

Isolation of Potential Dye Degrading Microorganisms and its Application in Bioremediation

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Abstract: The widespread usage of synthetic dyes has grown in various industries, especially the textile sector. An essential component for giving textile fabrics color is dye. The ecology is seriously threatened by the harmful inorganic and organic substances found in wastes produced during the dye's production and treatment. Therefore, it is crucial to put into place effective and affordable strategies to reduce these emissions to protect natural resources and habitats. In this regard, a reasonably priced and environmentally benign technique for breaking down or mineralizing dye compounds that are hardly or less degrading is biodegradation by fungi, also known as microembolization of dyes employing potential fungi. Through enzymes and mechanisms including absorption, adsorption, and aggregation, fungi are essential in the degradation and decolorization of organic dyes. Two methods were selected for biodegradation, viz. agar overlay and liquid media methods; stationary conditions at Room temperature. Present work was carried out to screen their decolorization ability of fungal strains isolated from Natural samples. It has significant 89.49% and 81% potential to decolorize the crystal violet and Congo red dye within ten days at room temperature.

Keywords: Biodegradation, Fungi, crystal violet, Congo red, Textile waste.

I. INTRODUCTION

Environmental pollution can occur due to wastewater from various industries such as textiles, cosmetics, food, plastics, leather, and more, which can cause health problems (Kishor et al., 2021; Raees et al., 2023; Shahzad et al., 2021). One of the main environmental issues is coloured water from the dyeing process (Berradi et al., 2019; Appiah-Brempong et al., 2022). Globally, the textile industry offers more than 100,000 types of commercially available dyes, with over 7050 tons produced annually. When dyeing 1 kg of cotton using reactive dyes, it usually requires about 30–60 grams of dye, 0.6–0.8 kg of sodium chloride, and a large amount of water, around 70–150 liters (Adamu, 2008; Abdel-Raheem and Shearer, 2002). This process produces wastewater that contains approximately 20–30% of the unused reactive dyes, along with dyeing helpers and high salt levels, usually around 2000 parts per million (ppm) (Kishor et al., 2021; Abdel-Raheem and Shearer, 2002). Pakistan is a major textile exporter in Asia and contributes about 8.5% to the country's GDP (Iqbal et al., 2010). Around 3.8 billion cubic meters of wastewater is produced each year from the textile industry in Pakistan. The textile sector includes 442 spinning units, of which 142 are large-scale, and 1221 ginning units (Adamu, 2008; Abdel-Raheem and Shearer, 2002; Iqbal et al., 2010). Over the past fifty years, the textile industry has been a key part of the country's economy, remaining a central industry with consistent significance. Its economic impact has varied depending on production levels and export demand. Managing information is a major challenge in the textile industry, and following international standards is essential for proper handling (Younis et al., 2023; Hunger, 2007). The range of synthetic dyes used in the industry includes various chemical types such as reactive, azo, triphenylmethane, polymer, and heterocyclic dyes (Guadie et al., 2017; Sen et al., 2016). Azo dyes are the most common type of synthetic dyes, making up up to 70% of total synthetic dyes produced, and are widely used in



