



Research article

Enzymatic profiles associated with the evolution of the lignocellulosic fraction during industrial-scale composting of anthropogenic waste: Comparative analysis



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ABSTRACT

In the new European Waste Law, composting is proposed as one of the best options to properly manage organic waste of anthropogenic origin. Currently, the massive generation of this type of waste, as well as its heterogeneity, makes difficult in many cases control this process of degradation on an industrial scale.

In this work, 15 facilities were selected based on 5 types of organic waste: Urban Solid Waste, Vegetable Waste, Sewage Sludges, Agrifood Waste and “Alpeorujo”. The samples were collected in different thermal phases. The results revealed very different physicochemical and enzymatic profiles, as well as different degrees of humification depending on the process and the raw materials. However, parameters such as β -glucosidase, amylase, lignin/holocellulose ratio and humification rate showed similar trends in all cases. All of them could act as important indicators to evaluate the quality of a composting process, despite the heterogeneity of the starting materials.

1. Introduction

In recent decades, the amount of municipal waste and sludge from wastewater treatment facilities has increased significantly due to the exponential growth of the world population and the consumer habits of the current society. In the same way, this has led to the mass generation of other types of organic waste, such as those derived from the agricultural and agrifood industries, as well as the extraction of olive oil (Albuquerque et al., 2009; Wei et al., 2019). Currently, many strategies have been proposed for the management of organic waste (biowaste) in a more sustainable manner. The composting process is considered one of the most attractive, in this sense, because it does not entail an environmental impact and does not imply a high cost. In fact, according to the recommendation of the European Waste Law (EU Waste Law, 2018), the composting process is positioned as the first and most appropriate alternative for the treatment and revaluation of biological waste.

Therefore, in order to control the quality of composting product, based on its maturity, each country has established its own regulations on the ideal characteristics that compost must possess to consider it with enough grade for soil application. In Spain, according to BOE A-2017-14332, the final product of composting should meet the following

properties: a minimum of 35–40% organic matter, no more than 40% humidity and C/N ratio values below 15–20. Additionally, several parameters have been established to determine the state of maturity of a compost, among which are temperature, odor, color, cation exchange capacity, humified fractions and other respirometric variables (Iglesias-Jiménez et al., 2008). Different studies have also correlated this state with other factors such as enzymatic indicators or biodiversity profiles during composting (Jurado et al., 2014; López-González et al., 2015). Therefore, at a legislative level, knowing the C/N ratio, moisture and organic matter content of a certain compost is considered fundamental to determine if that product is appropriate from an agronomic point of view. However, (i) could it be thought that a compost will have a different lignocellulosic content depending on the raw material? (ii) or that the biotransformation of organic waste occurs in the same way depending on the starting materials? In summary, is the composting process dependent or independent of the different raw materials?

Raw materials used in composting are composed of both assimilable compounds and complex polymers. The most recalcitrant fractions, such as lignocellulose, must be processed enzymatically before being used by microorganisms as carbon and nitrogen sources. This fraction, whose biodegradation is important for the process of humification and stabilization at the end of the composting process, is difficult to

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transform (Insam and de Bertoldi, 2007). However, although achieving an optimal degree of degradation is decisive to reach the appropriate maturity for a quality compost, the enzymatic strategies and the humification pathways could be very different in each process.

Enzymes produced by microorganisms participate in numerous biochemical transformations of organic matter occurred throughout the process (Mondini et al., 2004). Different studies have related the enzymatic biotransformations on lignocellulosic fractions with the compost stability degree (Ros et al., 2006; Jurado et al., 2015; Villar et al., 2016). In this sense, enzymatic evolution during the composting process could be considered an indicator of the material behavior and, therefore, of the state of maturity (Castaldi et al., 2008).

Since organic waste is constituted by a significant carbon fraction, enzymes related to carbon cycle are determinant during waste biotransformation. β -glucosidase, amylase, cellulase and xylanase are some of the most determinant enzymes in degradation of polymers and other saccharides during composting. Amylase is responsible for degradation of starch, resulting in glucose monomers. Xylanase is responsible for degradation of hemicellulose. Both, cellulase and β -glucosidase, participate in the degradation of cellobiose, and all of them play an important role in the degradation of lignin (Jurado et al., 2014, 2015).

In order to elucidate the influence of raw materials on the biodegradation dynamic of lignocellulosic fractions, and to determine the exclusiveness of each composting process, stabilization processes of 5 different type of materials, including Vegetable Waste (VW), Urban Solid Waste (USW), Agrifood Waste (AW), Sewage Sludges (SS) and olive mill waste "Alpeorujó" (ALP), were analyzed in this work. To verify this hypothesis, the following goals were set: (1) to analyse the conduct of physicochemical parameters related to the control and monitoring of the different composting processes, (2) to determine the evolution of lignocellulose, hemicellulose, cellulose and lignin, as well as different humification parameters, (3) to evaluate the effect of different enzymatic activities related to carbon fractions, and (4) to establish correlations between different groups of parameters on the basis of the diversity of the raw materials. This research will provide useful information about the way in which the processes behave, independently of the starting material and the operational activities.

2. Material and methods

2.1. Sampling strategy and experimental set-up

Industrial processes carried out in 15 companies dedicated to the stabilization of different organic materials were analyzed. Each one of them was chosen to obtain a collection of 270 samples (15 plants \times 6 stages \times 3 repetitions) made from different raw materials, and consequently to observe the influence of starting waste on all the biotransformation processes. The composting piles selected for this purpose were prepared outside, in rows of 7–15 m in length and 3–5 m in width, and turned periodically according to the periods of decrease of the internal temperature of the piles. Although each company works with mixtures of different organic materials and in very variable proportions, the main waste selected were as follow: Vegetable Waste (VW), Urban Solid Waste (USW), Sewage Sludges (SS), Agrifood Waste (AW), and Olive oil mill wastewater "Alpeorujó" (ALP). Samples were collected at 6 critical periods according to temperature evolution (including initial and final products): Raw Materials (RM), Mesophilic phase (MES), Thermophilic phase (THER), Cooling phase (COOL), Maturation phase (MAT) and Final Product (FP). The temperature was periodically measured in situ with long-handled PT100 probes (50 cm). During each stage, subsamples were taken from nine different locations, covering several depths of each pile, by means of a soil sampling probe capable of reaching about 1–2 m depth. Subsamples were mixed in equal amounts, in order to achieve a homogeneous and representative sample and, later, divided into three parts to work for analytical replicates.

2.2. Basic parameters of control and monitoring of composting processes

Several physicochemical parameters, based on those typically used for the control and monitoring of composting processes, were studied in this work.

The moisture content (M) was determined by drying at 105 °C for 24 h. The pH was analyzed in a 1:10 (w/v) water extract. Bulk density (BD) was measured according to the US Composting Council USCC, 2001. Elemental carbon (EC) 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). Organic matter content (OM) was assessed by determination of weight loss on ignition at 550 °C to a constant weight. Soluble organic carbon (SOC) was analyzed in extracts obtained from fresh samples according to method described by Hofman and Dusek (2003), and using a TOC-VCSN analyzer (Shimadzu, Japan). Extracts were prepared 1:4 (w:v) of fresh sample in 0.5 M K₂SO₄, shaken at 200 rpm for 30 min, and filtered through filter paper. The microbial biomass C (C_{bio}) was determined using the fumigation and extraction method according to Vance et al. (1987).

2.3. Lignocellulosic fractions

Cellulose (CEL), hemicellulose (HC) and lignin (LIG) fractions were determined by a fiber analyzer ANKOM 200/220 (Ankom Technology, Macedon, NY, USA). The methods applied in this case were those established by ANKOM Technology for fiber analyses (<http://www.ankom.com/procedures.aspx>). Holocellulose was expressed as the sum of CEL and HC values to calculate an additionally ratio, Lignin/Holocellulose.

Biodegradation ratios of HC, CEL and LIG were calculated according the following formula (Wang et al., 2011):

$$R_n(\%) = [(m_n - m_o)/m_n] \times 100,$$

where R_n is the degradation ratio (%) for nth sampling,

m_o : initial content (mg g⁻¹) of the lignocellulosic fraction (HC, CEL or LIG),

m_n : content (mg g⁻¹) of the lignocellulosic fraction (HC, CEL or LIG) for nth sampling.

