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Chemical Interaction of Flue Gas Components with the Growth of *Cyanidium caldarium*

Scientific Note

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INTRODUCTION

Flue gas released into the atmosphere from fossil fuel combustion contains carbon dioxide, a major contributor to possible global warming and the greenhouse effect. Photosynthetic processes result in the reduction of CO_2 to carbohydrate and the evolution of molecular oxygen from water. A photosynthetic organism growing in a process off-gas stream could effectively recycle CO_2 to nonpolluting products, especially if it could survive the high acid, sulfur dioxide, and low O_2 concentrations that are also present in flue gas. *Cyanidium caldarium*, a microalga isolated from acid sulfur hot springs in Yellowstone National Park, has been shown to be tolerant of such extreme conditions (*1*). It is thermophilic and acidophilic, and grows in 1*M* sulfuric acid at 70°C, as well as anaerobically in high concentrations of CO_2 (*2*). It is therefore a suitable candidate for the study of stress-tolerant algal CO_2 reduction in stack gas atmospheres. The purpose of the present work was to determine the conditions under which *Cyanidium* would grow and to measure the rate of its photosynthetic production of oxygen in a flue gas atmosphere.

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Table 1
The Composition
of Low Sulfur Flue Gas (3)

Component	% Volume
O_2	5.57
CO_2	11.7
SO_2	435 ppm
SO_3	5 ppm
HCl	56 ppm
H_2O	11.8
N_2	70.9

MATERIALS AND METHODS

Growth Experiments

Stock cultures of *Cyanidium caladarium* (strain III_{D2}) purchased from Carolina Biological Co., Burlington, NC were grown aerobically and aseptically in Allen's salts medium (*1*) (pH 3.5) shaking (100 rpm) in a water bath at 35°C. The growing algae were illuminated by fluorescent lights. During late exponential growth phase, aliquots of the algal suspension were transferred to sterile Erlenmeyer flasks containing fresh medium for growth in the flue gas atmosphere. Because these growth vessels were connected to air-permeable tubing, the atmosphere to which the algae were exposed was not 100% flue gas. The initial concentration of the algal suspension was approx 1×10^6 cells/mL. The medium was bubbled with synthetic flue gas (*see* Table 1 for flue gas composition) (*3*) purchased from Matheson, Morrow, GA. The algae were illuminated with fluorescent lights with 14-h on and 10-h off cycles. Cell growth was monitored by counting cells at regular intervals for 33 d using a phase-contrast microscope with hemocytometer.

In another set of experiments, *Cyanidium* growth rates were compared as a function of medium concentration. A stock solution of algae was transferred to sterile Erlenmeyer flasks containing medium at 1×, 2×, and 4× the concentration of the stock medium (*vide supra*). The initial concentration of cells in each suspension was approx 1×10^6 cells/mL. The media were bubbled with synthetic flue gas under the same conditions as described above, and the experiment was allowed to proceed for more than 30 d.

Measurement of Oxygen Production by *Cyanidium*

Determination of photosynthetic oxygen evolution was performed in a flow system using a laboratory-constructed Hersch electrogalvanic cell

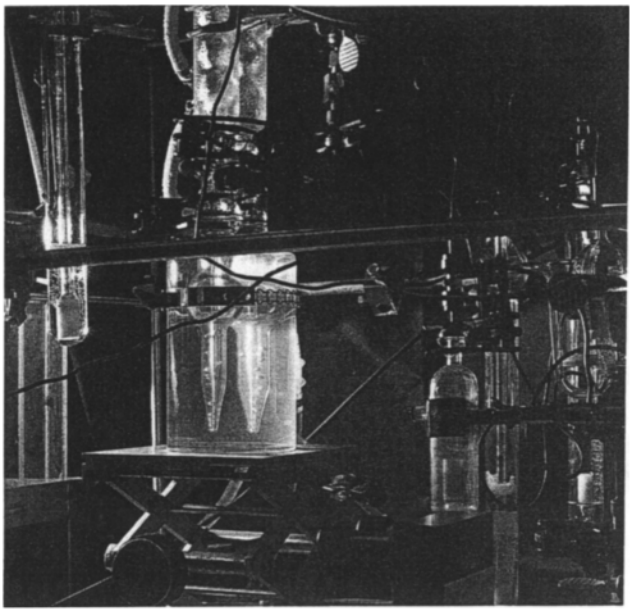

Fig. 1. Photograph of the water-jacketed algal suspension chamber containing *Cyanidium cadarium.*

