DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Multi-pronged approach to improving carbon utilization by cyanobacterial cultures

March 23, 2021
Advanced Algal Systems
WBS: 1.3.2.410

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Arizona State University

Project Overview

Problem statement: Bubbling cultures or ponds with CO_2-enriched air is very inefficient as most of the CO_2 escapes into the atmosphere

This contributes to cost (CO_2 is $50-100/tonne at scale) as well as to greenhouse gas emissions

Our approach: Test both physical/physicochemical and biological approaches to enhance carbon utilization, determine which one(s) are best, and then combine those. All are new approaches in the context of scalable microalgal production, so high risk

Where we are in the project:

- Start: October 2018
- Verification complete / start of project: January 2019
- Verification meeting, Go/No-Go at end of BP2: March 2020
- End of project: September 2021

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NanoGas™ TECHNOLOGIES

Project Overview

Test both physical/physicochemical and biological approaches to enhance carbon utilization, determine which one(s) are best, and then combine those

- **Task 2.** Enhance CO_2 absorption and retention in cyanobacterial culture media
  - CO_2 nanobubbles: greatly increased stability of gaseous CO_2 in the culture medium
  - Amines: react with CO_2 forming carbamates, thus pulling more CO_2 into the medium
- **Task 3.** Enhance bicarbonate uptake by the cyanobacteria
  - Overexpress bicarbonate transporters from different cyanobacterial strains in our strain
- **Task 4.** Introduce additional CO_2 fixation and conservation mechanisms in cyanobacteria
  - Overexpress enzymes that appeared to enhance carbon utilization in other studies
  - Minimize CO_2 production by cyanobacteria through pathway engineering
  - Enhance carbon utilization and cell growth with fermentation gas through adaptive laboratory evolution
- **Task 5.** Demonstrate improved CO_2 uptake efficiency in outdoor conditions
  - Outdoor cultivation trials at AzCATI
- **Task 6.** Techno-economic and life cycle assessments, and dynamic growth modeling
  - Assess economic viability and environmental impact of the system, and determine productivity estimates for different locations

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1 - Management

Team:

- ***Wim Vermaas*** (PI; ASU, Life Sciences)
    - Genetic modification of cyanobacteria; overall project oversight
- ***Al Darzins*** (Co-PI; Nano Gas)
    - CO_2 nanobubble development for cyanobacterial growth improvement
- ***Anna Keilty*** (ASU, Life Sciences)
    - Project coordinator; administration and financials
- ***John McGowen*** and ***Taylor Weiss*** (Co-PIs; ASU, AzCATI)
    - Outdoor cultivation
- ***David Nielsen*** (Co-PI; ASU, Chemical Engineering)
    - Improved CO_2 mass transfer with amines; bicarbonate transporters
- ***Jason Quinn*** (Co-PI; CSU, Mechanical Engineering)
    - Techno-economic analysis and life cycle assessments
- ***Xuan Wang*** (Co-PI; ASU, Life Sciences)
    - Metabolic engineering; adaptive laboratory evolution
- Two ***postdocs***, three ***graduate students***, several ***research staff*** members
- Biweekly two-hour meetings of the entire team to share results, discuss progress, risk and issues, and develop plans and mitigation strategies



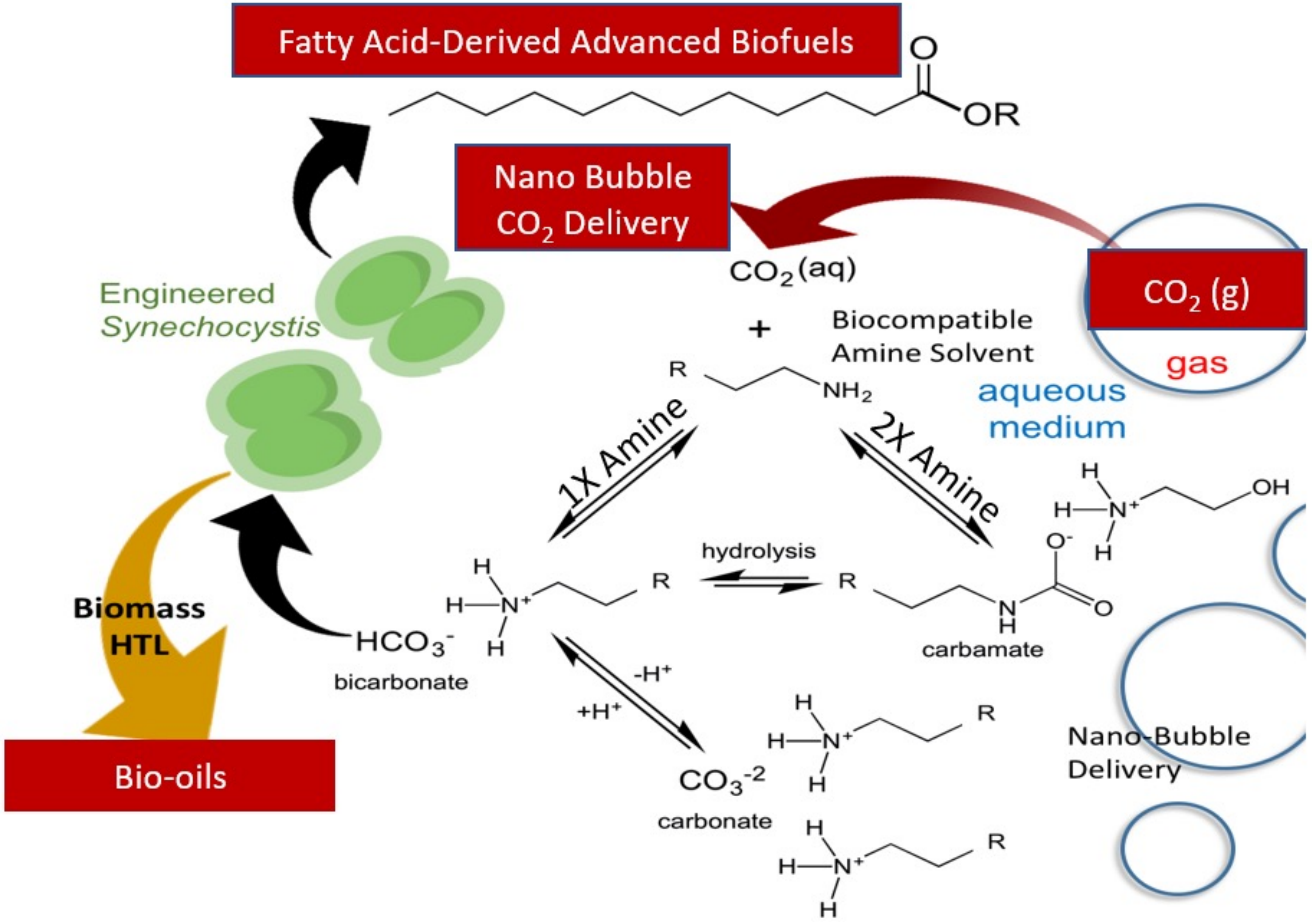





2 – Approach

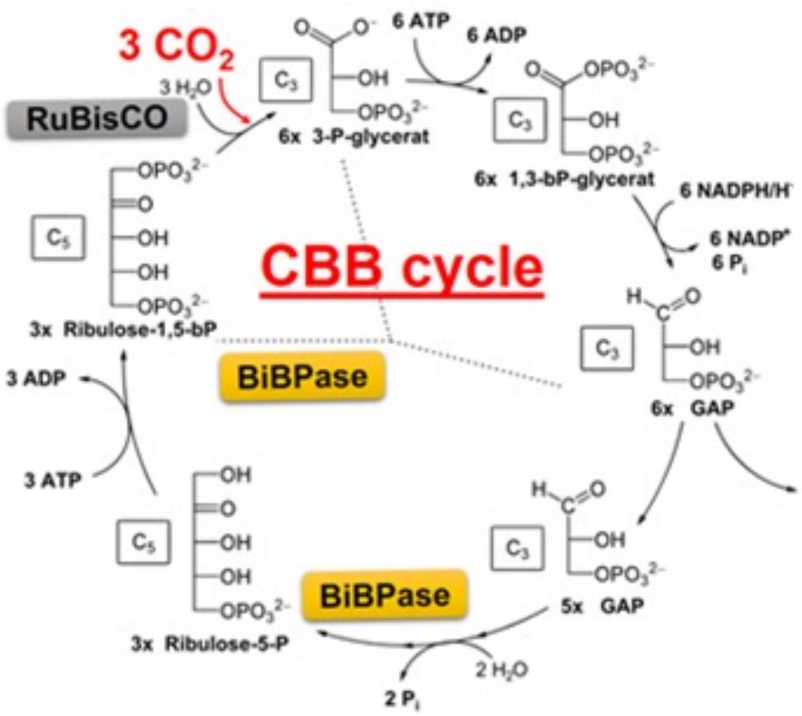
1. Improve CO_2 mass transfer into liquid using biocompatible amine solvents and nanobubbles

Integrate with TEA/LCA to determine the economic feasibility and impact on emissions

