

Phycoremediation of textile wastewater using indigenous microalgae

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Abstract

The recognition that environmental pollution is a worldwide threat to public health and environmental degradation has given rise to new initiatives for environmental restoration for both economic and ecological reasons. There are several methods to treat the dye contaminated industrial wastewater; of which biological treatment methods are economical and environmentally friendly. The bacteria and fungi remediation of dye pollutants has been well characterized over a period of more than 30 years. So, finding other biological methods in addition to bacteria and fungi is great important in the world. As a result, investigating and evaluating Phycoremediation techniques of dye wastewater (bioremediation using Microalgae) have gained a great deal of attention because of their versatility and capacity than bacteria and fungi. The aim of the research is to study Phycoremediation of Textile Wastewater Using indigenous Microalgae.

Physico-chemical parameters such as color, pH, total dissolved solid (TDS), biochemical oxygen demand (BOD) and chemical oxygen demand of the waste were determined with ASTM standard methods before and after bioremediation. Photo bioreactor systems were used for Phycoremediation treatment techniques. PH, incubation time and temperature effects were determined on a photo bioreactor treatment and optimal experimental condition was ascertained. Instrumental analytical techniques (UV-Vis, FTIR) were used to determine percent decolorizations of dye wastewater before and after bioremediation; and the actual break down of the dye functional groups.

The maximum reductions of the basic parameters; COD, BOD and TDS were obtained 91.50%, 91.90% and 89.10% respectively. The optimum operating conditions in the photo bioreactor system were found incubation time 20 days, 30°C; with 10% of inoculums at a pH of 8. Under these conditions, a maximum of 82.6% decolorization was achieved in 20 days. The experimental investigations evidently tell us algae undoubtedly have the potential to rapidly, efficiently and effectively remove dyes wastewater.

Key words: BOD, COD, dye wastewater, microalgae, percentage removal, phycoremediation

INTRODUCTION

Azo-dyes are an important class of synthetic organic compounds used as coloring agents in textile, paint, ink and plastic industries. Azo-dyes are one of the oldest industrially synthesized organic compounds and represent the major group (60–70%) of more than 10,000 dyes currently manufactured (Bras *et al.* 2005). However, large amount of these dyes remain in the effect after the completions of the dyeing process. Consequently, very small amount of dyes in wastewater is highly visible (Wang *et al.* 2005; Marungrueng & Pavasant 2006). A side from their negative aesthetic effects, certain azo-dyes and their biotransformation products has been shown to be toxic to aquatic life and mutagenic to humans (Brown & Hamburger 1987).

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Presently a wide range of physical and chemical methods is available to decolorize dye-contaminated effluents (Hao *et al.* 2000) but alternative processes based on biotechnological principles are attracting increasing interest (Kandelbauer & Gübitz 2005) since they often avoid consumption of high quantities of additional chemicals, energy and environmentally friendly. Different types of treatment methods have their own advantages and disadvantages and their range of applicability. Like most organic materials of animal and vegetable origin, dyes can be degraded into simpler compounds and are finally mineralized to water and carbon dioxide by a wide variety of aerobic or anaerobic organisms (McMullan *et al.* 2001; Binkley & Kandelbauer 2003).

Many bacterial, fungal and algal species have the ability to adsorb and/or degrade dyes wastewater. Moreover, microalgae decolorization is normally faster compared to bacterial and fungal systems with regard to the decolorization and mineralization and advantageous in terms of pathogenic to humans. Recently a substantial amount of research on the subject of dye wastewater removal has been carried out using single microalgae cultures like *Chlorella vulgaris*; *Scenedesmus* sp. has shown very promising results for the degradation. In the present investigation, one of the prominent dye wastewater used in textile industry was decolorized /or degraded using co-cultured freshwater microalgae species. Because mixed cultures are particularly useful in this area, as microalgae consortia can collectively carry out biodegradation tasks that no individual pure strain can undertake successfully.

