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Evolution of microbial dynamics during the maturation phase of the composting of different types of waste

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ABSTRACT

During composting, facilities usually exert greater control over the bio-oxidative phase of the process, which uses a specific technology and generally has a fixed duration. After this phase, the material is deposited to mature, with less monitoring during the maturation phase. While there has been considerable study of biological parameters during the thermophilic phase, there is less research on the stabilization and maturation phase. This study evaluates the effects of the type of starting material on the evolution of microbial dynamics during the maturation phase of composting. Three waste types were used: sludge from the fish processing industry, municipal sewage sludge and pig manure, each independently mixed with shredded pine wood as bulking agent. The composting system for each waste type comprised a static reactor with capacity of 600 L for the bio-oxidative phase followed by stabilization and maturation phase in triplicate 200 L boxes for 112 days. Phospholipid fatty acids, enzyme activities and physico-chemical parameters were measured throughout the maturation phase. The evolution of the total microbial biomass, Gram + bacteria, Gram − bacteria, fungi and enzymatic activities (β-glucosidase, cellulase, protease, acid and alkaline phosphatase) depended significantly on the waste type ($p < 0.001$). The predominant microbial community for each waste type remained present throughout the maturation process, indicating that the waste type determines the microorganisms that are able to develop at this stage. While fungi predominated during fish sludge maturation, manure and municipal sludge were characterized by a greater proportion of bacteria. Both the structure of the microbial community and enzymatic activities provided important information for monitoring the composting process. More attention should be paid to the maturation phase in order to optimize composting.

1. Introduction

Composting is a process of biological degradation of solid organic substrates under aerobic conditions through the action of different microbial populations, yielding a stable, humidified and suitable product to add to the soil (Insam and de Bertoldi, 2007). The organic material goes through different phases: a mesophilic phase, characterized by the proliferation of the microbiota, a thermophilic phase where a high rate of biodegradation, the growth of thermophilic organisms and the inhibition of non-thermotolerant organisms occur and the final phase that includes a period of cooling, stabilization and maturation, characterized by the growth of mesophilic organisms and the humification of the compost (Ryckeboer et al., 2003b). In the composting facilities the maturation phase is usually carried out with less control and monitoring than the bio-oxidative phase. The duration of the bio-oxidative phase that is carried out in bioreactors depends upon the type of substrate that is used but generally lasts from 7 to 15 days. After this phase, the material that exits the reactor generally is placed in windrows for a curing phase (Diaz et al., 2007). The time required for the maturation phase is a function of the substrate and environmental and operating conditions of the facility and can range from a few weeks to a year or two (Diaz et al., 2002). This lack of control over the process may cause environmental problems such as odours and leachates, in addition to adversely affecting the quality of the compost.

The maturation phase has mainly been studied in terms of the physico-chemical and biological parameters in order to determine when compost is mature enough to be added to the soil by establishing maturity and stability criteria and indexes of the final product (Bernal et al., 2009; Insam and de Bertoldi, 2007; Paradelo et al., 2010). In terms of biological parameters, several enzymatic studies have been carried out to determine microbiological activity during composting and provide indicators of the stability of different composts (Cayuela et al., 2008; Ros et al., 2006). Castaldi et al.

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(2008) proposed that the study of dynamics of certain enzymatic activities, without single point determinations, could be a suitable indicator of stability, although it was not possible to establish a threshold value. However, the study of enzyme activities provides information on the breakdown of organic matter and the metabolic processes that take place during composting and, therefore, on product stability.

Most studies on the structure of the microbial community in composting have specifically focused on the early stages of the process because, under aerobic conditions, temperature is the biggest selective factor of microbial populations (Ryckeboer et al., 2003b). Likewise, the nature of the organic substrates is also an important factor in determining the dynamics and microbial diversity during composting (Klammer et al., 2008; Ryckeboer et al., 2003b; Vargas-García et al., 2010). Ishii and Takii (2003) showed that the main factor affecting microbial communities was the concentration of dissolved organic substances, which depended on the type of starting material. López-González et al. (2015) showed that the composition of fresh materials and operating conditions determine how the microbiota behaves, as well as its structure and its biodiversity.

Phospholipid fatty acid (PLFA) profiles analysis is a technique that provides information about the structure of the microbial community and how it changes during composting. The total amount of PLFAs can be used as an indicator of viable microbial biomass. Furthermore, some PLFAs are specific to certain living organisms (e.g. bacteria, fungi, actinomycetes and plants) which means they can be used as biomarkers for the presence and abundance of specific microbial groups (Zelles, 1999). There have been studies of the evolution of PLFAs during the composting of different wastes, with greatest emphasis on the initial stages of the process and some authors including on time sampling during the maturation phase (Amir et al., 2010; Eiland et al., 2001; Hellmann et al., 1997; Klamer and Bååth, 1998). Jindo et al. (2012) found that after 12 weeks of composting, factor analysis based on the relative abundance of individual PLFAs revealed changes in the structure of the microbial community that depended on the original organic waste. Boulter-Bitzer et al. (2006) studied the microbial community of different composts during maturation and storage, noting that PLFA analysis was a valuable method for characterizing the microbial community structure during this phase of the composting process.

The study of biological parameters during stabilization and maturation and the influence of the source material can help improve the quality of compost and optimize the composting process, meaning that a better understanding of changes in the microbial dynamics is necessary for the maturation phase of composting. The objectives of this research were: (1) to study the development and structure of the microbial community using PLFAs and enzymatic activities during the maturation phase of the composting process; (2) evaluate the effect of the type of waste on the microbial structure and activity; and (3) improve and optimize the composting process by providing more information on the maturation phase.

2. Materials and methods

2.1. Composting materials

Composting experiments were performed using three different waste types whose main physico-chemical properties are detailed in Table 1:

- Sewage sludge from the food industry (FI), from precooked and frozen fish and cephalopods, obtained after separation of fats and treatment with coagulants and flocculants.
- Manure from a pig-breeding farm (PM), collecting the solid fraction of slurry after storage in manure pits.
- Municipal sewage sludge wastewater (MSS) obtained after aerobic digestion in lagoons and dewatering with band filter.

