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Technical Report

Basic Study on Development of Microalgal Biomass Production Utilizing By-products of the Steel Industry

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Abstract

Microalgal biomass production has attracted attention as a technology for CO_2 emission reduction. We started research on the development of the microalgal culture system utilizing by-products generated in steelworks. A unicellular microalga (NS001C strain) isolated from the wastewater treatment equipment in steelworks showed a high growth rate with high ammonia tolerance. In the experiments in which the NS001C strain was applied to the small-scale substrate surface culture, the maximum biomass productivity was 15.4 g/m^2/d. By the effective utilization of sunlight in the system, the biomass productivities of 20–30 g/m^2/d can be realized, an amount more than 5 times higher than that of the current pond system theoretically. We concluded that the substrate surface culture is a promising technique to achieve high biomass production in the large-scale culture of microalgae and shall undertake the development of a large-scale bioreactor.

1. Introduction

In recent years, the impacts of climate change due to global warming have emerged noticeably in the form of abnormal climate, and the reduction of greenhouse gas emissions has become an urgent issue. Although the development of various energy-saving technologies remains an important issue, the reduction of CO_2 is naturally limited. The global trend is to lower the dependence on fossil fuels, which are the main source of CO_2 emission, and to promote the use of renewable energies such as solar and wind power. Under such circumstances, attention is also focused on the use of biomass energy. Biomass was originally a term used in biological science to indicate the amount of the substance in living organisms. However, in recent years, it has been used as a term to indicate the resources derived from living organisms. The carbon contained in biomass is derived from CO_2 in the atmosphere that is fixed by the photosynthesis of plants and/or algae. Considering the process from biomass production to consumption as a whole, it is considered to be a carbon-neutral energy source because the CO_2 concentration in the atmosphere does not change.[1] However, the amount of the existing biomass such as the waste biomass usable without adversely affecting the natural environment is limited. Therefore, in order to sustainably use biomass as a renewable energy, it is necessary to artificially produce biomass. From this viewpoint, biomass production using microalgae has been attracting attention in recent years.

Microscopic sized oxygen-breathing photosynthetic organisms other than land plants are designated as microalgae. Compared with land plants, microalgae have a number of features that are appropriate for the production of biomass,[2] one of which is the high growth rate. The growth rates of microalgae greatly depend on species and/or strain, and the microalga with a high growth rate doubles the amount of biomass within hours. If such high growth rate is effectively utilized, the production of biomass with higher efficiency can be realized as compared with the production using land plants. Furthermore, since soil is not required for the culture, one of the advantages of microalgae is that it is less liable to conflict with the existing farming land. As an additional advantage, since there are algae that meet various environments, various culture conditions can be applied. Generally, land plants do not grow in seawater. However, regarding microalgae, there are various marine type species. The vast range of the applicability to the culture conditions means the possibility of adopting unused resources like wastewater. One study has reported that the wastewater treatment and biomass production

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were compatibly conducted using microalgae in the wastewater treatment process.[3] Furthermore, the high lipid content is also considered to be advantageous. Some of the species and/or the strains of microalgae can accumulate lipids exceeding 50% of the biomass weight.[2] This value is far above that of lipid-producing crops like palm. For this reason, the microalgal biomass is highly anticipated as the next generation material of biological fuel. Thus, the various characteristics of the microalgae that are appropriate for biomass production are attracting worldwide attention.

Nippon Steel Corporation is developing various technologies for reducing CO_2 emissions that include research on the biomass production using microalgae. We focused our attention on the microalgal biomass because, in addition to the above-described reasons, the by-products produced in the iron and steelmaking process are considered to be effectively utilized. Particularly, the wastewater from the steelworks is expected to be utilized as the culture medium for the culture of microalgae. Although most of the wastewater from the steelworks is circulated and reused within the steelworks, the reuse of the wastewater from coke ovens is difficult because it contains a high concentration of ammonia originating in coke. For microalgae, nitrogen is a major essential element. Utilizing ammonia in wastewater as a nitrogen source contributes not only to the effective utilization of the by-products, but also to the cost reduction in biomass production, and the improvement of the environmental load of the wastewater. In addition, the waste gas emissions in the steelworks that contain a high concentration of CO_2 gas are also convenient for promoting the growth of the microalgae. Furthermore, in a steelworks, there are a number of unused waste heat discharges, which can be utilized as the heat source to maintain the culture temperature of the microalgae in winter. In addition, the steelmaking slag, a by-product of the steelworks, is sold as fertilizer because it contains phosphorous in great quantities which is an essential nutrient for microalgae. Thus, we considered that, by effectively utilizing such in-house unused resources, a cost-saving and energy-saving-type biomass production system can be established.

