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Exploring indigenous Fungi for sustainable bioremediation of textile Dye

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ABSTRACT

Textile dyes are considered as major environmental pollutants due to their complex chemical structures and resistance to natural degradation. In the present work fungal isolates were isolated and evaluated for biodegradation and decolourization of textile dyes for remediation purpose. Two fungal isolates *Aspergillus niger* and *Aspergillus flavus* were tested for their dye degradation efficiency against Remazol Brilliant Blue R (RBBR) and Methyl Violet dyes. *Aspergillus niger* showed better decolourisation efficiency of Remazol brilliant blue R(96.05%) and methyl violet (95.16%) than *Aspergillus flavus*. FTIR analysis confirmed the structural changes of the treated dye samples by shifting, disappearance and presence of peaks of the functional groups which indicated the biodegradation of the dye molecules. Enzyme analysis showed positive laccase and lignin peroxidase activity in both fungal isolates whereas manganese peroxidase activity was not significant. Phytotoxicity analysis using *Vigna radiata* seeds showed increased germination percentage after fungal treatment compared with untreated dye samples, indicating reduced toxicity. Statistical analysis viz. ANOVA, DMRT and paired t-test supported efficacy of the fungal treatment. The study indicates the potential of *Aspergillus niger* and *Aspergillus flavus* for eco-friendly and sustainable remediation of textile dyes.

Keywords -Mycoremediation Textile dye degradation, Biosorption, Laccase, FTIR, Phytotoxicity.

1. INTRODUCTION

The growth of textile industries has led to the constant discharge of dye containing effluents in the environment. Synthetic dyes are widely used in textile processing due to high stability, good coloration and resistance to fading. However, their complex chemical structures, particularly aromatic rings and synthetic components, are very persistent and hard to degrade naturally [1,2,3]. The presence of these dyes in aquatic systems affects the water quality, reduces the light penetration, interferes with the photosynthetic processes and may cause toxic effects on living organisms. [4,5,6]

Dyes have been widely removed by conventional treatment methods, such as physical adsorption, chemical oxidation, coagulation and filtration [7,8,9]. But often these methods require high inputs of energy, costly chemicals and can create secondary waste products. Therefore, environmentally sustainable biological approaches have gained attention for the effective treatment of textile dye-contaminated wastewater [10,11]. Mycoremediation is an emerging biological technology that employs fungi to remove and transform a broad spectrum of environmental contaminants. The fungi have some unique characteristics that include the large mycelial networks, capability to adapt to different environmental conditions, and the ability to secret extracellular enzymes [12,13,14,15,16]. Fungi remove dyes by several processes such as biosorption onto fungal biomass, bioaccumulation and enzymatic degradation [17]. Functional groups of amino, hydroxyl, phosphate and carboxyl

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groups present in the cell walls of fungi increase the binding of dye molecules by electrostatic interactions and ion exchange mechanisms [18,19].

The dye-degrading potential of fungi belonging to the genus *Aspergillus* has been widely studied. *Aspergillus niger* is especially recognized for its synthetic dye removal ability owing to its powerful biosorption capacity and secretion of extracellular oxidative enzymes[20,21,22]. Similarly, *Aspergillus flavus* has also shown potential to degrade textile dye under suitable environmental conditions [23,24]. These fungi can survive in extreme conditions and this makes them good candidates for bioremediation of wastewater. The textile dye degradation is mainly due to ligninolytic enzymes such as laccase, lignin peroxidase and manganese peroxidase [25,6]. The enzyme catalyzes oxidative reaction that modify complex dye structure, degrading them to less toxic and simpler compounds. However, the enzyme production and dye degradation efficiency are highly dependent on environmental factor such as pH, temperature, dye concentration, incubation period and enzyme concentration [27,28]. Therefore, optimization of these parameters is important for improving fungal mediated dye removal.

Although several fungal species have been reported for dye decolorization, a complete evaluation of fungal isolates involves assessing degradation efficiency, enzymatic activity, structural transformation and reduction in toxicity after treatment. Decolorization alone cannot be taken as an evidence of degradation because decolorization may also occur by adsorption. Structural modifications in dye molecules upon treatment with fungi were identified using analytical techniques like Fourier Transform Infrared (FTIR) spectroscopy. Moreover, phytotoxicity evaluation gives important information about toxicity of degraded products.

