Isolation of Different Bacterial Isolates from Textile Effluent and Evaluation of Their Decolourization Efficacy Against Commercially Available Anthraquinone Blue 3R Dye

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ABSTRACT

Azo dyes are important chemical pollutants of industrial origin. Textile dye effluent sample was collected from Colombia dye chemical processing industry, Rayar Palayam, a dye industry at Palladam Taluk of Tirupur district in Tamilnadu. The sample was collected in a plastic pet bottle. Bio degradation of textile effluent and commercially available Anthraquinone Blue 3R textile dye was studied against the three bacterial isolates such as *Bacillus* sp., *E. coli*, and *Pseudomonas fluorescens*, which have been isolated from the dye effluent sample by pour plate method and the percentage decolourization, was estimated. The medium containing Anthraquinone Blue 3R dye sample (commercial dye) was inoculated with pre-grown bacterial isolates at 1×10^6 cfu/mL. The disappearance of the colour in the culture medium was monitored by measuring the absorbance wave length at viz. (493 nm, 572 nm, 413 nm, 574 nm, 664 nm) after 0, 4, 8, 12, 16 days of incubation period. Biodegradation of Anthraquinone Blue 3R by the different bacterial isolates at a dye concentration of 2.5% was estimated and the decolourization percentage was tabulated as *Pseudomonas fluorescens*, showed highest decolourization percentage of 82% and followed by *Bacillus* sp. (63%) and *E. coli* (59%) after 16 days of incubation period.

Keywords: Anthraquinone Blue 3R, Biodegradation, Bacteria, textile dye.

INTRODUCTION

Azo dyes are important chemical pollutants of industrial origin. Textile azo dyes with bio accessible groups for lignin degrading fungi, such as 2-methoxy phenol (guaiacol) and 2, 6-dimethoxy phenol (syringol) were synthesized using different aminobenzoic and aminosulphonic acids as diazo components. The inoculum of the best biodegradation assays was obtained from a pre-growth medium (PGM), containing one

of the synthesized dyes. The results of the dye biodegradation assays were evaluated every 8 days by the decrease of the absorbance at the maximum wave length of the dye of the increase of bio-mass during the 28 days of assay. It was observed that the extent of dye biodegradation dependent on the sucrose concentration, on the degraded dye structure and on the dye present in the PGM medium.

MATERIALS AND METHODS

Collection of the samples:

Textile dye effluent sample was collected from Colombia dye chemical processing industry, Rayar Palayam, a dye industry at Palladam Taluk of Tirupur district in Tamilnadu. The sample was collected in a plastic pet bottle. Prior to the collection the sample bottle was rinsed thoroughly with the sample water. Then the sample was brought to the laboratory. During transportation the sample bottles are kept in ice and were subjected for various microbiological analysis.

Isolation of bacteria from textile dye effluent

The dye sample containing organisms was isolated using pour plate technique. In this method, 1 mL of sample was thoroughly mixed with 9 mL of sterile distilled water, and then it was serially diluted by following standard procedure up to concentration of 10^{-6}. The 1 mL of serially diluted samples from each concentrations of sample were transferred to sterile petriplate and evenly distributed throughout the plate and sterile unsolidified nutrient agar was poured and it was allowed to solidify. The plates of nutrient agar medium were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated from the plates. Well grown colonies were picked and further purified by streaking, the isolated strains maintained on nutrient agar slants and stored at 4°C.

Identification of bacteria

The bacterial isolates were identified using the procedure given in Bergey's Manual of determinative Bacteriology and Cowan and Steel's manual for the identification of bacteria (Barrow and Fletham, 1993).

Based on Gram staining, Motility test, Spore staining, Catalase test (Slide test), Oxidase test and several other Biochemical tests such as Carbohydrate fermentation test, Indole test, MR-VP test, Citrate utilization test, Triple sugar iron test, Nitrate test, Urease test, Gelatin hydrolysis test and Casein hydrolysis test the four bacteria were identified as *Bacillus sp. E. coli* and *Pseudomonas fluorescens.*

Biodegradation studies

The dye samples were collected from the Colombia dye kem processing industry situated at Palladam area. The isolated bacteria were tested for its ability to decolourize Anthraquinone Blue 3R (λM = 572nm) textile dye was used at 200 mg/L concentration (Devi and Kaushik, 2005). Fifty milliliter of Nutrient agar sterile medium was amended with Anthraquinone Blue 3R textile dye and inoculated with 2% bacterial suspension. The suspension contained 2.5 x 10^6 cfu/mL (colony forming unit) spores. The flask was kept in mechanical shaker and incubated at 30±1°C for 8 days. Sample was drawn at 2 days intervals for observation. Sample was centrifuged at 10000 x s for 10 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of spectrophotometer at wave length maxima (λm) of Anthraquinone Blue 3R dye.

Two control flasks (dye + medium without inoculums and medium with inoculums without dye) were maintained.

