

ISOLATION AND CHARACTERIZATION OF MOLDS CAPABLE OF DEGRADING VARYING CONCENTRATIONS OF PHENOL RED DYE

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Abstract: This aim of this research was to isolate and characterize mold species capable of degrading Phenol Red. The soil sample was collected from an abattoir site in Amansea Awka and the fungal isolates were characterized by examining their colony morphology and the isolates were identified to be *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*. These isolates were further examined on their ability to degrade phenol red at different concentrations by the isolates. It showed that the three isolates are potential decolorizers of phenol red and are good biosorbents. *Aspergillus niger* showed higher degradability than *Aspergillus flavus* and *Fusarium oxysporum* in the degradation of phenol red. 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 *Fusarium oxysporum* demonstrated a high degradation efficiency across concentrations 0.01 % and 0.001% with an average degradation of 12 % and 15 % respectively by day ten whereas *Aspergillus flavus* showed effective degradation at 0.001 % concentration with an average of 22 % degradation by day ten but was less efficient at higher concentrations. These findings suggest that *Aspergillus flavus* is a promising candidate for the biodegradation of low-concentration dye effluents, while *Fusarium oxysporum* may be more suitable for environments with higher dye concentrations.

Keywords: Isolation, Characterization, Molds, Degradation, Phenol red and Dyes.

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INTRODUCTION

Environmental pollution resulting from industrial activities remains a major global challenge. Industries such as textile manufacturing, petroleum processing, agriculture, and laboratory services contribute significantly to the release of toxic substances into the environment. These pollutants contaminate soil and water resources, threatening ecosystem stability and public health (Awari et al., 2023; Agu et al., 2014). Among these pollutants, synthetic dyes are of particular concern because of their persistence, toxicity, and resistance to degradation.

Dyes are complex organic compounds characterized by high molecular weight and stable aromatic structures, making them difficult to remove from contaminated environments. Their release into aquatic systems reduces light penetration, inhibits photosynthetic activity, lowers dissolved oxygen levels, and disrupts ecological balance. The accumulation of dye-containing effluents in water bodies has therefore become an important environmental concern requiring effective remediation strategies (Agu et al., 2014; Ezenwelu et al., 2024).

Conventional methods for dye removal, including chemical oxidation, adsorption, membrane filtration, coagulation, and ion exchange, have been widely employed. However, these approaches are often expensive, generate secondary pollutants, and may be ineffective for complete dye mineralization. Consequently, attention has shifted toward biological treatment technologies, particularly bioremediation, due to their environmental friendliness, cost-effectiveness, and sustainability (Okafor et al., 2016; Orji et al., 2022).

Bioremediation involves the utilization of microorganisms to transform toxic compounds into less harmful substances. Numerous studies have demonstrated the ability of microorganisms to degrade environmental pollutants, including hydrocarbons, pesticides, plastics, heavy metals, and synthetic dyes. Agu et al. (2015) isolated and characterized microorganisms from oil-polluted soil and demonstrated their biodegradation potential. Similarly, Ifediegwu et al. (2015) reported the isolation of chlorpyrifos-degrading bacteria from agricultural soils, while Ojiagu et al. (2018) demonstrated the biosorption of hexavalent chromium by *Pseudomonas aeruginosa*. These findings highlight the versatility of microorganisms in environmental remediation.

Among the various microbial groups employed in bioremediation, fungi have attracted considerable attention because of their ability to degrade complex organic compounds through extracellular enzymatic systems. Fungi produce a broad spectrum of enzymes including lipases, proteases, cellulases, mannanases, peroxidases, and oxidoreductases that facilitate the degradation of recalcitrant pollutants (Agu et al., 2013; Agu et al., 2014; Chidi-Onuorah et al., 2015; Agu et al., 2023). Oparaji et al. (2024) further reported the production and kinetic properties of peroxidase by *Aspergillus* species isolated from hydrocarbon-spilled soils, emphasizing the importance of oxidative enzymes in pollutant degradation.

