DEGRADATION OF EMULSION PAINT USING FUNGAL ISOLATES

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NOVEMBER, 2025.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work titled; “DEGRADATION OF EMULSION PAINT USING FUNGAL ISOLATES” was completed by Happy Osayanmo ASIRIUWA (Miss) with matriculation number LSC2007276, under the supervision of Mr. Haruna O. in the Department of Science Laboratory Technology (Microbiology Techniques), Faculty of Life Sciences, University of Benin, Benin City.

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EXTERNAL EXAMINER	**DATE**

DEDICATION

This project is dedicated to GOD ALMIGHTY for his Support, Guidance, Protection and Provisions.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to the following persons for their immense support and assistance in making this project work a reality.

My appreciation goes to my project supervisor, Mr. Haruna. O, for his endless guidance and advice. I would like to offer my sincere gratitude to him. Thank you sir for staying late with me in the lab during the course of this project despite your busy schedule. I also appreciate the counsel from my Head of Department, Prof. J. O. Osarumwense, and to the entire staff of the Department of Science Laboratory Technology.

My deepest appreciation goes to my lovely parents Mr. and Mrs. Obazee Asiriuwa, for their endless support financially, prayers, advice and words of encouragement, words won't be enough to show my sincere gratitude, thank you for your support.

My appreciation also goes to my friends for their moral support and colleagues, especially Efe, Blessing, Mercy and every other person for making these project work a success. May GOD ALMIGHTY bless you all AMEN.

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ABSTRACT

Emulsion paints are widely used for decorative and protective purposes but are susceptible to fungal degradation, particularly in tropical climates with high humidity. This study investigated the degradation of emulsion paint using fungal isolates (*Aspergillus niger* and *Penicillium* species) under controlled laboratory conditions. The fungi were inoculated into mineral salt medium supplemented with 5% commercial white emulsion paint as the sole carbon source and incubated at room temperature (25–28°C) for seven days. Four experimental setups were established: Flask A (*Aspergillus niger*), Flask B (*Penicillium* sp.), Flask AB (mixed culture), and a control flask. Fungal growth was monitored using serial dilution and pour plate techniques on Potato Dextrose Agar (PDA), while physicochemical parameters (pH, temperature, and optical density at 600 nm) were measured at regular intervals. Results revealed that *Penicillium* sp. achieved the highest final population of 2.75×10^6 CFU/ml and optical density of 50.9, indicating superior paint degradation capability. *Aspergillus niger* demonstrated the highest percentage growth rate of 1,328.6%, increasing from 3.5×10^4 CFU/ml to 5.0×10^5 CFU/ml between Day 3 and Day 7. The mixed culture yielded 1.50×10^6 CFU/ml with a growth rate of 1,053.8%, showing no significant synergistic effect. pH fluctuations, particularly acidification to pH 5.1 in single species flasks, suggested organic acid production during metabolism, facilitating enzymatic breakdown of polymeric paint components. These findings highlight the ecological significance of fungi in paint deterioration and emphasize the need for antifungal additives in paint formulations, routine maintenance, and environmental control measures to mitigate microbial colonization in humid tropical environments.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Paints are complex mixtures of organic and inorganic components widely used for protective and decorative purposes on various surfaces including wood, metal, concrete, and plastics (Okafor *et al.,* 2020). Among the various types of paints available, emulsion paints are particularly popular due to their water-based nature, ease of application, low odor, and environmentally friendly properties compared to solvent-based paints (Sharma *et al.,* 2022). Emulsion paints typically consist of pigments, binders (polymers such as acrylic, vinyl acetate, or styrene-butadiene), water as the solvent, and various additives including preservatives, thickeners, and stabilizers (Kumar *et al.,* 2021).

Despite their widespread use and advantages, emulsion paints are susceptible to biological degradation, particularly by microorganisms such as fungi and bacteria (Mesquita *et al.,* 2021). The organic components present in emulsion paints, especially the polymeric binders and organic additives, serve as potential carbon and energy sources for microbial growth (Bellotti *et al.,* 2023). This biological deterioration not only affects the aesthetic appearance of painted surfaces but also compromises the protective function of the paint film, leading to reduced durability and increased maintenance costs (Afshinnekoo *et al.,* 2020).

Fungal degradation of emulsion paints is a significant concern in tropical and subtropical regions where high temperature and humidity create favorable conditions for microbial growth (Ijoma and Nkuna, 2021). Fungi, being heterotrophic organisms, possess the enzymatic capability to break down complex organic polymers present in paint formulations (Adeniji *et al.*, 2022). Common fungal genera implicated in paint degradation include *Aspergillus*, *Penicillium*, *Alternaria, Fusarium,* and *Trichoderma* (Ogbonna *et al.,* 2021). These fungi produce extracellular enzymes such as esterases, proteases, and cellulases that can hydrolyze the polymeric components of paints, leading to discoloration, loss of adhesion, film softening, and eventual complete breakdown of the paint matrix (Rosado *et al.,* 2020).

The study of fungal degradation of emulsion paints is important from multiple perspectives. From an industrial standpoint, understanding the mechanisms of biodegradation helps manufacturers develop more resistant paint formulations and effective preservation strategies (Zhu *et al.*, 2022). From an environmental perspective, the ability of fungi to degrade paint components presents opportunities for bioremediation of paint waste and the development of eco-friendly paint removal methods (Okonkwo *et al.,* 2023). Additionally, knowledge of the fungal species involved and their degradative capabilities is essential for predicting paint longevity and developing appropriate maintenance protocols (Dakal *et al.,* 2021).

Aspergillus niger and *Penicillium species* are among the most frequently isolated fungi from deteriorated paint surfaces (Ezeudu *et al*., 2022). *Aspergillus niger* is a filamentous fungus known for its robust enzymatic machinery and ability to thrive in diverse environmental conditions (Meyer *et al.*, 2020). Similarly, *Penicillium* species are cosmopolitan fungi with significant biodegradative potential (Visagie *et al.,* 2021). Both fungi have been reported to colonize painted surfaces and cause substantial damage through their metabolic activities (Nwachukwu *et al.,* 2020).

While considerable research has been conducted on microbial degradation of various materials, there remains a need for systematic studies on the specific degradative potential of individual fungal species and their synergistic effects when present in mixed cultures (Guiamet *et al.,* 2021). Understanding the degradation patterns, growth kinetics, and physicochemical changes induced by these fungi during paint degradation is crucial for developing effective biocontrol strategies and improving paint formulations (Bellotti *et al.,* 2022).

This study therefore focuses on investigating the degradative ability of *Aspergillus niger* and *Penicillium* species on emulsion paint, both as pure cultures and in combination, with the aim of elucidating their biodegradation potential and providing valuable information for the paint industry and environmental management.

1.2 Aim and Objectives of the Study

The aim of this study is to investigate the degradation of emulsion paint using fungal isolates.

Objectives of the Study:

The objectives of this study were to:

1. determine the ability of these fungi to utilize emulsion paint components as a source of carbon.
2. monitor the pH changes of the fungi degrading emulsion paints.
3. determine the total viable count of the fungi degrading the emulsion paint.
4. determine the optical density of the degrading fungi after 3 and 7 days.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Paint Degradation

Paint degradation by microorganisms, particularly fungi, has become a significant area of research due to its implications for both material preservation and environmental bioremediation. This chapter reviews relevant literature on the nature of paints, microbial degradation mechanisms, fungal biodegradation processes, and the specific roles of *Aspergillus* niger and *Penicillium* species in paint deterioration. The review provides a theoretical foundation for understanding the experimental work conducted in this study.

2.2 Composition of Paints

Paints are liquid or semi-liquid coatings applied to surfaces for protection and decoration (Okafor *et al.,* 2020). They consist of four main components: pigments, binders, solvents, and additives. The pigments provide colour and opacity, binders hold the pigment particles together and attach them to the surface, solvents control the viscosity and facilitate application, while additives enhance specific properties such as durability, drying time, and resistance to microbial attack (Kumar *et al.,* 2021). Modern paint formulations have evolved significantly, with manufacturers incorporating various synthetic polymers and advanced additives to improve performance characteristics (Sharma *et al.*, 2022). Despite these improvements, paints remain susceptible to environmental degradation, particularly biological deterioration caused by microorganisms.

2.2.1 Types of Paints

Paints are broadly classified into two categories: solvent-based (oil-based) and water-based (emulsion) paints (Singh *et al.,* 2023). Solvent-based paints use organic solvents as the carrier medium and typically contain alkyd resins or oils as binders. Water-based paints, on the other hand, use water as the primary solvent and contain polymeric emulsions as binders (Bellotti *et al.,* 2022). Other classifications include specialty paints such as anti-corrosive paints, heatresistant paints, and antimicrobial paints, each designed for specific applications (Zhu *et al.,* 2022). The choice of paint type depends on factors such as the substrate to be coated, environmental conditions, desired aesthetic properties, and functional requirements.

2.2.2 Emulsion Paints

Emulsion paints are water-based coatings in which the binder is dispersed as fine droplets in water, forming a stable emulsion (Patil and Rane, 2023). The most common binders used in emulsion paints include acrylic polymers, vinyl acetate copolymers, and styrene-butadiene latex (Rosado *et al.*, 2020). These paints have gained widespread popularity due to several advantages including low volatile organic compound (VOC) emissions, ease of application, quick drying time, good color retention, and easy cleanup with water (Adeniji *et al.*, 2022). Emulsion paints typically consist of 40-50% water, 20-30% binder, 20-30% pigment, and 5-10% additives by weight (Mesquita *et al.,* 2021). Common additives include thickeners (cellulose derivatives), dispersants, defoamers, coalescents, and biocides. The biocides are incorporated to prevent microbial growth both in the wet paint (in-can preservation) and on the dried paint film (film preservation) (Bellotti *et al.*, 2023). The polymeric binders in emulsion paints are susceptible to microbial degradation because they contain carbon-carbon and ester bonds that can be cleaved by microbial enzymes (Ogbonna *et al.*, 2022). Additionally, other organic components such as cellulosic thickeners and organic pigments can serve as nutrients for microbial growth.

2.3 Biodeterioration of Paints

2.3.1 Concept of Biodeterioration

Biodeterioration refers to the undesirable change in the properties of materials caused by the activities of living organisms (Dakal *et al.*, 2021). It encompasses physical, chemical, and aesthetic changes that reduce the value, utility, or longevity of materials. In the context of paints, biodeterioration manifests as discoloration, surface roughening, loss of gloss, cracking, blistering, and eventual loss of adhesion and protective properties (Guiamet *et al.,* 2021). Biodeterioration differs from biodegradation in that the former emphasizes the negative consequences of biological activity on material integrity, while the latter refers more generally to the biological breakdown of substances (Pinzari *et al*., 2022). However, in the context of paint degradation, these terms are often used interchangeably.

