Effect of Waste Separation on the Composting of Organic Waste Fraction from Domestic Solid Waste

Douglas, Salome Ibietela¹, Williams, Janet Olufunmilayo¹
and Ekeke, Joy Ijeoma¹

¹Department of Microbiology, Faculty of Science, Rivers State University, P.M.B. 5080, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Author DSI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors WJO and EJI managed the analyses of the study. All authors read and approved the final manuscript.

ABSTRACT

In Nigeria, due to lack of sanitary landfills and general poor attitude towards waste management, various types of wastes such as: commercial, hospital, construction and domestic wastes are mixed and transported in open trucks to unprotected open dumpsites. This is likely to interfere with degradation of biodegradable wastes by non-biodegradable components, which are non-permeable to water and gases. These factors are needed by biodegrading materials for adequate decomposition. This study therefore, evaluates the effect of waste separation on natural biodegradation of solid domestic waste. Domestic waste samples were obtained from three different households at Rivers State University campus senior staff quarters, Port Harcourt, Nigeria and were sorted and categorised into biodegradable (organic), mixed fractions (inorganic and organic) and non-biodegradable. These were composted in open containers and monitored. Samples were withdrawn biweekly and analysed for microbiological and some physicochemical parameters for a period of 12 weeks. Morphological and biochemical characteristics were used for identification of isolates. Statistical analyses of the results were performed using SPSS version 21. Results of total

*Corresponding author: E-mail: sallyakdougy@yahoo.com;
**1. INTRODUCTION**

Domestic solid waste, which is commonly referred to as garbage is a type of waste that consist of day to day used items being generated from house hold sources discarded by the public, incinerated, recycled or discarded into a solid waste municipal landfills. It consists of organic portions mainly, such as vegetable waste, food waste, animal waste, wastes from the garden etc. Inorganic domestic waste includes: clothing, paper, plastic, glass, wood and metal products like drink cans. These items are usually located on property and in people’s dwellings where all manner of domestic activities takes place [1]. The increased population density in modern Nigeria has resulted in increased volume of domestic wastes generated, leading to the mismanagement and mishandling of wastes which has affected the reduction in the aesthetic value of the environment [2]. Proper management of wastes and disposal practices help in boosting of a healthy environment.

Biodegradable or organic waste, is a type that originates typically either through plant or animal sources, and may be decomposed by other living organisms such as microbes (fungi and bacteria) and a few other factors including: oxygen, temperature, sunlight etc., and not add to pollution. Some of such wastes include food materials, plant wastes, animal waste and other natural wastes. A Non-biodegradable waste is a type of material which cannot be broken down by natural organisms and contributes as a source of pollution to the environment. Unlike biodegradable wastes, non-biodegradable waste is not easy to handle. They may remain on earth for thousands of years without undergoing any form of degradation: examples are cans, metals, plastics etc. [3]. Millions of tons of organic waste are produced annually resulting in the problem of safe disposal of such waste, which end up at dump sites or landfill. Food waste form a large amount of the organic waste produced from household. Microorganisms play very important role in the composting of organic food waste materials being broken down into simpler components, which will improve the texture of the soil, structure, aeration and water holding ability of the soil. Compost is produced, which is the simplest components that would be formed into humus [4]. The compost formed contains high amounts of nutrients from the microbial transformation of the organic matter through various biochemical pathways, producing fibre rich in carbon and other inorganic compounds such as phosphorus and nitrogen. The final products of composting can be used as fertilizers and for soil amendments. Composting involves three main stages; which are the characterization of microbial activity and the transformation of organic matter [4].

The first stage is the mesophilic stage where indigenous microorganisms start the composting process by the oxidation of organic matter, leading to increase in temperature, breaking down the biodegradable fractions and increasing the stability of the organic matter. The group of microorganisms involved at this stage includes: bacteria, actinomycetes, fungi and protozoa, and...
their populations and diversity depends on physical properties such as: oxygen, the moisture level, C/N ratio, nutrients, temperature and the nature of the organic substance [5]. Moisture content is very important and maintained between 40 to 60%, which is required for optimum microbial activity. Also, excess water could lead to anaerobic conditions which could result in the production of unpleasant smell (such as hydrogen sulphide, methane and other organic acids) and toxic volatile substances [6]. Many bacterial and fungal genera including: Pseudomonas, Bacillus, and Aspergillus initiate the decomposition process due to their hydrolytic potential and ability to degrade waste efficiently. In course of the composting, degradation of hemicelluloses, lignin and cellulose brings about the production of humic matter and the other degradable matters are degraded to ammonia and carbon dioxide [7]. Fungi acts more on the more resistant materials such as cellulose, lignins which are decomposed later. Oxygen is required for this stage to degrade the organic materials and the by-products are carbon dioxide, heat and leachate [8].

For the second stage, also known as the thermophilic stage where the remaining organic materials (hemicellulose, fats, lignin), in the humic matter is transformed through humification to increase the quality of compost. This process continues until all the organic matter is transformed to produce stable humic matter. This process is carried out by thermophilic organisms like Bacillus sp and Actinobacter sp, able to withstand high temperatures. The third stage is the maturation or cooling stage, where substrates are depleted and microbial activities reduced leading to a decrease in temperature. The activities of the thermophiles come to an end while mesophiles takes over to recolonize and other microbial spores that were able to survive the high temperature [9].

