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Decolorization and Degradation Studies on Dye Contaminated Effluent from Textile Industry using Aspergillus sp

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ABSTRACT

Availability, pollution and treatment of water are of major concern of present time. Many micro-organisms are capable for wastewater treatment. In the present study, feasibility of decolorization of dye contaminated effluents collected from different textile industries was examined using *Aspergillus tamarii* and *Aspergillus flavus* in batch and continuous reactor. *Aspergillus flavus* was found to be more efficient than *Aspergillus tamarii* to decolorize the effluents. Maximum decolorization of 85.3% was observed at optimized pH with 50% diluted effluent sample (ES1) using *Aspergillus flavus* (pH 4.5) as compared to 82.2% obtained using *Aspergillus tamarii* (pH 5). Chemical oxygen demand (COD) reduction has been observed to be 75% after decolorization of ES1 using *Aspergillus flavus*. Scanning Electron Microscopy (SEM) and Gas chromatography–mass spectrometry (GC-MS) analyses strongly supported biosorption as well as biodegradation of dye during decolorization.

1.Introduction

The textile industry accounts for usage of major part of the dyes manufactured and it has one of the largest water footprints across the globe [1-2]. Different processes in textile industry require varying amount of water and generate a substantial quantity of effluent containing high concentration of pollutants and inorganic salts. The toxic effluent is characterized by high chemical oxygen demand (COD), dissolved solids, soaps and detergents, finishing chemicals, sodium sulphate, sodium chloride, sodium hydroxide, traces of other salts and mixture of dyes. Discharge of untreated effluents in environment results in nuisance due to color, toxicity and carcinogenic or mutagenic forms of carcinogens such as benzidine, naphthalene and other aromatic compounds [3-5]. Quantity of effluent and concentration of pollutants depend upon the scale of production, chemicals used and technologies adopted. Textile wastewater is generally treated with physico-chemical processes, including flocculation-flotation, electro-flocculation, membrane filtration, ion-exchange, precipitation, etc. [6-7]. However, these processes are expensive, highly energy intensive, produce hazardous by-products and less effective for wide range of dyes. For small and medium scale industries these techniques become commercially non-viable. Therefore, the focus has recently been shifted towards bioremediation of dye contaminated wastewaters using bacteria, algae and fungi which have been reported to remove color and degrade dye in aqueous solution [8-15]. Fungi have advantages in bioremediation over other microbes due to their high tolerance level for contaminants and higher yield of biomass [16-18]. Most of the studies available on decolorization and degradation of dye by fungal species have been reported using synthetic dye solutions prepared in the laboratory in batch bioreactor [19-20]. The potential of both actively growing organism and non-living dead biomass for bioremediation has been explored by different researchers [21-23]. However, very little information is available on bioremediation of actual textile industry effluent.

In the present study, decolorization and degradation of dye have been studied for actual effluents procured from different textile industries using *Aspergillus* sp. in both batch and continuous modes of operation. *Aspergillus* sp. (*A. flavus* and *A. tamarrii*) isolated in the laboratory and used in the present study, were reported earlier to remove color and degrade various dyes in synthetic solutions prepared in the laboratory [24-25].

2. Materials and Methods

2.1 Effluents

The actual effluent samples (ES1, ES2, ES3 and ES4) were collected from four different textile industries located near Delhi, India. The effluents were stored in high-density polyethylene (HDPE) containers. The characterization of the effluents (shown in Table 1) for total dissolved solids (TDS), total solids (TS), total suspended solids (TSS), pH and chemical oxygen demand (COD) was done using standards methods of American Public Health Association (APHA 1989) [26].

Table 1. Summary of characteristic properties of ES1, ES2 and ES3 as-received.

Effluent Sample No.	pH	TSS (mg/L)	TS (mg/L)	TDS (mg/L)	COD (mg/L)
ES1	7.8	690	4540	3850	2000
ES2	7.6	610	3020	2410	1600
ES3	8.2	470	3760	3290	1200
Discharge Standards *	5.5 - 9.0	100	-	-	250

* For discharge in inland surface water.

