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The Role of Algae in Bioremediation of Textile Effluent

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Abstract—Dyes present in the effluent of textile industries are recalcitrant molecules difficult to be degraded biologically. The textile industry accounts for two thirds of the total dyestuff market. During dyeing process approximately 10-15% of the dyes used are released into the wastewater. In the present study Potential of Spirogyra sp. and Oscillatoria sp. for biodegradation of blue dye and red dye were investigated. Degradation was assayed using decolorization study, physico-chemical analysis and products formed during degradation were characterized through FTIR spectra as well as UV Spectrophotometry analysis. This present study was also including study of phytotoxicity and toxicity assay of untreated and treated dye effluents.

Keywords— Blue dye; FTIR analysis; *Oscillatoria sp.*; Physico-chemical parameters; Red dye; *Spirogyra sp.*;

INTRODUCTION

Dyes are the synthetic chemical compounds having aromatic structure and recalcitrant to biodegradation due to xenobiotic nature. Dyes are toxic to aquatic flora and fauna as they reduce the light penetration and obstruct photosynthesis process in aquatic system. Synthetic dyes are one of the toxic pollutants released by various industrial sources such as textile and dyeing industries, paper, paint, plastics, petroleum, electroplating and cosmetic industries.[27] It is estimated that total colorant production in world is 800,000 tons per year and at least 15% of the dyestuff is released into the environment through wastes. Millions of untreated effluents are discharged from textile industries which directly mixes into rivers and lakes and alters the pH, BOD, COD and colour of the water resources.[26] Various physico-chemical methods such as flocculation, sedimentation, precipitation, coagulation and reverse osmosis are commonly used for the treatment of textile dyeing effluents, but these conventional methods are generally cost effective, less efficient and disposal of the secondary pollutants are difficult. Biotechnological approaches are suggested by scientists and industrialists to remove the pollutants from wastewater using microorganisms often in combination with physicochemical processes. Eco-friendly microbial decolourization and detoxification has emerged as a viable attractive alternative to these physicochemical methods.[21][28]

Now-a-days, many investigators have made search for the feasibility of using low cost and efficient adsorbents. The use of biomass as adsorbents for the removal of dyes also offers a potential alternative to existing methods for detoxification. Bioremediation is a pollution control technology where the biological systems are used to drive the degradation or transformation of various toxic chemicals into less harmful forms. This natural process is expected to clean up the environment in an effective way, being an alternative to conventional remediation methods.[2][7] The main objective of this study is to investigate the effect of Spirogyra sp. and Oscillatoria sp. for decolorization and to reduce the physico-chemical levels of the solution containing a textile dye.

MATERIALS AND METHODS

Algal biomass:- The algae (without isolation) collected from natural pond. According to its morphology and microscopic observations it has been identified as *Spirogyra sp.* and *Oscillatoria sp.* belonging to green algae and blue green (brown green). Plate 4-12 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.

Dye effluent collection:- The areas adjoining to the industrial complex at Nandesari, Gujarat will be selected for the project study. After survey, two sides were selected for sample collection. The selected places were Sarika dye chem (A1) and Megha dye chem (A2). The collected samples were analyzed for pH, Color, Total Suspended Solid (mg/l), Total Dissolved Solid (mg/l), Biochemical Oxygen Demand (mg/l), Chemical Oxygen Demand (mg/l), Chloride (mg/l), Sulphate (mg/l), Total Chromium (Cr) (mg/l), Copper (mg/l), Iron (mg/l), Manganese (mg/l) and Nickel (mg/l).(Table-2)

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

The Blue & Red dye effluent used in this study. The absorbance was measured with a UV spectrophotometer at 220 nm. Decolorization was determined by absorbance reduction. The percentage of decolorization was performed by using the calculation as follow:

$$\textbf{Percentage of decolorizaton} = \frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial Absorbance}} \text{ x100}$$

Batch decolorization operation:- Experimental Set was conducted in 250 ml Erlenmeyer flasks containing respective dye solution by using diff. algal biomass (1% W/V, 2% W/V and 3% W/V) and diff. pH 4, 6, 8 & 10 (in 3% W/V algal biomass cond.) condition for 14 days duration.(Plate 2 & 3)

FTIR Analysis of decolorized samples:- In experiment, after 14 days incubation 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.

