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Phycoremediation of textile wastewater by unicellular microalga *Chlorella pyrenoidosa*

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Abstract

The potential application of microalga *Chlorella pyrenoidosa* was investigated for phycoremediation of textile wastewater. Two 15 days batch experiment containing autoclaved and unautoclaved textile wastewater were performed to measure the efficiency of alga to remediate the wastewater. Experiments were set at equivalent external conditions and pollutant load was measured on alternate of 5 days to determine the pollutant removal efficiency of alga. Alga was found to be more efficient in removal of pollutants load in autoclaved wastewater; agents of eutrophication such as nitrate and phosphate are reduced by 62% ±0.5 and 87% ±0.7 respectively while organic load in terms of BOD is reduced by 81% ±0.2 whereas, In unautoclaved wastewater in presence of algal-bacterial consortium, nitrate and phosphate were removed by 81% ±1 and 36% ±2.2 while BOD is reduced by 73% ±1.6 only. Another time dependent experiment of dye removal was also performed to measure the adsorption potential of selected dried algal biomass. An equal amount of dried algal biomass was introduced to various range of textile wastewater simulated with methylene blue (MB) dye. The maximum colour removal was observed afterduration of 30 minutes by dry algal biomass.

Key words: Adsorption, Methylene blue, Phycoremediation, Pollutants, Simulated wastewater.

Introduction

Dyes and coloring agents are frequently used in many industries such as food, pharmaceuticals, cosmetics and leather; however, they are most important in textile manufacturing industry. Textile industry and its dye containing wastewater are one of the major sources of severe water pollution (1). More than 100,000 commercially available dyes and approximate 7×10^5tonnes of dye stuffs are produced worldwide annually(2), in which 10% to 25% of textile dyes are lost during dyeing operations while 2% to 20% of it directly discharged in various environmental components as aqueous effluent (3). The dyes include various varieties such as reactive dyes, basic dyes, acidic, disperse, azo, diazo, anthraquinone based and metal-complex dyes.

Textile industries are very complex in nature as far as varieties of products, process and raw materials are concerned. During production, the cloth has to pass through various processes and chemical operations like sizing, desizing, scouring, mercerizing, bleaching, dying, printing, and finishing. In a textile industry, a number of dyes chemicals and auxiliary chemicals are used to impart desired quality in the fabrics. The wastewater of the textile industry discharged after dyeing process contains high concentration of BOD (0.01-1.8 g O_2/l), COD (1.1-4.6g O_2/l), TDS (50 mg/l) and variable pH (5-9) (4, 5). Composition of wastewater is depending on the different organic-based compounds, inorganic chemicals and dyes used in the industrial dry and wet-processing steps.

The coloured wastewater affects aesthetics, water transparency & gas solubility in water bodies and can be toxic to aquatic flora and fauna and this causes severe environmental problems worldwide (6). Most textile factories fail to adequately treat their wastewater. The textile dyes are hazardous and the presence of colour reduces the availability of life and reduces the biological activity in aquatic life.

Dye wastewater is generally treated by physical and chemical process such as flocculation combined with flotation, electro flocculation, membrane filtration, electro kinetic coagulation, electrochemical destruction, ion-exchange, irradiation, precipitation, ozonation, and katox treatment method , but these methods fail to achieve efficiency and not suitable for various types of dye wastewater (2).

Algal ponds are efficiently used to treat the agro-industrial wastewater by using high rate algal pond. Instead of pollutant removal microalgae also produce high quality animal feed. Microalgae such as *Chlorella* and *Spirulina* have shown their efficiency for pollutant removal from rubber effluent and sago starch factory wastewater, respectively (7, 8).

Microalgae are found to be potential biosorbent because of their large surface area, high binding affinity and availability in both fresh water as well as salt water (9). Algal cell wall takes place major role in the adsorption process provide site for electrostatic attraction and complexation during the adsorption process. Adsorption process has been proven efficient method for the color removal from the various type of dye containing wastewater, use of activated carbon for dye removal is well known but it is too expensive to use in long term. A number of studies have been done to investigate the low cost adsorbent such as peat, bentonite, steel-plant slag, fly ash, china clay, maize cob, wood shavings, and silica for color removal (10, 11) though the low cost adsorbents have potential application for adsorption process but they have low adsorption capacities thus there

Table 1. Initial characterization of collected textile wastewater.