The objective of this work was to investigate decolorization and biodegradability dye wastewater using mixed populations microalgal which are much more commonly applied than isolated cultures of single organisms because of their relative robustness and versatility against xenobiotic compounds

METHODOLOGY

Physico-chemical characterizations

Preservation

Samples were collected in a plastic bottle, before collecting it was thoroughly cleaned with hydrochloric acid and washed with tap water to render free of acid and preserved below 4°C.

All the glassware, burette and pipettes were first cleaned with tap water thoroughly and finally with deionized water. The chemicals and reagents were used for analysis were analytical reagent grade. PH, Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) were analyzed based on water and wastewater quality analysis ASTM standard methods before and after bioremediation.

Experimental setup

250 ml, 500 ml and 1,000 ml conical flask photobioreactor (PBR) were used as batch reactors in temperature incubation chamber and sealed with cap stoppers and glass tubes through which air fed, exhausted and screwed with plastic covers. Continuous Air flow (with 2.0 L/min volumetric air flow rate) from the air pump through polyvinylchloride pipes connected with the glass tubes on the top of PBR. The air feed tube immersed at the bottom of the growth container to allow mixing, to prevent sedimentations of the algae, to ensure that all cell of the populations are equally exposed to light and nutrients, and to improve gas exchange between the culture medium and the air.

Two red, two blue and one white fluorescent lamps were employed as the light source for growth with an average light intensity of 1,450 Lux and with 12 D:12 L hour photoperiod with negligible external light interference. The PBR temperature was monitored $25 \pm 2^\circ\text{C}$ with temperature controlled incubation chamber. The batch experiments were conducted in the medium protocol of bold basal medium (as seen in Figure 1 and 2).

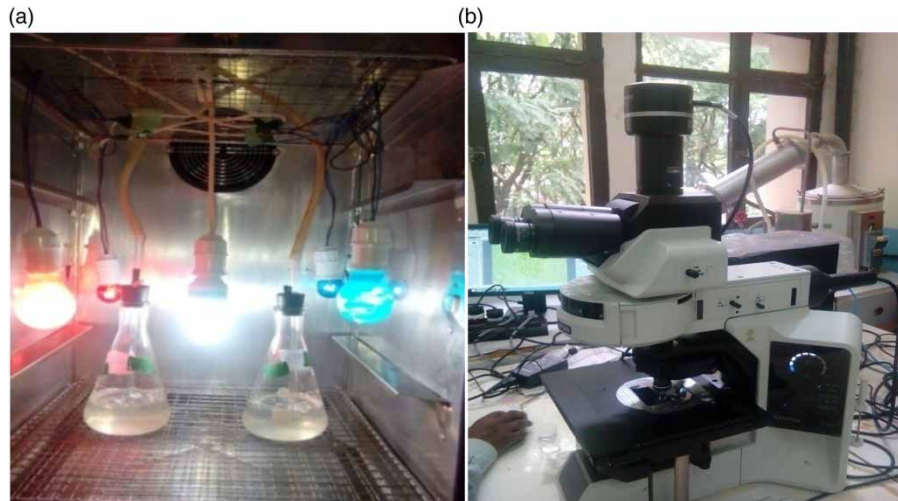


Figure 1 | (a) photobioreactor setup inside the incubation chamber (b) digital microscope.

