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RESEARCH ARTICLE

Phycoremediation of Dairy Wastewater by Microalgae for elimination of organic pollution load

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ABSTRACT

The present study aims to demonstrate the potential of microalgae *Chlorococcum humicola* for treatment of dairy industry effluent and reduction of its pollution load with the cultivation of microalgae in the same effluent. Dairy industry wastewater supplies the required nutrients for the growth of *C. humicola* and its growth was comparatively higher in 50 % dairy industry effluent as compare to Bold basal growth medium. Optimization of growth parameters of algae showed that growth of *C. humicola* was favoured by alkaline pH and optimum growth was observed at pH 8, whereas acidic pH does not favour the growth of selected algae. The exponential growth phase of *C. humicola* was achieved between 3-7 days, at 20˚C temperature, a further increase in temperature decreases the algal growth. The results for the effect of different concentration of dairy wastewater (0, 10, 25, 50, 75 and 100 %) on biochemical content (protein, chlorophyll *a*, carbohydrate) of *C. humicola* revealed that 50 % wastewater concentration was more efficient for enhancement of biochemical content of microalgae as compare to control. The result further showed considerable reduction in the organic pollution load of dairy wastewater as biological oxygen demand (BOD) and chemical oxygen demand (COD) reduced to 72 and 78 % respectively, after 15 days of treatment with *C. humicola*. The results also showed a reduction in the nutrient concentration of dairy wastewater such as nitrate, phosphate and sulphate by 92, 43, 62 % respectively, after 10 days of treatment and 96, 67 and 78 % respectively, after 15 days of microalgal treatment as compared to control (without microalgae). These findings suggested that dairy industry wastewater was a good nutrient supplement and can be directly used for mass cultivation of *C. humicola* without requiring additional nutrient supplements and also the microalgae *C. humicola* has a great potential for the treatment of dairy industry wastewater.

***Key words*:** Algae; Bioremediation; *Chlorococcum*; Industrial Pollution; Wastewater

1) INTRODUCTION

In India dairy industry is one among the key industries having vast economic value as it contributes about 13.1% of the total world milk production and 35% of the total Asian milk production [1]. It is the world's largest milk producer in the entire globe consuming almost 100% of its own milk production. There are about 286 large- and small-scale dairy industries in India, which are responsible for production of large quantities of solid and liquid waste along with milk [2]. It generally generates about 0.2 to 10 L of wastewater in processing of 1 litre of milk [3]. Dairy industry are called 'wet –industry' as they use large volumes of water for its diverse purposes, the major problem with dairy industry is that it is noted as one of the significant contributor of water pollution due the wastewater which it discharges. The wastewater released from such industries is the most odorous and polluted water in comparison to other industries as it contains a lot of organic matter like protein, fat and minerals [4] thus raising its biological oxygen demand (BOD) and chemical oxygen demand (COD) value. Dairy wastewater is characterized by strong colour, offensive odour, high BOD (40–48,000 mg l^{-1}), high COD (80–95,000 mg l^{-1}) and variable pH [5, 6, 7]. It also contains sufficient nutrient like N (14–830 mg l^{-1}) and P (9–280 mg l^{-1}) required for biological growth [8]. The effluent affects the aesthetic value of the receiving water and its alkaline pH causes damage to aquatic life [9]. An appropriate treatment of dairy wastewater is necessary to avoid eutrophication of surface and groundwater, aquatic life affection and also to minimize the effect of soil degradation by addition of effluent to soil.

Various physical and chemical treatment methods are being applied for wastewater, but these suffer with many disadvantages like need of high amount of chemicals,

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energy and manpower. Biological methods are considered more efficient, cost-effective and sustainable treatment methods for wastewater particularly for dairy industry. Composition of dairy wastewater allows for biological treatment as it contain organic matter, oil, grease and large amount of nitrates, phosphates, sulphates, chlorides which act as a nutrient for microorganisms [10]. There are a large number of studies on the treatment of industrial, municipal and agricultural wastewaters by microalgal culture systems [11, 12]. The use of microalgae as biological treatment and recycling of wastewater has attracted immense interest due to their role of carbon fixation and bioremediation [13]. Microalgae have potential to generate significant amount of biomass considered as third generation feedstock for biofuels and animal feed, while they remove organic content and minerals for building the biomass. The coupling of algal biomass production to wastewater treatment is one of the promising ways to fulfil multiple objectives of energy biomass generation, nutrient recovery and water management [14, 15]. The utilization of wastewater nutrients to microalgal growth will control eutrophication and also help in sustainable energy development. Microalgal wastewater treatment systems could also be a solution in remote areas and for small and medium scale farmers [16].

