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Baseline Study on the Dairy Wastewater Treatment Performance and Microalgae Biomass Productivity of an Open Pond Pilot Plant: Ethiopian Case

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Abstract

Increased urbanization and industrialization has given rise to serious water pollution and environmental problems. Discharge of industrial effluent as well as chemical spills, domestic sewage, and use of pesticides are the main cause of environmental pollution. Dairy industry is one of the important industries causing water pollution. There are many physico-chemical methods are available, but recent progress in bioremediation suggest that microalgae can play dual role to increase biomass by utilizing waste as nutrients and can be helped in treated wastewater that directly discharges to the environment. In general, dairy effluent contains huge amount of milk constituents such as casein, lactose, fat and high amount of BOD and COD. In the present study, an attempt has been made to treat the dairy effluent from anaerobic digestersby using the natural occurring microalgae in Ethiopia. This result revealed that the potential of the consortium of microalgae to reduce TDS (from 160 ± 1.53 to 103.67 ± 1.53), TSS (from 216.67 ± 1.53 to 47.00±5.57), VSS (from 113.67±3.06 to 22.33±3.06), nitrate (from 3.5±0.28 to 0.65±0.07), phosphate (from 8.25±0.07 to 1.03±0.3), BOD (from 235±14.14 to 64±4.24) and COD (from 665±8.49 to 270±1.41), and produce 0.209±0.017 mg/L of the biomass concentration and 31.33% of lipid.

Key words: Consortium Microalgae, Open pond, Wastewater, Dairy

1. **Introduction**

An increase in the number of small and large scale industries in Ethiopia has led to the production of a large volume of complex wastes. In abroad, various researchers have been carried out on the assessment of quality of freshwater pollution by the discharge of effluent from the industries and some domestic wastes (Eillis 1944; Rama Rao *et al.*, 1978). Dairy industry is noted as one of the significant contributor to water pollution. Dairy manure waste is basically biodegradable produces an undesirable odor. Also dairy waste contains sufficient nutrients for biological growth, biological treatment methods are considered more ideal and economical (Warner, 1976).

Phycoremediation is the process in which algae are employed to remediate environmental pollution. Phycoremediation involves the use of macroalgae or microalgae for effective removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and sequester CO_2 from waste air (Olguin, 2003; Olguın *et al*., 2004; Moreno-Garrido, 2008; Mulbry *et al*., 2008). Over the last few decades, efforts have been made to apply intensive microalgal cultures to perform the biological tertiary treatment of secondary effluents (Oswald and Gotaas, 1957; De la Noüe *et al*., 1992). The underlying assumption is that the microalgae will transform some of the contaminants into non-hazardous materials enabling the treated water to then be reused or safely discharged (Oswald, 1988).

Algae growth in wastewater treatment ponds contributes to treatment mainly through dissolved oxygen production and nutrient assimilation. However, the carbon: nitrogen and carbon: phosphorus ratios in dairy lagoon water (C: N 3:1; C: P 10:1) are low compared to typical ratios in rapidly-growing algae biomass (C: N 6:1; C: P 48:1) (Oswald and Golueke 1960; Metcalf and Eddy, 1991). Few studies however, have investigated algae-based treatment of dairy slurry from anaerobic digesters.

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Low-cost waste treatment methods one a prime need for the developing countries like Ethiopia. Although open ponds are of low cost to construct and easy to operate, their two major drawbacks are requirement of large amount of land and the algae-laden effluent which has a high potential of polluting receiving waters. In recent years, the importance of biological waste treatment system has caught attention of workers all over the world and has helped in developing relatively efficient, low cost waste treatment systems. To develop suitable and efficient treatment systems, it is obligatory to understand the mutual influence and interactions between the effluents and organisms, so that manipulations to improve the treatment systems become feasible.

Ethiopia, a Sub-Saharan (tropical) country has got a plenty of sun (13 months sunshine) and is rich in microalgal species. The present study was focused on the phycoremediation of dairy effluent from anaerobic digesters by using microalgae consortium that growing in Ethiopia climate condition. The role of microalgae consortium ideally fit to play a vital role in treating wastewater by utilizing different constituent's essential chemical as nutrients for its growth metabolism. The objective was to study the effectiveness of the consortium system of microalgae on the removal of nutrients and on the biomass productivity in Ethiopian context.

2. Materials and Methods

2.1 Experimental Design

The experiment was run in parallel to determine algae growth, nutrient removal, and lipid productivity in dairy wastewater. In the dairy effluent from anaerobic digesters experiment, lipid productivity and nutrient removal were monitored during 3-14 days of batch growth to study the effect of the growth cycle on lipid content. Control cultures (no CO_2) were used to simulate the carbon-limitation typical of wastewater ponds and to differentiate the effect of CO_2 addition on productivity. On an average, sampling was done about 2-3 days and includes daily *in-situ* measurements of pH, TDS and temperature.

2.2 Design of the small-scale open pond

Open pond systems designed strictly for algal biomass production typically use depths of 10-20 cm (Boussiba *et al*., 1988; Weissman *et al*., 1988). High Rate Algal Ponds for wastewater treatment utilize depths of 25-60 cm (Goldman and Ryther, 1975; Oron *et al*., 1981; Azov and Shelef, 1982; Oron and Shelef, 1982). Since shallow cultures have a greater tendency to overheat (Oswald, 1988). A final consideration for high algal productivity is mixing of the algal culture. In open pond systems, flow mixing and suspension of the algal culture are commonly achieved with a paddlewheel (Weissman *et al.,* 1988; Al-Shayji *et al*., 1994).

In the present study, each open pond was constructed from a rectangular tank (2.4 m x 0.98 m x 0.20 m depth) with a central baffle and a custom paddlewheel (Figure 1). Paddlewheel was fixed to a stainless steel shaft and powered by motor with a speed controller. The paddlewheels provided gentle mixing to prevent thermal stratification, prevent algae cell sedimentation and maintain carbonation and promote the exposure of the cells to the sunlight to optimize the biomass productivity.

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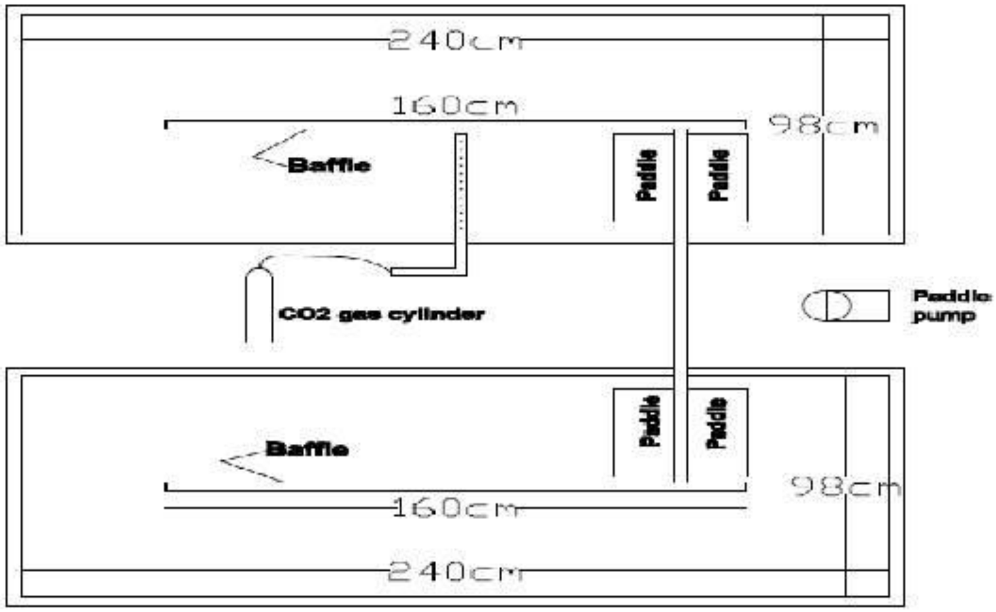

