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### Compost humic acid structure investigated by elemental analysis and Fourier transform infrared spectroscopy.

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#### ABSTRACT

Composting municipal solid waste (MSW) is a process through which organic matter is transformed. Several chemical methods were used to characterize the humic acids isolated from compost at several phases of development. It seemed that FTIR spectra were a valuable tool for gauging how quickly HA structure changed during MSW composting. According to elemental analysis, the percentages of nitrogen and sulfur both increased while hydrogen and carbon and their ratios both decreased. C percent and O percent showed the same values towards the end of composting as they did at the beginning, although increasing in the intermediate stage. The ratios of FTIR absorbance at 1230, 1053, and 1539 cm<sup>-1</sup> indicated an end product rich in etherified and peptidic chemicals. When organic matter decomposes, it creates new humic polymers that are largely made up of alkyl and other N-rich and oxidized resistant structures.

**Keywords:** MSW compost, FTIR, Fourier Transform-Infrared, Humic acids, Elemental analysis, microorganisms.

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## 1.Introduction

Particularly in underdeveloped nations, the proper disposal of organic waste has emerged as a pressing environmental concern (Onwosi et al., 2017). It is predicted that by 2030, the yearly production of livestock manure around the world would get up to 230 million metric tons of nitrogen, thanks to the rise characterized by extensive farming and animal husbandry (Wu et al., 2016). Manure from China's livestock and poultry accounts for almost 4 billion tons of the country's annual output. Inadequately treated manure, however, poses a risk to the well-being of people and the ecosystem as a whole because of the release of pathogenic microorganisms, releases of heavy metals and eutrophic components like nitrogen and phosphorus into the environment (Zhang et al., 2018). Thermophilic conditions, where the biological products generate heat, are ideal for the aerobic composting of organic wastes (Silva et al., 2014). Microorganisms, primarily humic substances, can transform unprocessed organic waste into more stable compounds during the aerobic composting process (HSs). Simultaneously, biodegradable substances are converted to carbon dioxide (CO<sub>2</sub>), ammonia (NH<sub>3</sub>), and water (H<sub>2</sub>O) (Zhang and Sun, 2017). Detoxifying the environment is one among the many benefits of recycling organic waste into HSs. One of the most crucial criteria for judging the success of an aerobic composting operation is the quality of the HSs produced. The primary components of HSs are Humins (Hus), Fulvic Acids (FAs), and Humic Acids (HAs), which together form a heterogeneous, complicated combination (Stevenson, 1994). In contrast to FAs and HUs, which are both soluble at neutral pH, Alkaline conditions are required for HA solubility (Rochfort et al., 2015). Raw material type, factors such as compost additions, microorganisms, heat, acidity, carbon to nitrogen ratio, wetness, oxygenation, and particle size are just few of the physical-chemical factors that might influence HS development (Silva et al., 2014). The output of aerobic composting can be used as a soil amendment or organic fertilizer (Jurado et al., 2015). The agronomic benefits of HSs are readily apparent: they can be employed in soils to improve aggregate formation, soil porosity, ability to retain water and nutrients (Guo et al., 2016); and in crops in order to supply food and a source of water (Guo et al., 2016). (Tahir et al., 2011). Additionally, HSs can reduce the dangers of using chemicals and decrease a wide range of soil-borne phytopathogens, making them a valuable natural pesticide (Mehta et al., 2014). Several different types of HSs' functional groups carboxylic, phenolic, hydroxylic, and quinonyl contribute to their positive outcomes (Guo et al., 2016). Because of this, aerobic composting aims to convert organic materials into HSs as quickly and effectively as possible. This is due to the fact that HSs are not only the compost's primary output but also its primary driver of the aforementioned advantages (Canellas et al., 2015). Predicting the results of applying organic fertilizers made from manure requires understanding the mechanisms by which HSs arise and classifying HSs into functional groups that correspond to distinct agronomic purposes. In this study, we use a variety of chemical and spectroscopic methods to compare and contrast the structural modifications made to humic acids during the composting process. We employed elemental

analysis to track shifts in the humic acids' base composition. As a qualitative method, Fourier Transform InfraRed analysis was used to identify the classes of chemicals that made up the humic structures.

