Carbon Dioxide Mitigation by Microalgal Photosynthesis

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Algal growth studies of *Chlorella* strains were conducted in a batch mode with bench type experiments. Carbon dioxide fixation rates of the following green microalgae were determined: *Chlorella* sp. H84, *Chlorella* sp. A2, *Chlorella sorokiniana* UTEX 1230, *Chlorella vulgaris*, and *Chlorella pyrenoidosa*. *C. vulgaris*, among other strains of microalgae, showed the highest growth rate (1.17 optical density/5 days). Cultivating conditions for *C. vulgaris* that produced the highest growth rate were at concentrations of 243 μg CO_2/mL, 10 mM ammonia, and 1 mM phosphate, with an initial pH range of 7-8.

Key Words : CO_2 fixation, *Chlorella vulgaris*, BioScrubber

Introduction

With global warming resulting from increased atmospheric carbon dioxide (CO_2) levels, this issue has become a major focus of the environmental agenda. The United States CO_2 production is on a rise from the already approximately 5.56 billion tons per year from coal-burning power plants.[1] This amount of CO_2 is equivalent to about 21% of the entire world's production. The flue gases' composition from power plants changes slightly with the specific fuel type and the amount of air used in combustion. Typical flue gas compositions with different fuel sources are approximately 10%.[2] CO_2 flue gas concentrations of up to 20% have been measured when coal is burned.[3]

Among many attempts to reduce the quantity of CO_2 in the atmosphere, biotechnology of using microalgae in a photobioreactor has extensively been studied since the beginning of the 1990's. With the biological approach, CO_2 is converted into algal biomass and then into value-added products such as proteins, vitamins, food, and feeds. However, processes for biological flue gas cleanups are still under development and there are problems such as low productivity of the biomass and inefficient CO_2 utilization to meet industry's challenge. Currently, there is no practical technology available in this direction.

The concept of using outdoor macroalgal cultures in ponds, lakes, oceans or land has been proposed as a method to reduce CO_2 emissions.[4] However, it is difficult to optimize algal growth in an open pond, resulting in low productivity of the process. For commercial operations, about 2% of the United States land would have to be converted to these ponds in order to capture enough CO_2 to make a difference in the CO_2 emissions in this country. Therefore, the applicability of this approach becomes questionable.

While outdoor systems mainly struggle with low productivity, closed photobioreactors can achieve higher productivity without causing any further environmental problems.[5,6] Closed photobioreactors can more readily control environmental conditions (temperature, oxygen, and nutrients) than open cultures. However, they cannot effectively control CO_2 from exhaust gases because CO_2 is usually bubbled through the reactor with the excess CO_2 being emitted to the atmosphere, and the CO_2 concentration and temperature vary with the combustion conditions of the power plant.

The ultimate goal of our research program is to develop an industrial BioScrubber as a closed photobioreactor system that can effectively and efficiently reduce green house gases from fossil-fueled power plants. In the BioScrubber system, we have a *Bioreactor* unit and a *Gas Scrubber* unit (not discussed in the present paper). In a Scrubber, CO_2 is absorbed into algal culture medium. In this approach there will be no excess CO_2 emitted to the environment. In a bioreactor unit, microalgal strains are cultivated in the CO_2 absorbed medium from the scrubber. We started to select microalgae that could be cultivated under high CO_2 concentration. In this paper, as the first step of series, preliminary assessment of algae for fixing CO_2 will be our focus. Detailed discussions on a gas scrubber system will be incorporated into the next paper of this series.

Materials and Method

Medium and cultivation conditions. *Chlorella sorokiniana* UTEX 1230 was obtained from the University of Texas, Austin. *Chlorella vulgaris* and *Chlorella pyrenoidosa* were purchased from Carolina Biological Supply Co. (Burlington, NC). *Chlorella* sp. H84 and *Chlorella sorokiniana* A2 were obtained from the Research Institute of Innovative Technology for the Earth (RITE, Tokyo, Japan).

