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Microalgal Removal of CO_2 from Flue Gases: Changes in Medium pH and Flue Gas Composition Do Not Appear to Affect the Photochemical Yield of Microalgal Cultures

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Abstract Our research objectives are to determine under what conditions microalgal-based CO_2 capture from flue gases is economically attractive. Specifically, our objective here was to select microalgae that are temperature, pH and f ue gas tolerant. Microalgae were grown under five different temperatures, three different pH and five different flue gas mixtures besides 100% CO_2 (gas concentrations that the cells were exposed to ranged 5.7-100% CO_2, 0-3504 ppm SO_2, 0-328 ppm NO, and 0-126 ppm NO_2). Our results indicate that the microalgal strains tested exhibit a substantial ability to withstand a wide range of temperature (54 strains tested), pH (20 strains tested) and flue gas composition (24 strains tested) likely to be encountered in cultures used for carbon sequestration from smoke stack gases. Our results indicate that microalgal photosynthesis is a limited but viable strategy for CO_2 capture from flue gases produced by stationary combustion sources.

Keywords: carbon capture, CO_2, flue gas, microalgae, NO_x, photosynthesis, SO_x

INTRODUCTION

Emissions of anthropogenic CO_2 have been predicted to increase for the foreseeable future leading to increased concentrations of carbon dioxide in the atmosphere. Most of the anthropogenic CO_2 emissions result from the combustion of fossil fuels for energy production. Electric utility plants represent 38% of the carbon dioxide emissions in the United States [1]. Projected increases in energy needs, particularly in the developing world, underlie the projected increase in CO_2 emissions. While there is much debate on the effects of increased CO_2 levels on global climate, it is generally agreed that the projected increases could have a profound effect on the environment. Meeting the demand for energy production without huge increases in CO_2 emissions will require more than conservation and increased efficiency of energy production. Carbon sequestration, *i.e.*, the capture and storage of carbon, could be a useful tool for reducing CO_2 emissions from fossil fuel usage.

Technologies (chemical and physical) exist today that can be used to capture CO_2 from smoke stacks. But the cost of removing CO_2 from flue gas ranges from several tens to hundreds of dollars per ton of CO_2 [2]. Adding this cost to the cost of producing electricity would result in a significant increase in the cost of power. The United States Department of Energy's goal is to reduce the cost of carbon sequestration to below $10/ton of avoided net cost.

Photosynthesis has long been recognized as a means, at least in theory, to capture anthropogenic CO_2. Aquatic microalgae have been identified as fast growing organisms whose carbon fixing rates are higher than those of land-based plants by one order of magnitude. Microalgae utilize CO_2 as one of their main building blocks and we propose that algal photosynthesis may be a viable option for anthropogenic CO_2 capture and sequestration (*e.g.*, from smokestack emissions). Microalgal sequestration of CO_2 consists of growing microalgae photoautotrophically utilizing anthropogenic CO_2 as the source of carbon for biomass production. While microalgal culturing is expensive, microalgae can also produce a variety of compounds that can be harvested, purified, and sold at a high value [3]. The revenue from the sale of those high value products could pay for the cost of carbon capture and sequestration.

Our vision of a viable strategy for carbon sequestration based on photosynthetic microalgae is shown conceptually in Fig. 1. In this figure, CO_2 from the fossil fuel combustion system and nutrients are added to a photobioreactor where microalgae utilize sunlight to photosynthetically convert the CO_2 into compounds of high commercial values or mineralized carbon for sequestration. The advantages of using a microalgal-based system are that

- High purity CO_2 gas is not required for algal culture. Flue gas containing varying amounts of CO_2 can be fed directly to the microalgal culture. This would simplify CO_2 separation from flue gas significantly.
- Some combustion products such as NO_x or SO_x can

