

Decolourization of Azo-Dyes by Bacterial Consortium

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Azo-dye polluted soil samples were used for the isolation of bacterial species. Among the isolates *Enterobacter aerogens*, *Proteus rettgeri*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* were the dominant bacterial species present and they are designated as indicator bacterial isolates. These indicator bacterial isolates are able to utilize the dye as nitrogen source and hence they are able to decolorize the azo-red and blue dyes. Decolourization was assayed colorimetrically at 540 nm for red and 580 nm for blue and percentage of decolourization was calculated. The optimum concentration for both the dyes was 100 ppm. The maximum decolourization of azo-red and azo-blue dye at the end of 96 hours of decolourization experiments were 90% and 80% respectively for consortium of indicator bacterial isolates. The individual bacterial isolates were less effective for decolourization. These bacterial consortium can be exploited as bioremediation agents to reduce dye pollutants.

Key words: Azo-red, Azo-blue dyes, Consortium, Decolourization, Bacterial isolates, Pollutant.

Water pollution control is at present one of the major thrust areas of scientific research. Colour removal in particular, has recently become an area of major scientific interest as indicator (Moosvi *et al.*, 2005). Dyes are released into the environment through industrial effluents from three major sources such as textile, dye stuff manufacturing and paper industry. One of the most pressing environmental problems related to dye effluents is the improper disposal of waste water from dyeing industry (Rajeswari *et al.*, 2011).

Dyes are chemical substances, which are used to colour textile fabrics. Dyes are synthetic, aromatic and water-soluble dispersible organic colorants, having potential application in various industries. Dyes include acidic, basic, azoic, chromic, diazoic dispersive, reactive sulphur and vat dyes. Approximately 1,00,000 commercial dyes are manufactured with an annual production of over 7×10^5 metric tons (Campos *et al.*, 2001; Mohan *et al.*, 2002).

In the azo dyes the aromatic moieties are linked together by azo ($-N=N-$) chromophores, and they are the largest class of dyes employed in dyeing and printing processes. The downstream processes, *i.e.*, after printing, washing and finishing, generate large quantities of coloured waste water (Othman *et al.*, 2011). Colour in any water is undesirable and is therefore considered a pollutant. Dyes are stable to light and oxidizing agents and are sometimes resistant to bio-oxidation.

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Partial degradation products of azo dyes are aromatic amines which have toxic effects. The strong colour of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength (Gondaliya and Pathak, 2005).

Coloured industrial effluents from the dyeing industries represent major environmental problems. Dye waste water discharged from textile and dyestuff industries have to be treated. Colour can be removed from waste water by physico-chemical methods. These include adsorption, coagulation, chemical transformation, flocculation, oxidation, filtration, photocatalysis, ozonation and electrochemical methods. These methods are often laborious and expensive (Sathiyamoorthi *et al.*, 2007). The success of biological process for colour removal from a given effluent depends in part on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures. Many bacteria, actinomycetes, yeast and fungi are able to decolorize dyes (Machado *et al.*, 2006). Biodecolourization constitutes an attractive alternative to physico-chemical methods as a low cost, eco-friendly and publicly acceptable technology. The removal of the polluting dyes is a serious problem, particularly for small scale textile industries where working conditions and low economic status do not allow them to treat their wastewater before disposal and they have no choice other than to dump all effluents into the main stream of water sources. Microbial decolourization and degradation is an eco-friendly and cost-competitive alternative to chemical decomposition processes (Verma and Madamwar, 2003). At present, bioremediation relies on the pollutant degrading capacities of naturally occurring microbial consortia in which bacteria play a control role (O'Neill *et al.*, 2000). The ability of microorganisms to carry out dye decolourization has received much attention as microbial decolourization of dyes is a cost effective method for removing them from the environment (Kalyani *et al.*, 2008). Currently, extensive research is being focused on finding an optimal microbial biomass that would be as cheap as possible for the removal of contaminating dyes from large volume of polluted water (Youssef *et al.*, 2008). In this study, an attempt was made to isolate bacterial consortium

from dye waste contaminated soil and their decolourization efficiency was studied.

MATERIAL AND METHODS

Isolation of Bacteria

Soil samples were collected from dye effluent contaminated sites at Karur in aseptic condition. Soil suspension was prepared by mixing 10 gm of soil in 100 ml of sterile distilled water. The samples were serially diluted and 0.1 ml of the suspension was spread plated on Nutrient Agar (Himedia) plates in sterile condition. The plates were incubated at $32^{\circ} \text{C} \pm 1^{\circ} \text{C}$ for 2 days. The bacterial colonies grown on the plates were further sub-cultured and pure colonies were isolated and stored on Nutrient Agar slants for further investigation.

