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CO_2 Removal from Industrial Flue Gas using *Botryococcus braunii* for Simultaneous Lipid Production

K. Pooja[1], V. Himabindu[2]

[1]Research Scholar, Centre for Environment, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad – 500 085, Telangana, India

[2]Associate Professor, Centre for Environment, Institute of Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad – 500 085, Telangana, India

Abstract: ***The aim of this research is to utilize flue gas from coal burning industrial plant as a carbon source to cultivate Botyrococcus braunii in laboratory flasks and outdoor open raceway ponds to achieve CO_2 reduction from the flue gases along with biomass and lipid yields. 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 and 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 biomass and lipid yields of 1.20 g/L and 0.19g/L respectively were obtained in 6% flue gas concentrations aerated into culture flasks at 0.1vvm for every one hour. On the other hand the CO_2 removal efficiency reached upto 73% along with SOx and NOx reductions upto 50%. Hence in the present study it was observed that the micro alga B. bauniiutilized the flue gas-air mixture for CO_2 reduction and in turn it produced biomass and lipids yields efficiently.***

Keywords: Flue gas, Botryococcus braunii, Open raceway pond, Biomass, Lipid.

1. Introduction

Fossil fuels now contribute most of the global energy demand, but these fuels are directly associated with the greenhouse effect and environmental pollution (Granite *et al.,* 2005]. Oil reserves may run out after 2050 due to the fast growth in fossil fuel requiring human activities worldwide(Chisti. 2007; Li *et al.,* 2008b). Therefore, huge efforts are being made in developing CO_2 fixation and reduction technologies and in finding alternative and renewable energy sources (Schenk*et al.,* 2008). Among those attempts, biodiesel has received significant attention since it is made from nontoxic and biodegradable materials, and its use leads to a huge decrease in the emissions of greenhouse gases (such as CO_2) and air pollutants (Kumar *et al.,* 2010). However, to produce enough biodiesel from oleaginous crops (such as soybean, palm, and rapeseed) to supply the existing demand for transportation in the United States alone, displacement of around 50% of its total crop land would be necessary. This would cause severe problems such as food shortages (Kanhaiya*et al.,* 2011). Therefore, there is an urgent need to identify more effective and sustainable oil feed stocks for making biodiesel to meet the global demand. Microalgae are considered a renewable alternative biodiesel feedstock due to their high growth rate, relatively high lipid content, and excellent CO_2 fixation ability (Kumar *et al.,* 2010; Lee *at al.,* 2002).

Microalgae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO_2, nitrogen, phosphorous, potassium) that can produce lipids in large amounts over short periods of time (Demirbas. 2011). Thus, microalgae and cyanobacteria biomass can also be used as feedstock for a variety of biofuels (De Morais&Costa. 2007; Ho *et al.,* 2011). Furthermore, four applications are achieved by using microalgae biomass production as a CO_2 reduction strategy: i) production of biofuels, ii) enhancement of the economic yield of the carbon capture and storage through production of commodities or by-products from flue gases, iii) utilization of bacteria-microalgae consortiums to reduce the energy required for aeration in wastewater treatment plants and iv) utilization of microalgae to reduce the total CO_2 emissions released by wastewater treatment plants (AciénFernández*et al.,* 2012)..

Microalgae can typically be used to capture CO_2 from three different sources: (1) atmospheric CO_2, (2) CO_2 emission from power plants and industrial processes, and (3) CO_2 from soluble carbonate (Brennan&Owende. 2010; Wang *et al.,* 2008). Capture of atmospheric CO_2 is probably the most basic method to sink carbon, and relies on the mass transfer from the air to the microalgae in their aquatic growth environments during photosynthesis. However, the potential yield from the atmosphere is limited by low CO_2 concentration in air (around 360 ppm) (Stepan*et al.,* 2002; Wang *et al.,* 2008), In contrast, CO_2 capture from flue gas emissions from power plants that burn fossil fuels achieves better recovery due to the higher CO_2 concentration of up to 20% (Bilanovic*et al.,* 2009). Since microalgae CO_2-fixation involves photoautotrophic growth of cells, CO_2 fixation capability of specific species should positively correlate with their cell growth rate and light utilization efficiency (Jacob-Lopes*et al.,* 2009a; Jacob-Lopes*et al.,* 2009b).Some other obstacles in flue gases utilization are related to its low pressure and consequent power requirement for supply it into the system, as well as the possible addition of dust or heavy metals to the system (AciénFernández*et al.,* 2012).

