RESEARCH PAPER

Chemically and Physically Stable Hydrogel Containing Spirulina as a Decarboxylation and an Oxygenation System

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An earlier version of this article was published, containing minor grammatical errors that did not influence the scientific content of the work. This corrected version replaces the original.

Abstract

Global warming is one of the most serious threats to the global environment ever faced by humans. Therefore, a decarboxylation system is urgently required to help reduce carbon dioxide emissions and mitigate the effects of global warming. In this study, we demonstrated the potential of a novel glucomannan hydrogel containing *Arthrospira platensis* (*Spirulina*) to be both a carbon dioxide absorber and an oxygen generator. The concentration of carbon dioxide in an air-tight space (1.3 L) reduced from 0.72% to 0.07% after 8 h of starting of light irradiation on 10 g of the hydrogel. We combined this hydrogel with phosphorescent pigments, magnetic powder, or inert gas. In a dark space after 10 min of light-irradiation using 3.5 g of hydrogel and 30 mL of SOT medium, the amount of dissolved oxygen decreased by 11.2%Sat in the regular hydrogel group and increased by 10.2%Sat in the phosphorescent hydrogel group. Hydrogel containing magnetic powder and that containing inert gas could be easily collected via magnetic adsorption (magnetic separation) and low specific gravity-based separation (floatation separation), respectively. Our findings also demonstrated a useful method for quantification of decarboxylation and oxygenation in a compact space using only small amounts of hydrogel.

Keywords: CO_2 capture utilization technologies, environmental engineering, microalgae-based products

1. Introduction

Over 75% of atmospheric oxygen, which is essential to life on Earth, is known to be produced through photosynthesis by microalgae, including *Arthrospira platensis* (*Spirulina*). *Spirulina*, which is a blue–green filamentous cyanobacterium, has been present on Earth for 3.5 billion years [1]. *Spirulina* grows in lakes with high sodium bicarbonate concentrations and high pH [2]. In *Spirulina* cultivation, sodium bicarbonate is generally used to raise the pH of the culture medium [3, 4]. A high pH

is deemed advantageous to create both a suitable environment for *Spirulina* and an unsuitable condition for microorganisms other than *Spirulina* [5]. When a small amount of sodium hydroxide is mixed with the culture medium as a pH-regulator, it reacts with carbon dioxide to produce sodium carbonate and water. Sodium carbonate and carbon dioxide are sequentially converted into sodium bicarbonate. Here, both sodium hydroxide and sodium carbonate serve as carbon dioxide absorbers [6], as in the following chemical formula:

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$
$$Na_2CO_3 + CO_2 + H_2O \rightarrow 2NaHCO_3$$

HCO_3– derived from $NaHCO_3$ increases the growth rate of *Spirulina* and oxygen production via photosynthesis.

Spirulina, under a microscope, displays a tiny, coiled or spiral filamentous form. It can be collected using a fine (e.g., 500 mesh (28 μm)) filter (figure 1) when changing the culture medium and when harvesting [7]. If we develop over a millimeter-sized hydrogel containing *Spirulina*, compared with *Spirulina* alone which will easily disperse, we can collect *Spirulina* in a shorter amount of time using a coarse strainer. The combination of *Spirulina*/hydrogel and sodium hydroxide/sodium bicarbonate-containing medium may be a potential approach for decarboxylation and oxygen-generation system. In terms of gel-stability and *Spirulina*-activity, it is important to prevent collapse of the gel and to continuously keep effective photosynthetic effects of the embedded *Spirulina* in the hydrogel for a long time. The utilization of alginate hydrogel for the culture of *Chlamydomonas reinhardtii*, a triacylglycerol-accumulating microalgae, has been previously reported [8]. However, alginate hydrogel is known to be converted into viscous sodium alginate solution in the presence of sodium carbonate or sodium bicarbonate.

In this study, we first decided on the appropriate hydrogel components and production procedure of chemically and physically stable live *Spirulina*/hydrogel in a sodium carbonate-containing culture medium. Then, we demonstrated the potential of the hydrogel by mixing several kinds of functional materials with the *Spirulina*/hydrogel. Finally, we measured the degree of decarboxylation and oxygenation of *Spirulina*/hydrogel using novel methods.