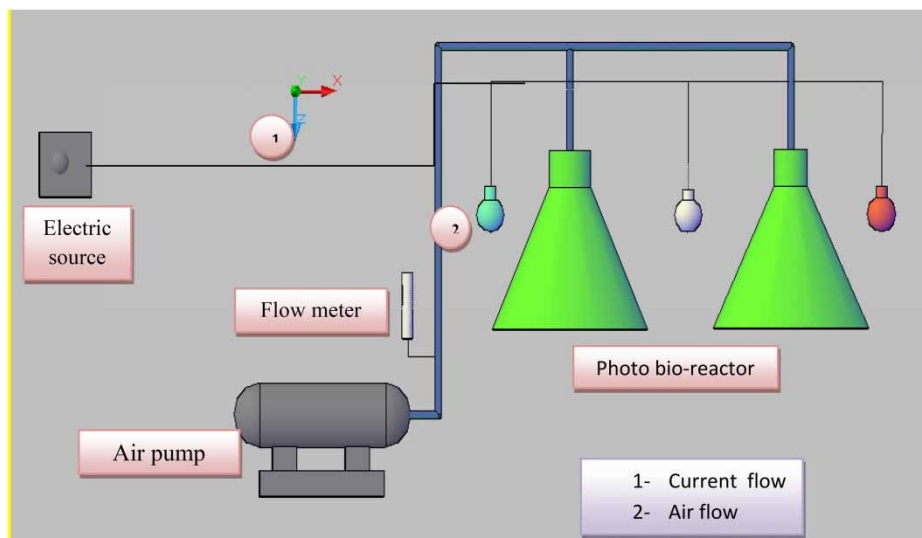


Figure 2 | Schematic diagram representations of the experimental set up.

Growth media and species identification

Algae were cultured first with 250 ml conical flask PBR in BBM media without inoculums and sterilization from the freshwater sample with continuous air bubble feeding and with several times a day CO₂ flashing (this keeping the pH below 10) until the medium turns green, signaling adequate algae growth. Once maximum density is attained, the predominant species were identified according to its morphology and microscopic observations as prominent genera using digital camera equipped microscopy Olympus DP 73, (Aragaw and Asmare, 2017).

Dye wastewater decolorization study

Wavelength of maximum absorbance (λ_{max})

The primary step for this study was the determination of maximum absorbance (λ) of the dye wastewater. Thus range of wavelength was scanned with diluted solution on the characteristic intensities of color and sensitivity of the instrument. The maximum wavelength absorbance (λ_{max}) was determined

Spectrophotometric determination

The dye wastewater containing 10% cultured microalgae from freshwater was taken for incubation. Decolorization of dyes wastewater was determined spectrophotometrically with 5 days interval up to optimum decolorizations. Treated waste was drawn at regular intervals and centrifuged at 3,500 rpm for 10 minutes in order to precipitate the cell mass and the supernatants were evaluated at respective maximum absorbance (λ_{max}) of the tested dyes wastewater and analyses were done in triplicate. The percentage decolorization or color removal efficiency achieved was determined by monitoring the decrease in absorbance at 592 nm, the λ_{max} . Percentage of decolorization was calculated as shown by (APHA 1995).

$$\text{Percentage decolorization (\%)} = \frac{(\text{Initial Absorbance} - \text{final Absorbance})}{\text{Initial Absorbance}} * 100$$

Also, measurements were performed the output of bioreactor were scanned in the range of 200–800 nm using Perkin-Elmer (lambda 35) spectrophotometer to observe the spectral shifts caused by biosorption.

Effect of temperature on decolorization

The effects of temperature on decolorization were investigated by incubating the reaction volumes at 20°C, 30°C, 40°C, 50°C and 60°C with 10% inoculum concentration.

Effect of PH on decolorization

The effects of pH on decolorization were examined; medium with dye wastewater was prepared at pH 4, 5, 6, 7, 8 and 10, where the temperature at which the maximum decolorizations obtained kept constant.

FTIR analysis

The range of Infrared region is 12,800 ~ 10 cm^{-1} and can be divided into near-infrared region (12,800 ~ 4,000 cm^{-1}), mid infrared region (4,000 ~ 200 cm^{-1}) and far-infrared region (50 ~ 1,000 cm^{-1}). The controls and samples obtained were mixed with KBr (0.02 g) with sample at a final weight of 0.4 g). The samples were then ground, desorbed and pressed to obtain IR transparent pellets. The absorbance FT-IR spectra of the samples were recorded using an FT-IR Spectrum Perkin-Elmer spectrophotometer. The spectra were collected within a scanning range of 450–4,000 cm^{-1} . The FT-IR was first calibrated for background signal scanning with a control sample of pure KBr and then the experimental samples was scanned.

Statistical data analysis

The triplicate data that collected on batch phobioreactor were subjected to SPSS software (version 20) to evaluate the factors that affect or not significantly on percentage decolorization. Factorial analyses were determined whether or not both main factors and their interactions have an effect on percentage decolorizations.

