ISOLATION AND IDENTIFICATION OF MICROORGANISMS ASSOCIATED WITH GROUNDED PALM KERNEL SHELLS USING CHICKEN DROPPINGS AS INNOCULUM AT RATIO 2:1.

BY

AYODELE GANIYAT BUKOLA

MCB/11/0330

A PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, FEDERAL UNIVERSITY, OYE EKITI, EKITI STATE, NIGERIA.

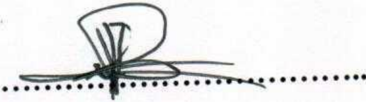
SUPERVISOR, DR(MRS) GABRIEL-AJOBIEWE

OCTOBER, 2015

CERTIFICATION

This thesis titled "Isolation and identification of microorganisms associated with palm kernel shell using chicken dropping as inoculum on sandy soil" was carried out and submitted by AYODELE GANIYAT BUKOLA and has satisfied the requirement for the award of Bachelor of Science (B.SC) Degree in Microbiology, in the Department of Microbiology, Faculty of Sciences, Federal University Oye Ekiti, Nigeria.

I AYODELE GANIYAT BUKOLA hereby undertake that the contents therein are outcome of an original research work undertaken by me and that I am responsible for all the contents.



……………………………… 07/12/2015

Dr (Mrs) R.A.O Gabriel-Ajobiewe Date

Supervisor

……………………………… 12/8/16

Prof Bryan Ogeneh Date

HOD Microbiology

DEDICATION

The success of this work is dedicated to the Glory of Almighty Allah for giving me life, strength, protection and opportunity and who has blessed me with all spiritual blessing, impacting me with wisdom, knowledge, and understanding. It is on this note that, I show my profound gratitude because He has, is and will always be the source of all creatures. Also I thank my parents who supported me all through, for their financial and moral support, their love, care and concern.

ACKNOWLEDGEMENTS

My warm and profound gratitude goes to Almighty Allah who is by his mercy, protection and guidance has led me so far and has seen me successfully through my years of study.

My sincere thanks and appreciation goes to my understanding supervisor, Dr Adefolakemi Ruth Gabriel-Ajobiewe for her guidance, mentoring and tutelage throughout the period of this research project.

A very big thank to my Head of Department- Prof. Bryan Ogeneh, and to all my Lecturers (Dr Ojo, Mrs Nwokeoma, Dr Akinyele, and Mr Osanyinusi) who through the years have improved me intellectually, morally, and whose achievements inspire me to continually improve myself and become excellent in the things I have learnt.

I owe profound gratitude to my beloved Parent, Mr and Mrs. Ayodele Baruwa who has morally and financially contributed to my success. I pray God to spare their life with good health so as to reap the fruit of their labour.

I also want to use this medium to express my profound gratitude to the love of my life, Raheem Nurudeen Ayodele, a real friend indeed who has a footprint in my life, May Almighty Allah be with you and continue to shower his blessing and favour upon your life.(Amen).

My appreciation goes to all the non-teaching academic staffs in the department of Microbiology. I specially thanks Mr Odun, Mrs Adebiyi, and Mrs Falade for putting me through the practical work.

To all my colleagues and final year students in the department, you are blessed and always remembered.

To my siblings, you are the best brothers and sisters I will always crave to have. Thank you so much for your support and love.

TABLE OF CONTENT

Contents

Title Page i

Certification ii

Dedication iii

Acknowledgements iv

Table of Content v

List of Table xi

Abstract xiv

CHAPTER ONE:

1.0 INTRODUCTION 1

1.1 Palm Kernel Shell (PKS) 2

1.2 Chicken Droppings 5

1.2.1 The chemical and microbial analysis 5

1.3 Sandy soil 6

1.4 Aims and Objectives of the study

CHAPTER TWO:

2.0 Literature Review 7

2.1 INTRODUCTION 7

2.1.1 Inoculum formulation 7

2.1.2 Inoculum development and maintenance

2.1.3 Pre-treatment of Empty fruit Bunches(EFB) 8

2.2 Biodegradation of Palm oil Effluent(POME) 9

2.2.1 Sampling 9

2.3 Characterization and identification of microbial isolates 10

2.3.1 Bacterial isolates 10

2.3.2 Mold Isolates 10

2.3.3 Yeast Isolates 10

2.3.4 Gas chromatography –mass spectroscopy (GS-MS) of palm oil Mill effluent and soil extracts 11

CHAPTER THREE:

3.0 Materials and Method

3.1 Materials 12

3.1.1 Chemical Reagent Used 12

3.1.2 Media Used 12

3.2 Collection of samples 12

3.2.1 Palm Kernel Shell Samples 12

3.2.2 Soil Samples 13

3.2.3 Chicken Droppings Samples 13

3.3 Sample Preparation 13

3.3.1 Palm Kernel Shell Preparation 13

3.3.2 Preparation of Chicken Droppings 14

3.3.3 Preparation of Sandy Soil 14

3.4 Media Preparation and Sterilization 15

3.4.2 Sabourand Dextrose Agar (SDA) 15

3.4.3 Nutrient Agar 15

3.4.4 Nutrient Agar Slant 15

3.4.5 Yeast Extract Agar 16

3.5 Microbiological Analysis 16

3.5.1 Enumeration of Microorganisms from samples 16

3.5.2 Isolation of Bacterial Isolates 17

3.5.3 Isolation of Fungal Isolates 17

3.5.4 Preservation of isolated microorganisms 17

3.6 Characterization of bacteria Isolates from Samples 18

3.6.1 The Gram Stain 18

3.6.2 Biochemical Characterization of Bacterial Isolates 18

3.6.2.1 Sugar fermentation 18

3.6.2.2 Production of acid from sugar and gas formation 19

3.6.2.3 Catalase Test 19

3.6.2.4 Gram's Reaction 19

3.7 Characterization of fungal Isolates from Samples 20

3.8 Biodegradation of Ground Palm Kernel Shell Inoculated 20

with Chicken Droppings at ratio 2:1

3.9 Physicochemical properties of degrading lnoculated Ground Palm Kernel Shells 21

3.9.1 pH measurement 21

3.9.2 Total Titratable Acid(TTA) 21

3.9.3 Microbial count of biodegrading lnoculated Ground Palm Kernel Shell and Chicken Droppings 21

CHAPTER FOUR:

4.0 RESULTS AND DISCUSSION

4.1 RESULT 22

4.1 Microbial viable Count of Samples 22

4.1.1 Total Viable Count of Uncrushed Palm Kernel Shells 22

4.1.2 Total viable Count of Chicken Droppings 22

4.1.3 Microbial viable Count of Sandy Soil 22

4.2: The total viable count of Degrading Bacteria, Yeast and Mold from Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings 26

4.2.1 Total viable bacterial count from Degrading Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings (CD) at ratio 2:1 26

4.2.2 Total viable Yeast count of isolates from Degrading Ground Palm Kernel Shell Inoculated with chicken droppings at Ratio 2:1 26

4.2.3 Total viable Mold count of isolates from Degrading Ground palm kernel shell (DGPKS) inoculated with chicken droppings at Ratio 2:1 26

4.3 The morphological characteristics of bacteria isolates from Uncrushed Palm Kernel Shell (UPKS) 30

4.3.1 The morphological characteristics of bacteria isolates from Chicken Droppings (CD) 30

4.3.2 The morphological characteristics of bacteria isolates from Sandy Soil 30

4.4: Morphological characteristics of bacteria isolates from degrading ground palm kernel shell (DGPKS) inoculated with chicken dropping (CD) at ratio 2:1 34

4.4.1 The morphological and Microscopic characteristics of fungal isolates from Uncrushed Palm Kernel Shell (UPKS) 34

4.4.2 The Morphological and Microscopic characteristics of Fungal isolates from Chicken Droppings (CD) 34

4.4.3 The Morphological and Microscopic characteristics of Fungal isolates from sandy soil (SS) 34

4.4.4 The Morphological and Microscopic characteristics of fungal isolates from Degrading Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings (CD) 35

4.5 Biochemical test and Morphological description of bacterial isolates from Uncrushed palm kernel shell (UPKS) 42

4.5.1 Biochemical test and Morphological description of bacterial isolates from Chicken Droppings (CD) 42

4.5.2 Biochemical test and Morphological description of bacterial isolates from Sandy Soil (SS) 42

4.5.3 The biochemical test and identification of probable Degrading bacteria isolates fromdegrading ground palm kernel shell (DGPKS) Inoculated with chicken droppings at 42

