

**ACCESS CARBON:*****Alkaline Carbon Capture and Expression-Streamlined Spirulina  
Cultivated in Air for Reliable Bioproducts, Oil, and Nutrition*****Applicant:** Lumen Bioscience, Inc.**Co-recipient:** National Renewable Energy Laboratory**Principal Investigator:** Damian Carrieri, PhD (Lumen)**Co-Principal Investigator:** Lieve Laurens, PhD (NREL)

Spirulina (*Arthrospira platensis*) is a widely cultivated microalga consumed for various purposes including human nutrition and animal feed. However, current cultivation techniques rely on purified CO<sub>2</sub> deliveries, so decoupling spirulina production from CO<sub>2</sub> point sources with direct air capture, or DAC, would both improve its scalability and improve its cost structure in existing algae markets (e.g., nutrition, animal feed). Spirulina readily tolerates alkaline media and it is already grown at commercial scale outdoors, so it is ideally positioned for DAC enhancement. This project aims to lessen spirulina cultivation dependence on concentrated CO<sub>2</sub>, increase its content of energy dense components, and develop genetically engineered spirulina strains with superior expression of valuable heterologous protein bioproducts. Achieving these goals will enhance the usefulness of spirulina for all products, including the spirulina-based products on the market today as well as future products like biomass for fuels and other novel applications.

The use of air as a major source of CO<sub>2</sub> for photosynthetic growth is challenged by physical gas-mass transfer limitations and physiological barriers to cultivating spirulina in carbon-limited conditions. This project will enact high-throughput optimization guided by machine learning to improve cultivation conditions and further enhance media formulation with the aid of computational fluid dynamics models of photobioreactor mixing. Genetic enhancement tools to boost productivity and biomass composition will be developed for general applicability to strains expressing valuable protein co-products. As an example, building on Lumen's experience in developing therapeutic proteins in spirulina, we will apply these tools in strains expressing antibodies that bind the receptor binding domain of SARS-CoV-2, representing a salient and urgent societal need. The tools and formulations developed will be integrated with a highly efficient combinatorial strain development pipeline called SOFAST, which was previously developed and successfully demonstrated by Lumen and NREL under a BETO-PEAK award. We will use the SOFAST technology to tune expression of traits that aid carbon uptake under high pH conditions, augmented by enhanced machine learning techniques previously pioneered at Lumen with separate funding support. The resulting pilot strains will be evaluated in outdoor growth systems by Lumen collaborator Arizona State University / AzCATI.

The projects goals are to (1) displace delivered CO<sub>2</sub> by at least 25%, (2) improve biomass productivity by at least 15%, and (3) double the proportion of energy dense components suitable for bioenergy generation within the biomass – all in an optimized platform strain producing valuable protein coproduct for progression to future development.