Although the large-scale microalgae culture has been put into practical application with respect to the several types of microalgae such as chlorella, euglena, and spirulina, their usages have been limited to the raw materials of high-value-added products such as health foods and pigments. The large-scale culture technologies intended for producing energy like biofuels have been researched and developed by many research institutes and companies around the world, particularly since the 2000s. However, they have not yet reached the practical application level. The practical production of the microalgae biomass is very difficult. However, if the practical production is realized in a steelworks, taking advantage of a number of its infrastructures applicable to the production, such a process can be an effective CO_2 gas reduction technology. Therefore, we are promoting research with the intention of developing a large-scale microalgae culture technology that can be introduced into steelworks. In the research conducted so far, we started by collecting and isolating the algae strains for culture, and then evaluated the characteristics of the obtained candidate algae. Then, in order to maximally utilize the growth rate of the candidate algae, we investigated the substrate surface culture method that is different from the conventional liquid culture method. The substrate surface culture is a culture method excellent in gas exchange and sunlight utilization efficiencies, and is expected to be a technology that greatly enhances the productivity of microalgae. This paper introduces this research.

2. Main Discourse

2.1 Isolation of strain for culture and evaluation of its characteristics

2.1.1 Background

Upon establishing a biomass production system using microalgae, the selection of the strain for culture is a crucial issue. Since the characteristics of microalgae vary greatly depending on the species and/or strain, it is required to select a strain with characteristics suitable for the purpose. For the biomass production, the selection of strains having the following characteristics is desired: adaptability to the culture environment, ease of culturing, high growth rate, and high accumulation of lipid and carbohydrates. Since our culture system is based on the utilization of coking wastewater as a prerequisite, we decided to first select strains that can grow in the coking wastewater.

The ammonia contained in coking wastewater is a nutrient for algae, and at the same time, toxic in high concentrations.[4] Furthermore, since the coking wastewater is diluted with seawater in the treatment process, the strains for culture must have resistance at least to ammonia and salt. There are two methods of selecting the candidate strains: one is the selection from algae strains owned by universities and/or by the microbiological strain preservation institutions (culture collections), and the other is the independent collection of new strains from the environments. Since there are no examples of separating algae from seawater containing high concentration ammonia, we considered that there are very few known algae strains applicable to coking wastewater. Therefore, we considered that it would be more efficient to first collect algae that can grow under the above conditions from the actual coking wastewater treatment facility, and then to select from among them the strain that grows rapidly. Thus, the algae that can be the candidate culture strains were isolated.

In establishing a culture system using the strain isolated as described in the previous section, we first conducted an experiment to grasp the characteristics of the strain. In particular, we decided to clarify the growth rate that is important as a characteristic directly related to the biomass productivity. Furthermore, in order to evaluate the ammonia resistance required for application to the coking wastewater, we studied the relationship between the ammonia concentration and the growth rate.

2.1.2 Material and method

Among the biofilms that adhered to the coking wastewater treatment facility in the steelworks, a part of the one visually tinged with green was scraped off, and recovered with a small amount of the wastewater as algae samples. The samples were spread onto an agar plate medium (filtered natural sea water added with Daigo's IMK Medium (FUJIFILM Wako Pure Chemical Corporation) to a final concentration of 250 mg/L, agar 1.5%), and stationary-cultured in the artificial climate chamber (Nippon Medical and Chemical Instruments Co., Ltd., LPH-411PFD-SP) under the condition of temperature: 25°C, 14L/10D light-dark cycles, and photosynthetic photon flux density: about 100 μmol/m^2/s. Collected with a micro loop, the microalgae in the green colony that appeared on the agar plate medium were subcultured on a new agar plate medium. This subculture was repeated. During this work, several strains were selected from the colonies that appeared in a short period of days and showed the high growth rate. By repeating the subculture, they were sterilized. One of the strains was nominated as the NS001C strain, and was observed by an optical microscope (Nikon Corporation, DS-Fi2). In addition, for identification of the species, analysis of the

18S rRNA genetic sequence was conducted (through outsourcing).