The green colonial hydrocarbon rich microalgae *Botyrcoccusbraunii* (Banerjee*et al.,* 2002), (Metzger*et al.,* 2005) is wide spread in freshwater, brackish lakes, reservoirs and ponds. It is also widely distributed in reservoirs at

temperature, tropical Antarctic latitudes. The alga *B.bruanii* has tremendous potential to be used as a renewable biomass feedstock and is recognized as one of the potent renewable resources for production of liquid hydrocarbons.*B. braunii* is a green micro alga that produces hydrocarbons up to 75% of its dry biomass and it has already been proposed as a future renewable source of fuel (Banerjee*et al.,* 2002). The species is notable for its ability to produce high amounts of hydrocarbons, especially oils in the form of Triterpenes, that are typically around 30-40 percent of their dry weight (Metzger*et al.,* 2005). Compared to other green algae species it has a relatively thick cell wall that is accumulated from previous cellular divisions; making extraction of cytoplasmic components rather difficult. Fortunately, much of the useful hydrocarbon oil is outside of the cell (Wolf *et al.,* 2004).

Botryococcus braunii has great potential for alga culture because of the hydrocarbons it produces, which can be chemically converted into fuels. Up to 86% of the dry weight of *Botryococcus braunii* can be long chain hydrocarbons. The vast majority of these hydrocarbons are botryocuccus oils: botryococcenes, alkadienes and alkatrienes. Transesterification cannot be used to make biodiesel from *Botryococcus* oils. This is because these oils are not vegetable oils in the common meaning, in which they are fatty acid triglycerides. While *Botryococcus* oils are oils of vegetable origin, they are inedible and chemically very different, being triterpenes, and lack the free oxygen atom needed for Transesterification. *Botryococcus* oils can be used as feedstock for hydrocracking in anoil refinery to produce octane (gasoline, a.k.a. petrol), kerosene, and diesel(Hillen*et al.,* 1982).

Flue gas is the gas exiting to the atmosphere via a flue, which is a pipe or channel for conveying exhaust gases from a fireplace, oven, furnace, boiler or steam generator. Combustion of flue gases refers to the emissions of combustion product gases resulting from the burning of fossil fuels such as coal, oil, and natural gas composition depends on what is being burned, but it will usually consist of mostly nitrogen (typically more than two-thirds) derived from the combustion air, carbon dioxide (CO_2), and water vapor as well as excess oxygen (also derived from the combustion air). It further contains a small percentage of a number of pollutants, such as particulate matter (like soot), carbon monoxide, nitrogen oxides (NOx), and sulfur oxides (SO_2).The composition of the coal-fired flue gas was 12.5-12.8% CO_2, 6.2% H_2O, ~ 4.4% O_2, 50 ppm CO, 420ppm NOx, 420 ppm SO_2, and 76-77% N_2 (Van den Hende*et al.,* 2012)

In the present study *Botyrococcusbraunii* was cultivated with coal burning industrial flue gas inputs and to produce lipids and studying the effect of parameters like pH, Alkalinity, escaped CO_2 using GC, Acqueous CO_2, Nitrates, sulphates, NOx, SOx and lipid content. M Guruvaiah and K Lee (2014) studied utilization of flue gas from coal burning power plant for microalgae cultivation for biofuel production. in this work,Microalgae studies were conducted in a batch mode experiments in different period (May to October, 2011) of time, at Power plant, Jefferson city, Missouri, USA.The genus Scenedesmussp was isolated from power plant habitat and used for this experiments and then comparative study done by flue gas ponds vs non flue gas treatment ponds. The microalgae were cultured with different simulated flue gases containing 1% –4% (volume fraction) of CO_2. The results show that Scenedesmus species were grown very efficient at 2% CO2 content. The maximal biomass productivity and lipid productivity were obtained when aerating with 2%of CO_2. The lipids content ranged from 10 to 18 % of dry mass of biomass. Scenedesmus species has a great potential for CO_2 mitigation, environmental tolerance and biodiesel production.

