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RESEARCH ARTICLE

BIODECOLORIZATION OF AZO DYE BY MICROBIAL ISOLATES FROM SOIL

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ABSTRACT

The dye decolourizing isolates, Klebsiella pneumonia, Pseudomonas florescence, Proteus mirabilis, Alcaligens sp, Serratia marscens and E.coli from garden soil. The present study confirms the ability of all isolates for decolourization of pink and orange dye used in textile. The isolates showed 91.14 % percentage decolourization for pink colour dye and 93.6 % for orange colour dye under optimum conditions. Among all the isolates, Proteus sp for Pink dye and Pseudomonas sp for orange dye were found to be most efficient in decolourization. All parameters studied in this paper were found to be effective for all isolates. The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as waste water treatment. High decolourization extent and facile conditions show the potential for this bacterial strain to be used in the biological treatment of dyeing mill effluents.

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INTRODUCTION

Environmental pollution caused by the release of a wide range of azo dyes through industrial wastewater is a serious problem in present days. Dyes are an important class of synthetic organic compounds, widely used in textile, leather, plastic, cosmetic and food industries and are therefore common industrial pollutants. Textile effluent released from industries is a complex mixture of many polluting substances such as organo chlorine based pesticides, heavy metals, pigments and dyes (Saraswathy and Balakumar, 2009) and must be treated before discharged into environment because of their recalcitrant nature and potential toxicity to animals and human (Levine *et al*., 1991; Hildenbrand *et al*., 1999; Martins *et al*., 2002). Dyes also obstruct light penetration and oxygen transfer that affects water bodies (Franciscon *et al*., 2009). In recent years, numerous studies were carried out for the decolorization of textile effluent, including various physicochemical methods such as filtration, coagulation, chemical flocculation, use of activated carbon, advanced oxidation processes, ion exchange, electrochemical and membrane process. Few of them are effective but with high cost, low efficiency and lack of selectivity of the process (Maier *et al*., 2004; Kurniawan *et al.,* 2006). The treatment processes are based on the microorganisms capable of decolorizing or degrading these recalcitrant compounds.

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These biological processes are environmental 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. The most widely studied white-rot fungus, in this regard is *Phanerochaete chrysosporium* (Reddy, 1995). Efforts to isolate bacterial culture capable of degrading azo dyes started in the 1970s with reports of a *Bacillus subtilis* (Horitsu *et al*., 1977). 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 Erio 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, 2000) are efficient dye decolorizer. The effectiveness of these treatment systems depends upon the survival and adaptability of microorganisms during the treatment processes. This part of the study was undertaken to isolate microorganisms capable of decolorization/degradation various textile azo dyes used in industry. 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.

MATERIALS AND METHODS

Bacterial Isolation and Cultivation

For isolation of bacteria, soil samples were collected as sources for bacteria. Numerous colonies were obtained through serial dilution method. Isolated colonies were then obtained through streaking method on nutrient agar. Each strain was then inoculated into nutrient broth and incubated for 24 h at 37 ^{0}C on a platform shaker at 150 rpm. A 10% (v/v) inoculum was transferred into 250 mL flask containing 100 mL LB media and incubated similarly. After 24 h, 10% (v/v) samples were sub-cultured into fresh LB media containing the respective dyes and further incubated as described above. Strains capable of utilising fresh dyes as a nutrient source were plated on MSM plates and incubated at 37 °C for 24 h. It was from these plates; isolated colonies were taken and repeatedly streaked on nutrient agar to obtain pure cultures. The pure bacterial cultures were subsequently transferred into nutrient broth.

Screening for Bacteria Decolourising Capability Using Selected Azo Dye

The bacterial isolates were cultivated in nutrient broth 24 hours before screening was done in MSM media. For initial screening, 0.1% (v/v) aliquot of each isolated strain in nutrient broth was inoculated into MSM, each containing 200 µL individual dye solutions. Decolourisation of the dye solution was monitored visually after 24 h incubation. Strains that showed high decolourising potential were chosen to be tested further using dye incorporated in MSM agar plate.

