

Study of Technology for Outdoor Stable Culture of Microalgae for Recycling of CO₂ in Atmosphere

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ABSTRACT

The optimization of culture tank structure and culturing conditions was investigated using Honda's own strain of microalgae and a flat panel photobioreactor for the purpose of creating carbon dioxide fixation technology using microalgae capable of repurposing carbon dioxide in the atmosphere as a valuable resource. The aim is to build a carbon-neutral society. The culturing conditions were configured in terms of the concentrations of the nutrient salt components of nitrogen, potassium, and phosphorus. By adopting a flow guide shape that discharges liquid and gas in a single direction, a flow-field velocity of 0.02 m/sec or higher was achieved for the culture solution. This optimization of the culturing conditions successfully increased microalgae production volume. Regarding the culture tank structure, the heat insulating layer structure made it possible to maintain temperatures within the control range throughout the year. The culture tank was also installed at a 45° tilt, which successfully increased the amount of incident light in the culture tank. Outdoor culture tests incorporating these factors yielded an expectation of algae production volume of 13.5 g/m²/day. These results indicate the possibility that DREAMO will be able to achieve ethanol production volume per planted area that is equal to or better than bioethanol from sugarcane, which is a carbon-neutral fuel.

1. Introduction

In the context of environmental issues and movements to build a carbon-neutral society, attention is being paid in recent years to carbon capture and utilization (CCU) technology, which collects carbon dioxide (CO₂) that has been discharged into the atmosphere and repurposes it as a valuable resource. Various methods for capturing CO₂ are being investigated, including direct air capture (DAC), which collects CO₂ directly from the atmosphere, technology targeting the capture of highly concentrated CO₂ from thermal power stations and other such sources, chemical absorption methods, physical adsorption methods, and so on. The captured CO₂ is used directly in dry ice, welding, and so on, and used as an ingredient in plastics and other chemical products, fuel, concrete, and other such products⁽¹⁾. CCU technology that makes use of microalgae absorbs CO₂ through photosynthetic reaction and converts CO₂ to a valuable substance through biological functions, and thus it is expected to provide conversion to valuable substances at low energy expenditure.

Compared with other biomasses, microalgae yielded greater annual CO₂ fixation volume per planted area than terrestrial plants. The light energy utilization efficiency

of common terrestrial plants 4.6%⁽²⁾ while microalgae are highly efficient with a top theoretical efficiency of 12.0%⁽²⁾ and they have better CO₂ absorption performance. As stated above, microalgae are capable of intracellular conversion of absorbed CO₂ into a variety of valuable substances. It is converted into the raw material of carbohydrates and other such valuable substances inside the microalgae and stored there. It is then possible to repurpose that raw material to make jet fuel⁽³⁾, bioethanol⁽³⁾, feedstock for plastics⁽⁴⁾, among other such uses in industry as well as in the food and medical fields.

Typical microalgae culturing methods that are commonly found are open pond systems, raceway systems, flat panel systems, and so on. The culture tank structure and research conducted for each system have been reported⁽⁵⁾. For the present research, a flat panel system was adopted in order to raise algae production volume per installed area. Figure 1 shows a conceptual image of the culturing equipment.

This paper introduces measures taken with respect to culturing conditions and culturing equipment for the creation of outdoor stable culture technology using a flat panel photobioreactor with the proprietary microalgae strain developed at Honda and multiple culture tanks in a row.

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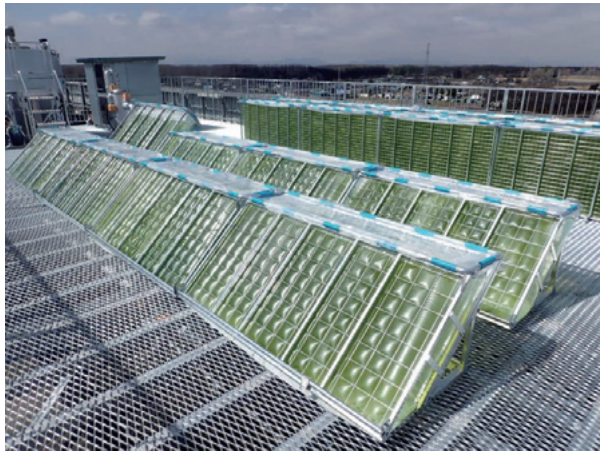


