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Identification of Bacteria and Fungi Isolated from Decomposed Domestic Solid Waste Samples of Rivers State University Campus

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ABSTRACT

This study aims to study the microorganisms involved in the biodegradation of domestic solid waste. Waste samples aged three to four days were collected from refuse bins in three different households within Rivers State University Campus, Port Harcourt. Five kilograms of the waste samples were sorted into biodegradable and non-biodegradable categories, placed in open containers, and allowed to decompose over a period of twelve weeks. Samples were withdrawn periodically from each waste category and analyzed using the spread plate technique on solid media. Total heterotrophic bacterial and fungal counts were monitored before microbial identification. The total heterotrophic bacterial counts ranged from $8.2\pm0.01 \times 10^5$ to $8.5\pm0.01 \times 10^6$ CFU/g, while the fungal counts ranged from $7.2+0.04 \times 10^2$to $7.9+0.0010^4$CFU/g. The bacterial genera identified included *Bacillus*, *Providencia*, *Proteus*, *Staphylococcus*, *Escherichia*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Streptococcus*, *Micrococcus*, *Citrobacter*, *Acetobacter*, *Arthrobacter*, *Serratia*, and *Chryseobacterium*. The fungal genera identified were *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Saccharomyces*, *Alternaria*, *Chlamydosporium*, *Fusarium*, and *Candida*. Throughout the biodegradation process, *Bacillus*, *Aspergillus*, and *Penicillium* species were consistently isolated. The results indicate a high population of active indigenous bacteria and fungi in the waste materials, which initiate and facilitate the degradation process. The waste was found to harbor microorganisms capable of decomposing both biodegradable and mixed waste categories. A significant difference was observed in the microbial population and diversity between the biodegradable and non-biodegradable waste categories, with the latter hosting fewer microorganisms. Properly harnessing the activities of these microorganisms could aid in the development of treatment plants for accelerating the bioconversion of waste into organic nutrients.

Keywords: Biodegradation, compost, domestic solid waste, total heterotrophic bacteria, heterotrophic fungi.

Introduction

Waste refers to all items that people no longer have any use for and either intend to discard or have already disposed of. Additionally, some waste materials must be discarded due to their hazardous properties. Waste is any unwanted or unusable material discarded after its primary use.

However, waste can sometimes be repurposed as a by-product—a joint product with relatively minor economic value—or transformed into a resource through innovations that increase its value beyond zero (Ogweleka, 2009).

Various materials can be classified as waste, including household refuse, sewage sludge, industrial by-products, packaging materials, discarded vehicles, outdated electronics, garden waste, and old paint containers. Daily human activities generate a vast array of different waste types from multiple sources.

The volume of waste continues to rise due to population growth, increased consumption, urbanization, and technological advancements (Douglas et al., 2020). In Nigeria, over 1.8 billion tonnes of waste are generated annually, equating to 3.5 tonnes per person. This waste originates from diverse sources, including households, commercial establishments (such as shops, restaurants, and hospitals), industries (such as pharmaceutical companies and textile manufacturers), agriculture, construction and demolition projects, mining, quarrying, and energy production (Vuk, 1995). Given the vast amount of waste produced, proper management is essential to prevent harm to human health and the environment (Vuk, 1995). Waste generation occurs at various stages of human and animal activities, from production processes to consumption. Managing waste remains a significant challenge, especially in developing countries, where inefficiencies result in higher waste production per unit of output compared to developed nations.

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Biodegradable waste consists of organic matter that can be broken down by microorganisms and other living organisms into carbon dioxide, water, methane, or simple organic molecules through composting, aerobic digestion, anaerobic digestion, or similar processes (Douglas et al., 2020). In some cases, certain inorganic materials, such as gypsum, are also biodegradable due to bacterial decomposition. In domestic waste collection, biodegradable waste is often limited to degradable materials that can be processed in local waste management facilities through composting (Davis and Song, 2015). Aerobic decomposition, widely used for waste stabilization, is known as composting. During composting, microorganisms break down biodegradable materials into simpler components. This process is characterized by intense microbial activity, leading to the complete or partial degradation of organic compounds in domestic solid waste through microbial metabolism (Jereb, 2004). A diverse microbial community is essential for effective composting, as these microorganisms naturally exist in compost feedstock and sustain active populations throughout the process (Karnchanawong and Nissaikla, 2014).

