

## Application of Techniques Derived from the Study of Soil Organic Matter to Characterize the Organic Matter during the Composting of Various Materials-A Review

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### Abstract

Organic compounds likely to be present in composts organic waste in municipal waste can be subdivided into five main classes: plant waste, kitchen waste of vegetable or animal origin, paper and cardboard, plastics, undesirable high in carbon and sludge from water treatment. A large variety of organic compounds constitute this initial waste in varying proportions. This review highlights the evolution of the characteristics of organic matter during the composting of different materials obtained from techniques derived from the study of soil organic matter. Biochemical fractionation of organic matter and spectral analysis revealed the significant degradation of cellulose and hemicellulose throughout composting. On the other hand, the slow degradation of lignin which is more resistant to biodegradation leads to its concentration in the organic matter. Since soluble compounds (mainly simple sugars and proteins) may be of microbial origin, they are present in composting. During composting, the characteristics of organic matter become increasingly independent from the origin of the waste.

**Keywords:** Composition; Organic matter; Waste

### Introduction

The compounds which can be found in composts throughout composting are; simple sugars, cellulose, lignin, proteins and carbohydrates. Carbohydrates, are polyhydroxyl compounds which include oses (simple sugars) and holosides resulting from the polycondensation of simple sugars (oligosaccharides comprising less than 10 oses and polysaccharides) and heterosides which are complex molecules obtained by combining simple sugars with non-polymerized substances. Simple sugars are also called mono-saccharides or oses, consist of aliphatic chains and have the crude formula  $(C_nH_{2n}O_n)$ . They have several alcohol functions (R-OH) and a carbonyl function (R-CHO with double bond C=O). Simple sugars which are present in plants, and in biowaste are abundant in decomposing matter, they are both synthesized and destroyed by microorganisms. Thus, their presence within a substrate is dependent on the moisture, temperature and energy resource of the microorganisms. Polysaccharides are the main form of carbohydrates in organic matter decomposition [1,2].

These are homopolymers which resulted from the condensation of a large number of simple sugars with a very high molecular mass and the polysaccharides are of vegetable or microbial origin. In organic matter with an advanced level of decomposition, polysaccharides are mainly derived from microbial metabolism. The complexity of their structure can make them resistant to enzymatic attacks and their adsorption on mineral compounds or their binding ability with humic substances also can give the polysaccharides a certain resistance to biodegradation [3].

The most abundant polysaccharides of plant origin are cellulose and hemicellulose. Cellulose is a macromolecule consisting of the n repeated sequence (n=1500) of gluco-pyranose  $((C_6H_{12}O_6)_n)$  rings. It forms the wall of plant cells and is one of the main constituents of wood. It is used in the manufacture of paper or for the manufacture of artificial textiles, cellulose is therefore abundant in the composition of urban waste. It exists in amorphous form, or in crystalline form that is more resistant to enzymatic and microbial degradation [3]. Cellulose is degraded by aerobic and anaerobic microorganisms (fungi and bacteria) [4]. Hemicelluloses are defined as alkaline-soluble

polysaccharides, they may be linear or branched and comprise from 2 to 6 monomers: xylose, galactose, glucose and mannose and glucuronic acids. Hemicelluloses are plant constituents which accompany cellulose in the formation of wood. Heterosides are heteropolymers consisting of the combination of simple carbohydrates and various non-carbohydrate substances and amino-sugars such as glucosamine belong to this family. The binding is carried out at the anomeric carbon level of the carbohydrate. The aglycone (non-carbohydrate part) of the heteroside which binds to the carbohydrate either by an oxygen atom or by a nitrogen atom is often a phenolic compound (hydroxyl group OH linked to a C of an aromatic ring). Heterosides are abundant in plants and animals. These include nucleic acids such as ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), which play an important role in nature. Lignins are high molecular weight aromatic polymers obtained from three phenolic monomers: hydroxyphenol, guaiacyl, and syringyl. These base units are connected by more than 12 possible links (C-O and C-C). The bio polymerization of lignin takes place in the cell walls of plants by enzymatic oxidation. After polysaccharides, lignins are the main constituents of plants. They are present in leaves and stems (up to 10% of the mass) and in wood (up to 30% of the mass) [5]. There are several natures of lignin depending on the type of plant. Thus soft woods (gymnosperms) mainly contain guaiacyl units, hard woods (angiosperms) contain lignins composed of equal amounts of guaiacyl and syringyl, and the herbs mainly contain syringyl units.

