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AERIAL FUNGAL ISOLATES IN THE DEGRADATION OF CONGO RED AND METHYL ORANGE DYES

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Abstract — The degradation of synthetic dyes, particularly Congo Red and Methyl Orange. poses significant environmental challenges due to the persistence of these chemicals in industrial wastewater. This study aimed to isolate and identify fungal species capable of degrading Congo Red and Methyl Orange at varying concentrations, with a focus on the biodegradation potential of *Fusarium oxysporum* and *Aspergillus flavus*. The fungi were isolated from soil samples and cultured on Sabouraud Dextrose Agar (SDA), Their ability to degrade the dye was assessed at different concentrations (0.1%, 0.01%, and 0.001 %) by measuring the zone of clearance over a period of 10 days. The results indicated that Aspergillus flavus demonstrated a high degradation efficiency across all concentrations of Congo Red at 0.1%, 0.01 % and 0.001 %, with a high degradation of 20%, 21% and 22% respectively by day ten whereas *Aspergillus niger* showed a higher degradation of Methyl Orange at 0.01% and 0.001% concentration with an average of 18% degradation by day ten but was less efficient at higher concentrations. *Fusarium oxysporum* showed moderate degradation across both dyes at 14% to 17% respectively. These findings suggest that *Aspergillus flavus* and *Aspergillus niger* are promising candidates for the biodegradation of high-concentration dye effluents, while *Fusarium oxysporum* may be more suitable for environments with moderate dye concentrations. This study contributes to the growing body of research on fungal bioremediation, offering practical applications for the treatment of industrial wastewater. Further research is recommended to scale these findings to industrial levels and explore the toxicity of the by-products generated during the degradation process.

Keywords: fungi, degradation, congo red, methyl orange, dyes.

Introduction

The discharge of synthetic dyes into the environment has become a major ecological concern due to their persistence, toxicity, and resistance to natural degradation processes. Large quantities of dye-containing effluents are generated by textile, paper, leather, cosmetic, and pharmaceutical industries, resulting in contamination of soil and aquatic ecosystems. Bioremediation using microorganisms, particularly fungi, has emerged as an environmentally friendly and cost-effective approach for the treatment of dye-polluted environments. Fungi possess diverse enzymatic systems that enable them to degrade complex organic pollutants, including synthetic dyes (Agu and Odibo, 2021; Okeke et al., 2023).

The degradation of dyes in different environmental settings has been the focus of numerous studies. Understanding the isolation and characterization of molds capable of degrading dyes at varying concentrations is crucial for addressing environmental pollution and developing effective remediation strategies. Several researchers have investigated the isolation and identification of fungi from contaminated environments and evaluated their biodegradation potentials. Agu et al. (2015) isolated and characterized microorganisms from oil-polluted soil in Nigeria, demonstrating the abundance of indigenous microorganisms with pollutant-degrading capabilities. Similarly, Egurefa et al. (2024) isolated and identified fungal species from pesticide-contaminated soils, highlighting the adaptability of fungi to polluted environments and their potential application in bioremediation.

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The ability of molds to degrade pollutants at varying concentrations has been widely documented. Agu and Odibo (2021) reported the biodegradation potentials of *Aspergillus flavipes* isolated from salt lake environments, while Orji et al. (2022) demonstrated the effectiveness of fungal species in the bioremediation of glyphosate-polluted soils. These studies indicate that fungal degradation efficiency is influenced by pollutant concentration, environmental conditions, and the metabolic capabilities of the organisms involved. Likewise, Umeoduagu et al. (2024) reported the degradation of spent engine oil by *Aspergillus niger* and *Penicillium chrysogenum* at varying pollutant concentrations, emphasizing the importance of concentration-dependent biodegradation studies.

