

Reutilization of Algal Supercritical Water Gasification Waste for Microalgae *Chlorella vulgaris* Cultivation

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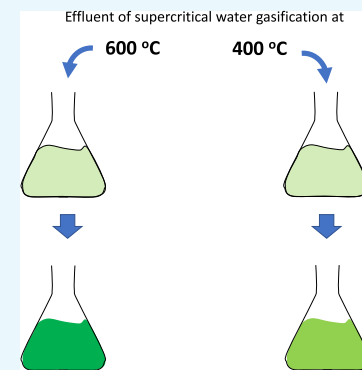
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ABSTRACT: Effluents obtained through a supercritical water gasification (SCWG) process at 400 and 600 °C were mixed with Bristol Medium to cultivate *Chlorella vulgaris*. Improvement of growth rate was observed only for the medium with the effluent at 600 °C. Low non-purgeable organic carbon implied that the inhibiting material was decomposed due to the high temperature of 600 °C. Thus, SCWG effluents might be more suitable for algae cultivation than hydrothermal liquefaction effluents. Phosphorus accumulation in *C. vulgaris* was improved in the SCWG mixed medium, irrespective of the treatment temperature. The media with SCWG effluents showed 2.5 times higher phosphorus accumulation in the algae, indicating the possibility of using a combination of *C. vulgaris* and SCWG for nutrient recycling processes.



1. INTRODUCTION

Supercritical water gasification (SCWG) can gasify various kinds of biomass quickly and almost completely.^{1–3} This very reactive state of water allows biomass components to be hydrolyzed, decomposed, and gasified to produce fuel gases including hydrogen and methane. To date, there have been several studies on SCWG for various kinds of biomass feedstocks,^{4,5} which report that carbon, hydrogen, and oxygen can be converted into fuel gases partly via reactions with water. However, studies on the behavior and utilization of heteroatoms such as phosphorus, nitrogen, sulfur, and potassium are very limited.

Heteroatoms are important, especially for development of sustainable processes. Biomass growth, whether related to trees, grasses, microorganisms, or algae, requires phosphorus, nitrogen, and potassium. Currently, a large amount of fossil fuel is used to produce fertilizers containing these heteroatoms.^{6,7} Thus, recovery and utilization of heteroatoms from an SCWG process is important; moreover, utilization of these elements is also effective for value addition to the SCWG process itself.

One desirable system is to utilize the SCWG effluent for growth of the feedstock biomass itself. Once the heteroatoms are recovered from an effluent and are used to cultivate a biomass feedstock, the heteroatoms that are retained into the system are then continuously used for biomass cultivation and recovered once again; no additional supply of a fertilizer is required.

Based on this concept, utilization of wastewater to grow microalgae by employing a hydrothermal treatment of biomass

has been investigated. To the best of our knowledge, the first study to cultivate microalgae using hydrothermal process effluents was conducted by Minowa and Sawayama.⁸ They gasified *Chlorella vulgaris* at 350 °C using a nickel catalyst and used the effluent obtained after filtration for cultivation of *C. vulgaris*. Their cultivation was successful, but the growth was one-eighth the yield of *C. vulgaris* using a standard medium. They attributed this slow growth to the lack of phosphorus in the effluent. Three years later, Biller et al. cultivated *C. vulgaris*, *Scenedesmus dimorphus*, *Spirulina platensis*, and *Chlorogloopsis fritschii* using an effluent produced by hydrothermal liquefaction of microalgae and cyanobacteria.⁹ By diluting the effluent, they achieved a similar growth rate to using a standard medium such as Bold Basal Medium with 3-fold nitrogen and vitamins (3N-BBM + V). Moreover, they found that if the effluent was not diluted, inhibition of gasification occurred possibly due to the presence of nickel, phenol, and fatty acids.

