

***Luffa cylindrica* Immobilized with *Aspergillus terreus* QMS-1: an Efficient and Cost-Effective Strategy for the Removal of Congo Red using Stirred Tank Reactor**

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Abstract

Microbial populations within the rhizosphere have been considered as prosperous repositories with respect to bioremediation aptitude. Among various environmental contaminants, effluent from textile industries holds a huge amount of noxious colored materials having high chemical oxygen demand concentrations causing ecological disturbances. The study was aimed to explore the promising mycobionme of rhizospheric soil for the degradation of azo dyes to develop an efficient system for the exclusion of toxic recalcitrants. An effluent sample from the textile industry and soil samples from the rhizospheric region of *Musa acuminata* and *Azadirachta indica* were screened for indigenous fungi to decolorize Congo red, a carcinogenic diazo dye, particularly known for its health hazards to the community. To develop a bio-treatment process, *Aspergillus terreus* QMS-1 was immobilized on pieces of *Luffa cylindrica* and exploited in stirred tank bioreactor under aerobic and optimized environment. Quantitative estimation of Congo red decolorization was carried out using UV-Visible spectrophotometer. The effects of fungal immobilization and biosorption on the native structure of *Luffa cylindrica* were evaluated using a scanning electron microscope. *A. terreus* QMS-1 can remove (92%) of the dye at 100 ppm within 24 h in the presence of 1% glucose and 1% ammonium sulphate at pH 5.0. The operation of the bioreactor in a continuous flow for 12 h with 100 ppm of Congo red dye in simulated textile effluent resulted in 97% decolorization. The stirred tank bioreactor was found to be a dynamic, well maintained, no sludge producing approach for the treatment of textile effluents by *A. terreus* QMS-1 of the significant potential for decolorization of Congo red.

Key words: *Aspergillus terreus*, biodegradation, Congo red, immobilization, stirred tank bioreactor, textile industry

Introduction

The microbiome of the rhizospheric area is generally viewed as a treasure trove to scrutinize the indigenous microbial communities in search of natural detoxification of xenobiotics and other biotechnological perspective. It is estimated that one gram of this enriched soil comprises approximately 10 billion microorganisms, while only 1% of soil microbial population has been explored (Kakirde et al. 2010), which is widely reported for deterioration of organic polymers and pollutants.

Among numerous ventures, textile materials are directly associated with ecological angles to be unequivocally considered. With 10–15% of the share, the textile industry ranks on the top among all the dye consuming sectors utilizing 100,000 different synthetic coloring agents for various processes (Daassi et al. 2014). Discharge of dye-contaminated waste to receiving water bodies may cause immense ecological threats due to disturbances in photosynthetic activities of aquatic life, creating adverse effects on the amphibian territory (Husseiny 2008). It has been estimated that there are

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more than 670 textile industries in Pakistan, and almost all are dumping their hued mechanical waste into natural water bodies without embracing the essential treatment strategies (Andleeb et al. 2010). Although the worldwide figure demonstrates 280,000 tons discharge of textile dyes and colored pigments into water bodies, per annum (Maas and Chaudhari 2005), this situation can be mulled over to comprehend the gravity of the matter. Azo dyes representing 60–70% of the commonly used dyes are characterized by the presence of one or more chromogenic azo groups. Separate chromophore (N=N, C=C, C=O) and the auxochrome (–OH, –NH₂, –NR₂) are present in these dyes. These are intended to oppose the chemical and microbial assaults and stay stable in light and washing systems. A considerable lot of these dyes are cancer-causing and may trigger allergic reactions in human beings (Adedayo et al. 2004). Therefore, industrial wastes carrying azo dyes must be pre-treated prior to its disposal to the environment.

The physical and chemical effluent-treatment strategies, for example, adsorption, synthetic precipitation, and flocculation, are inefficient because of the prerequisite of strong foundation, high cost, and generation of secondary-sludge (McMullan et al. 2001). Given the disadvantages of chemical treatments, microbial remediation procedures have gained strong consideration during recent decades. Fungi turned out to be the most reasonable species for the treatment of textile-effluents for the evacuation of toxic colors. Numerous fungal strains either in free-living or in the immobilized state have been utilized for the decolorization of various dyes (Prachi and Anushree 2009). The ability of fungi to decolorize dyes relies on three mechanisms, namely biosorption (Fu and Viraraghavan 2001), biodegradation (Conneely et al. 1999), and enzymatic mineralization (Wesenberg et al. 2003; Rojek et al. 2004). Bioreactors operated with immobilized fungal cells have shown enhanced productivity, and permit expanded process strength and resistance to shock loadings in comparison to free fungal cells for decolorization of dyes (Hao et al. 2000).

The reported potential of mycobiota of rhizospheric soil for bioremediation and ecological effect of azo dyes envisaged the current study to isolate fungal species and to evaluate their ability to develop an efficient system for removal of hazardous and toxic recalcitrants in stirred tank reactor.

Experimental

Materials and Methods

The chemicals used in the study include Congo red (Sigma-Aldrich), Sabouraud's Dextrose Broth (Oxoid) as a fungal growth medium, Technical Agar (Oxoid),

and 0.05% Tween 80 solution for the preparation of the spore suspension. Composition of Minimal Salt Media was [per l, Na₂HPO₄ · 2H₂O: 7.8 g; KH₂PO₄: 6.8 g; MgSO₄ · 7H₂O: 0.2 g; NaNO₃: 0.085 g; Ca(NO₃)₂ · 4H₂O: 0.050 g; C₆H₈FeNO₇: 0.01 g; trace elements solution: 10 ml]. Simulated textile effluent (STE) was formulated as [per l, acetic acid (99.9%): 0.15 ml; (NH₄)₂Cl: 108.0 mg; KH₂PO₄: 67.0 mg; NaHCO₃: 840.0 mg; MgSO₄ · 7H₂O: 38.0 mg; CaCl₂: 21.0 mg; FeCl₃ · 6H₂O: 7.0 mg; glucose: 860 mg].

Sampling and isolation of fungi. The rhizospheric soil samples of *Musa acuminata* (banana plant) and *Azadirachta indica* (neem tree) were collected from the garden area of Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan. The textile effluent sample was collected from local textile industry in Karachi. The spread plate technique was adopted for the isolation of fungi from soil samples. Each soil sample (1 g) was serially diluted 10⁴ times in sterile distilled water (10 ml), and 0.1 ml of the diluted sample was spread on Sabouraud dextrose agar (SDA) plates followed by incubation at 28°C for seven days. Fungal strains were also isolated from the sampled textile effluent by adopting a similar procedure. The fungal isolates were identified based on microscopic and colonial characteristics on SDA (Larone 1995).

Qualitative screening of fungal isolates for bio-removal of Congo red. All the fungal isolates were screened qualitatively for removal of dye using SDA supplemented with 0.01% Congo red. Fungal discs (1 cm) were cut from the periphery of each colony, inoculated on center of the modified SDA, and incubated for seven days at 28°C. The formation of the clear transparent zone around the fungal colony indicated the degradation of Congo red by fungi.