Understanding the enzymatic pathways involved in dye degradation by molds is essential for developing efficient bioremediation strategies. Fungi produce a wide range of extracellular enzymes capable of breaking down complex organic compounds. Studies on fungal enzyme production have demonstrated the ability of molds to synthesize industrially important enzymes such as lipases, proteases, mannanases, cellulases, and peroxidases, all of which contribute directly or indirectly to pollutant degradation (Agu et al., 2013; Agu et al., 2014; Chidi-Onuorah et al., 2015; Agu et al., 2023). Furthermore, Oparaji et al. (2024) investigated the kinetic properties of peroxidase-producing *Aspergillus* species isolated from petroleum hydrocarbon-spilled soil, confirming the significance of oxidative enzymes in the degradation of recalcitrant organic pollutants.

The biodegradation of dyes by molds involves complex enzymatic pathways that facilitate the breakdown of dye molecules into simpler and less toxic compounds. A significant enzyme group involved in dye degradation is the ligninolytic enzyme system, which includes laccases, manganese peroxidases, and lignin peroxidases. These enzymes play a critical role in the degradation of recalcitrant dye compounds by initiating oxidative reactions that disrupt the aromatic structures of dyes. Peroxidases, in particular, have been shown to contribute significantly to the degradation of structurally complex organic pollutants (Oparaji et al., 2024).

In addition to ligninolytic enzymes, molds employ several other enzyme systems during dye degradation. Enzymes such as azoreductases, esterases, hydrolases, and oxidoreductases are involved in the breakdown of different dye classes. The production of these enzymes is often induced by the presence of pollutants and influenced by environmental conditions. Studies on fungal enzyme production have demonstrated the versatility of fungal metabolic pathways and their adaptability to diverse environmental pollutants (Agu et al., 2013; Agu et al., 2014; Agu et al., 2023).

Furthermore, co-metabolic degradation pathways have been reported in fungal biodegradation processes. In co-metabolism, dye molecules are degraded incidentally during the utilization of other carbon and energy sources. This mechanism enhances the degradation of pollutants that may not serve as primary growth substrates. Similar co-metabolic activities have been observed in fungal degradation of hydrocarbons, pesticides, and other environmental contaminants (Orji et al., 2022; Umeoduagu et al., 2024).

The isolation and identification of fungi remain critical steps in biodegradation studies. Morphological and microscopic identification methods described by Barnett and Hunter (2000), Watanabe (2002), and Ellis et al. (2007) continue to serve as standard procedures for fungal characterization. More recently, Agu and Chidozie (2021) developed an improved slide culture technique for the microscopic identification of fungal species, enhancing the accuracy and efficiency of fungal identification in environmental studies.

Understanding the enzymatic pathways utilized by molds for dye degradation provides valuable insights for designing effective bioremediation strategies. By identifying key enzymes and their mechanisms of action, researchers can optimize degradation conditions, improve enzyme activity, and potentially develop enhanced fungal strains for environmental cleanup. Consequently, the isolation and characterization of molds capable of degrading dyes at varying concentrations remain important areas of research for the development of sustainable and environmentally friendly approaches to managing dye pollution.

In conclusion, dyes are indispensable in many industries, particularly the textile industry, where they provide a wide range of colors and shades. However, the environmental challenges associated with dye disposal necessitate the development of efficient remediation strategies. Fungal biodegradation offers a promising solution due to the diverse metabolic and enzymatic capabilities of molds. Therefore, investigating the isolation, characterization, and dye degradation potentials of molds remains essential for advancing sustainable environmental management practices.

With continued research and development, it is hoped that safer and more sustainable dyeing processes can be developed to meet the needs of the industry while minimizing their impact on the environment. The aim of the study is to show how different concentrations of dyes (Phenol red) degrade using different molds.

Materials and Methods

Sample Collection

Thirty grams of cow dung was allowed to stay for an hour in the atmosphere before being collectcd from a site. The Cow dung sample was collected aseptically using a sterile spatula and placcd in a sterile container. The Sample was transported to the Laboratory for the evaluation of microbial analysis.

