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ORIGINAL ARTICLE

Lab-scale degradation of leather industry effluent and its reduction by *Chlorella* sp. SRD3 and *Oscillatoria* sp. SRD2: a bioremediation approach

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Abstract
The present study focuses on treatment of tannery effluent samples using microalgae and isolated cyanobacteria. Different concentrations of both the effluent samples were treated with the algal isolates and a highest biomass of 0.295 g/l was attained in 50% of concentration on the 12th day in BHEL site. The biomass production in Walajapet site was found to be 0.387 g/l in the effluent treated with 50% of algae. The treated effluent was tested for growth and chlorophyll content was estimated, *Chlorella* sp. SRD3 showed higher chlorophyll content. The 50% treatment was processed at large-scale treatment in lab and biomass yield of 0.65 ± 0.04 g/l was achieved in BHEL site and 0.49 ± 0.028 g/l in Walajapet site. The treatment led to higher reduction rates in BOD and COD levels in treated effluents. The BOD in effluent from BHEL site was reduced to 83.41% and that from Walajapet site showed 87.46% reduction, whereas the COD values also showed 78% reduction. Based on the results, effluent sample serves as a medium for growth and bioremediation of tannery effluents by microalga, *Chlorella* sp. SRD3 revealed promising results which may prove efficient in the near future.

Keywords *Chlorella* sp. · Phycoremediation · Tannery · Fluorescent · Chlorophyll

Introduction

Microalgae serve as a better source for wastewater treatment as they are capable in utilizing the minerals by converting them to biomass (Amenorfenyo et al. 2019). Nutrients were consumed within the microbes for growth leading to a reduction in the minerals and the BOD, COD levels. Wastewater is considered to be discharged from industrial and domestic effluents consisting toxic suspended solid particles (Sara et al. 2012). When these toxic particles are released into waste systems, it leads to aquatic threat in the environment (Abdel-Raouf et al. 2012). The treatment by microalgae leads to nutrients removal, heavy metal ions and pathogens. It leads to reduction in BOD levels and production of oxygen by photosynthetic reaction in a variety of toxicant removal and biogas production techniques. The nutrients and water uptake by microalgae does not happen in a particular system as they are dependent on larger surface area for utilization of toxic minerals. Heavy metal toxicity in the environment is a major concern, because it affects the natural flora and fauna; hence the wastewater and sewage should be treated (Adefila et al. 2010). In the upcoming years, heavy metals are becoming a sole factor in polluting the environment (Sayin et al. 2011). Toxic metal pollutants such as chromium, lead and cadmium are abundantly present in the leather industry wastes (Karthi and Meenakshi 2014; Balaji et al. 2016). The wastewater treatment is a cost-intensive physicochemical means and cannot be employed in industries of most of the emergent countries. Hence, the importance of bio-based treatment systems is spreading all across the world and is becoming an efficient, cost effective system that attracted among many people (Vijayakumar and Manoharan 2012). As per the previous reports, cyanobacteria *Oscillatoria* (Vijayakumar et al. 2005), *Spirulina platensis* (Boominathan and Manoharan 2008) and *Westiellopsis* (Vijayakumar et al. 2005) are successfully used for the heavy metals removal from wastewater Wang et al. (2010) revealed in their study

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that algae can ingest the organic pollutants into cell constituents thereby reduction of pollutants an eco-friendly means. Leather industries exist from a longer time and environmental pollution has become a major problem in the recent decade (Dhal et al. 2013). The present study involves the algae-based treatment of tannery effluent collected from two sites namely BHEL and Walajapet area in Tamilnadu, and treating them with fresh-water microalgae and cyanobacteria. Treatment of tannery effluent with these isolates showed that *Chlorella* sp. SRD3 was more efficient in degrading the toxic metals present. The growth was monitored in terms of biomass and chlorophyll content. While the physiochemical parameters were analyzed both before and after treatment to observe the extent of degradation. Carbohydrate and protein content was tested during the large-scale treatment in the lab conditions. The metals analyzed in the effluents had Chromium and magnesium content present, that were degraded from 60 to 80% in both the sites.

Materials and methods

Culture collection

The microalgae *Chlorella* sp. SRD3 (NCBI Gene bank, accession number-MH879820) were obtained from department of microbiology and cyanobacterial strain was isolated from Mettur dam, Salem, Tamilnadu. The collected microalgae species were identified based on the monograph of Desikachary (1959); molecular identification of the cyanobacteria is done by 16 s rRNA sequencing. The cyanobacterium was grown in BG 11 medium and microalga in bold basal medium (BBM) kept at 28 °C for 16 h light: 8 h dark conditions under fluorescent light. Both the isolates were observed morphologically under compound microscope at 400× magnification.

Collection of tannery effluent

The raw leather industry effluents were collected from two different places in Vellore district in Tamil Nadu namely BHEL (Bharat Heavy Electronics Limited) (12.9321° N, 79.3335° E), and Walajapet (12.9250° N, 79.3669° E).

Morphological characterization of cyanobacteria

The genomic DNA was extracted from isolated cyanobacteria by CTAB method followed by Singh et al. (2011). The DNA was amplified by polymerase chain reaction using forward and reverse primers CYAN738F 5′ATACCCCWG TAGTCCTAGC3′ and CYAN1281R-5′GCAATTACTAGC GATTCCTCC3′, respectively. The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei 1987) as implemented using MEGA 6.06 software.

