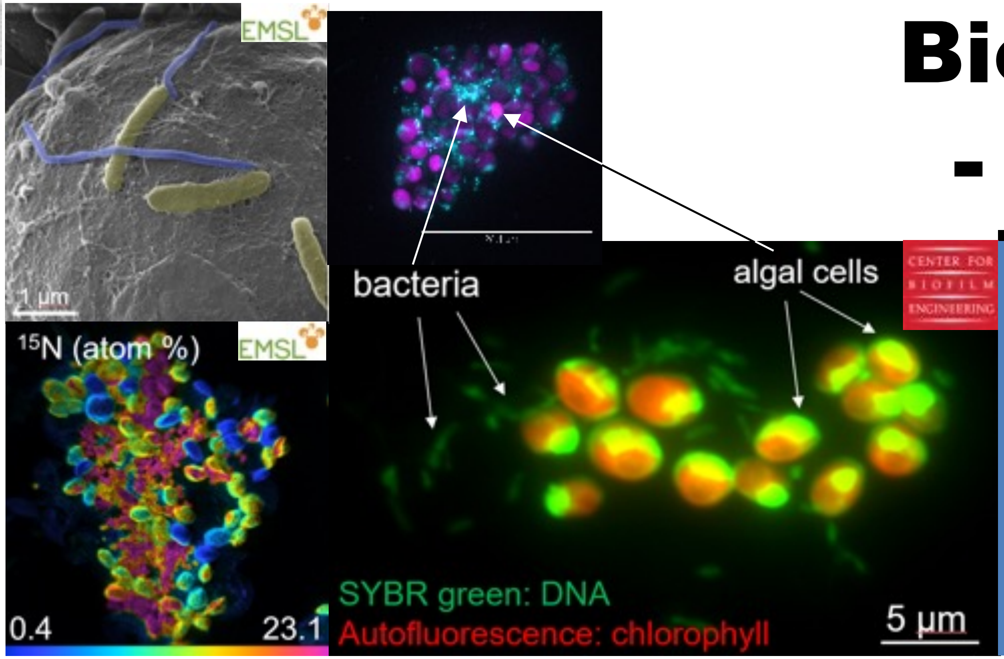
DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

High pH/High Alkalinity Cultivation for Direct Atmospheric Air Capture and Algae Bioproducts
- HiDAC -



Robin Gerlach
Center for Biofilm Engineering
Montana State University

Advanced Algal Systems

DE-EE0009273

April 04, 2023

Overview: Effective algal biomass cultivation with Direct Air Capture (DAC) will drastically increase location flexibility by decoupling from high concentration CO_2 sources.

Goals:

(i) Efficient DAC during raceway cultivation resulting in **increased carbon utilization from 15 to 18 g/m²/d AFDW** (with a stretch goal of 21 g/m²/d AFDW).

(ii) Improved and **more consistent biomass composition for fuel, foam and other high-value product generation** through microbiome engineering and improved cultivation strategies.

(iii) Development of **flexible design and operation strategies** allowing for changes in the product spectrum to accommodate the needs of customers **for** valuable algae **fuels and products.**

The project addresses the following **MYP Milestone**:

- **20AA25 Advanced Algal Systems**

By 2024, develop technologies that **increase** the mature modeled **value of cultivated algae biomass by 25% over the 2019 SOT baseline** minimum biomass selling price through incorporation of valuable coproducts and/or services

and the following **Multi-Year-Plan (MYP) barriers**:

- Aft-A Biomass Availability and Cost
- Aft-B Sustainable Algae Production
- Aft-G Algal Feedstock Material Properties

Quad Chart Overview

Timeline
- 10/1/2020
- 9/30/2024

	FY22 Costed	Total Award
DOE Funding	(10/01/2021 – 9/30/2022) ***$704,711.64***	(negotiated total federal share) ***$2,000,000.00***
Project Cost Share *	***$178,433.68***	***$511,124***

Project Goal (HiDAC)
To increase areal algal biomass productivity and biomass quality during DAC-based open raceway cultivation while maintaining or improving economic competitiveness through technical improvements (reactor design, growth and conversion) and the production of high value products.

End of Project Milestone
Reach an areal productivity of 18 g/m^2/d AFDW of algal biomass based on atmospheric CO_2 utilization, which would be an improvement of 20% over the baseline

Funding Mechanism
DE-FOA-0002203, Topic Area 3 Algae Bioproducts and CO_2 Direct-Air-Capture Efficiency (ABCDE), FY2020

Project Partners*
- Montana State University - Bozeman
- University of Toledo
- Clemson University
- University of North Carolina at Chapel Hill
- Sonoco

*Only fill out if applicable.

Fiscal Administration

Communications

Reporting

PI: Robin Gerlach
Responsible for all technical, management, and reporting activities
CO_2 Mass Transfer Modeling, Recarbonization Technology, Microbiome Engineering

Co-Investigator
Fields
MSU
Microbiome Engineering
Report preparation
Publication of results

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Carlson
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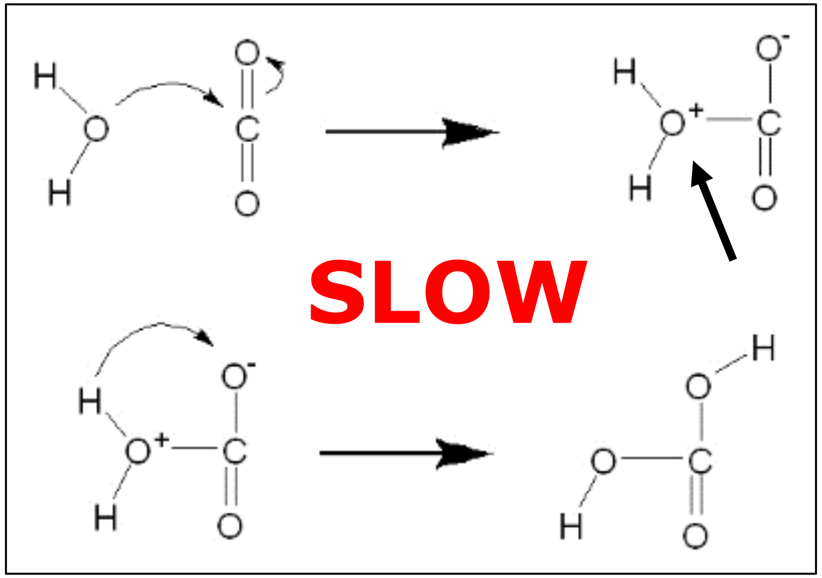
The University of Toledo
Ford Motor Company
Clemson University
Sonoco Protective Solutions

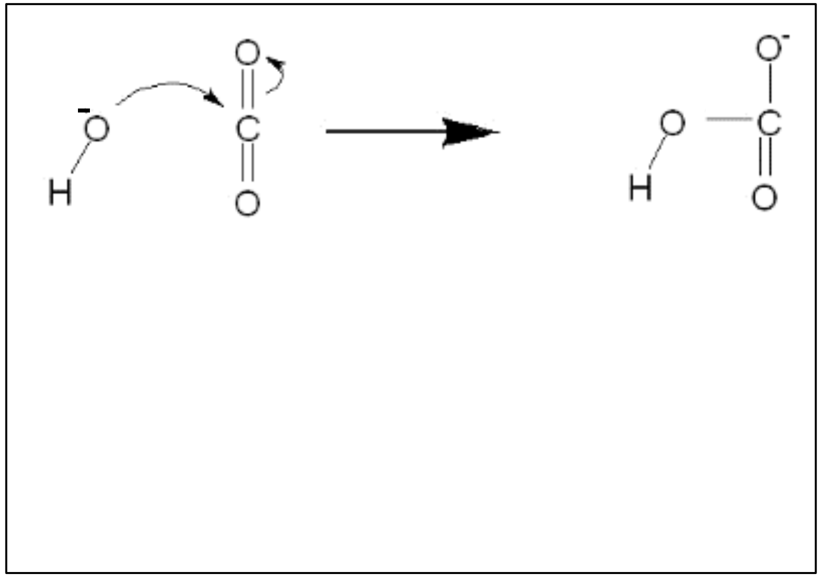
Guiding Question

Can we economically increase areal algal biomass productivity and biomass quality during DAC-based open raceway cultivation?*

- through technical improvements (growth and conversion)
- through strategic improvements (product spectrum and flexibility)

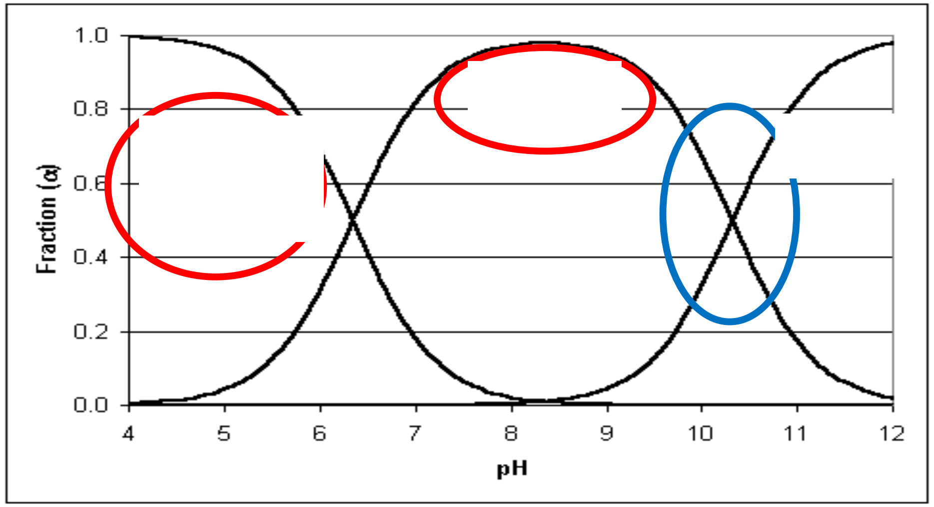
*DAC – Direct Air Capture (of CO_2)





