INVESTIGATION OF THE MICROORGANISM ASSOCIATED WITH PALM KERNEL SHELLS, CHICKEN DROPPINGS, CLAY SOIL AND NATURAL BIODEGRADATION OF PALM KERNEL SHELLS

BY

OLOGUN OLUSEGUN EMMANUEL
(MCB/11/0342)

A RESEARCH PROJECT SUBMITTED TO THE DEPATMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, FEDERAL UNIVERSITY OYE-EKITI. IN PARTIAL FULFILLMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE, IN MICROBIOLOGY.

OCTOBER, 2015

CERTIFICATION

This is to certify that Ologun Olusegun Emmanuel carried out this research work under my supervision in the department of Microbiology, Federal university Oye-Ekiti, Ekiti State.

.....

Dr. R.A.O. Gabriel-Ajobiewe

Supervisor

08/02/2017

.....

Date

.....

Prof. Bryan Ogeneh

Head of Department

9/2/16

.....

Date

DEDICATION

This project is dedicated to GOD Almighty, invisible and all sufficient for His loving kindness throughout my course of study.

ACKNOWLEDGEMENT

My sincere Appreciation goes to the Most High God for the strength, wisdom and grace sufficient for this academic achievement and above all His guidance throughout my period of academic career in this citadel of learning.

My unreserved gratitude goes to my hard working supervisor, Doctor Mrs Gabriel Ajobiewe who properly and meticulously guided me through my project work.

My heartfelt thanks goes to my beloved parents Elder Asegun Patrick and late Deaconess Asegun Yemi, they are indeed loving and caring parent. My deepest and profound gratitude goes to my siblings Asegun Toyin, Asegun Damilare, Asegun Femi, Asegun Olamide, Asegun Kemi, Gbajimi Abraham and Opeyemi Oluwatoyin.

I sincerely acknowledge and appreciate the effort Pastor Mrs Oladiti, Late Pastor Akanmu, my lovely brother Gbajimi Abraham and sister Opeyemi for their advice, prayers and financial support throughout my study.

My appreciation also goes to my friends who have been kind and supportive to me all my stay in the school, I love you all. I will not fail to give cudos to CACCF FUOYE Oye-Ekiti for your prayers and making me strong physically and spiritually through-out my study and to my fellow exco of 2012-2015 tenure. May God continue to protect, bless and supply all your needs according to His riches in glory. Finally my profound gratitude goes to my H.O.D, Prof Brayn Ogene, and all the lecturers in microbiology department especially Prof Ogundana, Dr. S.K Ojo, Dr. Ajayi, Mr Femi Bankefa, Dr. Oyerekua, Mrs Mukuoma and the entire laboratory technologist in the department, they have all helped me in building up to this stage. Thanks for making a good microbiologist out of me and I won't forget you all. To Yahweh be the glory because I came I saw and I conquer.

TABLE OF CONTENT

4.0 CHAPTER FOUR: RESULT AND DISCUSSION

LIST OF TABLES PAGES

ABSTRACT

Environmental pollution caused by palm kernel Shell has been an enormous problem from primordial age till present time and a threat to the world at large. This research is on the investigation of the microorganisms associated with palm kernel shells, chicken droppings, clay soil and natural biodegradation of palm kernel shells. Palm kernel shells were pulverized and samples are picked (5kg) for isolation and microbial assay after 5 days. Standard methods were used for pH and Total Titratable Acid (TTA), subsequently, occurrence of different microorganisms were grouped and used for the assessment of the frequency of occurrence. Major bacteria isolates tested positive to catalase, gram staining, gram reaction and fermented lactose, sucrose, fructose and Manitol. The pH started at acidic level of 5.02 at day 0 and maintained the acidic level at day 30 having a pH of 6.99 showing that organisms that were involve in degradation were alkaline friendly. Total Titratable acid of degrading ground palm kernel shell reduced during degradation indicting that the microorganisms present were not able to profuse the acid present in the sample. At the end of the study bacteria were the predominant organisms isolated from the naturally degrading ground palm kernel shell.

CHAPTER ONE

1.0 INTRODUCTION

While the oil palm industry has been recognized for its contribution towards economic growth and rapid development it has also contributed to environmental pollution due to the production of huge quantities of by-products from the oil extraction process. The waste products from oil palm processing consist of oil palm trunks, oil palm fronds, empty fruit bunches, palm pressed fibres and palm kernel shells, less fibrous material such as palm kernel cake and liquid discharge palm oil mill effluent. According to Thomas and Sampson, (2013), during processing in the palm oil mill more than 70% (by weight) of the processed fresh fruit bunch was left over as oil palm waste. Two major solid wastes are generated during palm oil processing, which include, the extracted fresh fibre (or called mesocarp) and seed shell (or called endocarp). Oil palm (*Elaeis guineensis*) is the most important species in *Elaeis* genus which belongs to the family *Palamae.* It is cultivated in West Africa and in all tropical areas especially in Malaysia, Indonesia and Thailand. The oil palm fruits is reddish in colour and has a size of large plum, and grows in large bunches. A bunch usually has the weight of 10 to 40kg. Each fruit consist of a single seed (the palm kernel) and surrounded by a soft oily pulp and the kernel. The oil extracted from fruit pulp is used for the manufacturing of soap. Palm oil has now become world's largest source of edible oil with 38.5 million tonnes or 25% of the world's total oil and fat production.

With the aid of biodegradation which is nature's way of recycling wastes, or breaking down organic matter into nutrients this huge quantities of by-products from the oil extraction process can be broken down. In the microbiological sense, "biodegradation" means that the decaying of all organic materials is carried out by a huge assortment of life forms comprising mainly bacteria and fungi, and other organisms. This organism can therefore serve as prospective degrading agents towards palm kernel shell waste which poses serious environmental threat. This pivotal, natural, biologically mediated process is the one that transforms hazardous toxic chemicals into non-toxic or

less toxic substances (Hartley, 1988). In a very broad sense, in nature, there is no waste because almost everything gets recycled. In addition, the secondary metabolites, intermediary molecules or any 'waste products' from one organism become the food/nutrient source(s) for others, providing nourishment and energy while they are further working-on/breaking down the so called waste organic matter. There are several reasons for which this process is better than chemical or physical processes. For example, this process directly degrades contaminants rather than merely transforming them from one form to the other, employ metabolic degradation pathways that can terminate with benign terminal products like CO_2 and water, derive energy directly from the contaminants themselves, and can be used in situ to minimize the disturbances usually associated with chemical treatment at the clean-up sites. Biological degradation of organic compounds may be considered an economical tool for remediating hazardous waste-contaminated environments. While some environments may be too severely contaminated for initial in situ treatment to be effective, most contaminated media will use some form of biological degradation in the final treatment phase.

1.1 Justification

Biodegradation is nature's way of recycling wastes or breaking down organic matter into nutrients that can be used and reused by other organisms. In the microbiological sense, ''biodegradation means that the decaying of all organic materials is carried out by a huge assortment of life-forms comprising mainly bacteria, fungi and other organisms. The by-products from the oil extraction process has create a huge pollution, hence the research work on the investigation of microbial community as prospective degradative organism in palm kernel shell maybe an added advantage of detecting methods that can solve the problem of the increase environmental pollution in the economy. Till date no investigation has been done on biodegradation of palm kernel. So this research will focus investigation of the microorganism associated with palm kernel shells, chicken droppings, clay soil and natural biodegradation of palm kernel shells.

1.2 Research objectives

The specific objectives are to:

A. isolate the microorganisms present in;

i. Palm kernel shell

ii. Clay soil

iii. Chicken droppings

B. identify the microorganisms

C. evaluate the microbial load

E. check degradation at day 0, 5, 10, 15, 20 and day 30

F. carry out natural biodegradation of the grounded palm kernel shell using the spontaneous organisms present in the palm kernel shell.

