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Systematic Investigation of Biomass, Fatty Acid Productivity and CO_2 Sequestration from Generator Gases by Fresh Water Microalgae in Photobioreactors for Biodiesel Application

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Abstract- The potential of microalgae as renewable energy feedstock for biofuel production is well recognized in developing countries and it is a large source of biomass for capturing CO_2 on non-arable lands. In the current studies photobioreactor with 2 L capacity was used and operated with generator exhaust gases for algal CO_2 sequestration. The reactor operated on generator exhaust gases with CO_2 ranges from 500-8000 ppm. As CO_2 concentration increased from 500 to 4000 ppm, there were steady increase in biomass 450 mg/l of dry weight till 2000 ppm, but above 2000 ppm there is decline in growth. The fatty acid profiles were more or less constant at all the CO_2 concentrations and maximum lipid content was 48%. The maximum reduction in CO_2 was 70% at 500 ppm whereas it was 52% at 4000 ppm. When CO_2 concentration increased to 8000 ppm without air, algae could not sustain and showed decline in biomass content. CO_2 fixation from generator gases not only reduces greenhouse gases but also help in getting fatty acid as biofuel.

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Abstract- The potential of microalgae as renewable energy feedstock for biofuel production is well recognized in developing countries and it is a large source of biomass for capturing CO_2 on non-arable lands. In the current studies photobioreactor with 2 L capacity was used and operated with generator exhaust gases for algal CO_2 sequestration. The reactor operated on generator exhaust gases with CO_2 ranges from 500-8000 ppm. As CO_2 concentration increased from 500 to 4000 ppm, there were steady increase in biomass 450 mg/l of dry weight till 2000 ppm, but above 2000 ppm there is decline in growth. The fatty acid profiles were more or less constant at all the CO_2 concentrations and maximum lipid content was 48%. The maximum reduction in CO_2 was 70% at 500 ppm whereas it was 52% at 4000 ppm. When CO_2 concentration increased to 8000 ppm without air, algae could not sustain and showed decline in biomass content. CO_2 fixation from generator gases not only reduces greenhouse gases but also help in getting fatty acid as biofuel.

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I. Introduction

The population of the world is increasing day by day; almost billion peoples are added in every 12-13 years. The population of the world was less than 1.6 billion at the beginning of 20th century, currently it is increased to 7.046 billion (PRB, 2014). In addition industries and transportation were increased to provide the needs of this population. As a result of this dramatic change the consumption rate of energy has increased significantly. This creates an uncertainty regarding over-consumption of energy, and also creates a threat of shortage in energy supply, rise in oil price and energy demand. In recent years many countries have initiated energy conservation policy to reduce the over-consumption of energy. However it is projected that the consumption of energy would increase 50 percent in next 20 years (EIA, 2008).

Fossil fuel supplies majority of energy requirement, even though it creates some serious problem of releasing CO_2 to the environment during combustion. The emission of CO_2 to the atmosphere is directly proportional to combustion of fossil fuel (Davis *et al.*, 2011). In last two decades the associated emission of CO_2 from combustion of fossil fuel is increased to 61 % (CDIAC, 2013; EIA, 2013). The continuous emission of CO_2 leads to is accumulated and retain as an extra carbon in the atmosphere, which took million years to mitigate by the oceans and by the earth biosphere.

Concerns about the risks of increasing energy demand, increasing CO_2 emissions and adverse climate changes are prompted to focus on microalgal CO_2 sequestration and biodiesel production. Algal biomass contain high amount of lipid content compared to conventional oil crops, the lipids could be used as a good renewable source of biodiesel. In addition microalgal biomass could be utilized for several applications such as feed for animals, food supplement, nutritional supplement, antioxidants, fertilizers, colorants, immune modulators and natural dyes(Pulz and Gross, 2004; Jhonson and Wen, 2010; Harun et al., 2010). The Omega-3-fatty acids and omega-6-fatty acids play an instrumental role in human health and have large application on medical fields. In particularly DHA is an essential nutrient has an integral role in human neural development, visual development and functioning of central nervous system (Bradbury, 2011).

The present research aimed to sequester CO_2 from generator exhaust gases and fatty acid production by fresh water microalgae in a closed photoreactor. The effect of CO_2 concentration on biomass production and polyunsaturated fatty acid content were investigated.

II. Materials and Methods

a) Algal feedstock

The algal culture was isolated from agricultural runoff grown in Bold's Basal medium (Bischoff and Bold, 1963) Cultures were routinely checked for purity by microscopic examination and plating. consortia of four fresh water microalgal strains were used in this experiment.