UV Spectrophotometry:- In experiment, after 14 days incubation 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

Phytotoxicity Studies:- In experiment, after 7 days incubation the phytotoxicity study was carried out at room temperature using plant seeds of *Triticum sp.* by using pot method. The plant seeds were tested with both the dyes (untreated Blue and Red dye efflluent) and its phytotoxic nature was analysed. Then the seeds were tested with the dye degraded metabolites and toxicity was analysed. In the experiments, analysis of Germination (%), length of root and plant height were recorded after 7 days.

Toxicity assay:- In experiment, untreated and treated effluents 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 untreated and treated dye effluent sample. The plates were incubated at 30^0C for 48 hours. Zone of inhibition surrounding the well represented the index of toxicity.

RESULTS

Physico-chemical characterization of textile dye effluents

The dye effluents of blue and red dyes shows turbid solutions of blue and brown color of sample respectively. These samples are odourless. The pH values of the effluents are 7.68 and 7.23 for blue and red dye respectively, which indicates the neutral nature of the effluents. The BOD and COD values are very high in both the textile dye effluent samples. The heavy metals such as chromium, copper, iron, manganese and nickel content in the textile dye effluent of both the samples were very high. Hence all the textile dye effluent samples collected from study area indicates high level of pollution.(Table -1).

The pH of the solution significantly affects the adsorption of dyes by algal biomass. Figure (1) shows % decolorization of Blue dye and Red dye at pH 4, 6, 8 & 10 respectively. At pH 10, the more effective dye adsorption capacity of algae was observed. At pH 10, *Spirogyra sp.* and *Oscillatoria sp.* showed about 78.29% and 76.48% decolorization respectively of blue dye for 14 days duration. Where as in case of red dye, 64.21% and 62.63% decolorization were monitored by *Spirogyra sp.* & *Oscillatoria sp.* respectively for the same period.

Figure (2) shows % decolorization of Blue dye and Red dye by different algal biomass (1.0%, 2.0% & 3.0%). There was an increased in the decolorization rate with an increase in algal biomass. The results obtained from present investigation revealed that the ability of *Spirogyra sp.* and *Oscillatoria sp.* in biodecolorization of both dyes. The 3.0% algal concentration of Spirogyra and Oscillatoria sp. showed about 78.28% and 74.30% decolorization of blue dye in 14 days duration. Where as in case of red dye, 63.68% and 59.73% decolorization were monitored by *Spirogyra sp.* & *Oscillatoria sp.* respectively for the same period.

Bioremoval of heavy metals- chromium, copper, iron, manganese and nickel from textile effluents

In all the samples the metal content were very high compared with standards of APHA Manual. The inoculation of textile dye effluent samples with Spirogyra sp. and Oscillatoria sp., significant decrease in copper, chromium, nickel, iron and manganese content were observed in all the samples even upto below detectable limit. At the same time treatment showed significance reduction in sulphate, chloride and chemical oxygen demand content. (Table - 1)

FTIR Analysis of Decolorized Sample

Figure 04-11 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, ≡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, ≡ 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.

UV-Visible analysis

UV Spectroscopy of unreated blue dye effluent showed peaks at 737, 223, 490 and 220.5 nm. After treatment of blue dye with *Spirogyra sp.* showed peaks at 736, 615, 720 and 492 nm. Whereas treatment of blue dye with *Oscillatoria sp.* showed peaks at

739, 615, 222, 726, 488 and 219 nm. In case of untreated red dye effluent showed peaks at 285 and 265 nm. After treatment of red dye with Spirogyra sp. showed peaks at 348, 282, 274, 234, 338, 280, 260 and 217 nm Whereas treatment of red dye with Oscillatoria sp. showed peaks at 348, 282, 274, 234, 337, 280, 260 and 217 nm with different absorption value. These obtained results of UV-Visible analysis proving that both dyes changed to other compound.(Figure 12-17)

