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Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium

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ABSTRACT

Removal of synthetic dyes is one of the main challenges before releasing the wastes discharged by textile industries. Biodegradation of azo dyes by alkaliphilic bacterial consortium is one of the environmental-friendly methods used for the removal of dyes from textile effluents. Hence, this study presents isolation of a bacterial consortium from soil samples of saline environment and its use for the decolorization of azo dyes, Direct Blue 151 (DB 151) and Direct Red 31 (DR 31). The decolorization of azo dyes was studied at various concentrations (100–300 mg/L). The bacterial consortium, when subjected to an application of 200 mg/L of the dyes, decolorized DB 151 and DR 31 by 97.57% and 95.25% respectively, within 5 days. The growth of the bacterial consortium was optimized with pH, temperature, and carbon and nitrogen sources; and decolorization of azo dyes was analyzed. In this study, the decolorization efficiency of mixed dyes was improved with yeast extract and sucrose, which were used as nitrogen and carbon sources, respectively. Such an alkaliphilic bacterial consortium can be used in the removal of azo dyes from contaminated saline environment.

Introduction

A number of colored effluents that contain dyes are released from textile, food, leather, dyestuff, and dyeing industries. The textile industry is one of the largest producers of effluents contaminated with dyes.[1] The residual dyes released from these effluents introduce different organic pollutants in the natural water resources and land.[2]

Approximately 80,000 tons of dyestuff and pigments are produced in India.[1] It has been estimated that 10,000 different textile dyes are commercially available worldwide and the annual production is estimated to be 7×10^5 metric tons; 30% of these dyes are used in excess that is 1000 tons per annum.[3–5] During the dying process, about 2% of these dyes fail to bind to the substrate and are discharged in aqueous effluents.[6] Azo dyes are the most widely used dyes in the industrial sector.[7] They contain one or more azo groups (—N=N) that can resist the breakdown and accumulate in the environment at high levels with high degree of persistence.[8,9]

The wastewater from textile when directly released in the surface water without treatment can cause a rapid

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depletion of dissolved oxygen and lead to a great environmental damage.[10] When dyes are available in the water system, the sunlight penetration into deeper layers is greatly reduced which disturbs photosynthetic activity resulting in deterioration of water quality, lowering the gas solubility, and finally causes acute toxic effects on aquatic flora and fauna. Most of the dyes that are released from wastewater, including their breakdown products, are toxic, carcinogenic, or mutagenic to humans and other life forms.[11,12]

Various physicochemical methods, such as adsorption on activated carbon, electrocoagulation, flocculation, froth flotation, ion exchange, membrane filtration, ozonation, and reverse osmosis, are used for the decolorization of dyes in wastewater. These methods are inefficient, expensive, have less applicability, and produce wastes in the form of sludge, which again needs to be disposed off.[13]

However, the microbial decolorization and degradation of azo dyes has gained considerable interest of researchers as it is inexpensive, eco-friendly, and produces less amount of sludge.[14,15] It has been reported that many organisms are capable of reducing dyes, such as purely anaerobic (e.g., *Bacteroides* spp., *Eubacterium* spp., *Clostridium* spp.), facultatively anaerobic (e.g., *Proteus vulgaris*, *Streptococcus faecalis*), aerobic (e.g., *Bacillus* spp., *Sphingomonas* spp.), several yeasts, and even tissues from higher organisms.[16–22]

Effluents released from textiles industries are toxic, which contain a high degree of color (from residues of reactive dyes and chemicals) along with acidic and alkaline contaminants and high concentrations of organic materials.[23] Extremophiles (alkaliphiles and halophiles) are metabolically diverse and can usually tolerate a greater amount of toxic metals and alkaline conditions in their environment.[24] This study focuses on the decolorization of azo dyes by a moderately alkaliphilic bacterial consortium isolated from saline soil samples. The isolated bacterial consortium was used in the decolorization of azo dyes Direct Blue 151 (DB 151) and Direct Red 31 (DR 31) at different concentrations. The growth parameters for the consortium were optimized. The bacterial strains present in the consortium were identified by 16S rDNA sequencing.

Materials and methods

Dyes and chemicals

The textile dyes (azo dye compounds), namely DR 31 and DB 151, were purchased from the textile industry. Nutrient agar media and all other chemicals used in mineral salt medium (MSM) preparation were of analytical grade and purchased from Merck, India.

Bacterial consortium and culture conditions

The bacterial consortium was isolated from soil samples of saline environment from three different regions of Chennai, namely Nagercoil, Tuticorin, and Pallavaram. The bacterial consortium was enriched in MSM amended with 100 mg/L of DB 151 and DR 31. The composition of the MSM (pH 9) used for enrichment and decolorization was as follows: Na_2HPO_4: 12.8 g/L; KH_2PO_4: 3 g/L; NH_4Cl: 1 g/L; NaCl: 0.5 g/L; 0.05 M $MgSO_4$: 10 mL/L; 0.01 M $CaCl_2$: 10 mL/L; and 20% glucose: 30 mL/L.[9] The medium was autoclaved, cooled, and then amended with 100 mg/L of filter sterilized DB 151 and DR 31 in a 250 mL Erlenmeyer flask. An amount of 10 g of soil sample was aseptically inoculated into the medium. Individual bacterial isolates were obtained from the enriched culture by plating on nutrient agar medium containing 100 mg/L of DB 151 and DR 31. The selected isolates were then purified by streaking on nutrient agar added with 100 mg/L of the dyes. The single colony pure cultures were stored in 15% glycerol at 20 °C.

Analytical techniques

All decolorization experiments were carried out multiple times. MSM added with azo dyes was used as a control to determine abiotic color loss during the experiment. A volume of 1 mL of precultured bacterial consortium was added to 50 mL of MSM added with different concentrations (100, 150, 200, 250, and 300 mg/L) of DB 151 and DR 31. The biodecolorization of DB 151 and DR 31 by bacterial consortium was observed for 5 days[25]. In order to monitor the decolorization process, the samples were withdrawn periodically, centrifuged at 10,000 rpm for 15 min, and filtered through syringe filter (PVDF, Millipore, Inc.); and decolorization was measured using UV/Vis spectra (Hitachi) at the corresponding λ_{max} of the dye and was compared with the uninoculated control. The total protein content was also estimated at every 24 h. The color removal efficiency of the bacterial consortium was determined as follows[26]:

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}}$$

Effect of pH and temperature on the decolorization of mixed dyes

In order to study the effect of pH and temperature, the sterilized MSM was amended with 200 mg/L of each of the DB 151 and DR 31 dyes. The medium was maintained at different pH: 8, 8.5, 9, 9.5, and 10. A volume of 1 mL of overnight culture was inoculated in the flasks and incubated in a shaker at 36 °C. The effect of temperature was studied by inoculating overnight culture and incubating in a shaker at 28 °C, 36 °C, and 45 °C. The medium was maintained at pH 9.5. The measurement of decolorization of the total dye concentration was performed at an interval of 24 h for 5 days.

Effect of carbon and nitrogen sources on the decolorization of mixed dyes

The effect of carbon sources was studied using various compounds, such as fructose, lactose, sucrose, and mannitol, at a concentration of 1% and they were added individually as a supplement to MSM for the decolorization of mixed dyes. A volume of 1 mL of the overnight culture was inoculated in the flasks and incubated in a shaker at 36 °C. Nitrogen sources, such as yeast extract, KNO_3, $NaNO_3$, and NH_4NO_3 were added