# DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

## ACCESS CARBON - Alkaline Carbon Capture and Expression-Streamlined Spirulina Cultivated in Air for Reliable Bioproducts, Oil, and Nutrition

April 3-7, 2023
Technology Area Session

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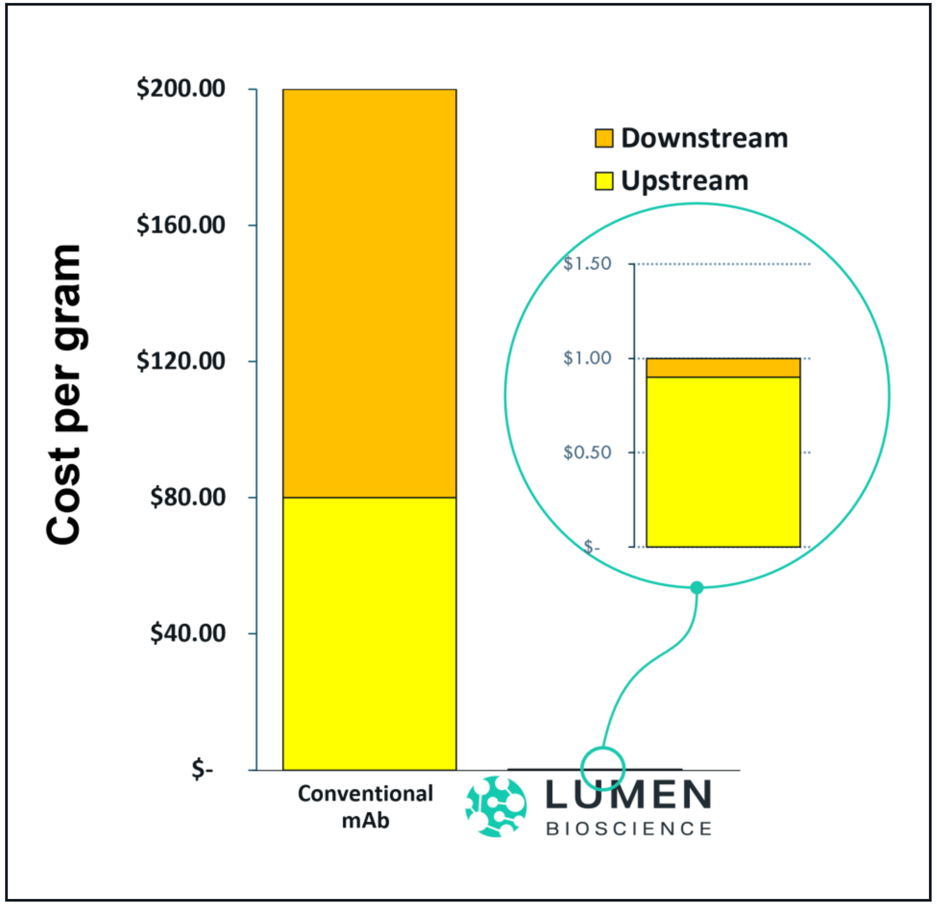
WBS - 1.3.4.008

This presentation does not contain any proprietary, confidential, or otherwise restricted information

# Project overview - Democratizing biologics




*https://www.earthrise.com/ecofriendly-farm*

nature biotechnology

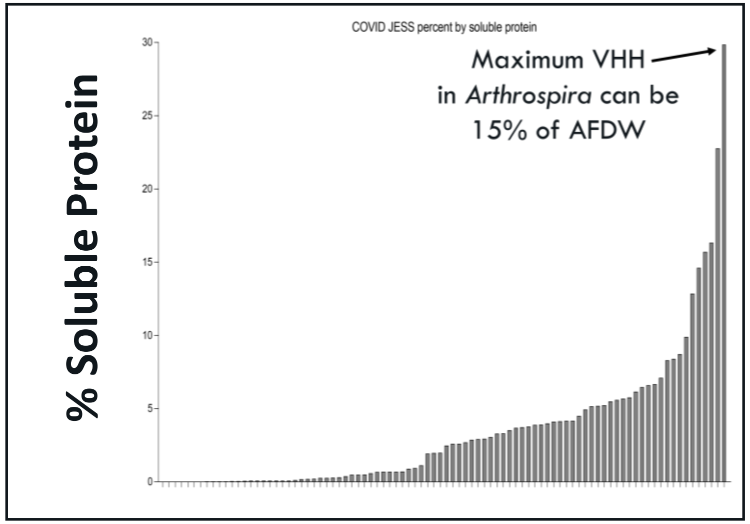
ARTICLES

https://doi.org/10.1038/s41587-022-01249-7

Check for updates

OPEN

**Development of spirulina for the manufacture and oral delivery of protein therapeutics**

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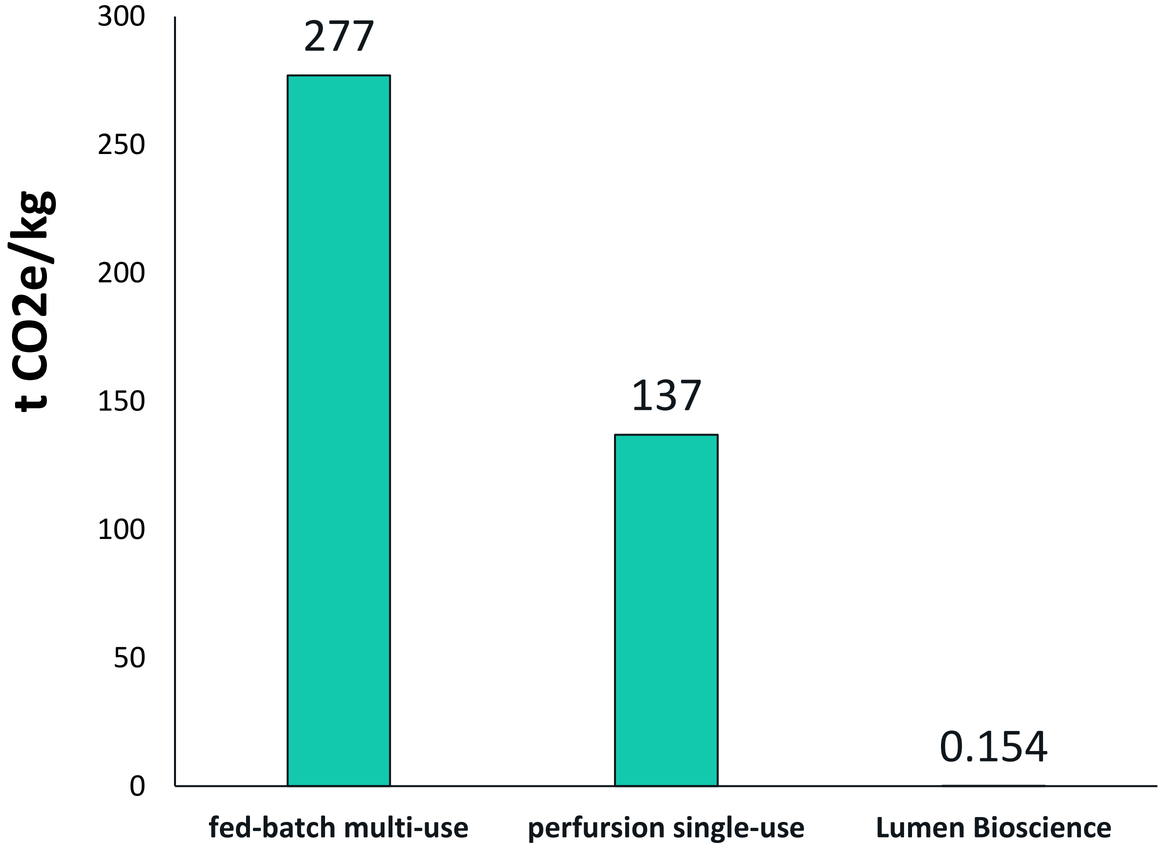
# Project Overview – Lumen's technology dramatically reduces the carbon footprint of antibody production