Table 1
Physico-chemical characteristics of the wastes used in the composting experiments: sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM).

	FI	MSS	PM
Moisture (%)	65.4	87.0	82.8
Organic matter (%)	93.0	73.1	79.6
Total carbon (mg g^{-1} dw)	532.5	364.0	402.1
Total nitrogen (mg g^{-1} dw)	26.8	46.5	31.9
Water soluble carbon (mg g^{-1} dw)	20.50	2.54	19.78
Dissolved organic nitrogen (mg g^{-1} dw)	10.05	9.07	10.18
Total phosphorus (mg g^{-1} dw)	7.16	20.01	16.07
Fats (% dw)	22.3	6.0	15.6

dw: dry weight.

Shredded pine wood passed through a 3 cm sieve was used as a bulking agent and each waste type was mixed with this agent to obtain a ratio 1:2 (v/v), a free air space (FAS) of 30–40% and a moisture content of around 60–70%.

2.2. Experimental design

After applying the bulking agent, each waste type was subjected to a composting process in which the bio-oxidative phase took place in a static reactor with forced ventilation and the stabilization and the maturation phase (hereinafter referred to as the maturation phase) were carried out in triplicate in 200 L batches (Fig. 1).

The adiabatic composting reactor had an effective volume of 600 L, a perforated floor and a ventilation system with the ability to introduce fresh or recirculated air through the top and bottom of the reactor. The temperature and oxygen level were recorded every minute using a Eurotherm controller with three temperature probes at different depths and a gas probe inside the mass with an oxygen sensor. A feedback loop of oxygen and temperature (ventilation when temperatures exceeded 60 °C or oxygen fell below 5%) and a time controller were used for aeration. The material was kept in the composting reactor until the temperature fell below 35 °C, requiring a total of 20, 18 and 17 days for FI, PM and MSS, respectively. After emptying the reactor, each waste type was mixed and placed in triplicate in maturation systems of 200 L. Wooden boxes of 70 × 54 × 54 cm with a perforated base and open top were used for the maturation systems to allow gas exchange with the outside, attempting to simulate the inside of a maturation pile by maintaining some isolation from the outside. Similarly, to take place correctly, composting requires moisture balanced conditions to prevent the occurrence of water stress that might generate biological inactivity and false compost stabilization. A layer of bulking agent was placed at the top and bottom of the box to provide thermal insulation for the waste and prevent moisture loss. Mesh was placed between the composted material and the bulking agent to prevent them mixing.

The beginning of the maturation process (day 0) started after emptying the reactor. Maturation systems were emptied and mixed by hand at 14, 28, 42, 56, 70 and 91 days to simulate the dynamics of turning a pile of compost under maturation. A composite sample was taken of each system during each turning. The total volume of the composite sample was 1500 mL. This was sieved through a 1 cm mesh to remove the bulking material. The temperature and oxygen level was monitored daily for the first 42 days and three times per week until the end of the process at 112 days. The moisture level was maintained above 60%, except

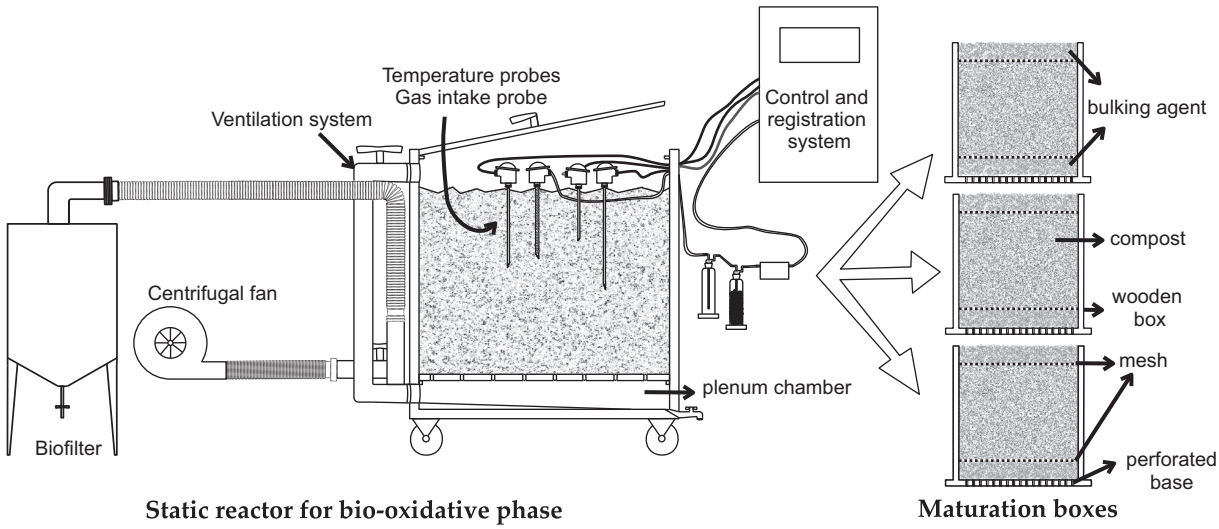

Fig. 1. Scheme of the reactor and the maturation boxes used during the composting process for each waste type.

for FI, which required periodic rewetting in the first weeks of the process related to its highly hydrophobic nature due to a high lipid content (Table 1) and the higher temperatures reached at the start of the maturation phase.

2.3. Chemical analysis

The moisture and organic matter contents of the samples were determined after drying at 105 °C until constant weight and ashing at 550 °C for 4 h, respectively. Fresh samples were extracted with 0.5 M K_2SO_4 in a ratio 1:10 (w/v) for analyses of inorganic nitrogen ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) (Sims et al., 1995) and dissolved organic nitrogen content (DON) (Cabrera and Beare, 1993). Total nitrogen content (TN) and total carbon content (TC) were determined by combustion of dried samples using a LECO 2000 CN elemental analyzer. Water soluble carbon content (WSC) was analyzed in aqueous extracts 1:5 (w/v) by dichromate oxidation in sulfuric acid solution (Sims and Haby, 1971). Electrical conductivity was determined in aqueous extracts 1:10 (w/v) using a conductivimeter Crison CM 35.

