Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 749 - 762

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

UTILIZATION OF FLUE GAS AS A CARBON DIOXIDE SOURCE FROM A COAL-BURNING POWER PLANT FOR THE CULTIVATION OF MICROALGAE IN OUTDOOR CIRCULAR PONDS

SWAMINATHAN PALANISAMI[1], THIRUVENKADAM VISWANATHAN[1,2], NICHOLAS DUDENHOEFFER[3], KEESOO LEE[1]* AND PAUL KI-SOUK NAM[3]

[1]Center for Bioenergy, Cooperative Research, Lincoln University in Missouri, Jefferson City, MO 65101, United States
[2]Department of Biological and Agricultural Engineering, University of Georgia, Athens, GA 30602, United States
[3]Department of Chemistry, Missouri University of Science and Technology, Rolla, MO 65409, United States

ABSTRACT

Flue gas originating from a coal-fired power plant was used as a carbon dioxide source for microalgae cultivation. Raw flue gas without scrubbing or desulfurization was diluted with compressed air in such a way as to attain 1%, 2%, and 4% CO_2. Throughout the 2010, from May to October, growth response was studied. While sparging pure CO_2, a proportionate increase in the biomass was observed corresponding to the increasing CO_2 percentage (1%, 2%, and 4%). CO_2 in the form of flue gas yielded less growth with increasing percentage; however, the lipid production revealed a steady elevation on sparging a higher percentage of flue gas. To investigate the toxic effects of flue gas entities, factors related to the stress-stabilization mechanisms were studied. Activity staining of stress stabilizing enzymes - superoxide dismutase and esterase showed increased activity. The used concentration of flue gas has not hindered the algal growth, which was evident by the organism's active nitrogen metabolism as revealed by the increased activity of glutamine synthetase. To understand the fate of CO_2 metabolism; Polyphenol oxidase activity was tested, which found decreased in activity.

KEY WORDS: **Flue gas; microalgae; biomass; stress; lipid**

KEESOO LEE
Center for Bioenergy, Cooperative Research, Lincoln University in Missouri, Jefferson City, MO 65101, United States.

INTRODUCTION

Microalgae are suitable feedstock for sustainable biofuel, bioproducts, and animal feed purposes. It has been projected that microalgae can generate a biomass of up to 100 to 150 tons ha $^{-1}$ year $^{-1}$, which is 10–15 times higher than the productivity of conventional agricultural crops, such as corn [1]. In general, photosynthetic microalgae have 50% of their weight as carbon; hence, 1.83 kg of CO_2 will be needed theoretically to produce a kilogram of biomass [2]. As the atmospheric CO_2 concentration (0.03%) is not sufficient to produce biomass at faster growth rates, an external source of CO_2 is usually required for intensive algal cultivation [3]. Consequently, CO_2 represents a considerable share of the operational cost of the microalgal biofuel production. It is also important to note that, an increasing concentration of atmospheric CO_2 levels is a major concern for worldwide climate and food sustainability. Available technologies for CO_2 removal/capture, such as physicochemical absorbents and injection into deep oceans and geological formations, are expensive, non-renewable, or inefficient for long-term storage. An alternate to physiochemical absorbents is biological CO_2 fixation, which can effectively recycle the CO_2 in the flue gas without increasing its net concentration in the atmosphere. Microalgae are considered ideal for biological CO_2 fixation due to their high photosynthetic activity when compared to terrestrial plants. Hence, it is reasonable to believe that the utilization of industrial flue gas for microalgae cultivation can concomitantly reduce the cost of microalgal production and remediate the flue gas. Mitigation of flue gas from power plants, especially coal-fired plants, presents a wide range of challenges, such as the presence of toxic gases (SOx, NOx) at growth-inhibitory concentrations and at a temperature high enough that it can heat/kill the microalgae. Nevertheless, several studies have reported the feasibility of remediating power plants with microalgae by using either direct industrial flue gas or by using pure gas mixtures that mimic flue gas composition [4-6]. Different growth systems, such as a thin layer photobioreactor [7], a membrane photobioreactor [8], a long tube photobioreactor, high rate ponds [9], and lab-scale experiments[5], were used in CO_2 mitigation studies at small scales. Further, the CO_2 mitigation strategies were optimized for the cultivation of monoculture strains, such as *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa*, and *Scenedesmus obliquus* [10], *Nannochloropsis salina* [7], and *Chlorella vulgaris* [11]. Monocultures can be cultivated only in a closed photobioreactor setup, which is cost-inhibitory for CO_2 mitigation at its current level of technological sophistication. Hence, an open pond system is the most economical option to fix industrial CO_2. The fact that the cultivation of a monoculture in open ponds is limited to extremophiles, such as *Spirulina*, has led the researchers to develop and mass cultivate a dominant and robust consortium of microalgae specific to a local environment [1]. It is also important to note that the above-mentioned studies were carried out at relatively small scales and have not addressed the issues of biomass harvest, biomass quality, and potential uses at larger scales. The aim of the present study was to evaluate the feasibility of utilizing the CO_2 (flue gas) from a coal-fired power plant for the cultivation of a microalgae native to Missouri. Additionally, this study investigated the impact of the composition of flue gas and weather changes on the growth of the algae. We also analyzed the fate of the lipid-producing capability of microalgae while sparging flue gas. Furthermore, the growth response of *Desmodesmus communis* LUCC 001 and its stress-stabilizing response against the flue gas (2% CO_2) were also studied.

