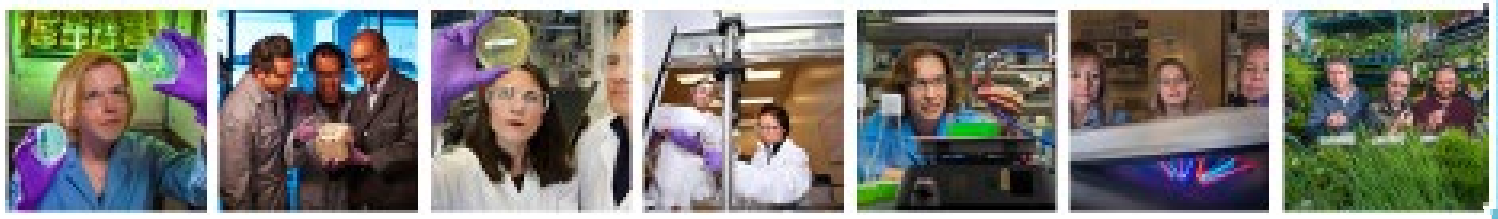
DOE Bioenergy Technologies Office (BETO)
2021 Project Peer Review

Sandia National Laboratories

Leveraging Algae Traits for Fuels (LEAF)



March 24, 2021

Advanced Algal Systems

Amanda N. Barry

Sandia National Laboratories

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SAND2021-1325 PE

Project Overview

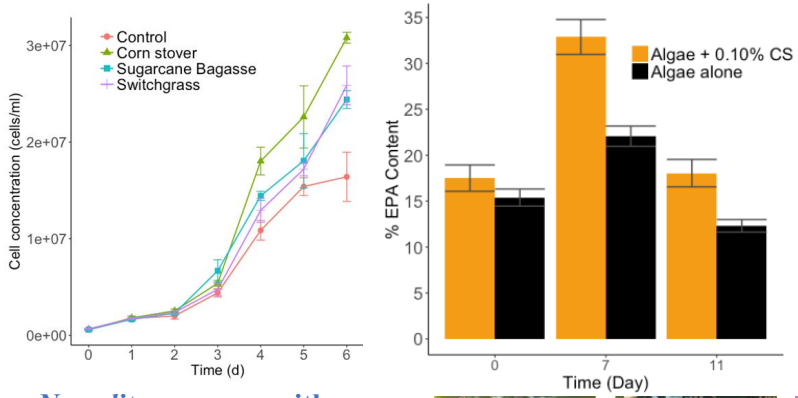
- In 2018, we reported the first example of degradation and utilization of whole native plant substrate (e.g. switchgrass) by a freshwater algae, *Auxenochlorella protothecoides*

- In preliminary work, saw same behavior in *Nannochloropsis* sp. with corn stover, indicating this trait was more widespread than previously known. Biochemical pathway is unknown.

- Partnering with USDA, performed preliminary Scanning Electron Microscopy (SEM) to examine plant substrate with and without algae

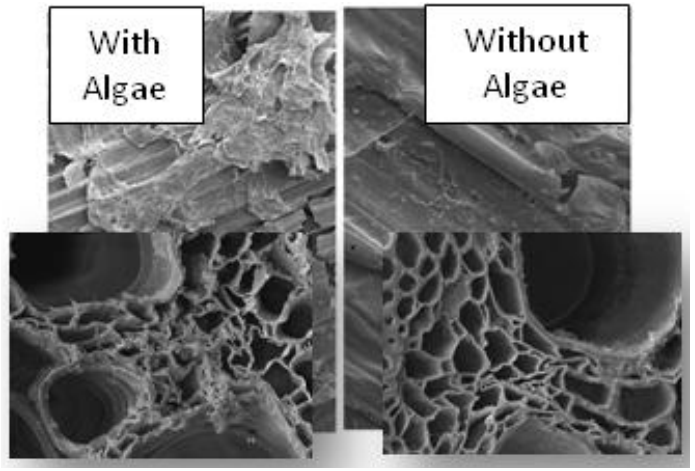
- Technical objectives by the end of the 3-year project:
  - Investigation of *Nannochloropsis* sp., *Picochlorum*, *Scenedesmus* and *Desmodesmus*, strains found to be top contenders for large-scale biofuel production by other groups.
  - Scale up and optimization of mixotrophic growth on plant substrates, demonstrating biomass and/or fuel yield improvement of 15% versus baseline (algae and plant alone).
  - Biochemical pathway mapping of plant substrate utilization in *Nannochloropsis* sp. and *Scenedesmus* sp.
  - Characterization of plant biomass changes after algae growth
  - Microbiome characterization in algae/plant substrate cultivation

Only group investigating biomass improvements with raw plant substrate. Other groups focus on hydrolysate sugars for mixotrophic growth.