Anaerobic decomposition is also possible where the organic materials are decomposed to produce biogas, producing nutrients as well as a way of waste management. Microorganisms are able to breakdown complex organic materials into macromolecules such as carbohydrates, proteins and lipids before being hydrolysed by enzymes; to such as cellulases, lipases, proteases and amylase into simple sugars, amino acids and long-chain fatty acids [10].

Waste sorting is the process of separating waste into different items and categories [11]. This sorting can either occur through hand picking (manually) at the household level and taken through curb side collection system or separated automatically in materials recovery facilities. In the history of waste sorting, the first method used was the use of hand to sort wastes [12]. This work is aimed at evaluating the effect of waste separation on biodegradation.

2. MATERIALS AND METHODS

2.1 Sample Collection

The study was conducted in Rivers State University, Nkpolo-Oroworukwo, Port Harcourt, Nigeria located at the South-South of Nigeria. Samples were collected from the refuse bins of three households of the senior staff quarters in Rivers State University Campus, Port Harcourt metropolis, according to the method described by Davis and Song [3] between 7.00am and 8.00am local time. The samples were sorted/separated into biodegradable (plants and animal products), and non-biodegradable [plastics, metals (cans of milk, tin tomato paste, drinks), bottles] and packed in sterile polythene bags and transported to the Department of Microbiology laboratory, Rivers State University for analysis.

2.2 Preparation of Samples

The solid domestic waste samples were sorted, separated and weighed (5000 g each) into six categories namely; vegetable and animal matter (biodegradable wastes), paper, plastics, metals, bottles, paper and finally mixture of the whole waste. The glass comprises of broken drinking glasses, bottles and plates; which were crushed using a sterile hammer while plastics including pure water sachets, polythene bags and plastic cans were shredded into smaller fragments using a sterile knife as well as the metals (cans of milk, tin tomato paste, drinks, were compressed using hammer). Each of the waste samples were kept in an opened bucket and allowed to decompose for a period of twelve weeks, with samples taken out every two weeks for analyses [13]. Each waste category was setup in duplicate.

2.3 Enumeration and Isolation of Total Heterotrophic Bacteria

Each of the decomposing waste samples were weighed out (10 grams each), and was dissolved into 90 ml of sterile normal saline. From the stock solution, ten-fold serial dilutions were made to $10^0$, 0. 1 ml aliquots of the diluents from $10^3$ and $10^5$ were inoculated in duplicate onto freshly
prepared Nutrient agar plates and spread using a sterile bent glass rod. The inoculated plates were incubated at 35°C for 24 hours. After incubation, the bacterial colonies that appeared on the agar plates were counted (plates containing between 30 and 300 colonies) and mean counts were obtained and recorded as colony forming units per gram (cfu/g) of waste sample. Characteristic colonies were sub-cultured several times using nutrient agar until pure isolates were obtained. The pure isolates were inoculated into Bijou bottles containing nutrient agar slant and incubated for 24 hours at 35°C for further identification, preserved and stored in the refrigerator at 4°C [14,15].

2.4 Enumeration of Total Heterotrophic Fungi

Another, 0.1 ml aliquots of dilutions from the $10^{-2}$ and $10^{-3}$ were inoculated in duplicate onto Sabouraud Dextrose agar (SDA) containing 0.05% (w/v) Chloramphenicol (added to prevent bacterial growth) and spread as described above for enumeration and isolation of total heterotrophic fungi. The inoculated plates were incubated at 22°C for 2 to 4 days, after which fungal counts that developed were counted, mean values calculated and recorded as colony forming units per gram. Discrete colonies were picked and sub-cultured onto fresh SDA plates to obtain pure isolates, which were stored for further characterisation and identification of the test organisms [16].

2.5 Identification and Characterization of Isolates

The purified isolates were characterized and identified on the basis of their morphological and biochemical characteristics including; Gram stain, catalase test, MR-VP (Methyl Red-Voges Proskauer test), fermentation of sugars (glucose, fructose and sucrose), citrate utilization, motility, oxidase, starch hydrolysis, urease and indole. The results obtained were compared with standard references of Bergey's Manual of Determinative Bacteriology [17].

2.6 Biochemical Characterisation was Carried out Using the Following Biochemical Tests

2.6.1 Gram stain

A smear was made using a sterilized wire loop; a loopful of 18-24 hours old cultured was mixed on the glass slide containing a drop of water and was spread evenly. The smear was air dried and was heat fixed by passing it under a blue flame severally. Crystal violet stain also known as the basic stain was applied in drops on the smear and allowed to remain on the slide for 1-2 minutes. The crystal violet stain was rinsed off with distilled water and Lugols iodine solution (mordant) was placed on the smear and was allowed for one minute to fix the organism. The iodine solution was poured off and the slide was washed with 95% alcohol (decolouriser) until no more violet runs from the slide. The slide was rinsed under a gentle running tap and counterstained with safranin for 1-2 minutes and was rinsed with water and blotted dried and examined under the microscope (Leica DM2500 model) using oil immersion objective lens. Gram positive cells appeared purple in colour this is as a result of not be decolourised by the alcohol and therefore retained the purple colour of the crystal violet-iodine complex. Gram negative cells were red or pink this is a result of the alcohol washing off the crystal violet stain and the organism were counter stained with the secondary dye safranin [17].

