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Dissolved inorganic carbon enhanced growth, nutrient uptake, and lipid accumulation in wastewater grown microalgal biofilms

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HIGHLIGHTS

- Bicarbonate added to enhance algal biofilm growth and nutrient uptake.
- Bicarbonate added to trigger lipid production in nitrogen stressed algal biofilms.
- Nutrient removal influenced by growth phase of algal biofilms.
- Bicarbonate addition enhanced photosynthesis in algal biofilms.

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ABSTRACT

Microalgal biofilms grown to evaluate potential nutrient removal options for wastewaters and feedstock for biofuels production were studied to determine the influence of bicarbonate amendment on their growth, nutrient uptake capacity, and lipid accumulation after nitrogen starvation. No significant differences in growth rates, nutrient removal, or lipid accumulation were observed in the algal biofilms with or without bicarbonate amendment. The biofilms possibly did not experience carbon-limited conditions because of the large reservoir of dissolved inorganic carbon in the medium. However, an increase in photosynthetic rates was observed in algal biofilms amended with bicarbonate. The influence of bicarbonate on photosynthetic and respiration rates was especially noticeable in biofilms that experienced nitrogen stress. Medium nitrogen depletion was not a suitable stimulant for lipid production in the algal biofilms and as such, focus should be directed toward optimizing growth and biomass productivities to compensate for the low lipid yields and increase nutrient uptake.

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1. Introduction

Cultivation of microalgae in wastewater streams has been proposed as a means of reducing competition for freshwater sources, as an inexpensive source of nutrients, and as a biological wastewater treatment alternative (Cai et al., 2013; Sturm and Lamer, 2011). Microalgae can utilize nutrients in wastewater for growth to generate considerable amounts of biomass. However, recovery of microalgae from the liquid medium is difficult and represents a substantial capital cost in suspended cultivation systems (Greenwell et al., 2010; Hoffmann, 1998), consequently there is a growing interest in attached algal growth platforms. Algal biofilm based systems such as the rotating algal biofilm reactor (RABR), algal turf scrubber (ATS™), revolving algal bioreactor (RAB), and Algaewheel® have been developed, and algal biofilm growth demonstrated in bench and pilot scale operations (Christenson and Sims, 2011, 2012; Gross et al., 2013; Kesaano and Sims, 2014; Pizarro et al., 2006). However, there is still limited fundamental information on algal biofilm physiological processes and growth especially in wastewater remediation.

Widespread application of algal biofilm-based systems is also limited but can be promoted through integration of wastewater treatment with the production of valuable bioproducts from the harvested algal biomass. Algal biomass composition (*i.e.*, lipid,

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carbohydrate, and protein content) is influenced by the chemical composition of the medium and the environmental growth conditions (*e.g.*, temperature, pH, and light), which subsequently determines the by-products that can be synthesized. Conventionally, microalgae grown as feedstock for biofuels require a two stage process where biomass accumulation occurs under nutrient-rich conditions followed by an environmental challenge to induce secondary byproduct accumulation (*e.g.*, tri-acylglycerols as energy storage compounds) (Su et al., 2011). Nutrient starvation is typically employed as an environmental stress to stimulate lipid biosynthesis in microalgae cultures (Devi et al., 2012; Rodolfi et al., 2009; Sharma et al., 2012). However, stimulation of lipid production in algal biofilms as a result of nutrient starvation has not been as successful as in suspended cultures (Bernstein et al., 2014; Schnurr et al., 2013).

Furthermore, information on the use of other lipid inducing techniques such as chemical addition, pH stress, and temperature either independently evaluated or in combination with nutrient starvation is limited in algal biofilm studies. For example, addition of bicarbonate salts (HCO_3^-) was reported as an effective trigger for lipid production in nutrient limited suspended microalgae cultures (Gardner et al., 2012, 2013; Peng et al., 2014; White et al., 2013). The bicarbonate salts not only induce lipid production, but also provide a stable and readily available source of inorganic carbon essential for photosynthesis and microalgae growth (Chi et al., 2013; Mus et al., 2013; Wensel et al., 2014). In addition, Glud et al. (1992) observed an increase in photosynthetic rates and a simultaneous reduction in respiration rates (17%) in a diatom-dominated biofilm community amended with bicarbonate.

The potential use of bicarbonate in minimizing photorespiration is especially of interest in algal biofilms because of the high O_2/CO_2 ratios due to localized supersaturated oxygen concentration from active oxygen photosynthesis (Bernstein et al., 2014; Glud et al., 1992). Photorespiration is a competing process to carboxylation, where ribulose-1,5-biphosphate carboxylase oxygenase (RuBisCO) acts as an oxygenase, thereby inhibiting carbon dioxide fixation and subsequently reducing photosynthetic efficiency. The study presented here evaluated the effects of adding dissolved inorganic carbon in the form of 2 mM HCO_3^- to synthetic wastewater medium to grow algal biofilms in order to:

(1) Enhance algal biofilm growth, nutrient uptake, and lipid accumulation during nutrient deplete culturing.
(2) Increase photosynthetic rates with biofilm depth within the photic zone.