Ratio 2:1

4.6 Total Titratable Acid from Ground palm kernel shell Inoculated with Chicken Droppings (CD) 49

4.7 pH reading from Ground Palm Kernel Shell(GPKS) Inoculated with Chicken Droppings(CD) 49

4.8 DISCUSSION 52

CHAPTER FIVE:

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion 53

5.2 Recommendations 53

REFERENCES 54

LIST OF TABLES

TABLES		PAGE
1	Total viable Microbial count isolated from Uncrushed Palm Kernel Shell	24
2	Total viable microbial count isolated from Chicken Droppings (CD)	25
3	Total viable microbial count isolated from sandy soil (SS)	26
4	Total viable Bacterial count from Degrading ground palm kernel shell (DGPKS)Inoculated with Chicken Droppings (CD) at ration 2:1	28
5	Total viable Yeast count of isolates from Degrading ground palm kernel Shell(DGPKS) Inoculated with Chicken Droppings at Ration 2:1	29
6	Total viable mold count of isolates from Degrading ground palm kernel Shell (DGPKS) inoculated with Chicken Droppings (CD) at Ration 2:1	30
7	Morphological characteristics of bacteria isolates from Uncrushed Palm Kernel Shell (UPKS)	32
8	Morphological characteristics of bacteria isolates from Chicken Droppings	33
9	Morphological characteristics of bacteria isolates from Sandy Soil(SS)	34
10	Morphological characteristics of Bacterial Isolates from Degrading Ground Palm Kernel Shell(DGPKS) inoculated with Chicken Droppings(CD) at Ratio 2:1	37
11	Morphological and microscopic characteristics of fungal isolates from	38

	Uncrushed palm kernel shell (UPKS)	
12	Morphological and Microscopic characteristics of fungal isolates from Chicken Droppings (CD)	39
13	Morphological and Microscopic characteristics of fungal isolates from Sandy Soil(SS)	40
14	Morphological and Microscopic characteristics of fungal isolates from Degrading Ground Palm kernel Shell(DGPKS) Inoculated with Chicken Droppings at Ratio 2:1	41
15	Biochemical test and Morphological Description of bacterial isolates from Uncrushed Palm Kernel Shells	43
16	Biochemical test and Morphological Description of bacterial isolates from Chicken Droppings	44
17	Biochemical test and Morphological Description of bacterial isolates from Sandy soil (SS)	45
18	Biochemical tests and identification of probable degrading bacteria isolates From Degrading Ground Palm kernel Shell (DGPKS) Inoculated with Chicken Droppings (CD) at Ratio 2:1	46
19	Total tritratable acid from Ground Palm Kernel Shell Inoculated with Chicken Droppings	49
20	pH reading from Ground Palm Kernel Shell Inoculated with Chicken Dropping	49

ABSTRACT

This research study is designed to determine the microbial count of Chicken Droppings and Ground Palm kernel shell in which the chicken droppings is used as inoculum on Ground Palm Kernel Shell at Ratio 2:1 in which the inoculum size is half of the Ground Palm Kernel Shell. Pollution of the environment due to oil production can be reduced through this study because is aimed at examining the microorganism responsible for the biodegradation that can be achieved in Palm kernel shell pollution after supplementing with organic manure(chicken droppings) .On every 5days of biodegradation 5g of the sample was picked and kept for microbial assay. The study demonstrated that chicken droppings contain more microorganisms that can breaks down quite rapidly. Bacteriological analysis was carried out on the samples for Isolation , characterization and identification of microorganisms associated with the biodegradation process as well as total aerobic plate count were carried out and compared over time with respect to the different concentrations. Biochemical test results on the isolates indicate that Palm kernel shell contained a variety of microorganisms which include *Klebsiella pneumoniae* ,*Bacillus sp*, *Proteus sp*, *Pseudomonas, Staphylococcus aureus* and *Rhizopus sp.* Analysis of Ground palm kernel shell mixed with chicken droppings revealed that the bacterial counts were encouraged while the proliferation of palm kernel shell degrading bacteria in the soil was depressed. The microorganism isolated are rod and coccibut rod are the dominating microorganism and most of the organism identified are gram(+) and gram(-) bacteria. The pH reading shows that Ground Palm Kernel inoculated with chicken droppings at day 0 to day 5 ranges from 4.59 pH – 6.02pH which is in acidic condition but from day 10- day 30 the alkalinity increases which indicate that the Degrading microorganism are in alkaline condition. From Day 0-Day 30 the pH ranges from 4.59pH – 12.50pH. The Total Titratable Acid (TTA) shows that the degrading microorganism are able to produce organic acid in which the acidity increases over time. The count of the bacteria from Uncrushed Palm Kernel Shell ranged from 1.8×10^6(cfu/g) to 2.2×10^6 (cfu/g), while that of soil ranged from 7.2×10^7 to 2.03×10^7 (cfu/g), the bacteria of the bacteria of chicken droppings ranged from 0.6×10^7 to 4.0×10^6 (cfu/g). The degrading bacteria of palm kernel shell inoculated with chicken droppings from Day 0- Day 30 ranged from 1.96×10^7 to 0.5×10^7(cfu/g) which indicate relatively high counts of bacteria and fungi in the samples.

CHAPTER ONE

1.0 INTRODUCTION

1.1 PALM KERNEL SHELL (PKS)

The oil palm (*Elaeis guineensis*) belong to the species of *Elaeis genus* and it is in the family Palamae. The palm kernel shells are the waste products from the oil palm processing. Oil palm nativity had been associated with the tropical rainforest of West Africa but has spread to most of the equatorial tropics of South-East Asia and America. In oil palm processing: palm oil fibre, effluent, palm kernel shell (PKS) and empty fruit bunch are regarded as wastes. Palm kernel shell as one of the wastes accruing from oil palm processing can be suitably converted to renewable energy by applying suitable thermochemical process (Abdullah *et al.*, 2011).

Palm kernel shell is characterized for its useful application in bio- energy production (Pansamut *et al.*, 2003). PKS is a potential feedstock for bio-oil, bio-char and biogas production through fast pyrolysis and/or gasification process through laboratory analytical methods. The fuel derived from it can be environmentally friendly since it possesses less sulphur and nitrogen (Mohammed *et al.*, 2011).

Palm kernel shell (PKS) was partially a waste in the 90s and early 2000, more than 300,000 tons were available for sale to be used in different commercial ventures. Palm kernel shell is used on environment for Green House Gases (GHG) emission reduction and also used for production of fuel being a neutral carbon fuel.

Biodegradation is the natural way of recycling wastes or breaking down organic matter into nutrients that can be used by other organism (Teoh. CH (2002). The palm kernel shell is broken down by microorganism in the soil through biodegradation and the parameters to be analyzed are: The surface area, moisture content, pH , bulk density, pore volume, porosity, tortuocity, ash content, and metal ions in the palm kernel shell (Parveen *et al.*, 2010).

The supply of PKS to the palm kernel crusher Rawang plant(RP), Selangor in late 2000 and Kanthan plant(KP), Perak in mid 2001, are reported to be using PKS as their fuels to generate power for their plants and steam for oil palm fruit processing. PKS is also used as neutral carbon fuel by replacing it with fossil fuel.

The palm kernel shell produce activated carbons which have so many important uses like clean-up of cane, beet and corn-sugar solutions. It is also use for the removal of tastes and odours from domestic and industrial water supplies, vegetable and animal fats and oils, alcoholic beverages, chemicals and pharmaceuticals and in waste water treatment (Prasertsan and Prasertsan 2005).

The activated carbon that is produced from the palm kernel shell is used for removing pollutant from gaseous, aqueous, and non-aqueous streams. During carbonization of the palm kernel shell most of non- carbon elements like oxygen, carbon dioxide, hydrogen, nitrogen, and sulphur are eliminated as volatile gaseous products by pyrolytic decomposition of the palm kernel shell (Singh *et al.*, 2010). The palm kernel waste degradation is given serious attention because its application concern treatment of drinking water and waste water. By producing activated carbon from the palm kernel shell help to reduce waste and reuse because the negative impact is cause by indiscriminate disposal of such wastes (Rincon and Gomez 2012).

1.2 CHICKEN DROPPINGS

Chicken droppings is a mixture of poultry excreta, spilled feed, feathers, and material used as bedding in poultry operations. Common bedding materials include wood shavings, sawdust, peanut hulls, shredded sugar cane, low-cost organic materials. The chicken dropping can be a valuable resource to optimize pasture production. It is mostly organic matter and supplies nutrients, helps hold moisture, improves soil structure and encourages the growth of organisms. The chicken droppings contain nutrients like nitrogen, phosphorus, and potassium in mineral and organic forms, and also contain some trace elements like copper, zinc, manganese, boron, calcium and chloride1, the availability of these elements is affected by soil pH and texture, with sandy soils having less nutrient build-up (Shreve *et al.*, 2007).