2.6.2 Catalase test

Catalase test was carried out by placing a loopful of bacterial cell on a clean grease free slide and was emulsified with a drop of hydrogen peroxide. The mixture was examined for the formation of oxygen bubbles which indicates a positive reaction [18].

2.6.3 Oxidase test

Oxidase test was performed by placing a few drops of tetramethyl-p-phenylenediamine hydrogen chloride reagent on a piece of No.1 whatman filter paper in a petridish. Using a glass rod, a loopful of the test organism was smeared onto the impregnated filter paper and was observed for a change in color. A purple colouration produced within 5 - 10 seconds signifies a positive reaction. This test was performed to differentiate between *Pseudomonas* from enteric bacteria and a positive reaction is dependent upon the presence of cytochrome C [18].

2.6.4 Motility test

Motility test was performed through the dispensing of 10ml of semi solid agar medium into tubes and autoclaved. The tubes were allowed to set in a vertical position and were
inoculated by stabbing at the middle of the semi solid agar using a sterile inoculating needle. The tubes were incubated at 37°C with a uninoculated tube serving as the control. Tubes were observed after 24 hours for motility. Motile organisms exhibited a diffuse hazy growth that spread through the medium rendering it slightly opaque [19].

2.6.5 Citrate utilization test

Citrate utilization test was performed by inoculating the test organism in a screw capped tube containing a solidified Simon’s citrate agar slant and was incubated at 35°C for 48 hours, after which the tubes was observed for colour change. Colour change from green to blue colour signifies citrate utilization. All positive changes were confirmed by subculturing into a freshly prepared Simon citrate agar [19].

2.6.6 Indole production test

Indole production test is performed to determine if bacteria can break tryptophan amino acids into indole, only bacteria with the enzyme tryptophanase can breakdown tryptophan. Tryptone broth was prepared and poured in a test tube and sterilised. Two to three (2 to 3) colonies of the isolates were inoculated into the broth and incubated at 35°C for 48 hours. After incubation, about 10 drops of Kovac’s reagent was added. A red/pink layer formed on the top of the media indicated that indole was produced [19].

2.6.7 Methyl red-vogues Proskauer (MRVP)

MRVP is a two in one test used to determine two things. MR (methyl red) is used to determine if glucose can be converted to acidic products such as, lactate, acetate and formate. VP (Vogues Proskauer) is used to determine if glucose can be converted to acetoin.

MRVP broth is a combination of Glucose (5 GL⁻¹), dipotassium phosphate (5 gl), peptone water or nutrient medium (7 gl⁻¹). The broth was sterilized after dispensing it in test tube, 2 to 3 colonies of the isolates were inoculated and incubated at 37°C for 48 hours. After incubation, approximately half of the culture was transferred to a clean test tube and was used for VP test and the remaining was incubated for another 48 hours, and then used for MR test [19].

2.6.8 VP (Proskauer mode) test

Ten drops of freshly prepared alpha-naphtol (Barratt’s reagent A) were added to the VP tube, after which 10 drops of 40% potassium hydroxide (Barritt’s reagent B) was added. The tube was shaken for 30 seconds. A positive test is determined by the gradual formation of red colour and a negative test is determined by the formation of a yellow or brown colour.

2.6.9 MR (Methyl-red) test

A few drops of MR solution was added to the MR tube. Yellow represents negative results, while red colour formations show positive results [19].

2.6.10 Sugar fermentation test

Acids and or gases are produced during fermentation using sugars (glucose, lactose, sucrose etc.). The sugar fermentation test detects a decrease in pH due to acid production during sugar fermentation. pH indicator is commonly used in phenol red. This indicator changes the colour of the fermentation medium from red to yellow during the production of acid. The Durham tube is placed in a broth tube to detect gas production.

The sugar broth is a combination of sugar, such as glucose (1% w/v), peptone water or nutrient medium (1% w/v) and phenol red (0.005% w/v). the Durham tubes were placed in a test tubes and the broth is dispensed into the tubes. The broth tubes were sterilized by autoclaving at 120°C for 10 minutes. After sterilization, the tubes were inoculated with 2 to 3 colonies of the isolate and incubated at 35°C for 24 hours. AG was used to record the production of acid and gas produced, A for acid and no gas production, and G no acid and gas is produced [19].

The pure fungal isolates were examined macroscopically. The colonial morphology such as, colour (pigmentation), texture and surface appearance were observed. The microscopic (Leica DM2500 model) examination was also done. A drop of lactophenol cotton blue was put on a clean, dry, grease-free slide and colonies of the pure isolate was emulsified into it and examined microscopically. Sexual and asexual reproductive structures like sporangia, conidial head and the vegetative mycelium were observed. Sugar fermentation (glucose, fructose, galactose, lactose, sucrose, maltose and mannose) were also carried out. The results of
the cultural, morphological and biochemical characteristics were compared with those of known taxa [19].

