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Influence of Flue Gas Components on the Growth Rate of *Chlorella vulgaris* **and** *Scenedesmus acutus*

INFLUENCE OF FLUE GAS COMPONENTS ON THE GROWTH RATE OF *CHLORELLA VULGARIS* AND *SCENEDESMUS ACUTUS*

C. Crofcheck, A. Shea, M. Montross, M. Crocker, R. Andrews

ABSTRACT. *Atmospheric carbon dioxide levels have increased since the industrial revolution due to the increase in combustion of fossil fuels. One possible alternative strategy is the use of microalgae for CO_2 capture and recycling. Major components in coal-derived flue gas that may accumulate and effect algae growth include both sulfur oxides and fly ash. However, in practical application, sulfur oxides will be converted quickly to the acid product (H_2SO_4) in the aerobic aqueous conditions of algae cultivation. In this article, the influence of elevated H_2SO_4 levels and the presence of coal-derived fly ash were investigated. As the H_2SO_4 level increased, algae growth was inhibited and finally ceased; however, this affect could be minimized by the addition of $NaHCO_3$ as a buffer. The effect of ash on the growth of algae was related to the type of coal-fired combustion and the amount of ash. For one of the ash types tested, the algae growth rate actually increased at a low ash concentration. In this work, major technical hurdles in the use of algal scrubbing systems are being addressed with respect to major contaminants in coal-derived flue gases.*

Keywords. Algae, Chlorella, CO_2 mitigation, Flue gas, Scenedesmus.

Coal-fired power plants alone emit about 1.9 billion metric tons of CO_2 annually, representing 33% of energy-related CO_2 emission from the U.S. in 2008 (EIA, 2009). Currently, the most promising technologies under development to capture CO_2 from coal-fired power plants include post-combustion, pre-combustion, or oxy-combustion, with CO_2 transportation to a storage site via pipeline. In addition to this high energy consumption, chemical absorption and geological storage of CO_2 have high capital cost and the potential problem of gas leakage. Consequently, these drawbacks are encouraging researchers to find alternative approaches for the mitigation of CO_2 emissions. One possible strategy is the use of microalgae for CO_2 capture and recycling (Sayre, 2010; Wang et al., 2008; Ho et al., 2011).

Although microalgae exist and thrive almost everywhere on Earth, not all strains are suitable for the capture of CO_2 from flue gas. Flue gas is emitted from coal power plants at a relatively high temperature, even after heat recovery, and usually contains 10% to 15% CO_2. If closed photobioreactors are chosen for the bio-mitigation system, the operating temperature often exceeds ambient. Therefore, two primary properties of the ideal strain should be tolerance of high

CO_2 concentrations and high temperatures. With these goals in mind, the search for ideal strains has been ongoing for decades. Researchers have isolated and selected various strains from high temperature (30°C to 70°C) and high CO_2 locations, such as warm natural carbonate springs (Sakai et al., 1995; Westerhoff et al., 2010), wastewater treatment facilities (de-Bashan et al., 2008), and artificial extreme conditions (Morais and Costa, 2007).

From a historical perspective, *Chlorophyta* is one of most studied groups of microalgae in terms of CO_2 bio-mitigation and biofuel production. Among the particularly promising genera, *Chlorella* and *Scenedesmus* have been the most studied to date. *Chlorella* occurs as 2 to 12 µm spherical or ellipsoidal unicells thriving in fresh and marine waters and soils (Graham and Wilcox, 2000). Molecular phylogenetic analyses indicate that *Chlorella* is polyphyletic and descended from multiple ancestors. *Scenedesmus* typically appears as a flat colony of 4, 8, or 16 linearly arranged cells growing in fresh and occasionally brackish water (Graham and Wilcox, 2000).

One crucial issue to be addressed is the toxicity of flue gas components on the growth of microalgae. Flue gas generated from coal combustion typically consists of ~75.8% N_2, 13.2% CO_2, 6.2% H_2O, and 4.8% O_2 (mole-fraction) (Desideri and Paolucci, 1999). In addition to these major components, flue gas also contains trace amounts of NO_x and SO_x (100 to 300 ppm) (Lee et al., 2002). Once dissolved in water, NO_x and SO_x become acids and account for the formation of acid rain when released into the atmosphere. When culture pH is not controlled within the tolerance range of algal growth (~7.0), high concentrations of SO_x and NO_x can show significant inhibition on the growth of some algae species. In a study of the effects of simulated flue gas with 200 ppm SO_2, the growth of *Chlorella vulgaris* was significantly inhibited, while *Chlorella*

caldarium was not dramatically affected due to its acidophilic characteristics (Hauck at al., 1996). When a series of SO_2 concentrations (0, 60, 100, and 150 ppm) were introduced to the cultivation media of *Chlorella* sp. KR-1, the growth rates (g L^{-1} d^{-1}) were accordingly decreased from 1.66 to close to zero with increasing SO_2 concentration (Lee et al., 2002). The growth rate decline is probably associated with a fall in the pH from 7 to 3. Therefore, appropriately buffering the medium can be a solution to maintain normal growth of algae with the addition of flue gas. Maeda et al. (1995) suggested the use of $CaCO_3$ to prevent the decrease in pH and subsequent death of algae (*Chlorella* sp. T-1). Without addition of $CaCO_3$, a gas mixture of SO_x (80 ppm) and NO_x (240 ppm) quickly acidified the culture medium, and growth ceased. After adding $CaCO_3$, the growth rate showed a marginal decrease. Similarly, it is reported that if the flue gas flow rate is constantly adjusted according to the medium pH (chemostat), algae exhibit a certain degree of tolerance of SO_x and NO_x (Zeiler et al., 1995; Olaizola, 2003). In addition to acidic components, the ash present in flue gases is also reported to alter the growth of algae, such that the algae productivity starts decreasing when the ash (containing V_2O_5, NiO, Fe_2O_3, Al_2O_3, and CaO) concentration is higher than 0.2 g L^{-1} (Matsumoto et al., 1997).

