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Growth of Microalgae in High CO_2 Gas and Effects of SO_x and NO_x

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ABSTRACT

Growth and lipid production of microalgae were investigated, with attention to the feasibility of making use of flue gas CO_2 as a carbon source. The effect of a high CO_2 level in artificial seawater differed from strain to strain. Three algal strains from the Solar Energy Research Institute (Golden, CO) collection were selected as good fixers of CO_2 when the level of CO_2 in the sparging gas was high. These algae also accumulated large amounts of crude lipids. SO_x and NO_x inhibited algal growth, but a green alga, *Nannochloris* sp. NANNO2 grew after a lag period, even when it received NO gas at the concentration of 300 ppm.

Index Entries: CO_2 elimination; flue gas; lipid production; marine algae; SO_x and NO_x.

INTRODUCTION

Global CO_2 emission, mainly from the combustion of fossil fuel, has been estimated to be 2×10^{10} tons/yr. Global warming is caused by the increase in the atmospheric CO_2 level and in the levels of other greenhouse gases, such as methane and chlorofluorocarbons. Power plants burn enormous amounts of fossil fuels, such as coal, oil, and LNG, for the generation of steam, and the CO_2 from these plants accounts for more than 16% of total CO_2 emissions. Possible engineering improvements

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and policy decisions have been widely discussed in an attempt to decrease the rate of the increase in the CO_2 concentration in the atmosphere. Elimination of CO_2 is possible by physicochemical methods, such as wet absorption, dry adsorption, and membrane separation techniques (*1*), but further disposal of the trapped CO_2 is a costly process.

The exhaust gas from power plants could be a useful source of CO_2 for the mass culture of microalgae (*2*). Carbon fixed by microalgae is incorporated into carbohydrates, lipids, and proteins, so energy, chemicals, or foods can be produced from algal biomass. Processes leading to the conversion of algal biomass to such useful products would be an economical method for CO_2 fixation and disposal, because they would indirectly decrease dependence on fossil fuels, which are not renewable. Fine chemicals, like vitamins and pigments, are commercially produced by the mass cultivation of microalgae (*3,4*). *Spirulina* is produced for use in algal health foods in Israel, the USA, Mexico, Taiwan, Thailand, and Japan (*5*). Liquid fuels, such as gasoline and diesel oil, can be produced from microalgae that accumulate lipids in great quantities when starved for nutrients (*6,7*). Gaseous fuels also can be produced from microalgae; microalgae grown on wastewater can be converted to methane by anaerobic digestion (*8*). Hydrogen is produced through biophotolysis, in which algal cells are used as biocatalysts for water splitting (*9*).

The process model postulated in this study is shown in the flowsheet in Fig. 1. Marine microalgae fix CO_2 in the flue gas from a power plant, and harvested cells are converted to liquid or gaseous flue, or else, sent directly to a combustion chamber in the form of slurry. Carbonation, reactor design, and harvesting are the major engineering problems encountered in the process. As the first step of our study to develop a bioprocess for the efficient elimination of CO_2, we investigated the basic growth characteristics of microalgae in a seawater medium with a CO_2 level similar to that in flue gas.

MATERIALS AND METHODS

Algal Strains

Ten strains of marine and halotolerant microalgae were cultured in the main part of these experiments. *Ankistrodesmus falcatus* ANKIS1, *Botryococcus braunii* BOTRY1, *Chlorella ellipsoidea* CHLOR2, *Monoraphidium* sp. MONOR2, *Nannochloris* sp. NANNO2, *Tetraselmis* sp. TETRA4, *Nannochloropsis* sp. NANNP2, *Chaetoceros muelleri* CHAET14, *Chaetoceros muelleri* var. *subsalsum* CHAET10, and *Phaeodactylum tricornutum* PHAEO2, were obtained from the Solar Energy Research Institute (SERI), Golden, CO (*10*). These species were selected on the basis of salinity tolerance, lipid accumulation, and taxonomic interests. Some marine species were isolated from enrichment cultures of marine samples collected in coastal environments of the Kinki area of Japan, but these were used only in preliminary experiments.

