DEGRADATION OF EMULSION PAINT USING BACTERIAL ISOLATES

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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 (HONOURS) DEGREE (BSC.) IN SCIENCE LABORATORY TECHNOLOGY

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CERTIFICATION

We certify that this thesis titled DEGRADATION OF EMULSION PAINT USING BACTERIAL ISOLATES with matriculation number LSC2007317 in the Department of Science Laboratory Technology, University of Benin, Benin City, in partial fulfillment of the requirements to be awarded a Bachelor in Science Laboratory Technology.

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DEDICATION

This project is dedicated to God Almighty for his guidance, protection, mercy and favor upon my life throughout my years of study.

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ABSTRACT

Microbial degradation of emulsion paints poses significant challenges in industrial and environmental settings, often leading to discoloration, structural breakdown, and reduced durability of coated surfaces. Among the key microbial agents, *Bacillus* spp. and *Escherichia coli* have been identified for their enzymatic capabilities and metabolic versatility in degrading paint components such as binders, pigments, and additives. These bacteria utilize organic compounds in paint as carbon sources, contributing to biodeterioration through acid production, biofilm formation, and enzymatic hydrolysis. This study presents a comparative analysis of the physiological and growth responses of *Bacillus* spp. and *Escherichia coli* over a 21-day incubation period under ambient laboratory conditions. Key parameters monitored included pH, optical density (OD), temperature, and total viable count (TVC), providing insights into microbial adaptation and proliferation trends. For *Bacillus*, the pH remained stable at 7.0 until Day 7, then declined to 6.0 by Day 14, suggesting increased metabolic activity and acid production, followed by a slight recovery to 6.2. OD values fluctuated, peaking at Day 14 (0.40), indicating active biomass accumulation, while TVC rose progressively from 0.675×10^7 CFU/mL to 9.60×10^7 CFU/mL by Day 21, reflecting robust growth. Temperature varied modestly between 24°C and 27°C, with no apparent inhibitory effect. In contrast, *E. coli* exhibited a sharper pH decline from 6.8 to 5.6 by Day 14, consistent with its fermentative metabolism. OD decreased steadily from 0.51 to 0.26, suggesting reduced cell density or viability over time. However, TVC increased significantly, from 0.5×10^7 CFU/mL to 8.35×10^7 CFU/mL, indicating sustained proliferation despite declining OD. Temperature ranged from 26°C to 30°C, with peak microbial activity observed at higher temperatures. The results highlight distinct metabolic and growth profiles between the two species. *Bacillus* demonstrated resilience and biomass recovery, while *E. coli* maintained high cell counts despite reduced optical density. These findings underscore the importance of multi-parameter monitoring in microbial ecology and have implications for bioprocess optimization, environmental microbiology, and industrial fermentation systems.

CHAPTER ONE

1.0 INTRODUCTION

In modern times paints where very common, and varieties of paints were widely available in the market. The two main function of paints are for surface protection and embellishment. Paint is used to protect the surface it is applied to from oxidation, erosion, and decoration (Kampman, 1972). Paints are substances that are used to coat an object in a liquid form to protect it from the environment and deterioration, or they can be used to adorn or protect surfaces (Caroline, 1967). According to Sharma (2006) and Constantino (1994), paints are stable mechanical mixtures of one or more pigments and various chemical components. Paints are applied as protective coatings to prevent materials from deteriorating due to the environmental factors and to enhance their aesthetic appeal. Stewart and Tekel (1998), state that paints are applied to surfaces to identify, decorate, and render them washable. There are many different types of paints available each with unique properties, such as Primer, house, masonry, marine, and fire-retardant paints (Kampman, 1972). Among their key characteristics are Consistency, opacity (hiding power), spreading ability, stickiness, and durability.

In addition to their primary purpose of shielding surfaces from oxidation, corrosion, weathering, and other forms of degradation, paints also provide an aesthetically pleasing finish (Briggs, 1980; Adeleye and Adeleye, 1999). To achieve both of these required qualities, the paint layer must be immune to microbial exploitation. Paint can decay, though, due to microorganism. It is impossible to maintain painted surfaces clean and free of organism spore exposure (Hare, 1998). Vehicle, pigment, additive, and solvent are all components of paints (Briggs, 1980). The different organic components of paints are used by almost all types of microorganisms as a source of carbon and as nutrients to encourage microbial growth. This severely compromises the paints adhesion, durability and aesthetic value (Da Silva, 2003). Unfortunately, production plant methods, packing materials, and raw materials can all contribute to microbial contamination of paints (Gillatt, 1992). In order to prevent bio-deterioration, one major challenge is managing microbial proliferation in paint.

Paint can be broadly divided between:

- Water-based coatings (such as emulsion paint and text coat paints)
- solvent-based materials (such as gloss paint).

Many paint materials, as well as the ensuing additives, glues, surfactant's, thickeners, etc. are biodegradable, which makes applying paint coats easier and improves the elegant quality of the finished product (Ogbulie and Obiajuru, 2004). The most prominent cause of the color fading is the use of inferior paint, which causes the paint system to deteriorate too soon. The greenish-black fuzzy looking growths known as mildew and or mold are the result of excessive moisture or inadequate air circulation. These growths affect the buildings fundamental structural integrity in addition to having detrimental effects on health.

The three main types of microorganisms that cause paint deterioration are fungi, bacteria and algae which can grow on paint films that have been applied as well as solvent and water-based coatings (Gaylarde, 2005).

These organisms can also arise on solvent and water-based coatings, applied paint films, and lichens (Stefanie *et al*., 2009). *Bacillus, Pseudomonas, Enterobacter, Proteus, Escherichia, Micrococcus*, *Serratia, Aeromonas*, and various other bacteria are commonly among the most often isolated bacterial species in paints. (Jakabowski *et al*., 1983; Opperman and Gull, 1984).

Paints have also been found to include a range of anaerobic bacteria, such as *Bacteroides, Clostridium, Desulphovibrio, and Bifidobacterium* (Opperman and Gull, 1984).