Dominant process at
low pH
produces H_2CO_3

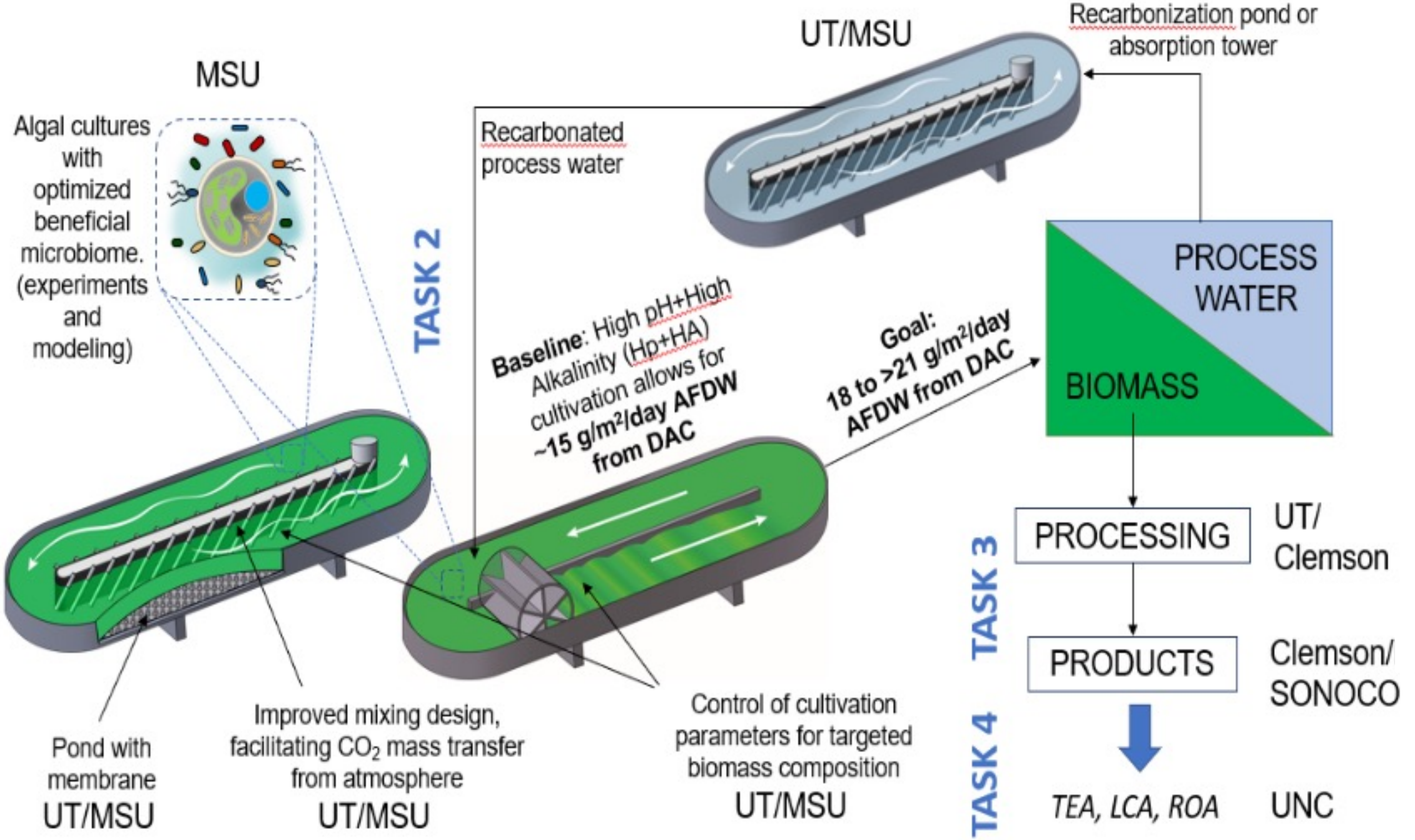
$H_2CO_3 \rightarrow HCO_3^- + H^+$



Dominant process
at **high pH**
produces HCO_3^-

$HCO_3^- + H^+ \rightarrow H_2CO_3$

$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O$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UT/MSU
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Recarbonated process water
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Goal: 18 to >21 g/m²/day AFDW from DAC
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Control of cultivation parameters for targeted biomass composition
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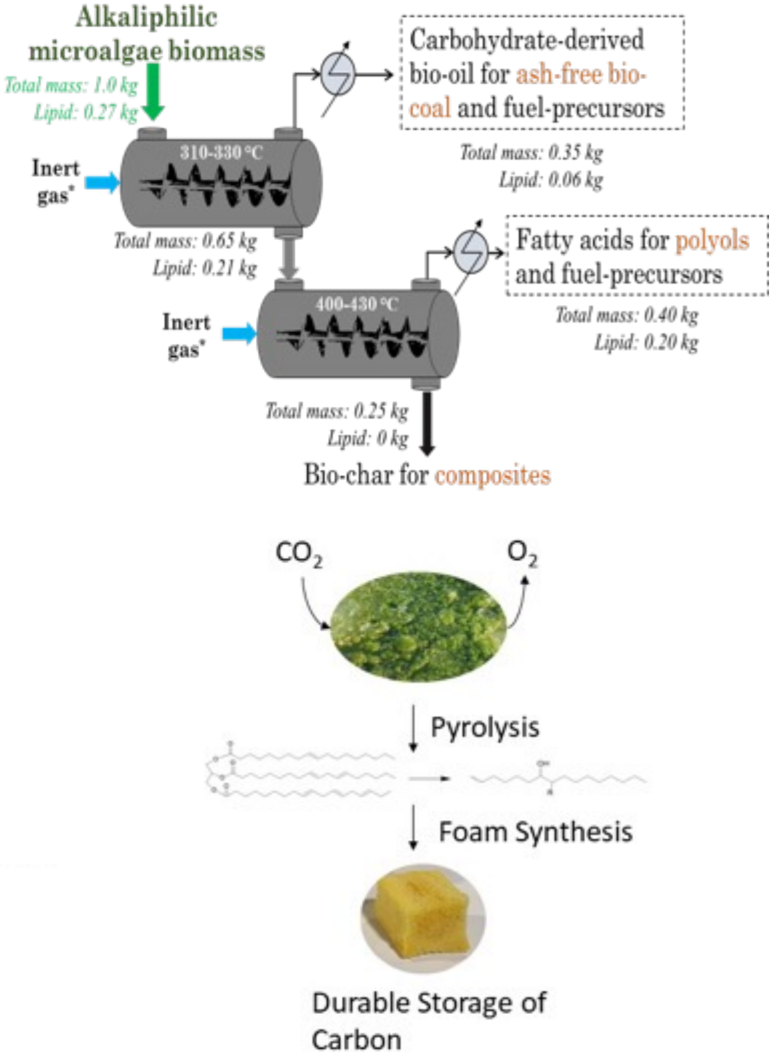
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310-330 °C
Inert gas*
Total mass: 0.65 kg
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Fatty acids for polyols and fuel-precursors
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O2
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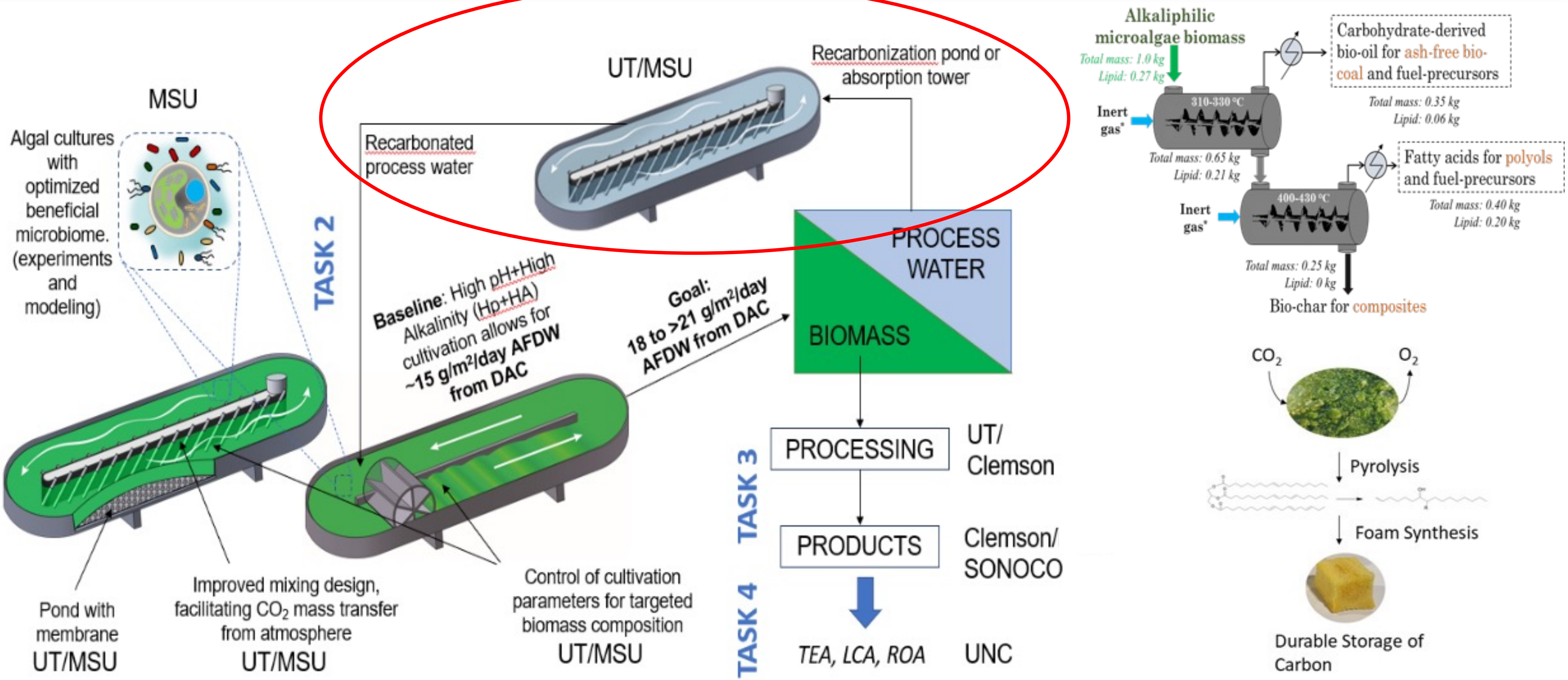
Foam Synthesis
Durable Storage of Carbon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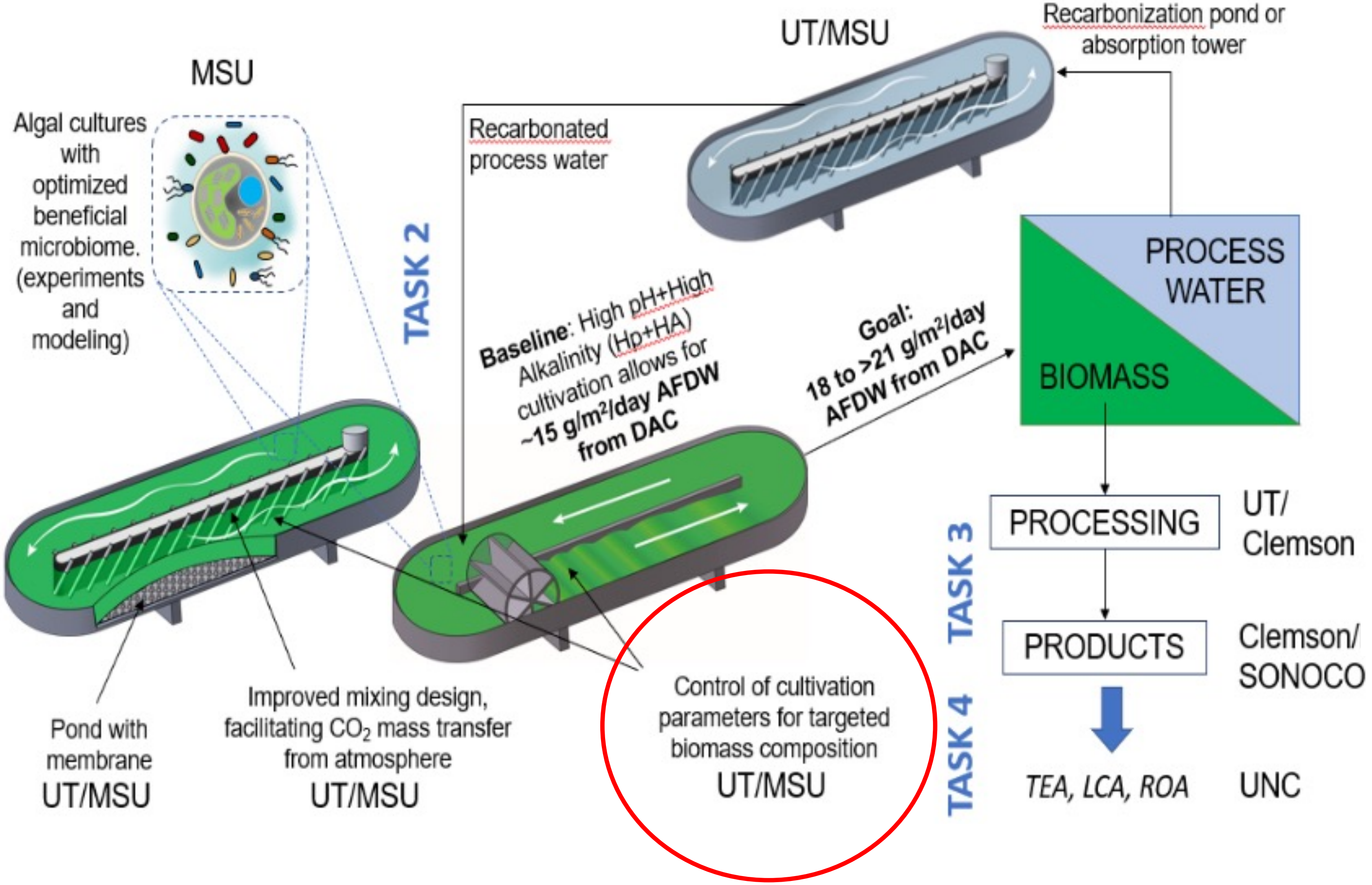

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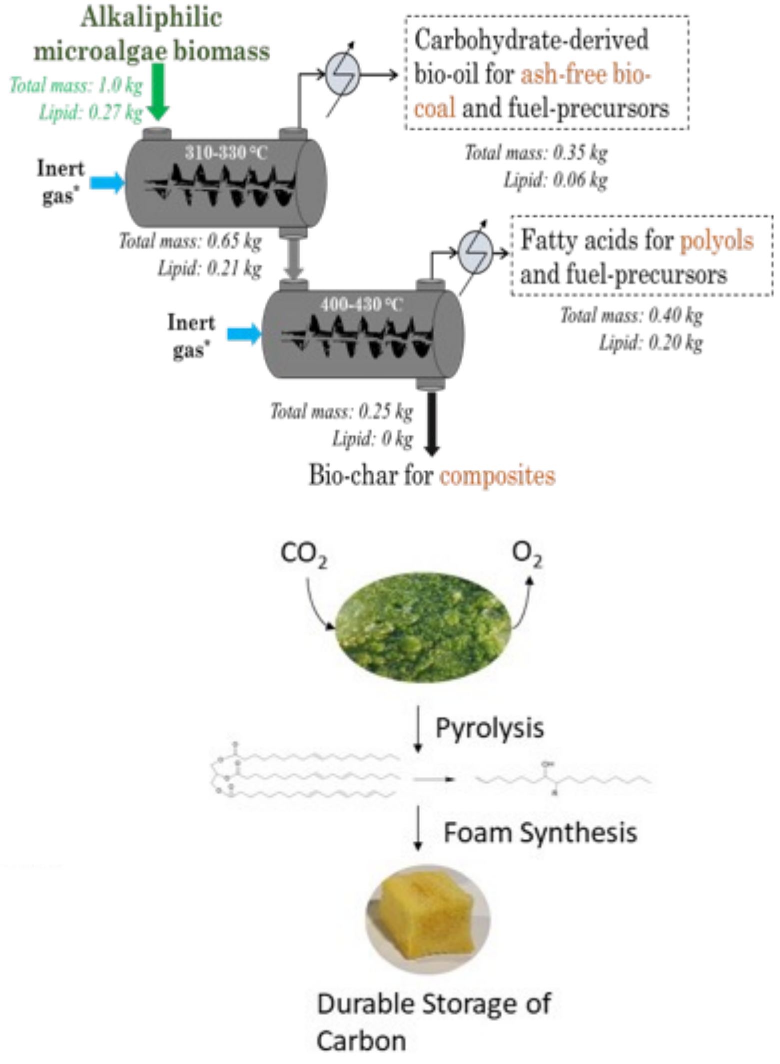

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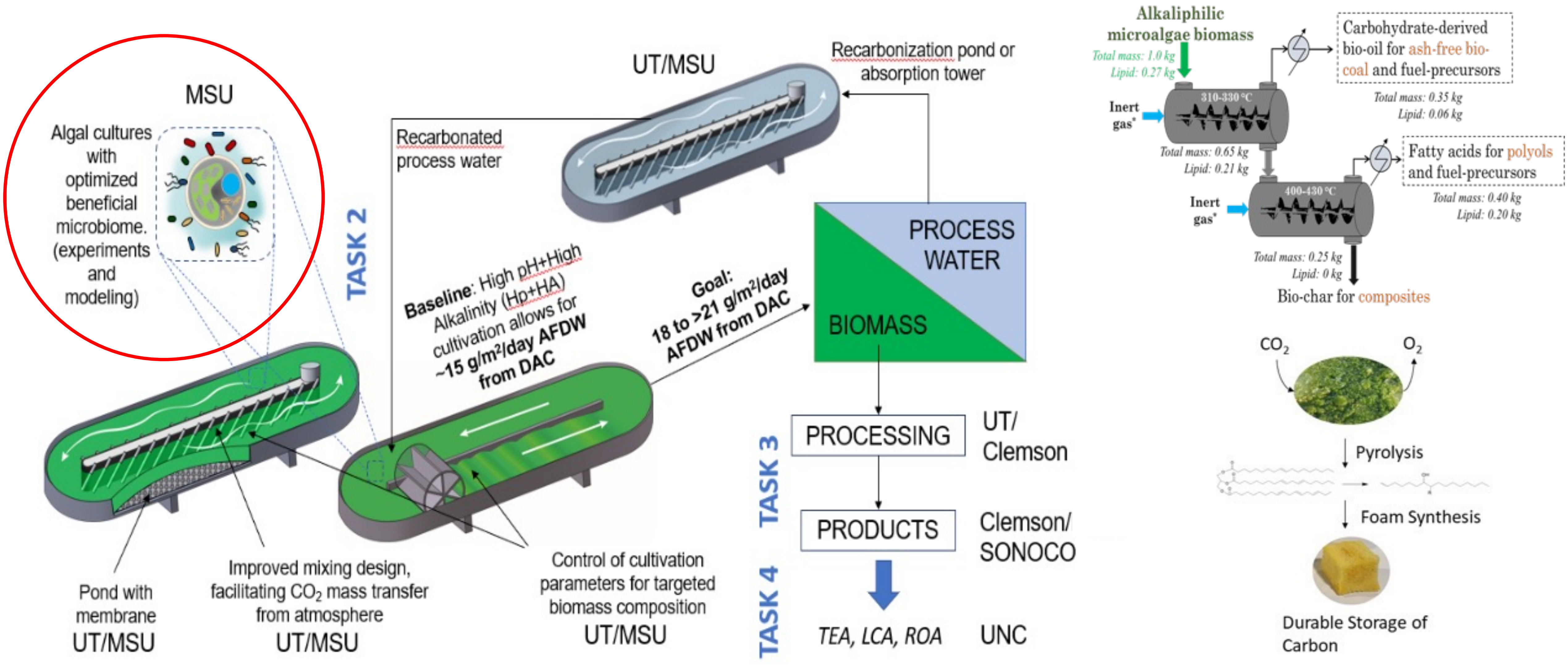

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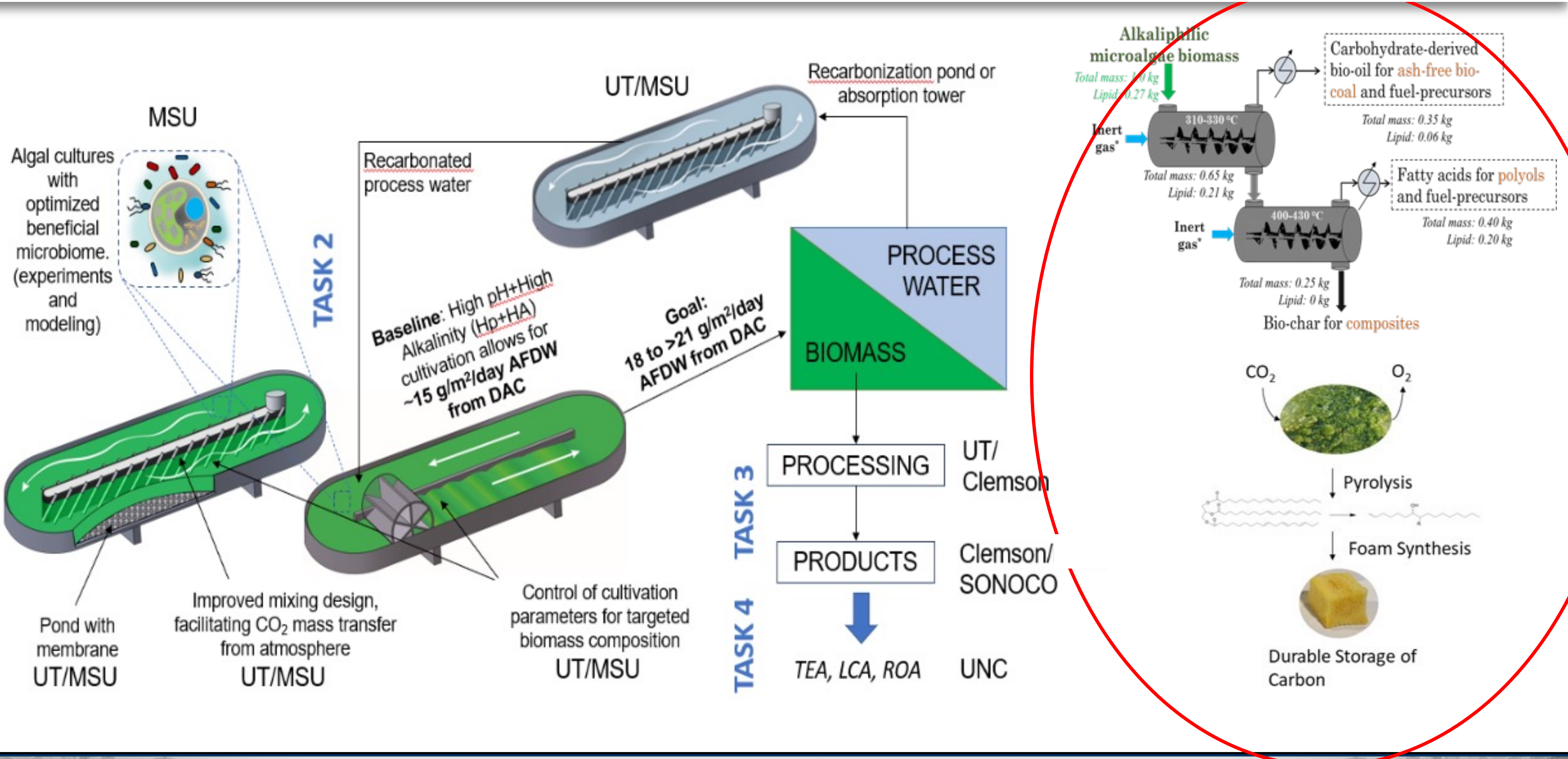

