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Isolation and screening of textile dye decolorizing bacteria from textile industrial effluent

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Abstract
Environmental pollution caused by the release of a wide range of dyes through industrial wastewater is a serious problem in present days. Removal of dyes through microorganisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent. The present study objective is the isolation and the screening of bacteria from textile industrial effluent and evaluation of their ability to decolorize commonly used dyes. Textile effluent sample were collected from the textile industries and isolated bacterial culture were evaluated for the decolorization of Jakafi red, Jakafi blue and Jackofix golden yellow dyes. Decolorization of three selected dyes by selected isolates was determined at their specific maximum wavelength in the culture supernatant using a U V spectrophotometer. Screening of dye decolorizing bacterial isolates showed that sixteen isolates appeared positive in response to decolorization of different dyes. Three bacterial strains were identified to be capable of decolorizing more than 45% of dyes up to species. The strains were *Pseudomonas* sp. (S3, S11 and S12), *Klebisiella* sp. (S4). and *Aeromonas* sp. (S2), Isolate *Pseudomonas* sp. (S3) was the most efficient bacterial isolates to decolorize dyes by 45% to 50% removal efficiency. The isolated and identified bacterial strains were found to be most effective and having enormous potential of textile dye degradation under versatile environmental conditions.

Keywords: Textile dye, decolorizing bacteria, textile industrial effluent

Introduction
Industrial effluents containing variety of dyes can be treated by a number of physicochemical approaches as, filtration, adsorption, coagulation, chemical precipitation, chemical oxidation, reduction, photolysis, use of activated carbon, chemical flocculation etc. (Verma *et al.*, 2012; Joshi *et al.*, 2015; Olukanni *et al.*, 2006) [22, 11, 15]. However, these approaches are expensive, less efficient and produces enormous amount of sludge that are difficult to dispose of and consequently create land pollution (Shah, 2013) [19]. Biological approach using microorganisms are gaining interest due to their low cost, less sludge production and eco-friendly nature. Microorganisms can decolorize and even completely mineralize azo dyes that are widely used in various industries under certain environmental conditions (Kalyani *et al.*, 2009) [12]. The treatment processes are based on the microorganisms capable of decolorizing or degrading these recalcitrant compounds. These biological processes are environmentally friendly and can lead to complete mineralization of xenobiotic compounds. Over the past decade, many organisms capable of dye decolorization at lab scale have been reported, but there are few reports available on their exploitation in treatment processes. Decolorizing of dyes through microorganisms has been identified as a cost effective and environment friendly alternative for disposal of textile effluent (Rani and Singh, 2015) [17]. A wide variety of microorganisms are reported to be capable of decolonization of dyes. The types of organisms being reported to have the ability to remove or degrade triphenylmethane dyes include fungi, yeast, actinomycetes and bacteria (Azmi *et al.*, 1998) [2]. Fungi such as *Phanerochaete chrysosporium* are able to produce enzymes that are able to degrade dyes (Bumpus and Brock, 1988). Several types of bacteria species such as *Citrobacter sp.*, *Bacillus sp.* and *Pseudomonas sp*. have been found to have the dye removing ability (Jang *et al.*, 2005) [10]. Bacterial isolates from soil and sludge sample belonging to *Bacillus* sp. *Alcaligenes* sp. and *Aeromonas* sp. were found to have high dye decolorization ability (Sharma and Saini, 2004). Decolorization of Direct yellow and Erie red dyes by bacterial and actinomycetes were studied by Waffa and Moawad, 2003. Other reports suggested that *Pseudomonas* sp. (Kothari, 2002) *Escherichia coli*, sulfate reducing bacteria (Yoo, 2002) are efficient dye decolorizer. The effectiveness of these treatment systems depends upon the survival and adaptability of microorganisms during the treatment processes. The study was undertaken to study the decolorization of textile dyes by Bacterial Consortium Screened from industrial effluent situated at Solapur MIDC, (Maharashtra, India). The use of isolated bacteria either individually or as consortium was envisaged to develop efficient biological process for the treatment of effluents containing different dyes.

Material and Methods
Sample collection
Textile effluent sample were collected from the textile industries which is situated at Solapur MIDC, (Maharashtra, India) using sterile bottles and soil sample were collected from near region of textile industry. The sample were transported to laboratory at 4 °C as in accordance with the standard methods.

Isolation of dye decolorizing bacteria
Bacterial isolations were carried out by serially diluting textile

effluent samples in sterile distilled water were subsequently plated onto nutrient agar plates (Cappuccino *et al.*, 1996) [5]. The plates were incubated at 37±2˚C for 24 h and colonies with distinct morphology were picked up and purified by regular subculturing. The strains were maintained on slants of nutrient agar.

