DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Integrating an Industrial Source and Commercial Algae Farm with Innovative CO_2 Transfer Membrane and Improved Strain Technologies

March 23, 2021
Advanced Algal Systems

Ken Reardon
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Project Overview

- Purchased CO_2 for pH control and added inorganic carbon (iC) is one of the major costs of industrial algal cultivation.
- CO_2 costs are high because traditional sparging leads to CO_2 utilization efficiencies (CUE) of only 10-15%.
- The objectives of this project are to increase CUE with a novel enzymatic membrane transfer system and to develop a faster-growing variant of *Nannochloropsis oceanica*.
    - 25% increase in CUE
    - 20% productivity increase
- Significance: Lower algal biofuel cost

1 – Management

- Project structure
  - Task 1 (enzymatic membrane)
    - Enzyme improvement (NREL)
    - Membrane and module development (CSU)

Collaboration within task:

- Desired enzyme properties
- Methods for rate measurement
- Production of improved variants for testing

1 – Management

- Project structure
  - Task 1 (enzymatic membrane)
  - Task 2 (improved strains)
    - Engineered carbon concentrating mechanism (CSU)
    - Strain adaptation (NREL)
    - Strain characterization (CSU)

Collaboration within task:

- Discussion of test conditions
- Shared genome sequence and annotation
- Proteomics analysis of variants

1 – Management

- Project structure
  - Task 1 (enzymatic membrane)
  - Task 2 (improved strains)
  - Task 3 (integration)
    - Pond modeling (CSU)
    - iC measurement system (NREL)
    - System performance evaluation (CSU, QH, NBB, NREL)
    - Process modeling (CSU)
    - TEA and LCA (CSU, NREL)

Collaboration within task:
- Data for modeling
- Testing of iC injection points from model
- TEA guidance for experiments

1 – Management

- Project structure
  - Task 1 (enzymatic membrane)
  - Task 2 (improved strains)
  - Task 3 (integration)

Collaboration across tasks:

- Data for all modeling
- Membrane modules in cultivation trials
- Strains for cultivation trials
- TEA guidance for experiments

1 – Management

- Project structure
- Communication
  - Biweekly project team meetings
  - Exchange of materials
  - Collaboration on experiments and modeling

1 – Management

- Project structure
- Communication
  - Biweekly project team meetings
  - Exchange of materials
  - Collaboration on experiments and modeling
- Decision-making
  - PI in consultation with task leads

1 – Management

- Project structure
- Communication
- Decision-making
- Project risks
  - Monitoring:
    - Project update meetings with follow-up
    - Review of quarterly progress reports
  - Mitigation:
    - Discussion and input
    - Evaluation of alternatives
    - Parallel efforts

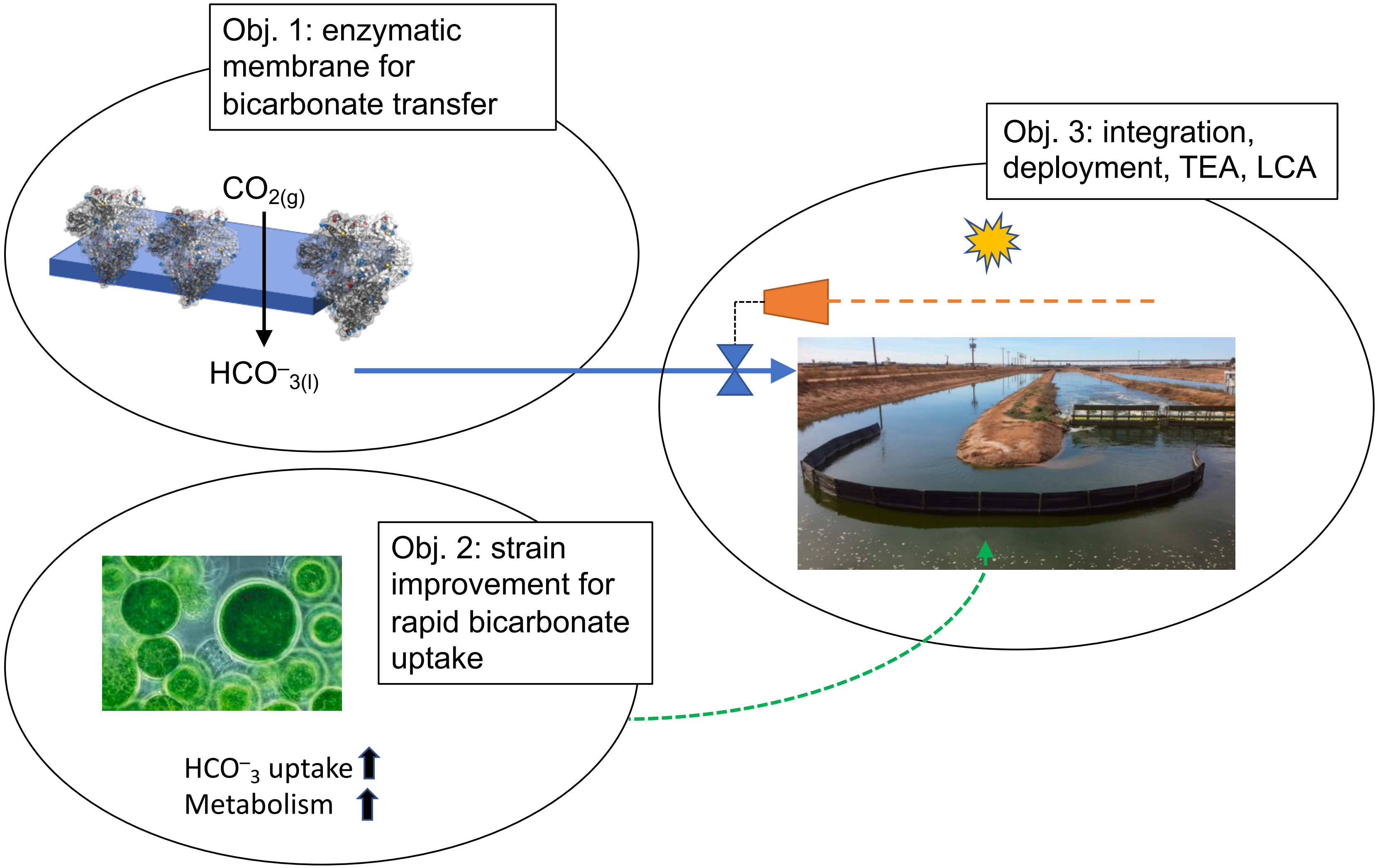
1 – Management

- Project structure
- Communication
- Decision-making
- Project risks
    - Monitoring:
        - Project update meetings with follow-up
        - Review of quarterly progress reports
    - Mitigation:
        - Discussion and input
        - Evaluation of alternatives
        - Parallel efforts

