



Biodegradation of Coloured Textile Industrial Wastewater using *Chlorella vulgaris*

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The main objective of this study is to reduce the pollutants by cultivating *Chlorella vulgaris* microalga in textile industrial wastewater that leads to remove up more than 80 % colour. This microorganism has the ability to reduce COD, total solids as pollutants to 55 % and remove TOC (61.98 %), NO₂-N (76.47 %), NO₃-N (92.59 %), NH₄-N (63.88 %) and PO₄-P (58.69 %). The present study showed the acceptable combination of biomass productivity content in indoor conditions by biodegradation of textile industrial wastewater as a culture medium.

Keywords: Microalga, *Chlorella vulgaris*, Biodegradation, Textile wastewater, Dye.

INTRODUCTION

Many industries such as textile, plastics, tile, enamel, paper, dyestuffs, etc. consume considerable volume of water and also they use chemicals throughout producing and dye to colour their products. Therefore, it created a substantial amount of contaminated wastewater. Their toxic effluents are a major source of aquatic pollution and will cause considerable damage to the receiving waters if discharged untreated [1,2]. The pollution is categorized by biological oxygen demand (BOD₅), chemical oxygen demand (COD), total solids (TS), high concentration of nutrients such as total organic carbon (TOC), NO₂-N, NO₃-N, NH₄-N and PO₄-P, especially colour and bad odour. Colour is the first contaminant to be identified in wastewater and the presence of very small amounts of dyes in water is highly visible and unpleasant [3]. Wastewater from dyeing and finishing processes in the textile industry comprises of a substantial source of pollution which show intense colour, high COD, low pH and suspended particles [4]. Indeed, textile industries utilize about 10000 pigments or dyes, but most of them are toxic substances to human and aquatic life [3,5] and it has been reported that upto 15 % of used dyes are released into wastewaters without any treatment [6]. The treatment of textile effluent involves mainly physical and chemical methods, which are often very costly [7]. Treatment of dye wastewaters by chemical and physical processes is hard due to the fact that the molecular structures of the colouring materials are complex. Physico-chemical methods include adsorption (e.g. on active carbon), coagulation flocculation (using inorganic salts or polymers)

[3], membranes (nanofiltration, reverse osmosis, etc.) [4], chemical oxidation (chlorination, ozonization, etc.), photodegradation (UV/H₂O₂, UV/TiO₂, etc.) [8,9] and novel electrochemical methods [10-12].

Conventional methods for removing dyes from industrial wastewater mainly consist of biological, physico-chemical treatments and/or their various combinations [9]. Biological methods for remediation of textile industrial wastewater often used particularly in colour removal [9]. Textile industrial wastewater is a mixture of colourants (dyes and pigments) and various organic compounds used as cleaning solvents and consist of high COD and BOD₅. Most studies [13-15] have concentrated on the use of fungi and bacteria to treat coloured wastewater. In recent years, the use of microalgae in biodegradation of coloured wastewater has a lot of attentions in their main role in carbon dioxide fixation [16]. In addition, microalgae biomass generated has great potential as feedstock for biofuel production [17,18]. Biological treatments are cheaper than other methods [19], but dye toxicity usually inhibits bacterial growth and limits therefore, the efficiency of decolorization [20].

Microalgae are useful bioindicators of physical-chemical stress because of their well-documented tolerance [21]. A change in the physical or chemical conditions will result in shifts in community composition [22]. The ability to remove colour by microalgae can be enhanced by stimulating their growth. For instance, the removal of reactive dye by cyanobacteria *Synechocystis* and *Phormidium* is enhanced with the addition of the plant growth regulator triacontanol hormone [23]. Recently, interest in using immobilized microalgae to remove colour from

textile dyes has surfaced. For instance, Chu *et al.* [24] showed that alginate-immobilized *Chlorella vulgaris* can remove a higher percentage of colour from textile dyes than suspension cultures [24]. Immobilized cultures of thermophilic strain of *Phormidium* can remove 8-50 % of textile dyes at high temperatures [25]. Microalgae such as *Chlorella* and *Spirulina* have the ability to grow in wastewater [18] and can be used for the treatment of textile industrial wastewater.

Therefore, the aim of this study is to examine the potential of *Chlorella vulgaris* strain, which was obtained from Iranian Academic Center for Education, Culture & Research (ACERC) of Shahid Beheshti University, Tehran, Iran to act as an affordable treatment of textile industrial wastewater and treated effluent's quality for agriculture reuse which remove colour and reduced the levels of pollutants such as COD, TOC, total solids, NO₂-N, NO₃-N, NH₄-N and PO₄-P, to a clear, apparently clean effluent which can be discharge into agricultural bodies.

