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Bioprecipitation and biodegradation of fabric dyes by using *Chara Sp.* and *Scenedesmus obliquus*

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ABSTRACT

The processes of biodegradation have widely used to remove hazardous material from aquatic and soil system is an environmental friendly tools. The present study describes biodegradation potential of a macroalgae Chara sp. and Scenedesmus obliquus against the fabric dyes used by dyer for dyeing cloths. The degradation monitored using UV-visible and FTIR spectroscopy techniques. The Chara extract has high potentiality towards the decolorisation and dye precipitation as compared to Scenedesmus obliquus extract and Chara immobilized beads treated dyes. Out of 6 tested dyes, Chara extract could precipitate more than 90% of dyes after 5th days of incubation. Maximum precipitation and decolorization 98.28% of FD2 dye occurred and it is relative low in FD5 (90.36%). On the basis of FTIR spectra, the Scenedesmus obliquus degraded more than 77.22 % of dyes after 5th days of incubation. Maximum degradation of FD2 dye occurred about 77.22% and lowest observed in FD3 about 38.12%. The Scenedesmus obliquus has more prospective for dye degradation as compared to Chara extract and Chara extract immobilized beads. The optimum pH and temperature for all the tested algal samples is 6.0 and 37^{0}C. Toxicity tests all pure dyes and degraded dyes reduced to maximum 31.25 % of FD2 and minimum 15 % of FD3 determined against E. coli. The present work gives insight for the uses of Chara sp. and Scenedesmus obliquus for the bioremediation of dye effluents to reduce water pollution.

Keywords: Biodegradation, Bioprecipitation, toxicity, fabric dyes, Congo red, Crystal violet, *Scenedesmus obliquus* and *Chara sp.*.

INTRODUCTION

Synthetic dyes extensively used in pulp and paper, food, pharmaceutical and rubber industries [1]. Textile industries are the major source of dye effluents and more than 10,000 different dyes are commercially available worldwide [2]. The methods of decomposition for the removal of organic and inorganic pollutants with the help of living organism like microbes and their enzymes called as biodegradation [3]. Biodegradation could happen in nature and used in wastewater treatment in recent years since humanity strives to find sustainable ways to clean up contaminated water economically and safely. The discharge of toxic effluents from various industries adversely affects water resource. Synthetic textile dyes discharge into the natural water bodies, causes water pollution, which is hazardous because of recalcitrant nature, toxicity and carcinogenicity of dyes [4]. Although, algae *Gracilaria verrucosa* used for sorption of the phenoxyalkanoic acid herbicide 2,4-D and the heavy metal Cr(VI) [5]. Algae are one of the most adaptive and diverse group of organisms on earth. Algae develop themselves to adapt in polluted environment and not only that they eventually flourish well. Some azo dyes present in water bodies exhibit high toxicity to aquatic life but

do not significantly reduce algal growth [6]. In available literature algae used in bioremediation for example, *Spirogyra sp*. [7-8], *Chlorella vulgaris* [9-10] and *Scenedesmus quadricauda* [11]. Since, microalgae may be a good option for the bioremediation of fabric dyes polluted wastewater. The one more advantage of using algae for bioremediation is that they do not required any carbon sources for their growth, derives energy from sunlight and carbon from the air, and scavenges atmospheric nitrogen [12]. The phenomena of degradation by algae are likely to associate with enzymatic activity either, absorption or both [13]. In literature rice husk could employed as low-cost and effective adsorbent for the removal of direct red 23 [14]. Algae used in both living and non-living form for dye degradation. The mechanism for degradation by living algae may be due to the enzymatic while non algae remove dyes by bio-absorption. *Cynobacteria* have a ubiquitous distribution but their role in functioning of ecosystems is not clear, including dyes remediation [15]. This study aims to investigate the potential of *Chara sp.* and *Scenedesmus obliquus* for decolorization dyes. In present study, the local market dyes, used by washer men for dyeing cloths, selected for the experiments rather than laboratory dyes. Although, due to the unavailability of chemical composition of local market dyes, only two laboratory dyes are also used for comparison. Usually local dyers use easily available dyes for dying purpose instead of pure and laboratory dyes. Since, laboratory dyes are not liberated in sewage water as compared to locally available dyes. Therefore, present study aims to study and solve the toxicity and degradation of dyes used by dyer.

