



Research Article

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**Isolation and Characterization of Microbial Strains from Textile Industry Effluents of Bhilwara, India: Analysis with Bioremediation**

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**ABSTRACT**

Waste water discharged from textile and dye industry causes serious environmental hazards. Many physical and chemical methods have been used to treat the waste water but unfortunately because of the high operating costs suitable alternative will be required. Therefore, present study deal with use of biological organism with potential bioremediation, which lead to the production of cost effective and nontoxic methods. In that view, present study was conducted on effluent discharge collected from textile industries of Bhilwara, Rajasthan-India. Our objective to examine potential biological strains isolated from untreated effluents discharged from textile industries in Banas river, Bhilwara Rajasthan. India. Based on the results obtained from morphological, biochemical and molecular datasets present investigation identified potential bacterial strains belonging to species *Bacillus cereus*, *Exiguobacterium sp* and *Acinetobacter sp*. All selected bacterial isolates showed decolourization of the dye effluent. Thus, present study reveals that textile effluents consisting of potential bacteria that utilize the dyes as the source of energy and exhibit the property of treating of industrial effluents.

**Keywords:** Textile effluents, Bioremediation, Morphological characterization, Molecular characterization, rDNA sequencing

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**INTRODUCTION**

One of the fast growing industries in India is textile industry, consuming large quantities of water and produces large volumes of wastewater during processing unit of textile manufacture. Continuous flow of textile industrial waste into river and cultivated land now is one of the major global problems. Although efforts for reducing water pollution are very limited. The discharge of dye-containing effluents, directly released into the water channels is serious problem for human and animal [1]. Furthermore, untreated industrial effluents flowed into the environment are also hazardous to the flora and fauna life, and is one of the reasons behind food contamination. These effluents are also the carrier of dyes, organic and inorganic materials such as high quantity of Cl, Pb, Fe, Fl and other heavy metals [2]. Heavy metals present in textile effluents (either in free form or in suspended solids) are also carcinogenic [3].

Bhilwara, Rajasthan India has more than 4000 textile manufacturing units which exports their production to approx 70 countries especially in Europe, South Africa and North America. This region is called Indian textile town which constitutes many dyeing and bleaching units. Bhilwara serves as one of the major exporters of textiles and is the second largest producer of polyester fiber in India [4]. Textile units in this area flowed dye consisting of heavy metal (Chromium, lead, Iron and Zinc) in open wells of villages near the Banas river above the norms set by the Bureau of Indian Standards. Besides, quality of drinking water in this region is also a not safe because water is contaminated with high levels of chlorides and fluorides. Agriculture practices of villages located downstream of textile processing unit are seriously affected [5]. The physiochemical methods to treat textile industry effluents are very difficult and also expensive. Therefore, continues the accumulation of large amount of sludge and toxic material in soil which

