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Methods to Enhance Tolerances of *Chlorella* KR-1 to Toxic Compounds in Flue Gas

Ju-No Lee,[1] Jin-Suk Lee,*[,1] Chul-Seung Shin,[1] Soon-Chul Park,[1] and Seung-Wook Kim[2]

[1]Biomass Team, KIER, 71-2 Jang-dong, Yuseong-ku, Taejeon 305-343, Korea, E-mail: bmjslee@kier.re.kr; and [2]Chemical Engineering Department, Korea University, 5 Anam-dong Sungbuk-ku, Seoul, 136-701 Korea

Abstract

Possible methods to minimize the toxic effects of SO_x and NO_x on the growth of a highly CO_2 tolerant and fast-growing microalga, *Chlorella* sp. KR-1, were investigated. Maintaining the pH of the culturing media at an adequate value was quite important to enhancing the tolerances of the microalgae to SO_x and NO_x. Controlling the pH by adding an alkaline solution, using a low flow rate of gas fed to the culture, and using a high concentration of inoculating cells were effective methods to maintaining the proper pH of the culture. Controlling the pH was the most effective method but could be applied only for some specific microalgae.

Index Entries: SO_x and NO_x tolerances; pH control; *Chlorella* KR-1; microalgae; flue gas.

Introduction

Biological methods, in particular using microalgal photosynthesis, have several merits, such as mild conditions for CO_2 fixation and no requirements for the further disposal of trapped CO_2. Carbon fixed by microalgae is incorporated into carbohydrates and lipids, and, therefore, energy, chemicals, or foods can be produced from algal biomass *(1,2)*. Several studies of CO_2 removal using microalgae have been reported in the literature *(3–5)*. Direct use of flue gas reduces the cost of pretreatment but imposes extreme conditions on microalgae such as high concentrations of CO_2 (10–15%) and the presence of inhibitory compounds such as NO_x and SO_x. The levels of CO_2 found in flue gas could be inhibitory to algal growth

*Author to whom all correspondence and reprint requests should be addressed.

(6). Studies have been conducted to isolate highly CO_2-tolerant microalgae *(7–9)*, and some microalgae isolated for high CO_2 tolerance exhibited a high growth rate at a 15% CO_2 concentration.

In addition to the high concentrations of CO_2, the inhibition by toxic compounds such as SO_x and NO_x in flue gas would be critical. If the flue gas is directly introduced into the microalgal culture, the medium pH should go down to 2.0 or 1.0 by the dissolution of NO_x and SO_x. To determine the effect of acidification by NO_x and SO_x, a few studies have been conducted *(5,10,11)*. Growth of some acidophilic microalgal strains were not inhibited by an NO_x-only flue gas consisting of 50 ppm of NO_x and 15% CO_2. Yoshihara et al. *(5)* also reported that the tolerance of a microalga to NO_x was dependent on the cell concentration to inoculate the culture, and that growth of a marine microalga, strain NOA-113, was not inhibited if the simulated flue gas containing 300 ppm of NO was supplied at a cell concentration of 1.5 g/L. The inhibition effect of SO_x was more remarkable. Yanagi et al. *(1)* reported that *Chlorella* HA-1, a highly CO_2-tolerant microalga, could not grow at 50 ppm SO_2. Kurano et al. *(11)* reported that *Galdieria partita*, an acidophilic unicellular red alga, showed growth at 50 ppm of SO_2 aeration. Hauck et al. *(10)* reported that *Cyanidium caladrium*, an acidophilic microalga, exhibited some growth in a simulated flue gas of 200 ppm of SO_2 for the first 20 h but that growth of *Chlorella vulgaris* was completely inhibited.

Since the inhibition effect of SO_x was less pronounced for acidophilic microalgae, both studies reported that inhibition was mainly owing to acidification of medium by the introduction of flue gas. Therefore, the studies of CO_2 removal from flue gas have been focused only on the isolation of the NO_x- and SO_x-tolerant microalgae or acidophilic algae *(10,11)*. But the acidophilic microalgae isolated until now was reported to exhibit stable growth only up to 50 ppm of SO_2, a stronger inhibitor *(10,11)*. Since flue gas from most industrial sources contains about 100–300 ppm of SO_x and NO_x, direct CO_2 fixation from flue gas using acidophilic microalgae could not to be successful. We propose a new strategy to overcome the inhibition of algal growth by SO_x and NO_x.

In the present study, a series of experiments were conducted to find a way to overcome the toxic effects of SO_x and NO_x by changing operating conditions. The effects of operating conditions such as gas flow rates, control of pH in the media, and inoculating cell concentrations on the growth of *Chlorella* sp. KR-1 were determined. *Chlorella* sp. KR-1 was selected from the viewpoint of its good growth with high concentrations of CO_2 *(9)*.

Materials and Methods

Strain and Culture Medium

Chlorococcum littorale, a marine microalga, and *Chlorella* HA-1, a freshwater microalga, were obtained, respectively, from Marine Biotechnology Institute (Kamaishi, Japan) and National Institute of Environmental Stud-

ies (Tokyo, Japan). *Chlorella* sp. KR-1, isolated in our laboratory *(9)*, was maintained by transferring the strain monthly on a Detmer agar plate. The Detmer agar plate had the following composition: 1000 mg/L of $Ca(NO_3)_2$; 250 mg/L of KCl; 250 mg/L of $MgSO_4 \cdot 7H_2O$; 250 mg/L of KH_2PO_4; and 0.002 mg/L of $FeCl_3$. The plate was cultured for 2 wk at 25°C. Illumination was provided by fluorescent light. Light intensity was about 90 $\mu M/m^2 s$. The *Chlorella* KR-1 strain was grown on modified M4N medium *(12)*, which contains 5000 mg/L of KNO_3; 2500 mg/L of $MgSO_4 \cdot 7H_2O$; 1250 mg/L of KH_2PO_4; 14 mg/L of NaFeEDTA; 2.86 mg/L of H_3BO_3; 2.5 mg/L of $MnSO_4 \cdot 7H_2O$; 0.222 mg/L of $ZnSO_4 \cdot 7H_2O$; 0.079 mg/L of $CuSO_4 5H_2O$; and 0.021 mg/L of Na_2MoO_4. The initial pH of the medium was adjusted with experimental conditions.