Thus, the utilization of the effluent of the hydrothermal process impeded the activities of the cultivation inhibitors. Si et al. employed ozonation or activated carbon for the direct liquefaction effluent of feedlot manure to feed the process of methane fermentation, thereby suppressing the activity of inhibitors.¹⁰ Bagnoud-Velasquez et al. cultivated *Phaeodacty-*

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lum tricorntum using a hydrothermal liquefaction effluent successfully by mixing it with a standard medium.¹¹

However, all these studies are based on a hydrothermal treatment using subcritical water. Apparently, most of the previous investigators preferred to use subcritical conditions; hence, reports on algae cultivation using SCWG are limited. Among these studies, Stucki et al. conducted SCWG of *S. platensis* at 400 °C with a ruthenium (Ru) catalyst¹² and Onwudili et al. gasified *Saccharina latissimi*, *C. vulgaris*, and *S. platensis* at 500 °C using alkali and Ni catalysts.¹³ However, they only mentioned the possibility of using the effluent for algae cultivation and analyzed it but did not cultivate algae with the effluent. Elliott et al. proposed a catalytic SCWG of microalgae with nutrient recovery for algae cultivation but did not carry out cultivation.¹⁴ It was Elsayed et al. who, for the first time, employed SCWG effluents to cultivate microalgae.¹⁵ They gasified *Acutodesmum abliquus* at 600–690 °C using a K₂CO₃ catalyst and used activated carbon and ultraviolet treatments to inactivate the inhibitors present in the effluent.

Thus, there are very few studies on the utilization of an SCWG effluent that contains residual heteroatoms to grow biomass. A detailed strategy of heteroatom utilization from an SCWG process can be developed by assessing its quantitative effects on growth using a growth model of microalgae. Hence, the purpose of this study was to employ an SCWG effluent to grow biomass and evaluate its effect quantitatively.

2. RESULTS AND DISCUSSION

Figure 1 shows normalized growth of *C. vulgaris* microalgae during cultivation for different initial concentrations of the

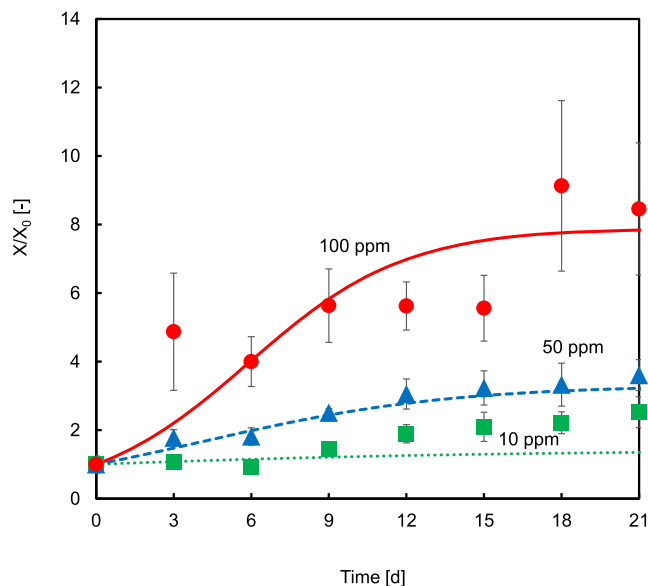


Figure 1. Algae growth with time for different concentrations of the fertilizer medium.

fertilizer. The cell density of each sample was divided by the initial density to compare the growth directly. A higher concentration of the fertilizer resulted in a faster growth rate. Error bars show the estimated standard deviations for the three runs. The relative errors are rather small, and good reproducibility is observed. The log phase and stationary phase are clearly observed.

