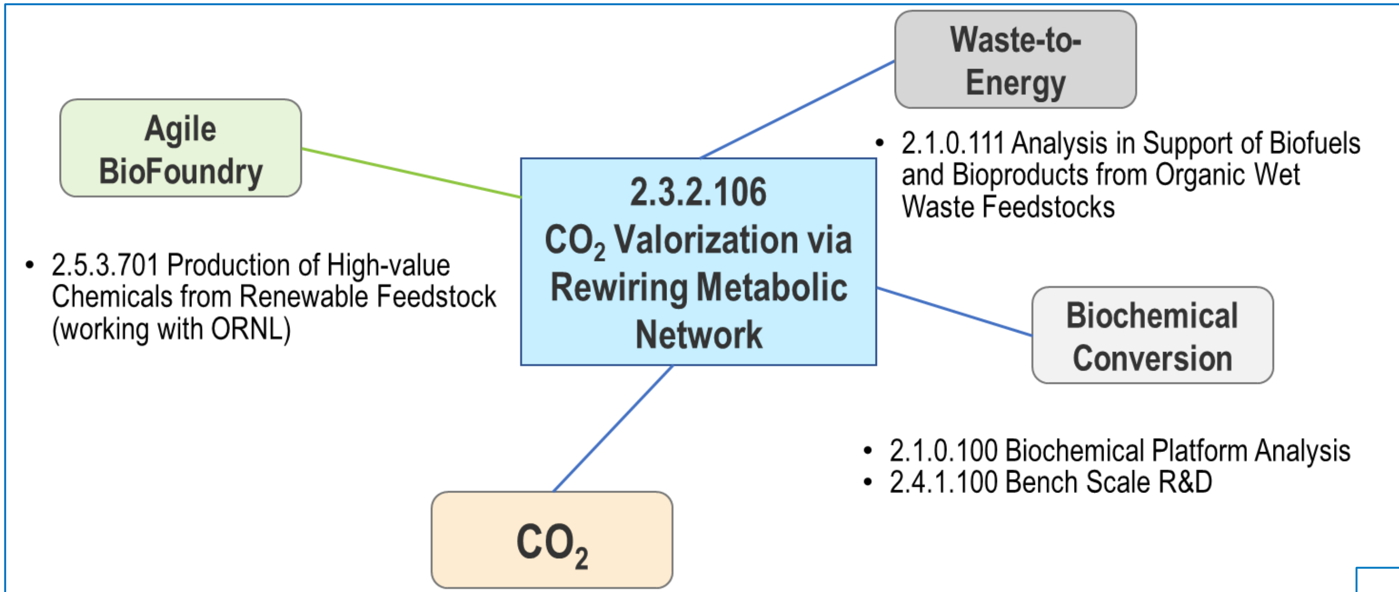


Gas Bioreactor



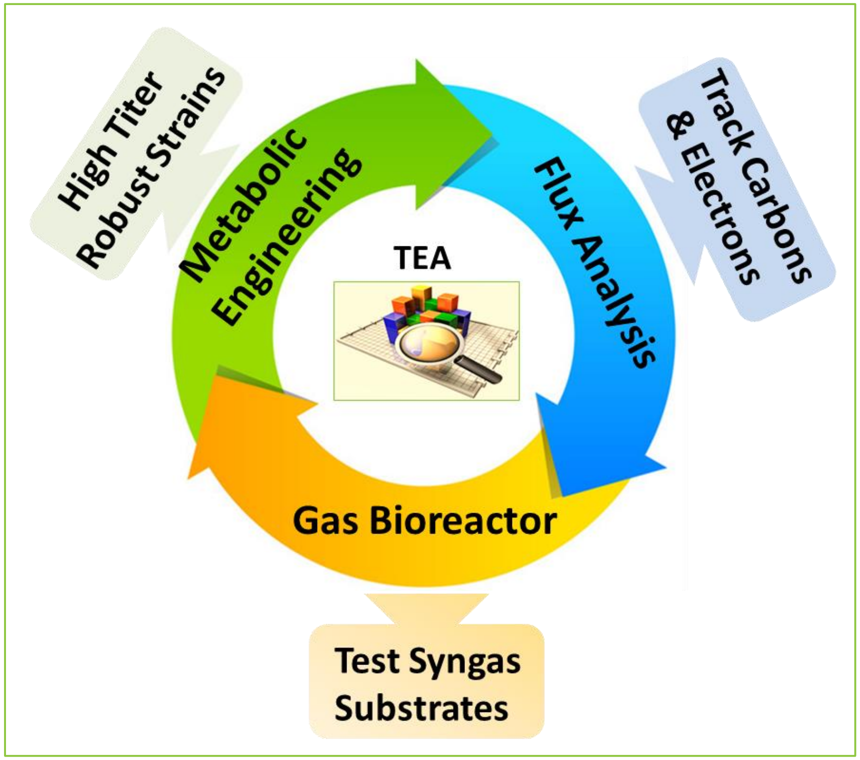
2 – Approach (Management)

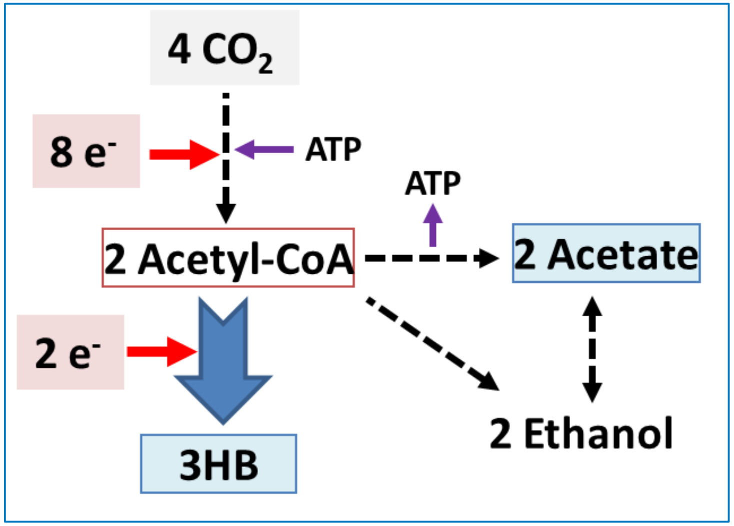
- **Interact with teams** across the Biochemical Conversion and CO_2 Platforms at NREL, ORNL, and industry.
- Participate in a **multi-lab Agile BioFoundry (ABF)** project teaming with ORNL.
- Team with industrial partner Visolis on ABF and DOE SBIR Phase 1 and Phase 2 awards, the latter producing intermediates from syngas fermentation (+/- sugar) for upgrading.



