U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2017 Project Peer Review

Microalgae Biofuels Production on CO_2 from Air

March 8, 2017
Advanced Algal Systems

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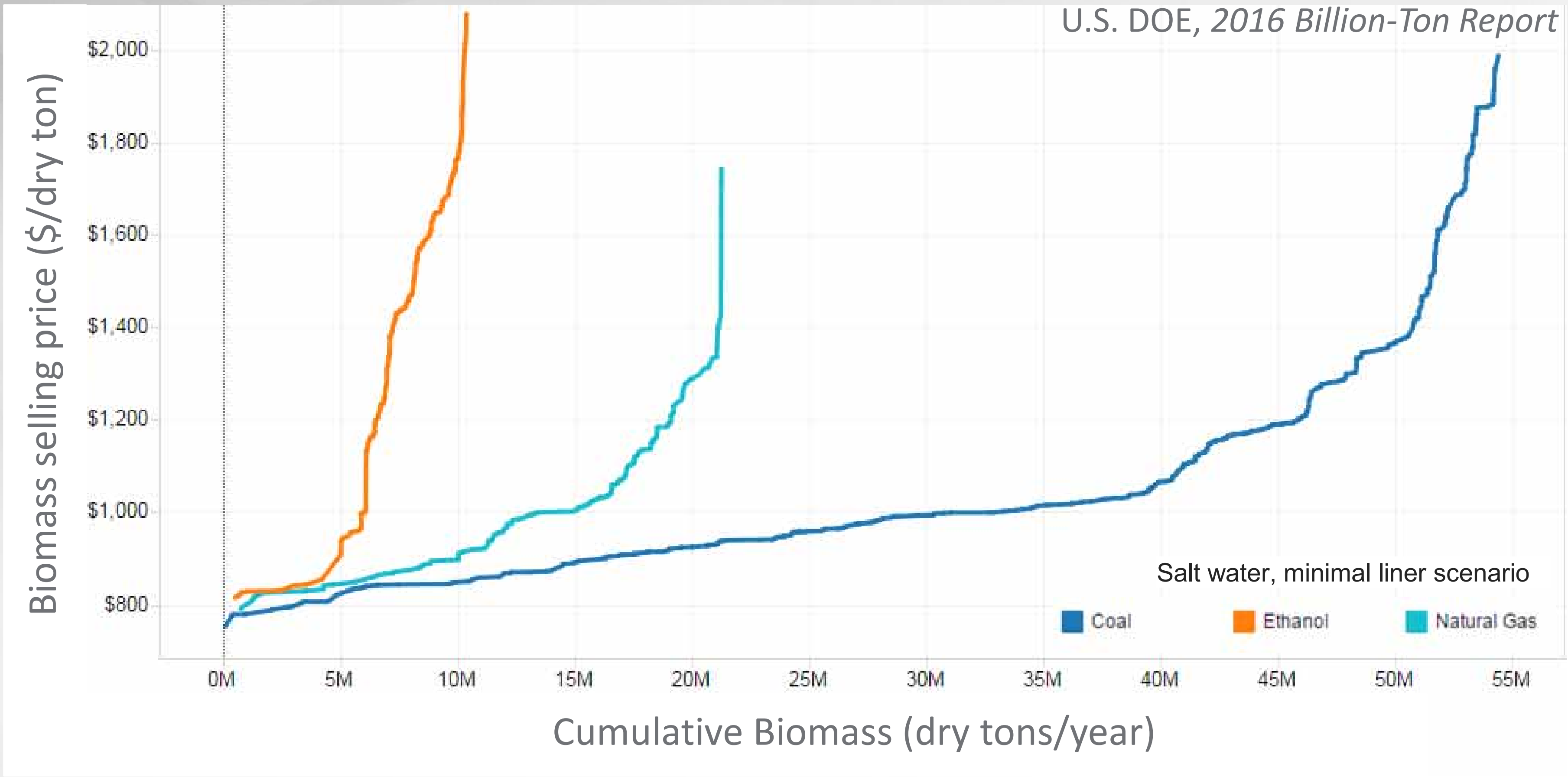
The Microalgae Biofuels Challenge

- Algae production with CO_2 co-location only displaces 5% of US petroleum imports (50 million dry tons/yr estimated)



- Production is limited by cost-prohibitive CO_2 pipeline distances, which force cultivation to unfavorable climates

The Microalgae Biofuels Solution

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- Uncoupling ponds from power plants expands algal biofuels production potential by 10x

Table 2. Summary of Results for Each of the Screening Scenarios

Venteris 2014

strain	scenario	number of sites	average annual productivity for the top 200 sites ($g\ m^{-2}\ day^{-1}$)
Arthrospira sp.	no screen	64412	15.38
Sphaeropleales	no screen	64412	17.5
Arthrospira sp.	base scenario	28638	14.56
Sphaeropleales	base scenario	3418	16.46
Arthrospira sp.	base, 10 mile infrastructure, 5 mile rail	4356	13.65
Sphaeropleales	base, 10 mile infrastructure, 5 mile rail	492	14.58

- 28,638 potential, 485 ha unit farms identified
- Assume 10 g VSS/m^2-day productivity
  - 36 dry ton/ha-yr with 330 operating days/yr)
- 28,638 sites X 485 ha/site X (36 dry tons/ha-yr) = **500 MDT/yr**
  - 1.4×10^7 Ha required (~Florida)
  - Requires optimistic assumptions regarding infrastructure

Goals and Outcomes

Goals

- Develop a process, **AlgaeAirFix^{TM}**, that would allow micro-algae **biofuels production with air-CO_2** with minimal change in productivity.
- Demonstrate that **CO_2 transfer from air to algal ponds** can be **enhanced** by **physical, chemical and biological** processes.
- **Decouple** algae production from **enriched sources of CO_2**

Outcomes

- **Significant increase in CO_2 resource potential, expanding microalgae biofuels production by over 10-fold the CO_2 co-location scenario.**
- Potential **reduction** in microalgae **biofuels cost**.

Quad Chart

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Timeline

- Start date: 10-1-2015
- End date: 9-30-2017
- Percent Complete: 75%

Barriers

- Aft-A Biomass Avail + Costs
  - Addresses potential locations and quantity of feedstocks
- Aft-B Sustainable Production
  - Addresses resource (industrial CO_2) limitations

Budget

	FY 16 Costs	FY 17 Costs	Total Funding
DOE Funded	$450K	$450K	$900K
Cost Share MBE, Inc.	$112K	$113K	$225K

Partners

- John Benemann, Co-PI (MicroBio Engineering)
- 22% of project budget for MBE, Inc.

Project Management

Meetings

- **Biweekly teleconferences** with the entire project team (5-6 members)
- **Annual** face to face **meetings**
- Other meetings on *ad hoc* basis

Quarterly Reports and Tracking of Milestones

- Data flows through the PI
- **PI tracks milestones and generates all reports**
- Synthesis of results into publications & solutions tracked & mediated by PI
- **Coordination with BETO**

Decision Making Process

- Decision making is through **consensus of PI and project team members**
- Technical leads at PNNL & MBE are responsible for achieving milestones
- PI retains ultimate decision-making authority

Approach: Increasing CO_2 Flux (F) from Air Into Pond via Carbonic Anhydrase

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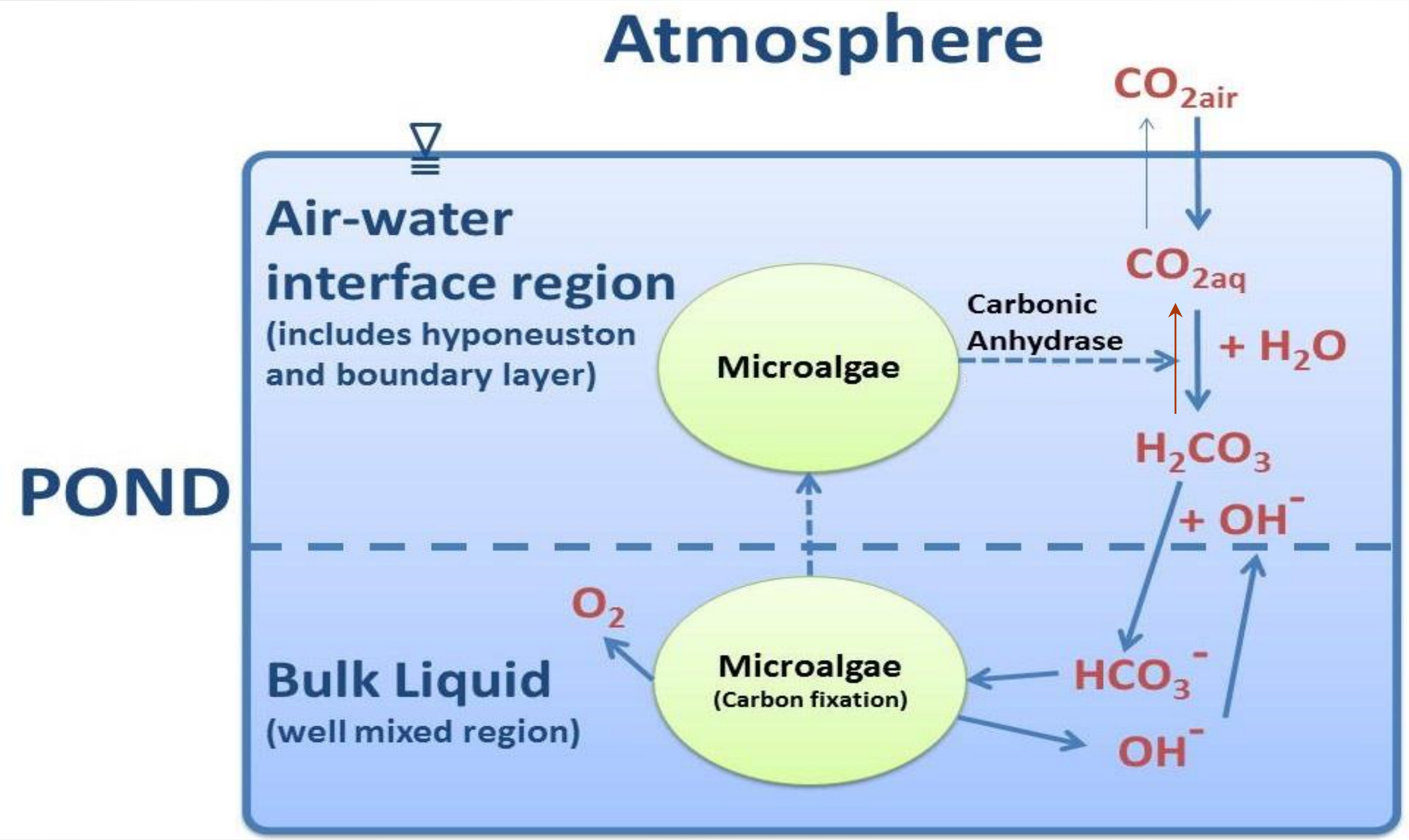
$$F = k_L \, A \, (C_{sat} - C_{bulk})$$

k_L = CO_2 mass transfer rate coefficient
A = air-water interface area
C_{sat} = CO_2 concentration at surface (equilibrium with air)
C_{bulk} = CO_2 concentration in bulk liquid

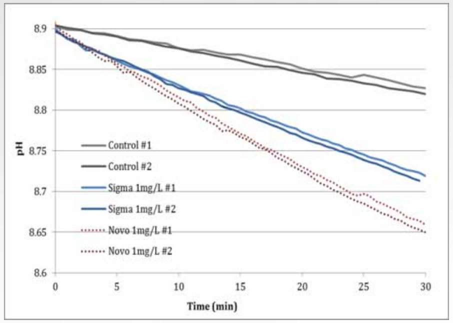


Approach: Specific Project Objectives

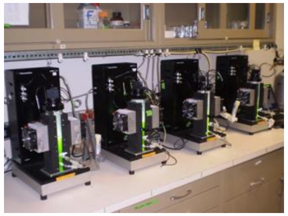
- Determine the **baseline biomass productivity** of outdoor microalgae pond cultures **grown on air CO_2**.
  - What increase in biomass productivity is needed to reach BETO targets?
  - What is the effect of culture depth, mixing speed, and alkalinity?
- Identify the **optimum concentration of carbonic anhydrase** (CA) and the range of suitable environmental conditions:
  - How much CA should be added to the cultures? How stable is CA?
  - What is the effect of salinity, alkalinity, temperature?
- Demonstrate that **addition of CA increases biomass productivity** in photobioreactor and pond cultures by **at least two-fold** relative to controls supplied with CO_2 from air.
- Conduct **techno-economic analysis** and **resource assessment** of **AlgaeAirFix™** process.