pharmaceuticals, food, paper, cosmetics, and fabrics (Sen et al., 2016). Methylene blue, scientifically known as 3,7-bis (Dimethylamino) phenothiazin-5-ium chloride, is a heterocyclic aromatic compound and a derivative of phenothiazine, with the chemical formula $C_{16}H_{18}N_3SCl$ (Saratale et al., 2007; Zhu et al., 2015). Research shows that 1 mg/mL of malachite green is harmful to fish. It is not allowed to be used in human medicine (Hidayah et al., 2013). If methylene blue is swallowed, inhaled, or absorbed through the skin, it can cause harmful effects such as methemoglobinemia, where oxygen cannot effectively bind to hemoglobin, leading to nausea, vomiting, stomach discomfort, headaches, dizziness, and breathing difficulties. Methylene blue is also used as a medicine for treating methemoglobinemia and as a color in some medical procedures. The usual dosage for treating methemoglobinemia is 1–2 mg/kg given intravenously over a few minutes (Tong and Xu, 2016). Nigrosin, a mixture of artificial black dyes (Solvent black 5, CI 50415), has the molecular formula $C_{22}H_{16}N_6O_3$ and a molecular weight of 616.49 g/mol (Kyzas et al., 2013). These dyes are toxic to both humans and aquatic species. Several physical and chemical methods have been introduced for breaking down these dyes, including adsorption, coagulation, chemical precipitation, flocculation, mineralization, flotation, and electrochemical destruction (Jaafar, 2017), as well as chemical oxidation, reduction, and photolysis (Sudha et al., 2014). However, these methods are often expensive, time-consuming, and difficult to use, and may result in harmful byproducts, which do not effectively reduce the toxicity of the treated materials (Kyzas et al., 2013; International Agency for Research on Cancer, 1990). In response, new eco-friendly technologies have emerged for dye degradation. These include the use of microorganisms. Among these methods, fungi show promise due to their low cost and effective absorption properties, making them valuable tools for environmental cleanup (Mian, 2021a). Fungi can break down dyes through processes like biodegradation, biosorption, bioaccumulation, and enzymatic mineralization, using enzymes such as lignin peroxidase, manganese-independent peroxidase, and laccase (Kaushik and Malik, 2009; Gupta et al., 2014; Chen et al., 2019a). This study focuses on using fungal isolates from textile wastewater to detoxify synthetic dyes. The research found that the fungal isolates, especially *Penicillium crustosum* and *Aspergillus iranicus*, had strong detoxification abilities. Their performance in detoxification was significantly better under shaking conditions compared to stationary conditions.

II. MATERIALS AND METHODS

The primary objective of this study was to demonstrate an affordable and environmentally sustainable method for dye degradation.

All experiments were conducted in the Microbiology Department laboratory at Willingdon College in Sangli, Maharashtra, India.

2.1. Sample Collection

Textile effluent samples were carefully collected from various industrial sources and stored in six separate polythene bags. These samples originated from well-established textile manufacturers. After collection, the samples were transported to the laboratory and placed in an incubator for further processing and analysis.

2.3. Isolation of Fungal Strain

Fungal strains were isolated from the textile dye effluent using a serial dilution method with 1 mL of effluent. Each sample was prepared in dilutions of 10^{-1} and 10^{-2} . Then, using the duplicate spread plate technique, a 0.5ml inoculum was spread onto pre-sterilized petri plates containing Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) media. The plates were incubated for 96 hours at 30°C. After incubation, visible fungal growth was observed, with differences noted between the SDA and PDA media. To obtain pure cultures, individual colonies from the SDA medium that displayed distinct fungal growth compared to the PDA were sub cultured using the streak plate method. Three strains, labelled S1, S2, and S3, were randomly selected from these isolates to evaluate their ability to decolorize dyes. These strains were chosen for a comparative study of their dye decolorization potential.



2.4. Microscopic Identification of Fungi

Fungal strains were examined for their cell and colony morphology using the methods outlined in the protocol by (Wesenberg et al., 2002). A clean glass slide was treated with a drop of 70% alcohol. A red-hot, sterilized wire loop was used to transfer a fungal colony onto the glass slide. Mounted needles were used to gently lift the fungal colony. Lactophenol cotton blue dye was applied for staining, followed by microscopic examination. The initial inspection was done using a low-power objective lens at 10X magnification, then higher magnifications at 40X, and finally 100X with cedarwood oil immersion for a detailed view of spores and other fungal structures. For each strain, the focus was on observing immature fungal hyphae, especially those in the early stage of development, typically three days old. The examination also included observation of the fungi's fruiting bodies and spores.

2.5 Biodetoxification Assay in Solid Medium

Potato Dextrose Agar (PDA) medium was prepared and sterilized at 121°C for 15 minutes. After cooling to approximately 45–50°C, selected textile dyes such as Congo red, Crystal violet were added separately to the medium at a final concentration of 100 mg/L. The dye-amended medium was poured into sterile Petri plates and allowed to solidify. Actively growing fungal cultures (5–7 days old) were used for inoculation. A mycelial disc (5 mm diameter) was aseptically cut and placed at the centre of each dye-containing agar plate. Control plates without dye were also maintained.

The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 5–7 days under static conditions. All experiments were performed in triplicates to ensure reproducibility.