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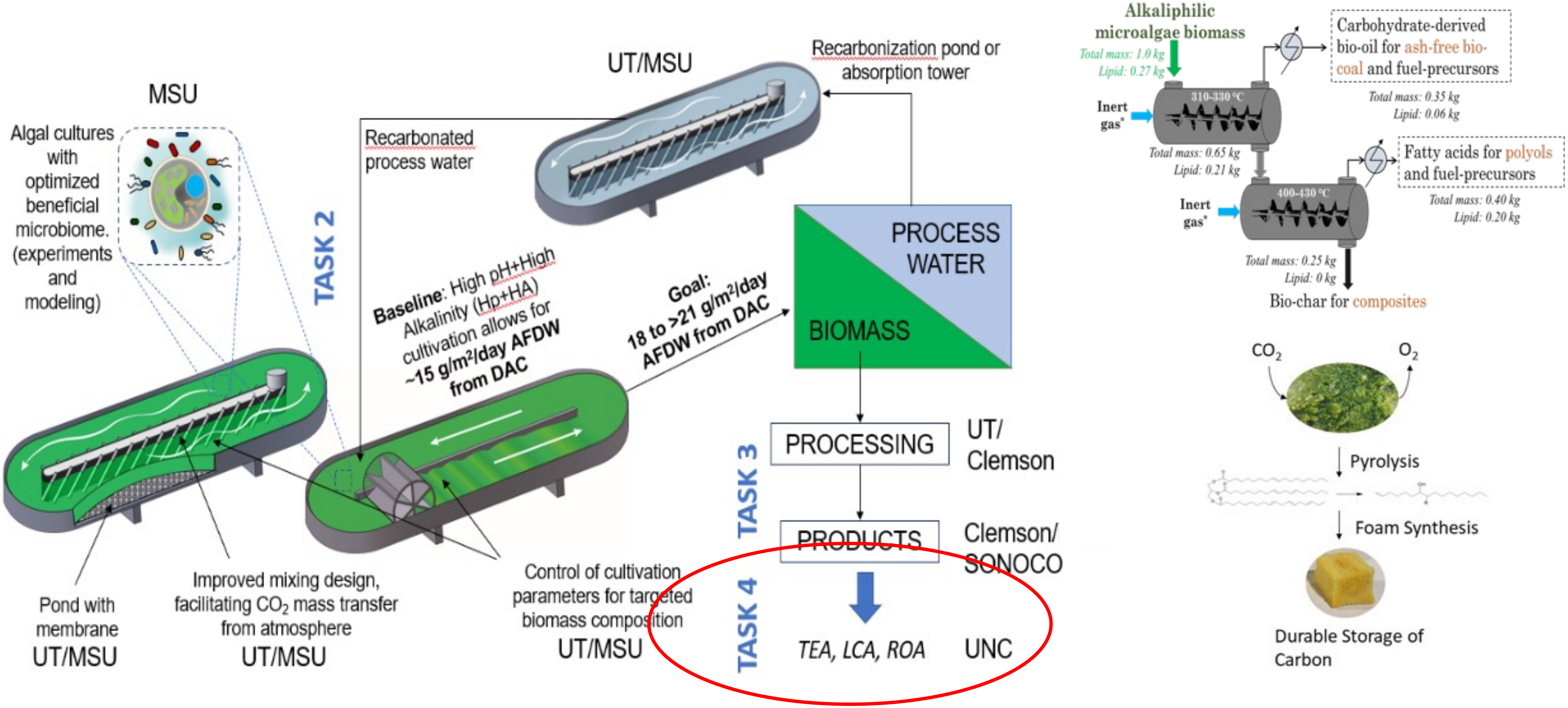
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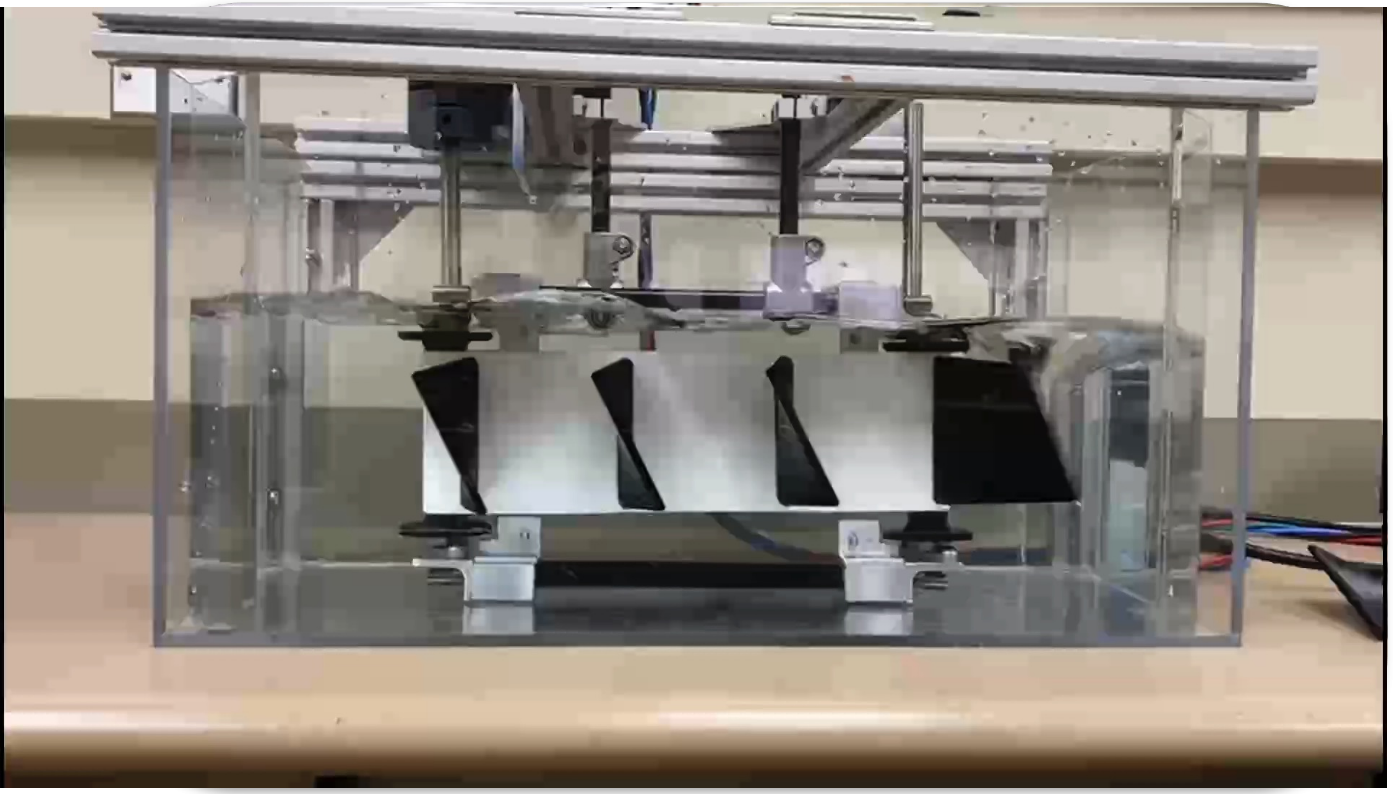
Belt-and-cleat 30L raceway tanks



Sridhar Viamajala

Project work **started late in FY 2021** hence **no peer review comments yet**. SOPO was revised through discussions during the verification process in April 2021.

Top Challenges

- **Belt-and-Cleat mixing is in its infancy**
- **Microbiomes are there**, they have been shown to be important in many biological systems (e.g., digestive systems), **interactions** in algal cultures **are challenging to observe**
- Creating algae polyols with similar reactivity to traditional polyols for polymer synthesis

Risk Analysis and Mitigation –
overlapping (and redundant) expertise and capabilities

e.g.,

- *Sonoco, Ford, and Clemson*
  - **Product Manufacturing, Testing**
- *Toledo and Clemson*
  - Toledo and Clemson could both do **conversion**, can both **produce foams**
- *MSU and Toledo*
  - Both have **cultivation expertise**
  - Toledo is expanding microbiological expertise and building **molecular expertise**
- *UNC, Toledo, and MSU*
  - **TEA/LCA expertise**

Risk Analysis and Mitigation (cont'd) –

Multiple strategies to achieve improvements in productivity

e.g.,

- New raceway designs, mixing, and re-carbonization strategies
- Microbiome improvements
- Cultivation media improvements

Go/No-Go decision points are SMART, build on the ***baseline of 15 g/m²/d AFDW*** *and are critical to reach the EPG.*

GNG1 *– Verification.* April 16, 2021

GNG2 *- Improved DAC and carbon utilization resulting in algal biomass* ***areal productivity*** *increase by Chlorella sorokiniana strain SLA-04 by* ***10% over baseline (16.5 g/m²/d AFDW)****.* March 31, 2023

EPG *- Reach an* ***areal productivity of 18 g/m²/d AFDW*** *of algal biomass based on atmospheric CO_2 utilization, which would be an* ***improvement of 20% over the baseline****.* September 30, 2024

Stretch goal: 21 g/m²/d AFDW

A ***combination of experiments and modeling*** *is being used to determine CO_2 mass transfer rate, algal growth potential/rate, media recycling potential, effect of microbiome, potential for foam, biocarbon and biochar utilization, prospective facility design and product spectrum.*

Communication

- *Monthly All-Hands Meetings, PM C. Sterner attends regularly, maintains awareness of other projects & DOE/BETO goals*
- *Monthly PI Meetings*
- *Weekly Meetings Gerlach-Viamajala*
- *Subgroup meetings on demand*
- *Weekly or more frequent meetings at participating institutions*
- *Ad-hoc meetings as necessary*
- *MS Teams/SharePoint based file exchange & communication on secure site*
- *Interaction and regular communication with belt-and-cleat pond operator*

Communication with other projects & entities

- *JGI*
- *FICUS (EMSL)*
- *NSF*

2- Progress and Outcomes

Project work started late in FY 2021 – so about 1.5 years of laboratory work. Project is mostly on schedule [a few (CoVID-related) delivery and personnel challenges].

Important Technical Accomplishments

- Belt-and-Cleat prototypes built and used (30L)
- Improved mass transfer modeling and comparisons
- Developed connections to larger scale belt-and-cleat pond operator
- Demonstrated that there are differences between day-and-night in activity of the algal microbiome
- Can recycle high alkalinity medium multiple times (12 times so far)
- Made foams from algal oil
- Completed initial analyses demonstrating quantitatively that utilizing as little as 31% of algal lipids/pyrolysis oil for polyurethane production can increase revenue potential by 10%

2- Progress and Outcomes (cont'd)

Key Milestones

<u>Pond operation and media composition</u>

Demonstrated through measurements that sufficient CO_2 can be transferred into cleat-and-belt-mixed ponds to enable an algal biomass areal productivity increase by **20% over baseline (i.e., to 18 g/m²/d AFDW)**

Compared belt-and-cleat-mixed ponds to paddlewheel-mixed ponds at equivalent fluid velocities (goal is based on equivalent **energy usage** – this **is proving to be challenging**)

GNG2 Improved DAC and carbon utilization resulting in algal biomass areal productivity increase by Chlorella sorokiniana strain SLA-04 by **10% over baseline (i.e. 16.5 g/m²/d AFDW)** --- March 31, 2023

<u>Microbiome Engineering</u>

Screened effect of **>15 bacterial isolates** on growth of axenic SLA-04 cultures under high pH/high alkalinity (Hp+HA) conditions – **did not find a clear effect**

Taxonomic characterization of microbiome of SLA-04 cultures cultivated under high pH/high alkalinity (Hp+HA) conditions – **see differences in activity between HA and LA & day and night**

2- Progress and Outcomes (cont'd)

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Key Milestones (cont'd)

In silico metabolic models built for bacterial cultures **and calibrated using experimental data** - **use of KBase**, conversations with JGI on how to better handle auxotrophy

Future: **In silico metabolic models of consortium level (algal-bacterial) interactions** will be built and calibrated. Goal is to predict and test strategies to improve culture robustness and productivity through optimized interactions (e.g., C- and N-exchange).

<u>Higher Value Products from Algae</u>

Synthesized bio-oil from pyrolysis of algae biomass grown in raceway ponds **used oil to produce PU foams**

Future:

Biocarbon electrode synthesis and testing and Biochar-based composites

2- Progress and Outcomes (cont'd)

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Key Milestones (cont'd)

Techno-Economic Assessment (TEA) and Life-Cycle Assessment (LCA)

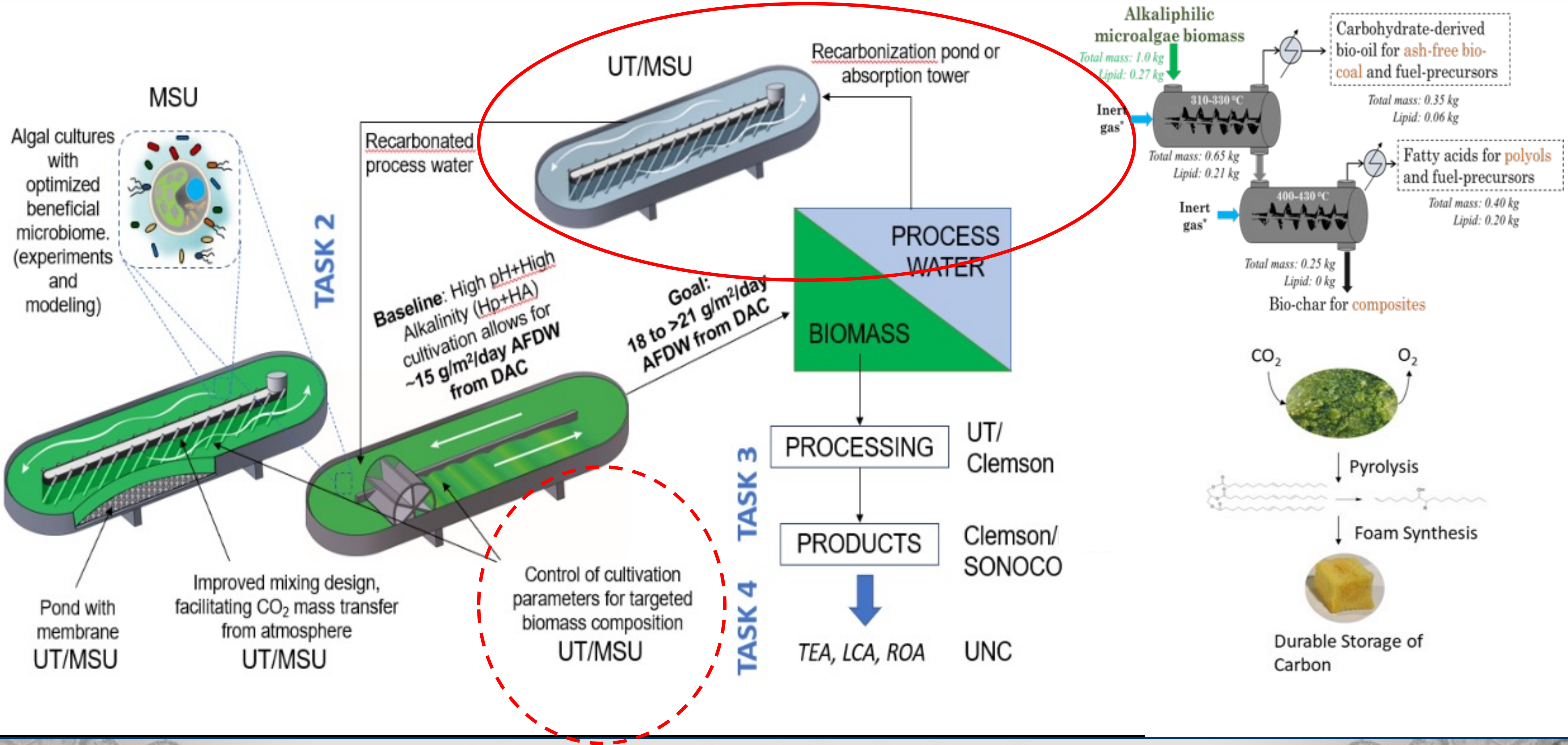
TEA and LCA for high pH/high alkalinity cultivation system, outlining designs that will enable **increased algal biomass revenue potential by 10% over the baseline of $300 per ton of algal biomass** at a productivity of 15 g/m^2/d AFDW

Future:

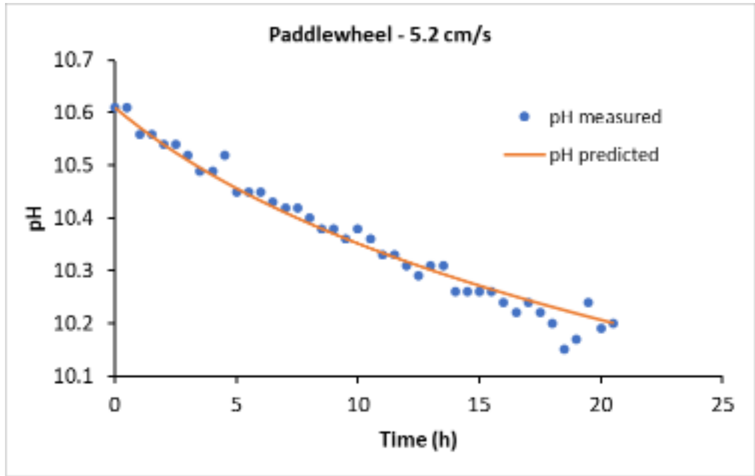
Develop flexible facility design and strategic operations to limit weather- and market-based financial risk by developing capital- & financial risk assessment-focused investment tools

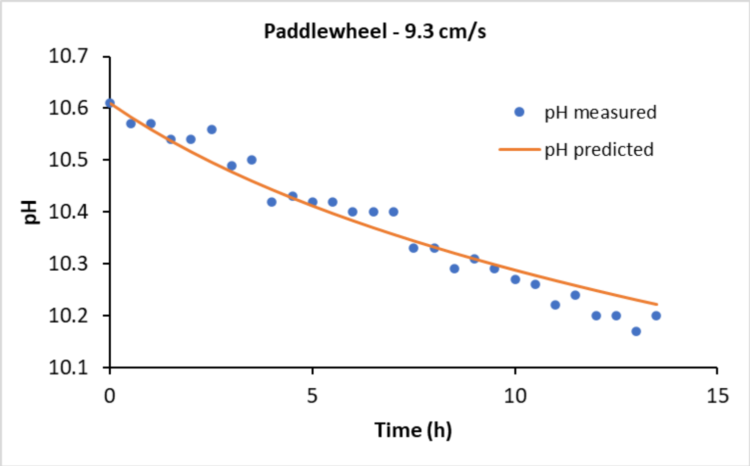
Incorporate seasonal and cultivation strategy-dependent production risks into dynamic systems modeling, and **development of novel risk mitigation strategies** (seasonal, strain specific production risks, temperature & irradiance variations, and market price volatility, etc.)

