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Microbial community structure and metabolic property of biofilms in vermifiltration for liquid-state sludge stabilization using PLFA profiles

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HIGHLIGHTS

- Microbial metabolic property of vermifilter (VF) biofilms has never been reported.
- Richer fungi diversity was featured in the biofilms of VF than biofilter (BF).
- Earthworms relieved microbial physiological and nutritional stress in VF biofilms.
- Aerobic microorganisms were predominant in VF due to earthworm burrowing action.
- Earthworms optimized treatment performance of VF on sludge stabilization.

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ABSTRACT

To investigate effects of earthworms on microbial community structure and metabolic properties of biofilms in vermifiltration for liquid-state sludge stabilization, a vermifilter (VF) with earthworms and a conventional biofilter (BF) without earthworms were compared. The Shannon index of fungi in VF was 16% higher than that in BF, which indicated earthworm activities significantly enhanced fungi diversity. The ratio of monounsaturated to saturated (mono:sat) PLFAs of VF biofilms was higher than that of BF biofilms, which indicated the physiological and nutritional stress for microbial community in VF was relieved due to the increasing of soluble substances caused by the earthworm ingestion. Further investigation showed that the burrowing action of earthworms promoted the aeration condition and led to aerobic microorganisms were predominant in VF. Those results indicated earthworms improved microbial community structure and metabolic properties of biofilms and thus resulted in the overall optimization of the vermifiltration system for liquid-state sludge stabilization.

1. Introduction

More and more municipal wastewater treatment plants (MWWTP) have been built in small towns in China due to the requirements of better quality water and the implementation of stricter environment laws (Chen et al., 2008). This leads to a sharp increase in sewage sludge production. Sludge management cost accounts for up to 60% of the total operation cost of MWWTPs, and most MWWTPs in small towns can not afford to construct and maintain conventional sludge treatment processes such as anaerobic and aerobic digestion (Wei et al., 2003; Xing et al., 2011). The application of vermifiltration (a liquid-state vermiconversion) for sludge treatment has turned out to be ecologically sound, economically viable and socially acceptable way to treat liquid-state sludge before dewatering (Xing et al., 2011; Zhao et al., 2010). Compared with the conventional biofilter (BF), the treatment performance of liquid-state sludge by vermifilter (VF) was improved significantly due to the presence of earthworms (Liu et al., 2012; Yang et al., 2013b).

Vermifiltration refers to an organic decomposition process involving the interactions between earthworms and microorganisms (Liu et al., 2012; Xing et al., 2012). Although the microorganisms are responsible for the bio-chemical degradation of the organics, compared with the microbial community developed in the conventional biofilters, microbial communities developed in the VF, which are mainly affected by the earthworm activities in the filter bed, are exposed to different conditions (Liu et al., 2012). Earthworms can modify microflora directly and indirectly by three main mechanisms: (1) comminution, burrowing and casting; (2) grazing; (3) dispersal (Brown, 1995). These activities may change the substrate's physico-chemical and biological status and cause drastic shifts in the density, diversity, compositions and activities of microbial communities in the VF biofilms (Li

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et al., 2013). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique has been applied to explore the microbial community structure of biofilms in VF and found that those biofilms were featured on richer diversity in their microbial community than BF biofilms (Li et al., 2013; Liu et al., 2012). However, due to the limitation of PCR-DGGE technique, the function of microbial community such as metabolic properties has not been fully studied (Leckie, 2005; Suzuki and Giovannoni, 1996). Therefore, a suitable technology needs to be applied to further discover the mechanism such as the diversity and the metabolic property of microbial community in biofilms during vermifiltration of liquid-state sludge.

Phospholipid fatty acids (PLFAs) is one of the most important biomarkers of microorganisms, which is useful for characterizing the microbial biomass in various environments, such as agricultural soils and various other systems (Amir et al., 2008). The membrane PLFA contents and composition in living bacterial cells are relatively unchanged under various growth conditions and PLFAs are rapidly degraded after the death of microorganisms (Amir et al., 2008). Hence, PLFAs can be used to estimate the total viable microbial biomass contained in a sample. Moreover, different microbial communities have different PLFA compositions. Thus PLFA composition analysis can provide the information on the compositions and overall changes in major groups, such as bacteria, actinomycetes and fungi. Furthermore, the analysis of characteristic PLFA ratio also provides more information on the metabolic properties of the microbial community structure. For example, the ratio of monounsaturated to saturated (mono:sat) PLFAs was used as an indicator of physiological or nutritional stress in microbial communities. This ratio is generally lower in microbial communities in the environment with limited organic carbon and nutrients (Gomez-Brandon et al., 2011). Generally, the PLFA approach is superior to PCR-DGGE in providing a quantitative measure of the microbial community structure composition (bacteria, actinomycetes and fungi) and reflecting the metabolic properties of microbial community (White et al., 1998).

