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Algae biofilm growth and the potential to stimulate lipid accumulation through nutrient starvation

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HIGHLIGHTS

- A system was developed to study the growth kinetics of algal biofilms.
- Algal biofilm growth kinetics are linear.
- Nutrient starvation caused sloughing and/or growth cessation of algae biofilms.
- Nutrient starvation did not increase in algal biofilm lipid concentrations.
- Algal biofilm lipid productivities were comparable to terrestrial biofuel sources.

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ABSTRACT

An algae biofilm growth system was developed to study the growth kinetics and neutral lipid productivities of *Scenedesmus obliquus* and *Nitzschia palea*, and to determine if algal biofilms can be starved of key nutrients to increase their neutral lipid concentrations. Linear growth curves were determined for each species until nutrient starvation commenced, at which point growth ceased and/or biofilms sloughed from their substratum. Nutrient starvation did not increase neutral lipid concentrations in any of the biofilms; however, it approximately doubled their lipid concentrations when grown in suspension. Biomass productivities of 2.8 and 2.1 g/m^2/d and lipid productivities of 0.45 and 0.18 g/m^2/d were determined for *N. palea* and *S. obliquus*, respectively. The results suggest that nutrient starvation of biofilms is not a desirable method of lipid production for algae biofilm biofuel production systems, but that lipid production rates compare favorably with conventional terrestrial biofuel sources.

1. Introduction

In recent years researchers around the world have been focusing their efforts on producing biofuels and biochemicals from the growth of algae cultures. This effort has been inspired by the need to develop renewable biofuels and other biochemicals in order to alleviate the environmental effects associated with the use of petroleum fuels and chemicals, and because conventional fuel resources are dwindling and are less secure (Li et al., 2008). In addition, algae produce some potentially high value chemicals such as β-carotene and astaxanthin (Barowitzka, 1992). Biofuel and biochemical development from culturing microalgae simultaneously addresses the above challenges if the technology can be economical and energy efficient, allowing nations to "grow" fuels and chemicals from inorganic nutrients through photosynthesis.

One of the most compelling advantages for algae as a source of renewable energy is the potentially high rates of productivity of many algal species compared to conventional biofuel stocks such as corn, soybean, rapeseed, and other plants (Chisti, 2007). It has been reported that some algae species can produce up to 30 times more bioenergy per area of land than the most productive terrestrial oil seed plants (Johnson and Wen, 2010; Mata et al., 2010). This is due to rapid growth with population doubling times as low as 3.5 h and to algae biomass lipid/oil concentrations in the order of 15–75% of the dry weight (Chisti, 2007). Algal systems also offer the potential to simultaneously treat wastewater and flue gas streams by utilizing the nitrogen, phosphorus and carbon dioxide within these streams.

Algal lipid yields may be enhanced by exposing cultures to a range of environmental stresses prior to harvest (Hu et al., 2008; Opute, 1974). Most of this increase in lipid concentration is in the form of neutral lipids, particularly triacylglycerol – the type generally used for biofuel production (Hu et al., 2008), aquaculture

Abbreviations: EPS, extracellular polymeric substance; PBR, photobioreactor; LED, light emitting diode; BSE, backscatter electron; SE, secondary electron; FAME, fatty acid methyl ester; GC, gas chromotography.

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feeds, and nutraceuticals (Sicko-Goad and Anderson, 1991). These neutral lipids form in the cytoplasm as dense lipid bodies (Hu et al., 2008; Sriharan et al., 1991), and arise from the assimilation of new carbon (from CO_2), and from the conversion of already assimilated carbon in carbohydrates, proteins and other internal biochemical molecules (Rodolfi et al., 2009; Roessler, 1988).

Although many types of environmental stress can trigger a lipid accumulation response, the most commonly researched are nutrient starvation stresses. Of the many potential types of nutrient starvation stresses, the majority of research has been done on nitrogen and silicon starvation. Silicon starvation is only relevant for diatom algae species because it is a macronutrient essential for cell division as it composes a significant fraction of the frustule exoskeleton unique to diatoms (Spilling et al., 2010). Nitrogen is a necessary macronutrient for all algae species because it is essential for cell division as it composes functional groups of nucleic acids, proteins and other biomolecules (Madigan et al., 2009).

