



BIODEGRADATIVE ACTIVITY OF BACTERIAL STRAINS ISOLATED FROM TEXTILE INDUSTRY EFFLUENTS FROM TIRUPUR, TAMIL NADU, UPON REACTIVE BRILLIANT RED X3B

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ABSTRACT

Untreated effluents from the textile industry contain very high levels of total dissolved solids (TDS), total suspended solids (TSS), COD, BOD and hardness. They also contain high levels of dyes which are recalcitrant and toxic to aquatic flora and fauna. As per some estimates, about 10-15% of dyes end up in the effluents during their synthesis and dyeing processes. The biological treatment of textile industry effluents by employing micro-organisms is a better alternative when compared to conventional physical and chemical methods of treatment. In the present study efforts were made to isolate bacterial strains with high degradative ability to degrade most commonly used azo-dye namely, “Reactive Brilliant Red X3b” which was selected as a representative dye of the textile industry effluent. The effluent samples were collected around Tirupur, Tamil Nadu, India bearing latitude-longitude co-ordinates as 11.1085° N, 77.3411° E. The wavelength maximum (λ_{max}) of the Azo-dye, Reactive Brilliant Red X-3b was determined to be 540 nm. Very high levels of COD of 833 mg/l, 845 mg/l and 798 mg/l and very high levels of BOD of 205 mg/l, 220 mg/l and 184 mg/l were recorded for the three effluent samples, T1, T2 and T3 respectively. A total of 52 bacterial strains with the dye degradative property were isolated from the three effluent samples. The inoculum volume of 15 ml exhibited highest degradation activity in all the three bacterial strains tested. In the present study, it was observed that as the duration of incubation increased, the absorbance values of the dye decreased considerably. The highest biodegradative activity was exhibited by the bacterial isolate TR-37 when compared to the other two isolates, TR-17 and TR-21. The bacterial isolate, TR-37, which exhibited the highest dye degradative activity, was identified to be *Bacillus cereus*.

KEYWORDS: Reactive Brilliant Red X3b, *Bacillus cereus*, Biodegradation, Azo dye, Wavelength Maximum, Inoculum volume.

INTRODUCTION

The textile industry generates huge volumes of wastewater and effluents containing dyes are very recalcitrant to treatment (Pearce *et al.*, 2003). Discharge of colored textile effluents into drainages and lakes results in reduced dissolved oxygen concentration and creates toxic conditions to aquatic flora and fauna (Liu *et al.*, 2004). Among many classes of synthetic dyes used in the textile and dyeing industries, azo dyes are used widely in many industries due to their bright color, excellent colorfastness and ease of application (Mahony *et al.*, 2002). Azo dyes, containing one or more azo bond (-N=N-), account for 60-70% of all textile dyestuffs used (Carliell *et al.*, 1995). It is estimated that about 10-15% of the total production of colorants is lost during their synthesis and dyeing processes (Easton, 1995; Maguire, 1992). Whereas, in the case of reactive dyes almost 50% of the initial dye load is found in the dye bath effluents. Although several physico-chemical methods have been used to eliminate the colored effluents in wastewater, they are generally expensive, produce large amounts of sludge. More often these conventional modes of treatment lead to the formation of some harmful side products. Physical and chemical methods include adsorption, chemical precipitation,

flocculation, photolysis, chemical oxidation and reduction, electro-chemical treatment and ion-pair extraction (Azmi *et al.*, 1998; Moreria *et al.*, 2000; Rajeshkannan *et al.*, 2010; Rajeshkannan *et al.*, 2011). Hence, biological mode of treatment of textile industry effluents using microbial biodegradation of dyes is a better alternative (An *et al.*, 2002). The biological mode of treatment of dye bath effluents offers distinct advantages over the conventional modes of treatment. This method is more economical and leads to less accumulation of relatively harmless sludge. Most importantly, biological treatment of dye bath effluents is ecofriendly. It causes mineralization of dyes to simpler inorganic compounds which are not lethal to life forms. Some microorganisms, including bacteria, fungi and algae, can degrade or absorb a wide range of dyes (Robinson *et al.*, 2001). Tirupur in Tamil Nadu state, popularly known as “Banian City” is located 60 kms away from Coimbatore city on the bank of Noyyal river. According to some estimates, Tirupur cluster comprises of around 5,000 units which are involved in one or the other activities of Textile value chain and Tirupur contributes to about 54.57% of total quantity of Indian textile exports (Rajkumar and Nagan, 2011). The Noyyal river gets polluted when it passes through Tirupur, due to discharges