2.4. Enzymatic analysis

β -glucosidase, amylase, cellulase and xylanase activities were analyzed and the results were expressed as $\mu\text{mol product g}^{-1} \text{ h}^{-1}$ on a dry weight basis.

The estimation of β -glucosidase activity was carried out following the method described by Tabatabai (1982). This method is based on the colorimetric estimation of p-nitrophenol released by the hydrolysis of the p-nitrophenyl- β -D-glucopyranoside (25 mM pNPG in MUB-HCl buffer, pH6) at 37 °C for 1 h. Cellulase activity was determined by a modified method described by Libmond and Savoie (1993), based on the colorimetric estimation of the glucose released in the reaction occurred between the enzyme and the substrate (1% carboxy methyl cellulose in 50 mM sodium acetate buffer, pH 5) at 37 °C for 2 h. The method described by He et al. (1993) was applied for the detection of xylanase activity; this protocol was based on the colorimetric estimation of the glucose released in the reaction occurred between the enzyme and the substrate (1% xylan solution in 50 mM citrate-phosphate buffer, pH 6.5) at 30 °C for 30 min. On the other hand, the amylase activity was carried out following the method described by Mishra et al. (1979), which consisted of a colorimetric estimation of the glucose released in the reaction occurred between the enzyme and the substrate (1% soluble starch in 0.5 M phosphate buffer, pH 5.5) at 35 °C for 24 h.

2.5. Humification parameters

Humic fractions were extracted and analyzed as described by Cavani et al. (2003). 2.0 g of sample were mixed with 100 mL of 0.1 M NaOH and 0.1 M Na₄P₂O₇ \times 10H₂O and incubated for 48 h at 65 °C

Table 1

Evolution of moisture (M), pH, bulk density (BD), organic matter (OM), C/N ratio, soluble organic carbon (SOC) and biomass carbon (C_{bio}) throughout composting of 5 types of organic waste (USW: Urban Solid Waste; VW: Vegetable Waste; SS: Sewage Sludge; AW: Agrifood Waste; ALP: Alpeorujó). Composting phases: raw materials (RM), mesophilic phase (MES), thermophilic phase (THER), cooling phase (COOL), maturation (MAT) and final product (FP). Nine repetitions have been taken into account in each sampling. Values followed by same letters are not significantly different ($P < 0.05$) according to Fisher's LSD test.

Organic Waste	Sampling	M (%)	pH	BD (g/cm ³)	OM (%)	C/N	SOC (%)	C_{bio} (%)
USW	RM	78.20 ^c	5.16 ^a	0.21 ^c	74.73 ^d	28.47 ^e	2.37 ^c	0.20 ^c
	MES	17.73 ^a	7.84 ^b	0.14 ^a	66.26 ^c	20.16 ^d	1.23 ^b	0.04 ^a
	THER	36.70 ^b	8.43 ^d	0.22 ^{cd}	54.14 ^a	14.03 ^c	0.62 ^a	0.12 ^b
	COOL	21.07 ^a	8.12 ^c	0.18 ^b	57.60 ^b	15.76 ^b	0.51 ^a	0.05 ^a
	MAT	13.05 ^a	8.64 ^d	0.25 ^d	51.88 ^a	12.09 ^a	0.63 ^a	0.05 ^a
	FP	11.31 ^a	8.66 ^e	0.22 ^{bc}	53.91 ^a	11.79 ^a	0.61 ^a	0.04 ^a
VW	RM	85.94 ^f	8.10 ^c	0.12 ^{ab}	74.06 ^e	12.63 ^{ab}	0.49 ^a	0.05 ^{ab}
	MES	77.05 ^e	8.43 ^d	0.10 ^a	70.33 ^d	14.18 ^{bc}	0.52 ^a	0.05 ^{ab}
	THER	46.97 ^d	5.57 ^a	0.16 ^{bc}	69.35 ^d	15.45 ^c	1.41 ^c	0.11 ^d
	COOL	53.42 ^c	6.93 ^b	0.18 ^{cd}	63.20 ^c	15.05 ^c	1.36 ^c	0.08 ^c
	MAT	43.92 ^b	8.87 ^e	0.19 ^{cd}	58.85 ^b	12.34 ^a	0.58 ^{ab}	0.06 ^b
	FP	41.05 ^a	9.18 ^e	0.22 ^d	48.43 ^a	11.73 ^a	0.68 ^b	0.03 ^a
SS	RM	81.87 ^e	7.95 ^b	0.28 ^b	71.97 ^e	9.29 ^e	0.25 ^c	0.06 ^a
	MES	67.09 ^c	8.47 ^d	0.19 ^a	58.05 ^d	7.32 ^b	0.05 ^a	0.10 ^b
	THER	73.68 ^d	8.59 ^{de}	0.23 ^a	57.18 ^d	8.42 ^c	0.13 ^b	0.10 ^b
	COOL	38.23 ^b	8.62 ^e	0.42 ^c	49.40 ^b	8.24 ^a	0.15 ^b	0.15 ^c
	MAT	35.74 ^{ab}	8.27 ^c	0.44 ^c	53.38 ^c	6.75 ^a	0.22 ^c	0.16 ^c
	FP	30.80 ^a	7.72 ^a	0.40 ^c	47.19 ^a	8.92 ^d	0.27 ^c	0.15 ^c
AW	RM	65.59 ^b	6.67 ^a	0.21 ^a	62.51 ^b	16.66 ^b	0.13 ^{ab}	0.14 ^c
	MES	66.60 ^b	7.41 ^b	0.20 ^a	72.95 ^c	19.16 ^d	0.18 ^{bc}	0.08 ^b
	THER	45.56 ^b	8.44 ^c	0.21 ^a	66.96 ^b	17.74 ^c	0.26 ^d	0.08 ^b
	COOL	33.16 ^a	8.60 ^d	0.27 ^b	50.64 ^a	12.51 ^a	0.22 ^{cd}	0.03 ^a
	MAT	35.15 ^a	8.72 ^e	0.30 ^b	52.73 ^a	12.69 ^a	0.10 ^a	0.03 ^a
	FP	30.45 ^a	8.67 ^{de}	0.29 ^b	52.10 ^a	11.83 ^a	0.14 ^{ab}	0.04 ^a
ALP	RM	62.48 ^c	5.52 ^a	0.18 ^a	74.85 ^c	19.02 ^b	1.55 ^c	0.05 ^b
	MES	57.36 ^d	5.91 ^{ab}	0.16 ^a	73.52 ^c	19.96 ^b	1.08 ^b	0.03 ^{ab}
	THER	51.17 ^{bc}	6.15 ^b	0.14 ^a	75.60 ^c	23.97 ^c	1.40 ^c	0.05 ^{ab}
	COOL	48.01 ^b	9.17 ^c	0.28 ^b	60.07 ^b	14.82 ^a	0.65 ^a	0.04 ^{ab}
	MAT	51.68 ^c	9.28 ^c	0.33 ^c	55.90 ^a	14.45 ^a	0.46 ^a	0.05 ^b
	FP	42.01 ^a	9.46 ^c	0.26 ^b	60.23 ^b	15.65 ^a	0.73 ^a	0.03 ^a

under continuous stirring (120 rpm) in a thermostatic water bath. After extraction, the samples were centrifuged at 10000 g for 10 min and the supernatants were filtered through a 0.8 µm filter (Standard MF-Millipore Membrane, EMD Millipore Corporation, Billerica, MA, USA). This filtered solution constituted the total extractable carbon (TEC) that was fractionated additionally in humified (humic and fulvic acids) and not humified fractions. Total Organic Carbon (TOC), Total extractable carbon (TEC), non-humified carbon (C_{NH}), humic-like carbon (C_{HA}) and fulvic-like carbon (C_{FA}) were evaluated by means of carbon analysis in the corresponding fractions (TOC-VCSN, Shimadzu, Japan). In addition, humification rate was calculated: $HR = \% ((C_{HA} + C_{FA})/TOC) \times 100$.