- Recent publications analyzed the carbon footprint of leading technologies for the production of monoclonal antibodies

- Lumen innovative approach was analyzed through a life-cycle assessment at NREL

- Far less carbon dioxide is produced in Lumen's process

Source: Amasawa et al (2021)
https://doi.org/10.1021/acssuschemeng.1c01435

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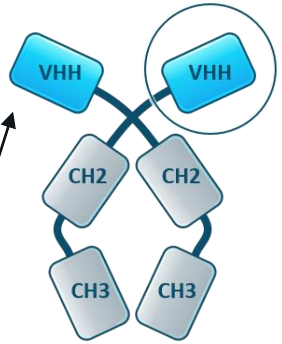
# Approach - Fuels could be a side product of our pharmaceutical approach

Camelid antibody




Camelid heavy-chain antibody (HCAb)

In contrast to the complicated human antibody, a single unit of the camelid antibody (VHH) can be expressed in bacteria including Spirulina

Single-domain VHH "nanobody"


VHHs can be overexpressed in Spirulina

VHH containing Spirulina

Purification

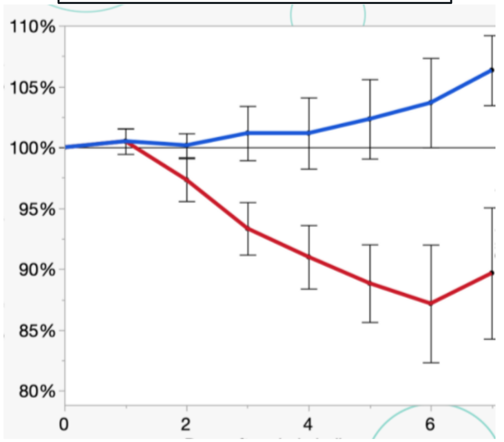

Respiratory Therapeutic

Lipids
Carbohydrates
PHAs

Fuel/Products

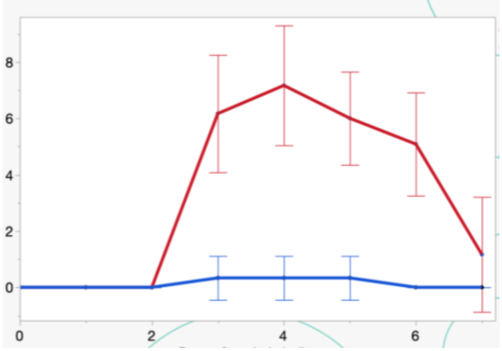
Mouse Trials

Body Weight




Time, days

Clinical Sign




Time, days

Therapeutic
Saline Soln.

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# Approach- How does Lumen fit with BETO mission Statement or the FOA?

**BETO mission statement** - The U.S. Department of Energy's (DOE's) Bioenergy Technologies Office (BETO) develops technologies that convert domestic biomass and other waste resources into fuels, products, and power to enable affordable energy, economic growth, and innovation in renewable energy and chemicals production – the bioeconomy.

**FOA for Algae Bioproducts and CO2 Direct Air Capture Efficiency** - Successful projects will capture $CO_2$ from the air, grow high quality algal biomass suitable for conversion to fuels and products, and develop fuels and/or products made from the algae biomass.

**ACCESS CARBON**– Lumen Bioscience develops strains that produce high-value therapeutics, in some cases this process produces biomass waste materials that could be used as fuel. This project seeks to increase Direct Air Capture for Spirulina, increase biofuel intermediates, Increase therapeutic level, build a CFD framework for scaling and build/apply TEA and LCA metrics. By partnering with the Department of Energy, we can lower $CO_2$ emissions of our process.

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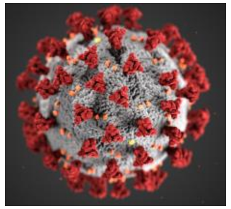
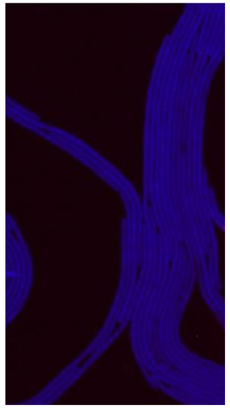
# Approach – The Go/No-Go for this project had six major parts. We have completed or made significant progress on all of them.



