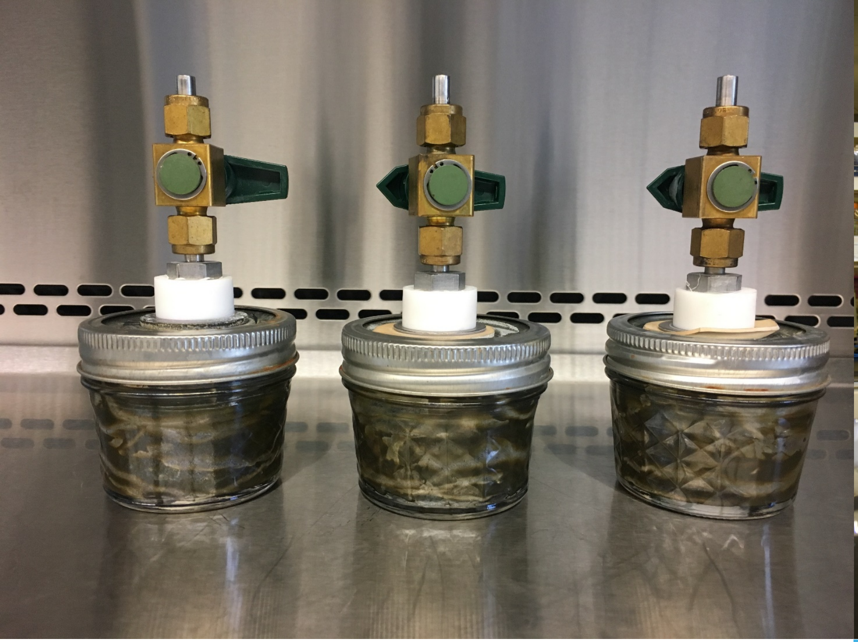
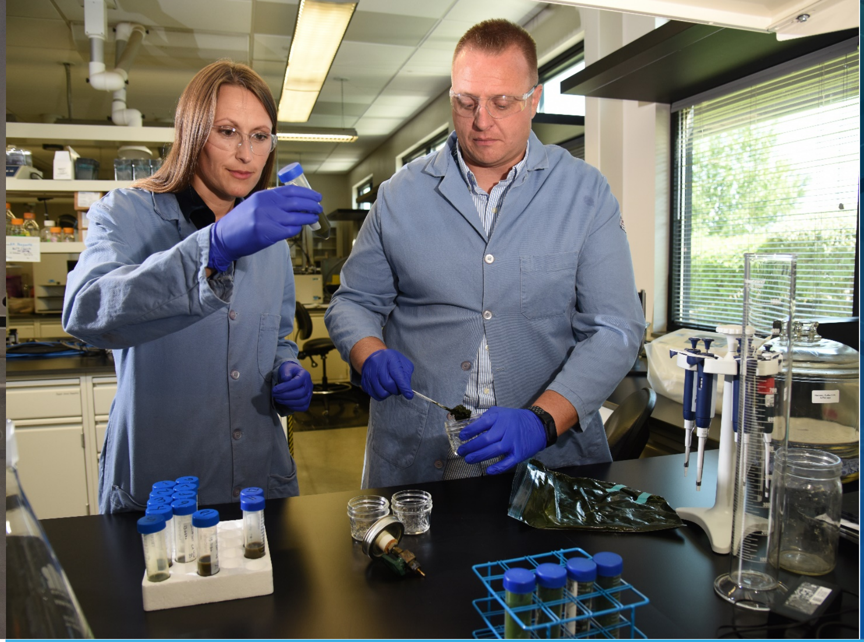
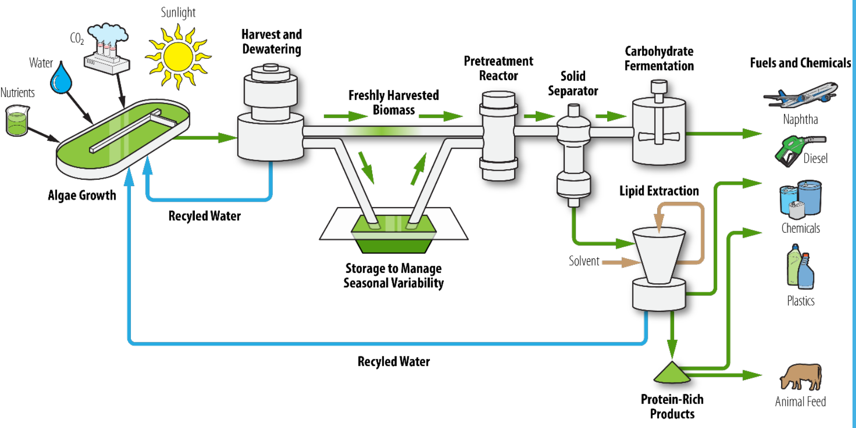




March 24, 2021

Advanced Algal Systems

Bradley Wahlen
Principal Investigator,
Idaho National Laboratory



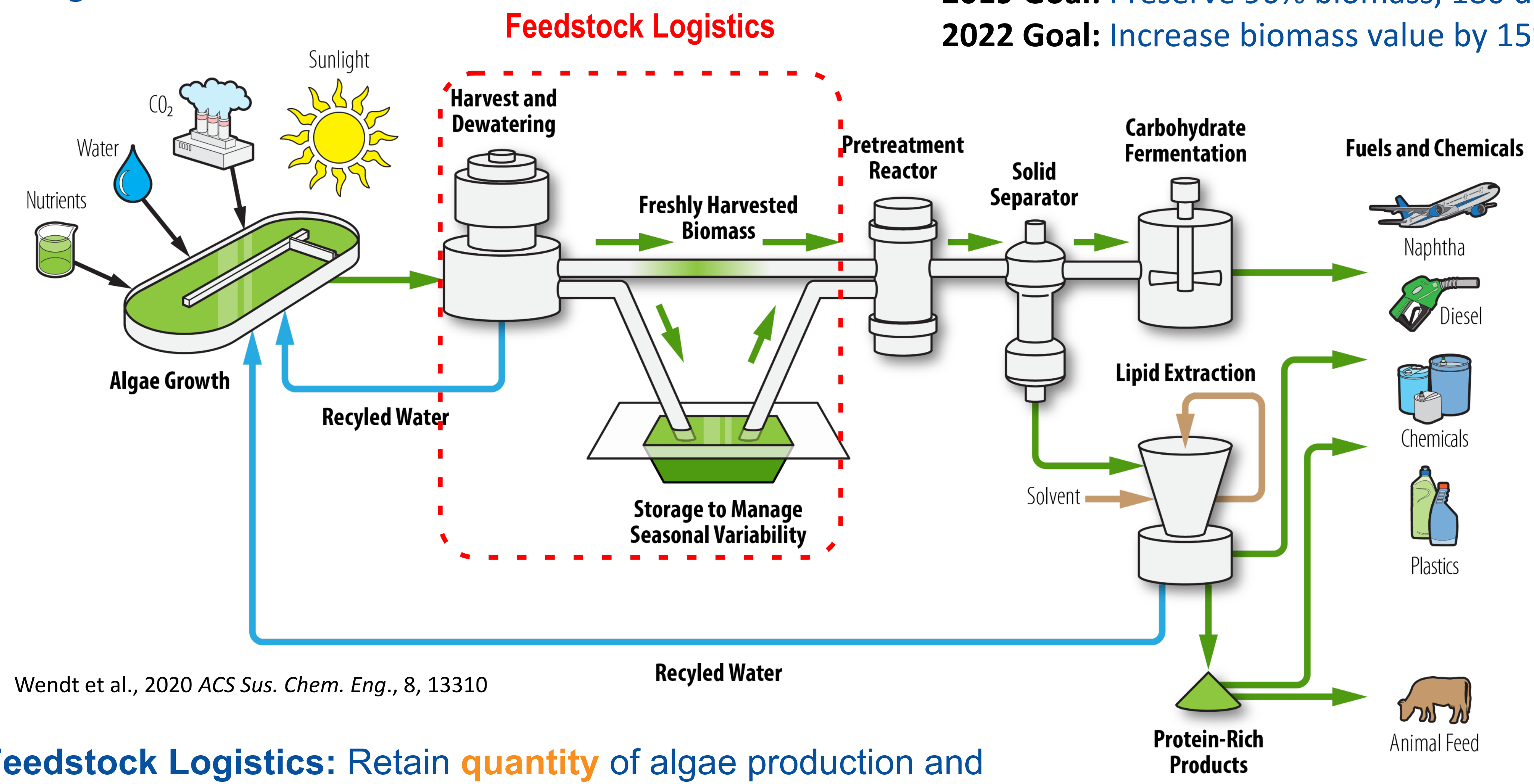
1.3.3.100: Algal Feedstock Logistics and Handling

DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Idaho National Laboratory

Project Overview

2019 Goal: Preserve 90% biomass, 180 days
2022 Goal: Increase biomass value by 15%



Wendt et al., 2020 *ACS Sus. Chem. Eng.*, 8, 13310

Feedstock Logistics: Retain **quantity** of algae production and maintain **quality** of post-harvest biomass for efficient conversion, **enabling $2.50 GGE^{-1}**

1 – Management

Engage diverse national laboratory **capabilities** through **collaboration**

- Measure cost impacts through TEAs (INL, NREL) to compare solutions to SOT (State of Technology)
- Measure impacts of storage treatments in multiple conversion approaches through collaboration (e.g. NREL, PNNL)

Quarterly, Annual and Go/No-Go Milestones provide framework for meeting **aggressive goals**

Interaction with BETO promotes **relevance** to DOE and industry

- Annual Operating Plans (AOPs) used to **define research path** and work scope
- Quarterly progress reports and milestones document **step-wise progression** of research
- Monthly presentations for BETO provide framework for information sharing and **feedback**
- Participation in biweekly calls with DISCOVR team to discuss outdoor cultivation for SOT efforts

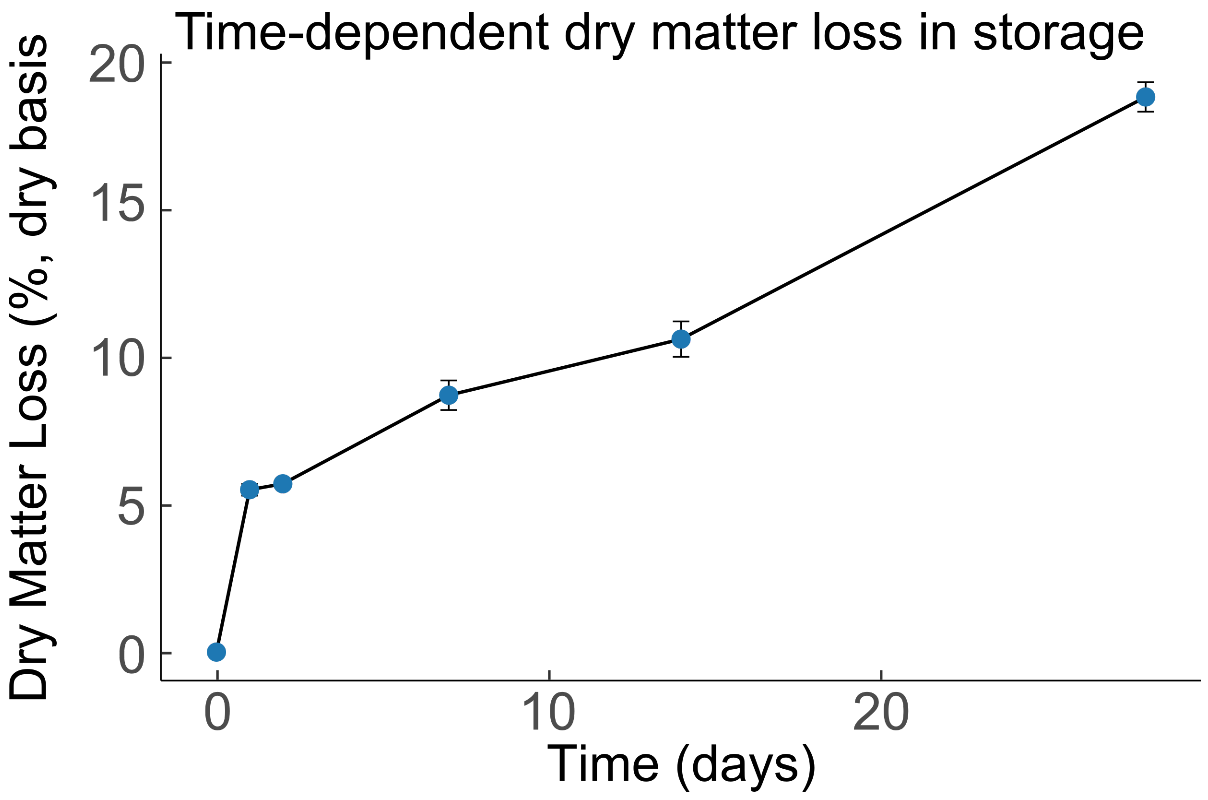
2 – Approach – Queuing Stability

Queuing Stability: Initial loss occurs rapidly due to metabolic activity.

- Queuing stability affects 100% of annual production; Long-term storage affects 16%
- Develop a short-term stabilization approach; **minimize initial loss**

Approach: Understand algae **post-harvest metabolism** and mode of **degradation**

- Utilize "omics" techniques to understand impact storage has on post-harvest metabolism
- Characterize chemical and structural impacts of degradation



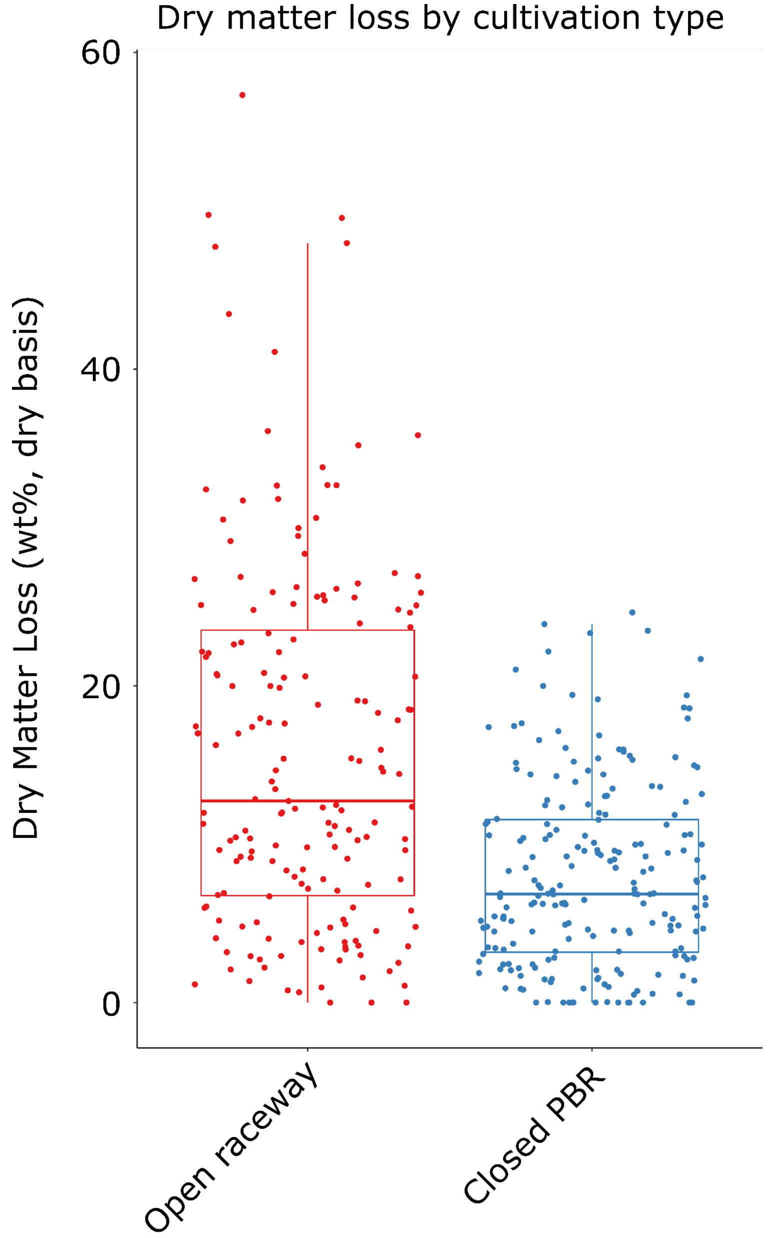
2 – Approach – Long-term Stability

Value-add in Long-term stabilization: Outdoor-cultivated algae is challenging to stabilize