ASM-1 medium[1] for *C. pyrenoidosa* and *C. sorokiniana* UTEX 1230 and N-8 medium[7] for *C. vulgaris* were used. ASM-1 medium was composed of (g/L) $Na_2HPO_4 \cdot 2H_2O$, 0.26; KH_2PO_4, 0.740; $CaCl_2$, 0.01; FeEDTA, 0.01; $MgSO_4 \cdot 7H_2O$,

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0.05; KNO_3, 1.0; and trace element stock solution, 1.0 mL/L. N-8 medium was composed of (g/L) $NaNO_3$, 0.17; $MgSO_4 \cdot 7H_2O$, 0.024; $MgCl_2$, 0.019; $CaCl_2$, 0.022; K_2HPO_4, 0.017; Na_2HPO_4, 0.014; and trace element stock solution, 1.0 mL/L. Cells were activated by inoculating a loop of a slant culture into 100 mL of the medium in a 250-mL Erlenmeyer flask and were precultured under continuous illumination at room temperature (20-25 °C) for a week. MC medium[4] was used for cultivating *Chlorella* sp. H84 and *C. sorokiniana* A2. MC medium was composed of (g/L) KNO_3, 1.25; KH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 1.25; Fe solution, 1 mL; A5 solution (composed of H_3BO_3, $MnSO_4 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$, H_2O), 1 mL. They were precultured and then mass cultured in a warm water bath at a temperature of 40 °C as recommended by Sakai *et al*.[3]

For an inorganic carbon source both CO_2 gas and sodium bicarbonate salt were employed; a prescribed ratio (using a mass flow controller) of CO_2 mixed in air was supplied to the cultures through an air diffuser (Aqua-Tech, Moorpark, CA). Also a known amount of bicarbonate salt was dissolved in the regular medium. Four cool-white fluorescent lamps (Econ-F40LW/RS/EW, 34 Watt, Philips, Underwriters Laboratories Inc.) were used as the light source at 3500 lux (maximum). Algae were cultured at room temperature, except for *Chlorella* sp. H84 and *C. sorokiniana* A2.

Analytical methods. The light intensity was measured by an analog photometer (Sper Scientific Ltd., Scottsdale, AZ). Cell counting was performed with a hemacytometer (Hausser Scientific, Horsham, PA). Optical density (OD) was measured by means of counting cell population of microalgae with a UV/Vis Spectrophotometer (model Lambda 3B, Perkin Elemer) at 680 nm based on a linear relationship between optical density and cell density. The pH levels of the cultivated solutions were measured by an Accumet 925 pH/ion Meter (Fisher Scientific) and adjusted with 1 M HCl and 1 M NaOH.

Results and Discussion

Assessment of microalgae. Among studied microalgae, *C. vulgaris* showed the highest cell growth during 5 days of batch cultivation (Figure 1). *Chlorella* sp. H84 and *Chlorella* sp. A2 were the next fast growing strains, and *C. pyrenoidosa* and *C. sorokiniana* UTEX 1230 showed the slowest cell growth.

When *C. vulgaris* was cultivated in our culturing media using the solutions obtained from our scrubbing operation, equivalent or higher growth rate to that given in the literature could be obtained (data not shown). This established the fundamental technical feasibility of our BioScrubber approach.

Although most organisms cannot survive under high temperatures, certain kinds of microalgae can thrive in hot springs. Their tolerance to high temperature could be beneficial to the biological CO_2 fixation, since the flue gas from power plants is about 40 °C after desulfurization and nitrogen oxide removal. In our study the growth of *Chlorella* sp. H84 and *C. sorokiniana* A2 at 40 °C was slower than at

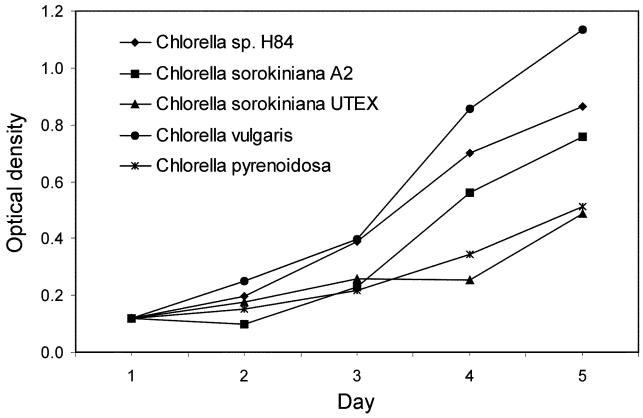

Figure 1. Different growth rates of microalgae.

25 °C, as approximately 250 ppm (or μg/mL) of carbon dioxide was supplied to all cultures. This result is not same to the result of Sakai et al.[3] Investigation of CO_2 fixation by microalgae who can survive at the temperature higher than 40 °C is under development in our laboratory with algal strains collected from hot springs.

Growth characteristics of *Chlorella vulgaris*. Further studies were performed to characterize the growth of *C. vulgaris*. The optimum starting cell density was investigated and the results are shown in Figure 2. With a cell density of 1.775×10^3 (cells/mL), *C. vulgaris* grew very slowly. At the range of 1.775×10^4 to 10^5 cells, *C. vulgaris* grew faster than with the lower initial cell density. Therefore, all cultivation of the alga was started at or above these cell densities.

