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Quantifying nitrogen transformation process rates using nitrogen functional genesin a multimedia biofilter under hydraulic loading rate constraints

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ABSTRACT

The present study explored the treatment performance and nitrogen removal pathways in a multimedia biofilter treating micro-polluted source water under hydraulic loading rate (HLR) constraints, ranging from 0.5 to 3.0 m^3/m^2 d. High and stable chemical oxygen demand (COD) (97.7–99.1%) and ammonium (NH_4^+-N) (76.3–90.9%) removal efficiency were simultaneously achieved. Results showed that an HLR exceeding 2.5 m^3/m^2 d was required to achieve complete denitrification without NO_3^--N accumulation in the biofilter. Molecular biological analyses showed that nitrification and anaerobic ammonium oxidation were the dominant NH_4^+-N removal pathways in the biofilter. Quantitative analysis demonstrated that the key functional gene groups for NH_4^+-N transformation process rate were *amoA*/archaea, *nxrA*/archaea, and (*nirS* + *nirK*)/anammox. Furthermore, *nxrA*/archaea contributed the most to the NH_4^+-N transformation rate, followed by (*nirS* + *nirK*)/anammox, and *amoA*/archaea. The results support that archaea potentially play vital roles in the nitrogen removal pathways, and nitrogen transformation pathways are coupled at the molecular level (i.e., the functional gene level).

1. Introduction

Worldwide, the drinking water supply is threatened by environmental pollution (Smarty et al., 2010). In particular, this issue has become a matter of great concern for areas with water sources in China (Li et al., 2011; Zhou and Zhu, 2003). Economic development throughout China has resulted in excess nitrogen being discharged into rivers, due to industrialization, civilization and large-scale emissions from sewage treatment plants. Yet, these rivers serve as the chief sources of drinking water supply for the nation. Most of these rivers are slightly polluted by chemical oxygen demand (COD) and ammonium (NH_4^+-N), which may reduce the quality of the aquatic environmental and threaten human health (Bhatnagara et al., 2011; Qin et al., 2009). Thus, it is becoming increasingly important to pre-treat micro-polluted water to remove excess nitrogen.

Compared with domestic wastewater, micro-polluted source water has a relatively low concentration of nitrogen and other pollutants (Piedrahita, 2003). To treat this type of water, bioreactors with high bacterial cell residence time are required (Eding et al., 2006). Fixed biofilm reactors, such as biofilters, have this characteristic. Biofilters have been successfully used for removing nitrogen, in addition to manganese and iron, from ground and surface water (Kornaros and Lyberatos, 2006; Nemade et al., 2009; Tekerlekopoulou and Vayenas, 2008; Van den Akker et al., 2008). However, under varying hydraulic loading rates (HLRs), the nitrogen removal efficiency of biofilters is subject to substantial fluctuation, leading to unsatisfactory results (Ji et al., 2013). Therefore, it is important to develop methods that improve nitrogen removal efficiency and the analysis of nitrogen removal pathways under varying HLRs, representing a research hotspot in the field of biofilters.

A biofilter is an attached growth bioreactor packed with plastic or mineral inert media as biofilm substratum. Water is distributed over a tower containing the packed media. Then, as the water trickles down, the microorganisms in the biofilm degrade organic matter, involving nitrification and denitrification pathways (Wik, 2003). These microorganisms play an important role in the nitrogen transformation and removal pathways in biofilters. These pathways include several ecological processes, such as nitrification, denitrification, and anaerobic ammonium oxidation (anammox) (Ji et al., 2013; Mulder et al., 1995; Satoh et al., 2004).

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Nitrogen removal in a biofilter primarily involves several microbiological processes, including nitrification, denitrification and anaerobic ammonium oxidation (anammox) (Ji et al., 2013; Satoh et al., 2004). These various nitrogen processes involve several functional genes, including ammonia monooxygenase (*amoA*), nitrite oxidoreductase (*nxrA*), periplasmic nitrate reductase (*napA*) and membrane-bound nitrate reductase (*narG*), nitrite reductase (*nirK*/*nirS*), nitric oxide reductase (*qnorB*), nitrous oxide reductase (*nosZ*), archaeal 16S rRNA (archaeal) and anaerobic ammonium oxidation (anammox) (Galloway et al., 2008; Ji et al., 2012). The *amoA* gene and *nxrA* gene are two functional genes involved in the nitrification process. Six other genes, nar*G*,*napA*, *nirK*, *nirS*, *qnorB* and no*sZ*, are six functional genes associated with denitrification (Ji et al., 2013). Anammox and archaeal are two functional genes involved in ammonium oxidation processes (Leininger et al., 2006; Rothrock et al., 2011). Published studies have demonstrated that nitrogen removal in biofilters is often restricted by increased HLR (Ji et al., 2011; Tan and Ji, 2010). A study by Li et al. (2011) on the effects of HLR on pollutants removal showed that both the effluent concentration of NH_4^+-N and COD increased with increasing HLR. Specifically, microbial ammonia oxidation, which is the first and rate-limiting step for subsequent nitrogen transformation and removal, is impaired by a higher HLR. This phenomenon arises because higher HLR causes organic loading (Ji et al., 2011; Tan and Ji, 2010). Van den Akker et al. (2011) studied the structure of nitrifying biofilms in a biofilter designed for potable water pre-treatment. The study found that nitrifiers in the biofilter were abundant under low organic loads, with small increases inorganic carbon promoting the rapid growth of heterotrophic bacteria, as well as the production of large amounts of polysaccharides. Petersen et al. (2012) investigated nitrogen transformation process rates across ecosystems using abundance of microbial genes, the results showed that functional gene abundance is a valuable index to predict nitrogen transformation process rates. Ji et al. (2013) investigated the spatial distribution of nitrogen removal functional genes in multimedia biofilters. The results showed that anammox, *napA*, *qnorB*, and *nosZ* genes exhibited partially or mutually beneficial cooperation in the nitrogen transformation process. The *nxrA* and *nirK* genes exhibited protocooperation, while *amoA* and *narG* genes exhibited partially beneficial cooperation in the nitrogen transformation process. To date, there have been no reports that quantitatively estimate the dynamics of nitrogen transformation process rates using nitrogen functional genes in biofilters under hydraulic loading rate constraints.

