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Effects of earthworms on physicochemical properties and microbial profiles during vermicomposting of fresh fruit and vegetable wastes

Kui Huang [a,*], Fusheng Li [a], Yongfen Wei [a], Xiaoyong Fu [b], Xuemin Chen [b]

[a] River Basin Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[b] School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou 730070, China

HIGHLIGHTS

- The investigation of fresh FVW decomposed by earthworms was carried out.
- Vermicomposting resulted in rapid losses of total carbon and nitrogen of fresh FVW.
- A greater volume of leachate was collected from earthworms' reactors.
- Earthworms promoted changes of bacterial and fungal densities and communities.

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ABSTRACT

This study aimed to investigate the effect of earthworms on physicochemical and microbial properties during vermicomposting of fresh fruit and vegetable wastes (FVW) by contrasting two decomposing systems of FVW with and without earthworms for 5 weeks. Compared to control treatment (without earthworms), vermicomposting treatment resulted in a rapid decrease of electrical conductivity and losses of total carbon and nitrogen from the 2nd week. Quantitative PCR displayed that earthworms markedly enhanced bacterial and fungal densities, showing the higher values than control, during the whole decomposition process. In addition, denaturing gradient gel electrophoresis combined with sequencing analysis revealed that earthworms pronouncedly modified bacterial and fungal community structures, through broadening the community diversities of Actinobacteria, Bacteroidetes, Proteobacteria, and Ascomycotina. These results suggest that the presence of earthworms promoted the activity and population of bacteria and fungi, and modified their communities, thus altering the decomposition pathway of fresh FVW.

1. Introduction

Fruits and vegetables are important healthy diet components that can help prevent multifarious chronic diseases. According to the recommendations of WHO, a minimum of 400 g of fruits and vegetables (excluding potatoes and other starchy tubers) is necessary per day to meet the requirement of the human health (WHO, 2003). However, large consumptions of fruits and vegetables are bound to bring about enormous quantities of fruit and vegetable wastes (FVW) because more than 50% of fruits and vegetables are lost or wasted during production, distribution and consumption (FAO, 2011). The FVW contain higher water content and a larger proportion of biodegradable organic components, and can easily get rotted under natural conditions. This leads to the pollution of air, water and soil, and can even induce the changes of climate and biological diversity. Considering that FVW contain many organic and inorganic nutrients, they are especially suitable for treatment with a recycling method that can convert FVW to such high value products as fuel ethanol, methane and compost.

The use of earthworms for composting treatment of organic biosolids, termed as vermicomposting, is a sustainable, cost-effective and ecological approach for effective management of biodegradable solid wastes. The final product of this technology is also considered as an environment friendly organic fertilizer for agricultural applications (Tajbakhsh et al., 2011). FVW, such as pineapple wastes (Mainoo et al., 2009), tomato wastes (Fernández-Gómez et al., 2010b), grape marc (Gómez-Brandón et al., 2011) and vegetable mixture (Fernández-Gómez et al., 2010a; Suthar, 2009; Huang et al., 2012; Huang et al., 2013) are important nutrient sources and can be treated by this technology.

* Corresponding author. Tel.: +81 058 293 2453; fax: +81 058 293 2398.
E-mail address: huangk1199@hotmail.com (K. Huang).

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Until now, several works as mentioned above have been carried out to demonstrate the feasibility of vermicomposting of FVW; however, little information is available on the effect of earthworms on the decomposition process of FVW, particularly for fresh FVW.

Vermicomposting is a biochemical process of degradation of organic materials through the joint actions of earthworms and microorganisms. According to Domínguez et al. (2010), the process of vermicomposting can probably be divided into two phases based on the activity of earthworms: (1) the direct functioning phase, in which earthworms process the organic materials via ingestion, digestion and assimilation, thereby partly modifying their physicochemical properties and the microbial profiles in the system; and (2) the indirect functioning phase, in which microorganisms that can coexist with earthworms and/or can favorably propagate under the existence of earthworms decompose the earthworms-processed materials till the end. Better understanding of the functions of earthworms in vermicomposting is necessary for further improving the process and clarifying the mechanisms involved. Previous studies have revealed that earthworms, as a driver in the composting system, could affect the microbial activity and population during decomposition of organic substances (Sen and Chandra, 2009; Castillo et al., 2013). The community structure of microorganisms could also be greatly altered by earthworms (Sen and Chandra, 2009; Vivas et al., 2009; Gómez-Brandón et al., 2013). A few researchers have reported that the presence of earthworms promoted the inhabitation of some specific phyla of bacteria such as Bacteroidetes (Héry et al., 2008; Bernard et al., 2012; Huang et al., 2013), Proteobacteria (Vivas et al., 2009) and Actinobacteria (Yasir et al., 2009; Huang et al., 2013). Recent literature by Pathma and Sakthivel (2013) even reported that the genus *Bacillus* was the predominant bacterial species, and was then followed by *Pseudomonas* and *Microbacterium* in the vermicompost of straw and goat manure. All these findings obtained through previous studies were based on comparison of the microbial community between the initial and final phases of vermicomposting. Information regarding the dynamic changes of microbial community during the entire vermicomposting process is very scarce, which need detailed investigations since they may closely associate with the speed and the final products of vermicomposting.