G. evaluate the microorganisms that has aided in biodegradation

1.3 Expected contribution

This study is expected to investigation of the microorganism associated with palm kernel shells, chicken droppings, clay soil and natural biodegradation of palm kernel shells. Evaluation of these microorganisms that have aided the biodegradation process and information on the microbial community can foster the degradation of palm kernel shell and other agro- waste.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Palm kernel shells (PKS)

Palm kernel shells are derived from the oil palm tree (*Elaeis guineensis*), an economically valuable tree, and native to the western. In Nigeria, the oil palm tree generally grows in the rain forest region close to the coastal areas and adjacent to some inland waterways. Palm kernel shells (PKS) are organic waste materials obtained from crude palm oil producing factories in Asia and Africa (Alengaram *et al.*, 2010). Palm Kernel Shell was partially a waste in the 1990s and early 2000 as more than 350,000 tons were available for sale and used for land-fillings, domestic cooking, etc. in Asia and Africa. Oil Palm trees grow in the coastal belt in Nigeria which varies in depth from 100 to 150 miles and a riverine belt which follows the valleys of the Niger and Benue for a distance of about 450 miles from the sea. The main palm oil producing states include Ogun, Ondo, Oyo, Edo, Cross River, Anambra, Enugu, Imo, Abia, Ekiti, Akwa-Ibom, Delta and Rivers. The Palm Kernel Shells is a virgin biomass with a high calorific value, typically about 3,800 Kcal/kg. Generally, palm kernel shell consists of 60 – 90% of particles in the range of 5 – 12.7mm (Okafor, 1988). The specific gravity of palm kernel shell varies between 1.17 and 1.37, while the maximum thickness of the shell was found to be about 4mm (Okpala, 1990). The density of palm kernel shell ranges from 1700 to 2050kg/m^3 and it depends on factors such as type of sand and palm kernel shell contents (Mohd *et al.*, 2008).

2.2 Uses of Palm kernel shells (PKS)

Palm kernel shells in the past had been used solely as fuelling material at home and for industries. Omange, (2001) wrote that palm kernel shells are used mostly as a source of fuel for domestic cooking in most areas where they occur. He stated further that the shells are often dumped as waste products of the oil palm industry. Palm kernel shells are not common materials in the construction industry. This is either because they are not available in very large quantities as sand or gravel, or because their use for such has not been encouraged. For some time now, the Nigerian government has been clamouring for the use of local materials in the construction industry to limit costs of construction. There has therefore been a greater call for the sourcing and development of alternative, non-conventional local construction materials (Omange, 2001). Palm kernel shells have been used as aggregates in light and dense concretes for structural and non-structural purposes (Uviasah, (1976); Ata *et al.,* (2006) compared the mechanical properties of palm kernel shell concrete with that of coconut shell concrete and reported the economy of using palm kernel shell as lightweight aggregate. Nuhu-Koko, (1990) went further to show that the 28 day compressive strength of concrete with palm kernel shells as aggregate range between 0.3 and 20.5N/mm^2 depending on the proportion in the mix. Generally, when the density of concrete is lower than 2000kg/m^3, it is categorized as light weight concrete. Thus, the palm kernel shell concrete can be produced within this target density of 2000kg/m^3, hence palm kernel shell concrete is a light weight concrete(Okafor, 1988). According to (Mohd *et al.*,2008), the 28 days cube compressive strength obtained was 15 – 25MPa while the structural behaviour of palm kernel shell is very limited. Ndoke, (2006) in his work observed the performance of palm kernel shells as partial replacement for coarse aggregate in asphalt cement.

2.3 Palm kernel

The palm kernel is the edible seed of the oil palm tree. The fruit yields two distinct oil palm derived from the outer parts of the fruit, and palm kernel oil derived from the kernel (Teo *et al.*, 2012). The oil palm *(Elaeis guineensis)* is a native of West Africa. It flourishes in the humid tropics in groves of varying density, mainly in the coastal belt between 10 degrees north latitude and 10 degrees south latitude. It is also found up to 20 degrees south latitude in Central and East Africa and Madagascar in isolated localities with a suitable rainfall. It grows on relatively open ground and, therefore, originally spread along the banks of rivers and later on land cleared by humans for long-fallow cultivation (Hartley, 1988). The palm fruit develops in dense bunches weighing 10 kilograms (kg) or more and containing more than a thousand individual fruits similar in size to a small plum. Palm oil is obtained from the flesh of the fruit and probably formed part of the food supply of the indigenous populations long before recorded history. A few written records of the local food use of a palm oil (presumably from *Elaeis guineensis*) are available in accounts of European travellers to West Africa from the middle of the fifteenth century. Red palm oil later became an important item in the provisioning trade supplying the caravans and ships of the Atlantic slave trade, and it still remains a popular foodstuff among people of African descent in the Bahia region of Brazil (Hartley, 1988).

2.3.1 African food uses

In West Africa, palm oil has a wide range of applications. It is employed in soups and sauces, for frying, and as an ingredient in dough's made from the various customary starch foods, such as cassava, rice, plantains, yams, or beans. It is also a condiment or flavouring for bland dishes such as *fufu* (cassava). Palm oil is also used in baked dishes, and one popular dish has different names according to the local language. Palm oil has been used as a traditional food in West Africa probably for thousands of years, which provides some evidence that it has good nutritional properties (Saman and Omidreza, 2011).

2.3.2 Nutritional properties

The specific nutritional properties of palm oil may be considered in relation to its chemical composition. The unsaturated acids present are mainly oleic, with a useful level of linoleic and a small amount of linolenic acid. In consequence, palm oil has a high stability to oxidation. Palm oil is readily absorbed and shows a digestibility of 97% or greater, similar to that of other common edible oils (Nganga, 2000).

2.3.3 Uses of palm kernel

Resembling coconut oil, palm kernel oil is packed with myristic and lauric fatty acids and therefore suitable for the manufacture of soaps, washing powders and personal care products (Faessler *et al.*, 2007). Lauric acid is important in soap making: a good soap must contain at least 15 per cent laurate for quick lathering, while soap made for use in sea water is based on virtually 100 per cent laurate. Derivatives of palmitic acid were used in combination with naphtha during World War II to produce napalm (aluminum *na*phthenate and aluminum *palm*itate) (Musa and John, 2009). Palm kernel cake is a high-fibre, medium-grade protein feed best suited to ruminants .Among other similar feedstock's palm kernel cake is ranked a little higher than copra cake and cocoa pod husk but lower than fish meal and groundnut cake, especially in its protein value. Composed of 16% fibre, palm kernel cake also has a high phosphorus-to-calcium ratio and contains such essential elements such as magnesium, iron and zinc (Tang Thin Sue, 2001).

2.4 Biodegradation

Biodegradation is a process where something breaks down into simple compounds as a result of the action of microorganisms (like bacteria, fungi, or algae). The first known use of the word in biological text was in 1961 when employed to describe the breakdown of material into the base components of carbon, hydrogen, and oxygen by microorganisms. Now biodegradable is commonly associated with environmentally friendly products that are part of the earth's innate cycle and capable of decomposing back into natural elements. The term biodegradation is actually a contraction, short

for "biotic degradation." Something is biodegradable if it *can* be broken down by this kind of process. In the microbiological sense, "biodegradation" means that the decaying of all organic materials is carried out by a huge assortment of life forms comprising mainly bacteria and fungi, and other organisms, these microorganisms do something to a material, usually the material has to be broken up into smaller pieces.

In order to say that something "biodegrades", it therefore has to meet the following requirements:

1. it has to break down (this is simply "degradation")
2. it's molecules have to break down from complex molecules into simpler ones (this is "chemical degradation")
3. the breaking down of its molecules has to be accomplished by microorganisms.