The application of fungi in environmental cleanup, commonly referred to as mycoremediation, has gained increasing recognition due to the adaptability of fungi to harsh environmental conditions and their remarkable degradative capabilities. Studies have shown that fungal species can effectively degrade petroleum

hydrocarbons, glyphosate, spent engine oil, and other environmental contaminants (Agu and Odibo, 2021; Orji et al., 2022; Umeoduagu et al., 2024). Furthermore, Okeke et al. (2023) demonstrated the degradative potential of *Aspergillus niger* and *Aspergillus flavus* against Congo red dye, indicating the potential of fungal species for dye bioremediation applications.

Fungi possess specialized ligninolytic enzyme systems comprising laccases, manganese peroxidases, and lignin peroxidases, which enable the breakdown of complex aromatic compounds. These enzymes facilitate the oxidation and mineralization of dye molecules, leading to decolorization and detoxification. The production of such enzymes has been widely reported among fungal isolates from polluted environments (Agu et al., 2013; Agu et al., 2014; Oparaji et al., 2024). Their enzymatic versatility makes fungi particularly suitable for the biodegradation of recalcitrant synthetic dyes.

Phenol Red is a synthetic dye extensively used in microbiological and biochemical laboratories as a pH indicator and tracking dye. Despite its usefulness, its release into the environment poses ecological concerns due to its resistance to natural degradation processes. Similar to many synthetic dyes, Phenol Red may accumulate in aquatic ecosystems, interfere with photosynthetic processes, and adversely affect microbial and aquatic life. Therefore, environmentally sustainable approaches are required for its removal from contaminated environments.

The successful application of fungi in biodegradation studies depends largely on the isolation and accurate identification of suitable fungal species. Conventional fungal identification methods described by Barnett and Hunter (2000), Watanabe (2002), and Ellis et al. (2007) remain important tools in mycological studies. More recently, Agu and Chidozie (2021) developed an improved slide culture technique for microscopic identification of fungal species, enhancing the accuracy of fungal characterization. Studies by Ejurefa et al. (2024) further demonstrated the occurrence of diverse fungal species in contaminated soils, providing evidence of their adaptability and potential application in bioremediation.

Although several studies have demonstrated the biodegradation potential of fungi against various environmental pollutants, information on the degradation of Phenol Red by molds such as *Aspergillus flavus* and *Fusarium oxysporum* remains limited. Therefore, this study seeks to isolate, characterize, and evaluate molds capable of degrading Phenol Red at varying concentrations. The findings will contribute to the development of environmentally friendly, cost-effective, and sustainable bioremediation strategies for dye-contaminated environments.

This study seeks to isolate and characterize mold species capable of degrading Phenol Red, thereby contributing to the development of cost-effective and eco-friendly bioremediation strategies.

MATERIALS AND METHOD

Sample collection

Thirty grams of soil sample were collected from an abattoir site at Amansea Awka. The sample was collected aseptically using a sterile spatula and placed in a sterile container. The Sample were transported to the Laboratory for the evaluation of microbial analysis.

Sample Analysis

One gram of the soil sample was weighed out aseptically. It was introduced into a 9 ml of peptone (diluent). A fivefold serial dilution was carried out. The test tube with the cow dung sample was centrifuged to enable it homogenize with the diluent. 10^{-5} and 10^{-4} folds were used for each of the samples. 0.1 ml of appropriate dilution were pour plated on a sterile agar media containing SDA. The culture plate was incubated at 37° C for 5-7 days. Subculturing was done to get pure fungi organism that will be able to degrade the dye. 9.75 g of SDA in 150 ml of water was prepared for the sub culturing. The plates were incubated at 37° C for about 3-5 days for growth.

Identification of Isolates

The isolates were identified based on morphological and microscopic view.

Preparation of Dye and Media

Chemical of phenol red was mixed with distilled water up to 200 ml in a conical flask. It was Shaked to homogenize the dye. Serial dilution of the dye was done to get different concentration (1, 0.1, 0.01, 0.001). 0.65 g of SDA were added to each concentration of the dye. The test tube was sterilized for 15 minutes in an autoclave, allow to cool for 5 minutes. Each constituent of the test tube was poured into a sterile petri dish that has been labelled according to the concentration of the dye.

Colony Morphology

The isolates were characterized morphologically based in their size, shape, colour margin, elevation and underneath colour.

