



Evolution of enzymatic activities and carbon fractions throughout composting of plant waste



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ABSTRACT

Many alternatives for the proper disposal of horticultural plant wastes have been studied, and composting is one of the most attractive due to its insignificant environmental impact and low cost. The quality of compost for agronomical use is related to the degree of organic matter maturation and stabilization. Traditional parameters as well as temperature, ratio C/N, cationic exchange capacity, extractable carbon, or evolution of humified substances have been successfully used to assess compost maturity and stability. However, microorganisms frequently isolated during composting release a wide range of hydrolytic enzymes, whose activity could apparently give interesting information on the rate of decomposition of organic matter and, therefore, on the product stability. The aim of this work was to study the evolution of some important enzymatic activities during composting of agricultural wastes and their comparison with other chemical parameters commonly employed as quality and maturity indexes, to establish a relationship between the degradation intensity of specific organic carbon fractions throughout the process. In this work, the chemical and biochemical parameters of plant wastes were studied along a composting process of 189 days to evaluate their importance as tools for compost characterization. Results showed an intense enzymatic activity during the first 2–3 weeks of composting (bio-oxidative phase), because of the availability of easily decomposable organic compounds. From a biological point of view, a less intense phase was observed between second and third month of composting (mesophilic or cooling phase). Finally, chemical humification parameters were more closely associated with the period between 119 and 189 days (maturation phase). Significant correlations between the enzymatic activities as well as between enzyme activities and other more traditional parameters were also highlighted, indicating that both kind of indexes can be a reliable tool to determine the degree of stability and maturation of horticultural plant wastes based-compost.

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1. Introduction

Soil organic matter plays a crucial role in soil fertility of horticultural systems (Kononova, 1966; Marinari et al., 2000). However, the continuous use of large quantities of chemical fertilizers has led to reduced levels of organic matter in most agricultural soils (Ayuso et al., 1997). This is the case of the southeast of Spain, where the intensive agriculture practiced during the last years has favoured the impoverishment of soil and the accumulation of different kinds of plant wastes. Many alternatives for the proper disposal of these wastes have been studied so far, being the use of composting one of

the most attractive due to its insignificant environmental impact and low cost (Bustamante et al., 2008; Lu et al., 2008; Khamforoush et al., 2013).

Composts are increasingly being used in agriculture since they contribute to the disposal of waste materials and thus to the environment preservation. Even though the incorporation of composted waste with high organic matter content could improve soil quality and fertility and reduce waste materials (García et al., 1991; Marinari et al., 2000), it could also produce toxicity problems that inhibit seed germination and plant development if inadequately matured composts are used (García et al., 1991; Marambe and Ando, 1992; Mitsuyo et al., 1986). For this reason several parameters have been established to determine compost maturity. Though recent studies on some compost parameters have been correlated with microbial biomass, activity and biodiversity

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changes during composting (Ayed et al., 2007; Mondini et al., 2003, 2004; Vargas-García et al., 2010), other more traditional parameters as well as temperature, odour, colour, ratio C/N, cationic exchange capacity, extractable carbon, phytotoxicity tests or evolution of humified substances have been successfully used to assess compost maturity and stability (Barrena et al., 2008; Iglesias-Jiménez et al., 2008; Pietro and Paola, 2004).

The population count and type of microorganisms that can be found in compost are closely related to the origin of the raw materials. Thus, when the substrates composted are of agricultural origin, lignocellulose usually constitutes the main fraction of the total organic matter. Some authors state that this is the most adequate component to conduct a proper humification process (Tuomela et al., 2000), since some compounds released from this fraction (polyphenols, sugar and aminocompounds) as a consequence of microbial activities, seem to promote the humus formation (Ji et al., 2005). Therefore, to know the changes in the lignocellulosic fractions during the composting process could be of interest to understand the evolution of the best maturity indicators (Iglesias-Jiménez et al., 2008) and definitively the humification process.

Microorganisms frequently isolated during composting release a wide range of hydrolytic enzymes (in particular, cellulases, xylanases, proteases, lipases, phosphatases and arylsulphatases) (Ben-David et al., 2011; Portillo et al., 2011; Shi et al., 2011), which depolymerize various organic waste compounds (Kandeler et al., 1999; Marx et al., 2001). Cellulases and β -Glucosidases are related to C mineralization, proteases and urease are involved in N cycle and phosphatases and arylsulphatases are implicated in P and S cycles, respectively (Mondini et al., 2004). Thus, enzymatic activities could apparently give interesting information on the rate of decomposition of organic matter and, therefore, on the product stability.

According to what has been stated above, the aim of this work was to study the evolution of some important enzymatic activities during composting of agricultural wastes and their comparison with other chemical parameters commonly employed as quality and maturity indexes during a composting process in order to establish, if possible, a relationship between the degradation intensity of specific organic carbon fractions throughout the process and the achievement of maturity in a compost pile.

2. Materials and methods

2.1. Composting process

The composting process was carried out using horticultural waste, specifically composed of tomato plants (lacking fruits) which were directly collected after cropping in green house facilities located at El Ejido (Almería, Spain). Three composting piles were built. Pile dimensions were 3.0 m length \times 1.5 m width \times 1.0 m height. Piles were prepared by mixing plant wastes with pine chips in order to get an appropriate C:N ratio (around 25). Prior to composting, some physical and chemical analyses were performed on the raw materials (Table 1). The piles were subjected to forced aeration at a rate of 7.5–9.0 L kg⁻¹ every 4 h in order to prevent the oxygen concentration inside the piles decrease below 10%. Piles were turned when the temperature inside them dropped for three

Table 1
Some physico-chemical properties of raw materials used for composting ($n = 5$).

Raw material	% TC	%N	C/N ratio	Density (g/cm ³)	Humidity (%)
Tomato plant (waste)	28.30	3.26	8.68	0.086	7.73
Sawdust	46.09	0.12	384.08	0.18	12.641

consecutive days. The moisture content was initially set between 50% and 55% (w/w) and it was maintained within this range by watering during turning operations. These management operations were applied during the bio-oxidative phase that lasted 63 days. After this period, the piles were statically maintained in maturation for an additional period of four months (126 days), so the process lasted for a total of 189 days. Temperature values inside the piles were continuously measured using a Pt 100 temperature probe connected to a data logger. pH, bulk density and electrical conductivity were monitored at sampling times throughout the whole process.

2.2. Sampling strategy

Three composite samples from each of the three different piles were collected at each sampling time. Composite samples were obtained by properly mixing and homogenizing sub-samples extracted from nine different locations inside each pile. Samplings were carried out at different stages of the composting process, according to the thermal values prevailing inside the piles. Samples were collected and named as indicated in Table 2.

Approximately 500 g of sample were collected (sum of 9 sub-samples) at each sampling time and then properly mixed for analysis. Each sample was divided into two equal parts, one of them was kept at 4 °C for further chemical analysis. The second portion was immediately used for the determination of enzymatic activities.

2.3. Total and reducing sugars

Samples were dried and milled to a particle size ≤ 0.2 mm. To evaluate the amount of total sugars, they were hydrolysed as follows: 25 mg of compost were mixed with 0.1 mL of 12 M SO₄H₂ in a test tube with screw cap and kept at room temperature for 16 h. Then, 2.4 mL of distilled water were added and the tube was heated in a boiling water bath for 8 h (Safarik and Santruckova, 1992). The total sugars concentration was determined by following the

Table 2
Sampling strategy.

Sampling no.	Sampling code	Description	Time (days) ^b
1	RM	Raw Materials	0
2	RMES1	Mesophilic phase (temperature increases)	1
3	T1A	Thermophilic phase	2
4	T1B	Thermophilic phase	5
5	DMES1 ^a	Mesophilic phase (temperature decreases)	7
6	RMES2	Mesophilic phase (temperature increases)	8
7	T2A	Thermophilic phase	9
8	T2B	Thermophilic phase	12
9	DMES2 ^a	Mesophilic phase (temperature decreases)	14
10	RMES3	Mesophilic phase (temperature increases)	15
11	T3A	Thermophilic phase	16
12	DMES3 ^a	Mesophilic phase (temperature decreases)	26
13	RMES4	Mesophilic phase (temperature increases)	28
14	C5	Cooling phase	42
15	C6	Cooling phase	56
16	C7 ^a	Cooling phase	63
17	MAT1	Maturation phase	119
18	MAT2	Maturation phase	168
19	FPR	Final product	189

^a Samplings at which turning operations were carried out.

