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Effects of NO and SO_2 on Growth of Highly-CO_2-Tolerant Microalgae

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Abstract The growth capabilities of three highly-CO_2-tolerant microalgae under high concentrations of CO_2 were compared for their tolerance to SO_2 and NO, the major inhibitory compounds present in flue gases. Although all strains showed good growth with 15% CO_2, *Chlorella* KR-1 exhibited the most desirable properties for CO_2 fixation among the strains compared. While *Chlorella* sp. HA-1 exhibited the best tolerance to NO among the algal strains tested, *Chlorella* sp. KR-1 showed higher tolerance to SO_2 than the other two strains tested.

Key words: SO_2-tolerant microalgae, CO_2 fixation, simulated flue gas, acidification

Biological methods, particularly the use of microalgal photosynthesis, have several merits such as mild conditions for CO_2 fixation and no requirements for further disposal of trapped CO_2. Carbon fixed by microalgae is incorporated into carbohydrates or lipids, therefore, energy, chemicals, or foods can be produced from the algal biomass [3, 10, 13]. Several studies on CO_2 removal using microalgae have been previously reported in the literature [1, 12].

The direct use of flue gas reduces the cost of pretreatment, yet imposes extreme conditions on the microalgae such as high concentrations of CO_2 (10–15%) and the presence of inhibitory compounds like NO_X and SO_X. The levels of CO_2 found in flue gas could be inhibitory to algal growth [7]. Some work has already been carried out to isolate highly CO_2 tolerant microalgae. The microalgae isolated for high CO_2 tolerance exhibit a high growth rate with a 15% CO_2 concentration [2, 4, 8, 12].

In addition to high concentrations of CO_2, inhibition by toxic compounds like SO_X and NO_X in flue gas is also critical. The combustion of fossil fuels generates nitrogen oxides (NO_X) and sulfur oxides (SO_X), the major constituents of which in flue gas are NO and SO_2. The acidification which results from solubilization of the toxic compounds in a culture medium has been reported to be a major cause for the inhibition of microalgal growth [4, 5, J.-H. Hauck *et al.* 1996. *Abstr. Annu. Meet. ACS.*, Orlando, USA, p.1391]. Several studies have been carried out to isolate a microalga which is tolerant to inhibitory compounds [5, 14, J.-H. Hauck *et al.* 1996. *Abstr. Annu. Meet. ACS.*, Orlando, USA, p.1391]. The biological elimination of nitric oxide (NO) and carbon dioxide from simulated flue gas has also been studied [14]. The growth of the marine microalga used in this work, strain NOA-113, was not inhibited in the flue gas containing up to 300 ppm NO when the cell concentration of the culture was about 1.5 g/l.

SO_X has been reported to be more toxic to the growth of algal cells than NO_X [5, 13]. Since SO_2 is easily dissolved in a culture medium, the pH of the medium is markedly reduced, thereby causing a strong inhibition of the algal growth. The screening work to identify a SO_X-tolerant microalgae has been focused on the isolation of an acidophilic microalga. Some microalgae collected from hot springs were reported to be more tolerant to SO_X [5, J.-H. Hauck *et al.* 1996. *Abstr. Annu. Meet. ACS.*, Orlando, USA, p.1391]. Kurano *et al.* [5] reported that only *Galdieria partita* could proliferate under 50 ppm of SO_2 aeration among three microalgal strains isolated from hot spring water samples, yet all three algae showed growth at 50 ppm of NO aeration. Hauck *et al.* also reported that *Cyanidium caldarium*, an acidophilic microalga isolated from hot spring water samples, exhibited some growth only during the first 20 h in a simulated flue gas containing 200 ppm of SO_2, however, the growth of *Chlorella vulgaris* was completely inhibited [J.-H. Hauck *et al.* 1996. *Abstr. Annu. Meet. ACS.*, Orlando, USA, p.1391].

Recently, several microalgal strains, which have been reported to include desirable properties for mass cultivation using flue gases, have been isolated [4, 8, 12]. However, the toxicity of the flue gas components on the growth of these microalgal strains has not been well-documented.

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The present study investigated the growth responses of the above-mentioned microalgal strains and compared their tolerances to NO and SO_2, the major toxic compounds contained in flue gas. Accordingly, an attempt was made to identify the most suitable microalga for direct CO_2 fixation from flue gas.