Model Flue Gas

Typical flue gas discharged from an oil-fueled boiler is estimated to contain 9.5–16.5% CO_2, 2–6.5% O_2, 280–320 ppm of SO_x, and 100–300 ppm of NO_x. In this study, several gas mixtures (Praxair Korea, Kiheung, Korea) were used for the experiments to evaluate the effects of the inhibitory compounds (SO_x and NO_x) in flue gas on the growth of the algal strains.

CO_2 Fixation Experiments

Microalgal culture experiments were conducted to determine the cultural characteristics of microalgae. All seed cultures were prepared with air bubbling in a 10-L illuminated jar (8 L medium) at 25°C and 200 $\mu mol/(m^2 \cdot s)$. Growth of *Chlorella* KR-1, when aerated with various gases, was conducted in a small bioreactor setup. All growth experiments were carried out in gas washing bottles (125 mL, Ace Glass, Vineland, NJ). Five bottles containing 50 mL of culture solution inoculated with *Chlorella* KR-1 were run in most experiments. The growth rates and pHs were monitored with different gas mixtures (Praxair Korea). The bottles were illuminated by fluorescent tubes. The seed culture was centrifuged and washed with sterilized water before inoculation. Samples were removed intermittently from the vessels to determine the algal growth and pH of the medium. The temperature of the culture medium was maintained at 25°C. The pH of the medium was not regulated and the gas flow rate was fixed to 0.5 vol gas/vol liquid/min (vvm) unless otherwise specified. In the culture aerated with 15% CO_2, the concentration of CO_2 was regulated by controlling the flow rates of air and CO_2 with a gas mass flow controller (905C-PS-BM-11, Sierra Instruments, Montrey, CA). Air-grown cells were inoculated into the medium to obtain an initial cell concentration specified in the results.

Assay

Algal growth was determined by measuring the absorbance at 660 nm using a spectrophotometer (HP8452A, Hewlett-Packard, Palo Alto, CA) and converting into dry cell weight. Light intensities were measured by a light sensor (LI-250, LI-COR, Lincoln, NE). CO_2 concentrations were on-line

monitored by a CO_2 analyzer (IR-8400, Summit Analyzers, Cleveland, OH). NO and SO_2 concentrations in gas mixtures were measured by an NO_x analyzer (NONOXOR II, Bacharach, Pittsburgh, PA) and an SO_x analyzer (DIOXOR II, Bacharach).

Results and Discussion

Controlling pH to Overcome Inhibitory Effect of SO_x and NO_x

Since SO_2 is known to inhibit the growth of microalgae significantly, controlling the pH was investigated as a means of overcoming the toxicity problem. In a simulated flue gas consisting of 150 ppm of SO_2, 15% CO_2, 3% O_2, and balance N_2, the growth of *Chlorella* KR-1 occurs initially but is inhibited after 12 h when pH was not controlled from the beginning of culturing. A significant decrease in pH from 5.8 to 3.0 was also observed. On the other hand, *Chlorella* KR-1 exhibited good growth in the simulated flue gas when pH was controlled to 7.0 by adding 1 *N* NaOH solution every 4 h for the first 8 h. Note that a few drops of NaOH solution were added only two times to the culture (*see* Fig. 1). The growth rate and cell yields were approximately equivalent to those of the control culture aerated with SO_2-free 15% CO_2-air gas mixture. This indicated that the toxicity of SO_2 itself or its aqueous products was not very significant for *Chlorella* KR-1. In contrast to this finding, Hauck et al. *(10)* reported that SO_2 or an aqueous reaction product of SO_2 was the toxic agent responsible for inhibition of growth of *Cyanidium caldarium*, an acidophilic strain, because the lowering of pH by SO_2 did not inhibit growth of the alga. However, they could not determine whether the SO_2 itself is toxic to *Chlorella vulgaris* because the drop in pH, caused by the solubility of SO_2 in aqueous solution, was remarkable and the inhibition of *C. vulgaris* should result from the low pH relative to the optimal pH of about 7.0 *(10)*. The results show that the mechanism of SO_x inhibition may be different with the algal strain employed. To investigate further, similar experiments have been conducted for other highly CO_2-tolerant microalgae, *Chlorella* HA-1 and *C. littorale*. Figure 2 shows that controlling the pH was also effective for *Chlorella* HA-1 and *C. littorale*. However, the degree of the inhibitory effect by SO_2 differed from one strain to another when pH was controlled.

Further experiments have been carried out with the simulated flue gases containing higher concentrations of SO_2. Figure 3 shows that *Chlorella* KR-1 could grow well with the simulated flue gas containing 250 ppm of SO_2 if the pH was controlled for about 8 h from the beginning. As expected, the growth of *Chlorella* KR-1 was completely inhibited from the beginning if pH was not controlled. However, *Chlorella* KR-1 showed stable growth when pH was controlled for the first 8 h. Therefore, direct CO_2 fixation using the algal strain from flue gas containing a high SO_2 concentration would be possible if pH were controlled in the culture.

