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Research Article

EFFECT OF FLUE GAS ON MICROALGAE POPULATION AND STUDY THE HEAVY METALS ACCUMULATION IN BIOMASS FROM POWER PLANT SYSTEM

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Abstract

Microalgae have high photosynthetic efficiency that can fix CO_2 from the flue gas directly without any upstream CO_2 separation, and concomitantly produce biomass for biofuel applications. Microalgae population studies were conducted in a batch mode experiments at power plant site of Chamois, Missouri in "USA". The experiments were conducted in different period (June to December 2011) of time. This study evaluated the effect of several heavy metals that are present in flue gases on the algae, focusing on the growth and accumulation of lipids in the algae that can be converted to biodiesel. The genus *Scenedesmus* presented the greatest richness of species and number of counted individuals in the flue gas ponds compare than non-flue gas treatment ponds. Among the diatomaceae the genus *Navicula* sp, *Nitizchia* sp and *Synedra* sp. presented the next subdominant richness in the ponds. The last results of counted green algae *Ulothrix* sp and *Coelastrum* sp were least number of cells reported in these ponds. The heavy metal-contaminated in flue gas and also enter into the microalgae biomass population. Comparative studies were carried out by flue gas and control system of open ponds. Control system of microalgae population was represented in less amount of heavy metals compare than flue gas ponds.

Keywords: Biofuels; CO2 fixation; Flue gas; Heavy metals; Microalgae.

Introduction

The commercial applications of microalgae include their use as food supplements, feedstuffs in agriculture, aquaculture and chemical industry. However, their photoautotrophic ability has recently been used for removal of carbon dioxide (CO_2) produced by combustion of fossil fuels in thermoelectric power plants with the aim of contributing to a reduction in greenhouse gases and global warming (Amit Kumar *et al*., 2010). Thermoelectric power plants based on fossil fuels are responsible for more than a third of the CO_2 emissions of the USA and about 7% of the total world emissions (Benemann, 1977; Chang and Yang 2003) producing as well sulfur and nitrogen oxides (SOx and NOx), which are known to inhibit the growth of microalgae. CO_2 fixation by photoautotrophic algal cultures has the potential to diminish the release of CO_2 into the atmosphere, helping alleviate the trend toward global warming.

To use micro-algae to fix CO_2 released from power plants via the exhaust gas and thereby mitigate the amount of carbon released into the atmosphere is an attractive design. Microalgae strains that grow well at CO_2 concentrations of 5-10% show drastic decreases in their growth rate above 20% (Watanabe *et al*., 1992; Ranga Rao *et al*., 2007). An important task therefore has been to identify strains that can cope with very high CO_2 concentrations and also have high growth rates. Screening has yielded microalgae strains that grow well in CO_2 concentrations between 30% and 70% saturation (Hanagata *et al*., 1992; Sung *et al*., 1999). Controlling the pH changes in the culture and releasing CO_2 to the algae on demand, growth could be sustained even at 100% CO_2 indicated that by Olaizola (2003). It has been suggested that the hot flue gases introduced in the algal cell cultures may influence the temperature (Ono and Cuello 2007). The vital need for substantive net reductions in CO_2 emissions to the atmosphere can be addressed via biological CO_2 mitigation, tied with transition to more extensive uses of biofuel, nuclear and renewable energy sources. Microalgae have concerned a great deal of attention for CO_2 fixation and biofuel production because they can convert CO_2 (and supplementary nutrients) into biomass via photosynthesis at much higher rates than conventional biofuel crops (Amit Kumar *et al*., 2010). In this paper, the

criteria used for microalgae population studies for CO_2 sequestration systems will be discussed.

Materials and Methods

The culture of green chlorococcales alga *Scenedesmus* sp was obtained from the collection of the power plant at Chamois, Missouri, "USA". The culture contained two and four-cellular coenobia. The number of cells in the culture doubled every 3-4 days. The flue gas from coal-fired power plant was used to cultivate the microalgae (*Scenedesmus* sp) in deep circular ponds. The pond diameter was 4 meters and approximately volume of each pond 4000 L. The flue gas from the power plant was diluted to 2% of CO_2 (air mixture) using compressed air and supplied as CO_2 source for 3 hrs daily in the morning. The treatment of flue gas in two ponds and without flue gas treatment (control) three ponds were tested.

