STUDIES ON DEGRADATION OF TEXTILE AZO DYE, MORDANT BLACK 17 USING *Pseudomonas Aeruginosa* SUB 7, ISOLATED FROM TEXTILE EFFLUENT

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Abstract

Improper discharge of colored effluents in aqueous ecosystems depicts acute toxic effects on aquatic flora and fauna and causing severe environmental problems worldwide. The present study concentrates on efficiency and potentiality of novel strain SUB 77 capable of degrading the azo textile dye Mordant Black 17, which was isolated from effluent disposal sites of textile industries, Tirupur and Gummidipoondi. Phenotypic characterization and phylogenetic analysis of the 16S rRNA sequence indicated that the bacterial strain-SUB 7 belonged to *Pseudomonas aeruginosa*. The strain was able to decolorize the azo dye (Mordant Black 17) efficiently up to 86% and 75% in shake and static conditions respectively, in a range of 100 mg L^{-1}, at temperature of 37°C, at pH 7.0. The spectrophotometric analysis of n-butanol cell extract authenticated the decolorization of dye was obtained because of the degradation of aromatic amines rather the inactive surface adsorption which is further substantiated with TLC and FTIR.

Keywords: Pseudomonas, decolorization, biodegradation, aromatic amines.

I. INTRODUCTION

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 low biodegradable and/or toxic industrial pollutants into the environment. The textile effluents contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper, 1995). The release of colored wastewaters represents not only a serious environmental problem, but also causes a public health concern [Dos Santos A.B. et al., 2007].

Although there are several wastewater treatment processes, such as chemical oxidation/coagulation, advanced oxidation, photocatalysis, adsorption and biodegradation, can be used to treat the dye containing effluent (Lin S.H, Peng F. 1996) (Kim T.H, et al., 2004) (Hai F.I., et al., 2007), they possess inherent limitations such as high cost, formation of hazardous by-products and intensive energy requirements (Aravindhan R, et al., 2007), (Sarioglu M, et al., 2007). Conversely, bio-processing can overcome these defects because it is cost saving and environmentally benign. Fungi (Jadhav, J.P et al., 2007) algae (N. Daneshvar, et al., 2007) (S.V. Mohan, et al., 2002) and bacteria (C. Valli Nachiyar and G. Suseela Rajkumar, 2003) have been used in dye decolorization. Adsorption rather than degradation plays a major role during the decolorization process by fungi and algae, as a result, the dyes remain in the environment.

It is well-known that bacteria can degrade and even completely mineralize many dyes under certain conditions (K.C. Chen, et al., 2003) (S. Moosvi, et al., 2005) (S. Asad et al., 2007) (I.K. Kapdan and B. Erten, 2007) Even better, the products of intermediate metabolism produces during the decolorization process, such as aromatic amines, can be degraded by the hydroxylase and oxygenase produced by bacteria (A. Pandey et al., 2007). The present work investigates the dye degradation potential of the developed aerobic bacteria, *Pseudomonas aeruginosa* SUB 7, isolated and identified from textile effluents with optimized physiological conditions (such as dye concentration, pH and temperature) to degrade Mordant Black 17.

II. MATERIALS & METHODS

A. Sources of organisms

Textile effluent-adapted bacteria were isolated from effluent samples collected from Suntex processing mills, Gummidipoondi; Professional fabrics, Tirupur and Kafer Textile mills, Tirupur. Numerous colonies were obtained through serial dilution. Isolated colonies were obtained through serial dilution on Nutrient agar with dye. Bacterial isolates were picked based on the colony

colour, zone formation and their texture and inoculated separately on to nutrient agar to obtain pure culture. Each strain was inoculated into nutrient broth for 24 h and cells after centrifugation was resuspended in 20% Glycerol and stored at – 20°C as stock cultures. To study the effect of pH, temperature and dye concentration on degradation, mineral salt medium having following composition was used (g/l) 1.73 K_2HPO_4, 0.68 KH_2PO_4, 01 $MgSO_4.7\ H_2O$, 0.1 NaCl, 0.03 $FeSO_4$, 1.0NH_4NO_3, 0.02 $CaCl_2.2H_2O$, 5.00 Glucose. Glucose was sterilized separately and added to the medium at the time of inoculation.