- **2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO_2 and Biomass**
- **2.3.1.316 CO_2 Utilization: Thermo- and Electrocatalytic Routes to Fuels and Chemicals.**
- **2.3.2.111 Improving formate upgrading by *Cupriavidus necator***
- **2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products**
- **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**
- **5.1.3.102 Biomethanation Upgrade**

2. Approach (Technical)

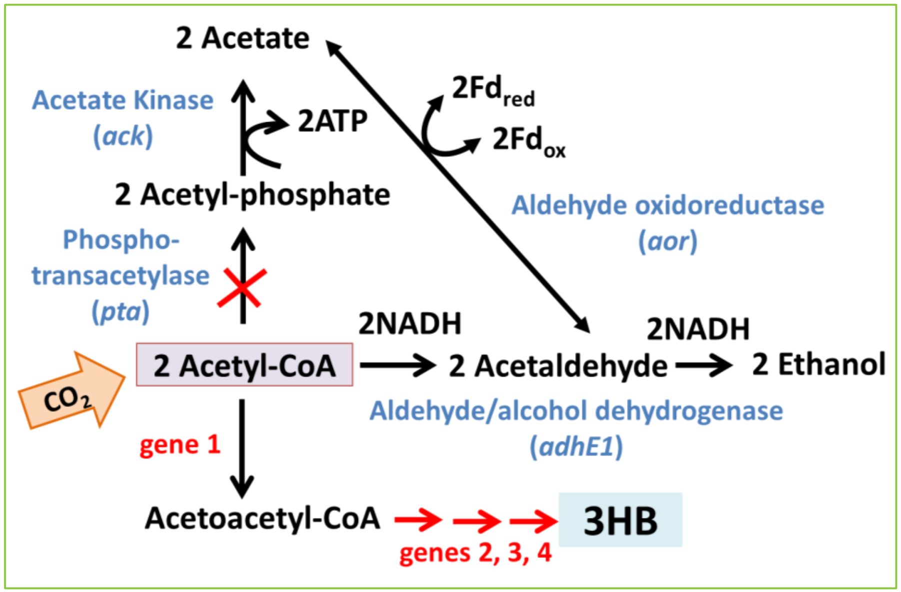


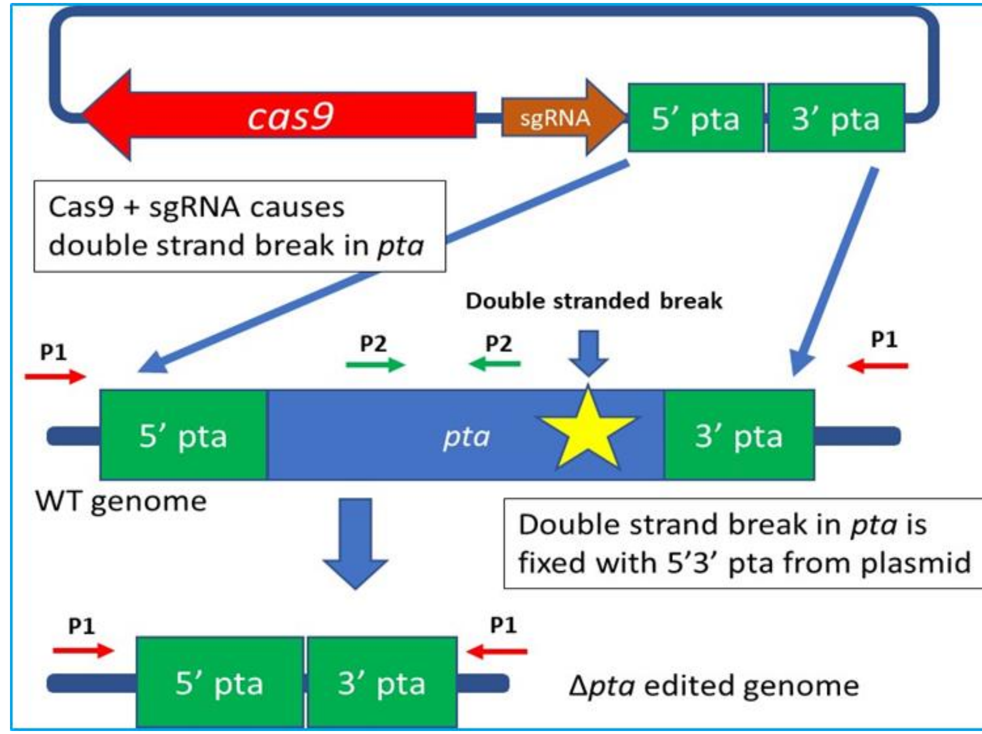


Approach: Develop genetic tools, pathway analysis, metabolic engineering, ^{13}C-metabolic flux analysis, reactor gas feeding strategy, and TEA to develop *C. ljungdahlii* as the robust host for CO_2 reuse.

- **Success factors:** (1) durable and robust microorganisms; (2) high biological productivity; and (3) enhanced gas-to-liquid mass transfer – all are critical to commercial viability.
- **Challenges:** (1) redox and energy balance for durable microbes; (2) pathway engineering to boost product titer and yield; and (3) mass transfer - gaseous substrate requires continuous operation which consumes energy.