^b Days after the process started.

procedure described by Dubois et al. (1956). Absorbance was measured at 485 nm in a spectrophotometer SHIMADZU UV-160A (Shimadzu Co., Kyoto, Japan) and a solution of glucose (0–100 µg/mL) was used as standard. Reducing sugars were evaluated by the method described by Somogyi (1951). 10 g of compost were extracted with 40 mL 0.5 M K₂SO₄ in a horizontal shaker at 200 rpm for 30 min. After filtration, 1 mL of the extract was mixed with 3 mL of DNS solution (3,5-dinitrosalicylic acid 7.49 g L⁻¹; NaOH 14 g L⁻¹; potassium sodium tartrate tetrahydrate 216.1 g L⁻¹; Phenol 90%; 5.37 g L⁻¹; sodium sulphite 0.7 g L⁻¹). The tube was heated in a boiling water bath for 15 min and absorbance was measured at 550 nm using glucose as standard.

2.4. Lignocellulosic fractions

To determinate cellulose, hemicellulose and lignin fractions, a fiber analyzer ANKOM 200/220 (Ankom Technology, Macedon, NY, USA) was used. The methods applied in this case were those established by ANKOM Technology for Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and Acid Detergent Lignin (ADL) (<http://www.ankom.com/procedures.aspx>). Holocellulose was expressed as the sum of Cellulose and Hemicellulose values. Additionally, the ratio Lignin/Holocellulose was calculated.

2.5. Humification parameters

Organic carbon was extracted according to the method described by Cavani et al. (2003). 2.0 g of sample were placed in a 250 mL centrifuge tube with 100 mL of 0.1 M NaOH and 0.1 M Na₄P₂O₇·10H₂O and incubated for 48 h at 65 °C in continuous agitation (120 rpm) in a thermostatic water bath under N₂ atmosphere. After extraction, samples were centrifuged at 5000 g for 15 min and supernatants were filtered through a 0.8 µm filter (Standard MF-Millipore Membranes, EMD Millipore Corporation, Billerica, MA, USA, 2013). This filtered solution constituted the total extractable carbon (TEC) which was further fractionated into humified (humic and fulvic acids) and non humified fractions following the method described by Ciavatta et al. (1990). Total Organic Carbon from both humified (C_{ha} and C_{fa}) and non humified (C_{NH}) fractions was measured using an organic carbon analyzer (Shimadzu UV-160 A). In addition, two humification parameters were calculated: Humification Index (HI = C_{ha}/TC × 100) and Non Humification Ratio (NHR = C_{NH}/C_{ha} + C_{fa}). Total carbon (TC) was determined in dried samples using an elemental analyzer LECO CNHS 923.

2.6. Enzymatic analysis

Fresh material was used for enzymatic analyses. Air-dried samples were analyzed in relation to β-Glucosidase, cellulase, xylanase, amylase, lipase, phosphatase (alkaline phosphomonoesterase), protease and urease activities and results were in each case expressed as µmol product g⁻¹ 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 colourimetric estimation of the p-nitrophenol (PNP) released by the hydrolysis of the p-nitrophenyl-β-D-glucopyranoside (PNG) at 37 °C for 1 h. The same reaction was used for the determination of the phosphatase activity, with the substitution of the PNG by p-nitrophenyl phosphate (PNPP) as suggested by Tabatai and Bremner (1969). Both activities, β-Glucosidase and phosphatase, were estimated using 0.5 g of sample. The estimation of cellulase activity was carried out with a modified method described by Libmond and Savoie (1993) based on the colourimetric estimation of the glucose released in the reaction with an acid reactive at 37 °C

for 2 h. The method described by He et al. (1993) was applied for the detection of xylanase activity, based on the colourimetric estimation of the glucose released in the reaction with an acid reagent at 30 °C for 30 min. The amylase activity was carried out following the method described by Mishra et al. (1979). In this case, the method consisted of the colourimetric estimation of the glucose released in the reaction with an acid reagent at 35 °C for 24 h. The estimation of lipase activity was carried out following the method described by Farnet et al. (2010). This method was based on the colourimetric estimation of the p-nitrophenol (PNP) formed by the hydrolysis of the p-nitrophenyl-laureate (pNPL) at 30 °C for 2 h. The 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). The method described by Bremner and Mulvaney (1978) was applied to 2 g of sample in order to quantify urease activity. The ammonia released after the incubation with urea at 37 °C for 1 h was estimated from the chromogenic complex produced in the presence of a basic solution of sodium hypochlorite and phenol, with nitroprussiate as catalyst.

2.7. Statistical analysis

Three independent replicates were used in all analyses, and the data obtained were subjected to statistical analysis using Statgraphics Centurion XVI.I (StatPoint, Inc., Virginia). One way analysis of variance (ANOVA) and multiple comparison tests (Fisher's Least Significant Difference) were performed to compare mean values for the different levels of sampling time ($P < 0.05$). The relationships between pairs of variables were analyzed by the Pearson correlation coefficient. In addition, to identify groups of interrelated variables, a principal components analysis was performed using the Varimax rotational method with normalization.

3. Results and discussion

3.1. Temperature evolution during the composting process

The temperature is an indicative factor of the composting process evolution. Changes in this parameter could be used to know the microbial activity along the entire process and to determine the organic material stability (Boulter et al., 2000).

In Fig. 1, forced turnings are indicated with black arrows. As it was expected, a thermal reactivation was promoted every time turning treatments were applied. An initial temperature of 20 °C

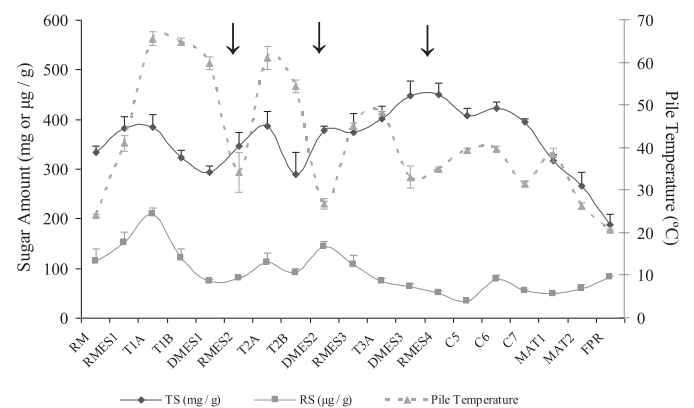


Fig. 1. Evolution of total and reducing sugars during a plant waste based-composting process. Values are the mean of five replicates (LSDTS = 23.4825; LSDRS = 0.0322). Non continuous curve represents the temperature evolution along the entire experimental period (forced turnings are indicated with black arrows).

was recorded at the start of composting and the highest temperature was observed at around 48 h after the beginning of the process. At this moment the temperature values were highest than 60 °C into the piles. The United States Environmental Protection Agency (EPA, 2003) recommends maintaining the compost piles at above 55 °C for 15 days or at least 5 consecutive days. These strict conditions were kept in this work for the period between T1A and DMES1 being similar to other experimental work achieved above by other authors in horticultural waste based composting (Elorrieta et al., 2003; Suárez-Estrella et al., 2007).

The temperature within the piles influences greatly on the composting process durability, since it directly affects on the organic matter degradation rate, whether determining a mature and stable product. Stentiford (1996) suggested that temperatures over 55 °C maximized the sanitation of the materials, those between 45 and 55 °C maximized biodegradation rates, and between 35 and 40 °C maximized diversity in the composting process. In this sense, the thermal profile inside the piles was proper in this work.

3.2. Content of total (TS) and reducing sugars (RS)

The evolution of TS content during the bio-oxidative phase generated a particular pattern, with consecutive increases and decreases during the first month of the process (RM-RMES4, Fig. 1). During this period, the highest TS contents paralleled the rising temperature, while the lowest ones matched with the end of the thermophilic phases. During this phase, the range of variation of TS content was between 300 and 400 mg g⁻¹ on a dry weight basis. After the stage of greater biological activity a slow increase was observed before the cooling phase. After 2 months from the beginning of the process the TS content fell gradually until the end of the process, when it reached the lowest level, nearby 200 mg g⁻¹.