Nutrient levels of chicken droppings vary within manure considerably, depending on the diet and age of the chickens, and the type of bedding manure it is mixed with. For instance, manure mixed with straw has a different nitrogen composition than pure manure. The major issue with the re- utilization of chicken droppings is the generation of ammonia. Ammonia is produce by microbial breakdown of fecal material in the droppings. It is well documented in the literature that higher moisture levels results in higher ammonia production (Kelleher *et al.*, 2002).

Application for used of poultry droppings is occasionally used as poultry material for the greenhouse and plant container industries, and also there has been an upsurge in the use of chicken droppings as a bio- fuel source for electrical cogeneration and gasification. Poultry droppings is used in Ireland as a biomass energy source, thus removing other fossil fuels. It is also used for producing valuable by –products including activated carbons and fertilizers (Bolan *et al.*, 2010).

During the application of chicken manure to the soil it breaks down rapidly through the process of microbial decay and it leaves little residue. The nutrients are mineralized within a week of application and also there is further release after two weeks. The application of chicken manure does not alter the sandy soil pH or soil water holding capacity (Oni *et al.*, 2013).

Chicken droppings contains significant amounts of nitrogen because of the presence of high levels of protein and amino acids. Chicken droppings is also usually recommended to control pathogens in the end products. Chicken droppings contains a large and diverse population of microorganisms. Microbial concentrations in chicken droppings can reach up to 10^{10} cfu/g, and Gram-positive bacteria, such as *Actinomycetes, Clostridial, Eubacteria, Bacilli/Lactobacilli*, account for nearly 90% of the microbial diversity. A variety of pathogens can be found in chicken droppings such as *Actinobacillus, Bordetalla, Campylobacter, Clostridium, Corynebacterium, Escherichia coli, Globicatella, Listeria, Mycobacterium, Salmonella, Staphylococcus, and Streptococcus*. While different microbes display different metabolic activities within the droppings environment, high levels of background microflora may interfere with the survival and growth of pathogens in chicken droppings (Lovett *et al.*, 1971).

The chicken waste promotes a very significant changes in soil microbial activity which lead to a slight rise in soil temperature and also human pathogenic bacteria were not found inside the chicken manure. Leaching columns were used to study what happens to chicken manure in the soil in the absence of plants. The chicken manure was applied at different rates, either it is mixed into the soil or it is placed on the surface, water was applied to the top of each column each day for up to three weeks and the leachate taken from the bottom of the column was analysed for nutrient ions. And at the completion of leaching, the soil was removed from the column and analyzed so that we can have the knowledge on how chicken manure breaks down to release nutrients to plants and how it changes the soil properties. The most nutrient release by the chicken manure were released within 14days of application and even the majority was released in the first 7days. The nitrogen released involved firstly the release of ammonium ion, but when it is after 7 days, most of the nitrogen was released in the form of nitrate (Shepherd *et al.*, 2007).

The chicken manure can be incorporated or applied into the soil but when applied on the surface of the soil the released nutrient will take longer to leach. The addition of incorporated chicken manure caused a very significant increase in soil microbial respiration, and it also increase carbon dioxide formation by 150-180% at a high application rate (Bansal and Goyal 2005). The main reason of applying it is that it provides the plant nutrients, increased the soil bacterial activity and it also elevate soil temperatures. But the environmental concerns of applying chicken manure to sandy soil include nitrate and phosphate leaching, carbon runoff, bacterial contamination, nuisance insect breeding and green house gas emissions (Moore *et al.*, 1998). And the Major constituent of poultry manure is organic matter with a significant energy content which becomes soluble as it is broken down by soil microbes. If such soluble organic matter enters waterways it can increase biological oxygen demand (BOD) and cause negative impacts on aquatic life a related problem is bacterial runoff, where it has been noted that fecal coliform levels from land receiving manure treatments are often high.

1.2.1 THE CHEMICAL AND MICROBIAL ANALYSIS

According to Rincon and Gomez (2012) a comparative study was done on physico-chemical properties of activated carbon of oil palm waste which is palm kernel shell, the properties that was evaluated were surface area, moisture content, pH, bulk density, pore volume, porosity, tortuocity, ash content and metal ions that are present in the palm kernel shell. The analysis of the physico- chemical properties form were, moisture content; 2.15% and 3.34%, ash content; 6.10% and 6.7%, surface area; $1080m^2/g$ and $1030m^2/g$, p H; 6.7 and 6.6, bulk *density*; 0.64 and 0.56, porosity; 63.6% and 57.9%, pore volume; $0.69cm^3/g$ and $0.53cm^3/g$ and tortuocity, 1.57 and 1.72. The analysis of metal ion revealed that palm kernel shell is composed of 47.60% of potassium.

The breakdown of the chicken manure organic matter, and the subsequent processes of mineralization of nutrients are mediated by soil bacteria and fungi and the changes in the soil properties are associated with microflora which improve the infiltration and porosity, increased soil respiration and co_2 evolution, the method of developing indicators of disease suppressive soils and it recognizes that bacteria which proliferate under conditions of high energy availability increase in numbers after organic amendment in soil and the new method also determines the ratio of copiotrophic bacteria to oligotrophic bacteria by plating soil on carbon rich and carbon poor media (Ringer *et al.*, 1997).

1.3 SANDY SOIL

Sandy soils are characterized by less than 18% clay and more than 68% sand in the first 100cm of the solum. These soils have developed in recently deposited sand materials such as alluvium or dunes. They are weakly developed and show poor horizonation. Soils characterized by a high proportion of sand in the first 100cm can also correspond to the upper part of highly developed soils formed in weathered quartz-rich material or rock, as evidence by the development of a highly depleted horizon (Bruand *et al.*, 2004).

Sandy soils are often considered as soils with physical properties easy to define: weak structure or no structure, poor water retention properties, high permeability, highly sensitivity to compaction with many adverse consequences (Hodnett and Tomasella., 2002).

According to Coquet (1995) measured the shrinkage properties of sandy soils, results obtained in the field and in the laboratory showed very small shrinkage: bulk volume variation was only 0.05%. when dried sandy soils develop very few thin cracks organized in a loose network.

Sandy soils in the tropics show a large range of porosities and consequently bulk density(D_b). porosity ranges from 33% (D_b =1-78gcm^{-3}) to 47% (D_b= 1.40gcm^{-3}) are commonly recorded. The porosity in sandy soils is usually smaller than in clayey and silty soils. Because of the very small inter-particle cohesion that results in a very small aggregate stability, sandy soils are highly sensitive to surface crusting whereas crusts protect the soil surface from wind and interrill erosion but they also favour runoff and consequently rill and gully erosion(Buand *et al.*, 2004)

1.4 AIMS AND OBJECTIVES OF THE STUDY

This work is therefore designed to achieve these objectives;

1) To determine the microbial count of Palm Kernel shell, chicken droppings and sandy soil during biodegradation.
2) To know how the consortium organism in chicken dropping and sandy soil break down Palm Kernel Shell.
3) To solve the problem of agro waste pollution in the environment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

There have been some efforts to recycle oil palm empty fruit bunches (EFB); some of the examples are composting, the generation of methane for energy, and the production of potting materials and mulching materials. EFB is a renewable biomass and contains fairly high cellulose. Fresh EFB contains 30.5% lignocellulose, 2.5% oil and 67% water. The lignocellulose consists of 45-50% cellulose, 32.8% hemicellulose and 20.5% lignin (Husin *et al.,* 2005). Lignin is difficult to degrade biologically; hence it reduces the bioavailability of the other lignocellulose components.

Controlled fermentation process can alter the hydrolysis route of degradation (Zeotemeyer 1982). As a result of the proliferation of specific group of microorganisms, specific compounds can be produced from such fermentation, EFB can be a classical candidate for a controlled fermentation process; the development of mixed microorganisms are suggested by Zeotemeyer (1982) may direct the fermentation to degrade the cellulose component. The objective of this study was to determine the effects of aeration rate in the biodegradation of pure cellulose and empty fruit bunches (EFB).