2.7 Physicochemical Analyses of the Samples

2.7.1 pH

Using the Jenway pH meter (3015 model) the pH of the waste was determined. The pH was calibrated first of all with solutions of known pH (sterile deionised water), before usage to ensure accuracy of measurement. Ten grams each of the wastes sample was soaked in 20 ml of sterile deionised water and allowed to stay for 10 minutes and the electrode was placed in the waste sample to be measured and the pH was displayed and allowed to stabilise before taking the reading. After taking the reading, the pH electrode was rinsed with deionised water to remove any traces of the sample measured, blot with scientific wipe and place back in the storage solution [20].

2.7.2 Temperature

The mercury thermometer (9313A38 model) was used to determine the temperature. The thermometer was held by the end opposite the coloured (red,) tip and was cleaned with a sterile cotton wool. The tip was placed in the sample and allowed to stay for about 5 minutes, after which readings was taken. The thermometer was finally rinsed and blotted dry with cotton wool mixed with alcohol [16].

2.7.3 Moisture content determination

Two grams of the waste sample was weighed into a metallic moisture can previously washed, dried and weighed using weighing balance (Model No. AE 223). The weighed sample was transferred to pre-heated oven (Model No. DHG 9140A), to dry for an hour at 130°C. The dried sample was brought out from the oven using forceps and transferred to the desiccator to cool for 15 to 20 minutes [15]. The sample was weighed as follows;

Sample weight = weight of Can + sample before drying – weight of empty can

Moisture loss = weight of can + sample before drying – weight of can + sample after drying.

Equation 1 was used to calculate the percentage moisture content

\[
\% \text{ Moisture content} = \frac{\text{Moisture loss}}{\text{Sample weight}} \times 100 \tag{1}
\]

2.7.4 Nitrogen content determination

Total nitrogen was determined by the Kjeldahl method in which the acid digested waste extract was subjected to the distillation, titration and digestion to get the percentage total nitrogen of the sample [20]. 0.5 gram of the sample was weighed into Kjeldahl Digestion flask, 0.3 g of copper sulphate (CuSO₄) and 3 g of sodium sulphate (Na₂SO₄) which serves as catalyst was added into the Kjeldahl flask containing the sample. Twelve millilitres (12 ml) of concentrated H₂SO₄ was added and mount on KDN – 04C Digest furnace and allowed to digest for 1 hour at 420°C (formation of a clear solution). The digest was diluted with distilled water and made up to 100 ml using a measuring cylinder; twenty millilitres (20 ml) of the digest was measured into a distillation flask. 20 ml of 45% NaOH was added and placed in Foss, Kjeltec 2100 Distillation unit. Ten millilitres (10 ml) of Boric acid indicator was measured into the receiving flask, and allowed to distil for 5 minutes. 0.1N HCL solution was used against the Distilled sample and titrated till a pink colour solution was observed (end point).

\[ N = \text{the normality of HCL} \]

2.7.5 Total organic carbon determination

Total organic carbon (TOC) in wastes was determined by the rapid wet oxidation method based on Walkley and Black [21] procedure. This is a trimetric method, which involves initial oxidation of the carbon content in the samples followed by a rapid back titration with ferrous sulphate solution [22].

One gram of the waste sample was weighed into dry 500 ml conical flask and 10 ml of K₂Cr₂O₇ was added into the flask with pipette and swirled. With a burette, 20 ml concentrated H₂SO₄ was rapidly added and swirled gently until the sample and reagents are mixed then more vigorously for one minute. The reaction proceeds for 30 minutes on asbestos sheet to avoid burning of table due to release of intense heat due to reaction of sulphuric acid. 200 ml of distilled water was slowly added, 10 ml of concentrated orthophosphoric acid and about 0.2 gm NaF (one
small teaspoon) was added and the sample was allowed to stand for 1.5 hours. The titration end point was clear in a cooled solution. Just before titration, 1 ml ferroin indicator was added into the conical flask. The excess K$_2$Cr$_2$O$_7$ with 0.5 N ferrous ammonium sulphate was titrated till the color flashes from yellowish green to greenish and finally brownish red at the end point. Simultaneously blank test was run without sample [22]. Equation 2 was used to calculate the percent organic carbon.

Calculations:

% Organic carbon = \( \frac{(B - S) \times N \times 0.003 \times 100}{\text{wt. of sample}} \)  
Equation (2)

Where, \( B = \) ml of std. 0.5 N ferrous ammonium sulphate required for blank.
\( S = \) ml of std. 0.5 N ferrous ammonium sulphate required for sample.
\( N = \) Normality of std. ferrous ammonium sulphate (0.5N).

Organic matter = Organic Carbon x 1.724.

2.8 Evaluation of Waste Degradation by Weight Loss Method

The waste degradation potential of the microbial population was studied by weight loss method. The composted dried waste samples were weighed using weighing balance (Model No. AE 223). The weight loss was calculated and from the data, the degradation potential of the microbes was determined after the 12 weeks of composting [23]. Percentage change in weight was calculated as presented in equation 3.

Sample weight (wt) = initial weight – final weight – volume used for analysis

\[ \% \text{ change in wt} = \frac{\text{Initial wt} x 100}{\text{Final wt}} \]  
Equation (3)

2.9 Statistical Analysis

The descriptive statistics of the data obtained from the different waste categories were computed in Microsoft Excel 2013 and analyzed using IBM SPSS version 22. The bacterial counts obtained from the samples were converted to logarithm base ten of number.

