KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

SOIL DEGRADATION OF BIOPLASTIC FILMS PRODUCED FROM BANANA (*MUSA PARADISIACA)* AND CASSAVA PEELS (*MANIHOT ESCULENTA*)

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(19/57MPB/00002)

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SOIL DEGRADATION OF BIOPLASTIC FILMS PRODUCED FROM BANANA AND CASSAVA PEELS

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DECLARATION

I hereby declare that this thesis titled “Soil Degradation of Bioplastic Films Produced from Banana and Cassava Peels” is a record of my research thesis. It has neither been presented nor accepted in any previous application for higher degree

Victor Jesulayomi Bamisaye **Signature/Date**

APPROVAL PAGE

This is to certify that this thesis, titled "Soil Degradation of Bioplastic Films Produced from Banana and Cassava Peels" by Victor Jesulayomi Bamisaye has been read and approved as meeting the requirement of the Department of Plant and Environmental Biology and for the award of the degree of Master of Science (M.Sc.) in Plant and Environmental Biology.

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DEDICATION

I dedicate this work to God for seeing me through the course of this research work

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Abstract

The world is currently faced with the problem of disposal of plastics due to its non-biodegradable nature. Biodegradable plastic has thus become a promising solution to solve this problem. This study assessed the rate of degradation of bioplastic films produced from two naturally renewable organic sources cassava (*Manihot esculenta*) and banana (*Musa paradisiaca)* peel. Cassava and banana peels were obtained from cassava tubers and unripe banana bunches purchased directly from a farm at Isan Ekiti, Ekiti State, Nigeria. The bioplastic films were produced and characterized for their physico-chemical and mechanical properties using standard laboratory procedures. The rate of degradation of the films was assessed using the soil burial method. Two soil types (sterilized and unsterilized soil) and 4 plastic types (banana-based, cassava-based, cling film, and no plastic as control) were used and was replicated 8 times. Bioplastic strips, 2 cm × 2 cm each were buried in 64 (2 × 4 × 8) polyethylene nylons filled with 1000 grams of soil at laboratory temperature for 40 days. Biodegradability of the samples was evaluated at varying intervals of 10, 20, 30 and 40 days. Soil was analyzed for physico-chemical and microbial properties pre- and post-burial using standard laboratory methods. Cassava-based film had a higher water absorption capacity and tensile strength of 48.79% and 4.67 Mpa respectively while banana-based film had a higher elongation at break of 31.72%. Higher rates of degradation were observed in non-sterile soils compared to sterile soils; with the highest rate of 71.75% observed with cassava film at 40 days in unsteriled soil. At 40 days, highly significant (p<0.05) fungal (162 x 10^3 CFU/g) and bacterial (2.50 x 10^6 CFU/g) loads were observed when cling film was buried in non-sterile soil. The probable fungal species identified in the soil include; *Aspergillus terreus, Aspergillus niger, Trichoderma spirale, Fusarium oxysporum* and *Penicillium griseofulvum* while bacteria species identified include; *Pseudomonas aeruginosa, Bacillus* spp., *Clostridium* spp., *Staphylococcus auerus, Enterobacter* spp., *Micrococcus* spp, *Rhizobium* spp., *Xanthomonas* spp. and *Klebsielela* spp. There was no significant (p < 0.05) correlation between the microbial count and the rate of degradation of the biofilms, but significant (p < 0.05) correlation occurred between the fungal count and bacteria count. The study established that the degradation of bioplastic films did not only depend on the composition of the bioplastics but also on time, physical and chemical properties of the environment. The study, therefore, concluded that cassava and banana peels will serve as good sources for production of eco-friendly bioplastics.

CHAPTER ONE
INTRODUCTION

1.1 Background to the Study

Bioplastic materials represent an alternative to the conventional plastics and their applications. The bioplastics market share is around 1% of the 370 million tons of total global plastic production but the annual growth rate of bioplastics is projected to be around 30% in 2025 (Vert *et al.*, 2012). Environmental pollution is increasing day by day due to more plastic application in the environment (Mariano *et al.*, 2021). According to Roser *et al. (*2018), 270 million tons of plastics were produced worldwide with an estimation of 10-100,000 tonnes floating on the ocean surface. Bioplastic according to European Bioplastics (EUBP) is defined as biodegradable plastic materials and plastics produced from renewable materials. International Union of Pure and Applied Chemistry (IUPAC) defined bioplastic as a derivative of "biomass or monomers with plant origin (Vert *et al.,* 2012).

Plastic materials comprise polymers with a relatively high molecular weight that are typically produced by chemical synthesis processes. The term bioplastics are used to distinguish polymers that originate from renewable sources of biomass. The synthetic polymers are made from monomers by polycondensation or polymerization, and most of them have a simpler structure than natural ones (Mariano *et al.,* 2021). The most common synthetic polymers are polypropylene (PP); polyethylene (PE), acrylonitrile–butadiene–styrene (ABS), polycarbonate (PC), polyamides (PAs), polystyrene (PS), polyethylene terephthalate; polyvinyl chloride (PVC), polytetrafluoroethylene (Tefon), poly (methyl methacrylate) (PMMA), acrylic polyurethane (PU, PUR). These plastics are traditionally petrochemically derived, but the demand for their production from renewable feedstocks is growing. Theoretically, all usual plastics are generally degradable, but they have a slow breakdown, hence considered non-(bio) degradable. Biodegradation of bioplastics depends on their physical and chemical structures in terms of polymer chains, functional

groups, and crystallinity, but also on the natural environment in which they are placed (i.e., moisture, oxygen, temperature, and pH) (Mariano *et al.*, 2021).

Biodegradation is an enzymatic reaction catalyzed by microorganisms, such as actinobacteria (*Amycolatopsis, Streptomyces*), bacteria (*Paenibacillus, Pseudomonas, Bacillus, Bulkholderia*) and fungi (*Aspergillus, Fusarium, Penicillium*) (Emadian *et al.*, 2017). There are different concepts of biodegradation. One very common degradation process is called hydrolysis. The hydrolysis mechanisms are exaggerated by diffusion of water through the polymer matrix (Thakur *et al.*, 2018). The main end-of-life choices for biodegradable plastics include recycling and reprocessing, incineration and other recovery options, biological waste treatments, such as composting, anaerobic digestion, and landfill (Song *et al.*, 2009; Mugdal *et al.*, 2012). The composting process represents the final disposition most favorable from an environmental point of view. The presence of ester, amide, or hydrolyzable carbonate increases biodegradation's susceptibility. Bioplastics also do produce less greenhouse gases than that of usual plastics over their period. Therefore, bioplastics contribute to a more sustainable society. Therefore, there are bioplastic alternatives to conventional plastic materials. Glass and carbon fibers are synthetic fbers commonly used to reinforce bioplastics, but they are not biodegradable. For this reason, they can be replaced by more environmentally friendly, abundant, and low-cost materials, such as lignocellulosic fibers and lignin (Yang *et al.*, 2019).

Starch is used as one of the major sources in the development of biodegradable bioplastic. Several studies have been conducted by using starch such as cassava peel and banana as natural biopolymer sources. Banana and cassava peel consist high sources of starch, which is about 18.5% (Astuiti and Erprihana, 2009). The abundance of this agriculture waste is deemed as a brilliant alternative to replace the synthetic plastic. As banana peels ripen, the glucose level increases. However, if the peels are too ripe, the starch will be converted into glucose while the least ripened peels, becomes too firm although high in starch

molecules (Soltani *et al.*, 2010). The cassava peel is rich in starch, which contains high amount of amylopectin. This can contribute to a greater binding for the plastics. Although, banana and cassava have been used as a suitable source for the manufacturing of biopolymeric film or bioplastics, there is paucity of research data on soil degradation properties of bioplastics from banana and cassava peel. Therefore, this research will attempt to address the effect of soil as a medium for the degradation of the bioplastics from agriculture waste in order to promote sustainable development.

1.2. Statement of Research Problem

Plastic pollution is a global and rising concern; and increasing demand and usage of plastics, and their disposal has resulted in several environmental pollution burdens on both land and water habitats, threatening the safety and health of wildlife, aquatic life and humans because most plastics are petrochemical-based, polymer derived, which are non-biodegradable. The world has produced more plastics in the last 10 years than in the whole of the 20th century, and every piece of plastic ever produced still exists today (Jamróz *et al.*, 2019). Unfortunately recycling or reuse does not eliminate toxic substances or non-biodegradability and incineration is a source of environmental pollution. With the high demand and increase in daily consumption of banana and cassava products, there is high waste accumulation of banana and cassava peels from households, agricultural areas and industrial areas. Many researches exist on the application of banana and cassava peel as raw material for bioplastic films, but there is paucity of information on the soil degradation properties of these bioplastic films.

1.3. Objectives of the Study

The broad objective of this study was to evaluate the rate of biodegradation of bioplastic films produced from banana and cassava peels, using the soil burial method.

Specific Objectives

The specific objectives of this study were to:

I. produce and characterize biodegradable plastic films from cassava and banana peels;

II. assess the effect of soil microorganisms on the rate of degradation of these bioplastics;

III. assess the effect of degradation on soil microbial composition

IV. assess the effect of degradation on soil physico-chemical properties.

CHAPTER TWO
LITERATURE REVIEW

2.1. Bioplastic Materials

Plastics are polymeric chains composed of repetitive units or monomers linked together. These macromolecules are conventionally synthesized by polymerization, polycondensation or polyaddition reactions from fossil sources. Interest in competitive biodegradable materials is growing to limit environmental pollution and waste management problems (European Bioplastics, 2020). Bioplastics are a new plastic generation, defined as plastics originating from a biological system and produced from renewable resources or by a range of microorganisms (Janssen *et al.*, 2009). Bioplastics significantly reduce the environmental impact in terms of greenhouse effect and energy consumption; therefore, contribute to a greener future. They possess different properties, and are classified in three main groups, as shown in Figure 2.1:

1. Bio-based or partially bio-based plastics;
2. Bio-based and biodegradable plastics;
3. Fossil resources and biodegradable plastics.

Bioplastics produce less greenhouse gases than that of usual plastics over their period and therefore, contribute to a more sustainable society (Janssen *et al.,* 2009). It serves as alternatives to conventional plastic materials and plays a vital part in different fields of application. Bioplastics that are bio-based have the same properties as general plastics and offer added advantages because they have a lesser carbon footprint on environment. Nevertheless, their low mechanical strength limits their application (Reddy *et al.*, 2013). Glass and carbon fibers are synthetic fibers commonly used to reinforce bioplastics, but they are not biodegradable. For this reason, they can be replaced by more environmentally friendly, abundant, and low-cost materials, such as lignocellulosic fibers and lignin (Yang *et al.,* 2019). Other physical strengthening methods are the mold temperature increase, dehydrothermal treatment, and ultrasounds

application. When applied to soy protein-based bioplastics, the thermal treatment enhanced the mechanical properties, the dehydrothermal treatment increased the superabsorbent capacity and ultrasounds led to a structure with smaller pores. As a consequence, the treated bioplastics could be used in different applications (Jiménez-Rosado *et al.*, 2020). A new green one-step water-based process was proposed to convert vegetable wastes into biodegradable bioplastic films having similar mechanical properties with other bioplastics (Perotto, 2018).

2.2. Non-biodegradable Bioplastics

Most of the current bioplastic market is non-biodegradable which has been a major problem for waste management (Algieri *et al.*, 2012). Bio-based /partially bio-based plastics include bio-based drop-in polyethylene (PE) and polypropylene (PP), polyethylene terephthalate (PET), and technical performance bio-based polymers, such as polytrimethylene terephthalate (PTT) or Thermoplastic polyester elastomers (TPC-ET), as well as bio-based polyamides (PAs). These non-biodegradable bioplastics are from renewable natural resources that are from biomass without having the bio-degradation characteristics (Rahman and Bhoi, 2021). Biopolyethylene is produced from bioethanol, as other common bioplastics such as polyethylene terephthalate (bio-PET), bio-PP or polypropylene (bio-PVC, polyvinyl chloride (bioPVC), bio-PET (Rujnić-Sokele and Pilipović, 2017). Bio-PE, bio-PET, and bio-PAs currently represent 40% around 0.8 million tonnes of global bioplastic production capacities. In these last years, the focus has shifted on polyethylene furoate (PEF), a novel polymer that is anticipated to enter the commercial market by 2023.This new polymer is comparable to PET, but it is completely bio-based and has superior barrier properties, which makes it an optimal material for beverage bottles.

