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ISOLATION AND IDENTIFICATION OF AZO DYE DEGRADING MICRO ORGANISM FROM EFFLUENT RUN OFF SITE

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ABSTRACT

Synthetic dyes are extensively used in textile dyeing, paper, printing, colour, photography, pharmaceutics, cosmetics and other industries. Among these, azo dyes represent the largest and most versatile class of synthetic dyes. Approximately 10-15% of the dyes are released into the environment during manufacture and usage. Traditional methods of treatment are found to be expensive and have operational problems. Biological decolourization has been investigated as a method to transform, degrade or mineralize azo dyes. In the present studies **bacteria** from soil and from effluent run off site were subjected for decolourisation in mineral salt media. The most promising bacterial isolate was found to be *Bacillus subtilis* and was used for further dye degradation studies. The strain showed complete decolourization of the selected dye (Astrazon yellow and Astrazon blue) within 36 hours in aerobic condition. The biodegradation was monitored by UV-Vis. The results suggest that the isolated organism *Bacillus subtilis* strain as a useful tool to treat waste water containing the azo dye (Astrazon yellow and Astrazon blue).

Key words: Azo dyes, Mineral salt medium, *Bacillus Subtilis*, decolourisation.

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INTRODUCTION

Dyes have a long history and constitute an important component in our daily lives. The dye industry began by using natural plant and insect sources, and then rapidly turned to synthetic manufacturing processes (Amit Bafana *et al*., 2011). Synthetic dyes are extensively used in textile, dyeing, paper printing, colour photography, food, cosmetic and other industries (Mathur and Bhatnagar, 2007; Pant *et al*., 2008; Laowansiri *et al*., 2008). An estimated 700000 tons of dyes are produced annually worldwide of which 60-70% are azo dyes (Soares *et al*., 2004). Azo dyes are color fast and many can be structurally modified to bind to a variety of natural and synthetic fibers (Bumpus, 2004). One of the most pressing environmental problems related to dye effluents is the improper disposal of waste water from dyeing industry. In recent years bacteria have been drawing a tremendous attention due to their ability to treat waste water and thereby improve the water quality (Rajeswari *et al*., 2011).

The azo-reactive dyes are used extensively in textile industries to their favourable characteristics of bright color, water-fast and simple application techniques with low energy consumption (Othman *et al*., 2011). Azo dyes usually have synthetic and complex aromatic molecular structures, which make them more stable and difficult to degrade (Padmesh *et al*., 2005). 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 (Husseiny, 2008). The presence of dyes or their degraded products in water can also cause human health disorders such as nausea, hemorrhage, ulceration of skin and mucous membranes and the presence of such compounds also resulted into severe damage to the kidney, reproductive system, liver, brain and central nervous system (Hai *et al*., 2007). The presence of very low concentration dye in water (10-20 mg L-1) is aesthetically undesirable (Mahmoud *et al*., 2007).

Various physiochemical methods can be used for the removal of dyes from the wastewater. Some of these methods are effective but are quite expensive because they generate significant amounts of chemical sludge waste whose disposal in a secure landfill increases process cost. Also, there is disposal

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problem of such waste material to a proper place that also limit the use of these methods (Hernandez *et al*., 2008). Therefore, in such situations, biological treatment may be a real hope. These methods have the advantages of being environment friendly. Over the last two decades considerable amount of research has been reported on the use of micro-organisms as bioremediation agents in the treatment of dye-containing waste water (Ramalho *et al*., 2004).

MATERIALS AND METHODS

Collection of dye samples

The azo dyes were collected from a dying unit -Tirupur. Selection of dyes was based on their extensive usage in local dyeing and textile industries around the study area. Two commonly used textile azo dyes (Astrazon yellow and Astrazon blue) were selected for the present study to check the decolourisation activity of selected microorganisms from the effluent run-off site.

