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Research article

Effects of nutrient ratios and carbon dioxide bio-sequestration on biomass growth of *Chlorella* sp. in bubble column photobioreactor

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ABSTRACT

Photobioreactor technology, especially bubble column configuration, employing microalgae cultivation (e.g., *Chlorella* sp.), is an ideal man-made environment to achieve sufficient microalgae biomass through its strictly operational control. Nutrients, typically N and P, are necessary elements in the cultivation process, which determine biomass yield and productivity. Specifically, N:P ratios have certain effects on microalgae's biomass growth. It is also attractive that microalgae can sequester CO_2 by using that carbon source for photosynthesis and, subsequently, reducing CO_2 emission. Therefore, this study aims to investigate the effect of N:P ratios on *Chlorella* sp.'s growth, and to study the dynamic of CO_2 fixation in the bubble column photobioreactor. According to our results, N:P ratio of 15:1 could produce the highest biomass yield (3568 ± 158 mg L^{-1}). The maximum algae concentration was 105×10^6 cells mL^{-1}, receiving after 92 h. *Chlorella* sp. was also able to sequester CO_2 at $28 \pm 1.2\%$, while the specific growth rate and carbon fixation rate were observed at 0.064 h^{-1} and 68.9 ± 1.91 mg L^{-1} h^{-1}, respectively. The types of carbon sources (e.g., organic and inorganic carbon) possessed potential impact on microalgae's cultivation.

1. Introduction

In recent years, the modernization accompanying high energy consumption has led to the rising demand for fossil fuels. Consequently, CO_2 emission from fossil fuels utilization worsens greenhouse effect and increases a considerable risk to human health (Marino et al., 2016). Several CO_2 remediation technologies have been promoted to satisfy the requirements of economic and sustainable criteria in long-term operation (Ji et al., 2015). These approaches are plentiful, such as physical processes (adsorption, membrane separation, geologic injection and oceanic injection), chemical processes (mineral carbonation and chemical adsorption) and biological processes (forestation, oceanic fertilization and photosynthetic microorganisms) (Kassim and Meng, 2017; Zhou et al., 2017). Amongst these options, bio-sequestration, employing photosynthetic organisms (e.g., microalgae), emerges as a promising method to deal with CO_2 pollutant (Nguyen et al., 2016).

Bio-sequestration involves microalgae in the photosynthesis process, which assimilates the nutrients (e.g., C, N and P) and exposes to the light source, subsequently generating biomass. Many benefits of using these pollutants for microalgae cultivation have been reported (Wiesberg et al., 2017). For instance, the cells' structures of microalgae is reported with substantial amounts of lipid, so that the its biomass is a promising feedstock for biofuel production rather than other traditional crops (e.g., rice, corn) (Vo Hoang Nhat et al., 2018). The algae feedstock also has productivity 23 times higher than oil palm feedstock (Wang et al., 2010b). The fuels produced by microalgae is also considered an alternative energy source (Guldhe et al., 2017). On the other hand, air pollution remediation through the algae bio-sequestration process reduces CO_2 level significantly (Wiesberg et al., 2017), and it is highly

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applicable to cement industry (Jang et al., 2016).

Although no optimal microalgae species have been found to sequester CO_2, *Chlorella* sp. possesses distinct commercial values thanks to its ability to grow in high level of CO_2 condition (40% in concentration). *Chlorella* sp., a genus of green unicellular microalgae, can reach to that of sufficient nutrients removal efficiency and biomass yield (Nguyen et al., 2016). It is spherical, about 2–9 μm in diameter and without flagella.

Biomass yield is a crucial role of the microalgae cultivation process; however, it is determined by many internal and external conditions. Nutrients (e.g., nitrogen and phosphorous) are the vital elements regulating microalgae's metabolism and decide the biomass productivity. Specifically, the ratios of nitrogen and phosphorus (N:P) have a strong effect on the levels of biomass generation (Vitova et al., 2015). However, the surplus or deficiency of N/P can reversely cause stress on algae's growth; therefore, the appropriate N:P ratios are important (Chu et al., 2013, 2014). The N:P ratios vary from 2:1 to 16:1 which rely on algae strains, types of wastewater and culture conditions (Cabanelas et al., 2013; Mayers et al., 2014). Carbon source for microalgae in the photobioreactor, which is supplied in the form of CO_2 gas, is also studied (Jana et al., 2017). Noticeably, limited works investigate the effect of N:P ratios, coupling CO_2 feeding, and examine the dynamic of CO_2 in the photobioreactor.

Photobioreactor, being operated in the form of a closed system, successfully pleases the above requirements (Lee and Han, 2016). It offers higher biomass concentration while the operational parameters (e.g., temperature, light and nutrients level) are strictly controlled, and less CO_2 loss is also observed (Singh and Sharma, 2012). Although many types of photobioreactors are applied, the bubble column reactor is a promising design because of advantages associated to high mass and heat transfer, accessible construction and high surface area to volume ratio (Chen et al., 2011).