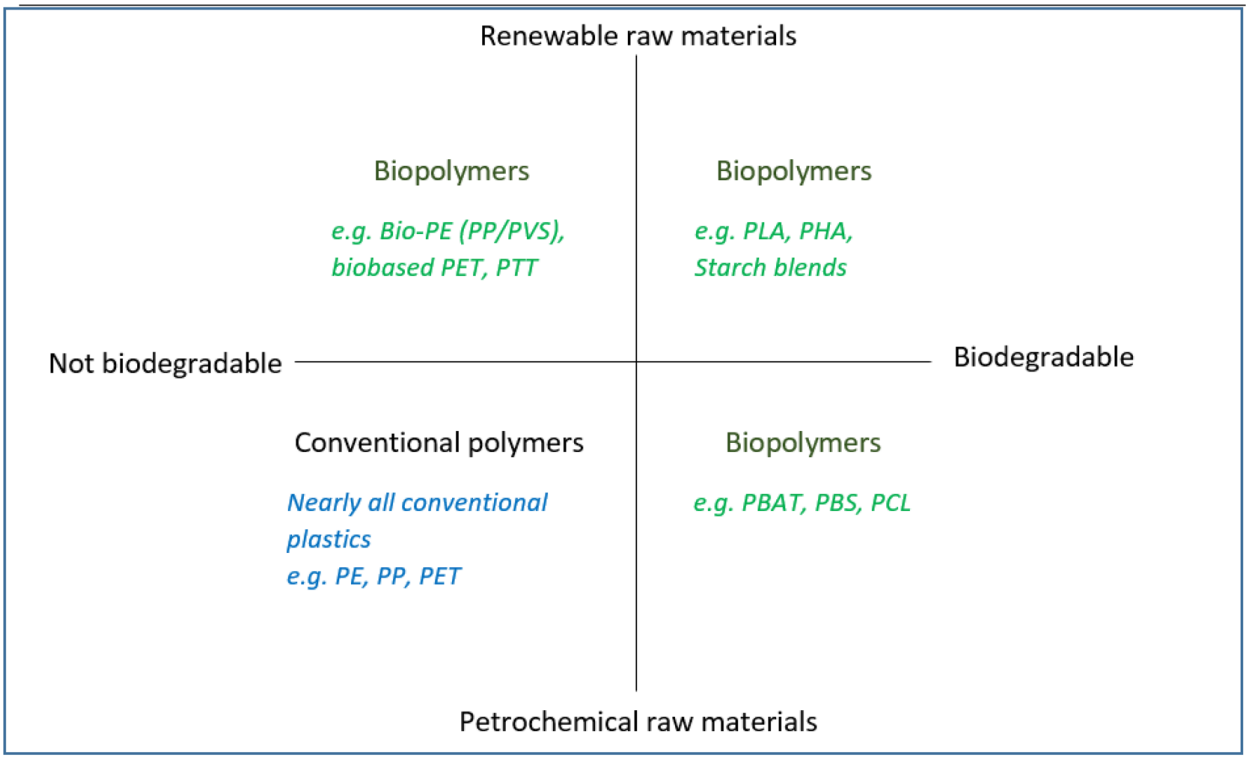


Figure 2.1: Type of bioplastics

Source: Philp *et al.* (2013)

2.3. Biodegradable plastics

Plastics that are both biodegradable and bio-based are produced from renewable natural resources; they possess biodegradation property at some stage. This group includes the thermoplastically modified starch as well as other bio-degradable polymers like polyhydroxyalkanoates (PHA), polylactide (PLA), and polybutylene succinate (PBS). Besides petrochemicals, PLA can be found in *Escherichia coli* (Jung and Lee, 2011) or with woven bamboo fabric (Porras and Maranon, 2012). Bioplastics are mostly produced from sugar or lipids by bacteria because PHAs represent an intracellular product of bacteria. Around 250 types of bacteria help to yield PHA. So, these bioplastics are collected with the demolition of bacteria and then disconnected from the microbial cell matter. Moreover, PHAs have good barrier characteristic and attractive in different biomedical applications. They also have the standard specification from marine degradability, which is ASTM D7081.

Polyhydroxyalkanoates have different attributes: fully bio-degradable either in water or even in soil (Meereboer *et al.,* 2020); good resistance as well as printability to oil and grease; until a temperature of 120 °C (Philp *et al.,* 2013). Moreover, PHAs came from agro- and food wastes, such as wheat bran, rice husk, potato peel, mango peel, straw and bagasse (Gowda and Shivakumar, 2014). They degrade at different rates in different media. Thus, as seen in the case of PHAs, in general, the property of biodegradability can be directly related to the structure of the polymer and can thus be benefited with specific applications, particularly in case of packaging. Polycaprolactones (PCL) is also a biodegradable polyester which has very low melting point (~60 °C). It has general application in the biomedical field, which includes the surgical structure (Mariano *et al.,* 2021).

2.4. Bioplastics Bio-Based Content

The term “bio-based” refers to material derived from biomass. The most common used biomass for bioplastic uses are corn, sugarcane, and cellulose. Bio-based plastics have the exceptional advantage over

general plastics materials because it can reduce the dependency on fossil resources, resulting in lesser amount of emission of greenhouse gas and therefore have positive environmental factor (Mariano *et al.*, 2021). The bio-based content of a material is the amount of the biomass-derived carbon, as compared to its total organic carbon content (TOC). The carbon content of biobased materials is determined independently and unequivocally as reported in international standard methods of the American Society for Testing and Materials (ASTM) and of the International Organization for Standardization (ISO). These ASTM D6866-20 and ISO 16620-2 methods report radiocarbon analysis as the technique to determine the bio-based content of solid, liquid, and gaseous samples.

Companies indicate their bio-based products with the word "bio-based carbon content" or with "biobased mass content", but some other standard certifications exist to identify this product. ^{14}C-method is an acceptable methodology to measure the bio-based carbon content in materials (Mariano *et al.*, 2021). This method is the basis for the European standard, and the corresponding USA standards exist. They are CEN7TS 16,137 and ASTM 6866, respectively, for EU standard and US standard. Moreover, a method to individuate a bio-based mass content was introduced by the French Association Chimie du Vegetal (ACDV) with a corresponding certification scheme. It consists to take chemical elements such as oxygen, nitrogen, and hydrogen into account, besides the bio-based carbon (Mariano *et al.*, 2021). The employment of the radiocarbon dating method is based on the significative difference in ^{14}C isotopic signature between the fossil derived (^{14}C-free) and the biomass derived (^{14}C-including) materials. In detail, the presence of ^{14}C in the bio-based materials is due to the fact that ^{14}C containing carbon dioxide formed in the atmosphere participates in the photosynthetic processes from which the biomass derives (Iwata, 2015). Thus, the ^{14}C content of biomass derived materials is the result, in a first approximation, of ^{14}C atmospheric levels (Quarta, 2013). ^{14}C measurements could be done by using Accelerator Mass Spectrometry (AMS) along with Isotope Ratio Mass Spectrometry (IRMS) or by using Liquid Scintillation

Counting (LSC) techniques (ASTM International, 2020). In order to define a bioplastic as bio-based, a biomass-derived carbon content not less than 25% is required (Iwata, 2015).

2.5. Biodegradability of Bioplastics

Measurement of biodegradability of bioplastics is another key bioplastic property, which refers to the ability of a material to decompose after interactions with biological elements. The biodegradation of polymers according to Lucas *et al.* (2008) involves three steps: bio-deterioration, bio-fragmentation, and assimilation as shown in Figure 2.2.

Bio-deterioration is the modification of mechanical, chemical, and physical properties of the polymer due to the growth of microorganisms on or inside the surface of the polymers. In the bio-fragmentation step, microorganisms fragment polymers in oligomers and monomers, which, in the next assimilation step, are available as their carbon, energy, and nutrient sources finally with CO_2, water, and biomass as by-products (Emadian, 2017).

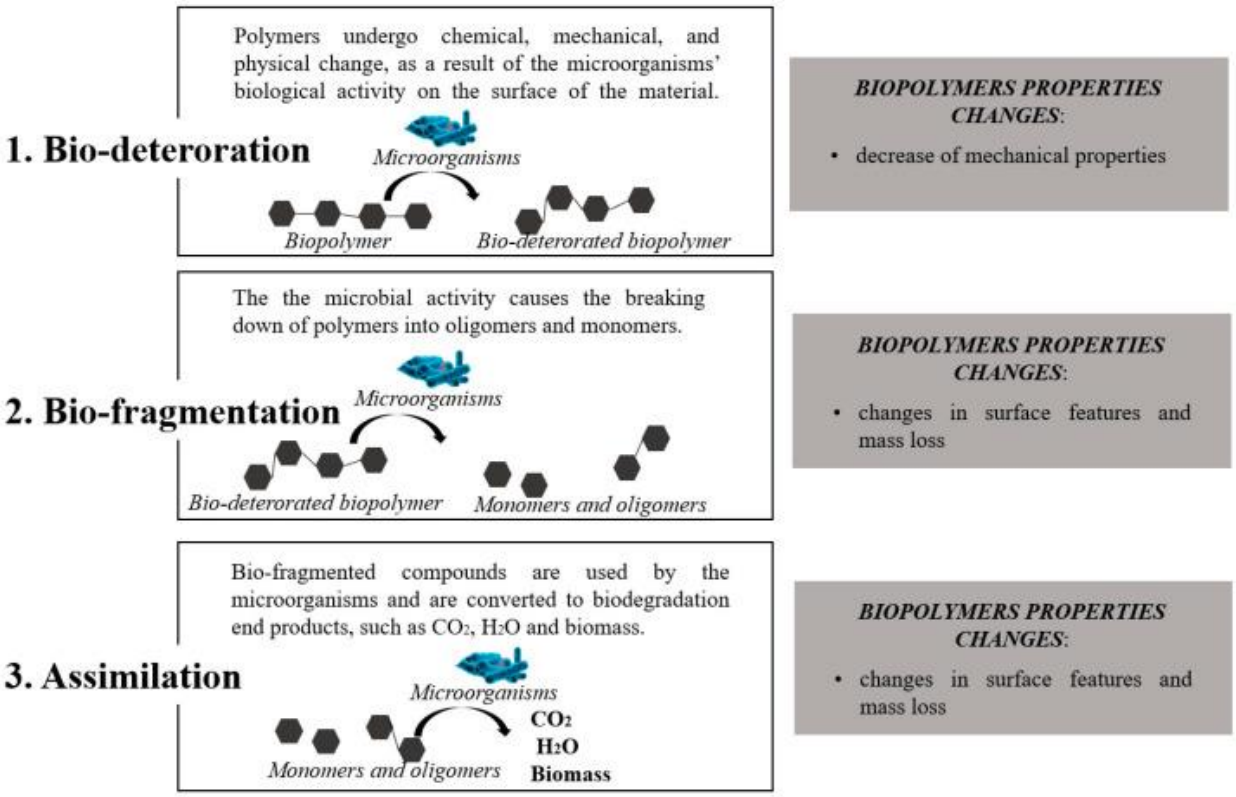


Figure 2.2: Steps involved in biodegradation of bioplastics

Source: Emadian (2017)

It should be pointed out that only specific microorganisms could degrade a given type of bioplastic. It has been reported that PCL can be degraded by bacteria isolates that exist in deep sea sediments, but these isolates are incapable of degrading other types of bioplastics, such as PLA, PHB, and PBS; however, there exist composting bacteria capable of degrading the latter (Emadian, 2017).

To date, a wide variety of methods for measuring the biodegradability of polymeric biomaterials have been developed and most of them are in agreement with ASTM, ISO, and European Standards (EN) standard methods in terms of environmental conditions, timings, and scales of the tests. Overall, all methods are focused on an indirect measure of degradation process, such as oxygen consumption or biogas generation (CO_2) by measuring differences of pressure in the test flasks and carbon dioxide production (Janssen and Moscicki, 2009). A biodegradation level higher than 90% in comparison with cellulose (positive standard) in 180 days, under conditions of controlled composting measured through respirometric methods has been established by the European Norm EN 13,432 as the level for a material/product to be defined as biodegradable and compostable. In addition, a disintegration level higher than 90% in three months and the respective eco-toxicity and chemical safety criteria should be kept. Then, only when the products meet the EN 13,432 standard criteria can the word "biodegradable" be reported on the packaging label.

2.5.1. Factors affecting the Biodegradability of Bioplastics

The biodegradation of bio-plastic materials is highly dependent on their chemical structures. Generally, polymers with a shorter chain, more amorphous parts, and less complex formula are more susceptible to biodegradation by microorganisms (Ruggero *et al.,* 2019). The presence of additives could influence the biodegradability of a matrix. As an example, polypyrrole, the archetype of polymers integrated in bio-sensing devices for biomedical applications, can acquire enhanced biodegradability if grafted onto

cellulose chains, thus forming bio-composite (Ciriello *et al.,* 2018a; 2018b). Moreover, the pH, temperature, and the oxygen content of the environment in which the polymers are placed or disposed of, could be key factors for their biodegradation (Massardier-Nageotte *et al.,* 2005; Kale *et al.,* 2007). For example, oxidative-degradable polymers accelerate their decomposition under the effect of oxidation through heat and/or UV light. UV radiation can disrupt polymer chains, since the radiation can be absorbed by oxygen-containing components to initiate a primary degradation; these polymers are known as photodegradable polymers. During photo-degradation, both molar mass and crystal structure are affected. The plastics that have the capacity to biodegrade by hydrolytic mechanisms such as biopolymers made of cellulose, starch, and polyesters such as PHA are known ad hydro-biodegradable bioplastics (Folino *et al.,* 2020).

The biodegradation of bioplastics has been extensively investigated in soil and compost environments, where they mainly showed high degradability. Anyway, the conditions of the experiments conducted to study the bioplastics biodegradability are highly variable, and to make a clear comparison among them is difficult. The experiments carried out in compost or in anaerobic digestion environments show a biodegradability over 50% in 65 and 68%, respectively. For those carried out in aquatic environments, this share is 44%, and for experiments carried out in soil, it is 33% of the cases (Folino *et al.,* 2020). It should be pointed out that, in addition to increasing bio-based content and biodegradability, bioplastics intrinsic properties often need to be improved to meet industrial expectations. The optimization can concern, for example, mechanical properties, increased material flexibility, increased rigidity, increased resilience, and improvement of water absorption capacity (Folino *et al.,* 2020). Water absorption can be defined as temperature for up to 24 hours in which water will fill the empty space contained in the plastic film (Junjie and Hanna, 2004). Liquid impermeability of bioplastic film is one of the conventional properties of plastic (Junjie and Hanna, 2004; Bonilla *et al*., 2015). The lower the amount of water

absorbed by plastic film in percent units, after being immersed in water at room temperatures, the better the quality of the plastic (Septiosari *et al*., 2014). Higher water affinity is demonstrated by adsorption and desorption isotherms (Septiosari *et al*., 2014). The fact that cassava and banana starch contain hydroxyl (OH), carbonyl (CO), and ester (COOH) functional group indicate the hydrophilic properties in their films.

2.5. Mechanical and Physical Properties of Bioplastics

The main mechanical properties that are typically tested after the production of a bioplastic are the ultimate tensile strength, the Young's Modulus, and the elongation at break. The ultimate tensile strength, or just tensile strength, indicates the maximum stress that a material can withstand before fracturing, while the Young's Modulus, also known as elastic modulus, refers to the stiffness of a material: the bigger is its value, the stiffer the material (Granda *et al.,* 2016). The elongation at break values is a measure of material ductility and depends on the rate (crosshead speed) and the temperature. The elongation at break value is, generally, very small and closes to zero for brittle materials. On the contrary, materials with a better capacity to handle an excessive load without failure show higher elongation than 100% (Palomba *et al.*, 2014). Clearly, all these properties are affected by the chemical structure, the orientation degree of the polymers, and the crystallinity of the material, as well as by the eventual presence of fibers that act as reinforcement, or plasticizer (Sanjay *et al.,* 2015).

