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Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR

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ABSTRACT

A developed consortium-GR, consisting of *Proteus vulgaris* NCIM-2027 (PV) and *Micrococcus glutamicus* NCIM-2168 (MG), completely decolorized an azo dye Scarlet R under static anoxic condition with an average decolorization rate of 16,666 μg h^{-1}; which is much faster than that of the pure cultures (PV, 3571 μg h^{-1}; MG, 2500 μg h^{-1}). Consortium-GR gave best decolorization performance with nearly complete mineralization of Scarlet R (over 90% TOC and COD reduction) within 3 h, much shorter relative to the individual strains. Induction in the riboflavin reductase and NADH–DCIP reductase was observed in the consortium, suggesting the involvement of these enzymes during the fast decolorization process. The FTIR and GC–MS analysis showed that 1,4-benzenediamine was formed during decolorization/degradation of Scarlet R by consortium-GR. Phytotoxicity studies revealed no toxicity of the biodegraded products of Scarlet R by consortium-GR. In addition, consortium-GR applied for mixture of industrial dyes showed 88% decolorization under static condition with significant reduction in TOC (62%) and COD (68%) within 72 h, suggesting potential application of this microbial consortium in bioremediation of dye-containing wastewater.

1. Introduction

Azo dyes account for the majority of all textile dyestuffs produced because of the ease and cost effectiveness of their synthesis, their stability and the variety of colors available compared to natural dyes. They are extensively used in the textile, paper, food, leather, cosmetics and pharmaceutical industries (Chang et al., 2001). Improper textile dye effluent disposal in aqueous ecosystems leads to the reduction in sunlight penetration which in turn decreases photosynthetic activity, dissolved oxygen concentration, water quality and depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems worldwide (Vandevivere et al., 1998). In addition to their visual effect, azo dyes also have adverse impact in terms of total organic carbon (TOC) and chemical oxygen demand (COD). Many synthetic azo dyes and their metabolites are toxic, carcinogenic, mutagenic, leading to potential health hazard to humankind (Nilsson et al., 1993).

Several physicochemical methods have been used in the treatment of textile effluents to achieve decolorization. The physical methods are based on coagulation/adsorption of dyes, whereas chemical methods enable destruction or decomposition of dye molecules via electrolysis, ozonation, advanced oxidation processes (e.g., photochemical and photocatalytic processes), etc. (Forgacs et al., 2004). However, implementation of physical/chemical methods have inherent drawbacks of being economically unfeasible (more energy consumption and chemicals uses), unable to remove the recalcitrant azo dyes and/or their organic metabolites completely, generating a significant amount of sludge that may cause secondary pollution problems, and involving complicated procedures (Zhang et al., 2004). The microbial decolorization and degradation of azo dyes has been of considerable interest since it is inexpensive, eco-friendly, and produces a less amount of sludge (Kalyani et al., 2008).

The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Wide range of microorganisms including bacteria, fungi, yeasts, actinomycetes and algae capable of degrading azo dyes have been reported (Chen et al., 2003; Daeshwar et al., 2007). The mechanism of microbial degradation of azo dyes involves the reductive cleavage of azo bonds (–N=N–) with the help of azoreductase under anaerobic conditions resulted into the formation of colorless solutions. The resulting intermediate metabolites (e.g., aromatic amines) are further degraded aerobically or anaerobically (Chang et al., 2000). Most studies on azo dye biodegradation have focused on bacteria and fungi in which various fungal cultures mainly belonging to white rot fungi has been used to develop bioprocesses for mineralization of azo dyes (Parshetti et al., 2007). However, a

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long growth cycle, requiring nitrogen limiting conditions and long hydraulic retention time for complete decolorization still limit the performance of the fungal decolorization system (Banat et al., 1996). In contrast, bacterial decolorization is more efficient and faster, but individual bacterial strain usually cannot degrade azo dyes completely and the intermediate products are often carcinogenic aromatic amines, which need to be further decomposed (Joshi et al., 2008). Thus the treatment systems composed of mixed microbial populations possess higher degree of biodegradation and mineralization due to synergistic metabolic activities of microbial community and offers considerable advantages over the use of pure cultures in the degradation of synthetic dyes (Khehra et al., 2005a). In microbial consortium the individual strains may attack the dye molecule at different positions or may utilize metabolites produced by the co-existing strains for further decomposition (Chang et al., 2004; Forgacs et al., 2004).

In the present study, a defined consortium of two organisms *Proteus vulgaris* NCIM-202 and *Micrococcus glutamicus* NCIM-2168, designated as consortium-GR was used for the decolorization of an industrial diazo dye Scarlet R as well as mixture of various industrial dyes under static anoxic condition. As both the bacteria are found to be efficient in the dye degradation, we used the consortium of these two microorganisms to achieve faster degradation by optimizing various physicochemical conditions. We have also studied the difference in the enzymatic status and the fate of metabolism of Scarlet R by individual organisms and with the consortium-GR. The various intermediates formed have been analyzed during the degradation of Scarlet R using HPLC, FTIR and GC–MS techniques. In addition, phytotoxicity study was used to evaluate toxicity of the biodegraded products of Scarlet R by the consortium-GR.

