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# Utilization of Flue Gas as Carbon Source to Grow *Scenedesmus quadricauda* for Simultaneous $CO_2$ Reduction and Biodiesel (FAME) Production

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**ABSTRACT:** In the present study *Scenedesmus quadricauda* was grown in laboratory flasks and outdoor open raceway ponds using industrial flue gas as carbon source to achieve $CO_2$ reduction from the flue gases along with biomass, lipid and FAME (biodiesel) yields. Three sets of experiments were carried out out of which two experiments were carried out in laboratory culture flasks using 6% flue gas-air mixture at a flow rate of 0.1vvm for a minute per every half an hour and per every one hour. The third experiment was planned in the outdoor open raceway ponds using 6% flue gas-air mixture at a flow rate of 0.1vvm for a minute per every one hour. The maximum algal biomass growth of 1.28 g/L, lipid yield of 0.20 g/L and fatty acid methyl esters (FAME) of 0.091 g/L were obtained in 6% flue gas concentrations aerated into culture flasks at 0.1vvm for every one hour. On the other hand the CO2 removal efficiency reached upto 75% along with SOx and NOx reductions upto 50%. Hence in the present study it was observed that the micro alga *S. quadricauda* utilized the flue gas-air mixture for $CO_2$ reduction and in turn it produced biomass, lipids and FAME yields efficiently.

**KEYWORDS:** Flue gas, *Scenedesmus quadricauda*, Open raceway pond, Biomass, Lipid, FAME, Biodiesel.

## 1 INTRODUCTION

The increase of world energy demand and greenhouse gas (GHG) emission has been concerning all sectors since last decades. An economic growth combined with a rising population has led to a steady increase in global energy demands. If the governments around the world stick to current policies, the world will need almost more percent of energy in 2030 than today. Of this 45% will be accounted by China and India together [1], [2]. Since the onset of the industrial revolution about 150 years ago, human activities such as the burning of fossil fuels and deforestation have accelerated, and both have contributed to a long-term rise in atmospheric burning fossil fuel (oil, natural gas and coal) releases carbon into the atmosphere far more rapidly than it is being removed, and this imbalance causes increase carbon dioxide ($CO_2$) concentrations in the atmosphere. Power generation, transport, industry and domestic uses are also contributing for increasing $CO_2$. However; $CO_2$ produced by combustion of fossil fuels is a major source of GHG

A typical coal-fired power plant emits flue gas from their stacks containing 5-15% $CO_2$. In India, In addition to high amounts of $CO_2$, flue gas also contains several other chemical species such as sulfur oxides (referred to as SOx and comprising mostly $SO_2$ and $SO_3$), nitrogen oxides (referred to as NOx and comprising mostly NO and $NO_2$) [8], heavy metal species including Ag, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, and Zn [9], [10], and carbon compounds including polycyclic aromatic hydrocarbons (PAHs) [11].

It is estimated that $CO_2$ emission may be expected to increase at an annual rate of 3% between 2005 and 2030. The long term demand for coal brings with it a demand for technologies that can mitigate the environmental problems associated with coal. While control technologies will be used to reduce air pollutants, no technologies exist today, which address the

problem of GHG emissions. A sustainable approach to the capture and removal of $CO_2$ and retain $CO_2$ from the atmosphere in a self-sustaining manner is the need of the hour. In addition, it is necessary to enhance the carbon sink capacity of the biosphere (e.g. forests). Meeting the energy demand without huge increases in $CO_2$ emissions requires more than merely increasing the efficiency of energy production. Since coal is much more plentiful than oil or gas, reducing coal emissions is absolutely essential to avoid dangerous effect of climate change.

The field of algal cultivation for $CO_2$ fixation and/or its conversion to biofuels is not new, having been suggested as early as 1955 [3], [4] and the earliest internal combustion engines were run on biologically derived molecules (plant oils for diesel engines and bioethanol for spark-ignition engines) until plentiful petroleum- derived fuel stock over due to their lower price. There are many reports on the potential and bio-economics of algal biomass to generate fuels and most of these are based on the premise that one would utilize the $CO_2$ emitted from fossil-fuelled power stations or other industrial sources of $CO_2$ such as cement processing [5], [6]. There are comparatively few, but valuable, studies in the literature exploring algal capture of either simulated or actual flue gas $CO_2$. Microalgae appear to represent the only current renewable way to generate biofuels [7], [13]. Microalgae biofuels are also likely to have a much lower impact on the environment and on the world's food supply than conventional biofuel-producing crops. *Scenedesmus quadricauda* is considered as one of the best candidates for biodiesel production among several microalgae species due to highest total fatty acid and lipid contents of 22.29 mg/g dry weight and 27.4 ± 0.75% respectively, and oleic acid (11.77 mg/g dry weight) [14]. Therefore, it is a promising approach to cultivate *Scenedesmus quadricauda* for nutrient removal and $CO_2$ fixation.

The aim of this research was to utilize the flue gas from coal burning industrial plant as a carbon source to cultivate *Scenedesmus quadricauda* in turn to produce lipids and fatty acid methyl esters (FAME) and to study the effect of various parameters like pH, alkalinity, carbonates, bicarbonates, aqueous $CO_2$, nitrates, sulphates, NOx, SOx, outlet flue gas $CO_2$ on algal biomass growth, lipid and fatty acids of methyl esters (FAME) yields.

## 2 METHODOLOGY

### 2.1 MICRO ALGAE CULTURE AND CULTIVATION

The presence of *Scenedesmus quadricauda* was identified in a local freshwater body by microscopic examinations under Olympus CX21 light microscope according to morphological properties [20] Pure colonies of *S. quadricauda* (Figure. 1) were then isolated and purified by micro-capillary pipetted method [21], serial dilution and plating techniques accordingly.

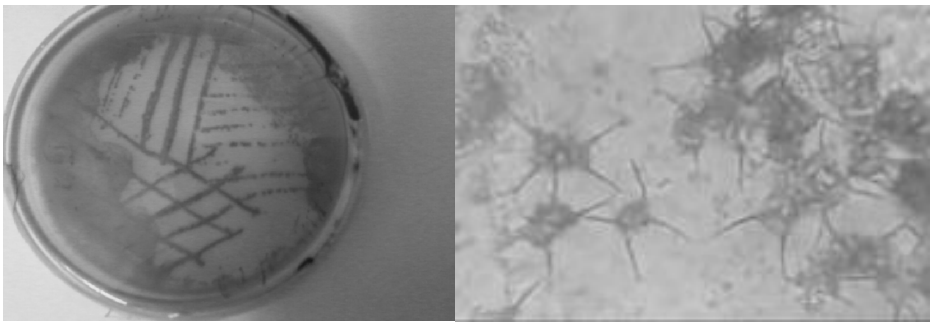

***Figure 1: Representing the streak plates and 100X microscopic view of S. quadricauda***

### 2.2 FLUE GAS SAMPLE COLLECTION

Flue gas sample was collected in 3 liters and 5 liter capacity tedlar bags from a coal burning boiler outlet at Sri Chaitanya Chlorides industrial plant, Isnapur, Hyderabad. The collected gas sample was analyzed for the presence of $CO_2$ (%) by using Gas Chromatography.

### 2.3 ALGAE CULTIVATION AND NUTRITION

The flue gas from coal-fired industrial plant was used to cultivate the microalgae *S. quadricauda* in indoor culture flasks of 500 mL capacity and in outdoor open raceway pond of 200L capacity (2m x 1m x 0.5m).

The nutrient media used to grow the algae was BG-11 and the composition was Citric acid 6.0 mg/L, Ferric ammonium citrate 6.0 mg/L, EDTA 1.0 mg/L, $NaNO_3$ 1.5 g/L, $K_2HPO_4.2H_2O$ 0.051 g/L, $MgSO_4.7H_2O$ 0.075 g/L, $CaCl_2$ 0.024 g/L, $Na_2CO_3$

0.02 g/L, $H_3BO_4$ 2.86 g/L, $MnCl_2.4H_2O$ 1.81 g/L, $ZnSO_4.7H_2O$ 0.222 g/L, $Na_2$ $MoO_4.2H_2O$ 0.391 g/L, $CuSO_4.5H_2O$ 0.079 g/L, $Co(NO_3)_2.6H_2O$ 0.049 g/L. The culture was maintained at 27 ºC temperature and 99 µmol.photons $m^{-2}$ $s^{-2}$ light intensity in a culture rack. Parameters like pH, alkalinity, carbonates, bicarbonates, aqueous $CO_2$, nitrates, sulphates, NOx, SOx, outlet flue gas $CO_2$, algal biomass growth, lipid and FAME content were monitored in flasks using 6% flue gas for every half an hour and one hour in the flasks.

