

Eco-Friendly Degradation of Blue Reactive Dye Enriched Textile Water by Floating Treatment Wetlands (FTWs) System (Part A)

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Abstract. Enriched effluents of textile dye are considered highly complex and containing hazardous components. Their discharge to nearby drains without treatment has high risk to environmental and human health. The physico-chemical strategies under practice to treat these effluents have technical and economical restrictions. Comparatively, biological treatment methods like floating treatment wetlands are less expensive and eco-friendly. Blue reactive dye enriched textile water was prepared synthetically and added to an artificial Floating Treatment Wetlands (FTWs) system vegetated with *Eichhornia crassipes* and *Pistia stratiotes* aquatic plants along with *Bacillus cereus* and *Bacillus subtilis* bacterial strains. Plant-microbe synergistic effect was studied by measuring the physico-chemical parameters i.e. pH, EC, TDS and TSS of dye enriched water after 0 (at the start of the experiment), 24, 48 and 72 h retention time. A substantial decrease in all these factors (11.34 %, 40.67 %, 64.37 %, 58.23 %, for pH, EC, TDS and TSS respectively) was noted for *E. crassipes* and *B. cereus* combination after 72 h retention time. This highlighted the fact that plant assisted microbial FTWs technique can be a unique approach to remediate the textile effluents.

Keywords: textile wastewater treatment, blue reactive dyes, floating treatment wetlands (FTWs), plant-microbe interaction

Introduction

Availability of fresh water has become a serious issue of this age. The pollution of water bodies is a burning issue of this age all over the universe. It is more serious in developing countries. Among all contributors, textile industry has its major share to pollute water in these countries due to the availability of cheap labour and less strict waste disposal norms (Valaria *et al.*, 2011). The wastewater generated from industrial sector especially from textile dyeing industry has been considered as most perilous in terms of its volume and composition (Wang *et al.*, 2017). In open market about more than 100,000 dyes have been documented as commercially available and the annual production of dyestuff has been reported about 700,000 tons (Mishra and Maiti, 2018). Among these, reactive dyes are most commonly used in dyeing processes especially for cotton product dyeing industry (Sudha *et al.*, 2014). In all available dyeing mechanisms, it has been recognized approximately more than 15% dye stuff remains unfixed

worldwide annually (Prasad and Aikat, 2014). This loss has been accepted because of the occurrence of unreactive hydrolyzed dyes in dye bath. This hydrolysis of dyes happens due to its reaction with water molecules in dye solution (Patil *et al.*, 2010). During washing this unfixed dye is removed from the fabric, becoming part of the wastewater. Many pre-dyeing and post dyeing processes like scouring, bleaching, finishing, washing etc. have been acknowledged to contribute different kinds of pollutants like detergents, bleaching agents, caustic, acids and salts in water (Hussein and Scholz, 2018; Ozturk *et al.*, 2015). When this highly contaminated water enters in nearby drains without treatment then it causes severe environmental and aquatic pollution (Ayed *et al.*, 2017a). Mutagenicity, allergic reactions, carcinogenicity and acute cytotoxicity of textile dyes on fishes, crop plants, rats, mollusks, human being and cultured mammalian cells are well documented evidences of hazardous effects of these dye enriched effluents (Khandare *et al.*, 2016) and when these effluents are leached into the ground, the ground water gets contaminated also. In dyeing industry

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especially in Pakistan (a major cotton producing country) cotton products are most widely processed and reactive dyes are commonly used. These dyes have been considered as having bright colors, easy application techniques and less energy consumption (Andrade *et al.*, 2007). Among these dyes, blue is one of the most commonly used primary reactive dyes. Blue dye has been observed as highly resistant to chemical oxidation and its aromatic anthraquinone structure makes it difficult to break down (Fig. 1), modified from Song *et al.* (2008).

Different physical, chemical and biological techniques like stabilization, coagulation, ion exchange, chemical oxidation, membrane filtration, adsorption etc. have been applied to degrade textile effluents (Chen *et al.*, 2009; Aslam *et al.*, 2004). But all these techniques have been reported to have limitations in respect of high cost, operation skill, regular maintenance and generation of high quantity of secondary waste like sludge that require further safe disposal (Sala and Gutierrez-Bouzan, 2012; Mohd Salleh *et al.*, 2011).