Furthermore, *Rhizopus arrhinus, Aspergillus niger, Aspergillus ustus, Penicillium citrinum, Chaetomium globosum, and Alternaria Altanata* were listed by Grant *et al.* (1993), as fungi linked to paint deterioration. Both on paint films and in liquid paint before application, microorganisms have the capacity to grow. Attacks by bacteria, fungi, and algae are a major issue for the paint business. Because of this, paint makers include biocides in their mixtures to stop the growth of these organisms. Chemical products known as biocides are made up of one or more active chemicals that, when present in paint, restrict or prevent the growth of microorganisms without negatively affecting the paints other characteristics. Nevertheless, a microbiological issue is typically not resolved by biocide alone (Woods *et al.*,1982). The characteristics of biocides can be anti-bacterial, anti-fungal or anti-algal.

Paint surfaces are harmed by microorganisms because they discolor the layers, increase their porosity, reduce their physical resistance, and facilitate the entry of moisture into the surface (Unger *et al*., 2001). Paints can get polluted during production and product storage, according to (La Rosa *et al*., 2008). Microorganisms and their activities can destroy water-based house paints, resulting in a reduction in the paints shelf life (Okunye *et al*., 2013). As a result, the quality of paints is severely compromised. This is as result of water and nutrient present in the paint which caused them to be prone to attack by both fungi and bacteria and some algae species which requires sunlight.

Microbial proliferation on paintings has the potential to compromise their aesthetics and structural integrity (Orio, 1999). Orio (1999) and (Stefenie *et al.,* 2009) define aesthetic damage as pigment discoloration, stains, and biofilm growth on the painted surface as well as paint layer separation from the support as a result of degradation of the support polymers or glues and binders. According to Orio (1999), the microbial populations challenges and the pace of microbial colonization are influenced by the characteristics of the substrate and environmental conditions such as temperature, light, humidity, and maybe pH. When paint is contaminated, whether it is still wet or has dried into a film, the painted surface can become physically and aesthetically degraded. Paint deterioration is caused by microbial

decomposition of the organic paint component. Many mold species, including Aspergillus,

Penicillium, Cladosporum, Pullularia, and Alternaria species, have been isolated from painted

surfaces. According to Michael (1993) and Los Bolton (1998), Pullularia species appear to be the

most frequent cause of paint deterioration and the blue-green algae (Cyanobacteria) (Adya, 1999).

The study's goal is to identify and characterize staphylococcus species that could be responsible for the deterioration of emulsion paints.

1.1 AIM AND OBJECTIVES

The aim of this experiment was to determine the degradation of emulsion paint using bacterial isolates.

The specific objectives were to:

1. determine the pH changes in the bacteria degradation after 21 days
2. determine the total viable count of the bacteria degrading the emulsion paint
3. determine the OD of the degrading bacteria for 21 days
4. monitor the temperature changes during degradation

CHAPTER TWO

2.0 LITERATURE REVIEW

In the context of this research project, this chapter carries out a review of literature relevant and related to the study. In particular, the primary issues talked about include the conceptual framework embraced for this research work, the empirical literature, and the theoretical framework backing up Isolation Characterization and Degradation Potential of Outdoor Emulsion Paint Using Staphylococcus species.

One of the earliest manmade materials used by humans, paint has a long history dating back to the Stone Age Clays and chalk were combined with animal fats in prehistoric times to create paints that were used to decorate cave walls with images of hunts. By 2500 BC, the Egyptians had made a clear blue pigment by combining azurite, gums, wax, and albumen and improving paint manufacturing technology (egg white), Moreover, during the first millennium BC, the Greeks discovered how to blend paints with hot wax rather than water, creating a paint that was both thicker and easier to apply, enabling the blending of colors (Clark, 1983). During this period, a wide variety of hues were accessible from both natural and artificial sources. One such color was a purple pigment created by heating yellow earth until it turned red and then submerging it in vinegar. After then, technology fell into disuse for a long time, with craftsmen on the road passing down their skills from generation to generation. This persisted up until the eighteenth century, when paint factories started to operate in Europe and America. As a result of mass production in the nineteenth century, houses started to be painted. Now that the chemistry of paint production and use is known, paint production has officially transitioned from an art to a science.

2.2.1 Types of Paint

Modern household paints fall into two broad categories: a) Orthodox, oil-based paints, thinned with mineral turpentine or other organic solvents and b) Emulsion paints, which may be vinyl or acrylic based and which are thinned with Water (Clark, 1983). Traditional oil-based paints are thinned with mineral turpentine or other organic solvents, while emulsion paints, which may be vinyl- or acrylic-based and are thinned with water, fall into two basic groups in modern household paints (Clark, 1983). The five main components of paint are the binder, which keeps the paint to the surface, the pigments, which decide the color and opacity of the paint and occasionally stop corrosion, the solvents, which make the paint easy to spread, the base, which gives hulk to the paint and the vehicle.

2.2.2 Components of Paints

Contemporary paints are made up of several different ingredients. Consequently, it is crucial to carefully mix them to ensure the paint's optimum consistency and stability. The use of dispersion agents is specifically for this objective. These not only give the paint the required performance characteristics, but also the desired color saturation and sufficient pigmentation. These items serve as very efficient dispersants in paintings that employ water as a solvent. The base is a substantial component of the paint that gives it structure and volume. White lead, red lead, zinc and iron oxides, solid titanium, lithopone, aluminum powder, etc. are frequently found in the base. This entire ingredient's main goal is to make a paint layer transparent, strong, and resilient while also preventing shrinkage from happening. It functions by fusing all the components, including the pigments, together and gives paintings their hardness, gloss, and flexibility.

Paint is essentially a mixture of :(i) binder, which adheres the paint to the surface, ii) pigments, which give the paint a color, opacity and occasionally prevent corrosion, and iii) solvents to make the paint spreadable. The chemistry of the components is outlined below.