Media and Nutrients used for the growth studies

The nutrient media used to grow the algae were F/2 (Guillard and Ryther 1962). The F/2 medium has two forms. F/2 A consists of ferric chloride, EDTA, cobalt chloride, Zinc sulfate, copper sulfate, manganese chloride, and sodium molybdate. F/2 B contains sodium nitrate, mono sodium phosphate, thiamine hydrochloride (Vitamin B1) vitamin B12, biotin. 129.03µl/L medium taken from part A and part B solution. Proline F/2 algae food was purchased from Aquatic Ecosystems (Apopka, Florida). Additional nutrients part A and part B were added alternation day required to avoid nutrient stress. Parameters like cell count were monitored on a 5 days once up to a month. The experiments were conducted in different period of time.

Preparation of sample

Make sure the cell suspension to be counted is well mixed by either gentle agitation of the flask containing the cells (or other appropriate container). A serological pipette may be used if required. Before the cells have a chance to settle take out about 1 ml of cell suspension using a serological pipette and place in an eppendorf tube. Using a 100 µl pipette, mix the cells in this sample again (gently to avoid lysing them).

Cell Count

Using the pipette, carefully fill the haemocytometer (Sigma Aldrich -Bright-Line,USA) by gently resting the end of the Gilson tip at the edge of the chambers. Take care not to overfill the chamber. The fluid should run to the edges of the grooves only. Keep track of how many squares you count. The whole chamber has 9 squares. The 4 corner squares have 4 x 4 subdivisions. The center square has 5 x 5 subdivisions which are further divided into 4 x 4. Each square is $1mm^2$ and the chamber depth is 0.1mm; therefore the volume overlying each square is $0.1mm^3$ (or 0.0001ml = 0.1µl). Calculate the average number of cells per square (total cells counted/#of squares used) and multiply by 10^4 and the dilution

Results

Effect on algal population growth in outdoor open pond conditions

The Microalgae *Scenedesmus* sp was inoculated in the ponds and the species variation according to local weather changes were also monitored using light microscopy. Microalgal samples were collected from June, July, August (summer) to October, November and December (fall) 2011 over a period of time sampling site at Chamios Power Plant. Microalgae population studied in pond with flue gas treatment and without flue gas treatment of ponds. Our strain *Scenedesmus* sp. was inoculated. After slowly grew other population of microalgae observed in our ponds. In the present study, minimal fluctuations in the population density of phytoplankton were observed. The experiment was observed June to July, 2011 first set of experiments. Here *Scenedesmus* sp. (Fig. 1) were dominant and subdominant species are *Navicula* sp. (Fig.2) and least dominant species are *Chlorococcum* sp. (Fig.3) In the next set of experiment observed in the month July to August, 2011. The observed slowdown population growth as compared to the control algae culture starting from 1^{th} – 30^{th} days. Analysis of algae distribution showed the appearance of large cells in 2-cellular *coenobia* to 4 cells of *Scenedesmus* sp was dominant (Fig.4).

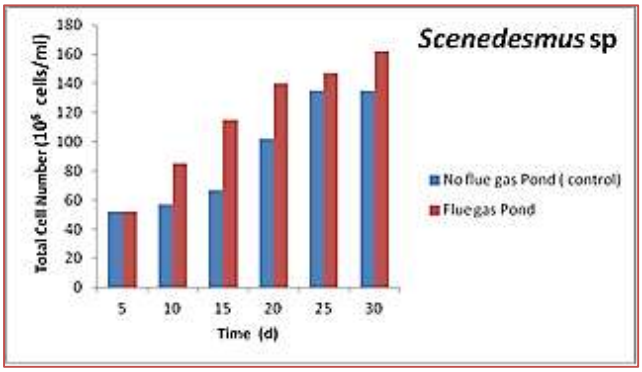

Fig. 1: Cell count of *Scenedesmus* sp. after 5^{th} day to 30 days in two different types of treatment.