There are several reasons for which this process is better than chemical or physical processes. For example, this process directly degrades contaminants rather than merely transforming them from one form to the other, employ metabolic degradation pathways that can terminate with benign terminal products like CO_2 and water, derive energy directly from the contaminants themselves, and can be used in situ to minimize the disturbances usually associated with chemical treatment at the clean-up sites. Biological degradation of organic compounds may be considered an economical tool for remediating hazardous waste-contaminated environments.

2.4.1 Microbial biodegradation

Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to clean up contaminated environments. Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of organisms to adapt to changing environmental conditions(Koukkou, 2011).The

elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to degrade or convert such compounds(Diaz and Eduardo, 2008).New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information. In the field of Environmental microbiology, genome-based global studies open a new era providing unprecedented in silico views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation strategies to changing environmental conditions(McLeod and Eltis,2008).

2.4.2 Aerobic biodegradation of pollutants

The burgeoning amount of bacterial genomic data provides unparalleled opportunities for understanding the genetic and molecular bases of the degradation of organic pollutants. Aromatic compounds are among the most recalcitrant of these pollutants and lessons can be learned from the recent genomic studies of *Burkholderia xenovorans* LB400 and *Rhodococcus* sp. strain RHA1, two of the largest bacterial genomes completely sequenced to date (Tor *et al.,* 2000). These studies have helped expand our understanding of bacterial catabolism, non-catabolic physiological adaptation to organic compounds, and the evolution of large bacterial genomes. First, the metabolic pathways from phylogenetically diverse isolates are very similar with respect to overall organization. Thus, as originally noted in pseudomonads, a large number of "peripheral aromatic" pathways funnel a range of natural and xenobiotic compounds into a restricted number of "central aromatic" pathways (Cupples *et al.,* 2005).

2.4.3 Anaerobic biodegradation of pollutants

Anaerobic microbial mineralization of recalcitrant organic pollutants is of great environmental significance and involves intriguing novel biochemical reactions. In particular, hydrocarbons and halogenated compounds have long been doubted to be degradable in the absence of oxygen, but the isolation of hitherto unknown anaerobic hydrocarbon-degrading and reductively dehalogenating bacteria during the last decades provided ultimate proof for these processes in nature. While such research involved mostly chlorinated compounds initially, recent studies have revealed reductive dehalogenation of bromine and iodine moieties in aromatic pesticides (Cupples *et al.,* 2005). Other reactions, such as biologically induced abiotic reduction by soil minerals (Tor *et al.,* 2000) has been shown to deactivate relatively persistent aniline-based herbicides far more rapidly than observed in aerobic environments. Many novel biochemical reactions were discovered enabling the respective metabolic pathways, but progress in the molecular understanding of these bacteria was rather slow, since genetic systems are not readily applicable for most of them. However, with the increasing application of genomics in the field of environmental microbiology, a new and promising perspective is now at hand to obtain molecular insights into these new metabolic properties (Heider and Rabus, 2008). Several complete genome sequences were determined during the last few years from bacteria capable of anaerobic organic pollutant degradation. The genome sequence revealed about two dozen gene clusters (including several paralogs) coding for a complex catabolic network for anaerobic and aerobic degradation of aromatic compounds. The genome sequence forms the basis for current detailed studies on regulation of pathways and enzyme structures. Characteristic for all these bacteria is the presence of multiple paralogous genes for reductive dehalogenases, implicating a wider dehalogenating spectrum of the organisms than previously known. Moreover, genome sequences provided unprecedented insights into the evolution of reductive dehalogenation and differing strategies for niche adaptation (Heider and Rabus, 2008).

2.4.4 Bioavailability, chemotaxis, and transport of pollutant

Bioavailability or the amount of a substance that is physiochemically accessible to microorganisms is a key factor in the efficient biodegradation of pollutants. O'Loughlin *et al.,* (2000) showed that, with the exception of kaolinite clay, most soil clays and cation exchange resins attenuated biodegradation of 2-picoline by *Arthrobacter* sp. strain R1, as a result of adsorption of the substrate to the clays. Chemotaxis or the directed movement of motile organisms towards or away from chemicals in the environment is an important physiological response that may contribute to effective catabolism of molecules in the environment. In addition, mechanisms for the intracellular accumulation of aromatic molecules via various transport mechanisms are also important (Parales, 2008).

2.4.5 Oil biodegradation

Petroleum oil contains aromatic compounds that are toxic for most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, about 250 million litters of petroleum enter the marine environment every year from natural seepages (Cupples *et al.,* 2005). Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB) (Yakimov *et al.*,2007). *Alcanivorax borkumensis* was the first HCB to have its genome sequenced. In addition to hydrocarbons, crude oil often contains various heterocyclic compounds, such as pyridine, which appear to be degraded by similar, though separate mechanisms than hydrocarbons (Sims and O'Loughlin, 1989).

2.4.6 Cholesterol biodegradation

Many synthetic steroidic compounds like some sexual hormones frequently appear in municipal and industrial wastewaters, acting as environmental pollutants with strong metabolic activities negatively

affecting the ecosystems. Since these compounds are common carbon sources for many different microorganisms their aerobic and anaerobic mineralization has been extensively studied (Koukkou, 2011). The interest of these studies lies on the biotechnological applications of sterol transforming enzymes for the industrial synthesis of sexual hormones and corticoids. Very recently, the catabolism of cholesterol has acquired a high relevance because it is involved in the infectivity of the pathogen *Mycobacterium tuberculosis* (*Mtb*) (Wipperman *et al.,* 2014). *Mtb* causes tuberculosis disease, and it has been demonstrated that novel enzyme architectures have evolved to bind and modify steroid compounds like cholesterol in this organism and other steroid-utilizing bacteria as well (Thomas and Sampson, 2013; Wipperman *et al.,* 2013). These new enzymes might be of interest for their potential in the chemical modification of steroid substrates.

2.4.7 Fungal biodegradation

In the ecosystem, different substrates are attacked at different rates by consortia of organisms from different kingdoms. *Aspergillus* and other moulds play an important role in these consortia because they are adept at recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some aspergilli are capable of degrading more refractory compounds such as fats, oils, chitin, and keratin. Maximum decomposition occurs when there is sufficient nitrogen, phosphorus and other essential inorganic nutrients. Fungi also provide food for many soil organisms (Machida *et al.*, 2010). For *Aspergillus* the process of degradation is the means of obtaining nutrients. When these moulds degrade human-made substrates, the process usually is called bio deterioration. Both paper and textiles (cotton, jute, and linen) are particularly vulnerable to *Aspergillus* degradation. Our artistic heritage is also subject to *Aspergillus* assault. To give but one example, after Florence in Italy flooded in 1969, 74% of the isolates from a damaged Ghirlandaio fresco in the Ognissanti church were *Aspergillus versicolor* (Bennett, 2010).

2.4.8 Categories of biodegradation

A. mineralization

B. biotransformation

Mineralization is the process by which micro-organisms work on organic compounds and by a chemical process reduces them to inorganic material such as water, carbon dioxide, and also possibly other such inorganic compounds. Mineralization involves total degradation of the organic matter. Biotransformation essentially differs from mineralization in that the organic matter is not degraded totally. While a part of it is degraded, another part is converted into other smaller chain organic compounds (Meyer and Panke, 2008).

2.4.9 Biodegradation Techniques

2.4.9.1 Hydro-biodegradable

Hydro-biodegradable materials are first broken down by interaction with water (a process called hydrolysis), and then are further broken down by microorganisms.

2.4.9.2 Photo-biodegradable

Photo-biodegradable materials are first broken down by interaction with sunlight (a process called photolysis), and then are further broken down by microorganisms.

