Oxygen removal efficiency from microalgal culture by gas-permeating photobioreactor

ガス透過型光バイオリアクターによる微細藻類培養液からの溶存酸素除去特性

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SYNOPSIS

微細藻類は健康食品・化粧品・天然着色料などの原料として近年研究・実用化が進んでいる。微細藻類の生産では、特に 純度の高いバイオマスを高効率で生産できる閉鎖型リアクターによる高密度培養において、溶存酸素の蓄積による生育 阻害が問題となる。そこで本論文では新たな低動力の溶存酸素除去法の確立を目指して、(1)拡散により溶存酸素を除 去可能な新規ガス透過型光バイオリアクターの開発、(2)新規リアクターに適した高濃度 CO2 供給法の開発、(3)それ らの実用化に向けたエネルギー性評価を実施した。ガス透過型光バイオリアクターの開発では、シリコーンや多孔 質膜な どの各種気体透過膜の酸素透過性や光学特性を比較した。その結果、疎水性多孔質膜がシリコーンの約 3000 倍高い酸素 透過性と 80%以上の高い光反射性を持っていることが明らかとなり、片側透明・片側多孔質膜の薄型バッグリアクター 形状が藻類培養に適していることを示した。また開発されたガス透過型光バイオリアクターが、既存の溶存酸素除去技術 である曝気法や従属性微生物共培養と比較して、エネルギー性および応用性において優位であることを示した。ガス透過 型光バイオリアクターにより微細藻類大量培養に必要な曝気エネルギーを削減できる一方で、曝気による CO2 供給量が 減少し、培養中に無機炭素が枯渇する可能性がある。そこで続く CO2 供給法の検討では、新規リアクターに適した手法 として、吸収塔で CO2 を重炭酸イオンに変換し、高濃度重炭酸溶液を培養槽に供給する 2 槽式 CO2 供給温の検討を行っ た。その結果、2 槽式 CO2 供給法は CO2 回収効率および無機炭素保持能が非常に高く、培養中の CO2 枯渇を解決出来る 運転法であることが明らかとなった。さらに 2 槽式 CO2 供給法を用いてガス透過型光バイオリアクターで Arthrospira platensis の連続培養を実施したところ、曝気・撹拌頻度を従来の 10 分の 1 に減少した条件で、従来リアクターの 1.6 倍 のバイオマス生産速度 1.98 gDW L⁻¹ d⁻¹が得られた。新規リアクターでの培養に 2 槽式 CO2 供給法を用いることで、曝気 動力を抑えた条件で微細囊類の高い生産性を維持可能であることを示した。さらに、本研究で開発されたりアクターの 2.6 倍 のメイオマス生産速度 1.98 gDW L⁻¹ d⁻¹が得られた。新規リアクターでの培養に 2 槽式 CO2 供給法を用いることで、曝気 動力を抑えた条件で微細嚢切られをころ、バイオマス生産にかかるエネルギー量が従来のチューブ型やフラットパネル型リアクター と比較して 4-20 分の 1 に削減出来ることを示した。本研究によってガス透過型光バイオリアクターで低動力での溶存 酸素除去が可能であり、曝気をほとんど行わずに高効率でバイオマス生産が可能であることが示された。

Keywords: algal mass cultivation, alkalihalophilic, bag reactor, CO₂ supply, cyanobacteria, dissolved oxygen, microalgae, gas-permeable membrane

INTRODUCTION

Microalgae is widely-cultured among the world to produce cosmetics, neutraceuticals, feed, and food supplements. While high-density culture in a closed bioreactor is desirable for a high biomass productivity with high purity, algal photosynthesis may lead to accumulation of dissolved oxygen (DO) over 600% (Vonshak 1997; Torzillo et al. 1998). The DO inhibition could lead to up to 30-60% decrease of biomass production (Marquez et al. 1995; Vonshak et al. 1996; Torzillo et al. 1998; Clausen and Junge 2005). Conventionally, DO has been removed through aeration. As a less energy-intensive alternative, a novel gaspermeating bag reactor may enable less energyconsuming DO removal through diffusion. Previous studies have suggested the utilization of gas-permeating films for internal DO removal structures (Willson et al. 2009; Oh et al. 2012). In this study, a bag reactor whose outer surface is constituted of a relatively cheap microporous gas-permeating film was suggested as a costeffective, energy-saving, oxygen-removing closed reactor.

