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RESEARCH ARTICLE

PHYCOREMEDIATION OF WASTE WATER AND DAIRY EFFLUENTS USING *CHLORELLA PYRENOIDOSA* AND *SCENEDESMUSABUNDANS*

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ABSTRACT

Wastewater is the spent or used water containing dissolved or suspended solids, microbes. Organic and inorganic substances which are released into the environment as a result of domestic, agricultural and industrial activities which leads to organic and inorganic pollution.Dairy industry plays a major role in nation economy with a share of 13.1% total milk produce in the world. About 286 large and several small dairy industries in India are producing large quantities of wastes and polluting the water bodies. The present study focuses on the use of microalgae to remove pollutants and nutrients from the wastewater by phycoremediation.Chlorella pyrenoidosa and Scenedesmusabundans are the chosen algal species for the study. To observe the efficiency of nutrient uptake by these algal species in sewage water and dairy industrial effluents. To compare the ability of the algal species to reduce the nutrients. To estimate the biochemical constituents of harvested algal biomass for further applications such as bio fuel production.The collected sewage and dairy samples were tested for the following standard physico-chemical parameters as per APHA-1998 and BIS 14543.About 35-90% reduction in different nutrients was noted though out the experiment, further the nutrients were utilised by these to strains to produce sugars 18-30%, protein 12.6-20% and lipids7.5-23.5% of dry cell mass,the gradual reduction in various physical and chemical parameters gives the evidence that *Chlorellapyrenoidosa* showed best removal capacity when compared to that of Scenedesmus abundans. These experiments confirm that *Chlorella pyrenoidosa* and Scenedesmus abundans may be considered efficient nutrient removers.

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INTRODUCTION

Today's world is facing problems related to with a wide range of pollutants and contaminates from various human developmental activities. The population explosion is the major cause that has resulted in an increase in polluted water bodies. The concern on the quantity and quality of waste generated and discharged into natural water bodies has recently indicated the need for different strategies to address water quality challenges. Bioremediation occurs through naturally occurring microorganisms and other aspects of the natural environment to treat wastewater for its nutrients or pollutants. Bioremediation also proves to be less expensive than other technologies that are used for cleanup of hazardous wastes (Validi M, 2001).

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Sewage water referred to as domestic wastes contains wide range of organic and inorganic wastes from residences, business houses and industries. The chemical composition of sewage varies considerably between and among different cities. Sewage water contains inorganic wastes which generally creates problems of disposal. The organic matter in the water is offensive and dangerous too. It has been reported that a number of serious diseases are transmitted through sewage water, for example: typhoid, gastrointestinal problems, cholera, dysentery, nematodes infection etc. The properties of the water are continuously being disturbed by using rivers and other water bodies as dumping grounds for human sewage and industrial wastes. Increase in population, industrialization and urbanization has further increased this sewage problem. Fast urbanization, industrialization resulting in discharge of untreated waste water into water bodies, coupled with massive abstraction of water for irrigation, industrial and domestic uses, lack of awareness and seasonal variations are the main cause of water quality degradation in the rivers (Trivedi, 2010;

Joshi,*et al*., 2006). The utility of river water for various purposes is governed by physico-chemical and biological quality of the water. The assessment of the changes in river communities as a result of the impact of pollution is particularly interesting issue within the framework of aquatic ecology, since the running waters are becoming increasingly affected by anthropogenic discharge (Whitton*et al*., 1991). Many workers have studied the impact of anthropogenic activities on the environmental conditions (Kang *et al*., 2004; Shirodkar *et al*., 2010). Wastewater is a general term used to represent the water with poor quality that contains more amounts of pollutants including microbes. If wastewater is discharged into the nearby water bodies, it can cause serious environmental hazards and health problems to human beings. Wastewater treatment is an important measure to reduce the pollutants and other undesirable contaminants present in waste water. The primary step in wastewater treatment involves the removal of solids and dissolved solutes from the wastewater. Secondary treatment or biological treatment exploits microorganisms to eliminate the chemicals present in wastewater. Final step, also called tertiary treatment, which eliminates the microbes from wastewater before discharging into the environment and river (Rawat*et al.*, 2010). Effluents produced from the secondary treatment plant contain more amounts of nutrients (nitrates and phosphates) and if these effluents are discharged into water bodies; it generally causes eutrophication and affects the ecosystem. To remove these undesirable nutrients, several processes are used, but the disadvantage of this type of treatment is high cost and increased sludge production (Yuan *et al*., 2011). As an alternative to the conventional treatment methods, microalgae have been suggested to eliminate the nutrients from wastewater (Mallick, 2002).