Fig. 1 Image of culture tank equipment

2. Distinctive Characteristics of Honda DREAMO and Outdoor Culture Technology

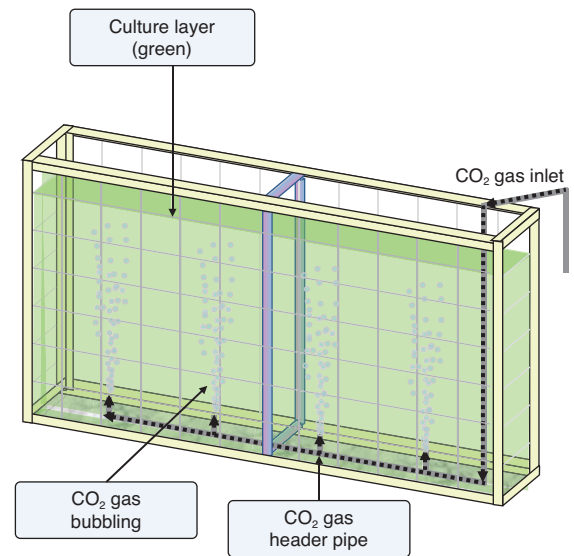
2.1. Distinctive Characteristics of Honda DREAMO Strain

The distinctive characteristics of the microalgae used for this research will be described. Honda's own strain (Honda DREAMO, hereinafter DREAMO) has the *Chlamydomonas reinhardtii* UTEX 90 (National Institute for Environmental Studies: contract NIES-2236) as its parent strain and was acquired by screening without genetic modification. The main distinctive characteristics^{(6), (7)} will be described. The first is that it has a higher production volume per unit area than other microalgae. Different kinds of microalgae have different production volumes, where *Chlamydomonas* of the same species as DREAMO yield from 0.10 to 0.25 g/L/day⁽⁸⁾; and in an ideal indoor environment that is unaffected by the influences of outdoor environmental disturbance, it has the potential to yield the high amount of 0.50 g/L/day. The second is that it is possible to increase its production volume of starch, which becomes the raw material for valuable substances. Generally, the algae production rate and starch content are in a trade-off relationship, but DREAMO can increase the starch content⁽⁶⁾ while maintaining the algae production rate. The third is that while the cell wall of *Chlamydomonas reinhardtii* UTEX 90, which is the parent strain of DREAMO, is 0.3 μm thick, the DREAMO cell wall is thinner, at 0.09 μm . This means that the valuable components stored in the DREAMO cells can be extracted⁽⁷⁾ by a short process.

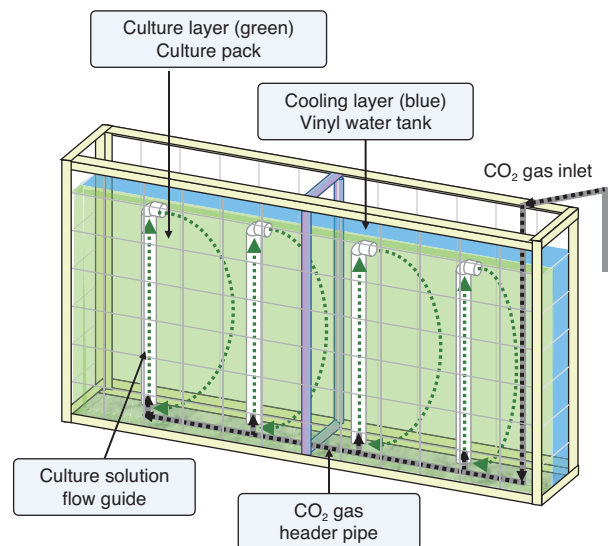
2.2. Flat Panel Photobioreactor

DREAMO culture is carried out in a flat panel photobioreactor in order to raise the algae production volume per installed area. Figures 2(a) and 2(b) show the structures of a general flat panel photobioreactor and of the distinctive Honda flat panel photobioreactor. In the general flat panel photobioreactor in Fig. 2(a), the tank is shallow,

with a depth ranging from 1 to 10 cm⁽⁵⁾, and structured for a water depth of 100 cm⁽⁵⁾ or less. Aeration takes place by discharging CO₂ gas from the piping installed at the bottom of the culture tank, stirring the culture solution. This arrangement suppresses the spontaneous sedimentation of the microalgae. On the other hand, the distinctive characteristics of the Honda original flat panel photobioreactor in Fig. 2(b) are the structure that retains cooling water in the culture tank to use the thermal capacity of the water to hold the temperature control energy for the culture solution down to the lowest possible level, and the structure equipped with flow guides for the culture solution. The culture tank has a



(a) General structure of flat panel type culture tank with culture tank and CO₂ gas bubbling



(b) Honda flat panel type culture tank

Fig. 2 Structural comparison of flat panel type culture tanks (a) and (b)

Table 1 Important factors and issues of DREAMO

Factor	Culture effect	Issue	Theme
Nutrients	Nutrient depletion	Contamination proliferation	Nutrient concentration optimization
Broth stirring	Algae subsidence	CO ₂ dissolution amount, O ₂ dissolved amount, reduced light reception	Stirring speed optimization and structure
Water temperature	Water temperature rise	Increased temperature control energy	Temperature control energy minimization
Incident light	Reduced production efficiency, water temperature rise	Low utilization of incident light, water temperature rise	Incident light utilization rate upward, temperature control energy minimization

vinyl water tank filled with cooling water and a culture pack into which culture solution is injected fixed in place inside the housing. In this structure, the culture pack is placed inside the cooling tank filled with cooling water. In order to reduce the energy used to stir the culture solution, stirring is done by air-lift using supplied gas.

