

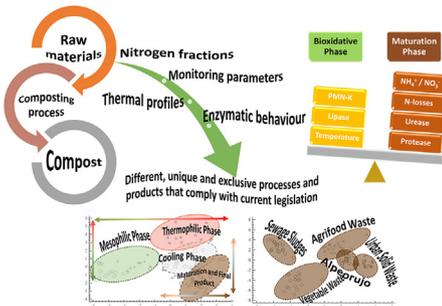


Evaluating the influence of raw materials on the behavior of nitrogen fractions in composting processes on an industrial scale

M.J. Estrella-González, J.A. López-González, F. Suárez-Estrella*, M.J. López, M.M. Jurado, A.B. Siles-Castellano, J. Moreno

Department of Biology and Geology, University of Almería, Agrifood Campus of International Excellence (ceiA3), Center for Research in Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), 04120 Almería, Spain

GRAPHICAL ABSTRACT



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ABSTRACT

Success of composting as an ecological technology for organic waste management has allowed its implementation in the current circular economy models. However, composting on an industrial scale often shows drawbacks and peculiarities. In this work, a comparative analysis of 15 industrial composting facilities was carried out in which different anthropogenic organic waste were processed. Results showed that composting process on an industrial scale did not always evolve in a standard way. Monitoring parameters as well as enzymatic activity depended largely on the raw materials and were strongly linked to the transformation of nitrogen fractions. Despite the heterogeneity of the processes and raw materials, microbial activity managed to the optimal biotransformation, obtaining products that comply with the agronomic quality standards. This work represents a breakthrough in composting and provides new knowledge for better management of this process on an industrial scale.

1. Introduction

Due to a great diversity of activities of anthropogenic origin, large quantities of organic waste are generated and accumulated today. This excessive production requires a proper management. In some cases, their elimination has been carried out through incineration, which is a

serious economic and environmental problem (Wei et al., 2019). To address these and other environmental sustainability problems, the concept of circular economy, although not entirely new, has recently gained importance. This is evident in the comprehensive European circular economy package of December 2015 (Geissdoerfer et al., 2017; Malinauskaite et al., 2017), since in regards to the management of

* Corresponding author.

E-mail address: fsuarez@ual.es (F. Suárez-Estrella).

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organic waste, composting stands out as the alternative closer to the previous economic model. However, one of the main problems arising from the composting of anthropogenic organic waste on an industrial scale is the heterogeneity of the starting materials, as well as the inadequacy of the starting conditions, or the inappropriate practices related to the mixing of fresh materials. Therefore, although composting is positioned as one of the most important tools for the future of biological waste management (EU Waste Law, 2018), the application of unstable and immature compost could release toxic substances, immobilize nitrogen in the soil and restrict the growth of plants that compete for oxygen in the rhizosphere (Guo et al., 2012).

Taking into account the above, monitoring the composting process is essential to obtain high quality products. So, the surveillance of the industrial processes is generally based on the study of parameters that allow to control the proper evolution of the process, while biological stability and maturity are generally evaluated in the final product (Cesaro et al., 2019).

Traditionally, some compost parameters have been considered to successfully evaluate the maturity and stability of the compost such as temperature, odor, color, C/N ratio or cation exchange capacity (Barrena et al., 2008; Iglesias-Jiménez et al., 2008). Nowadays, other parameters correlated with microbial biodiversity during composting are being taken into account (Mondini et al., 2004; Ayed et al., 2007; Vargas-García et al., 2010).

Nitrogen is one of the most important nutrients that control net primary production in ecosystems (Jones et al., 2004). In this sense, it would be logical to think that the relative populations of autotrophic and heterotrophic microorganisms inside a composting pile depend largely on the C/N ratio available to the microbial community (Sepehri and Sarrafzadeh, 2018). Thereby, since the transformation of the nitrogen fractions during composting is very complex and the improper management of the process could be accompanied by significant losses of nitrogen (Bao et al., 2008; Zhu et al., 2019), this work has focused on the study of a group of physicochemical and enzymatic parameters closely related to the transformation of the nitrogen fractions during composting on an industrial scale, and how the heterogeneity and diversity of raw materials could affect the standard evolution of them and the quality of the products obtained. Thus, the following objectives are established: (i) observe the evolution of the composting processes of organic remains of anthropogenic origin, based on indicators of maturity and stability related to the dynamics of the nitrogenous fractions (ii); establish the relevance of the enzymatic activity of microbial origin within composting piles in relation to the biotransformation of the different nitrogenous fractions and (iii) determine the possible differences or similarities between very different industrial composting processes, clarifying the possible correlation between physical-chemical and biological parameters related to the transformation of nitrogen fractions.

2. Material and methods

2.1. Sampling strategy

Samples were taken from 15 companies dedicated to the industrial stabilization of organic matter from different raw materials and operating procedures. Three companies dedicated to the composting of each of the following anthropogenic wastes were selected: Vegetable Waste (VW), Urban Solid Waste (USW), Sewage Sludges (SS), Agrifood Waste (AW) and olive mill waste named "Alpeorajo" (ALP). All of them were located at the Southeast of Spain (Almería, Granada, Murcia, and Jaén). Table 1 details the operating conditions and the composition of the mixtures used in each installation.

Samples were collected in each facility at significant composting phases as follows: mixture used for composting (Raw Materials, RM); material before reaching thermophile temperatures (Mesophilic phase, MES); material at thermophile temperatures (> 50 °C) (Thermophilic

phase, THER); material after the thermophilic phase, when the temperature decreases to environmental values (Cooling phase, COOL); material at the middle of Maturation phase (MAT); and Final Product (FP). Due to the seasonality of the raw material, a total amount of 90 samples were collected from November 2016 to November 2017. The entire sampling process took place on the same day, in those facilities where work was carried out continuously, i.e., plant waste (VR), sewage sludge (SS) and municipal solid waste (MSW). However, in those facilities where agrifood waste (AW) and alpeorajo (OMW) were processed, work was done discontinuously due to the seasonality of the raw materials. In this last case, the sampling was performed when each specific composting process reached the corresponding thermal phase.