Dairy industry plays a major role in Indian agriculture sector and nation economy with a share of 13.1% total milk produced in the world (Kumbhar Vijay, 2010). About 286 large and small dairy industries in India are producing large quantities of wastes and polluting the water bodies. Dairy wastewater is characterized by strong color, offensive odour, high BOD (40–48,000 mg/L), high COD (80–95,000 mg/L) and variable pH (Kothari *et al*., 2011). It also contains sufficient nutrient like N (14–830 mg/L) and P (9–280 mg/L) required for biological growth. Lipids are the precursor of biodiesel production. Not only quantity but also quality of produced lipid is very important during its conversion to biodiesel using lipid content for pure cultures of algae have been reported to range from 1%-85%, and the lipids exhibit varying carbon chain lengths, degrees of unsaturation, and polarity (Chisti 2007, Metting, 1996). The use of microalgae to remove pollutants and nutrients from the wastewater is called phycoremediation. Microalgae treatment is eco friendly and offers the advantage of a cost effective way of nutrient removal and biomass production (Mulbry *et al*., 2008). The microalgae grown in wastewater can be used as energy source, fertilizer, and fine chemicals production and as feed to animals (Vilchez*et al*., 1997; Mulbry*et al*., 2006). Several conventional biological methods have been used to remove nutrients (phosphates and nitrates) from wastewater. The principle objective of wastewater treatment is generally to allow human and industrial effluents to be disposed off without any danger to human health and no damage to the natural environment. Irrigation using waste water in both disposal and utilization indeed is an effective measure for waste water disposal. However some degree of treatment must normally be provided to raw municipal waste water before it can be used for agricultural or landscape irrigation or for aquaculture (Dominic, 2009). The present study focuses on the use of microalgae to remove pollutants and nutrients from the wastewater by phyco remediation.Chlorella pyrenoidosa and Scenedesmus abundans are the chosen algal species for the study.

MATERIALS AND METHODS

Collection of samples

Water Samples was collected periodically every month during morning hrs between 8.00 A.M and 9.00 A.M. Samples were collected in Polyethylene carbonyl bottles of 2 litre capacity, from 1 foot below the water surface for physico- chemical analysis. About 60 litres of surface water was filtered through standard plankton net. The final volume of the filtered sample was 200ml which were transferred to polyethylene bottles of 250 ml capacity and preserved by adding 8ml of 4% formalin.

Biological analysis

Algal species were studied under light microscope and identified with the help of standard references. Quantitative analysis was made using a plankton-counting cell (Sedgwick rafter). Identification and enumeration of algae were done as per the methods described by Adoni*et al*., (1985), Agarker*et al*., (1994), Welch (1948), Hosmani and Bharathi (1980). Identification was done by consulting the monographs by Philipose (1967), Gandhi (1998) and Prescott (1998).

Physico-chemical analysis

Temperature (air and surface water) was recorded on the spot using Centigrade thermometer. The pH of the water samples was measured by using the gun pH meter on the spot. Physico-chemical analysis was done before culturing and after of Domestic sewage sample and dairy effluent. Dissolved oxygen, B.O.D, C.O.D, TDS, Nitrate, Phosphate, calcium hardness, magnesium hardness and total hardness) of the sample was done as per standard methods followed by APHA, 1998 and Indian standard IS: 3025-1964.

Culturing of organism

Culturing was carried out using five litres of sample waste water and dairy effluent in 20 and10 lit PVC pet jar supplemented with sterile compressed air and kept under fluorescent light of 20 μmol m^{-2} s^{-1} intensity, with 16 h light photo period and at 25°C temperature for 21 days. Wet biomass was obtained by centrifugation. Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris.

Biochemical estimation

Estimation of carbohydrate by Anthrone method (Roe, 1955). Protein was estimated by Biurette method (Raymont*et. al*., 1964). Nile red staining for lipid granules as per methods followed by (Lee *et al*., 1998). The cells were observed in fluorescent microscope using excitation wavelength as 460 nm (Carl Zeiss, microimaging GmbH).

Extraction of lipid was done following the protocol of Bligh and Dyer 1959 and Lee *et al*., and Estimation by Gravimetric method.

RESULTS

The isolated organisms were morphologically identified as *Chlorella pyrenoidosa* and *Scenedesmus abundans*, microscopic image is been illustrated in fig 6 (Shakeel Ahmed Adhoni, *et al*., 2015). The physico chemical results are as follows. The initial pH of the untreated sewage sample and dairy effluent were noted as 6.6 and 6.8 respectively. In both the cases the rise in pH was observed. C. pyrenoidosa increased the pH of sewage to 8.1 (Table-1) and that of dairy effluent to 7.8 (Table-2). S. abundans showed the increase of pH upto 7.9 in sewage (Table-3) and 7.8 in dairy effluent (Table-4) in 21 days (Fig-4). Initial nitrate concentrations of sewage and dairy effluent were found to be 0.018 and 0.014 mg/L respectively.