2.6. Statistical analyses

Data were subjected to a statistical analysis using Statgraphics Centurion XVI.I (StatPoint Technologies Inc., Virginia). Analysis of variance (ANOVA) and multiple comparison tests (Least Significant Difference, LSD) were performed to compare the mean values of the analyzed variables and determine the influence of the different selected factors ($p < 0.05$). Discriminant analyses were used to separate groups of independent variables according to the stages of composting and the type of raw materials. Likewise, the relations between pairs of variables for each starting material were analyzed using the Pearson correlation coefficient.

3. Results and discussion

3.1. Monitoring of the control parameters during the composting processes on an industrial scale: moisture, pH, bulk density (BD), organic matter (OM), C/N ratio, soluble organic carbon (SOC) and biomass carbon (C_{bio})

The new legislation on waste is clearly oriented to the needs of the population in relation to the current concept of Circular Economy or

Bioeconomy (EU Waste Law, 2018). With this idea it is intended that materials do have a circular return to their origins. It means, to be transformed into others less harmful to the environment and living beings. The regulations that entered into force establish very ambitious targets for recycling, setting several periods for the fulfillment of very strict objectives related to the reduction of residues, including organic waste. Specifically, before 2023, the collection and transformation of so-called biological wastes should be managed in a very controlled way. In this sense, without a doubt, composting is positioned as one of the most important tools for the future of biological waste management (EU Waste Law, 2018).

Although the current European legislation on waste raises a series of recommendations on the management of organic waste through composting, each member state is governed by a series of specific regulations. In the particular case of Spain, this is included in the Official State Bulletin (BOE A-2017-14332 on fertilizer products). In above regulations, reference is made to the basic characteristics that a compost must have in order to be considered of quality, with special emphasis on parameters such as humidity ($< 40\%$), minimum content in organic matter (35–45%) or C/N ratio (< 15 –20).

The temperature inside the pile is one of the main factors to consider during a composting process, since it directly affects the rate of degradation of organic matter as well as determines the stability of the product. Stentiford (1996) suggested that temperatures above 55 °C maximize the sanitation of materials, while temperatures between 45 and 55 °C improve biodegradation rates. According to the Environmental Protection Agency (EPA, 2003), during the thermophilic phase of a composting process, temperatures above 55 °C must be reached for 3 consecutive days. In this research work, 15 industrial-scale composting plants dedicated to the management of different types of bio-waste were selected. The average temperature in the thermophilic stage for each installation met the minimum established by the EPA. The highest average temperature was reached in the VW piles (65 °C). In the

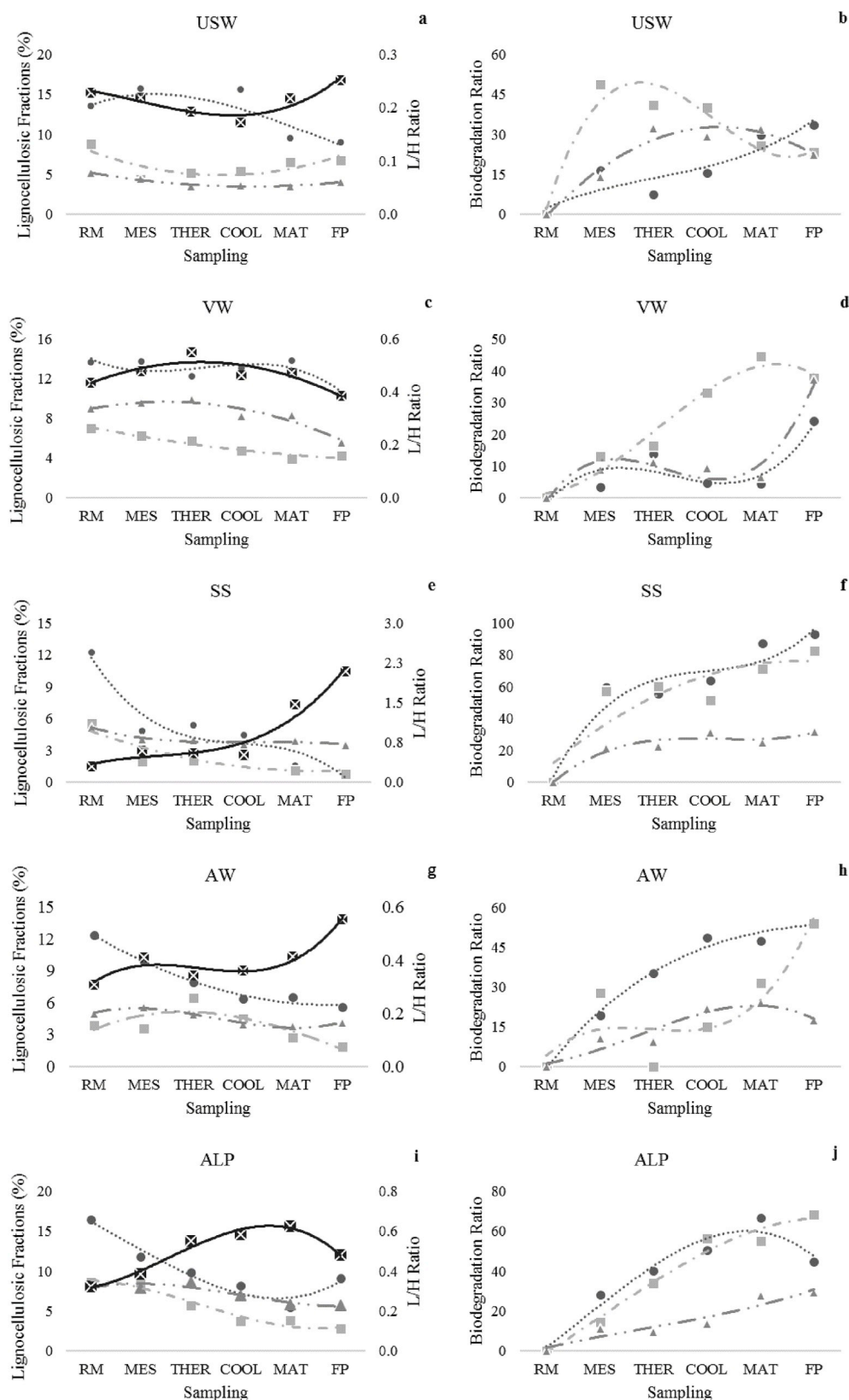


Fig. 1. Evolution (a, c, e, g, i) and biodegradation ratio (b, d, f, h, j) of cellulose CEL (●), hemicellulose - HC (■), lignin - LIG (▲) and lignin/holocellulose ratio - L/H (⊗) throughout composting of 5 types of organic waste. Values are the mean of nine replicates. LSD values are independently shown for each parameter and composting process: USW (LSD_{CEL}a = 0.9349; LSD_{Hc}a = 1.3582; LSD_{LIG}a = 0.2018; LSD_{L/H}a = 0.0083, LSD_{CEL}b = 5.1583; LSD_{Hc}b = 9.3066, LSD_{LIG}b = 3.0716), VW (LSD_{CEL}c = 0.7784; LSD_{Hc}c = 1.1319; LSD_{LIG}c = 0.5046; LSD_{L/H}c = 0.0275; LSD_{CEL}d = 2.5893, LSD_{Hc}d = 9.9096, LSD_{LIG}d = 3.9493), SS (LSD_{CEL}e = 0.9405; LSD_{Hc}e = 1.1049; LSD_{LIG}e = 0.2886; LSD_{L/H}e = 0.0828; LSD_{CEL}f = 4.0721, LSD_{Hc}f = 8.3252, LSD_{LIG}f = 2.9866), AW (LSD_{CEL}g = 1.0206; LSD_{Hc}g = 1.1029; LSD_{LIG}g = 0.3964; LSD_{L/H}g = 0.0313; LSD_{CEL}h = 5.7033, LSD_{Hc}h = 6.9002, LSD_{LIG}h = 3.0402) and ALP (LSD_{CEL}i = 0.6037; LSD_{Hc}i = 0.9257; LSD_{LIG}i = 0.3036; LSD_{L/H}i = 0.0284; LSD_{CEL}j = 1.8968, LSD_{Hc}j = 5.7578, LSD_{LIG}j = 4.3222).

rest of composting plants, the average temperature in the thermophilic phase ranged between 50 and 55 °C. All temperature data were provided by the responsible companies in each case. The mean values of temperature as a function of the starting materials are provided in Table S1 (see Supplementary material, Table S1).