Various microorganisms contribute to the specialized biodegradation process, including mesophilic, thermo-tolerant, and thermophilic aerobic bacteria, actinomycetes, yeasts, and molds. Composting occurs in three distinct phases: 1. Mesophilic phase – The initial stage where moderate-temperature microbes begin decomposition. 2 Thermophilic phase – A high-temperature phase lasting from days to months, during which more resistant organic materials are broken down; and the 3. Cooling and maturation phase – The final phase, where the compost stabilizes and reaches maturity. The duration of each phase depends on the nature of the organic matter being composted and process efficiency, which is influenced by aeration and agitation (Karnchanawong and Nissaikla, 2014). At the beginning of composting, the waste mass is at ambient temperature and slightly acidic. Microorganisms first consume easily degradable carbon sources such as monosaccharides, starch, and lipids. This process generates organic acids, lowering the pH. As composting progresses, proteins are degraded, releasing ammonia and increasing the pH. Once the easily degradable materials are exhausted, more complex compounds such as cellulose, hemicellulose, and lignin are broken down, forming humic acid, fulvic acid, and phenolic intermediates (Tuomela et al., 2000).

Humified substances formed during composting are categorized as follows: Humin – Not soluble in water at any pH; Humic acids – Soluble in water under alkaline conditions, and Fulvic acids – Soluble in water across all pH levels (Tuomela et al., 2000).

The complex symbiotic and synergistic interactions among microorganisms ultimately result in the humification of compost, producing a stable, earthy-scented material. Compost and manure have gained attention for their beneficial effects on soil structure stability, as well as nitrogen and carbon enrichment (Bouajila and Sanaa, 2011). The biodegradation of the organic fraction of waste enhances soil fertility, aeration, structure, and water retention, all of which are facilitated by microbial activity (Rastogi et al., 2020).

This study aims to identify the microorganisms involved in the biodegradation of domestic solid waste under controlled laboratory conditions. Understanding the microbial community responsible for waste decomposition can provide insights into optimizing composting processes and improving waste management strategies.

Materials and Methods

Sampling Site and Sample Collection

This study was conducted at Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria, located in the South-South region of Nigeria. Domestic solid waste samples, aged three to four days, were collected from refuse bins in three different households within the Rivers State University Staff Quarters. The collected waste samples were sorted into biodegradable and non-biodegradable categories, placed in clean polythene bags, and transported to the Department of Microbiology laboratory at Rivers State University for further analysis.

Sample Preparation

Five kilograms (5 kg) of domestic solid waste samples were sorted and categorized by weight into six groups:

1. Vegetable and animal matter (biodegradable waste).
2. Broken drinking glasses and bottles (non-biodegradable, crushed using a sterile hammer).
3. Paper waste (non-biodegradable).

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4. Plastic bags, pure water sachets, and polythene bags (non-biodegradable, shredded using a sterile knife).
5. Metal waste (e.g., aluminum cans and tin containers, compressed using a sterile hammer).
6. A mixture of all waste types.

The waste samples were placed in open containers and allowed to decompose for twelve weeks (Mahar et al., 2007).

Enumeration of Total Heterotrophic Bacteria

To determine bacterial populations, 10 g of decomposing waste was dissolved in 90 ml of sterile deionized water. A ten-fold serial dilution (10^{-1} to 10^{-8}) was prepared. From the 10^{-5} to 10^{-6} dilutions, aliquots were plated in duplicates onto freshly prepared nutrient agar using the spread plate technique with a sterile bent glass rod. The inoculated plates were incubated at 35°C for 24 hours. After incubation, bacterial colonies between 30 and 300 CFU were counted and multiplied by the reciprocal of the dilution factor to obtain the total bacterial count, recorded as colony-forming units per gram (CFU/g) of waste sample. Characteristic bacterial colonies were repeatedly sub-cultured on nutrient agar until pure isolates were obtained. Some pure isolates were inoculated into Bijou bottles containing agar slants and incubated at 35°C for 24 hours for further identification, while others were preserved at 4°C in a refrigerator (Odeyemi, 2012).