Phytotoxicity Assay

Phytotoxicity test was performed in order to assess the toxicity of the untreated and treated dye samples (Plate- 2). Triticum sp. seeds treated with untreated Blue and Red dye showed 60% and 30% germination, the mean plant height of 14.67 ± 1.12 cm & 13.72 ± 0.90 cm, respectively and the mean root length for both dyes 3.42 ± 0.66 cm & 2.9 ± 0.05 cm, respectively. Whereas the blue dye Sample treated with both *Spirogyra sp.* and *Oscillatoria sp.* showed 90 % & 90 % germination, the mean plant height of 15.77 ± 1.09 cm & 15.50 ± 0.91 cm, respectively and the mean root length for both samples 4.39 ± 0.61 cm & 4.05 ± 0.25 cm, respectively. The Red dye Sample treated with both *Spirogyra sp.* and *Oscillatoria sp.* showed 50 % & 50 % germination, the mean plant height of 16.97 ± 0.08 cm & 15.7 ± 1.25 cm, respectively and the mean root length for both samples 4.72 ± 0.41cm & 4.5 ± 0.62 cm, respectively. Phytotoxicity result indicates that the effluent treated with both *Spirogyra sp.* and *Oscillatoria sp.* gives better Wheat plant % germination, Plant height(cm) and Root height(cm). (Table-2)

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.

CONCLUSIONS

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 biodegrdation of blue dye and red dye by *Spirogyra sp.* and *Oscillatoria sp.*

OBSERVATION TABLES AND FIGURES

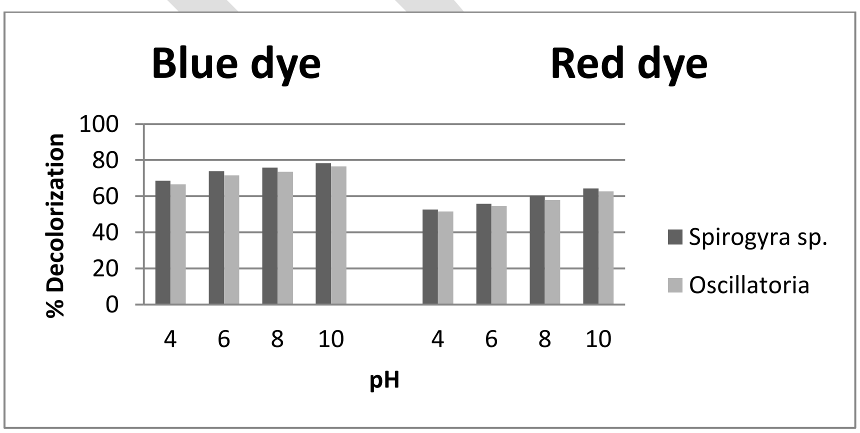

Fig.1 : % Decolorization of Blue and Red dye under different pH Conditions in set 1

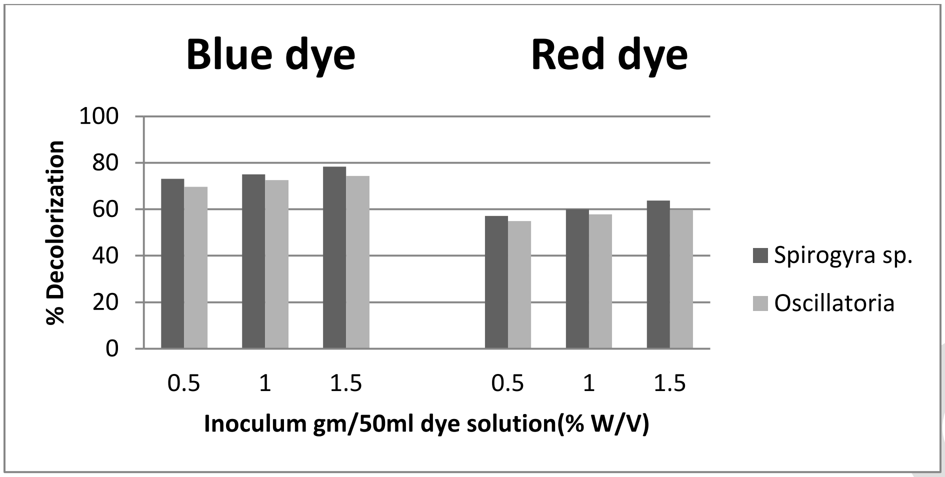

Fig.2 : % Decolorization of Blue and Red dye under different inoculum Condition in set 1