Table 1. Changes in physico-chemical parameters of sewage treated with *Chlorella pyrenoidosa*

Parameters (mg/L)	0^{th} day	7^{th} day	14^{th} day	21^{st} day
pH	6.6	6.7	6.9	8.1
Nitrate	0.018	0.0094	0.0048	0.0039
Phosphate	3.53	2.53	2.28	1.05
Calcium	25.28	24.54	22.31	9.37
Magnesium	15.84	14.01	12.53	8.39
Hardness)	128.2	118.8	107.0	64.1
TDS (mg/L)	1300	866.36	323.52	646.1
DO	2.13	3.23	4.92	5.23
BOD	3.34	2.89	1.91	0.971
COD	9.1	7.65	5.52	3.148

Higher reduction was observed after 14 days. On last day i.e. 21^{st} day the concentration was reduced to 0.0039mg/L (Table-1) and 0.0045mg/L (Table-2) by *C. pyrenoidosa* and *S. abundans* respectively in sewage. Whereas it was reduced to less extent in dairy effluent upto 74% by *C. pyrenoidosa* and 69% by *S. abundans* (Fig-1). Similarly phosphate reduction from the 3.21mg/L to 1.316mg/L and 1.15mg/L by *C. pyrenoidosa* (Table-3) and *S. abundans* (Table-4) in dairy effluent was observed. Higher percentage removal efficiency of 70% and 63% was noted in sewage sample (Fig-1) with both the organisms. C. pyrenoidosa removed up to 1.05mg/L and 1.306mg/L by S. abundans where before algal growth and inoculation it was noted to be 3.53mg/L(Table- 2 and 3).

Table 2. Changes in physico-chemical parameters of sewage treated with *Scenedesmusabundans*

Parameters (mg/L)	0^{th} day	7^{th} day	14^{th} day	21^{st} day
pH	6.6	7.5	7.9	7.9
Nitrate	0.018	0.0084	0.0058	0.0045
Phosphate	3.53	3.14	2.67	1.306
Calcium	25.28	20.89	14.85	12.13
Magnesium	15.84	13.14	10.23	9.71
Hardness	128.2	115.54	85.6	73.35
TDS	1300	900.48	875.32	689.26
DO	2.13	2.75	3.89	4.89
BOD	3.34	3.02	2.56	1.19
COD	9.1	8.87	6.34	4.12

Dissolved oxygen was significantly increased in both the effluents due to the photosynthetic activity of algae. 50% increase in DO was observed in sewage by C. pyrenoidosa while only 30% increase was found with S. abundans in the first week of inoculation that is on 7^{th} day.

Table 3. Changes in physico-chemical parameters of dairy effluent treated with *Chlorella pyrenoidosa*

Parameters (mg/L)	0^{th} day	7^{th} day	14^{th} day	21^{st} day
pH	6.8	7.0	7.7	7.8
Nitrate	0.014	0.0085	0.0046	0.0036
Phosphate	3.21	2.85	2.68	1.15
Calcium	125.62	105.5	98.32	57.78
Magnesium	27.21	25.63	21.45	14.69
Hardness	419	369	334	217.88
TDS	1533	1102	927.45	889.14
DO	1.4	1.5	2.4	2.7
BOD	6.20	5.45	4.89	2.11
COD	10.52	9.56	7.42	5.43

Higher significant increase was noted up to 21^{st} day (Fig-3). – BOD and COD were measured for the dissolved organic and inorganic contents in the waters. The organic and inorganic dissolved particles indirectly indicate the oxygen required for the oxidation of the dissolved solutes by simple iodometric titration. The BOD of the sewage water before treatment was 3.34mg/L. It was decreased to 70.9% and 64.3% when C. pyrenoidosaand S. abundans (Fig-1) were cultured in it after sterilization. The final BOD was found to be 0.971mg/L (Table-1) and1.192mg/L (Table-2) respectively with the two species. Similarly the initial BOD of dairy effluent was 6.20mg/L, and decreased to 2.58mg/L by S. abundans (Table-4) and 2.11mg/L by *C. pyrenoidosa* (Table-3). In dairy effluent the *C. pyrenoidosa*showed the 45% reduction in COD, while *S. abundans* showed 48.3% reduction (Fig-2). The initial calculated COD was 10.52mg/L. After culturing the algae in water for treatment it was found to be reduced up to 5.78mg/L by *S. abundans* (Table-4) and with *C. pyrenoidosa* to 5.438mg/L (Table-3). The COD of sewage was estimated to be 9.1mg/L before algal inoculation. Finally after 21 days it was found to be 3.148mg/L with *C. pyrenoidosa* (Table-1) and *S. abundans* reduced it to 4.123mg/L (Table-2). Total hardness was measured in terms of Calcium and magnesium hardness. Significant decrease was also noted in calcium, magnesium and total hardness along with other parameters on treatment in 21 days. Reduction of these was less in dairy effluent. The sewage total hardness was 128.2 mg/L with calcium and magnesium measuring to 25.28mg/L and 15.89mg/L respectively. *C. pyrenoidosa* showed higher reductions up to 50% in total hardness to 64.1mg/L (Table-1). Calcium hardness was 9.37mg/L and magnesium hardness was 8.39mg/L on 21^{st} day after algal treatment (Table-1).In dairy effluent, the concentration of calcium was 125.6mg/L and magnesium was 27.21mg/L whereas the total hardness was 419mg/L. Later after 21 days of growth of C. pyrenoidosa total hardness was reduced to 217.88mg/L and that of calcium and magnesium was 57.78mg/Land 14.69mg/L respectively (Table-3). S. abundans in sewage sample reduced the total hardness to 73.35mg/L. Similarly calcium and magnesium hardness was also reduced to 12.13mg/L and 9.71mg/L respectively (Table-2). The same organism reduced the dairy effluent total hardness to 247.21mg/L whereas calcium to 68.7mg/L and magnesium to 18.77mg/L (Table-4). After treatment, the algal mass was harvested on 21^{st} day by subjecting to centrifugation. The biomass wet weight, and dry weights were noted. Total proteins, lipids and carbohydrates were estimated from the harvested algal biomass. Confirmation for presence of lipid granules in both the strains was done by Nile red staining and the results are illustrated in fig 7. Lipid was estimated by gravimetric method. 10 ml of the *C. pyrenoidosa* algal culture yielded 18% of lipid when grown in sewage and 14.5% in dairy effluent. 7.5% and 12% of lipid yield was obtained from same amount of *S. abundans* culture. Protein was estimated by the Lowry's method. 20% and 17% protein was present in *C. pyrenoidosa* cultured in sewage and dairy effluents respectively. *S.abundans* contained 12.6% and 10.4% grown in sewage and dairy effluent. Good amounts of total carbohydrates were found in C. pyrenoidosa with 25% and 30% cultured in sewage and dairy effluent. *S. abundans* showed 18% and 20% in sewage and dairy effluent. All lipid, carbohydrate, and proteins are expressed as dry mass % (Table-5).