Approach: FY16 Tasks

- **PNNL: Demonstrate increase in CO_2 mass transfer rate coefficient (k_L) as a function of:**
  - CA concentration and type
  - Alkalinity
  - Salinity
  - Temperature



- **PNNL: Demonstrate increase in biomass productivity with exogenous CA enhanced air CO_2 mass-transfer in ePBRs**



- **MBE: Measure biomass productivity of algae pond cultures grown on air CO_2**

- **MBE: Conduct a techno-economic and resource analysis of the AlgaeAirFix™ process**

Approach: FY17 Tasks

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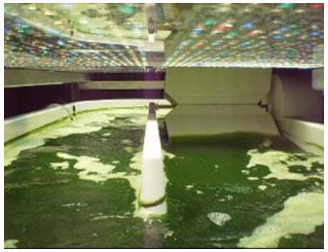
- **GO/NO GO Criteria: Demonstrate greater than <u>two-fold increase</u> in biomass productivity with exogenous CA enhanced air CO_2 mass-transfer in ePBRs**

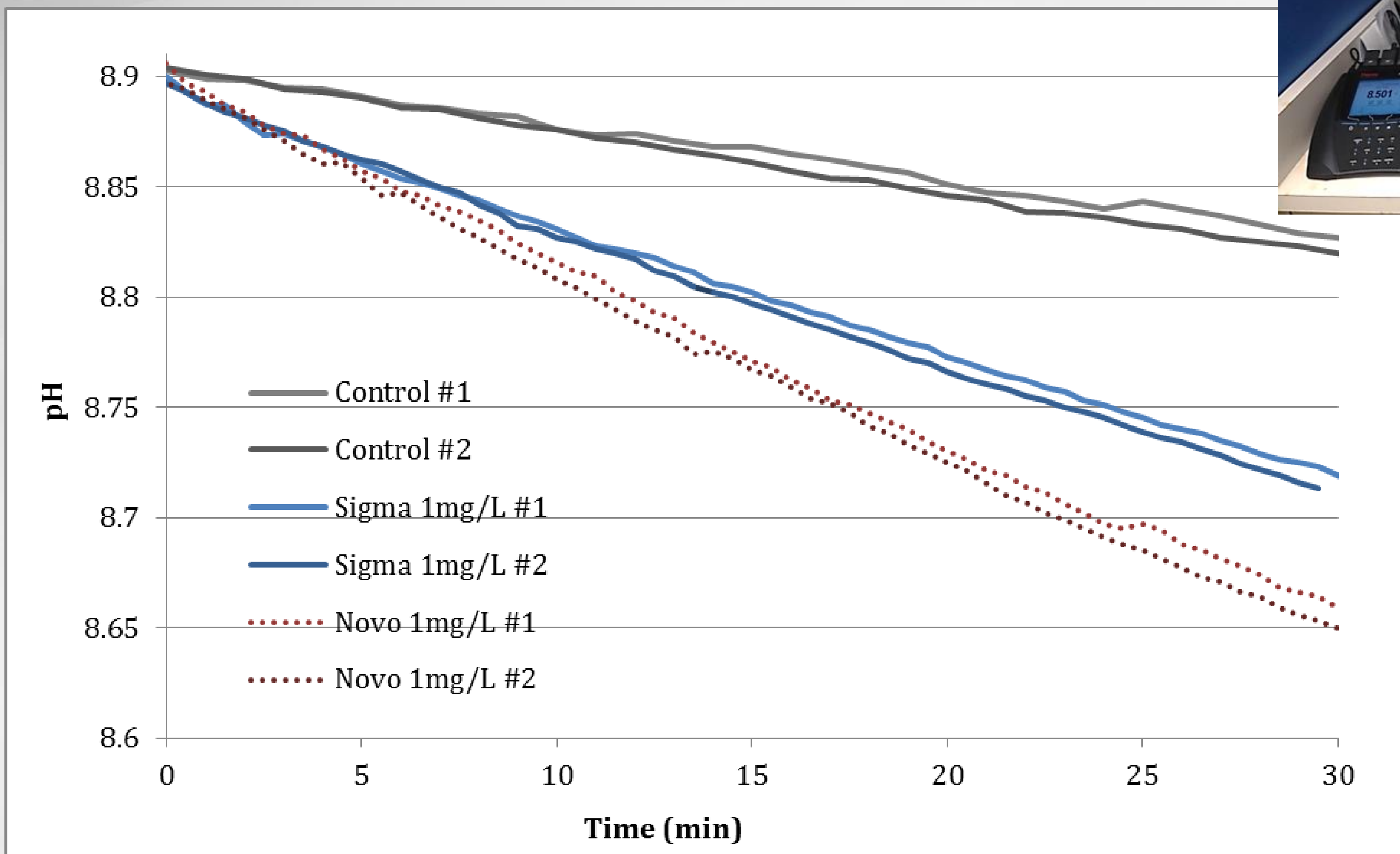
- **PNNL: Demonstrate increase in biomass productivity with exogenous CA enhanced air CO_2 mass-transfer in raceway ponds**



- **MBE: Selection of algal cultures for increased productivity under air CO_2:**
  - Productivity in shallow ponds with and without CO_2 supplementation
  - Measure CA activity and algal productivity in ponds operated with surface recycle

Progress: Measure CO_2 Mass Transfer Rate Coefficiencs (k_L)

pH versus Time Data Collected during typical CO_2 Mass Transfer Rate Experiment



Progress: Identify Optimal CA Concentration

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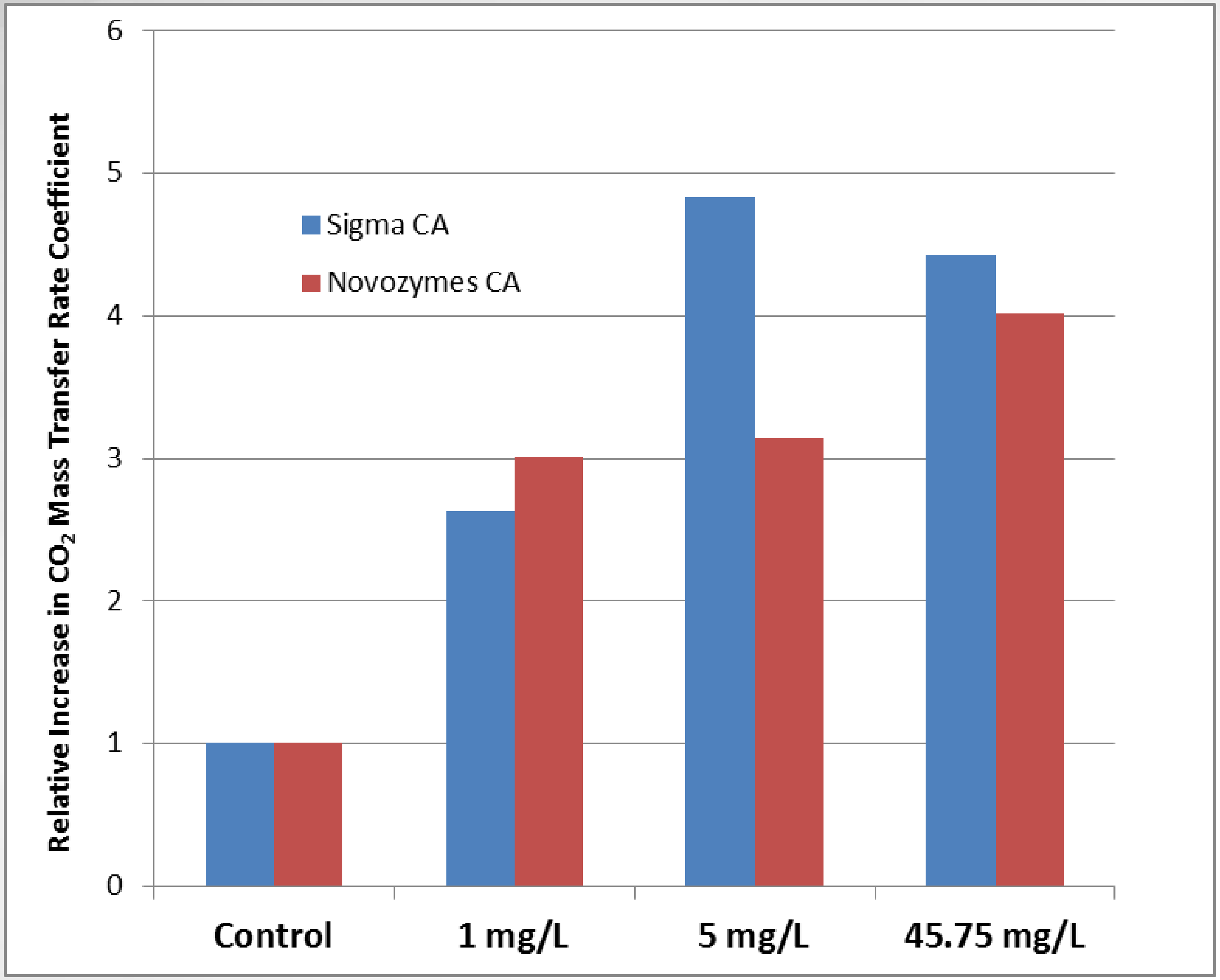
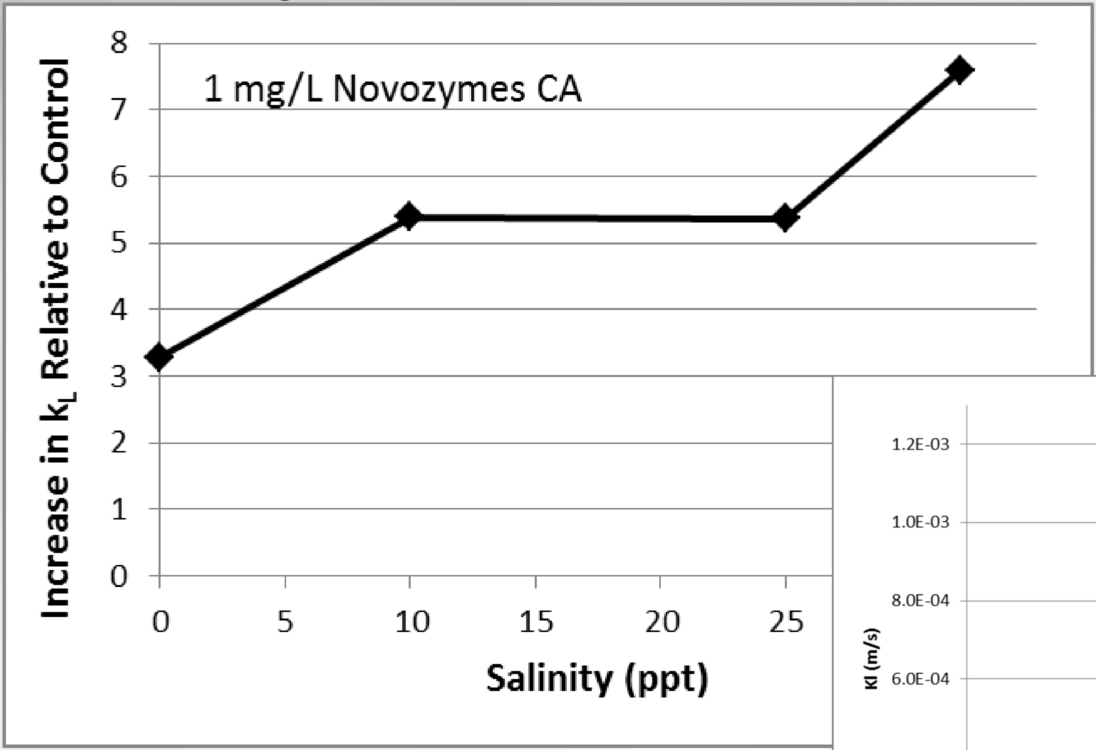
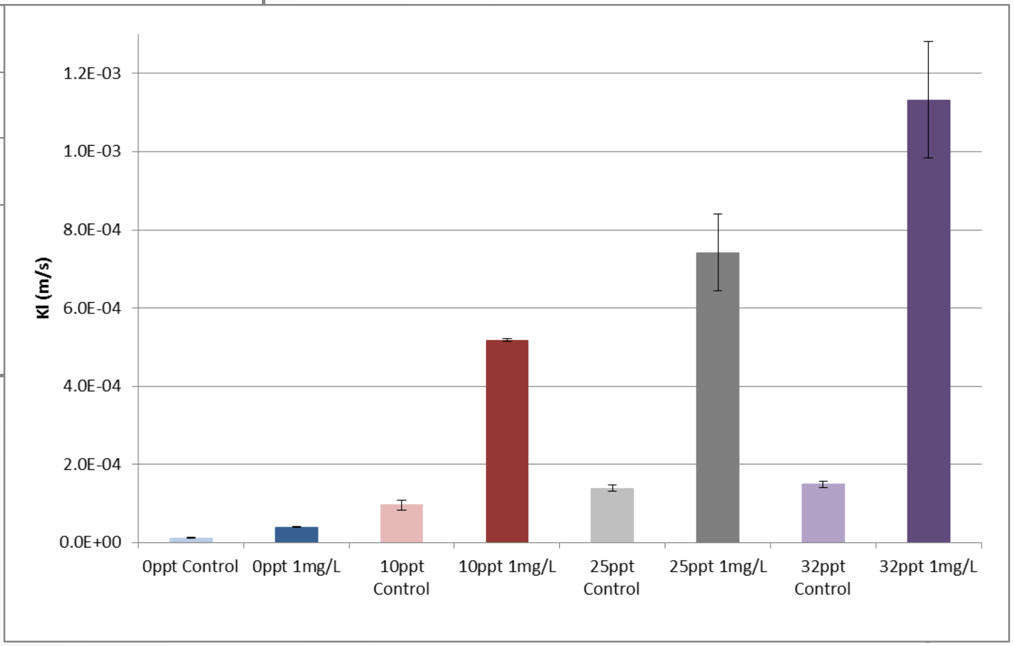
Addition of Carbonic Anhydrase (CA) Increases k_L Relative to Unamended Controls in Dose-Dependent Manner Exhibiting Saturation



Progress: Effect of Salinity on the CO_2 mass transfer rate coefficient (k_L)

Addition of CA at 1 mg/L results in a relative increase in k_L, increasing with salinity up to a 7.5x enhancement at seawater salinity.





Progress: Effect of CA Addition in ePBRs

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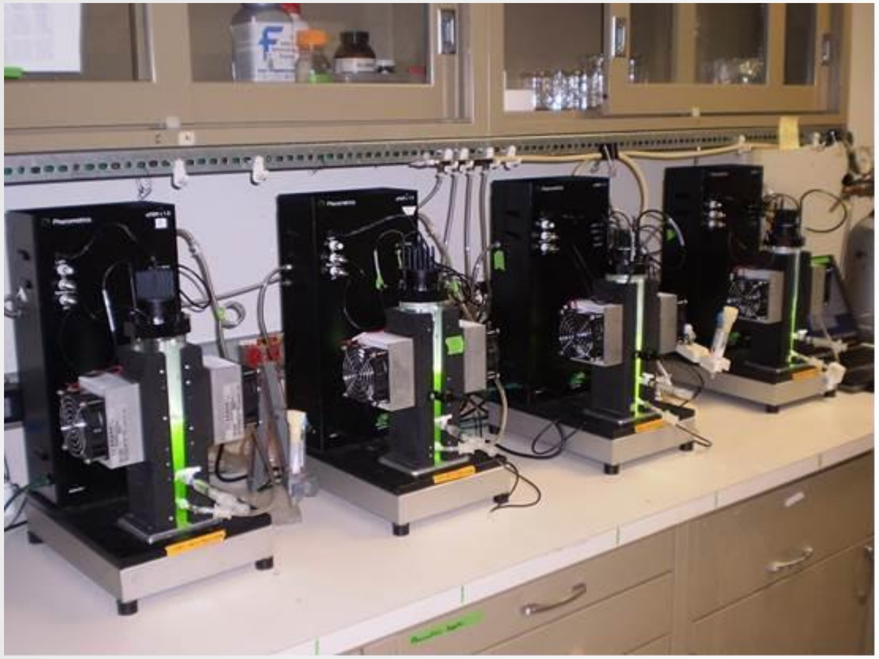
The Effect of Novozymes CA (18 mg/L or 60 Wilbur Anderson Units per mL) Addition on Biomass Productivity and pH in ePBR Cultures of *Chlorella sorokiniana* (DOE 1412)

- **Unsparged Control**: CO_2 from air in headspace (simulates pond with air flow)
- **Sparged Control**: Periodic CO_2 for pH control (Set-point = ca. pH 9)
- **CA Treatment (2x):** Add 18 mg/L Novozymes CA with initial pH of 8 (BG-11)

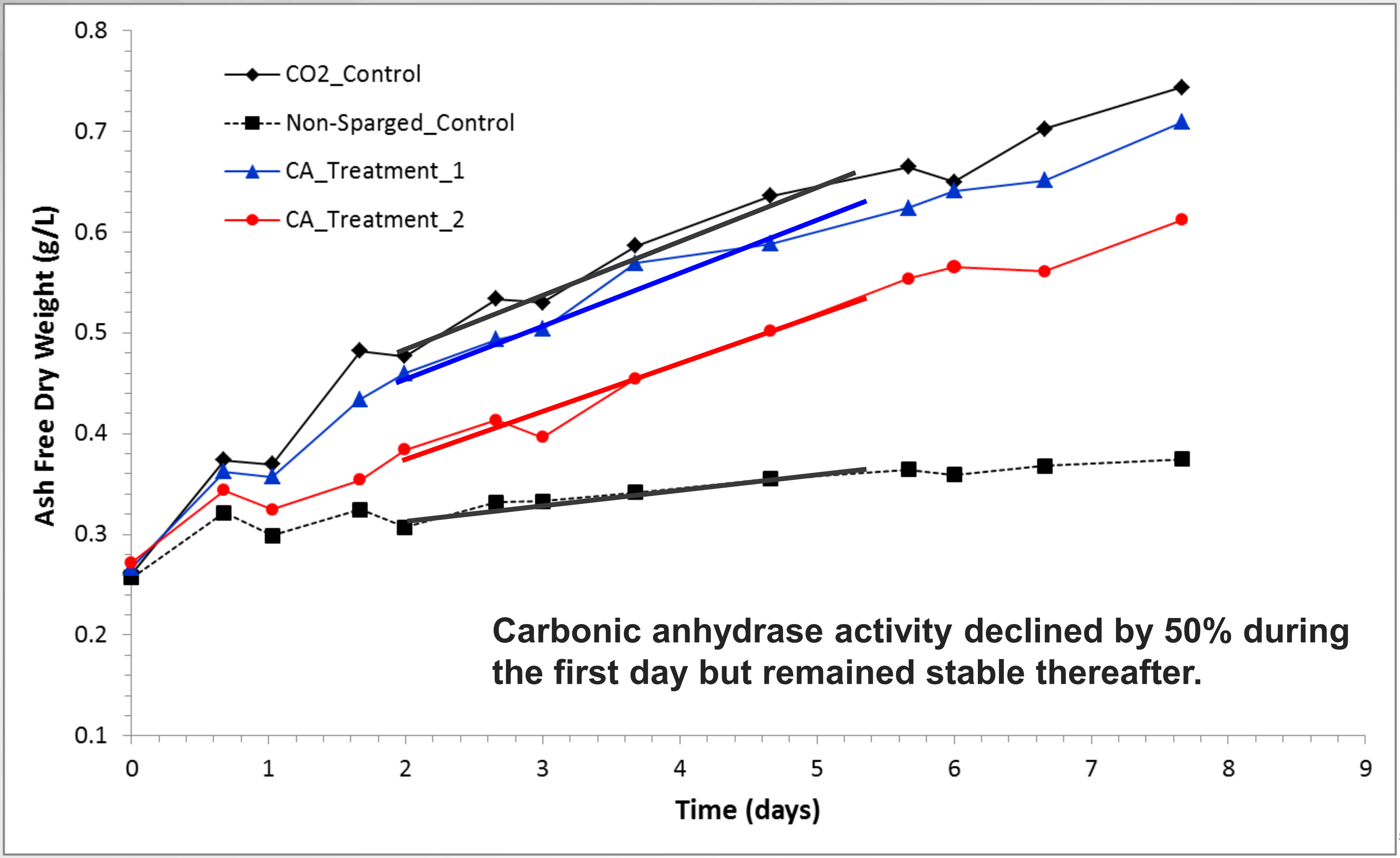


ePBR Culture Conditions:

- Sinosoidal (16:8 hr) temperatures (25-35 °C)
- Sinosoidal (16:8 hr) light intensities (0 – 2000 µmol/m^2-sec)
- Culture depth = 20 cm
- Mixing speed = 300 rpm
- Headspace purged with air at 844 mL/min
- Purged every 10 minutes for 10 sec with air to simulate paddlewheel mixing and remove foam on surface

Progress: Effect of CA Addition in ePBRs

The Rate of Biomass Growth in CA Treated Cultures is Almost as Fast as in CO_2-sparged Control



Achieved Go/No Go Decision Criterion
(>2-Fold Increase in Productivity due to CA Addition)