Table 1 shows the values of the control parameters that were used to verify the composting processes evolution. Although the raw material (RM) and the final product (FP) are not real phases of the composting process, it is important to highlight the relevance of both stages to evaluate the optimal evolution of the composting process. In fact, in this

work, the biooxidative phase involves raw materials (RM), mesophilic (MES) and thermophilic (THER) stages, while the maturation phase refers to the pre-maturation (MAT), cooling (COOL) and final product (FP) stages. With respect to the results shown in Table 1, in all cases, the average moisture values were higher during the biooxidative phase of the composting process. The initial humidity of the piles was optimal in the case of the facilities dedicated to AW and ALP, while this parameter was much higher than recommended in those companies dedicated to USW, VW and SS. During the process, the moisture content contributes to a better degradation of organic matter and maintenance of the temperature during a longer period of time, because it favors the microbial activity (Silva et al., 2013). However, at the end of composting, the reduction in moisture content is a positive sign of decomposition and compost maturity (Ameen et al., 2016). In all the analyzed processes the humidity of the piles decreased over time to reach values around 30–40% in the final products. Only in the case of products made from USW, moisture values were detected around 11%, which were considered excessively low (Table 1).

Bulk density (BD) is an important factor to condition the initial mixtures in the composting processes. BD also influences the mechanical properties of the compost, such as strength, porosity and ease of compaction (Agnew and Leonard, 2003). In this work, the values of pH and BD increased throughout the composting process (Table 1). The final pH values of all the compost oscillated around alkaline values. Singularly high were the pH values of the compost based on VW and ALP (López-González et al., 2015).

Regarding the percentage of organic matter (OM), it decreased considerably in all the processes, which supports the fact that in all cases the biotransformation of the starting materials was being carried out. The initial content in OM ranged between 62 and 74%, detecting the highest loss of organic matter at the end of the USW (27%), VW (34%) and SS (34%) processes. The percentage of loss of organic matter for the other samples (AW and ALP) ranged between 16 and 19% (Table 1). Although in some cases the final OM values were somewhat high, coinciding with what was obtained by other authors (Jurado et al., 2015; López-González et al., 2015).

At the beginning of composting, a C/N ratio around 25–30 is considered optimal; however, these initial values were only observed in the case of processes made from USW. This fact confirms that, in many cases, the conditioning of the starting materials in the industrial scale composting is not entirely adequate, due to the accidental mixing of fresh materials with other partially biotransformed ones, which negatively could affect the balance between C and N within the piles. Only in the case of USW samples, the decrease in this parameter was greater than 50% compared to the initial values. According to Guo et al. (2012), the C/N ratio directly influences the maturity of the product obtained, affecting other parameters. For example, when in a composting process the initial C/N ratio is not adequate, it evolves unexpectedly and influences the content of SOC and C_{bio} , which may even increase at the end of the process (Table 1). On the contrary, soluble organic carbon is a fraction that constitutes a rapid source of carbon and energy for growth and microbial activity and, in suitable composting, it is expected to decrease throughout the process (Jurado et al., 2014, 2015). Whilst, probably related to an incorrect C/N ratio at the beginning, C_{bio} did not show significantly lower values at the end of the process in the case of the VW and SS processes (Table 1).

3.2. Evolution of lignocellulosic fractions during the composting processes on an industrial scale

The evolution of lignocellulosic fractions and the changes in the degradation rates of lignocellulose during industrial-scale composting of different wastes are shown in Fig. 1. The biodegradation of lignin during the composting process generally occurs late and at very low rates (Malherbe and Cloete, 2002). In addition, it acts as a protective factor for the degradation of cellulose and hemicellulose. Both aspects complicate

the decomposition of this fraction in the materials that are being composted. In any case, closely monitoring the changes that this fraction undergoes during composting, can help to understand how the contribution of readily available compounds for growth and microbial activity occurs. Adequate degradation of the lignocellulose fraction is crucial to achieve a mature product, free of toxicity to be applied in agricultural soils.

In general terms, cellulose, hemicellulose and lignin decreased along the composting process, though this decrease was more noticeable in the case of the cellulosic fraction. The lignin/holocellulose (L/H) ratio shows a tendency to increase at the end of the process, as a result of the transformation of lignocellulose (Fig. 1a, c, e, g, i). Exceptionally, in the case of the processes elaborated from plant wastes (VW), cellulose remained practically constant in the intermediate stages, to then decrease at the end of the process. Meanwhile, hemicellulose and lignin decreased continuously. The L/H ratio showed a tendency to increase in the intermediate stages, although the final value was slightly lower than that obtained in the initial stage (Fig. 1c). This fact could be due to the accidental introduction of fresh material during the process.

The degradation degree of all the lignocellulosic fractions was very variable. Cellulose degradation reached values around 25–50% at the end of the processes made from USW, VW, AW and ALP (Fig. 1b, d, h, j) while it was higher than 80% in the case of SS processes (Fig. 1f). Hemicellulose degradation ranged between 20 and 80% approximately, being minimal in the case of USW processes and maximum in the case of SS processes. Lignin, on the other hand, did not exceed 20% degradation (Fig. 1b, f, h, j), excepting in the case of VW processes where lignin degradation almost reached 40% (Fig. 1d).

The starting material used in each process consisted of 12–15% cellulose, and 4–8% hemicellulose and lignin. In general, the fraction most affected by the composting process was cellulose, in contrast to lignin and hemicellulose, whose concentrations remained almost unchanged compared to their content in raw materials. Other authors observed this same effect in previous works (Jurado et al., 2014). In some cases, the degradation of cellulose became more noticeable when a substantial amount of hemicellulose had been degraded. This fact may be due to the fact that the lignin-hemicellulose complex between the cellulose fibers decreases the available surface area, and prevents easy access to cellulose by the microorganisms of the process and its enzymes (Komilis and Ham, 2003). According to other authors, however, hemicellulose is the fraction of lignocellulose that is easier to degrade and generally decomposes to a greater extent, in comparison with cellulose and lignin. Taking into account that hemicellulose is a polysaccharide with a lower molecular weight than cellulose and lignin, Wei et al. (2019) stated that in general that is subject to a greater loss during composting. These authors also stated that the high temperatures reached during the thermophilic phase are important for the degradation of hemicellulose, while the degradation ratio of cellulose/lignin increases from the cooling phase. In fact, Tuomela et al. (2000), previously reported that cellulose and hemicellulose degrading microorganisms require high temperatures, even above 60 °C, while most degradation of lignin generally occurs at lower temperatures (Tuomela et al., 2000).

Therefore, the results corresponding to the degradation of the lignocellulosic fractions showed different profiles depending on each type of raw material. This fact confirms that the transformation of polymeric carbon into soluble, simple, and available carbon compounds for the microorganisms present in the piles is unique and depends on the materials used. In spite of this, the global tendencies of biodegradation profiles were very similar in all the composting processes evaluated on an industrial scale, especially with regard to cellulose.

