Biodegradability traits of *Bacillus subtilis* and *Fusarium* sp. on composting of different nonconventional protein source

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Biodegradation of livestock processing waste is one of the more cost-effective, ecologically safe recycling methods that may preserve nutritional content and key amino acids useful in animal feed production. Here, we determined the biodegrading potentials of nonconventional feedstuffs using a combination of bacteria (*Bacillus subtilis*) and fungi (*Fusarium* sp.). Five nonconventional feed types (feather meal, hair waste, egg membranes, horn hoof and horn) were selected and replicated in a 10 L plastic biocomposter for 7 weeks and observed for degradation by measuring changes in total organic carbon, temperature, phosphorus, potassium, pH, microbial counts and rate of degradation. Bacteria and fungi were inoculated inside the substrate after isolation and incubation. The effects of physicochemical and microbial counts were assessed. The biodegradability and optimal activity were observed. From the results, physico-parameters measured during the 7 weeks showed significant differences (*P* <0.05) between substrates. The highest rate of decomposition, temperature, pH levels, total organic carbon, and total bacteria counts were recorded in feather meal, egg membrane, and hair waste. The weekly study showed that feather meal (68.79%), decompose faster than cow hoof (18.72%) egg membrane (60.89%) hair waste (62.82%) and cow horn (32.31%) during bio-composting. This finding has potential application in the treatment process of keratin wastes.

**Keywords:** Animal feed, Biodegradation, Egg membrane, Feather meal, Hair waste, Hoof, Horn, Keratin waste, Livestock

Solid wastes have become a serious challenge for populated cities throughout the world as rural-to-urban migration and globalization continue to grow¹. As a result of increased livestock consumption, slaughterhouse produces large amounts of animal waste on a regular basis. Feather, cow foot, and horn waste have all contributed to the daily increase in pollution. Due to its tenacious nature and improper management, animal waste, particularly feather, hoof, and horn, has become a major contaminant².

Furthermore, they serve as breeding grounds for many *salmonella* and other pathogenic micro-organisms, which release pollutants such as nitrous oxide, hydrogen sulfide, and heavy metal, all of which are harmful to human health and the environment¹. This waste material, on the other hand, contains high minerals including potassium, magnesium, calcium, iron, zinc as well as essential amino acids. The global daily build-up of feathers has reached five million tons³ which are normally disposed of in landfills, or by burning incineration, among other methods and pollute the environment in some parts of the globe.

Given their high protein content, feather, cow hoof, and horn wastes offer enormous promise for a variety of uses. Nevertheless, because keratin is insoluble and resistant to enzymatic digestion by various organisms such as animal, plant, and microbial proteases, its application as a source of value-added compounds is scare⁴.

Furthermore, biodegradation by composting is one of the more cost-effective and ecologically friendly methods of recycling waste generated⁵; as feathers alone contain 90% weight crude keratin protein and 15% N⁷. However, the keratin in discarded feathers is resistant to biodegradation and may necessitate the inclusion of bacterial inocula to speed up the decomposition process during composting. Many researchers have tried to increase and enhance the utilization of agronomics’ animal wastes through
composting. Here, we have studied the biodegradability trait of fungi and bacteria combination and its ability to degrade animal waste for wide application viz. animal feed, environmental management, etc.

**Materials and Methods**

**Experimental design**

Bio composting was used to degraded five nonconventional animal protein sources namely: cow hoof and horn, poultry feather, hair waste and egg membrane in a complete randomized design; the microbes used were *Bacillus subtilis* and *Fusarium* sp.

**Selection and collection substrate preparation for bio composting**

The various substrates were sourced in the University of Port Harcourt environment while the microbes (bacteria and fungi) were cultured and collected from a private microbiology laboratory in Port Harcourt, Rivers State, Nigeria.

The five unusual animal protein sources were collected, cleaned, oven dried, and ground into smaller particles. Ten grams of each of the five substrates were weighed and then autoclaved before being placed in a one-litre plastic capacity bio-composter. Each substrate and microbial inocula were piled in layers and evenly mixed over the course of the 7 weeks composting process at room temperature (28°C). To monitor the process, compost samples were collected at weekly intervals for physiochemical and microbiological analysis.

**Physicochemical and microbiological analysis of compost**

Temperature, total nitrogen (TN), phosphorus (P), potassium (K), pH, and total organ carbon (TOC) of all compost samples were determined weekly with methods as described earlier.