Composting piles had variable dimensions depending on composting method. Most of them were about 3 m high, 7–15 m length and 4–5 m width. To collect representative samples in each thermal phase of the processes, 300 g of each material were taken from nine different locations, covering various depths and surfaces of the composting piles. Three samples were taken superficially (0.5 m), other three were taken at a depth of 1.5 m and the last three as close as possible to the bottom of the piles. They were mixed in equal amounts, to obtain a homogeneous and representative sample (approximately 3 kg) and, subsequently, they were divided into three parts to work on three analytical repetitions. In the case of USW samples, all inadequate materials (plastic, glass, metal, etc.) were removed manually, only the biodegradable fraction (organic matter) was analysed. After sampling, materials were crushed in Moulinex Cousine Companion HF800A13 (Moulinex, Barcelona, Spain) before analysis. The samples were immediately stored in vacuum bags and frozen at -20 °C. These were thawed at room temperature for 24 h before analysis. The parameters studied were: Moisture (M), pH, Electrical Conductivity (EC), Organic Matter (OM), ashes (A), C/N ratio, $N-NH_4^+$, $N-NO_3^-$, Total Proteins (TP), Loss of Nitrogen (N-Losses), Relationship $N-NH_4^+/N-NO_3^-$ (Nitrification Index, NI), Proteolytic Activity (PRO), Urease Activity (URE), Alkaline Phosphomonoesterase Activity (PMN-K) and Lipolytic Activity (LIP) (see Sections 2.2 to 2.4). Based on the above, the experimental design used for this work resulted in the analysis of 270 samples.

2.2. Monitoring parameters of the composting processes on an industrial scale

The temperature inside the piles was measured *in situ* with a long-handled PT100 temperature probe, according to the selected sampling points. When necessary, temperature data were taken and provided by authorized company personnel, who had previously received instructions. The moisture content was determined by drying at 105 °C for 24 h. The pH and the conductivity were analysed in a 1:10 (w/v) water extract. Bulk density was measured according to the US Composting Council (2001). Ash content was calculated by ignition at 550 °C to a constant weight. On the other hand, organic matter (OM) content was determined by subtracting percent ash content from one hundred. Total carbon (C) and nitrogen (N) were determined in solid samples by dry combustion at 950 °C using a LecoTruSpec C–N Elemental Analyzer (Leco Co., St. Joseph, MI, USA).

2.3. Measurement of total proteins, $N-NH_4^+$, $N-NO_3^-$, nitrification index (NI) and N-losses during the composting processes on an industrial scale

For the measurement of nitrates, the wet sample was diluted 1:5 in distilled water. It was incubated for 30 min at 200 rpm. After the incubation period, the samples were filtered under vacuum. The resulting extract was used for the measurement of nitrates with the Nitracheck 404 system (KPG, Products Ltd., Hove, United Kingdom). For evaluation of ammonium content, the fresh sample was diluted 1:10 in distilled water and incubated for 30 min at 200 rpm. After this period, the sample was filtered under vacuum. To the supernatant, 0.1 M $MgSO_4$

Table 1
Characteristics of the industrial-composting processes.

Organic Waste	Code	Method of composting	Mixture for composting	Time ^a (months)	Temperature ^b (°C)
Vegetable Waste (VW)	1	Open air- Turned windrows	Mostly cucumber and zucchini crop residues: stalks, leaves	4	70
	2	Open air- Turned windrows	Mostly cucumber, zucchini crop residues: stalks, leaves	4	60
	3	Open air- Turned windrows	Mostly pepper crop residues: stalks, leaves	3	65
Urban Solid Waste (USW)	1	in-vessel Turned windrows in bays	Urban solid waste ^c	3.5	52
	2	in-vessel Turned windrows in bays	Urban solid waste ^c	4.5	55
	3	in-vessel Tunnel composting (turning by endless screw)	Urban solid waste ^c	3	50
Sewage Sludge (SS)	1	Open air- Turned windrows	Sewage sludge + straw (1:1 v/v)	3.5	48
	2	Open air Turned windrows	Sewage sludge + pruning wastes (1:1 v/v)	3	50
	3	in-vessel- Tunnel composting (turning by endless screw)	Dried sewage sludge (1:2 v/v)	3	52
Agrifood Waste (AW)	1	Open air- Turned windrows	Citric sludge ^d + palmtree prunings (1:3 v/v)	8	47
	2	Open air- Turned windrows	Cull tomatoes + tomato plant (stalks and leaves)	6	50
	3	Open air- Turned windrows	Citric sludge ^d + pig slurry + palmtree prunings (3:1:1.5 v/v)	4	55
"Alpeorujo" (ALP)	1	Open air -Turned windrows	Alpeorujo + chicken manure + straw (20.2:3.6:1 w/w)	8	50
	2	Open air Turned windrows	Alpeorujo + olive leaves + manure (12.5:3.5:1 w/w)	7	52
	3	Open air Turned windrows	Alpeorujo + manure + olive leaves (1:0.45:unknown w/w)	5	61

^a Total time of composting process.

^b Temperature of thermophilic phase.

^c USW: All facilities processed mixed urban solid waste.

^d Citric sludge: semi-solid residue in the form of juice centrifugation pulp obtained as waste products.

was added in a 1:10 ratio. The ammonium concentration was measured using the reference electrode Ref. 5044 (HACH, Loveland, Colorado, USA) and the ammonium ISE Ref. HI4101 (HACH, Loveland, Colorado, USA). The analysis of total proteins was carried out from an extract obtained in the following way: 10 g of sample were mixed with in 40 mL of 0.5 M K₂SO₄, and agitated at 200 rpm for 30 min. Final suspension was filtered through filter paper and the total proteins were analysed with Folin Ciocalteu reagent according to the method described by Herber et al. (1971).

The N-losses was estimated according to the following equation proposed by Cayuela et al. (2006) and Jolanun and Towprayoon (2010):

$$N - \text{losses (\%)} = 100 - 100 (A_1 \times N_2) / (A_2 \times N_1)$$

Where:

$$A_1 = \text{initial ash}; A_2 = \text{final ash};$$

$$N_1 = \text{initial N}; N_2 = \text{final N};$$

The Nitrification Index was calculated as the ratio of N-NH₄⁺/N-NO₃⁻ (Bernal et al., 1998).

2.4. Analysis of the enzymatic activities involved in the degradation of the nitrogen fractions

Fresh material was used to perform the enzymatic analyses. Protease activity was measured from the tyrosine derivatives generated from 1 g of sample after the incubation with sodium caseinate for 1 h at 37 °C and the subsequent reaction with Folin Ciocalteu reagent (Ladd and Butler, 1972). To determine Urease Activity, the method described by Bremner and Mulvaney (1978) was used. Thus, 2 g of sample were incubated in the presence of a basic solution of sodium hypochlorite and phenol with nitroprusside, as a catalyst, at 37 °C for 1 h. Ammonia was estimated by the chromogenic complex produced.