The percentage of decolourization was calculated using following formula.

$$\% \text{ Decolourization} = \frac{\text{Initial O.D} - \text{Final O.D}}{\text{Initial O.D.}} \times 100$$

EXPERIMENTAL RESULTS

Bio degradation of textile effluent and commercially available Anthraquinone Blue 3R textile dye was studied against the three bacterial isolates such as *Bacillus* sp., *E.coli*, and *Psedomonas fluorescens*, which have been isolated from the dye effluent sample by pour plate method and the percentage decolourization, was shown in the tables accompanying the results.

Isolation and identification of bacteria from the dye effluents

Three different bacteria were isolated from the dye effluent. Based on the Gram staining and biochemical test, they were identified as *Bacillus* sp., *E. coli*, and *Pseudomonas fluorescens* respectively.

Bio-degradation of dye effluent

In a defined nutrient agar sterile medium biodegradation was examined. The medium containing Anthraquinone Blue 3R dye sample (commercial dye) was inoculated with pre grown bacterial isolates at 1×10^6 cfu/mL. The disappearance of the colour in the culture medium was monitored by measuring the

absorbance wave length at viz. (493 nm, 572 nm, 413 nm, 574 nm, 664 nm) after 0, 4, 8, 12, 16 days of incubation period.

Biodegradation of Anthraquinone Blue 3R (at 572 nm)

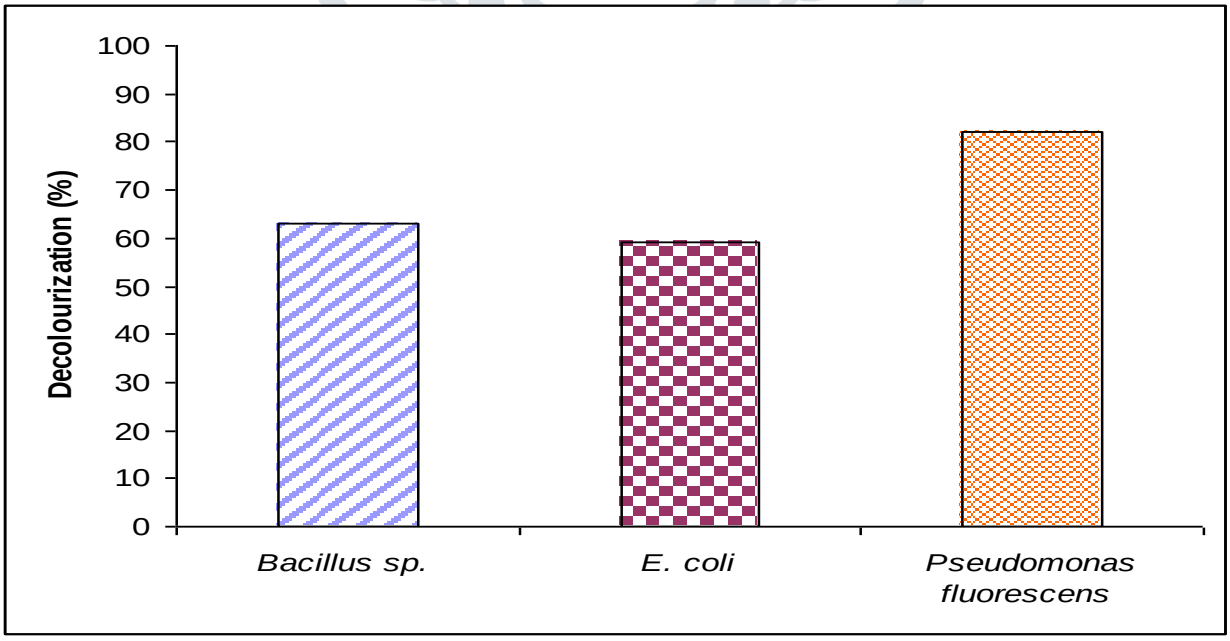
Biodegradation of Anthraquinone Blue 3R by the different bacterial isolates at a dye concentration of 2.5% was estimated and the decolourization percentage was tabulated in Table – 1. Here the *Pseudomonas fluorescens*, showed highest decolourization percentage of 82% and followed by *Bacillus* sp.(63%) and *E.coli* (59%) after 16 days of incubation period.