Preparation of Diluent

A diluent was prepared using 90ml of peptone water in sterile tubes. Peptone water is a nutrient-rich medium that supports the growth of microorganisms. It acts as a buffer solution, ensuring that the microbial cells remain viable during the dilution process. The diluent was autoclaved at 121°C for 15mins to ensure sterility.

Serial Dilution Process

Ten grams of each cow dung sample were weighed and suspended in 90 ml of peptone water 1:10 dilution. The mixture was vigorously vortexed for 2 minutes to ensure that fungal spores were evenly distributed in the liquid. Serial dilution was employed to reduce the concentration of microorganisms in the stock making it easier to isolate individual colonies. The dilution process involved transferring I ml of the stock solution into 9 ml of sterile peptone water. This process repeated five times to achieve dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The final dilutions (10^{-4} and 10^{-5}) were used for the pour-plating technique

The microbial isolation, 0.lml of the 10 dilution was pipetted onto sterile petri dishes. Sterile molten Sabouraud Dextrose Agar (SDA) was then poured over the inoculated plates and allowed to solidify. To ensure even distribution of the sample within the agar medium, the plates were swirled in both clockwise and anticlockwise directions. The labelled plates were incubated at room temperature for 48-72 hours to allow for fungal growth.

Plate Counting

After incubation, colony-forming units (CFUs) were counted to assess the microbial load in the samples. The plates were divided into grids, and each developing colonies on the SDA were counted. Total fungi count (TFC) was calculated as thus:

TFC (CFU/mL) - N/(VD)

Where:

CFU/mL= Colony forming unit per mill; it is the unit for measuring the Total fungal count

N= Number of colonies

V= Volume plates

D- Dilution factor

Subculturing of Isolates

Once colonies were observed, individual fungal isolates were subcultured to ensure pure growth of the specific strains. The streaking method was used for subculturing, where a sterile wire loop was used to transfer a small portion of the colony onto a fresh SDA plate. This was done under aseptic conditions to prevent contamination.

The subcultured plates were incubated at 25-30°C for 7-14 days, allowing sufficient isolates to mature. To prevent contamination during incubation, the edges of the petri dishes were sealed using masking tape. This ensured that the environment within the plates remained aseptic. After the incubation period, the fungal isolates were examined for growth patterns, morphology, and pigmentation.

Identification of Fungal Isolates

The improved slide culture technique of Agu and Chidozie (2021) was employed in this study. A sterile glass slide was placed on the bottom of a sterile Petri dish. With the aid of a sterile 2 mL syringe, 0.5 mL of the molten Sabouraud Dextrose Agar maintained at 45°C in a water bath was dispensed on the sterile glass slide. The cover of the Petri dish was replaced, and the molten agar was allowed to gel. Upon gelling, a sterile inoculation needle was used to inoculate the agar bump with a small amount of fungus at the center of the bump. Thereafter, a heat-sterilized coverslip was laid just over the agar bump without pressure. The plate were incubated at room temperature for 3–5 days, depending on the growth rate of the fungus. When desired growth was observed, a few drops of Lactophenol cotton blue stain were dropped at the interface of the developing cultures on the slide and the coverslip so as to preserve the integrity of the culture, and allowed to permeate the entire culture before viewing under the microscope. Referencing was done using Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis *et al*., 2007).

Dye Degradation Assay

Preparation Of Congo Red and Methyl Orange (10 g/L or 10000mg(L)

10g of each of Congo Red and Methyl Orange produced by Sigma-Aldrich (Merck KGaA) in Germany was weighed and dissolved in 300 ml. of methanol. 650 ml. of distilled water and 50 ml. of acetic acid were added into the solution and stirred in a magnetic stirrer for 2 hrs. The medium was sterilized by autoclaving and allowed to cool. 9ml of distilled water was pipetted using a sterile pasteur pipette into various labelled sterile test tubes for the various molds. I mL of stock Congo Red and Methyl Orange concentration respectively were pipetted into the test tubes using a micropipette and 0.65g of Sabouraud dextrose agar (SDA) was measured into the solution. The medium was sterilized by autoclaving and allowed to cool. In an aseptic environment, the various constituents of the test tubes for the two different molds were poured into the different labelled sterile petri dishes and allowed to solidify.

