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Enhanced carbon utilization efficiency and FAME production of *Chlorella* sp. HS2 through combined supplementation of bicarbonate and carbon dioxide

Manoranjan Nayak[a,b], William I. Suh[b], Bongsoo Lee[a,*], Yong Keun Chang[a,b,*]

[a] Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Advanced Biomass R&D Center, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

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ABSTRACT

The optimum concentration of dissolved carbon in the microalgal culture medium is a vital requirement for enhanced biomass production. The present study investigates the effect of supplying $NaHCO_3$ with CO_2 as the inorganic carbon source to enhance the utilization efficiency of CO_2 for maximum FAME productivity of *Chlorella* sp. HS2. The specific growth rate (0.615 d^{-1}), biomass productivity (530.1 mg L^{-1} d^{-1}), CO_2 biofixation rate (996.4 mg L^{-1} d^{-1}), FAME productivity (141.8 mg L^{-1} d^{-1}) and FAME content (26.76%) were found to be maximized at $NaHCO_3$ concentration of 0.5 g L^{-1} with 1% (v/v) CO_2 enriched air (0.25 vvm flow rate) supplementation in shake flask condition. The FAME productivity (169.37 mg L^{-1} d^{-1}) and FAME content (31.2%) were 1.19 and 1.16 times higher respectively in the flat panel photobioreactor than in shake flask condition. Fatty acid profile and biofuel properties show suitability for biodiesel production. The economic assessment revealed that combining supplementation of both carbon sources greatly reduces the carbon supply costs, from \$8.92 Kg^{-1} when only $NaHCO_3$ was used as the carbon source down to \$0.86 Kg^{-1} when 1% CO_2 was supplied alongside $NaHCO_3$. These findings show that combined application of $NaHCO_3$ and CO_2 is the more cost-effective approach of supplying carbon source to microalgae for FAME production.

1. Introduction

Growing concerns regarding sustainability of proven conventional petroleum reserves have led to an increased research interest for the development of an alternative renewable and sustainable energy resources. One of the alternative transportation fuel that have been suggested is the liquid fuels derived from microalgal biomass. Microalgae recently drew significant attention as a promising feedstock for sustainable biodiesel production and CO_2 mitigation, due to its high photosynthetic efficiency, high lipid content and fast growth rate [1,2]. Microalgae are unicellular organisms can utilize CO_2 as a carbon source for their autotrophic growth via photosynthesis, and is an effective method for CO_2 capture and storage [3]. The captured CO_2 is converted to organic carbon in the form of carbohydrate, protein, and lipids. Different components of the microalgal biomass can eventually be converted to biofuel and other additional products [4,5]. In addition microalgae do not compete for arable land resources and have several advantageous characteristics compared to other energy crops [6].

However, global transition from petroleum refinery based economy to microalgal biorefinery is not feasible yet due to high production cost and limited production scale of microalgal biomass [5]. A way to address both issues simultaneously is by increasing the overall biomass productivity of the microalgae and minimizing cultivation cost. The most effective way to improve the biomass yield of autotrophic cultivation is through supplementation of additional inorganic carbon. This is most commonly done via directly bubbling gaseous CO_2 or through supplementation of bicarbonate into the culture medium, which is often highly inefficient. Hence, to become a feasible option, it is necessary to optimize the carbon concentration for enhancing utilization efficiency of CO_2. This can enhance the CO_2 sequestration, increase biomass and FAME productivity, and decrease the cost of cultivation of microalgae.

The gaseous carbon dioxide (CO_2) or carbonate sources (HCO_3^-) are the two inorganic carbon species used by microalgae in the process of photosynthesis for its biomass growth. The distribution of inorganic carbon sources in aqueous environment is dependent on the pH. CO_2 is the predominant carbon specie in an acidic environment, while HCO_3^- is the most widely available in the pH range from 7 to 10. Most of the previous studies used to supply gaseous CO_2 through bubbling, to increase the carbon concentration in the culture [7]. However, the concentration of dissolved carbon resulting from CO_2 gas dissolution is limited due to low solubility, and as a result the carbon concentration in water is not high enough to meet the desired biomass productivity.

