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# Distribution and genetic diversity of microbial populations in the pilot-scale biofilter for simultaneous removal of ammonia, iron and manganese from real groundwater

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## HIGHLIGHTS

- Simultaneous removal of $NH_4^+$, $Fe^{2+}$ and $Mn^{2+}$ was achieved from real groundwater.
- AOB, NOB, AOA, IOB, IOA, MnOB were coexisted in the biofilter.
- Microbial populations varied along the biofilter depth.
- Microbial populations varied during the start-up process.
- Bacteria distribution could be the main factor to affect biofilter performance.

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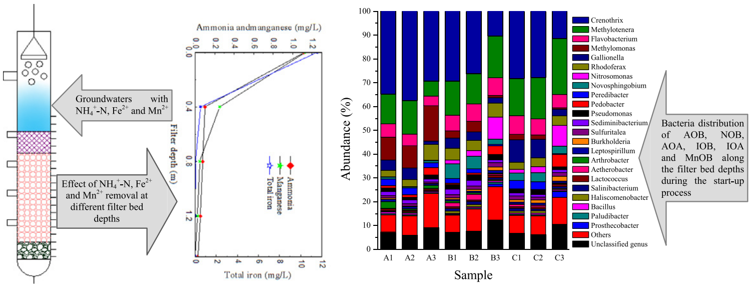
## GRAPHICAL ABSTRACT



## ABSTRACT

A pilot-scale biofilter treating real groundwater was developed in this study, which showed that ammonia, iron and manganese were mainly removed at 0.4, 0.4 and 0.8 m of the filter bed, respectively, and the corresponding removal efficiencies were 90.82%, 95.48% and 95.90% in steady phase, respectively. The variation of microbial populations in the biofilter during start-up process was also investigated using high-throughput pyrosequencing (HTP). Results indicated that the main functional microbes for ammonia, iron and manganese removal were *Nitrosomonas*, *Crenothrix* and *Crenothrix*, respectively, which was mainly distributed at 0.8, 0, and 0.8 m of the filter bed with a corresponding abundance of 8.7%, 28.12% and 11.33% in steady phase, respectively. Kinds of other bacteria which may be related to methane, hydrogen sulfide and organic matter removal, were also found. In addition, small part of archaea was also detected, such as *Candidatus Nitrososphaera*, which plays a role in nitritation.

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## 1. Introduction

Dissolved ammonia, ferrous iron and manganese exist widely in groundwater, especially in Northeast China (Qin et al., 2009; Cheng, 2016; Hasan et al., 2015). The presence of ammonia in drinking water treatment could affect the chlorination process (Cheng et al., 2017). Since ammonia would react with chlorine to form disinfection by-products (Richardson and Postigo, 2012; Du et al., 2017), which could produce deteriorate taste and odor of water (Cheng et al., 2016), reduce disinfection efficiency (Hasan et al., 2014), and damage human nervous system. In addition, ammonia can interfere with the manganese biofiltration process by consuming excessive oxygen during nitrification, resulted in mouldy and earthy tasting water (Hasan et al., 2014). The presence of iron and manganese can result in aesthetic and operational problems, such as giving water an undesirable color and odor, staining on laundry and accumulating in water distribution networks (Zeng et al., 2015). Therefore, the presence of ammonia, iron and manganese in drinking water should be avoided and the maximum contaminant levels (MCLs) for ammonia of 0.5 mg/L, total iron of 0.3 mg/L and manganese of 0.1 mg/L have been established in China (GB 5749-2006).

Chemical methods could be used to oxidize ammonia, iron and manganese, but it may produce potential hazardous by-products. Besides, it may also introduce other pollutants into the produced water (Cai et al., 2015). Thus, in the current circumstances, biological removal of ammonia, iron and manganese were emerged, and gradually replaced the conventional chemical treatments (Tekerlekopoulou et al., 2013). As demonstrated, simultaneous removal of ammonia, iron and manganese could be accomplished in biological systems (Han et al., 2013; Hasan et al., 2012), where iron and manganese removal through biological oxidation could be achieved by iron oxidizing bacteria (IOB) and manganese oxidizing bacteria (MnOB), respectively. Meanwhile, several groups of bacteria have been confirmed as IOB (*Gallionella*, *Leptothrix*, *Bacillus* and *Leptothrix discophora* (Yang et al., 2014; Li et al., 2013)) and MnOB (*Leptothrix*, *Gallionella*, *Pseudomonas*, *Siderocapsa*, *Crenothrix*, *Hyphomicrobium* and *Metalloaenium* (Hasan et al., 2012; Tang et al., 2016; Mckee et al., 2016; Granger et al., 2014)). Biological ammonia oxidation is carried out by two different consecutive microbial processes, nitritification and nitratification, thus ammonia removal through biological oxidation could be finished by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Currently, several groups of bacteria have been confirmed as AOB and NOB, such as *Nitrosomonas europaea*, *Nitrosomonas halophila*, *Nitrosomonas mobilis*, *Nitrospira*, *Comamonas* and *Acinetobacter* (Hasan et al., 2012; Li et al., 2013).