While the gas-permeating reactor may allow reduced aeration during algal culture, inorganic carbon could be limited due to the reduced CO_2 supply with aeration. Inorganic carbon supply in the form of bicarbonate (HCO₃⁻) has been recently gathering attention, because of over 1,000 times higher solubility of HCO₃⁻ than CO₂. The two-phase CO₂ supply process is one of those HCO₃⁻ based process that enables efficient CO₂ recovery and

prolonged algal inorganic supply (Chi et al. 2011; González-López et al. 2012). In the two-phase CO₂ recovery process, potentially inhibitory CO₂ is immediately converted into less harmful bicarbonate (HCO₃⁻) in a CO₂ absorption column (Eq. 1), which is filled with carbonate (CO₃²⁻)-rich alkaline solution (Chi et al. 2013). The CO₃²⁻ acts as a pH buffer in the absorption column and prevents pH decrease (Eq. 2):

$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$	(1)).
	(-)	<i>,.</i>

 $\mathrm{CO}_3^{2-} + \mathrm{H}^+ \rightleftharpoons \mathrm{HCO}_3^- \tag{2}.$

The carbon fraction converted into HCO_3^- is then transferred to an algal photobioreactor (PBR), in which HCO_3^- is utilized by algal photosynthesis (Eq. 3):

 $HCO_3^- \rightarrow CO_2(algal fixation) + OH^-$ (3) and used for pH counterbalancing (Eq. 4):

$$HCO_3^- + OH^- \rightleftharpoons CO_3^{2-} + H_2O$$
(4).

Note that the CO_3^{2-} used in Eq. 2 to buffer pH reduction is regenerated with the OH⁻ (Eq. 4) produced by photosynthesis (Eq. 3). The photosynthetic regeneration of CO_3^{2-} enables low-energy operation, unlike physicochemical processes in which absorbent is regenerated with heat (100-150°C). The two-phase CO_2 recovery process not only prevents inorganic carbon limitation during algal culture without aeration, but also may enable a high CO_2 recovery efficiency from waste gases such as flue gases from power plants, steel mills, and chemical plants. Furthermore, the process may also be applied for enhancing the gas quality of methane gas from anaerobic digestion, which contains CO_2 up to its 60%. This CO_2 supply process is suitable for alkalihalophilic microalgae, which can accept very high dissolved in organic carbon. *Arthrospira platensis* is a representative alkalihalophilic cyanobacterium with wide commercial applications, which grows optimally at an alkaline (pH 9.8), high dissolved inorganic carbon (DIC; 0.23 mol L⁻¹), and high temperature (34°C) (Kishi and Toda 2017). However, the physicochemical characteristics of this twophase CO_2 recovery process especially with *A. platensis* have not been demonstrated, and the optimum process parameters need to be studied.

Therefore, in this Ph.D. thesis, to improve the costeffectiveness and environmentally friendliness of *A*. *platensis* production, the following objectives were studied:

- 1) To develop a novel gas-permeating bag photobioreactor and to critically compare its performance with other existing oxygen removal techniques
- To construct an optimized operational model for nonaerated photobioreactor, combined with two-phase CO₂ supply system, and critically evaluate the performance of the process
- 3) To critically evaluate the energy and cost effectiveness of the developed reactor

MATERIALS AND METHODS

Study 1. Development of a novel gas-permeating photobioreactor and comparison with other oxygen removal technologies

Firstly, to develop an efficient gas-permeating bag reactor, the oxygen permeability of various gaspermeating films (silicone, fluoro resin, and microporous film) and non-permeating films (polyethylene [PE] and polypropylene [PP]) sheets were evaluated. The gas-gas oxygen exchange was measured with a pressure sensor method, with a maximum pressure difference between above and below films being 0.23 MPa. The liquid-gas oxygen permeation was measured using an acrylic reactor consisting of a liquid container filled with oxygensaturated water and a gas container separated by the respective films. The liquid-gas oxygen permeability was used to model DO removal. The optical characteristics of the films were evaluated with a spectrophotometer equipped with an integrating sphere.