3 – Technical Accomplishment: Developed CRISPR Genetic Tool

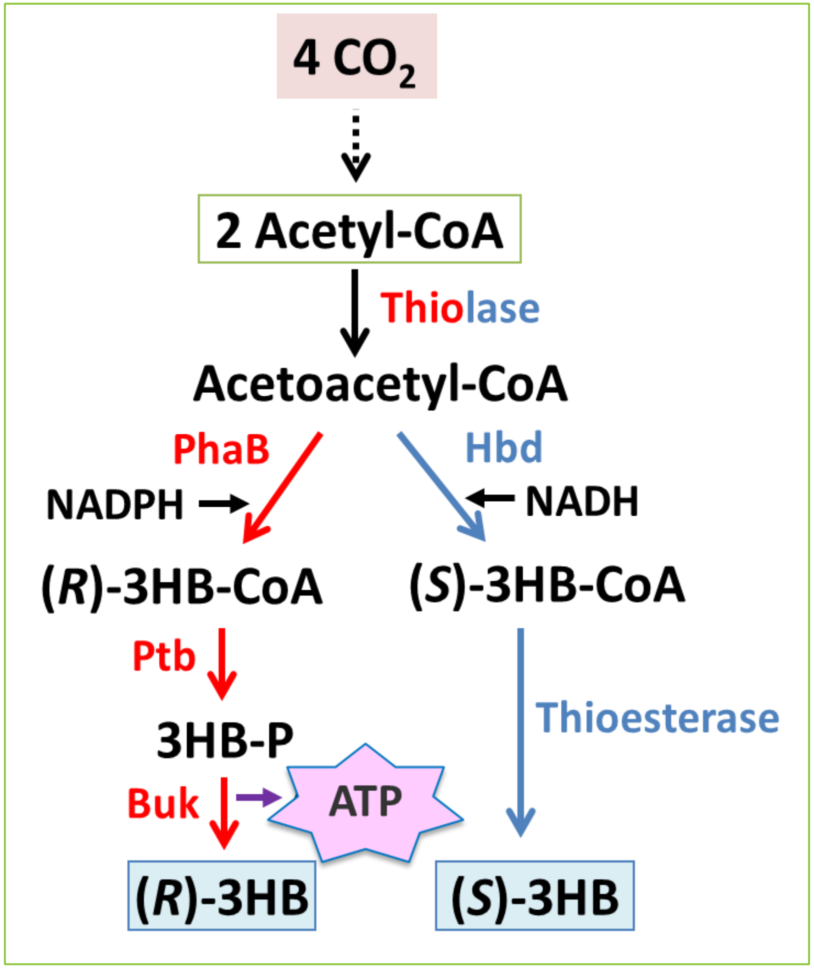




- **Developed CRISPR-Cas9 genome editing tool with <u>high efficiency, accuracy, and throughput, while leaving no antibiotic marker</u>.**
- Generated Δ*pta* knockout mutant, aimed to increase flux of acetyl-CoA toward more 3HB.
- The CRISPR tool is applicable toward "**organism development**."

3 – Technical Accomplishment: 3HB Pathway Analyses

- Conduct bioinformatics analysis and evaluate the best options for 3HB pathway: *completed FY18 Q1 Progress Measure*.
  - Evaluate both NADH (more abundant) route vs. NADPH (less abundant) route;
  - **Yet NADPH route yields more ATP**.
- Codon-optimized and synthesized 3HB pathway genes.
- Construct/express synthetic pathways using a combinatorial approach with varying promoters/genes/ribosome-binding sites.

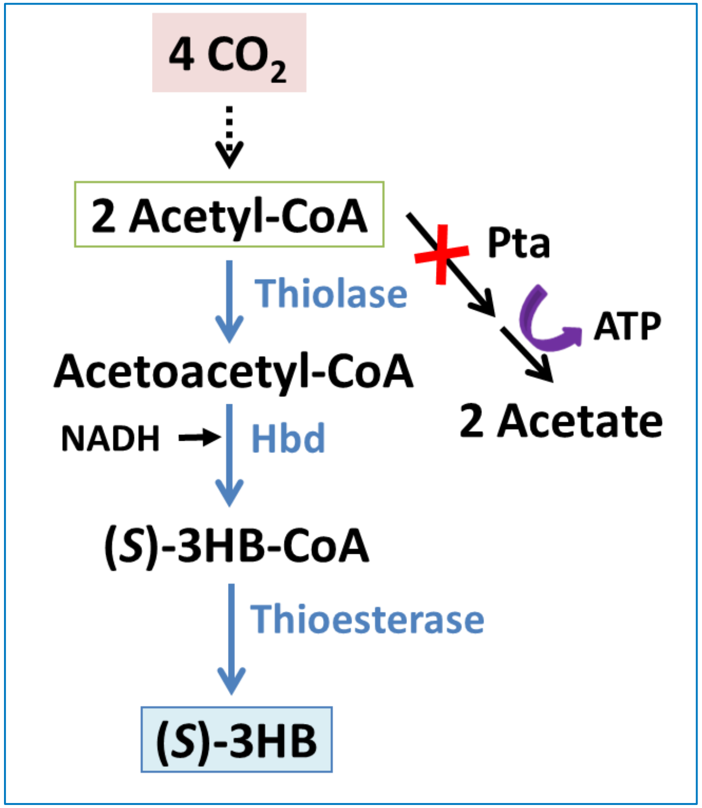


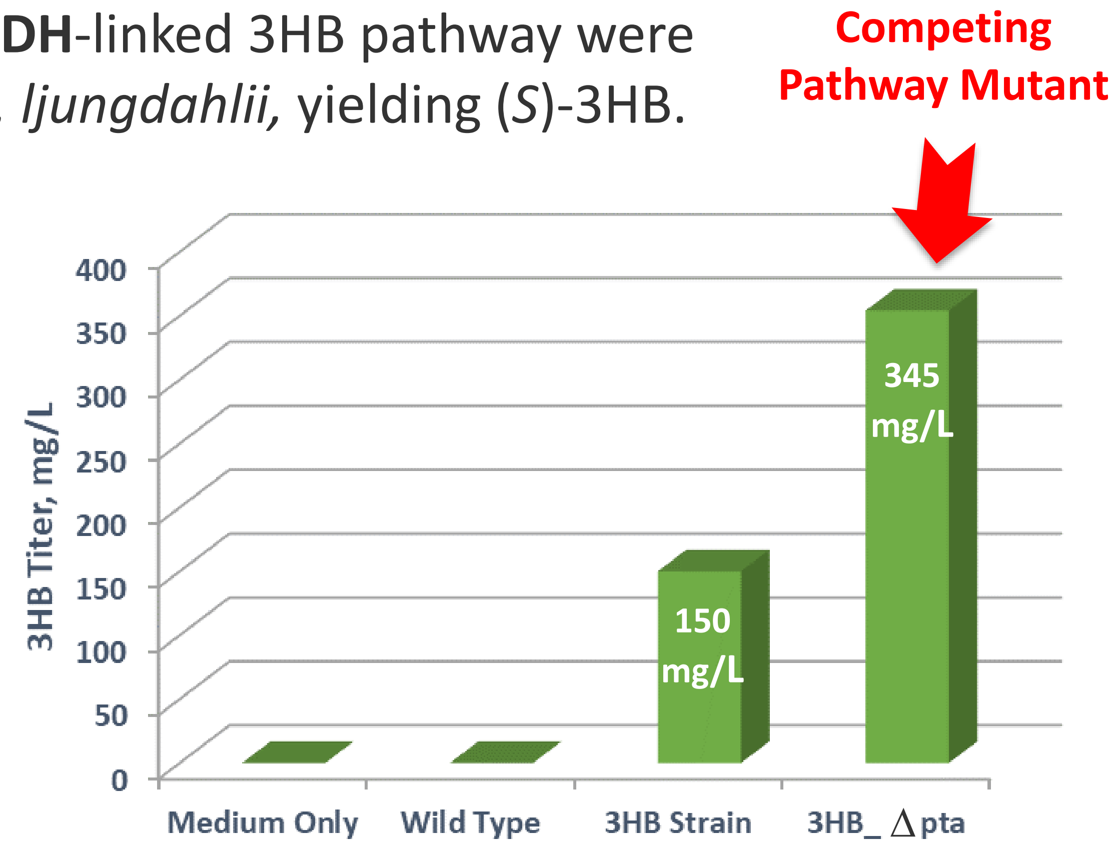
PhaB: 3-OH-CoA dehydrogenase (*Ralstonia eutropha*)
Ptb: phosphotransbutyralase (*C. acetobutylicum; Ca*)
Buk: butyrate kinase (*Ca*)
Hbd: 3-OH-CoA dehydrogenase (*Ca*)
Thiolase: *E. coli* or *Ca* origin

Comparing/contrasting NADH vs. NADPH route will guide the decision point to ensure redox and energy balance of the two routes for maximal 3HB productivity.