2.4. Biological and biochemical analysis

The microbial community composition and biomass were determined by phospholipid fatty acid (PLFA) analysis following the method described by Gómez-Brandón et al. (2010) for organic samples. Briefly, total lipids were extracted by stirring from 200 mg of each freeze-dried sample with 60 mL of chloroform–methanol (2:1, v/v). Phospholipid fraction was obtained after separation on silicic acid columns and was subjected to derivatization with trimethylsulfonium hidroxyde (TMSH). Fatty acid methyl esters (FAMEs) obtained were analyzed by gas chromatography and mass spectrometry (GC–MS). GC–MS analysis was performed on a column CP-Select FAME, 100 m × 0.25 mm. FAMEs were identified by comparison of their retention time and mass spectra with known standards (Larodan Fine Chemicals AB, Malmo, Sweden). The quantification was performed with methyl nonadecanoate fatty acid (C19:0) as internal standard. PLFAs were used to estimate the biomass of specific microbial groups: gram-positive bacteria (i14:0, i15:0, a15:0, i16:0, a17:0), gram-negative bacteria (16:1ω7, 17:1ω7, 18:1ω7, cy19:0) and fungi (18:2ω6, 18:1ω9, 20:1ω9). The total amount of PLFAs identified (totPLFAs) was used as an indicator of the viable microbial biomass (Zelles, 1999).

β-glucosidase was estimated by incubating 1 g of fresh sample with 1 mL of p-nitrophenyl-β-D-glucopiranoside (0.025 M) for 1 h at 37 °C and subsequent colorimetric measurement of p-nitrophenol released (Eivazi and Tabatabai, 1988). Alkaline and acid phosphatase were measured by incubating 0.5 g of fresh sample with 1 mL of p-nitrophenylphosphate (0.015 M) for 1 h at 37 °C and subsequent colorimetric measurement of p-nitrophenol released (Eivazi and Tabatabai, 1977). Protease was measured by colorimetric determination of the amino acids released, after the incubation of 1 g of fresh sample with 5 mL of sodium caseinate (2%) for 2 h at 50 °C, using Folin-Ciocalteu reagent (Ladd and Butler, 1972). Cellulase was assessed by colorimetric determination of reducing sugars released after incubation of 5 g of fresh sample with 15 mL of carboxymethyl cellulose sodium salt (0.7%) for 24 h at 50 °C (Schinner and von Mersi, 1990).

Germination index (GI) was calculated according to Zucconi et al. (1981) by determining seed germination and root length of *Lepidium sativum* growing in 2 mL of aqueous extracts 1:5 (w/v) in Petri dishes lined with paper filter during 48 h.

Static respiration rate (SR) was measured using manometric respirometers by OxiTop® system (WTW GmbH, Weilheim, Germany). Briefly, fresh weight equivalent to 4 g of dried sample was placed in a hermetic container with a 1 M NaOH trap to capture CO_2, and the pressure drop, due to microbial oxygen consumption, was recorded during 24 h at constant temperature. The self-heating test was carried out using 2 L Dewar flask for 10 days at room temperature (TMECC, 2002).

2.5. Statistical analysis

All statistical tests were performed using R software (R Development Core Team, 2014). The physico-chemical data was subjected to principal component analysis (PCA) after normalization to zero mean and unit variance, and the analysis was performed on the correlation matrix. The principal components with eigenvalues greater than one were retained. The PCA was performed using the prcomp function and the package ggbiplot (Vu, 2011). Enzyme and PLFA data was analyzed with linear mixed-effects models using the nlme package (Pinheiro et al., 2015). The waste type and time were fixed factors and the repeated measurement throughout time in each maturation box was treated as a random effect to address the non-independence of samples. Logarithmic and square root transformations of the data were necessary to ensure the normality and homogeneity of the variance of

residuals of models. For post hoc comparison between treatments, Tukey tests were carried out using the glht function of the multcomp package (Hothorn et al., 2008). Correlation analyses were also carried out to examine the relationships between PLFAs and enzyme activities with cor function of the stats package. All statistical tests were evaluated at the 95% confidence level and values are given as the mean ± standard error.

3. Results

3.1. Temperature evolution

The temperature inside the reactor increased rapidly at the beginning of the process (Fig. 2), reaching over 45 °C on days one and two for FI and PM, respectively, and both wastes maintained thermophilic temperatures for more than 10 days. For MSS, thermophilic temperatures were reached inside the reactor on day four and were maintained for seven days.

At the beginning of the maturation phase, the temperature of FI increased to 60 °C, falling on day four and reactivating after turning at 14 and 28 days, although in the latter cases, the temperature remained below 40 °C. Both PM and MSS generally remained below 25 °C during the maturation phase. In terms of the oxygen levels during the maturation phase, measurements remained above 17% for all waste types.

3.2. Composting parameters

The principal component analysis of the physico-chemical variables is presented in Fig. 3 and shows the three separate groups for the three different waste types. The samples taken during maturation of FI exhibited the greatest dispersion along the axes. The parameters responsible for the differences between waste types along principal component one (PC1) were WSC ($r = -0.97$, $p < 0.0001$) and moisture ($r = 0.89$, $p < 0.0001$), differentiating samples during the maturation of FI, PM and MSS. During the process, FI maintained a high concentration of WSC, with a maximum of 25.6 mg g^{-1} at the beginning of the maturation phase and a minimum of 9.1 mg g^{-1} at 112 days. Both PM and MSS reached a maximum of around 7.5 mg g^{-1}, with a drop throughout the maturation process to achieve significantly lower values in PM than MSS (Table 2). In terms of moisture, PM and MSS remained at around 60–70% throughout the process, while FI required periodic rewetting during the first seven weeks, obtaining minimum values of 35% by day 28 and remaining between 45% and 55% from day 42 until the end of the process. The parameter with the biggest contribution to the separation of the waste along principal component two (PC2) was the ratio C/N ($r = 0.92$, $p < 0.0001$), primarily