2.1. Fundamental Behavior of *C. vulgaris* Growth. The Monod equation well describes the growth of various kinds of microorganisms.¹⁸ In fact, many researchers have employed this equation to express the growth of *C. vulgaris*.^{19–23} The Monod equation is expressed by the simple equations shown below

$$\frac{dX}{dt} = \frac{\mu_{\max} C}{C + K_S} X \quad (1)$$

$$X - X_0 = -k(C - C_0) \quad (2)$$

which lead to

$$\frac{dX}{dt} = \frac{\mu_{\max} [C_0 - (X - X_0)/k]}{C_0 - (X - X_0)/k + K_S} X \quad (3)$$

where X is the biomass population [kg m^{-3}], X_0 is the initial biomass population [kg m^{-3}], μ_{\max} is the maximum specific growth rate of the cell [s^{-1}], C is the concentration of the medium [kg m^{-3}], C_0 is the initial concentration of phosphorus in the medium [kg m^{-3}], k is the growth yield constant [-], K_S is the substrate utilization constant [kg m^{-3}], and t is the time [s].

The obtained experimental data were fitted with the model using the least square method. The obtained curve is shown in Figure 1. The parameters were the same for these curves, and the initial concentration was at a ratio of 1:5:10. The curves well expressed the experimental data, although some discrepancy was observed for the 10 ppm fertilizer medium.

Figure 2 shows the change in growth rate for every 7 d obtained from the Monod equation for the 3 weeks of

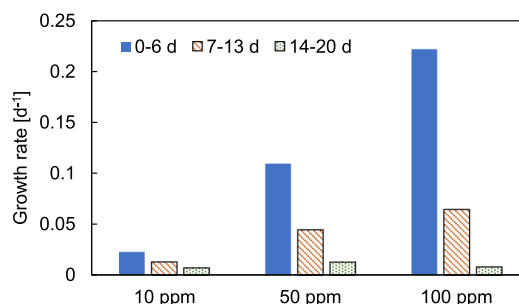


Figure 2. Growth rate for every 7 d for different concentrations of the fertilizer medium.

cultivation. The growth rate was larger for the higher-concentration medium. A rapid decrease in growth rate was observed for the 100 ppm medium. This rapid decrease can be explained by the fact that a higher concentration of the medium allows microalgae to grow rapidly and to consume the fertilizer nutrient rapidly.

Figure 3 shows the phosphorus yield in a liquid phase for this duration. As expected, the phosphorus yield in the liquid phase decreased with growth of the algae, and the reduction was faster for the higher concentrations. It should be noted that the amount of phosphorus in the liquid phase was almost 0 after 2 weeks in the 100 and 50 ppm media, suggesting that phosphorus is the limiting nutrient in such cases.

2.2. Effect of the SCWG Effluent on *C. vulgaris* Growth. Figure 4 shows the normalized increase of *C. vulgaris* for Bristol Medium (BM)-based media. Growth of *C. vulgaris* was observed in all the media. Pure BM was the standard

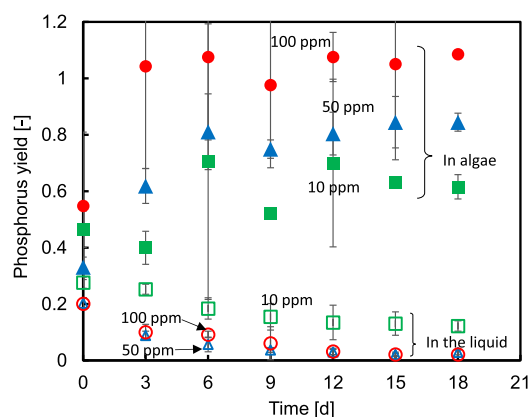


Figure 3. Phosphorus yield in the medium and the cell during cultivation for the fertilizer medium.