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**Received** April 13, 2017; **Accepted** May 09, 2017; **Published** May 16, 2017

**Citation:** Kalakodio L, Alepu OE, Zewde AA (2017) Application of Techniques Derived from the Study of Soil Organic Matter to Characterize the Organic Matter during the Composting of Various Materials-A Review. J Pollut Eff Cont 5: 184. doi: [10.4176/2375-4397.1000184](https://doi.org/10.4176/2375-4397.1000184)

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The diversity of their bonds and their aromatic nature give the lignins an important resistance against microbial degradation, especially for lignins containing mainly guaiacyl, which is stronger than syringyl. Biological degradation of lignins is carried out exclusively by fungi, the most effective of which are white rots.

This degradation requires aerobic conditions and is generally achieved by the action of phenol oxidases. White rots are more effective at degrading hardwood lignins than soft wood lignins because of the combined presence of guaiacyl and syringyl in the former and the absence of syringyl in the latter. The action of white rots can lead to the complete mineralization of the lignin. Certain external factors such as the acidity of the medium or the mineral matrix protecting the accessibility of lignin can make the biodegradation of lignins even more difficult. Lignins can also form a complex with cellulose and hemicellulose and can play a very important role in the theory of humification. The strong presence of lignin in plants and their resistance to biodegradation gives lignins an important place in the study of organic matter in composts.

## Characteristics of Organic Matter

### Proteins

Proteins are linear or cyclic amino acid chains of peptide bonding -CONH. Amino acids are compounds which combine an amine function, derived from ammonia  $\text{NH}_3$  or a (primary amine) to three (tertiary amine) hydrogen atoms are substituted by hydrocarbon groups, and a carboxylic acid function (R-COOH). There are twenty principal amino acids from which proteins are produced, differentiated by the number of amino acids which constitute them, the nature and order of the R groups carried laterally by the main chain.

Proteins are present in the vegetables and animal kingdom. Proteins are one of the main constituents of living organisms, both in their presence and role. Only plants are able to carry out the synthesis of proteins, which will be degraded into amino acids and then recondensed into new proteins at each stage of the food chain. The amino acids can also be associated with carbohydrates, giving the amino sugars, structural component of the microbial walls, or to lipids (lipoproteins). Proteins also play a large role in the ligno-protein theory of humic substance formation [6-8].

Note that proteins are not the only nitrogenous organic constituents likely to be present in composts. The nitrogen can also be present in the form of amide functional groups, which are carboxylic acids (R-COOH) in which the hydroxyl (OH) of the acids is replaced by an amino group (- $\text{NH}_2$ , for example). These amides may be derived from protein form, or from melanin's which are macromolecules produced at the end of the Maillard reaction, condensation of amino-sugars and simple sugars [9,10].

### Lipids

The term lipids do not refer to a specific type of compound but to a heterogeneous set of neutral and polar molecules having a common property: they are soluble in organic solvents (ethanol, acetone, ether, etc.). The molecular structures of the lipids are very variable but generally all contain linear carboxylic acids with a long hydrocarbon chain (12 to 40 atoms of C) called fatty acids. Lipids play an important role in nature both in animals and in plant soils, fats, waxes, resins, are lipids. Neutral lipid compounds such as ceres (waxes) and glycerides (oil, grease) are used to protect plant surfaces and store energy.

Lipids can be also found in bio-waste and residual sludge as well [11,12]. The biodegradability of lipids is variable, most are rapidly

decomposed by microorganisms but some as waxes are particularly resistant. In the decomposing organic matter of lipids can also be synthesized by microorganisms. Other natural compounds Terpenes and steroids Terpenes are polymers of the isoprene of the formula  $\text{C}_5\text{H}_8$ . Terpenes are very present in the plant kingdom. Monoterpenes ( $\text{C}_{10}\text{H}_{16}$ ) are mainly the fragrant constituents of plant essences (pinene, menthol, and limonene). Diterpenes ( $\text{C}_{20}\text{H}_{32}$ ) contain, in particular, the abietic acid (rosin) which is the main constituent of the pine resin and used for sizing paper. Elastomers such as rubber are part of the terpenes (more than 1000 isoprene units).

### Tannins

The tannins have polyphenolic structures. They comprise compounds of variable molecular structure and molecular weight. It is mainly mushrooms that are able to degrade tannins, but overall tannins are supposed to be an important source of compounds resistant to microbial degradation [13].

