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Increasing native microbiota in lignocellulosic waste composting: Effects on process efficiency and final product maturity

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ABSTRACT

Intensive agriculture produces more than one million tons of organic waste mainly composed of lignocellulosic compounds. Though some management strategies have been lately adopted, many problems related to time and surface required for a proper treatment and final product quality remain unsolved. In this work, a staggered bioaugmentation process is proposed for the improvement of horticultural waste composting. Inocula were composed of 30 microbial strains previously isolated from identical composting piles, operated under the same conditions used in this work. The high levels of biomass carbon at the beginning of the bio-oxidative phase and the parallel evolution of reducing sugars reflected a quick adaptation of microbial inocula to the high temperatures reached during the process. Reducing sugar content during the process in the uninoculated pile was below 200 $\mu g\,g^{-1}$, while a higher content was detected in the inoculated pile (2500 $\mu g\,g^{-1}$). Hemicellulose degraded at the end of the process in the inoculated pile was near to 85% whereas in the control pile was below 70%. Lignin degradation ratio was also higher in the inoculated pile (41% in comparison to 23% in the control pile). Moreover, several stabilization and humification indices supported that the bio-inoculation procedure here reported yields better quality products, earlier stabilized, from processes in which lignocellulosic carbon is more efficiently biotransformed.

1. Introduction

One the most important zones in the world devoted to intensive agriculture is located in the southeast of Spain, in the province of Almería. In 2013, more than three million tons of horticultural products were harvested from a land surface around 28,000 ha. As a direct consequence of this vast agricultural production more than one million tons of organic waste is yearly released [1]. In the last years, some management strategies have been started, composting being the treatment more enthusiastically proposed; however, land scarcity to place treatment plants and the speed at which waste is generated make difficult the production of good quality compost. Admitting that composting is probably the best alternative to recycle this kind of organic materials, it is yet mandatory finding the keystones allowing a quick process designed to use up huge amounts of waste and produce high quality compost that can be safely used as organic amendment. Otherwise, intensive agriculture waste problems will remain unsolved and treated waste will only be good for filling dumping sites.

In the last three decades, technical advances and knowledge on the stabilization of organic matter involved in the composting process have made it one of the most promising technologies for organic waste treatment. In fact, the most attractive aspects of composting are its low environmental impact and cost, and its capacity for generating a final valuable product used for increasing soil fertility or as a growing medium in horticulture [2]. However, time, space and manpower required to carry out the process are drawbacks of this eco-friendly technology [3]. Moreover, in regards to raw materials, agricultural by-products are characterized by their high content in lignocellulose which is consistently difficult to bio-transform during composting. As a whole, a high content in lignocellulose in starting materials makes difficult and delays the composting process. However, once composted, lignocellulosic agricultural by-products have been widely used as soil conditioners [4,5]. In general, pretreatment of raw materials is performed to break the lignocellulosic matrix, reduce the degree of crystalline cellulose, and increase the amorphous cellulose fraction, which is

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one of the most suitable forms for enzymatic attack [6]. Several pretreatments involve the application of alkaline and acid chemical solutions to remove lignin and some of the hemicelluloses [7,8]. Other different techniques have been recently developed to accelerate the process, such as co-composting using additives [4], rapid composting using accelerators or continuous thermophilic temperatures [5] as well as microbial inoculation at different phases of the process [9,10]. In this last case, although the microbial community naturally present in organic wastes usually carries out the process satisfactorily, inoculation with external microorganisms is a strategy that could potentially improve the way the process takes place and the properties of the final product [2,9,10]. Certainly, some contradictory results have been reported on the benefits of bio-inoculation, since composting closely depends on the original raw materials and the biological events that take place along the entire process. Taking into account all the biological, nutritional, physical and chemical aspects involved, it stands to reason the conditions are different in every situation, so it is really intricate to accurately estimate the efficacy of bio-inoculation in composting processes [2]. Then, successful composting depends on a number of factors that have both direct and indirect influence on microbial activities. Therefore, the importance of the different microbial communities involved in the process becomes an extensive object of study [11–14].

Inocula for composting have been usually composed of a few number of strains with selected enzymatic capabilities [15–17]. Inocula used throughout this work were composed of microorganisms isolated from an identical composting process to that described in this paper and employed at the same time points they were originally isolated. To the best of our knowledge, the proposed inoculation protocol has not been applied to lignocellulosic waste composting so far.

On the basis of the above cited, the objectives of this study were (i) setting up a staggered bioaugmentation process for the improvement of horticultural waste composting; (ii) studying the evolution of different chemical parameters throughout the process subjected to the inoculation protocol applied, looking for differences with respect to values found in the uninoculated pile; and (iii) determining maturation indices (as indicators of biological stabilization and humification) in both final products inoculated and control.

2. Materials and methods

2.1. Composting process

Composting process was carried out using post-harvest tomato plants (lacking fruits) and pine chips (50:50 w/w). Lignocellulosic fraction was consciously strengthened by the addition of wood chips in order to impose harder conditions for biotransformation. The starting mixture had an appropriate C/N ratio (around 25). Two identical piles were built, one of them (E1) was used as uninoculated control whereas the other pile (E2) was inoculated (see below). Pile dimensions were 3.0 m length × 1.5 m width × 1.0 m height. Raw materials were ground so particle size in the starting mixture was below 30 mm. 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 from decreasing below 10%. The piles were turned over when temperature inside them dropped for several consecutive days. Temperature values inside the piles were continuously measured using a Pt-100 temperature probe connected to a data logger. Besides temperature, pH, bulk density and electrical conductivity were also monitored at sampling times to assess the correct evolution of composting. The process was considered finished after 136 days.

2.2. Strain collection

Microorganisms used in this study were isolated at different stages during a previous composting process carried out with the same raw materials and under the same conditions described in this work (Table 1). They were selected because of their persistence throughout the composting process (they were isolated in most samples taken at different stages). Functionality and molecular identification of the microbial strains used as inoculants in this work have been previously reported [13,14]. Metabolic capabilities of the isolates have been included in Table 1 because of their special relevance to this work. The collection consisted of 30 strains including both mesophilic and thermophilic bacteria and fungi. The identification protocol was based on partial or nearly full length 16S rRNA gene (bacteria, including actinobacteria) and 5.8S-ITS region (yeast and fungi) sequence analysis. The 16S rRNA genes of bacteria were amplified using universal primers: 27F and 1492R. The 5.8S-ITS region of fungi and yeast was amplified using primers ITS1 and ITS4. Amplified PCR products were checked by gel electrophoresis on 1% agarose gel and purified using the Diffinity Rapid Tips (Sigma–Aldrich). The clean DNA was sequenced by capillary sequencer ABI Hitachi 3500 Genetic Analyzer (Applied Biosystems). The forward and reverse sequences were edited, assembled and aligned using the programs Sequence Scanner v1.0 (Applied Biosystem), Reverse Complement (www.bioinformatics.org/sms/rev_comp.html), Clustal X v2.0.11, and MEGA 5 v5.2. The sequences were then compared for similar nucleotide sequences with the BLAST search of the National Center of Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi). The strains closely related were retrieved from NCBI for further analysis.

These microorganisms were used as inocula in the pile E2 at the same composting stages they had been originally isolated. A guideline of inoculation is shown in Table 1.

2.3. Inocula preparation

Bacteria and actinobacteria were cultured in Nutrient Agar plates (CM0309 Oxoid Ltd., UK) for 24 and 72 h, respectively, whereas fungi were cultured in Rose-Bengal Chloramphenicol Agar plates (CM0549B Oxoid Ltd., UK) for 96 h. Incubation temperatures were set at 30 °C or 50 °C respectively for mesophilic or thermophilic strains. Each composting pile was inoculated with a volume of microbial suspension in sterile distilled water enough to reach a proportion of 10^6 colony-forming-units per gram of waste (CFU g^{-1}) (for each strain in the inoculum). The inocula were injected at many different locations in the pile E2 in order to spread the inoculated microorganisms. Pile E1 remained uninoculated.