2.6. Plasticizers

Plasticizers are low volatile molecules, added to bio-polymeric materials to ensure an increase of their extensibility, dispensability, flexibility, and elasticity (Vieira *et al.,* 2011). Several theories to explain the mechanisms of plasticization action have been proposed (Suyatma *et al*., 2015). The lubrication theory states that plasticizers, by interspersing themselves, act as internal lubricants by reducing frictional forces between polymer chains. The gel theory, instead, postulates that the rigidity of polymers comes from three-dimensional structures, and plasticizers take effect by breaking polymer-polymer interactions (e.g.,

hydrogen bonds and van der Waals or ionic forces). The free volume theory states plasticization as a study of ways to increase free volume and is useful in explaining the lowering of the glass transition temperature (Tg) by a plasticizer. Ideal plasticizers should be miscible and compatible in all proportions with plastic components, and they may be added to polymers in solution (dispersion technique) or after solvents have been removed (absorption technique). Water, oligosaccharides, polyols, and lipids are different types of plasticizers widely used for edible films and coatings (Ghasemlou *et al.*, 2010).

For hydrophilic polymers, polyols have been proven to be very efficient as plasticizers (Tihminlioglu *et al.*, 2010). In detail, for bio-based polymers obtained from fruits and vegetables waste, the recent researchers have focused on the usage of glycerol and sorbitol (Sultan *et al.*, 2017). Glycerol content has significant effects on the mechanical properties as well as on the dynamic rheological behavior of thermo-molded bioplastics. Indeed, it was demonstrated that the increasing of glycerol content decreases tensile strength and Young's modulus but improves ductility at room temperature (Sun *et al.*, 2008). Several studies on plasticization of chitosan films revealed that poly (ethylene glycol) (PEG) could improve the elastic properties of the chitosan biopolymer. Caner *et al.* (1998) observed that chitosan plasticization using PEG was stable until nine weeks of storage.

In addition to the mechanical properties, plasticizers also affect the physical properties of the biomaterials, which means water vapor permeability (WVP), oxygen permeability (OP), and water contact angle (WCA) (Delgado *et al.*, 2010). These parameters serve as indicators of how easily water vapor or oxygen can penetrate a biodegradable material and they are a function of the hydrophilicity and hydrophobicity ratio of the main components by which the biomaterial is made. As to water contact angle, which is measured as the angle between the baseline of a drop deposited on the surface of the material and the tangent at the drop boundary, it increases with increasing surface hydrophobicity (Tihminlioglu *et al.*, 2010). Since the surfaces degree of hydrophobicity is important to ensure good barrier properties, the

evaluation of WVP, OP, and WCA is demanded. Recently, Aguilar *et al.* (2020) found that different physical and mechanical properties could be achieved at room temperature for bioplastics based on a soy protein isolated as a byproduct of the soy oil industry and added with different polyols, i.e. (glycerol (GLY), ethylene glycol (EG), diethylene glycol (DEG) and triethylene glycol (TEG) (lonso-González *et al.*, 2020). In this sense, TEG-bioplastics were opaque, brittle, and also had a higher water uptake capacity, while EG-bioplastics were more ductile and translucent, absorbing much less water when immersed. Only GLY and TEG remained in the bioplastic after 9 days of storage at 50 °C, pointing out the volatility of EG and DEG causing a major ageing effect. On the other hand, it was also observed that sugars like sucrose and trehalose could act as plasticizers in presence of water. In detail, when water is included in the bioplastic formulation together with glycerol, sugars are solubilized within the aqueous fraction, and then play a plasticizer role in the bioplastics. In that case, lower viscoelastic properties and greater water absorption ability is generally detected (lonso-González *et al.*, 2020).

2.7. Sources of Bioplastics

2.7.1 Agricultural Source of Bioplastics

Bioplastics can be produced from polysaccharides (starch, cellulose, chitosan/chitin), proteins (e.g., casein, gluten), and other carbon sources (Nachwachsende and Agency, 2020). Presently, the most used bioplastic is thermoplastic starch, obtained through enzymatic saccharifcation and microbial fermentation (Figure 2.3) or by modifying starch with plasticizers with hydrophilic properties (Mojibayo *et al.*, 2020). However, starch-based bioplastics treated with plasticizers and stored for long time face recrystallization and consequent deterioration of mechanical properties.

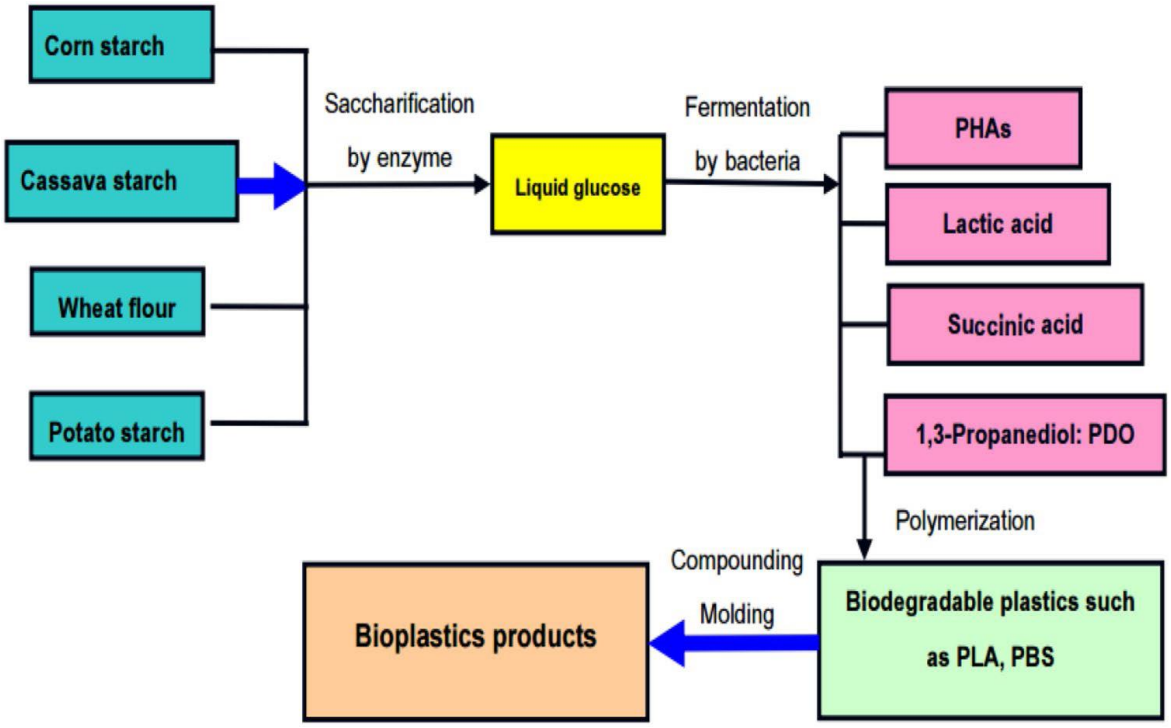


Figure 2.3: Bioplastic production from starch

Source: Chaisu (2016)

In order to overcome this problem, starch-based bioplastics' performance may be improved by the addition of nanoparticles to obtain nanocomposite bioplastics used in automotive components, packaging materials, and drug delivery (Mose and Maranga, 2011).

Starch is usually obtained from different plant sources. Cassava starch is used for production of bioplastics sheet through addition of distilled water, glycerol, and vinegar (Mojibayo *et al.,* 2020). Bioplastics from cassava starch were re-inforced also by coconut husk fibers (Babalola and Olorunnisola, 2019). Condensation polymerization was performed to produce bioplastic from corn starch and glycerin to obtain nanocomposites for packaging applications (Ateş and Kuz, 2020). Other starch sources are potatoes, wheat, and tapioca. The finest, smoothest, flexible and strong bioplastic was produced from tapioca starch (Gökçe, 2018), although the potato-derived starch showed the best properties in terms of extraction, ease of working, texture, and potential drying (Hamidon, 2018). Composite bioplastics from tapioca starch and sugarcane bagasse fiber were recently investigated and ultrasounds treatment improved properties by enhancing the tensile strength and decreasing the moisture absorption rate (Asrof *et al.,* 2020). Among proteins, wheat gluten can be processed to produce bioplastics (Rasheed, 2011; Jiménez-Rosado *et al.*, 2019). Sugarcane is exploitable for bioplastic production by bacterial sugar assimilation (Pohare *et al.*, 2017). Finally, oil is a good carbon source for the production of bioplastic. Cottonseed oil (Magar *et al.*, 2015), soybean oil (Park and Kim, 2011), crude palm kernel oil, jatropha oil, crude palm oil, palm olein, corn oil, and coconut oil were typically investigated (Wong *et al.*, 2012).

Lignocellulosic biomass is another promising resource for bioplastic production avoiding the consumption of food crops. Nevertheless, it requires suitable cost-effective pretreatments for decomposition into sugar monomers (Brodin *et al.*, 2017; Govil, 2020).

2.7.2. Organic Wastes as Sources of Bioplastics

Cassava and other staple crops require large land areas, water, and nutrients. Moreover, they contribute to global food security and their use to produce bioplastics is not sustainable. Instead, it is interesting to consider the organic waste source to valorize a residue and turn a problem into an opportunity in a circular economy approach and therefore, waste to wealth (Yadav *et al.,* 2019). Wastes from the food-processing industry are an important potential source of bioplastics (Tsang, 2019; Jõgi and Bhat, 2020). Vegetable wastes used to produce novel bioplastic films were carrots, radicchio, parsley, and cauliflowers (Perotto, 2018). Novel starch and/or cellulose-based bioplastics were produced from rice straw, an agricultural waste usually used for bioethanol production (Agustin *et al.,* 2014; Bilo, 2018), and other agricultural wastes (Chaisu, 2016). Extrusion of rice bran and kraft lignin that are industrial by-products of brown rice production and wood pulping process, respectively produced a bioplastic with good extrudability and mechanical properties (Klanwan *et al.*, 2016). A residual product of crude oil palm production is an empty fruit bunch, composed of cellulose, hemicellulose, and lignin. Having high cellulose content (36.67%), this abundant waste could be used to produce bioplastics (Isroi and Panji, 2016; Isroi *et al.,* 2017).

Ramakrishnan *et al.* (2018) and Sharma *et al.* (2018) reported the use of keratin from waste chicken feathers to produce biopolymeric films with the addition of microcrystalline cellulose and glycerol. Microcrystalline cellulose was a re-inforcing additive in bioplastic production also from avocado seeds (Sartika *et al.,* 2018), jackfruit seeds (Lubis *et al.,* 2018), and cassava peels (Maulida and Tarigan, 2016). Waste cassava peels were investigated in combination with kafr lime essential oil for future applications in industry and medicine (Masruri *et al.,* 2019). Cocoa pod husk and sugarcane bagasse, which are wastes from the chocolate industry and the sugar industry, respectively, are promising for the production of biodegradable plastic films (Azmin *et al.*, 2020). Bioplastics could be produced by injection molding from rapeseed oil production by-products, such as press cake or meal (Delgado *et al.*, 2018). New bioplastics

were also prepared from potato peels and waste potato starch with eggshells and/or chitosan (from exoskeleton seafood wastes) as additives (Kasmuri and Zait, 2018; Bezirhan and Bilgen, 2019). Also, banana peels were used to produce a bioplastic with the addition of corn starch, potato starch, sage, and glycerol (Sultan and Johari, 2017; Azieyanti *et al.*, 2020). Blood meal is a low-value protein-rich by-product from meat processing, that is convertible into a bioplastic material (Low *et al.*, 2014).

Bioplastic fibers were fabricated also from gum arabic by electrospinning method (Padil *et al.*, 2019). Polyhydroxyalkanoates (PHA) is a group of biodegradable plastics produced by microorganisms from renewable sources (Shraddha *et al.*, 2011). Among PHA sources, researchers investigated chicken feather hydrolysate (Benesova *et al.*, 2017), animal fat waste (Riedel, 2015), lignocellulosic biomass hydrolysate (Bhatia, 2019), grass biomass (Davis, 2013), fruit pomace, waste frying oils (Follonier, 2014), olive oil mill pomace (Waller *et al.*, 2012), saponified waste palm oil (Mozejko and Ciesielski, 2013), and low-quality sludge palm oil (Kang, 2017).Other source PHA sources investigated include waste oil palm biomass (Hassan, 2013), spent coffee grounds (Nielsen *et al.*, 2017) and other carbon sources (rice straw, maltose, glucose, sugarcane liquor, corn steep liquor, corn stover liquor, cheese whey, waste potato starch, sugar beet molasses, etc.) (Marjadi and Dharaiya, 2010; Tripathi *et al.*, 2012; Khatami *et al.*, 2021).

Another interesting resource is the organic fraction of municipal solid wastes convertible into PHAs by acidogenic fermentation of pre-treated and hydrolyzed biomass (Ivanov *et al.*, 2015; Ebrahimian *et al.*, 2020). Recent works investigated PHA production from volatile fatty acids, obtained by the anaerobic digestion of waste paper (Al-Battashi, 2019; Al Battashi *et al.*, 2020).

The most common PHA is polyhydroxybutyrate (PHB), produced from low-cost sugarcane molasses by *Bacillus cereus* (Suryawanshi *et al.*, 2020) or *Staphylococcus epidermidis* (Sarkar *et al.*, 2014), cheap agro-residues by *Bacillus sp*. (Getachew and Woldesenbet, 2016), date syrup by *Pseudodonghicola xiamenensis* (Mostafa *et al.*, 2020), non-food sugars from oil palm frond (Zahari *et al.*, 2015) or biodiesel

industry by-products (García, 2013) or used cooking oil (Martino, 2014) by Cupriavidus necator, wheat straw lignocellulosic hydrolysates by *Burkholderia sacchari* (Cesário*et al.,* 2014), wheat bran hydrolysate by *Ralstonia eutropha* (Annamalai and Sivakumar, 2016), bakery waste hydrolysate by *Halomonas boliviensis* (Pleissner, 2014). An innovative approach consists of PHB production from landfill methane by methanotrophs (Chidambarampadmavathy *et al.,* 2017).

2.7.3. Algae-Based Sources of Bioplastics

Microalgae are a promising alternative source for bioplastics production due to their fast growth and no competition with food (Rahman and Miller, 2017). Currently, several works have investigated the synthesis of bioplastics from microalgae (Beckstrom *et al.,* 2020; Simonic and Zemljic, 2020). It could be used directly as biomass to produce bioplastics or indirectly by the extraction of PHBs and starch within microalgae cells.