* Corresponding authors at: Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea.
E-mail addresses: bongsoolee@kaist.ac.kr (B. Lee), ychang@kaist.ac.kr (Y.K. Chang).

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Carbon availability is often the limiting factor on algal growth, and as such most microalgal chloroplast contains pyrenoid with high concentrations of Rubisco for effective carbon concentration mechanism [8]. In order to maximize the growth rate, increasing the carbon availability by supplying higher percentage of CO_2 in the culture system is necessary. However, this will result in a large percentage of injected CO_2 being lost to the atmosphere, resulting in algae being unable to sequester a high percentage of CO_2. In addition, the pH of the algal culture medium also dramatically decreases with increase in CO_2 (g) concentration, which also inhibits the growth of microalgae. Lastly, using gaseous CO_2 for industrial scale cultivation requires storage and transportation of large quantities of gas from the power plant to the microalgal cultivation site, which further adds to the cultivation cost.

Some of the previous studies have used a supplementation of sodium bicarbonate ($NaHCO_3$) to increase the carbon concentration in the culture medium for microalgae growth and to avoid CO_2 loss [9]. The supplementation of sodium bicarbonate in the culture medium resulted in enhanced biomass and lipid productivity of microalgae [10,11]. The microalgae can efficiently use sodium bicarbonate as carbon source either by converting it to carbon dioxide via the action of carbonic anhydrase (CA) enzyme or through direct uptake of bicarbonate by cell diffusion [10,12]. Sodium bicarbonate is readily available in large quantities as large number of coal fired power plant deploy CO_2 scrubbing system, and it is much easier to store and transport than gaseous CO_2. However, this system requires a large amount of bicarbonate in order to maintain an optimum concentration of dissolved carbon in the culture for algal cultivation. This is further complicated by the fact that the concentration of $NaHCO_3$ that can be added to the culture medium is limited by its salinity, due to the generation of Na^+ [13]. It also results in the pH increasing during the course of algal cultivation, since the OH^- will be generated as the algae consumes CO_2 during its growth [14].

In order to address these issues, we propose simultaneous supplementation of CO_2 and $NaHCO_3$ for synergistic effect, which would in theory reduce the overall carbon input requirement. This is achieved through enhancing the utilization efficiency of CO_2 as well as maximizing the dissolved carbon availability in the culture during microalgae cultivation. In this case, algae will consume the CO_2 from the $NaHCO_3$ which generates OH^- ions in the solution. The hydroxide will in turn increase the dissolution capacity of the CO_2 in the medium, and maintain the optimum dissolved carbon concentration in the solution. This also minimizes the loss of undissolved CO_2 that returns to the atmosphere. Lastly, the simultaneous use of CO_2 and bicarbonate also has an added benefit of stabilizing the pH of the culture by functioning as a form of a buffer system [13,15]. Supplying both carbon sources simultaneously will counterbalance the problem of consumed HCO_3^- increasing the pH and excess CO_2 decreasing the pH in the culture solution. As the algae steadily consumes HCO_3^- in the medium and generates OH^-, consistent input of CO_2 will form carbonic acid via reaction with water, which will neutralize the OH^- and regenerate HCO_3^-. Therefore, the medium can always maintain its optimum carbon concentration and also remain in a narrow pH range, which favors the biomass growth of microalgae. Improved carbon utilization efficiency resulting from above mentioned synergistic effects between bicarbonate and CO_2 will reduce the cultivation cost minimizing the carbon input while producing greater amount of biomass.

In this study, we demonstrated that the simultaneous addition of $NaHCO_3$ with supplementation of a low percentage of CO_2 in culture media can maintain an optimum concentration of dissolved carbon for improved biomass growth of *Chlorella* sp. HS2. The performance of this system was compared with those that involved the use of only one form of inorganic carbon sources. In addition, the economic assessment of the carbon supplementation was performed to determine the system which results in lowest carbon input cost per FAME produced from the biomass. With further optimization, this method may open up a window for efficient CO_2 sequestration with low-cost cultivation of microalgal for biofuels production.