Quantitative screening of fungal isolates for bio-removal of Congo red. Fungal isolates were screened quantitatively for removal of Congo red using SDB supplemented with 0.01% Congo red (modified SDB) through shake flask experiments. Fungal discs (1 cm) from SDA plates were cut from the periphery of each colony inoculated into the flask containing 20 ml of modified SDB and incubated at 28°C for three days on an orbital shaker at 150 rpm. The uninoculated flasks of the same medium served as controls in each case. A 4-ml sample was drawn aseptically from each flask after 72 h, filtered to separate biomass, and centrifuged at 5,000 rpm for 15 minutes. The degree of decolorization of Congo red by each fungal strain was determined in terms of percentage, measuring the absorbance of the cell-free culture supernatant (CFCS) spectrophotometrically at 498 nm (Mahbub et al. 2012).

Development of inoculum. The promising fungal strain, *A. terreus* QMS-1 was revived on the SDA plate

for a week at 28°C. The spores of *A. terreus* QMS-1 were harvested using a wire loop and shifted to the flask containing 50 ml of sterile distilled water with 0.05% Tween 80 (v/v). Following 30 minutes of vigorous shaking, 10 µl of the spore suspension was utilized for spore count. This inoculum was immediately used for further experimentation or stored in a refrigerator at 4°C for one week.

Optimization of physicochemical parameters for degradation of Congo red. The effect of agitation on biodegradation efficiency was studied by using SDB supplemented with 0.01% Congo red. Test flasks were inoculated with 1 ml spore suspension of *A. terreus* QMS-1, whereas uninoculated flasks served as control. One set of test and control flasks was incubated at room temperature in an orbital shaker at 100 rpm, while another set was incubated without shaking. Likewise, keeping shaking as constant, the effect of concentration of Congo red on biodegradation efficiency was investigated using 50 ml of Minimal Salt Medium (MSM) supplemented with different concentrations of Congo red (from 100–500 ppm) as described previously (Ali et al. 2008a). Similarly, the effect of carbon source in the medium on the ability of *A. terreus* QMS-1 to reduce Congo red was studied with different carbon sources like glucose, fructose, lactose, maltose, sucrose and starch in MSM containing a 100-ppm dye. When determining an optimum nitrogen source, the previously known factors were kept constant, and nitrogen source present in MSM (peptone, yeast extract, ammonium sulfate, and ammonium nitrate) was varied. The effect of pH was also investigated in a similar way by maintaining pH to 5.0, 7.0, and 9.0 with 1 M HCl or 1 M NaOH keeping other parameters unchanged.

In all the experiments, each test flask was inoculated with 1 ml spore suspension of *A. terreus* QMS-1, whereas uninoculated flasks served as controls. A 4-ml sample from each flask was taken aseptically after every 24 h up till 120 h, filtered and centrifuged at 5,000 rpm for 15 minutes, and used as cell-free culture supernatant (CFCS) to check the percent decolorization.

Immobilization of *A. terreus* QMS-1 on carrier materials. Seventeen natural and synthetic carrier materials were selected for fungal immobilization based on their stability and cost effectiveness, including polyethylene polymer, sand, gravels, natural loofah sponge, corn cob, used steel wool, orange peels, banana skins, nylon net, polyurethane foam (PUF), coconut bagasse, sugarcane bagasse, water beads, scouring pad, sandpaper, pistachio shells, and sunflower seed shells. All the supports were washed thoroughly with distilled water. Flasks containing 200 ml of SDB and 12–15 pieces of carrier material were autoclaved, inoculated with 10 ml

of spore suspension of *A. terreus* QMS-1, and kept on a rotary shaker at room temperature for 15 days.

Degradation of Congo red by immobilized cells in the stirred tank bioreactor. A benchtop stirred tank bioreactor (STR) comprised of an overhead impeller, and a vessel body (2l volume) was used. The reactor was run at room temperature, pH 5.0, with a working volume of 1l of simulated textile effluent (STE) containing 100 ppm of Congo red. The medium was inoculated separately with different carrier materials with immobilized *A. terreus* QMS-1. The hydraulic retention time was 12–120 h. The effluent samples were taken intermittently after every 12 h and analyzed for percent degradation of dye. When investigating the dye adsorption on the carrier materials, similar experiments were performed using carrier supports without immobilized cells.

Recycling of immobilized *A. terreus* QMS-1. In another experiment using the same procedure, STR was run and fed with immobilized carrier material yielding maximum decolorization of STE. The matrix with immobilized cells was checked for repeated use by reusing the previously withdrawn immobilized carrier supports from the reactor tank to fresh STE.

Quantitative estimation of decolorization. Decolorization was determined using a method described by Mahbub et al. (2012), measuring the absorbance of the CFCS at 498 nm by UV-Visible spectrophotometer. The extent of dye degradation was calculated in terms of percentage, using the following equation:

$$\% \text{ decolorization} = \frac{\text{initial absorbance} - \text{final absorbance}}{\text{initial absorbance}} \times 100$$

where, initial absorbance is the absorbance taken at 0 h after the inoculation, and final absorbance is the absorbance taken at after every 12–24 h.

Physico-chemical characterization of treated and untreated effluent samples under STR conditions. Treated and untreated STE samples were tested by using standard methods of Biological Oxygen Demand (APHA 5210 B), Chemical Oxygen Demand (APHA 5220 C), Total Dissolved Solids (APHA 2540 C), and Total Suspended Solids (APHA 2450 D).

Scanning electron microscope analysis. The surface morphology of the best carrier material (natural loofah sponge) before and after the immobilization and biosorption was investigated using scanning electron microscope (SEM) analysis (JEOL, Model number: JSM-6380A).

Statistical analysis. All the experimental attempts were executed in triplicate, and the findings were declared as mean ± standard deviation. Experimental data were analyzed by the one-way and two-way analysis of variance (ANOVA) using GraphPad Prism (version 6.0).

Results and Discussion

Based on the environmental impact of azo dyes as well as reported bioremediation potential of fungi, the present study was aimed to isolate and screen indigenous mycobiota for developing a process of bioremediation. For this purpose, 56 fungal strains isolated from different samples of soil and textile effluent were identified by standard mycological protocols (Table I). The identified fungal strains included *Alternaria* sp., *A. flavus*, *A. niger*, *A. terreus*, *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Helimentosporium* sp., *Mucor* sp., *Penicillium* sp., and *Rhizopus* sp.

This data also revealed that most of the fungal strains belonged to the genus *Aspergillus*. Amongst *Aspergilli*, *A. flavus* was found most frequent (19%) as compared

to the other fungal strains isolated from different soil and textile effluent samples followed by *A. niger* (13.4%), *A. terreus* (13.4%), *Mucor* sp. (11.5%), *Penicillium* sp. (11.5%), *Rhizopus* sp. (9.6%), *Curvularia* sp. (7.69%), *Cladosporium* sp. (5.7%), *Fusarium* sp. (5.7%), *Helimentosporium* sp. (5.7%), and *Alternaria* sp. (3.8%). Earlier, Cardoso Duarte and Costa-Ferreira (1994) have reported the abundance of *Aspergillus* in soil, whereas, Sohail et al. (2009) found *Aspergillus* as the predominant genus of the hydrolytic-enzyme producing fungi in soil.

Out of 56 fungal isolates, 42 strains showed decolorization of Congo red in the qualitative screening method. The ability of the fungi tested to remove dye was confirmed by the appearance of clear zones around the fungal colonies whereas, 14 strains were found unable to decolorize the Congo red, forming no zones of

Table I
Identification and screening of fungal isolates for biodegradation potential of Congo red.