## **2.Humic substances**

For a compost pile to be stable, humic compounds must be produced. Humification processes have thus been the subject of much study in an effort to identify relevant criteria or indicators that can aid in the assessment stability in compost (Gómez-Brandon et al., 2008). It's worth noting, too, that humification indices for composts made from different starting materials might have widely varying absolute values. This means that using such an index on a large scale is not recommended, and that individual applications should be carefully considered.

In general, the large diversity of humic compounds isolated and identified from various composts is a reflection of the myriad, poorly understood mechanisms that contribute to their genesis, evolution, and incomplete decomposition (Jouraiphy et al. 2008). Different aromatic rings (phenolic and quinonic) are present in humic compounds; these rings carry acidic functional groups and are surrounded by aliphatic, polysaccharide, and peptide chains. The final structure of humic compounds is determined by the range of methods in which these macromolecules are organized. Separation of the humic and fulvic fractions is necessary for accurate determination and/or quantification of humic compounds (Stevenson, 1994). (Stevenson 1994). The humified C content may be overstated if certain procedures are not followed. Since there are several carbon molecules that can be co-extracted early on in municipal solid waste composting, it is easy to overestimate the quantity of carbon that will be produced (proteins, lipids and intermediate microbial metabolites). This is perhaps why composting experts can't agree on the trajectory of humic and fulvic components (Bustamante et al. 2008). As the easily degradable compounds bound to humic substances are used up, the concentration of HA normally declines during the first stage of MSW composting (Genevini et al. 2002). Several authors have noted an uptick in HA levels, typically during the start of the "curing period" (Bustamante et al. 2008). The amount of the refractory fraction that is concentrated (Adani et al. 1999) and/or the synthesis of new humic compounds may account for the relatively modest size of these increases when compared to the preexisting humified fraction (Castaldi et al. 2005). According to Adani et al. (1999), HA's general trajectory during MSW composting reveals that a certain amount of HA is degraded when the rate is extremely high, followed by the simultaneous synthesis and degradation of an ex novo HA component. Last but not least, Lhadi et al. (2006) proposed relating humification in composting to the decomposition of more difficult, resistant materials (alkali insoluble fraction: humin).

## **3. Structural properties of humic substances**

HSs from dairy manure and sugarcane pressmud compost were reported to have an average particle size of 300-600 nm by Guo et al. (2016), demonstrating that HSs are

indeed tiny particles. It is challenging to determine a definitive structure of HSs due to raw material complexity, microbial community diversity, environmental factors, transformation period, and polydispersity are all factors (Stevenson, 1994). From a qualitative standpoint, though, it's clear that all HSs stand for the same basic categories of functions and organizational frameworks. Hydrogels (HSs) range from about 500 to 1,000,000 Da in molecular weight because of the tiny hydrophobic interactions between molecules and other heterogeneous polymers (i.e. van der Waals, - and CH-) and hydrogen bonds (Canellas et al., 2015). (Calvo et al., 2014). HAs are supramolecules that range in size from 10,000 to 100,000 Da (Diallo et al., 2003). The molecular weights of FAs, on the other hand, range from about 600 Da to over 1500 Da (Diallo et al., 2003). Figs. 1 and 2 illustrate The Temple-Northeastern-Birmingham model (TNB) model is used to construct structural representations of monomeric HAs and FAs, which has been proposed as a good approach of defining the structural features of HAs. These theories suggest that the hydrophilic groups, such as carboxylic and phenolic ones, in HAs and FAs structures are located toward the surface of the molecule. The hydrophobic components of HSs are aliphatic chains and aromatic rings (Aquino et al., 2009). The acidity of HSs is mostly composed of carboxylic and phenolic groups, with amino groups also being present in significant numbers. The overall acidity of HAs is higher than that of FAs, due to their increased levels of carboxyl and phenolic groups (Jindo et al., 2016).