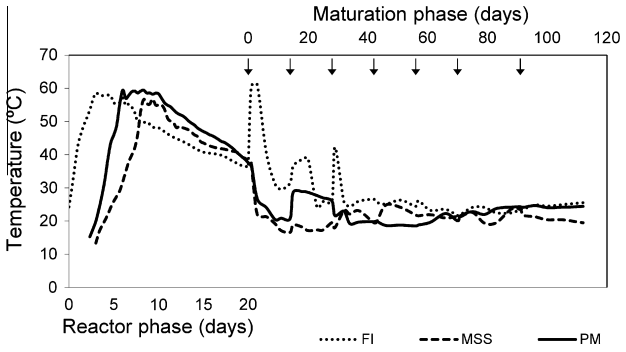

Fig. 2. Temperature evolution during the reactor phase and the maturation phase for sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM). Arrows correspond to the turnings after emptying the reactor (time 0) and during the maturation phase (14, 28, 42, 56, 70, 91 days).

differentiating between PM and MSS. In the latter one, values of C/N > 12 were not detected along the maturation phase, while PM and FI reached around 18 at the beginning of maturation, with significantly lower values for FI at the end of the process (Table 2).

3.3. Microbial community

Microbial biomass, Gram + bacteria, Gram − bacteria and fungi were significantly different between wastes ($p < 0.001$). Likewise, significant differences caused by time ($p < 0.0001$) and significant interactions between time and waste type ($p < 0.0001$) for all microbial biomass and microbial groups were observed.

The highest concentration of microbial biomass was observed in MSS at the beginning of maturation and fell progressively over time (Fig. 4). This reduction was also present in the group indicators, falling to about 97.8% in Gram + bacteria, 99.5% in Gram − bacteria and 99.3% in fungi. At the beginning of the process the proportion of PLFAs of a specific group with respect to total PLFAs group indicators was higher in Gram − bacteria (>40%), whereas by the end, the greater proportion was in Gram + bacteria (>60%). Hence, more PLFAs characteristic of bacteria than fungi ones were observed during the process. Finally, the microbial biomass of MSS during maturation was correlated with all microbial groups ($r > 0.98$, $p = 0.000$).

In the case of FI, there was an increase in microbial biomass during the first weeks of the process, declining in the following samplings and showing a significant increase during the last 21 days of maturation. The microbial biomass was strongly correlated with the concentration of fungi ($r = 0.915$, $p = 0.000$). In this case, the predominance of PLFAs characteristic of fungi was maintained at over 55% during the process.

Furthermore, PM presented less microbial biomass than the other ones in the first samplings, gradually falling until day 56 and later recovering to values slightly below the initial value. A high correlation between microbial biomass and the PLFAs characteristic of Gram + and Gram − bacteria ($r > 0.93$, $p < 0.0001$) were observed. During the maturation phase, bacteria predominated (values above 75%), especially Gram + bacteria.

3.4. Enzyme activities

The type of waste significantly affected the evolution of all hydrolytic activities studied ($p < 0.001$). Also, significant differences caused by time ($p < 0.0001$) and interaction between time and waste ($p < 0.0001$) were also observed for all the enzymes.

After intensive composting in the reactor, MSS presented the highest activity of β-glucosidase (Fig. 5a), which fell significantly in the first 14 days and remained stable from 70 days, resulting in a final reduction of 85%. In contrast, at the start of the maturation phase, PM and FI showed similar values of β-glucosidase, but from day 56, PM had significantly higher values than the other waste types.

With respect to cellulase (Fig. 5b), increased activity was observed in PM, except for the last sampling where similar results were found in FI. In the case of MSS, a continued reduction in cellulase activity was detected, while it increased slightly for FI in the final maturation sampling.

The acid and alkaline phosphatase activities exhibited similar trends for the same waste type (Fig. 5c and d, respectively), except the initial samplings of PM. Acid phosphatase activity was similar to β-glucosidase activity, with higher levels in MSS for initial samplings and high activity at the end of the process for PM. However, FI remained significantly lower throughout maturation. Despite the different activity levels observed at the reactor outlet, FI and MSS showed similar trends of alkaline phosphatase enzymes, with activity peaking on day 42, followed by a sharp decline to similar

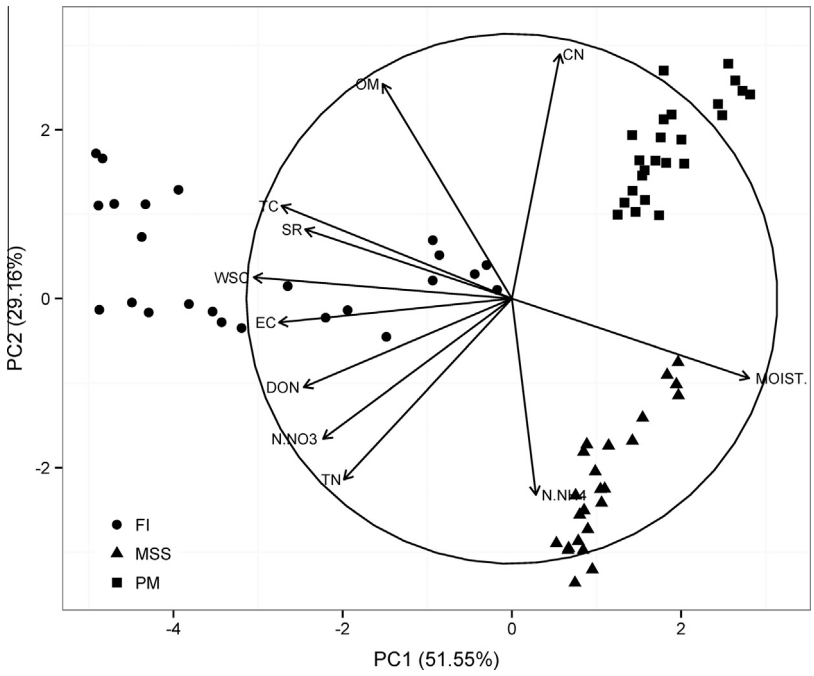

Fig. 3. Correlation biplot of principal component analysis where vectors correspond to the variables that define the components and points correspond to sampling during the maturation phase for sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM).