2. Optimizing the components and the preparation of hydrogels

In this study, we made four types of noodle-shaped *Spirulina*-containing hydrogels composed of (1) konjac glucomannan, (2) sodium alginate, (3) xanthan gum/locust bean gum, and (4) kappa-carrageenan/locust bean gum. These components are

generally known to form hydrogels of high strength [9–13]. Spirulina was cultured in SOT medium at 25 °C. The pH level of the SOT medium was regulated by adding a sodium hydroxide aqueous solution. SOT culture medium (100 mL) contains 99.9 mL of distilled water, 1.68 g of $NaHCO_3$, 50 mg of K_2HPO_4, 250 mg of $NaNO_3$, 100 mg of K_2SO_4, 100 mg of NaCl, 20 mg of $MgSO_4 \cdot 7H_2O$, 4 mg of $CaCl_2 \cdot 2H_2O$ 4 mg, 1 mg of $FeSO_4 \cdot 7H_2O$, 8 mg of Na_2EDTA and 0.1 mL of A5 solution. A5 solution was obtained by mixing 100 mL of distilled water, 286 mg of H_3BO_3, 217 mg of $MnSO_4 \cdot 5H_2O$, 22.2 mg of $ZnSO_4 \cdot 7H_2O$, 7.9 mg of $CuSO_4 \cdot 5H_2O$ and 2.1 mg of $Na_2MoO_4 \cdot 2H_2O$. All of these reagents were purchased from FujiFilm Wako Pure Chemical Corporation (Osaka, Japan). The pH level of SOT culture medium was regulated by adding NaOH aqueous solution [5]. Glucomannan and sodium alginate were purchased from Toshin Co., LTD. (Tokyo, Japan) and Fujifilm Wako Pure Chemical Corporation (Osaka, Japan), respectively. Xanthan gum, locust bean gum, and kappa-carrageenan were kindly donated by Unitech Foods Co., LTD. (Tokyo, Japan). The first and third ports of a three-way stopcock were connected to two 10 mL syringes. The *Spirulina*-dispersing SOT medium (6 mL, 6×10^6 cell body/mL) was introduced into the first syringe. Glucomannan (0.3 g) was added through the second syringe. The stopcock was opened to allow flow of the medium into the second syringe. The contents of the syringes were vigorously mixed by alternately pushing the pistons hard. This process was repeated 30 times until the *Spirulina*/glucomannan soft gel was obtained. One hour later, the gel had hardened and was extruded (3 mL/min) into the SOT/NaOH medium (pH 10.5) using a high-pressure syringe pump (Nexus 6000; Chemyx Inc., TX, USA). After leaving the mixture for 20 min, the gel had grown much harder, and was washed with 50 mL SOT medium (pH 8.6) twice. Finally, the *Spirulina*/glucomannan hard gel was obtained after the surplus liquid of the hydrogel was removed through filtration using a coarse strainer (30 mesh (535 μm)). The *Spirulina*/alginate hard gel was obtained when the *Spirulina*-dispersing SOT medium and sodium alginate (0.3 g) were mixed and extruded into 0.1 M calcium chloride. The *Spirulina*/xanthan gum/locust bean gum hard gel was then obtained when the *Spirulina*-dispersing SOT medium and xanthan gum (0.15 g)/locust bean gum (0.15 g) were mixed and extruded into SOT medium. The *Spirulina*/kappa-carrageenan/locust bean gum hard gel was obtained when the *Spirulina*-dispersing SOT medium and kappa-carrageenan (0.21 g)/locust bean gum (0.09 g) were mixed and extruded into SOT medium.

First, we measured the resistant temperature upto which *Spirulina* can remain alive for 15 min in SOT medium. As per our findings, we found that the color of *Spirulina* in the medium changed from green to yellow–green and that *Spirulina* was thermally killed when the temperature reached over 50 °C (figure 1). Therefore, we decided to prepare each *Spirulina*/hydrogel at 45 °C. Sequentially, we chose the optimal composition of *Spirulina*/hydrogel resistant to the sodium