MATERIALS AND METHODS

(i) Algal Cultivation and Nutrition

The flue gas from a 60 MW coal-fired power plant located at Chamois, Missouri (38.67 N, 91.76 W) was used to cultivate the microalgae in five deep circular ponds made of galvanized steel. The flue gas was used as provided without any scrubbing or desulfurization. Each pond's diameter was 4 m, and they were operated with a working volume of 3800 L. The effect of flue gas on microalgal cultivation was evaluated for four different sets of batch culture

experiments carried out in the months of May, July, August, and October of 2010. The flue gas from the power plant was diluted to different levels of CO_2 using compressed air (1%-4% v of CO_2/v of air-flue gas mixture) and supplied as the CO_2 source for three hours daily, from 9 am to 12 pm, for all the batch cultivation experiments in the study period (based on the optimal sunlight availability the flue gas sparging timing was selected). The mixing and aeration was provided by sparging the flue gas at a rate of 45 m^3/h. Once the flue gas supply was stopped after three hours, compressed air alone was used to keep the culture in suspension for the rest of the day. Nutrients were provided according to the recommendations of F/2 medium using a commercially available nutrient mix (Proline F/2 medium, Aquatic Ecosystems, Florida). Nitrate and phosphate concentrations were monitored using a HACH kit (Cat. No. 26053-45 nitrate and 27673-45 phosphate) to determine the nutrient limitation and additional nutrients were added as needed. Parameters such as pH, light intensity, and pond water temperature were monitored on a daily basis. *Desmodesmus communis* LUCC 001 was initially inoculated in the ponds at the beginning of May. One of the ponds was used as the mother inoculum for the subsequent batches, and the other species intrusion was monitored closely using light microscopy for each batch. In June 2012, the freshly scaled up *Desmodesmus communis* LUCC 001 was used to evaluate the growth response with and without flue gas.

(ii) Growth Evaluation

For the months of May, July, and August, the effect of 1% CO_2 and the monthly variation in the abiotic factors on the growth were monitored. In October, the effects of different concentrations of flue gas (1%, 2%, and 4%) on the algal productivity and biomass quantity were studied. For each batch, the growth in each pond was monitored using both optical density (OD) and ash-free dry weight. An aliquot of 20 ml of representative samples from each pond was filtered using pre-weighed, desiccant-cooled, glass fiber filter paper. The biomass (g/l) was determined gravimetrically after drying the filter papers at 80°C for 24 hours. The ash-free biomass content was determined by burning the biomass at 550°C. CO_2 fixation rate was calculated using the following de Morais and Costa 2007 [34]. The biomass yield was also calculated in terms of areal and volumetric productivity to easily compare the productivity across the summer.

(iii) Biomass Harvest and Lipid Analysis

At the end of each batch, the biomass produced after 15-18 days was harvested by flocculation with chitosan. This was accomplished by reducing the pH of the ponds approximately to 7.0 by adding CO_2; an aliquot of 0.4 ml of chitsoan solution (10 g of chitosan/l of 0.1 N HCL) was also added per liter of algal pond water. After the ponds were mixed vigorously, they were left undisturbed without any aeration overnight for efficient flocculation. The top water was either recycled for subsequent batch cultivation or sent to a water treatment facility. The flocculated slurry was collected in buckets and stored in a refrigerator for further use. The total lipid in the algae was extracted using a chloroform/methanol solvent system[12], and the lipid content was determined gravimetrically by evaporating the chloroform under N_2 stream.

(iv) Identification of Microalgae

Morphological characteristics of the experimental consortium were observed periodically by microscopic examinations using a Nikon Eclipse e800 at 60x magnification. Once the maximum domination was attained, the *Desmodesmus*-like species was made axenic. For 18S rRNA gene sequencing and analysis, genomic DNA of the microalgal strain was extracted using a MO BIO Laboratories Power Plant DNA Isolation Kit procedure, following manufacturer's instructions. PCR amplification and partial sequencing was undertaken at Genwiz, USA, using family-specific primers for *Chlorophyceae* ChloroF (5′-TGGCCTATCTTGTTGGTCTGT-3′) and ChloroR (5′- GAATCAACCTGACAAGGCAAC -3′)[13]. The 18S rRNA gene sequence of the isolate was compared with the 18S rRNA gene sequences available using the BLASTN search. Multiple sequence alignment and a phylogenetic dendrogram (see Fig. 4) were evaluated by using MEGA 5.1 (Molecular Evolutionary Genetic Analysis). The partial 18S rRNA gene sequence of the microalgal

strain was submitted to the GenBank database and obtained the accession number KC341713.

(v) Whole Cell Protein Preparation and Native PAGE

To study the response of various enzymes, cultures from the two types of ponds (with and without flue gas) were harvested by centrifugation at 6000 rpm for 10 minutes. Then the thoroughly washed algal pellets were homogenized with an extraction buffer (62.5 mM Tris–Cl, pH 6.8) using a porcelain mortar and pestle. Total soluble proteins, which served as the enzyme source, were obtained after three centrifugations, each for 20 minutes at 12000 rpm at 4°C. All the protein preparations were made inside an ice bath. Electrophoresis was carried out at 4 ± 1°C with 1.5 mm polyacrylamide gels in a Tris–glycine buffer (pH 8.3) under standard native conditions [14]. A uniform amount (50 µg) of protein, estimated by Lowry et al. (1951) [15] was loaded with the sample buffer, devoid of sodium dodecyl sulfate and b-mercaptoethanol. Samples were then electrophoresed at 50 V through the stacking gel (5%) and at 100 V through the resolving gel (8%). Gel images were scanned by a Canon scanner (Canoscan LIDE210), and the enzyme activity profiles were analyzed by software provided with the gel documentation system (GeneTools, Syngene). Based on the band densitometric difference, variations in the enzyme expression rate were calculated. Pixel quantification data corresponding to the band intensity was obtained as numerical values and used to compare samples with and without flue gas.