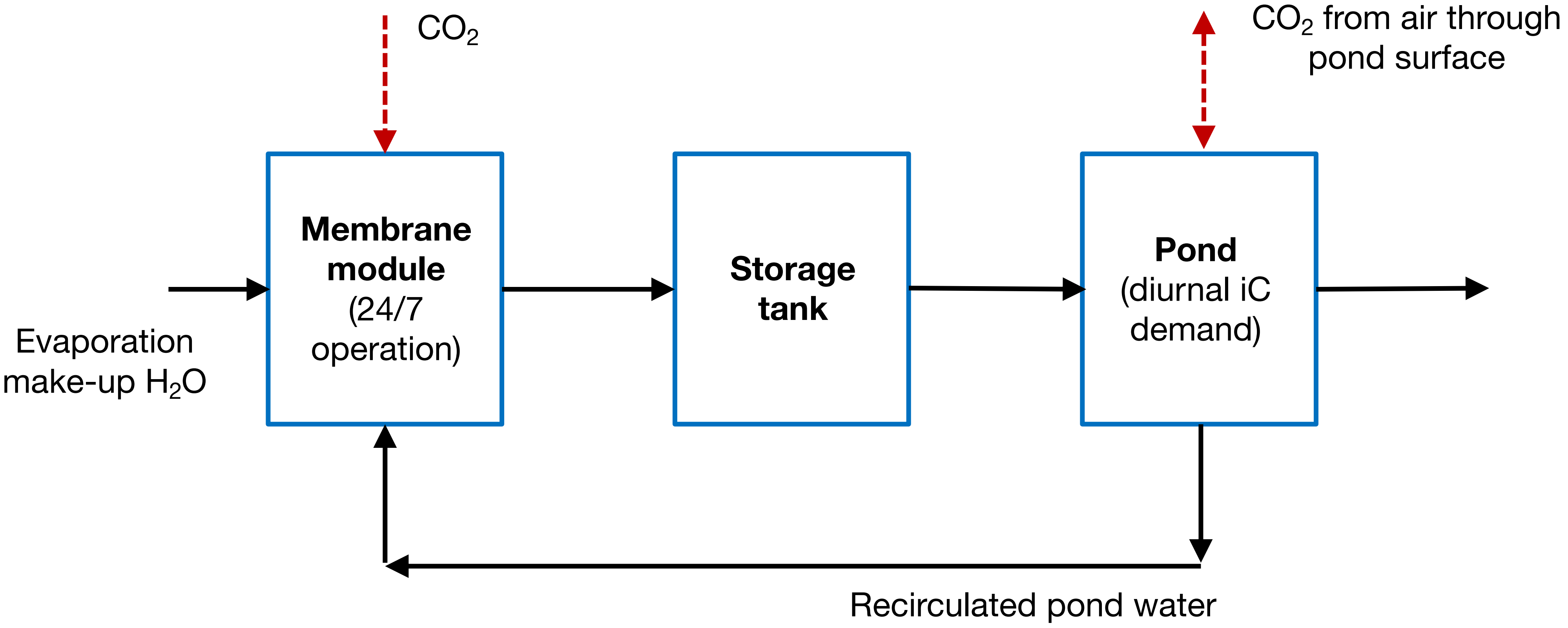
Examples:

- Development of improved strain: planned parallel efforts
- Development of improved enzyme: added effort to broaden options

2 – Approach


Obj. 1: enzymatic membrane for bicarbonate transfer
$CO_{2(g)}$
$HCO^{-}_{3(l)}$
Obj. 3: integration, deployment, TEA, LCA
Obj. 2: strain improvement for rapid bicarbonate uptake
HCO^{-}_{3} uptake ⬆
Metabolism ⬆

2 – Approach



CO_2
CO_2 from air through pond surface
Membrane module (24/7 operation)
Storage tank
Pond (diurnal iC demand)
Evaporation make-up H_2O
Recirculated pond water

		Y1				Y2				Y3			
	V	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
start of quarter	10/1/18	1/1/19	4/1/19	7/1/19	10/1/19	1/1/20	4/1/20	7/1/20	10/1/20	1/1/21	4/1/21	7/1/21	10/1/21
months in quarter		1–3	4–6	7–9	10–12	13–15	16–18	19–21	22–24	25–27	28–30	31–33	34–36
Validation Phase								G/NG					G/NG
Obj 1: Develop membrane system for transfer of CO_2 to water as bicarbonate													
Task 1.1: Protein engineering of carbonic anhydrase to improve pH tolerance (NREL)		M1.1.1			M1.1.2		M1.1.3	M1.1.4					
Task 1.2: Preparation and characterization of carbonic anhydrase membranes (CSU)			M1.2.1			M1.2.2							
Task 1.3: Modeling, construction, and demonstration of CO_2 transfer module (CSU)					M1.3.1		M1.3.2	M1.3.3					
Obj 2: Engineering of Nannochloropsis oceanica for enhanced carbon uptake													
Task 2.1: CCM engineering in *N. oceanica* (CSU)					M2.1.1	M2.1.2							
Task 2.2: Strain adaptation to high bicarbonate levels (NREL)					M2.2.1			M2.2.2					
Task 2.3: Characterize combined strain (photophysiology, -omics) CSU)							M2.3.1				M2.3.2		
Obj 3: System integration, deployment, and validation													
Task 3.1: Determine optimal carbon introduction location to raceway pond (CSU)				M3.1.1			M3.1.2		M3.1.3	M3.1.4		M3.1.5	
Task 3.2: Develop and test system for gas-phase CO_2 monitoring and control (NREL)						M3.2.1							
Task 3.3: Evaluate performance of C delivery and strain uptake (CSU, NREL, QH)				M3.3.1				M3.3.2					M3.3.3 M3.3.4
Task 3.4: Engineering Process Modeling (CSU, NREL)						M3.4.1				M3.4.2			
Task 3.5: Concurrent TEA and LCA (CSU, NREL)						M3.5.1							M3.5.2

2 – Approach

Task 1: Membrane system for efficient transfer of CO_2 to water

- Protein engineering of carbonic anhydrase (CA) to improve pH tolerance
    - Challenge: Find/create variant with durable activity at pH < 7
- Preparation of CA-containing membranes
- Construction, modeling, and testing of iC transfer module
    - Challenge: Developing test system

2 – Approach

Task 1: Membrane system for efficient transfer of CO_2 to water

- Protein engineering of carbonic anhydrase (CA) to improve pH tolerance
  - Challenge: Find/create variant with durable activity at pH < 7
- Preparation of CA-containing membranes
- Construction, modeling, and testing of iC transfer module
  - Challenge: Developing test system

✓ **Milestone 1.1.2**: Demonstrate a carbonic anhydrase variant with an active half-life of 10 hours at pH 6.

✓ **Go/No-Go:** Demonstration of enzymatic membrane module for inorganic carbon delivery into an aqueous phase at a minimum flux of 2×10^{-4} mol/s·m^2 at pH 7 and 30 °C.

2 – Approach

Task 2: Engineering of *N. oceanica* for enhanced carbon uptake

- CCM Engineering in *N. oceanica*
  - Challenge: Unstable transformants
- Strain adaptation to high bicarbonate levels
  - Challenge: Random nature of mutagenesis
- Characterize novel strains

2 – Approach

Task 2: Engineering of *N. oceanica* for enhanced carbon uptake

- CCM Engineering in *N. oceanica*
  - Challenge: Unstable transformants
- Strain adaptation to high bicarbonate levels
  - Challenge: Random nature of mutagenesis
- Characterize novel strains

✓ **Milestone 2.1.1**: Show expression of a reporter protein such as YFP in at least two separate transformation events.

✓ **Go/No-Go:** At least one developed variant of *N. oceanica* demonstrates at least 20% higher growth in laboratory-based high bicarbonate environments relative to parent strain.