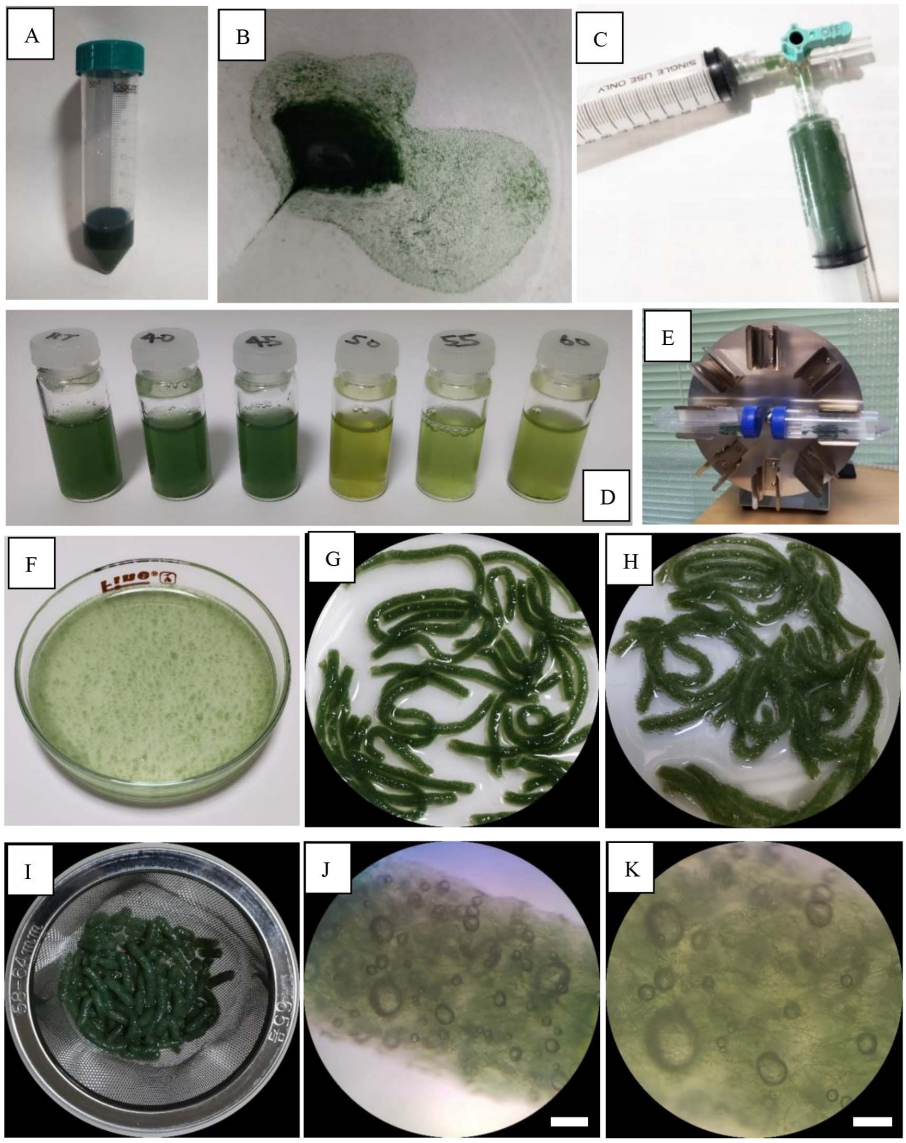

Figure 1. Preparation of *Spirulina*-containing hydrogels. (A) *Spirulina*-dispersing SOT medium. (B) Filtration of *Spirulina* using a filter paper. (C) Hydrogel-preparation using a syringe mixer. (D) Measurement of the heat-resistant temperature of *Spirulina*-dispersing SOT medium (3×10^5 cell body/mL), when non-heated (room temperature: 25 °C) and heated at 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C. (E) Mixing of hydrogel and SOT medium using a rotating mixer. (F) Dissolution of alginate-based hydrogel after mixing. (G) Appearance of the *Spirulina*/glucomannan hydrogel (SGG) right after preparation. (H) Appearance of the SGG 2 months after preparation. Several fine bubbles were generated through photosynthesis that covered the surface of the hydrogel. (I) Filtration of SGG using a coarse strainer. Microscopic evaluation of SGG 2 months after preparation (J: bar = 200 μm; K: bar = 350 μm). Hydrogel contains air bubbles produced through photosynthesis. No fungi were microscopically detected.

Table 1. Stability of each hydrogel after mixing with SOT.

Name of gel	Name of gel materials (amounts)	Dissolution after 24-h-mixing with SOT
Glucomannan	Konjac glucomannan (0.3 g)	–
Alginate	Sodium alginate (0.3 g)	+++
Xanthan gum/ locust bean gum	Xanthan gum (0.15 g)/ locust bean gum (0.15 g)	++
Carageenan/ locust bean gum	Kappa-carageenan (0.21 g)/ locust bean gum (0.09 g)	++

bicarbonate-containing SOT medium, because the culture medium for *Spirulina* mainly contains sodium bicarbonate. Each type of hydrogel was mixed with SOT medium for 24 h using a rotating mixer (TR-350; As One Corporation, Osaka, Japan). As per our finding, only the *Spirulina*/hydrogel composed of glucomannan retained its noodle shape without dissolving (table 1, figure 1). Glucomannan (2.5 to 3%) is commonly known to form stable hydrogels after treatment with an alkaline mixture and high temperature (over 90 °C). In this study, at a comparatively low temperature (45 °C), we confirmed that mixing a comparatively high concentration of glucomannan (5%) and *Spirulina*-dispersing SOT medium (pH 10.5) can effectively form a stable *Spirulina*/hydrogel against a rotating mixture. We believe that it is very important to prevent thermally killing *Spirulina* during the preparation of hydrogels.