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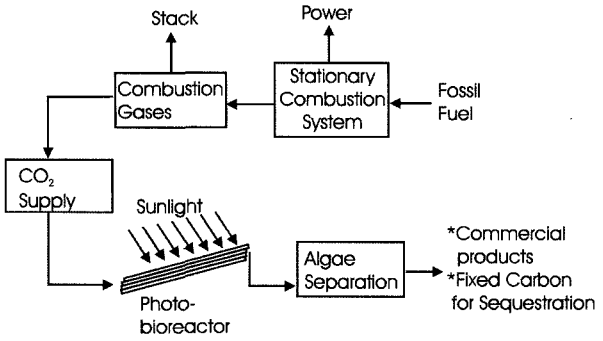

Fig. 1. Diagram showing a simple concept for a microalgal-based carbon capture scheme. High value products obtained from the microalgal biomass would offset the cost of CO_2 removal.

be effectively used as nutrients for microalgae. This could simplify flue gas scrubbing for the combustion system.

- Microalgae culturing may yield high value commercial products. Sale of these high value products can offset the capital and the operation costs of the process.
- The envisioned process is a renewable cycle with minimal negative impacts on environment.

The concept of using microalgae to ameliorate CO_2 emissions from stationary combustion sources is not new [4-6]. However, concerns exist as to the ability of microalgae, in general, to withstand the high CO_2 concentrations present in flue gas [7,8] as well as the potentially toxic accompanying SO_x and NO_x gases [9,10]. Thus, a number of efforts have been carried out to isolate microalgal strains that are especially adept to this application such as those by the RITE program [11] and others [12-14].

Here, we report on a series of experiments carried out to select microalgal species based on their temperature, pH, and flue gas tolerance. Specifically, we investigated whether changes in culture pH and combustion gas composition had any negative effects on the maximum quantum yield of photosynthesis of the organisms tested. Any decrease in photochemical efficiency translates into lower chemical energy available to the microalga to fix CO_2 into biomass and, thus, would lower the carbon capture potential of a microalgal culture.

MATERIALS AND METHODS

Selection of Strains

The microalgal strains used in this work were isolated from 71 water samples collected from aquaculture ponds, water treatment plants, birdbaths, puddles, and the seashore as well as obtained from microalgal culture collections. A total of 41 locally isolated strains and 13 strains imported from other collections were used. The local strains (Hawaii) were expected to have relatively high temperature tolerances. The imported strains were selected based on their ability to accumulate high value metabolites. The local strains were isolated from agar cultures grown on petri dishes using a proprietary medium formulation based on Bold's Basal medium [15]. For marine isolates, the same nutrient enrichments were used but added to deep ocean seawater obtained from the Natural Energy Laboratory of Hawaii Authority (NELHA, http://www.nelha.org/). Selected colonies of microalgae were plated repeatedly resulting in unialgal cultures. Here we identify the strains by our collection ID number (*e.g.*, AQ####) unless genus identification is available.

Temperature experiments

To determine the growth rates of microalgae at different temperatures, cultures were batch grown in 250-mL Erlenmeyer flasks. The flasks and medium were sterilized by autoclaving. Once inoculated, the flasks were immersed in temperature controlled water baths and were illuminated from below (fluorescent bulbs, 60 μE m^{-2} s^{-1}, 14:10 light:dark). The flasks were manually agitated three times daily.

Algal growth was estimated from daily changes in biomass concentration estimated from *in vivo* fluorescence. A Pulse Amplitude Modulated fluorometer (MINI PAM, Walz, Germany) was used to measure culture *in vivo* fluorescence of the cultures at the end of the dark period [16]. The cultures were manually shaken and the end of the MINI PAM fiber optic guide was placed directly against the bottom of the each flask in a darkened room. Following the *in vivo* fluorescence determination, the culture was shaken again and the procedure repeated for a total of three determinations of the maximum fluorescence yield (F_m). The three values for F_m thus obtained were averaged and assumed proportional to the biomass concentration in the flask. The growth rate (day^{-1}) was calculated as:

$$\mu = \ln\left[\frac{F_2 / F_1}{\Delta T}\right]$$

where μ is the growth rate (day^{-1}), F_2 is the fluorescence (F_m) at time 2, F_1 is the fluorescence (F_m) at time 1 and ΔT is the difference between time 2 and time 1 in days (1 day).