also creates the secondary level of land pollution [6, 7]. Moreover the cost effectiveness and ease in synthesis, azo dyes are much more used in industries compared to natural dyes. Usually dyes are aromatic and heterocyclic compounds and are toxic, carcinogenic and mutagenic especially azo dyes [8, 9]. Azo bonds (N=N) present in these compounds are resistant to break, with the potential for the persistence and accumulation in the environment [10]. Therefore, degradation of dyes by conventional methods is very difficult [11]. Other alternative method is being required to minimize the costs of treatment of effluents discharge [12]. A wide range of microorganisms (bacteria, fungi and yeast) has been reported capable of decolorization of textile dyes [13, 14]. Various study demonstrated the ability of bacteria to degrade and decolorize depends on its adaptability either aerobic or anaerobic and is also cost effective and environment friendly alternative for disposal of textile effluents [7, 15, 16]. Many microorganisms are used as bioremediative agents in the waste water treatment containing textile dyes. *Bacillus subtilis*, a dye degrading bacteria was first isolated in 1977, in 1978 *Aeromonas hydrophila* and *Bacillus cereus* in 1980. Recently, in several report using bacterial strains showed high efficacy for removal of azo dyes. Various bacterial pure culture has been reported that ensures the degradation of dyes such as *Pseudomonas sp.* SUK1[17], *Pseudomonas aeruginosa* NBAR12 for reactive Blue 172 [18], *Rhizobium radiobacter* MTCC8161 for reactive red 141[19]. Other bacterial strains such as *Bacillus cereus*, *Pseudomonas sp.* and bacterial consortium (*Aeromonas caviae*, *Proteus mirabilis* and *Rhodococcus globerulus*) showed dye decolorization [20, 21, 22]. Another consortia having 6 isolates (*Bacillus cereus*, *Bacillus myocoides*, *Bacillus subtilis*, *Bacillus sp.*, *Micrococcus sp.* and *Pseudomonas sp.*), have been used in the biodegradation of azo dyes [23]. Bacteria also exhibit tremendous potential to decolorize and detoxify azo dyes composed of phenylamine, benzenediazonium chloride or phenol [24, 25]. Some bacteria disintegrate azo bonds of the dyes, which result in the formation of colorless amines and subsequently simpler compounds [26]. The degradation process of these complex dyes lead to end products which are not toxic, under a given specified environmental conditions. Microorganisms have developed enzyme system for the decolourisation and mineralization of azo dyes under certain environmental conditions [27, 28, 29, and 30]. Primary characterization of the bacterial isolate including its morphological, physicochemical, biochemical characters and its decolourization activity and the molecular identity gives complete information with the reference to the further application of strain for bioremediation [31]. Therefore, present study with isolation, identification and characterization of indigenous bacteria from textile dye effluent and evaluation of their ability to decolorize dyes. Present research will also focus to analyze bio-degradative capacity of the selected bacterial strain using molecular methods. Major application of this work is to scale up microbial consortia in lab and exploit for commercial purpose. In future, our study will pave way for application of bioremediation techniques *in situ*, for decontamination of wastes of the textile industry. The treatment of the industrial wastes by the selected microorganisms will finally yield a non-toxic product(s), which can either be used further or as a final product. Therefore, the assessment of this approach, combining both scientific and socio-economic aspects will be performed at particularly Bhilwara, area of Rajasthan, India.

## EXPERIMENTAL SECTION

### 2.1 Sample collection site

The untreated and treated textile effluents were released into the Banas river by dyeing industries of Bhilwara, Rajasthan, India. Banas River Basin is located in east-central Rajasthan, between latitudes 24°15' and 27°20'N and longitudes 73°25' and 77°00'E. Sampling of effluents from three collection points of textile mill mention in Table (1) brought in a sterile screw capped bottles. The samples were brought to the laboratory within 1 hour of collection in an ice packed cooler box and stored at 4°C.

### 2.3 Physicochemical analysis

The physicochemical parameters such as color, pH, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TSS (Total Suspended Solids), TDS (Total Dissolved Solids) were determined as soon as brought to the laboratory as per the standard methods [32]. pH was determined by electronic digital pH meter (Scientech, India).

### 2.4 Isolation and screening of bacteria

One ml of the effluent was serially diluted ( $10^{-1}$ - $10^{-3}$ ) and loopful of each dilution was inoculated on nutrient agar ( $\text{gL}^{-1}$  Peptone -5, Beef extract- 3, NaCl - 5, Agar -15, pH - 7.0). The plates were incubated at 37°C for 24–48 hours. Morphologically different colonies were developed and transferred separately each colony into 10ml Luria broth culture (Hi-Media, India) and further sterilization was done by autoclaving at 121°C at 15 psi.

### 2.5 Decolourisation Assay

The decolorizing activity was observed using U.V spectrophotometer and absorbance expressed in terms of the percentage decolorization. Decolourization assay was carried out by inoculating 1ml. of pre-cultured bacterial isolates into 100ml of textile effluent and incubated on rotary shaker (130 rpm) at 37°C for 24h [33,17]. At regular intervals, 4 ml sample was withdrawn aseptically used to determine the percentage decolourization. Decolourization of dye was determined by monitoring the decrease in absorbance at maximum wavelength of 520 nm by using UV-

Visible spectrophotometer (UV-1100 Labtronics, India). The uninoculated dye consisting of yeast extract supplemented was used as blank [34]. Decolourization activity (%) was calculated by the following formula: % Decolorization =  $\frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$ .