**Phosphorus content determination**

Phosphorus was done by taking 1 g of the sample and digesting it in a ratio of 4:1 with nitric acid (HNO₃) and perchloric acid (HClO₄). The solution was made to a definite volume. Phosphorus content was determined by addition of vanado molybdate in phosphoric acid solution. The yellow colour developed was measured at 730 nm.

**Potassium content determination**

This was done by taking 1 g of the sample and digesting it at a 9:2:1 ratio with nitric, sulphuric, and perchloric acid, respectively. Thereafter, the solution was left for 24 h on a sand bath till a clear, colourless solution was obtained. A 5 mL digested solution was neutralised with ammonium hydroxide, and distilled water was used to make a 25 mL solution; a standard curve was prepared, and the potassium concentration was determined using a flame photometer.

**Organic carbon (Titrimetric method)**

Organ carbon was done by taking 1 g of the sample. A conical flask was used to weigh the samples. Sulphuric acid (20 mL) and potassium dichromate (10 mL) were added to the solution and gently stirred. The solution was left to sit for 30 min. Following that, 200 mL of water, 10 mL of phosphoric acid, and 1 mL of diphenylamine indicator were added. Ferrous ammonium sulphate (0.5N) was used to titrate the solution. The colour changes from dull green to turbid blue and eventually a sharp green colour. A blank titration was carried out as described above. The volume of 0.5N ferrous ammonium sulphate (FAS) consumed was noted.

**Rate of degradation**

The rate of degradation was calculated by the formula as described by Zhu et al.: Percentage rate of degradation = initial OCᵢ-final OCᵢ, divided by the initial OCᵢ, multiplied by 100%.

$$RD = \frac{OCᵢ - OCᵢ}{OCᵢ} \times 100$$

where OCᵢ is the initial organic carbon and OCᵢ is the final organic carbon at a particular time.

**Microbial analysis**

The bacteria were culturally verified using the biochemical test as described by Franco-Duarte et al. The bacteria and fungi load during the composting process was determined using cultural methods.

**Preparation of culture media**

The media were prepared according to the instructions provided by the manufacturer. In this experiment, nutrient agar and potato Dextrose agar were utilised.

**Nutrient agar**

This medium was made using commercially available dehydrated powder, which can be found at most culture media vendors. In a conical flask capped with cotton wool and aluminium foil paper, 28 g of nutritional agar powder was dissolved in 1 L of distilled water. It was properly blended before being autoclaved for 15 min at 121°C before being aseptically poured into sterile Petri dishes. The medium was later cooled to 45-50°C.

**Isolation of bacteria**

In a sterile beaker, one gram of the samples the results of the mean variation of biological and physicochemical parameters monitored using the combination of bacteria (*Bacillus subtilis*) and fungi...
(Fusarium sp.) composting of cow hoof, chicken feather, egg was weighed, and 9 mL of sterile distilled water was added to make a dilution of 10-1. The 10-1 suspension of the sample was then serially diluted up to 10-3 using 10-fold serial dilution. For bacteria isolation, aliquots of 1 mL of the appropriate dilution from each sample were placed on nutrient agar. The nutrient agar plates were incubated at 37°C for 24-48 h. The number of distinct colonies was counted in terms of colony forming units after incubation. The number of distinct colonies was counted in terms of colony forming units after incubation. The viable numbers were calculated using these values and the dilution factor.

Potato dextrose agar

This was utilized for fungus cultivation. A commercially available dehydrated powder was used to make the medium. In a sterile conical flask coated with cotton wool and aluminium foil paper, 39 g of potato dextrose agar powder were dissolved individually in 1 L of distilled water. It was properly blended before being autoclaved for 15 min at 121°C. After autoclaving; the medium was cooled to 45-50°C and aseptically placed into sterile Petri plates.

Isolation of fungi

In a sterile test tube, one gram of the sample was mixed with 9 mL of sterilized distilled water was added. This 10-1 suspension was then serially diluted up to 10-3 using a tenfold serial dilution. For fungus isolation, 1 mL of the appropriate dilution from each sample was placed in a Petri dish. The potato dextrose agar plates were incubated for 72 h at room temperature (28°C). The number of distinct colonies was counted in terms of colony forming units after incubation. Viable counts were calculated from these numbers by referencing the serial dilution or dilution factor utilized, viable counts were calculated from these numbers.

Enumeration of Bacteria and Fungi Counts

The total viable counts of the isolates were determined using bacterial and fungal counts. On the nutrient agar and potato dextrose agar plates, distinct colonies were picked and counted. The total viable counts for the samples were estimated in colony forming units per gram (cfu/g) using the mean colony count on nutrient agar and potato dextrose agar plates of each dilution.