Figure 1: Design of the Small-Scale Open Pond

Geomebrane was lined at the bottom of the pond. The illuminated area of culture is 2.352 m^2. To set the depth, it is important to avoid the shadowing effect where the microalgae cells do not allow getting into the culture, and then the cells down water column are not well illuminated (Qiang *et al*., 1996). For that reason, it was chosen the depth (0.2 m) according to other authors (Garcia et al., 2000). Finally, the volume of each pond is about 470 L.

A local made cost effective paddlewheel will be driven by 0.10 kW electrical motors kept the algae in suspension in algae open pond. Wastewater discharge was approximately 50 L with 2% inoculated microalgae consortium and the hydraulic residence time ranged from 3-14 days. For this study the ponds were constructed at Addis Ababa Institute of Technology, located in Addis Ababa, Ethiopia (8.98° N; 38.8° E at 2324 m elevation).

2.3 Development, observations and identifications of algal consortium

The microalgae used in this study include consortium different indigenous microalgae species. Developments of algal consortium were carried out from the wastewater samples were collecting from Akaki River, Ethiopia (Figure 2 Left). About 4 ml of wastewater sample was transferred into 250 Erlenmeyer flasks containing 100 ml BG-11 medium (Stanier *et al.*, 1971) and CHU 13 modified medium (Largeau *et al*., 1980) were cultured in the growth room at 25°C with 12 hr photoperiod (12 hr. light: 12 hr. dark in 24 hr. period) and 200-250 μmol $m^{-2}sec^{-1}$, light intensity provided by two 24 W full spectrum compact fluorescent light (Figure 2 Right). After a week of growth, identification of dominant algae types was done following monographs of algae (Smith, 1950; Prescott, 1951).

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Figure 2: Akaki River (Left) and Inoculated Microalgae (Right)

The consortium microalgae sample was subsequently cultured in a common algae growth medium, BG11, containing the following components (mg/L): $NaNO_3$ (1500), K_2HPO_4 (40), $MgSO_4{\cdot}7H_2O$ (75), $CaCl_2{\cdot}6H_2O$ (56), Citric acid (6), Ferric ammonium citrate (6), EDTA (1), $NaCO_3$ (20), 1 ml of trace metals within and distilled water. The other medium is CHU 13 medium that is a culture medium used in microbiology for the growth of certain algal species. Modified medium CHU 13 includes essential minerals and trace elements that are required by algae for growth, but does not include a carbon source and so is only appropriate for growth of photographs. It is containing the following components (mg/L): KNO_3 (400), K_2HPO_4 (80), $CaCl_2{\cdot}6H_2O$ (157), $MgSO_4{\cdot}7H_2O$ (200), Ferric Citrate (20), Citric acid (100), $CoCl_2$ (0.02), H_3BO_3 (5.72), $MnCl_2 \cdot 4H_2O$ (3.62), $ZnSO_4{\cdot}7H_2O$ (0.44), $CuSO_4{\cdot}5H_2O$ (0.16), Na_2MoO_4 (0.084) and 0.072N H_2SO_4 (1 drop). The composition of this consortium culture of algae was characterized roughly under the microscope. It was mainly composed of single cell (green algae), diatoms and filamentous green algae.

2.4 Substrates

Dairymanure effluent from anaerobic digesters was used for this experiment. Dairymanure effluent from anaerobic digesters was collected from a Dairy farm located at Genesis Dairy farm, Deberezeit. Wastewater for the research was collected from the biogas plant and homogenized by mechanical agitation, filtered and subsequently stored at 4^oC for further use.

2.5 Experimental Measurements

Periodic samples at the interval of 2-3 days within 14 days at 1:30 PM were drawn to measure total suspended solid (TSS), volatile suspended solid (VSS), soluble-orthophosphate (PO_4^{-3}-P), COD, BOD_5, nitrate (NO^-_3-N), algal cell, biomass, chlorophyll and crude lipid concentrations. Temperature, pH and TDS were monitored daily *in-situ*. The protocols to conduct these measurements are briefly described below. Each analysis was repeated in triplicate.

Identification and biomass concentration of the microalgae: Algae identification was performed by observation through the optical microscope (Optika Microscope at 100x). Algae were identified to the genera level using information in Standard Methods and other identification materials. The optical density (OD) of the samples were measured at 625 nm using a spectrophotometer (PerkinElmer, Lambda 950 UV/VIS Spectrometer) and optical path length of 0.01 m. Algae biomass

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concentration were estimated using the following equation (1) (Guillard and Ryther, 1962; Guillard, 1973; Levasseur *et al*., 1993), each analysis was repeated in triplicate.

$$B = 0.38 * OD_{625} \tag{1}$$

Where,

OD_{625} is the optical density at 625 nm.

B is the algae biomass concentrations (g/L).

The growth rate of a microalgal population is a measure of the increase in biomass over time and it is determined from the exponential phase. Growth rate is one important way of expressing the relative ecological success of a species or strain in adapting to its natural environment or the experimental environment imposed upon it. Biomass estimates need to be plotted over time, and logistical constraints determine their frequency but once every one to two days is generally acceptable. Specific growth rate was estimated using equation (2) (Levasseur *et al*., 1993).

$$Growth\ Rate\ (K) = \ln\left(\frac{B_t}{B_0}\right) / (T_t - T_0) \tag{2}$$

Divisions per day and the generation or doubling time can also be calculated once the specific growth rate is known. Divisions per day and the generation (doubling time) were estimated using equation (3) and (4), respectively, (Guillard, 1973):

$$\text{Division per day } (\mathrm{D_d}) = \frac{\mathrm{K}}{\ln_2} \tag{3}$$

$$\text{Doubling Time } (\mathrm{t_d}) = \frac{\ln_2}{\mathrm{D_d}} \tag{4}$$

Where,

B_0 and B_t are initial and final microalgae biomass concentrations (g/L) respectively.

T_0 and T_t are initial and final times (d) respectively.

K, D_d and t_d are biomass growth rate (d^{-1}), divisions per day (d^{-1}) and the generation (doubling time) (d^{-1}), respectively.