The evolution of RS content was similar to that of TS during the first month of the process, at which levels of this parameter were lower than 200 µg g⁻¹ (Fig. 1). After DMES2 sampling, this parameter steadily decreased. The minimum values were observed at the end of the bio-oxidative phase. However, at the end of the process, a rebound was detected in the maturation phase, although the content of RS were still low (100 µg g⁻¹).

The soluble organic carbon of immature compost mainly consists of total sugars (including hemicellulose or cellulose), phenolic substances, amino acids, peptides and other easily biodegradable compounds. In this sense, sugars are one of the main components present in the carbon fractions of composted materials, though one part of them is exclusively available for microbial growth (Hsu and Lo, 1999). Taking this into account, the typical evolution of this parameter along the bio-oxidative phase probably reflects the relationship among microbial activity and sugar content. On one hand, fungi, bacteria and actinomycetes have the ability to act on polysaccharide molecules, favouring the release of monomeric and oligomeric units and therefore increasing sugar levels. These sugars represent the available carbon source for most microbial species associated with composting. The dominant activity in each stage determines the levels of sugars, resulting in a fluctuating evolution of this parameter (Liu et al., 2011).

Though the evolution in both parameters was parallel up to RMES3, an increase in the TS values was observed after the bio-oxidative phase, contrary to what was detected in the RS values. This fact could be supported by growth on easily degraded substrates while acclimating to the degradation of more recalcitrant substrates (Charest et al., 2004) (Fig. 1). Finally, the low biological activity typical of the end of the process, corresponding to the stage of stabilization and maturation of the organic matter, explains the abrupt fall observed at this phase in relation to levels of TS (Fig. 1),

since there is no further input of oligosaccharides nor sugars, as described by other authors (Said-Pullicino et al., 2007).

3.3. Lignocellulosic fractions

The starting material was composed of 28.2% cellulose, 4.4% hemicellulose and 13.3% lignin. The fraction most affected by the composting process was cellulose, in contrast to lignin and hemicellulose, whose concentration in the final product remained almost unchanged in comparison to the content in raw material (Fig. 2). Cellulose declined sharply, mostly during the cooling and maturation phases. The highest values of around 250 mg g⁻¹ were recorded at thermophilic phases while cellulose contents fell below 100 mg g⁻¹ in the final product. The content of hemicellulose fluctuated during the bio-oxidative phase. The initial and final concentrations of hemicellulose were 44.4 mg g⁻¹ and 64.4 mg g⁻¹, respectively. Lignin levels remained stable throughout the process, showing a weak increase only at the beginning of the process, being this fact compensated by the decrease observed in the maturation phase. The lignin content at the end of the process was probably lower on the basis of continuous degradation of organic compounds and the consequent weight loss during the process, as it has been previously reported (Ait Baddi et al., 2004). Like lignin, L/H index (Lignin/Cellulose + Hemicellulose) was stable through the process, but with a pronounced rising in cooling and maturation phases (Fig. 2). The evolution of this indicator could be provoked by the loss of dry matter indicated above.

The results recorded in literature referring to the degradation of lignocellulosic fractions propose very diverse and contradictory information (Haddadin et al., 2009; Wang et al., 2011). Given the great diversity of materials that can be composted and their composition in relation with lignocellulosic content it is not surprising that the results offer a high heterogeneity (Francou et al., 2008). Generally, the biodegradation of the lignin fraction occurs at the late phases of the composting and a very low rate of decomposition is usually detected. The content of this polymer determines not only its own decomposition but also that of the other lignocellulosic components, cellulose and hemicellulose. Lignin is able to act as a protective factor for this other fractions. Specifically, a strong association exists between lignin and hemicellulose, favoured by the presence of phenolic compounds (Malherbe and Cloete, 2002). This intricate association hinders the access to the enzymes implicated in the biodegradation process. The entire molecule of lignocellulose therefore exhibits a great

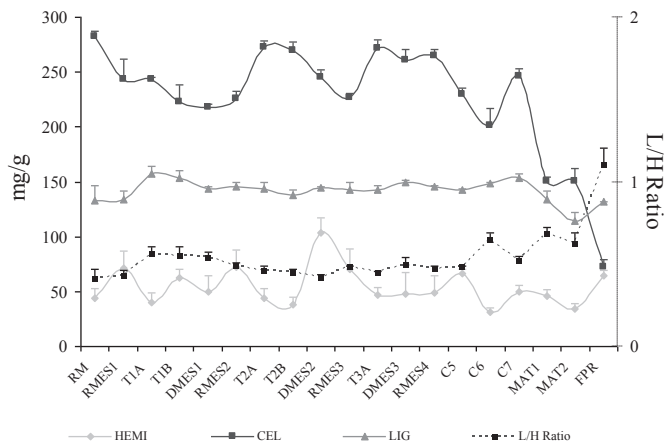


Fig. 2. Evolution of lignocellulosic fractions during a plant waste based-composting process. Values are the mean of five replicates (LSD_{HEMI} = 10.9738; LSD_{CEL} = 8.7961; LSD_{LIG} = 5.5687; LSD_{L/H} = 0.0436).

resistance to microbial degradation (Barrington et al., 2002). In this sense, and in order to achieve a more successful degree of involvement of lignocellulosic fractions in biotransformation processes such as composting, it has been needed to develop different indices relative to performance of different fractions. This is the case of the L/H Index), which values tend to increase during the process (Fig. 2), as described by others authors (Francou et al., 2008). In this work this parameter doubled its value at the end of the process due to the noticeable drop in the Holocellulose content as well as an important loss of dry matter occurred during the process.

3.4. Humification parameters

When an organic carbon source is added to agricultural soils, it is converted to humic substances and energy, taking an important effect on both the microbiota and physico-chemical parameters of the soil (Schnitzer, 1978; Suárez-Estrella et al., 2008a,b). This conversion corresponds to the stabilization of organic matter, which avoids adverse reactions occurring in the soil, such as the production of phytotoxic substances or development of anoxic environments (Sequi et al., 1991).

On the basis of the criteria stated above, detection and quantification of humic substances are very important to determine the degree of stabilization and humification during a composting process (Ciavatta and Govi, 1993). The main parameters tested to determine evolution of the humic fractions during the composting process are shown in Table 3. Additionally, some interesting humification indexes were calculated and they are depicted in Fig. 3.

Total carbon (TC) decreased throughout the composting and reached its lowest values at the end of the process (16.73%). This decrease was significant after 4 months from the beginning of the process. Carbon loss has been proposed by many authors as a parameter that may serve as an indirect indicator of the degree of compost maturity (Iglesias-Jiménez and Pérez-García, 1992). However, it must not be considered as a true humification indicator. Under our experimental conditions the product maturity reached optimal values when the material approximately contained 1.4 times less total carbon than the original material.

Contrary to what was observed in the case of TC, a significant increase was detected for the rest of the humification parameters, specifically in the maturation phase (Table 3). The increase in the content of extractable carbon (TEC) and humic acids (HA) matches the drop in TC. In our experience, the content of fulvic acids (FA) did not increase in the maturation phase. However, HA or even the sum of both fractions (HA + FA) were better maturation indicators

Table 3

Changes in the main humification parameters during the composting of horticultural wastes (dry weight basis). Values followed by different letters are significantly different ($P < 0.05$).

Composting Phase	Days	TC ¹	TEC ²	HA ³	FA ⁴	HA + FA ⁵	NH ⁶
Raw Material	1	24.55 ^{bc}	8.62 ^c	3.60 ^{bc}	1.70 ^{ab}	5.30 ^{bc}	3.33 ^{ab}
Thermophile-1	2	24.63 ^c	8.51 ^{bc}	3.47 ^{bc}	1.67 ^a	5.14 ^{ab}	3.36 ^{ab}
Thermophile-2	9	24.50 ^{bc}	8.21 ^{ab}	3.13 ^a	1.66 ^a	4.79 ^a	3.42 ^{bc}
Thermophile-3	26	22.50 ^{bc}	7.52 ^a	3.38 ^{ab}	1.30 ^a	4.68 ^a	2.84 ^a
Cooling	63	23.43 ^{bc}	7.46 ^a	3.31 ^a	1.46 ^a	4.77 ^a	2.69 ^a
Maturation	119	21.55 ^b	10.53 ^e	4.78 ^d	1.93 ^{bc}	6.71 ^{de}	3.82 ^d
Final Product	189	16.73 ^a	9.62 ^d	4.40 ^d	1.72 ^{ab}	6.12 ^d	3.50 ^{cd}

1: Total carbon %.