Statistical analysis

Various data obtained were analyzed using One-Way analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) version 21. The differences between group mean (±SE) was determined using Duncan multiply range test (DMRT) at 5% level of probability of the same software.

Results

Biological and physicochemical parameters during bacterial (Bacillus subtilis) and fungal (Fusarium sp.) bio-composting of substrates

Membrane, hair waste and cow horn is shown in Table 1. The results showed that there was significant (P <0.05) difference. Temperature rate for egg membrane (29.92±0.04a) and hair waste (29.86±0.04a) was significantly higher than cow hoof (29.63±0.04a), chicken feather (29.61±0.04b) and cow horn (29.66±0.04b). The pH range of cow horn was significantly higher than cow hoof, feather meal, egg membrane and hair waste (7.47, 7.56, 7.43 and 7.52 SE 0.07), respectively. The total organic carbon of cow hoof (3.31±0.99b) and chicken feather (4.00±0.99b) not significantly different but far different from egg membrane (7.06±0.99b), hair waste (7.57±0.99b) and cow horn (6.20±0.99b) though, cow horn was not significantly different from cow hoof and chicken feather substrates.

The percentage rate of degradation (ROD) observed from this study showed that substrates 2 (68.79±6.22b), 3 (60.89±6.22a) and 4 (62.82±6.22a) not significantly different (P >0.05) from each other; though substrates 1 (18.72±6.22b) and 5 (32.31±0.04b) not significantly different from each other but had the lowest degrading ability. Also, the total nitrogen presents in substrates 1 (6.27±0.43a) and 2 (5.43±0.43a) was significantly higher compare to substrates 5 (4.90±0.43b), 4 (3.05±0.43c) and 2 (0.18±0.43a), respectively, though in substrates 2 and 5 there was no significant different (P >0.05). Potassium content

Table 1 — Mean biological and physicochemical parameter monitored during biocomposting of substrates using microbes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CHF</th>
<th>FM</th>
<th>Substrates</th>
<th>EM</th>
<th>HW</th>
<th>CH</th>
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<tbody>
<tr>
<td>Temp</td>
<td>29.63±0.04a</td>
<td>29.61±0.04a</td>
<td>29.92±0.04a</td>
<td>29.86±0.04a</td>
<td>29.66±0.04a</td>
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<tr>
<td>pH</td>
<td>7.47±0.07a</td>
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<td>7.43±0.07b</td>
<td>7.52±0.07a</td>
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<tr>
<td>TOC</td>
<td>3.31±0.99b</td>
<td>4.00±0.99a</td>
<td>7.06±0.99a</td>
<td>7.57±0.99b</td>
<td>6.20±0.99b</td>
<td></td>
</tr>
<tr>
<td>ROD</td>
<td>18.72±6.22a</td>
<td>68.79±6.22a</td>
<td>60.89±6.22a</td>
<td>62.82±6.22a</td>
<td>32.31±6.22a</td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>±6.22a</td>
<td>±6.22a</td>
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<td>±6.22a</td>
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</tr>
<tr>
<td>T-N</td>
<td>6.20±0.43a</td>
<td>5.43±0.43b</td>
<td>0.18±0.43a</td>
<td>3.05±0.43c</td>
<td>4.90±0.43b</td>
<td></td>
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<tr>
<td>K</td>
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<td>1.36±0.12a</td>
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<td>0.29±0.12a</td>
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<tr>
<td>P</td>
<td>7.18±0.16b</td>
<td>8.14±0.16a</td>
<td>0.14±0.16c</td>
<td>0.21±0.16b</td>
<td>6.17±0.16c</td>
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<tr>
<td>THFC</td>
<td>4.32±0.25a</td>
<td>3.32±0.25a</td>
<td>4.02±0.25a</td>
<td>4.28±0.25a</td>
<td>3.47±0.25a</td>
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</tr>
</tbody>
</table>

[Mean values (mean±SE) in the same row with different superscript are significantly different (P <0.05). TOC, Total dissolve solid; ROD, Rate of degradation; T-N, Total nitrogen; K, Potassium; P, Phosphorus; THFC, Total fungi count; CHF, cow hoof; FM, chicken feather; EM, egg membrane; HW, hair waste; and CH, cow horn]
in substrate 1 (6.84±0.12a) was significantly higher compared to substrates 5 (2.85±0.12f), 2 (1.36±0.12b), 3 (0.15±0.12d) and 4 (0.29±0.12d) whereas, the phosphorus content of substrate 2 (8.14±0.16a) was significantly (P <0.05) higher compared to all other substrates, 1 (7.18±0.16b), 5 (6.17±0.16c), 3 (0.14±0.16d) and 4 (0.21±0.16d). The results of microbial counts in each substrate reveals that there were significant differences (P <0.05). However, the difference is not significant recorded in substrates 1 (4.32±0.25a), 3 (4.02±0.25b) and 4 (4.28±0.25b); substrates 2 (3.32±0.25c) and 5 (3.47±0.25bc) had the lowest bacteria count.

