Treatment of Simulated Textile Wastewater Containing Reactive Azo Dyes Using Laboratory Scale Trickling Filter

A. Irum, S. Mumtaz, A. Rehman, I. Naz, S. Ahmed

***Abstract*—**The present study was conducted to evaluate the potential applicability of biological trickling filter system for the treatment of simulated textile wastewater containing reactive azo dyes with bacterial consortium under non-sterile conditions. The percentage decolorization for the treatment of wastewater containing structurally different dyes was found to be higher than 95% in all trials. The stable bacterial count of the biofilm on stone media of the trickling filter during the treatment confirmed the presence, proliferation, dominance and involvement of the added microbial consortium in the treatment of textile wastewater. Results of physicochemical parameters revealed the reduction in chemical oxygen demand (58.5-75.1%), sulphates (18.9-36.5%), and phosphates (63.6-73.0%). UV-Visible and FTIR spectroscopy confirmed decolorization of dye containing wastewater was ultimate consequence of biodegradation. Toxicological studies revealed the nontoxic nature of degradative metabolites.

***Keywords*—**Biodegradation, textile dyes, waste water, trickling filters.

I. Introduction

During the past few decades, the textile industry has grown economically at national and international level and became a leading contributor to an alarming increase in pollution of water bodies both in terms of volume and composition [1], [2]. It is estimated that the number of commercially available dyes is more than 100,000 and world's annual production of colorants is over 7x 10^5 tons [3]-[5]. In general, textile wastewater is quiet complex and has a great amount of organic compounds and dyes that contribute to Chemical Oxygen Demand (COD) or cause considerable color to the effluent in the case of dyes [6]. Some quantity of dye is lost during its application and its discharge in surrounding water bodies can vary from 2 to 50% for basic and reactive dyes respectively and thus contribute to severe contamination of surface and groundwater table [7]. Inappropriate methods of disposal in water bodies greatly influence the physicochemistry of water by reducing the sunlight penetration that affects the photosynthetic activity. This in turn leads to decreased levels of dissolved oxygen, thereby negatively affecting the aquatic flora and fauna. Majority of dyes and their degradation products i.e. aromatic amines are toxic, mutagenic and carcinogenic [8].

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Reactive dyes constitute an important class of textile dyes. Due to their intensive application in textile industry, almost 45% of annually manufactured textile dyes belong to this class and they constitute a major proportion of textile wastewaters. Due to their extensive use, highly water soluble nature and limited biodegradability in an aerobic environment, these dyes are among the most problematic and challenging agents in textile effluents [9].

Over the last few decades, treatment of textile wastewater has emerged as a challenge [10]. Various methods i.e. physical, chemical and biological are used for removal of dyes from textile effluents. Due to chemical stability of synthetic dyes, conventionally practiced wastewater treatment techniques have not contributed towards promising outcomes [3]. Biological methods however are usually preferred over physical and chemical methods because they are specific, less energy consuming, cost effective and eco-friendly as they bring about partial or complete biotransformation of organic pollutants to stable and nontoxic end products [11], [12]. Different microorganisms such as bacteria, fungi, yeasts, algae and actinomycetes are capable of decolorization and even complete mineralization of wide range of dyes [11], [13].

Bacteria are among the most frequently used microorganisms for the bioremediation of textile wastewaters as they are adaptable to extreme conditions of temperature and salinity, are easy to cultivate and produce different types of oxidoreductases [14], [15]. Normally bacterial decolorization is relatively faster than fungal [16]. There are many reports of dye decolorizing bacteria either in pure culture or consortium [3], [4], [7], [11]-[16]. Use of bacterial consortia is advantageous over the use of individual strains as the dye is targeted at different positions and the metabolites generated by the coexisting strains are completely mineralized [17].

Biological trickling filters has been documented as reliable technology for the wastewater treatment from different sources including industrial, agricultural, municipal, swine wastewater and acid marine drainage etc. [18]-[23]. Basically, trickling filter is an attached growth system in which biofilm support media provides surface to biomass growth and microbial attachment, and contact with contaminants to be removed [24]. Lower energy requirement, lesser biomass production and relatively shorter treatment time are the advantages of attached growth systems over conventional activated sludge processes [25], [26].

Despite the significant number of reports demonstrating the potential of microorganisms (bacteria, fungi, yeast, protozoa) to successfully degrade different types of textile dyes in small-scale sterile batch tests [8], only few studies have been conducted to evaluate their proficiency and effectiveness in the biological trickling filter system under non-sterile conditions as would likely be experienced in real practice [27], [28].

The present work aimed at the development of a treatment system for the simulated textile wastewater using biological trickling filter. The biofilm of preselected bacterial strains proficient in dye decolorization was developed on stone carrier in a trickling filter reactor to decolorize three chemically different reactive dyes. The sand column filtration is added as a finishing step after treatment. The performance of the system was evaluated through detailed examination of physicochemical and microbiological parameters.

II. Materials and Methods

A. Chemicals and Microorganism

Most of the chemicals were purchased from Oxoid (USA) and Sigma-Aldrich (USA). The textile dyes Reactive Red (RR 195), Reactive Yellow (RY 145) and Reactive Blue (RB 221) subjected to investigation were courteously provided by an industrial unit.

