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ORIGINAL PAPER

Decolorization potential of mixed microbial consortia for reactive and disperse textile dyestuffs

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Abstract Four different aerobic mixed consortia collected from basins of wastewater streams coming out of dying plants of Crescent Textile (CT), Sitara Textile (ST), Chenab Fabrics (CF) and Noor Fatima Textile (NF), Faisalabad, Pakistan were applied for decolorization of Drimarene Orange K-GL, Drimarene Brilliant Red K-4BL, Foron Yellow SE4G and Foron Blue RDGLN for 10 days using the shake flask technique. CT culture showed the best decolorization potential on all dyestuffs followed by ST, NF and CF, respectively. CT could completely decolorize all dyes within 3–5 days. ST cultures showed effective decolorization potential on Foron Yellow SE4G and Drimarene Brilliant Red K-4BL but complete color removal was achieved after 4 and 7 days, respectively. NF culture showed 100% decolorization efficiencies on Foron Yellow SE4G and Foron Blue RDGLN but it took comparatively longer time periods (5–7 days). Where as, the NF culture had decolorized only 40% and 50% of Drimarene orange and red, respectively, after 10 days. CF caused complete decolorization of Foron Blue RDGLN and Drimarene Brilliant Red K-4BL after 4 and 8 days, respectively but it showed poor performance on other two dyes.

Keywords Mixed cultures · Textile dyestuffs · Decolorization potential · Industrial effluents

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Introduction

The two major sources of release of dyes into the environment are the effluents from textile processing units and dyestuff manufacturing industries (O'Neill et al. 1999). Discharge of highly colored dye containing effluents from textile and dyestuff industries to neighboring water bodies is causing significant health concerns (Chung et al. 1992; Hildenbrand et al. 1999; Chagas and Durrant 2001; Martins et al. 2002). During the past two decades, several physico-chemical decolorization techniques have been reported, few, however, have been accepted by the textile industries (Okazaki et al. 2002; da Silva and Faria 2003). Their lack of implementation has been largely due to high cost, low efficiency and generation of toxic by-products (Quezada et al. 2000; Selvam et al. 2003).

The ability of microorganisms to carry out dye decolorization has recently received much attention. Microbial decolorization of dyes is a cost-effective method for removing them from the environment (Verma and Madamwar 2003; Moosvi et al. 2005). Recent research has revealed the existence of a wide variety of microorganisms including white rot fungi, bacteria and mixed cultures capable of decolorizing a wide range of dyes (Banat et al. 1996; Asgher et al. 2006a, b). The present day bioremediation relies up on the pollutant degrading capacities of naturally occurring microbial consortia in which bacteria play central role (Liu and Suffita 1993; O'Neill et al. 2000). Microbial consortia are usually used without analyzing the constituent microbial populations for environmental remediation and complexity of the microbial consortium enables them to act on a variety of pollutants (Watanabe and Baker 2000).

The anaerobic microbial degradation of azo dyes results in the formation of aromatic amines, which are carcinogenic and mutagenic (Levine 1991; Robinson et al. 2001; Hildenbrand et al. 1999). Hence, the aerobic treatment is the only reliable and safe method for the biodegradation of textile dyes (Senan and Abraham 2004). Recently the aerobic decolorization of azo dyes has been reported by a number of investigators (Cripps et al. 1990; Wong and Yuen 1998; Adedayo et al. 2004).

In our previous studies we used a mixed aerobic culture for the biodegradation of 2,4,6-trichlorophenol (Synder et al. 2006) and white rot fungi for decolorization of reactive textile dyes (Asgher et al. 2006a). This study was aimed to investigate the textile dyes decolorization potential of mixed microbial consortia growing on the basins of effluent streams originating from dying plants of four major industrial units of Faisalabad, Pakistan as a part of efforts being made to develop indigenous technology for bioremediation of dye based textile industry effluents.

Materials and methods

Textile dyestuffs

Two reactive and two disperse textile dyestuffs used in this study were very generously provided by Clariant Pakistan (PVT) Limited, Canal Road, Faisalabad, Pakistan. The reactive dyestuffs were Drimarene Orange K-GL (C.I. RO 69) and Drimarene Brilliant Red K-4BL (C.I. RR 147) and disperse dyes included Foron Yellow SE4G (C.I. DY 211) and Foron Blue RDGLN (mixture).