2: Total extractable carbon (g/100 g).

3: Humic acids (g/100 g).

4: Fulvic acids (g/100 g).

5: Humic and fulvic Acids (g/100 g).

6: Non humified carbon (g/100 g).

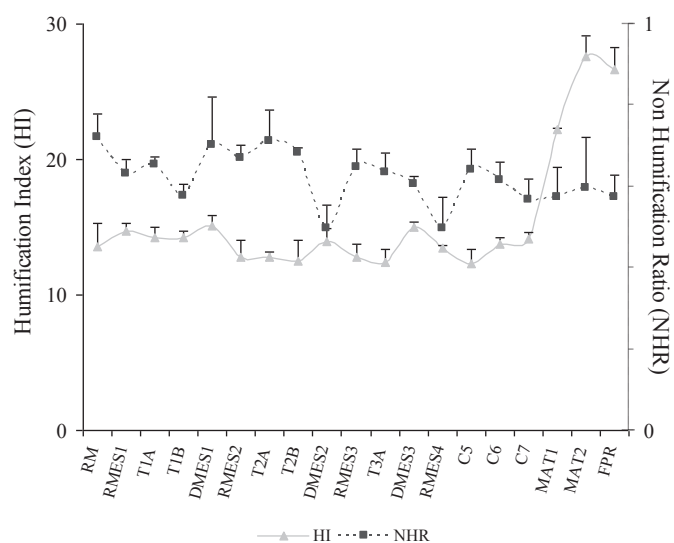


Fig. 3. Trends of the HI and NHR indices during the thermophilic, mesophilic and final phases of the organic matter stabilization in a plant waste based-composting process. Values are the mean of five replicates ($LSD_{HI} = 0.9209$; $LSD_{NHR} = 0.0613$).

(Cavani et al., 2003). Das (1988) found a gradual increase in the parameter HA/HF ratio during composting. However, a similar evolution of this parameter was observed in this work showing an increase in this factor (Final Product/Raw Material) around to 1.3, matching values detected by others authors (Vargas-García et al., 2006).

On the other hand, another non-humified carbon fraction (NH) was determined after the extraction protocol detailed in Section 2.4. (Ciavatta et al., 1990). Much of these substances were detected along the entire composting process, and especially interfering with fulvic component separation (Table 3). Several authors have supported their results on the basis of a humification pointer relative to the evolution of non humic substances (Ciavatta et al., 1990; Sequi et al., 1986). However, no humification ratio ($NHR = NH / (HA + FA)$), could not be considered in this work as a good maturation pointer, showing a very weak decrease along the composting process. Moreover, humification index (HI) was taken into account as good indicator of humification (Fig. 3). HI increased in maturation phase, matching the humification fractions mentioned previously. Certainly, the drastic increase detected in the HI at the end of the composting process was due to the fall of total carbon throughout the composting process, besides to the increase occurred in the humic acid content at the end of the process. Therefore HI could be considered, in this case, useful as an indicator in the maturation process.

Different values for HA, FA and HI were obtained in these experiments when compared to those described by other authors (Iglesias-Jiménez and Pérez-García, 1992; Vargas-García et al., 2006). This effect could be due to the different nature of raw material and protocols applied in each case. However, the increase factor HI (quotient between final and initial values) was in agreement with the value considered optimal for maturity (1.34 for HI) according to Iglesias-Jiménez and Pérez-García (1992).

3.5. Enzymatic analysis

The evolution of several hydrolytic enzyme activities throughout the composting developed is shown in Figs. 4–6. In general, the changes observed were more noticeable during the bio-oxidative phase, while the enzymatic activity was lowest at the end of the process. The enzymes related to carbon metabolism

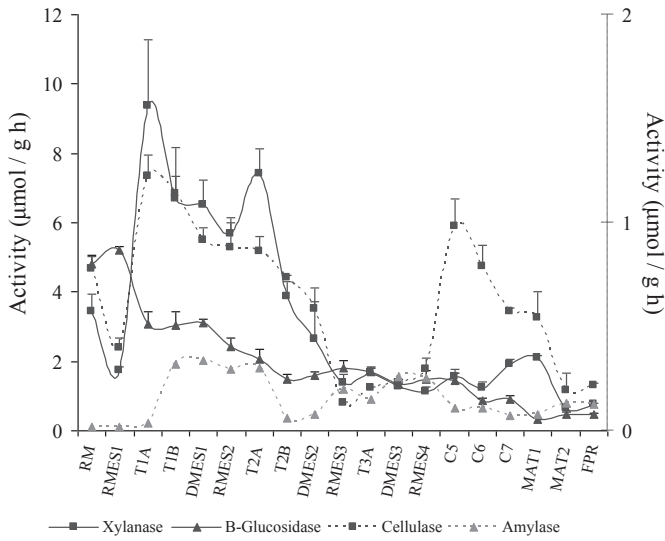


Fig. 4. Dynamics of β -Glucosidase, Cellulase, Xylanase and Amylase activities during a plant waste based-composting process. Values are the mean of five replicates ($LSD_{\beta\text{-Gluc}} = 0.1996$; $LSD_{\text{Cellulase}} = 0.0781$; $LSD_{\text{Xylanase}} = 0.7094$; $LSD_{\text{Amylase}} = 0.0094$).

are indicated in Fig. 4 (β -Glucosidase, Cellulase, Xylanase and Amylase), while those involved in the nitrogen metabolism (Protease and Urease) are shown in Fig. 5. Finally, phosphatase (FME-K) and lipase activities, related to the phosphorous and fat metabolism, respectively, are indicated in Fig. 6. β -Glucosidase showed the highest increase at the beginning of the process (between RM and RMES1) but then decreased constantly and reached a stable lowest value at the final of maturation phase (Fig. 4). β -Glucosidase is one of the key enzymes which govern the C-cycle. Its activity is indicative of the presence of labile organic matter easily usable by the microorganisms (Castaldi et al., 2008), which catalyzes the hydrolysis of cellobiose and other disaccharides (Fernández-Gómez et al., 2013). On the contrary, cellulase and xylanase activities decreased at the first, to reach the maxima levels during the thermophilic phase. One second peak was observed at the beginning of the cooling phase in the case of cellulase activity, while this peak occurred later for the xylanase (Fig. 4). The levels of amylase were

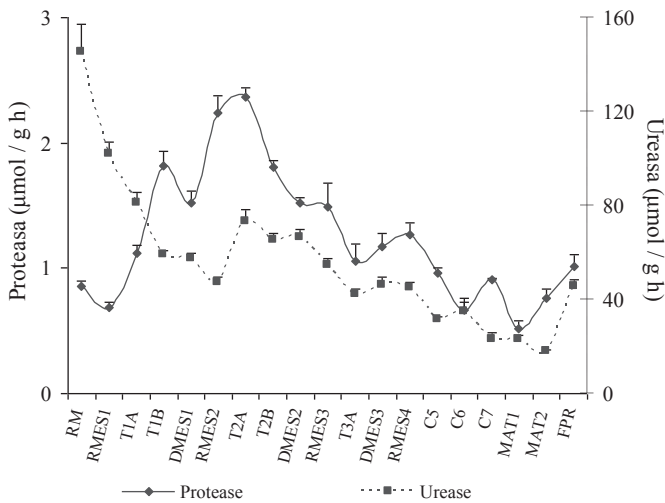


Fig. 5. Dynamics of Protease and Urease activities during a plant waste based-composting process. Values are the mean of five replicates ($LSD_{\text{Protease}} = 0.0904$; $LSD_{\text{Urease}} = 4.2097$).

