

ISOLATION AND CHARACTERISATION OF MOLDS CAPABLE OF DEGRADING VARYING CONCENTRATIONS OF CRYSTAL VIOLET DYE

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Abstract: The improper disposal of synthetic dyes, particularly crystal violet, poses significant environmental and health risks, necessitating the development of effective remediation strategies. This study aimed to isolate and characterize mold species capable of degrading varying concentrations of crystal violet dye. Soil and cow dung samples were collected and processed for fungal isolation using serial dilution and pour plating techniques. Three fungal species were identified: *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*. Morphological and microscopic analysis revealed distinct characteristics for each species, with *A. niger* exhibiting black, powdery colonies and biserrate conidial heads, *A. flavus* showing yellowish-green colonies with uniseriate columnar conidial heads, and *F. oxysporum* presenting whitish, velvety colonies with chlamydospores. The degradation potential of these isolates was evaluated at crystal violet concentrations of 0.001 %, 0.01 %, 0.1 %, and 1% over eight days. Results showed that *A. niger* exhibited the highest degradation efficiency, achieving 76% degradation at 0.01 % dye concentration by day eight. *A. flavus* demonstrated 80 % degradation at both 0.01 % and 0.001 % concentrations, while *F. oxysporum* showed a maximum degradation of 50 % at 1 % concentration. All isolates showed reduced degradation efficiency at higher dye concentrations, suggesting potential toxicity effects. The study highlights the potential of these fungal species, particularly *A. niger* and *A. flavus*, for bioremediation of crystal violet-contaminated environments. However, the challenges associated with high dye concentrations underscore the need for further research into optimizing fungal bioremediation processes, including the exploration of mixed fungal cultures and the effects of environmental parameters on degradation efficiency.

Keywords: Isolation, Characterisation, Molds, Degradation, Crystal Violet, Dyes.

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INTRODUCTION

The improper disposal of synthetic dyes, particularly from industries such as textiles, paper, cosmetics, and laboratories, has become a significant environmental concern. These dyes, including crystal violet, are persistent in the environment due to their chemical stability and resistance to biodegradation. The release of untreated dye effluents into water bodies poses a severe threat to aquatic ecosystems and human health. Crystal violet, a triphenylmethane dye, is widely used in textile dyeing and other industrial applications, but its improper disposal can lead to long-term contamination because it is toxic, mutagenic, and difficult to remove through conventional treatment methods (Ali et al., 2009; Mani and Bharagava, 2016). Environmental contamination by industrial wastes has become a major ecological challenge worldwide, necessitating the development of sustainable remediation strategies (Agu et al., 2014; Awari et al., 2023; Egreffa et al., 2024a). This environmental issue has prompted extensive research into eco-friendly methods capable of degrading and detoxifying synthetic dyes through biological processes.

Dyes are coloured substances that chemically bond to the materials they are applied to, distinguishing them from pigments, which do not chemically bind. They are widely used in various industries, including textile, paper, cosmetics, pharmaceutical, and food manufacturing (Gürses et al., 2016). The majority of dyes used today are synthetically produced, accounting for over 90% of industrial dyes due to their superior optical properties, durability, and cost-effectiveness compared to natural dyes (Zollinger, 2003). However, the extensive use of synthetic dyes has contributed significantly to environmental pollution and water contamination (Azimi et al., 2021; Rai et al., 2020). The global dyes and pigments market size was valued at USD 42.64 billion in 2023 and is projected to grow from USD 44.68 billion in 2024 to USD 56.91 billion by 2032, exhibiting a CAGR of 5.1% during the forecast period, with Asia-Pacific accounting for the largest market share (Fortune Business Insights, 2023).

Conventional methods for dye removal, including adsorption, coagulation, membrane filtration, and advanced oxidation processes, often suffer from high operational costs, incomplete degradation, and the generation of secondary pollutants (Ahmad et al., 2015; Solayman et al., 2023). Consequently, biological treatment methods have attracted increasing attention because of their environmental compatibility and cost-effectiveness. Bioremediation utilizes microorganisms to transform pollutants into less toxic substances and has been successfully applied in the treatment of hydrocarbon-contaminated soils, agricultural pollutants, and industrial wastes (Okafor et al., 2016a; Okafor et al., 2016b; Orji et al., 2022; Agu et al., 2022). Numerous bacteria have demonstrated the capacity to degrade synthetic dyes, including crystal violet, through enzymatic mechanisms involving reductases and oxidoreductases (Chen et al., 2007; Chen et al., 2008; Parshetti et al., 2011; Cheng et al., 2012). Nevertheless, the degradation pathways remain incompletely understood, and there is growing interest in identifying alternative microbial agents with superior degradative capabilities (Ashar et al., 2022).

