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# Physiological adaptation and metabolic property of earthworms in vermifiltration for liquid-state sludge stabilization using bulk stable isotope and specific fatty acid compound stable isotope values

Meiyan Xing, Chunhui Zhao*, Jian Yang, Xiaowei Li, Baoyi Lv

*Institute of Biofilm Technology, Key Laboratory of Yangtze Aquatic Environment (MOE), College of Environmental Science and Engineering, Tongji University, Shanghai, 200092, China*

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ABSTRACT

The presence of earthworms in vermifilter (VF) led to the significant stabilization of sludge by enhancing the reduction in volatile suspended solids by 20.9%. The proliferation of earthworms feeding on the sludge was reflected by the observed increase of earthworm biomass, which indicated that earthworms adapt to VF environment well. Further investigation conducted using bulk stable isotope combined with specific fatty acid compound stable isotope technologies to determine physiological adaptation and metabolic property of VF earthworms. The observed injury of cuticle and the dense of goblet cells demonstrated that the earthworm was suffering from environment stress. However, earthworms could adjust their antioxidant enzyme systems to maintain their normal physiological function. Analyses of the bulk stable isotope and specific fatty acid compound stable isotope showed that the physical metabolism of earthworm hatchlings was more active than that of earthworm adults, which suggested that the increasing proportion of earthworm hatchings might be an effective way to optimize earthworm population structure in the VF system. Consequently, the improved treatment performance of sludge stabilization by vermifiltration was realized.



## 1. Introduction

The production of excess sludge from the municipal wastewater treatment plants has been increasing dramatically due to expanding urbanization, the demand for better quality water and the imposition of stricter environment laws (Wei et al., 2003). Sewage sludge treatment and disposal represents one of the most critical environment issues today, with costs for these processes accounting for about half, even up to 60%, of the total wastewater treatment expense (Li et al., 2011). Most Chinese MWWTPs in small towns could not maintain conventional sludge treatment process, such as anaerobic and aerobic digestion. The introduction of earthworms into sludge treatment process, such as vermifiltration, an alternative way to treat liquid-state sludge before dewatering, has turned out to be ecologically sound, economically viable, and socially acceptable (Sinha et al., 2008).

Like vermicomposting, vermifiltration is a bio-oxidation process, in which earthworms interact intensively with microorganisms within the decomposer community to accelerate the stabilization of organic matters and greatly modify its physical and biochemical properties (Liu et al., 2012). More evidences indicate that the heterotrophic microorganisms, such as bacteria, fungi, and microfauna, play an important role in organic degradation. Earthworms were considered as the crucial driving forces of the vermifiltration. The ingestion of earthworms can convert insoluble organic matters into more soluble forms, thus enhancing the degradation of organic matters (Garg et al., 2006). Moreover, the enhancement of microbial community diversity by earthworms (i.e., aerobic and anaerobic microflora in their guts) should not be neglected (Gupta and Garg, 2009). Recent research also indicated that earthworms could improve the metabolic properties of microbial community in biofilm and result in the overall optimization of the vermifiltration system for liquid-state sludge stabilization (Zhao et al., 2014).

The inoculation of earthworms into the VF is a substantive change in living environment for earthworms. Therefore, earthworms may suffer a stress response while their physiological process adapt to the new environment. Furthermore, it is possible that changes in earthworm physiological adaption in response to the environmental stress may affect metabolic property of earthworms with consequential effects on the operation and treatment efficiency of the vermifiltration. The above adverse effects

* Corresponding author. Tel.: +86 531 82769233; fax: +86 531 82769233.
*E-mail address:* stu_zhaoch@ujn.edu.cn (C. Zhao).

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may respond to the imbalance between reactive oxygen species (ROS) and antioxidase activity. The excessive accumulation of ROS showed certain toxicity to organisms, which was mainly reflected in damage to cytoplasmic lipids, proteins, and other intermediates in cells (Mu and Chen, 2011; Sharma et al., 2009; Xia et al., 2008). The antioxidant enzymes such as SOD and CAT played a positive role in eliminating excessively accumulated ROD in organisms, thereby protecting the normal physiological and metabolic functions of biological cells (Lopez et al., 2006). Therefore, antioxidant enzymes can be used as the indicators to measure the responses of organisms suffering external environment stress.

Bulk stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have become a powerful tool for assessing the trophic structure and energy flow in ecological communities (Vander Zanden et al., 1999). Natural stable C and N isotope determinations have been used to explore the feeding ecology of earthworms in soil and grass system and thus some metabolic properties were uncovered (Curry and Schmidt, 2006). However, the disadvantage of the bulk stable isotope in exploring the metabolic properties of an organism should not be ignored. Researchers have reported that the variation of bulk stable isotope values might be caused by physiological changes or variations in body biochemical composition. Furthermore, bulk stable isotopic determinations provide an average value of many individual biochemical components. Each biochemical component will have characteristic isotopic compositions. The analysis of the fatty acids could reveal the feeding behavior of the organisms. Therefore, the isotopic determinations of the specific fatty acids could uncover insight information regarding the assimilation and ingestion metabolic properties of organisms.