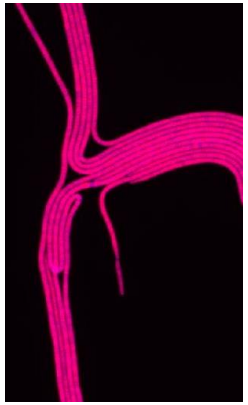
https://www.cdc.gov/

**Increase therapeutic by 50%**

***Arthrospira platensis***



DNA →



**Increase direct air capture by 50%**

*PowerPoint stock photo*

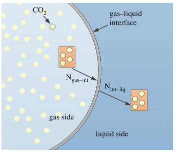
**Increase Biofuel intermediates by 50%**

*PowerPoint stock photo*

**LCA and TEA**


Amasawa et al (2021)

**Use CFD to scale to different dimensions**



$$\frac{dC}{dt} = kLa * (C^* - C)$$

$$\frac{C - C_0}{C^* - C_0} = 1 - \exp(-kLa(t - t_0))$$

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# Approach- Strategies and Risk Mitigation to the Go/No-Go Milestones

| Go/No-Go milestone | Strategy | Risk Identification | Risk Mitigation |
| --- | --- | --- | --- |
| Increase therapeutic by 50% | Optimized through Design of Experiments | Appropriate variables cannot be Identified | Simultaneously pursed genetic and screening strategies |
| Increase Biofuel intermediates by 50% | Nutrient Deprivation | Reduced therapeutic expression | Metabolomic analysis to identify bottlenecks |
| Increase direct air capture by 50% | Machine-learning directed discovery | Chemical mass transfer limitations | Genetic alteration that increase bicarbonate transport |
| Use CFD to scale to different dimensions | Reactor and media formulation specific CFD simulations | Scalability | Change reactor designs in outdoor reactors if necessary |
| Technoeconomic Analysis, Life-cycle assessment | Internal Analysis aligned with NREL's existing models | Unknown process have outsized effects | Anticipate process costs and carbon footprint that hurt economics |

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# Approach- Innovations relative to the state of the art, attempting to make a cleaner and cheaper therapeutic

| Go/No-Go milestone | Current State of the art | Innovation relative to the state of the art |
|---|---|---|
| Increase therapeutic by 50% | Sterile approaches that have high cost and carbon footprint | Therapeutic content increased over 50% |
| Increase Biofuel intermediates by 50% | | Biofuel intermediates increased over 100% |
| Increase direct air capture by 50% | | Capable of growing spirulina in 100% direct air capture system |
| Use CFD to scale to different dimensions | Heterotrophic systems with limited transferability between systems | Toolkit provided that gives optimum production parameters for different systems |
| Technoeconomic Analysis | | Toolkit provided that communicates cost |
| Life-cycle assessment | | Toolkit provided that communicates carbon footprint |

**Management Strategy** – biweekly meeting between NREL and Lumen navigate detailed milestones and Go/No-Go objectives. Monthly meetings with DOE to discuss this progress

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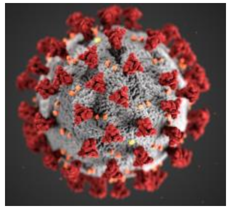
# Approach – The Go/No-Go for this project had six major parts. We have completed or made significant progress on all of them.



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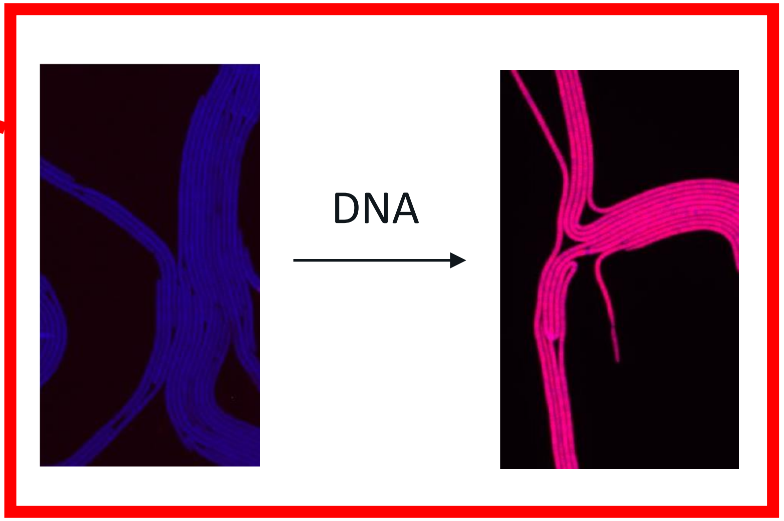