(vi) Activity Staining

2.6.1 Superoxide Dismutase (EC 1.15.1.1)

Activity staining for superoxide dismutase (SOD) on gel was carried out following the method of Palanisami and Uma (2011) [16]. The gels were soaked in staining solution containing 50 ml of Tris–Cl (50 mM, pH 8), 10 mg nitroblue trerazolium chloride, 1 mg EDTA, and 2 mg riboflavin for 30 minutes in the dark at 25°C and then illuminated on a light box with white fluorescent light (80 µM photons m^2 s^{-1}) for 30 minutes or until achromatic bands appeared.

(vii) Esterase (EC 3.1.1.1.)

Activity staining for esterases (EST) was performed by placing the gel in 100 ml of phosphate buffer (100 mM, pH 6.2) containing 50 mg α- napthyl acetate, 50 mg β -napthyl acetate and 100 mg fast blue RR salt. The gel was incubated at 25°C for one hour to develop black, red, or magenta color bands (Palanisami and Uma, 2011) [16]. Both the substrates (a-napthyl acetate and b-napthyl acetate) were dissolved quickly in 1 ml of acetone and mixed with the buffer solution just before the gel was transferred.

(viii) Polyphenol Oxidase (EC 1.10.3.1)

Activity staining for polyphenol oxidase was carried out following the method of Palanisami and Uma (2011) [16]. The resolved gel was incubated in 100 ml of phosphate buffer (20 mM, pH 7) containing 13.15 mg of DOPA (3,4-dihydroxy phenylalanine). The reaction was allowed to take place at 30°C in the presence of 233 µl of 30% hydrogen peroxide until the appearance of intense brown bands.

(ix) Glutamine Synthetase (EC 6.6.1.2)

Activity staining for glutamine synthetase (GS) was carried out based on the method of Gennady (2003) [17]; the concentration of substrates and buffer volume was modified as follows. Solution A was prepared with 50ml of 0.2 M Tris buffer pH 8, 200 mg of L-glutamic acid, 80 mg of ATP, 2 mg of MgCl2, and 0.2 ml of NH4 OH, and the pH was adjusted to 9.3. The prepared solution was applied to the gel surface and incubated at 37°C for one hour. Next, the gel was covered with 50% of acetone for 15 minutes at room temperature and rinsed with deionized water. The gel was then incubated in Solution B, which was comprised of 40 ml of distilled water and 1.1 ml of sulfuric acid, 125 mg of ammonium molybdate, and 1.2 g of L-Ascorbic acid; finally, the solution was made up to 50ml using distilled water. The incubation was extended until the appearance of blue bands.

RESULTS AND DISCUSSION

The CO_2, SO_x, and NO_x concentrations of the flue gas were analyzed periodically, and it was observed that the flue gas composition might

vary slightly with the quality of the coal used in the process. The detailed composition of the flue gas supplied to the ponds, along with the composition of flue gas generated from combustion of a different fuel, is given in Table 1.

Table 1
Flue gas composition for different fuel sources

	Our study	Subbituminous coal	Natural gas	Diesel fuel
CO2 (%)	14.4	24	13.1	62
SO_x (ppm)	282.1	929	0	113.1
NO_x (ppm)	589.5	240	22.1	169.7

It can be observed that the maximum CO_2, SO_x, and NO_x concentrations in the flue gas from the power plant were 14%, 250 ppm, and 550 ppm, respectively. The flue gas was cooled from 200°C to 15-30°C by mixing it with compressed air. The concentrations of SO_x present along with 1%, 2%, and 4% of CO_2 in flue gas were 20.1 ppm, 40.2 ppm, and 80.4 ppm, respectively. Similarly, the NO_x concentrations were 41 ppm, 82.5 ppm, and 165 ppm for the 1%, 2%, and 4% dilution of flue gas. The presence of SO_x and NO_x can instigate toxicity for microalgal growth[18,35]. For *Chlorella* species, the presence of NO_x had not inhibited the growth as reported by several researchers [7,18,9], while a lower initial inoculum concentration of less than 1 g/l can reduce the growth. However, SO_x did significantly affect the growth of the microalgae, depending upon its concentration in the flue gas in our study. It was reported that *C. cladirium* and *Galdeiria palidata* showed good tolerance for 50 ppm of NO_x but were inhibited by 50 ppm of SOx[19]. In fact, it is interesting to note that the SOx tolerance level for *Chlorella* sp. was reported to be 60 ppm [20], which falls within the range of SO_2 observed under the conditions of a 2% and 4% CO_2 supply in our study.

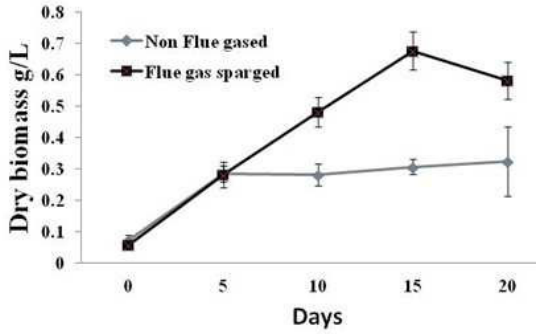

Figure 1
Effect of flue gas with 1% CO_2 on microalgae growth

The effect of adding flue gas at a 1% CO_2 concentration on the microalgal growth is represented in Figure 1. The biomass concentration of the ponds had increased significantly with the addition of flue gas after day 6. Growth conditions, biomass productivity, and the carbon fixation rate for the algal ponds supplied with flue gas containing 1% CO_2 for the months of May, July, August, and October are represented in Table 2.

