

Doctoral Dissertation Defense

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> > ABSTRACT

Algal biofilms and lipids: Bicarbonate amendment and nitrate stress to stimulate lipid accumulation in algal biofilms

Algal biofuels are compounds obtained by transesterification of algal lipids to fatty acid methyl esters (FAMEs) which can be used as biodiesel. Algal biofilms have a potential for commercial applications of algal biomass for biofuel production and provide concentrated biomass requiring less water removal to reduce biofuel production costs. Lipid production in algal biofilms is low as compared to planktonic algal growth systems and strategies for enhancing lipid content in algal biofilms need to be developed. The overarching goal of the studies presented herein was to develop lipid accumulation strategies in algal biofilms using nutrient stresses to increase triacylglycerides (TAGs) and FAMEs.

First, a reactor was designed for photoautotrophic biofilm growth incorporating a novel algal biomass harvesting mechanism. *Chlorella vulgaris* biofilm growth was demonstrated to establish reactor characteristics under three different inorganic carbon regimes and the presence of excess calcium to facilitate biofilm attachment and accumulation. Excess calcium resulted in precipitate formation and increasing ash content in biomass and caused difficulty in biofilm detachment. However, the highest biomass accumulation was observed in the bicarbonate and the bicarbonate with calcium treatments.

Second, two different algal strains were tested for lipid accumulation under two nutrient conditions: nitrate limitation and bicarbonate addition. Algal strains included, an extremophilic freshwater diatom RGd-1, a Yellowstone National Park (YNP) isolate, and oleaginous chlorophyte *C. vulgaris*. High bicarbonate content at low nitrate concentration in the bulk medium provided the highest lipid accumulation as determined by Nile Red fluorescence and Gas Chromatography Mass Spectrometry (GCMS) analysis of extracted FAMEs (7-22 % wt/wt). For prevention of biomass loss and quick response to nutrient stresses to stimulate lipid accumulation, the growth medium was exchanged after initial biofilm accumulation and operated in batch mode. This was implemented to quickly introduce nutrient stresses using fresh medium to vary bicarbonate and nitrate concentrations as needed.

Thus, enhanced lipid production in algal biofilms with nitrate stress and bicarbonate amendment is a viable strategy to increase lipid accumulation. Increased lipid content may help offset the cost for biodiesel production with more lipid product and lower processing requirements for water removal.