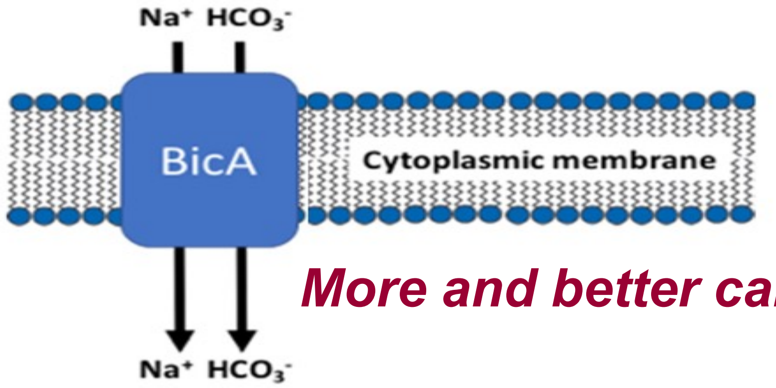


2 – Approach

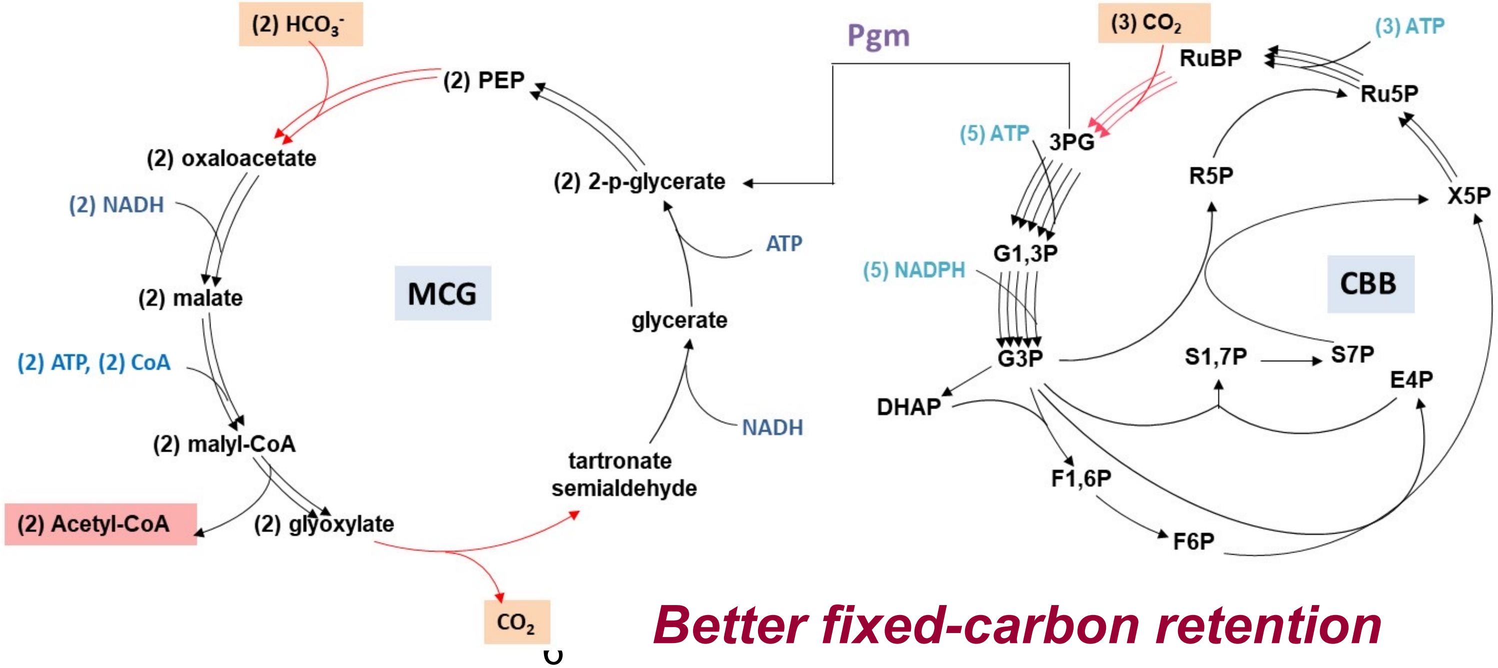
2. Improve bicarbonate uptake and carbon fixation in cyanobacteria



Better CO_2 fixation and storage



More and better carbon uptake



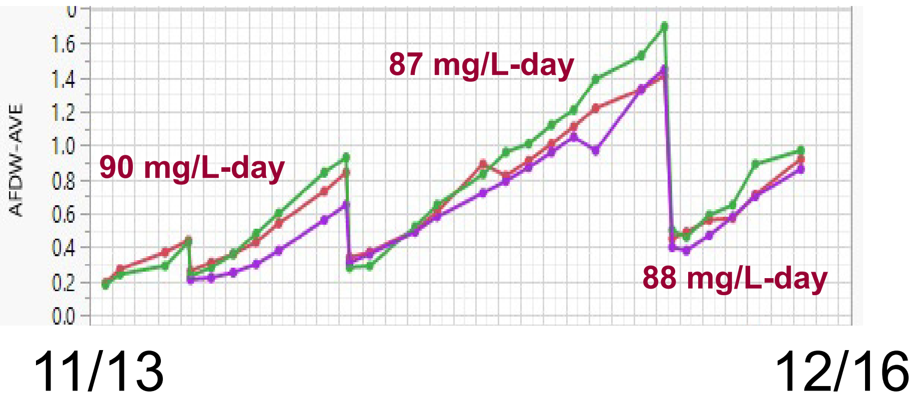
Better fixed-carbon retention

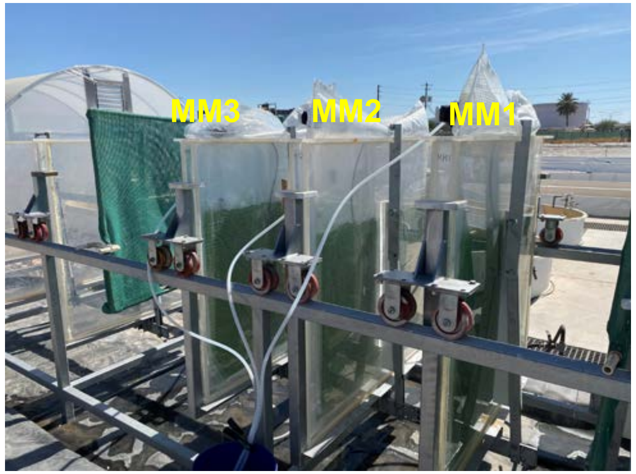
No decarboxylation to make acetyl-CoA

Integrate with TEA/LCA to determine the economic feasibility and impact on emissions

2 – Approach

3. Grow best strains in 50-L PBRs at AzCATI





Go/No-Go decision points:

Early 2020 (month 18): Demonstrate mechanisms resulting in 25% increase in CO_2 utilization efficiency by the biofuel-producing baseline strain under laboratory conditions. ✓

End of project (month 36): Isolate a biofuel-producing *Synechocystis* strain with at least a 15% increase in growth rate as measured by optical density at 730 nm at laboratory scale under conditions (light/dark cycling, temperature profile) relevant to outdoor culturing and supplemented with CO_2 from at least one industrial source

A challenge we encountered:

CO_2 utilization efficiency is hard to measure exactly:

We measure CO_2 in outlet gas from "blank" medium (no cyanobacteria) and from cultures; the difference between the two is CO_2 utilized. There is error in both measurements as the output from CO_2 probes is not very stable; distinguishing small gains (like 10%) in CO_2 utilization efficiency is difficult.

3 – Impact

- CO_2 utilization efficiency is an understudied field, yet it is key to economic considerations as well as to greenhouse gas removal
- In this project we test which approaches aid in improving CO_2 utilization efficiency:
  - Amines
  - CO_2 nanobubbles
  - Bicarbonate transporters in the cell
  - More CO_2 fixation in cells
  - Less decarboxylation in cells
  - Adaptive evolution using fermentation gas
  - Verify with 50-L PBRs at AzCATI
  - Use TEA/LCA to determine economics and greenhouse gas emission impacts
- Strategies that work will help CO_2 utilization in other projects: results are unlikely to be strain-specific
- Improving CO_2 utilization efficiency helps reaching BETO's 2030 \$2.50/gasoline gal equivalent goal as well as BETO's 2030 areal productivity target of 25 g m^{-2} day^{-1}