3. RESULTS

The total heterotrophic bacterial counts during biodegradation of the various waste categories are presented in Fig. 1. Total heterotrophic bacterial counts in the biodegradable fraction of the wastes showed higher bacterial load, followed by the mixture, paper, metal, plastics and glass in the following order, with a range of: 8.2 ±0.01 - 8.5 ±0.01 Cfu/g for biodegradable fraction, 6.4 ±0.01 - 8.1 ±0.02 Cfu/g for mixed fraction, 5.0 ±0.00 - 6.7 ±0.01 Cfu/g, 2.1 ±2.8- 4.3 ±0.02 Cfu/g, 4.0 ±0.00 - 4.7 ±0.01 Cfu/g, 4.1 ±0.01 - 4.9±0.03 Cfu/g for paper, metal, plastic and glass portion respectively.

The total heterotrophic fungal counts are presented in Fig. 2. The counts reveals that the biodegradable, mixture, paper, metal, plastic and glass fractions has fungal counts which ranged from: 7.2±0.04 - 7.9±0.00 Cfu/g, 6.2±0.01 - 7.0±0.00 Cfu/g, 3.3±0.01- 3.7±0.01 Cfu/g, 3.8±0.07 - 4.6±0.01 Cfu/g, 2.9±0.00 - 3.5±0.01 Cfu/g, 3.4±0.01- 4.4±0.00 Cfu/g for biodegradable, mixture, paper, metal, plastic and glass fractions respectively.

3.1 Microbial Types Isolated

In this study, various bacterial isolates were identified and they belong to the following genera: Bacillus, Providentia, Proteus, Staphylococcus, Esherichia, Enterobacter, Pseudomonas, Klebsiella, Streptococcus, Micrococcus, Citrobacter, Acetobacter, Serratia, and Chryseobacterium. Fungal isolates are: Aspergillus, Penicillium, Mucor, Rhizopus, Saccharomyces, Alternaria, Cladosporium, Fusarium and Candida species. The morphology and biochemical characteristics of the bacteria and fungi identified are presented in Tables 1 and 2 respectively.

The pH values recorded in this study are shown in Fig. 3. The various categories of domestic wastes had variations in the pH values. Biodegradable fraction ranged from 5.3 - 9.2, while mixture pH ranged from 5.9 - 6.9 and pH of paper ranged from 7.0 - 8.4.

The temperature values recorded during the biodegradation of the waste categories are presented in Fig. 4. The biodegradable fraction recorded temperature values that ranged from 30° - 35°C, and mixture ranged from 32°C - 35°C, then paper ranged from 25°C - 27°C, metal surged from 30°C - 32°C, plastic ranged from 30°C - 35°C, while glass recorded temperature value of 30°C.

The mixed fraction of the domestic waste was observed to have moisture content which ranged
from: 4.25 - 64.68% while the biodegradable fraction had a moisture content ranging from 6.07 - 79.74%. This is presented in Fig. 5.

The Nitrate content of the mixed fraction of the waste surged from 0.19 - 1.28 mg/kg whereas the biodegradable fraction ranged from 0.23 - 1.98 mg/kg. This is also presented in Fig. 6.

The results of the total organic carbon as presented in Fig. 7 shows that the mixed fraction ranged from 12.9 - 37.6 mg/kg while the biodegradable fraction ranged from 20.3 - 52.1 mg/kg.

The changes in weight of wastes following biodegradation are shown in Table 3. The initial weight of the various waste options was 5 kg, and the final weight of the biodegradable fraction of the waste 1150 g, while mixed fraction was 2540 g, and paper fraction recorded a value of 3745 g. The weight of plastic, metal and glass wastes remained the same at the end of the experiment.
4. DISCUSSIONS

The heterotrophic bacterial counts for the various categories of wastes samples are shown in Fig. 1. The total heterotrophic bacterial counts in the biodegradable fraction of the wastes showed higher microbial counts, followed by the mixture, paper, metal, plastics and glass in that order. Same trend was also observed for the total heterotrophic fungal counts; where the biodegradable category had the highest counts as seen in Fig. 2. This trend could be due to the higher organic carbon and nitrogen contents of the biodegradable fraction of the wastes, and increased availability of biodegradable organic substrates which serves as a source of food and energy to the microbial populations [24]. The biodegradable fraction is mostly composed of food wastes, which are the inedible or left-over foods such as fruits and vegetable peels, egg shell, fish and meat bones etc. Food waste materials contain proteins, carbohydrates, fats,
and some micronutrients whose concentrations and constituents depend on the type of food. Waste foods disposed of from the homes would contain about 60 - 80% moisture content, 40 – 60% carbohydrate, 10 – 30% protein, 15 – 40% fat, 18 – 30% volatiles and 3 – 5% ash [6]. All these components make the biodegradable category a suitable media for microbial growth.

