

Biodegradation of Reactive Dyes by Two Microalgal Species

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Abstract: Dye wastewater is one of the most difficult to treat. Meanwhile, there has been exhaustive research on biosorption of dye waste water and it is evolving as an attractive option to supplement conventional treatment processes. This paper examines the capacity of *Spirogyra* sp. and *Oscillatoria* sp. to decolorize two model dyes, Blue dye and Red dyes from their aqueous solutions. The effect of algal biomass on the rate of decolorization was studied. The rate of dye decolorization was found to increase with the increase of algal concentration. The biodegradation was monitored by UV-Vis and FTIR analysis. In conclusion, *Spirogyra* sp. showed high efficiency for color removal of the examined dyes.

Keywords: *Spirogyra* sp.; *Oscillatoria* sp.; Blue dye; Red dye; FTIR; UV Visible

1. Introduction

Global industrialization has resulted in the release of large amounts of potentially toxic, mutagenic and xenobiotic compounds into the biosphere. Cleaning up of the environment by the removal of hazardous contaminants is a crucial and challenging problem needing numerous approaches to reach long-lasting suitable solutions.[1] The textile industries are using synthetic dyes with ease of production, fastness and variety in color compared to natural dyes and daily discharging millions of liters of untreated effluent containing harmful dye wash into receiving water bodies posing serious health problems. An average textile mill produces 60×10^4 m of fabric and discharges approximately 1.5 million liters of effluent per day in India. The number of dyes presently used in textile industry is about 10,000. Among these dyes, Blue dye and Red dye constitute the largest and the most important class of commercial dyes. Both dyes are widely used in textile, plastic, leather, and paper industries as additives. The removal of both dyes in aquatic environment is important because some types of dyes are toxic to aquatic organisms. and are typically 5-10% of this amount is discharged into environment which is usually recalcitrant to conventional wastewater treatment methods.[3] Presence of dyes in aqueous ecosystem diminishes the photosynthesis by impeding the light penetration into deeper layer thereby deteriorating the water quality and lowering the gas solubility. To avoid these problems, the effluent from textile industries must be treated before their discharge. During the past three decades, several physical, chemical and biological decolorization methods have been accepted by the paper and textile industries.[5] There is a need to find alternative biodegradations that are effective in removing dyes from large volumes of effluents and are low in cost such as biological or combination systems.[6] Wide range of microorganisms including bacteria, fungi, yeasts, actinomycetes and algae capable of degrading dyes have been reported. Algae are microscopic, photosynthetic organisms, which typically inhabit aquatic environments, soil and other exposed locations.[11] So, the present study aims to investigate the potential of the

spirogyra sp. And *oscillatoria* sp. for degradation of the solution containing a textile dye. The dye degraded products after the microbial treatment would be analyzed by FTIR (Fourier Transform Infrared) and UV Visible analysis. [17][18]

2. Materials and Methods

Algal biomass :- The algae obtained from natural lake. According to its morphology and microscopic observations. It is identified as *Spirogyra* sp. and *Oscillatoria* sp. belonging to green algae and blue green algae. Fig.(21 & 22) shows the microscopic image of both algal sp. The algae *Spirogyra* sp. and *Oscillatoria* sp. were grown in several glass jars containing growth medium (Bold Basal Medium) in order to obtain stock algal cultures to be used in the experiments.[2][11]

Dye Analysis :- Dye analysis was performed at GREEN CIRCLE, INC [Recognized By Ministry of Environment and Forests. New Delhi under EPA 1986 and GPCB approved Environmental Auditor – (Schedule - 2)].