MATERIALS AND METHODS

Algal Strains and Culture Conditions

Chlorococcum littorale, a marine microalga, and *Chlorella* HA-1, a fresh water microalga, were selected due to their good growth in CO_2 enriched air, and were obtained respectively from the Marine Biotechnology Institute (Kamaishi, Japan) and National Institute of Environmental Studies (Tokyo, Japan), respectively. *Chlorella* KR-1, a fresh water algae isolated from water samples collected in a region nearby Youngwol, Korea, was also used in this work. The culture medium used for each alga is shown in Table 1. *Chlorococcum littorale* was cultured in medium B [4], *Chlorella* HA-1 in the M4N medium [12], and *Chlorella* KR-1 in a modified M4N medium [9]. The initial pH of the medium was adjusted depending on the experimental conditions.

Simulated Flue Gas

The 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 SO_X, and 100–300 ppm NO_X. Since the major portion (about 90%) of NO_X and SO_X is NO and SO_2, respectively, several gas mixtures (Praxair Korea Inc., Kiheung, Korea) containing various concentrations of NO or SO_2 were used as simulated flue gas for the experiments to evaluate the effects of the inhibitory compounds (SO_X and NO_X) on the growth of the algal strains.

Table 1. Chemical composition of various media (g/l).

	Medium B for *Chlorococcum littorale* [4]	M4N medium for *Chlorella* HA-1 [12]	Modified M4N medium for *Chlorella* KR-1 [9]
KNO_3	1.25	5	5
KH_2PO_4	1.25	1.25	1.25
$MgSO_4 \cdot 7H_2O$	1.25	2.5	2.5
NaCl	15	-	-
$FeSO_4 \cdot 7H_2O$	2.00×10^{-3}	3.00×10^{-3}	-
NaFeEDTA	-	-	0.014
H_3BO_3	2.86×10^{-3}	2.86×10^{-3}	2.86×10^{-3}
$MnSO_4 \cdot 7H_2O$	2.20×10^{-3}	2.50×10^{-3}	2.50×10^{-3}
$ZnSO_4 \cdot 7H_2O$	7.90×10^{-5}	2.22×10^{-4}	2.22×10^{-4}
$CuSO_4 \cdot 5H_2O$	-	7.90×10^{-5}	7.90×10^{-5}
Na_2MoO_4	2.10×10^{-5}	2.10×10^{-5}	2.10×10^{-5}
pH	5.7	6.0	5.6

CO_2 Fixation Experiments

The growth of the three strains of microalgae was conducted in a small bioreactor setup while aerating with various gases. Gas washing bottles (125 ml, Ace Glass Inc., Vineland, NY, U.S.A.) were used as the algal growth vessels. Three separate bottles containing 50 ml of the culture medium inoculated with the various microalgae were run in most experiments. The bottles were illuminated by white fluorescent tubes of 20 W and the light intensity at the surface of the reactor was 450 μmol/m^2-sec. The seed culture was centrifuged and washed before inoculation. Samples were removed daily from the vessels to determine the algal growth. The temperature of the culture media was maintained at 25°C. Air-grown cells were inoculated into the media to produce an initial cell concentration of 0.1 g/l. The various gases were bubbled through an inlet tube in each bottle, which was submerged to the bottom of the cultures. 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 Inc., Montrey, CA, U.S.A.) and monitored by an on-line CO_2 analyzer (IR-8400, Summit Analyzers Inc., Cleveland, OH, U.S.A.). Several gas mixtures containing various concentrations of SO_2 or NO were directly supplied to the culture vessel to evaluate the effects of the inhibitory compounds on the growth of the algal strains. The flow rate of the gas mixtures was measured with a flow meter. A flow rate of 0.5 vvm was used in the experiments. The culture pH was also monitored yet not controlled.

Assay

The algal growth was determined by measuring the optical density at 660 nm using a spectrophotometer (UV-1601, Shimadzu Inc., Kyoto, Japan). The spectrophotometric absorbance measurements of the cultures were related to the biomass concentration by drying the cell samples, which were collected on preweighed membrane filters, to a constant weight at 105°C. The light intensities were measured by a light sensor (LI-250, LI-COR Inc., Lincoln, NE, U.S.A.). The CO_2 fixation rate was estimated from the dry cell weight and carbon content in cells. The NO and SO_2 concentrations in the gas mixtures were measured by a NO_X analyzer (NONOXOR II, Bacharach Inc., Pittsburgh, PA, U.S.A.) and SO_X analyzer (DIOXOR II, Bacharach Inc., Pittsburgh, PA, U.S.A.).

