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An Innovative Approach to Biodegradation of Textile Azo Dyes by native bacterial strains in Iran

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Abstract. Wastewater effluents from the textile and other dye-stuff industries contain significant amounts of synthetic dyes that require treatment to prevent groundwater contamination. In research aimed at biotechnology for treatment of azo dyes, this study examined 4 strains of azo-dye degrading bacteria to identify efficient strains and determine incubation times required for decolorization. These bacteria were screened for decolorization of the textile azo dyes; Reactive Lanasol Black B (RLB), Eriochrome Red B (RN) and 1, 2 metal complexes I. Yellow (SGL). The screening results showed that isolates were able to decolorize the dyes, in 2h ranging from 57 to 100%.The biodegradation products of RLB (as a model of textile azo dyes) formed during anaerobic and sequential anaerobic/aerobic treatments were analyzed by HPLC. Peaks at different retention times were observed in the anaerobic stage, and these peaks completely disappeared at the end of anaerobic/aerobic incubation. This result clearly indicates that the dye had been catabolized and utilized by isolates. Azo dye degradation products were less toxic to growing Sorghum bicolor than the original azo dyes.

Keywords: Azo dyes; Safe biodegradation; HPLC analysis; Native bacteria

1. Introduction

Bioremediation is a pollution-control technology that uses natural biological species to catalyze the degradation or transformation of various toxic chemicals to less harmful forms.Xenobiotic compounds are not naturally available and hence the locally occurring microorganisms cannot readily degrade them. Hazardous materials may render harm to humans, livestock, wildlife, crops or native plants through handling, ingestion, application to land or other distributions of the contaminated materials into the environment. The textile industry leaves about 50 % of the textile azo dyes in free state to be discharged in the factory effluent and eventually to the surrounding environment.Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries such as textile, food, cosmetics and paper printing (Pandey et al., 2007). The reactive azo dyes-containing effluents cause serious environmental pollution. Therefore, industrial effluents containing azo dyes must be treated before discharging into the environment to remove the dye toxicity from textile effluents (Hao et al., 2000; Rajaguru et al., 2002; Wafaa et al. 2003; Umbuzeiro et al., 2005; Wafaa, 2006). However, the azo dyes are generally recalcitrant to biodegradation due to their xenobiotic nature. The microorganisms being highly versatile are expected to develop enzyme systems for the decolourization and mineralization of azo dyes under certain environmental conditions (Pandey et al., 2007). Anoxic degradation of various azo dyes by mixed aerobic and facultative anaerobic microbial consortia was reported (Kapdan et al, 2000; Padmavathy et al., 2003 a&b; Khehra et al., 2005; Moosvi et al., 2005). Although, many of these cultures were able to grow aerobically, degradation was achieved only under anaerobic conditions (Chen et al., 1999, 2003; Chang et al., 2001; Yu et al., 2001). The biodegradation of Reactive Red (RR) textile azo dye by the bacterial isolates Enterobacter cloacae, Pseudomonas spp. and Bacillus spp was investigated. The biodegradation of azo dyes starts with the cleavage of dye structure releasing aromatic amine (Khehra et al., 2006). Dye stuff toxicity (i.e.mortality, genotoxicity, mutagenicity and carcinogenicity) was studied on both aquatic organisms (fish, algae, bacteria,

etc.) and mammals. Chronic effects of dyestuffs, especially of azo dyes were seldom directly mutagenic or carcinogenic (Van der Zee, 2002). Perey (2002) and Pinheiro et al. (2004) reported that not all aromatic amines are toxic and carcinogenic, and found that some aromatic amines are non-toxic and non-carcinogenic. However, few studies reported the use of a single adaptable microorganism in a sequential anaerobic/aerobic treatment. Moreover, the available literature on sequential anaerobic/aerobic treatment with a single microorganism is extremely limited. Apparently there is a need to develop novel biological decolorization processes leading to the more effective clean up of azo dyes using a single and adaptable microorganism that is efficient under both anaerobic and aerobic conditions. Although degradation of azo dyes by microorganisms has been extensively documented, little is known about the biodegradation of azo dyes by lactic acid bacteria .Thus, this work aims to (I) screen and identify azo dye degrading lactic acid bacteria, (II) study the potential of these isolates in azo dye degradation, (III) detection of plasmids associated with degradation. (IV) determination of phytotoxicity of the azo dye degradation products.