B. PCR amplification and sequencing of the 16S rRNA gene

PCR amplification and sequencing of the 16S rRNA was performed to find the isolated organism.

C. Dyestuff and chemicals variations and Acronyms

The dyes Mordant black 17, (MB17) [CAS Registry Number 2538-85-4] was obtained from Dynasty Chemicals (Ningbo) Co., Ltd., China. The chemicals used for preparation of reagent, solutions and microbiological growth media were purchased from Hi-Media Laboratories Pvt. Ltd. Mumbai, India and SISCO Research, Mumbai, India. Solvents used in the studies were of AR grade, purchased from Merck Pvt. Ltd.

D. Optimization of parameters

A nutrient rich medium comprising dye solution, nutrient broth and mineral salt medium was inoculated with 2% of bacterial inoculum. Degradation was studied at different carbon source, nitrogen source, glucose concentration (0, 1, 2, 5, 8, 10 g/l), Ammonium nitrate concentration (0.1, 0.5, 1, 2, 5 g/l) pH (4, 6, 7, 8, 10), temperature (25, 30, 35, 40, 45, 50 °C), and dye concentration (50, 100, 200, 300, 1000 mg/L). The samples were careworn at 2 h time interval from 0 to 48 h, and the sample were analyzed for biomass, glucose and degradation products. Abiotic control without cultures was always included.

E. Degradation experiments

The 8 h (2%) grown culture of *Pseudomonas aeruginosa* SUB 7 were incubated with the textile dyes MB17 at the concentration of 100 mg L^{-1} and incubated at 32°C under shaking condition. 1 ml of dye containing culture medium was extracted with equal volume of n-butanol (Yatome et al., 1981). Supernatant was used to determine degradation by measuring the change in absorbance of culture supernatants at the maximum absorption wavelength (μ max) 520 nm. Rate of decolorization was calculated from the difference between the initial and the final absorption values of the supernatant at μ max for each dye (Ramya et al., 2007).

F. Extraction and analysis of degraded dye products

The Intracellular protein content of bacterial growth was estimated using the Lowry method (1951). Glucose utilization during dye degradation was evaluated using DNS method. Absorbance measurements were done using an UV/Visible spectrophotometer [Thermo Scientific, UV 2700]. Mordant Black 17 had μmax at 520 nm. Studies were carried out to assess the degradation products of Mordant Black 17.

50 ml of 28 and 48 h samples were centrifuged at 12,000 rpm for 20 min. The culture supernatant was lyophilized. The samples were subjected to TLC and FTIR analysis to identify the breakdown products.

G. Thin Layer Chromatography

TLC analyses for the breakdown products were done on fluorescent silica plates (Polygram Sil G/UV, 40 X 80 mm, Germany). For identification of intermediate products of the degraded dye, the solvent system used was Isolproponal: Acitic acid: water in the ratio of 19:9:1. The compounds were identified by comparing their R_f values with Standards. The samples were visualized by exposing the plates to Iodine vapor.

H. FTIR analysis

The spots obtained from TLC plates were scrapped and eluted using Ethyl acetate. The pellets as well as the ethyl acetate eluent were subjected to FTIR analyses using Perkin Elmer RX1 FTIR spectrophotometer.

III. RESULTS AND DISCUSSION

A. Isolation and Identification of decolorizing bacteria

Morphology of the isolated bacterium is Gram-negative, motile, rod shaped. Its green pigmented colonies are circular, flat, smooth and showed oxidase positive. Identification of the test organism through 16S rRNA analysis had showed the highest similarity with the species *Pseudomonas aeruginosa* (99%). Based on

the phenotypic characteristics, phylogenetic analysis and some positive biochemical characters strain SUB 7 was identified as *Pseudomonas aeruginosa*.