In contrast to all above drawbacks of conventional methods used for textile wastewater treatment, plant based treatment techniques also known as “*Phyto-remediation*” have been approved as an effective way to treat wastewater (Khandare *et al.*, 2013). Phyto-remediation has been recognized as operationally simple, economical, eco-friendly and natural way to treat contaminated water (Choo *et al.*, 2006). In addition to plants, bacterial treatment of textile waste water has also been reported as an effective way (Kabra *et al.*, 2013) because bacteria have capability to grow in short time and have direct interaction with dye molecules that enhance degradation process (Kalyani *et al.*, 2009). It has been accredited that the bacteria produce various

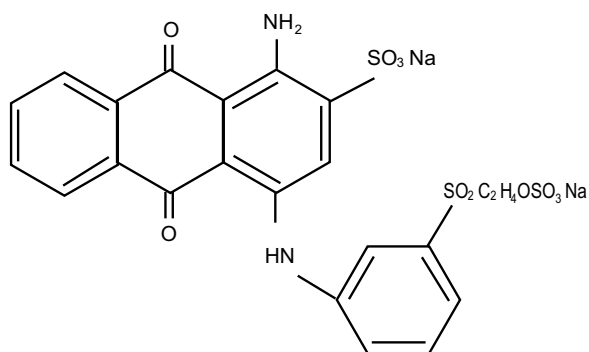


Fig. 1. Chemical structure of reactive blue.

enzymes of oxidative-reductive type like laccase, azoreductase, peroxidase, which enhance the mineralization and effective degradation of many reactive dyes (Bedekar *et al.*, 2014). However, the selection of dynamic bacterial species in order to achieve effective dye degradation is of supreme importance. Many studies have been reported in this respect such as Chang *et al.*, (2001) who observed 90% colour degradation of 300 mg/L reactive red-22 dye for 20 h incubation time by *Pseudomonas luteola* in static condition. In the same line Maqbool *et al.* (2016) made research study on mixed dye solution and concluded a simultaneous removal of 100 mg/L of these mixed reactive dyes (reactive black-5, reactive red-120, reactive orange-16 and reactive yellow-2) by *Pseudomonas aeruginosa* ZM130 after 180 h incubation time in static conditions. Many other works like Gulati and Jha (2014) investigated on *klebsiella* sp. BI-11, isolated from soil, to degrade reactive blue-19 (100 mg/L) and found 95% removal of dye, Shah (2014) made study on *Pseudomonas* sp for de-colourization of reactive black (100 mg/L) and noted up to 95% dye removal, Ayed *et al.* (2017a, b) explored *Staphylococcus aureus* consortium for degradation of reactive violet-5 dye 1000 mg/L and observed 99% removal of dye. In the light of available literature it has been disclosed that bacterium or bacterial consortium use would be a nice choice for reactive dye degradation from contaminated wastewater. *Bacillus subtilis* and *Bacillus cereus* bacteria belong to *Bacillus* species that have been renowned to degrade organic pollutants along with to promote plant growth by producing plant hormones like indole acetic acid (C₁₀H₉NO₂) and phosphorus solubilization (Asghar *et al.*, 2017; Zaidi *et al.*, 2006).

Plant microbial assisted technique is an emergent way that has been recognized as more proficient tool to cleanse contaminated water and having in *situ* applicability (Anuprita *et al.*, 2015). In this system plants take up nutrients from contaminated source and their nutrients up taking ability get stimulation by microbial communities (Afzal *et al.*, 2014; Shehzadi *et al.*, 2014). Plants provide habitat for microbes and their roots and shoots promote microbial activity.