The method described by Farnet et al. (2010) was used to quantify Lipase activity. This activity was estimated from 0.5 g of fresh sample. The protocol was based on the colorimetric estimation of the p-nitrophenol (PNP) formed by the hydrolysis of the p-nitrophenyl-laureate (pNPL) after mixing with the sample and incubating at 30 °C for 2 h. PMN-K activity was carried out following the method described by

Tabatabai and Bremner (1969). In this protocol, Alkaline Phosphomonoesterase Activity was estimated using 0.5 g of sample. This reaction was based on the colourimetric estimation of the p-nitrophenyl phosphate (PNPP) released by the hydrolysis of the p-nitrophenyl-β-D-glucopyranoside (PNG) at 37 °C for 1 h. Spectrophotometric measurements of all enzymatic activities were measured with an Eon Biotek model spectrophotometer (Winooski, VT, USA) using 96-well microplates. Wavelengths used in these cases were 640 nm for protease and urease activities, and 400 nm for lipase and PMN-K activities.

2.5. Statistical analyses

Data obtained were subjected to statistical analysis using Statgraphics Centurion XVI.I (StatPoint Technologies Inc., Virginia, USA). Analysis of variance (ANOVA) and multiple comparison tests (Least Significant Difference Fisher Test) were performed to compare mean values for the different factors and variables analysed (p < 0.05). A Discriminant Analysis was used to assess the adequacy of the classification in groups on the basis of the independent variables (Starting Material, Sampling Phase and Replicates). Finally, the relationships between pairs of variables were analysed by a Pearson correlation coefficient with a 99% confidence interval.

3. Results and discussion

3.1. Monitoring parameters of the composting processes on an industrial scale

In this work, 15 companies dedicated to the industrial-scale composting of organic wastes of anthropogenic origin were selected. The maximum temperature in the thermophilic stage was observed for VW processes (65 °C) (Fig. 1a). For the rest of raw materials, composting piles reached values between 50 and 55 °C in the thermophilic phase. According to the EPA (2003), during the thermophilic phase of a composting process, temperatures above 55 °C must be reached for at least 3 consecutive days in order to achieve the sanitation of materials. Taking this criterion into account, it cannot be emphatically affirmed that all the processes studied strictly comply with the thermal requirements necessary for the sanitation of the materials. However, the size of composting piles on an industrial scale does not always allow

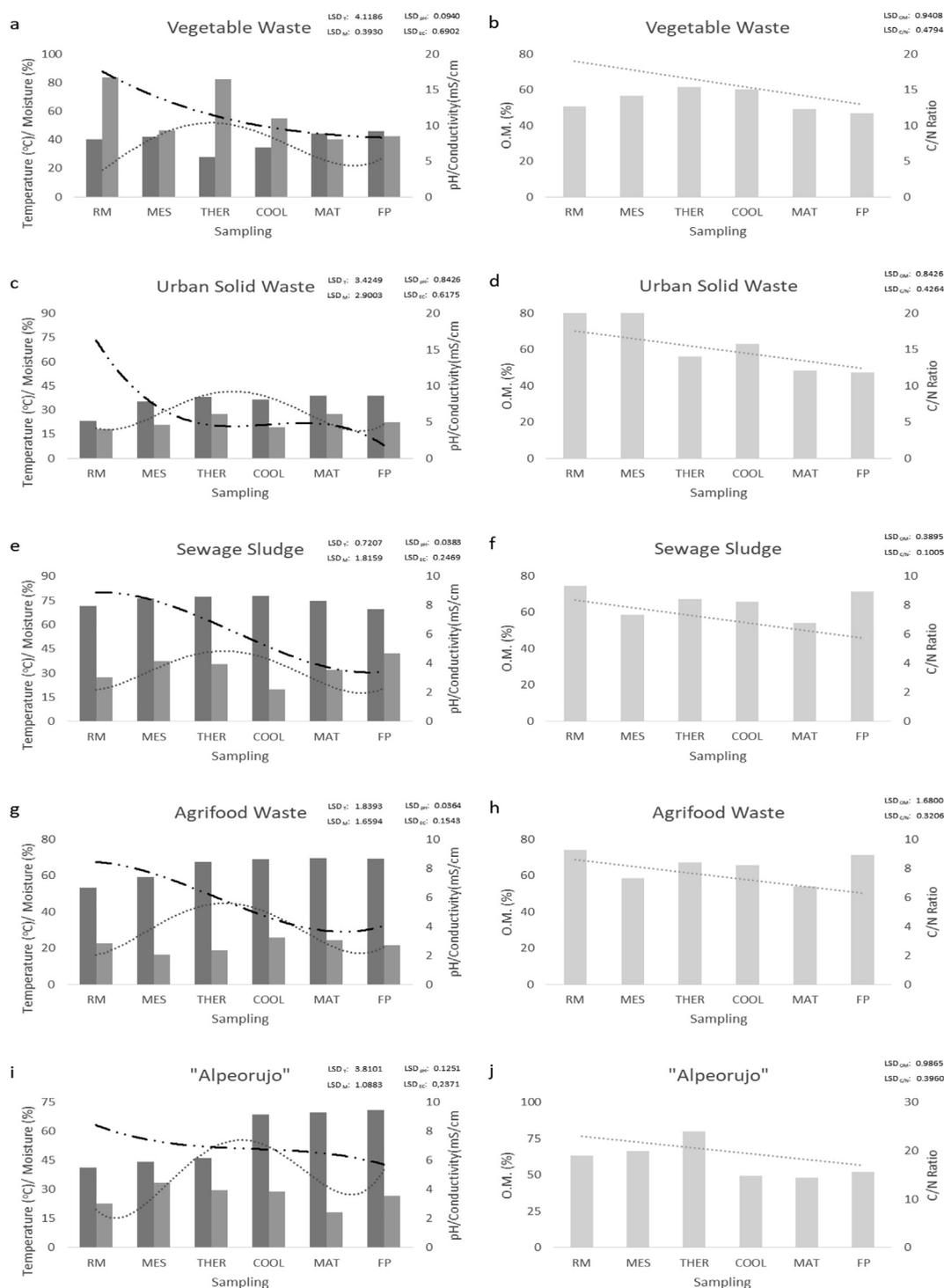


Fig. 1. Control and monitoring of composting processes on an industrial scale. Temperature (---), Moisture (— ·), pH (---), Conductivity (---), Organic Matter (---) and C/N ratio (---) throughout composting made of VW (a-b), USW (c-d), SS (e-f), AW (g-h) and ALP (i-j). Values are the mean of nine replicates. LSD values are indicated for each control parameter and raw material (Fisher LSD test at $p < 0.05$). VW: Vegetable Waste; USW: Urban Solid Waste; SS: Sewage Sludges; AW: Agrifood Waste; ALP: Alpeorujo; T: Temperature; M: Moisture, EC: Conductivity; MO: Organic Matter.

thermal data to be taken correctly, which could result in lower temperature measurements than those considered real within the piles. Anyway, the thermophilic temperatures remained active for more than three consecutive days in all the processes.