While bacterial degradation of crystal violet has received considerable attention, the role of filamentous fungi and molds remains relatively underexplored. Fungi are well known for their

ability to produce a wide range of extracellular enzymes, including ligninolytic and oxidative enzymes such as laccases, manganese peroxidases, lignin peroxidases, proteases, lipases, cellulases, and mannanases, which enable them to degrade complex organic compounds (Chia, 2008; Agu et al., 2013; Agu et al., 2014; Agu et al., 2023; Oparaji et al., 2024). Their extensive enzymatic systems allow them to mineralize recalcitrant pollutants that are resistant to bacterial degradation, making them promising candidates for environmental bioremediation.

Several studies have demonstrated the effectiveness of fungi in the biodegradation of environmental contaminants. *Aspergillus*, *Penicillium*, and other filamentous fungi have been reported to degrade hydrocarbons, pesticides, glyphosate, and synthetic dyes under diverse environmental conditions (Mbachu et al., 2014; Ifediegwu et al., 2015; Orji et al., 2022; Agu and Odibo, 2021). Specifically, *Aspergillus niger* and *Aspergillus flavus* have shown significant degradative potential against Congo red and other industrial dyes (Okeke et al., 2023). Furthermore, fungal species isolated from contaminated environments have demonstrated remarkable adaptability and biodegradation efficiency, highlighting their suitability for large-scale bioremediation applications (Egreffa et al., 2024b; Umeoduagu et al., 2024).

Previous studies have also shown that species such as *Aspergillus niger*, *Penicillium* spp., *Phanerochaete chrysosporium*, and *Trametes versicolor* can effectively decolorize and degrade a variety of synthetic dyes through enzymatic oxidation and mineralization processes (Fu and Viraraghavan, 2001; Sumathi and Manju, 2000; Chagas and Durrant, 2001; Reddy, 1995). The successful application of these fungi suggests that other mold species may possess similar or enhanced abilities for crystal violet degradation. Therefore, the isolation and characterization of indigenous molds capable of degrading crystal violet are essential for developing efficient and environmentally sustainable remediation technologies.

This study therefore aims to isolate, identify, and characterize molds capable of degrading varying concentrations of crystal violet dye. By evaluating their biodegradation potential, the study seeks to contribute to the growing body of knowledge on fungal bioremediation and provide environmentally friendly alternatives for the treatment of dye-contaminated wastewater.

MATERIALS AND METHODS

Soil and Cow Dung Sample Collection

Soil and cow dung samples were collected from areas contaminated with organic matter, which are likely to harbour fungal species capable of degrading dyes. The samples were collected in sterile containers to prevent contamination and transported to the laboratory for analysis. Approximately 1 g of each sample used for microbial isolation.

Preparation of Diluent

A diluent was prepared using 9 ml 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 microbial cells remain viable during the dilution process. The diluent was autoclaved at 121 °C for 15 minutes to ensure sterility.

Sample Measurement and Stock Preparation

To prepare the stock solution, 1 g of each sample (soil and cow dung) was added to 9 ml of sterile peptone water. The sample was thoroughly mixed to ensure homogeneity, creating the stock solution. This mixture served as the initial concentration for serial dilutions.

Serial Dilution Process

Serial dilution was employed to reduce the concentration of microorganisms in the stock solution, making it easier to isolate individual colonies. The dilution process involved transferring 1 ml of the stock solution into 9 ml of sterile peptone water, creating a 10^{-1} dilution. This process was 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.

Pour Plating Technique

For microbial isolation, 0.1ml of the 10^{-4} and 10^{-5} dilutions were 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 gently swirled in both clockwise and anticlockwise directions. The 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 colony was counted.

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 inoculating loop was used to transfer a small portion of the colony onto a fresh SDA plate. This was performed under aseptic conditions to prevent contamination.

Incubation and Maturation

The subcultured plates were incubated at 25-30°C for 7-14 days, allowing sufficient time for the fungal 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.

Preparation and Sterilization of Test Concentrations

To prepare the dye degradation experiment, test concentrations of crystal violet were prepared. The test tubes containing these concentrations were sterilized in an autoclave at 121°C for 15 minutes, ensuring that the test conditions were sterile and free from any contaminants. The sterilized tubes were allowed to cool for 5 minutes before further processing.

In an aseptic environment, the contents of the test tubes were poured into sterile petri dishes. The plates were allowed to solidify before being inoculated with the fungal plugs. This step was critical to ensure that the dye concentration in the test medium was consistent across all experimental replicates.