Other approaches include the production of microalgae-polymer blends through compression/ hot molding, melt mixing, solvent casting, injection molding, or twin-screw extrusion (Cinar *et al.,* 2020). The most investigated microalgae were *Chlorella* and *Spirulina*. *Chlorella* seems to have better bioplastic behavior, whereas *Spirulin*a showed better blend performance (Zeller *et al.,* 2013). Different species of *Chlorella* were used in biomass-polymer blends containing polymers and additives (Cinar *et al.,* 2020). Moreover, bioplastic may be produced from *Chlorella pyrenoidosa* (Das *et al.,* 2018) and *Chlorella* sorokiniana-derived starch granules (Gifuni *et al.,* 2017). Similar to *Chlorella, Spirulina* was investigated for bioplastic production (Cinar *et al.,* 2020). For example, a bioplastic-based film was produced from salt-rich *Spirulina sp*. residues with the addition of polyvinyl alcohol (Zhang *et al.,* 2020). Another bioplastic was prepared from *Spirulina platensis*, showing good biodegradability (Maheshwari and Ahilandeswari, 2011).

Other microalgae or cyanobacteria used to produce bioplastics were *Chlorogloea fritschii* (Monshupanee *et al.,* 2016), *Calothrix scytonemicola* (Johnsson and Steuer, 2018), *Neochloris oleoabundans* (Johnsson and Steuer, 2018), residual *Nannochloropsis* after oil extraction (Yan 2016), *Nannocloropsis gaditana* (Torres *et al.,* 2015; Fabra *et al.,* 2017), *Phaeodactylum tricornutum* (Hempel, 2011), and *Scenedesmus almeriensis* (Johnsson and Steuer, 2018). Ten green microalgae were screened for starch production and starch-based bioplastic development.

C. reinhardtii 11-32A resulted in the most promising starch producing strain with interesting plasticization properties with glycerol at 120 °C (Mathiot *et al.,* 2019). A microalgae consortium cultivated and harvested in a wastewater treatment plant was used as biomass to be mixed with glycerol as a plasticizer to obtain bioplastics (López-Rocha *et al.,* 2019). New composites were formed by combination of microalga biomass and petroleum. (Chia *et al.,* 2020; Cinar *et al.,* 2020). The PHB production is feasible in microalgae used as bioreactors by the introduction of bacterial pathways into microalga cells (Hempel, 2011). Besides microalgae, microalgae or seaweeds are aquatic plants rich in polysaccharides and potentially promising sources of bioplastics (Rajendran *et al.,* 2012; Thiruchelvi *et al.,* 2020). The whole red microalga *Kappaphycus alvarezii* was recently investigated to produce a bioplastic film with the addition of polyethylene glycol as a plasticizer for food packaging applications (Sudhakar *et al.,* 2020).

2.7.4. Wastewater Sources

Wastewaters are rich in organic matter and salts and are an important resource to be reused for different applications (Dasgupta *et al.,* 2016; Hoek *et al.,* 2016). Casein-rich dairy wastewater is a possible substrate for the manufacturing of bioplastics (Fricke *et al.,* 2019), but the physical properties of obtained brittle films were successfully improved by the addition of polysaccharides with proteins (Ryder *et al.,* 2020). Starch-based bioplastic was developed from potato processing industry wastewater (Arikan and Ozsoy,

2011). Activated sludge generated during the wastewater treatment is very abundant and could produce PHBs by thermal cracking (Liu *et al.,* 2019).

Mannina *et al.* (2019) recently implemented a new protocol to extract PHAs from mixed microbial cultures in a synthetic effluent simulating a fermented oil mill wastewater. PHAs were produced from municipal wastewater by a two-step process, consisting of anaerobic fermentation producing volatile fatty acids (VFA), and aerobic conversion of VFA to PHA by pure or mixed microorganisms (Pittmann *et al.,* 2013). Moreover, a two-step process was recently suggested to produce PHAs from cheese whey agro-industrial wastewater (Carlozzi *et al.,* 2020). Instead, a three-step process was proposed to accumulate PHAs in paper mill wastewater (Jiang *et al.,* 2012). Other wastewaters investigated for bioplastic production are wood mill effluents (Ben *et al.,* 2011) and municipal sewage sludge (Bluemink *et al.,* 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

Cassava tubers (*Manihot esculenta*) and unripe banana (*Musa paradisiaca)* with no injury or bruises on the skin were purchased directly from a farm at Isan Ekiti, Ekiti State, Nigeria, Latitude 7° 55′ 0″ North, and Longitude 5° 19′ 0″ East. Soil samples were collected from a farmland at Ekiti State Polytechnic (at a depth of 0 -15 cm). The soil samples were directly collected in a sterile polythene bag and transferred immediately to the laboratory for analyses.

3.2 Banana-based Bioplastic Film Production

Banana-based bioplastic films were produced according to the method described by Han *et al.* (2010) with slight modifications. Three kilograms (3 kg) bananas were peeled, washed and cut in to pieces. 1t was soaked in sodium metabisulphite (0.2 M) solution for 45 minutes. Two hundred grams of unripe banana peel was boiled in 400 ml of distilled water for 30 minutes, the water was decanted and the pieces of the banana peel was left to air dry on filter paper for 15 hrs. The resulting dried peel was placed in a beaker and purred using blender until uniform paste was formed. Calcium carbonate ($CaCO_3$ 6% w/w) was added to a beaker containing 25 g of banana peel paste. Thereafter, 2.9 ml of HCl (11.748% V/W) was added to the paste and stirred. 2.1 mL of plasticizer (Glycerol, 8.384 V/W) with purity of 99.9% was added and stirred for 14 minutes and 30 seconds. Thereafter, 2.9 mL of 0.5 N of NaOH was added to neutralize the mixture. Then the mixture was spread on plastic plate and placed in the oven at temperature of 52 °C for 24 hrs. After it was baked, the film was scrapped off from the surface and placed at room temperature.

3.3. Cassava-based bioplastic film Production

Cassava-based bioplastic films were produced according to the method described by Han *et al.* (2010) with slight modifications. The cassava peel was cleaned by separating brown peel part; the white cassava peel was cut into small pieces, and soaked in sodium metabisulphite for 45 minutes after which it was filtered. Water was added to the cassava peel in the ratio of 1:1 (cassava peel to water) and two hundred gram of the peel was grounded using blender. The resulting cassava peel slurry was filtered using muslin cloth, mixed and precipitated for 3 hours in order to obtain starch deposit. The Starch deposit obtained was dried in an oven at a temperature of 40^{o}C for 48 hours. Calcium carbonate ($CaCO_3$, 6% w/w) was added to a beaker containing 25 g of cassava peel paste. Thereafter, 2.9 ml of HCl (11.748% V/W) was added to the paste and stirred for hydrolysis. The mixture was oven dried at optimum temperature of 52^{o}C for 24 hrs.

3.4. Determination of Water Absorption Test of the Bioplastic films

The water uptake of films was measured according to the method described by Kuorwel *et al.* (2011). The film pieces were cut into smaller pieces of 4 cm × 4 cm size and they were directly immersed into a beaker containing a mixture of 50% water and 50 % glycerol (%v/v) at a temperature of 20 ± 1°C for 5 min. The samples were then removed from the beaker and wiped dried by placing them on filter paper for five minutes. The water absorption was calculated using the formula:

$$\text{Water absorption } = \frac{\boldsymbol{Wf - Wo}}{\boldsymbol{Wo}} * \mathbf{100}\%$$

Where, Wf= represents final weight of the film after 5 minutes

W_0= initial weight of the film

3.5. Determination of Tensile Strength of Bioplastic Films

The mechanical property of the bioplastic films was evaluated in terms of tensile strength (TS) and elongation at break (EB). Accordingly, The TS and EB values of the bioplastic films produced were investigated at the Central Research Laboratory, University of Ilorin, according to the method described by Tao *et al*. (2007). The laboratory condition was kept at 45% relative humidity (RH) and 24.5 ^{o}C temperature. The test parameters (thickness, width and length of the test samples) were fed to the tensile strength tester with the gauge length and the crosshead of 20 mm and 100 mm/min, respectively. The pre-prepared sample films were cut into strips with 2 cm length and 2 cm width in dumbbell shape. Finally, the test results were generated, that is, the mean of tensile strength at break in Mpa, elongation at break in percentage.

3.6. Fourier Transform Infrared Spectroscopy (FTIR) of Bioplastic Films

The FTIR measures of bioplastic films produced were investigated at University of Ilorin Chemistry laboratory, according to the method described by Ruhul *et al.* (2019). FTIR were carried out on a Perkin Elmer Spectrum One spectrometer coupled to an Auto Image light microscope. These analyses were performed on samples of the bioplastic film produced.

3.7. Soil Biodegradation experiment for of Bioplastic Films Produced

Soil burial method described by Nissa *et al.* (2019) was used to assess soil degradation of the bioplastics. Two soil types (non-sterile and sterilized soil) and 4 plastic types (banana-based, cassava-based, non-biodegradable plastic source: cling film, no plastic to serve as control) were used and each was replicated 8 times. Soil sterilization was carried out by autoclaving according to Adhikari *et al.* (2016) at 121°C at 2 atm for 60 minutes. The burials were conducted in polyethylene nylon filled with 1000 grams of soil at laboratory temperature. 64 vials (2 × 4 × 8) were used. Bioplastics film were cut into 2 cm × 2 cm and they were buried in the soil. Biodegradability of the samples were evaluated at varying interval of 10, 20,

30, 40 days. The non-sterilized soil used as the burial matrix for the bioplastic films in this study, is a loamy soil with humus. The biodegradability test of simple bioplastics was carried out over a period of 40 days. The films were removed from the soil, it was wiped clean with tissue paper and the weight loss was calculated by deducting the final weight after burial period from initial weight of the films before burial. The soil physico-chemical properties were determined after the bioplastics were removed at 40 days. Biodegradability was calculated using the formula:

$$\text{Weight loss (H) (\%)} = \frac{Initial\ mass - final\ mass}{Intial\ mass} * 100\%$$

3.7.Determination of Soil Physicochemical properties

3.7.1. Determination of Soil pH

The soil pH was determined using glass electrode pH meter make model 10PHM01. One gram of each air-dried soil sample was weighed into a clean beaker. Thereafter, 10 ml of distilled water was added (10:100) to each beaker to form a solution. The electrode of the pH which was calibrated with buffer 4, 7, 9 was introduced into the samples and the pH readings were taken three times the result was reported according to Wilke *et al.* (2005).

3.7.2. Exchangeable Cations determination (Ca, Mg, Na, K)

Exchangeable cations were determined in 1 N NH_4OAc (Ammonium Acetate) extract buffered at pH 7 (Bolan *et al.*, 2011), effective cations were obtained by calculation (Sen *et al.*, 2003).

Procedure: Five grams (5g) of air-dried soil samples passed through a 2 mm sieve were weighed into sample containers and 50 mL of 1 N NH_4OAc was added. The contents were shaken and left for an hour and then filtered (Sen *et al.*, 2003).

Calcium (Ca) and Magnesium (Mg) determination: Five milliliters (5 mL) of extracts each were prepared into (100 mL) conical flask. Five milliliters (5 mL) of each of the concentrated ammonium solution, 5 drops of 10% Hydroxylamine Hydrochloride and 2 drops of 5% K cyanate were added to the samples and finally titrated against 0.02 N EDTA (Ethylene Disodium Tetra Acetate) using Eryochrome Black T indicator which indicated the colour changes from red to blue (Ogunwale *et al*., 2005).

Calcium (Ca) Alone: Five milliliters (5mL) of extract were prepared into 100 mL conical flask and 5ml of 40% NaOH using Calcon indicator with colour change from red to colourless (Ogunwale *et al*., 2005).

Sodium (Na) and Potassium (K) determination: The ammonium acetate (NH_4OA) extracts were analyzed using flame photometer make model PFP-7. The standards of sodium and potassium were prepared with concentration in part per million (ppm), with Na in 10 ppm and K in 20 ppm according to Ogunwale *et al*. (2005).

Calculation:

$$\text{Ca} + \text{Mg} = \text{titre value} \times 0.02 \times \frac{50}{5} \times \frac{50}{5}$$

$$\text{Ca} = \text{titre value} \times 0.02 \times \frac{50}{5} \times \frac{50}{5}$$

Normality of EDTA = 0.02

Volume of extracts = 5 mL

Weight of sample = 5 g

$$\text{Na} = \frac{\text{standard ppm (10ppm)}}{\text{Standard reading (100)}} \times \text{sample reading (R)}$$

$$\mathbf{K} = \frac{20\text{ppm}}{100} \times \text{R}$$

3.7.3. Determination of Total Nitrogen in Soil Samples

Total nitrogen was determined using the Macro kjeldahl method described by Bremmer *et al.* (1965). One gram of air-dried soil samples was weighed into kjeldahl flasks and for each sample analysed was one kjeldahi tablet. Ten grams (10 g) of Potassium sulphate (K_2SO_4), 0.5g of copper sulphate ($CUSO_4$), and 25 mL concentrated sulphuric acid (H_2SO_4) were added and then heated until the clear mixtures changed to green colour which indicated digestion and were allowed to cool. The digests were made up to mark in 250 mL volumetric flasks with distilled water. Distillation was then done by preparing 5 mL of the digests each from the 250 mL into macro kjeldahl distillation apparatus and 5 ml of 40% sodium hydroxide (NaOH) was added (Bremmer *et al.,* 1965).

3.7.4. Determination of Organic Carbon in Soil Samples

Organic carbon was determined by Walkley Black Wet Oxidation according to method described by Schumacher (2002). One gram (1 g) of air-dried, finely ground soil samples passed through a 2 mm sieve was weighed into 250 ml conical flasks each. Five millilitres (5 mL) of 1N of potassium dichromate ($K_2Cr_2O_7$) solution was pipetted accurately into each conical flask and were gently swirled to disperse the soil. Ten millilitres (10 mL) of concentrated sulphuric acid (H_2SO_4) were rapidly added to the contents and were gently swirled immediately until the contents were properly mixed after which they were allowed to stand for 30 mins. Fifty millilitres (50 mL) distilled water was added to each suspension, 2-3 drops ferroin indicator was also added and were then titrated against 0.5 N of ferrous sulphate solution in the burette. At end point, colour changed from greenish to maroon (red) colour and blank titration was made in the same manner but without soil samples. Organic matter was also determined whose fate lies on the determination of organic carbon.