Among the various microalga, the green algal species used in this study is *Chlorococcum humicola* which is a distinct genus of Chlorophyta. Some of the main characteristics which set algae apart from other biomass sources are that algae can have a high biomass yield per unit area. Though it is a fresh water alga, it enjoys all kinds of habitat thus it can be used in wastewater treatment very smoothly. The present study focus on utilization of nutrients present in dairy wastewater for the growth of *C. humicola* and removal of organic pollution load from dairy wastewater by algae. Use of microalgae at dairy industry can fulfil the purpose of pollutant removal without any loss, since dairy wastewater is an excellent growth media for microalgae as it is enriched with nutrients which are needed by algae for its growth and survival.

2) MATERIALS AND METHODS

2.1) Wastewater collection

The secondary treated dairy wastewater sample was collected from Producers Cooperative Milk Union Limited, Lucknow, U.P., India. An oxidation pond is used by the industry for treatment of industrial wastewater. The industrial wastewater was collected in sterilized sampling bottles and was stored at 4˚C to avoid degradation before use. Different ratio of dairy industry wastewater and distilled water i.e.; 1:9, 1:3, 1:1, 3:1, and 1:0 was prepared to obtain various concentrations (10, 25, 50, 75 and 100 %) of dairy wastewater.

2.2) Microalgal culture

The microalgal strain *Chlorococcum humicola* was obtained from Culture laboratory of Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, U.P., India. The microalgal culture was further maintained in Bold Basal medium (BBM) under controlled laboratory conditions in the presence of 10 W/m^2 cool fluorescent light with 16 hours light/dark cycle.

2.3) Optimization of growth conditions

Growth of microalgae *C. humicola* was optimized for different environmental factors such as time, pH and temperature. Time dependent growth experiments were performed in 250 ml conical flasks containing 100 ml of Bold Basal medium, inoculated with *C. humicola* cells. It was then kept under favourable culture conditions and each day growth rate was measured in terms of increase in optical density (O.D.) at 665 nm for 15 days till the O.D. started to decline. For optimization of pH and temperature experiment was set under different pH range (5-10 pH) and different temperature range (15-30˚C). pH was maintained regularly with 0.1M HCl and 0.1M NaOH using a pH meter (Toshniwal Inst. Mfg. Pvt. Ltd., Ajmer). It was then kept under favourable culture conditions and each day growth was measured in terms of optical density (O.D.) at 665 nm by using UV–visible spectrophotometer (Schimadzu, 1601, Japan) for 7 days.

2.4) Batch Experiment

The experiment was set up in six different 250 ml conical flasks. The exponentially growing cells of *C. humicola* was inoculated (approximate cell density of 1 X 10^7 cells ml^{-1}) in different concentration of dairy industry waste water i.e. 10, 25, 50, 75, 100 % as well as in Bold Basal medium as control. The flasks were kept under controlled laboratory condition at 20±2˚C. The growth of microalgae was regularly monitored for 7 days in terms of optical density at 665 nm by using UV–visible spectrophotometer.

2.5) Biochemical content of microalgae

Proximate biochemical content (protein, chlorophyll *a* and carbohydrate) of *C. humicola* was analysed for 7 days to estimate the effect of different concentration (0, 10, 25, 50, 75, 100 %) of dairy wastewater on microalgae. The sample was taken regularly from batch experiment flasks and centrifuged for further procedure. Protein of the biomass was measured according to the method of Lowry et al. [17] as modified by Herbert et al. [18]. The total carbohydrate was measured by the Anthrone method as suggested by Hedge and Hofreiter [19]. The chlorophyll *a* was extracted using methanol and quantification was made by using the absorption coefficient of 12.5 µg ml^{-1} as suggested by McKinney [20].