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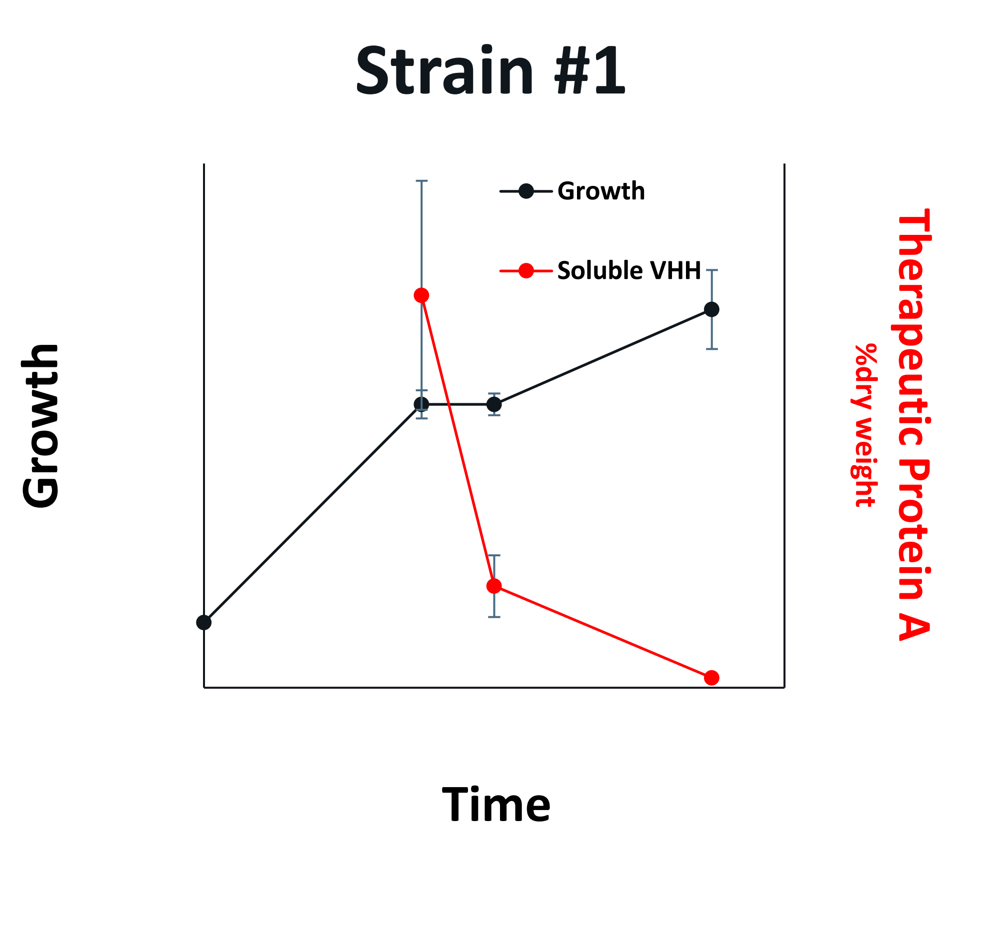
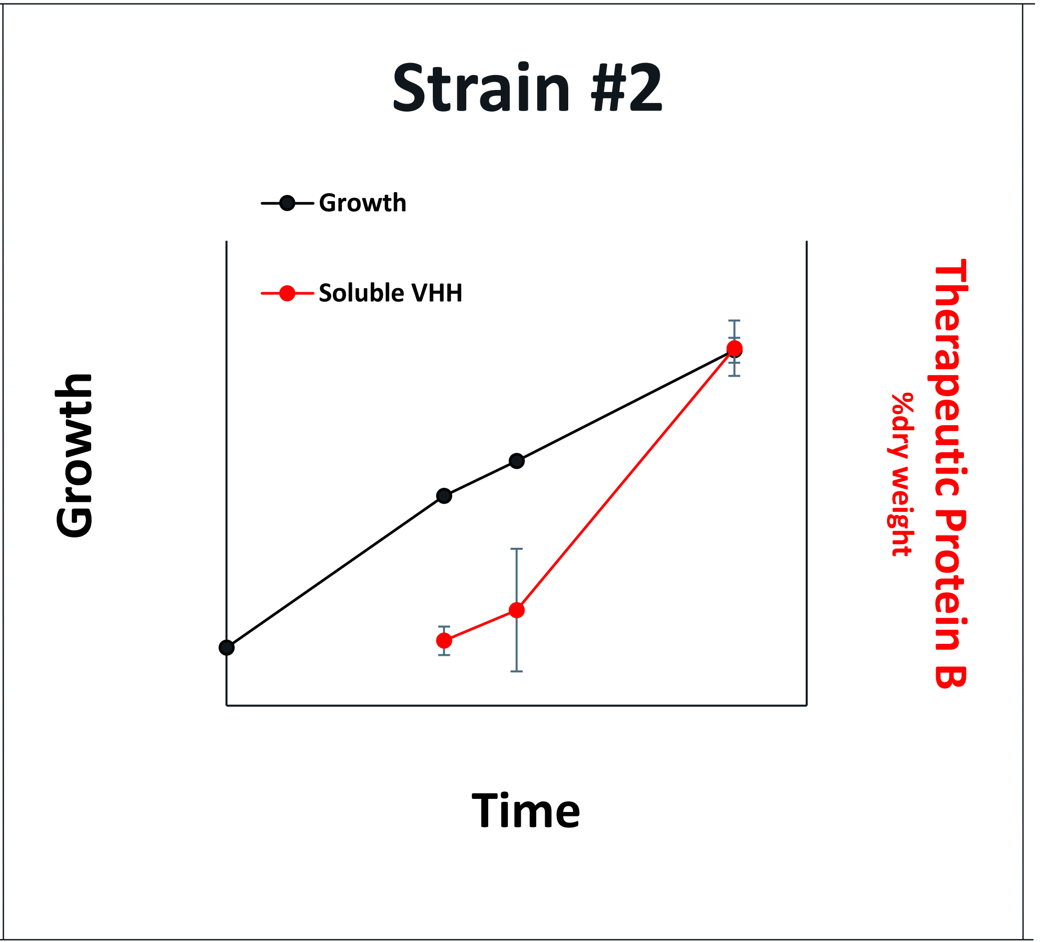
# Progress and Outcomes - Each strain needs a different set of parameters for optimization

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# Progress and Outcomes - Design of experiment approach identified conditions that increase potency

- Lumen has knowledge of conditions that optimize expression and the experimental rigour to detect small changes
- This allows for statistical parameter combinatorial space exploration and optimization
- Optimized parameters produced a 59% improvement in protein expression

| A Parameter (A.U.) | B Parameters (A.U.) | C Parameter (A.U.) | D Parameter (A.U.) |
|---|---|---|---|
| 0 | 1 | 0.5 | 0 |
| 1 | 0 | 0.5 | 1 |
| 1 | 0.5 | 0 | 0 |
| 0 | 0.5 | 1 | 1 |
| 0 | 0 | 0 | 0.5 |
| 1 | 1 | 1 | 0.5 |
| 0.5 | 1 | 0 | 1 |
| 0.5 | 0 | 1 | 0 |
| 0.5 | 0.5 | 0.5 | 0.5 |

