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Growth Characteristics of Microalgae in High-Concentration CO_2 Gas, Effects of Culture Medium Trace Components, and Impurities Thereon

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ABSTRACT

In order to reduce release of CO_2 contained in the flue gas from a power plant, we assumed a system in which the flue gas was directly blown into a pond and CO_2 was fixed on microalgae. We have experimentally examined the basic growth characteristics, such as trace components of culture medium, effects of impurities from exhaust gas, and light utilization rate of algal productivity, mainly for *Nonnochloropsis* sp. NANNP-2 from SERI collection. Although Ni and V contained in heavy oil burnt ashes dissolve into culture solution, their concentrations are low, and they have no particular adverse effect on growth as impurities.

Culture medium trace component (i.e., heavy metals and vitamins) are essential for the NANNP-2. However, for the PHAEO-2 (*Phaeodactylum* sp.), the growth rate hardly changes, even if vitamins and heavy metals other than Fe are eliminated.

Index Entries: CO_2 elimination; flue gas; marine microalgae; trace components of culture medium; Ni and V of burnt ash.

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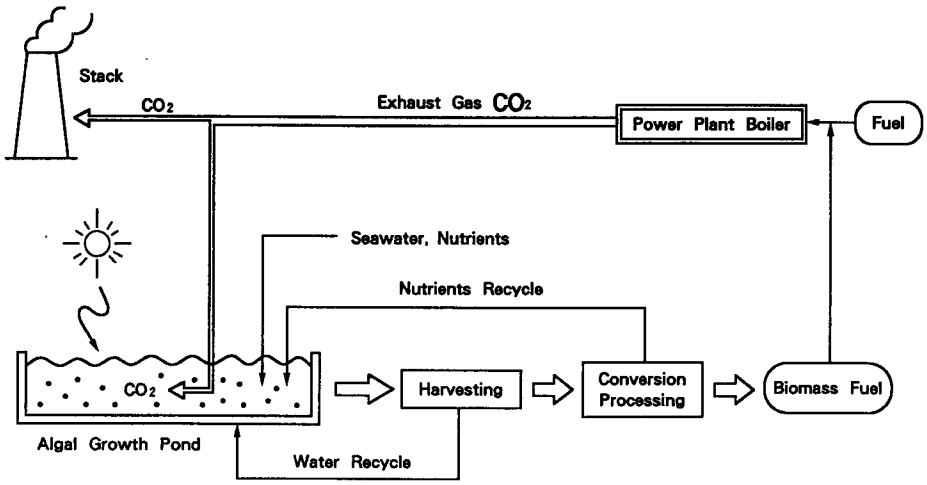

Fig. 1. Conceptual system of bioprocess for CO_2 elimination.

INTRODUCTION

In recent years, the phenomenon of global warming has been taken up as a serious environmental problem, and it is said to be owing to greenhouse gases, such as methane and chlorofluorocarbon, as well as to the increased concentration of CO_2 gas in the atmosphere. Release of CO_2 in vast amounts into the atmosphere caused by combustion of fossil fuels, such as coal, petroleum, and LNG, is considered to be the major cause of global warming.

Although technical means to reduce amounts of CO_2 gas to be released into the atmosphere are under research and development in many ways, this article deals with the fixation method of CO_2 on microalgae by photosynthetic reaction, which has already been researched by SERI (*1*) and EPRI (2) projects. Presented here is a system that converts cells harvested by growth of microalgae into biomass fuel for use as part of fuel in the power station combustion furnace by recycling.

Figure 1 shows the concept of the treatment system of CO_2 contained in combustion exhaust gas. To be more specific, it is a process in which all or part of exhaust gas is blown into an alga culture pond, followed by addition of sea water and nutrients into the pond, for conversion of CO_2 into biomass fuel by utilization of solar energy.

The fixation method of CO_2 by mass culture of microalgae presents difficulty in harvesting produced algae and, therefore, requires a large growth pond. However, this method can be thought to have many advantages, such as higher photosynthetic efficiency than that of plants and automation of the whole process.

Our last paper (3) reported on the experimental results about the effects of SOx and NOx, and also about the growth characteristics of sea-

water microalgae at high CO_2 concentration. Here we continue to report the test results on basic growth characteristics, such as components of culture medium, effects of impurities, and light utilization rate.