3 – Technical Accomplishment: Produce 3HB from *NADH* Pathway

Three genes encoding the **NADH**-linked 3HB pathway were expressed and functional in *C. ljungdahlii,* yielding (*S*)-3HB.



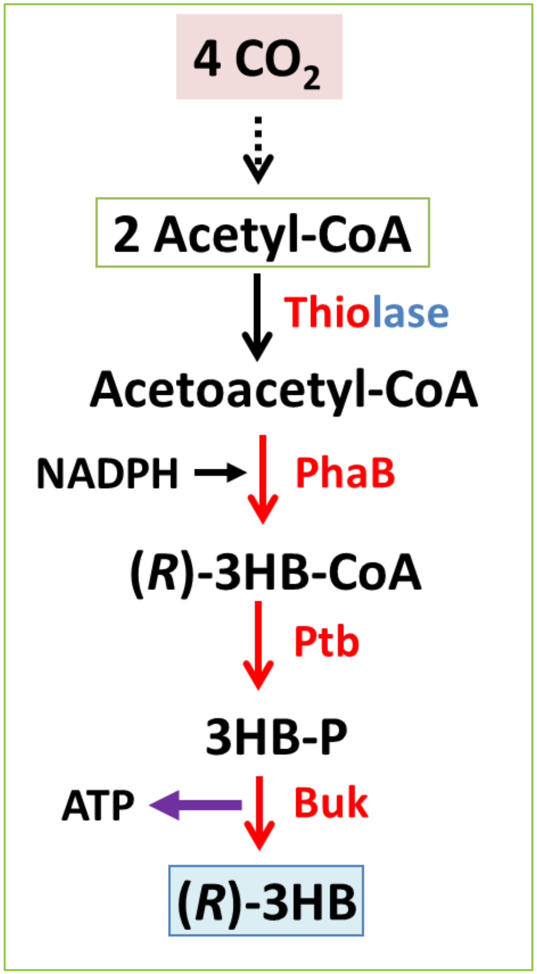


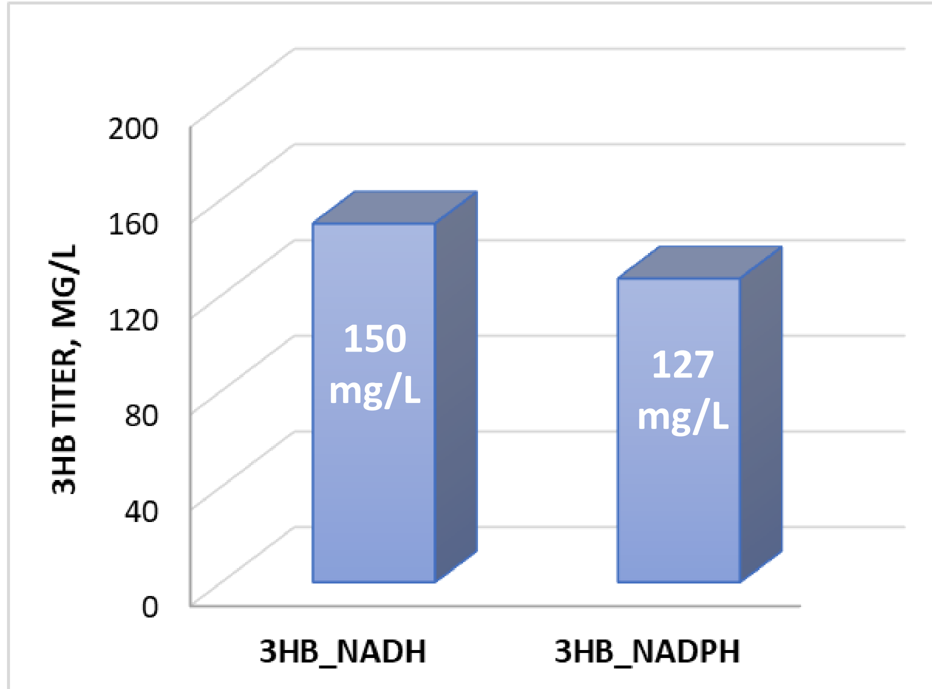
Pta: phosphotransacetylase

- **Obtain a 3HB titer of 150 mg/L in the transgenic line, completing and exceeding the <u>FY18 Q4 Milestone</u> technical target of 50 mg/L by 3-fold (Year 1).**
- **Redirecting flux of acetyl-CoA in Δ*pta* mutant led to 345 mg/L of 3HB - a <u>2.3-fold</u> increase, which validates <u>metabolic pathway redirection</u> strategy.**

3 – Technical Accomplishment: Produce 3HB from *NADPH* Pathway

Four genes encoding the **NAPDH**-linked 3HB pathway were expressed and functional in *C. ljungdahlii,* yielding (*R*)-3HB – FY19 Q1 Progress Measure.

