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RESEARCH ARTICLE

Decolourization of congo red and crystal violet by bacterial consortium

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SUMMARY: Dye polluted soil samples were used for the isolation of bacterial species. Among the isolates, *Bacillus subtilis, Clostridium butyricum, Enterobacter aerogens* and *Pseudomonas fluorescens* were the dominant bacterial species present and they are designated as the indicator bacterial isolates. These isolates are able to utilize the dye as nitrogen source and hence they are able to decolorize the congo red and crystal violet dyes. Decolourization was assayed colorimetrically at 495 nm 540 nm for congo red and crystal violet, respectively and percentage of decolourization was calculated. The optimum concentration for both the dyes was 100 ppm. The maximum decolourization of congo red and crystal violet dyes at the end of 96 hours of decolourization experiments were 85 per cent and 70 per cent, respectively for consortium of indicator bacterial isolates. The individual bacterial isolates were less effective for decolourization. UV spectroscopy analysis revealed the changes in the peaks before and after decolourization by bacterial consortium. These bacterial consortium can be exploited as bioremediation agents to reduce dye pollutants.

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Key Words :
Bacterial consortium, Congo red, Crystal violet dyes, Decolourization, Enzymatic degradation

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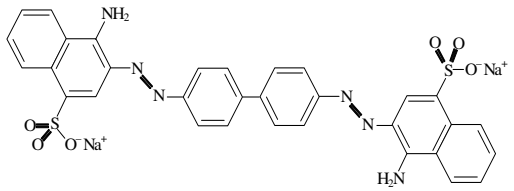
Water pollution control is at present one of the major thrust areas of scientific research. Colour removal in particular, has recently become an area of major scientific interest (Moosvi *et al*., 2005). Dyes are released into the environment through industrial effluents from three major sources such as textile, dye stuff manufacturing and paper industries. 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 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).

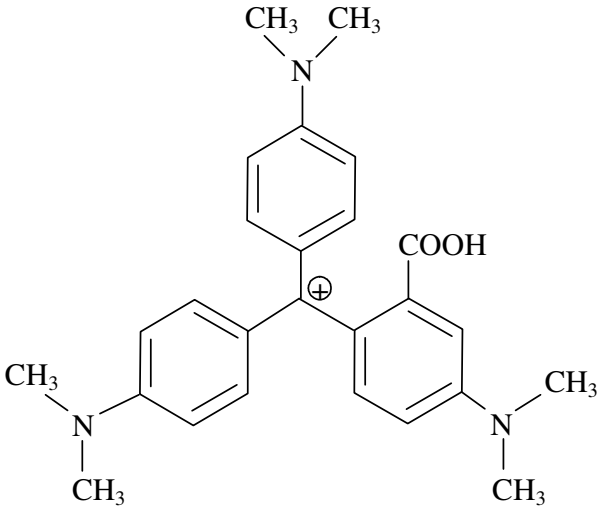
In the azo dyes the aromatic moieties are linked together by azo (– N = N –) chromophores, and they are the largest class of dyes employed in dyeing and printing processes. The downstream processes, *i.e.*, after printing, washing and finishing, generate large quantities of coloured waste water (Othman *et al.*, 2011).

Congo red (sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid) with molecular formula $C_{32}H_{22}N_6Na_2O_6S_2$ has been reported to be a carcinogenic direct diazo dye, used for colourization of paper products (Jaladoni-Buan *et al.*, 2010).



Crystal violet or gentian violet tris (4-dimethylamino) (phenyl) methylium chloride with

molecular formula $C_{25}N_3H_{30}Cl$ has been reported to be carcinogenic and it is used to dye paper and as a component of navy blue and black inks for printing, ball-point pens and ink-jet printers. The dye is also used as a histological stain, particularly in Gram's method for classifying bacteria (Titford, 2007).



Colour in any water is undesirable and is indicative of the presence of pollutants. Dyes are stable to light and oxidizing agents and are sometimes resistant to bio-oxidation. 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 due to turbidity and high pollution strength (Gondaliya and Pathak, 2005).

