# ISOLATION AND CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH BIODEGRADATION OF THE AFRICAN WALNUT SHELLS USING CHICKEN DROPPINGS AS INNOCULUM

**BY**

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**MCB/11/0323**

**A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY IN THE FACULTY OF SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOROF SCIENCE (B. Sc.) DEGREE OF FEDERAL UNIVERSITY, OYE EKITI, NIGERIA.**

**UNDER THE SUPERVISION OF**

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**OCTOBER, 2015.**

## CERTIFICATION

This is to certify that this project work was carried out presented by ABIODUN OLUWASANMI EZEKIEL with the Matriculation Number MCB/11/0323, of the Department of Microbiology, Faculty of Science, Federal University Oye Ekiti, Ekiti State, Nigeria. Under the supervision of Dr. (Mrs.) R.A.O. Gabriel- Ajobiewe.

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Head of Department.

## DEDICATION

This project is dedicated to Almighty God and to my entire family

**ACKNOWLEDGEMENT**

I lay forth all majesty, honor and glory to the almighty God, who always cares and makes way for me; who is the source of my wisdom, power and materials utilized in the accomplishment of this project work.

Firstly, I would like to extend my deepest gratitude to a great woman, my project supervisor, Dr. (Mrs.) R.A.O. Gabriel-Ajobiewe for her ardent interest in this research work, guidance, moral upliftment, constant attention, enthusiastic support and personal concern during the research and throughout the course of my study.

My special gratitude extends to the Head of Department, prof. B.O. Ogeneh for his guidance, encouragement, advice and care throughout the academic period.

My profound gratitude goes to Dr. S.K Ojo for his advice, contribution and corrections during the course of this study. I will not forget so soon, the effort of my lecturers Prof. S.K. Ogundana, Dr. A.O Ajayi, Dr. H.A. Akinyele and Mrs. Nwonkwoma who in one way or the other contributed to the success of this study.

Enormous appreciation to my lovely, sweet and caring friend, Adeniyi Oluwadunsin Nifemi for supporting me morally, materially and prayerfully, who is always there for me through thick and thin, for encouraging me to carry on. My appreciation also goes to my entire church most especially my pastor (pastor Oludare).

Special appreciation goes to my parent late Mr. and Mrs. S.O Abiodun and to my loving Family, especially my sisters and brothers who is always on my side, riding along with me in my ups and downs as well as giving me the encouragement to pursue my dreams and always reminding me that I have unfinished work. My siblings were indeed great, the success of this work wouldn't have been easy without you all, bearing it all moves me several paces forward.

My deepest gratitude goes to all the laboratory technicians, most especially to Mr. Adeleke, Mrs. Adebiyi, Mrs. Olatunde, Miss Foyeke and Mrs. Falade for helping me throughout my practical work.

I will not fail to mention my course mates and all my close friends in Federal University Oye Ekiti, Ekiti State, whose friendships will forever stay in my heart. May God bless you all and the Almighty will repay all my debts to you all, Amen.

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**Abstract**

Microbial analyses were carried out on the shell of the edible nuts of African walnut (*Tetracarpidium conophorum*). This study was aimed at determining the microbial load of boiled and raw African walnut shell and chicken dropping which can be used as inoculums in biodegradation process in order to solve the problem of agro waste pollution in the environment. The nuts were sorted and washed with tap water to remove flesh residues and other contaminants and divided into two lots. The first lot was boiled for 1.5 h. The second lot was used raw; the raw nut and the boiled nut were then unshelled and the shells were dried at 60◦C for 5days in the oven. The dried shells were grind and analyzed. Bacterial and fungal evaluations of the shell from nut were done using the agar diffusion technique with serial dilutions of the grind shell and the same process for chicken droppings. Forty Grams (40g) of the grind walnut shells were weighed and put in a sterile cover bowl and 20ml of sterile distilled water was added to the samples under a sterile condition and was gently mixed with sterile spatula. The grind walnut shell and chicken dropping was done in Ratio 1:1 and 2:1. Microorganisms were isolated and identified. The microbial analysis revealed that the boiled shell accommodate a large number of microorganism compared to that of raw. The result show that the microorganisms present in the walnut shell were increasing as the day is increasing and latter begins to decrease.

# CHAPTER ONE

## 1.0 INTRODUCTION

African walnut *Tetracarpidium conophorum* (also called *Plukenetia conophora*) belong to the family of Euphorbiaceae and is found in South east and South west Nigeria and Cameroon. Walnut (*Tetracarpidium conophorum*) is an important crop that is cultivated throughout the world's temperate regions for its edible nuts (Srinivasan and Viraraghavan, 2008). *T. conophorum* is a climbing shrub 10-20 ft. long, it is known in the Southern Nigeria as*ukpa* (Igbo), Western Nigeria as *awusa or asala* (Yoruba). It is known in the littoral and the Western Cameroon as *kaso or ngak* [Tchiegang *et al*., 2007]. They are usually planted under an indigenous tree that can provide strong support for the heavy weight of the climber when fully established on the crown of the tree, and in cases where they cannot be harvested manually; they are left for full maturation after which the pod falls off by itself and are picked, removed from the rotten pods, washed and sold in the market (Hemery, 2006).

*T. conophorum*, like many plants in Africa and other parts of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial values over the years. Conophor plants are cultivated principally for the nuts which are usually cooked and consumed as snacks (Enujiugha and Ayodele, 2003).

*Tetracapridium conophurum* contained in a pod which may house; one shelled nut (single), two shelled nut (double) and three shelled nut (triple). The walnut shells could be black or brown from the plant. The nut is whitish upon cracking from the shell. The nut has a thin layer in between two halves (when a nut is divided into two equal parts) of nut. The seed (subglobose) is about 2.5cm long and has wooly materials that attach the nut to the shell when cracked open. A bitter taste is usually observed upon drinking water immediately after eating the nuts. This could be attributed to the presence of chemical substances such as alkaloids [Ayodele, 2003]

Walnut shell is a waste generated in the walnut (*tetracarpidium conophorum*) harvest, containing natural compounds with antioxidant properties. The walnut shell has antioxidant compounds such as flavonoids which have been determined (Akbari *et al*., 2012). Production is growing every year because it is popular as a highly nutritious food. However, this causes problems, one being finding ways of using the walnut shells (WS) because they are waste. This causes environmental pollution it will take a long time for them to complete their natural cycles, and has a low utility value (Oliveira *et al*., 2008). It is therefore necessary to find new ways of using walnut shell. Burning agricultural residues causes environmental problems such as air pollutiose, soil erosion and decreasing soil biological activity [Copur *et al.,* 2007]. Utilizing agricultural residues not only prevents environmental concerns but also can mean farmers second income from plantation [Ayrilmis *et al.,* 2009].

A shell of the African walnut nut is formed by three basic substances, namely cellulose (40.5%), hemicellulose (23.8%) and lignin (20.3%) Various methods have been proposed for hydrolysis of cellulose and hemicellulose in wood biomass as a means of obtaining carbohydrates, which are good sources for bioethanol preparation. A large amount of walnut shell remains after harvesting. Currently, walnut shell is mainly used as fuel for incineration applications. The shell can be used as a carbonaceous sorbent to control of mercury from industrial liquid streams and activation of $co_2$ for waste water treatment (Zabihi *et al*., 2010).

*Tetracapidium conophorum* can be cooked, roasted or sun dried and the roasted seeds could be ground like melon seeds and used as a thickener in soup preparation. The plant is known in Africa especially in the Eastern and Western parts of Nigeria for its antibacterial efficacy (Okerulu and Ani, 2001). Decoction of leaves and seeds serve as beverage which relieves abdominal pains and fever (Malu *et al*., 2009). Dried walnuts can be ground and turned into flour which can be used as composite flour during baking or in-place of milk in tea preparation (Stevens and Domelam, 2003).

*T. conophorum*, like many plants in Africa and other parts of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial values over the years. A cardio protective dietary fat profile is recommended for the treatment of type 2 diabetes (Gillen *et al.,* 2005). The leaves, bark, and fruit of *T. conophorum* are used medicinally, and their uses include masticatory, giddiness, thrush, antihelminthic, toothache, syphilis, dysentery, and as an antidote to snakebite (Odugbemi and Akinsulire, 2008). In the Southern Nigeria ethno-medicine, African walnut is used as a male fertility agent and in the treatment of dysentery (Ajaiyeoba and Fadare, 2006)

Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Alexander, 2004). Indeed, biodegradation is the process by which organic substances are broken down into smaller compounds by living microbial organisms (Marinescu *et al., 2009*). When biodegradation is complete, the process is called "mineralization". However, in most cases the term biodegradation is generally used to describe almost any biologically mediated change in a substrate (Bennet *et al., 2002)*. Microorganisms can degrade numerous of organic pollutants owing to their metabolic machinery and to their capacity to adapt to inhospi environments. Thus, microorganisms are major players in site remediation. Several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process. Algae and protozoa reports are scanty regarding their involvement in biodegradation (Das and Chandran, 2011). However, their efficiency depends on many factors, including the chemical nature and the concentration of pollutants, their availability to microorganisms, and the physicochemical characteristics of the environment (El Fantroussi and Agathos, 2005)

Chicken litter is a waste by-product of poultry production and is comprised of feces, wasted feeds, bedding materials, and feathers (Kim *et al.,* 2012). Chicken litter is usually recycled as an organic fertilizer or soil amendment for direct application to agricultural land (Enticknap *et al.,* 2006). However, chicken litter may contain loads of human pathogens, such as Salmonella spp., that have

great potential to directly or indirectly contaminate fresh produce and cause food-borne disease outbreaks (Wilkinson *et al*., 2011). Raw chicken litter has been widely applied to arable land as organic fertilizer or soil amendment to improve the soil fertility and structure. Chicken droppings contain nitrogen and phosphorus (Adeleye, 1991), which are necessary for African walnut nut shell degradation. In addition, chicken droppings harbor bacteria and fungi that can utilize African walnut shell efficiently.

Previous studies have shown the feasibility of using agricultural waste to produce animal feeds and as substrate for mushroom production. Various agricultural by-products such as walnut waste, maize cobs, peanut shell, cassava waste, wheat bran, maize husk, coconut shell, bagasse, and banana peel are also utilized in the removal of heavy metals and toxic materials from wastewater (Thomas *et al., 2008*). Several works had been done on the walnut seed such as the determination of oxalate, phylates and tannin (Enujiugha and Ayodele, 2003). The present study is to determine the microbial load of boiled and raw African walnut shell and chicken dropping which can be used as inoculum in biodegradation process in order to solve the problem of agro waste pollution in the environment.

## 1.1 AIM OF THE STUDY

To assess the effectiveness of chicken droppings in enhance African walnut shell degradation

## 1.2 OBJECTIVES OF THE STUDY

1. To determine the microbial load of the walnut shell and chicken litter
2. To isolate the microorganism which responsible for the degradation of African walnut shell
3. To determine the pH. and acid production of the samples at different intervals of 5days
4. To identify the microorganism present on the samples

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Walnut comprises such families as *Juglandaceae* (English walnut), *Euphorbiaceae* (African walnut) and *Olacaceae* (African walnut). Each family has its own peculiar characteristics but they have some things in common such as the nuts. *Juglandaceae* is mostly found in the Southeast Europe, to Japan and more widely in the New world. *Tetracarpidium conophorum*, a woody perennial climber that belongs to the family Euphorbiacea is found in Nigeria and Cameroon while *Coulaedulis* (family Olacaceae) which is also referred to as African walnut is found in Congo, Gabon and Liberia (Wikipedia, 2008). Walnut, common name for small flowering plants are important for the nuts and timber most of them produce and for its representative genus.

### 2.1 Origin of Nuts

*Tetracarpidium conophorum*has a long history as food plant, grown by peasant farmers across West African rain forest. The climber i.e. *T.conophorum* bears capsules which are greenish yellow when fully ripe and greenish in color when young. The leaves are broadened and are rounded up to 53 inches with slender petioles up to 3inches long. The fruits are four winged ridged between wings and up to 3 inches in diameter. They can be eaten as raw, cooked or with roasted corn (Okerulu and Ani, 2000). Because of its origin in the West African Rainforest It is commonly referred to as African walnut and as Nigerian walnut by Nigerians.

It is usually cultivated as in different parts of Nigeria such as, Ondo, Abakiliki, Uyo, Etinan, Akamkpa, Akpabuyo, Lagos, Anambra, Ibadan e.t.c. It is known as ‘Ekporo’ by Efik and Ibibio’s of Cross River and Akwa-ibom,States, *‘ukpa’* (Igbo) ‘*awusa*’ or *‘asala’* (Yoruba), *‘kaso* or ‘*ngak*’ (littoral and the western Cameroon) ( Malu *et al.*, 2008).

It is interesting to note that the African Walnut though a climber and indigenous to Africa has similar nutritive, medical, physicochemical characteristics with especially the Black American walnuts *Juglansnigra*, *Juglansregia* respectively (Malu *et al.*, 2008; Ajaiyeoba *et al.*, 2006).

## 2.2 Storage and Selection of *T. conophorum*

The most important thing is that this valuable plant *T. conophorum* should be well selected and stored. Rubbery or shriveled shelled nuts should be avoided as this is an indication of age.

Shelled nuts should be brittle and snap easily. Nuts which grow on the summer side of the plant will have a darker skin and a richer flavour (more photosynthetic activity). It is best to buy unshelled nuts and store them in the refrigerator for 2 to 3 months or freeze up to 1 year, they can quickly turn rancid on storage, due to the high oil content of the nuts. (Ayankunbi *et al.,* 1991) Reported that Food materials are usually processed in order to improve palatability and reduce toxicity and as a means of preservation. Processing methods such as thermal processing, refrigeration, freezing, and fermentation have been applied to various food materials to achieve these purposes.

Shelled nuts are commercially available, packaged nuts are often treated with ethylene gas, fumigated with methyl bromide, dipped in hot lye or a solution of glycerine and sodium carbonate to loosen their skins and then rinsed in citric acid. Shelled walnuts should be kept refrigerated in an airtight container, and may be frozen up to a year. Walnut, *T. conophorum* oil is an excellent, albeit expensive oil and can be used to dress salads.