In view of the above, the major objectives of this study were to investigate the following two effects of earthworms during the entire process of vermicomposting treatment of fresh FVW: (1) the effect on the physicochemical properties of the vermicomposting products, and (2) the effect on the microbial communities. For the latter objective, the quantitative polymerase chain reaction (qPCR), denaturing gradient gel electrophoresis (DGGE) and sequencing were applied, and the populations of bacteria and fungi as well as their community compositions were analyzed.

2. Methods

2.1. Experimental set up

Non-clitellated juvenile *Eisenia foetida* with the mean individual weight of 0.2 g were randomly selected from the stock culture in the laboratory. The fresh products of banana peels, cabbage, lettuce, potato and watermelon peels were obtained from a supermarket in Gifu, Japan. In the laboratory, they were chopped into pieces with a size about 1 cm × 1 cm with a knife and then mixed at the weight ratio of 1:1:1:1:1 (wet basis) to form the mixture of FVW for experiment use. A bedding material of soil and vermicompost (produced and stored from a previous experiment) mixed in the weight ratios of 2:1 (dry basis) was used for providing a suitable inhabitation condition for earthworms. Before experiment, the bedding material was stored in a dark room at 25 °C and its moisture was kept at approximate70% by sprinkling with water. The properties of the FVW and the bedding material are displayed in Table 1.

Vermicomposting experiment was performed using perforated plastic containers as reactors. Each reactor, having a size of 255 mm × 355 mm × 200 mm (length × width × height), was consisted of three layers: a substrate layer, a bedding material layer and a leachate collecting layer. The schematic diagram of the vermicomposting reactor is shown in Fig. 1. In the reactor, 2 kg of the fresh FVW (wet basis) and 3 kg of the bedding material (wet basis) was placed onto the substrate layer and the bedding material layer, respectively. Between these two layers, a plastic mesh with the opening of about 10 mm × 10 mm was used to avoid mixing of the FVW with the bedding material. The bottom layer was designed for receiving leachate during the experiment. The leachate was subjected to its volume confirmation and was not recirculated for further decomposition treatment. Totally 6 reactors were prepared for this study, with 3 of them being used for vermicomposting treatment and the remaining 3 for control treatment without the addition of earthworms. For vermicomposting treatment, 100 juvenile earthworms were inoculated into the layer of the bedding material of each vermicomposting reactor. In order to provide an optimal habitat environment for *E. foetida*, all reactors were covered with wet towels to keep the moisture at 70–80%, and were operated in a temperature controlled room at 25 °C. During the experiment, sprinkling water was not permitted for all treatments. Samples were collected from all reactors at the time interval of one week. After composting for 5 weeks, the experiment was terminated because the residuals of FVW in the vermicomposting bins had been eaten up by earthworms. The collected samples were homogenized and each was then divided into two subsamples. One subsample was dried and finely pulverized for chemical analysis, and another subsample was stored at −20 °C for dehydrogenase activity and DNA related analyses.

2.2. Analytical methods

2.2.1. Physicochemical and enzymatic analysis

pH and electrical conductivity were measured in water extract (sample: water = 1:10, dry basis). Total carbon and total nitrogen were analyzed using an elemental analyzer (Yanaco CHN CORDER MT-6, Japan). Concentrations of nitrate, ammonia, phosphate and potassium ion were measured by an ion chromatograph system (SHIMADAZU, Japan). Dehydrogenase activity (DHA) was determined with the triphenyl-tetrazolium chloride (TTC) method, for which, 1 g (dry basis) of sample was cultured using TTC solution for 6 h, and the resulting formazan was extracted with toluene and then measured by the colorimetric method at 485 nm.

2.2.2. Real time quantitative PCR analysis

DNA extraction of each sample was performed with the MoBio UltraClean Soil DNA Isolation kit (MO BIO Laboratories, Inc.,

Table 1
Physicochemical properties of fresh FVW and bedding material. Values are means ± standard errors ($n = 3$).

Parameters	Fresh FVW	Bedding material
pH	4.9 ± 0.02	6.6 ± 0.02
Water content (%)	92.3 ± 0.1	76.5 ± 0.2
Electrical conductivity (s/m)	0.85 ± 0.01	0.14 ± 0.01
Total carbon (g/kg, dry basis)	392.6 ± 0.8	101.3 ± 1.9
Total nitrogen (g/kg, dry basis)	23.8 ± 0.1	7.2 ± 0.1
NH_4^+ (g/kg, dry basis)	1.4 ± 0.2	0.19 ± 0.00
NO_3^- (g/kg, dry basis)	3.1 ± 0.08	0.46 ± 0.01