of 96.1 million litres per day (MLD) of coloured effluent with high total dissolved solids (TDS) in the range of 6000 to 7000 mg/L, by the textile bleaching and dyeing units in Tiruppur (Rajkumar and Nagan, 2011). Hence in the present study, Tiruppur was selected as the place for the collection of the textile effluent samples. The present investigation was carried out with the following objectives-

- 1) Determination of the lambda max of the selected azo dye representative of the textile industry effluent
- 2) Isolation of bacterium from textile industrial effluent exhibiting highest degradability of the selected azo dye
- 3) Subjecting the bacterial isolate to aerobic biodegradation assays
- 4) Identification of bacterium exhibiting highest degrade ability of the selected azo dye

MATERIALS AND METHODS

Sampling station and sample collection

Three untreated textile effluent samples, T1, T2 and T3 were collected from three effluent discharge points within Tiruppur district surrounding Tiruppur town, Tamil Nadu, India bearing latitude-longitude co-ordinates as 11.1085° N, 77.3411° E. The samples were collected in 500 ml sterile, reagent glass bottles, sealed with adhesive tape and immediately stored on ice in an insulated box. The samples were brought to the laboratory as early as possible and stored in a refrigerator till further use. Determination of the wavelength maximum (λ_{max}) of the selected azo-dye representative of the textile industry effluent

Since "Reactive Brilliant Red X-3b" is one of the most commonly used Azo-dyes in the textile industry, it was selected in the present study as a representative of the Azo-dyes present in the textile industry effluent. The dye, Reactive Brilliant Red X-3b was purchased from Supremo Dyes Stuff Pvt. Ltd., Ahmedabad, and Gujarat. The molecular formula of the dye is $C_{19}H_{10}Cl_2N_6Na_2O_7S_2$ and the molecular weight of the dye is 615.324 g/mol. Its IUPAC name is "Disodium;4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]-5-oxido-6-phenyldiazanyl-7-sulfonaphthalene-2-sulfonate". Its molecular structure is given in Figure 1.

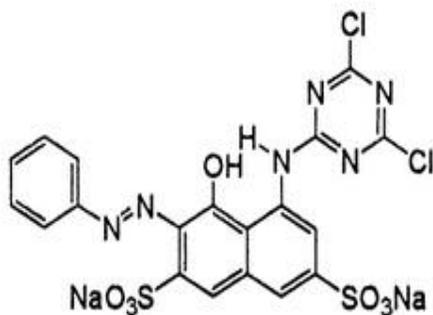


FIGURE 1: The molecular structure of the azo-dye, Reactive Brilliant Red X-3b

The working solution of the dye was prepared by preparing the solution of 100 ppm concentration in 500 ml distilled water using a volumetric flask. Initially, the dye solution was scanned for its absorbance within a wide

wave length range from 350–600 nm differing consecutively by 50 nm using a UV-VIS double beam Spectrophotometer of Systronics-2201. Once the maximum absorbance was noted down, the dye solution was scanned for its maximum absorbance within a narrow range of wavelength ranging from 500 nm – 600 nm differing consecutively by 10 nm following the standard protocol.

Analysis of physico-chemical properties of the effluent

The effluent samples were analyzed for various physico-chemical parameters *viz.*, Temperature, pH, Electrical Conductivity (EC), Colour, Odour, Total dissolved solid (TDS), Total suspended solids (TSS), Chemical oxygen demand (COD), Biological oxygen demand (BOD), Dissolved Oxygen (DO), Total Hardness, Chloride, Ca Hardness and Mg Hardness as per standard protocol (APHA, 1999).