To this end, the effect of N:P ratios on algae biomass yield and the dynamic of CO_2 fixation in the bubble column photobioreactor are investigated in this study. While previous works focus only on the impact of N:P ratios (Mayers et al., 2014) or the influence of CO_2 on biomass yield (Jacob-Lopes et al., 2008), this study tackles the drawbacks by investigating two aspects comprehensively. This research is important while the C:N:P stoichiometry offers an in-depth understanding of biomass yield in photobioreactor (Sardans et al., 2012). The objectives of this research are to (i) investigate the effect of N:P ratios and (ii) study the dynamic of CO_2 fixation related to *Chlorella* sp. in the bubble column photobioreactor.

2. Materials and methods

2.1. Microalgae strain and culture medium

The microalgae specie *Chlorella* sp. in this study was purchased from The Research Center of Aquaculture II (Ho Chi Minh City, Vietnam). The culture medium used for *Chlorella* sp. cultivation was prepared as described elsewhere (Li et al., 2011; Nguyen et al., 2016). The culture medium included 100 mg L^{-1} $MgSO_4 \cdot 7H_2O$; 50 mg L^{-1} $CaCl_2 \cdot 2H_2O$; 1 mL L^{-1} glacial acetic acid; 1 mL L^{-1} trace elements solution including 50 g L^{-1} Na_2EDTA; 22 g L^{-1} $ZnSO_4 \cdot 7H_2O$; 0.05 g L^{-1} $CaCl_2 \cdot 2H_2O$; 11.4 g L^{-1} H_3BO_3; 5.06 g L^{-1} $MnCl_2 \cdot 4H_2O$; 4.99 g L^{-1} $FeSO_4 \cdot 7H_2O$; 1.61 g L^{-1} $CoCl_2 \cdot 6H_2O$; 1.57 g L^{-1} $CuSO_4 \cdot 5H_2O$; 1.10 g L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 16 g L^{-1} KOH. All the chemicals were analytical grade and purchased from Merck (Singapore).

2.2. N:P mass ratio adjustment

The concentrations of NH_4Cl, K_2HPO_4 and KH_2PO_4 were calculated to meet the designed N:P ratios (10:1, 15:1, 20:1, 25:1), while only NH_4Cl concentration was varied to achieve these ratios. The final concentrations of NH_4Cl, K_2HPO_4, and KH_2PO_4 in the medium are presented in Table 1.

2.3. Bubble column photobioreactor

The experimental pilot reactor is demonstrated diagrammatically in Fig. 1. All experiments were operated systematically in batch mode from 96 to 100 h. In details, the photobioreactor was installed in a wooden wardrobe (thickness of 5 mm) to achieve a constant temperature and prevent light transparency outside in. The light source was supplied by the three 18 W lamps installed in the wooden wardrobe. The photobioreactor consisted of two identical columns, which the dimension of each column was 100 mm diameter and 600 mm height. The working volume of each column was 4 L. There was an air diffuser with 20 mm diameter located at the bottom of each column to introduce the mixture of CO_2 gas and air into the reactor. The operational conditions were temperature of 29 ± 2 °C, light irradiance of 3 klux and 24:0 light-dark cycles. The flowrates of air and air mixture were controlled at 2 L min^{-1} and 4 L min^{-1}, respectively; while the CO_2 (0.2 L min^{-1}) was injected via a 6-mm gas tube.

2.4. Physico-chemical parameter analyses

Light irradiance was determined by a digital Luximeter (Model LX1010B, Mextech, India). To control the operating conditions, a pH and temperature meter (IP 65, Milwaukee Instruments, USA) was used to record pH and temperature. The dissolved oxygen (DO) value was measured by a DO meter (HI 9146, Hanna Instruments, Canada). The flow rates of CO_2, air and air mixture (CO_2 enriched air) were controlled by the three rotameters (0.4–5.0 LPM, Cole-Parmer, USA). An electrical meter (HG-250, Heshbon, Korea) was used to measure CO_2 concentration of air flows for both inlet and outlet samples.

2.5. Relationship between cell density and dry biomass

The determination of *Chlorella* sp.'s productivity consists of measuring cell density with a counting method and dry biomass with a gravimetric method.

Cell density was determined with frequency of 4 times per d by a microscope (Eclipse E50i, Nikon, Japan). Initially, algae sample was put on a mirrored surface of Neubauer counting chamber. Afterwards, it was placed under the microscope for counting cells based on the instruction of Fuchs-Rosenthal and Burker producer. The cell density was calculated according to Eq. (1). The calculated formula was obtained from the guidance of manufacturer and pre-experiments. The samples were diluted to proper concentration to be counted and corresponded with the below formula:

Table 1
Concentrations of NH_4Cl, K_2HPO_4 and KH_2PO_4 in feed wastewater.

Components	N:P ratios			
	10:1	15:1	20:1	25:1
NH_4Cl (mg L^{-1})	668.8	1003.1	1337.5	1671.9
K_2HPO_4 (mg L^{-1})	59.5			
KH_2PO_4 (mg L^{-1})	30.3			