

**Improvement of Energy Network by Naturally Farming of Chlorophyll-bearing Algae : Effects of Culture Condition Changes for the Yield of Microalgae**

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**Abstract**

In this study, basic cultivation experiments were performed on microalgae *Desmodesmus* sp, then, the experiment results were deductively expanded, and the use of paddy in the off-season for farmers is advocated. As a result of the continuous culture experiments using 100 mL test tubes, 101.3 mg dry biomass was harvested in the aeration culture using the BG-11 medium. In the aeration culture with mixing CO<sub>2</sub>, the maximum harvest was obtained under the 10%-CO<sub>2</sub> concentration. Considering about CO<sub>2</sub> concentration, the condition of 1%-CO<sub>2</sub> was superior in CO<sub>2</sub> use efficiency. Based on the changes in the pH of the medium, algae such as *Desmodesmus* sp exchanges ions between the inside of the cell and the surrounding environment, and changes the surrounding environment in order to adapt themselves. In the proforma calculation of second crops in paddy fields, 26.4 million tons dry biomass is harvested in 120 days long of culture a year. Considering that the harvested dry biomass amount,  $187 \times 10^6$  GJ power energy is obtained by burning them. This amount of energy corresponds to about 5.2% of total electricity.

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## Introduction

Photosynthesis is the root of life on the earth. This paper presents the concept of converting carbon dioxide by solar energy into a flammable energy source, with emphasis on the biological properties of green algae. These properties, acquired through long geological ages, effectively result in a photosynthetic reaction by greener lives by consuming minimum amount of energy. In the earliest era of terrestrial life, plants were starting to live in the water and they are presumed to have developed a mechanism of photosynthesis that uses solar energy. It's thought that the life had adopted the condition of that era's water component, temperature, and acidity. At the beginning, primitive algae like the cyanobacteria spread, and eventually the green algae based on chlorophyll was born. By and by, living organisms have evolved to maintain environment in the body that is close to the aquatic environment, and have extended the living environment to land. Green algae are known for having the ability to live in a wide range of pH environments.

In this study, we experimentally found that algae are forced to live according to the external environment in order to survive. This adaptability is explained as an ability to endure the living environment. This is a way of saying that primitive organisms only have the ability to accept the environment's pH and not to regulate their internal environment. We focus on the environmental controllability of algae that we experimentally found, and propose coexistent use of rice cultivation and algae cultivation in the paddy field. The paddy field, which is a freshwater area, already has examples of coexistence with rice cultivation and breeding duck and carp farming [1-3]. Algae cultivation

in paddy fields is similar to rice production and suitable for the primary industry. By incorporating bioenergy production while maintaining rice production, it contributes to maintaining and improving food and energy self-sufficiency. Algae already have the environmental controllability under the clever mechanism of photosynthesis. The intuition that algae are undifferentiated is the haughtiness of Christian religious perspectives through which contemporary humanity has forgotten the awe of the respect of natural life.

## Materials and Methods

### *Sample Algae Selection*

For culturing experiments, microalgae *Desmodesmus* sp. (NIES-96, Japanese name:Ikadamo) distributed from the National Institute for Environmental Studies (NIES) was used. It's a freshwater microalgae which is in the shape of an ellipse or a rectangle with a body length of about ten and several micrometers. *Desmodesmus* sp. used in this study is a clone of a sample collected in Lake Shoji in Yamanashi Prefecture in 1981[4]. It's universally living in the freshwater area around the world. *Desmodesmus* sp. has a chlorophyll-*a/b* which are photosynthetic pigment in the chloroplast. In principle, *Desmodesmus* sp. consists of a fixed number of cells, and it's called Coenobium [5]. An overview picture of *Desmodesmus* sp. is shown in Figure 1 [6]. It's easy to cultivate *Desmodesmus* sp. because it has accommodation ability to changes in habitat. In general, *Desmodesmus* sp. has about 10% to 50% oil and fat content.