- Develop stabilization approaches that **limit losses** in outdoor cultures to 10% or add value to biomass (decrease MBSP by 15%)

Approach: Understand fundamentals of preservation and organic acid production

- Stabilize **industrially relevant algae** biomass from open raceways
- Optimize organic acid production to **increase biomass value**
  - Storage **co-products**, lactic, acetic & succinic acids



2 – Approach – Technology to Market

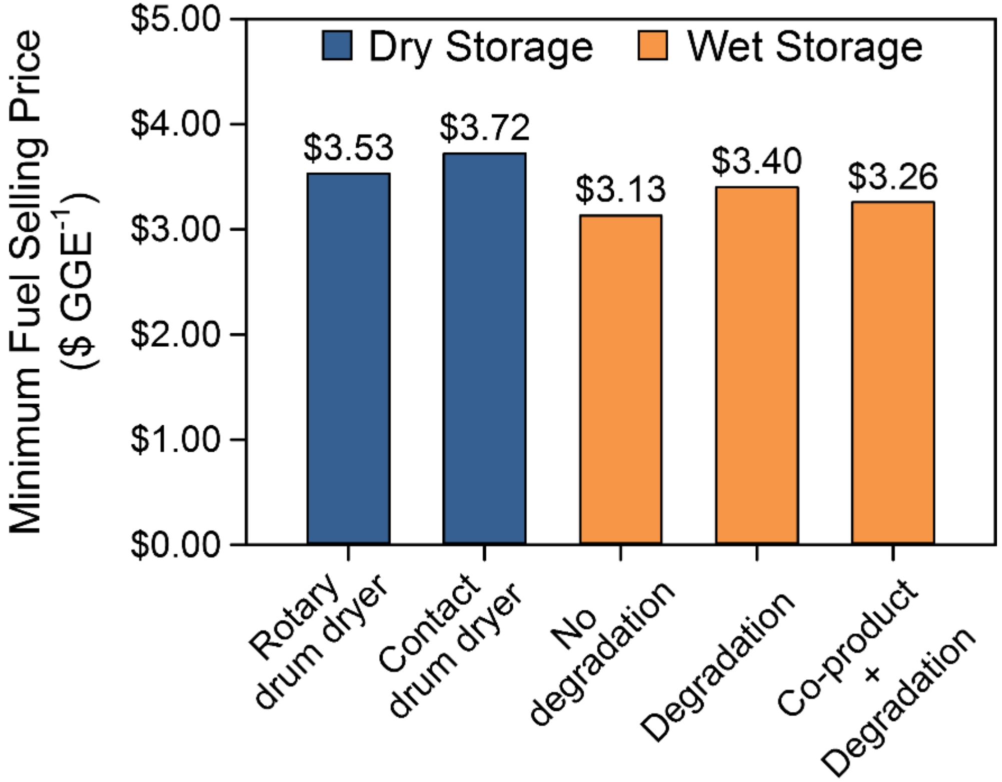
Enable commercial algae biofuels: Algae biomass productivity varies with season: Summer production 3x higher than winter

- Replacing drying with wet storage has been shown to **reduce fuel price**
- Costs are reduced further with **co-product credit**

Approach: Techno-economic models to extend laboratory results to industrial scales

- Cost impacts of storage treatments
- Cost of separation process for by-product recovery
- Physical models to characterize geographic-dependent storage performance, influence of temperature and other variables on stability

Go/No-Go Milestone: Increase value of microalgae biomass in storage (**decrease MBSP by 10%**), **reducing cost** of algae biofuels and contribute to achieving $2.50/GGE fuels



Minimum fuel selling price (MFSP) of dry and wet storage scenarios in CAP process

Modified from Wendt *et al. Biotechnol Biofuels* (2019)

3 – Impact

This project:

- **Reduces fuel production costs**
  - Long-term wet anaerobic storage **preserves >90%** of algae biomass for 180 days, is less costly than drying, co-products **add value**
  - Cost competitiveness, utilize sustainable designs that reduce energy and water
- Supports cultivation
  - Night-time losses in cultivation occur in similar conditions experienced in the first hours after harvest. Stability studies could lead to reduction in night-time biomass loss
- Supports crop protection efforts
  - Community analysis of healthy cultures can be used as a baseline to identify differences in pond microbiome of crashed cultures
  - Stability studies could provide insight into modes of degradation
  - Peer-reviewed publications and conference presentation
- Supports Conversion
  - Storage does not impact algae biomass **fuel yield** or quality
  - Application of approaches in industrially-relevant species and multiple end-uses

4 – Progress and Outcomes – Long-term storage

Goal: Wet anaerobic approach to storage that reduces cost and energy consumption compared to drying and dry storage, while retaining >90% of biomass in long-term storage (180 days).

Progress: Milestone completed, end of project FY19

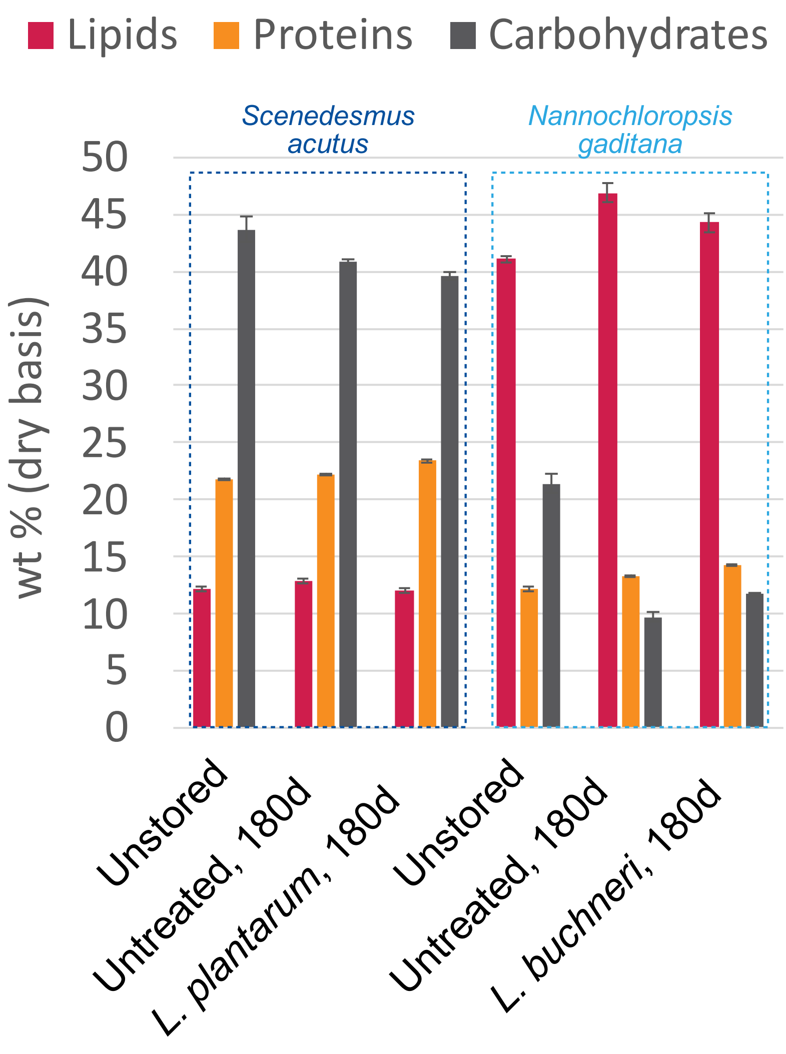
Outcomes: Storage of freshwater (*S. acutus*) and saline (*N. gaditana*) biomass with **losses ≤ 10%** after 180 days in storage

- **Carbohydrate** fraction impacted, protein and lipids relative increase

Impact: Mitigation of seasonal variation in productivity through wet anaerobic storage **decreases MFSP by \$0.32 GGE^{-1}** (Wendt-2019).