Preparation and Inoculation of Fungal Plugs

From 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 Petri 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 for 48 hours.

Measurement of Fungal Growth

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

Identification of Fungal Isolates

Wet Mount Method (Tease Mount)

To identify the fungal isolates, the wet mount method, also known as the tease mount technique, was employed. A small portion of fungal mycelium was transferred to a grease-free glass slide using a sterile teasing needle. A drop of lactophenol cotton blue stain was applied to the slide to stain the fungal structures. Excess stain was carefully dabbed off using filter paper. The slide was then covered with a cover slip and examined under a light microscope at 10x and 40x magnifications. The fungal structures, including hyphae, spores, and sporangia photomicrographs were taken to aid in the identification process.

Preparation of Crystal Violet Dye

Crystal violet dye was prepared by dissolving 1g of crystal violet in 300 ml of methanol. Methanol served as the solvent, ensuring the complete dissolution of the dye. To this solution, 650 ml of distilled water and 50 ml of acetic acid were added. The solution was stirred using a magnetic stirrer for 2 hours to ensure homogeneity.

A working solution of the dye was prepared by dissolving 0.2 g of the crystal violet dye in 60 ml of methanol. This solution was then diluted with 130 ml of distilled water and 10 ml of acetic acid. The mixture was stirred for 11 hour to ensure complete dissolution of the dye. This working solution was used for the dye degradation experiments.

Inoculation of Samples for Dye Degradation

Serial dilutions were prepared by transferring 1ml of the stock solution into test tubes containing sterile diluent (peptone water). Dilutions were made up to 10^{-5} to reduce the concentration of the dye. The test tubes containing cow dung samples were centrifuged to separate any particulate matter, and 0.1 ml of the supernatant was inoculated into Petri dishes containing the dye medium.

Sterile molten agar was poured into the petri dishes after inoculation, and the plates were gently swirled to ensure even distribution of the inoculum. The agar s allowed to solidify, and the plates were incubated under controlled conditions for 48 hours.

Test Tube Setup for Concentration Experiments

To test different concentrations of the dye, 12 test tubes were used. Each set of 3 test tubes contained varying concentrations of the dye: 0.1, 0.01, and 0.001. This allowed for the assessment of fungal growth and dye degradation at different dye concentrations.

Statistical Analysis

The data gathered from the plate counts, fungal growth measurements, and dye degradation experiments were subjected to

statistical analysis. Descriptive statistics such as mean, standard deviation, and percentage growth rate calculated to summarize the results. Analysis of variance (ANOVA) was used to compare the differences in fungal growth across different dye concentrations and treatments. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the total fungi count (CFU/ml) derived from soil and cow dung samples at different dilution levels.

SAMPLE	DILUTION FACTOR	TOTAL FUNGAL COUNT (CFU/ML)
Soil	10^{-1}	2.2×10^2
	10^{-2}	1.8×10^2
	10^{-3}	1.4×10^2
	10^{-4}	1.0×10^2
	10^{-5}	7.0×10^1
Cow Dung	10^{-1}	1.5×10^2
	10^{-2}	1.2×10^2
	10^{-3}	9.0×10^1
	10^{-4}	6.0×10^1
	10^{-5}	4.0×10^1

Table 2: Colony Morphology of the Molds

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 oxysporum</i>	Irregular	Whitish	Flat	Velvety	Lobate	Cream	Slow
<i>Aspergillus niger</i>	Circular	Black	Raised	Powdery	Entire	Pale yellow	Rapid

Table 3: Microscopic Morphology of the Molds

Isolate	Type of Asexual Spore	Specialized Structure	Genera
<i>Aspergillus flavus</i>	Conidiospore	Uniseriate columnar conidia head	<i>Ascomycota</i>
<i>Fusarium oxysporum</i>	Chlamydospore	Sclerotia	<i>Fusarium</i>
<i>Aspergillus niger</i>	Conidiospore	Biserrate conidia head with phialides	<i>Ascomycota</i>

Table 4: Degradation Rate of *Aspergillus niger*

Concentration of Dye (%)	Day 2	Day 4	Day 6	Day 8
1	5.00 ± 0.00	5.00 ± 0.00	55.00 ± 0.00	44.00 ± 0.00
0.1	5.00 ± 0.00	18.00 ± 0.00	13.00 ± 0.00	17.00 ± 0.00
0.01	20.00 ± 0.00	26.00 ± 0.00	35.00 ± 0.00	76.00 ± 0.00
0.001	25.00 ± 0.00	0.00 ± 0.00	60.00 ± 0.00	35.00 ± 0.00