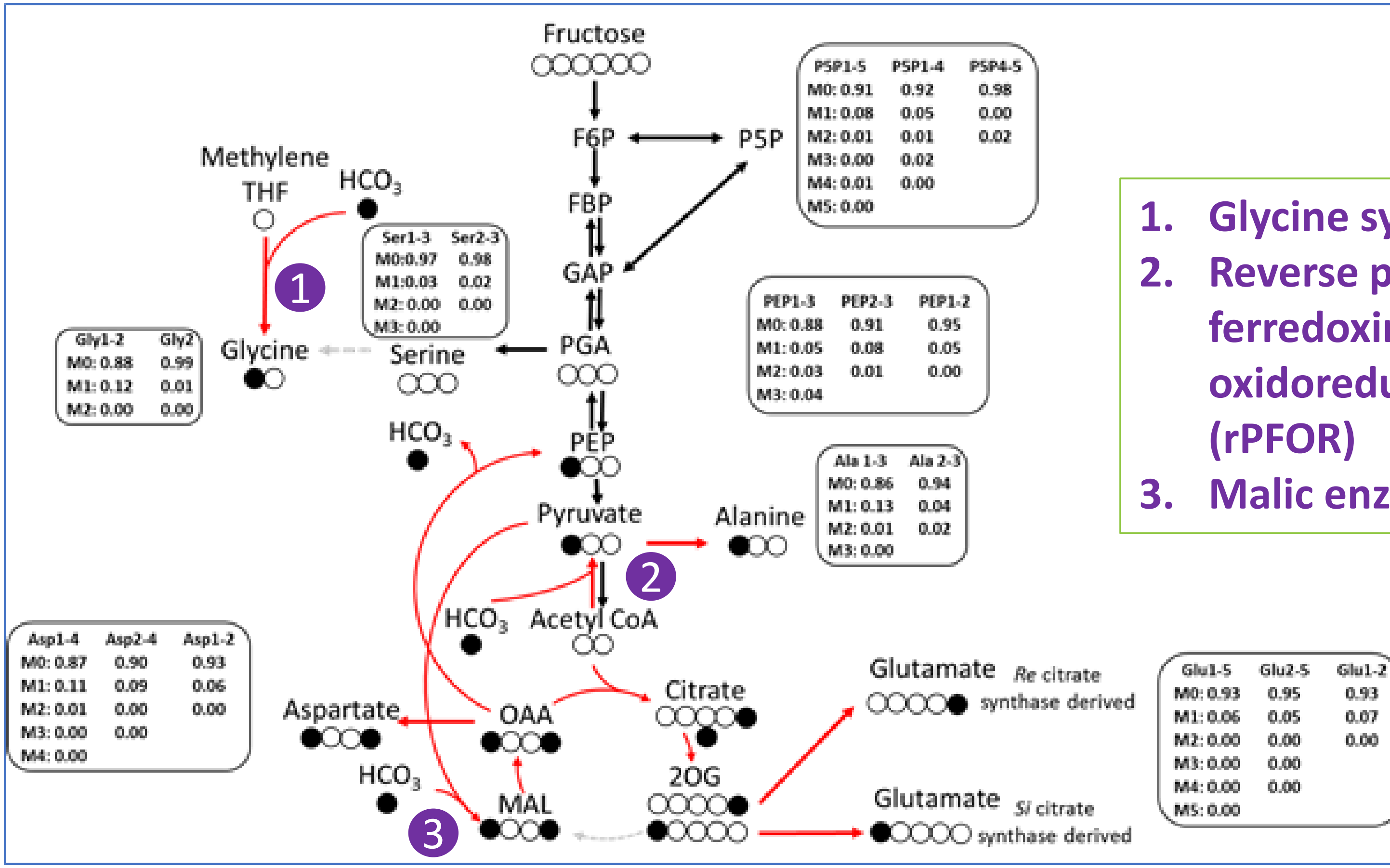




- NADH- or NADPH-linked pathway yields similar 3HB titer during growth on fructose where ATP is not limiting.
- **The extra ATP generated in the NADPH-route could be crucial for more robust organism under CO_2-fixation condition** where ATP is more limiting – ***Decision Point***.

3 – Technical Accomplishment: Develop Tool and Build Metabolic Flux Map using ^{13}C-tracer Analysis

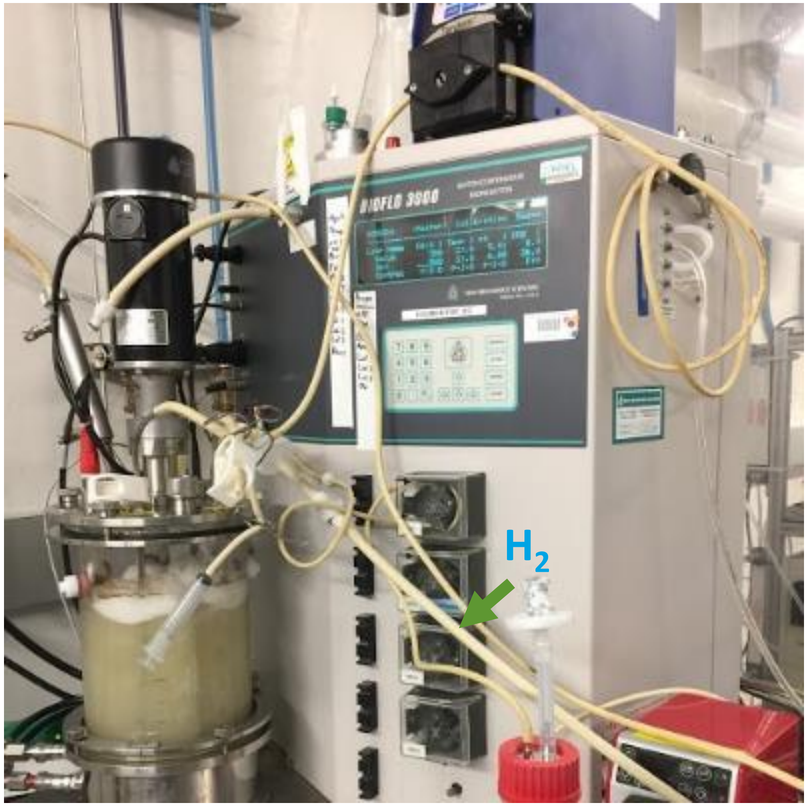
- Growth in fructose with **^{13}C-bicarbonate**, (OD ~ 0.8-1.0)

