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DECOLOURIZATION OF DIRECT ORANGE-102 AND MALACHITE GREEN BY BACTERIAL CONSORTIUM

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ABSTRACT

Dye polluted soil samples were used for the isolation of bacterial species. Among the isolates *Bacillus subtilis* (NCBT-012), *Clostridium butyricum* (NCBT-017), *Enterobacter aerogens* (NCBT-024) and *Pseudomonas fluorescens* (NCBT-046) 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 Direct Orange-102 and Malachite Green dyes. Decolourization was assayed colorimetrically at 245 nm 610 nm for Direct Orange-102 and Malachite Green, respectively and the percentage of decolourization was calculated. The optimum concentration for both the dyes was 100 mg/l. The maximum decolourization of Direct Orange-102 and Malachite Green at the end of 96 hours of decolourization experiments were 85% and 70% respectively for consortium of indicator bacterial isolates. The individual bacterial isolates were less effective for decolourization. The FTIR analysis revealed the changes in the vibration frequencies before and after decolourization by bacterial consortium. Hence this bacterial consortium can be exploited as bioremediation agents to reduce dye pollutants.

Key words: Bacterial consortium, Bacterial isolates, Decolourization, Direct Orange-102 dye, Enzymatic degradation, FTIR, Malachite Green dye, Pollutant.

INTRODUCTION

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[1]. 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[2].

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[3, 4].

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[5].

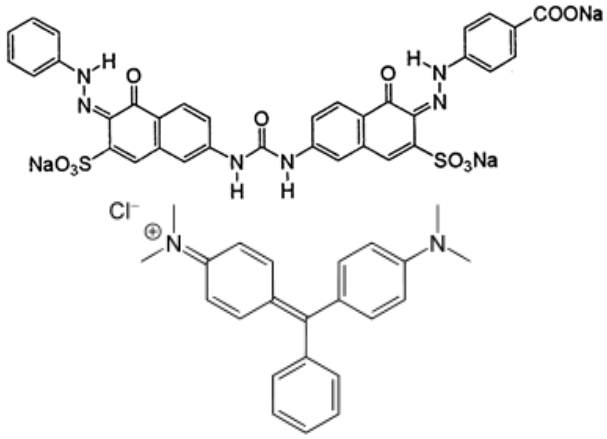
Colour in any water is undesirable and is indicative of the presence of pollutants. Dyes are stable to light and oxidizing agents and are sometimes resistant to bio-oxidation. Partial degradation products of azo dyes are aromatic amines which have toxic effects. The strong colour of discharged dyes even at very small concentrations has a huge impact on the aquatic environment due to turbidity and high pollution strength[6].

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[7]. 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[8-10].

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[11]. At present, bioremediation relies on the pollutant degrading capacities of naturally occurring microbial consortia in which bacteria play a control role[12]. 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[13]. 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[14].

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 by using commercial Direct Orange-102 and Malachite Green dyes.

Direct Orange-102 and Malachite Green



MATERIALS 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° C ± 1° 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 Direct Orange-102 and Malachite Green in Nutrient agar medium. Then the plates were incubated at 32° ±

1° 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, 200, 300 and 500 mg/l. The *p*H 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° ± 1° C and decolourization was observed for 96 hours. The content was assessed for decolourization by measuring absorbance at 245 nm (Direct Orange-102), 610 nm (Malachite Green) 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)[15].

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

where A_0 = Absorbance of the blank (dye solution)
A_t = Absorbance of the treated dye solution at specific time

Samples were removed after 96 hours and were centrifuged by high speed cooling centrifuge (Eppendorf 5804 R) at 10000 rev/min for 5 minutes at 4°C temperature. The decolourization experiments with cell-free extract before and after decolourization of the dye by bacterial consortium were performed spectrophotometrically.

Fourier Transfrom Infrared (FTIR) spectroscopy analysis :

FTIR spectroscopy was used to detect the vibration frequency changes in Direct Orange-102 and Malachite Green before and after decolourization by the bacterial consortium. The spectra were collected by Perkin Elmer spectrometer with the range 4000-400 cm^{-1} using chloroform as the Mulling agent.