MATERIALS AND METHODS

Algal Strain

In our present research, we have selected mainly algal strains that are of good salinity tolerance, as well as high in crude lipid accumulation and in growth. These strains were obtained mainly from SERI (*4*), and here we have examined the characteristics of NANNP-2 (*Nannochloropsis salina* from SERI). As reported in our last paper, the NANNP-2 was relatively high in growth rate (i.e., 300 mgdw/d at pH 7), and grew stably at high CO_2 concentration of 15% in a cycle of 16 h of light and 8 h of darkness.

Furthermore, the PHAEO-2 (*Phaeodactylum tricornutum*) also grew relatively stably in high CO_2 concentration conditions. Although its crude lipid accumulation level was high (50 ~ 60%), the effect of traces of culture medium components differed from that of the NANNP-2. Therefore, we present here experimental data of the PHAEO-2 only about this item.

Culture Methods

For stock culture and medium for growth experiments, we used a standard medium of f/2 sea water based on SERI. We also used "Instant Ocean" in place of natural sea water.

In the growth experiments, we used 40-mL test tubes, 1000-mL Roux bottles, and a cylindrical container (150 mm × 50 ~ 200 mm H) for examination of effects of solution depth in a clean room by taking care of microbial contamination.

A fluorescent light (with approx 10,000 lx) and a metal halide lamp (with approx 50,000 lx) were used for continuous lighting. Illuminance on the surface was measured with an illumination meter.

The solution temperature was between 20–25°C. CO_2 gas was mixed with air and supplied at the flow rate of approx 100 mL/min in a high concentration condition of 5% (v/v).

The growth of microalgae was monitored by taking measurements of changes in optical density at 680 nm (OD_{680}). The OD_{680} correlated with the dry wt measured after cells were washed and dried at 105°C for 3 h.

The total amount of crude lipids was estimated gravimetrically. Cells harvested by a centrifuge (at 5000 rpm for 5 min) were homogenized by glass beads in a blender and lipids extracted overnight by a chloroform/methanol mixture (mixture ratio being 2:1).

Table 1
Compositions of Harvested Algal Cells (wt%)

Ash	C	H	N	S	P	O
3.11	56.3	8.20	2.74	0.72	0.26	bal.

RESULTS AND DISCUSSION

Algal Cell Composition, Lipid Accumulation, and Calorific Value

For synthesis of microalgae by photosynthetic reaction, the following formula is, in general, established.

$$CO_2 + H_2O \xrightarrow[N \& P]{\text{light (400}\sim\text{700 nm)}} \text{algae (C,H,O,N,P, \& S)} + O_2 \uparrow \quad (1)$$

Algae were cultured in the conditions (i.e., 5% [v/v] CO_2 gas, f/2 sea-water culture medium, 25°C of solution temperature, and 10,000 lx), harvested when 1,500 mg/L of biomass concentration was reached after 7 d, centrifuged at 6000 rpm for 10 min, washed for removal of salts, dried at 105°C overnight, and then subjected to composition analysis (Table 1). As can be seen from this table, C is 56.3%, as also given in other reports (*5*). Furthermore, nitrogen (N) and phosphorus (P) were 4.9 and 0.5%, respectively, for C-100%. Our calculation from these composition analysis values has shown that approx 0.5 g of algal cell can be obtained from 1 g of CO_2. The total amount of crude lipids of the said biomass was 39%, and the total calorific value was 6310 kcal/kg. This calorific value is slightly high as compared with that of general algae, but is approx 2/3 of fuel oil.

Figure 2 shows the test results based on culture in a nitrogen-starved medium. A broken line in this figure indicates those cultured in a standard sea-water medium, and a solid line indicates those cultured in a medium in which only the nitrogen content is reduced to 1/4. The above test results have shown that, in the nitrogen-starved condition, the growth rate decreases, with the biomass concentration becoming approx 500 mg/L after 200 h. This concentration value is 1/3 of that (i.e., 1500 mg/L) obtained in the nonnitrogen-starved condition. On the other hand, its total amount of crude lipids was 54% and was found to become higher than that (47%) obtained in the nonnitrogen-starved condition.

Culture Solution Depth and Growth Rate

The growth rate of microalgae is largely influenced by intensity of light. For outdoor mass culture, a culture pond designed to have a large light-receiving surface area and a small solution depth of 10–20 cm is used.