## 2.3 Fungus Damage and Control of *T. conophorum* shell

Fungus damage to *T. conophorum* may be traced to three general causes: (a) lack of suitable protective measures when storing (b) improper seasoning, storing, or handling of the raw material produced from the *T. conophorum* (c) failure to take ordinary simple precautions in using the final product. The incidence and development of molds, decay, and stains caused by fungi depend heavily on temperature and moisture conditions (Yokoyama, 2012)

## 2.4 Medicinal Value of *T. conophorum*

*Tetracarpidium conophorum*is among the list of lesser known foodstuff (Achievement, 1998) recently in order to unravel the numerous benefits of the different parts of the plant more researches are being conducted regularly. One of the important benefits is its medicinal value.

Previous and present reports have shown that all the parts of the walnut (leaves, bark, root s, hull, and nuts) possess antimicrobial effect especially the leaves. However, polar solvents and soxhlet extracted extracts revealed more antimicrobial activity than the aqueous extract. Such organisms include *(Staphylococcus aureus, Bacillus subtilis* Gram negative *(Pseudomonas aeruginosa and Escherichia coli)* as well as fungi *(Candida albicans)* and mold *(Aspergillus flavus)* strains. Their susceptibility was concentration-dependent (Ajaiyeoba and Fadare, 2006). This justifies why the plant parts are used for the treatment of various ailments. Oyenuga (1997) reported on the amino acid and fatty components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups. The edible nut or kernel is used to strengthen the kidney functions, and therefore used to strengthen the lumber region of the back, the legs and organs. It is commonly combined with other kidney tonics to enhance this action. Also, walnuts are said to be beneficial to the brain since the brain is believed to be controlled by the kidney function. (olabinri*et al.,* 2010) Reported that African walnut has been known to possess antioxidant property which is as a result of its constituent bioactive polyphenols. There is evidence that phenolic substances act as antioxidant by preventing the oxidation of LDL-lipoprotein, platelet aggregation, and damage of red blood cells (cheynier, 2005); this explains the reason that made the nut to be listed among the plant foods that are of great health benefit to human body.

## 2.5 Nutritional Value

Ogunsua and Adebona [1983] have reported on the high nutritional potential of the nut. According to Dosunumu *et al.* (2008) the nuts of *T. conophorum* are good sources of ascorbic acid and the heavy metal content of the nut is also shown to be below WHO permissible limit which makes the nut safe for public consumption without any fear of heavy metal pollution. Nwokolo (1987) also reported on the impact of traditional processing on the nutrient and sensory qualities of the nut. Okpero (2001) reported on the methods of processing the *T. conophorum* nuts while, Okafor (2008) reported on the use of *T. conophorum* seeds and processing waste in livestock feed formulation (Azeredo*et al.*, 2008).

According to Edem *et al.*, 2009 reported *the* proximate composition, ascorbic acid and heavy metal content of (African walnut) *Tetracarpidium conophorum* were evaluated using chemical analysis. The result of the proximate composition showed the following; moisture (48.70%), carbohydrate (53.20%), crude protein (35.22%), crude fat (6.21%), crude fiber (3.34%) and ash (2.03%).It also contained 53.50mg/100ml of ascorbic acid. The heavy metal concentrations in the fruit is Fe (0.064ppm), Mn (0.012ppm), Cr (0.001ppm), Ni (0.005ppm) while the concentrations of Hg, Pb and Cd were not detected. The results revealed that the *T. conophorum* is rich in ascorbic acid and carbohydrate with moderate valves of crude protein while the ash content was shown to be very low. This result shows that *T. conophorum* nut is not polluted with heavy metals since the concentrations of the heavy metals were all below WHO permissible limits.

In research carried out by Kaur and Pramanik (2012) in the biodegradation of tiger prawn shell by lactic acid fermentation, *Lactobacillus plantarum* was found to be more efficient in term of % degradation. Detail investigation on biodegradation was done to study the efficiency of *Lactobacillus plantarum* for demineralization of tiger prawn shell waste. The fermentation experiments was carried out using 150, 200, 250 and 300 micron particle size of shell waste and 8% inoculation as the starter culture up to 5days.

## 2.6 Industrial value

Besides the nutritional, medicinal, agricultural etc. benefits of *T. conophorum,* several industrial benefits have been reported as well. The oil from the nut has been reported to be used in the formulation of wood varnish, stand oil and vulcanized oil (Ajaiyeoba and Fadare, 2006). Akpuaka and Nwankwor (2010) reported that heating of the oil with powdered Sulphur at a high temperature of about 150 - 160°C for 30 minutes gave rise to the production of vulcanized oil. Also, wood vanish was made from boiled conophor oil with other additives.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 study area:

The study was carried out in the microbiology laboratory, Federal university Oye-Ekiti, Ekiti State, South west Nigeria in Africa.

### 3.2. Materials

Analytical weighing balance, oven (Genlab), incubator (Genlab), autoclave (dixons), microscope (B series), fridge (refrigerator), deep fridge, water bath, electronic colony counter, petri dishes, test tube, slide, inoculating loops, bunsen burner, beakers, cotton wool, aluminum foil paper, syringe, conical flask, measuring cylinder

### 3.3 Media used:

1. Nutrient agar(Biomark, India )
2. Sabouraud's Dextrose Agar (Lab M , United kingdom)
3. Yeast Extract Agar (Lab M , United kingdom)
4. Peptone water (Bio Lab, Hungary )

### 3.3.1. Chemical reagent used:

Ethanol (75%), crystal violent, gram's iodine, acetone, safranin, distilled water and 250mg of chloramphenicol capsule (to inhibit bacterial growth on the SDA), 3% potassium hydroxide,

### 3.4. Collection of sample

#### 3.4.1 Walnut shell sample

African Walnut (*Tetracarpidium conophorum*) was purchased at a local market in Oye-Ekiti, Ekiti state, South west Nigeria in Africa in July, 2015. The nuts were sorted and washed with tap water to remove flesh residues and other contaminants and divided into two lots. The first lot was boiled for 1.5 h. The second lot was used as raw; the raw nut and the boiled nut were then unshelled and the shell is then dried in oven at 60◦C for 5days. The dry sample was crushed into fine powder and stored in an air tight zip lock bag prior to analysis.

#### 3.4.2 Chicken Droppings Samples

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

### 3.5 Media preparation and sterilization

Nutrient Agar ( Biomark, India.),Sabouraud's Dextrose Agar (lab M, united kingdom.) and Yeast Extract Agar (lab M, united kingdom) were weighed according to the manufacture's specifications 2.8g into 100ml of sterile distilled water, 6.5g into 100ml of sterile distilled water and 3.4g into 100m of sterile distilled water respectively and dissolved in distilled water and shaken for proper mixing.

I. Sterilizing of constituted medium was carried out by autoclaving at $121^0$C for 15 minutes. The Sabouraud's Dextrose Agar and the yeast extract agar were amended with chloramphenicol capsule (250mg per 500ml) to inhibit the growth of bacteria.

II. Sterilized media were dispensed into sterile petri dishes (about 20ml volume) aseptically at temperature of about $50^0$C (to avoid excessive evaporation and condensation)

III. The dispensed media were allowed to cool and solidify in the plates.

IV. To check for sterility, the prepared plates were incubated overnight and examined (18 hours for bacterial and 72hours for fungi).

### 3.6 Microbiological Analyses of Samples

#### 3.6.1 African walnut Shell

After grinding of the samples, analyses were carried out immediately. A setup for serial dilution was done in which 10g of grinded walnut shell was mixed with 90ml of 1% peptone water in Zip Lock Bag and was mixed thoroughly in order for the microorganism present in the walnut shell to be released inside the peptone water, labeled as stock and the sample were serially diluted to a dilution factor of Ten ($10^{-10}$). 9ml of 1% peptone water were dispense into a sterile test tube and Each dilution was done by sterilizing the test tubes containing 9ml of 1% peptone water in an autoclave after which 1ml from the stock was dispense into the 9ml peptone water inside the test tube ($10^{-1}$), and these process continues till the $10^{-10}$ dilution factor using 2ml and 5ml syringes. After the serial dilution, for the bacterial culture 1ml from each dilution factor of $10^{-6}$ and $10^{-5}$ the inoculum were enumerated by spread 1ml of inoculated into three sterile petri dishes, one plate from dilution 6 and 2 plates from dilution 5 and the agar was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface.

From dilution $10^{-2}$ and $10^{-3}$ the inoculum were enumerated by spread 1ml inoculated into three sterile petri dishes also, one plate from dilution 3 and 2 plates from dilution 2(for mold culture) and

the media was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface.

From dilution $10^{-4}$ and $10^{-5}$ the inoculum were enumerated by spread 1ml of inoculated into three sterile petri dishes, one plate from dilution 4 and two plates from dilution 5(for Yeast Culture) and the media was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface

### 3.6.2 Chicken Droppings

After collection of the samples, analyses were carried out immediately. A setup for serial dilution was done in which 10g of chicken droplet was mixed with 90ml of 1% peptone water in Zip Lock Bag and was mixed thoroughly in order for the microorganism present in the walnut shell to be released inside the peptone water, labeled as stock and the sample were serially diluted to a dilution factor of Ten ($10^{-10}$). 9ml of 1% peptone water were dispense into a sterile test tube and Each dilution was done by sterilizing the test tubes containing 9ml of 1% peptone water in an autoclave after which 1ml from the stock was dispense into the 9ml peptone water inside the test tube ($10^{-1}$), and these process continues till the $10^{-10}$ dilution factor using 2ml and 5ml syringes. After the serial dilution, for the bacterial culture 1ml from each dilution factor of $10^{-6}$ and $10^{-5}$ the inoculum were enumerated by spread 1ml of inoculated into three sterile petri dishes, one plate from dilution 6 and 2 plates from dilution 5 and the agar was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface.

From dilution $10^{-2}$ and $10^{-3}$ the inoculum were enumerated by spread 1ml inoculated into three sterile petri dishes also, one plate from dilution 3 and 2 plates from dilution 2(for mold culture) and the media was poured on it, to make the colonies spread throughout the medium instead of growing only on the surface.

From dilution $10^{-4}$ and $10^{-5}$ the inoculum were enumerated by spread 1ml of inoculated into three sterile petri dishes, one plate from dilution 4 and two plates from dilution 5(for Yeast Culture) and the media was poured on it (pour plating), to make the colonies spread throughout the medium instead of growing only on the surface.

### 3.7 Microbial counts:

Microorganisms in the African walnut shell were enumerated by pouring 1.0ml of serially diluted sample into 3 petril dishes and nutrient agar (NA) media was poured on it at angle $45^{o}$ and stirred gently for the enumeration of heterotrophic bacteria and was incubated at $37^{o}$ C for 24 hours. 1.0ml of serially diluted sample for the Sabouraud's Dextrose Agar (SDA) and Yeast Extract Agar (YEA) (for fungi) at $25^{o}$ C for 72 hours . Chicken litters degrading bacteria were enumerated on nutrient agar (NA). The inoculated plates were incubated at $37^{o}$C for 24h for bacteria and at $25^{o}$C for 72h for fungi. Colonies, which appeared on the plates, were counted and expressed as colony forming units per gram (CFU/g). The organisms were isolated and subculture to obtained pure culture.

#### 3.7.1 Isolation of pure isolates

Mixed colonies of isolates were observed after 18hours of incubation $37^{o}$c for bacteria and 72hours of incubation for fungi at $25^{o}$c which the respective plates were further examined morphologically and individual distinct colonies were sub-cultured for further characterization. A pure culture was made from an isolated colony, 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. Also distinct colonies of fungal isolates using inoculating needle were also inoculated into a freshly prepared sabouraud's dextrose agar and Yeast extract agar and it was amended with chloramphenicol.

Morphological and Cultural characteristics of the discrete bacterial colonies were observed such as shape, pigmentation, colors and opacity. This was followed by microscope examination for cell morphology characterization of the isolates.

### 3.7.2 Preservation of isolated microorganisms

Double strength agar were prepared by pouring 15ml of nutrient agar, into McCartney bottles and sterilized by autoclave at 121$^{o}$C for 15minutes. The McCartney bottles containing the media were allowed to cool and solidify in a sloppy position. After gelling, with a sterilized inoculating loop the pure cultured organism was picked on the plate and introduced into the slant. The inoculum was inoculated aseptically. The culture samples were incubated for 24hours at 37 $^{0}$C. After 24hours, the growths were seen and preserved at 4$^{0}$C in the refrigerator for further test.

Double strength sabouraud's dextrose agar were prepared, After which 15ml was poured into clean McCartney bottles and sterilized by autoclave at 121 $^{0}$C for 15minutes. The media were allowed to cool after which they were inoculated with each pure strain of fungi isolates with a sterilized inoculating needle. The cultured samples were incubated for 72hours at 25 $^{0}$C. This procedure was carried out under aseptic condition. After 72hours, growths were seen on the slant and preserved at 4$^{o}$c in the refrigerator for further tests.

## 3.8 Biochemical Characterization of Bacterial Isolates

These isolated organisms were further subjected to some confirmatory test. Staining technique such as the gram staining technique was used to differentiate between gram positive and gram negative bacteria. Some biochemical tests such as the catalase activity tests, sugar fermentation and Grams reaction were done to differentiate pathogenic organism from non-pathogenic strains of microorganism and to differentiate them respectively. Microscopy was also employed for morphological characteristic features of all the isolated organisms.

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

### 3.8.1 The Gram Reaction

#### 3.8.1.1 Gram Staining

This is the most important and frequently used staining technique in microbiology. 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 sterile loop and smear on 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 heat fixing by passing the slide over the flame thrice as quick as possible. The inoculating loop was flamed and the same steps were done on other bacterial colonies. The smear was flooded with crystal violet for 60 seconds, and was washed briefly with tap water, the dye were drained quickly and washed with 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 for 10 seconds. They were then rinsed with sterile water and later flooded with dilute safranin for 60 seconds. The stain was drained, the slides were washed and blot dried.

The prepared slide were examined under the oil immersion lens i.e. 100x. References were made with respect to the colours, shapes, sizes, and formation etc. of the bacteria.

#### 3.8.1.2 3% Potassium Hydroxide Grams reaction

This is a rapid non staining (KOH) method used for the determination of the Grams reactions of bacteria.

Ethanol and cotton wool was used to clean the surface of the slide and the clean glass slide was allowed to pass through the flame. A drop of 3% KOH was placed on the clean slide. Using a sterile loop a visible amount of bacterial growth on nutrient agar culture to the drop of KOH and it was mixed thoroughly on the slide, constantly stirring over an area of about 1.5cm in diameter for 60 seconds. After which the loop was raised for about 1cm upward to observe for stringiness.