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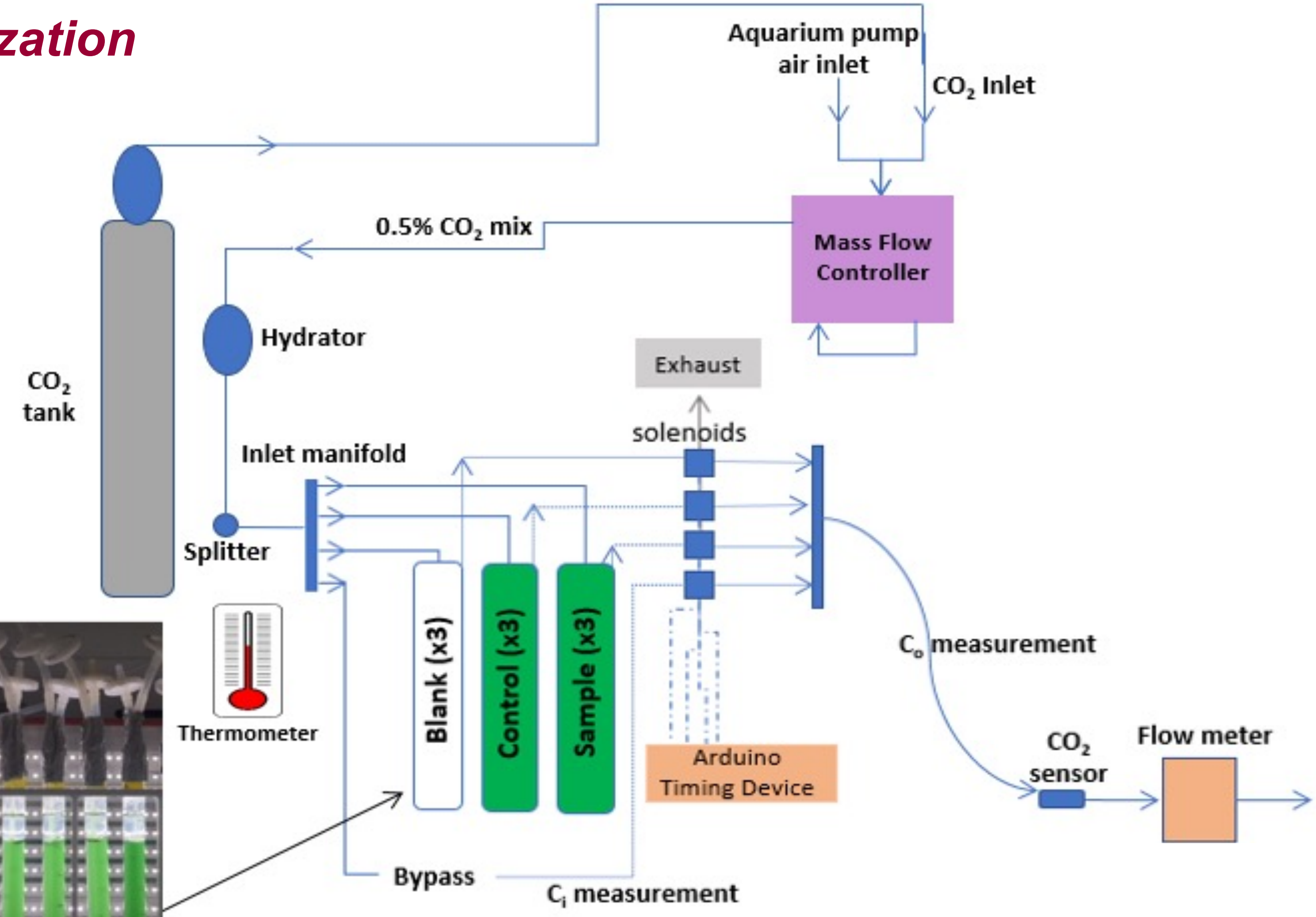
4 – Progress and Outcomes

General setup to measure CO_2 utilization by cells

Compare how much CO_2 is in the off gas from a culture vs. from a control with just medium

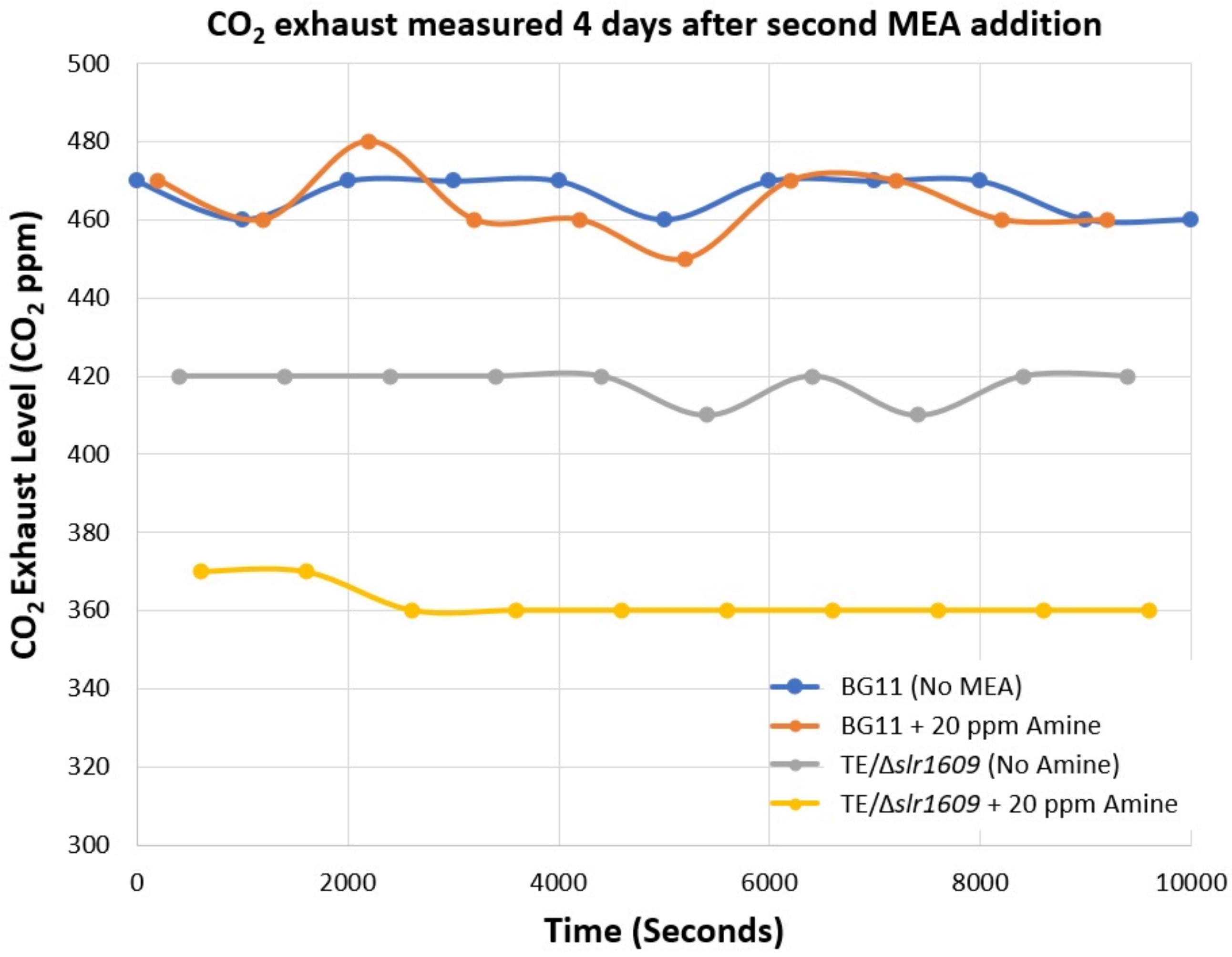


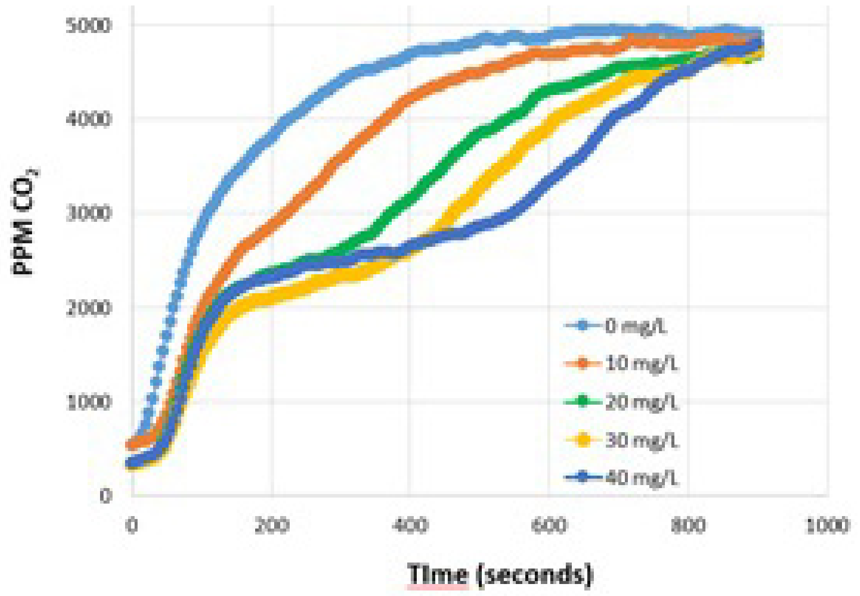
TysonTubes®
varies based on experiment, typically nine tubes total; multiple inlet manifolds can be connected to accommodate more sample tubes

4 – Progress and Outcomes

Task 2: CO_2 uptake in the presence of an amine in the culture

Early data:





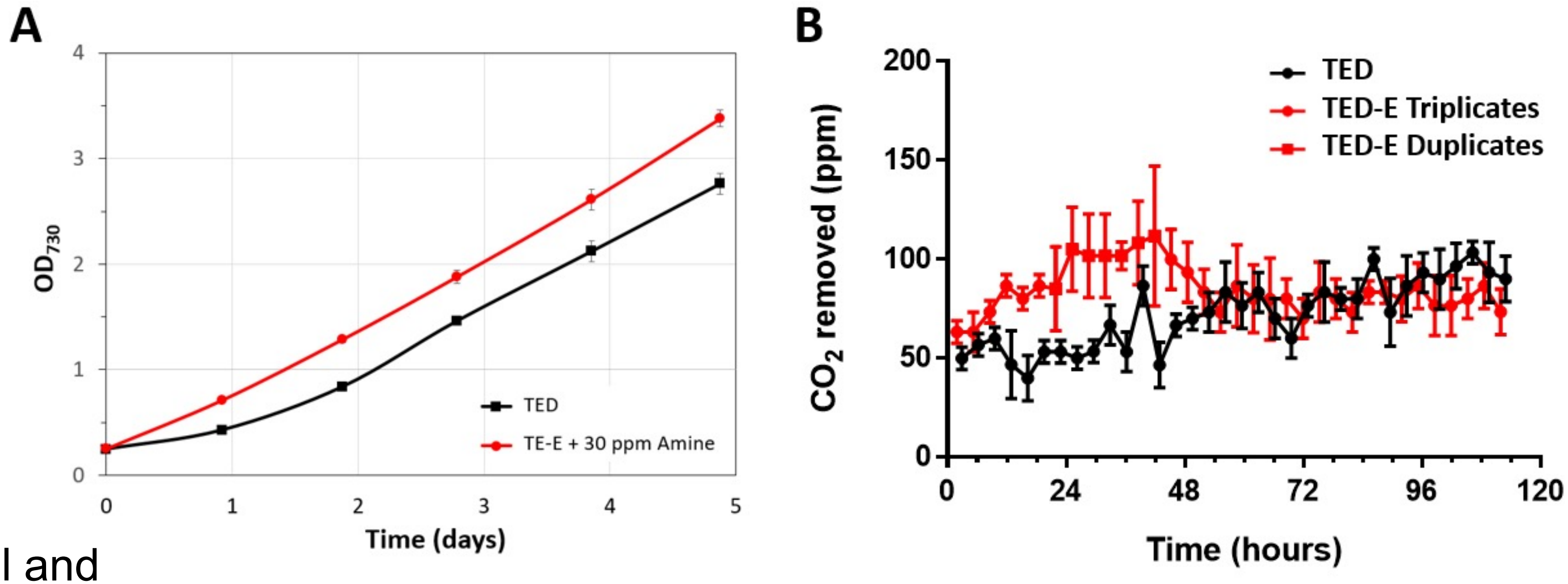
CO_2 breakthrough curves with BG-11 medium with 0-40 ppm of an amine. The area above each curve represents the CO_2 retained in the medium.