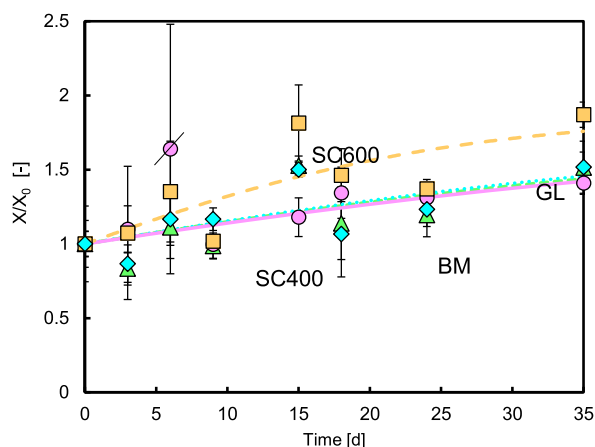


Figure 4. Algae growth with time for BM-based media.

medium used here. The growth rate of *C. vulgaris* in BM was slower than that in the fertilizer media. This may have been partly due to the initial ratio of the limiting nutrient and algae amount and partly due to the presence of some inorganic elements in the fertilizer, which was not clearly shown on the product package. Addition of glucose did not help to increase the growth rate as shown for that of the GL medium. When the SCWG effluent from the 400 °C reaction was added, it did not improve the growth rate. In fact, the growth curve was the lowest, although the difference was quite small. It should be noted that the data point at 6 d for the SC400 medium was omitted because of the large standard deviation; we failed to obtain reliable data here. Unfortunately, we did not have sufficient samples to repeat the analysis. Nevertheless, when

the SCWG effluent from 600 °C was added, a clear improvement in the growth rate was observed.

Potential nutrients that could be recovered from the SCWG process were in the form of dissolved inorganic components. The analysis of nutrients in the initial medium is shown in Table 1. Non-purgeable organic carbon (NPOC), determined using a total organic carbon (TOC) analyzer, in the liquid phase of BM should be from the inoculum. Notably, the NPOC of SC400 was clearly higher than that of other BM-based media. Moreover, the differences in the ion concentration among the BM-based media were not large. This is reasonable considering that for the GL, SC400, and SC600 media, only 10 vol % of BM was replaced with the corresponding solution.

The reason for the high growth rate observed for SC600 could have been the presence of components other than those shown in Table 1. Some minor components that are suitable for the growth of *C. vulgaris* could have been provided in the effluent. Considering that the SCWG effluent was obtained by the treatment of *C. vulgaris* itself, a component that assisted in the growth of the microalgae may have been present in the SCWG effluent.

The same component may have been present in the SC400 medium, but an improvement in the growth rate was not observed for SC400. This can be explained from the viewpoint of the existence of inhibitors in SC400. The treatment temperature of the SCWG effluent for the SC400 medium was 400 °C, which was close to the hydrothermal liquefaction temperature of 350 °C. Thus, it could have contained a similar inhibitor that had posed a problem related to using the effluent obtained by hydrothermal liquefaction. The high NPOC value of the SC400 medium supports the existence of some inhibitors in the SCWG effluent at 400 °C.

Based on the data mentioned above, performing SCWG at a high temperature is desirable because it results in decomposition of most of the organic compounds, including possible inhibitors. This implies that the SCWG effluent is superior compared to that obtained by hydrothermal liquefaction. In a previous study, Barreiro et al. treated the hydrothermal liquefaction effluent by SCWG before feeding to the algae culture, and no inhibitory effects were observed on the growth of *C. vulgaris*.²⁴ These findings also support our hypothesis. To date, there are no reports to determine exactly what the components are that inhibit the algal growth; hence, the inhibitors that should have been in SC400 but eliminated in the SC600 medium could not be determined.

Figure 5 depicts the growth rate of *C. vulgaris* for every week of cultivation for the BM-based media, obtained by fitting the experimental data with the Monod equation. The fitting curve is shown in Figure 4. It is clear that the growth rate of the algae

