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Cost-efficient cultivation of *Spirulina platensis* by chemical absorption of CO_2 into medium containing NaOH

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Abstract–*Spirulina* is one of the promising photosynthetic microorganisms as the source for food, cosmetic, and other value-added products such as phycocyanin. However, its production cost associated with cultivation is expensive because of high concentration of bicarbonate (76 wt%) in standard Zarrouk medium for *Spirulina*. Bicarbonate not only acts as a carbon source but also helps maintaining culture medium in alkaline condition, which is essential for growth of *Spirulina*. The present study demonstrates that bicarbonate (16.8 g/L) in standard Zarrouk medium was completely replaceable by chemical CO_2 absorption using 0.2 M NaOH and 154.2 mmol CO_2 gas could be absorbed in the form of $NaHCO_3$/Na_2CO_3 into 1 L medium during the process. This process was incorporated into *Spirulina* cultivation, which enabled us to reduce the total cost of preparation of Zarrouk medium by 34.3% without sacrificing biomass and pigments production. In addition, another important use of NaOH is achievement of cost-efficient and eco-friendly sanitization of culture medium.

Keywords: Chemical Absorption, Sodium Hydroxide, Carbon Dioxide, Zarrouk Medium, *Spirulina platensis*

INTRODUCTION

Spirulina platensis (hereafter *S. platensis*) is a blue-green algae with many beneficial properties. Used as a food source for centuries in Africa, America, and Asia, *S. platensis* has high quality proteins, pigments, carbohydrates, lipids, and many other essential nutrients such as vitamin, anti-oxidants [1]. Although mass production of *S. platensis* has been in progress, due to high usage of bicarbonate in growth medium for *S. platensis*, its production cost associated with cultivation is still relatively expensive. Bicarbonate is not only used as a carbon source for triacylglycerol and starch but also helps to maintain alkaline conditions, which are essential for the growth of *Spirulina* [2].

Carbon dioxide (CO_2), which constitutes up to 68% of total emissions of greenhouse gases (GHGs), has been an important issue in global economics and politics, as well as the environment [3,4]. Absorption is a common physical or chemical process for the treatment of industrial gas streams. Inorganic bicarbonate for cultivation of microalgae can be supplied by chemical absorption of gaseous CO_2, resulting in soluble inorganic carbon such as $NaHCO_3$ and Na_2CO_3 [5-7]. Among the chemicals used for CO_2 absorption, strong hydroxides such as sodium hydroxide have the advantages of high load capacity and fast reaction time due to high binding energy of chemical sorbents [6].

S. platensis is in general cultivated in Zarrouk medium, which contains sodium bicarbonate (76 wt%) as a major component (Zarrouk, 1966). The potential use of absorption of CO_2 using NaOH was investigated and presented in this research, in terms of chemical sterilization and replacement of $NaHCO_3$ by NaOH in standard Zarrouk medium for cultivation of *S. platensis*. The overall process is illustrated in Fig. 1. The production of biomass and photosynthetic pigments such as chlorophyll and phycocyanin was measured to validate sustainable growth of *S. platensis* in the prepared Zarrouk medium via chemical absorption of CO_2 using NaOH.

MATERIAL AND METHODS

1. Preparation of Cultivation Medium and Chemical Absorption of CO_2 by 0.2 M NaOH

S. platensis was obtained from the Korea Marine Microalgae Culture Center (KMMCC). Standard Zarrouk medium consists of 16.8 g/L $NaHCO_3$, 0.5 g/L K_2HPO_4, 2.5 g/L $NaNO_3$, 1.0 g/L K_2SO_4, 1.0 g/L NaCl, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.04 g/L $CaCl_2 \cdot 2H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.08 g/L EDTA, and 1 ml micronutrient solution. The micronutrient solution consisted of 2.86 g/L H_3BO_3, 1.81 g/L $MnCl_2 \cdot 4H_2O$, 0.222 g/L $ZnSO_4 \cdot 4H_2O$, 0.0177 g/L Na_2MoO_4, and 0.079 g/L $CuSO_4 \cdot 5H_2O$ (Zarrouk et al. 1966). For chemical absorption of CO_2, 0.2 M NaOH was added to the modified Zarrouk medium, lacking sodium