2. Methods

2.1. Microalgal biofilm culturing and sampling

The chlorophyte isolate *Botryococcus* sp. strain WC-2B, previously described in Bernstein et al. (2014), was cultured in 8 L laboratory scale rotating algal biofilm reactors (RABRs) operated at 12 rpm and 25 °C. Each reactor was comprised of two plastic cylindrical wheels (10 cm diameter) onto which 3/16 inch (diameter) untreated cotton cord was attached as the biofilm substratum. Synthetic wastewater was made to simulate typical medium strength domestic wastewater for total nitrogen (TN) and total phosphorus (TP) concentrations without a carbon source (Metcalf and Eddy, 2003). The medium consisted of 60 mg L^{-1} NH_4Cl, 150 mg L^{-1} $NaNO_3$, 16 mg L^{-1} Na_2HPO_4, 15 mg L^{-1} K_2HPO_4, 4 mg L^{-1} KH_2PO_4, 75 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 25 mg L^{-1} $CaCl_2{\cdot}H_2O$, and micronutrients (8.82 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 1.44 mg L^{-1} $MnCl_2{\cdot}4H_2O$, 0.71 mg L^{-1} MoO_3, 1.57 mg L^{-1} $CuSO_4{\cdot}5H_2O$, 0.49 mg L^{-1} $Co(NO_3)_2{\cdot}6H_2O$ and 4.98 mg L^{-1} $FeSO_4$).

The experimental set up consisted of four laboratory RABRs under fluorescent lights with a photosynthetically active radiation (PAR) of 227 ± 65 μmol m^{-2} s^{-1} on a 14:10 L/D cycle. Duplicate reactors were amended with 2 mM HCO_3^- in the form of $NaHCO_3$ and another duplicate set without HCO_3^- amendment was cultured for comparison. The reactors were operated in sequenced batch mode with a 5 day hydraulic retention time (HRT) for a period of 18 days, after which nitrogen stress was induced for an additional 5 days by replacing all liquid medium with synthetic wastewater without a nitrogen source. For each cycle of hydraulic retention time, the reactors were drained, cleaned, and filled with fresh medium. Prior to the start of the experiment, the medium was inoculated with microalgae and the RABRs operated for 3 days (seeding period) to allow the microalgae to attach to the rope strands. As shown in Fig. S1, after the seeding period, the RABRs with the exception of the substratum (rope strands) were covered with black polyethylene sheet to minimize microalgae growth in the liquid medium. Culturing and sampling was performed under non-aseptic conditions (open air).

Rope samples with attached microalgae were excised for oxygen microsensor measurements, microscopy characterization, biomass dry weight measurements, and lipid analysis. Biomass cell dry weights (CDW, gcdw m^{-2}) were obtained by removing the biofilm from a known length of cord into a pre-weighed aluminum weigh boat using a flat end spatula. The biomass was dried at 70 °C for 18 h until the biomass weight was constant. Biomass CDWs were calculated by subtracting the dry weight of the oven dried boat with biomass and normalizing by the total cylindrical surface area for the length of cotton cord substratum excised.

2.2. Water quality monitoring

Nitrate (NO_3^-), nitrite (NO_2^-), and orthophosphate (PO_4^{3-}) concentrations were monitored in the bulk medium and measured by ion chromatography (IC) using a Dionex IonPac AS22 carbonate eluent anion-exchange column set at a flow rate of 1.2 mL min^{-1}. IC data was analyzed by Chromeleon 7 Chromatography Data system (CDS) software. Ammonium (NH_4^+-N) concentrations were determined according to the 2-phenylphenol method (Rhine et al., 1998) with a BioTek PowerWave XS microplate reader (Vermont, USA) at an absorbance of 660 nm. The dissolved inorganic carbon (DIC) was measured on 8 mL filtered (0.2 μm pore size filters) medium samples using a Skalar Formacs$^{HT/TN}$ TOC/TN analyzer (model CA16, Netherlands) and Skalar LAS-160 autosampler. DIC was quantified using peak area correlation against a standard curve from a bicarbonate-carbonate mixture (Sigma Aldrich). Culture pH and optical density (OD) measurements were taken using a standard laboratory Accumet pH electrode (Fisher Scientific) and Genesys 10 UV-Model 10-S spectrophotometer (Thermo Electron Corporation), respectively.

2.3. Oxygen microsensor analysis

Clark-type oxygen microelectrodes (10 μm tip diameter; OX-10 Unisense) and specialized computer controlled hardware (Unisense) were used to analyze the reactive transport of dissolved oxygen with biofilm depth under steady-state diffusive conditions corresponding to light and dark conditions. Photosynthetic rates (coupled with photo-respiration) were estimated using the light/dark shift technique (Kühl et al., 1996; Revsbech and Jørgensen, 1986). The light/dark shift measurements are valid under the following assumptions: (1) initial steady state oxygen distribution is achieved before darkening, (2) oxygen consumption rates before and after dark incubation are identical, and (3) identical diffusive fluxes are maintained during the measurement time at each position. Two point calibrations were performed for the oxic conditions

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