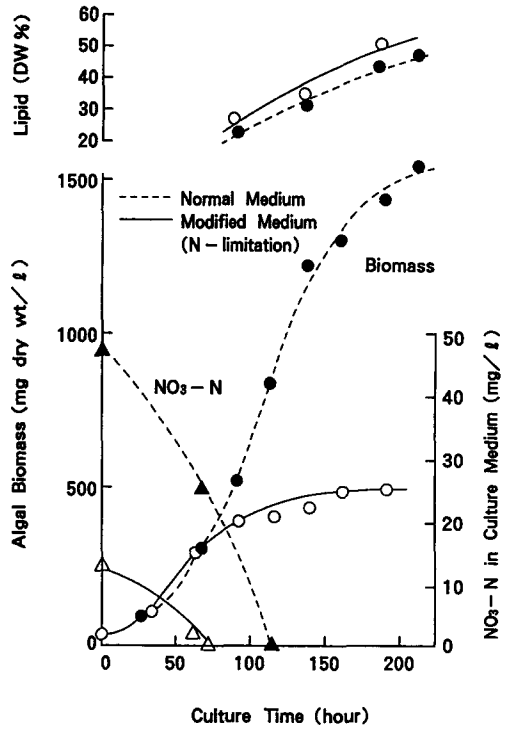

Fig. 2. Biomass and lipid in nitrogen limitation medium.

The reason for this is that solar light energy does not reach the deep part of a culture pond, because it is absorbed in the culture solution and is also blocked by algae.

We have examined the growth rate of algae per unit light-illuminating surface area and the light attenuation rate. Figure 3 shows an experimental apparatus for use in determining an alga growth by applying light from above with a metal halide lamp and also by varying a depth of culture solution at the same time.

Figure 4 shows changes in algal cell concentration for solution depths of 2, 5, and 10 cm in relation to lapse of time. Furthermore, Table 2 shows the growth rate determined from the slope of the straight line of this graph. Algae were allowed to grow while adding nutrients (N & P) to the culture solution halfway in the culture process. They grew constantly up to 2500 mgdw/L for a solution depth of 2 cm. The growth rate per unit volume was higher when the solution depth was small. However, the production rate per day was larger when the solution depths were 5 and 10 cm. In a solution depth of 2 cm, solar light energy can be thought to be not effectively used for alga growth because of transmission of the light. The relationship between light energy and an alga production rate can be expressed by the following formula.

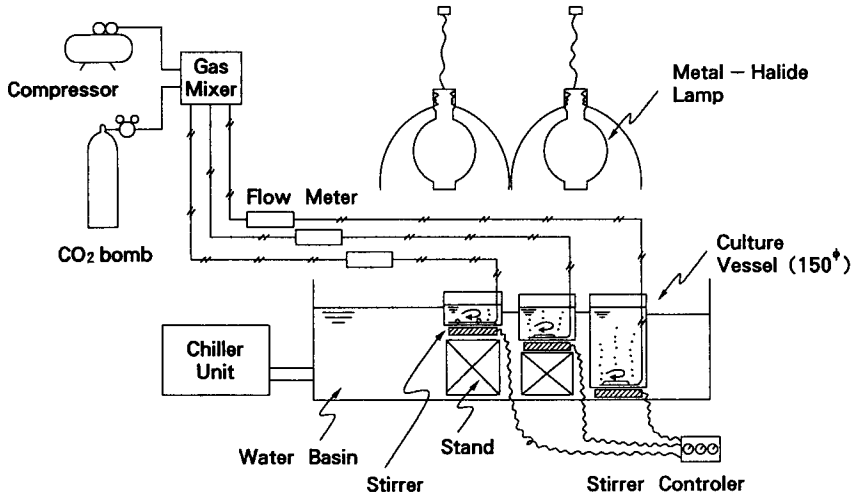

Fig. 3. Schematic apparatus for algal mass culture test.

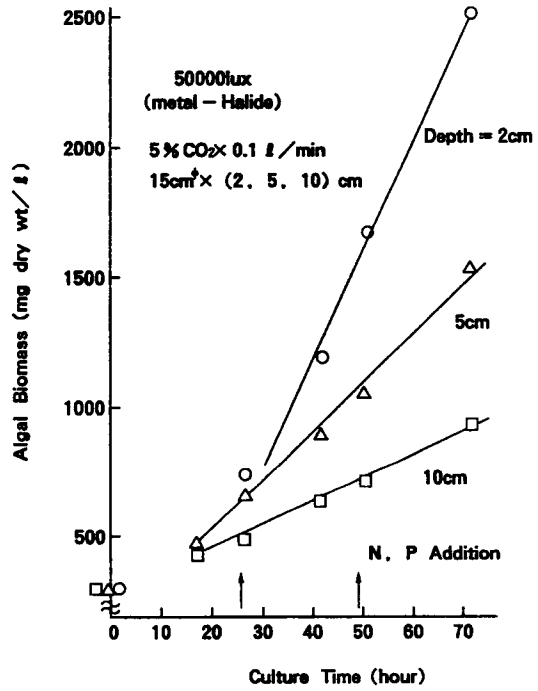

Fig. 4. Influence of water depth on algal productivity.

$$P = \alpha \cdot E \cdot I \tag{2}$$

where: P = production rate (gdw/m^{2}D), E = photosynthetic efficiency (−), I = light energy (kcal/m^{2}D), and α = conversion coefficient (g/kcal).

