Determination of the Bacterial Compositions in the Bio-Drying Process of Sewage Sludge from Wastewater

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Abstract

The aim of this study is to determine the bacterial compositions during the bio-drying process of sewage sludge. Bio-dried products and sewage sludge were randomly collected from the Wupa wastewater/sludge treatment plant in Idu Industrial area Abuja, Federal Capital Territory, Nigeria. A mixture of sewage sludge and sawdust that were bio-dried and cured before this experiment were composition of the bio-dried product. The bacterial communities were analyzed in the laboratory using Pour Plate Technique to examine the total bacterial present in the sewage sludge bio-drying material (SSBM) and the bacterial were later isolated using other techniques like Oxidase test, Catalase test, Grams Staining technique and Spread Plate Technique. Evaporation of free water and water formation was determined by humidity gauge and moisture-oven drying method. From the results obtained, *Acinetobacter* was the most abundant bacterial during the initial and final thermophilic phases (43% and 37%) respectively. *Bacillus* was the most abundant amid the cooling stage (53%). The water evaporation and water generation rate were credited to the degradation of organic content of the SSBM and heat produced by bacterial activities. From this study, there is a clear indication that the bacterial density grades that increase the temperature of the SSBM during the bio-drying process reshaped the bacterial communities.

1.0 Introduction

Sewage sludge can be described as an unavoidable semi-solid residual by-product produced during the treatment of industrial or municipal wastewater. Compositions of sewage sludge vary significantly based on wastewater constituents and the treatment methods being adopted. According to Wei et al, 2003, most of the wastewater treatment processes in use turn out a sludge that must be disposed of due to many reasons ranging from the environmental challenge, legal rules, land shortages, increasing cost, and public concern. The state of ecosystems on which all living organisms rely is also affected by the quality of water in nature (Chukwuma et al, 2016). Proper wastewater treatment is required to purify the water used by people for a variety of uses. All human activities, including indiscriminate waste discharge from industries such as effluents into waterways, contribute to water pollution, particularly of surface water (Ubah et al, 2021).

During water treatment process, sewage sludge is created in the septic tank after fresh sewage or wastewater enters a primary settling tank, after which approximately 50% of the suspended solid matter settles out in the settling tank. This assortment of solids is followed by anaerobic and the sludge becomes putrefying a little time which must be taken off from the settling tank before this process takes effect. Dewatering accounts for 49 to 53 percent of overall operating costs in Sewage Sludge management (Murray et al, 2008; Hospido et al, 2010), and poor treatment may result in re-pollution due to the enormous concentrations of toxins in Sewage Sludge (Poulsen and Bester, 2010; VerBerkmoes et al, 2009).

The effectual management and handling of these products have then turned into one of the most significant problems of our generation due 'to concerns regarding the preservation of lifestyles, environmental protection and the promotion of public health' (Ogwueleka and Balogun, 2020). Traditionally, wastes are only managed when the burdens to resolve the problem is more than the expediency of disposal (Seadon, 2010). As such, sustainability of Municipal waste management is among the factors that have not been met efficiently as observed in recent studies that have studied and reviewed waste management systems around the globe. Sewage sludge is composed of inorganic and organic matters, with monumental concentrations of some plant nutrients, abundant smaller concentrations of various plant nutrients and organic chemicals, and a few pathogens.

2.0 Material And Methods

Study area

The study location is the Wupa Sewage Treatment Plant, which is located close to the WUPA River in Abuja, Federal Capital Territory, Nigeria, within the IDU-Industrial Area WUPA River on coordinates 7°20'N, 9°20'E. It covers an area of 297,900 square meters (Source: Wupa Treatment Plant) with a surrounding rural settlement planned metropolis that was primarily built in the 1980s to replace Lagos, the country's most populated city, as the country's capital on December 12, 1991. Sewage sludge was collected from the Wupa wastewater and sludge treatment plant, Idu, Abuja. The city of Abuja is rated among the ten most populous cities according to United nation's estimate since it has a population of 776,298 people as of 2006. Sawdust was acquired from woodwork factories in Dei –Dei Timbre market Abuja while Bio-dried product was collected from the Wupa sewage treatment plant. Quality assurance procedures and precautions considered include; careful handling of samples and experimental equipment. The map showing the study area is as in Figure 1.