Strain	Treatment	Dry Matter Loss (%, db[a])	pH	Organic acids (%, db[a])
S. acutus	Untreated	6.1 ± 0.7	3.84 ± 0.04	11.4 ± 0.6
S. acutus	*L. plantarum*[b]	7.9 ± 1.0	3.95 ± 0.03	12.2 ± 0.4
N. gaditana	Untreated	9.3 ± 0.8	4.29 ± 0.16	14.0 ± 0.4
N. gaditana	*L. buchneri*[b]	7.1 ± 1.6	4.77 ± 0.12	13.2 ± 1.0

[a]dry basis, [b]algae biomass inoculated with lactic acid bacteria strain



4 – Progress and Outcomes – Queuing Stability

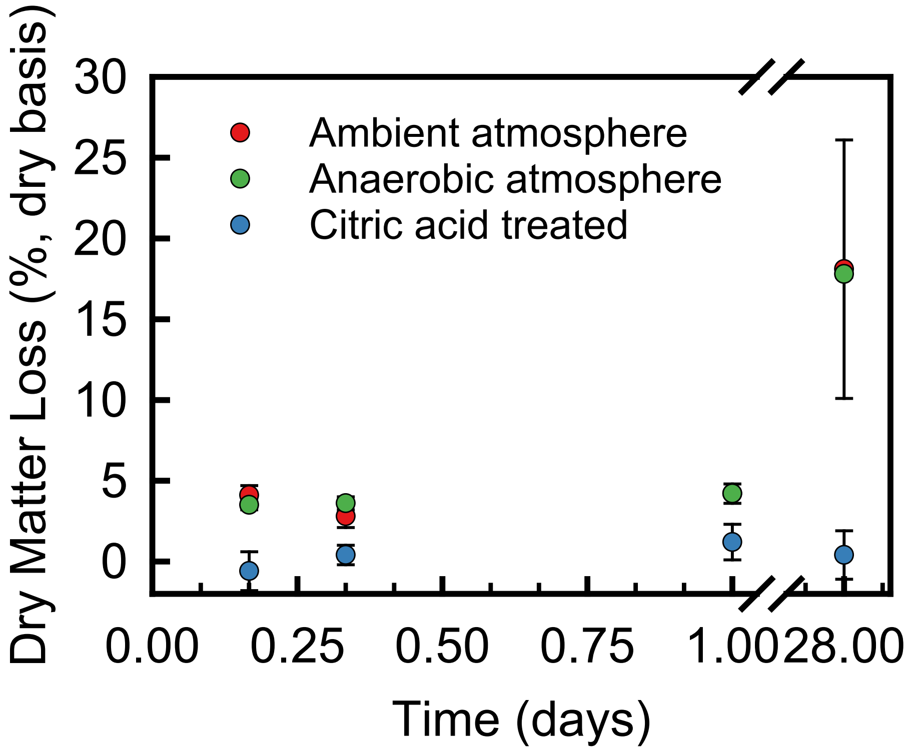
Goal: Understand risk of algae feedstock loss in queuing and develop methods of preservation

Progress: Queuing studies initiated at AzCATI with biomass directly from centrifuge.

Outcomes: Compared dry matter loss, organic acid production and bacterial community structure at 4, 8 and 24 hours (queuing) and 28 days (storage)

- Ambient & anaerobic: **4% loss within 24hrs**, similar to reported nighttime losses, 18% after 28d
- Citric acid treated: **Loss limited to 1%** after 28 d

Impact: Losses in ambient/anaerobic conditions demonstrate that post-harvest biomass is at risk. Impact of citric acid demonstrates that **active management mitigates losses**



Time dependent post-harvest dry matter loss in algae biomass under ambient, anaerobic atmospheres or treated with citric acid

4 – Progress and Outcomes – Queuing Stability

Goal: Understand risk of algae feedstock loss in queuing and develop methods of preservation

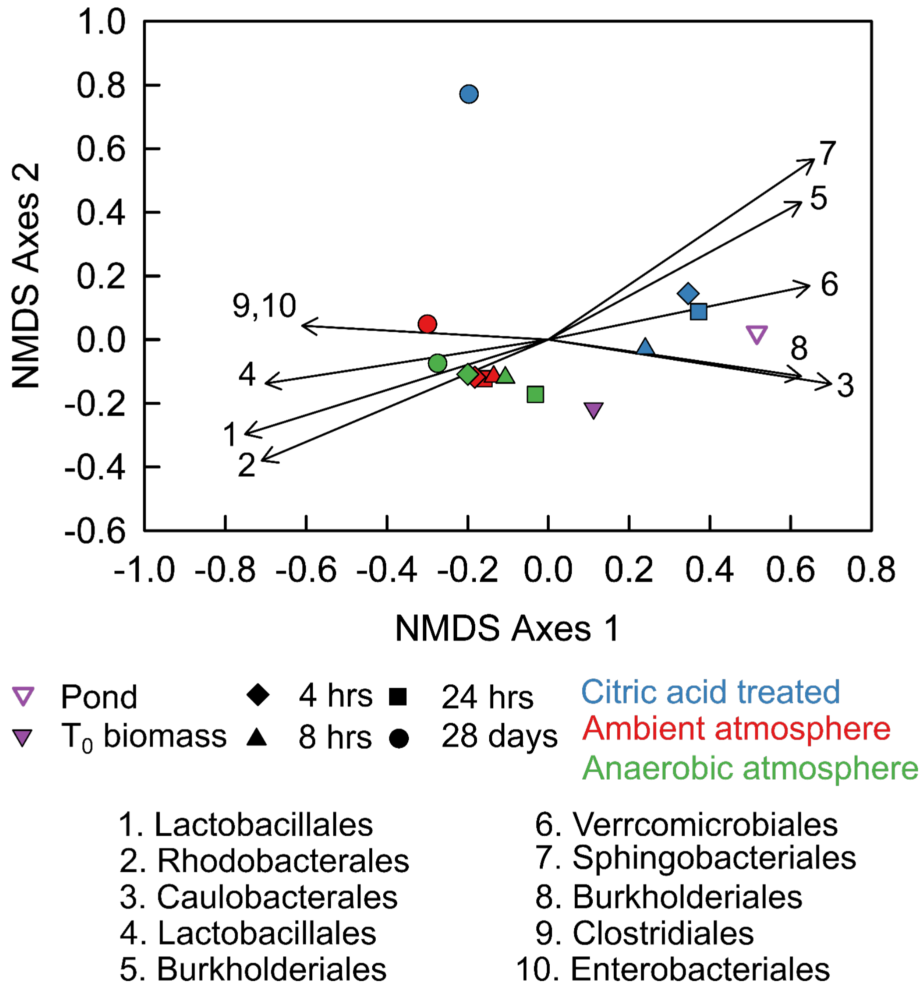
Progress: Assessed treatment and time dependent changes to bacterial community occurring in storage

Outcomes: Bacterial community structures varied by treatment

- Lactic acid bacteria were prominent OTUs in all conditions after 28 days
- **Clostridium is absent in citric acid** treated samples but present in ambient and anaerobically stored samples

Impact: Citric acid encouraged lactic acid bacteria and inhibited *Clostridia*.

- Future work: *Clostridia*-specific inhibitors



4 – Progress and Outcomes – Long-term Storage – Outdoor Cultivation

Goal: Assess stability of outdoor-cultivated strains (DISCOVR SOT effort), ensure **relevance to industry**

