IJBPAS, June, Special Issue, 2023, 12(6): 24-45

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIODEGRADATION OF ACID BLUE-25 DYE BY LOCAL FUNGAL CONSORTIUM FOR SUSTAINABLE ENVIRONMENT

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Received 19th Jan. 2023; Revised 20th Feb. 2023; Accepted 3rd March 2023; Available online 15th June 2023

https://doi.org/10.31032/IJBPAS/2023/12.6.1010

ABSTRACT

Due to increasing of population and industrial use of chemicals and manmade activity water resources are highly polluted with different harm full contaminants. Synthetic dyes are utilized extensively in various textile industries and found frequently in the effluent discharge of industrial wastes in excess quantity which can influence the ecological damage in the surrounding environment. Treatment is mandatory for those industrial effluents before being discharge. Mycoremediation by local fungal consortium is one of the sustainable and eco-friendly techniques for fast, realistic and complete degradation of Acid Blue-25 dye in to nontoxic metabolites. The present study deals with the isolation of dye degrading fungal strains from the dye amended soils. Irrespective of their molecular structure, during the decolorization process, observed that the Anthraquinone (Acid Blue-25) dye first undergo chromophore cleavage by anthraquinone reductase. It was confirmed by some of the released metabolites involved were analyzed by spectroscopy, TLC, HPLC, GCMS, LCMS, FTIR, and NMR. The derived intermediate metabolites are to be segregated in a specific pattern during the dye decolorization and degradation. But a few of the derivatives were fused to central metabolic pathway at the time of degradation. The phytotoxic impact of Acid Blue-25 along with the biodegraded compounds were proved that, the resulted and extracted derivatives were nontoxic in nature, stimulate in good germination of *Phaseolus mungo* seeds. Further, it is confirmed that the existing fungal consortium-10 was very efficient in decolorization and degradation of anthraquinone dyes by converting the toxic pollutants to non toxic which are eco-friendly.

Keywords: Acid Blue-25, Mycoremediation, Biotransformation, Dyes, Textile effluents

INTRODUCTION

A large array of manmade dyes such as azo and anthraquinone dyes are often used in numerous industries like textile, plastic, leather, food and pharmaceutical because of ease of synthesis with minimal cost as well as the feature of being more stable compared to natural colors **[1]**. Among the total industries two third of the total production of dye waste is expelled from the textile industries, that is approximately 10-15% of the dye stuff fails to adhere to the fibers and released in to the environment as it is without proper treatment **[2, 3]**. Thus the major types of pollutants in textile effluents are suspended particles, detergents, unbound dye stuff and the impurities that leads to reduce penetration of sunlight that simultaneously leads to reduction in photosynthetic activity, lowered dissolved O_2, and also decline of water quality, further it contributes to ecological damage **[4]**. Nearly 17-20% of freshwater impurities loaded with effluents from textile industry **[5]**. Due to the fused aromatic structure of the dyes, not responding to degradation owing to its stability exists for long time in the nature **[6]**. The dye and its metabolites are released in the effluents are both carcinogenic and mutagenic for microorganisms and develop potential risks to human health **[7]**.

The regular/natural or general physicochemical methods, like physical methods i.e., coagulation or adsorption on activated carbon and chemical methods (electrolysis, advanced oxidation, reverse osmosis, and ozonation process), in order to decolorizing textile wastewater **[8]**. The above said methods having several limitations to put for practical use, i.e., these are not being economically feasible, removal of incomplete recalcitrant's and their metabolites, and huge generation of sludge, developing secondary pollution, and are very tricky to implement **[9]**. Since, most of the industrial effluents charged with dyes cannot be treated properly by these conventional methods and they remain undegraded for a considerable span of time in the environment, hence they are stable against the temperature, oxidizing substances and light.

Numerous experiments are conducted worldwide which are directed to the dye degradation process, the main focus of the present work is to investigate the technology based on a cost-effective and eco-friendly strategy **[10-13]**. The popular technologies employed for eco-friendly methods use bioremediation process. Bioremediation is a friendly process, where toxic waste materials, with a dye component are biologically transformed into less lethal or even harmless using variety of microbes

such as bacteria, fungi, algae etc. Most of the work regarding microbes and plants are widely been reported as agents of bioremediation **[14-17]**. The advantages of bioremediation process, compared with physical and chemical methods are less cost and do not produce extended contaminants **[18]**. In general, the application of bacteria during bioremediation, the synthetic dyes can create deterioration of metabolic products in the configuration of their colorless aromatic amines they quietly extended in poisonous nature than the parent compounds **[19, 20]**. In addition, to the above another appropriate limitation bacteria implementation in bioremediation that the diffusion of the substrate into the cells, hence the results are less optimal than fungi **[21]**. Currently, the work is focused on fungi and its enzymes as one of the most promising dye bioremediating agents. The fungi are considered to change a crucial part in decolorization and degradation of dyes. Many studies have already carried out regarding fungi and their enzymes for the bioremediation of synthetic dyes considered as myco-remediation **[22-24]**.

The main objective of the study is to employ the emerging technology using mycodecolorization & degradation strategy which is one the prominent remediation process of contaminated wastewater with dye. In comparison, then the physicochemical approaches, and biological treatments are more economical and generates less sludge and non-toxic products as metabolites **[25]**. The activity of microorganisms and their adaptability has to determine the efficacy of color removal process. This biodegradation mechanism has evolved out to be a prominent method as it completely decolorizes dye materials and transform into a non-toxic chemicals **[26, 27]**. Under stable aerobic, as well as anaerobic conditions the microorganisms, has higher degree of decolorization activity and degradation **[28]**. A huge variety of organisms are capable of destructing textile dyes are reported in the literature, but most of the studies focused on bacteria but not on the fungi **[29, 30]**.

Hence the present study mainly addressed the use of eco-friendly process of decolorization of Acid Blue-25 anthraquinone dye by fungal consortium and extracted the biodegraded products and evaluated its toxic nature of eluted compounds. The fungal consortium consisting of six strains, *Penicillium, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigates, Aspergillus tamarii* and *Aspergillus niger* are employed in the present study. These organisms were evaluated decolorize Acid Blue 25 under aerobic conditions. All the fungal strains were tested individually and evaluated

separately, which were found to be efficient dye decolorizers, while the consortium showed enhanced dye degradation ability as compared to the individual strains. The dye degradation was evaluated using UV–vis absorption spectroscopy, FT-IR, GC-MS, LC-MS, HPLC and NMR analysis. The phytotoxicity study was also used to assess the toxic intensity of the degraded products of Acid Blue-25 by using fungal consortium.

MATERIALS AND METHODS

Materials: Variety of chemicals used for experiments were analytical reagent grade. Acid Blue-25 (sulphonated anthraquinone) dye purchased from Sigma Pvt Limited, India and all the culture ingredients were purchased from Himedia Laboratories, India.

Study sites and Sample Collection

The sampling sites were selected around the textile industries located at Madhavaram, Chirala, Bapatla, Dharmavaram, Madanapalle, Hyderabad and Bangalore, India where effluent wastes were discharged directly into the environment. Both dye effluent samples and contaminated soil samples collected in sterile glass bottles and transported to the laboratory for further analysis.

Physico Chemical Analysis of Effluents: methodology

It is very important to test the soil samples before and after dye percolation around the textile industries. For all the collected dye amended soils from textile industries analyzed through physico-chemical parameters such as pH, Electro conductivity, BOD, COD, TSS, TDS, Chlorides, Sulphates, Phosphates and nitrates tested regularly for monitoring the contamination levels by standard protocols **[31]**.