The NS001C strain cultured in advance in the enriched seawater medium (one liter of natural seawater added with 20 ml of PES culture medium[5]) was subcultured in a 30 ml enriched seawater culture medium, contained in a silicon-plug-sealed 100 ml baffled conical flask, so that the initial optical density of cells at the wavelength of 730 nm in the medium was 0.01. They were shaking-cultured at the rate of 120 rpm in an artificial climate chamber at temperature: 25°C, 14L:10D light-dark cycles, and photosynthetic photon flux density: about 150 μmol/m^2/s. At two-hour intervals, one ml of the culture solution was fractionated, and the optical density at a wavelength of 730 nm was measured with a spectrophotometer (Hitachi High-Tech Science Corporation, U-2910), and the growth curve in the logarithmic growth phase was plotted.

The PES culture medium that does not contain nitrogen (sodium nitrate removed from the PES culture medium, hereinafter referred to as N−PES culture medium) was prepared, and the N−enriched seawater culture medium (one liter of seawater added with 20 ml of N−PES culture medium) was formulated. Additionally, an ammonium chloride aqueous solution (20 000 mg/L as N) was prepared. Seven types of culture medium each having a different ammonia nitrogen concentration (0, 20, 40, 80, 150, 300, 1 000 mg/L as N) were prepared by creating a 30 ml N−enriched seawater medium and by adding thereto a proper quantity of the ammonium chloride aqueous solution in 100 ml baffled conical flasks. The NS001C strain, cultured in advance as described above, was subcultured in the respective culture medium so that the initial optical density of cells at a wavelength of 730 nm in the medium was 0.1, and cultured under the same condition as described above. One ml of the culture solution was collected from the respective flasks on the very day of the start, first, second and third days after the start of the culture and the optical density was measured at a wavelength of 730 nm to plot the growth curves.

2.1.3 Result and discussion

Figure 1 shows the results of the optical microscope observation of the NS001C strain. The NS001C strain is a green ellipsoidal unicellular alga with a cell diameter of approximately 3–8 μm. The growth due to autospore formation was observed. As a result of the analysis of the 18S rRNA genetic sequence, it was suggested that the strain is closely related to the *Trebouxiophyceae* class *Chlorella* genus or *Parachlorella* genus and can be a novel genus and novel strain.

We also succeeded in isolating several other unicellular algal strains in addition to the NS001C strain. Since the NS001C strain showed the best growth rate in the simple experiment on agar plate medium among those strains (data not shown), and many strains with excellent growth rates have been found in the algae closely related to *Chlorella*,[6] we selected the NS001C strain as a provisional culture strain for the subsequent research.

Figure 2 shows the growth curve of the NS001C strain in the logarithm growth phase. The optical density showed an exponential increase. The doubling time of the optical density calculated from the growth curve is 4.54 hours, which is 0.15 h^{-1} when converted to the specific growth rate. Although a direct comparison is difficult due to different experimental conditions, a previous research reported that the specific growth rate of *Chlorella sorokiniana* UTEX 1230, which has an excellent growth rate, is 0.12 h^{-1},[7] thus clearly indicating that the NS001C strain has an extremely high growth rate.

Figure 3 shows the growth curve at each ammonia concentration. The growth rate is highest when the ammonia nitrogen concentration is 20 mg/L, and gradually decreases as the ammonia concentration increases. In addition, linear growth is also observed at 1 000 mg/L. Since the growth is also observed at a very high concentration of ammonia, this demonstrates that the NS001C strain has sufficient ammonia tolerance for the application to coking wastewater. In addition, it was revealed that it has salt tolerance that enables the growth both in fresh water and seawater (data not shown). Based on these findings, the NS001C strain was considered to be suitable as a

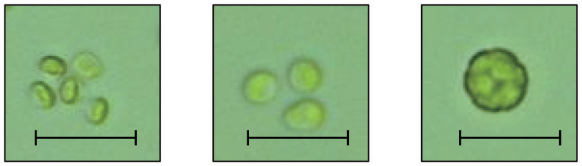

Fig. 1 Microphotographs of the NS001C strain
Cells of the NS001C strain cultured with 14 L/10 D light-dark cycles were observed at three different time points in a light period. Left: autospores. Middle: vegetative cells. Right: an autosporangium.