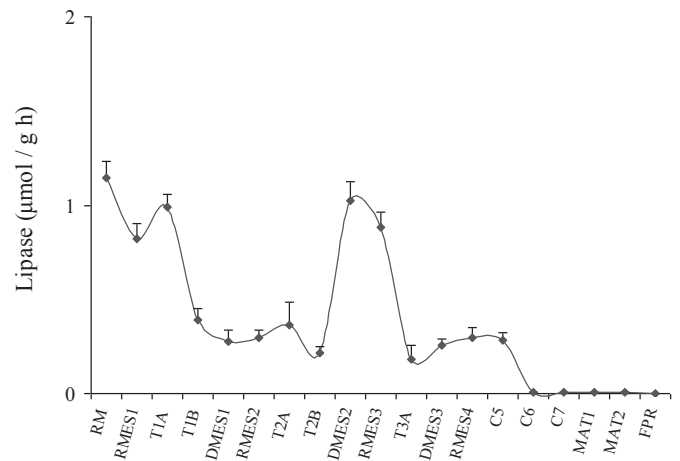
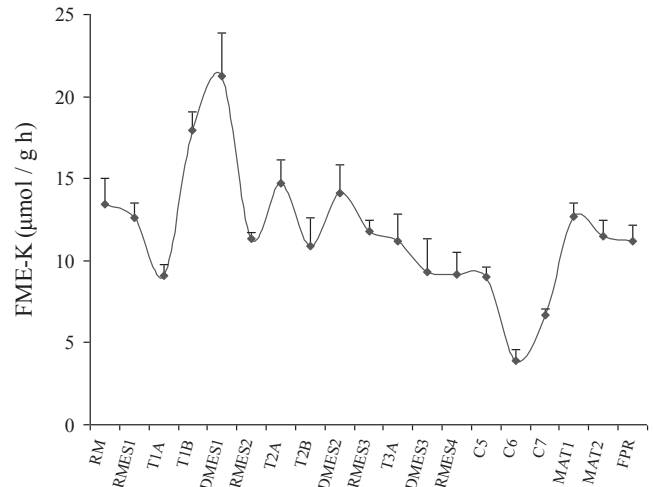


Fig. 6. Dynamics of Phosphatase (FME-K) and Lipase activities during a plant waste based-composting process. Values are the mean of five replicates ($LSD_{\text{FME-K}} = 1.0804$; $LSD_{\text{Lipase}} = 0.0602$).

very low both at the initial and final sampling, though two significant peaks were detected during the bio-oxidative phase (Fig. 4).

On the basis of cited above, the enzymes implied with the carbohydrates metabolism, xylanase, cellulase and amylase, markedly increased after the acclimation stage (24 h after preparation of composting piles), showing the highest values in the first thermophilic phase (between 2 and 5 days after starting the process) (Fig. 4). A temperature rise takes place at this phase, increasing the solubility the polymers, the alteration in their structure, and consistently favouring both the enzymatic process and the microbial growth (Singh and Sharma, 2002; Kaushik et al., 2013). The same profile was described by other authors during a composting process of plant residues (Zeng et al., 2010). On the contrary, some works describe a continuous decrease of these enzymatic activities from the beginning of the process (Castaldi et al., 2008). At the end of the third thermophilic stage (16 days after the beginning of the process) the enzymatic activities specified previously showed a significant fall (Fig. 4), coinciding with the end of the bio-oxidative phase (RMES4), in which the easily assimilable nutrients are depleted and more recalcitrant polymers remain (Castaldi et al., 2008; Cunha-Queda et al., 2007). In our work, this effect is corroborated on the basis of the results observed in Fig. 2, related to the lignocellulosic fraction. In general, at the end of the process, these enzymatic activities were minimal in the final product, indicating the noticeable decrease of the microbial activity and the

exhaustion of almost all available carbohydrate sources. Both facts indicated that the product had been stabilized (Cunha-Queada et al., 2007).

Protease activity showed a characteristic profile, oscillating between consecutive peaks and valleys during the entire composting process (Fig. 5). Similar to cellulase and xylanase activities, protease levels were descending at the acclimation phase of the process to reach the maximal levels between T1A and RMES4 (bio-oxidative phase). On the other hand, urease behaviour was different to the rest of activities analyzed, showing a drastic fall during the first five days after the beginning of the process (Fig. 5). After T2A sampling, a gentle but continuous decrease was observed until the maturation phase. However, a noticeable rebound was detected at the final sampling.

In summary, similarly to the results described for the enzymes involved in the metabolism of carbohydrates, whose activity levels were decreasing from thermophilic stages to cooling and maturation periods, protease and urease activities were maximal during bio-oxidative phase. However, these enzymes also peaked at the end of the process (Fig. 5). This profile has been described for the urease activity by Garcia et al. (1993), and Guo et al. (2012). In this sense, the peaks detected during the thermophilic stage may be attributable to the removal of ammonium because of high temperature and oxygen input generated by forced aeration. The ammonia can act as an inhibitor of hydrolytic activity catalyzed by proteases as well as by urease (Ros et al., 2006), so that its removal, together with the presence of nitrogenous substrates, could motivate the increase of the two enzymes in the thermophilic stage.

Alkaline phosphomonoesterase (FME-K) and lipase activities profiles are shown in Fig. 6. Two different evolution patterns were observed for both enzymes. FME-K showed a significant peak in T1B and DMES1 sampling, in contrast to the drastic drop occurred at the cooling phase. However, a rebound of this activity was detected at the end of the process, when the alkaline phosphatase activity reached similar levels to those found at the beginning of the process (Fig. 6). This enzyme is particularly relevant for the evaluation of the composting process, since it is only synthesized by microorganisms and does not originate from plant residues (Burns, 1982). The activity levels of this kind of enzyme suffer virtually no variations in poorly managed compost piles, while significant changes are detected in properly executed processes (Godden et al., 1986). The evolution of the FME-K during composting also varies greatly on the basis of raw materials and type of composting process. Similar to the findings in this study, several authors have described a downward trend in the activity of this enzyme after the bio-oxidative, following a weak peak at the end of the process (Vargas-García et al., 2010).

Finally, in the case of lipase activity, two significant peaks were detected during the bio-oxidative phase, after a light fall occurred at the start of the process. When the cooling phase was evaluated, this enzyme activity underwent an important decrease until it reached nearly undetectable values (Fig. 6). The presence of lipases is very low in plant residues, so that their role in the carbon cycle is limited in comparison to the role observed in other processes that use high-fat content substrates. This is the reason why lipase activity was very low at the beginning of the process ($<1.2 \mu\text{mol PNP/g h}^{-1}$) in comparison with the results described in high-fat substrate based-composting (animal fats, sewage sludge), where values higher than $60 \mu\text{mol PNP/g h}^{-1}$ have been described (Gea et al., 2007; Ruggieri et al., 2008). The lipase activity decreased at the beginning of the process probably due to the presence of the more easily biodegradable organic molecules such as sugars. Another hypothesis that could explain this initial decrease is the adsorption of lipases on the plant material interface (Boczar et al., 2001). Thus, the increasing temperature could facilitate the

release of lipases, as demonstrated by the peaks of activity found in the first and the second thermophilic peaks. Gea et al. (2007) found a similar behaviour during the composting of highly fat organic waste.

3.6. Pearson correlation

Table 4 shows the correlation matrix among the enzyme activities tested, total and reducing sugars and lignocellulosic fractions. Many interesting correlations have been indicated in Table 4 between sugar content and enzymes implied in the carbohydrate metabolism. In this work, β -Glucosidase, cellulase and xylanase were correlated with reducing sugars. The level of β -Glucosidase activity is determined by the presence of readily metabolizable substrates (at the beginning of the process and throughout the maturation phase). This last effect might be due to the presence of carbon compounds derived from the cellulolytic and hemicellulolytic activities, which are primarily achieved during the final thermophilic stage and the cooling phase that precedes the maturation (Yu et al., 2007). On the other hand, reducing sugar content was positively correlated with urease and lipase activities, while phosphatase activity (FME-K) showed an exclusive inverse correlation with this parameter.