Three previously isolated bacterial strains proficient in decolorization of three reactive dyes were selected for biofilm development on stone media and identified as *Pseudomonas aeruginosa* SAD1, *Alcaligenes* sp. SAD2, *Brevibacillus parabrevis* SAD3 through 16 S rRNA sequencing. The bacterial cultures were maintained on Nutrient agar slants at 4^0C. The media used in this study for the growth of seed culture was Nutrient broth medium (NB) supplemented with respective dye (50 mg/L). The simulated textile wastewater used for the study contain g/l; Na_2HPO_4 3.6, KH_2PO_4 1.0, $MgSO_4$ 1.0, $(NH_4)_2SO_4$ 1.0, $CaCl_2.2H_2O$ 0.1, Fe $(NH_4)_2$ citrate 0.01. To this medium, trace element solution (10 ml/L) of following composition was added; $ZnSO_4.7H_2O$ 10.0, $MnCl_2.4H_2O$ 3.0, $CoCl_2.6H_2O$ 1.0, $NiCl_2.6H_2O$ 2.0, $Na_2MoO_4.2H_2O$ 3.0, H_3BO_3 30.0, $CuCl_2.2H_2O$ 1.0. Final pH of the medium was adjusted by pH meter (Sartorious professional meter PP15) to 7.0 using 1N HCl and 1N NaOH solution [4].

B. Experimental Set-up and Operating Conditions

A laboratory-scale BTF was built from polyvinylchloride (PVC) pipe (height =36, outer diameter=14 and inner diameter = 12.4 inches) of 22 L capacity (Fig. 1). A steel cage with fixers (height = 24 and diameter = 11 inches) inside the pipe was used to hold filter media having effective volume of 12 L. To collect treated wastewater an under-drain system (height = 8 inches) with an outlet (sampling port) at a height of 3 inches was positioned. For the supply of wastewater, a shower rose (diameter = 8) was employed on the top of media bed. Treated wastewater was collected in a plastic container of 25 L capacity. A water pump (power, 220 V) was used for recirculation of water. A plastic pipe of 125 cm connected with pump was employed to facilitate flow of water. Passive down flow aeration through a space between outer court and inner core (steel cage) was utilized to ensure aerobic conditions. With the help of peristaltic pump, 30 L of simulated textile wastewater was passed through bed of BTF providing a retention time of 250 min/cycle. An electric dimmer connected to the water pump controlled the flow of water. The sand column filter was made up of plastic column having height 39 inches and 3 inches diameter filled with sand (0.2 mm). A peristaltic pump was used to pump treated water into sand column filter from the collecting tube.

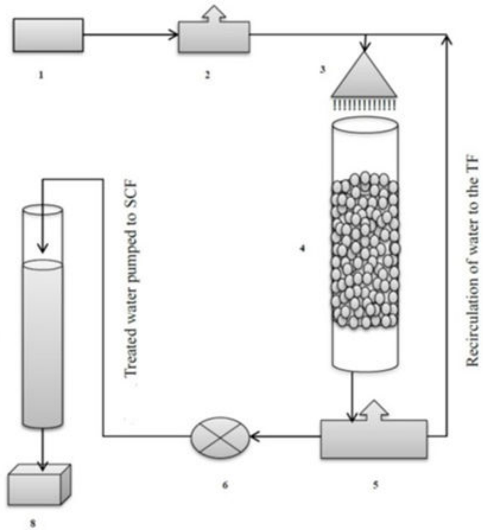

Fig. 1 Schematic diagram of treatment process, where; **1.** Sedimentation Tank for untreated wastewater, **2.** Plastic container with water pump, **3.** Shower rose, **4.** FBR filled with stone media, **5.** Plastic container with water pump, **6.** Peristaltic pump, **7.** Sand column Filter, **8.** Treated water

The three experimental trials were performed with three structurally different reactive textile dyes (Table I). Biofilm was developed on stone by seeding with enriched suspension of three bacterial strains for the development of biofilm before loading on the bioreactor for the reduction of start-up phase of the reactor.

TABLE I
Experimental Design

Trials	Incubation period (days)	Dyes	Dye Conc. (ppm)	Treatment Duration	
				Trickling Filter (Days)	Sand column filtration (Days)
1.	15	RB 221	50	9	2
2.	15	RY 145	50	9	2
3.	15	RR 195	50	10	2

C. Physicochemical Analysis

Physicochemical analysis of the influent and effluent at certain time intervals (24 hr) was carried out to study the performance of the reactor. The physicochemical parameters including chemical oxygen demand (COD), sulphates (SO_4^{2-}) and phosphates (PO_4^{2-}), Chloride (Cl^{1-}), Bicarbonates ($HCO_3^{\ 1}$)

were monitored using standard protocols (APHA). The change in concentration of dyes was determined by monitoring the shift in absorbance at the respective lamba maximum (λ max) of the dyes by using the spectrophotometer. The decolorization activity (%) was calculated by using [16]:

$$\text{Decolorization (\%)} = \frac{(I-F)}{I}\text{x}100$$

where, I = initial absorbance of coloured sample and F = Final absorbance of the decolorized samples.

D. Microbiological Analysis

To gain insight into microbial population proliferation, presence, degree of dominance and viability of the added microorganisms in the system CFU/ml was performed. Stones were collected from different zones of trickling filter, sonicated for 30 minutes in normal saline. Inoculated plates with diluted suspension were incubated at 37°C for 24 hrs. The colonies that appeared on plates were counted with the help of colony counter and the CFU/mL was calculated by multiplying with dilution factor and divided by volume plated in mL.