B. Effect of shake and static culture conditions

The dye decolorization of azo dye MB17 were studied under static and shake culture (200 rpm) with an initial dye concentration of 100 mg L^{-1} (fig. 1.(a)). It was observed that under shake culture, the decolorization of MB17 was efficient giving 75% within 24 h as compared to 86% decolorization under static culture. The degradation by *Pseudomonas aeruginosa* SUB 7 initiated at 6-7 h of its incubation. It was speculated that under agitation conditions, the aerobic respiration of the strain is not hindered by the utilization of NADH and the azoreductase is not deprived from obtaining electrons from NADH to decolorize azo dyes. There was a contradiction in the behavior of the organism toward the dyes from previous observations (Stolz, 2001).

C. Effect of different parameters on dye decolorization

The maximum decolorization was observed in neutral to slightly alkaline condition with the optimum pH being 7.5, 7.0 and 8.0 giving (92%) under still culture condition and (90%) under shake culture condition in 48 h (fig. 1.(b)). These results were comparable with other azo dye reducing species like *Bacillus, Citrobacter* and *Pseudomonas* reported so far (Kalme et al. 2007; Chang et al., 2001; Suzuki et al., 2001; Wang et al., 2009) which gave similar results. The optimum temperature was found to be 35°C (fig. 1.(c)) for the entire organism studied. It correlates well with the previous reports for the degradation of Disperse Blue 79 and Acid Orange 10 by Bacillus fusiformis KMK5 (Kolekar *et al.*, 2008). The decrease in decolorization at higher temperature may be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization at 40°C (Cetin and Donmez, 2006; Panswad and Luangdilok, 2000).