EXPERIMENTAL SECTION

2.1Materials

Chemicals used in our study of reagent grade in double distilled water at room temperature. All media and laboratory dyes used in the present work obtained from Merck, Qualigen or Himedia. The fabric dyes are purchased from local market which used by washer man for dying cloths.

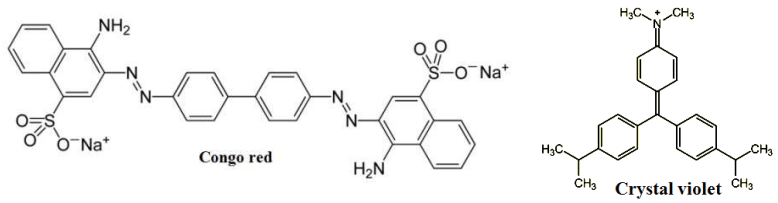

Figure 1 Structure of Congo red and Crystal violet dyes used for comparative analysis

2.2 Collection and cultivation of algae

The *Chara species* acquired from Waghur River at Sakegaon, Jalgaon (India) washed, purified and kept for experiment. The algal biomass washed with tap water and sterile distilled water to remove adhering particles like phytoplankton and kept in refrigerator below 0^0C. Whereas, the microalgae *Scenedesmus obliquus* isolated by strike plate method. Further, *Scenedesmus obliquus* cultivated in TAP medium at under 16-8 photoperiod.

2.3Immobilization of Chara sp. extract

The isolated algae crushed in distilled water and the supernatant collected, the supernatant mixed with 1.5% sodium alginate. The beads are prepared by dispensing in above mixture in 0.2 M chilled calcium chloride solution [16-17].

2.4Dye degradation experiment

The degradation experiments of dyes determined in following stages at (a) dye decolorization, (b) dye precipitation, (c) dye degradation and (d) toxicity determination.

2.5UV visible spectra determination

The UV–Vis absorption spectra of fabric dyes (0.01% solution) and laboratory dyes (0.01M) recorded with a UV Vis- 1800 Shimadzu, Japan Spectrophotometer. Dye decolorization measured in terms of reduced in color intensity of dye solution. Whereas, dye degradation determined by analyzing the FTIR spectra of precipitate obtained after treatment with algae.

2.6Dye decolorization assay

The decolorization process of the dyes determined by inoculating the wet algae and equal weight of beads (1g) in 0.1M laboratory dyes solution and kept at room temperature. After 24h of incubation, 2ml of aliquot withdrawn

from incubated samples and centrifuged. Further, the supernatant subject for λ_{max} determination and dye decolorization. The percentage of dye decolorization determined by using the below equation:

$$Percentage of decolorization = \frac{intial absorbance - observed absorbance}{intial absorbance} X\ 100$$

2.7 Optimization of parameters

In an attempt to study the effect of pH on decolorization, the algal extract (10ml) of both the algae and immobilized beads (10g) of Chara extract inoculated in conical flasks containing 100 ml 0.1M Congo red solution of varying pH (4-10 at 37^0C). The pH values adjusted using 1N NaOH and 1N HCl. In the similar fashion, the optimum temperature of dye decolorization by selected algae determined by evaluating the dye decolorization at 20, 30, 37, 40 and 50^0C. After three days of incubation, aliquot (5ml) of the dye solution withdrawn and supernatants obtained after centrifugation used for analysis of decolorization by Shimadzu double beam spectrophotometer [18].

2.8 Dye precipitation and dye degradation

The dye precipitation and degradation process, determined by collecting precipitation obtained after incubation of dyes with algae and algal beads by centrifugation. The precipitation collected and dried in oven and subjected for FTIR. IR spectra recorded on a Fourier transform infrared spectrometer (IR Affinity-1, Shimadzu, Japan).