Isolation and identification of efficient dye degrading fungal strains

The efficient dye degradable fungal cultures isolated from the collected samples by serial dilution method **[31]**. The appropriate dilutions were placed on SDA agar and incubated in BOD incubator at 28 ± 2^0C for 3-5 days. After incubation the selected fungal colonies were subjected for the pure culture development. The dye degrading efficiency of the pure culture was verified by following the standard protocols. The identified pure culture showing the maximum dye degradation efficacy and identified by its morphological as well as by molecular methods for conformation.

Consortium Development

Generally based on the performance a single culture was used for dye degradation studies, while a consortium comprising various fungal strains *A. terreus, A. flavus, A. fumigatus, A. tamarri, A. niger* and *Penicillium* sps developed by inculcating with similar ratio of fungal spores in to the medium. Meanwhile, we used to test the

antagonism activity between the identified fungi for the both growth and degradation activity process. Further a Petri-plate assay was carry out, in which the mentioned six fungal strains grown on a single petri-plate of SDA, incubated at 28^0C for 5 days.

Extraction and Analysis of Biotransformed Metabolites

The biotransformed byproducts of acid blue 25 dye by fungal consortium which was extracted by organic solvent extraction method using ethyl acetate and dried with anhydrous sodium sulphate and concentrated with rotor under reduced pressure and dried to powdered extract.

Dried extract material mixed in 10 ml of methanol and further analyzed using the different techniques like Uv-Vis spectrophotometer, (FTIR), Fourier Transform Infrared Spectroscopy (HPLC) High Performance Liquid Chromatography, (TLC) Thin Layer Chromatography, (GC-MS) Gas Chromatography–Mass Spectroscopy, (LC-MS) Liquid chromatography–Mass Spectrophotometer and (NMR) Nuclear Magnetic Resonance.

Phyto-Toxicity Studies

Phytotoxicity assay carried out with seeds of *Sorghum vulgare* and *Phaseolus mungo* prior to the treatment they were sterilized with 10% $HgCl_2$ for 10 min. It is conducted in three groups including pure dye treatment, as well as degraded dye metabolite treatment with control. Different concentrations were made for treatment for this assay was 100 to 1000ppm. All the seeds were initially kept for germinated in sterile petri plates containing filter paper soaked used for treatments (10 seeds/plate). All the germinated seeds were then planted in soil-containing clay pots, treatment continued for a period of 10 days and percentages of seed germination and phenotypic characteristics of plants including length of root and shoot etc. were observed for proximal changes **[32]**.

RESULTS AND DISCUSSION

Physico Chemical Analysis of Industrial Effluents

Industrial effluents were collected from different geographical sources such as Madhavaram, Chirala, Bapatla, Dharmavaram, Madhanapalle, Hyderabad and Bangalore. The results of the effluent obtained were compared with the standard values of the Federal Environmental Protection Agency (FEPA) for industrial effluents, World Health Organization (WHO) Guidelines for drinking water recommendations. **Table 1** contains the physicochemical properties of various water samples.

Isolation and Identification of Dye Degrading Fungi

Total 26 fungal cultures isolated from different study sites such as industrial areas

of Madhavaram, Chirala, Bapatla, Dharmavaram, Madhanapalle, Hyderabad and Bangalore, India. The selected fungal cultures were screened for dye degrading efficacy by inoculating in the medium enriched with different concentrations of acid blue 25 dye and incubated at 28-30^{0}C. The cultures which can degrade the high concentration of dye with less incubation time would be selected for future studies. The primary identification of fungal isolates was detected based on the microscopic and macroscopic characteristics. Lacto phenol cotton blue staining was performed so that better microscopic observations could be made instantly. Colony morphology, i.e., characteristics of mycelia, conidia, and conidiophores/fruiting bodies using light microscopy **[33]**. Later all the selected strains were confirmed at species level by 18s RNA sequencing patternand BLAST at NCBI. The Gene Bank Accession numbers of the selected strains arementioned in **Table 2.** The Scanning Electron Microscopic image analysis of identified fungal isolates in the present study is depicted in **Figure 1**. Aruna *et al*. **[34, 35]** isolated the bacterial strain which has high degrading nature and also constructed the bacterial consortium for the effective decolorization of Acid Blue-25.

Construction of Consortium

The present study was mainly focused at the development of fungal consortia by using the effective and potential strains isolated from dye amended effluents and their dye degrading optimization studies were conducted. Various parameters influenced on decolorization activity of anthraquinone dye, with these isolates, potential fungal consortia was constructed by using standard methods **[36]** and the optimization of several required parameters investigated according to the stands **[37, 38]**. Initially we tried with individual organisms and calculated their potentiality but the fungal consortium prominently showed maximum extent of dye decolourization with these lines we tried with several combinations at finally selected consortium-10. (Consortium-10 comprising of different organisms *Penicillium, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigates, Aspergillus tamarii* and *Aspergillus niger* and listed in **Table 3**.

Analysis of Acid Blue-25 Degradation Pathway by Fungal Consortium

The degradation studies of anthraquinone dye (AB-25) by fungal consortia were exclusively observed by TLC with pre coated silica gel. The gel plates were inoculated with Acid Blue-25 components obtained after the activity of consortium-10 by spotting and the results were shown in **Figure 2A**. From the **Figure 2A**, it was noticed that the screening of solvent system

(benzene: water 80:20) proved to be more effective in separating the dye metabolites. All the tested TLC plates were observed with different spots, the Rf values: 0.34, 0.40, 2.35, 3.40, 3.52 arevisualized under UV light (First TLC Plate). Henceforth these compounds were further confirmed based on the visibility. That is no visualized compounds of non-aromatic and none of them have amines. They are mostly preassumed as anthracenesulphonic acid, naphthalene dione, phenyl amine derivatives and mercapto anhtracenedione components having carbonyl group as chromophore and substitute of sulfonic acid. Further confirmation of these biodegradable products on TLC plates was obtained clear UV visible spectra by absorption spectrophotometer **(Figure 2B)**. No specific bands were observed for spots with un-inoculated sample (control) with only dye. Therefore, it has been an indication is that the obtained decolorization was only due to degradation of dye by fungal consortium.

FTIR is one of the prominent techniques usually employed for the detection of metabolites after degradation as shown in **Figure 3**. From the **Figure 3** it was observed that the acid blue-25 degradation by fungal consortium-10 showed very remarkable results by the development of peaks. The FTIR spectra of acid blue-25 with consortium-10 at 37^0C for 8 days incubation (**Figure 3A**), clearly showed with the control dye (**Figure 3B**) the specific peak observed at 3429.1cm^{-1} to 732.42cm^{-1} for the substituted benzene rings. In spite of these peaks other random peaks observed at 1031.03 cm^{-1} having C-N stretch aliphatic amines, 1224.59 cm^{-1} has also showed similarity to C-N stretch aliphatic amines. And 1411.06 cm^{-1} showed with C-H stretch bending alkanes, 1491.86 cm^{-1} C-C stretch aromatic compounds, 1585.36 cm^{-1} N-H bend primary amines 2099.63 cm^{-1} C triple bond & C stretching alkines.

As similar to the control, performed FTIR analysis for fungal consortium-10, in order to notify their maximum activity of decolorization and degradation. The peak at 1015.21cm^{-1} indicates C-N stretch amines, 1262.37 cm^{-1}alkayl halide, 1410.55, 1450.78 cm^{-1} for C-C stretching aromatic compounds and 1650.16 cm^{-1} showed the primary amine peaks. 2174.93 cm^{-1}(C-C stretch) alkines, 2836.13 cm^{-1} for (C=O stretch) aldehyde, 2947.13 cm^{-1} for (C-H stretching) alkines, 3338.2 cm^{-1} C-H stretching of alkines. Some of the peaks at C-N stretching of substituted with benzene ring derivatives showed the aromatic retained by the base dye, treated with fungal consortium-10. The change was also in peaks at 1015.2 cm^{-1} showed with untreated dye absorbent peak, but the similar peak observed after treatment with consortium

with a considerable shift of the peak (DATA NOT SHOWN).

Acid blue-25 anthraquinone dye treated with fungal consortia-10 for decolorization activity before and after decolorization and partial or complete degradation process results the biotransformation. Where the initial state of the dye components monitored by HPLC analysis and results were recorded in **Figure 4**. Similarly the **Figure 4** the HPLC analysis results of the control sample showed (**Figure 4B)** that there was a significant peak observed at 2.120 min of retention time and its corresponding retention and its 100% activity showed in the **Table 4**, in spite of these the other peaks were observed, which are negligible in nature. Similarly with the consortium-10 treated sample of acid blue-25 anthraquinone dye provides the degradation, monitored under shaking conditions. After 8 days of incubation the results were recorded in the **Figure 4A**. The HPLC chromatogram clearly showed that there are 6 to 8 prominent peaks observed at the retention time of 0.193 min, 2.602 min, 3.085 min, 3.517 min, 4.088 min, 4.398 min and 7.795 min showed in **Figure 4A**. In spite of the decolorization and degradation of acid blue-25 several polar derivatives viz aromatic, sulphonic and phenol complex were formed clearly. These components were confirmed by the unidentified peaks during the degradation because of partial or complete removal of dye/ chemical derivatives.

The complete analysis of the degradative compounds at the decolorization end of AB-25 by fungal consortium-10 was provided in the **Table 4**. It has been confirmed that the eluted compounds were extracted, purified and their bio-transformed compounds were analyzed by using GC-MS and LC-MS. The mass spectrum of the compound read at the peaks with the molecular mass in negative mode of ionization with the retention factors inclusive of time. The fragmentation peaks observed for all at different molecular mass of retention value with broad peaks of 149,207 (retention time 15.193) and 325,207 at (retention time 11.995). Similarly the small peaks at 73 (retention time-6.573) and 207, 101 at the retention time of 4.120 the reference to the control, analysis of the dye sample (AB-25) (**Figure 5B**). The degradative content of AB-25 after partial and complete decolorization observed for the biotransformed and their derived compounds by GC-MS and LC-MS analysis showed in **Figure 5 and 6.** It was observed that the chromatogram with varied retention time factors of individual fungi tested at the initial stage but consortium 10 ensuring the degradation peaks observed at 4.350 (346.08), 5.410 (421.6), 6.481 (394.8), 6.730 (365.6) and recorded the mass

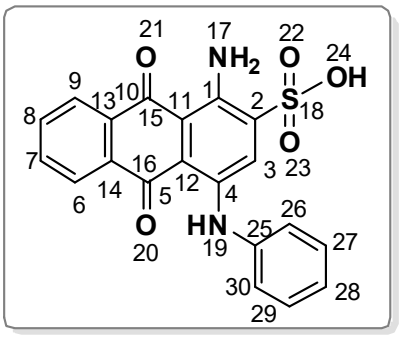
spectrum after 8 days of incubation.The mass spectrum of retention time peak at 4.350, instantly showed a molecular mass of 346.08 which corresponds to 1-amino, 2-mercapto-4(phenyl amino) anthracene 9, 10dione (DATA NOT SHOWN).

In comparison there is a drastic difference was observed at GC-MS analysis of individual and consortium respectively. Since the individual organisms degradative analysis resulted as mentioned below that is the molecular mass of 365.60 corresponding to 1, amino,5,8 dioxo 4-phenylamino,5,8 dihydro naphthalene 2 sulphonate, and the molecular mass of 394.8 (6.480) showed 1-amino, 9,10 dioxo-4-phrnyl amino, 9-10 dihydroanthracene-2-sulphonic acid. Similarly, the retention time of 5.402 peak observed with the molecular mass with corresponding structures of (M-Na) i.e. 1-amino, 9,10 di hydroxyl, 4-phenyl amino 9,10 diohydro anthracene-2 sulfonic acid with 421-60 molecular mass. The other compounds formed at the retention time of 4.350 with 346.0 it is corresponding to the structure of 1-amino 2 maercpto4-phenyl amino anthracene 9-10 dione with the same time (Data not shown). There are 1-amino 2-mercapto anthracene 9-10 dione, 1-amino 5,8 dioxo4-phenylamino 5-8-dihydo 6-7 dehydronapthalene-2-sulfonic acid compounds derived, from the dimerisation of 4-phenyl amino 9-10 dihydroanthracene 2-sulphonic acid (**Figure 8**). We performed the experiments with fungal consortium-10 which is an indicator agent for maximum decolorization and degradation. So the GC-MS and LC-MS analysis of the fungal consortium-10 placed in **Figure 5 and 6**. From the **Figure 6**, it has been identified that, the biotransformed compounds with their corresponding molecular mass at an appropriate retention time of the 3.280 (323.7) 5, 5a, 5b, 11, 11a, 11b, octahydro dihydrobenzo biphenylene 5, 611,12 tetrol, retention time 4.430 (316.9) has 5a, 5b, 11a, 11b tetrahydrodibenzo (b,h) biphenele 5, 6, 11, 12 tetrone, retention time 5.390 (367.9) sodium salt of 1 amino 5,8 dioxo 4-phenyl amino 5,8 dihydronapthalene-2-sulphonate, retention time at 6.48 (394.0) 1-amino 9,10 dioxo-4-phenyl amino 9,10 dihydro antracene-2-sulphonic acid, retention time of 6.730 (303.8) 1-amino 9,10 dioxo 9,10 dihydroanthracene -2-sulphonic acid formed. Meanwhile, there are two dimerised compounds been identified during the analysis and their corresponding structures were marked with 1-amino-2-marcapto-4-(phenyl amino) anthracene 9,10 dione and 1 amino 9, 10 dihydroxy 9, 10, dihydroanthracene 2-sulphonate-4 ammonium cation showed in **Figure 8**.

However, with these derived compounds and their possible state of retention time strategies were further confirmed by ^{1}H

NMR with negative mode of ionization. There are few identified structures of the dye fragments by *Aspergillus niger* are characterized by ^{1}H NMR spectral characterization (**Figure 7**).

Similarly observed with fungal consortium-10:



^{1}H NMR (400MHz, DMSO-d_6) δ: 8.00-7.96 (m, 1H, SO_3H), 7.25-7.21 (m, 6H, Ar-H), 6.38-6.30 (m, 4H, Ar-H), 4.63 (S, 1H, -NH), 4.35-4.24 (m, 2H, -NH_2);

Interpretation of ^{1}H NMR spectra reveals that the singlet peak for -NH-aryl (20) is observed at 4.63 (H-19 [-NH], 1H), a multiplet peak for -NH_2 (17) is observed at 4.35-4.24 ([-NH_2], 2H) and the remaining 10 aromatic protons were observed in their corresponding regions as multiplet at 7.25-7.21 (6H, Ar-H) and 6.38-6.30 (4H, Ar-H), finally the characterization peak of the sulfonic acid is observed as multiplet at 8.00-7.96 (1H, SO_3H) showed in **Figure 7B**. In case of acid blue 25 anthraquinone based compound, after treatment with fungal consortium 10, a strong peak was observed with molecular mass value of 394.0 showed the corresponding structure 1-amino-9, 10-dioxo-9, 10-di hydroanthracene-2-sulfonic acid which confirmed that the degradation was carried out by means of peroxidase enzyme (**Figure 8**). In the primary cleavage C=O groups were splits from the base structure having molecular mass of 416.38. Previously, **[39]** proposed the similar method of cleavage pattern, but no such peaks were observed with reactive black B and reactive orange 16, because of vinyl sulfone groups **[40, 41]** also studied on reactive orange 16, no metabolite could be observed. That indicates every microbial strain has its own substrate affinity, thus ensuring that different strain with same substrate could be different. Presence of O-H and amino groups shows the different reactivity for cleavage i.e. lower molecular weight aromatic compounds including phenols were detected and during dye decolorization by GC-MS.

