Bioresource Technology 196 (2015) 57–64

Contents lists available at ScienceDirect

Bioresource Technology

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

Enhanced long-term ammonium removal and its ranked contribution of microbial genes associated with nitrogen cycling in a lab-scale multimedia biofilter

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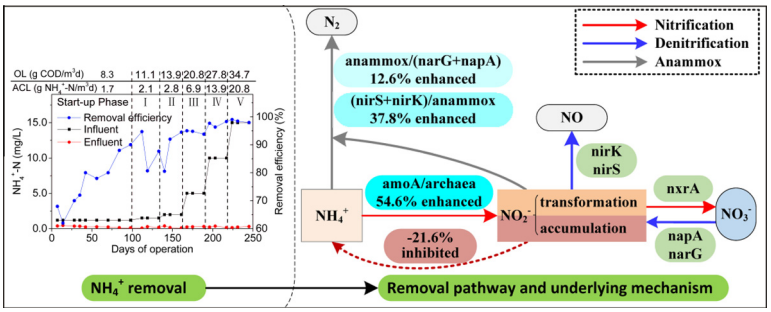
HIGHLIGHTS

- Multimedia biofilter achieved promising COD and NH_4^+ removal efficiencies.
- Increased COD and NH_4^+ loading showed significant effects on NO_3^- accumulation.
- The coupling of *amoA* and archaeal had the maximum effect (54.6%) on NH_4^+ removal.
- NO_2^--N accumulation had a significant negative effect (−21.6%) on NH_4^+ removal.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 15 July 2015
Accepted 17 July 2015
Available online 22 July 2015

Keywords:
Biofilter
Functional gene
Nitrogen transformation
Path analysis
Quantitative relationship

ABSTRACT

The multimedia biofilter achieved high and stable removal efficiencies for chemical oxygen demand (COD, 62–98%) and NH_4^+ (68–98%) without costly aeration. Results revealed that lower CL (less than 13.9 g COD/m^3 d) and ACL (less than 2.8 g NH_4^+-N/m^3 d) or a C/N ratio exceeding five was required to reduce NO_3^--N accumulation and NO/N_2O emission. Integrated analyses indicated that the coupling of simultaneous nitrification, anammox and denitrification processes (SNAD) were the primary reason accounted for the enhanced NH_4^+-N treatment performance. NH_4^+-N removal pathways can be ranked as follows: nitrification (*amoA*, archaeal) (54.6%) > partial denitrification (*nirS*, *nirK*) and anammox (37.8%) > anammox and partial denitrification (*narG*, *napA*) (12.6%). Specifically, NH_4^+-N removal was significantly inhibited by NO_2^--N accumulation in the system (−21.6% inhibition). Results from stepwise regression analysis suggested that the NH_4^+ removal rate was collectively controlled by *amoA*, archaeal, anammox, *nirS*, *nirK*, *narG* and *napA*.

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1. Introduction

The discharge of excessive organic matter and ammonium into water can cause serious ecological problems such as eutrophication, algae blooms and habitat degradation in lakes and rivers (Vörösmarty et al., 2010). More than 40% of the rivers in China fail to meet national standards for drinking water sources, being contaminated primarily by organic matter (chemical oxygen demand, COD) and ammonium (NH_4^+-N) according to monitoring data from recent years (Feng et al., 2012). Nevertheless, these rivers serve as China's main sources of drinking water. Thus, it is becoming increasingly important to pre-treat polluted water to remove excess NH_4^+-N.

Biofilters are engineered systems designed for NH_4^+-N removal from drinking water (Ji et al., 2013; Ryu and Lee, 2009; Van den

