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# Micoremediation of Leather Dyes by Novel Fungal Strains Isolated From Tannery Sludge

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**ABSTRACT**

The complex nature of leather dyes makes them resistant to conventional biological treatment processes. Fungi have proven to be a promising alternative due to their resilience in harsh environments and ability to remove dyes through bio-sorption, biodegradation, and bioaccumulation mechanism. This study explored the potential of unique fungal strains, Brown fungus (F1) and Green Fungus (F3) isolated from tannery solid waste to remediate leather dye biologically. A synthetic dye solution of acid brown was tested in the micoremediation process. Factors influencing dye removal by micoremediation were investigated using batch culture technique. Maximum dye removal achieved for the initial dye concentration of 50 mg/L and culture incubation time of 7 days for both the fungi. Optimal pH of dye removal was found to be 4 for F1 and 5 for F3. At the optimal experimental conditions fungus F3 remediated more dye in comparison with fungus F1 with the removal percentages of 92.32% and 88.24% respectively. Various carbon media to support fungal growth for dye removal were also tested where Potato Dextrose Broth (PDB) achieved the highest removal. The fungal species F1 and F3 demonstrated dye removal efficiencies of 52.03% and 55.13% respectively at a dilution factor of 10, for real wastewater from leather dyeing operation showcasing its practical applicability in industrial wastewater treatment. Alcohol wash was identified as the best decolorizing solution for fungus reusability where dry fungal biomass of F1 was better reusable than F3. The SEM studies provided insights into the structure of fungi involved in the micoremediation process. This study reveals that, the novel fungal isolates have a potential for micoremediation of leather dyes at its optimal growth conditions for both synthetic dye solution and tannery dyeing waste liquor.

Keywords: Micoremediation, Fungi, Leather Dyes, Carbon Source, Tannery Sludge.



## 1. Introduction (Top heading should be in bold)

Dyeing is a critical step in the wet finishing process of leather manufacturing, designed to impart the desired surface and interior colouration to leather [1]. Various chemical agents, including dyes, are used in this process to achieve the desired quality and physical properties. However, the addition of synthetic retanning agents, oils, and surfactants leads to complex effluents that not only cause visual pollution but also reduce the oxygenation capacity of water, introduce toxicities, and hinder water reuse in subsequent processes [2].

Traditional treatment technologies for dye bearing wastewater such as chemical degradation, electrochemical methods and membrane filtration are often costly and commercially impractical. Physico-chemical methods generate large volumes of sludge, complicating disposal and treatment. Adsorption is effective for certain dyes, but its economic viability is hindered by the need for frequent adsorbent regeneration and replacement. More advanced techniques such as ion exchange and photo catalysis are very complex and expensive as well, that limits their application [3-4].

Fungal bioremediation is a cost-effective alternative technology for dye decolorization. The ability of Fungi to remove organic and metal contaminants in an eco-friendly way makes them ideal candidates for wastewater treatment. Fungi survive in the harsh environmental conditions and are able to degrade various complex organic pollutants by utilizing both extracellular and intracellular enzymes that bacteria cannot [5-6]. Dyes require careful handling in micoremediation to avoid the formation of harmful byproducts due to their structural diversity. Optimizing fungal growth conditions, enhancing enzyme production, and selecting appropriate strains are the key to develop an effective fungal treatment process [7-8].

Very few research works were published earlier regarding the micoremediation of leather dyes where, in most of the cases, researchers utilized fungal strains that were previously used in other study for any other dye removal processes with significant efficiency. The researchers in their studies focused only on the preparation of synthetic dye solution to investigate the micoremediation performance of the tested fungi; no study on the real tannery dyeing wastewater of a complex nature was performed [8].

The main aim of the study was to utilize unique fungal strains isolated from polluted environments such as tannery sludge for micoremediation of commercially used leather dyes by preparing an acid brown dye solution in the laboratory. The novel fungal isolates were characterized morphologically and biochemically. Various operational parameters of the micoremediation process were optimized. This study also focused on the practical usability of this micoremediation process for dyeing wastewater collected from a tannery. Reusability study of the dried fungal biomass was also done to identify the proper reusable condition. Additionally, SEM analysis of the raw and dye-loaded fungi

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was conducted to explore the interactions between the dye and the fungi during the micoremediation process.