Table 1. Initial Carbon Organic, Nitrogen-Ammonium, Nitrogen-Nitrate, Phosphorus, and Potassium Content in the Cultivation Medium

medium	NPOC (mg L ⁻¹)	NH ₄ ⁺ (mg L ⁻¹)	K ⁺ (mg L ⁻¹)	PO ₄ ³⁻ (mg L ⁻¹)	NO ₃ ²⁻ (mg L ⁻¹)
fertilizer 10 ppm	n.d.	0.16 ± 0.03	2.14 ± 0.33	0.85 ± 0.02	2.69 ± 0.02
fertilizer 50 ppm	n.d.	0.70 ± 0.02	8.99 ± 0.10	4.47 ± 0.04	15.78 ± 0.01
fertilizer 100 ppm	n.d.	1.32 ± 0.10	16.40 ± 0.91	10.57 ± 1.87	32.03 ± 0.14
BM	28.22 ± 1.16	0.00 ± 0.00	84.60 ± 13.21	176.11 ± 4.01	139.54 ± 0.11
GL	32.07 ± 4.07	1.73 ± 0.05	73.04 ± 6.34	174.24 ± 0.20	122.71 ± 0.87
SC400	53.72 ± 2.95	2.22 ± 0.31	71.25 ± 2.22	178.26 ± 2.73	137.84 ± 0.93
SC600	34.92 ± 4.68	3.36 ± 0.44	67.97 ± 12.58	187.12 ± 0.07	141.27 ± 0.43

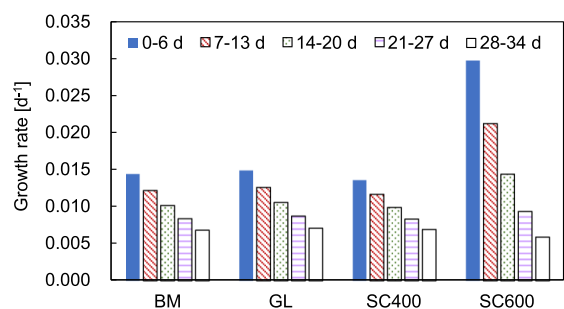


Figure 5. Growth rate for every 7 d for BM-based media.

in the SC600 medium exceeded that in other media. The decrease in growth rate was slower compared to the 100 ppm fertilizer medium, which could have occurred due to the ratio of the inoculum to the nutrient amount.

Figure 6 displays the change in the phosphorus yield in the liquid phase with time for algae cultivation in the BM-based

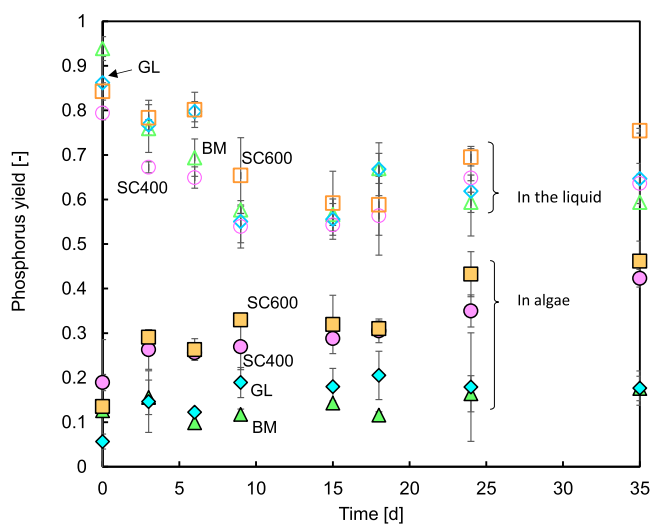


Figure 6. Phosphorus yield in the medium and the cell during cultivation for BM-based media.

media. Compared to the fertilizer media shown in Figure 3, the amount of phosphorus in the original media was sufficiently high for the algae to grow in the media. The amount of phosphorus in the liquid phase decreased as the algae grew, but even after 5 weeks, almost half of the amount was still in the liquid phase.

2.3. Phosphorus Yield Uptake by Cells during Cultivation. Phosphorus is an essential nutrient for biomass growth. However, the behavior of nutrient phosphorus removal from the medium and storage in cells during cultivation has not been reported. Figure 3 shows the accumulation of phosphorus in *C. vulgaris* cultured in fertilizer media. The yield of phosphorus that was taken up by the cells from the medium and stored in the cells increased with time. The yield increased much faster according to the higher initial concentration of phosphorus in the medium. This corresponded to the algae growth rate, which also occurred at a faster rate at the higher initial phosphorus concentration.

