EFFECTS OF SIMULATED FLUE GAS ON GROWTH OF MICROALGAE

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INTRODUCTION

Studies have demonstrated that the atmospheric carbon dioxide level is increasing on a global scale due to the emissions from increased combustion of fossil fuels. Current CO₂ emissions due to combustion of fossil fuels are estimated to be 2 x 10¹⁵ tons/yr (1). These emissions are implicated as a major contributor to the 1-2 ppm annual increase in atmospheric CO₂ concentration; the present level of atmospheric CO₂ is 360 ppm. Efforts are now under way to develop possible methods to minimize CO₂ emissions.

One method proposed for minimizing the CO₂ emissions from power plants is to grow microalgae in flue gas streams (1-7), converting CO₂ to algal biomass which could then be converted to fuels, chemicals, and foods. Algae can utilize CO₂ efficiently, yielding three to five times more biomass per land area than typical crops and terrestrial plants. Flue gas contains not only CO₂ but also oxides of sulfur and nitrogen that may be toxic to algal growth either by lowering the pH of solutions or by direct inhibition. High levels of CO₂ (10-15%) found in flue gas could also be inhibitory to algal growth.

For this work two strains of microalgae were obtained and cultured to measure their ability to fix CO₂ under realistic flue gas conditions. The selection of Chlorella vulgaris was based on previous work with various Chlorella species of microalgae for removal of CO₂ from flue gas streams (1,3). Cyanidium caldarium was selected because of its ability to thrive in an acidic environment and at slightly elevated temperatures.

Several studies of CO₂ removal using microalgae have been reported in the literature (1-4), but the toxicity of the flue gas components has not been well-documented. This study identifies SO₂ as an inhibitory component of flue gas and highlights the inhibition of algal growth in the presence of SO₂ by using cultivation conditions which accelerate the rate of SO₂ absorption in cultures.

MATERIALS AND METHODS

Algal Strains and Culture Conditions

Chlorella vulgaris strain 30581 was obtained from the American Type Culture Collection, Rockville, Maryland. The C. caldarium culture was obtained from an acidic seep in the vicinity of Frying Pan Creek north of the Norris Geyser Basin in Yellowstone National Park, Wyoming.

The C. vulgaris strain was grown on liquid algal proteose medium (8), containing 1.0 g bacto peptone (Difco) and one drop of a 1.0% FeCl₃ solution in 940.0 ml deionized water. In addition, the medium contained (g/L): NaNO₃ (0.250); CaCl₂ 2H₂O (0.033); MgSO₄ 7H₂O (0.075); K₂HPO₄ (0.075); KH₂PO₄ (0.175); NaCl (0.025). Prior to inoculation this solution was autoclaved at 121°C for 30 minutes. The pH of the final medium was adjusted to approximately 7.5 with sterile 1N NaOH.

The C. caldarium strain was grown on Allen's medium (9), which contains (g/L): (NH₄)₂SO₄ (1.3); KH₂PO₄ (0.28); MgSO₄ 7H₂O (0.25); CaCl₂ 2H₂O (0.07); and FeCl₃ 6H₂O (0.02). The pH was adjusted to 1.8 with 10N H₂SO₄. To this sterile autoclaved solution was added 1.0 ml of a 0.2 um filter-sterilized trace metals solution. The trace metals solution contained (g/L): Na₂EDTA (1.5); FeCl₃ 6H₂O (0.194); MnCl₂ 4H₂O (0.082); ZnCl₂ (0.01); CoCl₂ (0.004); Na₂MoO₄ (0.008).

Stock cultures of C. vulgaris were grown in 250-ml Erlenmeyer flasks under constant fluorescent illumination and static incubation at 20-25°C. Stock cultures of C. caldarium were grown at 45°C in 250-ml metal-capped baffled shake flasks under constant fluorescent illumination with shaking at 180 rpm.

