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Effects of nitrogen removal microbes and partial nitrification-denitrification in the integrated vertical-flow constructed wetland

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ABSTRACT

In this study, the treatment performance of the integrated vertical-flow constructed wetland (IVCW) and nitrogen (N) removal mechanism of microbes were explored by quantitative PCR (qPCR) technology. The results showed that the IVCW pilot system could achieve high removal efficiencies of NH_4^+-N (above 90%), TN (55–90%) and TOC (above 95%) under 2, 4, 6, 8 C/N ratios. A close relationship exists between the abundance of nitrogen-removing microbes and water quality parameters of effluents. The abundance of bacterial 16S rRNA genes, *amoA* of ammonia oxidizing bacteria (AOB), *nirS* of denitrification bacteria and *nxrA* of nitrite oxidizing bacteria (NOB) in the down-flow chamber were higher than those of the up-flow chamber in IVCW. The relative abundance of *amoA*, *nirS* and *nxrA* suggested that AOB and denitrification bacteria were predominant in the nitrogen removal process, and thus the partial nitrification and denitrification appeared to be the main N removal process in the IVCW test system.

1. Introduction

Constructed wetlands, simulating the purification function of natural wetlands, have become a widely applied technique and a valuable complement to traditional wastewater treatment systems in recent years. Low cost, convenient operation and maintenance and high wastewater treatment efficiency are the major advantages of constructed wetlands (Garfía et al., 2012; Vymazal, 2014; Wu et al., 2015a). Although the quantities of constructed wetlands have increased year by year, the nitrogen (N) treatment performance has always been unsatisfactory, which is a major challenge for constructed wetlands (Zhang et al., 2014; Liu et al., 2015).

Oxygen supply has been commonly perceived as a limiting factor to N removal efficiency (Ding et al., 2012; Li et al., 2014). The use of integrated vertical-flow constructed wetlands (IVCW) could overcome this problem because of its unique structure, consisting of a down-flow chamber and an up-flow chamber. Therefore, the U-shaped flow structure of IVCW gave rise to the alternating "aerobic-anoxic-anaerobic-anoxic-aerobic" multifunction layers, i.e., a gradient of physical, chemical and biological conditions favorable for microbial growth and reproduction. This is especially important for enhancing the function of N removal bacteria whose oxygen requirement is different (Xiao et al., 2010; Zhang et al., 2012; Peng et al., 2014).

The competition of N removal microbes in the environments is the key issue for the nitrogen removal pathway (Yang and Yang, 2011; Akizuki et al., 2015). Moreover, the influent C/N ratio also played a crucial role in denitrification which is usually restricted by the lack of organic carbon source (Liu et al., 2013; Wu et al., 2014). Very few studies have been focused on the relationship between N removal microbes and influent C/N ratio. Therefore, the objectives of this study were to detect the abundance of nitrogen removal microbes under the different C/N ratios (2:1, 4:1, 6:1, and 8:1) and to reveal the link between the functional genes of microbes and N conversion reactions. The qPCR technology was employed to quantify the abundance of key functional genes involved in N removal.

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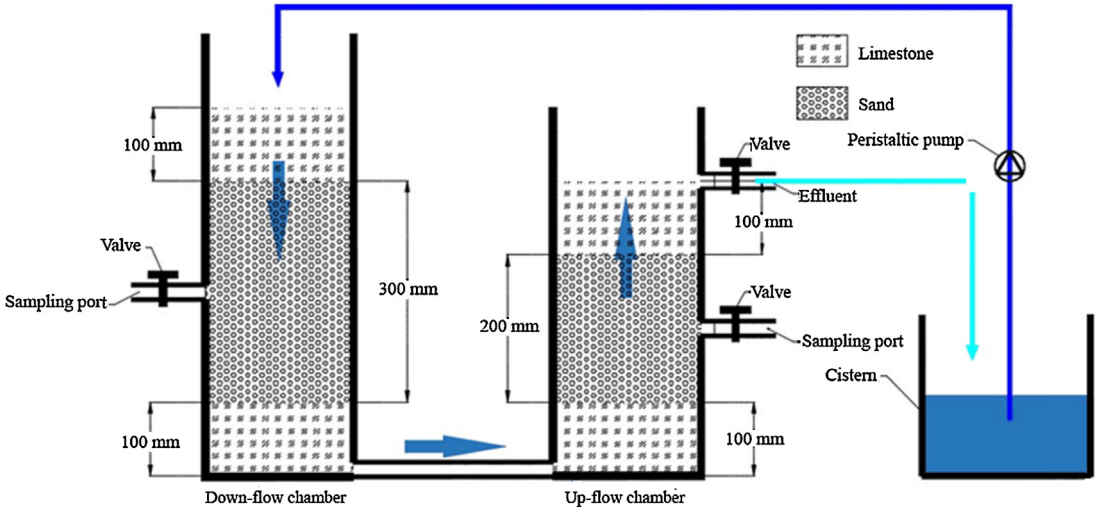

Fig. 1. The schematic diagram for small scale plot of IVCW.