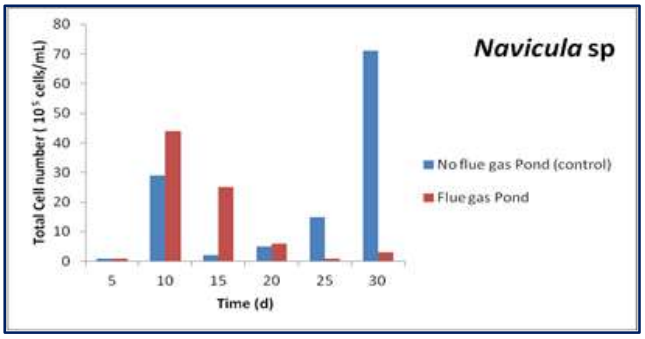

Fig. 2: Cell count of *Navicula* sp. after 5^{th} day to 30 days in two different types of treatment

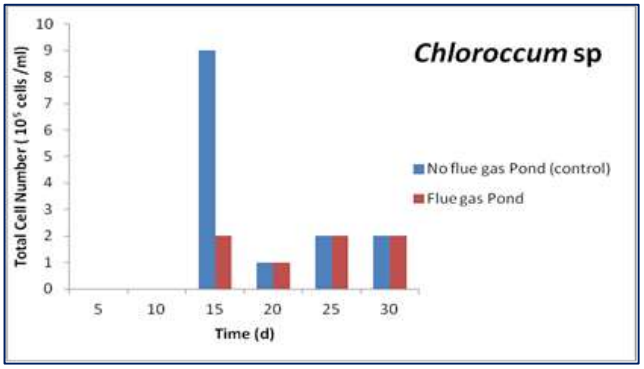

Fig. 3: Cell count of *Chloroccum* sp. after 5th day to 30 days in two different types of treatment.

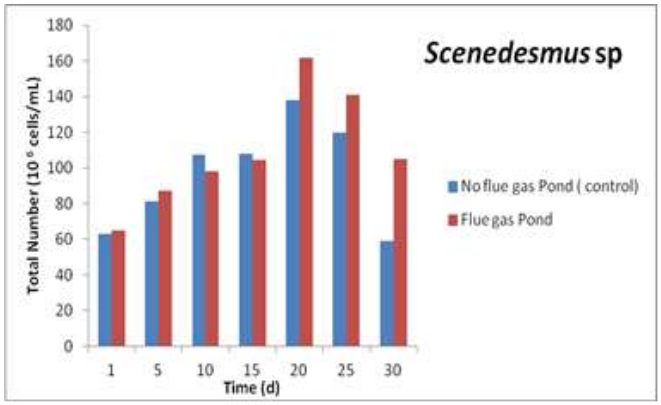

Fig. 4: Cell count of *Scenedesmus* sp. after First day to 30 days in two different types of treatment.

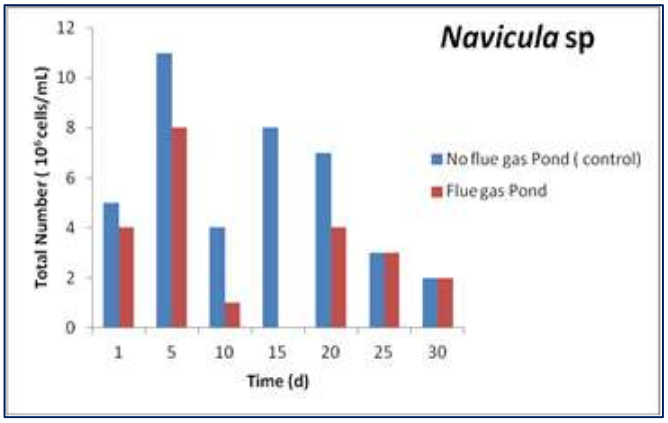

Fig. 5: Cell count of *Navicula* sp. after First day to 30 days in two different types of treatment

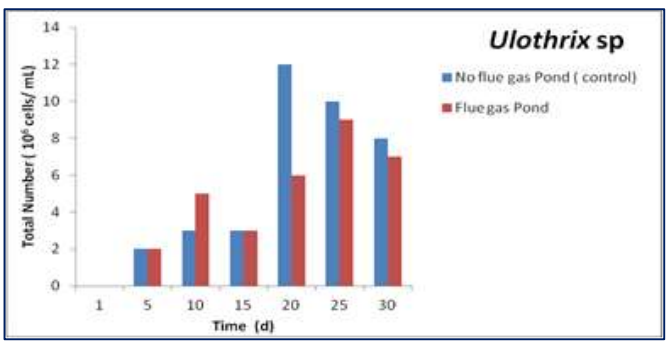

Fig. 6: Cell count of *Ulothrix* sp. after First day to 30 days in two different types of treatment.