Much less CO_2 is in the exhaust gas stream of cultures in the presence of amine, indicating that more CO_2 is taken up from the gas stream

4 – Progress and Outcomes

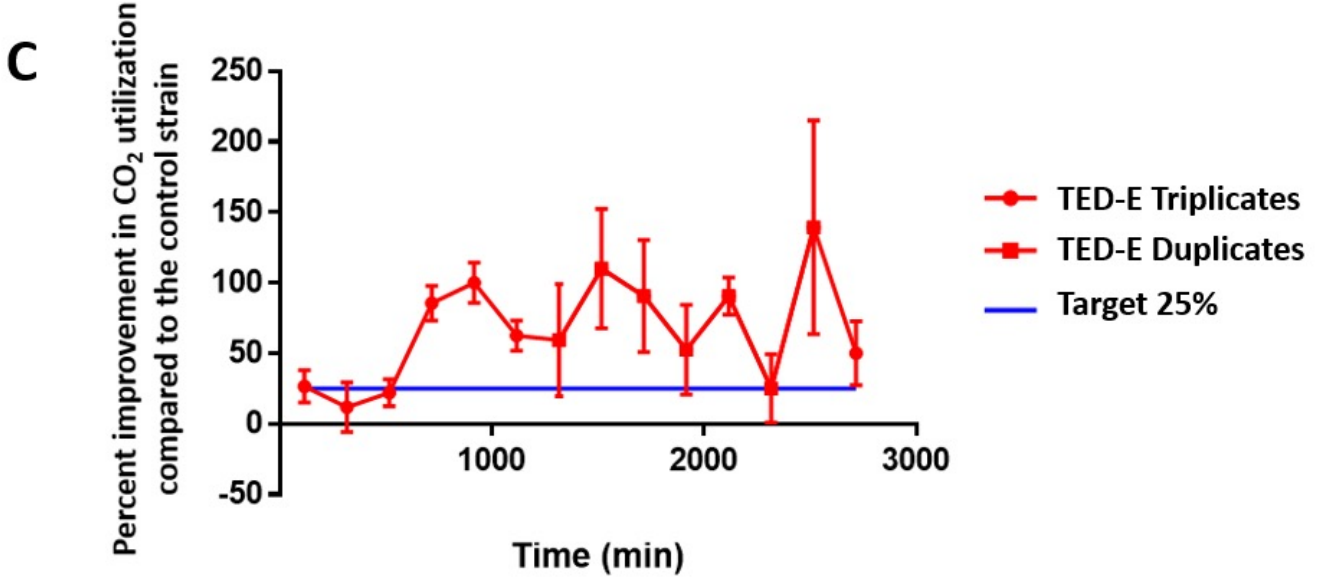
Go/No-Go between BP2 and BP3:

Demonstrate mechanisms resulting in 25% increase in CO_2 utilization efficiency by the biofuel-producing baseline strain under laboratory conditions.



In continuous light CO_2 removal and culture growth were greatly improved by the amine and in a spontaneously derived strain with better amine tolerance. CO_2 utilization was improved by much more than 25% in the first 2.5 days.

The amine advantage is more difficult to prove in diurnal light and temperature conditions, but we should have the answer after more replicates.