2.9 Toxicity testing of pure and degraded fabric dyes

Agar well diffusion method used for toxicity estimation of treated and pure dyes. The *E. coli* suspension spread on nutrient agar plate and a well created in the centre. Which further filled with standard dyes and degraded samples; Streptomycin used as a positive control for validation of experiments.

RESULTS AND DISCUSSION

The results of the experiments carriedout for the degradation of fabric dyes from aqueous solution using Chara and Chara beads discussed below. Various analytical techniques like UV-Vis, FTIR spectroscopy used to confirm the dye decolorization and degradation respectively [19-20]. It helps in determination of reaction progress step by step, as the decomposition and formation of new compounds during the reaction can be confirmed using this technique.

3.1 Dye degradation experiment

Total six fabric dyes and two laboratory dyes (Congo red and Crystal violet) used for the study. The λ_{max} of dyes determined and further the dyes incubated with *Chara sp.*, *Scenedesmus obliquus* (S6) and algal extract of Chara extract beads. The optical density of the mixture measured on 1st day and 5th day of incubation. Based on optical densities, the percentage of dye decolorization calculated. The λ_{max} of each dye is presented in table 1 and the percentage of dye decolorization is given in table 2. The maximum amount of dye precipitation or decolorization observed in Chara extract as compared to *Scenedesmus obliquus* extract and Chara extract immobilized beads. Out of 6 tested dyes, Chara extract precipitate more than 90% of dyes after 5 days of incubation (figure 2). Maximum precipitation of FD2 (98.28) dye occurred and lowest 90.36% in FD5. Even though, the similar extract of Chara algae used for beads preparation (Immobilization), different results observed. Where, the maximum amount of FD3 dye (93.67%) precipitated and minimum FD5 (61.04%). The differences may be attributed to the specific enzymes present in extracts with the dyes.

Table 1: Absorption characteristic of fabric dyes

Sr.No.	Abbreviation	Color of dye	Lambda max
1	FD1	Feroji Blue	662
2	FD2	Mango Green	623
3	FD3	Orange	494
4	FD4	Mehandi Green	596
5	FD5	Red	500
6	FD6	Black	501
7	CR	Red	510
8	CV	Violet	585

Note: CR = Congo red, CV= Crystal violet

Table 2: Dye degradation response to tested algae

	Chara extract			*Scenedesmus obliquus* extract			Chara beads		
Dye	1st day	5th day	% of D	1st day	5th day	% of D	1st day	5th day	% of D
FD1	0.14	0.004	97.14	0.148	0.047	68.24	0.15	0.012	92
FD2	0.175	0.003	**98.28**	0.18	0.041	**77.22**	0.176	0.016	90.90
FD3	0.165	0.015	90.90	0.16	0.099	**38.12**	0.158	0.01	**93.67**
FD4	0.163	0.005	96.93	0.164	0.052	68.29	0.163	0.032	80.36
FD5	0.166	0.016	90.36	0.169	0.101	40.23	0.172	0.067	**61.04**
FD6	0.164	0.014	**91.46**	0.161	0.096	40.37	0.174	0.053	69.54
CR	0.159	0.008	94.96	0.162	0.039	75.92	0.163	0.029	82.2
CV	0.167	0.013	92.21	0.171	0.047	72.51	0.169	0.037	78.1

Note: D= percentage of decolorization, CR = Congo red, CV= Crystal violet

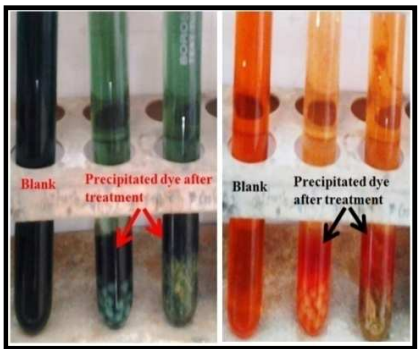

Figure 2 Fabric dye precipitation after treatment with Chara beads and Chara sp. (FD2 and FD3)