Enumeration of Fungi

For fungal analysis, aliquots from 10^{-2} to 10^{-4} dilutions were plated in duplicates onto Sabouraud Dextrose Agar (SDA) supplemented with 0.05% (w/v) chloramphenicol to inhibit bacterial growth. The plates were incubated at room temperature for 2 to 4 days, after which fungal colonies were counted and recorded.

Distinct fungal colonies were sub-cultured onto fresh SDA plates to obtain axenic cultures, which were further processed into stock and starter cultures for characterization and identification (Douglas, 2018).

Identification and Characterization of Bacterial and Fungal Isolates

The purified bacterial isolates were identified based on their colonial, morphological, physiological, and biochemical characteristics, following standard microbiological procedures. The tests performed included: Gram staining, Catalase test, Methyl Red-Voges Proskauer (MR-VP) test, Sugar fermentation (glucose, fructose, and sucrose), Citrate utilization test, Indole production test, and Starch hydrolysis test.

The obtained results were compared with standard references in Bergey's Manual of Determinative Bacteriology (Cheesbrough, 2006). Further identification was confirmed using the Advanced Bacterial Identification Software (ABIS) online database (Sorescu and Stoica, 2021).

For fungal identification, pure isolates were examined macroscopically for colony morphology, including surface appearance, pigmentation, texture, and growth pattern.

Microscopic examination was conducted using lactophenol cotton blue staining. A drop of lactophenol cotton blue was placed on a clean, grease-free glass slide, and a fungal colony was emulsified into the stain. The slide was examined under a microscope to observe reproductive and vegetative structures such as: Sporangia, Conidial heads, Arthrospores, and Vegetative mycelium. Additionally, sugar fermentation tests were performed using glucose, fructose, galactose, lactose, sucrose, maltose, and mannose. The results from cultural, morphological, and biochemical analyses were compared with standard fungal identification keys (Oyeleke and Istifanus, 2009).

Results

The results of total heterotrophic bacterial counts during the biodegradation of the various waste categories showed that the biodegradable fraction of the wastes had higher bacterial counts, followed by the mixture, paper, metal, plastics and glass in the following order, with a mean counts ranging from 8.2 ±0.01 to 8.5 ±0.01CFU/g for biodegradable fraction, 6.4 ±0.01 to 8.1 ±0.02CFU/g for mixed fraction, 5.0 ±0.00 to 6.7 ±0.01CFU/g, 2.1 ±2.8 to 4.3 ±0.02CFU/g, 4.0 ±0.00 to 4.7 ±0.01CFU/g, 4.1 ±0.01 to 4.9 ±0.03CFU/g for paper, metal, plastic and glass portion respectively.

The fungal counts revealed that the biodegradable fraction, mixed fraction, paper, metal, plastic and glass categories, had mean counts which ranged from: 7.2±0.04 to 7.9±0.00 CFU/g, 6.2±0.01 to 7.0±0.00 CFU/g, 3.3±0.01 to 3.7±0.01 CFU/g, 3.8±0.07 to 4.6±0.01 CFU/g, 2.9±0.00 to 3.5±0.01 CFU/g, 3.4±0.01 to 4.4±0.00 CFU/g for biodegradable, mixture, paper, metal, plastic and glass fractions, respectively.

Changes in the bacterial and fungal types observed during the period of the study are presented in Tables 1 and 2. The frequency of occurrence of the bacterial population is presented in figure 1 while the frequency of occurrence of the fungal population is presented in Figure 2.