RESULTS AND DISCUSSION

Comparison of the Algal Strain Growth Rates with High CO_2 Concentrations

To determine the influence of the elevated CO_2 levels found in flue gas, the growth of the algal strains was monitored while they were aerated with a gas mixture

composed of 15% CO_2 balance air. This gas composition was selected because it approximates the levels of CO_2 in a typical flue gas stream from a coal-fired power plant. None of the growths of the algal strains were suppressed in 15% CO_2 (Fig. 1). As shown in the figure, the *Chlorella* strains had about the same linear growth rate of 0.8 g/l-day. However, *C. littorale* exhibited a rather low growth rate (0.4 g/l-day). The growth patterns were also remarkably different. Whereas *C. littorale* had a long lag phase before growth, *Chlorella* HA-1 and KR-1 only exhibited a short lag phase. With regard to the capability of stable growth at high cell densities, *Chlorella* KR-1 showed a stable linear growth at a cell concentration of about 4 g/l. It has also been reported that *Chlorella* KR-1 can maintain a stable growth at a cell concentration up to 9.1 g/l [10]. In contrast, the growth rate of *Chlorella* HA-1 slowly decreased as the cell concentration was increased more than 3.3 g/l (data not shown). Therefore, among the strains investigated, *Chlorella* KR-1 exhibited the most desirable properties, *i.e.* a short lag phase and stable growth at high cell concentrations among the strains investigated.

Cultivation in Presence of NO

The ability of each alga to grow under aeration with NO-containing simulated flue gas mixtures was also examined. The growths of the algal strains, when aerated with a model flue gas (15% CO_2 and 100 or 300 ppm NO), were investigated. When the model flue gas containing 100 ppm NO was supplied at a cell concentration of 0.1 g/l, the resulting measurements of the cell concentrations and associated pH changes are shown in Fig. 2. For *C. littorale*, the lag period before starting growth tended to last longer with 100 ppm NO. The pH of the medium decreased during the initial stage and then rose slowly. However, the strain then exhibited stable growth after a long lag phase. Unlike *C. littorale*, the *Chlorella* strains showed a very stable growth, and the pH of the medium rose steadily

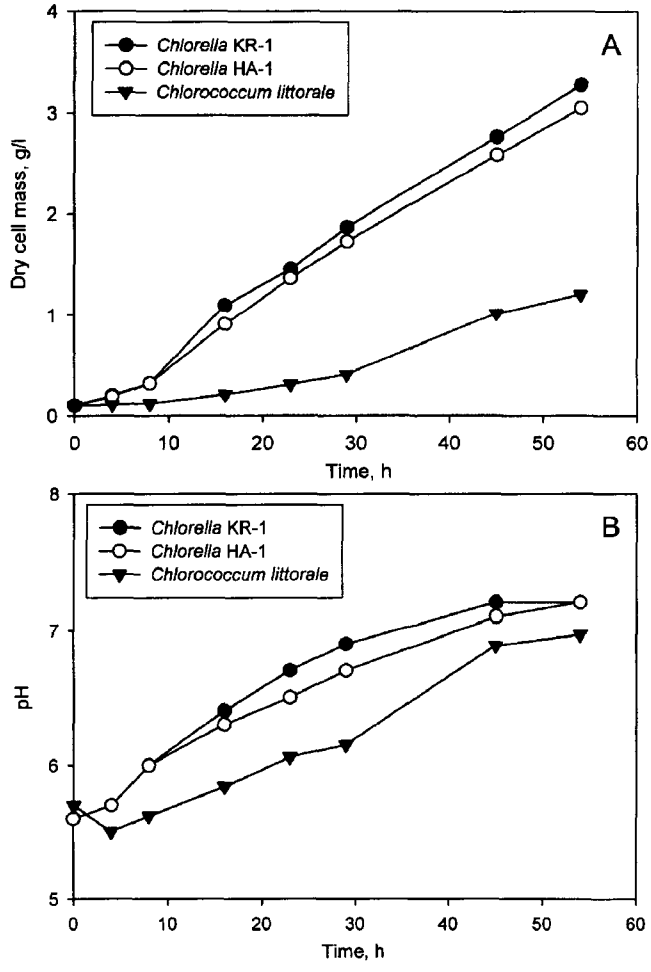

Fig. 1. Growth curves of highly-CO_2-tolerant microalgae (A) and pH change (b).
The cultures were illuminated at 450 μmol/m^2 · sec and bubbled at 25°C with 15% CO_2.