This article focuses on the influence of two of the major flue gas components, SO_x and ash, on the growth rate of two microalgae strains, *Chlorella vulgaris* and *Scenedesmus acutus*, both well suited for CO_2 capture.

MATERIALS AND METHODS

CULTURE PREPARATION

Chlorella vulgaris (#152075) was obtained from the algae collection at Carolina Biological Supply Company (Burlington, N.C.). *Scenedesmus acutus* (UTEX 72) was purchased from the algae culture collection at the University of Texas at Austin. Seed cultures of *Chlorella vulgaris* and *Scenedesmus* were grown and maintained in urea media containing urea (0.55 g L^{-1}), KH_2PO_4 (0.1185 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (0.055 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.109 g L^{-1}), and $Na \cdot EDTA \cdot Fe$ (0.02 g L^{-1}) (Crofcheck et al., 2012). Media were prepared with municipal drinking water (dechlorinated with 0.06 g sodium thiosulfate per 2 L of medium) and filtered through a 0.2 µm Nalgene nylon membrane filter (47 mm diameter). Once a week, microalgal cells were transferred into new media for subculture or used as inoculums for an experiment. Working in a laminar flow hood, 2 mL liquid stock culture was transferred to 400 mL fresh medium in a 500 mL Erlenmeyer flask. Newly transferred flasks were incubated under warm (F32T8/TL741 Alto, 32 W, Philips) and cool (F32T8/TL735 Alto, 32 W, Philips) white fluorescent lights (70 µmol m^{-2} s^{-1}) in a 16:8 h light:dark illumination period. Flasks were bubbled with 3% anaerobic-grade cylinder CO_2 (0.14 L min^{-1} CO_2 and 4.4 L min^{-1} air for the total system of flasks), the percentage of CO_2 being selected to achieve saturation of the flask with CO_2. All experimental flasks were placed on a shaking table (100 rpm) and kept at room temperature (22°C).

DRY WEIGHT AND GROWTH RATE

Chlorella and *Scenedesmus* biomass samples in suspension were filtered using Whatman binder-free glass microfiber filters (type 934-AH, 24 mm diameter). Ash free dry weight (AFDW) values were obtained by first drying algae samples at 105°C for 24 h and then ashing them at 550°C for 1 h (Becker, 1994). Since ash was added to some of the flasks, and the media may have contained some salts or minerals, an ash free dry weight basis was employed to ensure accurate measurement of biological growth. Growth curves of cultivation time versus AFDW were linear for the growth periods studied; hence, the slope of the growth curve was used as the growth rate (mg L^{-1} h^{-1}).

EXPERIMENTAL PROCEDURE

Chlorella and *Scenedesmus* were grown in triplicate in an environmental chamber (22°C). For both cultures, a target inoculum value of 0.1 g L^{-1} (dry weight) was cultured in 400 mL of medium with 3% enriched CO_2/air mixture. Gas flow rates of 0.14 L min^{-1} CO_2 and 4.40 L min^{-1} air were regulated with a mass-flow controller (model 5850E, Brooks Instrument, Hatfield, Pa.) and a flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, Ind.), respectively. The culture was illuminated with warm and cool white fluorescent light in a 16 h:8 h light:dark cycle (70 µmol m^{-2} s^{-1}). Algae dry weight and pH were measured every 24 h.

SO_x ACIDIFICATION EXPERIMENTS

Chlorella and *Scenedesmus* were both tested for their tolerance of SO_x. When SO_2 comes into contact with water, it forms sulfurous acid (H_2SO_3), which is rapidly oxidized to sulfuric acid (H_2SO_4) (Loerting et al., 2000). Therefore, to mimic the effect of SO_2, sulfuric acid was used. A sulfuric acid solution (1 M H_2SO_4) was added to urea media in varying amounts (0 to 120 ppm) while bubbling 3% CO_2. Next, *Chlorella* was incubated in the H_2SO_4-doped media with 3% CO_2 for 8 h. Using smaller increments of 0 to 120 ppm H_2SO_4, *Scenedesmus* was cultured in both the H_2SO_4-doped urea media and the same media prepared with addition of a buffer (urea medium with 1 g L^{-1} Na-HCO_3), using 3% CO_2. For H_2SO_4 levels above 120 ppm, the algae did not survive the 8 h culture time. The CO_2 level was maintained at saturation levels with the continuous introduction of CO_2, such that increasing the concentration further would not result in an increase in the CO_2 concentration in the flask.

ASH EXPERIMENTS

Ash from four different coal-fired plants in Kentucky was available for testing. Ash samples were taken from the ash silos at the plant as grab samples, following standard power industry sampling techniques for ash analysis. The samples were representative of what would be expected from the units they were collected from based on previous samples from these units taken over the last decade (Hower et al., 2009).

The ash samples were labeled based on the ash type and amount of residual carbon present. Details concerning the various ash samples are given in tables 1 and 2. Loss on

Table 1. Summary of ash samples used with *Scenedesmus* (SC) and *Chlorella vulgaris* (CV) tolerance tests.