Mixed microbial cultures

Mixed microbial consortia (along with some effluents) were obtained from the basins of outlet wastewater streams of dying units of Crescent Textile (CT), Sitara Textile (SF), Noor Fatima (NF) and Chenab Fabrics (CF) textile industries of Faisalabad, Pakistan. The pH values of CT, ST, NF and CF were 7.5, 8.2, 6.2 and 7.1, respectively. The mixed cultures were first activated by adding 0.5 g/l glucose and incubated in 500 ml shake flasks (120 rpm) of 100 ml working volume for 1 week at their respective pH values and 25°C. The cultures removed any effluent colors within 1 week. These activated cultures were used as inocula for shake flask experiments.

Culture media

All the mixed cultures were grown at 37°C in basal Bushnell and Haas medium (BHM) (Sigma, St. Louis, USA) having the following composition (g/l): $MgSO_4$, 0.2; K_2HPO_4, 1.0; $CaCl_2$, 0.02; $FeCl_3$, 0.05; NH_4NO_3, 1.0 supplemented with glucose (0.1% w/v) and yeast extract (0.05% w/v). The final pH of the medium was 7 at 25°C.

Decolorization procedure

Triplicate decolorization flasks were prepared each containing 50 ml of the BHM nutrient media receiving 0.5 g/l dyestuff concentrations. All media were adjusted to uniform pH 6 for all cultures. All flask cultures were sterilized (121°C) in autoclave (Sanyo MLS-3020U, Japan) for 15 min for sterilization. The flasks were inoculated each with 5 ml of the activated microbial mixed cultures and incubated for 10 days at 120 rpm in an incubator shaker (Sanyo-Gallenkemp PLC, London, UK). Temperature was controlled at 37°C for all

cultures. Triplicate samples were removed from shaker after every 24 h and centrifuged at 48,000*g* for 10 min in a refrigerated centrifuge (EYLA H-200NR, Tokyo, Japan). Carefully collected supernatants were analyzed for residual dyestuffs.

Dyestuff analysis

Absorbance measurements were done by using a UV/Visible spectrophotometer (Hitachi U2001, Tokyo, Japan). Wavelengths resulting in maximum absorbance (λ_{max}) were used for respective dyestuffs. A dilution of 1/10 was used for absorbance measurements. Drimarene Orange K-GL, Drimarene Brilliant Red K-4BL, Foron Yellow SE4G and Foron Blue RDGLN had λ_{max} values of 430, 520, 420 and 650 nm, respectively. The absorbance values for respective supernatants at each time period were corrected by subtracting the values for respective blanks (containing only medium but omitting the respective dye). The corrected absorbance values were compared with absorbencies of parent dye solutions to calculate percentage decolorization.

Results

Decolonization of drimarene K-GL

Results of time course study on Drimarene Orange K-GL decolorization by four mixed microbial cultures are presented in Fig. 1. The rate of color removal by CT was very high from the very beginning and it could completely decolorize the media in 4 days of incubation. ST cultures caused only 67% decolorization of the dye solution followed by 43% and 35% color removal by NF and CF cultures, respectively after 10 days.

Drimarene brilliant red K-4BL

Drimarene Brilliant Red K-4BL was effectively decolorized by most of the mixed cultures within the incubation period of 10 days (Fig. 2). CT cultures gave very exciting results in this case and 100% decolorization of the dye was recorded after only 3 days. Although the color removal with ST and CF cultures was not much appreciable within the first 2 days (10–36%), the decolorization proceeded gradually within the next 5 days resulting in complete decolorization in 7 and 8 days, respectively. NF proved to be a poor decolorizer; only 52% decolorization could be achieved at the expiry of 10 days trial.

Foron yellow SE4G

Time course versus decolorization curves for Foron Yellow SE4G are shown in Fig. 3**.** ST culture was found to be the best with 100% color loss observed in 4 days. CT and NF cultures caused gradually color removal and showed complete color removals after 5 and 7 days of incubation, respectively. CF culture was the poorest