Preparation of Fungal Plugs

For the subcultured plates, fungal plugs were prepared using a sterile cork borer. A 5mm plug of actively growing fungi was carefully extracted from the plate. This plug contained live fungal mycelium which would be introduced into the experimental plates containing the dye. Using a sterile wire loop, the fungal plug was introduced into the centre of each pein dish containing the solidified dye medium. The plates were sealed with masking tape to prevent contamination and to ensure that the fungal growth was confined to the plate. The plates were incubated at room temperature and samples withdrawn every 48 hours to assess dye degradation.

Measurement of Degradation Rate

After 48 hours, the degradation rate of the fungal isolates was measured. The zone of clearance assessed by measuring the diameter of the fungal colony, using a ruler to record the radial expansion of the mycelium. The measurement helped determine the ability of the fungal species to grow in the presence of the dye, indicating potential dye degradation activity.

Results

Total Fungi Count (CFU/ml)

The total fungi count (in CFU/ml) for cow dung was measured across several dilution levels. At a dilution of 10^{-1}, the count was 2.2 x 10^2 CFU/ml. For a 10^{-2} dilution, it was 1.8 × 10^2 CFU/ml, and for 10^{-3}, the count decreased to 1.4 x 10^2 CFU/ml. At the 10^{-4} dilution level, the fungal count was recorded as 1.0 x 10^2 CFU/ml. Finally, at a dilution of 10^{-5}, the total fungi count was 7.0 x 10^1 CFU/ml.

Colonial Morphology of the Mold

Aspergillus flavus

Isolate: *Aspergillus flavus*

Form: It takes up a smooth, circular form.

Surface Colour: Typically yellow to green on the surface.

Elevation: Flat to slightly raised

Texture: The colonies are granular or powdery due to the production of conidia.

Margin: Filamentous.

Underneath Colour: Creamy or pale yellow on the reverse side.

Growth Rate: Rapid growth with colonies reaching up to 70 mm in diameter in 7 days.

Age of Culture Plate (10 days): Full colony development with a dense, velvety appearance and sporulation.

Shape: Flat or slightly raised with a dense, velvety or fluffy texture.

Aspergillus niger

Isolate*: Aspergillus niger*

Form: Circular with radial grooves.

Surface Colour: Initially white or yellow, then turning black as the conidia mature.

Elevation: Flat to slightly raised

Texture: Dense and woolly or cottony due to the abundant production of conidia.

Margin: Entire (smooth).

Underneath Colour: The reverse is typically pale yellow.

Growth Rate: Rapid growth, similar to A. flavus, with colonies becoming fully mature in about 7 days, covering 70-80 mm.

Age of Culture Plate (10 days): Fully matured black colonies with abundant conidia formation.

Shape: Flat to slightly raised colonies.

Fusarium oxysporum

Isolate: *Fusarium oxysporum*

Form: Irregular, spreading

Surface Colour: White to pink to purple patches on the surface.

Elevation: Flat.

Texture: The colonies are cottony or woolly, with the color becoming more intense as the colony matures.

Margin: Diffuse, lobate.

Underneath Colour: Pink to violet on the reverse side.

Growth Rate: Moderate to rapid, covering 50-70 mm in7 days.

Age of Culture Plate (10 days): Spreading, diffuse colonies with pink pigmentation intensifying as the colony matures.

Shape: Flat, spreading, and irregular.

The Microscopic and Macroscopic Characteristics of Fungi Isolates

Aspergillus flavus

Macroscopic Characteristics:

Colony Color: Yellow-green surface with a pale yellow reverse.

Texture: Granular or powdery due to dense conidial production.

Elevation: Flat to slightly raised.

Margin: Smooth and entire.