Screening of Dye Degradation

The isolated pure bacterial colonies were screened for dye decolourization by incorporating 0.1 mg / 100 ml of azo-red and azo-blue in Nutrient agar medium. Then the plates were incubated at $32^{\circ} \pm 1^{\circ} \text{C}$ for 2 days to observe the clear zone formed due to the degradation of the dye. The bacteria which showed distinct zone of clearance were selected, identified and used for further study.

Decolourisation of Dye

The isolated bacterial cultures were incorporated in nitrogen limited medium (Glucose 15 g, malt extract 0.4 gm, manganese chloride 0.3 g, ferric sulphate 0.4 g, magnesium sulphate 0.04 g, pH 6.0, distilled water 1000 ml) and amended with dye at a concentration of 100 to 500 ppm. The pH of the dye solution was 6.5 to 6.8. The medium with dye solutions were sterilized and used for decolourization studies. A loop of pure culture was inoculated into the medium at $32^{\circ} \pm 1^{\circ} \text{C}$ and decolourization was observed for 96 hours. The content was assessed for decolourization by measuring absorbance at 540 nm (Red), 580 nm (Blue) using UV-visible spectrophotometer at the end of 24, 48, 72 and 96 hours. Decolourization was expressed in terms of percentage and calculated using the following formula used by Olukanni *et al.* (2006).

$$\text{Decolourization (\%)} = \frac{A_0 - A_t}{A_0} \times 100$$

where

A_0 = Absorbance of the blank (dye solution)

A_t = Absorbance of the treated dyes solution at specific time

RESULTS AND DISCUSSION

Dyes are aromatic compounds. They are degraded by bacteria during metabolism. It is important to ensure constant and complete removal of these dyes and their metabolites from the environment to prevent setting up of bioaccumulation.

Twelve bacterial cultures were isolated from the dye contaminated soil by spread-plate technique. Among them *Enterobacter aerogenes*, *Proteus rettgeri*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* showed maximum zone of

clearance in azo-red and azo-blue dye amended Nutrient Agar medium. Hence these bacteria were identified as indicator bacteria of dye degradation and were selected for this study. Maximum 70 per cent of decolourization at 96 h was shown by *P. fluorescens* for 100 ppm concentration of azo-red dye followed by 60, 55 and 50 per cent respectively for *E. aerogenes*, *P. rettgeri* and *S. aureus*. For azo blue dye maximum 65 per cent of decolourization at 96 h was shown by *P. fluorescens* followed by 50 per cent by *E. aerogenes* and *P. rettgeri* whereas 45 per cent by *S. aureus*. It was observed that a consortium of all these bacteria showed better result than the individual bacterial species and were able to decolorize 90% for azo-red dye and 80% for azo-blue at $32 \pm 1^\circ \text{C}$ in 96 hours (Tables 1-3 and Fig. 1-3).

Table 1. Decolourization of Azo-red dye (100 ppm concentration) by Bacteria

Bacteria	Percentage of Decolourization			
	24 hr	48 hr	72 hr	96 hr
<i>Enterobacter aerogenes</i>	35	45	50	60
<i>Proteus rettgeri</i>	40	50	55	55
<i>Pseudomonas fluorescens</i>	45	60	70	70
<i>Staphylococcus aureus</i>	35	40	45	50

Table 2. Decolourization of Azo-blue dye (100 ppm concentration) by Bacteria

Bacteria	Percentage of Decolourization			
	24 hr	48 hr	72 hr	96 hr
<i>Enterobacter aerogenes</i>	30	40	45	50
<i>Proteus rettgeri</i>	40	45	50	50
<i>Pseudomonas fluorescens</i>	45	60	65	65
<i>Staphylococcus aureus</i>	35	40	45	45

Table 3. Decolourization of Azo-red and Azo-blue (100 ppm concentration) by Bacterial Consortium (*Enterobacter aerogenes*, *Proteus rettgeri*, *Pseudomonas fluorescens*, and *Staphylococcus aureus*)

Dye	Percentage of Decolourization			
	24 hr	48 hr	72 hr	96 hr
Azo-red dye	45	60	70	90
Azo-blue dye	40	50	70	80