Binders: The binder is required to hold the pigment adhered to the surface. The binder is usually a polymeric substance, and is either dissolved in the paint or suspended in it by using emulsifiers. Paint technology has advanced very little until this century. Even as recently as the 1960s the 'drying oils' were the most common paint binders. Drying oils are substances that, when spread out as a film, will dry to form a continuous skin. Linseed oil, the most common example of a drying oil, will dry in 2 to 3 days while other oils, such as soya bean oil, may take up to 10 days (Clark, 1983). Linseed oil is a mixture of triglycerides of long chain carboxylic acids. Some of the major component carboxylic acids are: linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid. Many common drying oils contain these compounds and also include eleosteraric and recinoleic acids, in various ratios. The drying process is a complex one of polymerization, probably catalyzed by peroxides. Composition of the natural compounds varies widely and the proportions of the constituent triglycerides will vary from batch to batch of oil. Various processes have been used to improve the properties of oils; one among them is the increase in the molecular weight of the oil by controlled oxidation.

Pigments: Pigments perform three major tasks: an optical task of supplying color, opacity, and gloss; a protective task for the painted surface and the binder, which is susceptible to UV damage; and a strengthening task for the paint itself by assisting the binder in adhering. Pigments are made up of little solid particles with a diameter of less than 1m, which gives them the ability to refract light. The pigment must be evenly distributed throughout the solvent and in contact with it to be effective. A layer of wet air and, in some situations, other gases around pigment particles. Wetting is the process of displacing this layer to bring the pigment into contact with the solvent. It is important to choose solvents and pigments that result in a well-wetted pigment since improper pigment wetting might cause color streakiness in the finished paints. The wetting capabilities of the resin/solvent combination are enhanced by the application of wetting and dispersion agents.

Solvents: Solvents are necessary to ensure an even mixing of the paint components and to make them easy to apply. The solvents used differ with the way in which the paint will be applied as the drying rate differs on the manner of application, e.g., the solvents in spray paints need to evaporate much more quickly than those in brush-applied paints. In general, a blend of solvents is used to produce a paint that will surface and through dry (i.e., dry throughout) at the correct rate without uneven shrinkage. White spirit and mineral turpentine are probably the most widely used solvent, however many other compounds like toluene, methyl ethyl ketone, methyl isobutyl ketone, xylenes, butyl acetate and methoxy propyl acetate find its application in paint formulation

2.2.3 Bio-deterioration

The phrase "bio-deterioration" refers to the breakdown of materials that are economically useful but are typically resistant to biological attack, such as metals, plastics, pharmaceuticals, cosmetics kinds of a paint and are significantly influenced by its pigment. The final hue and the paints share determined by the pigments several pigments also offer extra volume, which when seeded can cause paint to congeal. Carbon, earth clay, ultramarine, etc. are common pigments und in paintings. Paints use a wide range of natural and synthetic pigments to provide a broad spectrum of colors and finishing patterns. Pigments can be roughly categorized as organic or inorganic Stoye and Freitag,1998.; Brock *et al.*, 2000; Smith, 2002). In general, organic pigments are preferred because they are more vibrant, robust, clear, and stable. Moreover, some of them absorb UV light to shield the binder from damage and have improved gloss development and tinting strength. Nonetheless, inorganic pigments are also frequently employed since they are significantly more affordable than organic pigments, do not bleed, and are heat- and light-stable. As it is impossible to obtain pure black or white organic pigments, they are also employed for some specialty pigments and for black and white pigments. It is generally accepted that titanium dioxide, one of these white pigments, is the most significant pigment now in use. Due to its pure white color, its tiny particle size, and the fact that it is the strongest known pigment in terms of opacity and tinting power, it can be employed as an opacifier to create films with a high hiding power and to lower the pigment content. Paints now have far better flexibility and, as a result, are more durable. In addition to color and concealing ability, pigments provide paints other qualities. Steel does not corrode as a result of anticorrosive pigments. Red lead has been used for many years as an anti-corrosive coloring (Pb304). The application qualities of the paint are improved by the addition of mineral ingredients. They are referred to as "extenders" and are a crucial component of the "tool kit" of the paint formulator. The extenders can be employed as "flatting agents" to produce flat or semi-gloss finishes, to stop pigments from settling, or to improve succeeding coatings' keying (sticking) capabilities.

The binder plays a key role in adhering a paint moisture while also providing paint resistance qualities that make and keeping the color in long-lasting Oil-derived and lates-dependent binders are the two types. Deyors facilitate quick drying of paint by oxidizing and hardening the substance; quick dying of paint is recommended to avoid painting with dust. Iron, lead, and organic zine salts make up most driers. Solvents are required to make the paint components easier to apply and to ensure an even mixing of the paint's components. In the simplest terms, binding agents and dyes are literally held to the wall's surface by the liquid component of paint. Thinner makes the paint smoother and less viscous. Paints can penetrate the porous surface for a smooth look by adding thinner.

Alcohol that is liquid-like is used as a solvent or paint thinner with oil-based paints. The solvents used vary depending on how the paint will be applied since the drying rate varies depending on the application method. For example, the solvents in spray paints must evaporate considerably more quickly than those in paints applied by brush. To create a paint that will surface and through dry (ie., dry throughout) at the right rate without uneven shrinkage, a blend of solvents is typically utilized. The most used solvents are probably white spirit and mineral turpentine. When other properties need to be altered or improved, additives have always been the alternative. Thickeners, for instance, are chemicals that have the tendency to thicken paint in order to make it even easier to apply some, wool plaques. (Raikum, 2012). Mineralization, or the final decomposition of organic substances into water, CO2, and some other inorganic end product, occur when biodegradation is complete (Alexander, 1981; Ravikumar *et al*., 2012). Nitrate and phosphate are used by microorganisms in the degradation process (Ebuchi*, et al*., 2005), which limits the availability of nutrients for microbial development (Odokuma and Smith, 2007).