The reduction in moisture content value at the end of composting is a positive sign of decomposition and compost maturity (Ameen et al., 2016). Based on the Spanish regulation this parameter must be less or around than 40% (BOE 999/2017) at the end of the composting

process, criteria that met all the processes analyzed in this work. Along the processes, the greatest moisture loss was observed in the USW processes (85.54%) while in ALP the moisture loss was less than 33% with respect to the starting materials (Fig. 1c, i). In the rest of the processes a loss of humidity was observed between 52 and 62% with respect to the initial raw materials (Fig. 1a, e, g). Only in the case of the USW compost, the humidity values were noticeably low in relation to the rest of the samples (Fig. 1c). In all the cases, the moisture of the

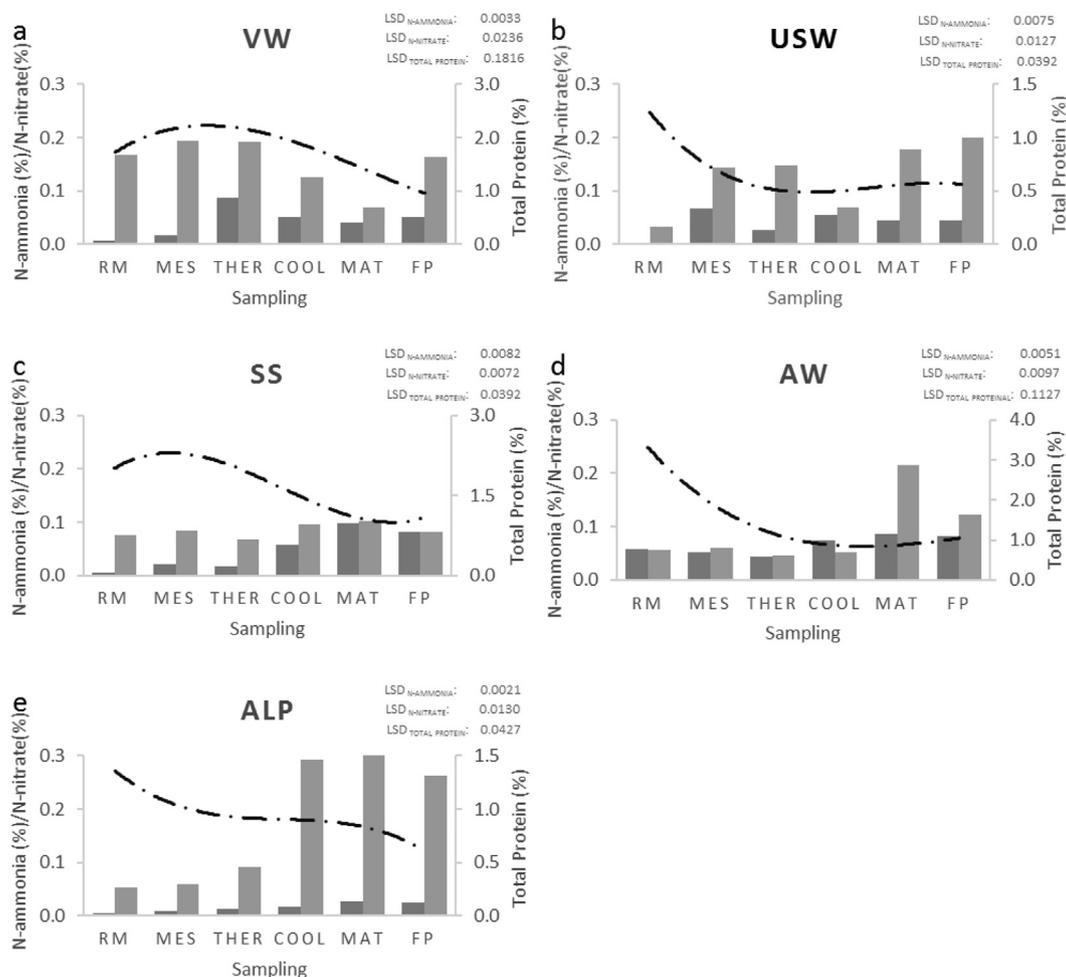


Fig. 2. Evolution of the soluble nitrogen fractions throughout the different composting processes: $N-NO_3^-$ (---), $N-NH_4^+$ (---) and Total proteins (— · —). Values are the mean of nine replicates. LSD values are indicated for each soluble nitrogen fractions and raw material (Fisher LSD test at $p < 0.05$). VW: Vegetable Waste; USW: Urban Solid Waste; SS: Sewage Sludges; AW: Agrifood Waste; ALP: Alpeorujo.

composts met the requirements indicated in current legislation.

With regard to the organic matter (OM), the greatest loss occurred in samples of VW and SS (OM loss near of 35%) (Fig. 1b, f). However, in all the processes, the requirement imposed by the Spanish regulation (BOE 999/2017) was met since OM values were around 40% (Fig. 1b, d, f, h, j). The C/N ratio tends to decrease due to the mineralization phenomena of organic matter (López-González et al., 2013) along the composting process. Initially, a C/N ratio around 25–30 is considered optimal. However, in this work, these initial values were only observed in the case of composting processes made from USW (Fig. 1d), while the rest of processes showed initial C/N ratio values lower than 20. According to Guo et al. (2012), the C/N ratio directly influences the maturity of the product obtained. However, despite the no optimal C/N ratio detected at the beginning of the different composting processes, it has been observed how the final value of the C/N ratio is adjusted to that described in Spanish regulation (BOE 999/2017) for compost (C/N ratio around 15–20).

The pH analysis of the compost samples of the different piles showed a gradual change from neutral to alkaline conditions, as described by Liu et al. (2011). In general terms, pH values at the end of the processes were higher than those obtained in the Raw Materials. Exceptionally, non-significant changes were detected from SS composting processes (Fig. 1e). Evolution of pH values for VW, USW and SS composting processes were similar to those obtained by Vargas-García et al. (2010). The pH value for ALP was similar to the results obtained by Alburquerque et al. (2011). According to Wang and Zeng (2017)

during the composting of agrifood waste the pH could increase at the end of the process due to the formation of NH_3 , being able to reach pH values around 8 or higher. These results agree with the results of this work in the case of processes elaborated based on agrifood remains.

The EC reflects the salinity of several substrates and several authors describe it as a good indicator of compost maturity (Liu et al., 2011). The highest values of EC corresponded to vegetable waste based-compost (VW) (Fig. 1a). These values could be due to the origin of the plant waste, since this type of material comes from intensive horticultural systems, where an excessive mineral fertilization is usually applied (Song and Lee, 2010). While the EC values decrease along the VW composting processes, this parameter slightly increased in the rest of the composting processes. Yadav and Garg (2011) and Liu et al. (2011) obtained the same results and indicated that the increase in EC could be due to the release of different mineral ions and the mineralization of organic matter.