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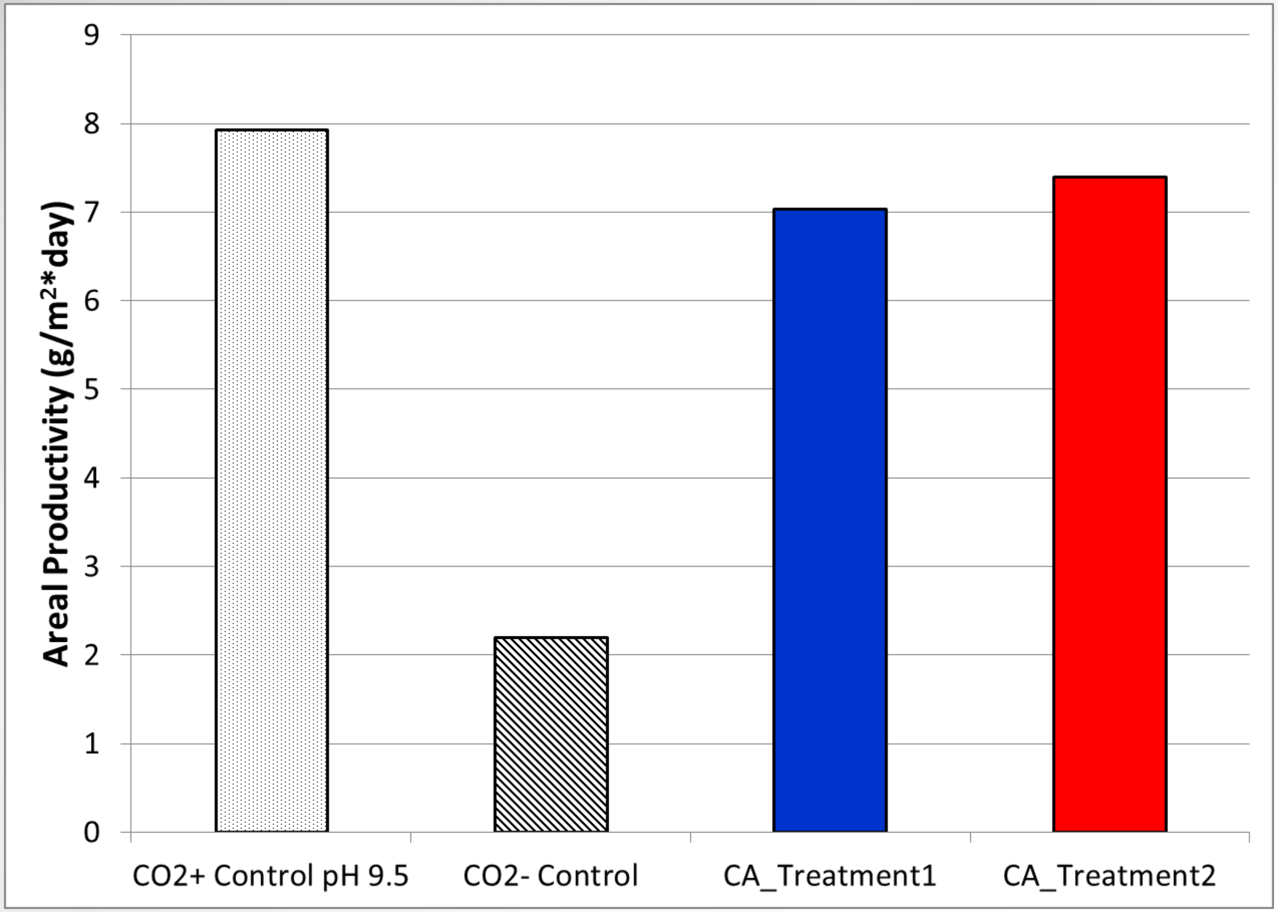
The Linear Phase Biomass Productivity in CA Treatments was More Than Three Times Higher than in the Non-sparged Control and Approached that of the Sparged Control.

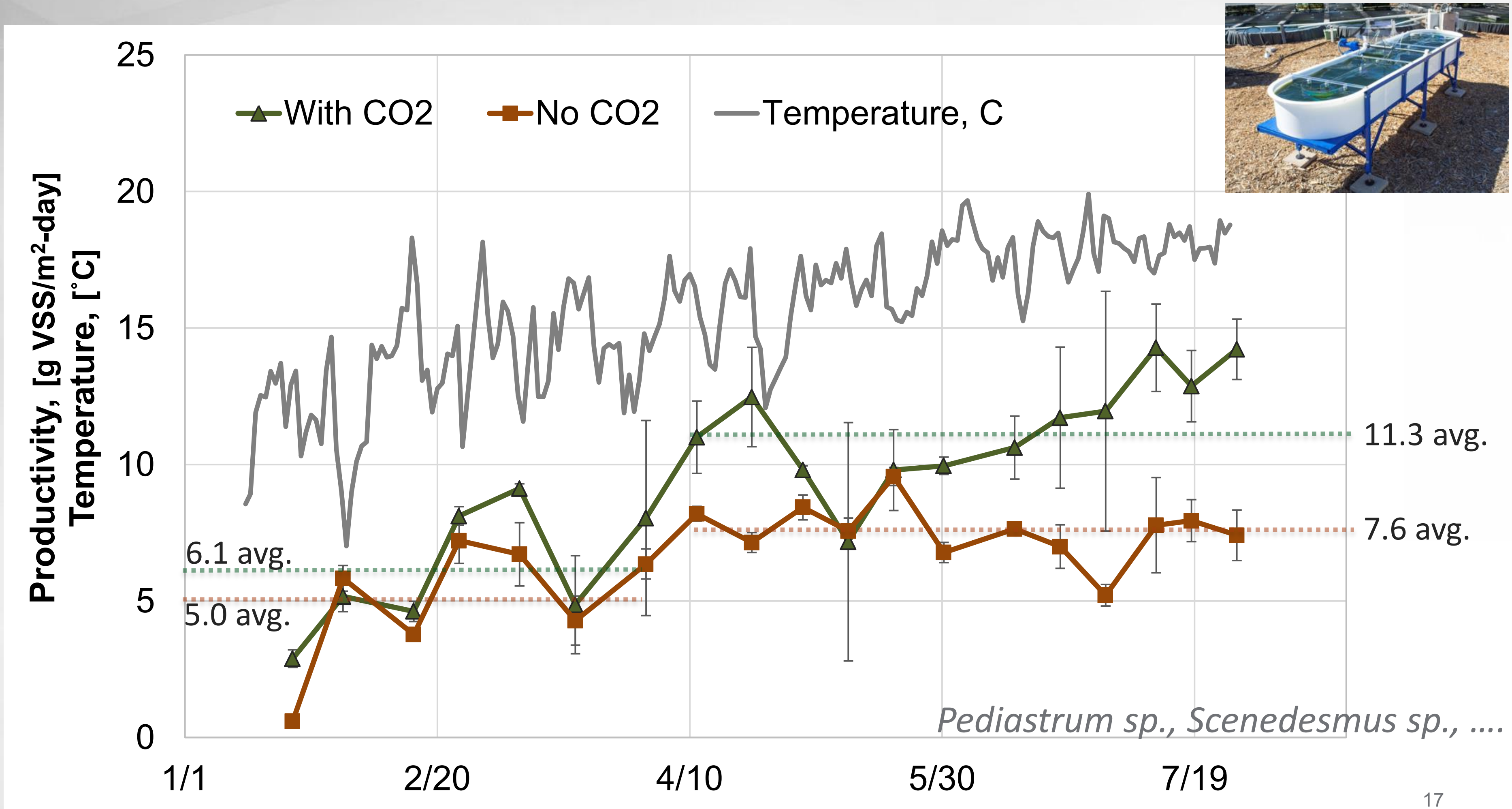


*Biomass productivities were calculated from linear regression AFDW vs. time slopes from day 2 to day 5.7.

Progress: Outdoor Pond air-CO_2 Productivity

Productivity saturates at ~8 g/m^2-day when grown on only air-CO_2 (native strain consortium)

Similar productivity in winter, 33% decrease in summer



*Error bars represent the standard deviation between duplicate ponds

Progress: Effect of CA Addition in Ponds

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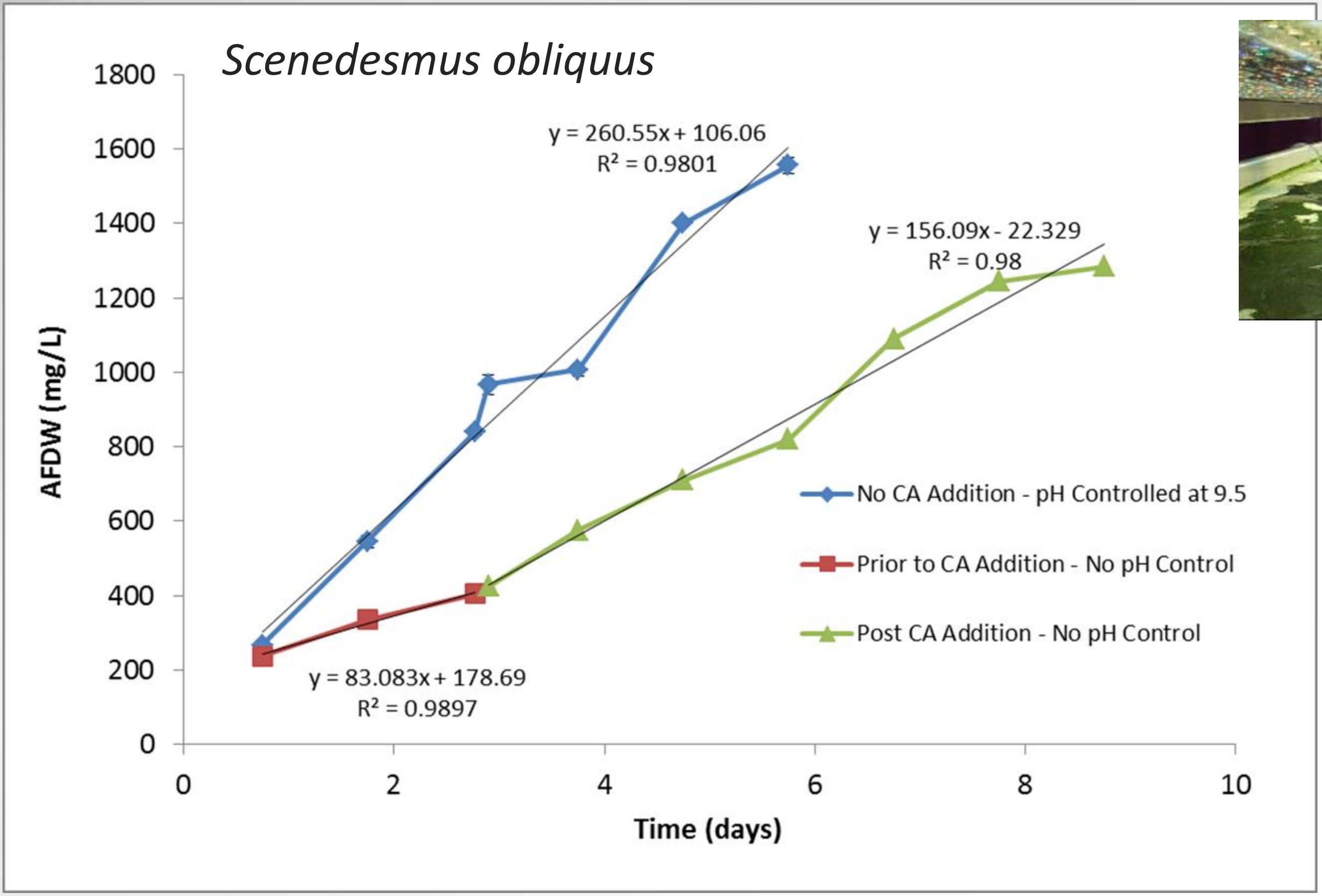
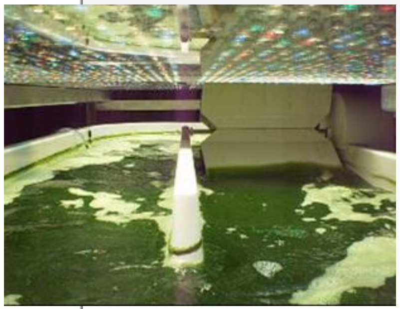
Addition of CA at Day 3 Increases Rate of Biomass Growth but Does Not Reach Rate Observed in CO_2-Sparged Control.