Fig. 5. Changes in moisture content of waste during biodegradation

Fig. 6. Changes in nitrate content of wastes during biodegradation
Table 1. Morphological and biochemical characterization of bacterial isolates

| Isolate | Shape     | Elevation | Opacity | Edge   | Colour          | Gram reaction and cell morphology | Catalase | Citrate | Oxidase | Motility | Glucose | Lactose | Mannitol | Sucrose | Amylace | Raftinose | Methy Red | Voges Proskauer | Indole | Probable identity     |
|---------|------------|-----------|---------|--------|----------------|------------------------------------|----------|---------|---------|----------|---------|---------|---------|---------|---------|-----------|-----------|----------------------|--------|------------------------|
| A       | Circular   | Flat      | Opaque  | Smooth | Rough          | +ve rod                            | +        | +       | -       | -        | +       | -       | -       | -       | +       | -         | -         | -         | Bacillus sp.          |
| B       | Circular   | Flat      | transparent | milky | -ve bacilli   | +        | +       | -       | +       | -       | +       | -       | -       | -       | -       | -       | +       | -         | Providencia sp       |
| C       | Circular   | Convex    | Opaque  | Smooth | Golden yellow | +ve cocci                         | +        | +       | -       | -       | +       | +       | +       | +       | -       | +         | +         | +         | Staphylococcus sp.    |
| D       | Round      | Flat      | translucent | red    | -ve rod       | +        | +       | -       | +       | +       | +       | +       | +       | -       | -       | +       | +       | Serratia sp.          |
| E       | Circular   | Convex    | Opaque  | Smooth | Cream         | -ve short rod                    | +        | +       | -       | -       | -       | -       | -       | -       | -       | +         | -         | +         | Citrobacter sp.       |
| F       | Circular   | Flat      | Opaque  | rough  | white         | +ve rod                          | +        | -       | +       | +       | -       | -       | +       | -       | -       | -         | +         | -         | Bacillus sp.          |
| G       | Circular   | Flat      | Opaque  | Entire | Cream         | -ve rod                          | -        | -       | +       | +       | -       | -       | +       | +       | -       | +         | +         | -         | Acetobacter sp.       |
| H       | Circular   | Convex    | Opaque  | Smooth | yellow        | +small cocci                     | -        | -       | +       | +       | +       | -       | +       | -       | -       | -         | -         | -         | Micrococcus sp.       |
| I       | Circular   | Raised    | translucent | smooth | milky         | -ve rod                          | +        | +       | -       | -       | -       | +       | +       | +       | -       | -         | +         | -         | Klebsiella sp.        |
| J       | Circular   | Raised    | translucent | smooth | Green         | -ve short slender rod           | +        | +       | +       | -       | -       | -       | -       | +       | -       | -         | +         | -         | Pseudomonas sp.       |
| K       | Circular   | Raised    | translucent | smooth | pink          | -ve bacilli                      | +        | -       | +       | +       | +       | +       | +       | +       | -       | -         | +         | -         | Enterobacter sp.      |
| L       | Circular   | Flat      | opaque  | smooth | creamy        | -ve rod                          | -        | +       | +       | -       | +       | -       | -       | -       | +       | -         | +         | -         | Enterobacter sp.      |
| M       | circular   | Raised    | translucent | smooth | pinkish      | -ve rod                          | +        | +       | -       | -       | -       | -       | -       | +       | -       | -         | +         | -         | Chrysobacterium sp.   |
| N       | Circular   | Raised    | translucent | smooth | milky         | -ve rod                          | +        | +       | +       | -       | -       | -       | -       | -       | +       | -         | +         | -         | Proteus sp.          |
| O       | circular   | Raised    | translucent | smooth | creamy        | +ve cocci in chains             | +        | +       | -       | -       | -       | +       | -       | +       | -       | +         | +         | -         | Streptococcus sp.     |
Table 2. Macroscopic and microscopic characteristics of the fungal isolates

| Isolates | Macroscopy | Microscopy | Probable Identity |
|----------|------------|------------|-------------------|
| A        | Black spores surrounded by cream background, brown reverse | Septate hyphae with aseptate conidiophore bearing conidia | **Aspergillus sp.** |
| B        | Green powdery surface surrounded by white lawn, brown reverse | Septate hyphae with conidiophore bearing conidia | **Penicillium sp.** |
| C        | Fluffy white to grey cottony, yellow reverse | Aseptate hyphae bearing sporangiospores | **Rhizopus sp.** |
| D        | Fluffy white cottony, white reverse | Aseptate hyphae bearing sporangiospores | **Mucor sp.** |
| E        | Creamy round and large | Oval budding blastoconidia | **Candida sp.** |
| F        | Light green lawn surrounded by white lawn-like growth | Septate hyphae with aseptate conidiophore bearing conidia | **Aspergillus fumigatus** |
| G        | Orange small round raised | Spherical budding blastoconidia | **Candida sp.** |
| H        | Blue-green velvety growth with white periphery and reverse white colour | Septate hyphae with sporangiospheres | **Mucor** |
| I        | Pale brown, smooth-walled or verrucose | Conidia are obclavate, ovoid or ellipsoidal | **Alternaria sp.** |
| J        | Black spores surrounded by cream background, brown reverse | Septate hyphae with aseptate conidiophore bearing conidia | **Aspergillus sp.** |
| K        | Pale green and conspicuously echinulate | Conidia heads are typically radiate | **A.flavus** |
| L        | White to yellow mycelial surface bearing black conidia heads | Conidial heads are biseriate with the phialides borne on brown | **A.niger** |
| M        | Greyish and suede-like to floccose | Branched acropetal chains and multicelled, obclavate conidia with short conical beaks | **Alternaria sp.** |
| N        | Deep pink surface and a carmine red reverse | Conidiophores scattered over the aerial mycelium | **Fusarium sp.** |
| O        | White to cream smooth, glabrous and yeast-like | Ellipsoidal budding blastoconidia | **Saccharomyces sp.** |
| P        | Fluffy white cottony, white reverse | Aseptate hyphae bearing sporangiospores | **Mucor sp.** |