EXPERIMENTAL

Microalga and culture medium: In this study, *C. vulgaris* used as a biological material to reduce pollution of textile industrial wastewater. For this purpose, 150 mL of textile industrial wastewater was added in two conical flasks (250 mL Erlenmeyer flask) and pH was fixed at 7. Eventually, 150 mL of Bold's basal medium (BBM) [26] was added to next flask as a culture medium. Then these conical flasks were autoclaved (for 15 min at 120 °C), cooled to room temperature. One flask consist of textile industrial wastewater sample mixed with *C. vulgaris* and another was blank sample (without *C. vulgaris*) and BBM culture flask was used as culture medium for growth *C. vulgaris*. Biomass of this microalga was measured by counting the number of cells by optical microscopy (Olympus, Japan) using a Neubauer Hemocytometer (Germany), 1.5×10^6 cells mL⁻¹. All the flasks were kept at 25 °C in laboratory shaker (GFL, 3015, Germany) at 120 rpm for 14 days, exposure period under continuous illumination of 2500 to 3000 Lux that was prepared by OSRAM cool white tubular fluorescent lamps. After every 2 days, the sample and BBM culture flasks were analyzed at defined time intervals and finally after 14 days, suspended particles from the flask samples were removed by centrifugation at 7000 rpm for 20 min using centrifuge (Kendro, Biofuge Stratos, D-37520 Osterode, Germany). Morphology of cultivated *C. vulgaris* in samples for cell count every 2 days was observed under a light microscope (400-1000X). Other parameters such as pH and electrical conductivity were recorded by using HACH Portable Multi-Parameter Meter model: HQ40d and optical density was determined using UV-visible spectrophotometer (JASCO V-570) based on standard methods APHA [27].

Quantitative and qualitative determination of textile industrial wastewater samples: Eventually, concentration of COD, BOD₅, PO₄-P in textile industrial wastewater, blank and treated textile industrial wastewater samples were determined by using an UV-visible spectrophotometer (JASCO V-570) [27]. Colour was determined based on APHA [27] standard of 1 colour unit (PtCo) being equal to 1 mg L⁻¹ platinum in the form of chloroplatinate ion. The percentage colour removal was calculated as follows:

$$\frac{(\text{Initial colour} - \text{Final colour})}{\text{Initial colour}} \times 100$$

Other physical chemical conditions included wastewater, total suspended solids (TSS), total dissolved solids (TDS), NO₂-N and NO₃-N concentrations were measured according to the standard methods [27]. Also, NH₄-N concentration by total Kjeldahl nitrogen (FOSS Kjeltac™ 2300) and TOC by TOC Analyzer (SGE Analytical Science, ANATOC Series II) were recorded.

Complexity of textile industrial wastewater not only have a whole range of dyes including, but also have other chemicals such as heavy metals and other elements. Therefore, 25 elements were recorded with inductively coupled plasma optical emission spectrometry (Perkin-Elmer Optima 8000 ICP-OES Spectrometer).

RESULTS AND DISCUSSION

Biodegradation of textile industrial wastewater: As shown in Table-1, the quality parameters of textile industrial wastewater were found to be high. The textile industrial wastewater contained much higher amounts of TDS, indicating high contents of soluble salts in the wastewater. The COD, total solids and electrical conductivity reduction in treated sample makes obvious that chemical pollutants were degraded, also increase of corroborated this statement based on conversion the chemical pollutant to intermediates. The almost uniformly BOD₅ in treated sample indicated that isolated *C. vulgaris* are growing (Table-1). However, it was observed that COD removal occurred in samples, in addition to those that presented cellular death. This can be explained by the fact that photosynthetic organisms and microalgae produce oxygen that enhances the biological degradation of organic matter in the wastewaters [28].