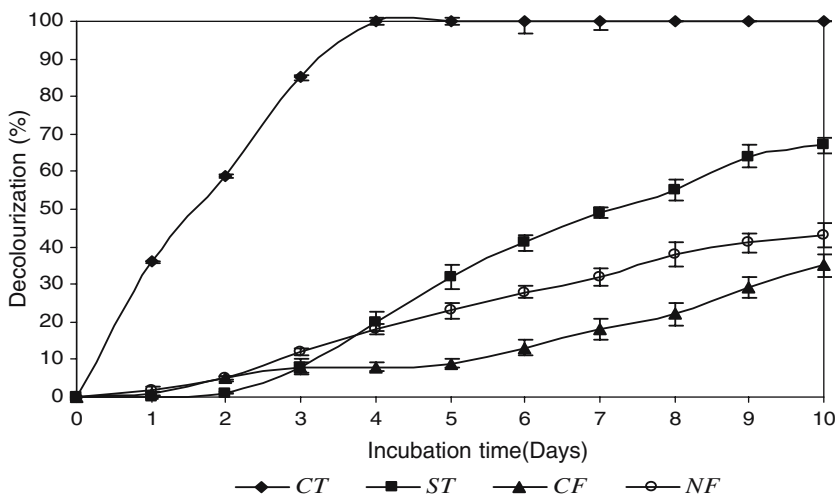

Fig. 1 Decolorization of Drimarene Orange K-GL by four mixed microbial cultures

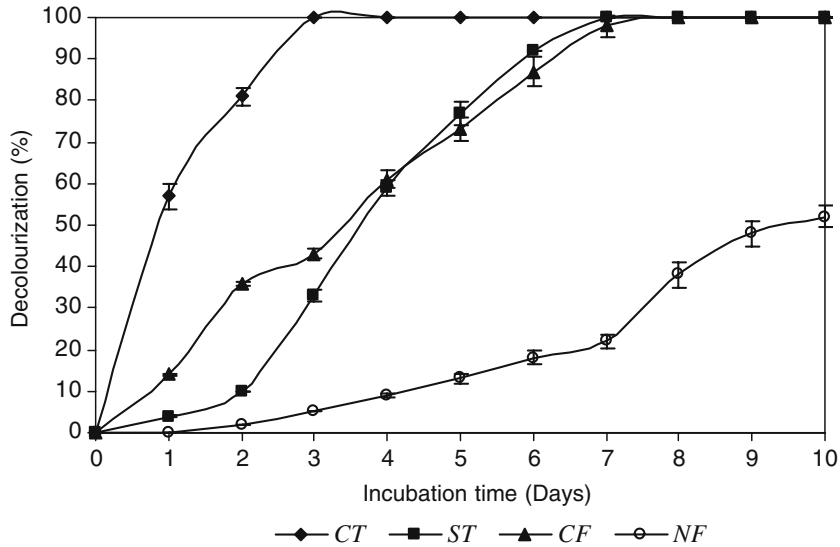

Fig. 2 Decolorization of Drimarine Brilliant Red K-4BL by mixed microbial cultures

decolorizer of Foron Yellow SE4G; it could cause only 48% decolorization in 10 days.

Foron blue RDGLN

Percentage color removals by different cultures for Foron Blue RDGLNH showed that there was a slow decolorization rate within the first 2 days for all the microbial consortia. CF was found to be the most effective culture resulting in 100% color removal after 4 days of incubation followed by CT (100% decolorization in 5 days). NF revealed a similar trend but took a longer time (8 days) for complete decolorization of the dye solution. The color removal efficiency of ST culture was very low (44%) at the end of 10 days trial (Fig. 4).

Discussion

Results of time course decolorization studies showed that CT culture was the most effective on all dyestuffs tested followed by ST, NF and CF, respectively. Complete color removals for all dyes were observed with CT within 2–5 days comparing favorably with previously reported results (Nigam et al. 1996) showing 76% textile dye decolorization in 3 days by mixed microbial consortia supplemented with cheap carbon and energy sources.

In the present study, different reactive and disperse dyes were found to be decolorized to different extents by the four mixed cultures. The duration of the lag phase (adaptation period) and