3.3. Evolution profiles of β -glucosidase, amylase, cellulase and xylanase activities during the composting processes

The biodegradation profiles of the lignocellulosic fractions described above depend absolutely on the activity of the microorganisms

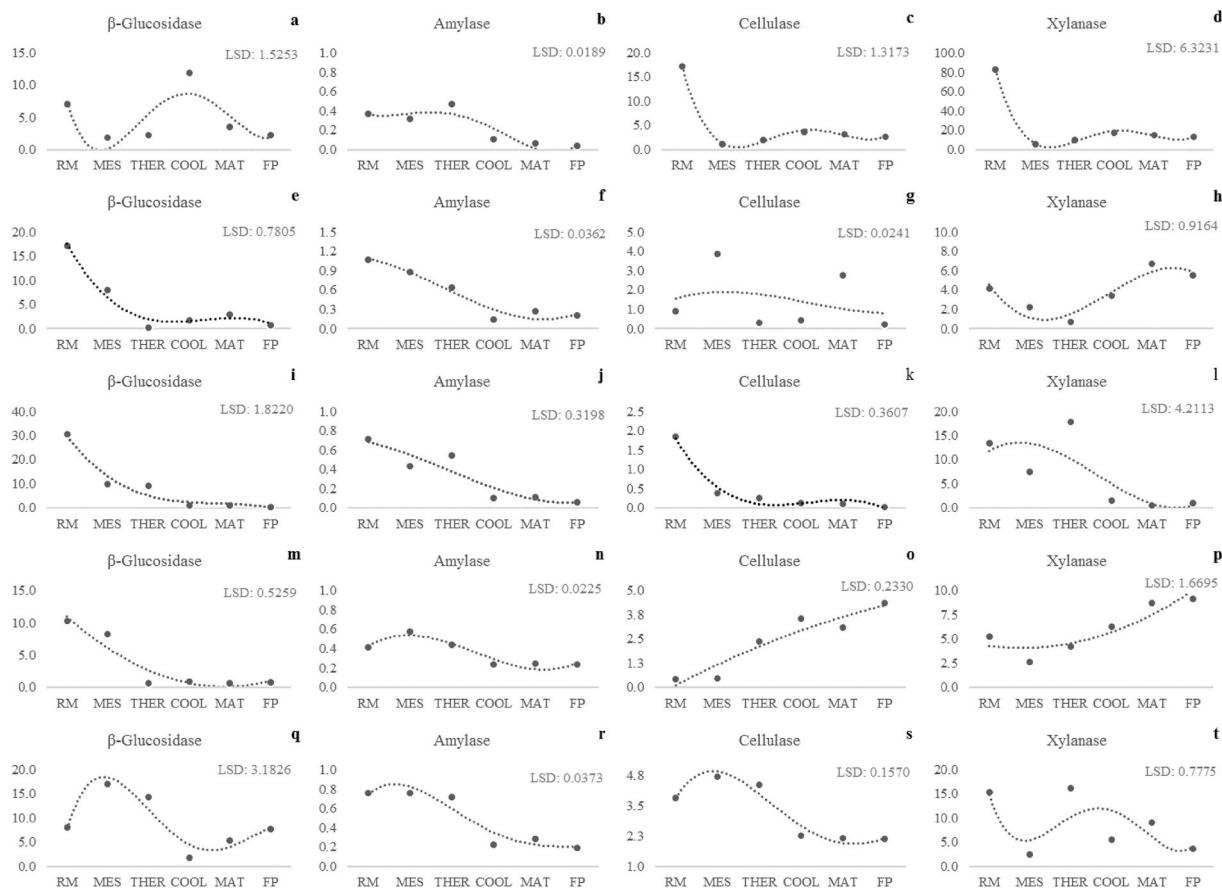


Fig. 2. Dynamics of β -glucosidase, Amylase, Cellulase and Xylanase activities throughout composting made of USW (a-d), VW (e-h), SS (i-l), AW (m-p) and ALP (q-t). Values are the mean of nine replicates. LSD values are indicated for each enzymatic activity and raw material (Fisher LSD test at $p > 0.05$).

present in the composting processes analyzed (Wei et al., 2019). This dependence refers more specifically to the activity of a group of enzymes of microbial origin directly related to the degradation of this type of molecules. Although culturable microbial communities have not been analyzed in this work, a group of enzymes highly involved in the degradation of very complex molecules has been selected. Such is the case of cellulose, hemicellulose and lignin, which can serve as bioindicators of the optimal evolution of the composting processes. In this way, Fig. 2 shows the evolution of the activities of β -glucosidase, amylase, cellulase and xylanase in the different composting processes analyzed, depending on each raw material and the different stages of composting.

Fig. 2a-d shows the trends of these four enzymatic activities in composting processes made from USW. All enzymatic activities analyzed decreased as the composting processes progressed. Even so, the activity of the β -glucosidase showed a more oscillating behavior, detecting important peaks of activity during the cooling phase of the process. In this type of waste, the levels of cellulase and xylanase activity at the beginning of the processes were maximum, probably due to the nature of the starting materials.

Fig. 2e-h correspond to the evolution of enzymatic activity in composting processes made from Vegetable Waste (VW). In general terms, the enzymatic activity of the samples decreased progressively throughout the composting process, although in a more discrete way in the case of the cellulase and xylanase activities, or even increased towards the end of the maturation phase (Fig. 2h). These activity profiles were very similar to those observed in the case of the processes made from AW as main residues (Fig. 2m-p). It should be noted that the increase in the xylanase activity at the end of both processes (VW and AW) is directly related to the biodegradation rates of cellulose and

hemicellulose represented in Fig. 1. Although in all cases an increase in the biodegradation towards the end of the composting process was perceived, a more pronounced degradation peak was detected in the cooling phase in the case of the processes made of VW and AW (Fig. 1g and i). This could be due to the complexity of the lignocellulosic material or the entry of semi-processed or fresh (untreated) materials in the intermediate stages of the composting process.

With regard to the plants elaborated from Sewage Sludges (Figs. 2i-1), a clear downward trend of all the enzymatic activities analyzed was observed. The most pronounced decrease was detected in the β -glucosidase and cellulase activities, whereas it was more gradual in the case of the amylase and xylanase activities. In the latter case, an increase in the activity was observed even during the thermophilic stage. It should be noted that the evolution profiles of the enzymes involved in the degradation of lignocellulosic fractions in Sewage Sludges plants coincide with the higher rates of biodegradation of these fractions at the end of the process (Fig. 1h).

With respect to the enzymatic activities corresponding to the processes elaborated from "Alpeorju", Fig. 2q-t shows identical profiles of β -glucosidase, amylase and cellulase activities. Those were significantly greater during the biooxidative phase of the processes and gradually decreased towards the end of them. However, the evolution of the xylanase activity (directly related to the degradation of hemicellulose) seemed to be controlled by the other three activities indicated above, since it was oscillating throughout the process, showing two peaks of maximum activity at the beginning and then in the cooling phase (Fig. 2t).

In view of the results obtained, it can be concluded that the selection of raw materials strongly influenced the enzymatic profiles of the different composting processes analyzed. In general terms, all enzymatic

activities tended to decrease at the end of the process (Fig. 2 a-p). However, in some cases, β -glucosidase activity increased during the biooxidative phase, showing an oscillating profile, which could be due to the continuous release of molecules more readily available for microorganisms (Fig. 2a, q). This fact coincided mainly with those processes in which the initial cellulase activity was remarkably high (USW and ALP, Fig. 2c and s). In all processes, amylase showed a similar activity profile, so that it was more or less constant during the first stages of the processes and then progressively decreased during the maturation phase to practically undetectable values at the end of the composting process (Fig. 2b, f, 2i, 2n, 2r). Although β -glucosidase and amylase activities showed the same trend in all processes, the highest values were observed in those made from Vegetable Waste (VW). Other authors have detected similar values of β -glucosidase in composting processes from urban solid waste (Zang et al., 2018).

Cellulase and xylanase activity profiles were generally downwards, except in the case of the processes made from VW and AW, where those activities increased towards the end of the composting process (Fig. 2g, h, 2°, 2p). In both cases, the activity profiles coincide with what was observed in Fig. 1, since it was in the VW and AW processes that a sudden change in the biodegradation rates of the lignocellulosic fractions was observed from the cooling phase onwards. In contrast to β -glucosidase, cellulase and xylanase activities were strongly influenced by the availability of the different lignocellulosic fractions, so their enzymatic profiles were highly variable. As described by Wei et al. (2019), xylanolytic activity in Sewage Sludges (SS) and “Alpeorujo” (ALP) showed maximum activity in the thermophilic stage. However, Amira et al. (2012), observed that xylanolytic and cellulolytic activity in Agrifood Waste increased at the end of the process.

3.4. Evolution of humification indicators during the composting process

During the maturation phase of a composting process, the stabilization and humification of the materials is carried out, obtaining a non-toxic and humified product, which is very useful from the agronomic point of view. On the contrary, the immaturity of a compost could cause serious phytotoxicity problems as well as the depletion of the cultivation soils. Therefore, determining the maturity degree of a compost through the appropriate indicators could be of great interest to control the quality of the final product (Jurado et al., 2015).