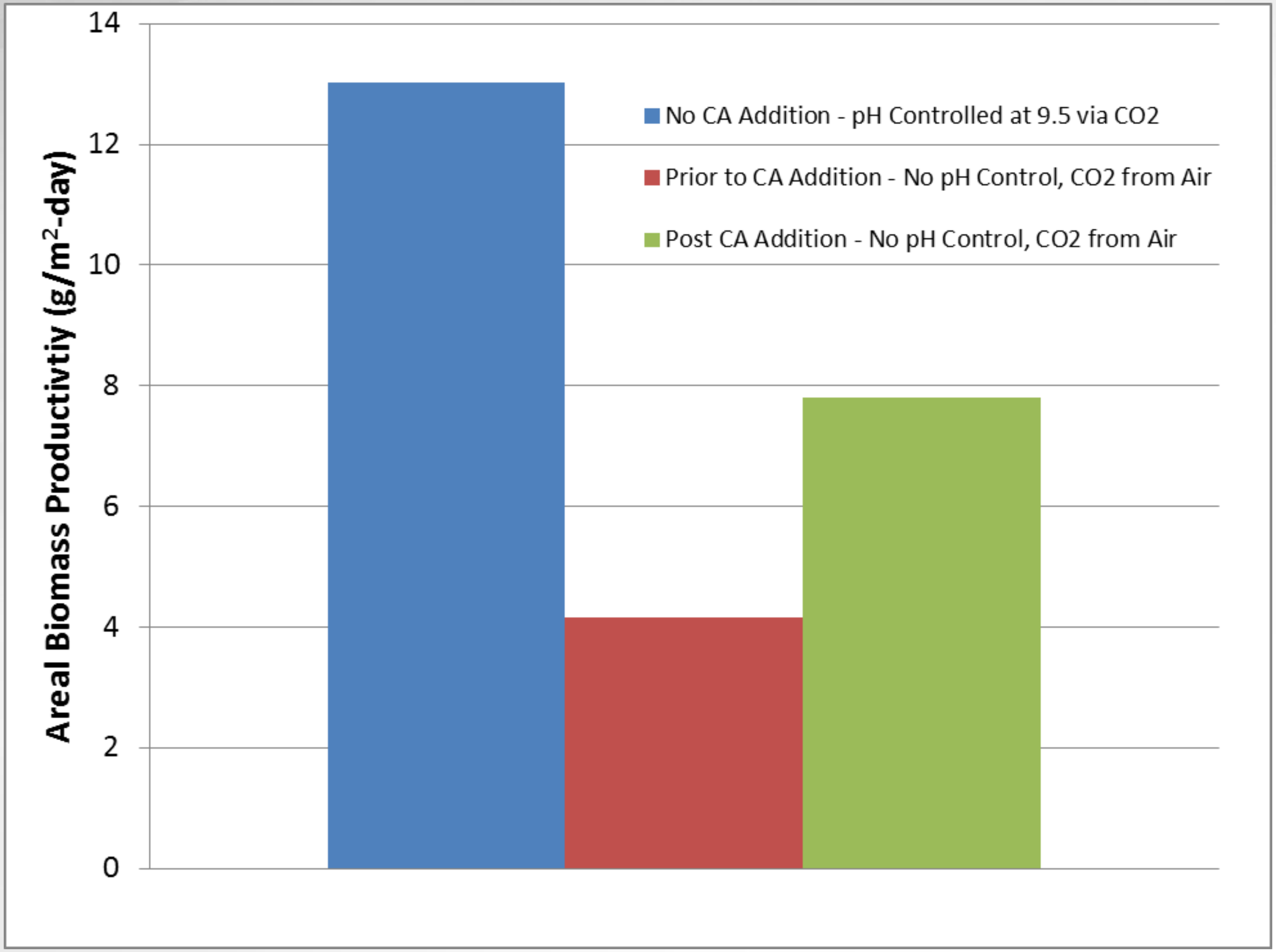




Key West, FL
July Script

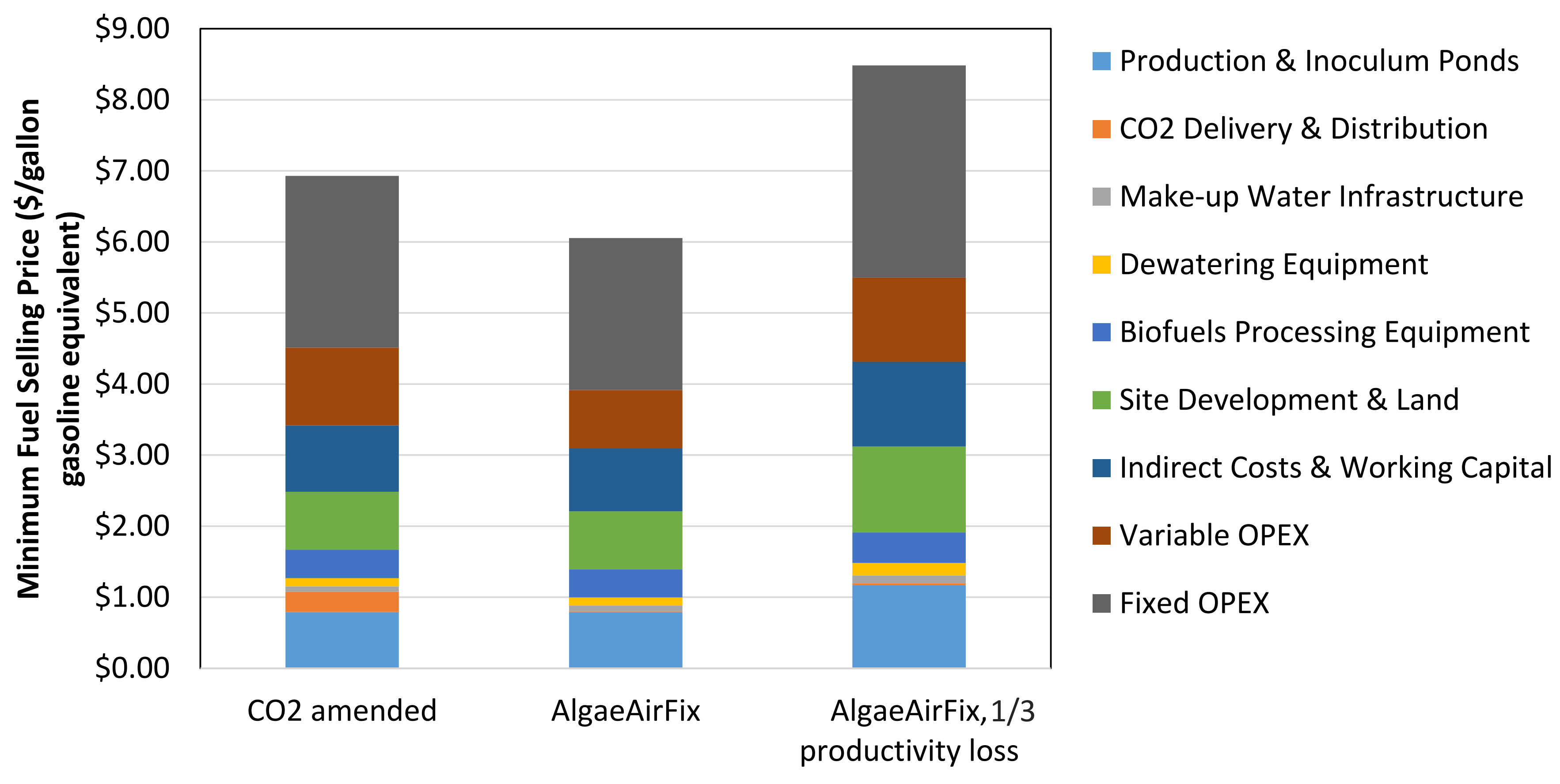
Progress: Effect of CA Addition in Ponds

Addition of CA Doubles Biomass Productivity (from 4 to 8 g/m^2-day) due to Increased CO_2 Mass Transfer and Lower pH



AlgaeAirFix™ TEA Implications

- **The AlgaeAirFix™ process decreases minimum fuel selling price by $1/gallon, if no change in productivity**



- **The main advantage of AlgaeAirFix™ is the 10x expansion in resource potential**

Relevance

- The goal of this project is to develop a process, **AlgaeAirFix™**, that would allow microalgae **biofuels production with air-CO_2.**
- We have **successfully demonstrated** that **CO_2 transfer from air** to *Chlorella* and *Scenedesmus* **cultures** can be **enhanced over two-fold** by addition of **carbonic anhydrase.**
- **Addition of carbonic anhydrase** can **increase** the **baseline** (CO_2 from air only) **productivity measured in outdoor ponds.**
- **AlgaeAirFix™ reduces fuel selling price by $1.00/gallon ($7.00 to $6.00/gallon)** if no reduction in productivity.
- **Endogenous CA production** avoids prohibitive enzyme cost
- By **eliminating the need for concentrated sources of CO_2** (e.g., flue gas CO_2), a fully improved AlgaeAirFix™ process will increase the **CO_2 microalgae biofuels potential over 10-fold.**

Summary

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- We have **demonstrated** that **CO_2 transfer from air to algal ponds** can be **enhanced <u>over two-fold</u>** with **carbonic anhydrase**
- An **<u>additional two to three-fold</u> improvement** is needed to **enable year-round outdoor pond cultivation using only air CO_2, at little to no reduction in productivity**
- **Future work for continued development of AlgaeAirFix™:**
  - Optimize mass transfer via exogenous carbonic anhydrase
  - Use of floating immobilized CA
  - Evaluate chemically enhanced CO_2 mass-transfer at high pH, using alkaliphilic strains
  - Assess marine strains, given k_L increases with salinity
  - Investigate endogenous carbonic anhydrase production by microalgae

Supplemental Viewgraphs

Future Work in FY17

- **PNNL: Demonstrate increase in biomass productivity with exogenous CA enhanced air CO_2 mass-transfer in raceway ponds:**
  - **Confirm** earlier ePBR results in raceway ponds.
  - **Evaluate whether winter biomass productivities** (10 g/m^2-day) can be achieved with **CA addition** and CO_2 from air only.
  - Evaluate **marine** and **alkaliphilic** strains (high alkalinity, high pH)
- **MBE: Selection of algal cultures for increased productivity under air CO_2:**
  - Productivity in shallow ponds with and without CO_2 supplementation
  - Measure CA activity and algal productivity in ponds operated with surface recycle
  - Select for strains with **endogenous CA production capability**
- **MBE: Update TEA/LCA/RA based on Year 2 results**

Effect of CA Concentration and Mixing Speed

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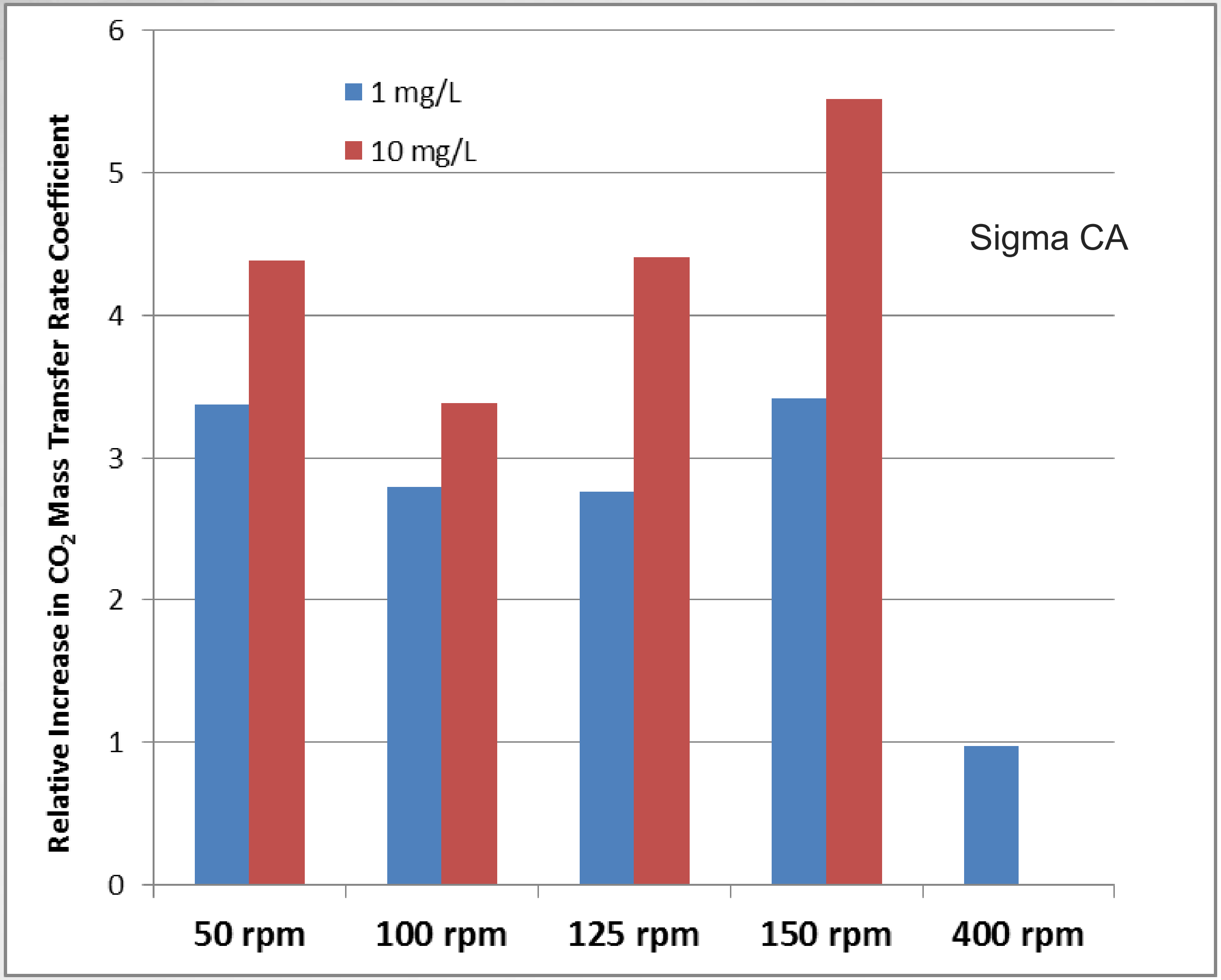
Addition of Carbonic Anhydrase (1 and 10 mg/L) at Mixing Speeds (50 to 150 rpm) Increases k_L Relative to Unamended Controls 3 to 5-fold .