The Blue dye & Red dye used in this study. The absorbance was measured with a spectrophotometer at the maximum absorption wavelengths (λ_{\max} =619 nm). Decolorization was determined by absorbance reduction.[11] The percentage of decolorization was performed by using the calculation as follow:

$$\text{Percentage of decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Batch decolorization operation :- The experiments were conducted in 250 ml Erlenmeyer flasks containing 50 ml of respective dye solution by using 3 % algal biomass for decolorization efficiency. The experiments were operated at static incubation.[8]

FTIR Analysis of Decolorized Samples :- The biodegraded dye samples were characterized by FTIR spectroscopy

(Perkin- Elmer, Spectrum one). The analysis results were compared with the control dye. The FTIR analysis was done in the mid IR region (400-4000 cm⁻¹) with 16 scan speed.[4][17]

UV spectrophotometry :- The UV and visible spectra of the samples were measured by UV-1800 Series . Quartz cells (1 cm square) having 1.0 cm path length were used for the determination. Hydrogen discharge tungsten filament lamp was used as a source of light and maximum absorbance was recorded. (Instrument Type: UV-1800 Series, Measuring Mode: Absorbance Slit Width: 1.0 nm ,Light Source Change Wavelength: 340.0 nm and S/R Exchange: Normal) [18][9]

Phytotoxicity Studies :- The phytotoxicity study was carried out at room temperature using *Triticum* sp. plant seeds. The plant seeds were tested with both of dyes (Blue dye and Red dye) and its phytotoxic nature was analyzed. Then the seeds were tested with the dye degraded metabolites and toxicity was analyzed. The control was carried out using plain water at the same time. Experiments were carried out in triplicates. Germination (%), length of root and plant height was recorded after 7 days.[7][17]

Toxicity assay :- The biodegraded products were tested for their effect on the agriculturally important soil bacterial flora. *Azotobacter* sp. and *Rhizobium* sp. were inoculated on Nutrient medium containing agar. Wells were made on the respective media containing plates and filled with decolorized sample. The plates were incubated at 30°C for 48 hours. Zone of inhibition surrounding the well represented the index of toxicity.[1]

