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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 ( $\beta$ -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.

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### 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.

**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 <sup>-1</sup> dw)	532.5	364.0	402.1
Total nitrogen (mg g <sup>-1</sup> dw)	26.8	46.5	31.9
Water soluble carbon (mg g <sup>-1</sup> dw)	20.50	2.54	19.78
Dissolved organic nitrogen (mg g <sup>-1</sup> dw)	10.05	9.07	10.18
Total phosphorus (mg g <sup>-1</sup> dw)	7.16	20.01	16.07
Fats (% dw)	22.3	6.0	15.6

dw: dry weight.

- 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.

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

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