2.4.9.3 Oxo-degradable

This degradation result from oxidative and cell-mediated phenomenon which allows plastic to return to the environment by addition of an additive that can be added to traditional plastics to make them biodegradable. These products become what is called oxo-degradable, and sometimes is incorrectly identified as oxo-biodegradable. Although, these products are not biodegradable, instead, the additive allows the plastic material to break down physically when exposed to water, into pieces small enough to be accidentally ingested by microbes. However, the microbes are not able to actually break this material down further. The end result is therefore a material that combines biomass with polymer residue. The plastic never decomposes as a result of interaction with the organisms

(Watanabe and Kasai (2008). Some organic materials will break down much faster than others, but all will eventually decay. By harnessing microbial communities, the natural "forces" of biodegradation, reduction of wastes and clean-up of some types of environmental contaminants can be achieved.

2.5 Clay soil

According to Guggenheim and Martin, (1995) Clay soil is a fine-grained natural rock or soil material that combines one or more clay minerals with traces of metal oxides and organic matter. Clays are plastic due to their water content and become hard, brittle and non–plastic upon drying or firing. (Guggenheim and Martin, 1995) Geologic clay deposits are mostly composed of phyllosilicate minerals containing variable amounts of water trapped in the mineral structure. Depending on the content of the soil, clay can appear in various colours, from white to dull grey or brown to a deep orange-red. Clays are distinguished from other fine-grained soils by differences in size and mineralogy. Silts, which are fine-grained soils that do not include clay minerals, tend to have larger particle sizes than clays. There is, however, some overlap in particle size and other physical properties, and many naturally occurring deposits include both silts and clay. The distinction between silt and clay varies by discipline. Geologists and soil scientists usually consider the separation to occur at a particle size of 2μm (clays being finer than silts. ISO 14688 grades clay particles as being smaller than 2μm and silt particles as being larger (Guggenheim and Martin, 1995).

2.5.1 History of clay soil

Knowledge of the nature of clay became better understood in the 1930s with advancements in x-ray diffraction technology necessary to analyse the molecular nature of clay particles (Bailey, 1980). Standardization in terminology arose during this period as well (Bailey, 1980) with special attention given to similar words that resulted in confusion such as sheet and plane (Bailey, 1980).

2.5.2 Formation of clay soil

Clay minerals typically form over long periods of time from the gradual chemical weathering of rocks, usually silicate-bearing, by low concentrations of carbonic acid and other diluted solvents. These solvents, usually acidic, migrate through the weathering rock after leaching through upper weathered layers. In addition to the weathering process, some clay minerals are formed through hydrothermal activity. There are two types of clay deposits: primary and secondary. Primary clays form as residual deposits in soil and remain at the site of formation. Secondary clays are clays that have been transported from their original location by water erosion and deposited in a new sedimentary deposit (Ehlers and Blatt 1982).

2.5.3 Structure of clay soil

Like all phyllosilicates, clay minerals are characterised by two-dimensional *sheets* of corner sharing SiO_4 tetrahedra and/or AlO_4 octahedra. The sheet units have the chemical composition $(Al,Si)_3O_4$. Each silica tetrahedron shares 3 of its vertex oxygen atoms with other tetrahedra forming a hexagonal array in two-dimensions. The fourth vertex is not shared with another tetrahedron and all of the tetrahedra "point" in the same direction; i.e. all of the unshared vertices are on the same side of the sheet. In clays, the tetrahedral sheets are always bonded to octahedral sheets formed from small cations, such as aluminium or magnesium, and coordinated by six oxygen atoms (Thomson, 2000). The unshared vertex from the tetrahedral sheet also forms part of one side of the octahedral sheet, but an additional oxygen atom is located above the gap in the tetrahedral sheet at the centre of the six tetrahedral. This oxygen atom is bonded to a hydrogen atom forming an OH group in the clay structure (Avé, 2000). Clays can be categorized depending on the way that tetrahedral and octahedral sheets are packaged into layers. If there is only one tetrahedral and one octahedral group in each layer the clay is known as 1:1 clay. The alternative, known as 2:1 clay, has two tetrahedral sheets with the unshared vertex of each sheet pointing towards each other and forming each side of the

octahedral sheet. Bonding between the tetrahedral and octahedral sheets requires that the tetrahedral sheet becomes corrugated or twisted; causing ditrigonal distortion to the hexagonal array, and the octahedral sheet is flattened. This minimizes the overall bond-valence distortions of the crystallite. Depending on the composition of the tetrahedral and octahedral sheets, the layer will have no charge, or will have a net negative charge. If the layers are charged this charge is balanced by interlayer cations such as Na^+ or K^+. In each case the interlayer can also contain water. The crystal structure is formed from a stack of layers interspaced with the interlayers (Moore and Reynolds, 1997).

2.5.4 Grouping of clay soil

Depending on the academic source, there are three or four main groups of clays: kaolinite, montmorillonite-smectite, illite, and chlorite. Chlorites are not always considered clay, sometimes being classified as a separate group within the phyllosilicates. There are approximately 30 different types of "pure" clays in these categories, but most "natural" clays are mixtures of these different types, along with other weathered minerals (Ehlers and Blatt, 1982).

2.5.5 Historical and modern uses of clay soil

Clays exhibit plasticity when mixed with water in certain proportions. When dry, clay becomes firm and when fired in a kiln, permanent physical and chemical changes occur. These changes convert the clay into a ceramic material. Because of these properties, clay is used for making pottery, both utilitarian and decorative, and construction products, such as bricks, wall and floor tiles. Different types of clay, when used with different minerals and firing conditions, are used to produce earthenware, stoneware, and porcelain. Prehistoric humans discovered the useful properties of clay. Some of the earliest pottery shards recovered are from central Honshu, Japan. They are associated with the Jomon culture and deposits they were recovered from have been dated to around 14,000 BC (Hillier, 2003). Clay, being relatively impermeable to water, is also used

where natural seals are needed, such as in the cores of dams, or as a barrier in landfills against toxic seepage (Koçkar *et al.*, 2012).

2.5.6 Medicinal uses of clay soil

A traditional use of clay as medicine goes back to prehistoric times. An example is Armenian bole, which is used to soothe an upset stomach, similar to the way parrots (and later, humans) in South America originally used it (Koçkar *et al.*, 2012). Kaolin clay and attapulgite have been used as anti-diarrheal medicines.

2.5.7 Clay soil as building material

Clay is one of the oldest building materials on Earth, among other ancient, naturally-occurring geologic materials such as stone and organic materials like wood (Biggs and Bruce, 1972). Between one-half and two-thirds of the world's population, in traditional societies as well as developed countries, still live or work in a building made with clay as an essential part of its load-bearing structure. Also a primary ingredient in many natural building techniques, clay is used to create adobe, cob, cordwood, and rammed earth structures and building elements such as wattle and daub, clay plaster, clay render case, clay floors and clay paints and ceramic building material (Koçkar *et al.*, 2012). Clay was used as a mortar in brick chimneys and stone walls where protected from water.

2.6 Chicken droppings poultry litter

In agriculture, poultry droppings or litter or broiler litter is a mixture of poultry excreta, spilled feed, feathers, and material used as bedding in poultry operations. This term is also used to refer to unused bedding materials. Chicken dropping is used in confinement buildings used for raising broilers, turkeys and other birds (Carter *et al.*, 2000). Materials used for bedding can also have a significant impact on carcass quality and bird performance (Carter *et al.*, 2000). Chicken droppings are traditionally used as fertilizer. As with other manures, the fertilizing value of poultry litter is excellent, but it is less concentrated than chemical fertilizers, giving it a relatively low value

per ton. This makes it uneconomical to ship long distances, and it tends to lose its nitrogen value fairly quickly. Extracting its value requires that it be used on nearby farms. This limits its resale value in regions where there are more poultry farms than suitable nearby farmland.