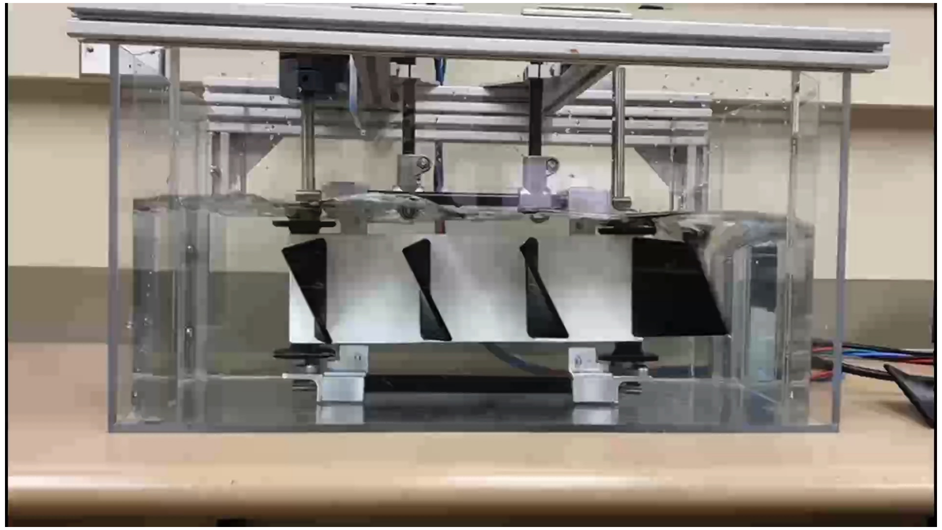
Project Overview & Approach

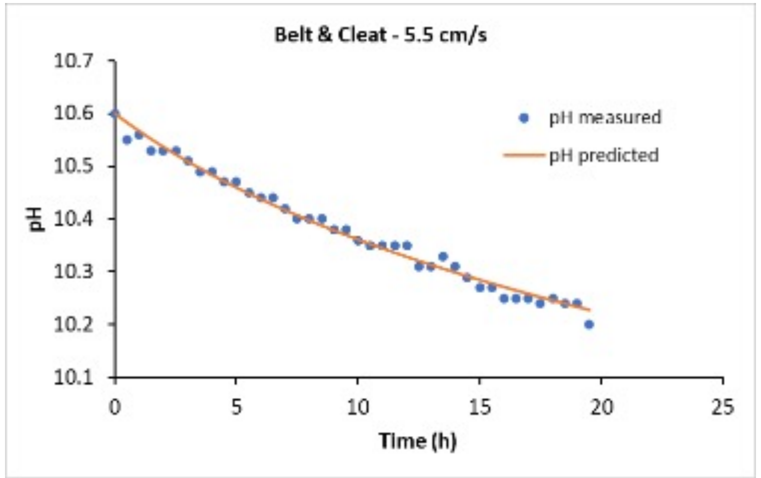


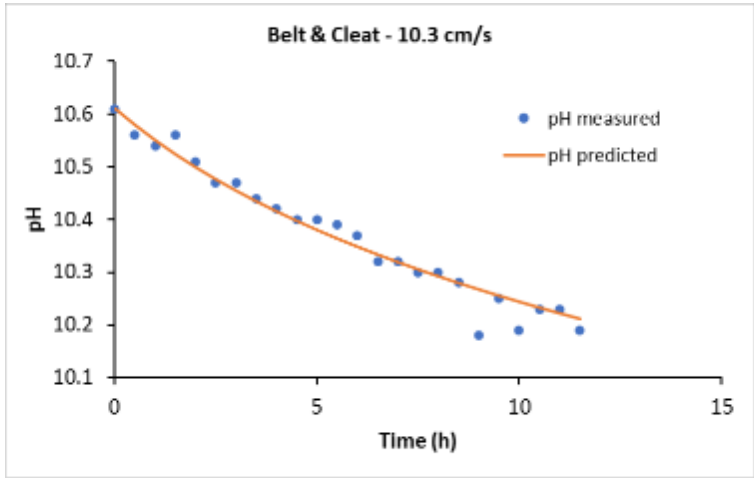
2- Progress and Outcomes (cont'd)
CO_2 Mass Transfer into Ponds











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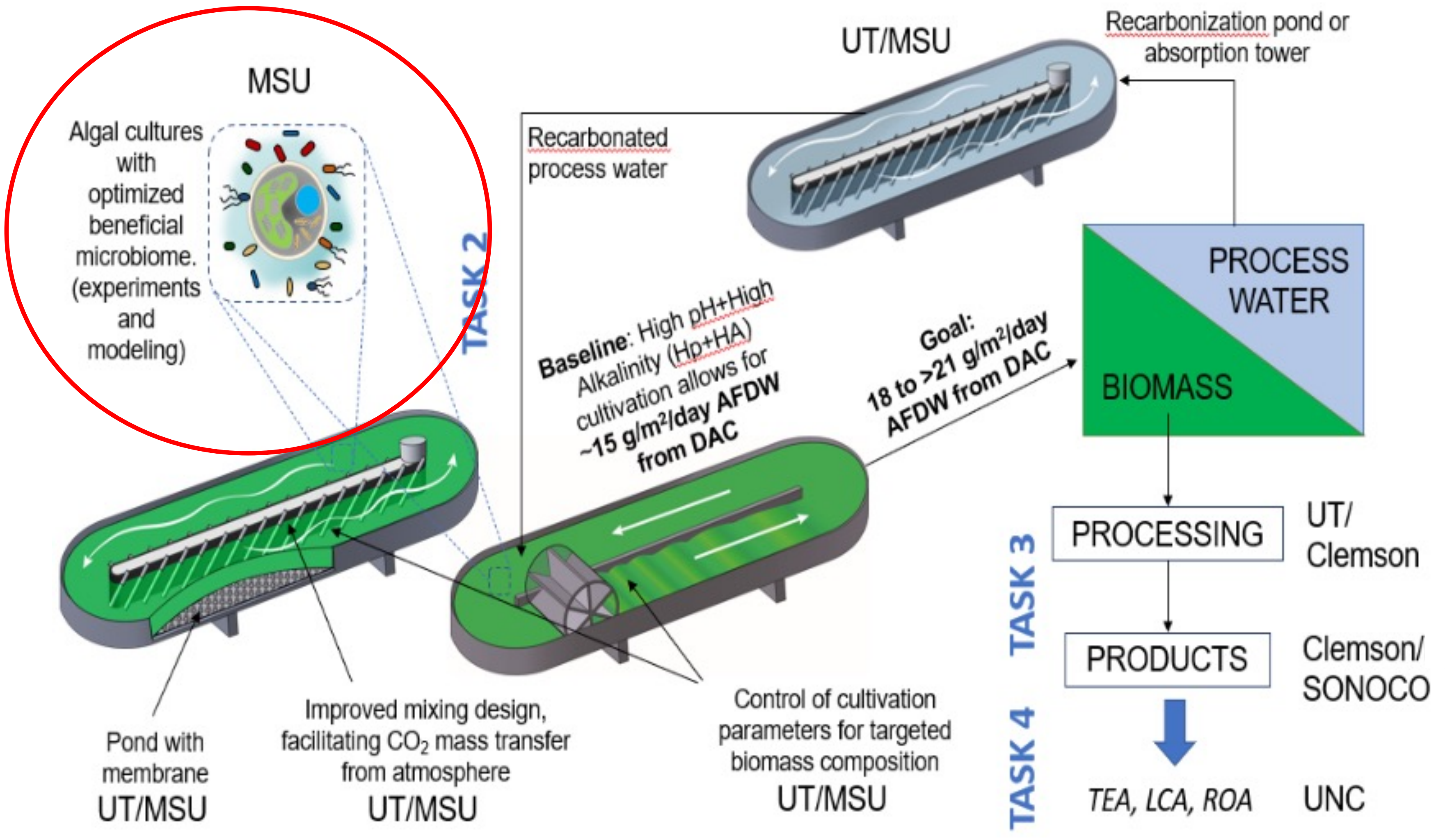

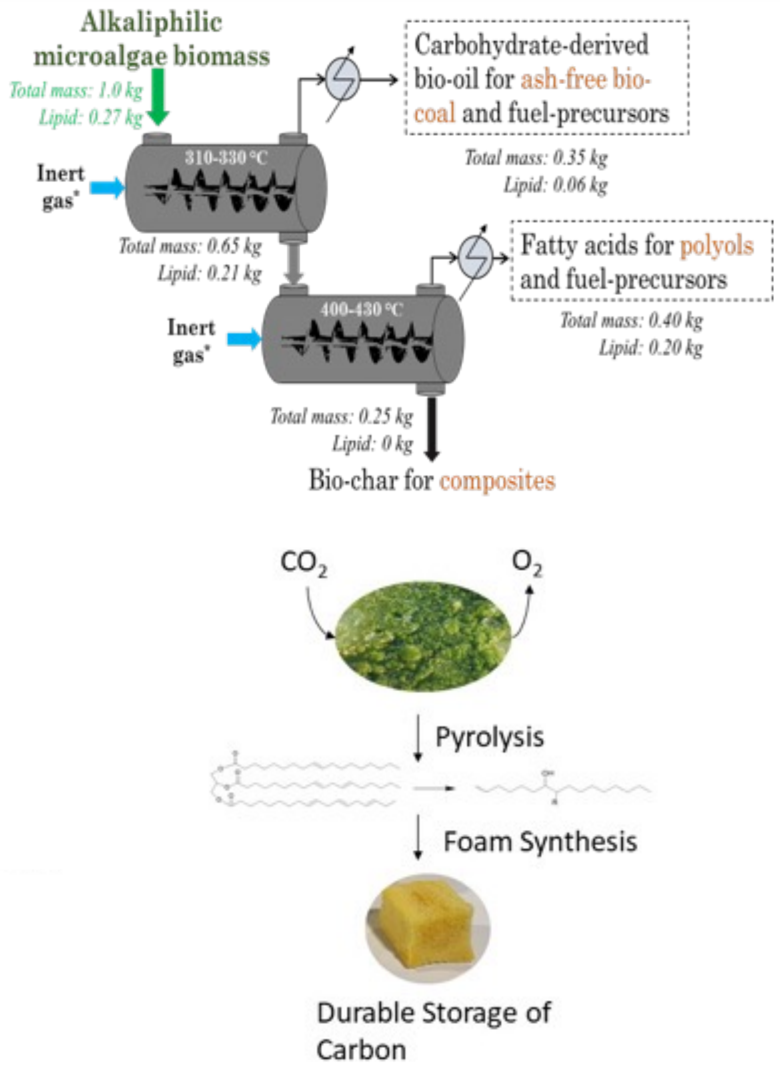

2- Progress and Outcomes (cont'd)
Metabolic Modeling of Algal Microbiome

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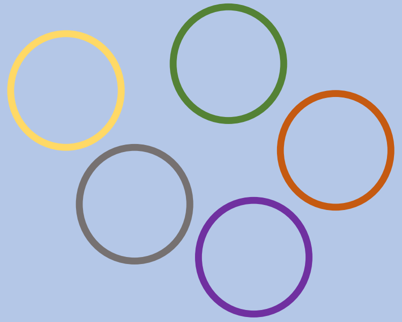
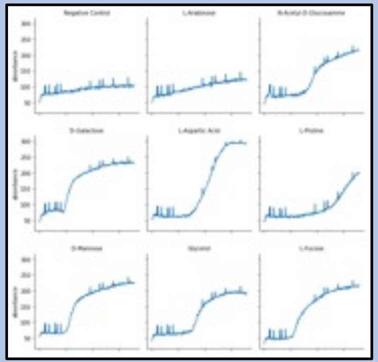
Integration of genomic information and experimental data





Genome and Metagenome sequencing - JGI

Experimental growth data e.g. Biolog

Adrienne Arnold

Analysis in KBase

KBase

Model SEED

RAST

DRAM

Construction and calibration of two metabolic models:

1. ***Paracoccus*** **sp.:** 1243 reactions, 1242 compounds
2. ***Microbacterium*** **sp.:** 1056 reactions, 1058 compounds

Predicted interactions:

- Turnover of algal detritus
- Glycolate degradation
- Mitigation of daytime O_2 stress

2- Progress and Outcomes (cont'd)
KEGG annotation – the MAGs encode for (almost) complete vitamin B synthetic pathways

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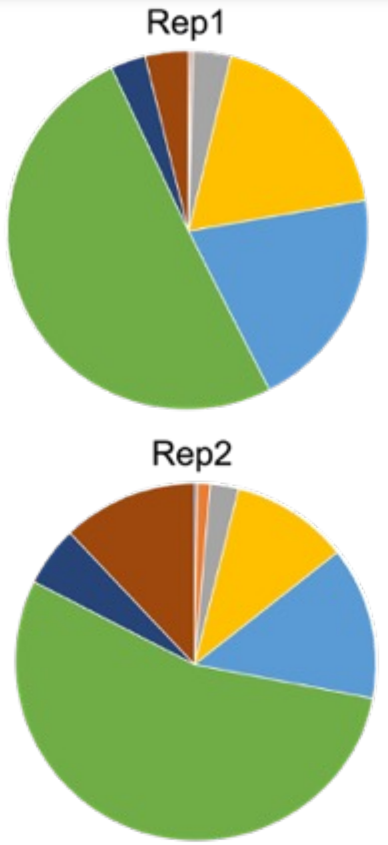


Genus/Species	Complete B vitamin synthetic pathways (KEGG)							
	B1	B2	B3	B5	B6	B7	B9	B12
Brevundimonas	NO	NO	NO	NO	NO	NO	YES	NO
Microbacterium sp001620065	NO	YES	NO	NO	YES	NO	NO	NO
Paracoccus sp000967825	NO	NO	NO	NO	NO	YES	NO	YES
Enterovigra rhinocerotis	NO	NO	NO	NO	NO	NO	NO	NO
Microbacterium	NO	YES	NO	NO	YES	NO	NO	NO
Methylobacterium	NO	YES	NO	NO	NO	YES	YES	YES
Mesorhizobium	NO	NO	NO	NO	NO	NO	NO	YES
Aeromicrobium sp001423335	NO	NO	NO	NO	YES	NO	NO	YES
SLA-04	NO	NO	NO	NO	NO	NO	NO	NO

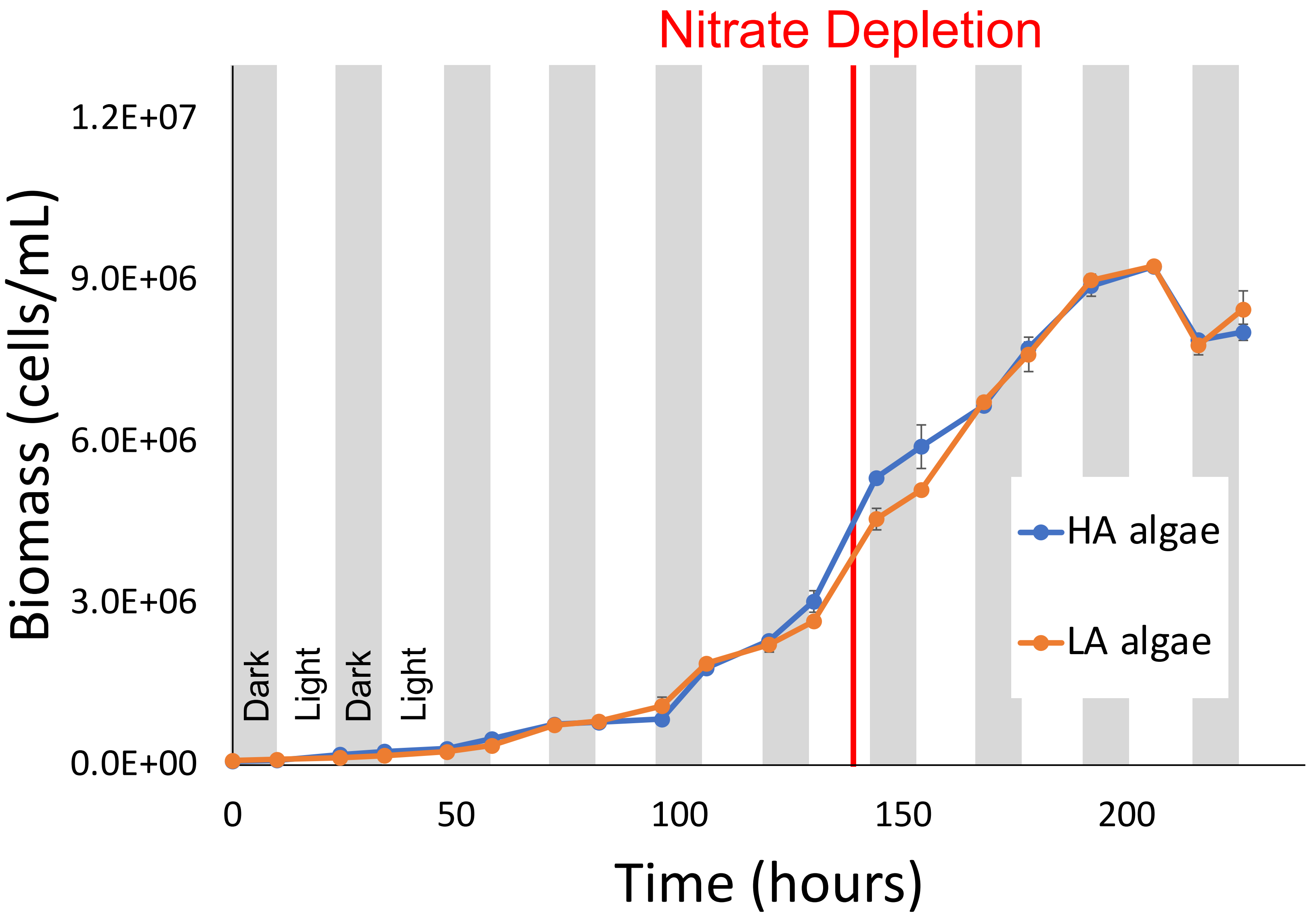
Thiamine - B1 Riboflavin - B2 Nicotinic acid - B3 Pantothenate - B5
Pyridoxal-P - B6 Biotin - B7 Tetrahydrofolate - B9 Cobalamin - B12