TABLE-1
CHARACTERISTICS DATA OF TEXTILE INDUSTRIAL WASTEWATER SAMPLES

Parameters	Textile industrial wastewater		
	Blank	Raw	Treated
EC (µs/cm)*	2777.15±12.11	2790.78±11.12	418.33±10.11
TDS (ppm)*	2845 ± 17.23	2630 ± 25.9	1244 ± 12.5
TSS (ppm)*	45 ± 3.99	304 ± 3.34	38 ± 0.88
TS (ppm)	2890	2934	1282
pH*	7.0 ± 0.09	4.16 ± 0.06	8.2 ± 0.11
DO (ppm)*	8.39 ± 0.21	7.24 ± 0.10	10.45 ± 0.31
BOD ₅ (ppm)*	997 ± 5.13	1005 ± 7.22	881 ± 4.21
COD (ppm)*	1598 ± 14.44	1670 ± 14.91	724 ± 14.44
TOC (ppm)*	521.376 ± 8.92	530.275 ± 8.21	198.221 ± 6.35
NO ₂ -N (ppm)*	51 ± 0.44	52 ± 0.60	12 ± 0.18
NO ₃ -N (ppm)*	27 ± 0.77	28 ± 0.27	2 ± 0.95
NH ₄ -N (ppm)*	108 ± 1.1	112 ± 1.2	39 ± 0.35
PO ₄ -P (ppm)*	46 ± 0.55	47 ± 0.52	19 ± 0.32
C:N:P ratio**	11:04:01	11:04:01	10:03:01

*Data are presented as mean ± standard deviation (n = 3);

**N = Sum of NO₂-N, NO₃-N and NH₄-N.

Removal of nutrients: The removal of TOC, NO₂-N, NO₃-N, NH₄-N and PO₄-P, by *C. vulgaris* was investigated and the data is listed in Table-1. The TOC concentration in the blank sample is from about 521 to 198 ppm, in other words *C. vulgaris* removes approximately 62 % of TOC. Also, *C. vulgaris* can reduce NO₂-N concentration from 51 to 12 ppm and NO₃-N concentration from 27 to 2 ppm, which means *C. vulgaris*

removes about 76 % of $\text{NO}_2\text{-N}$ and 93 % of $\text{NO}_3\text{-N}$. The range of $\text{NH}_4\text{-N}$ concentration in the treated sample is varied from 108 to 39 ppm. *C. vulgaris* removes about 64 % of $\text{NH}_4\text{-N}$. The $\text{PO}_4\text{-P}$ concentration in the treated sample is from 46 to 19 ppm, this means that *C. vulgaris* used more than 59 % of $\text{PO}_4\text{-P}$ concentration for growth them.

Furthermore, we used C:N:P ratio for interpretation of nutrient removal by *C. vulgaris*. The high C:N:P ratio for blank sample (11:04:01) in Table-1 indicates that there are high concentrations of carbon and low concentrations of nitrogen and phosphorus. This ratio is reduced for treated sample to 10:03:01. This means *C. vulgaris* trend to use carbon and nitrogen for nutrient source rather than phosphorus. In this respect, Acuner and Dilek [29] reported that several species of *Chlorella* were capable of degrading azo dyes to their aromatic amines and to further metabolize aromatic amines to simpler organic compounds or CO_2 and thereby detoxifying them. Furthermore, El-Sheekh *et al.* [30] also reported the ability of *C. vulgaris* to decolourize a variety of azo dyes via microalgal azo dye reductase enzyme.

Colour removal: As presented in Table-2, colour removal by *C. vulgaris* is achieved more than 80 %, which is found to be suitable for biological systems. Through biodegradation, some isolated microalgae can break down the dyes and other pollutants to more simple molecules. Acuner and Dilek [29] surveyed *Chlorella vulgaris* can remove 63-69 % of colour from the mono-azo dye tectilon yellow 2G by converting to aniline. The capability to remove colour by microalgae can be enhanced by stimulating their growth. Karacakaya *et al.* [23] showed that the removal of reactive dyes by cyanobacteria *Synechocystis* and *Phormidium* is enhanced with the addition of plant growth regulator triacontanol hormone.

TABLE-2
PERCENTAGE COLOUR REMOVAL IN SAMPLE AT 14th DAY*

Samples	Colour		Colour removal (%)
	Initial (PtCo unit)	Final (PtCo unit)	
Blank	1429 ± 12.89	1399 ± 11.37	2.09
Treated	1429 ± 12.89	279 ± 12.91	80.47

*Data are presented as mean ± standard deviation (n = 3).

Heavy metals and other elements: As shown in Table-3, the concentration of 25 elements for textile industrial wastewater samples were analyzed using ICP-AES and the values are under the permissible limits as per WHO guidelines for agricultural use.