Figure 4 summarizes the quantitative effects of SO_2 on the growth rate of *Chlorella* KR-1. When the model flue gas containing 150 ppm of SO_2 or

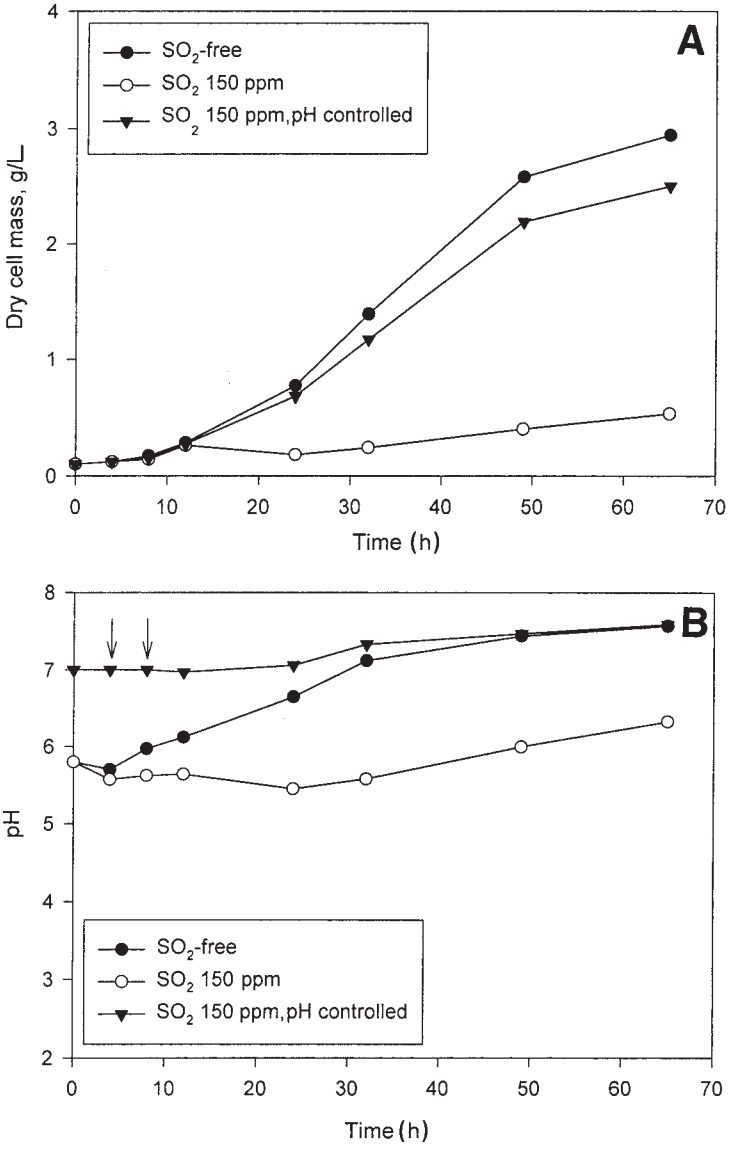

Fig. 1. Growth of *Chlorella* KR-1 **(A)** and pH change **(B)** under various cultural conditions. The cultures were illuminated at 350 μmol/(m^2 · s) and bubbled at 25°C. Arrows indicate the time when pH was controlled.

higher was supplied at a cell concentration of 0.1 g/L without pH control, cell growth was quite low or completely suppressed. However, the cells showed excellent growth when *Chlorella* KR-1 was cultured under identical cultural conditions if pH was controlled. The linear growth rate was about 1.5 g/(L · d), which is about equal to that of the culture at which SO_2-free gas was supplied at a cell concentration of 0.1 g/L (Table 1).

The growth of *Chlorella* KR-1 was totally inhibited when the model flue gas containing 300 ppm of NO was supplied at a cell concentration of