Bacterial community may provide B vitamins for SLA-04 growth

SLA-04 genome available

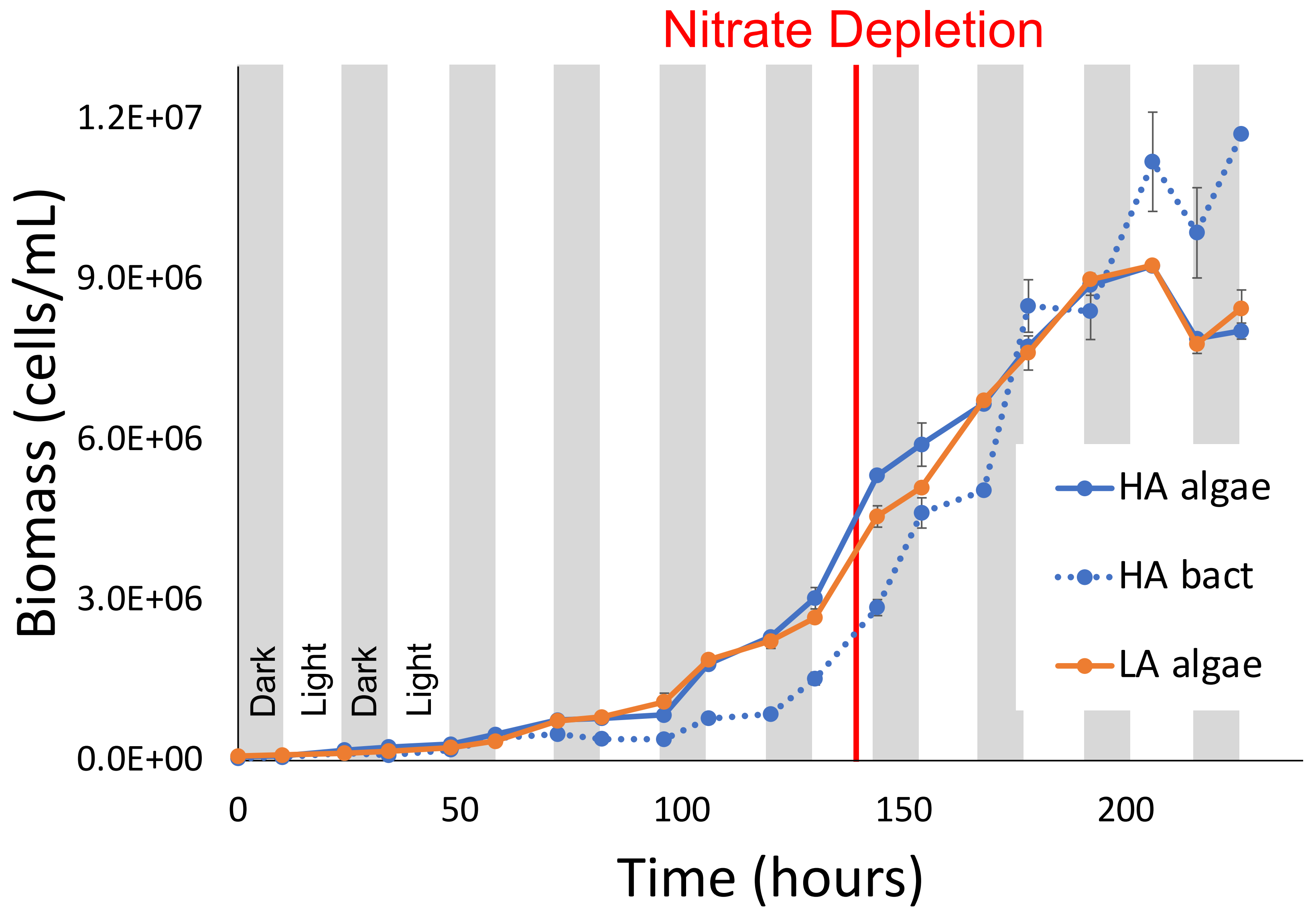
Goemann, Calvin L.C., Wilkinson, Royce, Henriques, William; Bui, Huyen; Goemann, Hannah M.; Carlson, Ross P.; Viamajala, Sridhar; Gerlach, Robin; Wiedenheft, Blake (2023). Genome sequence, phylogenetic analysis, and structure-based annotation reveal metabolic potential of *Chlorella* sp. SLA-04. Algal Research 69: 102943. DOI: 10.1016/j.algal.2022.102943

Diel cycling - phycosome growth

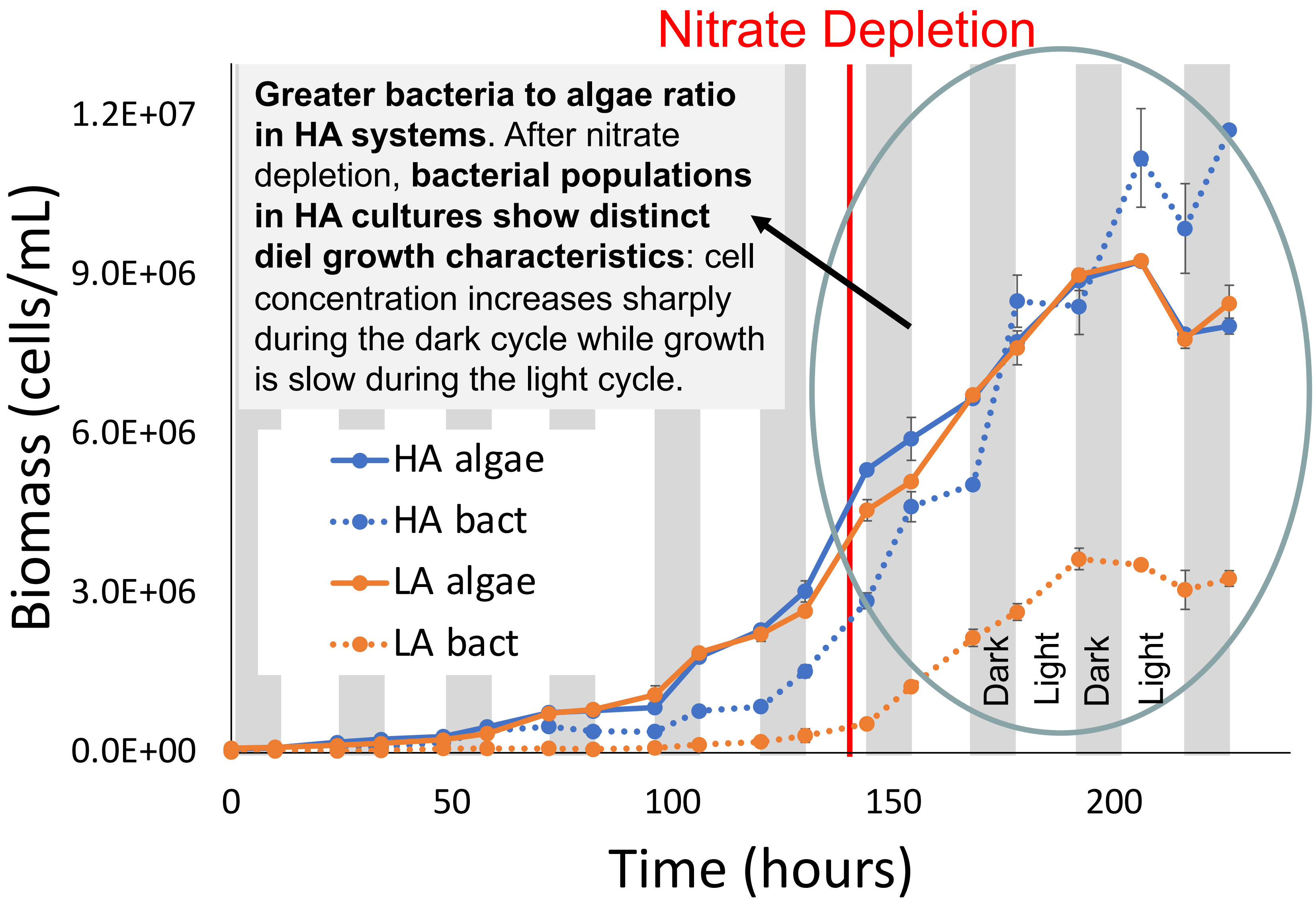


Isaac Miller

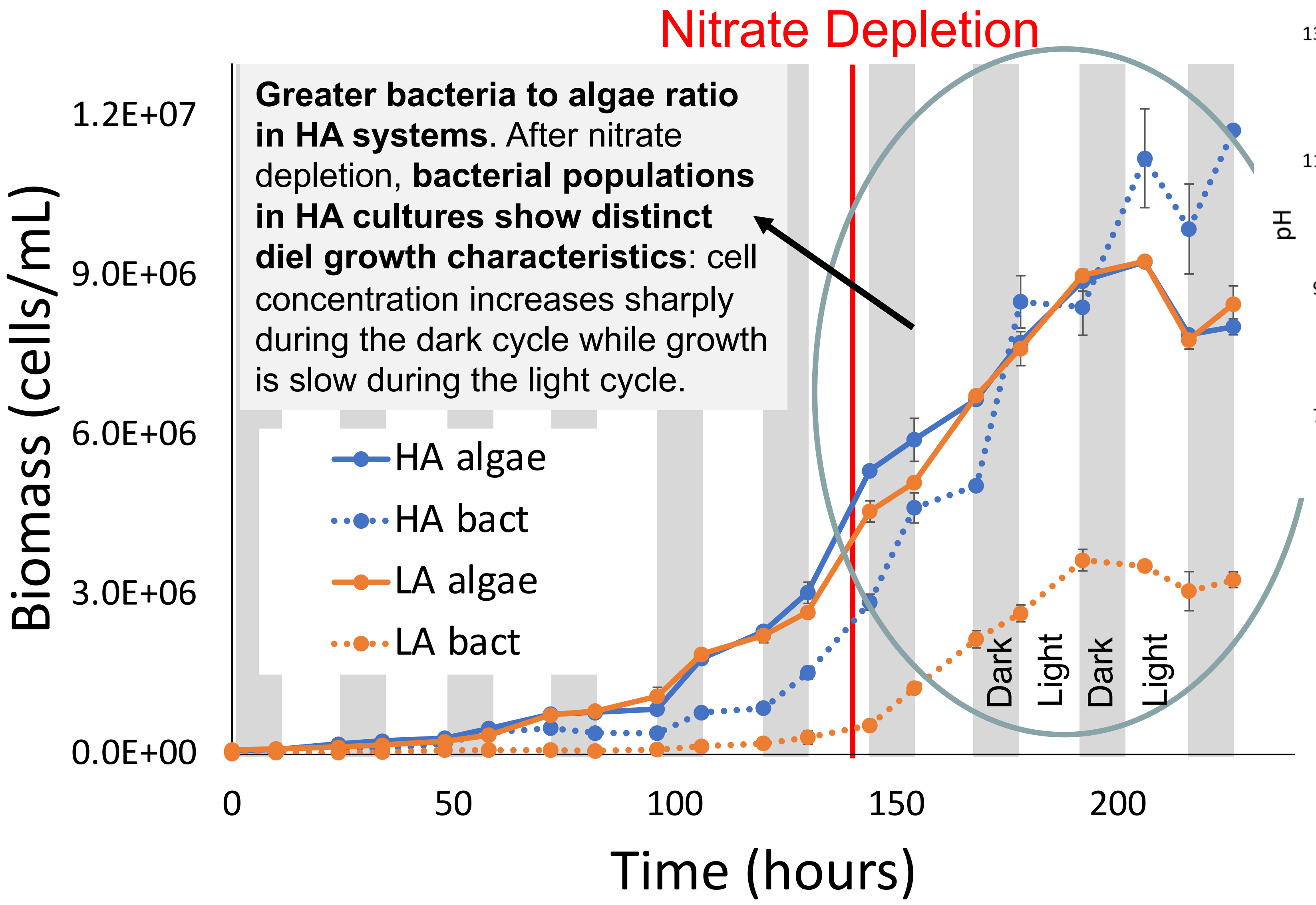
Diel cycling - phycosome growth

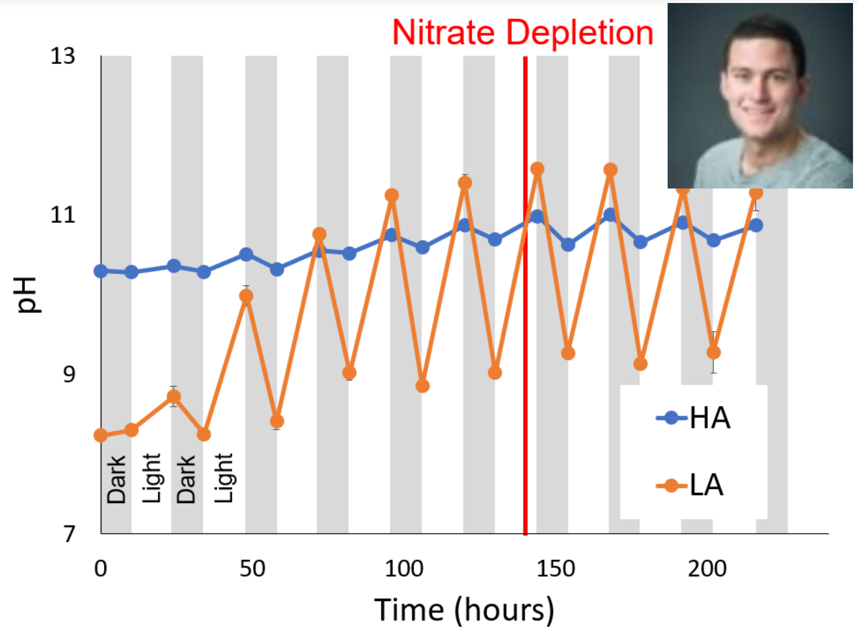


Diel cycling - phycosome growth



Diel cycling - phycosome growth





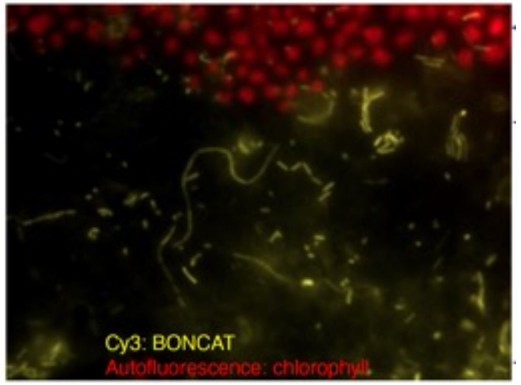
Hypotheses:

- Inhibition of bacteria in LA systems by high pH and/or pH fluctuations
- Release of storage compounds in HA cultures
- Higher bacterial numbers reduce oxidative stress in HA cultures

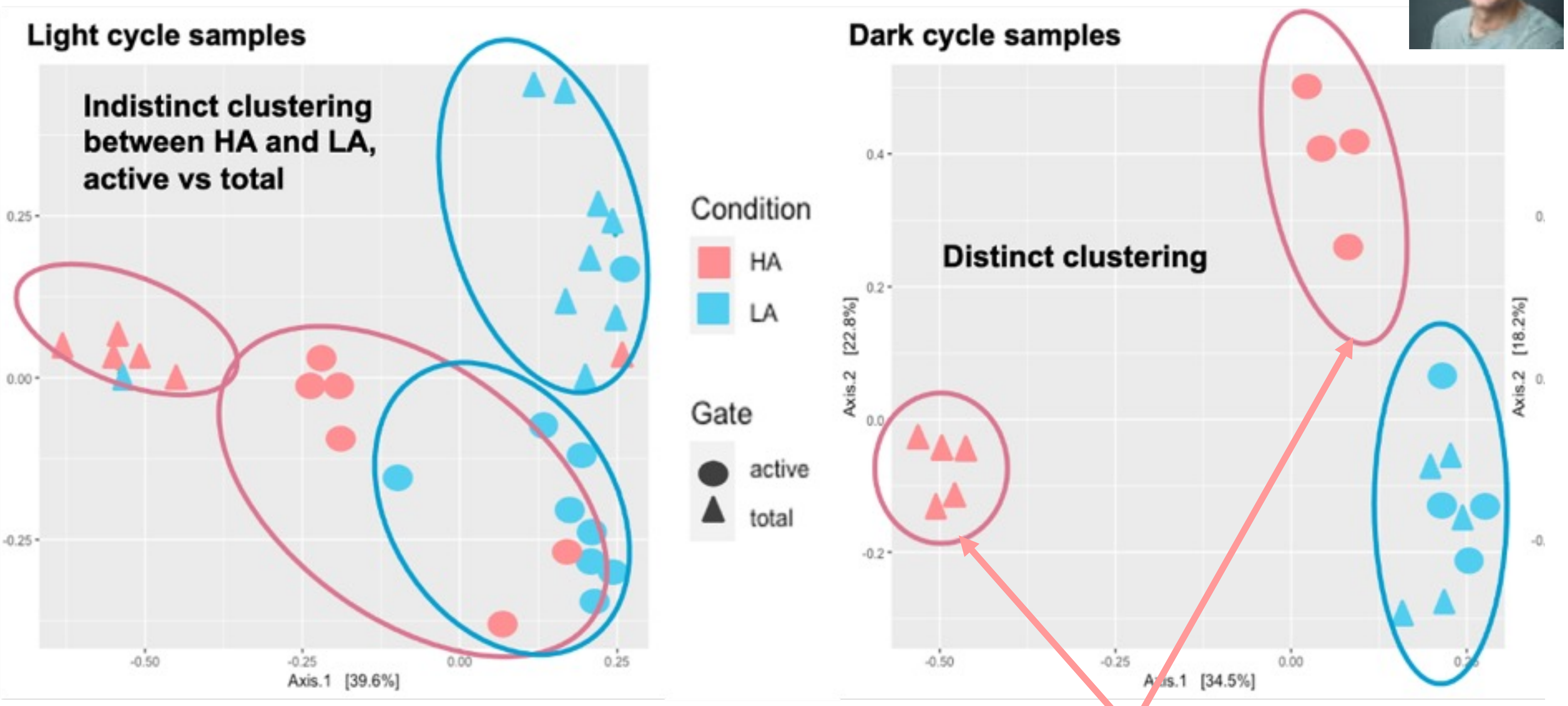
2- Progress and Outcomes (cont'd)
Characterization of active bacteria in SLA-04 culture using BONCAT