Standard inoculum preparation was applied to all treatments. Two carbon sources were used to compare the degradation rates of EFB and CMC (carboxyl methyl cellulose). The performance of batch fermentation on different carbon sources in different parameter effects was studied. For each studied the degradation rate of EFB and CMC was investigated based on the chemical oxygen demand of soild (COD_S) and dry matter (DM) measurement (Adamu *et al.*, 2008).The optimization studies of the fermentation process was carried out based on the parameters that yielded the best degradation rate as formulated below:

$$\text{Degradation rate (mg/litre/h)} = \frac{\text{Overall DM reduction (mg/litre)}}{\text{Fermentation time (hour)}}$$

2.1.1 Inoculum formulation

Standard inoculum preparation was used in the entire study. The inoculum was developed through enrichment in medium developed similar to the target fermentation process. The medium contained mainly POME (palm oil mill effluent) and EFB. The composition of the medium was developed at MARDI laboratory (Bioprocess Laboratory). The EFB was pre-treated for uniform size reduction and acid hydrolysis with 0.5M HCl. The same POME (palm oil mill effluent) and EFB were used for the growth and maintenance of the inoculum. Except for the initial samples, the subsequent raw materials for medium development utilized the same POME and EFB stored in the freezer (Ayob *et al.*, 2013)

2.5.4 Inoculum development and maintenance

The inoculum was enriched, developed and maintained in a 2 litre bioreactor (Biostat B, B. Braun Biotech International, Germany) independent of the actual fermentation study. Initially, raw materials used, POME and EFB, were taken fresh from the mills to assure the presence of mixed microorganisms. After several transfers, the same materials that had been stored in the freezer were utilized. This was to ensure the composition of the medium remained the same (Duan *et al.*, 2003).

The development of the inoculum was executed in a semi-continous process operated at 28^0C, agitated at 150 rpm, aerated at 0.5vvm, and pH of 6. The culture was maintained by replacing the culture with 10% (v/v) fresh medium once every 5 days. The inoculum was like a sludge, bad odour and brown colour.

2.1.2 Pre-treatment of EFB

EFB was dried under sunlight and shredded into loose fibrous materials by using a shredder (B & W Engineering , Angel Dove, Ely, Camb). The shredded EFB were hydrolyzed with 0.5M HCl at 121^0C for 30 min. After the hydrolysis process, the EFB was homogenized to 1-2 mm and the pH was adjusted to 6.0 with concentrated 2.0 M NaOH.

2.2 Biodegradation of palm oil mill effluent (POME)

POME effluent of 1- week-old was collected from the effluent pond at palm oil mill in Dengkil, Selangor and stored in a freezer at -2^{0}C. During the fermentation process, triplicate sample of 50ml each was taken twice daily to access the performance of the fermentation. A volume of 5ml of the sample was taken for microbial population analysis and 15ml for COD total (COD_t) measurement. The remaining 30ml sample was further centrifuged at 9,000 rpm (Universal 16R, Hettich, Hettich Zentrifugen, Germany) for 10 min at 5^{0}C. The pellet was used to measure the dry cell weight and COD soilds (COD_S) (Elorrieta *et al.*, 2002).

The soil samples used for biodegradation studies were collected from a demarcated area within the Kogi State University, Faculty of Agriculture farmland at Anyigba, bulked into a composite sample, poured into properly labeled clean polythene bags and transported from the site to the laboratory. The samples were air-dried, stones and unwanted materials were removed and the residue crushed to finer particles to ensure passage through a 2 mm mesh before use.

The pollution of soil with POME was done using a simple randomized block design with three replicates. Each treatment represented a block with three plastic buckets for the three replicates. 33 buckets were filled with soil from the 0-30 cm of top soil weighing 6 kg per bucket from the study area. 30 buckets were polluted with POME at a moderate pollution level of 20 %. (This translated to 1200 ml of POME being applied to 6 kg of soil). Biodegradation was done with chicken droppings at three different concentrations (10 %, 20 % and 30 %) in triplicates. Three buckets containing POME and soil and three buckets containing soil alone had no organic waste applied to them, and served as control. Sampling was done immediately after pollution and subsequently, after one month and two months.

Microorganisms in the soil samples were enumerated by spread inoculating 0.1ml ten-fold serially diluted samples onto nutrient agar (NA), Sabouraud Dextrose agar (SDA) and Palm oil agar (POA) for the enumeration of aerobic heterotrophic bacteria, fungi and palm oil utilizing bacteria respectively. The POA contained 10 ml of palm oil in 990 ml of mineral salts medium containing K_2HPO_4, 1.8g/l, KH_2PO_4, 1.2g/l, NH_4Cl, 4.0g/l, Mg

$SO_4.7H_20$, 0.2g/l, $FeSO_4.7H_2O$, 0.01g/l, NaCl, 0.1g/l, agar-agar, 20g/l and 1 % palm oil. The innoculated NA plates were incubated at 30 ^{0}C for 48hr while the SDA plates were incubated at 25^{0}C for 3-5 days. Observed colonies were counted and expressed as colony forming units per gram (cfu g^{-1}) of soil (Doyle *et al*., 2007).

2.3 Characterization and Identification of Microbial Isolates

2.3.1 Bacterial Isolates

The bacterial isolates were characterized based on their cultural and biochemical properties which included production of coagulase, catalase, indole, urease, motility test, citrate utilization test, starch hydrolysis, Methyl Red-Voges Proskaeur (MR-VP), triple sugar iron test, utilization of sodium azide and various carbohydrates (glucose, lactose, maltose, fructose, mannitol, sucrose, and arabinose). The isolates were identified to the species level by comparing their characteristics with those of known taxa, in Bergey's Manual of Determinative Bacteriology.

2.3.2 Mold Isolates

Mold isolates were characterized based on microscopic and macroscopic appearances which comprised pigmentation, colour of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, presence of special structures such as foot cell, sporangiophore or conidiophores and the characteristic of the spore head. The identities of the isolates were determined using the scheme of Domsch and Gams (1970).

2.3.3 Yeast Isolates

Yeast isolates were Gram stained and characterized based on colonial morphology, cell micromorphology, germ tube and blastosphore formation, gelatin liquefaction, starch hydrolysis, growth at 37^{0}C and on 50% glucose, and fermentation of the following carbohydrates: galactose, glucose, sucrose, maltose, and lactose. The identities of the isolates were determined using the scheme of Barnett and Pankhurst (1974).

2.3.4 Gas Chromatography-Mass Spectroscopy (GC-MS) of Palm Oil Mill Effluent and Soil Extracts

To obtain the POME extract, 1 litre of the POME was extracted with one litre of petroleum ether (40-60 ^{0} C) two consecutive times and evaporated to dryness. For the soil samples, 100 g of each was extracted using a Soxhlet extractor (Electrothermal) with 500 cm^3 of petroleum ether (40-60 ^{0}C) and evaporated to dryness. Gas chromatography (GC) analysis of the extracts was carried out using GC-MS-QP2010 PLUS (Shimadzu, Japan) which was equipped with a capillary inlet and mass selective detector set to scan from mz^{-1}45 to mz^{-1} 800 at a scan rate of 1.2 scans $second^{-1}$. The injection temperature was programmed from 80 ^{0}C to 250 ^{0} C at a total flow rate of 6.2 mL min^{-1} using helium as the carrier gas (Bek-Nielsen *et al.*, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS

Electronic weighing balance, 100ml volumetric flask, 500ml beaker, 100ml beaker, Aluminum foil paper, black polythene nylon, ice block nylon, zip lock bag, paper tape (Abro), Ethanol, Water bath(HH-SB) DIGITAL THERMOSTATIC WATER BATH , test tube, sterile distilled water, microscope(B1 Advanced series), inoculating loop, cotton wool, ice cube container, super bottle, Biggo bottle, The Vortex Mixer(XH-B) Techmel & Techmel China, 2ml and 5ml Syringes, petri dishes, Autoclave, Sterile nose mask, Sterile hand gloves, Oven, Bunsen Burner, Spatula, conical flask, grease free glass slide .

3.1.1 Chemical Reagent used

Potassium hydroxide (3%), Ethanol (75%), and 250mg of Chloramphenicol capsule (to inhibit bacterial growth on the yeast agar media and sabourand dextrose Agar).

3.1.2 Media Used

Nutrient agar (Manufactured by BIOMARK^{TM}, in India), Sabourand dextrose agar (Manufactured by BIOMARK^{TM}, in India), Yeast Extract agar(Manufactured by Lab M Limited, United Kingdom), Peptone water.