### 3.8.2 **Catalase test**

The catalase test is a test used to detect the enzyme catalase, which is produced by microorganism that mediates the breaking down of hydrogen peroxide into oxygen and water.

A drop of 3% hydrogen peroxide ($H_20_2$) was placed on a clean, dry glass slide using a sterile dropper and small amounts of young bacterial colony (18hours) was placed on it using a sterile inoculating loop and gently mixed with the H202 and observe for the present of bubble within a period of 10 seconds

### 3.8.3 Sugar fermentation test (sucrose, mannitol, lactose and fructose)

The sugars were weighed according to the manufacturer's specification (1g into 100ml of the broth). One gram of the sugars was added to 100ml of nutrient broth and it was labeled according to the sugars involved and phenol red was added to it until the colour changes. Ten (10 ml) of the solution was dispense into the sterile test tube, Durham tube were insert inverted into all tubes and the Durham tube was filled up with the broth inside the test tube. The test tubes were then sterilized at $121^0$c for 15 minutes. Aseptically the cultured organism was inoculated into each of the labeled

sugar present in the test tube, Un inoculated tube was used as control tubes and it was incubated at $37^{o}$c for 18hours. After 18 hours the reaction were observed.

### 3.8.4 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.

## 3.9 Biodegradation

### 3.9.1 Biodegradation of Natural Degrading Boiled African walnut Shell and Chicken Droppings

The biodegradation of Natural Degrading Boiled African walnut shell was done in which 40g of African walnut shell was weighed and put into covered biodegradation bowl and 10ml of sterile distilled water was added to the samples under a sterile condition so that the source of the organism present should be from a known source, and a sterile spatula was used to mixed the samples together gently which serves as the beginning of biodegradation process

Five gram (5g) of the natural and mixed sample (walnut shell and chicken droppings) was taking at 0 day of biodegradation for serial dilution in order to have the knowledge of the microorganism present in the mixture of the sample at initial stage and the pH.was also noted.

Five gram (5g) of the sample was also taking at 5th day for serial dilution analysis, in order to monitor the microorganism responsible for biodegradation process, changes in pH, also the same was done on 10 days, 15 days, 20 days and 30 days to have the full knowledge on microorganisms responsible for biodegradation at different level, the quantity of acid production and the pH of the sample at which the microorganism is operating was also observed.

### 3.9.2 Biodegradation of Natural Degrading Raw African walnut Shell and Chicken Droppings

The biodegradation of Natural Degrading Raw African walnut shell was done in which 40g of African walnut shell was weighed and put into covered biodegradation bowl and 10ml of sterile distilled water was added to the samples under a sterile condition so that the source of the organism present should be from a known source, and a sterile spatula was used to mixed the samples together gently which serves as the beginning of biodegradation process

Five gram (5g) of the natural and mixed sample (walnut shell and chicken droppings) was taking at 0 day of biodegradation for serial dilution in order to have the knowledge of the microorganism present in the mixture of the sample at initial stage and the pH.was also noted.

Five gram (5g) of the sample was also taking at 5th day for serial dilution analysis, in order to monitor the microorganism responsible for biodegradation process, changes in pH, also the same was done on 10 days, 15 days, 20 days and 30 days to have the full knowledge on microorganisms responsible for biodegradation at different level, the quantity of acid production and the pH of the sample at which the microorganism is operating was also observed.

### 3.9.3 **Biodegradation of Degrading African walnut Shell Inoculated with Chicken Droppings at Ratio 1:1**

The biodegradation of African walnut shell with chicken dropping was done in Ratio 1:1 in which 39g of African walnut shell was weighed and put into the covered biodegradation bowl and 39g of chicken droppings was incorporated into it and 10ml of sterile distilled water was added to the samples under a sterile condition so that the source of the organism present should be from a known source, and a sterile spatula was used to mixed the samples together gently which serves as the beginning of biodegradation process Five gram (5g) of the natural and mixed sample (walnut shell and chicken droppings) was taking at 0 day of biodegradation for serial dilution in order to have the knowledge of the microorganism present in the mixture of the sample at initial stage and the pH.was also noted.

Five gram (5g) of the sample was also taking at $5^{th}$ day for serial dilution analysis, in order to monitor the microorganism responsible for biodegradation process, changes in pH, also the same was done on 10 days, 15 days and 20 days to have the full knowledge on microorganisms responsible for biodegradation at different level, the quantity of acid production and the pH of the sample at which the microorganism is operating.

### 3.9.4 **Biodegradation of Degrading African walnut Shell Inoculated with Chicken Droppings at Ratio 2:1**

The biodegradation of African walnut shell and chicken dropping was done in Ratio 2:1 in which 39g of African walnut shell was weighed and put in covered biodegradation bowl and 78g of chicken droppings was incorporated into it and

10ml of sterile distilled water was added to the samples under a sterile condition so that the source of the organism present should be from a known source, and a sterile spatula was used to mix the samples together gently which serves as the beginning of the biodegradation process.

Five gram (5g) of the natural and mixed sample (walnut shell and chicken droppings) was taking at 0 day of biodegradation for serial dilution in order to have the knowledge of the microorganism present in the mixture of the sample at initial stage and the pH. was also noted.

Five gram (5g) of the sample was also taking at $5^{th}$ day for serial dilution analysis, in order to monitor the microorganism responsible for biodegradation process, changes in pH, also the same was done on 10 days, 15 days and 20 days to have the full knowledge on microorganisms responsible for biodegradation at different level, the quantity of acid production and the pH of the sample at which the microorganism is operating.

### 3.10 Physicochemical properties of samples

#### 3.10.1 The Total Titratable Acid (TTA)

The Total Titratable Acid was carried out by preparing the aliquot in which 5g of the sample was weighed and 45ml of 0.1 peptone water was added to it and was mixed gently and 20ml of the solution was put inside a beaker and 2drops of phenolphthalein was added to it. The 0.4 m of NAOH was prepared and poured into the burette and was titrated against the prepared solution and the readings was done on the burette.

#### 3.10.2 The pH measurement of the samples

The remaining aliquot from the above experiment was stirred for 3minutes for proper dilution. Three (3ml) was taking from the filtered solution was drown out using sterile syringe and its pH was determined using a digital pH meter.

# CHAPTER FOUR

## 4.0 RESULT AND DISCUSSION

### 4.1 RESULT

### 4.1.1 Total viable microbial count on the samples

#### *4.1.1.1. The viable microbial count from the boiled African walnut shell.*

The microbial count as indicated in Table 1 shows that the bacterial are predominant in the sample than the fungi. The viable bacterial count were higher in this sample ($1.5x10^6$ cfu/g) compare to the number of mold ($0.5x10^1$ cfu/g)and yeast ($0.7X10^3$ cfu/g) present in the sample. This is evidence that there is present of microorganism i.e bacteria, yeast and mold in the sample. This trends reveals heavy proliferation ratio of bacteria in the Boiled African walnut shell.

#### *4.1.1.2. The total viable microbial count isolated from Raw African walnut shell.*

The microbial count as indicated in table 2 shows that the total viable count of the bacterial present in this sample are of low number in comparable to the boiled African Walnut shell. The bacteria, mold and yeast are so few in number. The highest viable count for bacteria was $0.8x10^4$ cfu/g while that of mold and yeast were $0.2x10^1$ cfu/g and $0.4x10^3$ cfu /g respectively.

#### *4.1.1.3. The total viable microbial count isolated from chicken droppings (CD).*

The microbial count as indicated in Table 3 shows that the Chicken Droppings harbor a large number of microorganism compared to other samples used in this research work. The bacterial show the highest number of $8.5x10^6$ cfu/g followed by the mold with highest number of $2.0x10^3$ cfu/g and yeast $0.9x10^3$ cfu/g respectively. This trends reveals heavy proliferation ratio of bacteria in the chicken Droppings.

# Total viable microbial count on the samples

**Table 1: Total viable microbial count isolated from Boiled African walnut shell**

| **Sample Code** | **Viable Count ( cfu/g) at 24hours** |
|---|---|
| Bacterial viable count | |
| AWNSBB1 | $1.5x10^6$ |
| AWNSBB2 | $1.3x10^6$ |
| AWNSBB3 | $1.0x10^7$ |
| Mold viable count | |
| Sample Code | Viable Count ( cfu/g) at 72 hours |
| AWNSBM1 | $0.5x10^1$ |
| AWNSBM2 | $0.4x10^1$ |
| AWNSBM3 | $0.2x10^2$ |
| Yeast viable count | |
| Sample Code | Viable Count ( cfu/g) at 72 hours |
| AWNSBY1 | $0.7X10^3$ |
| AWNSBY2 | $0.5X10^4$ |
| AWNSBY3 | $0.5X10^4$ |

**KEY:** AWNSBB = African Walnut Shell Boiled Bacterial.

AWNSBY= African Walnut Shell Boiled Yeast.

AWNSBM= African Walnut Shell Boiled Mould.

cfu/g = Colony Forming Unit per gram

**Table 2: Total viable microbial count isolated from Raw African walnut shell**

| **Isolate code** | **Viable Count at 24 hours( cfu/g )** |
|---|---|
| Bacterial viable count | |
| AWNSRB1 | $0.6x10^4$ |
| AWNSRB2 | $0.8X10^4$ |
| AWNSRB3 | $0.5x10^5$ |
| Mold viable count | |
| Sample Code | Viable Count ( cfu/g) at 72 hours |
| AWNSRM1 | $0.2x10^1$ |
| AWNSRM2 | $0.2x10^1$ |
| AWNSRM3 | $0.1x10^2$ |
| Yeast viable count | |
| Isolate code | Viable Count ( cfu/g) at 72 hours |
| AWNSRY1 | $0.4X10^3$ |
| AWNSRY2 | $0.2X10^4$ |
| AWNSRY3 | $0.3X10^4$ |

**KEY**: AWNSRB= African Walnut Shell Raw Bacteria.

AWNSRM= African Walnut Shell Raw Mould,

AWNSRY= African Walnut Shell Raw Yeast

cfu/g = Colony Forming Unit per gram

**Table 3: Total viable microbial count isolated from Chicken Droppings (CD)**

| **Sample Code** | **Viable Count ( cfu/g) at 24hours** |
|---|---|
| Bacterial viable count | |
| CDB1 | $8.5x10^6$ |
| CDB2 | $8.0x10^6$ |
| CDB3 | $7.0x10^7$ |
| Mold viable count | |
| Sample Code | Viable Count ( cfu/g) at 72 hours |
| CDM1 | $2.0x10^3$ |
| CDM2 | $1.8x10^3$ |
| CDM3 | $1.5x10^4$ |
| Yeast viable count | |
| Sample Code | Viable Count ( cfu/g) at 72 hours |
| CDY1 | $0.9x10^3$ |
| CDY2 | $0.7x10^4$ |
| CDY3 | $0.6X10^4$ |

**KEY**: CDB = Chicken Droppings Bacteria

CDY = Chicken Droppings Yeast

CDM= Chicken Droppings Mold

cfu/g = Colony Forming Unit per gram

## 4.1.2 Total microbial Viable Count from Natural Degrading Boiled African Walnut Shell

### *4.1.2.1. The total bacterial viable count of isolate from natural degrading boiled African walnut shell.*

The microbial count as indicated in Table 4 shows that at day zero the bacterial count were very low but at the day of biodegradation increasing the bacteria count also increasing from the lowest number ($1.5x10^6$ cfu/g) to highest number of ($7.7x10^6$ cfu/g) at day 15 this shows that the sample create an appropriate condition for the growth of the bacterial. The growth of the bacterial begins to reduce at day 20 of the biodegradation ranging from highest number of $4.4x10^6$ cfu/g to highest number of $3.0x10^6$ cfu/g at day 30.

### 4.1.2.2. *The total yeast viable count of isolate from natural degrading boiled African walnut shell.*

The microbial count as indicated in Table 5 shows that the number of yeast present at day zero were so low but as it reaches day 5 of the biodegradation the number keep increasing but in a low state and at day 20 the number of the yeast begins to reduce from highest number of ($0.2x10^3$ cfu/g) and at day 30 there was no growth on any of the plate which may indicate that the yeast have completed it biodegradation process.

### 4.1.2.3. T*he total mold viable count of isolate from natural degrading boiled African walnut shell.*

The microbial count as indicated in Table 6 shows that The mold is the second largest microorganism present in this sample; it ranged from a higher rate of $0.5x10^1$ cfu/g at day zero to higher rate of $1.0x10^3$ cfu/g at day 10 of biodegradation but the number of the mold count begins to fall to a low level ($0.5x10^1$ cfu/g) at day 15 and at day 30 the number of the colonies were small ($0.2x10^1$ cfu/g).