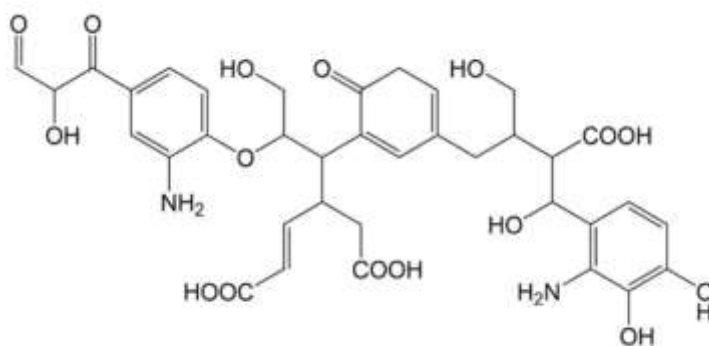


Fig. 1. Monomeric HA unit structure (with a molecular weight of 731 g/mol).(Pope et al., 2010)

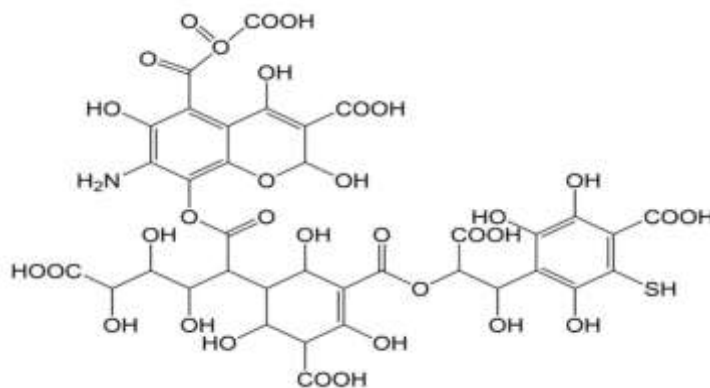


Fig. 2. FA structural model (MW = 1051 g/mol).(Alvarez-Puebla et al., 2006)

#### 4. Raw materials

The carbon source was agricultural waste (AW), and the nitrogen source was saw dust (SD), both of which were raw materials. The carbon source was agricultural waste (AW), and the nitrogen source was garbage food waste (GFW), sheep manure (SHM), horse manure (HGM), cattle manure (CM), laying hens manure (LHM), and raw activated sludge. Particle size is an area where compromise is required for optimal composting conditions. Raw materials include food scraps that have been manually chopped to a size of (0.5-1) inches with a knife, as well as dung from sheep, horses, cattle, laying hens, and raw activated sludge, whose particle size distribution lies somewhere between 1 and 5 cm. The table below details the characteristics of the raw ingredients (1).

Table (1). Description of the Raw Materials Used (Cornell Waste Management Institute., 2014)

Material	% Moisture	% Carbon	% Nitrogen	C/N Ratio
CM	81.0	45.6	2.4	19
SD	39.0	106.1	0.2	530.5
GFW	53.0	36.0	2.4	15
SHM	69.0	43.2	2.7	16
HGM	72.0	48.0	1.6	30
AS	78.0	33.6	5.6	6
LHM	69.0	48.0	8.0	6

The exact C:N ratios were determined using an Excel tool developed by the Cornell Institute for Waste Management (AL-Janabi., 2017), (AL-Saedi and Ibrahim., 2019), and (AL-Janabi., 2019). (AL-Saedi and Ibrahim., 2019). A1, A2, and A3 all started with 20:1, 25:1, and 30:1 carbon to nitrogen ratios, respectively. All the other mixtures (B1, B2, and B3) are 25:1 with varying amounts of manure added. The average weight per cell was 20 kg, and the percentage of moisture in the mixture was 60%.

$$G = \frac{M_1 Q_1 + M_2 Q_2 + M_3 Q_3}{Q_1 + Q_2 + Q_3} \quad \dots\dots (1)$$

$$R = \frac{Q_1(C_1 \times (100 - M_1)) + Q_2(C_2 \times (100 - M_2)) + \dots}{Q_1(N_1 \times (100 - M_1)) + Q_2(N_2 \times (100 - M_2)) + \dots} \quad \dots\dots (2)$$

If we rearrange and simplify the previous equation, we get that the mass of the third substance needed is Eq (3)

$$Q_3 = \frac{R Q_1 N_1 (100 - M_1) + R Q_2 N_2 (100 - M_2) - Q_1 (C_1 \times (100 - M_1)) + Q_2 (C_2 \times (100 - M_2))}{C_3 (100 - M_3) - R N_3 (100 - M_3)} \quad \dots (3)$$

C<sub>n</sub> is the carbon percentage in material n, N<sub>n</sub> is the nitrogen percentage in material n, and R is the carbon to nitrogen ratio of the compost mixture, where M<sub>n</sub> is the moisture

content (percent) of the material n, G is the moisture objective (percent), and Q is the mass of the material n (wet weight basis).