Previous studies were focused on the performance of the vermifilter on excess sludge and characteristics of microbial community on the biofilms (Xing et al., 2012). However, no information about the physiological adaptation or metabolic property of earthworms after being inoculating into the VF is currently available. In this study, the microstructure of body wall and antioxidant enzyme activities of earthworms were explored to assess the physiological adaptation of earthworms after being inoculating into VF. Moreover, the bulk stable isotope combining with specific fatty acid compound stable isotope was applied to uncover the metabolic property of earthworms in vermifiltration for liquid-state sludge stabilization. The objective was to determine the extent of adaptive response and the change of the metabolic property in earthworms introduced into the VF environment. Such information will provide insight into the adaption of earthworms to adverse environments, and it should realize the optimization of the treatment performance of VF for liquid-state excess sludge.

## 2. Materials and methods

### 2.1. Vermifilter setup and operation

Two sets (each set has three parallel reactors) of cylindrical filters were established. One set was the VFs (Fig. 1) with an initial earthworm density of 32 g/L (fresh weight), as suggested by Zhao et al. (2010), while the conventional biofilters (BFs) without earthworms were used as the control set. Each perspex filter with the diameter of 20 cm, the depth of 100 cm, and a working volume of 31.4 L was packed with ceramsite (10–20 mm in diameter). The earthworms (*Eisenia fetida*) used in this study were purchased from a farm in Yancheng City, China. The influent sludge was obtained from a secondary sedimentation tank of a municipal WWTP in Shanghai, China. The hydraulic load of the two sets of filters was maintained at 4 m/d and the organic load of the influent sludge was controlled within the range of 1.10–1.28 kg-VSS/($m^3$d). After passing through the filter bed, the sludge entered a sedimentation tank. These filters were operating continuously for 8 months after about 30-day acclimation.

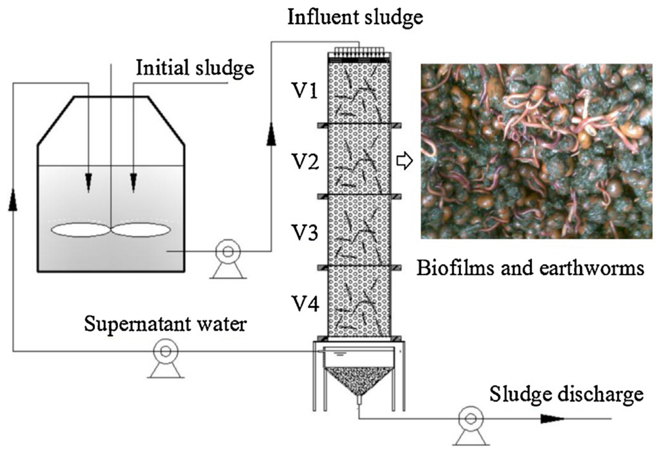


**Fig. 1.** Schematic diagram of the vermifilter treating excess sludge.

### 2.2. Sample pretreatment and chemical analysis

Samples of about 10 g earthworm adults (with clitellum) and earthworm hatchlings (without clitellum) survived in the VF were taken randomly from the VF each month during the experiment period and kept in dark for 24 h to empty their guts, and then their excrement was cleared with sterile water. After freeze-drying, all the samples (earthworm adults and earthworm hatchlings) were ground into power and then sieved through a 0.15-mm mesh for further analysis.

The sludge characteristics of the influents and effluents of BF and VF samples (e.g., SS and VSS) were assessed according to the Chinese Standard Methods (SEPA, 2002). Total chemical oxygen demand (TCOD) and soluble COD (SCOD) were measured with a NONA60 COD meter (Merk, Germany), and the samples for the SCOD measurement were firstly filtered through the 0.45-μm mixed cellulose ester membrane.

Superoxide dismutase (SOD) activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT), as described by Song et al. (2009). One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of the NBT photoreduction rate. Catalase (CAT) activity was determined as described by Saint-Denis et al. (1998). One unit of CAT activity was defined as the amount of the enzyme required to consume half of $H_2O_2$ at 25 °C.

### 2.3. Earthworm microstructure observation

The method of earthworm microstructure slices was with reference to Chuang et al. (2006). The initial earthworms and earthworms survived in the VF reactor were randomly selected and were dehydrated and embedded in paraffin. Slices (6 μm section) were obtained by microtome and then observed under a light microscope (Olympus BX53, Japan).

### 2.4. Bulk stable isotope analysis

Bulk $^{15}N$ and $^{13}C$ abundances of the earthworm samples (adults and hatchlings) were measured with a Thermo Finnigan Delta plus XP isotope ratio mass spectrometer coupled to a Flash EA 1112 elemental analyzer in the State Key Laboratory of Estuarine and Coastal Research at East China Normal University (Shanghai, China) according to the previous method (Sampedro and Dominguez,