Figure 6 also shows the phosphorus yield in the cells for the BM-based media. Clearly, the phosphorus uptake was higher for SC600 than that observed for BM or GL media. Interestingly, phosphorus uptake in the SC400 medium was

also high. Apparently, for SC400, some inhibitory activity resulted in a lower growth rate than for SC600, but the phosphorus uptake was not affected. The faster uptake of phosphorus in the media with the SCWG effluent indicates the possibility of the occurrence of a nutrient recycling process using a combination of *C. vulgaris* and SCWG. As presented in Table 1, the phosphorus content of all the BM-based media was almost equal. However, only the SCWG effluent showed a rapid accumulation of phosphorus compared to the BM or GL medium. The final yield at 35 d for the SCWG effluent medium was 2.5 times that for the BM and GL media. These results suggest that uptake of dilute phosphorus from the SCWG effluent is effectively achieved by *C. vulgaris*.

3. CONCLUSIONS

C. vulgaris was successfully grown in media containing the SCWG effluent and compared with those that did not contain the SCWG effluent. The growth of *C. vulgaris* with the SCWG effluent at 600 °C gave a higher rate than in the media without the effluent. We found that media with the SCWG effluent at 600 °C allowed a more rapid growth of *C. vulgaris* with the SCWG effluent at 600 °C for the first time. The lower growth rate in SC400 can be explained by the presence of inhibitors, which indicates that the SCWG effluent is superior to the hydrothermal liquefaction effluent. Phosphorus in the medium with the SCWG effluent was found to be accumulated in the microalgae 2.5 times as high as that in the medium without the SCWG effluent. This indicates the possibility of a nutrient recycling process that takes place by a combination of *C. vulgaris* with SCWG.

4. EXPERIMENTAL SECTION

4.1. Materials. To quantitatively evaluate biomass growth, simple biomass species are desirable. From this viewpoint, we used the microalgae *C. vulgaris* UTEX 395 purchased from the culture collection of algae at the University of Texas, Austin, for our study.

The SCWG effluent was obtained from an in-house reactor. Details of the reactor can be found elsewhere.^{16,17} The feedstock was the microalgae *C. vulgaris*, the reaction temperatures were 400 and 600 °C, the reaction pressure was 25 MPa, and the residence time was 30 s. These temperatures are two typical temperatures employed for SCWG. For the reference purpose, the typical temperature for hydrothermal liquefaction is 350 °C and the critical temperature of water is 374 °C. We fixed the pressure because for SCWG, it is known that pressure does not affect the result as much as temperature.

An inorganic fertilizer containing nitrogen, phosphorus, and potassium at concentrations of 65, 60, and 190 g/kg, respectively (HYPONeX Japan Co., Ltd.), was added to supply the nutrients required for growth of the algae. Chemical reagents were used as purchased without further treatment. D-glucose used as the standard for TOC analysis was a product of Nacalai Tesque, Inc., Japan. To adjust the pH of the medium, the JIS special grade of sodium hydroxide and hydrochloric acid (35%) procured from Nacalai Tesque, Inc., Japan, were used. Sodium phosphate and ammonium chloride used as standard compounds for phosphorus and ammonium analysis were provided by Nacalai Tesque, Inc., Japan. Nessler's reagent was prepared by mixing potassium iodide (Nacalai Tesque, Inc., Japan) and mercury (II) iodide (Sigma-Aldrich, Japan) in

a sodium hydroxide solution. For phosphorus analysis, ammonium molybdate tetrahydrate, potassium peroxydisulfate, and ascorbic acid (Nacalai Tesque, Inc., Japan) as well as 4-nitrophenol and potassium antimonyl tartrate trihydrate (Sigma-Aldrich, Japan) were used. Deionized (DI) water with a conductivity less than $1 \mu\text{S}/\text{cm}$ was used as a solvent.

