Microbial decolourization of textile dyes through isolates obtained from contaminated sites

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This study presents microbial decolourization of textile dyes through 3 bacterial isolates [CPE (bacterial isolate from Pali effluent), CPS (bacterial isolate from Pali soil) and CBE (bacterial isolate from Baddi effluent)] and a fungal isolate [FBE (fungal isolate from Baddi effluent)] obtained from effluent and effluent contaminated sites in Pali (Rajasthan, India) and Baddi (Himachal Pradesh, India). Removal of Acid Sulphone Blue dye (initial conc. 100 mg/l) after 50 h was: FBE (97.67%) > CBE (82.83%) > CPS (60%) > CPE (51.8%). Isolates (CBE and FBE) that displayed good decolourization of Acid Sulphone Blue could decolourize Acid Navy Blue (75.85-100%) and Fast Red A (46.42-99.33%) well but Acid Magenta was removed with lesser efficiency (51.57-69.93%). FBE could hold a good potential for removal of toxic azo dyes from industrial effluents.

Keywords: Azo dye, Decolourization, Shaking, Stationary

Introduction

Small-scale industries in India contribute 3900 million liters wastewater per day. Presence of very low concentrations of dyes in effluents is highly visible and undesirable. Some of these dyes are potentially mutagenic, carcinogenic and toxic. Dyes are resistant towards conventional methods of wastewater treatment. Biological methods using various microbes like bacteria, fungi and algae of dye removal could be a viable option as a low-cost and eco-friendly decentralized wastewater treatment system for small-scale industries. Bacterial degradation of azo dyes is often mediated by azoreductases, which are more efficient under static and anoxic conditions. Similarly, lignolytic enzymes secreted extracellularly by fungal strains also produce higher decolourization in static conditions. Aerobic decolourization of azo dyes by bacterial as well as fungal cultures results in more complete degradation and avoids accumulation of carcinogenic intermediates.

This study presents biological decolourization of textile effluents through microbial isolates obtained from contaminated sites. Further, studies with selected strains were conducted to determine effect of stationary and shaking conditions on dye removal.

Materials and Methods

Dyes and Chemicals

Three azo dyes [Acid Navy Blue (Acid Blue 120), Fast Red A (Acid Red 88) and Acid Sulphone Blue (Acid Blue 89)] and one triarylmethane dye [Acid Magenta (Acid Violet 19)] were kindly provided by Department of Textile Technology, IIT Delhi. These dyes (Fig. 1) are anionic and find extensive use in dyeing of woolen and silk fibers. Absorbance maxima for each dye were obtained by scanning dye solution over visible range of 450-750 nm. Stock solutions (5000 mg/l) were prepared for each dye in distilled water. All other chemicals were of analytical grade and obtained from Merck and Qualigens.

Microbial Isolates

Effluent was collected from Pali (Rajasthan, India) and Baddi (Himachal Pradesh, India). Effluent contaminated soil was collected from Pali. Cluster of many small scale dyeing and printing units are located at Pali while large-scale textile units are located at Baddi. Bacterial colonies were obtained on separate Petri plates containing Nutrient Agar media (Himedia) by serially diluting respective sample and subsequent plating (100 µl) of diluted effluent from both sources and contaminated soil. Plates were then
incubated at 30°C for 24 h and colony with most distinct morphology and repeatability was picked up, purified and named as CPE (bacterial isolate from Pali effluent), CPS (bacterial isolate from Pali soil) and CBE (bacterial isolate from Baddi effluent). Similarly, a fungal isolate from Baddi effluent was obtained through serial dilution and plating on to Petri plates containing Potato Dextrose Agar (PDA) (Himedia). Plates were incubated at 30°C for 24 h. Most distinct fungal colony was picked up and designated as FBE (fungal isolate from Baddi effluent) and maintained on slants of PDA. Freshly revived cultures were used for all experiments performed.

Comparing Dye Removing Efficiency of Microbial Cultures

Experiments with bacterial isolates (CPE, CPS and CBE) were performed on Bushnell and Haas medium with following composition: NH₄NO₃, 1; CaCl₂, 0.02; FeCl₃, 0.05; MgSO₄.7H₂O, 0.2; K₂HPO₄, 1 g/l; glucose, 0.1 w/v; yeast extract, 0.05 % w/v, and pH, 7. Flasks containing 100 ml media each were autoclaved for 20 min at 120°C and 15 lb. Inoculum (10% v/v) was added aseptically to experimental flasks with dye (Acid Sulphone Blue; initial dye conc., 100 mg/l) and incubated at 30°C for 24 h. Most distinct fungal colony was picked up and designated as FBE (fungal isolate from Baddi effluent) and maintained on slants of PDA. Freshly revived cultures were used for all experiments performed.