Chemostat Experiments

Microalgal cells were grown in 3.3-L chemostats. Temperature was maintained at 25°C by recirculating water baths (for jacketed chemostats, pH experiments, below) or at room temperature (22 ± 2°C, flue gas experiments, below). Light was provided by fluorescent bulbs (120 μE m^{-2} s^{-1}, 14:10 light:dark). pH was automatically maintained (using pure CO_2 injections) by a computerized data acquisition and control system developed in house.

Changes in biomass concentration in the chemostats were estimated from *in vivo* fluorescence measurements taken at the end of the dark period (see above). The end of the MINI PAM fiber optic guide was brought in contact

Table 1. Composition of simulated flue gases used in the flue gas tolerance experiments according to the combusted material

Fuel type	A. Bituminous coal	B. Sub-bituminous coal	C. Natural gas	D. Natural gas	E. Fuel oil
Gas (ppm)		Utility boilers		Gas Turb Comb	Diesel
CO2	181000	240000	131000	57000	62000
O2	66000	70000	76000	159000	170000
N2	719000	681000	793000	784000	767000
SO2	3504.0	929.7	0.0	0.0	113.1
NO	328.5	174.3	95.1	22.1	169.7
NO2	125.9	66.8	36.5	8.5	65.0

with the outside of the chemostat vessel wall and three measurements of the dark adapted maximum and minimum fluorescence yields (F_m and F_o) were taken and averaged. From these, we obtained estimates of the maximum quantum yield of photosystem II, F_v/F_m, where F_v is the difference between F_m and F_o [17].

Fluorescence-based biomass estimates during the initial growth phase (batch) of the chemostat cultures (*i.e.*, before medium additions were started) were used to estimate a maximal growth rate of the culture by averaging the three fastest daily growth rates measured during the logarithmic growth phase. These growth rates represent a near-maximum attainable since both nutrients and light are assumed not limiting during this period of culture growth.

pH Experiments

To test the pH tolerance of the different microalgal strains (20 strains tested), the cultures were grown in temperature controlled chemostats (25°C) and exposed to different pH conditions. Initially, the cultures were grown in chemostats at a nominal pH of 7.5. The pH of the cultures was automatically controlled by pure CO_2 injections into the growth medium in response to raises in pH. The system was programmed with set points at 7.4 and 7.6 pH. Thus, when the pH of the culture reached 7.6 in response to photosynthetic carbon uptake, a solenoid valve opened allowing the introduction of gaseous CO_2 into the culture. As the pH dropped and reached 7.4 in response to the injection of CO_2, the valve closed.

Once the flow of nutrient medium into the chemostats was started and steady state was reached (no change in fluorescence-based biomass estimates from day to day) the chemostats were allowed to grow using pH set points at 7.4 and 7.6 for a week. Next, the pH set points were changed to either 6.4~6.5 or 8.4~8.6 for another week. Changes in the fluorescence-based estimates of biomass and F_v/F_m in response to exposure to different pH conditions were used to indicate whether the cells were negatively affected by those conditions.

Flue Gas Experiments

To test the flue gas tolerance of the different microalgal strains, the cultures were grown in chemostats and exposed to different gas mixtures (BOC Gases, Lebanon, New Jersey, USA) selected to mimic the flue gas from power plants utilizing different fuels (Table 1). Initially, the cultures were grown at a pH range of 7.4~7.6. The pH of the cultures was automatically controlled by pure CO_2 injections into the growth medium in response to raises in pH (see above). Once the cultures reached steady state, the stream of pure CO_2 for pH control was substituted with commercial mixtures of gases specific to mimic different flue gas compositions (Table 1). Each culture was exposed to each gas mixture for 1 week of continuous culture. Addition of gas mixtures was controlled by the culture pH. As the cells photosynthesized and the pH of the culture rose to 7.6, a solenoid valve automatically injected the gas mixture into the chemostat. The valve closed when the pH of the culture dropped to 7.4 following the gas mixture injection.