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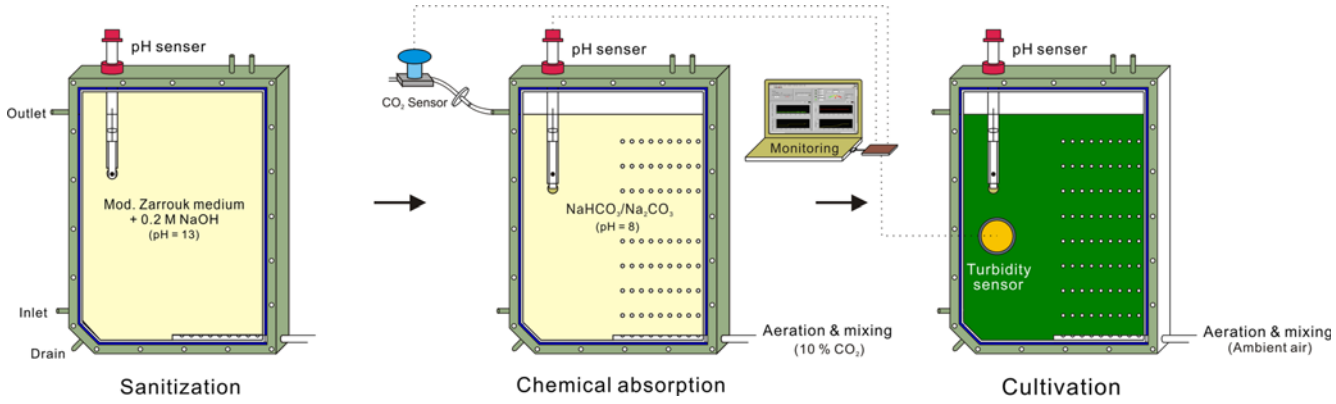

Fig. 1. Overall process diagram of chemical absorption by NaOH solution from sanitization to cultivation.

bicarbonate. The concentration of NaOH was set to match the amount of bicarbonate in standard Zarrouk medium. This modified medium was added to a closed 2 L flat-panel photobioreactor system, followed by aeration with a constant flow of 200 ml min^{-1} air enriched with 10% CO_2 (v/v) from a perforated plastic tube embedded in the bottom of the reactor. Two modified Zarrouk media at pH 8 and 9 were set by time-dependent CO_2 aeration, i.e., 10 and 7.5 hours, respectively, while standard Zarrouk medium has a pH of 8.7. During cultivation of *S. platensis*, temperature was maintained at 25 °C, and culture was continuously illuminated at a light intensity of 100 μmol photons m^{-2} s^{-1} and mixed with ambient air containing 0.04% CO_2 at a constant flow of 200 ml min^{-1}.

2. Sanitization by NaOH

Prior to chemical absorption of CO_2, the reactor system was chemically sterilized by incubation with 0.2 M NaOH solution (pH=13), while standard Zarrouk medium was sterilized in an autoclave. Disinfectant effect of 0.2 M NaOH as a function of incubation time was verified by CFU tests (n=10) using two different agars, TYG (5 g/L tryptone, 5 g/L yeast extract, 1 g/L glucose, 1 g/L K_2HPO_4, 15 g/L Bacto™ agar) and LB (25 g/L LB Broth, 15 g/L Bacto™ agar). Bacteria-contaminated medium was treated with 0.2 M NaOH for different incubation time: 1, 2, 3, and 4 hours. Medium treated with 0.2 M NaOH was plated onto the agars and incubated for 2 days at 37 °C under light intensity of 50 μmol photons m^{-2} s^{-1}. After this short incubation period, the number of individual bacterial colonies on each plate was counted. Bacterial populations were identified by DNA sequencing based on 16S rDNA blast library.