The next distribution of cells bacillariophyceae of *Navicula* sp. It was boat-shaped microbes (microorganisms) that are a type of Phytoplankton or algae primarily aquatic, eukaryotic, photosynthetic organisms, ranging in size from a single cell. (Fig. 5) after 5th day most little dominant species of filamentous *Ulothrix* sp. were reported. This is a genus of filamentous green algae, eukaryotic and unicellular generally grow in fresh and marine water. Its cells are normally as broad as they are long, and they thrive in the low temperatures of spring and winter. They become attached to surface by a modified holdfast cell. Reproduction is normally vegetative (Fig. 6).

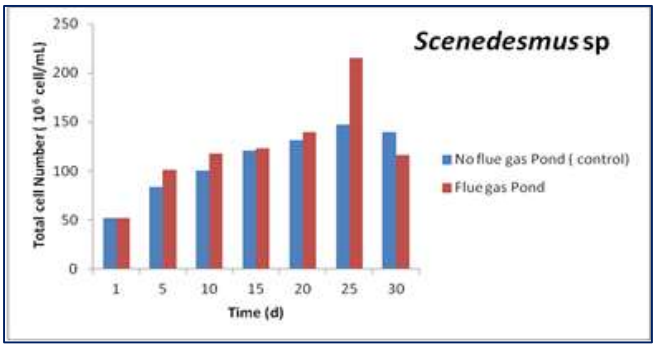

Fig. 7: Cell count of *Scenedesmus* sp. after first day to 30 days in two different types of treatment.

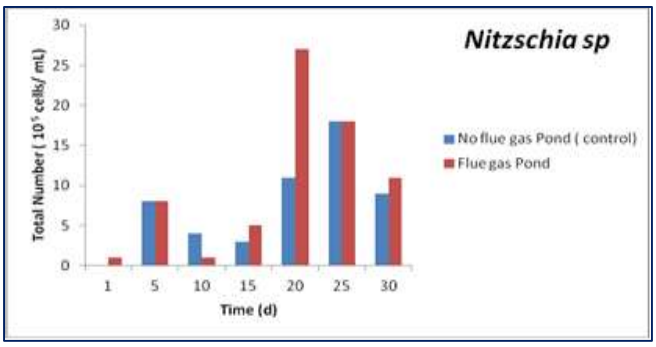

Fig. 8: Cell count of *Nitzschia* sp. after first day to 30 days in two different types of treatment.

The maximum cell number of genus (*Scenedesmus* sp) ranged from 50 cells to 210 (x10^6 cells/ml) within 30 days (Fig. 7). The experiment observed from October to November, 2011. The cell number rates varied ranges in other genus of *Nitzschia* sp. (Fig. 8). It was found mostly in colder weather and is associated with both arctic and antarctic polar sea ice where it is often found to be the subdominant diatom. Some *Nitzchia* species are also extremophiles by dent of tolerance to high salinity; for example, some halophile species of *Nitzchia* are found. Next least cell count of *Coelastrum* sp was reported. It was Colonies spherical, with inner empty space; cells attached to each other by a protrusion of cell wall, arranged in a single layer; cell body mostly spherical with some conical or globular bulges; chloroplasts cup-shaped with a pyrenoid; reproduction by asexual coenobium. But recorded lowest growth rate at 1 and 2.0 (x 10^5 cells/ml) respectively (Fig. 9).

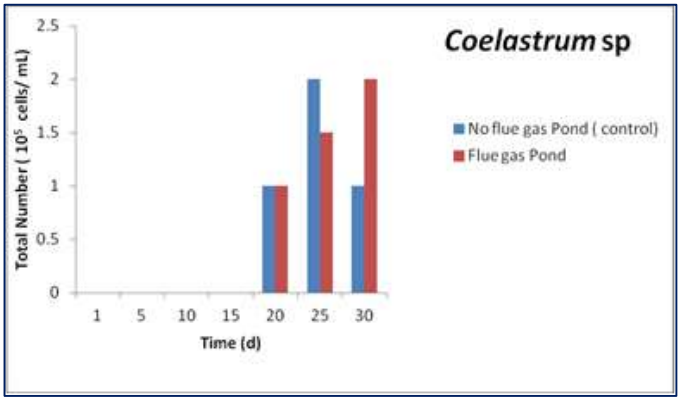

Fig. 9: Cell count of *Coelastrum* sp. after first day to 30 days in two different types of treatment.