2 – Approach

Task 3: System integration, deployment, and validation

- Determine optimal carbon introduction location to raceway pond
- Develop and test system for gas-phase CO_2 monitoring and control
- Evaluate performance of C delivery and strain uptake
- Engineering process modeling
- Concurrent TEA and LCA

2 – Approach

Task 3: System integration, deployment, and validation

- Determine optimal carbon introduction location to raceway pond
- Develop and test system for gas-phase CO_2 monitoring and control
- Evaluate performance of C delivery and strain uptake
- Engineering process modeling
- Concurrent TEA and LCA

Go/No-Go: Demonstration of 25% increase in baseline inorganic carbon utilization efficiency over a 6-week continuous cultivation demonstration run at QH test site and 20% growth rate increase in the integrated systems at CSU and NREL.

3 – Impact

- Increased CUE
  - Purchased CO_2 is major expense, yet nearly >85% is wasted
  - Project goal is >60% CUE
- Increased productivity
  - Major BETO strategy to achieve $2.50/GGE
  - Project goal is 20% baseline

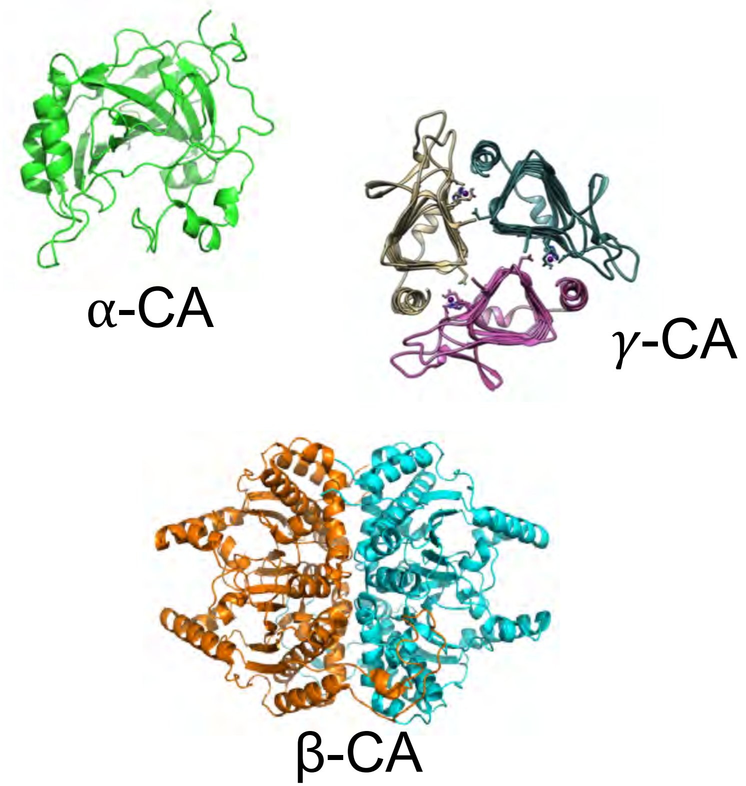
3 – Impact

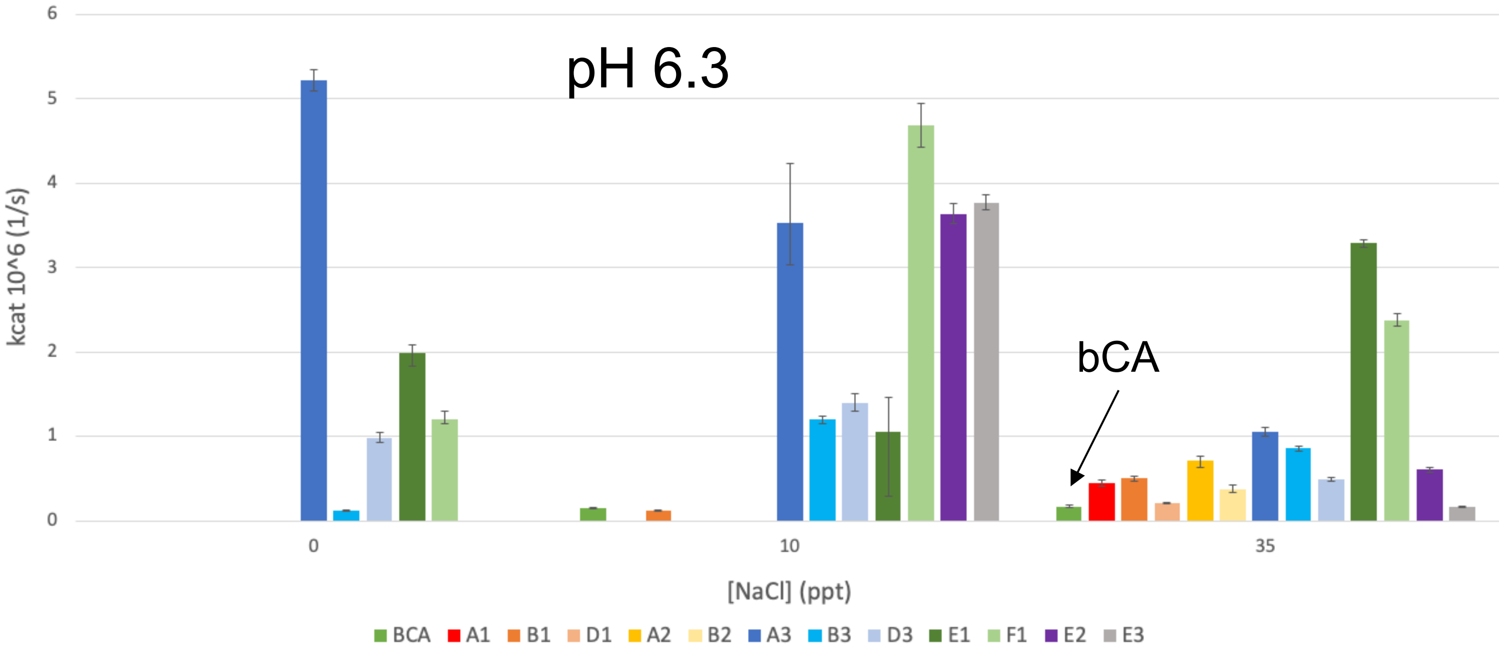
- Increased CUE
- Increased productivity
- Engagement with QH is extremely valuable
  - Realistic baseline and conditions
  - Feedback on approach
- Project provides value to QH
  - Technology for iC delivery
  - Flow modeling
- Dissemination
  - Planned publications and patents

4 – Progress and Outcomes

Foteini Davrazou, Deanne Sammond, Lieve Laurens (NREL)

- Task 1: Membrane system for efficient transfer of CO_2 to water
  - Highly active CA variants identified