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b) Photo bioreactor experiment

CO_2 sequestration studies were performed in a 2L closed borosilicate glass cylindrical photo bioreactor, at room temperature under continuous illumination of fluorescent light. Two fluorescent lamps of 40W were uniformly fitted on the sides of the photo bioreactor. The overall light intensity ranged from 1600 lux to 1800 lux. Fig. 1 showed a schematic diagram of the experimental setup. The air and CO_2 from generator exhaust gas was mixed in a mixing chamber to achieve stable and desired CO_2 concentration in the air stream before entering the photo bioreactor. The feed air stream and outlet air stream were analyzed for CO_2 concentrations. The algal samples were collected from an outlet at regular intervals and analyzed for various parameters.

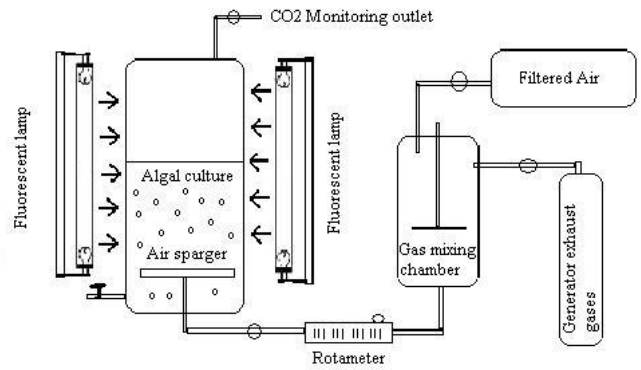

Figure 1 : Schematic diagram of photo bioreactor for sequestering CO_2 from generator exhaust gases by freshwater microalgae

III. Analytical Methods

a) Algal biomass

The concentration of algal biomass was measured by measuring the optical density of the algal suspension at 680 nm (Thermo spectronic, US). The dry weight of algae was estimated from a standard graph. Optical density was calibrated against dry weight measured gravimetrically on pre weighed GF/C glass fiber filters (R^2=0.981)

b) Fatty acid estimation

Algal cells were harvested by centrifugation (10000 rpm) for 10 min. The cell pellets were washed with distilled water and dried. Fifty mg of dried algal biomass was taken in 15 ml of test tube, 1.6 ml of double distilled water, 4 ml methanol and 2 ml of chloroform were added and mixed thoroughly for 30 S. Thereafter, an additional 2 ml of chloroform and 2 ml of double distilled water were added and solution was mixed for 30 S. Following this, the mixture was centrifuged, at 5000 rpm for 10 min. The upper layer decanted and the lower chloroform layer containing the extracted lipids was collected in another test tube. The extraction procedure was repeated again with the residual pellet and both the chloroform extracts were mixed to gather and evaporated till dryness. The dried total lipids were measured gravimetrically and lipid content was calculated as percentage of algal biomass.

Lipids were trans-esterified under nitrogen using BF_3/CH_3OH (12%) for 10 min at 100 °C. Fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (Agilent 7820A, US) equipped with an on-column injector, a DB-Wax (10 m×0.100 mm; 0.10 µm film thickness) capillary column and a flame ionization detector. Fatty acids were identified by comparing their retention times with known standards.

c) Results and Discussion

Since the objective of the study was to evaluate the sequestration of CO_2 from generator exhaust gases by isolated fresh water algae. The CO_2 removal efficiency, growth profile, lipid content and fatty acid profiling were measured at different CO_2 concentrations.

d) Growth of fresh water Algae

The growth profile of the freshwater algae under different CO_2 concentrations from generator exhaust gas is shown in Fig. 2. At all the concentrations, the growth increased steady with time reaching a steady state after about 3 days. The increasing CO_2 concentration from 500 to 4000 ppm enhanced the growth of microalgae, but further increase to 8000 ppm in CO_2 concentration resulted in decreased growth. Hu and Gao (2003) observed enhanced in growth of *Nanochloropsis* sp. when CO_2 concentration was increased from 350 to 2800 ul/l. The maximum specific growth and maximum productivity during CO_2 sequestration was given in Table1. In general, air stream with 500 ppm of CO_2 gave maximum biomass productivity of 54 mg/l with 0.14 g/l specific growth rate. Whereas at 4000 ppm of CO_2, biomass productivity has marginally decreased to 52 mg/l with specific growth rate of 0.16 g/l. Lopes and Franco (2013) have also shown that growth kinetics increased with increased in CO_2 concentration from 5% to 15%, but further increased there is decreased in biomass productivity and specific growth rate. The

microalgal culture reached the doubling time in 3 days and stationery stage was reached after 10 days for all the concentration of CO_2 Fig. 3 shows that pH of the fresh water microalgal culture was increased continuously in all the concentration of CO_2. The pH during the growth increased from 6.4 to 9.0– 9.5 after eleven days. This may be due to utilization of carbon dioxide which leads to accumulation of free OH- ions. The increasing CO_2 concentration from 500 to 4000 pm, the alkalinity during operation was 470-520 mg/l (Data not shown). This increased in alkalinity levels with the time may be due to formation of bicarbonates. The high alkalinity and alkaline pH are indicative of CO_2 fixation.