BONCAT (Bio-Orthogonal, Non-Canonical Amino acid Tagging)

Method to label metabolically active bacterial cells



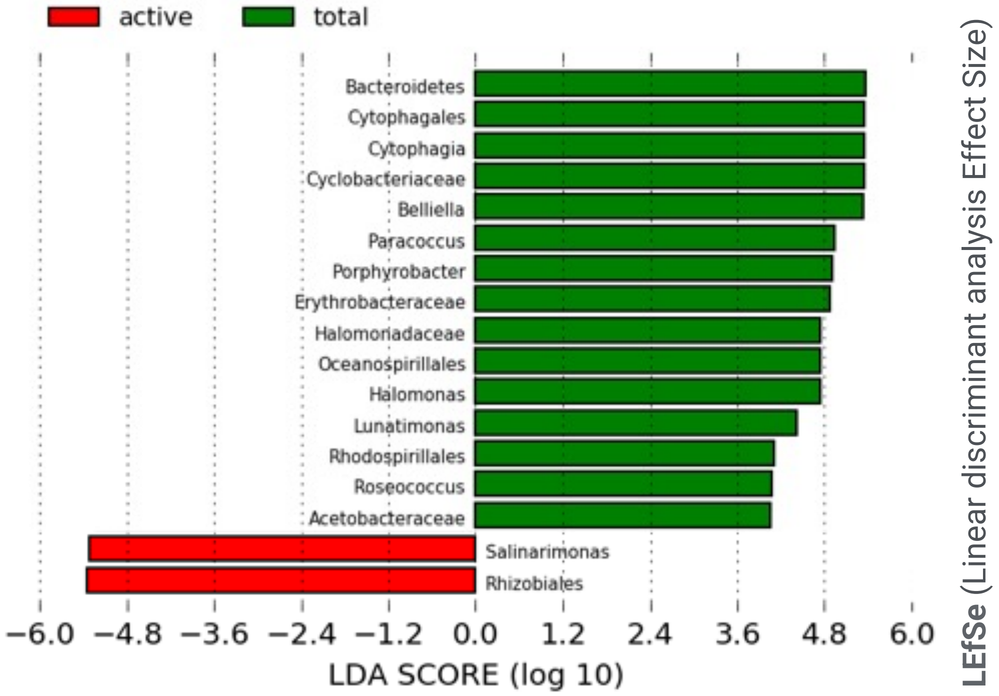
16S rRNA gene-sequencing

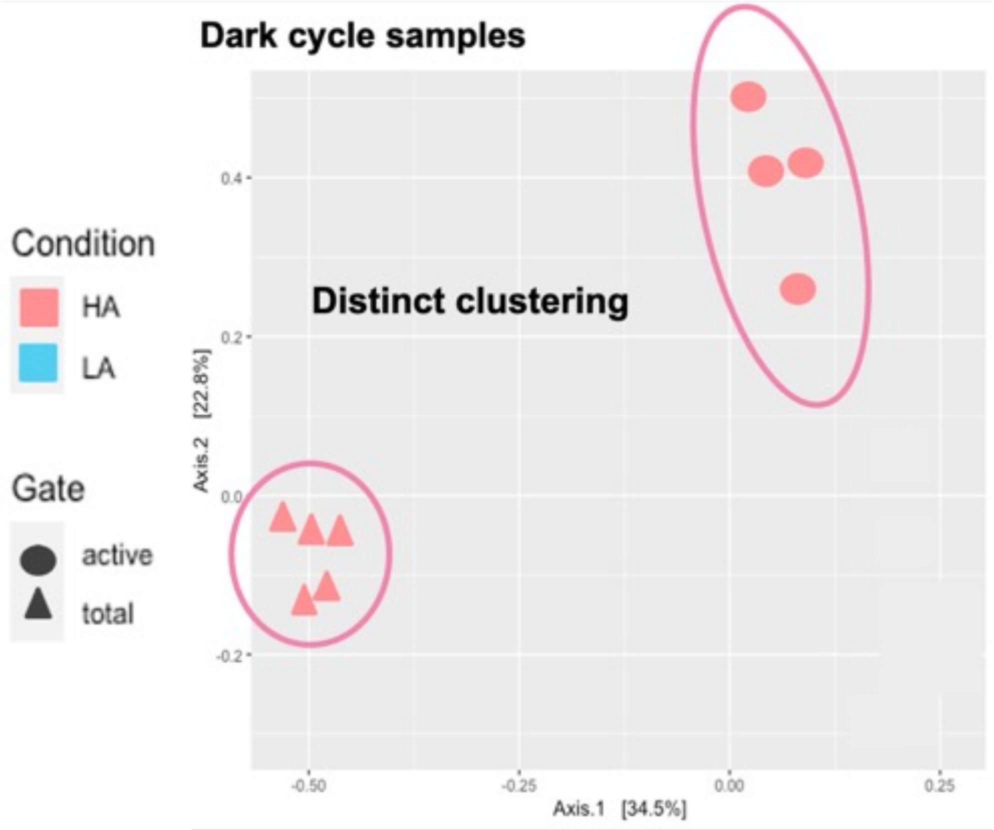


Significantly different, active populations during the dark cycle under HA conditions

Miller et al. unpublished

Phycosome activity during the diel cycle



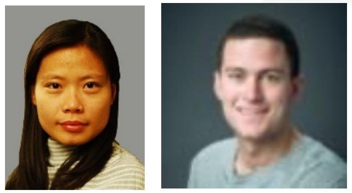


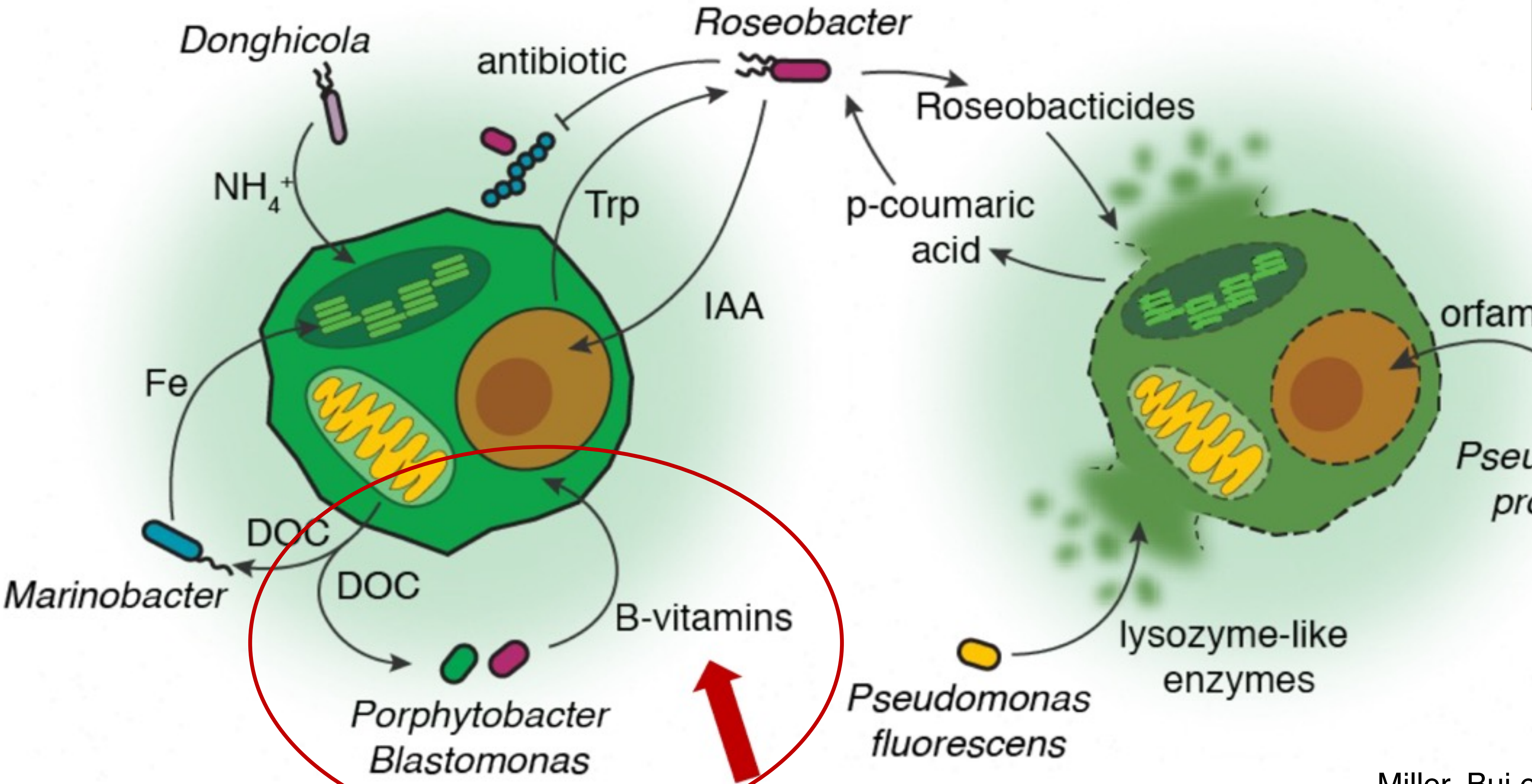
Salinarimonas:
halotolerant; compatible solute production

Rhizobiales:
known for association with plant growth, nitrogen fixation

Miller et al. unpublished

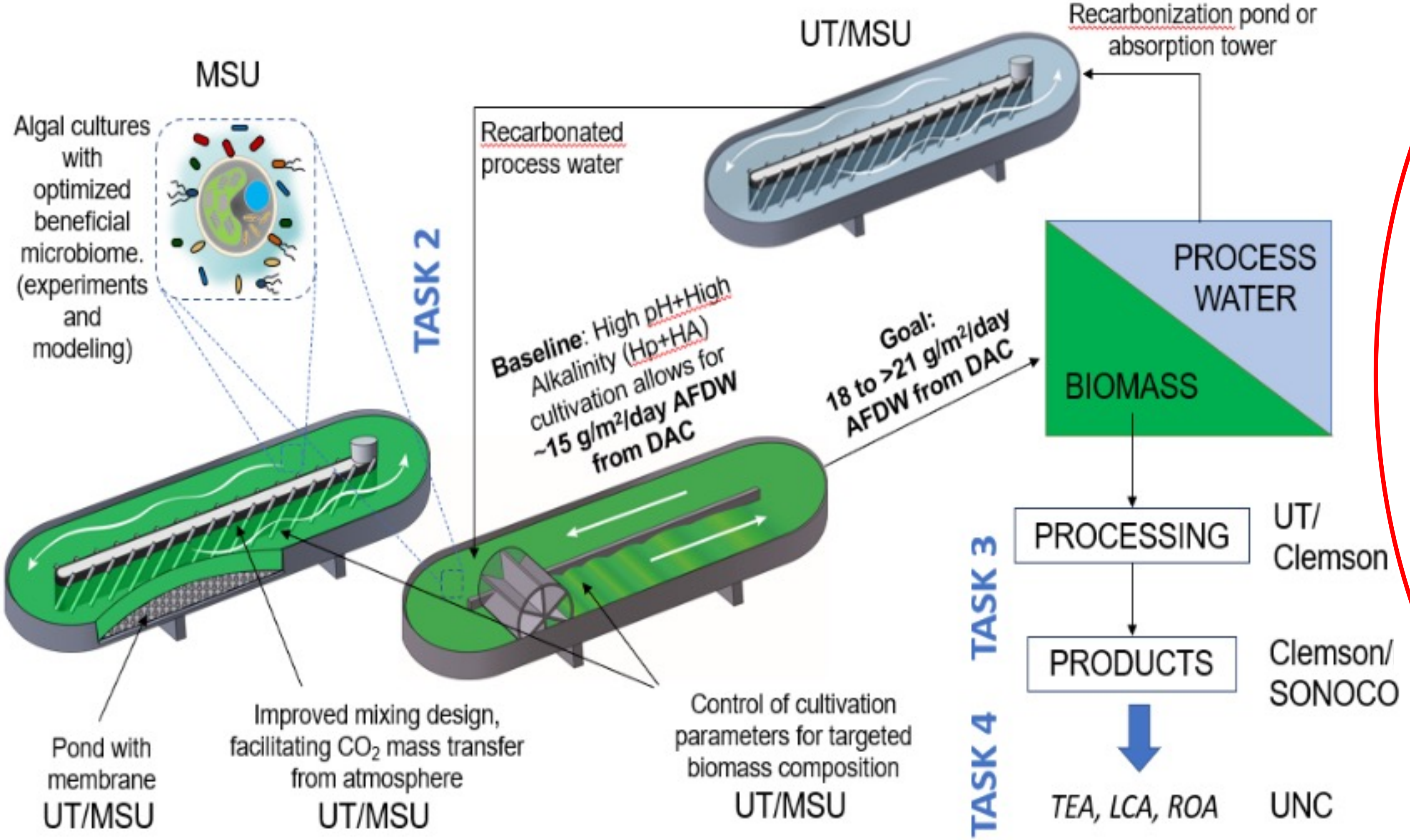
2- Progress and Outcomes (cont'd)
Metabolic Modeling of Algal Microbiome

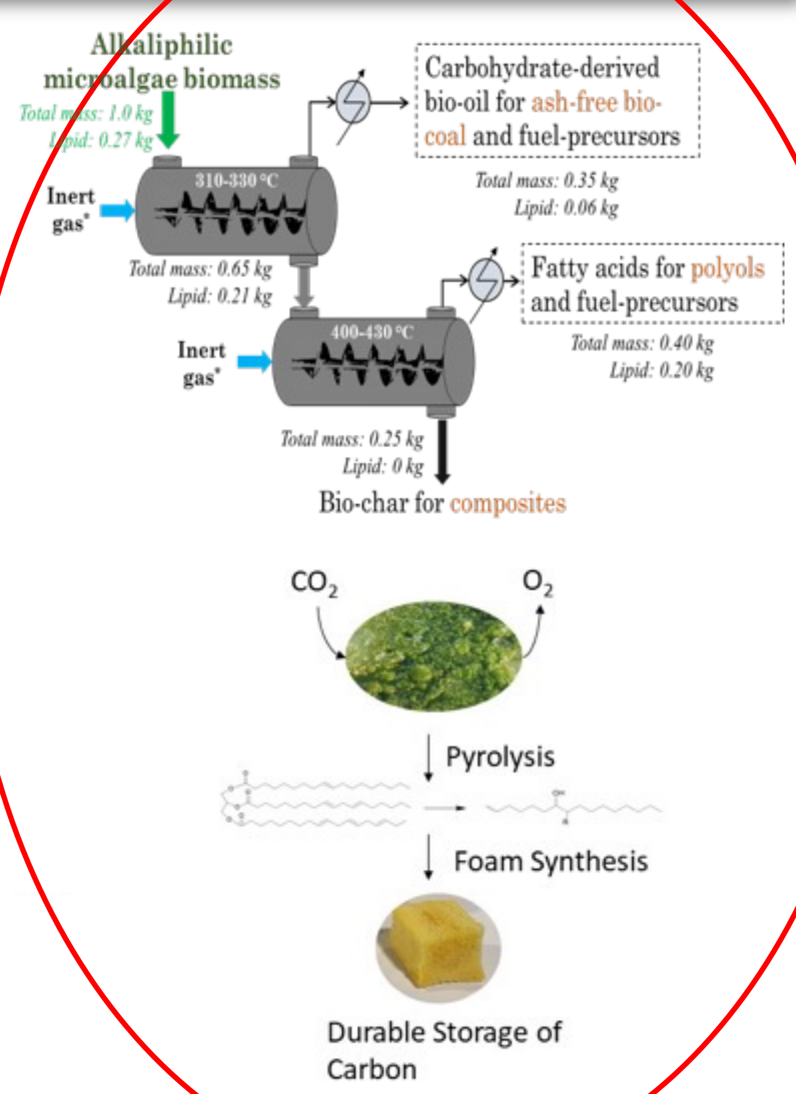




Miller, Bui et al. unpublished

Project Overview & Approach (cont.'d)