In this study, the application of PLFA analysis presented new insights into microbial community structure and metabolic properties of biofilms in the VF. The hypothesis that earthworms in the VF could result in the overall optimization of the vermifiltration system for liquid-state sludge stabilization could be proposed based on the results: (1) earthworm activities in the VF significantly enhanced the microbial activity and microbial community diversity; (2) the ingestion of earthworm increased the soluble substances and thereby relieved physiological or nutritional stress of microbial community; (3) the burrowing action of earthworms promoted the aeration condition and led to aerobic microorganisms were predominant in the VF.

2. Methods

2.1. Vermifilter setup and operation

Two sets (each set has three parallel reactors) of cylindrical filters were set up. One set was the vermifilters (Fig. 1) with an initial earthworm density of 32 g/L (fresh weight basis) as suggested by Zhao et al. (2010), while the conventional biofilters (BF) without earthworms were used as the control. Each filter (diameter of 20 cm and depth of 100 cm, made of perspex) had a working volume of 31.4 L and was packed with ceramsites (10–20 mm in diameter). A layer of plastic fiber was placed on the top of the filter bed to avoid the direct hydraulic influence on the earthworms and ensure an even influent sludge distribution. The earthworms, Eisenia fetida, used in this study were purchased from a farm in Yancheng City, China. The influent sludge was obtained from the secondary sedimentation tank of a municipal WWTP in Shanghai, China. The hydraulic load of the two sets of filters was kept at 4 m/d, and the organic load of the influent sludge was maintained within the range of 1.10–1.28 kg-VSS/(m^3 d). After passing through the filter bed continuously, the sludge entered into a sedimentation tank. These filters ran steadily for 8 months to investigate their treatment performances on liquid-state sludge stabilization after about 30-day acclimation.

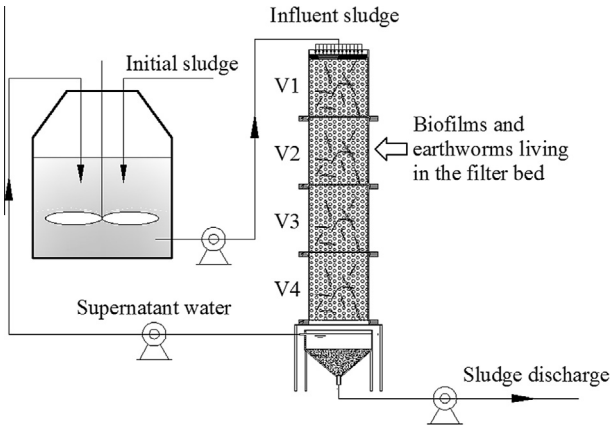

Fig. 1. Schematic diagram of the vermifilter (VF, with earthworms in the filter bed).

2.2. Sampling and chemical analysis

Biofilm samples were collected from the filter bed in both BF and VF reactors in the depths of 12, 37, 62 and 87 cm after the experiment completion to evaluate the microbial activities and PLFA profiles of microbial communities. Samples form the BF at the depths of 12, 37, 62 and 87 cm were, respectively designated as B1, B2, B3 and B4, while those from the VF were designated as V1–V4. The biofilms on the ceramsites were rinsed into centrifuge tubes with sterile water, and then centrifuged (9000 rpm) for 15 min at 4 °C. The dewatered biofilm samples were freeze-dried and grounded through the 0.15 mm mesh for further analysis.

Sludge characteristics such as the suspended solids (SS) and volatile suspended solids (VSS) were assessed according to Chinese Standard Methods. Total chemical oxygen demand (TCOD) and soluble COD (SCOD) were measured with a NOVA60 COD meter (Merck, Germany), and the samples for the SCOD measurement were firstly filtered through the 0.45 μm mixed cellulose ester membrane.

2.3. Dehydrogenase activity

Dehydrogenase activity has been adopted to assess the total microbial activity of sludge (Liu et al., 2012). Thus the total microbial activity of biofilms during vermifiltration of liquid-state sludge was evaluated according to the method proposed by Caravelli et al. (2004). One unit of dehydrogenase activity was defined as the catalysis capacity required for producing 1 mg INTF per hour.

2.4. Phospholipid fatty acid analysis

Fatty acids were extracted from 4 g freeze-dried biofilm samples with approximate 40 ml extraction mixture containing phosphate buffer, chloroform and methanol (0.9:1:2, V:V:V) (Bossio and Scow, 1998). The lipid extraction was separated through a solid phase extraction column (500 mg; 3 ml; Agilent Technologies Inc., UK). The neutral lipids, glycolipids and phospholipids were eluted with chloroform, acetone and methanol, respectively (Frostegard and Baath, 1996). The Phospholipid