

Eco-Friendly Degradation of Reactive Blue Dye Enriched Textile Water by Floating Treatment Wetlands (FTWs) System Applying the Strategy of Plant-Bacteria Partnership (Part-B)

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(received March 6, 2019; revised June 17, 2019; accepted June 26, 2019)

Abstract. Textile dye enriched effluents have been credited to be heavily polluted and highly hazardous to environment. Cleaning of textile wastewater from these dangerous pollutants is a deeply concerned issue of the industry. Various physico-chemical and biological techniques are being practiced to remediate these effluents. But all these strategies have limitations at any corner of their application. Floating treatment wetlands (FTWs) are acknowledged as economical treatment options for various kinds of wastewater. Their efficacy has been ascribed in many lab-scale and pilot-scale studies, however scarceness of published data lies in sense of application of this technique using free floating aquatic plants for treating major primary reactive blue dye enriched textile water. So, the present study attempted to evaluate the performance of this method using two free floating aquatic plants, "*Eichhornia crassipes*" and "*Pistia stratiotes*" to develop a lab scale FTWs system augmented with two pollutant degrading and plant growth promoting bacteria, "*Bacillus cereus*" and "*Bacillus subtilis*" after four retention times, 0, 24, 48 and 72 h. One control and eight FTW treatment reactors were established containing plants and bacteria separately or in combination. This system was applied to treat reactive blue dye enriched textile wastewater prepared synthetically. A remarkable reduction in pollutant indicating parameters, BOD, COD and colour concentration was observed for treatment reactor having *Eichhornia crassipes* and *Bacillus cereus* combination for 72 h retention time. All this stamped the authenticity of this plant-microbial enhanced FTWs technique to treat textile wastewater and opened an era of its on-site application.

Keywords: floating treatment wetlands, plant-bacteria partnership, textile wastewater treatment, reactive blue dye

Introduction

Textile industry is the key player in economy of many countries especially developing ones like Pakistan. However, it is high source of water pollution due to the discharge of its untreated or partially treated wastewater in nearby water bodies (Noreen *et al.*, 2017). Textile effluents are the complex mixture of variant organic and inorganic pollutants, chemicals and dyes which are highly toxic to aquatic and environment health. This hazardousness poses severe threat to developing countries, who lack capability to efficiently treat such toxicants cost effectively. This results in the form of contaminating their valuable water resources (Khandare *et al.*, 2013). Major sector of textile generating huge

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quantity of water is textile dyeing and processing industry. About 70 billion tons of wastewater is generated by this industry each year (Moga *et al.*, 2018). In dyeing industry reactive dyes are most commonly used especially to dye cotton related products (Sudha *et al.*, 2014). Among all reactive dyes blue dye is a major primary dye that has been observed highly resistant to chemical oxidation and difficult to break down due to its aromatic anthraquinone structure. It has been well recognized that more than 15% dye stuff do not bind to the textile product and is washed out resulting in dye enriched wastewater (Annuar *et al.*, 2009). These dye residues in wastewater become the source to increase BOD (biological oxygen demand), COD (chemical oxygen demand) and colour concentration in the receiving

water bodies (Lie *et al.*, 2012). Moreover, the polluted water when discharged in channels seeps and pollutes the ground water. When this contaminated water is used to irrigate the fields of various crops and vegetables, the pollutants enter the food chain. Ultimately, when human uses the polluted ground water and food, they cause serious health hazards by leading to diseases like cholera, typhoid, dysentery, guinea worm disease, cancer, tumor, brain diseases etc. Similarly, different kinds of dyes and pigments of organic nature, salts, alkalis, acids, bleaching and finishing agents also cause significant hazardous effects on the health of biota (Imtiazuddin *et al.*, 2012).