### Cutines

Cutines are considered to be rather aliphatic structures, although recent studies have shown the presence of aromatic compounds [13], they are present in the cuticles of plants [14]. The refractory character of microbial degradation is not well defined. High-polymers of industrial origin also interested in plastics because these materials are very present in urban waste, and selective collections and high-performance sorting processes do not allow them to be totally eliminated during composting. Plastics are synthetic macromolecular materials. They are obtained by polymerization or by polycondensation. Polymerization takes place largely from alkenes: ethylene, propene, styrene (packaging, films, bottles, molded objects, toys, etc.). Polymerization of vinyl chloride gives rigid PVC (pipes, bottles) or flexible (toys, shoes, films, cables). There is also the polymerization of acrylonitrile which gives acrylic textiles. Polycondensation involves the preparation of long chains by reacting molecules carrying different functional groups between them. The main plastic materials formed by polycondensation and which may concern our study are polyesters and polyamides, used for the manufacture of textile fibers. Evolutions of the characteristics of organic matter in compost during composting are reminders on humic substances and humification in soils. Composting is conventionally associated with the natural humification process observed for soil organic matter. For this reason, the study of the phenomena of humification or stabilization of organic matter during composting is based on the general theory of the formation of humic substances. This theme has been studied for more than two centuries and is the subject of many works, leading to sometimes divergent visions. The following is not intended to give a detailed and complete state of the art on the formation of humic substances but recalls the main theories concerning the processes of humification and the structures of humic substances and will serve as a basis for reflection, Interpretation of analyzes concerning the stabilization of composts.

Humic substances summarized the different studies on humic substances and proposed the following definitions [14]; humic substances: are organic molecules that are not biomolecules. Biomolecules include polysaccharides and sugars, proteins and amino acids, fatty acids, lipids and lignin whereas humic acids are organic compounds which are soluble in an alkaline solution but precipitated by acidification of the alkaline extract. Fulvic acids are organic compounds which are soluble in an alkaline solution and remain soluble by acidification of the alkaline extract and humane are organic compounds which are insoluble in an alkaline solution.

In 1786 Achard realized for the first time the extraction of humic substances by treatment with an alkaline solution, before being precipitated by acidification, thus obtaining a dark and amorphous precipitate [15,16]. Despite the profusion of studies on humic substances, their chemical structure is still not completely clarified. The lack of knowledge is due in particular to the fact that the extracts contain very varied compounds despite similar alkaline solubility's and by the existence of the reactions of the humic substances with other organic or inorganic compounds [17-20]. Traditionally, humic substances were considered as macromolecules of high molecular weight, with an aromatic nucleus surrounded by aliphatic chains, the nucleus being all the more imposing that the compound was humified. The progresses of the methods of investigation, in particular the use of the NMR, have called into question this vision. Humic substances are today defined as random and highly heterogeneous mixtures of organic molecules (lignin, polysaccharides, proteins) resulting from the decomposition of animal and vegetable residues. The strong molecular heterogeneity of humic substances makes them highly refractory to biodegradation and humic substances are no longer considered necessarily as molecules with a high molecular weight [21].

### Humification

The classical theories of humification present in the processes are either as purely biological processes or as biological processes followed by purely chemical processes. There are four proposed pathways for the formation of humic substances as shown in Figure 1. Lane 1 is chemical and is known as the Maillard reaction. This is the condensation of sugars and amino acid compounds, which can eventually lead to brown-colored melanoids. Lane 2 focuses on the formation of polyphenols from cellulose or other Lignic products. The oxidation of the polyphenols to quinones is followed by a polymerization giving the humic substances. Route 3 considers that the phenolic acids and aldehydes produced by the microbial degradation of lignin are transformed into quinones by the action of enzymes.

These quinones are then polymerized with or without amino compounds to give the humic substances. Lane 4 is called lingo-protein theory: it is the theory developed by Maheshwari et al. [22]. The amino compounds of the microorganisms react with the modified lignins. Lignins are incompletely degraded, causing loss of methoxyl ( $\text{OCH}_3$ ) groups, generation of O-hydroxyphenol, and oxidation of aliphatic leading to the formation of COOH groups.