Table 2
Parameters of composts after 112 days of maturation phase for sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM).

	FI	MSS	PM
Ratio C/N	12.25 ± 0.11^{a}	11.53 ± 0.20^{b}	16.31 ± 0.31^{c}
Ratio NH_4^+/NO_3^-	0.24 ± 0.03^{a}	0.09 ± 0.01^{b}	0.56 ± 0.03^{c}
SR (mg O_2 g^{-1} OM h^{-1})	0.53 ± 0.01^{a}	0.33 ± 0.02^{b}	0.78 ± 0.01^{c}
Self-heating test	Class V	Class V	Class V
GI (%)	91.3 ± 2.1^{a}	99.5 ± 1.1^{b}	94.1 ± 1.4^{a}
WSC (mg g^{-1} dw)	9.11 ± 0.15^{a}	3.50 ± 0.20^{b}	2.67 ± 0.15^{c}

SR: static respiration, GI: germination index, WSC: water soluble carbon, dw: dry weight.
In each parameter the different letters indicate significant differences between composts (Tukey post hoc test $p < 0.05$).

values for both waste types at the end of the process. Similar to acid phosphatase and β-glucosidase, PM showed significantly higher values of alkaline phosphatase than MSS and FI in the final samplings.

Only the protease enzyme (Fig. 5e) exhibited similar trends in all wastes, with reduction rates of 80%, 72% and 22% for PM, MSS and FI respectively. In all cases, activity stabilized in the final samplings.

As shown in Table 3, microbial groups for MSS were positively correlated with all enzymatic activities. In contrast, in FI, only PLFAs characteristic of fungi were positively correlated with the β-glucosidase and alkaline phosphatase enzymes. With respect to bacterial biomass for PM, both Gram + and Gram – bacteria were correlated with alkaline phosphatase, protease and cellulase activities, while fungal biomass was positively correlated with cellulase.

4. Discussion

4.1. Temperature evolution

Unlike FI, PM and MSS exhibited biological degradation of organic matter prior to composting, the former during storage in septic tanks for solid-liquid separation of the pig slurry and the latter during treatment of wastewater in aeration tanks. Consequently, both had a lower content of organic matter than FI (Table 1), allowing the full thermophilic phase to take place in the static reactor. The materials did not require turning to reactivate the process, showing that the forced ventilation was effective, and both fresh composts matured in boxes with stable and environmental temperatures. In the case of FI, however, turning favoured the increase in temperature at the beginning (day 0), and at 14 and 28 days, meaning that forced ventilation was not sufficient to allow full development of the bio-oxidative phase of the composting process in the reactor. Alburquerque et al. (2009) showed that forced ventilation was effective when performed together with mechanical turning during *alperujo* (olive waste) composting, concluding that turning improved the porosity and helped distribute the moisture, substrates and microorganisms. During treatment of wastewater from fish processing a digestion process was not performed, meaning that the organic load of the sludge was high (Table 1), and turning and watering during the first weeks allowed re-heating to provide biodegradable substrates for the microorganisms from the outside to the inside of the box. Despite this re-heating, FI kept the characteristics of a maturation process with temperatures similar to MSS and PM from the first month. So, in a composting facility, this highly organic waste should be monitored when it is disposed to mature, to prevent false stabilizations of compost, odours and other environmental problems.

4.2. Multivariate analysis

Multivariate analysis shows that the source material determined the physico-chemical development of the stabilization and maturation process for composting. Several authors have found that composts obtained from different organic waste types differ in their physico-chemical composition and, hence, in terms of their stabilities and qualities, depending on the composition of source material used in the composting (Bernal et al., 1998; Ranalli et al., 2001). Similarly, the physico-chemical properties during

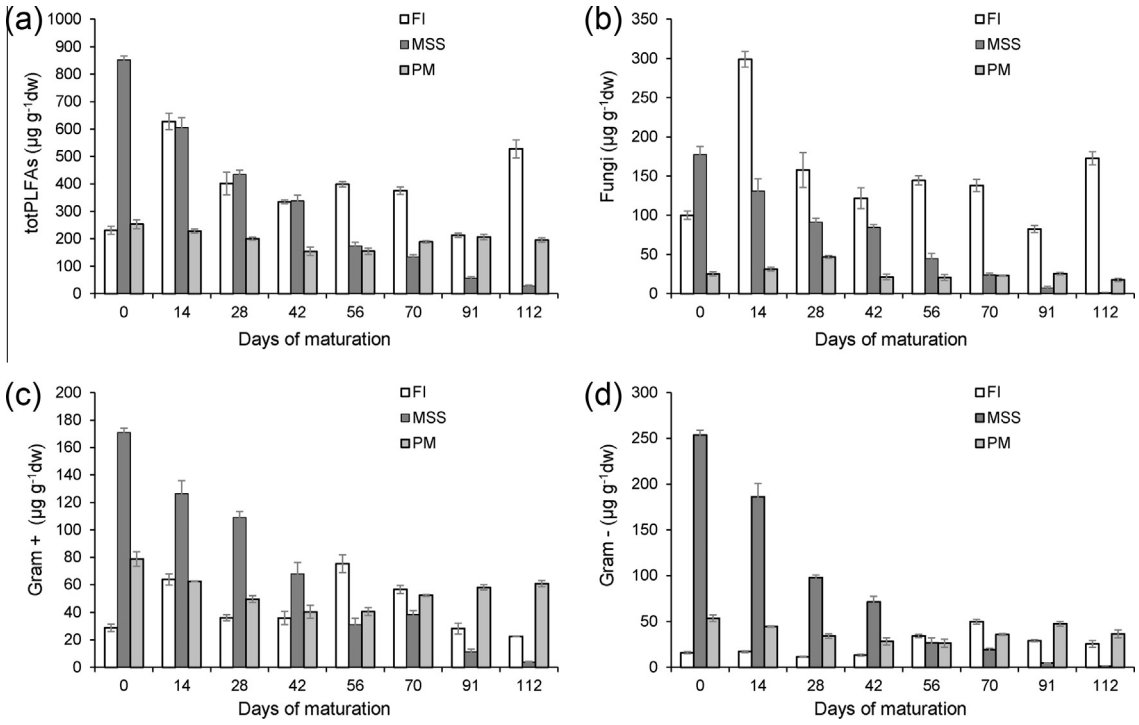

Fig. 4. Changes in (a) microbial biomass, (b) fungi, (c) Gram + bacteria and (d) Gram − bacteria estimated by phospholipid fatty acid (PLFA) analysis during the maturation phase for sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM).