Label	Feedstock (Bituminous Coal)[a]	Combustor[b]	Residual Carbon[c] (%)	Ash Type[d]	LOI[e] (%)	Strain Tested
Ash C-1%	High sulfur	CFBC	0.7	Class C	15	SC, CV
Ash F-19%	Compliance	PC	19.2	Class F	15	SC, CV
Ash F-3%	Compliance	PC	3.0	Class F	4	CV
Ash F-11%	Compliance	PC	11.2	Class F	5-7	SC

[a] High sulfur = >3 lbs S per million BTU; compliance = ~1.2 lbs S per million BTU.
[b] CFBC = circulating fluidized bed combustor; PC = pulverized coal combustor (standard technology).
[c] Residual carbon = residual unburned carbon particulate in ash.
[d] Ash type = classifications of Class C or F based on ASTM defined standards for fly ash.
[e] LOI = loss on ignition, i.e., mass loss due to complete combustion of sample in excess oxygen.

Table 2. Elemental composition of ash samples used in this study.

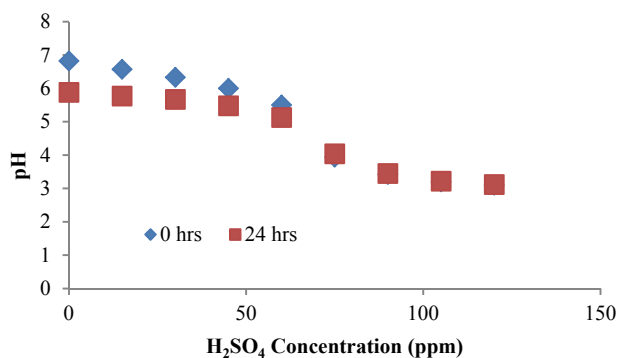|	Ash C-1%	Ash F-19%	Ash F-3%	Ash F-11%
	(%)			
C	0.7	19.2	3	11.2
SiO_2	15.1	52	53.9	54
Al_2O_3	6	31.7	29.3	31.2
Fe_2O_3	2.8	7.1	5.4	5.9
CaO	52.1	2	2.4	1.6
MgO	2	1	1.1	1
Na_2O	0.3	0.2	0.4	0.3
K_2O	0.5	2.3	3.2	2.6
P_2O_5	0.1	0.4	0.2	0.4
SO_3	22.4	0.3	0.6	0.2
	(ppm)			
V	0	408.5	468.2	450.7
Mn	19	166.5	310.5	156
Co	0	27	31.3	29.2
Ni	0	114	166	156.5
Cu	0	254.5	414.2	324.5
Zn	0	136.5	350.6	239.7
Mo	0	215	98.3	201.2

Figure 1. Effect of H_2SO_4 concentration on pH shown for the initial value and after 24 h of bubbling 3% CO_2. Standard error ranged from 0.00 to 0.06 (n = 2), all of which would be smaller than the data symbols.

ignition (LOI) was measured using a Leco TGA701 (Leco Corp., St. Joseph, Mich.) following ASTM Standard D7348. Percent carbon was measured using a Leco CHN628 following ASTM Standard 3178. Major and minor elements were measured using a Panalytical PW2404 XRF (Panalytical B.V., Almelo, The Netherlands) following ASTM Standard D4326. The Ash C-1% plant runs a circulating fluidized bed combustor (CFBC), which uses Illinois Basin coal as its feedstock and limestone in the combustor as a sorbent, producing a Class C ash (AS C-618). This ash is much coarser than the other ash samples since it contains calcined limestone; indeed, as the data in table 2 show, it contains a high proportion of Ca and S (the latter present as sulfate), along with minimal carbon (due to the design of the CFBC). The other three ash samples were from plants running a pulverized coal combustor (PC, standard technology).

Ash toxicity studies were done with a high loading of ash, 5 g L^{-1}, for both *Scenedesmus* and *Chlorella*. The ash tolerance studies were performed by adding no ash and 0.025, 0.25, and 0.5, g L^{-1} of Ash F-11%, Ash C-1%, and Ash F-19% (n = 3). Cultures were incubated in an environmental chamber at 22°C with 3% CO_2 (0.394 L min^{-1} CO_2 and 12.75 L min^{-1} air for 30 flasks).

RESULTS AND DISCUSSION

EFFECT OF SO$_X$ ACIDIFICATION

The addition of CO_2 during the H_2SO_4 study would be expected to decrease pH due to hydration to carbonic acid in solution. Therefore, an initial experiment without algae was performed to determine the combined effect of the addition H_2SO_4 and CO_2 on pH over a 96 h cultivation period in the absence of biological activity. Figure 1 shows the pH for varying levels of H_2SO_4 addition for 0 and 24 h (with continuous fixed CO_2 addition). After the initial 24 h, the pH remained approximately constant for all further times. At H_2SO_4 concentrations of 0 to 60 ppm, the pH remained above 5. For concentrations of 105 to 120 ppm, the pH leveled off at just above 3. Interestingly, the addition of CO_2 was observed to cause an additional decrease in pH in conjunction with H_2SO_4 levels up to 60 ppm. For H_2SO_4 additions of 75 ppm and above, the observed decrease in pH was dominated by addition of further H_2SO_4 only, resulting from limited acidification values for carbonic acid in equilibrium with gas phase CO_2.