RESULT AND DISCUSSION

Identifications of the predominant fresh water co-cultured species

A microalgae species having remarkable dye wastewater decolorization capacity was identified from freshwater co-cultured samples collected from Lake Tana. The specific characteristics found for algal strains under light microscopy were assessed and compared with Wehr & Sheath (2002) to find the respective algal genera. The obtained results were also confirmed on online algal database called 'Micrographia' available at (<http://www.micrographia.com/index.html>).

Alga cells cultivated on nutrient medium gave different species. The size and shapes of the microalgal species which identified was different with circular shapes, rod shapes and spherical shapes. The observation by light microscopy confirmed the predominant species was dominantly green algae. The identification of the species was done on the basis of microalgae morphology.

As a result, the Predominant consortium of microalgae species identified in this study was: *Scenedesmus sp.*, *Chlorella sp.*, *Synedra sp.*, *Achnanthydium sp.*

As can be seen from Figure 3 *chlorella sp.* dominantly grown but *Synedra sp.* and *Achnanthydium sp.* was found in small amount. This revealed that, mostly the freshwater microalgae species, the sample where taken from the study, are green algae.

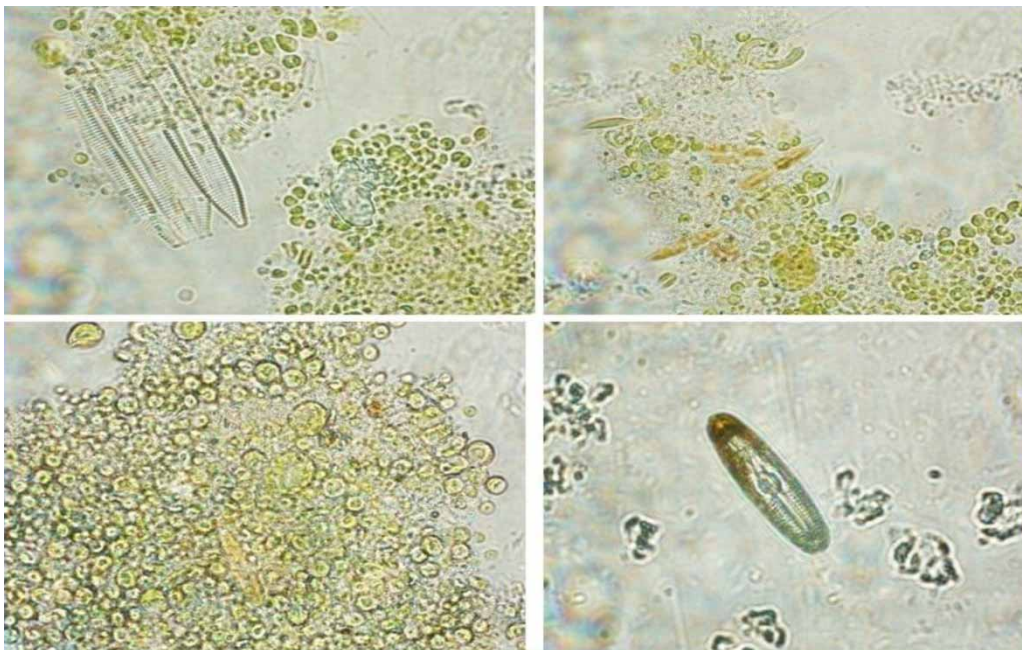


Figure 3 | Microscopic photograph of mixed culture microalgae species.

Effluent before treatment shown that the characteristics absorption peaks had band in the visible region with its maximum absorption near 592 nm. But in the case of test solution the absorption intensity become very weak. It can be assumed that the aromatic ring structure was destroyed or the intensity of the color of the dye wastewater was decreased by the action of microalgal species. The decrease in absorbance peak from Figure 4 indicated that there was optimum removal of dye wastewater. This decolorization is due to the biosorption as well as biodegradations with algal cells.