2.6.1 Uses of chicken drooping's

Traditionally used as fertilizer, it is now also used as a livestock feed as a cost-saving measure compared with other feedstock materials, particularly for beef animals (Bagley and Evans, 1995). There are currently several electrical generating plants in the UK, and recently in the US that are utilizing poultry and turkey litter as their primary fuel. The first three (and the world's first three of these plants) were developed by Fibrowatt Ltd in the UK, founded by Simon Fraser, who was awarded an OBE for his contribution to renewable energy. These are: Thetford (38.5 MWe), Eye (12.7 MWe) and Glanford (13.5 MWe - now switched to burning meat and bonemeal). The fourth, Westfield (9.8 MWe), was developed by Energy Power Resources, which now owns all four (Chikwendu and Okezie, 2000). On a smaller scale, poultry litter is used in Ireland as a biomass energy source. This system uses the poultry litter as a fuel to heat the broiler houses for the next batch of poultry being grown thus removing the need for LPG gas or other fossil fuels (Bagley and Evans, 1995). Hadjipanayiotou *et al.*, (1993) confirmed that some companies such as Advanced Fibers & Powders are also developing gasification technologies to utilize poultry litter as a fuel for electrical and heating applications, along with producing valuable by-products including activated carbons and fertilizers.

2.6.2 Microbiological properties of chicken droppings from different chicken types and production systems.

Poultry litter can support the growth of Salmonella and the production of aflatoxin, so care needs to be observed to assure that the poultry litter has been properly processed. Litter (droppings) collected from poultry operations. Fifty-two samples of poultry litter were evaluated bacteriologically and only eight were found to contain pathogenic organisms (E. coli 0157,

Campylobacter, Salmonella) and none were found to contain Salmonella (Jeffrey *et al.*, 1998). Chicken litter is produced in large quantities from all types of poultry raising activities. It's primarily used for land application analyse the properties before it is released to the environment. The microbiological analysis consisted of the enumeration of total bacteria, total coliforms, *staphylococcus species, salmoneella species and clostridium perfringes*. Chicken litter from layers reared under intensive and free range systems showed lower mean total bacterial count than the litter collected from chicken broilers reared under either of the two systems. The litter from intensive layers had the lowest mean total coliform counts while the lowest staphylococcus species count was observed in the litter from free range layers. The clostridium perfringes count was the lowest in chicken litter from intensively raised broilers and layers.

2.7 Sticky droppings: a feed-related poultry problem

Sticky droppings' describes an undesirable gummy consistency of poultry excreta (droppings) and are associated with secondary health problems. Sticky droppings pose a potential health risk to poultry, through respiratory stress from ammonia and potential increase of coccidiosis. Young birds (less than 3 weeks of age) are most susceptible to this feeding disorder while older birds (greater than 6 weeks of age) are the least susceptible. Cereal grains are widely known to cause sticky droppings as well as limited nutrient uptake and poor growth in poultry (Francesch and Brufau, 2004). Barley, in particular, is unpopular as poultry feed because it can cause sticky droppings due to its high amounts of a non-starch polysaccharide (NSP) called mixed-linked β-glucan. Mixed-linked β-glucan and other NSPs are important components of endosperm cell walls and are common in cereal grains and other seed crops (Harrold and Robert, 1999). In barley, 70% of the endosperm walls are made up of NSPs. Poultry is largely unable to digest these NSPs. The undigested NSPs bind with water in the intestinal tract, thus increasing the thickness of the intestinal contents. This results in gelatinous droppings which cause faecal matter to stick to the bird's vent or cloaca (McNab and Smithard, 1992). As a result, eggs are overly dirty and skin infections can occur, particularly on birds' feet.

Sticky droppings also impact litter moisture content, resulting in damp litter and causing increased disease incidence and reduced meat quality. Healthy chickens will pass faecal excretions twice a day in addition to the regular brown droppings that have a characteristic white cap of uric acid and which they pass 12–16 times each day. Poultry producers should be aware of the β-glucan content of any barley they are feeding to their birds, barley should be limited to no more than 20% in the ration for broilers and pullets younger than 3 weeks of age and enzyme supplements must be included in daily feed (Petersson and Åman, 1989).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study area is Oye- Ekiti, Ekiti state Nigeria [08.33N, 08.32E and 175m high]. Mean annual rainfall in the area is about 1150mm, minimum and maximum temperature range between 28.4^{oC} and 35^{oC}. The population of Oye- local government (2006) is over 134,210. There are no distinctive ethnic groups in the Local Government as a greater percentage of the people resident are of the Yoruba Language race and the major occupation in the community is farming.

3.2 Collection of samples

Palm kernel shell

Palm kernel shell was collected from a traditional palm kernel oil industry in Oyo, Oye-ekiti, Ekiti state.

Clay soil

Soil samples were collected from three locations around Oye-Ekiti (Irona, Ogbon-meta and FUOYE school gate) at depth of 0-15cm (top soil). Samples (1kg each) were taken in polythene bags to the laboratory and air dried for further studies.

Chicken droppings

Chicken droppings were collected at FUOYE poultry. Samples (1kg each) were collected into sterile plates to the laboratory.

3.3 Culture media

Culture media used for this research work were Nutrient agar (NA), Sabourand dextrose agar (SDA), and Yeast extract agar (YEA). Each of the culture medium was prepared and sterilized according to the manufacturer's recommendation. Culture media used were purchased from Chuks- Ezi. Media

used were sterilized by autoclaving at 121^{oC} for 15minutes at 15 pound pressure and air drying in the hot air oven at 160^{oC} for 2hours.

3.4 Culture media and manufacturer

Media	manufacturer	Batch no
SDA	Biomark laboratories	B057
NA	Biomark laboratories	B550
YEA	Lab M limited	106811/325

3.5 Diluents and reagents

1. Sterile distilled water.
2. Peptone water
3. Phenol red
4. Sodium hydroxide (NaOH)
5. Phenolphthalein
6. Potassium hydroxide
7. Hydrogen peroxide
8. Buffer
9. Ethanol

3.6 Equipment's

The apparatus used in this research work were water bath, colony counter, petri dishes, test tubes, conical flask, beakers, inoculating loop, syringes, cotton wool, spatula, bursen burner, measuring cylinder, super bottles, MacCartney bottles, binocular light microscope, incubator, oven, zip loc brand bag, foil paper, weighing balance, local grinding stone, voltex mixer, text tube rack, pH meter,

retort stand, burette, funnel, immersion oil, Durham tube, masking tape, beaker, magnetic hot plate stirrer and analytical weighing balance.

3.7 METHODS

3.7.1 Drying and grinding of palm kernel shell

Palm kernel shell [15kg] was collected into small sizes to separate palm kernel cake from its shell using a local crushing machine. Samples were stored in jute bag and brought down to the laboratory for hand- picking. Hand- picking was done to separate palm kernel shell (PKS) from cake. 1kg of PKS was washed using a little amount of detergent (sun light) and 5liter of distilled water. Palm kernel shells were oven dry at 70^{0C} for one (1) week using the hot air oven. PKS were grinded into powder form using local grinding stone. The powdered sample was then kept in air tight container for further analysis.

3.7.2 Isolation of microorganisms from the samples

Samples 10g was weighed from the uncrushed palm kernel shell. The initial dilution was prepared by adding 10 g of the pulverized sample to 90 ml of peptone water. Decimal dilutions (1 + 9 ml) were prepared down to 10^{10} using the serial dilution method . Using sterile pipettes, 1 ml of each dilution of the various samples was plated out by mixing with sterilized agar using the pour plate method. Before pouring plates, chloramphenicol was added at a concentration of 50 mg/L to (SDA) to inhibit the growth of bacteria. The plates were swirled round for the distribution of the inoculum and setting of the agar. After solidification, the Petri-dishes were incubated upright at room temperature for 24 – 48 hours, respectively. The colonies observed were counted and sub-cultured for identification. This was repeated for clay soil and chicken droppings.