Figure 3 shows the ancillary equipment for the device. This is made up of a feed pump for collecting and injecting the cultured DREAMO, a settlement tank for collecting the culture solution with included DREAMO, and a blower used to supply exhaust gas and other such gas that contains CO₂. The settlement tank is a device with the added function of spontaneous sedimentation of cultured DREAMO in the primary concentration.

2.3. Issues in Outdoor DREAMO Culture

Increasing microalgae production volume involves numerous issues⁽⁹⁾. Table 1 shows those culture factors^{(6), (7), (9)} and those culture effects⁽¹⁰⁾ that are important for DREAMO out of those factors that have an influence on general microalgae culture. Important factors include nutrient salts, culture solution (broth) stirring, culture solution (water) temperature, and light.

Regarding nutrient salts, it is possible to control microalgae production volume⁽¹¹⁾ by varying the concentrations of nutrient components. Consequently, how to set those concentrations is an important issue.

Culture solution stirring is an important factor in terms of suppressing the inhibition of production caused by the spontaneous sedimentation of DREAMO.

The culture solution temperature also has an effect on decreasing algae production volume and reduction of ecological activity. Figure 4 shows the results from tests of the production volume per week. These indicate that the optimal DREAMO temperature is in the range from 20°C to 35°C, and the limit temperature for not inhibiting growth is 40°C. In the low-temperature range, it is clear that production volume decreases at or under 10°C. In order to realize stable culture, therefore, control of the culture solution temperature to keep it in the range between 10°C and 35°C is an issue.

Light is the energy source for photosynthetic reaction, and as the intensity of the light cannot be readily increased, it is important to consider directing a greater amount of light onto the algae.

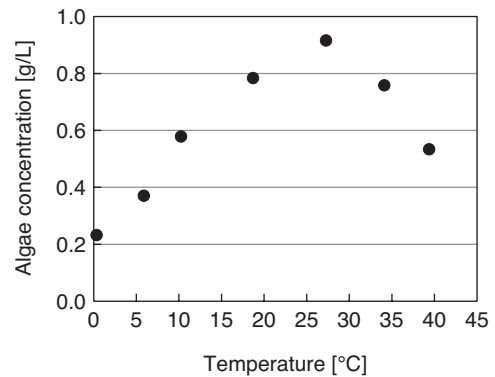


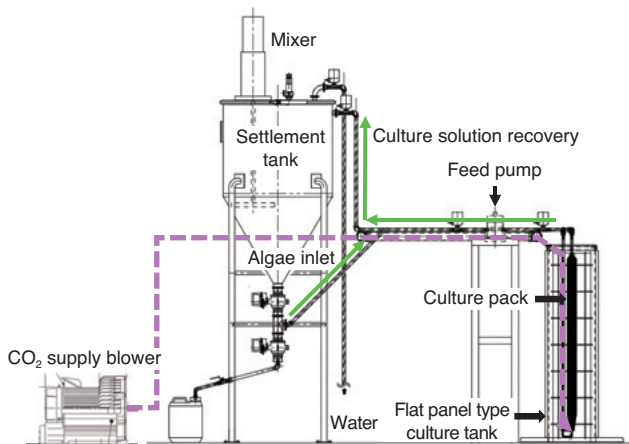
Fig. 4 Temperature dependence of DREAMO production

3. Development Goals

This research used the proprietary DREAMO developed at Honda and the flat panel photobioreactor to investigate the following items.

- (1) Microalgae culturing conditions (proportions of nutrient salts and culture solution stirring speed)
- (2) Culture tank structure to enable temperature control and the greatest possible amount of incident light

The raw material for bioethanol comes mainly from

Fig. 3 Ancillary equipment for harvesting and CO₂ supplying

corn and sugarcane. Ethanol production from sugarcane has a capacity per planted area that is three times greater than corn⁽¹²⁾ and is known to be more highly efficient than corn. The target value for the present research was therefore set as 10.0 g/m²/day (equivalent per occupied area), which is the amount of microalgae necessary in order to achieve efficiency that is on a par with ethanol from sugarcane, which has a production volume of 11.2 m³/ha/year⁽¹²⁾.

4. Investigation of Microalgae Culturing Conditions

4.1. Investigation of Nutrient Salts

Elements such as nitrogen (N), phosphorus (P), potassium (K), and so on are among the important nutrients in microalgae culture. Phosphorus is used in the form of adenosine tri-phosphate (ATP), which provides the energy in metabolic reactions. Potassium and other such minerals act to supplement chemical reactions inside cells, playing an important part in metabolic reactions. On the other hand, nitrogen is one of the important components that make up cells, and it regulates the culture medium⁽⁷⁾ that has already been optimized. This research investigated phosphate and potassium concentrations that are suited to DREAMO culture.