## Total microbial Viable Count from Natural Degrading Boiled African Walnut Shell

**Table 4: Total Bacterial viable count of isolates from Natural Degrading Boiled African Walnut Shells (NDBAWSB).**

| Isolate code | Viable Count ( cfu/g) at 24hours |
|---|---|
| DAY 0 | |
| NDBAWSB 1 | $1.5x10^6$ |
| NDBAWSB 2 | $1.3x10^6$ |
| NDBAWSB 3 | $1.0x10^7$ |
| DAY 5 | |
| NDBAWSB 1 | $6.5x10^6$ |
| NDBAWSB 2 | $5.8x10^6$ |
| NDBAWSB 3 | $4.6x10^7$ |
| DAY 10 | |
| NDBAWSB 1 | $6.8x10^6$ |
| NDBAWSB 2 | $6.0x10^6$ |
| NDBAWSB 3 | $5.1x10^7$ |
| DAY 15 | |
| NDBAWSB 1 | $7.7x10^6$ |
| NDBAWSB 2 | $7.2x10^6$ |
| NDBAWS 3 | $6.4x10^7$ |
| DAY 20 | |
| NDBAWS 1 | $4.4x10^6$ |
| NDBAWS 2 | $4.2x10^6$ |
| NDBAWS 3 | $3.8x10^7$ |
| DAY 30 | |
| NDBAWSB 1 | $2.0x10^6$ |
| NDBAWSB 2 | $1.8x10^6$ |
| NDBAWSB 3 | $1.5x10^7$ |

**KEY**: NDBAWSB = Natural Degrading Boiled African Walnut Shell Bacteria

cfu/g= Colony Forming Unit per Gram

**Table 5: Total Yeast viable count of isolates from Natural Degrading Boiled African Walnut Shells (NDBAWSY).**

| **Isolate code** | **Viable Count ( cfu/g) at 72 hours** |
|---|---|
| DAY 0 | |
| NDBAWSY 1 | $0.7X10^3$ |
| NDBAWSY 2 | $0.5X10^4$ |
| NDBAWSY 3 | $0.5X10^4$ |
| DAY 5 | |
| NDBAWSY 1 | $0.8X10^3$ |
| NDBAWSY 2 | $0.6X10^4$ |
| NDBAWSY 3 | $0.7X10^4$ |
| DAY 10 | |
| NDBAWSY 1 | $0.9X10^3$ |
| NDBAWSY 2 | $0.7X10^4$ |
| NDBAWSY 3 | $0.7X10^4$ |
| DAY 15 | |
| NDBAWSY 1 | $0.4X10^3$ |
| NDBAWSY 2 | $0.3X10^4$ |
| NDBAWSY 3 | $0.1X10^4$ |
| DAY 20 | |
| NDBAWSY 1 | $0.2x10^3$ |
| DAY 30 | |
| NDBAWSY | No Growth |

**KEY**: NDBAWSY= Natural Degradation of Boiled African Walnut Shell Yeast

cfu/g= Colony Forming Unit per gram

**Table 6: Total Mold viable count of isolates from Natural Degrading Boiled African Walnut Shells (NDBAWSM).**

| **Isolate code** | **Viable Count ( cfu/g) at 72 hours** |
|---|---|
| DAY 0 | |
| NDBAWSM 1 | $0.5x10^1$ |
| NDBAWSM 2 | $0.4x10^1$ |
| NDBAWSM 3 | $0.2x10^2$ |
| DAY 5 | |
| NDBAWSM 1 | $0.8x10^1$ |
| NDBAWSM 2 | $0.6x10^1$ |
| NDBAWSM 3 | $0.5x10^2$ |
| DAY 10 | |
| NDBAWSM 1 | $1.0x10^3$ |
| NDBAWSM 2 | $0.8x10^1$ |
| NDBAWSM 3 | $0.7x10^2$ |
| DAY 15 | |
| NDBAWSM 1 | $5x10^1$ |
| NDBAWSM 2 | $3x10^1$ |
| NDBAWSM 3 | $1x10^2$ |
| DAY 20 | |
| NDBAWSM 1 | $0.2x10^1$ |
| DAY 30 | |
| NDBAWSM 1 | $0.2x10^1$ |

**KEY**: NDBAWSM= Natural Degrading Boiled African Walnut Shell Mold

cfu/g= Colony Forming Unit per gram

### 4.1.3 Microbial Viable Count from Natural Degrading Raw African Walnut Shell (NDRAWS)

#### *4.1.3.1. The total bacterial viable count of isolates from natural degrading raw African walnut shells.*

The microbial count as indicated in Table 7 shows that the highest bacterial count at day zero of the biodegradation was at $0.8x10^4$ and at day 5 it keeps increasing until it reaches day 20 when the number of the bacterial begins to reduce from the highest value of $7.0x10^6$ cfu/g to the highest value of $3.0x10^6$at day 20, at day 30 the bacterial count was a bit higher than the day zero of the degradation. The highest bacterial count was observed at the day 15 ($7.0x10^6$ cfu/g) of the degradation. This implies that between day zero and day 15 the growth of the bacteria were increased rapidly.

#### *4.1.3.2. The total yeast viable count of isolates from natural degrading raw Africa walnut shells*.

The microbial count as indicated in Table 8 shows that between day zero to day 5 there was no difference in the yeast count. The count of the yeast at day zero remain practically unchanged with that of day 5. At day 10 there was a little changes in the yeast count which ranged from $0.4x10^3$ cfu/g to $0.6x10^3$ cfu/g,at day 15 the yeast count dropped from $0.6x10^3$ cfu/g to $0.3x10^3$ cfu/g but at day 20 to day 30 there was no yeast count.

#### *4.1.3.3. The total mold viable count of isolates from natural degradation of raw African walnut shell.*

The microbial count as indicated in Table 9 shows that the mold ranges from $0.2x10^1$ cfu/g at day zero to $0.4x10^1$ at day 5, the mold reaches its peak at day 15 with maximum mold count of $0.8x10^1$ cfu/g, at day 20 of the biodegradation the mold count reduced from $0.8x10^1$ cfu/g to $0.2x10^1$ and at day 30 $0.1x10^1$ cfu/g respectively.

## Microbial Viable Count from Natural Degrading Raw African Walnut Shell (NDRAWS)

**Table 7: Total Bacterial viable count of isolates from Natural Degrading Raw African Walnut Shells (NDRAWSB).**

| **Isolate code** | **Viable Count ( cfu/g) at 72 hours** |
|---|---|
| DAY 0 | |
| NDRAWSB 1 | $0.6x10^4$ |
| NDRAWSB 2 | $0.8X10^4$ |
| NDRAWSB 3 | $0.5x10^5$ |
| DAY 5 | |
| NDRAWSB 1 | $5.6X10^6$ |
| NDRAWSB 2 | $5.2x10^6$ |
| NDRAWSB 3 | $4.0x10^7$ |
| DAY 10 | |
| NDRAWSB 1 | $5.8X10^6$ |
| NDRAWSB 2 | $5.2x10^6$ |
| NDRAWSB 3 | $4.5x10^7$ |
| DAY 15 | |
| NDRAWSB 1 | $7.0x10^6$ |
| NDRAWSB 2 | $6.5X10^6$ |
| NDRAWSB 3 | $6.1x10^7$ |
| DAY 20 | |
| NDRAWSB 1 | $3.0x10^6$ |
| NDRAWSB 2 | $2.7X10^6$ |
| NDRAWSB 3 | $2.5x10^7$ |
| DAY 30 | |
| NDRAWSB 1 | $1.6x10^6$ |
| NDRAWSB 2 | $1.6x10^6$ |
| NDRAWSB 3 | $1.0x10^7$ |

KEY: NDRAWSB = Natural Degrading Raw African Walnut Shell Bacteria

cfu/g= Colony Forming Unit per gram

**Table 8: Total Yeast viable count of isolates from Natural Degrading Raw African Walnut Shells (NDRAWS).**

| Isolate code | Viable Count ( cfu/g) at 72 hours |
|---|---|
| DAY 0 | |
| NDRAWSY 1 | $0.4X10^3$ |
| NDRAWSY 2 | $0.2X10^4$ |
| NDRAWSY 3 | $0.3X10^4$ |
| DAY 5 | |
| NDRAWSY 1 | $0.4X10^3$ |
| NDRAWSY 2 | $0.2\ X10^4$ |
| NDRAWSY 3 | $0.3X10^4$ |
| DAY 10 | |
| NDRAWSY 1 | $0.6X10^3$ |
| NDRAWSY 2 | $0.4\ X10^4$ |
| NDRAWSY 3 | $0.2X10^4$ |
| DAY 15 | |
| NDRAWSY 1 | $0.3X10^3$ |
| NDRAWSY 2 | $0.2\ X10^4$ |
| NDRAWSY3 | $0.1X10^4$ |
| DAY 20 | |
| NDRAWSY | No Viable count |
| DAY 30 | |
| NDRAWSY | No Viable count |

KEY: NDRAWSY = Natural Degrading Raw African Walnut Shell Yeast

cfu/g= Colony Forming Unit per gram

**Table 9: Total Mold viable count of isolates from Natural Degrading Raw African Walnut Shells (NDRAWSM).**

| **Isolate code** | **Viable Count ( cfu/g) at 72 hours** |
|---|---|
| DAY 0 | |
| NDRAWSM 1 | $0.2x10^1$ |
| NDRAWSM 2 | $0.2x10^1$ |
| NDRAWSM 3 | $0.1x10^2$ |
| DAY 5 | |
| NDRAWSM 1 | $0.4x10^1$ |
| NDRAWSM 2 | $0.4x10^1$ |
| NDRAWSM 3 | $0.2x10^2$ |
| DAY 10 | |
| NDRAWSM 1 | $0.6x10^1$ |
| NDRAWSM 2 | $0.4x10^1$ |
| NDRAWSM 3 | $0.3x10^2$ |
| DAY 15 | |
| NDRAWSM 1 | $0.8x10^1$ |
| NDRAWSM 2 | $0.6x10^1$ |
| NDRAWSM 3 | $0.4x10^2$ |
| DAY 20 | |
| NDRAWSM 1 | $0.2x10^1$ |
| DAY 30 | |
| NDRAWSM 1 | $0.1x10^1$ |

**KEY**: NDRAWSM = Natural Degrading Raw African Walnut Shell Mold

cfu/g= Colony Forming Unit per gram

## 4.1.4 Total viable microbial count of degrading from African Walnut Shell Inoculated with Chicken Droppings at ratio 1:1(DAWSA)

### *4.1.4.1. The total bacterial viable count of isolates from degrading African walnut shell inoculated with chicken dropping at ratio 1:1*

The microbial count as indicated in Table 10 shows that at day zero of biodegradation the maximum bacterial count was $13.2x10^7$ cfu/g which reaches its peak at day 5 with bacterial count of $15.0x10^7$ cfu/g and at day 10 of the biodegradation the bacterial count begins to reduce in number with maximum value of $10.0x10^7$ cfu/g, $5.4x10^6$ cfu/g for day 15 and $5.1x\ 10^6$ cfu/g for day 20 respectively.

### *4.1.4.2. The total yeast count of isolate from degrading African walnut shell inoculated with chicken dropping at ratio 1:1*.

The microbial count as indicated in Table 11 shows that shows that the yeast count at day zero was very high compared to the yeast present in the previous samples at day zero. The yeast count at day zero was recorded to be $1.1x10^5$ cfu/g and at day 5 it reaches the peak level with yeast count of $15.0x10^5$ cfu/g. At day 10 there was a decrease in the number of the yeast present with a difference of $0.5x10^4$ cfu/g but at day 20 the number were so low.

### *4.1.4.3. The total mold viable count of isolates from degrading African walnut shell*.

The microbial count as indicated in Table 8 shows that the maximum count of mold at day zero was recorded to be $1.2x10^3$ cfu/g and at day 5 the highest value of the mold count $1.4x10^3$ cfu/g and at day 10 the mold count dropped from $1.4x10^3$ cfu/g to $0.5x10^1$ which shows that the mold obtained its highest count at day 5. At day 20 the mold count was so scanty with the highest number of $0.2x10^1$ cfu/g.

**Total viable microbial count of degrading from African Walnut Shell Inoculated with Chicken Droppings at ratio 1:1(DAWSA)**

**Table 10: Total Bacterial viable count of isolates from Degrading African Walnut Shells Inoculated with Chicken dropping at ratio 1:1 (DAWSAB).**

| **Isolate code** | **Viable Count ( cfu/g) at 24 hours** |
|---|---|
| DAY 0 | |
| DAWSAB 1 | $13.2X10^{7}$ |
| DAWSAB 2 | $13.4X10^{7}$ |
| DAWSAB 3 | $11.6X10^{8}$ |
| DAY 5 | |
| DAWSAB 1 | $15.0X10^{7}$ |
| DAWSAB 2 | $14.8X10^{7}$ |
| DAWSAB 3 | $12.4X10^{8}$ |
| DAY 10 | |
| DAWSAB 1 | $10.0x10^{7}$ |
| DAWSAB 2 | $8.7x10^{6}$ |
| DAWSAB 3 | $6.8x10^{7}$ |
| DAY 15 | |
| DAWSAB 1 | $5.4x10^{6}$ |
| DAWSAB 2 | $4.8x10^{6}$ |
| DAWSAB 3 | $4.0x10^{7}$ |
| DAY 20 | |
| DAWSAB 1 | $5.1x10^{6}$ |
| DAWSAB 2 | $4.4x10^{6}$ |
| DAWSAB 3 | $3.5x10^{7}$ |

**KEY**: DAWSAB = Degrading African Walnut Shell inoculated with Chicken Droppings at ratio 1:1 for Bacteria

cfu/g= Colony Forming Unit per gram

**Table 11: Total Yeast viable count of isolates from Degrading African Walnut Shells Inoculated with Chicken dropping at ratio 1:1 (DAWSAY).**

| **Isolate code** | **Viable Count ( cfu/g) at 72 hours** |
|---|---|
| DAY 0 | |
| DAWSAY 1 | $1.1x10^5$ |
| DAWSAY 2 | $0.8x10^4$ |
| DAWSAY 3 | $0.7x10^4$ |
| DAY 5 | |
| DAWSAY 1 | $1.5x10^5$ |
| DAWSAY 2 | $1.2x10^6$ |
| DAWSAY 3 | $1.1x10^6$ |
| DAY 10 | |
| DAWSAY 1 | $1.0x10^5$ |
| DAWSAY 2 | $0.5x10^4$ |
| DAWSAY 3 | $0.5\ x10^4$ |
| DAY 15 | |
| DAWSAY 1 | $0.2\ x10^3$ |
| DAWSAY 2 | $0.1\ x10^4$ |
| Day 20 | |
| DAWSAY 1 | $0.2\ x10^3$ |
| DAWSAY 2 | $0.1\ x10^4$ |

**KEY**: DAWSAY = Degrading African Walnut Shell inoculated with Chicken Droppings at ratio 1:1 for Yeast

cfu/g= Colony Forming Unit per gram

**Table 12: Total Mold viable count of isolates from Degrading African Walnut Shells Inoculated with Chicken dropping at ratio 1:1 (DAWSAM).**

| Isolate code | Viable Count ( cfu/g) at 72 hours |
|---|---|
| DAY 0 | |
| DAWSAM 1 | $1.2x10^3$ |
| DAWSAM 2 | $1.0x10^3$ |
| DAWSAM 3 | $0.7x10^2$ |
| DAY 5 | |
| DAWSAM 1 | $1.4x10^3$ |
| DAWSAM 2 | $1.2x10^3$ |
| DAWSAM 3 | $1.0x10^4$ |
| DAY 10 | |
| DAWSAM 1 | $0.5x10^1$ |
| DAWSAM 2 | $0.3x10^1$ |
| DAWSAM 3 | $0.2x10^2$ |
| DAY 15 | |
| DAWSAM 1 | $0.2x10^1$ |
| DAWSAM 2 | $0.2x10^1$ |
| DAWSAM 3 | $0.1x10^2$ |
| DAY 20 | |
| DAWSAM 1 | $0.2x10^1$ |
| DAWSAM 2 | $0.1x10^1$ |

**KEY**: DAWSAY = Degrading African Walnut Shell inoculated with Chicken Droppings at ratio 1:1 for Yeast

cfu/g= Colony Forming Unit per gram

### 4.1.5Total viable microbial count of Degrading from African Walnut Shell Inoculated with Chicken Droppings at ratio 2:1

#### *4.1.5.1. The total bacterial viable count of isolates from degrading walnut shells inoculated with chicken dropping at ratio 2:1*.

