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Enhancement of rural domestic sewage treatment performance, and assessment of microbial community diversity and structure using tower vermifiltration

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ABSTRACT

The performance of a novel three-stage vermifiltration (VF) system using the earthworm, *Eisenia fetida*, for rural domestic wastewater treatment was studied during a 131-day period. The average removal efficiencies of the tower VF planted with *Penstemon campanulatus* were as follows: chemical oxygen demand, 81.3%; ammonium, 98%; total nitrogen, 60.2%; total phosphorus, 98.4%; total nitrogen, mainly in the form of nitrate. Soils played an important role in removing the organic matter. The three-sectional design with increasing oxygen demand concentration in the effluents, and the distribution of certain oxides in the padding were likely beneficial for ammonium and phosphorus removal, respectively. The microbial community profiles revealed that band patterns varied more or less in various matrices of each stage at different sampling times, while the presence of earthworms intensified the bacterial diversity in soils. Retrieved sequences recovered from the media in VF primarily belonged to unknown bacterium and *Bacilli* of *Firmicutes*.

1. Introduction

Non-point source pollution of enclosed watersheds (e.g., lakes, reservoirs, and parts of rivers) is a global concern. The current deterioration of water quality in Lake Taihu in China is primarily due to pollution from domestic wastewater produced in rural areas (Li et al., 2009a). The essential issue regarding the increased incidence of eutrophication is the input of nitrogen (N) and phosphorus (P). Excessive amounts of these nutrients lead to a range of undesirable effects, including impairment of human health (algal toxins), reduced biodiversity of aquatic species, reduction in amenity value, and increased costs of treatment for drinking water (Withers et al., 2009). Therefore, nutrient inputs must be reduced and measures for effective reduction of nutrient concentrations in rural wastewater must be developed to protect these ecosystems from eutrophication.

Collecting and treating rural domestic wastewater is a large problem in China. Owing to the dispersed rural population and construction costs of sewage collectors, centralized wastewater treatment plants based on activated sludge or bacterial bed processes that are utilized in large and small cities are not suitable in rural areas (Ye and Li, 2009). Treatment systems that require relatively low costs, energy, and maintenance are preferable for the treatment of widely distributed rural domestic wastewaters. Several proposed solutions for the treatment of diffuse sources of domestic wastewater have been applied to on-site treatment in spacious rural areas, including constructed wetlands, soil infiltration trenches, vegetation-based wastewater treatment, and vermifiltration (VF) (Cuyk et al., 2001; Ham et al., 2007; Kaoru et al., 2010; Sinha et al., 2008). Among these technologies, VF, a process that separates wastewater solids by allowing wastewater to be gravity-fed over the filtration material, is the most promising economical method for treating point and diffuse sources of domestic wastewater.

VF has been studied extensively due to its effectiveness for removing pollutants in wastewater, and its positive effects on the environment. For example, Sinha et al. (2008) found that VF with earthworms increased the effectiveness of most wastewater contaminant treatments, whereas systems without worms showed poor performance. Similarly, Li et al. (2009b) reported that wastewater produced by more than 100 inhabitants per day and

Abbreviations: VF, vermifiltration; PCR-DGGE, polymerase chain reaction-denaturing gradient gel electrophoresis; COD, chemical oxygen demand; NH_3-N, ammonia nitrogen; BOD_5, 5 day BOD; HLs, hydraulic loads; TN, total nitrogen; TP, total phosphorus; DO, dissolved oxygen; PVC, polyvinyl chloride; NO_3-N, nitrate nitrogen; OM, organic matter; XRF, X-ray fluorescence; S1-1, 0–15 cm soil in the first stage; S1-2, 15–30 cm soil in the first stage; S1-3, silver sand in the first stage; S1-4, detritus in the first stage; S2-1, 0–15 cm soil in the second stage; S2-2, 15–30 cm soil in the second stage; S2-3, silver sand in the second stage; S2-4, detritus in the second stage; S3-1, 0–15 cm soil in third stage; S3-2, 15–30 cm soil in the third stage; S3-3, silver sand in the third stage; S3-4, detritus in the third stage.

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continuously treated by VF showed efficient reduction of chemical oxygen demand (COD), ammonia nitrogen (NH_3-N), and 5-day BOD (BOD_5). Fang et al. (2010) investigated the effect of hydraulic loads (HLs) on pollutant removal from synthetic domestic wastewater by an earthworm ecofilter. HLs exhibited varying influences on nutrient removals. Recently, the integration of VF with traditional sewage treatment technologies for the removal of excess nutrients from wastewater has appeared in the literature, including a laboratory-scale ceramsite-vermifilter and vermifilter enhancement using a converter slag–coal cinder filter for domestic wastewater treatment (Liu et al., 2009a; Wang et al., 2010). In addition to its usage in urban or rural domestic wastewater treatment, the design parameters and factors influencing earthworms have also been investigated (Hughes et al., 2009).