3.2 Collection of Samples

3.2.1 Palm Kernel Shell Samples

The palm kernel shell were collected from a traditional oil industry in Oye local government area Ekiti State, Nigeria. The palm kernel shell were washed severally with distilled water in order to remove dirt's and dusts. The sample was further dried in the oven for 7days and later grounded to obtain a powder form of the waste. The powder sample was then kept in air-tight containers for further analysis. The remaining Uncrushed palm kernel shell was kept in the fridge for microbial detection and identification.

3.2.2 Soil Samples

The Surface sandy soil samples were collected from three different sampling points coded A, B and C from the depth of 0-10cm using a sterile trowel. About 1000g of sandy soil samples was collected from point A was placed in a sterile labeled sampling bag and kept in the laboratory, as well as sample B was about 100 meters from point A and Sample C was taken at the School Physical Planning Unit building. All the soils samples were transported immediately to the laboratory for analysis. All the samples are in Triplicates.

3.2.3 Chicken Droppings Samples

The chicken droppings was collected to a sterile closed container from Federal University Oye Ekiti Poultry Site and the type of chicken are all layers and they the type of feed given to them is Metrovet Feeds, Hi-Yield diet, Fortified with selcon forte(Nutritional Insurance against viral infections)

3.3 Sample Preparation

3.3.1 Preparation of Palm kernel Shell

After collection of samples, analyses were carried out immediately. A setup for serial dilution was done in which the uncrushed palm kernel shell was mixed with 1% peptone water in Zip Lock Bag and was shaked thoroughly so that the organism present in the palm kernel shell will be release inside the peptone water and the sample were serially diluted to a dilution factor of Ten (10^{-10}). Each dilution was done by sterilizing the test tubes containing 9ml of 1% peptone water after which(under an aseptic environment) 10g of the samples(Palm Kernel Shell) was mixed with 90ml of peptone water inside Zip Lock Bag and inoculated into the 9ml peptone water(10^{-1}), and these process continues till the 10^{-10} dilution factor by the use of 2ml and 5ml syringes.

After the serial dilution, 0.1ml from each 10^{-6} and 10^{-5} was inoculated into three sterile petri dishes, one plate from dilution 6 and 2 plates from dilution 5 (for bacteria culture).
0.1ml from dilution 10^{-2} and 10^{-3} was inoculated into three sterile petri dishes, one plate from dilution 3 and 2 plates from dilution 2(for mold culture).

From dilution 10^{-4} and 10^{-5}, 0.1ml of the aliquot was inoculated into three sterile petri dishes, one plate from dilution 4 and two plates from dilution 5(for Yeast Culture) Spread plate method was done in which the suspended organism was spread on the petri dishes and the agar was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface

3.3.2 Preparation of chicken droppings

After collection of samples, analyses were carried out immediately. A setup for serial dilution was done in which the 10g of chicken droppings was mixed with 1% peptone water in Zip Lock Bag and was shaked thoroughly so that the organism present in the chicken droppings will be release inside the peptone water and the sample were serially diluted to a dilution factor of Ten (10^{-10}). Each dilution was done by sterilizing the test tubes containing 9ml of 0.1% peptone water after which(under an aseptic environment) 10g of the samples(chicken droppings) was mixed with 90ml of peptone water inside Zip Lock Bag and inoculated into the 9ml peptone water(10^{-1}), and these process continues till the 10^{-10} dilution factor by the use of 2ml and 5ml syringes.

After the serial dilution, 0.1ml from each 10^{-6} and 10^{-5} was inoculated into three sterile petri dishes, one plate from dilution 6 and 2 plates from dilution 5 (for bacteria culture). From dilution 10^{-2} and 10^{-3} of the aliquot was inoculated into three sterile petri dishes, one plate from dilution 3 and 2 plates from dilution 2 (for mold culture). From dilution 10^{-4} and 10^{-5} 0.1ml of the aliquot was inoculated into three sterile petri dishes, one plate from dilution 4 and two plates from dilution 5(for Yeast Culture). Spread plate method was done in which the suspended organism was spread into the petri dishes and the agar was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface

3.3.3 Preparation of sandy soil

After collection of samples, analyses were carried out immediately. A setup for serial dilution was done in which the 10g of sandy soil was mixed with 1% peptone water in Zip Lock Bag and was shaked thoroughly so that the organism present in the sandy soil will be release inside the peptone water and the sample were serially diluted to a dilution factor of Ten (10^{-10}). Each dilution was done by sterilizing the test tubes containing 9ml of 1% peptone water after which(

under an aseptic environment) 10g of the samples(sandy soil) was mixed with 90ml of peptone water inside Zip Lock Bag and inoculated into the 9ml peptone water(10^{-1}), and these process continues till the 10^{-10} dilution factor by the use of 2ml and 5ml syringes.

After the serial dilution, 0.1ml from each 10^{-6} and 10^{-5} was inoculated into three sterile petri dishes, one plate from dilution 6 and 2 plates from dilution 5 (for bacteria culture). From dilution 10^{-2} and 10^{-3} 0.1ml of the aliquot was inoculated into three sterile petri dishes, one plate from dilution 3 and 2 plates from dilution 2(for mold culture). From dilution 10^{-4} and 10^{-5} 0.1ml of the aliquot was inoculated into three sterile petri dishes, one plate from dilution 4 and two plates from dilution 5 (for Yeast Culture). Spread plate method was done in which the suspended organism was spread into the petri dishes and the agar was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface

3.4 Media preparation and sterilization

3.4.2 Sabourand Dextrose Agar(SDA)

Using the manufacturer's specifications (65g per 1000ml of distilled water), sabourand dextrose agar was prepared and sterilized inside autoclave at 121 ^{0}C for 15minutes; it was amended with chloramphenicol capsule(250mg per 500ml) to inhibit the growth of bacteria. This was prepare to isolate pure culture of Mold.

3.4.3 Nutrient Agar

Using manufacturer's specifications (28g per 1000ml of distilled water), nutrient agar was prepared and sterilized for the purpose of obtaining and isolating a pure culture of bacteria.

3.4.4 Nutrient Agar Slant

A double strength nutrient agar medium was prepared (11.2g per 200ml of sterile distilled water). After sterilization, 15ml were dispensed into different McCartney bottles; the bottles were slanted at an angle of 600 and allowed to solidify at room temperature. The slants were prepared for preservation of the pure bacterial isolates.

3.4.5 Yeast Extract Agar

Using manufacturer's specifications (34g per 1000ml of distilled water), Yeast extract agar was prepared and sterilized for the purpose inside autoclave at 121^{0}C for 15minutes for the purpose of obtaining and isolating a pure culture of Yeast

3.5 Microbiological Analysis

The microbiological analysis of the Palm Kernel shell, Chicken dropping and Sandy Soil were carried out. The bacterial isolates were identified and characterized using standard biochemical tests. The tests employed include colonial, morphological characteristics, gram stain, catalase, H_2S and gas production, sucrose, D- mannitol, fructose and lactose utilization tests. The fungal isolates were identified based on colour of aerial hyphae and substrate mycelium, arrangement of hyphae, conidial arrangement as well as morphology.

From the dilution of the samples, 0.1ml of aliquot was transferred aseptically into the respective plates and about 15ml of freshly prepared nutrient agar was pour plated with the aliquot.

It was allowed to solidify at room temperature and the plates was masked tape and turn upside down and incubated at 37 ^{0}c for 24hours.

The fungal plates were pour plated using a treated sabourand dextrose agar medium (250mg of chloramphenicol/500ml) to inhibit the growth of bacteria colonies.

Same pour plated procedure was carried out and the respective plates were incubated at 25 ^{0}C for 72hours.

3.5.1 Enumeration of microorganisms from samples

After 24hours for the bacteria culture and 120 hours for fungal culture respectively (due to the media used, which is selective for degradable microorganisms), a mixed colonies of isolates were observed after which the respective plates were further examined morphologically and individual colonies were sub-cultured for further enumeration from palm kernel shell, chicken droppings and sandy soil.

3.5.2 Isolation of bacterial isolates

The mixed colonies after morphological observation was further sub- cultured to get pure strains of bacterial and fungal isolates by inoculating each individual colony into a fresh agar respectively under an aseptic environment. A distinct colony of the bacterial isolates was inoculated into freshly prepared nutrient agar.

3.5.3 Isolation of fungi isolates

Also distinct colonies of fungal isolates were also inoculated into a freshly prepared sabourand agar and Yeast extract agar mixed with chloramphenicol.

