



Structural study of humic acids during composting of activated sludge-green waste: Elemental analysis, FTIR and ^{13}C NMR

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ABSTRACT

The humic acids extracted from a compost of activated sludge at different stages of maturity were characterized by various chemical techniques. Elemental analysis showed the reduction of H, and the H/C and C/N ratios and an increase in the proportion of N and S. At the end of composting C% and O% presented the same values as initially, although they increased in the intermediate stage. Based on the ratios of FTIR absorbance it was shown that the end product was enriched in etherified and peptidic compounds absorbing at 1384, 1034 and 1544 cm^{-1} . The alkyl and other N-rich and oxidized recalcitrant structures compose the new humic polymers produced during composting. In principal components analysis, the first axis, PC1: 49.75% considers the variability between structures in decomposition from the other parameters that concern the stable new humic polymers formed after composting. PC2 (40.5%) shows a negative correlation between (aromatic carbon, FA level) and (aliphatic carbon, HA level) during composting.

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1. Introduction

The recycling of waste by composting is a promising solution and has become the aim of waste managers the world over [1,2]. It enables the waste to be recycled in agriculture rather than being dumped as landfill, incinerated or applied directly to the soil—these practices are recognised as being a useless loss of organic matter as well as a source of pollution [2–4]. During composting, part of the organic matter is mineralised to yield carbon dioxide, ammonia and water, while the remainder is transformed into humic substances [5,6]. One of the most important factors influencing the success of agricultural recycling of compost is stability/maturity [7,8]. Stability is related to the level of biological activity of the compost and is dependent on the degree of degradation accomplished during composting while maturity is linked to the absence of phytotoxicity upon use of the compost [9]. The use of immature composts can retard the development of plants due to competition for oxygen, but it can also be directly phytotoxic due to insufficient degradation of the organic matter [10]. Several techniques have been used to identify stability/maturity parameters for compost intended for agricultural use [8], but as yet there is no standard way to assess the quality of compost although numerous methods (empirical, micro-

biological and physico-chemical) have been proposed. Sequi and Benedetti [11] recommended the use of the humification index, the level of humification and the degree of humification. Tomati et al. [12] suggested that the molecular weight of the humic substances should be followed as an index of stability/maturity. These parameters give a good indication of how humification is progressing but do not provide sufficient data concerning the composition of the humic substances. In general, chemical and spectroscopic techniques have been used to characterise the humic structures and the main transformations that fresh organic material can undergo during composting [1,3,5–7]. In the present study, several chemical and spectroscopic techniques are used to obtain comparative and complementary information on the changes occurring in the chemical structures of the humic acids on composting activated sludge. Elemental analysis was used to monitor variations in the elementary composition of the humic acids. Fourier Transform InfraRed analysis served as a qualitative tool to determine the chemical groups making up the humic structures, while nuclear magnetic resonance of carbon 13 (^{13}C NMR) was used to probe the chemical environment and the distribution of carbon among the chemical groups.

2. Material and methods

The samples of activated sludge were from the aerobic wastewater treatment plant in the town of Khouribga (Morocco). The composting trial was run on quite a large scale: 10 m^3 of sludge was

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Table 1
Variation of the physico-chemical characteristics of sludge-green waste mixture during composting.

Stage of composting	pH	C _{org} ^a	NTK ^a	C/N	Ash ^a	Dec %	HA level (g kg ⁻¹ dry matter)
RM	6.36	35.7 ± 0.5	1.2 ± 0.2	29.75	33.56 ± 0.5	–	26.5 ± 1.5
15	5.7	35.34 ± 0.8	1.36 ± 0.1	25.98	35.11 ± 0.1	6.6	30.0 ± 1.4
60	6.1	30.89 ± 0.7	1.3 ± 0.08	23.76	41.69 ± 0.2	29.3	39.7 ± 0.5
135	6.23	20.55 ± 0.3	1.69 ± 0.1	12.15	56.32 ± 0.2	60.8	39.5 ± 0.3

^a % dry weight; RM: raw material; C_{org}: organic carbon; TKN: total Kjeldahl nitrogen; Dec%: decomposition rate; HA: humic acids.

mixed with 5 m³ of green waste formed into a prism-shaped heap 8 m long and 1.5 m high, on a platform. When ready for composting, the mixture contained about 60% water and had a C/N ratio of 29.8. The compost was mixed every fortnight over a period of 135 days to keep the mixture well aerated. Samples of compost were taken at different stages of the process from raw mixture and after 15, 60 and 135 days. The sampling was done at each stage from different places within the heap (center, surface, core of the heap, sides) and after thorough mixing of the various sub-samples, a representative 500 g portion was collected and refrigerated (4 °C) until analysis [13]. Table 1 illustrates the variations of certain physical-chemical properties of the mixture at the various stages of treatment as reported in a previous study [14]. After ignition of the dry sample at 550 °C (16 h), the rate of decomposition (Dec) was calculated according to the formula [14]:

$$\text{Dec}(\%) = \left[\frac{(100 \times \text{Af} - \text{Ai})}{\text{Af}} \times (100 - \text{Ai}) \right] \times 100$$

where Af is final ash and Ai is initial ash.

2.1. Extraction of the humic acids

30 g of each sample were defatted and treated three times with distilled water to extract non-humic substances. Thus, the free fatty acids and lipids were removed as were the water-soluble substances (such as sugars and proteins) to prevent interference between these components and the humic acids [6]. The humic substances themselves were then extracted from each pre-treated sample using a 0.1 M solution of NaOH [1]. Extraction was repeated until a clear supernatant was obtained after centrifugation at 4000 × g for 15 min and filtration through Whatman paper (125 mm). The humic acids were then precipitated out of solution with 1.5 M H₂SO₄ for 24 h at 4 °C. They were separated from the fulvic acids in the supernatant by centrifugation and filtration through Whatman paper (125 mm) and then taken up again in 0.1 M NaOH. The solutions of humic acid obtained were then dialysed with Spectra Por (1000 Dalton MWCO) to eliminate the excess of salts and finally lyophilised for analysis. The humic acid content was calculated after freeze-drying a known volume (Table 1).

2.2. Elemental analysis

Elemental analysis was carried out using a Carbograph Auto-analyser (Fison Carlo Erba EA 1110) to analyse C, H and N and a type EA 1106 instrument to analyse the O of the humic acids.

Table 2
Elemental composition and atomic ratios of the humic acids extracted from the sludge-green waste mixture after different stages of composting.

Stage of composting	C ^a	N ^a	H ^a	O ^a	S	Ash	Atomic ratios		
							C/N	H/C	O/C
RM	47.9 ab	6.8 c	6.9 a	37.6 a	0.8	0	8.22 a	1.73 c	0.59 a
15	48.7 a	7.3 a	6.85 ab	36.5 b	0.7	2.3	7.82 b	1.69 b	0.56 b
60	48.9 a	7.2 b	6.75 b	36.2 b	1.0	2.3	7.97 c	1.66 a	0.56 c
135	47.5 b	7.3 a	6.55 c	37.5 a	1.2	2.3	7.62 d	1.66 a	0.59 a

Data with the same letter within the same column do not differ significantly at the 5% level according to least significant difference test.

^a % calculated on organic matter basis.

2.3. Spectroscopy

2.3.1. Fourier transform infrared (FTIR)

The infrared spectra (FTIR) were recorded from pellets containing 2 mg of the freeze-dried humic acids with 250 mg of dry KBr. The instrument used was a PerkinElmer 1600 FTIR spectrophotometer covering a wavenumber range of 400–4000 cm⁻¹ at 16 nm s⁻¹.

2.3.2. Carbon 13 nuclear magnetic resonance (¹³C NMR)

The solutions for NMR were prepared by dissolving 100 mg of the freeze-dried humic acids in 3 ml of 0.5 M NaOD/D₂O. The spectra were recorded on a Bruker AM WB 300 at 75.469 MHz. To suppress signal enhancement due to the nuclear Overhauser effect, a gated, impulse uncoupling program was used. The spectra were recorded in the following conditions: relaxation time 1.8 s, acquisition time: 0.98 s, pulse 35 degrees and total duration of acquisition, 72 h. The areas of integration of the spectra were 0–55 ppm (aliphatic carbon), 55–110 ppm (aliphatic carbon substituted with O or N), 110–165 ppm (aromatic carbon), and 165–200 ppm (carboxylic carbon).

2.4. Statistical analysis

Data were analyzed using ANOVA and Fisher's LSD with SPSS statistical software. Statistical significance refers to *P* < 0.05. Principal component analysis (PCA) was carried out between all above studied parameters at different times of the composting process.