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 *Bacillus subtilis* (NCBT-012), *Clostridium butyricum* (NCBT-017), *Enterobacter aerogenes* (NCBT-024), and *Pseudomonas fluorescens* (NCBT-046) showed maximum zone of clearance in

Table-1: Decolourization of Direct Orange-102 dye (100 mg/l concentration) by Bacteria

Bacteria	Percentage of Decolourization			
	24 hr	48 hr	72 hr	96 hr
B. subtilis NCBT 012	35	40	50	55
C. butyricum NCBT 017	40	50	60	65
E. aerogenes NCBT 024	35	45	50	60
P. fluorescens NCBT 046	45	60	70	75

Table-2: Decolourization of Malachite Green dye (100 mg/l concentration) by Bacteria

Bacteria	Percentage of Decolourization			
	24 hr	48 hr	72 hr	96 hr
B. subtilis NCBT 012	35	45	50	55
C. butyricum NCBT 017	40	50	60	60
E. aerogenes NCBT 024	40	45	50	50
P. fluorescens NCBT 046	45	55	65	65

Table-3. Decolourization of Direct Orange-102 and Malachite Green (100 mg/l concentration) by Bacterial Consortium (*B. subtilis* NCBT012, *C. butyricum* NCBT017, *E. aerogens* NCBT024 *and P. fluorescens* NCBT046)

Dye	Percentage of Decolourization			
	24 hr	48 hr	72 hr	96 hr
Direct Orange-102	50	65	75	90
Malachite Green	45	60	70	75

amended Nutrient Agar medium. Hence these bacteria were identified as indicator bacteria of dye degradation and were selected for this study. Maximum 75 per cent of decolourization at 96 h was shown by *P. fluorescens* for 100 mg/l concentration of Direct Orange-102 dye followed by 65, 60 and 55 per cent respectively for *C. butyricum, E. aerogenes and B. subtilis*. For Malachite Green dye maximum 65 per cent of decolourization at 96 h was shown by *P. fluorescens* followed by 60 per cent by *C. butyricum* whereas 55 per cent by *B. subtilis* and 50 per cent for *E. aerogenes.* It was observed that a consortium of all these bacteria showed better result than the individual bacterial species and were able to decolorize 90 per cent for Direct Orange-102 dye and 75 per cent for Malachite Green dye at 32° ± 1° C in 96 hours (Tables 1-3 and Fig. 1-5).

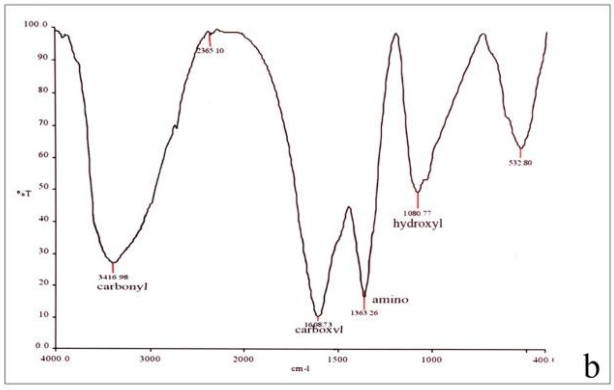
FTIR Analysis:

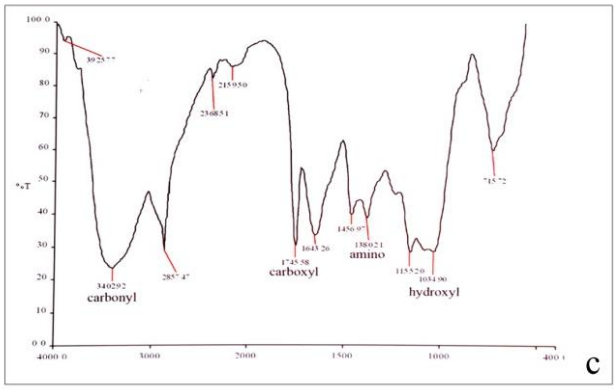
vibration frequencies were seen in carbonyl, carboxyl, amino and hydroxyl for both the dyes (Fig. 1 and 2).