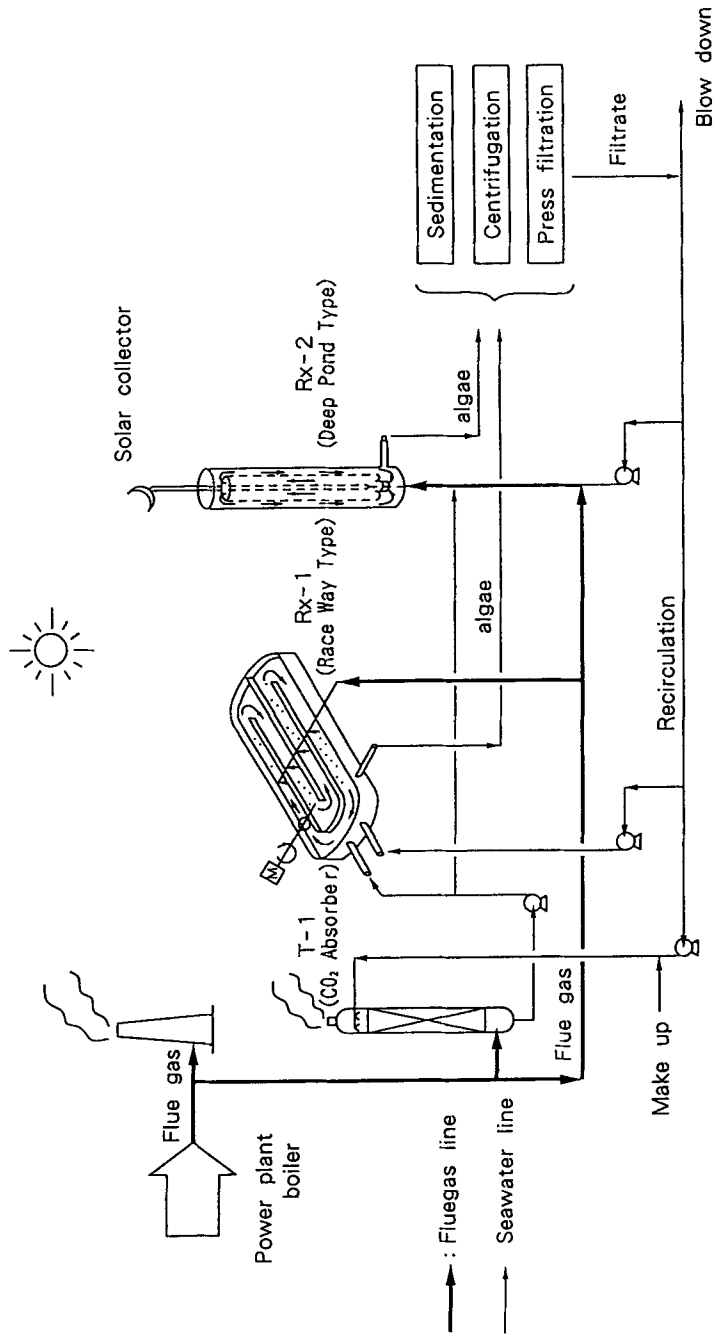

Fig. 1. Conceptual sketch of bioprocess for CO_2 elimination.

Culture Methods

The medium for stock cultures and growth experiments was a modification of the f/2 Seawater, described elsewhere (*10*). Nitrogen and phosphorus were enriched fourfold, and Instant Ocean (Aquarium System, Inc.) was used instead of natural seawater. Stock cultures were maintained at 10 mL of medium in 30-mL test tubes at 25°C under continuous illumination (about 300 lux). Cultures on a small scale (in 10-mL test tubes and 100-mL Erlenmeyer flasks) were done in the preliminary experiments. In most experiments, however, algae were grown at 25°C in Roux bottles (1000 mL), aerated with air supplemented with 5% (v/v) or 15% (v/v) CO_2. The cultures were illuminated continuously with fluorescent lights (10,000 lux) with the exception of one set of growth experiments where cultures were grown in a cycle of 16 h of light and 8 h of dark. The culture pH was not controlled, except in two sets of growth experiments in which the pH was adjusted to 7.0 or 8.0 by the addition of 1 *N* or 5 *N* NaOH.