3. Measurement of pH and CO_2 Concentration

CO_2 concentration absorbed in culture medium was calculated in terms of the detected partial pressure of CO_2 in emission gas by a BCP-CO_2 sensor (BlueSens GmbH, Herten, Germany). The sensor was directly connected with the exhaust-gas stream of the fermenter using a flow adapter. The gas flow rate was estimated as 200 mL min^{-1} to calculate a molar concentration of CO_2 in exhaust gas. The change of pH level was on-line monitored by a pH meter (Horiba Co. Ltd., Kyoto, Japan) equipped inside the photobioreactor. Measurements of pH value and CO_2 concentration continued until both reached a steady state.

4. Analytical Methods

To measure biomass concentration, equal volumes of biomass culture were filtered through GF/C glass fiber filter paper, which was pre-dried at 50 °C for 24 h. The differences between weights of filter paper before filtration and dried filter paper after filtration were calculated. Concentrations of chlorophyll *a* [8] and phycocyanin [9] were measured during cultivation.

RESULTS

1. Sanitization by NaOH

The antimicrobial activity of aqueous solutions of 0.2 M NaOH was investigated by CFU tests on LB and TYG agar plates. Medium contaminated by bacterial population indicated an initial colony-forming unit of $1.57\pm0.11\times10^5$ CFU mL^{-1} on LB agar plates and $1.24\pm0.14\times10^5$ CFU mL^{-1} on TYG agar plates. In contaminated medium, there were the majority of five bacterial strains detected; *Tristrella, Pseudomonas, Stappia, Labrenzia* and *Microbacterium*. After 1 hour incubation with 0.2 M NaOH solution, no colony was formed on either plate.

2. Chemical Absorption of CO_2 by Aqueous NaOH Solution in Modified Medium

Fig. 2 shows pH changes of medium and the concentration of CO_2 in the emitted gas over 12 hours, with both appearing as double logistic sigmoid curves. More than 95% of the infused CO_2 was converted to aqueous form of carbon dioxide for the first 2 hours, and half of the total infused CO_2 was still absorbed until the first sigmoid change appeared.

The pH value of the solution is of major importance in equilibrium of this reaction system. Different equilibrium conditions were observed following absorption of CO_2 gas by alkaline aqueous solution. The pH value decreased nonlinearly as a function of treatment time, and its value was not lower than pH 8.0 due to the formation of a carbonate-bicarbonate buffer (Fig. 2(a)). The fraction of bicarbonate among inorganic carbon sources decreased when the pH decreased. After 10 hours of CO_2 injection, the solution was completely saturated with aeration containing 9.87% of CO_2 (Fig. 2(a)).

Fig. 2(b) presents the amount of CO_2 absorbed into modified medium when aerated with 10 % CO_2 gas at a rate of 200 ml min^{-1}. While the pH decreased from 13 to 8, 154.2 mmol CO_2 gas was absorbed into 1 L of medium. Assuming working volume of 1,000 L for the preparation of modified medium, this process could cap-