2.4. Sampling strategy

Samples were collected throughout the process at 6 critical periods driven by temperature changes. Sampling times were named as follows: RM (day 0, raw materials mixture), IMES (8th day, mesophilic stage when temperature is increasing), TER (12th day, thermophilic stage, when temperature showed to be the highest), DMES (14th day, mesophilic stage when temperature is decreasing), COOL (56th day, cooling stage) and FPR (136th day, final product).

Composite sampling was employed. Sub-samples were taken from nine different locations (depth, length, width) all around the pile at each sampling time. Sub-samples were joined and thoroughly mixed to form a composite sample of approximately 2 kg. The composite sample weight was then reduced to 500 g

Table 1
Strains used as microbial inoculants at different composting stages in the pile E2.

Strain	Origin description[a]	Inoculation time (d)[b]	Accession no.[c]	Metabolic activities[d]
Bacillus licheniformis AM-2	Raw materials	0	JN013202.1	1,4,5,8
Bacillus subtilis AM-25	Raw materials	0	JQ403532.1	1,4,5,8
Microbacterium gubbeenense AM-28	Raw materials	0	EU863414.1	1,4,5
Bacillus licheniformis AT-1081	Raw materials	0	EU650317.1	4,8
Bacillus licheniformis BM-1988	Raw materials	0	EF472268.1	1,3,4,8
Brevibacterium halotolerans BM-2015	Raw materials	0	KC967073.1	1,3,4,5,8
Bacillus licheniformis BT-2928	Raw materials	0	HM753625.1	1,2,4,5,8
Bacillus subtilis BT-2938	Raw materials	0	FJ969738.1	4
Scopulariopsis brevicaulis HM-4299	Raw materials	0	EU436681.1	1,3,4,5,8
Gibellulopsis nigrescens HM-4232	Raw materials	0	HE972037.1	1,2,3,4,5,8
Scopulariopsis brevicaulis HM-4276	Raw materials	0	EU821476.1	2,4,5,8
Plectosphaerella cucumerina HM-4244	Raw materials	0	EU594566.1	1,8
Gibellulopsis nigrescens HM-4234	Raw materials	0	HE972037.1	1,2,3,4,5,8
Candida mycentagii HM-4205	Raw materials	0	FJ381698.1	2,3,8
Corynebacterium casei AM-52	Mesophilic phase (↑)[e]	1	JX966460.1	8
Microbacterium indicum AM-54	Mesophilic phase (↑)	1	NR_042459.1	1,4,8
Brachybacterium paraconglomeratum AM-62	Mesophilic phase (↑)	1	JN6495995.1	1,4,8
Bacillus thermoamylovorans AT-1163	Mesophilic phase (↑)	1	AB360815.1	1,4
Bacillus licheniformis BT-3008	Mesophilic phase (↑)	1	KC441778.1	3,4
Ureibacillus thermosphaericus BT-3010	Mesophilic phase (↑)	1	AB300774.1	8
Aspergillus fumigatus HT-5360	Mesophilic phase (↑)	1	HQ026746.1	3,5,8
Scopulariopsis brevicaulis HM-4378	Mesophilic phase (↑)	1	EU436681.1	3,4,5,6,8
Cladosporium lignicola HM-4334	Mesophilic phase (↑)	1	AF393709.2	7,8
Microbacterium gubbeenense AM-81	Thermophilic phase	2	EU863414.1	4
Pseudoxanthomonas taiwanensis BT-3042	Thermophilic phase	2	AB681369.1	8
Chelatococcus daeguensis BT-3147	Thermophilic phase	5	HM000004.1	8
Arthrobacter russicus BT-3271	Mesophilic phase (↑)	9	NR_024783.1	8
Ochrocladosporium frigidarii HM-4685	Mesophilic phase (↑)	9	FJ755255.1	8,9
Scopulariopsis brevicaulis HM-4784	Thermophilic phase	12	KC311514.1	1,2,3,4,5,8
Unidentified strain AM-321	Mesophilic phase (↓)	14	–	1,4,8

[a] Stage at which the strains were isolated in a previous composting trial.
[b] Days after the beginning of composting in E2 pile at which the strains were inoculated.
[c] National Center of Biotechnology Information (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi).
[d] Metabolic activities: amylolysis (1), pectin hydrolysis (2), lipolysis (3), proteolysis (4), hemicellulolysis (5), cellulolysis (6), lignolysis (7), ammonification (8), phosphate solubilization (9).
[e] Temperature was increasing from mesophilic to thermophilic stage (↑) or decreasing from thermophilic to mesophilic stage (↓).

via the quartile method and split into two fractions for analytical purposes. Samples for most chemical analyses were air-dried at 40 °C overnight and ground to <1 mm, while samples for analysis of soluble proteins (SP), reducing sugars (RS), phenolic compounds (PhC), ammonium-nitrogen (NH_4^+-N), nitrate-nitrogen (NO_3^--N), soluble organic carbon (SOC) and microbial biomass C (C_{BIO}) were immediately analyzed or stored in plastic bags at −20 °C until analysis.

2.5. Chemical parameters

2.5.1. Chemical analyses from solid compost samples

Organic matter (OM) content was assessed by loss on ignition at 550 °C to a constant weight. Total carbon (C) and nitrogen (N) were determined in solid samples by dry combustion at 950 °C using a LecoTruSpec C–N Elemental Analyzer (Leco Co., St. Joseph, MI, USA). Microbial biomass carbon (C_{BIO}) was determined using the fumigation–extraction method [18]. Soluble organic carbon (SOC) corresponded to total organic carbon (TOC) in non-fumigated samples. Every TOC analysis was performed in an organic carbon analyzer TOC-V CSN (Shimadzu Co., Kyoto, Japan).

Polymers (lignocellulose, total sugars, fats–oil–waxes and resins), humic-like and phenolic compounds were analyzed in solid compost samples. To determine cellulose (CEL), hemicellulose (HC) and lignin (LIG) 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).

Losses of OM and lignocellulosic fractions (HC, CEL and LIG) were calculated from the initial (A_1) and final (A_2) ash contents according to the equation of Paredes et al. [19]:

$$\%\text{loss} = 100 - 100 \times \left(\frac{A_1 \times P_2}{A_2 \times P_1}\right)$$

where P_1 and P_2 were the initial and final concentrations of OM or lignocellulose fraction (mg g^{-1}).

Biodegradation ratios of lignocellulosic fractions were calculated according to Wang et al. [20]:

$$R_n = \left(\frac{m_0 - m_n}{m_0}\right) \times 100$$

where R_n is the degrading ratio (%) for the nth sampling, m_0 is the initial content (mg g^{-1}) of lignocellulose (HC, CEL or LIG), m_n is the nth sampling content (mg g^{-1}) of lignocellulose (HC, CEL or LIG).

For total sugars (TS) analysis, compost samples (25 mg) underwent hydrolysis with 0.1 mL of 12 M H_2SO_4 for 16 h at room temperature, followed by addition of 2.4 mL of distilled water and heating in boiling water for 8 h [21]. Total sugars in the hydrolyzate were spectrophotometrically quantified [22]. Fats–oils–waxes (FOW) and resins (RES) fractions were gravimetrically determined by weighing Soxhlet diethyl ether and alcohol extracts, respectively obtained from 25 g compost samples [23]. Phenolic compounds (PhC) were extracted from solid samples and quantified as described by Marambe and Ando [24].

2.5.2. Soluble fraction analyses

Several soluble fractions were analyzed in extracts obtained from compost samples. Reducing sugars (RS) and soluble

proteins (SP) were analyzed in an extract of 10 g compost in 40 mL 0.5 M K_2SO_4 shaken at 200 rpm for 30 min and filtered through filter paper. A UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan) was used to measure these parameters [25,26].

NH_4^+-N and NO_3^--N were determined in aqueous extracts by Kjeldahl distillation in an Auto-Titration Kjeldahl Distiller Pro-Nitro A (Selecta S.A., Spain). In this case, the aqueous extracts were obtained by filtration of a mixture of 5 g sample in 100 mL distilled water after shaking at 200 rpm for 30 min.

2.5.3. Humic fractions and other maturation indices

The humic-like fractions were determined according to the method proposed by Ciavatta et al. [27]. Total extractable carbon (TEC) was further fractionated into humified (humic-like and fulvic-like acids – C_{HA}, C_{FA}) and non humified substances (C_{NHS}). TOC from both humified and non humified fractions was measured as previously mentioned.