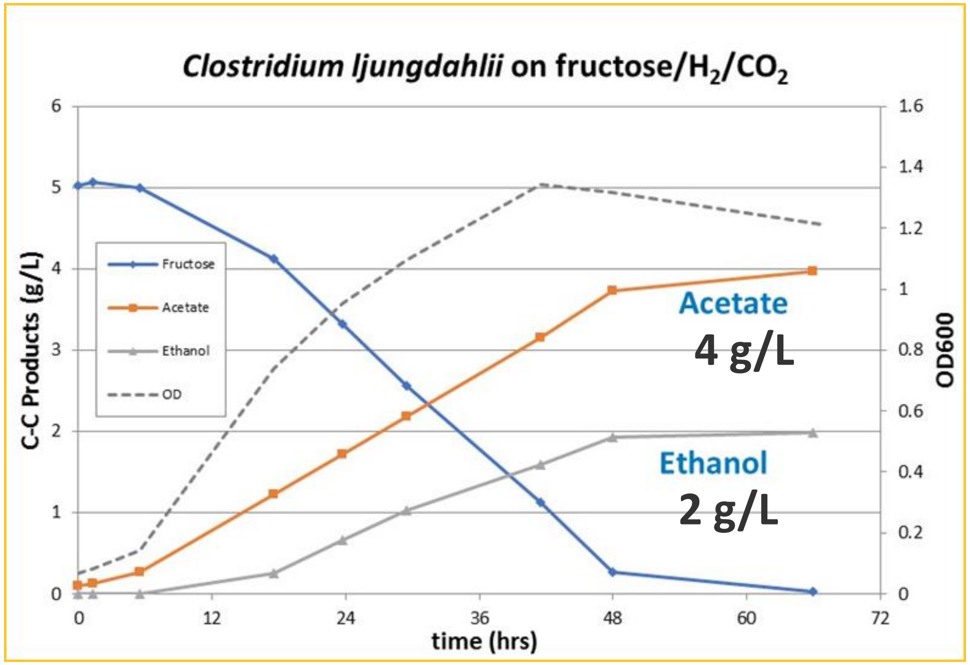


1. Glycine synthase
2. Reverse pyruvate-ferredoxin oxidoreductase (rPFOR)
3. Malic enzyme

- **Uncover three additional routes of CO_2 fixation into metabolic pathways, *in vivo*** – completed FY18 Q2 Progress Measure.
- New metabolic information will guide genetic engineering strategies.

3 – Technical Accomplishment: H_2-enhanced Carbon Yield during Mixotrophic Growth (fructose/H_2/CO_2)

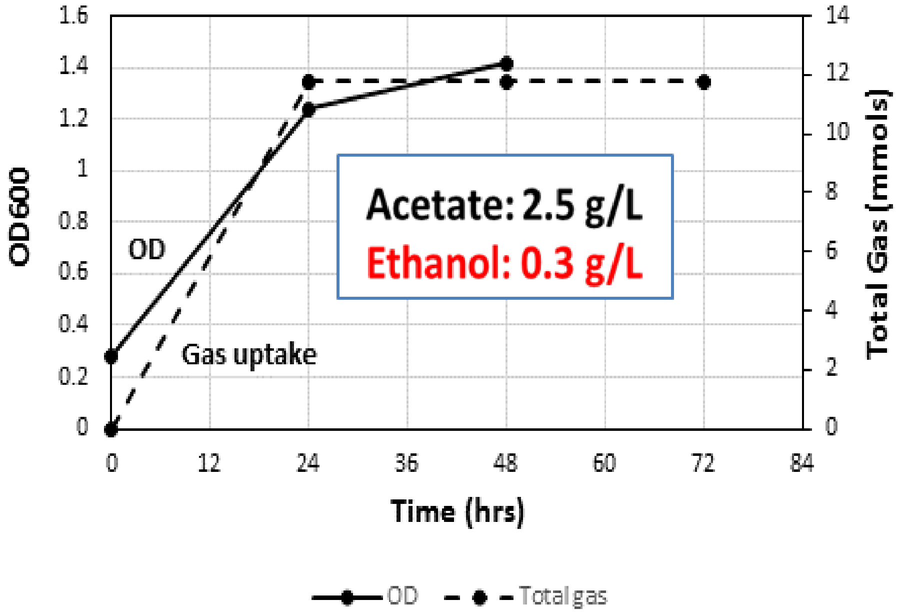




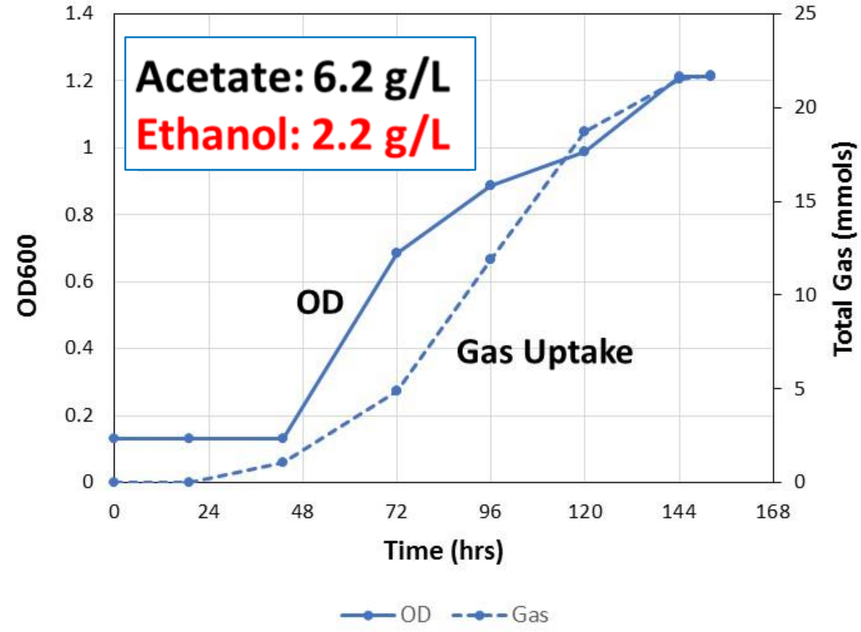
- Achieved a carbon yield of **132%** when the microbes metabolize sugar and **fix CO_2 *simultaneously*** using H_2.
- This high yield surpasses the <u>66%</u> theoretical maximum carbon yield in most microbes during glycolysis.

3 – Technical Accomplishment: Autotrophic Growth in H_2/CO_2 or $H_2/CO/CO_2$

Autotrophy in H_2/CO_2 (4:1)