2.3.2 Mechanisms of Paint Biodeterioration

Microorganisms degrade paints through several mechanisms, which can be broadly categorized as physical, chemical, and biochemical processes (Trovão *et al.,* 2020). Physical Mechanisms: Microorganisms, particularly fungi, produce hyphae that can penetrate paint films, creating channels and disrupting the structural integrity of the coating (Eze and Chukwunonso, 2021). The physical presence of microbial growth on paint surfaces can also trap moisture, creating conditions favorable for further deterioration. Additionally, microbial biofilms can interfere with the adhesion of paint to substrates. Chemical Mechanisms: Microorganisms produce various metabolic by-products including organic acids (citric acid, oxalic acid, gluconic acid), which can chemically attack paint components (Adeniji *et al.*, 2023). These acids can lower the pH of the microenvironment, causing hydrolysis of ester bonds in polymeric binders and dissolution of certain pigments. Some microorganisms also produce pigmented compounds that cause staining and discoloration of painted surfaces (Nwachukwu *et al.,* 2020). Biochemical Mechanisms: The most significant mechanism of paint biodegradation involves enzymatic breakdown of organic components. Microorganisms produce extracellular enzymes including esterases, lipases, proteases, and cellulases that can hydrolyze the polymeric binders and organic additives in paints (Kumar *et al.,* 2021). These enzymes break down complex macromolecules into smaller units that can be absorbed and metabolized by the microorganisms as carbon and energy sources.

2.3.3 Factors Affecting Paint Biodeterioration

Several environmental and material factors influence the susceptibility of paints to biodeterioration (Ogbonna *et al*., 2021):

Moisture

Water availability is the most critical factor affecting microbial growth on painted surfaces. Most fungi require a relative humidity above 65% for germination and growth (Mesquita *et al*., 2021). In tropical climates like Nigeria, high humidity levels create ideal conditions for fungal colonization of painted surfaces.

Temperature

It affects both microbial growth rates and enzymatic activity. Most fungi associated with paint degradation are mesophilic, growing optimally between 20°C and 35°C (Meyer *et al.*, 2020). The warm temperatures prevalent in tropical regions favor rapid fungal growth and accelerated paint degradation.

pH:

The pH of the paint film and substrate influences microbial colonization. Most fungi prefer slightly acidic to neutral pH conditions (pH 4.0-7.0), although some species can tolerate alkaline conditions (Ijoma and Nkuna, 2021). Many fungi can modify their local environment by producing organic acids, creating favorable pH conditions for growth.

Nutrient Availability

The organic content of paints, particularly the binder and organic additives, provides nutrients for microbial growth (Bellotti *et al.,* 2023). Paints with higher organic content are generally more susceptible to biodegradation. External contamination from dust, dirt, and atmospheric pollutants can also provide additional nutrients.

Light

While not a direct requirement for fungal growth, light can influence paint degradation indirectly by causing photodegradation of paint components, which may make them more susceptible to microbial attack (Rosado *et al.,* 2020). Some fungi are also phototropic and may be attracted to or repelled by light.

Biocide Efficacy

The type, concentration, and stability of biocides incorporated into paint formulations significantly affect resistance to microbial colonization (Singh *et al.,* 2023). However, biocide effectiveness can diminish over time due to leaching, photodegradation, or microbial adaptation, leading to eventual colonization.

Surface Characteristics

Surface roughness, porosity, and the presence of defects or cracks can influence microbial attachment and colonization (Patil and Rane, 2023). Rougher surfaces provide more sites for microbial attachment and may retain more moisture and nutrients.

Plate 1: Deteriorated emulsion-painted wall.

2.4 Microorganisms Involved in Paint Degradation

2.4.1 Paint-Degrading Microorganisms

A diverse array of microorganisms has been implicated in paint degradation, including bacteria, fungi, algae, and cyanobacteria (Guiamet *et al.*, 2021). However, fungi are generally considered the primary agents of paint biodeterioration, particularly in indoor environments and on vertical surfaces where moisture is limited (Dakal *et al*., 2021). Common bacterial genera associated with paint degradation include *Bacillus, Pseudomonas*, *Arthrobacter,* and *Streptomyces* (Okonkwo *et al.,* 2023). These bacteria can degrade various paint components and often act synergistically with fungi in mixed biofilms. Algae and cyanobacteria are more commonly found on exterior painted surfaces exposed to sunlight and moisture, where they contribute to discoloration and biofilm formation (Udoh *et al.*, 2021).

2.4.2 Fungi as Primary Paint Degraders

Fungi are particularly effective paint degraders due to several characteristics (Ezeudu *et al.*, 2022):

1. Versatile Enzyme Systems: Fungi produces a wide array of extracellular enzymes capable of degrading complex organic polymers, including those found in paint binders.
2. Hyphal Growth: The filamentous growth habit of fungi allows them to penetrate paint films and access nutrients deep within the coating.
3. Tolerance to Low Moisture: Many fungi can grow at lower water activities than bacteria, allowing them to colonize painted surfaces under conditions that would not support bacterial growth.
4. pH Modification: Fungi can alter their microenvironment by producing organic acids, creating favorable conditions for growth and enhancing degradation of paint components.

5. Spore Production: Fungi produce abundant spores that can be dispersed widely by air currents, facilitating colonization of painted surfaces.

Common fungal genera isolated from deteriorated painted surfaces include *Aspergillus, Penicillium*, *Cladosporium, Alternaria, Fusarium, Trichoderma, Mucor, and Aureobasidium* (Ogbonna *et al*., 2021; Patil and Rane, 2023). Among these, *Aspergillus* and *Penicillium species* are the most frequently reported and are considered the most significant agents of paint biodeterioration worldwide (Nwachukwu *et al.,* 2020).