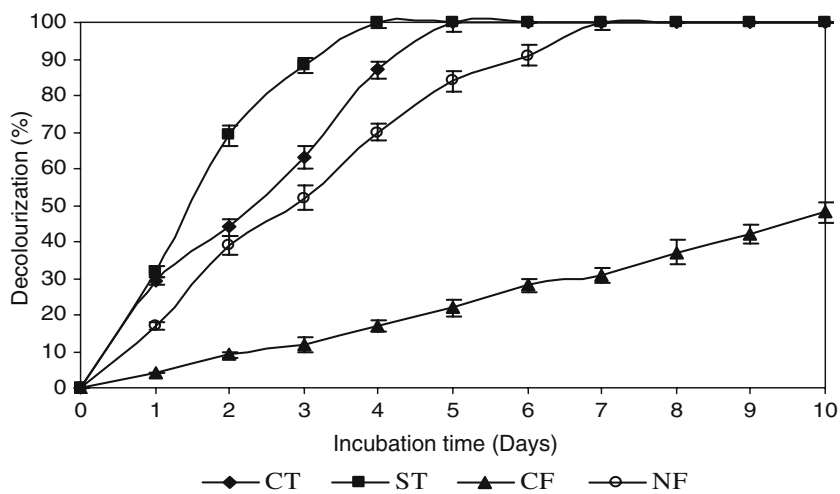

Fig. 3 Decolorization of Foron Yellow SE4G by mixed microbial cultures

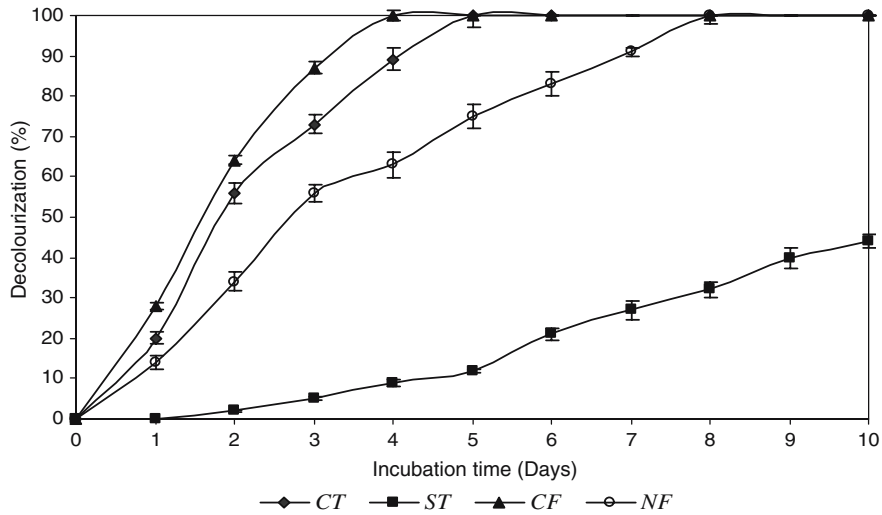

Fig. 4 Decolorization of Foron Blue RDGLN by mixed microbial cultures

the biotransformation rate in log/exponential growth phase varies significantly, depending on the origin of the mixed microbial culture and structural characteristics of the dyes (Martins et al. 2002; Senan and Abraham 2004; Moosvi et al. 2005). Decolorization of some dyes took longer for some cultures as compared to others, and in some cases it was incomplete even at the end 10 days study period suggesting the longer lag phase and delayed onset of log/exponential growth phase of components of the microbial consortia. However, decolorization times taken by our cultures to achieve 100% decolorization compares favorably with reports on dye decolorization by most of the white rot fungi which require 7–20 days period for 90% decolorization of a diverse range of synthetic dyes (Kirby et al. 2000; Boer et al. 2004; Asgher et al. 2006a) and other mixed microbial cultures **(**Senan and Abraham 2004; Adedayo et al. 2004).

Difference in microbial population in four different cultures may account for differences in their decolorization abilities. Microbial components of mixed microbial cultures are capable of decolorizing dyes via biotransformation and biodegradation (Banat et al. 1994; Chung and Stevens 1993) and efficiency of the decolorization process depends on the survival, adaptability and activities of enzymes produced by microorganisms present in the mixed cultures (Cripps et al. 1990; PastiGrigsby et al. 1992; Senan and Abraham 2004). Mixed cultures are better decolorizers than individual cultures (Moosvi et al. 2005), suggesting a synergistic role of the bacterial species of mixed cultures in dye decolorization (Knapp and Newby 1995; Senan and Abraham 2004). Normally the dye concentration in the effluent varies within a narrow range of 0.1–0.2 g/l (O'Neill et al. 1999). Our consortia could decolorize dyes above the reported dye concentrations in wastewaters, and thus could be successfully employed for treatment of textile dye-based industrial effluents.

Conclusions

Mixed microbial cultures collected from basins of wastewater treatment plants of different textile units of Faisalabad, Pakistan showed excitingly good potential in terms of time span for decolorization of the extensively marketed and used reactive and disperse dyestuffs. The results of the study will form the basis for development of a cost effective and robust indigenous process for bioremediation of textile dyes-based effluents.

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