Screening of dye decolorizing bacteria

Isolated bacteria were used for the decolorization and degradation of Jakafi red, Jakafi blue and Jackofix golden yellow. The dyes stock was prepared at a concentration of 1ppm in sterile distilled water, filter sterilized and stored for further use at 4 °C. In the dye degradation study, the initial concentration of dye was kept at 10 ppm. Fifteen morphologically distinct bacterial isolates were tested for their ability to degrade the textile dyes. The isolated bacterial strains were screened out by incubating them on Nutrient Agar containing 100 mg L-1 Jakofix red, Jakofix blue and Jackofix golden yellow. The nutrient agar medium incubated at 37° C for 24 hrs. After the incubation, plates were observed for clear zone. The screened culture was transfer to agar slant and store 4 °C for further study.

Identification of selected bacterial isolates

The isolated bacteria were subjected to Gram staining, morphological and biochemical characterization as described by the Bergey's Manual of Determinative Bacteriology, 8th edition. The tests performed were IMViC, starch hydrolysis, catalase, oxidase, H_2S production, fermentation of lactose, dextrose and sucrose.

Dye decolorization assay

Decolorization experiment of those three selected dyes was performed by using 0.002 g/l of dye in 15 ml test tubes containing 10 ml of nutrient broth. A 100 µl of 24 h old bacterial culture corresponding to Mcfarland standard 0.5 was used as inoculum to inoculate the dye supplemented broth. The inoculated test tubes were incubated at 37 ^{0}C for 1, 3 and 5 days to check the absorbance. Following incubation, decolorization of dyes by selected isolates was determined at their specific maximum wavelength in the culture supernatant using a U V spectrophotometer. After incubation at each time period, samples were centrifuged at 10,000 rpm for 10 min and the supernatants were subjected to UV-spectrometry and the absorbance was recorded. The uninoculated media with JR, JB and JG dyes were used as respective blank for the dye decolorization assay. The percentage of dye decolorization was calculated as stated before (Alalewi and Jiang, 2012) [1].

$$\text{Decolorization (\%)} = \frac{\text{(Initial OD-Final OD)} \times 100}{\text{Initial OD}}$$

Optimization of and conditions for degradation of Jackofix dye

A. Effect of pH on decolorization

Effect of pH on decolorization was observed by growing the isolate in the N.B medium containing dye having pH range from pH 5.0 to 10.0. Next day centrifuge all the cultures. Media and by O.D of Supernatant was taken on Spectrophotometer and percentage of dye decolorization was calculated.

C. Effect of temperature on decolorization

Effect of temperature on decolorization was observed by growing the bacterial isolates in the nutrient medium containing Jakofix Red dye and incubate it at the different temperature like 30 °C, 37 °C, 40 °C, 45 °C by keeping the pH of the medium 7.0 for 48 h. Centrifuge all the cultures and O.D was taken on spectrophotometer and percentage of dye decolorization was calculated.

C. Effect of dye concentration

Effect of dye concentration was checked by growing the isolate in the N.B medium containing different concentration like 10µl, 20µl, 30µl, 40µl, 50µl, 60µl 70µl, 80µl, 90µl, 100µl. of Dye and incubate 2 days. Centrifuge all the cultures. and O.D was taken on spectrophotometer and percentage of dye decolorization was calculated.

D. Effect of inoculum size

Take 20 flask each flask containing 20 ml of N.B medium. 20ul of jakofix red dye add in each flask. Then add inoculum of different size and then centrifuge it and take O.D was taken on spectrophotometer and percentage of dye decolorization was calculated.

E. Effect of Media Component

Take different media component such as lactose, yeast extract, mannitol, tryptone, peptone, beef extract, fructose, maltose, glucose, malt extract, etc. and autoclave all media component inoculate the isolate then incubate 2 days at room temperature and after incubation centrifuge it then collect the supernant and O.D taken on spectrophotometer and percentage of dye decolorization was calculated.

Results and Discussion

The discharge of these highly colored industrial effluents can be very dangerous to the receiving water resources, as these dyes in the water absorb sunlight, which in result decreases the intensity of light absorbed by water plants and phytoplankton, ultimately reducing photosynthesis and the oxygenation of water reservoirs. Also, physical appearances of the colored water badly impact its aesthetic value. These dyes are xenobiotic in nature and in some cases are mutagenic and carcinogenic (Daneshvar *et al.*, 2007; Dafale *et al.*, 2010). Allergic effects of these dyes have also been reported by several scientists (Saunders *et al.*, 2004; Sasaki *et al.*, 2008).