The components of the culture solution that serves as a base are shown in Table 2. Phosphorus and potassium make up the phosphate culture medium. The investigation thus sought to determine phosphorus and potassium concentrations that would yield the greatest DREAMO production volume using the potassium phosphate concentration as a benchmark and without changing the phosphorus/potassium ratio. The test conditions and test results are shown in Table 3 and Fig. 5, respectively. When potassium phosphate is at 2,000 ppm or lower, decreasing potassium phosphate concentration is accompanied by diminishing production volume. On the other hand, when the potassium phosphate concentration is at 2,000 ppm or above, production volume remains flat, so a potassium phosphate concentration of 2,000 ppm was set as the lower limit value.

4.2. Investigation of Culture Solution Stirring Conditions

Investigation of culture solution stirring suited to DREAMO production was carried out with the flow-field velocity of the culture solution as an index. The flow-field velocity requirements are set according to the relationship between flow-field velocity and photosynthesis efficiency (PE), which is an index of DREAMO production efficiency. As shown in Eq. (1), the PE is defined as the proportion of the energy equivalent value of DREAMO production volume and the amount of light received during the culture period.

$$\text{PE [\%]} = \frac{\text{DREAMO production volume [MJ/m}^2\text{/day]}}{\text{Photosynthetically active light energy [MJ/m}^2\text{/day]}} \quad (1)$$

The aim in raising culture solution stirrability is to suppress spontaneous sedimentation and thereby suppress

Table 2 Nutrient components of culture solution

Stock liquid	Chemical formula
UREA	CH ₄ N ₂ O
	MgSO ₄ ·7H ₂ O
	CaCl ₂ ·2H ₂ O
Phosphate	K ₂ HPO ₄
	KH ₂ PO ₄
Hutner's trace elements	ZnSO ₄ ·7H ₂ O
	H ₃ BO ₃
	MnCl ₂ ·4H ₂ O
	FeSO ₄ ·7H ₂ O
	CuSO ₄ ·5H ₂ O
	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O

Table 3 Typical conditions for lab-scale DREAMO culture

Factor	Unit	Value
Water temperature	[°C]	28
Incident light	[mol/day]	16
Light irradiation time	[hour-light / hour-dark]	10 / 14
Air-CO ₂ mixed gas airflow	[vvm]	0.15
CO ₂ concentration	[%]	5
Amount of culture medium	[L]	2

the inhibition of DREAMO production. In order to achieve the target algae production volume, the PE enhancement effect was allocated to each item for implementation, and it was found from the results that PE enhancement of 3.2% from stirring would be sufficient.

The flow field velocity of the culture solution was visibly quantified using the particle image velocimetry (PIV) method. Measurement was made by simulation of a culture tank, as shown in the photographs in Fig. 6, using a culture container (b) assembled with a flow guide (a). This was done using tracer particles of a specific weight and size equal to DREAMO in the culture solution. In the course

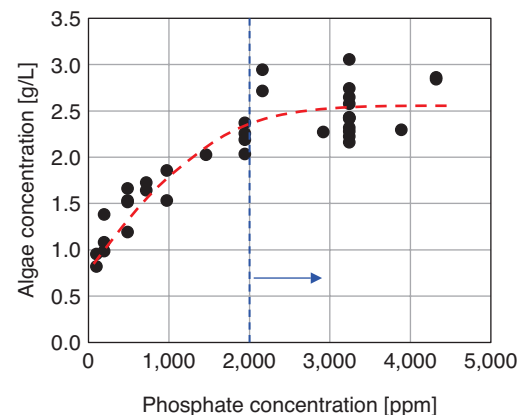


Fig. 5 Phosphate dependence of DREAMO production

of measurement, gas aeration and stirring of the tracer particles were performed. Particle velocities in the X-axis and Z-axis directions were measured by high-speed camera. It was anticipated that there would be variations in velocity inside the container as a result of the influence of bubble diffusivity due to water levels, and measurements were made in the height direction at three locations as enclosed by the red square in Fig. 7.

The relationship between the average velocity inside the culture container obtained by the above measurements and the PE enhancement margin is shown in Fig. 8. It was confirmed that when the necessary flow field velocity was 0.02 m/sec or greater, the PE would be enhanced by 3.2% or more.

Next, the culture solution stirring structure was investigated for the requirement of a flow field velocity of 0.02 m/sec.