**Weekly biological and physicochemical parameters during biocomposting**

The combination of bacterial and fungal decomposition of cow hoof, chicken feather, egg membrane, hair waste and cow horn temperature ranged from 29.5-29.6, 29.6-30.0, 29.6-30.2, 29.7-29.9 and 29.5-29.9ºC, respectively (Fig. 1A). There was a peak attainment in composting temperature of about 30ºC on the seventh week.

The pH values for substrates 1 to 5 ranged 6.8-7.4, 7.2-8.0, 7.7-8.3, 7.0-8.0 and 6.7-7.8, respectively (Fig. 1B). The range of total organic carbon using the combination of bacteria and fungi was from 2.20-4.06, 0.49-12.79, 2.15-18.02, 1.18-20.34 and 3.75-9.01 for cow hoof, chicken feather, egg membrane, hair waste and cow horn (Fig. 2). The rate of degradation using combination of bacteria and fungi ranged from 0 to 45.81, 96.7, 88.07, 94.20 and 58.38% for cow hoof, chicken feather, egg membrane, hair waste and cow horn (Fig. 3).

The range of total nitrogen using the combination of bacteria and fungi composting of substrates 1 to 5 ranged 4.15-9.72, 4.12-10.24, 0.08-0.35, 1.54-5.41 and 1.45-6.48%, respectively (Fig. 4A). The potassium concentration for cow hoof, chicken feather, egg membrane, hair waste and cow horn ranged 9.60-7.20, 1.13-1.30, 0.09-1.19, 0.27-0.30 and 1.25-6.8%, respectively (Fig. 4B). There was reduction of potassium content during the composting process. Total potassium was high in week 5 for cow hoof.

During microbial decomposition of substrates, phosphorus content inside the biocomposter for cow hoof, chicken feather, egg membrane, hair waste and cow horn using bacteria and fungi composting ranged 6.50-7.20, 8.20-8.51, 0.12-0.14 and 0.19-0.21%, respectively (Fig. 4C). There is an improvement observed during the composting process for all microbes used.

During decomposition of substrates, total bacteria count in the biocomposter of cow hoof, chicken feather, egg membrane, hair waste and cow horn
ranged 3.47-4.24, 3.75-5.31, 3.31-4.42, 2.06-6.7 and 2.5-4.4 × 10³ cfu/g for substrates 1-5, respectively (Fig. 5). There was an increase of microbial count in weeks 0 to 3 but declined in week 4 to 6 but then increased in the 7th week.

Discussion

In this study, the temperature range of substrates was elevated. Similar variations in temperature levels were found during keratin degrading experiments. There was a drop in temperature from week 4 to 6 which indicates depleting organic matters, lack of aeration inside the piles and enzyme production level by the decomposing microorganisms. There is usually an elevation of temperature during composting. Assandri et al. showed maximum temperature of 40 to 65.5°C while Nakasaki et al. had 40°C. Whereas, Jain et al. noted that keratinases from both bacteria and fungi activity exhibit under temperatures with a range of 45-70°C or even higher. This could be as a result of aerobic process, heat increase and heat retention capacity of the biocomposter because they are made of plastic which are poor conductor of heat, water vapor and the release of CO₂ during decomposition process. When microorganisms break down compost, they metabolic activities generate heat which increases the temperature of the composting materials. Although some were higher than others like substrates 3 (egg membrane) and 2 (chicken feather), which might be as a result of the surface area of the substrate.

