Ecological Engineering 71 (2014) 165–172

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

The effect of vermifiltration height and wet:dry time ratio on nutrient removal performance and biological features, and their influence on nutrient removal efficiencies

Longmian Wang[a,*], Zhaobing Guo[b], Yuxiao Che[b], Fei Yang[a], Jianying Chao[a], Yuexiang Gao[a], Yimin Zhang[a,**]

[a] Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection, No. 8 Jiang Wang Miao Street, Nanjing 210042, PR China
[b] Nanjing University of Information Science & Technology, No. 219 Ning Liu Road, Nanjing 210044, PR China

ARTICLE INFO

Article history:
Received 11 January 2014
Received in revised form 4 June 2014
Accepted 12 July 2014
Available online 3 August 2014

Keywords:
Domestic wastewater
Eisenia fetida
Microorganism
Nutrient removal efficiencies
Vermifilter

ABSTRACT

We investigated the effects of vermifiltration (VF) height and the wet:dry time (WD) ratio on nutrient removal from synthetic domestic wastewater, earthworm population characteristics, and microbial numbers in the substrate. We also evaluated the key factors influencing nutrient removal. Results showed that variation in VF height had a significant effect on chemical oxygen demand (COD) and total phosphate (TP) removal rates, earthworm population, and actinomycetes numbers, but had no effect on ammonia nitrogen (NH_3-N) and total nitrogen (TN) removal rates, and bacterial and fungi numbers. All parameters, except TP removal and fungi numbers, were significantly affected by variation in the WD ratio. Furthermore, when combined, the VF height and the WD ratio significantly influenced the earthworm reproduction rate and actinomycetes numbers. In particular, good nutrient removal efficiencies, vigorous earthworm activity and high microbial numbers were observed when the WD ratio was 1/3 and the VF height was 60 cm. Results suggest that earthworm growth and reproduction rates, fungi, and actinomycetes numbers are good indicators of the COD and TP removal efficiencies by VF. These factors, along with the bacterial numbers, are good indicators of NH_3-N removal efficiency; however, only the earthworm growth rate and actinomycetes numbers are useful indicators for the TN removal efficiency from wastewater by VF.

1. Introduction

Out of all the contaminants in wastewater, nutrients [nitrogen (N) and phosphorus (P)] give rise to much concern because of their capacity for complex transformations and interactions. In recent years, a large amount of untreated or minimally treated sewage from human and industrial activities has been directly discharged to land, or into water bodies, near its source. Water bodies that previously were important for recreation and leisure use have gradually become saturated, and even super-saturated, because of continuous nutrient inputs. High concentrations of nutrients have resulted in frequent algal blooms, and reduced drinking water safety for billions of people, such that both of these are considered as major global environmental issues. It is therefore important to find technical methods to decrease nutrient concentrations in domestic wastewater and to reduce nutrient discharges into water bodies, so as to reduce and prevent nutrient pollution.

Vermifiltration (VF) using earthworms is a recently developed novel technology that has been applied to rural and urban domestic sewage treatment (Tomar and Suthar, 2011; Wang et al., 2011). This technology is based on the ability of earthworms to ingest and break down organic wastes, heavy metals and solids from wastewater, and their ability to remove pollutants from wastewater by ‘absorption’ through their body walls (Sinha et al., 2008). In a previous study of nutrient removal, adding *Eisenia fetida* to a constructed wetland increased the total nitrogen (TN) and total phosphate (TP) removal efficiencies by 10% and 7%, respectively (Xu et al., 2013a,b). Similarly, removal of total suspended solids, total dissolved solids, nitrate nitrogen (NO_3-N), phosphate (PO_4^{3-}), and chemical oxygen demand (COD) were 38.8%, 20.8%, 80.6%, 50.8% and 144.6% higher, respectively, in a VF system than in a control

* Corresponding author. Tel.: +86 25 85287231; fax: +86 25 85287231.
** Corresponding author. Tel.: +86 25 85287127; fax: +86 25 85287127.
E-mail addresses: wlmian@sina.com (L. Wang), zym@nies.org (Y. Zhang).

http://dx.doi.org/10.1016/j.ecoleng.2014.07.018
0925-8574/© 2014 Elsevier B.V. All rights reserved.

(Tomar and Suthar, 2011). VF has also been integrated into existing methods for improved COD, ammonia nitrogen (NH_3-N), TN and TP removal from rural domestic, or swine, wastewater (Wang et al., 2010; Morand et al., 2011).

The operating and design features of the VF approach, and its mechanisms for nutrient removal, have been discussed previously in the literature, along with various VF applications in wastewater treatment. Zhao et al. (2012) found that VF systems with plants and earthworms achieved optimum nutrient removal in July when the influent C/N ratio was controlled at 6. Wang et al. (2013) demonstrated that the Shannon Index of Ammonia Oxidizing Bacteria diversity depended more on a constant NH_3-N degradation rate than temperature, thereby advancing our understanding of NH_3-N removal in VF wastewater treatment. Moreover, domestic wastewater P removal mechanisms were investigated by Fang et al. (2010). They found that P fixation, as aluminum (Al) and iron (Fe) phosphates through adsorption and co-precipitation in earthworm packing beds, was the main mechanism for P removal.