2. Materials and methods

2.1. Experimental set-up

The pilot system of the integrated vertical-flow constructed wetland (IVCW) was cylindrical in shape, comprising a down-flow chamber and an up-flow chamber made of resin (Fig. 1). The two chambers were connected at the bottom with polyvinylchlorid (PVC) tubes. Each chamber was 20 cm in diameter, with sand of 1.6–2.0 mm in diameter filled to a depth of 30 cm and 20 cm in the down-flow chamber and the up-flow chamber, respectively. Limestones of 1 cm in diameter were filled to a depth of 10 cm at the top and bottom layers of the sand to keep an alkaline environment. The sand layer was added with nitrogen-free activated sludge for microbial inoculation. The sludge was collected from the nitrogen removal chamber of Nanshan Wastewater Treatment Plant after dewatering by centrifugation. The sampling ports were set at the bottom of the 25 cm and 20 cm in the down-flow chamber and up-flow chamber, respectively.

2.2. Start-up and operation strategy

The study included 4 treatment groups and each treatment group included 3 replicates. The synthetic wastewater composed of 20 mg/L NH_4Cl, and 8 mg/L KH_2PO_4 and the C/N ratio was used as an indicator to control organic loading rates in the influent. The C/N ratios of 2:1, 4:1, 6:1, and 8:1 were made by adding 40 mg/L, 80 mg/L, 120 mg/L, 160 mg/L glucose. Hydraulic and nitrogen loading rates were maintained at 0.03 m^3/m^2 d and 0.57 g N/m^2 d. During the operation, water in the cistern was pumped with a peristaltic pump and the dissolved oxygen (DO) concentration reached 9 mg/L. The load of total organic carbon in each group was 2.54 gC/m^3 d, 5.08 gC/m^3 d, 7.62 gC/m^3 d and 10.16 gC/m^3 d accordingly.

2.3. Sample collection and chemical analyses

Samples of influent and effluent at different time intervals were collected from the IVCW system for the analyses of NO_3^--N, NH_4^+-N, and NO_2^--N by a CleverChem 380 discontinuous water quality analyzer, based on different standard methods (China, 2012). The concentration of total organic carbon (TOC) was calculated by subtracting inorganic carbon from total carbon, which was analyzed by a multi N/C 3000 TOC analyzer. Microbial samples were collected at the sampling ports (Fig. 1) in each of the down-flow chamber and up-flow chamber after draining the wastewater and then were placed in an ice incubator and immediately sent to the laboratory for DNA extraction.

2.4. Quantitative polymerase chain reaction (qPCR)

Total microbial DNA was extracted by using the E.Z.N.A. Soil DNA Kit (OMEGA) according to the manufacturer's protocols. The extracted DNA was stored at −20 °C until used for analyses. The quantity and quality of the extracted DNA were determined by NanoDrop 2000 analyzer.

qPCR was performed on a MyiQ2 Real-Time PCR Detection System (ViiA-7, Applied biosystems) in the final 20 mL volume of reaction mixtures containing the following components: 10 μL SYBR Premix, 2 μL template DNA (sample DNA or plasmid DNA for standard curves), forward and reverse primers (0.8 μL each), 0.4 μL ROX Reference Dye and 6 μL sterile water. The qPCR was conducted as previously described (Chen et al., 2014) and the specific operating procedures are shown in Table 1. Each qPCR amplification was performed in 40 cycles, followed by a melting curve analysis. Cycle thresholds were determined by comparison with standard curves constructed by using 10-fold serial dilution of the newly extracted plasmids containing the corresponding gene fragments.

2.5. Data analysis

The effluent quality parameters including TOC, NH_4^+-N, NO_2^--N, NO_3^--N and TN in different C/N ratio groups was tested by One-Way ANOVA analyses. The functional genes of microbial denitrification in different C/N ratio groups were analyzed by IBM SPSS 17. The relationships between effluent water quality (the concentration of TOC, NH_4^+-N, NO_2^--N, NO_3^--N and TN) and the genes of microbial denitrification were analyzed by Pearson correlation analysis.

3. Results and discussion

3.1. The TOC and nitrogen removal efficiencies in the IVCW system

The influent concentrations of TOC in 4 treatment groups were 40, 80, 120, 160 mg/L, respectively, while the effluent concentrations of TOC were all below 8 mg/L (Fig. 2a), indicating that the removal rate of TOC was above 95%. Removal efficiency of organic