Screening of different effluent concentration

The collected effluent samples were subjected to different concentration of algae that were initially screened for adaptability performed in test tubes. Concentrations used in the present study for effluent treatment are 50%, 60%, 70%, 80%, 90%, and 100%. To attain 50% concentration (5.0 ml of effluent sample + 5.0 ml of algae), 60% concentration (6.0 ml of effluent sample + 4.0 ml of algae), 70% (7.0 ml of effluent sample + 3.0 ml of algae), 80% concentration (8.0 ml of effluent + 2.0 ml of microalgae), 90% concentration (9.0 ml of effluent + 1.0 ml of microalgae) were added.

Evaluation of the growth study

The algae samples were collected during the treatment process and were analyzed for dry weight at every 4 days interval. 2.0 ml of algal culture was collected and centrifuged at 10,000 rpm for 10 min. The suspension was removed, the pellet was air dried and its weight was noted.

Chlorophyll estimation

Total chlorophyll estimation of the algae was performed on varying concentration of effluent as well as during the large-scale cultivation. For this assay (Hansmann 1973), 5.0 mg dry biomass was suspended in an 8% (90% v/v acetone) for extraction. The tube was vortexed, ultrasonicated for 10 min and incubated for 48 h at 4 °C. After incubation, the extract was centrifuged at 8000 rpm for 10 min. The supernatant was collected and its absorbance was read in three wavelengths 665, 645 and 630 nm in Shimadzu UV–Vis spectrophotometer (UV-1800). The amount of chlorophyll present in the algae was calculated by the following equations (Tredici 1999; Molina Grima et al. 1999):

$$Ch_a = 11.6 \times OD_{665} - 1.31 \times OD_{645} - 0.14 \times OD_{630}$$
$$Ch_b = 20.7 \times OD_{645} - 4.34 \times OD_{665} - 4.42 \times OD_{630}$$
$$Ch_c = 55.0 \times OD_{630} - 4.64 \times OD_{665} - 16.3 \times OD_{645},$$

[Ch_a, Ch_b and Ch_c represent different types of chlorophyll (mg/l) and OD_{xxx} is the optical density read at specified wavelength] Upon initial screening of two algae for the treatment of effluent samples. The concentration that revealed higher biomass and chlorophyll content was selected for further treatment process. In 1000 ml treatment process, the biomass and chlorophyll content was analyzed in both the effluent samples.

Optimization of tannery effluent for growth

The effluent samples collected from two sites were subjected to different effluent concentration (50%, 60%, 70%, 80%, 90% and 100%). The (setup) treatment was shaken thrice a day to enable complete mixing of the medium with the cultures. The faster algal growth in the effluent will lead to better degradation efficiency of effluent. The growth was visualized with high intensity of green color. Further confirmation can be made using the biomass determination and UV-spectrophotometer readings at 600 nm.

Physiochemical parameters

The physiochemical parameters such as pH, total suspended solids (TSS), total solids (TS), total dissolved solids (TDS), chloride, sulphate, chromium, total hardness, calcium, magnesium, total alkalinity, iron content, phosphate, biological oxygen demand (BOD), chemical oxygen demand (COD) for the two effluent samples were analyzed both prior and after treatment. The degraded parameters in effluent will be determined after the treatment process (APHA 2005).

Large-scale treatment of effluent sample (1000 ml)

The screening for different concentration of the effluent showed high growth rate at 50% and this was repeated twice to get standard values. For pilot-scale treatment process, 50% of the effluent was used that was diluted using BG 11 and BBM broth to make up the volume to 1000 ml (1:1 ratio). During the treatment process, the cells were analyzed for biomass weight at every 4 days interval for a period of 16 days and the chlorophyll content was evaluated.

Biochemical analysis during treatment process

Protein estimation

For estimating the protein content in algae, Lowry et al. (1951) was used. Different concentrations of BSA (1 mg/ml) were prepared with water. The total volume in each test tube was 5.0 ml with standards ranging from 0.05 to 1.0 mg/ml. From these dilutions, 0.2 ml solution was taken and added into different test tubes followed by addition of 2.0 ml alkaline copper sulphate that was mixed gently. This solution was incubated for 10 min at room temperature in dark condition and 0.2 ml of Folin ciocalteau reagent was added to each tube and incubated for 30 min. The absorbance of standards and unknown samples were read at 660 nm.

Total carbohydrates

To 100 mg of algal biomass 8.0 ml of perchloric acid (20% w/w) was added, ultrasonicated to 10 min and kept for hydrolyzing (12 h). This hydrolysate was filtered and diluted to 250 ml with distilled water. 5.0 ml of anthrone reagent was added to 1.0 ml of the filtrate, this mixture was boiled to 100 °C for 10 min. This green-colored solution was cooled by ice bath and its optical density was read at 630 nm. A calibration curve was plotted for each experiment, using D + glucose dissolved in distilled water (Miron et al. 2002). The glucose concentration (Cg, mg/ml) and the optical density had the following relationship:

$$Cg = 0.536 \times OD_{630} + 0.0028.$$

Fluorescence microscopy to detect lipids

The fluorescence microscopy analysis of the microalgal cells were stained with 5 µl of 500 µg/ml of nile red stain (Sigma, USA) (stock). The cells were fixed with 5% paraformaldehyde and examined at 400 × magnification under Novex, epi-fluorescence microscopy (Yilancioglu et al. 2014).