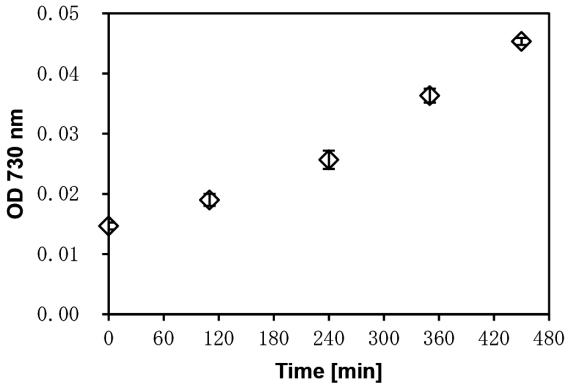

Fig. 2 Growth curve of the NS001C strain in the log phase
Optical density at 730 nm of culture solutions was measured by a UV-Vis spectrometer. The symbols indicate the mean value of the three cultures. The error bars indicate the standard deviations.

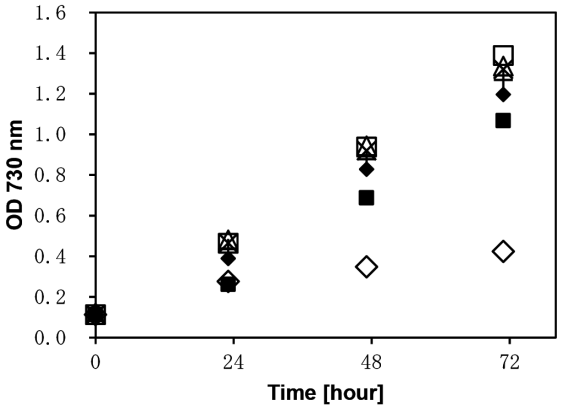

Fig. 3 Growth curves of the NS001C strain under various ammonia concentrations
Optical density at 730 nm of culture solutions was measured by a UV-Vis spectrometer. ◇: NH_4-N 0 mg/L, □: NH_4-N 20 mg/L, △: NH_4-N 40 mg/L, ×: NH_4-N 80 mg/L, +: NH_4-N 150 mg/L, ◆: NH_4-N 300 mg/L, ■: NH_4-N 1 000 mg/L.

culture strain in the algal culture system using the iron and steel making wastewater, and we decided to conduct further research using this strain. Based on the above characteristics, the NS001C strain was deposited as a patented microorganism (receipt number: FERM AP-22328) at the Patent Microorganism Depositary Center of the National Institute of Technology and Evaluation, an independent administrative institution.[8)]

2.2 Examination of substrate surface culture method

2.2.1 Background

Although we conducted small-scale experiments with the conventional liquid culture method using the NS001C strain aiming to establish a highly efficient biomass production/CO_2 recovery system, the biomass productivities obtained were lower than expected. In the liquid culture method, the NS001C strain exhibited the high growth rate only when the cell density was extremely low, and when the cell density increased by the culture, the growth rate significantly decreased. We considered that this is due to the lack of light and CO_2 required for the photosynthesis, and that it is necessary to review the culture method in order to solve this problem.

Conventionally, the liquid culture method in which algae are suspended in a liquid culture medium and cultured with stirring is generally used for microalgal culture. Even in large-scale culture, the liquid culture method using an open pond and/or a photo-bioreactor is used. The liquid culture method is an excellent culture method that can be applied to most algae. However, there are two major problems. One is that the gas exchange is difficult. The algae consume CO_2 in the medium by photosynthesis and discharge O_2. As the photosynthesis by the algae becomes active, the CO_2 concentration decreases and the O_2 concentration increases in the medium. As a result thereof, the photosynthetic rate is lowered. This problem can be improved by aerating a gas containing CO_2 through the medium. However, the rate of dissolution of CO_2 in water is low, and it is not easy to supply sufficient CO_2 to the algae. The other problem is the low light utilization efficiency. Since the algal cells themselves block the light, the light hardly reaches the inside of the culture solution when the cell density is high. On the other hand, when the use of sunlight is considered, there is another problem. Since strong light like sunlight cannot be used for photosynthesis, the light cannot be used efficiently even on the surface layer of the culture solution. For this reason, the efficiency of the light utilization in liquid culture is not so high.