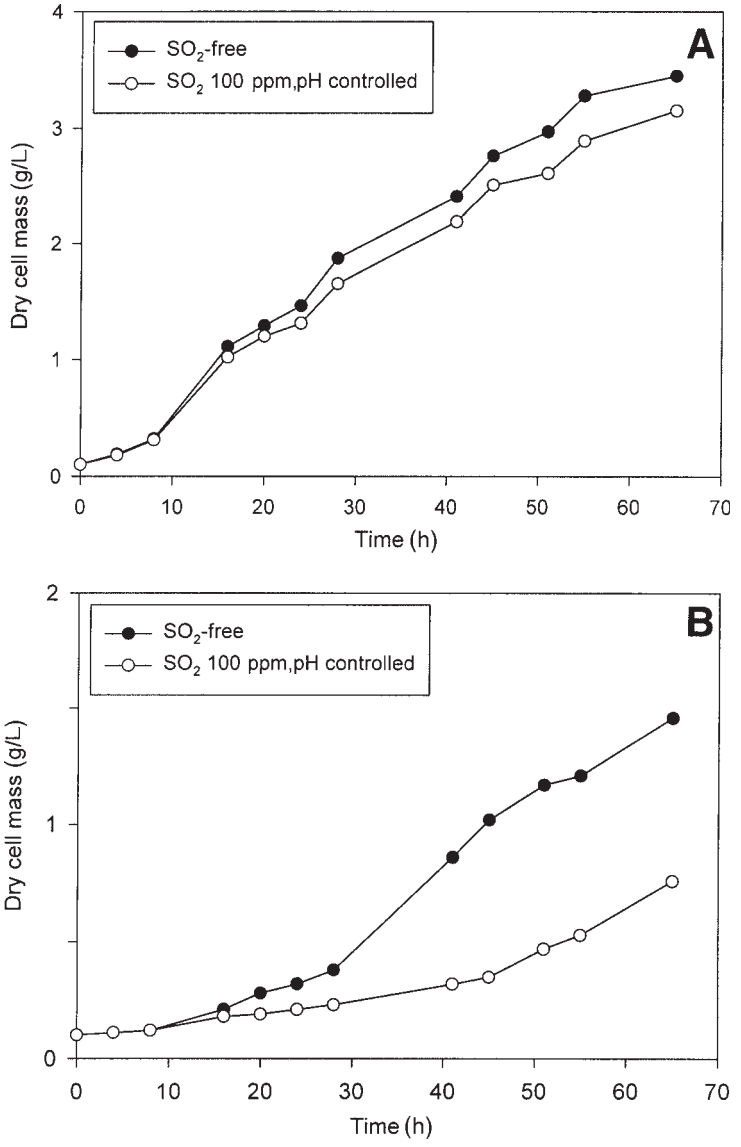

Fig. 2. Growth of *Chlorella* HA-1 **(A)** and *C. littorale* **(B)** under various cultural conditions. The cultures were illuminated at 350 μmol/(m^2 · s) and bubbled at 25°C.

0.1 g/L (Table 1). Control of pH was applied to enhance NO tolerances of *Chlorella* KR-1. As shown in Fig. 5, *Chlorella* KR-1 exhibited stable growth when it was cultured with gas containing 300 ppm of NO and pH was controlled for the first 8 h. The linear growth rate of *Chlorella* KR-1 with pH control was about 1.5 g/(L · d), which is equal to that of the control culture aerated with only CO_2-enriched gas; however, *Chlorella* KR-1 could not grow at all when pH was not controlled, as mentioned previously (Table 1). Therefore, it may be concluded that controlling pH was an effective method

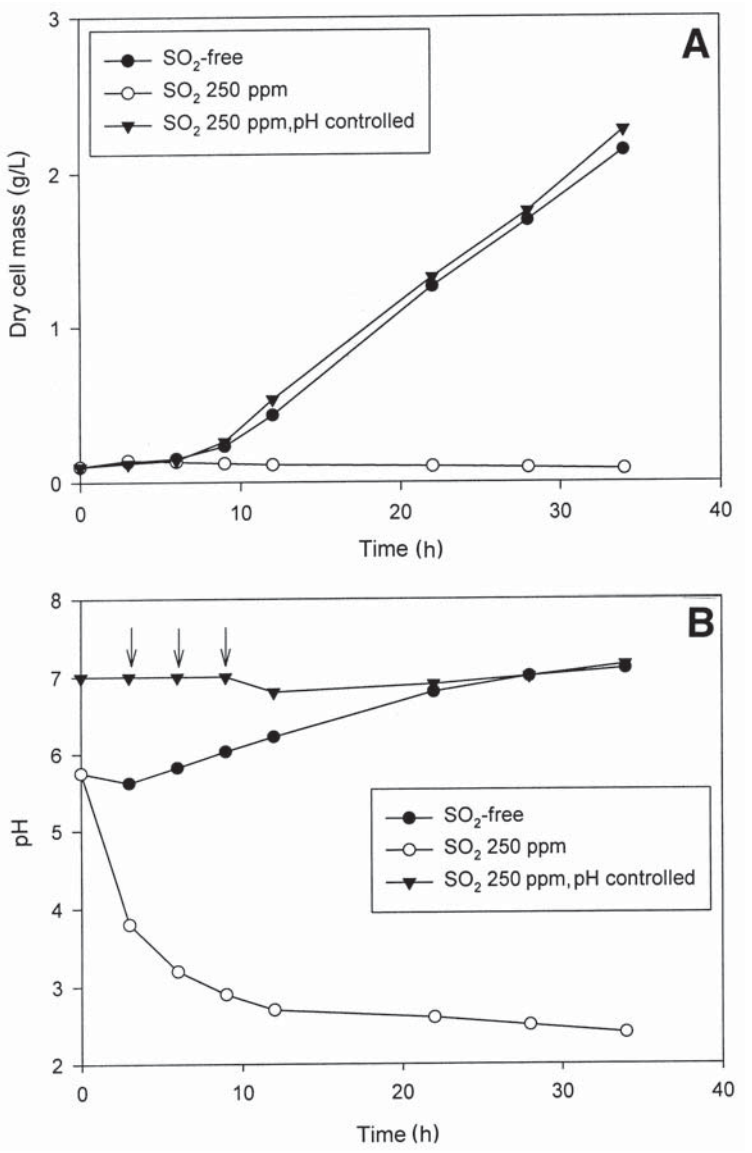

Fig. 3. Growth of *Chlorella* KR-1 **(A)** and pH change **(B)** under various cultural conditions. The cultures were illuminated at 350 μmol/(m^2 · s) and bubbled at 25°C. Arrows indicate the time when pH was controlled.

to enhance the tolerances of *Chlorella* KR-1 and some other microalgae to the toxic compounds in flue gas.

