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Studies on decolorization of textile dye by using *Pseudomonas* and *Bacillus* sp from the contaminated effluent soil samples of Kovilpatti, Thoothukudi district of Tamil Nadu

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Abstract
Textile industries releasing large amount of effluent which contains textile dyes and toxic chemicals and it is one of the major source of pollution also contaminating water bodies. To remove that, bacteria have been of great attention because of their ability to treat effluent. The present study was undertaken to exploit the ability of *Pseudomonas*sp and *Bacillus* sp from dye contaminated soil samples for bioremediation for dye effluent. Among the bacterial strains used in the study. *Pseudomonas* sp emerged out to be most potent decolorizer in comparison to *Bacillus* sp with the degree of decolorization of 90.0 %. Thus, it was concluded that the *Pseudomonas* sp had highest color removing capacity from contaminated effluent soil samples.

Keywords: Bacteria, Dye effluent, Decolorization, FT-IR analysis, Spectrophotometer

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INTRODUCTION

Synthetic dyes are polyaromatic, which gives permanent color to fabrics. Among the all synthetic dyes, Azo dyes are mostly used in textile industries and which has high affinity to bind with cellulosic fibre, stability, easiness, availability of colours and cost effectiveness of their synthesis (Singh *et al*., 2012; Babu *et al*., 2007). Most of the dyes do not bind to the textile fabrics and inefficiency during dyeing process, those unused dyestuff used to dispose into the environment. But these azo dyes are aromatic compounds but acute toxic to plants, microbes, other living things also affects aesthetic merit, obstructs light penetration, oxygen transfer, into the water bodies when it's released into the environment and recalcitrant to decolorization and degradation because dye effluent possesses benzene, anthraquinone, naphthalene, halogens, hydrocarbons, salts and other intermediate compounds (Pan *et al*., 2017; Birhanli and Ozmen, 2005; Couto, 2009). Moreover, some dyes may be decomposed to produce carcinogenic aromatic amines containing pyridine, cyanide, phenol and heavy metals such as mercury, cadmium, and chromium (Lumbaque *et al*., 2017). In the current scenario, environmental pollution has a foremost problem in worldwide due to rapid industrialization and its impact on huge waste generation into the biosphere (Andre *et al*., 2007). The textile industries has generated huge waste such as heavy metals, dyes, chlorinated compounds to the environment by discarding dye effluents into the water bodies as well as soils due to that its necessary to develop the effective way to treat the effluent for degrading the dyes (Mansour *et al*., 2012). Several physical, chemical and photochemical methods have been used for the treatment of textile wastewater, but these methods are have some limitation including cost effective, huge amounts of sludge which requires safe disposal (USEPA, 2012). Discharge of textile effluent without appropriate treatment may lead to bioaccumulation that may incorporate into food chain and also effect to the human health. A number of microorganisms have been already reported regarding dye decolorization, Whereas biological methods have stable effects and low operation cost (Khalid *et al*., 2010). Keeping in view of the above background, the present study was focused on the screening and characterization of potent indige-

nous bacterial isolates from textile dying effluents and utilization of these isolates as monoculture and consortium for decolorization of some commercially available textile reactive dyes as well as dye mixture.

MATERIALS AND METHODS

Sample collection: The untreated textile dye effluent soil sample were collected from dye contaminated soil resources from surroundings of dye industry in Kovilpatti, Tamilnadu, India, were used to isolate dye decolorizing bacteria. Effluent samples were collected by sterile screw-cap bottles and aseptically transported to the laboratory within an hour. The isolation of bacteria was carried out by serial dilution of the effluent sample in sterile distilled water and subsequently plated into Nutrient agar (Cappuccino *et al*., 1996).

Isolation of azodye degrading bacteria: For isolation of dye degrading bacteria, 0.1 ml of waste water sample was spread on the sterile Nutrient agar (NA) plates and incubated at 37^{o}C for 24h. After incubation, morphologically distinct and prominent colonies were picked up and purified through repeated streaking on the nutrient agar. The purified bacterial isolates were maintained in NA slants for further use at 4ºC.