## 2. Materials and Method

### 2.1 Materials collection

Tannery sludge was collected from a biological wastewater treatment plant in Jessore, Khulna, Bangladesh, serving as the source of fungi. Acid brown dye used in the micoremediation process was obtained from a leather chemical supplier in Dhaka and dissolved in distilled water to a concentration of 1000 mg/L, which served as the stock dye solution. Growth-promoting chemicals for fungi, including Potato Dextrose Broth (PDB), Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Dextrose, Yeast Extract, Starch, and Nutrient Agar were purchased from a scientific store in Dhaka (Himedia, India). Other chemicals for fungal characterization and analytical tests were procured from a local scientific store in Khulna.

### 2.2 Culture and isolation of fungi

Culture and isolation of fungi were done using the method explained by JH Warcup with slight modification [9]. In this modified process collected tannery sludge was diluted in distilled water to make 1% solution and the sludge solution was stirred overnight. In the next day fungus was cultured on the PDA plate from the sludge solution by spread plate technique and the plate was incubated at 30 $^0$C for 7 days. At the end of the incubation time a crowded culture plate with multiple fungus species were observed. From this plate three different fungal species were isolated in PDA media based on their color and macroscopic morphology. The primary isolates of the fungi named as F1 (brown), F2 (black) and F3 (green) are represented in the Fig. 1 and Fig. 2.

**Fig. 1** Primary culture of fungus from sludge source

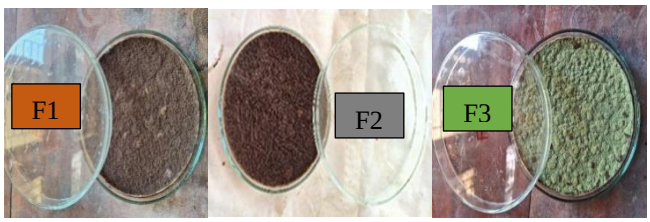



**Fig. 2** Isolated fungi from tannery sludge

### 2.3 Primary screening for selecting dye-removable fungus

The isolated fungi species (F1, F2 & F3) were primarily tested to identify their ability to accumulate acid brown dye in dye loaded solid culture media. In these experiments, three different PDA plates inoculated with the three different fungi were loaded with acid brown dye of 100 mg/L concentration. The dye loaded PDA plates were incubated at 30 $^0$C for 7 days. After the incubation period dye removable fungi were selected based on the visual observation. The result is given in the Fig. 3 from where based on the growth in dye loaded media and the formation of dye layers on the mycelium surface fungal strains F1 and F3 were selected as the dye removable species and were taken for the subsequent dye removal studies.

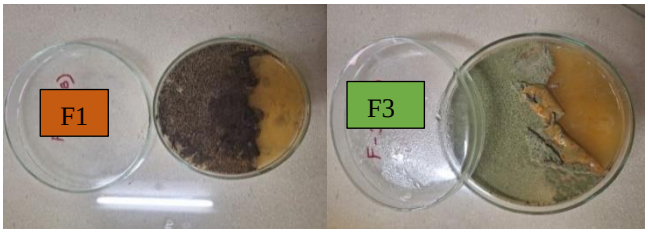



**Fig. 3** Primary screening for dye-removable fungi species

### 2.4 Characterization of the selected fungi species

Macroscopic features of the finally selected fungi were identified by visual observation using a magnifying glass. Microscopic morphology of the fungi was investigated by slide culture technique in PDA media where fungi were incubated in a moistened environment for 7 days at 30 $^0$C temperature [10]. After the incubation time the fungi were mounted on the glass slides, stained by Lacto phenol cotton blue dye and were observed under an optical microscope.

The biochemical properties of the fungal isolates were assessed using the Triple Sugar Iron (TSI) test, following the method described by Kones and his research team [11]. In this test fungus isolates were incubated at 30 °C for 7 days in presence of three sugars such as glucose, sucrose, and lactose. The result was made by the observation of acid, alkali, gas and sulfide production during the incubation period. The result of the TSI test is shown in Fig. 4.