About 35 days were spent on six separate composting trials, with the following raw materials Table (2):

Table (2) Details of the composting experiments.

## 5. Composting experiments

As seen in Figure 3, a composting system on a smaller, pilot-scale. There are six identical bio-reactors, each with a removable cover and dimensions of 55 centimeters (cm) 30 centimeters (cm) 30 centimeters (cm) (capacity, 60 liters). The duration of the experiment, in days, was 35, and it ran from March 2, 2023 to April 5, 2023. These reactors were pierced with small holes to promote aerobic conditions and increase energy conservation and

Experiment no.	Ingredients	Waste Mixture (kg)	Ratio%
Pile No. 1 (A1)	GFW:AS:SD:CM	10:4:1.75:4	50:20:0.8:20
Pile No. 2 (A2)	GFW:AS:SD:CM	12:3.3:2.9:3.5	55:15:13:16
Pile No. 3 (A3)	GFW:AS:SD:CM	12:1.5:3.5:5	54:0.68:15:22
Pile No. 4 (B1)	GFW:AS:SD: LHM	12:1:4.95:4	54:0.45:22:18
Pile No. 5 (B2)	GFW:AS:SD: SHM	10:2.7:3:6	46:12:13:27
Pile No. 6 (B3)	GFW:AS:SD: HGM	12:3:2.5:3	58:14:12:14

decrease emissions (5mm), There are openings in the reactors that allow air to enter, oxygen to be supplied, and gaseous emissions and reaction heat to escape. The reactor is tipped at an angle of 15 degrees, and at the end of the container is a hole of diameter (5mm). Composting is sped up and aerated by turning the materials by hand every two days. Each bio-reactor composted about 20 kilograms of organic solid waste. Full to 75 percent capacity, their practical volume is 45 L, as illustrated in figures (3) and (4).





Fig3. Fertilization system



Fig 4. Dimensions and design of the system

### 6.Sampling and analysis

Compost was collected at a variety of times, including immediately after mixing and again after 18 and 35 days. To ensure proper aeration of the compost, it was stirred every other day for 35 days. Following careful combining of the different sub-samples, a representative 450 g amount was taken and stored in the refrigerator (4 C) until analysis. Throughout the process, samples were taken from various spots inside the pile (center, surface, core of the heap, edges)(Jouraiphy et al., 2008). As described in a previous study, Table 3 shows how some physical-chemical features of the combination change across its various treatment stages (Jouraiphy et al., 2005). The rate of reduction (Red) was determined after incinerating the dry sample for 16 hours at 550 °C using the following formula (Jouraiphy et al., 2005):

$$Red(\%) = \left( \frac{C_f - C_i}{C_f} \right) \times 100$$

where  $C_f$  is final carbon and  $C_i$  is initial carbon.

**Table3**

Changes in the waste mixture's physicochemical properties as a result of composting.

Stage of composting	PH	$C_{org}^a$	Red%	NTK <sup>a</sup>	Red%	C/N
1	6.21	71.51	-	2.84	-	25.1
18	7.72	62.24	12.96	3.34	14.97	18.2
35	7.81	48.65	21.83	3.99	16.29	12.15

Red %: reduction rate; TKN: total Kjeldahl nitrogen; Corg: organic carbon.

## 7. Results and discussion

### 7.1.Elemental analysis

Table 2 lists the reported atomic numbers and elemental make-ups of humic acids recovered at different composting stages. C levels rise somewhat in the middle of the composting process and fall back to the starting point in the final phases. This may be due to the release of aliphatic chemicals during the high breakdown phase from more resistant structures like lignin. Composting is what ultimately breaks down these aliphatics. Other compounds, such as lignin breakdown leftovers, are generated during composting and may react similarly to the humic acids, precipitating at pH 2, as reported by (Sánchez-Monedero et al., 1999).