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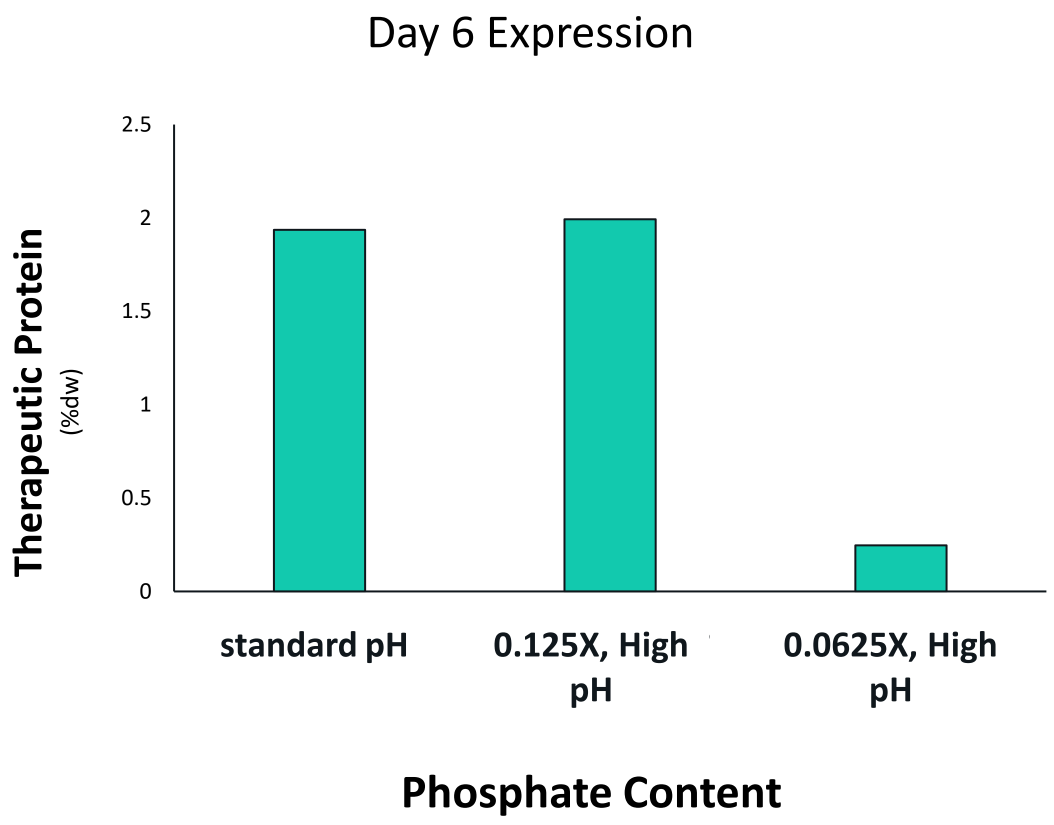
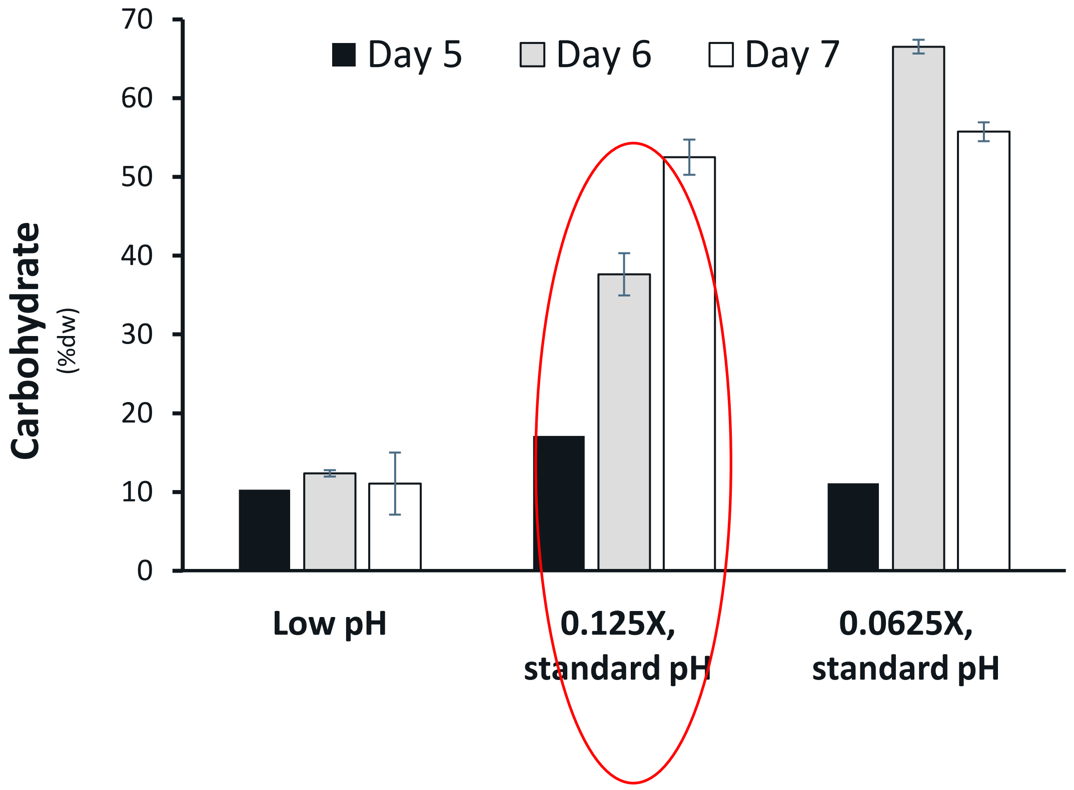
# Progress and Outcomes - Lowering phosphate 8-fold increased carbohydrate without reducing therapeutic production




**On day 6, 0.125X did not decrease growth or therapeutic accumulation**

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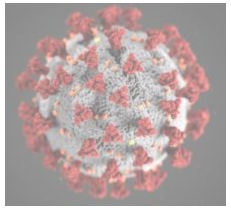

*https://www.cdc.gov/*

Increase therapeutic by 50%

Increased by 50%

Increase Biofuel intermediates by 50%

Increased by 117%

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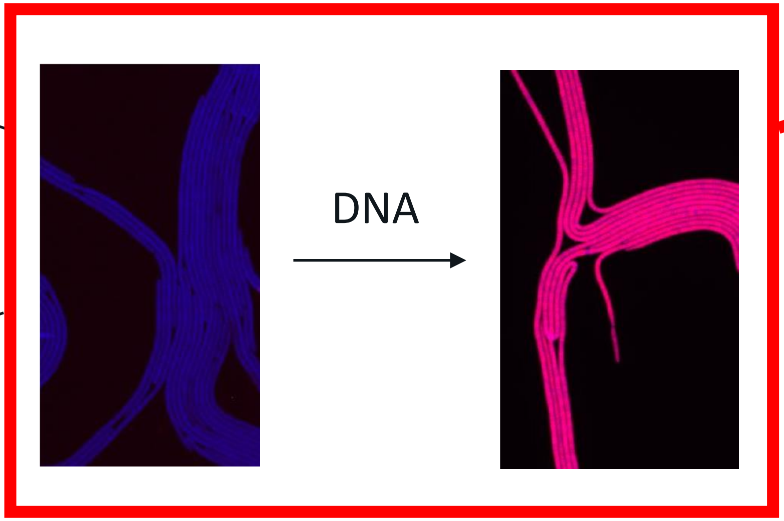
***Arthrospira platensis***




