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Fertilizer and sanitary quality of digestate biofertilizer from the co-digestion of food waste and human excreta

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ABSTRACT

This research was aimed at assessing the fertilizer quality and public health implications of using digestate biofertilizer from the anaerobic digestion of food wastes and human excreta. Twelve (12) kg of food wastes and 3 kg of human excreta were mixed with water in a 1:1 w/v to make 30-l slurry that was fed into the anaerobic digester to ferment for 60 days at mesophilic temperature (22–31 °C). Though BOD, COD, organic carbon and ash content in the feedstock were reduced after anaerobic digestion by 50.0%, 10.6%, 74.3% and 1.5% respectively, nitrogen, pH and total solids however increased by 12.1%, 42.5% and 12.4% respectively. The C/N ratios of the feedstock and compost are 135:1 and 15.8:1. The residual total coliforms of 2.10 \times 10⁸ CFU/100 ml in the digestate was above tolerable limits for direct application on farmlands. Microbial analysis of the digestate biofertilizer revealed the presence of Pseudomonas, Klebsiella, Clostridium, Bacillus, Bacteroides, Penicillum, Salmollena, and Aspergillus. Klebsiella, Bacillus, Pseudomonas, Penicillum and Aspergillus can boost the efficiency of the biofertilizer through nitrogen fixation and nutrient solubility in soils but Klebsiella again and Salmollena are potential health risks to end users. Further treatment of the digestate for more efficient destruction of pathogens is advised. 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Research into the development of alternative energy sources has been increasing as a result of the non-renewable nature of fossil energy sources and recent environmental challenges ([Alburquerque et al., 2012](#page-5-0)). Production of biogas through anaerobic digestion of organic waste materials is on the frontline of this alternative energy research. The major products of anaerobic digestion are biogas and digestate. Digestate comprises microbial biomass, semi-degraded organic matter and inorganic compounds, and therefore can be used as soil conditioners on farmlands ([Alburquerque et al., 2012\)](#page-5-0). It contains more readily available nutrients than the undigested products which make it better for crops fertilization [\(Goberna et al., 2011; Garfi et al.,](#page-5-0) [2011; Lansing et al., 2010\)](#page-5-0).

Large scale use of chemical fertilizers has resulted in soil quality and environmental degradation, eutrophication, and heavy metals pollution ([Owamah, 2013; Zhu et al., 2012](#page-5-0)). The importance of

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biofertilizer therefore is to provide socioeconomic and ecological benefits among which are improvements of soil quality, food quality and safety, human and animal health as well as environmental quality ([Johansen et al., 2013; Mohamed et al., 2009](#page-5-0)). There are different types of digestate biofertilizers and their differences are mainly in the raw materials used, forms of utilization, the source of microorganisms, and digester configurations, etc. [\(Garfi et al.,](#page-5-0) [2011; Higa and Parr, 1994\)](#page-5-0). The use of digestate biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and are renewable sources of plant nutrients for sustainable agriculture [\(Grigatti et al., 2011; Tamil](#page-5-0) [Nadu Agricultural University, 2008](#page-5-0)).

Anaerobic digestate usually contains microorganisms like Pseudomonas, Klebsiella, Samonella, Penicillum, Shigella, Bacteriodes, Aspergillus and Bacillus. These microorganisms can be exploited in the production of biofertilizers ([Tamil Nadu Agricultural](#page-5-0) [University, 2008\)](#page-5-0). Klebsiella and Clostridium spp. are free living nitrogen fixing biofertilizers while Bacillus and Pseudomonas spp. are phosphate solubilizing biofertilizers ([Alfa et al., 2014\)](#page-5-0). These organisms quicken the microbial processes in the soil and increase the availability of nutrients that can be assimilated by plants ([Tamil Nadu Agricultural University, 2008](#page-5-0)). Biofertilizers hold

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great promises for improving world food security through the enhancement of agricultural yield in developing continents such as Africa and Asia, which together hold approximately 50% and 74% of the total land mass and population of the globe, respectively ([Population Reference Bureau, 2012\)](#page-5-0).