To optimize the design, operation, and application of biofilters, it is first necessary to identify the key functional genes involved in these microbial-driven nitrogen removal pathways in a single biofilter system. Therefore, this study aimed to elucidate nitrogen transformation pathways of biofilters using abundance of nitrogen functional genes under different HLRs, in addition to the role of microbes in these nitrogen transformation pathways. Specifically, this study aimed to: (i) evaluate the treatment performance under varying HLRs, (ii) quantify the abundance of bacterial 16S rRNA, archaeal 16S rRNA, and anammox bacterial 16S rRNA, in addition to known nitrogen transformation functional genes (*amoA*, *nxrA*, *napA*, *narG*, *nirK*, *nirS*, *qnorB*, and *nosZ*), using real-time PCR, (iii) quantify the response relationships between the nitrogen transformation rates and functional genes under HLR constraints, and (iv) identify key functional genes that determine nitrogen removal pathways in biofilters. The findings of this study are expected to contribute to improving the efficiency of biofilter systems under varying HLRs.

2. Materials and methods

2.1. Multimedia biofilter setup and operation

A single laboratory scale multimedia biofilter was built with a metal framework and PVC board. The biofilter had the following dimensions: 40 cm length × 30 cm width × 240 cm height (working volume of 144 L). The biofilter was used to treat micro-polluted source water. The biofilter consisted of four functional layers. The dimensions of a single functional layer were 40 × 30 × 30 cm (length, height, and width, respectively). The distance between two functional layers was 20 cm. A sieve tray was installed between each layer. From top to bottom, the four functional layers were filled with sponge (polyurethane foaming plastic) (with5–8 mm apertures), porous lava rock (with a particle diameter of 2–5 mm), sponge (with 3–5 mm apertures), and porous lava rock (with a particle diameter of 2–5 mm), respectively. For the material roles, operation and performance of the sponge, please see the literatures (Guo et al., 2010; Ji et al., 2011). Synthetic wastewater was added to the top of the biofilter, and was allowed to flow via gravity through the four functional layers. Treated wastewater was discharged and collected from the bottom of the biofilter.

The experiment began on December 28, 2012. The experiment was divided into six stages (over a total of 245 days): (1) Start-up Stage (HLR = 0.5 m^3/m^2d), from December 28, 2012 to February 23, 2013; (2) Stage I (HLR = 1.0 m^3/m^2d), from February 24 to March 29, 2013; (3) Stage II (HLR = 1.5 m^3/m^2d), from March 30 to May 3, 2013; (4) Stage III (HLR = 2.0 m^3/m^2d), from May 4 to June 7, 2013; (5) Stage IV (HLR = 2.5 m^3/m^2d), from June 8 to July 12, 2013; and (6) Stage V (HLR = 3.0 m^3/m^2d), from July 13 to August 16, 2013. Synthetic wastewater was prepared daily in a feeding tank, and pumped into the biofilter through flumes in the distribution layers. The synthetic wastewater composition at each operational stage is summarized in Table 1. The biofilter was placed indoors, and the temperature range of the influents and effluents was 15.5 to 27.0 °C.

2.2. Sample collection and determination

Water samples were collected from the inlet and outlet of the biofilter three times during each stage. The samples were immediately analyzed at the Key Laboratory of Water and Sediment Sciences, Peking University, China. Four parameters were measured. COD was determined with an HACH DR2800 (HACH, USA). NH_4^+-N, NO_2^--N, and NO_3^--N were measured using a spectrophotometer UV-1800 (SHIMADZU, Japan). All variables

Table 1
Operational parameters of the multimedia biofilter.

Parameters	Start-up	Stage I	Stage II	Stage III	Stage IV	Stage V
COD ($C_6H_{12}O_6$, mg/L)	30	30	30	30	30	30
NH_4^+-N (NH_4Cl, mg/L)	1.5	1.5	1.5	1.5	1.5	1.5
TP (KH_2PO_4, mg/L)	0.3	0.3	0.3	0.3	0.3	0.3
Influent flow (L/d)	50	100	150	200	250	300
Hydraulic loading rate (m^3/m^2d)	0.5	1	1.5	2	2.5	3

All the chemical reagents used in this study were of Analytical Reagent (AR) grade.