2. Material and methods

Sample Collection: Soil sample was collected from textile industrial site of Kasare Vanya Silk Mill Pvt Ltd., located at Urkura Raipur, Chhattisgarh, India. The samples were collected from a depth of 5-10 cm below the surface using sterile hoe and spatula. The collected samples were aseptically transferred into Zip lock polythene bag and then transported to the laboratory. All samples were stored in refrigerator for further use. The Collected soil sample was brought to the laboratory and stored at room temperature for 24-48 hours to remove excess moisture. The dried soil sample was crushed using mortal and pestle to obtain fine powder and sieved through a sterile mesh and stored in sterile airtight containers.

Isolation and Purification of Fungal Isolates: Fungal isolates were obtained by the serial dilution agar plating technique. Approximately 1 g of pretreated soil was serially diluted, and aliquots of the diluted suspension were spread onto Potato Dextrose Agar (PDA) plates. The plates were incubated at 28 ± 1°C for 4–5 days. Morphologically distinct fungal colonies were selected and purified by repeated point inoculation on fresh PDA plates under aseptic conditions to obtain pure cultures.

Preparation of Dye Solutions: Two synthetic textile dyes, Remazol Brilliant Blue R (RBBR) and Methyl Violet (MV) were used. Stock solution of each dye (1000 mg/L) was prepared by dissolving 1 g of each dye separately in 1000 mL of distilled water. Working dye concentrations were prepared from the stock solutions by appropriate dilution.

Primary Screening for Dye Decolourizing isolate: Primary screening of fungal isolates was done on PDA plates containing either 20 mg/L of RBBR or Methyl Violet. Streptomycin was added to the medium to minimize bacterial contamination. Each purified fungal isolate was inoculated separately on PDA plates with the dye and incubated at 28 ± 1°C for 5–7 days. The decolorization zones around the fungal colonies demonstrated the potential for dye degradation. The isolates were identified using Lactophenol staining technique and observed under light microscope.

Quantitative Assay of dye decolorization: The quantitative dye decolorization was investigated in Potato Dextrose Broth (PDB) medium. Then, 500 µL dye stock solution was added to the broth and five actively growing mycelial discs (5 mm diameter) were inoculated. Cultures were incubated at 28 ± 1 °C for four days. The fungal biomass was separated by filtration after incubation. The filtrate was centrifuged at 5,000 rpm for 10 min. The decolorization of dye was determined by measuring the absorbance at λmax of each dye spectrophotometrically. The percentage of decolorization was calculated by the standard formula based on absorbance.

$$\%\text{Decolorization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Final OD}} \times 100$$

FTIR Analysis: The structural changes in the dye molecules after treatment with fungus were analyzed by Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of untreated and treated dye samples were compared to identify changes in functional groups and shifts in peaks,

Enzyme Activity Assays: The fungal isolates were cultured in Potato Dextrose Broth supplemented with rice husk for extracellular enzyme production and incubated at 28 ± 1°C for 4–5 days. The enzyme was isolated from the culture filtrate. Laccase activity was determined using ABTS as substrate. Manganese peroxidase activity was assayed by measuring the oxidation of phenol red in a mixture containing manganese sulfate, sodium lactate, bovine serum albumin, phenol red, and hydrogen peroxide. Lignin peroxidase activity was measured using methylene blue as substrate. Enzyme activity was determined spectrophotometrically according to the respective assay procedures.

3. Results and discussion

The serial dilution method led to the isolation of a total of ten fungal isolates from textile contaminated soil. After incubation, morphologically different colonies were isolated and tested for the ability to decolorize dyes in primary screening. Of the ten isolates, only two isolates produced distinct decolorization zones on PDA plates supplemented with Remazol Brilliant Blue R (RBBR) and Methyl Violet (MV) indicating that the isolates were able to degrade the txtile dyes. These two isolates were chosen for further study.