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[16]. Yatome *et al.* (1991)[17] reported the degradation of crystal violet by *Bacillus subtilis* IFO 13719. Rodriguez *et al.* (1999)[18] reported that several industrial dyes were decolorized biocatalytically by extracellular enzymes of microbes. Chang and Lin (2000)[19] reported that azo dyes are effectively decolorized by *Pseudomonas luteola* strain. Mabrouk and Yusef (2008)[20] reported decolourization of fast red by *Bacillus subtilis*.

Figure-1. Direct Orange – 102 Remediation by Bacterial consortium

a) Decolourization of different concentration
b) FTIR spectrum before decolourization
c) FTIR spectrum after decolourization

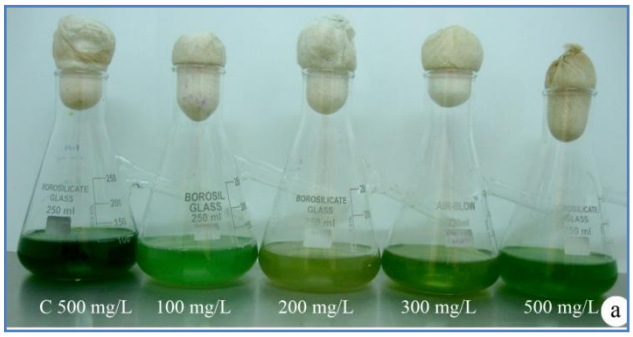


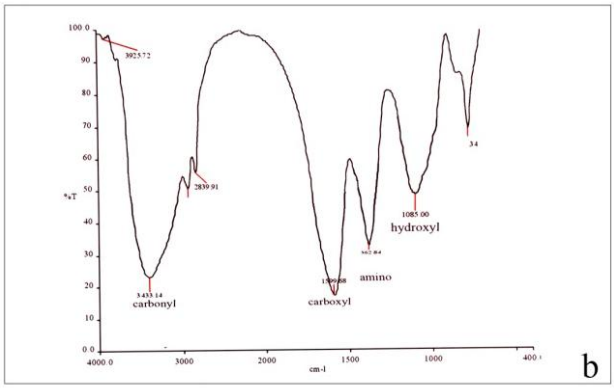


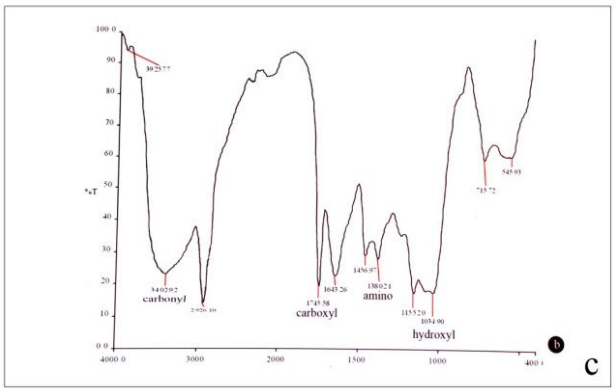
Olukanni *et al.* (2006)[15] reported decolourization of azo dyes by strain of *Micrococcus.* Nortemann *et al.* (1986)[21] revealed that bacterial communities were able to degrade amino and hydroxyl-naphthalene-sulfonates in azo dye. Hong *et al.* (2000)[22] reported that *Bacillus pulmilus* bacteria 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*[23,24].

Figure-2. Malachite Green Remediation by Bacterial consortium

a) Decolourization of different concentration
b) FTIR spectrum before decolourization
c) FTIR spectrum after decolourization







Pandey and Upadhyay (2010)[10] suggested that *Pseudomonas fluorescens* degraded Direct Orange-102 dye, the dye was first broken down into 3,7-diamino-4 hydroxy-naphthalene-2 sulfonic acid sodium salt, the breakdown compound was non-toxic in nature, such Malachite Green was enzymatically reduced to

leucomalachite green and also converted to N-demethylated and N-oxidized metabolites[8].