Effect of Shaking and Stationary Conditions on Dye Removal by CBE and FBE

Two best performing isolates were selected for further work. Effect of shaking and stationary conditions was determined on the removal of Acid Magenta, Acid Navy Blue, Fast Red A and Acid Sulphone Blue. Flasks having suitable media (Bushnell and Haas media for CBE and optimized fungal media for FBE), 100 ml each, were prepared in four series for each dye (experimental shaking and stationary, control shaking and stationary) and autoclaved as described earlier. Experimental flasks were inoculated with respective inoculum aseptically as previously stated. Shaking flasks were incubated in shaker at 30°C and 180 rpm and stationary flasks were kept on a tray in

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$
same shaker. Samples were withdrawn and analyzed as described earlier at absorbance maxima for respective dyes (Acid Magenta, 527 nm; Acid Navy Blue, 561 nm; Fast Red A, 506 nm; Acid Sulphone Blue, 572 nm).

**Results and Discussion**

**Comparing Dye Removing Efficiency of Microbial Cultures**

Bacterial isolate, CPE and CPS, showed substantial removal of Acid Sulphone Blue dye during 10-40 h of incubation with equilibrium reaching after 50 h (Fig. 2). Dye removal achieved using isolates at 50 h was as follows: FBE, 97.67; CBE, 82.83; CPE, 51.8; and CPS, 60 %. No dye removal was observed in control flask, suggesting that dye removal were solely due to presence of microbes. Further, CBE and FBE produce faster and more complete dye removal as compared to CPE and CPS. Dye removal achieved after initial 20 h was: FBE (91.77 %) > CBE (78.36 %) > CPS (36.72 %) > CPE (24.02 %). These results show that CBE and FBE have potential for decolourization of dye effluents.

Isolates from Baddi Textile Mill effluent were found more efficient than isolates obtained from Pali effluent/soil in decolourizing acid dyes. Hence in order to obtain an efficient dye removal, microorganism may be better obtained from similar effluent as these are more accustomed to the type of dyes present and extreme conditions of effluent like alkalinity, high temperature, BOD, TDS etc. Kilic et al. obtained bacterial consortium (comprising *Ochrobactrum* sp., *Salmonella enterica* and *Pseudomonas aeruginosa*) from tanning and textile wastewaters and reported bioaccumulation of 80% of Reactive Black B dye (initial conc. 59.3 mg/l) in presence of Cr (VI) (initial conc., 69.8 mg/l) in 7 days. Wong & Yuen reported 100% dye removal of Methyl Red (initial conc., 100 mg/l) in 24 h by *Klebsiella pneumoniae* RS-13. Hu also reported 93.2 % dye removal of RBB (initial conc., 100 mg/l) in 48 h by *Pseudomonas luteola*.

While comparing experimental isolates with these reports, it seems CPE and CPS did not perform sufficiently well but CBE and FBE are almost at par. Performance of FBE seems to be better than *Pleurotus ostreatus* sp.4, which causes 53% decolourization in 18 days with Poly R-478 dye (initial conc., 100 mg/l). Since CBE and FBE performed better than other two isolates, further experiments on decolourization of various dyes were performed with these strains.

**Effect of Shaking and Stationary Conditions on Dye Removal by CBE**

Under shaking and stationary conditions, CBE removed Acid Sulphone Blue and Acid Navy Blue more efficiently than Acid Magenta and Fast Red A (Fig. 3). In case of Acid Magenta (Fig. 3a), decrease in dye concentration in stationary cultures was almost similar to that observed in control. However, in shaking cultures, dye concentration significantly decreased during first 10 h, after which it remained almost constant. In case of Acid Navy Blue (Fig. 3b), during initial 10 h, dye concentration sharply reduced in shaking condition but it did not change substantially in stationary condition. However, between 10-30 h, a rapid decrease in Acid Navy Blue was observed in stationary flasks. As a result, Acid Navy Blue removal at the end of experiment (50 h) was at par in shaking and stationary condition. Efficient dye removal in stationary condition has a great significance in terms of low power requirement and provision of simpler reactor designs for large-scale applications.