Concentration of Chlorophyll *a* (µg ml^{-1}) = 12.5 X ΔA

where, ΔA = Absorption increase due to pigment at 665 nm

2.6) Physico-chemical analysis of dairy industry wastewater

The experiment for treatment of dairy industry wastewater by *C. humicola* was carried out in 1L conical flask containing 600 ml of 50% concentration of secondary treated dairy wastewater. The physico-chemical parameters of wastewater were analyzed on the 0th, 5th, 10th and 15th day to estimate the reduction in the pollution load of wastewater by *C. humicola* (Table 1). Physico-chemical parameters were analyzed according to the standard method given in APHA [21]. All parameters were analyzed in triplicate. The percent reduction in pollutant load was calculated as given below-

$$\text{Percent Reduction in Pollution load} = \frac{Initial\ value - Final\ Value}{Initial\ Value} X100$$

3) RESULTS AND DISCUSSION

3.1) Optimization of growth conditions of microalgae

Optimization of environmental growth conditions are necessary to achieve maximum growth and utmost utilisation of experimental microalgae. During the course of study of growth (15 days), the exponential growth phase was found to be between 3 to 7 days and thereafter, growth of *C. humicola* started to decline (Fig. 1A). Result showed that the overall increase in the cell density at the end of exponential phase was approximately 6 folds higher. The optimization of pH for growth of microalgae suggested that the growth of *C. humicola* initially increased with increasing pH and reached to a maximum level at pH 8, further increase in pH resulted into decline in growth of microalgae (Fig. 1B). It was apparent from the results that growth of selected microalgae was favoured by alkaline pH whereas acidic pH doesn't support the growth. Temperature is undoubtedly one of the most important factors controlling the growth of many algal species. It strongly influences cellular chemical composition, the uptake of nutrients, carbon dioxide fixation, and the growth rates for every species of algae. The optimum temperature for the growth of selected microalgae was observed at 20°C, further increase in temperature decreases the algal growth (Fig. 1C). In most Chlorococcales the optimum growth temperature was observed in the range of 20 -30°C and optimum pH in alkaline range by several workers [22, 23, 24].