3. Results and Tables

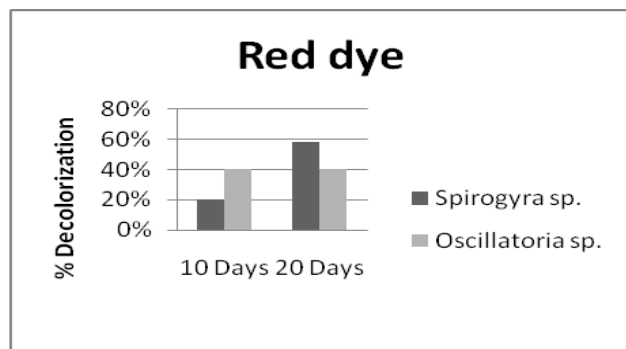


Figure 1

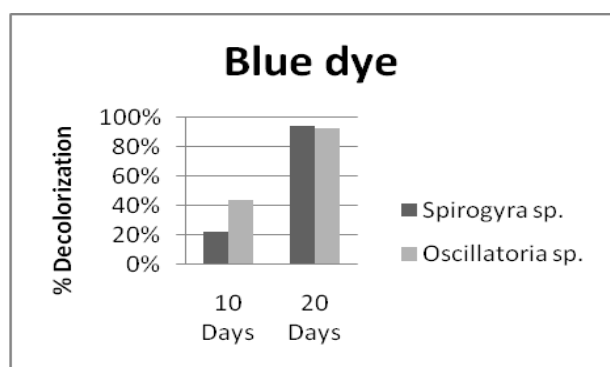


Figure 2

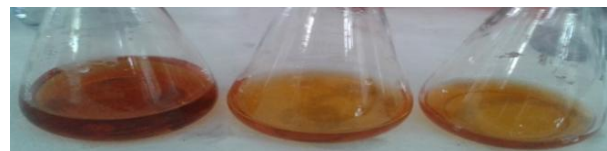


Figure 17



Figure 18

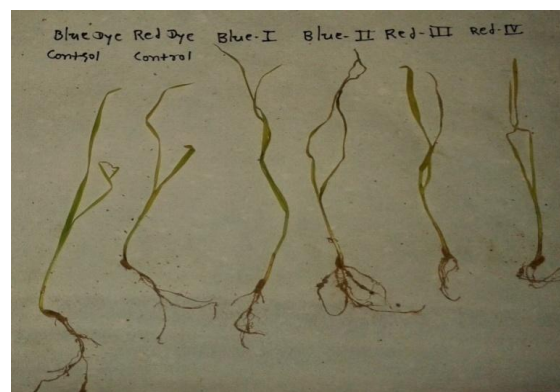
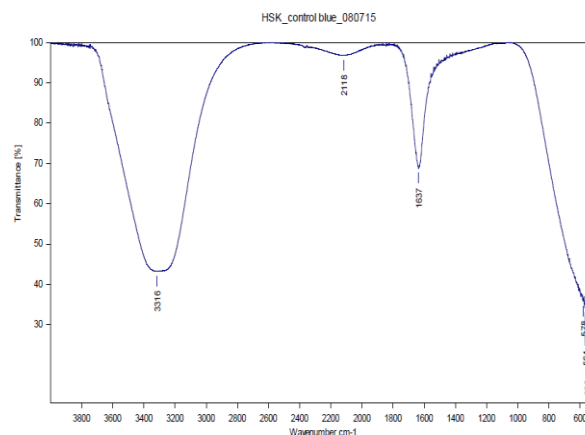
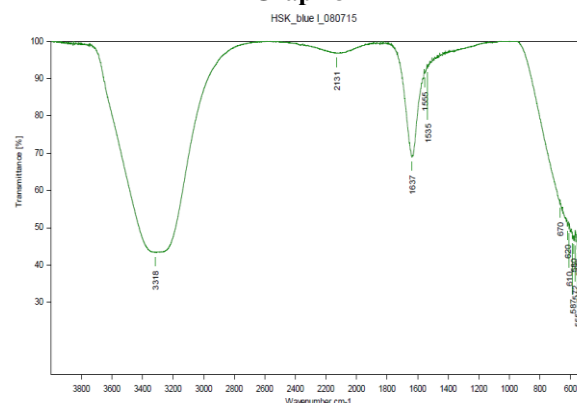