Sara P.cuellar-Bermudez *et al.,* 2014 has done work on "Photosynthetic bioenergy utilizing CO_2: an approach on flue gases utilization for third generation biofuels". In this work, the use of CO_2 released by the cement sector is described as potential gas for microalgae culture since their biofixation efficiency is higher than terrestrial plants. In addition, bulk applications such as wastewater treatment and biofuels production can be coupled. In this review, flue gas emissions coupled to microalgae cultures are described. In addition, microalgae lipids can be transformed into biodiesel to be used in the transport sector. However, design concepts, flue gas composition, temperature, microalgae culture and species must be evaluated for this application. However, since some studies have shown that microalgae cultured using flue gas from cement industry can accumulate heavy metals, final composition must be evaluated before considering commercial application of the produced biomass. Finally, it is expected that this review and perspective contributions have shown the starting point for research focused on integral management that can render cement industry to an environmental compatibility with energy production to achieve local and global sustainable goals.

AndriCahyoKumoro*et al,* 2013 has done the work on "Impact of hazardous components on CO2 biofixation from synthetic flue gas using Chlorella sp.JPR-1 in a raceway pond photobioreactor".This work aimed to investigate the effects of hazardous compounds (NO and SO2) in flue gas on Chlorella JPR-1growth in a raceway pond photobioreactor at ambient temperature (30 °C) without pH control. Chlorella JPR-1 exhibited its tolerant to CO_2 content in the flue gas as high as 50%. The maximum carbon fixation rate (1.84 g CO_2/L.day) observed when the flue gas contained 10% CO_2. Although the specific growth rate was 25.88% lower than the control culture when cultivated with 50 ppm SO2, Chlorella JPR-1 still could grow when cultured with 100 ppm SO2 with slightly longer lag phase period.A growth rate reduction of 3.53% of the control culture was observed when Chlorella JPR-1 was cultured with flue gas containing 50 ppm NO. This study has shown that Chlorella JPR-1 has a high potential to be used for CO_2fixation from flue gas.

Jiri Douchaet *al,* 2005 has done the work on "Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor". Flue gas containing 6–8% by volume of CO2 substituted for more costly pure CO_2 as a source of carbon for autotrophic growth of *Chlorella* sp .The degree of CO2 mitigation (flue gas decarbonization) in the algal suspension was 10–50% and decreased with increasing flue gas injection rate into the culture. NO*x* and CO gases (up to45 mgm−3 NO*X* and

3mgm−3 CO in flue gas) had no negative influence on the growth of the algae. On summer days the following daily net productivities of were attained in comparative parallel cultures:flue gas = 19.4–22.8; pure CO_2= 19.1–22.6. Net utilization (η) of the photosynthetically active radiant (PAR)energy was: flue gas = 5.58–6.94%; pure CO2 = 5.49–6.88%. It was estimated that about 50% of flue gas decarbonization can be attained in the photobioreactor and 4.4 kg of CO2 is needed for production of 1 kg (dry weight) algal biomass.

2. Methodology

2.1 Flue gas sample collection

Flue Gas sample was collected in 3lts, 5lts tedlar bags from a coal burning boiler outlet at Sri chaitanya chlorides industry, Isnapur, Hyderabad. The collected gas sample was analysed for the presence of CO_2 % in gas chromatography $_{(GC)}$ and it was found to be 6%.

2.2 Microalgae culture and cultivation

The presence of *Botryococcus braun*ii was identified in Nallakuntalake from Hyderabad and by microscopic examinations under Olympus CX21 light microscope according to morphological properties followed by isolation and purification (Philipose. 1967; Stein. 1973) by capillary tube method and streak plate methods respectively (figure 1).