Parameters	Measurements	Standard for emission of discharge of pollutant (Textile and Dye industry) CPCB
pH	6.8	6-8.5
Electrical Conductivity	6.89	----
Total Dissolved soild	5300	----
Total Susended Solid	100	100
Total Solids	5400	---
BOD	710	100
Phosphate	4.7	---
Nitrate	360	---
Chloride	900	1000
Alkalinity	1000	----

*Except pH and E.C (mS) all parameters are in terms of mg/l.

* Values are average of results of three replicates of experiment.

is need to found such a biosorbent which have low cost as well as potential adsorption capacity.

The primary objective of this study is to investigate the efficiency of *Chlorella pyrenoidosa* for removal of pollutant load as well as decolourization of textile wastewater.

Materials and methods

Collection of microalgal species

The culture of *Chlorella pyrenoidosa* was procured from the National Collection of Industrial Microorganism, (NCIM) Pune and maintained in recommended Fog's growth medium. The composition of medium includes macronutrients suchas $MgSO_4 7H_2O$ (0.2 g), K_2HPO_4(0.2 g), $CaCl_2H_2O$ (0.1 g), Fe-EDTA solution (5 ml) and micronutrients are H_3BO_3(286 mg), $MNCl_2.4H_2O$ (181 mg), $ZnSO_4 7H_2O$ (22 mg), $Na_2MoO_4 2H_2O$ (39 mg),$CuSO_4 5H_2O$ (8 mg) as described by NCIM, Pune. The cultures were provided with white fluorescent cool light (12:12 h) and optimum room temperature 25±2^oC. All cultures were manually shaken to avoid sticking of microalgal cell on wall of culture flask.

Collection and characterization of textile wastewater

Textile industry wastewater selected for study, was collected from Textile industry located, near Kundan Nagar Unnao (U.P.). The secondary treated wastewater was collected in plastic cane and brought to the departmental laboratory and keep at 4^oC to avoid further degradation. The physical and chemical parameters of textile wastewater were analyzed in departmental laborotary. Parameters like pH and Conductivity is measured by pH meter (Hanna HI96107), conductivity meter (Hanna HI98304) respectively, TS, TDS, TSS, BOD, Choride, Alkalinity, Nitrate and Phosphate were determined by method outline in the standard method given in APHA (1998) (12).

Study of growth pattern of alga at various concentration of textile wastewater

An experiment was set up for study the growth of alga in the effluent of textile wastewater at various concentrations to observe its ability to survive. For this *Chlorella pyrenoidosa* species was selected on the basis of its fast growing property. *Chlorella pyrenoidosa* was grown at various dilution of textile wastewater in effluent (25%, 50%, 75%, and 100%). The textile wastewater effluent was diluted with Fog's media and growth is measured in terms of protein content (µg/ml), monitored every alternate day by taking absorbance at optical density of 650 nm.

Removal of pollutant load from textile industry waste water

This experiment was set up for 15 days on the basis of growth pattern of alga observed at different concentration of textile wastewater. 75% of textile effluent inoculated with initial inoculums of algal suspension of 1%(v/v) equivalent to an optical density of 0.2 at 660 nm wavelength. Conical flask of 500 ml capacity was used for the experimental set up provided with 24 hour illumination of white cool fluorescent light at room temperature. The experiment was designed in such a way that pollutant removal efficiency of alga with no other microbial population and alga with naturally present microorganism could be determined, for this autoclaved and unautoclaved growth medium having 75% textile effluent was used.A control containing no algal population with same textile wastewater (TWW) composition is run parallel to the experiment to observe degradation of wastewater driven by pre-existed microorganism.

Decolourization of simulated Textile effluent

In this experiment alga *(Chlorella pyrenoisdosa)* was used for the colour removal from textile effluent simulated with Methylene Blue. Various concentrations of simulated textile wastewater were prepared (10, 20, 40 and 60 µg/ml). The dried (0.15 g) biomass of alga was used for adsorption of simulated textile effluent, alga was added in 100 ml textile effluent in 250 ml conical flask at room temperature (28^oC) and the mixture was stirred on an electric shaker at 200rpm. Decolorization of wastewater was monitored by taking absorbance (665 nm) of sample at different time intervals (15, 30 and 45 min).

Results

Characteristics of collected textile industry wastewater

pH of the wastewater was found slightly acidic whereas the other parameters like TDS and TSS were found above the permissible limit. The textile wastewater was found having significant organic load as the BOD was

Table 2. Removal of pollutant load at different time interval in Unautoclaved (75%) textile wastewater.