The growth curves for *Chlorella vulgaris* grown for 8 h with varying amounts of H_2SO_4 (0 to 150 ppm) are shown in figure 2, and the corresponding pH levels are shown in table 3. At concentrations above 150 ppm, the algae did not survive the 24 h cultivation time; therefore, only results from viable cultures within the initial 8 h are included. The growth curves for up to 90 ppm H_2SO_4 are all similar, although the pH decreased as expected with increasing additions of H_2SO_4. This indicates that *Chlorella vulgaris* is capable of compensating for the addition of up to 90 ppm of H_2SO_4, which afforded an average pH of 3.9. On the other hand, there was a noticeable decrease in the dry weight over time at H_2SO_4 concentrations of 120 and 150 ppm. The pH values associated with these two affected growth curves are 3.3 and 3.1, respectively.
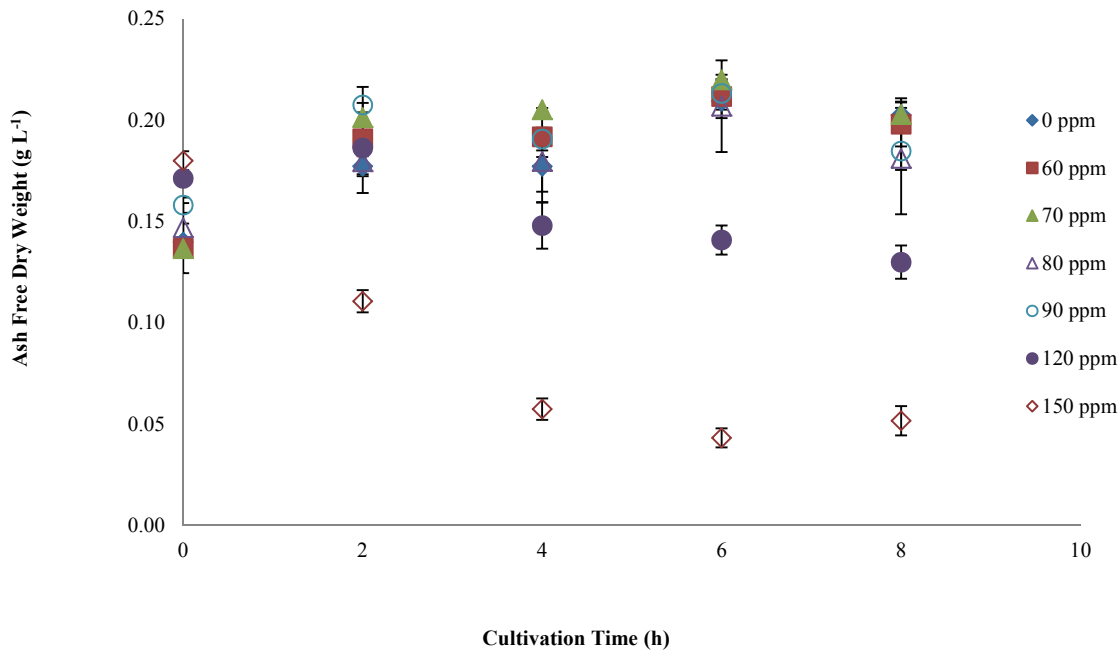
Figure 2. Growth curves for *Chlorella vulgaris* obtained at different H₂SO₄ concentrations. Error bars represent standard errors (*n* = 3).

The growth curves for *Scenedesmus* grown for 8 h with varying amounts of H_2SO_4 (0 to 150 ppm) are shown in figure 3, and the corresponding pH values are shown in table 3. Again, the growth curves for up to 90 ppm H_2SO_4 are all similar. The doubling time for *Scenedesmus* cultivated in 0 to 90 ppm H_2SO_4 is approximately 8 h, with the initial concentration of 0.04 g L^{-1}. In contrast, within 8 h, the mass gain for *Chlorella* is about 30%, with the initial concentration of 0.15 g L^{-1}. Moreover, the pH level again decreased as the amount of H_2SO_4 increased; however, it was not as drastic as the decrease seen with *Chlorella vulgaris*. This indicates that *Scenedesmus* is more capable of compensating for the addition of up to 90 ppm H_2SO_4, which produced an average pH of 5.5. There was a noticeable decrease in the *Scenedesmus* dry weight over time for 120 and 150 ppm of H_2SO_4, although not as drastic as that seen with *Chlorella vulgaris*. The pH values associated with the 120 and 150 ppm *Scenedesmus* growth curves were 3.7 and 3.3, respectively. The experiment with *Chlorella* had a four-fold higher concentration (0.15 g L^{-1} for *Chlorella* and 0.04 g L^{-1} for *Scenedesmus*), where typically a higher biomass concentration translates into a higher pH. However, the pH values for the *Chlorella* experiment, 3.3 and 3.1 for 120 and 150 ppm, respectively, were not higher. *Scenedesmus* appears to deal with the increase in H_2SO_4

better, as seen by the increase in the pH with less biomass. In either case, the 90 ppm level appears to be a threshold level at which a net decrease in algal mass is to be expected.

The initial growth rates (g mL^{-1} h^{-1}) for the first 6 h of cultivation for *Scenedesmus* and *Chlorella* are shown in table 4. These results show that overall *Scenedesmus* retained a higher growth rate with increasing acidity. There was a statistical difference between the growth rate at 150 ppm and at all the lower concentrations. For 120 ppm, all of the treatments were statistically different except 90 ppm for *Scenedesmus*. The negative growth rate for *Chlorella* at 120 and 150 ppm is most likely do to cell lysing.

For H_2SO_4 levels at 90 ppm and above, the effect of using a buffer (1 g L^{-1} NaHCO$_3$) was tested with *Scenedesmus*. The results are compared to the growth rates without H_2SO_4 in figure 4. The growth rates for *Scenedesmus* without buffer are similar up to 80 ppm, while there is a decrease for 90 and 120 ppm (fig. 3) during the 8 h cultivation period. However, for the buffered system, the growth rate is similar for H_2SO_4 concentrations up to and including 120 ppm for a cultivation period of 96 h.