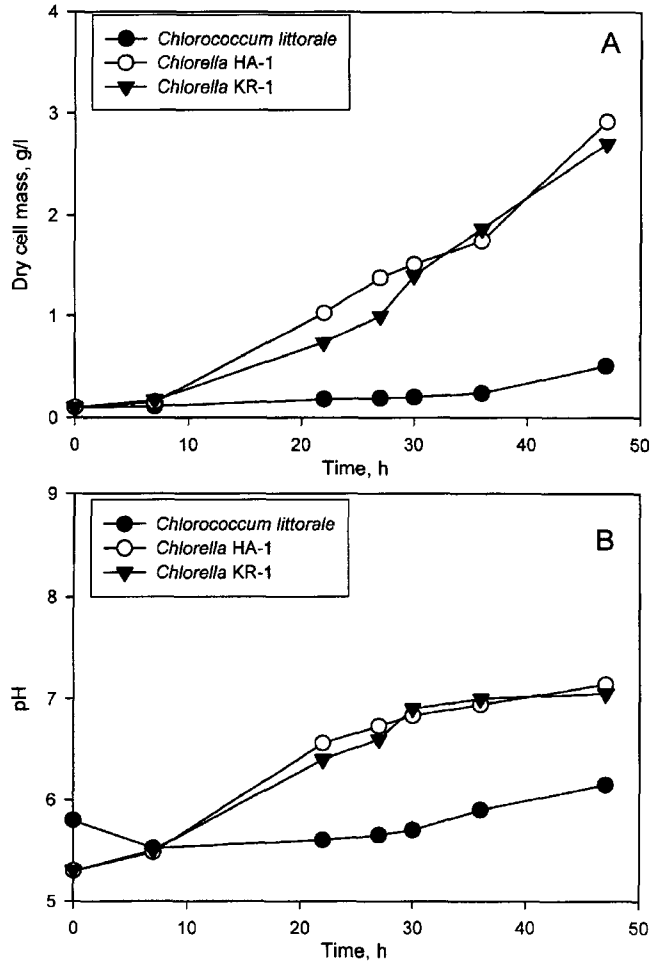

Fig. 2. Growth of algal strains (A) and pH change (B).
The cultures were exposed to 100 ppm NO, 15% CO_2 balance air, and illuminated at 450 μmol/m^2 · sec.

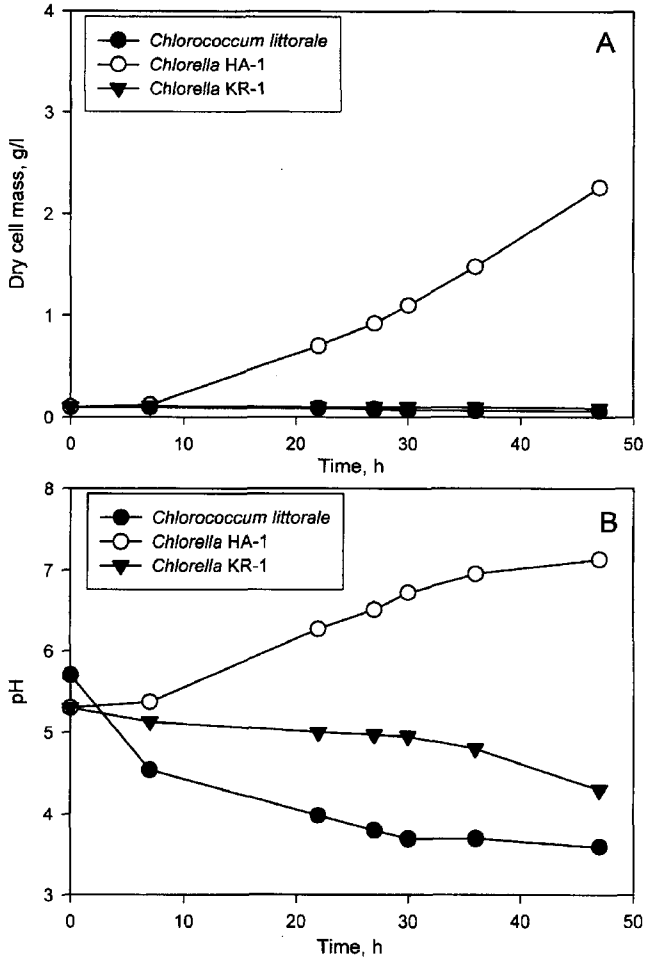

Fig. 3. Growth of algal strains (A) and pH change (B).
The cultures were exposed to 300 ppm NO, 15% CO_2 balance air and illuminated at 450 μmol/m^2 · sec.