Isolation of bacteria from textile industrial effluent exhibiting highest degradability of the selected azo-dye Tenfold serial dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} were carried out with sterile physiological saline as the diluent as per standard procedure. The serially diluted samples were spread plated onto Luria Bertani (LB) agar plates containing a final concentration of 100 ppm of the selected azo-dye, Reactive Brilliant Red X-3b. Triplicates were maintained for each dilution. The plates were incubated at room temperature for 24 hours. The bacterial colonies exhibiting clear zones around them were picked up and purified onto LB agar plates by streak plating. The purified bacterial cultures were maintained in LB agar slants in the refrigerator till further use.

Dye biodegradation assays using the standard azo-dye, Reactive Brilliant Red X-3b:

Dye biodegradation assays were carried out in 250 ml Erlenmeyer flasks. Each of the flasks was added with 100 ml of Nutrient Broth. The pH was adjusted to 7.0 ± 0.2 . The flasks with the medium were autoclaved at 121°C at 15 lbs pressure for 15 minutes. The autoclaved flasks were added with the required volume of filter sterilized Azo-dye, Reactive Brilliant Red X-3b to get a final concentration of 100 ppm from the 1000 ppm stock solution. The assay was designed to investigate the effect of three different inoculum volumes of 5 ml, 10 ml and 15 ml of three different bacterial isolates showing prominent degradative activity. The flasks were incubated in a mechanical shaker room temperature for 4 days. About 5 ml of the samples were drawn at every 12 hours intervals for observation. The samples were centrifuged at 12,000 rpm for 20 minutes and the degradation of the dye was assessed by measuring absorbance of the supernatant at the wavelength maximum (λ_{max}) of the Azo-dye, Reactive Brilliant Red X-3b.

Identification of selected bacterial isolate using Biochemical Tests

From the results of the dye biodegradation assay, only one bacterium among the five bacterial cultures showing the maximum dye degradative activity was selected and subjected to the standard biochemical tests of bacterial taxonomy protocol of Bergey's Manual [16]. The selected bacterial strain was also subjected to molecular taxonomy and the results of both taxonomy protocols were compared to ascertain the Genus and the Species of the isolated

bacterial strain with very prominent dye degradative activity.

RESULTS

Determination of the lambda max of the selected azo-dye representative of the textile industry effluent

The wavelength maximum (λ_{max}) of the Azo-dye, Reactive Brilliant Red X-3b was determined to be 540 nm. In the preliminary broad wavelength scan the maximum absorbance was recorded around 550 nm (Figure 2) and in the final narrow wavelength scan the maximum absorbance was recorded at 540 nm (Figure 3).