2.2.4 Bioremediation

Bioremediation is a process that utilizes biological systems to detoxify, degrade, or neutralize hazardous substances. Microorganisms, which lead simple lives, consume available nutrients, adhere to various surfaces, reproduce rapidly, and generate biomass. The natural occurrence of decomposition—where organic matter is broken down and recycled by diverse organisms, including microbes—is widely recognized. This beneficial process is known as **biodegradation**.

In contrast, **biodeterioration** refers to the microbial degradation of economically valuable materials and is considered detrimental. It is categorized into three types: mechanical biodeterioration, chemical assimilatory biodeterioration, and soiling.

Biodegradation encompasses two human-centered perspectives: bioremediation and biodeterioration. While bioremediation aims to eliminate toxic substances through biological means, biodeterioration involves the breakdown of materials typically resistant to microbial attack, such as metals, plastics, pharmaceuticals, cosmetics, artworks, wood products, electrical components, fuels, and oils (Sarkar et al., 1997).

Bioremediation strategies may involve native microbial communities, either with or without nutrient enhancement, or the introduction of external microorganisms—a method known as **bioaugmentation**. In both cases, the objective is to neutralize harmful chemicals without producing new toxins. Microorganisms follow a straightforward survival strategy: they utilize available resources, colonize surfaces, multiply, and contribute to biomass formation. The process of organic decay and recycling, termed biodegradation, is generally viewed as beneficial. Conversely, biodeterioration—defined as the microbial-induced degradation of economically important materials—is seen as harmful. Its classifications include mechanical biodeterioration, chemical assimilatory biodeterioration, and soiling.

2.2.5 Categories of Biodeterioration

1. **Mechanical Biodeterioration** This form of deterioration results from the physical actions of organisms, such as movement or growth, which directly damage materials. A common example is the destruction of electrical wiring due to insect or rodent activity.
2. **Chemical Assimilatory Biodeterioration** This is the most prevalent type, occurring when microorganisms degrade materials to extract nutrients. For instance, cellulolytic fungi breaking down cellulose-based items like wallpaper exemplifies this process.
3. **Chemical Dissimilatory Biodeterioration** In this case, damage arises from microbial metabolic byproducts that corrode or contaminate materials. Examples include the contamination of grains by mycotoxins and pigment release into plastic films.

2.2.6 Biodeterioration of Paintings

Paintings typically consist of three main components: a support (such as canvas, wood, paper, or parchment), a preparatory layer, and a paint layer. The chemical makeup of these layers varies depending on the painting technique and the type of paint used—whether oil, distemper, or watercolor.

- **Canvas paintings** often feature a preparatory layer made of lime or gypsum combined with animal or plant-based glue. Multiple layers of pigment mixed with binders like oil or egg-based distemper are applied over this surface, which is then sealed with a thin varnish.
- **Wooden panel paintings** share a similar layered structure.
- **Paper-based artworks**—including watercolors, gouaches, and pastels—are more vulnerable. Watercolors contain minimal binder (usually gum arabic), while pastels, made of pure pigment without binder, are particularly difficult to preserve.

Biological deterioration can affect any part of a painting—whether the reverse, the support, or the painted surface. Organic materials in paintings serve as nutrient sources for heterotrophic microorganisms. However, microbial growth typically occurs under favorable environmental conditions, such as those found in poorly regulated museum spaces, historic churches, or storage areas lacking humidity and temperature control.

Micro-fungi are the primary agents of biodeterioration due to their ability to utilize a wide range of organic compounds and their resilience to environmental fluctuations. They can thrive on condensation moisture, unlike bacteria, which require higher moisture levels.

Typically, the **support** is the first area affected. In canvas paintings, microbial invasion often begins on the reverse side, where glue sizing increases the vulnerability of the textile. The organisms then infiltrate the canvas, reaching the back of the paint layer, causing cracks and detachment. Cellulose breakdown further disrupts adhesion between the paint and canvas (Strzelczyk et al., 1987).

In **wooden panel paintings**, deterioration of the support may differ from that of the paint layer. Microorganisms involved are similar to those affecting materials of plant and animal origin. Additives like glue or lining paste used in treatment can increase susceptibility (Dhawan and Agrawal, 1986; Makies, 1981).

Biological damage to the **paint layer** is less common and depends on the pigment composition. Paints containing casein, egg distemper, emulsion distemper, and linseed oil are most vulnerable, in that order. Conversely, pigments with heavy metals such as lead, zinc, or chromium offer greater resistance to microbial degradation. Watercolors, with minimal organic binder, are as prone to biodeterioration as pastels (Dhawan and Agrawal, 1986).

Fungal growth from microscopic spores can develop rapidly, spreading radially and becoming visible within days

2.2.7 Biodeterioration of Emulsion Paints

Water-based paints are particularly vulnerable to microbial degradation during production, which can lead to issues within the container. One common problem is paint thinning, caused by cellulase enzymes produced by bacteria and fungi that degrade cellulose ether—typically used as a thickening agent. Contaminated ingredients, such as talc used as an extender, have been identified as potential sources of microbial intrusion. Signs of contamination include surface irregularities and the emission of unpleasant odors due to gas production.

Both oil- and water-based paint films are susceptible to microbial colonization on interior and exterior surfaces of buildings. This form of biodeterioration is not only aesthetically displeasing but may also pose health risks (Morton, 2001).

The term **biodegradation** refers to the biologically driven decomposition of chemical substances, encompassing a range of biochemical reactions. When this process reaches completion, it results in **mineralization**—the full conversion of organic compounds into water, carbon dioxide, and other inorganic substances (Alexander, 1981).

Emulsion paints are composed of pigments and polymeric particles suspended in water, where the particles remain insoluble. These paints are formulated using monomers, initiators, water, and emulsifiers. Acrylic and vinyl-based emulsions dominate the decorative paint market (Clark, 1983).