Total twenty-five industrial effluents were collected from the textile industries from solapur and Malegaon MIDC, Maharashtra that was enriched in Nutrient Broth medium for Acclamatized to particular environment to enhance the decolorization activity at 20 ppm concentration of dye Jakofix red, Jakofix golden yellow and Jakofix Blue.

Screening and isolation of Dye decolorizing Bacteria

In order to isolate dye decolorizing bacteria, the enriched culture broth was inoculated in nutrient agar medium amended with the dye mixture. A total of 16 morphologically distinct bacterial colonies were isolated and screened out by repeated streaking on dye supplemented NA agar medium. Based on

vigorous growth on the medium four bacterial isolates designated as S1, S2, S3, to S16 were selected for further studies. During screening process no zone of clearance around bacterial colonies was observed as reported previously (Rajee *et al.*, 2011; Mahbub *et al.*, 2012) [16, 14]. Hence, growth on dye supplemented NA agar medium as white colonies considered as a positive result for screening potent dye decolorizers. According to Chen *et al.*, 2003 [6] cell mat coloring occurs as a result of biosorption of dye, whereas retaining the original mat color indicates biodegradation. Thus, retaining the original colony color presumptively suggests that dye decolorization was an enzymatic process rather than adsorption of dyes.

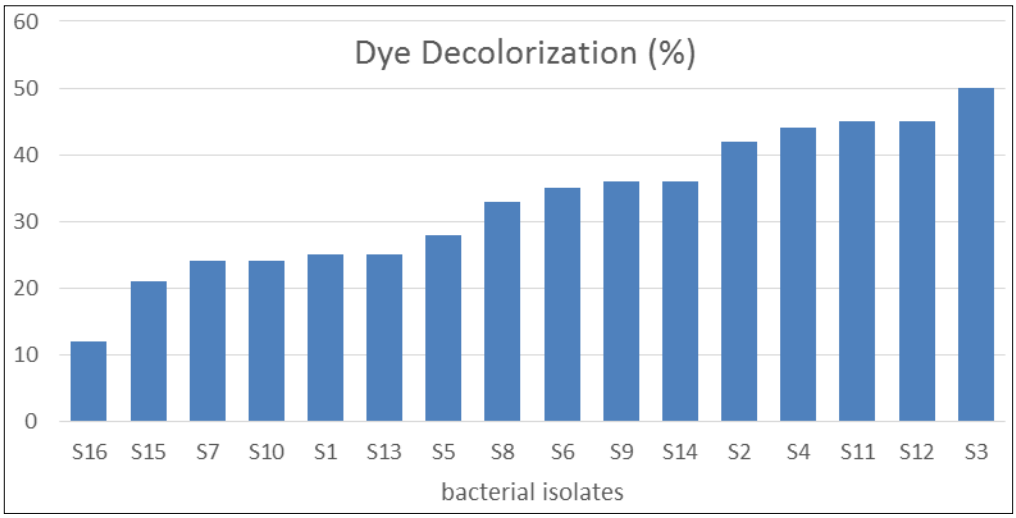

Fig 1: Screening and isolation of dye decolorizing Bacteria

Dye decolorization by bacterial monoculture and consortium

In the present study selected bacterial isolates and developed consortium was tested for their ability to decolorize three commonly used textile dyes Jakofix red, Jakofix golden yellow and Jakofix Blue at a final concentration of 100 mg L^{-1} [1]. Therefore, a final dye concentration of 100 mg L^{-1} was chosen for decolorization assay throughout the study. After 2, 4 and 6 days of incubation period, satisfactory result of dye decolorization by selected bacterial monoculture and consortium was recorded. Previous studies reported that only few researches were successful in isolating bacterial culture capable of utilizing dyes as sole source of energy (Sarnaik, 1999) [18].

This may be due to co-metabolic nature of microorganisms in natural environments. In co-metabolic process, specific co-substrates are added which can induce the biodegradation process and subsequently, reduce the overall process time (Jadhav *et al.*, 2008) [9]. It was reported that medium compositions are critical to the efficiency of microbial decolorization and the reduction of azo dyes depends on the presence and availability of a co-substrate because it acts as an electron donor for the azo dye reduction (Banat *et al.*, 1996) [3].