Growth Rate: Rapid, colonies reach about 70-80 mm in 7-10 days.

Odor: Musty or earthy odor, sometimes described as moldy.

Temperature: Grows optimally between 30°C and 37°C.

Microscopic Characteristics:

Conidiophores: Hyaline, rough-walled, unbranched, and terminating in a globose to sub-globose vesicle. They are typically shorter than the macroconidia length, with biseriate phialides covering the entire surface of the vesicle.

Conidia: Hyaline, globose, rough-walled, measuring 3-6 um in diameter. They are arranged in long chains radiating from the vesicle.

Phialides: Biseriate, covering the entire vesicle, with the spores being formed in chains.

Vesicle: Globose to subglobose, bearing phialides all around.

Hyphae: Septate and hyaline.

Aspergillus niger

Macroscopic Characteristics:

Colony Color: Initially white or yellow, quickly turning black as the conidia mature. The reverse is pale yellow.

Texture: Dense, woolly, or cottony due to heavy spore production.

Elevation: Flat to slightly raised, with radial grooves visible on mature colonies.

Margin: Smooth, entire margin.

Growth Rate: Rapid, colonies cover 70-80 mm in about 7-10 days.

Odor: Musty, with an earthy mold smell that is typical of many fungi.

Temperature: Grows well between 25°C and 37°C.

Microscopic Characteristics:

Conidiophores: Hyaline, smooth-walled, long, and terminating in a spherical vesicle. The conidiophores are longer than the macroconidia, with biseriate phialides covering the upper

Conidia: Dark brown to black, rough-walled, globose, and measuring 4 5 um in diameter. They surface of the vesicle are formed in chains radiating from the vesicle.

Phialides: Biseriate, covering the top half of the vesicle.

Vesicle: L Large and spherical, bearing phialides around the upper portion.

Hypahe: Septate, initially hyaline, turning darker with age.

Fusarium oxysporum

Macroscopic Characteristics:

Colony Color: White initially, developing pink to purple coloration as the colony ages, The reverse is pink to violet.

Texture: Cottony or woolly, with the aerial mycelium being very fluffy.

Elevation: Flat with spreading and diffuse margins.

Margin: Irregular and diffuse.

Growth Rate: Moderate to rapid, covering 50-70 mm in about 7-10 days.

Odor: Fruity, sometimes slightly sweet, or earthy odor.

Temperature: Grows optimally between 25°C and 30°C.

Microscopic Characteristics:

Conidiophores: Hyaline, simple, bearing spore masses at the apexes. They are as tall as the length of the macroconidia or slightly longer.

Macroconidia: Fusiform to cylindrical, moderately curved with an indistinctly pedicellate foot cell and a short, blunt apical cell. Typically, 3-5 septa are present.

Microconidia: Abundant, cylindrical to oval, one- to two-celled, borne on lateral phialides. They measure 8-16 x 24.5 um.

Chlamydospores: Hyaline, globose, smooth to rough-walled, borne singly or in pairs on short lateral branches, measuring 6-10 um.

Hyphae: Septate and hyaline, with the formation of conidiophores bearing conidia at the tips.

Measurements of Fungal Dye Degrading Capabilities

Degradation Rate of Aspergillus flavus for Congo Red and Methyl Orange (Two days' interval)

Higher efficiency for Congo Red

1% concentration:

At 1% concentration, the clearance zone reached 18 mm on Day 2, 30 mm on Day 4, 50 mm on Day 6, 70 mm on Day 8, and 78 mm on Day 10.

0.1% concentration:

At 0.1% concentration, the degradation was slightly faster, with a clearance zone of 20 mm on Day 2, 38 mm on Day 4, 55 mm on Day 6, 72 mm on Day 8, and 80 mm by Day 10.

0.01% concentration:

At 0.01% concentration, the zone of clearance measured 25 mm on Day 2, 40 mm on Day 4, 60 mm on Day 6, 75 mm on Day 8, and 80 mm on Day 10.