The selected microporous film (thickness 0.02 mm, pore size 0.2 μ m) was adopted for an gas-permeating bag reactor. The reactor was made into $600 \times 450 \times 20$ mm (height × width × thickness) with 0.02 mm PP film. The film was heat-sealed to a PP film (thickness 0.02 mm) to construct a reactor. Dissolved oxygen removal characteristics were evaluated with a decrease in DO concentration from oxygen-saturated water under continuous mixing of the water with a diaphragm pump. During the continuous culture, the bag reactor was aerated for 1 minutes in every 20 minutes to remove DO and to mix the culture. The medium was agitated for 12 seconds

in every 2 minutes with a small centrifugal pump. The medium supply and discharge were continuously implemented with a peristaltic pump. Dilution rate was set at 0.5 d⁻¹. Photosynthetic photon flux density (PPFD) was increased in steps from 100 to 325 and 800 μ mol m⁻² s⁻¹. The pH of culture was controlled at 9.8 with CO₂.

Study 2. Physicochemical characterization of the twophase algal CO₂ recovery process

A preliminary batch growth optimization study was conducted based on the central composite design (CCD). It was aimed to clarify the effect and the optimal values of temperature, pH, DIC, and NaCl concentration. The test range of each parameter was determined in preliminary experiments. A total of 30 runs were operated with 6 replicates for the center point. The specific growth rates at the exponential growth phase were analyzed for the elucidation of the optimal culture conditions.

The semi-continuous two-phase CO₂ recovery process was conducted using a set of two reactors: an absorption column and a PBR, under the optimized condition (Fig. 1). Three different CO₂ supply rates, 0.4, 0.8 and 1.2 L L-PBR⁻¹ d⁻¹ (Run 1-3), were tested to clarify their effects on CO₂ fixing rate, biomass productivity, and stability of the process.

The absorption column was a closed 1-L glass medium bottle with an active volume of 0.4 L. CO_2 was supplied by replacing the headspace of the absorption column. The absorption column was placed in an incubator at 25°C under dark, and the absorbent was stirred continuously with magnetic stirrers approximately at 200 rpm. The PBR was a glass column reactor with an active volume of 1 L and inner-diameter of 106 mm. The PBR was incubated at 35°C with 24-hour continuous light with the surface photosynthetically available photon flux densities (PPFD) of 300 µmol photons m⁻² s⁻¹.

Every 24-hour, the 0.2 L of the medium was exchanged between the two reactors aseptically (Fig. 1). The hydraulic retention times (HRT) of the absorption column, the PBR, and the entire system were 1.8, 4.75, and 18 days, respectively. The experiment was continued for 18 days.

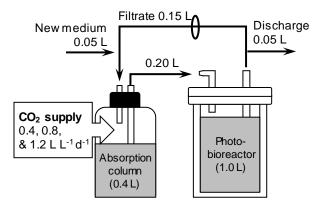


Fig. 1. Experimental set-up of the semi-continuous CO₂ recovery process. Exchange of medium between two reactors was conducted every 24 hours.

Based on ionic equilibrium, charge balance, and Fick's law of diffusion, models were created for the pH change during CO_2 absorption and the amount of maximum CO_2 absorption at different CO_2 supply rates. Carbon mass balance was calculated based on the CO_2 supply, DIC, and carbon fixation by *A. platensis*.

The two-phase CO_2 supply was integrated into the A. platensis culture with gas-permeating reactor. A CO_2 absorption column (effective volume 1.9 L) was supplied with $CO_2:N_2 = 40:60$ gas. The amount of the gas supplied was calculated from the carbon fixation on the previous day based on the model created in the previous experiments.

RESULTS AND DISCUSSION

Study 1. Development of a novel gas-permeating photobioreactor and comparison with other oxygen removal technologies

In this study, firstly, various gas-permeating films were comparatively evaluated for the bag material. The gas-gas oxygen permeabilities of silicone and fluoro resin were approximately 80 times higher than PE films, but the microporous film exhibited 3,000 times higher permeability than those. Thus, it was suggested that microporous film was a suitable material for creating gaspermeating bag reactor. However, the optical transmittance of the microporous film was found to be less than 20%, which was not suitable to be placed on the front surface of the bag reactor. On the other hand, the reflectance of the film was close to 80%, indicating that most light exposed to the film is not absorbed but reflected. Therefore, a bag reactor with the microporous film on its backside was suggested to be effective in fully utilizing the light provided to it, since the film can return the unutilized light back to the algal culture.