Recently, the distribution and genetic diversity of the microorganisms in the biofilter for ammonia, iron and manganese removal were investigated by some researchers (Hasan et al., 2012; Li et al., 2013), however, the microbial community was studied only in steady phase or one operational condition. There were few reports regarding to the microbial community in different conditions and the relationship between the operational conditions and the microbial community in the biofilter. In addition, synthetic drinking water source with model chemicals, which was used in most reports, is definitely different from the real water resources; therefore, the microbial community in biological removal process from the two kinds of waters should be also different.

In this study, a pilot-scale biofilter was established for the simultaneous removal of ammonia, iron and manganese using anthracite and manganese sand as the media. Compared with other studies, real feed groundwater was selected to carry out this experiment. Approximately 112 d long-term operation was evaluated with respect to ammonia, iron and manganese removal, and the microbial community structures from different depths of filter layer in different phases of the start-up process were analyzed and compared using 454 HTP. The main objectives of this study were to gain a deep insight into bacterial diversity in the biofilter, and the relationship between the microbial community and the removal efficiencies of ammonia, iron and manganese during the start-up process.

## 2. Materials and methods

### 2.1. Description of biofilter system

A pilot-scale biofilter system was developed in a groundwater treatment plant (GWTP), which is located in Harbin city, P.R. China (Fig. 1). The biofilter consisted of a 147.19 L transparent rigid plexiglass column with an inner diameter of 250 mm and a height of 3000 mm in which a height of 1500 mm was packed with support materials. Along the height of the column there were 20 water sampling ports at 100 mm intervals, and 3 media sampling ports at 0, 400 and 800 mm from media top to bottom. During the long-term running of the biofilter, it was backwashed according to the water head loss and effluent turbidity.

To avoid the redox reaction occurred between $Fe^{2+}$ and $Mn^{4+}$ after backwashing, two kinds of new support materials with different density were packed in the biofilter: the upper part of the media (300 mm) was columnar anthracite with a mean diameter of 1 mm and a height of 5 mm, and the lower part (1200 mm) was manganese sand with a mean diameter of 0.8–1 mm.

Real groundwater, which was extracted from the wells with a depth of 40–50 m, in Harbin city, P.R. China, was used throughout this experiment. The concentration of total iron, manganese and ammonia in raw groundwater was about 8–13, 0.9–1.3 and 0.9–1.4 mg/L, respectively, and the temperature was about 8 °C (Table 1).

### 2.2. Start-up process

The raw groundwater was sprayed to the tank, and then pumped to the biofilter with a flow rate of 2 m/h (Fig. 1). It should be noted that backwashing water obtained from the GWTP was inoculated into the biofilter as inoculum and weak backwashing intensity (5–7 $L/(s \cdot m^2)$) was adopted to shorten the start-up period of the biofilter. When the concentration of ammonia, total iron and manganese in effluent decreased to 0.1, 0.3 and 0.1 mg/L, respectively, the flow rate was promoted to 3 m/h and then promoted to 4 and 6 m/h in the same way. And the backwashing intensity increased to 12 $L/(s \cdot m^2)$, when the flow rate increased to 6 m/h. It was important to note that the concentration of ammonia, total iron and manganese was measured from the 6th day.

### 2.3. Sampling and analytical procedure

Water samples from the inlet and outlet were performed every day, and water samples along the height of the filter bed were performed twice a week. Ammonia, total iron and manganese in the water samples were measured by photometric method, according to standard methods for water and wastewater examination (Method NOs.: 4500-$NH_3$.B&C, 3500-Fe.B and 3500-Mn.B, respectively) (Li et al., 2013). The pH, dissolved oxygen (DO) and oxidation reduction potential (ORP) measurements were measured using a pH meter (pH 315i-WTW), a DO meter (Oxi 315i-WTW) and a ORP meter (pH 315i-WTW), respectively. The filter media (anthracite or manganese sands) were collected at three depths of the filter bed (0, 400 and 800 mm), and stored at −80 °C for further analysis. In addition, scanning electron microscopy (SEM, JSM-