Statistical analysis

All the experiments were performed in triplicates and their mean (±) standard deviation values were calculated using Microsoft excel 2007. The graphs were plotted using the Graph pad prism v.5.0 software. The standard values were plotted for the estimations performed in this study.

Results

Morphological observation and molecular identification

A drop of cyanobacteria and microalgae cultures was placed on a grease free slide and a cover slip was placed on it. This slide was observed under the compound microscope at 400 × magnification. On microscopic analysis, the organisms were found to be *Chlorella* sp. and *Oscillatoria* sp. as per key features (Fig. 1a, b). The genomic DNA isolated from the cyanobacteria was amplified by polymerase chain reaction and the length of the amplified product was 751 bp. The gene sequence of the isolated cyanobacteria was submitted to NCBI and the accession no. was found to be MH879820 (Fig. 2).

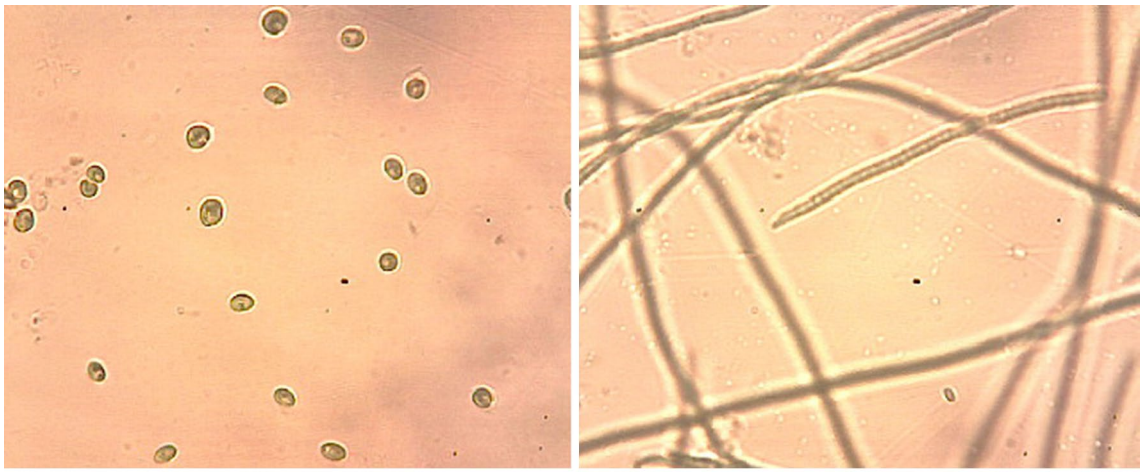


Fig. 1 Morphological appearance of **a** *Chlorella* sp. SRD3 and **b** *Oscillatoria* sp. SRD2

Scale up of test isolates culture

The cyanobacterial and microalgal culture were scaled up to 500 ml of BG11 and BBM in 1000 ml flask, respectively. Cultivated medium was scaled up regularly to provide nourishment to the algal cultures. After 20 days of growth, color change was observed for the intensity of green color (Fig. 3a, b).

Algal growth in effluent collected from BHEL

The treatment process was performed in 250 ml flasks in six different concentrations for 16 days and its biomass was recorded. The waste water collected from BHEL site was treated with both the algae at different concentrations and it was found that 50% concentration showed maximum growth in terms of biomass. During treatment, maximum biomass was obtained in *Chlorella* sp. SRD3 followed by *Oscillatoria* sp. used for the treatment of effluent sample collected from BHEL the growth was declined as the constituents present in the effluent could not support the growth of this cyanobacteria. The effluent sample treated with *Chlorella* sp. showed a highest biomass of 0.295 ± 0.021 g/l on 12th day of treatment as shown in Fig. 4a, b.

Algal growth in effluent collected from Walajapet

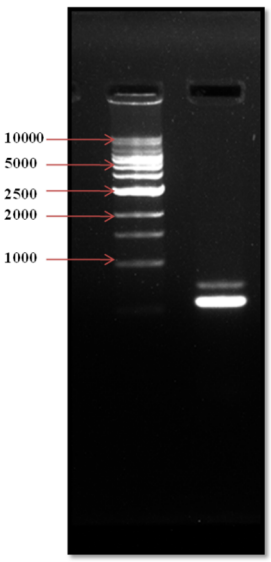
The effluent collected from Walajapet site was treated with both the cyanobacteria and microalgae at different concentrations and a maximum biomass was obtained in 50% effluent concentration. When *Chlorella* sp. was used at 50% concentration it was found that maximum biomass of 0.345 ± 0.049 g/l was present in this concentration, compared to the biomass obtained at other concentrations. The effluent water treated with *Oscillatoria* sp. showed low biomass at all concentrations proving that this cyanobacterium cannot remediate the effluent (Fig. 5a, b).