Calculation:

$$\% \text{ organic carbon} = \frac{(\text{blank} - \text{sample}) \times 0.5 \times 0.003 \times 1.33}{\text{Weight of sample}} \times 100$$

$$\% \text{ organiuc matter} = \ \% \text{ organic carbon } \times 1.729.$$

3.8.Microbiological Analysis of soil samples

The microbial counts and organisms present in the soil samples were determined before and after the experiment trial.

3.8.1. Microbial Soil Analysis

Ten-fold serial dilution of soil sample was done by adding 1 g of the soil sample to a test tube containing 9 ml of sterile distilled water; the dilution was done up to 10^{-10} aseptically. Dilution 10^{-2} and 10 were then used for fungal count while dilution 10^{-5} and 10^{-6} were used for heterotrophic bacteria count.

3.8.2. Inoculation and Incubation

Inoculation was done according to pour plate's method. Briefly, 0.1 mL of chosen dilution was added to sterile petri dishes. Thereafter, molten plate count agar was aseptically poured into each of the labeled petri-dishes for bacteria count and Potato Dextrose Agar (PDA) at 45˚C was poured into plates for fungi count. Bacterial agar plate was incubated at 37˚C for 24 hours while PDA plate was incubated at 28˚C for 72 hours. After incubation, the colony on each plate were counted and recorded. Also, distinct colony on each plate was sub-cultured to get pure cultures.

3.8.3. Identification of obtained fungal isolates from soil samples

Fungi isolates were characterized according to Jimoh *et al.* (2019) on the basis of colonial morphology and cellular morphology. The morphology was based on size, texture, spreading rate of the colonies on the PDA and colour of the isolates from the reverse side of the plates. Microscopic examination was done based on reproductive and vegetative structure. The inoculating needle was flamed until red hot and allowed to cool. It was then used to pick the colony and put on glass slide and then used to tease the

specimen apart. Thereafter, eosin methylene blue was added and a cover slip was placed on the specimen and observed under ×40 objectives under the microscope.

3.8.4. Identification of obtained bacteria isolates from soil samples

The bacteria isolates were characterized with respect to their colonial characteristics such as colour, shape and size of colonies, edge, elevation, surface texture, growth pattern, constituency and transparency (Fawole *et al.,* 1988). Representative colony of each isolate was sub-cultured for purity. The pure culture from plates were transferred to slants in McCartney bottles and stored as stock cultures.

3.8.5. Gram Staining

Gram staining was done according to the method described by Fawole and Oso (1988). A clean microscope slide was obtained, sterilized with alcohol-soaked cotton wool and allowed to dry. A loopful of sterile distilled water was dropped in the middle of a clean slide. A heat sterilized wire loop was used to pick an inoculum and a smear was made on the glass slide and allowed to air-dry. The reverse side of the slide was drawn quickly 3 times over flame to heat fix. The slide was flooded with Gram's crystal or methyl violet for 1-2 minutes, drained and washed with Gram/lugol's iodine. The iodine solution was left on the slide for 1 minute and washed under the tap. The slide was then washed with 95% ethanol until slide appeared free of stain (Between 10-15 seconds) and then rinsed under the tap. Slide was then flooded with dilute carbol fuchsin or safranin for 30 seconds. The stain was drained, slides washed and blotted dry. The slide was then observed under oil immersion objective lens; gram positive bacteria were stained purple while gram negative stained red.

3.8.6. Spore Staining

Spore staining was done according to the method described by Fawole and Oso (1988).. Smear of each isolate was made and heat fixed as for gram staining. The slide was flooded with malachite green solution

and steamed for about 10 minutes and frequently replacing the dye to prevent drying up. The slide was allowed to stand for 15 seconds. The slide was again washed with distilled water and safranin solution was added. The slide was allowed to stand for 10 seconds after which the slide was again washed with water. The wet stained slide was blotted dry with filter paper and examined under the oil immersion objective. Spore formers had their vegetative cells pink while spores appeared green Fawole and Oso (1988).

3.8.7. Catalase Test

Catalase test was carried out according to the method describe by Olutiola *et al.* (1991). A loopful of culture was emulsified on a clean microscope slide and a drop of 3.0% hydrogen peroxide was added. Effervescence bubbles indicated catalase positive organisms (Olutiola *et al.,* 1991).

$$2H_2O_2 \longrightarrow 2H_2O(i) + O_2\,(g)$$

3.8.8. Slides Coagulase test

This test is based on the ability of the organism to produce enzyme coagulase that will covert fibrinogen in plasma to fibrin clot. A colony of the test organism was emulsified on a drop up normal saline on a clean slide to produce a dense uniform suspension. A loopful fresh plasma was added to the suspension and mixed by continuous circular motion for 10 seconds. A positive reaction is indicated by easily visible clumps which usually appear immediately or within 5 seconds (Olutila *et al.,* 1991).

3.8.9. Motility Test:

The test was carried out using the hanging drop method with 24 hours old culture of the isolates. A thick emulsion of the isolate was prepared on clean dry cover slip and thin film of Vaseline was applied round the slide. The ring of Vaseline was gently pressed into the cover slip, ensuring that the drop of culture was in the centre of the cover slip without making contact with the slide. The slide and coverslip were pressed

down gently to make an airtight seal to prevent pseudo-motility and quickly inverted so that the coverslip was uppermost. The preparation was then examined for motility under x 40 objective lens of the microscope with reduced high intensity. The motile cells were seen moving rapidly in the field while non-motile cells were immobile (Albert *et al.,* 1991).

3.8.10. Gelatin Liquefaction Test:

Nutrient gelatin broth was used which was prepared according to the manufacturer's (Oxoid) prescription. It was aseptically poured into sterile test-tube, corked and sterilized at 121˚C for 15 minutes. After auto-claving, the gelatin was kept at low temperature of about 9˚C. Stabs of the isolates were made using inocula from the agar slope cultures. The inoculated test tubes were then incubated at 37˚C for 24-48 hours. Liquefaction was tested by removing the nutrient gelatin culture and holding them at 4˚C for 30 minutes or more. Non-resolidifiation of nutrient gelatin indicated liquefaction positive result.

3.8.11. Capsule Staining:

Thin smears of each isolate were made. The slide was initiated with crystal violet stain for 5 minutes, and then steamed over flame for 40 seconds. The slide was wash-off of the stain with aqueous cupric sulphate solution and blotted without rinsing and finally examined under oil-immersion lens. The capsules-stained blue violet while cells appeared dark blue Fawole and Oso (1988).

3.8.11. Methly Red Test

Peptone sugar broth were inoculated lightly with the isolates and incubated at 37 ˚C for 48 hours. After incubation, 5 drops of methyl red reagent were added to five ml of the culture and the reaction was read immediately. Positive results gave bright red colour while negative gave yellow (Olutiola *et al.,* 1991).

3.8.12. Voges-Proskaeur Test

The peptone dextrose broth was inoculated with the isolates and incubated at 37˚C for 48 hours. Thereafter, 0.5 ml of 16% potassium hydroxide and 0.5ml of 6% alpha-naphtol in ethanol was added to1ml of the inoculated broth after the period of incubation. Positive results were indicated by a bright pink colour and negative result by a reddish brown colour Fawole and Oso (1988).

3.8.13. Indole Test

The test organism was inoculated into a peptone broth containing tryptophan and incubated at 37˚C for 24 hours 0.5 ml of Kovac's reagent was added to the test organisms inside a bijou bottle. A deep red ring developed in the presence of indole released by the organism indicating a positive result Fawole and Oso (1988).

3.9.Statistical Analysis

Data from this study were subjected to analysis of variance (ANOVA), to assess the effect of soil type, plastic type and time on biodegradation. Differences between treatments means was separated using Duncan's Multiple Range Test at $P \leq 0.05$. Pearson correlation was calculated among all parameters to test for the significance of association between the percentage degradation and microbial count of the burial soil using SPSS 16.0.

CHAPTER FOUR
RESULTS

4.1. Appearance of Bioplastics

The simple and composite bioplastics obtained are presented in Plate 4.1. The simple bioplastics come in the form of flexible and continuous films. The banana-based (Plate 4.1a) bioplastics film is translucent and melon colour while the cassava-based simple bioplastic film (Plate 4.1b) is transparent and white in colour.

4.2. Physical and Mechanical Properties of Bioplastics Produced from Banana and Cassava Peel

4.2.1. Water absorption capacity

The water absorption capacity of the bioplastics is presented in Figure 4.1. Cassava-based bioplastic had the higher water absorption capacity 48.79%, banana-based bioplastic had a capacity of 41.41% while the cling films (control) had 7.92%.

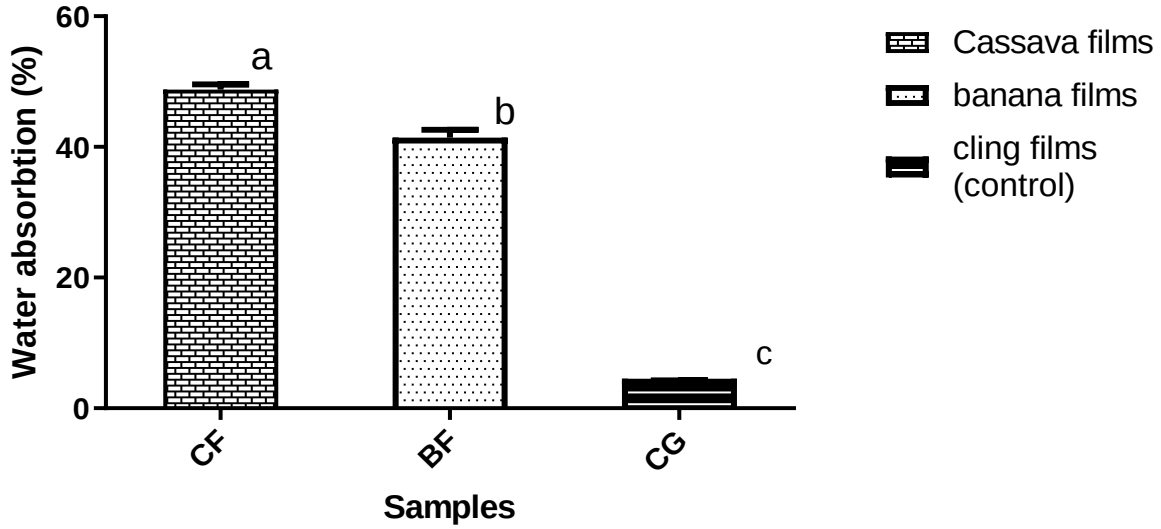
4.2.2. Tensile strength and elongation at break

Tensile strength and elongation at break of the bioplastics produced from cassava and banana peels are presented in Figure 4.2 and 4.3 respectively. The tensile strength of bioplastic from cassava, banana peels and cling films(control) is 1.46 Mpa, 4.67 Mpa and 7.00 Mpa respectively while the elongation at break of bioplastic from cassava, banana peels and cling films(control) is 31.72%, 27.86 % and 57.33% respectively.

(a) 

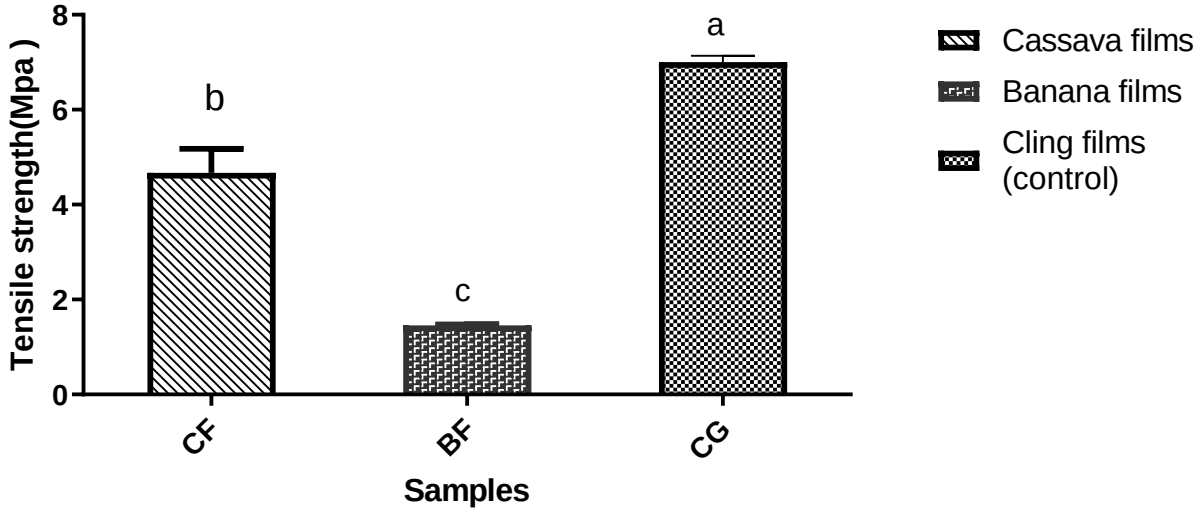
(b) 

plate 4.1: (a) Banana bioplastics; Image size (height; 0.87cm, width: 9.95 cm) (b) Cassava bioplastics; Image size (height: 6.93 cm, width:10.24 cm)



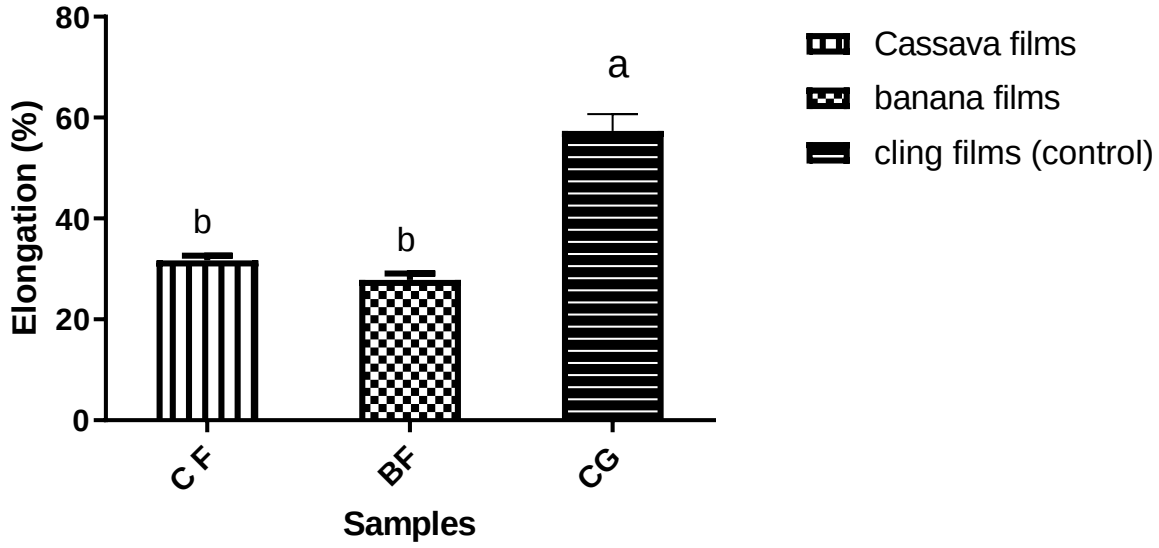
CF: Cassava films, BF: Banana films, CG: Cling films.

