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# Decolourization and Degradation of Dirct Azo Dyes and Biodegradation of Textile Dye Effluent by using Bacteria Isolated from Textile Dye Effluent

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***Abstract***

Bacterial cultures isolated from the waste water treatment plant have the capacity to decoluorize and degrade the toxic Azo dyes. The present study was conducted to investigate the decolourization and degradation of Direct azo dyes and biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Five different bacterial species were isolated from the textile dye effluent sample and the isolates were identified as *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli.* 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. The decolourization was expressed in terms of percentage decolourization. *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. *Klebsiella pneumoniae* (98.44%) was the best decolourizer of Viscose Orange – A. 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%). Bacterial biodegradation was assessed by physicohemical analysis.

## 1. Introduction

Textile dyes are of environmental interest because of their widespread use. Colours contributed by textile industry due to the usage of various dyes, is another form of pollution. Moreover, disposal of waste water on an open land contaminates the subsoil water in the area so much that drinking water gives colour as well as bad taste. Dyes contain chromophores, decolourized electron system with conjugated double bonds and auxochromes, electron-withdrawing or electron-donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system. Usual chromophores are – C = C, - C = N, - C = O, - N = N, - $NO_2$ and quinoid ring and auxochromes are –$NH_3$, -COOH, – $SO_3H$ and –OH (Van der Zee, 2002).

Azo dyes are largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries (Maximo *et al*., 2003). 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 during washing. Many are carcinogenic and may trigger allergic reactions in man. It is estimated that over 10% of the dye used in textile processing does not bind to the fibres and is therefore released to the environment. Some of these compounds cause serious threat because of their carcinogenic potential or cytotoxicity (Adedayo *et al* .,2004).

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 (Soares *et al*., 2004). 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 (Praveen Sharma *et al* ., 2009). 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 (Vidali, 2009).

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 term azo dye reduction comprises different mechanisms. It occurs either by direct specific (e.g azoreductase) and non-specific enzymes under anaerobic conditions by anaerobic and facultative aerobic bacteria or by indirect means by different enzymatically produced redox mediator compounds like $FADH_2$, $FMNH_2$, riboflavin, NADH and NADPH-generating system. It is also possible that azo dyes are purely chemically reduced by biogenic bulk reductants like sulfide. However, research has revealed that enzymatic anaerobic azo dye reduction is more or less a fortuitous reaction catalyzed by enzymes (e.g hydrogenases) which are usually used for other reaction(Van der Zee *et al* ., 2003). The present study was focused on decolourization and degradation of textile direct azo dyes and biodegradation of textile dye effluent by using bacteria and fungi isolated from textile dye effluent.

## 2. Materials and methods

### Sample collection and preservation

The dye house effluent was collected from a dyeing unit in Tirupur region (Tamil Nadu, India). It was refrigerated at 4°C and used without any preliminary treatment.

### Dyes

Direct azo dyes were used in this study. The dye samples were commercially graded and kindly supplied by the dealers of "ATUL Dyes". Direct azo dyes used in this research are, Congo Red (λm = 580 nm), Viscose Orange – A (λm = 480 nm), Direct Green PLS (λm = 580 nm), Direct Violet BL (λm = 550 nm), Direct Sky Blue FF (λm = 580 nm) and Direct Black – E (λm = 600 nm).

### Isolation and identification of dye decolourizing bacteria from textile dye effluent

### Isolation of dye decolourizing bacteria

Pour plate technique was used for the isolation of dye decolourizing bacteria.

### Maintenance of bacterial isolates

Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4°C.

### Identification of the bacterial isolates

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 were 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 (Benson, 1994).