3.2. Evolution of the soluble nitrogen fractions throughout the different composting processes on an industrial scale

During composting, nitrogen is distributed between insoluble and soluble fractions. The latter comprises a mixture of ammonium, NO_2^- , NO_3^- , amino acids and peptides. Proteolytic and ammonifying microorganisms prevail during the initial stages of composting, giving rise to the release of ammonium and soluble proteins, while nitrification predominates during the maturation phase (Guardia et al., 2010).

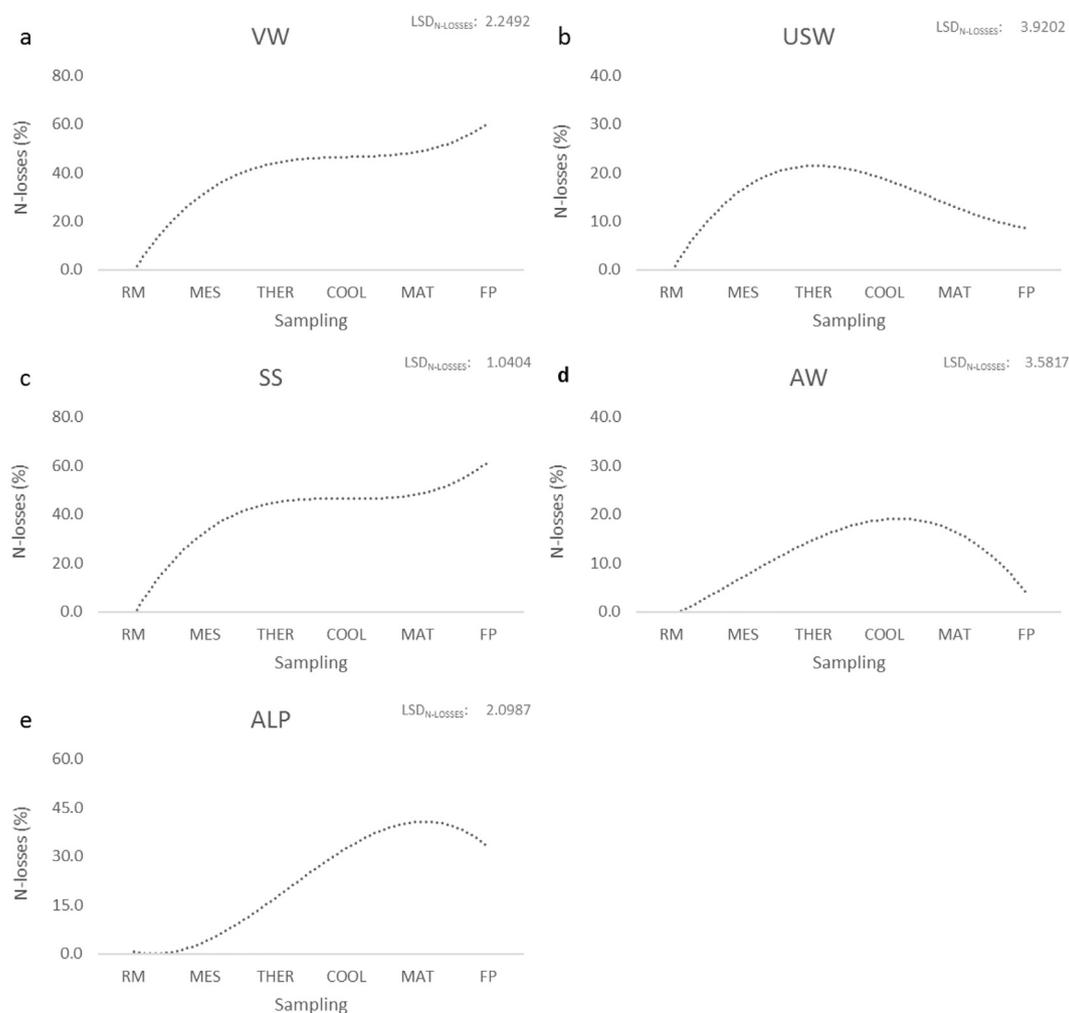


Fig. 3. Nitrogen Loss (%) throughout the different industrial-composting processes. Values are the mean of nine replicates. LSD values are independently shown for each raw material (Fisher LSD test at $p < 0.05$). VW: Vegetable Waste; USW: Urban Solid Waste; SS: Sewage Sludges; AW: Agrifood Waste; ALP: Alpeorrujo.

The results obtained for total proteins, $N-NH_4^+$ and $N-NO_3^-$ are shown in Fig. 2a–e based on each different raw material. The total proteins follow a pattern regardless of the raw material used. However, in general terms, this parameter decreased until the end of the process. The most pronounced decrease was observed in AW (Fig. 2d), where the maximum value of total proteins were also observed. These results follow a trend similar to that described by López-González et al. (2013).

On the other hand, there was a general increase in the $N-NH_4^+$ concentration at the end of the composting processes. This increase was progressive and constant for SS, AW and ALP processes (Fig. 2c, d, e), showing a more irregular behavior in the case of VW and USW processes. These values are within the same range of results obtained in López-González et al. (2013). According to Sánchez-Monedero et al. (2001) the level of $N-NH_4^+$ can be used, together with other parameters, as an indicator of the stability of the compost. In this sense, low concentrations of $N-NH_4^+$ must be detected in compost samples considered as fully mature.

Generally, during the composting process, $N-NO_3^-$ is found in higher concentrations than $N-NH_4^+$. According to Tognetti et al. (2007), $N-NO_3^-$ values increases along the composting process respect to the raw material. In Fig. 2a–e, the nitrate concentration in the different composting processes was representative for each raw material. In the composting processes described in this work, it is worth noting a significant increase in the fraction of $N-NO_3^-$ over time, although it was very noticeable in the case of the USW (Fig. 2b), AW (Fig. 2d) and ALP (Fig. 2e) processes. In the case of the VW (Fig. 2a) and SS (Fig. 2c)

processes, the increase in this fraction was almost undetectable with respect to the start of the process.