After inoculation (150–200 mg/L), growth was monitored by measurements of the change in optical density of 680 nm (OD_{680}) in a small sample from the bottle. For each strain, the OD_{680} was correlated with dry wt, measured after cells were washed, and dried at 105°C for 3 h.

Analytical

The total crude lipids were estimated as follows: First, 40–50 mg of algal cells were harvested by centrifugation (5000 rpm, 5 min) and homogenized with glass beads in a Waring blender (Nihon Seiki, 10000 rpm, 10 min). Lipids was extracted overnight with a 2:1 mixture of chloroform and methanol, and estimated gravimetrically.

Gas Analysis

Sulfur dioxide (SO_2) in the inlet and outlet gas mixtures was analyzed by the Kitagawa method, with use of a tubular detector. The nitric monoxide (NO) in the gas phase was assayed by a chemiluminescence method, with use of Portable NO_x analyzer (Shimadzu, NOA-305). The ranges of gas detection were 5–300 ppm and 0–500 ppm, respectively.

Concentrations of sulfate (SO_4^{2-}) and nitrite (NO_2^-) were measured according to testing methods for industrial waste water of Japanese industrial standard (JIS-K-0102).

RESULTS AND DISCUSSION

Algal Growth in Artificial Seawater at a High CO_2 Level

Sparging batch cultures with CO_2 at high partial pressures might change the pH and inhibit algal growth. However, in a pH-controlled

