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Dynamics of bacterial and eukaryotic community associated with stability during vermicomposting of pelletized dewatered sludge

Xiaoyong Fu [a, **, 1], Kui Huang [a, b, *, 1], Guangyu Cui [a], Xuemin Chen [a], Fusheng Li [b], Xiaoyu Zhang [a], Fei Li [a]

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

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ABSTRACT

This study aimed to investigate the dynamics of bacterial and eukaryotic community associated with stability during vermicomposting of pelletized dewatered sludge (PDS). For this, dewatered sludge was pelletized to the particle sizes of 4.5 mm and 14.5 mm and then vermicomposted for 60 days using earthworms *Bimastus parvus*. Physicochemical results showed that vermicomposting resulted in the decrements of organic matter, total nitrogen, ammonia-nitrogen and dissolve organic carbon and the increments of electrical conductivity, nitrate-nitrogen and available phosphorous. Principal component analysis of physicochemical and enzymatic characteristics revealed that the active decomposition stage occurred on day 20 and subsequent stable stage began from day 50–60 of vermicomposting. Moreover, the small PDS displayed a fast speed of stabilization than the large one. PCR and denatured gradient gel electrophoresis (DGGE) assay for communities of 16S rDNA and 18S rDNA ascertained that the microbes of the Flavobacteria and the Sphingobacteria and the Cercozoa predominated in vermicomposting system. This study suggests that earthworms combined with the diversified microbes could rapidly stabilize the fresh PDS within 60 days.

1. Introduction

In recent China, a large number of wastewater treatment plants are springing up along with the development of urbanization, which generates a mass of sewage sludge. Based on statistical data, the production of municipal sludge will reach to 46 million tons per year by the end of China plan of the 12th five year (Song et al., 2012). The municipal sludge is mainly comprised of the complex organic compounds and biological flocs, which not only contains lots of environment-unfriendly substances, but also possesses high content of organic matter that have a potential of being utilized as an organic fertilizer in agriculture. So far, the recycling methods, such as composting (Jouraiphy et al., 2005), sludge treatments wetland (Uggetti et al., 2010), vermifilter (Liu et al., 2012) and vermicomposting (Yang et al., 2014) have been deemed to be suitable for the treatment of sewage sludge. Among these methods, the vermicomposting has been becoming popular because it is cost-low, high effective and handling easily. More specially, it can ecologically convert a macro-weight non-degradable organic material into a stable and high valued product with an aesthetical appearance and beneficial components for plant growth and soil improvement (Domínguez, 2004). Until now, different types of sludge such as primary sludge (Hait and Tare, 2011), anaerobically digested sewage sludge (Kızılkaya and Türkay, 2014), and dewatered sludge (Yang et al., 2014) have been employed as the raw materials for the vermicomposting. Recently, the feasibility of vermicomposting of pelletized dewatered sludge (PDS) using an epigenic earthworm *Bimastus parvus* has been proved by Fu et al. (2015). And, they reported that this pelletized method could rapidly achieve the stabilization of sludge without the complicated pretreatment before vermicomposting. However, compared to the publications of vermicomposting of other sludges, the report regarding vermicomposting of PDS is still limited.

Vermicomposting is a biochemical degradation process of organic materials involving the interactions of earthworms and

* Corresponding author. School of Environmental and Municipal Engineering, Lanzhou Jiaotong University, Lanzhou 730070, China.
** Corresponding author.
E-mail addresses: fuxiaoyong7362@foxmail.com (X. Fu), huangk1199@hotmail.com (K. Huang).
[1] These authors contributed to the work equally.

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microbes. During vermicomposting, the organic substances are perceived to be decomposed by the digestive behavior of earthworms and microbes inhabited in the earthworms' gut or original substrate (Domínguez, 2004). Despite earthworm is a leader of fauna and microbes in vermicomposting system, the microorganisms play more important roles in degrading organic materials than earthworms, as revealed by Sen and Chandra (2009) and Ravindran et al. (2015). Hence, in vermicomposting system, the examination with respect to microbial profiles of activity, number and community is of significance and importance in the aspect of decomposition and stabilization of organic substances during vermicomposting process. After reviewing the relevant literatures, it was found that most reports were concentrated on the changes of microbial number and activity during vermicomposting process (Aira et al., 2002, 2007; Gómez-Brandón et al., 2013). But, for the change of microbial community, the available information is quite sparsity. As an example, to date, no studies have been carried out for the assessment of dynamic succession of microbial community during vermicomposting of fresh PDS.