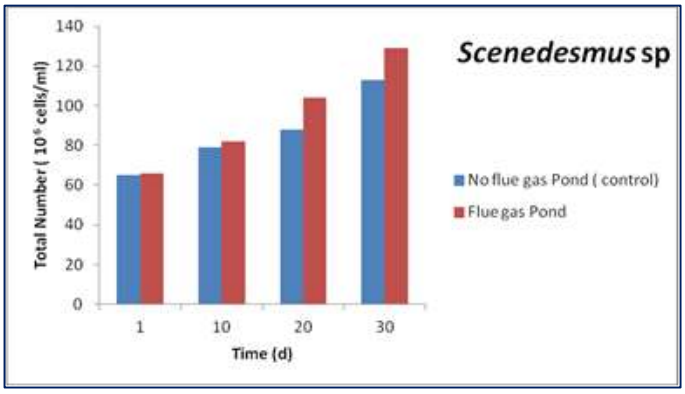

Fig. 10: Cell count of *Scenedesmus* sp. after first day to 30 days in two different types of treatment.

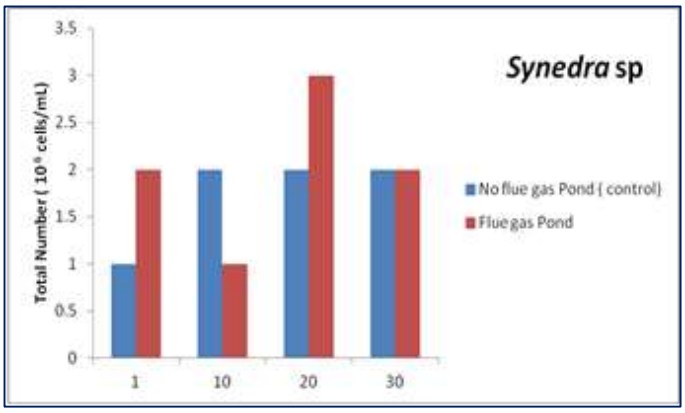

Fig. 11: Cell count of *Synedra* sp. after first day to 30 days in two different types of treatment.

According to the results of November to December 2011 experiment of microalgae analysis, *Scenedesmus* sp was observed with high values in almost all the Ponds. (Fig.10). But here samples were collected in every 10 days once. Because very cold weather and growth curve also reported in low conditions. Next sub dominant genus was *Synedra* has long, needle-like cells that exist singly or in radiate colonies. Certain species have two short horns or spines protruding from the valve face just above the pore field. The valves are covered by rows of round or elongated areolae. The cells appear rectangular when viewed from the girdle or side view. Each cell has two long, plate-like plastids. (Fig. 11). Diatomaceae were always observed in low percentages in samples.

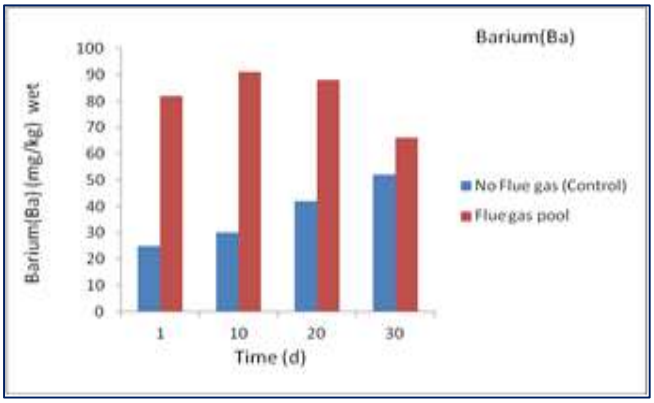

Fig. 12: Effect of barium from first day to 30 days in two different types of treatment

The experiments conclude that: The genus *Scenedesmus* presented the greatest richness of species and number of counted individuals in the flue gas ponds compare than non-flue gas treatment ponds. Among the diatomaceae the genus *Navicula* sp, *Nitizchia* sp and *Synedra* sp. presented the next subdominant richness in the ponds (Table 1). The last results of counted green algae *Ulothrix* sp and *Coelastrum* sp were least number of cells reported in these ponds. These organisms are evenly distributed in all the ponds

Statistical analysis

The abundances of microalgae from samples over the study period were analyzed by statistical method the average of duplicate results was used as data point with standard deviation.