The microbial count as indicated in Table 13 shows that the number of bacterial increased between day 0 to day 5 of the biodegradation. At day zero the bacteria count was very high with highest viable count of $15.0x10^7$ cfu/g and at day 5 it reached the peak rate with viable count of $18.0x10^7$ cfu/g and at day 15 it decreased to the highest viable count of $5.2x10^6$ cfu/g and $4.5 x10^6$ cfu/g at day 20 respectively.

#### *4.1.5.2. The total yeast viable count of isolates from degrading African walnut shells inoculated with chicken droppings at ratio 2:1*

The microbial count as indicated in Table 14 shows that the yeast count at day zero was so high compared to the day zero at ratio 1:1 of chicken droppings and walnut shell with viable count of $1.5x10^5$ cfu/g and at day 5 it reached the peak rate with viable count of $1.7x10^5$ cfu/g at the day 10 of the biodegradation process the yeast viable count reduces from $1.7x10^5$to $0.7x10^3$ cfu/g and at day 20 of the biodegradation the yeast count was very low with viable count of $0.2x10^3$ cfu/g.

#### *4.1.5.3. The total mold viable count of isolate from degrading African walnut shell, inoculated with chicken dropping at ratio 2:1*

The microbial count as indicated in Table 15 shows that the Mold show the lowest microbial count in this biodegradation process, at day zero the highest value was $1.0x10^3$ cfu/g and $1.3x10^3$ cfu/g at day 5, at day 10 there is decrease in the viable count which reduces from $1.3x10^3$ cfu/g to $0.8x10^1$ cfu/g and at day 20 the growth of the mold was minimal with viable count of $0.1x10^1$ cfu/g.

## Total viable microbial count of Degrading from African Walnut Shell Inoculated with Chicken Droppings at ratio 2:1

**Table 13: Total Bacterial viable count of isolates from Degrading African Walnut Shells Inoculated with Chicken dropping at ratio 2:1 (DAWSBB).**

| Isolate code | Viable Count ( cfu/g) at 24 hours |
|---|---|
| DAY 0 | |
| DAWSBB 1 | $15.0X10^{7}$ |
| DAWSBB 2 | $14.8X10^{7}$ |
| DAWSBB 3 | $13.5X10^{8}$ |
| DAY 5 | |
| DAWSBB 1 | $18.0X10^{7}$ |
| DAWSBB 2 | $16.0X10^{7}$ |
| DAWSBB 3 | $14.6X10^{8}$ |
| DAY 10 | |
| DAWSBB 1 | $6.0\ X10^{6}$ |
| DAWSBB 2 | $5.8\ X10^{6}$ |
| DAWSBB 3 | $5.2\ X10^{7}$ |
| DAY 15 | |
| DAWSBB 1 | $5.2\ X10^{6}$ |
| DAWSBB 2 | $4.0\ X10^{6}$ |
| DAWSBB 3 | $3.8\ X10^{7}$ |
| DAY 20 | |
| DAWSBB 1 | $4.5\ X10^{6}$ |
| DAWSBB 2 | $4.2\ X10^{6}$ |
| DAWSBB 3 | $3.3\ X10^{7}$ |

**KEY**: DAWSBB = Degrading African Walnut Shell inoculated with Chicken Droppings at ratio 2:1 for Bacteria

cfu/g= Colony Forming Unit per gram

**Table 14: Total Yeast viable count of isolates from Degrading African Walnut Shells Inoculated with Chicken dropping at ratio 2:1 (DAWSBY).**

| **Isolate code** | **Viable Count ( cfu/g) at 72 hours** |
|---|---|
| DAY 0 | |
| DAWSBY 1 | $1.5x10^{5}$ |
| DAWSBY 2 | $1.3x10^{6}$ |
| DAWSBY 3 | $1.0x10^{6}$ |
| DAY 5 | |
| DAWSBY 1 | $1.7x10^{5}$ |
| DAWSBY 2 | $1.5x10^{6}$ |
| DAWSBY 3 | $1.3x10^{6}$ |
| DAY 10 | |
| DAWSBY 1 | $0.7x10^{3}$ |
| DAWSBY 2 | $0.3x10^{4}$ |
| DAWSBY 3 | $0.1x10^{4}$ |
| DAY 15 | |
| DAWSBY 1 | $0.3x10^{3}$ |
| DAWSBY 2 | $0.1x10^{4}$ |
| DAWSBY 3 | $0.1x10^{4}$ |
| DAY 20 | |
| DAWSBY 1 | $0.2x10^{3}$ |

**KEY**: DAWSBY = Degrading African Walnut Shell inoculated with Chicken Droppings at ratio 2:1 for Yeast

cfu/g= Colony Forming Unit per gram

**Table 15: Total Mold viable count of isolates from Degrading African Walnut Shells Inoculated with Chicken dropping at ratio 2:1 (DAWSBM).**

| Isolate code | Viable Count ( cfu/g) at 72 hours |
|---|---|
| DAY 0 | |
| DAWSBM 1 | $1.0x10^3$ |
| DAWSBM 2 | $1.0x10^3$ |
| DAWSBM 3 | $0.8x10^2$ |
| DAY 5 | |
| DAWSBM 1 | $1.3X10^3$ |
| DAWSBM 2 | $1.0X10^3$ |
| DAWSBM 3 | $0.8X10^2$ |
| DAY 10 | |
| DAWSBM 1 | $0.8x10^1$ |
| DAWSBM 2 | $0.5x10^1$ |
| DAWSBM 3 | $0.4x10^2$ |
| DAY 15 | |
| DAWSBM 1 | $0.2x10^1$ |
| DAY 20 | |
| DAWSBM 1 | $0.1x10^1$ |
| DAWSBM 2 | $0.1x10^1$ |

**KEY**: DAWSBM = Degrading African Walnut Shell inoculated with Chicken Droppings at ratio 2:1 for Mold cfu/g= Colony Forming Unit per gram

### 4.1.6. Morphological and microscopic characteristics of isolate colonies from the samples

#### *4.1.6.1. The morphological and microscopic characteristics of bacteria isolate colonies from African walnut shell boiled.*

As indicated in Table 16: the morphological characteristics of bacteria isolates from Boiled African walnut Shell shows that the bacterial with rod shape dominate in the sample. Most of the bacterial forms circular shape with cream colour and few were white in color.

#### *4.1.6.2. The morphology and microscopic characteristics of bacteria isolate colonies from Raw African walnut shells.*

As indicated in Table 17: the morphological characteristics of bacteria isolates from Raw African walnut Shell shows that the bacterial with cocci shape dominate with cream color, raised in Elongation and some with smooth surface.

#### *4.1.6.3. The morphological and microscopic characteristics of the microorganism isolate from chicken droppings.*

As indicated in Table 18: the morphological characteristics of bacteria isolates from Chicken droppings shows that the predominant organism shows the following features, rod shape and white in color and some were rhizoid while some has irregular shape

**Morphological and microscopic characteristics of isolate colonies from the samples**

**Table 16: Morphological and microscopic characteristics of bacteria isolate colonies from African Walnut Shell Boiled Bacteria (AWNSBB).**

| Isolate Code | Forms | Shape | Colour | Elevation | Surface | Edge |
|---|---|---|---|---|---|---|
| AWNSBB1 | Circular | Rod | White | Raised | Smooth | Entire |
| AWNSBB2 | Circular | Bacillus | Cream | Raised | Margin | Dented |
| AWNSBB3 | Circular | Rod | Cream | Raised | Smooth | Entire |

**KEY**: AWNSBB= African Walnut Shell Boiled Bacteria

**Table 17: Morphological and microscopic characteristics of bacteria isolate colonies from African Walnut Shell Raw Bacteria (AWNSRB).**

| Isolate Code | Forms | Shape | Colour | Elevation | Surface | Edge |
|---|---|---|---|---|---|---|
| AWNSRB | Spindle | Rod | Yellow | Flat | Margin | Entire |
| AWNSRB | Circular | Cocci in pair chain | Cream | Raised | Smooth | Dented |
| AWNSRB | Circular | Cocci in chain | Cream | Raised | Margin | Lobate |

**KEY:** AWNSRB: African Walnut Shell Raw Bacteria

**Table 18: Morphological and microscopic characteristics of bacteria isolate colonies from Chicken Droppings (CDB).**

| Isolate code | Colour | Shape | Surface | Elevation | Form | Edge |
|---|---|---|---|---|---|---|
| CDB 1 | White | Cocci in cluster | margin | Raised | Rhizoid | Curled |
| CDB 2 | Cream | Rod | Gleaming | Convex | Irregular | Lobate |
| CDB 3 | Cream | Rod | Rough | Flat | Filamentous | Lobate |

**KEY**: CDB=Chicken Droppings Bacteria

### 4.1.7. Morphological and Microscopic characteristics of microbial isolate colonies from the Degrading African Walnut Shell.

#### *4.1.7.1. The morphological and microscopic characteristics of bacterial isolate from natural degrading boiled African walnut shell.*

As indicated in Table 19: the morphological characteristics of bacteria isolates from natural degrading Boiled African walnut Shell shows that the organism with cocci shape dominate over the rod-shaped bacteria and their colour ranges from cream to yellow and majority were dented while others were entire with a smooth surface.

#### *4.1.7.2. The morphological and microscopic characteristics of fungi isolate colonies from natural Degrading Boiled African walnut shell.*

As indicated in Table 20: the morphological characteristics of fungi isolates from Degrading Boiled African walnut Shell shows that the yeast present in this sample have an upright conidiophore that terminate in a deviate swelling bearing phliate, their mycelium are not extensive and some have apex radiating from the entire surface and their conidial are 1-celled. Majority of the mold have septate hyphae with sparse mycelia and conidiophore.

#### *4.1.7.3. The morphological and microscopic characteristics of bacterial isolate colonies from natural Degrading Raw African walnut shell.*

As indicated in Table 21: the morphological characteristics of bacteria isolates from Natural Degrading Raw African walnut Shell shows that the bacteria with rod shape predominant over the bacteria with cocci shape and majority of them forms circular and only few were gleaming at the surface.

**Morphological and Microscopic characteristics of microbial isolate colonies from the Degrading African Walnut Shell.**

**19: Morphological and Microscopic characteristics of bacteria isolate colonies from Natural Degrading Boiled African Walnut Shell (NDBAWSB).**

| Isolate Code | Forms | Shape | Colour | Elevation | Surface | Edge |
|---|---|---|---|---|---|---|
| DAY 0 | | | | | | |
| NDBAWSB 1 | Circular | Rod | White | Raised | Smooth | Entire |
| NDBAWSB 2 | Circular | Bacillus | Cream | Raised | Margin | Dented |
| NDBAWSB 3 | Circular | Rod | Cream | Raised | Smooth | Entire |
| DAY 5 | | | | | | |
| NDBAWSB 1 | Circular | Cocci | Cream | Raised | Smooth | Dented |
| NDBAWSB 2 | Circular | Cocci | Cream | Flat | Smooth | Entire |
| NDBAWSB 3 | Spindle | Rod | Yellow | Raised | Smooth | Entire |
| DAY 10 | | | | | | |
| NDBAWSB 1 | Circular | Diplococci | Cream | Flat | Margin | Entire |
| NDBAWSB 2 | Circular | Rod | Yellow | Raised | Smooth | Dented |
| NDBAWSB 3 | Circular | Streptococci | Cream | Raised | Gleaming | Dented |
| DAY 15 | | | | | | |
| NDBAWSB 1 | Circular | Rod | Yellow | Raised | Gleaming | Entire |
| NDBAWSB 2 | Circular | Cocci | Cream | Raised | Margin | Dented |
| NDBAWSB 3 | Circular | Cocci | Cream | Raised | Margin | Entire |
| DAY 20 | | | | | | |
| NDBAWSB 1 | Circular | Rod | Cream | Flat | Smooth | Entire |
| NDBAWSB 2 | Circular | Cocci | Yellow | Raised | Margin | Dented |
| NDBAWSB 3 | Circular | Rod | Yellow | Convex | Smooth | Dented |
| | | | DAY 30 | | | |
| NDBAWSB 1 | Circular | Cocci in pairs | Cream | Raise | Smooth | Dented |
| NDBAWSB 2 | Spindle | Cocci in pairs | Cream | Raised | Margin | Dented |
| NDBAWSB 3 | Spindle | Cocci in chain | White | Flat | Smooth | Entire |