the maturation phase determined the separation of the three waste types and affected the final composition of the composts. FI showed the greatest changes in physico-chemical composition during maturation, possibly due to its initial re-activation caused by its high organic load, as observed in the temperature profile. The highest respiratory activities and contents of C and N forms occurred in FI, meaning more time may be required for stabilization. The maturation process of PM was characterized by maintaining the C/N ratio at a low value (Bernal et al., 2009), suggesting a possible stabilization of the degradation process of organic matter. However, Fialho et al. (2010) have shown that the C/N ratio is not a good method for monitoring the composting process and that there is not an optimal ratio that characterizes humified compost. Finally, MSS was characterized by high levels of ammonia due to the low C/N ratio present throughout the whole process. De Guardia et al. (2008) have shown that an excess of aeration after the thermophilic phase could be responsible for the loss of N. Thus, stabilization of the composting parameters of MSS suggested that there were too much turnings or time processing for this type of waste, with the potential to increase the volatility and loss of N during the maturation phase.

4.3. Compost quality

All compost types presented parameters of stability and maturity as were highest rating during the self-heating test (class V, mature compost) and values of GI above 80% indicative of absence of phytotoxic substances for plant growth (Riffaldi et al., 1986). However, MSS showed a higher degree of maturity and stability because it had a respiratory rate below 0.5 mg O_2 g^{-1} SV h^{-1} (Iannotti et al., 1993), a ratio C/N below 12 and an ammonia/nitrate ratio below 0.16 (Bernal et al., 1998). Likewise, Garcia et al. (1991) proposed the use of WSC content as a useful method for determining the maturity of a compost, with a value below 5 mg g^{-1} for mature compost. In this experiment, FI exhibited higher values of this content. Both PM and FI may require more time to achieve similar maturity and stability parameters to MSS, even though both were classified as "mature" according to the rates in TMECC (TMECC, 2002). Although the waste types studied underwent the same treatment in composting, they exhibited different stability and maturity levels. As such, it is important to design and control the composting process as a whole, paying particular attention to the maturation phase, where it is possible to design an ad hoc process to yield higher quality compost in less time, depending on the physico-chemical properties of each type and its evolution over time.

4.4. Microbiological evolution

Changes in both enzyme activities and the microbial community during the maturation phase were determined by the individual features of organic waste types.

The decline in microbial biomass in MSS was consistent with previous studies (Garcia et al., 1992; Klamer and Bååth, 1998; Mondini et al., 2004; Ros et al., 2006) using different methods of quantification (Biolog, ATP, fumigation-extraction, PLFA) and microbial monitoring during the maturation phase. As mentioned, previous research has focused on the most intensive phase of composting, although some studies include occasional samplings during the maturation phase. Garcia et al. (1992) observed that the continued decrease in microbial biomass during the composting of municipal solid waste could be attributed to the slow and incomplete stabilization of organic matter. Here it should be noted that the low C/N ratio observed throughout the maturation of MSS could cause a shortage of available carbon, leading to a progressive decline in microbial biomass, slowing down the process for the degradation of organic matter. However, the results in Table 1 show optimal properties for compost, suggesting the drop in microbial biomass was the result of the maturity and stability of the organic matter. In terms of the structure of the microbial community, the abundance of PLFAs characteristic of bacteria detected throughout the maturation phase was high and could be attributed