Unlike chemical fertilizers, digestate biofertilizers can be cheaply produced through anaerobic digestion anywhere, utilizing a wide range of raw materials including agro, commercial and domestic wastes. Population growth and rising living standard have led to a great increase in food waste generation ([Curry and](#page-5-0) [Pillay, 2012\)](#page-5-0). Sewage sludge has also been predicted to increase continuously in the next decade as a result of increasing population connected to sewage networks ([Dai et al., 2013\)](#page-5-0). Direct landfiling of food wastes has created various problems such as putrid smell and leachate pollution of ground and surface waters ([Ming et al., 2008](#page-5-0)), and incineration has also been restricted due to its generation of greenhouse gases ([Donald, 1988](#page-5-0)). Anaerobic digestion as a sustainable waste treatment technology transforms organic matter into biogas and reduces the amount of pathogens in digestates [\(Martínez et al., 2012](#page-5-0)).

The demand for digestate biofertilizer is dependent on compliance with quality standards ([Alburquerque et al., 2012\)](#page-5-0). Though the use of digestate biofertilizer to increase agricultural food production, and soil improvement has been established, its safety as determined by the amount of pathogens contained is still of public health concern to end users ([Alfa et al., 2014](#page-5-0)). Reports on the fertilizer and sanitary quality of digestate from anaerobic digestion are scanty in scientific literature, despite the large volume of literature on biogas yield from various substrates. However, the fertilizer potential of digestate from farm and agro-industrial residues was investigated by [Alburquerque et al. \(2012\)](#page-5-0). [Johansen](#page-5-0) [et al. \(2013\)](#page-5-0) have also reported that digestate biofertilizer increases soil microbial community. [Alfa et al. \(2014\)](#page-5-0) have assessed the biofertilizer quality of digestate from the digestion of cow dung and chicken droppings. The properties of guinea pig manure digestate were reported by [Garfi et al. \(2011\).](#page-5-0)

Despite the numerous benefits of digestate biofertilizer to agricultural production, the relative abundance and ease of generation of chosen substrates within the particular region of proposed usage should also be given due consideration, inorder to meet with demands. Food wastes and excreta are among the most common wastes generated in Nigeria and are carelessly disposed into the environment to constitute public health risk. The objective of this research therefore is to assess the biofertilizer and sanitary quality of the digestate resulting from the mesophilic anaerobic co-digestion of food waste and human excreta.

2. Materials and methods

2.1. Digester design

A 40-l-biogas reactor of height 0.5 m and diameter 0.25 m was fabricated from galvanized steel. Galvanized steel was used as building material because of its strength and durability in acid or basic environment. Five different holes were bored on the lid of the digester for insertion of temperature and pH probes using threaded steel adapters and rubber stoppers to avoid gas leakage. The cylindrical shape was adopted to enhance better mixing. The tank was air tight and was clearly placed above the ground level where it was exposed to sunlight for partial heating. A 12.1 L gas holder tanks each of height 0.25 m and diameter 0.25 m were fabricated from thin sheet metal and was used to temporarily store the biogas until it was used to produce heat or used to replace or supplement the supply of cooking gas. Plastic hose was used to connect the digester to the gas collection system and the biogas stove burner while plastic valves were installed to control the gas flow. The gas holder stores the biogas and allows the volume of biogas produced to be measured through the indirect measurement of a liquid column height. The digester and gas holder were designed, built and operated by the methods described in [\(Fountoulakis et al., 2008; Karki, 2002\)](#page-5-0) with slight modifications. The composition of biogas ($CH₄$ and $CO₂$ contents) was determined using a gas chromatography (GC) (Hp 5890, Avondale, USA). Biogas composition measurement was taken two times a week in duplicate from each digester. A 100 µl gas tight syringe was used to take biogas samples from the digesters head space after releasing the gas. This was followed by injecting the biogas sample into the GC [\(Owen et al., 1979; Zhang et al., 2006\)](#page-5-0). The schematic of the setup is as shown in [Fig. 1.](#page-2-0)

2.2. Feedstock and materials

Carbohydrate food wastes (boiled rice, boiled cassava products, bread, boiled yam and boiled maize), human excreta, a forty litre size anaerobic digester, pH meter (HI 9024-C, Hanna Instruments, Smithfield, RI, USA), thermometer (HI 98517, Hanna Instr.), anaerobic jar (Oxoid), gas generating kit (Bio-oxoid), different media (Nutrient agar, Potato dextrose agar, MacConkey agar, Eosin methylene blue agar, and Fastidious anaerobic agar) were the materials used in this study.