3. Results and discussion

The elemental compositions and the atomic ratios of the humic acids extracted from sewage sludge compost at different stages of composting are reported in Table 2. The levels of C show some increase at the intermediate stages of composting and then decreased to the initial level at the end. This could be attributed to the release, during the phase of high decomposition, of some aliphatic compounds from other recalcitrant structures such as lignin. These aliphatics are decomposed later during composting. Sánchez-Monedero et al. [15] reported that in the course of composting, other materials such as lignin degradation residues are released and could behave in a similar way to the humic acids, and precipitate at pH 2.

The level of H and H/C decreased with the composting process, which could be attributed to an increase of aromatic or a decrease of aliphatic structures (Table 2). Garcia et al. [16] attributed the increase in the C/H ratio to an increase of the proportion of aromat-

Table 3
The main absorbance bands in FTIR spectra and their assignments.

Bands and peaks (cm ⁻¹)	Assignments
3300–3400 cm ⁻¹	H-bonded OH groups of alcohols, phenols and organic acids, as well as H-bonded N-H groups
2920–2930 cm ⁻¹	C–H stretching of alkyl structures
1660–1600 cm ⁻¹	Aromatic and olefinic C=C, C=O in carboxyl; amide (I), ketone and quinone groups
1550 cm ⁻¹	Amides II, aromatic C=C
1450 and 1380 cm ⁻¹	OH of phenols, COO ⁻ , –CH ₃ , amide II
1260–1200 cm ⁻¹	Amide III or aromatic ethers C–O–C
1070–1030 cm ⁻¹	–C–O–C of carbohydrates, aromatic ethers, Si–O–C groupments

The interpretations are based on data of numerous studies [1,23,34].

ics with composting. The O% decreased initially and then increased in the later stages. It seems that some readily decomposed compounds rich in oxygen such as polysaccharides are degraded in the early steps, while other, more oxidized, recalcitrant compounds are neo-formed in the course of humification or show increased intensity in the later stages of composting. Castaldi et al. [17] reported that during composting, a reduction of the aliphatic C took place with a progressive transformation of the polysaccharides to other oxygenated compounds, particularly carboxylic and ester groups, with an increase of aromatic structures suggesting the partial formation of neo-humic polymers. So, different oxidized compounds occurred in the raw and in restructured humic acids after composting. The levels of N and S increased with composting. The C/N ratio fell significantly, supporting our earlier suggestions, and those of other authors, concerning the richness of waste in N, becoming concentrated in stable N-rich structures as composting progressed [6,17]. Some authors showed the presence of numerous unidentified nitrogenous structures whose intensity increased during humification [18–20]. These authors suggest that the structures may derive from residual plant compounds or fungal and microbial remains that have been physically or chemically protected in humic structures against further microbial degradation.

The evolution of FTIR absorbance of humic structures (Fig. 1, Table 3) was followed by establishing the ratios between the main absorbance peaks (Table 4). The data from these ratios show an increase mainly of the intensity of structures absorbing at 1384, 1034, and 1544 cm⁻¹ (in decreasing order) with a relative decrease of absorbance at 2925 and 1235 cm⁻¹ as composting progresses. After composting, this suggests a richness of humic structures in etherified and peptidic compounds and a loss of readily available aliphatic compounds from these macromolecules.

The assignments of the main resonance areas in ¹³C NMR spectra (Fig. 2) are illustrated in Table 5. The integrated area of ¹³C NMR

Table 4
Ratios between main absorbance peaks of FTIR spectra.

	1652/2925	1652/1543	1652/1384	1652/1235	1544/2925	1544/1384	1384/2925	1034/2925	1034/1235
RM	2.09 a	1.55 a	2.29 a	2.13 a	1.35 a	1.48 a	0.91 b	1.61 b	1.64 ab
15	1.94 b	1.40 c	1.86 b	1.90 b	1.38 a	1.33 b	1.04 a	1.57 b	1.54 c
60	1.96 b	1.43 bc	1.86 b	1.96 b	1.37 a	1.30 b	1.06 a	1.63 b	1.63 bc
135	2.13 a	1.49 ab	1.97 c	2.13 a	1.43 a	1.32 b	1.08 a	1.73 a	1.73 a

Data with the same letter within the same column do not differ significantly at the 5% level according to the least significant difference test. RM: raw mixture.