From Table 2, P is 23 for a solution depth of 5 cm; a is a conversion coefficient of energy to biomass amount and is determined to be 0.16 by calculation of the caloric value. The illuminance of 5000 lx available from a

Table 2
Influence of Water Depth on Algal Productivity

Culture vessel			Growth rate		
Area, cm^2	Depth, cm	Vol, L	mg/L·D	mg/D	g/m^2D
	2	0.354	1000	350	20
177	5	0.89	450	400	23
15 cm^{ϕ}	10	1.77	220	390	22

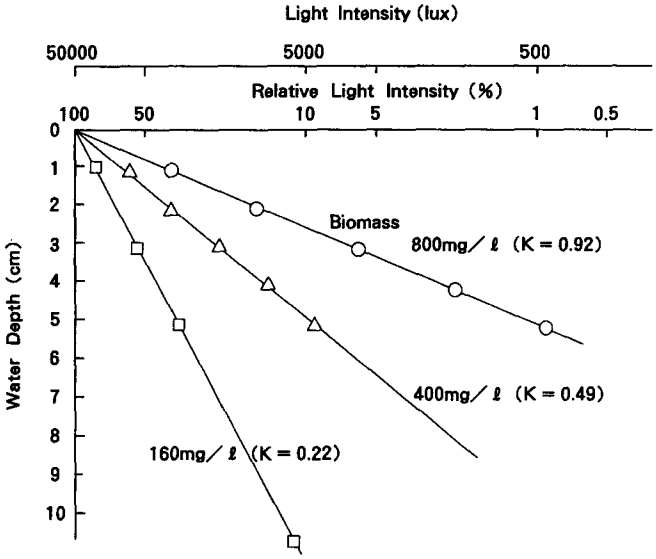

Fig. 5. Attenuation of light intensity on algal culture medium.

metal halide lamp is equivalent to 3670 $kcal/m^2D$ in terms of light energy (I). When the photosynthetic efficiency, E, is calculated using these values, one can obtain approx 0.04.

Light applied onto the culture solution surface is absorbed in the solution and is gradually attenuated. As the solution depth becomes larger, the light attenuation rate increases correspondingly, thus establishing, in general, the following relation.

$$I = Io \cdot e^{-KD} \tag{3}$$

where: I = quantity of light at solution depth (D) (lx), Io = quantity of light on solution surface (lx), D = solution depth (cm), and K = light absorption efficiency (cm^{-1}).

Figure 5 shows plots of actually measured values for algal cell concentrations of 160, 400, and 800 mg/L, and it can be seen that the above formula holds true. A light absorption coefficient, K, is a function of biomass concentration, and the larger the biomass concentration is, the larger the K becomes. The following experimental formula was obtained.

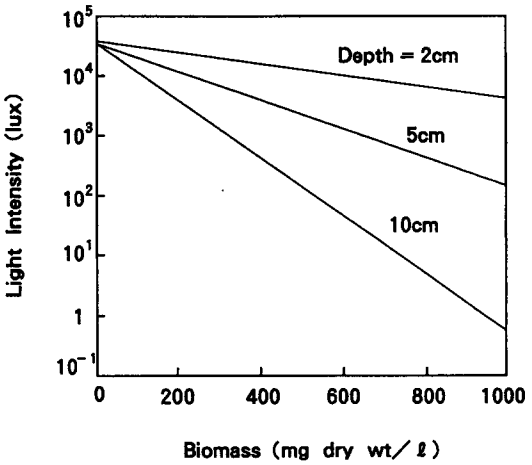

Fig. 6. Relation between biomass and light intensity.

$$K = 0.048 + 0.0011 \times C \quad (4)$$

where: C = biomass concentration (mg/L).

Figure 6 shows the relation between illuminance and biomass concentration, obtained by reducing the data given in Fig. 5. It can be seen from Fig. 6 that as the algal cell concentration increases, the illuminance deceases sharply.