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2.2 Micro-organisms and growth media

The micro-organisms used in decolorization experiments were *Aspergillus tamarii* and *Aspergillus flavus*, isolated in the laboratory from sludge of textile industry and effluent of electroplating industry, respectively[24-25]. The micro-organisms were grown in 250 mL conical flasks with 100 mL of growth media [24] [24]. The natural pH of the media was found to be 6.2. The media was sterilized and was inoculated with *Aspergillus tamarii* and *Aspergillus flavus*, separately. The flasks were incubated in Orbitek shaker at 27° C and 110 RPM for 84 hours. These cultures were preserved for further study. The pH was measured using Digital pH meter Systronics.

The pH was adjusted by using 0.1(N)HCl and 0.1(N)NaOH as per the requirement of the experiments. The effluent media was prepared by adding nutrients to the effluent collected from textile industries [24].

2.3 Batch decolorization

2.3.1 Using growing cells

To examine maximum decolorization in the effluents the experiments were carried out (i) using effluents as-received, (ii) by adjusting pH of the as-received effluents to the optimum values for maximum growth of *Aspergillus tamarii* (pH: 5) and *Aspergillus flavus* (pH:4.5) and (iii) by diluting the as-received effluents to 50% and keeping the pH at optimum. All the effluent-media were sterilized and were inoculated with *Aspergillus tamarii* and *Aspergillus flavus*, separately under above mentioned conditions (i, ii and iii). The flasks were incubated in Orbitek shaker at 27° C and 110 RPM for 84 hours. The liquid samples were withdrawn, centrifuged and analyzed for optical density to determine color intensity in the liquids using UV-VIS spectrophotometer (Systronics 117 UV-VIS). The optical density of each sample of effluents (as-received) was scanned for the visible spectrum in the range 300 nm to 900 nm. Peaks of absorbance were observed at different wavelengths. Peak absorbances were used to calculate percentage decolorization of the effluent using the formula (1) given below:

$$\% \text{ Decolorization} = [(C_0-C_1)/C_0] \times 100 \quad (1)$$

Where, C_0 = initial optical density of effluent and C_1 = optical density of effluent after decolorization. The fungal biomass was separated from the culture broth by filtration. The collected biomass samples were dried overnight (12 hours) in an oven at 60°C. The biomass concentration was determined gravimetrically. The decolorization study was monitored with respect to time for 84 hours with ES1 at optimized pH with 50% dilution.

2.3.2 Re-using desorbed biomass

The biomass of *A.tamarii* and *A.flavus* obtained from the above decolorization experiments was subjected to desorption using elutants. The benefits arising out of reuse of desorbed biomass for decolourization include reduction in cost of bioremediation process and more in-depth information of decolorization mechanism. The biomass containing color removed from the effluent was harvested from the culture broth, washed twice with distilled water and dried. The eluting reagents such as distilled water, (0.1-0.2N)H_2SO_4 and (0.1-0.2N)HCl were used in the present study to examine extracellular biosorption of dye in the cells. The dried biomass was taken in 250mL Erlenmeyer flasks containing 50mL of different concentrations of each eluting reagent. The flasks were agitated for 30minutes in shaker at 110 RPM. The liquid samples were withdrawn, centrifuged and analyzed for optical density to determine color intensity in the liquids using UV-VIS spectrophotometer. The desorbed biomass separated from the liquid phase was also mechanically destructed, grinded and washed with distilled water to remove the color from the cells. The liquid after washing the destructed cells was analyzed for optical density. This was done to examine intracellular bio-accumulation in the biomass. Further experiments were conducted using desorbed biomass (washed with distilled water and dried) of *A.flavus* in decolorization of ES1, 100mL of which was taken in 250 mL Erlenmeyer flask and agitated at 110RPM for 1hour.

2.3.3 Using non-living biomass

In order to explore whether decolorization process is growth associated or not, the experiments were also conducted using non-living *Aspergillus tamarii* and *Aspergillus flavus* as adsorbents. Fungal biomass was grown in synthetic media and separated from culture via filtration and dried. The dried biomass was grinded, sieved to size fraction between 0.5 – 1.0 mm and stored in air-tight containers. A weighed amount of dried biomass was added to 100 mL of effluent sample ES1 with 50% dilution for adsorption experiments and the mixture was agitated at 110 RPM for 1 hour to the reach the equilibrium.