4.2. Preparation of *C. vulgaris* Culture. *C. vulgaris* was cultured in fresh BM containing NaNO_3 $2.94 \text{ mol}/\text{m}^3$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ $0.17 \text{ mol}/\text{m}^3$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ $0.3 \text{ mol}/\text{m}^3$, K_2HPO_4 $0.43 \text{ mol}/\text{m}^3$, KH_2PO_4 $1.29 \text{ mol}/\text{m}^3$, and NaCl $0.43 \text{ mol}/\text{m}^3$. All chemicals were provided by Sigma-Aldrich, Japan. After sterilization at 121°C for 20 min, the culture was left for 24 h to achieve stabilization for gaseous equilibrium before the inoculation step. We carried out the cultivation at a total volume of 1 dm^3 using algae cultures at 10% v/v. Inoculation was continued for at least 14 d. If the growth of the algae within 14 d is in the log phase, the cell culture is presumed to be ready to be used for cultivation.

4.3. Reactor Assembly for Cultivation. A 1 dm^3 borosilicate glass bottle reactor was employed for cultivation. A silicone rubber plug was used as a lid to close the reactor. To transport the surrounding air for CO_2 circulation in the reactor, two silicone pipes, one for inlet airflow and another for outlet airflow, were inserted through the rubber plug. Before use, all the parts were sterilized in an autoclave at a temperature of 121°C for 20 min.

4.4. Medium Preparation for Microalgae Cultivation. We conducted two series of experiments. The first series employed a fertilizer medium to observe the fundamental characteristics of *C. vulgaris* growth. Different concentrations of the medium (10, 50, and 100 ppm) were used for a 21 d cultivation. The medium was prepared by dissolving the fertilizer in DI water and then filtrating it to remove the remaining solids.

The second series employed SCWG effluent-added BM. A mixture of 80 vol % of BM, 10 vol % of the SCWG effluent, and 10 vol % of algae culture was used. The medium using the effluent of SCWG at 400°C is designated as SC400 and that at 600°C is designated as SC600 in this study. In addition, cultivation with pure BM was also conducted for the reference purpose. We also employed 10 vol % of $500 \text{ g}/\text{m}^3$ glucose instead of the SCWG effluent to understand the effect of glucose during cultivation. This medium is designated as GL in this study.

All the experimental runs were conducted three times to check the reproducibility.

4.5. Sampling Methods. During the cultivation period, culture samples were taken twice weekly with a sterilized pipette. The cells and the medium were separated, employing a centrifuge at $80.5 \text{ km}/\text{s}^2$ (8217 G) for 20 min using a high-speed centrifuge (As-one As18S, Japan). The separation of supernatants was carried out manually by pipetting. Next, an equal volume of DI water as that of the removed medium was added to the remaining cells in the centrifuge tube to maintain cells at the same concentration.

4.6. Analysis Methods. The growth of algae was determined as the optical density using a spectrophotometer at a wavelength of 680 nm for the first set of cultivation. For the second set, the weight of algae in the sample was measured.

Analyses were performed for phosphorus in the cells and medium. Both the supernatants and cells were treated with 12% H_2SO_4 and 4% $\text{K}_2\text{S}_2\text{O}_8$ at 120°C for 30 min using an autoclave. Phosphorus determination was initiated by neutral-

izing the pH of each sample using a high concentration of sodium hydroxide with nitrophenol as an indicator. A yellowish color developed when the samples became neutral or basic. Later, the sample was diluted to the desired volume. To as much as 10 cm^3 of each sample, 2 cm^3 of the molybdenum reagent was added, and its absorbance was measured using a spectrophotometer at 880 nm. Moreover, the initial concentrations of ammonium, nitrate, phosphate, and potassium were analyzed by ion chromatography (Shimadzu, Japan).

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