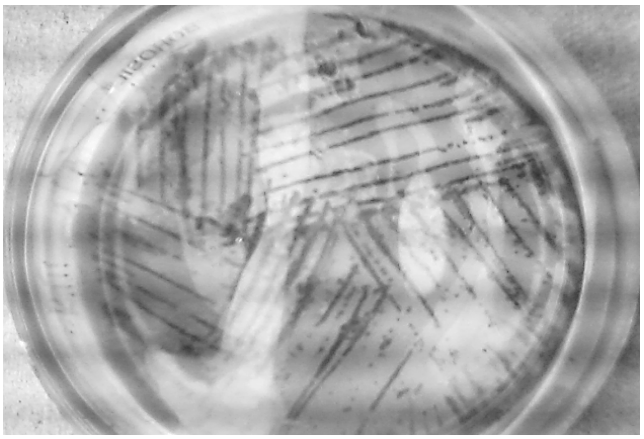

Figure 1: Quadrant streak plates of *Botrycoccusbraunii*

2.3 Algae Cultivation and Nutrition

The flue gas from coal-fired power plant was used to cultivate the microalgae *B. braunii*in indoor culture flasks of 500mL capacity and in outdoor open raceway ponds of 200L capacity (2mx1mx0.5m). The nutrient media used to grow the algae was CHU-13 whose composition was KNO_3 - 0.4g/L, K_2HPO_4 - 0.08g/L, $CaCl_2.H_2O$ - 0.107g/L, $MgS0_4.7H_2O$- 0.2g/L Ferric Citrate - 0.02g/L, Citric acid - 0.1g/L, $COCl_2$- 0.002g/L, H_3BO_3 - 0.00572g/L, $MnCl_2.4H_2O$ - 0.00362g/L, $ZnSO_4.7H_2O$ - 0.00044gr/liter, $CuSO_4.5H_2O$ - 0.00016g/L, Na_2MoO_4 - 0.000084g/L, 0.072 N H_2SO_4 - 1 drop. The cultures were maintained at 27°C temperature and 99umol.photons m^{-2} s^{-2} light intensity in the culture rack (figure 2). Parameters like Optical density, pH, Alkalinity, Acqueous CO_2, escaped CO_2 from outlet using GC – for culture flasks, Nitrates, sulphates, NOx, SOx and lipid content were monitored in flasks using 6% flue gas air mixture for every half an hour and one hour.

Figure 2: Growth of *B. braunii* in culture flasks

2.4 Experimental Design

The flue gas from the coal power plant (containing 6% CO_2) was diluted with air mixture (6ml flue gas in 100ml of air) using compressed air and supplied for a minute half an hour and for a minute one hour daily for in flasks as well as in raceway ponds. The mixing and aeration was provided by bubbling the flue gas in to microalgae pond systems with a 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 (Figure 3).

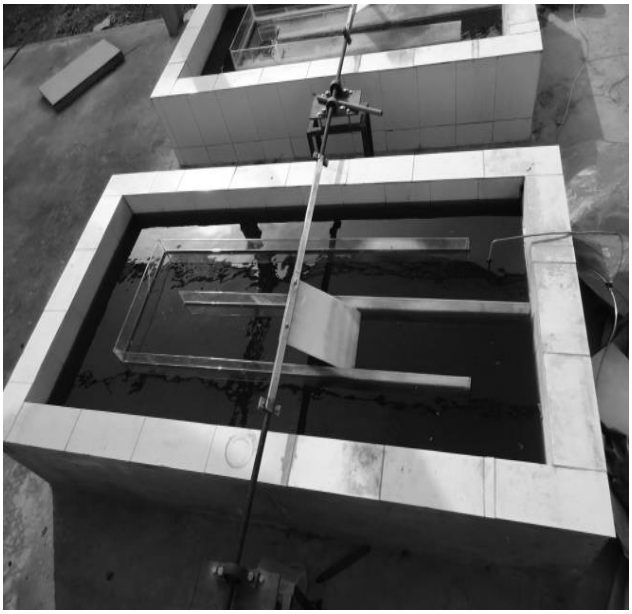

Figure 3: Representing the Growth of *B. braunii* in open raceway ponds

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 (OD) readings. Algal dry cell weight was determined daily by filtering 10 mL of the culture sample onto Glass Microfiber Filters, GFC (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.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 polyster cloth to trap the algae. The harvested algae from the ponds were spread evenly on stainless steel plates and let dry under direct sunlight.