Secondly, the DO removal rate (K_L) of the microporous film was compared with non-gas permeating PE film. It was found that the microporous film has approximately 30 times higher K_La than that of a PE film (Fig. 4). The K_L of the microporous film was calculated to be 0.069 m min⁻¹ in this setting. Based on assumption that the same moles of O₂ are produced as much as the moles of CO₂ fixed by the cyanobacteria, the required specific surface to maintain relatively less inhibitory DO 200% with biomass production rate 1.0 gDW L⁻¹ d⁻¹ was calculated to be 88 m⁻¹, which was assumed to increase K_La to 0.13 min⁻¹. Therefore, an gas-permeating bag reactor of specific surface 85 m⁻¹ was created (Fig. 2).

The DO removal efficiency of the constructed gaspermeating reactor was tested with oxygen-saturated water. Although the $K_{L}a$ was lower than the target value, probably due to the difference in mixing condition from the preliminary DO removal experiment, the $K_{L}a$ of the novel reactor was increased to 0.061 min⁻¹, which was over ten times higher than the non-gas-permeating PP bag reactor. It was demonstrated that the gas-permeating reactor composed of the microporous film was effective in removing DO without aeration.

The average A. platensis biomass production rate

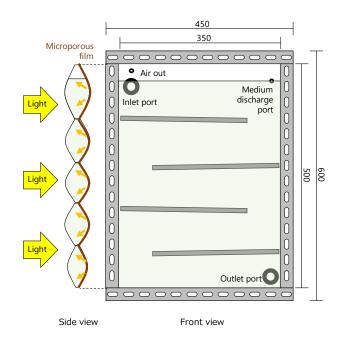


Fig. 2. Schematic diagram of the constructed bag reactor. The gas-permeable bag reactor was created by attaching the microporous film to polypropylene film on the backside of the reactor. The polypropylene and microporous bag reactors were designed to be horizontally flipped. All units are in millimeters.

using the gas-permeating reactor was 1.98 gDW L⁻¹ d⁻¹ under the PPFD of 325 μ mol m⁻² s⁻¹. This rate was nearly 1.5 times higher than that in the conventional plastic bag reactor under the same condition. It was suggested that, although significant DO differences were not observed between the developed reactor and PP reactor, the oxygen was likely removed in the backside of the reactor, and the change in DO was compensated by the increased oxygen production by *A. platensis* of higher density. While previous studies (Ito et al. 1998; Tan et al. 2005) reported DO removal with hollow fiber modules, this study demonstrated the possibility of the optically efficient,

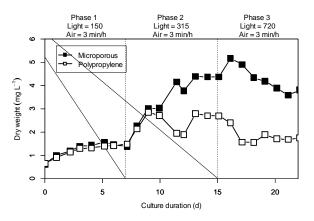


Fig. 3. Algal dry weight at different culture condition in microporous film and polypropylene film bag reactors.

simple, and cost-effective bag reactor that can remove DO without extensive aeration to increase the productivity.

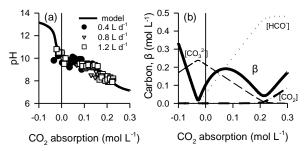


Fig. 4. Estimation of (a) pH (b) inorganic carbon species and buffer capacity β , from change in dissolved inorganic carbon (Δ DIC) with CO, absorption.

Study 2. Physicochemical characterization of the twophase algal CO₂ recovery process

The CCD analysis revealed the optimal culture conditions of *A. platensis* to be pH 9.8, DIC 0.23 mol L⁻¹, and temperature ca. 34°C, which is highly alkalihalophilic compared with common species such as *Chlorella* (pH 6.2, DIC 0.01 mol L⁻¹) and *Haematococcus* (pH 7, DIC 0.02 mol L⁻¹) (Kang et al. 2005; Yeh et al. 2010). Variations in pH and temperature were found to have relatively stronger effects on the growth than NaCl and DIC.