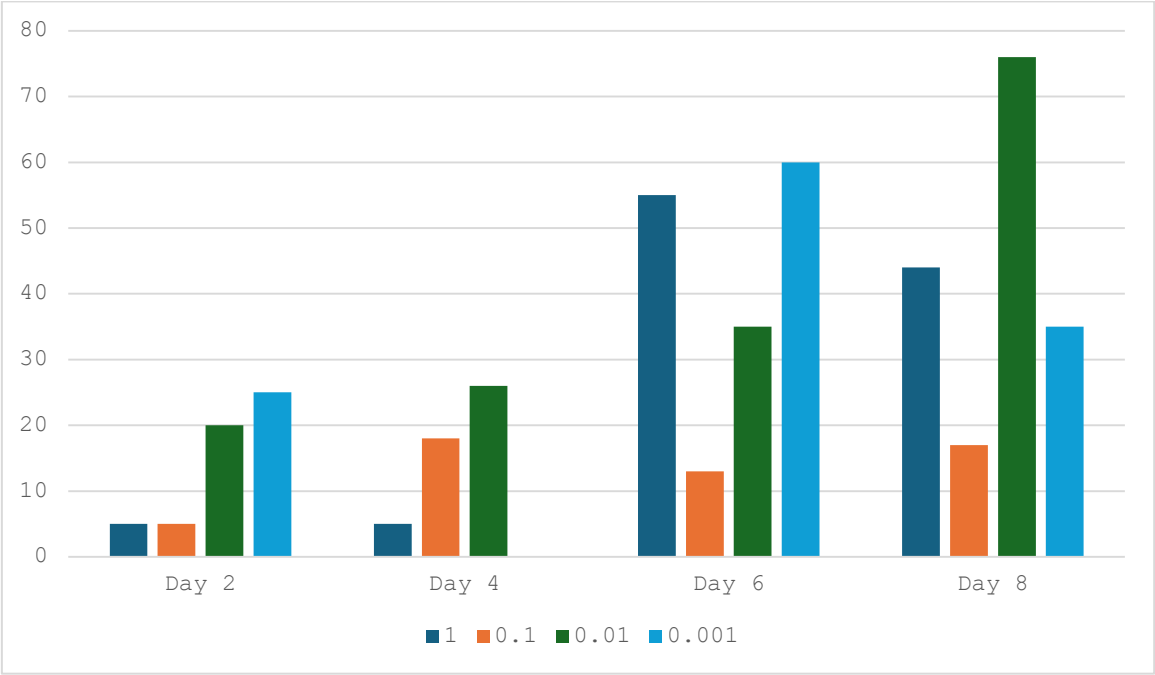


Figure 1: *Aspergillus niger* Degradation Chart

Table 5: Degradation Rate of *Aspergillus flavus*

Concentration of Dye (%)	Day 2	Day 4	Day 6	Day 8
1	5.00 ± 0.00	5.00 ± 0.00	75.00 ± 0.00	50.00 ± 0.00
0.1	11.00 ± 0.00	19.00 ± 0.00	12.00 ± 0.00	18.00 ± 0.00
0.01	14.00 ± 0.00	14.00 ± 0.00	80.00 ± 0.00	80.00 ± 0.00
0.001	14.00 ± 0.00	65.00 ± 0.00	80.00 ± 0.00	60.00 ± 0.00

