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Degradation of textile dyes using microbial isolates

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ABSTRACT

Microbial isolates including bacteria and fungi were chosen for degradation of textile dyes in soil samples collected from various spots. Based on the degradation of the diazo dye Congo Red, the isolates were chosen for degradation of four other commercial dyes. They were identified as *Aspergillus* spp. and *Penicillium* sp. The percentage of decolorization of the dyes by fungal cultures in Nitrogen limited media under still and shake conditions were studied. *Aspergillus* sp1was able to degrade all the four dyes, while only three dyes were degraded by *Aspergillus* sp2, *Penicillium* sp. The present study revealed that utilization of fungi for degradation is an easy and effective process.

KEYWORDS

Degradation;
Textile effluent;
Microbial isolates.

INTRODUCTION

Synthetic dyes are extensively used for textile dyeing, paper printing, and color photography and as additives in petroleum products. Approximately 10000 different dyes and pigments are used industrially and over 7×10^5 tons of these dyes are produced annually worldwide. Dyes are released into the environment from two major sources, the textile and dyestuff industries. It is estimated that 10-15 percent of the dyes are lost / released as effluent during the dyeing process.

The major classes of synthetic dyes used for textile dyeing and other industrial applications include azo, anthraquinone and trizryl methane dyes[1,2]. Azo dyes are typical recalcitrant pollutants of 'carcinogenicity, mutagenicity, teratogenicity'[3].

Incomplete treatment of azo dyes generates amines that can be transformed into highly reactive electrophiles which can for instance form covalent adducts with DNA thus posing a significant health risk[4]. Detoxification of herbicides by Glutathion S- transferase (GST) has been demonstrated to be a major factor in herbicide tolerance[5].

A necessary criterion is that for the use of the dyes, is that they must be highly stable during washing. They must also be resistant to microbial attack. Therefore they aren't readily degradable and shouldn't typically be removed from water by conventional methods or wastewater treatment systems. Most dyes aren't specifically toxic, yet they're considered to be potential pollutant.

Mechanism of color removal

Biodegradation process may be anaerobic, aerobic or involve a combination of the two. When considering the reaction between bacterial cells and azo dyes, it must be noted that there're significant differences between the physiology of microorganisms grown under aerobic and anaerobic conditions. In contrast, bacterial reduction under anaerobic conditions is relatively unspecific with regard to the azo compounds involved and is therefore, of more use for the removal of color in azo dye wastewater. All azo dyes contain at least one, but more usually two aromatic residues attached to the azo group. They exist in the more stable Trans rather than the cis form. Both the N_2 atoms are Sp^2 hybridized so that the Carbon-Nitrogen angles are 120°[6].

Color removal using microbial biocatalysts to reduce the dyes that are present in effluents, offers potential advantages over physico-chemical processes. Such systems are the focus of recent research. In particular, the ability of whole bacterial cells to metabolize azo dyes has been extensively investigated. Under aerobic conditions, azo dyes are not readily metabolized[7]. In an experiment, it was found that mutant strain of *E coli* was able to decolorize azo dye C I Reactive red 22[8]. Biodegradation of monoazo- sulfonic dye Acid Orange 7 by *Pseudomonas* sp OX 1 was achieved both in gas sparged flasks and internal airlift reactors wherein, cell growth took place in aerobic phase and dye conversion in anaerobic phase according to a Michaelis-Menten type kinetics[9].

However, under anaerobic conditions, many bacteria reduce the highly electrophilic azo bond in the dye molecule, reportedly by the activity of low-specificity cytoplasmic azoreductases, to produce colorless aromatic amines. These amines are resistant to further anaerobic mineralization and can be toxic or mutagenic to animals. Fortunately, once the xenobiotic azo component of the dye molecule has been removed, the resultant amino compounds are good substrates for aerobic biodegradation. Another experiment suggests that if a sequential anaerobic- aerobic system is employed for wastewater treatment, the amines can be mineralized under aerobic conditions by a hydroxylation pathway involving a ring opening mechanism[10]. In an experiment, aerobic degradation of *Galactomyces geotrichum* MTCC 1360 was much faster (10 min) than those reported for *Citrobacter* sp., *Enterobacter agglomerans*[11]. *Rhodopseudomonas palustris* could decolorize azo dye Acid red B and *Rhodobacter sphaeroides* was able to decolorize Methyl orange[12,13]. It was reported the effect of *Trametes hirsuta* laccase extract system on the chemical composition and strength properties of the fibrous fraction of an unbleached recycled softwood craft pulp[14]. However not much is known about recycling of unbleached pulp and its modification by treatment sequences involving enzyme preparations such as one with laccases from *G subrgentea*.[15].