### 2.4 EXPERIMENTAL DESIGN

The flue gas from the coal industrial plant (containing 6% $CO_2$) was diluted with air mixture (6 ml flue gas in 100 ml of air) using compressed air to produce 6% flue gas-air mixture and supplied for a minute once half an hour and for a minute once one hour daily in flasks as well as in open raceway pond. The mixing and aeration was provided by bubbling the flue gas in to microalgae pond systems with the flow rate of 0.1vvm. A pipeline was connected from flue gas filled tedlar bag to the rotameter inlet and another pipeline of air comes from the compressor connected to another rotameter inlet. The outlets of the two rotameters are connected to a common pipe where the flue gas and air are mixed according to the appropriate experiment and passed into the algae medium in both culture flasks and open raceway pond (Figure. 2).

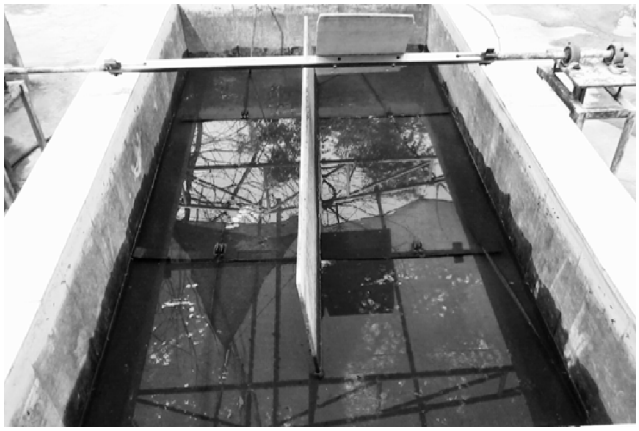

***Figure 2: Representing the Growth of S. quadricauda in open raceway pond***

### 2.5 DETERMINATION OF ALGAL GROWTH

Algal growth was analyzed in terms of optical density (absorption) which was daily read at 680nm using a spectrophotometer. A growth curve was generated based on the optical density readings. Algal dry cell weight was determined daily by filtering 10 mL of the culture sample onto Glass Microfiber Filters, GF/C (Whatman). The filtered sample was then washed with distilled water to remove adhering microalgae biomass, dried at 100°C for 24 h. The dried sample was immediately transferred to desiccators over silica gel for dehydration for at least 2 h before weighing. Cell or biomass dry weight productivity was calculated on a daily basis and/or at the end of the experiment.

### 2.6 DETERMINATION OF TOTAL ALGAL BIOMASS YIELD

Algae were harvested in the culture flasks after they reached late stationary phase by filtering on to a pre weighed dry Whatmann no. 1 filter paper. The algae cells trapped on the filter paper were dried under natural sunlight. Whereas from the raceway ponds the harvesting was carried out using a stainless steel harvester equipped with a double layer nylon polyester cloth to trap the algae. The harvested algae from the ponds were spread evenly on stainless steel plates and let dry under direct sunlight. The dried biomass from both indoor and outdoor cultures were collected and powdered in a mortar and pestle and weighed.

### 2.7 EXTRACTION OF LIPIDS

The lipids were extracted from powdered biomass with hexane. The dry algae was put directly into the solvent and heated under reflux inside a round bottom flask. A hot water bath was used because it makes controlling the temperature of the extraction easy and it ensured uniform heating. The water cooled condenser was connected directly to the round bottom

flask. Solvent (mL) to algae (g) ratio was taken to be 30:1 to ensure efficient extraction. The solvent, algae, and stir bar were combined in the round bottom flask and heated for 60 minutes at 70°C temperature. After the extraction time was up, the round bottom flask was removed from the hot water bath and allowed to cool. Cold water should be running through the condenser for the entire extraction and cool down processes. Once the round bottom flask cooled down, the algae cells were removed using filtration. Whatman no. 1 filter papers were used for the filtrations. After filtering, the lipids and solvent were in a flask. Then, the solvent was evaporated which left the lipids in the flask. The mass of lipids recovered were determined and used to calculate the extraction yield.

### 2.8 TRANSESTERIFICATION

The lipids obtained were subjected to transesterification process. This chemical process was carried out in a 100 ml Erlenmeyer flask. The lipid was first poured into the flask then a mixture of methanol and KOH (2.5 g in 100 ml methanol) was added to the lipid in a ratio of 1:5. The mixture was vigorously shaken for 15-30 minutes till an emulsion was formed. Then the flask was placed in an oven maintained exactly at 65 ºC and left for 12-24 hours until two distinct immiscible layers were formed. After cooling down the entire suspension was transferred into a separating funnel and shaken vigorously for 15-30 minutes for the two layers to settle down. The bottom layer was glycerol which settled down first due to its high density and the top layer was fatty acid methyl esters (FAME) termed as biodiesel. Glycerol was eluted first into a separate flask and the remaining FAMEs were washed twice with distilled and finally collected into a fresh clean 25 ml bottle.

### 2.9 LIPID AND FAME (BIODIESEL) CHARACTERIZATION USING GC-MS

The lipids and FAME obtained from extraction and transesterification were subjected to Gas Chromatography Mass Spectroscopy analysis to know the major components present in both the samples.

## 3 RESULTS AND DISCUSSION

$CO_2$ concentration present in the collected flue gas sample found to be 6% which was used in all the three experiments.

*Scenedesmus quadricauda* cultivated in laboratory culture flasks and outdoor open raceway pond with flue gas inputs delivered the biomass growth (Figure 3), lipid (Figure 4) and FAME (Figure 5) yields in spite of the toxicity of the flue gas components like SOx. The biomass growth, lipid and FAME yields reached maximum on $7^{th}$ day in culture flasks whereas on $12^{th}$ day in open raceway pond. Two different time intervals of flue gas air mixture inputs were studied in the culture flasks which are 0.1vvm of 6% flue gas air mixture inputs for every 30 minutes and for every 1 hour. Best result was noted at every one hour interval. Hence the one hour time interval was applied for raceway pond cultivation. Thus among the three experiments conducted, *S. quadricauda* grown in culture flask aerated with 6% flue gas mixture at 0.1vvm flow rate for every one hour showed little higher (biomass growth 1.28 g/L, lipid 0.20 g/L and FAME 0.091 g/L) as shown in the figures below:

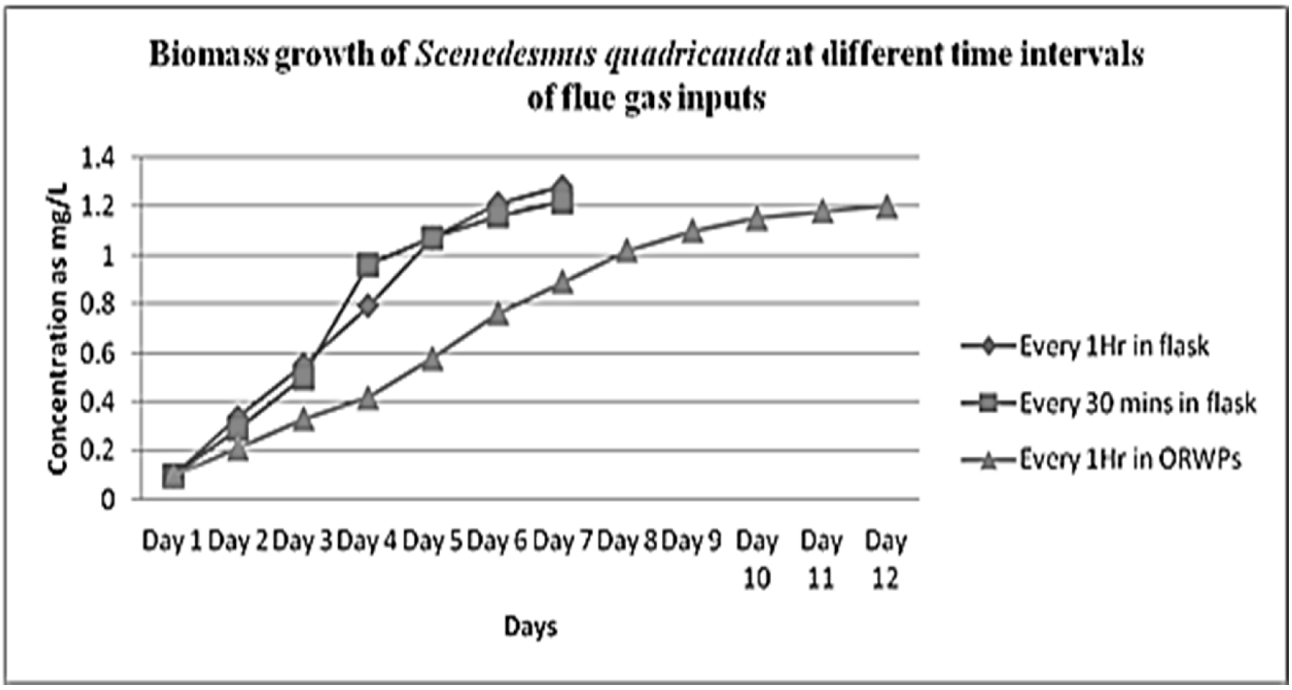


*Figure 3: Graph representing biomass growth of S. quadricauda at different time intervals of flue gas inputs*