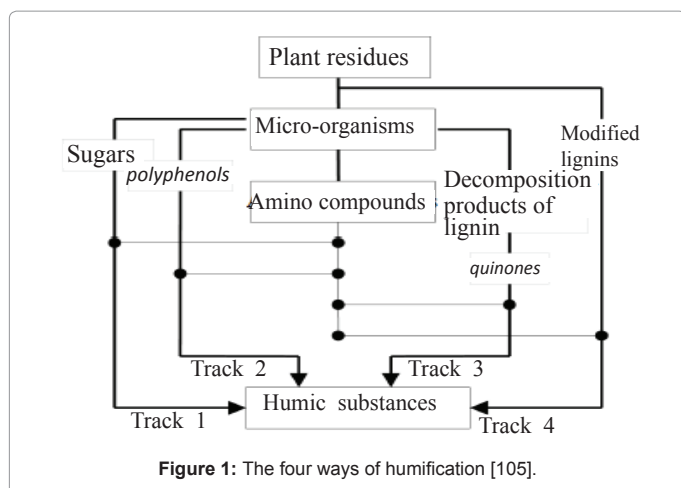


Figure 1: The four ways of humification [105].

### Stabilization of Organic Matter during Composting

During composting, the thermophilic phase is followed by a decrease in temperature due to the slowing of microbial activity. This slowdown is related to the stabilization of organic matter which can be defined as increasing resistance to biodegradation of organic matter, the more stable the organic matter the lower the microbial activity. The level of stability of the organic matter of compost can be estimated during compost incubations incorporated in a soil. The presence of soil offers the degrading micro flora whereas the compost offers the organic matter assimilable by this micro flora. The intensity of the activity of the micro flora will depend on the biodegradability of this carbon. It results in respiratory intensity (release of  $\text{CO}_2$  or consumption of  $\text{O}_2$ ). The material is incubated alone, or more generally mixed with soil. The duration of incubations varies from a few days to several months or even one year according to the objectives of the study.

Generally, the incubations are carried out under conditions corresponding to the optimum of the microbial activity, with a temperature of between  $25^\circ\text{C}$  and  $28^\circ\text{C}$  and humidity close to 70% [23,24]. But some authors prefer temperatures closer to annual averages under actual conditions of use around  $15^\circ\text{C}$  [25]. On household garbage compost measure mineralized carbon content after 38 days of incubation of 10% for initial waste, 2% after 3 months of composting and less than 1% after 7 months of composting. Anyasi and Atagana [26] observed similar results on livestock manure composts aged 0 to 16 weeks. The work of Ryan et al., Katheem et al. [27,28] with vegetable compost shows that stabilization is rapid at the beginning of composting. In fact, after 71 days of incubation, 25% of the compost carbon sampled at 0 days of composting is mineralized, compared with only 10% of the compost sampled at one month during the thermophilic phase. After 25 weeks of composting, the amount of mineralized carbon is less than 5% of the initial carbon. During incubations under optimal conditions of microbial activity of unstable compost, most of the carbon mineralization takes place during the first two weeks of incubation, reflecting the rapid mineralization of compounds with low refractoriness. Microbial biodegradation [29,30].

### Heated solubilized hemicelluloses in an acid detergent (ADF)

The residual lipids and proteins are also removed during this hydrolysis; the residue contains substantially the entire lingo cellulosic fraction. Cold solubilized by hydrolysis in concentrated sulfuric acid. The lignin corresponds to the residue after the hot acid hydrolysis (eliminating the hemicelluloses) and then the cold hydrolysis with the concentrated acid. For composts derived from the same type of waste, the range of variation of the measured fractions can be very wide. For soluble composts, the soluble fraction can vary from 12 to 21% of the dry matter, the hemicellulose is 0 to 10%, the cellulose is 4 to 40% and the lignin is 5 to 45%. Since lignin is a refractory compound for biodegradation, it is common to observe stability or an increase in the proportions of lignin in the organic matter during composting, while in contrast, cellulose is highly degraded [31-34]. For household garbage compost, Müller et al. [35] observed a halving of the proportion of organic matter present in cellulosic form, from 32% of MO to 16%, in 23 weeks of composting [36].

Kapetanios et al. [37] also measured 50% decreases in cellulose content after 25 weeks of biowaste composting initially at 30% cellulose, but the lignin content remains stable at 10%, reflecting its slight biodegradation. Note that Miller et al. [38] carried out an estimate of the evolution of the lignin concentration in humic acids extracted after 0 and 25 weeks of composting, using the Van Soest method and the

NMR. The two techniques yielded similar results, with respectively 52% and 58% in the initial state, and 65% and 62% in the final state.