Growth of the algae was monitored by measurement of change in optical density at 750 nm with time (Perkin-Elmer Lambda 3B UV/Vis spectrophotometer). The spectrophotometric absorbance measurements of cultures were related to biomass concentration by drying cell samples, collected on preweighed membrane filters, to constant weight at 105°C. A linear
relationship between culture absorbance at 750 nm and dry weight concentration for both *C. vulgaris* and *C. caldarium* was observed. For example, an absorbance of 0.3 corresponds to dry weight concentrations of 78 mg/L and 58 mg/L for *C. vulgaris* and *C. caldarium*, respectively. Absorbance values greater than approximately 0.3 were diluted appropriately to allow for accurate spectrophotometric measurement. In addition, small samples of the cultures were withdrawn regularly and observed by phase contrast and fluorescence microscopy employing UV (365 nm) illumination to determine cell concentration and viability. Culture pH was also monitored but was not controlled.

**Experimental Reactor Setup**

Growth of the two strains of microalgae, when aerated with various gases, was conducted in a small bioreactor setup. These growth tests were conducted in a five-gallon aquarium maintained at either 25°C or 45°C, depending on the algae being tested. The 45°C temperature was maintained using a 300 W laboratory immersion heater. The lower temperature was maintained by the room temperature and fluorescent lighting. The water in the aquarium was circulated using a laboratory stirrer to maintain uniform temperature.

Glass bubbler tubes (40-ml, Ace Glass Company, Vineland, NJ) were used as the algal growth vessels. Five separate tubes containing 30 ml of culture solution inoculated with the various microalgae were run in most experiments. A five-port gang valve was used to split the incoming gas from the cylinder so that five bubbler tubes could be used concurrently to determine reproducibility of algal growth in each experiment. The bubbler tubes were suspended in the aquarium and illuminated continuously with fluorescent lights mounted outside two sides of the aquarium. The light intensity was measured with a digital light meter (Model D-2000, Sylvania Light Company, West Seneca, NY) or a digital flowmeter (Humonics Optiflow 520, Fairfield, CA). A flow rate of approximately 15 ml/min through each bubbler tube was used in all experiments. Several gas mixtures was also examined (Figure 1). This gas composition was selected because it approximates the levels of CO₂ and O₂ found in ambient air in industrial settings.

To determine the influence of elevated CO₂ levels found in flue gas, growth of the algal strains were monitored when aerated with a gas mixture composed of 15% CO₂, 3% O₂, balance N₂ (Figure 1). This gas composition was selected because it approximates the levels of CO₂ and O₂ in a typical flue gas stream from a coal-fired power plant. Both *C. vulgaris* and *C. caldarium* grown in 15% CO₂ exhibited a significant increase in biomass as compared to that with air aeration. The growth rate and cell yields were approximately equivalent to that observed using the 5% CO₂ gas mixture. The ability for growth of this strain of *C. caldarium* using elevated CO₂ levels supports previous findings by Brock indicating enhanced growth in high CO₂ environments (10).

In contrast to *C. vulgaris*, *C. caldarium* initially exhibited some growth in the simulated flue gas containing SO₂ (Figure 2). It should be noted that this growth was approximately ca. 130 times less than that observed in an enriched CO₂ atmosphere. The different patterns of inhibition for the two algae depends to some extent on the values of optimum pH for their growth relative to the final culture pH.

The change in pH of five replicate bubbler tubes as a function of gas exposure time in Figure 1. It should be noted that this growth was approximately ca. 130 times less than that observed in an enriched CO₂ atmosphere. The different patterns of inhibition for the two algae depends to some extent on the values of optimum pH for their growth relative to the final culture pH.

A significant decrease in pH from 7.3 to 2.3 was observed for *C. vulgaris*. The
effect of the simulated flue gas on the pH was less pronounced for \textit{C. caldarium} cultures. Upon introduction of the simulated flue gas, the pH was lowered to ca. 1.3 which is within the optimal range for growth of \textit{C. caldarium}. Thus, the lowering of the pH by the flue gas does not inhibit growth of this alga but rather can provide the acidic environment needed for its growth. The growth inhibition of \textit{C. caldarium} may have resulted from exposure to the SO$_2$ or some hydrolysis product.

The drop in pH, caused by the solubility of SO$_2$ in aqueous solutions, was particularly evident in the initially neutral pH cultures of \textit{C. vulgaris}. Thus, inhibition of \textit{C. vulgaris} appears to have resulted from the low pH created relative to the optimal value of about pH 7, since the growth medium was not effectively buffered to prevent acidification. The SO$_2$ in the simulated flue gas almost certainly had a negative effect on the growth of this alga just as was shown for \textit{C. caldarium}. However, this was not proven in this study and further experiments using buffered media are needed to determine if the SO$_2$ itself is toxic to \textit{C. vulgaris}.