The simplest mechanism of color removal by whole bacterial cells is that of the adsorption of the dye on to the biomass. In an experiment, it was suggested that sorption is one of the most promising strategies either using activated carbons or other non- conventional adsorbents like clays or some industrial and agricultural wastes[16]. Bioassociation between the dye and the bacterial cells tends to be the first step in the biological reduction of azo dyes, which is a destructive treatment technology. Biodegradation processes may be anaerobic, aerobic or involve a combination of the two.

Two possible bottlenecks in anaerobic bioreduction[17] process of azo dyes are:

1 High salinity causes plasmolysis and / or loss of cell activity and low BOD removal performance of some traditional aerobic and anaerobic-biological treatments.
2 Anaerobic bioreduction is more time-consuming

Adaptation of a microbial community towards toxic or recalcitrant compounds is very useful to improve the rate of decolorization process[18]. Acclimatized bacterial consortium was capable of decolorization, biotransformation and detoxification of the toxic benzidine-based dyes.

When considering the reaction between bacterial cells and azo dyes, it must be noted that there are significant differences between the physiology of microorganisms grown under aerobic and anaerobic conditions. In contrast, bacterial reduction under anaerobic conditions is relatively unspecific with regard to the azo compounds involved and is therefore, of more use for the removal of color in azo dye wastewater.

In a survey in Argentina (in a subtropical area), for

isolating lignin degraders, a highly active fungus, *Grammothele subargentea* (speg) Rajeh strain LPSC 436[20] showed highest laccase activity among several fungi[21]. Laccase is currently investigated for litter mineralization, dye detoxification and decolorization[22].

Lignin degrading ability of *G subargentea* LPSC 436 strain was studied[23]. It can decolorize different chromophore types as well as it can improve the strength properties of a recycled high-kappa-number pulp after treating it with the organism's laccase extract / HBT system, suggesting that it can be used in pulp industry and bioremediation.

In an experiment, original seed sludge was taken from a municipal wastewater treatment plant to decolorize C.I. Acid Red 42, C.I. Acid Red 73, C.I. Direct Red 80 and C.I. Disperse Blue 56[24]. In an experiment, bacterial isolate *Pseudomonas aeroginosa* NBAR 12 was capable of decolorizing 12 different dyes with varying decolorization efficiency. Among diazo reactive 172 was found to be decolorized when glucose and yeast extract was supplied in the medium[25]. Wide range of azo dyes is decolorized under anaerobic conditions while they're mostly persistant under aerobic conditions. Previous studies show that bacterial azo dye biodegradation proceeds in 2 stages:

1 Azo dyes are cleaved under anaerobic conditions, yielding colorless and hazardous aromatic amines that require aerobic conditions for their biodegradation.
2 Aromatic amines are mineralized under aerobic conditions. Therefore combination of both is the best option for effective decolorization. Sequencing batch reactors (SBR) have been used in recent studies to achieve desired dye removal[26-29].

Mixed microbial cultures decolorized textile dye under anaerobic conditions[30]. Contribution of aerobic phase on color removal is almost negligible. By decreasing anaerobic cycle time, the system performance is increased.