As in the pH tolerance experiments (above), changes in the fluorescence-based estimates of biomass and F_v/F_m in response to exposure to different gas mixtures were used to indicate whether the cells were negatively affected by the flue gases.

RESULTS AND DISCUSSION

Temperature Experiments

These experiments were designed to quickly test the tolerance of the different microalgal strains to different temperatures. As such, the cultures were grown in batch mode. The data indicated that there is a large degree of uncertainty about the mean growth (not shown). This is the case for two reasons. First, these experiments were designed to quickly provide information on temperature tolerances for the different strains. Cultures in batch mode show different growth rates at different stages of the cultures' growth curve. Second, a number of these cultures are of a filamentous and clumping nature. Thus, the cells are not uniformly distributed throughout the growth medium. This translates into inherently noisy data. However, the results from the experiment allow us to determine the temperature tolerances of the different

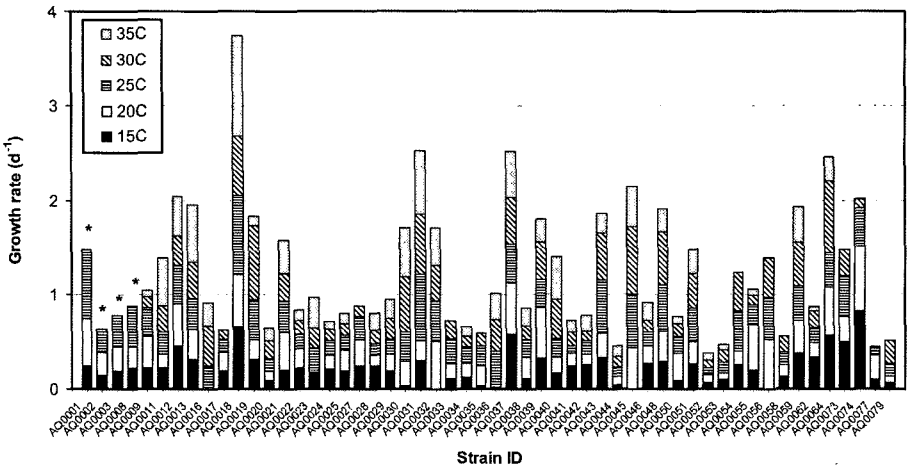

Fig. 2. Growth rate estimates for 54 strains of microalgae at five different temperatures. * Incubations for these strains were not carried out at 30 and 35°C.

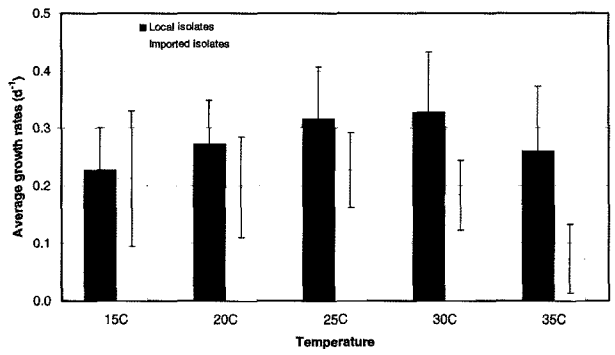

Fig. 3. Average growth rates under 5 different temperatures for locally isolated *vs.* imported strains. Error bars are one standard deviation wide .