In addition to the chemical parameters described, some stabilization and humification indices were calculated: polymerization index (C_{HA}/C_{FA}), percent humic acids $P_{HA} = (C_{HA}/TEC) \times 100$ [28], degree of humification $DH = (C_{HA} + C_{FA}/TEC) \times 100$, humification index $HI = C_{NHS}/C_{HA} + C_{FA}$ [27], nitrification index (NH_4^+-N/NO_3^--N) and lignin/holocellulose index (LIG/HOL). Holocellulose was expressed as the sum of cellulose and hemicellulose values.

2.6. Statistical analyses

Data obtained were subjected to statistical analysis using Statgraphics Centurion XVI.I (StatPoint Technologies Inc., VA). 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 treatment and sampling time ($p < 0.05$) provided that data came from a normal distribution (verified by the Shapiro–Wilk's test) and variances were homogeneous (verified by the Levene's homocedasticity test). On the other hand, several statistical multivariate analyses were performed. The presence of categories within samples collected from different composting treatments (E1 and E2) was investigated using linear discriminant analysis (LDA). The method used for variable selection was to minimize the λ_{Wilks} statistics since this parameter indicates the significance of the discriminant function ($p < 0.05$) [29]. Hierarchical cluster analyses (HCA) were carried out to group observations (individual combinations of treatment, sampling time and replicate). Between-groups linkage was used as clustering method and the squared Euclidean distance was the measured interval. Finally, Principal Component Analyses (PCA) was used for data reduction in each treatment (E1 and E2). Data matrix was standardized (based on correlations) and the criterion used to determine the number of principal components to extract was based on eigenvalues (an eigenvalue = 1 was selected).

3. Results and discussion

Results presented here were part of an extensive study on the composting microbiota. Several sets of paired piles (control and inoculated) were studied using always the same raw materials and the same operating conditions (moisture, pH, aeration, etc.). Only the identity of microorganisms, the doses of inocula or the proportions of microorganisms in the inocula were changed in order to find out the best pattern of inoculation. Specifically the results presented here corresponded to the final attempt in optimizing the bioaugmentation process and were repeated two times. Results were absolutely comparable.

3.1. Background

Results presented here are part of an extensive study on the composting microbiota that lasted three years. Several sets of experiments were conducted in order to elucidate physico-chemical and microbiological trends throughout composting. In all experiments, composting piles with identical composition and identically operated were used. In the first experiment (with 3 replicated piles) composite samples were taken at 6 critical periods according to temperature evolution (see Section 2.4). At each sampling time, physico-chemical, and enzymatic determinations were performed in samples. Besides, culturable microbiota was isolated and counted. Initially, more than 5400 strains were obtained, but after an in depth study at cellular and biochemical level, some 1400 strains were finally selected. Strains were functionally characterized (a total of nine important enzymatic activities mostly related to biodegradation of polymeric carbon, were analyzed). At that point, valuable information about the evolution of strains throughout the process and their biochemical capabilities was available. Two additional sets of experiments were carried out, using some of the strains as inoculants for composting. In both cases, the influence of inoculation was followed by determining physico-chemical parameters, humification and stabilization indices. Seven strains were selected on the basis of their enzymatic capabilities and were inoculated in composting piles after each turning operation. On the other hand, 30 strains were selected on the basis of their persistence (they had been isolated at several sampling times throughout the process in significant amounts). They were inoculated at the same times they had been previously isolated (see Section 2.2). The experiments were repeated two additional times while looking for the best proportion of microorganisms to inoculate piles. This last study with strains selected for their persistence is here presented. Other results have already been published [12–14,30].

Since this paper deals exclusively with chemical aspects of the biotransformation of organic matter in inoculated composting piles, it may result convenient to briefly summarize some microbiological results related to the process here described. Fig. 1a and b shows information about the evolution of mesophilic and thermophilic actinobacteria, bacteria and fungi throughout composting in control and inoculated piles. It could be observed a similar pattern for most microbial groups, although counts were in general higher in the inoculated piles. No matter the microbial groups, counts were greater at the mesophilic phases in comparison to those at the thermophilic stage. Fungi were less abundant than bacteria and actinobacteria, and a significant proportion of strains seemed to be thermotolerant. These results have been partially published [11]. Strains from original isolations were fully identified by molecular methods. Microbial community was composed of *Actinobacteria* (68 species), *Bacteroidetes* (1 species), *Firmicutes* (59 species), *Proteobacteria* (59 species) and 70 fungal species (unpublished data).

3.2. Temperature of composting piles

In general, temperature inside the piles (Fig. 1c) followed the typical evolution of composting processes subjected to turning operations and was closely related to that found by other authors using horticultural wastes as starting materials [2]. Thermal profiles in piles E1 and E2 were quite similar although some differences could be observed. An initial temperature of 25 °C was recorded in both piles at the start of composting and the highest temperature (65–70 °C) was observed at around 24–48 h after the beginning of the process. As it was expected, a thermal reactivation was promoted every time turning operations were applied to the piles. Temperature reached three thermophilic periods, being two of them over 55 °C, although temperature values were slightly lower

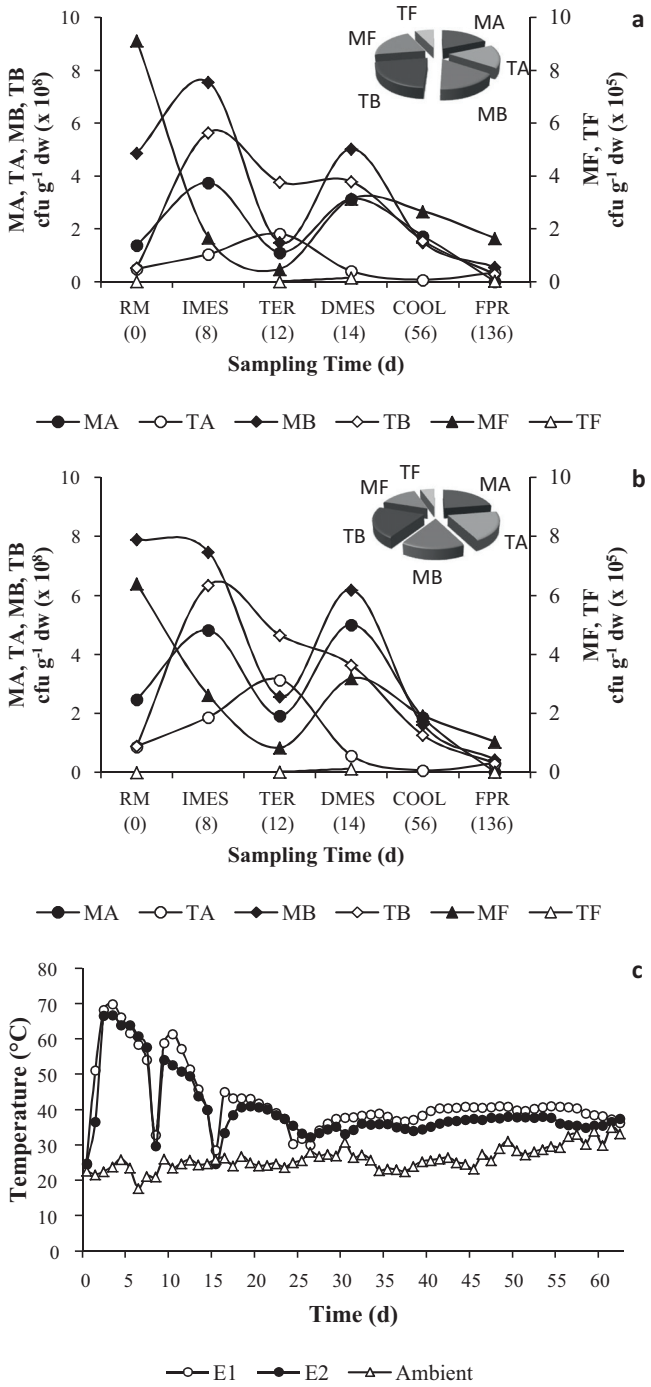

Fig. 1. Overview of microbial groups evolution during composting (a) in the control pile, (b) in the inoculated pile and (c) temperature evolution in the piles E1 and E2 during the first 60 days of composting. Ambient temperature is also depicted. MA and TA (mesophilic and thermophilic actinobacteria), MB and TB (mesophilic and thermophilic bacteria), MF and TF (mesophilic and thermophilic fungi).

in the pile E2. Probably, the periodical introduction of inocula suspensions (see Table 1) could be responsible for these small thermal differences. The United States Environmental Protection Agency [31] recommends maintaining the compost piles above 55 °C for 15 days or at least 5 consecutive days. These strict conditions were kept in this work on the first week after composting started. Both intermediary samples and final products (RM, IMES, TER, DMES, COOL and FPR) were intensively searched for both plant and human pathogens. As expected, after high temperatures (close to 70 °C) reached and maintained in composting piles, pathogens were not detected (data not shown).

