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Effects of pH on cell growth, lipid production and CO_2 addition of microalgae *Chlorella sorokiniana*

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ABSTRACT

Microalgae have emerged as one of the most promising alternative energy feedstocks. Some advantages include the simple cellular structure, short production cycle, high lipid content, and fast growth. However, high production costs due to high CO_2 usage and low lipid productivity have been some of the major challenges impeding the commercial production of algal biodiesel. Here, cell growth and lipid content of *Chlorella sorokiniana* DOE1412 were first evaluated at different pH in flask cultivation. Culture pH was manipulated by CO_2 addition. The optimal pH for DOE1412 is approximately 6.0 when only accounting for cell growth and lipid production and not considering the CO_2 efficiency. A flat panel airlift photobioreactor (PBR) was used for scale-up cultivation at five different pH levels (6.5, 7, 7.5, 8 and 8.5). Data of pH values and CO_2 addition was collected by a data logger. Biomass productivity increased with decreasing pH. By taking into account not only the cell growth and lipid production but also CO_2 addition, the lowest value of CO_2 addition was achieved at pH 8 (2.01 g CO_2/g biomass). The fatty acid profiles and biodiesel properties, such as iodine value (IV), saponification value (SV), cetane number (CN), degree of unsaturation (DU), long-chain saturated factor (LCSF), and cold filter plugging point (CFPP), were determined as a function of pH. CN of biodiesel produced at pH 6.5, 7 and 7.5 satisfied the US standard ASTM D6751; among them, the pH 6.5 products met the European standard EN 14214. Finally, protein content in microalgal biomass increased with increasing pH, while C/N ratio in cells decreased.

1. Introduction

Microalgae can fix CO_2 efficiently from industrial exhaust gasses during photosynthesis, providing a very promising alternative for the mitigation of CO_2, the most prominent greenhouse gas [1–6]. Microalgae have the ability to utilize nutrients (especially nitrogen and phosphorus) from polluted municipal, industrial and agricultural wastewater, which allows for use of non-potable water for growth and provides a promising option for the bioremediation of wastewater [7–10]. However, the production cost of microalgal biodiesel is currently not competitive with petroleum-based products. Many significant technical challenges need to be tackled before this pathway is ready for commercial-scale implementation [11]. Some example research and development areas include: studying the molecular biology of microalgae; selecting and bioengineering microalgae strains optimized to regional climate conditions; developing large-scale microalgae cultivation systems; optimizing cultivation conditions (e.g., nutrient, temperature, light, pH, salinity, and mixing); developing efficient techniques for lipid extraction, biomass harvesting, and downstream processing; reducing and controlling operating costs and energy consumption; and utilizing residues to make valuable co-products [12,13]. This work specifically focuses on optimizing the pH and the amount of CO_2 required for efficient algal growth.

Chlorella sorokiniana DOE1412, a member of the Trebouxiophyceae class, also known as UTEX B 3016, was the microalgae strain used in this study. It has been reported as an important microalga with potential for biodiesel production, accumulating high amounts of lipids and proteins [14,15]. Moreover, it can grow robustly at temperatures of up to 40 °C and in impaired water [5,16].

Microalgae cultivation can be optimized by regulating a variety of environmental parameters, such as light, temperature, nutrients, salinity, temperature, and pH [17,18]. These parameters not only affect the accumulation of biomass profoundly but also influence the biochemical composition of microalgal biomass. The pH is one of the most critical environmental conditions in microalgal cultivation since it determines the solubility and availability of CO_2 and nutrients, and has a significant influence on microalgal metabolism [19]. Each microalgal species has an optimal pH range for biomass and lipid production,

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which is narrow and strain specific [20–23]. Specifically, pH is critical in determining the relative concentrations of the carbonaceous species in water [24]. The pH of microalgal cultures rises gradually during the day due to the uptake of inorganic carbon by microalgae. Higher pH limits the availability of CO_2, thus, inhibiting cell growth [19,24]. On the other hand, algal cultivation at high pH can suppress undesired biological contaminants [25].

Methods for controlling pH include CO_2 injection, buffer addition, and acid/base adjustment. The former two are more commonly used in algae cultivation [20,25,26]. However, the presence of buffers can be a potential pitfall for research because, to date, effects of buffers on microalgal metabolism are not entirely clear. Also, it is not cost-effective or realistic at large scale. The present study focuses on regulating pH by controlling and measuring the amount of CO_2 injected during cultivation. CO_2 addition is a significant economic consideration for biodiesel production. However, carbon balances are not typically performed on algal bioreactors and many economic studies do not provide detailed analyses of the costs associated with producing and transporting CO_2 [27]. A common simplifying assumption is based on co-locating an algal production system with a facility that produces waste CO_2, without considering the potential negative effects associated with toxic substances in flue gas on algal growth, the availability of land near the flue gas, or the possibility for scalability. More recently, the need to understand the costing for CO_2 was highlighted by some studies [28,29]. CO_2 and land were identified as two barriers for the large-scale potential of microalgae [28].

In this study, both Erlenmeyer flasks and flat panel photobioreactor (PBR) cultivation systems were used. Moreover, real-time data of temperature, dissolved oxygen, pH and CO_2 flow rate were monitored during PBR cultivation. An optimal culturing pH range was determined by taking into account not only the cell growth and lipid production but also CO_2 addition. The implications of this research demonstrate the tradeoffs between growth and CO_2 addition that can be implemented in the field to enhance the economical and sustainable viability of algal cultivation.