Table 4. Changes in physico-chemical parameters of dairy effluent treated with *Scenedesmus abundans*

Parameters (mg/L)	0^{th} day	7^{th} day	14^{th} day	21^{st} day
pH	6.8	7.5	7.7	7.8
Nitrate	0.014	0.0091	0.0054	0.0043
Phosphate	3.21	2.65	1.95	1.316
Calcium	125.62	98.65	78.85	68.7
Magnesium	27.21	25.23	20.25	18.77
Hardness	419	398.26	352.31	247.21
TDS	1533	1352	1159	919.8
DO	1.40	1.46	1.81	2.35
BOD	6.20	5.79	4.12	2.52
COD	10.52	9.79	7.64	5.78

Table 5. biochemical composition of *Chlorella pyrenoidosa* and *Scenedesmusabundans*

Component (as dry matter %)	In sewage effluent		In dairy effluent	
	C. pyrenoidosa	*S. abundans*	*C. pyrenoidosa*	*S. abundans*
Protein	20	12.6	17	10.4
Carbohydrate	25	18	30	20
Lipid	18	7.5	14.5	12

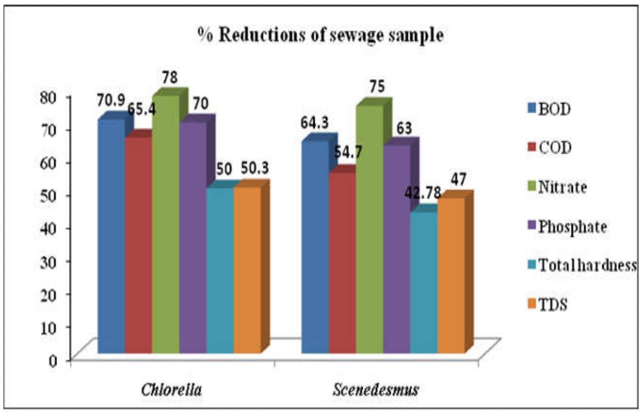


Figure 1: Comparative percentage reduction in domestic sewage by *C. pyrenoidosa* and *S.abundans*

DISCUSSION

Phycoremediation is a novel technique that uses algae to clean up polluted water and soil. It takes advantage of the algae's natural ability to take up, accumulate and degrade the undesirable constituents that are present in their growth environment. Algae based waste water treatment technology offer more simple and economical as compared to the other environmental protection systems. In this system the process of photosynthesis can be effectively exploited to generate oxygen from waste water remediation by algae (Dominic, et al., 2009). In our study the ability of micro algae, *Chlorella pyrenoidosa* and *Scenedesmus abundans* have been used to reduce the nutrient load of polluted water sample collected from two sources namely domestic sewage and dairy effluents.

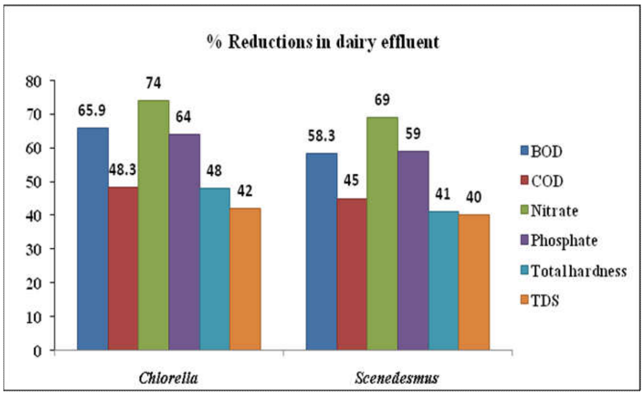


Figure 2. Comparative percentage reductions by *C. pyrenoidosa*and *S. abundans* in dairy effluent

The results obtained using domestic sewage and dairy effluent were compared and discussed. Wastewater contains different types of materials like soluble organic, inorganic, insoluble inorganic materials, macro solids, toxins, etc. By processing, it is ensured that only the soluble wastes like carbon, nitrogen and phosphate are used for the microalgae cultivation. Besides pollutants, bacteria and protozoa are also present in polluted water. If this water is used as a culture medium, microbes present in it will compete for microalgae growth and nutrition. Hence, ultra violet light source, filtration, autoclaving and centrifugation are the different types of pre-treatment methods are used to remove suspended solids and algae feeding microorganisms like protozoa and bacteria. Li *et al*., (2011) reported the *Chlorella* sp. cultivated in the autoclaved centrate showed higher growth rate than the raw centrate. In our experiment we autoclaved the waste water media before inoculation.