On the other hand, temperature is an indicative factor of the composting process evolution. Changes in this parameter are crucial to describe the microbial activity along the entire process and achieve the organic material stability [32]. The temperature within the piles influences greatly the composting process duration, since it directly affects the organic matter degradation rate. Stentiford and de Bertoldi [33] suggested that temperatures between 45 and 55 °C maximized biodegradation rates so higher rates of organic matter degradation could be expected.

3.3. Changes in chemical parameters during composting

The evolution of some chemical parameters throughout composting can help assess differences between treatments (E1 and E2). In this sense, changes observed in the carbon and nitrogen fractions as well as in other interesting chemical parameters (resins, fat–oil–waxes, soluble proteins and phenolic compounds) are shown in Table 2 and Fig. 2. In each case, a comparative study between treatments (E1 and E2) and the different levels of sampling time was carried out.

3.3.1. Analysis of biomass carbon (C_{BIO}), soluble organic carbon (SOC), total and reducing sugar (TS and RS)

Fig. 2a and b shows the evolution of C_{BIO}, and TS and RS in piles E1 and E2 throughout the process. SOC evolution is presented in Table 2. In all cases (C_{BIO}, SOC, TS and RS), there were significant differences between mean values for treatments E1 and E2 and sampling time in each treatment ($p < 0.05$) with the exception of SOC in pile E1 which maintained the same levels during the whole process. As a direct consequence of the microbial inoculation C_{BIO} values in the pile E2 were higher than those in the pile E1. This parameter reached its maximal values at the beginning of the process in pile E2, and decreased from that moment (Fig. 2a). The same profile could be observed for SOC in pile E2 (Table 2). A slower decrease was observed during the cooling phase to reach similar values to those measured in composting E1 at the end of the process. C_{BIO} and SOC variations were similar to the thermal trend in pile E2. This behavior clearly indicates that dynamics of carbon soluble fractions is not only strongly related to microbial activity but also to the temperature evolution (that is indeed a direct consequence of microbial activity). The high levels of C_{BIO} measured at the beginning of the bio-oxidative phase and the parallel evolution of RS (see Fig. 2b) reflects a quick adaptation of microbial inocula to the high temperatures reached during the process. Similar results have been previously published [34].

Readily degradable organic matter of immature compost mainly consists of total sugars, phenolic substances, amino acids, peptides and other easily biodegradable compounds. In this sense, sugars are among the main components present in the carbon fractions of composted materials, though only RS are straightaway available for microbial growth [35]. According to our results, the effect of bio-inoculation significantly affected the evolution of TS and RS values (Fig. 2b). TS detected in the pile E1 increased until the cooling phase in which the TS content was higher than 450 mg g^{-1}. Another evident peak was detected during the thermophilic phase, with values around 350 mg g^{-1}. After two months of composting, the TS content fell gradually until the end of the process, when it reached the lowest level, near 250 mg g^{-1}. The evolution of this parameter in the pile E2 was very different, since the bio-inoculation noticeably affected the initial TS values. At the beginning of the composting process the TS content was higher than 400 mg g^{-1}. However this value slowly decreased throughout the entire process until it reached 300–350 mg g^{-1} in the final product (Fig. 2b).

Table 2
Evolution of organic matter (OM), C/N ratio, soluble organic carbon (SOC), phenolic compounds (PhC), soluble proteins (SP), fats–oils–waxes (FOW) and resins (RES) throughout composting under treatments (T) E1 and E2. Values (mg g^{-1}) are means ± standard deviations (n = 3).

Sampling (d)	T	OM*	T*	C/N*	T*	SOC*	T*	PhC*	T*	SP*	T*	FOW*	T*	RES*
RM[a] (0)		^{C}659.35 ± 17.06		^{C}27.67 ± 4.12		^{AB}1.04 ± 0.21		^{C}9.86 ± 1.31		^{BC}1.25 ± 0.23		^{E}24.80 ± 1.05		^{E}71.57 ± 3.73
IMES[b] (8)		^{B}590.34 ± 39.32		^{BC}24.53 ± 2.35		^{A}0.85 ± 0.13		^{A}7.42 ± 0.87		^{BC}1.21 ± 0.11		^{B}11.71 ± 0.96		^{CD}62.22 ± 2.07
TER[c] (12)	^{A}E1	^{BC}619.45 ± 19.29	^{A}E1	^{BC}22.94 ± 3.22	^{A}E1	^{A}0.87 ± 0.24	^{B}E1	^{BC}9.20 ± 0.98	^{A}E1	^{CD}1.39 ± 0.13	^{B}E1	^{BC}12.53 ± 1.67	^{B}E1	^{DE}66.54 ± 3.05
DMES[d] (14)		^{BC}636.90 ± 25.67		^{BC}23.55 ± 3.15		^{B}1.24 ± 0.15		^{BC}9.14 ± 0.71		^{D}1.75 ± 0.57		^{A}9.65 ± 0.84		^{C}59.92 ± 3.27
COOL[e] (56)		^{B}592.76 ± 32.28		^{B}21.61 ± 1.42		^{A}0.80 ± 0.12		^{AB}7.72 ± 0.93		^{AB}0.81 ± 0.18		^{D}19.85 ± 1.27		^{A}41.74 ± 2.94
FPR[f] (136)		^{A}480.51 ± 42.61		^{A}14.43 ± 0.92		^{A}0.90 ± 0.06		^{A}6.74 ± 0.20		^{A}0.54 ± 0.17		^{C}13.81 ± 0.79		^{B}53.35 ± 1.18
RM (0)		^{D}673.54 ± 13.99		^{C}25.41 ± 1.81		^{C}7.14 ± 1.75		^{C}4.99 ± 0.24		^{E}9.12 ± 0.10		^{E}20.22 ± 0.12		^{B}52.71 ± 0.78
IMES (8)		^{C}632.07 ± 4.98		^{B}21.46 ± 0.31		^{B}3.07 ± 0.25		^{B}3.77 ± 0.16		^{D}5.37 ± 0.30		^{D}16.85 ± 0.83		^{A}47.99 ± 2.04
TER (12)	^{A}E2	^{D}657.57 ± 5.32	^{A}E2	^{B}21.69 ± 0.59	^{B}E2	^{A}1.47 ± 0.16	^{A}E2	^{B}3.62 ± 0.01	^{B}E2	^{C}4.65 ± 0.22	^{A}E2	^{C}7.23 ± 1.48	^{A}E2	^{C}57.54 ± 2.00
DMES (14)		^{C}634.86 ± 9.66		^{B}21.14 ± 0.42		^{A}1.07 ± 0.16		^{A}3.00 ± 0.17		^{B}4.08 ± 0.41		^{B}5.15 ± 0.43		^{B}53.86 ± 2.81
COOL (56)		^{B}553.44 ± 12.50		^{B}20.46 ± 0.29		^{A}0.89 ± 0.11		^{A}3.23 ± 0.05		^{A}2.43 ± 0.05		^{A}3.17 ± 0.32		^{BC}54.57 ± 1.36
FPR (136)		^{A}470.56 ± 11.13		^{A}16.31 ± 1.68		^{A}0.49 ± 0.02		^{B}3.58 ± 0.18		^{A}2.04 ± 0.03		^{A}3.09 ± 0.48		^{BC}55.19 ± 2.54

* Indicates significant differences (p < 0.05) between treatments or sampling times.
[a] RM: raw materials.
[b] IMES: mesophilic stage when temperature is increasing.
[c] TER: thermophilic stage, when temperature shows the highest values.
[d] DMES: mesophilic stage when temperature is decreasing.
[e] COOL: cooling stage.
[f] FPR: final product.