Many physical, chemical and biological techniques are successful in treating textile wastewater but mostly they have limitations in sense of their high demand of operational costs, labour administration, engineering skills and production of additional waste in form of sludge that requires further safe disposal (Srinivasan *et al.*, 2014). Contrary to all these draw backs of existing physico-chemical mechanisms to treat textile effluents, phyto-remediation has been acknowledged as an effective tool to treat textile wastewater in simple, less expensive and green way (Choo *et al.*, 2006). Application of floating treatment wetlands (FTWs) as a well designed and engineered way of phyto-remediation has been measured to be an effective, innovative, reliable, cost effective and eco-friendly technique to treat different types of wastewaters like municipal, sewage, domestic and industrial (Arshad *et al.*, 2017; Ijaz *et al.*, 2015; Faulweter *et al.*, 2011). In this technique plants roots hang down into water column and provide both biological and mechanical filtering of wastewater. Biological filtering is the result of bacterial degradation of pollutants, while mechanical filtering is carried out by dense root system of plants in the form of sedimentation, adsorption, filtration, etc. (Zhang *et al.*, 2016; Merkhali *et al.*, 2015). Additionally this system provides aesthetic beauty to the area along with acting as habitation for fish and many other life forms (Ijaz *et al.*, 2015). Moreover, this is the source of generating significant amount of biomass that can be used for bio-energy purposes (Shahid *et al.*, 2018). Many additional approaches have been proposed for the performance enhancement of FTWs, among of them inoculation of pollutant degrading and plant growth promoting bacteria has been found more effective (Saleem *et al.*, 2018). Under this plant-bacteria synergism, plants provide habitat and nutrients to bacteria; while in return, bacteria

boost up plant growth and degrade organic pollutants due to their metabolic activities. Many studies regarding augmentation of FTWs through plant-microbe interactive mechanism have been carried out for remediation of different wastewaters (Rehman *et al.*, 2018; Watharkar *et al.*, 2015). In most of them emergent plants were vegetated on free floating raft. However, application of this technique using free floating aquatic plants assisted with plant growth promoting and pollutant degrading bacteria, for remediation of pure reactive blue dye enriched textile wastewater, finds paucity. Hence the present study endeavored to have performance evaluation of this system using two free floating aquatic plants (*Eichhornia crassipes* and *Pistia stratiotes*) with augmentation of two pollutant degrading and plant growth promoting bacteria (*Bacillus cereus* and *Bacillus subtilis*) to treat purely reactive blue dye enriched textile wastewater.

Materials and Methods

Synthetic solution of reactive blue (Bezaktiv HP-R) was prepared at lab scale following standard recipe for 0.1% shade depth. Then this solution was transferred to transparent polyethylene containers (39 cm × 28 cm × 20 cm) of 10-12 liter capacity. FTWs system was designed by vegetating two free floating aquatic plants *Eichhornia crassipes* and *Pistia stratiotes* in these containers. The system was amplified by inoculating two bacteria, “*Bacillus cereus*” and “*Bacillus subtilis*” in these containers Fig. 1 in order to develop 9 treatment reactors as per the decided detail given in section “Designing of FTWs Experimental reactors”. The capability of these bacterial strains regarding plant growth promotion and pollutant degradation has been well reported in many previous studies (Asgar *et al.*, 2017; Nair and Swarnalatha, 2015). The interaction of these bacteria with plants was made by dipping plants in 500 mL broth of each bacterium for 40 min. Then these plants were transferred to each treatment reactor according to their decided number.

Different aspects of experimental study have been explained below:

Reactive blue dye enriched solution preparation.

Reactive blue (Bezaktiv HP-R) dye enriched textile wastewater solution of 0.1% shade depth was prepared synthetically at lab scale according to the following recipe:

Dye 1 g/L, Glauber’s salt 5 g/L and soda ash 0.4 g/L



Fig. 1. Various components and designing of FTWs system.

Collection of effluent degrading bacterial strains.

Two type of bacterial strains “*Bacillus cereus*” and “*Bacillus subtilis*” were taken from Soil Microbiology and Biochemistry lab of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan, where they were isolated (Rafique, 2015) using general purpose agar media (glucose peptone agar media) applying dilution plate technique. Media plates were inoculated with soil solution and incubated at 28 ± 2 °C for 72 h. Colony forming units (CFU/g soil) from each soil sample were calculated. The bacterial isolates were assayed for their potential to biodegrade polycyclic aromatic hydrocarbon using Bushnell-Haas broth in 24-well microtiter plates (Hanson *et al.*, 1993). For the verification of bacteria properties regarding plant growth promoting, they were also tested for ACC-deaminase activity through method (modified) described by Jacobson *et al.* (1994). These bacteria have also been reported by many previous researchers (Ijaz *et al.*, 2015; Nair and Swamalatha, 2015) to have characteristics of textile effluent degrading and plant growth promotion.

Plant selection. Locally available plants having immunity with the native environmental conditions are appreciable to use for this treatment technique. So, two abundantly occurring plants, in and surrounding of Lahore and Faisalabad (The textile industry hub of Pakistan) were selected for conducting this research study. Adult plants were collected from nearby fresh and wastewater bodies and then stored in circular tubs of diameter 76 cm with 30 cm depth under ambient conditions at the Department of Fibre and Textile Technology, University of Agriculture Faisalabad. For making them able to survive in pure textile water, they were watered with textile wastewater and tap water in increasing ratio i.e. 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0, respectively. The plants flourished well after getting immunity within 10 days and doubled their population.