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 was 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 #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 Lipid characterization using GC-MS

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

3. Results and Discussion

Botryococcusbrauni cultivated in laboratory culture flasks and outdoor open raceway ponds with flue gas inputs delivered potential biomass (Figure 4) and lipid yields (Figure 5) in spite of the toxicity of the flue gas components like sulphur. The biomass and lipid yields peaked on 7th day in culture flasks whereas on 12th day in open raceway ponds. Two different time intervals of flue gas air mixture inputs were studied in the culture flasks which are 0.01vvm of 6% flue gas air mixture inputs for every 30 minutes and for every 1 hour. Best result was noted at every one hour time interval. Hence the one hour time interval was applied for raceway pond cultivation. Thus among the three experiments conducted, *B. braunii* grown in culture flask aerated with 6% flue gas air mixture at 0.1vvm flow rate for every one hour showed little higher (biomass 1.2g/L and lipid 0.19g/L) as shown in the graphs below.

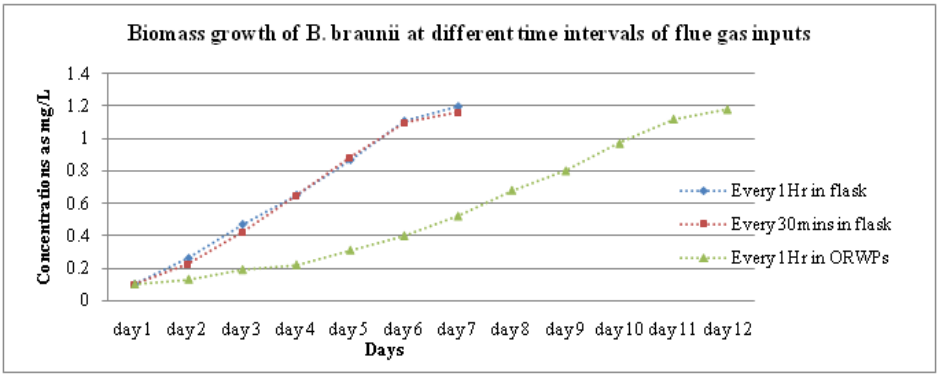

Figure 4: Graph representing the Biomass growth of *B. braunii* at different time intervals of flue gas inputs

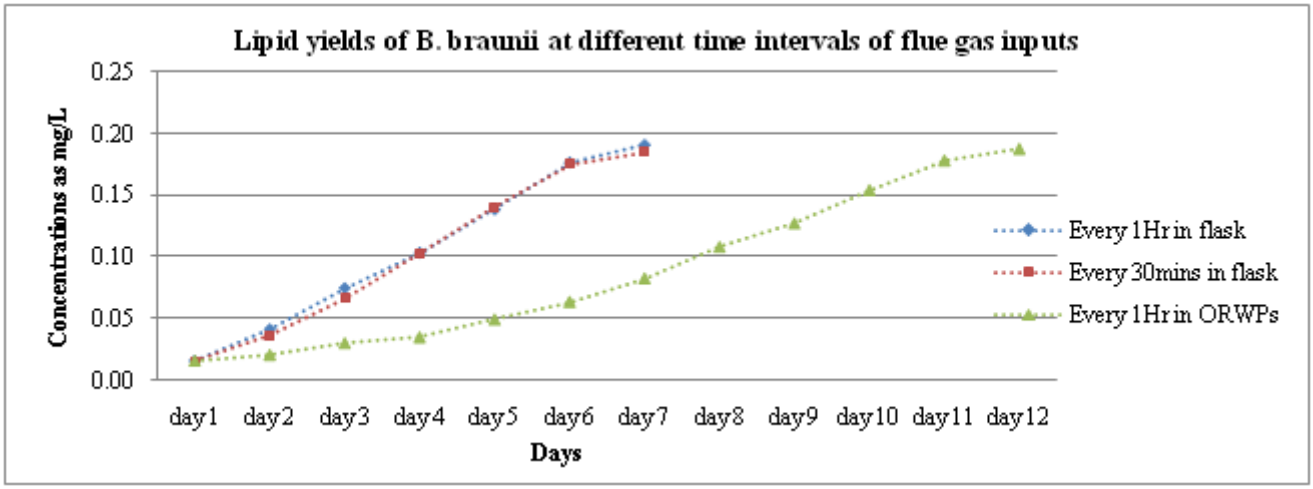

Figure 5: Graph representing the Lipid yields of *B. braunii* at different time intervals of flue gas inputs

The SOx (Figure 6) and NOx (Figure 7) concentrations of the flue gas were analyzed initially and finally 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 dissolution in the culture medium which were also reduced upto 65% in all the three experiments.