Determination of chlorophyll content: Algal cells were extracted with ethanol and the chlorophyll content was determined spectrophotometrically by measuring the absorbance of Ethanol extract against an ethanol blank at 649 and 665 nm using a spectrophotometer (PerkinElmer, Lambda 950 UV/VIS Spectrometer). The chlorophyll *a, b* and total chlorophyllconcentrations (g/L) were determined using the following equations (Minocha *et al*., 2009), and estimated using equation (5). Each analysis was repeated in triplicate.

$$\begin{aligned} &Chlorophyll\ a = 13.36 * A_{665} - 5.19 * A_{649} \\ &Chlorophyll\ b = 27.43 * A_{649} - 8.12 * A_{665} \\ &Total\ Chlorophyll = Chlorophyll\ a + Chlorophyll\ b \end{aligned} \tag{5}$$

Where,

A_{649} *= the absorbance for chlorophyll at 649*

A_{665} *= the absorbance for chlorophyll at 665*

Lipid content analysis: The algal lipid content was determined according to the method by Bligh and Dryer (1959). A sample of algae suspension was centrifuged at 3,800 rpm for 10 minutes to obtain a concentrated algae paste. The dry weight (W_d) of the

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cake was determined gravimetrically after drying at 60°C. A 2-mL sample of algae solution was mixed with 4 ml of a 2:1 methanol/chloroform solution in a glass vessel. The suspension was left for 24 hr. Thereafter, 1 mL of chloroform was added and the solution was mixed on a vortex for 1 min. 2 mL of water was then added and the mixture was again agitated for 2 min. The layers were separated by centrifugation at 2,000 rpm for 10 min. The lower layer was extracted with a glass syringe and filtered through a Whatman no. 1 filter into a previously weighed glass vessel (w_1). The solvent was dried in a water bath at 98°C and the vessel was weighed again (w_2) to obtain the lipid content of the sample. The solvent layer was evaporated to dryness and the total lipids were measured gravimetrically.

$$Lipid\ content\ \% = \frac{w_2 - w_1}{w_d} X100 \tag{6}$$

Water Quality Analyses:The method for COD, BOD_5, TSS and VSS measurement is based on the correlation between mass loss after exposure to high temperature and the amount of organic material present in the sample (APHA, 2005). The TSS and VSS concentrations were determined gravimetrically as per the Standard Method 2540 D (APHA, 2005) immediately after sampling. Orthophosphate (PO_4^{-3}-P), and nitrate (NO_3^--N) were measured based on the ascorbic acid and cadmium reduction methods (method 4500-P-D) (APHA 2005) using HachPhosVer TM reagent and NitraVer TM reagent respectively (Hach Company, Loveland, CO). Removal rates for those parameters were calculated using equation (7), each analysis was repeated in duplicate.

$$Removal\ Effeciency(\%) = \frac{Intitial\ Concentration - Final\ Concentration}{Intitial\ Concentration} X\ 100 \tag{7}$$

2.6 Operating Conditions

The experiments were conducted at March, 2013. Temperature, conductivity, TDS and pH were recorded at the time of each sampling after mixing. Weather data, such as daily solar irradiation, air temperature and other climatic information were obtained from NASA RETScreen database, Addis Ababa at Latitude 8.98° N at and Longitude: 38.8°E. The elevation is 2324 m.

2.7 Statistical Analysis and software

Some of the experiments were carried out in duplicate and some of them are in triplicate, and all data represent the mean and statistical significance of replicate measurements except where noted. Microcalorgin (8.0) and excel softeware were used for graph and analysis.

3. Results and discussion

The experiment was conducted in the March (spring) in greenhouse conditions at the Addis Ababa Institute of Technology, Addis Ababa. Weather data, such as daily solar irradiation, air temperature and other climatic information were obtained from the NASA RETScreen. The weather data for Addis Ababa during the experiment at the month of March (spring) for study site (NASA, 2008).

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3.1 Light conditions

Sunlight intensity and temperature variations, which affect biomass productivity, are a challenging problem in the use of open ponds in many areas. Figure 3 shows the daily insulation and air temperature of the study area included the period of cultivation. The values are higher in spring season and it decreases in summer season, therefore, it leads higher biomass productivity in spring season rather than summer or other seasons. The light intensity also affects the photosynthetic efficiency, the productivity of cell biomass and the activity of cellular metabolism. Average daily insulation was 6.34 KWh/m^2/d in the March (spring) during this study to cultivate microalgae.

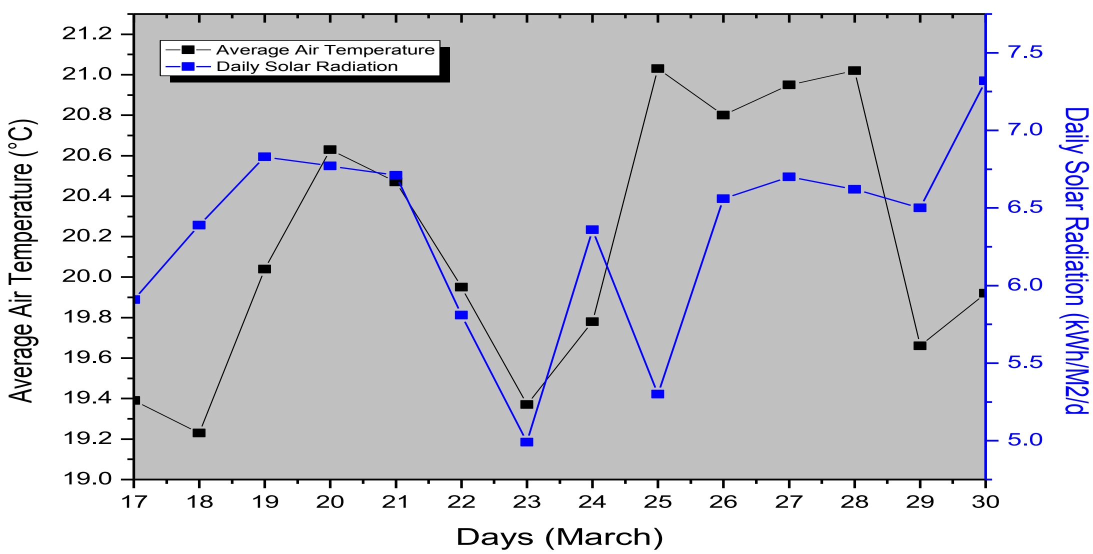

Figure 3: Air Temperature and Solar Radiation Obtained from the NASA RETScreen

Microalgae are more efficient at utilizing sunlight than terrestrial plants (Pirt, 1986), consume harmful pollutants, and have minimal resource requirements and do not compete with food or agriculture for precious resources (Searchinger, 2008). Microalgae have higher growth rates than terrestrial plants, allowing a large quantity of biomass to be produced in a shorter amount of time in a smaller area. Microalgae growth rates of 10 to 50 g/m^2/d have been in the literature (Chaumont, 1993). Compared to terrestrial plants such as corn and soy, Microalgae have shorter harvest times because they can double their mass every 24 hours (Chisti, 2007).

3.2 Temperature and pH

As Figure 4 shows, at the 0.05 level, the difference of the population mean is significantly different with the test difference occurred in the average water temperature on both ponds during the whole study. During the cultivation (March) the air temperature ranged from 14.16 °C to 27.33°C, with an average value of 20.16 °C. As a consequence of such low air temperature variability, the average water temperature reached the maximum value of 25°C and the minimum value of 21°C for both ponds (Figure 4).