Composting led to a drop in H and H/C, which may have been caused by a shift toward more aromatic or less aliphatic structures (Table 4). Composting raises the fraction of aromatics, which raises the C/H ratio (Garcia et al., 1989). The O percentage went down at the start, then up at the end. Some oxygen-rich molecules, such as polysaccharides, appear to be destroyed early on in the composting process, as opposed to other, more oxidized, refractory. During humification, new compounds can emerge, or existing ones can become more potent. In the process of composting, aliphatic C is depleted and polysaccharides are gradually converted to various compounds with oxygen, such as carboxylic or ester groups; a rise in aromatic structures may indicate some synthesis of neo-humic polymers (Castaldi et al., 2005). Thus, the raw humic acids and the restructured humic acids produced during composting contained different oxidized components. Composting resulted in a rise in N and S concentrations. Supporting our and other writers' claims that garbage is rich in N, the C/N ratio dropped dramatically as the process of composting advanced, with the N becoming concentrated in stable structures (Castaldi et al., 2005). The intensity of various, unexplained nitrogenous structures was shown to rise during the humification process by certain writers (Adani et al., 2006). These scientists hypothesize plant compounds or fungal and microbial remains chemically or physically preserved in humic structures are the source of the formations.

**Table 4.** Elemental composition and atomic ratios of the humic acids extracted from the waste mixture after different stages of composting.

Stage of composting	C	N	H	O	S	Ash	Atomic ratios		
							C/N	H/C	O/C
1	47.6	6.7	6.8	37.5	0.7	0	8.33	1.74	0.58
18	48.9	7.2	6.73	36.3	0.6	2.2	7.98	1.68	0.57
35	47.4	7.4	6.45	37.4	1.12	2.2	7.64	1.65	0.59



## 7.2 Fourier Transform Infrared

Table 5 summarizes information about the composition, behavior, and band assignments in FTIR spectra of HA extracted from municipal solid waste compost. Similar peaks were seen in the FTIR spectra of HA recovered from all three composting stages, however their intensities varied (Fig. 5). Bands at 3832.29 to 3097.47  $\text{cm}^{-1}$  were the most prominent absorbance feature (H-bonds, OH groups). In addition, there are often two peaks at 2918.10 and 2846.74  $\text{cm}^{-1}$  (representing the stretching of aliphatic C-H bonds) (Castaldi et al. 2005; Spaccini and Piccolo 2007; Carballo et al. 2008). This latter set of signals has attributed to CH<sub>2</sub> group bending and expanding waves in long chain aliphatic molecules, both symmetrically and asymmetrically (Spaccini and Piccolo 2007). An abundance of lipids in the HA extract, a strong adsorption in this range may indicate indicating the compost is immature and/or could be contaminated influenced by the quantity of fat present in the starting mixes. one that can be found between 2574.79 and 2092.62  $\text{cm}^{-1}$  (Asymmetric C-H bonds; -CH carbon atom elongation), An intense band at 1539  $\text{cm}^{-1}$  (the Amide II band) indicates the presence of peptidic molecules in an immature compost, and a peak at 1650.95  $\text{cm}^{-1}$  is associated with the ring vibration of aromatic compounds (C=C in aromatic structure, C=O with a COO- and a H bond) (Spaccini and Piccolo 2007). a notable maximum at 1407  $\text{cm}^{-1}$  (CH<sub>2</sub>, COO-), A peak at 1230.50  $\text{cm}^{-1}$  (single C-O bond stretching in ethers, esters, and carboxylic acids) is also commonly observed (Castaldi et al. 2005). Polysaccharides, polyalcoholics, ethers, and a broad peak at 1053.06  $\text{cm}^{-1}$  have all been implicated by these scientists (Spaccini and Piccolo, 2007). (Carballo et al. 2008). Finally, the C-O stretch of polysaccharide Carbonate salts deformed out of plane during composting may be attributed to a narrow band at 867.91 to 428.17  $\text{cm}^{-1}$  (Spaccini and Piccolo 2007; Carballo et al. 2008; Spaccini and Piccolo 2008). Multiple studies have found that during MSW composting, polysaccharide and aliphatic peak intensities in their FTIR spectra decreases. This is because the organic molecules in the composting matrix undergo varying degrees of breakdown and condensation at various stages of the process (Castaldi et al. 2005; Senesi et al. 2007). After 35 days of stabilization, Spaccini and Piccolo (2008) found a drop in alkyl signals in the 2926-2853  $\text{cm}^{-1}$  region, indicating the depletion of the degradable lipid component. They indicated low quantities of polysaccharide and significant levels of aromatic compounds, as indicated by a broad peak at 1650.95  $\text{cm}^{-1}$ . The peak at 1550  $\text{cm}^{-1}$  decreases in intensity and then increases toward the end of composting, and a measure of the strength of the band that indicates the presence of alcoholic and ether functional groups (1053.06-1110.92  $\text{cm}^{-1}$ ) decreases over the course of composting (Spaccini and Piccolo 2008). Because of this, FTIR spectroscopy shows that composting breaks down the coating components including components such as polysaccharides, peptides, and lipids that are bound to the HA framework, resulting in more aromatic humic structures (Castaldi et al. 2005; Senesi et al. 2007).