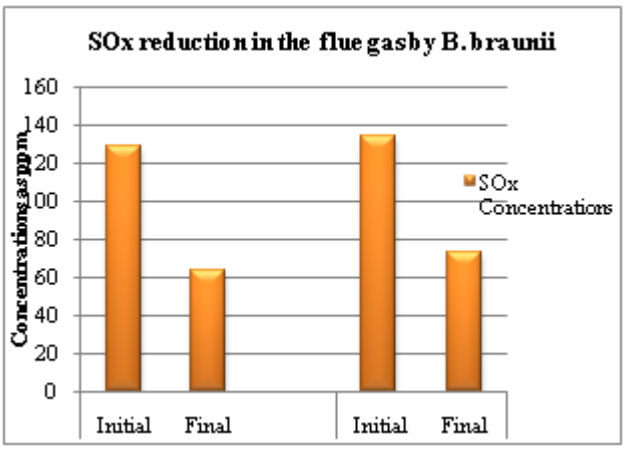

Figure 6: Graph representing the Sox reduction in the flue gas by *B. braunii*

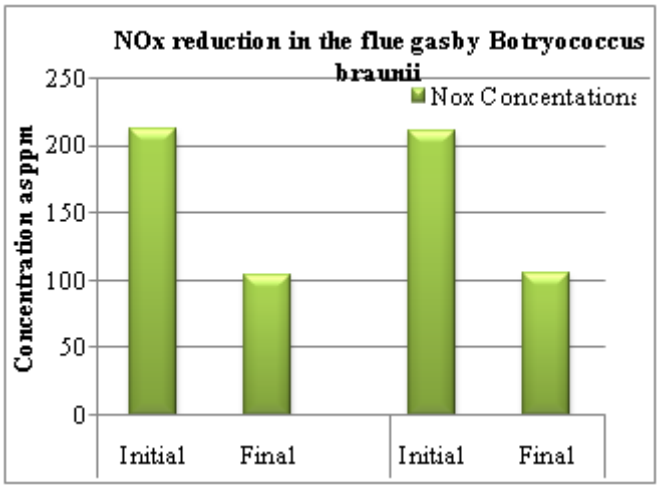

Figure 7: Graph representing the NOx reduction in the flue gas by *B. braunii*

Parameters like Alkalinity, carbonates, bicarbonates and aqueous co_2 was measured in culture flasks and in open raceway ponds. Alkalinity was increased gradually due to the algae growth in both culture flasks as well as in the open race way ponds as shown in the (Figure 8, 9 and 10).

The bicarbonate ions showed a decreasing pattern with the growing algal biomass while carbonate ions were increasing. The aqueous CO_2 decreased gradually as the algae growth increased in culture flasks and in open raceway ponds. In culture flasks the percentage of CO_2 escaped out during flue gas inputs was analyzed using GC. Both the aqueous CO_2 and escaped CO_2 experiments were correlated to find the amount of CO_2 utilized and reduced. However the correlation was not applied to open raceway ponds as the escaped CO_2 couldn't be captured. The overall CO_2 reduction achieved was 73% in the culture flask aerated with 6% flue gas air mixture at 0.1vvm flow rate for every one hour time interval in culture flasks. However the CO_2 reduction was above 70% in the other two experiments.

The results obtained in the present study were in accordance with MahendraperumalGuruvaiah and Keesoo Lee, 2014 [27] who studied on utilization of flue gas from coal burning power plant for cultivation of *Scendesmussp* in open ponds. They achieved a maximum algal biomass of 1.20 g/L in 2 % flue gas CO_2 concentrations for 3 hours daily.

