



Autotrophic nitrogen removal process in a potable water treatment biofilter that simultaneously removes Mn and $\text{NH}_4^+\text{-N}$



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HIGHLIGHTS

- ANAMMOX bacteria were detectable in a biofilter for treating potable water.
- ANAMMOX bacteria could adapt to extreme low $\text{NH}_4^+\text{-N}$ concentration.
- Both CANON process and nitrification participated in $\text{NH}_4^+\text{-N}$ removal.
- The contributions of CANON to the low $\text{NH}_4^+\text{-N}$ removal could reach 48.5% and 46.6%.

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ABSTRACT

Ammonia ($\text{NH}_4^+\text{-N}$) removal pathways were investigated in a potable water treatment biofilter that simultaneously removes manganese (Mn) and $\text{NH}_4^+\text{-N}$. The results indicated a significant loss of nitrogen in the biofilter. Both the completely autotrophic nitrogen removal over nitrite (CANON) process and nitrification were more likely to contribute to $\text{NH}_4^+\text{-N}$ removal. Moreover, the model calculation results demonstrated that the CANON process contributed significantly to the removal of $\text{NH}_4^+\text{-N}$. For influent $\text{NH}_4^+\text{-N}$ levels of 1.030 and 1.749 mg/L, the CANON process contribution was about 48.5% and 46.6%, respectively. The most important finding was that anaerobic ammonia oxidation (ANAMMOX) bacteria were detectable in the biofilter. It is interesting that the CANON process was effective even for such low $\text{NH}_4^+\text{-N}$ concentrations.

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1. Introduction

Mn and $\text{NH}_4^+\text{-N}$ usually exist together in water in a dissolved state. In drinking water resources, the soluble Mn usually causes undesirable color, bad taste, the clogging of pipelines, and additional operational problems (Li et al., 2013). When $\text{NH}_4^+\text{-N}$ is present in the water, the disinfection process consumes a significant amount of chlorine (Pressley et al., 1972), which is especially problematic, as the disinfection process could also produce by-products. It is essential to remove these contaminants from drinking water, and the problems mentioned could be solved by using a biological water purification method.

Biotechnology has been widely applied worldwide for potable water treatment because extra chemical oxidizing agents are not needed, and, most important, these contaminants could be simultaneously removed by a one-stage biofilter (Gouzinis et al., 1998;

Han et al., 2013; Tekerlekopoulou and Vayenas, 2008). Recently, the technique of biologically removing Mn has become well known. In this technique, the soluble Mn is oxidized to insoluble metallic Mn oxides by microorganisms (Das et al., 2011). The carbon sources in drinking water are usually insufficient for the conversion of $\text{NH}_4^+\text{-N}$ to nitrogen gas by the conventional nitrification–denitrification process. Therefore, $\text{NH}_4^+\text{-N}$ is believed to be converted to nitrate by nitrification (Kihn et al., 2002; Reeves, 1972; van den Akker et al., 2008). In such an instance, the nitrogen in the form of nitrate will continue to exist in the water. However, for the simultaneous Mn and $\text{NH}_4^+\text{-N}$ removal potable water treatment biofilter, the operational results in the laboratory showed a significant nitrogen imbalance between the influent and the effluent of the biofilter. Although nitrogen could be used as a nutrient for bacterial growth (Yu et al., 2007), it could not cause such a remarkable loss of nitrogen. Therefore, in addition to the nitrification process, there had to be other nitrogen transformation paths in the biofilter, and it seems likely that the nitrogen loss was autotrophic.

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Currently, since the discovery of the anaerobic ammonia oxidation (ANAMMOX) bacteria, autotrophic nitrogen removal processes have received considerable attention (Strous et al., 1999; van der Star et al., 2007). In such processes, $\text{NH}_4^+\text{-N}$ could be converted to nitrogen gas by cooperation between the ammonia oxidizing bacteria and the ANAMMOX bacteria (Khin and Annachhatre, 2004), without any addition of a carbon source. However, almost all the research focusing on autotrophic nitrogen removal is related to wastewater containing high $\text{NH}_4^+\text{-N}$, while not much is being reported in the field of water supply on the autotrophic ANAMMOX bacteria.

Therefore, in this paper, the autotrophic removal nitrogen process was identified in accordance with the operational results of the biological Mn and $\text{NH}_4^+\text{-N}$ removal biofilter, and, in addition, the influence of the biofilter on the removal of $\text{NH}_4^+\text{-N}$ was calculated. Moreover, the functional ANAMMOX bacteria were also analyzed using polymerase chain reaction (PCR) amplification technology.

2. Methods

2.1. Device and synthetic water

A laboratory-scale biofilter was used to carry out a series of experiments, and the experimental equipment of the biofilter is shown in Fig. 1. A Plexiglas column with a diameter of 15 cm was used for the laboratory-scale biofilter. The supporting layer of cobblestone at the bottom of the biofilter was 5 cm high, and the particle size of the cobblestones was 0.5–1 cm. Quartz sand, with a particle size of 0.6–1.2 mm, was used as a filtration material, and the filter bed was 32 cm high. The other reactor components consisted of an influent tank, a backwashing tank, and two peristaltic pumps.