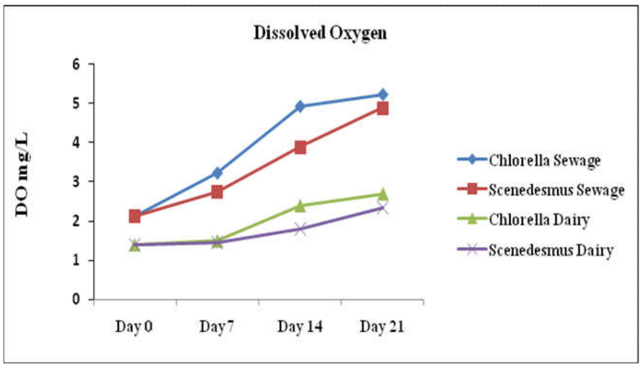


Figure 3. Increase in dissolved oxygen of the effluent during treatment

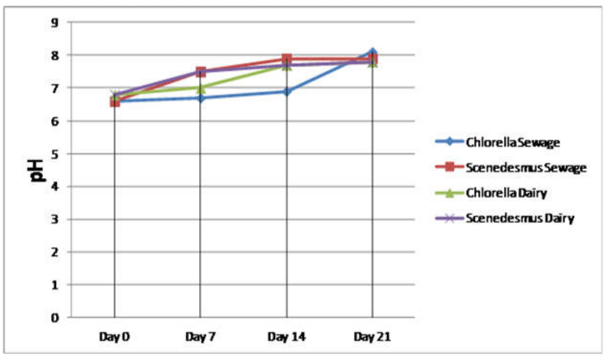


Figure 4. Increase in pH of the effluents during treatment

The microalgae cultures were inoculated at 10% (v/v) of the working volume (SiraneeSreesaia, 2007). And on subsequent growth, monoculture of algae was observed without any contamination by other algal species or micro organisms, as observed under the microscope. Expansion of urban populations results in increased coverage of domestic water supply and sewage in greater quantities of municipal wastewater. With the current emphasis on environmental health and water pollution issues, there is an increasing discharge of waste water in developing countries.When the domestic sewage was treated with algae *Chlorellapyrenoidosa*, the pH was increased from 6.6 to 7.8 and with that of Scenedesmus abundans it was found to be increased from 6.6 to 7.7. Similar observation of increasing pH was also recorded byKotteswari M*et al*., (2012) and AyodhyaKhirsagar (2013). It suggests that carbon dioxide and bicarbonate has been removed from the water by the photosynthetic process of aquatic plants raises the pH.