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The pH of the Dairy effluent from anaerobic digesterswas mostly acidic in nature due to decomposition of lactose into lactic acid under aerobic conditions and may cause corrosion of sewers (Joseph, 1995). When the effluent treated with microalgae the pH was increased and became alkaline Hence, the pH effect on the treatment efficiency was investigated. Manohara and Subramanian (1993) have also reported rise in pH value up to 10th day of growth. In this study interestingly, the pH of the dairy effluent increased to an average 9.09±0.14 and 9.27±0.1 in the Carbon sparged pond and in the Non-Carbon sparged pond, respectively, treated with the consortium of microalgae (Figure 4). Changes in pH are shown in Figure 4 in both ponds and were more variable ranging between 6 and 10. There was an excursion of pH above 9 for both ponds from days 6 to 14.The temperature range needed to support algal growth is specific to the species and strain cultured. The optimal temperature for phytoplankton is within the range of 20−30°C. Temperatures lower than 16°C will slow growth, and temperatures higher than 35°C are normally lethal for a number of species (Hanagata et al., 1992; Andersen and Andersen, 2006; Graham et al., 2008). In the present study the water temperature (20°C) and pH (9) of the systems were within the optimum growth range suggested for most strains of the algae (Soeder 1981; Grobbelaar 1982; Fontes *et al*., 1987; Borowitzka 1998; Chevalier *et al*., 2000).

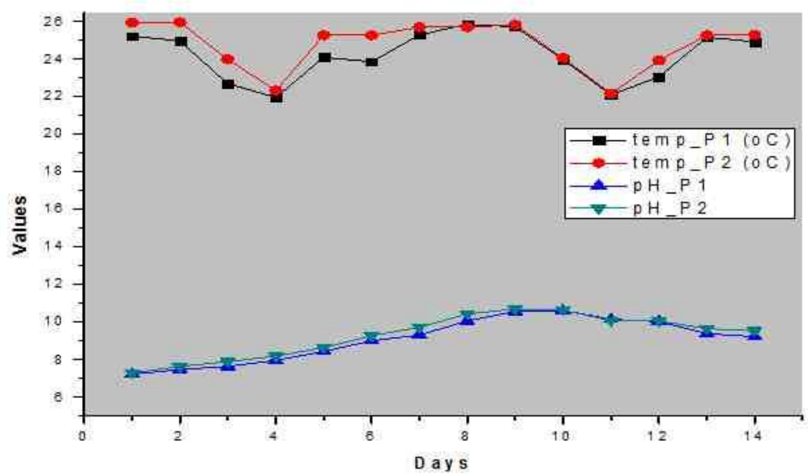

Figure 4: Water Temperature and pH Value during Pond Cultivation

3.3 Microalgae Identification, Growth Rate, Biomass Productivity and Lipid Production from Wastewater Effluent

The mass production of algae has historically been for use as a food supplement or wastewater treatment (Johnson and Sprague, 1987). The technology for production of biomass from wastewater has been present since the 1950s. Microalgae are efficient in the removal of nutrients from wastewater. Thus many microalgal species to grow by rapid production of new cells in wastewater due to the abundance of carbon, nitrogen and phosphorus that act as nutrients for the algae. Microalgae have shown great efficiency in the uptake of nutrients and have been found to show dominance in oxidation ponds (Pittman *et al*., 2010).

In the present study, the ponds have been previously inoculated with a community of different algal species derived from samples collected from Akaki River (highly polluted river) in Ethiopia. The prominent algae genera were *Actinastrum, Ankistrodesmus,Coelastrum, Spirogyra, Micractinium, Golenkinia, Gleocystis, Westella,Chlorella, Closterium, Chlorococcum, Scenedesmus, Synechoccystis, Gloeocapsa, Chroococcus, Anabaena, Lyngbya, Oscillatoria,* and*Spirulina* (see Figure 5).

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Figure 5: Some of the Identified Microalgae

This study was conducted to assess the potential of cultivating algae using wastewater as a nutrient medium. The dominant consortium of micralgal species that obtained at the end of fourteen days in the system were *Scenedesmus sp. Chlorella sp.Ankistrodesmus sp. andOscillatoria sp.* grew favorably on dairy effluent from anaerobic digesters treatment. There was relatively high nutrient uptake for phosphorous and nitrate.

The microalgae growth for each pond was observed throughout the treatment period of 14 days. In the two treatments, Carbon Sparged pond (Pond_1) had higher biomass densities compared to Non-Carbon Sparged pond (Pond_2). However, it was observed that the biomass densities of the consortium exceed the average biomass densities of the Carbon Sparged pond at all sampling times indicating a synergistic interaction between the strains (Table 1).

Table 1: Algae Biomass (g/L) and Crude Lipid Content (%)

Treatment	Biomass Concentration (mg/L)		Lipid content (%)
	Initial	*Final*	*Final*
Carbon Sparged pond (Pond 1)	0.016±0.008	0.209±0.017	31.33
Non-Carbon Sparged pond (Pond 2)	0.015±0.002	0.137±0.002	28.05

Biomass concentration at the end of fourteen days growth period for the media with dairy manure as the sole nutrient sources, the algal biomass densities were 0.209±0.017 and 0.137±0.002 for Carbon Sparged pond and Non-Carbon Sparged pond, respectively. Based on their biomass concentrations, the specific growth rates of the Carbon Sparged pond were 0.185d^{-1}at, and 0.160 d^{-1} at Non-Carbon Sparged pond, respectively (Table 2). These growth rates were lower compared to the growth rates in the Bristol medium (0.3644 d^{-1}) under axenic conditions (Lau *et al*., 1997). In all cases, average biomass growth rates were significantly higher while doubling time was correspondingly lower for the Carbon Sparged pond (2.599 d^{-1}) compared to Non-Carbon Sparged pond (2.997 d^{-1}), further strengthening the hypothesis that the Carbon Sparged pond have a better growth profile compared to Non-Carbon Sparged pond.

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Table 2: Average Specific Growth Rate, Division per Day and Doubling Time of Algae

Treatment	Specific Growth Rate (K)	Division per day (d^{-1})	Doubling time (d^{-1})
Carbon Sparged pond (Pond 1)	0.185	0.267	2.599
Non-Carbon Sparged pond (Pond 2)	0.160	0.231	2.997

Green algae contain chlorophyll a and b. Presence of these pigments makes green color of the green algae (Vonshak and Maske, 1982; Tomaselli, 2004). There are a few reports about second metabolites of green algae (Gamal, 2010). Green algae species can access higher sugar levels and this makes them useful energy sources. They also have high cellulose content (Bruton *et al*., 2009).

Chlorophyll content of cells is an important determinant of their overall autotrophic growth efficacy. Chlorophyll a concentration at the end of fourteen day growth period for the media with anaerobic digester dairy effluent as the sole nutrient were 0.444±0.05 and 0.318±0.04 g/L for Carbon Sparged pond and Non-Carbon Sparged pond, respectively (Table 3). Chlorophyll b and total chlorophyll concentrations were significantly higher for Carbon Sparged pond, 0.329±0.07 and 0.773±0.05 g/L than Non-Carbon Sparged pond 0.277±0.06 and 0.596±0.07 g/L, respectively. Figure 6 shows Chlorophyll a, b total chlorophyll (g/L) for both treatments.