The influent water in this study was simulated by tap water to which the agents $\text{MnSO}_4\cdot\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, and NaNO_2 had been added. No microelements were added to the influent water since they were already present in the tap water. The water solution was prepared every other day. The water temperature, pH, and the dissolved oxygen (DO) were not controlled during the operational period of the biofilter. The tap water characteristics are shown in Table 1.

2.2. Biofilter operation

“Matured” quartz sand was used as a filtration material; therefore, when the biofilter started to function, it performed well in

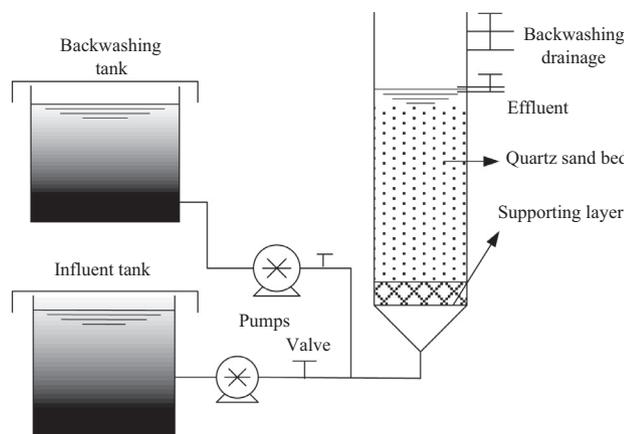


Fig. 1. Schematic diagram of the biofilter used in this study.

Table 1
Tap water characteristics in the experiments.

Parameters	Units	Tap water
Temperature	°C	17–23
Chromaticity	degree	<10
Turbidity	NTU	<1.0
pH		7.3–7.9
DO	mg/L	5–8
$\text{NH}_4^+\text{-N}$	mg/L	None
Nitrite ($\text{NO}_2^-\text{-N}$)	mg/L	None
Nitrate ($\text{NO}_3^-\text{-N}$)	mg/L	4.06–6.51
Total nitrogen (TN)	mg/L	4.19–6.65
COD_{Mn}	mg/L	<1.5

removing Mn and $\text{NH}_4^+\text{-N}$. Additionally, the change in the color of the quartz sand from white to gray was an indication of a “mature” biofilter (see supplementary material, Fig. S1). In order to keep the water flow constant, upflow filtration was used. The value of the empty bed contact time (EBCT, i.e., the time needed for the water to fill the empty column) was twenty minutes. The backwashing procedure was carried out once a week to wash out metallic oxides and excess organisms.

2.3. Analytical methods

Mn and nitrogen were detected by spectrophotometry in accordance with the standard methods (SEPA, 2002), while DO measurements were done using an electrode (pH/oxi340i, WTW). Since the major portion of the nitrogen in the tap water was in the $\text{NO}_3^-\text{-N}$ form, the following equations were referenced to assess the loss of nitrogen in this paper:

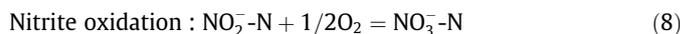
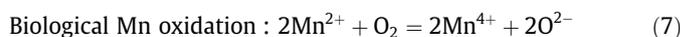
$$\text{TN}_{\text{influent}} = \text{NH}_4^+\text{-N}_{\text{influent}} + \text{NO}_2^-\text{-N}_{\text{influent}} + \text{NO}_3^-\text{-N}_{\text{influent}} \quad (1)$$

$$\text{TN}_{\text{effluent}} = \text{NH}_4^+\text{-N}_{\text{effluent}} + \text{NO}_2^-\text{-N}_{\text{effluent}} + \text{NO}_3^-\text{-N}_{\text{effluent}} \quad (2)$$

$$\text{TN loss} = \text{TN}_{\text{influent}} - \text{TN}_{\text{effluent}} \quad (3)$$

$$\begin{aligned} \text{Actual nitrate production(ANP)} \\ = \text{NO}_3^-\text{-N}_{\text{effluent}} - \text{NO}_3^-\text{-N}_{\text{influent}} \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Theoretical nitrate production(TNP)} \\ = \text{NH}_4^+\text{-N}_{\text{influent}} + \text{NO}_2^-\text{-N}_{\text{influent}} \end{aligned} \quad (5)$$



According to Eqs. (6)–(8), about 0.29, 4.57, and 1.14 mg DO are needed per mg of Mn, $\text{NH}_4^+\text{-N}$, and $\text{NO}_2^-\text{-N}$, respectively.

Therefore,

$$\text{Actual DO consumption(ADOC)} = \text{DO}_{\text{influent}} - \text{DO}_{\text{effluent}} \quad (9)$$

$$\begin{aligned} \text{Theoretical DO consumption(TDOC)} \\ = 0.29\text{Mn}_{\text{influent}}^{2+} + 4.57\text{NH}_4^+\text{-N}_{\text{influent}} + 1.14\text{NO}_2^-\text{-N}_{\text{influent}} \end{aligned} \quad (10)$$

2.4. Bacterial DNA extraction and PCR amplification

Bacterial genomic DNA was extracted from a wet sand sample of 5 g by a soil biological genomic extraction kit (Sangon, China), in accordance with the manufacturer’s protocol, and the products were then detected by 0.8% (w/v) agarose gel electrophoresis.

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