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Biological Degradation of Reactive Dyes by Using Bacteria Isolated from Dye Effluent Contaminated Soil

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Abstract: The textile industry is one of the industries that generate a high volume of waste water. Strong colour of the textile waste water is the most serious problem of the textile waste effluent. The disposal of these wastes into receiving water causes damage to the environment. Dyes may significantly affect photosynthetic activity in aquatic habitat because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics, metals, chlorides and other toxic compounds. The present study was carried out to degrade the textile Reactive azo dyes by using bacteria isolated from dye contaminated soil. Three different bacterial isolate such as, *Bacillus* sp*., Escherichia coli* and *Pseudomonas fluorescens* were isolated from textile dye effluent contaminated soil sample and used for the degradation study. It was noticed that there was a decrease in the OD in all the three species of all the five dyes as the incubation period increased. *Pseudomonas fluorescens* was more effective followed by *Bacillus* and *Escherichia coli.* It was found that all the isolated bacteria were efficient decolourizers of Reactive textile azo dyes.

Key words: Degradation · Decolourization · Reactive Azo Dyes · Effluent Contaminated Soil · Bacteria

INTRODUCTION

Dyes are recalcitrant by design and not readily amendable to common treatment methods, imposing a challenge for closed water systems. Extensive research in the field of biological azo dye decolourization has shown promising results, but much of this work has been done with single model compounds [1]. However, industrial textile wastewater presents the additional complexity of dealing with unknown quantities and varieties of many kinds of dyes [2], as well as low BOD/COD ratios, which may affect the efficiency of the biological decolourization. India's dye industry produces every type of dyes and pigments. Production of dye stuff and pigments in India is close to 80,000 tones. India is the second largest exporter of dye stuffs and intermediates after China. The textile industry accounts for the largest consumption of dyestuffs, at nearly 80%. Industrialization is vital to a nation's economy because it serves as a vehicle for development. However, there are associated problems resulting from the introduction of industrial waste products into the environment.

Dyeing of textile request water and generates a substantial quality of effluents containing mineral salts and dyes at high concentration. An estimated 700000 tons of dyes are produced annually worldwide of which 60-70% are azo dyes [3]. Chronic effects of dyestuffs, especially of azo dyes, have been studied for several decades. Azo dyes in purified form are mutagenic or carcinogenic, except for some azo dyes, leads to formation of aromatic amines and several aromatic amines are known mutagens and carcinogens to human beings. In mammals, metabolic reduction of azo dye is mainly due to bacterial activity in the anaerobic parts of the lower gastrointestinal tract. Various organs, especially the liver and the kidney also reduce azo dyes. After azo dye reduction in the intestinal tract, the released aromatic amines are absorbed by the intestine and are excreted in the urine. The acute toxic hazard of aromatic amines is carcinogenesis, especially bladder cancer. International Agency for Research on Cancer (IARC) summarized the literature on suspected azo dyes, mainly amino-substituted azo dyes, fat soluble dyes and benzidine azo dyes, also a few sulphonated azo dyes [4].

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Dyes are stable against breakdown by many microorganisms and most dyes do not biodegrade under the aerobic biological treatments in a municipal sewage plant. Many dyes, including the azo dyes, degrade under anaerobic conditions and the aromatic amines thus formed have been found to degrade further aerobically. Out of several methods that are used in the treatment of textile effluents to achieve decolourization, including physiochemical methods like filtration, specific coagulation, use of activated carbon and chemical flocculation some of the methods are effective but quite expensive. Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluent [5]. Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. This natural process, bioremediation, includes bioengineering the capabilities of intrinsic microorganisms, to clean up the environment is an effective alternative to conventional remediation methods [6].