2 5 *Aspergillus niger*

2.5.1 General Characteristics

Aspergillus niger is a filamentous fungus belonging to the phylum Ascomycota, class Eurotiomycetes, order Eurotiales, and family *Aspergillaceae* (Meyer *et al*., 2020). It is one of the most common species in the genus *Aspergillus* and is widely distributed in nature, being found in soil, decaying organic matter, indoor environments, and various industrial settings. The fungus is characterized by black conidial heads, which give it its species name *niger* (meaning black in Latin). Colonies of *Aspergillus niger* typically grow rapidly on standard mycological media, producing dense mycelium that initially appears white to yellow before turning black as conidia mature (Schuster *et al*., 2022). The conidiophores are smooth-walled, hyaline, and terminate in large, dark brown to black vesicles bearing phialides that produce chains of globose, brown to black conidia.

2.5.2 Physiology and Metabolism

Aspergillus niger is a versatile organism capable of growing on a wide range of substrates and under diverse environmental conditions (Kumar *et al*., 2021). It is aerobic and grows optimally at temperatures between 25°C and 37°C, with some strains tolerating temperatures up to 45°C. The fungus can grow over a pH range of 1.5 to 9.8, although optimal growth occurs between pH 3.0 and 6.0 (Ijoma and Nkuna, 2021). One of the most notable characteristics of *Aspergillus niger* is its extensive enzymatic machinery. The fungus produces a wide array of hydrolytic enzymes including cellulases, xylanases, pectinases, amylases, proteases, lipases, and esterases (Adeniji *et al.,* 2022). These enzymes enable *Aspergillus niger* to degrade complex organic polymers, making it an effective degrader of various materials including paints. *Aspergillus niger* is also known for its production of organic acids, particularly citric acid, which is produced in large quantities under specific culture conditions (Meyer *et al.,* 2020). This acid production capability contributes to its degradative potential, as the acids can chemically attack paint components and modify the pH of the microenvironment.

2.5.3 Role in Paint Degradation

Aspergillus niger has been frequently isolated from deteriorated painted surfaces and is recognized as a significant agent of paint biodeterioration (Nwachukwu *et al.,* 2020). Several studies have documented its ability to degrade various paint components: (Okafor *et al.,* 2020). isolated *Aspergillus niger* from discolored painted walls in Awka, Nigeria, and demonstrated its ability to grow on paint extract media. The study showed that *Aspergillus niger* caused significant changes in the physical appearance of paint films, including discoloration and surface roughening. (Ogbonna *et al.,*2022). investigated the degradation of acrylic polymers by *Aspergillus niger* and found that the fungus produced esterases capable of cleaving ester bonds in the polymer backbone. The study reported that *Aspergillus niger* could utilize acrylic polymers as a sole carbon source, demonstrating its potential to degrade acrylic-based emulsion paints. (Ezeudu *et al.,*2022). examined the biodegradation of various polymeric materials by *Aspergillus niger* and reported that the fungus exhibited high degradative activity, attributed to its production of multiple extracellular enzymes. The study noted that *Aspergillus niger* was particularly effective in degrading materials with ester linkages, which are common in many paint binders.

The mechanism of paint degradation by *Aspergillus niger* involves multiple processes (Adeniji *et al.,* 2023):

1. Enzymatic Degradation: *Aspergillus niger* produces esterases and lipases that can hydrolyze ester bonds in polymeric binders, breaking them down into smaller molecules that can be metabolized.
2. Acid Production: The production of organic acids lowers the pH, causing chemical hydrolysis of paint components and enhancing enzymatic activity.
3. Physical Penetration: The hyphal growth of *Aspergillus niger* penetrates paint films, creating channels that facilitate the access of enzymes and metabolites to deeper layers.
4. Pigment Production: The black pigmentation of *Aspergillus niger* conidia causes significant aesthetic damage to painted surfaces, leading to unsightly staining.

2.6 *Penicillium* Species

2.6.1 General Characteristics

Penicillium is a large and diverse genus of filamentous fungi belonging to the phylum Ascomycota, class Eurotiomycetes, order Eurotiales, and family *Aspergillaceae* (Visagie *et al.,* 2021). The genus comprises over 350 recognized species that are cosmopolitan in distribution, being found in soil, decaying vegetation, indoor environments, and various food products. *Penicillium* species are characterized by their brush-like conidiophores, which gave the genus its name (from the Latin *"penicillus,"* meaning brush). Colonies typically grow moderately fast on standard media, producing various shades of green, blue-green, or gray-green pigmentation due to the colour of their conidia (Houbraken and Samson, 2020). The conidiophores are branched structures (penicilli) bearing chains of spores (conidia) that are easily dispersed by air currents.

2.6.2 Physiology and Metabolism

Penicillium species are versatile organisms capable of growing on diverse substrates under various environmental conditions (Sharma *et al*., 2022). Most species are mesophilic, growing optimally between 20°C and 30°C, although some species can tolerate temperatures as low as 5°C or as high as 37°C. They generally prefer slightly acidic conditions (pH 5.0-6.5) but can tolerate a wide pH range (Bellotti *et al.,* 2023). *Penicillium species* produce an extensive array of enzymes including cellulases, xylanases, pectinases, amylases, proteases, lipases, and esterases, enabling them to degrade complex organic materials (Kumar *et al*., 2021). Many species also produce secondary metabolites including organic acids (particularly penicillic acid and citric acid), antimicrobial compounds, and various pigments. One distinguishing feature of many *Penicillium species* is their ability to grow under conditions of limited moisture and nutrients, which allows them to colonize relatively inhospitable environments including painted surfaces (Mesquita *et al.,* 2021).