### 2.6 Morphological Analysis

Selected colonies showed decolourization was picked and purified by repeated subcultures on Luria agar (Hi Media, India) plate. The colonies were further used for morphological analysis by gram staining and shape analysis methods [35].

### 2.7 Biochemical characterization

Biochemical analysis using selected bacterial strains were carried out by the methods described in Bergey's manual of determinative bacteriology [35]. Biochemical tests (Indole, Methyl Red, Voges-Proskauer test, Citrate, Catalase, Oxidase, Nitrate Reduction test, Hydrolysis of Casein, Starch, Urea and Gelatin) was carried out for the identification of bacterial isolates. Utilization of various sugars by same bacterial isolate such as D-glucose, D-fructose, galactose, mannitol and D-maltose as sole carbon source was determined by inoculating isolates into carbohydrate broth supplemented with respective carbon source.

### 2.8 Molecular Characterization

Genomic DNA of selected bacterial strain was extracted using genomic DNA isolation kit (InstaGene™ matrix, Biorad- USA). The 16S region of ribosomal rRNA gene was amplified using the universal primers 27F and 1492R primer [36]. The PCR amplification was done by initial denaturation at 94°C for 2 min followed by 35 cycles of 94°C for 45 seconds, 55°C for 60 sec, 72°C for 60 sec and final extension at 72°C for 10 min. PCR purification was done by PCR purification kit (Qiagen, USA). The purified PCR product was sequenced in both directions using an automated sequencer by ABI 3730x1 sequencer (Applied Biosystems, USA). The sequencing was performed on an automated multicapillary DNA sequencer, namely ABI Prism 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the Big Dye Terminator ver. 3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) at the sequencing laboratories of (Yaazh xenomics, New Delhi, India). The sequences were assembled and edited using Bioedit software and deposited in the NCBI database for accession numbers. Sequence similarities were determined using the Blastn similarity search algorithm available at the NCBI home page [37]. The phylogenetic tree was constructed using MEGA v.4.0 [38].

## RESULTS

### 3.1 Physicochemical Characterization of Textile Effluents

Effluent collected from textile Industry, of Bhilwara, India, was reddish brown in colour due to presence of mixture of various dyes and chemicals used during dyeing process with pungent smell and pH above permissible limits [39]. TSS and TDS in the textile effluent were very high as compared with normal permissible level. COD and BOD values were also high in the effluent sample (Table. 2)

### 3.2 Isolation of Dye Decolorizing Bacterial Strains

Potential decolorizing bacteria was isolated from the textile industry effluent. Colonies surrounded by a nearly decolorized zone were isolated and then tested for dye removal capability using yeast extract media. Three bacterial strains exhibiting decolorizing activity were chosen for molecular and biochemical characterization. Potential bacterial strains with decolorizing ability are shown in Figure 1 (A-B).

Decolorization assay was performed in yeast extract media to check dye effluent decolorization by bacterial strains, in which the bacterial colonies isolated from pure culture were transferred. Five colonies were isolated as B1, B2, B3, B4 and B5 showed decolourization. U.V Spectrophotometer analysis suggested that, strain B1 showed maximum (80%) dye decolorization in yeast extract however strain B2, strain B3, B4 and B5 showed moderate level of dye degradation in same broth shown in (Figure. 1,A-B).

### 3.3 Morphological Identification

Gram staining tests showed that out of five bacterial colonies three bacterial strain (B1, B2, and B3) showed positive gram positive and shape colonies were circular and smooth. However, two colonies B4 and B5 showed negative in gram reaction with rough and cocco-bacillus in shape as shown in Figure 2.

### 3.4 Biochemical Identification

Bacterial strain B1 showed negative results of Methyl Red, Voges-Proskauer test, Urease, Citrate utilization, Indole, Gelatin hydrolysis, Catalase, Caesin hydrolysis and positive for Oxidase and Nitrate reduction. Strain B1 utilizes

starch and produces acid from Maltose, Fructose and Mannitol. Colony B1, B2 and B3 utilize nitrogen source whereas bacterial colony B4 and B4 not utilizes nitrogen source as shown in Table 3.