2.4 Decolorization using growing cells in continuous mode

The continuous system can treat a larger volume of effluent in a short duration of time as compared to the batch system. Based on the decolorization data generated from the batch reactors, further studies were conducted in continuous mode using growing *Aspergillus flavus* and effluent sample ES1 with 50% dilution at optimized pH of 4.5 under non-sterile condition. The schematic diagram of the continuous reactor assembly is shown in Figure 1. The 1L continuous stirred tank reactor (CSTR) with 400 mL working volume was operated at room temperature (27-30°C). Air was continuously supplied through an aerator at the bottom of the reactor. The media holding tank was connected to the reactor through a peristaltic pump for continuous feeding. The overflow from the reactor was collected in another flask. All the connections were made using the silicon rubber tube. The flow rate of the peristaltic pump was adjusted for 60 hours hydraulic retention time (HRT) in order to have maximum removal of color. The growth media components were added and inoculated with growing *Aspergillus flavus* in continuous operation. Initially, the reactor was operated in batch mode till steady state condition was reached, after which continuous feeding of effluent media was started. The process in continuous mode was monitored for 8 days. The samples collected at the outlet of the reactor at regular intervals were analyzed to determine color removal.

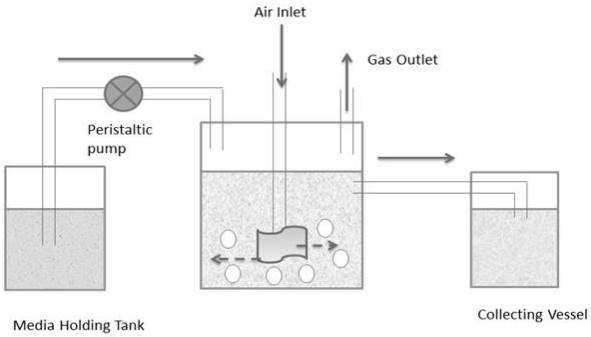

Figure 1. Schematic diagram of Continuous Stirred Tanks Reactor (CSTR).

2.5 Chemical Oxygen Demand (COD)

Since maximum decolorization was observed in ES1 using A. flavus, cicaheml oxygen demand (COD) was determined for ES1 after decolorization by open reflux method according to standards methods of American Public Health Association (APHA 1989)[26]. This COD value was compared with that determined in the ES1 before decolorization.

2.6 Scanning Electron Microscopy (SEM)

In the present study, scanning electron microscopy (SEM) was carried out to examine the cell surface and morphological changes of the *Aspergillus flavus* under different conditions as described below for ES1 using ZEISS EVO Series Scanning Electron Microscope EVO 50. The samples were kept on a stainless steel stub for analysis and the SEM pictures of *Aspergillus flavus* were taken at three magnifications i.e. 1 KX, 5 KX and 10 KX [27].

a) Biomass grown in media without any effluent
b) Biomass after batch decolorization of ES1using growing cells
c) Biomass after batch decolorization of ES1 using dead cells
d) After desorption of color from biomass obtained in (b)
e) Biomass after batch decolorization of ES1 reusing desorbed biomass
f) Biomass after decolorization of ES1 in CSTR using growing cells

2.7 Gas Chromatography-Mass Spectrophotometry (GC-MS)

The ES1 effluent sample used in the present study contained numerous dyes, chemicals and salts with unknown concentration. Therefore, to estimate the composition of ES1, GC-MS analysis was carried out for ES1 (as-received) before and after decolorization using Aspergillus flavus. The GC-MS analysis was conducted using GCMS-QP2010Ultra.

3.Results and discussion

3.1 Characteristics of the effluents

Preliminary batch-decolorization experiments conducted with four effluent samples ES1, ES2, ES3 and ES4 using Aspergillus tamarii and Aspergillus flavus upto an extended period of 84 hours to check the capability of micro-organisms for decolorization of the samples. The experiments indicated no growth of the micro-organisms in ES4 possibly due to presence of toxic components. The characteristic properties (such as pH, TSS, TS, TDS and COD) of ES1, ES2 and ES3 (as-received), therefore were determined and are shown in Table 1. Initial pH value was found to be lower and the values of TSS, TS, TDS and COD were found to be higher for ES1 than those observed with ES2 and ES3. The pH values of the prepared effluents media were found to be 6.8, 6.6 and 6.93 for ES1, ES2 and ES3 respectively after adding the growth nutrients. The peak absorbance (λmax) was observed from scanning through UV-VIS spectrophotometer at 598 nm, 552.8 nm and 580.0 nm for ES1, ES2 and ES3, respectively.