***N. gaditana* grown with corn stover increases biomass productivity compared to other plant substrates and algae alone.**

***N. oceanica* grown without (left) and with plants (right) at 50 L scale. Greener color with plants.**



SEM of corn stover with and without algae. Behind: Ripped epidermis and exposed parenchyma beneath in plants with algae. Forefront: Note collapsed cells and degraded cell walls of phloem in the treated plant. Also note the algae cells in the phloem.

I – Management

Los Alamos National Lab [Shawn Starkenburg and Jenna Schambach]– Strain growth (green algae), lipid analyses, and sequencing

Sandia National Labs (PI moved; Year 2-3) [Amanda Barry and Chuck Smallwood]– Strain growth (stramenopiles), biomass tracking, and examining the microbiome

USDA/U.S. Forest Service [Chris Hunt and Peter Kitin] – Plant substrate changes with algae growth

National Renewable Energy Lab [Jake Kruger and Nick Nagle] – Sugar recovery comparison with/without plant utilization

Industry partners:

Qualitas Health [Jake Nalley] – Consultation and scale-up support

Heliae Development LLC [Steven Pflucker] – Consultation and strain supply

Meetings every other week with LANL/SNL/USDA project teams. Frequent communication with NREL with timely delivery of samples.

Consultation with industry partners with regular data presentation.

I – Management - Task Structure

Task 1: Scale up and optimization of mixotrophic growth on plant substrates for several strains of microalgae, demonstrating biomass and/or fuel yield improvement of 15% versus baseline (algae and plant alone). LANL, SNL

Task 2: Biochemical pathway mapping of plant substrate utilization in algae. LANL, SNL

Task 3: Characterization of plant biomass changes after algae growth. USDA

Task 4: Microbiome characterization in algae/plant substrate cultivation. SNL, LANL

DISCOVR Project Integration

Screen DISCOVR strains for plant substrate utilization at flask scale. SNL

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I – Management - Project Risks & Mitigation

Name	Severity	Mitigation Response	Description
Fewer than 2 new strains will utilize plant substrates	Low	Focus on known strains (Auxenochlorella and Nannochloropsis) in future studies in microbiome and pathway mapping	This would potentially affect our Annual SMART milestone, as we would have <3 strains with improved growth on plant substrate.
Productivity benefits observed with mixotrophic growth muted in presence of CO_2 (industry standard)	High	Test with and without CO_2 delivery to cultures at multiple scales.	This would potentially impact the end of project goal: Demonstrated biomass and/or fuel yield improvement of 15% versus baseline (algae and plant alone) in 700 L and larger ponds at Qualitas Health industrial site
Pandemic causes limited access to labs and delayed progress	High	Prioritize experiments to productivity goals forward.	This would potentially impact timely sequencing, plant substrate analyses, and sugar analyses.

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2 – Approach

Strains and plant substrates to examine:

- *Nannochloropsis* sp. (multiple *gaditana* and *oceanica* strains), *Picochlorum*, *Scenedesmus* and *Desmodesmus*
- From Idaho National Lab Feedstock Library, corn stover, switchgrass, sugarcane bagasse
- From the Los Alamos EcoStation, yard waste (grass clippings and mulch)
- From USDA partner, stem and leaf sections

Determining productivity:

- Multiple scales
- Calculate growth rates by cell count and ash-free dry weight (AFDW)
- Quantify total lipids and lipid content by FAME
- Measure sugar recovery and yield with NREL protocols

Biochemical pathways involved in utilization:

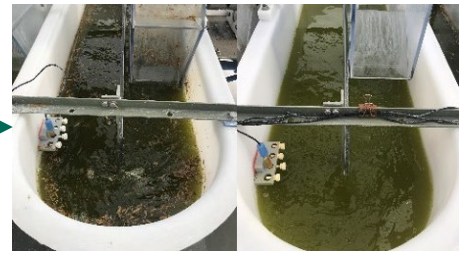
- ^{13}C plant label tracking experiments
- Transcriptomic sampling at multiple timepoints

Effects on plant substrate:

- Examine plant structure with SEM
- Total sugar analyses before and after algae growth

Role of the microbiome:

- 16S analyses at multiple timepoints
- ^{13}C plant label tracking experiments



QUALITAS HEALTH

Year 1 Year 2 Year 3

2 – Approach

Examine growth at multiple scales to meet productivity targets.