2.3. Sampling, physico-chemical analysis and experiment

Carbohydrate food wastes were collected from a university cafeteria in two batches (10 am in the morning and 7 pm in the evening) and sorted out for ease of pre-treatment. The periods of collection were selected to approximately match the periods of either peak consumption or defecation. The food wastes were thoroughly homogenized using a blender (BLG-401-18N) to achieve minimal particulate size suitable for easy digestion. After this, they were seeded with the human excreta which have also undergone thorough mixing. The mixture was a combination of 12 kg of food wastes and 3 kg of human excreta serving as an easy source of microbes. This was further mixed with water in a 1:1 w/v to make approximately 30-l slurry. The feedstock was fed into the digester (the digester was not in operation before the fermentation experiment) and the fermentation process lasted for 60 days. Parameters monitored and or determined during the fermentation are: (a) daily recording of volume of gas produced, (b) the temperature of the digester content was taken twice daily, (c) the pH of the digester content was taken weekly, (d) weekly collection of samples for the isolation and assessment of the microbial population causing the bio-conversion at different stages, (e) analysis of the gas to separate it to its different components and (f) physico-chemical analysis of the digestate at the end of the experiment.

After the 60 days retention period, the slurry was removed from the digester, dewatered by filtration, using geo-textile tubes and cured for 20 days to form compost. This was then applied to a demonstration farmland for the cultivation of maize and vegetables. However, the experiment on the effect of the cured digestate on the growth and yield of the maize and vegetables is still on going. The physico-chemical characteristics of the feedstock and the digestate were evaluated before and after fermentation respectively using standard procedures ([Owamah et al., 2013;](#page-5-0) [APHA, 2012](#page-5-0)). The physiochemical parameters analyzed include pH, temperature, organic carbon, moisture content, total solids, total nitrogen, ash content, biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Three replicates were used and the mean values of the parameters recorded. Mesophilic fermentation was preferred to thermophilic as it has been reported

Fig. 1. Schematic view of the digester set up.

that most of the nitrogen fixing and phosphate-solubilizing microorganisms survive best at mesophilic temperature range ([Ghazifard et al., 2001\)](#page-5-0). The digestate was cured for 20 days at the prevailing ambient temperature of 31 $^\circ\textsf{C}$ inoder to form a simple compost of the digestate. Constituent elements of the compost were determined using standard methods [\(APHA, 2012\)](#page-5-0).

2.4. Isolation of mesophilic microbes

Microbial population of the feedstock and digestate was enumerated by standard plate count. Potato Dextrose agar (PDA) plus Chloramphenicol was used for fungi while MacConkey agar, Fastidious Anaerobic agar, Eosin Methylene Blue (EMB) agar, and Nutrients agar plates were used for bacteria enumeration. Incubation of MacConkey, EMB and Nutrient agar plates was done for 24–48 h at 37 °C. PDA plates were incubated within 3–5 days at room temperature. The incubation of Fastidious Anaerobic agar plates was done a 37 °C for 7 days in an anaerobic jar (Oxoid) that contained a moistened pack of gas generating kit (Bio-oxoid). Purification and identification of individual colonies was done by morphological and biochemical techniques ([Jolt et al., 1994\)](#page-5-0). Isolates of fungal were identified by the microscopic and macroscopic features of the hyphal mass, nature of the fruiting bodies, morphology of cells and spores, among other criteria ([Tsuneo, 2010](#page-5-0)). Details of the isolation methods are contained in ([Dahunsi and Oranusi, 2013](#page-5-0)). Three counts were used to determine each mean value reported in this study.

3. Results

From the physicochemical characteristics of the feedstock and the resultant digestate as shown in Table 1, the amount of BOD, COD, organic carbon, and ash content in the feedstock was found to be reduced by 50.0%, 10.6%, 74.3% and 1.5% respectively. Again, there was an increase in total solids (by 12.4%), total suspended solids (by 12.6%) and nitrogen content (by 12.1%) of the digestate when compared with the original feedstock. The carbon/nitrogen ratio of the feedstock was 139:1. Fig. 2 gives the mean daily records

Table 1

The physico-chemical parameters of the digester feedstock before and after digestion.