Table 6
Levels of alkyl, O/N alkyl, aromatic, and carboxylic carbon of humic acids after different stages of composting of the sewage sludge-green waste mixture.

Stage of composting	C-alkyl	O/N-alkyl	C-aliphatic	C-aromatic	C-carboxyl	Aroma/aliph
RM	8.7	19.8	28.5	48.4	23.1	1.7
15	26.4	31.5	57.9	16.8	25.3	0.23
60	46.7	20.1	66.8	12.5	20.7	0.19
135	16.4	13.7	30.1	45.6	24.3	1.51
LSD	0.54	0.42	0.92	0.66	0.44	–

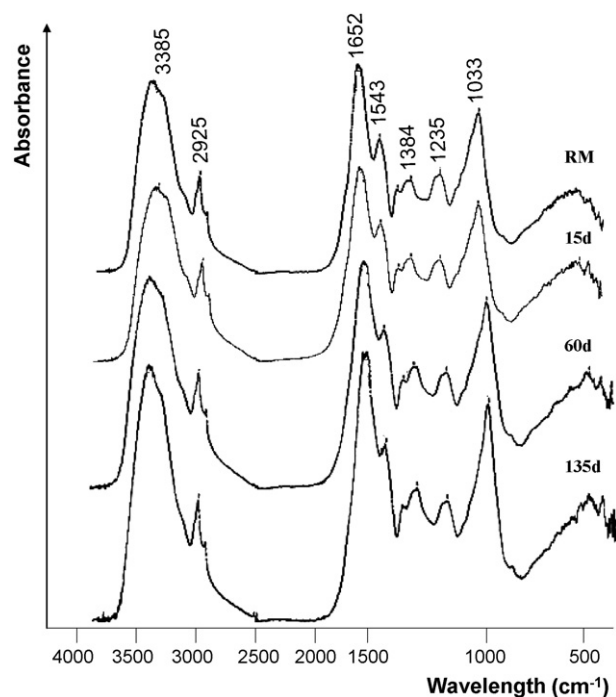


Fig. 1. FTIR spectra of the humic acids isolated from the composted sludge-green waste mixture at different stages of treatment.

Table 5
Resonance signals in ¹³C NMR spectra and attributed chemical groups.

Signal (ppm)	Attributed chemical groups
0–50	Paraffinic C in alkyl chains
50–110	Aliphatic carbons substituted by oxygen and nitrogen, around 78 ppm mainly arised the carbohydrates
110–130	Olefinic carbons; unsubstituted aromatic C
130–145	Carbon-substituted aromatic carbons
145–160	Oxygen or nitrogen substituted aromatic carbons
160–200	Carboxylic carbons, ester or amide

The interpretations are based on data of numerous studies [1,23,34].

(Table 6) showed, early on in composting, the increase of aliphatic carbon with a relative decrease of aromatic carbon, but at the end of composting the opposite had occurred. The end product contained twice the initial values of alkyl carbon, while aromatic carbon was slightly decreased, but remained high compared to other carbon