Fungal isolate number	The fungi identified	Qualitative screening for Congo red dye	Quantitative screening for Congo red dye (%)
01	<i>Alternaria</i> sp QMS-1	-	-
02	<i>Alternaria</i> sp QMS-2	+	66
03	<i>Aspergillus flavus</i> QMS-1	+	60
04	<i>Aspergillus flavus</i> QMS-2	+	55
05	<i>Aspergillus flavus</i> QMS-3	+	80
06	<i>Aspergillus flavus</i> QMS-4	+	17
07	<i>Aspergillus flavus</i> QMS-5	+	80
08	<i>Aspergillus flavus</i> QMS-6	-	-
09	<i>Aspergillus flavus</i> QMS-7	-	-
10	<i>Aspergillus flavus</i> QMS-8	+	26
11	<i>Aspergillus flavus</i> QMS-9	+	42
12	<i>Aspergillus flavus</i> QMS-10	+	28
13	<i>Aspergillus niger</i> QMS-1	-	-
14	<i>Aspergillus niger</i> QMS-2	+	54
15	<i>Aspergillus niger</i> QMS-3	+	26
16	<i>Aspergillus niger</i> QMS-4	+	53
17	<i>Aspergillus niger</i> QMS-5	+	18
18	<i>Aspergillus niger</i> QMS-6	+	89
19	<i>Aspergillus niger</i> QMS-7	+	54
20	<i>Aspergillus terreus</i> QMS-1	+	92
21	<i>Aspergillus terreus</i> QMS-2	+	35
22	<i>Aspergillus terreus</i> QMS-3	+	12
23	<i>Aspergillus terreus</i> QMS-4	-	-
24	<i>Aspergillus terreus</i> QMS-5	+	12
25	<i>Aspergillus terreus</i> QMS-6	+	31
26	<i>Aspergillus terreus</i> QMS-7	+	79
27	<i>Cladosporium</i> sp QMS-1	+	65
28	<i>Cladosporium</i> sp QMS-2	+	66
29	<i>Cladosporium</i> sp QMS-3	+	62
30	<i>Curvularia</i> sp QMS-1	-	-
31	<i>Curvularia</i> sp QMS-2	+	56
32	<i>Curvularia</i> sp QMS-3	-	-
33	<i>Curvularia</i> sp QMS-4	-	-
34	<i>Fusarium</i> spQMS-1	+	43
35	<i>Fusarium</i> sp QMS-2	+	66
36	<i>Fusarium</i> sp QMS-3	+	81
37	<i>Helimentosporium</i> sp QMS-1	+	74
38	<i>Helimentosporium</i> sp QMS-2	+	62
39	<i>Helimentosporium</i> sp QMS-3	+	54
40	<i>Mucor</i> sp QMS-1	+	31
41	<i>Mucor</i> sp QMS-2	-	-
42	<i>Mucor</i> sp QMS-3	+	62
43	<i>Mucor</i> sp QMS-4	-	-
44	<i>Mucor</i> sp QMS-5	-	-
45	<i>Mucor</i> sp QMS-6	+	82
46	<i>Penicillium</i> sp QMS-1	-	-
47	<i>Penicillium</i> sp QMS-2	+	43
48	<i>Penicillium</i> sp QMS-3	+	83
49	<i>Penicillium</i> sp QMS-4	+	79
50	<i>Penicillium</i> sp QMS-5	+	65
51	<i>Penicillium</i> sp QMS-6	-	-
52	<i>Rhizopus</i> sp QMS-1	+	11
53	<i>Rhizopus</i> spQMS-2	+	25
54	<i>Rhizopus</i> sp QMS-3	-	-
55	<i>Rhizopus</i> sp QMS-4	+	56
56	<i>Rhizopus</i> sp QMS-5	+	43

(+) - it indicates the zone of Congo red decolorization around the fungi
(-) - it indicates no zone of Congo red decolorization around the fungi

decolorization. Since the screening was mainly carried out using SDA that supports the growth of fungi and, therefore, growth-linked removal of the dye was investigated. The absence of zones around 14 strains may be linked with the inhibitory effect of the dye on the growth rate of fungi due to which degradation of dye proceeded more slowly as compared to radial growth of fungi; consequently, zone of decolorization did not appear around the fungal mycelia (Machado et al. 2006).

The findings of quantitative screening assay showed that fungal isolates namely *A. flavus* QMS-3, *A. flavus* QMS-5, *A. niger* QMS-6, *Fusarium* sp. QMS-3, *Mucor* sp. QMS-6, *Penicillium* sp. QMS-3 and *A. terreus* QMS-1 were found to possess excellent dye removal potential. However, amongst all the outstanding dye degraders, the strain QMS-1 of *A. terreus* exhibited promising potential for degradation of Congo red (92%) as compared to other fungal strains (Table I).

The dye removal ability of fungi is attributed to different phenomena such as bio-sorption/ bio-adsorption followed by bio-degradation (Knapp and Newby 1995; Fu and Viraraghavan 2000; Sumathi and Manju 2000; Ali et al. 2008b). Bio-sorption is regarded as the primary process of dye removal, which enables the fungi to bind with molecules of dyes in the vicinity of fungal biomass due to electrostatic attraction between the positively charged cell walls and negatively charged dye molecules (Aksu and Tezer 2000). Bio-degradation enables the fungi to break down the dye molecule enzymatically into various products (Saranraj et al. 2010). It has been reported that some species belong to genus *Aspergillus* can produce enzymes like laccase, lignin peroxidases, and manganese peroxidases (Kanayama et al. 2002). In this context, it may be suggested that high electrostatic attractions due to the increased number of positively charged receptors on the cell wall of *A. terreus* QMS-1 were responsible for the efficient removal of Congo red in comparison with other fungal strains.

The strain *A. terreus* QMS-1 was found capable of exhibiting maximum degradation of Congo red (95%) under shaking (100 rpm) conditions after 24 h of cultivation, although degradation was increased to 96% when cultivation was extended to 48 h. In contrast, the cultivation of *A. terreus* QMS-1 under static conditions caused a decrease in decolorization, and only 62% and 63% reduction was noted in 24 h and 48 h, respectively (Fig. 1). The aeration has its recognizable effect on the growth and metabolic activities of all the obligate aerobes including fungi by expanding oxygenation and dissemination of nutrients in the medium that lead to the increased fungal mass and might be heightened expression of oxidative enzymes for the bio-transformation of dye (Rani et al. 2014). However, static cultural medium causes a reduction in decolorization potential of the fungal strains by limiting oxygen just

Effect of agitation and static Conditions on biodegradation efficiency of *Aspergillus terreus* QMS-1

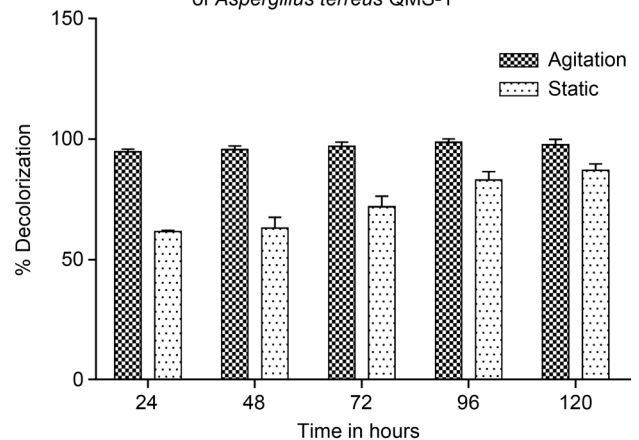


Fig. 1. Effect of agitation and static conditions on biodegradation efficiency of *Aspergillus terreus* QMS-1.

in inward layers of fungal mycelia triggering suppression of oxidative enzymes and considerable reduction in biodegradation activities.