Fig. 2. Temperature changes during the anaerobic digestion.

of temperature during the anaerobic digestion. The temperature remained at mesophilic range throughout the study. The lowest temperature reading of 22 °C was obtained on the 49th, 57th and 58th day while the highest of 31 \degree C was obtained on the first day of the digestion process. The pH of the anaerobic digestion fluctuated between 4.5 and 6.5 [\(Fig. 3\)](#page-3-0) with higher biogas production occurring when pH approached the neutral status. The mean microbial count in the feedstock before anaerobic digestion was 2.4 \times 10¹⁰ for coliform, 2.0 \times 10¹² for total aerobic plate and 1.9×10^8 for fungal. The mean microbial count for the biofertilizer digestate was 2.0 \times 10 8 for coliform, 1.0 \times 10 4 for total aerobic plate and 2.0×10^3 for fungal. The microbial population found in the feedstock includes species of Escherichia, Citrobacter, Bacillus, Pseudomonas, Proteus, Klebsiella, Clostridium, Bacteroides, Enterobacter, Staphylococcus, Salmonella, Streptococcus, Aspergillus, Mucor, Rhizopus and Penicillium while those isolated from the biofertilizer digestate include species of Pseudomonas, Klebsiella, Clostridium, Bacillus, Bacteroides, Penicillium and Aspergillus, Salmonella [\(Table 2](#page-3-0)). The minimum and maximum biogas production of 200 cm^3 and 6000 cm^3 occurred on the 60th and 23rd day of the anaerobic digestion, respectively ([Fig. 4](#page-3-0)).

The average percentage distribution of the microflora of the digester feedstock during the period of digestion is shown in

Number of replicates $n = 3$; ±standard deviation.

Fig. 3. pH changes during the anaerobic digestion.

Fig. 5. The cumulative total volume of biogas generated within the 60 days retention period is 85,200 cm³. The gas chromatography analysis of the biogas revealed CH₄ to be 58%, CO₂ 24% and other impurities 19%. Table 3 shows the properties of the compost obtained from the digestate and indicates a modification in pH. From Table 3, the carbon, nitrogen and phosphate contents of the compost were found to be 37.91%, 2.49% and 3.49% respectively. The C/N ratio and pH of the resulting compost are 15.9:1 and 7.2. Curing improved pH, carbon and nitrogen contents as found in the composted digestate (Table 3) in comparison to the uncured digestate (Table 2). The mean microbial count per week of the species of microorganisms found in the digester during the anaerobic digestion, shows that the methanogens had the least growth rate over the retention period ([Table 4\)](#page-4-0).

4. Discussion

The 50.0% reduction in BOD, 10.6% in COD, 74.3% and 1.5% in organic carbon and ash content in the digestate when compared to the feedstock could be traced to the biodegradation of the organic matter in the substrate due to the activities of mesophilic microorganisms and the high initial C/N ratio of the feedstock ([Yun et al., 2000\)](#page-5-0). Rapid and entire humification of a substrate essentially depends on its initial C/N ratio [\(Beck-Friis et al., 2001\)](#page-5-0). The 10.6% reduction in COD is in agreement with the report of [Wei et al. \(2011\)](#page-5-0) in which a high COD removal from the supernatant of hydrothermally treated municipal sewage sludge by upflow anaerobic sludge blanket reactor was obtained. The two groups of bacteria isolated from the digester during the anaerobic digestion include the acid-formers (Bacillus, Escherichia, Clostridium, Klebsiella, Proteus and Bacteroides) and a methane former Methanococcus species. The correct balance between these two groups of microorganisms determines the successful operation of anaerobic digesters for biogas production. The methane formers

Table 2

Microbial counts of feedstock and digestate biofertilizer.

Fig. 5. Percentage distribution of microorganisms in the digester.

Number of replicate conuts $n = 3$; ±standard deviation.

however multiplied at a slower rate than the acid formers and were found to be very sensitive to environmental changes, such as pH ([Table 4\)](#page-4-0). Fungal isolates include Aspergillius, Rhizopus, Penicillium and Mucor; their major source was the human excreta. [Pritchard](#page-5-0) [et al. \(2009\)](#page-5-0) reported a similar result when they isolated Escherichia coli, Aspergillius, Clostridium botulinum, Clostridium chavoie from water contaminated by human excreta in Malawi.

