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Properties of biofilm in a vermifiltration system for domestic wastewater sludge stabilization

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HIGHLIGHTS

- The VF biofilms had less organic matter and microbial biomass than the BF biofilms.
- The VF biofilms had higher microbial enzyme activities than the BF biofilms.
- *Proteobacteria* seemed to be the important contributors in the VF system.
- The relationships between some indexes in VF biofilm was different from BF biofilm.
- The presence of earthworm was a main reason to change the VF biofilm property.

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ABSTRACT

Vermifiltration is an alternative and low-cost technology for stabilizing excess sludge from domestic wastewater treatment plants. The biofilm properties of a vermifilter (VF) with earthworms, *Eisenia fetida*, for domestic wastewater sludge (DWS) treatment were studied. A biofilter (BF) without earthworms served as the control. VF biofilms had lower levels of suspended solids (SSs), volatile SS, C, H, N and S contents, protein-like groups, and total viable cell numbers and larger humic acid-like fractions and protease, dehydrogenase, lipase, and amylase activities compared with BF biofilms. Furthermore, VF biofilms featured richer diversity in their microbial community and more populations of *Proteobacteria* than BF biofilms. The relationships between organic matter and microbial eco-physiological indices in VF biofilms were significantly different from those in BF biofilms. Overall findings indicated that earthworm presence remarkably decreases organic matter contents and microbial biomass and improves microbial enzyme activities and the community structure of VF biofilms.

1. Introduction

The introduction of earthworms to filtration systems, termed vermifiltration systems, was first advocated by José Toha [1,2]. As an extension of vermicompost for solid wastes, vermifilters (VFs) were developed to treat a mixture of solids and liquids from household or animal wastes with high organic pollution [3,4]. Several studies have been conducted to investigate the use of vermifilters in wastewater treatment, including synchronous treatment of sewage and sludge [2,5–11]. Recent studies have shown that VF is a feasible and efficient technology for stabilizing excess sludge from domestic wastewater treatment plants (WWTPs) [12–14]. The reduction of volatile suspended solids (VSSs) using VF reaches 56.2–66.6%, meeting up with the criteria for aerobic and anaerobic sludge stabilization (>40%) [14]. The capacity of VF to treat domestic wastewater sludge (DWS) can be attributed to the vermicomposting process that occurs within the system and earthworm consumption of solid organic waste on the bed surface [3].

DWS treatment and disposal pose challenges for domestic WWTPs worldwide due to environmental, economic, social, and legal concerns [12,15]. Several mechanical, physical, and chemical treatment processes, including ultrasonic, thermal, and ozone pre-treatment, require large amounts of capital and high operational costs [14]. Compared with other technologies used in DWS treatment, such as anaerobic and aerobic digestion [12,15], VF is a low-cost "bio-safe" technique, and thus is more suitable for wastewater and DWS treatment of WWTPs in developing countries [4,13,14]. Previous studies have focused on treatment performance, earthworm–microorganism interactions, and organic-matter distribution and transformation in vermifiltration systems [10–14]. However, biofilm properties in the vermifiltration system have not been fully investigated.

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The functions of conventional biofilters (BFs) rely heavily on the metabolism of microorganisms present in the biofilm, which is the most fundamental characteristic of BFs [14]. In a vermifiltration system, the reduction and stabilization of DWS is involved by the joint action of earthworms and microorganisms in the biofilm [14]. The relationship between microorganisms and earthworms during the vermicomposting process has been widely investigated [17]. In a vermicomposting system, microbes are responsible for the biochemical degradation of organic matter of sewage sludge, whereas earthworms drive this process by conditioning the substrate and altering its biological activity [18,19]. However, little information about the organic matter composition and microbial eco-physiological properties of biofilm in vermifiltration systems, as well as the influence of earthworm inoculation on VF biofilm properties, is available.

A range of parameters, such as suspended solids (SSs), VSS, viable cell numbers, and activities of protease, dehydrogenase, lipase, glucosidase, and amylase, is indicative of biofilm development and activity. VSS corresponds to the quantity of biofilm and represents the non-ash part of the total SS [20]. Viable cells are quantified by extracting phopholipids and analyzing phosphate contents (cleaved from phospholipids); this parameter correlates to viable biomass [20]. Phospholipids have the advantages that their concentration remains fairly constant in relation to cell biomass and once a cell dies the phospholipids have a short half-life. The protease activity arising from the depolymerization of dissolved organic nitrogen from N-containing compounds is assumed to be a critical point in the N cycle because polymers are not accessible to microorganisms [21]. Dehydrogenase activity is essential in both mineralization and transformation of organic C [22,23] and thus plays an essential role during the initial stages of oxidation of organic matter by transferring hydrogen electrons from substrates to acceptors [20]. Lipase, glucosidase, and amylase are associated with carbon turnover in a wide range of ecosystems [24]. Thus, enzymes play an essential role in the biochemical transformation that involves the decomposition of organic matter in soil [25] and biological wastewater treatment processes [26]. Enzyme activities serve as indicators of microbiological activities in the biofilm.