**Increase direct air capture by 50%**

*PowerPoint stock photo*

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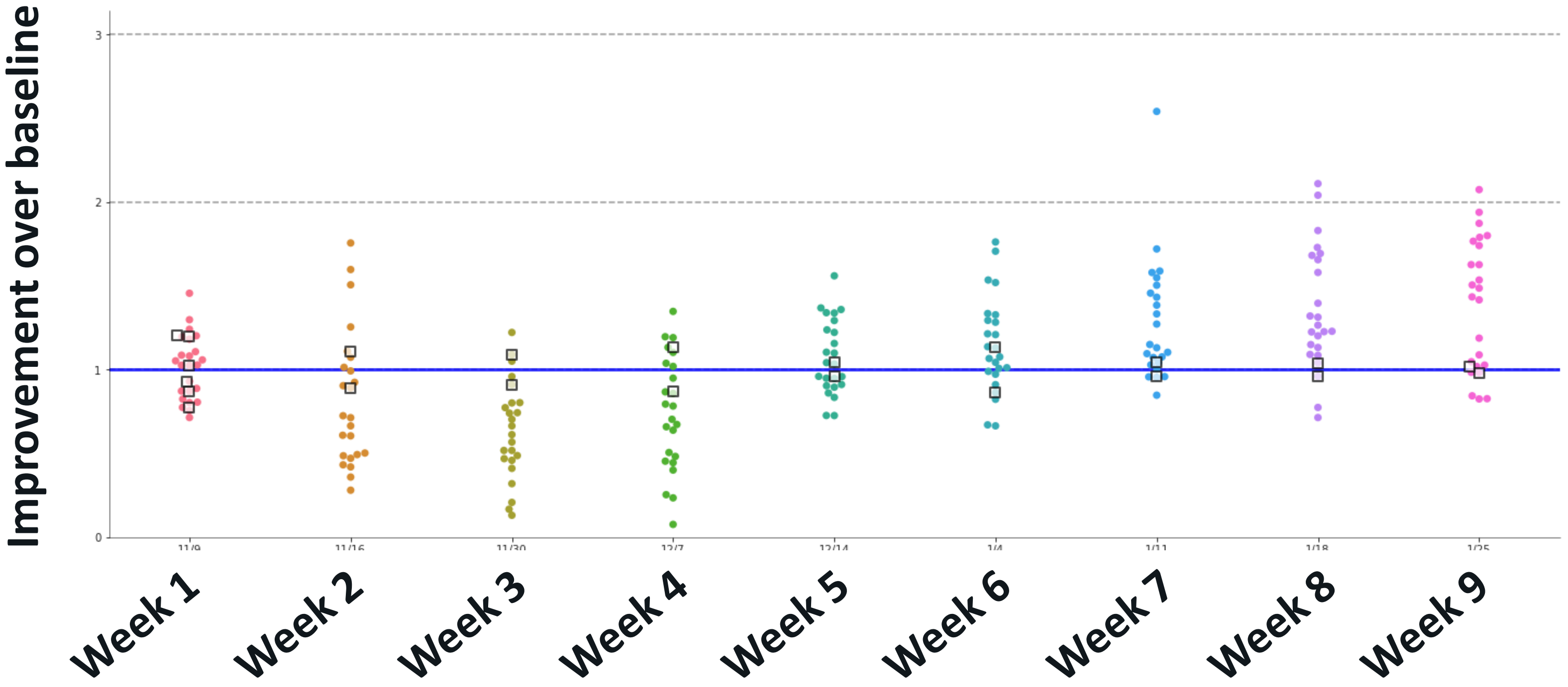
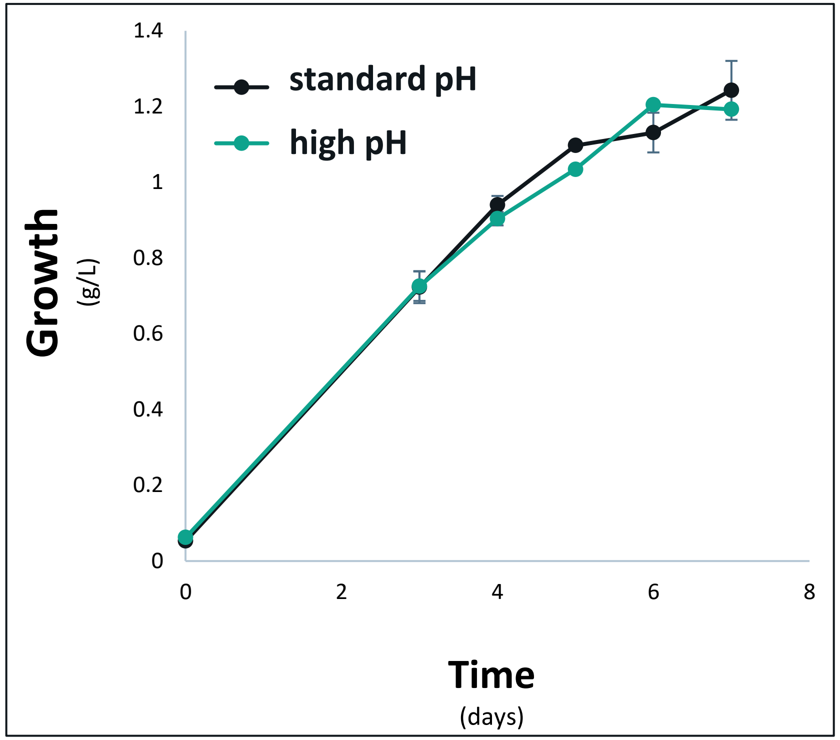
# Progress and Outcomes - Raising pH dramatically decreases the $CO_2$ taken from the tank source (75-90%)




**Outdoor Simulated conditions**

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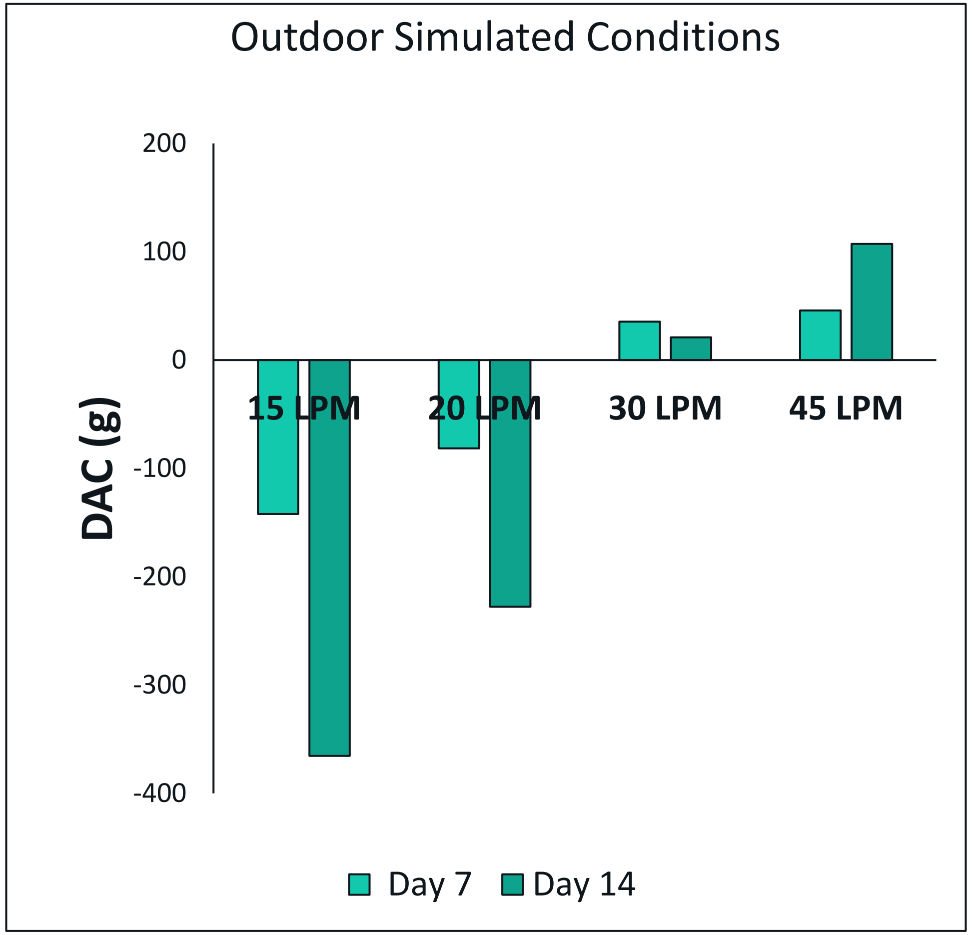
# Progress and Outcomes Increased airflow at elevated pH allows cultures to grow mainly on direct air capture