The two types of guide shape shown in Figs. 9(a) and 9(b) were investigated. Both proposed shapes supplied gas from the bottom of the culture pack to the inside of the flow guide, placing an upward velocity through the flow guide to create a flow in the culture solution. Shape (a) is structured

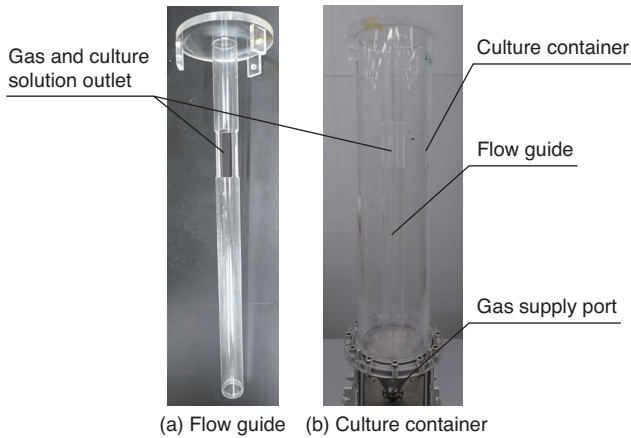


Fig. 6 Simulated culture tank test container

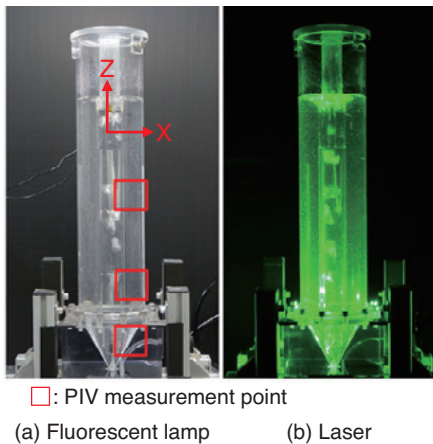


Fig. 7 Flow velocity measurement by (a) and (b)

so that gas and liquid are discharged perpendicularly to the culture tank installation surface. The aim of this structure is to create leftward and rightward flows from the installed flow guide in the middle. Shape (b) is structured so that gas and liquid are discharged in a single direction. The aim here is to increase the flow velocity by causing the flow to go in a single direction.

The flow field velocities when using flow guide shapes (a) and (b) were predicted by computational fluid dynamics (CFD) using the Fluent solver from Ansys, Inc. This simulation predicts the flow field for the culture tank as a whole. It reflects the body force of the culture solution due to the air-lift, obtained by the PIV method, that has the gas supply as its driving force, together with the dispersibility of particles of the same specific weight and size as the algae.

Figure 10 shows the results from simulation of guide shapes (a) and (b). The flow velocity due to the air-lift inside the flow guide is 0.10 m/sec or higher. With shape (a), the only location where that flow velocity could be maintained was in the vicinity of the flow guide gas supply port. The flow velocities around the base of the culture pack and at the back of the guide were found to be extremely low. However, shape (b) was found to be able to maintain the

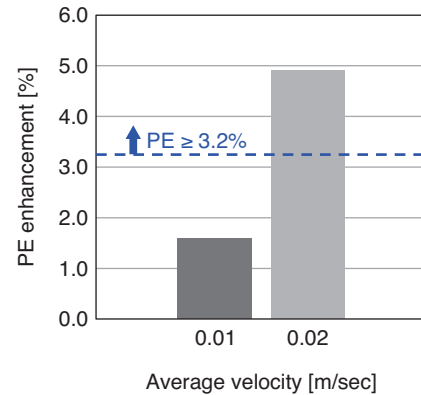


Fig. 8 Relationship between flow velocity and PE

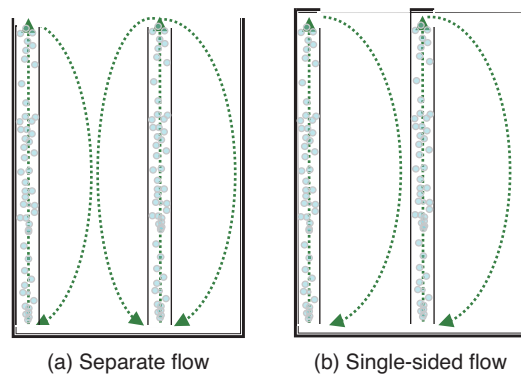


Fig. 9 Guide shape designs to make flows (a) and (b) in culture tank perpendicular to gas exhaust port

flow velocity in the culture pack as a whole. This is thought to occur because the supply ports for water and gas from the flow guide are oriented in a single direction so that the flow velocity due to air-lift is not dispersed and stirring occurs without reduction in the flow velocity. Given the above results, shape (b) was chosen for the flow guide shape.

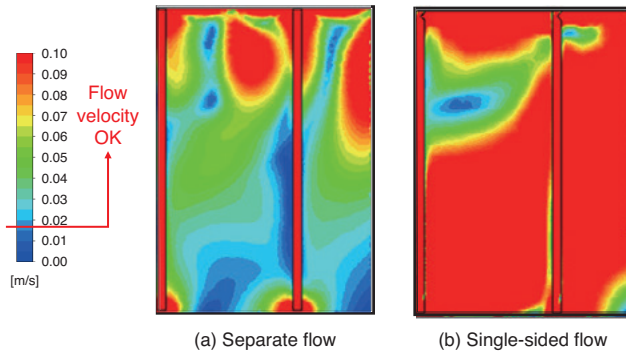


Fig. 10 Results of culture fluid flow simulations for guide shape effect

5. Investigation of Culture Tank Structure

5.1. Temperature Retention Due to Thermal Insulation Layer Structure

The control temperature for DREAMO culture is known to be from 10°C to 35°C. Culture tank structure that enables control within the control temperature range was investigated.