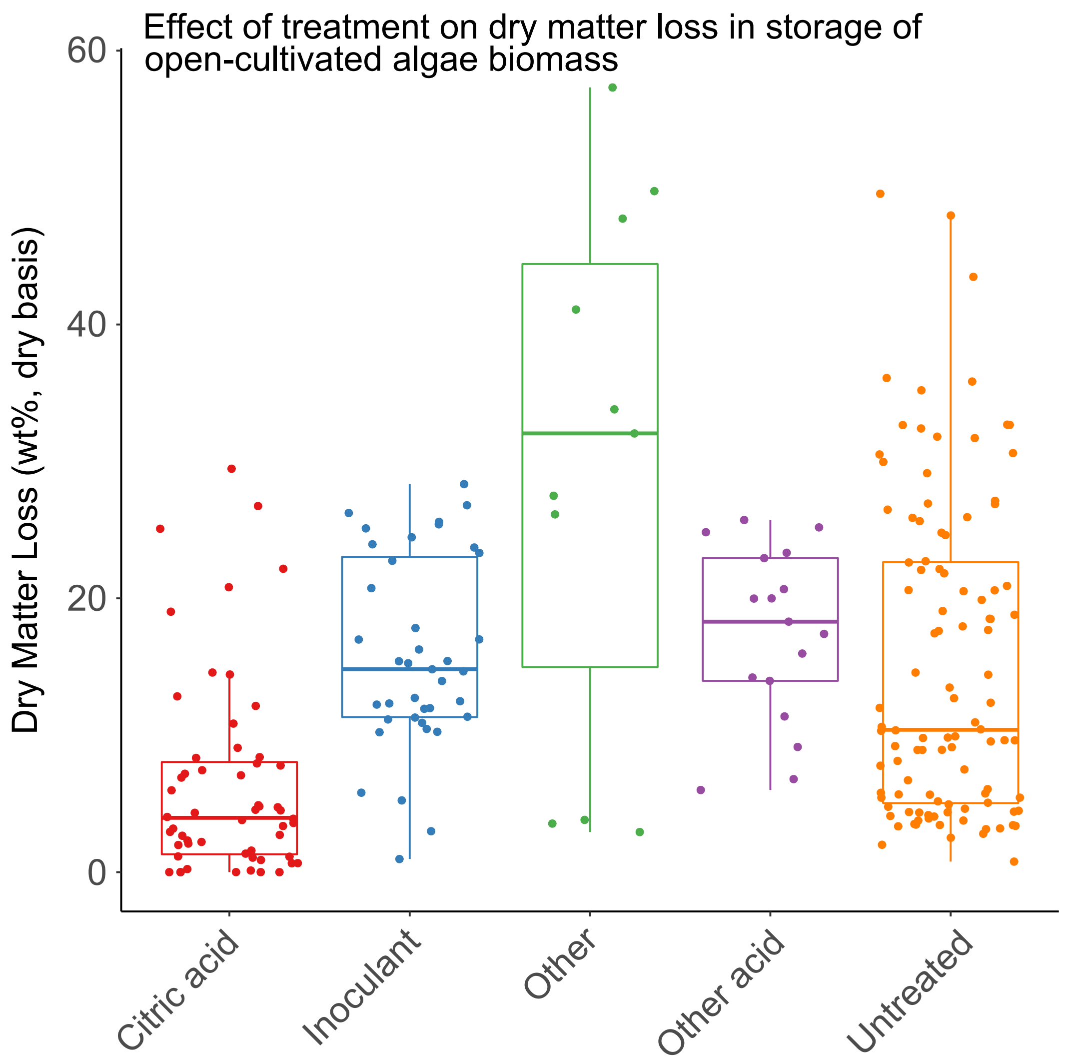
Progress: Assessed stability of 15 unique strains from 34 unique cultivations

Outcomes: Citric acid significantly ($p<0.01$) **reduces loss** relative to inoculation, other treatments

- Other additives show promise

Impact: Small molecules that modify environment can promote stability in storage, reduce loss and **preserve value**

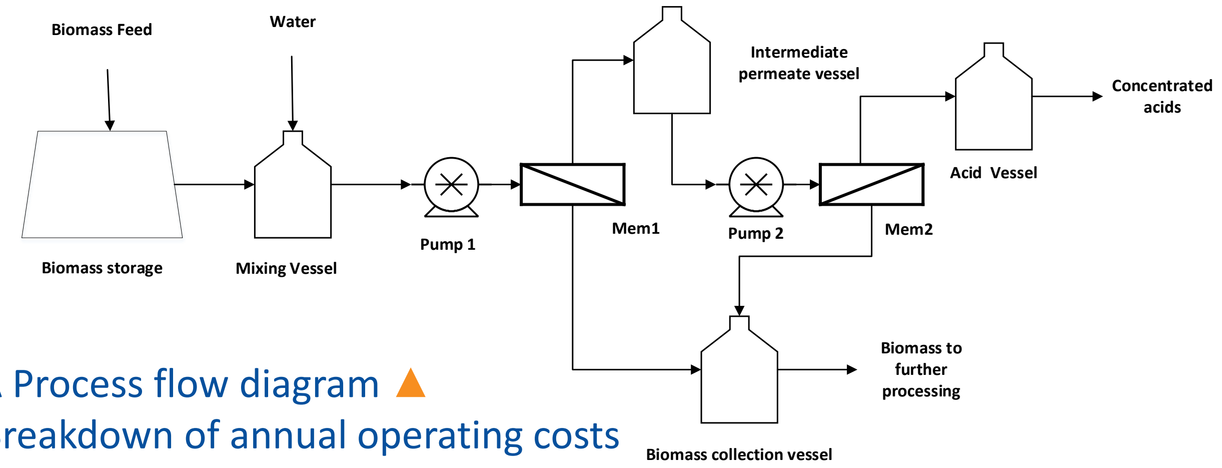
- Future work: Identify other low-cost small molecules that promote stability



4 – Progress and Outcomes – Techno-economic analysis

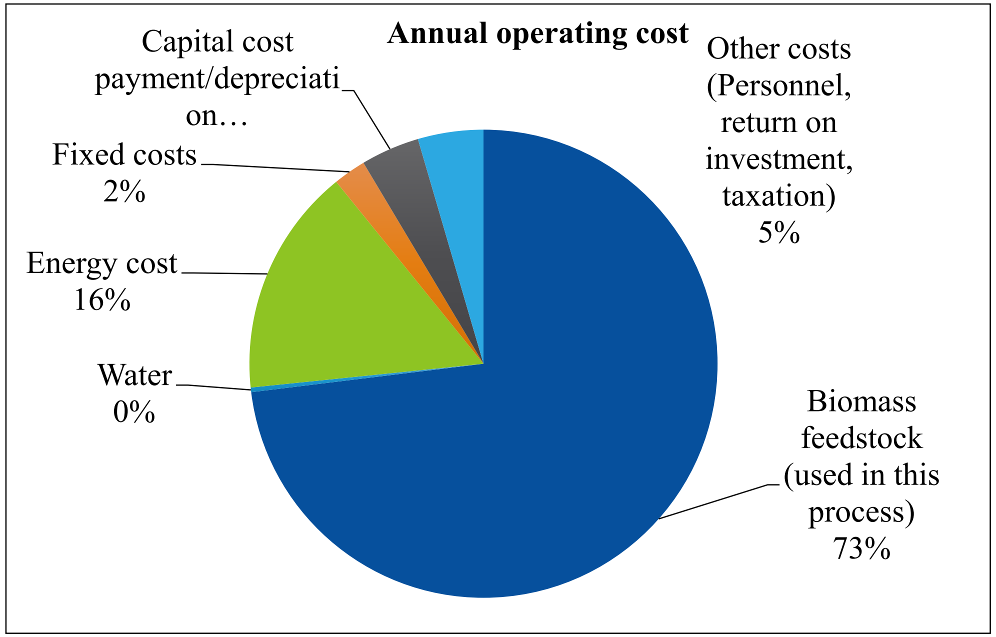
Goal: Develop techno-economic model to quantify costs of a **membrane-based separation process** to recover organic acids from stored algae biomass

Progress: Developed initial TEA model, and evaluated the **recovery of organic acids** from stored biomass containing 7% succinic acid (wt%, dry basis)



TEA Process flow diagram ▲

◀Breakdown of annual operating costs



Outcomes: TEA model that quantifies costs, calculates values in terms of MFSP

- Based on lab organic acid recovery data from stored biomass high in succinic, lactic and butyric acids.

Impact: Evaluate organic acid recovery impact on minimum biomass selling price (MBSP, FY21 Q2 Go/No-Go)

- Future work: Model refinement and sensitivity analysis to guide R&D of storage approach/product recovery

Summary

Overview

- Wet anaerobic storage to mitigate seasonal variation in algae productivity to reduce the risk of feedstock loss prior to conversion
- Storage period can be used to add value to algae biomass without affecting final fuel yield or quality

Management

- Collaborate with production and conversion researcher to ensure wet storage is industrially relevant

Approach

- Stability research addresses seasonal variability in production and provides opportunities to add value to algae biomass prior to conversion
- Gain fundamental understanding of post-harvest algae physiology to support cultivation gains

Impact

- Optimization of algae feedstock logistics and handling can assist in meeting or lowering conversion cost targets and can facilitate the integration of algae production and conversion

Progress and Outcomes

- Long-term storage (180d) preserves >90% of algae feedstock and decreases fuel costs by as much as \$0.42 GGE^{-1} without impacting fuel yield or quality
- Citric acid treatment enhances stability of difficult to preserve cultures by modifying the microbial community, reducing dry matter loss in queueing (24hr) and long-term storage (>28 days).