Effect of Cell Concentrations

Increasing the amount of inoculating cells is reported to be helpful in enhancing tolerances of microalgae to the toxic compounds in flue gas. Yoshihara et al. *(5)* reported that growth of a newly isolated marine

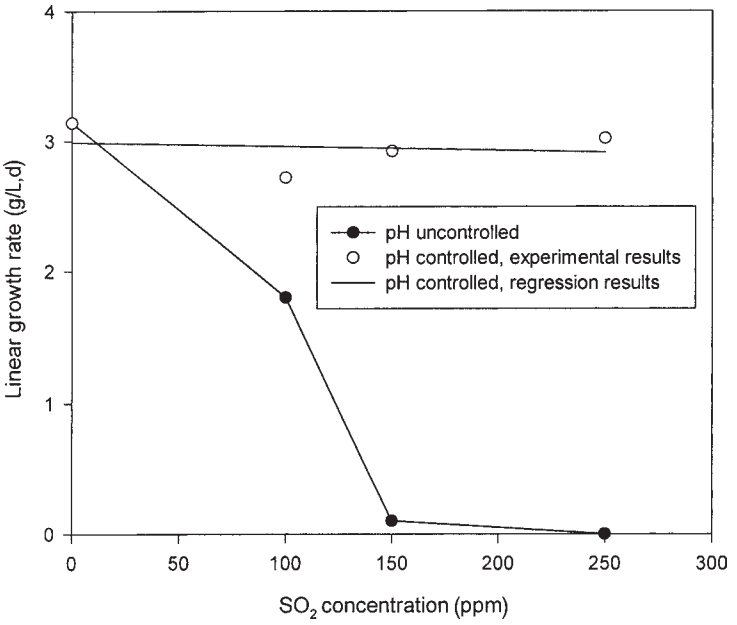

Fig. 4. Effects of pH control on the growth rate of *Chlorella* KR-1 cultured with the model gas containing various SO_2 concentrations.

Table 1
Linear Growth Rates of *Chlorella* KR-1 Under Various Cultural Conditions (g/L · d)

Toxic compounds in model gas	Cell concentration (g/L)		
	0.1		0.5
	pH controlled	pH free	pH free
Control[a]	—	1.6	1.6
150 ppm SO_2	1.5	0.05	1.5
250 ppm SO_2	1.5	0	0
300 ppm NO	1.5	0	1.5

[a]Aerated with only CO_2-enriched gas (15% CO_2 in gas).

microalga named NOA-113 was completely suppressed when the model flue gas containing 100 ppm of NO was supplied at a cell concentration lower than 1 g/L. However, the algal cells grew well if the model flue gas was introduced into the reactor at a cell concentration of 1.5 g/L. To investigate the inhibition effect of SO_2 on the growth of *Chlorella* KR-1 at higher cell concentrations, the model flue gas containing 150 ppm of SO_2 was supplied at a concentration of 0.5 g/L. When *Chlorella* KR-1 was cultured with the simulated flue gas containing 150 ppm of SO_2, algal cell growth was completely suppressed with an initial cell concentration of 0.1 g/L but exhibited good growth with an initial cell concentration of 0.5 g/L (Fig. 6).

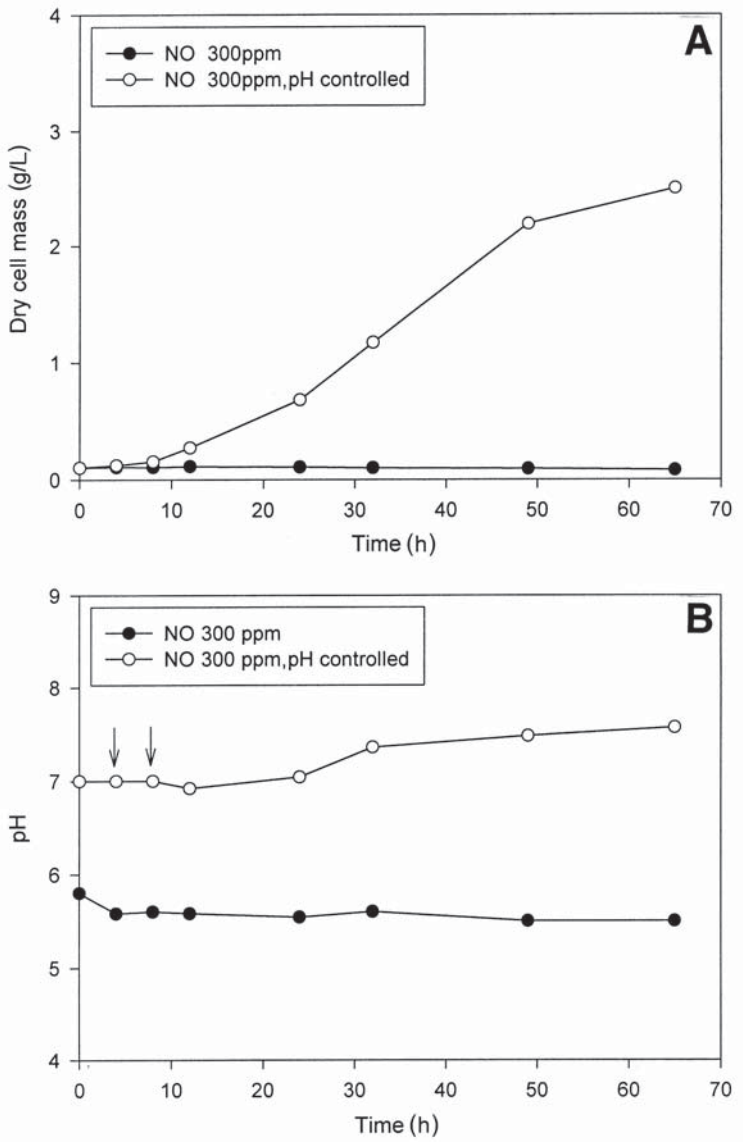

Fig. 5. Growth of *Chlorella* KR-1 **(A)** and pH change **(B)** under various cultural conditions. The cultures were illuminated at 350 μmol/(m^2 · s) and bubbled at 25°C. Arrows indicate the time when pH was controlled.