Investigations to bacterial dye biotransformation have so far mainly been focused to the azo dyes. The electron withdrawing nature of the azo linkages obstructs the susceptibity of azo dye molecules to oxidative reaction. Therefore, azo dyes generally resist aerobic bacterial biodegradation. Only bacteria with specialized azo dye reducing enzymes (Azoreductase) were found to degrade azo dyes under fully aerobic conditions. This anaerobic reduction implies decolourization of the dyes are converted to usually colourless but potentially harmful aromatic amines. Aromatic amines are generally not further degraded under anaerobic conditions. Anaerobic treatment must therefore be considered merely as the first stage of the complete degradation of azo dyes. The second stage involves conversion of the produced aromatic amines. For several aromatic amines, this can be achieved by biodegradation under aerobic conditions. The present study was carried out to degrade the textile Reactive azo dyes by using bacteria isolated from dye contaminated soil.

MATERIALS AND METHODS

Sample Collection and Preservation: The dye house effluent soil was collected from the surroundings of dye industry, Tirupur region, Tamil Nadu, India. The sample was collected in a plastic container. Then the sample was brought to the laboratory as early as possible and was subjected for various microbiological studies.

Dyes: Reactive azo dyes used in this research are, Reactive Orange – M2R (λm = 493 nm), Reactive Blue – M58 (λm = 572 nm), Reactive Yellow – M4G (λm = 413 nm) and Reactive Black - B (λm = 574 nm).

Isolation and Identification of Dye Bacteria from Dye Effluent Contaminated Soil: Pour plate technique was used for the isolation of dye decolourizing bacteria from dye effluent contaminated soil. Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored in refrigerator at 4°C. Identification of the bacterial isolates was carried out by the routine bacteriological methods *i.e.*, By the colony morphology, preliminary tests like Gram staining, capsule staining, endospore staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests.

Screening of Bacterial Isolates for Textile Direct Azo Dye Degradation

Inoculum Preparation: The suspension of 2 days old cultures of bacteria was used to investigate their abilities to decolourize dyes. They were prepared in saline solution (0.85% Sodium chloride). A loopful of bacterial cultures were inoculated into 50 ml of saline and incubated at 37°C for 3 hours.

Dye Decolourization Experiment: Fifty milliliter of Nutrient agar sterile medium was amended separately with each of the textile dyes (200 mg/l) and subsequently inoculated with 2% bacterial suspension. The suspension contained 2.5 x 10^6 cfu/mL spores. The flasks were kept in mechanical shaker and incubated at 30±1°C for 8 days. Samples were drawn at 2 days intervals for observation. The samples were centrifuged at 10000 rpm for 10 minutes and decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wavelength maxima (λm) of respective dye. Two control flasks (Dye + medium without inoculums and medium with inoculums without dye) were maintained.

Dye Decolourization Assay: Dye decolourization assay was measured in the terms of percentage decolourization using UV-Spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{Intitial OD - Final OD}}{\text{Initial OD}} \times 100$$

RESULTS AND DISCUSSION

Azo dyes are the largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries [7]. Azo dyes are characterized by the presence of one or more azo groups – N = N -, which are responsible for their colouration and when such a bond is broken the compound loses its colour. They are the largest and most versatile class of dye, but have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water system. Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and washing.

A number of azo dyes including reactive dyes are used in textile dying operations. This leads to effluent streams containing intense colour due to the presence of azo dyes. The removal of azo dyes from effluents is important due to their mutagenicity and carcinogenicity together with their intense colouration. Both physicochemical and biological methods for the removal of dyes have been investigated widely. The physicochemical dye removal techniques such as flocculation–coagulation, adsorption, electrochemical oxidation, photocatalytic oxidation, electro- Fenton oxidation appear to face several technical and economic limitations [8]. On the other hand, biological methods such as activated sludge process and anaerobic treatment have been applied to control pollution of aquatic environment. Lower cost of treatment and amenability to scale up easily are the merits of biological methods. The present study was focused on decolourization of textile azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent contaminated soil.

In Tamil Nadu many of the districts are known for textile industries. Tirupur is one among them. These industries discharge the coloured effluents with dyes and toxic compounds into the open environment. Textile and dyeing industry are among those which contribute much to water and soil pollution. They consume substantial volumes of water and chemicals. Further, about 10,000 different dyes and pigments are being used. Among these azo-dyes are widely used. Apart from chemicals nearly 10-15% of the dye is lost as effluent during the dyeing process [9].