3. Additional effects of the optimized hydrogel mixed with functional materials

We tested whether the *Spirulina*/glucomannan hydrogel has an advantage when mixed with various kinds of materials, which can provide another function to the hydrogel. We made three types of *Spirulina*/glucomannan hydrogels (SGG) mixed with (1) phosphorescent pigment, (2) magnetic powder, and (3) inert gas, respectively (figure 2). Phosphorescent powder LumiNova GLL, magnetic powder RK-200, and C_3F_8 gas were purchased from Nemoto & Co., LTD. (Tokyo, Japan), Dowa IP Creation Co., LTD. (Okayama, Japan) and TOMOE SHOKAI Co., LTD. (Tokyo, Japan), respectively. Phosphorescent *Spirulina*/glucomannan gel was obtained when glucomannan (0.3 g) and LumiNova GLL (0.06 g) were mixed with *Spirulina*-dispersing SOT medium and extruded into SOT/NaOH medium (pH 10.5). Magnetic *Spirulina*/glucomannan gel was obtained when glucomannan (0.3 g) and RK-200 (0.1 g) were mixed with *Spirulina*-dispersing SOT medium and extruded into SOT/NaOH medium (pH 10.5). Floating *Spirulina*/glucomannan gel was obtained when glucomannan (0.3 g) and C_3F_8 gas (0.6 mL) were mixed with

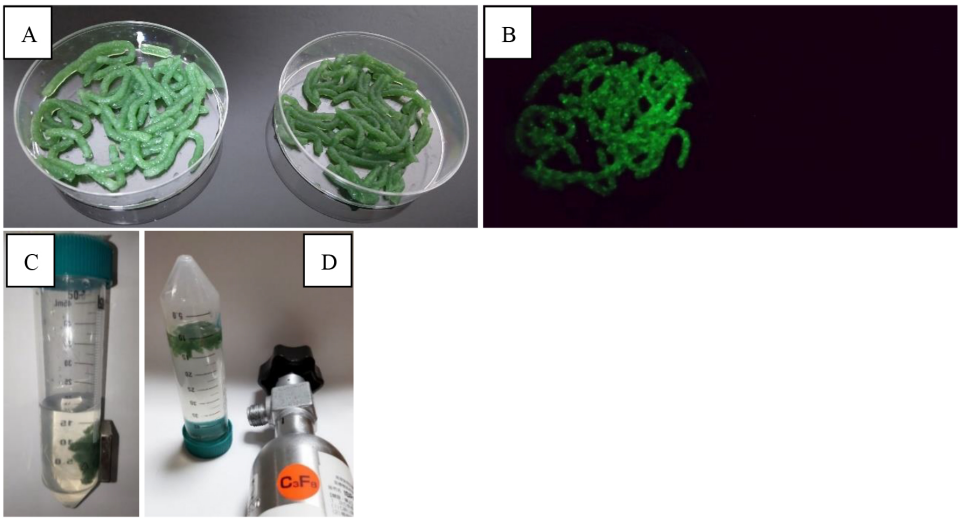

Figure 2. SGG containing each functional material. (A) Phosphorescent (*left*) and regular (*right*) SGG. (B) Light emission of light-irradiated phosphorescent (*left*) and regular (*right*) SGG in the darkroom. (C) Magnetic separation of SGG containing iron powder. (D) Flotation separation of SGG containing C_3F_8 gas.

Spirulina-dispersing SOT medium and extruded into SOT/NaOH medium (pH 10.5). Strontium aluminate [14], zinc sulfide [15], magnesium silicate [16] and strontium silicate [17] are well-known phosphorescent materials. Among these materials, strontium aluminate is known to exhibit the greatest chemical stability, the highest brightness, and the longest afterglow. We fabricated phosphorescent SGG containing strontium aluminate. Phosphorescent materials are known to absorb and accumulate light energy and emit light; thus, this phenomenon is useful for the illumination of the hydrogel in dark places. We have also tested the potential for the enhancement or the assistance of photosynthesis in SGG (figure 4). White LED lamps (Luminshonor, 10 W) were purchased from Zhongshan Yuqui Lighting Co., Ltd. (Guangdong, China). Light intensity was measured using a digital light meter (KEW 5204; Kyoritsu Electrical Instruments Works, LTD (Tokyo, Japan)) and was regulated by changing the distance between the LED lamp and the irradiation field. After the irradiation of LED light (10,000 lux, 10 min), the phosphorescent material-containing hydrogel continued emitting light for at least 5 h. We have previously reported the merits of a porous-structured magnetic material composed of nickel ferrocyanide and iron nanoparticle for magnetic separation of radioactive cesium [18]. Iron powder displayed a certain degree of chemical stability in alkaline conditions. Iron oxide has been a major material for magnetic separation [19] and is also used as the black toner in copying machines. When we prepared the prototype hydrogel which contained *Spirulina*, glucomannan, and iron oxide powder, the color of this type of gel was black and it exhibited a strong light shielding effect. In fact,