Table - 1

Decolourization percentage of Anthraquinone Blue 3R by different bacterial isolates

S. No.	Incubation Period (Time)	Organisms	OD value at 572 nm		Decolourization (%)
			Initial	Final	
1.	0	*Bacillus sp.*	0.800	0.515	63
2.	to	*E. coli*	0.789	0.474	59
3.	16	*Pseudomonas fluorescens*	1.329	1.116	82
	days				

Fig. 1. Decolourization of Anthraquinone Blue 3R by different bacterial isolates



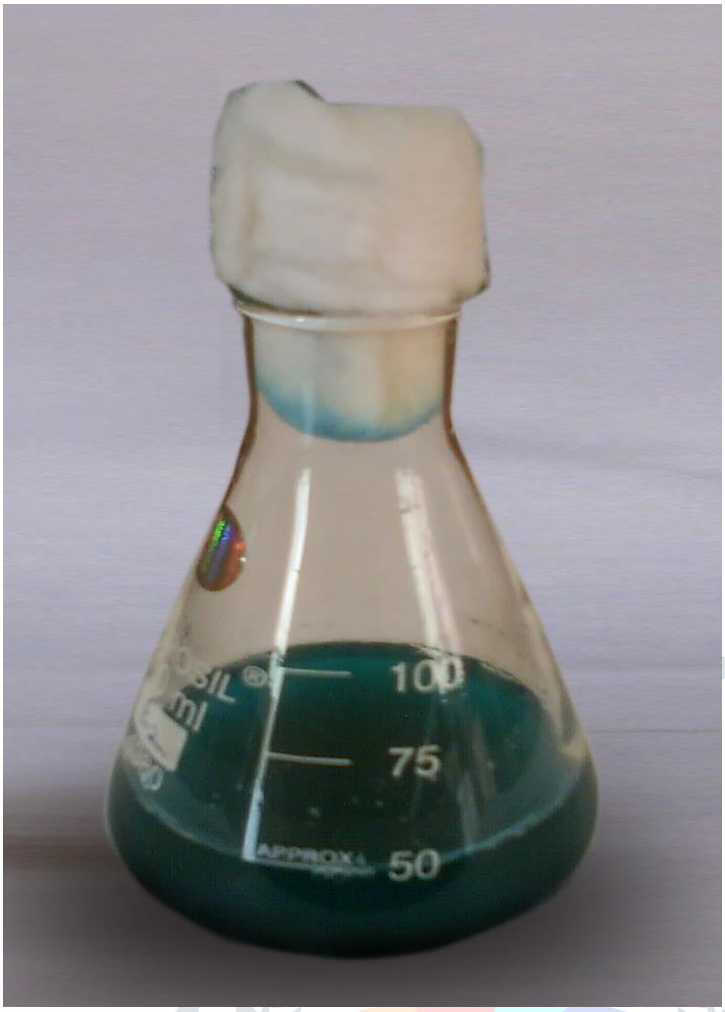


Plate - 1: Decolourization of Anthraquinone Blue 3R by *Pseudomonas fluorescens*

DISCUSSION

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 (Jothimani *et al.*, 2003). Senthinathan and Azeeze (1999) have analyzed the effluents from dyeing units for their physico-chemical properties and reported that the effluent exceeded the tolerance levels for irrigation and public usage.

Coloured textile dye effluents cause environment problem not only by affecting the aesthetic value of water but also due to their hazardous effects, as it is reported that many azo-dyes are found to be carcinogenic. Hence it is necessary that dye should be removed from the waste water.

Few industries have been provided with chemical treatment plants. But even after treatment the effluent possesses enormous soluble salts (Jothimani *et al.*, 2003) several researchers have demonstrated the possibility of utilizing microorganisms for bio treatment of textile waste water

Physiochemical methods like, adsorption chemical precipitation, flocculation, photolysis, chemical oxidation and reduction. Electrochemical treatment and ion-pair extraction have proved to be costly and less effective to treat these coloured effluent and also have operational problems and do not provide satisfactory results, but biological treatment methods are cheap and other the best alternative with proper analysis and environmental control. So the more effective microorganisms capable of specifically degrading the toxic compounds present in the textile effluent are cultured on a large scale and introduced into effluent treatment plants.

The present study was carried out to isolate dye decolourizing microorganisms and to study the dye decolourizing ability. Different bacterial isolate such as, *Bacillus, E. coli, Pseudomonas fluorescens* were isolated from textile dye effluent sample were collected from dying industry in Tirupur in Palladam Taluk. The textile dye effluent from the dyeing industries was screened for bacterial isolates by serial dilution and plating methods. The bacterial isolates were isolated and identified by its cultural and biochemical test with the help of standard manual. The isolates were *Pseudomonas fluorescens, Bacillus* sp. and *E. coli* which were commonly present in the collected dye effluent.

The decolourization efficiency of *Pseudomonas, Bacillus* and *E. coli* was studied by measuring the optical density after 0, 4, 8, 12, 16 days of incubation. It is noticed that there was a decrease in the OD in all the three species in Anthraquinone Blue 3R as the incubation period increased. *Pseudomonas fluorescens* was more effective followed by *Bacillus,* and *E. coli.* The percentage of decolourization of colour by the bacteria was also calculated. *Pseudomonas fluorescens,* showed highest decolourization percentage of 82% and followed by *Bacillus* sp. (63%) and *E. coli* (59%) after 16 days of incubation period.

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