In an elaborated experiment, it was observed that the nitrification rate in the activated sludge system exposed to magnetic field was higher compared to the system without the magnetic field influence and the rate of oxygen uptake by the nitrifiers of the second phase of nitrification was increased almost twice[31]. In an experiment, anthraquinone immobilization beads were used to decolorize azo dyes where the results of repeated-batch operations showed that anthraquinone immobilization beads could accelerate bio- decolorization of the azo dyes and incorporation of an immobilized redox mediator and the salt-tolerant bacteria in the biotreatment system was a great improvement of the redox mediator application and the biotreatment concept. Also the beads were reusable[32]. It was reported that enzymes like ligninases, laccases, lignin peroxidases and manganese peroxidases, which function together with H_2O_2 producing oxidases and secondary metabolites can be used to decolorize wide range of synthetic dyes[33-35]. *Aspergillus* sp mixed with 200 mg/l of Reactive blue (200 mg/l) in minimal broth for decolorization whose HPLC analysis showed that a major peak appeared at a retention time of 3.69 min, which represented the retention time of pure Reactive blue, the dye selected conforming the degradation of the dye[36]. White rot fungus *Pleurotus ostreatus* was employed for degradation of disperse azo dyes, disperse orange 3, disperse yellow 3. Decolorization products were determined using UV spectrophotometer and High Performance Liquid Chromatography. This study showed that both were removed more than 50 % in 5 days[37].

In another study, a high concentration of commercial dyes (mixture) could be decolorized using a fungi *Funalia trogii* with the interactive enzymes extracellular laccase and manganese peroxidase. Also suggests that it'd be a great advance in the treatment of dye containing waste water[38].

One more positive study with the fungi Dichomitus squalens suggested that if the organism immobilized on polyurethane foam or pinewood in a fixed bed reactor improved laccase production and was able to decolorize an anthraquinone dye Remazol Brilliant Blue R and an azo dye Reactive Orange 16[39]. Also different dye decolorization capacities of Lc1 and Lc2 (2 different chromatographical forms of laccases isolated from pinewood cultures) were observed.

Experiments with *Shewanella decolorationis* S 12 reported that azo dye Acid Red G R was completely degraded by the organism under microaerophilic conditions (mostly in anaerobic)[40,41]. Also formation of H2S indicated that strain S 12 could replace polar sulfonate groups of Fast Acid Red G R to avoid the product of naphthylamine sulfonic acid, a xenobiotic compound.

In the light of the above facts, the study was proceeded to find the fungal species that certainly and virtually degrades dyes in aerobic environments from the soil. As the results were significant, the present investigation was carried out. Objective of the present study is isolation of fungi from soil and screening of fungi for dye degrading ability, thereby suggesting the procedure for pilot level process.

Study area

In South India, the worst affected part in connection with pollution due to dye effluents are Tiruppur, Erode, Karur and Coimbatore. Tirupur, referred as 'textile valley of south India' has nearly 720 dyeing units, is ranked topmost in terms of generating hazardous waste. It discharges nearly 100 million litres of effluent water containing bleaching powder, sulphonic dyes, chemicals and other inorganic catalysts. These solid, semi solid and liquid wastes are dumped into the Noyyal River or in the open wasteland. The entire Tirupur depends on Bhavani River, for both drinking and discharging which is just over 100 kilometres from the textile city. The ground water is being contaminated by the hazardous waste. The discharge of untreated effluent has damaged over 80,000 acres of farmland along the Noyyal River. It has also brought a decline in the yield of crops like turmeric and bananas.

Collection of soil sample was done from the areas of effluent discharge of various textile dyeing units situated in Tiruppur and Kondalampatty bye pass road area in Salem city. To isolate dye degrading fungi from soil, samples were collected at various areas (sampling points) from the effluent discharge point. The soil along the banks of the effluent flow was chosen because it is always damp and continuously bathed in the dye effluent.

MATERIALS AND METHODS

Sample collection

After selecting the sampling point the surface soil on the selected area was dug to about an inch depth & the sub - surface soil was collected in sterile polythene bags using a clean metal rod. Sampling was done at various points in different dyeing units. After sampling, the polythene bags were sealed tightly, brought to lab and stored in refrigerated condition.

Media used: Potato Dextrose Agar, Czapek - Dox Broth, Nitrogen Limited CDB.

Analytical grade chemicals and Borosil type glassware were used for all the experiments.

The following dyes used in the experiments were obtained from the Department of Chemistry, SITRA, Coimbatore-14.

1 Cold Brand Procion Turquoise-MGN
2 Hot brand Procion Turquoise-HEG
3 Hot Brand Procion Brilliant Red-HE7B
4 Cold brand Procion Magenta-MB

All the media and glassware used were sterilized at 121°C for 15 minutes at 15 psi in an autoclave. The soil samples collected were serially diluted aseptically and were plated onto sterile PDA plates.

