Journal of Environmental Management 226 (2018) 83–94

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Biophysiological and factorial analyses in the treatment of rural domestic wastewater using multi-soil-layering systems

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A R T I C L E I N F O

Keywords:
MSL system
Microbial species diversity
Factorial analysis
Operating factors
Field design and application

A B S T R A C T

Multi-soil-layering (MSL) system was developed as an attractive alternative to traditional land-based treatment techniques. Within MSL system, the environmental cleanup capability of soil is maximized, while the soil microbial communities may also change during operation. This study aimed to reveal the nature of biophysiological changes in MSL systems during operation. The species diversity in soil mixture blocks was analyzed using Illumina HiSeq sequencing of the 16S rRNA gene. The interactive effects of operating factors on species richness, community diversity and bacteria abundance correlated with COD, N and P removal were revealed through factorial analysis. The results indicated the main factors, aeration, bottom submersion and microbial amendment, had different significant effects on microbial responses. The surface area and porosity of zeolites in permeable layers decreased due to the absorption of extracellular polymeric substances. The findings were applied for the design and building of a full-size MSL system in field and satisfied removal efficiency was achieved. The results of this study can help better understand the mechanisms of pollutant reduction within MSL systems from microbial insights. It will have important implications for developing appropriate strategies for operating MSL systems with high efficiency and less risks.

1. Introduction

In recent years, more attention is being paid to the environmental problems in the rural and remote areas (Sorensen et al., 2016; Zhi et al., 2016). A large amount of domestic wastewater is often discharged into the environment directly without effective treatment, resulting in the deterioration of water and groundwater quality (Qin et al., 2007; Zhang et al., 2015). It can also contribute to non-point source pollution of water bodies (Ding et al., 2017; Khan and Valeo, 2017). For the small and remote areas without centralized wastewater treatment facilities, it is urgent to develop a suitable strategy to address such environmental issues. One of the major concerns for determining suitable technique used in rural areas of developing countries is cost. Land-based treatment system can help address such concern with reasonable cost (Massoud et al., 2009). Some treatment techniques such as soil trench system and constructed wetland, have been developed and applied for decentralized wastewater treatment (Yu et al., 2012). However, some problems such as low loading rate, large land space requirement and clogging risk still exist in the operation of these treatment systems.

Multi-soil-layering (MSL) system was developed as an attractive alternative to traditional land-based treatment techniques (An et al., 2016a; Khaoula et al., 2017). It is featured by high hydraulic load rates and small land space requirement, which are ideal for application in the rural areas of developing countries. MSL system shows good performance in the reduction of organic matter and nutrients (Latrach et al., 2016). The reduction of organic matter and nutrients in wastewater through MSL is a complicated process involving various chemical, physical and biological processes (Luanmanee et al., 2002b; Sato et al., 2005). The performance of MSL is mainly attributed to its unique brick-wall style of structure including soil mixture blocks (SMBs) and permeable layers (PLs) (Fig. S1). Soil within SMBs plays an important role in treatment process. Due to its local availability, a low build-up cost of MSL system can be achieved (Ho and Wang, 2015). Soil also serves as a habitat for a wide variety of microorganisms, which have close relation with the removal and transformation of pollutants in wastewater (Guan et al., 2015).

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https://doi.org/10.1016/j.jenvman.2018.08.001
Received 21 April 2018; Received in revised form 25 July 2018; Accepted 1 August 2018
0301-4797/

In previous studies, the MSL system has been successfully used for treatment of domestic wastewater (Luanmanee et al., 2002a), livestock wastewater (Chen et al., 2007), polluted river water (Masunaga et al., 2003), dairy effluent (Pattnaik et al., 2008) and leachate (Yidong et al., 2012). The influence of operating conditions such as hydraulic loading and filter materials on wastewater treatment were also studied (Ho and Wang, 2015). Although various types of MSL have been applied for wastewater treatment in China, Thailand and USA, the biological processes in MSL system and their influencing factors have not been fully elucidated. The findings in literature are limited to the analysis of removal rates and mass balance (Sato et al., 2005). COD, BOD, TP, TN and NH_4^+ −N were usually used as the performance indicators for MSL systems. There is a lack of in-depth discussion of the internal causes of treatment process. It is still necessary to obtain more quantitative information for pollutant removal in MSLs (An et al., 2016a). In addition, for the applications of MSL system, there are some problems that need to be resolved. For instance, high hydraulic loads and prolonged operation could cause system blockage and reduced efficiency. Clogging of MSL has been a major problem that results in system failure (Yidong et al., 2012). The lack of the appropriate aeration will result in less aerobic environment, and excessive aeration will affect the denitrification rate (Luanmanee et al., 2001). More detailed studies on MSL systems will facilitate broader applicability.

The soil microbial groups are not immutable and they may change as the surrounding environment varies (Peralta et al., 2012). Within MSL system, the environmental cleanup capability of soil is maximized, while the soil microbial communities may also change during operation. Although the performance of MSL system have been reported, the microbial nature within MSL system is not clear. It is still a challenge to determine the relation between internal MSL characteristics and operating conditions. Few studies focused on the analyses of microbial communities within the MSL system and the interaction of multiple condition factors. Based on our research regarding the interactive effects of operation conditions on MSL's performance, this study will give a further insight into the MSL treatment from a microbial perspective. In detail, (1) species diversity and abundance in SMBs system will be characterized and their interactive relation with treatment process will be revealed, (2) the variation of physiochemical properties of PLs will be explored, and (3) a full-size MSL system for practical rural wastewater treatment on site will be designed and built based on the findings in this study. The results can help obtain a better understanding of the inherent mechanisms of treatment process in MSL.