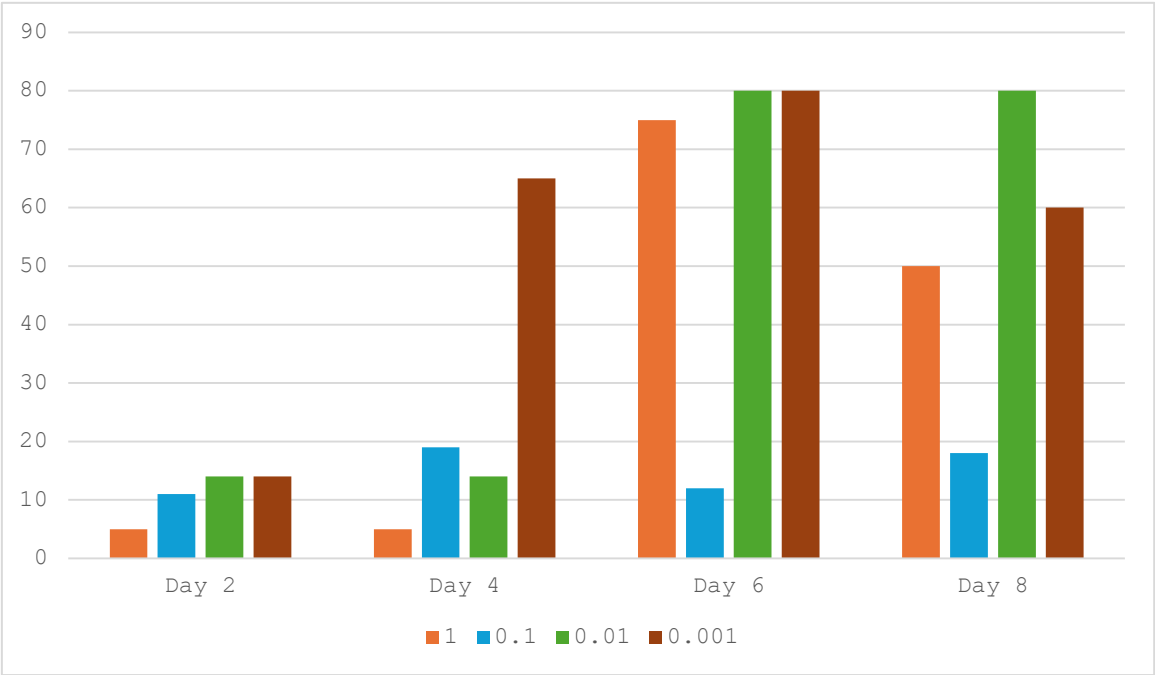
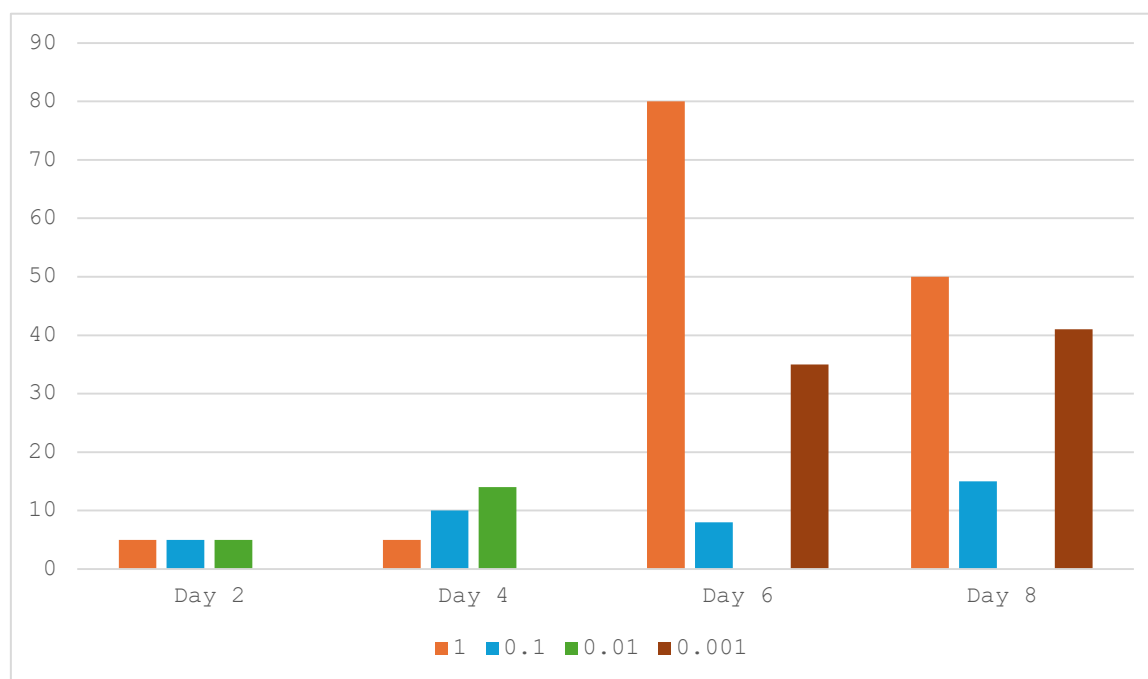


Figure 2: *Aspergillus flavus* Degradation Chart

Table 6: Degradation Rate of *Fusarium oxysporum*

Concentration of Dye (%)	Day 2	Day 4	Day 6	Day 8
1	5.00 ± 0.00	5.00 ± 0.00	80.00 ± 0.00	50.00 ± 0.00
0.1	5.00 ± 0.00	10.00 ± 0.00	8.00 ± 0.00	15.00 ± 0.00
0.01	5.00 ± 0.00	14.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.001	0.00 ± 0.00	0.00 ± 0.00	35.00 ± 0.00	41.00 ± 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 crystal violet 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 crystal violet 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 CPU 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, 2009). 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 of crystal violet, 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 179% degradation, respectively, by day eight. This suggests that high concentrations of crystal violet 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., 2008). 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, 2016).