Table 3: Chlorophyll a, b Total Chlorophyll (mg/L) and Biomass Normalized Total Chlorophyll (mg /g Biomass) Concentrations

Treatment	Chlorophyll a Concentration (mg/L)		Chlorophyll b Concentration (mg/L)		Total Chlorophyll Concentration (mg/L)		Biomass Normalized Total Chlorophyll (mg/g biomass)	
	Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	*Initial*	*Final*
Carbon Sparged pond (Pond 1)	0.199±0.01	0.444±0.05	0.154±0.06	0.329±0.07	0.353±0.06	0.773±0.05	22.55	3.71
Non-Carbon Sparged pond (Pond 2)	0.164±0.04	0.318±0.04	0.113±0.02	0.278±0.06	0.277±0.06	0.596±0.07	19.04	4.34

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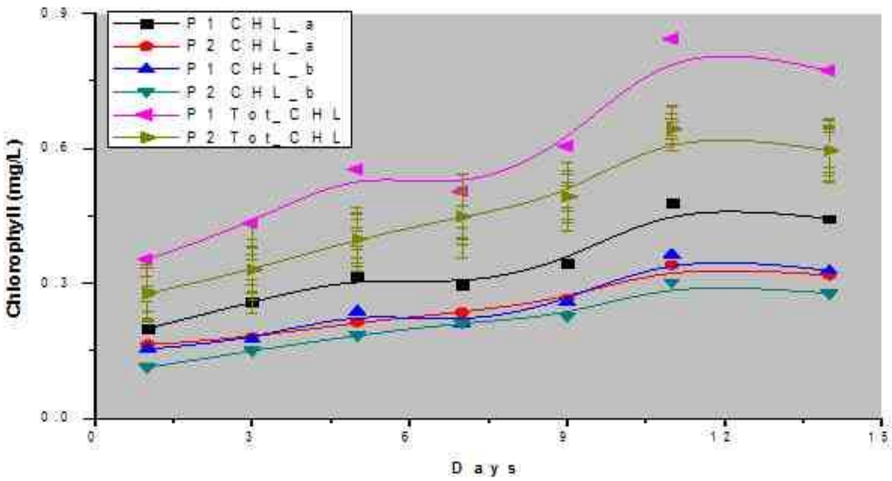

Figure 6: Chlorophyll a, b Total Chlorophyll (mg/L) for Both Treatments

The theoretical biomass productivity was calculated using solar radiation and the biomass that obtained in both ponds during cultivation as shown Table 4; from the theoretical biomass productivity the area obtained an average of 99.00 $g/m^2/d$ at the month of March (Figure 7). In the present study the biomass productivity results obtained a value of 58 $g/m^2/d$ and 50 $g/m^2/d$ in the Carbon sparged pond and in the Non-Carbon sparged pond, respectively.

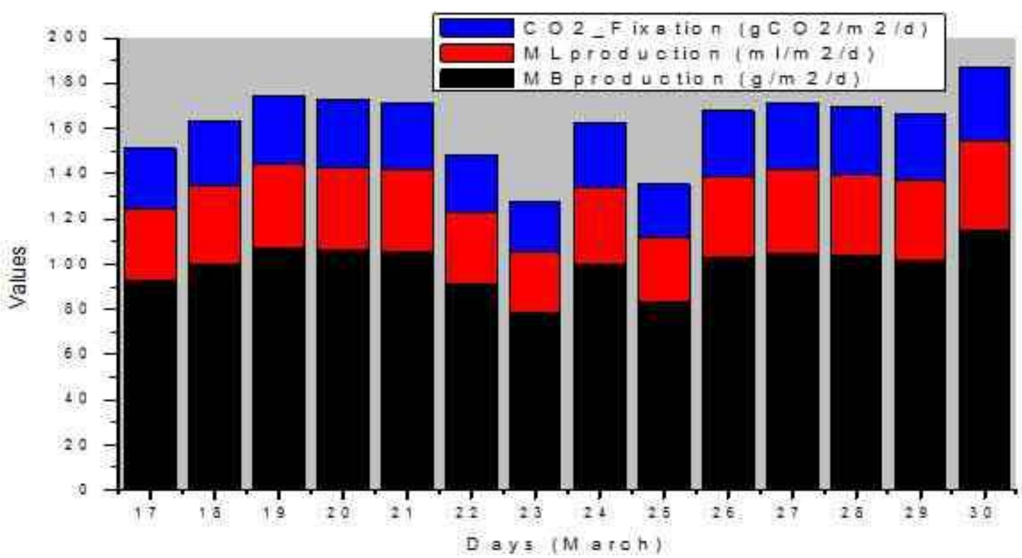

Figure 7: Theoretical Estimation of Algae Biomass Productivity, Oil Productivity and Carbon Fixation of the Study Site

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Table 4: Actual and Theoretical Estimation of Biomass Productivity of the Study Site

Pond type	Average Solar radiation kWh/m^2/d	Average Air temperature ^{0}C	Average Water temperature ^{0}C	Average pH	Theoretical Estimation Biomass productivity (g/m^2/d)	Actual Biomass productivity (g/m^2/d)	Actual Dry weight Biomass (g/m^2/d)
Pond 1 without CO_2	6.34	20.16	24.14±0.32	9.09±0.14	99.4	58	5.2 ±1.3
Pond 2 with CO_2	6.34	20.35	24.70±0.22	9.27±0.09	99.4	50	4.8±1.2

In this study, the theoretical biomass productivity is larger than the actual biomass productivity, this is because of the actual amount of irradiance is greatly reduced from the theoretical by clouds, ozone thickness, thickness of water vapor and other absorptive atmospheric conditions.

The mean productivity obtained for the entire cultivation period on both ponds were 5.2 ±1.3 and 4.8±1.2 gDW/m^2/d in the Carbon sparged pond and in the Non-Carbon sparged pond, respectively. Li et al. (2011) report a biomass production rate of gDW/m^2/d for algae grown on anaerobic sludge. The total amount of algal biomass produced may be estimated by considering the total flows of nitrogen. Nitrogen is assumed to be the limiting nutrient since phosphorous is generally considered to be an abundant nutrient in this effluent due to the numerous phosphate deposits. The growth rate and lipid production for algae grown on wastewater with moderate nitrogen levels (~30 mg/L) were adopted from Woertz et al. (2009) as 3 gDW/m^2/d and 30% lipids by dry weight respectively Zhou et al. (2012), the result of this study is approximately similar to those studies. The lipid content reported by Li et al. (2011) was 11%, less than to these results. This is a downside of growing algae in less strength nitrogen media. In general, high lipid content is achieved when the organisms are starved of nitrogen (Illman *et al*., 2000; Frac *et al*., 2004; Chisti, 2007).

Production in high rate algal ponds is possible and has shown commercial production rates as high as 40 gDW/m^2/d (Laws *et al*., 1988). Craggs et al. (2011) provide a good summary of production in high rate algae pond. There is a wide variability of production rates achieved based on wastewater source, type, location and culture conditions. Algae growth in high rate algae pond has also been shown to achieve greater than 75% nutrient removal (Park, 2011). Production was shown to improve with CO_2 addition from 10.6 to 15.2 gDW/m^2/d. Li et al. (2011) and Zhout et al. (2012) scaled up their wastewater grown algal with 25-L BIOCOIL reactors and obtained net biomass productivity of 13 and 12.8 gDW/m^2/d, respectively.