E. Biodegradation Analysis

FTIR spectra of samples (control and decolorized effluent obtained after bacterial treatment) were recorded by using FTIR (Perkin Elmer, spectrum 65 FTIR spectrometer, equipped with ATR) and transmission spectrum was recorded. Ethyl acetate extracted residues were analyzed at mid IR region of 400-4000 $cm^{-1.}$

F. Toxicity Assay

By using *Raphanus sativus* (radish) seeds, phytotoxicity of the untreated and treated samples was determined [29] and cytotoxicity of samples was tested by Brine Shrimp (*Artemia salina*) Lethality Assay [30].

III. Results and Discussion

An efficient treatment technology for degradation of dyes must use a combination of aerobic and anaerobic steps to achieve maximum remediation of textile effluent [31]. Use of trickling filter as treatment technology also fulfilled this requirement as different zones of trickling filter provide room for aerobic and anaerobic microbial degradation activity which is necessary for complete mineralization of dye. In the present study stones were used as trickling filter media. It is a cheap, inert and readily available media that is very efficient in developing biofilm i.e. bacterial and fungal [32]-[34]. For wastewater treatment, use of bacterial and fungal species for biofilm development is a common and old practice [35]-[37]. Fungal systems are usually less efficient due to relatively longer growth cycles as compared to bacteria [38]. Moreover, fungi show rigorous growth in wastewater. Therefore it gets hard to assure stable treatment efficacy [39]. Compared to pure cultures, microbial consortia generally demonstrated better decolorization ability [4]. Microbes in consortium can complement each other and collectively carry out biodegradation and in some cases complete mineralization that cannot be achieved by pure cultures [8], [40]. An improved decolorization rate by SDS bacterial consortium, consisting of *Pseudomonas aeruginosa* strain BCH and *Providencia* sp. was demonstrated [31] due to coordinated metabolic interaction. The present finding corroborates with the report by [13], who observed high decolorization performance of azo dye Scarlet R by consortium-GR, consisting of *Proteus vulgaris* NCIM-2027 (PV) and *Micrococcus glutamicus* NCIM-2168 (MG), which could mineralize the dye to near completion within 3 hr. In the present study, due to the enhanced decolorization rate demonstrated by biofilm of microbial consortium, it can be proposed that synergistic interaction and metabolic activities among the three bacterial strains might be responsible for efficient decolorization potential.

Bacteriological analysis of trickling filter media via CFU/ml serves as an important indicator of strength of bacterial population [41]. The results indicated that the microbial strains that were initially inoculated for biofilm development were the dominant one and involved in the treatment of wastewater (Table II). Of the three stains used, *Brevibacillus parabrevis* SAD3 principally dominated the biofilm micro flora.

TABLE II
CFU/ML OF BIOFILM ON STONE MEDIA

Experimental Trials		**CFU/ml**		
		SAD1	**SAD2**	**SAD3**
RB 221	Before Treatment	3×10^5	24×10^5	63×10^5
	After Treatment	1×10^3	18×10^3	45×10^3
RY 145	Before Treatment	5×10^5	20×10^5	72×10^5
	After Treatment	4×10^4	12×10^4	56×10^4
RR 195	Before Treatment	6×10^6	32×10^6	45×10^6
	After Treatment	4×10^5	11×10^5	23×10^5

Moreover, various strains of *Brevibacillus* have been well documented for their dye decolorizing potential by several other studies [42]-[44]. A considerable decline in the microbial load was observed at the end of treatment process. The possible cause for decline in microbial load might be the sloughing off of microbial cell during the treatment process.

From textile wastewater treatment perspective, COD removal is considered as an important parameter. However, the extent of COD reduction alone cannot be taken as an indicator of dye degradation, as glucose is the major source of COD. Therefore, the performance of the reactor for the actual degradation of dye was also monitored from the color removal profiles of the reactor. The decolorization percentage in case of reactive blue 221, reactive yellow 145, Reactive red 195 was 96.20%, 99.88%, 99.88% respectively (Fig. 2). The results revealed that the consortium reactor was efficient in terms of color removal under non-sterile conditions. Initially the decolorization percentage was low in case of all three dyes but progressive increase was observed till it reached the stable steady state. The carbon source functions as electron donor in the degradation of dye so its requirement is inevitable [45]. Electrons released from the oxidation of electron donor (carbon source i.e. glucose) are transferred by carriers of the electron transport chain to terminal electron acceptor i.e. dye molecule [46]. The color and COD removal efficiency became more proficient due to the mutually beneficial physical and physiological interactions among microorganism present in biofilm systems [47].

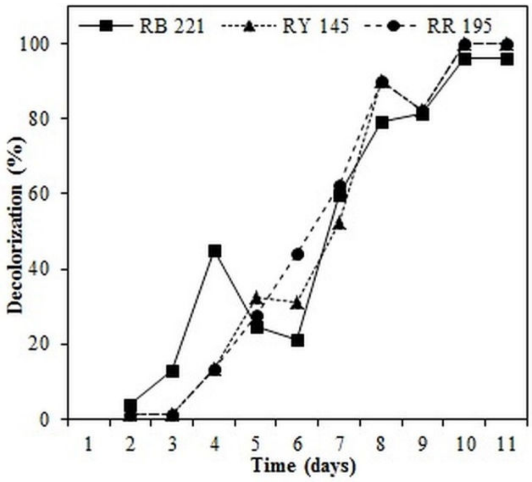

Fig. 2 Percentage decolorization profile of structurally different dyes

Chemical oxygen demand is an indirect indicator of organic pollutant level of water. Water is said to be waste if its COD lies above 1000 mg/l [48]. Chemical oxygen demand is an indirect indicator of organic pollutant level of water. COD test is a standard method that helps to determine the amount of pollution in water that cannot be oxidized biologically. In the initial trials with the single dye, the reduction in COD value was very low i.e. Reactive Blue 221 (59.93%), Reactive Yellow (67.14%), reactive Red (69.70%) (Fig. 3). Progressive increasing trend in COD removal rate was observed. The expected reason is the acclimatization of biomass to the dye substrate resulting in the increased COD removal efficiency until it reaches to stable value by the end of reactor operation [49]. The COD removal was found to be the same with three dyes indicating no significant effect of dye structure on COD removal.