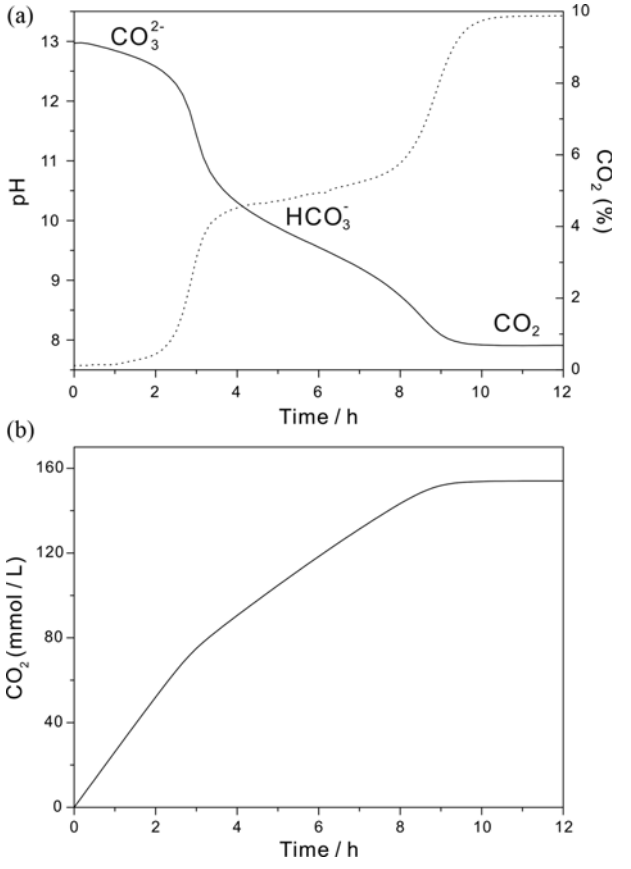

Fig. 2. (a) Chemical absorption of 10% (v/v) CO_2 gas into modified Zarrouk medium containing 0.2 M sodium hydroxide. Changes in pH value inside the reactor are indicated as solid lines. CO_2 concentration in the gas emitted from the reactor is recorded as dashed lines. (b) Total absorbed inorganic carbon concentration in medium.

ture 6.78 kg of CO_2 gas prior to cultivation of *S. platensis*. In addition, the emitted CO_2 gas into air could be recycled and reused for additional chemical absorption process.

3. Growth of *Spirulina platensis*

Cultivation conditions such as pH, temperature and nutrients have an effect on biomass accumulation by *S. platensis*; 1.5-3 g/L biomass accumulation by *S. platensis* was reported in previous research [10,11]. In this research, 2.5 g/L of biomass was accumulated in photoautotrophic conditions. From the cultivation results, no significant change in biomass and pigment production was monitored among cells grown in the media prepared by chemical absorption and in the standard Zarrouk medium (Fig. 3). In conclusion, this research demonstrated that bicarbonate, essential for growth of *S. platensis*, could be successfully replaced by chemical absorption of CO_2 using 0.2 M NaOH without loss in production of biomass and pigments.

DISCUSSION

Post-combustion CO_2 capture can be accomplished by chemical absorption, adsorption, membrane separation, cryogenic distillation, and chemical looping combustion [12-14]. Although these