Effect of Alkalinity with 1 mg/L CA on the CO_2 Mass Transfer Coefficient

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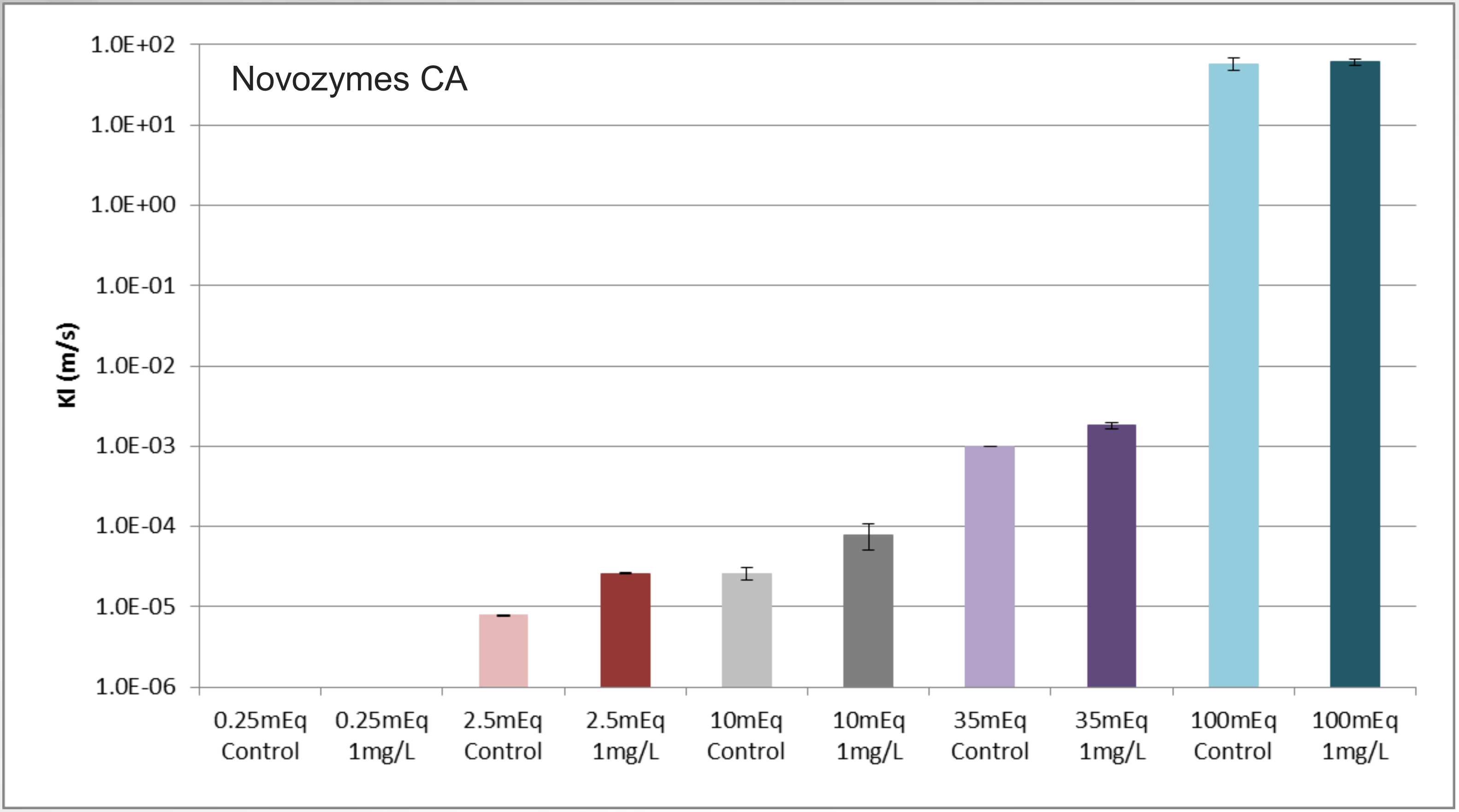
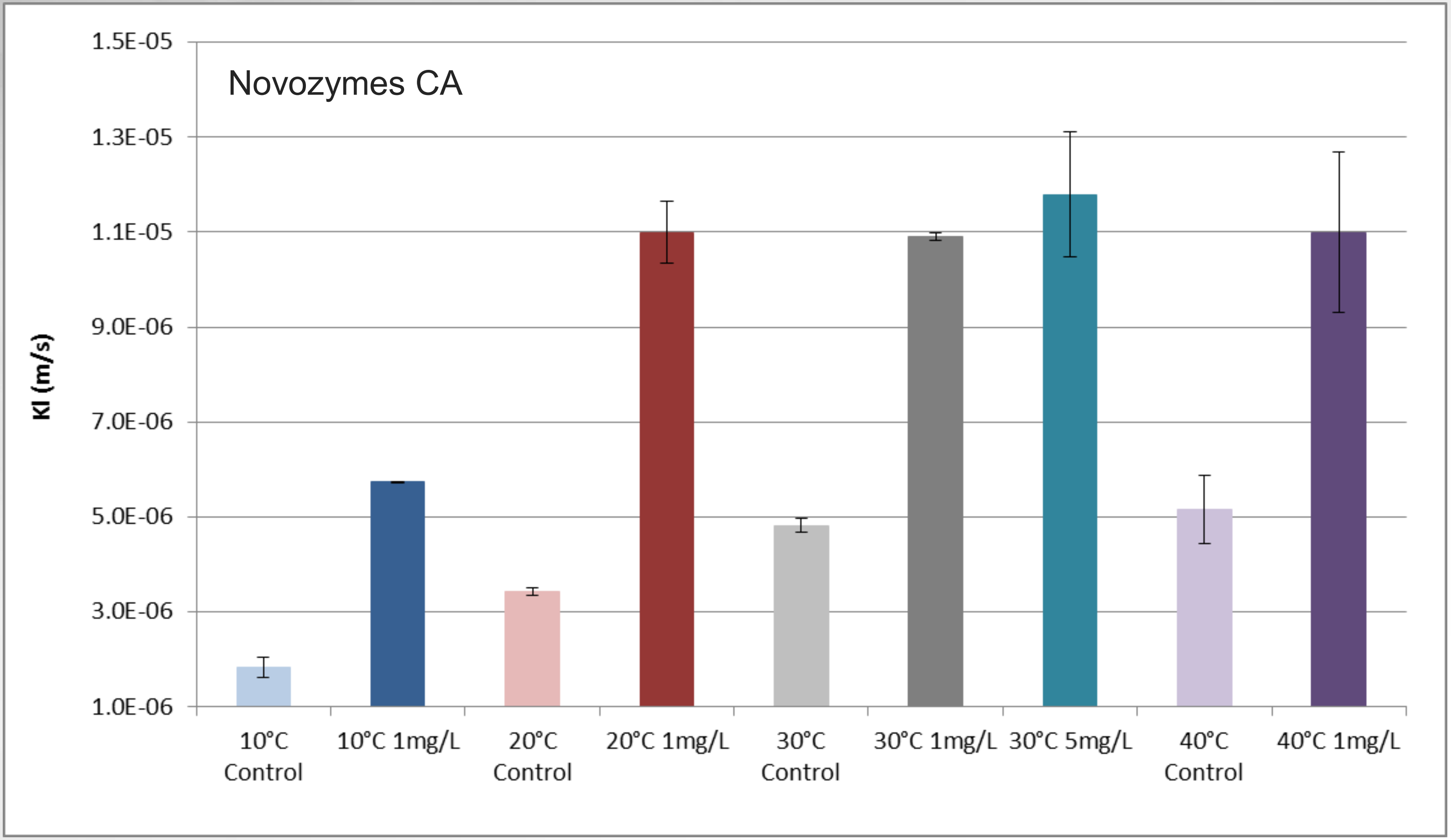


Effect of Temperature with 1 mg/L CA on the CO_2 Mass Transfer Coefficient



Progress: Effect of CA Addition in ePBRs

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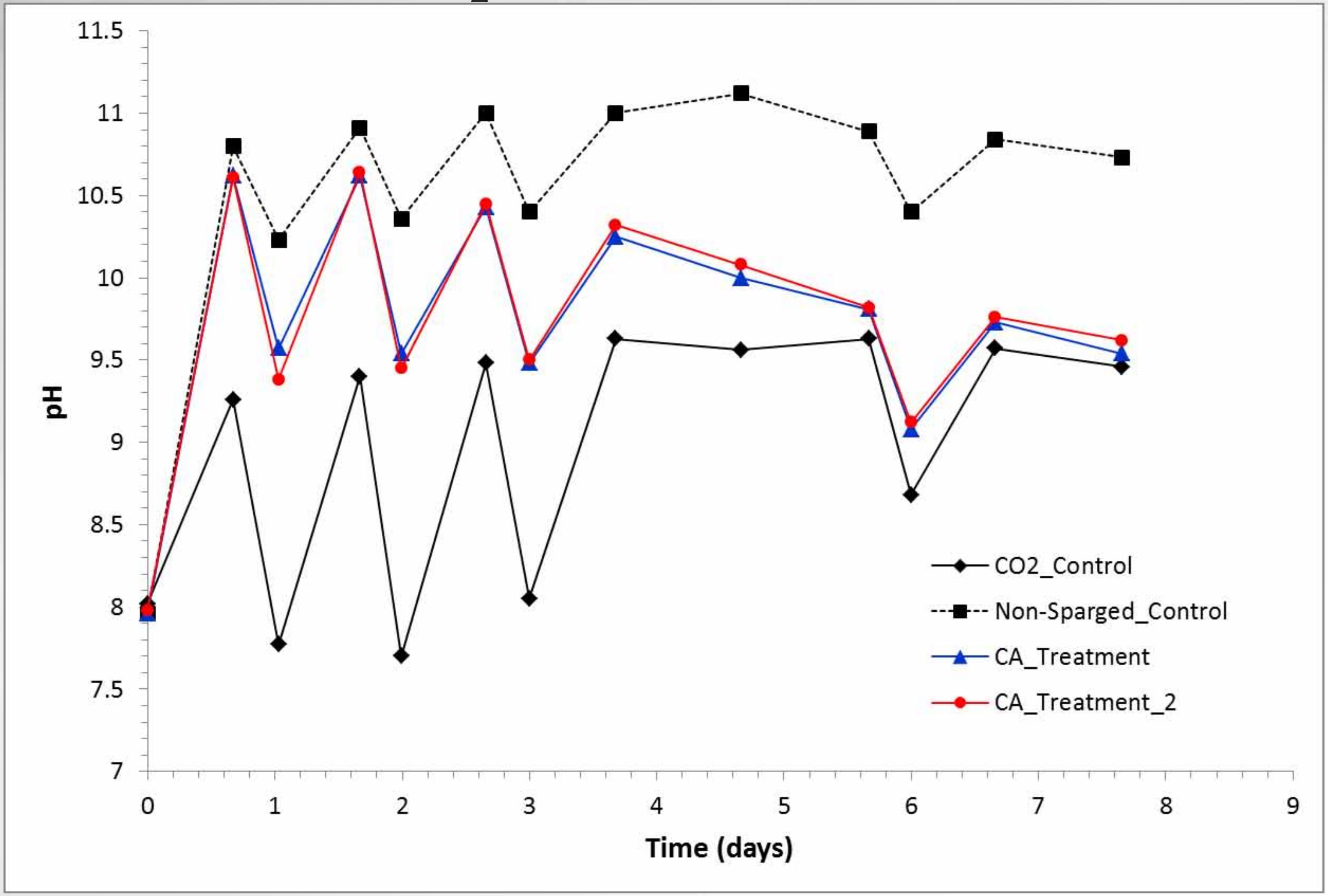
The pH in the CA Treated Cultures was Lower than in the Non-sparged Control, Indicating Effectiveness of CA to Increase Transfer of CO_2 from Headspace Air into Cultures.