Common monomers used in emulsion paints include styrene, vinyl acetate, methyl acrylate, butyl acrylate, and acrylonitrile. These undergo polymerization—typically initiated by compounds like per-sulfates—to form acrylic polymers. The reaction, which is exothermic, is conducted under controlled temperature conditions. Initiators such as ammonium per-sulfate trigger free-radical polymerization, while activators like ferrous ammonium sulfate enhance the dissociation of initiators, increasing radical concentration. Emulsifiers or surfactants are added to stabilize the emulsion, resulting in polymer micelles ranging from 0.1 to 1.0 μm in diameter. Each micelle is enveloped by an emulsifier layer that anchors to the particle and extends into the water, maintaining suspension stability.

2.2.8 Degradation Potential

Soiling This form of biodeterioration is visually apparent and occurs when the presence of microorganisms or their waste renders a material unacceptable. In some cases, the functionality of the material is compromised, such as when barnacles and algae foul ship hulls. It is important to recognize that multiple forms of biodeterioration may occur simultaneously.

A wide range of economically significant materials are susceptible to microbial degradation, including stored agricultural goods, archival documents, paper, wood, textiles, leather, construction materials, fuels, pharmaceuticals, metals, cosmetics, paints, polymers, rubber, stone, buildings, glass, adhesives, and sealants. Surprisingly, even seemingly resistant materials like glass, metal, and stone can be affected.

Bacteria Bacteria contribute to biodeterioration primarily through biofilm formation, which can compromise the structural integrity of materials. Unlike fungi and algae, which are often visible, bacteria can exist in large numbers on surfaces that appear clean, causing hidden damage. Their metabolic activities can lead to corrosion of concrete and metals through the production of inorganic acids (Cragnolino and Tuovinen, 1984; Bock and Sand, 1986; May et al., 1993), or cause paint blistering (Johannessen and Norgaard, 1991).

Certain bacteria, such as chemolithotrophs and oligotrophs, thrive in low-nutrient environments and can alter surfaces in ways that make them more susceptible to colonization by other microorganisms (May et al., 1993).

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CHAPTER THREE

3.0 MATERIALS AND METHODS:

3.1 Materials/Equipment/media used

Autoclave, Dry Oven, Bunsen burner, Conical flask, measuring cylinder, Cotton wool, Aluminum foil paper, weighing balance, Petri-dishes, Micropipette and tips, Hand gloves, Microscope, Microscopic slides, pH meter, Incubator, McCartney bottles, Irish potato, inoculating loop, Stirring loop, Pot, Beaker, Rotary shaker, Spectrophotometer, Sterile test tube, Sterile beaker, Sterile pipette, nutrient agar, Mineral salt medium.

3.2 Sample of Emulsion Paint Collected

A fresh emulsion paint sample was collected directly from the production line of a local paint manufacturing factory to ensure its purity and consistency. The sample was obtained in an airtight container to prevent contamination and preserve its chemical integrity. The sample was then transported under controlled conditions to the laboratory for subsequent analysis and biodegradation experiments.

3.3 Bacterial isolates Used

Bacterial isolates were obtained from previously cultured strains from deteriorated emulsion paint known for their ability to degrade emulsion paint. These isolates were cultivated on nutrient agar plates and incubated at 30°C for a period of 24 to 48 hours to allow for optimal growth. After incubation, selected colonies were suspended in sterile water and adjusted to an optical density of approximately 0.5 at 600 nm to prepare a standardized inoculum.

3.4 Preparation of Mineral Salt Medium

The Mineral Salt Medium: Dipotassium hydrogen phosphate (K_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4), Sodium chloride (NaCl), Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), Ammonium chloride (NH_4Cl), Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), Ferrous sulphate heptahydrate ($FeSO_4$. $7H_2O$) with pH 7.5 was used for the isolation. The medium was prepared with fresh emulsion paint sample as the only carbon source which was used to monitor the growth of the bacterial isolates. The medium was sterilized by autoclaving at 121 ^{0}C for 15 min.

3.5 Bacteria Degradation Test Using Emulsion Paint

A bacteria degradation test utilizing newly opened emulsion paint was used to monitor the isolate paint utilization rate. For the degradation experiment, 250 mL Erlenmeyer flasks were used, each containing 100 mL of sterilized mineral salt medium (MSM). Emulsion paint was added to each flask in a volumes of 5 mL, serving as the sole carbon source. The flasks were then inoculated with 1 mL of the standardized bacterial suspension. A negative control setup consisting of MSM and paint without bacterial inoculum was also carried out. All flasks were shaken on a shaker at 150 revolutions per minute (rpm) at intervals for a duration of 3 to 21 days. During the incubation period, pH, optical density (OD) using a spectrophotometer set at 600 nm, total viable counts (TVC) and temperature were monitored to assess the progress of paint degradation for the bacteria to grow in the medium.

3.5.1 pH Determination

At each sampling point, the pH of the culture medium was measured to assess changes in acidity or alkalinity resulting from microbial metabolic activity. A 5 mL aliquot was withdrawn from each flask, was analyzed using a calibrated digital pH meter.

3.5.2 Total Viable Count (TVC) Determination

Total Viable Count (TVC) was determined to estimate the bacterial population during degradation. Serial dilutions of the culture were prepared in sterile saline, and 100 µL of appropriate dilutions were plated on nutrient agar. The plates were incubated at 30°C for 24 hours, after which colonies were counted and expressed as colony-forming units per milliliter (CFU/mL).

3.5.3 Temperature Determination

Temperature was monitored throughout the experiment using a digital thermometer to ensure consistent incubation conditions. Although the flasks were maintained in a controlled environment, temperature readings were recorded at each sampling point to detect any fluctuations that might influence microbial activity or degradation rate.

3.5.4 Optical Density (OD) Determination

Optical Density (OD) was measured at 600 nm using a spectrophotometer to monitor bacterial growth over time. A 1 mL sample was taken from each flask, and OD readings were recorded to evaluate biomass accumulation and correlate it with paint degradation progress.

All measurements were performed in triplicate to ensure accuracy and reproducibility. The collected data were analyzed to determine the relationship between microbial growth, paint degradation, and physicochemical changes in the medium over the 21-day period.