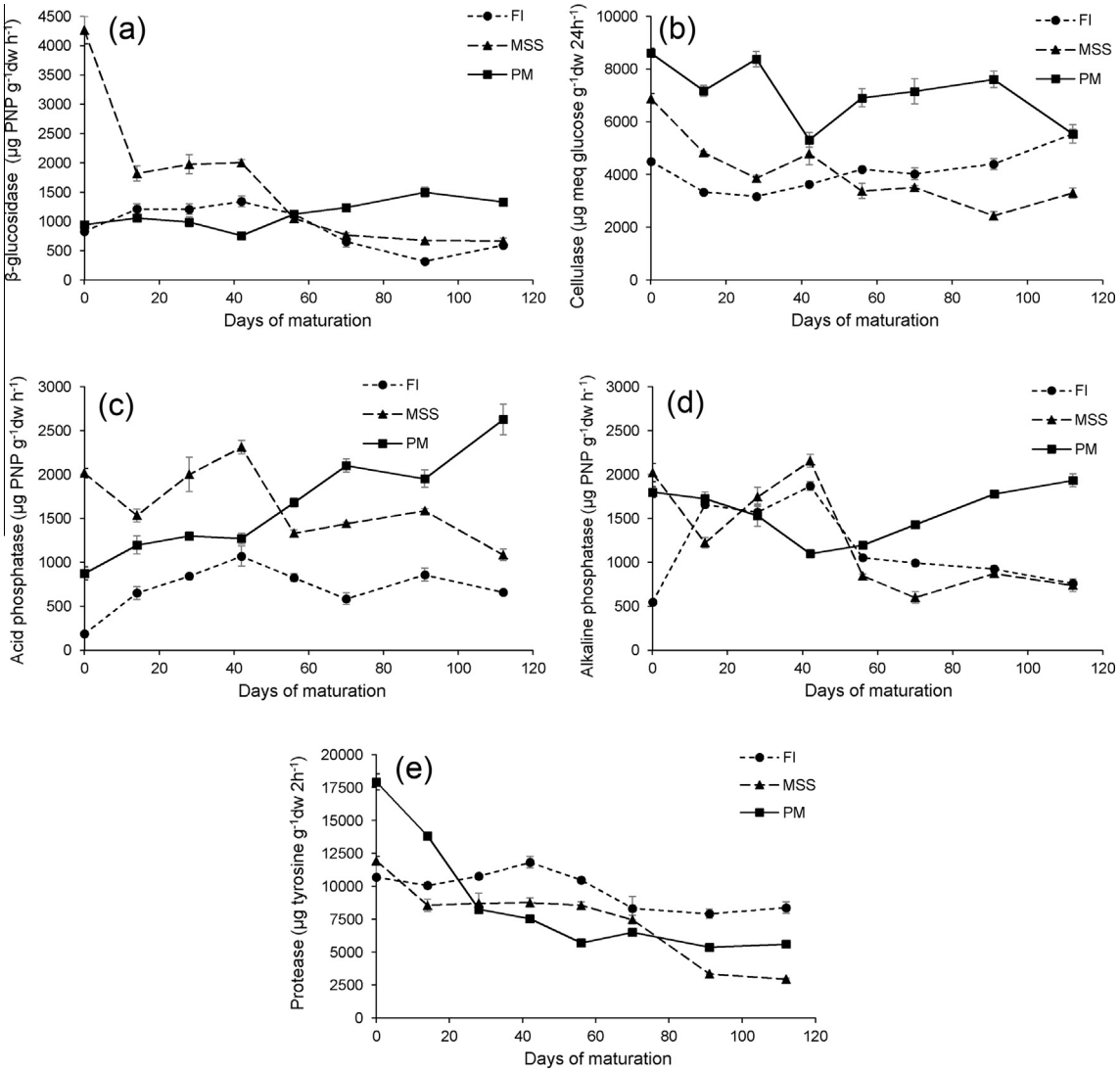

Fig. 5. Changes in (a) β-glucosidase, (b) cellulase, (c) acid phosphatase, (d) alkaline phosphatase and (e) protease during the maturation phase for sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM).

to the origin and physico-chemical properties of the waste. The main and most important microbial population that develops in wastewater treatment systems is bacteria, especially Gram – bacteria such as *Proteobacteria* and *Bacteroidetes*, and Gram + bacteria such as *Actinobacteria* (Wagner and Loy, 2002). Furthermore, following the phase of thermophilic composting, the recolonization of the compost with mesophilic microorganisms of the environment occurs, dominated by the bacteria of the phylum *Bacteroidetes* (Insam and de Bertoldi, 2007; Ryckeboer et al., 2003b). Eiland et al. (2001) have found that bacteria dominated the microbial community throughout the composting process when C/N was low, although all treatments studied showed a fungi/bacteria ratio of less than 0.5. The waste used (straw with various additions of slurry) was a predominantly bacterial medium for all tested mixtures and throughout composting. Ishii and Takii (2003) observed similar bacterial communities in different composting sewage sludge processes, including Bacillus, *Actinobacteria* and Gram – bacteria. These authors have suggested that microorganisms that proliferate in composting processes adapt to the composting environment and are selected by factors within the composting materials. Furthermore, in a previous study using sewage sludge from the same wastewater plant, a predominance of PLFAs typical of bacteria were observed in both vermicomposting and the combined process composting-vermicomposting (Villar et al., 2016). So, the physico-chemical factors exhibited by MSS characterized the microbial biomass that developed during the maturation process.

The microbial biomass for FI developed in a similar way to previous studies on PLFAs (Boulter-Bitzer et al., 2006; Hellmann et al., 1997), with an increase in the abundance of PLFAs in the final stages of composting. The microbial biomass growth in FI was caused by an increase in PLFA fungi biomarkers. It is normal for fungi to increase during the maturation phase of composting as a result of the breakdown of substrates of difficult degradation and less aggressive environmental factors (Albrecht et al., 2010; Hassen et al., 2001) and there is evidence of an increase in fungal diversity (Shemekite et al., 2014). However, FI showed greater abundance of PLFA fungi biomarkers than bacterial ones throughout the maturation process. The lipidic nature of the starting material and its high WSC content throughout maturation might involve the proliferation of saprophytic sugar fungi, such as *Zygomycetes* species, in early stages of the maturation and the proliferation of cellulolytic fungi during the final phase (Richardson, 2009; Ryckeboer et al., 2003b). Similarly, Amir et al. (2010) found that

Table 3
Correlation matrix between enzyme activities and PLFAs during the maturation phase for sludge from fish processing industry (FI), municipal sewage sludge (MSS) and pig manure (PM).

	totPLFAs	Gram +	Gram −	Fungi
FI				
β-Glucosidase	0.358*	*NS*	−0.531**	0.384*
Cellulase	*NS*	−0.411*	*NS*	*NS*
Acid phosphatase	*NS*	*NS*	*NS*	*NS*
Alkaline phosphatase	*NS*	*NS*	−0.421*	0.409*
Protease	*NS*	*NS*	−0.627**	*NS*
MSS				
β-Glucosidase	0.921**	0.887**	0.900**	0.911**
Cellulase	0.892**	0.850**	0.889**	0.888**
Acid phosphatase	0.549**	0.582**	0.464**	0.564**
Alkaline phosphatase	0.702**	0.704**	0.634**	0.719**
Protease	0.821**	0.827**	0.756**	0.831**
PM				
β-Glucosidase	*NS*	*NS*	*NS*	*NS*
Cellulase	0.581**	0.459*	0.506**	0.590**
Acid phosphatase	*NS*	*NS*	*NS*	−0.401*
Alkaline phosphatase	0.644**	0.715**	0.633**	*NS*
Protease	0.720**	0.702**	0.601**	*NS*

NS: not significant.
* Indicates significance at the 0.05 probability level.
** Indicates significance at the 0.01 probability level.

the presence of fungi was greater in waste with a high fatty acid content, which impeded the stabilization and maturation of the compost. Hence, the maintenance of high content of fungi could be indicative of the lack of stabilization during the maturation phase of this type of waste.