With respect to RS content, important data were compiled, since very different profiles were observed in both processes (Fig. 2b). RS content during the whole process (E1) was below 200 μg g^{-1}, while a much higher RS content was detected in the pile E2 where values fluctuated between 2500 μg g^{-1} (at the beginning) and 1000 μg g^{-1} (at the end of the process).

At first glance, it is obvious that differences detected in both parameters (TS and RS) were mainly due to bio-inoculation. The evolution of these parameters throughout composting reflects the relationship between microbial activity and sugar content. Microorganisms have the ability to act upon polysaccharide molecules, favoring 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 [9]. Therefore, the inoculation of the pile E2 with the selected microorganisms (Table 1) over a period of two weeks after the starting of the process was determinant to achieve a significant increase in the RS levels with respect to those observed in the pile E1 (Fig. 2b). It is especially noteworthy the great difference between RS concentrations in piles E1 and E2 at the first sampling time (RM). Inocula suspensions were made in distilled water, however, some remnants of the culture medium could be dragged together with cells (slimy extracellular material and cellular debris) so this material could have been added to the pile at the moment of inoculation. Taking into account that 15 strains at a final concentration each of 10^6 CFU g^{-1} were initially inoculated in the pile E2 (see Table 2), it would be reasonable to conclude that there could be a significant increase of carbonaceous materials including soluble sugars as a result of massive inoculation performed at the beginning of the process. On the other hand, elapsed time between piles inoculation and the analytical procedures corresponding to the first sampling time was at least 5–6 h, so there could be a substantial release of soluble sugars as a result of microbial activity over this period of time.

3.3.2. *Inorganic forms of nitrogen (NH_4^+-N and NO_3^--N)*

Knowledge about the availability of the primary mineral nutrient in composts, in whichever of its forms (NH_4^+-N and NO_3^--N) is of major importance to determine the end use for the final product [36]. In this work, most of the mineral N in the composts was in the nitrate (NO_3^--N) form, whereas very low ammonium nitrogen (NH_4^+-N) was detected. This last nitrogen form weakly increased in the first 2 weeks after the beginning of the process and gradually decreased to minimal values at the end of the composting period (Fig. 2c). The evolution of this parameter was similar in both experiments and agreed with the results previously reported by other authors [9]. However, the entire NH_4^+-N content along the process was globally lower in the pile E2, probably as a consequence of microbial growth demands.

On the contrary, the levels of NO_3^--N were generally higher in the pile E2, although at the end of the process NO_3^--N concentrations were quite similar in both piles (Fig. 2c). Nitrification is a bio-oxidative process, requiring oxygen for the normal functioning of the nitrifying bacteria [37]. Additionally, high amounts of ammonium and extreme temperatures could inhibit the nitrification process by microbial inactivation [33]. In this work, the lowest amount of ammonium and the highest levels of nitrate were detected in the bio-inoculated pile during the bio-oxidative phase. Although temperature has been reported to negatively affect nitrification, NO_3^--N content was higher in the thermophilic stage in both piles, but possibly this increase was achieved in the previous mesophilic (IMES) phase and detected later. In any case, recent studies have revealed the existence of thermophilic ammonium oxidizers [38], whose activity could support higher nitrate levels under thermophilic conditions. Probably, microbial augmentation activated the nitrification process avoiding excessive losses of NH_3 by volatilization [39].

3.3.3. *Lignocellulose evolution*

In this work, microbial strains used as inocula were selected on the basis of their persistence throughout the composting process. Obviously, this high persistence is a consequence of their enzymatic capabilities. Detailed studies on the functionality of these strains (including enzymatic activities related to lignocellulose degradation) have been recently published [13,14].

Results recorded in literature referring to the degradation of lignocellulosic fractions offer a high heterogeneity and sometimes they are contradictory [28,40]. Some authors stated that lignin fraction is the most adequate component to conduct a proper humification process since polyphenols, sugars and amino compounds released after its biodegradation, seem to promote humus formation [41]. In this sense, microorganisms isolated during composting are able to release a wide range of hydrolytic enzymes which favor or even improve the degradation of the organic waste [42]. Thus, bioaugmentation of microorganisms involved in the polymeric

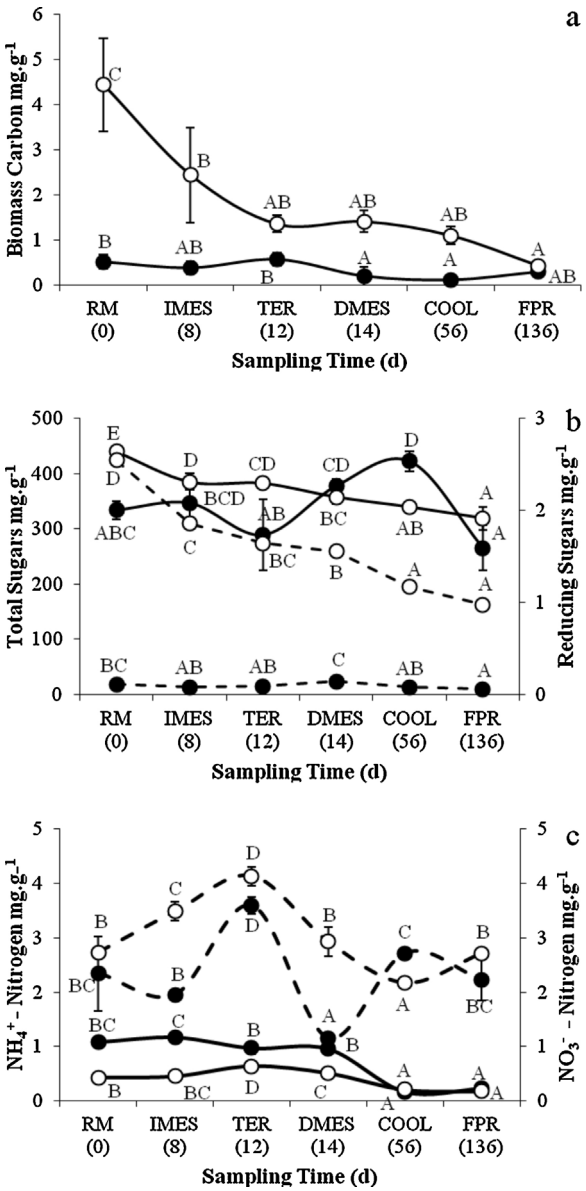

Fig. 2. Evolution of different chemical parameters throughout composting under treatments E1 (●) and E2 (○): (a) C_{BIO}, (b) TS (solid lines) and RS (dashed lines), (c) NH_4^+-N (solid lines) and NO_3^--N (dashed lines). Bars represent standard deviations ($n = 3$). Points with the same letter in a given line are not significantly different according to Fisher's LSD test.

