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Phycoremediation of Tannery Dye Wastewater using Green Microalga: Bioremediation of Tannery Dye Wastewater

Shamshath Begum[1]*, Vedaraman N[2], Srinivasan SV[3], Rengasamy R[4], Kurinji Malar[5], Kavitha G[6], Vijayarani S[7]

1.Department of Polymer Engineering, B.S.Abdur Rahman University, Vandalur, Chennai, India; Email: msshamu@gmail.com
2.Chemical Engineering Division, Central Leather Research Institute (CLRI), Adyar, Chennai, India
3.Environmental Technology Division, Central Leather Research Institute (CLRI), Adyar, Chennai, India
4.Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India
5.Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India
6.Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India
7.Department of Polymer Engineering, B.S.Abdur Rahman University, Vandalur, Chennai, India

***Corresponding author:** Department of Polymer Engineering, B.S.Abdur Rahman University, Vandalur, Chennai, India; Email: msshamu@gmail.com

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ABSTRACT

The present study is on the treatment of pretreated tannery dye wastewater (PTDW) by green microalga *Chlorella vulgaris*. The growth of *Chlorella vulgaris* in PTDW was assessed with the pigment content Chlorophyll *a* and Chlorophyll *b*. Maximum content of

pigment Chlorophyll *a* and Chlorophyll *b* was found to be 6.5 µg/mL and 3.3 µg/mL respectively on 12th day. Colour, chemical oxygen demand (COD) and biological oxygen demand (BOD) removal efficiency was found to be 99 %, 88.5 % and 90.17 % respectively on the 3rd day of alga treatment. The confirmation of presence of functional groups based on the optimal growth period of *Chlorella vulgaris* was carried out using Fourier Transform Infrared Spectroscopy (FTIR). The morphology of *Chlorella vulgaris* was also examined by scanning electron microscope (SEM). The present investigation shows that colour, COD and BOD can be removed effectively and efficiently by microalga *Chlorella vulgaris*.

Key words - Tannery Dyes, *Chlorella vulgaris*, Microalgae, Tannery Wastewater, BOD, COD and Colour removal.

1. INTRODUCTION

The leather industry holds an important position in the Indian economy and is one of the top ten foreign exchange earners of our country. This division maintains constant profit in export earnings. In one hand it adds reputation to our country and on the other hand process wastewater generated from the tannery units pose serious environmental problems. In India, there are more than 1500 tanneries which processes 0.7 million tones of wet salted hides and skins per year. Each ton of the leather processing approximately generates 30-50 m^3 of wastewater [1].

The wastewater generated from the tanneries during the different types of operation and processes are increasing and which in turn demands the appropriate treatment. This leads to the development of system which can treat wastewater and meet the standards imposed by the pollution control boards. The general treatment processes adopted in any industry are primary, secondary and tertiary methods. Secondary processes are biological processes and may be further classified into two main categories i.e., aerobic and anaerobic processes. In both the processes, organic content like COD and BOD present in the wastewater will be oxidized by the microorganisms and photosynthesis of microalgae. In aerobic processes the most commonly adopted system in the tannery premises is activated sludge process (ASP), which requires continuous supply of energy for aeration which converts the organic carbonaceous matter to carbon dioxide (CO_2) with the microorganisms present in the system. In case of algae treatment, algae take organic pollutants from the wastewater and convert it into lipid and carbohydrates. This type of biological treatment (bioremediation) is less expensive than the other methods to treat the high organic content.

Microalgae are photosynthetic organisms which normally present in marine and fresh water systems. In one hand it serves as nutritional source for human kind and animal feed as well [2,3]. On the other hand, microalgae wastewater treatment is appreciably attractive because of its photosynthetic ability to trap the solar's energy into useful biomass in taking the nutrients in the wastewater in the form of nitrogen, phosphorous, heavy metals, COD and BOD [4,5]. The most important unique characteristic of microalgae is converting the toxic wastewater to non-toxic one which may be safely discharged in the inland surface water bodies [6].

Of many microalgae species, C*hlorella* species is one of the indicators of water pollution and the most tolerant genera which can sustain in the polluted wastewater [7]. Recent studies shows that microalgae biological wastewater treatment is the promising one and this has been extensively studied by many researchers [8,9]. The growth of *Chlorella vulgaris* on the recalcitrant wastewater was found to be increased from 5 x 10^5 to 2 x 10^6 cells/mL and reduction of ammonium ion, phosphorous and COD to 71.6 %, 28 % and 61 % respectively [10]. Increasing *Chlorella vulgaris* from 1 g/L to 10 g/L caused an increase in the removal rate of BOD from 80.41 % to 82.92 %; COD from 78.33 % to 82.30 % [11]. COD, BOD, nitrate and phosphate removal of wastewater were found to be 80.64%, 70.91%, 78.08% and 62.73%, respectively using *Chlorella vulgaris* upto 15 days [12].