In case of Fast Red A (Fig. 3c), dye concentration decreased almost simultaneously with time in both stationary and shaking conditions, although as compared to other dyes, dye removal was less pronounced. In case of Acid Sulphone Blue (Fig. 3d), dye concentration was similar in stationary and shaking conditions but reduction was more pronounced in shaking condition. While comparing performance of CBE in the removal of four dyes in shaking and stationary condition after 50 h, it was seen that except Acid Magenta, remaining three dyes could be decolourized in stationary conditions as well.
Thus, in shaking conditions, dye removal for various dyes after 50 h was: Acid Sulphone Blue (82.83 %) > Acid Navy Blue (75.85 %) > Acid Magenta (51.57 %) > Fast Red A (46.42 %). Whereas trend in stationary condition was: Acid Navy Blue (68.96 %) > Acid Sulphone Blue (48.50 %) > Fast Red A (40.89 %) > Acid Magenta (9.28 %). Dye removal in stationary condition was less as compared to that in shaking condition, suggesting that dye removal by CBE is an aerobic process. This also necessitates provision of agitation of cultures during dye removal thus increasing contact between dye and biomass. This is in contrast to results by Chen et al\textsuperscript{18}, who reported that although \textit{Aeromonas hydrophila} displayed good growth in aerobic or agitated cultures, color removal (80% at initial dye conc. 50 mg/l within 1.5 days) was best in anoxic or anaerobic cultures; however, there was no significant reduction in agitated/ aerobic cultures. Shifting of agitated cultures to stationary condition produced quick decolourization. Bacterial degradation of azo dyes under anaerobic conditions is often attributed to enzymatic reactions\textsuperscript{6}. Sometimes alteration from anaerobic to aerobic conditions is also required to achieve complete degradation. Haug et al\textsuperscript{19}, reported that a bacterial consortium was capable of mineralizing Mordant Yellow dye when alteration of anaerobic to aerobic conditions were provided. Alteration was necessary because different members of consortium needed different conditions for optimum reaction and also enzyme responsible for main azo bond cleavage (reductase) is functional in anaerobic conditions. Similarly Kudlich et al\textsuperscript{20} employed both anaerobic and aerobic treatments through co-immobilized \textit{Sphingomonas} bacteria and an uncharacterized 5-Aminosalicylate degrading isolate on alginate beads to degrade Mordant Yellow 3 dye. Nevertheless, there are several recent reports on aerobic

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**Fig. 3— Effect of stationary and shaking conditions on removal of different dyes by CBE** [■ and □ indicate static control and shaking control, respectively; ▲ and △ indicate static experimental (inoculated) and shaking experimental, respectively; Initial dye conc., 100 mg/l; temp., 30°C; pH, 7]: A) Acid Magenta ($\lambda_{max}$: 527 nm); B) Acid Navy Blue ($\lambda_{max}$: 561 nm); C) Fast Red A ($\lambda_{max}$: 506 nm); D) Acid Sulphone Blue ($\lambda_{max}$: 572 nm)
degradation of azo dyes indicating that microbes possess more than one mechanism of dye degradation.

Performance of CBE for removal of Acid Navy Blue and Acid Sulphone Blue seems to be almost at par with that of recombinant *Escherichia coli* SS125 strain that produced 80% removal for Remazol Red (initial dye conc. 50 mg/l) in 72 h in shaking conditions. Asgher *et al.* reported decolourization of Drimarene Orange K-GL using mixed cultures CT (60 %) and ST, NF and CF (below 10%) in 2 days. Therefore, CBE appears to be more efficient than these cultures for removal of tested dyes.

**Effect of Shaking and Stationary Conditions on Dye Removal by FBE**

Removal of different dyes in shaking condition by FBE (Fig. 4) after 50 h was: Acid Navy Blue (100%) > Fast Red A (99.3%) > Acid Sulphone Blue (97.67%) > Acid Magenta (69.93%). In stationary condition, trend was: Acid Sulphone Blue (58.9%) > Fast Red A (54.1%) > Acid Navy Blue (41.75%) > Acid Magenta (34.18%). In shaking conditions, fungi grew in spherical pellets (diam, 2-3 mm), which were uniformly suspended during agitated cultures leading to greater contact with dye and nutrients as well. However, in stationary condition, fungi grew in the form of a mat of mycelia, which eventually covered whole interface of media and air, thus blocking exchange of air. Lower dye removal observed in stationary condition was due to limited binding of dyes at lower surface of mycelial mat, which was in contact with dye present in the media. Hence, higher dye removal in shaking condition appears to be greater due to degree of contact between fungal biomass and dye. Rigas & Dritsa compared removal of Poly R-478 dye by 16 strains of basidiomycetes fungi under shaking (50.31±6.72-93.4±5.64%) and stationary conditions (26.14±1.02-52.95±0.97%). Parshetti *et al.* reported that shaking conditions are better for faster and complete biosorption (7 h) and decolourization (20 days) of Reactive Blue-25 (100 mg/l) as compared to static conditions (biosorption, 15 h; decolourization, 25 days) by fungus *Aspergillus ochraceus* NCIM-1146. Lignolytic enzymes, produced by white-rot fungi (*Trametes versicolor* and *Phanerochaete chrysosporium*), aid in...
degradation of various dyes in static conditions and agitation of cultures leads to decreased ligninase activity\textsuperscript{23}. Stationary cultures of a highly degradative strain \textit{Irpex lacteus} exhibited 380-fold increase in manganese peroxidase (MnP) levels compared to submerged cultures. In present study, FBE requires agitation and better contact for optimal dye decolourization.