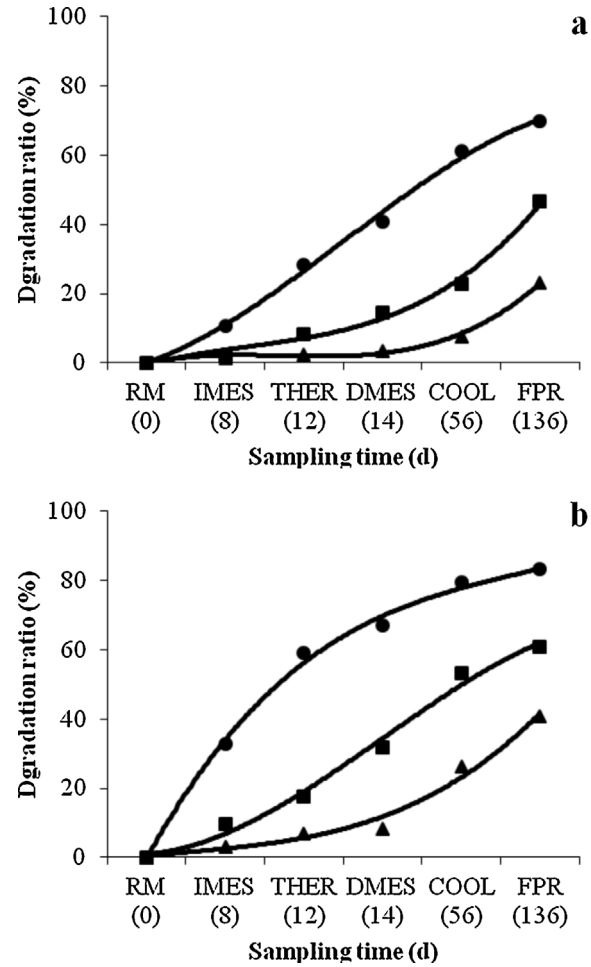

Fig. 3. Biodegradation ratio of HC (●), CEL (■) an LIG (▲) throughout composting under treatments E1 (a) and E2 (b).

carbon metabolism can potentially accelerate biodegradation and consequently, the humification process [2].

As usually, analyzed data came from immediate measures (i.e. concentrations at each sampling time). However, instant measures of concentration for a given parameter do not consider losses of dry matter mainly due to microbial activity (organic matter mineralization and CO_2 production). In this sense, significant weight losses are recorded throughout any composting process [43,44]. Actually, the only parameter that theoretically does not vary during the process is the ash content; however, instant measures of ash concentrations indicate an apparent increase from the beginning to the end of composting. Bearing in mind this fact and using the appropriate calculations [19], it is possible to ascertain real changes for a given parameter in relation to initial values.

The evolution of lignocellulosic fractions throughout composting is illustrated in Fig. 3 in which degradation ratios on an ash-free basis for HC, CEL and LIG both in control (Fig. 3a) and inoculated (Fig. 3b) piles are shown. HC degradation profiles showed this fraction was noticeably used throughout composting. The amount of HC degraded at the end of the process in the inoculated pile was near 85% (of the initial amount) whereas in the control pile the degraded proportion was below 70%. On the other hand, most of HC degradation was achieved during the bio-oxidative stage in the inoculated pile (E2), while in the uninoculated pile (E1), the utilization of this polymer was constantly accomplished along the whole process, though more slowly than in pile E2. In regards to CEL, a similar pattern was found. Degradation profile in the control pile, showed that most of CEL was degraded at the end of the process (45%) after the bio-oxidative stage had finished. In the inoculated pile, biodegradation ratios started earlier and accounted for a higher amount (61%). Finally, LIG degradation ratio was also higher in the inoculated pile (41% in comparison to 23% in the control pile). Negligible biodegradation ratios were found throughout the bio-oxidative stage in the control pile and most of LIG degradation was attained at the end of composting; however, LIG biotransformation in the inoculated pile started to be significant after the thermophilic phase. A comparison of lignocellulose biodegradation ratios between treatments, revealed that final values were earlier reached in the inoculated pile than in the control pile, so it is not adventurous to affirm that the bioaugmentation strategy here employed speeded the utilization of the hardest to degrade polymeric fractions during composting.

These results were confirmed by calculating the percentage of change of each lignocellulosic fraction in the final composts

Table 3
Initial (RM) and final values (FPR) (mg g^{-1}), and % change produced throughout composting under treatments E1 and E2. Values of total organic matter (OM_T), hemicellulose (HC), cellulose (CEL), lignin (LIG) and non-lignocellulosic organic matter (OM_{NCL}) are shown as percentages of OM_T on an ash-free basis.

Treatment	Sampling (d)	OM_T	HC	CEL	LIG	OM_{NLC}
E1	RM (0)	100.00	15.72	42.76	22.53	18.98
	FPR (136)	47.79	3.11	14.98	11.33	18.36
	% change	52.21	12.61	27.78	11.20	0.62
E2	RM (0)	100.00	14.80	41.59	21.88	21.74
	FPR (136)	43.08	1.50	9.74	8.28	23.56
	% change	56.92	13.29	31.85	13.60	−1.82

obtained from both piles in relation to initial values on ash-free basis.

Table 3 includes initial (RM) and final (FPR) concentrations (mg g^{-1}) for total organic matter (OM_T), HC, CEL, LIG, non-lignocellulosic organic matter (OM_{NLC}) and changes of each parameter (as % of its initial value) in piles E1 and E2. In all cases, values are expressed as percentage of OM_T. These calculations come in very useful to achieve real ratios of variances between two sampling times. According to results shown in Table 3, the organic fraction of raw materials was effectively used throughout composting in both piles, although some important differences were detected (52.21% in the control pile and 56.92% in the inoculated pile). Losses in organic matter were unevenly shared by the different lignocellulosic fractions (HC 12.61%, CEL 27.78%, LIG 11.20% in the control piles and HC 13.29%, CEL 31.85%, LIG 13.60% in the inoculated pile) but in every case % Change was higher in the inoculated pile. Values of OM_{NLC} do not come from a direct measure; they are actually calculated by subtracting (HC + CEL + LIG) from OM_T. Non-lignocellulosic OM (OM_{NLC}) in the raw materials (RM) represents the amount of readily available OM. Obviously, some non-lignocellulosic organic compounds are produced throughout the process at the expense of lignocellulose biotransformation. These compounds, together with other soluble carbon substances initially detected in raw materials are exhausted as a consequence of microbial growth throughout the process. On the other hand, some of lignocellulosic materials are used to build humic substances. Composted lignocellulosic wastes have a high agronomic value due to the amount and quality of their humic-like substances, since the degradation products from lignocellulose (polyphenols and carbohydrates) actively participate as precursors in the formation of humic substances [23]. Thus, at the end of the process (FPR), OM_{NLC} does not represent a remaining amount of undegraded soluble carbon, on the contrary, it accounts for humified organic matter.

3.3.4. Other polymeric fractions

Other polymers such as fats–oil–waxes, resins, phenolic compounds and soluble proteins were analyzed in solid compost samples. There were significant differences between mean values of all these parameters for treatments (E1 and E2), and sampling time in each treatment ($p < 0.05$). The evolution of RES was similar in both processes, but a significant decrease concerning the amount of FOW and PhC was detected in the pile E2 (Table 2). FOW accounted for a small fraction of starting material (2–3% dw). This hydrophobic fraction dropped during bio-oxidative phase until it reached a value below 0.5% at the end of the composting E2. This significant fall was much more obvious in the pile E2 and it mainly occurred during the thermophilic phase. This decreasing trend has been also reported in composting of oily materials such as olive mill waste [45] and suggests lipolytic activity among composting microbiota.

PhC exhibited a general decreasing trend once thermophilic phase was reached in composting E1 (Table 2). On the other hand, a weak increase of the PhC amount was detected at the end of the process in the pile E2. This final increase has been previously observed by other authors [46], suggesting a polyphenol re-polymerization during the maturation phase that would contribute to the formation of humic-like substances.

Nitrogen is found during composting processes as insoluble (mainly some proteins) and soluble fractions (NH_4^+, NO_2^-, NO_3^-, amino acids and peptides). Both forms are released by ammonification and proteolysis mechanisms. Proteolytic microorganisms are usually present at the initial stages of composting. This microbial group favors the release of soluble proteins during the bio-oxidative phase, while this fraction is detected at low levels at the end of the process [47]. This was the general profile found for SP concentration during the composting process in the pile E1 (Table 2) where maximal levels of this parameter were around 2 mg g^{-1}. The amount of SP was much higher in the pile E2. The bio-inoculation process favored the release of SP in this pile, reaching values between 5 and 9 mg g^{-1} during the first week after the beginning of the process. The evolution of this fraction was parallel to that of C_{BIO} (see Fig. 2a). In fact, a strong and significant correlation between both parameters was found ($r = 0.9$, $p < 0.001$).

3.4. Humification and stabilization indices

Though the incorporation of composted waste with high organic matter content could improve soil quality and fertility and reduce waste materials [48], it could also produce toxicity problems that inhibit seed germination and plant development if inadequately matured composts are used [24]. Traditional parameters, such as temperature, odor, color, C/N ratio, cationic exchange capacity, extractable carbon, phytotoxicity tests or evolution of humified substances have been successfully used to assess compost maturity and stability [28,49].

