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Feeding behavior and trophic relationship of earthworms and other predators in vermifiltration system for liquid-state sludge stabilization using fatty acid profiles

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HIGHLIGHTS

- Feeding behavior and trophic level of VF earthworms have never been reported.
- Earthworm activity increased the percentage of protozoa on VF biofilm.
- Fatty acid composition of earthworms was changed due to their feeding behavior.
- The trophic level order of predators was leeches > earthworms > lymnaeidaes > limaxes.
- Earthworms optimized microbial community structure and extended VF food web.

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ABSTRACT

The sludge reduction capability (VSS reduction) of vermifilter (VF) was 14.7% higher than that of conventional biofilter (BF) due to the fact that there was a net loss of biomass and energy when the food web in VF is extended. Therefore, feeding behavior and trophic relationship of earthworms and other predators (leeches, lymnaeidaes and limaxes) in VF were investigated using fatty acid (FA) profiles for the first time. Compared with BF biofilm, microbial community structure of VF biofilm got optimized by earthworms that the percentage of protozoa increased from 14.2% to 20.4%. Furthermore, analysis of specific microbial FAs composition in each predator suggested different trophic level of predators resulted from their selective ingestion of different microorganisms, and earthworms were at the second high trophic level in VF food web. Overall findings indicated earthworms modified microbial community and extended the food web of VF and thus enhanced the sludge reduction.

1. Introduction

The activated sludge process is the most widely used biological wastewater treatment for both domestic and industrial plants in the world (Wei et al., 2003). Excess sludge produced during the activated sludge process is considered as an inevitable drawback, because its treatment and disposal to avoid repollution accounts up to 60% of the total operating cost of municipal wastewater treatment plants (MWWTP) (Yan et al., 2013). Moreover, it is noteworthy that sludge dewatering has been identified as one of the most expensive and least understood process in excess sludge treatment and disposal. Therefore, it is considerable essential to explore and develop cost-effective and environmental friendly method for excess sludge treatment (Alam and Fakhru'l-Razi, 2003). 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., 2014, 2010).

It is well accepted that the inoculation of earthworms into conventional biofilter (BF) could enhance the stabilization of excess sludge (Yang et al., 2013; Zhao et al., 2010), and the earthworms were considered as the crucial drivers of the vermifiltration (Liu et al., 2012). Researchers have reported that the ingestion of earthworms were able to convert the important plant nutrients such as N, P, K, Ca present in the excess sludge into the much more soluble and available forms (Garg et al., 2006). Moreover, the enhancement of microbial community diversity caused by earthworms (i.e. aerobic and anaerobic microflora in their gut) should not be

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neglected (Gupta and Garg, 2009). Recent study also indicated that earthworms could improve the metabolic properties of microbial community in biofilm and thus resulted in the overall optimization of the vermifiltration system for liquid-state sludge stabilization (Zhao et al., 2014).

Previous studies have focused on the performance of the vermifilter (VF) and characteristics of microbial community on the biofilms as well as the physiological adaptation of earthworms in VF (Xing et al., 2011; Zhao et al., 2010). Besides the microorganism and earthworms, there were considerable quantities of other predators such as leeches, lymnaeidaes and limaxes living in the VF. All these predators interrelate with each other in the relations such as competition, antagonism and predation action in VF ecosystem, forming a complicated artificial ecosystem, in which bacteria, fungi, protozoa, earthworms and other predators are concomitant. In this ecosystem, excess sludge reduction was realized by the net loss of biomass and energy in term of food web relation among those creatures.

Few investigations have advanced knowledge of the feeding behavior and trophic relationship of earthworms and other predators during vermifiltration of liquid-state excess sludge. Moreover, the competition mechanism and the trophic level of earthworms and other predators cannot be visually judges. Food source greatly influenced the fatty acid (FA) composition of creatures since it was energetically more efficient to incorporate the FAs of food into creature tissue without modification, and then the composition of FAs in creatures and their food source were similar (Ruess and Chamberlain, 2010). Therefore the relations between the creatures and their possible food source could be determined by analyzing their FA composition and the trophic level of each creature could also be estimated as well. Since trophic transfer of intact lipids was firstly demonstrated in microbial-based tritrophic cascades (Ederington et al., 1995), fatty acids and lipid signature biomarkers have been used widely to reveal the feeding behavior and trophic relationship in the food webs of marine, freshwater and soil (Kainz et al., 2004; Pond et al., 1997; Ruess et al., 2004). In this study, the FA profile technology was used to investigate the FA composition of the substrates (influent sludge, effluent sludge and biofilm) and the predators (earthworms, leeches, lymnaeidaes and limaxes) in vermifiltration system for liquid-state sludge stabilization, and the feeding behavior and trophic relationship of earthworms and other predators were also established.