Microalgae biomass results mainly from photosynthesis, which utilizes inorganic compounds (including CO_2). In simple terms, algal biosynthesis can be described by the following chemical equations where ammonium and nitrate are the nitrogen sources respectively (Stumm and Morgan, 1996; Ebeling *et al*., 2006)

$$16NO_3^- + 124CO_2 + 140H_2O + HPO_4^{-2} \rightarrow hv\ C_{106}H_{263}O_{110}N_{16}P + 138O_2 + 18HCO_3^-$$

In the above equations, the chemical formula $C_{106}H_{263}O_{110}N_{16}$ represents algal biomass (Stumm and Morgan, 1996). According to the stoichiometry, 1 g of nitrate-nitrogen (NO_3^--N) produces about 15.8 g of biomass and consumes 18.1 and 24.34 g of CO_2 in the process, respectively. In addition to nutrient availability, algal biomass production also depends on light energy (hv). In the absence of nutrient limitation, photosynthesis increases with increasing irradiance until the maximum algal growth

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rate is attained as described my Michaelis-Menten kinetics (Qiang and Richmond, 1996; Richmond, 1999; Richmond *et al*., 1999) (Figure 7).

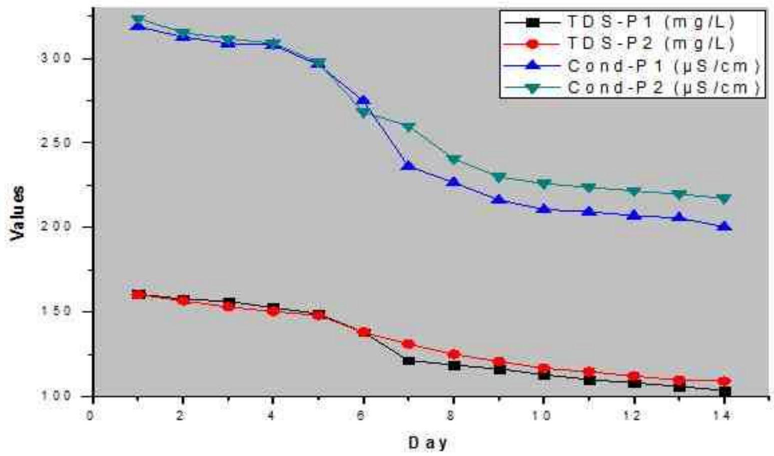

Figure 8: Reduction level of both Electro Conductivity and TDS

Actual CO_2 supply to a raceway pond was found to be 2.2 kg per kg biomass (Weissman and Goebel, 1987). For an inclined flow-way reactor with a carbonation sump, 4.4 kg CO_2 per kg biomass was required since more outgassing can occur in a thin layer of moving water than in a pond (Doucha, *et al*., 2005).

3.4 Physico-Chemical Parameters Before and After Treatment

The primary anaerobic digester effluent dairy wastewater characteristics were monitored weekly and the average, maximum and minimum values are reported in Table 5. The organic matter, represented as BOD_5 concentration, and the COD, nitrate and phosphate were the most abundant compounds in the influent.

Table 5: Characteristics of Primary Treated Wastewater Used on Experiments Pond_1 and Pond_2

Parameter	***Carbon Sparged pond (Pond_1)***			***Non-Carbon Sparged pond (Pond_2)***		
	Minimum Value	Maximum Value	Average	Minimum Value	Maximum Value	Average
TDS (mg/L)	59.00	162.00	160.67	159.00	162.00	160.67
COD (mg/L)	659.00	671.00	665.00	659.00	671.00	665.00
BOD (mg/L)	225.00	245.00	235.00	225.00	245.00	235.00
PO_4^{-3}-P (mg/L As P)	8.20	8.30	8.25	8.20	8.30	8.25
$NO3^-$-N (mg/L As N)	3.30	3.70.00	3.50	3.30	3.40	3.35
TSS (mg/L)	215.00	218.00	216.67	213.00	217.00	214.67
VSS (mg/L)	111.00	117.00	113.67	109.00	116.00	112.67

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A large number of pollutants can impart color, taste and odor to the receiving water there by making them unaesthetic and unfit for domestic consumption. The present study revealed that the diary anaerobic digester effluent was blackish gray in color with disagreeable odor which may be due to decomposition of organic matter or presence of various aromatic and volatile organic compounds (Singh *et al*.,1998) and it may also be due to microbial activity (Nagarajan and Shasikumar, 2002). Therefore, in the present study, color of the effluent treated with the consortium microalgae changed from blackish gray on the 4th day yellowish green and on 14th day it was completely turned to green. These changes in color and odor of the dairy anaerobic digester effluent may be due to the organic matter present in the effluent and made the water clear (Verma and Madamwar, 2002).

Total Dissolved Solids (TDS) and Electrical Conductivity: Untreated effluent were found to have high levels of TDS and this high levels of TDS may be due to salt content present in the same and also renders it unsuitable for irrigation. A wide variety of industries wastewaters (dairy) may contribute dissolved solids to the environment. Murugesan et al., (2007) reported 36.19 percent reduction of total dissolved solids, when the oil refinery effluent was treated with *Spirulinaplatensis*. Similarly, Veeralakshmi et al., (2007) reported 19.16 percent reduction of total dissolved solids, when the petroleum effluent was treated with Oscillatoria sp. Total dissolved in the present study was reduced to 35.48±1.37 and 32.01±1.26 percent in the Carbon sparged pond and in the Non-Carbon sparged pond, respectively, when the effluent was treated with microalgae consortium (Figure 8).

Electrical conductivity of the treated the dairy effluent from anaerobic digesters was reduced to 37.2±2.89 and 32.85±1.2 percent in the Carbon sparged pond and in the Non-Carbon sparged pond, respectively, using the consortium of microalgae (Figure 8). The higher level of electrical conductivity in raw effluent could be attributed to the use of inorganic chemicals in diary manufacturing. Electrical conductivity were found to be within the permissible limits (3000 μS/cm) issued by irrigation guidelines (Hamoda and Al-Awadi, 1996).

Reduction of Total Suspended Solids and Volatile Suspended Solids: Wastewater treatment also involves reduction of total suspended solids (TSS) and volatile suspended solids (VSS) which were also measured throughout the course of the experiments. Higher VSS was desirable as the values represent concentration of organic and provide more accurate representations of the biomass in the samples than TSS. As expected the initial values for TSS and VSS increase with increasing concentration of wastewater. In all treatments, significant reduction in the TSS and VSS was achieved (Table 6). High reductions in TSS and VSS were achieved for Carbon sparged pond rather than for the Non-Carbon sparged pond dairy manure concentration. However, when normalized with respect to the biomass concentrations, highest TSS (1255.05 mg/g) and VSS (676.4 mg/g) was achieved with the Non-Carbon sparged pond of dairy manure (Table 8).