### 3.5 Molecular Identification

Out of bacterial 05 bacterial strain 03 bacterial strains was belonged to genera *Bacillus*, *Exiguobacterium* and *Acinetobacter*. Sequence analysis using ribosomal DNA revealed that strain B1 > 98% similar with *Bacillus cereus*. Molecular phylogeny using maximum parsimony methods showed that bacterial strain B3 was clustered with *Exiguobacterium*. However, bacterial strain B5 was clustered with *Acinetobacter sp.* (Figure 3). The 16 S rDNA sequences of these bacteria were deposited in NCBI database with accession numbers (KU561544-46).

## DISCUSSION

The process of color removal from textile effluents discharge is very challenging for both textile industry as well as the waste water treatment plants. However, the result of present study revealed a great potential of bacteria to be used to decolorize the dye from textile wastewaters. Result based on physio chemical analysis suggested that colour of the effluent was reddish brown. In present study result also revealed that pH of the effluent was alkaline (9.58) as compared to the pH of the dyeing effluent which was acidic in a previous study [40]. Present investigation reveal that temperature of the effluent collected from textile industry was high (50°C) in compared with the temperature of textile effluent in one study [41]. High temperature decreases the solubility of gases in water which is ultimately expressed as high BOD/COD [41, 42]. Several report suggested that biological treatment methods have many advantages over physical or chemical methods [43, 44].

In the present study, a strain of bacterium *Bacillus cereus* showed strong decolorizing ability upto 80% within 48h under shaking. However *Exiguobacterium indicum* and *Exiguobacterium aurantiacums* showed percentage decolorization by 71% and 61% respectively under same condition. Furthermore, *Acinetobacter baumannii* showed decolorization activity by 53%. Many bacteria capable of reducing azo dyes were isolated from contaminated sites of textile effluent [42]. In one study, *B.cereus* decolorized cibacron red P4B dye by 81% when combined with ammonium nitrate and sucrose and 75% cibacron black PSG using yeast extract and lactose (45).

The percentage decolorization of crystal violet by *Bacillus subtilis* strain was 90% under static condition within 24h of incubation similar study reported with 35h of incubation period [22]. Percentage decolorization of the dye recorded for *Bacillus cereus* was 95% at pH 7, 37°C [46]. Another report showed that *Bacillus sp1* decolorised dye by 72.17% and *Bacillus sp2* by 58.3% where as 75.58% decolourisation by *Acinetobacter* [47]. Effluent adapted strains of *Acinetobacter*, *Bacillus* and *Legionella* were found to remove colour and chemical oxygen demand (COD) removal activities were possessed by the strains of *Acinetobacter*, *Bacillus* and *Pseudomonas* [48]. *Exiguobacterium* spRD3 isolated from dye contaminated soil exhibit dye degradation activity by cleaving azo bond and sulfonate bond of reactive blue 172 and also showing lignin peroxidase and laccase activity during degradation process [49, 50]. *B. cereus* KVGNV1 isolated from saline environment has been identified by 16S rDNA gene sequence [51, 52]. Based on the phylogenetic analysis of 16 S rRNA sequence *Bacillus sp.* VITABR13, a textile azo dye degrading bacteria was isolated [53]. Another report of biodegradation of reactive dyes by a bacterium *Lysinibacillus sphaericus* RSV-1 using MEGA-4.0 software [54]. Actinomycetes is also potent group of bacteria has being use for remediating heavy metals from effluents polluted soils [55]. Hence the present study was undertaken for the isolation of effective bacteria for the treatment of textile dye effluent. Based on the morphology, biochemical and phylogenetic analysis bacterial strains were identified as *Bacillus cereus*, *Exiguobacterium sp.*, *Acinetobacter baumannii* and showed potential bioremediation property. However, this is only possible if in the future strains are applied in treatment of waste waters using appropriate bioreactors [56].