The inoculated plates were incubated at room temperature for one week. NL and CDB cultures of fungi were incubated in the laboratory conditions (still culture) unless otherwise mentioned, till the desired period, where necessary aeration was provided by placing the Erlenmeyer flasks in an Emenvee shaker with a speed of 180-220 RPM (Shake culture). Three fungal isolates obtained, were then subcultured onto sterile PDA agar slants. After considerable growth the slants were stored in refrigerated condition, serving as stock cultures for further subculturing and experimental work.

Inoculation

From the margin of an actively growing colony, a plug of mycelium was transferred to the experimental media. To ensure equal inoculum a sterile cork borer (5mm) was used to cut the colony. Modified Cripps et al (1990) method was followed for identification of screening of fungi for dye degrading ability.

Congo red dye (dye of choice) was added to Nitrogen limited CDB medium to give a final concentration of 50 ppm (0.25 g in 25 ml-stock) 50 ml each of the prepared medium was dispensed in 250 ml Erlenmeyer flasks. After sterilization fungal isolates were inoculated and allowed to grow in still and shake conditions for 5 to10 days.

On the 5^{th} and 10^{th} days of incubation, dye disappearance was determined spectrophotometrically (using Bausch & Lomb spectronic 20 Spectrophotometer) by monitoring the absorbance at 505 nm. The uninoculated medium served as control. CDB without

TABLE 1 : Congo red degradation by fungal cultures in NL medium (Shake condition)

S. No.	Cultures	Percentage of dye decolourization by fungal cultures		
		5 Days	10Days	12 Days
1	1	80.83	80.83	84.17
2	2	57.5	58.3	60.83
3	3	87.5	95.83	95.83
4	4	85	88.3	88.3
5	5	56.67	56.67	65
6	6	75	75	80
7	7	29.16	80	80.83
8	8	60.83	82.5	85.83
9	9	89.16	82.16	89.16

dye served as blank. To measure optical density an aliquot of culture was withdrawn aseptically and was centrifuged at 5000 RPM for 10 minutes. The clear supernatant was used.

Based on the percentage of degradation of congo red dye, fungal isolates were chosen and used for further studies. The selected fungal isolates were identified by comparing with authentic cultures.

The dyes used in the experiments were obtained as solutions of 5 percent concentration. Each dye was added to the NL and CDB to give an initial Optical Density value of I *i* 0.2. The absorption maxima for each dye were determined. Uninoculated dye containing medium served as control. The fungal cultures were inoculated and incubated as mentioned in the above method. Dye disappearance was determined on the 5th and 10th day spectrophotometrically.

RESULTS AND DISCUSSION

Degradation of congo red dye and selection of fungal isolate

The cultures incubated were monitored for dye degradation by measuring the absorbance of the cultures. The percentage of dye decolourization on 5, 10 and 12 days of incubation was calculated. Of the twenty five isolates tested, nine were found to degrade the congo red. The results of dye degradation by the nine isolates are given in TABLE 1. Based on the percentage of dye decolourization three best degraders were selected for further studies.

Based on microscopical morphology, cultural characters and comparison with authentic cultures, the fungal isolates that degraded the congo red dye upto 95 % were identified as *Aspergillus* sp1, *Aspergillus* sp2 and *Penicillium* sp. These cultures were selected for degradation of 4 other dyes.

Degradation of the dye cold brand procion turquoise blue - mgn

The dye was added to NL CDB medium so as to give an initial OD value of 1±0.2. The fungal cultures were inoculated and incubated both under still and shake conditions. The absorption maxima of the dyes were determined. The dye disappearance was observed and determined on 5 and 10 days of incubation. The same procedure was followed for all the other dyes.

Figure 1 shows the results of degradation of Cold Brand Procion Turquoise Blue – MGN by fungal cultures.

From TABLE 2 it is clear that the dye was extensively biodegraded by the fungal cultures as evidenced by decrease in absorbance of culture media. *Aspergillus* spl completely decolourised the dye after 10 days of incubation both in still and shake cultures. Over 90 percent of the initial color from the dye had disappeared from the culture medium of the Nitrogen limited cultures of *Aspergillus* sp-2, and *Penicillium* sp. after 3 days of incubation. No decrease in absorbance was observed in sterile control cultures.