Physiological and biochemical characterization: The physiological properties of selected isolates (*Psudomonas* sp and *Baillus* sp) were tested for their ability to decolorize textile effluent and determined through standard procedures like motility test, Gram staining, spore staining, capsule staining**.** Bacterial cultures were characterized by making use of biochemical tests like Motility, IMViC, catalase test, oxidase, starch hydrolysis and H_2S using textual procedures.

Measurement of dye decolurization: Different concentrations of textile effluent were added into the nutrient broth (10, 20, 30, 40, 50, 60, 70, 80, 90, 100ml) and 3 ml of 24 hours selected isolate of *Bacillus sp* were subsequently added into the nutrient broth. In another set of effluent treatment flask another selected isolate of *Pseudomonas* sp were added and control were maintained. All the conical flasks were kept for incubation along with decolorization for 48 hours. After incubation, samples were subjected to centrifugation at 10,000 rpm for 15 min. The supernatant was measured using spectrophotometer at 460nm. The efficiency of decolorization given in percentage (Sriram *et al*., 2013)

$$\text{Decolorization} = \frac{\text{Initial absorbance- final absorbance}}{\text{Initial absorbance}} X100$$

….Eq. 1

Fourier Transform-Infrared Spectroscopy analysis (FT-IR): Decolorized culture medium by microbial consortium was centrifuged at 7000 rpm for 20 min. The metabolites present in the culture supernatant were extracted using equal volume of Ethyl acetate (Shah, 2016).

RESULTS AND DISCUSSION

In the present study five different (S1, S2, S3, S4 and S5) dye contaminated samples were processed. The effluent contaminated soil samples were collected from local field of Kovilpatti, Thoothukudi district. From these, S3 sample exhibited maximum bacterial isolates. Hence it was selected for further enumeration process. Murthy *et al*., (2012) documented six bacterial isolates from dye contaminated soil samples of Ahmedabad, Gujarat. Out of these, who also stated that SpNB6 expressed maximum (85%) decolourization deficiency against azo dye A total of 20 bacterial isolates (SB1- SB20) were enumerated by sterile Nutrient agar. Based on the morphological distinct characteristics, only two isolates S3 (*Pseudomonas* sp) and S5 (*Bacillus* sp) were selected for further taxonomical studies. Similarly Liu *et al* (2017) studied the characterization of methyl orange decolorizing isolate namely strain *Bacillus circulans* BWL1061 from salt plan and also he reported the efficiency of decolorization of the strain was 50mg/L methyl orange and 50mg/L of chromium under anaerobic condition with the presence of 60g/L sodium chloride.

Many researchers focus on the utilization of microbial catalysts to remove die from the effluent. Especially bacterial decolorization and degradation of Azo dyes has been of considerable interest since it achieves a higher degree of biodegradation and mineralization is applicable to a wide variety of azo dyes (Pan *et al*., 2017). Khan and Srivastava (2014) isolated and identified five types of bacterial strains namely KN1, KN2, KN3, KN5, KN6 for textile dye decolorization process from rhizosphere region of garden soil and reported that among the five strains KN3, KN5 and KN6

Table 1. Biochemical characterization of selected potent bacterial strains.

S.N.	Characteristics	S3	S5
1	Gram's Staining	-	+
2	Morphology	Rod	Rod
3	Motility	-	-
4	Spore staining	-	+
5	Indole test	+	-
6	Methyl red test	-	-
7	Voges-Proskauer test	+	-
8	Citrate test	+	+
9	Catalase test	+	+
10	Oxidase test	+	-
11	H_2S production	-	-

Table 2. *Decolorization ability of azo dye using selected potent bacterial strains.*

Concentration of Dye (ppm)	OD (460nm)	Percentage of decolorization
Pseudomonas	0.035	90%
Bacillus	0.062	82%

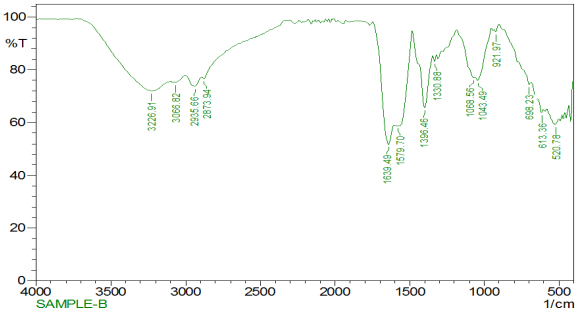

Fig. 1. *FT-IR spectral analysis of dye decolorization ability of potent Pseudomonas sp.*

shows maximum ability of decolorization against methyl orange.