Table 2
Growth conditions and productivity across the summer of 2010 (with 1% CO_2)

	pH	Water temp °C	light (lux)	g/m^2/day	g/l/day	CO_2 fixation rate
May	9.43	23.90	966.40	11.39	0.031	0.05
July	8.79	24.00	1106.00	8.23	0.022	0.04
August	8.64	29.95	1139.57	12.02	0.032	0.05
October	8.62	16.20	1099.48	6.31	0.017	0.03

In our study period, the month of August showed the maximum yield. From analyzing the species variation across the summer (data not shown), it can be postulated that the predominant species in a particular batch determined the yield and the rate of CO_2 fixation for that specific batch. The pH value in Table 2 represents the pH of the pond at the beginning of the CO_2 sparging. After three hours of CO_2 supply, the pH had dropped by approximately 0.4-0.6 units, and it rebounded to its initial value in another three hours. While it is common to observe productivities like 15-30 g/m^2/day [3,21] in raceway ponds, our system had a productivity ranging from 6.3 to 11.3 g/m^2/day depending upon the monthly weather conditions. The CO_2 fixation rate was calculated from the carbon content of the algae and the amount of carbon present in the flue gas. The maximum CO_2 fixation rate was 0.05 g of carbon/l/day or 0.18 g of CO_2/l/day. The fixation rate was observed to be a function both of the species and the growth system. The tolerance of a high percent of CO_2 is also dependent on the species cultivated in the ponds, Chiu et al. (2008) [22] reported complete growth inhibition of *Chlorella* sp. and *Nannochloropsis oculata* at 5% CO_2 concentration. In contrast, *Chlorella kessleri*, *Scenedesmus obliquus*, *Spirulina* sp., *Chlorella vulgaris*, and *Chlorella sorokiniana* grew best under 6% CO_2 concentration [23]. Hanagata et al. (1992) [24] grew *Scenedesmus* sp. and *Chlorella* sp. and reported that both organisms presented a productivity rate of 0.15 g/l/d with 10% carbon dioxide and 0.18 g/ l/ d with 40% carbon dioxide.

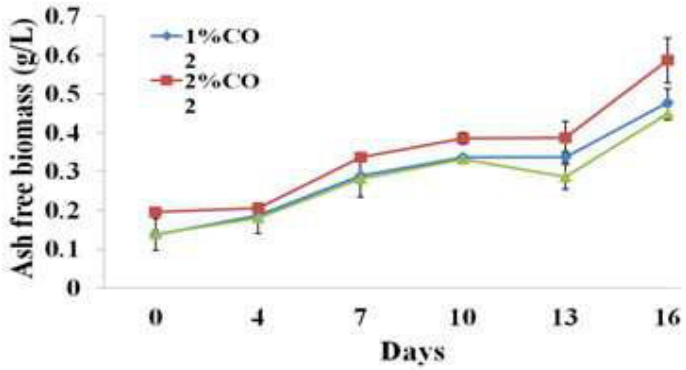

Figure 2a
Effect of different concentrations of CO_2 in flue gas on productivity

To determine the effect of higher concentrations of CO_2 in the flue gas on the biomass yield, the flue gas mixture was diluted with air to final concentrations (1%, 2%, and 4%) of CO_2. It can be observed from Figure 2a that the flue gas containing 2% CO_2 performed better than the ponds containing 4% CO_2 flue gas. Possible explanations for this anomaly are either that the drastic pH change associated with the 4% CO_2 supply hindered growth or that the relatively higher concentrations of SO_x and NO_x associated with the 4% CO_2 supply were detrimental. The pH drop of 1.6-1.7 units (from 8.5 to 6.8) over the three-hour duration of 4% CO_2 feeding might have stressed the culture when compared to the 2% CO_2 supply, where the pH drop was only 0.9-1 unit (from 8.5 to 7.5) over the same three-hour duration. To further investigate the inhibition of growth due to higher concentrations of SO_x and NO_x, we conducted a lab-scale study mimicking the conditions observed at the power plant site in a small aquarium-type growth system. The depth of the tanks was maintained at 30 cm. The growth curve for different concentrations of pure CO_2 alone (1%, 2%, and 4%), without SO_x and NO_x is shown in Figure 2b.

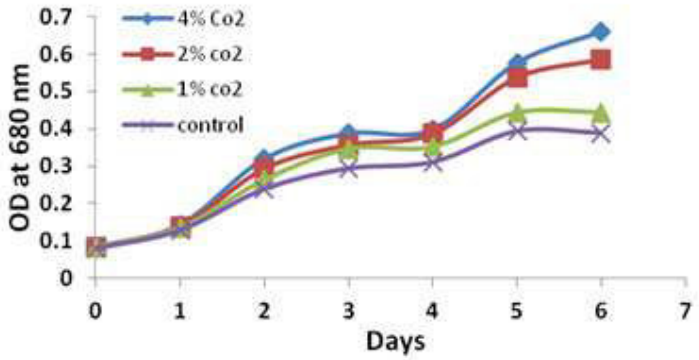

Figure 2b
Effect of different concentrations of pure CO_2 on productivity

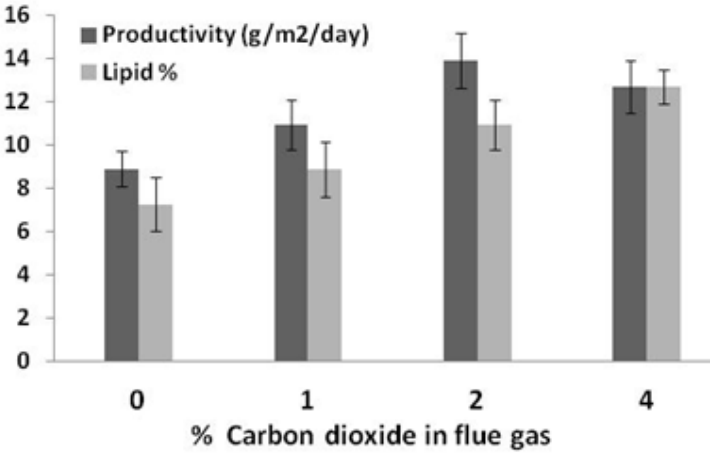

Figure 3a
Lipid increase and biomass productivity decrease upon an increase in CO_2 concentrations of flue gas