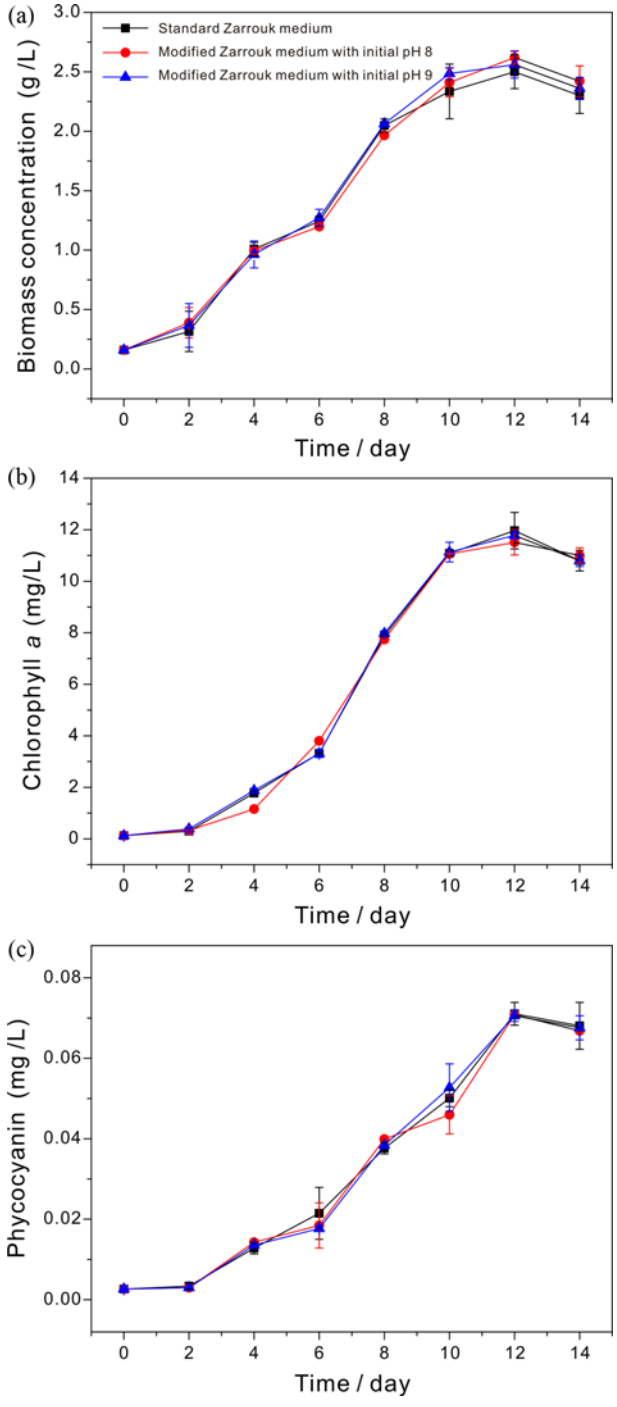

Fig. 3. Biomass (a), C-phycocyanin (b), and chlorophyll *a* (c) contents of *S. platensis* cells grown in modified Zarrouk media (pH 8 and 9) and standard Zarrouk medium. The plotted values indicate the average value of three independent measurements within standard errors.

physical and chemical methods are well-developed and show high efficiency and capacity for CO_2 capture, they have a few drawbacks in high energy consumption, large space requirements, and difficulties in utilizing the captured CO_2 for valuable products [15]. Moreover, additional energy penalties can be incurred from compression and disposal of concentrated CO_2 complexes in these pro-

cesses [16]. Conventional methods such as underground CO_2 storage, fertilizer production, and usage in food and beverage industry are limited in their ability to store all CO_2 captured from fossil fuel power plants [17]. New alternatives of CO_2 capture technology, including biological fixation using microalgae and cyanobacteria, have recently received attention for their ability to biochemically convert CO_2 into biomass, which can be utilized as a platform for the production of biofuels, foods, cosmetics, and medications [18]. However, biological CO_2 fixation rate by microalgae is much lower than those of physical and chemical technologies of CO_2 capture. Furthermore, additional methods are required to drastically reduce the amount of CO_2 lost to the atmosphere due to its low solubility in pure water.

These limitations of respective treatment of CO_2 can be overcome by a method involving combinations of biological methods and chemical absorption. NaOH present in medium will react with dissolved CO_2 gas, to the extent determined by the pH of culture medium. This will cause consumption of OH^- by CO_2, decrease of pH, and conversion of CO_2 gas into inorganic carbon in growth medium, presented in Fig. 2. Chemically captured CO_2 can later be biologically converted into organic compounds by photosynthetic microorganisms (*S. platensis* in this study). The economically valuable products by photosynthetic microorganisms would offset the costs of CO_2 capturing absorbents and plant maintenance, although other costs such as isolating valuable product from remaining biomass mixtures have to be considered. CO_2 absorption capacity (kg CO_2/kg absorbent) by several absorbents is presented in Table 1. From our experimental results, the chemical absorption by NaOH showed high CO_2 absorption capacity of 0.85 (kg CO_2/kg NaOH). This CO_2 convergence process using NaOH for *S. platensis* cultivation has several advantages: fast chemical absorption of CO_2 gas, reduction of cultivation costs by replacing $NaHCO_3$ with NaOH in standard Zarrouk medium for growth of *S. platensis*, biological conversion of fixed CO_2 into valuable compounds, and decreased sterilization costs using alkalinity of NaOH solution. Depending on the cultivation place of *S. Platensis*, there may be either a carbon tax or carbon credit, which provides the minus value of carbon dioxide in conjunction with economic analysis. In this study, it is assumed that the place of *S. Platensis* cultivation is located in Korea, which at the moment provides the carbon trading market at approximately US$10 per CO_2 ton (Table 2). Based on the industry sources (http://www.kosep.co.kr and http://kospo.co.kr), the emitted gas from coal powered plant has the approximate composition of CO_2 10.2%, O_2 2.8%, and N_2 87%. The emitted CO_2 from coal-powered plant can be directly applied for reaction with sodium hydroxide. Although a comprehensive analysis of including labor cost, *Spirulina* product price, raw material, interest rate, capital investment etc., will be considered in this work for the purpose of small-scale economic feasibility, we limited the economical viewpoint within the advantages due to substitution of nutrient related with CO_2 absorption. In conclusion, the emitted gas composition of coal-powered plant can be used directly on a commercial scale. This work presents not only the reduction in preparation cost of culture medium for *Spirulina* by 34.3% but also a distinct advantage involved with global warming.