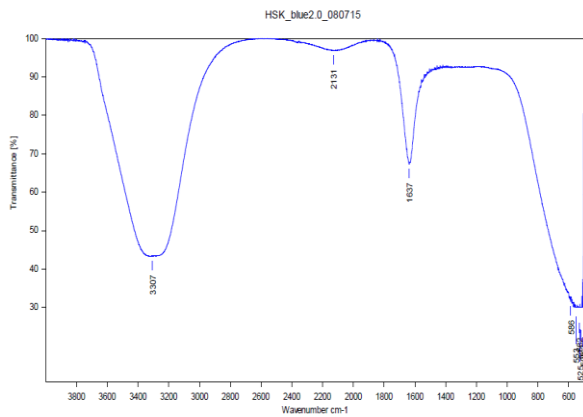
Figure 19



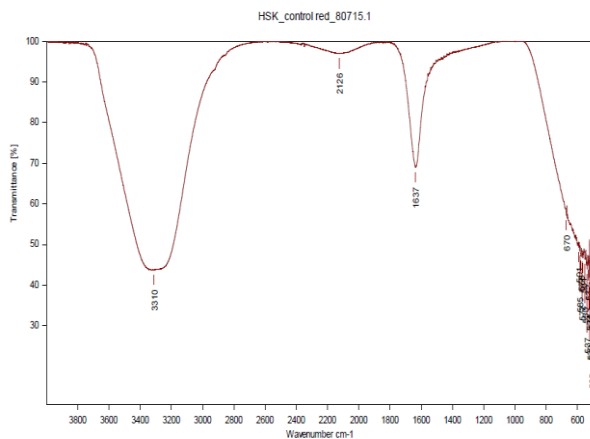
Graph 3



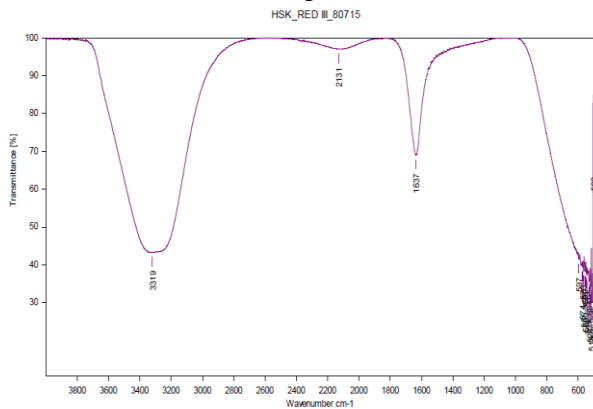
Graph 4



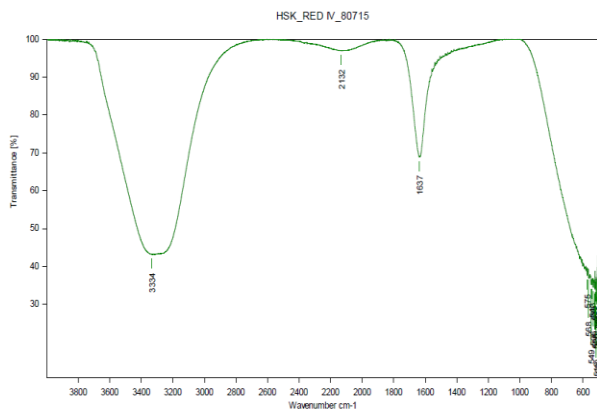
Graph 5



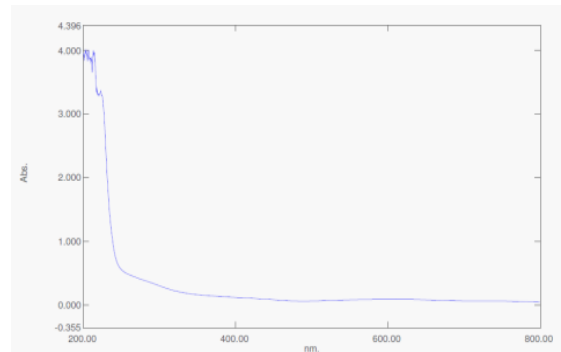
Graph 6



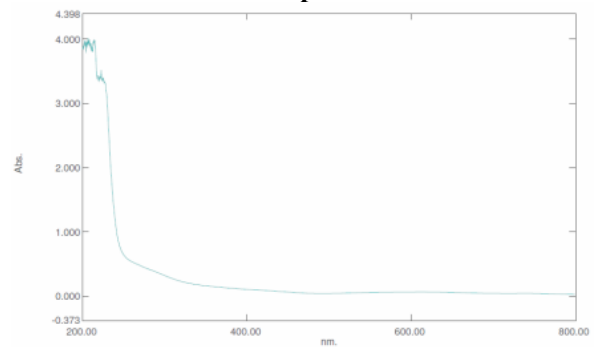
Graph 7



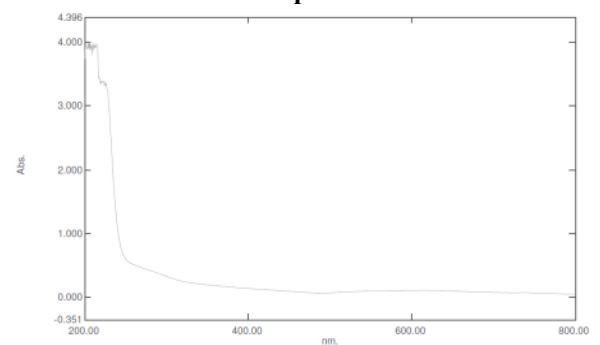
Graph 8



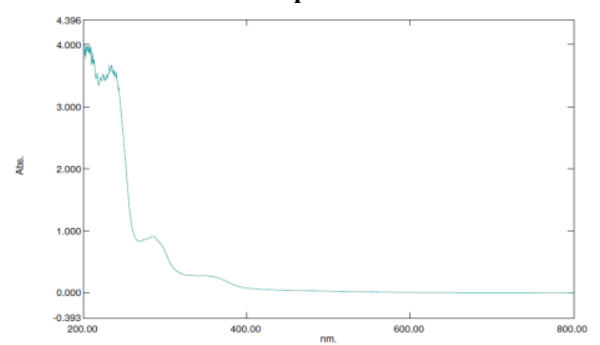
Graph 9



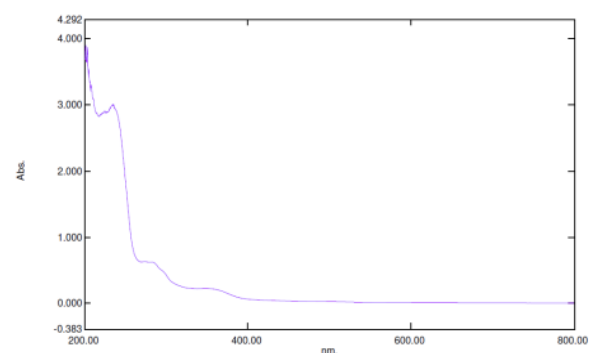
Graph 10



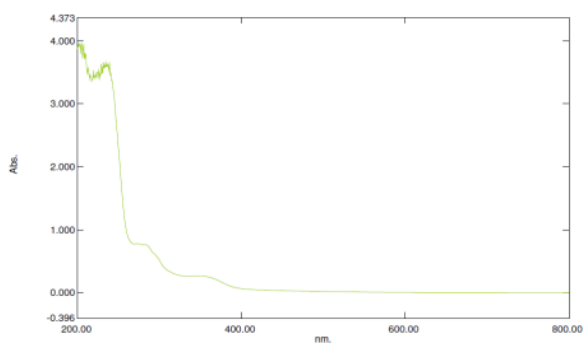
Graph 11



Graph 12



Graph 13



Graph 14

Table 15

	% Dry Matter	% Water
Control Blue	55.07 %	44.93 %
Blue 1	53.98 %	46.02 %
Blue 2	50 %	50 %
Control Red	64 %	36 %
Red 3	46 %	54 %
Red 4	50.27 %	49.73 %

Table 16

	% Germination	Plant height(cm)	Root height(cm)
Control Blue	90 %	15.77 ± 1.09	4.39 ± 0.61
Blue 1	90 %	15.50 ± 0.91	4.05 ± 0.25
