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Exploiting the potential of plant growth promoting bacteria in decolorization of dye Disperse Red 73 adsorbed on milled sugarcane bagasse under solid state fermentation

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ABSTRACT

Bioremediation of textile dyes adsorbed on agricultural solid wastes under solid state fermentation (SSF) using rhizospheric plant growth promoting microorganisms pose an ecofriendly, economically feasible and promising treatment approach. The purpose of this study was to adsorb azo dye Disperse Red 73 (DR73) on sugarcane bagasse (SCB) and its further bioremediation using consortium-RARB under SSF. The particle size of SCB 0.002 mm showed maximum adsorption (65%) for DR73. Kinetics of adsorption of DR73 on milled SCB follows pseudo-second order kinetics. The individual cultures of *Rhodobacter erythropholis* MTCC 4688, *Azotobacter vinelandii* MTCC 1241, *Rhizobium meliloti* NCIM 2757 and *Bacillus megaterium* NCIM 2054 showed 44, 28, 50 and 61% decolorization of DR73 in 48 h respectively; while the consortium-RARB showed complete decolorization in 48 h. Optimum moisture content, temperature and pH for decolorization of DR73 was found to be 90%, 30 °C and 6 respectively. DR73 biodegradation analysis was carried out using HPTLC, FTIR and HPLC. Phytotoxicity and genotoxicity studies revealed detoxification of DR73. Tray bioreactor study for decolorization of adsorbed DR73 on SCB suggests its implementations at large scale. Use of plant growth promoting bacteria's consortium under SSF for bioremediation of adsorbed dyes gives a novel ecologically sustainable approach.

1. Introduction

Textile dyeing processes are known to be most environmental unfriendly industrial processes, because of the wastewaters produced by them are of heavily polluted with different dyes, textile auxiliaries and chemicals (Sanghi et al., 2007). About 7×10^5 ton and approximately 10,000 different textile dyes are produced annually world-wide and 10% of these dyes may found in wastewater (Couto, 2009). Presence of color in textile wastewater causes decrease in penetration of sunlight into waters, retards photosynthesis, inhibits aquatic biota (growth) and interferes with gas solubility in water bodies which ultimately causes to the strong impacts on aquatic ecosystem (Banat et al., 1996). In addition to this, carcinogenic nature of dyes has been seriously consideration regarding human health (Kariminiaae-Hamedaani et al., 2007). Hence, it is essential to provide a textile wastewater treatment approach.

Textile effluent treatment using biological methods were more suitable than chemical and physical methods (Saratale et al., 2009). Biological methods for decolorization mainly involve the use of bacteria, fungi and plants (Lade et al., 2012; Khandare et al., 2013). Decolorization of textile dyes by using number of microorganisms has been already reported (Saratale et al., 2009). Textile wastewater treatment using environmental friendly, non-pathogenic and ecologically sustainable microorganisms put an additional insight over existing techniques of microbial bioremediation. The rhizobium-legume symbiosis offers an ability to convert atmospheric molecular nitrogen into forms usable by the plant, a process called biological nitrogen fixation (Mahadi et al., 2010). Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria which actively colonize plant roots and benefit plants by providing growth promotion (Bashan et al., 2011). In view of this, use of PGRR for different pollutants bioremediation suggests environmental friendly treatment approach (Bashan et al., 2011). There are few reports available which suggest the use of PGPR for

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bioremediation process (Ahmad et al., 1997; Ju et al., 2007), but the combination of different plant growth promoting microorganisms for degradation of textile dye was still scarce.

Sugar and alcohol industries produce sugarcane bagasse (SCB) as an agro-industrial waste. It is a largest natural fiber resource because it mainly contains high cellulose content, higher regeneration capacity and yield (Huang et al., 2012). Thousands of tons of SCB have been produced daily by the sugarcane processing industries (Darani and Zoghi, 2008). Removal of dyes using adsorption on SCB and then adsorbed dye decolorization under the solid state fermentation using PGPRs bacterial consortium ensure safe, ecofriendly and economical approach. Many agro-industrial wastes are found to have good potential to replace the standard medium and the commonly used peat in rhizobial inoculant production (Rebah et al., 2007). Hence, growth of PGPRs on waste biomass leads to dyes removal and generated biomass may be further used as rhizobial inoculants.

Large volume of textile wastewater was produced daily from textile industries, hence to use submerged culture conditions for waste treatment is not practically applicable. However, removal of dyes by adsorption process and SSF approach for biodegradation was preferred for dye treatment (Murugesan et al., 2007). Therefore, use of SCB as a cheap agricultural waste for dye adsorption and microbial consortium PGPRs for biodegradation of dye gives environmental friendly and complete treatment approach.

This study shows the potential of rhizospheric bacterial consortium developed using *Rhodobacter erythropholis* MTCC 4688, *Azotobacter vinelandii* MTCC 1241, *Rhizobium meliloti* NCIM 2757 and *Bacillus megaterium* NCIM 2054 for biodegradation of SCB adsorbed azo dye DR73 under SSF. FTIR, HPLC and HPTLC analysis used to study biodegradation. Phytotoxicity and genotoxicity confirms detoxification of DR73. Tray bioreactor study suggests that to apply this treatment at large scale.