Flask experiments:

- Calculate growth rates of *Nannochloropsis* sp. (multiple *gaditana* and *oceanica* strains), *Picochlorum celeri*, *Scenedesmus,* and *Desmodesmus* with corn stover, switchgrass, sugarcane bagasse, and yard waste by cell counts
- Quantify total lipids and lipid content by FAME
- With top performing substrates, examine growth in the presence of CO_2
- ^{13}C plant label tracking experiments
- Sample for transcriptomic and microbiome analyses
- Send samples to USDA for SEM and total sugar analyses

50 L greenhouse raceway ponds:

- Examine top performing substrates and strains with and without CO_2
- Sample for transcriptomic and microbiome analyses
- Quantify total lipids and lipid content by FAME
- Send samples to NREL for sugar recovery comparison

700 L Qualitas ponds:

- Examine Qualitas strain with top performing plant substrate under usual industrial conditions

- **End of project goal:** Demonstrated biomass and/or fuel yield improvement of 15% versus baseline (algae and plant alone) in 700 L and larger ponds at Qualitas Health industrial site; Biochemical pathway mapping of plant substrate utilization in algae.

- **Go/No-Go (12/31/2020):** Demonstrated biomass and/or lipid yield improvement of 15% versus baseline (algae and plant alone) at 50 L minipond scale with at least 1 strain; Initial TEA of mixotrophic algae growth with plant substrate for fuel production shows an improvement over phototrophic growth alone.

3 – Impact

- If productivity targets are met, strategy could be employed immediately at industrial scale.
- Strains and plant substrates are selected for their industry relevance.
- Examination of productivity (both biomass and lipid) at multiple scales ensures relevance.
- Communication and consultation with Heliae and Qualitas Health, two companies with very different cultivation methodologies, throughout the project maintain our responsiveness.

Biology discoveries have impacts across the field:

- Redefining strain biology
- Microbiome dynamics
- Mapping alternative carbon substrate utilization
- Carbon storage under mixotrophic conditions
- Methodology for examining novel strategies at multiple scales

4 – Progress and Outcomes

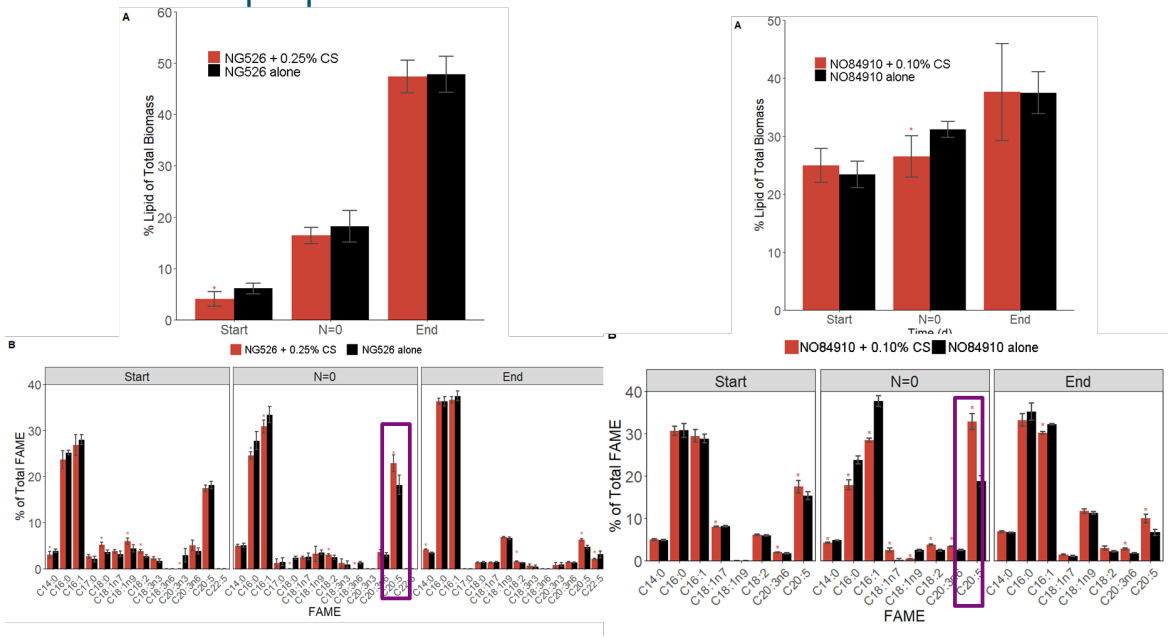
Major findings:

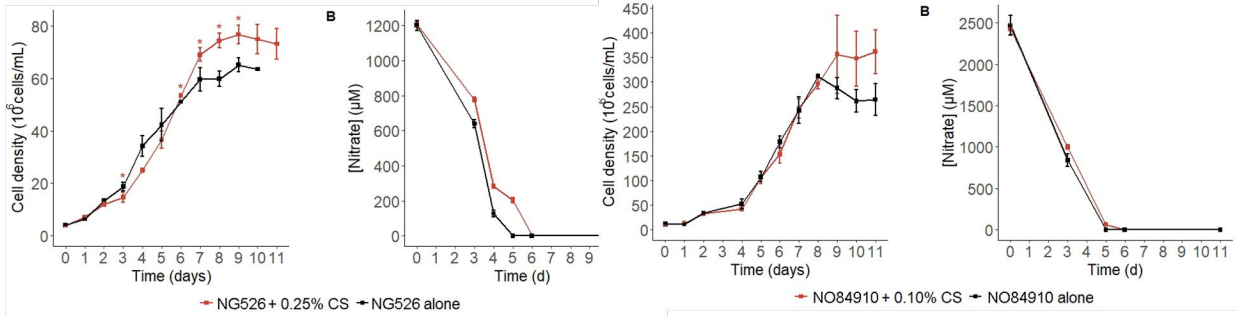
Nannochloropsis sp., *Scenedesmus*, and *Desmodesmus* all indicate biomass and/or lipid improvement with plant substrate addition at flask scale with one or more plant substrates tested. *Picochlorum* did not show improvement.

Scale-up of multiple *Nannochloropsis* strains showed mixed performance in 50 L raceways.

- With CO_2 input, biomass improvements are less significant than observed in flasks, while lipid improvements are generally maintained (increase in coproduct EPA); cultures appear greener in color over time
- AFDW of algae alone could not be calculated accurately due to algae colonization of the plant material even after washing.

Mini pond experiments show no difference in total lipids but a shift in the lipid profile at N starvation





Mini raceway outdoor pond experiments demonstrate improved growth for *N. gaditana* with plant substrates with a delay in N starvation

N. oceanica shows no difference with plant substrates at mini raceway pond scale

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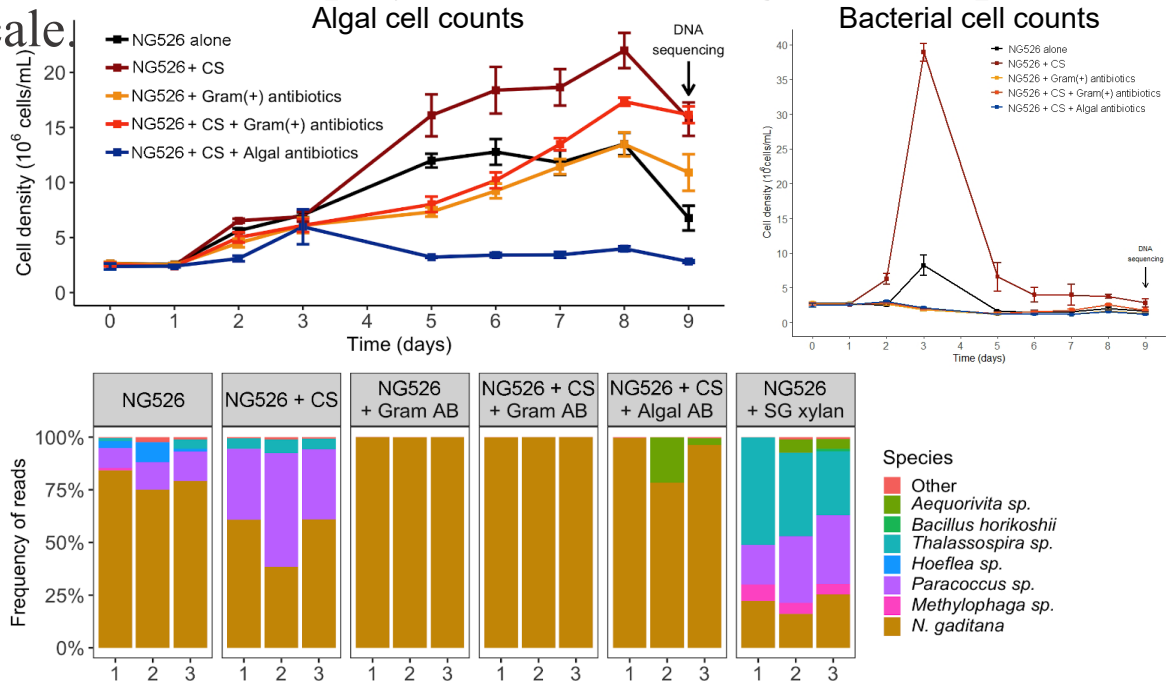
Microbiome dynamics indicate microbes play a role in initial growth improvements, but are not necessary for plant utilization at flask scale.