Chemicals and media

The microorganisms present in soil samples from effluent run–off site of a textile dyeing house located in Tirupur, Tamil Nadu were enriched in a growth medium according to the modified method of Li *et al*., 2008. The enrichment medium consisted of: glucose 0.1%, yeast extract 0.05%, peptone 0.5%, NaCl 0.5%, $(NH4)_2SO_3$ 1%, K_2HPO_4 0.02%, KH_2PO_4 0.5% and $MgSO_4.7H_2O$ 0.5%, amended with 50 mg/L of the test dyes for adaptation of the microorganisms. 10 g of the soil sample was aseptically inoculated into 250 ml conical flask containing 100 ml of the enrichment media. Nystatin (0.1 g/ml) was used in the flask to inhibit fungal growth for the development of the bacterial consortium. The flask was incubated under shaking conditions at 180 rpm and at a temperature of 30°C. The most effective decolorizing species were screened by 48 hours incubation on Nutrient Agar plates amended with 50 mg/L of the test dyes (Astrazon yellow and Astrazon blue). Bacterial colonies that showed a clear decolourisation zone around them were picked and reintroduced into 100 ml of freshly prepared enrichment media.

For initial screening,0.1%(v/v) aliquot of each isolated strain in nutrient broth was inoculated into 20 well microtitre plate, each containing 200 µL individual dye (Astraon yellow and Astrazon blue) solutions. Decolourisation of the dye solution was monitored visually after 24 hours incubation. Strains that showed high decolorizing

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potential were chosen to be tested further using selected dyes incorporated in Mineral salt medium Agar plates In secondary screening using dye incorporated Mineral salt medium agar plates, the selected isolates were first inoculated into the nutrient broth for 24 hours. The culture was then lawned on to the agar and left for another 24 hours. After incubation decolourisation zone was observed in the dye incorporated agar plates.

Final screening using selected dyes in mineral salt liquid media were initially done using smaller volume of samples. Each selected strain was inoculated in to several bottles containing10 ml nutrient broth and allowed to grow for 24 hours. A sample of 10% (v/v) of the aliquot was then transferred in to bottles containing 10 ml of mineral salt media. A final concentration of 50 mg/l of each dye was added in to each bottle and the absorbance at their respective maxima was taken initially (t_o) and after a period of 24 hours (t_{24}).

Based on the reduction of the absorbance, the percentage of colour removal was determined. Aliquots (3 ml) of the culture media were withdrawn at time intervals of 6 hours over 48 hours and centrifuged at 7000 rpm for 15 min. Decolourisation was quantitatively analyzed by measuring the absorbance of the supernatant using a UV–visible spectrophotometer at maximum wavelength, λmax, of 354 nm for azo yellow and 510 nm for azo blue. The efficiency of colour removal was expressed as the percentage ratio of the decolorized dye concentration to that of initial one based on the following equation (Chen *et al.*, 2003).

Colour removal (%) = Dye (i) – Dye (r) x 100%

Dye (i)

Where Dye (i) = initial dye concentration (mg/L), Dye (r) = residual dye concentration (mg/L).

Identification of Bacteria present in the soil sample

Identification of Selected Microorganisms

The screened bacterial strains from the broth decolourization assay which showed high reduction pattern were selected and identified by using standard biochemical and microscopic techniques (Cappuccino and Sherman, 1999).

RESULT AND DISCUSSION

Nearly 15 isolates (Table 1) were identified and checked for the decolourisation activity of the selected azo dyes, astrazon yellow and astrazon blue. The organism which

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showed better decolourisation effect was *Bacillus subtilis* (isolate 6).

Table 1.1 Morphological and biochemical characteristics of identified bacterial strains

Organism	*S. lactis*	*M.luteus*	*C. xerosis*	*Azospirillum*	*Rhizobium*
Gram stain	Gram +ve cocci	Gram +ve cocci	Gram +ve rod	Gram –ve	Gram –Ve pleomorphic rods
Agar slant cultural characteristics	Thin even growth	Soft, smooth yellow growth	Grayish, granular, limited growth	Pellicle formation with change of colour from green to blue on malate medium	Large gummy colony on YEMA medium
Fermentation					
Lactose	A	AG	-Ve	-	-
Dextrose	A	AG	A	-	-
Sucrose	A	AG	A+/-	-	-
Indole production	-Ve	-ve	-ve	-	-
MR production	+ve	-ve	-ve	-	-
Vp reaction	-Ve	-ve	-ve	-	-
Ctrate use	-Ve	-ve	-ve	-	-
Urease activity	-Ve	+ve	-ve	-	-
Catalase activity	-Ve	+ve	+ve	-	-
Oxidase activity	-Ve	-ve	-ve	-	-
Gelatin liquefaction	-Ve	+ve slow	-ve	-	-
Starch hydrolysis	-Ve	-ve	-ve	-	-

