

**Title:** Microscale Characterization Tools for Algal-Bacterial Interactions and Cell Sinking

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**Project Goals:** Algal and plant systems have the unrivaled advantage of converting solar energy and CO<sub>2</sub> into useful organic molecules. Their growth and efficiency are largely shaped by the microbial communities in and around them. The  $\mu$ Biospheres SFA seeks to understand phototroph-heterotroph interactions that shape productivity, robustness, the balance of resource fluxes, and the functionality of the surrounding microbiome. We hypothesize that different microbial associates not only have differential effects on host productivity but can change an entire system's resource economy. Our approach encompasses single cell analyses, quantitative isotope tracing of elemental exchanges, 'omics measurements, and multi-scale modeling to characterize microscale impacts on system-scale processes. We aim to uncover cross-cutting principles that regulate these interactions and their resource allocation consequences to develop a general predictive framework for system-level impacts of microbial partnerships.

**Abstract Text:** Previous studies have documented that different algal associated bacteria influence the aggregation of algal cells in culture [1], which has implications for harvesting large-scale algal cultures for biofuels. Sinking aggregates, consisting of microbial cells including photosynthetic algae and heterotrophic bacteria, also are major contributors to biological carbon sequestration in aquatic environments [2], which could be used as an engineering solution to combat climate change [3]. Cells within these particles are physically connected via an exuded, sticky polymer called extracellular polymeric substances (EPS). For decades, it has been hypothesized that algal-bacterial interactions modulate the biosynthesis of EPS and leads to a change in formation and sinking rate of the aggregates [4]; however, its detailed mechanism remains unanswered due to a limited number of tools that characterize this microscale interaction.

Here we report a new phenomenon where the sinking rate of algal cells is influenced by the presence of a host-associated bacterial strain. Based on our observation that a cell culture stage (log or stationary phase) is the main driver that leads to different sinking rates, we develop a hypothesis that algal EPS production increases due to the presence of bacteria and this facilitates algal aggregation and increases cell sinking. To test this hypothesis, we employ several imaging-based characterization methods. First, we establish an optical setup that tracks vertical movement of single cells and compare sinking rates between different culture conditions. Second, we measure the surface charge of individual algal cells via electrokinetic microfluidic tools [5] and test if host-associated bacteria can influence algal surface charge. Third, we examine EPS

production from algae at both single-cell (using conjugate fluorescein that binds to a cell surface) and bulk (using polysaccharide extraction). Finally, as a proxy of nutrient exchange we report differential algal incorporation rate of carbon and nitrogen across bacterial taxa based on NanoSIMS [6] and compare it to the algal sinking speed under bacterial co-culture. Taken together, these characterization tools will provide a fundamental understanding on the biophysical role of microbes in microalgal aggregate dynamics critical for biofuel production and carbon sequestration.

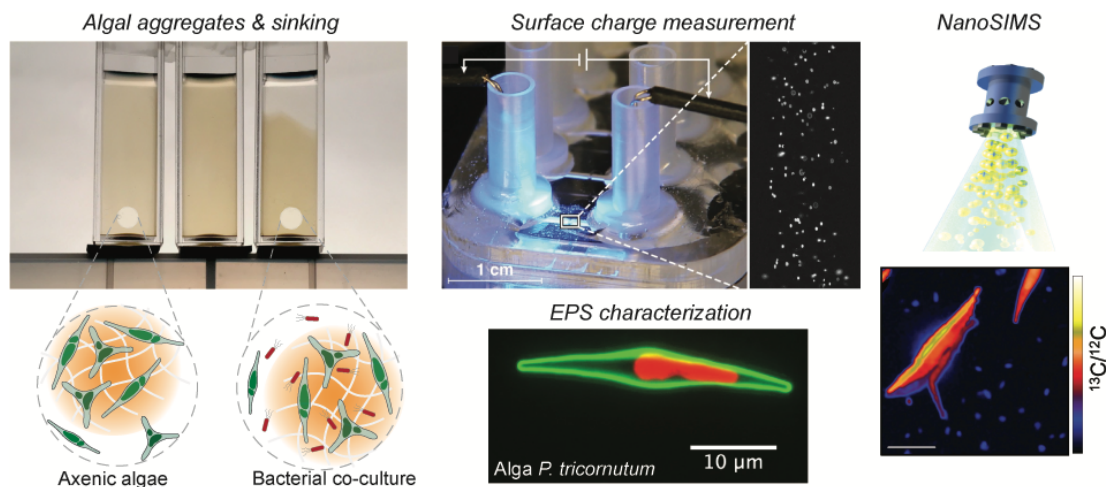


Figure. Imaging-based methods to characterize microscale interaction between algae and bacteria and their sinking.

## References/Publications

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