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Effects of dissolved inorganic carbon and mixing on autotrophic growth of *Chlorella vulgaris*

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ABSTRACT

Carbon dioxide (CO_2(aq)) and bicarbonate ion (HCO_3^-) are the two inorganic carbon species used for the autotrophic growth of microalgae. An effective supply of the two dissolved inorganic carbon (DIC) species in the culture medium is critical when sparingly soluble CO_2 gas is added within a narrow pH window suitable for the growth. In this study, sodium bicarbonate ($NaHCO_3$) was used as an excellent buffer that can keep the DIC concentration high without its significant loss for open systems within a benign pH window for the growth of *Chlorella vulgaris*. The use of $NaHCO_3$ along with CO_2 gas under agitation could significantly enhance the growth rate by overcoming the DIC limitation for photosynthesis. The photosynthesis reaction was found to be limited by DIC concentration at an initial growth stage and subsequently by light availability at a later growth stage. A high concentration of $NaHCO_3$ helps increase a DIC concentration for photosynthesis, but was found to be limited by its salinity generated by Na^+ for this freshwater green alga, *C. vulgaris*.

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1. Introduction

Microalgae recently drew significant attention due to a growing demand for petroleum [1]. They are reported to have a substantial amount of lipids (approximately 15–50% dry cell weight), faster growth rates than terrestrial plants owing to their high photosynthesis rates, and a large fraction of solar energy utilization (3–10%) [2–4]. Microalgae can utilize CO_2 gas, which is a major contributor to global warming, as an inorganic carbon source for their autotrophic growth [5,6].

Open and closed systems can be used to cultivate microalgae using CO_2(g) [7]. An open system is simple and easy to scale up at a relatively low cost for large-scale cultivation. However, the production of dissolved inorganic carbon (DIC: $HCO_3^- + CO_2$(aq)) species derived from CO_2(g) dissolution is limited in the open pond system due to a low solubility of CO_2(g) under an atmospheric pressure and the DIC concentration available in water may not be high enough to meet a desired microalgal cultivation rate. Meanwhile, a closed system can readily increase the DIC concentration at a desired level by increasing the partial pressure of CO_2 (pCO_2). However, the cost of such a closed system is expensive for a large-scale cultivation system [8]. In addition, the pH of the culture medium decreases with an increase in DIC concentrations as a result of CO_2(g) dissolution, which interferes with an allowable pH window for microalgal growth [9,10]. In this respect, it is important to study whether microalgal growth can be limited by DIC concentrations for large-scale cultivation.

In this study, sodium bicarbonate ($NaHCO_3$) was used as a buffer that could keep the DIC concentration level high in the culture medium. The primary objectives of this study were to examine the feasibility of using $NaHCO_3$ as a buffer to overcome potential DIC limitation for open systems and to keep the pH of the medium within a desired pH window particularly for closed systems during the autotrophic growth of *Chlorella vulgaris* (*C. vulgaris*). The effects of DIC concentrations with $NaHCO_3$ on the growth were investigated by varying different mixing speeds for the consideration of mass transfer and photosynthetic reaction.

2. Materials and methods

2.1. Culture media and conditions

C. vulgaris (Beij. [K&H]) used (UTEX #2714) in this study was obtained from UTEX at the University of Texas at Austin. A culture medium was prepared by following Shuisheng-4 medium [11] and 1800 mL of the prepared medium was added to a 2-L bottle (21 cm (height) × 11 cm (diameter)). The cell density of *C. vulgaris* in the medium was found to be 117.1 ± 0.6 (C1, C2, C3, C4, C5 and C6) and

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Table 1
DIC concentrations generated from CO_2(g) absorption and $NaHCO_3$ dissolution at 1 atm.

pCO_2 (%)	DIC (mg/L)	pH	$NaHCO_3$ (mg/L)	DIC (mg/L)	pH
0.037	0.2	5.6	1.3	0.2	7.7
25	101.9	4.2	713.2	101.0	8.3
50	201.8	4.1	1411.6	199.8	8.3
75	300.0	4.0	2097.9	296.9	8.3
100	396.4	3.9	2772.7	392.4	8.3

46.6 ± 0.2 (D1, D2, D3, D4, and D5) mg/L after inoculation. $NaHCO_3$ was not added to the medium during this step. Then, an initial DIC concentration in a culture medium was controlled by adding a different amount of $NaHCO_3$: 100 mg of $NaHCO_3$/L for C1, C2, and C3 cultures; 1000 mg of $NaHCO_3$/L for C4, C5, C6, D1 and D3 cultures; 5000 mg of $NaHCO_3$/L for D2 and D4 cultures; and 10,000 mg of $NaHCO_3$/L H_2O for D5 culture. Then, CO_2(g) was added to all the culture media to control the initial pH of the media at 7 and this pH control was repeated once daily. During the culture, C1, C4, D1 and D2 culture media were not mixed. C2, C5, D3, D4 and D5 culture media were mixed by using a magnetic stirrer at a speed of 125 rpm. C3 and C6 media were mixed at a speed of 550 rpm.