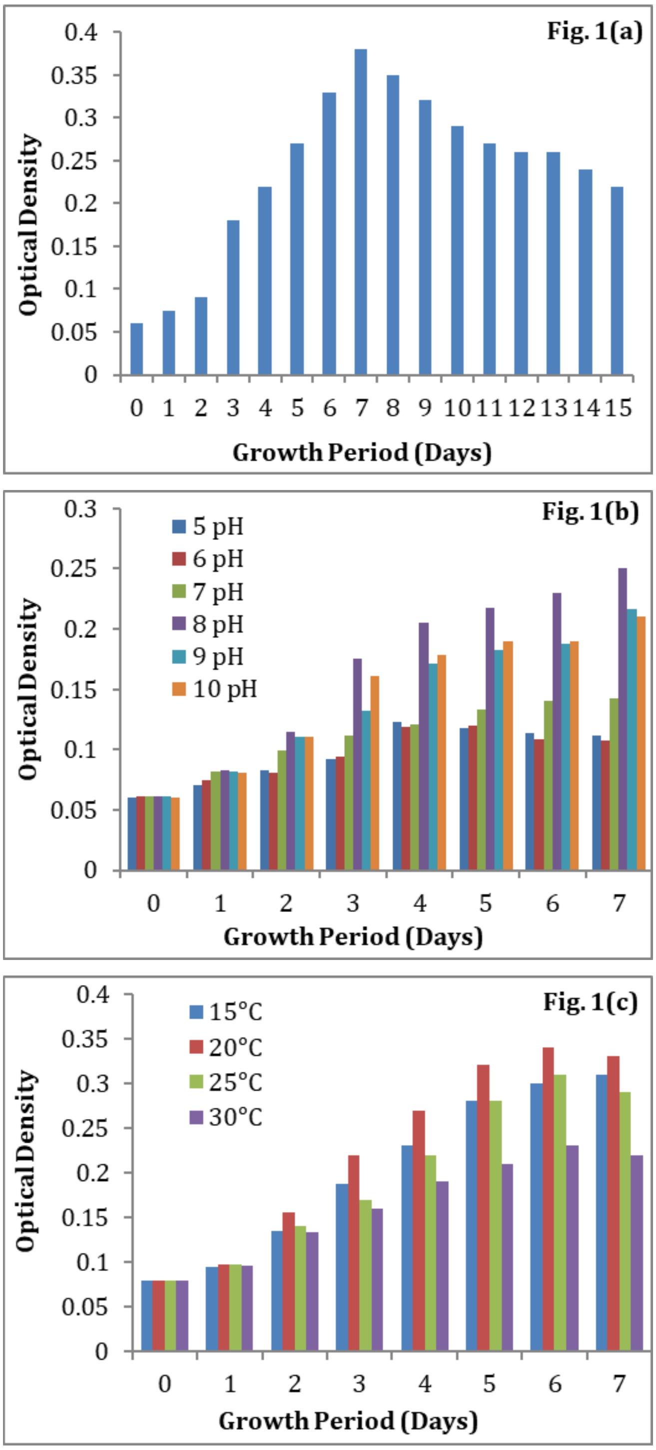

Figure 1: Optimization of growth conditions of *Chlorococcum humicola* under different **(a)** time, **(b)** pH and **(c)** temperature.

3.2) Effect of dairy wastewater on growth of microalgae

Growth of *C. humicola* was monitored in terms of increase in the optical density at 665 nm at different concentration (10, 25, 50, 75, and 100 %) of dairy industry wastewater and control (Bold Basal Medium). The results (Fig. 2) showed that microalgal growth increased with increasing concentration of wastewater and maximum growth was obtained at 50 % concentration of dairy wastewater. The wastewater dependent growth of microalgae was when compared with control (Bold basal medium), it was observed that *C. humicola* showed better growth response in 50 % wastewater as compare to control (Fig. 2 inset). Lower concentrations of wastewater (10 and 25 %) were not able to sustain better growth of microalgae as compared to control, perhaps due to low nutrient availability at lower concentrations of wastewater. However, algal growth at higher concentration of wastewater (75 and 100 %) was almost similar as it was observed with control. Similar results were obtained for cultivation of microalage *Botrycoccus* [25] and *Chlamydomonas polypyrenoideum* [7] on dairy industry wastewater. Previous studies have indicated possible microalgal cultivation on dairy and other wastewater also [24, 26, 27, 28, 29, 30, 31]. However, various microalgae suffered from inhibition of photoautotrophic growth on dairy wastewater owing to its high levels of turbidity [26, 29, 31]. Some microalgae showed enhanced growth on the highly diluted dairy wastewater while avoiding possible inhibition from high turbidity of wastewater [27, 28].

3.3) Effect of dairy wastewater on biochemical content of microalgae

Biochemical contents (protein, chlorophyll *a* and carbohydrate) of *C. humicola* was evaluated at different concentration (10, 25, 50, 75, and 100 %) of dairy industry wastewater and control with respect to time (0 to 7 days). The result showed that all the three tested components i.e.

protein, chlorophyll *a* and carbohydrate increases with increasing concentration of wastewater and maximum level of all these was obtained at 50 % wastewater concentration. The pigment concentration depicted the healthy status and provides the survival index of the any plant system [32]. The high concentration of chlorophyll *a* in *C. humicola* grown in dairy wastewater is the indicator of low stress in microalgae. Almost similar pattern was observed for protein, chlorophyll *a* and carbohydrate in *C. humicola*. Several other workers [33, 34] observed an increase in carbohydrate level of algae with increasing concentration of industrial waste water. The results also demonstrate that 50 % wastewater concentration was more efficient for enhancement of biochemical content of microalgae as compare to control (synthetic growth medium). A very marginal increase in protein, chlorophyll *a* and carbohydrate level of microalgae was observed with increasing time (0-7 days) at lower concentration (10 and 25 %) of dairy wastewater which clearly state that lower concentrations are not very effective for enrichment of biochemical content of microalgae, may be due to low nutrient availability at lower concentrations of wastewater. Biochemical compositional analysis of microbial biomass is a useful tool that can provide insight into the behaviour of an organism and its adaptational response to changes in its environment.