Constructed wetlands have been measured as reliable plant based wastewater treatment techniques (Vymazal., 2011). Floating treatment wetlands (FTWs) are one of them. FTWs have been considered as the integration of agronomy and ecological engineering and a novel approach to detoxify textile dye enriched effluents. In

this system aquatic plants remain floating on surface of contaminated water, while their roots hang freely in the water (Hwang and LePage, 2011). FTWs system has been agreed upon to treat the polluted water by physical, biological and chemical ways (Chang *et al.*, 2013). It has been explored that physically plants create hindrance in water flow and reduce its velocity resulting better situation for sedimentation of dissolved and suspended solids; biologically plants do photosynthesis, respiration, phyto-extraction, rhizodegradation, while chemically the contaminants are killed by plant roots due to their generation of antibiotic material (Arshad *et al.*, 2017). Plant selection for this technology is of utmost importance. The plants that have dense root system are most commonly used in floating wetland technologies. The contaminant removal efficiency and the oxygen generation of the floating wetland have been observed to increase with the vegetation of the plant in the wetland (Li *et al.*, 2010).

E. crassipes (water hyacinth) is an aquatic plant having broad, thick leaves and can rise about 1 m above the water surface. Its roots and shoot system is very dense and have been reported to be supportive for wastewater treatment (Mishra and Maiti, 2018; Saha *et al.*, 2017). Similarly *P. stratiotes* (water lettuce) is a free floating aquatic plant that doubles its biomass in 6 days. It has been observed very effective to treat contaminated water applying FTWs technique (Arshad *et al.*, 2017; Pawan *et al.*, 2015). FTWs system has been accepted as a highly operative technique to remediate different types of wastewater (Lynch *et al.*, 2015). However, rare work is available on plant-bacteria assisted FTWs technology for desalination and decolorization of reactive blue dye enriched textile wastewater with respect to hydraulic retention time (average length of time for which solution remained in bioreactor). So, this study was planned to assess the potential of aquatic plants (*E. crassipes* and *P. stratiotes*) and bacteria (*B. cereus* and *B. subtilis*), in synergy, to treat the reactive blue dye enriched textile effluents under the umbrella of FTWs technology.

Materials and Methods

FTWs system was designed in transparent polyethylene containers (39 cm x 28 cm x 20 cm) of 10 liter capacity having synthetically prepared reactive Blue dye enriched solution vegetated with *E. crassipes* and *P. stratiotes* aquatic plants (Fig. 2). Two bacteria, "*B. cereus*" and "*B. subtilis*" that have been acknowledged for their capability to degrade contaminants in wastewater

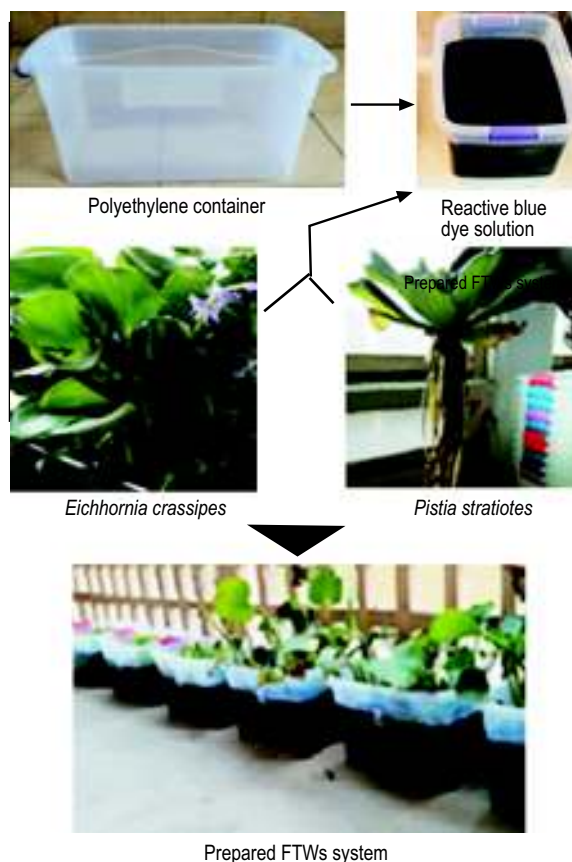


Fig. 2. Various components and designing of FTWs system.

(Asghar *et al.*, 2017; Nair and Swarnalatha, 2015), were also pooled with these plants to develop plant-bacteria synergism in order to treat textile effluents. 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 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; Soda ash = 0.4 g/L (at fixed 8.91 pH)

Collection of effluent degrading bacterial strains.