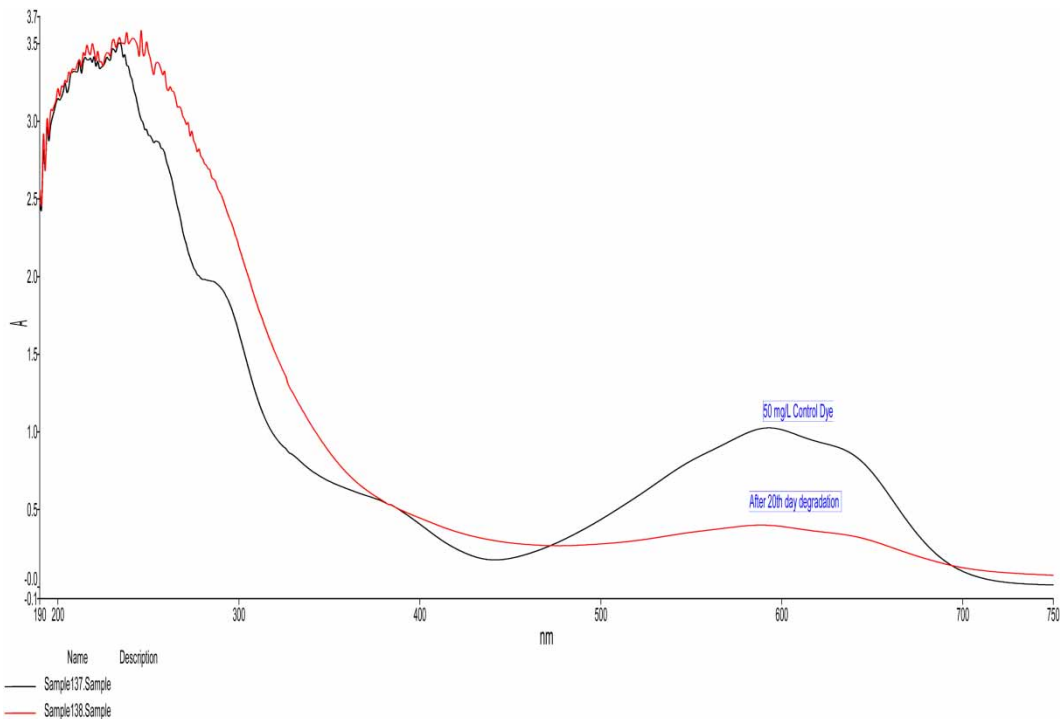


Figure 4 | UV-Vis Spectra of before and after bioremediation dye wastewater.

Effect of temperature on phycoremediation

The temperature and pH of the cultures was within the optimum microalgae growth range suggested for most strains of the algae and the wastewater temperature values (Grobbelaar *et al.*, 1981; Fontenot *et al.* 2003; Grobbelaar 2004; Borowitzka 2010). Those ranges are mostly 20–60°C to understand the effect of low temperature, room temperature and high temperature on the decolorization.

Among different temperature tested, 30°C was found an optimum decolorization. It is noted that the percentage decolorization increased with an increase in temperature from 20°C to 30°C. The percentage decolorization decreased with further increase in temperature up to 60°C. An increase in the temperature from 20 to 30°C had a positive impact on the decolorization of dye wastewater (Figure 5).