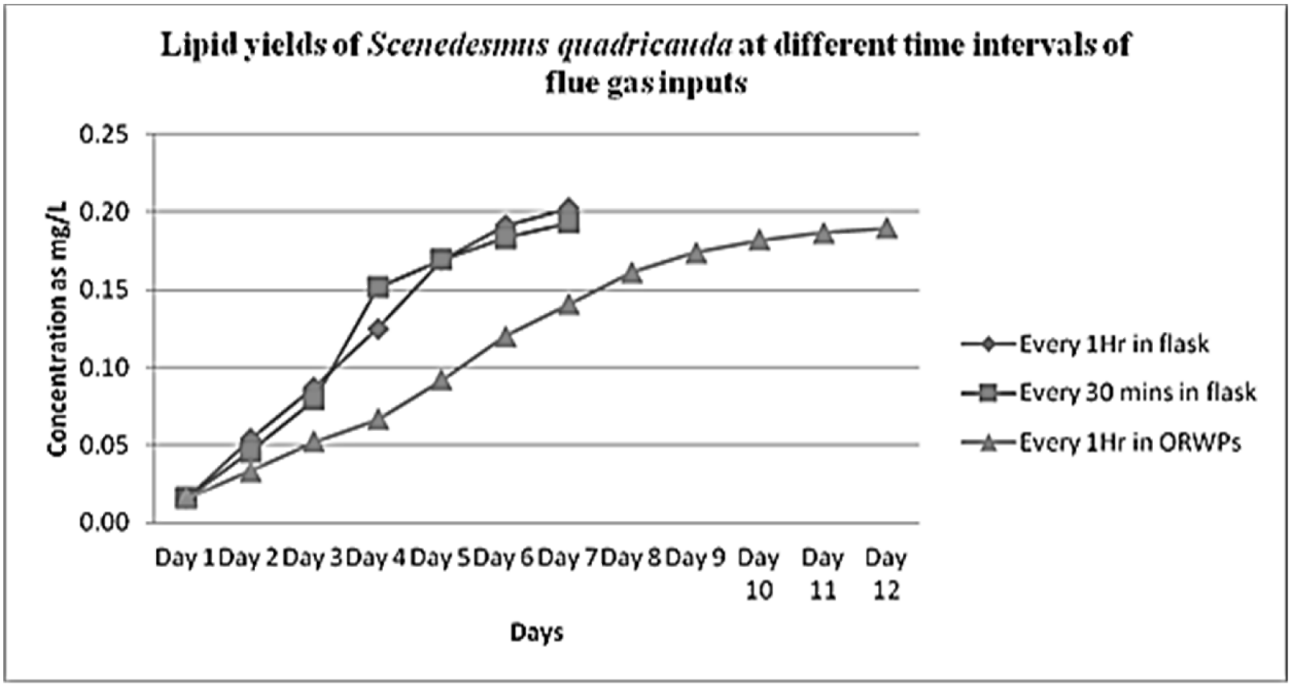


*Figure 4: Graph representing the lipid yields of S. quadricauda at different time intervals of flue gas inputs*

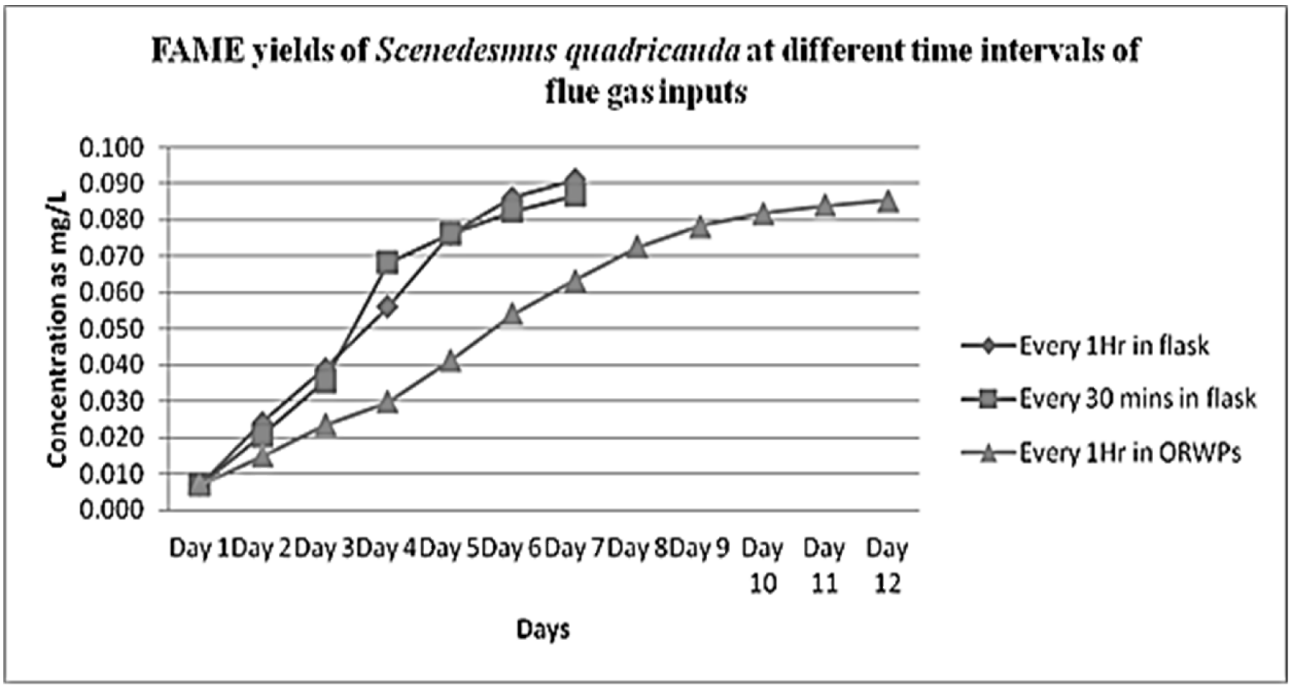


*Figure 5: Graph representing the FAME yields of S. quadricauda at different time intervals of flue gas inputs*

Initial and final concentrations of SOx (Figure 6) and NOx (Figure 7) present in the flue gas were analyzed and it was observed that the concentrations were reduced up to 50% in all the three experiments. The nitrate and sulphate concentrations were monitored daily relating to the sulphur and nitrogen dissolved in the culture medium which was also reduced up to 65% in all the three experiments.