EFFECT OF ASH

For ash toxicity tests using *Scenedesmus*, 5 g L^{-1} of each ash sample (Ash F-19%, Ash C-1%, and Ash F-11%) was added to the algae culture. The resulting growth curves are shown in figure 5. In each case, the starting pH of the culture was adjusted to 6.7 before the addition of the ash samples, while the initial biomass content ranged between 0.07 and 0.31 g L^{-1}. For Ash C-1%, the pH increased to 11.25 upon addition of the ash sample but decreased to 6.84 after 28 h. Visually, the increase in pH appeared to kill the algae culture. Algae appeared pale yellow-green to opaque white
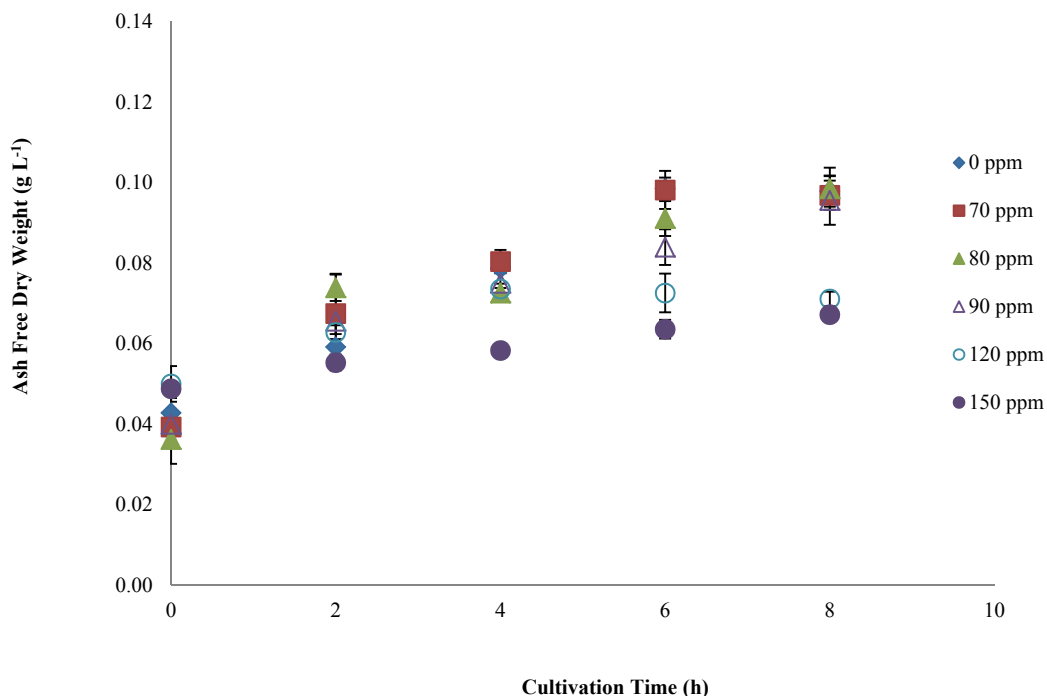
Table 3. Average pH and the beginning (*t* = 0) and end of the 8 h experiment with varying amounts of H₂SO₄.

H₂SO₄	Culture pH ± standard error (*n* = 3)			
	Scenedesmus		*Chlorella*	
(ppm)	*t* = 0	*t* = 8	*t* = 0	*t* = 8
0	7.13 ±0.02	6.2	6.68 ±0.02	6.05 ±0.01
70	6.2 ±0.01	5.7	5.58 ±0.04	5.06 ±0.06
80	5.95 ±0.01	5.6	4.61 ±0.05	4.12 ±0.27
90	5.73 ±0.05	5.4	3.93 ±0.05	3.73 ±0.03
120	3.66 ±0.03	3.8	3.23 ±0.04	3.34 ±0.03
150	3.27 ±0.01	3.3	3.01 ±0.01	3.11 ±0.01

Figure 3. Growth curves for *Scenedesmus* obtained at different H$_2$SO$_4$ concentrations. Error bars represent standard errors (*n* = 3).

Table 4. Average initial growth rate based on the first 6 h of growth for *Scenedesmus* and *Chlorella* with varying amounts of H$_2$SO$_4$.[a]

H$_2$SO$_4$	Initial Growth Rate (mg L^{-1} h^{-1})	
(ppm)	*Scenedesmus*	*Chlorella*
0	9.56 ±0.99 a	11.61 ±0.42 wx
70	9.81 ±1.10 a	13.87 ±0.59 x
80	9.14 ±0.78 a	9.98 ±1.03 wx
90	7.34 ±0.62 ab	9.19 ±1.08 wx
120	3.77 ±1.05 b	-5.07 ±1.24 y
150	2.48 ±0.24 c	-22.78 ±0.35 z

[a] Values are means ± standard error (*n* = 3). Means followed by the same letter are not statistically different (α = 0.05).
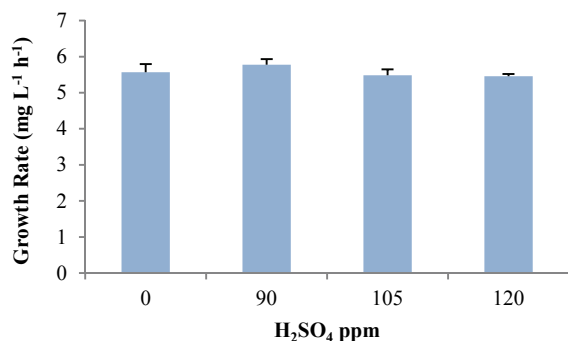

Figure 4. Growth rate of *Scenedesmus* as a function of H$_2$SO$_4$ concentration during 96 h culturing in the presence of 1 g L^{-1} NaHCO$_3$ as a buffer. Error bars represent standard errors (*n* = 3).