### Dye decolourization experiments

Dye decolourization experiments were carried out in 100 ml flasks containing 50 ml of Direct azo dyes (500 mg/l), traces of yeast extract, sucrose and glucose. The pH was adjusted to 7±0.2 using sodium hydroxide and hydrochloric acid solution. Then, the flasks were autoclaved at 121°C for 15 minutes. The autoclaved flasks were inoculated with 5ml of bacterial inoculums of each isolates. The flasks were kept in mechanical shaker and incubated at 37°C for 4 days. Samples were drawn at 24 hours intervals for observation. 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wavelength maxima (λm) of respective dye.

### Decolourization assay

Decolourization assay was measured in the terms of percentage decolourization using UV-Spectrophotometer. The percentage

decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{InitialOD} - \text{FinalOD}}{\text{InitialOD}} \times 100$$

**Biodegradation of textile dye effluent by bacterial consortium**

Bacterial biodegradation of textile dye effluent was carried out in 1000 ml flask containing 800 ml of dye effluent. The dye effluent was enriched with Minimal medium containing Dextrose, 1g/l; Dipotassium phosphate, 7g/l; Monopotassium phosphate, 2g/l; Sodium citrate, 0.5 g/l; Magnesium sulphate, 0.1 g/l and Ammonium sulphate, 1g/l and then sterilized by autoclaving at 121°C for 15 minutes. The pH was adjusted to 7 ± 0.2. The autoclaved flask was inoculated with 5 ml of bacterial inoculums of each microorganism. The flask was kept in mechanical shaker and incubated at 37°C for 7 days. Biodegradation was assessed by physico-chemical analysis.

## 3. Results and Discussion

Biodegradation of textile dye effluent and commercially available textile dyes namely, Congo Red, Viscose Orange-A, Direct Green- PLS, Direct Violet-BL, Direct Sky Blue-FF and Direct Black-E 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 graphs accompanying the results. Finally physico-chemical analyses were done with untreated and bacterially treated textile dye effluent. Five 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 subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli*.