It was observed that the higher concentration of CO_2 resulted in an increased biomass (measured as OD) in contrast to our result at a large scale with direct flue gas, confirming the impact of SO_x and, NOx on the growth. It is interesting to find that the increase in CO_2 concentration had increased the lipid concentration of the biomass in spite of lesser productivity, as shown in Figure 3a. A possible explanation for the increase in lipid concentration is the increased production of unsaturated fatty acids at higher CO_2 concentrations. The increase in CO_2 concentration might have affected the enzymatic desaturation [25] and increased the content of polyunsaturated fatty acids. On the other hand, increasing the concentration of CO_2 results in a lower pH that adversely affects the lipid accumulation in some species. The higher pH also has a higher amount of available bicarbonate for carboxylation of lipid synthesis, suggesting that a high concentration of CO_2 decreases the lipid accumulation. Sheng et al. (2009) [26] also observed a decrease in the lipid content of *Nannochloropsis oculata* at elevated CO_2 levels of 5%, 10%, and 15%. A detailed lipid profile of the consortium cultivated in our pilot plant would yield further information that might explain the increase in lipids. A literature search reveals scant research on the use of open circular ponds for microalgal cultivation with the aid of flue gas as the CO_2 source. Our notion was to use the locally dominated indigenous algal species for the biomass production and to remediate the toxic entities in the flue gas. In this context, the indigenous organism might have the ability to grow at a

preferred locality and withstand the prevailing climatic conditions. Reports show evidence that organisms such as *Senedesmus dimorphus* are able to tolerate CO_2 concentrations of 10–20%, NOx concentrations of 100–500 ppm, and SO_2 at 100 ppm from simulated flue gas using indoor cultivation [27]. Instead, our tested experimental consortium tolerated 20.1-40.2 ppm SOx and 41-82.5 ppm NOx. The consortium underwent an initial lag time to acclimatize and then continued growing (see Fig. 1). The initial similarity between the flue-gassed and non-flue-gassed cultures until day 6 might result from the fact that cell density until day 6 was not high enough to make use of the additional CO_2 supplied to the ponds. This result parallels earlier reports [4,5,6] that had tested direct flue gas from various industries as a CO_2 source for algal production. The pH of the medium was determined by the ionic concentration of different species, namely aqueous CO_2 $CO_2(aq)$, carbonic acid (H_2CO_3), bicarbonate (HCO^{-3}), and carbonate (CO_2^{-3}), which are formed when the CO_2 dissolves in water. Depending upon the water temperature, pressure, and the concentration of the above-mentioned ions, the following chemical equilibrium would be reached as soon as the flue gas entered the pond: $CO_2(aq) + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H+ + HCO^{-3} \leftrightarrow 2H+ + CO^{-2}$. The addition of CO_2 decreased the pH of the pond. As microalgae grow, $CO_2(aq)$ is removed from the surrounding media, which causes the equilibrium to shift to the left and the pH to rise. Similarly, the breakdown of bicarbonate with the release of the OH^- during photosynthetic CO_2 uptake also increases the pH. The optimum pH level for microalgae is species specific. For example, the optimum pH level for *Chlorella* sp. and the *Scenedesmus* sp. is about 7, and the pH maintained in our ponds was clearly not optimal. Maintaining an optimal pH using feedback-controlled automated CO_2 addition might increase the productivity. Other than the CO_2, the presence of SO_x and NOx after they dissolve in the water might further complicate the pH equilibrium and hence the overall productivity. While it is common to observe productivities like 15-30 g/m^2/day [3,21] in raceway ponds, our system had a productivity ranging from 6.3 to 11.3 g/m^2/day depending upon the monthly weather conditions. The productivity values given in Table 2 are typical for a circular open pond system when mixing is done infrequently. Deep circular pond systems without mixing are intrinsically low productive systems due to light penetration, gas transfer, and temperature control issues. Nevertheless, these growth systems can be applied at large-scale cultivation if land costs are low, water is free, there are low pumping costs, and the climate is close to optimum, so that production can be achieved year round. The applicability of deep open ponds to mitigate CO_2 from power plants needs to be further validated using detailed economic evaluations and efficient low cost mixing equipment. A deep pond system can also be a viable option when the ponds are operated at low cell density, by continuously harvesting and diluting the culture, which can achieve high growth rates comparable to raceway ponds. A direct correlation between the productivity and water temperature was observed, except for the month of July. The optimal temperature for microalgal growth can vary with species but it was observed that *Chlorella* sp. revealed maximum growth around 30°C [5]. Evidently the lower temperature (16°C) and lower light intensity during October reduced the productivity. Efforts to use waste heat from a power plant to heat the ponds during winter are needed to maintain an optimum temperature for growth. To obtain a supply of warm water year round, it has been proposed that the waste heat in the cooling water needed for nuclear plants be used for algal cultivation. Light intensity is a crucial factor for the growth rate; a lack of this intensity had reduced the biomass productivity during all the months tested, except August (see Table 2). The CO_2 fixation rate was calculated from the carbon content of the algae and the amount of carbon present in the flue gas. The maximum CO_2 fixation rate was 0.05 g of carbon/l/day or 0.18 g of CO_2/l/day. The fixation rate was observed to be a function of species and the growth system. At lab scale, Watanabe and Hall (1995) [2]8 reported a carbon fixation rate of 14.6 g C m^2/day, [19] reported a range of 0.65–4.0 g CO_2 l/day at growth rates of 0.4–2.5 g dry wt/l/day, and for *Scenedesmus obliquus*, the maximum rate was reported to be 0.288 g/l/day. As the rate of CO_2 fixation is directly related to the biomass productivity of the system, controlled system