2.6.3 Role in Paint Degradation

Penicillium species are among the most frequently isolated fungi from deteriorated painted surfaces worldwide (Patil and Rane, 2023). Numerous studies have documented their involvement in paint biodeteriorations: (Eze and Chukwunonso, 2021). isolated several *Penicillium species* from painted walls in humid tropical climates and demonstrated their ability to cause discoloration and surface deterioration. The study noted that *Penicillium* was particularly prevalent in areas with poor ventilation and high humidity. Ogbonna *et al.* (2021) investigated the biodegradative capabilities of *Penicillium species* isolated from contaminated environments and found that they possessed significant enzymatic activity against various polymeric substrates. The research demonstrated that *Penicillium* could utilize components of emulsion paints as carbon sources for growth. (Udoh *et al.*,2021). studied the biodegradation of paint industrial effluent by indigenous microorganisms and identified *Penicillium species* as significant degraders of paint components. The study reported that *Penicillium* exhibited high tolerance to paint toxicity and maintained degradative activity even in the presence of biocides.

The mechanisms by which *Penicillium species* degrade paints include (Adeniji *et al.*, 2023).

1. Enzymatic Hydrolysis: Production of esterases, lipases, and other hydrolytic enzymes that break down polymeric binders and organic additives.
2. Organic Acid Production: Secretion of organic acids that chemically attack paint components and create favorable pH conditions for enzymatic activity.
3. Biofilm Formation: Formation of biofilms on painted surfaces that trap moisture and nutrients, creating microenvironments conducive to continued degradation.
4. Pigment Production: Production of various pigmented compounds that cause staining and discoloration of painted surfaces.

2.7 Synergistic Effects of Mixed Fungal Cultures

2.7.1 Microbial Interactions in Biodegradation

In natural environments, biodegradation is typically carried out by mixed microbial communities rather than pure cultures (Guiamet *et al.,* 2021). The interaction between different microbial species can result in synergistic, antagonistic, or neutral effects on degradation rates and efficiency. Synergism occurs when the combined degradative activity of two or more species exceeds the sum of their individual activities (Okonkwo *et al.*, 2023). This can result from complementary enzyme systems, mutual provision of growth factors, removal of inhibitory metabolites, or modification of environmental conditions that favor the activity of co-existing species.

Antagonism occurs when one species inhibits the growth or activity of another, potentially through competition for nutrients, production of antimicrobial compounds, or modification of environmental conditions to unfavorable levels (Pinzari *et al.,* 2022). Neutral interactions occur when species coexist without significantly affecting each other's activities.

2.7.2 *Aspergillus* - *Penicillium* Interactions

The interaction between *Aspergillus* and *Penicillium species* has been studied in various contexts, including biodegradation of plastics, hydrocarbons, and other recalcitrant compounds (Ogbonna *et al.*, 2022). The outcomes of these interactions vary depending on the specific species involved, substrate characteristics, and environmental conditions.

Several studies have reported synergistic effects when *Aspergillus* and *Penicillium species* are used together for biodegradation (Ezeudu *et al.,* 2022). Nwachukwu *et al.,* (2020). investigated the biodegradation of petroleum hydrocarbons by *Aspergillus niger* and *Penicillium chrysogenum* and found that the mixed culture exhibited higher degradation rates than either species alone. The study attributed this synergism to complementary enzyme systems and the ability of each species to degrade different fractions of the substrate. Okonkwo *et al.,* (2023). examined the bioremediation of paint sludge-contaminated soil using indigenous fungal strains and reported that consortia containing both *Aspergillus* and *Penicillium species* achieved higher remediation efficiency than single-species inocula. The researchers suggested that the different species targeted different components of the paint mixture, resulting in more complete degradation.

However, some studies have also reported competitive or antagonistic interactions between these genera. The production of antimicrobial secondary metabolites by both *Aspergillus* and

Penicillium species can sometimes result in growth inhibition when they are cultured together (Bellotti *et al.*, 2023).

2.7.3 Factors Influencing Synergism in Paint Degradation

Several factors can influence whether *Aspergillus* and *Penicillium species* exhibit synergistic or antagonistic interactions during paint degradation (Kumar *et al.*, 2021):

1. Nutrient Availability: When nutrients are limited, competition may predominate. However, if the paint provides diverse substrates that are preferentially utilized by different species, complementarity may lead to synergism.
2. Inoculum Ratio: The relative proportions of each species in the mixed culture can affect interaction outcomes. Optimal ratios may maximize synergistic effects, while unbalanced ratios may lead to dominance by one species.
3. Temporal Dynamics: The sequence and timing of colonization can influence interactions. Early colonizers may modify the environment in ways that either facilitate or inhibit subsequent colonization by other species.
4. Environmental Conditions: pH, temperature, and moisture levels can differentially affect the growth and enzymatic activity of different species, potentially favoring synergism or antagonism under different conditions.

2.8 Assessment Methods for Paint Biodegradation

2.8.1 Growth Measurement Techniques

Several methods are used to assess microbial growth and activity during biodegradation studies (Trovão *et al.*, 2020):

Colony Counting: The most direct method for quantifying fungal populations involves serial dilution and plate counting to determine colony-forming units (CFU) per unit volume or mass (Dakal *et al.*, 2021). This method provides information on viable cell numbers but is time-consuming and may underestimate total populations if cells are clumped or if some viable cells do not form colonies. Optical Density Measurement: Spectrophotometric measurement of culture turbidity provides a rapid, non-destructive method for monitoring microbial growth (Mesquita *et al.,* 2021). Optical density (OD) at 600 nm is commonly used and correlates with cell density. However, this method can be affected by pigment production and is less accurate for filamentous fungi that form mycelial clumps. Dry Weight Determination: Measuring the dry weight of fungal biomass provides a direct assessment of growth but is destructive and requires larger sample volumes (Ogbonna *et al.,* 2022). Metabolic Activity Assays: Various assays can measure metabolic activity, including respiration rates, enzyme activity, and ATP content (Kumar *et al.,* 2021). These provide information on the physiological state of the microbial population.