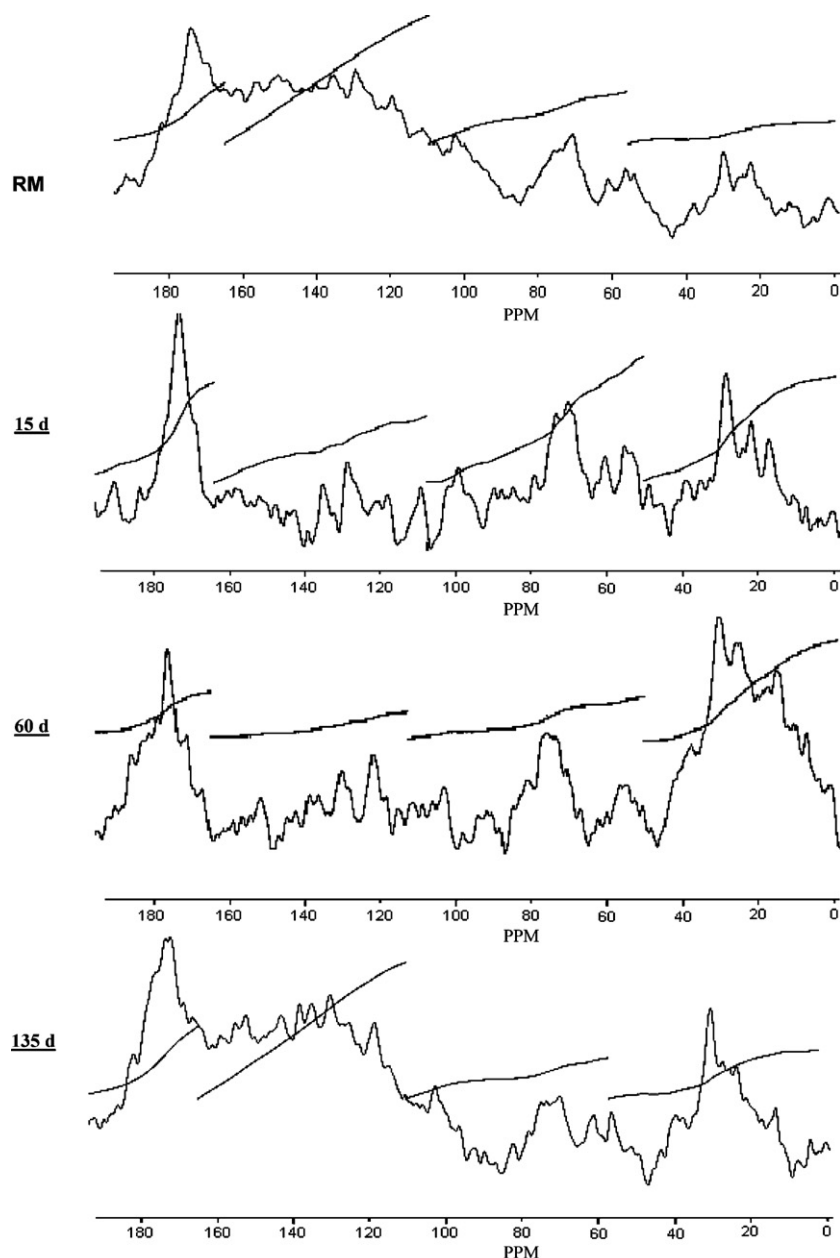


Fig. 2. ^{13}C NMR spectra of the humic acids extracted from the composted sludge-green waste mixture at different stages of treatment.

types. This is an agreement with the results found by many authors such as Sánchez-Monedero et al. [15] who suggest that during composting the extensive degradation suffered by the main organic components (lignin, cellulose, hemicellulose and proteins) leads to the release of a great variety of simple organic compounds such as carbohydrates, amino acids, simple peptides and phenols of low structural complexity. These can be then either degraded by the microorganisms as a source of carbon and energy or used by the microorganisms for the synthesis of new, more humified, materials. Granit et al. [21] reported that white-rot fungi, isolated and identified from thermophilic composts, are capable of degrading and transforming refractory substances such as lignin and humic acid. Indeed, aromatization correlates well with oxidation processes [22]. Sánchez-Monedero et al. [15] found significant correlations between phenols and the humification indices HAC/TOC and the HAC/EXC ratios, whereas no significant correlations were recorded with the carbohydrate fraction. Chefetz et al. [23], following laccase during composting, reported that HS formation involves condensa-

tion or polymerisation of quinones or of another free radical phenol 3,5-dimethoxy-4 hydroxyacetophenone originating from the oxidation of polyphenol. These latter molecules can undergo self- or cross-coupling to form oligomers [24].

A well-fitted relationship has been demonstrated between the ratio of aromatic/aliphatic carbon (^{13}C NMR) and the ratios obtained from FTIR absorbances 1652/2925 (linear $R^2 = 0.89$; polynomial $R^2 = 0.92$); 1652/1235 (linear $R^2 = 0.91$; polynomial $R^2 = 0.92$); 1652/1384 (polynomial $R^2 = 0.94$); 1544/2925 (polynomial $R^2 = 0.86$); 1544/1384 (polynomial $R^2 = 0.8$). Indeed, Castaldi et al. [17] attributed the increase from 0.922 to 1.104 of the ratio 1650/2930 in the FTIR spectra of HA to an increase of aromatic C/aliphatic C in course of composting. In our case, this ratio decreased at the initial times of composting and then increased. These changes confirm the above suggestions on the increase in the medium during the initial phase of intense biodegradation of the large amounts of aliphatic compounds. The subsequently formed structures could either be released through degradation of lignins

Table 7
The aromatic to aliphatic index by ^{13}C NMR of HA + FA (I ar/al) and proportion of decomposition obtained after current activated sludge composting (AS), as well as in previous composting trials, and after aerobic digestion of olive mill wastewater.