### Humification of organic matter

The conventional method for monitoring the humification of organic matter during composting is used for studying soils, i.e. the extraction of fulvic acids, humic acids and humine, and their quantification by determining their carbon content. The relative distribution of carbon in these different classes is often used to assess the degree of stability of organic matter in composts. This method is a transposition to the study of composts, a method defined within the framework of the study of the soils. This transposition must be used with vigilance. In fact, unlike soils, composts are made up of a high proportion of young

organic compounds, thus rich in biomolecules. These biomolecules are more or less extractable during the humic fractionation. The terms “humic acids” and “fulvic acids” do not therefore refer to humic substances in the strict sense. It is a collection of compounds, including humic substances and biomolecules, soluble in alkaline solution. Low molecular weight polysaccharides and amino acids will be found, for example, in the fulvic fraction [39,40].

Presents some of the values mentioned in the literature on composts of different origins and ages are presented in Table 1. The share of extractable carbon (humic acids plus fulvic acids) generally represents less than 25% of the total carbon. This means that the vast majority of the carbon in the composts is in non-extractable form. This fraction is the subject of little attention in the bibliography in relation to humic acids.

Reference	Composted primary waste	Degree of volution	COT	C-AH	C-AF
Sugahara and Inoko et al. [41]	Household waste	Initial	41	0.6	9.7
		1 month	41	1.3	6.2
		3 months	33	7.9	4.5
Stevenson and Cole [42]	Household waste	Advanced	18	1.4	2.3
	Effluents	Advanced	32	4.5	2.8
	Step sludge	Advanced	27	2.5	3.8
Riffaldi et al. [43]	Household waste	Initial	30	0.6	2.1
		Stable	21	2.4	1.3
	Effluents	Waste	39	2.1	2.7
		Stable	36	4.2	2.4
Atiyeh et al. [44]	Step sludge	Advanced	28	3	0.9
	Effluents	Advanced	31	3	0.9
Roletto et al. [45]	Plants	6months	33	7.9	1.9
	Household waste	9months	25	6.8	2.4
	Household waste	18 months	15	4.2	0.7
	Sludge	4 months	31	2.1	1.9
Paul et al. [16]	Sawdust	3months	43	1.3	1.6
		6months	42	1.5	1.4
Forster et al. [46]	Effluent	Initial	44	6.2	1.5
		2months	36	8.4	1.4
		3months	34	9.4	2.1
	Peel	--*	43	20.5	2.5
	Step sludge	--	25	11.5	1.5
Serra-Wittling et al. [47]	Bio waste	3months	14	1.6	0.3
	Bio waste	7months	17	2.7	0.5
Gavilanes-Terán et al. [48]	Sludge industry cotton	Initial	44	7.2	3.7
		Average	36	7.8	3.1
		Advanced	36	7.9	2.6
Zhu et al. [49]	Household waste	3days	28		5.7
	Household waste	15days	26		5.1
	Household waste	2months	32		6.2
	Household waste	1year	23		6.4
Provenzano et al. [50]	Household waste	20days	45*	1	10.2
		2months	35*	1.4	7.6
		6months	23*	3.6	5
		1year	15*	3.1	1.7
St. Marti et al. [51]	Green waste	1month	17	4.2	1.3
	Green waste	6months	14	3.3	1.2
	Green waste	1year	12	6.4	0.8
	Green waste reactor	2weeks	43	3.2	8.4
5weeks		37	4.4	2.6	
30weeks		31	5.2	2.2	
Zaccheo et al. [52]	Household waste and sludge	6months	24	7.2	1.9
	Green waste	6months	21	2.1	4

**Table 1:** Some bibliographic data on the distribution of organic carbon in the humic and fulvic fractions for different composts.

Generally, the fulvic fraction predominates over the humic fraction at the start of composting and represents up to 10% of total dry matter (or 25% of total carbon). During composting, the humic fraction becomes progressively predominant relative to the fulvic fraction and represents, for example, up to 4% of the dry matter (25% of carbon) of a household waste compost ages 18 months, (5% of carbon) for the fulvic fraction.

This reduction in the proportion of carbon in fulvic form in conjunction with the increase in the proportion of carbon in humic form corresponds to the humification of the organic material of the compost, or more precisely to the increase in the concentration of stable compounds in the compost. But the comparison of these results is difficult because the distribution of the carbon is dependent on the nature of the extractant, on the number of extractions and on the extraction ratio. For example, Serra-Wittling et al. [47] carry out alkaline extraction for two hours with an extraction ratio of 2% (mass/volume), while with the same extractant, cause the extraction to last twenty-four hours at a ratio of 4% and repeat this extraction five times [33,53,54].