Table 1: List of collection site with bacterial strain used in the study

Sample	Aeration	Outlet
Collection unit	Dilution	Bacterial isolates
Effluents (Direct discharge )	No	B1
(Recycled) Aeration tank	1:10	B2
	0.100	B3
Outlet tank	1:10	B4
	1:100	B5

Table 2: Physical and Chemical Parameters of Textile Waste Water

Parameter	Standard	Effluent Value
Colour	-	Reddish Brown
Odour	-	Pungent
pH	5.5- 7.0	9.58 (Aeration tank), 8.8 (outlet unit)
Temperature	40° C	50° C
TSS(mg/l)	50 to 150	180
TDS(mg/l)	3000	3284
BOD(mg/l)	20 to 40	49
COD(mg/l)	120 to 400	303

Table 3: Biochemical Characteristics of the Five Bacterial Strains Isolated from Textile Effluents

CHARACTERS	Strain B1	Strain B2	Strain B3	Strain B4	Strain B5
Methyl Red Test	-	-	-	-	-
Voges-proskauer	-	-	-	-	-
Indole Test	-	-	-	-	-
Citrate Test	-	-	-	+	+
Urease Test	-	-	-	-	-
Nitrate Reduction Test	+	+	+	-	-
Starch Hydrolysis	+	-	-	-	-
Gelatin Hydrolysis	-	-	-	-	-
Catalase Test	-	-	-	-	-
Caesin Hydrolysis	-	-	-	-	-
Oxidase Test	+	+	-	-	-
Carbohydrate Fermentation					
Maltose	Acid / NG	Acid / NG	Acid / NG	-- / NG	-- / NG
Fructose	Acid / NG	Acid / NG	-- / NG	-- / NG	-- / NG
Mannitol	Acid / NG	Acid / NG	-- / NG	-- / NG	-- / NG
Dextrose	-- / NG	Acid / NG	-- / NG	Acid / NG	Acid / NG
Triple sugar Iron Test					
Slant	Yellow	Yellow	Yellow	Yellow	Yellow
Butt	Yellow	Red	Yellow	Red	Yellow
H <sub>2</sub> S Production	-	-	-	-	-
Gas Production	-	-	-	-	-

--Negative Test    + Positive Test    NG No Gas Production

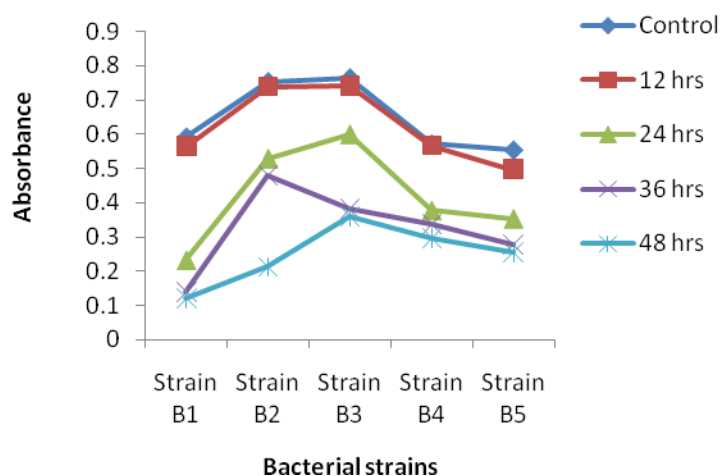


Figure-1 (A): Absorbance of dye decolorization by bacterial strain culture from textile effluent

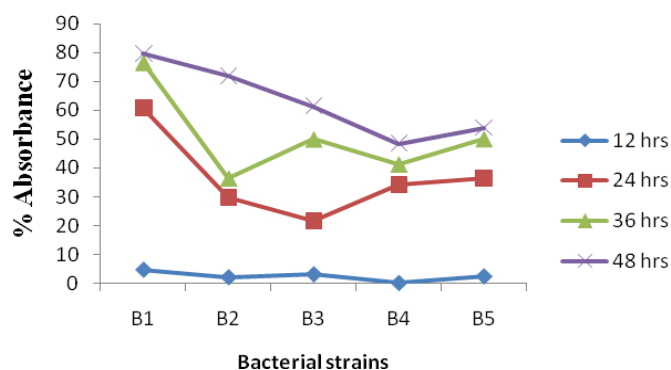


Figure-1(B):Percentage of dye decolorization by bacterial strain culture from textile effluent