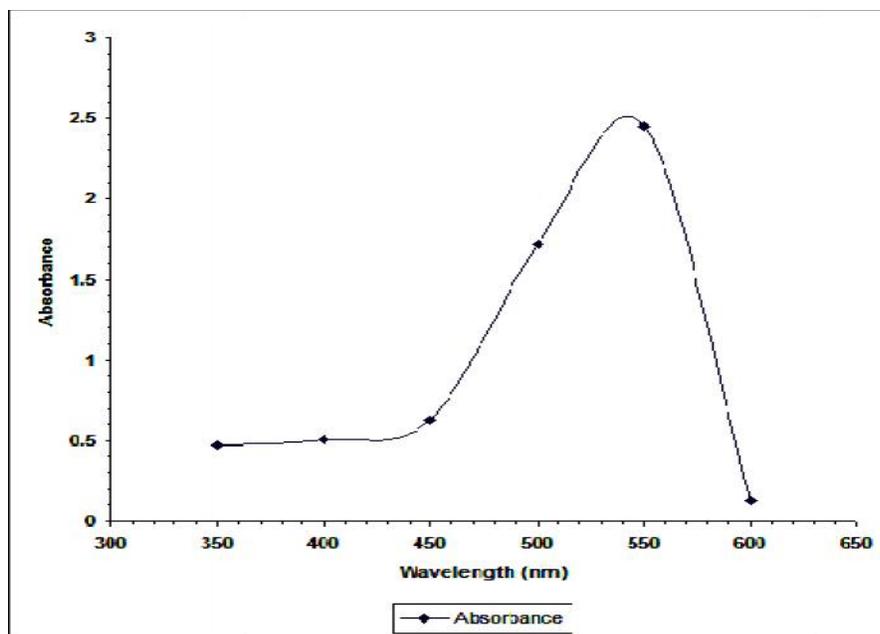


FIGURE 2: Determination of wavelength maxima (λ_{max}) of the Azo-dye, Reactive Brilliant Red X-3b at a broad wavelength scan

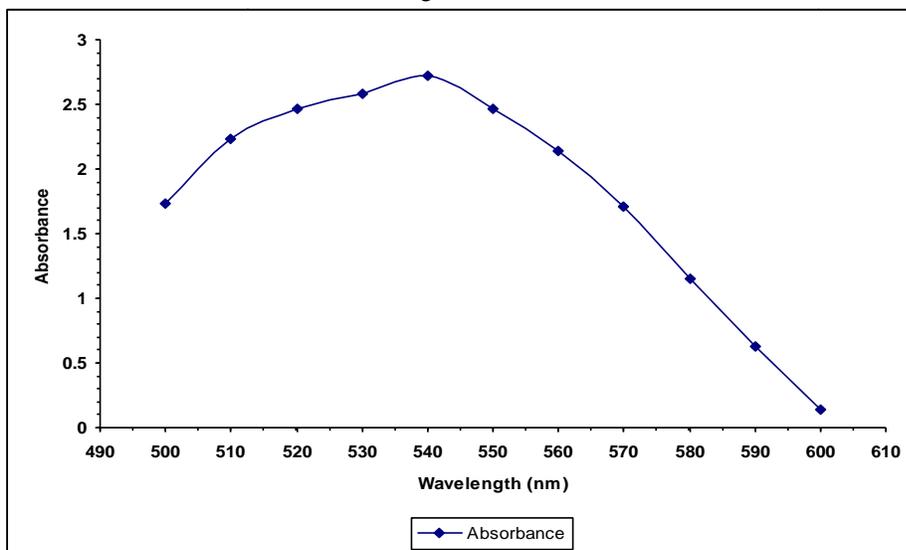


FIGURE 3: Determination of wavelength maxima (λ_{max}) of the Azo-dye, Reactive Brilliant Red X-3b at a narrow wavelength scan

Analysis of Physico-Chemical properties of the effluent

The physico-chemical properties of the three untreated effluent samples, T1, T2 and T3 were analysed and the results are given in Table 1. The Total Dissolved Solids

(TDS), Total Suspended Solids (TSD), Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) were observed to be very high in all the three untreated effluent samples.

TABLE 1. Physico-chemical properties of the untreated textile industry effluent samples

Parameter	Textile Industry Untreated Effluent Sample		
	T1	T2	T3
Color	Black	Black	Black
Odour	Pungent	Pungent	Pungent
Temperature	35 ⁰ C	35 ⁰ C	35 ⁰ C
pH	8.3	8.5	7.8
Total dissolved solids (mg/l)	2210	2155	2317
Total suspended solids (mg/l)	148	165	139
Chemical Oxygen Demand (mg/l)	833	845	798
Biochemical Oxygen Demand (mg/l)	205	220	184
Total Hardness (mg/l)	312	308	285
Chloride (mg/l)	1185	1210	1156
Ca-Hardness (mg/l)	193	216	195
Mg-Hardness (mg/l)	68.16	71.84	66.29

Isolation of bacteria from textile industrial effluent exhibiting highest degradability of the selected Azo-dye

A total of 52 bacterial strains with the dye degradative property were isolated from the three effluent samples. About 18 bacterial strains were isolated from the effluent

sample T1, 13 bacterial strains from the effluent sample T2, 21 bacterial strains from the effluent sample T3 were isolated (Table 2).