3.2 Optimization of parameters

The effect of initial pH on the decolorization of dye by algal strains is shown in figure 3. The results revealed that Chara specie and *Scenedesmus obliquus* capable of decolorizing this dye over a pH range of 4.0 –8.0 efficiently. The Chara extract degraded the dye (42.6%), immobilized beads (50.8%) and Scenedesmus *obliquus* (39.11%) at an acidic pH 6. The incubation temperature had a significant effect on the dye decolorization ability and maximum decolorization (59.22%) by Chara extract (69.7%) by immobilized beads; whereas *Scenedesmus obliquus* reduced the color (52.52%) at 37°C as shown in figure. 4. The results shows contradiction with the results of *Chara vulgaris* maximum at pH between 7.0-8.0 and when pH lower than 6, the phytoremediation ability of *Chara vulgaris* decreases [21] and *Valoria bryopsis* maximum at pH 5.0 [22].

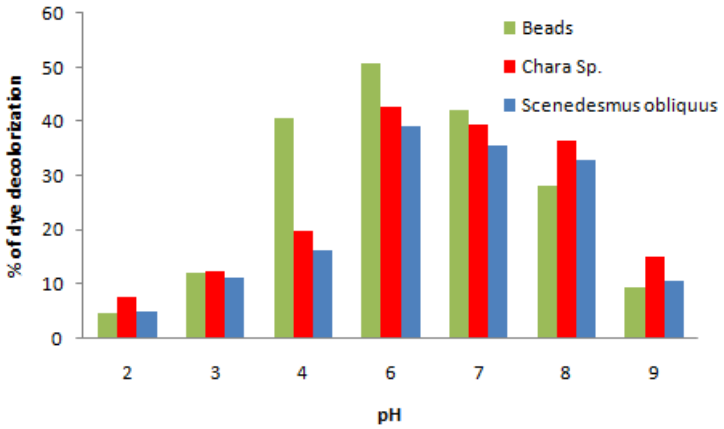

Figure 3 Effect of pH on Congo red decolorization

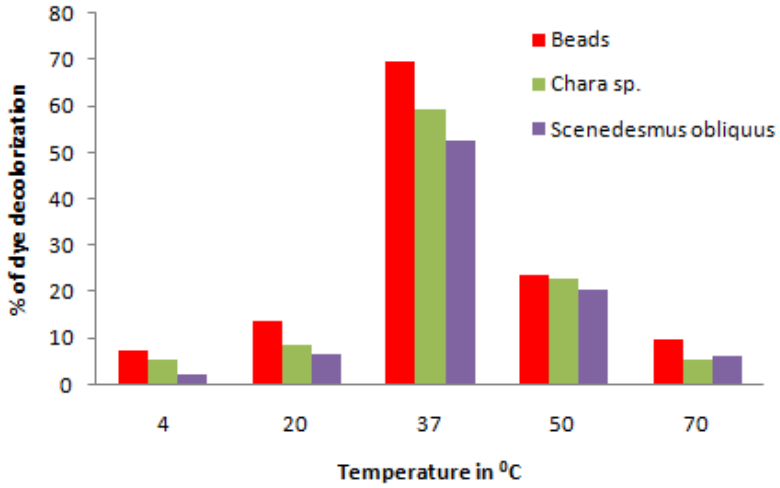

Figure 4 Effect of Temperature on Congo red decolorization

3.3 FTIR spectra of degraded dyes

Fourier transforms infrared spectroscopy (FTIR) used to determine the functional groups on the carbon surface. The spectra measured within the range of 400 - 4000cm^{-1} in IR Affinity-1, Shimadzu, Japan spectrophotometer. The absorption frequencies are shifted to higher wave numbers with the absorption of all the dyes after degradation. Due to unknown nature of fabric dyes, the results compared with laboratory dyes like Congo red and Crystal violet.The maximum amount of dye degradation observed in *Scenedesmus obliquus* as compared to Chara extract and Chara extract immobilized beads. Out of 6 tested dyes, *Scenedesmus obliquus* degraded more than 77.22 % of dyes after 5th days of incubation. Maximum degradation of FD2 dye occurred about 77.22% and lowest percentage observed in FD3 about 38.12%.