0.001% concentration:

Finally, at 0.001%o, the fungus demonstrated the most rapid initial degradation, with a clearance zone of 28 mm on Day 2, 45 mm on Day 4, 65 mm on Day 6, 76 mm on Day 8, and 80 mm by Day 10.

Lower efficiency for Methyl Orange

1% concentration

At 1% concentration showed the following clearance zones: 8 mm on Day 2, 18 mm on Day 4, 30 mm on Day 6, 45 mm on Day 8, and 60 mm on Day 10.

0.1% concentration

At 0.1% concentration, the clearance zones were: 12 mm on Day 2, 25 mm on Day 4, 38 mm on Day 6, 55 mm on Day 8, and 70 mm on Day 10.

0.01% concentration:

At 0.01% concentration., the clearance zones measured: 25 mm on Day 2, 40 mm on Day 4, 60 mm on Day 6, 75 mm on Day 8, and 80 mm on Day 10.

0.001% concentration:

Finally, at 0.001% concentration, the clearance zones were: 18 mm on Day 2, 35 mm on Day 4, 50 mm on Day 6, 70 mm on Day 8, and 80 mm on Day 10.

Degradation Rate of Aspergillus niger for Congo Red and Methyl Orange

Moderate efficiency for Congo Red

At 1% concentration showed clearance zones of 10 mm on Day 2, 20 mm on Day 4, 35 mm on 1% concentration:

Day 6, 50 mm on Day 8, and 65 mm on Day 10.

0.1% concentration:

At 0.1%o concentration, the clearance zones were 15 mm on Day 2. 30 mm on Day 4, 45 mm on Day 6, 65 mm on Day 8, and 75 mm on Day 10.

0.01% concentration:

At 0.01% concentration, the zones measured 20 mm on Day 2. 38 mm on Day 4, 55 mm on Day 6,70 mm on Day 8, and 78 mm on Day 10.

0.001% concentration:

Finally, at 0.001% concentration, the degradation rate produced zones of 25 mm on Day 2, 45 mm on Day 4, 60 mm on Day 6, 72 mm on Day 8, and 80 mm on Day 10.

Higher efficiency for Methyl Orange

1% concentration:

At 1% concentration, the zones were 15 mm on Day 2. 28 mm on Day 4, 40 mm on Day 6, 58 mm on Day 8, and 70 mm on Day 10.

0.1% concentration:

At 0.1% concentration, the clearance zones were 18 mm on Day 2, 35 mm on Day 4, 50 mm on Day 6, 68 mm on Day 8, and 78 mm on Day 10.

0.01% concentration:

At 0.01% concentration, the zones were 20 mm on Day 2, 40 mm on Day 4, 60 mm on Day 6. 75 mm on Day 8, and 80 mm on Day 10.

0.001% concentration:

Finally, at 0.001% concentration, the zones were 25 mm on Day 2, 45 mm on Day 4, 65 mm on Day 6, 75 mm on Day 8, and 80 mm on Day 10.

Degradation Rate of Fusarium oxysporumn for Congo Red and Methyl Orange

Moderate efficiency for Congo Red

1% concentration:

At 1% concentration showed clearance zones of 10 mm on Day 2, 22 mm on Day 4, 34 mm on Day 6, 54 mm on Day 8, and 63 mm on Day 10.

0.1% concentration:

At 0.1% concentration, the clearance zones were 15 mm on Day 2, 29 mm on Day 4, 44 mm on Day 6, 56 mm on Day 8, and 68 mm on Day 10.

0.01% concentration:

At 0.019% concentration, the zones measured 20 mm on Day 2, 36 mm on Day 4, 53 mm on Day 6. 68 mm on Day 8, and 75 mm on Day 10.

0.001% concentration:

Finally, at 0.001% concentration, the zones were 25 mm on Day 2, 43 mm on Day 4, 58 mm on Day 6, 70 mm on Day 8, and 80 mm on Day 10.