The concentration of dye has a marked impact on the dye removal efficiency as a higher concentration may inhibit fungal growth. The strain was found capable of degrading the dye at all concentrations after 72 h; however, maximum degradation (95%) was observed with 100 ppm of dye followed by 84%, 78.6%, 70%, and 76% at 200, 300, 400, and 500 ppm, respectively (Fig. 2). Hence, it could be stated that the decolorization ability of *A. terreus* QMS-1 decreased with the increasing concentration of dye, which may be attributed to the toxicity of Congo red at higher concentrations as reported for some other fungi (Sponza and Isik 2005; Siddiqui et al. 2009). Nonetheless, the dye at a concentration equal to 90 ppm was being removed within 24 h regardless of the initial concentration used. In the studies by Gharbani et al. (2008), 60 ppm of Congo red was used as 'simulated loaded textile wastewater' implicating the importance of dye removal ability by *A. terreus* QMS-1 and its possible application for *in situ* dye removal.

The strain, *A. terreus* QMS-1 showed maximum degradation of Congo red in the presence of 1% glucose (95.2%) followed by maltose (90.3%), fructose (76%), sucrose (73%), lactose (66.6%), and starch (56.70%) after 24 h of cultivation under shaking conditions in the medium containing 100 ppm of Congo red (Fig. 3). These findings showed that glucose was the most suitable catabolizable carbon source for *A. terreus* QMS-1 that accelerated the decolorization of Congo red. Nevertheless, monosaccharides like glucose are decomposed readily as compared to the disaccharides or polysaccharides. Earlier research findings suggested that 5.0 g l⁻¹ glucose was sufficient to achieve maximum decolorization of Congo red as a further increase in

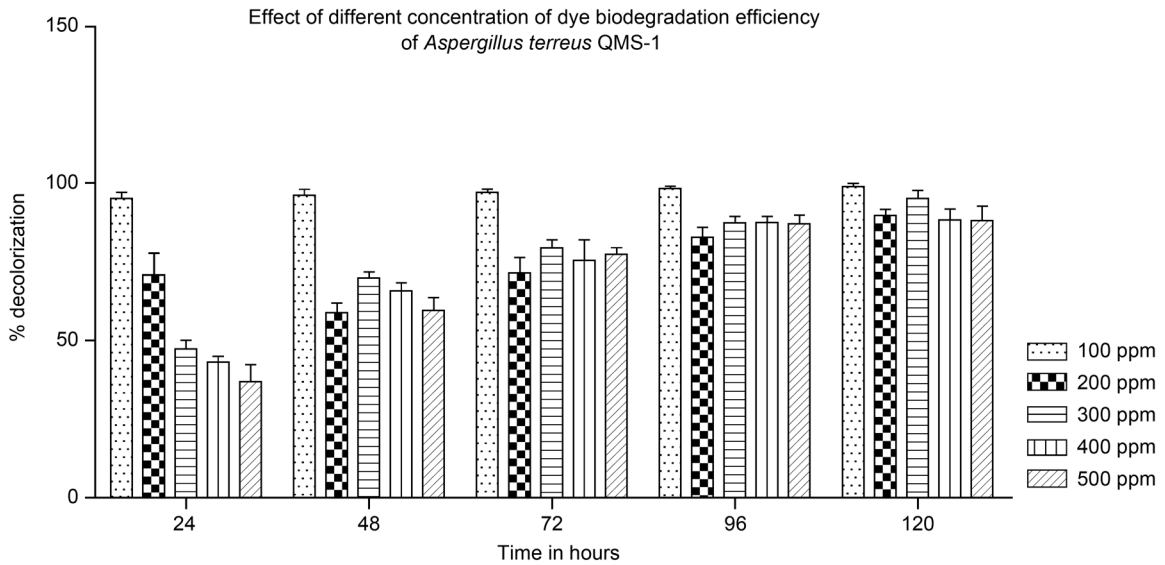


Fig. 2. Effect of different concentration of dye on biodegradation efficiency of *Aspergillus terreus* QMS-1.

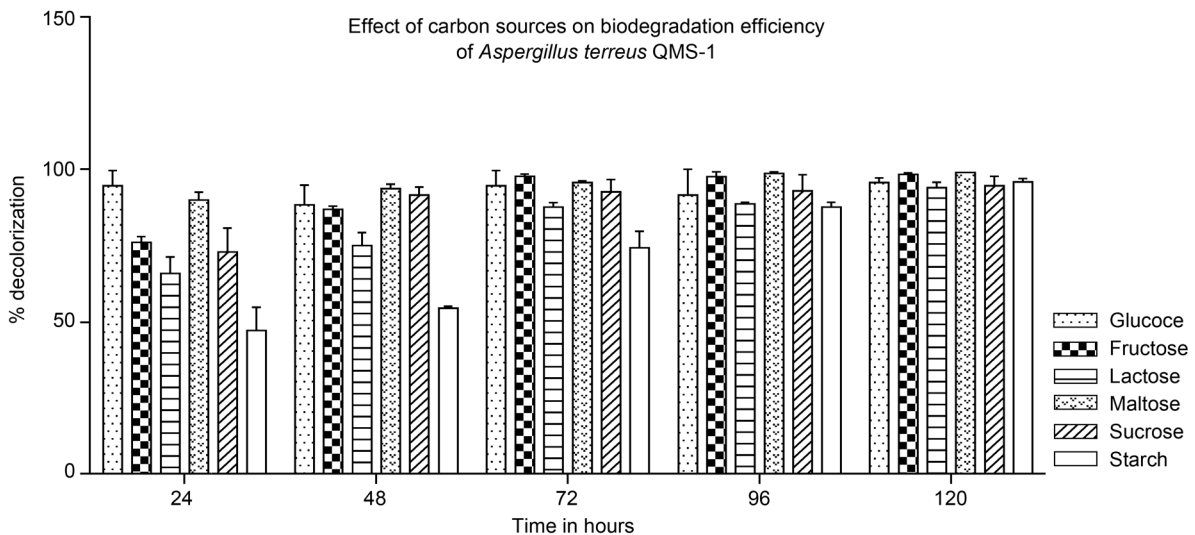


Fig. 3. Effect of carbon sources on biodegradation efficiency of *Aspergillus terreus* QMS-1.

glucose concentration resulted in a decline in the rate of dye decolorization (Radha et al. 2005). Furthermore, it is also reported that polysaccharides such as starch, cellulose, and its derivatives did not support the dye decolorization process by fungi (Vaidyanathan et al. 2011).

