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Changes in microbial dynamics during vermicomposting of fresh and composted sewage sludge

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ABSTRACT

Municipal sewage sludge is a waste with high organic load generated in large quantities that can be treated by biodegradation techniques to reduce its risk to the environment. This research studies vermicomposting and vermicomposting after composting of sewage sludge with the earthworm specie *Eisenia andrei*. In order to determine the effect that earthworms cause on the microbial dynamics depending on the treatment, the structure and activity of the microbial community was assessed using phospholipid fatty acid analysis and enzyme activities, during 112 days of vermicomposting of fresh and composted sewage sludge, with and without earthworms. The presence of earthworms significantly reduced microbial biomass and all microbial groups (Gram+ bacteria, Gram− bacteria and fungi), as well as cellulase and alkaline phosphatase activities. Combined composting–vermicomposting treatment showed a lesser development of earthworms, higher bacterial and fungal biomass than vermicomposting treatment and greater differences, compared with the control without earthworms, in cellulase, β-glucosidase, alkaline and acid phosphatase. Both treatments were suitable for the stabilization of municipal sewage sludge and the combined composting–vermicomposting treatment can be a viable process for maturation of fresh compost.

1. Introduction

Municipal wastewater treatment plants produce significant amounts of sewage sludge, amount to about 11 million dry tonnes per year in the EU, which needs suitable and environmentally accepted management before final disposal (Kelessidis and Stasinakis, 2012). Sewage sludge can harm the environment when it is deposited directly on soil due to its fermentative capacity and the presence of hazardous substances, both organic and inorganic, including pathogenic organisms and heavy metals (Williams, 2005). Due to its high organic load, sewage sludge is a suitable waste for being treated by biological techniques such as composting and vermicomposting aimed at obtaining a stable product with a high agronomic value.

Vermicomposting is a bio-oxidation and stabilization process of organic matter as a result of the interaction between microorganisms and earthworms. Microorganisms are mainly responsible for the degradation of organic material, although earthworms stimulate microorganisms due to the modification of substrate properties through feeding, aeration and cast excretion, which leads to the acceleration of mineralisation of organic matter and the improvement of nutrient availability for plants (Domínguez, 2004). Vermicomposting has been successfully applied on the treatment of municipal sewage sludge. Most research on sewage sludge vermicomposting has focused on the study of physical–chemical parameters such as nutrients (Domínguez and Gómez-Brandón, 2013; Fu et al., 2015), humic and fulvic substances (Zhang et al., 2015) and heavy metals (Suthar, 2010). Nevertheless, less is reported on the microbiological and biochemical changes that occur during the vermicomposting of municipal sewage sludge. Benitez et al. (1999) observed a reduction in β-glucosidase, protease, urease and dehydrogenase activities related to the decline in available substrates, in the first 6 weeks of vermicomposting of municipal sewage sludge mixed with paper mill sewage sludge. Domínguez and Gómez-Brandón (2013) found that the presence of the earthworm *Eisenia andrei* increased microbial biomass, measured as N-microbial biomass by fumigation-extraction method, from week 1 to week 16 of vermicomposting of sewage sludge compared to the control without earthworms. On the contrary, Fu et al. (2015) observed a decrease of C-microbial biomass in the first 40 days of vermicomposting of pelletized dewatered sludge, with subsequent low and constant values that indicated the stability of the final products.

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The integration of composting and vermicomposting has been considered a suitable method for waste management (Ndegwa and Thompson, 2001). The inoculation of earthworms in the material that passed through the thermophilic phase of composting has been used as a pre-treatment before vermicomposting, in order to remove compounds harmful for earthworms, such as ammonium (Domínguez, 2004). Several authors have investigated the combined use of composting and vermicomposting for the treatment of different organic materials, showing that prior composting can accelerate degradation and improve the stabilization of the final product (Frederickson et al., 1997; Lazcano et al., 2008). Fornes et al. (2012) studied the evolution of composting, vermicomposting and the combined composting–vermicomposting process of horticulture waste, focusing their research on the physical–chemical changes over time. These authors showed that vermicomposts had better properties as growing media than as compost. Also, Lazcano et al. (2008) compared the products of these processes, but not the evolution over time, by the study of microbiological and biochemical parameters, noting that the combined process was the most effective method for the stabilization of the cattle manure. Hait and Tare (2011) reported that the combined composting–vermicomposting process of sewage sludge made it possible to obtain a good quality pathogen-free product. Likewise, Sen and Chandra (2009) showed that earthworms changed the dynamic of the bacterial community in the combined process compared with composting of sugar waste, but they did not study the vermicomposting process. So, no research on microbiological evolution over time of the vermicomposting process and the combined composting–vermicomposting process for the same waste was found.