The complexity of the fractionation techniques used depends on the objectives of the studies. Successive and repeated extractions followed by purification phases are necessary for a fine characterization by spectroscopy of constituents of humic substances in compost [50,55]. Several reports have been used to assess degrees of humification; the most commonly used ratio is the CAH/CAF ratio, which increases during composting. This ratio is close to 0 for very young composts and reaches values of between 2 and 5 for highly stabilized composts (around one year of composting) [49,56,57], and even 10 for some green waste composts. Other ratios such as percentage of extractable carbon on total carbon, ratio of carbon in humus were also mentioned in the literature [58-60]. The differences in fractionation techniques used mainly concern alkaline extraction that is the joint extraction of humic acids and fulvic acids. It is therefore probable that the respective proportions of humic acids and fulvic acids are only slightly altered by these operative differences. Therefore, the CAH/CAF ratio should be minimally altered. This may explain the consistency of the CAH/CAF results reported in the literature [47,60-62].

## Evolution of the Chemical Composition of the Organic Matter Demonstrated by Spectral Analyzes

The use of spectral analytical methods for humic substances has developed in recent years. These methods allow a better understanding of the degradation processes of organic matter during composting. The main techniques used today to study the stabilization of composts during composting are infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and pyrolysis coupled to a mass spectrometer [63]. Only the results of the infrared analyzes are reported herein, therefore we will only mention pyrolysis and NMR. Infrared spectrometry is a method of identification and non-destructive testing. It consists of several techniques based on the absorption or reflection by the sample studied of electromagnetic radiation in the range of infrared (1 to 1000  $\mu\text{m}$ ). Only the range of the medium infrared (2.5 to 25  $\mu\text{m}$ ) is generally studied.

Absorption in this area is a kind of fingerprint of the main classes of chemical groups that make up organic matter and provides information on the nature and arrangement of functional groups of humic acids. Infrared spectrometry has been used for about sixty years, but its use has developed considerably since the appearance in 1970 of spectrometers with transformed Fourier. The technique of Fourier Transform Infrared Spectrometry (FTIR) is widely used for the study of humic substances

in soil. Its application to the study of stabilization of the organic matter of composts is more recent, and often complements NMR analyze [63].

The IRTF gives spectra whose interpretation is not always easy. A mass or a peak does not correspond to a precise compound but to energy of rotation or vibration of a bond. The interpretation of a spectrum is based on the existence of a correlation between the positions of certain peaks and the presence of certain organic compounds. Each type of organic function absorbs at particular wavelengths. The assignment of bands to organic functions has been defined by correlating the positions of certain peaks with the presence of certain organic compounds [64-68]. The main absorption bands in the medium infrared range are shown in Table 2. Infrared analyzes carried out directly on total compost matter are rare [69-72]. The degradation of organic matter during composting is accompanied by an increase in the proportion of aromatic compounds resistant to biodegradation compared to short aliphatic chains, polysaccharides and alcohols, which are readily degradable found that this increase is high in the case of composting household refuse whereas it is practically insignificant in the case of composting based on sawdust [50]. Spaccini et al. [69] showed, in their study on manure composting, that changes in the IRTF absorbance ratios at several distinct frequencies (1385/2930, 1650/2930, 1425/1050) are significantly correlated with compost age, The C/N ratio or the proportion of humic substances. The IRTF spectra of household garbage composts are distinguished from those of green waste by a peak predominant at 1450  $\text{cm}^{-1}$  (acid CH) instead of 1650  $\text{cm}^{-1}$  (C=O aromatic and amides) or 1050  $\text{cm}^{-1}$  (CO polysaccharides) in the case of other composts. But it also appears that when the compost reaches a high level of stability it becomes a fairly homogeneous mixture whose characteristics are independent of the nature of the waste Composites [50]. There are many studies on the characterization of compounds present in humic acids extracted from composts [73-75]. This work mainly shows a decrease in aliphatic compounds and an increase in aromatic compounds in the constitution of humic acids. Sugahara et al. [41] also found that the spectra of humic acids extracted from three compost households are different at the beginning of composting and very similar at the end of composting.