Since coloured industrial effluents from the dyeing industries pose major environmental problems, they need to be treated. Colour can be removed from waste water by physico-chemical methods. These include adsorption, coagulation, chemical transformation, 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 (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 it 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).

The present study was carried out to isolate and screen microbial strains for their ability to decolourize azo dyes aerobically and optimize the pH and temperature required for effective decolourization of the individual bacterial isolate and bacterial consortium.

EXPERIMENTAL METHODOLOGY

Isolation of bacteria :

Soil samples were collected from dye effluent contaminated sites at Karur in aseptic condition. Soil suspension was prepared by mixing 10 g 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 32^0 C ± 1^0 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 congo red and crystal violet in Nutrient agar medium. Then the plates were incubated at 32° ± 1^0 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 g, 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 32^0 ± 1 C and decolourization was observed for 96 hours. The content was assessed for decolourization by measuring absorbance at 495

nm (congo red), 540 nm (crystal violet) 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 dye solution at specific time

Analytical method :

Samples were removed after 96 hours and were centrifuged by high speed cooling centrifuge (Remi C-24) at 10000 rev/min for 5 minutes at 4^0 C temperature. The decolourization experiments with cell-free extract before and after decolourization of the dye by bacterial consortium were performed spectrophotometrically.

UV Spectrophotometry :

The UV and visible spectra of the samples were measured in ethanol with a Perkin Elmer double beam UV-vis spectrophotometer 117. Quartz cells (1 cm square) having 1.0 cm path length were used for the determination. Hydrogen discharge tungsten filament lamp was used as a source of light and maximum absorbance was recorded.

EXPERIMENTAL FINDINGS 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, *Bacillus subtilis, Clostridium butyricum, Enterobacter aerogenes* and *Pseudomonas fluorescens* showed maximum zone of clearance in congo red and crystal violet 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 congo red dye followed by 60, 55 and 50 per cent, respectively for *E. aerogenes, C. butyricum and B. subtilis*. For crystal violet dye, maximum 55 per cent of decolourization at 96 h was shown by *P. fluorescens* followed by 45 per cent by *E. aerogenes* and *C. butyricum* whereas 40 per cent by *B. subtilis.* It was observed that a consortium of all these bacteria showed better result than the individual bacterial species and were able to decolorize 85 per cent for congo red dye and 70 per cent for crystal violet dye at 32 ± 1^0 C in 96 hours (Table 1-3 and Fig. 1-3).

Table 1 : Decolourization of congo red dye (100 ppm concentration) by bacteria

Bacteria	Percentage of decolourization			
	24 hr	48 hr	72 hr	96 hr
Bacillus subtilis	35	40	45	50
Clostridium butyricum	40	50	55	55
Enterobacter aerogenes	35	45	50	60
Pseudomonas fluorescens	45	60	70	70

Table 2 : Decolourization of crystal violet dye (100 ppm concentration) by bacteria

Bacteria	Percentage of decolourization			
	24 hr	48 hr	72 hr	96 hr
Bacillus subtilis	30	35	35	40
Clostridium butyricum	35	40	40	45
Enterobacter aerogenes	35	40	45	45
Pseudomonas fluorescens	40	40	50	55

Table 3 : Decolourization of Azo-red and Azo-blue (100 ppm concentration) by bacterial consortium (*Bacillus subtilis, Clostridium butyricum, Enterobacter aerogens* and *Pseudomonas fluorescens*)

Dye	Percentage of decolourization			
	24 hr	48 hr	72 hr	96 hr
Congo red	45	60	70	85
Crystal violet	40	50	65	70