Biodegradation of commercially available textile dyes namely Reactive Orange – M2R, Reactive Blue - MR, Reactive Yellow – M4G and Reactive Black-B were studied against five bacterial isolates which have been isolated from the dye effluent sample by Pour plate method and percentage decolourization was shown in the figures accompanying the results. Three different bacteria were isolated from the textile dye effluent. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Bacillus* sp., *Escherichia coli* and *Pseudomonas fluorescens*.

Olukanni *et al.* [10] isolated eighteen textile effluent adapted bacterial isolates belonging to the genera, *Bacillus, Acinetobacter, Staphylococcus, Legionella* and *Pseudomonas* were investigated for the potential of textile effluent adapted bacteria in decolourizing it. *Bacillus* and *Legionella* were found to have use in effluent treatment. Ajibola *et al.* [11] checked the ability of *Staphylococcus aureus, Bacterioides fragilis, Bacillus subtilis, Bacillus cereus, Clostridium perifringens, Escherichia coli* and *Peptostreptococcus* sp. to reduce and stabilize textile effluents containing predominantly Indigo Blue.

Saranraj *et al.* [12] isolated five different bacterial isolates from the textile dye effluent sample and identified as *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli.* Manivannan *et al*. [13] also isolated three different bacteria were isolated from the textile dye effluent. Based on preliminary tests, plating on selective media and biochemical tests, they were identified as *Bacillus* sp., *Escherichia coli* and *Pseudomonas fluorescens*.

The decolourization efficiency of *Bacillus* sp., *Pseudomonas fluorescens* and *Escherichia coli* was studied by measuring the optical density after 0, 4, 8, 12, 16 days of incubation and the results were showed in Figure – 1, 2, 3 and 4. It was noticed that there was a decrease in the optical density (OD) in all the three species in all the five colours as the incubation period increased. *Pseudomonas fluorescens* was more effective followed by *Bacillus* sp. and *Escherichia coli.* The percentage of decolourization of colours by the bacteria was also calculated. It was found that all the isolated bacteria were efficient decolourizers of Reactive Orange – M2R. The decolourization of dye amounted to 59, 77 and 79 respectively within 16 days. Reactive Yellow – M4G was recalcitrant to decolourization, the O.D. value from an initial value of 0.6912 was reduced only to 0.303 and from 0.746 to 0.218, 1.236, to 1.33 by *Pseudomonas fluorescens, Bacillus* sp. and *Escherichia coli* respectively. Percentage decolourization was 43%, 15%, 90% respectively.

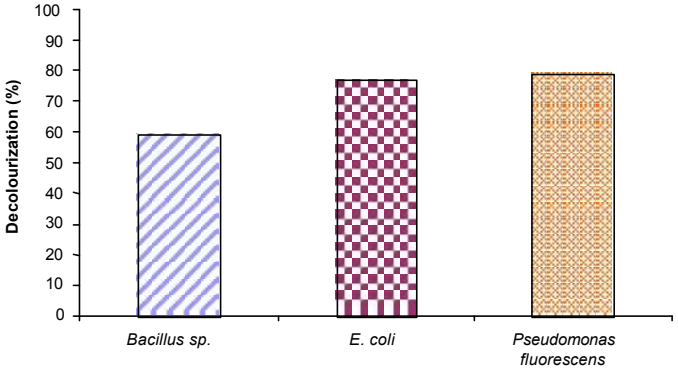

Fig. 1: Decolourization percentage of Reactive Orange – M2R by bacterial isolates