Chlorophyll estimation

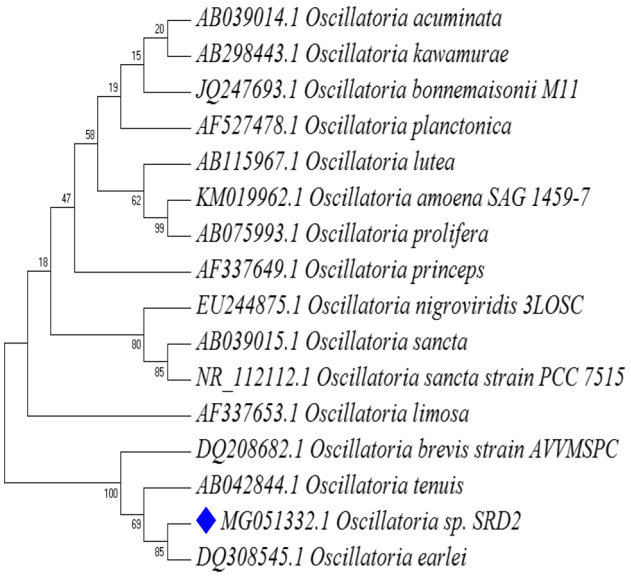
The chlorophyll estimation of both the treatment involving effluent collected from BHEL area and Walajapet area with the algal cultures to analyze the growth in the effluent samples. In the BHEL, effluent sample on the 12th and 16th day chlorophyll a and c content was high at 50% effluent concentration but chlorophyll b was high in 60% effluent concentration. Whereas in the Walajapet, sample on 12th day the chlorophyll a, b and c content was high in 50% compared to all other concentrations used, when the same readings were taken on the 16th day chlorophyll a and c content were higher in 50% concentration but chlorophyll c was higher in 60% concentration (Fig. 6a, b).

Large-scale treatment of effluent sample with algae (1000 ml)

The large-scale treatment of the two effluents collected from BHEL and Walajapet area were subjected to continuous aerator for providing CO_2 to enhance the growth of algae. Their growth was monitored periodically for 10 days and its biomass yield, chlorophyll and biochemical analysis was performed. An increased biomass and chlorophyll content reveals the adaptability of microalgae and cyanobacteria to tannery effluent. Based on the extent of degradation in the leather effluent treatment process, *Chlorella* sp. was found to be dominant in growth conditions and chlorophyll yield. Further studies involving large-scale treatment,



(a)



(b)

Fig. 2 **a** PCR amplified product of *Oscillatoria* sp. 16 s rRNA gene and **b** phylogenetic tree of the *Oscillatoria* sp.

physiochemical parameters, biochemical analysis will be done with the potent strain *Chlorella* sp.

Biomass production in large-scale treatment

The treatment was carried out for a period of 10 days and it was found that in the effluent sample collected from BHEL area the biomass weight was 0.432 g/l on 5th day which gradually increased to 0.65 ± 0.04 g/l on 10th day. The effluent sample collected from Walajapet area showed an increase in biomass weight from initial 5th day to the 10th day as 0.49 ± 0.028 g/l (Fig. 7).

Fig. 3 Cultivation of algae in broth **a** *Chlorella* sp. SRD3 and **b** *Oscillatoria* sp. SRD2

(a)

(b)

Chlorophyll estimation of potent isolate

The Chlorophyll estimation (Chlorophyll a, b and c) of *Chlorella* sp. obtained from treatment of both the effluent samples showed increased chlorophyll values on 10th day in both the treatment samples. On 5th day, chlorophyll a, b and c content in biomass treated with BHEL effluent showed chlorophyll content ranging from 4.4 to 5.8 mg/l and that of Walajapet site showed chlorophyll content in rage 3.6–4.3 mg/l. This content gradually increased to 6.2–7 mg/l and 7.2–8.1 mg/l in BHEL and Walajapet sites, respectively (Fig. 8a, b).

Physiochemical parameters

The physiochemical parameters of the effluent collected from two sites were analyzed both before and after treatment. The results showed drastic decrease in some parameters. The effluent collected from the two sites namely BHEL and Walajapet area showed a decline in TDS, total solids, Total suspended solids, total alkalinity, BOD, COD and total hardness levels. The metal concentrations such as chromium, calcium and magnesium values have also decreased, the other elements also have shown decrease in results pertaining to before treatment (Table 1a, b).

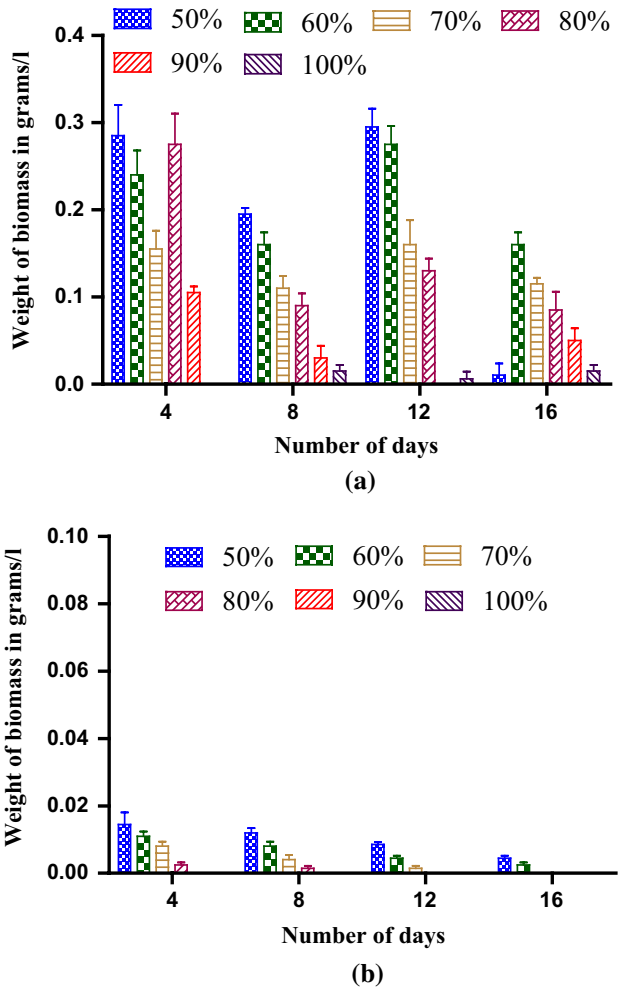


Fig. 4 Treatment of different effluent concentration of BHEL area using **a** *Chlorella* sp. SRD3 and **b** *Oscillatoria* sp. SRD2