TABLE 2. Number of bacterial strains isolated from the three effluent samples

Effluent Samples	T1	T2	T3
Number of Bacterial Isolates	18	13	21

Dye Biodegradation Assays using the standard Azo-dye, Reactive Brilliant Red X-3b

The optical density values were recorded for the dye degradation assay at three different inoculum volumes of 5 ml, 10 ml and 15 ml for the three bacterial strains, TR-17, TR-21 and TR-37 (Tables 3,4 and 5) (Figures 4,5 and 6) respectively. The absorbance values decreased considerably with increasing inoculum volume in all the three isolates. The inoculum volume of 15 ml exhibited

highest degradation activity in all the three bacterial strains tested. In the present study, it was observed that as the number of days of incubation increased, the absorbance values of the dye decreased. In the present study, the highest biodegradative activity was exhibited by the bacterial isolate TR-37 when compared to the other two isolates.

TABLE 3. Absorbance values reflecting biodegradation of Reactive Brilliant X3b by the bacterial strain, TR-17

Time of Incubation (Hrs)	Inoculum Volume of the selected bacterial culture		
	5 ml	10 ml	15 ml
0	0.61	0.61	0.62
12	0.59	0.57	0.54
24	0.56	0.54	0.51
36	0.52	0.51	0.49
48	0.48	0.46	0.44
60	0.45	0.43	0.41
72	0.42	0.40	0.38
84	0.38	0.37	0.35
96	0.36	0.34	0.31

TABLE 4. Absorbance values reflecting biodegradation of Reactive Brilliant X3b by the bacterial strain, TR-21

Time of Incubation (Hrs)	Inoculum Volume of the selected bacterial culture		
	5 ml	10 ml	15 ml
0	0.62	0.63	0.63
12	0.58	0.59	0.56
24	0.55	0.54	0.52
36	0.52	0.50	0.49
48	0.50	0.48	0.46
60	0.47	0.45	0.43
72	0.45	0.43	0.41
84	0.42	0.41	0.38
96	0.39	0.37	0.34

TABLE 5. Absorbance values reflecting biodegradation of Reactive Brilliant X3b by the bacterial strain, TR- 37

Time of Incubation (Hrs)	Inoculum Volume of the selected bacterial culture		
	5 ml	10 ml	15 ml
0	0.61	0.61	0.62
12	0.53	0.49	0.45
24	0.48	0.43	0.38
36	0.42	0.39	0.33
48	0.39	0.34	0.27
60	0.35	0.31	0.21
72	0.32	0.29	0.18
84	0.27	0.24	0.13
96	0.22	0.19	0.08

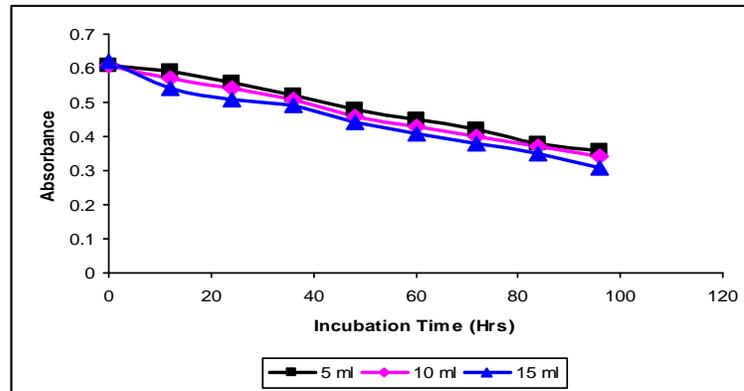


FIGURE 4: Optical density values reflecting biodegradation of Reactive Brilliant Red X-3b by the bacterial strain TR-17