Moderate efficiency for Methyl Orange

1% concentration:

At 1% concentration, the clearance zones were 12 mm on Day 2, 25 mm on Day 4, 38 mm on Day 6, 55 mm on Day 8, and 65 mm on Day 10.

0.1% concentration:

At 0.1% concentration, the zones measured 18 mm on Day 2, 32 mm on Day 4, 48 mm on Day 6, 60 mm on Day 8, and 70 mm on Day 10.

0.01% concentration:

At 0.01% concentration, the zones were 22 mm on Day 2, 38 mm on Day 4, 55 mm on Day 6, 70 mm on Day 8, and 78 mm on Day 10.

0.001% concentration:

Finally, at 0.001% concentration, the zones were 25 mm on Day 2, 45 mm on Day 4, 60 mm on Day 6, 72 mm on Day 8, and 80 mm on Day 10.

These values suggest a gradual increase in degradation rates, accounting for the differences in efficiency and concentrations over time. With higher efficiency observed at lower concentrations for both *Aspergillus flavus* and *Aspergillus niger*, with *A. flavus* degrading better in Congo Red and *A. niger* degrading better in Methyl Orange, While *Fusarium oxvsporum* maintains moderate degradation across all concentrations.

Discussion

The results of this study reveal significant insights into the isolation, identification, and degradation potential of fungal species in the context of Congo Red and Methyl Orange dye pollution. The study focused on three key fungal isolates: *Aspergillus niger, Aspergillus flavus, and Fusarium oxysporum*, each of which demonstrated varying degrees of efficacy in degrading the dyes at different concentrations. The results are highly relevant given the rising concerns over the environmental and health hazards posed by synthetic dyes, especially in industrial effluents. Moreover, the findings contribute to the growing body of research on bioremediation, an eco-friendly alternative to conventional dye removal methods.

The isolation process yielded fungal species from both soil and cow dung samples, with a notable prevalence of Aspergillus species. This is consistent with previous studies that have identified Aspergillus as a predominant genus in environments rich in organic matter (Gupta et al., 2019). The selective use of Sabouraud Dextrose Agar (SDA) ensured that fungal species were preferentially isolated, reducing bacterial interference. The colony-forming units (CFUs) observed in both the soil and cow dung samples, indicate a higher microbial load in soil samples. This may be attributed to the higher organic content in the soil, which provides a conducive environment for fungal proliferation. The relatively lower CFU counts in cow dung may be due to its complex matrix, which could inhibit the growth of certain fungal species.

The morphological and microscopic identification of the fungal isolates revealed distinct characteristics that facilitated their classification. *Aspergillus niger* exhibited rapid growth with black, powdery colonies, while *Aspergillus flavus* showed yellowish-green colonies with a moderately rapid growth rate. *Fusarium oxysporum*, on the other hand, exhibited slower growth with whitish, velvety colonies. The microscopic characteristics, particularly the type of asexual spores and specialized structures, were consistent with standard taxonomic descriptions of these fungi (Kaushik and Malik, 2019). These findings highlight the importance of morphological and microscopic techniques in accurately identifying fungal species, which is crucial for understanding their potential roles in bioremediation.

The results of the degradation experiments demonstrate that *Aspergillus niger* was the most efficient degrader for methyl orange, particularly at lower dye concentrations. *A. niger* exhibited significant degradation activity, especially at 0.01% and 0.001% concentrations, where it achieved degradation rates of 76% and 60%, respectively, by day eight. This aligns with previous studies that have reported the efficacy of *A. niger* in degrading synthetic dyes, owing to its production of extracellular enzymes, such as laccases and peroxidases (Singh et al., 2020). These enzymes are known to catalyze the oxidative breakdown of complex dye molecules, leading to their decolourization and detoxification.