As expected, cellulose and lignin contents showed some interesting correlations with β -Glucosidase, cellulase, xylanase, lipase as well as the rest of the enzymes implicated in N-cycle (Table 4). On the contrary, hemicellulose was neither correlated with the enzymes directly implied in the N nor carbohydrates metabolism. As indicated above, a strong association between lignin and hemicellulose exists, favoured by the presence of phenolic compounds (Malherbe and Cloete, 2002). This fact hinders the access to the enzymes implicated in the hemicellulose biodegradation process. However, lipase was the only activity lightly correlated with this fraction.

Enzymatic activities implied in the carbohydrates metabolism were strongly correlated among them. This effect was specially observed in the case of xylanase, which showed a positive correlation both with β -Glucosidase, cellulase and amilase activities (Table 4).

The protease activity was strongly related to the enzymatic activities implied in the C-cycle, (excepting β -Glucosidase) as well as cellulose content and phosphatase activity. Urease activity showed a very close relationship with β -Glucosidase activity, suggesting that both enzymes worked together during the early phase of composting (Castaldi et al., 2008; He et al., 2012). This correlation contradicts results obtained by other authors probably due to the use of different raw materials (He et al., 2012). On the other hand, protease correlated with cellulase, xylanase and amylase being more important after the acclimation phase of the composting. Finally, lipase was related to cellulosic and hemicellulosic fractions, as well as β -Glucosidase, xylanase and urease activities.

In summary, the activities of the eight enzymes analyzed were strongly intercorrelated (Table 4), as previously described by other authors (He et al., 2012; Vargas-García et al., 2010). This fact could corroborate the presence of multiple interactions between different microbial groups and organic substrates like plant wastes.

3.7. Multivariate analyses

A principal component analysis (PCA) was carried out for several of the parameters analyzed ($n = 14$). The parameters considered in the statistical analyses were: Total Sugars (TS), Reducing Sugars (RS), Cellulose (Cel), Lignin/Holocellulose Index (LH), Humic acids (Cha), β -glucosidase (β -Gluc), Cellulase, Xylanase, Amylase, Protease, Urease, Phosphatase (FME-K) and Lipase. Establishing three

Table 4
Correlation matrix between the different lignocellulosic fractions and enzymatic activities during a composting of plant waste. Coefficient correlation values with * are significant at $P < 0.05$.

	Total sugars	Reducing sugars	Hemicellulose	Cellulose	Lignin	β -Glucosidase	Amylase	Cellulase	Xylanase	FME-K	Protease	Urease
Reducing Sugars	0.0929											
Hemicellulose	0.0031	0.0282										
Cellulose	0.6027*	0.1463	-0.0275									
Lignin	0.4647*	0.1292	0.0366	0.2777*								
β -Glucosidase	0.0904	0.3605*	0.1438	0.4504*	0.0736							
Amylase	0.0411	-0.2429	0.0151	0.0585	0.2001	-0.0565						
Cellulase	0.0317	0.3934*	-0.0712	0.2492	0.3349*	0.3216*	0.0690					
Xylanase	-0.1076	0.4029*	-0.0744	0.2660*	0.3532*	0.3911*	0.2689*	0.6873*				
FME-K	-0.2835*	-0.0046	0.2473	-0.0122	-0.0593	0.3978*	0.3832*	0.1477	0.3984*			
Protease	-0.0279	0.0559	0.1310	0.3078*	0.2382*	0.0991	0.6268*	0.3193*	0.5445*	0.3621*		
Urease	-0.0065	0.4131*	0.0642	0.4339*	-0.0577	0.8201*	-0.2405	0.2608	0.3186*	0.2637*	0.1253	
Lipase	0.1676	0.5181*	0.3075*	0.4312*	0.1182	0.6677*	-0.2579	0.1960	0.2865*	0.1819	0.1182	0.7645*

PCs, the model was able to explain 70.3% of the variability, with the following contribution of each PC: PC1 36%, PC2 18.5 and PC3 15.8% (Fig. 7). In PC1, the variables cellulose, β -glucosidase, urease and lipase were grouped, while humic acids, HI and LH too grouped but negatively (Fig. 7). PC2 was associated with humic acids, xylanase and phosphatase (FME-K) while total sugars were negatively associated with this principal component. Finally, amylase and protease were grouped in PC3, but lipase was negatively associated in this case (Fig. 7). Several of the enzymatic activities analyzed in this work, showed a slow increase at the end of the process. The peaks in activity observed during the process could be attributed to the protection of extracellular enzymes due to the formation of complexes with humic-like substances. Formation of such complexes is indicated by authors on the basis the significant correlations between some humification indexes and specific enzymatic activity (Cayuela et al., 2008; Mondini et al., 2004). The formation of humo-enzymatic complexes should be considered as a process with a direct link with compost stability, as it is closely related to changes in the compost matrix and, consequently, in the formation of humic-like substances, which is the main purpose of the composting process.

Also, PCA revealed a clear differentiation of groups of objects depending on the sampling times. PC1 discriminated the sampling days having positive loadings on this axis for the nearly entire bio-oxidative phase (RM-DMES3, 26 days), while they were negative for the rest of the process (28–189 days). On the other hand, PC2 grouped initial and final sampling on the positive side of this axis, while central period was grouped on the negative side (9–63 days). In general, a more intense enzymatic activity was associated with the initial sampling between RM and RMES3 (15 days). However, as

expected, humification parameters were more closely associated with maturation and final sampling (between 119 and 189 days).

Enzymes are important indicators of various degradation processes (Goyal et al., 2005). In this sense, the presence of a high content of degradable organic compounds in the initial mixture may stimulate enzymatic synthesis and activity, while the enzymatic activity drops when available substrate decreases. In general, studying the results obtained, all the enzymatic activities measured in the present work increased during the first 16 days of composting, excepting β -Glucosidase and urease which were highest during first 48 h. Similar results were observed by Castaldi et al. (2008) showing that the enzymes required for the hydrolysis of various organic compounds, including complex molecules, were synthesized during the first 2 or 3 weeks of the composting process (Margesin et al., 2006).

4. Conclusions

Therefore, on the basis of the data above indicated, differences in enzymatic profiles must be expected in parallel to the decomposition progresses and temperature rises. The thermal profile inside the piles was proper in this work. Though the evolution in total and reducing sugar content was parallel during the first 15 days after the beginning of the process, an increase in the TS values was observed after the bio-oxidative phase, contrary to the fall detected in the RS values. This fact corroborates that the degradation of more recalcitrant substrates occur after bio-oxidative phase. In relation to lignocellulosic fraction, the fraction most affected by the composting process was cellulose, in contrast to lignin and hemicellulose, whose concentration in the final product remained almost unchanged in comparison to the content in raw material. Like lignin, L/H index (Lignin/Cellulose + Hemicellulose) was stable through the process, but with a pronounced rising in cooling and maturation phases. Moreover, Humification Index increased in maturation phase, and therefore it could be considered useful as an indicator in the maturation process.

In our experience, the enzymatic diversity has been consistent with the temperature pattern shown in this work. In general, at the early stage of the process, the presence of the readily available carbon substrates, as well as the prevalence of mesophilic temperatures, favoured the highest rates of enzymatic activities overall during the first 15–16 days of the beginning of the process. On the contrary, after bio-oxidative phase, when piles begin to cooling the availability of easily available nutrients was minimal and therefore, the microbial and enzymatic activity decreased noticeably. At this stage, other humification parameters as humic acids, HI or LH were prominent.

Bearing in mind our findings and those described by other authors, it is obvious that the evolution of the parameters analyzed in

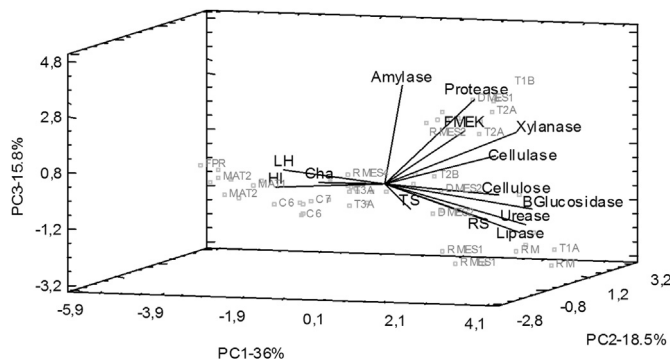


Fig. 7. Results of principal component analysis (PCA) based on data of several parameters studied from day 0–189. Parameters and sampling time are separated along principal components.

this work can be different depending of the started material, considering its nature and origin. However, a thorough understanding of the dynamic in the biological activity of organic waste based-composting could become very useful to improve the efficiency of composting processes.

