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Research article

Microbial enzyme and biomass responses: Deciphering the effects of earthworms and seasonal variation on treating excess sludge

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ABSTRACT

This paper reports on a seasonal pattern comparison of microbial enzymatic activities and biomass responses based on a conventional biofilter (BF, without earthworm) and a vermifilter (VF, with earthworm, *Eisenia fetida*) for excess sludge treatment. The volatile suspended solids (VSS) reduction, viable cell number and enzyme activities were assayed to probe what made the VF operate stably. The results indicated that the earthworm activities can polish the VSS reduction with 27.17% more than the BF. Though the VF had a lower level in the viable cell number compared with the BF, the earthworm strongly improved the microbial enzymatic activities such as INT-dehydrogenase, protease, β-glucosidase and amylase, which can explain the excellent performance of VSS reduction. The correlation analysis documented that the VSS reduction was positively correlated with microbial enzyme activities. More importantly, the earthworm enabled the VF to avoid the detrimental influence of temperature, which guaranteed a stable performance during seasonal variations.

1. Introduction

With the rapid economic growth, industrialization, urbanization and inadequate investment in infrastructures, (Jin et al., 2014). A sharp increase of excess sludge has been captured more and more attentions (Chen et al., 2012). Currently, production of excess sludge from wastewater aerobic treatment is one of the most serious problems encountered in China, especially for small MWWTP. It has been estimated that approximately 30–60% of sewage treatment plant operating costs are related to sludge treatment activities (Chen et al., 2012; Murray et al., 2008).

Anaerobic digestion and biogas utilization are suitable for large scale MWWTP (Wei et al., 2000). Most MWWTP in small towns cannot afford to construct and maintain conventional sludge treatment processes such as anaerobic and aerobic digestion in China. In this regard, excess sludge treatment and disposal using vermifiltration (VF, a liquid-state excess sludge vermiconversion) may guide an opportunity for an ecologically sound, economically viable and socially acceptable method for small and mid-scale MWWTP.

Vermifiltration is a relatively novel technology that works on the principle of earthworm-microorganisms symbiotic and synergistic interactions to treat excess sludge. Zhao et al. (2010) advocated it for the first time that VF can reduce VSS in the range of 56.6–66.6%, which met the requirement of discharge standard of pollutants for municipal wastewater treatment plant. The removal contribution of organic matter depends not only on organic matter characteristics but also on microbial activity. The earthworms digested the sludge into smaller particles which can be better utilized by microorganisms (Wang et al., 2013a). Earthworms achieved greater utilization of organic matter by microbial enzymatic activity living in the filter bed. Enzymes played an essential role in speed of organic matters decomposing and nutrient cycling. It has been demonstrated that the VF biofilms had less microbial biomass but higher microbial enzyme activities than the BF biofilms (Li et al., 2013; Parthasarathi and Ranganathan, 2000; Tejada and Masciandaro, 2011). Thus, the enzymatic activity can be used as a sensitive index to monitor treatment efficiency. Many studies about enzyme activities have focused on the biofilm hydrolases and dehydrogenase over a short time period or in isolated conditions. There still have no research concerning the effects in temporal

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dynamics, which mainly supports the essence of the vermifiltration.

Thus the objective of the present work was to assess the variations of biomass and enzyme activities in a lab-scale vermifiltration on excess sludge treatment with the seasonal changes. Some important microbial parameters i.e. total viable cell number, dehydrogenase activity and hydrolases (β-Glucosidase, Protease, Amylase, Lipase) were measured at different intervals in reactors. Furthermore, data was analyzed to find whether there is a relationship between the parameters and the vermifiltration performance.

2. Materials and methods

2.1. Experimental design

Two sets (each set has three parallel reactors) of cylindrical filters were set up (Fig. 1). The liquid excess sludge was pumped via a peristaltic pump into the cylindrical vermifilter that was naturally ventilated and distributed by a distributor. The vermifilter (30 cm in diameter and 90 cm in depth) made of perspex was operated in two layers with a total working volume of 56.5 L, packed with ceramsites (10–13 mm in diameter). The hydraulic loading of the two sets of filters was both kept at 4 $m^3/(m^2 \cdot d)$ and the organic loading was maintained within the range of 1.0–1.2 Kg-VSS/($m^3 \cdot d$). After passing through the filter bed, the treated sludge entered into a sedimentation tank at the bottom of the reactor. Both the BF and the VF were operated stably (without clogging) in continuous mode for approximately 12 months.

2.2. Excess sludge and earthworm used

The influent sludge was obtained from the secondary sedimentation tank of a municipal waste water treatment plant in Shanghai. The sludge was diluted to a constant concentration of approximately 300 mg/L using tap water in an equalization tank, then the mixture was pumped into the VF and the BF system.

Earthworms (*Eisenia fetida*) with well-developed clitella were purchased from a farm in Yancheng City, China. Adult earthworms weighed between 300 and 600 mg per worm were acclimatized and fed with the excess sludge for one week in the VF. The initial earthworm density was 32 g/L (fresh weight basis).

2.3. Sampling

Ceramic pellet samples were collected from the filter bed in the BF and the VF at depths of 10, 25, 35, 40, 50, 65 and 75 cm, respectively. Then the samples taken at different depths in the BF and the VF were mixed together defined as compound sample, respectively. The picked ceramsite sample was placed in beaker and stirred with glass rod. This process was repeated three times and then merged the wash 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.

2.4. Physico-chemical analysis

Suspended solids (SS), volatile suspended solids (VSS) were measured every three days according to standard methods (SEPA, 2002). Filter temperature and room temperature were recorded daily by thermometer. Polysaccharides were measured by the Anthrone method (Gaudy, 1962), using glucose as the standard. Protein contents were determined by the modified Lowry method (Lowry et al., 1951), using casein as the standard.

2.5. Total viable biomass

Measuring lipid phosphate or phospholipid ester-linked fatty acids provides a quantitative measure of bacterial and eukaryotic biomass containing intact cellular membranes. The viable biomass can be determined by quantifying organic phosphate from the phospholipid fraction of the lipid extract using a relatively simple colorimetric analysis. Phosphate concentration was then correlated to viable biomass using the conversion factor, 1 nmol phosphate from phospholipid is proportional to 3.4×10^7 cells as determined by Findlay et al. (1989).

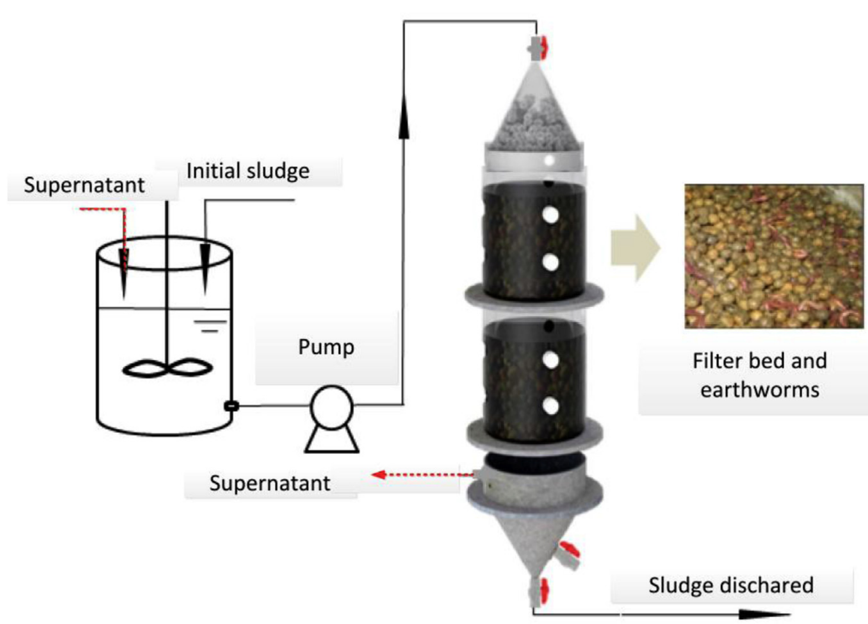

Fig. 1. Schematic diagram of a small scale reactor (VF, with earthworms).