Therefore, in order to exploit the high growth rate of the microalgae, and to improve the biomass productivity thereby, among the culture methods called substrate culture or surface culture in which the algae are carried and cultured on the surface of a substrate, the culturing method of algae in a gaseous phase has garnered attention (hereinafter referred to as the substrate surface culture method).[9, 10)] In this method, the algae are carried on the substrate in a paste-like state containing a small amount of water, and are in contact with both the gaseous phase and the solid phase (substrate) (**Fig. 4**). By making the substrate a porous body like sponge, and impregnating it with a liquid medium, the algae can absorb water and nutrients from the substrate.

We consider that this substrate surface culture method has three major advantages. The first is that the gas exchange rate is superior to that of the liquid culture method. Since the algae are in contact with the gaseous phase, it is possible for the algae to take in CO_2 from the gaseous phase and discharge O_2 into it. The second is that the light utilization efficiency can be improved. Although the use of the sunlight is a prerequisite for algal biomass production, it is difficult to utilize all of the sunlight through photosynthesis. The Photosynthetic Photon Flux Density (PPFD) of the sunlight near the ground exceeds 2 000 μmol/m^2/s at the maximum. On the other hand, the photosynthetic rate is a constant rate above a certain PPFD, which is called the light saturation point. The value, though dependent on the species, has been reported to be approximately 50 to 500 μmol/m^2/s.[11)] When algae are exposed to strong sunlight, the light exceeding the light saturation point is not effectively utilized for the photosynthesis, and does not contribute to the biomass production. To solve this issue, a method of utilizing sunlight effectively by increasing the sunlight receiving area and decreasing the strength of the sunlight is considered. In the substrate surface culture method, the substrate supporting the algae can be arranged at arbitrary angles. Therefore, it is possible to adjust the strength of the sunlight onto the algae on the substrate surface by adjusting the installation angle of the substrate (**Fig. 5**). Thus, the substrate surface area per the culture equipment footprint means that the area used for

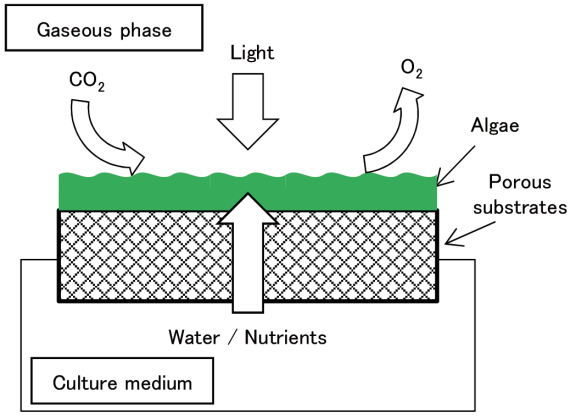

Fig. 4 Conceptual drawing of substrate surface culture
Algae are cultured on porous substrates in a gaseous phase. Light is supplied to algae through the gaseous phase, while water and nutrients are supplied from the substrates filled with a culture medium. Algae can take up CO_2 from the gaseous phase and discharge O_2 into it.

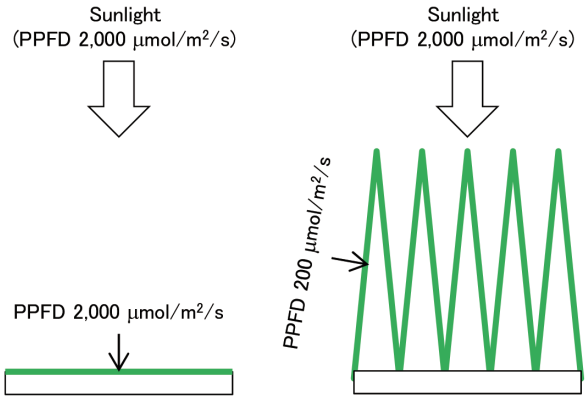

Fig. 5 Conceptual drawings of effective utilization of sunlight by substrate surface culture
When substrates are arranged vertically in the direction of the sunlight, algae cannot utilize the sunlight efficiently because PPFD on the substrates exceeds their light saturation point (left). When the substrates are arranged diagonally in order to decrease PPFD on the surfaces of substrates to the saturation point or lower, algae can utilize the sunlight more efficiently with the wider area of substrate surface than in the situation on the left (right).

culture can also be increased. As a result thereof, the sunlight is utilized efficiently in a wider area, and can contribute to the enhancement of biomass productivity. In the liquid culture method wherein the algae are disorderly suspended in the liquid medium, it is impossible for such a method to adjust the strength of light.