Table 3. Changes in weight following degradation

| Waste Categories | Initial Weight (Wt) | Final Wt | Change in Wt | Change in Wt (%) |
|-----------------|---------------------|----------|--------------|------------------|
| BIODEGRADABLE   | 5000                | 1150     | 3850         | 50               |
| MIXTURE         | 5000                | 2540     | 2460         | 31               |
| PAPER           | 5000                | 3745     | 1255         | 17               |
| PLASTIC         | 5000                | 4940     | 60           | 1                |
| METAL           | 5000                | 4940     | 60           | 1                |
| GLASS           | 5000                | 4940     | 60           | 1                |

The following bacterial genera were isolated from the domestic wastes which include: **Bacillus, Providentia, Proteus, Staphylococcus, Escherichia, Enterobacter, Pseudomonas, Klebsiella, Streptococcus, Micrococcus, Citrobacter, Acetobacter, Serratia, Chryseobacterium**, as well as the following fungal genera: **Aspergillus, Penicillium, Mucor, Rhizopus, Saccharomyces, Alternaria, Chlamydosporium, Fusarium** and **Candida** were identified in this study. These microorganisms were identified based on cultural, morphological and biochemical characteristics of the organisms. These organisms were able to convert complex
organic materials into valuable plant nutrients, hence play very important role in the recycling of inorganic nutrients in the biogeochemical cycle and maintain ecological balance in the environment. These microbes produce enzymes such as cellulases, endoglucanases, xylanases, dhnases, hyaluronidase, staphylolysin, streptokinase among others which converts organic materials into essential metabolites for growth [25]: [26]. Proteins are broken down to amino acids, finally to ammonia, nitrates, and free nitrogen. Also to urea, uric acid reduced to form nutrients. Some of the bacterial isolates reported in this study have been previously reported to be associated with waste and waste biodegradation. Studies by Obire et al. [27] indicated that all the bacterial isolates reported in this study except Chryseobacterium, Acinetobacter and Providentia have been reported to be associated with waste. However, Pseudomonas, Bacillus, E.coli, Proteus, Micrococcus, Staphylococcus, Serratia and Streptococcus genera has been reported by Ekundayo [28]. Among others, Pseudomonas and Bacillus genera have been reported widely to be associated with waste due to their hydrolytic potentials [7]. Williams and Hakam, [25] reported Arthrobacter, Micrococcus, Proteus, Serratia and Streptococcus genera. The isolation of Bacillus, Staphylococcus, Streptococcus and others from the domestic waste is an indication that microbes are not only ubiquitous in nature but also populates our environment. However, in the present study the predominant genera which are in the spectra of Bacillus, Pseudomonas, and Klebsiella are considered as opportunistic pathogens that can cause wound infection which could lead to sepsis [29].

The fungal genera identified in this study are almost impossible to control in the environment because they produce large amount of spores which are airborne, their spores remain viable in the environment for some time and germinates when conditions are favourable. Ekundayo, [28], reported Aspergillus, Penicillium, Mucor, Rhizopus, Fusarium and a variety of yeasts species to be associated with biodegradation of waste. Ikpedu [30], also indicated that Aspergillus and Saccharomyces was involved in waste biodegradation while, Sanni [31] reported Saccharomyces and Rhizopus species. However, this finding was in agreement with the fungal genera isolated and identified in this study. Furthermore, most of the fungal genera reported in this present study with the exception of Penicillium are also potential pathogens; they are implicated in various disease conditions such as dermatitis and others, and therefore, could pose a serious health risk in the environment [32]. However, these microorganisms and their various activities if properly harnessed can be utilised in future treatment plants in Nigeria for the acceleration of the bioconversion of waste compost into organic fertilizer use in gardening, horticulture and Agriculture [33].

![Fig. 7. Changes in total organic carbon of wastes during biodegradation](image-url)
The low pH values observed at the beginning of the study could be as a result of the easily metabolizable organic contents that serve as food source to the decomposing microbial population present in the domestic waste. Smars et al. [34] suggested that the initial phase that is characterised by a low pH is always observed during organic waste composting especially of energy-rich material such as household wastes that is easily degradable. The pH in house-hold waste often has a low pH when it arrives at the composting plant. Smars et al. [32] found that a considerable gain in time and process activity could be obtained by a mesophilic control of temperature during the initial low pH phase of the compost. An initial degree of acidity recorded in this study for the biodegradable, mixed and paper wastes is in agreement with Obire et al. [27], who reported a pH range of 5.4 -7.9. Hagerty et al. [35] also reported that the initial pH of solid waste is between 5.0 and 7.0 for refuse that is about 3 days old while Paroni et al. [36] also found that in the first to three days of composting, the pH drops to 5.0 or less then begins to rise to about 8.5 for the remaining aerobic process which is in agreement with this study. In general, an obvious pH drop would be observed in the initial stage of composting, especially in food waste composting because of low molecular weight organic acids were substantially produced [37].