2. Material and Methods

The commercial textile azo dyes, RLB, RN and SGL were purchased from the local market in Iran. These dyes were selected on the basis of their structural diversity and frequent use in local textile industries. The chemical structures of used dyes and other information were shown in (Table 1). The stock solution of dyes (1g/100 ml) was prepared by dissolving in distilled water and filtration through Whatmann No. 5 filter paper. Isolation of efficient azo-dye decolorizing bacteria isolated from activated sludge and turf grass soil. Isolates from each inoculum source that were capable of growth on azo dyes were first enriched using MSM amended with a mixture of four dyes as the sole source of C and N. Each dye was added to the medium at 50 mg l−1 to achieve a final concentration of 200 mg l−1. This value is typical of those used in studies on treatment of azo-dye waste water effluent (Zhao and Hardin 2007). The cultures containing 200 ml of MSM with dyes in 500 ml Erlenmeyer flasks were inoculated with 10 ml volumes of activated sludge, soil, or asphalt-soil and incubated at 30°C for 5 days under static conditions. After incubation, cell suspensions from each flask were plated onto MSM agar medium and incubated at 30°C for 24 h. Microbial colonies that appeared on the agar medium were washed gently with sterile water and resuspended into the flasks containing fresh MSM broth spiked with the mixture of dyes. After a second transfer of the cell suspensions onto MSM agar plates containing 0.1% yeast extract, 100 actively growing colonies with different colony growth characters were selected from each source and were purified by streaking twice on agar medium. The purified cultures were preserved at −20°C in 15% (w/v) glycerol for subsequent study. Bacterial isolates from the enrichment cultures were individually tested for their abilities to grow on agar plates with MSM using each dye separately as a sole source of C and N. The bacterial cells were centrifuged and washed twice with autoclaved MSM broth before cultivation on MSM agar plates containing each dye. On the basis of the screening in liquid medium, the isolates were selected and further compared in megatiter plates inoculated with uniform cell densities. The bacterial isolates were first cultured in MSM containing 0.1% yeast extract (without dye) for 24 h at 30°C with shaking at 150 rpm. The cells were harvested, and a uniform cell density (0.6 OD at 550 nm) was maintained. The mean cell counts of these cultures were between 108 and109 cfu ml−1. The cell density standardized decolorization experiment was performed using the same protocol above. Isolates capable of degrading azo dyes were further characterized by using the API 50 CHL System identification kit (Bio Mérieux, France) and the species were preliminarily identified from the API database. Isolates of lactic acid bacteria were grown anaerobically at 35°C in MRS broth for 48 h for use as starters. A loopful of each starter was cultured in Hungate tubes (16 ml vol) containing 14 ml of MRS medium at 35°C for 48 h .Individual azo dye stock solutions were added to the cultures at final concentration of 100 mg l-1, and the cultures were incubated at 35°C under anaerobic conditions for 48 h. Dye decolorizing activity was expressed in terms of decolorization percentage and was determined by measuring the absorbance at 555 nm against the original color of the medium. Decolorization activity (%) was calculated according to the equation:

Decolourization = Re adding of(C) decolourization –Re adding of (S) decolourization / Re adding of(C) decolourization×100

3. Microorganisms

In this research, four bacterial isolates active in dye removal including Bacillus cereus CCM 2010, Pseudomonas sp. B13T, Shewanella algae strain ATCC 51192, Shewanella algae strain ATCC 51192 were identified from NCBI data.