(*4*). This two-electrode device is comprised of a silver screen (cathode) and a thin lead sheet (anode). The silver and lead electrodes are separated by a filter paper membrane that is impregnated with 5*N* acetic acid. This sandwich-like assembly is wrapped around a glass cylinder and placed in a hermetically sealed chamber, which is incorporated downstream from the algal chamber as an integral component of the flow system. Two thin platinum wires, which pierce the main *o*-ring comprising the hermetic seal, are used to make electrical contact with the lead and silver electrodes (*5*).

In this galvanic cell, lead is oxidized to lead oxide in the presence of oxygen, with the silver screen acting as a catalyst. Electrons generated in this redox reaction are forced to flow in an external circuit. The electric current produced by this chemical reaction is measured with a digital multimeter. The response of the sensor is linear with respect to gas phase oxygen concentration. This latter fact was proved by inserting an electrolysis cell in tandem with the Hersch cell, and using Faraday's Law of Electrochemical Equivalence to electrolyze water to oxygen and hydrogen.

Experiments and apparatus for the physiological studies were designed to measure the production of O_2 in a closed system devoid of atmospheric O_2. A stock solution of *Cyanidium* (8 mL) was introduced into the jacketed cell chamber (*see* Fig. 1) at 35°C, through which was bubbled CO_2/He mixtures (1.0%–1.05% CO_2) at 50 mL/min. The cell chamber was illuminated by a lamp on 2-h on/off cycles with an irradiance of 2.7 W/m^2. The gas streams were humidified, and condensers cooled to 5°C were installed

above the cell chamber to prevent evaporative losses from the algal suspension. Baseline values for rates of photosynthetic oxygen production were determined in CO_2/He. Oxygen-free flue gas, containing 11.7% CO_2 and 434.8 ppm SO_2 in N_2, was then introduced into the gas stream through a rotameter at 10, 20, or 40% of the total gas flow. Total gas flow was maintained at 50 mL/min. A sodium hydroxide sparger downstream from the cell chamber neutralized the flue gas and prevented damage to the galvanic cell.

It was observed during the growth experiments that the pH of the medium decreased as the length of exposure to flue gas increased. To minimize this additional stress imposed on *Cyanidium*, the medium was made up in 0.1*M* potassium tartrate buffer (pH 3.5) and 0.1*M* HCl/KCl buffer (pH 2.2), and the observed effects of flue gas on *Cyanidium* were compared relative to the unbuffered medium. O_2 production is expressed per milligram chlorophyll per minute in the 8-mL preparation. Chlorophyll concentration was determined spectrophotometrically at 665 nm after sonication and extraction in methanol.

RESULTS AND DISCUSSION

Growth Experiments

The *Cyanidium* grew in a flue gas/air mixture from a concentration of approx 1×10^6 cells/mL to 1.77×10^8 cells/mL over a 33-d period, with the onset of stationary growth after 20 d (Fig. 2). In another growth experiment, the medium concentration (1×, 2×, and 4×) was found to have little effect on the rate of growth of *Cyanidium* (Fig. 3) in flue gas within the 30- to 40-d time period studied. However, the relatively long lag phase before the onset of exponential growth in the 1× solution (~ 8 d) suggests that the stress of the flue gas components was more acute in the 1× than in the 2× and 4× samples, suggesting that the 2× and 4× medium concentrations may have buffered some of the effects of the flue gas.