**K**

**EY**: NDBAWSB: Natural Degrading Boiled African Walnut Shell Bacteria

**Table 20: Morphological and Microscopic characteristics of fungi isolate colonies from Natural Degrading Boiled African Walnut Shell (NDBAWSF).**

| SAMPLES | CULTURAL MORPHOLOGY | MICROSCOPIC OBSERVATION | Suspected organism |
|---|---|---|---|
| DAY 0 | | | |
| NDBAWSY 1 | Yellow mycelia growth | An upright conidiophores that terminates in a davate swelling bearing phliades | *Candida parapsilosis* |
| NDBAWSY 2 | White mycelium | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| NDBAWSY 3 | Yellow mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Saccharomyces pastorianus* |
| NDBAWSM 1 | White mycelia | A single, simple and narrow conidiophores | *Aspergillus fumigatus* |
| NDBAWSM 2 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Candida albicans* |
| NDBAWSM3 | White cotton-like mycelia | Sparse mycelia, non-septate hyphae, conidia hyaline single | *Rhizopus oryzae* |
| DAY 5 | | | |
| NDBAWSY 1 | Yellowish mycelia growth | The apex radiating from the entire surface; conidia are 1-celled and globose | *Trichosporan spp* |
| NDBAWSY 2 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| NDBAWSY 3 | Yellowish mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| NDBAWSM 1 | yellow mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| NDBAWSM 2 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| NDBAWSM3 | Grey mold | Sparse mycelia, septate hyphae, conidia hyaline single | *Zoophage nitospora* |
| DAY 10 | | | |
| NDBAWSY 1 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Pichia guilliermondii* |
| NDBAWSY 2 | Yellowish mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Candida tropicalis* |
| NDBAWSY 3 | White mycelium | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| NDBAWSM 1 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| NDBAWSM 2 | White- greenish growth | Conidia have a globose shape with rough surface wall | *Alternaria alternate* |
| NDBAWSM3 | Dark mycelium | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAY 15 | | | |
| NDBAWSY 1 | Yellowish mycelia growth | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| NDBAWSY 2 | White mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| NDBAWSY 3 | Cream mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| NDBAWSM 1 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| NDBAWSM 2 | White mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and non septate hyphae | *Acremonium spp* |
| NDBAWSM3 | White mycelium | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| DAY 20 | | | |
| NDBAWSY 1 | Cream mycelia growth | The conidia have a globose surface serrated to be forming smoothened surface. | *Yarrowia spp* |
| NDBAWSM 1 | White mycelium | Septate hyphae, thin sporagiophore with a sporangium in a club-like form | *Aspergillus terreus* |
| DAY3O | | | |
| NDBAWSM 1 | White mycelium | A single, simple and narrow conidiophores | *Cladosporidium spp* |

**Key:** NDBAWSY= Natural Degrading Boiled African Walnut Shell Yeast

NDBAWSM= Natural Degrading Boiled African Walnut Shell Mold

**Table 21: Morphological and Microscopic characteristics of bacteria isolate colonies from Natural Degrading Raw African Walnut Shell (NDRAWSB).**

| Isolate Code | Forms | Shape | Colour | Elevation | Surface | Edge |
|---|---|---|---|---|---|---|
| DAY 0 | | | | | | |
| NDRAWSB 1 | Spindle | Rod | Yellow | Flat | Margin | Entire |
| NDRAWSB 2 | Circular | Cocci in chain | Cream | Raised | Smooth | Dented |
| NDRAWSB 3 | Circular | Diplococcic | Cream | Raised | Margin | Lobate |
| DAY 5 | | | | | | |
| NDRAWSB 1 | Circular | Cocci-bacilli | Cream | Raised | Smooth | Dented |
| NDRAWSB 2 | Rhizoid | Rod | Cream | Raised | Rough | Undulate |
| NDRAWSB 3 | Irregular | Rod | Yellow | Umbonate | Rough | Entire |
| DAY 10 | | | | | | |
| NDRAWSB 1 | Circular | Diplococci | White | Flat | Smooth | Entire |
| NDRAWSB 2 | Circular | Rod | Cream | Raised | Smooth | Dented |
| NDRAWSB 3 | Circular | Rod | Cream | Raised | Rough | Dented |
| DAY 15 | | | | | | |
| NDRAWSB 1 | Circular | Rod | Cream | Raised | Smooth | Entire |
| NDRAWSB 2 | Circular | Cocci | White | Flat | Gleaming | Entire |
| NDRAWSB 3 | Circular | Cocci | Cream | Raised | Smooth | Dented |
| DAY 20 | | | | | | |
| NDRAWSB 1 | Circular | Rod | Cream | Pulvinate | Smooth | Entire |
| NDRAWSB 2 | Circular | Rod | Cream | Raised | Margin | Entire |
| NDRAWSB 3 | Circular | Rod | Cream | Flat | Margin | Entire |
| DAY 30 | | | | | | |
| NDRAWSB 1 | Circular | Cocci in clusters | Yellow | Raised | Smooth | Dented |
| NDRAWSB 2 | Spindle | Rod | Cream | Raised | Smooth | Dented |
| NDRAWSB 3 | Circular | Cocci in pairs | Yellow | Raised | Smooth | Dented |

**KEY:** NDRAWSB= Natural Degrading Raw African Walnut Shell Bacteria

### 4.1.8. Morphological and Microscopic characteristics of microbial isolate colonies from the Degrading African Walnut Shell

#### *4.1.8.1. The morphological and microscopic characteristics of fungi isolate colonies from natural Degrading Raw African walnut shell*

As indicated in Table 22: the morphological characteristics of fungi isolates from Degrading Raw African walnut Shell shows that the yeast in this category were non septate hyphae with a thin sporangiophore with a sporangium in club-like form. The hyphae of the yeast present lack cross wall. The mycelium of the mold form a circular colony of small diameters with septate hyphae profusely branched. The mold produced a relatively small circular mycelium, which have a brush-like arrangement of conidia.

#### *4.1.8.2. The morphological and microscopic characteristics of fungi isolates from chicken Droppings*

As indicated in Table 23: the morphological characteristics of fungi isolates from Chicken droppings shows that the yeast present in this samples some were forming short chain by budding which are produced on mycelium epically and were non septate hyphae, thin sporangiophore. The mold conidiophore hyaline, slender with sparing upper part, branched conidia and septate hyphae

#### *4.1.8.3. The morphological and microscopic characteristics of bacteria isolate colonies from degrading African walnut shell inoculated with chicken Droppings at ratio 1:1*

As indicated in Table 24: the morphological characteristics of Bacteria isolates from Degrading African walnut Shell inoculated with chicken droppings shows that the bacteria obtained at day zero were rod shaped bacteria, at day 5 of the biodegradation day majority were cocci in shape why few are rod shape bacterial, these trends reveal highly proliferation of bacterial with cocci shape.

**Morphological and Microscopic characteristics of microbial isolate colonies from the Degrading African Walnut Shell**

**Table 22: Morphological and Microscopic characteristics of fungi isolate colonies from Natural Degrading Raw African Walnut Shell (NDRAWSF).**

| ISOLATE CODE | CULTURAL MORPHOLOGY | MICROSCOPIC OBSERVATION | Probable organism |
|---|---|---|---|
| DAY 0 | | | |
| NDRAWSY 1 | Yellowish mycelia growth | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| NDRAWSY 2 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| NDRAWSY 3 | Yellowish mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| NDRAWSM 1 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| NDRAWSM 2 | White- greenish growth | Conidia have a globose shape with rough surface wall | *Alternaria alternate* |
| NDRAWSM3 | Dark mycelium | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAY 5 | | | |
| NDRAWSY 1 | Yellow mycelia growth | An upright conidiophores that terminates in a davate swelling bearing phliades | *Candida parapsilosis* |
| NDRAWSY 2 | White mycelium | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| NDRAWSY 3 | Yellow mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| NDRAWSM 1 | White mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| NDRAWSM 2 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| NDRAWSM3 | Grey mold | Sparse mycelia, septate hyphae, conidia hyaline single | *Zoophage nitospora* |
| DAY 10 | | | |
| NDRAWSY 1 | Yellowish mycelia growth | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| NDRAWSY 2 | White mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| NDRAWSY 3 | Cream mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| NDRAWSM 1 | White mycelia | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| NDRAWSM 2 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| NDRAWSM3 | White cotton-like mycelia | Sparse mycelia, non-septate hyphae, conidia hyaline single | *Rhizopus oryzae* |
| DAY 15 | | | |
| NDRAWSY 1 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| NDRAWSY 2 | Yellowish mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| NDRAWSY 3 | White mycelium | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| NDRAWSM 1 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| NDRAWSM 2 | Brown mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and non septate hyphae | *Acremonium spp* |
| NDRAWSM3 | White mycelium | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| DAY 20 | | | |
| NDRAWSM 1 | White mycelium | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| DAY3O | | | |
| NDRAWSM 1 | White mycelium | Septate hyphae, thin sporagiophore with a sporangium in a club-like form | *Aspergillus terreus* |

**KEY:** NDRAWSY= Natural Degrading Raw African Walnut Shell Yeast
NDBAWSM= Natural Degrading Raw African Walnut Shell Mold

**Table 23: Morphological and Microscopic characteristics of Fungi isolates from Chicken Droppings (CD).**

| Isolate code | CULTURAL MORPHOLOGY | MICROSCOPIC OBSERVATION | Probable organism |
|---|---|---|---|
| YEAST | | | |
| CDY 1 | Yellowish mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| CDY 2 | White mycelium | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| CDY 3 | Cream mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| MOLD | | | |
| CDM 1 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| CDM 2 | Grey mold | Sparse mycelia, septate hyphae, conidia hyaline single | *Zoophage nitospora* |
| CDM 3 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |

KEY: CDY: Chicken Dropping Yeast

CDM: Chicken Dropping Mold

**Table 24: Morphological and Microscopic characteristics of Bacteria isolate colonies from Degrading African Walnut Shells Inoculated with Chicken Dropping at ratio 1:1 (DAWSAB).**

| Isolate Code | Forms | Shape | Colour | Elevation | Surface | Edge |
|---|---|---|---|---|---|---|
| DAY 0 | | | | | | |
| DAWSAB 1 | Circular | Rod | Yellow | Raised | Smooth | Dented |
| DAWSAB 2 | Spindle | Rod | White | Flat | Smooth | Lobate |
| DAWSAB 3 | Circular | Rod | White | Raised | Margin | Dented |
| DAY 5 | | | | | | |
| DAWSAB 1 | Circular | Cocci in cluster | Cream | Raised | Smooth | Dented |
| DAWSAB 2 | Circular | Rod | Yellow | Raised | Margin | Entire |
| DAWSAB 3 | Circular | Cocci in chain | Cream | Flat | Smooth | Dented |
| DAY 10 | | | | | | |
| DAWSAB 1 | Circular | Rod | Cream | Convex | Smooth | Erose |
| DAWSAB 2 | Circular | Cocci in chain | Yellow | Raised | Gleaming | Entire |
| DAWSAB 3 | Spindle | Streptococci | Yellow | Raised | Gleaming | Entire |
| DAY 15 | | | | | | |
| DAWSAB 1 | Filamentous | Rod | Cream | Raised | Smooth | Dented |
| DAWSAB 2 | Circular | Cocci | White | Flat | Smooth | Entire |
| DAWSAB 3 | Circular | Cocci | Cream | Raised | Smooth | Entire |
| DAY 20 | | | | | | |
| DAWSAB 1 | Circular | Cocci | Cream | Raised | Smooth | Dented |
| DAWSAB 2 | Spindle | Rod | Yellow | Flat | Smooth | Entire |
| DAWSAB 3 | Circular | Cocci | Cream | Raised | Smooth | Entire |

**Key:** DAWSAB= Degrading African Walnut Shells Inoculated with Chicken Dropping at ratio 1:1

### 4.1.9 Morphological and Microscopic characteristics of microbial isolate colonies from the Degrading African Walnut Shell

#### *4.1.9.1. The morphological and microscopic characteristics of fungi isolate colonies from degrading African walnut shell inoculate with chicken droppings at ratio 1:1.*

As indicated in Table 25: the morphological characteristics of fungi isolates from degrading African walnut shell inoculate with chicken droppings at ratio 1:1 shows that The mycelia of the yeast present in this category were not extensive, non septate hyphae with a sporangium in club like form. The molds are simple, single and narrow conidiophore while some are slender with sparing upper part, branched conidia and septate hyphae

#### *4.1.9.2. The morphology and microscopic characteristics of bacterial colonies from degrading African walnut shell inoculate with chicken dropping ratio 2:1*.

As indicated in Table 26: the morphological characteristics of Bacterial isolates from degrading African walnut shell inoculate with chicken droppings at ratio 2:1 shows that the majority of the bacterial present in the sample were rod shaped bacteria while some are cocci and the little were streptococci, this reveals that the bacterial with rod shape dominate over others.

#### *4.1.9.3. The morphology and microscopic characteristics of fungi isolate colonies from degrading raw African walnut shell inoculate with chicken dropping at ratio 2:1*

As indicated in Table 27: the morphological characteristics of fungi isolates from degrading African walnut shell inoculate with chicken droppings at ratio 2:1 shows that the fungi (yeast) were observed in this categories are short forming chain yeast produced on the mycelium epically and some of their conidia have globose surface serrated to be forming roughened surface. The mold present in this categories some were septate and some were non septate but majority are septate hyphae.

**Morphological and Microscopic characteristics of microbial isolate colonies from the Degrading African Walnut Shell**

**Table 25: Morphological and Microscopic characteristics of fungi isolate colonies from Degrading African Walnut Shell Inoculate with chicken droppings at ratio 1:1 (DAWNSAF).**

| ISOLATE CODE | CULTURAL MORPHOLOGY | MICROSCOPIC OBSERVATION | Probable organism |
|---|---|---|---|
| DAY 0 | | | |
| DAWNSAY 1 | Yellowish mycelia growth | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| DAWNSAY 2 | White mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| DAWNSAY 3 | Cream mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| DAWNSAM 1 | White mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| DAWNSAM 2 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| DAWNSAM 3 | Grey mold | Sparse mycelia, septate hyphae, conidia hyaline single | *Zoophage nitospora* |
| DAY 5 | | | |
| DAWNSAY 1 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| DAWNSAY 2 | Yellowish mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAWNSAY 3 | White mycelium | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| DAWNSAM 1 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Penicillium chrysogenium* |
| DAWNSAM 2 | White- greenish growth | Conidia have a globose shape with rough surface wall | *Alternaria alternate* |
| DAWNSAM 3 | Dark mycelium | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAY 10 | | | |
| DAWNSAY 1 | Yellowish mycelia growth | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| DAWNSAY 2 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| DAWNSAY 3 | Yellowish mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| DAWNSAM 1 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| DAWNSAM 2 | White mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and non septate hyphae | *Acremonium spp* |
| DAWNSAM 3 | White mycelium | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| DAY 15 | | | |
| DAWNSAY 1 | Yellow mycelia growth | An upright conidiophores that terminates in a davate swelling bearing phliades | *Candida parapsilosis* |
| DAWNSAY 2 | White mycelium | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| DAWNSAY 3 | Yellow mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAWNSAM 1 | White mycelia | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| DAWNSAM 2 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| DAY 20 | | | |
| DAWNSAY 1 | white mycelia growth | Having a white septate mycelium, conidiophore, and short cylindrical conidia with truncate ends formed by segmentation of hyphae | *Torula spp* |
| DAWNSAY 2 | Yellow mycelia growth | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAWNSAM 1 | Grey mold | Sparse mycelia, non-septate hyphae, conidia hyaline single | *Rhizopus oryzae* |
| DAWNSAM 2 | White mycelia | Single, simple and narrow conidiophores. | *Cladosporidium spp* |