<u>Set-2</u>:- Fig. 4-11 Shows FTIR analysis & Fig. 12-17 Shows UV-Visible analysis results

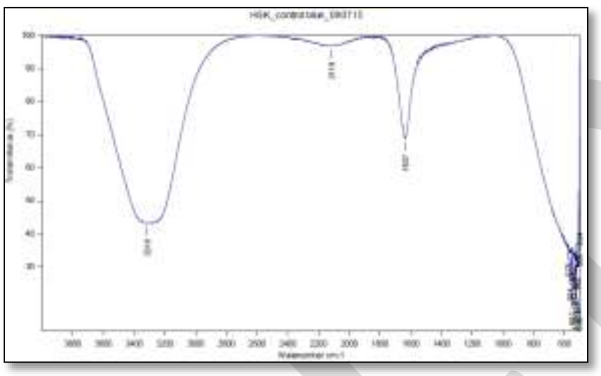

Fig.4 (Untreated Blue dye effluent)

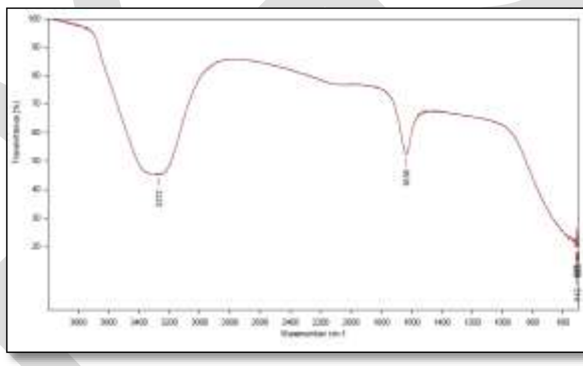

Fig.5 (Blue dye treated with *Spirogyra sp.*)

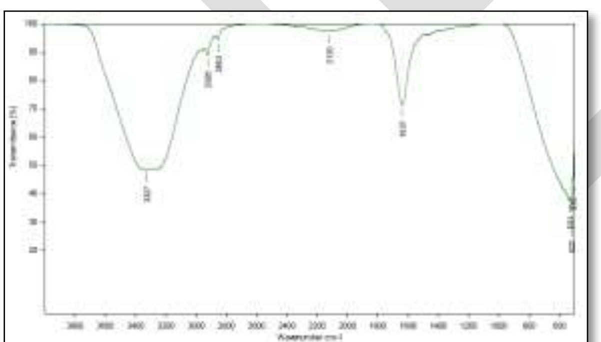

Fig.6 (Blue dye treated with *Oscillatoria sp.*)

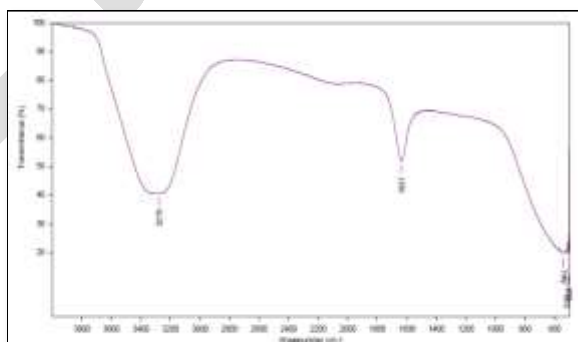

Fig.7 (Blue dye treated with *both* algal sp.)