Progress: Effect of CA Addition in ePBRs

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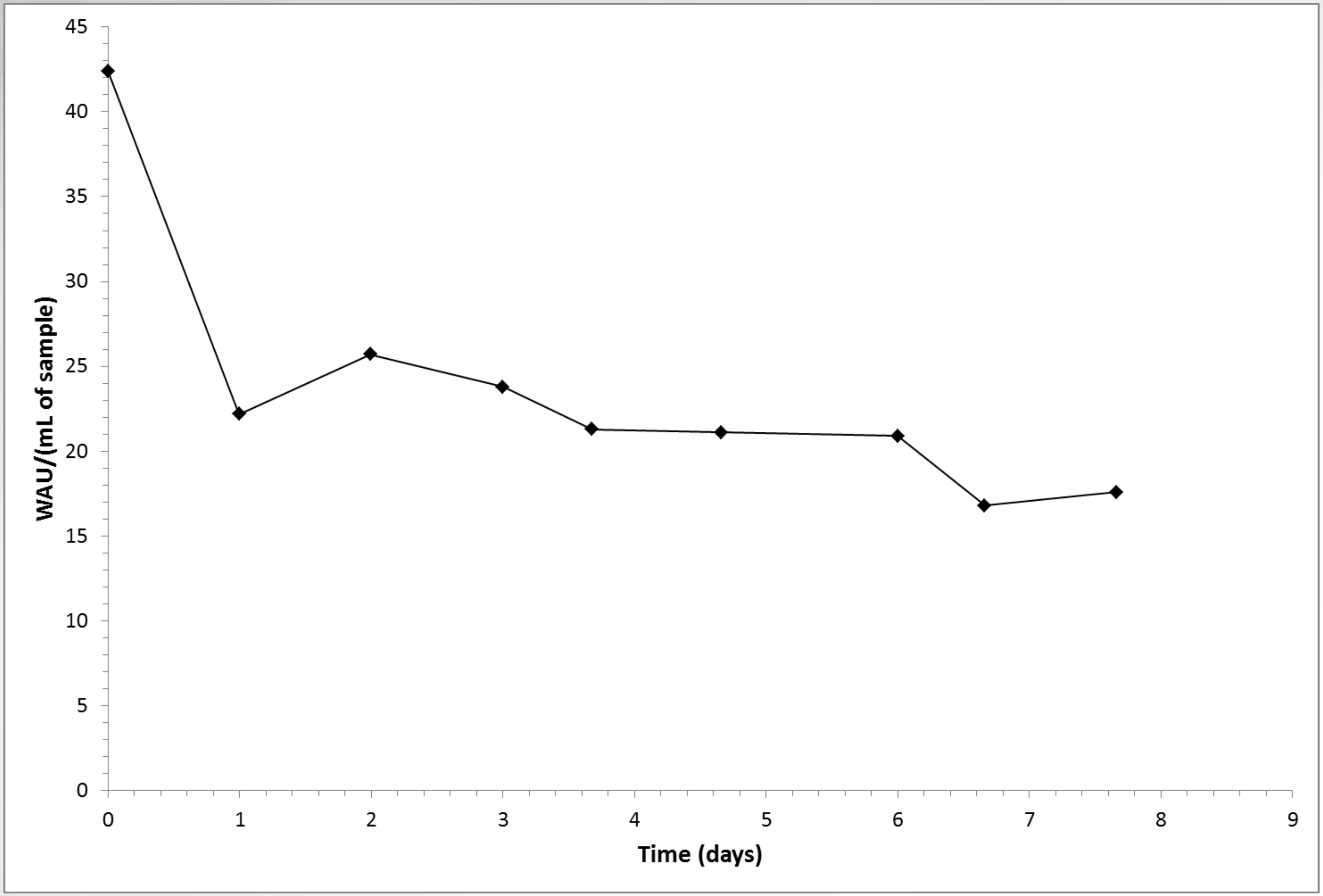
Carbonic anhydrase activity declines sharply during the first day but then remained stable for the duration of the experiment.



Progress: Effect of CA Addition in Ponds

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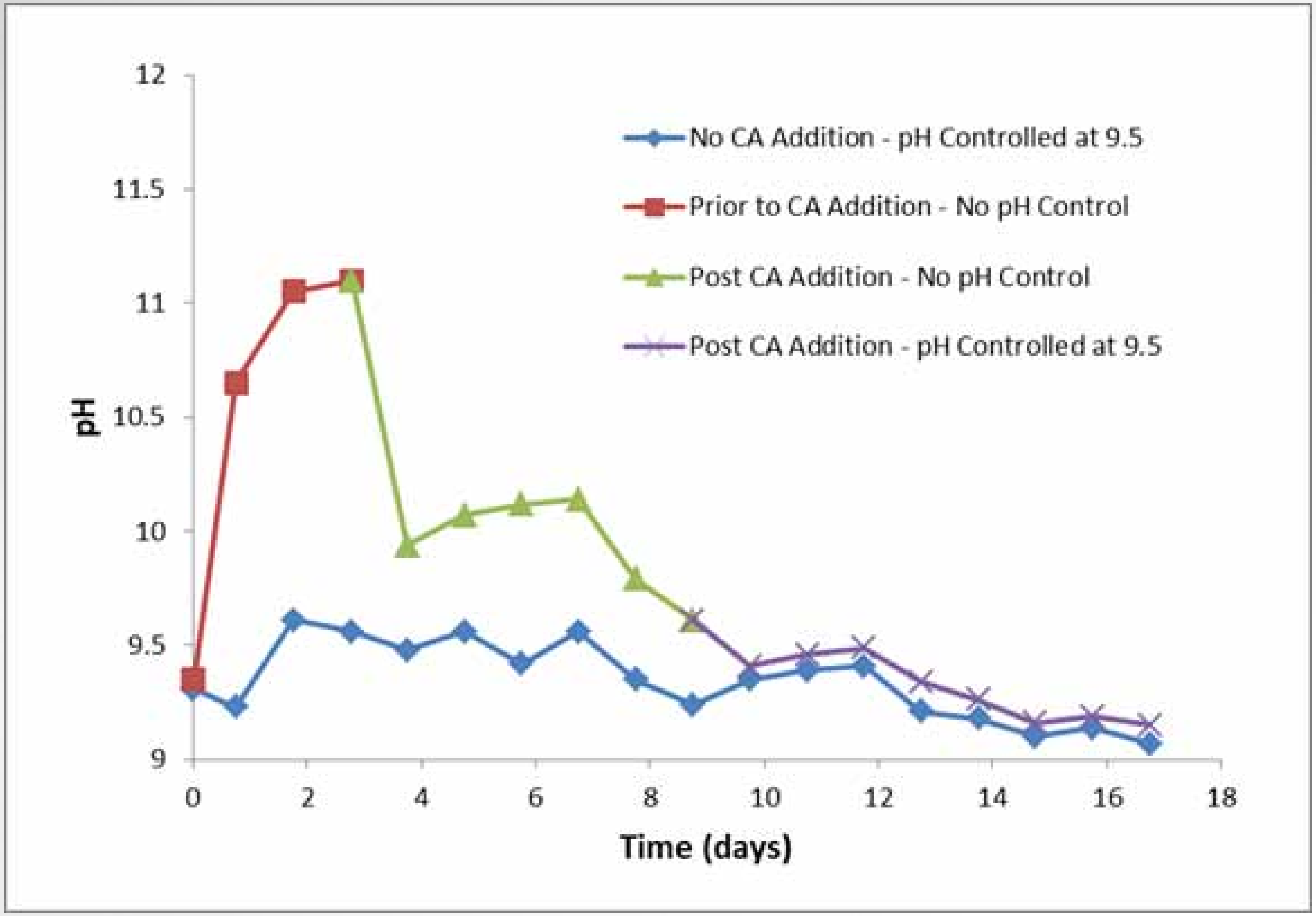
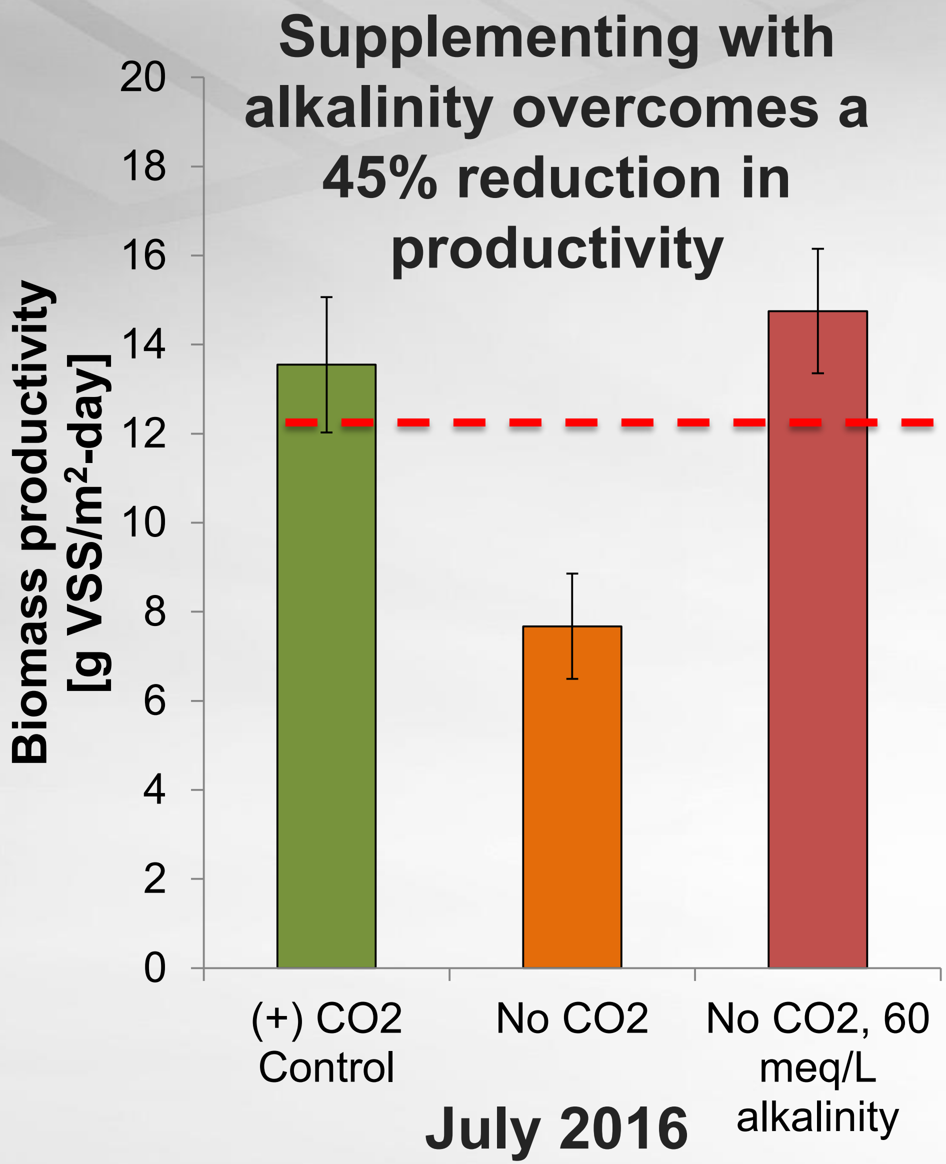
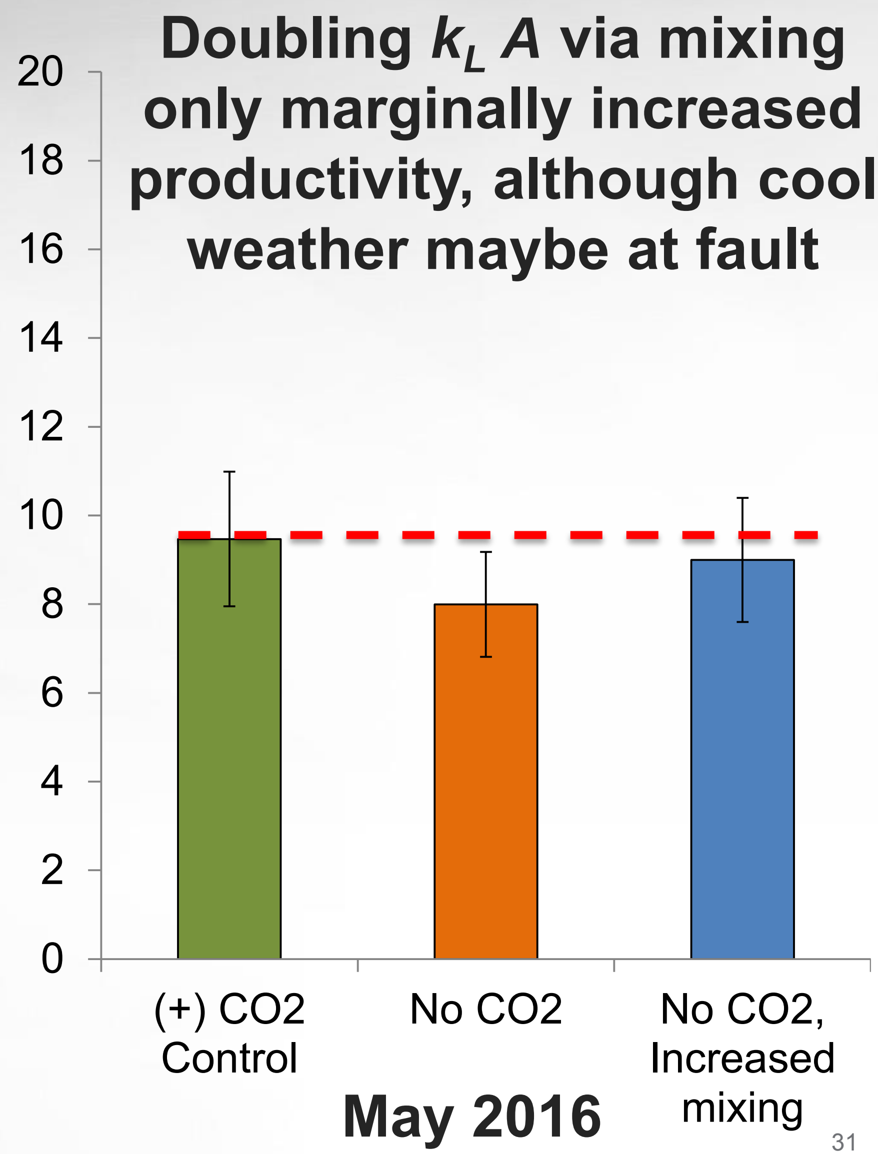
Addition of CA at Day 3 Sharply Reduces the Culture pH, Indicating Effectiveness of CA in Transferring CO_2 from Air into Climate-Simulation Pond Culture