**Determination of Possible Growth Inhibitors**

FTIR spectrophotometric analysis of the simulated flue gas indicated that no measurable amounts of contaminants, in particular the presence of SO$_2$, or CO. This indicates that SO$_2$ or an aqueous reaction product of SO$_2$ is the toxic agent responsible for inhibition. This is particularly evident with \textit{C. caldarium}; growth occurs initially, but is inhibited after 20 hours. This pattern would be consistent with the accumulation of dissolved SO$_2$ or an aqueous oxidation product which occurs due to the high solubility of SO$_2$ in water (11).

To test this hypothesis one of the replicate bubbler tubes inoculated with \textit{C. caldarium} was equipped with a "pre-bubbler" tube filled with deionized water. This pre-bubbler served as a scrubber for the flue gas prior to the gas being bubbled through the experimental culture tube. Initially, good growth occurred, similar to that when aerated with the CO$_2$ mixture lacking SO$_2$, but inhibition prevailed after about two days (Figure 4). Apparently, the SO$_2$ is accumulated initially in the pre-bubbler solution, decreasing the concentration of SO$_2$ entering the experimental growth tube. The pre-bubbler tube delayed inhibition of the culture from 20 h to approximately 50 h but could not prevent it.

Figure 4 may also explain results of short-term studies in which operating conditions, such as flow rate (v/v, min), may delay the inhibitory effect of SO$_2$ containing flue gas (5). Since our experiments were designed for rapid saturation of SO$_2$ by using high flow rates, the inhibitory effect is clearly noticeable even with short exposure times.

Growth of \textit{C. caldarium} on a NO$_2$-only flue gas consisting of approximately 50 ppm NO$_2$, 15% CO$_2$, 3% O$_2$, in balance N$_2$ was also tested. The growth of \textit{C. caldarium} did not appear to be significantly inhibited by the NO$_2$ flue gas which was tested only on this alga (Figure 5). This is an important finding since NO$_2$ is a potentially harmful agent to growth of biological systems. Thus, \textit{C. caldarium} may have considerable advantages for its use as an agent for CO$_2$ removal from flue gas. The apparent tolerance of this alga to nitrogen oxides and low pH environments make it a logical candidate for further studies of growth in flue gas streams.

**CONCLUSIONS**

A major problem in the large-scale farming of unicellular algae is the control of competing organisms. \textit{C. caldarium} has the advantage, although not unique, of having the ability to grow in highly acidic media and at elevated temperature, where competitors are not viable. It has been shown that this alga is capable of growth in an environment with temperatures up to 57°C and as acidic as 1N H$_2$SO$_4$ (10). Because of this tolerance to acidity and elevated temperatures that may accompany flue gas streams, further investigation of \textit{C. caldarium} for the removal of CO$_2$ from flue gas appears to be warranted. These studies should include assessing the possibility of adaptation of cultures to SO$_2$-containing flue gas to obtain further insight to the nature of inhibition for development of SO$_2$-resistant strains.

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**DISCLAIMER**

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REFERENCES


Figure 1. Growth of the strain, *C. caldarium* and *C. vulgaris*, exposed to 5% CO₂/air (*O*.*, *•*) and 15% CO₂, 3% O₂ balance N₂ (*○*, *□*).

Figure 2. Growth of *C. caldarium* and *C. vulgaris* exposed to 200 ppm SO₂, 15% CO₂, 3% O₂ balance N₂.

Figure 3. Change in *C. caldarium* and *C. vulgaris* culture pH as a function of simulated flue gas exposure. Cultures were vented with 15% CO₂, 3% O₂ balance N₂ (*□*, *○*) and 200 ppm SO₂, 15% CO₂, 3% O₂ balance N₂ (*○*, *□*).
Figure 4. The effect of an SO$_2$ scrubber (pre-bubbler) on the growth of *C. caldarium* exposed to 200 ppm SO$_2$, 15% CO$_2$, 3% O$_2$, balance N$_2$.

Figure 5. Growth of *C. caldarium* exposed to NO$_x$-only flue gas.