**Table. 5** Analysis of the FTIR spectra of HA isolated from municipal solid waste compost at various stages, their functional group assignment, and the HA's behavior during the composting process.

Wavenumber position (cm <sup>-1</sup> )	Vibration	Functional groups	Behaviour of peak relative intensity (I= increase; D= decrease; S= stable)
3832-3257	O-H stretch	Bonded and non-bonded hydroxyl groups	D
3190-3097	NH <sub>2</sub> stretch	and water	D
2918	C-H stretch	Primary amides	I
2846	C-H bend	Symmetrical and asymmetrical stretching of CH <sub>2</sub> groups	D
1650	C=O; C=C	Bending vibrations of CH <sub>2</sub> groups	I
1539	N-H in plane	C=O conjugated, C=C in aromatic structure	S
1407	C-H deformation; CO <sub>2</sub>	Secondary Amides	D
1230	stretch; C-O stretch	Alkanes; carboxylates and carbonates	D
1053	C-O	Stretching of single C-O bond in carboxylic acids, esters and ethers	D
867-428	C-O	Polysaccharides	I
	C-O stretch	Carbonate	

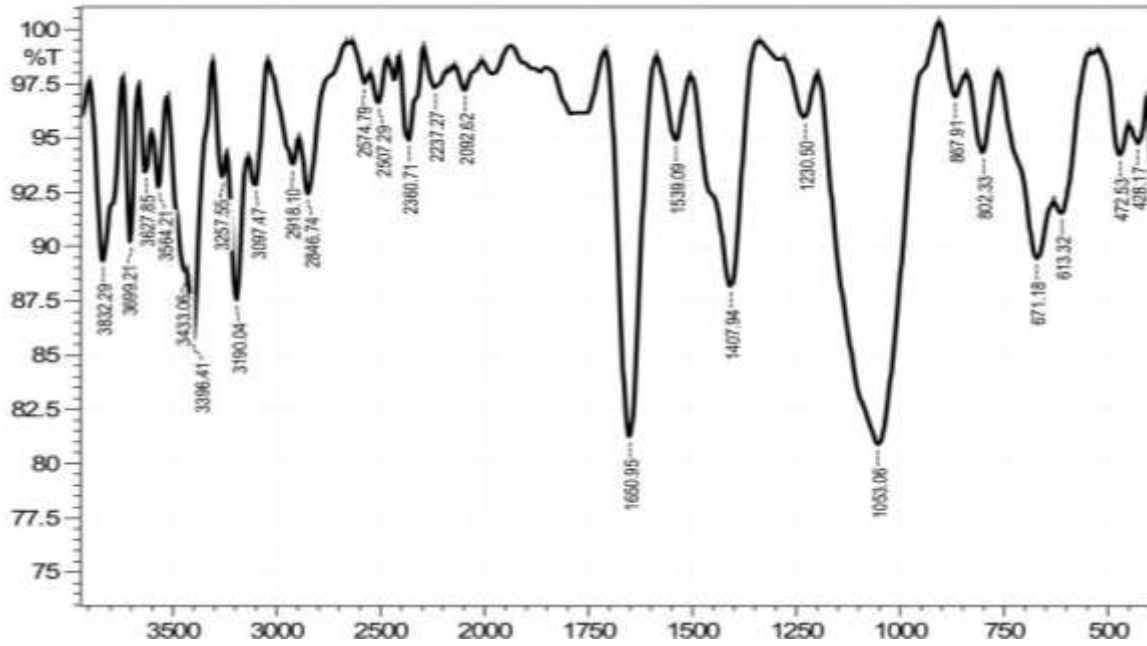


Figure 5(a) FTIR spectrum at 1 stage for compost converted from municipal waste.