- Airflow was increased in production reactors to increase direct air capture when grown in alkaline pH
- When grown in this condition, point source $CO_2$ is not utilized
- The majority of biomass in this condition comes direct air capture, with a small amount coming from inorganic carbon in the media




$$DAC = \Delta biomass - CO_{2,pure} - \Delta carbon\ in\ media$$

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# End of project milestone

- We have modeled our DAC work on conditions determined in Arizona (spring 2021)
- Final milestone is to use our technology to increase DAC while maintaining high therapeutic content
- This will be done in the AzCATI facility in June 2023


*Figure 1* MM FP-PBR setup for the Summer 2021 run. Nine (9) reactors in total were used in groups of three (3) with one of each strain per group.

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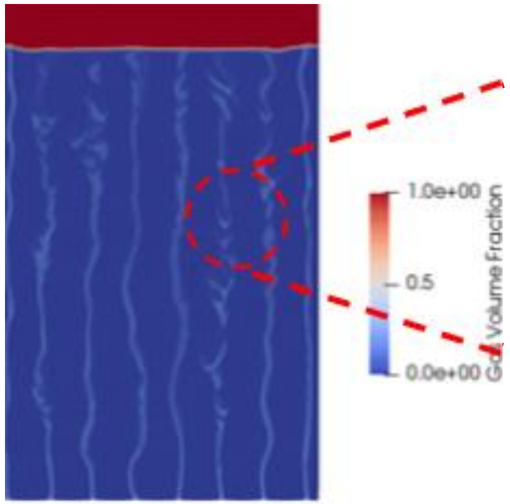
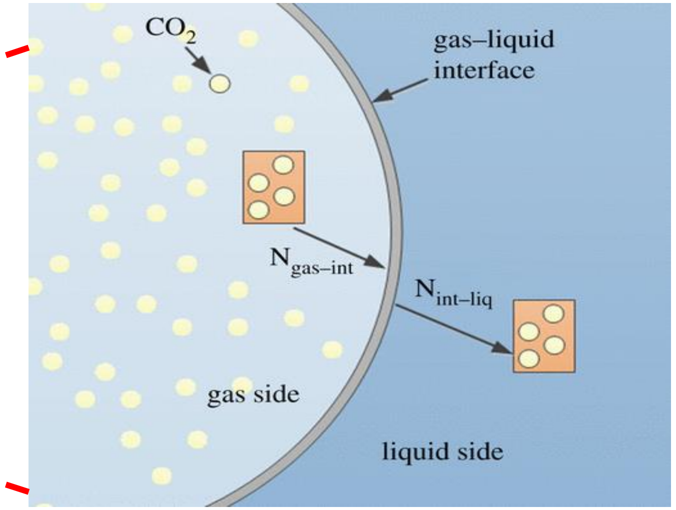
# Project Overview - Simulation computational fluid dynamics models were validated and ready for scaled deployment




- CFD models built to mimic Lumen PBR, incorporate temperature, pH, light availability, and mass transfer efficiency
- Chemical inorganic carbon equilibria underpin CUE and DAC models
- Models simulating Lumen reactors validated the experimentally observed Carbon Utilization Efficiency (CUE) and direct air capture carbon (DAC-C)

| **Reactor** | **Air flow (L/min)** | **Initial Biomass concentration (g/L)** | **Point Source carbon (g)** | **CUE (%) Exp \| Model** | | **DAC-C (g) Exp \| Model** | |
|---|---|---|---|---|---|---|---|
| 1 | X | 0.085 | 177.85 | 35.9 | 30.79 | -141.9 | -173.9 |
| 2 | 1.33X | 0.161 | 113.57 | 41.6 | 54.04 | -81.59 | -70.71 |
| 3 | 2X | 0.129 | 0.0 | 66.8 | 69.23 | 35.52 | 24.34 |
| 4 | 3X | 0.1435 | 0.0 | 56.3 | 58.97 | 45.76 | 28.14 |



Interactions between gas–liquid mass transfer and bubble behaviors, Volume: 6, Issue: 5, DOI: (10.1098/rsos.190136)

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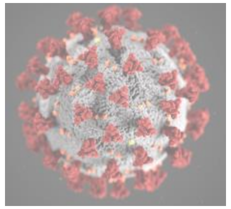

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Increase therapeutic by 50%

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Increase Biofuel intermediates by 50%

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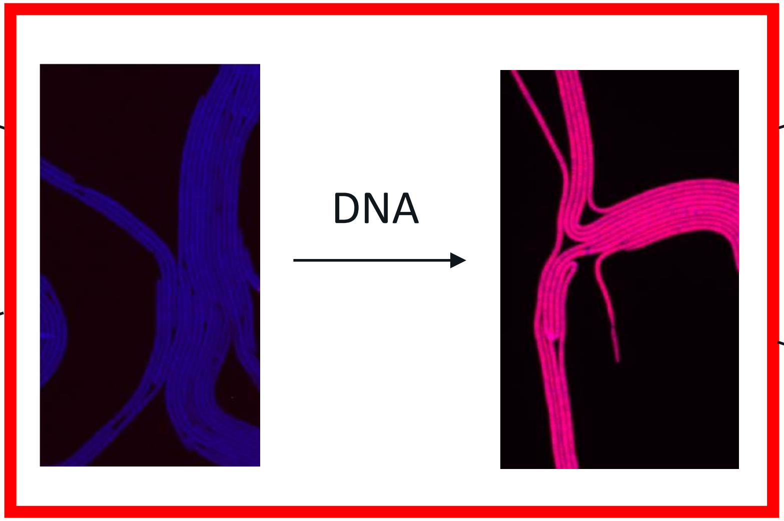
Increased by 117%