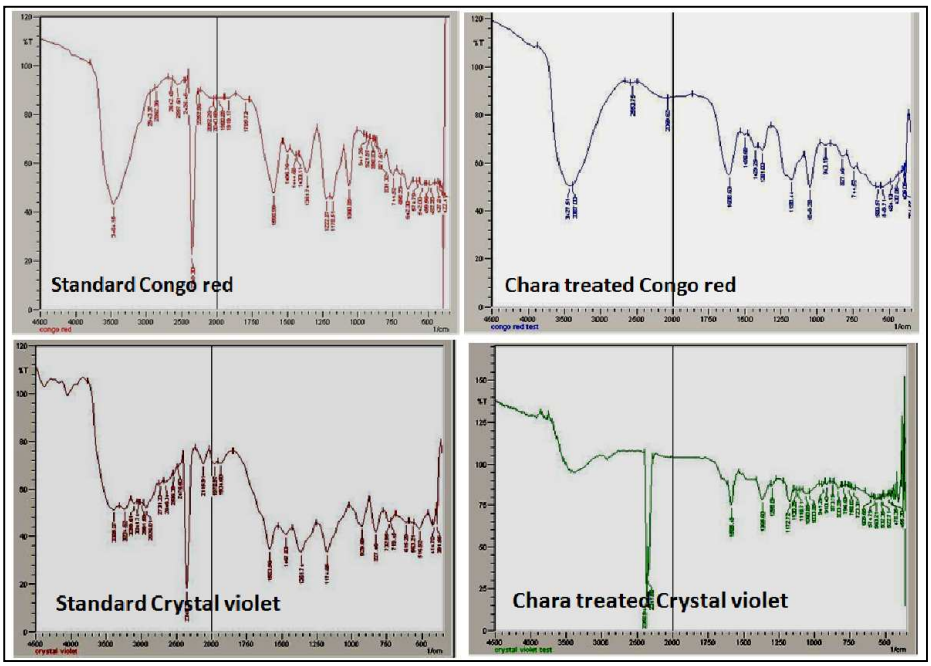

Figure 5 FTIR spectra of pure and bio-degraded Congo red and Crystal violet by Chara extract beads