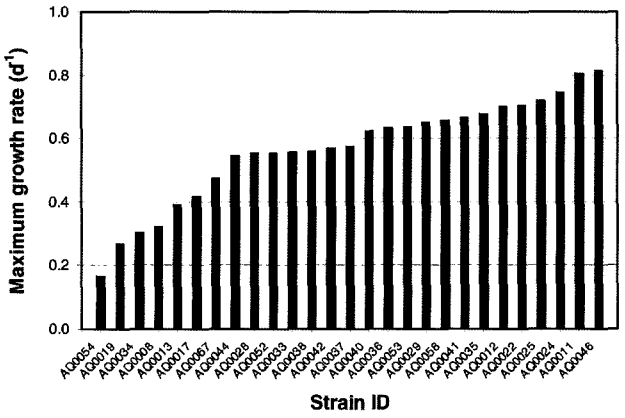

Fig. 4. Maximum growth rates obtained during the log phase of growth in chemostat cultures for 27 microalgal strains.

microalgal strains. Fig. 2 summarizes the results of culture growth for 54 strains at up to five different temperatures. The results show that for the tested strains five did not grow at 15°C, one did not grow at 20°C, one did not grow at 30°C and eleven did not grow at 35°C. In general, local isolates were able to better tolerate the higher temperatures than the imported strains (Fig. 3).

Chemostat Experiments

Maximum Growth Rates

Biomass data from the exponential growth phase of all chemostats grown for pH and gas tolerance experiments was pooled to estimate the maximal growth rate of the individual strains under our standard conditions (7.5 pH, pH controlled via pure CO_2 injections). For the 27 microalgal strains grown in chemostat cultures, the calculated maximum growth rates ranged between 0.17 and 0.81 day^{-1} (Fig. 4). We consider these rates to be indicative of potential growth and, thus carbon capture potential, in industrial scale applications of microalgal photosynthesis.

pH Tolerance Experiments-Biomass

Fig. 5 illustrates the controlled changes in pH during a chemostat experiment. In this example, Strain AQ0022 (a locally isolated *Scenedesmus* sp.) was grown for 3 months and exposed to pH conditions of 6.5, 7.5, and 8.5. Twenty different strains of microalgae were successfully grown at the three different pH levels.

Fig. 6 summarizes the fluorescence-based biomass levels maintained by the different strains at 6.5, 7.5 and 8.5 pH. Lower biomass levels at high pH (8.5) could be interpreted as CO_2 limitation of the cultures. Lower biomass levels at low pH (6.5) could be interpreted as a detrimental effect on the cells due to the acidity of the medium. The biomass estimates, however, must be interpreted with caution. Several strains produced clumps of cells and some strains fouled the inside of the chemostat vessels. Thus, the biomass estimates obtained may not have always reflected the biomass concentration of equivalent

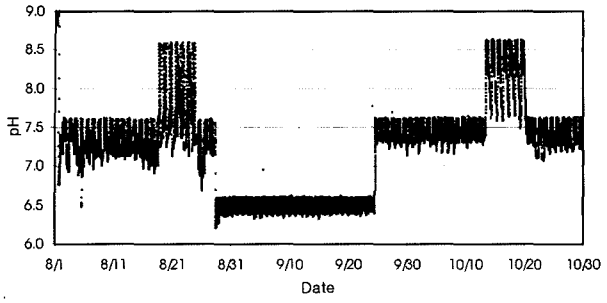

Fig. 5. Computer generated trace of culture pH measured in a chemostat culture of strain AQ0022 (a locally isolated *Scenedesmus* sp.). This chemostat was maintained for 3 months and it clearly shows the periods of time during which the culture's pH was maintained at 6.5, 7.5, and 8.5.

homogeneous cultures.

pH Tolerance Experiments - Maximum Quantum Yield of Photosystem II

Fig. 7 shows the results of the F_v/F_m measurements, averaged over several days, for the chemostat cultures grown at three different pH levels. The values are normalized to the maximum F_v/F_m value obtained for each specific strain and, thus, reflect relative changes in the maximum quantum yield of photosystem II in response to the changes in pH for each strain.

