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Sodium Bicarbonate as Inorganic Carbon Source for Higher Biomass and Lipid Production Integrated Carbon Capture in *Chlorella vulgaris*

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ABSTRACT

Chlorella vulgaris was isolated from sewerage treatment plant and grown in the presence of sodium bicarbonate as carbon source at 0.25, 0.5 and 1.0 g L^{-1}. Highest specific growth rate (0.653 μ d^{-1}) was obtained with 1 g L^{-1} bicarbonate followed by 0.5 g L^{-1} (0.641 d^{-1}) on 15th day culturing. Total chlorophyll content of microalgae has increased in a dose dependent fashion with bicarbonate addition and maximum level recorded in 1 g L^{-1} (0.769 ± 0.09 g L^{-1}). The biomass productivity was in the range of 0.237–0.996 g d^{-1} L^{-1}. Rate of CO_2 fixation and carbon content, in terms of quantity was estimated. Results showed that at 1 g L^{-1} sodium bicarbonate concentration, maximum CO_2 fixation (0.497 g/dry weight) and carbon content (0.69 g mL^{-1} day^{-1}) was found. Biomass concentration was significantly higher ($p < 0.05$) in cultures (1.54 g L^{-1}) supplemented with 1 g L^{-1} bicarbonate whereas there was no much difference in cellular lipid concentration (16 mg mL^{-1}). GC–MS analysis of fatty acids showed highest amounts of palmitic acid, myristic and stearic acid. In summary, the addition of sodium bicarbonate increases cellular abundance, chlorophyll content and to some extent in the case of lipid content in *C. vulgaris* integrated with CO_2 sequestration.

Introduction

Among the atmospheric pollutants, CO_2 contributes significantly to the greenhouse effect. Global warming attributed primarily to the elevated CO_2 level in the atmosphere has led to various CO_2 mitigation strategies. As chemical reaction based CO_2 capturing is relatively costly and energy consuming, it is necessary to develop cost effective and sustainable alternatives. Biological CO_2 mitigation leads to the production of biomass energy and is an alternative strategy of CO_2 fixation through photosynthesis (Kondili and Kaldellis, 2007; de Morais and Costa, 2007). Microalgae are more efficient than terrestrial plants in photosynthesis from ambient air and are important for the prevention of increase in atmospheric CO_2 concentration. It converts CO_2 into biomass energy and thus recycles CO_2 (Demirbas, 2004). Coupling of CO_2 sequestration with algal cultivation reduces the carbon footprint and sustainable environment.

Microalgae have drawn more attention as a promising source for the production of biodiesel because they possess high growth rate and provide lipid fraction for biofuel production. Microalgal growth and biochemical composition are governed by environmental conditions (Guiheneuf et al., 2008; Pal et al., 2011). Sodium bicarbonate has been demonstrated to enhance lipid accumulation in both freshwater and marine microalgae (Gardner et al., 2012, 2013; White et al., 2013; Peng et al., 2014). Microalgae utilize bicarbonate

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as the external source of carbon for photosynthesis and derive CO_2 via the action of carbonic anhydrase (Dixon et al., 1987; Nimer et al., 1997; Bozzo et al., 2000). This work established the growth of *Chlorella vulgaris* in a medium where sodium bicarbonate serves as a source of inorganic carbon by assessing growth rate, chlorophyll content, biomass and lipid production in batch cultures. Further, rates of inorganic carbon concentration and CO_2 fixation were determined for CO_2 sequestration using bicarbonate.

Materials and Method

Sample Collection and Identification

Algal samples were collected from Bangalore Water Supply and Sewerage Board (BWSSB), Bengaluru (13°04′N, 77°58′E) and poured into a closed 250 mL bottle and exposed in sunlight for 3 weeks. The upper layer of the water was inoculated in agar plates enriched with BG11 medium containing 200 μg mL^{-1} ampicillin to control the growth of bacteria as the sample used was sewage water. Agar plating technique was used to isolate the microalgae and the plates were incubated at 25 ± 2 °C under cool white fluorescent light (40 μmol photons m^{-2} s^{-1}; 15 h light/9 h dark) until algal growth was detected. The isolates were purified by streak plating and individual colonies were diluted in distilled water. Species of single cells were obtained using capillary pipette under a microscope followed by inoculation into fresh media. After appropriate growth, cells were observed to confirm the single culture and the capillary method was repeated as many times as required to obtain axenic cultures. Identification of *Chlorella* was using standard protocols as described by Anderson (2005), Stanier et al. (1971) and the database http://web.biosci.utexas.edu/utex/default.

Growth Under Different Concentrations of Sodium Bicarbonate

Analytical grade sodium bicarbonate was used as the source of bicarbonate in all experiments. Batch cultures (100 mL) of *C. vulgaris* (BG 11 medium; n = 3) were grown under different levels of bicarbonate supplementation (0.25, 0.5 and 1 g L^{-1}) into early stationary growth phase (10–15 days), where samples were taken for growth rate, biomass productivity, chlorophyll content and cellular lipid analyses. Media without the addition of bicarbonate were served as control.

Analytical Methods

Biomass Concentration and Productivity

Biomass concentration (g L^{-1}) of *C. vulgaris* grown under different bicarbonate concentrations was determined by measuring the optical density (OD_{680}) using UV–Vis spectrophotometer. The result was converted to biomass concentration using the calibration curve relating OD_{680} (Xia et al., 2014) using the following Eq. (1)

$$\text{Biomass concentration} = 320 \times OD_{680}. \tag{1}$$

The biomass productivity (mg L^{-1} d^{-1}) was calculated according to Eq. (2).

$$\text{Biomass productivity} = (B_2 - B_1)/T \tag{2}$$

where B_2 and B_1 represent the dry weight biomass densities at the time T (days), at the end and start of the experiment, respectively.

Specific Growth Rate

Specific growth rate (μ) of the microalgae was calculated (Guillard and Ryther, 1962) according to the following formula.

$$\mu = \frac{\ln(N_t/N_0)}{T_t - T_{0.}}$$

where, N_t and N_0 are the total cells at the end of log phase (T_t) and start of log phase (T_0), respectively.

Chlorophyll Estimation

Chlorophyll a and b of microalgae were estimated according to Mackinney (1941). Algal suspension was filtered and extracted with methanol in water bath at 60 °C for 30 min. The suspension was cooled, added equal volume of 96% methanol and centrifuged for 6500 *g* for 10 min. Pigment content of the supernatant was analyzed in a UV–Vis spectrophotometer at 650 nm and 665 nm using 96% methanol as blank and the total chlorophyll was determined using Eq. (3)

$$\text{Total chlorophyll} = 2.55 \times 10^{-2}.E650 + 0.4 \times 10^{-2}.E665 \text{ mg ml}^{-1} \tag{3}$$

where, E650 and E665 are the absorbance at 650 and 665 nm wavelengths respectively.