Materials

The Wupa wastewater/sludge treatment plant in the Idu Industrial District of Abuja, Federal Capital Territory Nigeria, was randomly sampled for bio-dried products and sewage sludge. A Timber mill in Deidei, Abuja provided the sawdust. The bio-dried product is made up of a mixture of sewage sludge and sawdust that has been biodried and cured prior to the experiment. The materials used were sawdust (SD) and bio-dried product (BDP). The bulk agents were sawdust (SD) and bio-dried product (BDP) (BA). Sawdust (SD) and bio-dried product (BDP) were the bulk agents (BA). The sewage sludge bio-drying material was made by combining 3:2:1 volumes of sewage sludge (SS), bio-dried product (BDP), and sawdust (SD) (Cai et al, 2016). The moisture content, bulk densities, and volatile solids (VS) content of the SS, SD, BDP, and SSBM respectively, were measured.

Experimental Procedure

To form a heap, the mixture (SSBM) was placed in a fermentation tank. The bio-drying heap was 1.10m in height and an unsealed cylinder with an internal diameter of 1.13m and a cross-sectional area of 1.0m² (Cai et al, 2016) made of metal sheet, was installed vertically within the heap to minimize the interference caused by external factors. The volume of the heap for the experiment was measured in kg.

To collect data for water computation, a moisture gauge and temperature were installed in the center of the cylinder 0.30m from the top to the heap surface at a depth of 0.30m from top to bottom. The amount of air aerated into the material was measured using an airflow gauge mounted in the ventilation duct.

During the 20-day bio-drying period, the material was mechanically turned four times and aerated by an air blower through the air chamber beneath the heap (d9, d12, d15, and d18).

Legend for the Research Design:

Moisture gauge – Mini LCD digital moisture meter

Thermometer – Soil thermometer

Unsealed cylinder – Made of metal sheet

Fermentation tank – Made of metal sheet

Air flow meter – HHIP 7600 – 0185; Air flow regulator gauge

Air blower –

- Pressure of air from the blower – 120Psi

- Amount of air aerated – 8.44kg/cm²

Research Design

Sample Collection

During the mesophilic stage (collected on d0 and d2 at different temperatures), Samples collected for analysis were sewage sludge (SS), bulk agent (BA), and sewage sludge bio-drying material (SSBM). All of the temperatures were recorded at the time of collection. The initial thermophilic stage (collected on d6), the secondary thermophilic stage (collected on d16), the cooling stage (collected on d18) and the final product (collected on d20)

Bacterial analysis

Before and during the experiment, the total bacterial presence in the sewage sludge was examined using the Pour Plate Technique. Later, the bacteria were isolated utilizing the Oxidase, Catalase, Grams staining, and Spread Plate techniques.

Water analysis

A Humidity gauge and Moisture-Oven drying were used to assess water evaporation and formation. The oven-dried samples were dried for 24 hours at 105°C.

Organic content analysis

By assessing the volatile solids (VS) contents of the sewage sludge bio-drying material, the organic contents of the sewage sludge bio-drying material were examined as reported by (USADCC, 2001).

SSBM- Sewage Sludge Bio-Drying Material

Standard Used

Test methods for the examination of Compost and Composting (TMECC), 2001. US Composting Council.

Safety Measures

During the experiment, the following precautions were taken: Rubber hand gloves were used to prevent against direct contact with the sewage sludge, and hand washes with soap and disinfectants were performed on a regular basis.