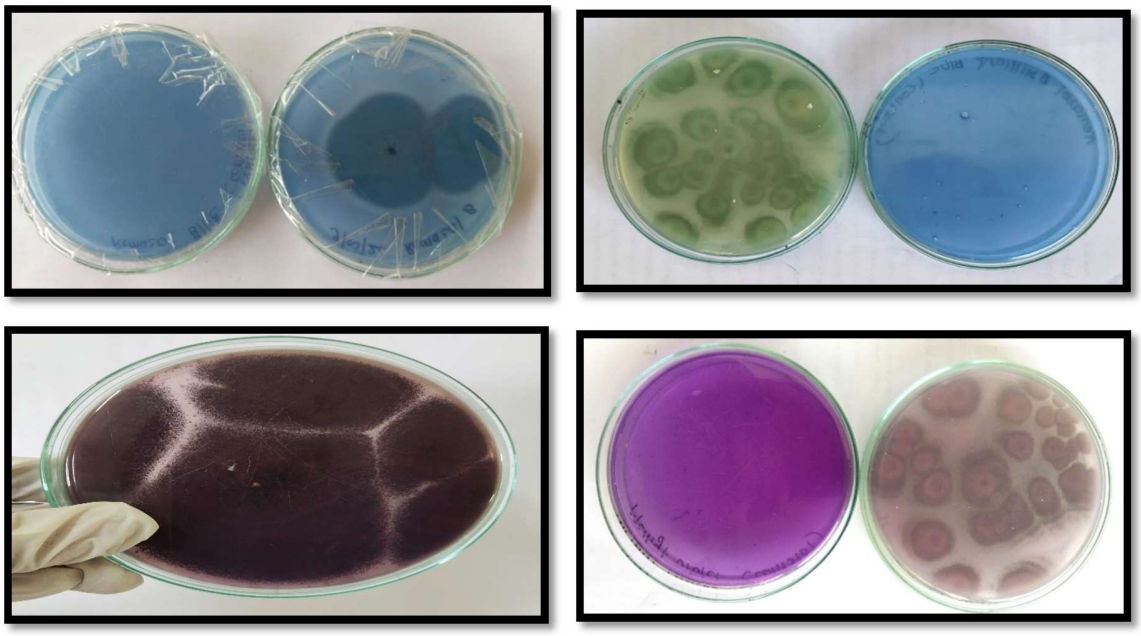


Fig 1 :Fungal isolates decolorizing dye

The two efficient dye degrading isolates were identified on the basis of colony morphology and microscopic characteristics. The isolates were identified as *Aspergillus niger* and *Aspergillus flavus* which are known ligninolytic fungi and can produce extracellular oxidative enzymes useful in the degradation of dyes.

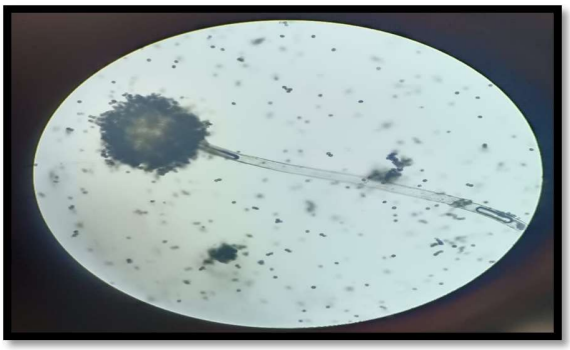


Fig 2. *Aspergillus Niger*

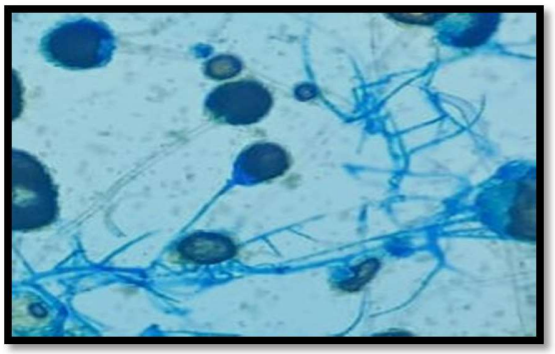
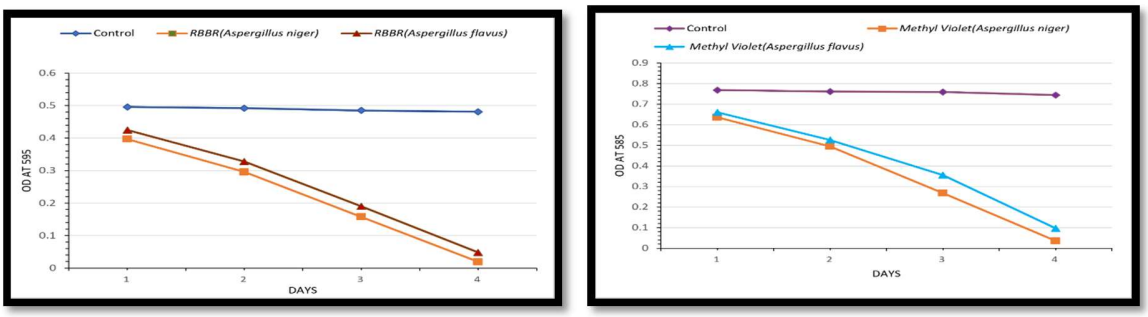


Fig 3. *Aspergillus Flavus*

Both fungal isolates demonstrated efficient decolorization of RBBR during the incubation period. Decolorization increased progressively from day 1 today 4. Among the tested isolates *Aspergillus niger* showed the highest decolorization efficiency. In the present study, *Aspergillus niger*. showed 96.05% decolorization of RBBR dye, while *A. flavus* showed 92.10%. In previous study reported where *Aspergillus niger*. showed 100%, reported 90% decolorization by *Aspergillus flavus.* For Methyl Violet*, Aspergillus niger* showed 95.16% and *A.flavus* showed 86.97% decolorization in the present study . Mahmoud *et al* in 2007 also reported similar result around 95% decolorization using *Aspergillus* species [29].



Graph 1: Graphical representation RBBR and Methyl Violet decrease in OD by Fungus *Aspergillus niger and Aspergillus flavus* at 595 and 585nm

Fig 5: Decolorization of RBBR by *Aspergillus niger and Aspergillus flavus*

Fig 4 : Decolorization of Methyl violet dye by *Aspergillus niger* and *Aspergillus flavus*

FTIR Analysis

FTIR analysis revealed that the structure of RBRR and methyl violet was changed significantly after treatment with fungi. The chemical transformation of the dye molecules is confirmed by the shift in the peak position, disappearance of the characteristic peaks, decrease in peak intensity and formation of new peak[30,31] These spectral changes indicate cleavage of aromatic structure and formation of intermediate metabolites and provide strong evidence for fungal mediated biodegradation. Both by *Aspergillus niger and Aspergillus flavus* modified the chemical structure of dye, but the spectral changes were more evident in the sample treated with *Aspergillus niger.*

