Waste Management 69 (2017) 498–507

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Product quality and microbial dynamics during vermicomposting and maturation of compost from pig manure

Iria Villar *, David Alves, Salustiano Mato

Department of Ecology and Animal Biology, University of Vigo, 36310 Vigo, Spain

ARTICLE INFO

Article history:
Received 3 May 2017
Revised 7 August 2017
Accepted 16 August 2017
Available online 23 August 2017

Keywords:
Hydrolytic enzymes
PLFAs
Microbial evolution
Compost maturation
Earthworms

ABSTRACT

This research evaluates, through microbial dynamics, the use of earthworms *Eisenia andrei* for maturation of pre-composted pig manure in comparison with maturation under static conditions and with vermicomposting of fresh pig manure. Therefore, two substrates were used (fresh and pre-composted pig manure) and four treatments were developed: fresh manure vermicomposting, control of fresh manure without earthworms, pre-composting followed by vermicomposting and static maturation of pre-composted manure. In order to determine the microbial dynamics, the enzymatic activities and profiles of phospholipid fatty acids (PLFAs) were evaluated over a 112-days period. Physicochemical and biological parameters of the obtained products were also analyzed. The presence of earthworms significantly reduced ($p < 0.05$) microbial biomass and all the microbial groups (Gram + bacteria, Gram – bacteria, and fungi) in both substrates. The enzymatic activities (cellulase, β-glucosidase and acid phosphatase) behaved in a significantly distinctive manner ($p < 0.05$) depending on the treatment. Microbial communities had significant correlations ($p < 0.05$) with hydrolytic activities during static maturation of pre-composted manure. This indicates a direct effect of microbiota evolution on the degradative processes; however, complex earthworm-microbiota interactions were established in the presence of *E. andrei.* After earthworms' removal from vermicompost of fresh substrate at 70 day, an increase in Gram + (4.4 times), Gram – (3.8 times) and fungi (2.8 times) were observed and, although the vermicompost achieved quality values, it is necessary to optimize the vermicompost aging phase period to improve the stability. Static maturation presented stability on microbial dynamics that indicated a slow degradation of organic compounds so that, maturation of pre-composted manure through vermicomposting is better option.

1. Introduction

Spain is the second largest pork producer in the European Union and generates more than 50 million m^3 of pig slurry per year as a result of this activity (MAGRAMA, 2012). This waste must be properly managed to prevent environmental problems. The separation of the slurry's liquid phase from its solid one is a treatment process regularly carried out on pig farms (Hjorth et al., 2010). The solid portion is mainly composed of excrement, straw, and the remains of food with a high content of biodegradable organic matter, which allows for it to be treated through biological techniques, such as vermicomposting and composting. Vermicomposting is a process of waste biodegradation using earthworms to decompose the organic substrates. The microorganisms are the ones principally responsible for breaking down the biodegradable organic material, although the earthworms stimulate the microorganisms as a result of them modifying the substrate's properties through feeding, aeration, and the excretion of casts (Domínguez, 2004). Various authors have studied the effects of epigeic earthworms have on pig manure through studies on microbial dynamics. Aira et al. (2007) investigated the interaction between the earthworms and microbial biomass and enzymatic activity by using continuous feed vermireactors, with different doses of manure in the presence and absence of *Eisenia fetida.* In a later study Aira and Domínguez (2009) compared microbial stabilization and nutrients of the casts produced by *E. fetida* fed with pig manure with those fed with cow manure. Similarly, Gómez-Brandón et al. (2011a) studied the impact of the species *E. andrei* on the structure and function of the microbial community in the casts from three types of animal manure, among them pig manure. Sometimes it is necessary to reduce noxious compounds for earthworms' survival before their inoculation in the waste. For example, Chan and Griffiths (1988) were faced with the impossibility of vermicomposting the pig manure without treating it. So they prepared it through a thermophilic composting stage and the addition of calcium sulphate.

* Corresponding author.
E-mail address: iriavillar@uvigo.es (I. Villar).

http://dx.doi.org/10.1016/j.wasman.2017.08.031
0956-053X/

Pre-composting has been used as a pre-treatment prior to vermicomposting to reduce pathogenic organisms and potentially toxic components (Gunadi et al., 2002). Composting is a process of biological degradation of solid organic substrates under aerobic conditions through the action of different microbial communities, resulting in a stable and humified product and apt for adding to the soil (Insam and de Bertoldi, 2007). Composting can be divided into different phases: a mesophilic stage, characterized by microbiota proliferation; a thermophilic stage, which produces a high level of biodegradation, the growth of thermophilic organisms and the inhibition of non-thermo-tolerant organisms; and the final phases that consist of a cooling period, which is characterized by the growth of mesophilic organisms, and a maturation period during which the organic material is stabilized and turned into humus, obtaining a product suitable for use as a soil amendment (Ryckeboer et al., 2003b). Composting facilities pay special attention to the thermophilic phase that is carried out with a specific technique or technology. When this intensive process ends, the pre-composted material is deposited in the maturation area which in many cases is simply and erroneously considered to be a storage space for compost (Diaz et al., 2002; Rynk, 2000). Control and handling during maturation of pre-composted waste results in obtaining quality compost and optimizing the composting process (Villar et al., 2016b). Research on earthworms' inoculation in pre-composted waste with the aim of study changes through maturation phase and product quality has not been found. The comparison of vermicomposting with regards to composting pig manure was dealt with by Zhu et al. (2016) who compared both treatments with the objective of studying the variation of heavy metals and dissolved organic matter. No studies comparing vermicomposting and composting process of pig manure through microbial dynamics have been found.