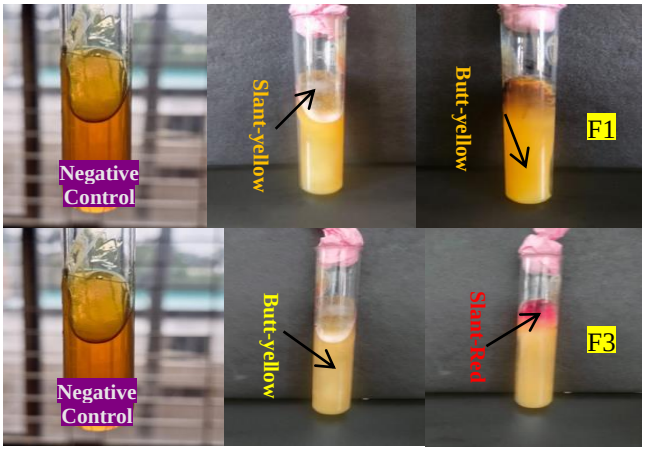



**Fig. 4** TSI test of fungal isolates

### 2.5 Micoremediation process for dye removal

The micoremediation experiments were conducted using a batch culture technique by following the liquid state dye decolorization method illustrated by Sheam and the co-authors with minor modifications where fungi were inoculated into dye solutions with Potato Dextrose Broth (PDB) at a ratio of 80:20 [12]. Various influencing factors on the micoremediation process were evaluated and the optimal conditions were identified based on the maximum dye removal percentages. The initial dye concentration ranging from 10 to 500 mg/L, pH from 2 to 8 and incubation time varying from 3 to 12 days at a temperature of 30 °C were investigated in this optimization process. Various

carbon sources such as PDB, glucose, lactose, sucrose and starch were also tested to identify their role in fungal growth and dye removal. After each set of experiment dye removal efficiency was measured by spectrophotometric analysis by taking absorbance at 454 nm. The percentage removal was calculated from the initial and final absorbance values. Every measurement was performed in triplicates and average value was taken for analysis.

2.6 Micoremediation of tannery dyeing wastewater

Collected wastewater from a local tannery in Jessore was diluted with PDB medium in different batches with the dilution factors of 1.25, 2, 4, 6, and 10 to minimize inhibitory effects. One set of the batches was inoculated with F1 and the other set with F3 and the batches were incubated under optimal temperature conditions as determined by earlier experiments. The treated wastewater samples were then centrifuged and absorbance was measured at 514 nm and percentage removal was calculated. Each test was done in triplicates and average was used for analysis.

2.7 Reusability study of fungal isolates

Fungal biomass (F1 and F3) after micoremediation process was collected by filtration through a cotton cloth and was washed with various chemical agents such as 0.5% H2SO4, 0.5% NaOH, 0.5% hydrochloric acid alcohol, and distilled water for decolorization. The biomass was then dried in an oven at 105 $^0$C temperature for overnight. Dye bio-sorption by the dried fungus biomass was then conducted in batches using 0.5 g of biomass for 50 mL of acid brown dye solution. The pH, temperature and contact time were maintained as 5 (physiological pH of dye solution), 30 $^0$C (room temperature) and 1 hour respectively. After bio sorption the batches were filtered, absorbance was measured and percentage removal was calculated.

## 3. Results and Discussion

3.1 Characteristics of fungal isolates

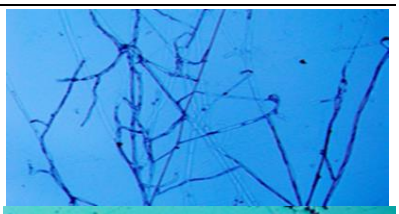
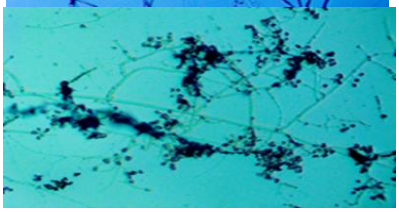
The macroscopic and microscopic morphological features of the dye removable fungi (F1 and F3) were given in the following tables.