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References

- Ait Baddi, G., Albuquerque, J.A., González, J., Cegarra, J., Hafidi, M., 2004. Chemical and spectroscopic analyses of organic matter transformation during composting of olive mill wastes. *Int. Biodeter. Biodegrad.* 54, 39–44.
- Ayed, L.B., Hassen, A., Jedidi, N., Saidi, N., Bouzaine, O., Murano, F., 2007. Microbial C and N dynamics during composting process of urban solid waste. *Waste Manag. Res.* 25, 24–29.
- Ayuso, M., Moreno, J.L., Hernández, T., García, C., 1997. Characterisation and evaluation of humic acids extracted from urban waste as liquid fertilisers. *J. Sci. Food Agric.* 75, 481–488.
- Barrena, R., Vázquez, F., Sánchez, A., 2008. Dehydrogenase activity as a method for monitoring the composting process. *Bioresour. Technol.* 99, 905–908.
- Barrington, S., Choiniere, D., Trigui, M., Knight, W., 2002. Effect of carbon source on compost nitrogen and carbon losses. *Bioresour. Technol.* 83, 189–194.
- Ben David, E.A., Zaady, E., Sher, Y., Nejidat, A., 2011. Assessment of the spatial distribution of soil microbial communities in patchy arid and semiarid landscapes of the Negev Desert using combined PLFA and DGE analyses. *FEMS Microbiol. Ecol.* 76, 492–503.
- Boczar, B.A., Forney, L.J., Begley, W.M., Larson, R.J., Federle, T.W., 2001. Characterization and distribution of esterase activity in activated sludge. *Water Res.* 35, 4208–4216.
- Boulter, J.I., Bolaand, G.J., Trevors, J.T., 2000. Compost: a study of the development process and end-product potential for suppression of turfgrass disease. *World J. Microbiol. Biotechnol.* 16, 115–134.
- Bremner, J.M., Mulvaney, R.L., 1978. Urease activity in soils. In: Burns, R.G. (Ed.), *Soil Enzymes*. Academic Press, London, UK, pp. 150–196.
- Burns, R.G., 1982. Enzyme activity in soils: location and possible role in microbial ecology. *Soil Biol. Biochem.* 14, 423–427.
- Bustamante, M.A., Moral, R., Paredes, C., Vargas-García, M.C., Suárez-Estrella, F., Moreno, J., 2008. Evolution of the pathogen content during co-composting of winery and distillery wastes. *Bioresour. Technol.* 99, 7299–7306.
- Castaldi, P., Garau, G., Melis, P., 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions. *Waste Manag.* 28, 534–540.
- Cavani, L., Ciavatta, C., Gessa, C., 2003. Identification of organic matter from peat, leonardite and lignite fertilisers using humification parameters and electro-focusing. *Bioresour. Technol.* 86, 45–52.
- Cayuela, M.L., Mondini, C., Sánchez-Monedero, M.A., Roig, A., 2008. Chemical properties and hydrolytic enzyme activities for the characterisation of two-phase olive mill wastes composting. *Bioresour. Technol.* 99, 4255–4262.
- Charest, M.H., Antoun, H., Beauchamp, C.J., 2004. Dynamics of water soluble carbon substances and microbial populations during the composting of de-inking paper sludge. *Bioresour. Technol.* 91, 53–67.
- Ciavatta, C., Govi, M., 1993. Use of insoluble polyvinylpyrrolidone and isoelectric focusing in the study of humic substances in soils and organic wastes. *J. Chromatogr.* 643, 261–270.
- Ciavatta, C., Govi, M., Vittori, L., Antisari, L., Sequi, P., 1990. Characterization of humified compounds by extraction and fractionation on solid polyvinylpyrrolidone. *J. Chromatogr.* 509, 141–146.
- Cunha-Queada, A.C., Ribeiro, H.M., Ramos, A., Cabral, F., 2007. Study of biochemical and microbiological parameters during composting of pine and eucalyptus bark. *Bioresour. Technol.* 98, 3213–3220.
- Das, A., 1988. City garbage compost as a source of humus. *Biol. Waste* 26, 65–69.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28 (3), 350–356.
- Elorrieta, M.A., Suárez-Estrella, F., López, M.J., Vargas-García, M.C., Moreno, J., 2003. Survival of phytopathogenic bacteria during waste composting. *Agric. Ecosyst. Environ.* 96, 141–146.
- EPA (Environmental Protection Agency, USA), 2003. Environmental Regulations and Technology. Control of Pathogens and Vector Attraction in Sewage Sludge. EPA, 625-R-92–013.
- Farnet, A.M., Qasemian, L., Goujard, L., Gil, G., Guiral, D., Ruauadel, F., Ferre, E., 2010. A modified method based on p-nitrophenol assay to quantify hydrolysis activities of lipases in litters. *Soil Biol. Biochem.* 42, 386–389.
- Fernández-Gómez, M.J., Díaz-Raviña, M., Romero, E., Nogales, R., 2013. Recycling of environmentally problematic plant wastes generated from greenhouse tomato crops through vermicomposting. *Int. J. Environ. Sci. Technol.* 10 (4), 697–708.
- Franco, C., Linères, M., Derenne, S., Le Villo-Poitrenaud, M., Houot, S., 2008. Influence of green waste, biowaste and paper-cardboard initial ratios on organic matter transformations during composting. *Bioresour. Technol.* 99 (18), 8926–8934.
- García, C., Hernández, T., Costa, F., 1991. Changes in carbon fractions during composting and maturation of organic wastes. *Environ. Manag.* 15, 433–439.
- García, C., Hernández, T., Costa, C., Ceccanti, B., Masciandaro, G., Ciardi, C., 1993. A study of biochemical parameters of composted and fresh municipal wastes. *Bioresour. Technol.* 44, 17–23.
- Gea, T., Ferrer, P., Alvaro, G., Valero, F., Artola, A., Sánchez, A., 2007. Co-composting of sewage sludge: fats mixtures and characteristics of the lipases involved. *Biochem. Eng. J.* 33, 275–283.
- Godden, B., Penninckx, M., Castille, C., 1986. On the use of biological and chemical indexes for determining agricultural compost maturity. Extension to the field scales. *Agric. Wastes* 15, 169–178.
- Goyal, S., Dhull, S.K., Kapoor, K.K., 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresour. Technol.* 96, 1584–1591.
- Guo, X., Gu, J., Gao, H., Qin, Q., Chen, Z., Shao, L., Chen, L., Li, H., Zhang, W., Chen, S., Liu, J., 2012. Effects of Cu on metabolism and enzyme activities of microbial communities in the process of composting. *Bioresour. Technol.* 108, 140–148.
- Haddadin, M.Y.S., Haddadin, J., Arabiyat, O.I., Hattar, B., 2009. Biological conversion of olive pomace into compost by using *Trichoderma harzianum* and *Phanerochaete chrysosporium*. *Bioresour. Technol.* 100, 4773–4782.
- He, L., Brickerstaff, G.F., Paterson, A., Buswell, J.A., 1993. Purification and partial characterization of two xylanases that differ in hydrolysis of soluble and insoluble xylan fractions. *Enzyme Microb. Technol.* 15, 13–18.
- He, Y., Xie, K., Xu, P., Gu, W., Zhang, F., Tang, S., 2012. Evolution of microbial community diversity and enzymatic activity during composting. *Res. Microbiol.* 164 (2), 189–198.
- Hsu, J.H., Lo, S.L., 1999. Recycling of separated pig manure: characterization of maturity and chemical fractionation of elements during composting. *Water Sci. Technol.* 40, 121–127.
- Iglesias Jiménez, E., Pérez García, V., 1992. Determination of maturity indices for city refuse composts. *Agric. Ecosyst. Environ.* 38, 331–343.
- Iglesias Jiménez, E., Barral, M.T., Marhuenda, F.C., 2008. Indicadores de la estabilidad y madurez del compost. In: Moreno, J., Moral, R. (Eds.), *Compostaje*. Mundi-Prensa, Madrid, Spain, pp. 243–283.
- Ji, R., Chen, Z., Corvini, P.F.X., Kappler, A., Brune, A., Haider, K., Schäffer, A., 2005. Synthesis of [¹³C]- and [¹⁴C]-labeled phenolic humus and lignin monomers. *Chemosphere* 60, 1129–1181.
- Kandeler, E., Stemmer, M., Palli, S., Gerzabek, M.H., 1999. Xylanase, invertase and urease activity in particle-size fractions of soils. In: *Effect of Mineral Organic Microorganism Interactions on Soil and Freshwater Environment*. Kluwer Academic/Plenum Publishers, New York, pp. 275–286.
- Kaushik, P., Malik, A., Sharma, S., 2013. Vermicomposting: an Eco-Friendly Option for Fermentation and Dye Decolourization waste disposal. *Clean Soil, Air, Water* 41, 616–621.
- Khamforoush, M., Bijan-Manesh, M.J., Hatami, T., 2013. Application of the Haug model for process design of petroleum hydrocarbon-contaminated soil bioremediation by composting process. *Int. J. Environ. Sci. Technol.* 10 (3), 533–544.
- Kononova, M.M., 1966. *Soil Organic Matter*. Pergamon Press, London, UK, p. 544.
- Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol. Biochem.* 4, 19–30.
- Libmond, S., Savoie, J., 1993. Degradation of wheat straw by a microbial community-stimulation by a polysaccharidase complex. *Appl. Microbiol. Biotechnol.* 40, 567–574.
- Liu, D., Zhang, R., Wu, H., Xu, D., Tang, Z., Yu, G., Xu, Z., Shen, Q., 2011. Changes in biochemical and microbiological parameters during the period of rapid composting of dairy manure with rice chaff. *Bioresour. Technol.* 102, 9040–9049.
- Lu, A.L., Kumar, M., Tsai, J.C., Lin, J.G., 2008. High-rate composting of barley dregs with sewage sludge in a pilot scale bioreactor. *Bioresour. Technol.* 99, 2210–2217.
- Malherbe, S., Cloete, T.E., 2002. Lignocellulose biodegradation: fundamentals and applications. *Environ. Sci. Biotechnol.* 1, 105–114.
- Marambe, B., Ando, T., 1992. Phenolic acids as potential seed germination inhibitors in animal-waste compost. *Soil Sci. Plant Nutr.* 38, 727–733.
- Margesin, R., Cimadom, J., Schinner, F., 2006. Biological activity during composting of sewage sludge at low temperatures. *Int. Biodeter. Biodegrad.* 57, 88–92.
- Marinari, S., Masciandaro, G., Ceccanti, B., Grego, S., 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. *Bioresour. Technol.* 72, 9–17.
- Marx, M.C., Wood, M., Jarvis, S., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol. Biochem.* 33, 1633–1640.
- Mishra, P.C., Mohanty, R.K., Dash, M.C., 1979. Enzyme activity in subtropical surface soils under pasture. *Indian J. Agr. Chem.* 12, 19–24.
- Mitsuyo, F., Hirai, A., Kubota, H., 1986. Effect of compost maturity on plant growth. *Biocycle* 27, 58–61.
- Mondini, C., Dell'Abate, M.T., Leita, L., Benedetti, A., 2003. An integrated chemical, thermal and microbiological approach to compost stability evaluation. *J. Environ. Qual.* 32, 2379–2386.
- Mondini, C., Fornasier, F., Sinicco, T., 2004. Enzymatic activity as a parameter for the characterization of the composting process. *Soil Biol. Biochem.* 36, 1587–1594.