3.2 Batch Decolorization

3.2.1 Using growing cells

In the growth media containing no dye, the maximum biomass concentration of 5.2 g/L for Aspergillus tamarii and 6.3g/L for Aspergillus flavus has been reported earlier at pH values 5 and 4.5 respectively [24, 25]. In the present study, biomass concentration was found to be decreased in the actual textile industry effluents (ES1, ES2 and ES3) due to the presence of dye and other salts in the effluents, which inhibited the growth of the fungus. Table 2 shows a comparison of percentage decolorization and biomass concentration during growth of Aspergillus tamarii and Aspergillus flavus in ES1, ES2 and ES3 (as-received), at optimized pH and at optimized pH with 50% dilution. Higher percentage decolorization and higher biomass concentration were observed in case of Aspergillus flavus than those observed with Aspergillus tamarii under all the conditions in all the effluent samples. Also, the percent decolorization using both the fungi increased with increased biomass concentration. The increased decolorization of the effluents at optimized pH as compared to that obtained in as-received conditions is due to the increased biomass concentration. Table 2 also represents increased decolorization with dilution of effluents to 50% and at optimized pH. Dilution with water reduces the concentration of dyes and other salts present in the effluents. This results in improved growth of the fungi, higher biomass concentration and thus better decolorization of all the effluents.

Table 2. Percentage decolorization of effluents and biomass concentration using Aspergillus tamarii and Aspergillus flavus at as-received conditions, at optimized pH and at optimized pH with 50% dilution.

Effluent sample	Aspergillus tamarii		Aspergillus flavus	
	Decolorization (%)	Biomass concentration (g/L)	Decolorization (%)	Biomass concentration (g/L)
ES1 (as-received)	71.5	2.6	74.2	4.13
ES2 (as-received)	45.4	2.17	72.1	3.86
ES3 (as-received)	37.5	2.05	38.4	3.82
ES1 at optimized pH	76.6	3.68	79.8	4.72
ES2 at optimized pH	56.4	3.52	67.2	4.39
ES3 at optimized pH	52.7	3.50	67.0	4.38
ES1 at optimized pH and 50% dilution	82.2	3.92	85.3	5.22
ES2 at optimized pH and 50% dilution	58.4	3.73	73.8	4.69
ES3 at optimized pH and 50% dilution	55.3	3.74	71.4	4.40

The maximum decolorization was observed with ES1 at optimum pH values with 50% dilution of ES1 using both the organisms and hence further study was continued with this combination. Figure 2 shows percent decolorization with time using Aspergillus tamarii and Aspergillus flavus. The percent decolorization increases with time and reaches maximum at approximately 30 hours and then decreases for both Aspergillus tamarii and Aspergillus flavus. The maximum percent decolorization observed for Aspergillus tamarii was 82.2% as compared to 85.3% obtained with Aspergillus

flavus. The total biomass concentration was 3.92 g/L for Aspergillus tamarii and 5.22 g/L for Aspergillus flavus for duration of 84 hours. The results of experiments conducted in batch mode clearly indicate that both the fungi are capable of decolorization of textile effluent during their growth phase.

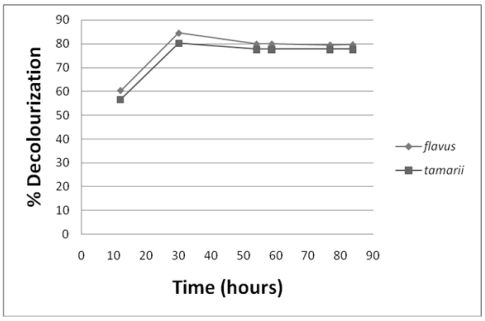

Figure 2. Percent decolorization of textile effluent sample in batch mode with time using Aspergillus tamarii and Aspergillus flavus.