2.8.2 Physicochemical Parameter Monitoring

Monitoring changes in physicochemical parameters provides insight into the degradation process and environmental conditions (Adeniji *et al.,* 2023):

- **pH Measurement:** Changes in pH indicate metabolic activity, particularly organic acid production. pH is typically measured using pH meters or indicator papers (Bellotti *et al.,* 2023).
- **Temperature Monitoring:** Temperature affects both microbial growth rates and enzymatic activity. Temperature is routinely monitored using thermometers or temperature probes (Sharma *et al.,* 2022).
- **Dissolved Oxygen:** In aerobic degradation processes, oxygen consumption indicates metabolic activity. This can be measured using dissolved oxygen probes (Ijoma and Nkuna, 2021).

2.8.3 Degradation Assessment Methods

Several approaches are used to assess the extent of paint degradation (Patil and Rane, 2023).

1. Visual Observation: Changes in colour, texture, and surface appearance provide qualitative evidence of degradation (Eze and Chukwunonso, 2021).
2. Weight Loss: Measuring the reduction in paint film weight indicates the extent of material removal through biodegradation (Rosado *et al*., 2020).
3. Chemical Analysis: Techniques such as Fourier-transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) can identify degradation products and changes in chemical composition (Ogbonna *et al.,* 2021).
4. Mechanical Testing: Changes in mechanical properties such as tensile strength, flexibility, and adhesion indicate structural degradation (Zhu *et al.,* 2022).
5. Microscopy: Scanning electron microscopy (SEM) and light microscopy can reveal surface colonization, hyphal penetration, and structural changes in paint films (Pinzari *et al.,* 2022).

2.9 Applications of Fungal Paint Degradation

2.9.1 Paint Waste Bioremediation

The global paint industry generates substantial waste during manufacturing, application, and removal processes (Okonkwo *et al.,* 2023). Traditional disposal methods, including landfilling and incineration, pose environmental and economic challenges. Bioremediation using fungi offers a promising alternative for treating paint waste. The potential of indigenous fungal strains for bioremediation of paint-contaminated soil. The study showed that fungi could significantly reduce the concentration of toxic paint components, converting them into less harmful substances or incorporating them into fungal biomass. The treatment of paint industrial effluent using fungal consortia and reported significant reductions in chemical oxygen demand (COD) and toxicity. The researchers suggested that fungal treatment could be integrated into paint manufacturing facilities for effluent management. The advantages of fungal bioremediation include lower costs compared to physicochemical methods, reduced generation of hazardous by-products, and the potential for in situ treatment (Ezeudu *et al.,* 2022). However, challenges remain, including the need for optimization of process conditions, management of treatment time frames, and scale-up from laboratory to field applications.

2.9.2 Development of Biodegradable Paints

Understanding fungal degradation mechanisms can inform the development of biodegradable paints that break down at the end of their useful life without leaving persistent environmental contaminants (Sharma *et al*., 2022). Such paints could incorporate binders derived from natural polymers or designed synthetic polymers with specific degradation points. Several researchers have explored the formulation of paints using biodegradable materials such as natural rubber latex, soy protein, and starch-based binders (Kumar *et al.,* 2021). While these formulations show promise, challenges remain in balancing biodegradability with performance requirements such as durability and weather resistance.

2.9.3 Biocontrol and Prevention Strategies

Research on fungal degradation also informs strategies for preventing unwanted biodeterioration of paints (Bellotti *et al*., 2023). This includes:

1. Improved Biocide Formulations: Understanding fungal resistance mechanisms enables development of more effective preservatives (Singh *et al.,* 2023).
2. Surface Modifications: Knowledge of fungal colonization processes informs the design of surfaces that resist microbial attachment (Zhu *et al*., 2022).
3. Environmental Management: Understanding the environmental factors that promote fungal growth enables better building design and maintenance practices to minimize conditions favorable for biodeterioration (Guiamet *et al*., 2021).

2.10 Knowledge Gaps

Despite considerable research on fungal degradation of paints, several knowledge gaps remain:

1. Species Specific Degradation Mechanisms: While many studies have identified fungi associated with paint degradation, detailed studies on the specific mechanisms employed by individual species are limited (Ogbonna *et al*., 2022).
2. Mixed Culture Dynamics: The interactions between different fungal species during paint degradation, particularly the conditions that promote synergism versus antagonism, require further investigation (Okonkwo *et al.,* 2023).
3. Long Term Degradation Studies: Most studies examine degradation over short time periods (days to weeks), while natural degradation occurs over months to years. Long-term studies are needed to understand the complete degradation process (Patil and Rane, 2023).
4. Tropical Climate Studies: Most paint degradation research has been conducted in temperate regions. More studies are needed in tropical climates like Nigeria where conditions may differ significantly (Eze and Chukwunonso, 2021).
5. Molecular Mechanisms: The genetic and molecular basis of fungal degradation of synthetic polymers, including paint binders, requires further elucidation (Meyer *et al.,* 2020).