**Key:** DAWNSAY= Degrading Raw African Walnut Shell Yeast at ratio 1:1, DAWNSAM= Degrading Raw African Walnut Shell Mold at ratio 1:1

**Table 26: Morphological and Microscopic characteristics of Bacteria isolate colonies from Degrading African Walnut Shells Inoculated with Chicken Dropping at ratio 2:1 (DAWSBB).**

| Isolate Code | Forms | Shape | Colour | Elevation | Surface | Edge |
|---|---|---|---|---|---|---|
| DAY 0 | | | | | | |
| DAWSBB 1 | Circular | Rod | White | Raised | Smooth | Dented |
| DAWSBB 2 | Circular | Cocci in cluster | White | Raised | Smooth | Dented |
| DAWSBB 3 | Spindle | Rod | Yellow | Flat | Smooth | Entire |
| DAY 5 | | | | | | |
| DAWSBB 1 | Circular | Rod | White | Flat | Smooth | Entire |
| DAWSBB 2 | Circular | Coccobacillus | Yellow | Raised | Smooth | Dented |
| DAWSBB 3 | Circular | Rod | White | Raised | Margin | Dented |
| DAY 10 | | | | | | |
| DAWSBB 1 | Circular | Rod | Cream | Pulvinate | Gleaming | Entire |
| DAWSBB 2 | Circular | Cocci in chain | Cream | Convex | Smooth | Dented |
| DAWSBB 3 | Spindle | Streptococci | Cream | Convex | Gleaming | Dented |
| DAY 15 | | | | | | |
| DAWSBB 1 | Spindle | Cocci in chain | Cream | Raised | Margin | Dented |
| DAWSBB 2 | Spindle | Diplococci | Yellow | Flat | Margin | Dented |
| DAWSBB 3 | Circular | Cocci | Cream | Raised | Smooth | Entire |
| DAY 20 | | | | | | |
| DAWSBB 1 | Circular | Rod | Cream | Raised | Smooth | Dented |
| DAWSBB 2 | Irregular | Cocci | Cream | Raised | Margin | Entire |
| DAWSBB 3 | Circular | Diplococci | White | Flat | Smooth | Dented |

**KEY:** DAWSBB= Degrading African Walnut Shells Inoculated with Chicken Dropping at ratio 2:1

**Table 27: Morphological and Microscopic characteristics of fungi isolate colonies from Degrading Raw African Walnut Shell Inoculate with chicken droppings at ratio 2:1 (DAWNSBF).**

| ISOLATE CODE | CULTURAL MORPHOLOGY | MICROSCOPIC OBSERVATION | Probable organism |
|---|---|---|---|
| DAY 0 | | | |
| DAWNSBY 1 | Cream mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| DAWNSBY 2 | White mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| DAWNSBY 3 | Yellowish mycelia growth | Mycelium are not extensive, conidia are 1-celled, ovoid to fusoid. | *Candida oleophila* |
| DAWNSBM 1 | White mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| DAWNSBM 2 | Grey mold | Sparse mycelia, septate hyphae, conidia hyaline single | *Zoophage nitospora* |
| DAWNSBM 3 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| DAY 5 | | | |
| DAWNSBY 1 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| DAWNSBY 2 | Yellowish mycelia growth | The conidia have a globose surface serrated to be forming roughened surface. | *Saccharomyces bayanus* |
| DAWNSBY 3 | White mycelium | The apex radiating from the entire surface; conidia are 1-celled and globose | *Candida stellate* |
| DAWNSBM 1 | Dark mycelia growth | A single, simple, dark and narrow conidiophores | *Cladosporidium spp* |
| DAWNSBM 2 | White- greenish growth | Conidia have a globose shape with rough surface wall | *Alternaria alternate* |
| DAWNSBM 3 | Dark mycelium | Non- septate hyphae, thin sporagiophore with a sporangium in club- like form | *Aspergillus glaucus* |
| DAY 10 | | | |
| DAWNSBY 1 | Yellowish mycelia growth | Forming short chains by budding which are produced on mycelium epically or laterally | *Rhodosporidium toruloides* |
| DAWNSBM 1 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| DAWNSBM 2 | White mycelium | Conidiophore hyaline, slender with spairing upper part, branched conidia and non septate hyphae | *Acremonium spp* |
| DAWNSBM 3 | White mycelium | A single, simple and narrow conidiophores | *Cladosporidium spp* |
| DAY 15 | | | |
| DAWNSBY 1 | Yellow mycelia growth | An upright conidiophores that terminates in a davate swelling bearing phliades | *Candida parapsilosis* |
| DAWNSBM 1 | Greenish mold | Conidiophore hyaline, slender with spairing upper part, branched conidia and septate hyphae | *Penicillium chrysogenium* |
| DAWNSBM 2 | White cotton-like mycelia | Sparse mycelia, non-septate hyphae, conidia hyaline single | *Rhizopus oryzae* |
| DAY 20 | | | |
| DAWNSBY 1 | Yellow mycelia growth | Forming short chains by budding which are produced on mycelium laterally | *Rhodosporidium toruloides* |
| DAWNSBM 1 | White mycelia growth | Thin sporangiophore, septate hyphae and branched conidia with a rough surface wall | *Tricoporum spp* |
| DAWNSBM 2 | Grey mold | Septate hyphae and the conidia have a globose shape with rough surface wall | *Alternaria alternate* |

**KEY:** DAWNSBY= Degrading Raw African Walnut Shell Yeast at ratio 2:1
DAWNSBM= Degrading Raw African Walnut Shell Mold at ratio 2:1

### 4.1.10 BIOCHEMICAL CHARACTERISTICS OF THE ISOLATE

***4.1.10.1. The biochemical test on bacterial isolates from natural degrading boiled African walnut shell.***

As indicated in Table 28: The biochemical test on bacterial isolates from natural degrading boiled African walnut shell shows that virtually all the bacterial present produces acid and some produces gas while some do not produce gas and majority are gram positive bacterial with catalase positive. For the sucrose the organism produces gas were not much.

***4.1.10.2. The biochemical test on the bacterial isolates from naturally degrading raw African walnut shell***.

As indicated in Table 29: The biochemical test on the bacterial isolates from naturally degrading raw African walnut shell shows that virtually all the microorganisms were acid producing organism. For the sugar (fructose) the organism produces gas were so small compared to the organism with no production gas.

***4.1.10.3. The biochemical test on the bacterial isolates from chicken droppings.***

As indicated in Table 30: The biochemical test on the bacterial isolates from chicken droppings shows that the bacterial present in the chicken droppings were acid producing bacterial and majority produces gas were as little as those that does not producing gas**.**

## BIOCHEMICAL CHARACTERISTICS OF THE ISOLATE

**Table 28: Biochemical test of Bacterial isolates from Natural Degrading Boiled African Walnut Shell (NDBAWSB).**

| ISOLATES CODES | NDBAWSB 1 O HOUR | NDBAWSB 2 O HOUR | NDBAWSB 3 0 HOURS | NDBAWSB 1 DAY 5 | NDBAWSB 2 DAY 5 | NDBAWSB 1 DAY 10 | NDBAWSB 2 DAY 10 | NDBAWSB 1 DAY 15 | NDBAWSB 2 DAY 15 | NDBAWSB 1 DAY 20 | NDBAWSB 2 DAY 20 | NDBAWSB 1 DAY 30 | NDBAWSB 2 DAY 30 | NDBAWSB 3 DAY30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GRAM STAIN** | + | + | + | + | + | + | + | + | + | + | + | + | - | + |
| **SHAPES** | **R** | **B** | **R** | **C** | **C** | **C** | **R** | **R** | **C** | **R** | **C** | **C** | C | C |
| **CATALASE** | + | _ | - | + | - | + | - | + | + | + | + | - | + | + |
| **GRAM'S REACTION** | + | + | + | + | + | + | + | + | + | + | + | + | - | + |
| **SUGAR FERMENTATION** | | | | | | | | | | | | | | |
| **LACTOSE** | A/G | A/-G | A/G | A/G | A/G | A/G | A/G | A/-G | A/-G | A/G | A/-G | A/G | A/G | A/G |
| **SUCROSE** | A/-G | A/G | A/-G | A/-G | A/-G | A/-G | A/-G | A/-G | A/G | A/-G | A/G | A/G | A/G | A/-G |
| **MANNITOL** | A/-G | A/G | A/G | A/G | A/G | A/-G | A/G | A/-G | A/G | A/G | A/G | A/G | A/-G | A/G |
| **FRUCTOSE** | A/G | A/G | A/G | A/G | A/G | A/G | A/G | A/G | A/-G | A/-G | A/-G | A/G | A/G | A/G |
| **PROBABLE ORGANISM** | *Listeria spp* | *Bacillus species* | *Lactobacillus species* | *Enterococcus species* | *Staphylococcus species* | *Pneumococcus species* | *Bacillus species* | *Mycobacterial species* | *Staphylococcus species* | *Listeria spp* | *Enterococcus species* | ***Actinomycetes sp.*** | ***Lactobacilli sp.*** | ***Clostridium sporogenes*** |

**KEY:** NDBAWSB = Natural Degrading Boiled African Walnut Shell Bacteria, B= Bacillus, R= Rod, C= Cocci, += Positive, -= Negative, A/G= Acid/ Gas production,A/-G= Acid/ No gas

**Table 29: Biochemical test of Bacterial isolates from Natural Degrading Raw African Walnut Shell (NDRAWSB).**

| ISOLATES CODES | NDRAWSB 1 O HOUR | NDRAWSB 2 O HOUR | NDRAWSB 3 0 HOURS | NDRAWSB 1 DAY 5 | NDRAWSB 2 DAY 5 | NDRAWSB 1 DAY 10 | NDRAWSB 2 DAY 10 | NDRAWSB 1 DAY 15 | NDRAWSB 2 DAY 15 | NDRAWSB 1 DAY 20 | NDRAWSB 2 DAY 20 | NDRAWSB 1 DAY 30 | NDRAWSB 2 DAY 30 | NDRAWSB 3 DAY30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **GRAM STAIN** | + | + | + | - | - | + | + | + | + | + | - | + | - | + |
| **SHAPES** | R | C | C | C | R | C | R | R | C | R | R | C | R | C |
| **CATALASE** | + | + | + | + | - | + | - | + | + | + | + | - | + | + |
| **GRAM'S REACTION** | + | + | + | - | - | + | + | + | + | + | - | + | - | + |
| **SUGAR FERMENTATION** | | | | | | | | | | | | | | |
| **LACTOSE** | A/G | A/-G | A/-G | A/-G | A/G | A/-G | A/-G | A/G | A/G | -A/-G | A/G | A/G | A/G | A/G |
| **SUCROSE** | A/G | A/-G | A/-G | A/G | A/G | A/-G | A/-G | A/-G | A/-G | A/G | A/G | A/-G | A/G | A/G |
| **MANNITOL** | A/-G | A/G | A/G | A/-G | A/G | A/-G | A/G | A/-G | A/G | A/G | A/-G | A/G | A/-G | A/G |
| **FRUCTOSE** | A/G | A/G | A/-G | A/-G | A/-G | A/-G | A/-G | A/-G | A/-G | A/-G | A/-G | A/G | A/G | A/G |
| **PROBABLE ORGANISM** | *Corynebacterial species* | *Enterococcus species* | *Pneumococcus species* | *Hemophilia species* | *Campylobacter species* | *Streptococcus species* | *Gardnerella species* | *Mycobacterial species* | *Staphylococcus species* | *Corynebacterial species* | *Mycobacterial species* | *Pneumococcus species sp.* | *Pseudomonas ssp.* | *Clostridium sporogenes* |

KEY: NDRAWSB = Natural Degrading Raw African Walnut Shell Bacteria, B= Bacillus, R= Rod, C= Cocci, += Positive, -= Negative, A/G= Acid/ Gas production, A/-G= Acid/ No gas, -A/-G= No Acid and Gas

**Table 30: Biochemical test of bacterial isolates from Chicken Droppings (CD).**

| **Isolate code** | CDB 1 | CDB 2 | CDB 3 |
|---|---|---|---|
| **Gram staining** | + | - | + |
| **Catalase test** | + | + | + |
| **Gram reaction** | + | - | + |
| **Shape** | C | R | R |
| **Sugar fermentation** | | | |
| **Lactose** | A/G | A/G | A/-G |
| **Sucrose** | A/-G | A/G | A/G |
| **Mannitol** | A/G | A/G | A/G |
| **Fructose** | A/G | A/-G | A/G |
| **Probable organism** | *Enterococcus species* | *Mycobacterial species* | *Corynebacterial species* |

**KEY**: CDB= Chicken Dropping Bacteria,R= Rod, C= Cocci,,+= Positive,-= Negative,A/G= Acid/ Gas production,A/-G= Acid/ No Gas production

### 4.1.11 BIOCHEMICAL CHARACTERISTICS OF THE ISOLATE

#### *4.1.11.1. The biochemical test on bacteria of bacteria isolates from degrading raw African walnut shell inoculate with chicken droppings at ratio 1:1*

As indicated in Table 31: The biochemical test on bacteria of bacteria isolates from degrading raw African walnut shell inoculate with chicken droppings at ratio 1:1shows that the bacterial present in this sample produce acid and majority produces gas at each sugar and the bacteria produces gas much compared to those with no gas and majority are catalase positive bacteria**.**

#### *4.1.11.2. The biochemical test on bacterial isolate from degrading raw African walnut shell inoculate with chicken dropping at ratio 2:1*

As indicated in Table32:The biochemical test on bacterial isolate from degrading raw African walnut shell inoculate with chicken dropping at ratio 2:1shows that the bacterial were acid producing bacteria with low production of gas. The bacteria were catalase positive bacterial while some are catalase negative.