Although the underlying mechanism is not well understood, researchers have clearly demonstrated that starvation of key macronutrients can significantly increase lipid concentrations within algae biomass of many different species. Opute (1974) determined that the lipid concentration of *Nitzschia palea* could be increased from 22% to 35% after 5 days of silicon starvation. Roessler (1988) reported an increase from 20% to 28% lipid concentration when starving the marine diatom *Cyclotella cryptica* of silicon for just 12 h. Sriharan et al. (1991) starved *C. cryptica* until lipid concentrations peaked (unreported amount of time) increasing the lipid concentrations from 21% to 42%, and from 17% to 45%, for silica deficiencies and nitrogen deficiencies, respectively. Rodolfi et al. (2009) starved a marine algae *Nannochloropsis sp.* of nitrogen and increased the lipid concentration 4-fold (from ~15% to ~60%) after 3 days of starvation. Lastly, Shifrin and Chisholm (1981) starved 30 different algal species of nitrogen and silicon for 4 to 9 days and observed a 2 to 3-fold increase in lipids for green algae, and both increases and decreases for diatoms. Thus, nutrient starvation can be an important industrial strategy to control and increase the yield of neutral lipids prior to biomass harvest.

Although there are many attractive attributes to the production of biofuels and biochemicals from algae cultures, there are significant economical challenges to overcome before commercialization of these technologies can occur. One of the most significant limitations to the economical use of algae is the high cost of harvesting and concentrating the biomass (Christenson and Sims, 2011; Gudin and Therpenier, 1986; Uduman et al., 2010). In their review article, Uduman et al. (2010) state that algal suspensions are often between 0.02% and 0.06% total suspended solids (TSS), and that significant energy is spent to harvest and concentrate the cells to 5–25% TSS. Gudin and Therpenier (1986) report that energy for harvesting and de-watering accounts for 20–30% of the total biomass production costs.

To circumvent these biomass-processing costs, we have been investigating the utility of growing algae as a biofilm, rather than in a conventional suspension culture. The idea is that by growing algae as a biofilm the biomass can effectively be concentrated and immobilized on a solid subsurface. Typically, biofilms range from 6–16% total suspended solids (TSS) (Christenson and Sims, 2011; Johnson and Wen, 2010; Ozkan et al., 2012) thereby minimizing the effort required to concentrate and de-water the biomass during harvest. Additionally, the immobilized and concentrated biomass can potentially reduce difficulty in downstream processing i.e. removing substratum-biomass structure and directly immersing in solvent, or removing growth medium from the reactor and adding solvent.

To date, there has been very little research on biofilm systems for commercial production of algae and lipids (Christenson and Sims, 2012; Irving and Allen, 2011; Johnson and Wen, 2010; Ozkan et al., 2012). Most of the algal biofilm literature focuses on using them as a means of wastewater treatment. In this paper the dynamics of a laboratory-scale algal biofilm growth system were explored to determine algal biofilm growth kinetics and lipid production. Additionally, the hypothesis that nutrient starvation (nitrogen and silicon) would elicit enhanced lipid production in biofilm cultures, as is the case in algal suspensions, was tested.

2. Methods

An algal biofilm growth system was designed to characterize and control many algal biofilm growth parameters while maintaining the ability to manipulate the bulk medium nitrogen and silicon concentrations part way through the experiment to elicit nutrient starvation. Additionally, the system allows for the collection of growth kinetics data while doing destructive lipid analysis on the biofilms.

2.1. Biofilm culturing system

The semi-continuous flat plate parallel horizontal photobioreactor (PBR) system (Fig. 1) had significant re-circulation (~95% by volume) to re-use the large amounts of medium going through the reactors. The fresh feed medium (~5% by volume of total to

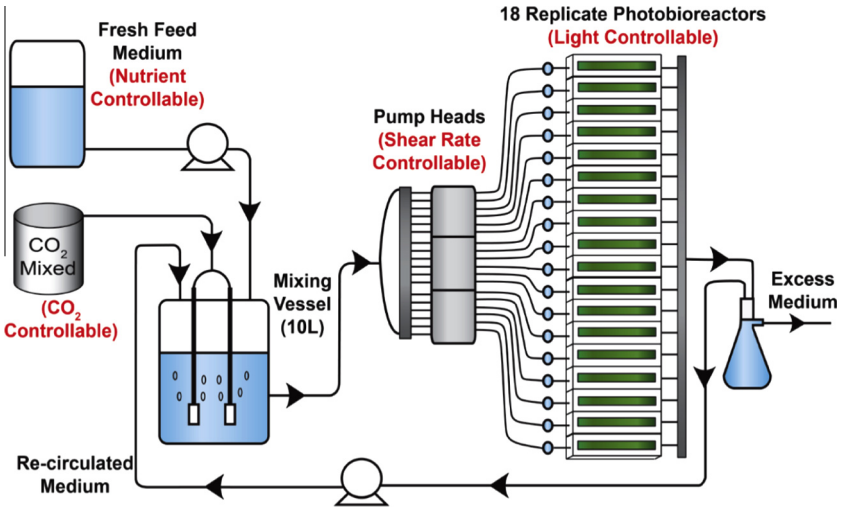

Fig. 1. Schematic of the algae biofilm culturing system used to grow algae biofilms with controls on key growth parameters.