During the culture, fluorescent lamps with 6500-K color temperature similar to natural sunlight color temperature were used as a light source. The incident light intensity at the reactor surface was set to 6000 lux (100.8 μmol/(m^2 s)), and a 16-h light and 8-h dark cycle was applied to all the cultures. All the culture media were open to an atmosphere at 25 °C.

2.2. Determination of cell mass and number densities of C. vulgaris

The cell mass density of *C. vulgaris* in the culture medium was determined by measuring the optical density at 682 nm by using a UV–vis spectrophotometer (UV-1800, Shimadzu Scientific Instruments). The absorbance of the UV–vis spectrophotometer at 682 nm was calibrated by measuring the weight of dried *C. vulgaris* [12]. Then, the weight of the dried biomass was obtained from the prepared calibration curve.

A hemocytometer counting chamber was used to determine the cell number density of *C. vulgaris* (i.e. the number of cells per unit volume of a sample) for the estimation of a total external surface area of *C. vulgaris* in the culture mediums [13,14]. The volume of each rectangular chamber in the hemocytometer was 0.1 mm^3 (1 mm × 1 mm × 0.1 mm), and the number of *C. vulgaris* cells in each chamber was counted under a microscope (Nikon Labophot-2). Then an average cell number density was obtained by taking an average of the cell number densities in all the rectangular chambers.

2.3. Determination of DIC, nitrogen (NH_3/NH_4^+), and orthophosphate (PO_4^{3-}) concentrations

An acid–base titration method was used to determine the concentrations of DIC species present in the aqueous phase [15]. This titration method determines a total inorganic carbon (TIC) concentration (=$[HCO_3^-]+[CO_3^{2-}]+[H_2CO_3]+[CO_2(aq)]$) in a 15-mL sample using 0.1 N and 0.01 N HCl solutions for the titration of high and low carbon concentrations, respectively. Then, the DIC concentrations were calculated by using the equilibrium relations shown in the next section among HCO_3^-, CO_3^{2-}, H_2CO_3, and CO_2(aq).

The concentrations of ammonia/ammonium ion (NH_3/NH_4^+) were determined using an ammonia probe (Model: 9512HPBNWP Orion Thermo Scientific) and orthophosphate ion (PO_4^{3-}) was determined using Phosver 3 phosphate regent (Hach company) [16].

2.4. Determination of carbon content in C. vulgaris

The carbon content in *C. vulgaris* was analyzed by an elemental analyzer (Vario Macro cube, Elementar Americas Inc.). The content was used to estimate the specific DIC uptake rate by *C. vulgaris* with respect to different DIC concentrations in the culture media.

3. Results and discussion

3.1. Comparison of equilibrium DIC concentrations with and without sodium bicarbonate

The DIC concentration in the aqueous phase is dependent on the partial pressure of CO_2(g) (pCO_2) based on Henry's law. Then the dissolved carbonic acid ($H_2CO_3^*$) will further dissociate into bicarbonate and carbonate ions, and the equilibrium concentrations for all species can be calculated by the following equilibrium and charge balance Eqs. (1)–(5):

$$CO_2(g) + H_2O \overset{H'_{CO_2}}{\longleftarrow} H_2CO_3^*, \quad H'_{CO_2}(= 3.4 \times 10^{-2}\ \text{mol/(L atm)}) = \frac{[H_2CO_3^*]}{P_{CO_2}} \tag{1}$$

$$H_2CO_3^* \overset{K_1}{\longleftarrow} H^+ + HCO_3^-, \quad K_1(= 4.5 \times 10^{-7}\ \text{mol/L}) = \frac{[H^+][HCO_3^-]}{[H_2CO_3^*]} \tag{2}$$

$$HCO_3^- \overset{K_2}{\longleftarrow} H^+ + CO_3^{2-}, \quad K_2(= 4.7 \times 10^{-11}\ \text{mol/L}) = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \tag{3}$$

$$H_2O \overset{K_W}{\longleftarrow} H^+ + OH^-, \quad K_W(= 1.0 \times 10^{-14}\ \text{mol}^2/\text{L}^2) = [H^+][OH^-] \tag{4}$$

$$[H^+] = [HCO_3^{2-}] + 2[CO_3^{2-}] + [OH^-] \tag{5}$$

where all the equilibrium constant values were obtained at 25 °C. Here, $H_2CO_3^*$ consists of H_2CO_3 and CO_2(aq) as shown in Eq. (6), and these two components have the following equilibrium relationship in Eq. (7):

$$[H_2CO_3^*] = CO_2(aq) + H_2CO_3 \tag{6}$$

$$CO_2(aq) + H_2O \overset{K_3}{\longleftarrow} H_2CO_3, \quad K_3(= 2.6 \times 10^{-3}\ \text{mol/L}) = \frac{[H_2CO_3]}{[CO_2(aq)]} \tag{7}$$

where K_3 was obtained at 25 °C. Then, the equilibrium concentrations $H_2CO_3^*$, H_2CO_3, CO_2(aq), H^+, OH^-, HCO_3^- and CO_3^{2-} can be determined, and the equilibrium pH values and DIC concentrations with respect to pCO_2 are summarized in Table 1. When CO_2(g) is used to increase the DIC concentration, pH decreases with an increase in the partial pressure of CO_2(g) (pCO_2). A maximum DIC

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