The ranges in temperature in all waste options fall between the mesophilic range of temperatures. Most microbial species are mesophilic. Obire et al. [27], reported a lower temperature range in a soil sample obtained from Eagle island waste dump site. However, Hagerty et al. [35], reported that at the initial stage of composting, the mesophilic flora predominates and are responsible for most of the metabolic activities which takes place. The elevation of the temperature of the compost as a result of the increased microbial activities results in decrease in pH and production of organic acids with the release of a stench odour and the subsequent replacement of mesophilic population by thermophilic flora such as Aspergillus, Mucor and Bacillus reported in this study. The temperature changes in the course of composting is closely correlated to the microbial activities and is normally considered to be one of the main parameters used to monitor the composting efficiency [38].

Results of this work showed a significant difference in the moisture contents of the mixture and the biodegradable fraction of the waste sample. A decline in the moisture content was observed in both the mixed and biodegradable fractions, though the biodegradable wastes tend to hold more moisture. The biodegradable fraction of the sample showed higher moisture content. This could be as a result of the ability of the organic portion which includes materials of plants and animal origin ability to contain and hold moisture. While the mixed fraction was observed to have moisture content lower than the biodegradable fraction. This is in agreement with Amrita and Subhas, [23], who recorded a similar moisture content in a waste dumpsite in Dhapa. Although a decline in the moisture content was observed in both the mixed and biodegradable fraction over time in this study. However, this could be because of the aerobic composting which enables the incorporation of oxygen which is one of the environmental factors that enhances the evaporation of moisture and ensuring the degradation of the organic substrates. This observation is in line with Zhaol et al. [39], who opined that the decrease of moisture is influenced by the presence of air during the process of mineralisation as a metabolising element of microorganism. The reduction of moisture content in solid waste is due to exothermic reaction of biochemical oxidation supported by the contact of airflow into the solid waste mass [39]. However, the moisture content of the paper, plastic, metal and glass was not done because of the nature of the waste sample.

Results of this study showed that higher nitrogen production occurred in the biodegradable waste fraction with the highest concentration. This process is characterised by high temperature, high organic content and intense biodegradation that took place during the composting process because of microbial activity. The mixed waste fraction recorded lower nitrogen content. It was observed that the concentration of nitrogen started to decrease until the end of the experiment. This is because the release of nitrogen into the air is faster than its accumulation, and the accumulation of nitrogen/simple amines results in the increase in pH which makes the composting materials alkaline. This was observed in the mixed and biodegradable options, which is closely related to the activity of microorganisms participating in the process. Because at the alkaline stage, the microorganisms use the amines to build their own body mass. This is-in-agreement with Kucic and Briski, [40], who observed during the
biodegradation of nitrogen containing compounds, that nitrogen is transformed to ammonium ions and the composting mass becomes alkaline. This could be attributed to the microbial activity participating in the process of compost formation.

The results of the total organic carbon (TOC) showed variations in the values of biodegradable waste options. The breakdown of carbon compounds and release of CO$_2$ which accumulates increases the acidity in the waste which results in the lowering of the pH values to acidic level [41]. This was observed in the initial stage of the biodegradable and mixed fraction. However, the TOC of the paper, plastic, metal and glass was not done because of the nature of the waste sample.

The changes in weight during biodegradation process as shown in Table 3, indicates the highest weight reduction for the biodegradable category. After the 12 weeks monitoring there was 50% weight loss for the biodegradable fraction while the mixed fraction was 31%, and paper fraction recorded 17% loss. This implies that sorting of waste into different categories before disposal over time have a significant impact on degradation as seen in the weight variation in biodegradable fraction. Similar observations were made by Pan et al. [4]; Andrea et al. [6], who also observed higher, weight loss for the biodegradable fraction than the others. The results revealed that the biodegradable fractions decomposed faster within a short period than others and there was correlation between microbial colonization and rate of degradation. However, the final weight of the plastic, metal and glass remained the same, as biodegradation did not take place in them during the monitoring period, since they are recalcitrant and can persist in the environment over a long period of time. The amount of waste in the biodegradable fraction was reduced over time because of microbial transformation. Microorganisms play very crucial role in the degradation process, breaking down large organic compounds into simpler nutrient rich in organic materials by various biochemical processes [7].

The non-biodegradable categories were recalcitrant and are not prone to microbial attack, they persist longer in the environment and are not easily degraded by the microorganisms. This is because they are not recognised by naturally occurring microorganisms, therefore cannot enter common metabolic pathways or produce the required metabolic enzymes to break them down. Their physical and chemical characteristics also affect their biodegradability [42].

5. CONCLUSION

This study has revealed that at the end of the 12 weeks, there was 50% weight loss for the biodegradable fraction which showed a significant decrease in waste volume, while the non-biodegradable fraction did not show any significant weight loss. Therefore, separation of waste into different categories such as biodegradable from non-biodegradable before disposal enhances the rate of decomposition and transformation of the organic portion of the waste by the degrading microorganisms and other environmental factors. This in turn reduces the mass and volume of wastes in the dumpsite, also improving the aesthetic value of the environment and leads to sustainability. Then, the non-biodegradable fractions could be moved to treatment plant or recycled to prevent environmental pollution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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