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Table 1: Changes in Bacterial Types during Biodegradation of Domestic Solid Wastes

Week	Biodegradable	Mixture	Paper	Metal	Plastic	Glass
1	*Bacillus* sp., *Bacillus cereus, Enterobacter xiangfangensis, Staphylococcus* sp. *Pseudomonas* sp	*Bacillus* sp., *Enterobacter xiangfangensis, Staphylococcus* sp. *Pseudomonas* sp.	*Bacillus* sp. *Pseudomonas* sp., *Staphylococcus* sp	*Bacillus* sp. *Staphylococcus* sp, *Pseudomonas* sp	*Bacillus* sp. *Pseudomonas* sp, *Staphylococcus* sp	*Bacillus* sp., *Staphylococcus* sp, *Enterobacter* sp
2	*Providencia vermicola, Bacillus* sp., *Bacillus subtilis, Proteus* sp., *Citrobacter* sp., *Klebsiella* sp., *Staphylococcus* sp. *Pseudomonas* sp	*Providencia vermicola, Bacillus* sp., *Proteus* sp., *Citrobacter* sp. *Bacillus subtilis, Pseudomonas* sp.	*Bacillus* sp., *Proteus* sp., *Staphylococcus* sp., *E.coli, Pseudomonas* sp.	*Bacillus* sp., *Proteus* sp.,	*Bacillus* sp.	*Bacillus* sp. *Streptococcus* sp., *Enterobacter xiangfangensis*
3	*Bacillus* sp., *Proteus* sp., *Pseudomonas* sp. *Providentia vermicola*., *Klebsiella* sp., *E.coli, Acinetobacter* sp. *Arthrobacter* sp. *Micrococcus* sp	*Bacillus* sp., *Providentia* sp., *Pseudomonas* sp., *E.coli, Acinetobacter* sp.	*Bacillus* sp. *Providentia* sp., *Staphylococcus* sp., *Bacillus circulans, E.coli.*	*Bacillus* sp. *Proteus* sp., *Bacillus cereus*,	*Bacillus* sp. *Bacillus cereus, Acinetobacter* sp.	*Bacillus* sp., *Pseudomonas* sp., *Enterobacter xiangfangensis Staphylococcus* sp.
4	*Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Proteus* sp., *E.coli, Arthrobacter* sp., *Acinetobacter* sp. *Micrococcus* sp.	*Bacillus sp., Pseudomonas sp., Staphylococcus sp., Micrococcus sp., E.coli, Acinetobacter* sp.	*Bacillus* sp., *Staphylococcus* sp., *Providentia* sp., *Proteus* sp., *Micrococcus* sp., *Acinetobacter* sp. *Arthrobacter* sp.	*Bacillus* sp. *Chryseobacterium indologens, Bacillus velezensis, Micrococcus sp., Bacillus circulans , Staphylococcus sp.,*	*Bacillus* sp., *Citrobacter* sp., *Proteus* sp., *Pseudomonas* sp., *Chryseobacterium indologens*	*Proteus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp.
5	*Chryseobacterium indologenes, Bacillus sp., Bacillus cereus, Bacillus subtilis, Pseudomonas* sp, *Micrococcus* sp.	*Chryseobacterium indologens, Bacillus subtilis, Bacillus cereus Pseudomonas* sp	*Bacillus* sp. *Bacillus subtilis*, *Bacillus cereus*	*Bacillus* sp., *Micrococcus sp., Bacillus velezensis, Bacillus subtilis, Acinetobacter* sp	*Bacillus* sp., *Providentia vermicola Bacillus velezensis, Bacillus* sp.,	*Bacillus* sp. *Staphylococcus* sp., *Proteus* sp. *Micrococcus* sp.
6	*Serratia sp., Bacillus sp., Bacillus velensis, Bacillus cereus, Micrococcus* sp, *Pseudomonas* sp.	*Bacillus velensis, Bacillus subtilis, Bacillus cereus*	*Bacillus* sp., *Bacillus velensis, Bacillus sp.*	*Bacillus* sp., *Bacillus velensis*	*Bacillus* sp. *Serratia* sp.	*Bacillus* sp., *Bacillus velensis*

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Table 2: Changes in Fungal Population During Biodegradation of Domestic Solid Wastes