### *Culture Experiment*

Culture experiment was conducted by placing a 100 mL test tube (A-30, Maruemu, Osaka, Japan) in a



Figure 1. *Desmodesmus* sp.

stainless steel test tube stand (SS30-10, Sanwakaken, Osaka, Japan) in the water tank (Dax C60, Kotobuki-kougei, Nara, Japan). For the turbid solution of algae and medium, an initial capacity of 75 mL was used, and five samples were prepared each conditions. All test tubes were stoppered with Biosilico (N-32, Shin-Etsu Polymer, Tokyo, Japan). Air in the test tube is always ventilated through biosilico. The water temperature was set at 25 °C with a heater (Metal heater SH80, Gex, Osaka, Japan) and a thermostat (NX003, Gex). Figure 2 shows the experimental apparatus diagram. As a light source, the white tape LED (5050 SMD, 3 m 43.5 W) was used and the quantity of photons was set up 100  $\mu\text{mol}/\text{m}^2/\text{s}$  by using a quantum meter (MQ-200, Apogee Instruments, Utah, United States). The measurement mode was ELEC. The 12 hours light period and the dark period were set by program timer (DT-01, Asona, Osaka, Japan). 6 o'clock to 18 o'clock is the light period, and 18 o'clock to 6 o'clock is the dark period. In the aeration culture experiment, in addition to the above-mentioned apparatus, a gas cylinder of air and CO<sub>2</sub> was used. Both of them were depressurized to 0.2 MPa with a regulator (for air: NPR-1B, Yamato, Osaka, Japan, for CO<sub>2</sub>: FCR-807K, Yutaka, Tokyo, Japan) and mixed so as to achieve the target CO<sub>2</sub> concentration. In the aeration culture with air only, the CO<sub>2</sub> cylinder wasn't used, so the CO<sub>2</sub> concentration is about 400 ppm (0.04%) similar to that in the atmosphere. Thereafter, the flow rate was

adjusted to 0.1 L/min with a flowmeter (RK1710, Kofloc, Kyoto, Japan), and the mixed gas was supplied to the cultivation test tube through a distributor, an air filter (13JP020AN, Advantec, Tokyo, Japan), and a glass tube ( $\phi 5 \text{ mm} \times \phi 3 \text{ mm}$ ). Aeration was done for many hours regardless of CO<sub>2</sub> concentration. The duration of the culture experiment is 14 days. 1 mL of algal turbid solution was collected once a day at regular time, then, pH (LAQUAtwin B-712, Horiba, Kyoto, Japan) and concentration (PD-303S, Apel, Saitama, Japan) was measured. The concentration was measured by wavelength of 620 nm and 678 nm. Table 1 shows the main conditions of the culture experiments.

#### Filtration Recovery Experiment

After the 14-days cultivation experiment, the remaining amount of the algal turbid solution was measured with a measuring cylinder (Graduated cylinder Custom A, 100 mL, Shibata, Saitama, Japan), and cultured algae were collected by suction filtration. For vacuum filtration, a 500 mL filter flask (5340FK500R, AGC Techno Glass, Shizuoka, Japan), filter holder for this filtration (KG-47, Advantec) and a membrane filter (A500A047A, Advantec) were used. The collected algae was measured with a wet weight immediately after filtration, and then a dry weight was measured using an electronic balance (AUX220, Shimadzu, Kyoto, Japan) after air drying at room temperature for about 1 week. The amount of water contained in the algae after air drying was determined by a thermo-gravimetric analyzer