Sulphates are an important constituent of wastewater and responsible for hardness of water [50]. According to world health organization, the permissible sulphate range is 25 mg/L. Water is said to be safe for irrigation purpose if its sulphate concentration ranges between 18-26 mg/L.

Simulated textile wastewater which was used as influent had sulphates concentration ranging from 44-46 mg/L (Fig. 4). Using stone as the trickling filter media, appreciable removal in sulphates concentration was not achieved and maximum reduction (36%) was achieved in the third trial dealing with 50 ppm of Reactive Red 195 dye (Fig. 4).

Phosphate content of wastewater is principle contributor to eutrophication. Therefore, phosphates must be efficiently targeted and subsequently utilized by degrading microorganism increasing water quality, making it safe for disposal. The phosphate content of simulated textile wastewater was 8.71-14.85 mg/L. Phosphate concentration was reduced to 4.09-2.03 in the treatment of three structurally different dyes (Fig. 5). Phosphorous is a vital constituent of bio molecules regulating metabolism and growth of organisms [51]. Phosphates that can be utilized by microorganisms are in the form of orthophosphates [52]. Utilization of phosphorous enhanced microbial growth, especially in stress condition [53], resulted in improved mineralization of dye leading to enhanced reduction in color and COD of the wastewater.

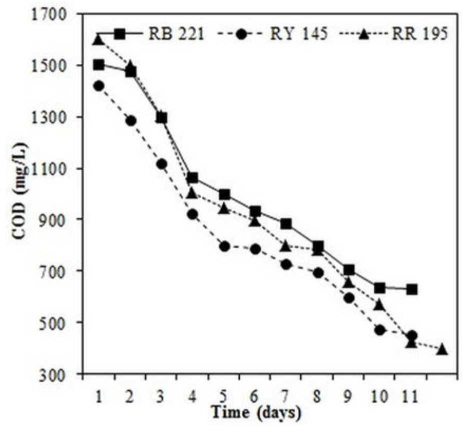

Fig. 3 Reduction Pattern of COD

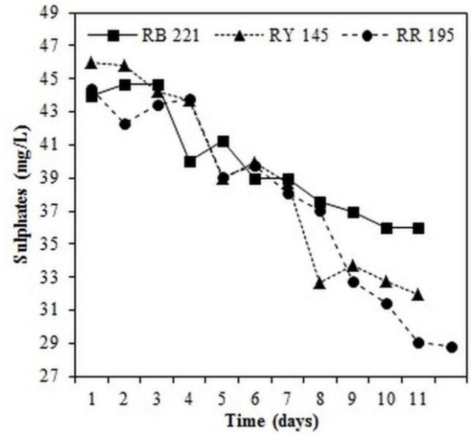

Fig. 4 Reduction profile of sulphate

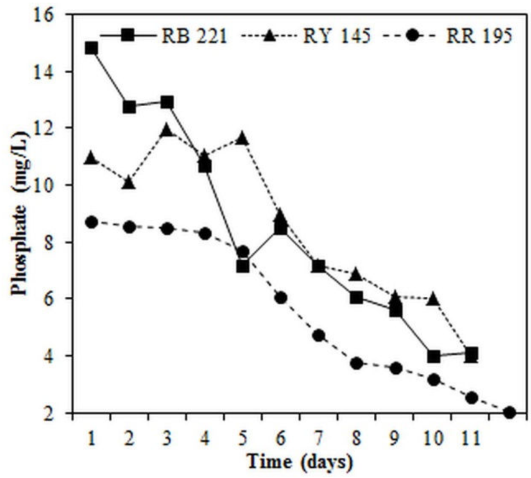

Fig. 5 Phosphate reduction profile during the treatment of waste water

Fig. 6 shows the results of different parameter which play an influential role in performance of the reactor. Alkalinity and pH are interrelated parameters which affect the economic feasibility of fixed film reactor [54]. A change in the values of effluent pH was observed from 7.6-8.0 to 6.5 to 6.9. The most likely reason in pH reduction might be the acidogenesis in the trickling filter by the microorganisms under reduced conditions. Therefore, pH is within the acceptable range for proper reactor operation and is a good indicator of the performance of the system. Most the dyes used in textile industry are alkaline in nature, but the initial pH of the system was 7.0 to maintain neutral conditions [55]. Major sources of alkalinity in a solution are carbonates and bicarbonates [56]. Textile effluent is rich in carbonates and bicarbonates. Carbonates were completely absent in all the samples and reduction in bicarbonate concentration was observed in treated samples. Dissolved oxygen is also referred as oxygen saturation. It is the relative measure of amount of oxygen present in water. Water that is rich in chemicals and nutrient is low in dissolved oxygen [57]. Recirculation technology employed during the trickling filter operation continuously aerated water. Chemical load is also reduced with the passage of time as microbes utilize and degrade nutrients so dissolved oxygen level rises. Textile effluent if disposed of improperly in any water body raises the chloride content. The permissible chloride concentration is <250 mg/L [58]. High chloride content reduces agricultural yield and adversely effects aquatic organisms. The simulated textile wastewater once processed through trickling filter and sand column, considerable reduction in chloride content was achieved.