2. Methods

2.1. Dyestuff and chemicals

An analytical grade and of highest purity chemicals were used for study. The textile azo dye Disperse Red 73 (DR73) (I.U.P.A.C Name – Disperse Rubin GFL, CAS Number – 16889-10-4) was obtained from Mahesh textile processing industry Ichalkaranji, India. Dimethyl sulphoxide were taken from S D Fine-Chem Ltd., India. Microbiological medium such as nutrient broth was taken from HiMedia Laboratories Pvt. Ltd., India.

2.2. Milling of SCB

SCB was taken from Shri Chatrapati Rajaram Coperative Sugar Industry, Kolhapur, India. It was washed with tap water and dried in sunlight. After drying it was ground in mixer and sieved to obtain desired particle size using Micro-Mesh sieves (Industrial Netting, USA).

2.3. Microorganism and culture conditions

The microorganisms which already known for their rhizospheric plant growth promoting activity was collected. The microbial cultures of *Rhodobacter erythropholis* MTCC 4688 and *Azotobacter vinelandii* MTCC 1241 were received from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. *Rhizobium meliloti* NCIM 2757 and *B. megaterium* NCIM 2054 were received from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. Nutrient medium containing (g l^{-1}; peptone 10, beef extract 2 and sodium chloride 10) was used for maintaining the stock cultures at 4 °C.

2.4. Adsorption of textile dyestuff from textile effluent

Batch study experiments were carried out for adsorption of DR73 on SCB. In 250 ml Erlenmeyer flasks 2 g of a SCB and 50 ml solution of DR73 (300 mg l^{-1}) was added. This mixture was kept at shaking 120 rpm for time 20 min. Then it was centrifuge for 5000 rpm for 15 min to collect clear supernatant. Maximum absorbance wavelength (520 nm) of DR73 was selected for measurement of color removal using UV–vis spectrophotometer (Hitachi U-2800, Japan). The adsorption percentage was calculated as reported earlier by Kadam et al., (2011, 2013a). Further, dye adsorbed to SCB was used for the decolorization study. The effect of particle size of SCB on adsorption of DR73 (300 mg l^{-1}) was studied taking the particle size of the SCB as 2, 0.2, 0.02 and 0.002 mm.

2.5. Adsorption kinetics study

The DR73 adsorbed on milled SCB (2 g, 0.002 mm) and its adsorption kinetics was carried out using DR73 concentrations as 100, 200, 300, 400 and 500 mg l^{-1} and keeping it for 5, 10, 15, 20 and 25 min agitation time (Kadam et al., 2013a,b). Non-linear regression method was used to study pseudo-first order and pseudo second-order kinetics (Khambhaty et al., 2008; Lin and Wang, 2009; Kadam et al., 2013a,b).

2.6. Consortium development and decolorization experiment

One loop full culture of *Rhodobacter erythropholis* MTCC 4688, *Azotobacter vinelandii* MTCC 1241, *Rhizobium meliloti* NCIM 2757 and *B. megaterium* NCIM 2054 culture was inoculated into 3 ml nutrient medium separately and then it was incubated for 30 °C at static condition. In the 250 ml Erlenmeyer flasks DR73 adsorbed on the SCB (2 g) were added. These flasks were sterilized after pH adjustment (7.5–8). The flasks were inoculated with 3 ml of (0.5 OD at 530 nm) culture for decolorization study. The moisture content was maintained between 85 and 90%. All flasks were incubated for 30 °C under static condition.

In order to develop the consortia RARB of *Rhodobacter erythropholis* MTCC 4688, *Azotobacter vinelandii* MTCC 1241, *Rhizobium meliloti* NCIM 2757 and *B. megaterium* NCIM 2054, each of these bacterial culture were grown at 24 h in 3 ml of nutrient medium, and 0.75 ml from each bacterial culture were added aseptically in decolorization medium.

SCB adsorbed textile dye DR73 was desorbed using dimethyl sulphoxide (DMSO) and used for measurement of decolorization as suggested by Kadam et al. (2011, 2013a,b). Decolorization percentage was calculated by formula as described by Kadam et al. (2013a,b). All experiments were done in triplicates and abiotic controls were included for each set.

2.7. Optimization of a carbon and nitrogen sources, pH and temperature for decolorization

SCB was supplemented with 3 ml of 1% glucose, starch, beef extract, ammonium chloride (NH_4Cl), urea, peptone, rice bran, yeast biomass or lactose in order to study its effect on a decolorization of DR73. The pH was kept to 2, 4, 6, 8 and 10 to analyze its effect on DR73 decolorization by consortium-RARB. Different temperatures as 10,20, 30, 40 and 50 °C used to analyze their effect on DR73 decolorization with the pH of 6.5–7 and at static condition. Moisture content of 80, 85, 90 and 95% were evaluated for decolorization of DR73 by consortium-RARB.