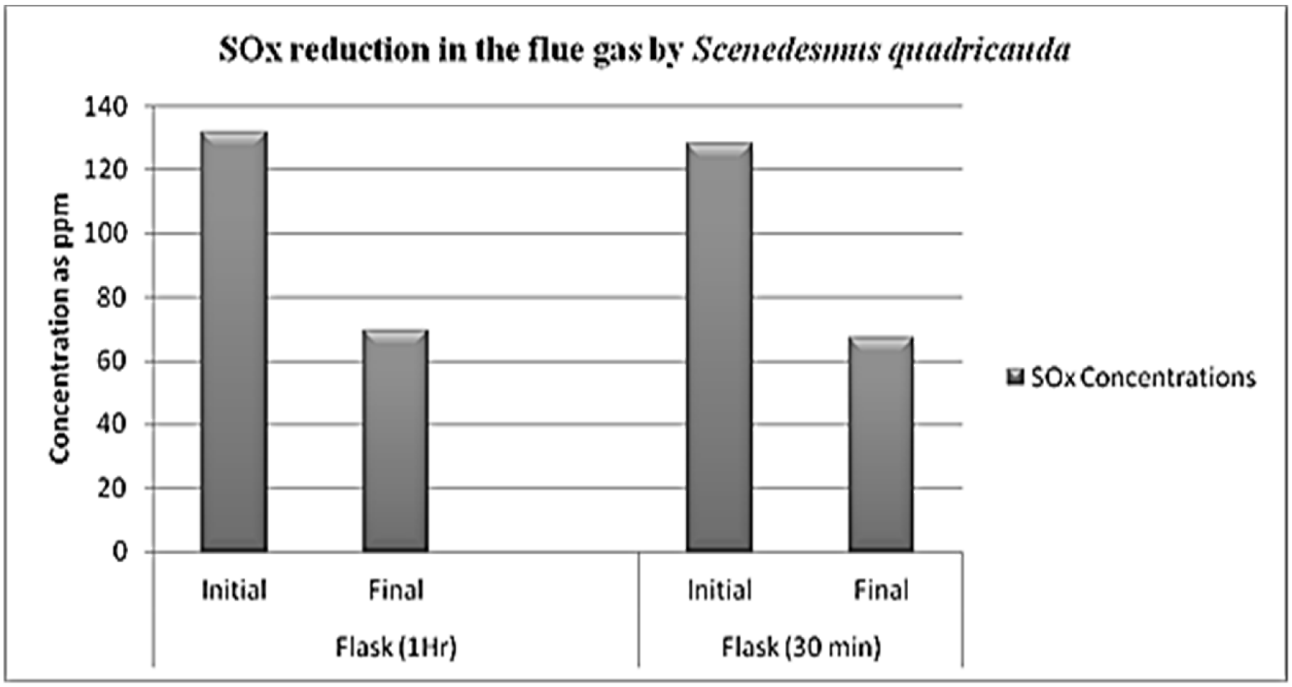


*Figure 6: Graph representing the SOx reduction in the flue gas by S. quadricauda*

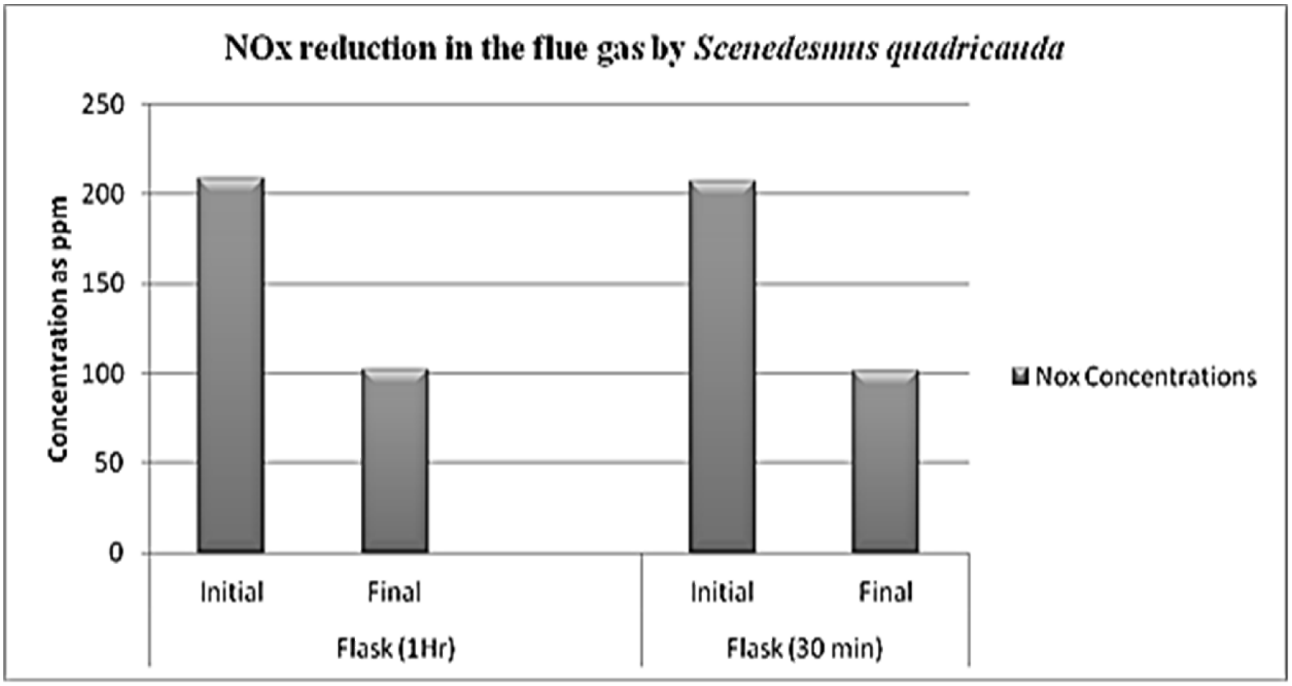


***Figure 7: Graph representing the NOx reduction in the flue gas by S. quadricauda***

In all the three experiments various parameters like alkalinity, carbonates, bicarbonates and aqueous $CO_2$ were analyzed. These parameters concentration varied from day 1 to day 7 in flasks (Figures 8 and 9) and day 1 to day 12 in open raceway pond (Figure 10).

Alkalinity and carbonate ions concentration gradually increased with the increasing growth of *S. quadricauda* during the exponential phase. Bicarbonate ions concentration gradually decreased due to the rapid uptake of $CO_2$ by the fast growing micro algae (Figures 8, 9 and 10).

In all the three experiments, the concentrations of nitrates and sulphates (Figures 8, 9 and 10) were reduced upto 65% from initial day to final day due to their utilization by *S. quadricauda* for its growth and lipid generation. On the other hand the concentration of $CO_2$ in aqueous state reduced up to 70% from initial day to final day due to the utilization of carbon dioxide by the micro algae *S. quadricauda.*

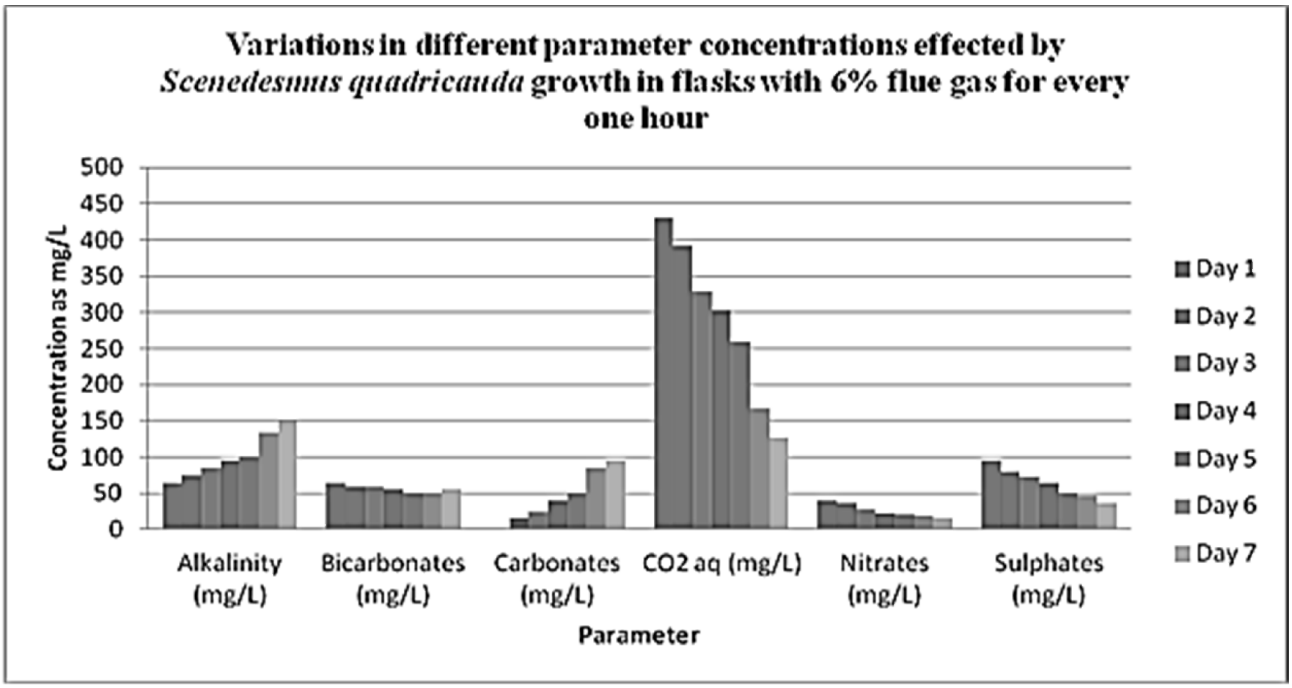


*Figure 8: Graph representing the variations in different parameter concentrations effected by S. quadricauda growth in flasks with 6% flue gas for every one hour*