3.7.2.1 Isolation of microorganisms from Naturally Degrading Ground Palm Kernel Shell (NDGPKS).

Considering the design of the experimental set up of 0, 5, 10, 15, 20 and 30 days at the same degradation conditions, Samples 5g was weighed from the different containers. The initial dilution was prepared by adding 5 g of the pulverized sample to 45 ml of peptone water. Decimal dilutions (1+ 9 ml) were prepared down to 10^{10} using the serial dilution method. Using sterile pipettes, 1 ml of each dilution of the various samples was plated out by mixing with sterilized agar using the pour plate method. Before pouring plates, chloramphenicol was added at a concentration of 50 mg/L to (SDA) to inhibit the growth of bacteria. The plates were swirled round for the distribution of the inoculum and setting of the agar. After solidification, the Petri-dishes were incubated upright at room temperature for 24 – 48 hours, respectively. The colonies observed were counted and sub-cultured for identification. This was also repeated at day 5, 10, 15, 20 and day 30.

3.7.3 Identification of isolates

All the isolates were identified by microscopic, biochemical and morphological characteristics and by reference to identification manuals. Subsequently, occurrence of different organisms were grouped and used for the assessment of the frequency of occurence.

3.7. 3.1 Staining

3.7. 3.2 Preparation of Smear

- A loopful of sterile water was placed on the grease free glass slide.
- wire loop was flamed
- A very small amount of a distinct colony from 18hours old culture was aseptically transferred onto the centre of a clean grease free slide and emulsify with the wire loop.
- Culture was heat- fixed by passing through the Bunsen flame.

3.7. 3.3 Staining Procedure

- A drops of crystal violet was added over the fixed smear and allow to stand for 60seconds
- Stain was gently poured-off and gently rinse off the remaining stain with distilled water to remove unbound crystal violet.
- Lugol's iodine was added on the smear, enough to cover the fixed smear and allow to stand for 60seconds and then washed with water.
- 95% alcohol was applied and allowed to drain off until no more color was seen leaving the smear
- Safranin solution was added on the slide which is the secondary stain, was allow to stand for 60seconds and rinsed off.

3.7. 3.4 Catalase test

Catalase test was carried out for the detection of the enzyme catalase in bacteria. The presence of the enzyme catalase is evident in most aerobic organism and it neutralizes the bactericidal effects of hydrogen peroxide.

- A loopful of sterile water was placed on the grease free glass slide.
- wire loop was flamed
- A sterile wire loop was used to pick a colony of the organism of 18 hours old culture and was aseptically transfer onto the centre of a clean grease free slide
- With the help of a dropper a drop of 3% hydrogen peroxide was added to the slide
- The slide was then observed for formation of effervescence (bubbles).

Effervescence caused by the liberation of oxygen as a gas bubble indicates the production of catalase by the bacterium while a negative result indicates the absence of catalase.

3.7. 3. 5 Gram reaction

- A loopful of 3% potassium peroxide was placed on the grease free glass slide.
- wire loop was flamed
- A sterile wire loop was used to pick a colony of the organism of 18 hours old culture and was aseptically transfer onto the centre of a clean grease free slide
- The organism was smeared on the slide and then pull-up.

If organism smeared on the slide pulls-up (stingy) it indicates a gram positive test and no pull indicate a negative test.

3.7. 3.6. Sugar fermentation

The sugars used were sucrose, fructose, lactose and Manitol. 1g of each sugar was differently dissolved in a 100ml of nutrient broth and 2 drops of phenol red were added to each solution. 10ml of the mixture was dispensed into test tubes, and then a Durham tube was inverted and placed in each test tube to detect any gas production after fermentation might have occurred. The sugars were sterilized at 121 ^{O}C for 15 minutes in an autoclave, allowed to cool and then inoculated aseptically with each isolates and incubated at 37 ^{O}C for 24 hours.

3.7. 3.7 Total Titratable Acid and pH analyses

Total Titratable Acid and pH analyses were determined according to the recommended Standards. Total Titratable Acid was obtained by weighing 5g of NDGPKS from the container after every 5 days. 45ml of peptone was added to form aliquot. 20ml of the aliquot is pipetted and 2-3 drop of phenolphthalein was added, this was titrated against 0.1mole NaOH. Colour change to purple indicates acid production. The pH values were obtained by weighing 5g of NDGPKS from the container after every 5 days. Suspensions of the sample in distilled, de-ionized water was obtained and used to determine the pH values.

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 Microbial viable Count of Samples

4.1.1 *Total Viable Count of Uncrushed Palm Kernel Shells*

The viable microbial count of bacteria as represented in Table 1 shows that the total viable count of bacteria (22) were higher than those of yeast and mould (11). This trend reveals heavy proliferation of bacteria of the uncrushed palm kernel shell.

4.1.2 *Total viable Count of Chicken Droppings*

The viable microbial count of bacteria in chicken as represented in Table 2 shows an increase in bacterial count to palm kernel shell as bacteria count has the highest count of 3.0×10^6.

4.1.3 Microbial viable Count of Clay soil

Table 3 shows the least count of yeast (0.1×10^5). The result reveals that clay soil used for the experiment has few fungi. It was shown from each table that chicken droppings have the highest consortium of organisms of 1.6×10^6.

Table 1: Total viable microbial count isolated from uncrushed Palm kernel Shell (UPKS)

Isolate code	**Count at 24 hours (Cfu/g)**
Bacterial viable count	
UPKSB 1	0.8×10^{6}
UPKSB 2	0.7×10^{6}
UPKSB 3	0.4×10^{7}
Yeast viable count	
Isolate code	Count at 72 hours (Cfu/g)
UPKSY 1	0.4×10^{5}
UPKSY 2	0.2×10^{6}
UPKSY 3	0.3×10^{6}
Mould viable count	
Isolate code	Count at 72 hours (Cfu/g)
UPKSM 1	0.5×10^{3}
UPKSM 2	0.5×10
UPKSM 3	0.3×10^{4}

KEY: UPKSB = Uncrushed Palm Kernel Shell Bacteria

UPKSY = Uncrushed Palm Kernel Shell Yeast

UPKSM= Uncrushed Palm Kernel Shell Mould

CFU = Colony Forming Unit

Table 2: Total viable microbial count isolated Chicken Droppings (CD)

Isolate code	**Count at 24 hours (Cfu/g)**
Bacterial viable count	
CDB 1	3.0×10^6
CDB 2	2.6×10
CDB 3	0.5×10^7
Yeast viable count	
Isolate code	Count at 72 hours (Cfu/g)
CDY 1	0.4×10^5
CDY 2	0.4×10^6
CDY 3	0.1×10
Mould viable count	
Isolate code	Count at 72 hours (Cfu/g)
CDM 1	0.6×10^3
CDM 2	0.4×10^3
CDM 3	0.2×10^4

KEY: CDB = Chicken Droppings Bacteria

CDY = Chicken Droppings Yeast

CDM= Chicken Droppings mould

CFU = Colony Forming Unit

Table 3: Total Viable Microbial Count Isolated from Clay Soil (CS).

Isolate code	Count at 24 hours (Cfu/g)
Bacterial viable count	
CSB 1	1.5×10
CSB 2	1.0×10^{6}
CSB 3	0.5×10^{7}
Yeast viable count	
Isolate code	Count at 72 hours (Cfu/g)
CSY 1	0.3×10^{5}
CSY 2	0.1×10^{6}
CSY 3	0.2×10^{6}
Mould viable count	
Isolate code	Count at 72 hours (Cfu/g)
CSM 1	0.4×10^{3}
CSM 2	0.4×10^{3}
CSM 3	0.2×10^{4}

KEY: CSB = Clay Soil Bacteria

CSY = Clay Soil Yeast

CSM= Clay Soil mould

CFU = Colony Forming Unit

4.2 Microbial Viable Count of Naturally Degraded Ground Palm Kernel Shells (NDGPKS)

4.2.1 *Total bacterial viable count of isolates from naturally degrading ground palm kernel shell*

The viable microbial count of bacteria isolated from naturally degrading ground palm kernel shell as represented in Table 4 shows that the results increased at day 0(0.8×10^6) and (3.0×10^5) at day 15 and drop from(1.0×10^5) day 20 to (0.1×10^5) at day 30 which also had the least count. The drop in count indicates that the organisms involve in the degradation process declined from day 15 to day 30.