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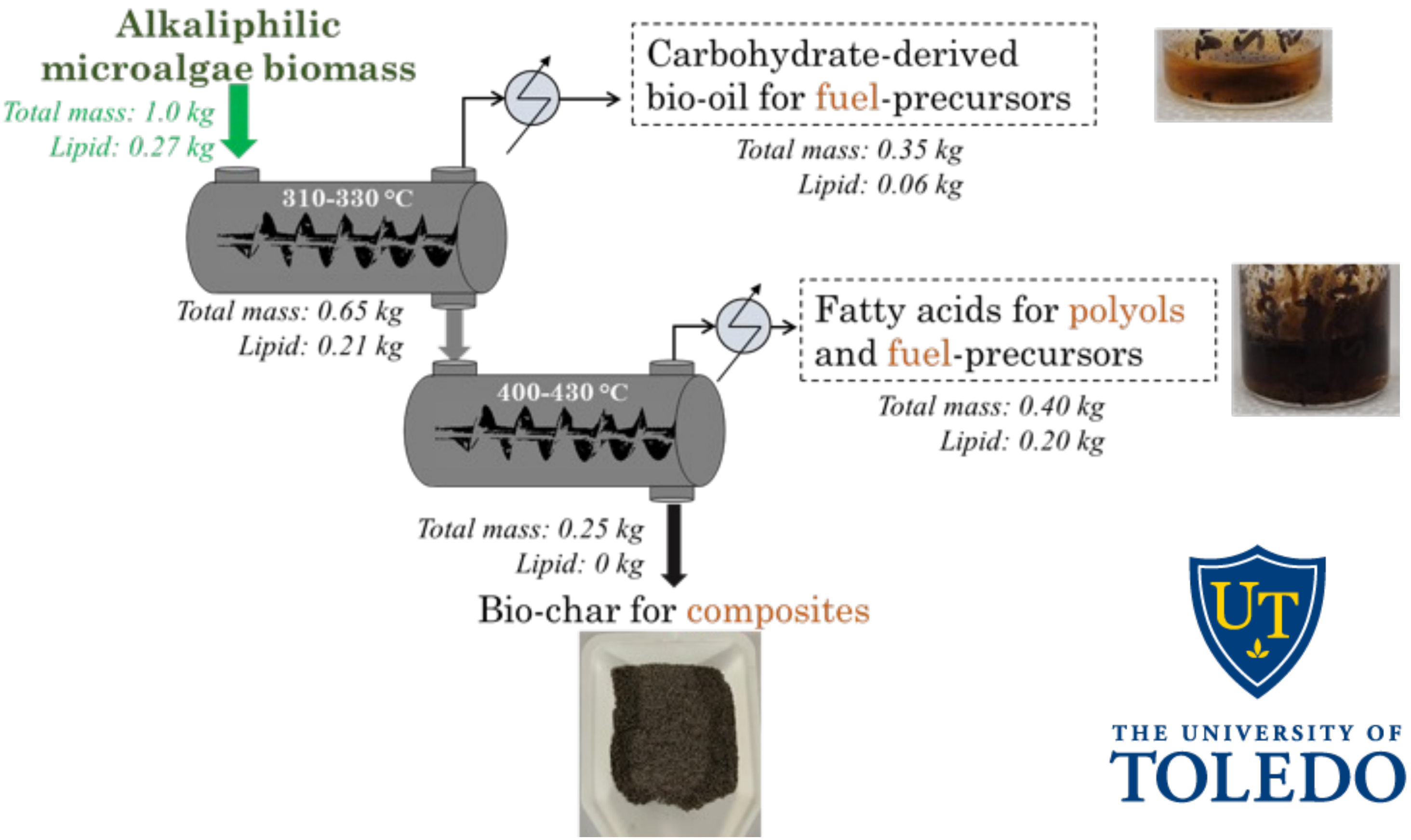
Pyrolytic Fractionation

Two step pyrolysis

- First step at T ~ 300–320 °C
  - Bio-oils from degradation of proteins and carbohydrates
- Second step at T ~ 420–430 °C
  - Triglycerides to fatty acids
  - Free of N, water, short-chain organic acids, and other carbohydrate degradation products.



THE UNIVERSITY OF TOLEDO

Sridhar Viamajala

2- Progress and Outcomes (cont'd)

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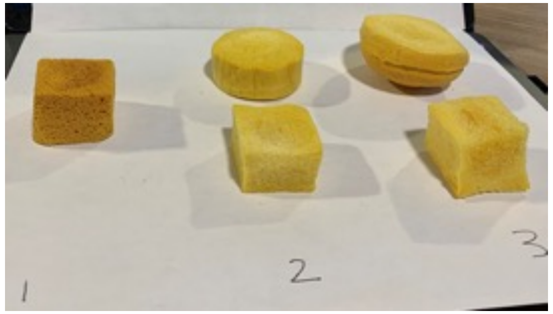
Mountains & Minds

Polyurethane Foam Production

(Left to Right) 0, 10, 30, 50% Polyol Replacement with Pyrolysis Oils



50% polyol replacement with (1) raw algae oil and (2,3) algal polyol

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AWARDS

EPA Green Chemistry Challenge Awards announced

Winners recognized for developing greener products and processes

by Sam Lemonick
June 15, 2021

non-isocyanate polyurethane (NIPU) foam

Credit: Srikanth Pilla

Clemson University researchers work on a biobased polyurethane foam. The research won a 2021 EPA Green Chemistry Challenge Award.

Srikanth Pilla
CLEMSON UNIVERSITY

2021 EPA Green Chemistry Award
https://www.epa.gov/greenchemistry/green-chemistry-challenge-2021-academic-award

2- Progress and Outcomes (cont'd)

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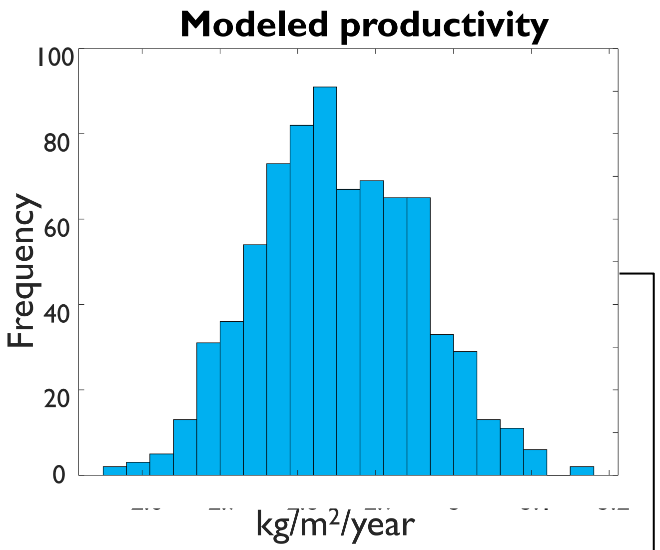
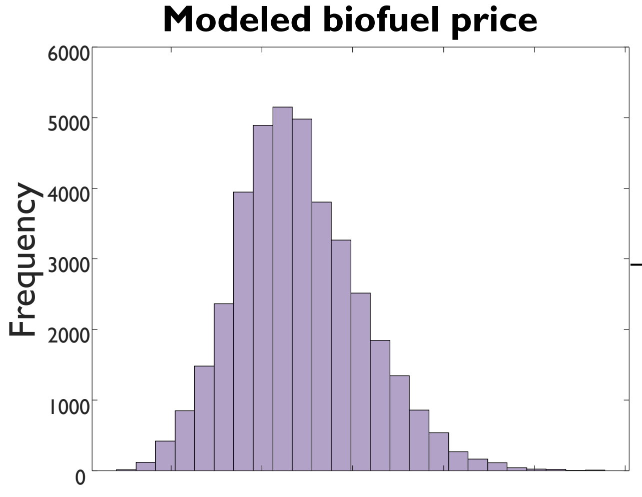
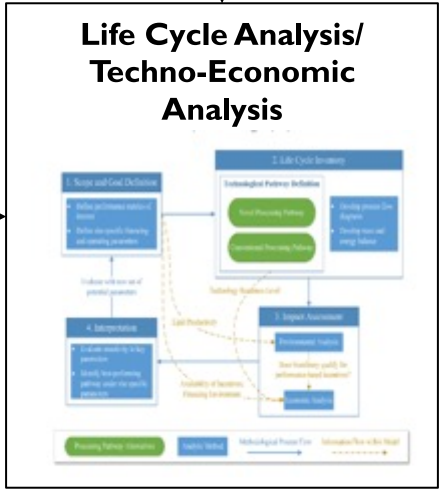




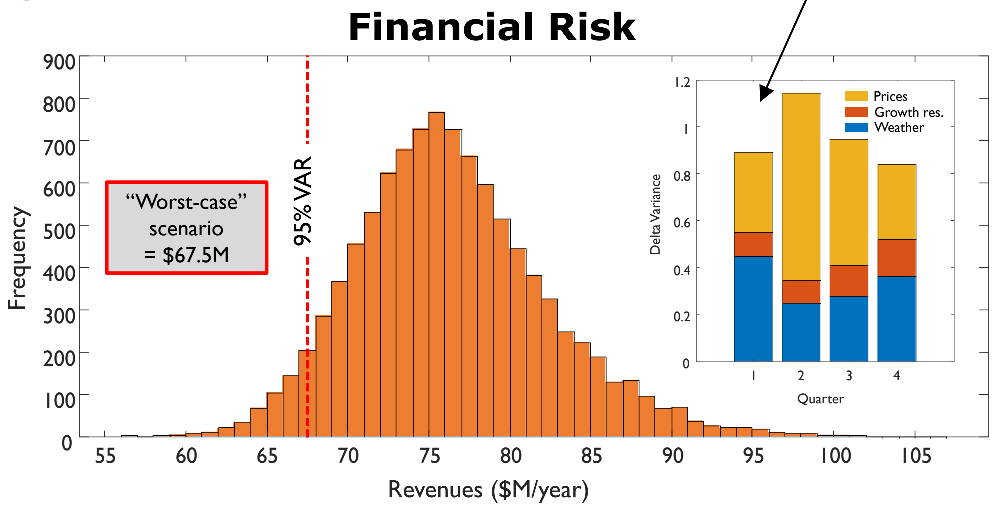
-Capital costs*
-Operational costs*
-Co-product assumptions
-Financing assumptions



Characterizing the Financial Risk of Algal Biofuel Production

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Drivers of Revenue Variability (i.e., Financial Risk)



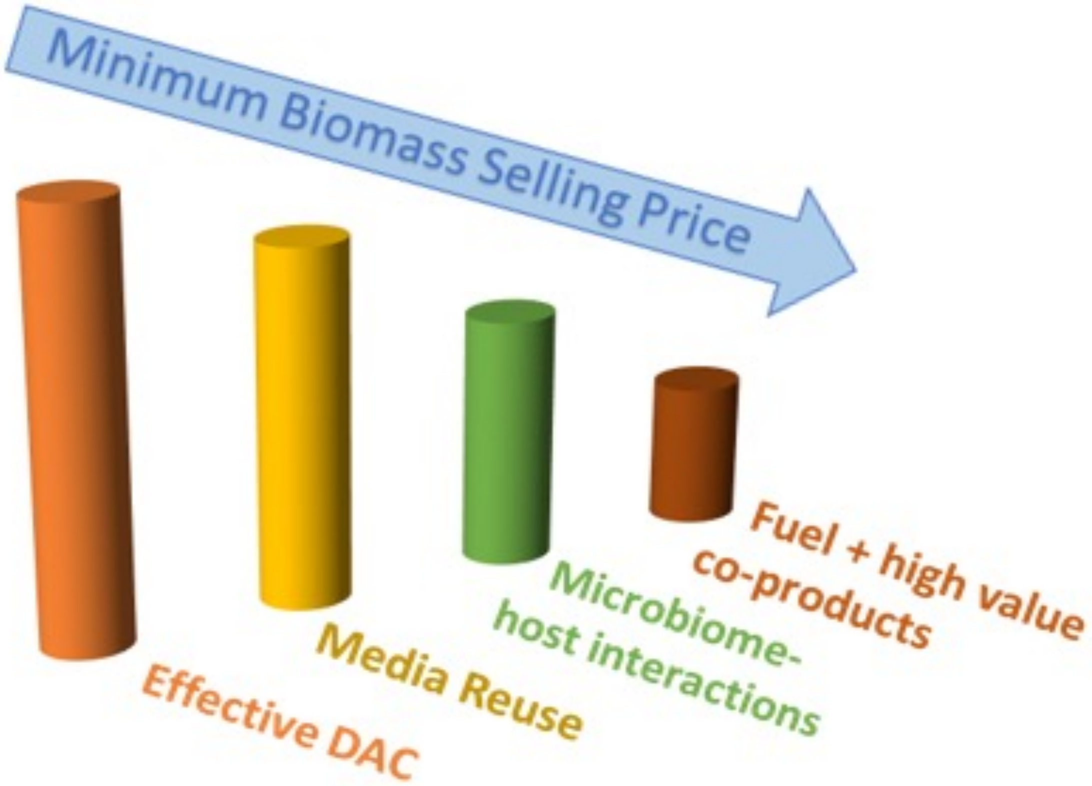
- Kleiman, Characklis and Kern (2022): Managing Weather- and Market Price-Related Financial Risks in Algal Biofuel Production. j.renene.2022.09.104
- Kleiman, Characklis et al. (2021): Characterizing Weather-Related Biophysical and Financial Risk in Algal Biofuel Production. j.apenergy.2021.116960
- Kern, Hise, Characklis et al. . (2017): Using Life Cycle Assessment and Techno-Economic Analysis in a Real Options Framework to Inform the Design of Algal Biofuel Production Facilities. j.biortech.2016.11.116
- Hise, Characklis, Kern, et al. (2016) Evaluating the relative impacts of operational and financial factors on the competitiveness of an algal biofuel production facility. j.biortech.2016.08.050

Greg Characklis

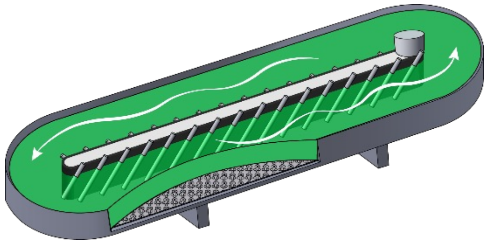
3 - Impact

- Demonstrated **ability to recarbonize high pH/high alkalinity medium**
- Ability to **recycle high pH/high alkalinity medium repeatedly**
- Microbiome/**microbiome activity changes between low/high alkalinity & day/night**
- **Foams** have been **produced** from algal oil

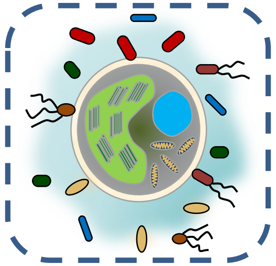
- Overall, we are **providing data demonstrating that the economics of algal production facilities can be improved** (and that modelling can be used to predict best strategy based on needs (e.g., low revenue potential fuels for transportation vs. higher value/higher volume products such as foams vs. high(est) value products (e.g., dyes, electrode materials, etc.))



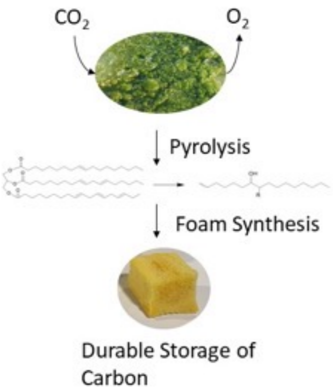
Summary



- Fabricated small belt-and-cleat ponds
- Demonstrated that we can effectively recarbonize Hp/HA medium to support >18 $g/m^2/d$ productivities
- Demonstrated that we can recycle Hp/HA medium (at least 12 times)



- There are differences in microbiome/ microbiome activity
- Have metabolic models for in silico experiments



- Can convert algal biomass into fuels and polyols, which can be used to produce foams using isocyanate- and non-isocyanate-based pathways
- TEA to support economic improve-ments by selecting competitive product spectra (e.g., foams & fuels)

future

Plans for Future Work
- Work on other high value products (biocarbon, biochar)
- Identify specific algal microbiome interactions and understand why Hp/HA SLA-04 cultures seem to be so robust and productive
- Identify and address challenges that might be arising through repeated media recycling
- Continue TEA/LCA work to develop strategies to increase revenue and lower MBSP/MFSP

DE-EE0009273

Additional Slides

(Not a template slide – for information purposes only)

- *The following slides are to be included in your submission for evaluation purposes, but <u>will **not** be part of your oral presentation</u> –*
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

Responses to Previous Reviewers' Comments

- N/A, Project work **started late in 2021** hence **no peer review comments yet**. SOPO was revised through discussions during the verification process in April 2021

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.