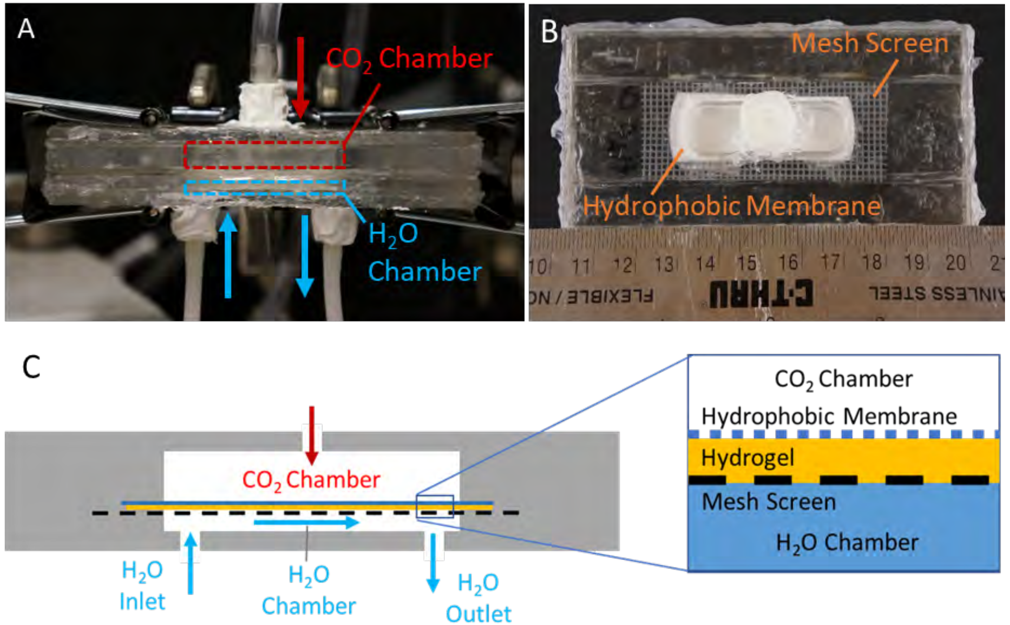




4 – Progress and Outcomes

Jaclyn Adkins, Boston Morris, Travis Bailey, Ken Reardon (CSU)

- Task 1: Membrane system for efficient transfer of CO_2 to water
  - Highly active CA variants identified
  - Lab-scale membrane modules developed with milestone flux



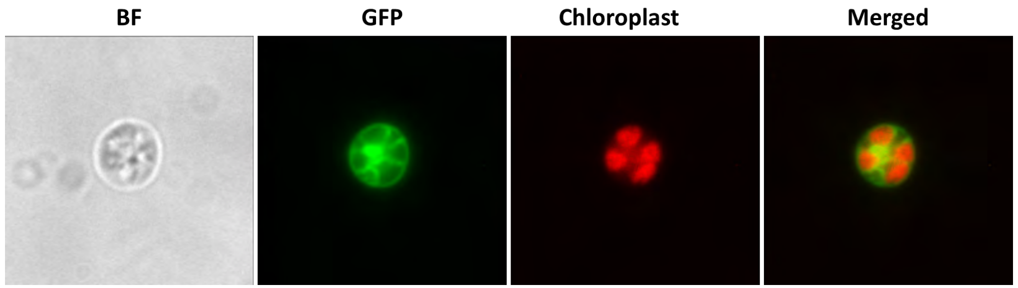
4 – Progress and Outcomes

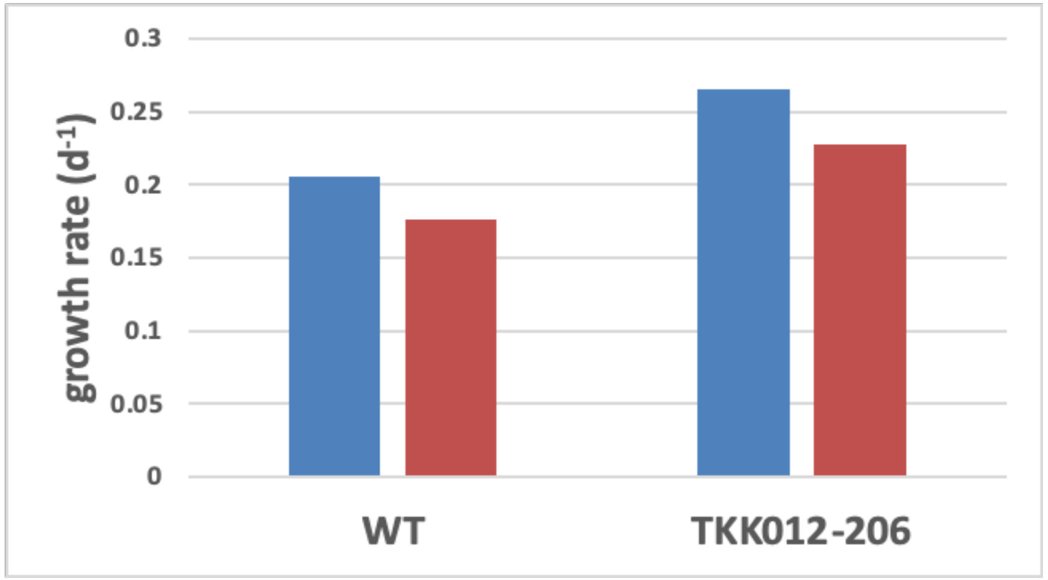
- Task 1: Membrane system for efficient transfer of CO_2 to water
  - Highly active CA variants identified
  - Lab-scale membrane modules developed with milestone flux
  - All milestones achieved

Tessema Kassaw, Graham Peers (CSU)

4 – Progress and Outcomes

- Task 2: Engineering of *N. oceanica* for enhanced carbon uptake
  - Engineered carbon concentrating mechanism mutant
    - Stable
    - Demonstrated 29% average increase in specific growth rate over WT in elevated CO_2 conditions