Traditionally, the quality of a compost has been determined through a group of physical-chemical parameters, such as temperature, CO₂, humic/fulvic acid ratio, NH₄-N/NO₃-N ratio, C/N ratio, pH, microbial biomass, enzymatic activity, germination of seeds and concentration of heavy metals (Castaldi et al., 2008). However, due to the great diversity of raw materials and the different composting protocols, it is necessary to establish more precise maturity indices (Martínez et al., 2016).

Taking into account that the degree of maturation is more relevant at the end of the composting process, the humification parameters were analyzed in this work only in the final stage (PF) of the different processes. However, the analysis of the humification parameters in the raw materials at the beginning of the composting processes was also included, to compare the results between the initial and final phase (RM-FP) in terms of maturity. Based on the above, the main parameters analyzed to determine the evolution of the humified fractions during the selected composting processes are shown in Table 2. The initial and final values of elemental and organic carbon (EC and TOC) as well as the proportion of non-humified substances (C_{NH}), are shown in this table. However, the data related to the humic/fulvic ratio (C_{HA}/C_{FA}), as well as the humification rate (HR), were parameters of greater interest when determining the humification dynamic of the processes.

With respect to the different parameters related to humification, it should be noted that very different trends were observed in terms of raw materials (Table 2). As expected, elemental and organic carbon (EC and TOC) values decreased in all composting processes. In some cases (USW, VW and SS), the decrease meant a total carbon reduction of more

Table 2

Change produced in humification parameters throughout composting of different residues (USW: Urban Solid Waste, VW: Vegetable Waste, SS: Sewage Sludge, AW: Agroindustrial Waste and ALP: Alpeorujo). Nine repetitions have been taken into account in each sampling. Values of elemental carbon (EC), total organic carbon (TOC), non humic carbon (C_{NH}), humic/fulvic carbon ratio (C_{HA}/C_{FA}) and Humification Ratio (HR) followed by same letters are not significantly different ($p > 0.05$) by Fisher's LSD test (RM: Raw Material, FP: Final Product).

Organic Waste	Sampling	EC (%)	TOC (%)	C _{NH} (%)	C _{HA} /C _{FA}	HR
USW	RM	40.33 ^b	43.45 ^b	0.86 ^a	1.41 ^a	6.55 ^a
	FP	27.99 ^a	31.34 ^a	0.83 ^a	1.78 ^a	5.69 ^a
VW	RM	33.74 ^b	43.06 ^b	0.20 ^a	0.45 ^a	9.16 ^a
	FP	24.51 ^a	28.16 ^a	0.62 ^b	0.54 ^a	14.45 ^b
SS	RM	35.45 ^b	41.84 ^b	1.08 ^b	1.25 ^a	6.05 ^a
	FP	23.17 ^a	27.44 ^a	0.47 ^a	1.32 ^a	9.27 ^b
AW	RM	35.52 ^b	36.34 ^b	0.67 ^b	0.59 ^a	7.14 ^a
	FP	30.54 ^a	30.29 ^a	0.52 ^a	0.74 ^a	12.69 ^b
ALP	RM	36.06 ^b	43.51 ^a	0.73 ^a	1.40 ^a	6.48 ^{ab}
	FP	31.75 ^a	35.02 ^b	0.50 ^a	2.85 ^a	4.40 ^a

than 30% over the initial value, while in others it did not exceed 14%. Many authors have proposed the loss of carbon as a parameter that can serve as an indirect indicator of the degree of maturity of the compost (Jurado et al., 2015).

In general terms, non-humified carbon (C_{NH}) values tended to decrease during all the composting processes analyzed, with the exception of the process elaborated from Vegetable Waste (Table 2). On the other hand, C_{HA}/C_{FA} ratio increased in the final phase, mainly and significantly in the case of the USW, AW and ALP processes. The positive trend of the values of the C_{HA}/C_{FA} relationship could be explained by the typical humification process that occurs during the maturation phase of a composting process (Iglesias-Jiménez et al., 2008). C_{HA}/C_{FA} ratio has been proposed as an important indicator of compost stability, being higher in mature compost (Azim et al., 2017). Compared to fulvic acids, humic acids are derived mainly from a more mature fraction of organic material. C_{HA} include compounds that increase the cation exchange capacity in soils (Mindari et al., 2014). They are related to soil fertility, crop yield and improve the C, N, P, K and S contents (Zhang et al., 2017). Meanwhile, C_{FA} are characterized by having a lower molecular weight, a high level of oxidation and a lower amount of C, N and P, but they also improve the capacity of cation exchange in soil. Therefore, the C_{HA}/C_{FA} ratio reflects the degree of polymerization in materials of humic nature. According to Iglesias-Jiménez et al. (2008), C_{HA}/C_{FA} values above 1 indicate that humification is occurring, while values of C_{HA}/C_{FA} above 1.6 indicate that the product is already mature. Our data revealed that the best degree of humification was obtained in the case of the processes elaborated from ALP.

In addition to the C_{HA}/C_{FA} ratio, humification rate (HR) was calculated. During composting, thanks to a combination of chemical and biological transformations, the total organic carbon content decreases, while the relative content of humic compounds increases. According to several authors, it has been demonstrated that the humification rate (HR) is an effective indicator of the formation of humic substances during the composting process, being able to establish with precision the moment of the complete stabilization (Tittarelli et al., 2002). Based on the results shown in Table 2, the humification rate (HR) was increased in the case of the processes elaborated from VW, SS and AW. In the case of the processes elaborated from “Alpeorujo” (ALP), contrary to what was observed in the case of the C_{HA}/C_{FA} ratio, a decrease in the humification rate was detected. This could be due to the fact that the HR values depend on the total organic carbon content of the samples and, probably, there could have been an entry of fresh material towards the end of the process, which stopped the mineralization process.

Results described for all the composting processes analyzed, agree that during the degradation of different materials, the amount of non-

humified compounds (C_{NH}) extracted in alkaline conditions was significantly reduced over time, except in the case of the processes elaborated from vegetable remains (Table 2). During the composting process, the release of compounds that are incorporated into the humification routes takes place through numerous reactions of reorganization and molecular condensation (Alburquerque et al., 2009). This fact leads to the formation of more humified compounds. In this sense, it is known that lignin plays an essential role in the processes of humification of lignocellulosic residues, since the partial degradation of this complex molecule can give rise to the basic structure of humic substances, as well as to the liberation of aromatic and phenolic by-products (Alburquerque et al., 2009; Jurado et al., 2015). All these phenomena contribute to increase the content of humified substances during the maturation phase of the composting process. However, the type of humified substances (humic or fulvic acid) and the generation speed of them can vary greatly depending on the starting materials.

3.5. Establishment of the differential profiles based on the diverse raw materials: discriminant analysis and pearson correlation

On the basis of the results described and above discussed, it is almost evident that different physical-chemical and enzymatic scenarios can be found depending on the composted materials or even on each process analyzed. Numerous recent scientific studies highlight the important relationship between the enzymatic activities involved in the biodegradation processes of the lignocellulosic fractions and the evolution of the humification markers during the composting process (Awasthi et al., 2018; Song et al., 2018; Gao et al., 2019). However, the verification of the actual influence of the raw material as a starting point to obtain compost in different pathways was one of the most important challenges addressed in this work.

Fig. 3 shows the discriminant analysis that help to understand how distinctive or similar the composting processes are depending on the starting materials (Fig. 3a), the final products obtained (Fig. 3b) and the thermal phases of the processes (Fig. 3c).