Dye decolorization was monitored visually by observing the formation of clear or decolorized zones around the fungal colony. The extent of decolorization was recorded by measuring: Diameter of fungal growth (mm) Diameter of decolorized zone (mm) The Decolorization Efficiency (DE%) was calculated using the formula:

$$\text{DE (\%)} = \frac{\text{Diameter of decolorized zone}}{\text{Diameter of colony}} \times 100$$

2.6. Biodetoxification assay in liquid medium

Spores and mycelia were aseptically extracted from pure cultures using a sterile wire loop and combined with 1 mL of sterile distilled water to assess the decolorization effectiveness of fungal strains in a liquid medium. After that, three flasks containing 50 mL of optimized (PDB) were autoclaved at the designated temperature and duration. After sterilization, each flask holding 50 mL of the PDB medium was filled with 1 mL of the prepared spore and mycelia mixture. To promote the growth of fungal biomass, these 100 mL flasks, which were initially filled with 50 mL of media, were incubated for seven days. Following this incubation time, each flask showed signs of fungal growth. The fungal biomass was then collected and filtered through a Whatman No. 44 filter paper. Three sets of 50 mL PDB medium were used in a series of tests where different dyes were added: 0.01% Crystal violet in the first set and 0.01% Congo red in the second. Each flask was filled with the pre-weighed fungal biomass. Daily observations of dye decolorization were documented while triplicate culture vials were incubated. Spectrophotometric analysis was used to measure the decolorization process following a 10-day incubation period. In particular, the spectrophotometric analysis at a particular wavelength for both dyes. The formula was used to determine the extent of dye decolorization caused by the fungal strains in the liquid media.

$$\text{PDD (\%)} = \frac{(\text{Absorbance ci} - \text{Absorbance cf})}{\text{Absorbance ci}} \times 100$$

By comparing the absorbance of a control sample with the absorbance of an inoculation sample, this formula determines the decrease in dye concentration and displays the result as a percentage. The dye's initial concentration is represented by the control absorbance, but the concentration following a treatment or reaction is represented by the inoculation absorbance.



III. RESULTS AND DISCUSSION

The purpose of this study was to isolate fungal strains that could survive azo dyes while investigating the characteristics and interactions of these dyes. The goal of this study was to determine whether these isolated fungal strains may be used to solve the problems caused by the persistence of synthetic colours in environmental effluents that come from the textile industry. A total of six textile effluent samples were successfully collected from different textile industrial sources for the present study. The samples were obtained in sterile polythene bags to avoid external contamination and were properly labelled according to their respective collection sites. The collected textile effluent samples served as the primary source for subsequent microbial isolation and screening for dye decolorization potential in the present investigation.

3.2. Identification of fungal strains

Morphological techniques, including both macroscopic and microscopic analyses, were carried out according to the protocols outlined by (Ram et al., 2011; Dewi et al., 2019). In this study, certain characteristics such the fruiting body, culture, pores, and spores were morphologically characterized. Colonies identified as belonging to strain S1 had a dark hue, a velvety texture, and noticeable radial folds, all of which were suggestive of *Aspergillus* sp. Solidifying PDA medium were used to cultivate and preserve the isolated fungi. On the other hand, using the procedures described in the earlier work by [30 and 31], the fungal inoculum obtained from these cultures was employed for further biodegradation investigations. Colonies were obtained on potato dextrose agar medium (supplemented with 0.5g/L 1 chloramphenicol) after 48 hrs at 30°C. The microscopic characteristics reveal a sporulation exhibiting white to bluish-green coloration, devoid of white margins, presenting as mono-vericillate, spherical strains.

3.4. Biodegradation study of dyes in solid medium

The decolorization capacity against specific fungal strains in a solid media was assessed in the current study. After eight to ten days of incubation at room temperature, (S1) showed the greatest decolorization capacity, as shown in fig.1 (A,B,C) (Muhammad et al., 2012). Additionally, another study used the fungus *Aspergillus niger*, which was isolated from soil contaminated by textile industry output, to detoxify dye industrial effluent. According to the study (Muhammad et al., 2012; Lopez et al., 2006),