3.2.2 Re-using desorbed biomass

The extent of desorption of color from the cell mass depends upon the hydration of metal ions, functional groups of the cell wall and their respective binding strength. Desorption experiments were conducted with both Aspergillus tamarii and Aspergillus flavus by using maximum upto 0.2(N) HCl and 0.2(N) H2SO4 so as not to destruct biomass structure. A marginal desorption (0.9%) of the sorbed colour was observed with distilled water. Table 3 shows that maximum desorption was 2.8 % for 30 minutes contact time using 0.2(N) HCl for both Aspergillus tamarii and Aspergillus flavus. No significant increase in desorption of color was observed with increased contact time between the biosorbent and the eluting agent. This indicates intracellular uptake of dye by the fungi. Effective desorption of the color could only be possible after complete destruction of the fungi cells. The percent color desorbed due to mechanical grinding of the cells has been estimated to be 4.3% of the sorbed color. These results suggest that total 8% color could be desorbed. Lower percentage of the color desorbed suggests biodegradation apart from extra and intra-cellular uptake of dyes by the organisms. Less biosorption and mainly bio-degradation of dye might have taken place during the biotreatment with Aspergillus tamarii and Aspergillus flavus. Also, the lower value can be due to destruction of color during acid treatment.

Table 3. Percentage color desorbed with respect to effluents as-received.

Eluting Agents	% Desorption	
	Aspergillus tamarii	Aspergillus flavus
Distilled Water	0.9	0.9
Sulphuric Acid (H_2SO_4)		
0.1 N	0.3	0.3
0.2 N	0.9	0.9
Hydrochloric acid (HCl)		
0.1 N	0.4	0.4
0.2 N	2.8	2.8

The desorbed biomass of Aspergillus flavus was reused as adsorbent for decolorization of ES1 (as-received). The percent decolorization observed with desorbed biomass using Aspergillus flavus was 31% as compared to 74.2% under as-received conditions using growing Aspergillus flavus. Decolorization has been observed to be reduced as compared to decolorization using living micro-organisms which involves extra-cellular biosorption, intra-cellular biosorption and biodegradation processes. Decolorization due to dead biomass suggests only adsorption on the surface of the dead biomass due to lack of growth activity, whereas decolorization using growing cells appears to be growth associated.

3.2.3 Using non-living biomass

The decolorization in the batch studies using non-living Aspergillus flavus has been observed to be 39%. Lower decolorization using dead biomass as compared to that obtained using growing biomass indicates only biosorption on the cell surface of the dead biomass. The decolorization has been observed to be within very close range of 39% for non-living and 31% for desorbed biomass using Aspergillus flavus. Marginal increase in decolorization using non-living biomass in comparison to the desorbed biomass may be due to the non-availability of certain sites for biosorption in the former.

3.3 Decolorization using growing cells in continuous mode Figure 3 shows the percent decolorization with time in continuous mode of operation at Hydraulic Retention Time (HRT) of 60 hours. During transient stage of operation of the reactor the percent decolorization was observed to increase. The decolorization increased till the reactor stabilized and reached a steady state condition. The commencement of steady state operation was suggested by the constant value of decolorization. The maximum percent decolorization was observed to be 63.9% initially in batch mode. Subsequent to which the decolorization decreased to 50% due to onset of continuous mode. As per the Table 3, the percent decolorization during batch studies using Aspergillus flavus at optimized pH and 50% dilution was observed to be 85.3%. Lower percent decolorization has been obtained due to operation in continuous mode in non-sterile conditions and ambient temperature in comparison to batch studies conducted in sterile and controlled conditions.

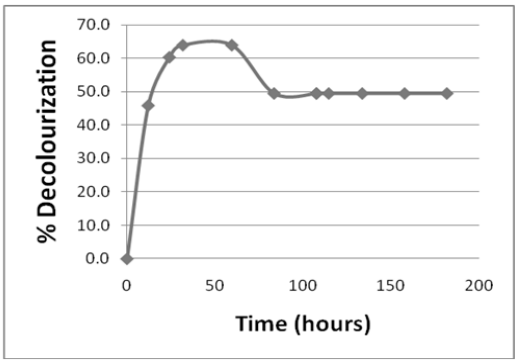

Figure 3. Percent decolorization with time for continuous mode using Aspergillus flavus.