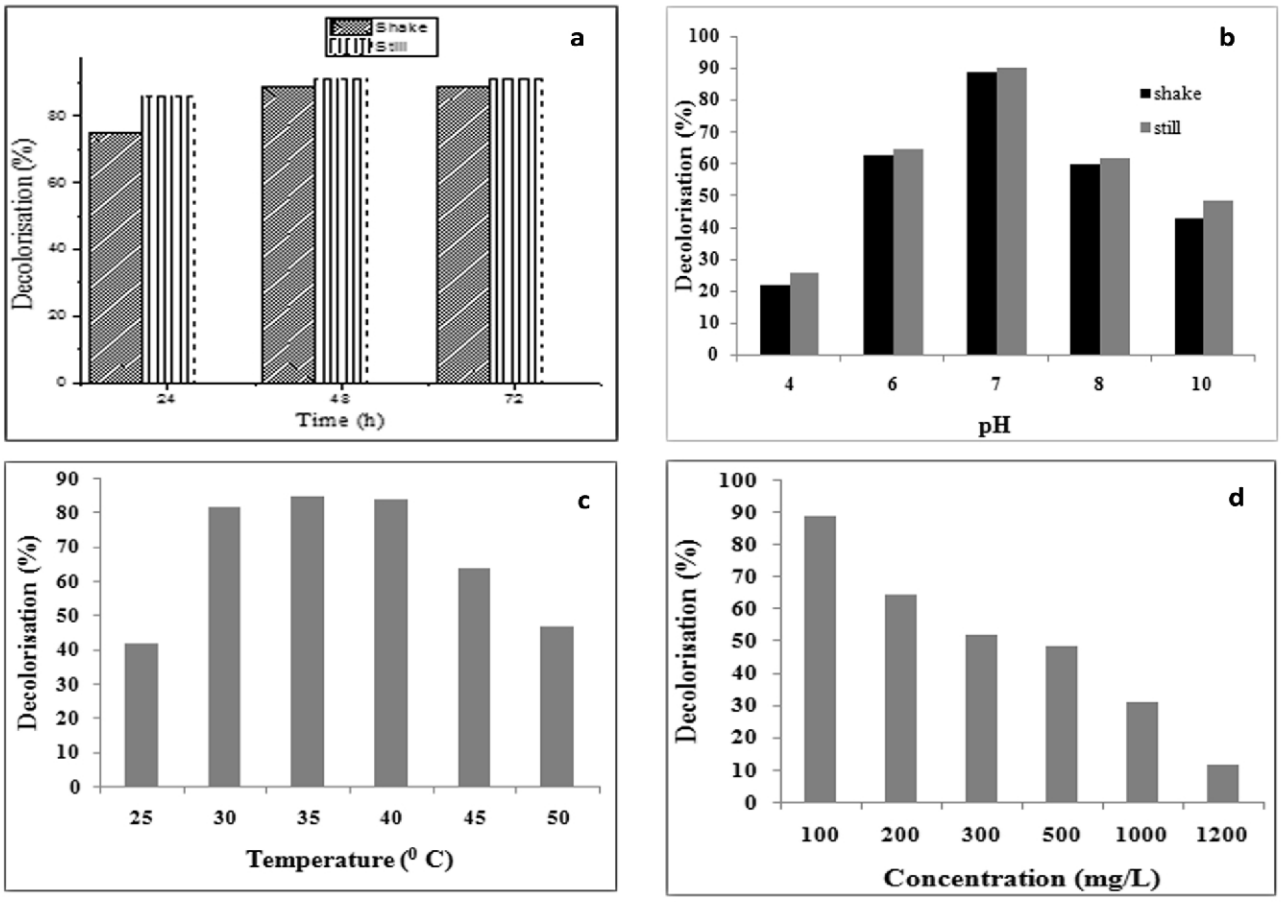

Fig. 1. Effect of different parameters on dye decolorisation

Efficiency of the organism was checked by studying the degradation of MB17 at different concentration in the range of (100-1200 mg/L) and the results are given in fig. 1.(d). *Pseudomonas aeruginosa* exhibited about 90% and 86% degradation in MB17 at 100 and 200 mg/L concentration respectively and above this concentration there was only a minor changes with decrease in the degradation (< 65%) percentage. The similar case was seen with Valli Nachiyar and Suseela Rajkumar, (2003) have reported that after 48 h, the dye was completely decolourized up to 200 mg/L, by *Pseudomonas aeruginosa* but above this concentration there was not much change in the decolourization level.

D. Degradation experiments

Pseudomonas aeruginosa SUB 7 was isolated from the textile effluent and were compared it with some other referred organism which has ability to degrade aromatic pollutants was purchased from IMTECH, Chandigarh for biodegradation studies. The results obtained from degradation studies are presented in Table I.

Table 1. Decolorization of Mordant Black 17 by different bacterial strains

Bacterial strains	Concentration (mg L^{-1})	Decolorisation (%)	
		24 h	48 h
Pseudomonas aeruginosa SUB 7	100	86	89
Pseudomonas flurescence	100	67	72
Flavobacterium mltvorumu	100	81	85
Aeromonas hydrophila subs	100	78	82
Proteus vulgaris	100	45	58
Proteus rettgeri	100	61	64
Proteus mirabilis	100	73	75

It is evident from the results that different organisms degraded the dye at different time intervals. All the organisms studied were able to degrade about 45-85% degradation was noticed after 24 h of incubation. Whereas, only *Pseudomonas aeruginosa* SUB 7 could degrade mordant Black 17 even up to 86% degradation was observed after 24 h. Increasing the incubation period beyond 24 h did not show much variation in the decolorization rate.

E. Biomass, dye decolorization and glucose uptake by Pseudomonas aeruginosa SUB 7

The time course of degradation was followed in terms of decolorization percentage, protein concentration (as an index of growth) and glucose utilization (Fig. 2). The organism started to degrade in the late log phase reaching the maximum when they reached their stationary phase (Jo-Shu Chang et al., 2001).