The strain, *A. terreus* QMS-1, exhibited maximum dye removal, i.e., 92.3% and 91% in the presence of ammonium nitrate and ammonium sulfate after 24 h of cultivation, respectively. However, a noticeable decline in the removal of dye was observed, i.e., 55.2% and 39.2% in the presence of organic sources of nitrogen, i.e., peptone and yeast extract, respectively (Fig. 4). The results obtained are in agreement with those of Kashif et al. (2011) who reported the maximum decolorization of dye, Sollar Golden Yellow R by *Pleurotus ostreatus* species in the presence of ammonium sulfate that facili-

tated in the regeneration of NADH in the medium as electron donor, causing reduction of the dye.

A. terreus QMS-1 showed maximum mineralization of Congo red at pH 5.0, i.e., 95% and 99% after 24 h and 48 h, respectively. In contrast, an increase in pH (alkaline pH) proved to have an undesirable effect on the biodegradation potential of *A. terreus* QMS-1, and decolorization was decreased considerably to 39% (Fig. 5). In this regard, it was reported that the effective dye biotransformation/decolorization process and fungal growth usually required pH between 3 and 6 (Mansur et al. 2003; Baldrian 2004). Low pH enables the fungi to show maximum dye removal potential by providing an increased number of H^+ ions located on the fungal cell wall and facilitating the uptake of negatively charged dye molecule (Won et al. 2009). It has

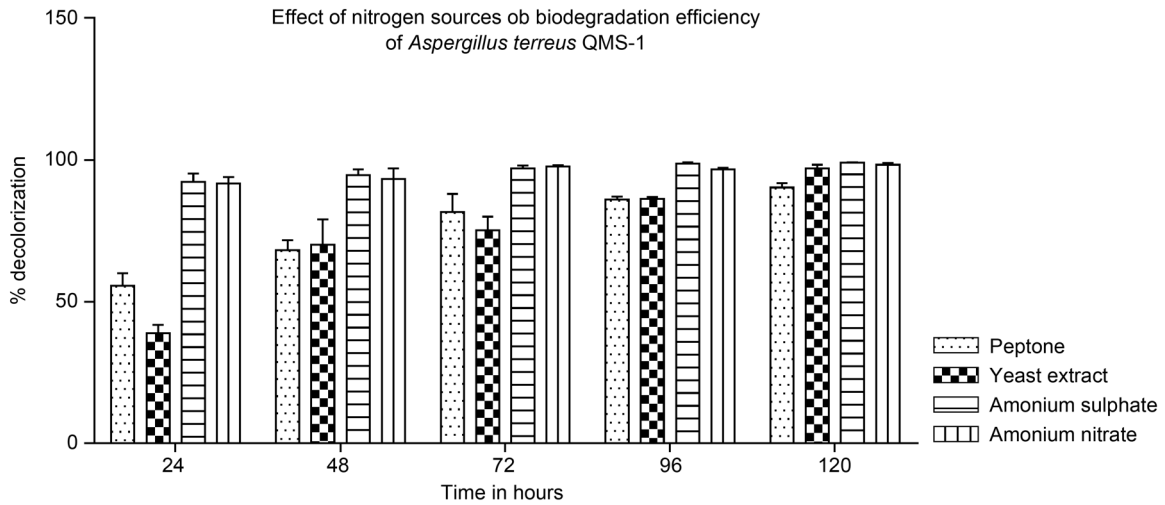


Fig. 4. Effect of nitrogen sources on biodegradation efficiency of *Aspergillus terreus* QMS-1.

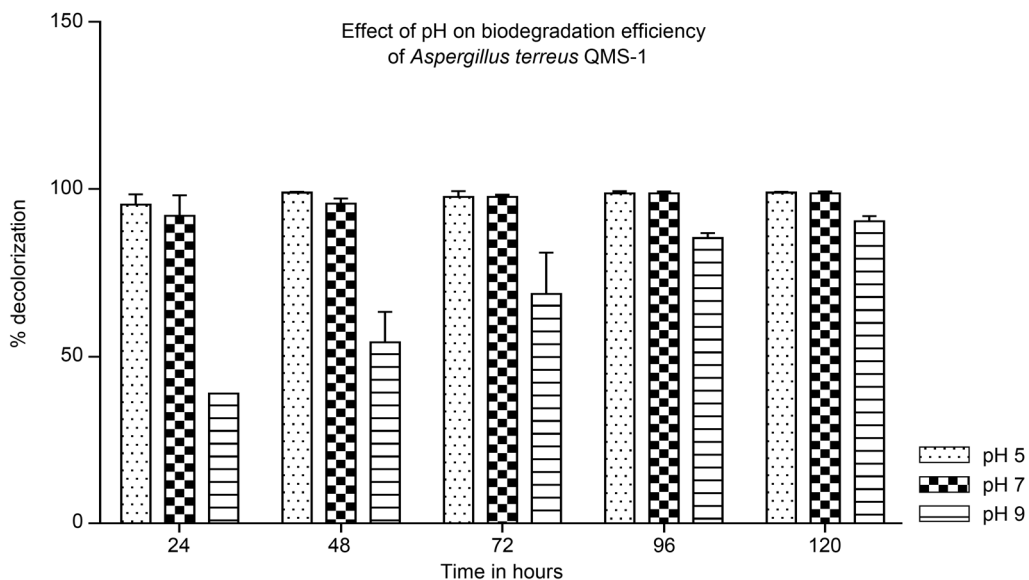


Fig. 5. Effect of pH on biodegradation efficiency of *Aspergillus terreus* QMS-1.

also been observed and suggested that a further increase in pH greater than 5.0 leads to the disintegration of fungal mycelia and reduction in fungal growth, therefore, the dye removal process decreases.

Using the STR system, the effect of various natural and synthetic immobilization support matrices on Congo red removal efficiency was investigated. *A. terreus* QMS-1 was immobilized over 17 different support matrices to select the one best for decolorization/degradation of dye. The selection of support matrices was based on high porosity, inert nature, easy availability, and low cost. It was found that *A. terreus* QMS-1 immobilized over natural loofah sponge showed maximum removal of Congo red, i.e., 97%, followed by polyethylene polymer (92%), sugarcane bagasse (86.3%), nylon net (83%), used steel wool (83%), orange peels (81%), scouring pad (80%), sand (77.3%), coconut bagasse (77.3%),

polyurethane foam (74.87%), banana skins (67.8%), water beads (63%), corn cob (62%), sand paper (62%), pistachio shells (65%), sunflower seed shells (55.5%) and gravels (48.9%) at a hydraulic retention time of 12 h under STR conditions. However, complete decolorization, i.e., 100% removal of Congo red dye, was achieved at a hydraulic retention time of 48 h by employing *A. terreus* QMS-1 immobilized over natural loofah sponge (Fig. 6). The results of removal of Congo red dye (100 mg/l) under STR conditions revealed the superiority of natural loofah sponge as an immobilization support matrix for *A. terreus* QMS-1, as compared to the other immobilization supports (Fig. 7), in terms of decolorization of dye and reduction in the treatment time. Natural loofah sponge is derived from the vegetable fibre of *Luffa cylindrica* or *Luffa aegyptiaca*. The spatial structure of natural loofah sponge is formed