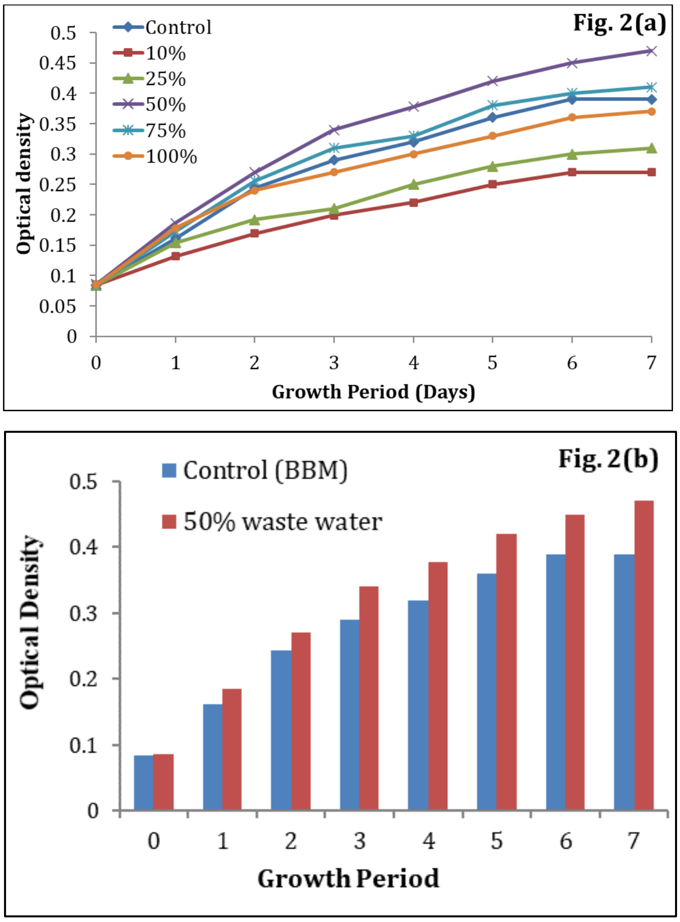

Figure 2: (a) Effect of different concentration (0-100 %) of dairy industry wastewater on growth of *Chlorococcum humicola* and **(b)** comparison of growth between 50 % wastewater and Control (Inset)