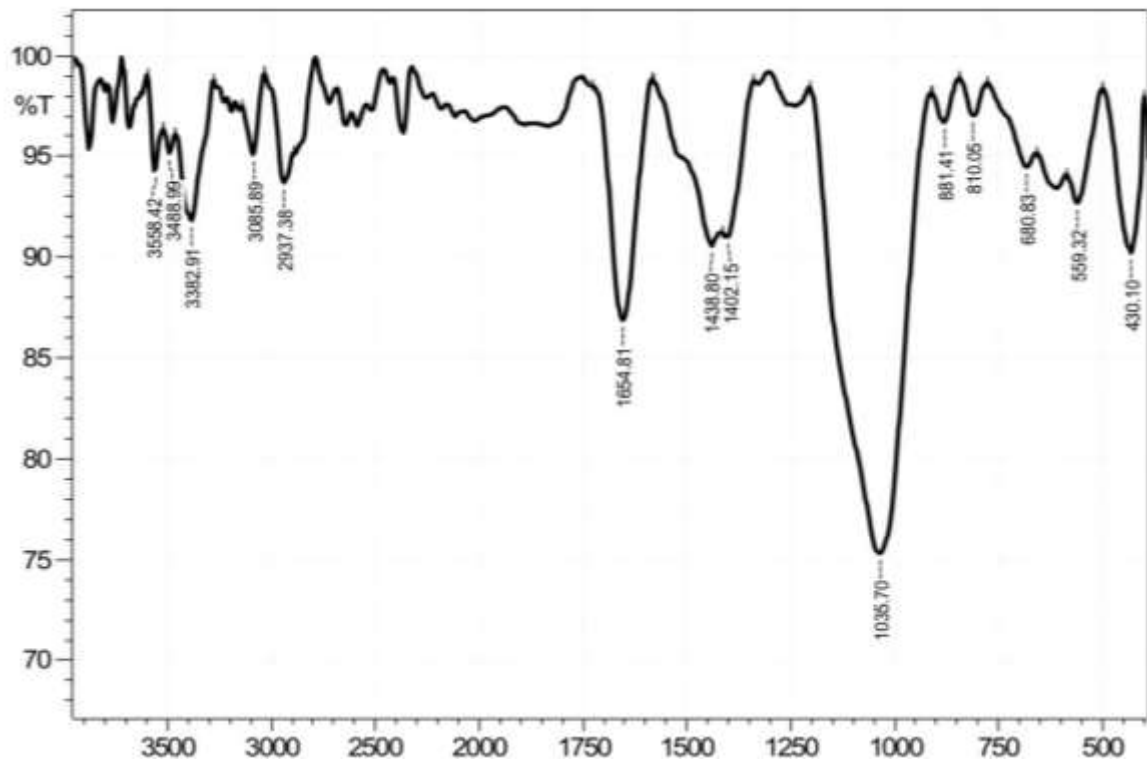


Figure 5(b) FTIR spectrum at 2 stages for compost converted from municipal waste.