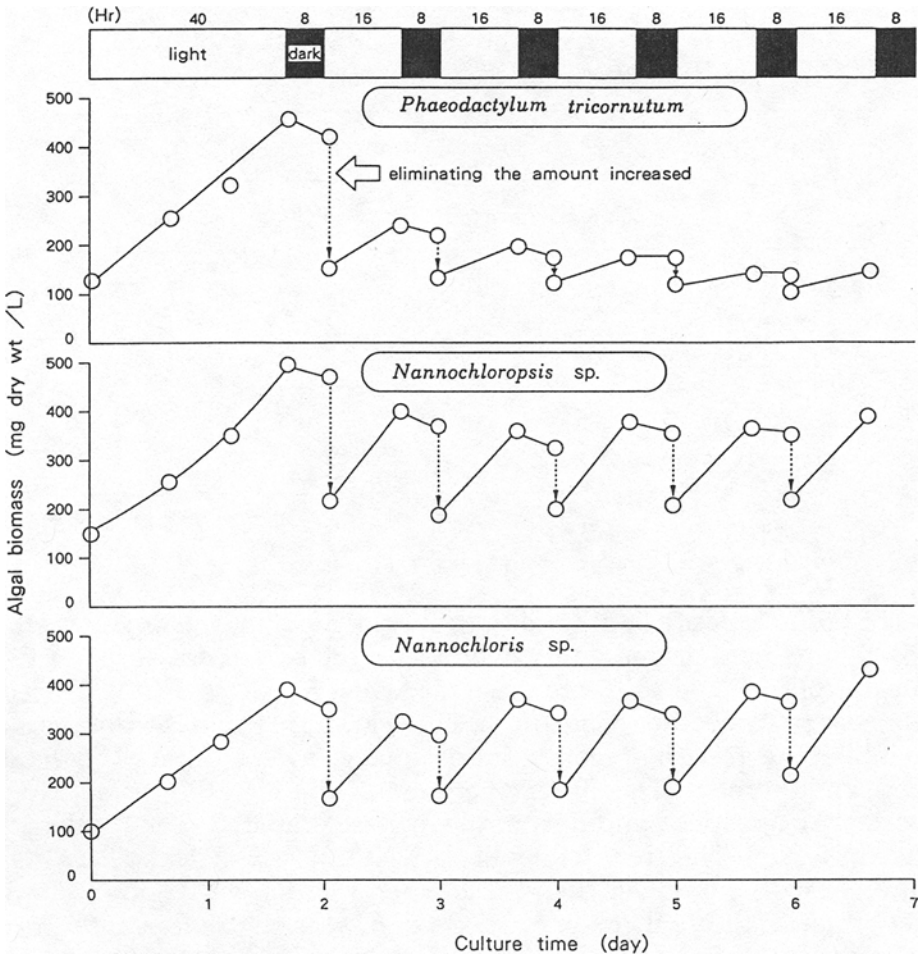

Fig. 2. Daily changes of algal biomass in a 16 h light and 8 h dark cycle with a high CO_2 concentration.

chemostat culture, growth of *Chlorella* cells adapted to high CO_2 are little affected by increases in CO_2, up to 0.65 atm (*11*). Here, ten strains of microalgae, all from the SERI collection (*10*), were cultured at an estimated flue-gas CO_2 level of 15%. Compared with results from cultures sparged with air or 5% CO_2, the effects of 15% CO_2 in artificial seawater were not uniform; half of the algal strains exhibited no, or very poor, growth, and the others grew with extended lag periods or decreased growth rates. A Chlorophyceae, *Nannochloris* sp. NANNO2, a Eustigmatophyceae, *Nannochloropsis* sp. NANNP2, and a Bacillariophyceae, *Phaeodactylum tricornutum* PHAEO2, grew best. These three algae were cultured semicontinuously in a cycle of 16 h of light and 8 h of dark for testing of culture stability at high CO_2 (Fig. 2). The growth of PHAEO2 slowed for an unknown reason, but the growth of NANNO2 and NANNP2 was very stable.

Table 1
Effect of pH Control on the Growth Rate of NANNO2, NANNP2, and PHAEO2

Algal strain	Growth rate (mg dry wt/L·day)		
	pH not controlled[a]	pH 7.0	pH 8.0
Nannochloris sp. NANNO2	320	290	290
Nannochloropsis sp. NANNP2	270	310	200
Phaeodactylum tricornutum PHAEO2	150	160	-[b]

[a] pH ranged from 6.1 to 6.2.
[b] not measured because of wall growth.

The buffer capacity of the artificial seawater medium was small, so, the pH in the medium decreased, depending on the partial pressure of CO_2. Values of pH 6.8 and 6.3 were observed when 5% or 15% CO_2, respectively, was sparged into the medium. These lower pH values would probably inhibit the growth of marine species that grow at around pH 8 in the natural environment. Therefore, algal growth at a high CO_2 level (15%), with the pH controlled and uncontrolled, was compared. The results are shown in Table 1. The growth rates of NANNO2, NANNP2, and PHAEO2 at low pH were not lower than those when the pH was kept at 7 or 8. These results indicate that some species of marine or halotolerant microalgae could be cultured without strict pH control. Control of pH in an outdoor mass culture system would not be economically feasible. Thus, the algal strains selected here may be particularly useful because of their pH tolerance.

Lipid Accumulation

Elimination of CO_2 from flue gases is technically feasible with the physicochemical methods mentioned before (*1*). However, all of these methods give rise to the problem of the disposal of large amounts of separated CO_2. Biological fixation of CO_2 incorporates it into complexes that are potentially converted to useful compounds. The SERI has investigated the production of lipids by the mass culture of microalgae, followed by the conversion of the lipids to liquid fuels, such as gasoline and diesel fuel (*6*).

The total amount of lipids is affected by various environmental factors. Nitrogen limitation can enhance the lipid concentration in most microalgal cells (*7,12*). The three strains selected here, which accumulate large

Table 2
Biomass and Lipid Production with High CO_2 Level[a]

Algal strain	Initial NO_3^--N (mg/L)	Final biomass[b] (mg dry wt/L)	Crude lipid content[b] (% of dry wt)
Nannochloris sp. NANNO2	12	510	56
Nannochloropsis sp. NANNP2	47	1430	45
	12	520	53
Phaeodactylum tricornutum PHAEO2	12	640	45

[a] Air supplemented with 15% CO_2 was used.