Hot brand procion turquoise - heg dye degradation by fungal cultures

The shake culture proved more effective for the *Aspergillus* spp and *Penicilluim* sp while the *Aspergillus* sp.2 could not degrade the dye even under shake conditions. 99.09 % decolourization by *Aspergillus* sp2 was achieved after 10 days of incubation. Although no decolourization was observed after 5 days of incubation for *Penicillium* sp. over 98 % of the dye was decolourized by the end of 10 days. The results are presented in TABLE 3.

Hot brand procion brilliant red - he7b dye degradation by fungal isolates

This Brilliant Red Procion dye is also one among commercially important textile dyes, contributing to the coloration of discharged effluents. Studies on their degradation is an important aspect of pollution control.

TABLE 2 : Cold brand procion turquoise blue-MGN dye degradation by *Aspergillus* Spp. and *Penicillium* in NL medium

S. No.	Cultures	Incubation			
		Still culture		Shake culture	
		Percentage of decolourisation		Percentage of decolourisation	
		5 Days	10 Days	5 Days	10 Days
1	*Aspergillus* sp 1.	47.5	100	100	100
2	*Aspergillus* sp2.	97.65	97.65	98. 82	98.82
3	*Penicillium* sp 1	98.8	98.8	94.11	98.82

TABLE 3 : Hot brand procion turquoise-HEG dye degradation by cultures of fungi in NL medium

S. No.	Culture	Incubation			
		Still culture		Shake culture	
		Percentage of decolourisation		Percentage of decolourisation	
		5 Days	10 Days	5 Days	10 Days
1	*Aspergillus* sp 1	0	0	0	0
2	*Aspergillus* sp 2	0	0	49.09	99.09
3	*Penicillium* sp	0	0	0	98.18

TABLE 4 : Hot brand procion brilliant red-HE 7 B dye degradation by cultures of fungi in NL medium

S. No.	Cultures	Incubation			
		Still culture		Shake culture	
		Percentage of decolourisa tion		Percentage of decolourisa tion	
		5 Days	10 Days	5 Days	10 Days
1	*Aspergillus sp* 1	0	0	0	33.33
2	*Aspergillus sp* 2.	0	0	0	0
3	*Penicillium* sp	0	0	0	0

TABLE 5 : Cold brand procion magenta-M8 dye degradation by cultures of fungi in NL medium

S. No.	Cultures	Incubation			
		Still culture		Shake culture	
		Percentage of decolourisation		Percentage of decolourisation	
		5 Days	10 Days	5 Days	10 Days
1	*Aspergillus* sp 1	0	0	0	54.35
2	*Aspergillus* sp 2	67.4	67.4	23.9	86.95
3	*Penicillium*	0	45.66	10.86	71.73

The fungal isolates were incapable of degrading this dye both in still and shake cultures, except for the *Aspergillus* sp.1 which was able to decolorize only 33.33 percent under shake culture after 10 days of incubation. The results are presented in TABLE 4.

Cold brand procion magenta - m8 decolourisation by fungal cultures

The procedure taken to observe degradation of other 3 dyes was followed. The Absorption maxima of the dye were determined. The disappearance of the dye was determined on 5 and 10 days of incubation.

Figure 2 shows the degradation of Cold Brand Procion Magenta-M8 by fungal cultures.

The dye has been considerably degraded by the fungal isolates. In still cultures of *Aspergillus* sp1, no decolourisation was observed. The *Aspergillus* sp.2 and *Penicillium* sp. decolourized 67.4 percent and 45.66 percent respectively after 10 days of incubation, although *Penicillium,* could not degrade the dye initially. In shake conditions all fungal cultures exhibited decolourisation greater than 50 percent, of which 86.95 percent, 71.73 percent and 54.35 percent were decolourized by *Aspergillus* sp.l, *Aspergillus* sp.2 and *Penicillium* sp. respectively. The results are presented in TABLE 5.