Sample Collection

Samples collected for analysis were sewage sludge (SS), bulk agent (BA), and sewage sludge bio-drying material (SSBM) all through the mesophilic stage (collected on d0 and d2 at a different temperature), the initial thermophilic stage (collected on d6), the secondary thermophilic stage (collected on d16), the cooling stage (collected on d18) and the final product (collected on d20). All the temperatures at the time of collection were recorded respectively.

Bacterial analysis

Pour Plate Technique was used to examine the total bacterial present in the sewage sludge before and during the experiment. The bacterial were later isolated using Oxidase test, Catalase test, Grams staining technique, and Spread Plate technique.

Water analysis

Water evaporation and formation were determined by a Humidity gauge and Moisture – Oven drying. The oven-dried samples were dried at 105°C for 24 hours.

Data computation

During the sludge bio-drying process, water evaporation (WE) represent the total water output, while the water formation (WF) i.e. water produced by microbial metabolism and the aeration water input (AWI)

make up the water input (WI). The difference between WI and WE equals the apparent moisture reduction (AMR), (Cai, et al, 2001)

Organic content analysis

The organic contents of the sewage sludge bio-drying material were analyzed as described by (USADCC, 2001), by determining the volatile solids (VS) contents of the sewage sludge bio-drying material.

Standard Used

Test methods for the examination of Compost and Composting (TMECC), 2001.

US Composting Council.

Safety Measures

The following precautions were taken during the experiment:

- Rubber hand gloves were used for protection against direct contact with the sewage sludge.
- Hand washes with soap and disinfectants were constant throughout the experiment.

3.0 Results And Discussion

The results of the study are presented and discussed considering the objectives of the study which is to determine the bacterial compositions in the bio-drying process of sewage sludge from wastewater

Raw Materials

The Tables of values below are the characteristics of Raw materials, Sieve analysis of sawdust and Micro Organism present in the mixture (SSBM) before experiment.

Total Weight of Mixed material (SSBM) = 1,408.5kg

Table 1
– Characteristics of Raw Materials (In situ) before experiment

Material	Moisture Content (%)	Volatile Solid Content (%)	Bulk Density (g/cm ³)	PH
Sewage Sludge (SS)	78	69.80	1	8.5
Saw Dust (SD)	36	77.80	0.20	
Bio – Dried Product(BDP)	19	64	0.30	
Mixture (SSBM)	63.10	67	0.67	

Table 2 – Sieve Analysis of Saw Dust

Total Weight of Sample 200g

B.S Sieve Size	Weight Retained (g)	Percentage Retained (%)	Total Passing (%)
12.5mm	-	-	100
10.0mm	0.1	0.05	99.9
6.3mm	-	-	-
5.0mm	3.7	1.9	98.1
3.35mm	-	-	-
2.36mm	9.0	4.5	95.5
1.18mm	57.5	28.8	71.2
600µm	91.6	45.6	54.4
425µm	124.1	62.1	37.9
300µm	156.2	78.1	21.9
212µm	176.5	88.3	11.7
150µm	189.7	94.9	5.1
75µm	199.8	99.9	0.1

Table 3 – Micro Organism present in the Mixture (SSBM) before experiment

Micro Organism	Micro – Biological Density (cfu/g)
Mesophilic Bacteria	483×10^4
Thermophilic Bacteria	2.50×10^4
Fungi	8.0×10^3
Clostridium Perf	60.0×10^2
Enterobacteria CA	300×10^2
Salmonella	Nil

Bacterial Communities

Table 4
– Density of Bacterial Communities during the 20 day's bio – drying Process

Samples	Phase	Temperature When collected (⁰ C)	Mesophilic Bacterial (cfu.10 ⁴ /g)	Thermophilic Bacterial (cfu.10 ⁴ /g)
D0	Mesophilic	26.7	483	2.50
D2	Mesophilic	27.5	825	35.10
D6	Thermophilic	41.9	201	159
D16	Thermophilic	59.7	28	277
D18	Cooling	35	180	58
D20	Cooling	26.8	15	0.10

Table 5
Relative Abundance of Bacterial Communities present during the Bio –
drying process