Effect on heavy metals accumulate in biomass from outdoor open pond conditions

The biomass was harvested after the cultivation by centrifuging the cell suspension at 5,000 rpm for 5 min and then freeze dried. The heavy metals like Barium, Chromium, Lead and Mercury were analyzed by PDC Laboratories, Inc. Florissant, MO, USA. The concentration of heavy metals compounds in the biomass of microalgae population cultivated on the actual flue gas and control (mixture air and carbon dioxide). The various microalgae population was indicated. The level of the toxic heavy metals was varied in the flue gas.

The heavy metal-contaminated in flue gas and also enter into the microalgae biomass population. Comparative studies were carried out by flue gas and control system of open ponds (Fig. 12-15). Control system of microalgae population was represented in less amount of heavy metals compare than flue gas ponds

Table 1. Shows that abundances of microalgae from samples over the study period in Chamios Power Plant, 2011.

Month	Microalgae genus	Class	Dominant types
June – July	*Scenedesmus* sp. *Navicula* sp. *Chlorococcum* sp.	*Chlorophyceae* *Bacillariophyceae* *Chlorophyceae*	+++ ++ +
July – August	*Scenedesmus* sp. *Navicula* sp. *Ulothrix* sp.	*Chlorophyceae* *Bacillariophyceae* *Ulvophyceae*	+++ ++ +
October – November	*Scenedesmus* sp. *Nitzschia* sp. *Coelastrum* sp.	*Chlorophyceae* *Bacillariophyceae* *Chlorophyceae*	+++ ++ +
November - December	*Scenedesmus sp.* *Synedra sp.*	*Chlorophyceae* *Bacillariophyceae*	+++ ++

+++ : More abundance; ++ : Moderate abundance' + : Least abundance

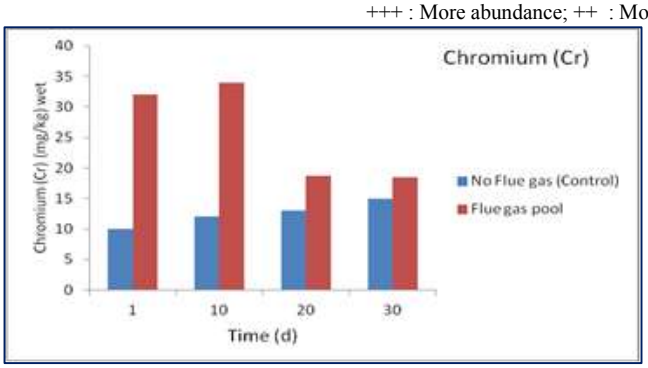

Fig. 13: Effect of Chromium from first day to 30 days in two different types of treatment

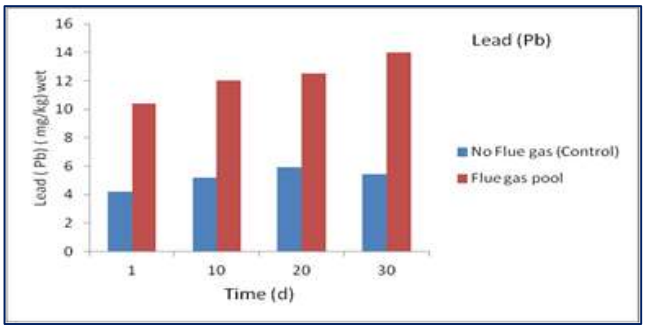

Fig. 14: Effect of Lead from first day to 30 days in two different types of treatment

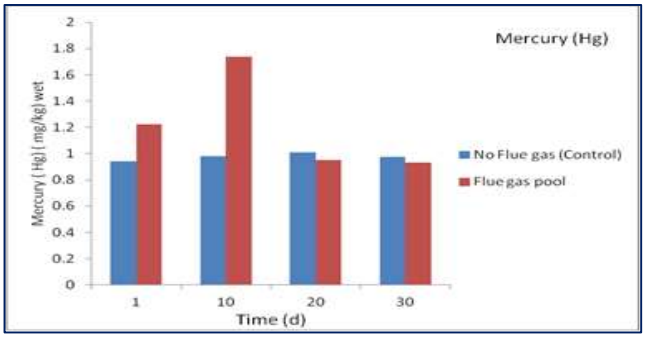

Fig. 15: Effect of Mercury from first day to 30 days in two different types of treatment

Discussion

Thus, analyzing the Microalgae population cell count and that showed the differences between the population states that level of cell number increasing under the flue gas treatment in ponds. Previous analysis has shown that CO_2 cost plays a central role in process economics, making the minimization of CO_2 cost a top priority (Kadam and Sheehan, 1996). A rigorously derived CO_2 recovery-cost model is available in the context of microalgae cultivation using flue gas emitted by a typical 500 MW power plant located in the Southwestern United States (Kadam, 1997).