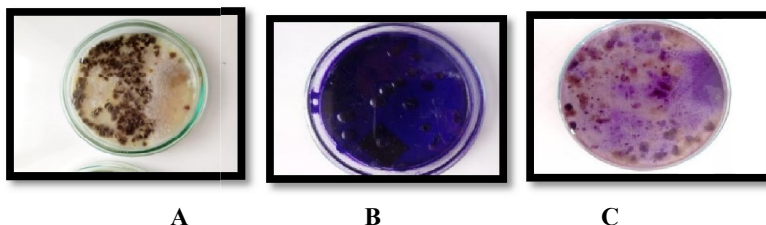


Fig.1. A) Fungal strain grown on PDA B) Fungal strain grown on PDA with dyes c) Fungal strain with decolourization zones on plates

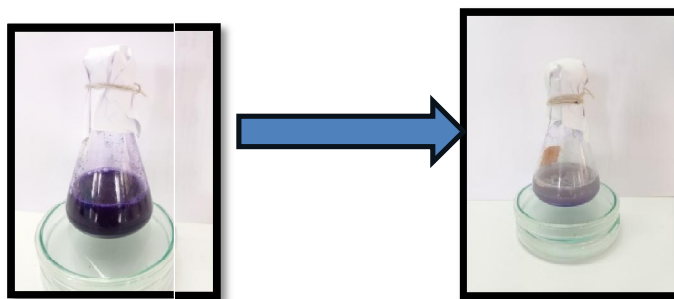


Fig.2. Decolorization of Crystal viole



TABLE 1. Decolorization Efficiency (%) OF Crystal Violet

Times in Day	Colony Diameter (mm)	Decolorized Zone (mm)	Decolorization Efficiency (%)
5	15	10	66.6
6	29	21	70.40
7	45	35	77.70
8	60	48	80.0
10	60	53	89.49

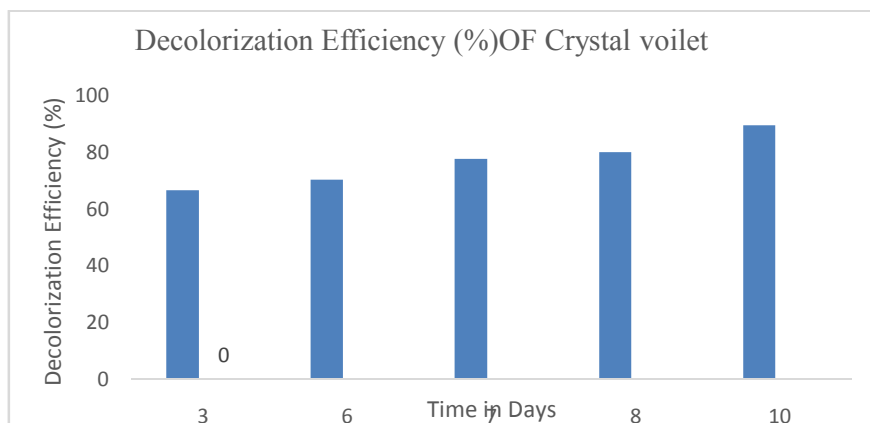


Fig .3. Decolorization Efficiency (%) Of Crystal violet

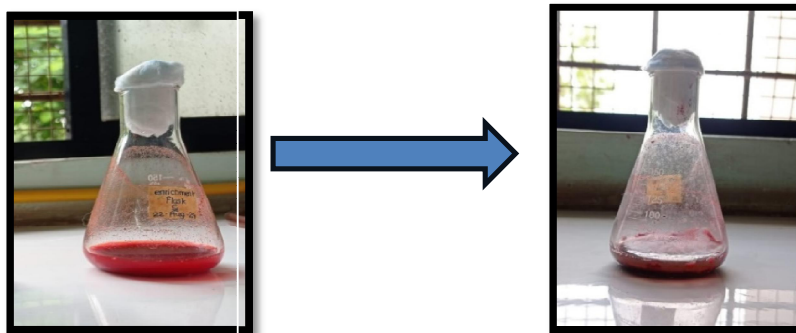


Fig.4. Decolorization of Congo red

Table 2. Decolorization Efficiency (%) Congo Red

Times in Day	Colony Diameter (mm)	Decolorized Zone (mm)	Decolorization Efficiency (%)
5	12	5	41.6
6	25	15	60.0
7	38	25	65.7
8	52	35	67.3
10	56	45	81