Previous studies have primarily focused on the use of VF or its combined processes in the treatment of different types of wastewater, and the related factors contributing to its efficiency in removing pollutants. However, the effluent quality concerning nitrogen (N) and phosphorous (P) do not always meet the I-class A criteria specified in the *Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants* (GB18918-2002) in China. To enhance the nutrients removal, a three-stage microbial-earthworm ecofilter was designed with a continuous flow configuration. This system provided excellent aerobic conditions for nitrification, and increased the removal rates for COD, NH_3-N and P. Therefore, to obtain a more detailed understanding of the nutrient degradation processes in the tower VF, the present study was conducted with the following objectives: (1) evaluate the performance of the three-stage tower earthworm ecofilter for the treatment of rural domestic wastewater, mainly in terms of COD, total nitrogen (TN), total phosphorus (TP), and NH_3-N removal at each VF phase under a certain hydraulic load; (2) assess the nutrient removal with respect to dissolved oxygen (DO) in each effluent and the change in chemical components before and after the operation periods; and (3) investigate the microbial community diversity and compositions in media at different sampling times by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE).

2. Methods

2.1. Experimental system

Fig. 1 presents the schematic diagram of the tower VF system, which was constructed at a village in Changzhou, Jiangsu Province, China. The system consists of three cubic stages and a wastewater containment tank, all of which were made of polyvinyl chloride (PVC) and fixed by an iron base. The tank and pump were installed to collect and transfer the influent from rural domestic wastewater to the apparatus. The first and second stages were 50 cm long and 50 cm wide, with a depth of 60 cm and packed padding of 55 cm. Their filter beds both consisted of four layers from top to bottom to a height of 30 cm with soil (diameter 400–800 μm), 10 cm with silver sand (diameter 100–800 μm), 10 cm with fine detritus (diameter 3–4 mm), and 5 cm with cobblestones (diameter 4–5 cm), which were used as the supporting layer. The third stage was filled with the same substrates with dimensions of 110 cm in length, 65 cm in width, and 120 cm in depth. The upper, middle, and third layers had equal depths of 30 cm and were filled with soil, silver sand, and detritus, respectively. The lowest layer of cobblestones had a depth of 20 cm. All padding came from Pukou in the suburb of Nanjing, Jiangsu Province, China. The characteristics of the padding used in this study can be found in Fang et al. (2010). To achieve homogeneous water distribution, the raw wastewater was introduced to the first stage through a rotating PVC pipe with holes, and the distribution of wastewater from the up-level to the down-level stage was achieved using perforated PVC pipes that ran along the entire length in the second and third phases. The vertical distances among stages were 40 cm. All PVC pipes, which were drilled with holes 1.5 mm in diameter evenly distributed on the lateral surface, were kept 15 cm above each stage surface to ensure the creation of drop-overflow and thereby increase aerobic conditions.

Eisenia fetida (Savigny), a common earthworm, was selected as the vermifilter for wastewater treatment. The vermifilter had a density of 12.5 g/L in soil for every step, and its activity ensured that the microbial-earthworm ecofilter was not in a state of inundation. The average soil C to N ratio was 18.6. In March 2010, all surfaces of the stages were planted with fully matured *Penstemon campanulatus* (transplanted from the suburb of Yixing, Jiangsu Province, China) at a density of approximately 20 plants per m^2. No mortality occurred during the running periods. Introduction of *P. campanulatus* into the system improved the plant uptake of nutrients and aesthetics.

The start-up of the tower VF process was initiated by seeding domestic wastewater in batch mode for acclimatization of earthworms and plants, colonization, and accumulation of microorganisms in the medium. From March 27 to July 31, the process was operated in continuous inflow mode using the 1 m^3/m^2 d hydraulic load. The raw wastewater was controlled using a liquidometer, and the treated sewage passed through the stages in sequence by gravity flow. During the operation period, the average extreme air temperature was 19.6 °C (ranged = 7.8–32.9 °C).

2.2. Analytical procedure

2.2.1. Water quality analysis

Wastewater samples were collected weekly from the inlet of the first stage and outlet of the first to third stage. COD, NH_3-N, TN, TP, and nitrate nitrogen (NO_3-N) were analyzed according to the standard methods (APHA, 1998). COD was measured using the potassium dichromate method. NH_3-N, TN, TP, and NO_3-N were determined using the Nessler's reagent spectrophotometric method, potassium persulphate oxidation-ultraviolet spectrophotometry, molybdenum-antimony anti-spectrophotometric method, and ultraviolet spectrophotometric screening method, respectively. The DO, pH, and temperature of the effluent of each stage were measured *in situ* using a DO meter, including temperature measurement (YSI Model No. 550A, USA) and a pH meter (Shanghai Kangyi Instrument Co. Ltd., PHS-2C, China). All analyses, including the control, were conducted in triplicate.

2.2.2. Matrix chemical composition analysis

At the end of the experiment (July 31, 2010), different types of filter materials in the packed tower were collected at different depths. In each stage, five padding sampling holes 50 mm in diameter were distributed on the lateral surface. Soil samples, namely, topsoil (0–10 cm), middle-level soil (10–20 cm), subsoil (20–30 cm), silver sand, and detritus including the initial medium were air-shipped to the laboratory. The earthworms and trash were removed from the plant roots on the day of collection. All samples were freeze-dried, sieved (<2 mm) (except detritus), and stored at −20 °C for analysis. Organic matter (OM) was determined by the loss of ignition after treatment at 550 °C for 2 h (Liu et al., 2009b). Chemical compositions were analyzed using an ARL-9800 XRF spectrometer (X-ray fluorescence spectroscopy-XRF, Switzerland).

2.2.3. Microbial analysis

Filters from 0–15 cm soil (S1/2/3-1), 15–30 cm soil (S1/2/3-2), silver sand (S1/2/3-3), and detritus (S1/2/3-4) collected in every