The worth of collected bacterial strain and plants was tested for degrading textile wastewater at lab scale. Various operational parameters like hydraulic retention time, bacterial strains, plants types and their interaction were analyzed for getting their optimum levels. These factors were selected in the range as given in Table 1.

Designing of FTWs experimental reactors. One control and eight experimental FTWs treatment reactors were designed with the following specifications:

- C = control (only reactive blue dye enriched solution)
- T1 = dye solution + P1 (plant 1: *Eichhornia crassipes*)

Table 1. Parameters and their values selected for the current study

Textile effluent source	Retention time (h)	Bacterial strains	Plant types	Reactors description
Reactive blue	TM1 = 0	<i>Bacillus cereus</i>	<i>Eichhornia crassipes</i>	R ₁ = effluent + plant
	TM2 = 24	<i>Bacillus subtilis</i>	<i>Pistia stratiotes</i>	R ₂ = effluent + bacteria
	TM3 = 48			R ₃ = effluent + plant + bacteria
	TM4 = 72			

T2 = dye solution + P1 + B1 (bacteria 1: *Bacillus cereus*)

T3 = dye solution + P1 + B2 (bacteria 2: *Bacillus subtilis*)

T4 = dye solution + P2 (plant 2: *Pistia stratiotes*)

T5 = dye solution + P2 + B1

T6 = dye solution + P2 + B2

T7 = dye solution + B1

T8 = dye solution + B2

Testing of textile effluent. 500 mL sample was taken from each reactor in transparent plastic bottles after washing them thoroughly with distilled water and analyzed for determining the BOD, COD and colour concentration (CC) according to standard procedures (APHA, 2005).

Statistical analysis. The data obtained were statistically analyzed for checking overall significance of mean values, while the difference among treatment means was calculated by using least significant difference (LSD) test. Experiment was arranged according to completely randomized design (CRD) with three replications. SAS program, version STAT 9.1 of SAS Institute (Clark, 2004) was operated for all statistical applications.

Results and Discussion

Physico-chemical parameters of reactive blue dye enriched textile wastewater as affected by selected

treatments (T) and retention times (TM). The data regarding analysis of variance (ANOVA) presented in Table 2 indicated significant effects ($\alpha=0.05$) of all treatments (T) and retention times (TM) on BOD, COD and CC values of selected solution.

Effect of various treatments and hydraulic retention time on BOD value of reactive blue dye enriched textile wastewater. All the selected treatments and times had significant effects on BOD of reactive blue dye enriched textile wastewater. There was noted remarkable reduction in BOD value of the treated water with increasing retention time. The BOD value of wastewater for control reactor (C) at initial time TM1 (0 h) was found 90 mg/L that reduced to 38 mg/L for combined treatment of the water with *Eichhornia crassipes* and *Bacillus cereus* bacteria (T2). This treatment combination made 57.78% reduction in BOD value of the treated water after 72 h retention time (TM4) as shown in Fig. 2.

The ANOVA for regression Table 3 indicated that all the retention times (TM) had significant effects ($\alpha=0.05$) on BOD of the solution. Regression model developed disclosed that time inversely affected BOD values at the rate of 0.47. The high degree of certainty ($r^2 = 0.56$) ensures the best representation of the data observed by

Table 2. ANOVA of treatment and retention time for reactive blue dye enriched textile water

Source	DF	Dep var BOD		Dep var COD		Dep var CC	
		MS	P>F	MS	P>F	MS	P>F
Model	35	807.6262	0.0001	995.4696	0.0001	0.0009	0.0001
T	8	1012.7083	0.0001	1403.8357	0.0001	0.0001	0.0001
TM	3	5696.0833	0.0001	6652.0093	0.0001	0.0064	0.0001
T*TM	24	128.2083	0.0001	152.2801	0.0001	0.0002	0.0001
Error	72	29.6667		34.0648		0.00002	
Corr Total	107						

T = treatment; C = control (only solution); T1 = solution + plant 1 (*Eichhornia crassipes*); T2 = solution + plant 1 + bacteria 1 (*Bacillus cereus*); T3 = solution + plant 1 + bacteria 2 (*Bacillus subtilis*); T4 = solution + plant 2 (*Pistia stratiotes*); T5 = solution + plant 2 + bacteria 1; T6 = solution + plant 2 + bacteria 2; T7 = solution + bacteria 1 and T8 = Solution + bacteria 2; TM = retention time (TM1=0; TM2=24; TM3=48; TM4=72 h).