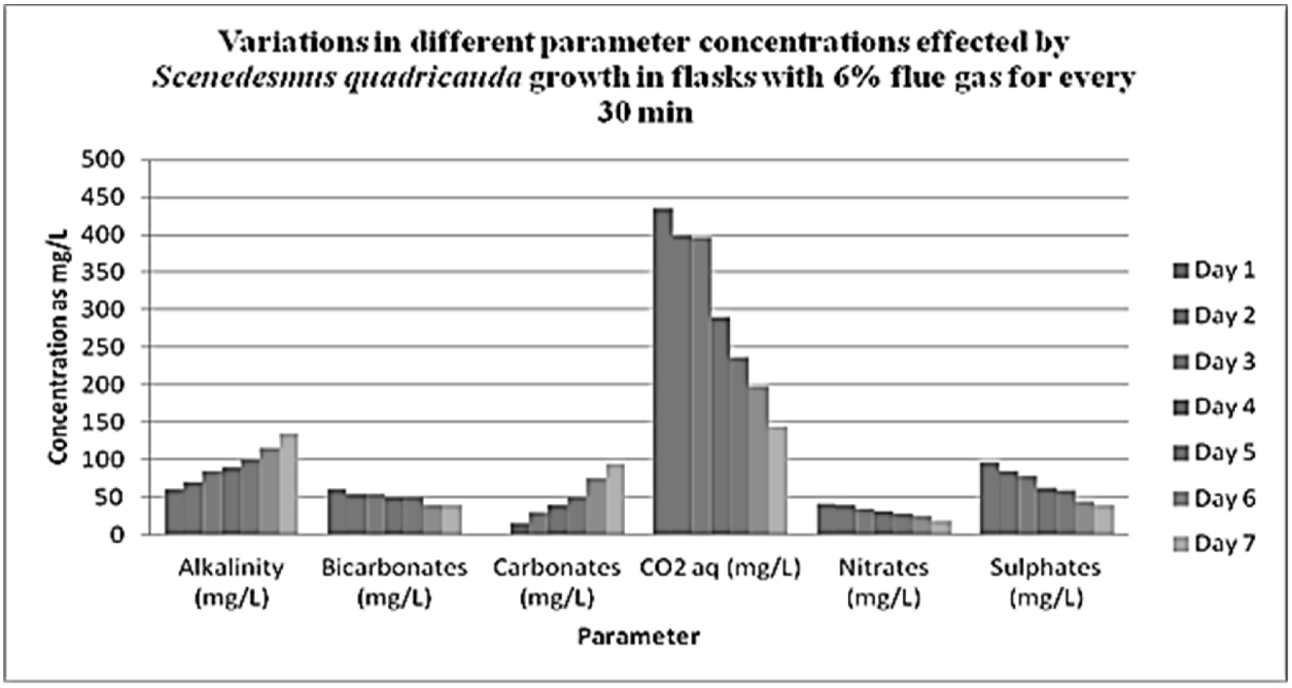


*Figure 9: Graph representing the variations in different parameter concentrations effected by S. quadricauda growth in flasks with 6% flue gas for every 30 min*

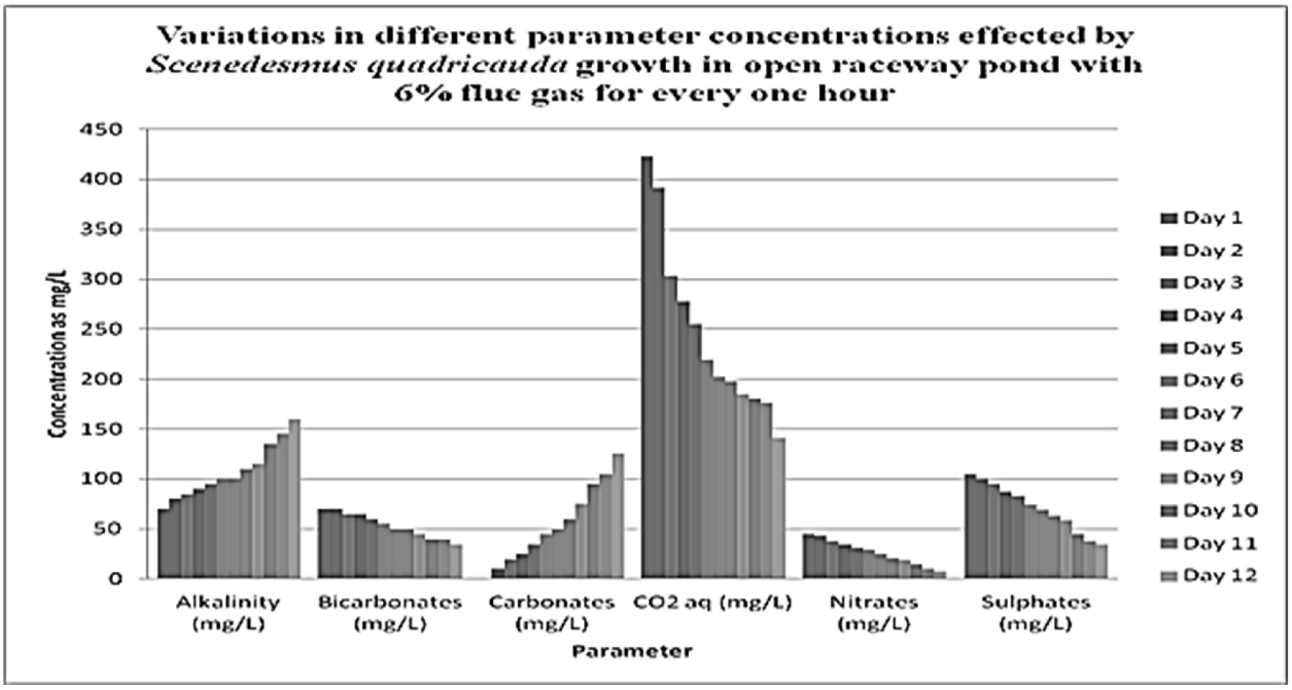


***Figure 10: Graph representing the variations in different parameter concentrations effected by S. quadricauda growth in open raceway pond with 6% flue gas for every one hour***

The outlet flue gas-$CO_2$ percentages in the flasks (1 Hr and 30 min) were analyzed by using Gas Chromatography (Figures 11 and 12) showed slight reduction from initial day to final day in the two experiments. This is due to the utilization of the $CO_2$ from the flue gas by *Scenedesmus quadricauda* for its growth.

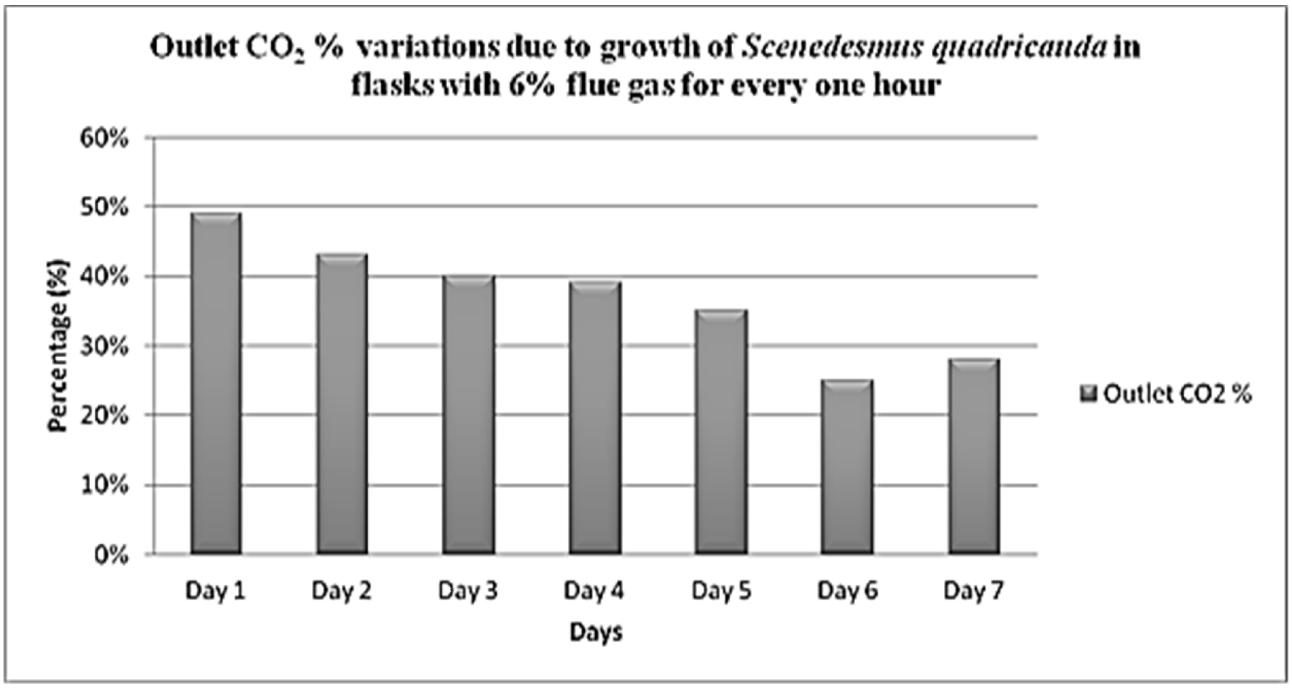


***Figure 11: Graph representing the outlet flue gas $CO_2$ % variations due to growth of S.quadricauda in flasks with 6% flue gas for every one hour***

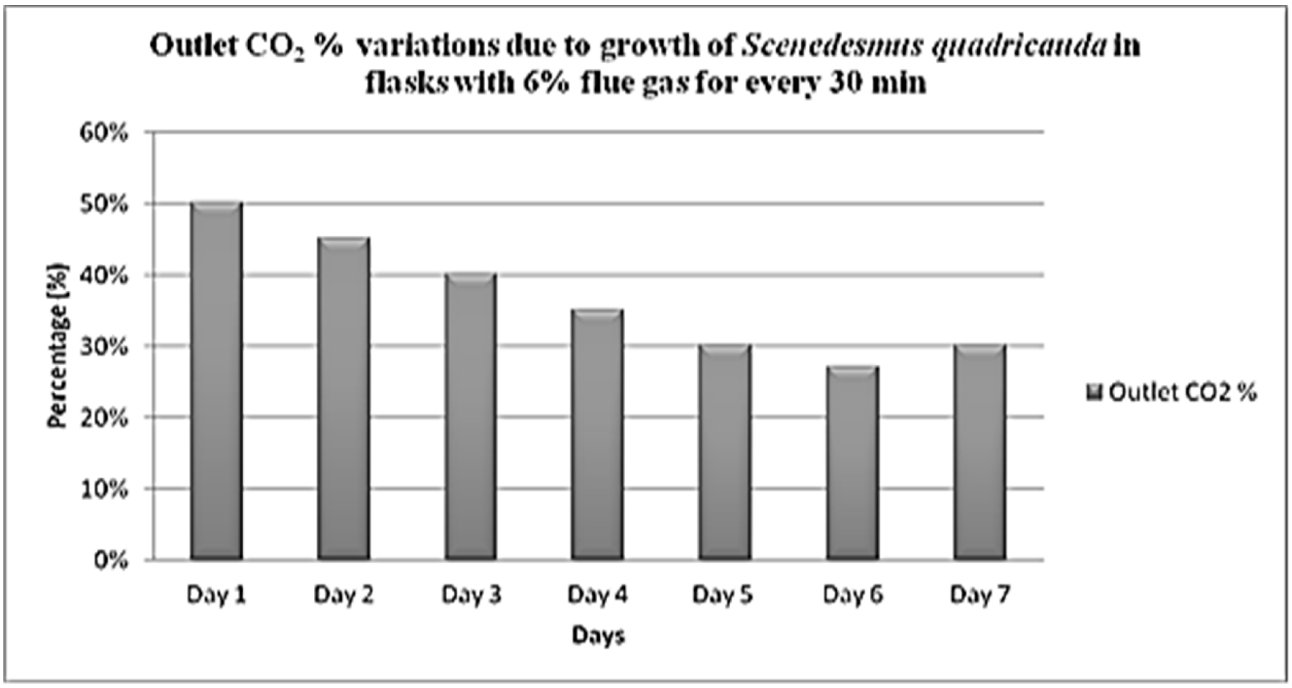


***Figure 12: Graph representing the outlet $CO_2$ % variations due to growth of S. quadricauda in flasks with 6% flue gas for every 30 min***

In all the three experiments, pH variations (Figures 13, 14 and 15) were observed from initial day to final day. The pH scale varied from slight acidic from initial day to slight alkaline on the final day due to the accumulation of carbonate ions.

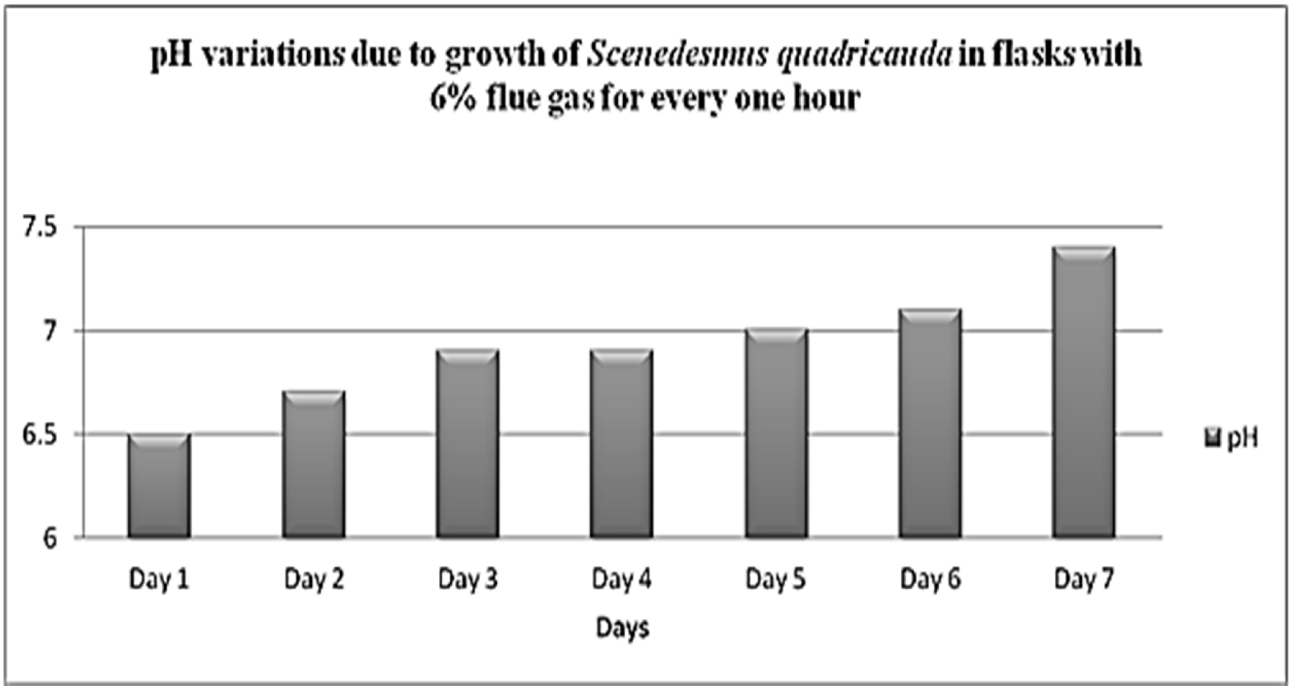


***Figure 13: Graph representing the pH variations due to growth of S. quadricauda in flasks with 6% flue gas for every one hour***

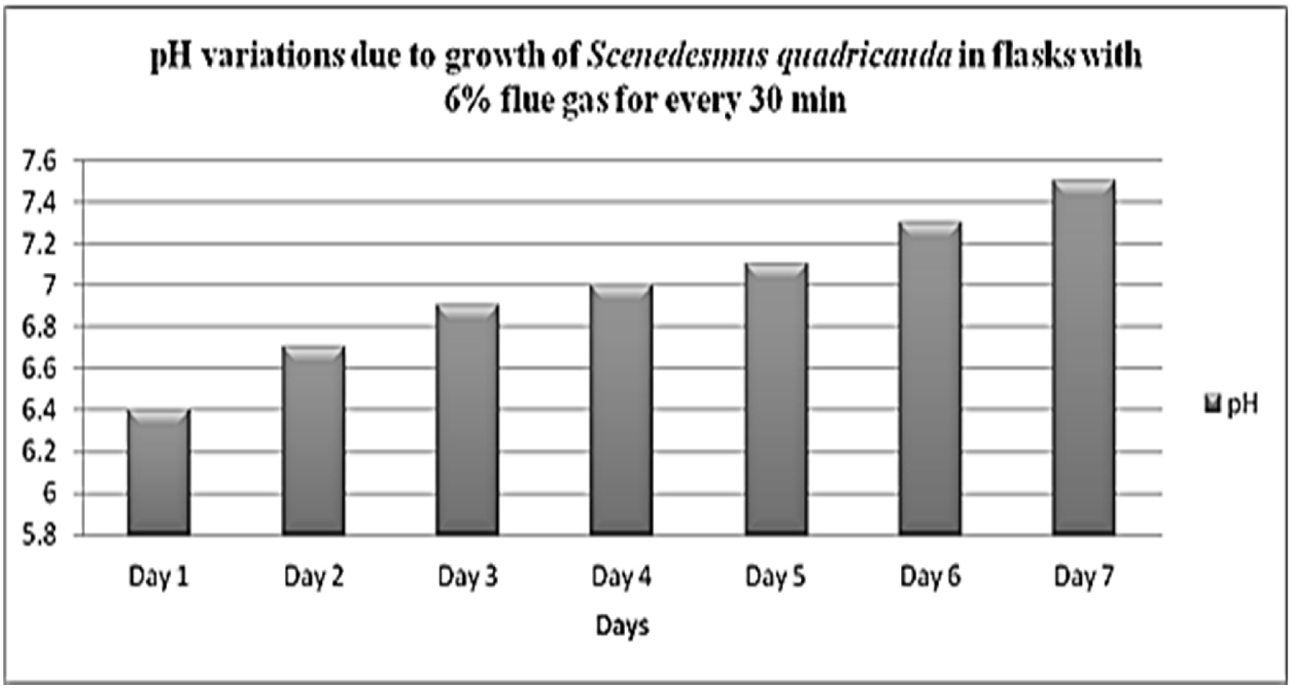


***Figure 14: Graph representing the pH variations due to growth of S. quadricauda in flasks with 6% flue gas for every 30 min***

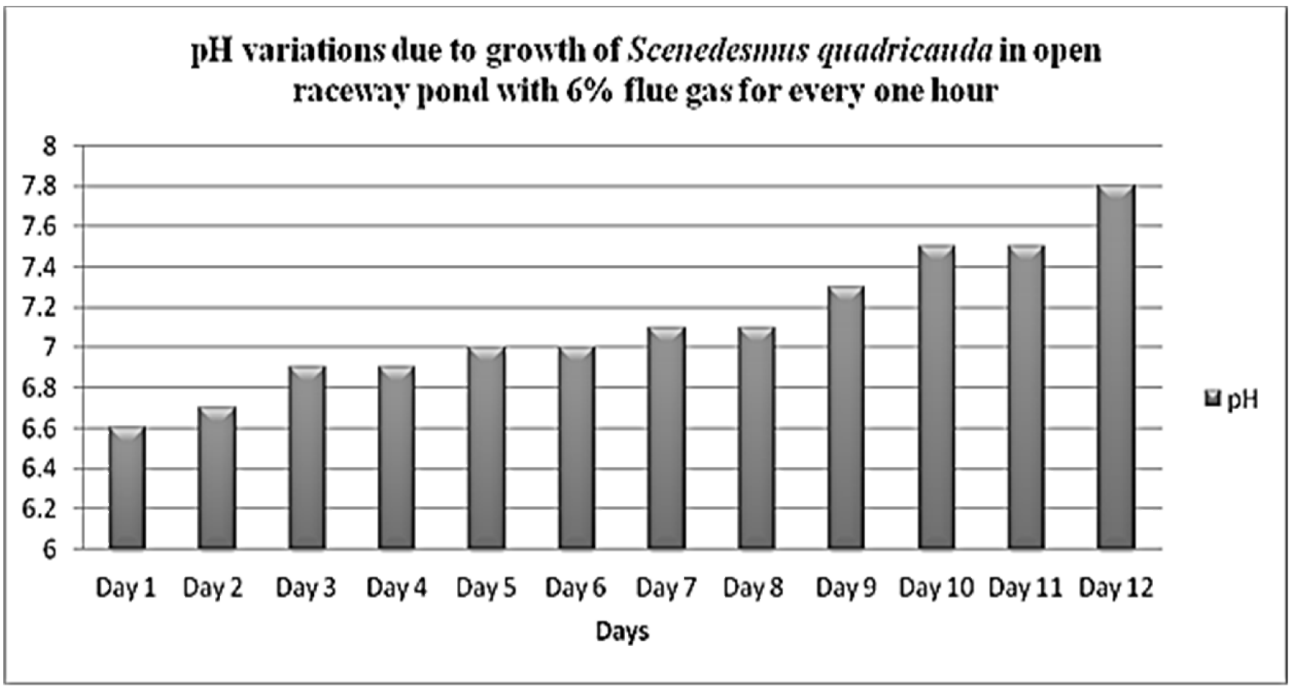


***Figure 15: Graph representing the pH variations due to growth of S. quadricauda in open raceway pond with 6% flue gas for every one hour***

In the present study, 6% flue gas air mixture was used for the cultivation of micro alga *S. quadricauda* which showed efficient growth in accordance with the previous studies by De Morais et al., [16] who observed that *Chlorella kessleri, Scenedesmus obliquus*, *Spirulina* sp., *Chlorella vulgaris*, and *Chlorella sorokiniana* grew best under 6% $CO_2$ concentration.

The present results observed indicates that micro alga *S. quadricauda* can efficiently utilize $CO_2$ from flue gas which was in accordance with the studies of Yoo et al. [17] who found *Scenedesmus* sp. to be the most suitable for $CO_2$ reduction due to high rates of biomass production (0.218 g/l per day).

In the present study, the results which were obtained pertaining to biomass growth and lipid yields were closely related to previous study done by Guruvaiah et al., [15] who studied on utilization of flue gas from coal burning power plant for microalgae cultivation for biofuel production and they obtained the maximum biomass growth of 1.20 mg/L at 2% flue gas $CO_2$ concentrations and lipid productivity more than 15%.

The present results obtained pertaining to the parameters like pH variation, increase in carbonate ions, decrease in bicarbonate ions and decrease in aqueous $CO_2$ were in accordance with Palanisami et al., [18].

In the present study, it was observed that the presence of NOx in flue gas did not inhibit the growth of microalgae, in accordance with the findings of Hauck et al., [19] and Matsumoto et al., [12].

### 3.1 GC-MS ANALYSES

The GC-MS analysis reports showed that In the lipid samples the presence of Palmetic acid, Stearic acid, oleic acid and linolenic acid were noted (Figure. 16). Whereas in the FAME sample the presence of long chain methylated fatty acid esters were seen such as: Palmetic acid phenyl methyl ester, Stearic acid phenyl methyl ester, oleic acid phenyl methyl ester and linolenic acid phenyl methyl ester (Figure. 17).