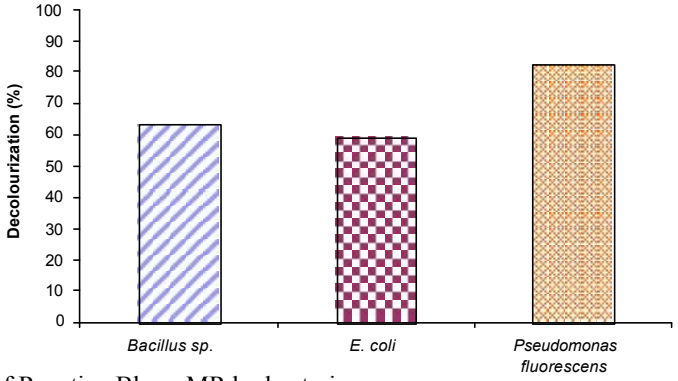

Fig. 2: Decolourization of Reactive Blue - MR by bacteria

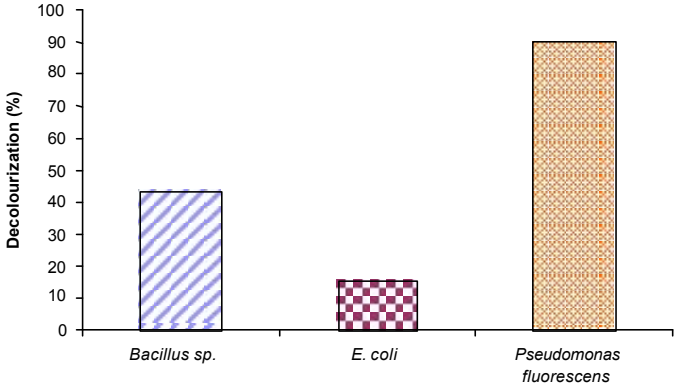

Fig. 3: Decolourization of azo dye Reactive Yellow – M4G by bacteria

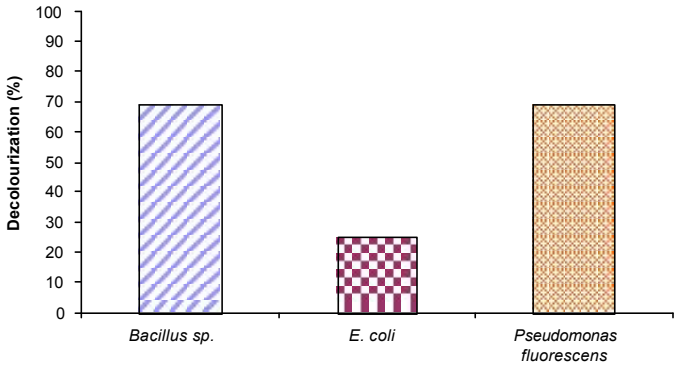

Fig. 4: Decolourization of azo dye Reactive Black - B by bacteria

The decolourization of textile reactive azo dyes by *Clostridium biofermentans* isolated from a contaminated site was studied under aerobic conditions. *Clostridium biofermentans* decolourized the dyes Reactive red 3B-A, Reactive black 5 and Reactive yellow 3B-A, by over 90% after 36 hours post-inoculation spectrophotometric analyses of the reactive dyes showed no distinct peak indicating aromatic amines. The results suggested that *Clostridium biofermentans* was a suitable bacterium for the biological processing of dye-contaminating waste water [14]. Under anaerobic conditions, the decolourization of many azo dyes takes place via reduction of the azo bond for both aerobic as well as facultative anaerobic bacteria [15].

Saranraj *et al*. [12] investigated the decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. They isolated five different bacterial species from the textile dye effluent sample and the isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli.* In our study also we have isolated three same genus among five. The bacterial inoculums were inoculated into flasks containing Direct azo dyes (500 mg/l) with trace amounts of yeast extract, glucose and sucrose and then sterilized and incubated for 4 days. In their research, *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. Similar results we also obtained in our present study. The best decolourizer of Direct Green-PLS was *Bacillus subtilis* (99.05%). *Klebsiella pneumoniae* (87.27%) highly decolourized the Direct Violet-BL. *Escherichia coli* (61.56%) was the best decolourizer of Direct Sky Blue-FF. The best decolourizer of Direct Black-E was *Klebsiella pneumoniae* (92.03%). Recently, Silveira *et al*. [16] checked the ability of *Pseudomonas* sp. to remove colour from textile industrial dyes *Pseudomonas cepacia* exhibited no growth at all on the plates containing dyes (1 g/l), whereas *Pseudomonas aeruginosa, Pseudomonas oleovorans* and *Pseudomonas putida* exhibited considerable growth. Decolourization in a liquid culture revealed that *Pseudomonas oleovorans* was more viable for decolourizing textile dyes.