in color and settled to the bottom. There was a slight decrease in biomass from 0 to 27 h, after which the biomass content increased. For Ash F-11%, the pH increased to 6.97 after the addition of ash but returned to approximately the same pH as the control after 50 h. There was a noticeable decline in the biomass health at the beginning of the culture

period, although the culture recovered, such that an increase in biomass mass was seen over the last 36 h of the culture period. For Ash F-19%, the pH increased to 7.1 after the addition of ash and returned to approximately the same pH as the control after 75 h. The growth rates for the control, Ash C-1%, Ash F-11%, and Ash F-19% treatments were 7.0, 1.9, 2.0, and 6.0 mg L^{-1} h^{-1}, respectively (calculated from the data shown in fig. 5). While all three ash samples appeared to inhibit the growth of *Scenedesmus*, the growth observed for Ash F-19% was not significantly different from that of the control (p = 0.155). The growth rates for Ash C-1% and Ash F-11% were statistically the same (p = 0.137), and they were both statistically different from the control (p-values of 0.0007 and 0.001, respectively). Ash F-19% has the highest amount of residual carbon (19%). The presence of other components in the ash most likely inhibited growth the same as the other ashes did, but the excess carbon offset that effect by enhancing growth. However, this would not be the ideal case because this would mean the algae was not consuming as much of the CO$_2$.

Calculation of the atomic Ca:S ratio for Ash C-1% gives a value of 3.3:1 (assuming that all of the sulfur is present as CaSO$_4$). The Ca:S ratio indicates that a significant fraction of the calcium (70%) is not bound up as sulfate, i.e., is present as CaO (given that there are no other anions present in significant concentration that could balance the Ca^{2+} ions). This explains why the pH of the *Scenedesmus* culture increased to 11.25 upon addition of Ash C-1%. Consequently, highly efficient ash removal from the flue gas would be required before algae could be used as a CO$_2$ scrubbing strategy for the Ash C-1% plant.
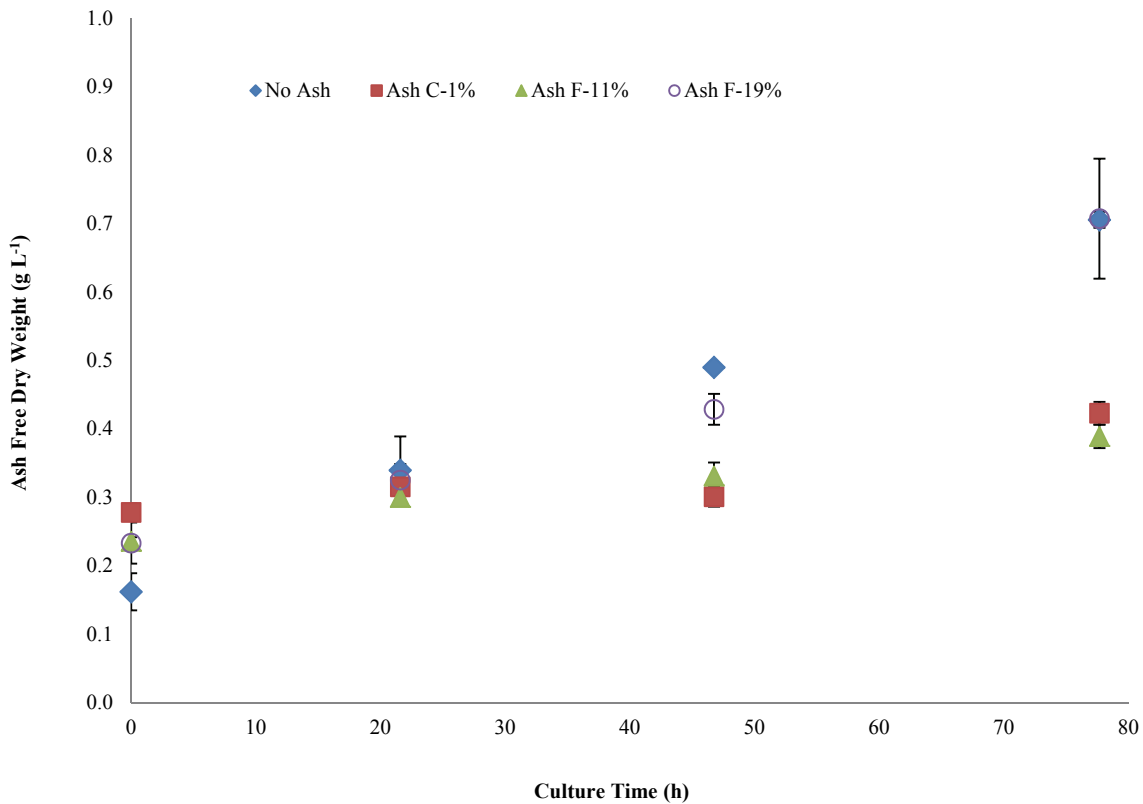
Figure 5. Growth curves for *Scenedesmus* obtained with the addition of 5 g L⁻¹ of ash from various coal-fired power plants in Kentucky. Error bars represent standard errors (*n* = 3).