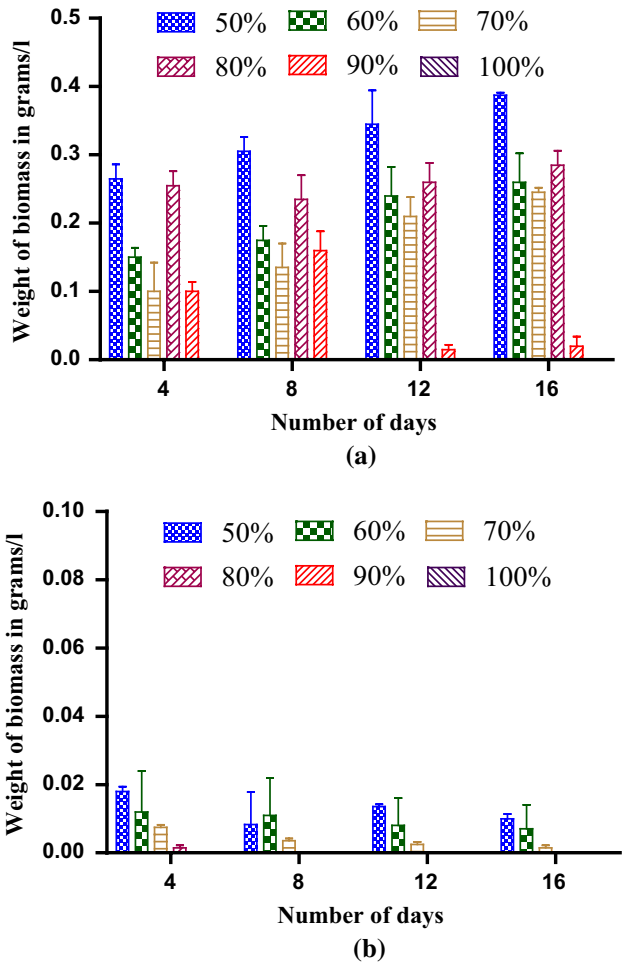


Fig. 5 Treatment of different tannery effluent concentration from Walajapet by **a** *Chlorella* sp. SRD3 and **b** *Oscillatoria* sp. SRD2

Protein and carbohydrate content

The intracellular protein content of the *Chlorella* sp. SRD3 was determined at varying stages of effluent treatment process. Initially, the protein content was low during the treatment process that kept increasing till the 10th day. On 10th day, the intracellular protein content was maximum in the *Chlorella* sp. SRD3 that reveals that during the treatment process intracellular proteins were synthesized that enables the degradation of the constituents in tannery effluent. The carbohydrate content also started to raise 410 µg/ml on the 6th day that gradually decreased to 200 µg/ml on 10th day (Fig. 9).

Lipids observed in fluorescent microscope

The intracellular lipids were observed under epi-fluorescence microscope and the cells with lipid content were visualized as yellow-colored cells. This normal view (Fig. 10a) of the microalgal cells when visualized in fluorescence mode showed lipid accumulated bodies (Fig. 10b).

Discussion

This current study on phycoremediation is on testing the efficacy of microalgae and cyanobacteria involved in reduction of toxic components contained in the collected tannery effluents. Initially, different concentrations of the algal inoculum were treated with tannery effluent to determine the degradation and growth survivability in terms of biomass.

Sharma and Khan (2013) worked on treatment of primarily wastewater treatment by microalgae named *Chlorella minutissima, Nostoc* sp. and *Scenedesmus* sp. It was observed that magnificent results were attained by reducing the physiochemical parameters of sewage wastewater by employing the three microalgae. Sengar et al. (2011) in