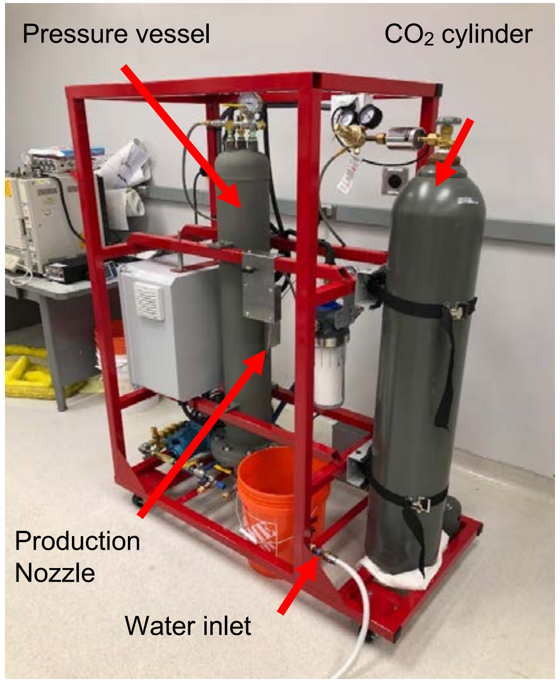


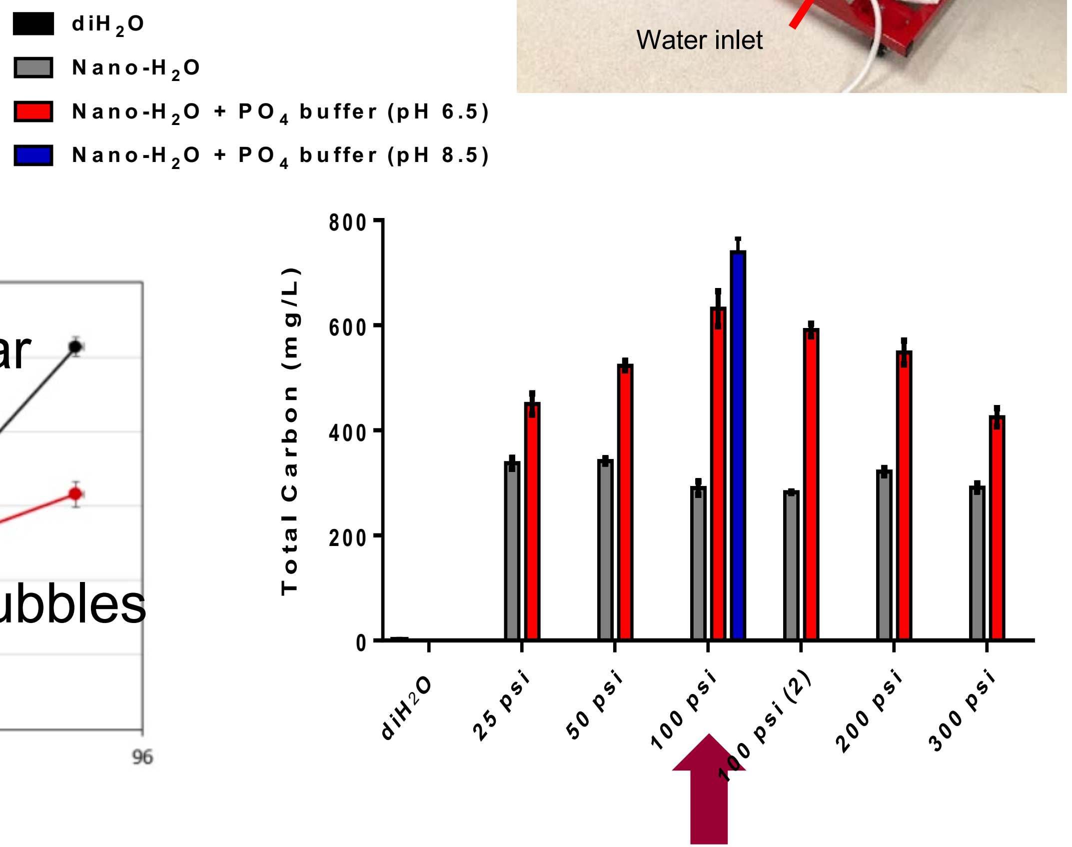
4 – Progress and Outcomes

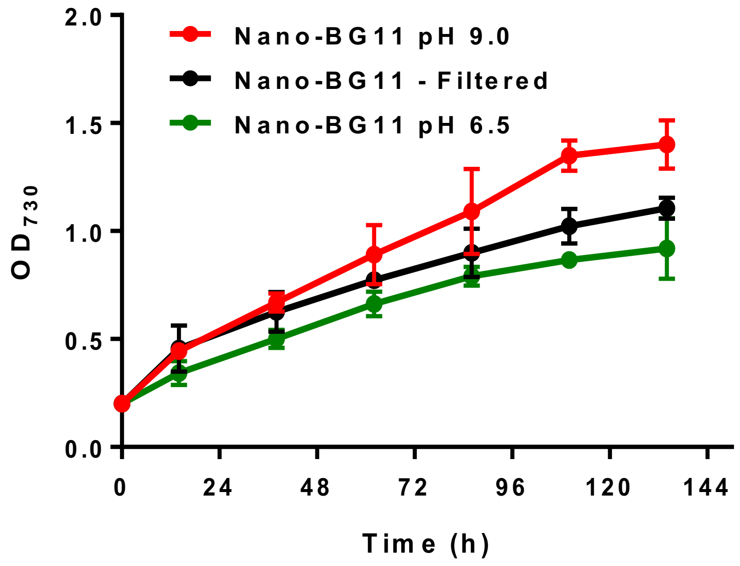
Task 2: Nanobubbles

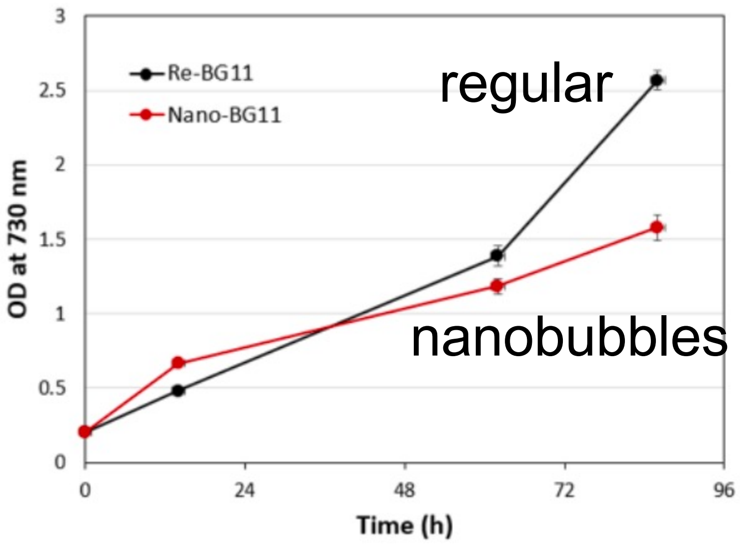
- A lab-scale nanobubble generator was fabricated
- Optimal operating pressure for CO_2 nanobubble production is 100 psi – saves on energy costs
- More carbon retained under alkaline conditions
- Typical CO_2 nanobubble size range: 50-200 nm
- Nanobubble concentration: about 10^8 per mL
- Cyanobacterial growth with nanobubbles exceeds that in normal growth conditions for the first day, but not after that
- Nanobubble generation procedures are now being modified

Lab-scale nanobubble generator



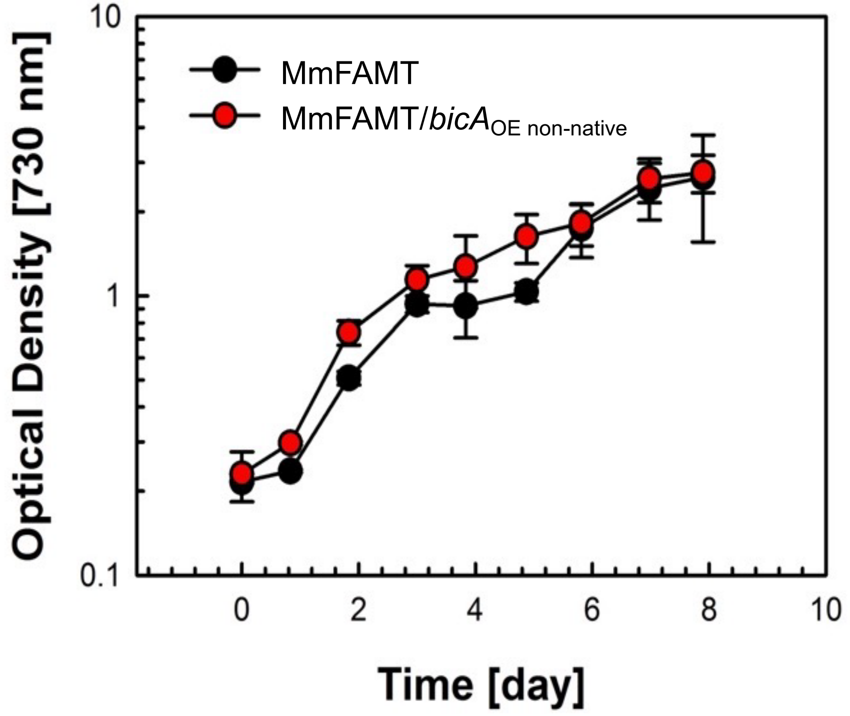






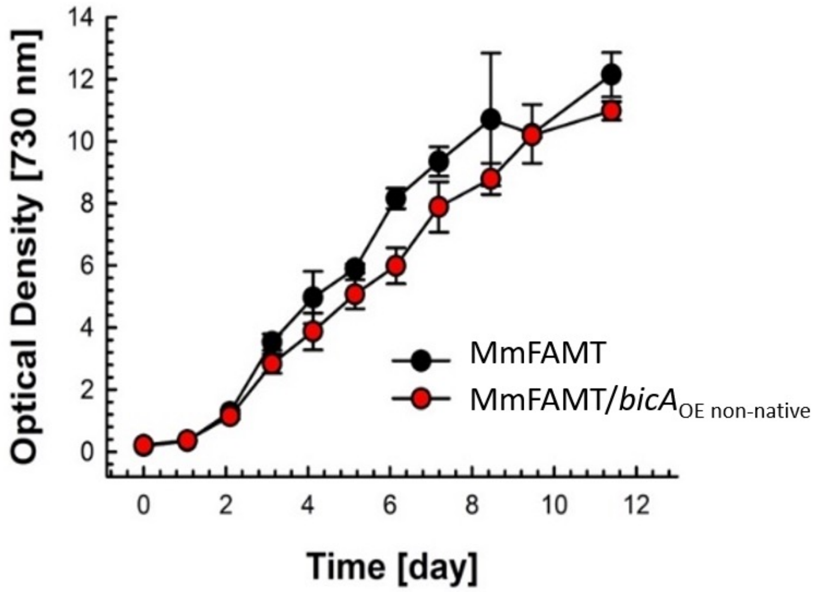
4 – Progress and Outcomes

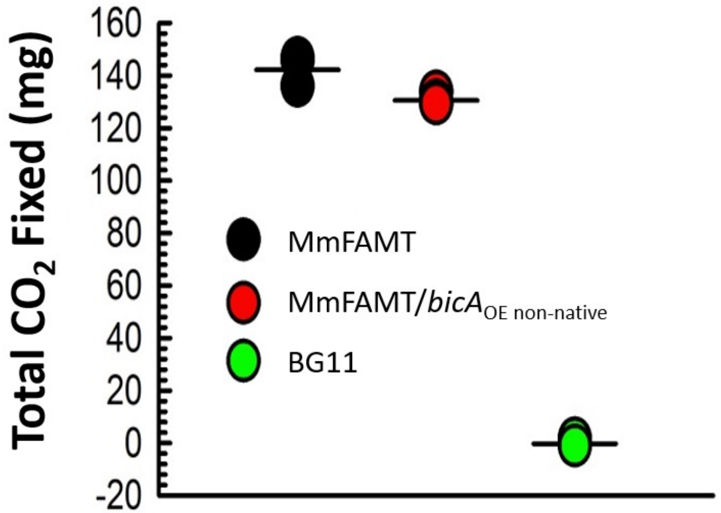
Task 3: Add bicarbonate transporters to strain



Overall, much less of a positive effect than sometimes is seen in the literature. Under ambient CO_2 conditions under continuous illumination at 150 µmol photons m^{-2} s^{-1} sometimes a small improvement is seen…

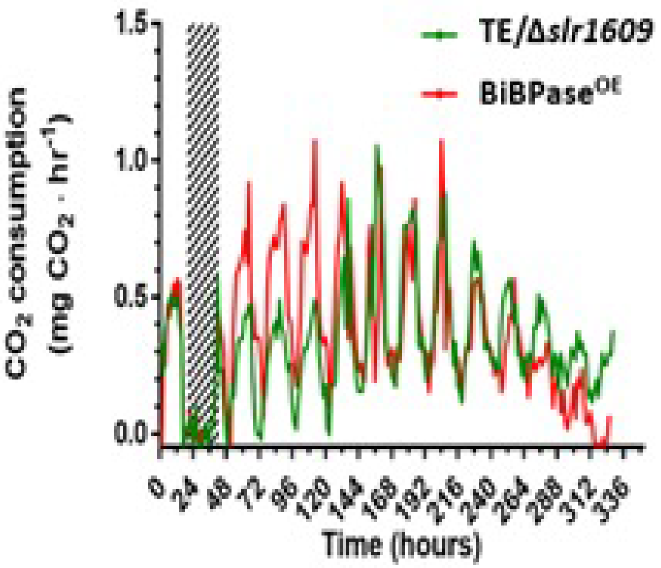
But this positive effect evaporates under standard diurnal conditions (14 hours 150 µmol photons m^{-2} s^{-1} light at 35 °C followed by 10 hours of darkness at 20 °C). Error bars represent one standard deviation of biological triplicate.

