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Bioremediation of textile azo dyes by aerobic bacterial consortium

Aerobic degradation of selected azo dyes by bacterial consortium

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Abstract

An aerobic bacterial consortium consisting of two isolated strains (*BF1*, *BF2*) and a strain of *Pseudomonas putida* (MTCC1194) was developed for the aerobic degradation of a mixture of textile azodyes and individual azodyes at alkaline pH (9–10.5) and salinity (0.9–3.68 g/l) at ambient temperature (28 ± 2 °C). The degradation efficiency of the strains in different media (mineral media and in the Simulated textile effluent (STE)) and at different dye concentrations were studied. The presence of a H_2O_2 independent oxidase – laccase (26.5 IU/ml) was found in the culture filtrate of the organism BF2. The analysis of the degraded products by TLC and HPLC, after the microbial treatment of the dyes showed the absence of amines and the presence of low molecular weight oxidative degradation products. The enzymes present in the crude supernatant was found to be reusable for the dye degradation.

Abbreviations: DB – Direct blue; PNB – Procion Navy Blue; PR –Procion Green; SR – Supranol Red; STE – Simulated Textile Effluent; VMM – Vogel's Mineral Media

Introduction

Rapid industrialization and urbanization results in the discharge of large amount of waste to the environment, which in turn creates more pollution. Majority of colored effluents consist of dyes, released to the environment from textile, dyestuff, and dyeing industries. Azo dyes represent a major group of dyes causing environmental concern because of their color, biorecalcitrance and potential toxicity to animal and human (Levine 1991). It is very difficult to treat the effluents from textile and dyeing industries by the commonly used physical and chemical methods mainly because of its high BOD, COD, heat, color pH and the presence of metals. Moreover the physical and chemical methods have disadvantages of being highly expensive, coupled with the formation of large amount of sludge and the emission of toxic substances (Johnson 1978).

The general approach of bioremediation is to improve the natural degradation capacity of the native organisms. But azo dyes are xenobiotics and its degradation in nature is rather difficult. Moreover the anaerobic degradation of azo dyes produces aromatic amines, which are carcinogenic and mutagenic (Levine 1991). Hence the aerobic treatment is the only safe method for the biodegradation of textile azo dyes. Microbial species of bacteria, actinomycetes, fungi and algae are capable of removing azo dye via biotransformation, biodegradation or liberalization (Banat et al. 1994; Chung et al. 1993; Glenn & Gold, 1983) and the effectiveness of microbial treatment depends on the survival, adaptability and activity of the selected organism (Cripps et al. 1990; PastiGrigsby

et al. 1992). The decolorization rate of the azo dyes also depends on the oxidation potential of the azo dyes.

Environmental biotechnology relies up on the pollutant degrading capacities of naturally occurring microbial consortium in which bacteria play central role (Liu & Suffita 1993). Under aerobic conditions the azo dyes are nondegradable by most of the bacteria. A few studies have been conducted to identify bacterial species that are capable of aerobically degrading azo dyes. Certain bacteria like *Bacillus sterothermophilis* are able to produce microperoxidases. A degradative pathway has been elucidated for sulfonated azo dyes using *Pseudomonas* strains (Kulla et al. 1983). *Flavibacterium* also produces peroxidase, which can degrade azo dyes aerobically (Cao 1993). Microbial consortia are used as black boxes without analyzing the constituent microbial populations for environmental remediation. The complexity of microbial consortium enables them to act on a variety of pollutants (Watanabe & Baker 2000).

The present study deals with the isolation of bacteria, and development of a bacterial consortium for the aerobic degradation of textile azo dyes at high pH and salinity conditions. The identification of the oxidative enzyme involved in the azo dye degradation, and the reuse of the consortium for degradation of textile dyes are discussed.

Materials and methods

Substrates used

Seven commercially available textile azo dyes (ATUL, India) were selected for decolorization studies. The specification of dyes are given in Table 1. ABTS (2,2′ azinobis- 3-ethyl benzthiazoline-6-sulphonate) and Sodium dithionate was from Sigma Chemicals, St. Louis, USA. All the other chemical used are standard analytical grade, procured from E-Merck, BDH and SD fine chemicals, India.

Organisms used

A microbial consortium consists of two isolated strains (*BF1* and *BF2*) and a known strain *Pseudomonas putida* (MTCC 1194, Chandigarh, India) was used for degradation studies.