S. NO.	Parameters	Unautoclaved control				Unautoclaved Treated				CPCB (Textile and Dye industry)
		0 Day	5th Day	10th Day	15th Day	0 Day	5th Day	10th Day	15th Day	
1.	pH	8.5	8.5	8.5	8.0	8.5	8.8	9.0	9.0	6-8.5
2.	EC	6.1	6.5	6.5	6.5	6.1	6.0	5.5	5.4	---
3.	TDS	5000	4100	3900	3500	5000	3450	1600	720	---
4.	TSS	1500	1400	1300	1280	500	350	300	120	100
5.	TS	6500	5500	5200	4800	5500	3800	1900	800	---
6.	BOD	600	575	530	500	600	302	202	161	100
7.	Phosphate	0.44	0.40	0.24	0.20	0.44	0.31	0.30	0.28	---
8.	Nitrate	475	381	339	216	457	337	258	87	---
9.	Alkalinity	800	780	600	436	800	580	400	348	---
10.	Chloride	660	650	648	640	660	106	100	90	1000

*Except pH and E.C (mS) all parameters are in terms of mg/l.
* Values are average of results of three replicates of experiment.

Table 3. Removal of pollutant load at different time interval in Autoclaved textile (75%) wastewater.

S. NO.	Parameters	Autoclaved control				Autoclaved Treated				CPCB (Textile and Dye industry)
		0 Day	5th Day	10th Day	15th Day	0 Day	5th Day	10th Day	15th Day	
1.	pH	8.5	8.5	8.5	8.5	8.5	8.8	9.0	9.0	6-8.5
2.	EC	6.5	6.5	6.5	6.5	6.5	6.7	6.7	6.7	---
3.	TDS	4850	4800	4000	4000	4850	4000	3800	2800	---
4.	TSS	1880	1200	1100	800	1880	1000	800	744	100
5.	TS	6730	6000	5100	4800	6730	5000	4600	3544	---
6.	BOD	308	301	295	290	308	201	100	56	100
7.	Phosphate	0.80	0.76	0.70	0.60	0.80	0.37	0.10	0.10	---
8.	Nitrate	453	387	363	281	453	343	268	171	---
9.	Alkalinity	800	740	600	416	800	600	340	272	---
10.	Chloride	661	650	630	640	661	138	124	106	1000

*Except pH and EC (mS) all parameters are in terms of mg/l.
* Values are average of results of three replicates of experiment.

twenty times higher than the discharge limit. Agents of eutrophication such as nitrate and phosphate were also present in significant amount (Table 1).

Growth pattern of alga at various concentration of Textile Wastewater

The growth of alga can be negatively correlated with increase in the concentration of textile effluent. At 100% concentration alga enters in the death phase earlier in comparison to death phase of alga at lower concentrations (75%, 50% and 25%). Alga showed its efficiency to tolerate a concentration of 75 % of wastewater as shown in figure 1, while at lower concentration it showed significant growth in comparison with culture growth medium. In present study alga was found to have potential to grow at 75 % of textile wastewater concentration, however Lim *et al*. (13) found the potential of *Chlorella vulgaris* to grow on 100 % textile wastewater but they achieved lower yield of biomass at that concentration. Presence of carbon, nitrogen, phosphorus and salt in textile wastewater support algal growth whereas it also containstoxicants which cause the limited growth of alga (14).

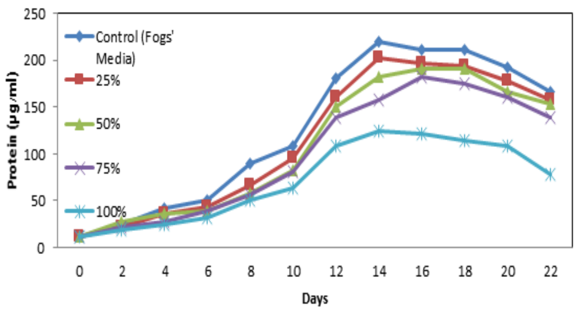

Figure 1. Growth pattern of alga at different concentration of textile wastewater incubated for 22 days.