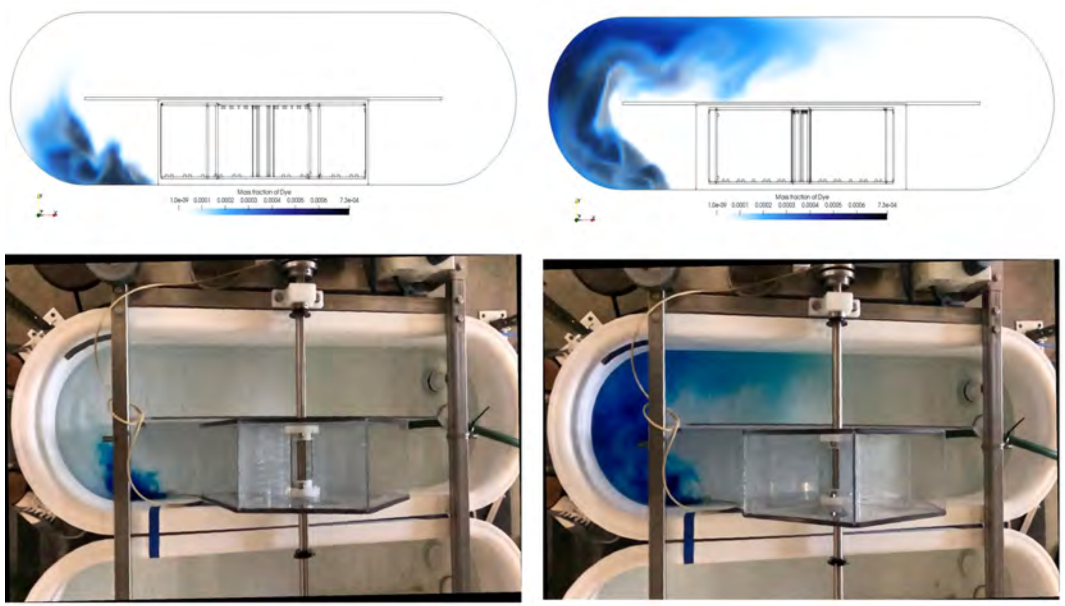
4 – Progress and Outcomes

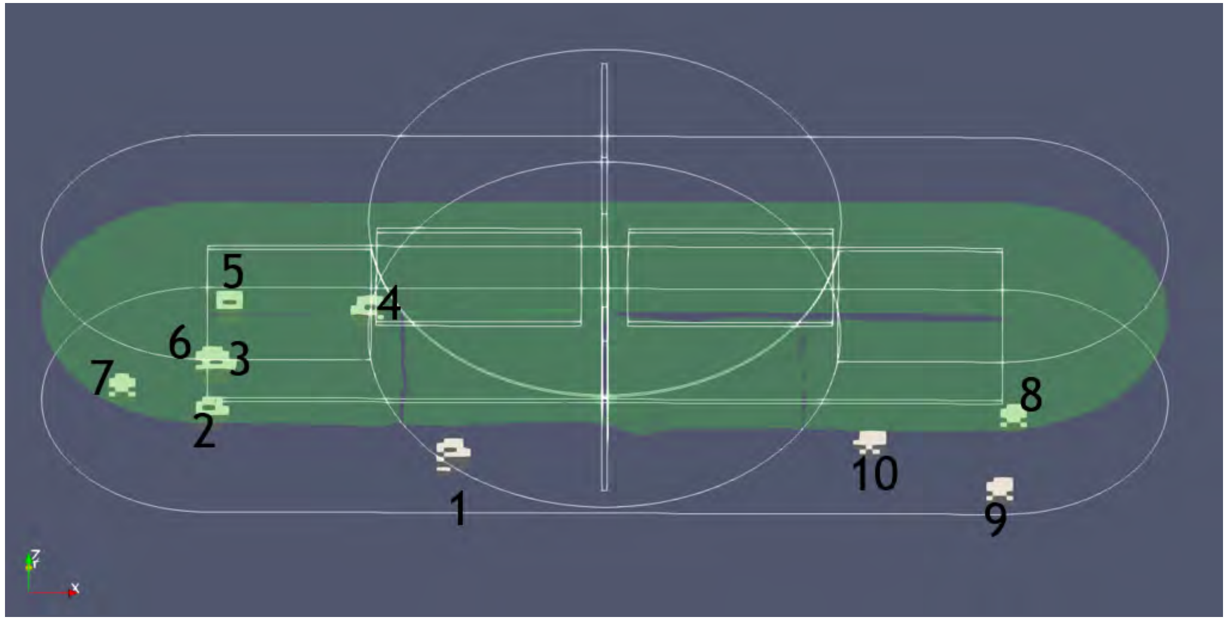
- Task 2: Engineering of *N. oceanica* for enhanced carbon uptake
  - Engineered carbon concentrating mechanism mutant
    - Stable
    - Demonstrated 29% average increase in specific growth rate over WT in elevated CO_2 conditions
  - Genome sequenced and annotated
  - Go/No-Go milestone achieved

Chen Shen, David Dandy (CSU)

4 – Progress and Outcomes

- Task 3: System integration, deployment, and validation
  - Computational fluid dynamics model matches mixing and growth; predicts best locations for iC stream injection

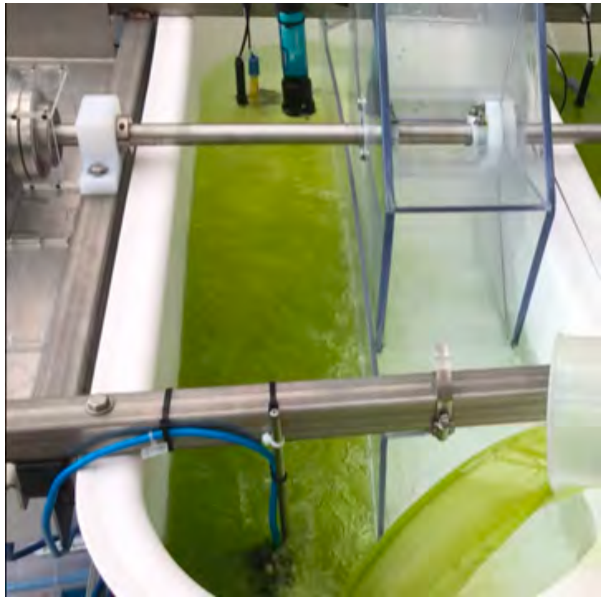


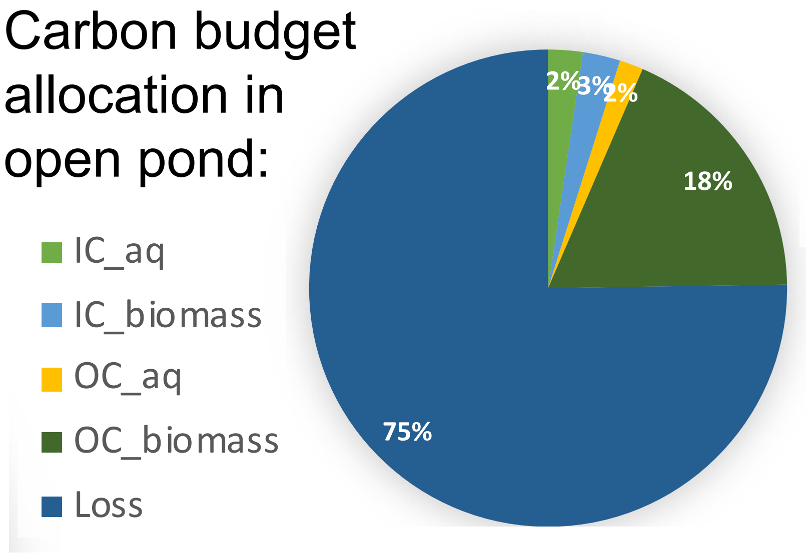


4 – Progress and Outcomes

Damien Douchi, Foteini Davrazou, Bonnie Panczak, Alicia Sowell, Mauro Lua, Nick Sweeney, Kaitlin Lesco, Lieve Laurens (NREL)

- Task 3: System integration, deployment, and validation
  - Computational fluid dynamics model matches mixing and growth; predicts best locations for iC stream injection
  - C tracking system for ponds developed and applied

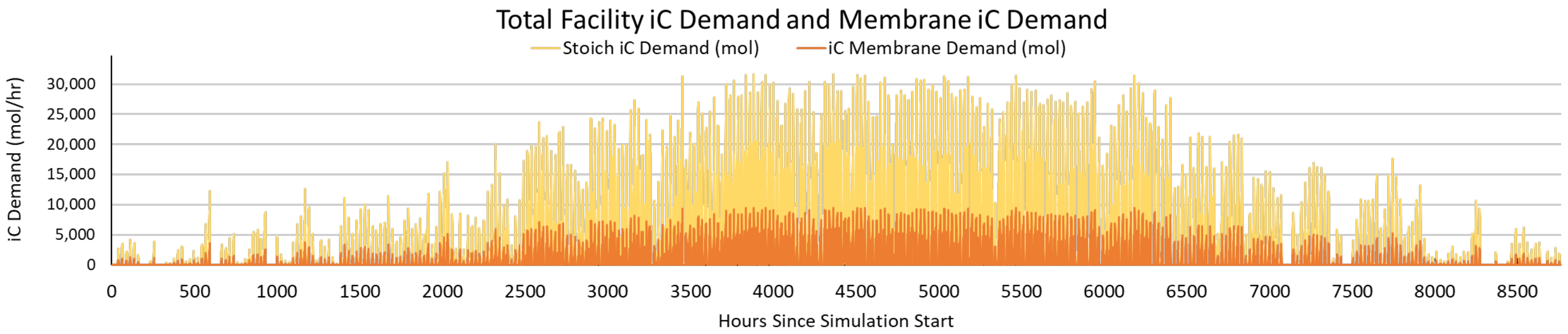




4 – Progress and Outcomes

Jonah Greene, Jason Quinn (CSU)
Bruno Klein (NREL)

- Task 3: System integration, deployment, and validation
  - Computational fluid dynamics model matches mixing and growth; predicts best locations for iC stream injection
  - C tracking system for ponds developed and applied
  - Process model developed to integrate system components



Summary

- Important technical goals achieved
  - Improved enzyme variants
  - Improved *N. oceanica* strain
  - CFD and process models developed
- Moving to system integration and evaluation
- Schedule delays owing to COVID-19 shutdowns/restrictions; otherwise on track

Quad Chart Overview

Timeline
- 10/01/2019
- 3/31/2022 (NCE)

	FY20 Costed	Total Award
DOE Funding	(10/01/2019 – 9/30/2020) *$799,490*	(negotiated total federal share) *$2,145,600*
Project Cost Share	$267,940	$752,018

Project Partners
- NREL
- Qualitas Health
- New Belgium Brewing

Project Goal
The goal of this project is to increase CUE and productivity through development and application of a novel enzymatic membrane transfer system and a faster-growing variant of *Nannochloropsis oceanica*.