Total suspended solids in the present study were reduced to 78.31 and 71.74 percent when the effluent was treated with microalgae consortium in the case of Carbon sparged pond and in the Non-Carbon sparged pond, respectively (Figure 9). This study confirmed due to the reduction of suspended solids the effluent is suitable for safe disposal on land through irrigation. The higher amount of suspended solids present in raw effluent may be due to the presence of higher concentration of biodegradable organic matter in the wastewater dairy effluent which is accordance with earlier reports. The high suspended solids in different industrial effluent were also reported by Sinha (1993), Amudha and Mahalingam (1999) and Sundaramoorthy *et al*., (2000). Kotteswari et al., (2007) reported 74.37 percent reduction of total suspended solids, when the dairy effluent was treated with

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Spirulinaplatensis, but in the present study the removal efficiency is better than the above study this is due to the presence of consortium of microalgae in the system.

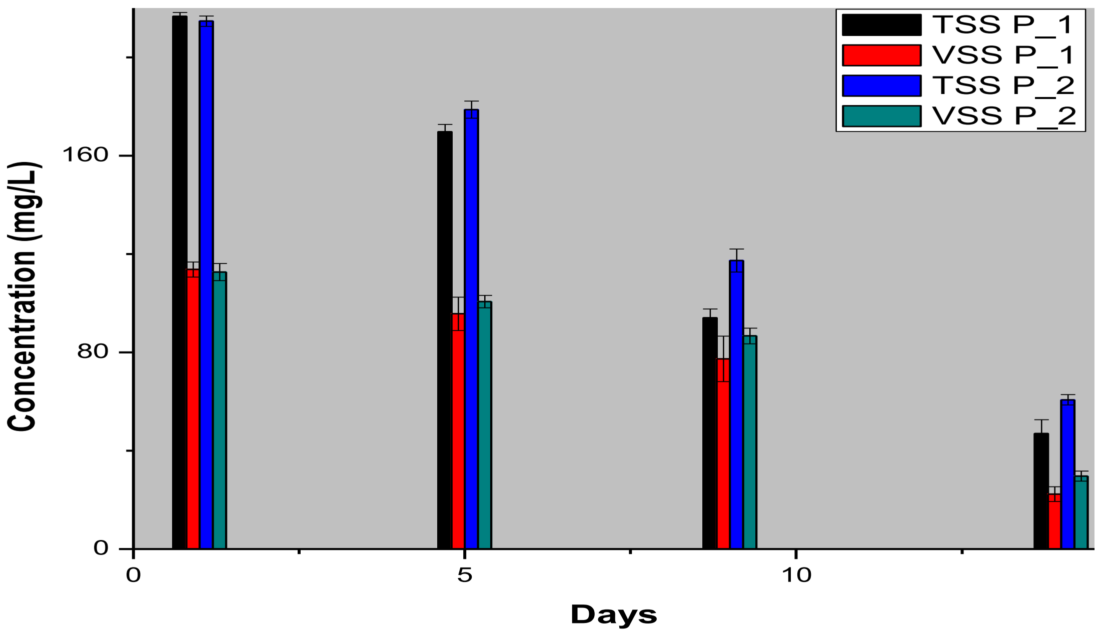

Figure 9: Reduction of pollution load TSS and VSS

VSS in the present study were reduced to 80.35 and 73.67 percent when the effluent was treated with microalgae consortium in the case of Carbon sparged pond and in the Non-Carbon sparged pond, respectively (Figure 9). The open pond effluent VSS was a mixture of microalgae, and its trend estimated both the heterotrophic and autotrophic microalgae growth. The VSS average reduced from 113.67±3.06 mg/L to 22.33±3.06 mg/L in the Carbon sparged pond; while the VSS average concentration was lower and reduced in the Non-Carbon sparged pond from 112.67±3.5 mg/L to 29.67±2.08 mg/L.

Reduction of Chemical Oxygen Demand and Biological Oxygen Demand: The Chemical Oxygen Demand (COD) determines the organic content of wastewater (Torzillo, et al., 1986). The high content of organic matter results high value of COD of wastewater because COD measures the recalcitrant organic matter in biologically treated industrial effluents (Malaviya and Rathore, 2001).

The Biological Oxygen Demand (BOD) is the amount of oxygen required to stabilize the organic matter in the water. The BOD_5 is commonly used to determine the efficiency of treatment processes, and the size of wastewater treatment facilities (Torzillo, et al., 1986). It is important that treatment processes decrease the BOD_5 for environmental reasons. If water with a high BOD_5 is discharged into a river, it could consume all oxygen in the water killing living organisms including fish. The incorporation of algae production into the wastewater treatment process decreases the BOD_5 in the wastewater (Lee and Low, 1991; Chaumont, 1993). The effects of algae production on the wastewater at Genesis should be within a similar range as (Lee and Low, 1991). Figure 10 shows the average reduction in COD and BOD_5 that could occur during month (March) algae production.

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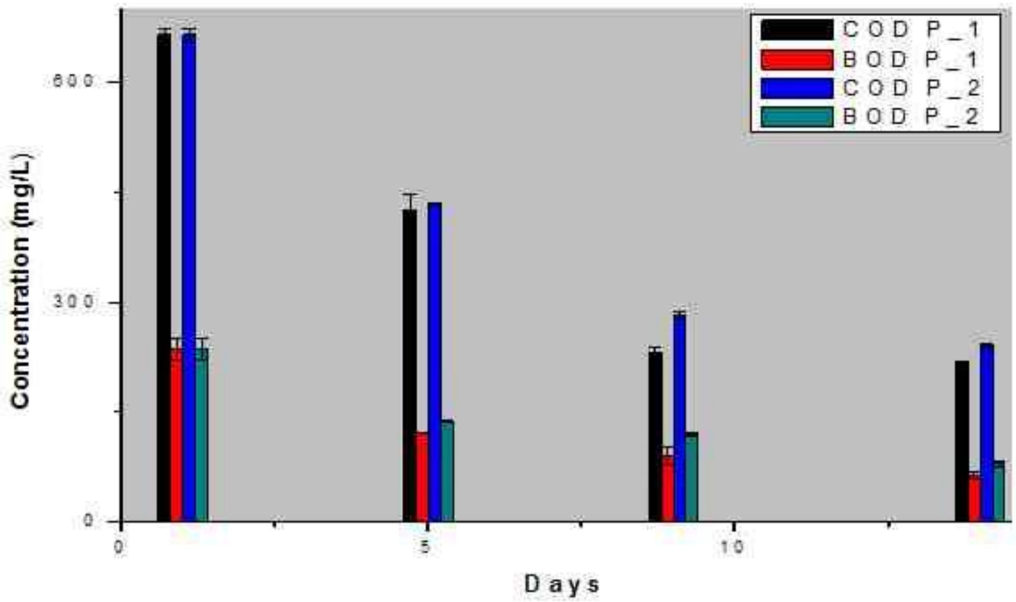

Figure 10: Reduction of pollution load COD and BOD

The measurement BOD_5 is an indicator measurement of substances that can bedegraded biologically, consuming dissolved oxygen in the process, over a test period of 14 days. BOD_5 and COD levels of treated effluent were reduced significantly. The COD and BOD_5 removal efficiencies for both ponds (carbon sparged pond and non-carbon sparged pond) are summarized in Table 7 and Figure 10. In this study the BOD_5 level was reduced to 72.8 and 66.4 percent and the BOD_5 concentration ranged during the algaecultivation period from 235 mg/L to 64 mg/L and from 235 mg/L to 79 mg/L in the carbon sparged pond and in the non-carbon sparged pond, respectively, after 14 days (Table 6), similarly, the COD in the wastewater decreased by 59.4 and 55 percent the COD concentration ranged during the algae cultivation period from 665 mg/L to 270 mg/L and from 665 mg/L to 299 mg/L in the carbon sparged pond and in the non-carbon sparged pond, respectively (Table 6).