Maximum absolute F_v/F_m values ranged between 0.37 and 0.39 for Cyanobacterial strains (AQ0012, a locally isolated filamentous strain, and AQ0038, tentatively identified as *Merismopedia* sp.), between 0.58 and 0.65 for Rhodophytes (three *Porphyridium* strains: AQ0033, AQ0035, and AQ0036) and between 0.65 and 0.78 for all other strains (mostly Chlorophytes and including one diatom).

Interestingly, different pH growth conditions resulted in (for most cases) negligible changes in F_v/F_m. Only in Strain AQ0013 did we measure a significant reduction in F_v/F_m in response to 6.5 pH. Strains AQ0012 and AQ0038 are representatives of the Cyanophyceae, or blue-green algae. In this case there appears to be a reduction in photochemical efficiency at the higher pH values (equivalent to lower levels of dissolved CO_2 in the culture medium).

Previous reports have indicated that addition of high concentration CO_2 gas to microalgal cultures may reduce their productivity [7,14]. In our system, pure CO_2 gas is used as the carbon source in microalgal cultures without

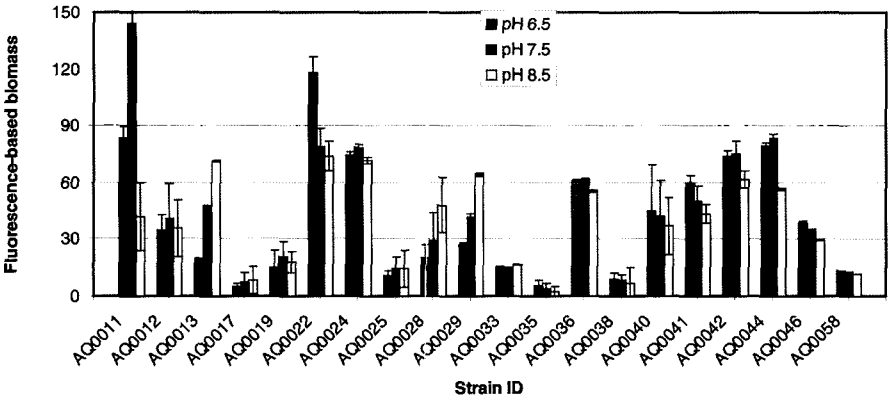

Fig. 6. Fluorescence-based estimates of biomass in chemostat cultures grown at three different pH. Error bars are one standard deviation wide.

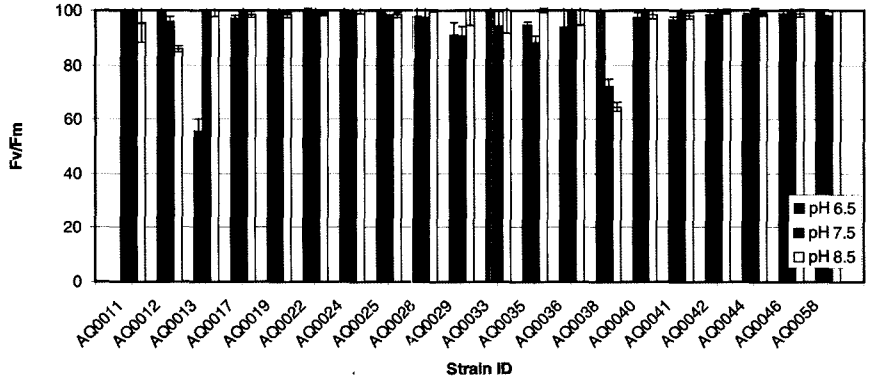

Fig. 7. Fluorescence-based estimates of photochemical efficiency normalized to the maximum measured. Error bars are one standard deviation wide.