Figure 1. Decolourization of Congo Red by bacterial isolates

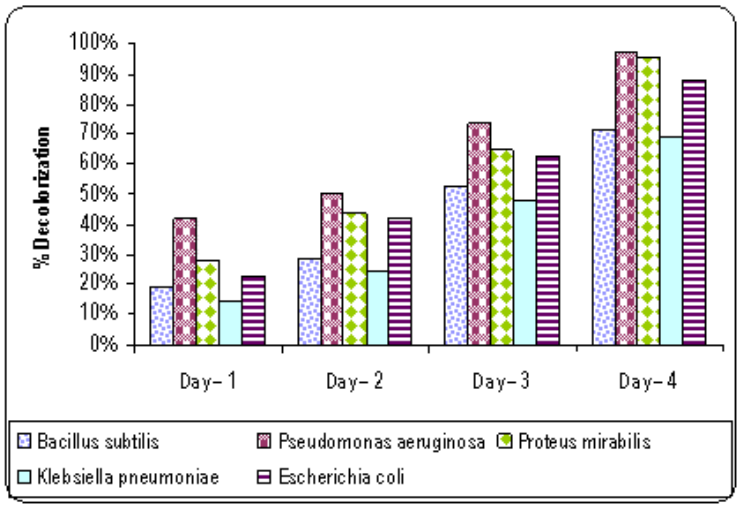



Figure 2. Decolourization of Viscose Orange-A by bacteria

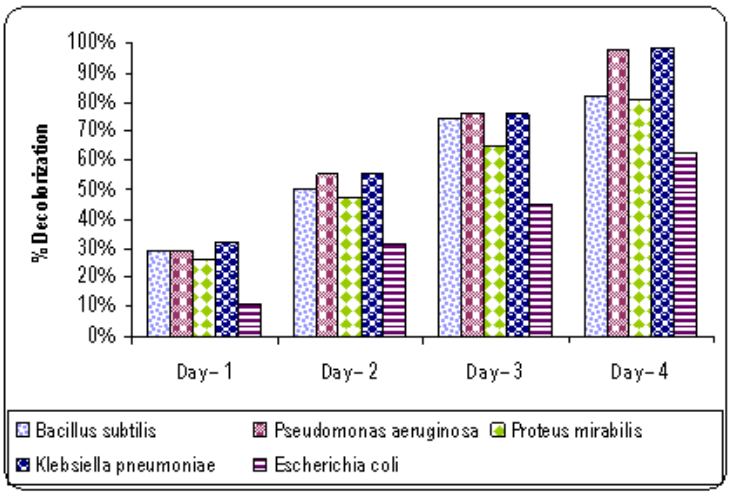


Figure 3: Decolourization of Direct Green - PLS by bacteria

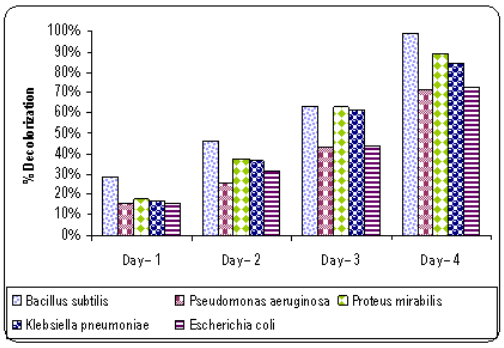



Figure 4: Decolourization of Direct Violet- BL bacteria

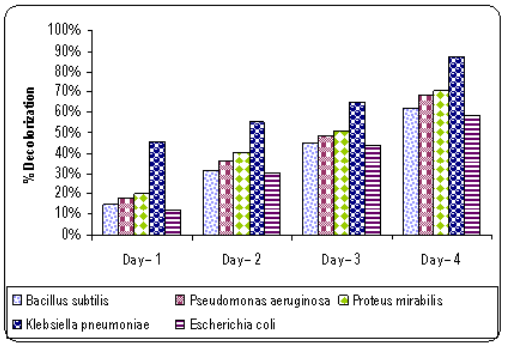



Figure 5: Decolourization of Direct Sky Blue-FF by bacteria

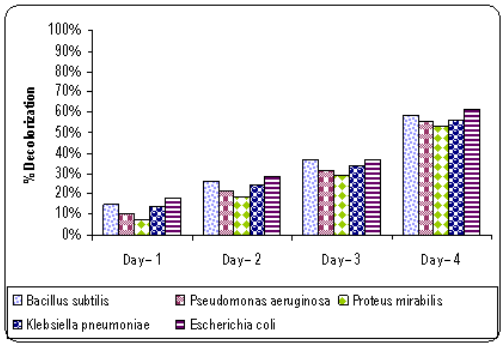
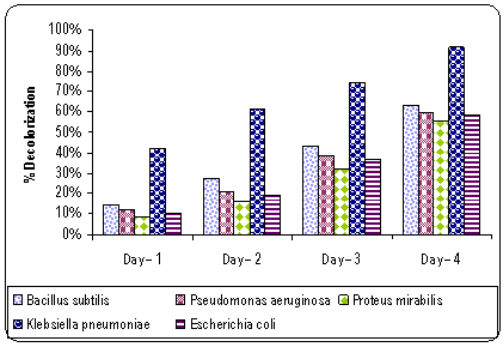



Figure 6: Decolourization of Direct Black-E by bacteria




Olukanni *et al.*, (2005) 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.*, (2005) 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.

In the present study, bacterial dye decolourization was studied using spectroscopic analysis. The bacterial inoculums were inoculated into the flasks containing Direct azo dyes with trace amounts of yeast extract, glucose and sucrose and incubated for 4 days. The decolourization was expressed in terms of percentage decolourization. *Pseudomonas aeruginosa* (97.33%) was identified as the best decolourizer of Congo Red. *Klebsiella pneumoniae* (98.44%) was the best decolourizer of Viscose Orange – A. The best decolourizer of Direct Green – PLS was *Bacillus subtilis* (99.05%). *Klebsiella pneumoniae* (87.27%) highly decolourized 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%).