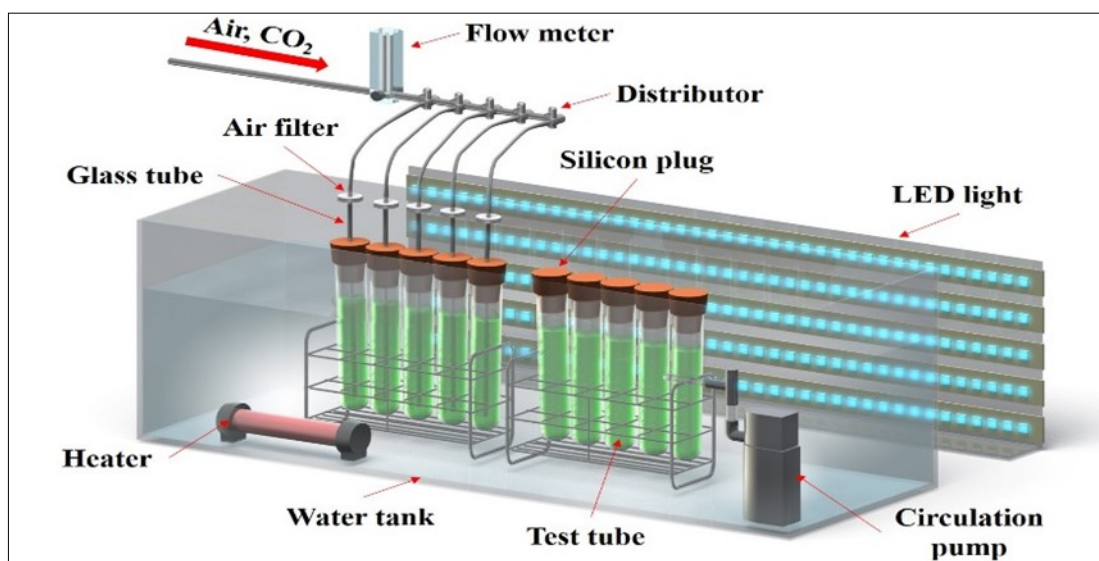


Figure 2. Culture apparatus

Table 1. Culture conditions

Microalgae	Desmodesmus sp.
Medium	BG-11
Initial pH	7.4
Initial Volume [mℓ]	75
Temperature [°C]	25
CO <sub>2</sub> Concentration [%]	0.04, 1, 5, 10, 20
Light irradiation [h]	12/12
Photon amount [μmol/m <sup>2</sup> /s]	100

(TGA-50, Shimadzu). The rate of temperature rise was set at 10 °C/min and the sample was heated to 1,000 °C with a platinum cell. The initial temperature of the thermo-gravimetric measurement is about 25 °C, which is equivalent to room temperature.

### Fertilizer

In the culturing experiment, BG-11 was used as a fertilizer. At the NIES, C medium is used as the recommended medium for the culture. C medium is supplemented with vitamins, so it's suitable for most of green algae cultivation including *Desmodesmus sp.* The compositions of BG-11 medium and C medium are shown in Table 2.

### Results

#### *Algae Growth Rate*

Figure 3(a) shows the results of culture experiment of *Desmodesmus sp.*, which is a typical example of the green algae containing chlorophyll. The graph shows the amount of transition of chlorophyll-*a* which is determined from the absorbance. In the 14-days cultivation, algae concentration continued increasing until experiments ended. It is thought that the algae in the test tube got stirred with bubbles generated by aeration, and light was supplied to the algae that was precipitated at the time of static culture to encourage the growth. For estimating growth of algae culture, CO<sub>2</sub> concentration is changed of 1%, 5%, 10%, and 20%, and the algae concentration became maximum under the condition of CO<sub>2</sub> concentration of 10%. Growth rate under low CO<sub>2</sub> concentration is somewhat slowed after 8<sup>th</sup> day of cultivation.

#### *pH Changes*

Changes in pH over time, a chlorophyll-*a* amount under each culture condition are shown in Figure 3(b). The initial pH of the medium is 7.4 in the BG-11 medium, but at the stage when the algae is turbidified in the medium, the pH became around 8.0. After one day, it settles to a certain pH value depending on the conditions. After that, it changes to a slight alkaline with all conditions. Microalgae like *Desmodesmus sp.* is thought to exchange ions between the inside of the cell and the surrounding environment and adapt itself to the optimum environment in order to adapt to the surrounding environment. Normally, since the surrounding environment is infinitely large, there is not enough power to change the surrounding environment. However, in the closed narrow area like the test tube used in this experiment, there is enough power to protect itself from the surrounding environment. *Desmodesmus sp.* creates an optimum environment and grows freely.