Samples	Temperature (⁰ C)	Bacterial Present	Relative Abundance (%)
D0	26.7	Ferribacterium	8
		Flavobacterium	5
		Bacillus	1
		Acinetobacter	0.5
		Pseudomonas	0.4
		Clostridium Perf	3
		Enterobacteria	6
D2	27.5	Acinetobacter	28
		Bacillus	3
		Ferribacterium	0.7
		Clostridium Perf	4
		Enterobacteria	8
		Flavobacterium	8.5
		Others	47.8
D6	41.9	Acinetobacter	43
		Bacillus	4
		Pseudomonas	0.5
		Enterobacteria	5
		Others	47.5
D16	59.7	Acinetobacter	37
		Bacillus	7
		Pseudomonas	1
		Others	55

Samples	Temperature (⁰ C)	Bacterial Present	Relative Abundance (%)
D18	35	Bacillus	53
		Pseudomonas	5
		Acinetobacter	2
		Others	40
D20	26.3	Flavobacterium	10
		Bacillus	9
		Pseudomonas	5
		Others	76

Bacterial Density

The results showed that increasing the temperature of the material during the bio-drying process changed the bacterial communities (Fig. 10). The bacterial density was highest during the mesophilic phase, day two (D2), totaling 860.1 x10⁴cfu/g (mesophilic and thermophilic bacteria's) at a temperature of 27.5⁰c. At 41.9⁰C and 59.7⁰C, respectively, a decrease in total 360 x 10⁴ cfu/g and 305 x 10⁴ cfu/g (mesophilic and thermophilic bacteria) was seen during the thermophilic phase, day six (D6) and day sixteen (D16), demonstrating a selective effect of high temperature. Additionally, turning bacteria, including potential pathogens, promotes their death, and when there is no turning, the majority of microorganisms survive [USADCC, 2001). The bacterial diversity recovered when the temperature dropped below 59.7⁰C and the bio-drying process entered its cooling phase, but it was still lower than before the bio-drying. On days eighteen (D18) and twenty (D20), the bacterial diversity totaled 238 x 10⁴ cfu/g and 15 x 10⁴ cfu/g at temperatures of 35⁰C and 26.8⁰C, respectively (D20).

Relationships amongst Bacterial Communities

The composition of the bacterial communities showed distinct variation during the Sewage Sludge bio-drying as indicated in Table 6, Table 7, Figs. 11 and 12 (Cluster analysis), all known microbial communities were assembled into three groups. Considering all microbial communities, bacterial clustered in group 3 were highly abundant, group 2 were moderately abundant, and while the cluster in group 1 was of low abundance. Flavobacterium, Clostridium Pelf, Ferribacterium, Enterobacteria were clustered as group 1, Bacillus, Pseudomonas were clustered as group 2, Acinetobacter and others were clustered as group 3.

Bacillus and Acinetobacter were the two most dominant during the entire process, collectively accounting for 1.5% on D0, 38% on D2, 46.5% on D6, 44% on D16, 55% on D18, and 9% on D20. The percentages of Bacillus and Acinetobacter increases from 1–3% and 0.5–28% on D2, 4% and 43% on D6, 7% and 37% on D16, 53% and 2% on D18, 9% and Nil on D20.

Table 6
Agglomeration Schedule

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	2	6	.258	0	0	2
2	1	2	.949	0	1	3
3	1	7	2.398	2	0	6
4	3	5	4.240	0	0	6
5	4	8	8.607	0	0	7
6	1	3	13.136	3	4	7
7	1	4	25.467	6	5	0

Table 7
Cluster Membership

Case	4 Cluster	3 Cluster	2 Cluster
1:Ferribacterium	1	1	1
2:Flavobacterium	1	1	1
3:Bacillus	2	2	1
4:Acinetobacter	3	3	2
5:Pseudomonas	2	2	1
6:Clostridium Perf	1	1	1
7:Enterobacteria	1	1	1
8:Others	4	1	2

Effects of Bacterial Communities on Evaporation of Free Water, Water Formation and Organic Degradation.