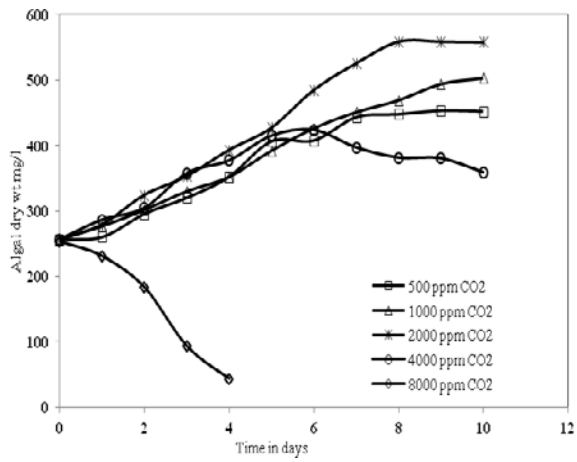

Figure 2 : Growth of fresh water microalgae in photo bioreactor under different concentration of CO_2 from generator exhaust gases

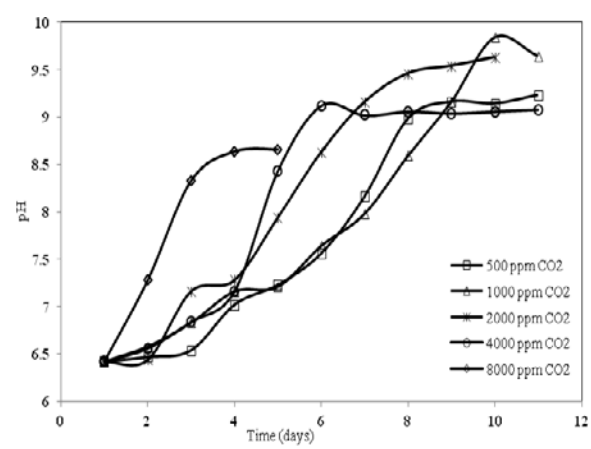

Figure 3 : pH increase of fresh water microalgae in photo bioreactor under different concentration of CO_2 from generator exhaust gases

Table 1 : Maximum biomass and specific growth rate of fresh water micro algae under different concentration of CO_2 from generator gases

CO_2 Concentration of generator gases (PPM)	Maximum biomass productivity mg/l	Maximum specific growth μ
500	54	0.14
1000	40	0.10
2000	58	0.14
4000	52	0.16
8000	-	-

e) CO_2 removal efficiency of fresh water microalgae

The carbon dioxide removal efficiency of the fresh water microalgae in the photo bioreactor at 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm and 8000 ppm of carbon dioxide are shown in Fig 4. The results of the present study show comparatively higher removal percentage at high CO_2 concentrations. The maximum removal efficiency of fresh water microalgae was 70 %, 63 %, 60 % and 52 % for 500- 4000 ppm of CO_2 respectively. The freshwater algae was observed to be more efficient at higher CO_2 concentration. When the concentration of CO_2 increased to 8000 ppm the maximum removal efficiency declined to 33%. Morais and Costa (2007) reported the similar pattern of CO_2 removal efficiency in cultivating *Spirulina* sp 6 % and 12 % of CO_2, the CO_2 reduction rate was was reduced to 53.29 % to 45.61% at 12 % of CO_2. Keffer and Kleinheinz (2002) was observed CO_2 removal efficiency of Chlorella was reached to a maximum of 74 % at lowere concentration of (1850 ppm) CO_2 than the higher concentration.

According to Lopes et al (2009) longer retention time increases the removal efficiency of the CO_2, it has been reported the removal efficiency increased to 45% by providing longer retention time in sequential reactor. In the present experiment, the retention time has been increased by providing low flow rate. The low flow rate (20 ml/ min) increases the retention time in the photo bioreactor and help the freshwater algae to sequester high amount of CO_2 and decrease the loss of CO_2 from the photo bioreactor.