Throughout the composting process, nitrogen losses can occur due to ammonia volatilization and leaching phenomena (Zhang and Sun, 2016). Fig. 3 shows the percentage of nitrogen loss in the different composting processes. According to Sánchez-Monedero et al. (2001) the percentage of nitrogen loss depends on the initial composition of the raw material used. In this work, the largest nitrogen losses occurred at the maturation phase in the VW (61%) and SS (65%) processes (Fig. 3a, c). These results are endorsed by Sánchez-García et al. (2015), whose study analysed different mixtures that show high percentages of nitrogen loss. In the case of USW, AW and ALP processes, the greatest losses were observed in the THER, COOL and MAT phases, respectively. During the most advanced stages of maturation, nitrogen losses were less remarkable in USW and AW processes. In these last cases, the decrease in nitrogen losses could be due to the transformation of $N-NH_4^+$ into $N-NO_3^-$ avoiding losses due to ammonium volatilization (Zhang and Sun, 2016), or even to the entry of new fresh materials (this last statement is only a conjecture). Anyway, this last aspect would be a clear reflection of bad practices in composting on an industrial scale.

In recent years, many physical-chemical and biochemical parameters associated with the composting process have been studied. This study has mainly sought to combine different criteria with which to establish maturity and stability criteria for compost. One of these criteria is based on the calculation of the $N-NH_4^+/N-NO_3^-$ ratio (nitrification index) to know the degree of maturity of the compost

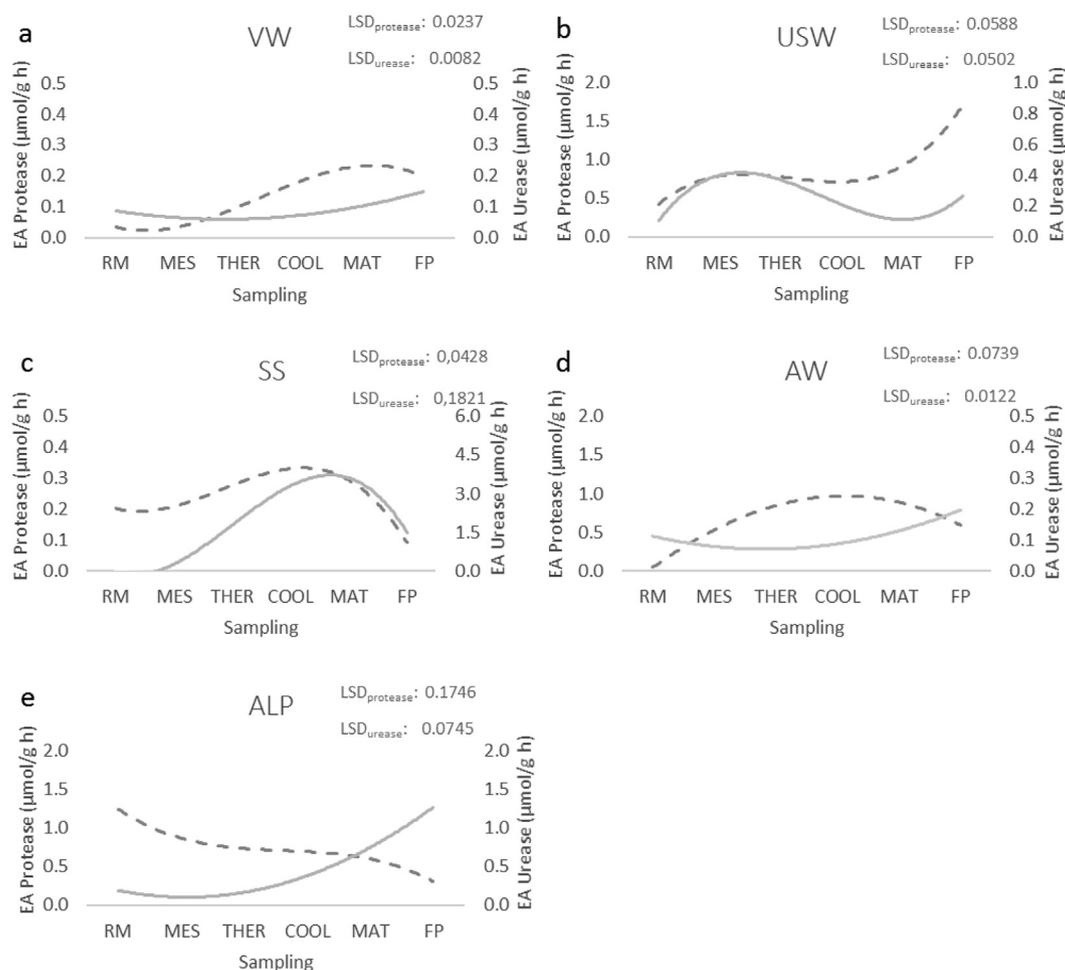


Fig. 4. Dynamics of Protease (---) and Urease (—) activities throughout composting processes made from VW (a), USW (b), SS (c), AW (d) and ALP (e). Values are the mean of nine replicates. LSD values are indicated for each enzymatic activity and raw material (Fisher LSD test at $p < 0.05$). VW: Vegetable Waste; USW: Urban Solid Waste; SS: Sewage Sludges; AW: Agrifood Waste; ALP: Alpeorjuo.

(Onwosi et al., 2017). In this same work, it was tried that the nitrification, can be an index of maturity of the compost, which should have a level as low as possible at the end of the composting process. A nitrification index value is generally used to classify the compost as fully mature (nitrification index of 0.5); mature (above 0.5 to 3.0) and immature (above 3.0). According to this classification, final products derived from VW (0.396), USW (0.225) and ALP (0.097) processes could be considered as fully mature composts, while those from SS (1.016) and AW (0.716) processes could be considered as mature composts. However, according to the stricter rules of Bernal et al. (1998) ($\text{N-NH}_4^+/\text{N-NO}_3^-$, ratio < 0.16 means fully mature products), only the products obtained from ALP processes could be considered as fully mature compost.

3.3. Enzymatic activity involved in the transformation of the nitrogen fractions during the composting processes on an industrial scale

Protease and urease activities take part in the process of nitrogen mineralization during the composting process. Both of them hydrolyze nitrogen compounds into ammonia, using low molecular weight proteins and urea as substrates, respectively (Liu et al., 2011). In general, both activities tend to increase throughout the composting process, but their evolution can vary according to the starting materials. In Fig. 4a–e, these activities are represented for each different raw material.

The highest value of proteolytic activity was detected in the USW,

AW and ALP processes while the lowest one was reached in VW and SS samples (Fig. 4). Protease activity increased over time except for SS and ALP processes where a remarkable decrease occurred at the end of the composting process. The data obtained in Fig. 4 a-d showed trends similar to those obtained by Jurado et al. (2014), while the evolution observed in the ALP samples was similar to that obtained in some of the processes studied by Cunha-Queda et al. (2007), in which the decrease in the protease activity was associated with the origin of the raw material used.