Table 2. Summary of results from the flue-gas experiments: fluorescence based biomass estimates and F_v/F_m of the cells under pure CO_2 and five different flue gas mixtures. For an explanation of the gas mixtures see Table 1

	Fluorescence-based biomass						F_v/F_m					
Strain	CO2	MIX A	MIX B	MIX C	MIX D	MIX E	CO2	MIX A	MIX B	MIX C	MIX D	MIX E
AQ0008	14	32	30	38	41	32	0.72	0.73	0.73	0.72	0.72	0.73
AQ0011	33	27	29	32	36	29	0.73	0.72	0.74	0.73	0.74	0.74
AQ0012	15	22	23	15	19	21	0.41	0.38	0.42	0.36	0.38	0.35
AQ0013	21	43	21	23	27	33	0.77	0.78	0.77	0.78	0.78	0.79
AQ0017	3	33	29	8	16	28	0.76	0.71	0.66	0.73	0.71	0.70
AQ0022	35	33	39	40	35	32	0.76	0.76	0.77	0.77	0.76	0.77
AQ0024	28	24	27	28	24	21	0.73	0.72	0.72	0.73	0.72	0.73
AQ0025	31	41	40	44	39	40	0.72	0.72	0.73	0.71	0.72	0.74
AQ0028	25	67	88	25	29	49	0.79	0.79	0.79	0.80	0.79	0.79
AQ0033	3	3	4	3	3	3	0.63	0.54	0.59	0.63	0.61	0.64
AQ0034	2	4	2	2	3	4	0.57	0.56	0.56	0.53	0.54	0.63
AQ0035	7	11	10	9	9	11	0.55	0.55	0.52	0.58	0.58	0.52
AQ0036	13	18	18	17	19	22	0.61	0.60	0.61	0.60	0.59	0.58
AQ0037	11	41	16	20	26	34	0.50	0.51	0.53	0.54	0.51	0.51
AQ0038	12	14	12	13	11	13	0.31	0.49	0.52	0.32	0.31	0.32
AQ0040	17	22	27	24	16	10	0.73	0.72	0.74	0.73	0.73	0.68
AQ0041	31	41	42	25	26	31	0.69	0.69	0.69	0.71	0.70	0.69
AQ0042	28	21	29	26	25	23	0.74	0.75	0.75	0.75	0.75	0.74
AQ0044	22	17	19	25	21	18	0.73	0.73	0.73	0.74	0.72	0.74
AQ0046	37	37	39	37	37	41	0.73	0.74	0.74	0.73	0.73	0.74
AQ0052	N/D	22	28	26	31	25	N/D	0.67	0.68	0.67	0.69	0.69
AQ0053	N/D	31	39	38	33	23	N/D	0.69	0.69	0.70	0.70	0.67
AQ0054	3	2	3	3	2	2	0.70	0.64	0.68	0.63	0.71	0.69
AQ0067	26	33	31	37	40	35	0.71	0.72	0.72	0.71	0.71	0.71

any negative effects. However, pure CO_2 is added to the culture on demand, that is, when photosynthetic carbon uptake results in an increase in pH, which triggers the addition of the gas. The three pH conditions that we used in our experiments (6.5, 7.5 and 8.5 pH) correspond to dissolve CO_2 concentrations ranging over 2 orders of magnitude (from 0.7 to over 70 mg/L). However, in most cases, the photochemical efficiency was found near the maximum (Fig. 7). We conclude that as long as the pH of the system is controlled and CO_2 is fed on demand (such as with automatic pH control), no deleterious effects on photochemical efficiency will be found from using gases of different CO_2 content.

Flue Gas Tolerance Experiments

Twenty four microalgal strains were grown in chemostat cultures while exposed to pure CO_2 and five different gas mixtures representing five different flue gas sources (Table 1). The gases were injected into the chemostat cultures in response to increases in pH. The range of gas concentrations that the cells were exposed to were 5.7~100% CO_2, 0~3,504 ppm SO_2, 0~328 ppm NO, and 0~126 ppm NO_2.