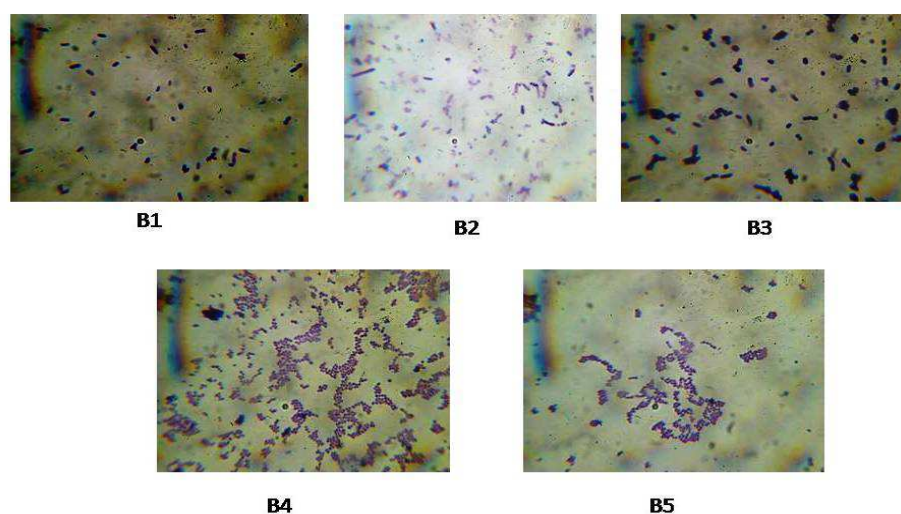


Figure 2: Microscopic view of *Bacillus cereus* strain B1, *Exiguobacterium indicum* strain B2, *Exiguobacterium aurantiacum* strain B3, *Acinetobacter baumannii* strain B4, *Acinetobacter baumannii* strain B5

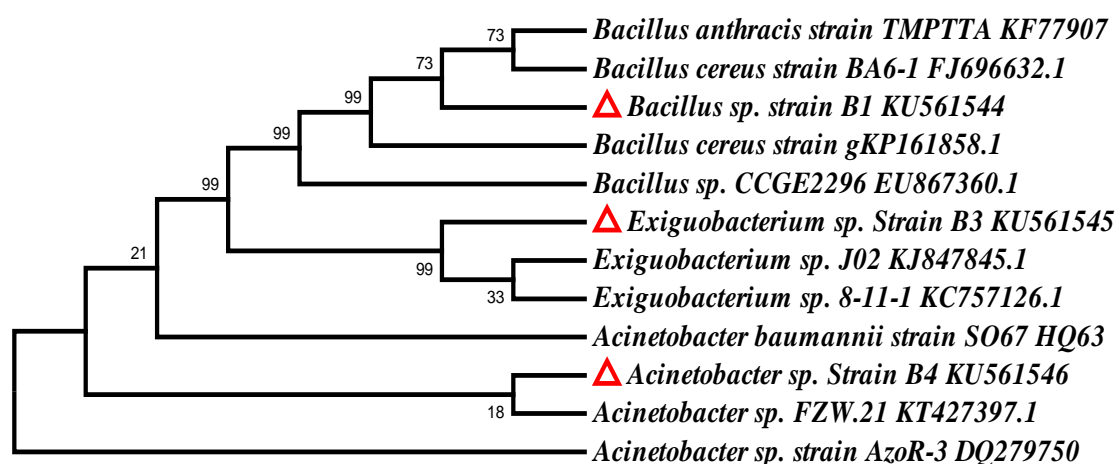


Figure3: Sequences generated from amplicon of primer set 27F and 1492R primer used for phylogenetic analysis. Maximum Parsimony tree obtained from alignment of 3, 16s SSU rDNA (1300 bp) used in present study along sequences retrieved from GenBank. Percentage bootstrap support (out of 1000 trials) is indicated. Names followed by accession no. represent sequences retrieved from GenBank. Names preceded by a shape represent the sequence obtained in this work. Non-significant value (<50%) were omitted. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1142 positions in the final dataset

## CONCLUSION

The isolated bacterial consortium had four bacterial isolates which were identified as *Bacillus cereus*, *Exiguobacterium aurantiacum*, *Exiguobacterium indicum* and *Acinetobacter baumannii* by 16S rRNA gene sequence analysis and biochemical test. Thus these observations provide hope for the establishment of efficient and cost effective methods for treatment of textile industry waste waters.

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