Autotrophy in Syngas H_2/CO (57%:35%) balanced CO_2

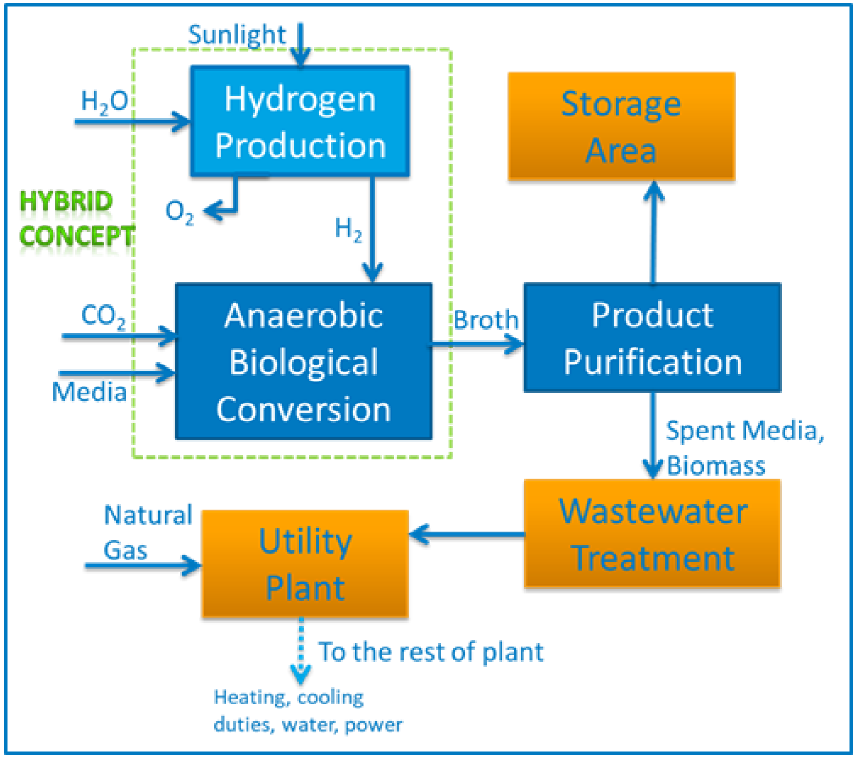


- Obtain an acetate titer of **2.5 – 6.2 g/L** under varying ratio of H_2/CO_2, completing and exceeding FY18 Q4 milestone of 1 g/L acetate (Year 1) .
- **Adding CO leads to more reduced product** using ethanol as a proxy (**7.2-fold increase**), which guides gas fermentation strategy to tune product profile.

3-Technical Accomplishment: Preliminary TEA

Designed Conceptual Process Concept

- H_2 supply system
- Biological H_2/CO_2 conversion
- Product purification and balance of plant
- Completed FY18 Q3 Progress Measure.



- **TEA Outcomes guide research directions.**
- **A 3HB minimal Product Selling Price of \$2.05/kg** was projected based on:
  - A H_2 cost of \$1.57/kg
  - 51% CO_2-to-3HB carbon efficiency
  - **3HB Productivity of 0.2 g/L/h**

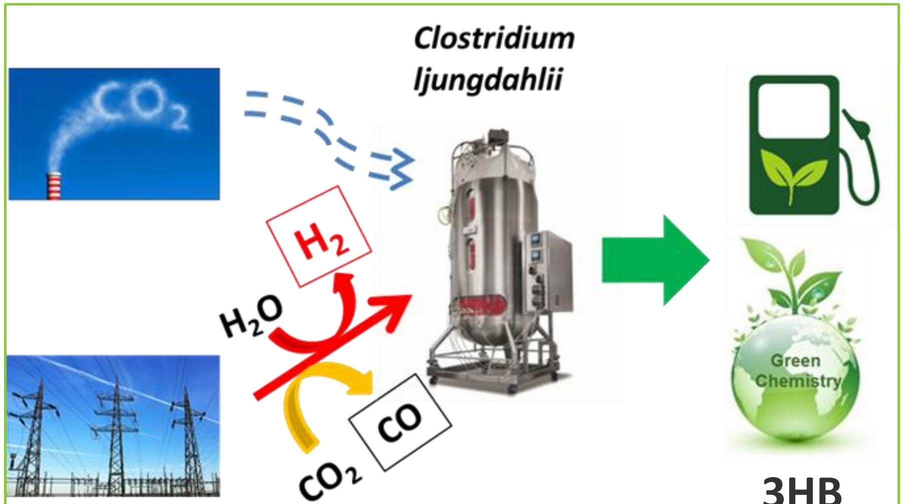
Primary cost drivers are H_2 cost and CAPEX of the <u>biological conversion step, the latter is the early-stage R/D focus</u>.

4 - Relevance

Lowering the cost of waste CO_2 reuse via developing CO_2-relevant bioenergy technology to rewire the carbon economy.

- Our fuel infrastructure is energy-dense and carbon-based. CO_2 is the most abundant carbon feedstock on earth and its innovative reuse will transform and revolutionize the biofuels industry.
- **Directly support BETO's mission to**
  - ***"Develop industrially relevant, transformative, and revolutionary bioenergy technologies..."***
- **Directly support BETO's strategic goal to**
  - ***"Enable use of America's abundant waste resources, i.e., CO_2 for advanced biofuels/bioproducts."***

- Project success will interface with various industrial sectors including fossil power plants, biofuels industry, and various industrial manufacturing processes (i.e., iron/steel, cement, fertilizers) to reuse their waste CO_2 and reduce overall cost.



4 - Relevance

BETO MYPP recognizes that "Organism development is an <u>enabling technology</u> to address research barriers aimed to decrease development time for industrially relevant CO_2-fixing microbes."

- Guided by TEA to "increase biological productivity", this project focuses on ***organism development*** to achieve targets/milestones as outlined in the BETO MYPP and is relevant to its "CO_2 Utilization Technology."
- Develop a cross-cutting technology to transform a new carbon-based economy, monetize waste CO_2, and better manage carbon footprint, which collectively will create jobs and stimulate US economy.

Technology Transfer/Marketability:

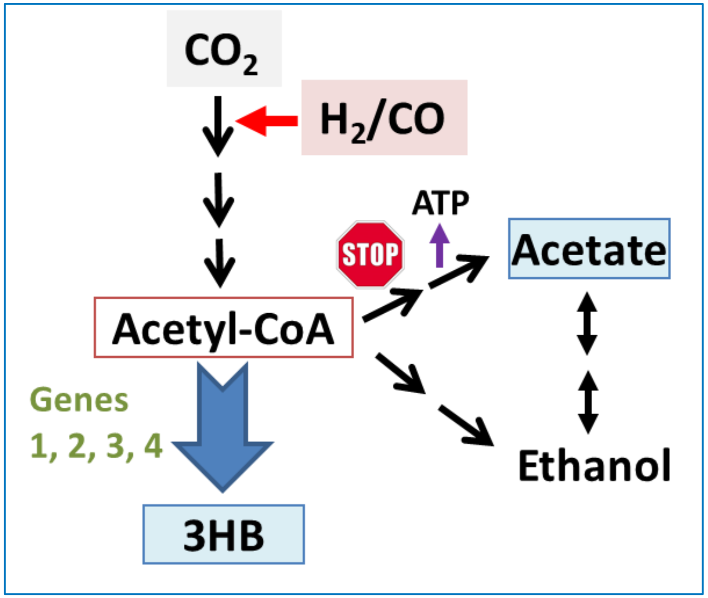
- Ongoing collaboration with **<u>Visolis</u>** on two synergistic projects: (1) Agile BioFoundry project and (2) DOE SBIR Phase 1/2 projects.
- ***This early industry engagement will guide R&D directions and address the needs of industry and market place.***

VISOLIS
CARBON NEGATIVE MATERIALS

5 – Future Work: FY19

- **Go/no-Go (18 mo.) Decision:** obtain a 3HB titer of **400 mg**/L in *C. ljungdahlii* cultured in $H_2/CO/CO_2$ enriched atmosphere…via deleting carbon- or electron-competing pathway……
- **FY19 Q4 Milestone:** Obtain a 3HB titer of **800 mg**/L in an engineered *C. ljungdahlii* lacking at least one competing pathway, in an atmosphere enriched in $H_2/CO/CO_2$.