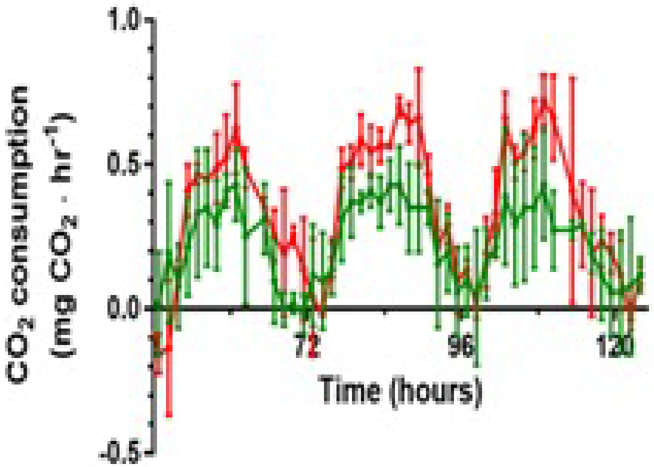


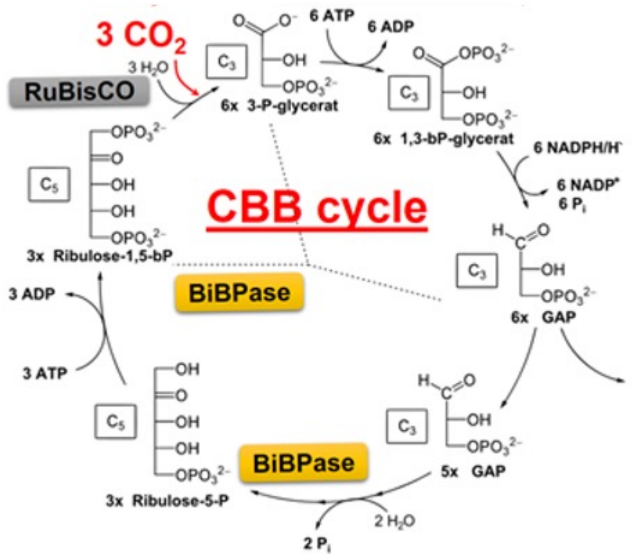


4 – Progress and Outcomes

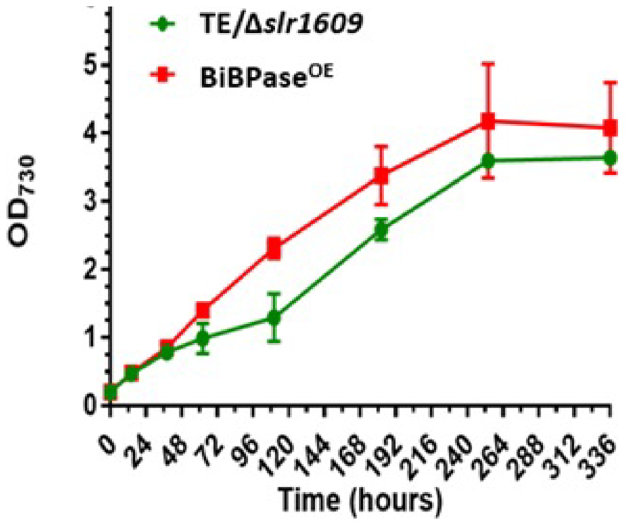
Task 4. Overexpress BiBPase

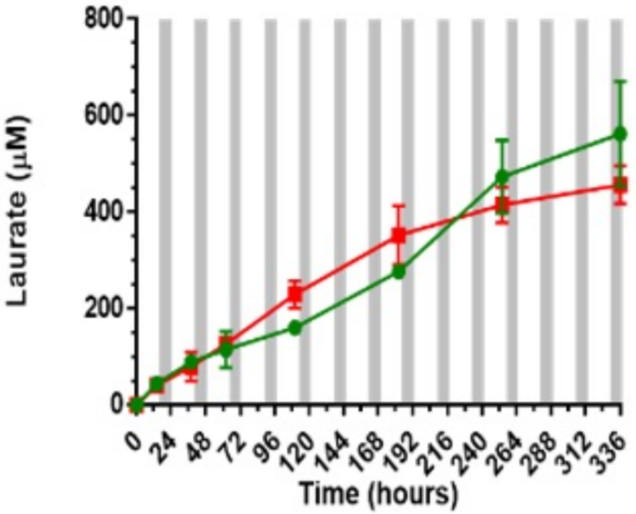


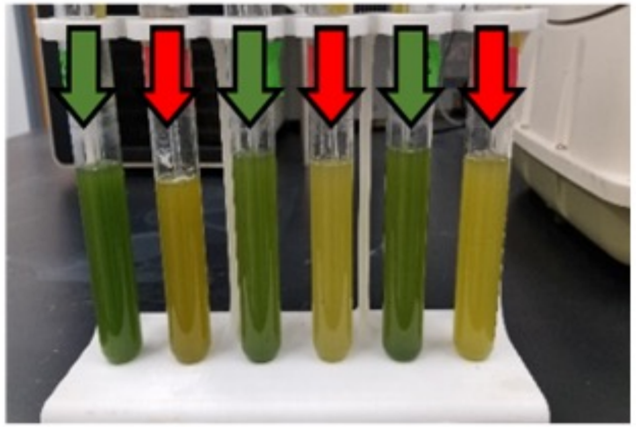




- Helps in CO_2 consumption and growth when bubbled with ambient air, at least in the first five days; requires further testing to confirm





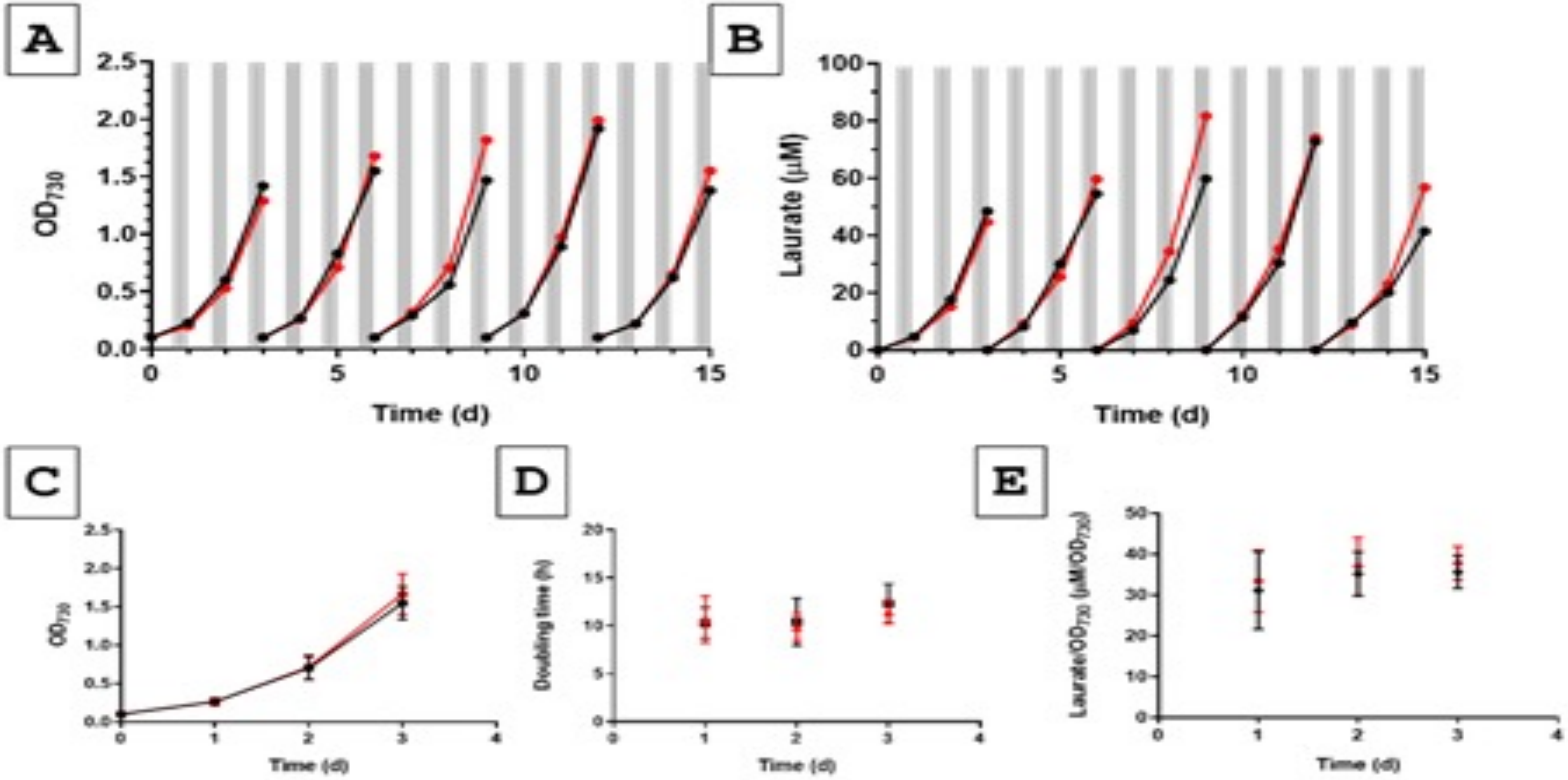


4 – Progress and Outcomes

Task 4. Adaptive laboratory evolution using fermentation off-gas

This improved BiBPase overexpression strain is used for adaptive laboratory evolution to determine if a faster-growing strain can be evolved. The strain is grown under standard diurnal conditions, and CO_2 is provided in the form of fermentation off gas from a brewery.

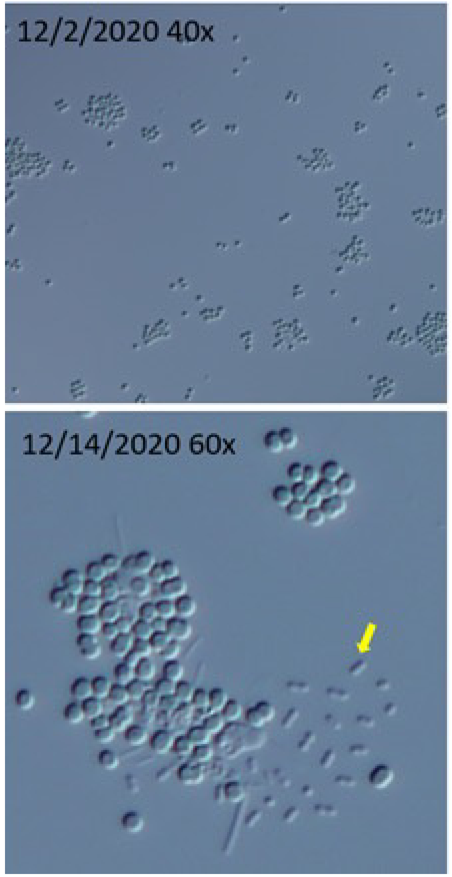
Thus far no significant changes yet, but the experiment only has been going on for a month or so.