2. Methods

2.1. Organism and culture conditions

The strain *P. vulgaris* (NCIM-2027) and *M. glutamicus* (NCIM-2168) were obtained from National Chemical Laboratory (NCL, Pune, India). Pure culture was maintained on nutrient agar slants and stored in test tubes at 4 °C and sub-cultured monthly. The pure culture of *P. vulgaris* and *M. glutamicus* were grown in 250 ml Erlenmeyer flask, containing 100 ml nutrient broth containing (g l^{-1}): beef extract, 3; peptone, 10; NaCl, 5, pH 6.6 at 37 °C and 30 °C for 24 h, respectively under static anoxic condition. To study the effect of carbon and nitrogen sources on decolorization of Scarlet R, semi-synthetic medium having following composition was used (g l^{-1}): Scarlet R, 0.050; $(NH_4)_2SO_4$, 0.28; NH_4Cl, 0.23; KH_2PO_4, 0.067; $MgSO_4 \cdot 7H_2O$, 0.04; $CaCl_2 \cdot 2H_2O$, 0.022; $FeCl_3 \cdot 6H_2O$, 0.005; yeast extract, 0.2; NaCl, 0.15; $NaHCO_3$,1.0 and 1 ml/l of a trace element solution containing (g l^{-1}) $ZnSO_4 \cdot 7H_2O$, 0.01; $MnCl_2 \cdot 4H_2O$, 0.1; $CuSO_4 \cdot 5H_2O$, 0.392; $CoCl_2 \cdot 6H_2O$, 0.248; $NaB_4O_7 \cdot 10H_2O$, 0.177; and $NiCl_2 \cdot 6H_2O$, 0.02 with different carbon and nitrogen sources (1% each) such as starch, sucrose, glucose, malt extract, peptone, urea, beef extract, casein, lactose, yeast extract and NH_4Cl. In addition, 1 g of rice husk, rice straw, bagasse powder and wood shavings were mixed with 100 ml distilled water individually and autoclaved at 121 °C for 20 min. Then, 5 ml extract of each agricultural waste was added in semi-synthetic medium and check the decolorization performance of Scarlet R by consortium-GR to make the process more economically feasible.

2.2. Development of consortium-GR

The consortium-GR was developed by aseptically transferring the 0.5 ml suspension of 24 h grown culture of each individual strains; *P. vulgaris* NCIM-2027 (PV) and *M. glutamicus* NCIM-2168 (MG) in 250 ml Erlenmeyer flasks containing 100 ml nutrient broth, pH 6.6 and incubated at 30 °C for 24 h under static anoxic condition. Individual strains used in this study *P. vulgaris* (NCIM-2027) and *M. glutamicus* (NCIM-2168) were inoculated with 1 ml suspension of 24 h grown culture, respectively, to maintain the same cell count in the pure culture and in the consortium.

2.3. Dyestuff and chemicals

Scarlet R, Navy blue HER, Red HE7B, Green HE4BD, Orange HE2 R, Navy blue G, Red HE3B, Navy blue HE2 R, Golden yellow 24 D, Brilliant blue G, Direct Brown MR and Direct Blue GLL were obtained from local Manpasand textile industry, Ichalkaranji, India. Yeast extract and glucose were obtained from Hi Media (Mumbai, India). ABTS [(2,2-azinobis(3-ethylbenzothiazolin-6-sulfonic acid)] was purchased from Sigma–Aldrich (Mumbai, India). Tartaric acid, *n*-propanol, 3′3′-diaminobenzidine tetrahydrate and riboflavin were purchased from Sisco Research Laboratories, India. All chemicals used were of the highest purity available and of the analytical grade.

2.4. Decolorization experiments

The 24 h grown consortium-GR and individual strains cells were incubated with different textile dyes such as Scarlet R, Navy blue HER, Red HE7B, Green HE4BD, Orange HE2R, Navy blue G, Red HE3B, Navy blue HE2R, Golden yellow 24 D, Brilliant blue G, Direct brown MR and Direct blue GLL at concentration, 50 mg l^{-1}, individually and incubated at 37 °C under static condition. Decolorization performance of mixture of above industrial dyes with concentration (30 mg l^{-1}) each was studied in 250 ml Erlenmeyer flask containing 100 ml nutrient broth at 37 °C under static condition. An aliquot (3 ml) of the culture media was withdrawn at the different time intervals. Aliquot was centrifuged at 8000 rpm for 15 min to separate cell mass. Supernatant was used to determine decolorization by measuring the change in absorbance of culture supernatants at the maximum absorption wavelength (λ_{max}) of the respective dyes.

The individual (PV and MG) strains and the developed consortium-GR cells grown in the nutrient broth for 24 h were used to monitor decolorization of Scarlet R at 37 °C under static anoxic and shaking (150 rpm) condition. Decolorization at different initial concentrations of Scarlet R (50–250 mg l^{-1}) were tested by using individual strains as well as developed consortium-GR at 37 °C under static condition. Studies on the effect of temperature (30–50 °C) and pH (5–12) were carried out in the nutrient broth (dye concentration, 50 mg l^{-1}) under static anoxic condition. Further, decolorization of repeated addition of dye aliquots (50 mg l^{-1}) to culture media was also studied in nutrient broth under static condition without supplement of additional nutrients. Studies on the effect of various carbon and nitrogen sources were carried out in the synthetic medium (dye concentration, 50 mg l^{-1}) at 37 °C under static condition by using developed consortium-GR. For this 10% inoculum with an optical density of 1.0 (at 620 nm), grown in the nutrient broth for 24 h was used for inoculation of synthetic medium. All decolorization experiments were performed in triplicates. Abiotic controls (without microorganism) were always included. The percentage decolorization was calculated (Saratale et al., 2006) as follows:

$$\%\ \text{Decolorization} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100\%$$

The average decolorization rate (μg h^{-1}) was calculated (Jadhav et al., 2008b) as follows: