



Study on the biomass and size spectra of bio-particles in vermifilter biofilms

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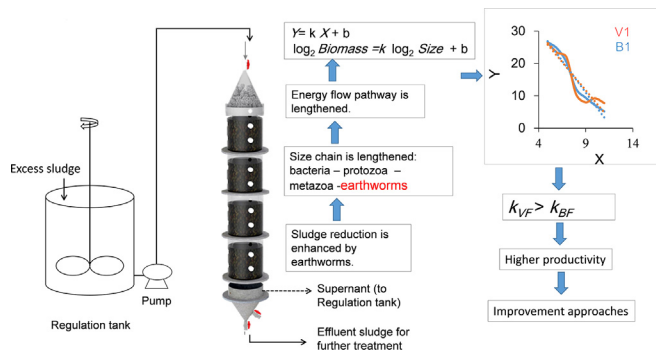
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HIGHLIGHTS

- Large organisms increased while small ones decreased by the presence of earthworms.
- Good linear models are found between abundance and size of bio-particles.
- The VF has a higher productivity level of large organisms than the BF.
- Temperature and humidity affect the VF performance.

GRAPHICAL ABSTRACT



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ABSTRACT

In biological processes of sludge treatment, the sludge yield is closely related to the energy dissipation of entire microbial system. The vermifilter (VF), a novel biofilter, works efficiently due to the introduction of earthworms, which modifies the energy flow pathway through the variations of microbial size structure. For a deep insight into the sludge reduction in the VF, the biomass size spectrum (BSS) was employed to map the energy dissipation in the VF. The results indicated that bio-particles in the size class of [31, 63] μm were reduced most in the excess sludge after the VF treatment. In biofilms, bio-particles in the size class of [31, 63] μm varied most with the filter depth and earthworm density. Eight biomass and size spectra (BSS) were established for all beds of the VF and BF (the control of the VF, without earthworms). The normalized BSS were all linear both in the VF and BF, and their linear regression parameters, the slopes (k) and intercepts (b), varied with the filter depth and the earthworm density. The k and b of the VF were both significantly different from those of the BF. According to the k , the productivity level of largest bio-particles was higher in the VF than in the BF. According to the b , bio-particles at the bottom of size structure could be taken faster in the VF than in the BF. At last, some improvement approaches with some tries were proposed to enhance the sludge treatment capacity of the VF.

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Abbreviations: WWTP, wastewater treatment plants; VF, vermifilter; BF, traditional biofilter; IES, influent excess sludge; VFS, the VF effluent sludge; BFS, the BF effluent sludge; VSS, volatile suspended solids; TSS, total suspended solids; COD_{cr} , chemical oxygen demand measured by the potassium dichromate; Sig., the significance level; RT, room temperature; RAH, relative air humidity; BSS, biomass and size spectrum; NBSS, normalized BSS; V1, the top bed of the VF; V2, the second top bed of the VF; V3, the second bottom bed of the VF; V4, the bottom bed of the VF; B1, the top bed of the BF; B2, the second top bed of the BF; B3, the second bottom bed of the BF; B4, the bottom bed of the BF; AL, the auxiliary line; RPM, round per min; Ind., individual; ESD, the equivalent spherical diameter.

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1. Introduction

With the increment of WWTPs in China, total sludge production in China had an average annual growth of 13% recently (Yang et al., 2015). In WWTPs, the sludge yield is 0.5–1.0% of treated wastewater in volume, and the sludge treatment could take 25–50% of all the operation cost (Molinos-Senante et al., 2013), which causes that most small WWTPs in developing nations cannot afford to construct and maintain conventional sludge treatment processes (Zhao et al., 2010). Therefore, there is a need for low-cost techniques to treat sewage sludge. At present, vermifiltration is recommended as an environmentally sustainable and economically viable technology, including vermi-composting, vermi-filtration, vermi-remediation, et al. (Sinha et al., 2010). Thereinto, the vermifiltration is widely applied for the treatment of wastewater (Wang et al., 2016; Samal et al., 2018) and liquid-state sludge (Mudziwapasi et al., 2016; Zhong et al., 2017; Zheng et al., 2017; Furlong et al., 2015), et al. Currently, the VF enables to stably and efficiently reduce the VSS of excess sludge by more than 40% through the earthworm-microbe interactions (Di et al., 2016). How earthworms and microbes work together is a scientific problem. As we all know that the sludge yield is closely related to the energy dissipation, less sludge yield means more energy dissipation, vice versa. We have demonstrated that there was more energy dissipated in the VF than in the BF (Xing et al., 2016). How and where did the energy dissipate? It deserves a further study, which could facilitate the optimization of the VF process in terms of organic reduction. Zhao et al. (2010) indicated that excess sludge could be reduced by the earthworm ingestion and the earthworm-microbe interactions. Xing et al. (2014) thought that earthworms could extend the trophic level of predation chains (limaxes, lymnaeidae, earthworms, leeches) in the VF using fatty acid profiles.

Study about predation chains of the VF will be a very complicated, costly and time-consuming project according to the taxonomic approach. The analysis of BSS is an approach to the study of the structure and function of the aquatic ecosystem without rigorous microbial taxonomy (Quinones et al., 2003; Yurista et al., 2014), which can be employed in the VF system to map the energy flow pathway.