The Ash F-19% and Ash F-11% plants use mid-Appalachian coal as a feedstock in typical combustors, producing Class F ash, which usually contains residual carbon. The Ash F-19% plant is small and usually produces an ash containing a substantial amount of unburned carbon content. As shown in table 2, this value can approach 20% by weight, whereas the value for the larger Ash F-11% plant is typically close to 10%. Due to the high carbon content of these ashes, they are black in color; hence, the presence of high ash concentrations during culturing leads to darkening of the culture medium and decreased light penetration into the culture. For Ash F-19% and Ash F-11% this effect is noticeable, whereas the change in culture pH upon addition of the ashes was minor (as described above). The latter effect reflects the relatively low concentrations of free alkali metal oxides and alkaline earth oxides present in the ashes. Other than the carbon content, the compositions of these two ashes are similar, and both contain useful amounts of trace elements (V, Mo, Co, Zn, etc.), which are frequently required for algae growth (Kaplan et al., 1986). Although the dissolution of these metal oxides is expected to be appreciable only at low pH values (<5), the fact that they are present in ppm levels suggests that sufficient amounts may dissolve during culturing to supply the ppb levels typically required for these micronutrients.

Additional studies were performed to examine the effect of lowering the concentration of the different ashes during algae culturing (fig. 6). At the lowest ash concentration examined (0.025 g L⁻¹), Ash F-11% had a growth rate that was not statistically different from the control (without ash). Moreover, for all three ash types tested at the 0.025 g L⁻¹ level, the growth rates were not statistically different from each other or the control. For the two higher concentrations (0.25 and 0.5 g L⁻¹), there was a significant difference in each case between the ash samples and the control, but there was no significant differences between the measured growth rates for the different ash types at the same loading. Vocke (1979) found a significant change in the growth of a *Scenedesmus* algae using as little as 0.0001 g L⁻¹ of an extract from a western coal-fired generation plant. In contrast, for all three of our ash samples, there were no statistically significant differences in growth rate at the 0.025 g L⁻¹ level. However, one of the conclusions from the work of Vocke (1979) was that toxicity is very algae- and ash-dependent, which may explain the discrepancy.

For the toxicity tests with *Chlorella vulgaris*, 5 g L⁻¹ of each ash sample (Ash F-19%, Ash C-1%, and Ash F-3%) was added to the algae culture and grown for five days (fig. 7). The starting pH of the culture was adjusted to 6.7 before the addition of the ash samples. For Ash C-1%, the pH increased to 9.57 upon addition of the ash sample and increased to as high as 11.08 after 24 h. Visually, the increase in pH appeared to kill the algae culture. Although there was a slight decrease in biomass from 0 to 20 h, after 20 h the biomass content remained constant. For Ash F-3%,
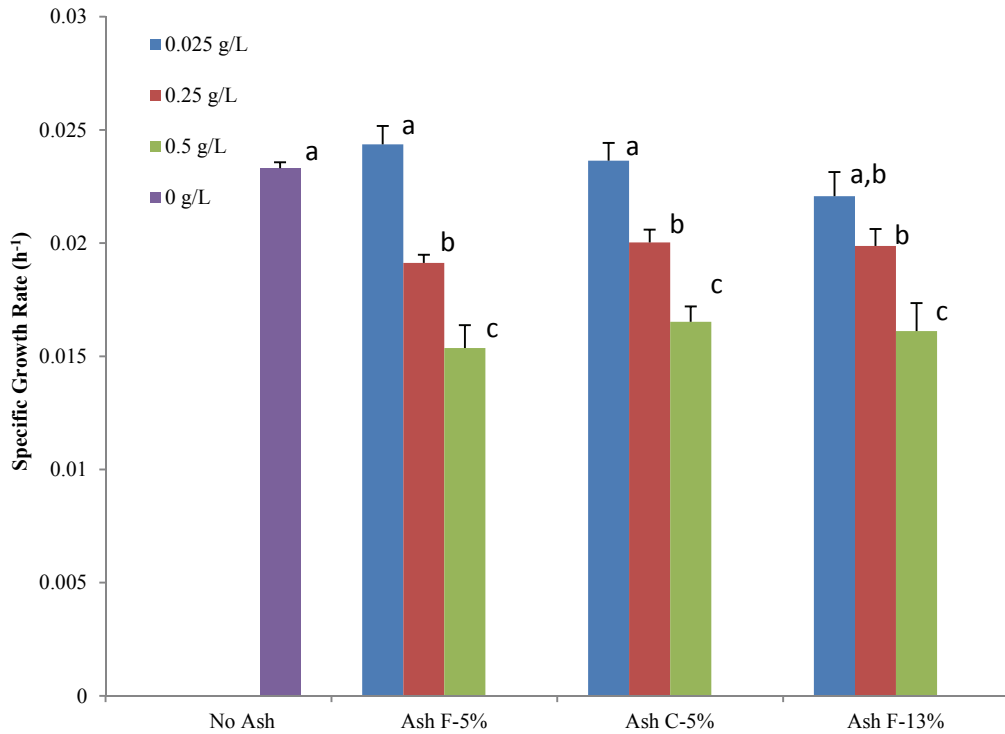
Figure 6. Growth rate of *Scenedesmus* grown with three different ash types at three different loadings compared to a control. Error bars represent standard errors (*n* = 3), and treatments with the same letter are not statistically different with α = 0.05.
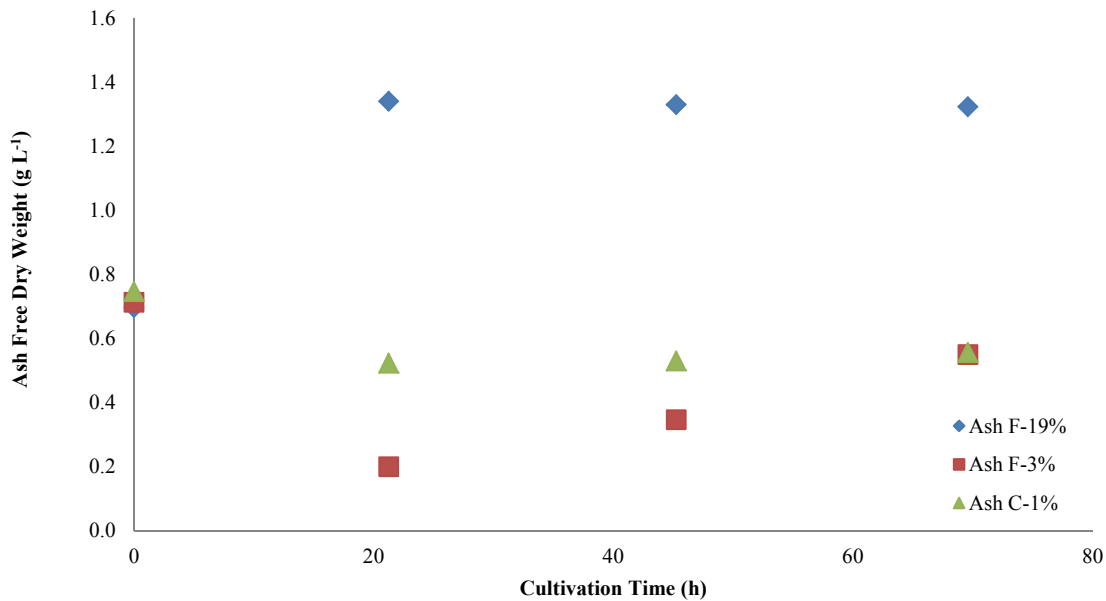