we could not microscopically observe the internal structure of this black gel. Another black powder, that is, carbon fine particles, also displays a light shielding property and is not suitable for the development of light transmission materials. Entirely green-colored magnetic SGG containing gray-colored iron powder could be rapidly separated using a neodymium magnet. Such a hydrogel has potential for use in magnetic separation while maintaining photosynthesis. Octafluoropropane (C_3F_8), a nontoxic and inert gas, expands to at least twice the volume of the gas mixed because of its lower water solubility than nitrogen. C_3F_8 is one of the most frequently used intraocular injected gases. The half-life of C_3F_8 is 3.5 to 5 times longer than that of air after injection [20]. We prepared SGG containing C_3F_8 and found that it continued to float on the surface of SOT medium for at least 3 weeks. Such a hydrogel has potential for floating separation and can be advantageous for floating accumulation at the water surface, where light intensity is deemed strongest.

4. Decarboxylation ability of SGG

In a closed space (1.3 L) of an airtight container (LBF-809, Lock & Lock Co., Seoul, Korea), we arranged a plastic box with multiple slits through which air can enter and exit, a CO_2 generator, a CO_2 measurement apparatus, a circulating fan (IC-UFAN2001-FN, IconShop Co., LTD., Tokyo, Japan), an air pump (Useekoo Inc., Shanghai, China), a gas introduction tube, and a 100 mL plastic bottle (Nikko Hansen & Co., LTD., Osaka, Japan), which contained the SOT/NaOH medium (pH 10.5) with *Spirulina* gel (figure 3). The bottle had two holes (diameter: 2.5 mm) for the insertion of the tube and for the exhaust of the hydrogel-treated air. One end of the gas introduction tube was connected to an air pump, while the other end was inserted into the bottom of the bottle. The plastic bottle was installed in a plastic box with slits. A CO_2 generator, a CO_2 measurement apparatus, a circulating fan, and an air pump were installed on the plastic box with slits. We used a COZY-1 (Ichinen Manufacturing Co., LTD., Tokyo, Japan) and an AnaeroPouch-CO_2 (Mitsubishi Gas Chemical, Tokyo, Japan) as the CO_2 measurement apparatus and as the CO_2 generator, respectively.

We measured the degree of decarboxylation of SGG (10 g) dispersed in 50 mL of SOT medium. In a darkroom, one pack of AnaeroPouch-CO_2 increased the CO_2 concentration level of the airtight apparatus (figure 3) that reached 0.72% 18 to 24 h after the beginning of the experiment. At 24 h after the beginning of the experiment, the white LED light (10,000 lux) started to irradiate. At 4, 8, 12, and 18 h after the start of irradiation, the CO_2 concentration decreased to 0.28%, 0.07%, 0.04%, and 0%, respectively (figure 4). After the experiment, we confirmed that the hydrogel maintained a noodle shape without dissolving, in spite of the continuous mixing by