Effects of Traces of Culture Medium Nutrient Components

It goes without saying that nutrient salts are essential for growth of microalgae. However, in order to process a large amount of CO_2 gas, large amounts of sea water and nutrient salts are also required. Therefore, it can be thought desirable to use a medium that has as few nutrient components to be added as possible and that are added, if possible, with only N and P sources that are minimally required for natural seawater.

On this subject, we are scheduled to perform experimental verification in the future. Here we would like to discuss the effects of elimination of heavy metals and vitamins on the growth rate of microalgae regarding nutrient component traces of an f/2 standard sea-water culture medium.

The f/2 standard sea-water medium (1000 mL) contains $NaNO_3$ as an N source, NaH_2PO_4 as a P source, and Na_2SiO_3 as an Si source, along with traces of thiamine (100 μg), biotin (0.5 μg), and B_{12} (0.5 μg) as vitamins, and trace elements of $FeCl_3$, $MnCl_2$, $CuSO_4$, $5H_2O$, $ZnSO_4$, and so forth, as heavy metals. Figure 7 shows the results obtained by examining the effects of trace nutrient components of NANNP-2 and PHAEO-2.

The experiments were performed at 10,000 lx, 5% of CO_2 concentration, and 25°C of solution temperature, using a 40-mL test tube, by allowing algal strains to grow in each culture medium for about a week and by

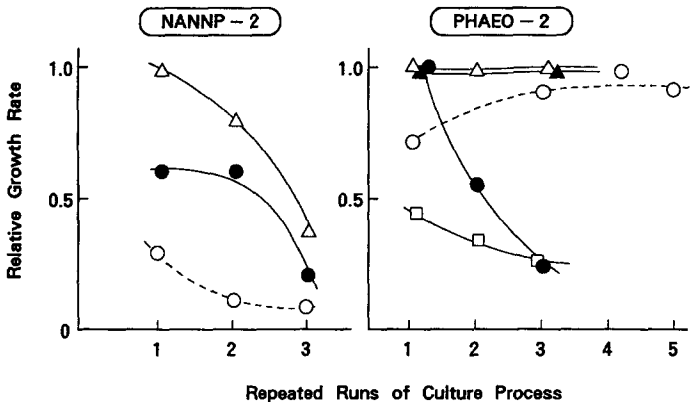

Fig. 7. Influence of trace elements eliminated on algal growth. Trace elements removed from standard medium: —●— Fe, —△— heavy metals except Fe, —▲— Si, —□— all heavy metals, --○-- vitamins.

repeating culture processes while adding N and P to the new culture medium. The experimental results were evaluated and expressed in a ratio of growth rate in the said culture media to that in the f/2 standard sea-water culture medium.

For the NANNP-2, the microalga growth rate decreased even when trace components of both heavy metals and vitamins were eliminated. Elimination of vitamins in particular caused a sharp decrease in growth rate. Furthermore, the more the culture process is repeated, the larger the decrease in growth rate becomes, and at the third repetition, an extremely sharp decrease was observed.

For the PHAEO-2, the growth rate decreased largely when Fe of heavy metals was eliminated. However, even if other heavy metals than Fe were removed, no such a large decrease took place. Furthermore, even if the culture process was repeated five times with vitamins eliminated, a decrease in growth rate hardly occurred. The same situation as this was also observed when other heavy metals than Fe or Si were eliminated.

Effects of Dust on Growth Rate

This research is based upon the idea of blowing combustion exhaust gas directly into a culture pond to fix CO_2 gas contained in the exhaust gas on microalgae. Table 3 shows an example of exhaust gas compositions obtained when heavy fuel oil, coal, and LNG are burned. Although LNG has < 0.1 mg/m^3 of dust concentration, heavy fuel oil and coal have approx 50 mg/m$_3$ even at the outlet of an electric precipitator installed as an exhaust gas treatment facility. We have examined whether introduction of dusts contained in exhaust gas into a culture pond will form a factor of hindering the growth rate of microalgae.

Table 4 shows a composition example of heavy fuel oil burnt ashes. The components considered to be harmful to growth of algae are Al, V, and

Table 3
Compositions of Power Plant Flue Gas

Fuel	CO_2, %	O_2, %	SOx, ppm	NOx, ppm	Dust, mg/m^3
Heavy oil (S: 0.35%)*	14.1	1.3	185	125	(50)
Coal (S: 0.34%)*	13.6	5.4	232 (30)	275 (177)	(50)
LNG (CH_4: 85%)*	11.2	1.8	-	47 (15)	<0.1

*( ): After desulfurization, denitrification, and dust collecting by electric precipitator.