The pH is carried out to ascertain acidic or alkaline nature of the compost. During composting, the pH is usually increased for faster decomposition. After composting pH values increase. From this study we discovered an increase in pH range. Observed pH value is increased due to degradation of keratin, similar trends were reported by Kumar et al. The initial pH value of composts was neutral in all treatments. During week 1 to 4 composting process, the pH values increased and decreased in weeks 4 to 7. Joshi et al. who compost chest nut burr/leaf, litter and poultry manure reported similar observations. Abdallah et al. found that it may be due to digestion of keratinaceous waste. At pH 6 to 9 and at 30-50°C, most keratinases are optimally active, bacteria producing keratinases by exhibiting a wide pH (5.8-11). This might be that the initial stage of composting produces more organic acids which make the compost pile more acidic as a result fungi grow better than bacteria. Bacteria produces organic acid and don’t perform well in a reduce pH because the acid will kill them and reduce their performance. These can also be attributed to the production of CO₂ from organic acids and loss of nitrogen. Furthermore, during the time of composting, compost temperature...
and pH were increased, which may be due to higher microbial activity.

The total organic carbon reduced during microbial composting, however, during the degradation process, total organic carbon content was reduced. It varied from 0.49 to 20.34%. Organic carbon loss was significantly affected by composting. The results not in line with Sekar et al.\textsuperscript{24} who recorded a range of 11.56-4.34%. There was a decrease in organic carbon throughout the week which indicates a decrease of microbial population decomposition of waste, high organic content means a corresponding increase in the growth of microorganisms that contribute to the lack of oxygen. Butnariu et al.\textsuperscript{25} have also shown that part of the carbon in the decomposing residues were assimilated by the microbial biomass and a part grows as CO\textsubscript{2}. Furthermore, carbon loss accounted for initial total carbon during the composting process\textsuperscript{26}. Similarly, Park & Kim\textsuperscript{27} have noted that organic matter particularly decreases at the initial stages of degradation. During composting, organic materials are mixed to create a moist, aerobic environment where organic matter decomposition and humification occur at rapid rates.

Additionally, there was also a corresponding reduction in percentage rate of degradation. This shows that the microbes were able to breakdown the substrates. Some substrates degraded faster than others depending on their physical properties. It was observed that the degrading ability was faster in feather meal. There is an increase in microbial population of the substrate with higher composting ability. The lowest degrading ability was observed in cow hoof and horn. This could be attributed to the hard nature of the substrate.

The total nitrogen was highest in cow hoof and feather meal. This might be as a result of the degrading method used. Similar result was observed by Chang et al.\textsuperscript{28} with a decreased of nitrogen content during composting. The nitrogen concentration decreases during composting; this may be caused by the slow degradation of organic substrate which contains amino sugars and proteins. Nitrogen is an essential component of amino acid; it is one of the important primary nutrients, which serves as the basic structural units of proteins\textsuperscript{29}. It is required for plant cell division and reproduction as a component of nucleic acids. Total nitrogen content measurement includes both organic and inorganic forms of nitrogen in compost. In mature composts, most of the nitrogen is in organic form.

Potassium in cow hoof was higher compare to other substrates. There was also an increase in potassium. Although, they had similar potassium increased during composting period. This might be due to an increase of potassium in the compost which is often due to degradation of organic cellular components. However, fibrous materials like, straw or wood chips which can absorb relatively large quantities of water and still maintain structural integrity and porosity which could prevent the loss of potassium from the compost formed\textsuperscript{30}. Potassium leaches easily because it is highly soluble in water. The insoluble potassium salts can be solubilized by the decomposition of the compost.

Phosphorous total concentration in feather meal was observed to be the highest in our present study similar to Wan et al.\textsuperscript{32} who have also observed higher total phosphorous in all the samples studied. Jalal et al.\textsuperscript{33} noted that total phosphorous content increased gradually during the process of composting and there is a decrease with humification of water solubility of phosphorous because phosphorous solubility during the decomposition was subjected to further immobilization factor. This is not in accordance with our observation, where only feather meal showed increase in phosphorous content.

Feather meal and cow horn had the lowest count using the combination of bacteria and fungi. The microbial count is very essential in that the population of microbes will determine the rate of decomposition. There was a continuous increase of microbial count for hair waste. It was observed that the use of bacteria and fungi inoculants enhanced the rate of substrate composting.

**Conclusion**

The study revealed that composting with both bacteria (\textit{Bacillus subtilis}) and fungus (\textit{Fusarium sp.}) resulted in the maximum degradability after the 7\textsuperscript{th} week of composting. Furthermore, it has been categorized as environmentally safe, offers moderate reaction conditions as well as low-cost in production. Degradation employing microorganisms may give a feasible option for enhancing their usage. The above demonstrated microbial process of degradation can be used for feedstuff preparation as well. More research is needed to understand the mechanism of action of feather degradation and other non-conventional protein sources utilized to develop an economic approach for large-scale processing.
Conflict of interest
Authors declare no competing interests.

References