Blue 2	60 %	14.67 ± 1.12	3.42 ± 0.66
Control Red	50 %	13.72 ± 0.90	4.72 ± 0.41
Red 1	50 %	15.7 ± 1.25	4.5 ± 0.62
Red 2	30 %	16.97 ± 0.08	2.9 ± 0.05

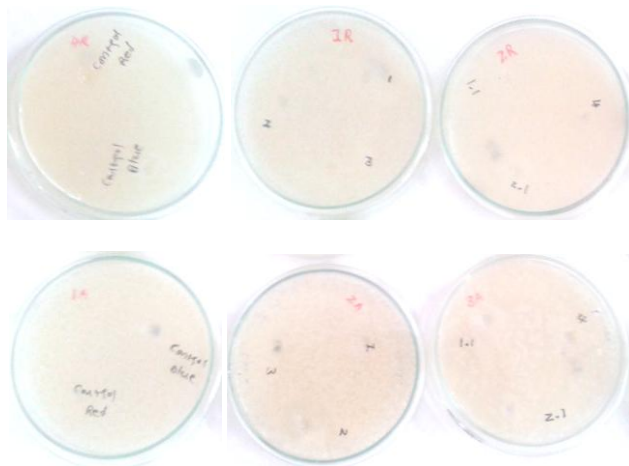


Figure 20



Figure 21



Figure 22

4. Results and Discussion

Decolorization study

Figures (1-2) shows % decolorization of Blue dye and Red dye by algal biomass. There was an increased in the decolorization rate with an increase time duration. The results obtained from present investigation revealed the ability of *Spirogyra* sp. and *Oscillatoria* sp. in biodegradation of both dyes. The 3 % algal concentration of *Spirogyra* sp. and *Oscillatoria* sp. showed about 94.44% and 92.77 % decolorization of blue dye in 14 days duration. Where as in case of red dye, 58 % and 40 % decolorization was monitored by *Spirogyra* sp. & *Oscillatoria* sp. respectively for the same period.(Figure 17-18)