In general, the urease activity reached the maximum value in the maturation phase, except for the USW processes. The results of several investigations have been contradictory regarding the behavior of urease activity during the composting process. Jurado et al. (2014) showed how the urease activity decreased throughout the composting, while Vargas-García et al. (2010) and Sudkolai and Nourbakhsh (2017) observed that urease activity increased over time in composting processes based on plant waste and sewage sludge, supporting the results obtained in this work.

In addition to protease and urease activities, other enzymes are indirectly related to the nitrogen cycle. Phosphatases catalyse the hydrolytic cleavage of phosphoric acid esters. Depending on their pH optimum they are classified as alkaline or acid phosphatases (Linhardt and Walter, 1965). PMN-K is considered, together with acidic phosphomonoesterase, one of the predominant phosphatases in most soils and residues (Albrecht et al., 2010). Phosphatases are N-rich enzymes, which means that there is a coupling between P and N cycles (Marklein

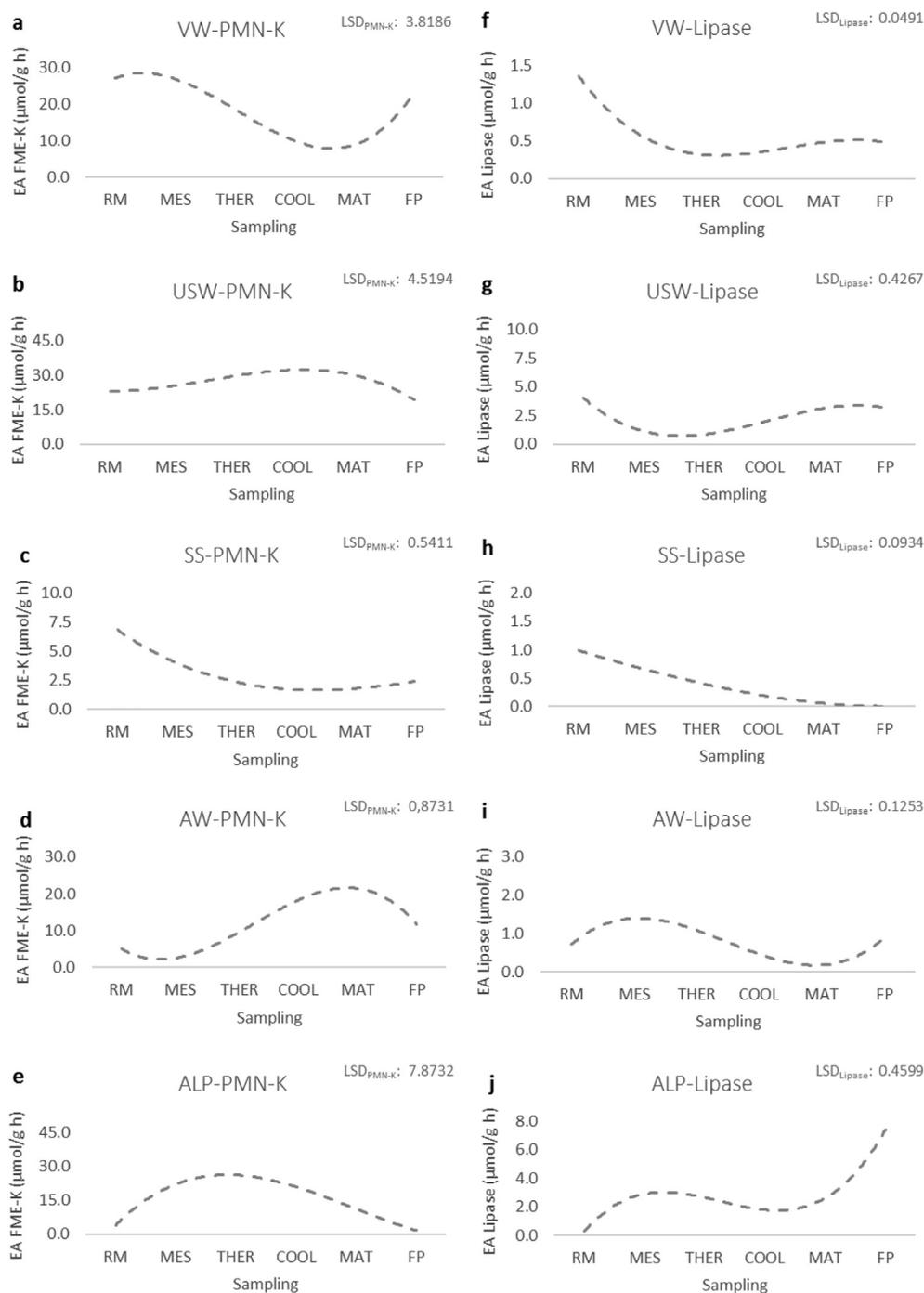


Fig. 5. Dynamics of PMN-K (a-e) and Lipase (f-j) activities throughout composting processes made from VW (a), USW (b), SS (c), AW (d) and ALP (e). Values are the mean of nine replicates. LSD values are indicated for each enzymatic activity and raw material (Fisher LSD test at $p < 0.05$). VW: Vegetable Waste; USW: Urban Solid Waste; SS: Sewage Sludges; AW: Agrifood Waste; ALP: Alpeorujo.

and Houlton, 2012). PMN-K activity showed an irregular behavior based on the different raw materials (Fig. 5a-e). Results indicated that the highest PMN-K values were detected during the biooxidative phase for VW, SS and ALP processes (Fig. 5a, c, e), while this effect was detected during maturation phase in the case of AW processes (Fig. 5d). Contrary to the above, PMN-K activity did not vary significantly when USW were processed. This irregular behavior of the PMN-K activity was supported by Jurado et al. (2014), who revealed peaks of this activity in thermophilic phase and after, at the end of the process. On the other hand, according to Cunha-Queda et al. (2007), this activity increased at the end of the process due to the nature of the raw material, as occurs with VW and AW samples (Fig. 5a, d), while Albrecht et al. (2010)

detected that the PMN-K activity trend declined as the process progressed. Microbial lipases catalyze both hydrolysis and the synthesis of long chain acylglycerols. In general, lipase production is influenced by the type and concentration of carbon and nitrogen sources and other physicochemical parameters (Andualema and Gessesse, 2012). In this work, lipase activity tended to decrease at the end of the composting process. Jurado et al. (2014) and Cunha-Queda et al. (2007) detected this same behavior. Only when the lipase activity was analysed in the ALP processes an important increase was detected during the maturation phase (Fig. 5j). This effect could be due to an accidental contribution of organic matter at the end of the process.