Pigments are present in the plants and other autotrophs especially for the transportation of sunlight's energy into chemical energy which is nothing but the process of photosynthesis. In general the content of pigment present in microalgae can be used to evaluate the measure of cell growth indirectly [13]. Pigments are of mainly classified as primary and accessory types. Chlorophyll *a* comes under the category of primary, whereas Chlorophyll *b* and carotenoids fall in the accessory pigments. Photosynthesis in the microalgae is achieved by the pigment Chlorophyll *a* thus by transferring the electrons which is present around the porphyrin ring. Carotenoids transfer their stored energy to the Chlorophyll and does not involve in the photosynthetic reaction directly.

The effective and efficient treatment of PTDW using microalgae demands good growth of microalgae and understanding the various influential factors as well. Integrated methods of treating the wastewater is gaining interest currently like oxidation processes combined with biological treatment. This present study focused on the treatment of PTDW by ozonation process further by biological treatment i.e., phycoremediation using microalgae *Chlorella vulgaris*. *Chlorella vulgaris* was chosen for the present research investigation because of its fast and rapid growth in simple and cheap low cost medium Pigment content, colour, BOD and COD removal with respect to the growth of the *Chlorella vulgaris* was studied.

2. MATERIALS AND METHODS

The culture of microalga was obtained from Centre for Advanced studies in Botany, University of Madras, Chennai 25. Experiments were carried out in triplicate in 1 L erlenmeyer flasks and their growth was checked.

Chlorella vulgaris was grown in modified bold basal medium (BBM) [14]. Algae growth chamber were designed with horizontal racks at 30 µmol m^{-2} s^{-1} light intensity, 12/12 light/dark photo period. The growth chamber was maintained at 25 ± 1 °C. To prevent the cells from clumping, the samples along with the cultures taken for the study were shaken intermittently during the study period.

5 ml of the samples were withdrawn from the culture in the laminar UV chamber carefully for the stipulated period of time to measure the concentration of the photosynthetic pigment viz., Chlorophyll *a* and Chlorophyll *b* [15]. The respective sample was read at maximum absorption wavelength (λ_{max}) of 661.6 nm and 644.8 nm for pigment Chlorophyll *a* and Chlorophyll *b* respectively in UV-Visible spectrophotometer (Hitachi U-1900, Japan). The pigment concentration in terms of µg/mL was calculated by using the formula given in equation 1 and 2.

$$Chlorophyll\ a\ \left(\frac{\mu g}{mL}\right) = 11.24\ X\ A661.6 - 2.404\ X\ A\ 644.8 \rightarrow (1)$$

$$Chlorophyll\ b\ \left(\frac{\mu g}{mL}\right) = 20.13\ X\ A\ 644.8 - 4.19\ X\ A\ 661.6 \rightarrow (2)$$

COD and BOD were estimated according to the standards [16]. COD, BOD and colour removal was calculated by using the equation 3.

$$\text{Removal of colour or COD or BOD (\%)} = \left(\frac{(\text{Colour or COD or BODconcentration})_{\text{in}} - (\text{Colour or COD or BODconcentration})_{\text{out}}}{(\text{Colour or COD or BOD concentration})_{\text{in}}}\right) * (100) \rightarrow (3)$$

FTIR spectra of the alga grown in PTDW was collected at the optimum conditions and was analysed in the range of 400 - 4000 cm^{-1} using JASCO 6300 model (Japan). SEM digital images were recorded using JEOL JSM-6390 (Japan).

3. RESULTS AND DISCUSSIONS

The content of Chlorophyll *a*, Chlorophyll *b* indicates the growth of *Chlorella vulgaris*. Since these two pigments play a vital role in confirming the growth of *Chlorella vulgaris*, the quantitative analysis of Chlorophyll *a* and Chlorophyll *b* content for the study period was assessed.

Chlorophyll *a* is used generally to estimate the algal biomass. Chlorophyll *a* was found to be increased with the increase in the number of days with respect to the control (BBM). Maximum amount of Chlorophyll *a* was found to be 6.5 µg/mL on the 12th day. Growth was found till 12th day and after 12th day the growth rate was found to be almost constant indicating the growth reaches the stationary phase. This is shown Fig. 1.