**Table 1** Macroscopic morphology of fungal isolates

| Colony characteristics | Morphology for F1 | Morphology for F3 |
|---|---|---|
| Color | Brown | Green |
| Surface | Dry, velvety | Dry, velvety |
| Margin | Entire | Entire |
| Form | Circular | Irregular |
| Elevation | Flat | Umbonate |

It is observed from Fig. 2 and Table 1 that the macroscopic morphology of fungal isolates exhibits distinct characteristics. The colonies of F1 were brown in color with a dry velvety surface, an entire margin and a circular form along with a flat elevation. In contrast, F3 displayed green-colored colonies with a similarly dry, velvety texture and entire margins, but the form was irregular, and the elevation was umbonate. These morphological differences particularly in color, form, and elevation highlight the inherent diversity between the two fungal isolates. Such variations are often indicative of their environmental adaptability and effectiveness in bioremediation applications [13].

**Table 2** Microscopic morphology of fungal isolates

| Fungi isolates | Microscopic description | Microscopic appearance |
|---|---|---|
| F1 | Septate hyphae |  |
| F3 | Sporulated septate hyphae |  |

It is observed from Table 2 that the microscopic morphology of fungal isolates F1 and F3 displayed distinct structural features. F1 is characterized by the presence of septate hyphae, which were uniformly shaped and exhibited consistent branching patterns. These structural attributes indicated the robust growth and adaptation potential. On the other hand, F3 demonstrated sporulated septate hyphae with abundant spore production. The spores were well-formed, with a defined shape and thick walls, suggesting their resilience and suitability for environmental stress conditions. [14].

Biochemical features of the isolates were checked through TSI test and the results are shown in the Table 3.

**Table 3** TSI test results of the fungal isolates

| Fungal isolates | Result (slant/butt) | Symbol | Interpretation |
|---|---|---|---|
| F1 | Yellow/yellow | A/A* | Glucose, lactose and sucrose fermentation; No gas |
| F3 | Red/yellow | K/A* | Glucose fermentation only; Peptone catabolized; No gas |

***A=acid production; K=alkaline reaction**

It is noticed from Fig. 4 and Table 3 that for F1, the slant and butt both turned yellow, indicating an acid/acid (A/A) reaction. This result confirmed that F1 ferments the sugars. In contrast, the TSI results for F3 revealed a red slant and a yellow butt, signifying an alkaline/acid (K/A) reaction. This was the indication of the capability of F3 for fermenting glucose only, with the catabolism of peptone contributing to the alkaline environment in the slant. The absence of gas bubble and blackening in the tested slants for both F1 and F3 further suggests that no gas and no hydrogen sulfide was produced during the fermentation process.

3.2 Dye removal analysis

3.2.1 Effect of dye concentration on removal process

The effect of initial concentration of dye on micoremediation process of dye removal was analyzed by plotting the initial dye concentration versus removal percentage graph presented in the Fig. 5.

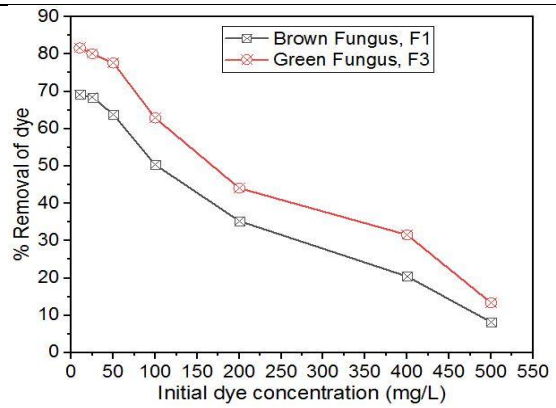



**Fig. 5** Effect of initial concentration on dye removal

It is seen from Fig. 5 that at a lower initial dye concentration, higher removal occurred for both the fungal isolates. Dye removal percentages were very close to dye concentration of 10 to 50 mg/L. The removal was 69.18%, 68.43% and 63.83% for 10mg/L, 25 mg/L and 50 mg/L, respectively, for F1, whereas for F3 at similar concentrations, the removal percentages were 81.66%, 80.11% and 77.63%. After 50 mg/L of initial concentration, removal percentages declined sharply for both the fungi. The removal percentages were the minimum at 500 mg/L dye concentration, such as 8.18% and 13.34% for F1 and F3 accordingly. For any concentration, higher dye removal was experienced by F3 than F1. Fungal dye removal operates through three primary mechanisms such as bio-sorption, biodegradation and bioaccumulation [15]. The sporulated morphology of F3 signifies its actively growing cells and relative abundance of cellular biomass, so F3 showed higher removal in comparison with F1 following any of the above mechanisms. The decrease in removal above 50 mg/L dye concentration may be due to the lower biodegradation due to enzyme inhibition at higher dye concentrations. In the subsequent experiments, initial dye concentration was maintained at 50 mg/L.