The linear growth rate was about 1.5 g/(L · d) (Table 1). However, the growth of *Chlorella* KR-1 with an initial cell concentration of 0.5 g/L was totally inhibited when cultured with the model gas containing 250 ppm of SO_2 (Table 1). Therefore, increasing cell concentration was useful for enhancing tolerances of *Chlorella* KR-1 for only some limited range of SO_2 concentrations.

The effect of NO on the growth of *Chlorella* KR-1 at high cell concentrations was also determined. As shown in Fig. 7, *Chlorella* KR-1 exhibited

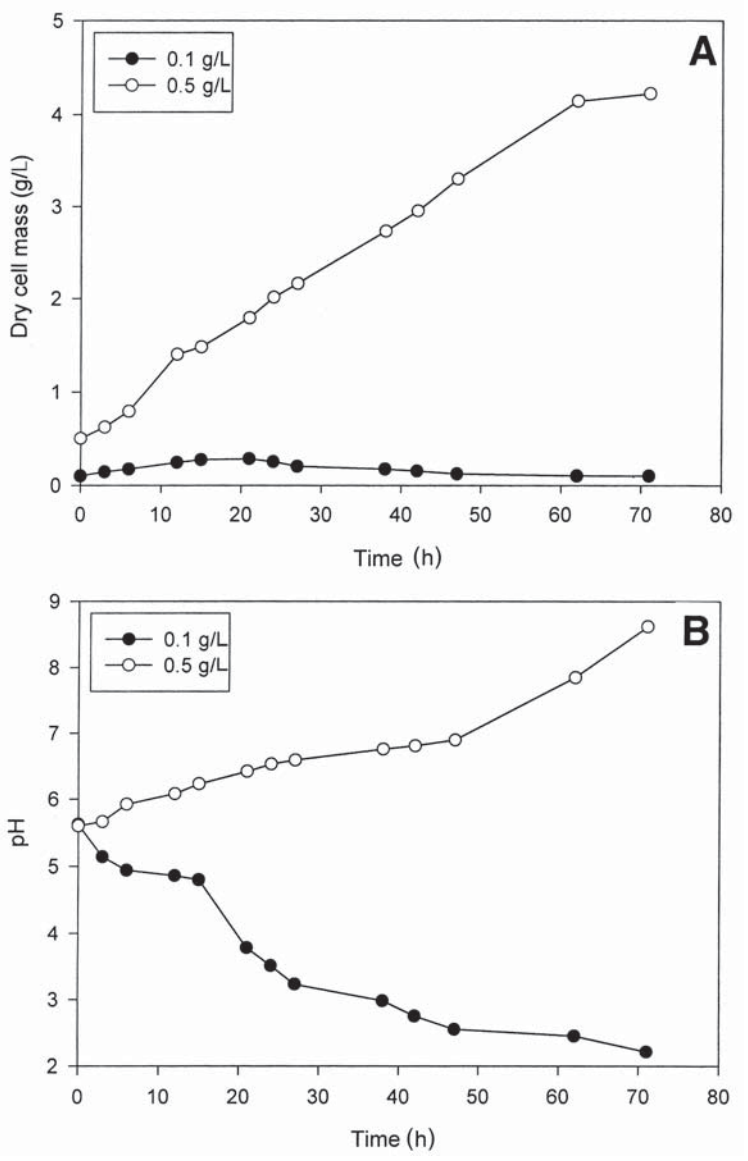

Fig. 6. Effect of inoculating cell mass on growth of *Chlorella* KR-1 **(A)** and pH change **(B)**. The cultures were illuminated at 350 μmol/($m^2 \cdot s$) and aerated with the simulated flue gas containing 150 ppm of SO_2 at 25°C.

good growth even with the gas mixture containing 300 ppm of NO when the initial cell concentration was increased to 0.5 g/L. The linear growth rate was about 1.5 g/(L · d), which showed that the growth of KR-1 with the initial cell concentration of 0.5 g/L was not inhibited at all when cultured with the model gas containing 300 ppm of NO (Table 1). With regard to NO, increasing the cell concentration is good for the detoxification of the culture.