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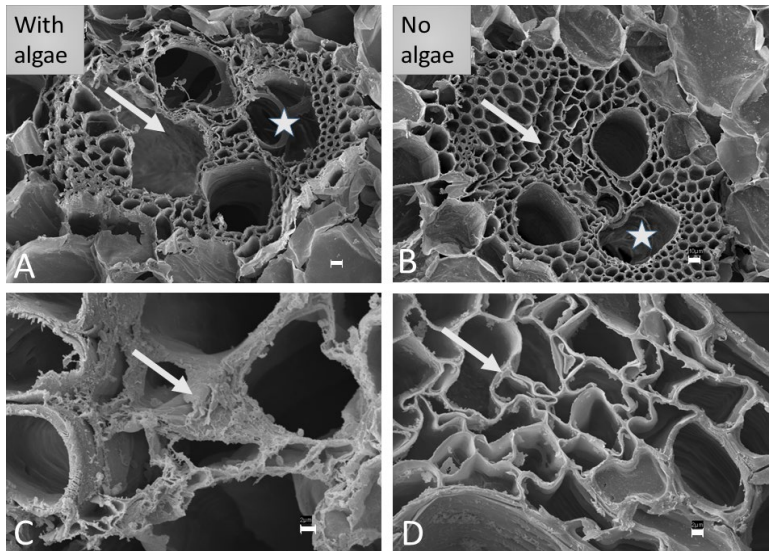
Plant substrate analysis after algae growth show structural and sugar content changes when compared to plant in media alone.



	Arabinan		Galactan		Glucan		Xylan		Mannan	
	Avg	95% C.I.	Avg	95% C.I.	Avg	95% C.I.	Avg	95% C.I.	Avg	95% C.I.
NG526 alone	0.0	N/A	54.6	18.6	49.1	37.3	9.0	8.6	16.0	11.4
CS alone	30.9	19.9	55.3	43.8	109.5	30.1	59.2	30.1	0.0	N/A
Sum	31.0	19.9	110.0	37.5	159.0	31.4	68.0	27.4	16.0	11.4
NG526 + CS	29.5	16.9	100.9	64.7	78.5	11.5	44.0	18.3	20.2	43.8
p value	0.797		0.656		0.002		0.051		0.716	

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Plant substrate analysis after algae growth show structural and sugar content changes when compared to plant in media alone.