Number of replicate conuts $n = 3$; \pm standard deviation.

Table 4 Mean microbial count per week ($\times 10^6$ CFU/ml).

Week	Aerobes	Anaerobes	Fungi	Methanogens
	1.4	0.6	0.6	
2	1.6	0.8	0.8	
3	1.9	0.8	0.7	
4	1.1	0.8	7.0	
5	0.9	0.9	0.8	
6	1.0	0.9	0.9	
7	1.2	9.0	0.7	0.5
8	1.4	9.0	0.7	0.7
9	1.5	1.2	0.9	0.9

There was an increasing trend in the aerobic count within the first three weeks of the anaerobic digestion possibly due to the richness of the digester feedstock in carbon, providing nutrients for the micro-aerophillic organisms to utilize (Table 4). This may also be due to the acidic nature of the feedstock over the first three weeks which supports the proliferation of acid-producing organisms. The observed increase in fungal isolates over the weeks is in contrast with fungal general physiology and metabolism which is known to be purely aerobic and therefore calls for further research. The methanogenic bacteria were the least populated in the digester representing 8% [\(Fig. 5\)](#page-3-0).

The pH data obtained ([Fig. 3](#page-3-0)) shows an initial fall to a more acidic level before assuming stable values toward neutrality. By the 4th week, a pH of 6.4 was obtained and thereafter remained within 6.0–6.5 throughout the fermentation period thus accounting for the scanty population of the methanogens, which could have contributed to the reduction in gas production in the latter period of the anaerobic digestion. The initial drop in pH is important since the activities of aerobes and facultative aerobes are essential to produce relevant acidic metabolites, which are later acted upon by methanogenic bacteria to produce methane. Methanogenesis is known to occur best within a pH range of about 6.0 and 7.8. In the present study maximum biogas production corresponds with pH 6.4 of the 4th week ([Figs. 3 and 4\)](#page-3-0). This is inline with the report of [Alkan-Ozkaynak and Karthikayan \(2011\)](#page-5-0) where the highest biogas yields were observed at pH 8. The observed increase in pH could have contributed to the reduction in pathogens in the biofertilizer digestate as most pathogens cannot tolerate high pH levels. [Yun et al. \(2000\)](#page-5-0) have also reported that a large amount pathogen is destroyed by the metabolic heat generated by microorganisms during anaerobic digestion. Temperature was observed to maintain mesophilic range (22–31 °C) throughout the period of the anaerobic digestion indicating that the biofertilizer can be produced within such temperature range ([Fig. 2](#page-2-0)).

There were increases in nitrogen content (12.1%), total solids (12.4%) and total suspended (12.6%) after the anaerobic digestion ([Table 1\)](#page-2-0). The physicochemical analysis of the compost [\(Table 3\)](#page-3-0) shows that the compost had nitrogen (2.4%) and phosphate (3.49%). While nitrogen is needed by plants for vegetative growth and enzymatic reactions, phosphate is required for seed production and root development. The nitrogen in the compost was mainly ammonium nitrogen and could be lost by ammonia volatisation. The storage and application of the composted digestate should therefore be carefully controlled to prevent negative environmental impacts.

Species of bacteria and fungi isolated from the biofertilizer digestate include Pseudomonas, Klebsiella, Clostridium, Bacillus, Salmolena, Bacteroides Penicillum and Aspergillus. Klebsiella and Clostridium species are known to be free-living nitrogen-fixing organisms ([Tamil Nadu Agricultural University, 2008](#page-5-0)). The presence of these organisms in the biofertilizer would enhance the fertility of soil for crop production [\(Tamil Nadu Agricultural](#page-5-0) [University, 2008\)](#page-5-0). Bacillus and Pseudomonas species are phosphate

solubilizing biofertilizers. Bacillus species also act as solubilizers for trace elements like silicates and zinc as well as plant growth promoters. Pseudomonas species are known for promoting plant growth. Species of Aspergillus and Penicillum are also phosphate solubilizing fungi ([Alfa et al., 2014\)](#page-5-0). The presence of all these organisms makes the digestate useful as biofertilizer. Biofertilizers are not only suitable for use as soil conditioners and fertilizers, but can also suppress soil-borne and foliar plant pathogens ([Hadar and](#page-5-0) [Mandelbaum, 1992](#page-5-0)).