Week	Biodegradable	Mixture	Paper	Metal	Plastic	Glass
1	*R. stolonifera, A. flavus, A. fumugatus, A.niger, Saccharomyces* sp., *Penicillum* sp., *Alternaria* sp.	*Aspergillus* sp. *A. fumugatus, A.flavus, Saccharomyces* sp., *Penicillum* sp., *A. niger*	*Aspergillus sp., Saccharomyces* sp.,	*Rhizopus sp., A. flavus*	*Aspergillus* sp.	*Penicillum* sp.
2	*Rhizopus* sp., *Aspergillus* sp., *A. fumugatus, A.niger, Saccharomyces* sp., *Penicillum* sp., *Alternaria* sp. *Cladosporium* sp.	*Mucor* sp., *A.fumugatus, A.niger,, Penicillum* sp., *Alternaria* sp.	*Aspergillus* sp., *Penicillum* sp.,	*Penicillium* sp., *P. notatum*	*Penicillium* sp. *Aspergillus* sp., *A. Niger*	*Penicillium* sp, *Aspergillus* sp.
3	*A.niger, Saccharomyces sp., Penicillum sp., Alternaria sp., Candida* sp.	*A.niger, Saccharomyces* sp., *Penicillum* sp., *Candida* sp.	*Saccharomyces* sp., *A. niger, Candida* sp.	*Aspergillus* sp. *Penicillium* sp.	*Aspergillus* sp., *Penicillium* sp.,	*Penicillium* sp., *Aspergillus* sp.
4	*A.niger, P. notatum., Penicillum sp., Fusarium* sp.	*A.niger, P. notatum., Saccharomyces* sp.	*Aspergillus sp., Penicillum sp, Saccharomyces* sp.,	*Aspergillus sp., Alternaria* sp., *Penicillium* sp.	*Alternaria* sp., *Cladosporium* sp.,	*A.fumigatus* sp., *Penicillium* sp.
5	*A.niger,, Penicillum* sp.. *Saccharomyces* sp., *Fusarium* sp.	*Aspergillus* sp.., *Penicillum* sp..	*Aspergillus* sp., *Aspergillus niger, P. notatum,*	*Aspergillus niger, Penicillium* sp.	*Alternaria* sp. *Penicillium* sp	*Penicillium notatum, Penicillium* sp.
6	*Saccharomyces* sp *Aspergillus* sp.	*Aspergillus* sp., *Penicillium* sp	*Penicillum sp., Aspergillus* sp.	*Aspergillius* sp., *A niger.*	*Alternaria* sp.	*Penicillium* sp.