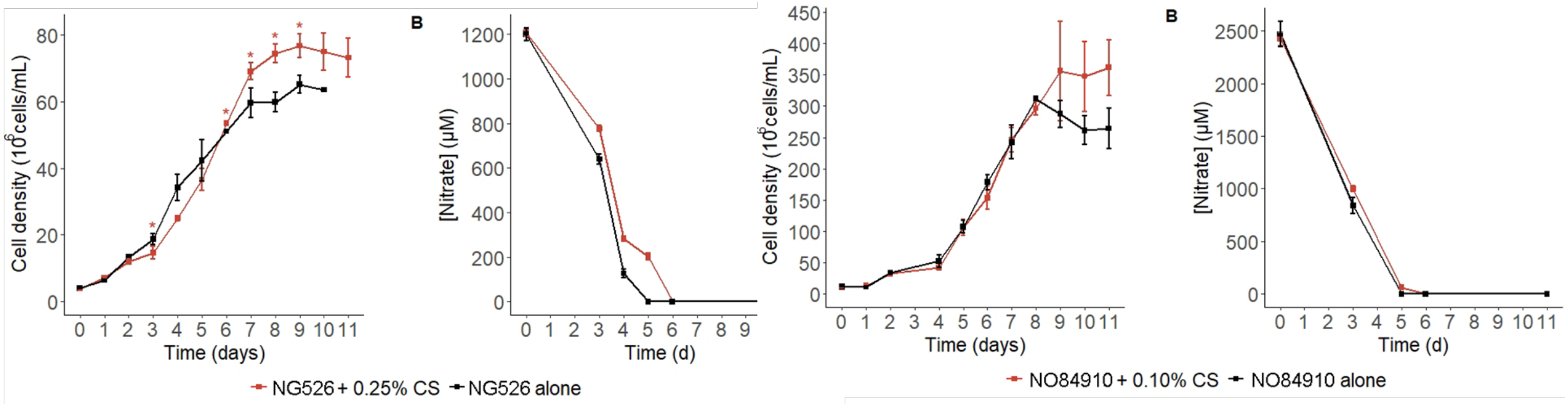
Transcriptomic sequencing, plant structure analyses in *Scenedesmus* cultures, and sugar recovery delayed due to COVID-19. Expect to be on schedule by end of fiscal year.

4 – Progress and Outcomes

Go/No-Go (12/31/2020): Demonstrated biomass and/or lipid yield improvement of 15% versus baseline (algae and plant alone) at 50 L minipond scale with at least 1 strain; TEA of mixotrophic algae growth with plant substrate for fuel production shows an improvement over phototrophic growth alone.

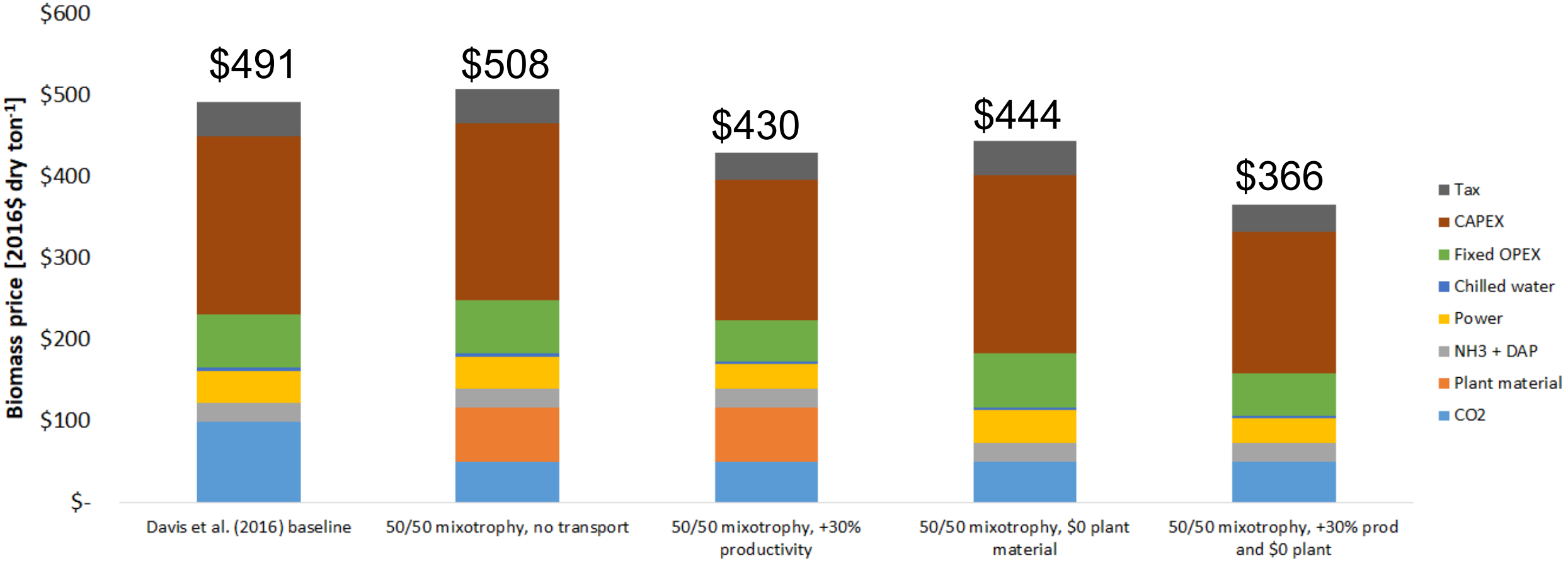
At 50 L scale with CO_2, the average specific growth rate for N. gaditana *cultures with corn stover (0.39 ± 0.02) was* ***30% greater than those grown without*** *(0.27 ± 0.02). This was statistically significant (p=0.0021). The average specific growth rate for* N. oceanica *cultures with corn stover (0.48 ± .04) was only 8% greater than those grown without (0.44 ± 0.04).*