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Quad Chart Overview

Timeline
- Project start date: 04/01/2015
- Project end date: 9/30/2022

	FY20	Active Project
DOE Funding	$600K	$1,800K

Project Partners
- **NREL**: 1.3.4.201 – CAP process research;
  1.3.4.201 – Algal biofuels techno-economic analysis
- **PNNL**: 1.3.4.101 – Thermochemical interface;
  1.3.2.501 – DISCOVR
- **Arizona State University (AzCATI)**

Barriers addressed
Aft-F – Algae Storage Systems

Aft-G – Algal Feedstock Material Properties

Project Goal
Use feedstock supply chain logistics to enable cost-effective, consistent, high-quality biomass supply for a biorefinery

End of Project Milestone
FY19: A 6-month wet stabilization approach that limits losses to 10% for harvested microalgae, reducing costs and energy consumption compared to drying and dry storage

FY22: Solve the problem of post-harvest biomass instability by developing robust queuing and long-term stabilization approaches for outdoor cultivated microalgae in freshwater and saltwater that limit losses and even improve biomass quality to increase MBSP by 15% (stretch goal of 20%) to enable $2.50/gge biofuels

Funding Mechanism
Annual Operating Plan (AOP).

Additional Slides

Milestone Table

Type	Milestone Description	End Date
Regular Quarterly Milestone	Evaluate recovery strategy for organic acids that are produced in post-harvest biomass to obtain value-added product. Report on potential market size and value of storage-produced metabolites.	3/31/2021
Go/No-Go Milestone	Obtain 10% reduction in MBSP through a combination of any of the following storage degradation products: (1) CO_2, (2) acid-containing biomass provided to conversion, or (3) capture of acid stream in storage and delivery of low acid biomass to conversion.	3/31/2021
Regular Quarterly Milestone	Assess the proteome in 2 stored algae samples cultivated in outdoor ponds to understand the functional metabolism of microbial communities associated with well-preserved algae biomass and degraded algae biomass.	6/30/2021
Annual Milestone	Assess post-harvest stability in 2-3 advanced cultivation approaches in collaboration with partner labs. Achieve 10% or lower dry matter loss over 30 days.	9/30/2021
End of Project Milestone	Solve problem of post-harvest instability by developing a robust queuing and long-term stabilization approach for outdoor-cultivated microalgae in freshwater and saltwater that limits losses to 10% or improves biomass value by 15% (stretch 20%) in order to enable $2.50/gge biofuels.	9/30/2022

Responses to Reviewers' Comments

Wet storage of algae biomass is a novel concept that needs to be further explained. This project has made great progress in addressing many questions, but it is lacking additional results from large scale harvests. Their collaboration with AzCATI should be expanded to include multiple strains grown in different seasons to see if there is a variability. The data already is showing significant variability from different strains, further evaluation of seasons and how growing conditions effect the storage will be also important.

- We have been collaborating with AzCATI since Spring 2018 on assessing the storage stability and ash content of multiple strains grown outdoors from each season, and will continue this effort to understand strain to strain and seaonal variability and the fundamental aspects of preservation through wet storage with a goal to make wet storage approaches universally applicable.

4 – Progress and Outcomes – Stored biomass conversion

Goal: Determine impact of storage of 20% solids microalgae on yield in biochemical conversion process

Progress: *S. acutus* biomass (20% solids) was stored at 100 mL scale for pretreatment screening and 1 L for fermentation studies conducted at NREL

Outcomes: *S. acutus* biomass in both 100 mL and 1 L reactors were well ensiled after 30-day storage period

- For 1 L – low dry matter loss, minimal organic acids, and low pH
- Stored biomass was representative of well-preserved biomass for conversion studies

Scale	Dry Matter Loss	pH	Total organic acids
100 mL	8.8 ± 1.0%	3.8 ± 0.1	8.0 ± 0.1%
1 L	3.5 ± 0.1%	4.3 ± 0.1	6.5 ± 0.3%

4 – Progress and Outcomes – Stored biomass conversion

Goal: Determine impact of storage of 20% solids microalgae on yield in biochemical conversion process

Progress: *Clostridium butyricum* fermentation

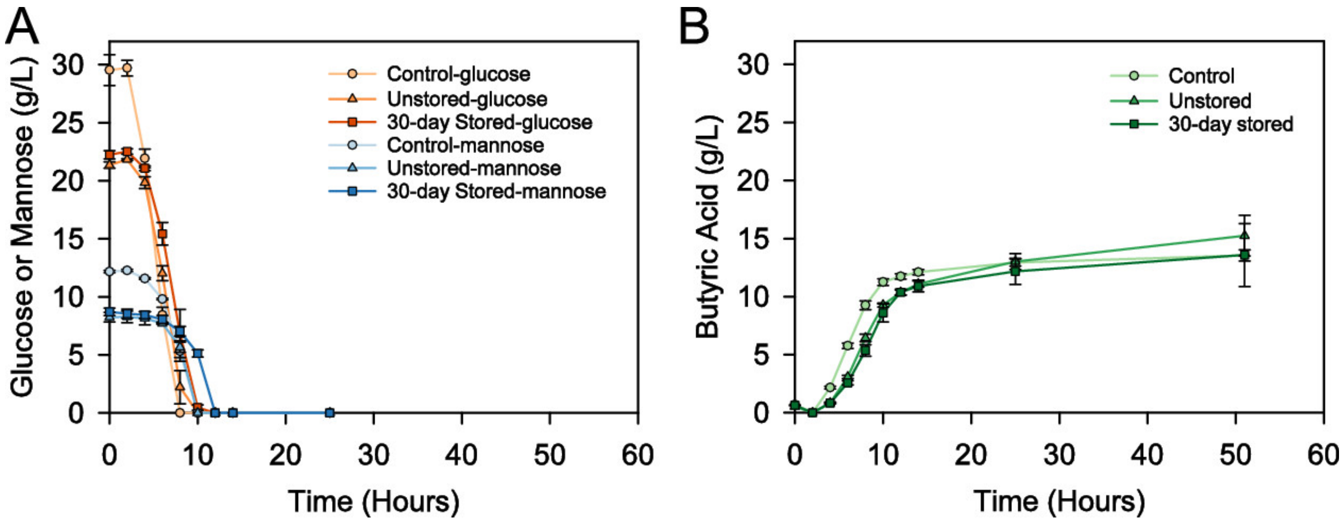
- Butyric acid production from unstored and stored algae hydrolysate compared with glucose media control

Outcomes: Conversion of 30-day stored *S. acutus* biomass compared favorably to unstored and media control

- Similar yield and identical productivity
- FAME recovery greater for 30-day stored

Impact: Storage does not affect butyric acid yield or productivity and **enhances lipid recovery**

Fermentation Feedstock	Yield (g butyric acid/ g sugar)	Productivity (g/L h)	Total FAME recovery
Media control	0.32 ± 0.01	0.98 ± 0.02	NA
Algae unstored	0.47 ± 0.05	0.86 ± 0.02	79.6 ± 2.0%
Algae 30-day stored	0.38 ± 0.08	0.86 ± 0.02	88.8 ± 1.7%



Glucose and mannose consumption (A) and butyric acid production (B) in control media and with unstored and 30-day stored *S. acutus* hydrolysate. DOI: (10.1021/acssuschemeng.0c03790)