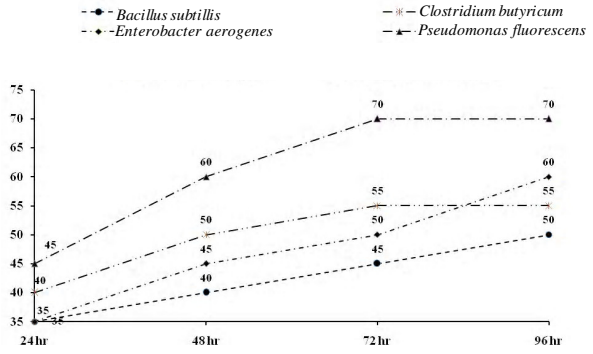


Fig. 1 : Decolourization of congo red dye (100 ppm conc.) by bacteria

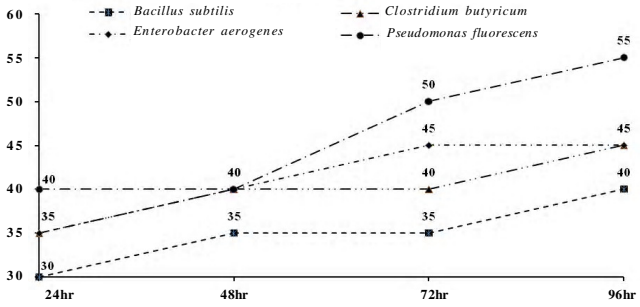


Fig. 2 : Decolourization of crystal violet dye (100 ppm conc.) by bacteria

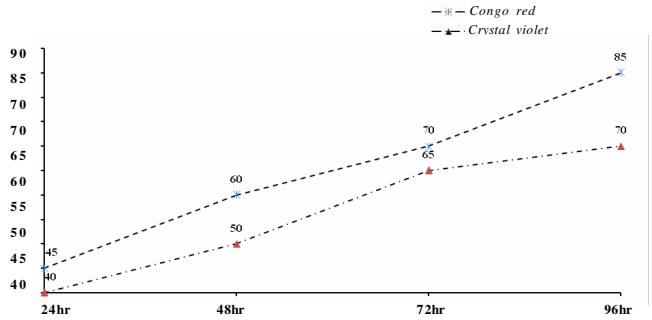
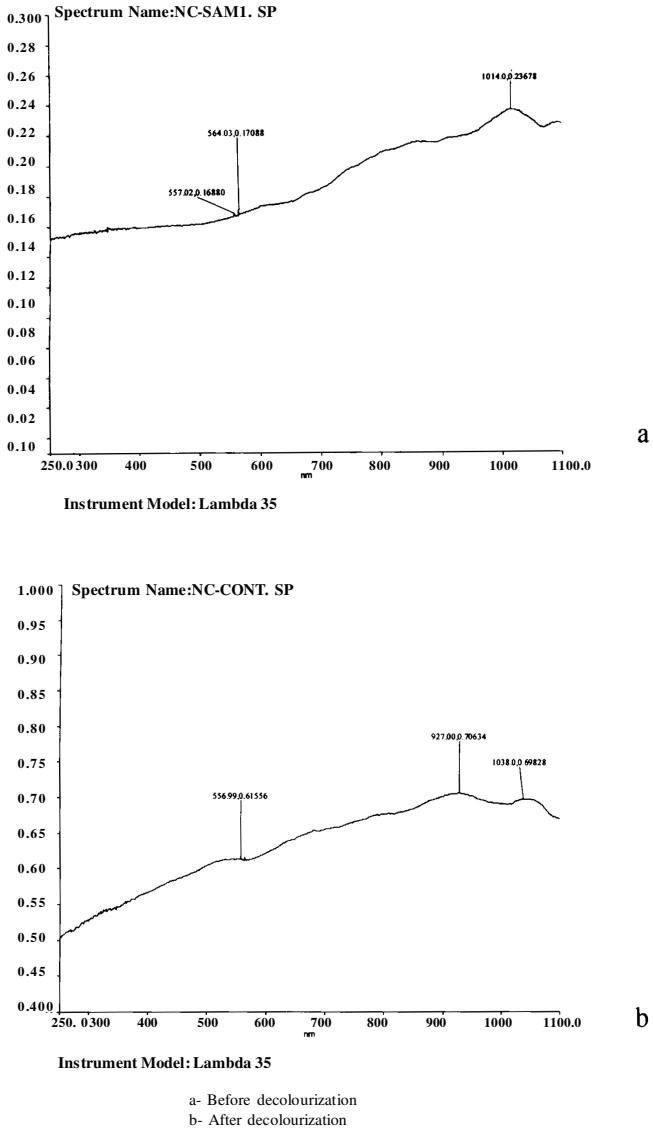