Previous studies have mainly compared (1) the nutrient removal efficiencies of systems with VF and control systems without VF, and (2) the biochemical characteristics of the filter media that contribute to the nutrient removal. Studies on how earthworm packing bed height and the wet:dry time (WD) ratio affect nutrient removal, earthworm characteristics and microbial populations are scarce. In addition, little is known about the effects of variation in the earthworm population and microbial substratum numbers on nutrient removal efficiency within domestic sewage. The aim of this study, therefore, was to obtain an improved understanding of the effects of earthworm packing bed height and the WD ratio on system operation, and the key factors influencing nutrient wastewater treatment using VF. The four main goals of the study were: (1) to determine the COD, NH_3-N, TN and TP removal efficiencies under different WD ratios in three different earthworm packing bed VF heights; (2) to analyze earthworm growth and reproduction rates, microbial numbers (bacteria, fungi and actinomycetes) in packing filters in response to varying WD ratios at different VF heights; (3) to evaluate the relationships between COD, NH_3-N, TN and TP removal efficiency, earthworm growth and reproduction rate, and substrate microbial numbers with different earthworm packing bed heights and WD ratios; and (4) to examine the correlation between earthworm population characteristics and substratum microbial numbers in VFs with nutrient removal for synthetic domestic wastewater treatment.

2. Materials and methods

2.1. Experimental design

The experimental VF at Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection, Nanjing, China (32°03′N, 118°47′E) is displayed in Fig. 1. The experimental setup was protected from the sun and the rain. A pipe with small holes (1.5 mm in diameter) drilled in its underside was installed. This pipe rotated constantly at an even speed to distribute wastewater uniformly. A polyvinyl chloride (PVC) VF column (35 cm in diameter) was filled (from bottom to top) with a supporting layer of 10 cm of cobblestones (diameter 6–10 cm), and a specific thickness of earthworm packing bed material, which was composed of soil, sawdust and earthworms (*E. fetida*). The earthworm packing bed heights were set at 40 cm, 60 cm and 80 cm, respectively. The physicochemical properties of the system filter and the soil:sawdust volume ratio can be found in Fang et al. (2010) and Wang et al. (2013).

The synthetic sewage, composed of tap water, glucose, urea, $NaHCO_3$, NH_4Cl, KH_2PO_4, K_2HPO_4, $CaCl_2$, and $MgSO_4·7H_2O$, was supplied by a peristaltic pump (Longer pump, basic type BT300-2J, Hebei, China). It was prepared every day and agitated manually for 5 min before being pumped into the apparatus. In the experiment, the surface loading of the synthetic wastewater was adjusted to $0.2\ m^3/m^2\ d^{-1}$. Three parallel devices were installed, one for each VF height, to compare the effects of earthworm packing bed heights on nutrient removal. Each operation cycle involved 2 h of wastewater flow, 1 h of retention, water drainage, and drying. The WD ratio in the operation was changed such that the wastewater flow and retention times were fixed but the water drainage and drying times were 6, 9 and 12 h. For each WD ratio and earthworm packing bed height, there were three parallel VFs. During the experimental period (June 2013 to August 2013), the mean summer air temperature was 28.4 °C (range 20.5–39.6 °C).

2.2. Water quality, earthworm and microorganism analyses

The influent was sampled immediately after the synthetic sewage was prepared, and effluents were sampled every 7 days, at the end of the retention time when wastewater drained from each VF. All samples were stored at 4 °C for less than 24 h before analysis, and three replicates were assessed for every indicator in each sample. Standard methods (APHA, 1998) were used to determine COD (potassium dichromate method), NH_3-N (Nessler's reagent colorimetric method), TN (potassium persulfate oxidation-ultraviolet spectrophotometric method) and TP (molybdenum–antimony anti-spectrophotometric method). Dissolved oxygen (DO) content was measured *in situ* using a portable DO meter (YSI Model no. 550A, USA) at the same time as water samples were collected. pH was measured using a PHS-2C pH meter (Shanghai Kangyi Instrument Co. Ltd., Shanghai, China).

Approximately 70 clitellated *E. fetida* with a live weight of 50 g were purchased from a Nanjing earthworm culturist and introduced into each VF. Growth and cocoon production in each apparatus were measured at the end of the experimental period. Earthworms and cocoons produced during the experiment were separated from the substrate material by hand sorting, after which they were counted, examined for clitellate development, and weighed after washing with tap water to remove material that had adhered to their bodies (Suthar, 2009). The worms were weighed without voiding their gut content. Based on biomass change and cocoon number data, the growth rate (mg/worm·day) and reproduction rate (cocoon/worm·day) were calculated with the help of recorded data for the different treatments.

The filter materials at different depths (upper, middle, and lower parts) in the column of earthworm packing bed from different VFs were analyzed for microbial populations at the end of the experiment. The samples collected from the same VF under an equal WD ratio were mixed thoroughly to form a composite sample to avoid an accidental value at any sampling point. 1 g of sample from the different depths of the same VF under an equal WD ratio was transferred to sterilized test tubes containing sterilized distilled water, and mixed thoroughly using a horizontal shaker for 50 min. The composite sample was then diluted using serial dilution methods and 1 mL aliquots were placed in autoclaved Petri plates according to the APHA method (1998). All culture mediums were kept in triplicate. The pour plate method was used to enumerate bacteria, fungi and actinomycetes in nutrient agar media, Rose Bengal agar, and Kenknight's media, respectively (Singh and Suthar, 2012). The colony forming units (CFU), expressed in microbial counting, were also measured using the method described in Singh and Suthar (2012). The mean bacterial, fungi and actinomycetes numbers in the initial VF substrates were 7.85 CFU × 10^5/g, 4.16 CFU × 10^5/g and 3.29 CFU × 10^4/g, respectively.