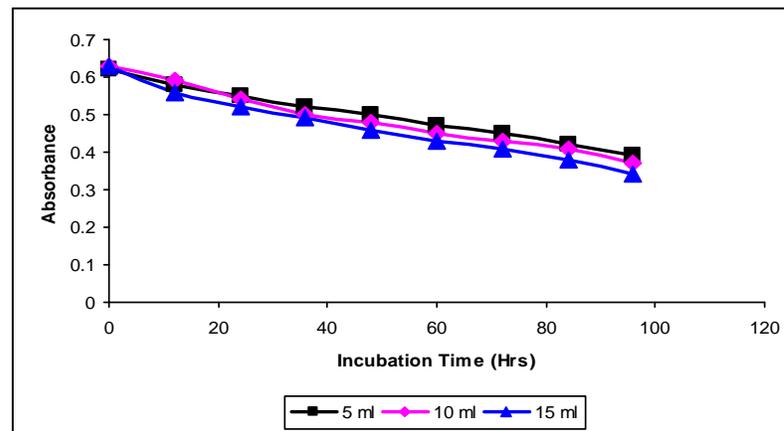


FIGURE 5: Optical density values reflecting biodegradation of Reactive Brilliant Red X-3b by the bacterial strain,TR-21

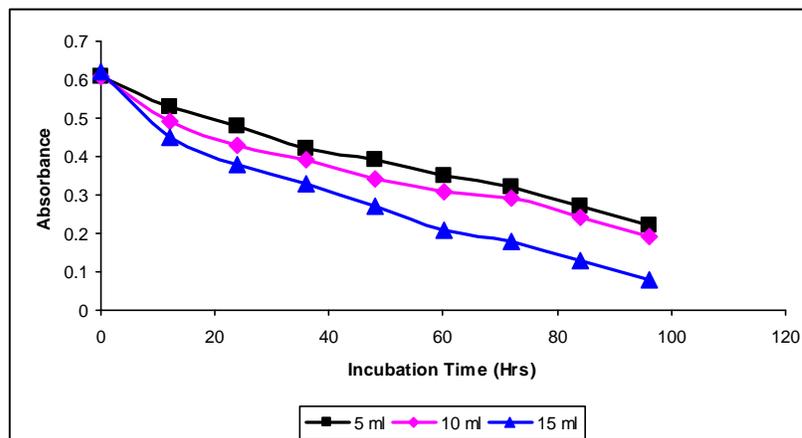


FIGURE 6: Optical density values reflecting biodegradation of Reactive Brilliant Red X-3b by the bacterial strain, TR-37

Identification of selected bacterial isolate using Biochemical Tests- TR-37

In the present study, the bacterial isolate, TR-37, which exhibited the highest dye degradative activity, was identified to be *Bacillus cereus* (Table 6).

TABLE 6. Results of the biochemical tests for the identification of the bacterial strain TR-37

Biochemical Tests/Staining	Result
Gram Staining	Gram +ve Rod
Motility	+
Spore	+
Indole	-
Methyl Red	-
Voges Proskauer	-
Citrate	+
Catalase	+
Oxidase	-
Nitrate Reduction	-
Urease	-
Glucose -Fermentation	+
Lactose	+
Sucrose	+
H ₂ S Production	-

DISCUSSION

Determination of the lambda max of the selected azo-dye representative of the textile industry effluent

In the present study, the wavelength maximum (λ_{max}) of the Azo-dye, Reactive Brilliant Red X-3b was determined to be 540 nm (Figure 2 and 3). The results of the present study are in agreement with those of Zhang *et al.* (2017) who also observed in their study that the Azo-dye, Reactive Brilliant Red X-3b had the absorption maximum of 540 nm.

Analysis of physico-chemical properties of the effluent

It is observed in the present study that, Total Dissolved Solids (TDS), Total Suspended Solids (TSD), Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) were observed to be very high in all the three untreated effluent samples (Table 1). The textile industry waste water contains acids/alkalis, common salt (NaCl), heavy metals, sulphides, chlorine and mineral oils. As a result, the dye wastewaters are extremely toxic to both aquatic fauna and flora, crop plants, including human beings (Sharma *et al.*, 1999). Observations of high concentration of TDS and TSS in the textile industry effluent elsewhere have also been reported (Kaur *et al.*, 2010). The presence of high levels of BOD and COD affect the aquatic flora and fauna and destroys the ecosystem (Shah and Patel, 2014).