To evaluate if the flue gas could be directly utilized, an alternative case was devised that only includes compression, dehydration, and transportation to the ponds. This option delivers CO_2 to the ponds at a concentration of only 14%. Processing of flue-gas equivalent to a 50 MW capacity was also analyzed since it matches well with the CO_2 mitigation capacity of a 1000 ha pond system. The algal mass produced from power plant CO_2 is a useful product with application in food, fuels, etc. (Kadam and Sheehan, 1996). The production of lipids from microalgae is a possibility because plant storage lipids could be among the best biomass feed stocks for producing renewable, high-energy liquid fuels such as diesel fuel. Growth and lipid production of microalgae were investigated, with attention to the feasibility of making use of flue gas CO_2 as a carbon source. The effect of a high CO_2 level in artificial seawater differed from strain to strain. SOx and NOx inhibited algal growth (Masaaki, 1991).

Many different culture systems that meet these requirements have been developed over the years. More recently, others have also confirmed that flue gas can be used to grow algae without harmful effects and at least one commercial algae cultivator on Hawaii is using CO_2 from a small power plant (Pedroni *et al.*, 2001). In addition,

dissolved NOx can be used by algae as a nitrogen source. The amount of flue gas needed per hectare will differ per species of algae, and will also vary throughout the day with light intensity and temperature, thus needs to be optimized for each specific application. High dissolved concentrations of CO_2 (and also SO_2) will affect the pH, thus need to be controlled or buffered (Moheimani and Borowitzka, 2007). To determine the viability of using microalgae as a carbon dioxide sequestration option, carbon dioxide fixation and microalgae population have been studied.

The microalgae growth rates that occur in bioreactors and small scale ponds cannot be expected in full-scale operating sequestration ponds. The conversion efficiencies of microalgae are not expected to exceed those of the higher plants (Benemann, 1997). And to achieve those efficiencies would require development of techniques to maintain the original algal strains and ward off invasion by other algae, zooplankton and infections. This principle has since been utilized in increasing the efficiency of high rate oxidation ponds. Likewise, microalgae having high affinity for polyvalent metals are effectively used to reduce the concentration of heavy metals present in water and flue gas (Trevieso, 2002). The toxicological of the heavy metals accumulated in biomass using the flue gas ponds was evident in the excellent flue gas treatment and actual possibility of using this source of carbon dioxide for large-scale microalgae production facilities (Douskova *et al*., 2008).There is a great potential for its exploitation in the field of biofuels because of the important advantage of the microalgae in possibly increasing the relative content of lipids or starch via different culturing conditions.

Conclusion

As our investigations have shown, these changes take place in the presence of different weather conditions. Population survival was in unfavorable conditions. The limits of algal cells resistance (spores) to long-term duration to survival of population as whole. The genus *Scenedesmus* presented the greatest richness of species and number of counted individuals in the flue gas ponds compare than non-flue gas treatment ponds. Among the diatomaceae the genus *Navicula* sp, *Nitizchia* sp and *Synedra* sp. presented the next subdominant richness in the ponds. The last results of counted green algae *Ulothrix* sp and *Coelastrum* sp were least number of cells reported in these ponds. Our results indicate that there were differences in heavy metals concentration in microalgae production from power plant unit. The heavy metal-contaminated in flue gas and also enter into the microalgae biomass population. Flue gases contain several chemical compounds, which even at concentration levels of treated flue gas can affect the growth, biochemical composition and excretion of microalgae population. Vice versa, microalgae can also directly and indirectly affect the removal of flue gas compounds such as CO2, NOx, SOx, heavy metals, and unburned carbohydrates. Comparative studies were carried out by flue gas and control system of open ponds. Control system of microalgae population was represented in less amount of heavy metals compare than flue gas ponds.

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