Table 1: Percentage of dye decolorization by bacteria isolated for effluents

Name of Dye	%Dye Decolorization
Jakofix red	86
Jakofix Blue	24
Jakofix Golden	32
Crystal violet	26
Methyl violet	14
Safranine	16
Basic Fuschin	15
Methyl Red	18

Table 2: Dye decolorization by bacteria isolated for effluents and probable species of bacteria

Isolates Code No	Dye Decolorization (%)	Probable bacteria
S1	25	*Aeromonas sp.*
S2	42	*Aeromonas sp.*
S3	50	*Pseudomonas sp.*
S4	44	*Klebisiella sp.*
S5	28	*Pseudomonas sp.*
S6	35	*Klebisiella sp.*
S7	24	*Pseudomonas sp.*
S8	33	*Aeromonas sp.*

S9	36	*Pseudomonas sp.*
S10	24	*Klebisiella sp.*
S11	45	*Pseudomonas sp.*
S12	45	*Pseudomonas sp.*
S13	25	*Klebisiella sp.*
S14	36	*Aeromonas sp.*
S15	21	*Pseudomonas sp.*
S16	12	*Aeromonas sp.*

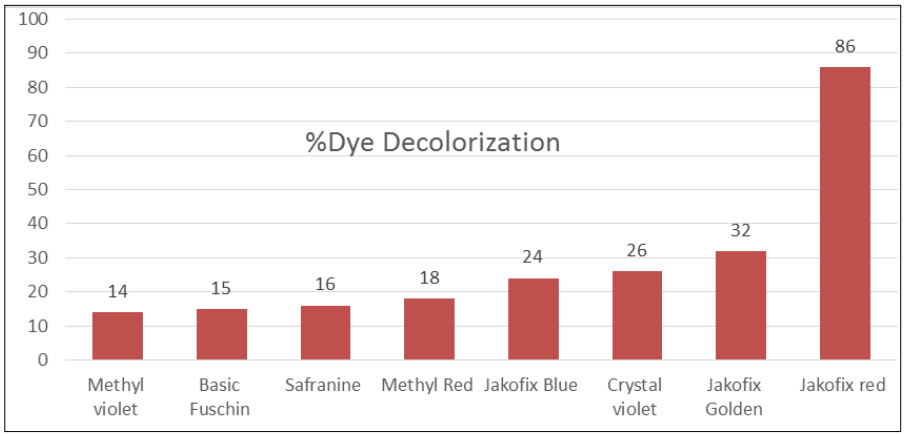

Fig 2: Percentage of dye decolorization by bacteria isolated for effluents

Identification of selected bacterial isolates

The selected bacterial isolates were characterized on the basis of their cultural, morphological physiological and biochemical characteristics. All these characteristics were then compared with the standard description of Bergey's Manual of Determinative Bacteriology and the isolates were identified up to the genus as *Pseudomonas* sp. (S3, S11 and S12), *Klebisiella* sp. (S4). and *Aeromonas* sp. (S2)