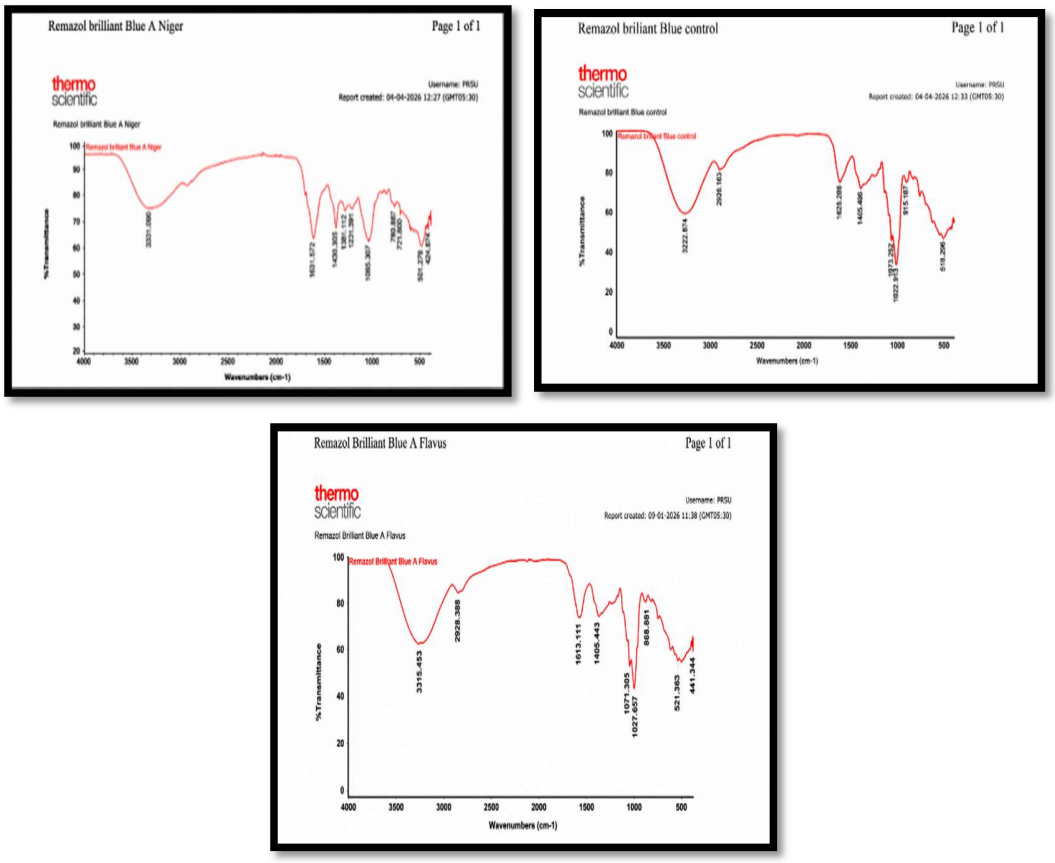
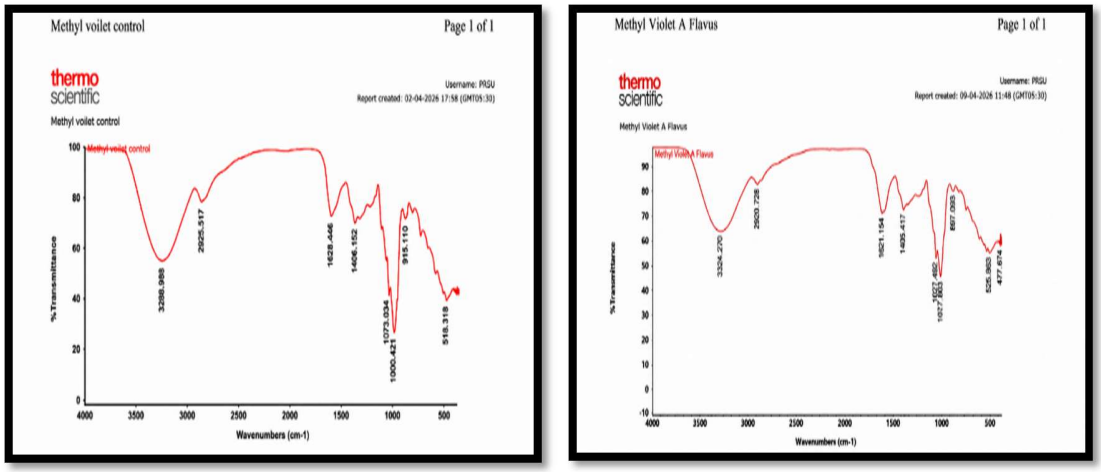


Fig 6: FTIR Result Of Remazol Brilliant Blue

In RBBR peaks shifted to 3331.48 cm-1, 2924.67 cm-1, 1631.20 cm-1, 1407.36 cm-1, 1056.39 cm-1, 780.45 cm-1 and 721.18 cm-1. $^{-1}$ After treatment with *Aspergillus niger.* In previous study absorption bands at 3474.22,2977.49,2092.85, 1728.09, 1389.74, 1256.58, 1051.36, 616.93and 457.94 cm-1[32]



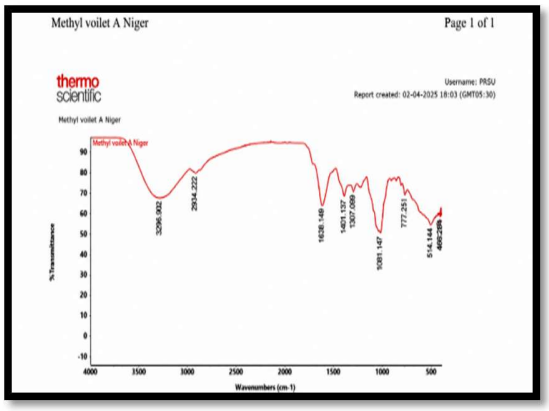


Fig 7: FTIR Result of Methyl violet

In the fingerprint region, the peak at 915.11 cm^{-1} shifted to 777.52 cm^{-1}, indicating breakdown of aromatic compounds. In previous study showed variation in fingerprint wave number region 1500-500 cm-1 which indicate decolorization of MV by *Aspergillus niger* [33].