CHAPTER FOUR

4.0 RESULTS

Figure 1 illustrates the variation in temperature and pH over the 21-day degradation period by *Bacillus* species. The temperature remained relatively stable around 30°C, indicating consistent incubation conditions. However, the pH showed a gradual decline from neutral to slightly acidic, suggesting metabolic activity and the production of acidic intermediates during paint degradation.Figure 2 presents the growth profile of *Bacillus* species, measured through optical density (OD_{600}nm) and total viable counts (TVC). Both OD and TVC increased steadily from day 3 to day 14, indicating active bacterial proliferation. A slight plateau or decline by day 21 may reflect nutrient depletion or accumulation of toxic byproducts, signaling the end of the exponential growth phase. This figure 3 shows the temperature and pH trends for *Escherichia coli* during the degradation process. The temperature remained constant throughout the experiment, while the pH exhibited a more pronounced drop compared to *Bacillus*, indicating higher acid production. This suggests that *E. coli* may metabolize paint components differently, possibly producing more acidic compounds. Figure 4 highlights the microbial growth dynamics of *E. coli* during paint degradation. OD and TVC values increased significantly between days 3 and 14, reflecting robust growth. However, by day 21, both parameters showed signs of stabilization or decline, which may be attributed to reduced substrate availability or inhibitory effects of accumulated degradation products.

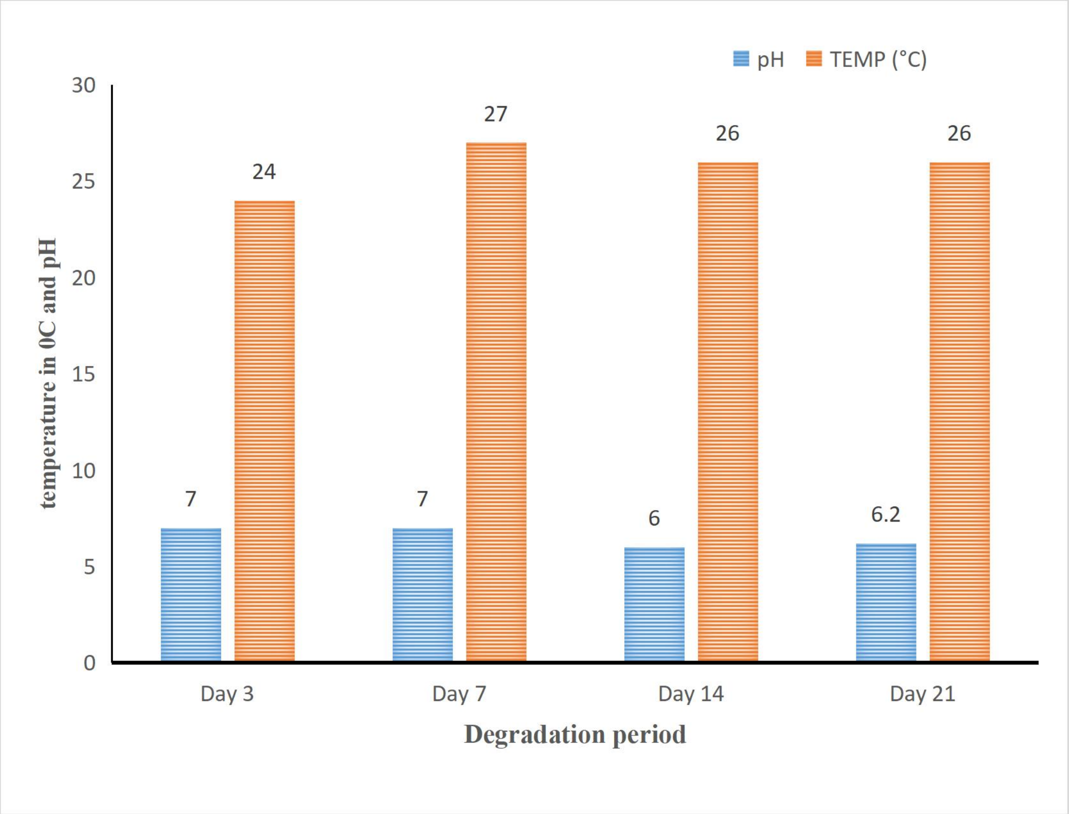

Figure 1: Temperature and pH changes of *Bacillus* specie undergoing degradation of emulsion paint.