**Table 31: Biochemical test of Bacterial isolates from Degrading Raw African Walnut Shell Inoculate with chicken Droppings at ratio 1:1 (DRAWSAB).**

| Isolate code | Gram staining | Catalase test | Gram reaction | Sugar fermentation | | | |
|---|---|---|---|---|---|---|---|
| | | | | L | S | M | F |
| DAY 0 | | | | | | | |
| DRAWSAB 1 | + | + | + | A/G | A/-G | A/G | -A/-G |
| DRAWSAB 2 | + | + | + | A/G | A/G | A/G | A/-G |
| DRAWSAB 3 | + | + | + | A/-G | A/-G | A/G | A/-G |
| DAY 5 | | | | | | | |
| DRAWSAB 1 | + | + | + | A/-G | A/G | A/G | A/-G |
| DRAWSAB 2 | + | - | + | A/-G | A/-G | A/G | A/G |
| DRAWSAB 3 | - | + | - | A/G | A/G | A/-G | A/G |
| DAY 10 | | | | | | | |
| DRAWSAB 1 | + | + | + | A/G | A/-G | A/G | A/G |
| DRAWSAB 2 | + | _ | + | A/-G | A/-G | A/G | A/-G |
| DRAWSAB 3 | + | + | + | A/G | A/G | A/G | A/-G |
| DAY 15 | | | | | | | |
| DRAWSAB 1 | + | + | + | A/-G | A/G | A/G | A/G |
| DRAWSAB 2 | - | + | - | A/-G | A/-G | A/G | A/G |
| DRAWSAB 3 | + | + | + | A/G | A/G | A/-G | A/-G |
| DAY 20 | | | | | | | |
| DRAWSAB 1 | + | + | + | A/-G | A/G | -A/-G | A/G |
| DRAWSAB 2 | + | + | + | A/G | A/-G | A/-G | -A/-G |
| DRAWSAB 3 | - | + | - | A/-G | A/-G | A/G | A/-G |

**KEY:** DRAWSAB = Degrading Raw African Walnut Shell inoculated with chicken dropings at ratio 1:1 Bacteria, L= Lactose, S= Sucrose, F= Fructose, M= Manitol, += Positive, -= Negative, A/G= Acid/ Gas production, A/-G= Acid/ No gas, -A/-G= No acid, No gas

**Table 32: Biochemical test of Bacterial isolates from Degrading Raw African Walnut Shell Inoculate with chicken droppings at ratio 2:1 (DRAWSBB).**

| Isolate code | Gram staining | Catalase test | Gram reaction | Sugar fermentation | | | |
|---|---|---|---|---|---|---|---|
| | | | | L | S | M | F |
| DAY 0 | | | | | | | |
| DRAWSBB 1 | + | + | + | A/G | A/G | A/-G | A/G |
| DRAWSBB 2 | + | + | + | A/-G | A/-G | A/G | A/G |
| DRAWSBB 3 | + | + | + | A/G | A/G | A/G | A/-G |
| DAY 5 | | | | | | | |
| DRAWSBB 1 | - | + | - | A/-G | A/G | A/-G | A/-G |
| DRAWSBB 2 | - | - | - | A/-G | A/-G | A/G | -A/-G |
| DRAWSBB 3 | + | + | + | A/-G | A/-G | A/-G | A/-G |
| DAY 10 | | | | | | | |
| DRAWSBB 1 | + | + | + | A/G | A/G | A/G | A/-G |
| DRAWSBB 2 | + | _ | + | A/-G | A/-G | A/G | A/G |
| DRAWSBB 3 | | | | A/G | A/G | A/-G | A/-G |
| DAY 15 | | | | | | | |
| DRAWSBB 1 | + | - | + | A/-G | A/-G | A/-G | A/G |
| DRAWSBB 2 | - | - | - | A/G | A/-G | A/G | A/G |
| DRAWSBB 3 | + | + | + | A/G | A/G | A/-G | A/-G |
| DAY 20 | | | | | | | |
| DRAWSBB 1 | + | + | + | A/G | A/G | A/-G | A/-G |
| DRAWSBB 2 | - | - | - | A/G | A/-G | A/G | A/G |
| DRAWSBB 3 | - | + | - | A/G | A/-G | A/-G | A/-G |

**KEY**: DRAWSBB = Degrading Raw African Walnut Shell inoculated with chicken droppings at ratio 2:1 Bacteria, L= Lactose,S= Sucrose, F= Fructose, M= Manitol, += Positive,-= Negative

A/G= Acid/ Gas production, A/-G= Acid/ No gas, -A/-G= No acid, No gas

### 4.1.12: Physiochemical properties of the degrading African walnut shell samples

#### *4.1.12.1. The total titratable acid of natural degrading boiled African walnut shell*

As indicated in Table 33: The total titratable acid of natural degrading boiled African walnut shell shows that there is decrease in the flow rate of Acid. At day zero the sample have the highest value of (19.00), as the day increases the value began to reduce at day 30 of the biodegradation the value was very low compared to the day zero with the lowest value (7.60)

#### *4.1.12.2. The total titratable acid of natural degrading raw African walnut shell.*

As indicated in Table 34: The total titratable acid of natural degrading raw African walnut shell shows that at day zero the samples obtain the highest value of total titratable acid of (20.20) and it begins to reduce as the numbers of days were increasing at day 30 the sample obtain the lowest value of (8.45).

#### *4.1.12.3. The total titratable acid of degrading Africa walnut shell inoculated with chicken dropping at ratio 1:1*

As indicated in Table 35: The total titratable  acid of degrading Africa walnut shell inoculated with chicken dropping at ratio 1:1 shows that at day zero of the biodegradation the sample obtain the lowest value of (13.00) and as the days increased the value begins to increase at day 20 of biodegradation the samples obtain its highest value of (21.18)

**Table 33: Total Titratable Acid (TTA) of Natural Degrading Boiled African Walnut Shell (NDBAWS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| NDBAWS | 0 | 19.00 |
| NDBAWS | 10 | 14.10 |
| NDBAWS | 15 | 11.60 |
| NDBAWS | 20 | 9.00 |
| NDBAWS | 30 | 7.60 |

**KEY:** NDRAWS= Natural Degrading Raw African Walnut Shell

**Table 34: Total Titra Acid (TTA) of Natural Degrading Raw African Walnut Shell (NDRAWS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **NDRAWS 1** | 0 | 20.20 |
| **NDRAWS 2** | 10 | 18.50 |
| **NDRAWS 3** | 15 | 12.30 |
| **NDRAWS 4** | 20 | 10.70 |
| **NDRAWS 5** | 30 | 8.45 |

**KEY:** NDRAWS= Natural Degrading Raw African Walnut Shell

**Table 35: Total Titra Acid (TTA) of Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 1:1 (DAWNAS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **DAWNAS** | 0 | 13.00 |
| **DAWNAS** | 5 | 16.50 |
| **DAWNAS** | 10 | 19.00 |
| **DAWNAS** | 15 | 21.10 |
| **DAWNAS** | 20 | 21.18 |

**Key: DAWNAS= Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 1:1**

### 4.1.13: Physiochemical properties of the degrading African walnut shell samples

#### *4.1.13.1 . The total titratable acid of degrading Africa walnut shell inoculated with chicken droppings at ratio 2:1*

As indicated in Table 36:The total titratable acid of degrading Africa walnut shell inoculated with chicken droppings at ratio 2:1 shows that at day zero of the biodegradation the value was so low (9.00) compare to the value obtained at day zero of ratio 1:1 which is (13.00) but as the biodegradation days were increases the value begins to increase as well and at day 20 of biodegradation the sample obtained the highest value of (21.53)

#### *4.1.13. The pH of natural degrading raw African walnut shell.*

As indicated in Table 37: The pH of natural degrading raw African walnut shell shows that at day zero of the biodegradation the pH was at the range of 7.18 but soon as the day of biodegradation is increasing the pH is increasing towards alkaline level of (9.00) at day 30.

**36: Total Titra Acid (TTA) of Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 2:1 (DAWNBS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **DAWNBS** | 0 | 9.00 |
| **DAWNBS** | 5 | 12.50 |
| **DAWNBS** | 10 | 17.20 |
| **DAWNBS** | 15 | 21.50 |
| **DAWNBS** | 20 | 21.53 |

**Key: DAWNBS= Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 2:1**

**Table 37: PH of Natural Degrading Raw African Walnut Shell (NDRAWS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **NDRAWS** | 0 | 7.18 |
| **NDRAWS** | 5 | 7.12 |
| **NDRAWS** | 10 | 7.60 |
| **NDRAWS** | 15 | 8.70 |
| **NDRAWS** | 20 | 8.90 |
| **NDRAWS** | 30 | 9.00 |

**KEY**: **NDRAWS= Natural Degrading Raw African Walnut Shell**

### 4.1.14 PH reading of Degrading African walnut shell

#### *4.1.14.1 38 shows the pH of natural degrading boiled African walnut shell*

As indicated in Table 38: The pH of natural degrading boiled African walnut shell shows that at day zero of the biodegradation the ph was at 7.20 and as the days of biodegradation were increases, the pH begins to increase at each day of biodegradation and the sample obtained a highest pH of (9.50)

#### *4.1.14.2 . The pH of degrading African shell inoculated with chicken Droppings at ratio 1:1*

As indicated in Table 39: The pH of degrading African shell inoculated with chicken Droppings at ratio 1:1 shows that at day zero of the biodegradation of the sample, the sample was at the acidic level of 6.80 and changes from the acidity level to basic level of 9.52 at day 20 0f the biodegradation day

#### *4.1.14.3 . The PH. of Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 2:1*

As indicated in Table 40: ***The PH. of Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 2:1 shows that a***t the day zero of the degradation the pH of the sample was at 7.10 and as the days were increasing the level of the pH keep increasing and the highest pH value 0f 10.30 was obtained at day 20 0f the biodegradation

**Table 38: PH of Natural Degrading Boiled African Walnut Shell (NDBAWS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **NDBAWS 1** | 0 | 7.20 |
| **NDBAWS 2** | 5 | 7.25 |
| **NDBAWS 3** | 10 | 8.40 |
| **NDBAWS 4** | 15 | 8.44 |
| **NDBAWS 5** | 20 | 9.50 |
| **NDBAWS 6** | 30 | 9.56 |

**KEY: NDBAWS= Natural Degrading Boiled African Walnut Shell**

**Table 39: PH. of Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 1:1 (DAWNAS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **DAWNAS** | 0 | 6.80 |
| **DAWNAS** | 5 | 7.54 |
| **DAWNAS** | 10 | 9.32 |
| **DAWNAS** | 15 | 9.45 |
| **DAWNAS** | 20 | 9.52 |

**KEY:** DAWNAS= Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 1:1

**Table 40: PH. of Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 2:1 (DAWNBS)**

| SAMPLE | DAY | RESULT |
|---|---|---|
| **DAWNBS 1** | 0 | 7.10 |
| **DAWNBS 2** | 5 | 7.75 |
| **DAWNBS 3** | 10 | 10.11 |
| **DAWNBS 4** | 15 | 10.22 |
| **DAWNBS 5** | 20 | 10.30 |

**KEY: DAWNBS= Degrading African Walnut Shell Inoculated with Chicken Dropping at Ratio 2:1**

## 4.2 Discussion

The microbial counts of the microorganism (both bacteria and fungi) obtained indicated that the walnut Shell and Chicken dropping is contaminated with the presence of microorganisms which shows that the present of this microorganism in a large number will aid the biodegradation process. The presence of this microorganism in these samples revealed a high level of microbial activities in the contamination especially in the chicken dropping than the walnut shell. This was because the effluents may contain many growth factors that could be easily utilized by the organisms which are not available in the natural biodegradation process.

1 shows that the numbers of microorganism present in the sample are much in the boiled shell than the raw shell and this shows that the boiled shell accommodates and support the growth of microorganism than the raw shell this is because the raw shell contain antibacterial and antifungal which can be reduced drastically by boiling Although freshly crushed shell do have antimicrobial properties, these have been observed to diminish a little after a 3-day period as the polyphenols oxidize (Blessington *et al*., 2014). The result shows that several microorganisms, including fungi, bacteria and yeasts are involved in biodegradation process.

In most cases, there were fewer than 10 colonies per sample at the lowest dilution, demonstrating that it was possible to have shell with very low-level contamination and suggesting that the shell provides reasonable protection to the nut from microbial contamination while the nut is on the tree. Microbial loads on the kernels increased after the walnuts were harvested even when the

shell was visibly intact. Both boiled and raw shell can support microbial growth especially when crushed to release cell nutrients which support the view of (Blessington*et al*., 2014)

The presence of a variety of microorganisms in chicken droppings with the ability of breaking down the shell helps to speed up the process of biodegradation. Besides, these wastes have been found to be rich in nitrogen and phosphorus (potter, 2012), which are crucial in the biodegradation of organic pollutants.

6 shows that the boiled walnut shell have high microbial load of fungi this may be due to the fact that fungi usually grow on a saprophytic material and provide nutrient that support the growth of microorganism which can help in quick biodegradation of the boiled shell than the raw shell.

Walnuts collected directly from the tree may have been contaminated with microorganisms from the decomposing hull or from other orchard sources such insects, animals, or aerosols. The observed increase in microbial loads after the walnuts were harvested is likely due to a combination of exposure to orchard soil and cross-contamination among nuts and from equipment during harvest and hulling. The study revealed that *T. conophorum*nuts shell of the boiled walnut is susceptible to bacteria and fungi compared to the raw similar case was also reported by Okeke and Elekwa (2006). Most of the fungal isolates were also walnut Shell inhabiting microorganism as well as common spoilage organisms associated with biodegradation. A detailed comparism from both walnut Shell and chicken droppings incubates both bacteria and fungi that are able to degrade the walnut Shell which are in conformity with the chemical composition of the Nutrient agar medium, Sabourand dextrose agar and yeast extract agar.

The crude fiber contents present in the nut shell of the samples to support the growth of microorganism ranged from higher rate for nuts shell boiled to lower rate for raw nuts shell. The two processing methods employed in this study show a significant reduction in the microorganism present in the raw African walnut shell; the microorganisms reported for all the walnut samples in this study were higher than that reported for cashew nut shell as it is reported by [Akinhanmi*et al., 2008*]. In the experiment of carbohydrate fermentation both (raw and cooked) shell microorganism show reaction there was change in colour and some produces gas while some don't produces gas.

# CONCLUSION AND RECOMMENDATION

From the results of this study, it can be concluded that chicken droplet possess the ability to enhance the biodegradation of African walnut shell, hence chicken waste can serve as good materials for degrading walnut shell and to solve the problem of solid waste disposal in the Nigerian environment. The demonstration of microbial load of the sample is an indication that there is possibility of biodegradation of the walnut shell. It is thereby recommended that for a quick degradation of walnut shell chicken droplet should always employ.

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