Oxygen Production Experiments

The results of the photosynthetic O_2 production experiments are shown in Figs. 4, 5, and 6. O_2 was detected during the 2-h light-on cycle in all buffered and nonbufferred media that were bubbled with the CO_2/He mixture. These results confirm that *Cyanidium* is able to thrive under anaerobic, high CO_2 concentrations. When 10% flue gas was introduced into the gas stream, the cells in tartrate buffered (in two out of three cases) and nonbuffered solutions continued to produce O_2 at the same or at a slightly elevated rate. O_2 production in HCl/KCl buffer solutions, however, steadily decreased in 10% flue gas and ceased altogether when

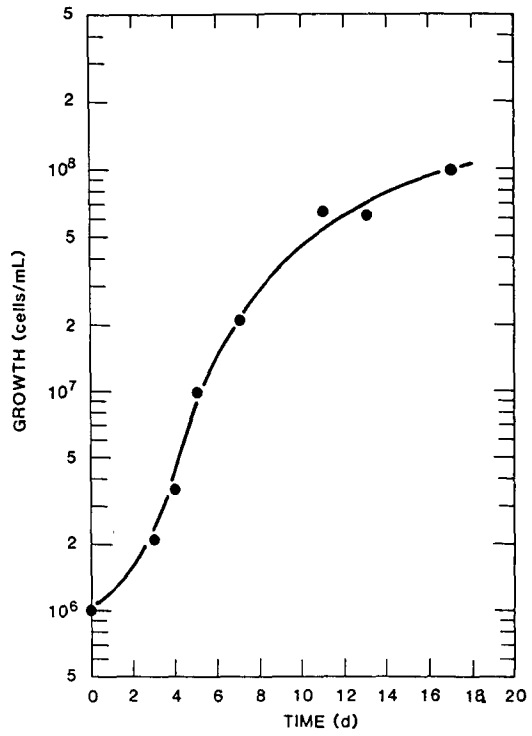

Fig. 2. Growth of *Cyanidium caldarium* III_{D2} on synthetic flue gas. Since the tubing connecting the growth flasks to the flue gas cylinders was air-permeable, the atmosphere to which the algae were exposed was not 100% flue gas.

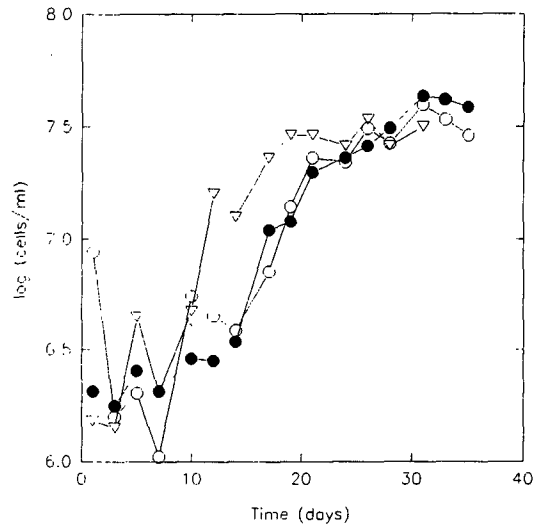

Fig. 3. *Cyanidium* growth curves in 1× (○), 2× (●), and 4× (▽) medium in synthetic flue gas.

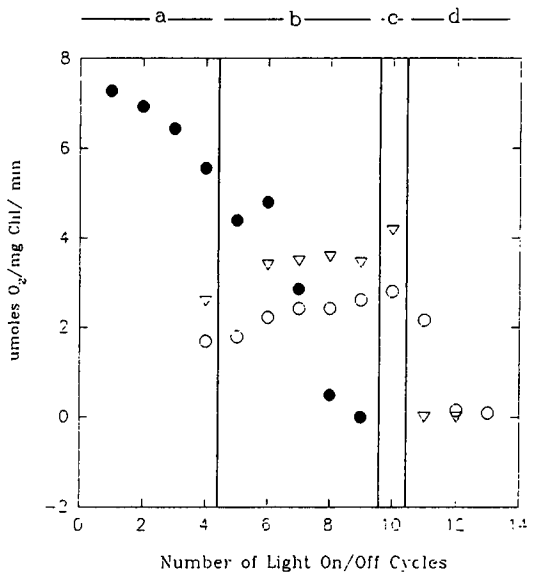

Fig. 4. Rates of photosynthetic oxygen evolution from *Cyanidium caldarium* in synthetic flue gas blends for culture media containing 0.1*M* tartrate buffer. a: CO_2/He only; b: 10% flue gas blend; c: 20% flue gas blend; d: 40% flue gas blend; ○ 1.0% CO_2/He; ● 1.0% CO_2/He; ∇ 1.05% CO_2/He.