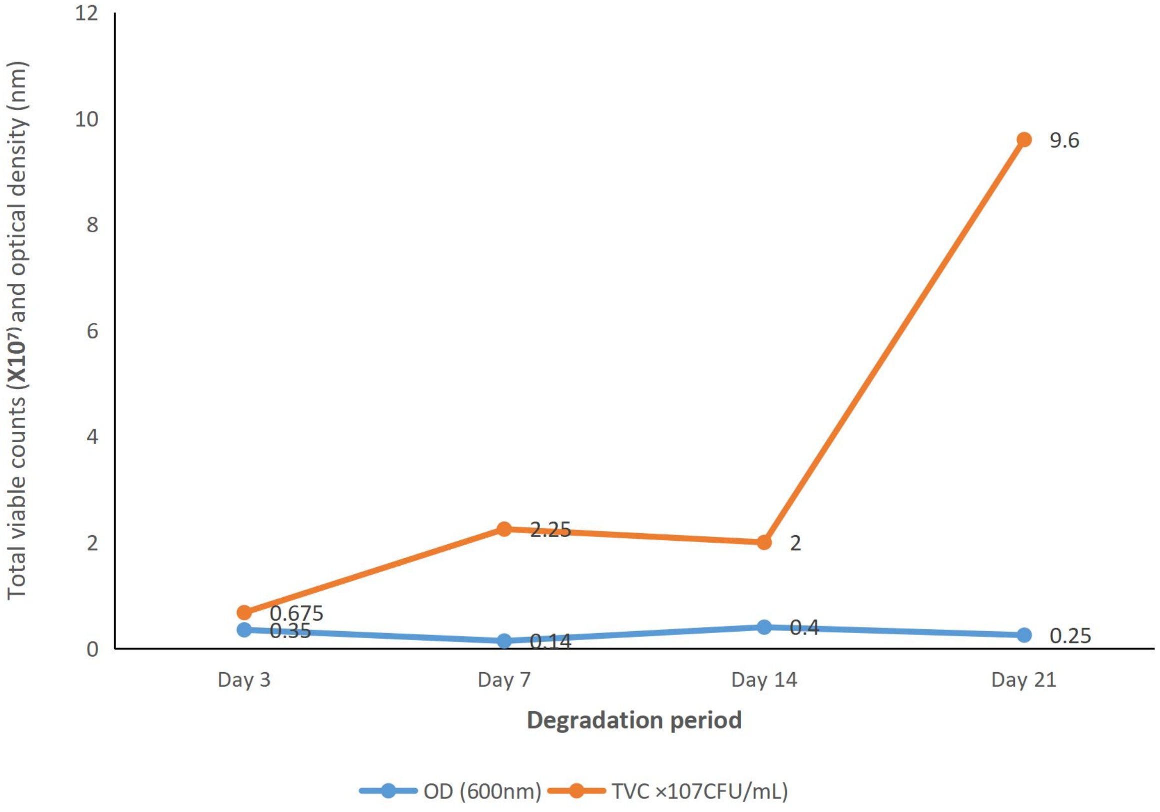

Figure 2: Optical density and total viable counts of *Bacillus* specie undergoing degradation of emulsion paint.

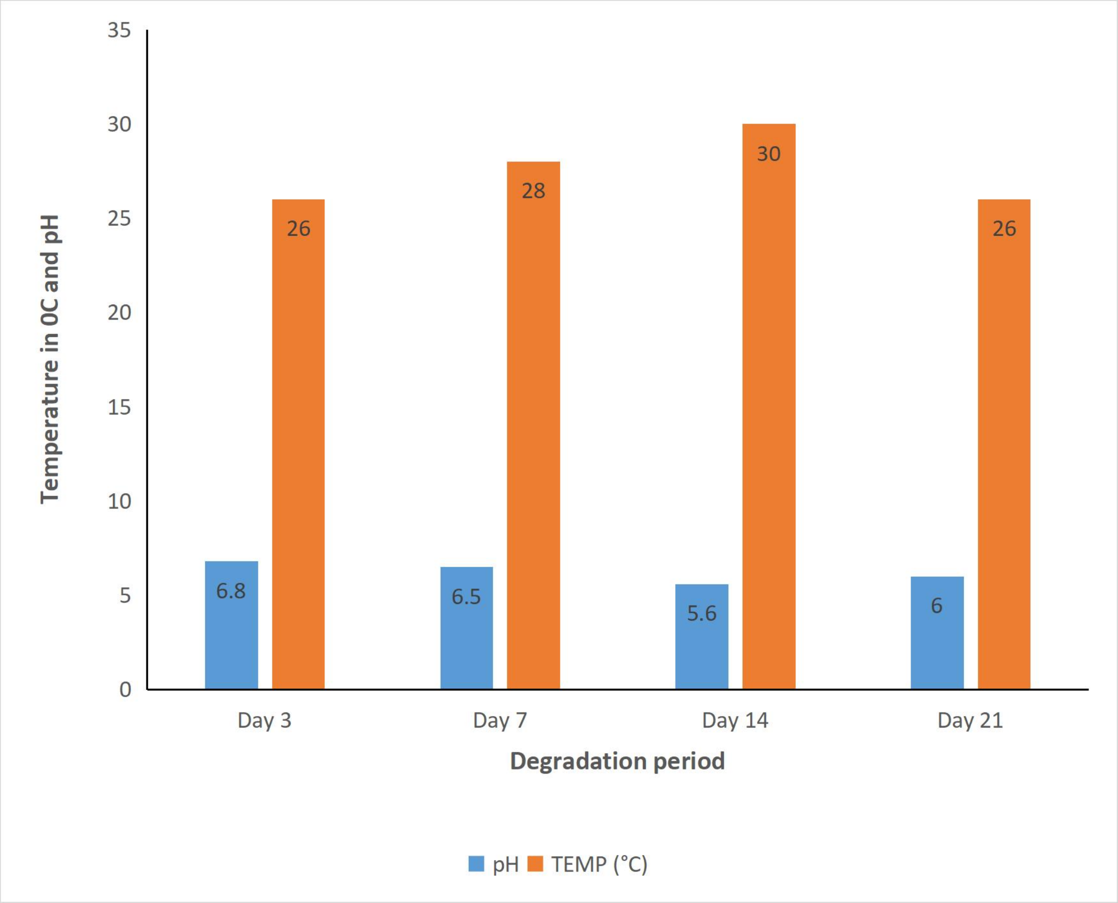

Figure 3: Temperature and pH changes of *Escherichia coli* specie undergoing degradation of emulsion paint.