None of the similar compounds were detected after biodegradation by fungi **[42]**. In fact some of the investigations were reported that high molecular weight polymer formed by coupling reaction by formed by products of chromophores groups. In the present study we observed few of dimeric coupling products. These results are in contrast with **[43]** that oxygen can interact

with free radical mechanism with aromatic compounds. Kandelbaurer*et al*. **[44]**, Kalme*et al*. **[45]** have reported the similarly with different fungal strain. According to the Tan *et al*. **[46]** reported that fungal degradation did not require oxygen for reeducates as a key factor.

In many studies noticed that despite of oxygen present in the environment the degradation could be performed linkage of oxygen was insensitive to reductases adopted in aerobic fungal degradation. But in anaerobic conditions aromatic amines sulphonic acids as substrate, due to resistance. No further degradation was processed. As a result of these reactions catacheol sulfonates have been identified as intermediates **[47, 48]**.

The present study was concluded that peroxidases acts as first part and amino derivatives are formed on one side and anthracene derivatives on other side. With further aerobic degradation C-N bond would be splitted to the phenolic compounds naphthyl derivatives. Phenolic compounds were easily mineralized to aromatic carboxylic acid through destruction of ring opening. Further these carboxylic acids could be changed to carbondioxide, water leading to complete mineralization of organic dye molecules.

Phytotoxic Studies

Phytotoxic effect of dyes and its degradative products of fungal consortium-10 was studied by measuring the plumule and radical in both control as well as dye and dye metabolites treated groups. We observed the maximum plumule and radical in plant i.e. 15cm, 2cm and 6 cm, 9 cm in *Phaseolus mungo and Sorghum vulgare* respectively in water control group. The size of the plumule and the radical was drastically decreased with 5.1 cm, 0.5 cm and 3cm, 6.5 cm in *Phaseolus mungo and Sorghum vulgare* respectively in acid blue-25 treated group. Whereas the size of plumule and radical was normal like in control group. In case of dye degradative products treated plants i.e. 12 cm, 1.4cm and 5.3 cm, 8 cm in *Phaseolus mungo and Sorghum vulgare* respectively **(Figure 9 and Table 5)**.

The bioassay for dye toxicity was based on measuring the effect of acid blue 25 dye on agriculturally beneficial microorganisms and on plant growth [49]. Similarly Parshetti *et al*. **[50]** reported that *Azotobacter vinelandii, Pseudomonas aeuroginosa* were nontoxic nature of the degradation products. Ayed *et al*. **[51]** reported the phytotoxicity of different soluble textile dyes estimated by measuring the relative changes in germination of seeds. The results showed the selected dye concentration of degradative products was nontoxic to seed germination when compared to that of

control. Rene*t al*. **[52]** reported the toxicity of polycyclic aromatic hydrocarbons (PAHs), Anthracene (ANT) and Fluoranthene to the duckweed *Lemna gibba L*. and *Brassica napus L.* seeds, used for the germination efficiency, root and shoot growth and chlorophyll content, as a base measurement for toxicity. The results were suggested that the low concentration of dye was found less toxic to seed germination and more toxic when dye concentration increased significantly **[53]**. Chen *et al*. **[54]**, intimated that the antimicrobial activity of crystal violet using degradation produces by *E. coli*. From their reports it was earmarked that after appropriate incubation the products were separated from >98% decolorized material, because of no toxicity. *E.coli* grown a 37^0C for 48hrs and showed the extent of culture mass as good as with normal bacteriological media **[55]**. Saratale *et al*. **[56]** showed the phytotoxic effect of Navy Blue HER on the germination of *Phaseolus mungo* and *Sorghum vulgare*. Both the plant seeds inhibited 90% germination when seeds were treated with 1500ppm concentration of Navy blue HER. On the contrary, no phytotoxic effect (100% germination) was observed at the same concentration of degradation products.

The developed consortium-(*Bacillus flexus* TS8 (BF), *Proteus mirabilis* PMS (PM), and *Pseudomonas aeruginosa* NCH (PA) showed an increased decolorization of Indanthrene Blue RS dye with an Average decolorization rate of 11,088 µg h^{-1} within 9h, compared to the individual strains under aerobic conditions **[57]**. Rania *et al*. **[58]** *S. halophilus* SSA1575 was isolated from the wood-feeding termite *Reticulitermes chinenesis* and evaluated its dye degrading efficiency which degrade of Reactive Black 5 in elevated concentrations with high salinity conditions and might be promising halo tolerant yeast valued for the treatment of various textile effluents with high salinity. We, therefore, conclude from this study that the textile effluents containing Acid Blue-25 dye treated with fungal consortium cultures resulted in the complete degradation of the dye with the treated effluent being used for agri-irrigation that indicates the nontoxic nature of metabolite products.

Table 1: Analysis of physico-chemical parameters of textile effluents

S/N	pH	EC S/cm	BOD Mg/L	COD Mg/L	TSS Mg/L	TDS Mg/L	Chloride Mg/L	Sulphates Mg/L	Phosphates Mg/L	Nitrates Mg/L
S1	7.8	2.3	2300	5300	130	4652	1780	4300	51.3	124.3
S2	8.9	5.4	3202	4600	250	2364	950	2530	24.3	202.4
S3	9.0	4.3	4210	1300	378	3876	2750	4100	13.5	133.5
S4	7.8	3.0	8440	800	290	7072	1540	3800	35.4	403.2
S5	7.1	1.8	1050	700	650	4312	2167	3400	55.6	523.6
S6	7.0	4.1	1600	1600	340	2260	1020	2342	52.4	143.4
S7	6.5	2.5	2312	2800	430	8090	1575	2700	35.4	205.4
S8	7.0	1.3	1200	2652	463	3428	1245	3220	24.0	222.5
S9	7.4	4.9	1502	2500	290	3980	1800	2302	21.3	523.5
S10	8.0	5.2	1000	2400	260	3618	1548	2500	38.7	444.5
S11	9.0	4.0	2300	3602	320	2530	1800	3400	45.0	742.4

S12	7.5	2.9	700	2512	420	3200	2000	4100	24.5	123.2
S13	8.0	4.1	1200	1600	850	2130	1880	4200	45.0	326.3
S14	7.5	2.7	3400	3200	1230	1860	1500	1820	56.1	128.3
S15	8.0	3.2	5100	800	880	1400	1300	2344	45.0	222.4
WHO	6-9	5.2	50	150	100	1200	20	20	10	9
Mean	7.72	3.44	2634.4	2424	478.7	3651.4	1657.2	3137.3	37.8	297.9

Table 2: The selected dye degrading fungal strains and their accession numbers

S/N	Description	Accession number
1	*Aspergillus terreus* 31TUHQ219673.1	HQ219673.1
2	*Aspergillus flavus strain AJ* 31	HQ324118.1
3	*Aspergillus fumigatus strain YHYS*	KC986235.1
4	*Aspergillus tamarii strain SRRC 1088*	AY373870.1
5	*Aspergillus niger strain MCAS2*	KM103363.1
6	*Penicillium sps*	MCC 1031

Table 3: Decolorization of Acid Blue-25 by different potential fungal consortia. P= *Penicillium, 1= Aspergillus terreus, 2= Aspergillus flavus, 3= Aspergillus fumigates, 4= Aspergillus tamarii and 5= Aspergillus niger.*

S/N	Members of Fungal Consortium	% Degradation of Acid Blue-25
1	5+P+1+3	86.6
2	P+1+2+4	76.7
3	P+1+2+5	87.2
4	1+2+3+4+5	89.0
5	1+2+3+4+6	90.0
6	2+3+4+5+6	92.0
7	3+4+5+6+1	91.0
8	4+5+6+1+2	91.0
9	5+6+1+2+3	94.0
10	1+2+3+4+5+6	96.0