FTIR Analysis of Decolorized Sample

Graph (3-8) shows IR spectra of Control and Decolorize blue & Red dyes. Comparison of FTIR spectrum of the control dye with after complete decolorization clearly indicated the biodegradation of Blue dye and Red dye by both species. The results of FT-IR analysis of both parent dye and sample obtained after decolorization showed various peaks. The FT-IR spectra of Blue parent dye displayed peaks at 3316, 2118, 1637, 578, 552, 504, 564, 534, 524, 505, 522, 524 and 508 cm^{-1} , for OH stretching (alcohol, phenol) vibration, $\equiv\text{C-H}$ stretching (terminal alkynes) vibration, N-H bending (primary amines) vibration, C-X (X= Cl, Br) stretching (Chloroalkanes, bromoalkanes) vibration, respectively. However the FT-IR spectra of degradation product displayed peaks at different positions indicating the breakdown of Blue dye and the result of red parent dye displayed peaks at 3310, 2126, 1637 and 670 cm^{-1} , for OH (alcohol, phenol) stretching vibration, $\equiv\text{C-H}$ stretching (terminal alkynes) vibration, N-H bending (primary amines) vibration, C=O stretching (ketone) vibration, C-H stretching (vinyl) vibration C-X(X= Cl, Br) stretching (chloroalkanes, bromoalkanes) vibration, respectively. The FT-IR spectra of degradation product displayed peaks at different positions indicating the breakdown of red dye.

Phytotoxicity Assay

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated both dye samples (Fig.19). *Triticum* sp. seeds treated with parent Blue and Red

dye showed 90% and 50% germination, the mean plant height of 15.77 ± 1.09 cm & 13.72 ± 0.90 , respectively and the mean root length for both dyes 4.39 ± 0.61 cm & 4.72 ± 0.41 cm, respectively. Whereas the blue dye Sample 1 & 2 showed 90 % & 30 % germination, the mean plant height of 15.51 ± 0.91 cm & 14.67 ± 1.12 cm, respectively and the mean root length for both samples 4.05 ± 0.25 & 3.42 ± 0.66 , respectively. And Red dye Sample 1 & 2 showed 50 % & 30 % germination, the mean plant height of 15.7 ± 1.25 cm & 16.97 ± 0.08 cm, respectively and the mean root length for both samples 4.5 ± 0.62 & 2.9 ± 0.05 , respectively. Phytotoxicity result indicated that Blue dye showed good effect than Red dye on Wheat plant % germination, Plant height(cm) and Root height(cm). (Figure 19)

UV-Visible analysis

UV Spectroscopy of Blue dye before degradation showed maximum absorbance at 223 and 220.5 nm (Fig. 1). After degradation the maximum absorbance were obtained at 615.5 nm & 720.5 nm, 222 nm & 219 nm from both samples respectively. Whereas red dye before degradation showed maximum absorbance at 285 nm & 269 nm. After degradation the maximum absorbance were obtained at 234 nm & 217 nm, 239 nm & 217 nm from both samples respectively. The peaks were completely from parent blue and red dye proving that both dyes changed to other compound. (Graph 9-14)

Toxicity assay

No zone of inhibition observed in surrounding the wells containing decolorized dye water, indicated that the biodegraded or decolorized product was non toxic to beneficial soil bacteria.

5. Conclusion

In this research study, both algae has sufficient biodegradation potential for removing blue dye and red dye from its aqueous solution under optimized conditions. It has been also found that *Spirogyra* sp. has more potential to biodegradation than *Oscillatoria* sp. Keeping in view of this research study, concludes that both species of algae can be used for removing blue and red dye from its aqueous solution. Knowledge from present work may be employed on large scale at actual contamination sites. Our future study aims to find out the mechanism of this biodegradation of blue dye and red dye by *Spirogyra* sp. and *Oscillatoria* sp.

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10.5897/AJMR 12.1539 ISSN 1996-0808 ©2013
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