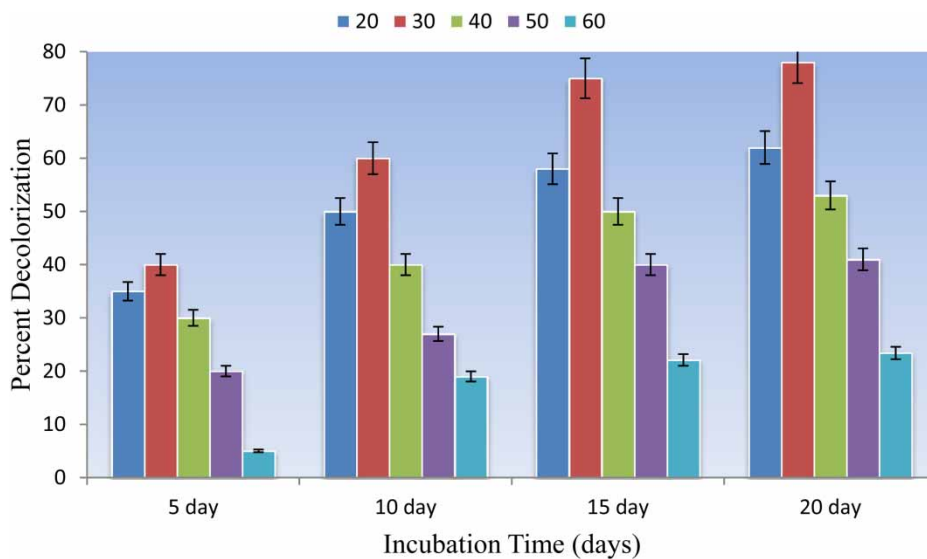


Figure 5 | Effect of temperature (°C) on phycoremediation for 20 days incubation time with 5 day interval.

Decolorization of dye wastewater was found optimal at temperature 30°C as the species were able to decolorize by 78% for 20 day cultivation time. However, decolorization rate dropped gradually as the temperature increased from 40°C–60°C.

The decrease in decolorization at high temperature is attributed due to the decline in microalgal activity that led to the deactivation of the enzyme and eventually the loss of cell viability. High temperature probably caused thermal deactivation of algal enzyme(s) responsible for decolorization. Previously, *Guo et al. (2010)* reported that 28 to 35°C may be an optimal temperature for the decolorization of dyes wastewater. Therefore, the species could acclimatize to broad range of temperature of practical dyeing wastewater.

Effect of PH on Phycoremediation

Optimum value of temperature was used as constant to determine the effect of PH on the biodegradations ability of microalgae. Effect of pH on reaction volume was 82.6% of decolorization (*Figure 6*). The mixed culture algae were able to decolorize the over a wide range of pH. Maximum decolorization in this study was recorded at pH 8. A rapid increase in decolorization was observed as the pH increased from 4 to 5 and 6 to 8 for the 15 and 20 day. However, a relative decrease in decolorization was found when pH increases from 9 to 10.

At pH 10, the species showed 72.5% decolorization of dye wastewater. Whereas at pH 4, the species showed only 42.4% decolorization. Rate of decolorization decreases at lower pH and at higher pH. The study showed that pH 8 is more favorable for decolorization of the dyes wastewater and is suitable for industrial applications. From the result it can be conclude that the more acidic and basic media affects the biodegradation ability of microalgae species. This is because, the activity of species decline consequently deactivate their enzymes. An increase in pH from 6 to 8 caused significant increase in the rate of decolorization with algae. Most likely pH affects the enzymatic activity involved in decolorization in addition to cellular growth of algae. (*Prasad and Aikat, 2014*) reported that biological treatment can effectively decolorize over a wide range of pH (6–9).

FTIR analyses

Infrared analysis was applied to identify the structural variation of the tested compounds. *Figure 7* shows the presence of detectable differences in the IR peaks resulted from the microalgae species treatment of dye wastewater and before treatment as a control.

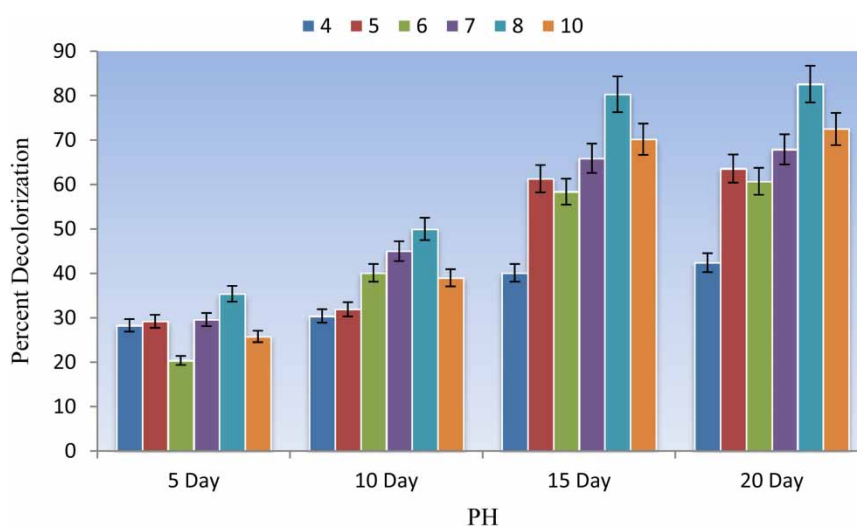


Figure 6 | Effect of PH on phycoremediation for 20 days incubation time with 5 day interval.