Table 4: Analysis of LCMS fragments of acid bue-25 formed by the fungal consortium-10

S/N	Fragment	Retention Time	Height	Area	%	Molecular Weight
1	A	3.28	45601	4914	1.67	323.7
2	B	4.43	269605	20060	6.84	316.9
3	C	5.39	1304377	129746	44.23	367.9
4	D	6.47	915933	81735	27.86	394.0
5	E	6.73	96336	8935	3.05	303.8

Table 5: Phytotoxic effect of Acid Blue-25 dye and its metabolite on growth of *Phaseolus mungo and Sorghum vulgare*

S/N	Parameter	*Phaseolus mungo*			*Sorghum vulgare*		
		Water	Acid Blue-25	Biotransformed products	Water	Acid Blue-25	Biotransformed products
1	Germination (%)	90	30	70	100	60	90
2	Plumule (cm)	15	5.1	12	6	3	5.3
3	Radical (cm)	2	0.5	1.4	9	6.5	8

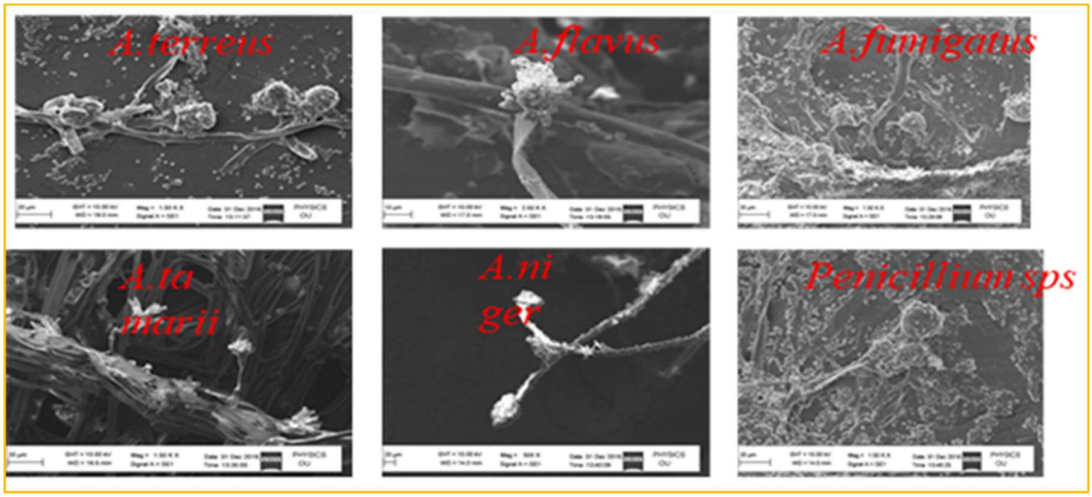


Figure 1: Scanning Electron Microscopic image analysis of selected fungal isolates in the present study. A) *Aspergillus terreus*, B) *Aspergillus flavus strain AJ*, C) *Aspergillus fumigatus strain YHYS*, D) *Aspergillus tamarii strain SRRC 1088*, E) *Aspergillus niger strain MCAS2*, F) *Penicillium* sps