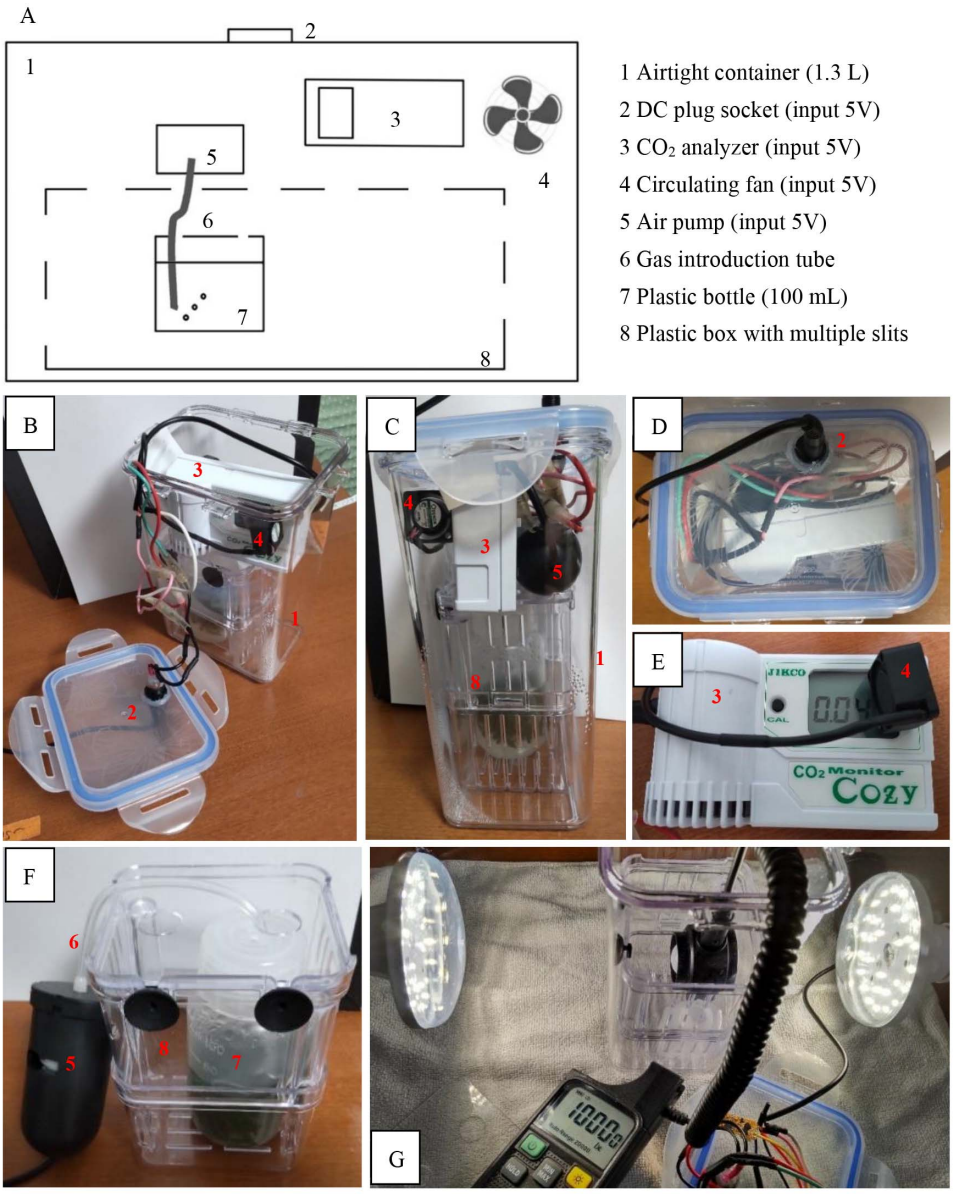

Figure 3. CO_2 analysis system in a closed small-space. (A) Schematic diagram. (B) General view. (C) Lateral view. (D) Top view. (E) CO_2 analyzer and circulating fan. (F) Air pump and gel-containing bottle in a plastic box with multiple slits. (G) Adjustment of light intensity of the white LED lamp using a digital light meter.

air bubbling for 48 h. These results demonstrated that the aeration and light irradiation of the alkaline medium with dispersed SGG can be a potential decarboxylation approach.