3.5.4 Preservation of isolated microorganisms

Double strength agar were prepared by pouring 15ml of nutrient agar, into McCartney bottles and sterilized by autoclave at 121^0C for 15minutes. The McCartney bottles with the agar were allowed to cool and solidify in a sloppy position. After cooling to 40 ^{0}C, McCartney bottles with the agar were inoculated with each colony, with a sterilized inoculating loop. The streaking method was used to introduce the inoculums on the agar in a slanting position. The inoculum was inoculated aseptically. The culture samples were incubated for 24hours at 37^0C. After 24hours, the growths were seen and preserved at 40^0C in the refrigerator for further test.

Double strength sabourand dextrose broth media were prepared by removing the agar agar with the use of a filter paper and cotton wool to trap the agar agar that would have escaped into the conical flask. After which, 15ml of the broth was poured into clean McCartney bottles and sterilized by autoclave at 121^0C for 15minutes. The broths were allowed to cool to about 40^0 after which they were inoculated with each pure strain of fungi isolates with a sterilized inoculating loop. The cultured samples were incubated for 72hours at 25^0C. The entire procedure were carried out in an aseptic environment, after 72hours growths were seen and preserved at 40C in the refrigerator for further tests.

3.6 Characterization of isolated microorganism

Several methods were implemented in the identification of the isolated microorganisms. Below are various methods:

3.6.1 The Gram Stain

This is the most important and frequently used staining technique in bacteriology. It aids in the differentiation of bacteria into two main groups, known as Gram-positive and Gram-negative bacteria.

A smear of the 24hour isolates were prepared by placing a drop of sterile water in the middle of the clean glass slide, after which, the inoculating loop was sterilized by flaming and then cool. The bacteria colony were picked with the loop and rubbed in the drop of water on the slide and spread into a thin smear along the slide. The smear were allowed to air-dry and then passed over the flame thrice as quick as possible in order to fix the bacteria. The inoculating loop was flamed and the same steps were done on other bacterial colonies. The smear were flooded with methyl violet for 60 seconds, and the dye were drained quickly and washed with lugol's iodine which was left on them for 60 seconds. The iodine was drained and the slides were washed gently with sterile distilled water, the slides were then washed with 95% ethanol until it appeared free of violet stain. They were then rinsed with sterile water and later flooded with dilute safranin for 30 seconds. The stain was drained, the slides were washed and blot dried.

The prepared slide were first examined without a coverslip under the high power of the microscope and then with a coverslip under the oil immersion lens. References were made with respect to the colors, shapes, sizes, etc. of the bacteria.

3.6.2 Biochemical characterization of bacterial isolates

3.6.2.1 Sugar fermentation

The test was carried out to determine the type of sugar the organisms will ferment or utilize. One gram of each of these sugars were used; D-mannitol, sucrose, fructose and lactose. Each of them were weighed and introduced into different 50ml conical flask containing peptone (which stands as basal media) was added to each conical flask and mixed together with distilled water.

Phenolphthalein was then added to the mixture by using clean pipette, 5ml of each mixture was dispensed into each test tube with Durham tube, put into the tubes by inverting them according to the number of organisms of samples. The test tubes were covered with cotton wool and aluminum foil paper, sterilized in the autoclave at 121^0C for 15 minutes. The cultured microorganisms under test were then smeared across the impregnated paper with an inoculating loop. A positive reaction is indicated by the appearance of a yellow coloration within 10-20 seconds, while no coloration indicates a negative reaction.

3.6.2.3 Production of acid from sugar and gas formation

A nutrient broth containing 0.5% of glucose, lactose and 0.01% bromcresol purple, 1% phenolphthalein indicators were prepared in eight test tubes of ten sets in which Durham's tubes were inverted and sterilized in an autoclave. After sterilization, on cooling, the unknown cultures were inoculated into the indicator-sugar-broth, and one tube was left uninoculated to serve as control. The test-tubes were incubated at 35^0C for 2-5days and the results were recorded.

3.6.3 Catalase test

The enzyme catalase acts as a catalyst in the breakdown of hydrogen peroxide (H_2O_2) and water (H_2O). Catalase test was used to know the specie of a particular bacterium that relates with the hydrogen peroxide to produce oxygen and water. The culture used for this test was not more than 24-hours old. Drops of 3% hydrogen peroxide were applied on the colony of organisms that was 24-hours culture, which was on the slide. The presence of catalase is shown by the formation of gas bubbles.

3.6.4 Gram's Reaction

A drop of 3% aqueous Potassium hydroxide (KOH) was put on a slide. A sterile loop is used to transfer a visible amount of bacterial growth from an agar culture to the drop of KOH. The cell is mixed with the KOH thoroughly on the slide, constantly stirring over an area about 1.5cm in diameter. if the bacterium –KOH suspension becomes markedly viscid or gels within 5 to 60s, the isolate is gram positive and the best way to determine viscosity is to raise the loop about 1cm from the slide. If an obvious stinginess is present, then the culture is gram negative

3.7 Characterization of Fungi Isolates

Cotton blue-in-lactophenol is the popular strain used in staining fungal hyphae. This stains the cytoplasm light blue. It is used for mounting fungal specimens.

A drop of cotton *blue-in-lactophenol* was placed on clean glass slides, using a sterile needle. Thereafter, a small piece of mycelium free of medium was picked and transferred to the stain on the slide carefully. They were then covered with a cover slip with care to avoid bubbles. The slides were observed under the low power first and then the high power of the microscope. And the observations were recorded.

3.8 Biodegradation of Ground Palm Kernel Shell using Chicken Droppings Inoculated with Chicken Droppings at Ratio 1:2

The biodegradation of grounded palm kernel shell and chicken dropping was done in Ratio 2:1 in which 100g of palm kernel shell was weighed and put in covered biodegradation bowl and 50g of chicken droppings was incorporated into it and 20ml of sterile distilled water was added to the samples, and a sterile spatula was used to mixed the samples together which serves as the beginning of biodegradation.

The mixed sample was weighed and 5g (Palm kernel shell and chicken droppings) was taking at 0 day of biodegradation for serial dilution in order to have the knowledge of the new microorganism present in the mixture of the new sample.

The mixed sample was weighed and 5g of the sample was also taking on the 5th day for serial dilution analysis, in order to monitor the microorganism responsible for biodegradation process at different level.

The mixed sample was weighed and 5g was also taken on the 10th, 15th,20th and 30th day of biodegradation for microbial analysis in order to have the full knowledge on the microorganisms present and responsible for the biodegradation process of Palm Kernel Shell.

3.9 Physicochemical properties of degrading Inoculated Ground Palm Kernel Shells

3.9.1 pH Measurement

1 g of the sample were weighed and dissolved in 3 ml of de-ionized water. The mixture was heated and stirred for 3 minutes to ensure proper dilution of the sample. The solution was filtered out and its pH was determined using a digital pH meter.

3.9.2 Total Titratable Acid (TTA)

The mixed sample was weighed and 5g of the sample was mixed with the 45ml peptone water and 20ml of the aliquot was taken for analysis by adding 3 drop of phenolphthalein and titrated against sodium hydroxide and observe the colour changes.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

Table below shows the result obtained from the experiment carried out on the respective Uncrushed Palm Kernel Shell samples from the traditional oil processing industry.

$$\text{CFU/g of original sample} = \frac{\text{No. of Colonies}}{\text{Inoculum size (ml) x Dilution Factor}}$$

4.1.1. *Microbial viable Count of Samples*

The table shows the number of colony count of bacteria at 18hrs and 24hrs of incubation and the fungi viable count at 72hrs of incubation which also include the dilution factor of the cultured plate.

4.1.2. *Total Viable Count of Uncrushed Palm Kernel Shells*

The microbial count as indicated in Table 1: shows the isolates codes and the colony count of Uncrushed palm kernel shell at 18hrs and 24hrs (cfu/g) of incubation of the bacterial isolates and the fungi viable count which include mold and yeast at 72hrs of incubation in which the total viable count of mold (8.1×10^{-3}) isolates have higher count than those of bacteria(1.8×10^{6}) and mold (3.3×10^{6}). This trends reveals heavy proliferation ratio of bacteria in the uncrushed Palm Kernel Shell.

4.1.3. *Total viable Count of Chicken Droppings*

The microbial count as indicated in Table 2: shows the bacterial and fungi (mold and yeast) isolated from chicken droppings in which bacterial isolates 1(4.0×10^{6}) have higher count at 24hrs and yeast isolates 2($3.3 \text{ X } 10^{-6}$) have higher count at 72hrs. This trends reveals heavy proliferation ratio of bacteria in the Chicken droppings.