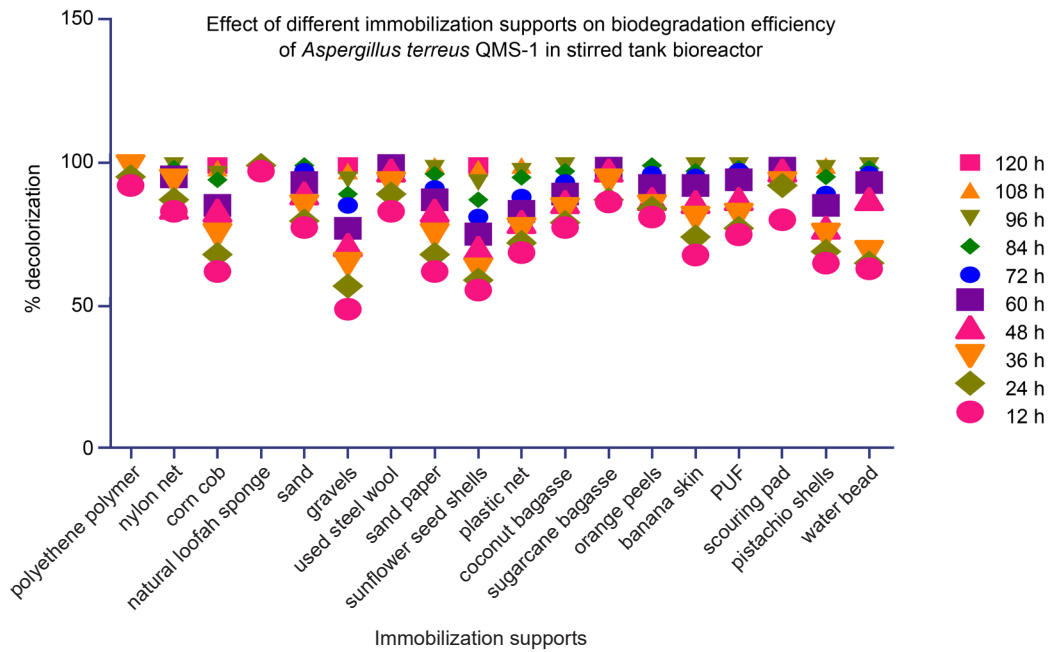


Fig. 6. Effect of different immobilization supports on biodegradation efficiency of *Aspergillus terreus* QMS-1 in the stirred tank bioreactor.

by the parallel and antiparallel arrangement of fibres, thereby creating open and free spaces for the exchange of nutrients. Moreover, it provides enough space for the diffusion of oxygen and contains sites for the physical confinement or localization of fungal hyphae. Other advantages of this carrier include its high porosity (85–95%) with simultaneous low density (0.018–0.05 g/cm³) and mechanical strength. Previously, natural loofah sponge has been applied as a Microalgal-luffa sponge immobilized discs for biosorption of nickel (Akhtar et al. 2003). Furthermore, due to the robustness, sta-

bility, and regenerating capability of this matrix, the immobilized culture can be recycled for at least seven times. Besides, loofah sponge is also used as a nutrient source for white-rot fungi providing carbon and energy contributing towards long-term bioremediation (Mazmanci et al. 2005).

The decolorization of Congo red dye in simulated textile effluent was also investigated in the bioreactor with entire pieces of natural loofah sponge without fungal cells to determine the physical adsorption capacity of the support matrix. It was found that removal of

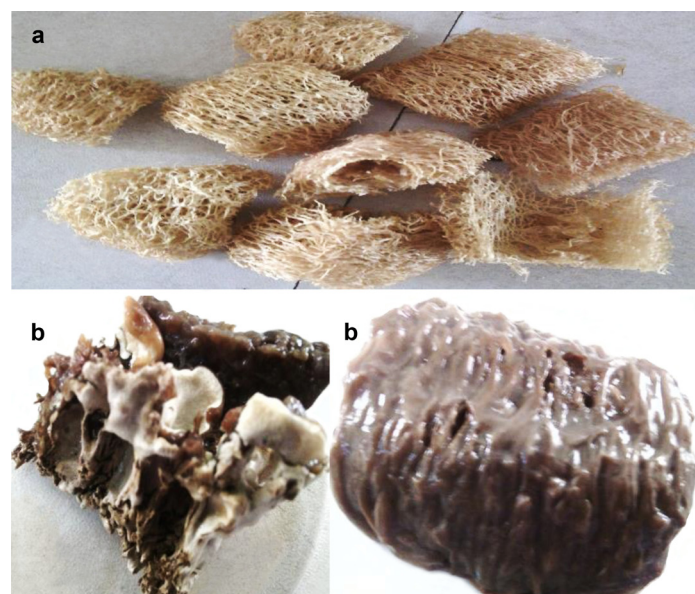


Fig. 7. *Luffa cylindrica* (a) before and (b) after with immobilization of *Aspergillus terreus* QMS-1.

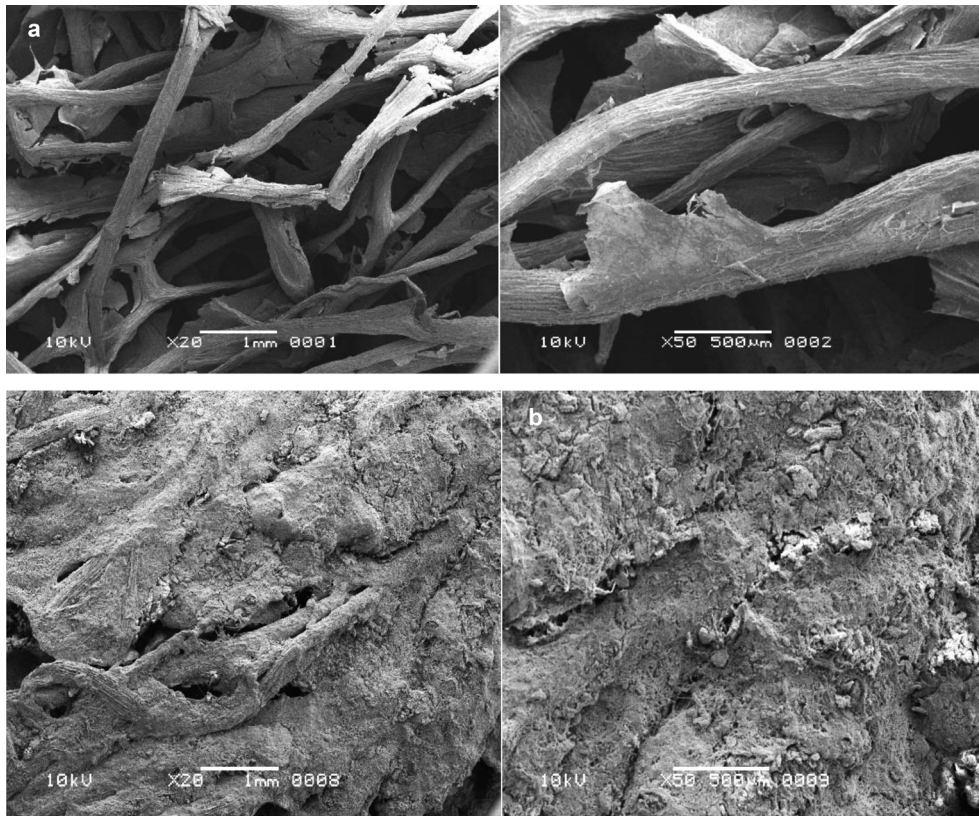


Fig. 8. SEM micrographs of *Luffa cylindrica*, a) before and b) after immobilization of *Aspergillus terreus* QMS-1.