Publications, Patents, Presentations, Awards, and Commercialization

- Nancy Chen, Srikanth Pilla, A comprehensive review on transforming lignocellulosic materials into biocarbon and its utilization for composites applications., Composites Part C: Open Access, Volume 7, 2022, 100225, ISSN 2666-6820, https://doi.org/10.1016/j.jcomc.2021.100225
- Numerous publications in various stages of preparation

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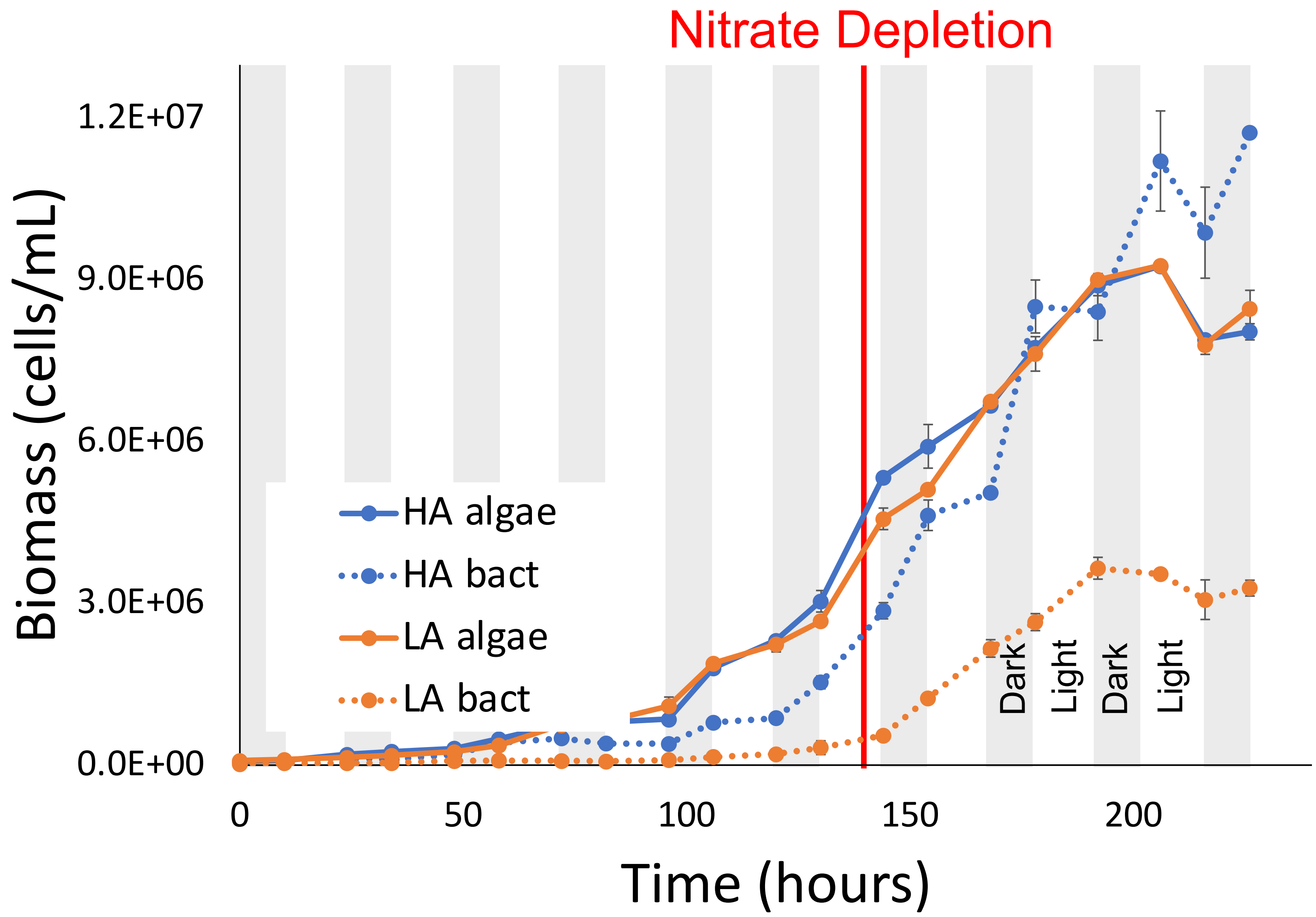
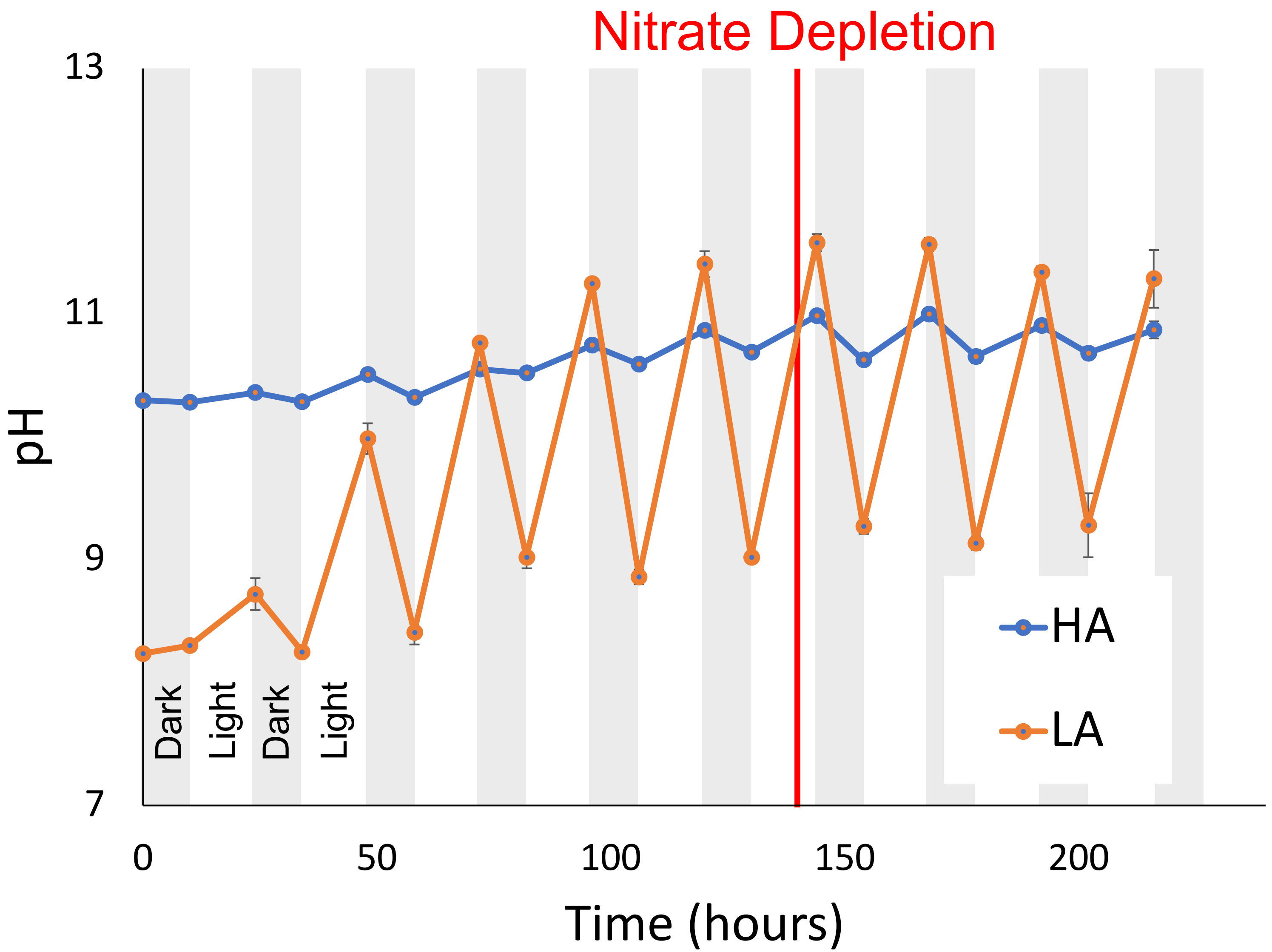
Diel cycling - phycosome growth



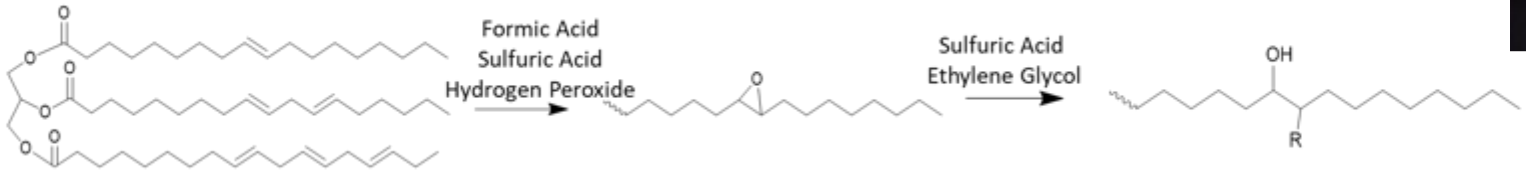
Diel cycling - phycosome growth

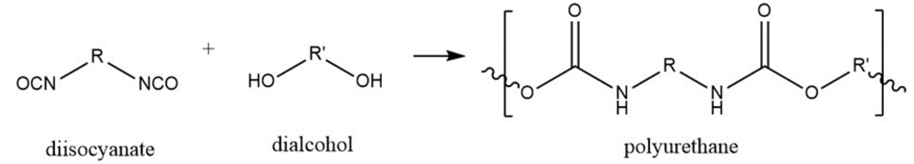


2- Progress and Outcomes (cont'd)

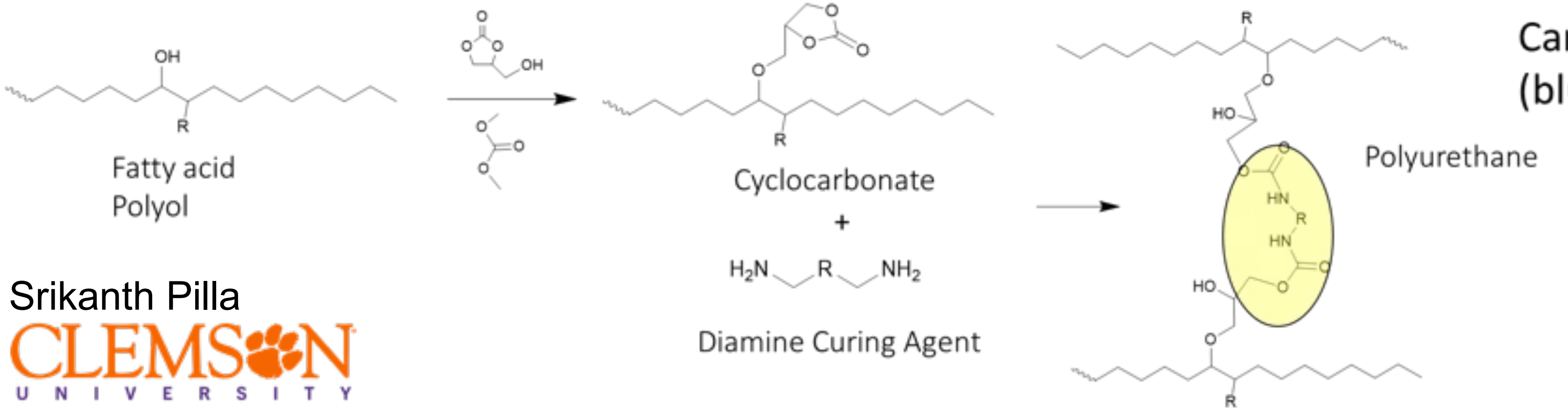
Polyurethane Foam Production

Synthesis of Polyol from unsaturated hydrocarbons





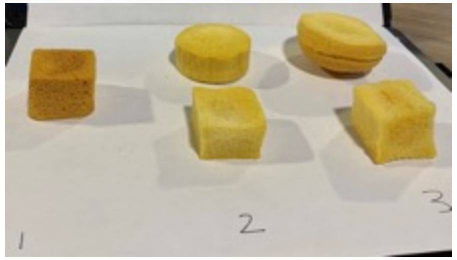
Non-isocyanate Pathway to Polyurethanes with use of Cyclic Carbonates



Srikanth Pilla

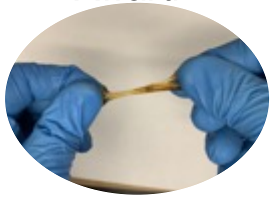
CLEMSON UNIVERSITY

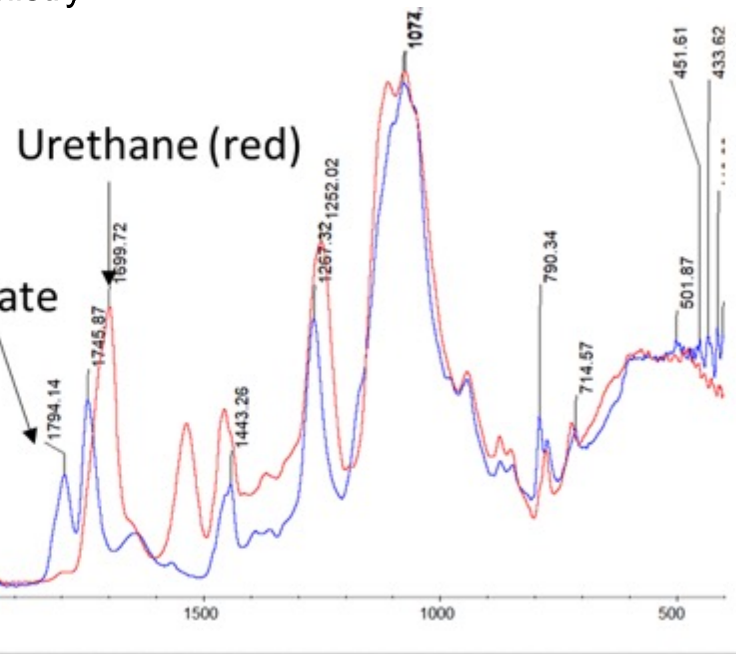
(Left to Right) 0, 10, 30, 50% Polyol Replacement with Cottonseed Oil



50% polyol replacement with (1) raw algae oil and (2,3) Algae polyol

2021 EPA Green Chemistry Award



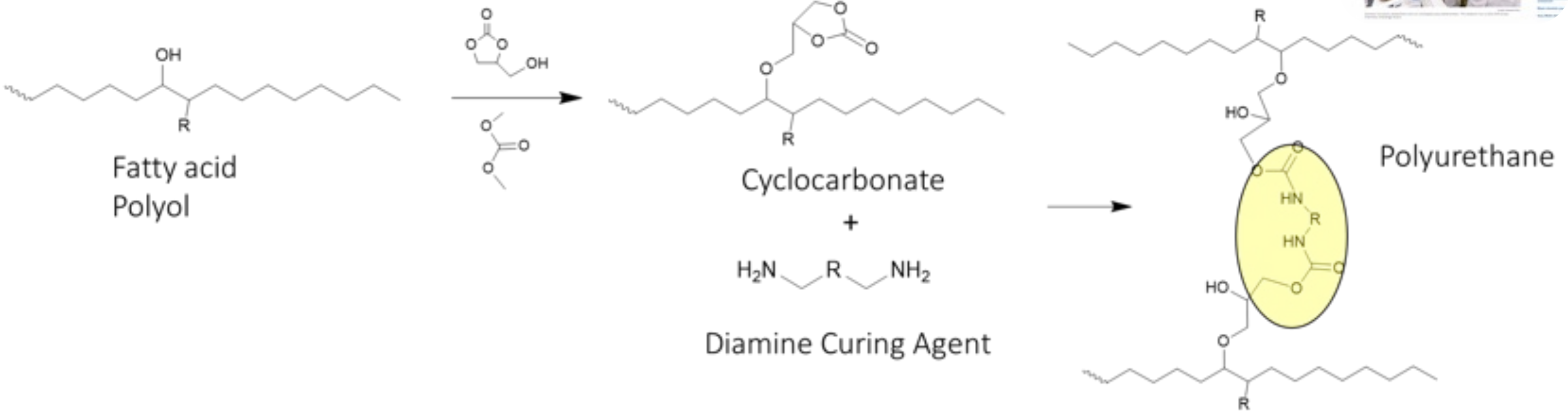


FTIR Spectrum: proof of concept

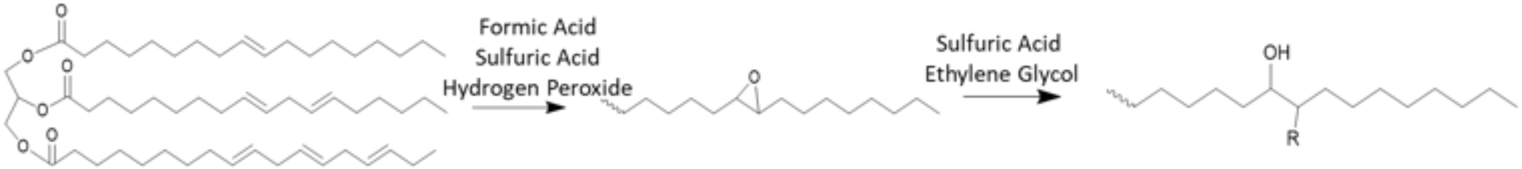
2- Progress and Outcomes (cont'd)

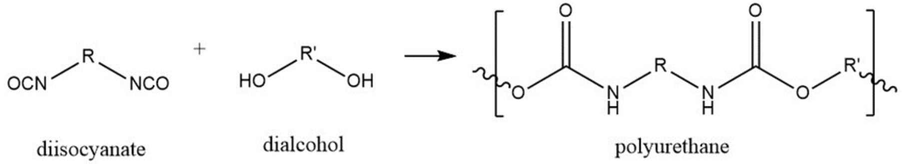
Polyurethane Foam Production

Non-isocyanate Pathway to Polyurethanes with use of Cyclic Carbonates



Isocyanate Pathway for Polyurethane Production





Srikanth Pilla

CLEMSON UNIVERSITY

2021 EPA Green Chemistry Award

https://www.epa.gov/greenchemistry/green-chemistry-challenge-2021-academic-award

Nonisocyanate polyurethane (NIPU) foam

A team led by Professor Srikanth Pilla of Clemson University is being recognized for creating the first lignin-based non-isocyanate polyurethane (NIPU) foam. This new technology replaces traditional polyurethane foams, one of the most commonly used plastics in the industry. Traditional polyurethane foams are typically manufactured from diisocyanates, a known carcinogen that has significant health and safety effects in its manufacturing.

Summary of Technology:

While the lignin-based NIPU foams have the same mechanical properties as traditional polyurethane foams, they are specifically designed for chemical recycling at their end of life. Clemson University's innovation uses lignin directly to bypass fractionation and purification steps that typically complicate a sustainable application. The Clemson team first created a reactive, carbonated lignin precursor by using organic carbonates as a reagent. The application of organic carbonates creates a unique opportunity to break down the polymer structure and regenerate the lignin by creating "molecular zippers." These zippers break down the structure of the foam and recover the lignin. Recycled lignin can be readily used to create a new NIPU foam. The sustainability of the process is also enhanced by the ability to recycle the catalyst (e.g., potassium carbonate) used in multiple synthetic steps. The true innovation of this chemistry lies in the formation of reactive precursors using non-toxic and 100 percent biobased reagents. In the past, lignin has been made into a reactive precursor using oxypropylation with propylene oxide and added diisocyanates to mimic the synthesis of conventional polyurethane foams. With the lignin-based NIPU foams, the curing agent is derived from environmentally friendly vegetable oils.

Dipl.-Ing. Robin Gerlach (Ph.D.)

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