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 (Min-Ho Joe *et al.*, 2008). 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 (Bragger *et al.*, 1997)

In this study, after inoculation of isolated bacterial consortium in textile dye effluent, the colour was changed from black to light brown. The pH was brought from 9.3 to 6.1. The biological oxygen demand was reduced from 1646 mg/l to 433 mg/l and the chemical oxygen demand was reduced from 3279 mg/l to 794 mg/l.

The *Klebsiella* sp. has the capacity to reduce chemical oxygen demand upto 53.06% using glucose as co-substrate Chen Shiong Chong *et al.*, 2006). The BOD and COD reduction occurs during the logarithmic growth phase. BOD and COD reduction was maximum during the maximum stationary growth phase (Hu, 1998). The

bacterial isolates like *Acinetobacter* sp., *Bacillus* sp. and *Legionella* sp. had potential for colour removal and strains of *Acinetobacter* sp., *Bacillus* sp. and *Pseudomonas* sp. had potential for COD removal activities (Olukanni *et al*., 2005).

## 4. 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 I concluded that the bacterial isolates like *Bacillus subtilis, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* and *Escherichia coli* were used as a good microbial source for waste water treatment.

## Bibliography


Adedayo, O., Javadpour, S., Taylor, C., Anderson, W.A., and Moo-Young. 2004. Decolourization and detoxification of Methyl Red by aerobic bacteria from a waste water treatment plant. *World Journal of Microbiology*. 20: 545-550.

Ajibola, V.O., Oniye, S.J., Odeh, C.E., Olugbodi, T., and Umeh, U.G. 2005. Biodegradation of Indigo containing texile effluent using some strains of bacteria. *Journal of Applied Sciences*. 5(5): 853-855.

Benson, W.J. 1994. Microbiology applications: laboratory manual in general microbiology. Wm. C. Bron Commication., U.S.A.

Bragger, J.L., Lloyd, A.W., Soozandehfar, S.H., Bloomfield, Marriot, C., and Martin, G.P. 1997. Investigation on the azo reducing activity of a common colonic microorganisms. *International Journal of Pharmacy*. 157: 61-71.

Chen Shiong Chong, Zaharah Ibrahim, Madihah Md Salleh, Noor Aini Abdul Rashid, Adibah Yahya and Wui Jin Wong. 2006. Decolorization of azo dye Direct Blue-15 using batch culture of *Klebsiella sp. Petroleum and Natural Resource Process:* 595-600.

Hu, T.L. 1998. Degradation of azo dyes by *Pseudomonas luteola*. *Water Science Technology*. 38: 299-306.

Maximo, C., Amorim, M.T.P., and Costa Ferreira, M. 2003. Biotransformation of industrial reactive azo dye by *Geotrichum* sp. *Enzyme and Microbial Technology*. *32*: 145-151.

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.

Olukanni, O.D., Osuntoki, A.A., and Gbenle, G.D. 2005. Textile effluent biodegradation potentials of textile effluent-adopted and non-adopted bacteria. *Applied Environmental Microbiology*: 837-844.

Praveen Sharma, Chaudry, G.R., and Thomes Edison. 2009. Mutagenicity testing of some commonly used azo dyes. *Applied Environmental Microbiology*. 42(4): 641-648.

Soares, G.M., Amorim, R., Hardina, and Ferreire, M.C. 2004. Studies on the Biotransformation of novel diazo dyes by laccase. *Process Biochemistry*. 37 : 581-587.

Van der Zee, F.P. 2002. Anaerobic azo dye reduction. *Environmental Science Technology*. 37(2): 402-408.

Van der Zee, F.P., Bisschops, I.A.E., Blanchard, V.G., Lettinga, G., and Field, J.A. 2003. Characterization of azo reduction activity in a novel Ascomycete yeast strain. *Water Science Technology* : 97-104.

Vidali, M. 2009. Bioremediation – an overview. *Pure Application Chemistry*. 73 (7): 581-587.