2. Materials and methods

2.1. Strains and growth medium

Chlorella sorokiniana DOE1412 [30] was obtained from the National Alliance for Advanced Biofuels and Bioproducts (NAABB) cultivation team. Currently it is available from UTEX. The seed culture (500 mL in a 1 L Erlenmeyer flask) was continuously shaken on a platform at 120 rpm under a 12:12 h light/dark period at ambient temperature. Culture medium was replaced every 14 days. Algae cells were re-suspended in fresh medium after old medium was removed by centrifugation.

The standard growth medium used for freshwater *Chlorella* sp. is BG-11 [31]. For this work, an optimized medium PE-001A was used. *Chlorella sorokiniana* DOE1412 grew as well on the PE-001A medium as on the BG-11 medium; however, the PE-001A medium recipe is ten times less expensive [32]. The PE-001A medium consists of the following: 100 mg/L $(NH_2)_2CO$, 12 mg/L $MgSO_4{\cdot}7H_2O$, 25 mg/L $NH_4H_2PO_4$, 75 mg/L Potash, 3.15 mg/L $FeCl_3$, 20 mg/L Na_2CO_3, 4.36 mg/L EDTA and 1 mL/L trace metal solution. The trace elements in the solution consist of the following: 2.86 g/L H_3BO_3, 1.81 g/L $MnCl_2{\cdot}4H_2O$, 0.22 g/L $ZnSO_4{\cdot}7H_2O$, 0.39 g/L $Na_2MoO_4{\cdot}2H_2O$, 79 mg/L $CuSO_4{\cdot}5H_2O$ and 49.4 mg/L $Co(NO_3)_2{\cdot}6H_2O$. Based on pH titration of PE-001A medium, a pKa = 6.2 is observed between pH 6 and pH 9.

2.2. Flask cultivation

Chlorella sorokiniana DOE1412 was cultured in a 1 L Erlenmeyer flask using sterile PE-001A medium at room temperature (25 °C). The culture volume was 500 mL. The artificial light source consisted of four 61 cm long 54 watt fluorescent light tubes (FLT5464, Hydrofarm, Inc., Petaluma, CA) illuminating the top of the flasks, with a light intensity of 200 μmol photons $m^{-2}s^{-1}$ on the flask surface, measured by a quantum meter (MQ-200, Apogee Instruments, Logan, UT). The light cycle was 12 h-on/12 h-off. The initial biomass concentration was approximately 0.06 g/L. Cultures were grown at four different pH levels (6, 7, 8 and 9) using a pH controller to adjust injection of CO_2. Air at a flow rate of 1 L/min (LPM) was added to improve culture mixing. For the control group, a CO_2/air mixture (5/95, v/v) was continually injected into each flask at 1 LPM. All the gasses were saturated with water before entering each flask to decrease water evaporation.

2.3. PBR cultivation

Airlift flat-panel PBRs were used for scale-up cultivation. They were built using 6.35 mm thick transparent acrylic panels with dimensions of 122 (H) × 91 (L) × 10 cm (W). The working volume was 90 L. The air was continually injected into the PBR via three air bubbling stones (Discard-A-Stones 12,526, Lee's Aquarium & Pet Products, San Marcos, CA) placed at the bottom of the PBR to achieve proper mixing of the microalgae culture. An air compressor (3Z355H, W. W. Grainger, Inc., Lake Forest, IL) was used to maintain the proper injection pressure. Air flow was kept constant at 6 LPM to ensure the culture was well mixed. CO_2 was injected discontinuously (0.3 LPM when it was on) via a micro bubble diffuser (1PMBD075, Pentair Aquatic Eco-Systems, Inc., Apopka, FL), controlled by a data logger (CR1000, Campbell Scientific, Inc., UT, Logan, UT) to maintain the desired culture pH. The pH (HI1006-2005, Hanna Instruments, Inc., Woonsocket, RI), dissolved oxygen (DO6400/T, Sensorex, Garden Grove, CA) and temperature (109SS-L, Campbell Scientific Inc., Logan, UT) probes were placed in the PBR and scanned every second. The data logger was also connected to a digital mass flow meter (TopTrak 820, Sierra Instruments, Monterey, CA), to monitor CO_2 addition. The 10-minute averaged data were stored. The PBR was illuminated by eight 61 cm long 54-watt fluorescent light tubes (FLT5464, Hydrofarm, Inc., Petaluma, CA) providing 250 μmol photons $m^{-2}s^{-1}$ on the surface.

Chlorella sorokiniana DOE1412 was grown in 4 L Erlenmeyer flasks provided with a 8 LPM flow rate of a CO_2/air mixture (5/95, v/v) each, at room temperature (25 °C), for seven days before being transferred to the PBRs. The initial biomass concentration was approximately 0.3 g/L. Cultures were grown at five different pH levels (6.5, 7, 7.5, 8 and 8.5), in duplicates, in the PBRs.

2.4. Biomass concentration

The biomass concentration, in dry weight, was determined by measuring the optical density of samples at wavelength 750 nm (OD_{750}) using an ultraviolet-visible spectrophotometer (Genesys 10S UV–Vis, Thermo Fisher Scientific, Inc., Waltham, MA). Daily samples were taken from the reactors during the daylight hours. They were diluted by appropriate ratios to ensure that the measured OD_{750} values were in the linear range of 0.1–0.7. The relationship between OD_{750} and biomass concentration was determined experimentally. Biomass was centrifuged, washed, and dried. A linear regression relationship was obtained:

$$\text{Biomass concentration} = \text{conversion factor} \times OD_{750} \times \text{dilution factor} \quad (1)$$

The conversion factor was validated periodically for all the treatments.

2.5. Lipid content

Algae samples were harvested by a centrifuge (J2–21, Beckman Coulter, Inc., Brea, CA) at relative centrifugal force (RCF) of 4400 × *g* for 10 min. The pellets were dried at 37 °C in a forced air oven (414005-

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