In secondary screening using dye incorporated MSM agar plates, the selected isolates were first inoculated into the nutrient broth for 24 h. The culture was then lawned onto the agar and left for another 24 h before any decolourisation zone was noted. Respective dye incorporated agars without any inoculums were used as controls and the decolourisation were estimated visually by comparing the inoculated plates with those of the control plates after 24 to 72 h. Final screening using selected dyes in MSM liquid media were initially done using smaller volume of samples. Each selected strain was inoculated into flasks containing 10 mL nutrient broth and allowed to grow for 24 h. A sample of 10% (v/v) of the aliquot was then transferred into flasks containing 10 mL of MSM media.

In secondary screening percent decolorization was measured as *decrease in optical density using spectrophotometer*. Percentage decolorization was calculated as follows:

$$\text{Decolorization (\%)} = \frac{(\text{Initial Absorbance} - \text{Observed Absorbance})}{\text{Intital Absorbance}} \text{x } 100$$

RESULTS AND DISCUSSION

A total of 18 cultures of bacteria were isolated, purified and screened for the degradation of azo dyes from textile effluent and sludge sample. Of all the cultures tested, 6 bacterial isolates (were further screened for dyes degradation on the basis of dyes tolerance i.e., resistance in minimal medium containing 2% of reactive light red dye.Table 1 shows different bacterial strains isolated from the Soil which were screened for their ability to decolorize textile dye and the potential strains were morphologically and biochemically characterized for identification. Based on preliminary tests and secondary screening, plating on selective media and biochemical tests, they were identified as *Klebsiella pneumonia, Pseudomonas florescence, Proteus mirabilis, Alcaligens sp, Serratia marscens* and *E.coli* and were selected for pink and orange dye degradation. The rate of decolourization increased with increase in initial dye concentration from 10 to 100 mg/L, showed 91.14 % percentage of decolourization for pink colour dye and 93.6 % for orange colour dye.

Table 1. Biochemical characteristics of isolates from Soil

Isolates No.	MR	VP	I	Ci	TSI Slant	Mid	Butt	H2S	Gas	Cat	Identified bacterial sp.
S1	+ve	-ve	-ve	+ve	P	Y	Y	+ve	-ve	+ve	*Alcaligenes sp.*
S2	+ve	+ve	+ve	+ve	Y	Y	Y	+ve	-ve	+ve	*Serratia marscens*
S3	-ve	-ve	-ve	-ve	P	Y	Y	+ve	-ve	+ve	*Pseudomonas florescens*
S4	+ve	-ve	-ve	+ve	Y	Y	Y	+ve	-ve	+ve	*Klebsiella pneumonia*
S5	+ve	-ve	-ve	-ve	P	Y	Y	+ve	-ve	+ve	*Proteus mirabilis*
S6	-ve	-ve	-ve	+ve	P	Y	Y	-ve	+ve	+ve	*E.coli*

Table 2. % dye decolorization by bacterial isolates from textile effluent and sludge

Bacterial sp.	Pink	Orange
	% Decolorization	% Decolorization
Alcaligenes sp.	83.33%	54.30%
Serratia marscens	69.47%	56.00%
Pseudomonas florescens	42.40%	93.60%
Klebsiella pneumonia	42.90%	68.50%
Proteus mirabilis	91.10%	63.00%
E.coli	88.10%	75.20%

Similar results were mentioned by Khalid *et al.* (2008). Dye concentration can influence the efficiency of microbial decolourization through a combination of factors including the toxicity imposed by dye at higher concentration (Sahasrabudhe and Pathade, 2011).

Conclusion

Soil is rich source of dye decolorizing bacterial population. The ability of isolated bacterial culture showing 91.1% decolourization for Pink dye in soil isolates thus suggesting

their application for decolourization of dye in industrial waste waters. These indigenous bacterial strains could be utilized for treatment of dye present in wastewater with high degrading and decolorizing activity against various reactive dyes commonly used in the textile industries. It is proposed that these bacterial species has a practical application potential in the biodegradation of various dye effluents.

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