*50 L experiments with other strains currently underway.



4 – Progress and Outcomes

Go/No-Go (12/31/2020): Demonstrated biomass and/or lipid yield improvement of 15% versus baseline (algae and plant alone) at 50 L minipond scale with at least 1 strain; **TEA of mixotrophic algae growth with plant substrate for fuel production shows an improvement over phototrophic growth alone.**



(no plant biomass logistics costs [storage, unloading, grinding, etc.])

Peter Chen, CSU

Summary

- Plant substrate addition to some algae strains (not all!) can result in improved biomass and/or lipid production.
- Productivity improvements can vary across scales.
- Algae utilize specific carbon substrates within the plant matter.
- The microbiome may play a role in substrate accessibility.
- Tracking the plant substrate carbon and transcriptomics will give a better understanding of algae biology and biochemistry.
- Industry partnerships are important to show technology applicability and relevance at large scales.

- Project is on-track with major milestones and should be up-to-date after pandemic delays by the end of FY21.

Quad Chart Overview (AOP Project)

Timeline

- October 1, 2019
- September 30, 2022

	FY20	Active Project
DOE Funding	$350,000 (LANL/SNL) $60,000 to USDA (for all 3 years at $20K/year)	$1,110,000

Project Partners*

- SNL/LANL
- USDA
- NREL
- Qualitas Health
- Heliae

Project Goal

The goal of this project is to design, develop, and understand mixotrophic growth cultivation with plant substrates for industrially relevant algae strains to improve biomass and lipid productivity.

End of Project Milestone

- Demonstrated biomass and/or fuel yield improvement of 15% versus baseline (algae and plant alone) in 700 L and larger ponds at Qualitas Health industrial site; Biochemical pathway mapping of plant substrate utilization in algae.

Barriers addressed

Aft-B. Sustainable Algae Production
Aft-C. Biomass Genetics and Development

Funding Mechanism

AOP USDA/BETO partnership

*Only fill out if applicable.

Additional Slides

Responses to Previous Reviewers' Comments

- Project has not been presented at BETO Peer Review previously.
- Proposal review in June, 2019

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

Publications:

Schambach, J., Finck, A., Kitin, P., Hunt, C., Hanschen, E., Starkenburg, S., Vogler, B.W., and Barry, A.N. 2020. Growth, total lipid, and omega-3 fatty acid production by Nannochloropsis gaditana cultivated with raw plant substrate. Algal Research 51:102041.

Project presentations:

Chen, P. Mixotrophic algae cultivation: Economics and life-cycle sustainability, 2020 LANL Virtual Student Symposium and CSU Graduate Student Showcase. **Won $500 prize for the "Drivers of Innovation" award.**

Barry, A.N. Turning Over a New Leaf: Cultivation of Multiple Algae Strains for Biofuels and Bioproducts with Plant Waste, Algae Biomass Summit, Virtual Conference, August 12-October 2, 2020

Barry, A.N. Tapping into Algal Biodiversity: Plant Carbon Utilization by Diverse Algal Genera, Medicinal Plant Chemistry Group, Northern Michigan University, March 25, 2020

Schambach, J. Investigation of enhanced growth, total lipids, and coproduct production by Nannochloropsis sp. Cultivated with raw plant substrate, Algae Biomass Summit, October, 2019

Commercialization:

Heliae and Qualitas Health have both completed initial tests under their own cultivation systems with plant substrates. Experiments are ongoing. Heliae has a patent pending for a *Scenedesmus* mixotrophic growth process.