Pre-isolated and characterized pollutant degrading bacteria "*B. cereus*" and "*B. subtilis*" were taken from Soil Microbiology and Biochemistry Lab of Institute

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>	R1 = effluent +plant
	TM2= 24	<i>Bacillus subtilis</i>	<i>Pistia stratiotes</i>	R2 = effluent+ bacteria
	TM3= 48			R3 = effluent+ plant + bacteria
	TM4= 72			

of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. The efficacy of these bacteria regarding pollutant degradation and plant growth promotion had been already testified (Rafique, 2015).

Plants selection. Native plants for such treatments are suitable due to their ability to get immunity with the local climatic conditions. So, locally available aquatic plants, *E. crassipes* and *P. stratiotes*, were selected for the present research study. These plants exist in abundance in many fresh and waste water bodies located in the surroundings of Lahore and Faisalabad (Hub of textile industry), Pakistan. Fully grown plants were collected and stored in water tubs of circular shape having 76 cm diameter and 30 cm depth under ambient conditions (28-43 °C temperature and 35 -50 % humidity). For making them to survive in pure textile water they were immune by giving them textile wastewater collecting from Paharrung drain, Faisalabad, near the out let of Kalash textile dyeing and processing mill, for one week with increasing ratio of tap water and textile water i.e. 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0. After getting immunity the plants flourished well in pure textile water and increased their population about in double within 10 days. Then they were transferred to containers having synthetically prepared reactive blue dye enriched effluent in order to develop FTWs reactors. Five plants of nearly equal mass were vegetated in each FTWs experimental reactor. 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. Eight experimental FTWs treatment reactors were designed with the following specifications.

C = control (only reactive blue dye enriched effluent);
T1 = blue dye effluent + *Eichhornia crassipes* plant;
T2 = blue dye effluent + *Eichhornia crassipes* + *Bacillus*

cereus; T3 = blue dye effluent + *Eichhornia crassipes*+
Bacillus subtilis; T4 = blue dye effluent + *Pistia stratiotes*
plant; T5 = blue dye effluent + *Pistia stratiotes*+ *Bacillus*
cereus; T6 = blue dye effluent + *Pistia stratiotes*+
Bacillus subtilis; T7 = blue dye effluent + *Bacillus*
cereus; T8 = blue dye effluent + *Bacillus subtilis*

Sample collection.

Samples were collected in plastic bottles thoroughly washed with detergent (prepared by adding 1 L of concentrated H₂SO₄ by stirring with 35 mL saturated sodium dichromate solution) then rinsed comprehensively with tap water. Finally, bottles were rinsed with distilled water before adding sample and stored in refrigerator at about 4 °C for 24 h before analysis.

Testing of textile effluent. 500 mL sample was taken from each reactor after each selected retention time and analysed for determining the pH, EC, TDS and TSS according to standard procedures (APHA, 2005). pH was measured by ML. 1010 pH meter of HANNA instruments after calibrating it with 4 and 9 pH buffer solutions, while EC and TDS were measured by CO150 conductivity meter of HACH after proper calibration, while TSS was measured by filtration method using 1.2 µm pore size filter.

Statistical analysis. All the selected water quality parameters were statistically analyzed in order to check the overall significance of data. The difference between treatment means was compared by employing the least significant difference (LSD) test. Complete randomized design (CRD) was applied to arrange this experiment. Moreover, the effect of time on selected parameters was analyzed by using regression analysis. The statistical tests were conducted by operating the SAS program version STAT 9.1 of SAS Institute (Clark, 2004).

Results and Discussion

Physico-chemical properties of reactive blue dye enriched textile water. *Effect of various treatments and hydraulic retention time on pH value of reactive blue dye enriched textile water.* There was found

significant impact of all selected treatments and retention time on pH value of the water under treatment. The maximum value of pH (8.91) was noted for control treatment (C) i.e. at the start of the experiment (TM1= retention time of 0 hour). This reduced to 7.90 after 72 hours retention time (TM4) under combined treatment of water with *E. crassipes* plant and *B. cereus* bacteria. This treatment combination made 11.34% reduction in pH of blue dye enriched wastewater with maximum retention time as shown in Fig. 3. These findings disclosed that with the increase of time, *E. crassipes* and *B. cereus* synergistic treatment reduced the pH of the solution significantly.