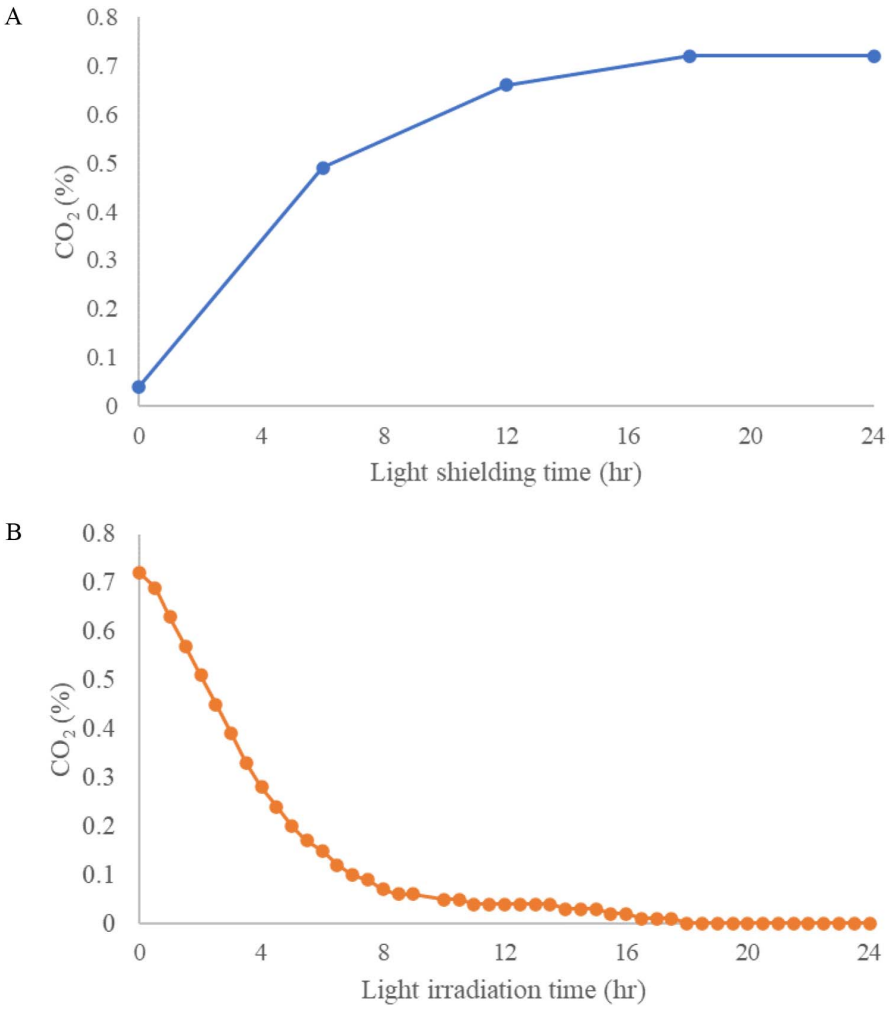

Figure 4. Measurements of (A) CO_2 production by CO_2 generator and of (B) decarboxylation by SGG in a closed small-space of CO_2 analyzing system. $N = 3$.

5. Enhancement and assistance of the oxygen production-ability of phosphorescent SGG

We compared the degree of oxygen production of regular SGG and phosphorescent SGG. In the closed space of a syringe (30 mL; Terumo Corporation, Tokyo, Japan), we arranged an optical dissolved oxygen probe (sHI764113; Hanna Instruments, Inc., RI, USA), a rubber gasket around the sensor, and a magnetic stirrer bar. The rubber piston head of a syringe was removed, and a hole (diameter: 18 mm) was punched out at the center using a hollow punch (Niigata Seiki Co., LTD., Niigata, Japan) to serve as a rubber gasket. The syringe was filled with SOT/NaOH medium (pH 10.5) and contained each type of SGG (figure 5), and was plugged with a closing cap (Terumo Corporation). An oxygen sensor was connected to an optical dissolved oxygen meter (HI98198; Hanna Instruments, Inc.). The syringe with the optical