A prominent difference in the peak of FTIR spectrum of untreated and treated water samples provide a clue for structural deformation of the dyes after treatment. FTIR spectrum obtained for control dye sample RB 221 showed peaks at 611 cm^{-1} corresponded to S=O, peak at 1406, 1626 and 2922 cm^{-1} indicated presence of aromatic amine, C=O region and C-H bond stretching respectively.

Peaks at 982, 1138, 1340 and 3364 cm^{-1} probably indicated C-O bonding, asymmetric N-H stretching, C-N stretching and formation of aniline respectively (Fig. 7). Similar peak pattern was reported by [59].

For Reactive Red 195 control peak obtained at 3442 cm^{-1} indicated aromatic ring. Several peaks in region between 3300 -3500 cm^{-1} showed presence of N-H and O-H bonds, carbonyl group was absent as no peak was observed at 1600-1680 cm^{-1}. For treated sample peaks obtained at 3270, 2789 and 1407 cm^{-1} indicated formation of aromatic amines, C-H and C-N stretching as shown in Fig. 8. Similar results were interpreted by [60].

In case of Reactive Yellow 145, prominent peaks at 3446, 1550, 1108, 1038 and 669 cm^{-1} indicated presence of aromatic ring, asymmetric C=O stretching, N-H bonds, C-N vibration in aliphatic amines and C-Cl bond respectively. Analysis of treated samples showed peak variation as shown in Fig. 9. Peaks at 3003, 1530, 1407 and 619 cm^{-1} represented formation of ketone, N=O vibration in aromatic nitro compound, aromatic tertiary amine and deformation in benzene ring [61].

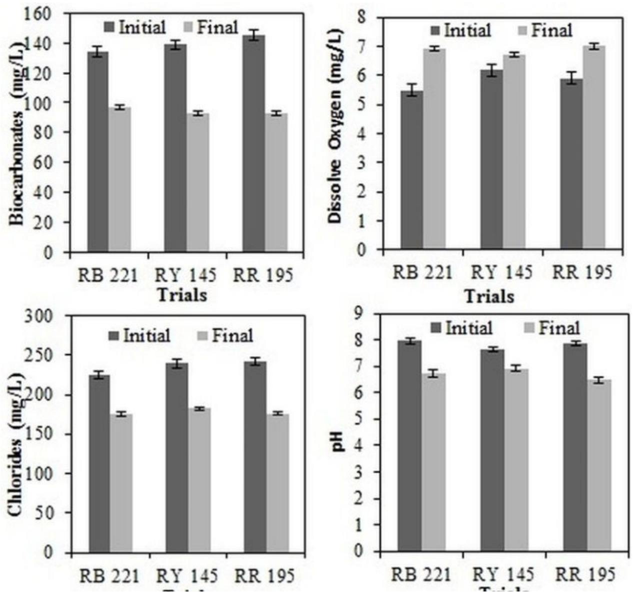

Fig. 6 Comparison of different physicochemical parameters before and after treatment

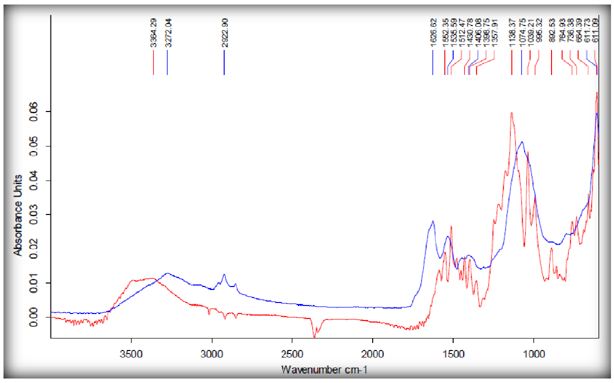

Fig. 7 FTIR overlay of untreated and treated water sample containing 50 ppm of Reactive Blue dye (RB 221)

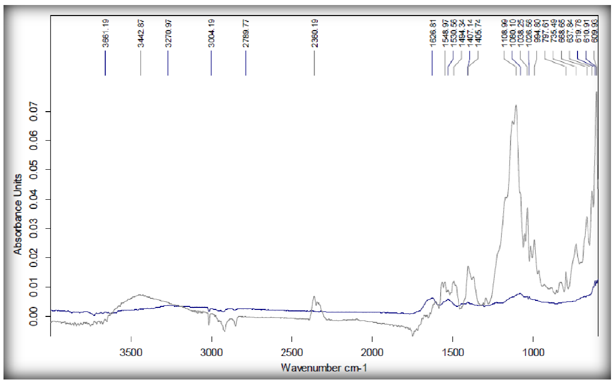

Fig. 8 FTIR spectrum of untreated and treated sample containing of Reactive red 195 (50 ppm)

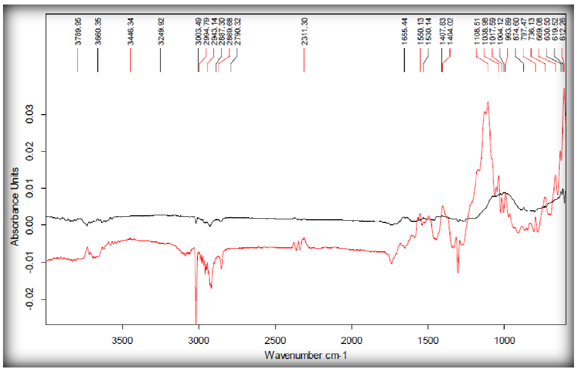

Fig. 9 FTIR spectrum of control and treated sample containing of Reactive red 195 (50 ppm)