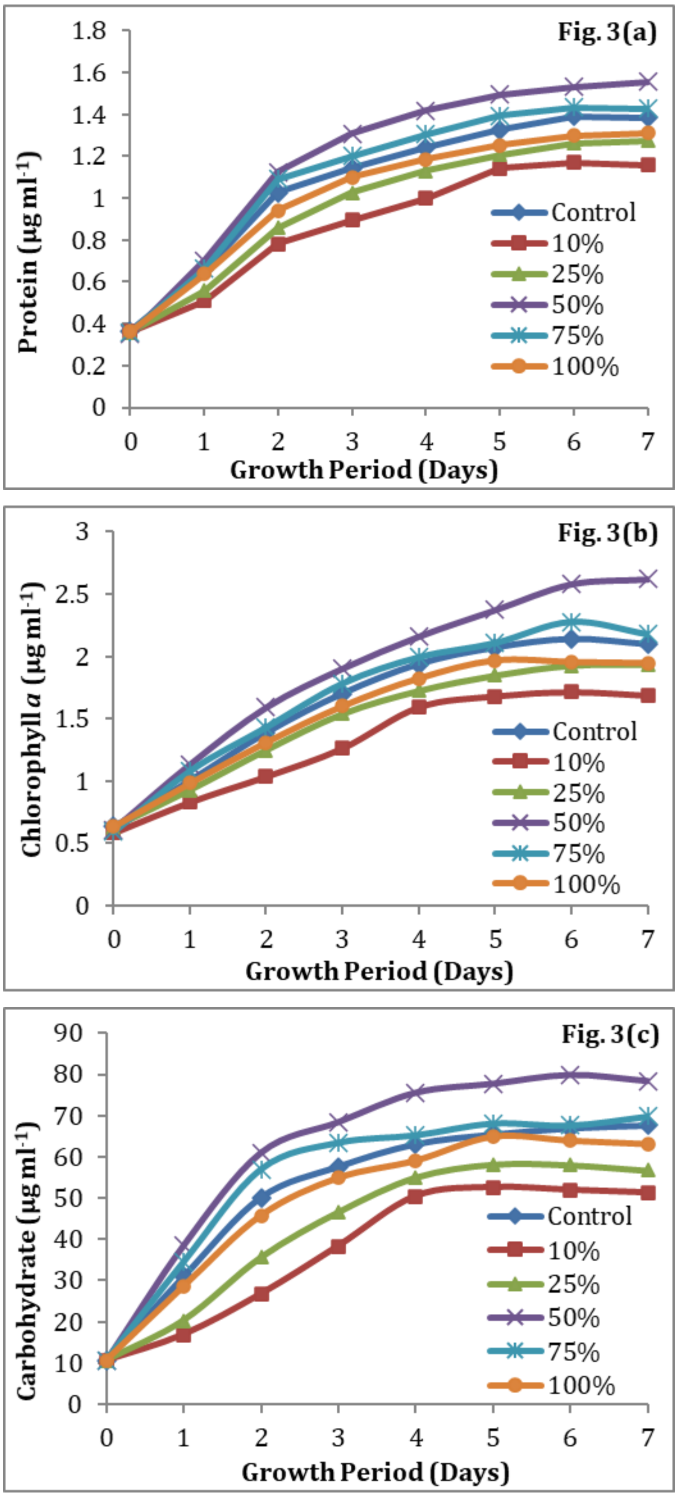

Figure 3: Effect of different concentration (0-100 %) of dairy industry wastewater on protein **(a)** chlorophyll *a* **(b)** and carbohydrate **(c)** content of *Chlorococcum humicola* with respect to time (0-7 days)

3.4) Removal of pollutant from industrial wastewater by microalgae

Based on the growth response of *C. humicola* this experiment was performed at 50 % dairy wastewater concentration, which was the optimum concentration of wastewater for microalgae growth. The pollution load of the wastewater after inoculation by algal cells was measured on 0th, 5th, 10th and 15th day after algal inoculation (Table 1).

The initial values of pH, TS, TDS, TSS, hardness, BOD, COD, nitrate, phosphate and sulphate recorded on the first day (0th days) of treatment were found to be in the higher range than the permissible limit given by CPCB [35]. The algal cells used the nutrients present in the wastewater for their growth, an increase in algal biomass resulted in gradual reduction in the excessive load of nutrients. The untreated dairy effluent has an acidic pH value initially (5.9 pH) but after algal treatment the treated effluent is shifted to slightly alkaline pH range (7.5 pH). The pH of the untreated effluent was mostly acidic in nature due to decomposition of lactose into lactic acid under aerobic conditions and may cause corrosion of sewers [36]. These results are supported by the findings of other workers [7, 37] who observed increase of pH in industrial wastewater treated with microalgae. The total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) in the dairy effluent was reduced to 80.9, 82.4 and 71.9 %, respectively, after 15 days of microalgal treatment (Fig. 4). Results are in agreement with the findings of other workers [38, 39] who reported reduction in total dissolved solids of oil refinery and petroleum effluent treated with cyanobacteria *Spirulina platensis* and *Oscillatoria* sp. The presence of inorganic nutrients degrades the quality of water and depletes the dissolved oxygen present in water. Our study reveals that nitrate, sulphate and phosphate of dairy wastewater reduced by 96.3, 78 and 67 %, respectively, after 15 days of treatment with *C. humicola* (Fig. 4). The reduction in nitrate was noticed due to loss in denitrification process. Kshirsagar [40] found nitrate removal of 78.1 % from dairy wastewater using *C. vulgaris*. The study by Hena et al. [29] reported upto 99.4 % removal of nitrate and 98.8 % removal of phosphate. The reduction in phosphate level was found due to utilization of phosphate during metabolic processes. The reported phosphate uptake efficiency varies depending on the media composition and environmental conditions, such as the initial nutrient concentration, light intensity, nitrogen/phosphorus ratio and the light/dark cycle or algae species [41]. Nutrient uptake, assimilation and absorption by algal cell are key processes for nutrient removal in wastewater [40, 42]. The high concentration of nitrate and phosphate down regulate protein and chlorophyll synthesis [42]. The nutrient requirement increases with algal biomass growth, therefore, the relation is nearly exponential between nutrient removal and treatment duration [14]. Wastewater of dairy industry contains large quantities of milk constituents such as casein, lactose, fat, inorganic salts. All these components contribute largely towards their high biochemical oxygen demand. High BOD and COD values lead to the deprival of oxygen for aquatic life in water [9]. BOD value decreased from 286.2 to 79.2 mg L^{-1} and COD value decreased from 3232.2 to 720.6 mg L^{-1} after 15 days of treatment with *C. humicola* (Table 1). Since the microalgae can effectively grow in the nutrient-rich environment [43, 44] and also accumulate nutrients [27, 45] and metals from the wastewater [46, 47, 48], these attributes make them attractive, efficient, economical and eco-friendly tool to remediate the wastewater.