It is also recorded that the alteration of dissolved oxygen content where oxygen drops during respiration and decomposition and it rises with photosynthetic activity (Aarti*et al*., 2008). One of the objectives of wastewater treatment is the reduction of total dissolved solids (TDS). Highest TDS reduction was achieved with the C. pyrenoidosa cultures than that of the S. abundans. It is clearly indicated that both the algae have caused significant effect on TDS at final stage. The TDS was reduced to about 50.3% when treated with *Chlorella pyrenoidosa* where it is 47% when treated with Scenedesmus abundans. The total dissolved solids (TDS) was reported to be slightly reduced by 1.3% (Job Gopinatahan, *et al*., 2014).Total Dissolved solids also considerably reduced from 850 mg/L to 540mg/L (Nandini*et al*., 2013). The BOD is an indicator measurement of substances that can be degraded biologically consuming dissolved oxygen. BOD was recorded and its level was reduced to 70.91 % by *Chlorella pyrenoidosa* and 89.21 % by Scenedesmus abundans in 21 days. Aziz and Nag (1993) studied the feasibility of using an activated-algal process to treat wastewater and found that it was able to remove 80-88 % of BOD, 70-82 % of COD with a retention period of 15th days using *C. vulgaris*. In the present study, the COD level was highly reduced to 65.4% and 54.7% by *C. pyrenoidosa* and *S. abundans*upto 21st days respectively. *Chlorella pyrenoidosa* proved to be the best removal organism of COD from wastewater. The study of Valderramna*et al*., (2002) showed 61% removal efficiencies for COD by *C. vulgaris* in the treatment of diluted ethanol and citric acid production industry wastewater. *Chlorella* sp. induced progressive reduction in both BOD and COD values of the effluent and this could be attributed to the high algal growth rate and maximum photosynthetic activity (Colak and Kaya, 1988). The study of Zhang *et al*., (2008) using Scendesmus sp. showed high removal efficiency of inorganic nutrients from the domestic effluents. COD and BOD removal efficiency were found to be 88 and 89.60%, respectively using *C. vulgaris* (Azeez, 1993). On mg/L basis, the BOD level was reduced from 3.34mg/L to 1.23mg/L when treated with *Chlorella pyrenoidosa* and with *Scenedesmus abundans* it was reduced from 3.34mg/Lto 1.84mg/L. Similarly the COD level was reduced from 9.1mg/L to 4.93mg/L when treated with *C. pyrenoidosa* and with that of *Scenedesmus abundans*, it was found to be reduced from 9.1mg/L to 5.2mg/L. Similar reduction was reported in BOD and COD levels by 31.2% and 13.9% respectively with *Chlorella* sp. after the treatment (Nandini, et al., 2013). In our study, in the domestic sewage with *C. pyrenoidosa* the DO level was significantly increased from 2.13mg/L to 5.23mg/L and with that of *S.abundans* was increased from 2.13mg/L to 4.89mg/L. Similar observations were reported by the authors where initial Dissolved Oxygen level was 0.335mg/L and it increased to 2.75mg/L made by Whitton *et al*., (1991) using *Chlorella*sp. The present study reveals that nitrates and phosphates content in raw sewage was 0.018mg/L and 3.53mg/L respectively, after treatment these pollutants were significantly reduced by 78% and 70% which amounts to 0.0039mg/L and 1.05mg/L respectively when treated with *C. pyrenoidosa*. The presence of higher phosphate level in the raw sewage may be due to the usage of detergents in the daily life, and other human activities. The presence of phosphate generally enhances the biological activity of microorganisms and as a result the dissolved oxygen depletes. The work of Nandini N, *et al*., (2013), reveals that nitrate and phosphate contents in raw sewage was recorded 7.9mg/L and 89mg/L respectively and after the treatment these pollutants were significantly reduced to 97.5% and 85.8% which was recorded as 0.28mg/L and 13.53mg/L respectively. Our observation showed on S. abundans showed nitrate reduction up to 75%. The present study also revealed the removal of 63% phosphate in wastewater, on 21st day of experiment using S. abundans. Such a high percentage of removal was found 70% by *C.pyrenoidosa*.

Almost similar results were reported by Granter *et al*., (1984) who concluded that *Chlorella* sp. and *Scenedesmus*sp. were the most efficient algal species to eliminate or reduce the phosphate from the wastewater of municipal and refinery wastes. Phosphate was efficiently removed from the wastewater by *S. quadricauda* within 15th days. The wastewater treatment using *S. quadricauda* found higher removal rates of phosphate as recorded by Colak O and Kaya Z (1988). Similarly Kim *et al*., (2007)observed over 83% of removal of phosphorus by Scenedesmus in fermented swine wastewater with phosphorus concentration of 120 mg/L. Phosphate removal by *C. vulgaris* during phycoremediation is due to the utilization of phosphorus for growth (Rao *et al*.,2011).Job Gopinatahan et al., (2014) reported the significant reduction in total hardness by about 50%. Calcium and magnesium also followed a similar trend with 63% and 50% reductions respectively using *C. vulgaris*. In our study C. pyrenoidosa efficiently reduced the total hardness to 50%. Simultaneously the reduction in calcium and magnesium were noted to be 60% and 52% respectively. *S. abundans* showed less reduction up to 73.35% of total hardness and 52% and 38.9% reduction in calcium and magnesium respectively. The initial pH of the untreated dairy effluent was 7.2 and on 21st day of the treatment it was 0increased to 8.2 with *C. pyrenoidosa* and 8.1 with *S. abundans*. A pH change from 5.62 to 9.82 was reported by earlier work of Kotteswari *et al*., (2012). In the present study using *C. pyrenoidosa* and *S. abundans* TDS was reduced by 42% and 40% respectively in the dairy effluent water. Similar reduction was also reported by many researchers (Kotteswari *et al*., 2012, Ahmad *et al*., 2013 and Elumalaei*et al*., 2013) using different species of Cyanophyceae and Chlorophyceae for wastewater treatment, the observations revealed the average reduction of TDS up to 60 %. The unique mechanism of bio absorption / adsorption of different types of dissolved solids in wastewater are responsible to reduce TDS to lowest level (Nanda *et al*., 2010). Results of present investigation are in close agreement with the earlier observations. The reason for reduction in TDS by both the algal species may be the same as reported by previous workers. The removal of nitrate and phosphate from effluent was influenced by both manure concentration and algae biomass density (Pizarro *et al*., 2002). In the concentrated dairy effluent the nitrate waste reduced by 74% with Chlorella and 69% with Scenedesmus. The phosphate reduction was 18% on 14the day 64% on 21st day in the Chlorella culture, while, 59% reduction was observed by Scenedesmus on 21stday. These results agreed with other studies, such as that by Weerawattanaphong who cultured Chlorella in poultry wastewater at 10,000 lux with a retention time of eight days, found nitrate and phosphate removal efficiency were 72–85% and 57–77% respectively. Likewise, Gonzales also found that the microalgae Chlorella and Scenedesmus removed 95% of ammonium-nitrogen and 50% of phosphorus in wastewater. Nutrient removal is dependent upon a direct mechanism involving algae uptake and harvesting of the produced biomass and upon two indirect mechanisms, ammonia-nitrogen volatilization and orthophosphate precipitation (Nurdogan and Oswald, 1995).