## *Arthrospira platensis*




Increase direct air capture by 50%

*PowerPoint stock photo*

Increased by 117%

LCA and TEA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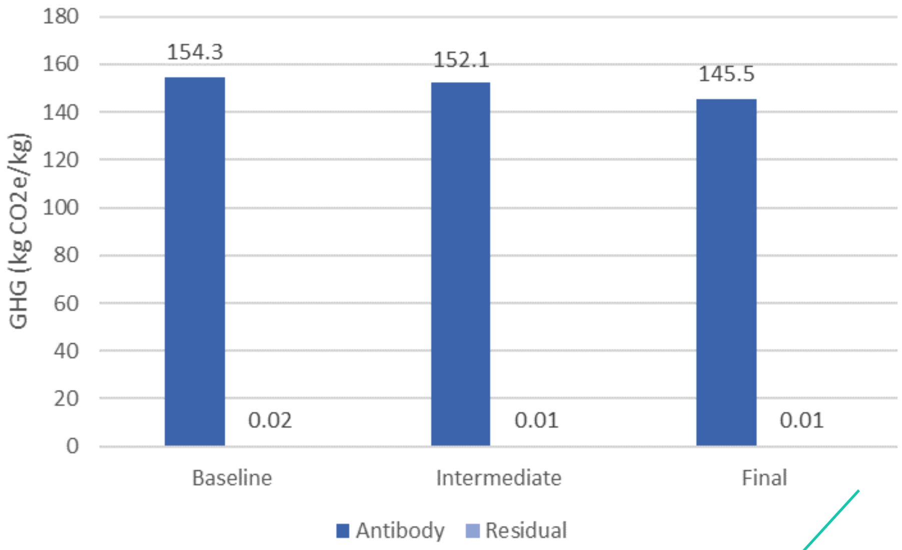
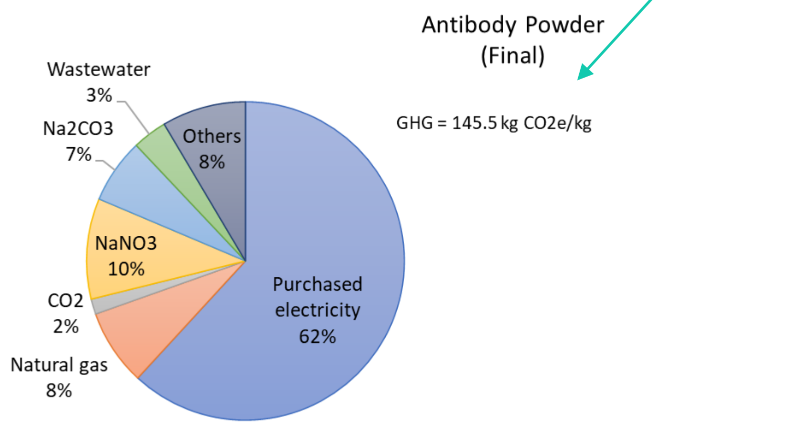
# Project Overview – Lumen's technology dramatically reduces the carbon footprint of antibody production

**Literature monoclonal antibody production GHGs**:

- 277 t CO2e/kg via fed-batch/multi-use technology
- 137 t CO2e/kg via perfusion/single-use technology
- 0.154 tCO2e/kg via Lumen's process

Source: Amasawa et al (2021) https://doi.org/10.1021/acssuschemeng.1c01435




Electricity consumption is the biggest GHG driver.

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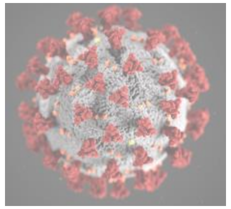

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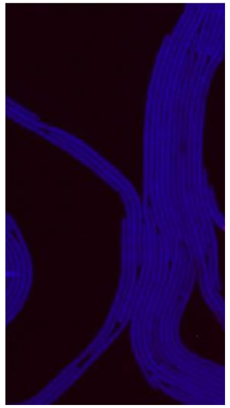
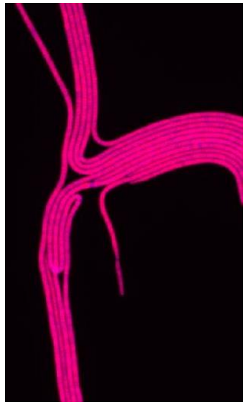


DNA



Increase direct air capture by 50%

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LCA and TEA

Innovations improve TEA/LCA


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# Impact

- Lumen’s technology dramatically reduces the cost of using biologics (protein therapeutics) demonstrating clear commercialization potential.

- Nutrient limitation increased carbohydrate content, this opens the opportunity for concomitant biofuel production from residual biomass

- The Access carbon  project has found ways to grow our strain almost exclusively on direct air capture, reducing the overall carbon footprint

- Computational fluid dynamics, LCA and TEA increase the translatability of this technology, creating models and toolkits that can expand the use of this approach

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# Summary

- Lumen's technology uniquely democratizes therapeutics
- In this project, we have used direct air capture to totally replace point source $CO_2$ .This meets the major goal of the Access Carbon project.
- Have shown the value of "Definitive Screening Design" in improving strain potency
- Conditions have been used to dramatically increase biofuel intermediates through nutrient depravation. This has been done in a way that does not interfere with direct air capture or potency.
- Computational fluid dynamics simulations models developed in this project allow for reliably scaling of the direct air capture technology

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# Quad Chart Overview

## Timeline

- *Project start date: 10/01/2020*
- *Project end date: 9/30/2023*

| | FY22 Costed | Total Award |
|---|---|---|
| **DOE Funding** | *(10/01/2021 – 9/30/2022)* | *$1,368,000* |
| **Project Cost Share** * | | *$500,000* |

TRL at Project Start: 2
TRL at Project End: 5

## Project Goal

To decrease the reliance of spirulina biomass generation on point sources of $CO_2$ by 25% while boosting productivity by 15% and doubling the amount of biofuel intermediates in the biomass of a strain producing valuable protein coproduct.

## End of Project Milestone

Demonstrate that at least 1 improved platform spirulina strain achieves at least a 15% improvement in biomass productivity relative to wild type baseline, displaces 25% of delivered $CO_2$ with DAC, and has a biomass composition containing at least 2% therapeutic protein and achieves a 100% relative increase in components of biomass that can be used for bioenergy production relative to baseline strain and conditions.

## Funding Mechanism

FOA Number: DE-FOA-0002203 / 000001
Award Number: DE-EE0009277

## Project Partners*

- NREL – award program funding $632,000

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*Only fill out if applicable.