3.1. HPLC analysis of decolorization metabolites

For HPLC analysis, isolate Lab 2 was grown anaerobically at 35°C for 48 h in Hungate tube containing 14 ml MRS broth, and then RLB was added to the culture at final concentration of 100 mg l-1. The culture was incubated anaerobically at 35°C for 10 h, and subsequentially incubated aerobically for 4h (anaerobic/aerobic sequential system).Ten ml culture were withdrawned after 2 and 10 hours of anaerobic incubation and after 4 hours of aerobic incubation which followed the 10 hours of anaerobic incubation and centrifuged at 12,000 rpm for 15 min. The supernatants were clarified by passing through a 0.45 µ mmembrane filter. Degradation products were extracted thrice with diethyl ether, then extracts were pooled and evaporated to dryness, and the final residue was dissolved in methanol and analyzed using HPLC according to Zhao and Hardin.

4. Results and Discussion

4.1. Decolorization of azo dyes

Although, several bacteria and fungi are capable of catabolizing and mineralizing azo dyes, information on the ability of safe microorganisms to degrade azo dyes is scant. In this work, the first screening experiments indicated that 2 out of 4 isolates were able to decolorize the three tested dyes RLB, RN and SGL completely. These isolates showed decolorization percentages ranging from 57 to 100 % within2 h, indicating that degradation of azo dyes seems to be widespread among tested bacteria. Based on the screening results, the two most effective isolates, which showed the highest decolorization potential for all tested azo dyes in anaerobic condition, were selected for further studies. These isolates exhibited complete decolorization of 100, 93.2 and 94.4 %, for RLB, RN and SGL, respectively. The decolorization efficiency of other isolates was 65.2, 53.8 and 52.96 %, respectively. Hu reported; 37, 88, 92.4 and 93.2 % decolorization of reactive azo dyes Red G, V2RP, RP2B and RBB, respectively by Pseudomonas luteola after 42 h incubation under static conditions. However, compared to degradation rates previously reported, decolorization of the textile azo dyes was lower than what obtained in the present study. It was mentioned that, most azo dyes are reduced anaerobically to the corresponding amines with cleavage of azo bonds by bacterial azoreductase, but they are difficult to degrade aerobically. In general microbial degradation of azodyes involves the reductive cleavage of azo bonds with the help of an azoreductase enzyme under anaerobic conditions, and this involves a transfer of four electrons, which acts as a final electron acceptor, short period of time by selected bacteria in textile azo dye bioremediation. It is worth mentioning that rapid initial decolorization of all the three tested textile azo dyes was achieved within the first two hours. This suggest that isolates may have an efficient enzymatic system for the cleavage of azo bonds, which caused rapid decolorization, and consequently, could successfully be employed in the treatment of waste waters contaminated with azo dyes, resulting in dye decolorization and the formation of colorless solutions. The results obtained in this study are very promising for the treatment of wastewater streams since bacterial isolates used could able to achieve complete mineralization of textile azo dyes for safe degradation products in rapid and short time under sequential anaerobic/aerobic system. This methodology using a single microorganism in azo dyes decolorization was shown to be very effective. However, further work is needed to identify the gene(s) responsible for this kind of textile azo dyes decolorization.

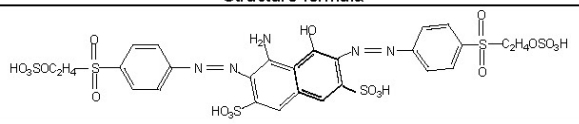
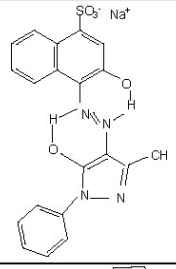
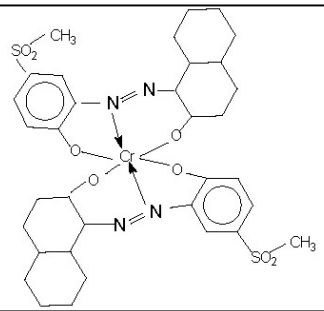
Dye name	Structure formula	Color index (CI)	MW
Lanasol black B		Reactive Black B	991.82
Eriochrom red B		Acid Mordant Red B	446.41
1, 2 metal complex 1. Yellow		Acid Yellow M_2GLN	620

Table 1: Chemical structures and some information of textile azo dyes used in this study.

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