Toxicity assessment of treated wastewater for determination of their environmental safety is very important, as the ultimate aim is reduction of environmental pollutant as well as their toxicity [7], [17]. Results of phytotoxicity assay depicted moderate detoxification following treatment in the biological trickling filter. Good germination rate as well as significant growth in the plumule and radical with treated wastewater, as compared to untreated dye sample was observed. The considerable reduction in percentage mortality of *Artemia salina* larvae with treated sample is attributed to detoxification of dyes after treatment.

IV. Conclusion

The biological trickling filter showed a great potential for the treatment of simulated textile wastewater containing structurally different dyes with variation in dye concentration. The preselected bacterial strains proficient in dye decolorization were remained as dominant strain of the biofilm involved in dye decolorization under non sterile conditions till the end of operation.

Acknowledgments

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References

[1] T.M. Uddin, A.M. Islam, S. Mahmud and M. Rukanuzzaman, "Adsorptive removal of methylene blue by tea waste," *J. Hazard. Mater.* vol. 164, pp. 53-60, 2009.

[2] T. Robinson, B. Chandran and P. Nigam, "Studies on the decolorization of an artificial textile-effluent by white-rot fungi in N-rich and N-limited media," *Appl. Microbiol. Biotechnol.* vol. 57, pp.810, 2001.

[3] S. A. Khehra, S. H. Saini, K. D. Sharma, S. A. Chadha and S. S. Chimni, "Decolorization of various azo dyes by bacterial consortium," *Dyes Pigm.* vol. 67, pp. 55, 2005.

[4] S. Moosvi, X. Kher and D. Madamwar, "Isolation, characterization and decolorization of textile dyes by a mixed bacterial consortium JW-2," *Dyes. Pigm.* vol. 74, pp. 723-729, 2007.

[5] R. M. Saeed, J. M. Mughal, S. S. Naeem, Nizami, H. Tanzile and Usmani, "Decolorisation of Remazol vinyl sulphone reactive dyes by potassium permanganate," *Colouration Technol.* vol. 125, pp. 277-283, 2009.

[6] N. Ali, A. Hameed and S. Ahmed, "Physicochemical characterization and bioremediation perspective of textile effluent, dyes and metals by indigenous Bacteria," *J. Hazard. Mater.* vol. 164, pp. 322-328, 2009.

[7] G. R. Saratale, G. D. Saratale, S. J. Chang and S. P. Govindwar, "Bacterial decolorization and degradation of azo dyes: A review," *J. Taiwan Inst. Chem. Eng.* vol. 42, pp. 138-157, 2011.

[8] Ö. Tunc, H. Tanaci and Z. Aksu, "Potential use of cotton plant wastes for the removal of Remazol Black B reactive dye," *J. Hazard. Mater.* vol, 163, pp.187-198, 2009.

[9] K. Sarayu, and S. Sandhya, "Current technologies for biological treatment of textile wastewater: a review," *Appl. Biochem. Biotechnol.* vol. 167, no. 3, 645-661, 2012.

[10] A. Pandey, P. Singh and L. Iyengar, "Bacterial decolorization and degradation of azo dyes," *Int. Biodeter. Biodegr.* vol. 59, pp. 73-84, 2007. R. Khan, P. Bhawana and M. H. Fulekar, "Microbial decolorization and degradation of synthetic dyes: a review," *Rev. Environ. Sci. Biotechnol.* vol. 12, pp. 75-97, 2012.

[11] R. G. Saratale, G. D. Saratale, D. C. Kalyani, J. S. Chang and S. P. Govindwar, "Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR," *Bioresour. Technol.* vol. 100, pp. 2493-2500, 2009.

[12] A. Khalid, M. Arshad and D. E. Crowley, "Decolorization of azo dyes by *Shewanella* sp. under saline conditions," *Appl. Microbiol. Biotechnol.* vol. 79, pp. 1053-1059, 2008.

[13] I. O. Ola, A. K. Akintokun, I. Akpan, I. O. Omomowo, V. O. Areo, "Aerobic decolorization of two reactive azo dyes under varying carbon and nitrogen source by *Bacillus cereus*," *Afr. J. Biotechnol.* vol. 9, pp. 672-677, 2010.

[14] D. C. Kalyani, A. A. Telkedhanve and J. P. Jadhav, "Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas sp.* SUK1," *J.Hazard. Mater.* vol. 163, no. 2-3, pp.735-742, 2009.

[15] U.U. Jadhav, V. V. Dawkar, G. S. Ghodake and S. P. Govindwar, "Biodegradation of Direct Red 5B, a textile dye by newly isolated *Comamonas* sp. UVS," *J. Hazard. Mater.* vol. 158, no, 2-3 pp. 507-516, 2008.

[16] M. Sudha, A. Saranya, G. Selvakumar and N. Sivakumar, "Microbial degradation of Azo Dyes: A review, *Int. J. Curr. Microbiol. App. Sci.* vol. 3, no. 2, pp. 670-690, 2014.

[17] F. J. Szogi, J. M. Humenik, and R. Hunt, "Swine wastewater treatment by Media Filtration," *J. Environ. Sci. Health.* Vol. 832, no. 5, pp. 831–843, 1997.