Congo red from simulated textile effluent by the intact pieces of natural loofah sponge was not significant, and hence the dye removal was not because of adsorption by the natural loofah sponge.

These experimental findings suggested that natural loofah sponge possessed all the required characteristics to be utilized as an ideal bio-support. Therefore, it was further subjected to SEM analysis at various magnifications ($\times 20$ and $\times 50$) to observe the morphological changes before and after the immobilization of *A. terreus* QMS-1, and was later applied in STR system. SEM micrographs of intact natural loofah sponge indicated porous, hollow, and rough topography with a greater number of interstitial voids and inner channels (Fig. 8a). This was converted into a compact, tighter, and smooth material as fungal mycelia covered interstitial voids, inner channels, and superficial surfaces (Fig. 8b). The channeled structure has an identifiable effect as it allows respiratory gases and essential elements effectively through fungal mycelial mass, increasing metabolic efficiency of the strain. Therefore, immobilized fungi are viewed as more proficiently than suspended mycelia in many frameworks (Villena et al. 2010).

Effect of repeated use of *A. terreus* QMS-1 immobilized over the most promising carrier matrix (natural loofah sponge) on decolorization of Congo red dye in STE was also evaluated to make the use of bioreac-

tor more affordable and economical. Moreover, it was found that loofah sponge was an extremely stable carrier and can be reused for at least eight times with the same rate of decolorization, i.e., 97% (12 h). However, further recycling for more than eight cycles resulted in a gradual reduction in the rate of decolorization by the immobilized *A. terreus* QMS-1. In this regard, several factors including higher molecular mass, structural complexity, a saturation of receptors on fungal cells, and the presence of inhibitory functional groups may cause desorption or removal of dye from fungal cells, especially at the long contact time, thereby declining the recyclability of immobilized fungus and affecting the process of decolorization (Ali and Muhammad 2008b).

Although microbes can remove color-producing recalcitrant compounds by a wide variety of mechanisms, it has also been indicated by several research studies that detoxification of reactive group of azo dyes leads to the generation of robust and even more lethal intermediates during biodegradation processes (Gottlieb et al. 2003). Therefore, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), and Total Suspended Solids (TSS) analysis of the dye-containing effluent samples (before and after treatment under STR) were carried out to evaluate and confirm the extent of degradation of Congo red dye by *A. terreus* QMS-1 through

quantifying and comparing their organic loads. In case of STR treatment system, the BOD, COD, TDS and TSS values decreased from 1,260, 2,657, 3,000, and 500 mg/l to 300, 1,180, 2,160, and 160 mg/l at HRT of 12 h respectively, showing the acceptable values according to National Environmental Quality Standards (NEQs) range. The drastic reduction of BOD, COD, TDS, and TSS values suggests the significance of STR treatment technology, even though it is crucially imperative to explore final residues of dye mineralization to render this system as an ecologically perfect and economical alternative to conventional treatment methods.

Conclusions

This research study proclaimed the excellent attempt of immobilized *A. terreus* QMS-1 under STR conditions for the reduction of Congo red, i.e., 97% decolorization within 12 h, reinforcing its potential for a safer environment by reducing the hazardous effects of textile dyes and proposing this technology as an adaptable, proficient and sustainable way for dealing with textile effluents.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Adedayo O, Javadpour S, Taylor C, Anderson WA, Moo-Young M. Decolorization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant. *World J Microbiol Biotechnol.* 2004;20(6):545–550. <https://doi.org/10.1023/B:WIBI.0000043150.37318.5f>

Akhtar N, Iqbal J, Iqbal M. Microalgal-luffa sponge immobilized disc: a new efficient biosorbent for the removal of Ni(II) from aqueous solution. *Lett Appl Microbiol.* 2003 Aug;37(2):149–153. <https://doi.org/10.1046/j.1472-765X.2003.01366.x>

Aksu Z, Tezer S. Equilibrium and kinetic modelling of biosorption of Remazol Black B by *Rhizopus arrhizus* in a batch system: effect of temperature. *Process Biochem.* 2000 Dec;36(5):431–439. [https://doi.org/10.1016/S0032-9592\(00\)00233-8](https://doi.org/10.1016/S0032-9592(00)00233-8)

Ali H, Muhammad SK. Biodecolorization of acid violet 19 by *Alternavia solani*. *Afr J Biotechnol.* 2008b;7:831–833.

Ali N, Ikramullah, Lutfullah G, Hameed A, Ahmed S. Decolorization of Acid red 151 by *Aspergillus niger* SA1 under different physicochemical conditions. *World J Microbiol Biotechnol.* 2008a Jul;24(7):1099–1105. <https://doi.org/10.1007/s11274-007-9581-6>

Andleeb S, Atiq N, Ali MI, Razi-Ul-Hussnain R, Shafique M, Ahmad B, Ghumro PB, Hussain M, Hameed A, Ahmed S. Biological treatment of textile effluent in stirred tank bioreactor. *Int J Agric Biol.* 2010;12:256–260.

Baldrian P. Purification and characterization of laccase from the white-rot fungus *Daedalea quercina* and decolorization of synthetic dyes by the enzyme. *Appl Microbiol Biotechnol.* 2004 May 1;63(5):560–563. <https://doi.org/10.1007/s00253-003-1434-0>

Cardoso Duarte JÁ, Costa-Ferreira M. Aspergilli and lignocelluloses: enzymology and biotechnological applications. *FEMS Microbiol Rev.* 1994 Mar;13(2–3):377–386.

<https://doi.org/10.1111/j.1574-6976.1994.tb00057.x>

Conneely A, Smyth WF, McMullan G. Metabolism of the phthalocyanine textile dye remazol turquoise blue by *Phanerochaete chrysosporium*. *FEMS Microbiol Lett.* 1999 Oct;179(2):333–337.

<https://doi.org/10.1111/j.1574-6968.1999.tb08746.x>

Daássi D, Rodríguez-Couto S, Nasri M, Mechichi T. Biodegradation of textile dyes by immobilized laccase from *Corioloopsis gallica* into Ca-alginate beads. *Int Biodeterior Biodegradation.* 2014 May;90:71–78. <https://doi.org/10.1016/j.ibiod.2014.02.006>

Fu Y, Viraraghavan T. Fungal decolorization of dye wastewaters: a review. *Bioresour Technol.* 2001 Sep;79(3):251–262.

[https://doi.org/10.1016/S0960-8524\(01\)00028-1](https://doi.org/10.1016/S0960-8524(01)00028-1)

Fu YZ, Viraraghavan T. Removal of a dye from an aqueous solution by the fungus *Aspergillus niger*. *Water Qual Res J Can.* 2000;35:95–111.

Gharbani P, Tabatabaai SM, Mehrizad A. Removal of Congo red from textile wastewater by ozonation. *Int J Environ Sci Technol.* 2008 Sep;5(4):495–500. <https://doi.org/10.1007/BF03326046>

Gottlieb A, Shaw C, Smith A, Wheatley A, Forsythe S. The toxicity of textile reactive azo dyes after hydrolysis and decolourisation. *J Biotechnol.* 2003 Feb;101(1):49–56.