#### *Dry Algae Yield*

Table 3 shows the yield of algae, which is recovered by vacuum filtration using a membrane, after completion of each culture experiment. The numerical values in the table are average values of five samples under the same condition. The average dry algae obtained by the stationary culture as the reference was 10.9 mg on average. In aeration of the culture with air, an average 101.3 mg dry algae was yielded. This corresponds to approximately 10 times that during static culture without aeration. In the aeration of the culture with CO<sub>2</sub>, there

Table 2. Recipe of culture media BG-11 and C

(a) BG-11			(b) C		
	Distilled water indication	100mL		Distilled water indication	100mL
1	NaNO <sub>3</sub>	150mg	1	Tris	50mg
2	K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	4.0mg	2	Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O	15mg
3	MgSO <sub>4</sub> · 7H <sub>2</sub> O	7.5mg	3	KNO <sub>3</sub>	10mg
4	CaCl <sub>2</sub> · 2H <sub>2</sub> O	3.6mg	4	β-Na <sub>2</sub> glycerophosphate · 5H <sub>2</sub> O	5.0mg
5	Citric acid	0.6mg	5	MgSO <sub>4</sub> · 7H <sub>2</sub> O	4.0mg
6	Ferric ammonium citrate	0.6mg	6	Vitamin B <sub>12</sub>	0.01μg
7	Na <sub>2</sub> EDTA-Mg	0.1mg	7	Biotin	0.01μg
8	Na <sub>2</sub> CO <sub>3</sub>	2.0mg	8	Thiamine HCl	1.0μg
9	Trace metal mix A5 + Co	0.1mL	9	PIV metals	0.3mL
*pH7.4			*pH7.5		
Trace metal mix A5 + Co			PIV metals		
	Distilled water indication	100mL		Distilled water indication	100mL
1	H <sub>3</sub> BO <sub>3</sub>	286mg	1	Na <sub>2</sub> EDTA · 2H <sub>2</sub> O	100mg
2	MnCl <sub>2</sub> · 4H <sub>2</sub> O	181mg	2	FeCl <sub>3</sub> · 6H <sub>2</sub> O	19.6mg
3	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	22.2mg	3	MnCl <sub>2</sub> · 4H <sub>2</sub> O	3.6mg
4	Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	39mg	4	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	2.2mg
5	CuSO <sub>4</sub> · 5H <sub>2</sub> O	7.9mg	5	CoCl <sub>2</sub> · 6H <sub>2</sub> O	0.4mg
6	Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	4.9mg	6	Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O	0.25mg

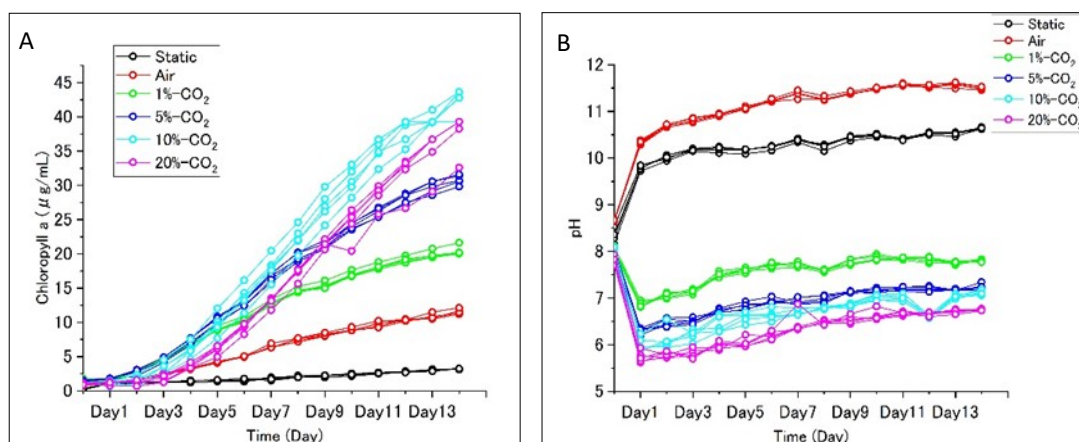


Figure 3. The experimental results of *Desmodium* sp. culture under several conditions. (a) The time-dependent change of Chlorophyll-a concentration. (b) The time-dependent change of pH of water.