References in the literature on total compost matter or humic extracts show that there may be qualitative differences (location of main bands) depending on the type of composted waste, during composting alone. The intensities of the absorption bands vary and other methods of spectral analysis are also used in studies of organic matter in composts. Appearing about 50 years ago, NMR starts to be widely used in organic chemistry. It consists in the joint action of two electromagnetic fields, one intense and constant, the other weak and oscillating. The absorption by certain atomic nuclei of the sample of frequencies presented in the electromagnetic source leads to the NMR spectrum. This property is related to the spins of the atoms. NMR:  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$  for example  $^{13}\text{C}$ -NMR is an analytical technique widely used in studies of soil organic matter [76] also applied to composts [77-81].

The analytical pyrolysis consists of a thermal degradation, between 650°C and 750°C of organic materials in the absence of oxygen. The present invention relates to a chromatograph and a mass spectrometer (pyrolysis-GC/MS), which allows the characterization of many organic macromolecules. On the pyro chromatograms the different peaks corresponds to the compounds produced during the pyrolysis. This technique allows a semi-quantitative approach [82,83]. According to Chen et al. [63], the results of pyrolysis are not representative of the sample because of their high sensitivity to the presence of the most

Frequency (cm-1)	Assignment	Vibration	Reviews
3700-3050	O-H free of phenols (3650-3600)	Elongation	Water introduced by moisture of the sample absorbs in this range of frequencies.
	And bound (3400-3200). Aromatic and Aliphatic N-H amides and amines, primary And secondary (3500-3180)	Elongation	This broadband centered at 3400 is hardly interpretable.
	C-H aromatic (3100)	Elongation	
2930-2920	C-H aliphatic, asymmetric -CH <sub>2</sub>	Elongation	
2850	C-H aliphatic, symmetric -CH <sub>2</sub>	Elongation	
2500	O-H carboxylic acids	Elongation	Frequency absorbed by Carbonates CaCO <sub>3</sub>
1720	C = O carboxylic acids	Elongation	
1650-1600	C = C aromatics	Elongation	
	C = O amides and quinones	Elongation	
	N-H primary amines and amides	Deformation	
1525	C = C aromatics	Elongation	Typical of lignin
	N-H secondary amines and amides	Deformation	
1430-1460	C-H aliphatic, symmetric and Asymmetric groups -CH <sub>2</sub> and -CH <sub>3</sub> , Aromatic, and fatty acids	Deformation	Frequency absorbed by Carbonates CaCO <sub>3</sub>
1384	-NO <sub>3</sub>	Deformation	Very narrow peak
1320-1250	C-O phenolic	Elongation	
	C-O carboxylic acids	Elongation	
	O-H carboxylic acids	Deformation	
	C-C aliphatic	Elongation	
1170	C-O aliphatic	Elongation	
1100-1020	C-C aliphatic	Elongation	Frequency absorbed by the Silica Si
	C-O polysaccharides	Elongation	
874		Deformation	Frequency absorbed by Carbonates CaCO <sub>3</sub>
870-600	C-H aromatic s		Massif not interpretable (Many bands due to
			Aromatic C-H and absorption by minerals)

Table 2: Infrared absorption bands of organic matter.

volatile compounds, such as lipid compounds. The use of this technique in the study of composts is recent and still undeveloped [84,85].

## Biological Activities

### Measures of breathing

Breathing measurements are based on the respiratory activity of the microorganisms present in the compost. Immature compost is characterized by high O<sub>2</sub> demand and high CO<sub>2</sub> production due to an intense microbial activity caused by the high biodegradability of the substrates [86,87] in contrast to a more stable mature compost and active month. Many authors have studied the relationship between microorganism activity and composting time by different respirometric methods [88-91].

### Enzymological activities

The important enzymes involved in the composting process include cellulases, hemicellulases, phenol oxidases, proteases, lipases, phosphatases, and their activities evolve according to the degree of decomposition of organic matter. Thus, high levels of protease, lipase and cellulase activities were measured during the active phase of composting [92-94]. However, since composting is carried out from very different organic substrates and by numerous processes, Kazemi et al. [95] estimate that the establishment of general threshold values for enzymatic activities as an indicator of maturity is extremely difficult. Indeed, the enzyme activities measured at maturity are specific to the composts tested. Thus, these authors claim that it is necessary to use several enzymes and prefer to carry out a dynamic study of the enzymatic activities throughout compost.