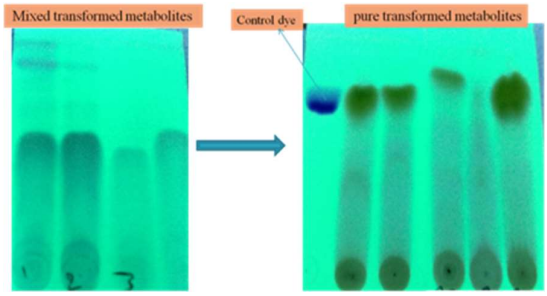


Figure 2: A) Identification of Acid Blue 25 dye metabolites by TLC

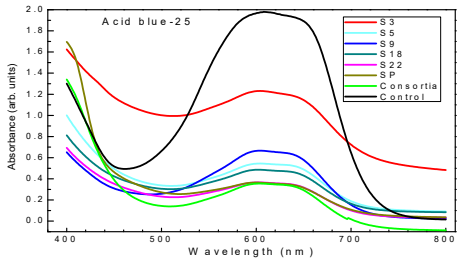


Figure-2: B) UV-Visible spectra showing degradation of Acid Blue 25

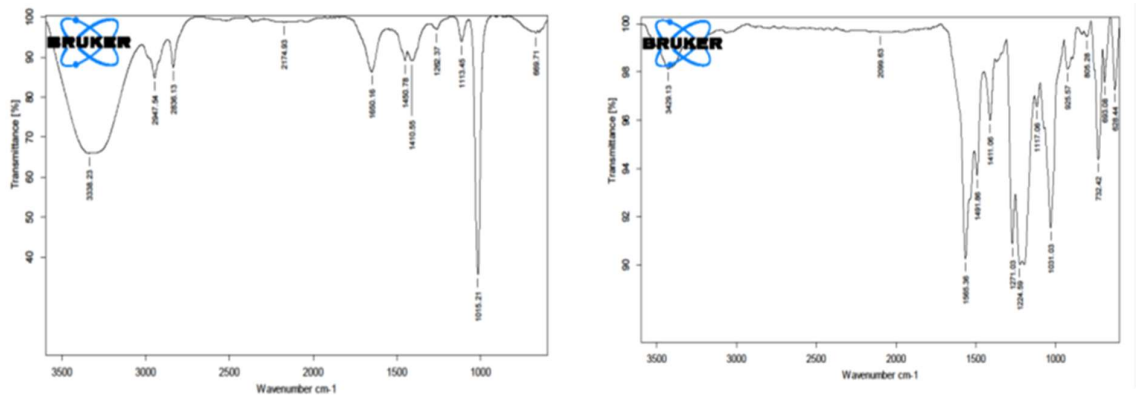


Figure 3: FTIR spectrum of Acid Blue 25 dye

A) Fungal consortium **B) Control dye**

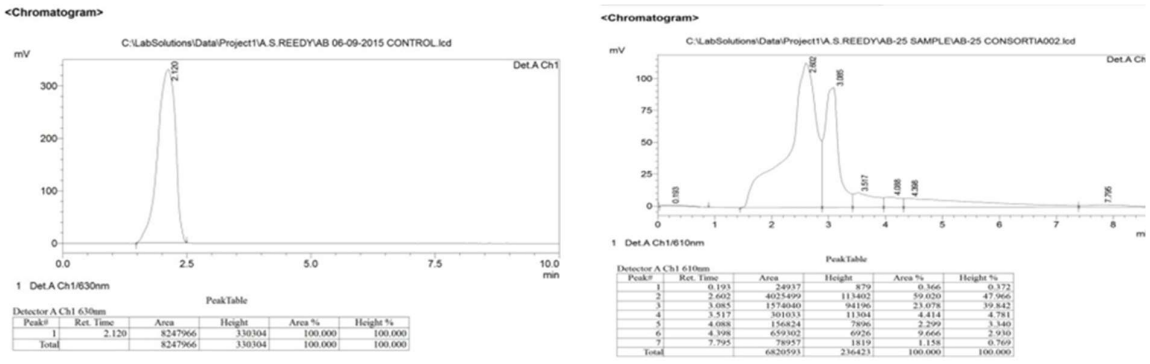


Figure 4: HPLC analysis of Acid Blue 25 dye

A) Fungal consortium **B) Control dye**

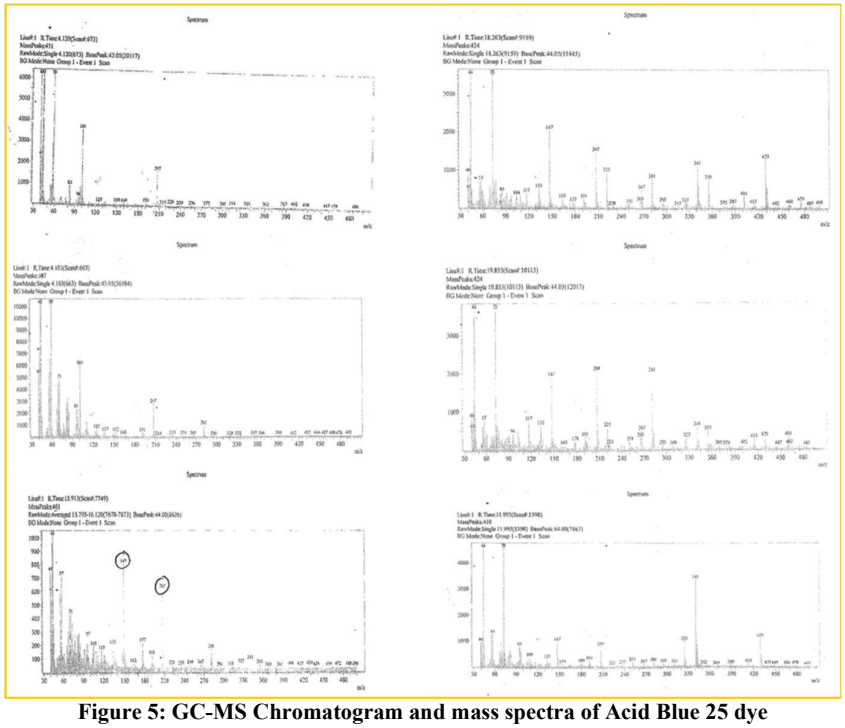


Figure 5: GC-MS Chromatogram and mass spectra of Acid Blue 25 dye
A) Control dye B) Degraded by fungal consortium

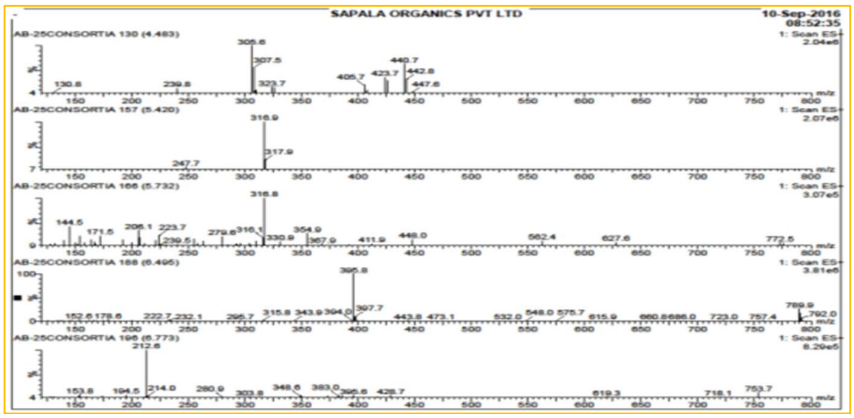


Figure 6: LC-MS Chromatogram and mass spectra of Acid Blue 25 by fungal consortium

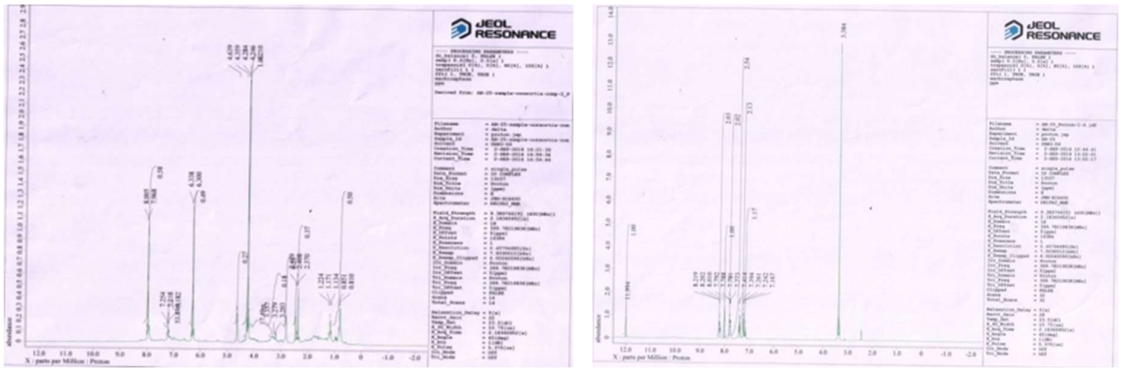


Figure 7: NMR analysis of Acid blue 25 dye
A) Control Dye B) Degraded by fungal consortium

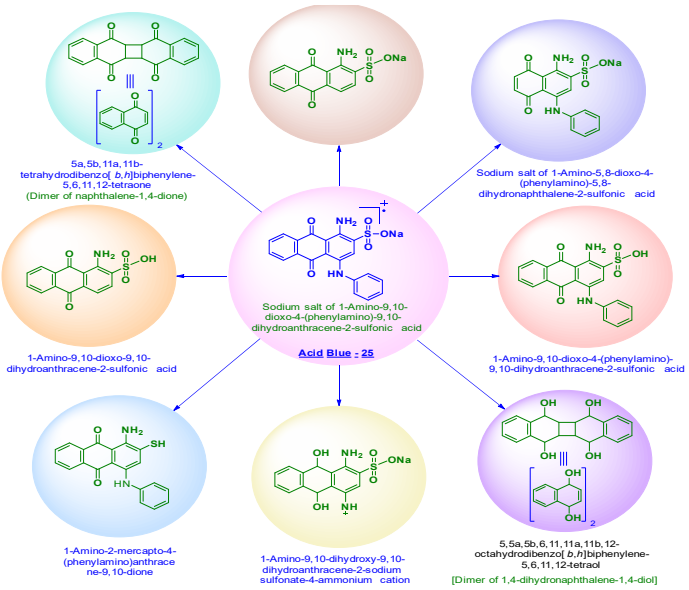


Figure 8: Proposed degradation metabolites of Acid Blue 25 by fungal consortium

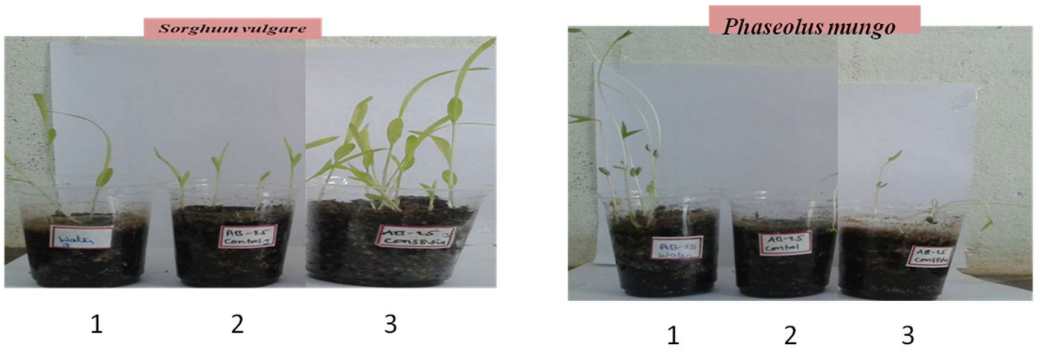


Figure 9: Phytotoxic effects of Acid blue-25 dye and its metabolite on plant growth
A) *Sorghum vulgare* **B) *Phaseolus mungo***
1) Water control, 2) Acid Blue-25 dye control, 3) Dye metabolites

CONCLUSIONS

Mycoremidiation is one of the broad techniques which employ the fungai and its derivatives used to decontaminate the dye amended environments of textile industry. This technique has been proven an extensive chief/cost effective ecofriendly. It has the advantage over other conventional methods that is microbial agents (fungi) degrading dye elements such as poly cyclic aromatic compounds. Future strategies are employed to improve mycoremidiation as suggested frame work to address the global challenges. In the present study the developed fungal consortium which consists of *Aspergillus terreus, Aspergillus flavus strain AJ, Aspergillus fumigatus strain YHYS, Aspergillus tamarii strain SRRC 1088, Aspergillus niger strain MCAS2, Penicillium* sps are efficient degradative

combination proven the biologically significant one. The derivatives of the anthraquinone dye Acid Blue-25 are tested as nontoxic.