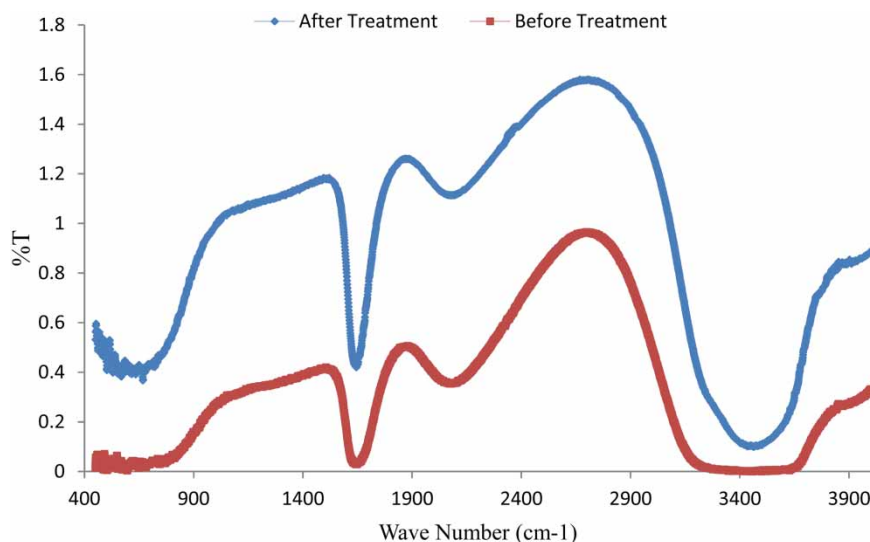


Figure 7 | Fourier Transformation Infrared Spectroscopy before and after treatment.

The changes observed in the spectrum indicate the possible involvement of different functional groups in the process. Before biodegradation peaks were detected at $2,078\text{ cm}^{-1}$ and the conjugated aromatic rings or amide functional groups at $1,648\text{ cm}^{-1}$ (Figure 7). FTIR spectrum of algae before biodegradation showed two prominent peaks and shows one peak after biodegradation and also existing peaks showed peaks shifts. It was found that the presence of new peaks at $3,477\text{ cm}^{-1}$ (from Figure 7) attributes to -N-H- vibration; other peaks correspond to the same functional groups which are seen in the control. The results revealed that the large conjugated chromophore structure of dye wastewater was destroyed yielding smaller organic molecule.

The obtained variations in IR spectra of the dyes could be attributed to the cleavage of azo linkage of the dye and the subsequent formation of aromatic amine. These results are in agreements with that obtained by Urushigawa & Yonezawa (1977); Kirso *et al.* (1988); Jinqi & Houtian (1992) and Mohan *et al.* (2006). Additionally, the obtained differences in spectral intensity and the occurrence of stretched vibration in IR figures of algae treated also manifest possible biosorption besides the algal biodegradation activities as reported by Kirso *et al.* (1988).

Determination of physicochemical parameters of the wastewater before and after treatment

As can be seen from Figure 8, BOD₅, COD and TDS value have decreased dramatically from 5 to 15 remediation time. Also it can be seen that from Table 1, the color is not offensive, TDS, COD and

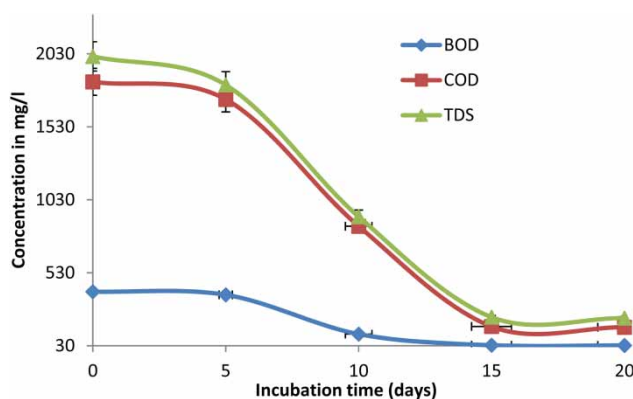


Figure 8 | Determination of physicochemical parameters of the wastewater before and after treatment.