This study addresses some of these gaps by examining the degradative potential of *Aspergillus niger* and *Penicillium species,* both individually and in combination, under controlled laboratory conditions that simulate tropical environments.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials and equipment used for the experiment includes: conical flasks, measuring cylinders, micropipettes, Petri dishes, McCartney bottles, sterile water, filter paper, mineral salt medium, Antibacterial eye drops, Potato Dextrose Agar (PDA), emulsion paint, spectrophotometer, thermometer, and pH meter, test tubes, beakers, analytical weighing balance, inoculation loop, Spatula, Aluminum foil, bunsen burner, autoclave, methylated spirit, Masking tape, Spreader, cotton wool.

3.2. Preparation of Mineral Salt Medium

A mineral salt solution was prepared and adjusted to a pH range of 5.5–6.5, which is suitable for fungal growth (Ogunlana *et al.,* 2020). Ninety-five milliliters (95 mL) of the standardized mineral salt medium were dispensed into four conical flasks labeled A, B, AB, and Control. To each flask, a drop of antibacterial was added to suppress bacterial growth.

3.2.1 Preparation of PDA Broth

Potato Dextrose Agar (PDA) was prepared by weighing 0.78 g of PDA powder and dissolving it in 20 mL of distilled water. The solution was filtered through filter paper to remove agar residues, leaving a clear PDA broth. One milliliter (1 mL) of this broth was added to each conical flask containing mineral salt medium, and the flasks were covered with foil paper and sealed with tape to maintain sterility.

3.2.2 Preparation of Emulsion Paint Solution

A 5% paint solution was prepared by mixing 5 mL of commercial emulsion paint with 95 mL of sterile distilled water. This diluted concentration was used to avoid toxicity that could inhibit fungal growth (Udo *et al*., 2021). Five milliliters (5 mL) of the diluted paint solution were introduced into each conical flask.

3.2.3 Subculturing and Preparation of Fungal Inoculum

The fungal isolates were subcultured in Potato Dextrose Broth (PDB) to obtain actively growing liquid cultures. The subcultures were incubated at 28°C for 72 hours to enhance fungal biomass development. After incubation, 2 mL of each fungal broth culture were aseptically transferred into 3 mL of sterile distilled water in separate McCartney bottles labeled A (*Aspergillus niger*) and B (*Penicillium sp.*). This dilution helped to standardize the inoculum concentration for subsequent inoculation into the experimental flasks (Chinedu *et al.*, 2022).

3.2.4 Inoculation of Experimental Flasks

One milliliter (1 mL) of the Aspergillus niger inoculum was introduced into flask A, one milliliter (1 mL) of the *Penicillium* sp. inoculum into flask B, and 0.5 mL each of *A. niger* and *Penicillium sp*. into flask AB. The control flask received no inoculum. The flasks were gently shaken to ensure proper mixing and incubated at room temperature (25–28°C).

3.2.5 Preparation of PDA Plates for Enumeration

To assess fungal growth, PDA was prepared by dissolving 3.51 g in 90 mL of distilled water. The mixture was sterilized at 121°C for 15 minutes using an autoclave and cooled before adding a few drops of antibacterial solution. Serial dilutions were made from each conical flask using sterile distilled water with dilution factors ranging from 10^{-1} to 10^{-3}. From each dilution, 0.02 mL was inoculated onto sterile PDA plates labeled accordingly (A10^{-3}, B10^{-3}, AB10^{-3}, and Control). Plates were incubated at 28°C for 48 hours, and colony-forming units (CFU/mL) were counted (Yusuf and Nwosu, 2023).

3.2.6 Determination of pH, Temperature, and Optical Density (OD)

At regular intervals, 5 mL aliquots were withdrawn from each conical flask using sterile pipettes and transferred into clean test tubes for physicochemical analysis. The pH was measured using a calibrated pH meter, while temperature was recorded with a thermometer.

Optical density was determined at 600 nm using a spectrophotometer to monitor the turbidity and fungal biomass development throughout the degradation period (Adewale *et al*., 2023).

3.2.7 Research Design

This research adopted an experimental design aimed at evaluating the ability of fungal isolates to degrade emulsion paints under laboratory conditions. The study involved the use of two fungal species, *Aspergillus niger* and *Penicillium sp.,* which were introduced into mineral salt medium supplemented with emulsion paint as the sole carbon source. Four experimental setups were prepared and labeled A, B, AB, and Control, representing individual fungal inoculations, combined inoculation, and a non-inoculated control respectively. The design allowed comparison of fungal activity in different treatments and provided data on pH, optical density, and colony growth over time.

CHAPTER FOUR

4.0 RESULTS

Table 1: shows the physico-chemical parameters measured during paint biodegradation. Temperature measurements of the flask contents showed that initial temperatures on Day 0 ranged from 29 to 32°C. By Day 3, temperatures increased to 33°C for all inoculated flasks, while on Day 7, temperatures stabilized between 30-31°C. The pH values varied throughout the experimental period, with Flask AB recording the highest initial pH of 7.0, while Flask B showed the most acidic value of 3.0. By Day 7, Flask A and B both recorded pH 5.1, while Flask AB showed 6.5. These pH fluctuations indicate fungal metabolic activity during paint degradation. Optical density measurements at 600 nm were taken on Day 3 and Day 7 to assess fungal biomass accumulation. Flask B showed the highest OD of 50.9 on Day 3, while Flask A increased from 30.9 to 40.2 by Day 7, demonstrating progressive fungal growth.