In order to analyse in a global way the implication of the different

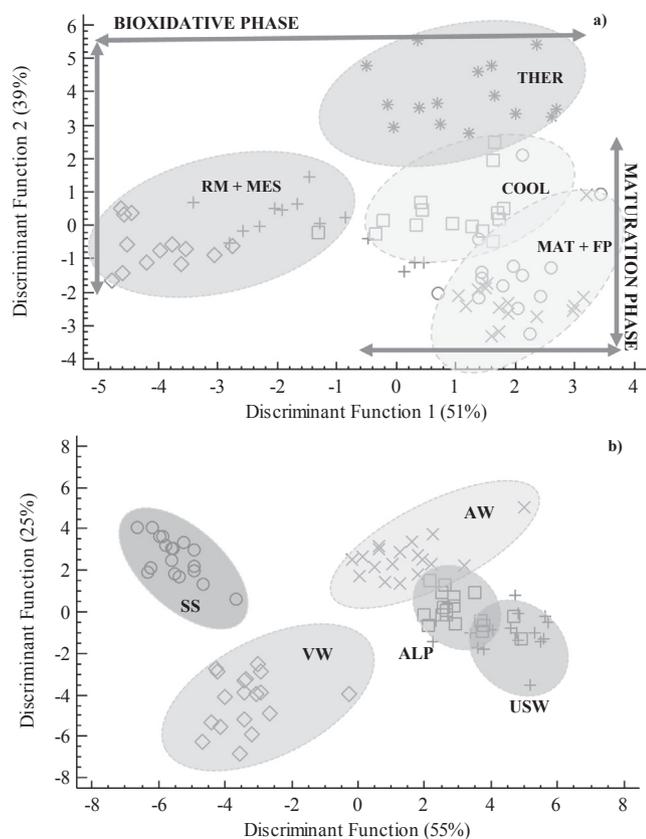


Fig. 6. Discriminant analysis based on composting of different processes, taking into account the distribution of principal thermal phases (Biooxidative Phase = RM + MES + THER; Maturation Phase = COOL + MAT + FP) a), and the raw materials b). VW: Vegetable Waste; USW: Urban Solid Waste; SS: Sewage Sludges; AW: Agrifood Waste; ALP: Alpeorujo.

enzymatic activities in the biotransformation of the nitrogenous fractions, a Pearson correlation test including the data derived from all the analysed samples was carried out. So, the temperature factor was significantly correlated with nitrogen losses ($R = 0.2319$), as well as with the production of $N-NO_3^-$ ($R = 0.2138$). In addition, the greatest losses of nitrogen occurred in parallel to the greater urease activity ($R = 0.4007$) but contrary to the protease one ($R = -0.2814$). On the other hand, the total protein content not only correlated negatively with protease activity ($R = -0.4986$), but also with PMN-K and lipase ($R = -0.2785$ and -0.3152 , respectively), which supports the proximity of both activities with the N biotransformation processes. Lipase was also negatively correlated ($R = -0.4048$) with the nitrification index ($N-NH_4^+/N-NO_3^-$) and, consequently, with lower ammonia production and higher nitrate production ($R = -0.3936$ and 0.2972 , respectively). Although protease and urease activities tend to increase throughout the composting process, it is clear that their progress depends largely on the starting materials. In fact, both activities did not correlate with each other when all the processes were analysed together. On the other hand, despite the chaotic behavior of the PMN-K activity, the results showed an important link between this activity and the two activities mentioned above, protease and urease, although in the opposite direction ($R = 0.3006$ and -0.3114 , respectively).

To analyse globally the evolution and coherence of all the composting processes, two discriminant analyses were performed (Fig. 6). In both cases, the parameters included in the analysis were temperature, moisture, pH, organic matter, C/N ratio, conductivity, protease, urease, PMN-K and lipase activities, $N-NH_4^+$, $N-NO_3^-$, total proteins, nitrification index and nitrogen losses. Fig. 6a shows the general composting profile depending on the succession of thermal phases. Two

functions explained around 90% of the discrimination in the results obtained. Data were located in two main groups: Biooxidative Phase I (RM + MES + THER) and Maturation Phase (COOL + MAT + FP). Biooxidative, stabilization and humification (maturation) phases appeared well separated (Fig. 6a). Likewise, the stage in which the highest temperatures were reached (thermophilic stage) appeared clearly defined and differentiated from the rest of the mesophilic stages (RM and MES). On the other hand, the cooling stage (COOL) appears as a very limited transition period between the biooxidative and maturation phase, as well as independent of the final products (FP). Thereby, it could be concluded that there was a temporal coherence regarding the evolution of composting processes, regardless of the starting materials (Fig. 6a). However, when a discriminant analysis was performed based on the nature of the original organic matter, two functions were obtained that served to explain up to 80% of the differences between the different composting processes (Fig. 6b). Surprisingly, although the processes elaborated from ALP and USW, showed a slight overlap, the rest of the processes appeared in the discriminant graph as totally independent groups. This fact confirmed the exclusive nature of composting processes carried out with raw materials of different nature.

In view of the results obtained, it should be taken into account that certain control parameters of the composting process, do not evolve in a standard way when it comes to composting processes on an industrial scale, which could be related to the type of raw material and the inappropriate conditioning of the initial mixtures. Consequently, both aspects could influence the evolution of the enzymatic activities related to the dynamics of the nitrogen fractions, since the behavior of these, in some cases, was random and practically specific for each type of material. These results confirm what is indicated in recent works (Estrella-González et al., 2019), in which it is concluded that very different and exclusive composting processes from the point of view of the starting materials, can be uniform with respect to the evolution of the basic stages of the composting process, obtaining compost that conform to the parameters previously established at the legislative level.

4. Conclusions

It could be concluded that the basic parameters of monitoring and the evolution of enzymatic activity depended largely on the raw materials, being strongly linked to the transformation of the nitrogen fractions. However, despite the apparent lack of parallelism between industrial composting processes performed with different anthropogenic wastes, biotransformation occurred as expected in relation to the final quality parameters. Therefore, despite the need to standardize composting practices on an industrial scale, microorganisms fulfill their function and manage to biotransform substrates of a very diverse nature, obtaining products that meet the agronomic quality standards established at the legislative level.

CRedit authorship contribution statement

M.J. Estrella-González: Formal analyses, Investigation, Writing - original draft, Visualization. **J.A. López-González:** Investigation, Resources, Writing - original draft. **F. Suárez-Estrella:** Formal analyses, Investigation, Writing - review & editing, Visualization. **M.J. López:** Conceptualization, Methodology, Resources. **M.M. Jurado:** Investigation, Resources. **A.B. Siles-Castellano:** Investigation, Resources. **J. Moreno:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2020.122945>.

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