[18] M. R. Evangelho, M. M. Goncalves, M. S. Anna, G. L. Jr. and R. C. Villas Bo, "A trickling filter application for the treatment of a gold milling effluent. *Int. J. Miner. Proc. vol.* 62, pp. 279–292, 2000.

[19] Y. Tanaka, "A dual purpose packed-bed reactor for biogas scrubbing and methane-dependent water quality improvement applying to a wastewater treatment system consisting of UASB reactor and trickling filter," *Bioresource Technol.* vol. 84, no. 1, pp. 21-28, 2002.

[20] C. Wang, J. Li, B. Wang and G. Zhang, "Development of an empirical model for domestic wastewater treatment by biological aerated filter," *Process Biochem.* vol. 41, no. 4, pp. 778-782, 2006.

[21] E. Dermou, A. Velissariou, D. Xenos, and D. V. Vayenas, "Biological chromium (VI) reduction using a trickling filter," *J. hazard. Mater.* vol. 126, no. 1, pp.78-85, 2005.

[22] E. Dermou, and D. V. Vayenas, "A kinetic study of biological Cr (VI) reduction in trickling filters with different filter media types" *J. hazard. Mater.* vol. 145 no. 1, pp. 256-262, 2007.

[23] M. Mahmoud, A. Tawfik and F. El-Gohary, "Simultaneous organic and nutrient removal in a naturally ventilated bio tower treating presettled municipal wastewater," *J. Environ Eng.* vol. 136, pp. 301–307, 2010.

[24] Z. Shareefdeen and A. Singh, "Biotechnology for odor and air pollution control," Berlin, Springer, 2005.

[25] W, Guo, H. H, Ngo, F. Dharmawan and C. G. Palmer, "Roles of polyurethane foam in aerobic moving and fixed bed bioreactors," *Bioresource Technol.* vol. 101, pp. 1435–1439, 2010.

[26] M. Kornaros and G. Lyberatos., "Biological treatment of wastewaters from a dye manufacturing company using a trickling filter," *J. Hazard. Mater.* vol. 136, pp. 95–102, 2006.

[27] C. Novotny, K. Svobodova, O. Benada, O. Kofronova and A. H. W. Fuchs, "Potential of combined fungal and bacterial treatment for color removal in textile wastewater," *Bioresource Technol.* vol. 102, pp. 879–888, 2011.

[28] A. U. Turker and N. D. Camper, "Biological activity of common mullein, a medicinal plant," *J. Ethnopharmacol.* vol. 82, pp. 117-125, 2002.

