



Performance and microbial community profiles in pilot-scale biofilter for ammonia, iron and manganese removal at different dissolved oxygen concentrations

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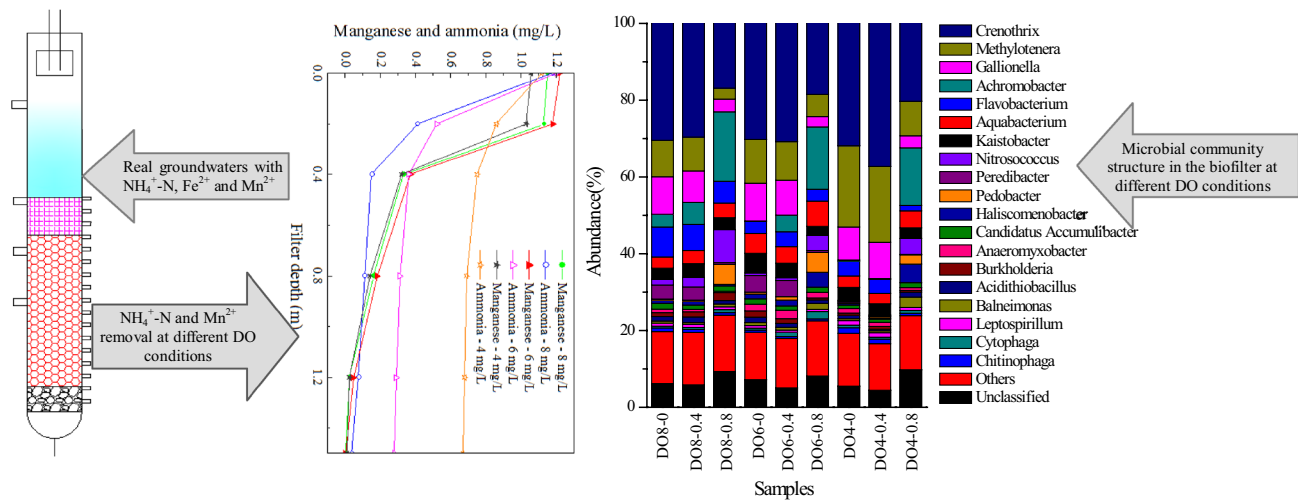
Abstract

Dissolved oxygen (DO) is a significant operational parameter in biological systems. In this study, a pilot-scale biofilter was constructed to investigate the removal efficiency of ammonia, iron and manganese, as well as the microbial community structure evolution at different DO concentrations. Results indicated that when DO decreased from 8 to 4 mg/L, iron and manganese were still completely removed, however the concentration of ammonia in the effluent increased, and exceeded the permitted limit of 0.5 mg/L when DO was about 4 mg/L. The main functional microbes for ammonia and manganese removal were *Nitrosomonas* and *Crenothrix*, which was mainly distributed at 0.8 and 0.8 m of the filter bed with a corresponding abundance of 8.61% and 16.87% in sufficient DO condition, respectively; while iron was mainly removed by *Crenothrix* and *Gallionella* in 0 m with a corresponding abundance of 30.45% and 9.77%. With the decreasing of DO concentration, iron oxidizing bacteria (IOB, *Crenothrix* and *Gallionella*) was not affected, while the abundance of manganese oxidizing bacteria (MnOB, *Crenothrix*) increased to completely oxidize manganese. However, the amount of ammonia oxidizing bacteria (AOB, *Nitrosococcus*) at 0.4 and 0.8 m of the filter depth obviously decreased with increased ammonia in the effluent. 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* and *Ferroplasma*, which could oxidize ammonia and ferrous iron, respectively.

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Extended author information available on the last page of the article

Graphical Abstract



Keywords Ammonia, iron and manganese removal · Biofilter · Microbial community · Dissolved oxygen · High-throughput sequencing