When various microorganisms degrade various substances, bio-heat was produced which prompted the bio-drying material to increased temperature and resulted in different phases. The early period of the mesophilic stage shows no dominant bacteria in the SSBM exist in high proportions. However, the self-heating of the material plummeted bacterial diversity as well as increasing water evaporation. After getting into the thermophilic phase, *Acinetobacter* that degrades bio-degradable substances became the most abundant bacteria. Followed by the increase in the abundance of the prevailing bacteria, the water evaporation rate increased, this was credited to organic matter degradation and heat production. The microorganism that produced bio-heat to increase water evaporation generated metabolic water during the organic matter degradation. The evaporation of free water, water formation, and the rate of organic matter degradation declined as the bacterial communities decreases due to the increased temperature of the material.

Through the cooling stage, the volatile solid degradation, water formation, and water evaporation rate all declined. The low water evaporation, water formation, and Volatile degradation rates indicated a decline in microbial metabolism (Cai et al, 2016).

Water Evaporation in material

Table 8
 –Daily water evaporation in bulk
 (obtained by oven dried for 24hrs at
 105°C)

Time (days)	Evaporation (kg/d)
D1	0.87
D2	1.66
D3	2.02
D4	2.53
D5	3.29
D6	2.33
D7	2.12
D8	2.11
D9	1.92
D10	2.53
D11	3.13
D12	2.97
D13	2.95
D14	2.41
D15	2.39
D16	2.98
D17	2.06
D18	1.89
D19	0.97
D20	0.43

Table 9
– Water Evaporation during each Phase

Phase	Water Evaporation (kg d ⁻¹)
Mesophilic Phase (2 days)	2.53
Thermophilic Phase (15 days)	37.74
Cooling Phase (3 days)	3.29

4.0 Conclusion

The bacterial density indices from the study as shown that the material temperature (SSBM) reshapes the bacterial communities and that the bacterial density started decreasing after entering the thermophilic phase and recovered amid the cooling stage but less than that of the mesophilic phase. Acinetobacter was the most abundant bacterial during the initial and final thermophilic phases (43% and 37%). The water evaporation rates reached its peak which was credited to the organic content degradation and heat produced by microbial activities. Bacillus was identified amid the cooling stage as the most abundant. Its relative abundance was 53%.

5.0 Abbreviations

SS- Sewage Sludge

BA- Bulk Agent

VS- Volatile Solids

TMECC- Test Methods for the Examination of Compost and Composting

Declarations

6.0 ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

7.0 CONSENT FOR PUBLICATION

Not applicable

8.0 AVAILABILITY OF DATA AND MATERIALS

Obtained data are contained in the work

9.0 COMPETING INTERESTS

There are no competing interest in this work

10.0 AUTHORS' CONTRIBUTIONS

Ubah J.I and Ogwueleka T.C conducted the experiment and generated data while Ifeoshi C.I computed the data and also served as an assistant during the experiment. Ubah J.I developed the introduction and materials/methods of the article while Ogwueleka T.C. and Ifeoshi C.I. developed the remaining part. Ubah J.I. reviewed the article and did the final corrections and submission to the Editor-in-Chief of this journal.

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Figures

Figure 1

Map showing study area. (Source: Audu, 2016)

Figure 2

Structure of the bio – drying of sewage sludge experiment (Cai et al, 2016)

Figure 3

Humidity Gauge

Figure 4

Humidity Gauge

Figure 5

Thermometer

Figure 6

Saw Dust Sample

Figure 7

Wet Sludge Sample

Figure 8

Preparation of material

Figure 9

Bio-drying material (3 components Mixed)

Figure 10

Density of bacterial communities during the bio – drying process

Figure 11

I Circle Plot

Figure 12

Cluster analysis of Relative abundance of bacterial communities

Supplementary Files

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