Isolates

Industrial Dye

Fig 3: Optimization of Different media

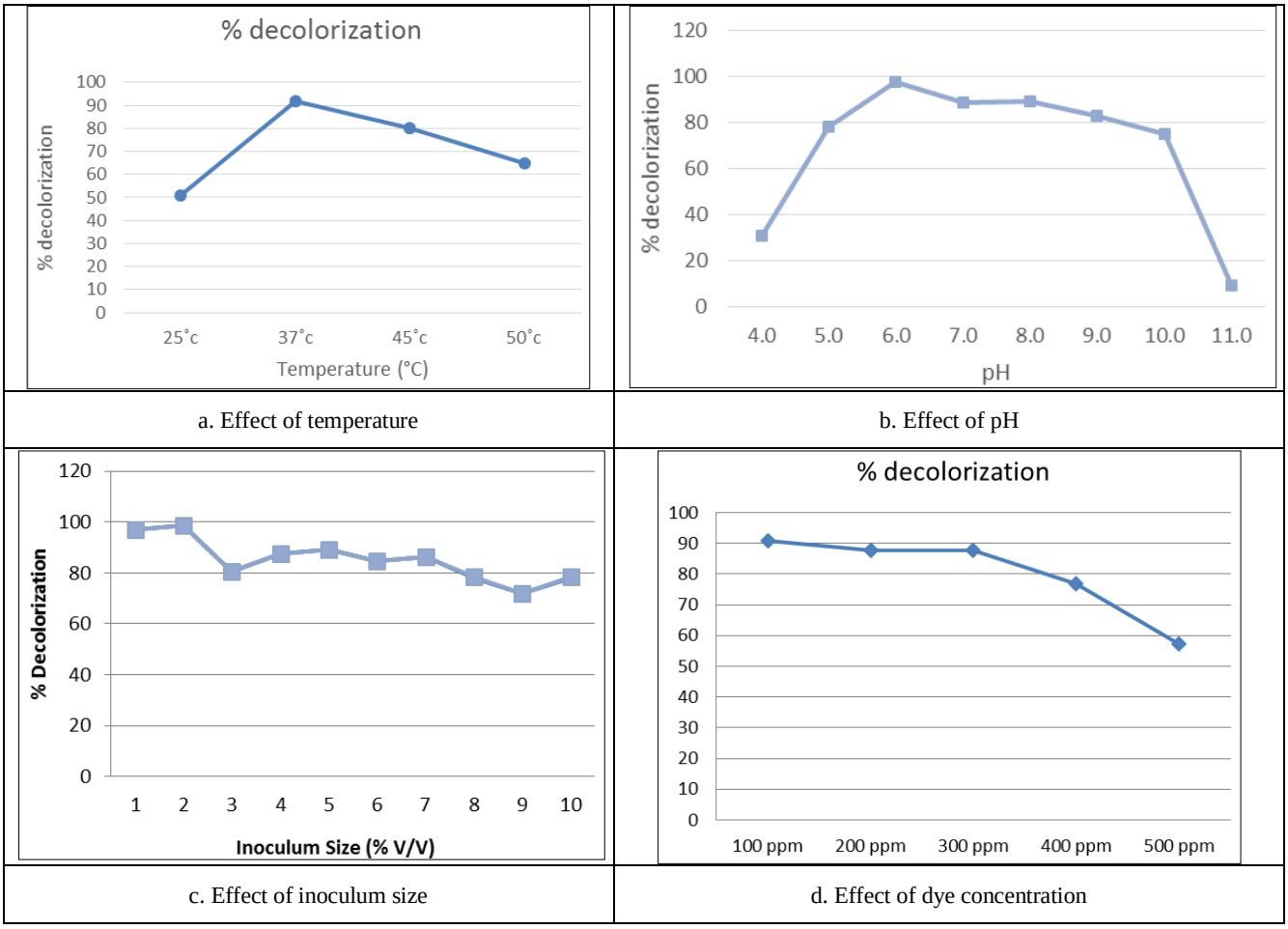

Fig 4: Optimization of Different Media for dye decolorization

Factors like substrate concentration, temperature and pH were optimized during the experimentation for maximizing decolorizing efficiency of the isolates. Different carbon sources (glucose, yeast extract, mannitol and maltose) at the concentration of 4 g L^{-1} were also tested as co- substrate in the decolorization process. Optimization studies included various concentration of dye (50, 100, 150, 200 and 250 mg L-1), pH values (5, 6, 7, 8, 9) and temperatures (25, 30, 35, 40, 45ºC). All the bacterial isolates S1, S2, …… were tested to optimize their decolorization efficiency. While culture conditions were the same as used in decolorization experiment i.e., minimal salt medium was used along with the 100 mg L-1 of dye (Figure 4). Uninoculated blanks were run to check the abiotic decolorization during the experimentation.

It was indicated that increase in substrate concentration from its optimum level had negative effect on decolorization capacity of isolates. Investigations with different dye concentrations in other experiments also reported higher net color removal efficiencies at lower dye concentrations (Cruz and Buitron, 2001; Kapdan and Oztekin, 2003; Sponza and Isik, 2005) [7, 13, 20]. Dyes generally contain one or more sulphonic-acid groups on aromatic rings, which might act as detergents to inhibit the growth of microorganisms (Chen *et al.*, 2003) [6]. Another reason of the toxicity at higher concentration may be due to the presence of heavy metals (metal-complex dyes) and/or the presence of nonhydrolyzed reactive groups which may retard the bacterial growth (reactive dyes) (Sponza and Isik, 2005) [20]. Similarly, reduction in decolorization at low concentration of the substrate might be due to the decrease in enzyme ability to recognize the substrate efficiently. Similar results were also reported by Guo *et al.*, (2008) [8]. The mesophilic range is traditionally used (Varel *et al.*, 1980) [21] since it is generally thought that maintaining high temperature would be uneconomical, while degradation within the psychrophilic range is too slow. Overall, one of the selected isolates (S3) of bacteria was able to completely remove color of the dye in textile effluents. However, these isolates should be tested at large scale treatment system to examine their potential for bioremediation of dye-polluted wastewaters.

Conclusion

The present study concludes that the bacterial strains of this study, *Pseudomonas* species, *Klebisiella* species., and *Aeromonas* species, can be used as a good microbial source for removal of the dyes form the effluents from textile industries. The isolated and identified bacterial strains were found to be most effective and having enormous potential of textile dye degradation under versatile environmental conditions.

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