End of Project Milestone
Demonstration of 25% increase in baseline inorganic carbon utilization efficiency over a 6-week continuous cultivation demonstration run at QH test site (mini-ponds plus membrane module plus CO_2 monitoring) and 20% growth rate increase in integrated systems at CSU and NREL.

Funding Mechanism
DE-FOA-0001908, Efficient Carbon Utilization in Algal Systems
Topic Area 1 – CO_2 Utilization within Algae Cultivation Systems

Project Team

Colorado State University
Jaclyn Adkins, Travis Bailey, Jonah Greene, Tessema Kassaw, Boston Morris, Seijin Park, Graham Peers, Kennalyn Peterson, Jason Quinn, Ken Reardon, Chen Shen, Maxwell Ware

National Renewable Energy Laboratory
Ryan Davis, Foteini Davrazou, Damien Douchi, Bruno Klein, Lieve Laurens, Kaitlin Lesco, Mauro Lua, Bonnie Panczak, Deanne Sammond, Alicia Sowell, Nick Sweeney

Los Alamos National Laboratory
Blake Hovde, Shawn Starkenburg

Qualitas Health
Jake Nalley, Eneko Ganuza

New Belgium Brewing
Chris Keogan, Shane Roberts

Additional Slides

Responses to Previous Reviewers' Comments

- Go/No-Go (Interim Verification) Review
  - Delayed 4 months by COVID-19 pandemic
  - Held on January 15, 2021
  - All milestones accomplished
  - Approved for BP3

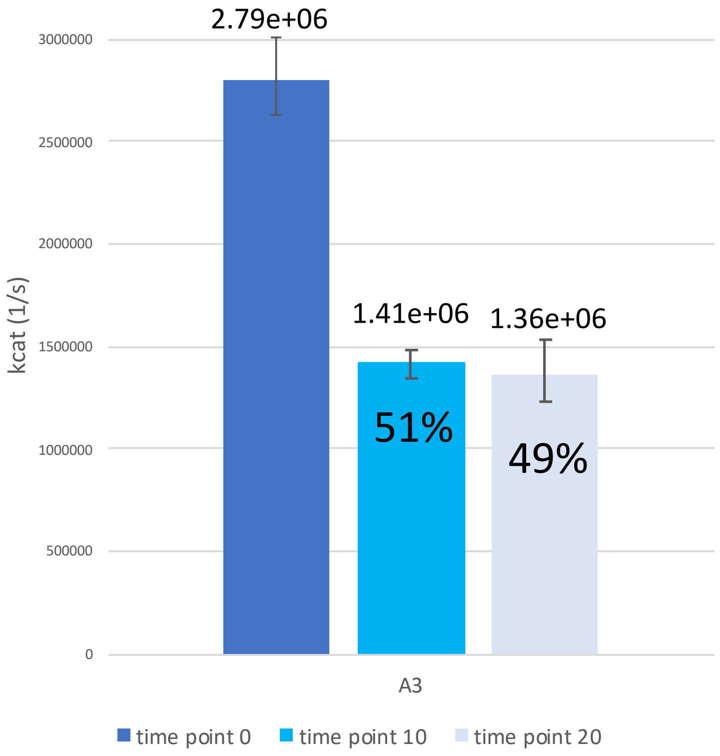
Publications, Patents, Presentations, Awards, and Commercialization

- NREL Laboratory Analytical Procedure: "Determination of Total, Organic, and Inorganic Carbon in Biological Cultures in Liquid Fraction Process Samples" (https://www.nrel.gov/docs/fy21osti/78622.pdf)
- Publications in preparation include:
    - Carbonic anhydrase engineering
    - CCM engineering
    - *N. oceanica* genome sequencing and annotation
    - Algae cultivation computational fluid dynamics modeling
    - iC measurement system design and application
    - Engineering process modeling

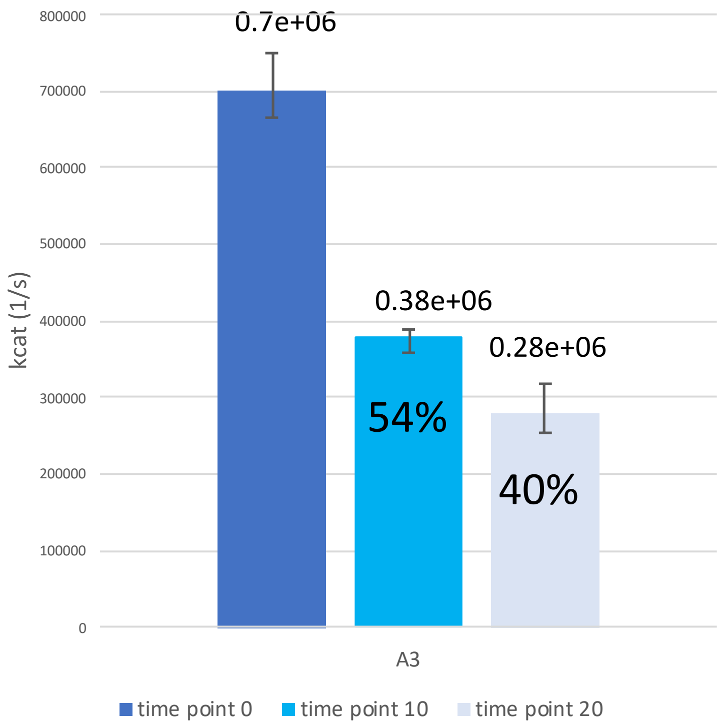
4 – Progress and Outcomes (Task 1.1)

Foteini Davrazou, Deanne Sammond, Lieve Laurens (NREL)

Activity Retention of Carbonic Anhydrase Variant at pH 6



10ppt NaCl



35ppt NaCl

4 – Progress and Outcomes (Task 2.3)

Seijin Park, Ken Reardon, Graham Peers (CSU)
Shawn Starkenburg, Blake Hovde, (LANL)

- Sequencing and annotation
  - Genome is 30.96 Mbp
    - 3144 scaffolds
    - 1546 scaffolds >1000 bp
  - Paired end-read Illumina sequencing
    - 3.94M reads; 118kM bases

4 – Progress and Outcomes (Task 2.3)