Isolation and screening

Isolation of bacterial species was carried out from soil contaminated with dye and from textile industry effluent and lignocellulosic wastes. The composition of media used for isolation was as follows, glucose 3%, yeast extract 0.6%, KH_2PO_4 0.6%, $MgSO_4$ $7H_2O$ 0.02%, Na_2 CO_3 1.0%, agar 2.0%, dye concentration 20–100 μg/ml and at neutral pH. For the screening of the microorganism the same media having higher concentration of dye (200 μg/ml) and a higher pH of 9.5 was used.

Development of a consortium of bacteria

Individual strains of bacteria were inoculated to a dye mixture having a concentration of 50-ppm (50 μg/ml) in Vogel's Mineral Medium (VMM), at a pH of 9.0. It was subjected to shaking at 120 rpm at ambient temperature (28 $\pm$ 2 °C). The degradation of the dyes was monitored at 24-hour intervals. The rate of decolorization was measured

Table 1. Substrates used

Dye	C.I.name	Type	Chemical nature	Structure
Sirius yellow	Direct Yellow 86	Direct dye	diazo	Known
Chrysoidine	Basic orange 2 C.I.11270	Basic dye	Monoazo Cationic	Known
Procion green H-E4BD	Reactive green 19	Reactive dye	Azo dye	UnKnown
Direct blue	Direct Blue 54 C.I.27960	Direct dye	Azodye Anionic	Known
Procion Navy Blue HER	Reactive Blue 171	Reactive dye	Azo-monochlorotriazinyl	Unknown
Procion Brilliant red H-E7B.	Reactive Red 141	Reactive dye	Disazo	Unknown
Supranol red	Acid Red 260	Acid dye	Diazo	Unknown

spectrophotometrically. Organisms that are capable of degrading more than 50% of the dyes were selected and mixed in different combinations and their ability to degrade a mixture of seven azodyes in alkaline condition with a salinity of 0.9%, which is usually encountered in the textile effluents was studied. The bacterial combination, that showed maximum dye degradation, was selected as the potent consortium for further studies.

Biodegradation

The biodegradation of individual dyes (25–100 μg/ml) and azo dye mixture (50 μg/ml) was studied using 1% of the inoculum in VMM and in simulated textile effluent (STE) (Basibuyuk & Forster 1997).

Analytical determinations

Assay of decolorization

The suspension was centrifuged at 10,000 rpm for 15 min and the absorption spectrum of the clear supernatant from 180 to 700 nm was recorded using a spectrophotometer (UV 2100, Shimadzu, Japan). Supernatant incubated without the inoculum was taken as control for the dyes and the rate of decolorization/degradation was calculated.

Laccase assay

Laccase assay was carried out by oxidation of 2,2′ azinobis-(3-ethyl benzthiazoline-6-sulphonate) (ABTS). The reaction mixture contained 500 μM ABTS buffered with 50 mM sodium phosphate buffer of pH 4.5 and 1 ml of culture filtrate. Oxidation of ABTS was followed by an increase in absorbance at 420 nm (Nikku-Paavola et al. 1990). Presences of H_2O_2 dependant enzyme were also checked using the same substrate.

Analysis of degraded products

Supernatant of the degraded dye components were extracted with dichloro methane and concentrated in a rotavapour (Büchi, Germany). The concentrated products were seperated by using TLC (Silica gel G, solvent-benzene:glacial acetic acid ::70:30 V/V, Rf values were compared) and HPLC (Shimadzu, reverse phase C-18 column, 4.6 mm diameter and 25 cm length), 25 °C, Eluent-acetonitrile:water (80:20V/V) flow rate 1 ml/min. The products were monitored by their absorbance at 254 nm with a UV detector.

Results

The isolation and the screening gave four fungi and nine bacterial cultures. Degradation of dyes with fungal cultures were carried out separately. The nine bacterial cultures were used for the consortium development along with the known strains of *Pseudomonas* and *Bacillus*. The presence of H_2O_2 dependent and independent peroxidase in the culture media was also monitored.

Development of a consortium of bacteria to degrade azo dyes

Selected organisms were inoculated individually to the dye mixture (seven textile azo dyes 50 μg/ml) in VMM at a pH of 9.0 and shaken at 120 rpm at (28 ± 2 °C) and the rate of decolorization was monitored. From these experiments a total of four organisms were selected which are capable of degrading 50% of the azo dye mixture at a pH of 9.0. These organisms were then mixed in different combinations and their degradation ability of the azo dye mixture was studied. The strain *EAP3* could not survive in the consortium even though it was a good organism and degraded 82.6% of the dyes in 3 days. The selected consortia consist of three organisms, two isolates *BF1*, *BF2* (both are Gram negative cocci.) and a known organism *Pseudomonas putida (*MTCC 1194) (Gram negative rod), and was found to degrade the mixture of dyes by co-metabolism.