Figure 4.1: Water Absorption Capacity of the Film



CF: Cassava films, BF: Banana films, CG: Cling films.

Figure 4.2: Tensile Strength of the Films



CF: Cassava films, BF: Banana films, CG: Cling films.

Figure 4.3: Elongation at Break of the Films

4.2.3. FTIR Analysis of Banana and Cassava Biofilm

The FTIR analysis of banana and cassava biofilms is presented in Table 4.1, Figures 4.4 and 4.5. The absorption of banana biofilm is at a wavelength of 3350.92 cm^{-1} while the wavelength for cassava biofilm is 3309.76 cm^{-1}. This indicates the presence of O-H groups of free hydroxyl groups and bonded O-H in polymeric compounds such as alcohol and phenols. A wavelength range of 3000 – 2500 $cm^{-1}$and 2000 – 1500 cm^{-1}indicates the presence of aliphatic saturated hydrocarbon chain (C-H) and carboxyl group respectively in both films. Carbonyl group was only present in banana biofilm at wavelength range of 2540 – 1000cm^{-1}, while functional group of alkenes was only present in cassava film at wavelength range of 1950 – 1600 cm^{-1}. Both films had the functional group ester, ether, carboxylic acid and anhydrides (C-O) which was in the wavelength range of 1450-1000 cm^{-1} and the functional group Ca-O which was in the wavelength range of 950 – 700 cm^{-1}.

4.3. Physicochemical Properties of the Burying Soil Prior to Burial

The physicochemical properties of the sterilized and un-sterilized soil prior to burial of the films are presented in Table 4.2. The pH sterilized and non-sterilized soil was 7.27±0.025 and 7.57±0.006, electrical conductivity of the soil samples was 16.0±0.10 and 15.40±0.30 µs/cm, total nitrogen, available phosphorus of the sterilized and non-sterilized soil was, 0.12±0.01% and 0.41±0.18%, and 30.17±0.06 mg/kg and 56.20±0.10 mg/kg respectively. TOS, TDS, Na and K of sterilized and non-sterilized soil was 1.21±0.10 and 1.86±0.25%, 78.80±0.50 and 74.00±0.00 mg/L, 1.12±0.36 and 1.12±0.06 mol/kg, and 0.93±0.10 and 1.17±0.27 mol/kg respectively while calcium and magnesium level of the sterilized and non-sterilize soil was 2.2±0.2 and 2.3±0.1 mol/kg, and 1.80±0.2 and 1.90±0.10 mol/kg.

Table 4.1: FTIR Analysis of Banana and Cassava Biofilm

Cassava biofilm (cm^{-1})	**Banana biofilm (cm^{-1})**	**Wavelength range (cm^{-1})**	**Functional Group**
3309.76	3350.92	3450 – 3200	Hydroxyl groups in alcohol and phenols (O-H)
2920.32 2844.33 2524.54	2929.82 2888.65 2138.26	3000 – 2500	Aliphatic saturated hydrocarbon chain (C-H)
1729.82 1625.33 1539.85	1627.99	2000 – 1500	Carboxyl group
_	1473.35 1365.70 1156.73	2540 – 1000	Carbonyl group (C=O)
1729.82 1625.33 1539.85	_	1950 – 1600	Alkene (C=C)
1454.34 1378.36 1321.37 1232.72 1159.89	1365.70 1245.38 1156.73	1450 – 1000	Ester, ether, carboxylic acid, anhydrides (C-O)
707.12 764.12 434.83	916.09 868.60 707.12	950 – 700	Ca-O

Table 4.2: Physicochemical Properties of Soil before Burying the Bioplastics

Parameters	**Sterilized soil**	**Non-sterilize soil**
PH	7.27± 0.025	7.57±0.006
Conductivity (µs/cm)	16.0±0.10	15.40±0.30
Total Nitrogen (%)	0.12±0.01	0.18±0.18
Available Phosphorous (mg/kg)	30.17±0.06	36.20±0.10
Total organic carbon (%)	1.21±0.10	1.26±0.25
Total dissolved solid (mg/L)	78.80±0.50	74.00±0.00
Sodium (mol/kg)	1.12±0.36	1.12±0.06
Potassium (mol/kg)	0.93±0.10	1.17±0.27
Calcium (mol/kg)	2.2±0.2	2.3±0.1
Magnesium (mol/kg)	1.80±0.2	1.90±0.10

Results are means ± standard deviation (SD) of independent triplicate determinations.

4.4. Biodegradation of Bioplastic films Produced from Banana and Cassava Peels

Figure 4.6 shows the rate of biodegradation of the bioplastics. The biodegradation rates were closely observed and compared with the synthetic polythene. On day 10, the size and colour of the bioplastic films changed and started to degrade slightly. On day 40, the degradation of films in sterilized soil were in order of cassava film (48.77%) > banana film (31.65%) > cling film (0.00%) while degradation in non-sterilize soil were in order of; cassava film (71.75%) > banana film (55.22%) > cling film (0.00%). The same order was obtained for degradation of films in both soils on days 10, 20 and 30. The highest rate of degradation of 71.75% was obtained when cassava film was buried in non-sterilize soil.

Overall burial in the sterile soil presents a very weak degradation while burial in non-sterilize soil present a very strong degradation. It was also observed that the period of degradation had an effect on the degradation rate; that is, the longer the period of burial the higher the degradation (in order of 40 days> 30 days> 20 days> 10 days).

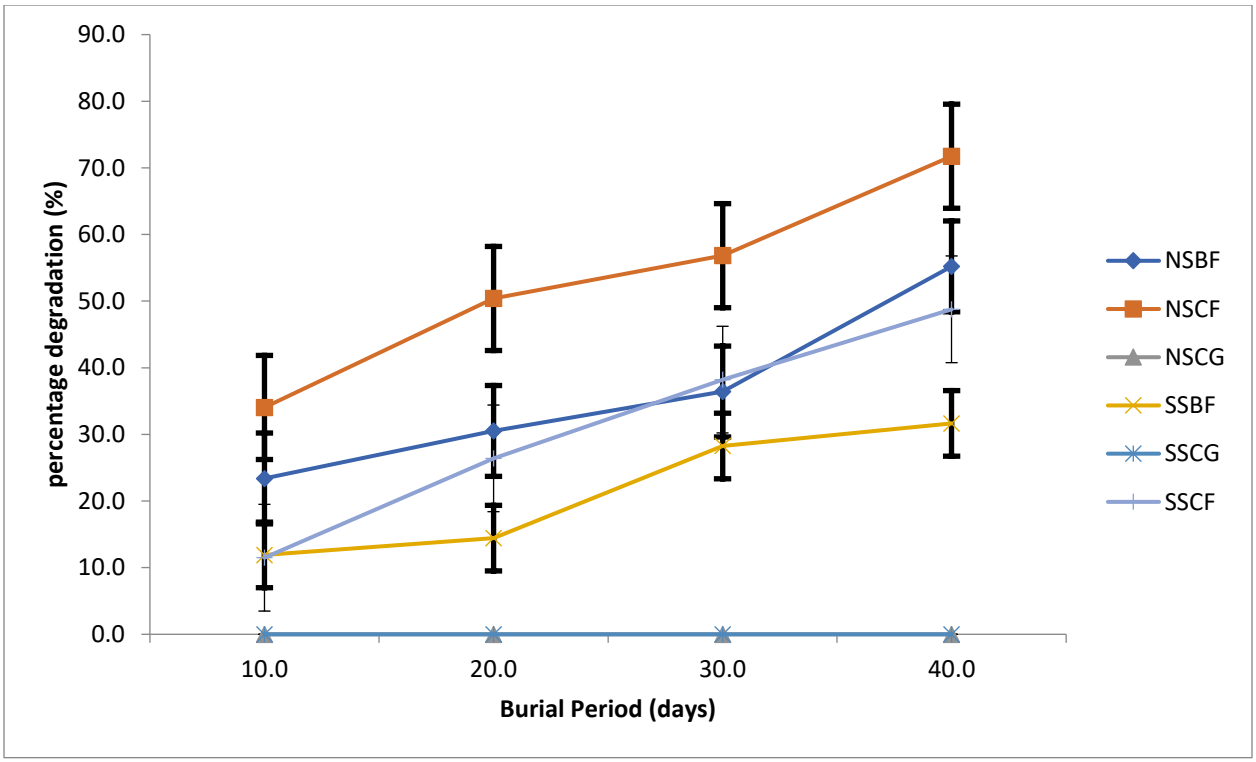


Figure 4.6: Biodegradation of Bioplastics

SSBF: sterilized soil with banana film, SSCF: sterilized soil with cassava film, SSCG: sterilized soil with cling film, NSBF: non-sterilized soil with banana film, NSCF: non-sterilized soil with cassava film, NSCG: non-sterilized soil with cling film.

Table 4.3: ANOVA table showing the effect of soil type, biofilms, and burial time on biodegradation

Source of variation	DF	SS	F value	P Value Pr > F
ST	1	8.06	18837.00	<.0001
FT	2	130.52	152557.00	<.0001
Time	4	46.07	26926.40	<.0001
ST*FT	2	4.73	5529.77	<.0001
ST*Time	4	2.37	1382.65	<.0001
FT*Time	8	26.80	7832.50	<.0001
ST*FT*Time	8	1.56	456.02	<.0001

Legend = ST: Soil type (sterilized and unsterilized soil), FT: Film type (cassava film, banana film, cling film), Time: Days of burial (0,10,20,30,40), DF: Degree of freedom, SS: Sum of Squares

4.5. Effects of Soil type, biofilms, and burial time on biodegradation

The effects of the different variables (soil type, biofilms and burial time), their interactions and significant differences are shown in Table 4.3. Significant interaction ($p < 0.005$) exists among the three variables.

4.6. Microbial Analysis of the Burial Soil

The fungal and bacterial load of the soil samples before and after burying the biofilms are presented in Figures 4.7 and 4.8, respectively. Among all the soil, non-sterilize soil with cassava film (NSCF) had the highest fungal load (162×10^3 CFU/g) which was significantly ($p<0.05$) different from all the other experimented soils. NSCF also had the highest bacterial load (2.50×10^6 CFU/g) which was significantly ($p<0.05$) different from other soil except NSBF in contrast, there are no fungi or bacteria in the sterilized soil without films (SSF).

The biochemical identification and distribution of bacteria and fungi species in the soil samples is presented in Table 4.4 and Table 4.6 respectively while the morphological identification of bacteria species and fungi identified in each of the soil is presented in Table 4.5 and Table 4.7 respectively.

4.6.1. Physical Properties of the Microorganism (Bacteria) Isolates

Table 4.4 shows the results of the physical properties of the bacterial isolates. 4 Reaction test (*Pseudomonas aeruginosa, Enterobacter* spp, *Xanthomonas* spp, *Klebsielela* spp) out of the 9 isolates were gram negative while the other 5 (*Bacillus* spp, *Clostridium* spp, *Staphylococcus aureus, Micrococcus* spp, *Rhizobium* spp) were gram reaction positive; Indole test; 5 (*Clostridium* spp, *Enterobacter* spp, *Micrococcus* spp, *Xanthomonas* spp, *Klebsielela* spp) out of the 9 isolates tested negative to indole test while 4 isolates (*Pseudomonas aeruginosa, Bacillus* spp, *Staphylococcus aureus, Xanthomonas* spp) tested negative to indole; Motility; 5 (*Staphylococcus aureus, Clostridium* spp, *Micrococcus* spp, *Rhizobium* spp, *Klebsielela* spp) out of the 9 isolates were non-motile while 4 isolates (*Pseudomonas aeruginosa, Bacillus* spp, *Enterobacter* spp, *Xanthomonas* spp) were motile; Methyl red; 5 (*Pseudomonas*

aeruginosa, Clostridium spp, *Staphylococcus aureus, Rhizobium* spp, *Klebsielela spp*) tested positive to methyl red while 4 isolates (*Bacillus spp, Enterobacter spp, Micrococcus spp, Xanthomonas* spp) tested negative to methyl red; Voges Proskauer test; 5 isolates (*Pseudomonas aeruginosa, Bacillus* spp, *Clostridium* spp, *Staphylococcus aureus, Rhizobium* spp) tested negative to Voges Proskauer while 4 isolates (*Enterobacter* spp, *Micrococcus spp,Xanthomonas spp,Klebsielela* spp) tested negative to Voges Proskauer; Oxidase test; 3 isolates (*Bacillus* spp, *Clostridium* spp, *Xanthomonas* spp) tested positive to oxidase test while 6 isolates (*Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter* spp, *Micrococcus* spp, *Rhizobium* spp, *Klebsielela* spp) tested negative to oxidase test; Glucose and Maltose test; all the 9 isolates tested positive to Glucose and Maltose test; Sucrose test; 1 (*Rhizobium* spp) out of the 9 isolates tested negative to sucrose test while other isolates tested positive to sucrose test.