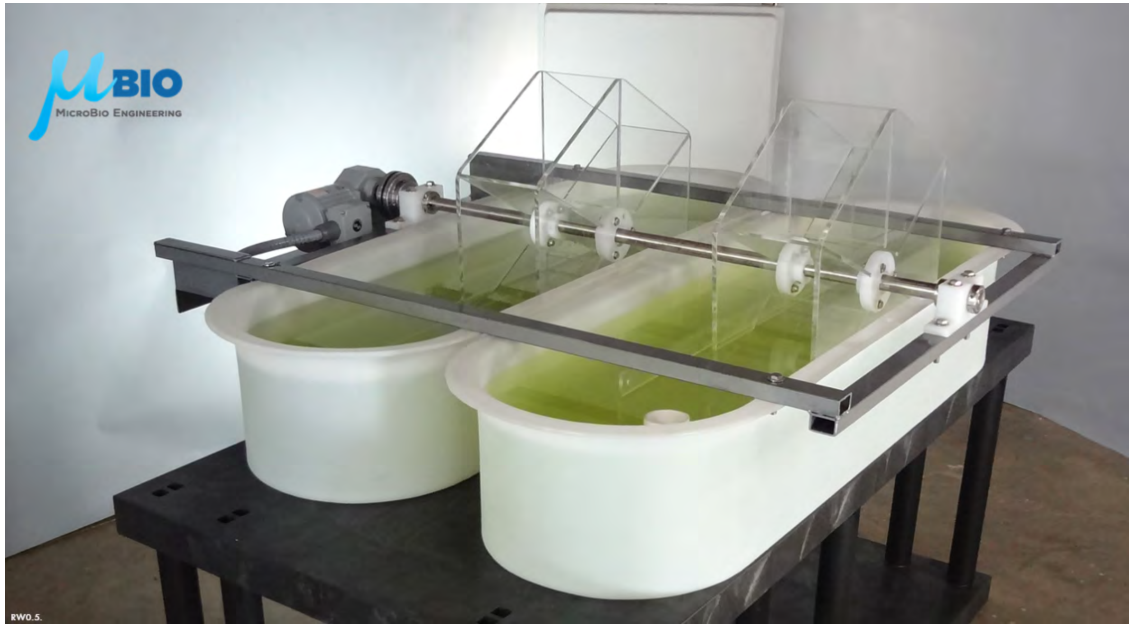
Seijin Park, Ken Reardon, Graham Peers (CSU)
Shawn Starkenburg, Blake Hovde, (LANL)

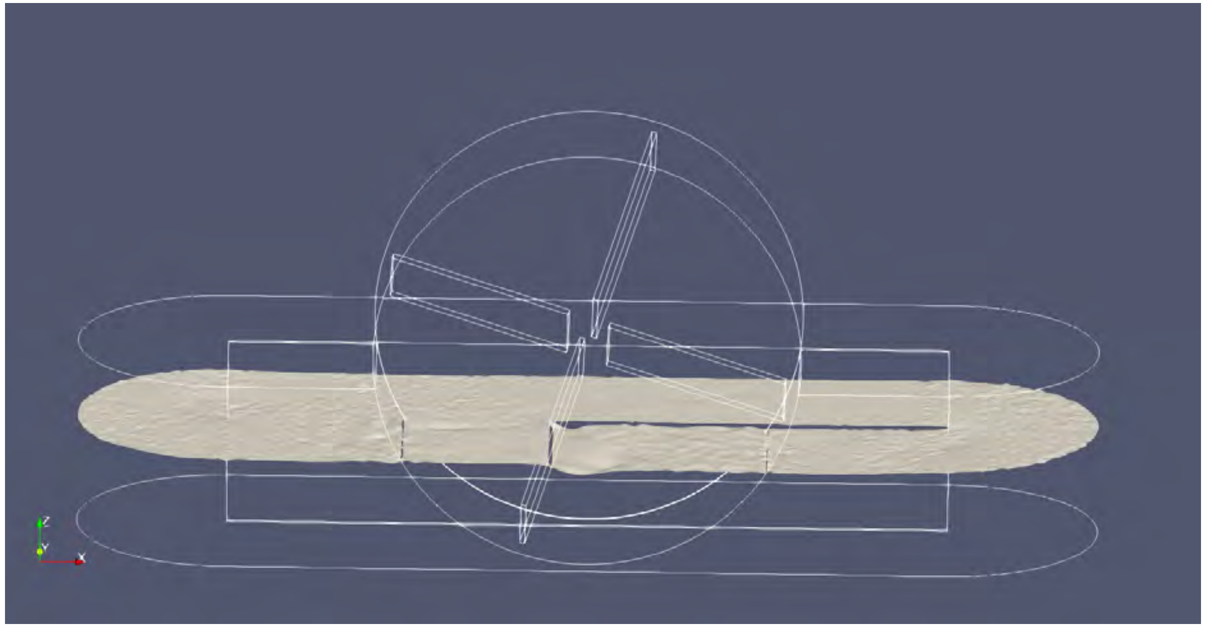
- Sequencing and annotation

	IMET1 (2014)	LANL-CSU (2020)
entries	9915	11131
unknown function	9915	4958

Chen Shen, David Dandy (CSU)

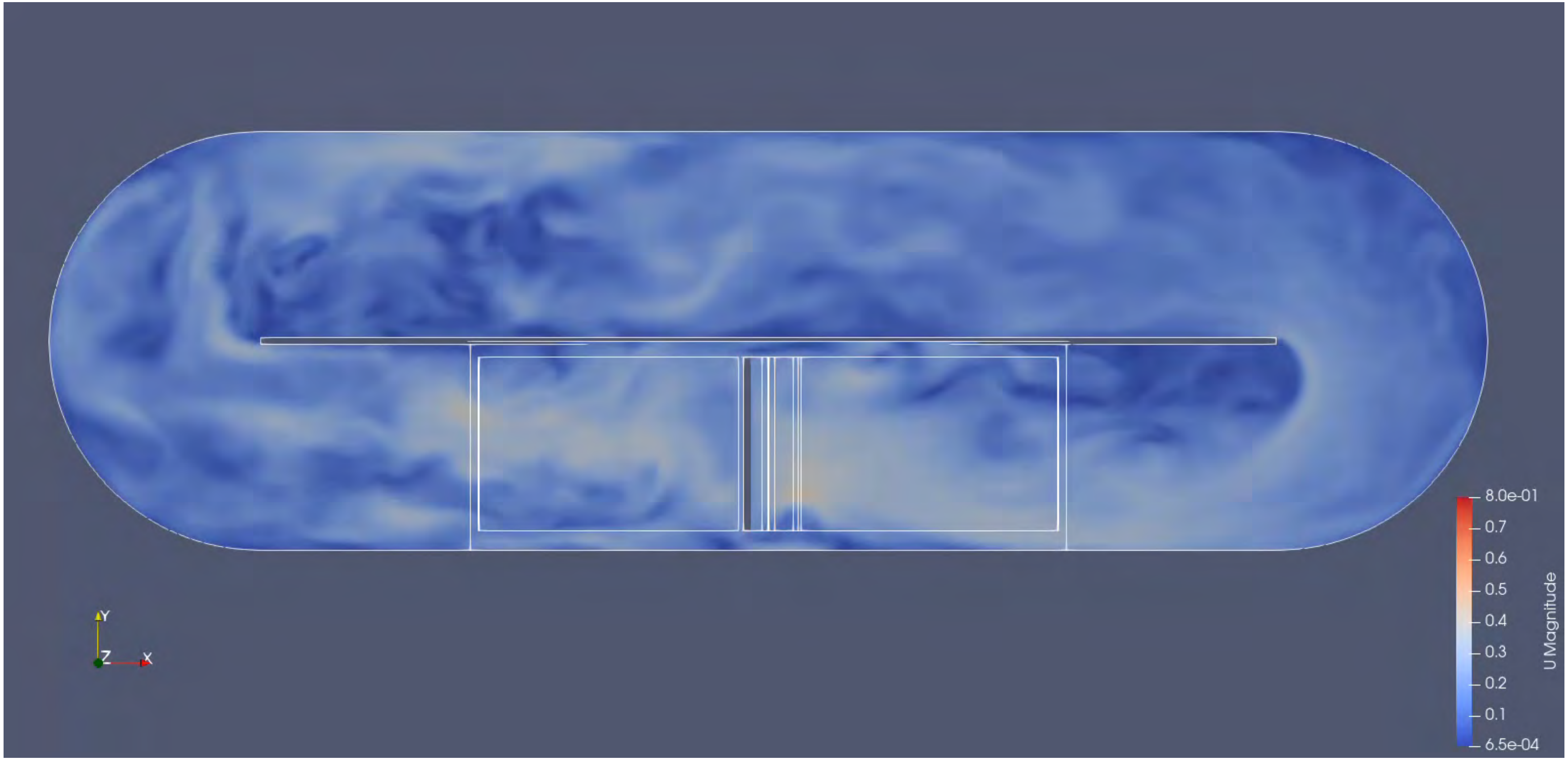
4 – Progress and Outcomes (Task 3.1)