- Pietro, M., Paola, C., 2004. Thermal analysis for the evaluation of the organic matter evolution during municipal solid waste aerobic composting process. *Thermochim. Acta* 413, 209–214.
- Portillo, M., Villahermosa, D., Corzo, A., González, J., 2011. Microbial community fingerprinting by differential display-denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 77, 351–354.
- Ros, M., Garcia, C., Hernández, T., 2006. A full-scale study of treatment of pig slurry by composting: kinetic changes in chemical and microbial properties. *Waste Manag.* 26, 1108–1118.
- Ruggieri, L., Artola, A., Gea, T., Sánchez, A., 2008. Biodegradation of animal fats in a co-composting process with wastewater sludge. *Int. Biodeter. Biodegrad.* 62, 297–303.
- Safarik, I., Santruckova, H., 1992. Direct determination of total soil carbohydrate content. *Plant Soil* 143, 109–114.
- Said-Pullicino, D., Kaiser, K., Guggenberger, G., Gigliotti, G., 2007. Changes in the chemical composition of water-extractable organic matter during composting: distribution between stable and labile organic matter pools. *Chemosphere* 66, 2166–2176.
- Schnitzer, M., 1978. In: Schnitzer, M., Khan, S.U. (Eds.), *Soil Organic Matter*. Elsevier, Amsterdam, pp. 1–58.
- Sequi, P., De Nobili, M., Leita, L., Cercignani, G., 1986. A new index of humification. *Agrochimica* 30, 175–179.
- Sequi, P., Ciavatta, C., Antisari, L.V., 1991. Lewis, Chelsea, MI. In: Baker, R.A. (Ed.), *Organic Substances and Sediments in Water-humic and Soils*, pp. 351–367.
- Shi, S., Richardson, A.E., O'Callaghan, M., DeAngelis, K.M., Jones, E.E., Stewart, A., Firestone, M.K., Condon, L.M., 2011. Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiol. Ecol.* 77, 600–610.
- Singh, A., Sharma, S., 2002. Composting of a Crop residue through treatment with microorganisms and subsequent vermicomposting. *Bioresour. Technol.* 85, 107.
- Somogyi, M., 1951. Notes on sugar determination. *J. Biol. Chem.* 73, 599–612.
- Stentiford, E.I., 1996. Composting control: principles and practice. In: de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), *The Sciences of Composting*. Blackie Academic and Professional, Glasgow, UK, pp. 49–59.
- Suárez-Estrella, F., Vargas-García, C., López, M.J., Capel, C., Moreno, J., 2007. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. *Crop Prot.* 26, 46–53.
- Suárez-Estrella, F., Vargas-García, M.C., López, M.J., Moreno, J., 2008a. Effect of humic substances from compost to plant growth and soil microorganisms. *Dyn. soil Dyn. Plant* 2 (1), 96–102.
- Suárez-Estrella, F., Vargas-García, M.C., López, M.J., Moreno, J., 2008b. Changes in carbon fractions during composting of plant wastes and the influence of a humic extract on soil microorganism growth. *Dyn. soil Dyn. Plant* 2 (1), 90–95.
- Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analyses, Part 2, Agronomy*, second ed., vol. 9. Am. Soc. Agron, Madison, Wis, pp. 903–947.
- Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenol phosphate in assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301–307.
- Tuomela, M., Vikman, M., Hatakka, A., Itävaara, M., 2000. Biodegradation of lignin in a compost environment: a review. *Bioresour. Technol.* 72, 169–183.
- Vargas-García, M.C., Suárez-Estrella, F., López, M.J., Moreno, J., 2006. Influence of microbial inoculation and co-composting material on the evolution of humic-like substances during composting of horticultural wastes. *Process Biochem.* 41, 1438–1443.
- Vargas-García, M.C., Suárez-Estrella, F., López, M.J., Moreno, J., 2010. Microbial population dynamics and enzyme activities in composting processes with different starting materials. *Waste Manag* 30, 771–778.
- Wang, H.Y., Fan, B.Q., Hu, Q.X., Yin, Z.W., 2011. Effect of inoculation with *Penicillium expansum* on the microbial community and maturity compost. *Bioresour. Technol.* 102, 11189–11193.
- Yu, H., Zeng, G., Huang, H., Xi, X., Wang, R., Huang, D., Huang, G., Li, J., 2007. Microbial community succession and lignocellulose degradation during agricultural waste composting. *Biodegradation* 18, 793–802.
- Zeng, G., Yu, M., Chen, Y., Huang, D., Zhang, J., Huang, H., Jiang, R., Yu, Z., 2010. Effects of inoculation with *Phanerochaete chrysosporium* at various time points on enzyme activities during agricultural waste composting. *Bioresour. Technol.* 101, 222–227.