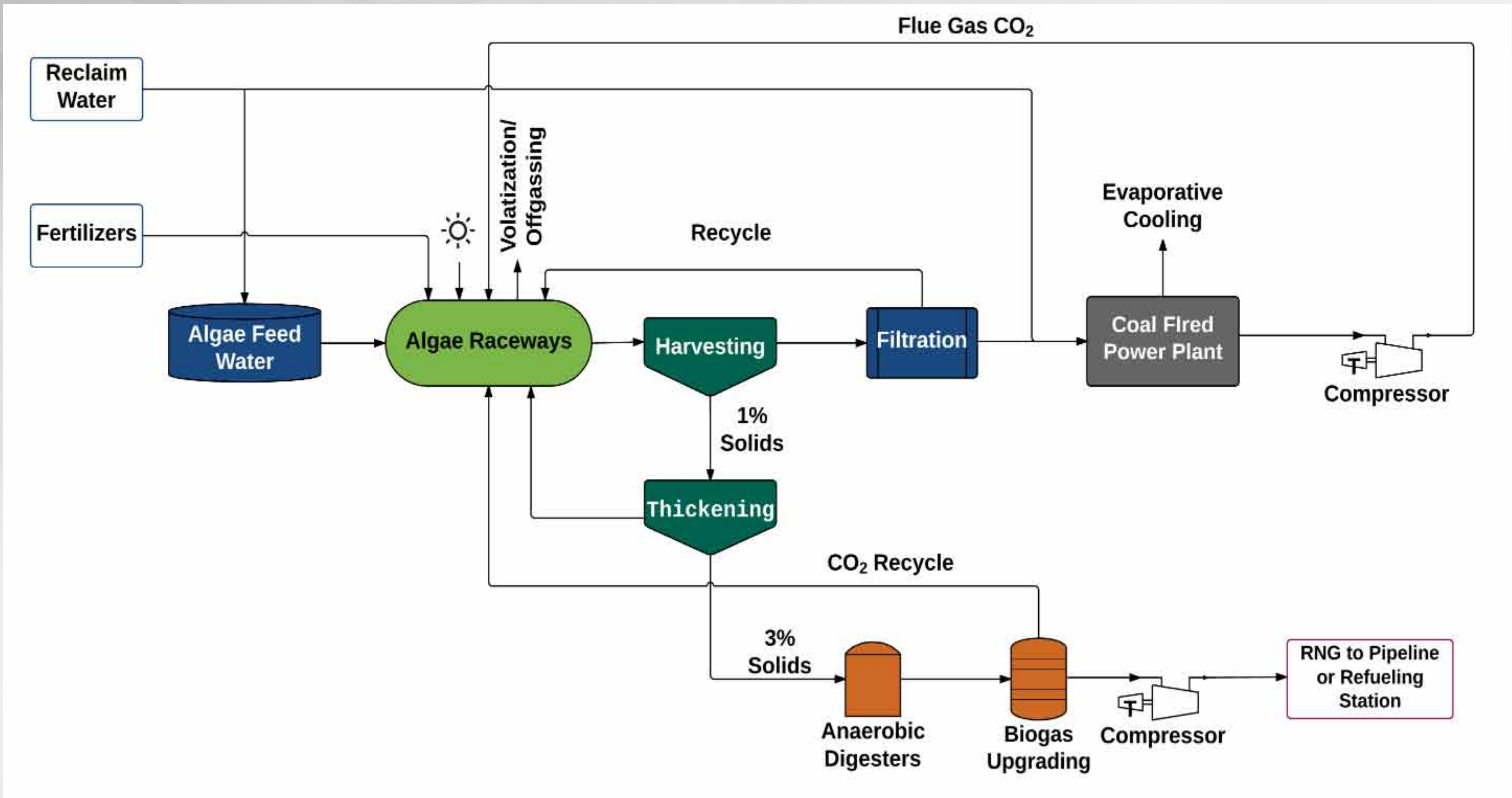
Progress: Outdoor pond air-CO_2 Productivity





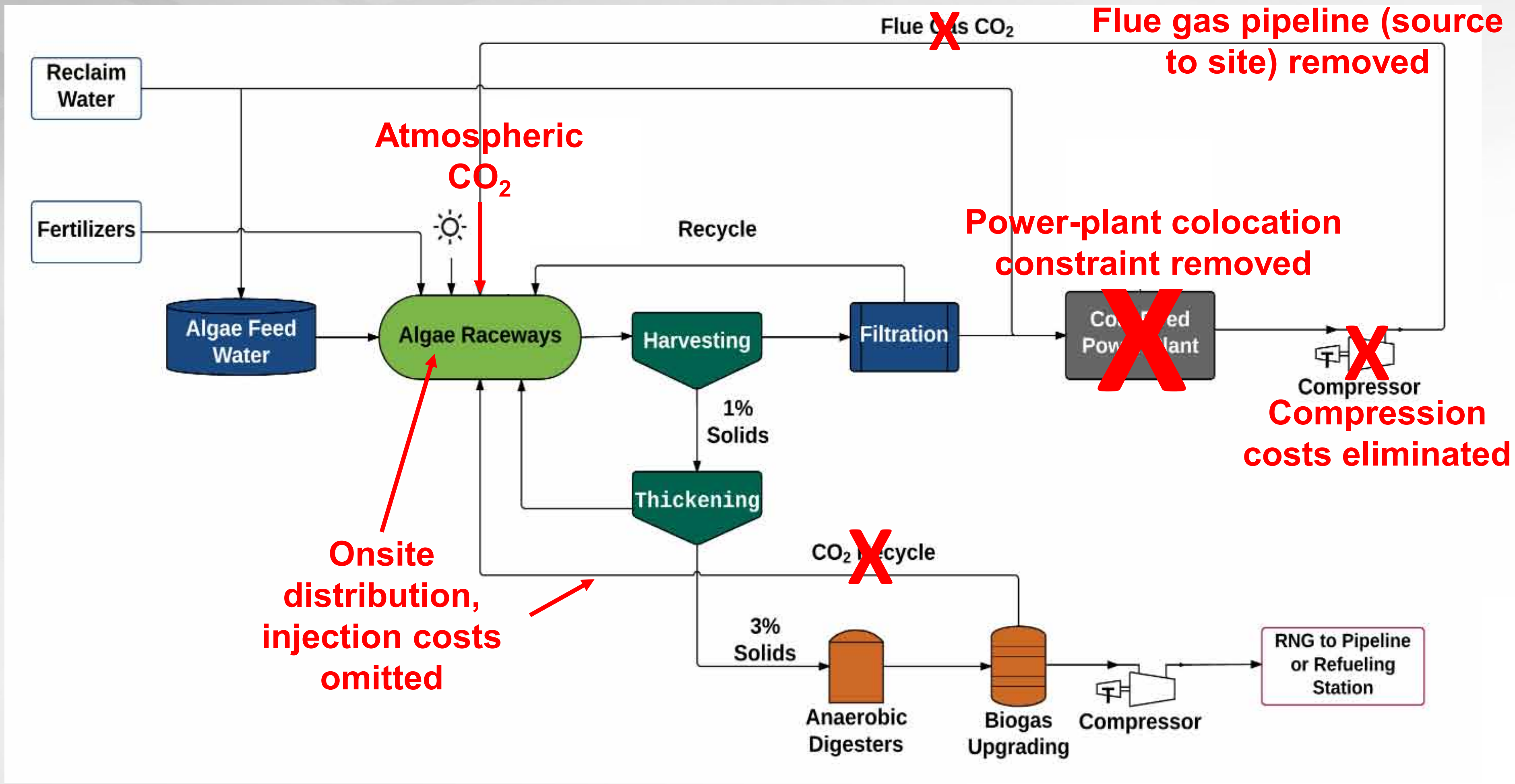
Progress: TEA and Resource Assessments

Baseline Scenario (+CO_2) Process Flow:



Progress: TEA and Resource Assessments

AirFix Process Flow:



Endogenous enzyme production assumed