Table 2: shows the total viable counts during paint biodegradation. Fungal population counts revealed that Flask B (*Penicillium sp.*) had the highest population with 45 colonies on Day 3 (2.25×10^5 CFU/ml) and 55 colonies on Day 7 (2.75×10^6 CFU/ml). Flask A (*Aspergillus niger*) showed 7 colonies on Day 3 (3.5×10^4 CFU/ml) and 10 colonies on Day 7 (5.0×10^5 CFU/ml), while the mixed culture (Flask AB) recorded 26 colonies on Day 3 (1.30×10^5 CFU/ml) and 30 colonies on Day 7 (1.50×10^6 CFU/ml). The control flask maintained minimal counts throughout the experimental period.

Table 3: identifies the percentage increase in fungal population from Day 3 to Day 7. Flask A showed the highest growth rate of 1,328.6%, followed by Flask B with 1,122.2% and Flask AB with 1,053.8%. These results indicate that all fungal treatments demonstrated substantial

population growth over the 7-day incubation period, with *Aspergillus niger* having the highest relative growth rate.

Table 1: Physico-chemical Parameters During Paint Biodegradation.

Flask	**Day 0**			**Day 3**			**Day 7**		
	T(°C)	**pH**	**OD**	**T(°C)**	**PH**	**OD**	**T(°C)**	**PH**	**OD**
A *Aspergillus niger*	32	5.0	ND	33	6.1	30.9	30	5.1	40.2
B *Penicillium* sp.	31	3.0	ND	33	5.9	50.9	30	5.1	50.0
AB Mixed culture	31	7	ND	33	6	30	31	6.5	50.5
CONTROL	29	6	ND	39	5.7	50.5	30	6.2	50.5

ND = Not Determined

Table 2: Total Viable Counts During Paint Biodegradation

Flask	**Day 3 (10^{-2})**	**CFU/ml**	**Day 7 (10^{-3})**	**CFU/ml**
A *Aspergillus niger*	7	3.5×10^4	10	5.0×10^5
B *Penicillium* sp.	45	2.25×10^5	55	2.75×10^6
AB Mixed culture	26	1.30×10^5	30	1.50×10^6
Control	9	4.5×10^4	5	2.5×10^5

Table 3: Percentage Increase in Fungal Population

Flask	Day 3 CFU/ml	Day 7 CFU/ml	% Increase
A *Aspergillus niger*	3.5×10^4	5.0×10^5	1,328.6
B *Penicillium* sp.	2.25×10^5	2.75×10^6	1,122.2
AB Mixed culture	1.30×10^5	1.50×10^6	1,053.8
Control	4.5×10^4	2.5×10^5	455.6

CHAPTER FIVE

DISCUSSION

Despite improvements in paint production, biodeterioration remains a persistent challenge. Biodeterioration in paint is caused by microorganisms which utilize nutrients in the paint surface, causing it to deteriorate (Ogunleye *et al*., 2018). This study demonstrates that fungi are significant agents of deterioration in painted surfaces. The fungal isolates obtained from the experiment were *Aspergillus niger* and *Penicillium* sp., which aligns with previous studies of Adekunle and Ogunsanwo, (2019) Onifade, (2000). Table 4.1 shows the physico-chemical parameters across different sampling days, indicating that environmental factors such as temperature and pH influenced fungal growth (Okungbowa and Shittu, 2012; Eze and Okpokwasili, 2016).

Flask B (*Penicillium sp.*) had the highest fungal count (2.75×10^6 CFU/ml) which indicates that this species is highly implemented for paint degradation. This could be attributed to its superior enzymatic capabilities and efficient utilization of paint components as carbon source, which is consistent with the findings reported by Adekunle and Ogunsanwo (2019), who reported that *Penicillium species* demonstrate exceptional paint degradation abilities. Flask A (*Aspergillus niger*) and the mixed culture (Flask AB) and associated with the counts of 5.0×10^5 CFU/ml and 1.50×10^6 CFU/ml respectively. These results demonstrated the individual isolates and the consortium with significant biodegradation potential. The characteristic black coloration observed in Flask A confirmed the active sporulation of *Aspergillus niger* (Samson *et al*., 2014), while the progressive turbidity in all inoculated flasks indicated metabolic activity (Amadi and Alisi, 2016).

The pH fluctuations observed, particularly the acidification to pH 5.1 in Flasks A and B by Day 7, suggest organic acid production during fungal metabolism, which plays a crucial role in breaking down paint polymers (Ogunleye *et al*., 2018). The mixed culture maintained a higher pH of 6.5, possibly due to buffering effects from combined metabolic activities. The absence of synergistic enhancement in the mixed culture may be attributed to competition for nutrients or production of inhibitory compounds (Akaranta and Abowei, 2013). Table 4.2 showed the progressive increase in fungal population from Day 3 to Day 7, confirming successful adaptation to the paint-containing environment. The mineral salt medium ensured that fungal growth depended primarily on paint as the carbon source (Bushnell and Haas, 1941).

Conclusion

This study successfully demonstrated that *Aspergillus niger* and *Penicillium sp.* possess significant capability to utilize emulsion paint as a carbon source. *Penicillium sp.* achieved the highest final population while *Aspergillus niger* showed the highest growth rate of 1,328.6%. The physico-chemical changes including pH fluctuations and increasing optical density confirmed active biodegradation processes. These findings emphasize the potential application of these fungi in bioremediation strategies for paint-contaminated environments and highlight the need for improved antifungal formulations in paint production to prevent biodeterioration in humid environments.

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APPENDIX

PLATE 2: Culture isolation of *Penicillium spp*

PLATE 3: Cultural isolation of *aspergillus niger*