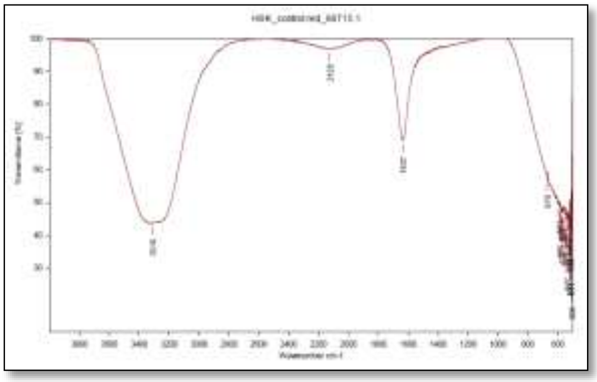

Fig.8 (Untreated Red dye effluent)

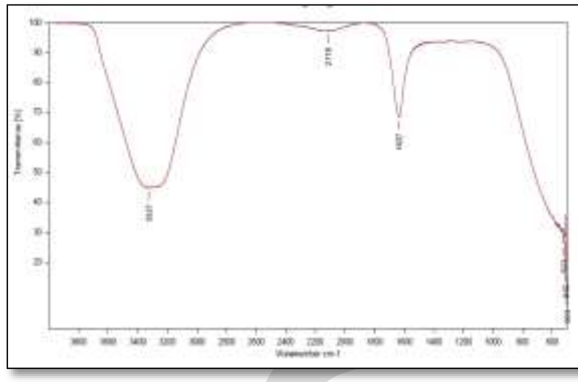

Fig.9 (Red dye treated with *Spirogyra sp.*)

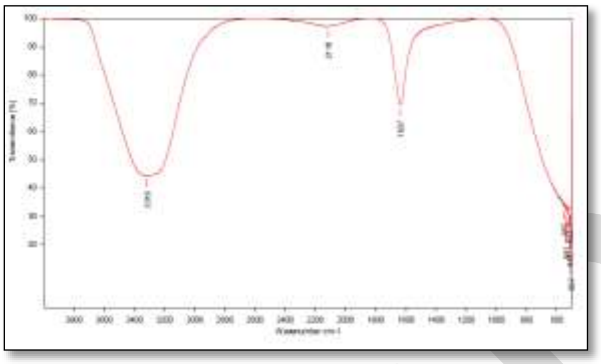

Fig.10 (Red dye treated with *Oscillatoria sp.*)

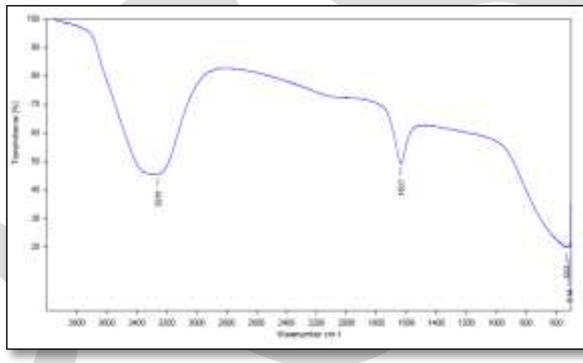

Fig.11 (Red dye treated with *both* algal sp.)

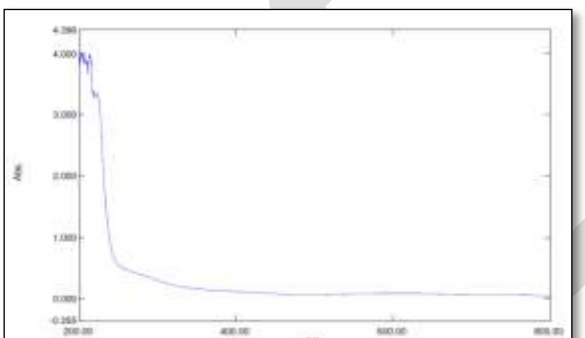

Fig.12 (Untreated Blue dye effluent)