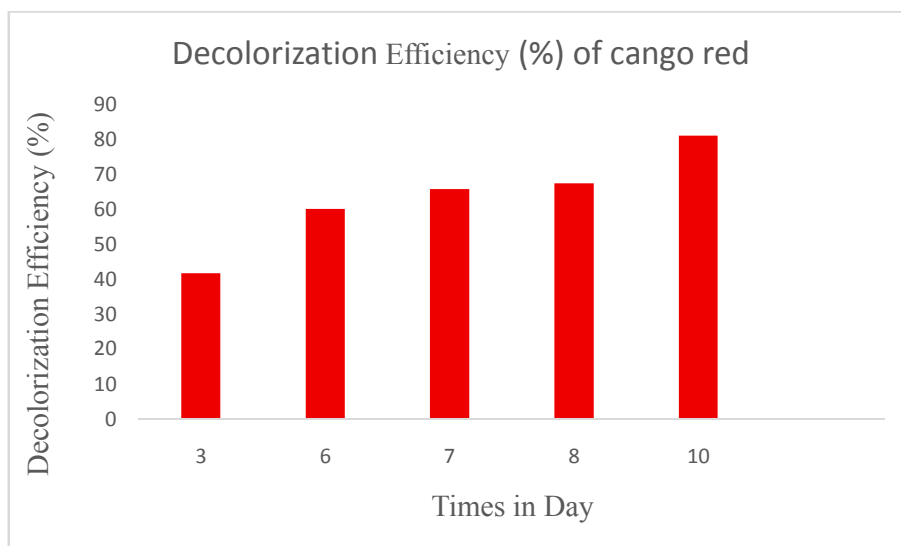


Fig.5. Decolorization Efficiency (%) OF Cango red.

3.5. Biodegradation of dyes in liquid medium

Over the course of a 10-day experiment, the isolated strains were submitted to a biodegradation screening against crystal violet and Congo red dyes, with frequent monitoring of their biodegradation capability. The decolorization of approved dyes was assessed in this study using 0.123 g of S1 biomass, and the biodegradation potential of the strains was evaluated using spectrophotometric analysis. According to the study's findings (Gao et al., 2010; Asgher et al., 2013), the maximum biodegradation was reached on the seventh day for each of the azo dyes, with a slight rise shown up until the tenth day. Crystal violet showed the greatest decolorization at 89.49% (at 590 nm) according to spectrophotometric measurements as seen in Fig., followed by 81% for Congo red (at 497 nm). This finding aligns with the research conducted by Lopez et al. (2006). Under both stationary and shaking situations, S1 noticed changes in pH. Therefore, using this fungus could provide a considerably more affordable and effective alternative treatment for wastewater that is highly contaminated with textile dyes. However, while qualitative assays employing the tube overlay method are effective tools for screening fungi for the production of extracellular enzymes, they are not definitive because a negative reaction does not prove that a species cannot produce a specific enzyme (Abdel-Raheem and Shearer, 2002). Therefore, the tube agar overlay method (Lopez et al., 2006) merely offers a quicker and simpler way to screen a large number of fungal isolates for more research on dye decolorization activities. For effluent soil fungal organisms to be used as bioremediation agents in wastewater treatment facilities and runoff waters, the dye colour must be eliminated. Therefore, testing dye-contaminated soil is crucial.



Fig 6. Biodegradation of dyes in liquid medium A) Decolorization of Crystal violet B) Decolorization of Congo red



IV. CONCLUSIONS

The transformation, degradation, and decolorization of azo dyes are mostly dependent on fungal-assisted processes. *Aspergillus* species have shown promise in both liquid and solid media dye degradation investigations. These fungal strains are viable options for creating fungus-based treatment systems intended to clean up dye-contaminated water sources and remediate dye industry effluents. According to the current research, this strain emerged as a leader and shown excellent performance, underscoring its potential as a key component in the biological treatment of industrial effluents contaminated with dyes. *Aspergillus* species demonstrated notable decolorization of crystal violet and Congo red dye, reaching up to 89.49% and 81%, respectively, according to the study, which also shown a convincing degradation potential for other dyes. These results not only add to the increasing amount of evidence that fungal bioremediation is a feasible and sustainable substitute for traditional treatment techniques, but they also identify fungal strains that may be used to create wastewater treatment systems that are more effective and economical. These results therefore point to potential uses of fungal culture in the biological treatment of azo dye-contaminated industrial effluents. This conclusion is based on the study's findings.

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