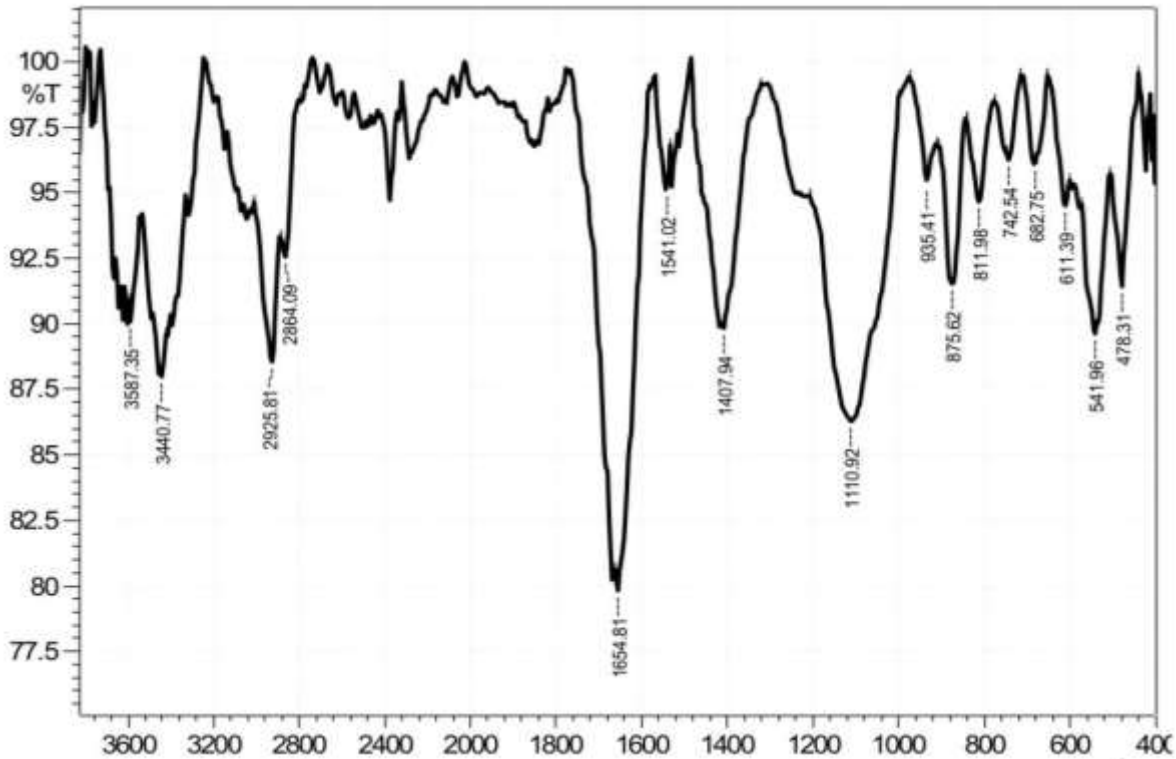


Figure 5(c) FTIR spectrum at 3 stages for compost converted from municipal waste.

### 7.3 The Microorganisms

The bacteria discovered at each of the three temperatures are listed in Table 6. Bacteria identified during the mesophilic, thermophilic, and cooling down phases of the reactors showed that the microbial diversity was highest during the thermophilic stage of decomposition. Some of the bacteria recovered at mesophilic temperatures were *Bacillus* and *Klebsiella pneumoniae* ssp, with the total number of mesophilic bacteria ranging from 1.75105 to 1.93103. *Bacillus* and *Klebsiella pneumoniae* ssp recognized from the mesophilic stage were rediscovered at the thermophilic stage, with a significant rise in numbers between  $(1.15 \times 10^7)$  and  $(1.87 \times 10^3)$ . These organisms may have been active at this time, allowing the process to reach the cooling down stage. They continued on throughout the time when everyone else gave up. Other bacteria present at this high temperature may have been affected by the heat generated by microbial activity. Thermophilic *Pseudomonas* were found in large numbers  $(8.5 \times 10^6)$  after being isolated. At the cooling-down stage, the presence of *Bacillus*, *Klebsiella pneumoniae* subsp. was again found, with numbers ranging from 610106 to 2.45108. The total count of bacteria is shown in (Table 7). demonstrated the tremendous complexity of compost's microbiology and the impossibility of isolating every bacterium species present. Culture medium used for bacterial isolation are intrinsically selective, meaning that only a small proportion of the original sample may be grown in them (Hassen et al., 2001).

**Table 6.** Bacterial at mesophilic, thermophilic and cooling down stages of composting.

Bacterial	(Mesophilic stage) (30-39) C°	(Thermophilic stage) (40-60) C°	Cooling downstage
Bacillus	P	P	P
Klebsiella pneumoniae ssp	P	P	P
Pseudomonas	A	P	A

A=Absent

P = Present

**Table 7.** Number of Bacterial at mesophilic, thermophilic and cooling down stages of composting.

Bacterial at the mesophilic stage	
Bacillus	1.75*10 <sup>5</sup>
Klebsiella pneumoniae ssp	1.93*10 <sup>3</sup>
Bacterial at thermophilic stage	
Pseudomonas	8.5*10 <sup>6</sup>
Bacillus	1.15*10 <sup>7</sup>
Klebsiella pneumoniae ssp	1.87*10 <sup>3</sup>
Bacterial at cooling down stage	
Bacillus	6*10 <sup>6</sup>
Klebsiella pneumoniae ssp	2.45*10 <sup>8</sup>

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