4.6.2. Physical Properties of the Microorganism (Fungi) Isolates

Different and similar properties of the isolates (fungi) were observed during the microbial degradation analysis of each treatment as presented in Table 4.6. Most of the fungi were pinkish in color; 3 out of 5 isolates obtained from the samples were creamy green and the remaining 2 were purple and dark brown; Shape; All of the isolates were Globuse in shape; Elevation properties, 3 out of 5 isolates were raised in elevation, while *Aspergillus terreus* and *Penicillium griseofulvun* were raised fluffy; Margin; 3 had curled margin while *Trichoderma spirale* and *Penicillium griseofulvun* had entire margin; Surface; 3 out of the 5 isolates had smooth surface, smooth surface while other either had irregular and rough surface.

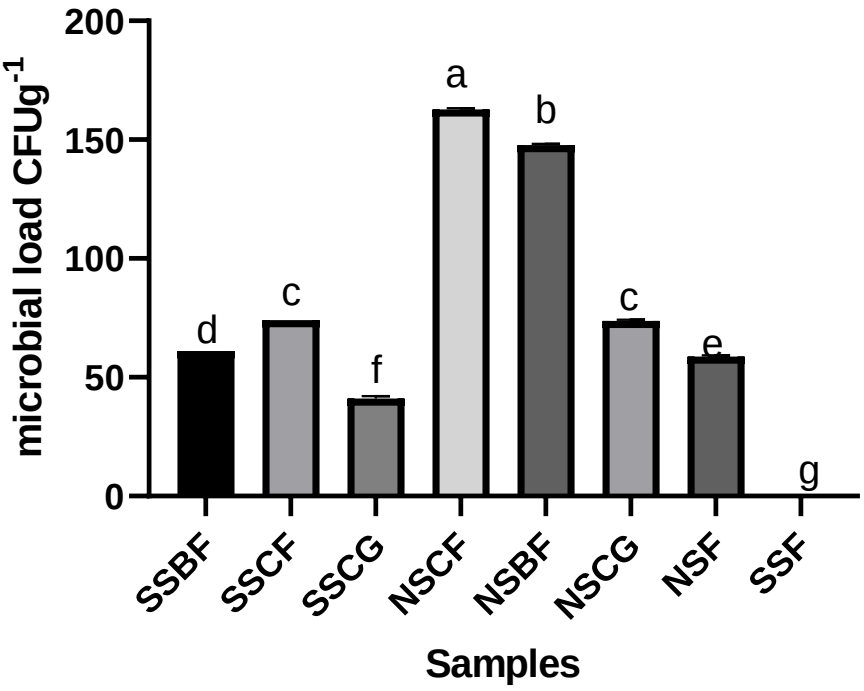


Figure 4.7: Fungi load of the Soil

Results are means ± standard deviation (SD) of independent triplicate determinations. SSBF: sterilized soil with banana film, SSCF: sterilized soil with cassava film, SSCG: sterilized soil with cling film, NSBF: non-sterilized soil with banana film, NSCF: non-sterilized soil with cassava film, NSCG: non-sterilized soil with cling film, NSF: non-sterilized soil without film, SSF: sterilized soil without film

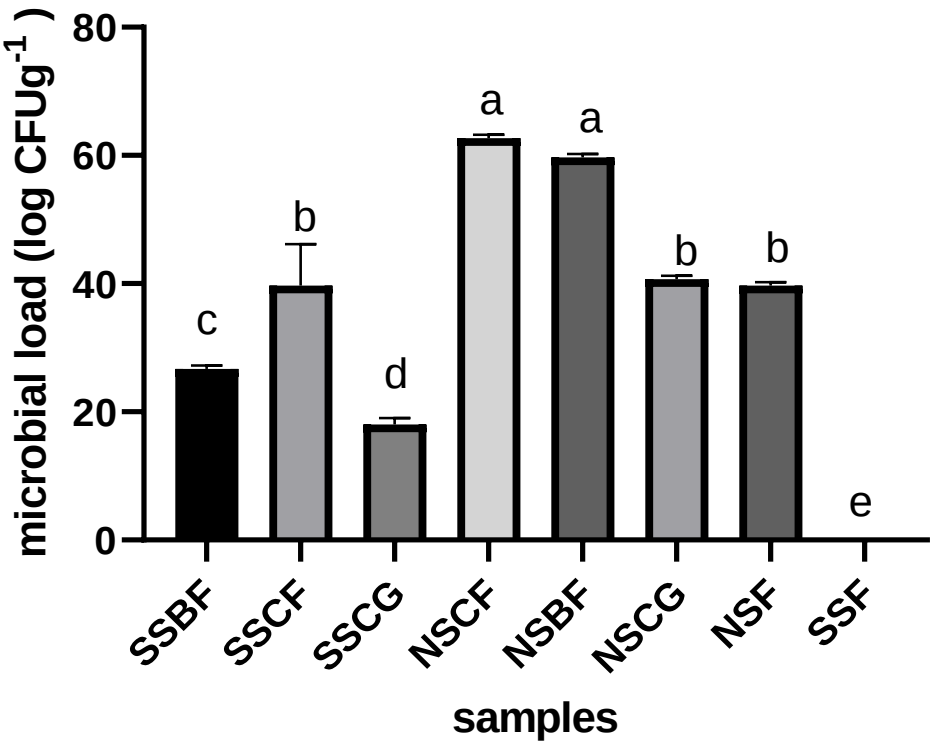


Figure 4.8: Bacteria load of the Soil

Results are means ± standard deviation (SD) of independent triplicate determinations.

SSBF: sterilized soil with banana film, SSCF: sterilized soil with cassava film, SSCG: sterilized soil with cling film, NSBF: non-sterilized soil with banana film, NSCF: non-sterilized soil with cassava film, NSCG: non-sterilized soil with cling film, NSF: non-sterilized soil film, SSF: sterilized soil without film.

Table 4.4: Biochemical identification of bacterial isolates

Isolate	Grams Reaction	Motility	Catalase	Indole Test	V. P Test	M.R. Test	Oxidase	C.U	N.R	Glucose	Sucrose	Maltose	Tentative identification
1	-	+	+	+	-	+	-	-	+	+	+	+	*Pseudomonas aeruginosa*
2	+	+	+	+	-	-	+	+	-	+	+	+	*Bacillus* spp.
3	+	-	+	-	-	+	+	-	+	+	+	+	*Clostridium* spp.
4	+	-	+	+	-	+	-	-	-	+	+	+	*Staphylococcus auerus*
5	-	+	+	-	+	-	-	+	-	+	+	+	*Enterobacter* spp.
6	+	-	+	-	+	-	-	+	-	+	+	+	*Micrococcus* spp.
7	+	-	+	+	-	+	-	+	+	+	-	+	*Rhizobium* spp.
8	-	+	+	-	+	-	+	-	-	+	+	+	*Xanthomonas* spp.
9	-	-	+	-	+	+	-	+	-	+	+	+	*Klebsielela* spp.

V. P - Voges Proskauer, M. R. – Methyl red, C.U – Citrate Utilization, N.R- Nitrate Reduction, +VE = Present, -VE = Absent.

Table 4.5: Distribution of bacterial isolate in soil samples

Soil Sample	Bacterial distribution in the soil sample
SSBF	*Micrococcus* spp., *Bacillus* spp.,
SSCF	*Staphylococcus aureus, Bacillus* spp., *Clostridium* spp. *Enterobacter* spp.
SSCG	*Bacillus* spp., *Pseudomonas aeruginosa*,
NSCF	*Bacillus* spp., *Pseudomonas aeruginosa, Enterobacter* spp. *Rhizobium* spp. *Klebsielela* spp.
NSBF	*Micrococcus* spp. *Rhizobium* spp. *Bacillus* spp. *Enterobacter* spp. *Xanthomonas* spp.
NSCG	*Bacillus* spp. *Pseudomonas aeruginosa. Staphylococcus aureus*
NSF	*Pseudomonas aeruginosa. Bacillus* spp. *Staphylococcus aureus*
SSF	Nill

SSBF: sterilized soil with banana film, SSCF: sterilized soil with cassava film, SSCG: sterilized soil with cling film, NSBF: non-sterilized soil with banana film, NSCF: non-sterilized soil with cassava film, NSCG: non-sterilized soil with cling film, NSF: non-sterilized soil film, SSF: sterilized soil without film

Table 4.6: Morphological Identification of Fungi Isolate from Soil Samples

Sample	**Colour**	**Reverse side colour**	**Shape**	**Elevation**	**Margin**	**Surface**	**Conidia surface**	**Isolates**
1	Pinkish brown	Creamy green	Globuse	Raised, fluffy	Curled	Smooth walled	Smooth	*Aspergillus terreus*
2	Dark Brown	Cream	Globuse	Raised	Curled	Smooth walled	Irregular and rough	*Aspergillus niger*
3	Greyish brown	Dark brown	Globose	Raised	Entire	Smooth	Rough	*Trichoderma spirale*
4	Pink whitish	Purple	Globose	Raised	Curled	Oval	Smooth	*Fusarium oxysporum*
5	Dark green	Cream green	Globose	Raised, Fluffy	Entire	Spherical	Smooth	*Penicillium griseofulvum*

Table 4.7: Distribution of fungi isolate in soil samples

Soil Sample	Fungi distribution in the soil sample
SSBF	*Aspergillus niger. Fusarium oxysporum*
SSCF	*Aspergillus niger. Aspergillus terreus*
SSCG	*Aspergillus niger. Aspergillus terreus*
NSCF	*Aspergillus niger. Fusarium oxysporum. Trichoderma spirale Penicillium griseofulvum*
NSBF	*Aspergillus terreus. Penicillium griseofulvum*
NSCG	*Fusarium oxysporum. Penicillium griseofulvum*
NSF	*Aspergillus niger. Aspergillus terreus. Penicillium griseofulvum.*
SSF	Nill

SSBF: sterilized soil with banana film, SSCF: sterilized soil with cassava film, SSCG: sterilized soil with cling film, NSBF: non-sterilized soil with banana film, NSCF: non-sterilized soil with cassava film, NSCG: non-sterilized soil with cling film, NSF: non-sterilized soil without film, SSF; sterile soil without film

4.7. Physicochemical Properties of the Burying Soil after Burial of the Films

The physicochemical properties of both the sterilized and non-sterilized soil after the burial of the films is presented in Table 4.8. The conductivity, total nitrogen and available phosphorus ranged from 15.00±0.10 to 27.37±0.15 µs/cm, 0.18±0.01 to 0.27±0.01% and 32.00±0.02 to 48.77±0.21 mg/kg respectively across all the burial soil. TOC, TDS, Na and K ranged from 1.21±0.10 to 1.86±0.25%, 11.57±0.58 to78.80±0.50 mg/L, 1.07±0.04 to1.16± 0.06 mol/kg and 0.70± 0.80 to 1.17±0.27 mol/kg respectively. These parameters were significantly different ($p<0.05$) across all the soil samples.

4.8. Relationship between the biodegradability of the biofilms and the microbial count of the burial soil

The relationship between the biodegradability of the biofilms and the microbial count of the burial soil is presented in Table 4.9. As obtained in this study, there was significant positive ($p < 0.05$) correlation among days of biodegradation and no significant ($p < 0.05$) correlation was observed between the microbial count and the biodegradability of the biofilms, but significant ($p < 0.05$) correlation occurred between the fungi microbial count and bacteria.

Table 4.8: Physicochemical Properties of the Soil used Burying the Films

Samples	pH	Conductivity (µs/cm)	Total Nitrogen (%)	Available Phosphorous (mg/kg)	Total organic carbon (%)	Total dissolved solid (mg/L)	Sodium (mol/kg)	Potassium (mol/kg)	Calcium (mol/kg)	Magnesium (mol/kg)
SSBF	8.42 ± 0.25^{b}	27.37 ± 0.15^{a}	0.25 ± 0.01^{b}	$44..40\pm0.10^{b}$	1.61 ± 0.10^{b}	13.57 ± 0.58^{c}	$1.16\pm0.06^{a,b}$	0.76 ± 0.10^{b}	2.5 ± 0.10^{d}	2.4 ± 0.20^{a}
SSCF	8.31 ± 0.10^{c}	$23.60\pm0.20^{b,c}$	0.21 ± 0.01^{d}	41.87 ± 0.25^{d}	1.51 ± 0.10^{d}	11.57 ± 0.58^{e}	1.12 ± 0.05^{b}	0.84 ± 0.00^{b}	2.3 ± 0.10^{e}	1.80 ± 0.00^{b}
SSCG	8.64 ± 0.45^{a}	15.80 ± 0.10^{c}	0.18 ± 0.01^{e}	34.50 ± 0.40^{d}	1.39 ± 0.30^{e}	78.47 ± 0.45^{a}	1.07 ± 0.04^{b}	$0.99\pm 0.08^{a,b}$	2.2 ± 0.20^{f}	1.6 ± 0.00^{b}
NSCF	8.27 ± 0.30^{d}	23.46 ± 0.50^{c}	0.27 ± 0.01^{a}	48.77 ± 0.21^{a}	1.65 ± 0.01^{a}	12.67 ± 0.25^{d}	1.25 ± 0.09^{a}	0.70 ± 0.80^{b}	3.4 ± 0.20^{a}	1.9 ± 0.10^{b}
NSBF	7.83 ± 0.10^{e}	23.80 ± 0.10^{b}	0.23 ± 0.01^{c}	42.77 ± 0.21^{c}	1.57 ± 0.15^{c}	12.09 ± 0.58^{d}	1.09 ± 0.90^{b}	$0.91\pm 0.31^{a,b}$	3.0 ± 0.00^{b}	2.3 ± 0.10^{a}
NSCG	7.84 ± 0.005^{e}	15.00 ± 0.10^{d}	0.16 ± 0.01^{f}	32.00 ± 0.20^{e}	1.32 ± 0.20^{f}	74.50 ± 0.50^{b}	1.14 ± 0.40^{b}	0.75 ± 0.14^{b}	2.6 ± 0.20^{c}	1.70 ± 0.30^{b}
SSF	8.70 ± 0.45^{a}	16.0 ± 0.10^{c}	0.17 ± 0.01^{e}	33.17 ± 0.50^{d}	1.53 ± 0.10^{b}	78.80 ± 0.47^{a}	1.12 ± 0.03^{b}	$0.95\pm0.08^{a,b}$	2.2 ± 0.20^{f}	1.80 ± 0.00^{b}
NSF	7.87 ± 0.005^{e}	15.40 ± 0.20^{d}	0.18 ± 0.01^{f}	34.20 ± 0.20^{e}	1.30 ± 0.21^{f}	74.00 ± 0.48^{b}	$1.27\pm0.50^{a,b}$	0.73 ± 0.14^{b}	2.6 ± 0.20^{c}	1.72 ± 0.30^{b}

Results are means ± standard deviation (SD) of independent triplicate determinations. Along the same column, values having different superscript letters vary significantly ($p < 0.05$). SSBF: sterilized soil with banana film, SSCF: sterilized soil with cassava film SSCG: sterilized soil with cling film, NSCF: non-sterilized soil with banana film, NSCF: non-sterilized soil with cassava film (NSCF), NSCG: non-sterilized soil with cling film, SSF: sterilized soil without film, NSF: non-sterilized soil without film.