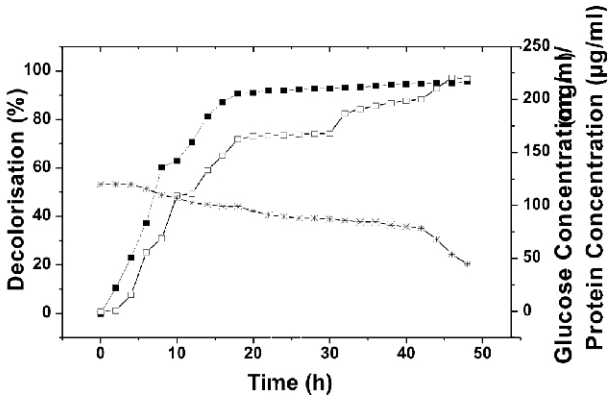

Fig. 2 Dye decolorisation studies

F. Identification of intermediate product

FTIR analysis of the dye showed a peak at 3447 cm^{-1} corresponding OH stretching vibrations. A slight change in this peak approximately to 3467 can be noticed in samples containing degradation products of the dye. This may be due to the overlapping of NH stretching vibration of aromatic amines produced due to azo reduction. Another new peak at 2127 cm^{-1} present in the degraded sample is comparable to peak found in the FTIR spectrum of Naphthaquinone. FTIR spectrum of the spot with the Rf value of 0.66, obtained from the TLC plates showed prominent peaks at 2950 - 2900 cm^{-1} and at 2340 cm^{-1} characteristics of Naphthoquinone confirming that this is one of the intermediate product.

The GC Mass spectra of the ethyl acetate extract containing degradation products of the dye degraded by all the organisms were found to be identical and showed many peaks of which peaks with RT values of 7.71 and 12.48 were emphasized. Mass spectra

corresponding to RT value of 7.71 indicates a fragmentation with m/z 120, 92 and 65 signals indicate the presence of methyl salicylic acid. Methyl salicylic acid might have formed from salicylic acid during the extraction process with ethyl acetate. Fragments with m/z 144 and 115 for the peak with RT value of 12.48 confirmed the presence of α / β naphthol. A small peak with the RT value of 15.04 gave mass spectra with m/z 115, 101 and a strong signal at 43 may be due to the presence of β ketoadipic acid.

G. Degradation of different azo dyes by Pseudomonas aeruginosa SUB 7.

Decolorization of different azo dyes at a concentration of 100 mg L-1 was studied. Another set of flasks was incubated under static culture condition. Growth and dye decolorization were monitored after 48 h of incubation. The results are given in Table II.

It is apparent from the table that mordant black 17 was almost completely decolorized (both at static and shake culture condition) followed by moderate levels of dye decolorization with Congo red and Eriochrome black under both the culture conditions and moderate level of degradation by Amaranth at the static culture condition. Atul Acid Black and Navitan Red were poorly degraded by Pseudomonas aeruginosa SUB 7 under both the culture conditions.

Table 2. Degradation of different azo dyes by Pseudomonas aeruginosa SUB 7

Different Azo Dyes	Concentration (mg L^{-1})	Decolorisation (%)	
		Shake	Static
Mordant Black 17	100	86	75
Eriochrome black	100	83	79
Congo red	100	78	75
Amaranth	100	51	66
Navitan Red	100	53	47
Atul Acid Black	100	54	42

H. Analytical methods

The TLC pattern showed the absence of amines (3-hydroxy 4 amino 1 naphthalenesulfonic and 2-diazo-1 naphthol-4 sulfonic acid 1 hydrate). Further substantiated by FTIR analysis also did not show any peak corresponding to azo bond 1639 cm^{-1} which was present in the 0 h and not in 24 h. (Model- Perkin Elmer RX1 FTIR spectrometer).

IV CONCLUSIONS

In this study, a decolorizing bacterial strain *Pseudomonas aeruginosa* SUB 7 was isolated from textile effluent. The maximum decolorization efficiency against Mordant Black 17, achieved in this study was 86% within 24 hr and the color changes starts from 6-7 h. *Pseudomonas aeruginosa* SUB 7 found to be potential candidate for the degradation of dye rather than adsorption. TLC and FTIR analysis also confirm the removal of azo dyes. The ability of the strain to tolerate, decolorize and degrade azo dyes at high concentration gives it an advantage for treatment of effluent from textile industries.

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