like photobioreactors and strains that are tolerant of high CO_2 can increase the fixation rate. For example, CO_2 fixation in a thin layer bubble column photobioreactor growing *Chlorella* sp. occurred at the rate of 4.4 g/l/day [7], and it was also reported that growth in the membrane-photobioreactor was 0.95–5.40 times higher than that in the airlift, bubble column, and membrane-contactor reactor for *Chlorella* sp., under optimal conditions [8]. The maximum CO_2 biofixation rates of *C. pyrenoidosa* SJTU-2 were over 0.223 g/l/d with 5% CO_2 concentrations and that of *C. vulgaris* was 0.252 g/l/day at 5% CO_2 using flask cultures [29]. The fixation rate also depends on the flow rate of the gas used in sparging. It was also observed that a lower flow rate had increased the gas retention rate while a high flow rate had increased the mixing and light exposure; nevertheless, lower flow rates had proven to increase the productivity [8].

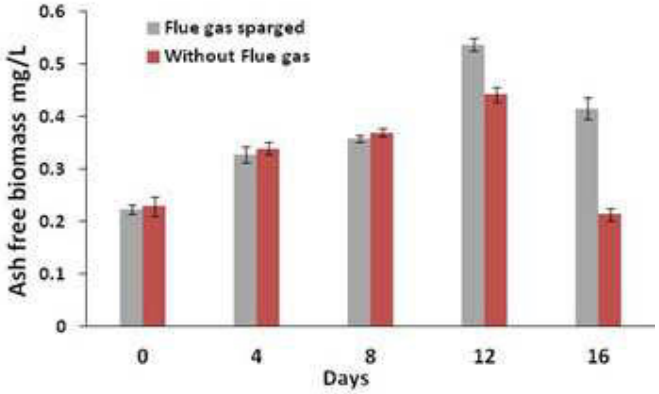

Figure 3b

Effect of flue gas with 2% CO_2 on the growth of Desmodesmus communis LUCC 001

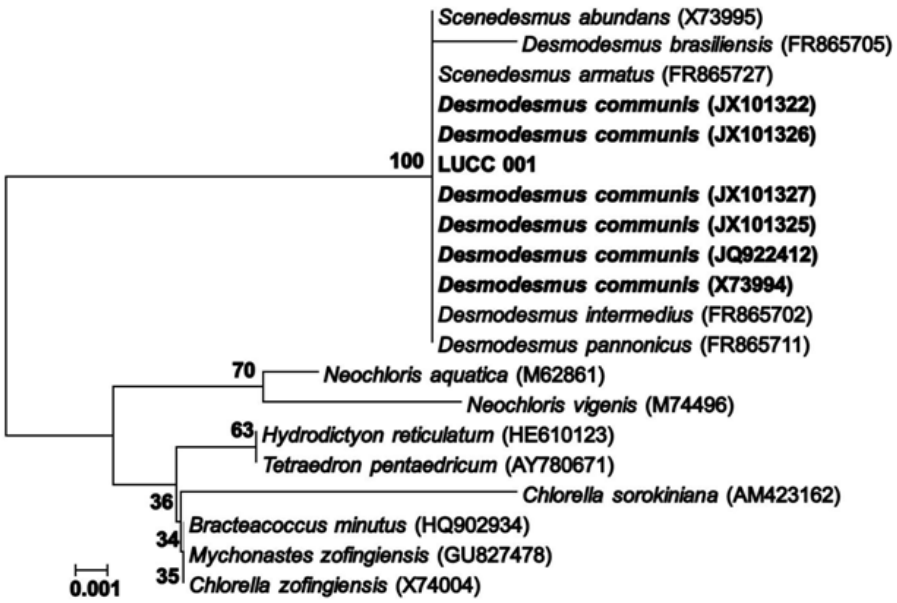

Figure 4

Neighbor-joining phylogenetic tree inferred from 1000 replicates based on the 18S rRNA gene sequence of Desmodesmus communis LUCC 001 with phylogenetic neighbors. Bootstrap values are indicated at nodes. Scale bar represents the observed number of changes per nucleotide position.

Based on the morphological and 18S rRNA gene sequence analysis, the microalgal strain used in this study was identified and designated as *Desmodesmus communis* LUCC 001. This is clearly evident using phylogenetic analysis (see Fig. 4) that shows a 100% similarity with type strains of *Desmodesmus communis.* The unialgal inoculum of *Desmodesmus communis* LUCC 001 showed increased growth when compared to the non-flue-gassed control (see Fig. 3b). While checking the growth rate every four days, up to day 8, the growth was similar to the non-flue-gassed control. The boost in growth and a distinct increase (17.6%) in biomass content was evident at day 12. Besides CO_2, flue gas can contain up to 142 different compounds [30] such as NO_x, sulphur oxides (SO_x), unburned carbohydrates, heavy metals, halogen acids, and particulate matter. Flue gas treated to current emission threshold values still contains non-negligible concentrations of several compounds, some of which can be toxic to microalgae, e.g., SO_2 [20], while others can be metabolized by microalgae. However, *Desmodesmus communis* LUCC 001 demonstrated an ability to grow at the provided concentration of flue gas, which was comprised of 2% CO2.

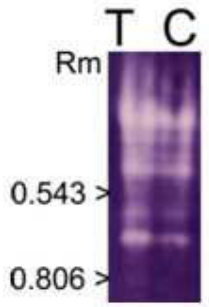

Figure 5a
Effect of flue gas on the activity of superoxide dismutase. T represents flue-gassed and C represents the non-flue-gassed control.