Isolation of bacteria from textile industrial effluent exhibiting highest degradability of the selected azo-dye

In the present study, among the total of 52 bacterial strains with the dye degradative property, 18 bacterial strains were isolated from the effluent sample T1, 13 bacterial strains from the effluent sample T2, 21 bacterial strains from the effluent sample T3 were isolated and all of them exhibited dye degradation ability (Table 2). The sites contaminated with the textile industry effluents and the effluent water can be rich sources of micro-organisms with ability to degrade the dyes associated with the textile industry waste water and many workers isolated micro-

organisms with dye degradative abilities from them (Dawkar *et al.*, 2009; Selvakumar *et al.*, 2013; Shah, 2014; Bouraie and Din, 2016). It is well known that bacteria can degrade and even completely mineralize many reactive dyes under certain conditions, (Asad *et al.* 2007 ; Kapdan and Erten, 2007). Tripathi and Srivastava (2011) reported that *Bacillus cereus* MTCC 3105 was the second best degrader of Azo dye Acid Orange 10 in their study.

Dye biodegradation assays using the standard azo-dye, Reactive Brilliant Red X-3b

In the present study, the absorbance values decreased considerably with increasing inoculum volume in all the three isolates of TR-17, TR-21 and TR-37 (Tables 3,4 and 5) (Figures 4,5 and 6). The inoculum volume of 15 ml exhibited highest degradation activity in all the three bacterial strains tested. Hence, the present study clearly reveals that the process of biodegradation of the dye is directly proportional to the inoculum volume. On the contrary, it was observed in a study that maximum biodegradation activity was recorded at 3 % of inoculum volume (v/v) for the dye reactive red M8B, but further increase in the inoculum volume to 4% did not increase the biodegradation and it can be due to nutrient limitation (Nikhil *et al.*, 2012). In the present study, it was observed that as the number of days of incubation increased, the absorbance values of the dye decreased and this clearly demonstrates that biodegradation process is also directly proportional to the duration of incubation. Shah and Patel (2014) also observed that the degradation of the representative dye "Reactive Red 195" was highest at the end of 5 days of incubation by the selected micro-organisms. In the present study, the highest biodegradative activity was exhibited by the bacterial isolate TR-37 when compared to the other two isolates.

Identification of the selected bacterial isolate, TR-37 using biochemical tests

In the present study, the bacterial isolate, TR-37, which exhibited the highest dye degradative activity, was

identified to be *Bacillus cereus* (Table 6). Many different groups of microorganisms, namely, fungi, bacteria including actinomycetes, have the ability to degrade the dyes from the textile industry effluents (Chang *et al.*, 2001; Khehra *et al.*, 2005). Among bacteria, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Clostridium biofermentans* and *Aeromonas hydrophila* were reported to have the dye-degradative ability (Shah, 2014; Bouraie and Din, 2016). In the present study, the bacterial isolate TR-37, exhibiting the highest biodegradative activity upon the Azo-dye Reactive Brilliant Red X3b was subjected various biochemical tests for identification and was identified to be *Bacillus cereus* (Table 6). It was observed by many workers that *Bacillus spp.* has better degradative ability when compared to other bacterial species with respect to the biodegradation of the synthetic dyes in the textile industry effluents (Prasad and Rao, 2011; Tripathi and Srivastava, 2011). In another study, Sriram and Reetha (2015) also identified the bacterial isolate with second highest degradative activity against the dyes present in the textile industry effluents as *Bacillus cereus*.

CONCLUSION

The results of the present study, clearly demonstrate that among micro-organisms, bacteria have great ability to biodegrade the azo-dyes present in the untreated textile industry effluent. It is evident from the results of the present study that as the inoculum volume and the duration of treatment increase, the rate of biodegradation also increases. It is also clear from the results of the present study that *Bacillus cereus* can be employed as one of the most effective biological agents among bacteria, during the biodegradation of the Azo dyes in the effluents of the textile industry.

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