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Table 1.2 Morphological and biochemical characteristics of identified bacterial strains

Organism	*B. subtilus*	*E. coli*	*Eterobacter. acrogens*	*K. pneumoniae*	*Shigella. dysenteriae*
Gram stain	Gram +ve rod	Gram –ve rod	Gram –ve rod	Gram –ve rod	Gram –ve rod
Agar slant cultural characteristics	Abundant opaque whitewaxy	Abundant thick, white glistening	Abundant thick, white glistening	Slimy, white, translucent raised growth	Thin, even, grayish growth
Fermentation					
Lactosee	-ve	AG	AG	AG	-
Dextrose	Acid productin	AG	AG	AG	A
Sucrose	Acid productin	AG +/-	AG +/-	AG	A +/-
Indole production	-ve	-ve	-ve	-ve	-ve/+ve
MR production	-ve	-ve	-ve	-ve/+ve	-ve/+ve
Vp reaction	+ve	+ve	+ve	+ve/-ve	-ve
Ctrate use	+ve	+ve	+ve	+ve	-ve
Urease activity	+ve	-ve	-ve	+ve	-ve
Catalase activity	+ve	+ve	+ve	+ve	+ve
Oxidase activity	-ve	-ve	-ve	-ve	-ve
Gelatin liquefaction	+ve	-ve	-ve	-ve	-ve
Starch hydrolysis	+ve	-ve	-ve	-ve	-ve

Table 1.3 Morphological and biochemical characteristics of identified bacterial strains

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Organism	*S. typhimurium*	*P. vulgaris*	*P. aeroginosa*	*A. faecalis*	*Staph. aureus*
Gram stain	Gram –ve rod	Gram -ve rod	Gram –ve rod	Gram –ve rod	Gram +ve cocci
Agar slant cultural characteristics	Thin, even grayish groth	Thin, blue gray, spreading growth	Abundant thin,white groth, with medium turning green	Thin,white, spreading, viscous growth	Abundant opaque,golden growth
Fermentation					
Lactose	-Ve	-Ve	-Ve	-Ve	A
Dextrose	AG	AG	-Ve	-Ve	A
Sucrose	A+/-	AG	-Ve	-Ve	A
Indole production	-Ve	+ve	-Ve	-Ve	-Ve
MR production	+ve	+ve	-Ve	-Ve	+ve
Vp reaction	-Ve	-Ve	-Ve	-Ve	-Ve/+ve
Citrate use	+ve	+ve/-ve	+ve	+ve/-ve	-Ve
Urease activity	-Ve	+ve	-Ve	-Ve	-Ve
Catalase activity	+ve	+ve	+ve	+ve	+ve
Oxidase activity	-Ve	-Ve	+ve	+ve	-Ve
Gelatin liquefaction	-Ve	+ve	+ve	-Ve	+ve
Starch hydrolysis	-Ve	-Ve	-Ve	-Ve	-Ve

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Almost 90% of decolourisation was observed under aerobic condition at 36 hours duration. Effluent-adapted *Bacillus* sp. gave 35.68% reduction in colour. The results agree with that of Olukanni *et al*. (2006). 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 **(**Saranraj 2010). An NADH-dependant azoreductase of the strain *Bacillus* sp. SF was found to be responsible for decolourization of azo dyes (Maier *et al*., 2004). The result obtained by us was in correlation with that of Mannivannan *et al.*, 2011. *Bacillus subtilis* to decolorize the leather Acid Blue 113 with decolorization efficiency of 90%, thus suggesting its application for decolorization of dye bearing of industrial wastewaters (Mannivannan *et al*., 2011).Therefore, effective and economical waste water treatment facility is required to overcome health and environmental hazardous problems (Kothari *et al.*, 2006). Biological treatment is the only way for ultimately controlling pollution generate by textile and dyes stuff industries. Thus, by this present study we conclude that the bacterial isolates like *Bacillus subtilis* can be used as a good microbial source for azo dye waste water treatment.

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