Table 1 | Physico-chemical properties textile wastewater samples before and after remediation

Parameters	Before treatment	After treatment	Percentage removal	Guide line limit
Color	Blue black	Light blue	–	Offensive colors not accepted
TDS	2012 ± 5.751811	220 ± 2.753785	89.10%	≤250 mg/L
PH	10.5 ± 0.2	8.3 ± 0.220303	–	6–9
COD	1838 ± 7.00	157 ± 2.00	91.50%	≤160 mg/L
BOD ₅	401 ± 5.00	32.5 ± 3.00	91.90%	≤160 mg/L

BOD have 89.10%, 91.50% and 91.90% percentage removal respectively. This much of removal COD, BOD and TDS conforms as acceptable ranges of the guide line limit.

Statistical data analysis

About 100% of the variability in the percent decolorization is explained by the incubation time, temperature and temperature-incubation time interaction as shown from Table 2. For each, P -value = $0.000 < 0.05$, this shows that there is a significant effect both on the main effect and their interaction on the percentage decolorization.

About 100% of the variability in the percent decolorization is explained by the incubation time, PH and PH-incubation time interaction as shown from Table 3. For each, P -value = $0.000 < 0.05$, this shows that there is a significant effect both on the main effect and their interaction on the percentage decolorization.

Table 2 | Effect of temperature and remediation time on percentage decolorization

Tests of Between-Subjects Effects

Dependent Variable: Percent Decolorization					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21529.780 ^a	19	1133.146	22361.699	.000
Intercept	102805.145	1	102805.145	2028773.961	.000
Temperature	14915.260	4	3728.815	73585.060	.000
Time	6063.451	3	2021.150	39885.721	.000
Temperature * Time	551.069	12	45.922	906.241	.000
Error	2.027	40	.051		
Total	124336.952	60			
Corrected Total	21531.807	59			

^aR Squared = 1.000 (Adjusted R Squared = 1.000).

CONCLUSION

Microalgae have capability of bio-transforming and biodegrading dye contaminants commonly found in natural and wastewaters. Furthermore, microalgae have the ability to enhance the biodegradation potential of the microbiota present and therefore contribute to the elimination of pollutants from the respective ecosystem. Mostly the green microalgae, from freshwater, were used to treat the dye wastewater.

Biodegradation activity is significantly suppressed at high temperature, the basic and acidic PH range. This can be due to the loss of cell viability or deactivation of the enzymes responsible for Biodegradation.

Table 3 | Effect of PH and remediation time on percentage decolorization

Tests of Between-Subjects Effects

Dependent Variable: Percent Decolorization

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	24107.012 ^a	23	1048.131	567836.193	.000
Intercept	171402.198	1	171402.198	92858978.336	.000
PH	4790.941	5	958.188	519108.762	.000
Time	17459.910	3	5819.970	3153031.094	.000
PH * Time	1856.161	15	123.744	67039.690	.000
Error	.089	48	.002		
Total	195509.299	72			
Corrected Total	24107.101	71			

^aR Squared¼ 1.000 (Adjusted R Squared¼ 1.000).

Generally, it can be concluding that algae undoubtedly have the potential to rapidly, efficiently and effectively remove dyes wastewater to acceptable ranges of guide line limits at environmental temperature and neutral PH range. Moreover, the biosorption process could be adopted as a cost effective and efficient approach for decolorization of effluents and it may be an alternative to more costly materials.

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Since we are from Ethiopia which is categorized under low-income countries list, we need to kindly request the waiver.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no conflict of interest.

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

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