The discriminant analysis shown in Fig. 3a revealed two discriminant functions responsible for more than 80% of the variability observed among the data. In view of what is observed in Fig. 3a, it is clear that data related to the lignocellulosic fractions, enzymatic activities and humification process are grouped separately on the basis of the raw materials. In this Figure, the set of data corresponding to the compost elaborated from Vegetable Waste (VW) stands out as being the most distant group in relation to the rest (Fig. 3a). When the same analysis was exclusively performed bearing in mind the data set corresponding to the final products (FP), two discriminant functions influenced the variability of the data by more than 85% (Fig. 3b). Fig. 3b supports the diversity of compost due to different raw materials, since 5 significantly different groups were established based on that criterion. While products (compost) made from AW, SS and VW were more similar in terms of biological activity as well as content in lignocellulosic and humic fractions, compost from ALP and USW were very different from the rest and from each other (Fig. 3b). Despite the remarkable differences detected among compost made from different organic waste, all the analyzed samples complied with the basic monitoring parameters (Moisture, Organic Matter and C/N Ratio) indicated at the legislative level (BOE A-2017-14332). In this sense, humidity data relative to the compost samples ranged approximately between 30 and 40%, excepting the final products made from USW, where a very low humidity was detected at the end of the process (Fig. 3d). The organic matter content was greater than 35–45% in all the final products analyzed but this parameter was significantly higher in the case of samples from ALP. Both circumstances could be involved in the differences observed in the compost ALP and USW with respect to the rest of products from different origins (Fig. 3b).

On the other hand, in order to establish the global differences or similarities between the different phases of the 15 composting processes

analyzed, Fig. 3c shows the existence of two functions that explained more than 94% of the variability of the data. Thus, surprisingly, the results were grouped into 3 data sets, one of them corresponding to the initial phase of the processes (RM = Raw Materials), a second group formed by the data related to the biooxidative phase (Phase I = MES + THER) and a third group including the data corresponding to the stabilization and humification phase (Phase II = COOL + MAT + FP). Therefore, notwithstanding the heterogeneity of the data in relation to the starting materials and working conditions of each company, the data are suitably grouped from an evolutionary point of view, establishing continuity and logical limits between the three main stages of the process: Initial phase (RM), Biooxidative phase (Phase I) and Maturation phase (Phase II). The above statement may be irrelevant when working with small-scale or semi-heavy composting. However, the control of the different thermal phases of the process is crucial when working on composting on an industrial scale, since the accidental (or voluntary) mixing of raw materials with others that are already biotransformed or that are in an intermediate phase of transformation could happen.

In order to analyse the composting profiles from each kind of material, five discriminant and Pearson correlation analyses were performed for each process independently scrutinized (Fig. 4). Fig. 4 a-e showed the profiles observed for each group of material, depending on the succession of thermal phases. In all cases, discriminant function explained near or even more than 90% of the variability results. Each profile was apparently different from the rest, which supported that each process is exclusive. Data were located in three main groups: RM, Phase I (MES + THER) and Phase II (COOL + MAT + FP). In general, biooxidative (Phase I) and stabilization-humification phases (Phase II) appeared well separated in all the cases (Fig. 4 a-e). However, only in the composting process performed from AW (Agrifood Waste), a logical succession among COOL, MAT and FP was observed (Fig. 4d). On the contrary, during the processes performed from USW and SS, the MAT and FP stages appeared very closed between them (Fig. 4b-c), while COOL stage appeared very near of FP in the case of composting processes made from VW and ALP (Fig. 4a, e).

To establish significant links between the parameters analyzed in this study (lignocellulosic-humic fractions and enzymatic profiles), Pearson correlation maps were made for each type of material (Fig. 4f-j). Many surprising relationships were detected, but different in each case. In the case of the VW and USW composting processes, there were no significant correlations between the humification parameters and the rest of the variables (Fig. 4g). However, only in the case of compost samples from plant remains was a close negative relationship detected between the hemicellulose content and the C_{HA}/C_{FA} ratio (Fig. 4f). Something similar happened in the case of the samples from ALP, although in this case the C_{HA}/C_{FA} ratio was negatively correlated with the cellulose fraction and the xylanase activity (Fig. 4j). On the contrary, the Pearson correlation profiles were very different in the case of samples made from SS and AW, since the humification rate (HR) significantly correlated with the different enzymatic activities concerning the biodegradation of the lignocellulosic fractions (Fig. 4h and i). Pearson correlation map between lignocellulosic and enzymatic parameters was remarkable in the case of samples made from SS, since positive links were detected among the group integrated by β -glucosidase-amylase-cellulase activities (Fig. 4h). A more discrete correlation was established in the case of samples made from AW and ALP (Fig. 4i and j).

In view of the results shown in the correlation maps (Fig. 4f-j), it is obvious that the USW-based composting processes did not develop orthodoxly, since during a biotransformation process of this nature, it is expected that some type of relationship will be established between the degradation of the lignocellulosic fractions, the degree of humification and the enzymatic activity (Fig. 4f). This connection did not occur in this case.

Some of the enzymatic activities showed homogeneous and

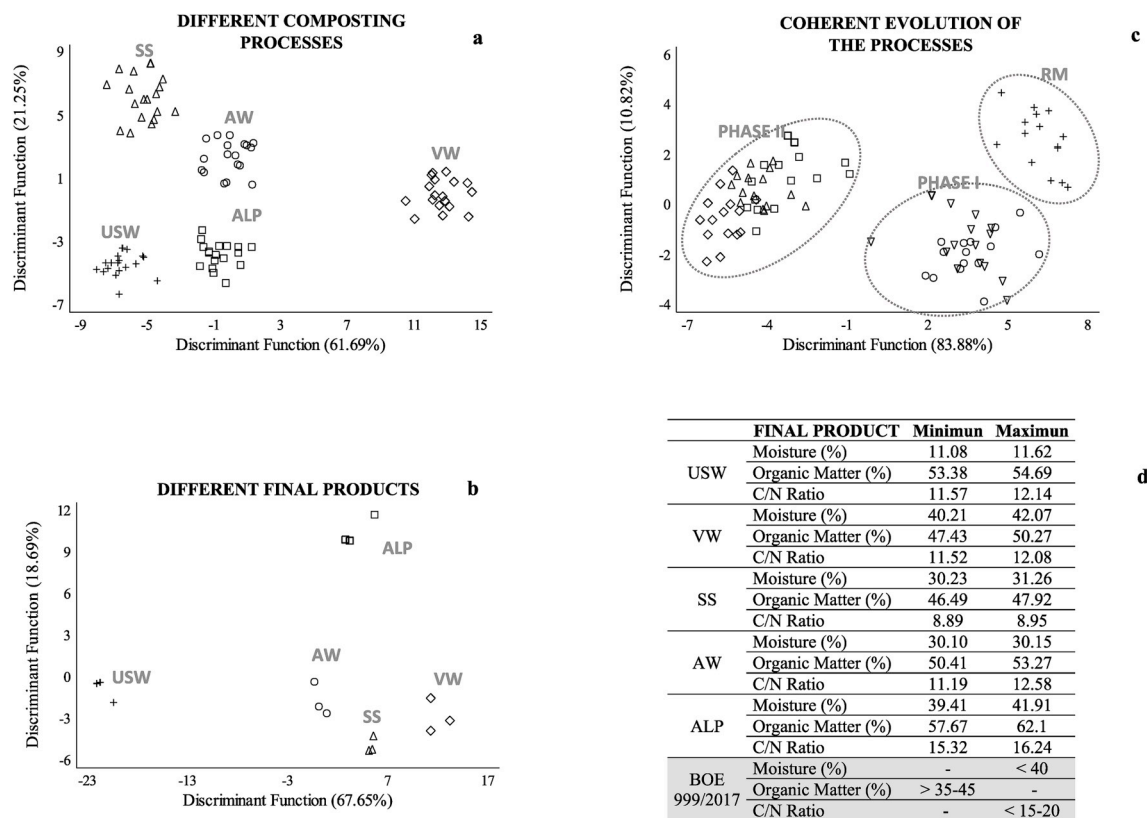


Fig. 3. Discriminant analysis based on composting of different raw materials, taking into account the complete processes (a), the final products (b) and the distribution of principal thermal phases (RM = Raw Material; Phase I = MES + THER; Phase II: COOL + MAT + FP) (c). Range (minimum and maximum values) corresponding to moisture, organic matter and C/N ratio for each type of residues are shown compared with those included in the Spanish regulation as the main parameters of compost quality (d).

independent profiles of the type of raw material (section 3.2.). Such is the case of β -glucosidase and amylase activities, which established close links with each other and with other related enzymatic parameters. Surprisingly, the correlations did not always occur in the same direction. In the case of plants dedicated to Sewage Sludges composting (Fig. 4h), Pearson P values were positive and very close to 1 in the β -glucosidase-amylase-cellulase-xylanase block. This correlation did not happen in the case of plants dedicated to AW composting, where very strong and negative correlations were established between cellulase activity and the β -glucosidase-amylase pair (Fig. 4i). A totally opposite effect to the previous one was observed in the case of the ALP samples (Fig. 4j).