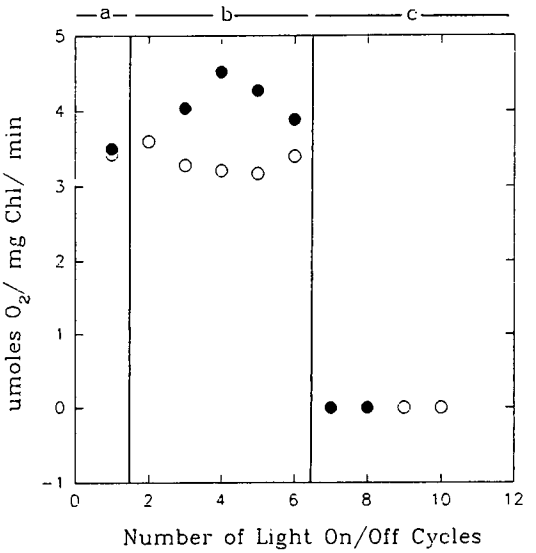

Fig. 5. Rates of photosynthetic oxygen evolution from *Cyanidium caldarium* in synthetic flue gas blends for unbuffered culture media. a: 1.05% CO_2/He only; b: 10% flue gas blend in 1.05% CO_2/He; c: 20% flue gas blend in 1.05% CO_2/He; ○ experiment #1; ● experiment #2.

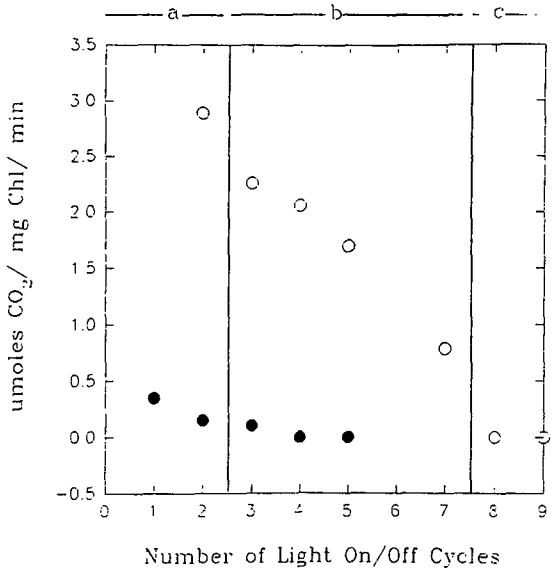

Fig. 6. Rates of photosynthetic oxygen evolution from *Cyanidium caldarium* in synthetic flue gas blends for culture media containing HCl/KCl buffer. a: 1.05% CO_2/He; b: 10% flue gas blend in 1.05% CO_2/He; c: 20% flue gas blend in 1.05% CO_2/He; ○ experiment #1; ● experiment #2.

the concentration reached 20%. Tartrate buffered cells maintained a steady evolution of O_2 for 4 h in 20% flue gas and abruptly stopped when the concentration was increased to 40%. Further incubation of cells in tartrate buffered solutions in 40% flue gas for up to 7 d did not show a return of measurable O_2 in the gas stream (data not shown). These data may be explained by an interaction between tartrate and SO_2, which neutralized the toxic effects of flue gas until a critical concentration and time of exposure were reached.

CONCLUSIONS

Normal photosynthesis by *Cyanidium* results in O_2 evolution, suggesting that the uptake and subsequent reduction of CO_2 also occur. This process, therefore, has potential for the clean-up of pollution from power plant stack exhaust. *Cyanidium* is hardier than other photosynthetic organisms, and our studies suggest that, when grown in a stress-reducing buffer, this alga can continue the photosynthetic removal of CO_2 in an atmosphere containing up to 40% flue gas. Further studies are currently under way to optimize the conditions under which *Cyanidium* will grow in high concentrations of flue gas as well as to measure the rates of CO_2 uptake.

ACKNOWLEDGMENTS

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