3.4 COD reduction

The COD was analyzed for ES1 before and after decolorization. After decolorization of ES1 at optimized pH and with 50 % dilution, the maximum percent decolorization (85.3%) was observed using *Aspergillus flavus*. The COD value of ES1 as-received was 2000 mg/L before decolorization (Table 1). The reduction of COD in the treated effluent was found to be approximately 75% at optimized pH and 50% dilution for ES1.

3.5 Scanning Electron Microscopy (SEM)

Scanning electron micrographs of *Aspergillus flavus* with ES1 is shown in Figures 4 (a-f) for magnification of 5 KX. Figure 4 (a) shows micro-organism having smooth mycelia in the absence of textile effluent i.e. before decolorization. Figure 4(b) shows biomass after batch decolorization of ES1using

growing cells. The mycelia appeared to be distorted in the effluent. This is due to the fact that the components present in the effluent inhibited the growth of the micro-organism. Figure 4(c) shows biomass after batch decolorization of ES1 using dead cells. The surface of the micro-organisms appeared to be smooth to have added components due to adsorption phenomenon. Figure 4(d) shows biomass obtained in (b) after desorption of color. The micro-organism surface appeared to be fragmented due to mechanical grinding and strong eluting reagents i.e. H_2SO_4 and HCl used during desorption studies. Further, Figure 4(e) shows biomass after batch decolorization of ES1 reusing desorbed biomass. The surface of the micro-organism appeared to be more distorted due to combined action of mechanical grinding, eluting agents and adsorption of the textile effluent. Figure 4(f) shows biomass after decolorization of ES1 in CSTR using growing cells. The surface of the micro-organism appeared to be distorted as compared to micro-organism grown in absence of the effluent.

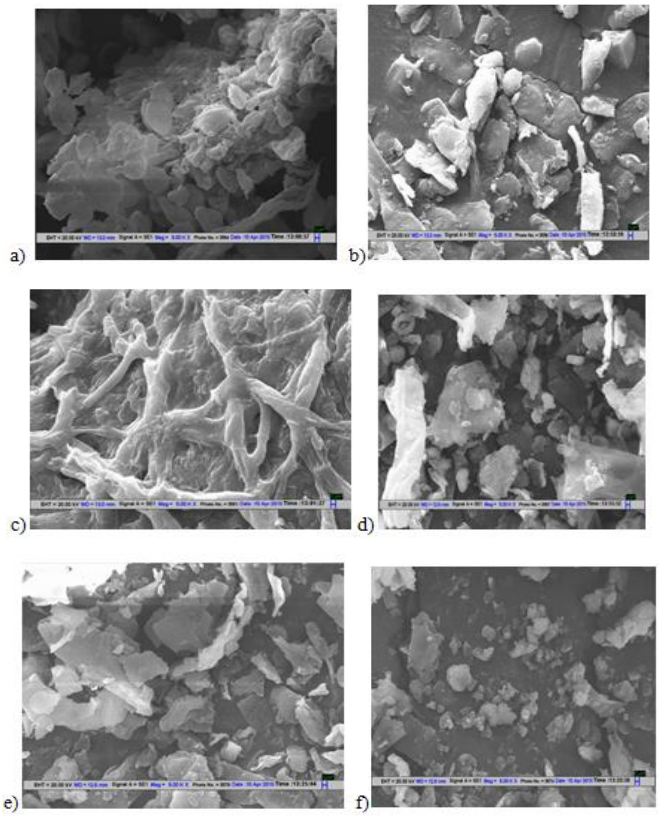

Figure 4. a) Biomass grown in media without any effluent, b)Biomass after batch decolorization of ES1using growing cells, c) Biomass after batch decolorization of ES1 using dead cells, d) After desorption of color from biomass obtained in (b), e) Biomass after batch decolorization of ES1 reusing desorbed biomass, f)Biomass after decolorization in CSTR of ES1 using growing cells

3.6 Gas Chromatography and Mass Spectrometery (GC-MS)

Textile effluent contains numerous components due to various processes involved in textile processing. Gas Chromatographic analysis was done for ES1 to assess the components present in the textile effluent samples collected from the industries. Chromatograms generated through GC-MS analysis has been shown in Figures 5 and 6. Reduction of peaks or appearance of new peaks indicates generation of new components [28-29].