It has been observed that the study of enzyme activities is a reliable index of the evolution of organic matter during vermicomposting (Benitez et al., 1999; Aira et al., 2007a). Enzyme activities provide information on the conversion of complex organic compounds into more readily assimilable substances and, hence, enzymes are of interest to evaluate stabilization throughout waste biodegradation. Likewise, enzyme activities have been related to earthworm growth. Thus, they have been proposed as indicators to optimise vermicomposting process (Fernández-Gómez et al., 2010). Benitez et al. (1999) demonstrated that hydrolytic enzyme activities tended towards stability in the course of sewage sludge vermicomposting, but however, the lack of controls makes it difficult to distinguish between the effect caused by earthworms and the effect of the microbiota present in the waste.

Conversely, phospholipid fatty acid analysis (PLFAs) is a useful tool for monitoring the microbial community dynamics. The total amount of PLFAs can be used as an indicator of viable microbial biomass and some PLFAs are specific to certain living organisms and, therefore, can be used as biomarkers for the presence and abundance of microbial groups (Zelles, 1999). Thus, analysing PLFAs during vermicomposting makes it possible to know the changes in the microbial community composition over time. Fernández-Gómez et al. (2013) observed a reduction in total PLFAs of different organic wastes after 24 weeks of vermicomposting. In the same way, Gómez-Brandón et al. (2011a, 2013) reported that the activity of earthworms reduced the PLFAs characteristic of bacterial and fungal biomass. This reduction was more pronounced between week 21 and week 36 of rabbit manure vermicomposting and pig slurry vermicomposting.

In this work, we studied the microbiological evolution during vermicomposting compared with vermicomposting after composting, for the treatment and stabilization of sewage sludge. The main hypothesis was that earthworms cause a different effect on microbial community structure, depending on whether they feed on fresh or previously composted material. To this end, enzyme activities (cellulase, β-glucosidase, protease, alkaline and acid phosphatase) and the structure of the microbial community by analysing PLFAs were assessed throughout the vermicomposting of fresh and composted sewage sludge with the earthworm specie *E. andrei*. In order to discern the effects due to the different microbiological composition of the waste from the effects caused by earthworms, the same substrates incubated without earthworms were studied.

2. Materials and methods

2.1. Substrates and earthworms

Sewage sludge was collected from a municipal wastewater treatment plant in Cangas (Pontevedra, NW Spain) after an aerobic biological treatment and subsequent dehydration. The sludge was mixed with wood chips as a bulking agent, adjusting the ratio to 1:2 (v/v). A part of this mixture was used for the vermicomposting treatment (V). Another part of the mixture was subjected to composting in a static adiabatic reactor with a 600 L capacity and automatic control of temperature and oxygen. Forced aeration was applied, using a centrifugal fan intermittently and depending on the controlled variables. The temperature was maintained above 45 °C for 7 days with maximum values of 60 °C. The process ended after 15 days when the temperature in the composting mass reached values below 35 °C. The fresh compost was removed from the reactor, mixed and used as a substrate for vermicomposting in the combined composting–vermicomposting treatment (CV). The earthworm species *E. andrei* was used for the vermicomposting due to its high tolerance to environmental factors and its high rate of organic matter processing (Domínguez, 2004). In order to determine if the substrates affected the growth and maturation of the earthworms, juvenile specimens with an average weight of 310 ± 25 mg were collected from a laboratory culture fed with horse manure.

2.2. Experimental design

Vermicomposting was carried out in rectangular culture systems of 14 L capacity, which were filled with a layer of sieved and moistened vermiculite as refuge for earthworms, with the advantage of being a biologically inert material. A plastic mesh (5 cm mesh size) was placed between the vermiculite and the substrate to prevent their mixture and facilitate the sampling. Two kilograms of substrate sludge or compost (2000 ± 6 g) and 115–120 earthworms according to the feed rate of 0.75 kg feed/kg worm/day (Ndegwa et al., 2000), were introduced. Each substrate was replicated three times. Controls involving the same materials (vermiculite, mesh and sludge or compost) incubated without earthworms were included in triplicate. Culture systems were kept in darkness under the same conditions. The moisture content was controlled and maintained above 70% by watering throughout the process. After 70 days, cocoons, earthworms and hatchlings were removed by hand from the cultures, counted and weighed. The culture systems were maintained until day 112 to enable the maturation of the vermicompost. Samples were taken at 0, 14, 28, 42, 56, 70, 91 and 112 days. In order to remove the bulking agent, samples were sieved (less than 10 mm) and several parameters were determined, as detailed below.

2.3. Physical–chemical analysis

Organic matter content was measured by the loss on ignition of dried samples at 550 °C for 4 h. Inorganic nitrogen (N-NH_4^+ and N-NO_3^-) was determined in 0.5 M K_2SO_4 extracts in a ratio of 1:10 (w/v) applying the modified indophenol blue colorimetric