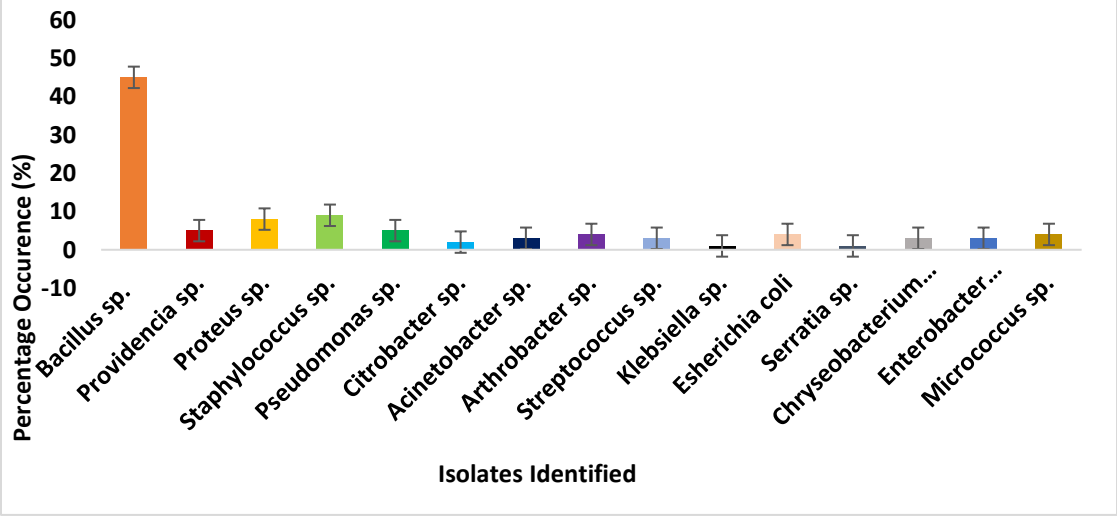


Figure 1: Frequency of Occurrence of Bacterial Isolates

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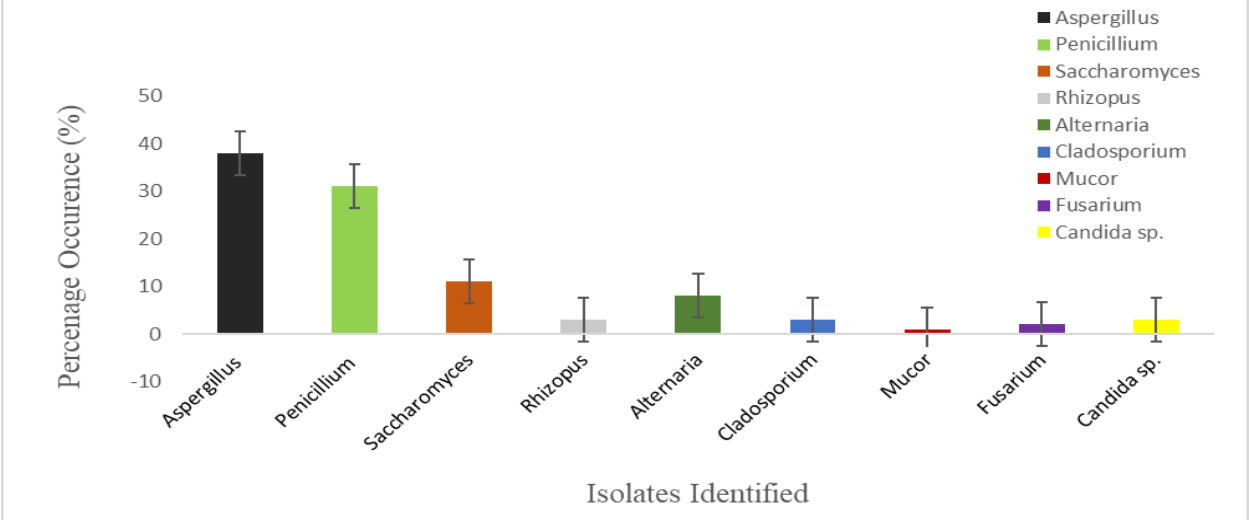


Figure 2: Frequency of occurrence of fungal Isolates

Discussion

The total heterotrophic bacterial counts in the biodegradable fraction of the waste exhibited the highest microbial load, followed by the mixture, paper, metal, plastics, and glass. This pattern aligns with previous studies conducted in various waste dumpsites across Nigeria. For instance, Obire et al. (2002) reported bacterial counts ranging from 4.2×10^5 to 1.60×10^6 CFU/g, while Ebe et al. (2015) recorded the bacterial counts between 1.3×10^8 to 9.2×10^8 CFU/g in domestic waste. The high bacterial load in the biodegradable fraction can be attributed to its higher organic carbon and nitrogen content, which serves as a rich source of nutrients and energy for bacterial populations (Osazee et al., 2017). This fraction predominantly contains food waste, which is highly degradable due to its high moisture content, abundant nutrients, and soft structure that facilitates microbial colonization and breakdown (Siciliano et al., 2019). Generally, food waste consists of 60–80% moisture, 40–60% carbohydrates, 10–30% proteins, 15–40% fats, 3–5% ash, and 45–65% carbon (Paritosh et al., 2017).

Overall, bacterial counts were consistently higher than fungal counts across different waste categories. This trend is not unexpected, as the pH of the waste materials, particularly in the biodegradable fraction, ranged from 5.2 to 9.2, while paper waste had a pH range of 5.9 to 6.9 and 8.4 to 8.7. This pH range favors bacterial growth over fungal proliferation (Obire et al., 2002).

Fungal counts varied across different waste categories, with the biodegradable fraction recording the highest counts, followed by the mixture, metal, paper, glass, and plastic. This finding is consistent with Obire and Nwaubeta (2002), who reported similar fungal populations in a waste dumpsite in Port Harcourt. Ekundayo (1977) identified *Aspergillus, Penicillium, Mucor, Rhizopus, Fusarium*, and various yeast species as key fungi involved in waste biodegradation. Other researchers have also reported similar findings, with Ikpedu (1980) isolating *Aspergillus* and *Saccharomyces*, while Sanni (1980) documented the presence of *Saccharomyces* and *Rhizopus* species in waste decomposition.