3.2.2 Effect of pH on dye removal

The influence of pH on dye removal is represented in the Fig. 6.

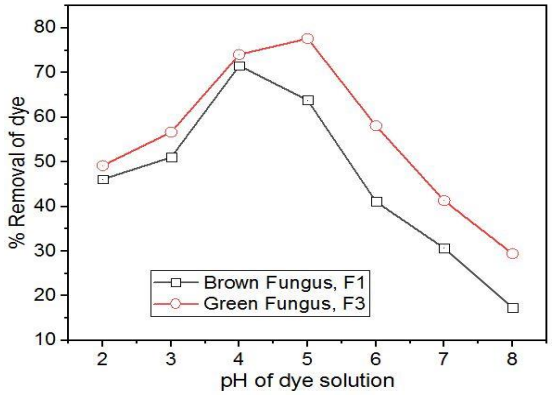



**Fig. 6** Effect of pH on dye removal

It is observed from the figure that for both the fungal isolates, dye removal was the maximum at acidic pH condition such as 71.56% at pH 4 for F1 and 77.63% at pH 5 for F3. Beyond this pH values removal percentage started to decrease and lower removal was viewed at alkaline ranges. The minimum removal was experienced at 17.36% and 29.44% for F1 and F3, respectively at pH 8. For any mechanism of micoremediation, the more fungus grows, the more dye is removed. Fungal growth and multiplication depends on pH [15]. In this study, the optimum pH for the growth of F1 and F3 fungus were 4 and 5, so the maximum removal was observed at theses pH conditions. At any pH, higher removal was noticed for F3 than F1 due to the higher growth of F3.

3.2.3 Effect of incubation time on dye removal

The graph in the Fig. 7 shows the influence of incubation time on dye removal.

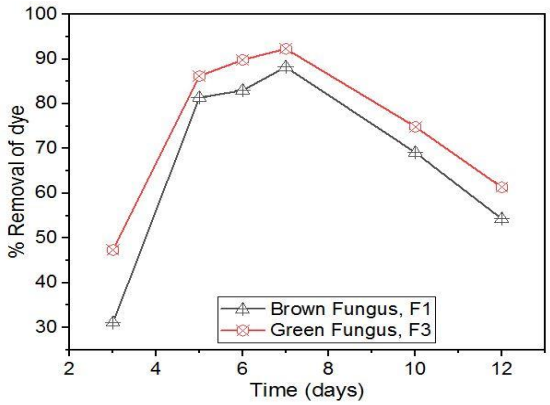



**Fig. 7** Effect of incubation time on dye removal

Fig.7 illustrates that fungal isolates displayed rapid dye removal in the first 4 days, reaching maximum efficiency by day 7. F3 achieves a slightly higher removal rate (92.32%) compared to F1 (88.24%) during this incubation duration. After day 7, the removal efficiency declined. This is due to the fact that with increasing time fungal biomass increases that leads to an increase in the removal efficiency. After the optimal time due to the unavailability of foods fungal growth starts to decrease, so the dye removal efficiency also decreases because of the inadequate bioaccumulation and biodegradation. In case of bio-sorption the dead cells may starts to desorption after certain period, so dye concentration again increases in the solution that signifies the lower removal efficiency [15]. In this study the drop in performance of the fungi after day 6 was may be due to the inadequate nutrient after this period that affected fungal growth. If consider bio-sorption mechanism, reaching the equilibrium at day 7 may be another cause of the maximum dye removal at this point.

3.2.4 Effect of carbon source for fungal dye decolorization

A column chart in the Fig. 8 represents the impact of carbon sources on dye removal. The chart demonstrates that PDB (Potato Dextrose Broth) was the most effective one among the tested carbon sources. Dye removal efficacy of fungus F1 & F3 was 88.24% and 92.32% respectively. Glucose ranked second, showing considerable efficiency but slightly lowers than PDB. Sucrose and Lactose provided moderate dye removal, with F3 performing slightly better than F1 in these cases. Starch, however, is the least effective carbon source, yielding the lowest dye removal percentages, particularly for F1. These results highlighted the importance of carbon source selection in enhancing fungal growth and maximizing dye decolorization efficiency. The findings

emphasized that optimizing the growth medium can significantly influence the bioremediation potential of fungal strains.