[b] Biomass and lipid levels were measured on day 8.

amounts of lipids (45–65%) under optimum conditions (*10*), were cultured with 15% CO_2. Nitrate was completely consumed during the first 3 d of cultivation, and thereafter, the crude lipid concentration increased up to 45–55% of dry wt in nitrogen-starved algal cells under conditions of high CO_2, and thus, low pH (Table 2). When NANNP2 were grown in two different concentrations of nitrate, the final crude lipid concentration was about the same in the two cultures. However, because the biomass yield is roughly proportional to the initial nitrate concentration, crude lipid production was higher at the higher nitrate concentration.

Effects of SO_x and NO_x

The flue gases from power plant boilers contain sulfur and nitrogen oxides (SO_x and NO_x). Emissions of these oxides and their derivatives cause so-called acid rain, which disrupts the balance of the natural ecosystem (*13*). Therefore, the possibility of decreasing these oxides and CO_2 simultaneously by the use of algal cultures was investigated by evaluation of the effects of SO_x and NO_x on algal growth.

Sulfur dioxide (SO_2) was fed to cultures of NANNO2, NANNP2, or PHAEO2 at 0, 50, or 400 ppm (v/v), with the CO_2 level kept at 15%. Results are summarized in Fig. 3. Sparging of SO_2 at an inlet concentration of 50 ppm had no effect on the growth of NANNO2 or NANNP2. The growth of PHAEO2 was slightly inhibited by SO_2, though the pH changed little. At the elevated level of 400 ppm, however, the pH dropped and growth ceased after 20 h of cultivation. It was found in a parallel experiment with artificial seawater that did not contain algal cells that the absorbed SO_2 was oxidized to sulfate, even at an actual level of 1% O_2 in the flue gas.

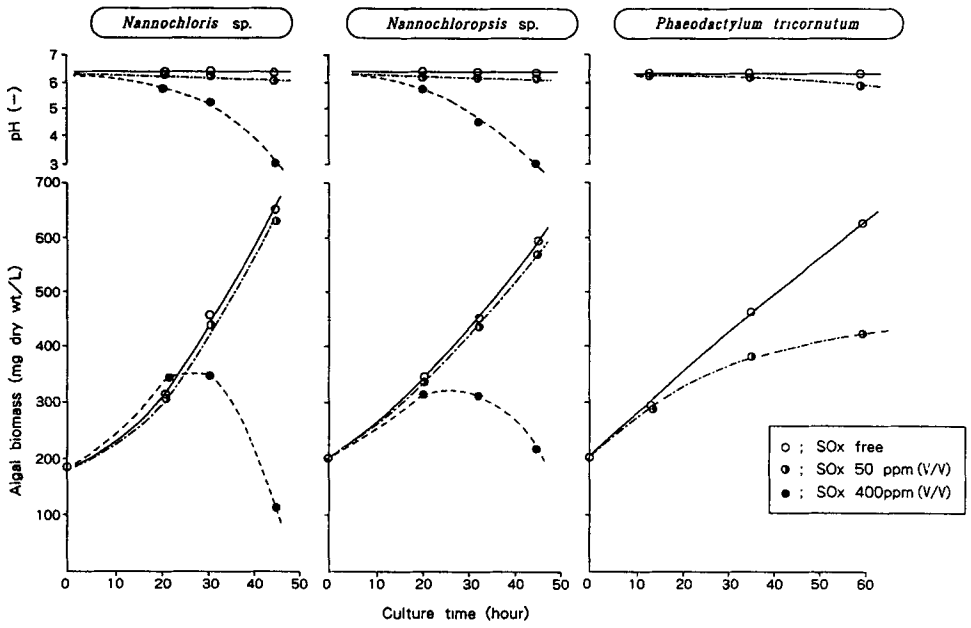

Fig. 3. Effect of SO_x on algal growth with a high CO_2 level.