Table 4.9: Correlation between the percentage biodegradation of bioplastics and the microbial count of the burial soil.

Variables	**10 days (%)**	**20 days (%)**	**30 days (%)**	**40 days (%)**	**Fungi ($CFUg^{-1}$)**
20 days (%)	**0.97***				
30 days (%)	**0.93***	**0.97***			
40 days (%)	**0.95***	**0.98***	**0.99***		
Fungi ($CFUg^{-1}$)	0.01	0.10	0.31	0.23	
Bacteria ($CFUg^{-1}$)	0.06	0.16	0.34	0.25	**0.95***

*Significant at $p < 0.05$

CHAPTER FIVE

DISCUSSION

The water absorption capacity of the cassava and banana biofilms is associated with hydrophilic properties of their starch (Junjie and Hanna, 2004; Bonilla *et al*., 2015). Banana bioplastic films shows higher liquid impermeability and better quality than cassava and cling films. Hence it will have a long-term performance in final products.

The Tensile strength and elongation at break are essential mechanical characters of a plastic. It shows the ability of film's integrity under stress and it is important to determine their application (Aznury *et al.*, 2019). It is also referred to as the maximum pull that can be achieved until the film can survive before breaking up. The tensile strength can be observed to decrease when starch percentage increases due to the filler-filler interaction becoming more prominent (Azahari *et al*., 2011). This might be the reason why the results observed in this study showed that banana biofilm has lower tensile strength (1.46 Mpa) than cassava biofilm (4.67 Mpa) due to the high percentage of starch composition. The result obtained in this study was lower than the range reported for the cling films (7.00 Mpa) used as control and low-density polyethylene (LDPE) (8–58 MPa) which is used extensively for packaging and bags (Neves *et al*., 2020). The tensile strength of banana observed in this study was significantly higher than that reported by Santana *et al*. (2012) for starch-based bioplastic from jackfruit (3.12 MPa) and the one reported for potato starch (0.6 MPa) and yam starch (1.9 MPa) by Ismail *et al*. (2016). Smaller tensile strength observed for banana biofilm indicates that the material can easily deform in plastic behavior (Jaramillo *et al*., 2016).

Elongation at break is the maximum length of the bioplastic before rupture. It is the ability of a material to resist change in shape when subjected to force (Fan and Fu, 2017). Higher elongation value means that the bioplastic sample is more deformable. The reduction of the elongation at break percentage of material is related to the increment percentage of the fiber content (Rozman *et al*., 2000). In this study, cassava has

higher percentage elongation than banana biofilm and this can be attributed to higher fiber contents in banana which implies that casava based bioplastic film is more deformable. The two biofilms produced also had lower elongation than the cling films (control). Samples with larger dimensions are known to have higher elongation values than that with a smaller dimension, indicating that the sample with larger size can hold its shape better than the smaller size under the same elongation (Warsiki and Bawardi, 2018). The values of elongation were significantly higher than those reported by Aznury (2019), who reported on the elongation of starch bioplastic (15.15%). Overall, the cassava and banana biofilms can hold their shape better than the control films.

Presence of hydroxyl group (-OH) in cassava and banana biofilm indicates the formation of a hydrogen bond as there was an interaction between hydrogen atoms with an electronegative atom, either oxygen, fluorine or nitrogen (Chen *et al.,* 2012). Hydrogen atoms have partial positive charge while oxygen atoms have partial negative charge in which they are attracted to each other and thus form a hydrogen bond. As hydroxyl groups have hydrophilic properties which lead to the formation of hydrogen bond, it enhances solubility of the sample in water. Therefore, the production of bioplastic from cassava peel can absorb some water as it contains hydroxyl group that increases solubility in water (Mason *et al.,* 2005). In addition, hydrocarbon chain (C-H) and alkene (C=C) were found to be present in cassava and banana. Hydrocarbon chain is a basic unit in the production of plastic in which the carbon atom in hydrocarbon chain acts as backbone for every organic material in polymer while alkene group is reactive and commonly used in production of polymer or plastics such as polyethene, which is produced from polymerizing the simplest alkene, ethane (C_2H_4). Other than that, calcium oxide (Ca-O) acts as desiccant in polymer in order to prevent dryness and is known as hydroscopic substance.

To assess the rate of deterioration of bioplastics in the environment, biodegradability test was carried out on the films, as a parameter of an environmentally friendly material. On Day 40, the mass of the two films were reduced more than 50% in the non-sterilized soil. This is in line with the report of Darni (2014) who stated that the longer the burial time, the higher the weight loss of bioplastic; that means the higher the degradation of bioplastic. This study revealed a better biodegradability of cassava biofilm compared to banana biofilm in the non-sterilized soil. This result is confirmed by the relatively high degradation of the cassava-based burial soil. The mass loss observed in the two films was because they were composed from natural materials which were easily digested by microbes (Hartatik *et al*., 2014). Biological transformations are mainly connected with the activity of earthworms, bioturbation by plant roots and microbiological decomposition (Rillig *et al*., 2017).

The other factor that can be attributed to the degradability of these two films is their hydrophilic nature, which caused bioplastics to be degraded more easily (Harnist and Darni, 2011; Darni, 2014), making bioplastics an eco-friendly packaging material. The significant high biodegradability rate observed in cassava biofilm can be attributed to the high moisture contents and hydrophilicity of cassava, as both solubility and diffusion of water in the bioplastics favored disintegration and biodegradation. The hydroxyl group present in the starch initiated the hydrolysis reaction; due to this reaction, cassava starch was decomposed into small units and quickly disappeared. Similar results were reported by Balaguer *et al*. (2015). The crystalline structure of cassava starch according to Van Soest *et al*. (1992) is arranged in the form of double helixes in a hexagonal system containing thirty-six (36) molecules of water. As for banana starch crystalline structure, it is arranged in a monoclinic system with 8 water molecules per cell. The control film (cling film) a non-biodegradable plastic derived from petroleum, shows only colour change but no disintegration or degradation.

The rate and mechanism of biodegradation of plastic materials are strongly influenced by temperature, pH, oxygen, humidity and microbial conditions of polymeric materials and not only on the durability of the molecules, but also the pH, temperature, humidity, and oxygen content in the environment (Massardier-Nageotte, 2006; Emadian, 2016). The surrounding environment can affect pH, redox potential, presence of suitable microorganisms, availability of adequate nutrition, and concentration of the compound (Nurfauzi *et al.*, 2018). The difference observed between the degradation rate of the films in the sterilized and non-sterilized soil may be attributed to variation in the aforementioned characteristics.

To gain a comprehensive biogeochemical understanding of bioplastic residues in soil, it is important to examine the effect of degradation of bioplastics on soil physicochemical properties (Machado *et al*., 2018). Soil physicochemical properties are basic indicators for estimating the level of soil nutrient contents and characteristics (Theuerl *et al*., 2010). These physical and chemical characteristics such as pH (Stursova and Sinsabaugh, 2008) and metal (Wahsha *et al*., 2017) have been shown to be principal factors affecting both microbial community composition and their enzyme activities in the soil.

Studies have revealed that pH had a significant impact on soil microbial community composition and enzyme activities in different soils (e.g., forest and grassland) (Nicol *et al*., 2008: Stursova and Sinsabaugh, 2008; Lauber *et al*., 2009). Sinsabaugh *et al*. (2008) studied the impact of soil acidic and alkaline pH on microbial activities and their findings showed that pH was one of the major factors that affect microbial enzyme activities and the degradation of bioplastics. The increase in pH of the soil after burial might be attributed to production of basic compounds by these microorganisms, since alkaline soil pH enhances biodegradation, while acidic environments pose limitations to biodegradation (Singh and Walker, 2003; Stursova and Walker, 2006; Pawa, 2015).

Soil electrical conductivity (EC) can serve as a measurement of soluble nutrients and it is useful in monitoring the mineralization of organic matter in soil (Ingole, 2015; Sde *et al*., 2000). EC is an important indicator of soil health. The significant increase in EC in the treated soil is an indication of the biodegradation of the biofilm in the soil.

The management of Soil Organic Carbon is complex, and it is the only factor responsible for the overall maintenance of the soil environment and productive capacity (Nunes *et al.,* 2021). Hence, the loss of soil organic carbon is suggested as an indicator of soil degradation (Aryal *et al.,* 2018). The significant increase of the total organic carbon in both the sterilized soil and non-sterilized soil after burying the biofilm is an indication of biodegradation of the biofilm thus, improving the soil quality.

The increase in the total nitrogen, available phosphorus, total organic carbon and total dissolved solid might be associated with the biodegradation of the film, thus, improving the soil quality. This finding is in consonance with the finding of Chowdhury and Das (2011) for starch-based film. The buried film being rich in starch content can act as a potential substrate for the growth of soil microflora (Chandani and Madhusweta, 2018). This, with addition of adequate moisture and regular aeration, helped the microorganisms to increase organic carbon and Nitrogen content of the soil. Metals such as Na K, Ca, and Mg are essential for the activity of microorganisms in the soil as they serve as important cofactor that aids the production of most enzymes responsible for degradation of bioplastics in the soil (Granger and Ward, 2003: Glass and Orphan, 2012).

Microorganisms such as bacteria and fungi are involved in the biodegradation of both natural and synthetic plastics (Gu *et al*., 2000). These plastic polymers, especially bioplastics, are potential substrates for these microorganisms (Glass and Swift, 1989). The high degradation rate observed in non-sterilize soil compared to the sterile soil can be attributed to high microbial load in the non-sterilize soil compared to

sterile soil; this implies that microorganism has significant impact on the degradation rate of the biofilm and influences bioplastics biodegradability (Szumigaj *et al*., 2008; Sihaloho, 2011). An increase in the bacterial population during biodegradation is expected in studies involving degradation or disintegration of biodegradable materials in the soil (Adhikari *et al*., 2016), as related to this present study. Hayase *et al*. (2004) and Altaee *et al*. (2016) reported an increase in the microbial load in soil subjected to biodegradation of bioplastics. The presence of fungal hyphae and bacteria is a qualitative indication of disintegration and biodegradation of film (Ummah *et al.*, 2013). Microbial growth was observed in regions containing cassava-based and banana-based biofilm; however, no fungal and bacterial growth were detected in the control. The higher microbial load in the cassava biofilm both in the normal and sterile soil can be attributed to its high degradation rate compared to banana biofilm. It also indicated that microorganisms have an important role to play in the degradation of biofilm.

Biodegradation of organic polymer by action of microorganism leads to production of carbon at the end of this process without the release of harmful compounds into the environment (Hashimoto *et al.,* 2002). These biodegradable polymers are degraded by microorganisms in a single step (Karamanlioglu *et al.*, 2017). Bacteria and fungi are more commonly involved in bio-polymers' biodegradation (Karamanlioglu *et al.*, 2017).

Five (5) fungal species (*Aspergillus terreus, Aspergillus niger, Trichoderma spirale, Fusarium oxysporum and Penicillium griseofulvum*) belonging to Ascomycete group were isolated and identified in the burial soil. The obtained result was in accordance with Shah *et al.* (2008) who stated that *A. niger,* and *Penicillium griseofulvum* were starch plastic degrading species. This is also in consonance with their activities in nature as organic polymer degraders and evidence has also shown that most of the fungi that degrade plastic are predominantly Ascomycota (Geweely and Ouf, 2011) and only active in aerobic environment as well as in compost and soil.

These plastic degrading fungi in the soil produce a wide range of enzymes that have the potential to break down the chemical bonds of the plastic polymers e.g., manganese peroxidase (MnP) and lignin peroxidase (LiP), which are commonly associated with lignin degradation (Xu *et al.*, 2013). These enzymes catalyze oxidation-reduction reactions, involving free radicals, transforming several compounds into oxidized or polymerized products (Wei and Zimmermann, 2017). Nine (9) bacteria species *(Pseudomonas aeruginosa, Bacillus spp., Clostridium spp., Staphylococcus auerus, Enterobacter spp., Micrococcus spp., Rhizobium spp., Xanthomonas spp. and Klebsielela spp.)* were isolated and identified in the burial soil (except in the control sterilized soil). All these bacteria are found in the soil and are known to be plastic degrading bacteria. Since water is the medium of these microorganisms, the absorption of water from the soil initiates the hydrolysis reaction (Wahyuningtyas and Suryanto, 2017). Fungi and bacteria identified in this study are similar to that reported by Lee *et al*. (2005), Kumarave*et al*. (2010) and Accinelli *et al*. (2012). These microorganisms produce intracellular or extracellular enzymes that are responsible for enzymatic degradation of bioplastics. They degrade bioplastics through a process known as depolymerization, by producing depolymerases enzymes that play a significant rule in bioplastics biodegradation (Tokiwa and Calabia, 2004; Chua *et al*., 2013).

Conclusion

The biodegradation and mechanical properties of bioplastic produced from cassava and banana peel was evaluated in this study, although these bioplastics was produced without additional reinforcing components. The results obtained in this study showed that the biofilms from the two sources had good biodegradability properties but mechanical properties of the films have not yet reached standard value for the moderate grade of bioplastic. This study further established that the degradation of bioplastic did not only depend on the composition of the bioplastics but also on the physical and chemical properties of their environment. Hence, the study concluded that cassava and banana peels will serve as good source for production of eco-friendly bioplastics

Recommendation

Based on the findings of this study, the following are recommended:

- Further study should be carried out that will involve the addition of reinforcement materials to the film, so as to improve their mechanical properties.
- Additional study to check the morphological properties of the two biofilms.

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