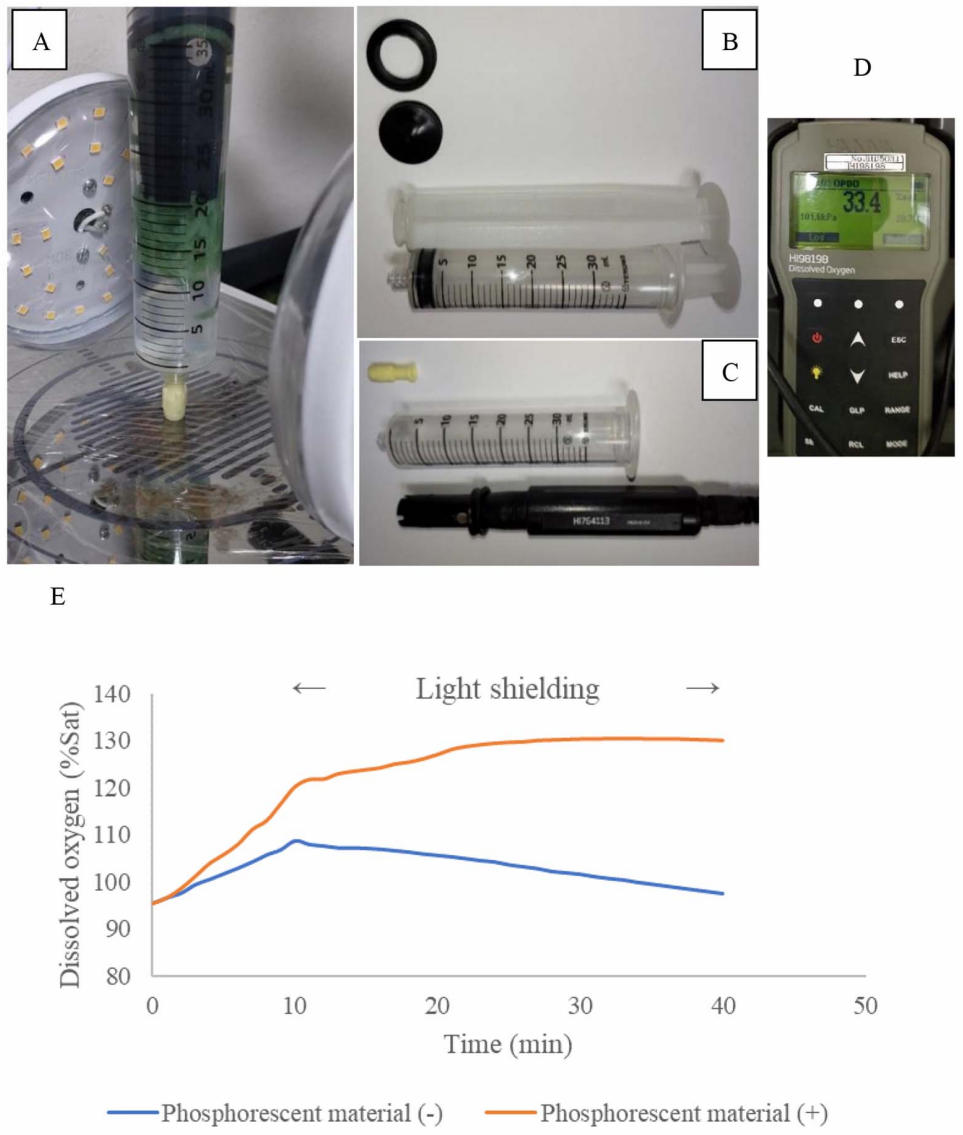

Figure 5. Measurements of dissolved O_2 produced by SGG in a closed small-space. (A) Dissolved O_2 sensor, SGG dispersed in SOT medium and a magnetic stirrer bar in a syringe cylinder. Hydrogel and SOT medium were mixed in a syringe using a magnetic mixer. (B,C) Rubber piston head was removed, and its center was punched out. Piston head with a hole was used as an airtight rubber gasket between the O_2 sensor and the outer cylinder. (D) Dissolved O_2 analyzer. (E) Dissolved O_2 production by phosphorescent and regular SGG. The degree of % saturation of SOT medium was measured when LED light (10,000 lux) was irradiated for 10 min and was shielded for 30 min in a darkroom. $N = 3$.

probe was then fixed using a clamp stand, and the stirrer bar was rotated using the magnetic mixer (RP-1AN, As One Corporation). Each gel (3.5 g) and a magnetic stirrer bar were placed in a cylindrical tube (30 mL). A rubber gasket was fitted

around the optical oxygen sensor probe, and the probe was inserted into the cylindrical tube while pressing the gasket against the inner wall of the cylindrical tube. Then, 25 mL of SOT medium was added into the cylinder from an outlet nozzle using an 18 G needle-attached injector while removing air, and the outlet was finally plugged with a syringe cap (figure 5). In a darkroom, this apparatus was set on a magnetic mixer, and the stirrer bar was rotated (150 rpm). The LED lamp (10,000 lux) was irradiated to the outer cylinder for 10 min. The amount of dissolved oxygen in the regular SGG group and phosphorescent SGG group were 108.7%Sat and 120.2%Sat, respectively, at 10 min after the start of the experiment. After light-irradiation was stopped, the amount of dissolved oxygen of each group was measured for 30 min. The amount of dissolved oxygen in the regular SGG group was noted to decrease to 97.5%Sat from 108.7%Sat. On the other hand, the amount of dissolved oxygen in the phosphorescent SGG group increased to 130.4%Sat from 120.2%Sat and reached a plateau. These results demonstrated that light-irradiation of the SGG dispersed in an alkaline medium can be a potential approach for oxygenation. Moreover, the phosphorescent material enhanced and assisted oxygenation in a light space and in a dark space, respectively.

6. Conclusions

In conclusion, we were able to determine that SGG was a chemically and physically stable composition of photosynthetic hydrogels. Moreover, we demonstrated that SGG containing strontium aluminate, iron powder, and C_3F_8 gas can be potential approaches for the enhancement of photosynthesis, magnetic accumulation, and floating separation, respectively. We have also developed the apparatus to measure the degree of decarboxylation- and oxygenation-ability using only small amounts of photosynthetic hydrogel in a small space.

Patents

A patent resulted from the work reported in this manuscript. Patent application has been completed.

Conflict of interest

The authors declare no conflict of interest.

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