4.1.4. *Microbial viable Count of Sandy Soil*

The microbial count as indicated in Table 3: shows the total viable count of bacterial isolates code 3($2.03 \text{ X } 10^{7}$) have higher count than bacterial isolates 1(4.0×10^{6}) and 2($2.03 \text{ X } 10^{7}$) while mold isolate 3($6.0 \text{ X } 10^{4}$) have higher count than isolates 1($1.5 \text{ X } 10^{3}$) and 2($1.1 \text{ X } 10^{3}$) and yeast isolate 3($2.3 \text{ X } 10^{5}$) have higher count than isolates 1($1.3 \text{ X } 10^{6}$) and 2($1.7 \text{ X } 10^{6}$). This trends reveals heavy proliferation ratio of bacteria in the sandy soil.

TABLE 1: TOTAL VIABLE MICROBIAL COUNT ISOLATED FROM UNCRUSHED PALM KERNEL SHELL (UPKS).

ISOLATES CODES	COLONY COUNT @ 18h(cfu/g)	COLONY COUNT @ 24h(cfu/g)
BACTERIAL VIABLE COUNT		
UPKSB-1	0.8×10^6	1.8×10^6
UPKSB-2	0.6×10^6	2.2×10^6
UPKSB-3	0.4 x 10	1.7 x 10
MOLD COUNT @ 72hrs		
UPKSM-1	3.5×10^{-3}	
UPKSM-2	8.1×10^{-3}	
UPKSM-3	2.1×10^{-4}	
YEAST COUNT @ 72hrs		
UPKSY-1	2.2×10^{-5}	
UPKSY-2	3.3 X 10	
UPKSY-3	1.4×10^{-6}	

KEY: UPKSB- Uncrushed palm kernel shell Bacteria
UPKSY – Uncrushed palm kernel shell yeast.
UPKSM – Uncrushed palm kernel shell mold.

TABLE 2: TOTAL VIABLE MICROBIAL COUNT ISOLATED FROM CHICKEN DROPPING (CD).

ISOLATE CODES	@ 18hrs (cfu/g)	@ 24hrs (cfu/g)
BACTERIAL VIABLE COUNT		
CDB- 1	3.6×10^6	4.0×10^6
CDB- 2	1.5×10^6	1.8×10^6
CD – 3	0.2×10^6	0.6×10^6
YEAST COUNT @ 72hrs		
CDM-1	3.5×10^6	
CDM-2	3.7×10^6	
CDM-3	4.5×10^5	
MOLD COUNT @ 72hrs		
CDY-1	3.01×10^4	
CDY-2	2.3×10^3	
CDY-3	1.1×10^4	

KEY: CDB – Chicken dropping bacteria
CDY – Chicken dropping yeast
CDM – Chicken dropping mould

TABLE 3: TOTAL VIABLE MICROBIAL COUNT ISOLATED FROM SANDY SOIL (SS).

ISOLATE CODES	COLONY COUNT @ 18h (cfu/g)	COLONY COUNT @ 24h (cfu/g)
	BACTERIAL VIABLE COUNT	
SSB-1	6.8 X 10	7.2 X 10
SSB-2	1.08×10^{7}	2.03×10^{7}
SSB-3	8.3×10^{6}	2.03×10^{7}
	MOLD COUNT @ 72hrs	
SSM-1	1.5×10^{3}	
SSM-2	1.1×10^{3}	
SSM-3	6.0×10^{4}	
	YEAST COUNT @ 72hrs	
SSY- 1	1.3×10^{6}	
SSY- 2	1.7×10^{6}	
SSY-3	2.3×10^{5}	

KEY: SSB – Sandy soil bacteria
SSY – Sandy soil yeast
SSM – Sandy soil mould

4.2. *The total viable count of Degrading Bacteria, Yeast and Mold from Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings*

The tables show the microbial count of degrading bacteria, yeast and mold from the ground palm kernel shell.

4.2.1. *Total viable bacterial count from Degrading Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings (CD) at ratio 2:1*

The microbial count as indicated in Table 4: shows the bacterial count from degrading Ground Palm Kernel Shell that was inoculated with chicken droppings at 2:1 at every 5days of biodegradation, Degrading bacteria isolates code 2(2.23×10^7) have higher count at Day 0 and on Day 5 degrading bacteria isolates code 1($3.45 X 10^7$) have higher count and on day 15 degrading isolate code 2($5.3 X 10^6$) have higher count than isolate 1($1.7 X 10^6$) and 3($1.1 X 10^7$) and on 20th day isolate code 1($1.6 X 10^6$) have higher count while on 30th day of biodegradation isolate 1($1.2 X 10^6$) have higher count than isolates 2($0.8 X 10^6$) and 3(0.5×10^6). This trends reveals heavy proliferation ratio of bacteria in the ground palm kernel shell inoculated with chicken droppings.

4.2.2. *Total viable Yeast count of isolates from Degrading Ground Palm Kernel Shell Inoculated with chicken droppings at Ratio 2:1*

The yeast count as indicated in Table 5: was done on every 5days of biodegradation starting from Day 0 – Day 30. Degrading yeast isolates($3.54 X 10^6$) have higher count on Day 0 while Degrading yeast isolates 1(9.4×10^5) have higher count on Day 5 and isolates 1 have higher count on Day 10, 15 ,20 and 30. This trends reveals heavy proliferation ratio of yeast.

4.2.3. *Total viable Mold count of isolates from Degrading Ground palm kernel shell (DGPKS) inoculated with chicken droppings at Ratio 2:1*

The mold count as indicated in Table 6: the total viable mold count at 72hrs of incubation at every 5 days of biodegradation which is from Day 0- Day 30.

Table 4: Total viable bacterial count from Degrading Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings (CD) at ratio 2:1

ISOLATES CODES	COLONY COUNT @ 18h (cfu/g)	COLONY COUNT @ 24h (cfu/g)
	DAY 0	
DGPKSB-1	1.06 x 10^7	1.96 x 10^7
DGPKSB-2	1.56 x 10^7	2.23 x 10^7
DGPKSB-3	8.6 x 10^7	1.02 x 10^8
	DAY 5	
DGPKSB-1	2.67 X 10^7	3.45 X 10^7
DGPKSB-2	8.6 X 10^6	1.05 X 10^7
DGPKSB-3	1.56 X 10^7	2.14 X 10^7
	DAY 10	
DGPKSB-1	2.8 X 10	4.0 X 10
DGPKSB-2	1.8 X 10^6	2.0 X 10^6
DGPKSB-3	1.1 X 10^7	1.9 X 10^7
	DAY 15	
DGPKSB-1	1.0 X 10^6	1.7 X 10^6
DGPKSB-2	2.0 X 10^6	5.3 X 10^6
DGPKSB-3	0.5 X 10^7	1.1 X 10^7
	DAY 20	
DGPKSB-1	1.0 X 10^6	1.6 X 10^6
DGPKSB-2	0.6 X 10^6	1.0 X 10^7
DGPKSB-3	0.2 X 10^7	0.6 X 10^7
	DAY 30	
DGPKSB-1	0.6 X 10^6	1.2 X 10^6
DGPKSB-2	0 .4X 10^6	0.8 X 10^6
DGPKSB-3	0.1 X 10^7	0.5 X 10

KEY: DGPKSB- Degrading Ground Palm Kernel Shell Bacteria

TABLE 5: TOTAL VIABLE YEAST COUNT OF ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INOCULATED WITH CHICKEN DROPPINGS (CD) AT RATIO 2:1

ISOLATE CODES	**COUNT @ 72h(cfu/g)**
DAY 0	
DGPKSY-1	2.50×10^{7}
DGPKSY-2	1.02×10^{7}
DGPKSY-3	3.54×10
DAY 5	
DGPKSY-1	9.4×10^{5}
DGPKSY-2	7.3×10^{6}
DGPKSY-3	4.5×10^{6}
DAY 10	
DGPKSY-1	3.1×10^{5}
DGPKSY-2	1.4×10^{6}
DGPKSY-3	2.3×10^{6}
DAY 15	
DGPKSY-1	2.0×10^{5}
DGPKSY-2	1.6×10^{6}
DGPKSY-3	0.6×10^{6}
DAY 20	
DGPKSY-1	1.2×10^{5}
DGPKSY-2	0.9×10^{6}
DGPKSY-3	0.4×10^{6}
DAY 30	
DGPKSY-1	4.0×10
DGPKSY-2	-
DGPKSY-3	0.1×10^{6}

KEY: DGPKSY – Degrading Ground Palm Kernel Shell Yeast

TABLE 6: TOTAL VIABLE MOLD COUNT OF ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPINGS (CD) AT RATIO 2:1.