Fig. 3 : Decolourization of congo red and Crystal violet by bacterial consortium (*Bacillus subtilis*, *Clostridium butyricum*, *Enterobacter aerogens* and *Pseudomonas fluorescens*)

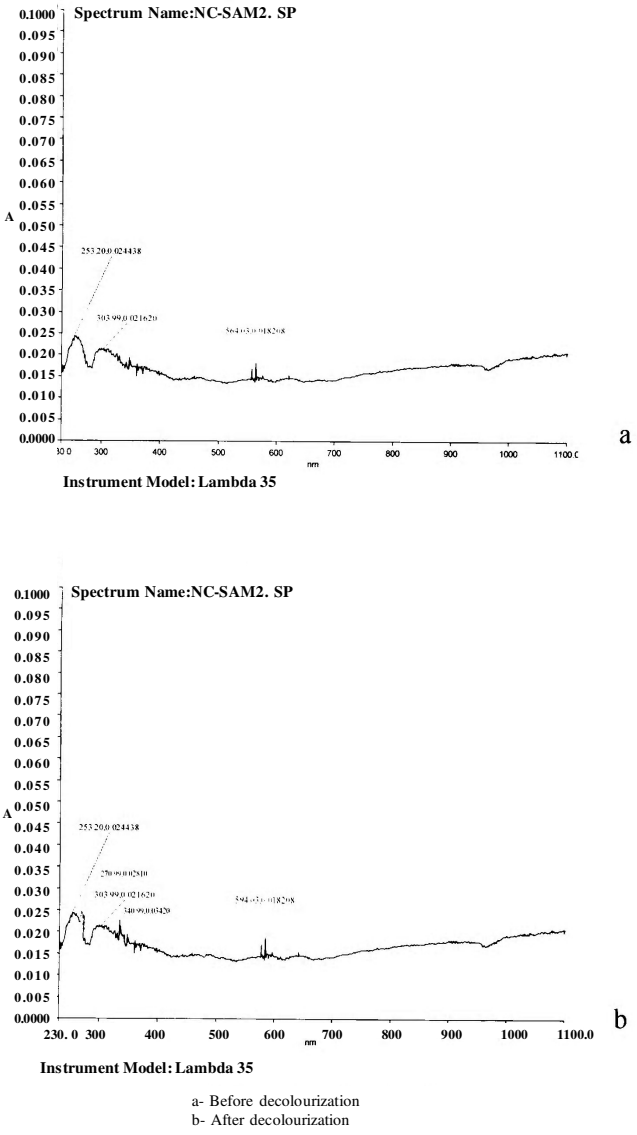


a- Before decolourization
b- After decolourization

Fig. 4 : Decolourization of congo red dye by bacterial consortium

UV spectroscopy of congo red before decolourization of bacterial consortium showed absorbance at 557.02, 564.03 and 1014.00 nm (Fig. 4) and after decolourization was at 556.99, 927.00 and 1038.00 nm (Fig. 5). As far as crystal violet was concerned, the UV spectroscopy before decolourization showed absorbance at 253.20, 303.99 and 564.03 nm (Fig. 5a), and after decolourization at 253.20, 270.99, 303.99, 340.99 and 594.03 nm (Fig. 5b). The new peaks of both the dyes proved that congo red and crystal violet changed to some other compound due to decolourization.

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). Yatome *et al.* (1991) reported the degradation of crystal violet by *Bacillus subtilis* IFO 13719. 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 were 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* bacterium is involved in biosorption of 1,2,3, 4-tetrachlorodibenzo-p-dioxin of azo dye. The decolourization ability is attributed to the presence of azo reductase enzyme in bacterial species such as *Enterobacter agglomerans, Staphylococcus aureus* (Montaonakkil *et al*.,



a- Before decolourization
b- After decolourization

Fig. 5 : Decolourization of crystal voilet dye by bacterial consortium

2003, Chen *et al*., 2005). The present study revealed 100 ppm as optimum concentration, pH 5.8, temperature at 32° ± 1^{0}C as optimum condition for maximum decolourization by bacterial consortium for both congo red and crystal violet 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).

conclusion :

The present study is thus an effort to develop a bacterial consortium 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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