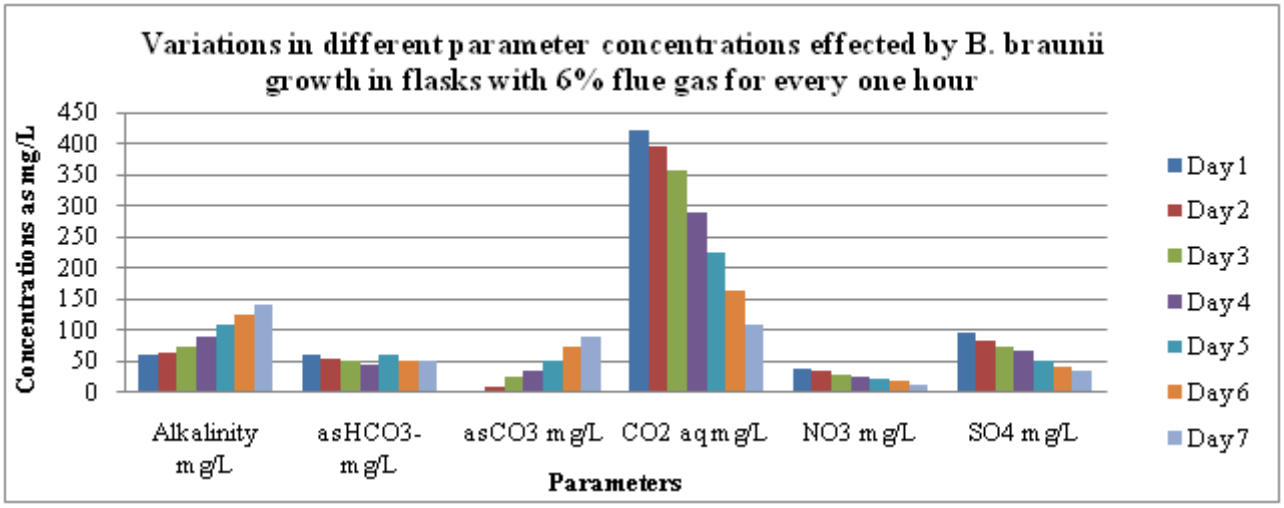

Figure 8: Graph representing the variations in different parameter concentrations effected by *B. braunii* grown in flasks with 6% flue gas for every one hour.

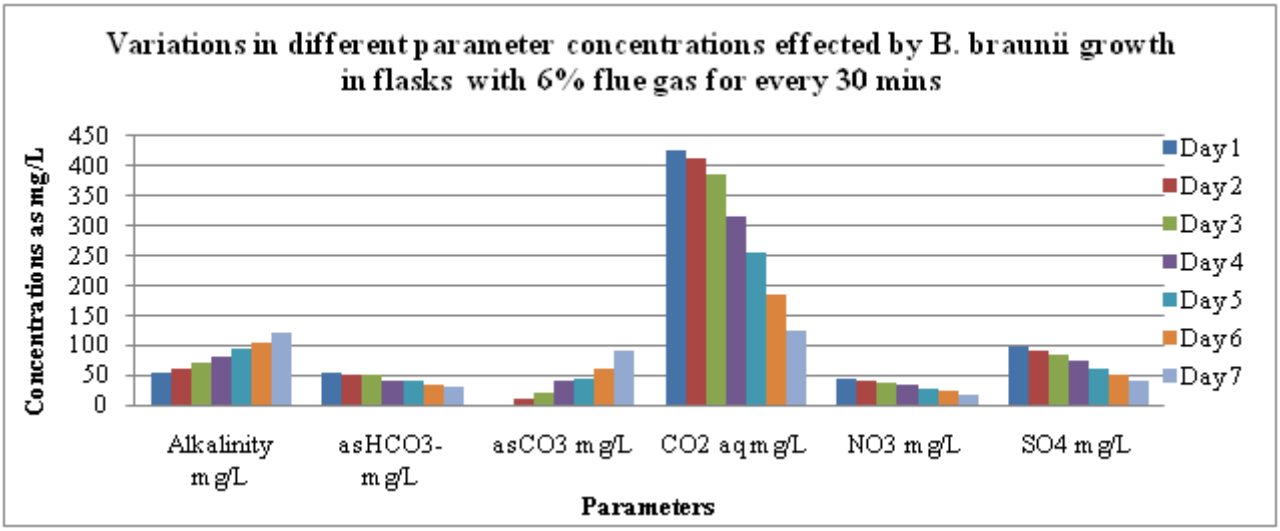

Figure 9: Graph representing the variations in different parameter concentrations effected by *B. braunii* grown flasks with 6% flue gas for every 30 minutes.