2. Materials and methods

2.1. Algae strains and culture media

Three freshwater green microalgae strains of *Chlorella* species (*Chlorella sorokiniana* HS1, *Chlorella* sp. HS2 and *Chlorella vulgaris* UTEX-265) were obtained from the Korea Research Institute of Bioscience and Biotechnology, South Korea. These microalgae were cultivated in BG11 medium, which consisted of the following nutrients (g L^{-1}): $K_2HPO_4{\cdot}3H_2O$, 0.04; $MgSO_4{\cdot}7H_2O$, 0.075; $CaCl_2{\cdot}2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; $NaNO_3$, 1.5; Na_2CO_3, 0.02 and 1 mL A5 trace mental solution per liter. The A5 trace mental solution contains 2.86 g H_3BO_3, 1.81 g $MnCl_2{\cdot}4H_2O$, 0.222 g $ZnSO_4{\cdot}7H_2O$, 0.391 g $NaMoO_4{\cdot}2H_2O$, 0.079 g $CuSO_4{\cdot}5H_2O$ and 0.05 g $CoCl_2{\cdot}6H_2O$ in per liter deionized water. The medium was sterilized in an autoclave for 20 min at 121 °C before use for microalgae cultivation. The pH change of the algal culture medium was measured using a standard benchtop pH/Ion meter (Mettler-Toledo SevenCompact Model).

2.2. Inoculum preparation and culture conditions

For inoculum preparation, the algal strains were grown for 3 days in BG11 media until the exponential phase and then centrifuged; the pellet was transferred to fresh culture media with different experimental conditions. Microalgae cultivation was carried out in a 500 mL baffled culture flask with 250 mL working volume. A pure seed culture of microalgae was used for inoculation to the culture medium and the initial culture density for each experiment was maintained at 0.05g L^{-1}. The cultivation was performed in batch mode under continuous illumination of 130 µmol photons m^{-2} s^{-1} and at a temperature of 25 ± 2 °C. Aeration to the algal culture was provided by bubbling into the flask with a flow rate of 0.25 vvm and shaking at 110 rpm. The microalgae were cultivated for 7 days, and these conditions were kept constant for all the experiments. Each set of experiment was done in triplicate. The flasks were placed on a continuous orbital shaker (110 rpm) throughout the experimental period.

2.3. Experimental design under flasks conditions

To obtain an ideal strain for high biomass and FAME productivity, three *Chlorella* species were screened using the BG11 medium in the first part of the study. The selected microalgal strain was used for subsequent experiments. In the next part of the study, to understand the growth profile of microalgae using only bicarbonate as the carbon source, the culture was supplemented with different concentrations of sodium bicarbonate (0, 1, 2.5, 5, 7.5 & 10 g L^{-1}) without aeration of carbon dioxide. In order to determine the synergistic effects of combining bicarbonate and carbon dioxide, different concentrations of sodium bicarbonate (0, 0.1, 0.25, 0.5, 0.75 & 1 g L^{-1}) were supplied with additional aeration of CO_2 (1%). The best conditions obtained from the shake flask study, (0.5 g L^{-1} of $NaHCO_3$ with 1% (v/v) CO_2) was further up-scaled in a 2 L flat-panel photobioreactor. Each experiment was performed in triplicate.

2.4. Experiments under photobioreactor conditions

Scaled up cultivation was performed in a 2 L flat-panel photobioreactor with height, width, and thickness of 335, 220, and 30 mm, respectively [16]. The reactor components included a plastic frame made of polyvinyl chloride (PVC) and two flat panels made of transparent polycarbonate sheets with a working volume of 2 L. The cultures of microalgae were mixed by circulating air bubbles, which were supplied through a bubble tube at the bottom of each FP-PBR at a