Microbial communities play a crucial role in the degradation of organic waste, particularly bacteria such as Pseudomonas and Bacillus species, which possess hydrolytic enzymes capable of breaking down organic materials efficiently (Pant et al., 2012). These microorganisms degrade complex organic matter into proteins, lipids, carbohydrates, amylase, and cellulose, which are then further broken down into simpler forms such as carbohydrates, amino acids, and long-chain fatty acids (Lauwers et al., 2013).

As waste decomposition progresses, microbial succession occurs, where initial microbial populations are gradually replaced by new species as the waste composition changes. This dynamic microbial ecosystem plays a significant role in determining the rate and extent of waste degradation.

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However, the presence of microbial pathogens in waste dumpsites poses potential health risks, particularly when waste is improperly disposed of in residential areas.

Several bacteria and fungi produce extracellular enzymes that facilitate waste degradation. Williams and Hakam (2015) reported that microbial enzymes such as DNase, hyaluronidase, staphylokinase, staphylolysin, cellulases, endoglucanases, xylanases, and streptokinase play a vital role in organic waste decomposition at dumpsites. Fungal species, in particular, secrete enzymes like cellulases, endoglucanases, and xylanases, which help convert waste materials into metabolites essential for microbial growth (Chukwu et al., 2014 ; Wemedo et al., 2012). These enzymes further break down complex organic compounds into plant nutrients, thereby playing a key role in nutrient recycling and maintaining ecological balance. The composition of microorganisms during biodegradation depends on several factors, including moisture content, initial microbial diversity, and the composition of organic matter. Over time, nutrient depletion, toxic metabolite accumulation, and environmental changes cause a decline in microbial population and diversity.

Bacillus species recorded the highest frequency of occurrence and was isolated throughout the sampling period across all waste categories. This finding aligns with the study by Amrita and Subhas (2014), which reported the dominance of Bacillus strains in domestic waste from Barrackpore Municipality. The ability of *Bacillus* species to form endospores allows them to survive harsh environmental conditions and remain dormant until favorable conditions return. *Bacillus* > *Staphylococcus* > *Proteus* > *Providencia* > *Pseudomonas* > *Arthrobacter* > *Escherichia coli* > *Micrococcus* > *Enterobacter* > *Chryseobacterium* > *Streptococcus* > *Acetobacter* > *Citrobacter* > *Klebsiella* > *Serratia*.

Among the fungi, *Aspergillus* species had the highest frequency of occurrence in domestic waste, consistent with Obire et al. (2002), who found *Aspergillus fumigatus* to be the most dominant fungal species in waste dumpsites. The order of fungal prevalence was: *Aspergillus* > *Penicillium* > *Saccharomyces* > *Alternaria* > *Rhizopus* > *Chlamydosporium* > *Candida* > *Fusarium* > *Mucor*.

Conclusion

The order of bacterial prevalence was: *Bacillus*, *Providencia, Proteus, Staphylococcus, Escherichia, Enterobacter, Pseudomonas, Klebsiella, Streptococcus, Micrococcus, Citrobacter, Acetobacter, Arthrobacter, Serratia,* and *Chryseobacterium*. The fungal genera identified were *Aspergillus, Penicillium, Mucor, Rhizopus, Saccharomyces, Alternaria, Chlamydosporium, Fusarium,* and *Candida*. Among these, *Bacillus, Aspergillus,* and *Penicillium* species were the most dominant organisms throughout the biodegradation process, highlighting their crucial role in organic waste degradation. Statistical analysis confirmed a significant difference in the microbial population and diversity between biodegradable and mixed waste samples compared to non-biodegradable waste. This difference is attributed to the potent microbial activity responsible for breaking down organic components in the waste. Conversely, the non-biodegradable waste category harbored fewer microorganisms due to the limited availability of degradable organic substrates. These findings suggest that the microbial communities present in domestic waste can be harnessed and utilized in waste treatment plants in Nigeria. By leveraging these microbial degradation processes, bioconversion of waste compost into organic fertilizer can be enhanced, promoting sustainable waste management and agricultural productivity.

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