The removal efficiencies were correlated with the growth of algae and as well as effluent characteristics (SiraneeSreesaia, 2007).Aarti *et al*., (2008) reported that dissolved oxygen in the water sample treated with *Chlorella vulgaris* had been found increasing by 247.83% and in water sample treated with *Synechocystissalina* 178.26 % increase was noted. 152.9% increase in the water sample treated with *Gloeocapsagelatinosa* was observed. It rises with the photosynthetic activity of algae. Our study did not show any such efficiency. The dissolved oxygen was increased by 68% by S. abundans and 73.5% by *C. pyrenoidosa*. The reason for such variations may be the quality of effluent as we used, for our work we used untreated and undiluted dairy effluent. Good results were reported with diluted dairy effluents by Asmare *et al*., (2014). BOD was reduced by 58.3% with *S.abundans* and higher reduction was seen by *C. pyrenoidosa* up to 65.9%. Similarly COD total hardness, magnesium and calcium hardness was also reduced significantly. *C. pyrenoidosa* showed 48.3%, 48%, 46%, 54% reductions respectively. *S. abundans* was less efficient with results of 45% COD, 41% total hardness, 31% magnesium and 45.3% calcium hardness reductions.

Analysis of various physio-chemical parameters before and after treatment revealed that the micro algae *Chlorella pyrenoidosa* and *Scenedesmus abundans* could effectively improve water quality by removal of pollutants. The studies on the nutrient uptake by these micro algae clearly reveal the efficiency of algae to remove nutrients from polluted water bodies. Both the algae showed high efficiency in the removal of nutrients. Biological treatment processes accomplish oxidation of organic materials in wastewater by microbial activity such as activated sludge, lagoons or anaerobic processes and photosynthesis of micro algae which have been used efficiently to reduce some physico-chemical parameters such as pH, BOD and COD (Tarlan*et al*., 2002; Goel, 2006). Thus the present study is collaborating the observations made by some of the earlier authors. Although much of the details are to be obtained based on further studies.Carbohydrates exist in the forms of monosaccharides, oligosaccharides, and polysaccharides (Mathews *et al*., 2000). Hexoses are produced as a result of photosynthesis and the molecule can further be converted to storage polymers, polysaccharides, such as starch and glycogen and used to build cell material (Mulbry, 2006).

The carbohydrate contents were found to be similar between the species at around 10-18 % g/g in the work carried by Mathias Bark who cultivated 11 different algae in varied conditions, and concentrations of CO_2 using flue gas. The carbohydrate was estimated as % of dry matter content. 25% and 18% of carbohydrate was found in C. pyrenoidosa and S. abundans harvested from the domestic sewage after treatment. The mass of Chlorella and Scenedesmus showed the presence of 30% & 20% of carbohydrate, obtained from dairy effluent (Fig-5). Similar results were recorded by Rothermel *et al*., (2011).Lipids exist within algal cells and lipid productivity is thought as a key desirable characteristic when choosing algal species due to the possibility of producing biodiesel (Dominic, 2009). The lipids and fatty acids exist in the membrane, as storage products, or metabolites.

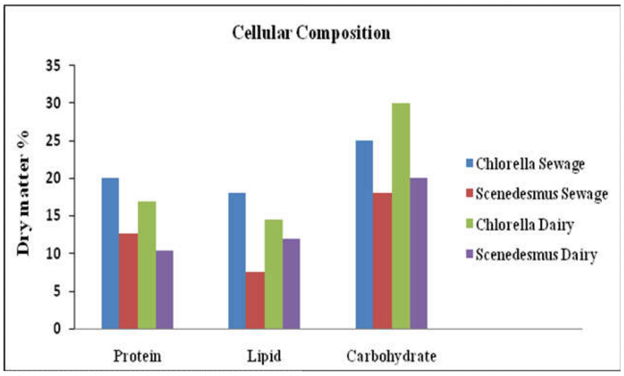
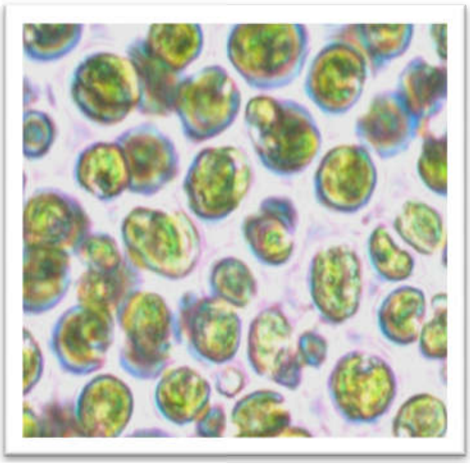
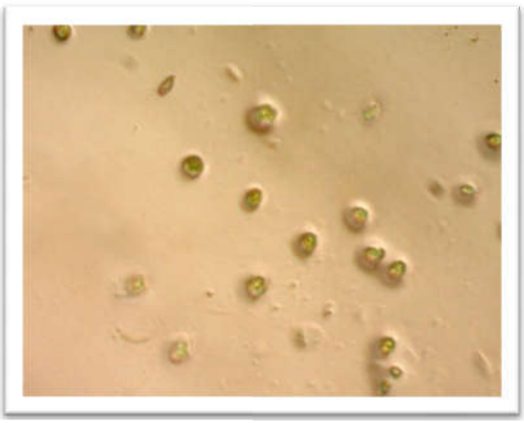