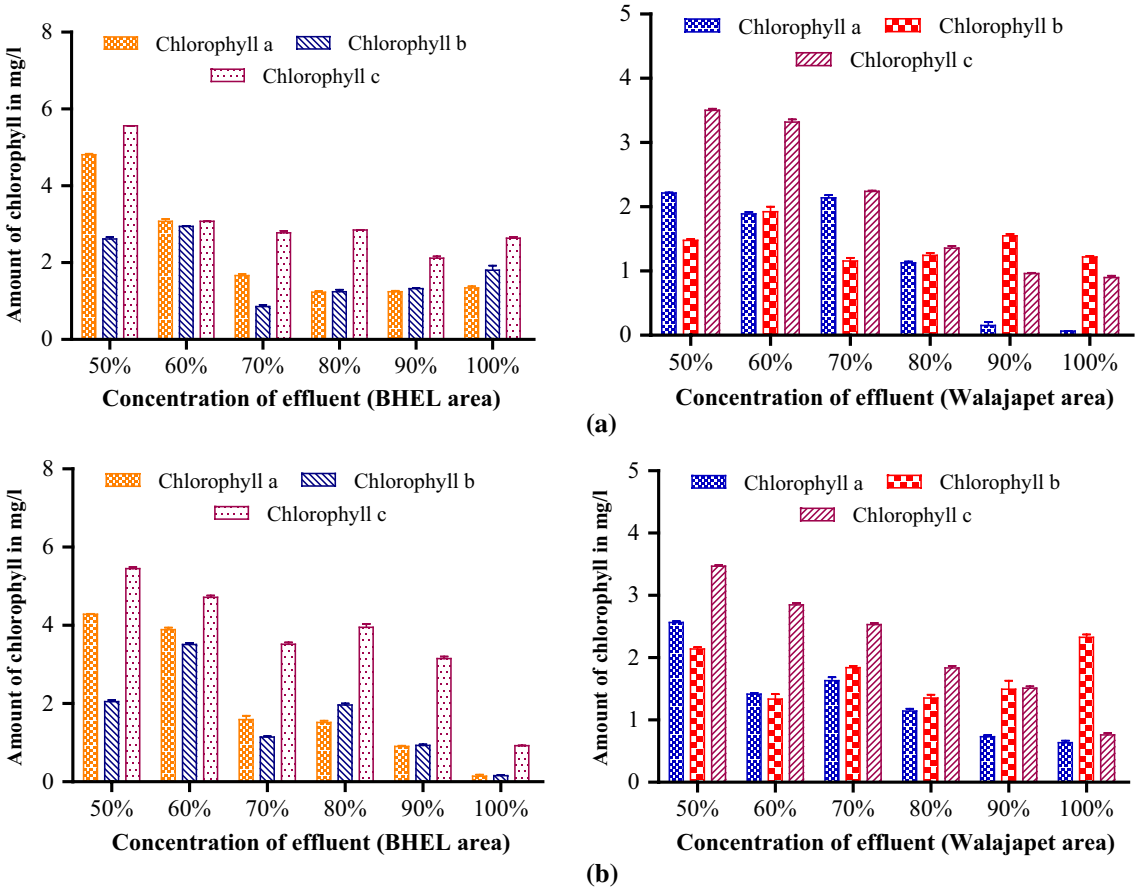


Fig. 6 Chlorophyll content of biomass treated effluent on **a** *Chlorella* sp. SRD3 and **b** *Oscillatoria* sp. SRD2

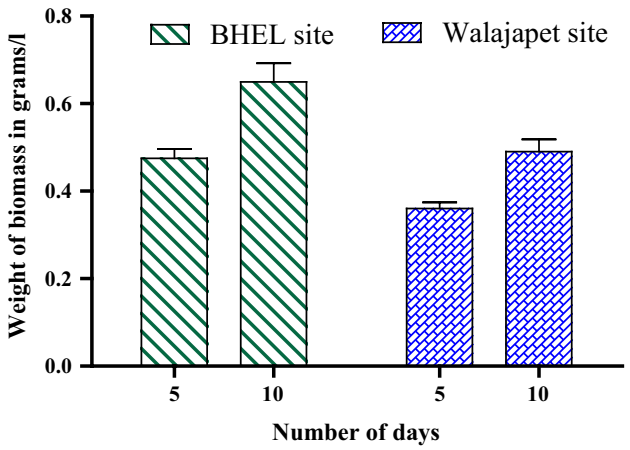


Fig. 7 Biomass of the potent strain during large-scale effluent treatment

his work revealed that algae may hinder by reducing the pH value of open drain wastewater from 8.1 to 7.1 and increase DO to about 87.5% on the 25th day of cultivation. Bioremediation of thermal waste water by *Pithosphora* sp. showed a better result in removal of physiochemical parameters from 32 to 92% (Murugesan and Dhamotharan 2009). Based on literature review, it is evident that microalgae are highly potential in degradation and remediation of wastewater. The microalgae *Botryococcus* sp. had prevailed in the urban wastewater treatment study carried out by Orpez et al. (2009) and in greywater as reported by Gokulan et al. (2013). Gani et al. (2015) employed phycoremediation for treatment of greywater and dairy wastewater using 1000 cells/ml as initial inoculum. Their results proved *Botryococcus* sp. to be effective in the reduction of physicochemical constituents up to 73.3% of BOD in dairy wastewater thereby removal of 88% for COD in greywater. The gap in the literature is realized; much more research is needed on *Botryococcus* sp. upon treatment of wastewater such as domestic and industrial. A major environmental threat to human health is a pollutant that is considered to be a heavy metal (Chekroun et al. 2013). In the present study, also the BOD and COD levels of the effluents was decreased from 76.13 and 83.4%, respectively, in BHEL effluent sample compared to the previous reports using *Chlorella* sp. SRD3 and *Oscillatoria* sp. SRD2. The effluent sample collected