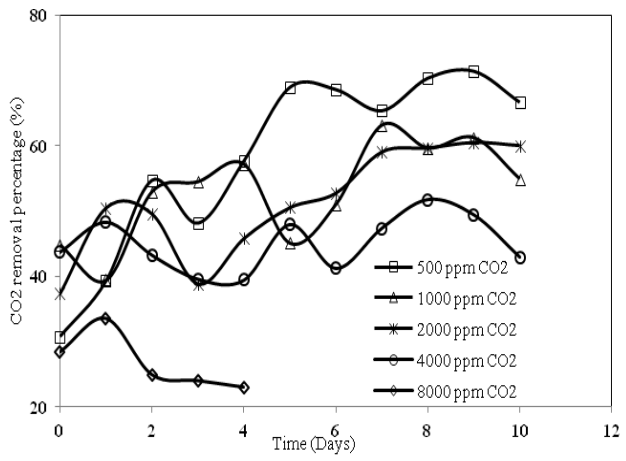

Figure 4 : CO_2 removal efficiency of fresh water microalgae in photobioreactor under different concentration of CO_2 from generator exhausts gases

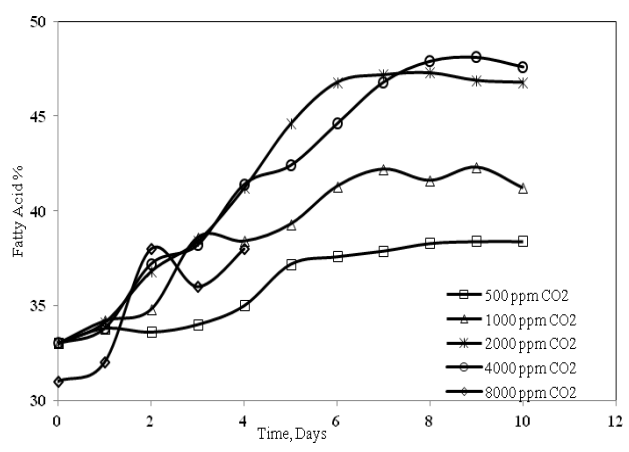

Figure 4 : Fatty acid percentage of fresh water microalgae in photo bioreactor under different concentration of CO_2 from generator exhausts gases

f) Lipid and fatty acid content of fresh water algae

The fresh water alga was not only evaluated for its CO_2 sequestration potential based on its growth, but also for its potential use as a feedstock for biodiesel. This was determined from the fatty acid content of the algal cell given in Fig 5. It was observed that as the cell growth increased with the time according to the concentration of the CO_2 and its fat content was also

increased in 5 to 10 days. The maximum lipid content was 48.1%, 47.3%, 42.3% and 38.4% for 500 ppm, 1000 ppm, 2000 ppm and 4000 ppm of CO_2 concentration respectively. In general the combinations of high biomass growth accompanied with high lipid content consider as a good lipid producer, however the lipid content and the growth of the microalgae is inversely proportionate (Francisco et al., 2009).

Table 2 : Fatty acid composition of fresh water micro algae under different concentration of CO_2 from generator gases

CO_2 Concentration of generator gases (PPM)	SFA (g/100g of FA)	MUFA (g/100g of FA)	PUFA (g/100g of FA)	TFA (g/100g of FA)
500	56.05	34.27	9.68	<0.01
1000	45.20	36.05	18.75	<0.01
2000	46.08	34.18	19.74	<0.01
4000	49.78	28.85	21.37	<0.01
8000	64.26	26.43	9.31	<0.01

In the present work the results clearly indicate that the fresh water microalgal growth and lipid content was comparatively high in higher concentration of CO_2. However the very high concentration at 8000 ppm of the CO_2 the fresh water microalgae showed declined growth but the lipid content was reached 38%.

The fatty acid profiling was given in Table 2. The polyunsaturated fatty acid proportion was increased when the concentration of CO_2 increased. The PUFA content were increased dramatically from 9.68% to 21.37% with increased in CO_2 concentration from 500 to 4000 ppm. It is noted that the increase in concentration of CO_2 increases the concentration of saturated fatty acids (SFA) and monounsaturated fatty acid (MUFA) content. In all the concentration of CO_2, microalgal lipids were mainly composed of 26-35% MUFA, 9-22 % of PUFA and 45-65% of SFA. The present study demonstrates that elevation of CO_2 concentration could raise the production of PUFA, MUFA, and SFA on dry mass basis. The conversion of CO_2 from generator gas gives "wealth from waste".

IV. Conclusion

Microalgae with fast growing capacity and high lipid content fixes more CO_2 and provide a promising alternative technology for reducing greenhouse gases and fulfil the future energy demand. Our results showed the removal efficiency, fatty acid content under different concentration of CO_2 (500 ppm to 8000 ppm) introduced to freshwater microalgae in a photobioreactor. Algal biomass and lipid productivity was increased in all the concentration, and the removal efficiency CO_2 remained constant in the photo bioreactor. Considering the greenhouse gas emission by the fossil fuel and limited availability, algal biofuels are only the current renewable source for biodiesel.

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