- **Increase Metabolic Flux from CO_2 to 3HB**
  - Compare NADH- vs. NADHPH-linked route in varying $CO/H_2/CO_2$ gas compositions.
  - Down select best strains
  - Conduct ^{13}C-fluxomics to identify bottlenecks
- The best strains and growth conditions will help complete the 18-mo. Go/No-go Decision and FY19 Q4 Milestone.



- **Perform TEA**
  - Evaluate economic potentials of a variety of products such as acetate, 3HB, etc.
  - Rank R&D criteria using inputs from R&D on carbon flux and energy efficiency potentials, as well as market assessment and environmental benefit potentials.

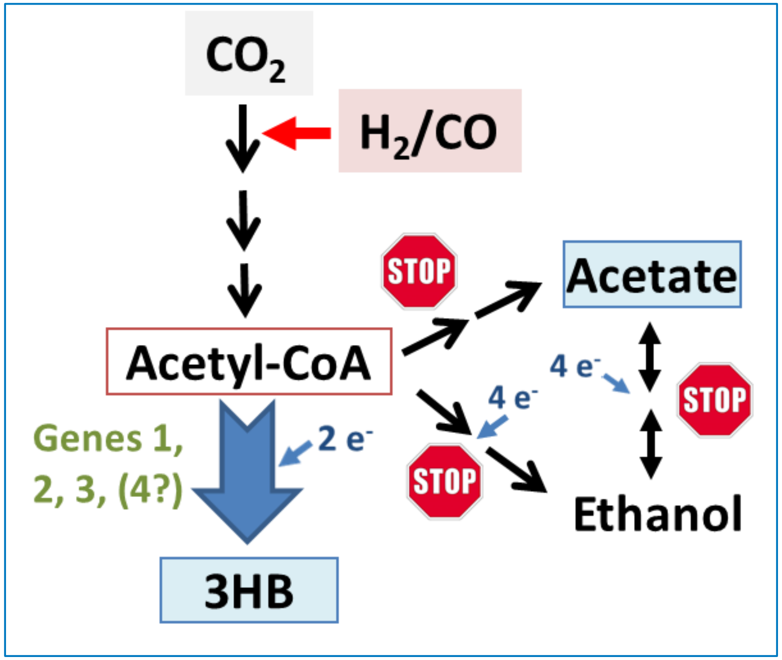
5 – Future Work: FY20

FY20 Q4 Milestone: Perform detailed TEA for the integrated concept from CO_2 to the down-selected products (acetate or 3HB) including biological conversion, ..report key cost drivers, integration strategies…and a path forward to achieve cost target of \$2/GGE.

- **TEA Effort**
  - Evaluate overall economic potentials for integrated process concept
  - Identify key cost drivers
  - Define innovative and relevant pathways to achieve cost competitiveness

Governing Equation	Condition	[H] Yield	[C] Yield
$9H_2+4CO_2 \rightarrow C_4H_8O_3 + 5H_2O$	Anaerobic	44%	100%
$5H_2+4CO \rightarrow C_4H_8O_3 + H_2O$	w/CO	80%	100%

- **Increase Metabolic Flux from CO_2 to 3HB (2 g/L)**
  - Down-select the best 3HB strains.
  - Overexpress key 3HB-pathway genes.
  - Block additional competing pathways (e.g., ethanol).
  - Conduct ^{13}C-fluxomics to uncover flux redirections and identify bottlenecks to yield 3HB at 2 g/L.



Summary

- **Overview:** Using electricity to power CO_2 reduction will expand the renewable energy feedstock portfolio, bypass land-use/water requirement, and kickstart **an economy based on CO_2 to rewire the carbon cycle**.
- **Approach:** *C. ljungdahlii* is a model non-photosynthetic CO_2-fixing microbe with **inherent high carbon- and energy-conversion efficiency**, both are important premises to generate sustainable bioenergy for BETO mission and industry needs.
- **Accomplishments:**
  - Developed **CRISPR** and generated 3HB strains with a titer of 345 mg/L .
  - Generated a metabolic flux map and uncovered new CO_2-fixation pathways.
  - Adding CO could tune product profiles, which guides gas feeding strategy.
  - TEA identified **biological conversion step** as the key cost driver and focus of early-stage R&D.
- **Relevance:** Provide an efficient pathway for CO_2 reuse that will benefit biofuels, fossil fuels, and manufacturing industries and mitigate CO_2 emission.
- **Future work:** increase 3HB titers to 2 g/L via metabolic engineering, flux analysis, and CO_2/CO/H_2 gas feeding, with data input into a TEA to provide a research path forward to achieve cost target .

Acknowledgements

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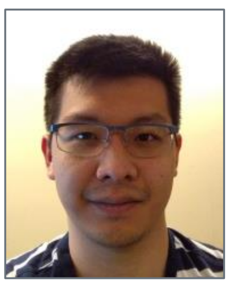
BIOENERGY TECHNOLOGIES OFFICE

NREL
NATIONAL RENEWABLE ENERGY LABORATORY



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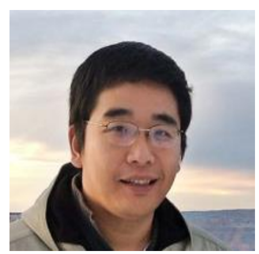
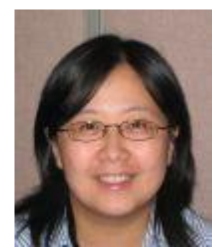


Wei Xiong



Ling Tao

Lauren Magnusson

Extra Slides

Responses to Previous Reviewers' Comments

None – this project was not reviewed previously.

Publications, Patents, Presentations, Awards, and Commercialization

- **Patents:** filed a Record of Invention (ROI-19-40): Biological Methanol Condensation to Higher-order Alcohols by Engineering a Non-photosynthetic C1-troph.

- **Commercialization Efforts**
  - Shell International Exploration & Production **awarded** NREL a project (upon their request) entitled "Hybrid CO_2 Valorization to High Titer Isopropanol." This work is in collaboration with Princeton University to co-develop a biohybrid approach for CO_2 upgrade. BETO can leverage industrial funding and technical progress to accelerate DOE research.
  - Teamed with **Visolis** and received DOE SBIR Phase I and Phase II awards, work for the latter is ongoing.