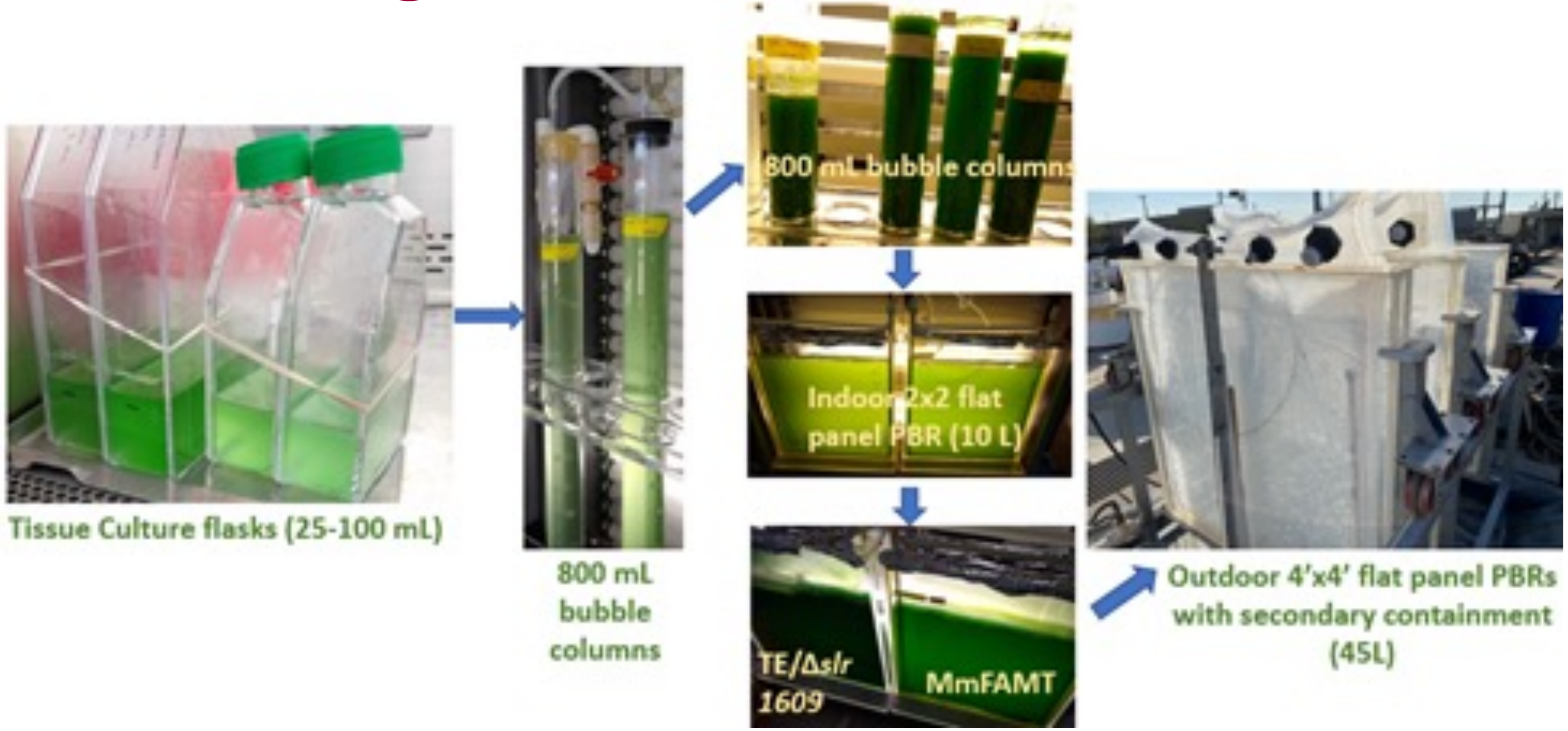
End of Project Goal: Isolate a biofuel-producing *Synechocystis* strain with at least a 15% increase in growth rate as measured by optical density at 730 nm at laboratory scale under conditions (light/dark cycling, temperature profile) relevant to outdoor culturing and supplemented with CO_2 from at least one industrial source

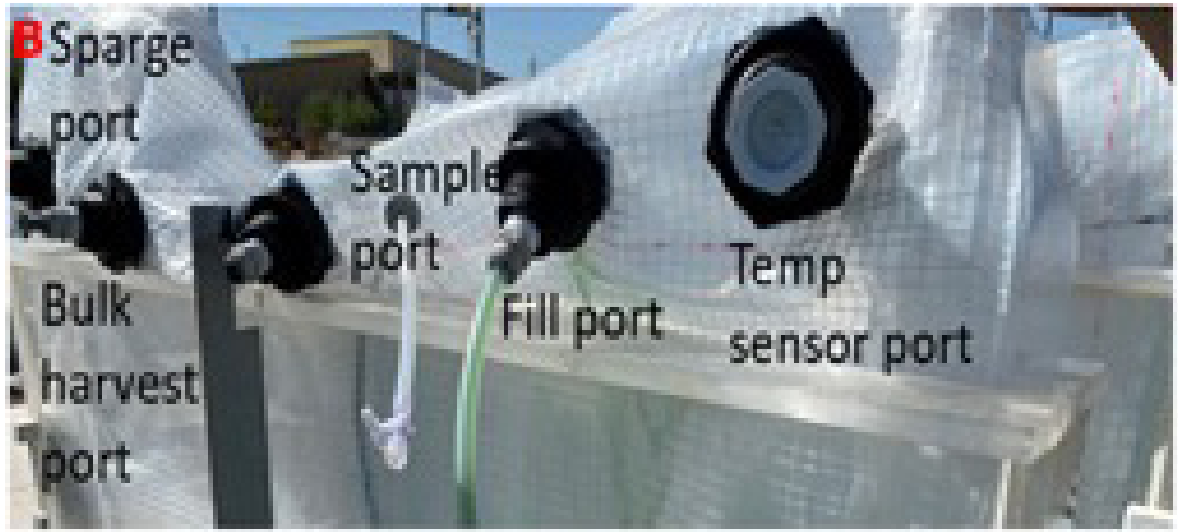
4 – Progress and Outcomes

Task 5: Grow strains outdoors at AzCATI and evaluate CO_2 utilization



Reasonable productivity was observed (see approach slides)



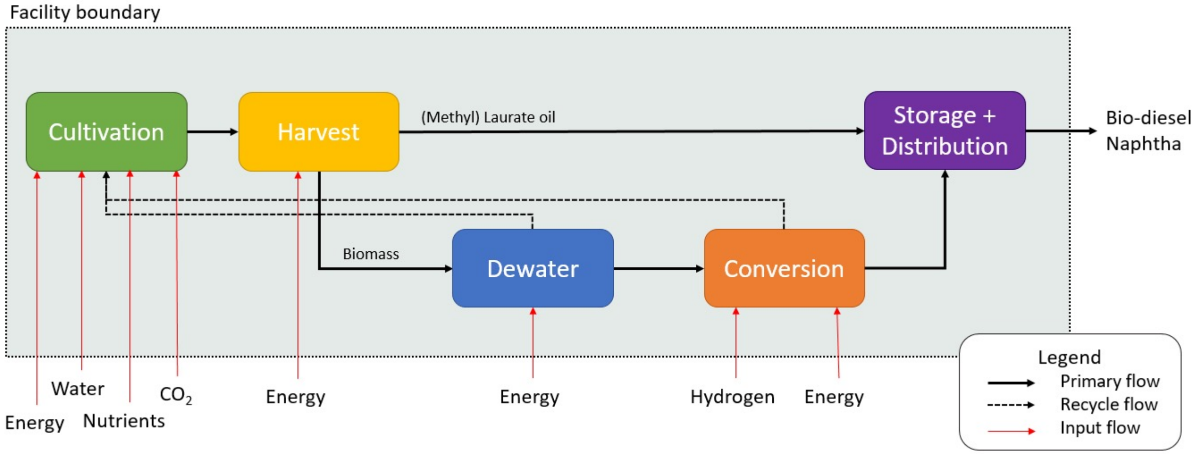


4 – Progress and Outcomes

Task 6. TEA and LCA methods

1. Process Modeling

Mass and energetic demands are captured for every unit and sub-unit process, such as hydrothermal liquefaction conversion or carbon dioxide delivery.

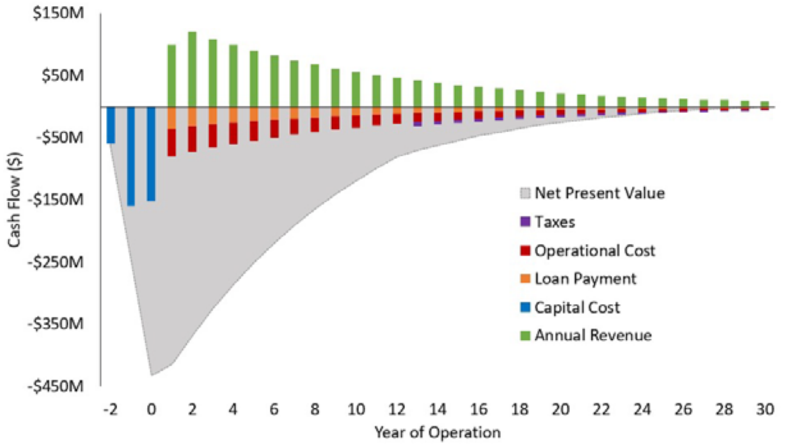


4. First-order growth modeling

Incoming photosynthetically active radiation (PAR) is correlated with growth, to determine how much fixed carbon can be expected from a given amount of radiation. Outdoor growth data from the Fall 2020 run is now being processed.