Ligninolytic Enzyme Activity

Qualitative enzyme assay demonstrated positive laccase and lignin peroxidise activities in both fungal isolates. Development of green colour in the laccase assay confirmed extracellular laccase production, while the colour changes observed during lignin peroxidise assay indicated the secretion of ligninolytic enzymes involved in oxidative dye degradation. Manganese peroxidase activity was not detected under the experimental conditions, suggesting either its absence or very low production.

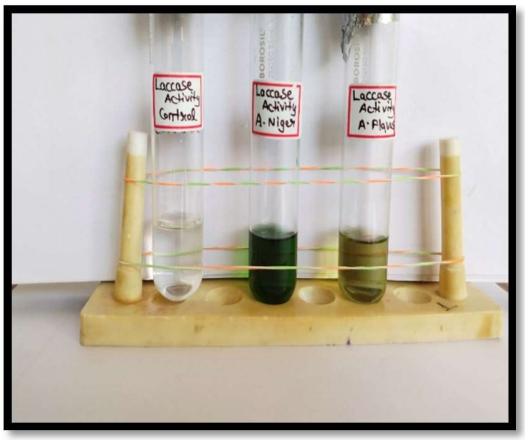


Fig:Laccase activity

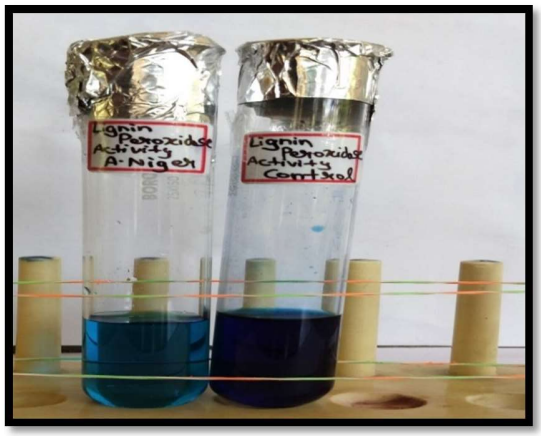


Fig:Lignine peroxidase activity

Statistical Analysis

Two-way ANOVA showed that fungal species and type of dye had significant effect on decolorization of dye. The effect of fungal species was significant ($F = 42.56$, $p < 0.0001$), and the effect of dye type was more pronounced ($F = 265.84$, $p < 0.0001$). Furthermore, the significant interaction between fungal species and dye type ($F = 4.87$, $p = 0.042$) suggested that the degradation efficiency was dependent on both fungal isolate and dye used. In general *Aspergillus niger* showed significantly better efficiency in dye removal than *Aspergillus flavus.*

The Duncan's Multiple Range Test (DMRT) also showed significant differences among treatments during the incubation period.

The lowest absorbance values obtained on Day 4 were grouped separately which showed significantly higher decolorization than earlier incubation periods. *Aspergillus niger* showed least absorbance values for both the dyes confirming dye degradation efficiency. Paired t-test analysis showed that fungal treatment significantly reduced dye concentration compared with the untreated control. Significant reductions were observed for *Aspergillus niger* in both Methyl Violet ($p = 0.050$) and RBBR ($p = 0.041$). In contrast, reductions achieved by *Aspergillus flavus* were statistically non-significant ($p > 0.05$). These findings further confirmed the superior dye degradation efficiency of *Aspergillus niger*.

Conclusion

The present study demonstrated that indigenous fungal isolates have great potentials for the biodegradation of textile colour . Among the two selected isolate as *Aspergillus niger* showed better decolourisation efficiency of Remazol brilliant blue R(96.05%) and methyl violet (95.16%) than *Aspergillus flavus*. FTIR analysis confirmed structure transformation of the dye molecule indicating that dye removal occurred through biodegradation rather than simple adsorption. The involvement of extracellular ligninolytic enzymes in the breakdown process is further indicated by the synthesis of laccase and lignin peroxidase. Optimization tests also showed that the dye decolorization efficiency was increased at acidic pH, moderate temperature, low dye concentration and higher enzyme concentration.

Overall, the findings indicate that *Aspergillus niger* is an efficient and eco friendly fungal candidate for the bioremediation of textile dye contaminated wastewater. This study highlights the potential application of indigenous fungal isolates as sustainable biological alternatives to conventional physicochemical treatment methods and provides a basis for future scaleup studies and industrial wastewater treatment applications.

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