4.2.2 *Total yeast viable count of isolates from naturally degrading ground palm kernel shell*

Yeast count as shown in table 5 shows isolates at day 0 (0.4×10^5) having the highest count but decreased at each day of degradation from day 5 (0.2×10^5) to day 30 (0.1×10^5). Day 15 shows the highest count of (0.3×10^5) during degradation process.

4.2.3 *Total Mould viable count of isolates from naturally degrading ground palm kernel shell.*

Mould count according to table 6 shows an appreciable count at day 0 (0.5×10^3) and (0.4×10^3) at day15. Day 15 has the highest count of (0.3×10^5) during degradation process. Day 15 has the highest count during degradation process.

Table 4: Total Bacterial viable count of isolates from Naturally Degrading Ground Palm Kernel Shells (NDGPKS).

Isolate code	Count at 24 hours (Cfu/g)
DAY 0	
NDGPKSB 1	0.8 x 10
NDGPKSB 2	0.7×10^6
NDGPKSB 3	0.4×10^7
DAY 5	
NDGPKSB 1	0.7×10^6
NDGPKSB 2	0.3×10^6
NDGPKSB 3	0.1×10^7
DAY 10	
NDGPKSB 1	2.0×10^6
NDGPKSB 2	3.0×10^6
NDGPKSB 3	0.5×10^7
DAY 15	
NDGPKSB 1	2.0×10^6
NDGPKSB 2	3.0 x 10
NDGPKSB 3	1.0×10^7
DAY 20	
NDGPKSB 1	1.0×10^6
NDGPKSB 2	0.7×10^6
NDGPKSB 3	0.2×10^7
DAY 30	
NDGPKSB 1	0.1×10^6
NDGPKSB 2	0.1×10^6

KEY: NDGPKSB= Naturally Degrading Ground Palm Kernel Shell Bacteria

CFU= Colony Forming Unit

Table 5: Total Yeast viable count of isolates from Naturally Degrading Ground Palm Kernel Shells (NDGPKS).

Isolate code	Count at 72 hours (Cfu/g)
DAY 0	
NDGPKSY 1	0.4×10^5
NDGPKSY 2	0.2×10
NDGPKSY 3	0.3×10^6
DAY 5	
NDGPKSY 1	0.1×10^5
NDGPKSY 2	0.2×10
DAY 10	
NDGPKSY 1	0.1×10^5
DAY 15	
NDGPKSY 1	0.3×10
NDGPKSY 2	0.2×10^6
DAY 20	
NDGPKSY 1	0.1×10^5
NDGPKSY 2	0.1×10
DAY 30	
NDGPKSY 1	0.1×10^5
NDGPKSY 2	0.1×10^5

KEY: NDGPKSY= Naturally Degrading Ground Palm Kernel Shell Yeast

CFU= Colony Forming Unit

Table 6: Total Mould viable count of isolates from Naturally Degrading Ground Palm Kernel Shells (NDGPKS).

Isolate code	Count at 72 hours (Cfu/g)
DAY 0	
NDGPKSM 1	0.5 x 10
NDGPKSM 2	0.5×10^3
NDGPKSM 3	0.3 x 104
DAY 5	
NDGPKSM 1	0.4×10^3
NDGPKSM 2	0.4×10^3
NDGPKSM 3	0.2×10^4
DAY 10	
NDGPKSM 1	0.2×10^3
NDGPKSM 2	0.3×10^3
NDGPKSM 3	0.1×10^4
DAY 15	
NDGPKSM 1	0.4×10^3
NDGPKSM 2	0.3×10^3
NDGPKSM 3	0.1×10^4
DAY 20	
NDGPKSM 1	0.3 x 10
NDGPKSM 2	0.2×10^3
NDGPKSM 3	0.1×10^4
DAY 30	
NDGPKSM 1	0.2×10^3
NDGPKSM 2	0.2×10^3
NDGPKSM 3	0.1×10^4

KEY: NDGPKSM = Naturally Degrading Ground Palm Kernel Shell Mould

CFU= Colony Forming Unit

4.3 Morphological and Microscopic Characteristic of Samples

4.3.1 Morphological and microscopic characteristics of bacterial isolate colonies from uncrushed palm kernel shell.

All bacteria colonies in table 7 had the same morphological and microscopic characteristics. They were cream in colour, rod in shape and had clear forms respectively.

4.3.2 Morphological and microscopic characteristics of bacteria isolate colonies from clay soil

Table 8 shows bacteria isolates colonies which possess the same morphological and microscopic characteristics. All isolate colonies were rod like and cream in colour. CSB2 and CSB3 were not transparent but have the same characteristics in surface, form and elevation respectively.

4.3.3 Morphological and microscopic characteristics of bacteria isolate colonies from chicken droppings.

Bacteria isolate colonies in table 9 had similar characteristics but were different in surface and colour. CDB1 has glistering surface, CDB2 and CDB3 had dull surfaces respectively.

4.3.4: Morphological and microscopic characteristics of bacteria isolate colonies from naturally degrading ground palm kernel shell.

Bacteria isolate colonies as shown in table 10; Day 0, 15 and 30 has similar characteristics in colour, shape, elevation, form and opacity with differences in surface. NDGPKSB day 0 have glistering surface and NDGPKSB day 5 having dull surface respectively. Day 5, 10, 20 and 30 were different in colour and surface but had similarities in form and elevation. The major dominating characteristics of bacteria isolated colonies were in colour (cream) and in shape (rod).

4.4.1: Morphological and microscopic characteristics of fungal isolates from chicken droppings.

The fungi isolates in table 11 had similar characteristics in colour. Yeast isolated had the same characteristics, CDY1 was different in colour. Moulds isolated also had similar characteristics in colour, form and opacity only CDM3 had a rough surface.

4.4.2: Morphological and microscopic characteristics of fungal isolates from clay soil.

The isolated colonies had the same characteristics. Mould isolates CSM3 and CSM2 were different in colour and elevation.

4.4.3: Morphological and microscopic characteristics of fungal isolates from uncrushed palm kernel shell.

All fungi isolates shown in table 12 had similar characteristics. Yeast isolated was all white in colour, raised, clear and dull in surfaces respectively. Mould isolate UPKSM1 was the only different isolate in colour and shape.

4.4.4 Morphological and microscopic characteristics of fungal isolates from naturally degrading ground palm kernel shell.

Yeast and Mould isolated at day 0 as in table 13 have the same characteristics. At day 5 and 10 similar characteristics were also observed. Yeast isolated at day 20 and 30 also had similar characteristics respectively. Mould isolated at each day had similar characteristics and NDGPKSM3 day 30 has wrinkled surface and was rhizoid in form.

Table 7: Morphological and microscopic characteristics of bacteria isolates colonies from Uncrushed Palm Kernel Shell (UPKS).

Isolate code	Colour	Shape	Surface	Elevation	Form	Opacity
UPKSB 1	Cream	Rod	Glistering	Elevated	Circular	Clear
UPKSB 2	Cream	Rod	Glistering	Elevated	Circular	Clear
UPKSB 3	Cream	Rod	Glistering	Elevated	Circular	Clear

KEY: UPKSB= Uncrushed Palm Kernel Shell Bacteria

Table 8: Morphological and microscopic characteristics of bacteria isolates colonies from Clay Soil (CS).

Isolate code	Colour	Shape	Surface	Elevation	Form	Opacity
CSB 1	Cream	Rod	Glistering	Elevated	Circular	Clear
CSB 2	White	Rod	Glistering	Convex	Circular	Opaque
CSB 3	Yellow	Rod	Glistering	Convex	Circular	Opaque

KEY: CSB= Clay Soil Bacteria

Table 9: Morphological and microscopic characteristics of bacteria isolates colonies from Chicken Droppings (CD).