	AS	LS	Tan (Ca^{2+})	Tan (NH_4^+)	OMWS
I ar/al raw waste	1.83	1.90	0.45	0.87	1.23
I ar/al compost	1.82	2.62	0.92	1.12	1.79
% In	-0.73	37.8	104.7	29	45.5
% Dec	60.8	40	27.4	18.5	56

AS: activated sludge composting; LS: lagooning sludge + straw [6,33]; Tan (Ca^{2+}): tannery sludge neutralised by lime; Tan (NH_4^+): tannery sludge neutralised by ammonia (data not published); OMWS: olive mill wastes plus straw [28].

or could originate from microbial tissues [25]. These aliphatic structures then decomposed during the last step of composting due to the activities of other microbial communities proliferating after the death of the original ones [26,27].

Comparing this trial to our previous composting studies (Table 7), it can be seen that all the others show an increase in the aromaticity/aliphaticity index of (HA + FA) with composting. In contrast, during composting of activated sludge (AS) the ratio I ar/al did not show significant change and also, in this trial, intense biodegradation largely predominated – high % of decomposition of about 60% – compared to other composting trials where the values were of the order of 18.5–27.4% in tannery waste composting and 40% in lagooning sludge composting. The biodegradation process in activated sludge composting remained higher in the maturation phase (increasing from 29.3 in the stabilization stage to 60% at the end of the maturation step) in contrast to other trials (LS: lagooning sludge from 36% to 40%; tannery waste (Ca^{2+}): from 21% to 27.4%; tannery waste (NH_4^+): from 7.4% to 18.5%, olive mill waste sludge: from 44% to 56%). This suggests that degradation of substrate continues in this later step of activated sludge composting which maintains unusually high temperatures over a large part of this phase [14]. The increase of ash content in the compost indicates a mineralization of organic components during the whole period of composting (Table 1). Except in the composting of tannery sludge neutralised by lime or composting of activated sludge, a linear relationship was found between In % and Dec % which are positively correlated ($R^2 = 0.998$), even in olive mill waste plus straw, where high levels of decomposition and humification have been recorded [28]. Thus, in these trials both the biodegradation and humification processes are approximately at the same level in each compost. So, the “biodegradation and humification” processes are dependent on the structures or the compositions of the initial waste to be composted.

Indeed, it may be thought that the increase of aliphatic components and carbon percent in the intermediate step originates from heterogeneity although great attention was focused on obtaining representative samples. However, the steady variation of some parameters and the stability of fulvic acid ash (2.3%) after the initial step of composting suggests that the fluctuations are not random or caused by heterogeneity of sampling. It most likely originates from the release of compounds during the intense decomposition. This intense decomposition which continues even in late steps of composting is apparently due to the presence of more abundant easily assimilated components in the activated sludge compared to in the substrate of our previous trials.

Vincelas-akpa and Loquet [29] suggest that the humification process depends on the balance between mineralization, polycondensation and neosynthesis. These authors showed more mineralization of the organic fraction during the first month of vermicomposting. At the end of composting, a net production of aromatic compounds, as well as neosynthesis of polysaccharides occurred particularly in the vermicompost. Wei et al. [30] reported that mixed inoculation of MSW with complex microorganisms plus ligno-cellulolytic fungi during composting gave a greater degree of HA aromatization and humification than inoculation either with complex microorganisms or ligno-cellulolytic

organisms alone. Vargas-Garci et al. [31] report that despite *in vitro* studies, the results show that inoculation contributes to the degradation process if good microbial selection is made, i.e. selecting a combination of microorganisms showing different abilities in relation to ligno-cellulose degradation and humification. Other authors, like Steger et al. [32], reported that temperature was an important selective factor for the development of Actinobacteria populations in late stages of composting. They showed that the actinobacterial PLFAs reached higher values towards the end of composting in trials where the maximum temperature reached was only 40–55 °C, compared to a trial where it reached 67 °C. In the current experiment, the maximum temperature reached was 72 °C, so, these microbial entities, considered by many authors [26,27] to be responsible for humification processes, could be only weakly represented at the end of our compost. Future research in this subject must be carried out to confirm these hypotheses.