Potential of alga to remove pollutant load from textile wastewater

Two experiments were run simultaneously to investigate the potential of alga to remove pollutant load from unautoclaved and autoclaved textile wastewater with the selected wastewater concentration (75%), experimental parametric analysis given in table 2 and table 3 respectively. In both set of experiments alga showed significant potential for removal of pollution load as depicted from figure 2 (a, b) and 3 (a, b) for unautoclaved and autoclaved textile wastewater, respectively. In autoclaved effluent inorganic contaminant like phosphate and nitrate is removed by 62% ±0.5 and 87% ±0.7 respectively whereas in unautoclaved effluent nitrate and phosphate is removed by 81% ±1and 36% ±2.2 respectively after 15th day of batch experiment. Organic load in autoclaved is removed by 81% ±0.2 and from unautoclaved wastewater it was 73% ±1.6 in terms of BOD.

Other parameters like alkalinity and chloride was also reduce by the alga in both set of experiments. The retention time of alga showed significant importance on pollutant removal and depend upon the algal growth pattern. The slow removal in early days was due to slow algal growth and less population. Once the alga achieved higher population (exponential phase), pollutants

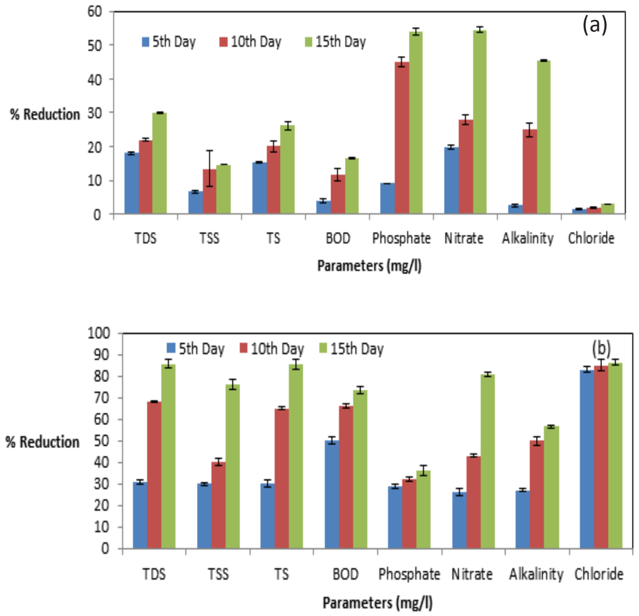

Figure 2. Percentage removal of pollutant load from 75% textile effluent **(a)** unautoclaved without treated with algae **(b)** Unautoclaved treated with algae at different time intervals.

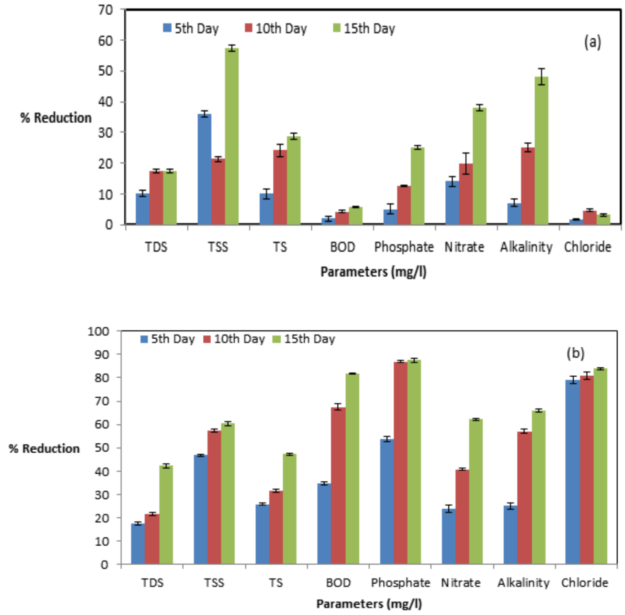

Figure 3. Percentage removal of pollutant load from 75% textile effluent (a) autoclaved without treated with algae (b) autoclaved treated with algae at different time intervals.