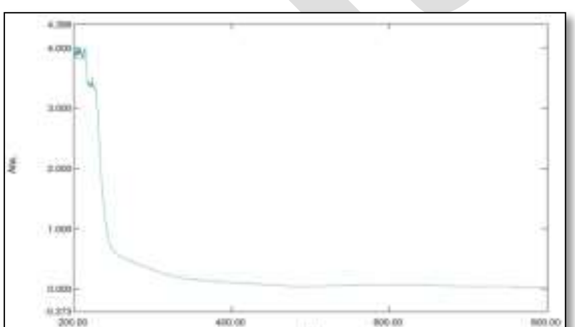

Fig.13 (Blue dye treated with *Spirogyra sp.*)

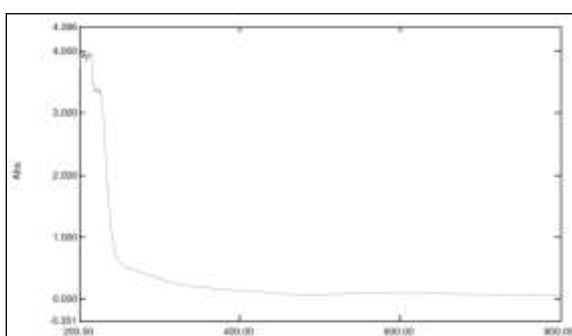

Fig.14 (Blue dye treated with *Oscillatoria sp.*)

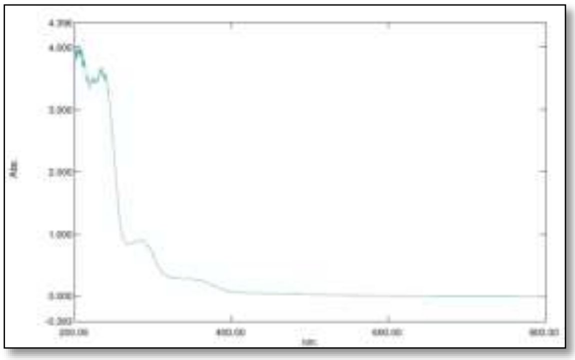

Fig.15 (Untreated Red dye)

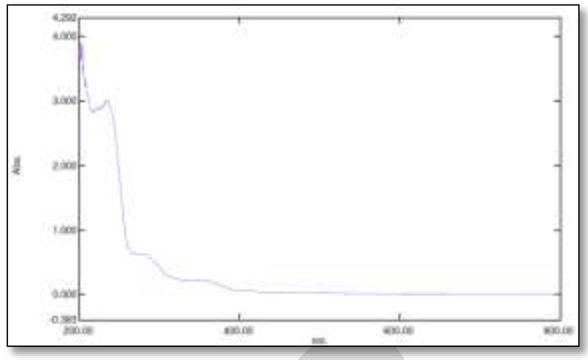

Fig.16 (Red dye treated with *Spirogyra sp.*)

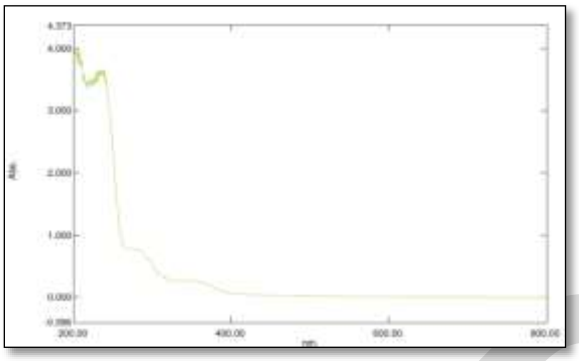

Fig.17 (Red dye treated with *Oscillatoria sp.*)

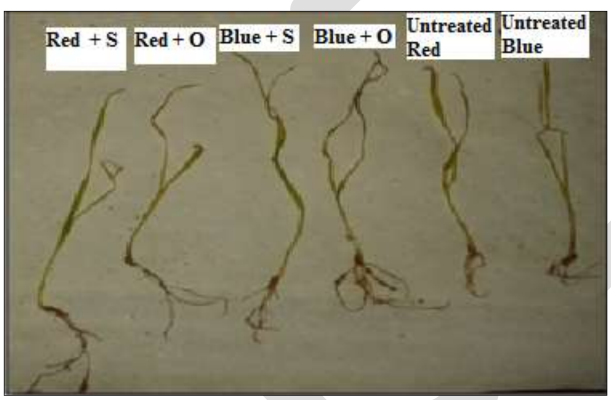

Plate-1: Phytotoxicity study
(S:- *Spirogyra sp.*, O:- *Oscillatoria sp.*)

Table-1: Physico-chemical analysis of untreated and treated effluent.