The degradation of individual dyes using consortium in VMM was carried out. The degradation profile of Procion Brilliant red, Sirius Yellow, Supranol Red and Chrysoidine are represented in Figure 1. Chrysoidine and Procion Brilliant Red have shown more than 80% of degradation by the 7th day of the inoculation of the consortium and have got completely decolorized thereafter. The degradation pattern of Direct Blue, Procion Green and Procion Navy Blue is given Figure 2. All the three dyes were degraded better by the consortia. The degradation of dye mixture at different concentrations in VMM is given in Figure 3. The dye concenftration dropped to 3.42 μg/ml from 25 μg/ml, and that of 50 μg/ml was reduced to 15.68 μg/ml and that of 100 μg/ml was only reduced to 65.22 μg/ml. The result for degradation of dye was given in Figure 4. In the case high pH

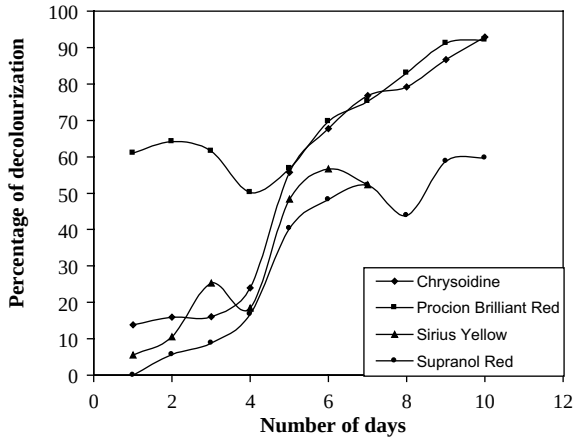

Figure 1. Decolorization of individual dyes in VMM. The decolorization of individual dyes Chrysoidine, Sirius Yellow, Supranol Red and Procion Brilliant Red at an initial dye concentration of 50 μg/ml in Vogel's mineral media inoculated with bacterial consortium, grown for 7 days and was assayed at every 24 h interval.

and salinity, the degradation was less as expected. The degradation of dye mixture at pH 9.0 and salinity of 900 mg/l was 60%, while at pH 10.5 and salinity of 3.68 g/l was only 46.08%.

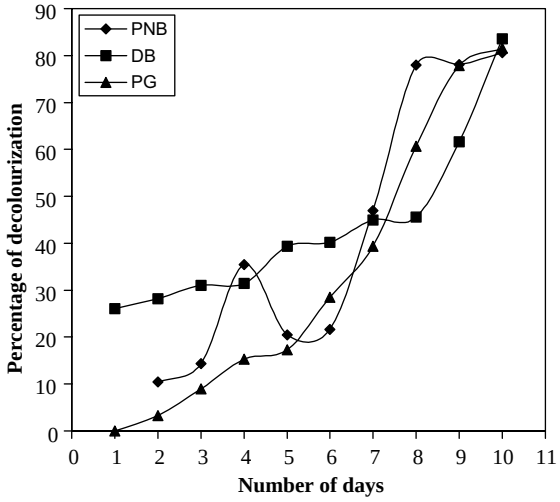

Figure 2. Decolorization of individual dyes in VMM. The decolorization of individual dyes Procion Navy Blue, Procion Green and Direct Blue at an initial dye concentration of 50 μg/ml was in Vogel's mineral media inoculated with bacterial consortium, grown for 7 days and assayed at every 24 h interval.

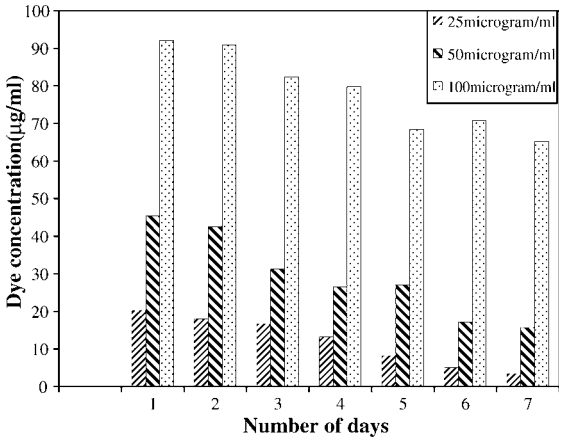

Figure 3. Decolorization of mixture of azodye by the bacterial consortium in VMM. Decolorization of mixture of azo dye by the bacterial consortium in VMM at three different initial azo dye concentrations [25, 50, 100 μg/ml].