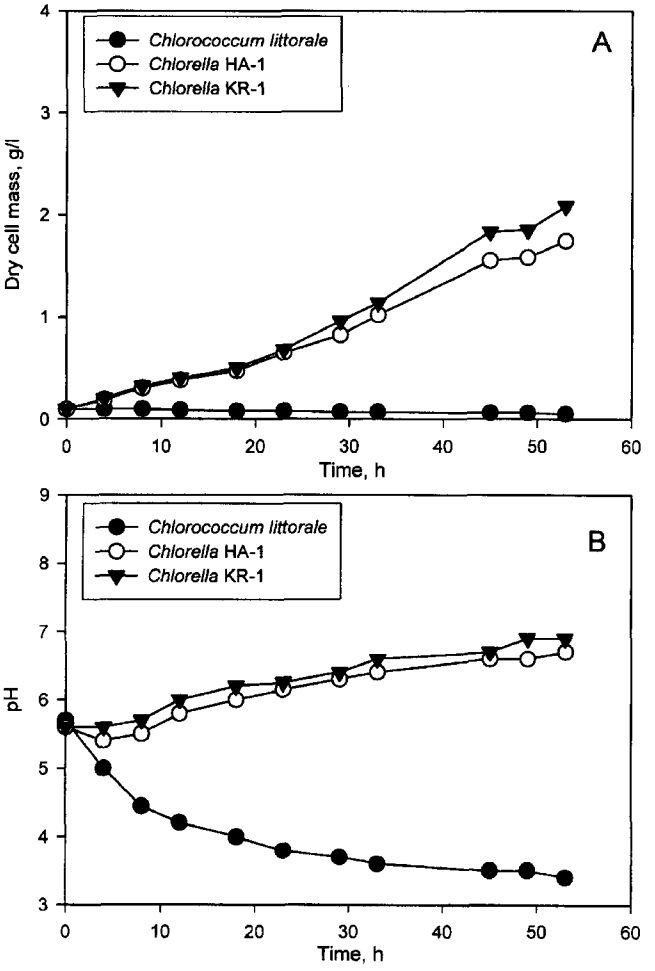

Fig. 4. Effect of 60 ppm SO_2 on growth of algal strains (A) and pH change (B).
The cultures were illuminated at 450 μmol/m^2 · sec.

from the beginning of the culture. Since flue gas from a boiler fueled by LNG does not contain SO_X and the concentration of NO_X in the flue gas is usually less than 100 ppm, these algal strains could be applied for direct CO_2 fixation from LNG flue gas.

With a higher concentration of NO, the algal strains showed different tolerances to NO. When the model gas containing 300 ppm NO was introduced to the cultures, the growths of *C. littorale* and *Chlorella* KR-1 were completely inhibited as indicated by the steady decrease in the cell concentrations (Fig. 3). The pHs of the media also dropped consistently. Under these culture conditions, *Chlorella* HA-1 showed a slightly retarded growth, but it still exhibited a good overall growth. The growth rate and maximum cell concentrations were approximately 80% of those obtained using the 15% CO_2 balance air as a NO-free control.

Effect of SO_2 on Growth of Algal Strains

The toxicity of SO_X on the growth of microalgae has been reported to be remarkably pronounced. Accordingly, the ability of each alga to grow under aeration with SO_2-containing simulated flue gas mixtures was also examined. As shown in Fig. 4, the growth of *C. littorale* was completely suppressed with the supply of the model gas containing 60 ppm SO_2. A significant decrease in pH from 5.6 to 3.2 was observed for *C. littorale*. *Chlorella* HA-1 and KR-1 showed a stable growth when the strains were cultured under aeration of the model gas containing 60 ppm SO_2 (Fig. 4). This finding is contrary to previous results which reported that *Chlorella* HA-1 was intolerant to 50 ppm of SO_2. [14]. Although the reason for the different result is not clear, it may be due to the different culture conditions (higher light intensity to obtain high growth rate) employed in this study. If the growth rate of the microalgae was maintained high enough, it was found that tolerance of the algae to SO_X and NO_X were enhanced (results not shown). The pHs of the media rose steadily from 5.6 to 6.4 for *Chlorella* HA-1 and to 6.7 for *Chlorella* KR-1.