## Study of microbial communities

The successions of microbial communities during composting are well [96] and many methods have already been used in their study, such as the analysis of 16S or 18S RNA, (PLFA) or the analysis of Quinone profiles [97-99]. One tool seems very promising: the study of metabolic profiles by Biologic micro plate. This technique consists in studying microbial growth on different sources of carbon, thus making it possible to demonstrate different physiological profiles or community level physiological profiles (CLPP). Gryta et al. [100] argued that this technique appears to be a promising indicator of the degree of maturity, but add, nevertheless, that methodological improvements on the standardization of the inoculum used are necessary.

### Self-heating test

The intense activity of microorganisms in immature composts results in the production of heat during degradation of the simplest and most accessible compounds. Thus, it is possible to measure the degree of decomposition of the organic matter of compost by measuring the rise in temperature after re-wetting. Several studies have shown the relevance of this test to evaluate maturity of compost [101,102].

### Solvita® test

The Solvita® test is based on carbon mineralization and volatilization of ammonia marketed under the Solvita® test name (Woods Research® Management, USA). This test is carried out on composts whose humidity is adjusted to a level corresponding to the optimum microbial activity. This test combines an estimate of carbon mineralization and an estimate of volatilization of ammonia. A global index, based on two

color indicators, of 6 or more, on a scale of 1 to 8, corresponds to mature compost. Awasthi et al. [103] showed that this test provides useful information about the excessive and toxic concentrations of ammonia in composts, and concluded that the Solvita® method is a simple and inexpensive test for composts stability and NH<sub>3</sub> emission. Biological stability of organic matter, a necessary condition for maturity whatever the approach considered, mature compost must have a stable organic matter. As we saw earlier, stability is the opposite of biodegradability. The reference method for evaluating the stability of compost is the monitoring of the respiratory activity of a soil which the compost is incorporated. This respiratory activity is generally estimated by the evolution of CO<sub>2</sub> during incubations at 25-30°C of previously dried and homogenized composts [104]. Immature composts, rich in readily biodegradable compounds, will result in rapid carbon mineralization during the first days of incubation. This suggests that incubations of 21 or even 7 days may be sufficient to assess the degree of stabilization of the compost. The amounts of mineralized carbon during incubation depend on the origin of the compost (process and waste) and the age of the compost. For uncomposted urban waste, 50% of the carbon can be mineralized after 5 months of incubation, while a 17% mineralization obtained with 4-month-old household refuse compost makes it possible to consider mature compost and [105] measures mineralization from 40% to 90% of the initial carbon for household composting sampled at the start of composting and considered to be immature, and from 10% to 40% for the same Composts sampled at the end of composting and considered mature. Mekan et al. [106] measured only a 10% mineralization of carbon from waste from domestic refuse and sludge in 70 days of incubation. It should be noted, however, that no bibliographic references establishing stability levels have been found. The work generally highlights the highly unstable composts whose incorporation to the soil causes a very great increase of the microbial activity and the very stable composts whose incorporation to the soil does not modify the microbial activity. The situations between these two extremes correspond to a continuous range of stabilization. These intermediate situations make it difficult to compare results from separate studies.

### Biological activities measures of breathing

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### Conclusion

During composting, the organic matter of the composts evolves from a material rich in readily biodegradable compounds to a material offering greater resistance to microbial degradation. The rate and magnitude of stabilization depends on the nature of the composted waste, different methods of investigation make it possible to account for and explain this stabilization of organic matter during composting. The study of carbon mineralization during the incubation of compost makes it possible to evaluate the degree of biodegradability of the compost carbon. The results obtained by chemical fractionation in humic acids, fulvic acids and humine show a humification of the organic matter during composting. The interpretation of the results obtained by this fraction of the organic matter, however, must be done with caution in the context of the study of composts. Biochemical fractionation of organic matter and spectral analyzes revealed the significant degradation of cellulose and hemicellulose throughout composting. On the other hand, the slow degradation of lignin which is more resistant to biodegradation leads to its concentration in the organic matter. Since soluble compounds (mainly simple sugars and proteins) may be of microbial origin, they are present in composting. During composting, the characteristics of organic matter become increasingly independent from the origin of the waste.

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**Citation:** Kalakodio L, Alepu OE, Zewde AA (2017) Application of Techniques Derived from the Study of Soil Organic Matter to Characterize the Organic Matter during the Composting of Various Materials-A Review. *J Pollut Eff Cont* 5: 184. doi: [10.4176/2375-4397.1000184](https://doi.org/10.4176/2375-4397.1000184)

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