4 – Progress and Outcomes – Long-term Storage – Outdoor Cultivation

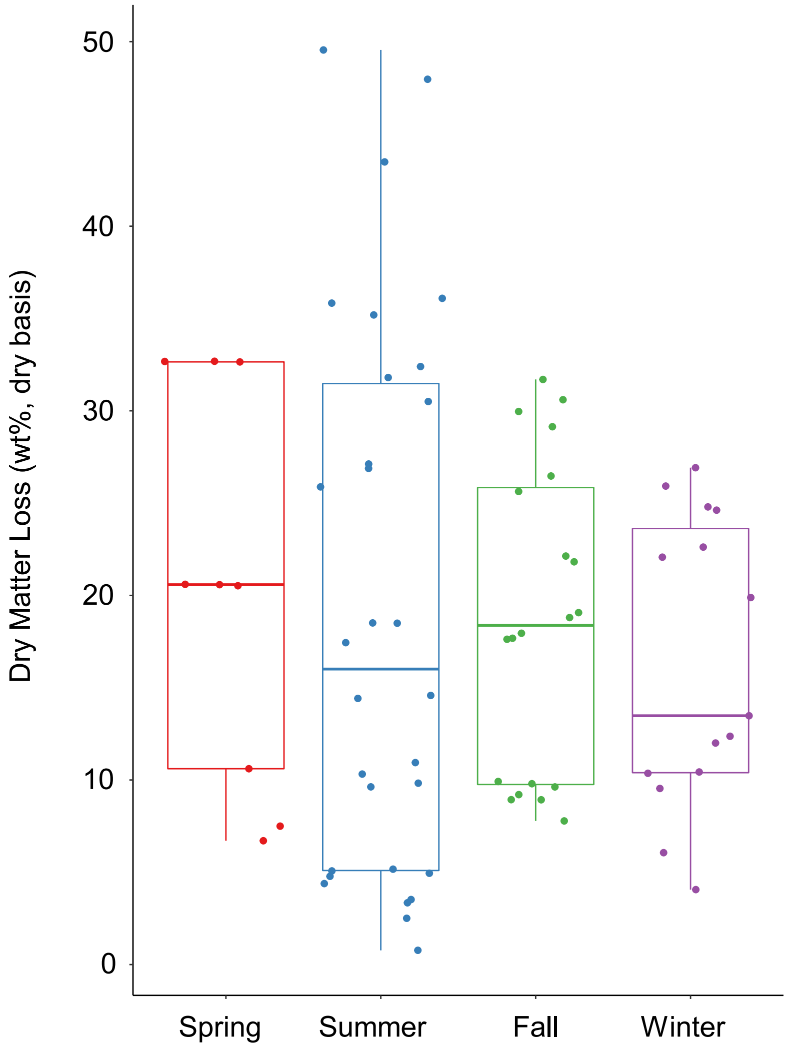
Goal: Assess stability of strains from DISCOVR SOT effort and novel cultivation approaches, ensure **relevance to industry**

Progress: Assessed stability of 11 unique strains from 20 unique cultivations across all 4 seasons

Outcomes: Growth season affect stability?

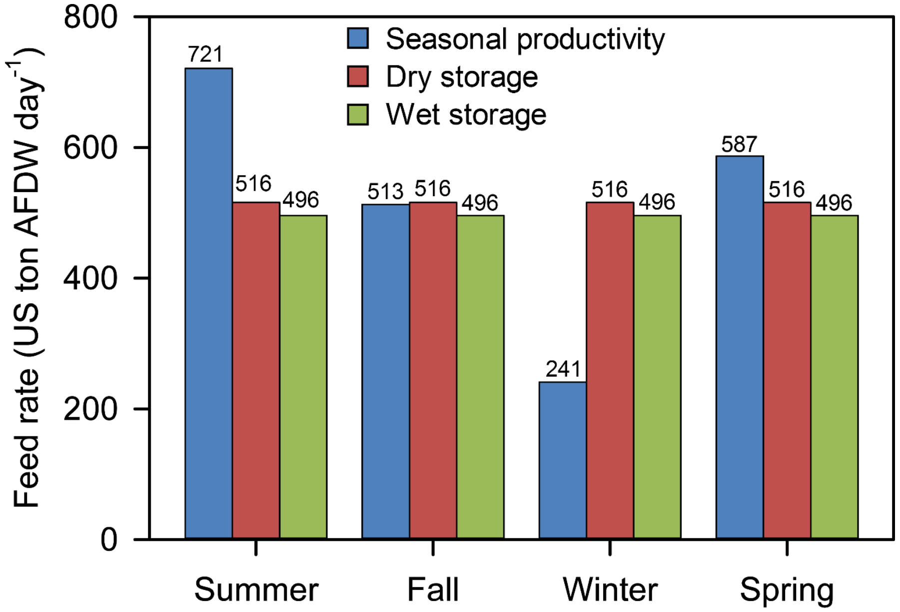
- No difference in mean dry matter loss of untreated, stored biomass when cultivated in different seasons, p=0.087

Impact: Biomass cultivated in spring, summer or fall can be stored using the same approach



Project Overview

- Project Goals
  - 2019: Develop a wet storage approach that **reduces costs relative to drying** and dry storage and **preserve 90% of biomass** in long-term storage (180 days).
  - 2022: Develop a robust long-term stabilization approach for outdoor-cultivated microalgae; limit losses to 10% or **improve biomass value by 15%** in order to enable \$2.50 GGE^{-1} biofuels
  - 2022: Queuing studies to minimize losses between harvest and conversion
- Long-term storage needed to mitigate seasonal variability in production: maximize conversion efficiency
- Drying, state-of-the-art method of preservation, is energy intensive and expensive for high moisture algae
- Wet anaerobic storage can be used to preserve algae biomass for long-term stability to **manage seasonal variation** and **add value** to biomass
- Short-term stability: **enable queuing**, as a **tool to understand mechanism** of degradation and physiology
  - Better understanding to inform crop protection and nighttime losses in cultivation



Seasonal productivity of algae growth

Wendt, Kinchin et al., 2019 *Biotechnol Biofuels*, 12:80

4 – Progress and Outcomes – Stored biomass conversion

Goal: Determine impact of storage of 20% solids microalgae on yield in biochemical conversion process

Progress: Sugar and FAME yield was measured for *S. acutus* biomass without storage and after 30-day storage.

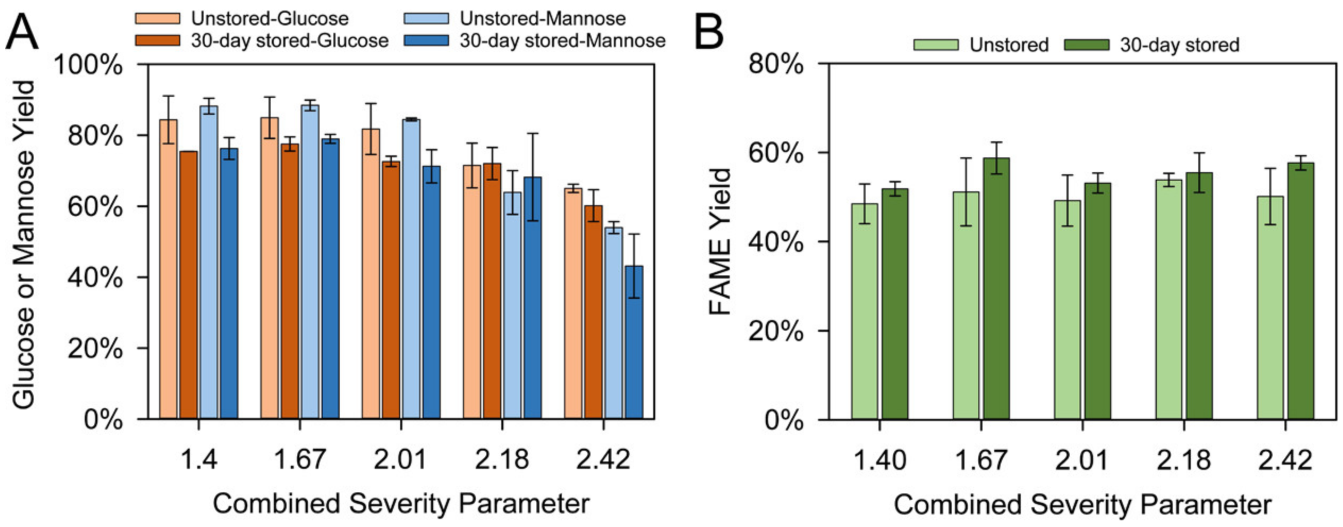
- Screened at multiple severity levels

Outcomes: Comparison of sugar release and lipid (FAME) recovery from fresh and 30-day stored *S. acutus* biomass

- **Similar sugar yield** (slightly decreased in stored biomass) and **FAME yield** (slightly increased in stored biomass)

Impact: Similar pre-treatment yields demonstrate the compatibility of storage with conversion

Biomass	FAME (lipids)	Carbo-hydrates	Protein	Mass Balance
Unstored	35.7	30.0	9.7	77.5
30-day Stored	35.9	28.3	9.9	76.7