CONCLUSION

Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using bacteria can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. Thus, by this present study it is concluded that the bacterial isolates like *Bacillus* sp*., Pseudomonas fluorescens* and *Escherichia coli* can used as a good microbial source for waste water treatment.

REFERENCES

1. Cervantes, F.J., F.P. Van Der Zee and G. Lettinga, 2001. Enhanced decolourisation of Acid Orange 7 in a continuous UASB reactor with quinones as redox mediators. Water Science and Technology, 44: 123-128.
2. Bisschops, I.A.E. and H. Spanjers, 2003. Literature review on textile wastewater characterization. Environmental Technology, 24: 1399-1411.
3. Safia Moosvi, Xama Kher and Datta Madamwar., 2006. Isolation, characterization and decolourization of textile dyes by a mixed bacterial consortium. Water Science Technology, 36: 65-72.
4. Praveen Sharma, G.R. Chaudry and Thomes Edison, 2009. Mutagenicity testing of some commonly used azo dyes. Applied Environmental Microbiology, 42(4): 641-648.
5. Olukanni, O.D., A.A. Osuntoki and G.D. Gbenle, 2005. Textile effluent biodegradation potentials of textile effluent-adopted and non-adopted bacteria. Applied Environmental Microbiology, pp: 837-844.
6. Vidali, M., 2009. Bioremediation – an overview. Pure Application Chemistry, 73(7): 581-587.
7. Maximo, C., M.T.P. Amorim and M. Costa Ferreira, 2003. Biotransformation of industrial reactive azo dye by *Geotrichum* sp. Enzyme and Microbial Technology, 32: 145-151.
8. Rao, N.N., G. Bose and S.N. Kaul, 2006. Fenton and electro- Fenton methods for oxidation of H-acid and Reactive Black 5. Journal of Environmental Engineering, 132: 367-376.
9. Jothimani, P. and A. Bhaskaran, 2003. Assessing the azo reductase enzyme activity of bacterial cultures used for Decolourization. Journal of Ecotoxicology and Environmental Monitoring, 13: 179-183.
10. Olukanni, O.D., A.A. Osuntoki and G.D. Gbenle, 2005. Textile effluent biodegradation potentials of textile effluent-adopted and non-adopted bacteria. Applied Environmental Microbiology, pp: 837-844.

11. Ajibola, V.O., S.J. Oniye and C.E. Odeh, 2005. Biodegradation of Indigo containing textile effluent using some strains of bacteria. Journal of Applied Sciences, 5(5): 853-855.
12. Saranraj, P., V. Sumathi and D. Reetha, 2010. Decolourization and degradation of Direct azo dyes and Biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Journal of Ecobiotechnology, 2(7): 7-11.
13. Manivannan, M., D. Reetha and P. Ganesh, 2011. Decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Journal of Ecobiotechnology, 2(7): 7-11.
14. Min-Ho Joe, Sang-Young Lim, Dong-Ho Kim and In-Soo Lee, 2008. Decolourization of reactive dyes by *Clostridium bifermentans* SL186 isolated from contaminated soil. World Journal of Microbiology and Biotechnology, 24: 117-121.
15. Bragger, J.L., A.W. Lloyd and S.H. Soozandehfar, 1997. Investigation on the azo reducing activity of a common colonic microorganisms. International Journal of Pharmacy, 157: 61-71.
16. Silveira, E., P.P. Marques and S.S. Silva, 2011. Selection of *Pseudomonas* for industrial textile dyes decolourization. International Biodegradation and Biodeterioration, 63: 230-235.