The role of enzymes in the azodye degradation

It was found that the cell free supernatant was degrading the azo dyes faster than the bacterial biomass and also, the supernatant could be reused many times to degrade fresh textile effluent. The

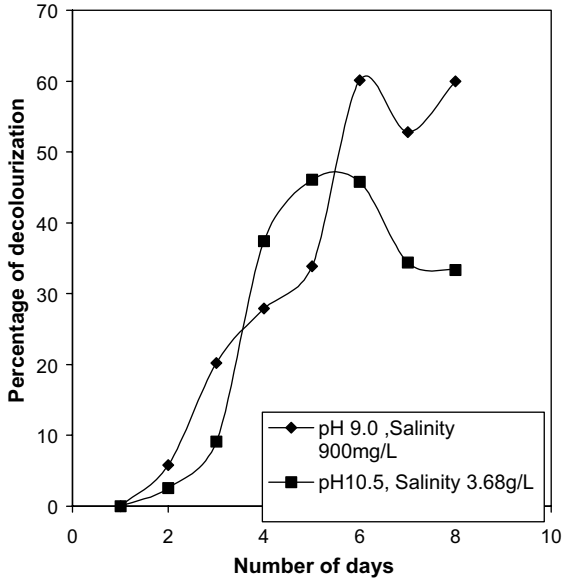

Figure 4. Decolorization of mixture of azodye by bacterial consortium in STE. Decolorization of mixture of azo dye in STE by the bacterial consortium at two different conditions [pH 9.0 and salinity 900 mg/l, pH 10.5 and salinity 3.68 g/l]. The initial concentration of dye mixture was 50 μg/ml.

analysis of culture supernatant for oxidative enzymes revealed the presence of H_2O_2 independent oxidase mainly laccase. Laccase was produced by the bacterial strain *BF2* (26.5 IU/ml). The role of laccases in dye degradation has been already reported (Chivukala & Renganathan 1995).

The products obtained after complete decolourization of dye mixture was analyzed for the presence of amines. The dyes were chemically reduced to corresponding amines with Sodium dithionate and compared with the biodegraded end products of the dyes by TLC. The TLC pattern showed the absence of amines and presence of four to five low molecular weight end products in the effluent. The HPLC analysis also did not show any peak for amines. There were three to four peaks by the oxidative degradation products like 4-hydroxy benzene sulphonic acid, benzene sulphonic acid, quinones and aryl nitrates.

Discussions

Bioremediation is cost effective and environmental friendly, as it is an enhanced way of biodegradation. Under aerobic conditions the azo dyes are non-degradable by most of the bacteria and the isolation of bacteria, which use dye as a sole source of carbon, is proved to be difficult (Zimmermann et al. 1982). In the present study glucose was provided as an extra carbon source to isolate the aerobic bacteria prior to the development of the consortium . It is a mixture of organisms; and can act on a variety of pollutants (Watanabe & Baker 2000) and this was exactly happening in the case of *in situ* biodegradation. The consortium developed by us was found to produce the H_2O_2 independent enzyme laccases as major oxidative enzyme involved in the azo dye degradation. Laccases are already identified as the enzymes involved in the degradation of azo dyes (Chivakula & Ranganathan 1996). Laccase is not stable at high pH and hence the dye degradation at high pH was substantially lower. During the process of degradation of the dyes by the bacterial consortium it was found that there was simultaneous adsorption and desorption of the dyes onto the microbial biomass and onto the starch in case of STE. This is seen from the random increase and decrease in the percentage of dye during the effluent treatment.

Conclusions

An aerobic bacterial consortium was developed for the decolorization of textile azo dyes. The decolorization efficiency was evaluated in mineral media as well as with simulated textile effluent having mixture of azodyes.

This bacterial consortium was able to decolorize the textile azo dyes at alkaline pH of 9–10.5 and salinity of 0.9–3.68 g/l in mineral media and simulated textile effluent. Laccase was found in the culture supernatant. The analysis of degradation products showed that dyes are converted to low molecular weight compounds and amines were absent.

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