Table 4
Compositions of Heavy Oil Dust (%)

C*	V_2O_5	NiO	Fe_2O_3	Na_2O	A L_2O_3	CaO	NH_3	SO_3	H_2O
39.49	1.95	0.66	2.30	0.95	0.34	0.21	9.00	31.80	0.36

*Unburned carbon.

Ni. For coal burnt ashes, it is known from separate analysis results that these ashes contain very small amounts of Ni and V, and approx 99% of water-insoluble components. It is therefore estimated that a mixture of coal ashes into a culture pond will cause no hindrance in microalgae growth.

Using heavy fuel oil burnt ashes shown in Table 4, testing on dissolution of ashes into artificial sea water was performed by putting 5 g of the said ashes into 1000 mL of artificial sea water to analyze dissolved components in the solution after a lapse of 120 h. The results showed 3.2 ppm of V, 25 ppm of Ni, and <0.1 ppm of Al, respectively. These ashes were then put in the culture solution in 0.1, 0.25, 0.5, 1, and 5 g/L, respectively, to compare microalgae growth rates for the solution with no addition of ashes and that which is added with ashes in the above manner, after which the results were evaluated for growth-hindrance rate.

Figure 8 shows charging amounts of dusts (or Ni and V concentrations in test solution) and growth hindrance rate. It has been found that the growth of algae is hindered when 0.2 g/L of dusts is put into culture solution. This condition is equivalent to the case where approx 1 ppm of Ni ion is dissolved in culture solution.

As shown in Table 3, the dust concentration of exhaust gas is estimated to be approx 50 mg/m^3. Therefore, the case where dusts accumulate in culture solution until 0.2 g/L of concentration, at which growth of microalgae is hindered, is reached is limited to the situation where microalga culture solution is used by recycling for an extended period of time because

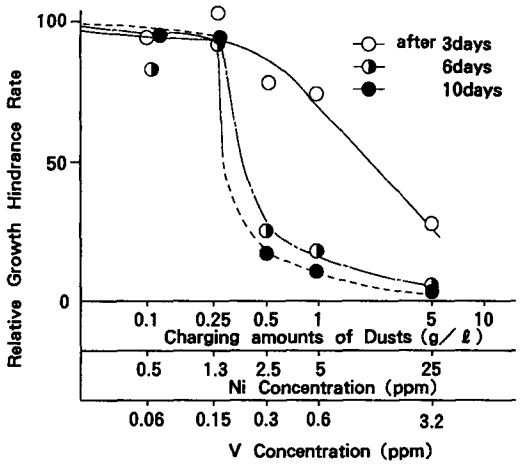

Fig. 8. Growth hindrance rate by charging amounts of dusts.

ash accumulation rate is approx 0.165 g/m^{3}D and time to reach 0.2 g/L is about 1200 d. Therefore, it can be thought that growth hindrance owing to dissolution of heavy metals into solution caused by mixing of dusts creates no particular problem.

CONCLUSION

We have obtained the following results:

1. The NANNP-2 stably grew even at high CO_2 concentrations (i.e., 5 and 15%). The total crude lipid content and calorific value of the harvested cells were approx 40% and 6,310 kcal/kg, respectively. Although the total crude lipid content increases (i.e., to over 50%) if N of the culture medium is limited, the growth rate showed a tendency to decline.
2. Approximately 0.5 g of algal cell was obtained from 1 g CO_2. According to the alga composition analysis results, C of biomass was 56%, and the ratios of N and P to C were 4.9 and 0.5%, respectively.
3. We have given an experimental formula about attenuation of light in culture solution in which a light absorption coefficient, K, is proportional to the biomass concentration. Furthermore, our findings are that, when the biomass concentration increases, the illuminance correspondingly decreases and also sharply decreases, particularly when a solution depth increases, as well as that approx 5 cm of solution depth is appropriate in view of light utilization rate.

4. Although Ni and V contained in heavy fuel oil burnt ashes dissolve into culture solution, their concentrations are low, and it has been made clear that they have no particular adverse effect on growth of microalgae.
5. Culture medium trace components (i.e., heavy metals and vitamins) are essential for the NANNP-2. However, for the PHAEO-2, it was found that the growth rate of microalgae hardly changes, even if vitamins and heavy metals other than Fe are eliminated.

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