This pH reduction might be credited to the production of carbon dioxide by plants through their photosynthesis process. Moreover, the presence of bacteria in the system has been reported to degrade organic pollutants in wastewater that resulted in the formation of organic acids like formic and acetic acids etc (Somasiri *et al.*, 2008).

Effect of various treatments and hydraulic retention time on EC value of reactive blue dye enriched textile water. The EC value of blue dye enriched solution was influenced significantly by all selected treatments and retention times. The value of EC of solution was noted highest (9.91 dS/m) for control treatment (C) at the initial stage of experiment i.e. (TM4 = 0 hour retention time). There was observed prominent reduction in EC up to value of 5.88 dS/m after 72 h retention time for *E. crassipes* plant treatment in synergy with *B. cereus* bacteria. A considerable reduction (40.67%) was noticed

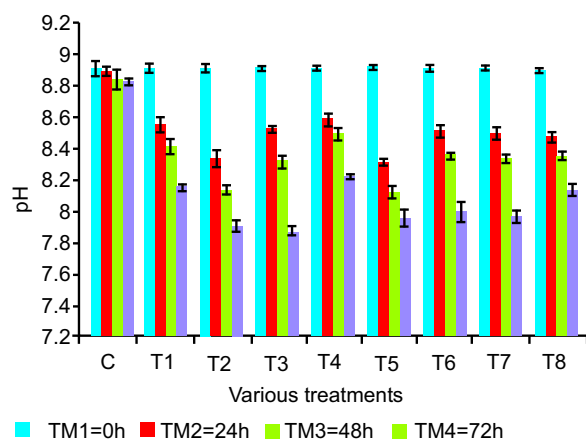


Fig. 3. Effect of various treatments for different retention times (TM) on pH value of reactive blue dye enriched textile water.

for this combination of plant and bacteria after maximum hydraulic retention time (Fig. 4). Hence the combine effect of plant and bacteria significantly reduced the EC value of blue dye enriched solution with increasing retention time.

This reduction in EC has been acknowledged due to decrease in soluble salts that might have been taken by plants (Seenivasan *et al.*, 2015) and bacterial addition has been reported to enhance this factor by promoting plant growth resulting in adding up the nutrient up taking ability of the plants through their roots and shoots (Ugya *et al.*, 2015).

Effect of various treatments and hydraulic retention time on TDS value of reactive blue dye enriched textile water. All selected treatments and retention times affected significantly the TDS value of the blue dye enriched textile wastewater. Maximum value of TDS (5997 mg/L) was noted for control treatment (C) at start of the experiment (TM1= 0 h) that reduced significantly to 2137 mg/L after 72 h when the water was treated with *E. crassipes* in partnership with *B. cereus*. There was found 64.37% reduction in TDS of wastewater under this combination of treatment after maximum retention time (Fig. 5). The results reflected that TDS value of the dye enriched textile water reduced significantly when it was treated with *E. crassipes* plant in partnership with *B. cereus* bacteria with increasing hydraulic retention time.

This substantial reduction in TDS of the effluents is attributed to plant-microbe interactive mechanisms

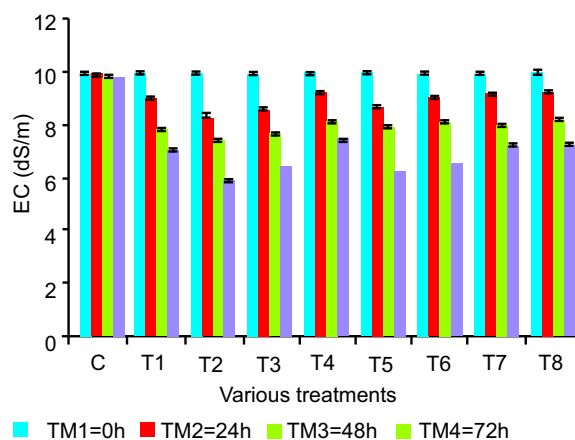


Fig. 4. Effect of various treatments for different retention times (TM) on EC value of reactive blue dye enriched textile water.