To determine the tolerance of the *Chlorella* strains to higher concentrations of SO_2, the model gas containing 100 ppm SO_2 was introduced to the cultures. The growth of *Chlorella* HA-1 was completely inhibited. Since the pH of the medium was not controlled, it consistently dropped from 5.6 to 3.2 from the beginning. The color of the HA-1 strain changed from green to white within 10 h and then it died. In contrast, *Chlorella* KR-1 showed a remarkable tolerance to SO_x compared to the other algal strains employed in this work. Although *Chlorella* KR-1 showed a longer lag phase in the initial stage, it then exhibited a stable growth phase after the lag phase. It should also be noted that the growth rate with the simulated flue gas was about half that observed in the culture aerated with only CO_2 enriched gas (Fig. 5).

Considering the fact that none of the previously isolated algal strains of *Chlorella* sp. have been reported to have a tolerance to 50 ppm of SO_2, it is interesting to observe that *Chlorella* KR-1 would have such high tolerance to SO_2.

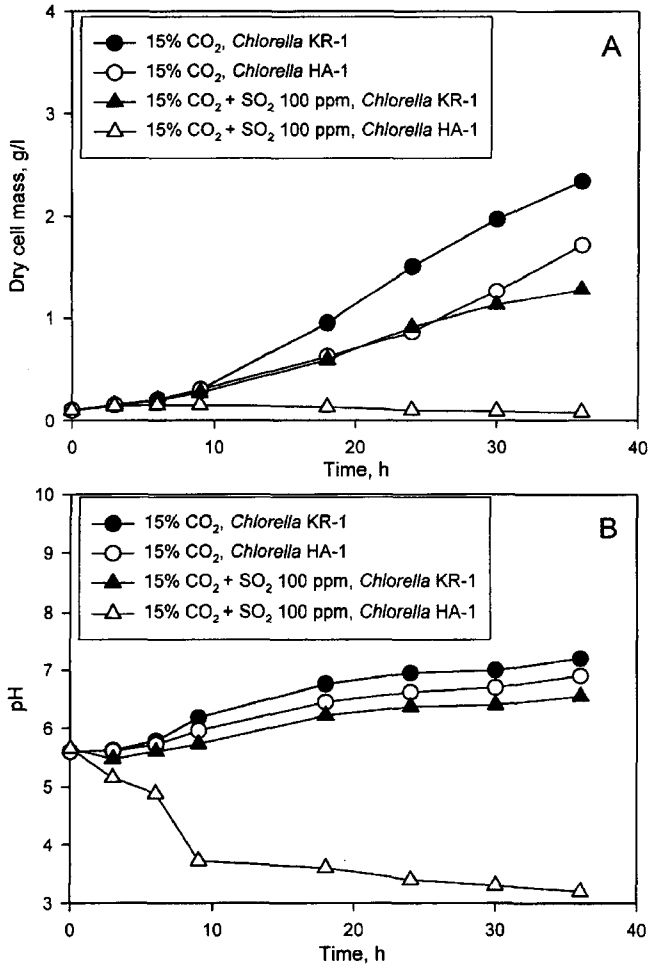

Fig. 5. Effect of 100 ppm SO_2 on growth of *Chlorella* HA-1 and KR-1 (A) and pH change (B).
The cultures were illuminated at 450 μmol/m² · sec.

The reason for the excellent tolerance of KR-1 to SO_2 is still not clear. However, there is a thermal power plant which uses anthracite, a coal containing high content of sulfur, and the plant emits flue gas containing high concentrations of SO_x. This thermal power plant has been in operation in Youngwol for over 10 years and has emitted large amounts of flue gas containing high concentrations of SO_x (about 800 ppm) over this period. Therefore, the Youngwol area, where the *Chlorella* KR-1 was isolated, is probably highly contaminated with SO_x. Since the flue gas emitted from some thermal power plants with advanced facilities for treating flue gas has a SO_x and NO_x content of less than 100 ppm, *Chlorella* KR-1 could be applied for direct CO_2 fixation from this flue gas.

The remarkable growth capabilities of *Chlorella* sp. KR-1 with gas containing SO_2 and NO indicate that it is an ideal strain to fix CO_2 directly from stack gases with low concentrations of these toxic compounds. Some further work has been carried out by us to enhance tolerances of microalgae to SO_x and NO_x. With newly developed methods, *Chlorella* strains have exhibited stable growth even with 250 ppm of SO_2. These results will be presented elsewhere. Further work will also be conducted using microalgae to directly fix CO_2 from typical flue gas containing high concentrations of SO_x and NO_x.

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