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Molecular characterization of microbial populations in full-scale biofilters treating iron, manganese and ammonia containing groundwater in Harbin, China

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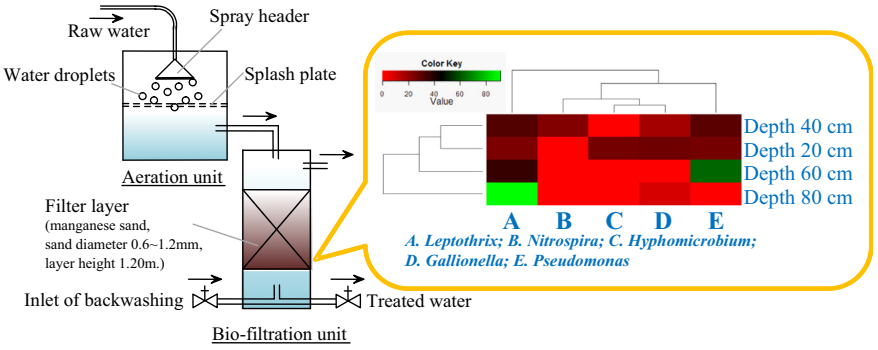
HIGHLIGHTS

- Simultaneous removal of Fe^{2+}, Mn^{2+} and NH_4^+ was achieved in a full-scale biofilter.
- IOB, MOB and NOB dominated in the biofilter.
- AOA instead of AOB was responsible for the oxidation of NH_4^+.
- Microbial populations varied along the depth of the biofilter.

GRAPHICAL ABSTRACT



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ABSTRACT

In iron and manganese-containing groundwater treatment for drinking water production, biological filter is an effective process to remove such pollutants. Until now the exact microbial mechanism of iron and manganese removal, especially coupled with other pollutants, such as ammonia, has not been clearly understood. To assess this issue, the performance of a full-scale biofilter located in Harbin, China was monitored over four months. Microbial populations in the biofilter were investigated using T-RFLP and clone library technique. Results suggested that *Gallionella*, *Leptothrix*, *Nitrospira*, *Hyphomicrobium* and *Pseudomonas* are dominant in the biofilter and play major roles in the removal of iron, manganese and ammonia. The spatial distribution of microbial populations along the depth of the biofilter demonstrated the stratification of the removal of iron, manganese and ammonia. Additionally, the absence of ammonia-oxidizing bacteria in the biofilter implicated that ammonia-oxidizing archaea might be responsible for the oxidation of ammonia to nitrite.

1. Introduction

Dissolved iron (Fe^{2+}) and manganese (Mn^{2+}) exist widely in groundwater, especially in Northeast China (Qin et al., 2009). This can result in aesthetic and operational problems such as undesirable color, odor and staining of laundry when the groundwater is intended for potable water (Sharma et al., 2005). Therefore, the presence of iron and manganese in drinking water should be avoided and the maximum contaminant levels (MCLs) for Fe^{2+} of 0.3 mg/L and Mn^{2+} of 0.1 mg/L have been established in China (GB 5749-2006).

Physicochemical treatment methods such as aeration and filtration have been used to remove Fe^{2+} and Mn^{2+} for a long time (Ellis et al., 2000). However, they always fail to remove manganese while obtain high removal efficiency for iron, mostly because abiotic

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homogenous manganese oxidation by oxygen is very slow at pH values below 9 (Stumm and Morgan, 1996). Afterwards, chemical oxidants such as $KMnO_4$ and Cl_2 were introduced to achieve high removal of manganese (Knocke et al., 1991). Nevertheless, the use of chemical reagents not only causes the increase of operating costs, but also generates potentially hazardous by-products (Gallard and von Gunten, 2002).

In the circumstances, the biological removal of iron and manganese emerged and gradually replaced the conventional physico-chemical treatments (Mouchet, 1992; Pacini et al., 2005; Tekerlekopoulou et al., 2013). The removal process involves passing aerated water vertically through a filter containing Mn-oxidizing bacteria (MOB). So far, several groups of bacteria have been confirmed as MOB, such as *Leptothix*, *Gallionella*, *Siderocapsa*, *Crenothix*, *Hyphomicrobium* and *Metalloaenium* (Abu Hasan et al., 2012; Das et al., 2011; Katsoyiannis and Zouboulis, 2004b; Tebo et al., 2005). However, the characteristics of groundwater containing iron and manganese vary greatly from site to site, such as low iron and high manganese in Shenyang (Liaoning Province, China), high iron and high manganese coupled with ammonia in Harbin (Heilongjiang Province, China) and so on. So there is an urgent need to improve the biological filtration technology to satisfy different processing demands.

Up to now, most studies related to biological manganese oxidation have been focused on lab-scale experiment (Burger et al., 2008; Han et al., 2013; Hope and Bott, 2004; Pacini et al., 2005), while detailed information about full-scale application, especially microbial ecology of full-scale biofilters, has been rarely reported. So in this paper, a full-scale biofilter for removing Fe^{2+}, Mn^{2+} and NH_4^+, which is located in Harbin, China, was investigated and microbial community structures from different depths of filter layer were analyzed and compared using T-RFLP. The main objectives of this study were to gain a deep insight into bacterial diversity in the biofilter, and to provide a guidance for the optimization of biofilter.