In the BSS formulation, each organism in the system is assigned to its corresponding size class, which is proposed based on the trophic-level concept and a continuous flow of energy from small to large organisms (Kerr, 1974; Platt and Denman, 1977). When BSS are exhibited in the logarithms of sizes and biomass, a linear spectrum would be present, called NBSS (Quinones et al., 2003; Zhou et al., 2015). The construction of the VF NBSS requires following two hypotheses: (1) the ecosystem should be stable and relatively closed; (2) the food chains are dominated by predation chains. The VF is a semi-artificial microecosystem, and the earthworms could keep the constant biomass during most of the year (Xing et al., 2012), so it has the functional diversity and ecological integrity of the community. The size range of bio-particles could cover the range of 2 μm –2 mm (Zhao et al., 2010). The VF is essentially an improved trickling filter, and the organisms are dominated by the aquatic organisms. The food chains of aquatic organisms are usually dominated by the predation chains, which are characterized by the size superiority of predators (Silvert, 1984). Hence, food chains in the VF could also be predation chains. In this way, NBSS model could be established for the VF ecosystem.

In the BF, the introduction of earthworms could lengthen original predation chains (Xing et al., 2012; Xing et al., 2014). The production of organisms at the top of the trophic level is intimately associated with the biomass of organisms at the bottom of the trophic level (Hunt Jr. and McKinnell, 2006), so the introduction of earthworms could increase the biomass input of small organisms, and then the biomass output of large organisms could be kept theoretically. Li et al. (2014) showed that earthworm biomass increased in upper beds and decreased in lower beds in the VF at the organic load of about 1.12 kg-VSS $\text{m}^{-3} \text{d}^{-1}$. Therefore, VF ecosystem could hold earthworms with certain biomass. Being similar to the evaluation method of fisheries yield

(Boudreau and Dickie, 2011), NBSS in the VF beds could also be used to guide the VF management if earthworm biomass needs to be optimized.

In this study, the size structure of excess sludge and VFS was analyzed, and NBSS in biofilms were established. According to the parameters of NBSS and the comparisons of the VF and BF, clues of improvement approach could be reminded for the present VF.

2. Material and methods

2.1. Vermifilter setup and experimental design

Two sets of reactors packed with ceramsites (15–20 cm in diameter) were set up. One was the VF inoculated with *Esenia fetida*, and the other one was the BF as the control. The VF was cylindrical, and consisted of 4 beds in the same diameter and height. Each bed was 20 cm in inner diameter, 30 cm in height, and 25 cm in thickness as suggested by Zheng et al. (2017). A plastic water distributor was put on the top of each filter to ensure an even influent distribution. Excess sludge was passed through 1.00 m high filter media when it was introduced into the VF reactor. The supernatant of the sedimentation tank was recycled to the regulation tank, and the treated sludge was used for vermicomposting.

The major parameters of the VF and BF are listed in Table 1. The hydraulic load was kept at about 4.00 m d^{-1} , and the organic load was kept at about 1.33 kg-VSS $\text{m}^{-3} \text{d}^{-1}$. Excess sludge was taken from the secondary settling tank of Quyang WWTP, Shanghai, China. The VF was inoculated with healthy adult *E. fetida* (Wangjun earthworm farm, Jiangsun Province, China) at an initial density of about 32.0 g-fresh weight L^{-1} as suggested by Zhao et al. (2010). Reactors had been operated continuously and stably in a shed before the experiment started.

2.2. Sampling and chemical analysis

Each bed of the VF and BF had sampling ports at depths of 5 and 15 cm under the bed surface. Sampling began in March 2017. Samples of the effluent and influent sludge were got once a week and biofilm samples were got fortnightly. Adult earthworms and filter flies were excluded in biofilm samples. The contents of VSS were determined according to Chinese Standard Methods (SEPA, 2002). Hygrometers (testo 608-H1, testo AG, Germany) were used to detect AT and RAH in the shed daily.

2.3. Bio-particle analysis

The assay was conducted in the summer when earthworms and biofilms had constant biomass according to the experience of Xing et al. (2012). A fresh biofilm sample was fixed and dispersed at a final concentration of 5% formaldehyde, 5% polysorbate and 0.1% rose bengal sodium salt; then it was shaken continuously for 24 h at 140 RPM. The dyed red bio-particles were screened through series of sieves with sieve pores as 2000, 1000, 500, 250, 125, 63 and 31 μm , respectively (Shimanaga and Shirayama, 2000). Bio-particles on sieves with sieve pores larger than 500 μm were picked up manually and identified individually under an anatomical microscope (Zoom-690C, Shanghai

Table 1
Main parameters of the VF and BF.

Main parameters	VF	BF
Total height of filter beds (m)	1.00	1.00
Diameter of filter beds (cm)	20	20
Porosity of filter beds (%)	48 ± 2	48 ± 2
Excess sludge (mg-VSS L^{-1})	5550 ± 417	5550 ± 417
Hydraulic load (m d^{-1})	4.00 ± 0.02	4.00 ± 0.02
Earthworm density (g-fresh weight L^{-1})	32.0 ± 0.1	0.0
Organic load (kg-VSS $\text{m}^{-3} \text{d}^{-1}$)	1.33 ± 0.02	1.33 ± 0.02

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