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http://dx.doi.org/10.1016/j.biortech.2015.07.055
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Akker et al., 2008). However, in practice, both domestic and industrial effluents released into rivers are subject to fluctuations in both quality and quantity, leading to variations in COD and NH_4^+-N loading. Due to the different responses of microorganisms associated with nitrogen cycling to such inconsistent loading, NH_4^+-N removal in biofilters fluctuates substantially, and the results of treatment are often unsatisfactory. Previous studies have showed that COD and NH_4^+-N loading significantly influence nitrogen transformation and removal processes in biofilters (Ji et al., 2011; Ling and Chen, 2005; Tan and Ji, 2010). Nitrogen removal in biofilters is often inhibited by increased organic carbon concentrations due to competition between autotrophic and heterotrophic nitrifying bacteria (Guerdat et al., 2011; Michaud et al., 2006). This competition between different microbial groups can result in a spatial distribution of microorganisms within the biofilm matrix that affects mass transfer processes and thus nitrification performance (Lee et al., 2004; Michaud et al., 2006). Several studies have reported the impacts of COD loading on nitrification processes and NH_4^+-N removal. Ji et al. (2011) investigated the impacts of COD loading on NH_4^+-N removal in the different portion of a biofilter (from top to bottom). NH_4^+-N removal slightly decreased following an increase in COD loading. Higher COD loading provided supplemental nutrients primarily to the autotrophic nitrifying bacteria in the upper portion of the biofilter, causing their rapid growth, to the detriment of heterotrophic nitrifying bacteria in the lower portions, regions characterized by fewer nutrients and reduced space for expansion; NH_4^+-N removal, therefore, was compromised. Guerdat et al. (2011) reported that NH_4^+-N removal rates exhibited substantial fluctuations with increased concentrations of organic carbon. Competition between the heterotrophic and autotrophic nitrifying bacterial communities for dissolved oxygen, media space, and nutrients reduced the NH_4^+-N removal performance of biofilters (Nogueira et al., 2002). Recently, Jeong et al. (2013) investigated the bacterial community structure in a biofilter (from top to bottom). Bacterial community structure showed a dynamic shift at the top parts of the biofilters, while relatively stable bacterial community structures were observed in the middle and bottom parts of biofilters. Wang et al. (2015) reported nitrogen transformation processes in a trickling filter. The results indicated that ammonia monooxygenase (*amoA*) and anammox bacterial 16S rRNA (anammox) were the key functional gene driving the major processes for NH_4^+-N. Although the importance of the effects of organic matter on nitrogen removal processes is widely accepted, no efforts have been made to investigate the detailed information about the underlying molecular mechanism that governs nitrogen transformation and removal, and very little is known about the ranked contribution of microbial genes associated with nitrogen cycling to long-term ammonium removal in biofilters. The lack of a quantitative link between nitrogen removal processes and nitrogen functional genes limits the ability to optimize treatment processes and to reliably predict long-term effluent quality.

This study presents as the first attempt to explore the operational adaptability/ feasibility for treating NH_4^+-N under varying COD loading and NH_4^+-N capacity loading, as well as to the ranked contribution of microbes in nitrogen removal processes. Specifically, The following four specific objectives were pursued: (1) to evaluate the long-term (245 days) treatment performance of COD and NH_4^+-N removal; (2) to determine the dynamic populations of microbial functional genes associated with nitrogen removal and identify the key functional genes that shape the nitrogen removal rates; (3) discern the primary nitrogen removal pathways and determine the quantitative link between nitrogen transformation processes and functional genes; and (4) quantify the ranked contribution of nitrogen functional genes to nitrogen transformation and removal.

2. Methods

2.1. Multimedia biofilter

A single laboratory-scale multimedia biofilter with working volumes of 144 L ($L \times W \times H = 40 \times 30 \times 240\ cm^3$) was built (see Fig. S1 in Supplementary information). The biofilter beds consisted of four treatment layers. The distance between the two treatment layers was 20 cm. A sieve tray was installed between each treatment layer. Forty-eight holes were evenly drilled into each sieve tray to allow for the wastewater/air contact. From top to bottom, the four treatment layers were filled with polyurethane foaming plastic of 5–8 mm apertures, lava rock of 2–5 mm in diameter, polyurethane foaming plastic of 3–5 mm apertures, and lava rock of 2–5 mm in diameter, respectively. The biofilter was fed with NH_4^+-N wastewater to investigate the long-term treatment performance and NH_4^+-N removal pathway. Synthetic wastewater was made from tap water (Beijing groundwater). The wastewater composition in each operation stage is summarized in Table 1. The organic loading rate varied between 8.3 and 34.7 g-COD/m^3 d, and the NH_4^+-N loading rate varied between 1.7 and 20.8 g-NH_4^+-N/m^3 d, depending on the operation phase. The hydraulic loading rate in all phases was maintained at 2.0 m^3/m^2 d. The biofilter was placed indoors, and influents and effluents ranged in temperature from 10.3 to 26.9 °C.

2.2. Sample collection and determination

Water samples were collected from the biofilter at least three times in each phase. 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 a HACH DR2800 instrument (HACH, USA). NH_4^+-N, NO_2^--N, and NO_3^--N were measured using a UV-1800 spectrophotometer (SHIMADZU, Japan). All variables were analyzed according to standard analytical procedures (APHA, 1995). The pH of the influent was measured by an 828 Orion pH meter. Dissolved oxygen (DO) and temperature of the solution were measured with a 52 YSI DO meter.

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)	6	8	10	15	20	25
NH_4^+-N (NH_4Cl, mg/L)	1.2	1.5	2	5	10	15
C/N ratio	5.0	5.3	5.0	3.0	2.0	1.7
TP (KH_2PO_4, mg/L)	0.2	0.2	0.3	0.4	0.5	0.6
Nutrient loading rate (g-N/m^2 d)	1.7	2.1	2.8	6.9	13.9	20.8
COD loading rate (g-COD/m^2 d)	8.3	11.1	13.9	20.8	27.8	34.7
Time (day)	0–98	99–133	134–161	162–189	190–217	218–245

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

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