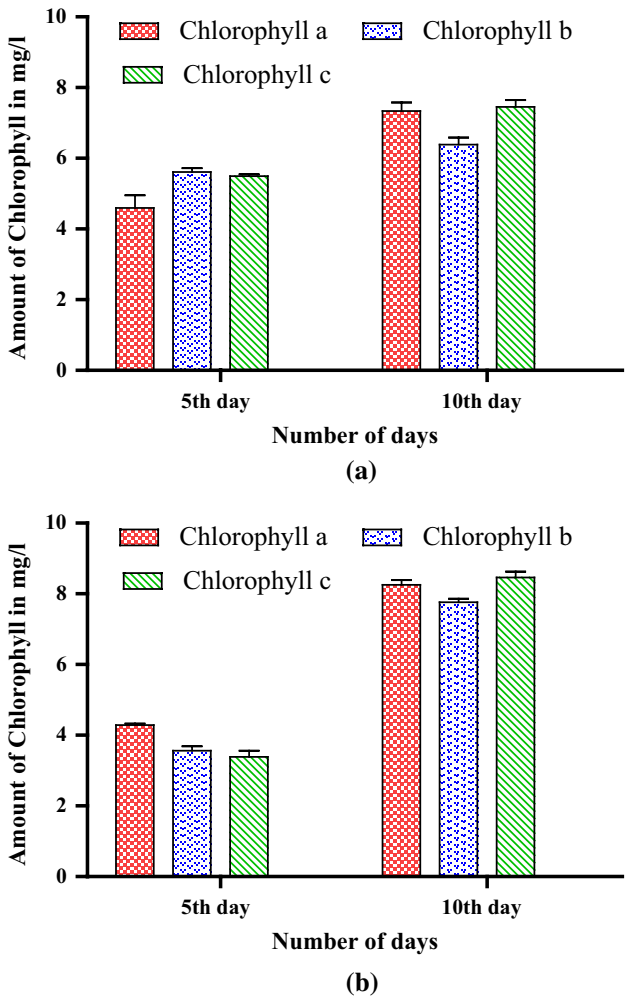


Fig. 8 Chlorophyll estimation of *Chlorella* sp. SRD3 treated effluent **a** BHEL area and **b** Walajapet area

from Walajapet site also the BOD and COD levels were found to be reduced to 83.41% and 76.13%, respectively, in the 10 days treated effluents.

The increased anthropogenic activity is caused due to water bodies getting contaminated by toxic metals and organic pollutants. Therefore phycoremediation is used to assimilate the toxic content present in wastewater. Bioremediation is a process by which specific microbes are transformed to hazardous contaminations in water to non-hazardous waste products (Dwivedi 2012). Dwivedi (2012) proclaimed on surface charge studies showed that availability of free sites depends on pH. As pH increases the surface charged sites of calcium alginate became more negative, thereby uptaking the metal by increasing the pH. Worku and Sahu (2014) took an effort in culturing *Synechocystis salina* in groundwater for reduction of heavy metals and total within 15 days treatment. After the treatment, *S. salina* was able to remove 60% of Cr, 66% of Fe, 70% of Ni, 77% of Hg, 65% of Ca^{2+}, and 78% of total hardness. However, Kumar et al. (2013) had tested the Zinc removal by immobilized and powdered *Chlorella marina* thereby highest removal of 97% compared to immobilized with 55.3% removal. The optimum pH required for the adsorption of heavy metals by algae is 8. In phycoremediation of industrial wastewater (Soeprobowati and Hariyati 2013), *Porphyridium cruentum* isolated from brackish water was employed for assimilating lead, cadmium, copper and chromium. For the above experiment, pH (7–8), temperature (28–32 °C), salinity (32–34 ppt) and light (4200 lx) were maintained. In our study, also the degradation of metals was observed in Calcium as 82.12%, magnesium 82.51%, iron content 60% and chromium as 61.90% from the test sample and control. The same parameters were analyzed for the Walajapet effluent samples and it was found that there was a degradation in the following metals by *Oscillatoria* sp., as Calcium as 77.22%, magnesium 77.69%, iron content 40% and chromium as 61.11% compared to the before treatment analysis.

The wastewater treatment by microalgal bacterial flocs in their study showed significant results in removal of turbidity, BOD, TCOD, TOC, TC, TN and TP are 96%, 87%, 80%, 71%, 48%, 58% and 8%, respectively. Their study also proved the alkaline pH nature of the final effluent and the DO was 6.06 mg/l (Van Den Hende et al. 2014). Azarpira et al. (2014) compared two cyanobacterial strains namely *Oscillatoria limosa* and *Nostoc commune* for the removal of nutrients using polluted river water. Their results gave average reduction efficiency of between 84 and 98%. In their study, the cynobacteria, *Oscillatoria limosa* was proven to be better than *Nostoc commune*. In the present study, microalgae and cyanobacteria namely *Oscillatoria* sp. and *Chlorella* sp. were used in the treatment of tannery effluent collected from BHEL and Walajapet area in the vicinity of vellore district. These two effluents were treated with both the cyanobacteria in different concentrations ranging from 50 to 100% and it was found that in both the sites, only one cyanobacteria was able to grow and produce biomass at higher rates compared to all different concentrations used. The chlorophyll estimation of both the cyanobacteria which showed a better biomass weight were performed and it was found that in both the sites the chlorophyll content was higher in cyanobacteria grown in 50% concentration of the effluent.

The intracellular protein and carbohydrate was reduced due to nutrient deprivation that led to the higher lipid accumulation in the *Chlorella* sp., which is observed in nile red staining observed in fluorescent microscope. As per previous reports during stress condition, the protein content tends to decrease thereby increasing the lipid level which is similar to the present study (Pushpakumari Kudahettige et al. 2018).