Table 2 summarizes the fluorescence-based biomass levels maintained by the different strains under the six different growth conditions as well as the F_v/F_m values measured in those cultures. Our fluorescence-based biomass estimates are, however, to be taken with caution. As was the case in the chemostat pH experiments, clumping of cells and fouling on the inside of the chemostats mean that the biomass estimates obtained may not have always reflected the biomass concentration of equivalent homogeneous cultures. In the case of the flue gas experiments, this is more so since the experiments were of longer duration than that of the pH experiments.

Table 2 also summarizes the measured F_v/F_m values av-

eraged for 7 consecutive days, at each gas condition. As opposed to the biomass estimates, it is expected that changes in detected biomass concentration (*e.g.*, caused by fouling or clumping, above) does not affect the measured F_v/F_m. Our data indicate that changes in flue gas composition did not induce reductions in the measured F_v/F_m. Thus, the photochemical efficiency of the cells was not negatively affected by the presence of combustion products in the gas mixtures. In two instances we observed a deviation from the norm: the F_v/F_m values for AQ0038 (tentatively identified as *Merismopedia* sp.) appeared to increase under gas mixes A and B (Table 2). Microscopic observations indicated that the increase was probably caused by the presence of a Chlorophyte contaminant in the culture.

Our results indicated that wide ranging concentrations of SO_x and NO_x in the gas streams (0~3,504 ppm SO_2, 0~328 ppm NO, and 0~126 ppm NO_2, Table 1) did not negatively affect the photochemical efficiency of microalgal cultures. Previous reports have shown that SO_x and NO_x can inhibit algal growth [10]. It has been shown that as little as 60 ppm SO_2 decreased microalgal productivity by 25% [9]. It appears, however, that pH control could be effective in controlling that toxicity [18]. Furthermore, it has been suggested that NO_x species present in the flue gas can be used as a nitrogen source by the algae [19]. NO could oxidize in the medium before being assimilated by the cells [20] or could diffuse directly into the cells where it might be oxidized before being used in the cell's metabolism [21]. Our results support the conclusion that SO_x and NO_x components in flue gas do not represent any negative impacts in microalgal photochemical efficiency, as long as the pH of the medium is under control. This can easily be done by buffering the culture medium (*e.g.*, with bicarbonate) and controlling the addition of flue gas to the culture on an on-demand basis as in our system.

Significance of These Results and Conclusion

Cost-effective microalgal-based carbon capture and fixation is done outdoors to take advantage of natural sunlight to drive the photosynthetic reactions. Because the amount of light energy available per surface area unit is limited, the amount of carbon that can be fixed is limited by the amount of area available for cultivation and the culture's light utilization efficiency. It has been estimated that outdoor microalgal productivity maximizes at about 60 g dry biomass m^{-2} day^{-1} [22] which, assuming that about 50% of the algal biomass is carbon, is equivalent to 30 g C m^{-2} day^{-1} or 110 g CO_2 m^{-2} day^{-1}. A one MW capacity generating plant, working at full load 24 h per day and using bituminous coal produces about 24 tons of CO_2 day^{-1} at present day conversion efficiencies, resulting in an area requirement of 2.2×10^5 m^2.

To attempt to reach the maximum estimated productivities, the following must happen. First, microalgal cultivation schemes must not waste any of the available sunlight: the culture needs to be optically black, assimilate 100% of the light radiation available and avoid non-photochemical quenching [17,23]. Second, the photochemical efficiency of the algae must be maximized. This will be the case as long as the cells are light limited and as long as other factors do not reduce the photochemical efficiency of photosystem II such as nutrient deficiencies [17]. Here we have shown that changes in pH and flue gas composition, for the most part, do not negatively influence the photochemical efficiency of the tested organisms. We conclude that, with proper management of outdoor cultures (nutrients, light, pH), microalgal photosynthesis represents a viable alternative for direct CO_2 capture from smoke stack gases produced by combustion of different fuels.

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