2a. Techno-economic analysis (TEA)

Process model data are super-imposed with financial data and evaluated on minimum fuel selling price with a discounted cash flow rate of return (DCFROR).



2b. Life cycle assessment (LCA)

Environmental burdens related to energy use and feedstocks are accounted for using OpenLCA and Ecoinvent data.

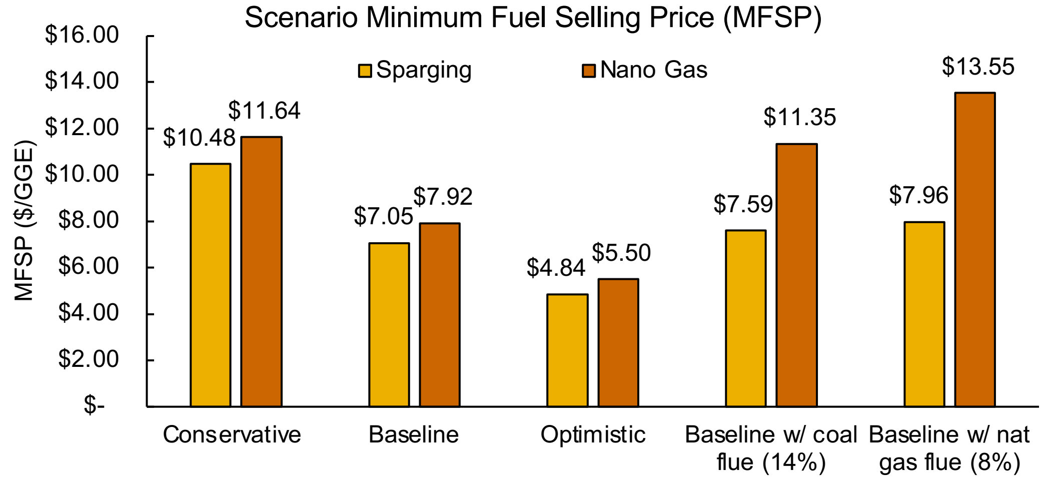
3. Monte Carlo Assessment (MCA)

A method of dealing with uncertainty, MCA replaces static inputs with stochastic probability curves using @Risk software and VBA for data processing.

4 – Progress and Outcomes

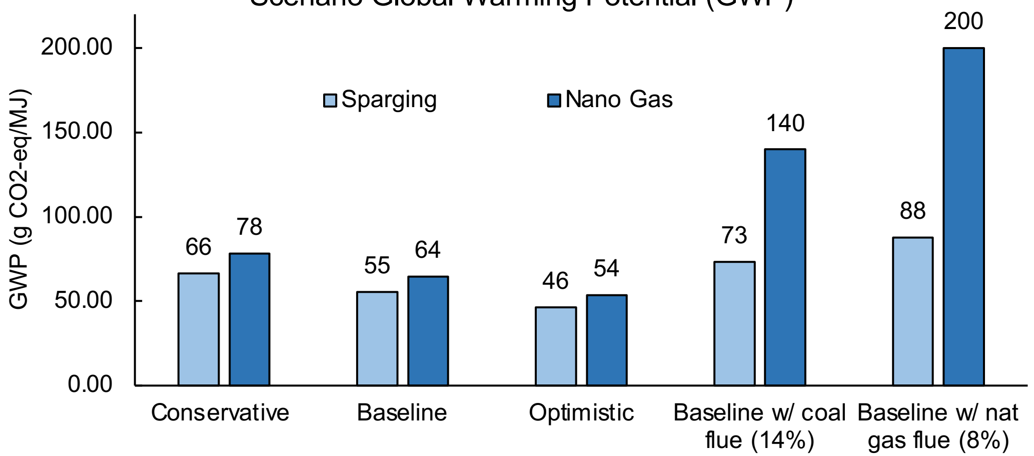
Task 6, selected results

Techno-economic analysis



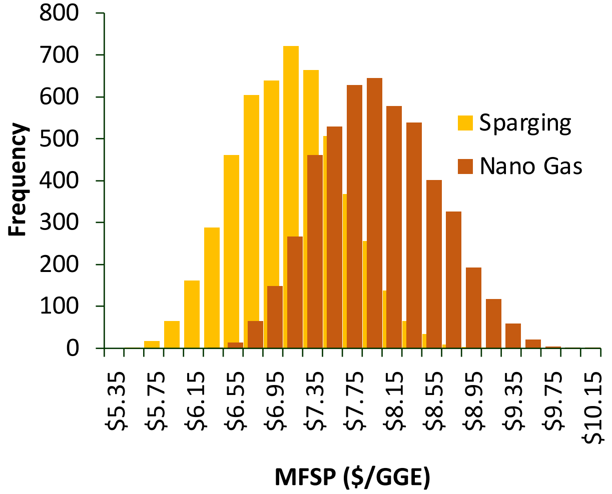
TEA results using sparging and Nano Gas carbon delivery systems

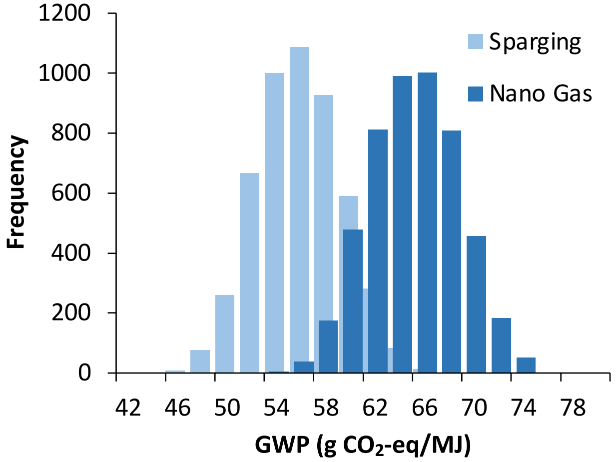
Life cycle assessment



LCA results using sparging and Nano Gas carbon delivery systems

Monte Carlo Analysis





Preliminary MCA results; see above for abbreviations

The scenario results include the conservative, baseline and optimistic scenarios using sparged and nanobubbled pure CO_2, as well as the two flue gas scenarios (coal and natural gas). The MCA results only include the pure CO_2 scenarios with input distributions spanning the conservative, baseline and optimistic range.

Summary

A number of approaches to enhance CO_2 utilization by biofuel (laurate/methyl laurate)-producing cyanobacteria were investigated in parallel. We knew in advance that some were going to work and some might not, and the challenge was to figure out which of the approaches enhanced CO_2 utilization.

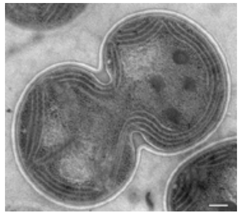
The ones that work best:

- Addition of amines was particularly effective in enhancing CO_2 utilization by cyanobacteria, enhancing carbon utilization by well over 25% under some conditions
- Overexpression of BiBPase enhances CO_2 utilization at non-saturating CO_2 levels
- TEA and LCA methods

Promising and in progress:

- Nanobubble optimization
- Adaptive laboratory evolution to further enhance growth using fermentation off gas
- Outdoor growth: They grow well even in winter, and can be stably maintained for >1 month

Overall, the best methods to enhance CO_2 utilization efficiency are being identified, and will contribute to yield and cost goals of BETO's MYPP



Quad Chart Overview

Timeline

- Project start date: October 1, 2018
- Project end date: September 30, 2021

	FY20 Costed	Total Award
DOE Funding	(10/01/2019 – 9/30/2020) *$799,911*	(negotiated total federal share) *$2,500,000*
Project Cost Share	*$217,085*	*$626,580*

Project Partners

- Colorado State University
- Nano Gas Technologies

Project Goal

Determine which physicochemical and biological mechanisms are most effective to enhance carbon utilization by cyanobacteria

End of Project Milestone

Isolate a biofuel-producing *Synechocystis* strain with at least a 15% increase in growth rate as measured by optical density at 730 nm at laboratory scale under conditions (light/dark cycling, temperature profile) relevant to outdoor culturing and supplemented with CO_2 from at least one industrial source

Other relevant milestones:

Identify conditions and biofuel-producing strain improving CO_2 utilization efficiency by $\geq$50% compared to biofuel-producing baseline strain at lab scale under conditions (light/dark cycling, temperature) relevant to outdoor culturing

Complete an outdoor cultivation trial in a relevant outdoor situation and establish performance capability at larger scale.

Assess economic viability of the proposed process with future recommendations

Funding Mechanism

DE-FOA-0001908 (Efficient Carbon Utilization in Algal Systems)

Additional Slides

Responses to Previous Reviewers' Comments

- The project was not presented in an oral presentation two years ago as it had just started
- Change in emphasis based on a Go/No-Go Review by the verification team in March 2020:
  - Nanobubble work does not need to be included in Task 5 as currently CO_2 nanobubbles are not helping growth sufficiently
- The SOPO was revised accordingly

Publications, Patents, Presentations, Awards, and Commercialization

- No publications resulting from this particular project yet, but a number of poster or student presentations have been given