Identification of selected potent strain: The selected two bacterial (S3, S5) isolates characterized by using standard procedures and the results are represented in Table 1 which expressed both strains appeared as rod shape, non-motile and showed negative results for H_2S production and methyl red reduction test. One of the isolate S3 exhibited Gram negative, Non – spore forming family organism and also expressed positive results for indole, Voges-Proskauer, citrate utilization, catalase and oxidase test. The selected bacterial strain S5 exhibited as Gram Positive and spore forming organisms and respectively showed the positive result for citrate utilization test.

Likewise Khan and Srivastava (2014) studied the biochemical characterization of six different (KN1, KN2 …KN6) bacterial isolates through standard procedure. They also observed that all the six bacterial isolates showed positive results for oxidase test and carbohydrate fermentation test and casein hydrolysis test. Based on the results, a dominant strain was selected and identified as *Pseudomonas* sp. through Bergey's manual of systemic bacteriology. Based on the characterization part of this study the two isolates were identified as *Pseudomonas* sp. (S3) and *Bacillus* sp. (S5) respectively. Similarly dye decolorizing microorganisms *Bacillus circulans* was isolated and identified up to species level through standard procedure by Liu *et al* (2017).

Decolorization of Dye: The degradation efficiency of the study using the selected bacterial strains *Bacillus* sp and *Pseudomonas* sp were determined by spectrophotometric assay method and results are described in the table 2. It is expressed that the highest degree of decolorization (90%) was observed using *Pseudomonas* sp and another bacterium *Bacillus* sp exhibited the degree of decoulrization is 82% using 5 ppm of Azo dye. Extensive studies have been carried out to determine the role of diverse groups of bacteria in the decolorization of azo dyes (Pandey *et al*., 2007). Likewise, Carliell *et al* (1996) and Razo – Flores *et al.,* (1997) have associated the decolurization with methonogens, whereas studies by other investigation showed that acidogenic as well as methonogenic bacteria contribute to dye decolurization with the degree of decolourization 62 % and 2% respectively.

FT-IR Analysis: The FT-IR spectrum of the decolorization effect using the candidate strains was analyzed and the results are represented in Fig. 1. It expressed that the basic functional groups were observed in between the wavenumber of 3226.91 and 520.78cm^{-1}. The FTIR spectrum of dye decolorizing sample displayed a peak with the wavenumber of 3226.91cm^{-1} exhibited the intramolecular hydrogen bonding aromatic -OH and O-H stretching; a peak at 2,935cm^{-1} expressed C-H stretching of alkyl acetals and a peak at 2,873cm^{-1} denoted N-H stretching of amines; a peak at 1639cm^{-1} showed C = N stretching of alkane group; a peak with the wavenumber of 1,068 cm^{-1} expressed S = O stretching of sulfonic acid; a peak with the wavenumber of 921.00 and 613.36 cm^{-1} described aromatic nature and C-Cl stretching, respectively. Notably there was absence of the peak responsible for azo (NON) group proposing that removal of azo bond after dye decolorization (Yu and Wen, 2005) Overall both these analytical study point out that there was structural degradation of the dye molecule which leads to the decolorization.

Conclusion

The results, thus obtained the efficiency of *Pseudomonas* sp (S3) and *Bacillus* sp (S5) for decolorization of azo dyes and to tolerate at high concentration gives an advantage for treatment of textile industry effluent. Current investigation confirmed that efficiency of maximum decolorization obtained by *Pseudomonas* sp under ideal condition was 90 % and by *Bacillus* sp was 82%. The results of the present study indicated that *Bacillus* sp., and *Pseudomonas* sp., can be used for the treatment of effluent contaminated waters.

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