Table 3. Dry algae biomass yield

	①	②	③	④	⑤	⑥
Conditions	Stationary	Air	1%-CO <sub>2</sub>	5%-CO <sub>2</sub>	10%-CO <sub>2</sub>	20%-CO <sub>2</sub>
Dry biomass yield [mg]	10.9	101.3	260.2	317.4	355.2	322.4
Dry biomass yield [mg/CO <sub>2</sub> -%]	—	2543	260.2	63.48	35.52	16.12
Chlorophyll- <i>a</i> [µg/mL]	3.21	11.57	20.45	30.84	42.42	37.75

was an average yield of 260.2 mg when the CO<sub>2</sub> concentration was 1%, and a yield of 317.4 mg on average at the CO<sub>2</sub> concentration of 5%. When the CO<sub>2</sub> concentration was 10%, 355.2 mg dry algae as an average was obtained. This yield is greatest through these experiments. When the CO<sub>2</sub> concentration is 20%, the average yield is 322.4 mg which is almost same as the yield of 5%-CO<sub>2</sub> concentration.

### Discussion

#### *Relationships Between Dry Biomass Yield and CO<sub>2</sub> Efficiency*

Normalizing by the supplied CO<sub>2</sub> (wt%), the highest efficiency of CO<sub>2</sub> (dry mass (g)/total CO<sub>2</sub> (g) ×100%) was obtained under the natural air blowing in which CO<sub>2</sub> concentration is 0.04%. It suggests that biomass captures most effectively CO<sub>2</sub> from the natural air, as illustrated in Figure 4. At 0.04% of CO<sub>2</sub>, *Desmodesmus* sp. yielded dry weight of about 0.1 g in 14 days. If it got much more CO<sub>2</sub> of 10%, the product rate reached to the maximum value of about 0.37 g in 14 days. When CO<sub>2</sub> concentrations increased to two orders (from 0.04 to 10%), the yields increased less than a half order only. By thermo-gravimetric analysis exhibit the moisture content of the algae to be about 10% after natural drying at room temperature for 1 week. For natural drying with the sunlight and air, we do not need expensive external energy from industrial processes.

#### *Comparison of Fertilizer*

The microalgae *Desmodesmus* sp. was cultured in BG-11 medium in this study, because it has almost equal concentration of the constituents that present in the paddy field water. In paddy fields, rice cultivation is usually carried out. In the rice cultivation, nitrogen,

phosphorus and potassium are used as fertilizer [7]. Table 4 shows an excerpt from the results of measuring the quality of agricultural water passing through paddy fields[8]. NH<sub>4</sub><sup>+</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> are decreasing with the passage of time and thought to be used for the growth of rice plants. BG-11 medium contains three major nutrients such as nitrogen, phosphorus and potassium as well as plant fertilizer. Comparing the representative value of the ingredients between BG-11 medium and paddy water, the types of main components are similar, but the component's concentration is lower in paddy water. For example, NaCO<sub>3</sub> contains nitrate ion 12.84 mmol/L in BG-11 medium (Table 5). On the other hand, nitrate ion contains 0.12 mmol/L in paddy water, thus actual nitrogen concentration in the paddy water is about 1/100 or less.

Water filled in paddy fields after rice cultivation was collected, then preliminary experiments were carried out in glass containers larger than test tubes. As a result, algae cultivated in paddy water showed about half the growth rate as compared with BG-11 medium. This test water was collected after filling the water more than 100 days after draining the water post harvesting the rice. Therefore, it's assumed that the content of ingredients is lower than the actual paddy field water. It clearly indicates that the BG-11 medium is excessive in nutrition, and the algae requires no high concentration nutrients such as BG-11 medium.