Although the relationships established between many of the analyzed parameters were variable depending on the raw materials, there is no doubt that some of the enzymatic activities, such as β -glucosidase and amylase, showed similar trends during the composting process (section 3.3). This fact makes them very useful bioindicators to evaluate the correct or incorrect evolution of the composting processes independently of the starting materials. Something similar happened in the case of the humification rate (HR) since, although in general it showed a logical tendency to increase toward the end of the process (Table 2), the Pearson values observed were highly variable because of the type of materials. In the case of the SS samples (Fig. 4h), HR showed a negative correlation with the β -glucosidase-amylase-xylanase package, whereas in the case of AW plants, the HR correlated negatively with the β -glucosidase-amylase pair, but in a positive way with the cellulase-xylanase block. Despite the different established links, the control of the evolution of the humification rate (HR), in combination with other enzymatic parameters, could be useful to determine the correct or incorrect evolution of the composting processes.

To establish the most suitable recipe for composting, some authors

have tried to select the most appropriate waste mixtures and the best indicators to control the start-up of the process (Ghinea et al., 2019). In this sense, some of the most interesting physical-chemical parameters used for this purpose are pH, electrical conductivity, humidity, ash, content of N and C, C/N ratio, total organic carbon or the presence of heavy metals. Other authors have tried to establish the biodegradable character of the starting materials in order to optimize the bio-transformation process as much as possible (Gutiérrez et al., 2017). However, the results described here have revealed the exclusive nature of composting processes on an industrial scale. This exclusivity is fundamentally based on the different raw materials and operating conditions. Therefore, similar products could have been obtained thanks to different enzymatic and humification profiles. In spite of this, in all cases, the tendencies of some enzymatic indicators such as β -glucosidase and amylase were very homogeneous.

4. Conclusions

Although different compost could be similar in relation to their agronomic quality, the enzymatic strategies and the humification pathways could be very different in each process. The results derived from this work admit that each composting process is unique and runs independently, even showing similar trends.

Parameters such as β -glucosidase, amylase, lignin/holocellulose ratio or the humification degree could act as important indicators of the process. This fact confirms the efficiency and versatility of microbial enzymes and their role as transforming agents of organic matter, even when the heterogeneity of materials and operating conditions on industrial scale are very diverse.

Taking into account the initial considerations regarding the singularity of each composting process, it seems evident that there are not

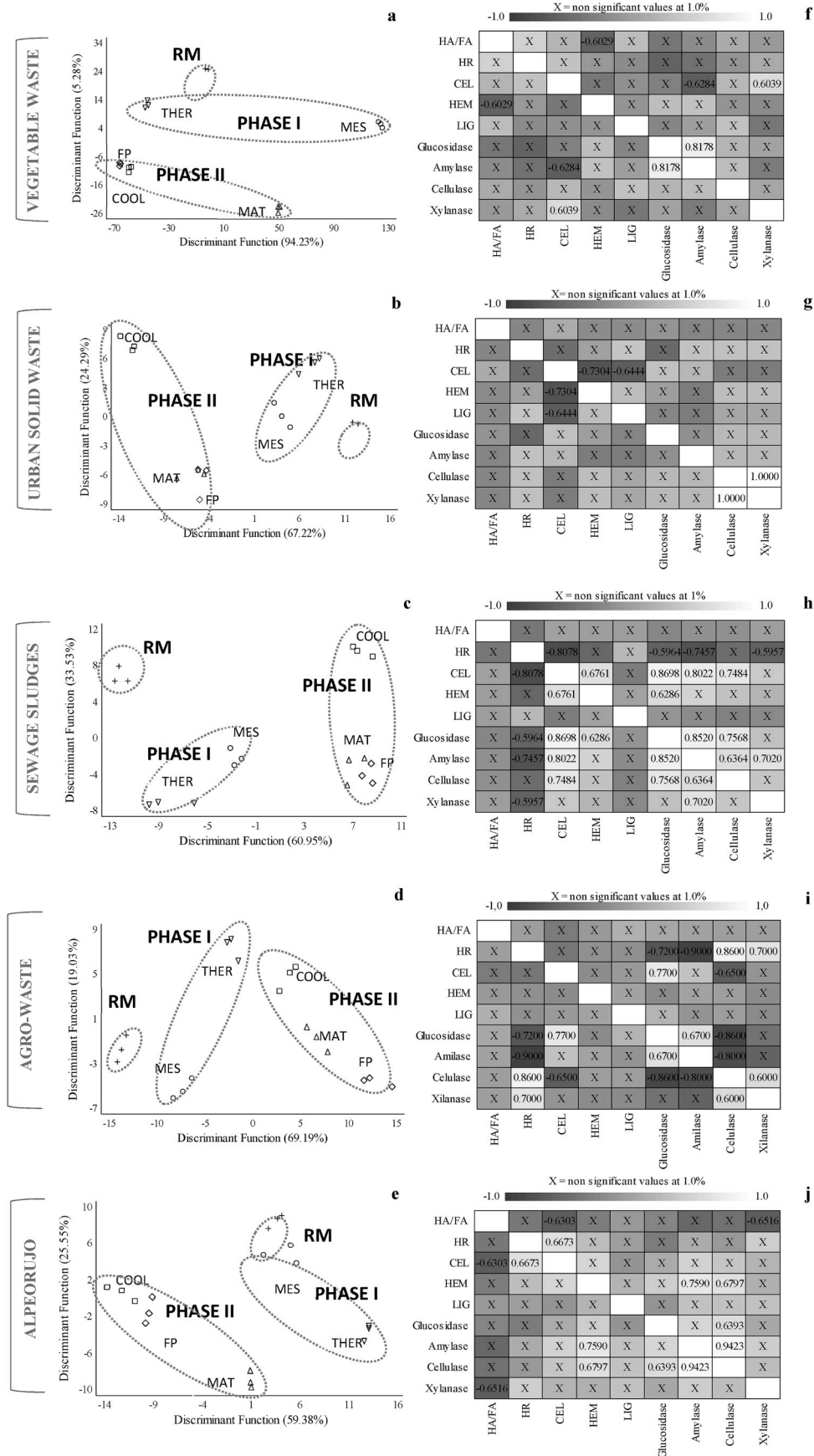


Fig. 4. Comparative analysis of the different composting processes according to the starting materials (USW: Urban Solid Waste; VW: Vegetable Waste; SS: Sewage Sludges; AW: Agrifood Waste, ALP: Alpeorujo). Discriminant analysis according to the distribution of the main thermal phases (a-e) and Pearson tables showing positive (light grey) or negative (dark grey) correlations between the different analyzed parameters (f-j). HA/FA: CHA/CFA; HR: Humification Rate; CEL: Cellulose; HEM: Hemicellulose; LIG: Lignin; β -glucosidase, Amylase, Cellulase and Xylanase.

two equal composting processes. This can be directly related to the starting materials, the physical-chemical and enzymatic profiles that in each process are outlined. This fact can be considered, therefore, as the main impact derived from this work. There is no recipe or a fixed protocol for the practice of composting, but different starting situations must be control from the beginning.

Although different composting processes follow different paths, it is possible to reach to final products that comply with the legally established quality criteria. So, in this work, final products that complied with the legal recommendations (minimum content in organic matter, humidity and C/N ratio) obtained.

On the other hand, we must highlight the role of certain enzymatic activities, such as β -glucosidase and amylase, as well as the ratio lignin/holocellulose, as bioindicators to monitor the composting process. The profiles observed in this sense followed a characteristic and independent pattern of the starting materials.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2019.109312>.

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