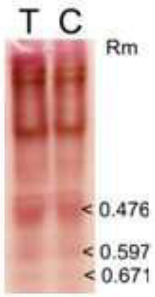

Figure 5b
Effect of flue gas on the activity of esterase. T represents flue-gassed and C represents the non-flue-gassed control.

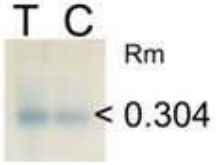

Figure 5c
Effect of flue gas on the activity of glutamine synthetase. T represents flue-gassed and C represents the non-flue-gassed control.

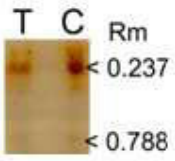

Figure 5d
Effect of flue gas on the activity of polyphenol oxidase. T represents flue-gassed and C represents the non-flue-gassed control.

Furthermore, while checking the stress-stabilizing ability of the species against the other toxic entities in flue gas, a native PAGE analysis of superoxide dismutase pronounced an overall activity increase in all the isoforms of the flue-gas-sparged condition. Especially, the bands appeared at the relative mobility (Rm) 0.543 and 0.806 are newly expressed (see Fig. 5a), as evidence of the capability of the organism to persevere and its ability to overcome oxidative stress. In the case of esterase, the three isoforms stained at Rm 0.476, 0.597, and 0.671 showed an increase in activity. The nitrogen-metabolizing enzyme, glutamine synthetase, showed elevated activity at the flue-gassed condition (see Fig. 5c). Unlike the other three tested enzymes, polyphenol oxidase showed a decrease in activity in the flue-gas-sparged sample. It is essential to understand the stress-stabilizing physiology, which will pave the way to improve the biomass yield by altering the growth conditions. It is also obvious that flue gas contains a number of other chemical entities, which may act as inducers or inhibitors for certain enzyme systems. One unexpected effect was that the nitrogen metabolizing enzyme, glutamine synthetase, increased its activity while sparging flue gas. This consequence might have occurred individually or by the synergistic action of various flue gas entities. As a net result, this was advantageous to the organism by enhancing its nitrogen utilization. The enhanced activity of superoxide dismutase, which revealed the stressed status of the organism, also revealed the organism's capacity to overcome obstacles via its newly expressed isoform, which is a mechanism of stabilization. It is convincing when correlating the SOD expression of *Desmodesmus communis* LUCC 001 with the glutathione peroxidase of *Phormidium valderianum* BDU 140441 [16], the two newly expressed isoforms of SOD might have played a pivotal role in stabilizing the oxidative stress instigated by the toxic flue gas entities. Esterase isoform expression is a critical biomarker for heavy metal toxicity [31]. *Desmodesmus communis* LUCC 001 did not produce new isoforms of esterase; instead esterase regulated the stabilization mechanism by the existing constitutive isoforms, which was evident by the isoforms at the Rm 0.476, 0.597, and 0.671, which had 16.8%, 5.88%, and 14.28% increased activity, respectively (see Fig. 5b). CO_2 is a competitive inhibitor of polyphenol oxidase [32] ; this characteristic feature of polyphenol oxidase makes it one of the biomarkers to check the fate of the CO_2 effect (see Fig. 5d). Activity of polyphenol oxidase found decrease on the flue gas sparged condition. The interference or reactivity of chitosan on the activity of polyphenol oxidase can be suspected since chitosan is a kind of inhibitor to polyphenol oxidase [33]. For the purpose of testing the enzyme activity; algal biomass has been harvested by centrifugation, not by chitosan flocculation, hence it is obvious that the decreased activity could be influenced by other flue gas chemical entities.

CONCLUSION

Carbon dioxide is the major cost constraint for large-scale microalgal cultivation. Utilization of flue gas is an ideal practice that has dual benefits, being used for mitigation and for waste utilization. For outdoor cultivation, indigenous strains are the perfect candidates as they can withstand the local weather conditions without allowing invasion by other algal species. Further investigations into the influence of flue gas heavy metals, water quality used in the outdoor medium and the effect of other microbial communities are in

progress. Furthermore, formulating a perfect outdoor medium is essential for avoiding invasion and successful outdoor cultivation. As the present study is a venture of selecting a dominant indigenous species, commercially available F/2 media (meant for marine organisms) was used, so as to keep the determined nutrient concentrations. Overall, this study paves the way to create a safe method that simultaneously deals with waste utilization and biomass yield.

ACKNOWLEDGEMENT

This study was financially supported by the United States Department of Energy. The authors are indebted to the Chamois power plant employees for their excellent support in conducting the outdoor experiments. The authors are also thankful to Mr. Gregory Bradford, Mrs. Jamie Hill, and Mr. Jon Haslag for their proofreading to improve the manuscript.