For PM, the stabilization of microbial biomass at the end of maturation suggested nutrients were available to the microorganisms, probably due to the high content of cellulosic materials, such as straw and remnants of seeds that are normally present in pig slurry and degrade more slowly. Tiquia et al. (2002a) found ATP stabilization during pruning composting, indicating the maturity of the compost and suggesting a change in the microbial community to more specialized microbial groups, such as fungi and *Actinomycetes*. However, as observed for MSS, bacterial biomass was greater than fungal biomass during the maturation process, particularly PLFA biomarkers of Gram + bacteria. Elouaqoudi et al. (2015) have suggested that the increase in Gram + bacteria in the final phase of composting indicates the availability of organic substrates due to the breakdown of lignocellulosic compounds. Moreover, the origin of waste, which characterizes its physico-chemical properties, may influence the development of the microbial community during the maturation phase, since pig manure is an environment rich in bacteria with a high content of fermentative microbial groups, especially Gram + bacteria, and bacteria dominate during the initial phase of the decomposition of manure due to the high availability of water and compounds that can be easily broken down (Domínguez et al., 2010; Snell-Castro et al., 2005).

The change in the microbial community during the maturation process appears to be affected by the adaptation of microorganisms to mesophilic conditions and the different physico-chemical properties of different sources of waste, meaning there is a continuous turnover of the microbial groups while maintaining the influence of the nature of the starting material and the predominant microbial groups.

The study of enzymatic activities during maturation showed the dynamics of the metabolic processes of the carbon, nitrogen and phosphorus cycle and thus hydrolytic enzymes were indicative of the evolution of organic matter and the biological activity during the final phase of the composting process.

In terms of MSS, the high correlation between all enzymes and microbial biomass indicates the enzyme activities during maturation were a direct result of the microbial community, both bacterial and fungal. The sharp decline of enzymes and PLFA content over time suggests a decrease of available substrates for microorganisms. At the beginning of the process, MSS had the highest β-glucosidase and phosphatase activities. The low C/N of this waste may require the synthesis of β-glucosidase, since the limitation of carbon with respect to the available nitrogen and phosphorus provides a strong incentive for microorganisms to invest in the acquisition of carbon (Allison and Vitousek, 2005). Likewise, García et al. (1993) indicated that high values of phosphatase activity in sewage sludge can be induced by the presence of phosphate compounds from detergents in wastewater. β-glucosidase, protease and acid phosphatase enzymes showed stabilization in the final samplings while alkaline phosphatase continued to decline and cellulase increased slightly in the final sampling. Castaldi et al. (2008) observed a decrease in both enzyme activities and water-soluble fractions during composting of the organic fraction of municipal solid waste with plant waste, indicating a stabilization of hydrolytic enzymes and hence the organic matter during the maturation phase.

In contrast to MSS, no correlation was observed between the measure of microbial biomass and enzymes, except for β-glucosidase during maturation of FI, suggesting the enzyme activities were not directly associated with the development of the microbial biomass. Burns (1982) proposed that the enzymes may be located in dead cells, cell debris or stabilized in organic complexes and remain as active hydrolyzing substrates. A further study by Mondini et al. (2004) found no correlation between the microbial biomass carbon and different enzyme activities during the composting of different wastes, indicating stabilization of extracellular enzymes due to the formation of complexes with humic substances. During maturation, FI exhibited low enzyme activities, compared to MSS and PM. The high content of nutrients directly available for microorganisms, such as WSC and DON, could inhibit the production of enzymes. Cellulase and protease activities increased at the end of the process, indicating that the fall in soluble organic matter is accompanied by an increase in the hydrolysis of more complex organic compounds (Tiquia et al., 2002b).

In terms of the hydrolytic activity of PM, particularly high cellulase activity was observed throughout the maturation. However, this was in line with expectations of high cellulosic activity during composting of pig manure due to the predominance of cellulose and hemicellulose in this type of waste (Iannotti et al., 1979). Both bacteria and fungi determined cellulase activity and the predominance of bacteria throughout the process resulted in the presence of a significant cellulolytic bacterial community during maturation of PM, as was observed by Ryckeboer et al. (2003a) during the composting of vegetable, fruit and garden waste. Moreover, β-glucosidase and phosphatases increased at the end of the maturation process, with a notable progressive increase in alkaline phosphatase over time. The highest level of enzymatic activity in the PM compost was similar to that shown by other authors (Cayuela et al., 2008; Tiquia and Tam, 1998) and was indicative of the presence of available nutrients for the microbial biomass, especially bacteria, and/or the protection of enzymes in humic complexes, as indicated by the lack of correlation between β-glucosidase and acid phosphatase with the microbial community. The protease enzyme was of particular importance, since it decreased during the maturation of PM, FI and MSS and stabilized at the end of the process. This was the only enzyme to be positively correlated with WSC ($p < 0.01$) in the three waste types, suggesting the availability of carbon regulates the synthesis of proteases (Allison and Vitousek, 2005). Indeed, Lazcano et al. (2008) suggested that protease may be indicative of the degradation of organic matter because of an extreme dependence on the availabil-

ity of substrate. Hence, the increased availability of substrates and therefore the higher protease activity at the end of the process was observed in FI, PM and finally MSS.

The different correlation between the microbial groups and enzymatic activities during maturation provided information on the state of the degradation of organic matter and compost quality, as well as the requirement for more or less processing time and handling. The authors recommend more research on different maturation systems and waste types with particular attention to biological parameters during the maturation phase.

5. Conclusions

Taken together, the structure of the microbial community and enzymatic activities provide important information for monitoring the composting process and on the stability and maturity of compost. Low enzymatic activity is not indicative of stabilization, as observed by the high microbial community present in sludge from the fish processing industry, although a decrease in both enzymatic activity and microbial community may indicate stability, as was the case during the maturation of municipal sewage sludge. The predominant microbial community for each type of waste remained present during the maturation process, indicating that the different origin of the waste and the different physico-chemical properties determine the microorganisms that are able to develop at this stage. Although waste types were subjected to the same composting process, the level of stability and maturity was different and this represents an important factor for designing ad hoc processes and controlling the composting process according to waste type, paying particular attention to the process as a whole, including maturation. It is important to monitor microbial communities and their activity over time to determine if and when compost is stable enough to be applied to soil, or whether more time or alternative process management is required.

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