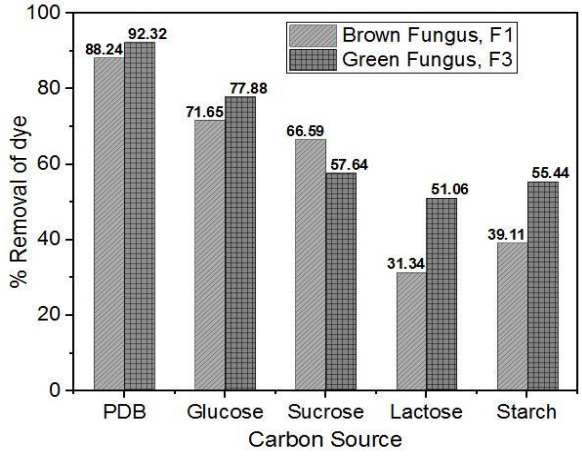



**Fig. 8** Effect of carbon sources on dye removal

3.3. Micoremediation of tannery dyeing wastewater

The stacked column chart in Fig. 9 illustrates the percentage removal of dye from tannery dyeing wastewater using two fungal isolates, F1 and F3, across varying dilution factors.

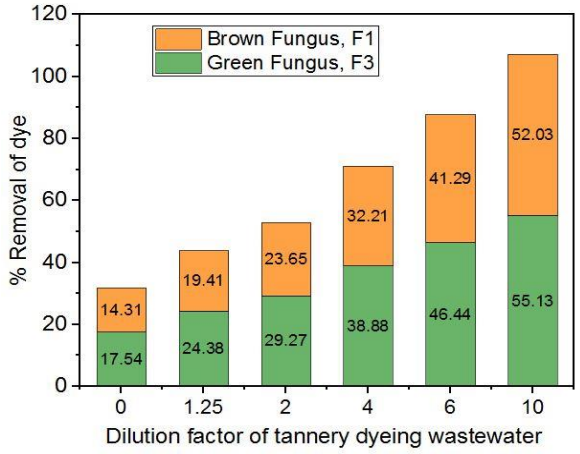



**Fig. 9** Micoremediation of tannery dyeing wastewater

As the dilution factor increased, the dye removal efficiency improved significantly for both the isolates. Without dilution F1 showed dye removal efficacy of 14.31% while for F3 the removal was 17.54%. These values progressively increased, with the highest efficiency observed at a dilution factor of 10, where F1 and F3 remove 52.03% and 55.13% of the dye, respectively. The chart clearly shows that F3 consistently outperformed F1 in dye removal across all dilution levels, indicating it as the more effective isolate. This disparity could be attributed to F3's potentially superior mechanisms, such as higher adsorptive or chemical reactivity. Additionally, the trend suggested that increasing the dilution factor reduces the concentration of interfering substances in the wastewater, enabling better dye removal for both the fungi.

3.4 Reusability of the fungal strains

Dye removal percentages of fungal biomasses, F1 and F3 for different washing conditions is presented in the Fig. 10.