No.	Parameter	Unit	$R_{original}$	$B_{original}$	R_1	R_2	B_1	B_2	R_{12}	B_{12}
1	pH	-	7.23	7.68	6.83	6.81	8.61	8.85	6.12	8.46
2	Sulphate	mg/L	708	240	206	362	233	199	550	337.5
3	Chloride	mg/L	3480	840	3122	2989	832	758	1139	1343
4	Chemical Oxygen Demand	mg/L	2640	960	2200	2553	920	800	2352	880
5	Copper(Cu) PPM	mg/L	0.56	0.84	BDL	BDL	BDL	BDL	BDL	BDL
6	Chromium(Cr)PPM	mg/L	0.76	BDL	BDL	BDL	BDL	BDL	BDL	BDL
7	Nickel(Ni)PPM	mg/L	1.60	0.26	0.0353	BDL	BDL	BDL	0.0491	BDL
8	Iron(Fe)PPM	mg/L	1.26	0.52	0.3597	0.5439	0.341	0.486	0.4166	0.2722
9	Manganese(Mn)PPM	mg/L	0.27	1.02	0.243	BDL	BDL	BDL	BDL	BDL

R:- Red dye effluent, B:- Blue dye effluent

1:- treatment with Spirogyra sp., 2:- treatment with Oscillatoria sp., 12:- treatment with both Spirogyra sp. and Oscillatoria sp.

Table -2: Phytotoxicity study of *Triticum astivum* (1:- *Spirogyra sp.*, 2;- *Oscillatoria sp.*)

DYE	% Dry Matter	% Water	% Germination	Plant height(cm)	Root height(cm)
Control Blue	50 %	44.93 %	60 %	14.67 ± 1.12	3.42 ± 0.66
Blue 1	55.07 %	50 %	90 %	15.77± 1.09	4.39 ± 0.61
Blue 2	53.98 %	46.02 %	90 %	15.50 ± 0.91	4.05 ± 0.25
Control Red	46 %	36 %	30 %	13.72 ± 0.90	2.9 ± 0.05
Red 1	64%	54 %	50 %	16.97 ± 0.08	4.72 ± 0.41
Red 2	50.27 %	49.73 %	50 %	15.7 ± 1.25	4.5 ± 0.62

Plate-2: Untreated and treated effluent of red dye after decolorization

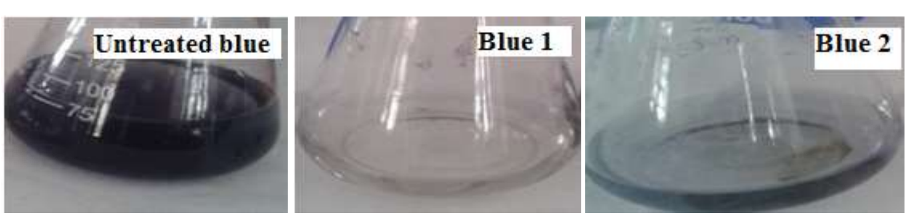

Plate-3: Untreated and treated effluent of blue dye after decolorization

1:- treatment with Spirogyra sp., 2:- treatment with Oscillatoria sp.

Plate.4

Plate.5

Plate.6 (Before)

Plate.7 (After)

Plate.8 (After)

Plate.9 (After)

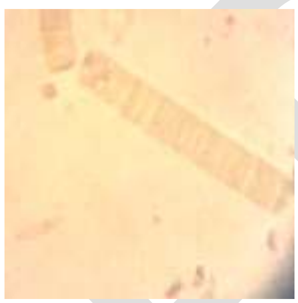

Plate.10 (Before)

Plate.11 (After)

Plate.12 (After)

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