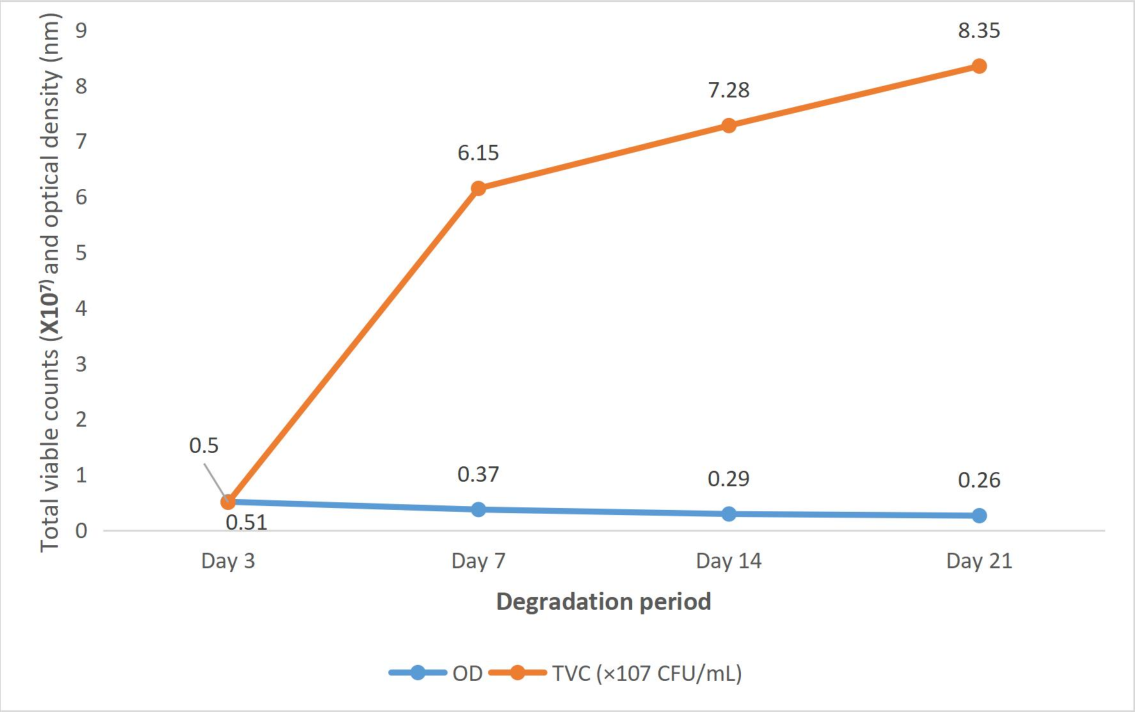

Figure 4: Optical density and total viable counts of *Escherichia coli* specie undergoing degradation of emulsion paint.

CHAPTER FIVE

5.0 DISCUSSION

The degradation of emulsion paint by microbial agents is a well-documented phenomenon, particularly in tropical environments where humidity and organic residues create favorable conditions for microbial colonization. This study examined the physiological responses of *Bacillus* spp. and *Escherichia coli* over a 21-day period, focusing on pH, optical density (OD), temperature, and total viable count (TVC) as indicators of microbial activity and paint degradation potential.

Emulsion paints are composed of synthetic polymers (e.g., polyvinyl acetate), pigments, surfactants, and plasticizers—many of which serve as potential carbon sources for microbes. Microorganisms such as *Bacillus* and *E. coli* can metabolize these compounds, leading to discoloration, surface roughening, and eventual structural breakdown of the paint film. According to Onuoha (2018), microbial colonization of painted surfaces in humid regions like southern Nigeria is common, with "infected patches" often linked to microbial degradation of paint constituents.

Bacillus spp. are known for their enzymatic versatility, producing proteases, lipases, and esterases that can hydrolyze paint binders and additives. Zaharaddeen *et al*. (2023) reported that *Bacillus* strains isolated from deteriorated paint surfaces exhibited strong enzymatic activity and were capable of surviving in harsh chemical environments. Similarly, *E. coli*, though less commonly associated with paint degradation, can ferment organic additives and produce acids that contribute to biodeterioration.

The observed pH decline in both microbial cultures reflects acid production during metabolism of paint components. *Bacillus* maintained a relatively stable pH until Day 7, followed by a drop to 6.0 by Day 14, suggesting moderate acidogenesis. In contrast, *E. coli* exhibited a sharper pH decline to 5.6, indicating more aggressive acid production. This aligns with findings by Ajayi *et al*. (2021), who noted that *E. coli* isolated from painted walls produced significant amounts of organic acids during growth on paint substrates.

Acidification not only signals metabolic activity but also contributes to the chemical breakdown of paint films, weakening polymer bonds and facilitating microbial penetration. OD measurements provide insight into microbial biomass and turbidity. *Bacillus* showed a peak OD of 0.40 at Day 14, indicating active growth and biomass accumulation. The subsequent decline may reflect sporulation or nutrient exhaustion. *E. coli*, however, showed a steady OD decline from 0.51 to 0.26, despite increasing TVC. This suggests that while viable cells persisted, biomass density decreased—possibly due to pigment toxicity or cell aggregation, which can reduce light scattering.

These trends are consistent with previous studies where microbial growth on painted surfaces led to biomass accumulation followed by stabilization or decline due to substrate limitation or environmental stress.

Temperature ranged from 24°C to 30°C across the study period. *Bacillus* maintained stable growth across this range, consistent with its mesophilic nature. *E. coli* showed peak TVC at 30°C, aligning with its optimal growth temperature. Environmental temperature plays a critical role in microbial metabolism and enzymatic activity, influencing the rate of paint degradation.

TVC data revealed robust microbial proliferation. *Bacillus* increased from 0.675×10^7 to 9.60×10^7 CFU/mL, while *E. coli* rose from 0.5×10^7 to 8.35×10^7 CFU/mL. These increases confirm that both organisms utilized paint components as nutrient sources. The more gradual increase in *Bacillus* may reflect its slower but sustained enzymatic degradation, while *E. coli*'s rapid growth suggests efficient exploitation of readily available organics.

Bacillus exhibited **controlled acid production**, **stable biomass**, and **gradual proliferation**, suggesting a structured degradation mechanism possibly involving biofilm formation and enzymatic hydrolysis. *E. coli* showed **rapid acidification**, **declining OD**, and **sharp TVC increase**, indicating a more aggressive but potentially stress-prone degradation strategy.

These differences highlight the diverse ecological roles and metabolic strategies of bacteria in paint biodeterioration. The findings support the assertion by Onuoha (2018) that microbial degradation of emulsion paint is a multifactorial process involving both enzymatic and physicochemical interactions.

5.1 CONCLUSION

This study confirms that both *Bacillus* spp. and *E. coli* are capable of degrading emulsion paint through distinct metabolic pathways. *Bacillus* demonstrated enzymatic resilience and biomass stability, while *E. coli* exhibited rapid proliferation and acidogenic stress. These insights contribute to the understanding of microbial paint degradation and underscore the need for antimicrobial additives in paint formulations, especially in humid, microbe-rich environments.

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