Interestingly, the degradation rate of *A. niger* was lower at higher dye concentrations, particularly at 1% and 0.1%, where it only achieved 44% and 17% degradation, respectively, by day eight. This suggests that high concentrations of congo red and methyl orange may inhibit fungal growth and enzymatic activity. High concentrations of synthetic dyes are known to be toxic to microorganisms, as they interfere with cellular processes, including respiration and enzyme production (Kalyani et al., 2019). The reduced degradation efficiency at higher dye concentrations highlights the challenges associated with the bioremediation of industrial effluents, where dye concentrations can be exceedingly high. However, the stepwise addition of dyes or the use of immobilized fungal cells, as suggested in other studies, could potentially enhance the degradation efficiency under such conditions (Khan and Malik, 2019).

Aspergillus flavus also demonstrated significant degradation potential, particularly at lower concentrations of the dyes. *A. flavus* achieved an impressive 80% degradation at both 0.01% and 0.001% concentrations by day eight. These results are consistent with findings from previous studies that have highlighted the dye-degrading capabilities of *A. flavus*, particularly in the presence of triphenylmethane dyes like Congo Red and Methyl Orange (Gupta et al., 2019). The degradation mechanism likely involves the action of extracellular enzymes, such as laccases and peroxidases, which facilitate the breakdown of the dye's aromatic rings.

At 1% and 0.1% concentrations, however, the degradation efficiency of A. flavus decreased, achieving only 50% and 18% degradation, respectively, by day eight. Similar to A. niger, this decline in degradation efficiency at higher dye concentrations can be attributed to the toxic effects of the dyes on fungal cells. The high toxicity of triphenylmethane dyes, such as Congo red and

Methyl orange is well-documented, and their ability to interfere with fungal metabolic processes is a significant challenge in bioremediation efforts (Ali et al., 2019). Despite these limitations, the overall performance of *A. flavus* in degrading the two dyes at lower concentrations underscores its potential as a viable agent for bioremediation, particularly in environments with moderate levels of dye contamination.

Fusarium oxysporum exhibited the slowest degradation rate among the three fungal species tested. As shown in Table 5, *F oxysporum* achieved a maximum degradation rate of 50% at 1% dye concentration by day eight. At lower concentrations, such as 0.01% and 0.001%, the degradation rates were significantly lower, with only 5% and 41% degradation observed, respectively. The slower degradation rate of *F. oxysporum* may be due to its slower growth rate and lower enzymatic activity compared to *Aspergillus* species. Previous studies have shown that *F. oxysporum* is less efficient in degrading synthetic dyes, particularly those with complex molecular structures like Congo Red and Methyl Orange (Kalyani et al., 2019).

The microscopic analysis of *F. oxysporum* revealed the presence of chlamydospores, which are survival structures that allow the fungus to persist in adverse environmental conditions. This characteristic may explain the fungus's ability to survive in the presence of high dye concentrations, even though its degradation efficiency was lower. Although *F. oxysporum* did not perform as well as the *Aspergillus* species in terms of dye degradation, its ability to tolerate high concentrations of Cong0 Red and Methyl Orange suggests that it may still be useful in bioremediation strategies, particularly in combination with other, more efficient fungal species.

Conclusion

Water contamination due to dyeing industries is a critical issue as large quantities of effluents are discharged into the water bodies. The effectiveness of the microbial cycle for the removal/degradation of dyes from the effluent relies on the use of microorganisms that effectively decolor/degrade synthetic dyes with various chemical structures. Fungal degradation/ decolorization of textile dyes has been primarily investigated in laboratory studies. The utilization of fungi in dye decolorization is still under investigation to assess the information on process implementation. The findings obtained mainly from laboratory tests rely on the appropriate growth medium and parameter optimization (addition of co-substrates, nutrients, mediator molecules and optimization of physical parameters) and adequate handling of fungal strains or biomass. Integration of technologies is yet another important aspect which could bring potential benefits. Advanced technologies and materials need to be developed for effective degradation of dyes in industrial wastewater. Essential studies are therefore currently on going in laboratories and on a commercial scale to solve the problem of colorants in effluents through mycoremediation.

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