#### *Calculation of Yield*

In this study, we propose that the case of using paddy field in Japan be considered as an example of the cultivation of *Desmodesmus* sp. in the future and calculated for calculations of the yield. The most

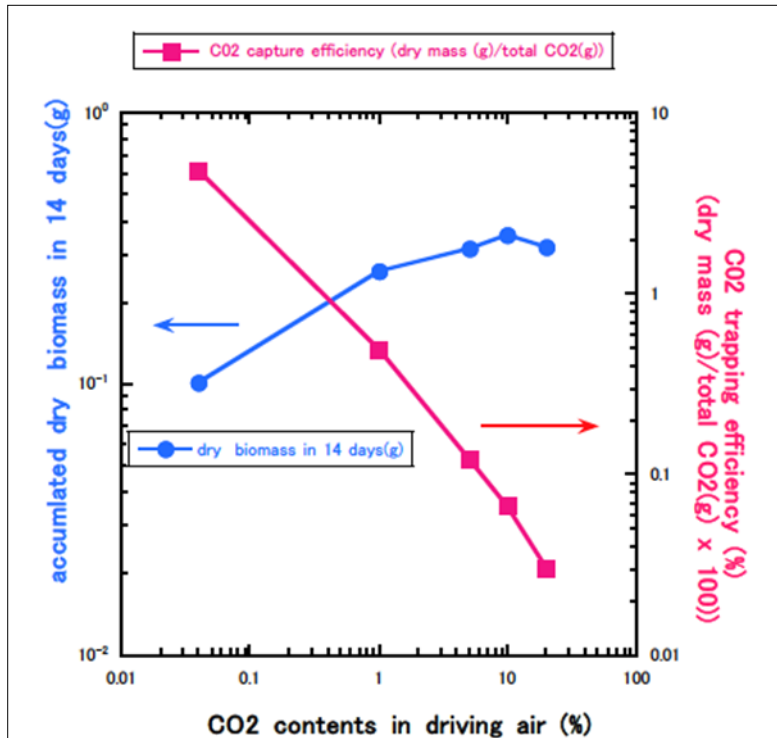


Figure 4. CO<sub>2</sub> capture efficiency vs. CO<sub>2</sub> content in the feeding air. *Desmodium* sp. works with highest efficiency in CO<sub>2</sub> content around 0.04%.

Table 4. Paddy water quality inspection

Period	pH	T-N	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>
		[mmol/L]						
Transplant period	7.6	0.161	0.119	0.005	0.0016	0.06	0.46	0.18
Tillering period	7.3	0.139	0.126	0.001	0.0006	0.05	0.47	0.19
Sprouting period	7.5	0.166	0.128	0.000	0.0003	0.05	0.50	0.21
Total average	7.5	0.159	0.122	0.001	0.0006	0.05	0.48	0.20

Table 5. Main components of BG-11 medium

Component	NaNO <sub>3</sub>	K <sub>2</sub> HPO <sub>4</sub> · 3H <sub>2</sub> O	CaCl <sub>2</sub> · 2H <sub>2</sub> O	MgSO <sub>4</sub> · 7H <sub>2</sub> O
Amount [mmol/L]	17.6	6.57	10.2	6.08

important condition is that algae should be cultivated in the more natural state. The harvested algae is not supposed to extract fuel at all, it is assumed that it is burned fully after sun drying. In Japan, rice cultivation is carried out from May to September. Therefore, for the algae cultivation in paddy fields, we assume about 120 days in the period from October to April. During for this period, it should be considered making soil for rice cultivation and giving time to recondition of the paddy fields. Wide shallow paddy fields are about 10 cm water deep and suitable for cultivation of algae. When the concentration of algae increases due to the growth light does not reach into the water, and photosynthesis is inhibited. In some previous studies, there are cases reported that the effective penetration depth of sun light is about 30 cm in the paddy field.

In the main cultivation experiment, *Desmodesmus* sp. was cultivated in a 100 mL test tube. Under the aeration condition of 1%-CO<sub>2</sub> concentration, the biomass was about 260 mg in 14 days as described in Section 3.3. As an off-season crop of paddy fields, it is assumed that *Desmodesmus* sp. can grow in the paddy field for 120 days/year. So this yield is calculated,  $A = 22 \text{ kg/m}^3/\text{year}$ . The total amount of paddy field area in Japan is 24,050 km<sup>2</sup>, of which approximately 12,000 km<sup>2</sup> excluding Hokkaido, Tohoku, Hokuriku and Sanin area[9]. Assuming that they can be cultivated at depths of up to 0.1 m in these paddy fields, the effective water volume  $W$  (total culture volume) of paddy fields is  $12,000 \times 10^6 \text{ (m}^2) \times 0.1 \text{ (m)} = 1,200 \times 10^6 \text{ m}^3$ . The total yield of algae will be,  $A_{total} = A \times W = 26.4 \times 10^9 \text{ kg/year}$ . Production volume of rice is 8 million tons per year in Japan[10]. Rice straw and paddy are added, it is about 15 million tons per year yield, which was invented by photosynthesis during the 5 months from May to September in summer. Algae yield is about 6 million tons per month which is about twice compared with rice.