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 crystal violet on fungal cells. The high toxicity of triphenylmethane dyes, such as crystal violet, is well-documented, and their ability to interfere with fungal metabolic processes is a significant challenge in bioremediation efforts (Ali et al., 2009). Despite these limitations, the overall performance of *A. flavus* in degrading crystal violet 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 6, *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 have shown that *F. oxysporum* is less efficient in degrading synthetic dyes, particularly those with complex molecular structures like crystal violet (Kalyani et al., 2008).

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 crystal violet suggests that it may still be useful in bioremediation strategies, particularly in combination with other, more efficient fungal species.

CONCLUSION

In conclusion, this study has demonstrated the potential of *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum* in degrading crystal violet dye. The degradation rates observed, particularly at lower dye concentrations, suggest that these fungi could be valuable agents in bioremediation strategies aimed at mitigating dye pollution. However, the challenges associated with high dye concentrations and the toxicity of synthetic dyes underscore the need for further research into optimizing fungal bioremediation processes. Future studies should focus on exploring the effects of environmental parameters, nutrient supplementation, and the use of mixed fungal cultures to enhance the degradation efficiency of fungal species in various environmental conditions.

REFERENCES

1. Agu, K.C. and Chidozie, C.P. (2021). An improved slide culture technique for the microscopic identification of fungal species. *International Journal of Trend in Scientific Research and Development*, 6(1): 243–254.
2. Agu, K.C. and Odibo, F.J.C. (2021). Biodegradation Potentials of *Aspergillus flavipes* Isolated from Uburu and Okposi Salt Lakes. *International Journal of Trend in Scientific Research and Development*, 5(5): 1160–1170.
3. Agu, K.C., Ogbue, M.O., Abuchi, H.U., Onunkwo, A.U., Chidi-Onuorah, L.C. and Awah, N.S. (2013). Lipase Production by Fungal Isolates from Palm Oil-Contaminated Soil in Awka, Anambra State, Nigeria. *International Journal of Agriculture and Biosciences*, 2(6): 386–390.
4. Agu, K.C., Okafor, F.C., Amadi, O.C., Mbachu, A.E., Awah, N.S. and Odili, L.C. (2014). Production of Mannanase Enzyme Using *Aspergillus* spp. Isolated from Decaying Palm Press Cake. *Scholars Academic Journal of Biosciences*, 2(12A): 863–870.
5. Agu, K.C., Orji, M.U., Ikele, M.O., Uwanta, L.I. and Onyencho, V.I. (2022). Hydrocarbon Biodegradation Potential of Cyanobacteria in Oil Polluted Soil. *International Journal of Trend in Scientific Research and Development*, 6(7): 733–737.
6. Agu, K.C., Orji, M.U., Onuorah, S.C., Egufofa, S.O., Anaukwu, C.G., Okafor, U.C., Awah, N.S., Okafor, O.I., Mbachu, A.E. and Anyaegbunam, B.C. (2014). Influence of Solid Waste Dumps Leachate on Bacteriological and Heavy Metals Contamination of Ground Water in Awka. *American Journal of Life Science Researches*, 2(4): 450–457.
7. Agu, K.C., Umeoduagu, N.D., Victor-Aduloju, A.T., Uwanta, L.I., Adepeju, D.M., Udenweze, E.C., Awari, V.G., Chidubem-Nwachinemere, N.O., Nwosu, J.C. and Udeh, K.C. (2023). Isolation and Characterization of Proteolytic Enzyme Produced from Fungi. *Cognizance Journal of Multidisciplinary Studies*, 3(6): 485–493.

8. Agu, K.C., Bassey, E.E., Iloanusi, C.A., Awah, N.S., Okeke, B.C., Anaukwu, C.G., Ezenwa, C.U., Orji, M.U. and Okafor, A.C. (2015). Isolation and Characterization of Microorganisms from Oil Polluted Soil in Kwata, Awka South, Nigeria. *American Journal of Current Microbiology*, **3**: 46–59.
9. Anaukwu, C.G., Ezemba, C.C., Anakwenze, V.N., Agu, K.C., Amechi, S.N., Okeke, B.C. and Awah, N.S. (2016). Influence of Anionic, Cationic and Non-Ionic Surfactants on Growth of Hydrocarbon Utilizing Bacteria. *American Journal of Current Microbiology*, **4**: 10–16.
10. Anaukwu, C.G., Ezemba, C.C., Anakwenze, V.N., Agu, K.C., Okeke, B.C., Awah, N.S. and Ekwealor, I.A. (2016). Effect of biosurfactant produced by *Citrobacter murlinae* AF025369 and a synthetic surfactant on degradation of crude oil. *Edorium Journal of Microbiology*, **2**: 1–6.
11. Awari, V.G., Umeoduagu, N.D., Agu, K.C., Okonkwo, N.N., Ozuah, C.L. and Victor-Aduloju, A.T. (2023). The Ubiquity, Importance and Harmful Effects of Microorganisms: An Environmental and Public Health Perspective. *International Journal of Progressive Research in Engineering Management and Science*, **3**(12): 1–10.
12. Barnett, H.L. and Hunter, B.B. (2000). In: Laskin, A. and Lechevalier, H.A. (Eds.), *Illustrated Genera of Imperfect Fungi* (4th ed., pp. 1–197). West Palm Beach, Florida: CRC Press.
13. Chidi-Onuorah, L.C., Onunkwo, A.U., Agu, K.C., Ogbue, M., Kyrian-Ogbonna, E.A., Awah, N.S., Okeke, C.B. and Nweke, G.U. (2015). Optimization of Reaction Time for the Assay of Protease Activity in a Local Strain of *Aspergillus niger*. *International Journal of Research Studies in Biosciences*, **3**(9): 1–5.
14. Egrefa, S.O., Awari, V.G., Okinedo, J.I., Ogbonna, U.S.A., Obianom, A.O., Aniekwu, C.C., Agu, K.C., Umeoduagu, N.D., Nwosu, J.C. and Aji, S.M. (2024). Isolation and identification of fungal species in pesticide-contaminated soil from Abo-Obosi, Anambra. *Research Journal of Life Science, Bioinformative, Pharmaceuticals and Chemical Science*, **5**(10): 1–12.
15. Egrefa, S.O., Okinedo, J.I., Awari, V.G., Ogbonna, U.S.A., Obianom, A.O., Victor-Aduloju, A.T., Abana, C.C., Umeoduagu, N.D., Uwanta, L.I., Agu, K.C., Nwosu, J.C. and Onoyima, L.C. (2024). Assessment of the bacteriological and heavy metal contamination in drinking water from borehole sites in Enugu metropolis. *Research Journal of Life Science, Bioinformative, Pharmaceuticals and Chemical Science*, **10**(5): 13–26.
16. Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R. (2007). *Descriptions of Medical Fungi*. Retrieved September 13, 2013.
17. Ezeokoli, C.M., Agu, K.C., Nwosu, J.C., Orji, M.U., Uwanta, L.I., Umeoduagu, N.D., Victor-Aduloju, A.T. and Ikenwa, B.O. (2023). Bacteriological Evaluation of Kwata Abattoir Waste Water, Awka, Nigeria. *Innovare Journal of Science*, **11**: 1–4.
18. Ezenwelu, C.O., Okeke, C.M., Duruamaku, P.U., Udemezue, O.I., Agu, K.C. and Oparaji, E.H. (2024). Ecological Significance of Dynamism of Physiologic Variance on Properties of Palm Oil Mill Effluents. *Asian Journal of Microbiology and Biotechnology*, **9**(1): 60–67.
19. Ifediegwu, M.C., Agu, K.C., Awah, N.S., Mbachu, A.E., Okeke, C.B., Anaukwu, C.G., Uba, P.O., Ngenegbo, U.C. and Nwankwo, C.M. (2015). Isolation, Growth and Identification of Chlorpyrifos Degrading Bacteria from Agricultural Soil in Anambra State, Nigeria. *Universal Journal of Microbiology Research*, **3**(4): 46–52.
20. Mbachu, A.E., Onochie, C.C., Agu, K.C., Okafor, O.I. and Awah, N.S. (2014). Hydrocarbon Degrading Potentials of Indigenous Bacteria Isolated from Auto-Mechanic Workshops at Mgbuka-Nkpor, Nigeria. *Journal of Global Biosciences*, **3**(1): 321–326.
21. Ojiagu, K.D., Odibo, F.J.C., Ojiagu, N.C., Agu, K.C. and Okafor, A.C. (2018). Biosorption of Hexavalent Chromium by *Pseudomonas aeruginosa* Strain ANSC: Equilibrium Isothermic, Kinetic and Thermodynamic Studies. *Bioengineering and Bioscience*, **6**(1): 1–10.
22. Okafor, U.C., Orji, M.U., Agu, K.C., Awah, N.S., Okeke, B.C., Okafor, O.I. and Okoro, N.C.N. (2016). Bioremediation of Crude Oil-Polluted Soil Using Broiler-Chicken Droppings. *Journal of Applied and Environmental Microbiology*, **4**(4): 75–84.
23. Okafor, U.C., Orji, M.U., Nwankwegu, A.S., Anaukwu, C.G., Onuorah, S.C., Archibong, E.J., Obika, I.E. and Agu, K.C. (2016). Effect of Chicken Droppings Amendment on Bioremediation of Crude Oil Polluted Soil. *European Journal of Experimental Biology*, **6**(4): 62–68.
24. Okeke, B.C., Uwanta, L.I., Odibo, F.J.C., Agu, K.C. and Victor-Aduloju, A.T. (2023). Monoculture Solid Phase Degradative Potential of Congo Red by *Aspergillus niger* and *Aspergillus flavus*. *International Journal of Trend in Scientific Research and Development*, **7**(3): 684–688.
25. Okonkwo, N.N., Okoli, F.A., Agu, K.C., Okeke, C.M., Awari, V.G., Ifediegwu, M.C. and Umeoduagu, N.D. (2023). Identification of Indigenous Bacteria from Soil Contaminated with Cassava Mill Effluents. *International Journal of Progressive Research in Engineering Management and Science*, **3**(9): 393–399.
26. Oparaji, E.H., Ogana, J., Ngwu, R.O., Agu, K.C. and Ezenwelu, C.O. (2024). Studies on Kinetic Properties of *Aspergillus* Producing Peroxidase from Petroleum Hydrocarbon Spilled Soil. *Rafidain Journal of Science*, **33**(1): 49–67.
27. Orji, M.U., Agu, K.C., Ikele, M.O., Uwanta, L.I. and Ugwuoke, G. (2022). Bioremediation of Glyphosate Polluted Soil using Fungal Species. *International Journal of Trend in Scientific Research and Development*, **6**(7): 726–732.
28. Orji, M.U., Ezenekwe, F.N., Odibo, F.J.C., Agu, K.C. and Mbachu, A.E. (2014). Utilization of Used Global System for Mobile Communication (GSM) Recharge Cards for Cellulase