2. Methods

2.1. Vermifilter setup and operation

Two sets of cylindrical filters (each set has three parallel reactors) that consisted of perspex tubing was set up, which was assembled as previously described by Xing et al. (2011). One set was the vermifilters with an initial earthworm density of 32 g/L (fresh weigh basis) as suggested by Zhao et al. (2010), while the conventional biofilter (BF) without earthworms was used as the control. The earthworms, *Eisenia fetida*, used in this study were purchased from a farm in Yancheng City, China. The influent sludge was obtained from secondary sedimentation tank of a MWWTP in Shanghai, China. After passing through the filter bed continuously, the sludge entered into a sedimentation tank. These filters were operated continuously for 240 days. Details about parameters and treatment performance of each reactor are listed in Table 1.

Table 1
Parameters and treatment performances of the BF and VF.

	BF	VF
Filter media	Ceramsite	Ceramsite
Filter media diameter (mm)	10–20	10–20
Filter diameter (cm)	20	20
Filter depth (cm)	100	100
Working volume (L)	31.4	31.4
Hydraulic load ($m^3/(m^2\ d)$)	4	4
Organic load (kg-VSS/(m^3 d))	1.10–1.28	1.10–1.28
Earthworm density (g/L)	32	32
VSS reduction (%)	35.2 ± 2.08	49.9 ± 2.80
VSS/SS	0.68 ± 0.037	0.63 ± 0.033

2.2. Sampling and pretreatment

The influent and effluent sludge samples of BF and VF were withdrawn and centrifuged at 6000 rpm for 5 min, and the residues were freeze-dried. Biofilm samples were collected from the filter beds in both BF and VF reactors after the experiment completion. The biofilms on the ceramsites were rinsed into centrifuge tubes with sterile water and treated the same as the sludge samples. The initial earthworms (living in cattle dung), the earthworms and other predators (leeches, lymnaeidaes and limaxes) surviving in the VF reactor were randomly picked up, kept in dark for 24 h to empty their gut and then using deionized water to clear their excrement prior to freeze-dry. All samples were grounded to powder, and then sieved through 0.15 mm mesh prior to further analysis.

2.3. Fatty acid analysis

FAs of substrates (influent sludge, BF and VF effluent sludge, BF and VF biofilms) and creature tissues (earthworms, leeches, lymnaeidaes and limaxes) were extracted with extraction mixture containing phosphate buffer, chloroform and methanol (0.9:1:2, V:V:V) according to Macnaughton et al. (1997). The FAs were dried with a stream of nitrogen gas and transesterified into fatty acid methyl esters (FAMEs) through mild alkaline methanolysis reaction (Amir et al., 2010). Then FAMEs were analyzed in split mode (100:1) with a Trace DSQ gas chromatography mass spectrometer (Thermo, USA). FAMEs were separated with a VF23MS column (60 m × 0.32 mm, 0.15 μm thickness; Varian, Palo Alto, USA). The detailed GC–MS experimental conditions were obtained according to the method proposed by Dungait et al. (2008).The adopted carrier gas was He (10 psi head pressure) and the oven temperature rose according the program (rising from 50 °C to 150 °C at 15 °C min^{-1}, rising from 150 °C to 240 °C at 4 °C min^{-1}, and held at 240 °C for 20 min). The FAMEs were quantified through the comparing the peak areas with those of an internal standard nonadecane (19:0) peak. The retention times were compared with the FAME standards (Bacterial Acid Methyl Esters Mix 47080-U, PUFA NO. 1 47033, PUFA NO. 2 47015-U and Supelco™ 37 component FAME Mix 18919-1 AMP, Sigma–Aldrich, USA) to identify the FAMEs.

2.4. FA signature biomarkers

Interpretation of the FA profiles was aided by the use of FA biomarkers, since certain lipid compounds are known to be associated with specific groups of organisms (Frostegard and Baath, 1996; White et al., 1997). The equivalent chain length of FAs that shorter than 20C is considered as the microbial origin (Sampedro et al., 2006). In particular, 14:0, i15:0, i16:0, i17:0, cy17:0, cy19:0, 16:1ω7c, 18:1ω7, 18:1ω9t have been used as bacterial FAs biomarker (Frostegard and Baath, 1996; Ringelberg et al., 1997). The FAs 18:1ω9c, 18:2ω6, 18:3ω3 and 20:1ω9 were considered as fungal origin (Madan et al., 2002; Zelles, 1997) and the 20:3ω6, 20:4ω6 and 20:5ω3 were mainly from microfauna such as protozoa (Dungait et al., 2008; Sampedro et al., 2006).