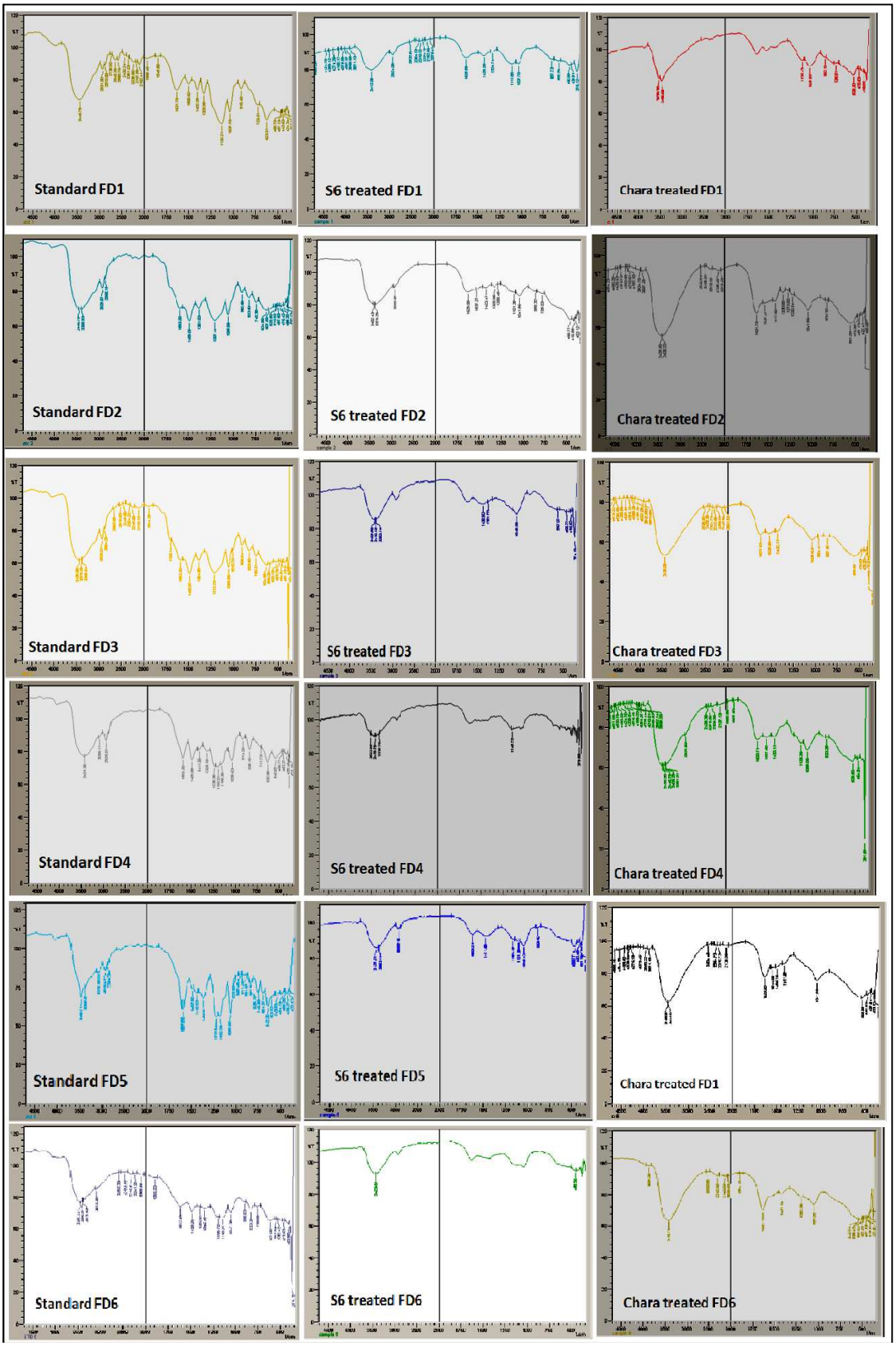

Figure 6 FTIR spectra of pure and bio-degraded all six Fabric dyes

To further, confirm the results of dye degradation ability of algae, laboratory dyes like Congo red and crystal violet used. The FTIR spectra of Congo red and crystal violet are shown in figure 5 whereas, the FTIR spectra of all the fabric dyes are shown in figure 6. The IR vibrational spectra of Congo red exhibit characteristic peak in fingerprint region associated to un-substituted, multi-substituted napthalene or benzene ring. This supported by peaks at 642 cm^{-1} for C-H bending vibration, 644 cm^{-1} that corresponds to the C-C bending vibration, 698 cm^{-1} for C-H stretching vibrations for disubstituted aromatic compound, 744 cm^{-1} for CH_2 bending vibrations, 831 cm^{-1} corresponding to P-disbstituted ring vibration, 1060, 1361 cm-1 for S=O stretching vibrations of sulfonic acid, 1444 cm^{-1} for aromatic C=C stretching vibrations 1598 cm^{-1} for N=N stretching vibrations, and 1611 cm^{-1} to stretching vibrations of C=C while 3464 cm^{-1} for NH stretching of NH_2 group. Whereas, the FTIR of metabolite obtained after decolorisation showed reduction in 642 cm^{-1} for C-H bending vibration, 644 cm^{-1} that corresponds to the C-C bending vibration, 698 cm^{-1} for C-H stretching vibrations for disubstituted aromatic compound. Similar, results recorded after decolorisation of Congo red by *Fusarium sp.* TSF-01 [23].