The third advantage concerns the possibility of collecting high-density biomass. In the substrate surface culture method, the algae are in a paste-like state containing only a small amount of water. Accordingly, the energy cost required for the dehydrating treatment of the biomass after its collection is expected to be significantly reduced. In the microalgal biomass production, the high energy cost required for the processes of dehydration and drying after its collection was pointed out.[12] Therefore, the availability of low water content biomass is a significant advantage in improving the energy balance.

Based on the above, we considered that the substrate surface culture method is a feasible technology to solve the productivity issue in the large-scale microalgal culture. On the other hand, studies on this are scarce as compared with those of the liquid culture method, and the culture method can hardly be referred to as being established. Furthermore, there are few examples of applying the marine microalgae to the substrate surface culture method and the applicability of the NS001C strain to the substrate surface culture method remained unknown. Therefore, we first employed a small-scale substrate surface culture system, and examined the applicability of the NS001C strain to the substrate surface culture method. Simultaneously, we conducted the experiment to measure the biomass productivity quantitatively.

2.2.2 Material and method

An experimental system of the substrate surface culture method was used, wherein the culture was conducted by placing a glass filter carrying an alga on a sponge that contains a liquid culture medium (**Fig. 6**). The cell suspension of the NS001C strain which was liquid-cultured in advance in the enriched seawater medium (filtered sterilized seawater added with Daigo's IMK Medium to a final concentration of 250 mg/L) was filtered through a 47 mm in diameter glass filter (GE Health Care, GF/F), the tare weight of which had been measured in advance, and the algal cells were carried on the filter so as to make the biomass density about 1 mg/cm^2. The following seawater medium was used as the culture medium: artificial seawater (Tomita Pharmaceutical Co., Ltd., Marine Art SF-1) added with Daigo's IMK Medium to a final concentration of 500 mg/L to avoid the depletion of the nutrients. A PVA sponge sheet (AION Co., Ltd. AION PVA sponge sheet D-series) 10 mm thick and of a size slightly smaller than that of the plastic case inner base area was laid on a transparent polystyrene plastic case (148 mm long, 84 mm wide and 32 mm deep). 120 ml of the seawater culture medium was added into the case, and the sponge sheet was soaked with the culture medium. The three filters each carrying the NS001C strain were placed on the sponge sheet in the case, inserted into a transparent plastic bag with a fastener and were sealed while the case was uncovered. This was held in the culturing chamber at 25°C under the LED illumination condition of a light period of 14 hours and a dark period of 10 hours, and air or the air mixed with CO_2 at a ratio of 100:1 (hereinafter referred to as 1%CO_2) was supplied to the bag through a silicone tube after the aeration in the sterile distilled water in a gas washing bottle (flow rate per case: about 150 ml/min). By adjusting the distance between the LED light and the filters, the light intensity on the filter surface was changed. PPFD was measured with a light quantum meter (Nippon Medical and Chemical Instruments Co., Ltd., LA-105). The entire culture medium was changed after 2 days of culture. After 3 days of culture, the filters were collected and were placed on a suction-filtration equipment, and the filter and microalgal cells on the filter were rinsed with 5 ml distilled water while being suctioned by a vacuum pump. These filters were dried at 110°C in the drying oven overnight and their weight after drying was measured. From the difference between the weight of the filter after culture and the tare weight, the growth amount of the NS001C strain per substrate surface area and the areal biomass productivity were calculated.

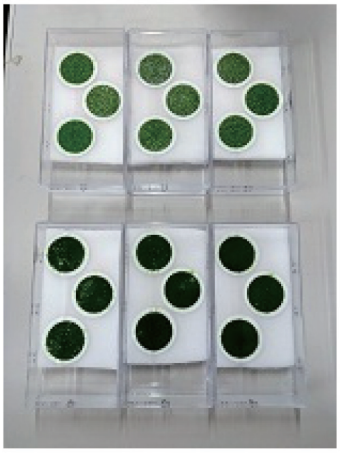

Fig. 6 Photograph of the substrate surface cultures
Algae are cultured on glass filters placed on sponges filled with a culture medium in plastic cases. Upper: three-day cultures supplied with air. Lower: three-day cultures supplied with 1%CO_2.