Figure 3 shows the growth rate of *C. vulgaris* at different concentrations of CO_2. Without any addition of CO_2, *C. vulgaris* grows very slowly by only utilizing CO_2 (0.035% in air). Both 10% (v/v) and 20% (v/v) of CO_2 (mixed with air) showed similar results with medium growth rates. With 30% (v/v) of CO_2 in air, the growth rate was higher after four days than the growth rate obtained under the other conditions four days after the cultivation was initiated. Higher than 30% of CO_2 concentration was not tested because the expected composition of CO_2 from the flue gas is about 15%.

To simplify the cultivating procedure, known amounts of sodium bicarbonate salt was dissolved into the standard

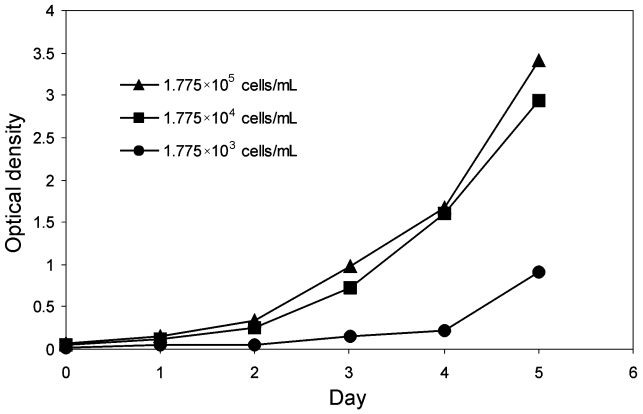

Figure 2. *Chlorella vulgaris* growth with different starting cell densities.

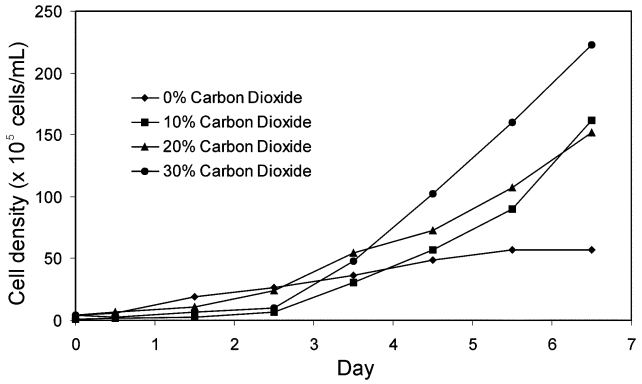

Figure 3. *Chlorella vulgaris* growth with different CO_2 concentration (vol %) in air.

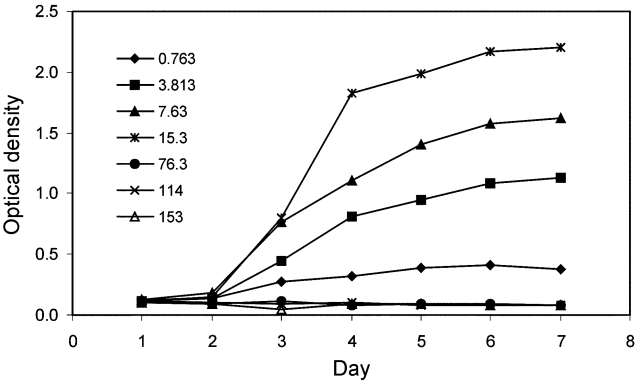

Figure 4. Effects of CO_2 concentrations on the growth of *Chlorella vulgaris*. The legends describe the CO_2 concentration as sodium bicarbonate ($NaHCO_3$), ppm.

medium as an inorganic carbon source. As can be seen in Figure 4, at a concentration of 15.3 ppm of bicarbonate (equivalent to 243 ppm as CO_2), the *C. vulgaris* strain exhibited its fastest growth rate (1.8 optical density/4 days).

At the optimum concentration of CO_2, concentrations of other species in the algal broth were calculated by a software program, called MINEQL$^{4.0}$ (calculation program based on thermodynamics). It was found that at the conditions of pH 5.7 (initial pH of *C. vulgaris* cultivation) and 243 ppm of CO_2, the algal broth contains 4.61×10^{-3} M of aqueous CO_2 (H_2CO_3) and 8.92×10^{-4} M of bicarbonate (HCO_3^-). This indicates that *C. vulgaris* utilizes both aqueous CO_2 (*ca*. 80%) and bicarbonate (*ca*. 20%) for its biological CO_2 fixation.