ACKNOWLEDGMENTS

The authors are gratefully acknowledged Yogi Vemana University for facility and the Department of Biotechnology (DBT) for funding major research project. No.BT/PR13941/BCE/08/807/2010.

REFERENCES

[1] Chang, J.S., & Lin, C.Y. (2001). Decolorization kinetics of a recombinant *Escherichia coli* strain harboring azo-dye-decolorizing determinants from Rhodococcus sp. *Biotechnology Letters*, *23*, 631-636.

[2] Solis, M., Vaughn, S., Swanson, E., & Mcculley, L. (2012). Collaborative models of instruction: The empirical foundations of inclusion and co-teaching. *Psychology in the Schools*, *49*(5), 498-510.

[3] Uddin, F. (2021). Environmental hazard in textile dyeing wastewater from local textile industry. *Cellulose*, *28*(17), 10715-10739.

[4] Dhruv Patel, D., & Bhatt, S. (2022). Environmental pollution, toxicity profile, and physico-chemical and biotechnological approaches for treatment of textile wastewater. *Biotechnology and Genetic Engineering Reviews*, *38*(1), 33-86.

[5] Al Abbad, E., & Alakhras, F. (2020). Removal of dye acid red 1 from aqueous solutions using chitosan-iso-vanillin sorbent material. *Indonesian Journal of Science and Technology*, *5*(3), 352-365.

[6] Xu, A., & Wang, F. (2020). Carboxylate ionic liquid solvent systems from 2006 to 2020: Thermal properties and application in cellulose processing. *Green Chemistry*, *22*(22), 7622-7664.

[7] Bhatnagar-Mathur, P., Devi, M.J., Reddy, D.S., Lavanya, M., Vadez, V., Serraj, R., ... & Sharma, K.K. (2007). Stress-inducible expression of At DREB1A in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. *Plant Cell Reports*, *26*, 2071-2082.

[8] Forgacs, E., Cserháti, T., & Oros, G. (2004). Removal of synthetic dyes from wastewaters: a review. *Environment International*, *30*(7), 953-971.

[9] Zhang, F., Yediler, A., Liang, X., & Kettrup, A. (2004). Effects of dye additives on the ozonation process and oxidation by-products: a comparative study using hydrolyzed CI Reactive Red 120. *Dyes and Pigments*, *60*(1), 1-7.

[10] Lim, S. L., Chu, W. L., & Phang, S. M. (2010). Use of *Chlorella vulgaris* for

bioremediation of textile wastewater. *Bioresource Technology*, *101*(19), 7314-7322.

[11] Ji, Q., Liu, G., Zhou, J., Wang, J., Jin, R., & Lv, H. (2012). Removal of water-insoluble Sudan dyes by *Shewanella oneidensis* MR-1. *Bioresource Technology*, *114*, 144-148.

[12] Lade, H., Govindwar, S., & Paul, D. (2015). Low-cost biodegradation and detoxification of textile azo dye CI reactive blue 172 by *Providencia rettgeri* strain HSL1. *Journal of Chemistry*, *2015*.

[13] Molla, A. H., & Khan, H. I. (2018). Detoxification of textile effluent by fungal treatment and its performance in agronomic usages. *Environmental Science and Pollution Research*, *25*(11), 10820-10828.

[14] Merino-Restrepo, A., Mejía-Otálvaro, F., Velásquez-Quintero, C., & Hormaza-Anaguano, A. (2020). Evaluation of several white-rot fungi for the decolorization of a binary mixture of anionic dyes and characterization of the residual biomass as potential organic soil amendment. *Journal of Environmental Management*, *254*, 109805.

[15] Toker, S. K., Evlat, H., & Koçyiğit, A. (2021). Screening of newly isolated marine-derived fungi for their laccase production and decolorization of different dye types. *Regional Studies in Marine Science*, *45*, 101837.

[16] Syafiuddin, A., & Fulazzaky, M.A. (2021). Decolorization kinetics and mass transfer mechanisms of Remazol Brilliant Blue R dye mediated by different fungi. *Biotechnology Reports*, *29*, e00573.

[17] Pecková, V., Legerská, B., Chmelová, D., Horník, M., & Ondrejovič, M. (2021). Comparison of efficiency for monoazo dye removal by different species of white-rot fungi. *International Journal of Environmental Science and Technology*, *18*, 21-32.

[18] Ergene, A., Ada, K., Tan, S., & Katırcıoğlu, H. (2009). Removal of Remazol Brilliant Blue R dye from aqueous solutions by adsorption onto immobilized *Scenedesmus quadricauda*: Equilibrium and kinetic modeling studies. *Desalination*, *249*(3), 1308-1314.

[19] Saratale, R.G., Gandhi, S.S., Purankar, M.V., Kurade, M.B., Govindwar, S.P., Oh, S.E., & Saratale, G.D. (2013). Decolorization and detoxification of sulfonated azo dye CI Remazol Red and textile effluent by isolated Lysinibacillus sp. RGS. *Journal of Bioscience and Bioengineering*, *115*(6), 658-667.

[20] Dellamatrice, P.M., Silva-Stenico, M.E., Moraes, L.A.B.D., Fiore, M.F., & Monteiro, R.T.R. (2017). Degradation of textile dyes by cyanobacteria. *Brazilian Journal of Microbiology*, *48*, 25-31.

[21] Munck, C., Thierry, E., Gräßle, S., Chen, S.H., & Ting, A.S.Y. (2018). Biofilm formation of filamentous fungi Coriolopsis sp. on simple muslin cloth to enhance removal of triphenylmethane dyes. *Journal of Environmental Management*, *214*, 261-266.

[22] Kulla, H.G., Klausener, F., Meyer, U., Lüdeke, B., & Leisinger, T. (1983). Interference of aromatic sulfo groups in the microbial degradation of the azo dyes Orange I and Orange II. *Archives of Microbiology*, *135*, 1-7.

[23] Banat, I. M., Nigam, P., Singh, D., & Marchant, R. (1996). Microbial decolorization of textile-dyecontaining effluents: a review. *Bioresource Technology*, *58*(3), 217-227.

[24] Tomasini, A., & León-Santiesteban, H.H. (2019). The role of the filamentous fungi in bioremediation. In *Fungal Bioremediation* (pp. 3-21). CRC Press.

[25] Kalyani, D.C., Patil, P.S., Jadhav, J.P., & Govindwar, S.P. (2008). Biodegradation of reactive textile dye Red BLI by an isolated bacterium Pseudomonas sp. SUK1. *Bioresource Technology*, *99*(11), 4635-4641.

[26] Thakur, M. C., Khan, A., & Doshi, H. (2012). Isolation and screening of dye degrading micro-organisms from the effluents of dye and textile industries at Surat. *American Journal of Environmental Engineering*, *2*(6), 152-159.

[27] Deepti, G., & Isha, J. (2014). Microbial decolourization of dye reactive blue 19 by bacteria isolated from dye effluent contaminated soil. *International Journal of Current Microbiology and Applied Sciences*, *3*(9), 913-922.

[28] Daneshvar, N., Ayazloo, M., Khataee, A.R., & Pourhassan, M. (2007). Biological decolorization of dye solution containing Malachite Green by microalgae Cosmarium sp. *Bioresource Technology*, *98*(6), 1176-1182.