Table 6: Removal Efficiencies of TDS, COD, BOD, Nutrients, TSS and VSS

Parameter	**Carbon Sparged pond (Pond 1)**			**Non-carbon sparged pond (Pond 2)**		
	Initial Concentration (mg/L)	Final Concentration (mg/L)	Removal Efficiency (%)	Initial Concentration (mg/L)	Final Concentration (mg/L)	Removal Efficiency (%)
TDS	160±1.53	103.67±1.53	35.40	160±1.53	109±1.00	32.02
COD	665±8.49	270±1.41	59.40	665±8.49	299±2.83	55.04
BOD_5	235±14.14	64±4.24	72.77	235±14.14	79±2.83	66.38
PO_4^{-3}-P	8.25±0.07	1.03±0.3	87.52	8.25±0.07	1.89±0.03	77.09
NO_3^--N	3.5±0.28	0.65±0.07	81.43	3.5±0.07	0.91±0.01	72.84
TSS	216.67±1.53	47.00±5.57	78.31	214.67±2.08	60.67±2.08	71.74
VSS	113.67±3.06	22.33±3.06	80.35	112.67±3.51	29.67±2.08	73.67

Table 7: Total Chlorophyll Accumulation, Nitrate, Phosphate, TSS and VSS Removal Normalized by Biomass Growth

Treatment	Total Chlorophyll Accumulation (mg/g biomass)	Nitrate Removal (mg/g biomass)	Phosphate Removal (mg/g biomass)	TSS Removal (mg/g biomass)	VSS Removal (mg/g biomass)
Carbon Sparged pond (Pond 1)	2.175	14.00	37.43	879.64	423.54
Non-Carbon Sparged pond (Pond 2)	2.596	19.89	51.83	1255.05	676.41

Reduction of BOD_5 and COD levels might be occurred due to the removal of dissolved organic compounds and derivatives to some extent from the effluent during the treatment process. (Verma, *et al*., 1998). The greater BOD_5 removal efficiency in the carbon sparged pond may have been due to the significant concentration of carbon dioxide in the pond (Table 7).

The discharged of BOD_5 in the receiving environment with the limited assimilative aptitude sometimes reduce the dissolved oxygen concentration to the levels of below those required for aquatic life and it is in this condition that BOD_5 and COD levels of the treated effluent were reduced significantly.

COD is usually considered as a major indicate of organic pollution in water (Dash and Mishra, 1999). Reduction of BOD_5 and COD levels high might occur due to the removal of dissolved organic compounds and derivatives to some extent from the effluent during the treatment process. It thus becomes evident that reduction in COD was less as compared to reduction in BOD_5. Thus it is obvious that the degradation sought was through biological activity and not through a chemical agent. Kotteswariet al., (2007) reported 47.34 percent of BOD_5 and 24.69 percent reduction of COD when the dairy effluent was treated with single strain, which means less than in the removal efficiency from this study (Table 6)

Removal of Nitrate and Phosphate:Total nitrogen is the sum of organic, ammonia and nitrate concentration in wastewater. The total nitrogen removal in wastewater is important because it prevents environmental damage, especially eutrophication. Nitrogen is one of the most important nutrients necessary for algae growth. The algae production process is an effective way to decrease the total nitrogen in wastewater (Martinez, et al., 2000; Shengbing and Gang, 2010). The algae only consume Nitrogen during photosynthesis. The overall results of the average total nitrogen removal of the algae production system is 36% (Shengbing and Gang, 2010). Nitrogen is mostly supplied as nitrate (NO_3^-), but often ammonia (NH_4^+) and urea are also used, therefore, nitrate is the only one reported in this paper.

One main factor that could determine the removal rate of nitrate is the relatively constant pH maintained throughout the algae production process. The conditions given by Martinez et al. removed the total nitrogen by 76% at a water temperature of 25°C (Martinez, et al., 2000). However, the pH of the system was not constant throughout the process. In this study the dairy anaerobic digester effluent was treated with the consortium of microalgae nitrate as nitrogen was decreased by 80 and 73 percent, the average nitrate concentration ranged during the 14 day of algae cultivation period from 3.35±0.07 mg/L to 0.61±0.07 mg/L and from 3.35±0.07 mg/L to 0.91±0.14 mg/L in the carbon sparged pond and in the non-carbon sparged pond, respectively (Figure 11). Figure 11 shows the removal of nitrate from wastewater through algae production over 14 days period and Table 8 shows the normalized of nitrate with biomass concentration of the systems.

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Phosphate is an important nutrient for algae production. Algae production effectively removes phosphorous from wastewater (Martinez, et al., 2000; Shengbing and Gang, 2010).

In this study the dairy anaerobic digester effluent was treated with the consortium of microalgae, phosphate content was decreased by 88 and 77 percent, the average phosphate concentration ranged during the 14 day of algae cultivation period from 8.25 mg/L to 1.03 mg/L and from 8.25 mg/L to 1.89 mg/L in the carbon sparged pond and in the non-carbon sparged pond, respectively (Figure 11). Tam and Wong (1990) have reported over 90 percent removal in total phosphorus within 10 days of algal cultivation, and other studies suggest that the maximum removal of phosphorous from wastewater is about 51% (Boyd and Musig, 1981). Doran and Boyle (1979) reported that 90 percent of phosphorus removal by activated algae was due to chemical precipitation. Garrett and Allen (1976) have shown that accumulation of phosphorus accounts for 96 percent of that removed during the growth of a strain of *Chlorella vulgaris* on animal slurry. However, algae production could result in a much higher reduction in total phosphate. The removal of NO^{3-}-N and PO_4^{-3}-P from manure effluent was similarly influenced by both manure concentration and algae biomass density (Table 6) (Pizarro, 2002).

Conclusions

From the present study, it was clear that when the growth rate of the consortium of microalgae in the dairy anaerobic digester effluent wastewater increases, the rate of reduction of different pollutants/nutrients also increases. Based on the experimental results the following specific conclusions have been made. Therefore, it was found that the land application of the treated dairy effluent provides an effective and environmentally acceptable option for waste disposal, which not only recycles valuable nutrients into the soil-water-plant system but also improves soil and water quality.

The result of the present study indicates that the treatment of dairy effluent by consortium of microalgae is very efficient and it also proved to be cost effective and eco-friendly treatment. In this study, the consortium of microalgae play a vital role in the removal of nitrate (81.43%), phosphate (87.52%), COD (59.40%), BOD (72.77%), TSS (78.31%), VSS (80.35%) and TDS (35.40%) in the sparged of carbon dioxide into the system. Microalgae can be used for tertiary treatment of wastewater due to their unique capacity to assimilate nutrients. Employing this technology in the treatment of industrial effluents presents an alternative tool to the current practice of using conventional methods, including physical and chemical methods. The timely and cost-effective remediation of metal and organic contaminated sites mandate an understanding of the extent and mechanisms by which toxic metals inhibit organic biodegradation.

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