The stability is usually utilized to reflect the degree of decomposition and transformation of organic matter in vermicompost (Zmora-Nahum et al., 2005), which determines the vermicomposting quality for the purpose of agriculture. It is well known that a large variety of parameters were adopt for the assessment of stability from different viewpoints, rather than single parameters (Nikaeen et al., 2015). So for, representative physiochemical properties combined with microbial activity/numbers (Nikaeen et al., 2015; Castillo et al., 2013; Sen and Chandra, 2009; Huang et al., 2014; Fu et al., 2015) were often chosen for the comprehensive assessment of composting/vermicomposting stability. However, the available method through combining microbial activity, number and community for the evaluation of vermicomposting stability is limited.

Consequently, in this regard, the first objective of present study was to investigate the changes of bacterial and eukaryotic community during vermicomposting of PDS. Secondly, this study aimed to assess the change of the stability using the microbial activity, number and combined with bacterial and eukaryotic community during vermicomposting of PDS. Given that particle size may affect the decomposition process, two particle types of 4.5 mm PDS and 14.5 mm PDS were manufactured for the experiment, respectively.

2. Methods

2.1. Material preparation

Earthworms *Bimastus parvus* were cultured in laboratory. The adult and active *B. parvus* with the weight of approximate 1 g were randomly selected for vermicomposting experiment. Fresh dewatered sludge was collected from the sludge dewatering workshop of Lanzhou Qilihe wastewater treatment plant (Lanzhou, China) in Oct. 2013. Subsequently, the fresh dewatered sludge was placed on wire meshes with sizes of 4.5 mm × 4.5 mm and 14.5 mm × 14.5 mm and then gently squeezed by hand in laboratory, respectively. As a result, the ellipsoidal fresh PDS with the sizes of the 4.5 mm and 14.5 mm were obtained in this study. A steel pot, without a hole on the bottom, having a size of 36 cm × 12 cm (diameter × depth), was used as vermireactor. The physicochemical properties of dewatered sewage sludge are given in Table 1.

2.2. Vermicomposting process

Two vermicomposting treatments were built with the PDS of small and large particles, respectively. For each treatment, three replicates were set up in parallel. A total of 4 kg fresh 4.5 mm and 14.5 mm PDS used as substrate and simultaneously utilized as vermi-bedding materials of earthworms were added into each vermireactor, separately. Then, vermicomposting was launched with the inoculation of 200 *B. parvus* into each reactor. The temperature was maintained at 20 °C. To keep constant moisture and dark environment for earthworms, a plastic film was used to cover each reactor. The constant moisture was maintained under 70–75%. The vermireactors were allowed to be turn over manually every day. After 60 days, vermicomposting was stopped and earthworms were picked out from the reactors by hand. The samples collected at the interval of 10 days were separated into triplicate. The fresh one was submitted for analyzing the microbial biomass carbon and enzymatic activity within 24 h. Another one was air-dried in a dark room and then pulverized for physicochemical analysis. The others were stored at −40 °C for DNA relating analysis.

2.3. Physicochemical and enzymatic activity analysis

All parameters were detected in triplicate. The pH value and electrical conductivity were measured in a mixture of sample and water at the ratio of 1/50 (dry weight basis). The suspension of fresh sample and water (fresh sample/water = 1/5, wet weight basis) was filtrated with 0.45 μm pore for the DOC determination by the titration with ferrous sulfate after the oxidation of sulfuric acid potassium dichromate. The organic matter was measured by oven at 550 °C for 5 h. The total nitrogen was analyzed by the ultraviolet spectrophotometry after the digestion of alkaline potassium persulfate. The available phosphorous, ammonia-nitrogen and nitrate-nitrogen were measured based on the method of Fu et al. (2015). To gain the homogenized sample for analyzing microbial biomass carbon and microbial activity, fresh samples were dissolved into the

Table 1
Changes of physicochemical parameters during vermicomposting of pelletized dewatered sludge with particle sizes of 4.5 mm and 14.5 mm. Values are mean and standard (n = 3). The different letters (a, b and c) behind the values represent the difference among three treatments were significant (P < 0.05).

Parameters	Initial pelletized dewatered sludge	Final vermicomposting products of pelletized dewatered sludge	
		4.5 mm PDS	14.5 mm PDS
pH	6.9 a	6.67 b	6.67 b
Electrical conductivity (μs/cm)	302 a	884 b	794 c
Water content (%)	76.63 a	78.32 a	78.87 a
Organic matter (%)	51.8 a	31.8 b	36.1 c
Total nitrogen (g/kg)	35.1 a	24.8 b	32.4 c
Ammonia-nitrogen (mg/kg)	14.2 a	0.1 b	0.12 b
Nitrate-nitrogen (g/kg)	0.009 a	1.39 b	0.7 c
Available phosphorous (g/kg)	1.42 a	4.36 b	2.84 c