The percentage of humic substances is expected to increase in relation to total organic matter throughout the composting process. When the composting pile was bio-inoculated, a higher content of extractable carbon was detected in the final compost E2 (Table 4) in comparison to that in the uninoculated control E1 (102.18 and 77.89 mg g^{-1}, respectively). Similarly, humic acids concentration was significantly higher in the inoculated pile. On

Table 4
Humification parameters in final composts after treatments E1 and E2. Total extractable carbon (TEC), humic acids (C_{HA}), fulvic acids (C_{FA}), humic substances ($C_{HA} + C_{FA}$), non-humic substances (C_{NHS}) and humification indices (C_{HA}/C_{FA} ratio, P_{HA}, DH and HI). Values (mg g^{-1}) are means ± standard deviations ($n = 3$).

Humification parameter	Control (E1)	Inoculated (E2)
TEC (mg g^{-1})	^{A}77.89 ± 1.34	^{B}102.18 ± 3.75
C_{HA} (mg g^{-1})	^{A}34.95 ± 1.75	^{B}59.21 ± 1.29
C_{FA} (mg g^{-1})	^{A}12.66 ± 0.73	^{A}11.54 ± 0.60
$C_{HA} + C_{FA}$ (mg g^{-1})	^{A}47.61 ± 2.15	^{B}70.75 ± 1.19
C_{NHS} (mg g^{-1})	^{A}30.28 ± 2.03	^{A}31.43 ± 2.60
C_{HA}/C_{FA}	^{A}2.76 ± 0.16	^{B}5.14 ± 0.33
P_{HA} %	^{A}44.87 ± 2.32	^{B}57.96 ± 0.94
DH %	^{A}61.12 ± 2.56	^{B}69.27 ± 1.42
HI	^{B}0.64 ± 0.07	^{A}0.44 ± 0.03

Within a row, values with the same letter are not significantly different ($p < 0.05$).

the contrary, fulvic acids fraction was slightly lower in the inoculated pile, although significant differences could not be detected. Total amounts of humified substances ($C_{HA} + C_{FA}$) were obviously greater in the inoculated compost. Many of the substances included in NHS fraction are detected along the entire composting process, especially interfering with fulvic component separation [50]. As indicated in Table 4, an appreciable amount of C_{NHS} was measured in both products. Some compounds such as those measured as TS, RS, SP, RES, HC, CEL and LIG can easily be included in the extractable carbon pool.

Das [51] found a gradual increase in the parameter C_{HA}/C_{HF} ratio during composting. C_{HA}/C_{FA} reflects the degree of polymerization, in other words, the formation of complex molecules (HA) from more simple molecules (FA) [52]. This parameter was used to evaluate compost maturity (Table 4). C_{HA}/C_{FA} values over 1 indicate humification is taking place. Since the index represents the ratio between more polymerized carbon contained in HA and less polymerized carbon (more easily degradable for microorganisms) contained in FA, the higher the index, the more stable the product is. In both treatments, this index gave rise to values greater than 1, and consequently, the 4 month-old composts produced in this study could be considered mature [10]. Nevertheless, the ratio C_{HA}/C_{FA} reached higher values in composting E2 than those observed in composting E1 (5.14 and 2.76, respectively). P_{HA} and DH represent the proportion of C_{HA} or $C_{HA} + C_{FA}$ respectively, in relation to TEC. According to Iglesias-Jiménez et al. [28], P_{HA} values greater than 62% are considered as indicative of an optimal maturation of city refuse materials after composting. In regards to DH, Ciavatta et al. [27] suggested a value of 60% for humified materials such as soils and organic amendments. Both indices P_{HA} and DH showed values for compost from the inoculated pile very similar to those considered as indicative of maturity. Compost from the control pile also reached a correct degree of maturity according to DH index but not according to P_{HA}. Finally, the humification index (HI) is based on the assumption that during humification, non-phenolic compounds are decomposed to produce polyphenolic (humic) substances [23]. According to Ciavatta et al. [27], HI decreases continuously during humification processes, and reaches values lower than 1 at the end of the stabilization. Our results showed HI was 0.44 in compost from the inoculated pile, indicating this material was highly stabilized. Compost from the control pile was also stabilized although HI was greater (0.64).

Many attempts have been made to relate inoculation of composting piles to an enhancement of humification parameters, although results have shown to be controverted [2,10]. However, this controversy should not be surprising since one of the most influential factors affecting the humification process is the chemical and biological composition of raw materials which can be itself considered quite heterogeneous [53]. Similarly, results recorded in literature referring to the degradation of lignocellulosic fractions in relation to the humification parameters propose very diverse information, given the great diversity of materials that can be composted and their composition in relation to lignocellulosic content [28,40]. Generally, the biodegradation of the lignin fraction occurs at the late phases of composting and a very low rate of decomposition is usually detected. Lignin acts as a protective factor for the cellulosic and hemicellulosic fractions. Specifically, there exists a strong association between lignin and hemicellulose, favored by the presence of phenolic compounds [54]. It is obvious that the entire molecule of lignocellulose exhibits a great inertia to microbial degradation [55]. Some indices have been developed to relate two or more lignocellulose fractions and help interpret more accurately the evolution of these fractions. This is the case of the Lignin/Holocellulose index (LIG/HOL), whose values tend to increase during the process (Fig. 4a) [40]. An ascending trend for this parameter could be detected in both composting piles. Nevertheless, the LIG/HOL index

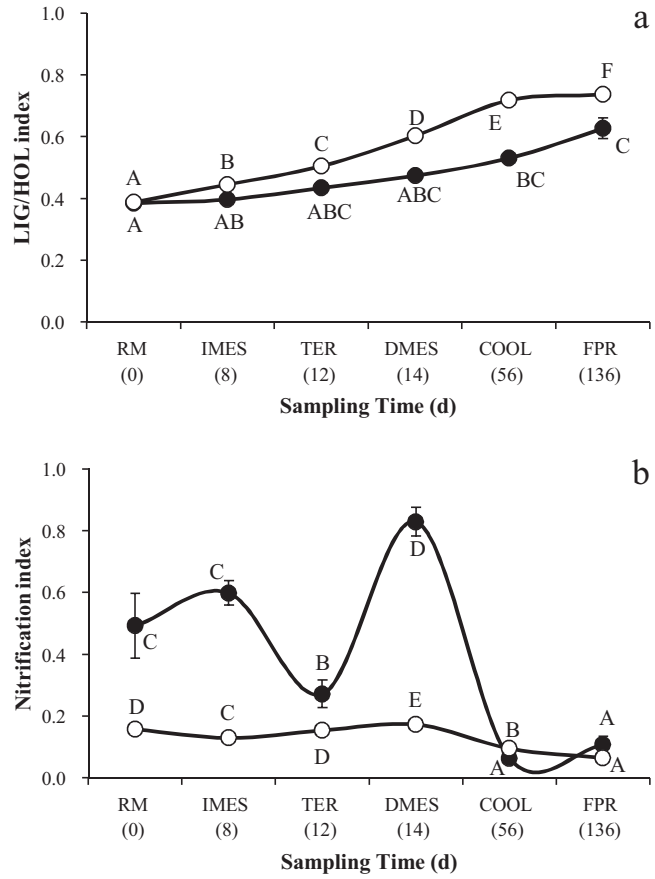

Fig. 4. Evolution of stability indexes throughout composting under treatments E1 (●) and E2 (○): (a) LIG/HOL index and (b) NH_4^+/NO_3^- index. Bars represent standard deviations ($n = 3$). Points with the same letter in a given line are not significantly different according to Fisher's LSD test.

almost doubled its value at the end of the process E2, and went well above that observed in the process E1 (0.74 and 0.63, respectively).