Chen Shen, David Dandy (CSU)

4 – Progress and Outcomes (Task 3.1)



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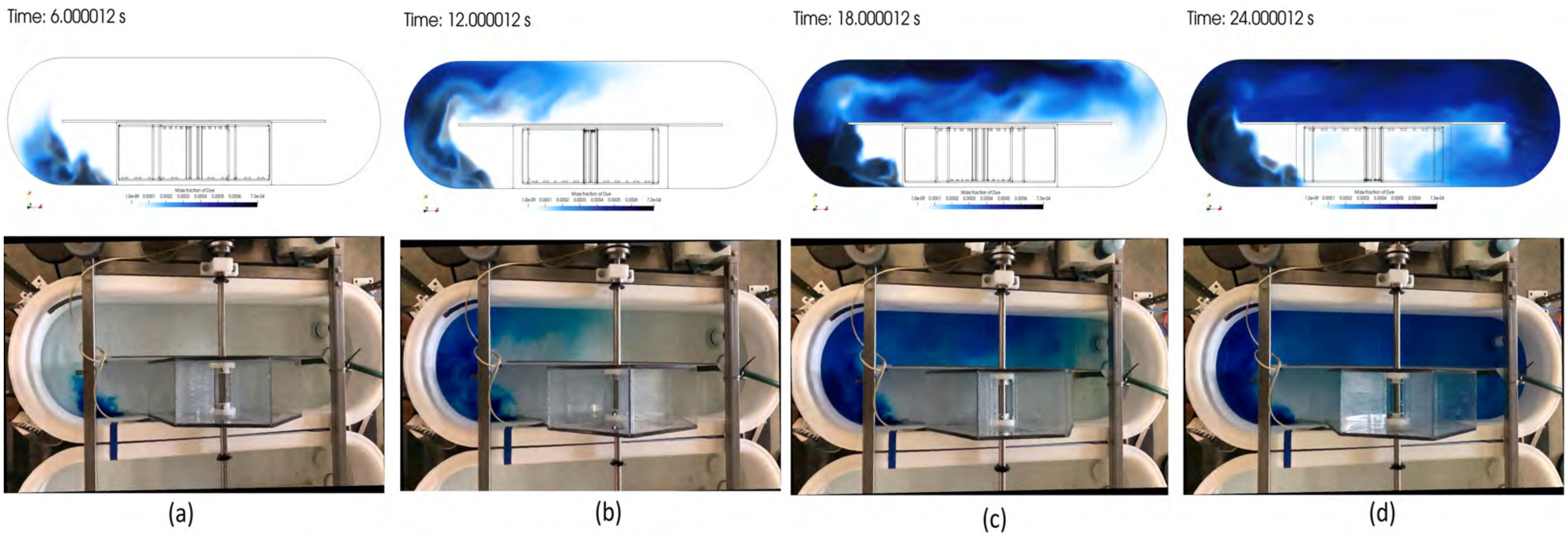
4 – Progress and Outcomes (Task 3.1)



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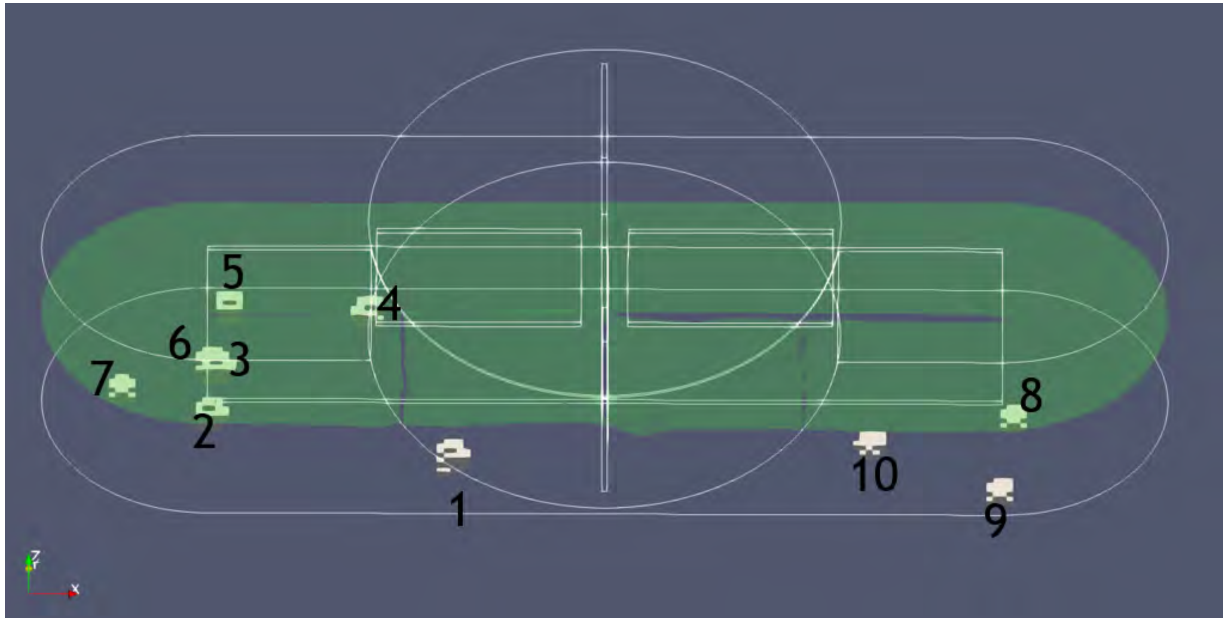
4 – Progress and Outcomes (Task 3.1)



Case Number	Standard Deviation of HCO3 Concentration
1	0.001499
2	0.001537
3	0.001512
4	0.001629
5	0.001648
6	0.001583
7	0.001417
8	0.001534
9	0.001596
10	0.001490

4 – Progress and Outcomes (Task 3.2)

Damien Douchi, Foteini Davrazou, Bonnie Panczak, Alicia Sowell, Mauro Lua, Nick Sweeney, Kaitlin Lesco, Lieve Laurens (NREL)

Improved Carbon Budget in Ponds: Medium composition and pH affect efficiency of CUE