TABLE-3
ANALYSES OF 25 ELEMENTS IN TEXTILE INDUSTRIAL WASTEWATER SAMPLES BY ICP-AES

Element	Value (ppm)	Element	Value (ppm)	Element	Value (ppm)
Ag	0.20	Fe	0.60	Sb	4.00
Al	0.10	Hg	< 0.01	Sc	< 0.01
As	< 0.01	K	< 0.01	Se	< 1.00
Bi	< 0.01	La	< 1.00	Sn	< 0.01
Ca	15.00	Mg	5.00	Sr	< 1.00
Cd	< 0.01	Mn	0.04	Tl	< 0.01
Co	< 0.01	Mo	< 0.01	Zn	0.04
Cr	< 0.01	Ni	< 0.01	—	—
Cu	0.02	Pb	< 0.01	—	—

Microalgal growth parameters: As can be seen from Fig. 1, the growth of *C. vulgaris* was significantly affected by Bold's basal medium (BBM) and TWM culture. The maximum growth after 14 days for TWM 9.2×10^6 cells mL^{-1} and for BBM 9.5×10^6 cells mL^{-1} was obtained. Furthermore, microscopic images indicated that the cell size became bigger in TWM than BBM. It is also found that *C. vulgaris* cultured at two medium had the highest optical density of cells (1.1 for TWM and 1.15 for BBM) (Fig. 1). In 12 days, *C. vulgaris* reached maximum concentration of biomass for TWM (9.2×10^6 cells mL^{-1}) while that is 8 days for BBM (10.1×10^6 cells mL^{-1}). This result can be related to presence of colour in TWM that prevent light from reaching microalgae. As shown in Fig. 2 pH range changes at 14 days for two culture medium. It is well-known that the growth of microalgae is a process of alkalinity production [31], which means *C. vulgaris* can be growth in TWM as culture medium similar to BBM.

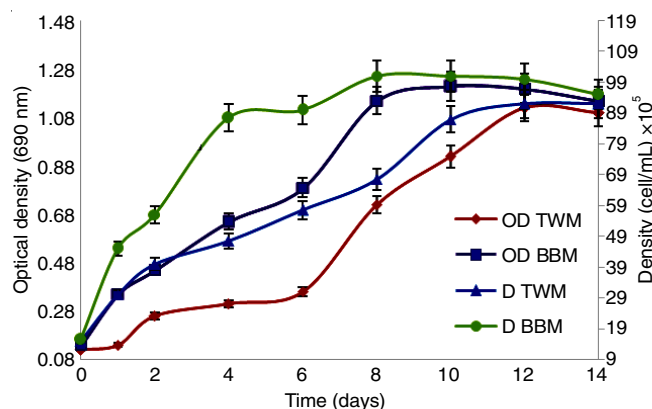


Fig. 1. Cell concentration (OD) and density in 14 days for biomass producing *C. vulgaris* in TWM and BBM culture

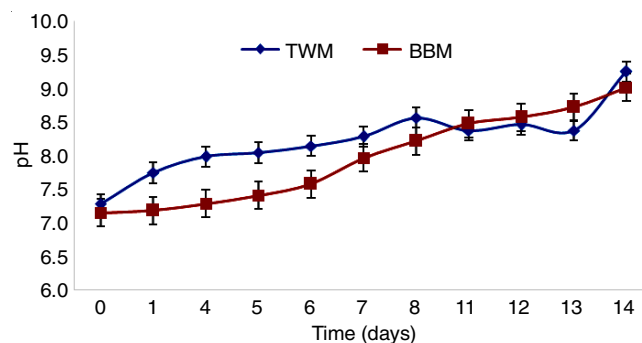


Fig. 2. Increase of pH and alkalinity production in 14 days for biomass producing *C. vulgaris* in TWM and BBM culture

The biomass of *C. vulgaris* have been attracting attention as a source of high lipid material to produce biofuel. In addition to biodegradation of textile industrial wastewater, a methodology combining experiments in lab scale and pilot plant can be used to predict biomass and lipid productivity for the systematic investigation of the potential of *C. vulgaris* for biofuel production. Maximum specific growth rate, production and productivity biomass were obtained after 14 day culture by TWM and BBM as culture.

Conclusion

This study shows that use of *C. vulgaris* is considered as one of the suitable microalgae as it could remove more than 80

% of colour, also this microorganism has the ability to reduce COD and total solids upto 55 % and also efficiently remove TOC (61.98 %), NO₂-N (76.47 %), NO₃-N (92.59 %), NH₄-N (63.88 %) and PO₄-P (58.69 %). It is also anticipated that microalgal biomass can trap some heavy metals.

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