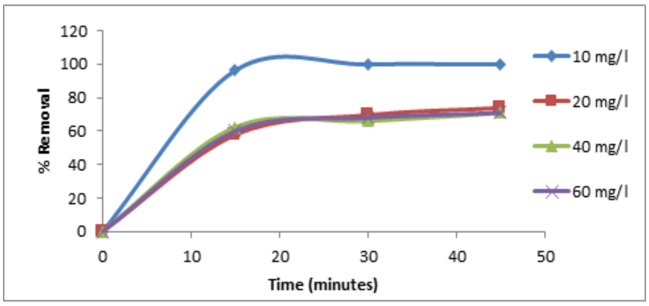

Figure 4. Percentage removal of MB dye by dry algal biomass.

are removed more rapidly from the effluent on the other side, in un-autoclaved effluent pollutant removal was carried out in presence of alga as well as pre existing microorganism (i.e. algal-bacterial consortium). This show the potential of alga for removal of pollutant load in association with the other microorganism. The algal-bacterial mediated remediation was also found potential as compare to control.

Potential of alga as an adsorbent for removal of dye from simulated textile wastewater

Algal potential as an adsorbent is measured using dried algal biomass. Dried algal biomass (0.15g) was applied at different concentration of methylene blue (10 to 60 ppm). Removal of dye was found to be negative correlate with higher concentration of dye. This can be explain by the fact that at lower concentration of Mb solution adsorption occurs mainly on the surface of the adsorbent, more time is needed for adsorption to take place in the pores. Effect of time also play significant role in the dye removal. As the time increases the rate of dye removal was also increases. The colour removal efficiency have a break through at 30 minute duration, after that there is no colour removal takes place. The maximum colour removal occurs after 30 minutes duration by dried algal biomass.

Discussion

From the characteristics of textile wastewater it is clear that the textile effluent is highly polluted and major toxicants are present above the permissible limit that can cause serious environmental impact on water bodies and aquatic organism. The coloured wastewater affects aesthetics, water transparency and gas solubility in water bodies and can be toxic to aquatic flora and fauna and this causes severe environmental problems (15). Dye containing wastewater contains carcinogenic, mutagenic and other aromatic compounds such as benzidiene and naphthalene (16, 17). Without adequate treatment discharged dyes remain in aquatic bodies for a long period of time as they have long half –life (18).

Results obtained from the present study can be supported by the data obtained by Elumalai *et al.* (14) using *Chlorella vulgaris* and *Scendesmus obliquus* grown on textile wastewater. All forms of nitrogen were significantly reduced by both of the alga with highest reduction in dissolved solids by 61%. Lim *et al.*(13) achieved 33% of Phosphate removal by using *Chlorella vulgaris* from 100% of textile wastewater. Efficiency of alga for nutrient removal also varies with the type of wastewater and species selected for the remediation. *Chlorella pyrenoidosa* was reported to remove nitrate and phosphate from dairy wastewater by 60% and 87% (19) whereas a removal of 90% of nitrate and 70% of phosphate from the dairy wastewater is reported by using *Chlamydomonas polypyrenoideum*(20).

The dye removal using alga may be attributed to the accumulation of dye ions on the surface of algal biopolymers and further to the diffusion of dye molecule from aqueous phase on to the solid phase of the biopolymer (21). The biodegradation of azo dyes by the algae (*Chlorella pyrenoidosa, Chlorella vulgaris* and *Oscillatoriatenuis*) has also been assessed and it was observed that azoreductase present in such algal species is responsible for the degradation. Application of dried biomass of alga *Spirogyra rhizopus*was observed by Ozer*et al.*(22) for removal of acid Red 274 dye. They achieved higher rate of adsorption with increased dose of biosorbent. Green alga of *Cosmarium Sp.* was also reported as a viable biological material for treatment of Triphenylmethane dye and Malachite green (23).

The treatment of textile effluent mainly involves physical and chemical method which are often less efficient (24). In this context alga as a biological agent for the treatment of textile wastewater have good potential and economic, effective adsorbent and can be effectively use for the remediation of textile wastewater. It was examined in present study that the growth of algae on 75% concentration of textile was satisfactory and partially fulfills the requirement for the growth.

The alga and algal -bacterial consortium not only removes pollutants from the wastewater but both also reduce the simulated methylene blue dye.Although wastewater grown algae may not be suitable for use as animal feed due to the presence of toxic dyes and heavy metals but there has been increased interest in using such algal biomass for biodiesel production could be a solution for clean energy production. In present study it is concluded that unautoclaved textile wastewater with algal inoculum was found more efficient to remove dissolved soilds and organic pollutant load in terms of BOD in comparison to control. Whereas autoclaved wastewater having algal inoculum shown its efficiency for better nutrient removal and organic load in compare to all other treatments.

Other articles in this theme issue include references (25-40).

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