Table 1: Different physico-chemical parameters of Dairy industry wastewater measured before (0th days) and after treatment (5th, 10th and 15th days) with *Chlorococcum humicola.*

Parameters	0th days	5th Days	10th Days	15th Days
Colour	Greyish	Greyish	-	-
Odour	Foul Smell	Foul Smell	-	-
pH	5.9	6.3	7.2	7.5
EC	1.3	1.6	2.0	2.1
TS	1171	1012	645	224
TDS	1003	880	558.1	176.8
TSS	168	132	86.9	47.2
Hardness	184.3	118.3	67.2	27.4
BOD	286.2	226.5	120.2	79.2
COD	3232.2	2799.2	1172.2	720.6
Nitrate	56.4	23.9	4.2	2.1
Sulphate	45.2	32.2	17.2	9.9
Phosphate	13.4	10.1	7.6	4.4
(EC in terms of dS m^{-1}, all other parameters are in terms of mg L^{-1} except colour, odour, pH)				

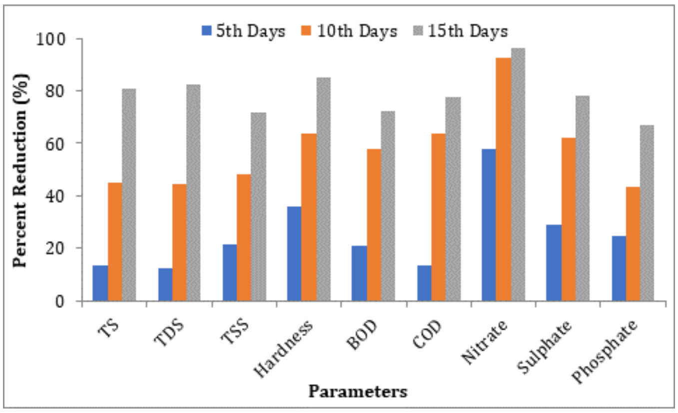

Figure 4: Percent reduction in the pollution load of Dairy industry wastewater after 5th, 10th and 15th days of treatment with *Chlorococcum humicola*

4) CONCLUSION

The findings of present study suggest that dairy industry wastewater can be directly used for mass cultivation of microalgae without requiring additional nutrient supplements which decreases the microalgae production costs. The microalgae also have immense potential to minimize the organic pollutant load of the dairy industry wastewater which showed its application for phycoremediation. Reduction of considerable amount of nitrate (96 %) and phosphate (67 %) which are the main cause of water eutrophication is another benefit of using these microalgae for wastewater treatment. The study conducted on assessment of lipid content in *C. humicola* (results not shown) revealed a high percentage of lipid (53.42 % of the dry weight of algal biomass) which proof this algae can be used as an alternative for production of biofuel.

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