RESULTS

Table 1: Total fungal count of the soil sample

Sample	Dilution Factor	Total Fungal Count (CFU/ml)
Soil	10^{-1}	2.2×10^{-3}
	10^{-2}	1.8×10^{-3}
	10^{-3}	1.4×10^{-3}
	10^{-4}	1.0×10^{-3}
	10^{-5}	7.0×10^2

Table 2: Colonial Morphology of the Mold Isolates

Isolate	Form	Surface Colour	Elevation	Texture	Margin	Underneath Colour	Growth Rate
<i>Aspergillus flavus</i>	Irregular	Yellowish green	Flat	Powdery	Filamentous	Cream	Moderately rapid
<i>Fusarium oxysporon</i>	Irregular	Whitish	Flat	Velvety	Lobate	Cream	Slow

Table 3: Microscopic and Macroscopic Characteristics of Fungal Isolates

Isolate	Macroscopic Characteristics	Microscopic Characteristics
<i>Aspergillus flavus</i>	Yellowish-Green colonies with granular texture. Musty odor	Septate, hyaline hyphae with roughened, unbranched and yellowish-green conidiophores that end in a spherical vesicle (uniserate)
<i>Fusarium oxysporon</i>	Light-orange and cottony colonies. Fruity odor	Septate hyphae, sickle-shaped macroconidia that are multi-celled, thin-walled and slightly curved. Thick-walled chlamydospores that appear in clusters.

Table 4: Degradation Rate of *Aspergillus flavus*

Concentration of Dye (%)	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
0.1	5.00 ± 0.00	10.00 ± 0.00	20.00 ± 0.00	47.00 ± 0.00	55.00 ± 0.00	65.00 ± 0.00
0.01	5.00 ± 0.00	15.00 ± 0.00	35.00 ± 0.00	55.00 ± 0.00	67.00 ± 0.00	75.00 ± 0.00
0.001	5.00 ± 0.00	25.00 ± 0.00	50.00 ± 0.00	75.00 ± 0.00	78.00 ± 0.00	80.00 ± 0.00