The culture solution temperature performance of the flat panel photobioreactor is shown in Fig. 11. For summer verification, this testing was carried out in Okinawa, where Japan has high insolation and outdoor temperatures. For winter, it was carried out in the northern Kanto region, where the temperatures drop below freezing.

In the summer, the control temperature was confirmed to be at or below the upper limit value of 35°C. On the low-temperature side, however, there was a large difference from the control temperature. A device structure that would raise the culture solution temperature by making use of solar energy was therefore investigated.

The method, as shown in Fig. 12, was a structure that maintains the culture solution temperature by adding a thermal insulation layer around the culture tank.

For the thermal insulating effect, the insolation⁽¹³⁾ and outside air temperature⁽¹⁴⁾ data provided by the New Energy and Industrial Technology Development Organization (NEDO) was used to calculate the annual temperature of the culture solution⁽¹⁵⁾. Changes were made to the culture solution temperature to reflect the heat input from solar energy together with the amount of heat released into the air and the ground surface in each time period. The rate of heat release into the outside air and some other such coefficients

reflect measurements made with the culture tank installed outdoors.

Figure 13 shows the annual predicted culture solution temperature. The thermal insulating structure was attached from October to March and removed from April to September. This yielded the expectation that the temperature could be kept within the control temperature range throughout the year.

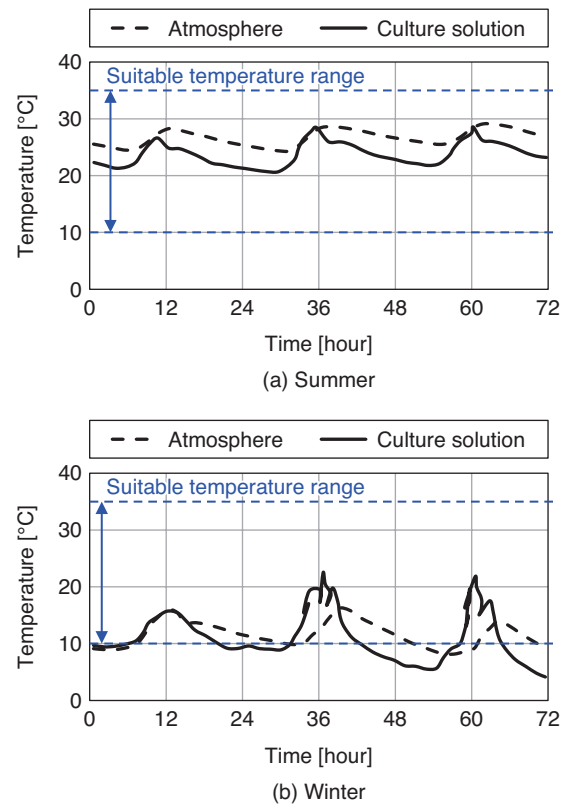


Fig. 11 Temperatures of culture tank culture solution and ambient air measured in summer and winter

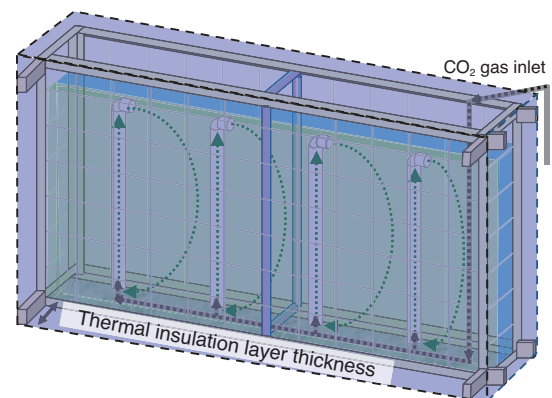


Fig. 12 Schematic representation of flat panel culture tank with heat insulating layer

5.2. Culture Tank Structure that Increases the Amount of Incident Light

Light is the source of energy for photosynthetic reaction, and a culture tank structure that would increase the amount of incident light was investigated.

The requirement for the amount of light was set at 20 mol/day or more as the necessary amount of light calculated from the target DREAMO production volume.

Figure 14 shows the path of incident light and the amount of light loss up to when solar energy is received by the DREAMO. The amount of light is determined by the area of culture tank installed and the culture tank height. The culture tanks were also placed at uniform intervals (below, separation distance) in order for each culture tank to have equal light exposure. For this study, a fixed separation distance was made a precondition. As shown by the proposed structures for investigation in Fig. 15, two models were investigated, one structure (a) with a reflector installed on the culture tank and another structure (b) that has the culture tank installed at an angle (below, a tilt).