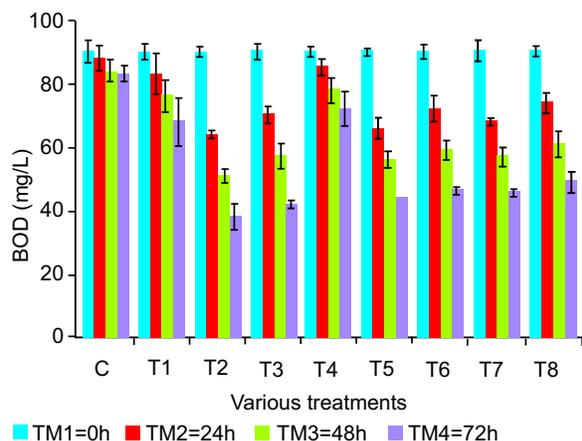


Fig. 2. Effect of various treatments for different retention times (TM) on BOD value of reactive blue dye enriched textile water.

the predicted equation (1):

$$\text{BOD}(\text{mg/L}) = 87.178 - 0.47 \times \text{TM} (\text{h}) \dots\dots (1)$$

It can be derived from these observations that plant-bacteria coalition degraded the textile dye enriched effluent more effectively. These results are in line with the findings of earlier studies that plant bacteria synergic mechanism reduced BOD of the solution more efficiently (Tara *et al.*, 2019). This reduction has been credited to the increase of dissolved oxygen of the wastewater because of the presence of plants. Plants increased the oxygen concentration in the solution resulting oxidation reaction due to which the degradation of organic pollutants took place. Moreover, the bacterial inoculation enhanced this removal ability because of their aptitude to transform and decompose organic matter (Vymazal, 2010).

Effect of various treatments and hydraulic retention time on COD value of reactive blue dye enriched textile wastewater. The COD value of reactive blue

dye enriched textile wastewater under treatment was found to be affected significantly by all selected treatments and times. With increase in hydraulic retention time there was found increase in reduction of COD. The maximum value of COD (183 mg/L) was noted for control reactor (C) at start of the experiment TM1 (0 h). This value reduced considerably up to 125 mg/L when this wastewater was treated by *Eichhornia crassipes* plant in synergy with *Bacillus cereus* bacteria (T2) after 72 h retention time (TM4). In Fig. 3 the calculated 31.69% reduction in COD of treated water for this treatment combination.

The ANOVA for regression Table 3 indicated that all the retention times (TM) had significant effects ($\alpha=0.05$) on COD of the solution. Regression model so developed depicted an inverse relation between time and COD values at the rate of 0.49. The value of degree of certainty was found high ($r^2 = 0.49$) that ensured the best representation of the data observed by the predicted equation (2):

$$\text{COD}(\text{mg/L}) = 177.76 - 0.49 \times \text{TM} (\text{h}) \dots\dots (2)$$

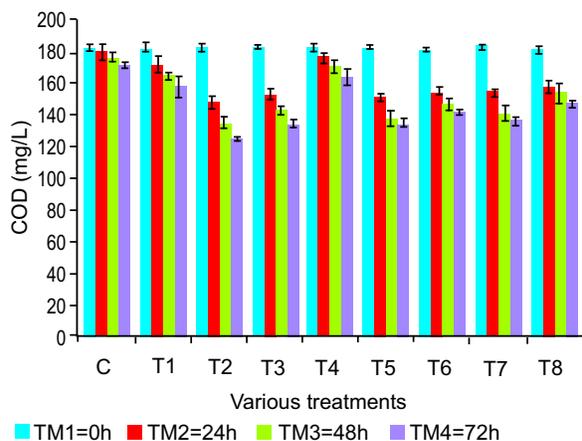


Fig. 3. Effect of various treatments for different retention times (TM) on COD value of reactive blue dye enriched textile water.

Table 3. ANOVA for regression analysis

Source	DF	Dep var BOD		Dep var COD		Dep var CC	
		MS	P>F	MS	P>F	MS	P>F
Model	1	16968	0.0001	18317	0.0001	0.01930	0.0001
Error	106	126.74		179.032		0.00012	
Corr Total	107						

The reduction in COD of effluent is related to the fact as acknowledged previously (Patel and Adhvaryu, 2016) that the presence of plants in wastewater could deplete dissolved CO₂ during the time of high photosynthetic activity. Because of this photosynthetic activity the dissolved oxygen of water increased and resulted in creation of aerobic conditions in wastewater that favoured the aerobic bacterial activity to reduce the COD. The presence of plant growth promoting bacteria in reactor booted up this reduction process. The application of plants in combination of pollutant degrading and plant growth promoting bacteria applying floating wetland technology has also been considered as an auspicious approach for BOD and COD reduction by earlier researchers (Arslan *et al.*, 2017; Abed *et al.*, 2017; Ijaz *et al.*, 2016).

Effect of various treatments and hydraulic retention time on colour concentration value of reactive blue dye enriched textile wastewater. The data disclosed significant impact of all treatments and retention time on color degradation of reactive blue dye enriched textile wastewater. The results for colour concentration percentage (CC %) of wastewater Fig. 4 exposed off its maximum value (0.1%) at the start of the experiment (TM1) before any kind of treatment. This value reduced to 0.043% for *Eichhornia crassipes* and *Bacillus cereus* combined treatment (T2) of the wastewater for 72 h retention time (TM4). This treatment combination resulted in 57% degradation in colour concentration of the treated water.

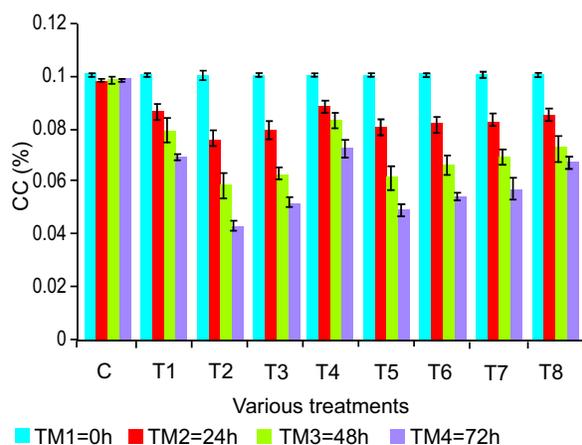


Fig. 4. Effect of various treatments for different retention times (TM) on CC of reactive blue dye enriched textile water.

The regression analysis Table 3 indicated significant ($\alpha=0.05$) effect of time (TM) on color concentration (CC) of the solution. The developed regression model in this respect showed that time inversely affected CC values at the rate of 0.00050. Degree of certainty resulted high ($r^2 = 0.60$) in value depicted the best representation of the data observed by the predicted equation (3):

$$CC(\%) = 0.097 - 0.00050 \times TM \text{ (h)} \dots\dots\dots (3)$$

This colour degradation has been considered due to the presence of plants and microbes in treatment reactor. As plants played significant role in dye removal by mineralization and decomposition of contaminants due to the presence of microbes at the rhizosphere of plants (Lehl *et al.*, 2017), while the presence of bacteria decolourized the azo dye with the reductive breakage of its azo bond (-N=N), chromophore group, resulting in the formation of aromatic amines (Sing *et al.*, 2015). Additionally, these bacteria have been reported to enhance the growth of plant roots which reciprocally resulted in increasing the growth and metabolic activities of microbes in the rhizosphere of plants in which these microbes utilized dye as a carbon and nitrogen source (Padmanaban *et al.*, 2013) that boosted up the biodegradation of pollutants. So, plant bacteria combination in this system has been accepted to accelerate the contaminants degradation process (Bais *et al.*, 2003).

Conclusion

A substantial decrease in all pollutant indicating parameters *i.e.* BOD, COD and color concentration, of reactive blue dye enriched textile wastewater was found under the treatment of plant-bacteria assisted floating treatment wetlands system. Among all combinations, *Eichhornia crassipes* and *Bacillus cereus*, interactive treatment proved its supremacy to make maximum reduction of pollutants. Hence it can be concluded that *Eichhornia crassipes* and *Bacillus cereus* synergism can be a plausible approach to treat dye enriched textile wastewater. The existence of *Eichhornia crassipes* plant in abundance in local climate conditions of Faisalabad and Lahore (Textile industry hubs of Pakistan) increase its credibility to be used for treatment of textile wastewater. Furthermore, this research study unrevealed plant-microbe amplified FTWs technique as an efficient tool to clean textile effluents in green and ecofriendly way and paved the path of its *in situ* application.

Conflict of Interest. The authors declare that there is no conflict of interest.

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