ISOLATE CODES	COUNT @ 72h(cfu/g)
DAY 0	
DGPKSM-1	8.5×10^3
DGPKSM-2	4.1×10^3
DGPKSM-3	2.7×10^4
DAY 5	
DGPKSM-1	5.6×10
DGPKSM-2	3.7×10^3
DGPKSM-3	1.8×10^4
DAY 10	
DGPKSM-1	4.5×10^3
DGPKSM-2	3.2×10^3
DGPKSM-3	1.5×10^4
DAY 15	
DGPKSM-1	3.4×10
DGPKSM-2	2.0×10^3
DGPKSM-3	1.0×10^4
DAY 20	
DGPKSM-1	2.7×10^3
DGPKSM-2	1.6×10^3
DGPKSM-3	0.9×10^4
DAY 30	
DKPKSM-1	1.2×10^3
DGPKSM-2	0.8×10^3
DGPKSM-3	0.4×10^4

KEY: DGPKSM- Degrading ground palm kernel shell mold

4.3. *The morphological characteristics of bacteria isolates from Uncrushed Palm Kernel Shell (UPKS)*

As indicated in Table 7: the morphological characteristics of bacteria isolates from Uncrushed Palm Kernel Shell in which bacteria isolates code 1 is rod shape, white coloured and smooth surfaced, Bacterial isolates 2 is rod shape, cream colour and margin surfaced and bacteria isolates 3 is cocci shaped, cream coloured and having smooth surface.

4.3.1. *The morphological characteristics of bacteria isolates from Chicken Droppings (CD)*

As indicated in Table 8: the morphological characteristics of bacteria isolates from chicken droppings sample in which bacterial isolates code 1 is rod shape, cream colour, flat with smooth surface microorganism, bacterial isolate 2 is cocci shape, cream colour, raised with margin surface organism while chicken droppings isolates 3 is cocci shape, white colour, raised microorganisms with rough surface.

4.3.2. *The morphological characteristics of bacteria isolates from Sandy Soil*

As indicated in Table 9: the morphological characteristics of bacteria isolates from Sandy Soil in which bacteria isolate 1 and 3 are rod shape and flat microorganism and bacteria isolate 2 is cocci shape with rough surface and raised microorganism

TABLE 7: MORPHOLOGICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM UNCRUSHED PALM KERNEL SHELL (UPKS).

ISOLATES CODES	SHAPE	COLOUR	SURFACE	EDGE
UPKSB-1	Rod	White	Smooth	Rhizoid
UPKSB-2	Rod	Cream	Margin	Entire
UPKSB-3	Cocci	Cream	Smooth	Entire

KEY: UPKSB – Uncrushed palm kernel shell bacteria

TABLE 9: MORPHOLOGICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM SANDY SOIL (SS).

SAMPLE	SHAPE	COLOUR	SURFACE	ELEVATION
SSB-1	Rod	White	Smooth	Flat
SSB-2	Cocci	Cream	Rough	Raised
SSB-3	Rod	Cream	Rough	Flat

KEY: SSB – Sandy Soil Bacteria

4.4. *Morphological characteristics of bacteria isolates from degrading ground palm kernel shell (DGPKS) inoculated with chicken dropping (CD) at ratio 2:1*

As indicated in Table 10: the morphological characteristics of bacteria isolates from degrading ground palm kernel shell Inoculated with chicken droppings at Ratio 2:1 from Day 0- Day 30 in which at Day 0 Degrading isolates 1 and 3 are cocci, smooth surface and flat microorganism while degrading isolate 3 is white colour, bacillus shape, rough and raised microorganism. The microorganism isolated are cocci or bacillus shape with rough or smooth surface which are flat or raised microorganisms.

4.4.1. *The morphological and Microscopic characteristics of fungal isolates from Uncrushed Palm Kernel Shell (UPKS)*

As indicated in table 11: the morphological and microscopic characteristics of fungal isolates from uncrushed palm kernel shell (UPKS) which include the Yeast and Mold morphological description and microscopic observation and the isolates are dark, greenish, light grey, brown and white coloured microorganisms and which also indicate microscopically that some are single, simple conidiophore, some have sparse mycelia, non-septate hyphae and thin sporagiophore.

4.4.2. *The Morphological and Microscopic characteristics of Fungal isolates from Chicken Droppings (CD)*

As indicated in Table 12: the morphological and microscopic characteristics of fungal isolates from chicken droppings which have brown, white and cotton like mycelia and showing microscopically that some have upright conidiophore at their apex, while some are non- septate hyphae.

4.4.3. *The Morphological and Microscopic characteristics of Fungal isolates from sandy soil (SS)*

As indicated in table 13: the morphological and microscopic characteristics of the isolates from sandy soil in which the isolates are characterized morphologically with some having white

mycelium, black mycelia growth, white fluffy and moist growth and which under microscope was observed to have branched septate conidiophore, and an upright conidiophores.

4.4.4. *The Morphological and Microscopic characteristics of fungal isolates from Degrading Ground Palm Kernel Shell (DGPKS) Inoculated with Chicken Droppings (CD)*

As indicated in Table 14: the morphological and microscopic characteristics of fungal isolates from degrading ground palm kernel shell inoculated with chicken droppings at Ratio 2:1 at every 5 days of biodegradation from Day 0- Day 30 and the table also indicates some suspected organism like *Aspergillus flavus, Candida albicans, Aspergillus fumigatus, and Zoophage nitospora.*

TABLE 10: MORPHOLOGICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM DEGRADING GROUND PALM KERNEL SHELL (DGPKS) INNOCULATED WITH CHICKEN DROPPING (CD) AT RATIO 2:1

ISOLATE CODES	COLOUR	SHAPE	SURFACE	ELEVATION
DAY 0				
DGPKSB-1	Cream	Cocci	Smooth	Flat
DGPKSB-2	White	Bacillus	Rough	Raised
DGPKSB-3	Cream	Cocci	Smooth	Flat
DAY 5				
DGPKSB-1	Cream	Baccilus	Smooth	Raised
DGPKSB-2	Cream	Cocci	Rough	Flat
DGPKSB-3	Cream	Cocci	Smooth	Raised
DAY 10				
DGPKSB-1	White	Cocci	Smooth	Raised
DGPKSB-2	Cream	Cocci	Smooth	Flat
DGPKSB-3	Cream	Cocci	Rough	Raised
DAY 15				
DGPKSB-1	Cream	Cocci	Smooth	Flat
DGPKSB-2	Grey	Cocci	Smooth	Raised
DGBKSB-3	Cream	Cocci	Smooth	Raised
DAY 20				
DGPKSB-1	Green	Cocci	Smooth	Flat
DGPKSB-2	Cream	Bacillus	Smooth	Raised
DGPKSB-3	Cream	Cocci	Smooth	Raised
DAY 30				
DGPKSB-1	Cream	Cocci	Rough	Flat
DGPKSB-2	Cream	Cocci	Smooth	Flat
DGPKSB-3	Cream	Cocci	Smooth	Flat

KEY: DGPKSB – Degrading Ground Palm Kernel Shell Bacteria

TABLE 11: MORPHOLOGICAL AND MICROSCOPIC CHARACTERISTICS OF FUNGAL ISOLATES FROM UNCRUSHED PALM KERNEL SHELL (UPKS).

ISOLATES CODES	CULTURAL MORPHOLOGY	MICROSCOPIC OBSERVATION	Suspected Organisms
UPKSM-1	Brown mycelia growth	An upright conidiophores that terminates in a davate swelling bearing phliades at the apex or radiating from the entire surface; conidia are 1-celled and globose.	*Aspergillus fumigatus*
UPKSM-2	Cotton-like mycelia at 24hrs turning dirty with development of black spores on mycelium	Non-septate hypha, thin sporagiophore with a sporangium in club-like form	*Rhizopus stolonifer*
UPKSM-3	Light Grey mycelia	Sparse mycelia, non-septate hyphae, conidia hyaline single	*Zoophage nitospora*
UPKSY-1	Greenish mold	Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid, forming short chains by budding which are produced on mycelium epically or laterally	*Candida albicans*
UPKSY-2	White fluffy and moist growth	It has a white septate mycelium, no conidiophore, and short cylindrical conidia with truncate ends formed by segmentation of hyphae	*Torulopsis spp*

KEY: UPKSM- Uncrushed Palm Kernel Shell Mold
UPKSY- Uncrushed Palm Kernel Shell Yeast