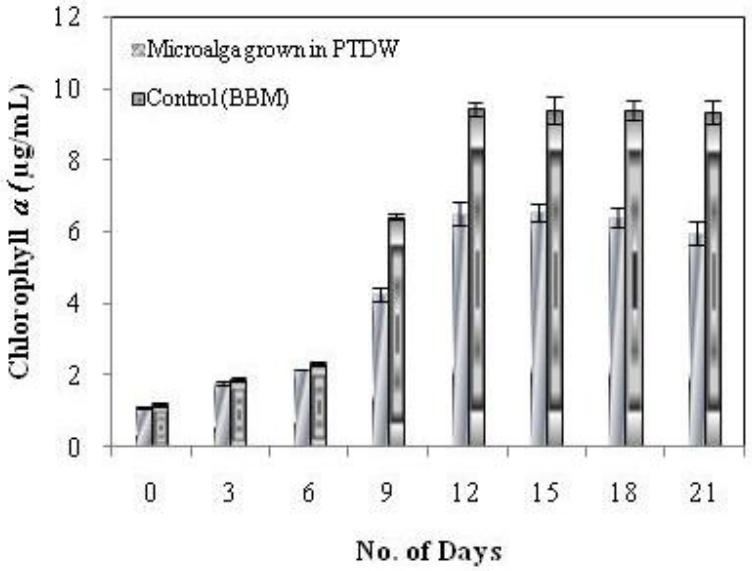

Figure 1
Chlorophyll *a* content (µg/mL) of the microalga grown in PTDW at different intervals

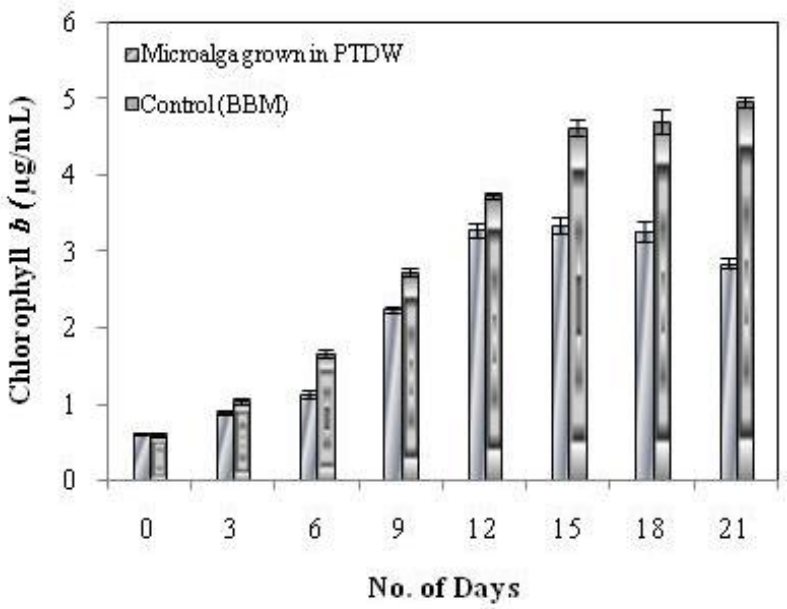

Figure 2
Chlorophyll *b* content (µg/mL) of the microalga grown in the PTDW at different intervals

Photosynthetic reaction in the pigment chlorophyll *a* happens directly. On the other hand, photosynthetic reaction also happens indirectly in the pigment chlorophyll *b*. Maximum amount of Chlorophyll *b* was found to be 3.3 µg/mL, on 12th day. In general there will be maximum chlorophyll content at the end of the exponential phase of growth. After 12th day the chlorophyll *b* content was found to be constant shows the end and the beginning of exponential and the stationary phase respectively. This is shown Fig. 2.

Yield of pigments production Chlorophyll *a* in mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortum* microalgae tested in culture media Nitrofoska, Poliverdol and Guillard was found to be 0.66 µg/mL (24 days), 2.21 µg/mL (18 days) and 3.07 µg/mL (18 days) respectively [17]. Yield of pigments production chlorophyll *b* in mixed culture of *Chlorella vulgaris* and *Hyaloraphidium contortum* microalgae tested in culture media Nitrofoska, Poliverdol and Guillard was found to be 0.35 µg/mL (24 days), 0.73 µg/mL (18 days) and 1.10 µg/mL (18 days) respectively [17]. In the present study, Chlorophyll *a* and Chlorophyll *b* content of the PTDW was found to be more than the study stated above.