This research addresses the study of the microbial dynamics during the vermicomposting of pig manure and during the maturation process of pre-composted pig manure, with and without epigeic *E. andrei* earthworms. The main hypothesis of this study are (1) that the presence of earthworms determines the changes to the microbiota and thus the changes in the enzymatic activity and that (2) maturing the pre-composted manure with earthworms improves the product obtained in terms of physic-chemistry and biology. For this, enzymatic activity (cellulase, β-glucosidase, protease, and acid phosphatase) and the microbial community's structure were studied by analyzing PLFAs throughout the vermicomposting and compost maturation process. The stability and maturity parameters in the products obtained were also analyzed.

2. Materials and methods

2.1. Substrates

The solid fraction of manure was collected from a pig breeding farm after storage in manure pits for liquid separation. The manure was mixed with pine wood chips as a bulking agent, adjusting the ratio to 1:2 (v/v). The initial properties of this feedstock mixture can be seen in Table 1. This mixture was composted using a static adiabatic reactor with 600 l capacity and automatic control of temperature and oxygen by forced aeration. No turning and moistening was carried out during the stay in the reactor. Details are described in Villar et al. (2016a). The reactor phase, during with the temperature rose 60 °C, ended after 14 days when the temperature reached values below 35 °C. The pre-composted material was removed from the reactor and turned. The initial properties of the pre-composted feedstock mixture are shown in the Table 1.

Table 1
Physico-chemical characteristics of the substrates used in the treatments.

	Feedstock mixture	Pre-composted feedstock
Moisture (%)	76.2 ± 0.5	68.8 ± 0.3*
Organic matter (%)	87.3 ± 0.2	85.7 ± 0.8*
pH	8.40 ± 0.03	6.86 ± 0.03*
Electrical conductivity (mS cm^{-1})	0.86 ± 0.02	0.64 ± 0.02*
Total carbon (mg g^{-1} dw)	433.1 ± 11.2	419.0 ± 6.9*
Total nitrogen (mg g^{-1} dw)	23.2 ± 0.4	22.5 ± 1.3
C/N ratio	18.9 ± 0.5	18.3 ± 0.3
P_2O_5 (mg g^{-1} dw)	11.3 ± 0.3	11.7 ± 0.4
Ammoniacal nitrogen (mg g^{-1} dw)	3.02 ± 0.10	1.40 ± 0.05*

The asterisk indicates significant differences between substrates (paired-sample Student's *t*-test, $p < 0.05$) dw: dry weight

2.2. Experimental design

Specimens with an average weight of 325 ± 34 mg of the earthworm species *E. andrei* were collected from a laboratory culture fed with horse manure.

Vermicomposting treatments were carried out according with previous work (Villar et al., 2016c). Briefly, rectangular culture systems of 14 l capacity were filled with a layer of sieved and moistened vermiculite, a plastic mesh of 5 cm mesh size and 2010 ± 8 g of substrate: feedstock mixture fresh (V treatment) or pre-composted (CV treatment). Each substrate was replicated three times and 115–120 earthworms were introduced in each system. Controls involving the vermiculite, mesh and fresh feedstock mixture without earthworms were included in triplicate ($V_{control}$ treatment). After 70 days, cocoons, earthworms and hatchlings were removed by hand, counted and weighed and the culture systems were maintained until day 112 to enable the maturation of the vermicompost.

Static maturation of the pre-composted feedstock without earthworms (C treatment) was done in triplicate in wooden boxes of 200 l as has been described in Villar et al. (2016a). The material was left for 112 days without being turned and it was not necessary to moisten it. Thus in total four different treatments were carried out:

- V treatment: vermicomposting of fresh feedstock mixture
- CV treatment: vermicomposting of pre-composted feedstock mixture
- $V_{control}$ treatment: fresh feedstock mixture without earthworms
- C treatment: maturation of pre-composted feedstock mixture without earthworms.

Samples were taken at 0, 14, 28, 42, 56, 70, 91 and 112 days in all treatments. In order to remove the bulking agent, samples were sieved (10 mm mesh) and several parameters were determined from fraction <10 mm, as detailed in the following sections.

2.3. Physico-chemical analysis

Organic matter content was measured by the loss on ignition of dried samples at 550 °C for 4 hours. Total carbon content (TC) and total nitrogen content (TN) were determined by combustion of dried samples using a LECO 2000 CN elemental analyser. N-NH_4^+ and N-NO_3^- were determined spectrophotometrically in 0.5 M K_2SO_4 extracts in a ratio of 1:10 (w/v) according with Sims et al. (1995). Water soluble carbon content (WSC) was analyzed in aqueous extracts 1:5 (w/v) by dichromate oxidation in sulfuric acid solution and spectrophotometric measurement of reduced chromium (Benitez et al., 1999a). The pH and electrical conductivity