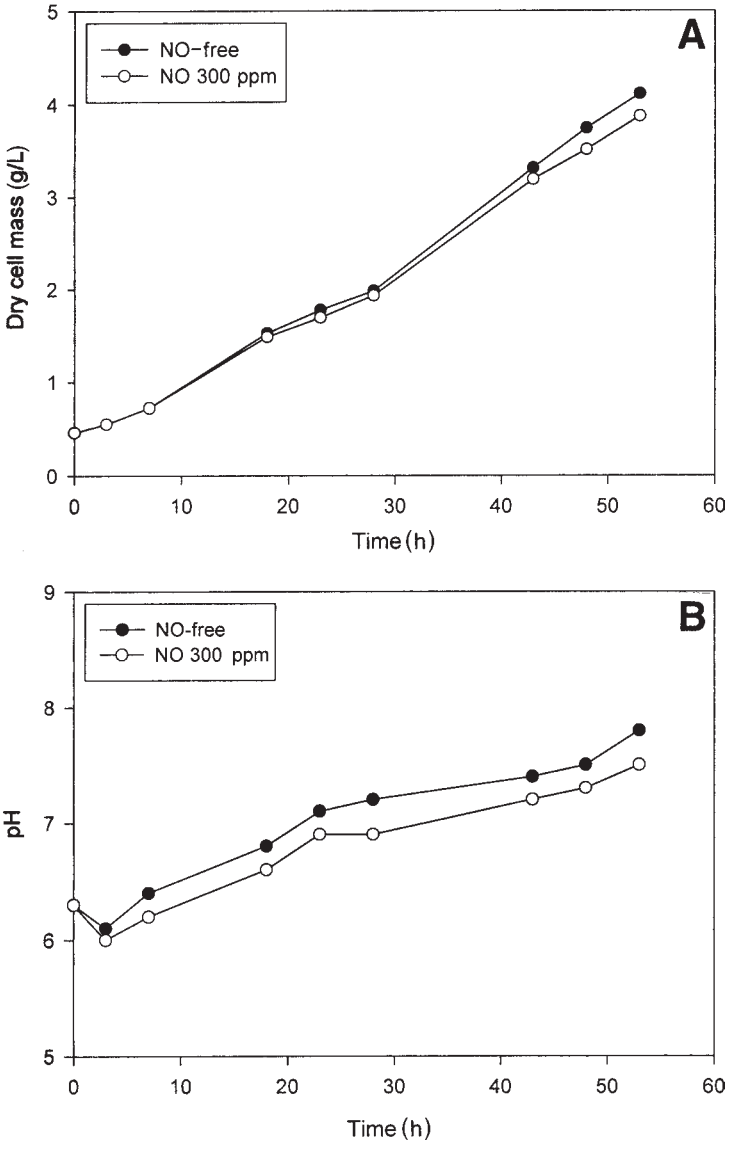

Fig. 7. Effect of NO on growth of *Chlorella* KR-1 **(A)** and pH change **(B)**. The cultures were cultured with inoculating cell mass of 0.5 g/L.

Controlling Gas Flow Rates

The effects of the flow rates of the model flue gases containing various SO_2 concentrations on the growth of *Chlorella* KR-1 have been investigated. Although the growth rates of microalgae were reported not to be affected by the gas flow rate when only CO_2-enriched gas was used *(13–15)*, the gas flow rate significantly affected the growth of *Chlorella* KR-1 when model gas was supplied, as shown in Fig. 8. *Chlorella* KR-1 exhibited stable growth even with the model gas containing 250 ppm of SO_2 when the aeration rate

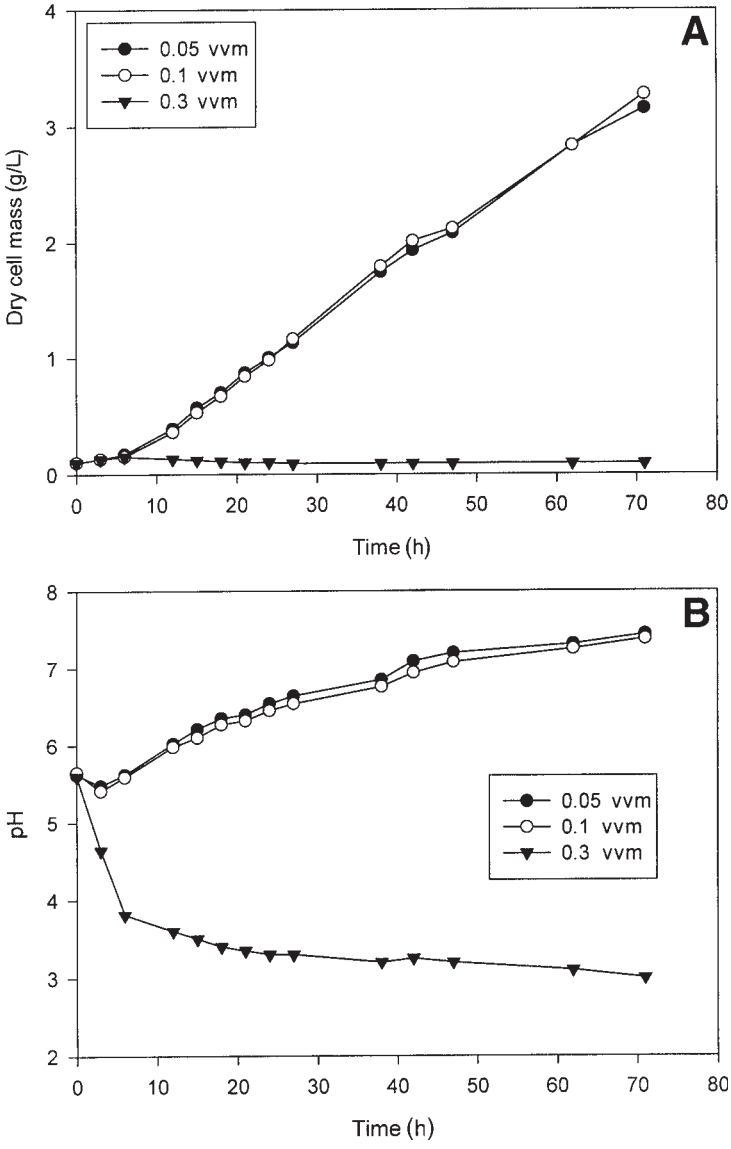

Fig. 8. Growth of *Chlorella* KR-1 **(A)** and pH change **(B)** under various gas flow rates. The cultures were illuminated at 350 μmol/(m^2 · s) and bubbled at 25°C with the gas containing 250 ppm of SO_2.

was decreased to 0.1 vvm or lower. Growth rate was 1.2 g/(L · d), which is about 80% of the control culture that was aerated at 0.5 vvm with the gas containing no toxic compounds, SO_2, and NO. Therefore, maintaining a low gas flow rate is another important means to prevent the growth inhibition of *Chlorella* KR-1 when the cells are cultured with the flue gas containing high SO_2.