Some advanced analytical techniques, such as fluorescence analysis of humic acid-like (HAL) fraction, polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE), and scanning electron microscopy (SEM), are often used to further understand the microbial characteristics contained within the biofilm. Unlike the traditional plate-counting method, PCR–DGGE can provide a more comprehensive analysis of bacterial compositions, including culturable and non-culturable microbes [27]. SEM can directly reveal the microorganism profile and biofilm microstructure from magnifications of 10 times to more than 500,000 times [12,28].

The objectives of the study are (1) to determine the organic matter composition and microbial eco-physiological characteristics of VF biofilms using various techniques and (2) to investigate the effect of earthworm presence on biofilms by comparing biofilm properties in VFs with and without earthworms.

2. Materials and methods

2.1. Vermifilter system

A cylindrical VF consisting of a Perspex tubing (20 cm in diameter and 120 cm in depth) was set up (Supporting information Fig. S1) and assembled as previously described in Zhao et al. [14]. The tubing contained a 100 cm filter bed of ceramic pellets (10–20 mm in diameter). A layer of plastic fiber was placed on top of the filter bed to avoid direct hydraulic influence on the earthworms and ensure even influent distribution.

The VF was inoculated with *Eisenia fetida* at an initial earthworm density of approximately 40 g L^{-1}, while a conventional BF was set up without earthworms as the control. The hydraulic loadings of the filters were kept at 4 m d^{-1} during the experimental period. The influent sludge was obtained from the aeration tank of a domestic WWTP (Quyang WWTP, Shanghai) and diluted to a constant organic load of approximately 1.12 kg VSS m^{-3} d^{-1} using tap water. After passing through the filter bed, the treated sludge entered into a sedimentation tank below the VF, and the supernatant in the sedimentation tank was recycled. The ratio of VSS to SS and the pH in the initial sludge were 70.4 ± 3.3% and 7.5 ± 0.4%, respectively. The initial earthworms were randomly picked from a Donghai farm, Shanghai, China, and cultured with cow dung as substrate. After acclimation for approximately 30 d, the filters were continuously operated for 330 d.

2.2. Biofilm analyses

2.2.1. Sampling

Ceramic pellet samples were collected from the filter bed in the BF and VF at depths of 12.5, 37.5, 62.5, and 87.5 cm at the end of the experiment to evaluate biofilm properties. Samples from the BF were designated as B1, B2, B3, and B4, while those from the VF were designated as V1, V2, V3, and V4; all samples were rinsed with sterile water. The biofilm in the wash water was collected and centrifuged for 10 min at 8000 rpm and 4 °C. Settled biofilm samples were then used for further analysis.

2.2.2. Organic matter composition

The SS and VSS contents of the biofilm samples in the BF and VF were assessed according to Chinese standard methods [14].

Settled biofilm samples were freeze-dried and filtered using 0.15 mm sieves. C, H, N, and S contents in the samples were measured in triplicate using the elemental analyzer Vario EL III (Germany).

2.2.3. Fluorescence analysis

HAL fractions were extracted from freeze-dried and sieved biofilm samples as previously described [16], and fluorescence excitation–emission matrix (EEM) spectra from aqueous solutions of the HAL fractions were obtained. The HAL fractions had a concentration of 100 mg L^{-1} after overnight equilibration at 25 °C. The pH was adjusted to 8 using 0.05 mol L^{-1} NaOH and measured by an F-4600 fluorescence spectrophotometer (Hitachi, Japan). Emission and excitation slits were set to 5 nm bandwidths, and a scan speed of 12,000 nm min^{-1} was selected for both monochromators. EEM spectra were recorded by scanning the emission wavelength between 250 nm and 500 nm, while the excitation wavelength was sequentially increased from 200 nm to 400 nm. Surfer 8.0 software was used to analyze the fluorescence spectral data.

2.2.4. Total viable cell number

The total viable cell number is represented as the quantity of phospholipids. Extraction of phospholipids and release of phosphate from phospholipids, as well as measurement of nanomole concentrations of phosphate released from phospholipids, were performed as previously described by Findlay et al. [29].

2.2.5. Enzymatic activities

Biofilm samples from the two filters were analyzed for protease, dehydrogenase, glucosidase, lipase, and amylase activities. Protease activity was measured from the tyrosine derivatives generated from 1 g of sample after incubation with sodium caseinate for 10 min at 40 °C and subsequent reactions with Folin Ciocalteau reagent [21]. Dehydrogenase activity was quantified according to the method previously described by Zhang et al. [20], which involves