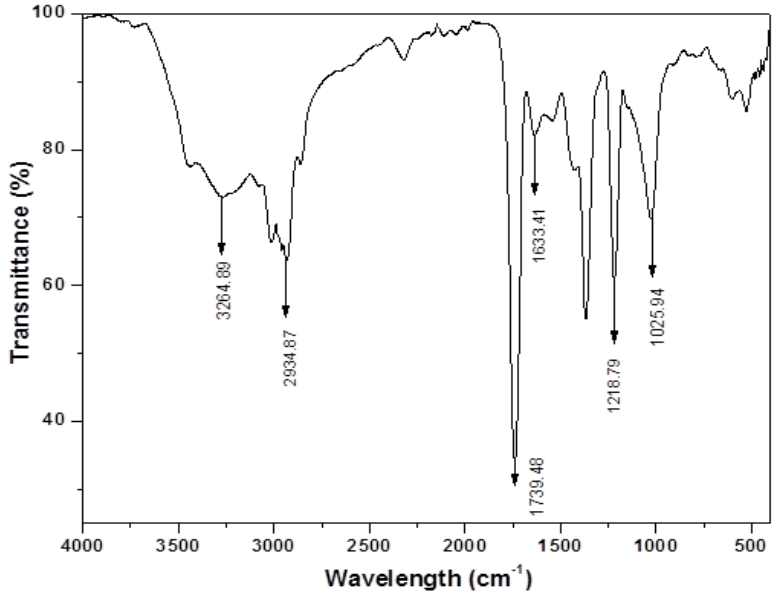

Figure 3
FTIR Spectrum of microalga grown in PTDW (12th day)

The increase in the both pigment shows the growth of the microalga in the PTDW efficiently. Invariably it justifies the increase in cell concentration. Literature shows that variation of chlorophyll concentration follows the same pattern as the growth of cells [18].

The pollutant concentrations were usually measured in terms of COD, BOD and additionally aesthetic view, i.e., colour. Colour was developed slightly in PTDW samples because of the adjustment of pH to 7-7.5 which is the favorable pH for the growth of *Chlorella vulgaris*. It was found that colour developed because of the change in pH were also removed completely i.e 99.99 %. COD and BOD removal was found to be 88.5 % and 90.17 % respectively on the 3rd day of microalga treatment.

FTIR of the microalga samples is useful in identifying the presence of functional groups of proteins, carbohydrates, lipids, polysaccharides and nucleic acids. The functional groups in general are C-O-C, $-CH_2$, $-CH_3$, N-H, C = O, -O-C, = C-H and O-H. The presence of functional groups in alga grown in PTDW was confirmed with the FTIR spectra and is shown in Fig. 3.

The presence of peaks at 3264.89 cm^{-1} represents the function O-H and N-H stretching. The peaks 2934.87 cm^{-1} shows the presence of lipid and carbohydrate. The peak 1633.41 cm^{-1} represents the protein amide I functional group of the alga grown in PTDW. The presence of nucleic acids generally lies in the band 1191-1356 cm^{-1}. The peak 1218.79 cm^{-1}, shows the presence of nucleic acids. Figure 3 exhibits adsorption bands at 1025.94 cm^{-1} shows the presence of C-O-C group. The same has been observed in the earlier studies [19]. The functional groups present in the control were found in the microalga grown in the PTDW which shows and confirms the growth of *Chlorella vulgaris* in terms of its biochemical composition (protein, carbohydrates and lipids).

The morphology of the microalga grown in the PTDW and in the control (BBM) was studied using SEM. The SEM image of the alga grown in PTDW shows the similar morphology of *Chlorella vulgaris* with respect to the control. This is shown in Fig. 4.

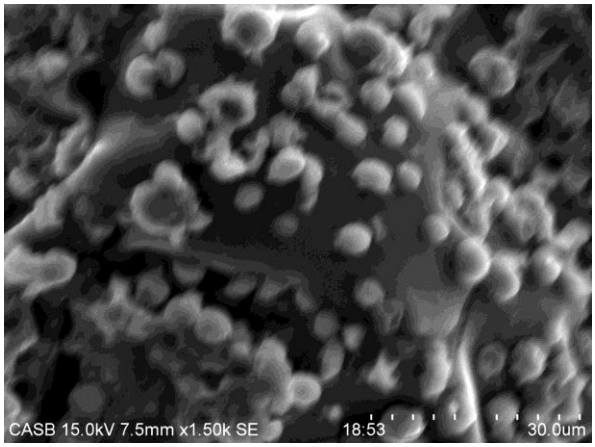

Figure 4
Scanning electron microscopy images of *Chlorella vulgaris* grown in PTDW

The present investigation shows the maximum pigment content of 6.5 µg/mL and 3.3 µg/mL of Chlorophyll *a* and Chlorophyll *b* respectively on 12th day. It was observed that there is no significant change in the pigment content after 12th day. Colour, COD and BOD removal efficiency was found to be 99.99 %, 88.5 % and 90.17 % respectively on the 3rd day of alga treatment. FTIR analysis confirms the presence of main functional groups (protein, carbohydrate and lipid) based on the optimal growth of *Chlorella vulgaris*. The morphology of *Chlorella vulgaris* was also examined by SEM. The present study shows that colour, BOD and COD can be removed effectively and efficiently by microalga *Chlorella vulgaris*.

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