The semi-continuous operation of the two-phase algal CO_2 recovery process was operated with *A. platensis* at three different CO_2 supply rates (Run 1: 0.4; Run 2: 0.8; and Run 3: 1.2 L L-PBR d⁻¹) for 18 days. The CO_2 recovery efficiencies in Run 1, 2, and 3 were 115, 94 and 63%, respectively. While most of the supplied CO_2 was recovered in Run 1 and 2, only half was recovered by Run 3, owing to the pH reduction in the absorption column.

The pH of the absorption column and the PBR fluctuated with CO₂ supply and CO₂ utilization by the cyanobacteria. In Run 2 and 3 with high CO₂ supply rates, the pH of the absorption column largely decreased with CO₂ absorption during 24-hour of incubation. However, the pH recovered back to over 9.5 with a supply of PBR medium owing to the strong buffer function of the SOT medium. The pH in Run 1 relatively remained stable, because the CO₂ supply and algal CO₂ fixation were more balanced than the other two. Since the biomass production rate of A. platensis remained in the same range (on average 0.27-0.31 gDW L⁻¹ d⁻¹) regardless of the CO₂ supply rate, the higher CO₂ supply resulted in the excess of CO₂ supply. The carbon mass flux analysis revealed that, while 59% of the supplied CO_2 was fixed by A. platensis, in Run 1, only 34 and 21% were fixed in Run 2 and 3, respectively. Instead, 60 and 70% of absorbed CO₂ remained in the form of DIC in Run 2 and 3, respectively. The imbalance of carbon supply and fixation led to the accumulation of DIC and caused drastic pH reduction.

The successfully constructed model (Fig. 4-a) revealed that the modified SOT medium can buffer pH with the CO₂ absorption of up to 0.17 mol L⁻¹ (half of the maximum β ; Fig. 4-a,b). When the CO₂ absorption exceeded this buffer range, the pH dropped drastically. The reduction in pH not only interferes the CO₂ recovery but also damages the growth of *A. platensis*; e.g., over

50% decrease with pH reduction from 9.8 to 8. While the balance between CO_2 supply and the carbon fixation rate is highly demanded, the current medium can buffer at least 1.6 and 4.1-fold fluctuations of the CO_2 supply rate and the fixation rate, respectively. Although some other freshwater algal CO_2 recovery studies suffer from large pH fluctuation (Kao et al. 2012; Bahr et al. 2014), the two-phase CO_2 recovery with *A. platensis* achieved successful CO_2 recovery with high pH stability.

The two-phase CO₂ supply was combined with the developed gas-permeating reactor. The supplied CO₂ reached equilibrium and the CO₂ concentration at the exit was less than 1.5% continuously. There was pH gradient between CO₂ absorption column and the photobioreactor, but higher circulation rate improved the stability. The two-phase CO₂ supply system allowed virtually no aeration system in the gas-permeating reactor.

The cost analysis revealed that, in a 100-ha scale cultivation, the energy cost for biomass production can be reduced to 4 to 20 times lower than conventional tubular and flat panel reactors at the maximum. The analysis also revealed the relatively high cost of microporous film in the production cost, but the total cost can be lower than other reactors if the film cost is reduced.

This study demonstrated that the combination of the gas-permeating photobioreactor and the two-phase CO_2 recovery process can be successfully operated, and that it enables a high production efficiency and with less energy consumption than the conventional method.

SUMMARY

The present study developed (1) the novel gas-permeating bag photobioreactor anad (2) the two-phase CO₂ supply process. The key findings were that (a) Microporous film exhibited a higher oxygen permeability than other gaspermeating sheet made of silicone or fluoro resin; (b) The gas-permeating bag reactor enabled higher carbon fixation than a normal plastic photobioreactor; and (c) High-DIC alkaline medium of *A. platensis* allows an efficient CO₂ recovery. The above findings demonstrate that gaspermeating photobioreactor is a promising technology for improving energy-efficiency with maintaining high biomass productivity. Moreover, the key findings not only contribute to the commercial microalgal cultivation, but also to CO₂ recovery process and biogas upgrading system.

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