Table 1 Physiochemical analysis of the (a) effluent sample (BHEL area) and (b) effluent sample (Walajapet area) before and after treatment

S. no	Parameters	Experimental value		Percentage of removal
		Before treatment	After treatment	
(a)				
1	pH	9.27	9.50	–
2	Total dissolved solids	5849 ppm	1960 ppm	66.48
3	Total solids	5910 ppm	1988 ppm	66.36
4	Total suspended solids	54 ppm	14 ppm	74.07
5	Chloride Cl^-	3190 ppm	1389 ppm	56.45
6	Sulphate SO_4^{2-}	1423 ppm	398 ppm	72.03
7	Chromium Cr^{3-}	21 ppm	8 ppm	61.90
8	Total hardness $CaCO_3$	1180 ppm	170 ppm	85.59
9	Calcium Ca	179 ppm	32 ppm	82.12
10	Magnesium Mg	143 ppm	25 ppm	82.51
11	Total alkalinity CaCO3	623 ppm	512 ppm	17.81
12	Iron content	0.05 ppm	0.02 ppm	60
13	Phosphate	Traces	Traces	Traces
14	Chemical oxygen demand	4269 ppm	1019 ppm	76.13
15	Biological oxygen demand	398 ppm	66 ppm	83.41
(b)				
1	PH	9.44	9.31	–
2	Total dissolved solids	3075 ppm	1219 ppm	60.35
3	Total solids	3124 ppm	1228 ppm	60.69
4	Total suspended solids	45 ppm	6 ppm	86.66
5	Chloride Cl^-	1276 ppm	771 ppm	39.57
6	Sulphate SO_4^{2-}	598 ppm	269 ppm	55.01
7	Chromium Cr^{3-}	18 ppm	7 ppm	61.11
8	Total hardness $CaCO_3$	1150 ppm	180 ppm	84.34
9	Calcium Ca	180 ppm	41 ppm	77.22
10	Magnesium Mg	130 ppm	29 ppm	77.69
11	Total alkalinity CaCO3	644 ppm	507 ppm	21.27
12	Iron content	0.05 ppm	0.03 ppm	40
13	Phosphate	Traces	Traces	Traces
14	Chemical oxygen demand	4128 ppm	912 ppm	77.90
15	Biological oxygen demand	351 ppm	44 ppm	87.46

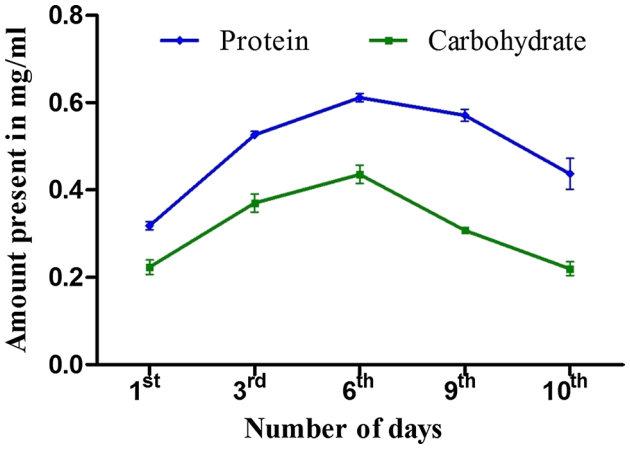
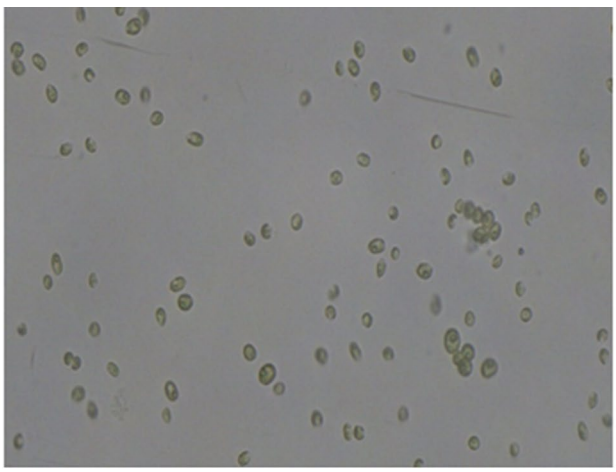
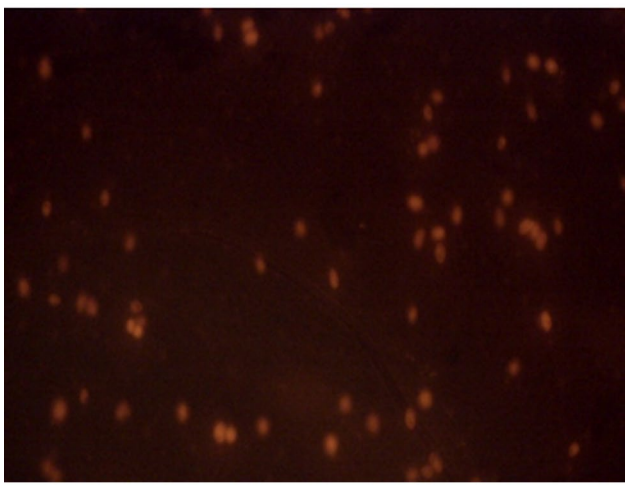


Fig. 9 Changes in the protein and carbohydrate content of *Chlorella* sp. SRD3 during effluent treatment



(a)



(b)

Fig. 10 Morphological appearance of *Chlorella* sp. on fluorescent microscope—nile red staining **a** white light and **b** UV light

Conclusion

Tannery effluent waste was initially treated by microalgae and cyanobacteria at 50% concentration that procures its maximum growth. The chlorophyll was estimated initially as well as during lab scale treatment at larger quantity and physiochemical parameters showed higher reduction rates. As *Chlorella* sp. was able to degrade the effluents efficiently, it may be employed as a natural source for bioremediation in leather industries before discharging the effluent into environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interests to disclose.

Ethical approval This article does not contain any studies with human participants or animals, performed by the authors.

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