Figure 5. Biochemical composition of *Chlorella pyrenoidosa* and *Scenedesmusabundans*

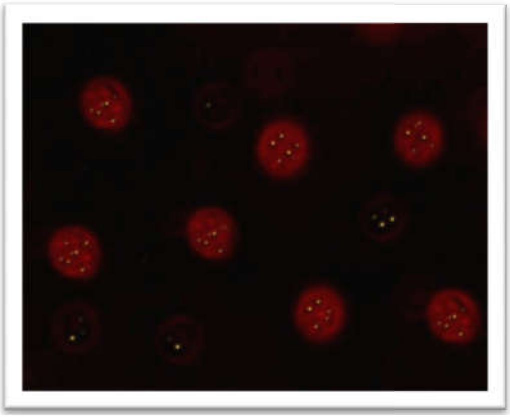


(a)

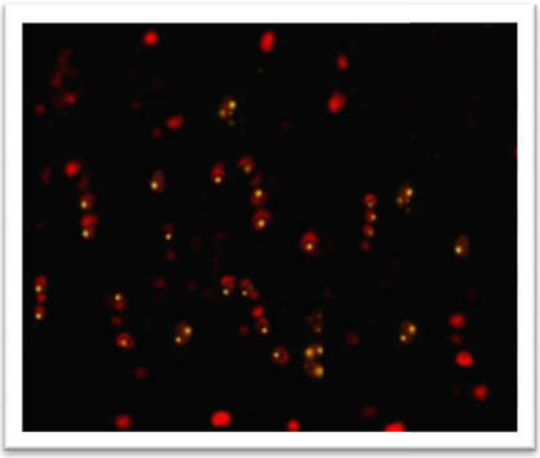


(b)

Figure 6: Microscopic image of pure cultures of (A) *Chlorella pyrenoidosa* and (B) *Scenedesmus abundans*.



(a)



(b)

Figure 7: Nile red staining image of pure cultures of (A) *Chlorella pyrenoidosa* and (B) *Scenedesmus abundans*

The largest portion of lipids often exists as a part within membranes (Mathews *et al*., 2000). Primarily lipid droplets exist in the cytoplasm, not in other organelles within the cell, and can therefore be extracted quite easily from the cells (Oilgae, 2009). The components and amounts of lipids differ between species and classes (Huang and Chen, 2009). And the growth phase and environmental conditions affect the composition and productivity (Huang X F, 2006).In our study we estimated the lipid content of the algae after their growth in the effluents on 21^{st} day by gravimetric method. 18 % of the lipid was obtained from *C. pyrenoidosa* when grown in sewageand14.5% which was grown in dairy effluent. S.abundans showed 7.5% and 12% cultured in sewage and dairy effluent. The crude lipid content was significantly higher in *Scenedesmus abundans*. (23.5%) compared to C. pyrenoidosa (7.9%) (Fig-5). According to literature studies several species of *chlorella* sp. can have higher lipids contents up to 60%. Mehrotra *et al*., (2010) and Asmare *et al*., (2014) have reported the higher lipid accumulation by *S. dimorphus* compared to *C. vulgaris* cultivated in dairy manure. Micro algae are widely used as a source of protein, and used in several forms like tablets, powder, etc. The protein contents of the microalgae vary widely depending on the organism and its culture conditions and may measure up to 50-60%. (Oilgae, 2010).The proteins are estimated as % of dry matter. In our study the protein content of *C. pyrenoidosa* was 20% in sewage and 17% in dairy effluent. 12.6 % of protein was present in the mass obtained from sewage S. abundans while the mass from dairy effluent contained 10.4% of the protein. Similar results have been noted by Cynthia*et al*., (2010).

Conclusion

The two strains *Chlorella pyrenoidosa* and *Scenedesmus abundans* showed excellent nutrient removal capability from sewage and dairy effluent. Both organic as well an inorganic contaminants were reduced at a safe level of standards. Hence these organisms can be cultivated invitro and employed in water treatment plants for bioremediation of industrial effluents and sewage water, hence preventing eutrophication of lakes and other water bodies.After phytoremediation the algal biomass can be separated by flocculation, centrifugation, sedimentation, filtration and lipids can be extracted for biodiesel production, as bio fertilizer in crop fields, as feed stock for cattle, other energy sources such as bio hydrogen, bio ethanol can also be produced.

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