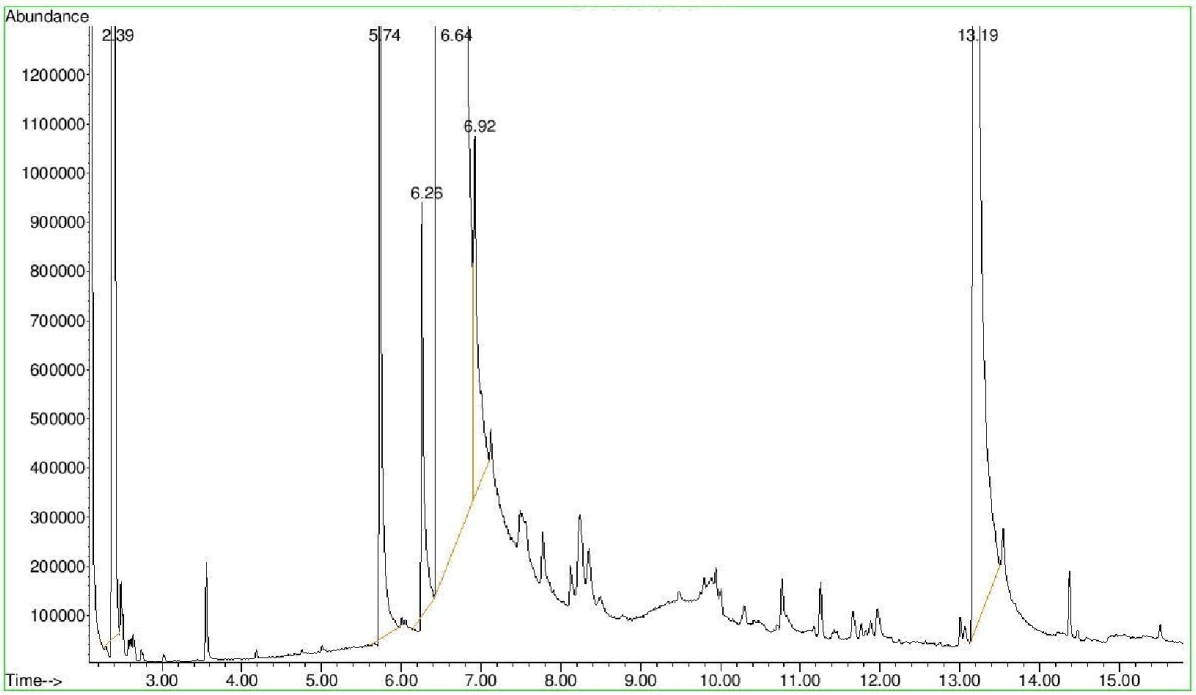


***Figure 16: GC-MS graph of lipid components derived from S. quadricauda***

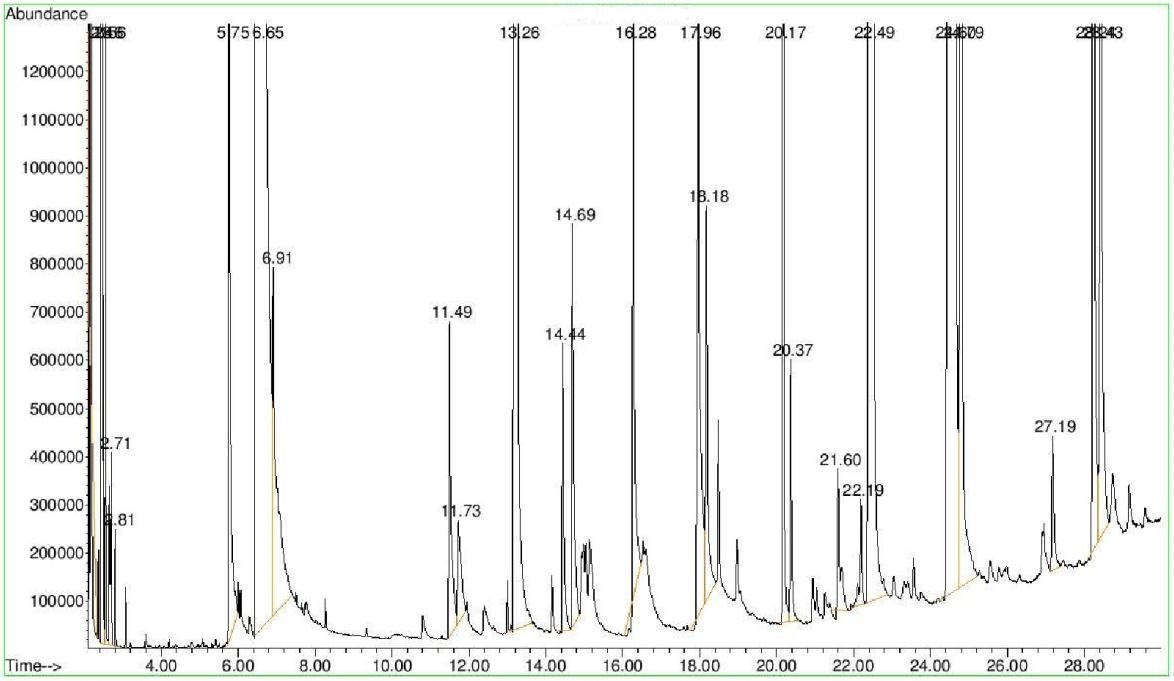


***Figure 17: GC-MS graph of FAME components derived from S. quadricauda***

## 4 CONCLUSION

The present study results revealed that *Scenedesmus quadricauda* was efficiently grown in the presence of flue gas obtained from the coal-burning industrial plant. It was also observed that the microalga *S. quadricauda* efficiently utilized the $CO_2$ from the flue gas for its biomass growth and in turn with the production of lipids and fatty acid methyl esters (FAME). It was also recorded that the microalga *S. quadricauda* had a potential for the reduction of carbon dioxide from the flue gas up to 75%. Hence these experiments are recommended to be carried out at industrial scale for the dual purpose of remediation and energy production followed by commercialization.

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## REFERENCES

[1] IEA. (2007) International Energy Agency (IEA), World Energy outlook, China and India Insights International Energy Agency publication, France.
[2] Patil V., Tran K.Q. and Giselrod H.R. (2008) International Journal Molecular Science, 9: 1188-1195.
[3] Packer M. (2009) Energy Policy, 37: 3428-3437.
[4] Benamann J.R. (1993). Energy Conservation Management, 34: 999-1004.
[5] Benamann J. (1997) Energy Conversion and Management, 38: 475-479.
[6] Grequede Morais M. and Costa J.A. (2007) Journal of Biotechnology, 129: 439-445.
[7] Chisti Y. (2007) Biodiesel from microalgae. Biotechnol. Adv. 25: 294-306
[8] Reddy M.H. (2002*)* Applications of Algal Culture Technology for Carbon Dioxide and Flue Gas Emission Control. M.S. thesis, Arizona State University, Tempe, AZ.
[9] Jakob A., Stucki S., and Kuhn P. (1995) Evaporation of Heavy Metals during the Heat Treatment of Municipal Solid Waste Incinerator Fly Ash. Environ. Science and Tech., 29(9): 2429-2436.

[10] Wang J., Ban H., Teng X., Wang H. and Ladwig, K. (2006) Impacts of pH and Ammonia on the Leaching of Cu(II) and Cd(II) from Coal Fly Ash. Chemosphere, 64: 1892-1898.
[11] Mastral A.M. and Callen M.S. (2000) A Review on Polycyclic Aromatic Hydrocarbon (PAH) Emissions from Energy Generation. Environ. Science and Tech., 34(15): 3051-3057.
[12] Matsumoto H., Shioji N., Hamasaki A., Ikuta Y., Fukuda Y., Sato M., Endo N and Tsukamoto T. (1995) Carbon-Dioxide Fixation by Microalgae Photosynthesis using Actual Flue-Gas Discharged from a Boiler. App. Biochem. and Biotech., 51/52: 681-692.
[13] Schenk P., Thomas-Hall S., Stephens E., Marx U., Mussgnug J., Posten C., Kruse O and Hankamer B. (2008) Second Generation Biofuels: High-Effiency Microalgae for Biodiesel Production. Bioenergy Res., 1:20-43.
[14] Prabakaran P and Ravindran A.D. (2012) *Scenedesmus* as a Potential Source of Biodiesel among selected Microalgae. Current Science, 102(4): 616-620.
[15] He L., Chen AB., Yu Y., Kucera L and Tang Y. (2013) Optimize flue gas settings to promote microalgae growth in photobioreactors via computer simulations. J Vis Exp, (80)..
[16] De Morais M.G and Costa J.A.V. (2007) Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Conversion and Management, 48(7): 2169-2173.
[17] Yoo C., Jun S.Y., Lee J.Y., Ahn C.Y and Oh H.M. (2010) Selection of microalgae for lipid production under high levels carbon dioxide. Bioresource Technology, 101: S71-S74.
[18] Palanisami S., Viswanathan T., Dudenhoeffer N., Lee K and Nam P.K.S. (2013) Utilization of Flue gas as a Carbon dioxide source from a Coal-Burning Power Plant for the Cultivation of Microalgae in Outdoor Circular Ponds. International Journal of Pharma and Bio Sciences, 4(4): 749-762.
[19] Hauck J.T, Olson G.J, Scierka S.J, Perry M.B and Ataai M.M. (1996) Effects of simulated flue gas on growth of microalgae. Abstracts of Papers. American Chemical Society, 212 Meeting, Pt.1, Fuel 118. Orlando, FL.
[20] Philipose M.T., 1967. Chlorococcales monographs on algae. Indian Council of Agricultural Research Publication, New Delhi, India. 1-365.
[21] Stein J.R., 1973. Handbook of phycological methods: Culture methods and Growth measurements. Cambridge University Press, London and New York. 448pp.