The accumulation of sulfate and the resulting drop in pH would explain the complete inhibition of algal growth.

The effect of another pollutant in flue gases is presented in Fig. 4. With the CO_2 level high, cultures of NANNO2 and NANNP2 received NO gas at the concentration of 300 ppm. The growth of both strains was greatly affected by the supply of NO, in spite of the pH changing very little. NANNP2 did not grow at all, and NANNO2 grew after a prolonged lag period. NO_2^- was detected in the culture broth and in cell-free seawater medium, suggesting that NO can be nonbiologically converted to NO_2^-. The accumulation rate of NO_2^- in the cell-free medium depended on the O_2 tension in the sparging gas. The concentration of NO_2^- increased linearly with time in the culture of NANNP2. On the other hand, the increase in the NO_2^- concentration in the NANNO2 culture slowed after the start of growth, suggesting that some nitrogen oxides were assimilated by the NANNO2 cells. NO gas cannot be completely removed with use of an alkaline solution. Thus, it is of interest that some NO gas was absorbed and assimilated by an algal culture. The form of the nitrogen oxide utilized by this alga is not known.

CONCLUSIONS

The growth of the three stains we selected was affected little by 15% CO_2. Lipid accumulation in these algae was as high as in the conditions the SERI has used before, probably because the supply of the carbon

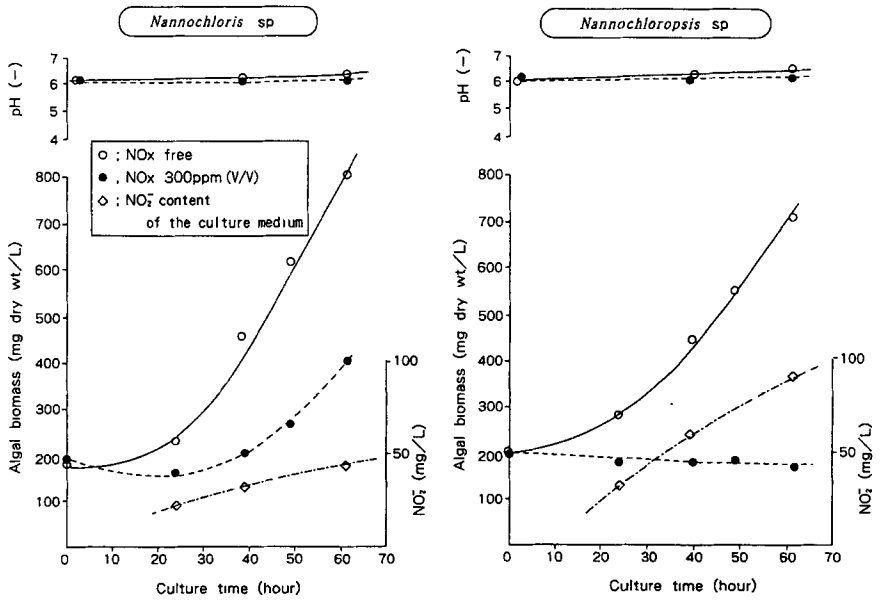

Fig. 4. Effect of NO_x on algal growth with a high CO_2 level.

source was adequate. A well-designed carbonation system, e.g., timed supply of CO_2, should increase the growth rates and accumulation of lipids. Both SO_2 and NO inhibited algal growth at a high level of CO_2. The use of NO_x as a nitrogen source in the culture of NANNO2 might be possible. Screening of algal strains would make an effective biological de-NO_x system feasible. It seems difficult for algal cultures to assimilate a large amount of sulfate; SO_x would have to be previously eliminated if algal growth is to be satisfactory. In Japan, a unique lime-limestone process for desulfurization has been developed that has decreased the concentration of SO_x to below the regulation level (*14*). Stringent control of the emission of SO_x are now planned in other industrial countries, and a large amount of SO_x-free CO_2 should become available in these countries. In the meantime, we plan to continue to try for improvements in the elimination of CO_2, and possibly NO_x, by use of the capabilities of microalgae.

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