Isolate code	Colour	Shape	Surface	Elevation	Form	Opacity
CDB 1	Cream	Rod	Glistering	Elevated	Filamentous	Not clear
CDB 2	Cream	Rod	Dull	Elevated	Filamentous	Not clear
CDB 3	White	Rod	Dull	Elevated	Filamentous	Not clear

KEY: CDB=Chicken Droppings Bacteria

Table 10: Morphological and microscopic characteristics of bacteria isolates colonies from Naturally Degrading Ground Palm Kernel Shell (NDGPKS).

Isolate code	Colour	Shape	Surface	Elevation	Form	opacity
DAY O						
NDGPKSB 1	Cream	Rod	Glistering	Elevated	Circular	Clear
NDGPKSB 2	Cream	Rod	Glistering	Elevated	Circular	Clear
NDGPKSB 3	Cream	Rod	Glistering	Elevated	Circular	Clear
DAY 5						
NDGPKSB 1	Cream	Rod	Dull	Convex	Filamentous	Opaque
NDGPKSB 2	White	Rod	Glistering	Convex	Circular	Clear
NDGPKSB 3	White	Rod	Dull	Convex	Circular	Clear
DAY 10						
NDGPKSB 1	Yellow	Rod	Glistering	Elevated	Circular	Translucent
NDGPKSB 2	Yellow	Rod	Glistering	Elevated	Circular	Translucent
NDGPKSB 3	Yellow	Rod	Glistering	Elevated	Filamentous	Translucent
DAY 15						
NDGPKSB 1	Cream	Rod	Dull	Elevated	Circular	Clear
NDGPKSB 2	Cream	Rod	Dull	Elevated	Circular	Clear
NDGPKSB 3	Cream	Rod	Dull	Elevated	Circular	Clear
DAY 20						
NDGPKSB 1	Cream	Rod	Glistering	Convex	Filamentous	Opaque
NDGPKSB 2	Cream	Rod	Glistering	Convex	Circular	Clear
NDGPKSB 3	Cream	Rod	Glistering	Elevated	Circular	Opaque
DAY 30						
NDGPKSB 1	Cream	Rod	Glistering	Elevated	Filamentous	Opaque
NDGPKSB 2	Cream	Rod	Glistering	Elevated	Circular	Opaque
NDGPKSB 3	Cream	Rod	Glistering	Elevated	Circular	Opaque

KEY: NDGPKSB= Naturally Degrading Ground Palm Kernel Shell Bacteria

TABLE11: Morphological and Microscopic characteristics of Fungal isolate from Chicken Dropping.

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
YEAST			
CDY1	Rough creamy colony growth	Growing a spherical to sub- spherical budding yeast- like cell or blastoconidia	*Candida tropicalis*
CDY2	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
CDY3	Whitish colony growth	The apex radiating from the entire surface, conidia are 1-celled and globose	*Candida stellate*
MOULD			
CDM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
CDM2	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
CDM3	Yellowish growth colony	A single, simple, dark and narrow conidiophores	*Aspergillus herbarious*

KEY: CDY – Chicken dropping yeast

CDM- Chicken dropping mould

TABLE12: Morphological and microscopic characteristics of Fungal isolate from Clay Soil

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
YEAST			
CSY1	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
CSY2	Rough creamy colony growth	Growing a spherical to sub-spherical budding yeast- like cell or blastoconidia	*Candida tropicalis*
CSY3	Smooth white coloured colony	Mycelium are not extensive. Conidia are 1-celled ovoid to fusoid.	*Candida oleophila*
MOULD			
CSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
CSM2	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
CSM3	White mycelia	Sparse mycelia, non- septate hyphae conidiophore	*Rhizopusstolonifera*

KEY: CSY – Clay soil yeast

CSM- Clay soil mould

TABLE13: Morphological and Microscopic characteristics of Fungal isolates from Uncrushed Palm Kernel Shell

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
	YEAST		
UPKSY1	Whitish colony growth	The apex radiating from the entire surface, conidia are 1-celled and globose	*Candida stellate*
UPKSY2	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
UPKSY3	Smooth white coloured colony	Mycelium are not extensive. Conidia are 1-celled ovoid to fusoid.	*Candida oleophila*
	MOULD		
UPKSM1	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
UPKSM2	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
UPKSM3	White mycelia	Sparse mycelia, non- septate hyphae conidiophore	*Rhizopusstolonifera*

KEY: UPKSY – Uncrushed palm kernel shell for yeast

UPKSM- Uncrushed palm kernel shell for mould

TABLE 14: Morphological and Microscopic Characteristics OF Fungal Isolates from Naturally Degrading Ground Palm Kernel Shell (NDGPKS).

ISOLATE CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	PROBABLE ORGANISM
(DAY 0)			
DGPKSM1	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
DGPKSM2	White mycelia	Sparse mycelia, non-septate hyphae conidia	*Rhizopus stolonifera*
DGPKSY	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
(DAY 5)			
DGPKSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
DGPKSM2	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
DGPKSY	Smooth white coloured colony	Mycelium are not extensive. Conidia are 1-celled ovoid to fusoid.	*Candida oleophila*
(DAY 10)			
DGPKSM1	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
DGPKSM2	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
DGPKSY	Rough creamy colony growth	Growing a spherical to sub-spherical budding yeast- like cell or blastoconidia	*Candida tropicalis*
(DAY 15)			
DGPKSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
DGPKSM2	Black coloured colony	Conidia head are large, globose, becoming radiate and tending to split into several loose columns with age.	*Aspergillus niger*
DGPKSY	Whitish colony growth	The apex radiating from the entire surface, conidia are 1-celled and globose	*Candida stellate*
(DAY 20)			
DGPKSM1	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
DGPKSM2	Brown mycelial growth	Mycelium are not extensive	*Aspergillus fumigatus*
DGPKSY	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*
(DAY 30)			
DGPKSM1	Yellow-green coloured colony	Conidia head are typically radiate, later splitting to form loose columns. Conidia are globose to sub-globose	*Aspergillus flavus*
DGPKSM2	Light gray mold	Sparse mycelial, non-septate hyphae conidia	*Aspergillus tamari*
DGPKSY	Cream coloured, smooth and glabioues	Spherical budding yeast- like cell or blastoconidia	*Candida albican*

KEY: DGPKSY – Degrading ground palm kernel shell yeastDGPKSM –Degrading ground palm kernel shell mould

4.4 Biochemical Characterization of Bacterial Isolates from Samples

4.4.1 Biochemical Characterization of Bacterial Isolates from uncrushed palm kernel shell.

All isolates in table 15 tested positive to catalase, gram staining, gram reaction and fermented lactose, sucrose, fructose and Manitol.

4.4.2 Biochemical Characterization of Bacterial Isolates from chicken droppings.

All isolates in table 16 tested positive to catalase, gram staining, gram reaction and fermented lactose, sucrose, fructose and Manitol.

4.4.3 Biochemical Characterization of Bacterial Isolates from clay soil.

All isolates tested as shown in table 17 tested positive to catalase, gram staining, gram reaction and fermented lactose, sucrose, fructose and Manitol.

4.4.4 Biochemical Characterization of Bacterial Isolates from naturally degrading ground palm kernel shell.

Bacterial isolates at day 0 and 5 as shown in table 18 tested positive to catalase, gram staining, gram reaction and fermented lactose, sucrose, fructose and Manitol respectively. Isolates at day 10, 20 and 30 tested positive to catalase, gram staining and grams reaction but NDGPKSB2 day 10 and 20 didn't ferment the sugar. Bacterial isolates at day 15 tested positive to catalase, gram staining, gram reaction and fermented lactose, sucrose, fructose and Manitol respectively.