Next, the total calorific value  $C$  due to burning of algae is obtained. The fever amount of green algae like *Desmodesmus* sp. is about 20 GJ/t[11]. On the other hand, since the harvested algae contains about 10% of moisture, it is necessary to consider subtracting the latent heat of evaporation of water. The latent heat of evaporation of water at 100 °C is 2.257 kJ/g[12], so the net calorific value of algae is about 17.75 GJ/t. As mentioned earlier, considering that the harvested

*Desmodesmus* sp. burns entirely, the total calorific value  $C_{total} = 17.75 \text{ (GJ/t)} \times 26.4 \times 10^6 \text{ (t/year)} = 468 \times 10^6 \text{ (GJ/year)}$ . The power generation efficiency  $\eta$  of the thermal power plant is about 40%, the power energy obtained by burning algae is  $187 \times 10^6 \text{ GJ/year}$ . According to the Energy White Paper 2018 of the Ministry of Economy, Trade and Industry, the total energy generated in 2016, is about 1 trillion kWh[13]. In other words,  $P = 10^{12} \text{ kWh/year} = 3,600 \times 10^6 \text{ GJ/year}$ . *Desmodesmus* sp.'s replaces the thermal energy production by burning of fossil fuels that corresponds to 5.2 % of electric power consumed in Japan in a year.

In this calculation, laboratory-level figures are deductively converted into a large area of paddy fields. As future research subjects, we must conduct demonstration experiments using real paddies. In the demonstration experiment, the difference between the spectrum of sunlight and artificial light and the photon number density (illuminance) becomes clear. Demonstration experiment reveals the difference from uniform irradiation. Also, the influence of water depth and the effect of stirring by wind waves will become clear.

## Conclusions

Microalgae *Desmodesmus* sp. was cultured in the test tubes under several conditions. For estimating growth of algae culture, the algae concentration became maximum under the condition of CO<sub>2</sub> concentration of 10%. Growth rate under low CO<sub>2</sub> concentration is somewhat slowed after 8<sup>th</sup> day of cultivation. Normalizing by the supplied CO<sub>2</sub>, the highest efficiency of CO<sub>2</sub> was obtained under the natural air blowing in which CO<sub>2</sub> concentration is 0.04%. It suggests that biomass captures most effectively CO<sub>2</sub> content from the natural air.

Talking about pH changes, in the closed narrow area like this experiment, *Desmodesmus* sp shows enough power to protect itself from the surrounding environment. However, since the surrounding environment is infinitely large, there is not enough power to change the surrounding environment. Even though it is a local paddy field, raising carbon dioxide concentration is inconsistent with the purpose of reducing greenhouse gas emissions in the environment.

In the calculation for the future use of algae as



an energy source, algae's contribution to electric energy is about 5.2%. For the country which has nearly 90% overseas dependence of primary energy like Japan, it's significant to be able to produce 5% of primary energy for electric power domestically. For industrialized countries like Japan, raising the domestic production rate of primary energy not only increases the domestic economic cycle index (GDP), but also has a positive impact on the world economy. Similarly, for developing countries, reducing fossil fuel consumption and mitigating deforestation will greatly contribute to the preservation of the global environment, and furthermore it will make an increasing the GDP in those countries. Since the paddy field area of developing countries is larger than that of Japan, and the temperature of these countries is higher, so the supply of primary energy by algae is larger than that of Japan. Also, energy consumption of these countries is less than that of Japan, with the exception of China. Therefore, primary energy production in developing countries has greater effect on GDP of those countries than in Japan.

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