DISCUSSION

Pollution of surface and ground water bodies due to indiscriminate discharge of industrial effluents is a topic of great public and government concern. Forced by legislation, the units are now looking forward for cost effective solutions to arrive at the required reduction of pollution load to meet regulatory requirements. Coloured textile dye effluents cause environmental problems not only by affecting aesthetic value of water, but also due to their hazardous effects, since most of the azo dyes are reported to be carcinogenic. Hence, the dye should be removed from the waste water. Azodyes, though restricted, its use is obvious. Conventional waste water treatment systems are not efficient in removal of dyes, since the dyestuffs are completely dissolved in the waste water. Whenever treated, the Total Dissolved Solids (TDS) of the treated water is challenging where they finally settle.

Congo red was used by many workers for their biodegradation studies by micro organisms. The ability of fungi to decolourise Congo red in nitrogen limited medium under both still and shake cultures was used for screening. Three isolates were found to degrade the dye at a faster rate than the rest of the fungi. So they were selected for further studies (TABLE 1).

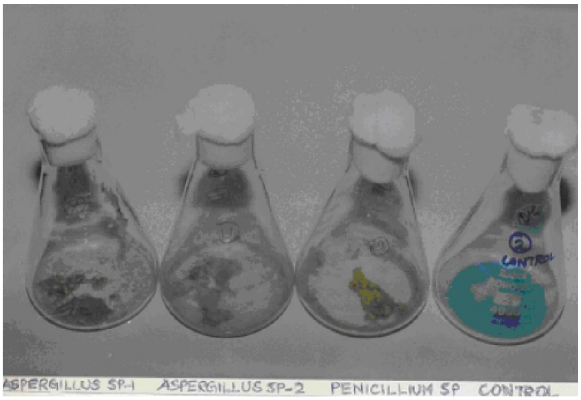

Figure 1 : Degradation of cold brand procion turquoise blue-MGN

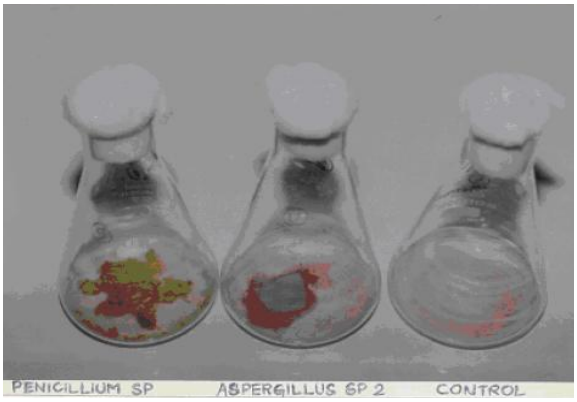

Figure 2 : Degradation of cold brand procion magenta-M8 by fungal cultures

The three fungal isolates were identified as *Aspergillus* sp.l and 2 and *Penicillium* sp. in the Department of Botany, Bharathiar Univerisity, Coimbatore. The identification was based on the microscopical and morphological characters. Comparision was also made with authentic cultures. In this study, azo dye degradation by *Aspergillus* and *Penicillium* was first reported.

Advanced Oxidation Process is a recent progress in feasibility of dye degradation that involves chemical production of highly reactive hydroxyl radicals which then react with dyes to break them into small and simpler products[42-44].

Several reports have been published on the decolorization of industrial dyes by laccase and laccase producing fungi, in the course of which also different groups of dyes were examined as well as the environmental conditions of their bioconversion and the kinetic characteristics of laccase oxidation[45-48].

CONCLUSIONS

- Twenty five fungal isolates were isolated from soils collected from different environments.
- Out of the twenty five isolates three were found to be potential degraders of the azo dye Congo red. They were preferred and cultured for further studies. The fungi were identified as *Aspergillus* sp 1 and *Penicillium* sp.
- The fungi were identified as *Aspergillus* spp. and *Penicillium* sp.
- The 3 species were used for degradation of 4 other dyes.
- All the four dyes used were degraded by *Aspergillus* spl, while only three dyes were degraded by *Aspergillus* sp.2 and *Penicillium* sp.
- Utilization of fungi for degradation of textile azo dyes can be considered as an effective process.
- Obtaining fungal culture for the experiments is not a tedious step and can be conducted easily.
- Experiment with fungal cultures are successful than those conducted with bacterial species due to the absence of growth in a low pH.
- Up to 98% color removal was achieved in shake culture of a particular fungal species.
- Though it is a slow process (time consumed for the growth was nearly 3 weeks), the results obtained are authentic and optimistic.

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