2.2.3 Results and discussion

Figure 6 shows the actual aspect of the substrate surface culture experiment. The results of the three experiments conducted at different CO_2 concentrations and light conditions are summarized in **Fig. 7**. PPFD during culture is shown on the horizontal axis, and the biomass productivity is shown on the vertical axis. Under any conditions, the growth of the NS001C strain was observed visually and indicated by increments of the biomass weight, and it was confirmed that the NS001C strain can grow with the substrate surface culture method using seawater medium. Under the condition with the supply of air, a trend of increased productivity up to a PPFD of 131 μmol/m^2/s is observed, above which point, the productivity becomes nearly constant and stays within 2.9–3.8 g/m^2/d. On the other hand, under the condition with a supply of 1%CO_2, the following trend is observed: the productivity increases until a PPFD of 264 μmol/m^2/s, above which PPFD, productivity becomes constant and stays within a range of about 12.4–15.4 g/m^2/d (values at PPFD 1 357 μmol/m^2/s lie in a wide range of error bars, therefore, we considered that these values should be outliers). The above result shows that the supply of 1%CO_2 improves the maximum value of the biomass productivity by about 4–5 times, and approximately doubled the PPFD at which the biomass productivity levels off and becomes constant.

This result is interpreted from the relationship between PPFD and the photosynthetic rate. As described earlier, the photosynthetic rate linearly increases with respect to the photosynthetic photon flux density while PPFD is low. However, above the light saturation point, the photosynthetic rate becomes constant.[13] Furthermore, with respect to the CO_2 in the air (about 400 ppm concentration), CO_2 is the rate-limiting factor of the photosynthesis, and the in-

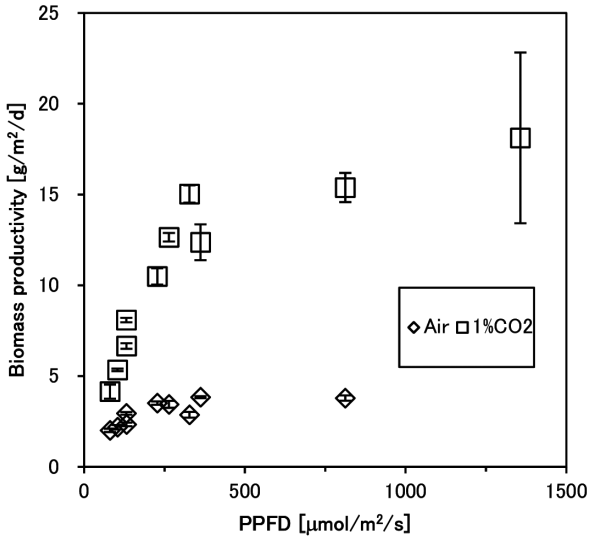

Fig. 7 Biomass productivities of the NS001C strain cultured with lights of various PPFDs
◇: supplied with air, □: supplied with 1%CO_2. The results of the three independent experiments are shown in this graph. The symbols indicate the mean values of three cultures. Error bars indicate the standard deviations.

Table 1 Theoretical biomass productivities calculated from measured values
Theoretical biomass productivities were calculated from the measured values obtained in the experiments of substrate surface culture with 1%CO_2 supply (data are shown in Fig. 7).

Experimental PPFD[*1] [μmol/m^2/s]	Measured biomass productivity[*2] [g/m^2/d]	Theoretical biomass productivity[*3] [g/m^2/d]
80	4.1	25.6
104	5.3	25.5
131	8.1	30.6
132	6.7	25.1
227	10.5	22.9
264	12.6	23.8
328	15.0	22.8
362	12.4	17.0
812	15.4	9.4
1357	18.1	6.6

[*1] PPFD used in the experiments.
[*2] Measured values of biomass productivities obtained from the experiments.
[*3] Theoretical biomass productivities at 25 mol/m^2/d of daily light integral calculated from the measured values.

crease of the CO_2 concentration is linked to the increase of the photosynthetic rate and the light saturation point. Under the condition wherein the algae grow actively, the biomass productivity is considered to be approximately proportional to the photosynthetic rate. Therefore, we consider that the above result was obtained.