The effects of nitrogen (N) and phosphorus (P) nutrients on *C. vulgaris* were investigated. The cell growth was higher and faster with N-8 medium containing both N and P than a medium without N and P (Figure 5). The optimum concentrations of phosphate and ammonia were determined for *C. vulgaris* at the optimized CO_2 level. Similar *C. vulgaris* growth was resulted with both ammonium ion and nitrate ion for a nitrogen source (data not shown). It was, therefore, decided to use ammonium hydroxide (NH_4OH) as the nitrogen source for all *C. vulgaris* cultures, since NH_4OH is much less expensive than KNO_3, which is of common nitrogen source.

The highest cell growth of *C. vulgaris* was achieved with

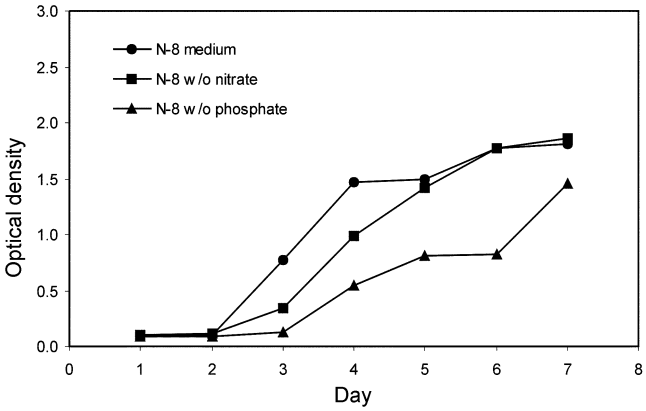

Figure 5. Effects of nitrate and phosphate ions on the growth of *Chlorella vulgaris* at an optimized CO_2 concentration.

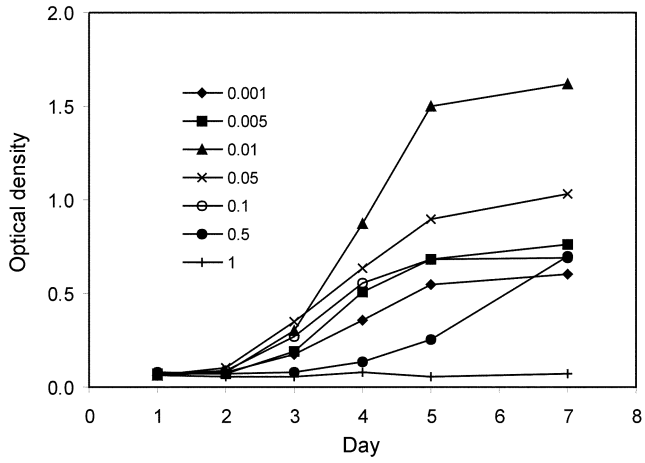

Figure 6. Optimizing ammonia concentration for a *Chlorella vulgaris* culture. The legend indicates the concentration of ammonia as Molarity (M).

10 mM of ammonia and 1 mM of phosphate (Figures 6 and 7). There was a great increase in optical density between day 4 and 5, while other cultures showed slower growth. A very high concentration of ammonia (1 M) and phosphate (100 mM) appeared to inhibit the *C. vulgaris* growth.

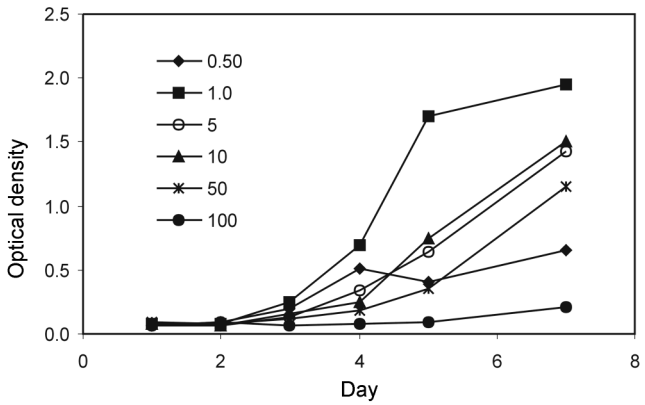

Figure 7. Optimizing phosphate concentration for a *Chlorella vulgaris* culture. The legend indicates the concentration of phosphate as mM.

Conclusions

Among five algal strains tested in the present study, *Chlorella vulgaris* showed the highest growth rate. Therefore, this alga was selected as our primary candidate for our BioScrubber system, whose detailed design and testing are under way in our laboratory. *C. vulgaris* produces a range of high-value substances, and the biomass itself can be used in aquaculture for feeding purposes and as an additive for animal feed that is rich in vitamins.[8,9] These preliminary studies prove a great deal of potential for a successful BioSrubber approach. Investigation of new candidates of different algal strains (including marine algae) for this study is now under way in our laboratory.

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