To investigate the effects of gas flow rates and SO_2 concentrations in the model gas on pH changes, a series of experiments were carried out.

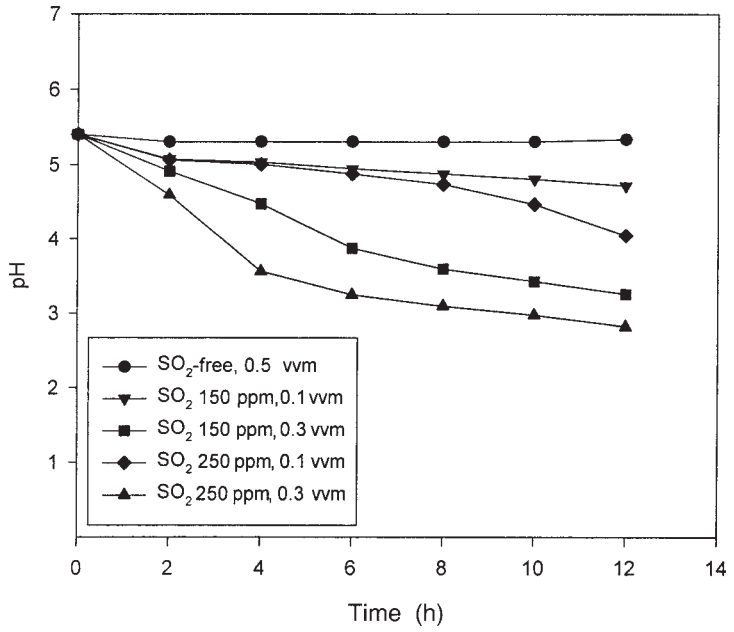

Fig. 9. Change in pH of the modified M4N medium as a function of simulated flue gas exposure. The media were aerated with 15% CO_2, 3% O_2, and various concentrations of SO_2, and balance N_2.

Figure 9 presents the pH changes that occurred in the media. The initial pH of all media was 5.4. With the supply of the model flue gas containing various SO_2 concentrations, the pH decreased. As shown in Fig. 9, the drop in pH was most noticeable when the media was aerated with the model flue gas of 250 ppm of SO_2 supplied at 0.3 vvm.

A major problem in the CO_2 fixation from flue gas using microalgae is the inhibitory effect by oxides of sulfur and nitrogen contained in the gas. It has been shown that controlling pH, increasing the inoculating cell concentration, and lowering the gas flow rate are effective methods to prevent growth inhibition. Our study proposes that direct CO_2 fixation from SO_2 containing flue gas emitted from typical industrial sources should be possible by culturing *Chlorella* KR-1 if operating conditions are properly controlled.

Acknowledgment

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References

1. Yanagi, M., Watanabe, Y., and Saiki, H. (1995), *Energy Convers. Mgmt.* **36,** 713–716.
2. Kishimoto, M., Okamura, T., Nagashima, H., Minowa, T., Yokoyama, S., and Yamaberi, K. (1994), *J. Fermentation Bioeng.* **78,** 479–482.

3. Negoro, M., Shioji, N., Ikuta, Y., Makita, T., and Uchiumi, M. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 681–692.
4. Hamasaki, A., Shioji, N., Ikuta, Y., Hukuda, Y., Makita, T., Hirayama, K., Matuzaki, H., Tukamoto, T., and Sasaki, S. (1994), *Appl. Biochem. Biotechnol.* **45/46,** 799–809.
5. Yoshihara, K., Nagase, H., Eguchi, K., Hirata, K., and Miyamoto, K. (1996), *J. Fermentation Bioeng.* **82,** 351–354.
6. Silva, H. J. and Pirt, S. J. (1984), *J. Gen. Microbiol.* **130,** 2833–2838.
7. Hanagata, N., Takeuchi, T., Fukuju, Y., Barnes, D. J., and Karube, I. (1992), *Phytochemistry* **31,** 3345–3348.
8. Kodama, M., Ikemoto, H., and Miyachi, S. (1993), *J. Marine Biotechnol.* **1,** 21–25.
9. Sung, K. D., Lee, J. S., Shin, C. S., and Park, S. C. (1998), *Kor. J. Ch. E.* **15,** 449, 450.
10. Hauck, J. T., Olson, G. J., Scierka, S. J., Perry, M. D., and Ataai, M. (1996), in *212th ACS National Meeting*, vol. 41, no. 4, 25–30 Aug., Orlando, FL, pp. 1391–1396.
11. Kurano, N., Ikemoto, H., Miyashita, H., Hasegawa, T., Hata, H., and Miyachi, S. (1995), *Energy Convers. Mgmt.* **36,** 689–692.
12. Sung, K. D., Lee, J. S., Shin, C. S., and Park, S. C. (1998), *J. Microb. Biotechnol.* **8,** 409–411.
13. Miyamoto, K., Wable, O., and Benemann, J. R. (1988), *Biotechnol. Lett.* **10,** 703–708.
14. Yamada, H., Ohkuni, N., Kajiwara, S., and Ohtaguchi, K. (1997), *Energy* **22,** 349–352.
15. Suzuki, T., Matsuo, T., Ohtaguchi, K., and Koide, K. (1995), *J. Chem. Tech. Biotechnol.* **62,** 351–358.