Specific dye uptake by fungal biomass for different dyes was: Fast Red A (37.0 mg/g) > Acid Navy Blue (32.7 mg/g) > Acid Sulphone Blue (27.9 mg/g) > Acid Magenta (21.5 mg/g). This suggests that fungal biomass has highest uptake capacity for Fast Red A and Acid Navy Blue, while least uptake capacity was obtained for Acid Magenta. These values are considerably high as compared to specific dye uptake (14.5 mg/g) by \textit{Trametes versicolor}\textsuperscript{24} while removing Remazol Black B (initial conc., 100 mg/l) suggesting that efficiency of dye removal by fungal isolate FBE is higher as compared to widely employed fungal strain \textit{Trametes versicolor}. Sumathi & Manju\textsuperscript{13} indicated specific dye uptake (28.7-30 mg/g) with \textit{Aspergillus foetidus} for Drimarene Red (initial conc., 100 mg/l) after 72 h of incubation. These results further establish superiority of FBE in removing dyes more quickly and efficiently.

In both cultures, CBE and FBE, higher dye removal was observed in shaking condition as compared to stationary condition (Fig. 5). Also, except for Acid Navy Blue, dye removal achieved by FBE in stationary condition was higher as compared to that by CBE, indicating that FBE is superior to CBE in removing 4 test dyes. Both CBE and FBE were not efficient enough to remove Acid Magenta from the solution but were able to remove other 3 dyes. Further, in case of CBE, biomass produced was colorless. However, in case of FBE, biomass appeared to bind dye on its surface, in turn reflecting colour of dye. Chen \textit{et al}\textsuperscript{18} found that dye biosorption or biodegradation can be judged by the colour of cell mat. If cell mats get deeply colored by the dye then mechanism of dye removal is biosorption but if, cell mat retains its original colour then phenomenon of biodegradation can be attributed for dye removal. Hence, mechanism of dye removal by CBE seems to be biodegradation whereas dye removal by FBE is principally through biosorption. To further consolidate this observation, under biosorption experiments, it was observed that almost complete dye removal from the solution containing 100 mg/l dye occurred at 3 g/l dose within 4 h at pH 6.0 and 30°C. This suggests that dye solution is decolourized primarily through biosorption on fungal pellets. Fungal cell wall is rich in chitin, in which hydroxyl and amino groups are present which makes it an efficient adsorbent for dye effluents\textsuperscript{25}. These results also imply that if condition of industrial effluent is not conducive for fungal growth, separately cultivated biomass can also be used for biosorption and complete colour removal from the effluent. This process and hence retention time of effluent for treatment will be quite faster (4 h) as compared to dye decolourization during growth phase (24-48 h). Fungal biomass is often produced as a byproduct of industrial fermentations or enzyme production processes. If fungal isolate obtained in this study can be employed for such dual purpose, whole process can become very economic and efficient.

**Conclusions**

Microbial isolates (4) obtained from effluent or effluent contaminated site were able to cause substantial removal (52-98\%) of Acid Sulphone Blue dye (initial conc., 100 mg/l). Isolates obtained from Baddi effluent (CBE and FBE) were more efficient in removing acid dyes as compared to those from Pali site. CBE and FBE performed well with 3 other dyes (Acid Magenta, Acid Navy Blue and Fast Red A). In both cases, dye was better removed in agitated cultures. By comparing performance of bacterial consortia, FBE was found more efficient in removing dyes than CBE, attributed to fungal property of producing more biomass as compared to biomass produced by bacteria. Since bacterial and fungal isolates cause dye removal through potentially different
mechanisms, it is possible that a consortium of two isolates could be more effective in complete dye removal. CBE and FBE are potential candidates for development of a decentralized process for treating textile effluent containing tested azo dyes.

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References