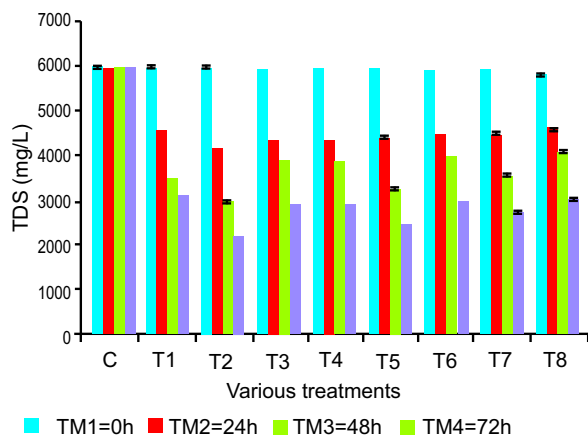


Fig. 5. Effect of various treatments for different retention times (TM) on TDS value of reactive blue dye enriched textile water.

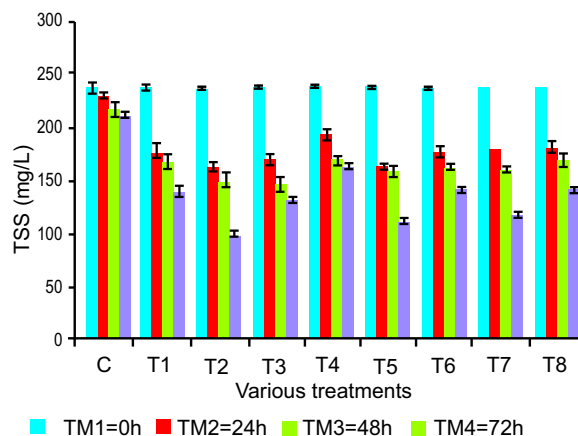


Fig. 6. Effect of various treatments for different retention times (TM) on TSS value of reactive blue dye enriched textile water.

(Abou-Elela *et al.*, 2017) that improved pollutant degradation process of wetlands. According to this, plant's roots provide more spaces for bacteria attachment, settlement and trapping of suspended particles in the water. These spaces also motivate the adsorption and accommodation in plant tissues for both organic and inorganic matters, while the bacteria presence enhance the efficacy of these processes due to having ability to degrade, transform and mineralize the contaminants.

Effect of various treatments and hydraulic retention time on TSS value of reactive blue dye enriched textile water. TSS value of the wastewater was found to be affected significantly by all treatments and time selected for the present study. TSS value of the wastewater was maximum (237 mg/L) at start of the treatment (TM1=0 h) for control reactor (C). This value got maximum reduction (58.23 %) after 72 h retention time under combined treatment of plant and bacteria (*E. crassipes* and *B. cereus*) and after reducing up to 99 mg/L (Fig. 6). These outputs narrated a significant impact of both *E. crassipes* plant and *B. cereus* bacteria to reduce TSS value of the wastewater with increasing time.

This significant decrease in TSS value due this plant-bacteria partnership might be credited towards the root structure of plants in this system that provides more sites for microbial attachment, filtering, settlement and trapping of suspended particles. Moreover the presence of plant growth promoting and pollutant degrading bacteria enhanced this factor by growing dense root

system of plant along with degrading and mineralizing the pollutants (Hussein and scholz, 2018).

Conclusion

All pollutant indicating parameters like pH, EC, TDS, TSS, of reactive blue dye enriched textile effluent were significantly decreased by the combined use of plants and microbes in floating treatment wetlands system. *E. crassipes* plant and *B. cereus* bacterial strain partnership (T₂) was found to be the most effective combination as compared to other selected treatment combinations for degradation of reactive blue dye enriched textile water. So, it can be concluded that *E. crassipes* plant in partnership with *B. cereus* bacteria may be a remarkable choice for treating reactive dye enriched textile wastewater. This plant is found in abundance in the surroundings of Faisalabad (the textile industry hub of Pakistan) showing its suitability in native climate of this city. Moreover, this study disclosed FTWs as an effective platform for plant-bacteria interaction to cleanse textile effluents in eco-friendly way. This study paved the path for *in situ* application of this technology to treat textile wastewater.

Conflict of Interest. The authors declare no conflict of interest.

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