By supplying CO_2 instead of air, a significant improvement in productivity was observed. The maximum value of the biomass productivity obtained this time is 15.4 g/m^2/d (excluding the outlier values). However, if the use of sunlight is taken into consideration, the productivity can be further improved. As described earlier, in the substrate surface culture method, there is a possibility of using sunlight more efficiently by devising the installation of the substrate. If the substrate is installed diagonally with respect to sunlight, PPFD of the sunlight on the surface of the substrate is kept below the light saturation point, and the surface area of the substrate vs. the installation area of the culture equipment is increased. Therefore the substrate surface culture method can utilize almost all the sunlight in theory. In reality, the altitude and the direction of the sun change depending on the season and time, and furthermore, the culture equipment itself blocks the sunlight, so the discussion above is overly simplified. However, herein, disregarding such restrictions, the theoretical biomass productivity under sunlight was calculated from the following Equation (1) using the experimental values under the condition of supplying 1%CO_2.

$$P_t = P_m / L_e \times L_s \quad (1)$$

where P_t: Theoretical biomass productivity [g/m^2/d]
P_m: Biomass productivity obtained in the experiment [g/m^2/d]
L_e: Daily light integral used in the experiment [mol/m^2/d]
L_s: Daily light integral used in sunlight [mol/m^2/d]

Herein, the daily integrated light (DLI) is the value obtained by converting PPFD into the daily integrated amount, and the unit is mol/m^2/d. The daily light integral used in the experiment (L_e) was calculated by integrating PPFD as to the length of the light period (14 hours) under the light conditions used in the experiment. The daily light integral under sunlight (L_s) was set to 25 mol/m^2/d referring to previous studies. This is considered to be close to the annual average value of the daily light integral in Japan.[14] **Table 1** shows the theoretical biomass productivity calculated from each measured experimental value. Theoretically, it is suggested that a productivity of about 20–30 g/m^2/d is feasible. In the preceding study, the ideal biomass productivity under sunlight is estimated to be 33–42 g/m^2/d.[15] The fact that the estimated values close to the ideal values were obtained indicates the high biomass productivity of the substrate surface culture using the NS001C strain. Furthermore, the productivity can be expected to be improved further by optimizing the culture conditions such as the CO_2 concentration, medium, and the substrate. On the other hand, in order to realize the estimated values, the culture equipment that realizes the efficient utilization of sunlight as conceptually shown in Fig. 5 is required. Several types of such culture equipment have been proposed in the preceding studies, and relatively large-scale experiments have been conducted.[9] However, it can hardly be said to be technically established. Therefore, the development of the culture equipment that can be scaled up remains a future subject of investigation.

3. Conclusion

In order to develop new technologies that contribute to CO_2 reduction, we are conducting research with the aim of developing a large-scale microalgal culture system that can be introduced into steelworks. This paper introduced a series of studies from the isolation of algae in steelworks, via the characterization evaluation of the cultured strains, and to the examination of their applicability to the substrate surface culture. The unicellular green alga NS001C strain isolated from the wastewater treatment facility in a steelworks had a high growth rate and ammonia resistance. Furthermore, this strain can be applied to the substrate surface culture using a seawater culture medium, and achieved a high biomass productivity of 12.4–15.4 g/m^2/d under the 1%CO_2 supply conditions. Theoretically, it is suggested that a productivity of about 20–30 g/m^2/d can be achieved in the substrate surface culture using sunlight. From these results, it

was clarified that the substrate surface culture is an effective means for achieving high biomass productivity. However, in order to achieve the estimated value, the culture equipment that enables the effective utilization of sunlight is indispensable. We consider that the development of such culture equipment is one of the major future research issues.

Many research groups around the world are engaged in the research concerning microalgal biomass production due to the growing concerns about global warming. However, there are still many challenges to be addressed before it can be put into practical use. However, the development of the innovative CO_2 fixation technologies is indispensable for solving the global warming problem, and we consider that the large-scale microalgal culture system in a steelworks wherein there are various infrastructures available to the culture is one such innovation. We are determined to continue to promote research toward the practical application of the large-scale microalgal culture system that can be introduced into steelworks.

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