PCA analysis was carried out between all parameters studied in the course of composting (Fig. 3) to demonstrate the correlation between parameters of biodegradation and humification. The majority of the parameters are located in the circular-arc, this explains that they are highly correlated and that there is a strong relationship between the way they vary. Two principal components can explain the majority of the convergence and divergence between the parameters. The first component PC1 (49.75%) separates two groups of parameters, the first gathering C.Org, NH_4^+ , C/Nhum, C/N, H/C, FA, O-/N-alkyl represents the structures which undergo decomposition. Indeed, these parameters correlate negatively with decomposition (% of decomposition on the negative side of axis PC1). The second group containing parameters such as HA, N content, NTK, 1544/2925, NO_3^- and S are attributed to the stable neo-formed structures or the structures

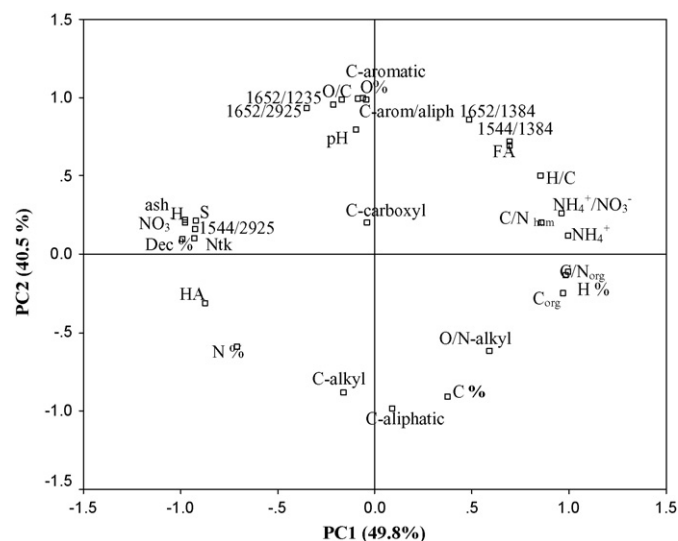


Fig. 3. Principal components analysis between all the physico-chemical and humic parameters followed during activated sludge composting.

remaining after biodegradation during composting. They correlate positively with decomposition (they are present on the same negative side). The aromatic to aliphatic index which is near to zero on axis PC1, it is located high on axis PC2, and it is positively correlated with % oxygen, but negatively with aliphatic carbon located on the same axis but on the opposite side. The last component (axis) which presents (40.5%) shows a negative correlation between parameters HA, N%, C-aliphatic (C-alkyl, O/N-alkyl) versus C-aromatic, aromatic/aliphatic degree, O%, O/C, FA and the other groups of parameters including ratios of humification (1652/2925; 1652/1235; 1652/1384, 1544/1384). Both HA and FA levels are located on the circular arc, but on opposite sides. This demonstrates that they are negatively correlated, so change in opposite directions during composting.

This suggests that during composting, the aliphatic components (O/N alkyl) in the FA fraction suffer microbial attack which leads to the increase of the intensity of oxidized aromatic structures in this fraction [13]. In contrast, the humic fraction appeared to be less decomposed or varied, but it was more enriched in some alkyl structures containing N elements. In fact, the appearance of N-aliphatic structures in the course of composting has been reported in previous works [33]. Many authors have proposed a microbial origin for certain aliphatic polymers [34,35]. Moreover, depolymerisation, occurring during the decomposition of the organic structures of a compost, could bring about an increase in the prevalence of peripheral alkyl structures.

4. Conclusion

During composting of activated sludge, humic acid structures undergo profound changes. This has been demonstrated by a number of chemical analyses (elemental composition, FTIR, ^{13}C NMR) showing that, in the intermediate step of composting, a large release of aliphatic compounds occurs. The latter are produced either through degradation of lignin structures or from microbial tissue proliferation in the initial step. The aliphatic compounds then decomposed. However, at the end of composting, the levels of N and S increased with alkyl carbon exhibiting twice the initial values, the O/N-alkyl carbon decreased, and aromatic carbon was slightly reduced but remained high in comparison to other forms of carbon.

PCA analysis revealed a positive relationship between oxygen and C aromatic, but a negative one between oxygen and C aliphatic. This agrees with the aromaticity correlating well with oxidation. So, different oxidized compounds occur between the raw and the restructured humic acids after composting. After composting, the other stable N-rich structures – alkyl, peptidic and oxidized recalcitrant compounds – composed partially humic polymers.

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