The mean microbial count revealed decreasing trend in total coliform, total aerobic plate and fungal in the biofertilizer digestate as against their higher values in the feedstock ([Table 2\)](#page-3-0). This agrees with ([Shu-Hsien et al., 2007\)](#page-5-0) that microbial population has a tendency to decrease within the first seven days of anaerobic digestion due to acidic environment and then remains steady during the biofertilizer preparation. Though anaerobic digestate can be used to efficiently improve the fertility of soil and boost crop production, its safety still remains a source of concern to end users due to pathogens ([Alfa et al., 2014\)](#page-5-0). In this study, anaerobic digestion was found to reduce the microbial load in the digestate but the residual total coliform content of 2.10×10^8 CFU/100 ml is however above tolerable limits for use as direct fertilizer on farmlands ([Tsuneo, 2010; Yun et al., 2000\)](#page-5-0). Similar reduction in total coliforms after anaerobic digestion was reported by [Goberna et al. \(2011\).](#page-5-0) The presence of Salmonella and Klebsiella spp. in the digestate calls for concern in its use on farmlands. Salmonellae are known pathogens and could be transmitted to man and animals via contaminated food, feed and water [\(Chen et al., 1998\)](#page-5-0). Klebsiella spp. have been reported to be infectious to humans amidst its usefulness as nitrogen fixing bacteria in a biofertilizer ([Chin et al., 1999; Nakasaki et al.,](#page-5-0) [1993\)](#page-5-0). The presence of Salmonella spp., Klebsiella spp. and total coliforms only suggests that the digestate may not be safe for direct application as fertilizer for crops that are eaten raw without further treatment of the digestate. This however does not discourage the use of digestate biofertilizer for improvement of soil fertility but implies that care should be taken in the use of digestate biofertilizer to safeguard public health.

[Fig. 4](#page-3-0) is the graph of the daily gas production; the production started on the 9th day of fermentation with 600 cm^3 of biogas and followed an increasing trend. It reached its peak (6000 cm^3) on the 23rd day before a gradual fall in production rate was recorded for the rest of the study period. The least volume of biogas (200 cm^3) was obtained during the last four days of the experiment. The fertilizer quality of the digestate biofertilizer obtained in this study is comparable to those reported earlier using pure microbial inocula of phosphate solubilizing Aspergillus species ([Tiquia and Tam, 2002; El-Azouni, 2008](#page-5-0)). The 20 day curing period for the compost preparation led to improvements in the pH from 6.46 to 7.2 and in the C/N ratio. The pH and C/N ratio $(15.8:1)$ of the compost obtained in this study are good for crop production as the uptake of available nutrients by plants is enhanced at these pH and C/N ratio ([Hartmann et al., 2002](#page-5-0)). It is therefore advisable to convert digestate from anaerobic digestion to compost by curing before utilization as biofertilizer.

5. Conclusion

Digestate biofertilizer from the anaerobic digestion of food wastes and human excreta can be used to improve soil fertility. Results from this study show significant reductions in BOD, COD and organic carbon content in the digestate when compared to the feedstock. Temperature was observed to maintain mesophilic range throughout the period of digestion indicating that the biofertilizer can be produced at such temperature. The presence of nitrogen fixing and phosphate solubilizing organisms in the digestate shows that it could be utilized as an efficient biofertilizer for crop production. The mean microbial count revealed decreasing trend for total coliform, total aerobic plate, and fungal in the digestate as against their higher values in the feedstock. However, the presence of Salmonella ., Klebsiella and total coliforms in the digestate suggests that it may not be safe to apply the digestate as fertilizer without further treatment. Because of the easy loss of ammonia nitrogen to volatisation, the storage and application of the composted digestate should be carefully controlled to prevent negative impacts on the environment. This study recommends longer retention period of 90 days (mesophilic) and shorter retention period of 30 days (thermophilic) for a better quality biofertilizer than obtained in this study in terms of pathogens destruction.

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