2. Methods

2.1. Description of songbei water treatment plant

Songbei Water Treatment Plant (WTP), which is located in Songbei District (Harbin, China), has a water-production capacity of 40,000 m^3/d. The raw water of Songbei WTP is derived from underground water of the overburdened aquifer (the depth of 40–60 m). Characteristics of the groundwater are summarized in Table 1. The most salient feature of the raw water is high dissolved iron (~15 mg/L) and manganese (~1.2 mg/L), and slight ammonia contamination (~1.3 mg/L). In addition, the low temperature of the raw water (8 °C) makes the treatment more difficult.

Songbei WTP employs the "aeration-biofiltration" process to gain high removal efficiencies (see Fig. 1). The theoretical oxygen demand to completely oxidize Fe^{2+}, Mn^{2+} and NH_4^+ of the raw water is calculated as follows (Stumm and Morgan, 1996),

$$[O_2] = 0.14[Fe^{2+}] + 0.29[Mn^{2+}] + 4.57[NH_4^+] = 7.98 \text{ mg/L}.$$

The oxygen demand is met by the unit of spraying and dropping, which is designed with perforated pipes so that small water droplets are formed to get air dissolved. Subsequently, the aerated water flows through a down-flow filter at the rate of 5 m/h to remove Fe^{2+}, Mn^{2+} and NH_4^+ simultaneously. The filter is filled with manganese sands, which is the optimum packing according to previous study (Li et al., 2006). Backwashing is performed as needed when the filter becomes clogged.

Table 1
Physicochemical characteristics of the raw water from the deep wells of Songbei WTP, Harbin, China (the data were kindly provided by Songbei WTP).

Properties	Value	Properties	Value (mg/L)
Temperature	8.0 °C	SO_4^{2-}	5.5
Turbidity	<2.5 NTU	F^-	0.54
Color	19~21°	Cl^-	2.04
pH	~6.9	NO_3^-	<0.12
Fe^{2+}	~15 mg/L	NH_4^+	~1.3
Mn^{2+}	~1.2 mg/L	COD_{Mn}	1.2~1.6 mg O_2/L
Total As	<0.002 mg/L	DO	~0.7 mg/L
Total hardness	165~175 mg $CaCO_3$/L	Alkalinity	218.96 mg $CaCO_3$/L

2.2. Sampling and analytical procedure

A filter was taken as study object. Dissolved iron, manganese and ammonia in water samples were measured by photometric method, according to standard methods for water and wastewater examination (Method NOs.: 3500-Fe.B, 3500-Mn.B and 4500-NH3.B&C, respectively) (Eaton et al., 2005). After the operation of the filter for four months, the filter media (manganese sands) was collected at four depths of the filter layer (20, 40, 60, 80 cm) using a self-made soil sampler, and stored at −20 °C for further analysis. Additionally, scanning electron microscopy analysis (SEM, JSM-5610) was used to observe the morphology of the microorganisms in backwashing sludge and matured manganese sands from the filter, according to previous studies (Katsoyiannis and Zouboulis, 2004a).

2.3. DNA extraction and PCR amplification

DNA was extracted from matured manganese sands (~0.25 g) taken from the filter using Powersoil® DNA Isolation Kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. 16S rRNA gene fragments of domain bacteria were PCR-amplified with bacterial primer set 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) (Weisburg et al., 1991), using a thermocycling program consisting of 5 min of denaturation at 95 °C and 30 cycles of 45 s at 95 °C, 45 s at 55 °C and 1 min at 72 °C, followed by an extension step at 72 °C for 10 min. PCR products were electrophoresed in 1.5% (w/v) agarose gel and recovered by EasyPure Quick Gel Extraction Kit (TransGen, China).

2.4. Cloning and sequencing of 16S rRNA gene

Purified PCR products were ligated into pMD18-T vectors (TaKaRa, Japan) and transformed into competent *Escherichia coli* DH5α (TaKaRa, Japan), as described in the manufacturer's protocol. Transformants were selected by ampicillin resistance and blue–white screening was performed to identify clones with inserts. Thirty white colonies were randomly selected for further analysis. The inserts were confirmed by colony PCR, and then plasmids containing the target gene were extracted using EasyPure Plasmid MiniPrep Kit (TransGen, China).

16S rRNA gene inserts (~1450 bp) were sequenced using an ABI 3730XL Genetic Analyzer (Applied Biosystems, USA) at Sangon Biotech (Shanghai, China). Sequence data were processed using the pipeline available on the Greengenes (http://www.greengenes.lbl.gov) (DeSantis et al., 2006) and also compared with the GeneBank database sequences (http://www.ncbi.nlm.nih.gov) using nucleotide BLAST (Altschul et al., 1990). Sequences with more than 97% sequence similarity were grouped into the same operational taxonomic unit (OTU) by MOTHUR (Schloss et al., 2009). The sequences of each representing OTU and closely related bacterial species were aligned with SINA (Pruesse et al., 2012). A phylogenetic tree was