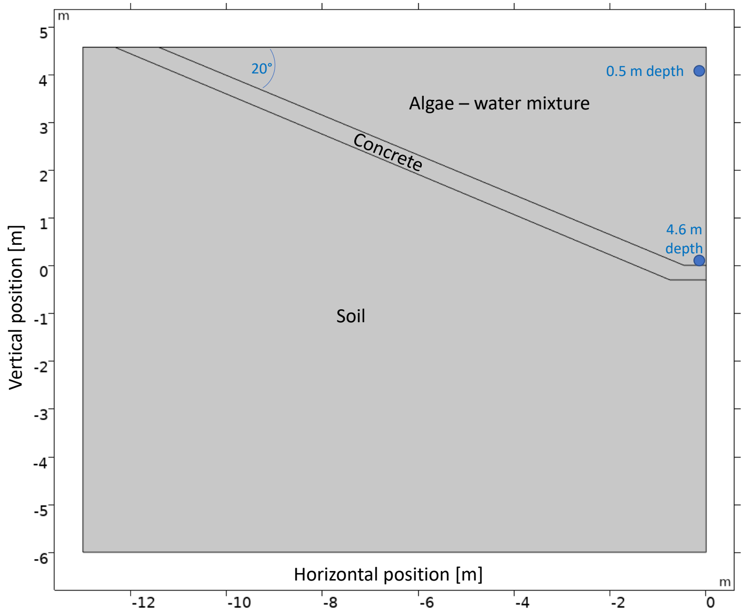


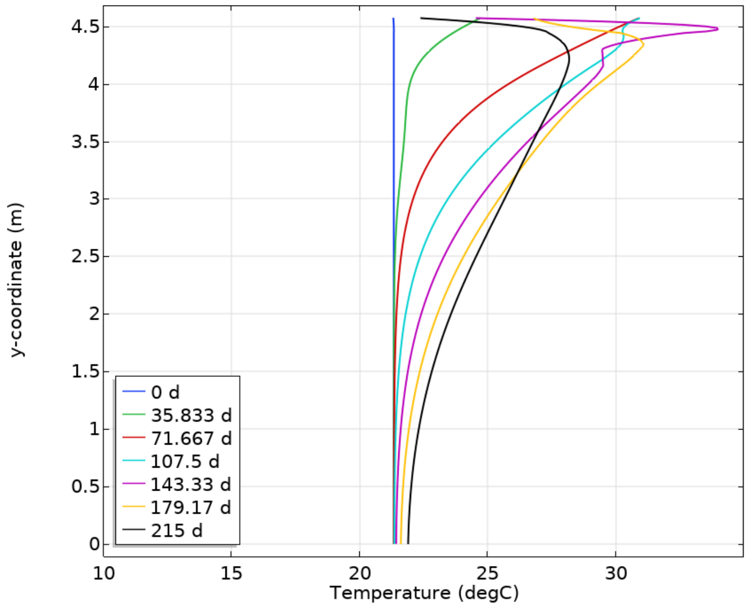
Monomeric glucose and mannose yield (A) and FAME yield (B) at varied combined severity parameters in *S. acutus* biomass that was unstored and after 30 days of wet, anaerobic storage. DOI: (10.1021/acssuschemeng.0c03790)

4 – Progress and Outcomes – Physical Storage Model

Goal: Model algae storage at commercially relevant scale with lab biomass stability data at temperatures expected at algae cultivation sites

Progress: Initial model constructed, considers ambient temperature, solar irradiation, wind speeds and soil temperature





Model domain – in ground algae ▶ storage

◀Simulated temperature profile from April to November at ~36 day intervals

Outcomes: Model can estimate time-dependent dry matter loss based on climate inputs, dry matter loss varies by depth due to surface heating

Impact: Model will be used in future studies to estimate storage losses at geographic locations that support algae cultivation

2 - Approach

Challenges

- Microalgae is **metabolically active** at the time of storage. Response to storage may vary by species
- Bacteria in outdoor growth systems can destabilize harvested cultures
- Nutrients in media (e.g. ammonia, phosphate, etc) will be variable and can influence microbial community

Go/No-Go Milestone

- Increase value of microalgae biomass in storage (**decrease MBSP by 10%**), ultimately **reduce the cost of algae biofuels** and contribute to achieving $2.50/GGE fuels

Critical Success Factors

- Cost competitiveness
- Sustainable designs to reduce water and energy requirements
- Peer-reviewed publications and conference presentation
- Application of approaches in industrially-relevant species and multiple end-uses
- Modeling to ensure approach translates to relevant commercial scale

Publications, Patents, Presentations, Awards, and Commercialization

Publications

Wendt, LM, et al. (2020). Anaerobic storage and conversion of microalgal biomass to manage seasonal variation in cultivation. ACS Sus Chem Eng **8**(35): 13310-13317. DOI: 10.1021/acssuschemeng.0c03790

Wahlen, BD, et al. (2020). Preservation of microalgae, lignocellulosic biomass blends by ensiling to enable consistent year-round feedstock supply for thermochemical conversion to biofuels. Front Bioeng Biotechnol **8**(316). DOI: 10.3389/fbioe.2020.00316

Wendt, LM, et al. (2019). Assessing the stability and techno-economic implications for wet storage of harvested microalgae to manage seasonal variability. Biotechnol Biofuels **12**(1): 80. DOI: 10.1186/s13068-019-1420-0

Wahlen, BD, et al. (2019). Mitigation of variable seasonal productivity in algae biomass through blending and ensiling: An assessment of compositional changes in storage. Algal Research **42**: 101584. DOI: 10.1016/j.algal.2019.101584

Hess, D, et al. (2019). Techno-economic analysis of ash removal in biomass harvested from algal turf scrubbers. Biomass Bioenergy **123**: 149-158. DOI: 10.1016/j.biombioe.2019.02.010

Wendt, LM, et al. (2017). Evaluation of a high-moisture stabilization strategy for harvested microalgae blended with herbaceous biomass: Part II — Techno-economic assessment. Algal Research **25**: 558-566. DOI: 10.1016/j.algal.2017.04.015

Wendt, L. M., et al. (2017). Evaluation of a high-moisture stabilization strategy for harvested microalgae blended with herbaceous biomass: Part I— Storage performance. Algal Research **25**: 567-575. DOI: 10.1016/j.algal.2017.05.016

Wahlen, B. D., et al. (2017). Managing variability in algal biomass production through drying and stabilization of feedstock blends. Algal Research **24**: 9-18. DOI: 10.1016/j.algal.2017.03.005

Publications, Patents, Presentations, Awards, and Commercialization

Patents

Wendt LM, Wahlen BD, Li C, inventors; Battelle Energy Alliance LLC, assignee. Methods of preserving a microalgae biomass and a preserved microalgae biomass. United States patent application US 15/495,625. 2018 Oct 25

Presentations

Wendt LM, Wahlen BD. Utilization of post-harvest storage to increase value of algae biomass. 2020 Algae Biomass Summit, held virtually.

Wahlen BD, Wendt LM, You Y, McGowen J. Impact of microbial community on post-harvest algae biomass. 2020, Rules of Life: Complexity in Algal Systems Virtual Summer Symposium.

Wahlen BD, Wendt LM, Dempster T, Gerken H. Compositional changes to *Nannochloropsis gaditana* biomass in long-term wet anaerobic storage. 2019, 9th International Conference on Algal Biomass, Biofuels and Bioproducts, Boulder, CO.

Wendt LM, Wahlen BD, Pienkos P, Nagle N, Kinchin C, Davis R, Knoshaug E, Dong T, Dempster T, Gerken H, Stabilizing algae to provide a consistent, cost-effective feedstock supply for chemicals and fuel production. 2019 Algae Biomass Summit, Orlando, FL.

Wendt LM, Wahlen BD, Fornes B, Dempster T, Gerhken H. Ensiling microalgae: compositional changes as a result of long-term storage. The 8th International Conference on Algal Biomass, Biofuels and Bioproducts, Seattle, WA.

Wendt LM, Wahlen BD, Dempster T, Ogden, KA. Fate of total dry matter and composition in long-term storage of microalgae and herbaceous biomass blends. The 7th International Conference on Algal Biomass, Biofuels and Bioproducts, Miami, FL.