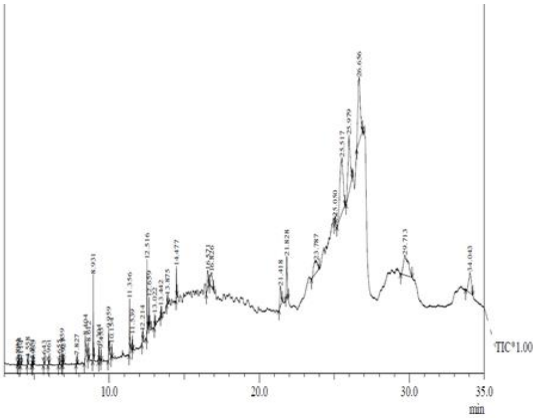

Figure 5: Chromatogram of ES1 as-received

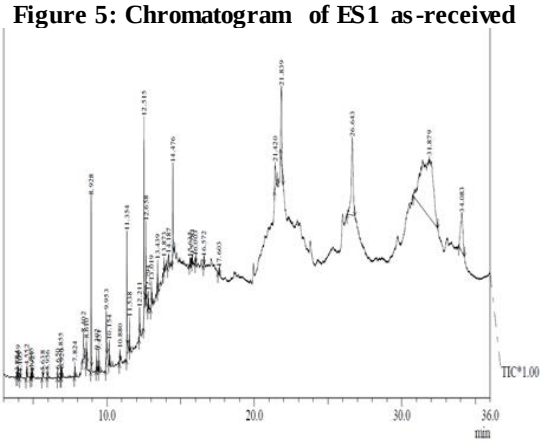

Figure 6: Chromatogram of ES1 after decolorization using Aspergillus flavus

Different components with maximum retention time and % area in chromatogram of ES1 before and after decolorization with *Aspergillus flavus* have been presented in Table 4. Retention time (RT) and percent area of the peaks obtained in ES1(as-received) were observed to be different from the peaks obtained in ES1 after decolorization using *Aspergillus flavus*. It is observed that multiple small peaks are obtained (as shown in the Figure 6) for effluent after decolorization. This implies that during decolorization using *Aspergillus flavus* the composition of effluent has been changed through biodegradation.

Table 4. Different components, their retention time(RT) and % area in chromatogram of ES1 as-received and after decolorization.

Components	ES1 as-received		ES1 after decolorization	
	RT	% Area	RT	% Area
Hexadecanoic Acid, Octadecyl Ester	33.39	5.75	33.772	3.67
Dodecanoic acid, ethenyl ester	21.418	2.64	21.42	1.64
Heptadecafluorononanoic acid, pentadecyl ester	23.787	4.89	---	---
Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester	76.14	32.49	17.603	0.29

4. Conclusions

The study was carried out to ascertain the potential of Aspergillus tamarii and Aspergillus flavus in treating effluent samples collected from different textile industries. Aspergillus flavus was found to be more efficient than Aspergillus tamarii for decolorization of the effluents with maximum decolorization of 85.3 % and 82.2 % respectively for ES1. The desorption studies indicated that decolorization of the effluents was due to both biosorption (intra and extra-cellular) and

biodegradation by the micro-organism. The maximum percent color desorbed was found to be 2.8% using 0.2(N) HCl as eluting reagent and the percent color desorbed after mechanical grinding of the desorbed biomass was 4.3%. This suggests bio-degradation of dye during the decolorization with Aspergillus flavus. Also, the SEM analysis clearly shows the changes in morphology of Aspergillus flavus after decolorization of the effluent in different conditions. The outcomes of GC-MS study supports that the biodegradation of the dye has taken place during decolorization as the peaks have been reduced and new peaks arrived in the chromatogram after decolorization of effluent. In the continuous mode the percent decolorization was observed to be 50 %. The desorbed biomass resulted into decolorization of 31%, whereas non-living biomass resulted in decolorization of 39%. It indicates that the biosorbed biomass can be reused for decolorization after desorption and Aspergillus flavus can treat efficiently industrial effluent contaminated with dye.

5. References

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