[https://doi.org/10.1016/S0168-1656\(02\)00302-4](https://doi.org/10.1016/S0168-1656(02)00302-4)

Hao OJ, Kim H, Chiang PC. Decolorization of wastewater. *Crit Rev Environ Sci Technol.* 2000 Oct;30(4):449–505.

<https://doi.org/10.1080/10643380091184237>

Husseiny M. Biodegradation of the reactive and direct dyes using Egyptian isolates. *J Appl Sci Res.* 2008;4:599–606.

Kakirde KS, Parsley LC, Liles MR. Size does matter: application-driven approaches for soil metagenomics. *Soil Biol Biochem.* 2010 Nov;42(11):1911–1923.

<https://doi.org/10.1016/j.soilbio.2010.07.021>

Kanayama N, Tohru S, Keiichi K. Purification and characterization of an alkaline manganese peroxidase from *Aspergillus terreus* LD-1. *J Biosci Bioeng.* 2002 Apr;93(4):405–410.

[https://doi.org/10.1016/S1389-1723\(02\)80075-5](https://doi.org/10.1016/S1389-1723(02)80075-5)

Kashif J, Muhammad A, Haq NB, Zahid M. Shake flask decolorization of direct dye solar golden yellow R by *Pleurotus ostreatus*. *J Chem Soc Pak.* 2011;33:209–214.

Knapp JS, Newby PS. The microbiological decolorization of an industrial effluent containing a diazo-linked chromophore. *Water Res.* 1995 Jul;29(7):1807–1809.

[https://doi.org/10.1016/0043-1354\(94\)00341-4](https://doi.org/10.1016/0043-1354(94)00341-4)

Kumar Praveen GN, Bhat SK. Fungal Degradation of Azo Dye-Red 3BN and optimization of physico-chemical parameters. *ISCA Res. J.Biol. Sci.* 2012;1:17–24.

Larone DH. Medically Important Fungi. A Guide to Identification. Washington (USA): ASM Press; 1995.

Maas R, Chaudhari S. Adsorption and biological decolourization of azo dye Reactive Red 2 in semicontinuous anaerobic reactors. *Process Biochem.* 2005 Feb;40(2):699–705.

<https://doi.org/10.1016/j.procbio.2004.01.038>

Machado KMG, Compart LCA, Morais RO, Rosa LH, Santos MH. Biodegradation of reactive textile dyes by basidiomycetous fungi from Brazilian ecosystems. *Braz J Microbiol.* 2006 Dec;37(4):481–487. <https://doi.org/10.1590/S1517-83822006000400015>

- Mahbub KR, Mohammad A, Ahmed MM, Begum S.** Decolourisation of synthetic dyes using bacteria isolated from textile industry effluent. *Asian J Biotechnol.* 2012 Mar 1;4(3):129–136. <https://doi.org/10.3923/ajbkr.2012.129.136>
- Mansur M, Arias ME, Copa-Patiño JL, Flärdh M, González AE.** The white-rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities. *Mycologia.* 2003 Nov;95(6):1013–1020. <https://doi.org/10.1080/15572536.2004.11833017>
- Mazmanci MA, Ünyayar A.** Decolourisation of Reactive Black 5 by *Funalia trogii* immobilised on *Luffa cylindrica* sponge. *Process Biochem.* 2005 Jan;40(1):337–342. <https://doi.org/10.1016/j.procbio.2004.01.007>
- McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat IM, Marchant R, Smyth WF.** Microbial decolourisation and degradation of textile dyes. *Appl Microbiol Biotechnol.* 2001 Jul 1;56(1–2):81–87. <https://doi.org/10.1007/s002530000587>
- Prachi K, Anushree M.** Effect of the nutrient composition on dye decolorization and extra cellular enzyme production by *Lentinus edodes* on solid medium. *Enzyme Microb Technol.* 2009;30:381–386.
- Radha KV, Regupathi I, Arunagiri A, Murugesan T.** Decolorization studies of synthetic dyes using *Phanerochaete chrysosporium* and their kinetics. *Process Biochem.* 2005 Oct;40(10):3337–3345. <https://doi.org/10.1016/j.procbio.2005.03.033>
- Ramya, M.B., S. Anusha, Kalavathy and S. Devilaksmi.** Biodecolorization and biodegradation of Reactive Blue by *Aspergillus* spp. *Afr J Biotechnol.* 2007;6:1441–1445.
- Rani B, Kumar V, Singh J, Bisht S, Teotia P, Sharma S, Kela R.** Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability. *Braz J Microbiol.* 2014 Sep;45(3):1055–1063. <https://doi.org/10.1590/S1517-83822014000300039>
- Saranraj P, Sumathi V, Reetha D, Stella D.** Fungal decolorization of direct azo dyes and biodegradation of textile dye effluent. *J. Ecobiotechnol.* 2010;2:12–16.
- Siddiqui MS, Andleeb S, Ali N, Ghumro PB, Ahmed S.** Up-flow immobilized fungal column reactor for the treatment of Anthraquinone dye Drimarene Blue K2RL. *Afr J Biotechnol.* 2009; 8: 5570–5577.
- Sohail M, Naseeb S, Sherwani SK, Sultana S, Aftab S, Shahzad S, Ahmad A, Khan SA.** Distribution of hydrolytic enzymes among native Fungi: aspergillus the pre-dominant genus of hydrolase producer. *Pak J Bot.* 2009;41:2567–2582.
- Sponza DT, Işık M.** Reactor performances and fate of aromatic amines through decolorization of Direct Black 38 dye under anaerobic/aerobic sequential. *Process Biochem.* 2005 Jan;40(1): 35–44. <https://doi.org/10.1016/j.procbio.2003.11.030>
- Sumathi S, Manju BS.** Uptake of reactive textile dyes by *Aspergillus foetidus*. *Enzyme Microb Technol.* 2000 Sep;27(6):347–355. [https://doi.org/10.1016/S0141-0229\(00\)00234-9](https://doi.org/10.1016/S0141-0229(00)00234-9)
- Vaidyanathan VK, Selvaraj DK, Premkumar P, Subramanian S.** Screening and induction of laccase activity in fungal species and its application in dye decolorization. *Afr J Microbiol Res.* 2011 Jun 04;5(11):1261–1267. <https://doi.org/10.5897/AJMR10.894>
- Villena GK, Fujikawa T, Tsuyumu S, Gutiérrez-Correa M.** Structural analysis of biofilms and pellets of *Aspergillus niger* by confocal laser scanning microscopy and cryo scanning electron microscopy. *Bioresour Technol.* 2010 Mar;101(6):1920–1926. <https://doi.org/10.1016/j.biortech.2009.10.036>
- Wesenberg D, Buchon F, Agathos SN.** Degradation of dye containing textile effluents by the agaric white-rot fungus *Clitocybula dussenii*. *J Biotechnol.* 2002;24:989–993.
- Won SW, Vijayaraghavan K, Mao J, Kim S, Yun YS.** Reinforcement of carboxyl groups in the surface of *Corynebacterium glutamicum* biomass for effective removal of basic dyes. *Bioresour Technol.* 2009 Dec;100(24):6301–6306. <https://doi.org/10.1016/j.biortech.2009.07.063>