# Efficient $CO_2$ Use for Robust Marine Microalgae Biomass Yields (MASS)

We recently isolated a marine alga, *Picochlorum celeri*, from the Gulf of Mexico that has exceptional productivities (~40 gAFDW $m^{-2}$ $d^{-1}$) under diel culturing and has been grown outdoors for three consecutive years at the Arizona State University testbed (DOE DISCOVR program). In 2020, *P. celeri* averaged productivities of ~32 gAFDW $m^{-2}$ $d^{-1}$ for over three months in a seawater medium without a pond crash. Several single day productivities reached ~40 gAFDW $m^{-2}$ $d^{-1}$. These are the highest sustained productivities reported to date for a marine (non-potable water) alga at this site. Importantly, *P. celeri* was highly stable across three summer campaigns without crashes or the need for biocides for crop protection. The exceptional carbon uptake rates of *P. celeri* will now be combined with innovative and efficient $CO_2$ transfer and pond operation strategies. These strategies were first conceptualized and successfully tested by members of this team at Roswell NM (DOE-Aquatic Species Program). Using 1000 $m^2$ raceway ponds they demonstrated the importance of measuring $CO_2$ outgassing through the pond surface and efficiently injecting $CO_2$ into open ponds. The latter led to the installation of a shallow sump (0.91 m depth) with gas spargers 0.25 m from the bottom, which resulted in $CO_2$ injection efficiencies of >90% when operated counter-currently. When these designs were combined with rapid carbon uptake by the algae, $CO_2$ utilization efficiencies, accounting for outgassing losses, exceeded 90% in the summer months. However, the overall Carbon Utilization efficiency (CUE) was only 60% due to the lack of medium recycling and high overnight outgassing of $CO_2$ produced by respiration. We propose to increase CUE further by incorporating medium recycling, improving the operation of carbon transfer in sumps, and developing rapidly growing (efficient C uptake) strains of *Picochlorum* under the pH cycling regimes that occur in large ponds. These efforts will enable exceeding the FOA targets of 70% CUE at 20 gAFDW $m^{-2}$ $d^{-1}$ productivity for two summer 30-day growth cycles (40 $m^2$ raceway ponds). We will develop custom-built harvesting membrane filtration units and pH-based cell flocculation for media clarification, cell concentration and full medium recycling. $CO_2$ and C, organic and inorganic, mass balances will be determined. Lastly, we will screen *Picochlorum* cells (random mutants and natural isolates) for improved growth under pond-relevant pH cycling (7.0->8.0) and for strains with higher lipid content for conversion to sustainable aviation fuels. In process scale-up, we propose to use concentrated $CO_2$ from direct air capture. Experimental data will be integrated into system modeling for the evaluation of the advancements through sustainability modeling with data feedback used to identify performance targets throughout the project. In summary, we have already shown that *P. celeri* has robust outdoor yields and that highly efficient $CO_2$ injection can be achieved in relatively large (1000 $m^2$) ponds. We now propose a multifaceted approach that we are confident will exceed the current FOA goals. Improved CUEs and high areal productivities will enable the algal biofuels community to move towards a more sustainable future. The tools developed in the proposal are translatable to other organisms and will be of broad use to the algal biotechnology community.

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