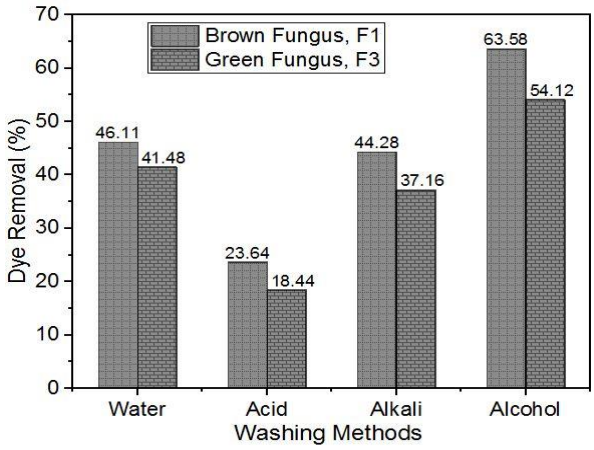



**Fig. 10** Reusability of the tested fungi

It is noticed from the figure that for alcohol washed biomass samples maximum dye removal was observed for both the fungi. The values were 63.58% and 54.12% for F1 and F3 respectively. Lower removal percentages for acid and alkali washed samples may be due to the structural damage of the pores present in the fungal biomass for dye adsorption. Moderate removal (46.11% for F1 & 41.48% for F3) was noticed for water washed biomasses likely due to the inadequate number of pores caused by improper decolorization. Fungal biomass of F1 showed better removal than F3 at any condition whereas in micoremediation process by living fungus F3 performed better, this is because bio sorption mechanism was not well fit for the fungus F3.

3.5 SEM analysis

For SEM analysis both raw and dye-loaded fungal isolates were prepared by drying at 105 °C overnight in an oven. The prepared samples were analyzed using a benchtop scanning electron microscope (SEM) (JCM 700, JOEL, Japan). To improve conductivity during SEM analysis, the fungal samples were gold-coated prior to imaging. The SEM images of both the raw and dye loaded fungal isolates were shown in the Fig. 11.

The SEM analysis revealed that the raw fungal structure comprised spores and branched mycelium with numerous pores, which play a vital role in facilitating the interaction between the dye and the fungal surface. For F1, dye accumulation was observed on the spores and mycelium, but no distinct dye layer was formed, indicating that the interaction at this stage primarily involved adsorption onto the fungal surface. However, for F3, under dye-loading conditions, a smooth and uniform dye layer was observed on the fungal surface. This smooth layer suggests enhanced interaction and a higher degree of dye degradation, which is attributed to the active enzymatic processes facilitated by the fungal structure. These findings underscore the significance of the porous mycelium and surface morphology in enabling efficient dye adsorption and subsequent enzymatic breakdown, as discussed in the study [16].

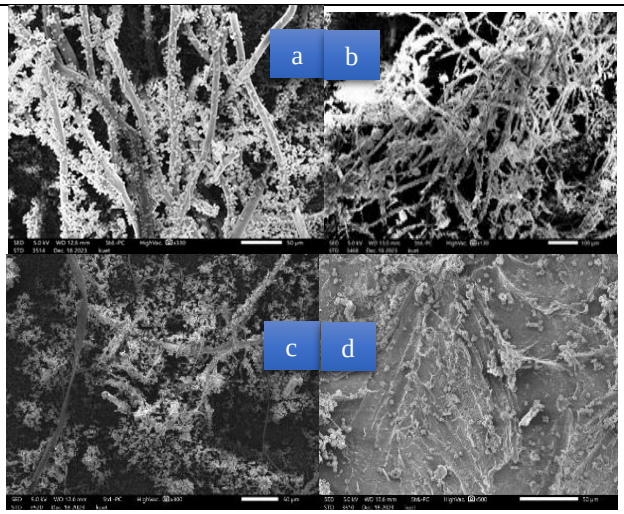



**Fig. 11** Raw fungus isolates F1 (a) and F3(c) and dye loaded F1 (b) and F3 (d)

## 4. Conclusion

This study explored the potential of two novel fungal strains, brown fungus (F1) and green fungus (F3) for their dye removal efficacy for both the synthetically prepared dye solution and for the tannery dyeing waste liquor. Micoremediation experiments revealed that F3 demonstrated the highest dye removal efficiency of 92.32% for prepared dye solution under the optimal conditions of an initial dye concentration of 50 mg/L, pH 5, and a treatment period of 7 days using Potato Dextrose Broth (PDB) as the carbon source. In contrast F1 showed relatively lower removal efficacy than F3 as 88.24% at the similar operational conditions, except pH that was 4 as optimum for F3. When tested on real tannery wastewater, the fungal strains achieved a dye removal efficiency of 52.03% and 55.13% for F1 and F3 respectively. Upon bio sorption study using fungal dead biomass, F1 showed better results than F3 with the dye removal of 63.58% and 54.12% accordingly. Scanning Electron Microscopy (SEM) confirmed the structural attributes made F3, the Green Fungus, an incredibly effective candidate for dye removal. This study highlighted the potential of fungal-based micoremediation as a cost-effective and environmentally sustainable method for treating dye-laden industrial effluents.

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