2. Materials and methods

2.1. Factorial design of MSL treatment experiments

The synthetic rural wastewater containing COD, BOD, TP, TN, NH_4^+ −N and $N0_3^-$ −N of 477 mg L^{-1}, 288 mg L^{-1}, 3.7 mg L^{-1}, 45 mg L^{-1}, 23 mg L^{-1} and 3.8 mg L^{-1} respectively was used throughout this study. The lab-scale MSL systems made of acrylic were designed with the dimension of 52 × 10 × 85 cm (L × W × H). There were two 3-cm holes in the top for influent distribution pipe and 2-cm outlet hole in bottom for effluent pipe. The influent enters the system from the upper pipe. The SMBs consisted of planting soil, charcoal, sawdust and iron powder with a ratio of 7:1:1:1 and the mixture was put in jute bags. The bulk density of SMBs was 0.9 g/cm^3. The zeolites with a size of 4–6 mm were filled as PLs. SMBs had the dimensions of 5 × 10 × 5 cm and 10 × 10 × 5 cm (L × W × H). The horizontal distance between SMBs was 3–4 cm and each PL between SMBs was 5 cm in height.

Aeration in MSL systems was conducted by air pumps at a rate of 26000 L/(m^3•d). The aeration pipe was made of PVC with an inner diameter of 10 mm. The aeration pipe was arranged under the fourth soil layer. The aeration hole with an interval of 50 mm was evenly distributed at the top of the aeration pipe. The operation of bottom submersion was conducted by submerging the bottommost SMBs under the outlet position, and the submerged one was controlled via adjusting outlet which was 20 cm higher than MSL. The microbial amendment was prepared using the microbial product of BZT Waste Digester from Bio-Form Co., Ltd (Foshan, China). The inoculum bacteria mainly contained *Lactococcus* with a percentage of 51%, *Bacillus* with a percentage of 26%, *Entertococcus* with a percentage of 18% and some other species. In detail, 100 g product was mixed with 10 L synthetic wastewater, with continuous aeration of 24 h for activation, as well as further growth and reproduction of microbial amendment. Microbial growth during cultivation was monitored by measuring its optical density at a wavelength of 600 nm (OD600) (Lim et al., 2016). The microbial amendment was further inoculated into MSL systems when achieving OD600 0.9 approximately.

In the present study, three factors were examined in two levels. Through preliminary experiments, those factors and levels were selected because of their important impacts on the system response. It is essential to reveal the potential interrelationships among these factors and their impacts on system performance. A 2^3 full factorial design was applied for investigating the interactive effects of these factors (Table S1). Factorial design can reveal not only the effects of individual parameters but also their relative importance in given process. The interactive effects of two or more variables can also be revealed (Xin et al., 2016). All experiments were conducted within the MSL systems as shown in Fig. S2.

2.2. Analysis of microbial communities in SMBs

The microbial communities in SMBs were analyzed to investigate the relation of MSL performance and its microbial features. In order to reflect the changes of microbial communities in MSL systems, the nature soil samples M1 was analyzed besides 8 soil samples M2-M9 from MSL systems. To ensure the repeatability in experiments, the soil samples have been collected from multiple places in the same system and mixed before testing (Chiaia-Hernández et al., 2017). A 20 g fresh sample in SMBs at the same layer was collected from each original system and systems at the end of operation. Samples were snap-frozen in liquid nitrogen and stored at −80 °C. DNA was extracted from the SMBs samples using a PowerSoil DNA Isolation Kit. The V3 + V4 region of the bacteria 16s RNA gene was amplified by PCR using the primes 338F 5' (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTA AT-3′) (Yu et al., 2017). The PCR cycle conditions included an initial denaturation at 94 °C for 2 min, 25 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 40 s, and 72 °C for 7 min. Then, the mixture of PCR products were purified for sequencing analysis. The sequencing was performed using Illumina Hiseq 2500 (Illumina Inc., San Diego, CA, USA). The overlapping regions between the paired-end reads acquired from sequencing were merged using FLASH v1.2.7 (The Center for Computational Biology, Johns Hopkins University, USA). The merged reads and raw tags were filtered to obtain high-quality clean tags using Trimmomatic V0.33 (Secretariat AG Usadel RWTH Aachen University, Institute for Biology, Germany). Finally, the chimera sequences were detected and then removed. The optimal clean tags with ≥97% similarity were grouped into the same operational taxonomic units (OTUs). A series of analysis about species composition, abundance and difference between species in different samples were also carried out by comparing with the bacteria 16s RNA database of Silva (Quast et al., 2013).

2.3. Specific surface area and porosity of zeolites in permeable layers

The information on zeolite structures can help reveal the mechanism of the system performance through physiobiochemical response. Prior to analysis, 0.5–0.6 g zeolite sample was taken from PLs of each MSL system. The zeolite samples were dried at 110 °C in the electrothermal blowing dry box for 2 h before analysis, then degassing