[29] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. J. Nichols and J. L. Mclaughlin, " Brine shrimp: a convenient general bioassay for active plant constituents," *Planta Medica.* vol. 45, pp. 31-34, 1982.
[30] C. Cripps, J. A. Bumpus and S. D. Aust, "Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*," Appl. Envir. Microbiol. vol. 56, no. 4, pp.1114-1118, 1990.
[31] K. Chung and C. E. Cerniglia, "Mutagenicity of azo dyes: Structure-activity relationships," Mutat Res-Rev Genet, vol. 277, no. 3, pp. 201–220, 1992.
[32] F. P. van der Zee and S. Villaverde, "Combined anaerobic–aerobic treatment of azo dyes—A short review of bioreactor studies, Water Res. Vol. 39, no. 8, pp. 1425–1440, 2005.
[33] S. Mohanty, N. Dafale and N.N. Rao, "Microbial decolorization of reactive black-5 in a two-stage anearobic–aerobic reactor using acclimatized activated textile sludge," *Biodegr.* vol. 17, pp. 403–413, 2006.
[34] K. T. Chung and S. E. Stevens Jr., "Degradation of azo dye by environmental microorganisms and helminthes," *Environ. Toxicol. Chem.* vol. 12, pp. 2121–2132, 1993.
[35] M. F. Coughlin, B. K. Kinkle, A. Tepper and P. L. Bishop, "Characterization of aerobicazo dye-degrading bacteria and their activity in biofilms," *Water Sci. Technol.* vol. 36, pp. 215–220, 1997.
[36] L. S. Clesceri. A. E. Greenberg, M. P. eaton, "APHA, AWWA, WEF, Standard method for examination of water and wastewater," 20th ed. 1998.
[37] J. Swamy and J. A. Ramsay, "The evaluation of white rot fungi in the decoloration of textile dyes," *Enzyme Microbiol. Tech.* vol. 24, no.3, pp. 130-137, 1999.
[38] I. M. Banat, P. Nigam, D. Singh and R. Marchant, "Microbial decolorization of textile-dye containing effluents: a review, "*Biores. Technol.* vol. 58, no. 3, pp. 217-227, 1996.
[39] J. Deng, G. Guo and G. Zeng, "Decolorization of anthraquinone, triphenylmethane and azo dyes by a new isolated *Bacillus Cereus* strain DC11. *Int. Biodeter. Biodegr.* vol. 62, no. 3, pp. 263-269, 2008.
[40] S. S. Phugare, D. C. Kalyani, S. N. Surwase and J. P. Jadhav, "Ecofriendly degradation, decolorization and detoxification of textile effluent by a developed bacterial consortium," *Ecotoxicol. Environ. Safe.* vol. 74, pp. 1288–1296, 2011.
[41] W. Haug, A. Schmidt, B. Nortemann, D. C. Hempel, A. Stoiz and H. J. Knackmuss, Mineralization of the sulfonated azo dye mordant yellow 3 by 6-aminonaphthalene-2-sulfonate-degrading bacterial consortium," *Appl. Environ. Microbiol.* 57, pp. 3144–3149, 1991.
[42] H. A. Alhassani, M. A. Rauf and S. S. Ashraf, "Efficient microbial degradation of Toluidine Blue dye by *Brevibacillus* sp.," *Dyes Pigments.* vol. 75, no. 2, pp. 395–400, 2007.
[43] S. U. Jadhav, M. U. Jadhav, A. N. Kagalkar and S. P. Govindwar, "Decolorization of Brilliant Blue G dye mediated by degradation of the microbial consortium of *Galactomyces geotrichum* and *Bacillus* sp.," Journal of the Chinese Institute of Chemical Engineers, vol. 39, no. 6, pp. 563–570, 2008.
[44] S. S. Gomare and, S. P. Govindwar, *"Brevibacillus laterosporus* MTCC 2298: a potential azo dye degrader," *J. Appl. Microbiol.* vol. 106, no. 3, pp. 993–1004, 2009.
[45] S. V. Mohan, P. Suresh and S. Srikanth, "Azo dye remediation in periodic discontinuous batch mode operation: evaluation of metabolic shifts of the biocatalyst under aerobic, anaerobic and anoxic conditions," *Sep. Purf. Technol.* vol. 118, pp. 196-208, 2013a.
[46] A. N. Kumar, C. N. Reddy, R. H. Prasad and S. V. Mohan, "Azo dye load-shock on relative behavior of biofilm and suspended growth configured periodic discontinuous batch mode operations: Critical evaluation with enzymatic and bio-electro catalytic analysis," Water Res. vol. 60, pp. 182-196, 2014.
[47] S. V. Mohan, C. N. Reddy, A. N. Kumar, J. A. Modestra, "Relative performance of biofilm configuration over suspended growth operation on azo dye based wastewater treatment in periodic discontinuous batch mode operation," *Bioresour. Technol.* vol. 147, pp. 424-433, 2013b.
[48] APHA, "Standard methods for examination of water and wastewater," American public health association (APHA). Washington D. C., 21st ed., 2005.
[49] R. Mass and S. Chaudhari, "Adsortion and biological decolorization of azo Reactive Red 2 in semi continuous anaerobic reactors," *Process Biochem,* vol. 40, no. 2, pp. 699-705, 2005.
[50] P. Lens, A. Massone, A. Rozzi and W. Verstraete, "Effect of sulfate concentration and scraping on aerobic fixed biofilm reactors," *Water Res.* vol. 29, pp. 857-70,1995.
[51] P. A. Alphenaar, R. Sleyster, P. D. Reuver, G. J. Ligthart and G. Lettinga, "Phosphorous requirement in high rate anaerobic wastewater treatment," *Water Res*. vol. 27, pp.749-756, 1993.
[52] S. Soo and K. Chang-Jin, "Decolorisation of artificial dyes by peroxidase," *Biotech. Letters*. vol. 20, no.6, pp. 569-572, 2003.
[53] M. R. Atlas, "Bacterial growth and reproduction in principles of microbiology," edited by james M Smith (Hoffmann Press Inc, Missouri) pp. 327-349, 1995.
[54] K. Balapure, N. Bhatt and D. Madamwar, "Mineralization of reactive azo dyes present in simulated textile waste water using down flow microaerophilic fixed film bioreactor, *Bioresource Technol*. vol. 175, pp. 1–7, 2015.
[55] B. Saba, A. Khalid, A. Nazir, H. Kanwal, T. Mahmood, "Reactive black-5 azo dye treatment in suspended and attach growth sequencing batch bioreactor using different co-substrates. *Int. Biodet. Biodegr.* vol. 85, pp. 556-562, 2013.
[56] P. K. Gupta, Methods in Environmental Analysis Water, Soil and Air, 1st ed., UpdeshPurohit for Agrobios, India Jodhpur Agro House, 2004, pp. 47–48.
[57] M. A. Belmont, E. Cantellano, S. Thompson, M. Williamson, A. Sanchez, C. D. Metcalfe, "Treatment of domestic wastewater in a pilot-scale natural treatment system in central Mexico," *J. Ecol. Eng.* vol. 23, 299-08, 2004.
[58] H. Wu, S. Wang, H. Kong, T. Liu and M. Xia, "Performance of combined process of anoxic baffled reactor-biological contact oxidation treating printing and dyeing wastewater," *Bioresource Technol.* vol. 98, no. 7, pp. 1501-1504, 2007.
[59] S. D. Kalme, G. K. Parshetti, S. U. Jadhav and S. P. Govindwar, "Biodegradation of benzidine based dye Direct Blue-6 by *Pseudomonas desmolyticum* NCIM 2112," *Bioresource Technol.* vol. 98, no. 7, 1405-1410, 2007.
[60] F. Elisangela, Z. Andrea, D. G. Fabio, R. M. Cristiano, D. L. Regina and C. P. Artur, "Biodegradation of textile azo dyes by a facultative *Staphylococcus arlettae* strain VN-11 using a sequential microaerophilic/aerobic process," *Int. Biodeter. Biodegr*. vol. 63, no. 3, pp. 280-288, 2009.
[61] M. B. Kurade, T. R. Waghmode, A. N. Kagalkar and S. P. Govindwar, "Decolorization of textile industry effluent containing disperse dye Scarlet RR by a newly developed bacterial-yeast consortium BL-GG," *Chem. Eng. J.* vol. 184, pp. 33-41, 2012.