The decolourization effect of different bacterial species is variable. It has been reported that the adsorption of chromatophores on the cell surface of the microorganisms results in the decolourization of dyes. The possible mechanism is biosorption which is dependent on functional groups in the dye molecule on bacterial biomass, which also plays a role in the biosorption of dyes (Fu and Viraraghavan, 2002). Rodriguez *et al.* (1999) reported that several industrial dyes were decolorized biocatalytically by extracellular enzymes of microbes. Chang and Lin (2000) reported that azo dyes are effectively decolorized by *Pseudomonas luteola* strain. Mabrouk and Yusef (2008) reported decolourization of fast red by *Bacillus subtilis*. Olukanni *et al.* (2006) reported

decolourization of azo dyes by strain of *Micrococcus*. Nortemann *et al.* (1986) revealed that bacterial communities were able to degrade amino and hydroxyl-naphthalene-sulfonates in azo dye. Hong *et al.* (2000) reported that *Bacillus pulmilus* bacteria is involved in biosorption of 1,2,3,4-tetrachlorodibenzo-p-dioxin of azo dye. The present study revealed 100 ppm as optimum concentration, pH 5.8, temperature at $32^{\circ} \pm 1^{\circ} \text{C}$ as optimum conditions for maximum decolourization by bacterial consortium for both azo-red and azo-blue dyes. These results are on par with the previous works of Chang and Lin (2000), McMullan *et al.* (2001), Olukanni *et al.* (2006) and Mabrouk and Yusef (2008).

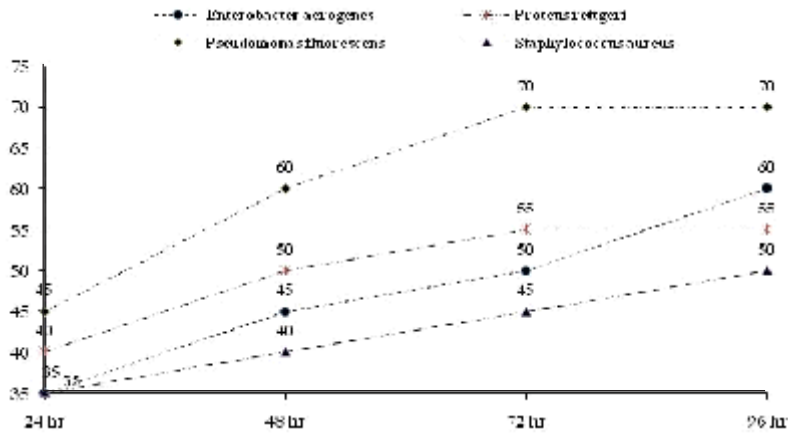


Fig. 1. Decolourization of Azo-red dye (100 ppm concentration) by Bacteria

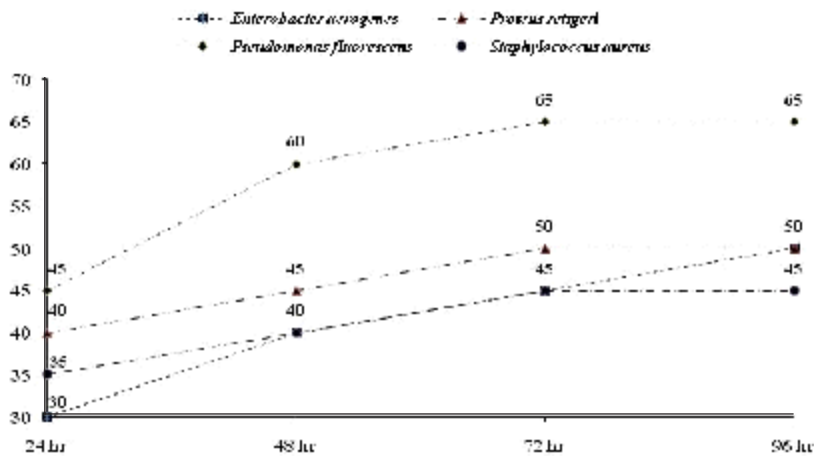


Fig. 2. Decolourization of Azo-blue dye (100 ppm concentration) by Bacteria

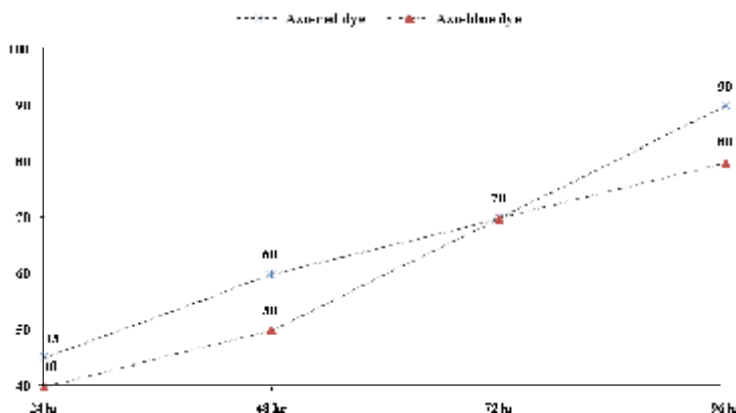


Fig. 3. Decolourization of Azo-red and Azo-blue by Bacterial Consortium

CONCLUSION

The present study is thus an effort to develop a bacterial-based treatment system for the cleaning up of dye industry effluents and for bioremediation of dye contaminated soil using bacterial-based consortium.

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