Sterile conditions in a closed system, which is important for reliable mono-cultivation, can be usually achieved by heat- and chemical-treatment. Autoclave is the most reliable method to eliminate population of fungi, bacteria, viruses and spores, although it is energy-consuming and inappropriate for heat-sensitive materials such as plastics and electronic sensors. Chemical sterilization, widely used medically, avoids the problem of heat damage. However, its proce-

Table 1. CO_2 absorption capacity by several chemical absorbents

Absorbent	CO_2 absorption capacity (kg CO_2/kg absorbent)	
NaOH	0.85	In this study
Monoethanol amine (MEA)	0.40	[17]
NH_3 (Aqua ammonia)	1.20	〃
Dual alkali (MEA+NaCl)	0.54	〃
Na_2CO_3	0.73	〃
Aminoethylethanolamine (AEEA)	0.42	[7]
Piperazine (PZ)	0.51	〃
Methyldiethanolamine (MDEA)	0.37	〃
2-Amino-2-methyl-1-propanol (AMP)	0.49	〃
Diethylenetriamine (DETA)	0.43	〃

Table 2. Cost advantage by CO_2 absorption at the preparation of culture medium

	Concentration (g/L)	Cost ($/kg)[a]
$NaHCO_3$	16.80 (Zarrouk medium)	0.503[a]
NaOH	8.00 (used amount in the study)	0.701[a]
CO_2 (Carbon Tax, Korea)	6.79 (absorbed amount in the study)	−0.009[b]

[a]ICIS.COM (2008) Indicative Chemical Prices A-Z, retrieved on August 20, 2014, from http://www.icis.com/chemicals/channel-info-chemicals-a-z/

[b]Estimated Carbon Emission Trading Price, Korea, http://www.gir.go.kr

dures require multiple washes with sterile water after treatment with sterilizing agents. These chemicals are often toxic and can be harmful to the environment. Therefore, a cost-efficient and eco-friendly sterilization process is necessary for validating the economic feasibility and achieving sustainable technology of microalgae-based biorefinery. Sodium hydroxide is widely used for cleaning and sanitization [19]. In the present study, incubation of culture medium with NaOH for 1 hours resulted in successful reduction of bacterial populations. High alkalinity induced by NaOH was neutralized to pH 8 or pH 9 by chemical absorption of CO_2 gas prior to cultivation (Fig. 2). The applied chemical absorption process using NaOH could avoid additional cost-consuming steps caused by unavoidable neutralization and washing steps when other chemical sterilants were used. This approach of sanitization can be applied to cultivations of other microalgae and cyanobacteria, which require large amount of bicarbonate for their growth. In this work, *S. platensis* served as a model organism.

CONCLUSIONS

This work describes a sequential process involving chemical absorption of CO_2 gas and cultivation of *S. platensis*. Bicarbonate in standard Zarrouk medium could be completely replaced by inorganic carbonic ion, formed by chemical absorption of 154.2 mM CO_2 using 0.2 M NaOH. In conjunction with the carbon emission trading market in Korea, it is assumed that the cultivation place of *S. Platensis* is located in Korea and, accordingly, this process enabled us to reduce the preparation cost of Zarrouk medium by 34.3% via complete replacement of $NaHCO_3$ by NaOH (Table 2). Moreover, high alkalinity induced by NaOH solution could be used for cost-efficient and eco-friendly sterilization of culture medium. In this research, the use of inorganic carbon sources via chemical absorption of CO_2 does not decrease the amount of biomass and pigments in *S. platensis* compared to using standard Zarrouk medium.

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