Fig. 3: Decolourization of Direct Orange-102 dye (100 mg/l conc.) by Bacteria

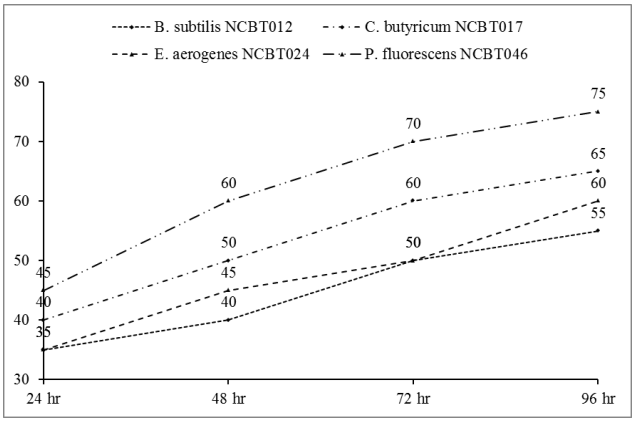


Fig. 4: Decolourization of Malachite Green dye (100 mg/l conc.) by Bacteria

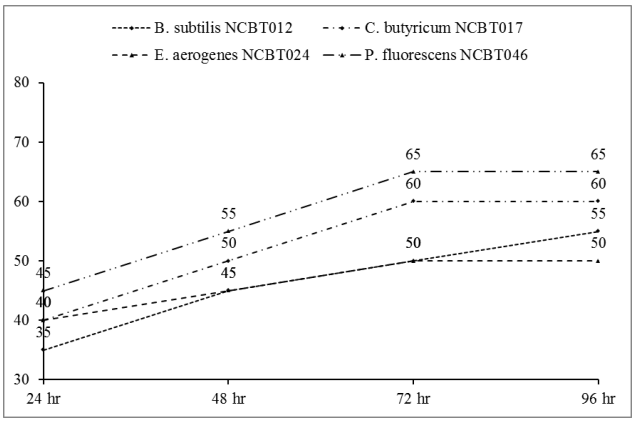
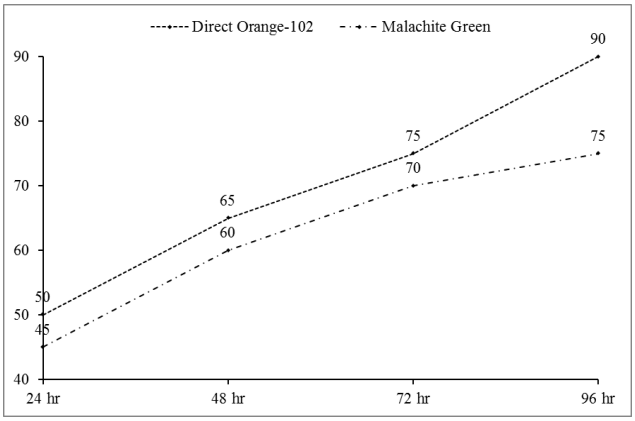


Fig. 5: Decolourization of Direct Orange-102 and Malachite Green (100 mg/l) by Bacterial Consortium (*B. subtilis* NCBT012, *C. butyricum* NCBT017, *E. aerogens* NCBT024 *and P. fluorescens* NCBT046).



Such degradation mechanisms might have occurred in the present study by the bacterial consortium in effectively decolourization of Direct Orange-102 and Malachite Green. The present study revealed 100 mg/l as optimum concentration, pH 5.8, temperature at 32° ± 1° C as optimum conditions for maximum decolourization by bacterial consortium for both Direct Orange-102 and Malachite Green dyes. These results are on par with the previous works of McMullan *et al.* (2001)[25] and Abubacker and Mehala (2012)[26].

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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