Figure 7. Growth curves for *Chlorella vulgaris* obtained with the addition of 5 g L⁻¹ of ash from various coal-fired power plants in Kentucky.

the pH increased to 6.95 after the addition of ash and peaked at 9.41 at 48 h. There was a noticeable decline in the biomass health in the beginning of the experiment, but interestingly the culture appeared to recover, and an increase in biomass was seen over the last three days of culture. For Ash F-19%, the pH increased to 7.05 after the addition of ash and peaked at 8.35 after 24 h of culturing, decreasing to 8.19 at 72 h. The increase in pH did not appear to impair the growth of the culture. In fact, the culture grew well, reaching a maximum concentration of 1.2 g L⁻¹

after the first 24 h. These results support the conclusions from the ash toxicity test with *Scenedesmus*, i.e., that Ash F-19% does not greatly affect the growth rate, whereas Ash C-1% exerts a significant inhibitory effect.

These data can be used to evaluate the potential impact of ash from different power plant designs. If a biomitigation strategy is to be employed at a facility that produces a Class F ash, such as Ash F-19% and Ash F-3%, few problems associated with ash contamination would be expected. Indeed, the foregoing results suggest that it is possible that the presence of minute amounts of ash has the potential to improve the growth rate, as seen with Ash F-5%, but at the same time it might also adversely affect the growth rate, as seen with Ash C-1%. Further ash tolerance studies will need to be done with the ash specifically used in each installation, unless the exposure level can be limited to below 0.025 g L^{-1}.

SO_x and ash components were chosen for this study because they are likely to be the most influential components of flue gas on algae growth in an algal CO_2 mitigation system. Obviously, other components will likely need to be addressed before commercialization, but most are in such trace amounts (or likely beneficial, e.g., NO_x) as to be of little primary concern. Studying both SO_x and ash together is a logical approach, as any system without SO_x scrubbers will release both ash and SO_x together, whereas a scrubbed system will still introduce ash. By looking closer at the most influential component in the gas phase (SO_x) and the effect of the solid particles in the flue gas, we have actually found counter influences. This suggests that the analysis of the entire flue gas system will involve several connected influences.

CONCLUSIONS

The influence of possible flue gas contaminants on the growth rate of two microalgae strains, *Chlorella vulgaris* and *Scenedesmus acutus*, was investigated. These two strains of algae are interesting candidates for large-scale cultivation to mitigate CO_2 emissions from coal-fired power plants. To study the influence of SO_x and ash on the viability of an algal scrubber system, elevated H_2SO_4 (as surrogate for SO_x) levels and the effect of ash were investigated. While the H_2SO_4 concentration influenced the pH and subsequent growth, the addition of a buffer ($NaHCO_3$) was found to minimize the effect. These results indicate that between standard power plant SO_x management practices and carbonate buffering of the growth media, the introduction of SO_x and its subsequent hydration products are manageable without deleterious effect on the algal system.

The introduction of ash had varying effects depending on the source of the ash. In all cases, it appears that ash addition had some negative effect on algae growth. However, in one case, due to a high carbon content, the ash addition was seen to overcome this inhibitory effect and enhance growth. Based on the broad set of ash samples (both Class C and Class F) from various power plant types used in this study, the results indicate that no catastrophic outcomes result from the carryover of ash into the algae sys-

tem at concentrations of 0.025 g L^{-1} or less.

Overall, this study indicates that the primary components of the flue gas from a coal-fired power plant will not prevent use of an algal scrubbing system. While the results indicate that different plant configurations will require adaptation of the growth media to some extent, they do not show any major obstacles to the next development stages of this technology. Rather, these results indicate the ability of an algal system to serve as a multi-component scrubbing system, handling both residual ash carryover and SO_x. Under scaled-up conditions, this could allow for reduced burdens on upstream emissions control units and thereby reduce the overall energy parasitism and capital costs for the plant.

With the growing desire to address carbon dioxide emissions, which are associated with increasing rates of climate change, innovative processes are needed to capture carbon dioxide from major point sources such as fossil fuel power plants. In this work, major technical hurdles in the use of algal scrubbing systems are being addressed with respect to major contaminants in coal-derived flue gases. With such knowledge, mitigation systems can be developed to reduce overall capital and operating costs of the emissions controls for a plant, and make algal scrubbing economically viable.

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