[29] Junnarkar, N., Murty, D.S., Bhatt, N.S., & Madamwar, D. (2006). Decolorization of diazo dye Direct Red 81 by a novel bacterial consortium. *World Journal of Microbiology and Biotechnology*, *22*, 163-168.

[30] Joshi, T., Iyengar, L., Singh, K., & Garg, S. (2008). Isolation, identification and application of novel bacterial consortium TJ-1 for the decolourization of structurally different azo

dyes. *Bioresource Technology*, *99*(15), 7115-7121.

[31] Aneja, K.R. (2007). *Experiments in microbiology, plant pathology and biotechnology*. New Age International.

[32] Rao, K.B., & Prasad, A.A. (2014). Biodecolourisation of azo dye reactive red 22 by *Bacillus infantis* strain AAA isolated from seawater and toxicity assessment of degraded metabolites. *Nature Environment and Pollution Technology*, *13*(2), 369.

[33] St-Germain, G., & Summerbell, R. (1996). Identifying filamentous fungi: a clinical laboratory handbook. Star Publishing Company, Singapore.

[34] Aruna, B., Rathna Silviya, L., Shiva Kumar, E., Srinu, A., Roja Rani, P., Vijaya Lakshmi, D. & Durbaka V.R Prasad. (2015). Biodecolorization of Anthraquinone Textile (Acid Blue 25) dye by Klebsiella Sp. *International Journal of Recent Scientific Research, 6*(3), 3216-3222.

[35] Aruna, B., Silviya, L.R., Kumar, E.S., Rani, P.R., Prasad, D.V., & Vijaya Lakshmi, D. (2015). Decolorization of Acid Blue 25 dye by individual and mixed bacterial consortium isolated from textile effluents. *International Journal of Current Microbiology and Applied Sciences*, *4*(5), 1015-1024.

[36] Karunya, A., Rose, C., & Valli Nachiyar, C. (2014). Biodegradation of the textile dye Mordant Black 17 (Calcon) by *Moraxella osloensis* isolated from textile effluent-contaminated site. *World Journal of Microbiology and Biotechnology*, *30*, 915-924.

[37] Ottoni, C., Simões, M.F., Fernandes, S., Santos, C.R., & Lima, N. (2016). High laccase expression by Trametes versicolor in a simulated textile effluent with different carbon sources and pHs. *International Journal of Environmental Research and Public Health*, *13*(8), 778.

[38] Durruty, I., Fasce, D., González, J.F., & Wolski, E.A. (2015). A kinetic study of textile dyeing wastewater degradation by *Penicillium chrysogenum*. *Bioprocess and Biosystems Engineering*, *38*, 1019-1031.

[39] Telke, A., Kalyani, D., Jadhav, J., & Govindwar, S. (2008). Kinetics and Mechanism of Reactive Red 141 Degradation by a Bacterial Isolate *Rhizobium radiobacter* MTCC 8161. *Acta Chimica Slovenica*, *55*(2).

[40] Svobodová, K., Senholdt, M., Novotný, Č., & Rehorek, A. (2007). Mechanism of Reactive Orange 16 degradation with the white rot fungus *Irpex*

lacteus. *Process Biochemistry*, *42*(9), 1279-1284.

[41] Novotný, Č., Trošt, N., Šušla, M., Svobodová, K., Mikesková, H., Válková, H., ... & Pavko, A. (2012). The use of the fungus Dichomitus squalens for degradation in rotating biological contactor conditions. *Bioresource Technology*, *114*, 241-246.

[42] Novotný, Č., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., & Šašek, V. (2004). Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. *Soil Biology and Biochemistry*, *36*(10), 1545-1551.

[43] Field, M.S., Wilhelm, R.G., Quinlan, J.F., & Aley, T.J. (1995). An assessment of the potential adverse properties of fluorescent tracer dyes used for groundwater tracing. *Environmental Monitoring and Assessment*, *38*, 75-96.

[44] Kandelbauer, A., Maute, O., Kessler, R.W., Erlacher, A., & Gübitz, G.M. (2004). Study of dye decolorization in an immobilized laccase enzyme-reactor using online spectroscopy. *Biotechnology and Bioengineering*, *87*(4), 552-563.

[45] Kalme, S., Ghodake, G., & Govindwar, S. (2007). Red HE7B degradation using desulfonation by Pseudomonas desmolyticum NCIM 2112. *International Biodeterioration and Biodegradation*, *60*(4), 327-333.

[46] Tan, N.CG., Prenafeta-Boldu, F.X., Opsteeg, J.L., Lettinga, G., & Field, J.A. (1999). Biodegradation of azo dyes in cocultures of anaerobic granular sludge with aerobic aromatic amine degrading enrichment cultures. *Applied Microbiology and Biotechnology*, *51*, 865-871.

[47] Mohorčič, M., Teodorovič, S., Golob, V., & Friedrich, J. (2006). Fungal and enzymatic decolourisation of artificial textile dye baths. *Chemosphere*, *63*(10), 1709-1717.

[48] Li, J., Galley, M., Brockett, C., Spithourakis, G.P., Gao, J., & Dolan, B. (2016). A persona-based neural conversation model. *arXiv preprint arXiv:1603.06155*.

[49] Novotny, V., Drozd, P., Miller, S.E., Kulfan, M., Janda, M., Basset, Y., & Weiblen, G.D. (2006). Why are there so many species of herbivorous insects in tropical rainforests?. *Science*, *313*(5790), 1115-1118.

[50] Parshetti, G., Kalme, S., Saratale, G., & Govindwar, S. (2006). Biodegradation of Malachite Green by *Kocuria rosea* MTCC 1532. *Acta Chimica Slovenica*, *53*(4).

[51] Ayed, L., Cheriaa, J., Laadhari, N., Cheref, A., & Bakhrouf, A. (2009). Biodegradation of crystal violet by an isolated Bacillus sp. *Annals of Microbiology*, *59*, 267-272.

[52] Ren, Z., Chen, B., Ma, Z., & Xu, G. (1996). One-proton halo in P 26 and two-proton halo in S 27. *Physical Review C*, *53*(2), R572.

[53] Durve, A.A., Gupta, A.R., & Naphade, S.R. (2012). Decolourisation of Textile Dyes and Biological stains by Bacterial strains isolated from Industrial effluents. *Advances in Applied Science Research*, *3*(5), 2660-2671.

[54] Chen, J.Y., Bottjer, D.J., Oliveri, P., Dornbos, S.Q., Gao, F., Ruffins, S., ... & Davidson, E.H. (2004). Small bilaterian fossils from 40 to 55 million years before the Cambrian. *Science*, *305*(5681), 218-222.

[55] Joshi, C., Mathur, P., & Khare, S.K. (2011). Degradation of phorbol esters by Pseudomonas aeruginosa PseA during solid-state fermentation of deoiled *Jatropha curcas* seed cake. *Bioresource technology*, *102*(7), 4815-4819.

[56] Saratale, R.G., Saratale, G.D., Chang, J.S., & Govindwar, S.P. (2009). Decolorization and biodegradation of textile dye Navy blue HER by *Trichosporon beigelii* NCIM-3326. *Journal of Hazardous Materials*, *166*(2-3), 1421-1428.

[57] Mohanty, S.S., & Kumar, A. (2021). Enhanced degradation of anthraquinone dyes by microbial monoculture and developed consortium through the production of specific enzymes. *Scientific Reports*, *11*(1), 7678.

[58] Rania, M., Petersen, L. V., Benros, M. E., Liu, Z., Diaz, L., & Bulik, C. M. (2020). Psychiatric comorbidity in individuals with bullous pemphigoid and all bullous disorders in the Danish national registers. *BMC Psychiatry*, *20*(1), 1-10.