The ratio between the inorganic forms of nitrogen (nitrification index NH_4^+-N/NO_3^--N) has been used as a decisive factor for evaluating compost maturity [56]. In this work, the highest significant values were obtained during the bio-oxidative phase followed by a significant decrease during the cooling and maturation phases (Fig. 4b). The resulting nitrification index was less than 1, in accordance to that previously proposed [57]. The evolution of the nitrification index was significantly different ($p < 0.05$) between both treatments, though similar values were reached at the end of the process. Concerning composting E1, the values detected for the nitrification ratio were much higher than those observed in process E2. This fact was consistent with both the lower levels of NH_4^+-N and the higher levels of NO_3^--N detected in the pile E2 in relation to those measured in the pile E1. The evolution of this parameter in the bio-inoculated composting processes could reveal a decrease in the NH_3 volatilization rate as well as an increase in the nitrification rate during the bio-oxidative phase [38,39].

3.5. Statistical multivariate analyses and final considerations

According to data previously presented, it is obvious that inoculation implies variations in the chemical parameters studied (as confirmed by typical analyses of variance and multiple comparison). In order to ascertain which parameters were more affected and the extent of the influence of bio-inoculation on them, some multivariate statistical analyses were performed.

In the first place, Linear Discriminant Analyses were carried out. Initially, no restrictions were imposed, so all chemical parameters were included. In the resulting model, 21 variables were involved and using the resulting discriminant function, all the 36 observations (combinations of treatment, sampling times and replicates) employed to adjust the model, were correctly classified as belonging to E1 or E2 treatments ($\lambda_{Wilks} = 0.005$, $p < 0.05$). Then, a stepwise discriminant analysis was carried out in order to decrease the number of variables involved and identify the minimal set of them needed to discriminate between treatments E1 and E2. The stepwise discriminant analysis only used 5 steps in which 4 variables were involved (PhC, RS, C_{HA}/C_{FA}, SOC), although finally, PhC was removed to minimize the λ_{Wilks} value. The discriminant function in this case was −1,42*SOC + 2.49*RS + 1.31*CAH/CAF ($\lambda_{Wilks} = 0.017$, $p < 0.05$) and all the observations were correctly classified as belonging to E1 or E2 treatments. Thus, it seems these 3 variables could be used to characterize samples from both treatments. To confirm these results, a Hierarchical Cluster Analysis was carried out using as grouping variables RS, SOC and C_{HA}/C_{FA} and the resulting dendrogram showed two clearly separated groups of observations corresponding to E1 and E2 treatments (data not shown).

Finally, Principal Component Analyses were performed for each set of results (E1 and E2) in order to identify orthogonal combinations of variables accounting for as much of the variability in the data as possible. When E1 results were analyzed, five principal components, accounting for 87.44% of the variance, could be extracted (eigenvalue = 1). Principal components 1 and 2 (PC1 and PC2) are graphically represented in Fig. 5a. The most influential variables in PC1 (48.69% of variance) were CEL, HC, LIG/HOL, AN and C/N. On the other hand, PC2 accounted for 15.00% of variance being C_{BIO}, SOC, OM_{NLC}, TS and RS. Notwithstanding the above, most of variables in Fig. 5a are quite far away from the origin (0.0) so it can be concluded that a large number of variables contribute significantly to total variability in treatment E1; in other words, there is a reasonably high correlation between variables, and the first extracted component (PC1) is responsible for more than half of the total variance. Qualitatively, chemical parameters corresponding to gross sources of carbon and nitrogen seem to be determinant to characterize events occurring under treatment E1.

Regarding to results from E2 treatment, the Principal Component Analysis extracted four components accounting for 92.38% of total variance. PC1 (67.03%) and PC2 (13.31%) are graphed in Fig. 5b. For PC1, the most influential variables were CEL, RS, SP, LIG/HOL and HC. PC2 showed that NH_4^+-N, NO_3^--N, NH_4^+/NO_3^-, PhC and OM seemed to contribute more significantly to total variance. In general, the number of variables located close to the origin (0.0) was higher in E2 (when compared to E1), so a lower number of chemical parameters significantly contributed to variance. In addition, RS and SP were among the variables with higher coefficients in PC1. This fact should not result surprising, since these parameters had already proven to be involved in discriminant and clustering analyses. Besides, RS and SP represent a lighter fraction (readily degradable) of carbon and nitrogen sources needed to support higher amounts of microorganisms present in the pile E2, at least at the first stages of composting. On the other hand, NH_4^+-N, NO_3^--N and NH_4^+/NO_3^-, were among the variables with higher absolute coefficients (PC2). This is consistent with results presented in Table 2, Figs. 2b and 4b according to which RS and SP evolve in parallel throughout E2 composting and higher amounts of NH_4^+-N are demanded as a consequence of microbial growth.

Finally, in light of results obtained, and being aware that composting can be carried out without any supplementary addition of microorganisms, it is evident that the inoculation strategy here employed causes clear upgrades in the process. The main goals to be accomplished for the improvement of composting are always related to the time required to complete the process (and ground surface occupied in the meanwhile) and quality of final products. On the other hand, when inoculants are introduced inside a composting pile they have to compete with autochthonous microbiota and most of times they end up disappearing. The bioaugmentation protocol here proposed uses the own autochthonous microbiota which is intensified at several key moments of the process and produces better quality products in shorter periods of time. Whether the time saved and the enhanced quality make up for the economic investment derived from the inoculating operations or not, is something still unknown and deserves more attention.

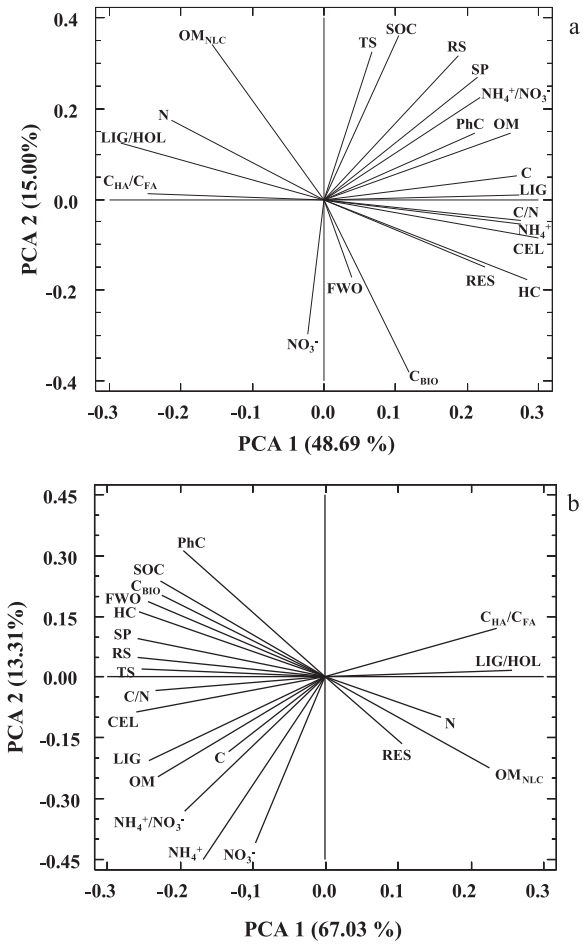

Fig. 5. Principal component analysis of data showing variable loadings plots for each treatment. (a) E1 and (b) E2.

4. Conclusions

The proposed bioaugmentation protocol, using native microorganisms isolated from identical composting processes, induced statistically significant changes in the values of more than 25 typically analyzed chemical parameters. Dynamics of carbon soluble fractions was strongly related both to microbial activity and temperature evolution. Higher levels of biomass measured throughout the bio-oxidative phase and the parallel evolution of soluble organic carbon and reducing sugars in the inoculated pile were absolutely evident. Favored by forced aeration and lower ammonium concentrations, higher rates of nitrification occurred throughout the process in those stages in which temperature was softer in the bio-inoculated pile.

Lignocellulosic fractions were degraded more efficiently in the inoculated pile (quantitatively more lignocellulose was

transformed); but more importantly, the level of biodegradation achieved in the control pile was reached much earlier in the inoculated pile, suggesting that the process could be shortened if the raw materials are suitably enriched with appropriate microbiota. Both composts achieved a correct maturity degree, however, several stabilization and humification indices showed the final product from the inoculated pile had a better quality to be potentially applied as an organic amendment. To sum up, bioaugmentation with native composting microbiota allows a more effective and shorter process, and yields final products of better quality.

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