REFERENCES

1. Chinnasamy S, Bhatnagar A, Hunt and Das K. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications, Bioresour Technol, 101: 3097–3105, (2010).
2. Buehner MR, Young PM, Willson B, Rausen D, Schoonover R, Babbitt G and Bunch S, Microalgae growth modeling and control for a vertical flat panel photobioreactor. IEEE 2301-2306, (2009).
3. Benemann J and Oswald W, Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. Technical report, Department of Energy Pittsburgh Energy Technology Center. Final report (1996).
4. Douskova I, Doucha J, Livansky K, Machat J, Novak P, Umysova D, Zachleder V and Vitova M, Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs, Appl Microbiol and Biotechnol, 82: 179-185, (2009).
5. Maeda K, Owada M, Kimura N, Omata K and Karube I, CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae. Energ Conver and Manage, 36: 717-720, (1995).
6. Negoro M, Hamasaki A, Ikuta Y, Makita T, Hirayama K and Suzuki S, Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Appl Biochem and Biotechnol, 39: 643-653, (1993).
7. Doucha J, Straka F and Lívanský K, Utilization of flue gas for cultivation of microalgae *Chlorella* sp. in an outdoor open thin-layer photobioreactor, J of Appl Phycol, 17: 403-412, (2005).
8. Fan LH, Zhang YT, Cheng LH, Zhang L, Tang DS and Chen HL, Optimization of carbon dioxide fixation by *Chlorella vulgaris* cultivated in a membrane photobioreactor, Chemical Eng and Technol, 30: 1094-1099, (2007).
9. Matsumoto H, Shioji N, Hamasaki A, Ikuta Y, Fukuda Y, Sato M, Endo N and Tsukamoto T, Carbon dioxide fixation by microalgae photosynthesis using actual flue gas discharged from a boiler. Appl Biochem and Biotechnol, 51: 681-692, (1995).
10. Yang Y and Gao K, Effects of CO_2 concentrations on the freshwater microalgae, *Chlamydomonas reinhardtii, Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). J of Appl Phycol, 15: 379-389, (2003).
11. Fan LH, Zhang YT, Zhang L and Chen HL, Evaluation of a membrane-sparged helical tubular photobioreactor for carbon dioxide biofixation by *Chlorella vulgaris,* J of Membrane Sci, 325: 336-345, (2008).
12. Bligh E and Dyer W, A rapid method of total lipid extraction and purification, Canadian J of Biochem and Physiol, 37: 911-917, (1959).
13. Moro CV, Crouzet O, Rasconi S, Thouvenot A, Coffe G, Batisson I and Bohatier J, New design strategy for development of specific primer sets for

PCR-based detection of *Chlorophyceae* and *Bacillariophyceae* in environmental samples. Appl and Environ Microbio, 75: 5729–5733, (2009).
14. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 277: 680–685, (1970).
15. Lowry OM, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. J of Biol Chem, 193: 265–275, (1951).
16. Palanisami S and Uma L, Role of copper in poly R-478 decolorization by the marine cyanobacterium *Phormidium valderianum* BDU140441. World J of Microbiol and Biotechnol, 27: 669–677, (2011).
17. Gennady PM, Handbook of Detection of Enzymes on Electrophoretic Gels. CRC Press, Washington, DC, 2003, pp. 531.
18. Hauck JT, Olson GJ, Scierka SJ, Perry MB and Ataai MM, Effects of simulated flue gas on growth of microalgae. Abstract (1996) 25–29. American Chemical Society, Orlando, Florida, United States.
19. Kurano N, Ikemoto H, Miyashita H, Hasegawa T and Miyachi S, Carbon dioxide uptake rate of *Chlorococcum littorale,* Oceanographic Literature Review, 43: 953, (1996).
20. Lee JS, Kim DK, Lee JP, Park SC, Koh JH, Cho HS and Kim SW, Effects of SO_2 and NO on growth of *Chlorella* sp. KR-1. Bioresour Technol, 82: 1-4, (2002).
21. Sheehan J, Dunahay T, Benemann J and Roessler P, A look back at the US Department of Energy's Aquatic Species Program—biodiesel from algae. National Renewable Energy Laboratory, Golden, CO; Report NREL/TP 580–24190, (1998).
22. Chiu SY, Kao CY, Chen CH, Kuan TC, Ong SC and Lin CS, Reduction of CO_2 by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor, Bioresour Technol, 99: 3389-3396, (2008).
23. De Morais MG and Costa JAV, Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide, Energ Conver and Manage, 48: 2169–2173, (2007).
24. Hanagata N, Takeuchi T and Fukuju Y, Tolerance of microalgae to high CO_2 and high temperature, Phytochemistry, 31: 3345-3348, (1992).
25. Vargas MA, Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. J of Phycol, 34: 812–817, (1998).
26. Sheng YC, Chien YK, Ming TT, Seow CO, Chiun HC and Chih SL, Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. Bioresour Technol, 100: 833–838, (2009).
27. Yinli J, Wei Z, Junfeng W, Yu C, Shuhua S and Tianzhong L, Utilization of simulated flue gas for cultivation of *Scenedesmus dimorphus* Bioresour Technol, 128: 359–364, (2013).
28. Watanabe Y and Hall DO, Photosynthetic CO2 fixation technologies using a helical tubular bioreactor incorporating the filamentous cyanobacterium *Spirulina platensis.* Energ Convers and Manage, 36: 721–724, (1995).
29. Sydney EB, Wilerson S, Julio CC, Vanete TS, Christian L, Ashok P and Carlos RS, Potentialcarbon dioxide fixation by industrially important microalgae. Bioresour Technol, 101: 5892-5896, (2010).
30. Simoneit BRT, Rogge WF, Lang Q and Jaffe R, Molecular characterization of smoke from campfire burning of pine wood (*Pinus elliottii*). Chemosphere, 2: 107–122, (2001).
31. Suman M, Swati M, Bhattacharyya P and Duttagupta AK, Heavy metal levels and esterase variations between metal-exposed and unexposed duckweed *Lemna minor*: Field and laboratory studies. Environment International, 30: 811–814, (2004).
32. Jon SC, Murat OB, Cheng IW, Maurice RM and Wei YH, Inactivation of polyphenol oxidase by high-pressure carbon dioxide, J of Agri and Food Chemist, 40: 2345–2349, (1992).
33. Jiang Y and Li Y, Effects of chitosan coating on postharvest life and quality of

longan fruit, Food Chemist, 73: 139–143, (2001).

34. De Morais MG and Costa JA, Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. J. Biotechnol, 129: 439–445, (2007).
35. Gandhi N, Sirisha D, Mary Priyanka V, and Arthisree SR, Adsorption studies on mixed algae to control SO_2 and NO_2 pollution, Int J of Pharma and Bio Sci, 3: Oct; 304 – 310, (2012).