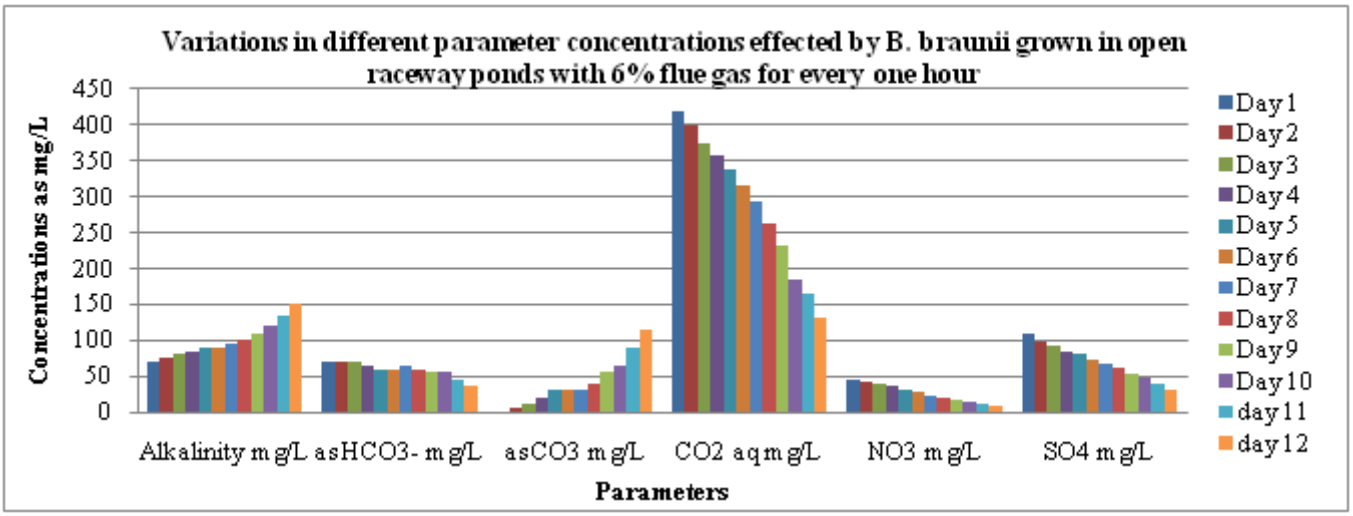

Figure 10: Graph representing the variations in different parameter concentrations effected by B. braunii grown in open raceway ponds with 6% flue gas for every one hour.

3.1 GC-MS Analyses

The GC-MS analysis reports showed that in the lipid samples 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. 11).

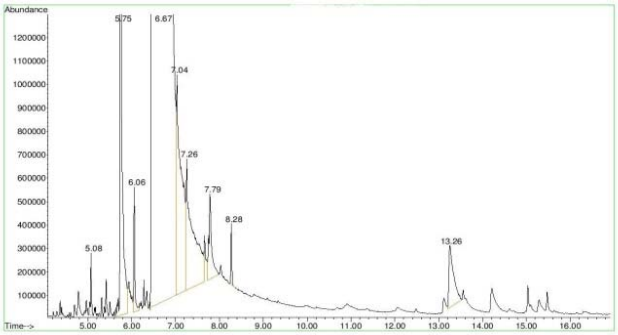

Figure 11: GC-MS graph of lipid components derived from *B. braunii*

4. Conclusion

The present study results showed that *Botryococcus braunii* was able to grow in the presence of flue gas. *B. braunii* are able to utilize CO_2 from the flue gases and yielded biomass and lipids. Maximum algal biomass of 1.20 g/L and lipid yield of 0.19g/L were obtained in 6% flue gas concentrations aerated into culture flasks at 0.1vvm for every one hour. *B. braunii* also had a potential for CO_2 reduction from flue gases up to 73%.

Hence it is recommended that this study may be carried at industrial vicinities to reduce the pollution as well as to generate energy products followed by commercialization. Coupling flue gas treatment with biomass yield for energy generation is an economically feasible technology which can be practiced to conserve the exhausting conventional environmental resources. There is certainly a future in fuels from algae provided timely technological advances are made. Ultimately, algae is the only sustainable source of biofuels and CO_2 sequestration at present and it is up to the human race to make the most of this available resource and use it efficiently to our advantage sustainably.

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