Structure (a) has the reflector installed at the front surface (south side) of the culture tank. It is a structure that directs incident light at the culture tank by reflecting the

light received within the separation distance. Structure (b) places the culture tank itself at a tilt. It is a structure that aims to increase the light intake efficiency of the culture tank. Both structures were considered at angle levels of 30°, 45°, and 60°.

The amount of light received by each culture tank structure was investigated using light simulation. OpticStudio from Zemax was used and the solar orbit with respect to the installed culture tank based on the solar altitude and the change in time per day from sunrise to sunset according to the season was incorporated in the model to predict the amount of incident light on the surface of the culture pack. The simulation conditions for this investigation are shown in Table 4 and Fig. 16. The light source was defined as 1 sun as used in solar cell performance evaluation standards. The solar altitude and orientation as affected by the season and time⁽¹⁶⁾ were incorporated into the model for the assumed culture tank installation location in Utsunomiya City, Tochigi Prefecture, Japan.

Figure 17 shows the results of simulations conducted for structures (a) and (b). The tilt structure in which the

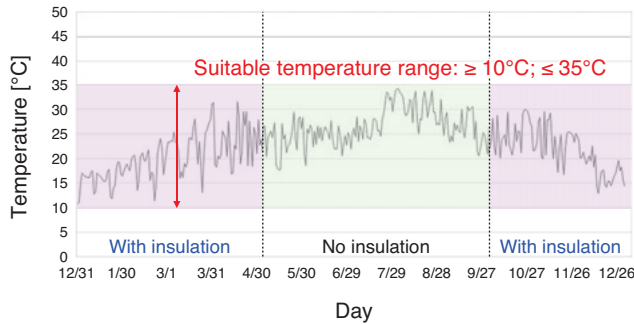


Fig. 13 Estimated annual temperatures of culture solution in North Kanto region with thermal insulation for winter period considered

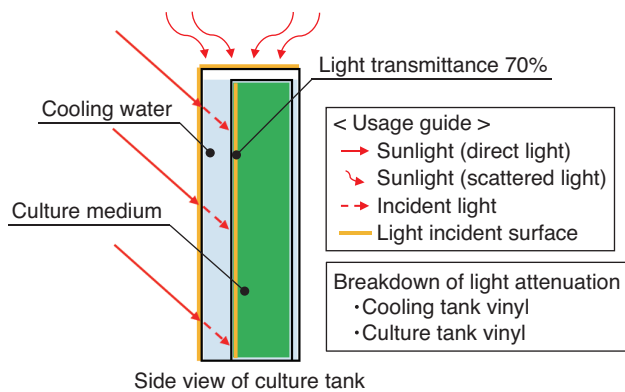
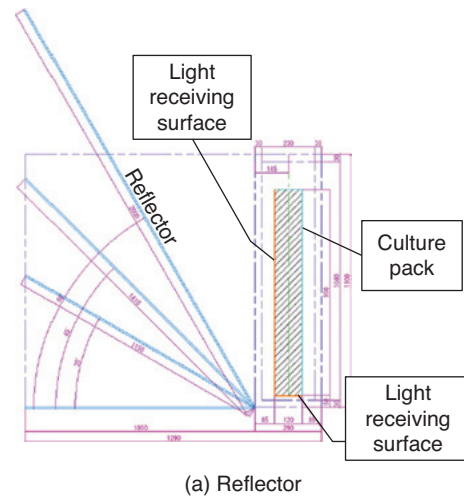
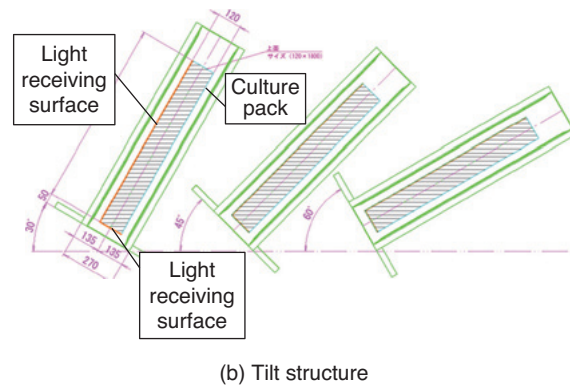


Fig. 14 Schematic representation of light loss caused by culture medium and culture tank structure



(a) Reflector



(b) Tilt structure

Fig. 15 Schematic representation of simulation models used for estimation of effects of (a) and (b) on flat panel photobioreactor

Table 4 Basic shape, light source, and incident light conditions for simulation model

Factor	Unit	Value
Simulation software	[-]	Zemax OpticStudio
Housing conditions		
Housing size	[mm]	L 1,800 × W 210 × H 1,000
Separation distance	[mm]	1,000
Light loss conditions		
Light transmittance	[%]	72.3
Solar conditions		
Light source	[Sun] (W/m ²)	1 (1,000)
Direct light	[%]	70
Scattered light	[%]	30