The FTIR spectra of pure crystal violet shows characteristic absorption bands at 1582, 1358 and 1164 cm^{-1}[24], broad bands in the 3600 - 3099 cm^{-1} region, which is due to the OH stretching vibration gives considerable information concerning the hydrogen bonds, band 2926 cm^{-1} corresponding to the C-H vibration, FTIR absorption band at 1467 cm^{-1} assigned to a symmetric CH_2 bending vibration, 1978, 1583 and 1361 cm^{-1} which are indicative for C=C in aromatic ring and to C-N stretching in aromatic tertiary amine respectively. The metabolites remaining after decolorisation showed the reduction in 3600-3099, 2926, 1978 and 1467 cm^{-1} peaks which represent OH stretching vibration, C-H vibration, C=C in aromatic ring and CH_2 bending vibration respectively. Similar results obtained for crystal violet decolorisation by *Spirulina platensis* [25]. Reports are available on degradation of dyes by use of algae. The macroalgae *Chara vulgaris* used as a viable biomaterial for biological treatment of congo red [21]. The Malachite green removed by *Chlorella vulgaris* (100%) of the color in 10 days the *Scendesmus quadriquada* in 8 days [26]. The highest percentage of degradation of naphthalene by *Nostoc linckia* after 7 days 47.71%, while the highest percentage of degradation of anthracene by *Elkatothrix viridis* after 7 days 92.28% [27]. The *Listeria sp.* found to be the best candidature for biodegradation of azo dyes [28]. The *Cosmarium sp*. doable biomaterial for removal of triphenylmethane dye, malachite green [29].

Table 3: Reduced percentage of toxicity after dye degradation

Fabric dyes	Antibiotic	Degraded dye	Non- Degraded dye	Reduced percentage of toxicity
FD1	21	09	35	25.71
FD2	22	10	32	31.25
FD3	20	06	>40	15
FD4	21	06	29	20.68
FD5	18	08	40	20
FD6	20	09	34	26.47
CR	17	11	>40	27.5
CV	19	07	26	26.92

**Unit of zone of hydrolysis is in mm*

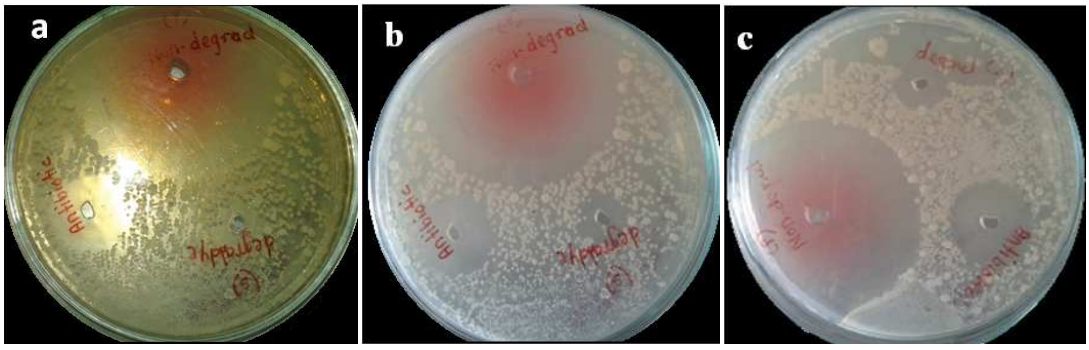

Figure 7 Toxicity testing of non degraded, degraded dye and streptomycin as a positive control against *E. coli*, a) Fabric dye 3, a) Fabric dye 5, and a) Fabric dye Congo red

3.4 Toxicity testing of pure and degraded fabric dyes

The toxicity against *E.coli* of degraded and standard dyes can be determined by observing the restricted zone of hydrolysis. The result reveals the toxicity of dyes reduced to maximum 31.25 % of FD2 and minimum 15 % of FD3. The results are given in table 3 and shown in figure 7.

CONCLUSION

Earlier researcher describe the dye degradation, none of them have discussed about dye precipitation. However, in our studies we have found the precipitation which may be suitable for easily removal of dye from large scale samples. The algae must have some factors which are responsible for the precipitation of dyes, whereas the dye degradation may be due to the enzymes like peroxidase and laccase etc. To understandfully, the mechanism, further study is essential. Coincidentally a single reference obtained on precipitation of dye, which has used approximately 0.06 M ionic liquid 1-butyl-3-methylimidazolium hexafluoro phosphate to aqueous solutions of six popular cationic dyes resulting in their precipitation [30]. From these findings, it is presumed that the dye not only degraded but precipitated by the *Chara sp*. and *Scenedesmus obliquus* through interaction with the active functional groups. The molecular physiology and chemical reaction within algal molecules and dyes remain unanswered and need further study.

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