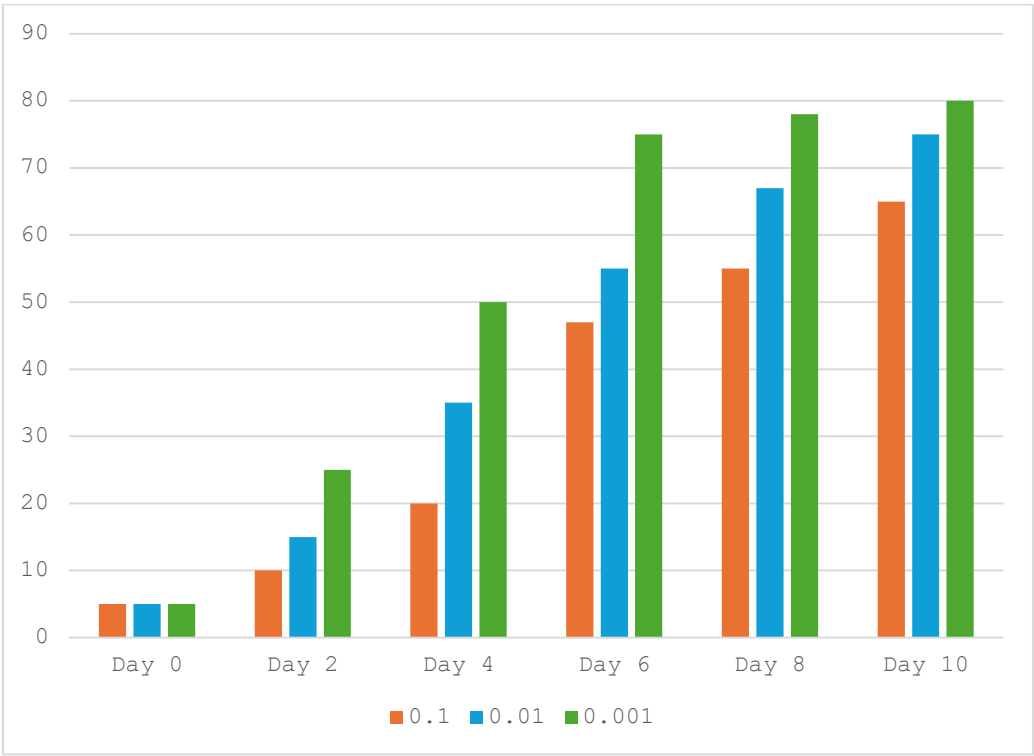


Figure 1: *Aspergillus flavus* Degradation Chart

Table 6: Degradation Rate of *Fusarium oxysporum*

Concentration of Dye (%)	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
0.1	5.00 ± 0.00	18.00 ± 0.00	38.00 ± 0.00	45.00 ± 0.00	58.00 ± 0.00	65.00 ± 0.00
0.01	5.00 ± 0.00	28.00 ± 0.00	43.00 ± 0.00	56.00 ± 0.00	67.00 ± 0.00	72.00 ± 0.00
0.001	5.00 ± 0.00	38.00 ± 0.00	55.00 ± 0.00	70.00 ± 0.00	75.00 ± 0.00	80.00 ± 0.00

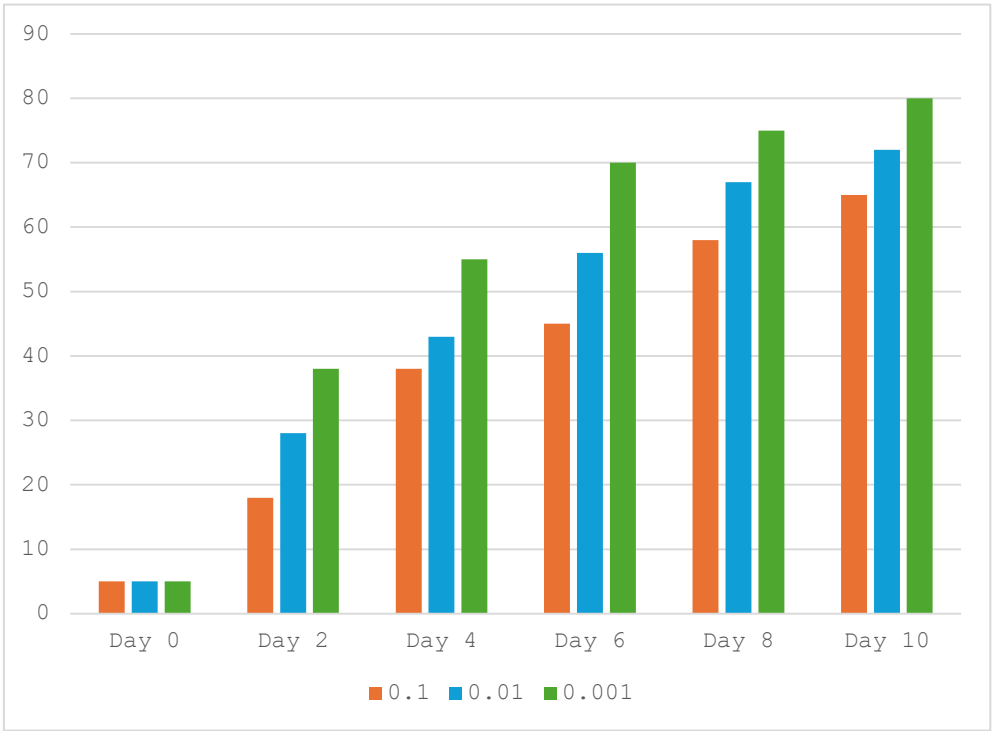


Figure 3: *Fusarium oxysporum* Degradation Chart

DISCUSSION

The results of this study reveal significant insights into the isolation, identification, and degradation potential of fungal species in the context of Phenol Red 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 phenol red 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, as shown in Table 1, 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 phenol red, particularly at lower dye concentrations. As shown in Table 4, *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 phenol red 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 crystal violet. As shown in Table 5, *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 crystal violet (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 phenol red on fungal cells. The high toxicity of triphenylmethane dyes, such as Phenol Red 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 phenol red 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 crystal violet (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 Phenol red 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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