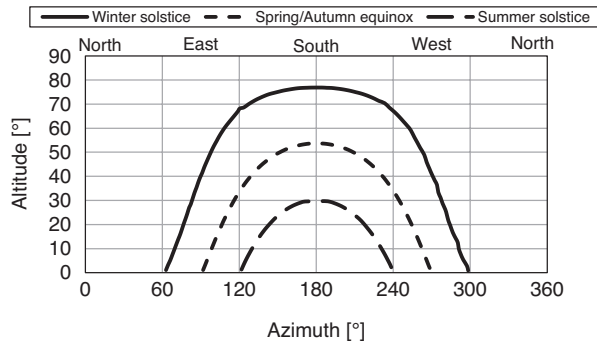
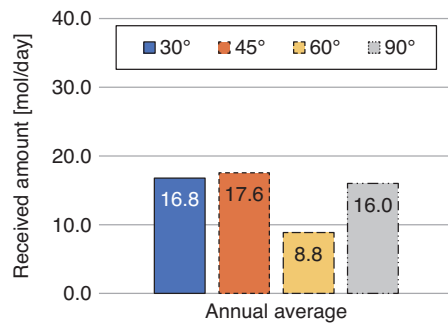
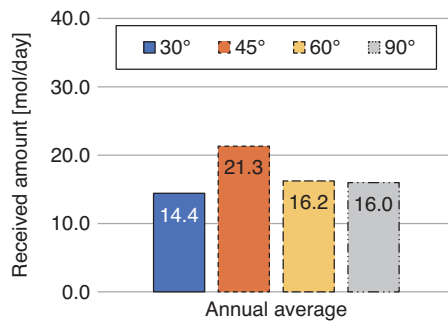


Fig. 16 Sun orientation and altitude used for optical simulations for spring and autumn, summer, and winter conditions



(a) Reflector



(b) Tilt structure

Fig. 17 Estimation of effects of (a) and (b) to enhance incident light on culture tank surface

culture tank itself is installed at an angle received the greatest amount of light under the 45° condition. This was found to exceed the target of 20 mol/day.

6. Enhanced DREAMO Production Volume Results

Outdoor culture of DREAMO was carried out in Tochigi Prefecture, as shown in Fig. 18, reflecting the optimization of nutrient salt component concentrations and the results from investigation of culture solution flow guides. Figure 19 shows the average annual production volume (a) calculated from these test results and the estimated value for annual average production volume (b) when the effects of the thermal insulating structure and tilt structure are included. The configured culturing conditions yielded a production volume of 8.6 g/m²/day. In addition, the verification results described above yielded the prospect that production volume of 13.5 g/m²/day which exceeded the target of 10.0 g/m²/day could be obtained by using a thermal insulating layer structure on the culture tank to keep it within the culturing temperature control range and by using a tilt structure to increase the amount of light received on the culture tank.



Fig. 18 Flat panel photobioreactors placed outdoors

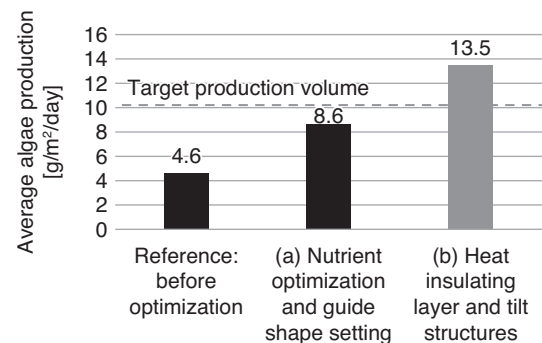


Fig. 19 Results of annual production of DREAMO estimated from outdoor experiments and public climate database

7. Conclusion

DREAMO and a flat panel photobioreactor were used to conduct an investigation of the optimization of culturing conditions and of the culture tank structure with the objective of creating technology for outdoor stable culture in large quantities. The following results were obtained.

- (1) Production volume was increased under culturing conditions by optimizing the concentrations of nutrient salt components phosphorus and potassium.

In addition, by raising the flow field velocity of the culture solution to 0.02 m/sec or higher and adopting a flow guide shape that discharges liquid and gas in a single direction, efficiency was enhanced and production volume increased.

- (2) It was verified that by adoption of the thermal insulating layer structure, the culture tank temperature is controllable to within the control range throughout the year even in Tochigi Prefecture. This verified that culturing can be conducted without the growth of DREAMO being inhibited. In addition, installing the culture tank with a 45° tilt made it possible to increase the amount of incident light received by the culture tank.

Based on the outdoor culture tests reflecting the above findings and culture tank structure, prospects for a DREAMO production volume of 13.5 g/m²/day were gained.

This indicated the possibility that DREAMO could achieve ethanol production volume per planted area that is equal to or better than that of bioethanol from sugarcane, which is a carbon neutral fuel.

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