- Production by *Penicillium* Species and *Aspergillus* Species. *American Journal of Life Science Researches*, **2**(4): 548–556.
29. Umeoduagu, N.D., Uwanta, L.I., Agu, K.C., Ozuah, C.L., Anazodo, C.A., Okoli, F.A., Orji, C.C., Chukwujekwu, A.G. and Anene, C.C. (2024). Randomized Genetically Modified Monoculture Degradation by *Aspergillus niger* and *Penicillium chrysogenum* on Varying Concentration of Spent Engine Oil. *International Journal of Research*, **11**(6): 162–173.
 30. Uwanta, L.I., Orji, M.U., Agu, K.C., Udenweze, E.C., Umeoduagu, N.D., Egrefa, S.O. and Awari, V.G. (2023). *Pseudomonas aeruginosa* Isolated from Vermicompost in the Degradation of Varying Concentration of Polyvinyl Chloride (PVC) and Polyvinyl Alcohol (PVA). *International Journal of Research Publication and Reviews*, **4**(8): 547–553.
 31. Uwanta, L.I., Okoli, F.A., Anazodo, C.A., Ozuah, C.L., Agu, K.C., Ezeokoli, M.C. and Ezenwelu, C.O. (2024). Comparative Mechanical Properties of Cassava (*Manihot esculenta*) and Potato (*Ipomoea batatas*) Peel Starch Biodegradable Plastics in Food Packaging. *International Journal of Research Publication and Reviews*, **5**(4): 4376–4379.
 32. Victor-Aduloju, A.T., Okonkwo, N.N., Okoli, F.A., Agu, K.C., Okoye, C.W., Awari, V.G. and Umeoduagu, N.D. (2023). Comparative Analysis of Microbial Load of Water in Selected Hostels in Ifite, Awka. *International Journal of Progressive Research in Engineering Management and Science*, **3**(9): 400–408.
 33. Watanabe, T. (2002). In: Haddad, S., Norwitz, D.E. and Lewis, B.E.R. (Eds.), *Pictorial Atlas of Soil and Seed Fungi* (2nd ed., pp. 1–486). Boca Raton, Florida: CRC Press LLC.