Introduction

Ferrous iron and bivalent manganese associated with ammonia are widely exist in groundwater, especially in Northeast China (Qin et al. 2009; Cheng 2016; Zeng et al. 2015). The presence of iron and manganese in drinking water treatment could result in aesthetic and operational problems, such as staining on laundry and sanitary facilities, giving water an unpleasant metallic taste, and reducing pipe diameter when iron and manganese deposits build up in pipelines (Zeng et al. 2015). Meanwhile, ammonia present in drinking water treatment could affect the chlorination process, since ammonia could react with chlorine to form disinfection by-products (Hasan et al. 2013; Richardson and Postigo 2012; Du et al. 2017), which could produce deteriorate taste and odor of water (Cheng et al. 2017a, b), reduce disinfection efficiency (Hasan et al. 2014), and damage human nervous system (Cheng et al. 2017a). Furthermore, ammonia would interfere with the manganese biofiltration process by consuming excessive oxygen during nitrification stage, resulted in mouldy and earthy tasting water (Hasan et al. 2014). Therefore, the presence of iron, manganese and ammonia in drinking water should be limited and the maximum contaminant levels (MCLs) for total iron of 0.3 mg/L, manganese of 0.1 mg/L and ammonia of 0.5 mg/L have been established in China (GB 5749-2006).

Biological removal of ammonia, iron and manganese is preferable compared with chemical methods, since it is not necessary to add extra chemicals which may introduce other pollutants into the produced water and produce potential

hazardous by-products (Cai et al. 2015; Tekerlekopoulou et al. 2013). As demonstrated, ammonia, iron and manganese could be simultaneously removed in biological systems (Tekerlekopoulou and Vayenas 2008; Hasan et al. 2012). Biological ammonia oxidation could be achieved by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), such as *Nitrosomonas halophila*, *Nitrosomonas europaea*, *Comamonas*, *Nitrospira*, *Nitrosomonas mobilis* and *Acinetobacter* (Hasan et al. 2012; Li et al. 2013). Iron and manganese could be biologically oxidized by iron oxidizing bacteria (IOB) and manganese oxidizing bacteria (MnOB), respectively. So far, several types of bacteria have been confirmed as IOB [*Crenothrix*, *Leptothrix discophora*, *Leptothrix*, *Gallionella* and *Bacillus* (Li et al. 2013; Yang et al. 2014)], and MnOB (*Leptothrix*, *Gallionella*, *Hyphomicrobium*, *Pseudomonas*, *Crenothrix*, *Siderocapsa* and *Metalloaenium* (Hasan et al. 2012; Tang et al. 2016; Mckee et al. 2016; Granger et al. 2014)).

Dissolved oxygen is a significant operational parameter in biological systems, since it was utilized to oxidize ammonia, iron and manganese. Moreover, energy is needed in general when oxygen dissolves in water, so the higher DO in water, the higher energy consumption and operational cost (Cheng 2016). In recent years, researches had been conducted to investigate the ammonia, iron and manganese removal from different perspectives, such as interactions among ammonia, iron and manganese removal (Tekerlekopoulou and Vayenas 2007), reaction dynamics of biological ammonia, iron and manganese removal (Tekerlekopoulou and Vayenas 2008), and the microbial community profiles of the microorganisms

in the biofilter for iron, ammonia and manganese removal (Li et al. 2013). It should be noted that DO in their biological systems was sufficient for ammonia, iron and manganese oxidation, or even 7–8 mg/L. However, few researchers focused on the removal efficiency of ammonia, iron and manganese, as well as the distribution and genetic diversity of the microorganisms in biofilter when DO was insufficient.

In this study, a pilot-scale biofilter system was constructed for ammonia, iron and manganese removal from real groundwater. The biofilter was operated approximately 160 days to evaluate removal efficiency of ammonia, iron and manganese, and the microbial community structures at different DO concentrations were analyzed and compared using 454 HTP (DO in the influent was about 8, 6 and 4 mg/L, respectively, and theoretical oxygen demand to completely oxidize Fe^{2+} , Mn^{2+} and NH_4^+-N in raw water is calculated as follows (Li et al. 2013), $[\text{O}_2] = 0.14[\text{Fe}^{2+}] + 0.29[\text{Mn}^{2+}] + 4.57[\text{NH}_4^+-\text{N}] = 7.23 \text{ mg/L}$, where the concentration of Fe^{2+} , Mn^{2+} and NH_4^+-N was calculated as average concentration in the formula). The work herein aimed at gain to a deep insight into the relationship between the microbial community structures and the removal efficiency of the pollutants, which would provide significant guidance for optimization of operation parameters for biofilter to reduce energy consumption and operational cost.

Materials and methods

Description of biofilter system

A pilot-scale biofilter system employed to simultaneously remove ammonia, iron and manganese was established in a groundwater treatment plant (GWTP) located in Harbin city, P.R. China (Fig. 1). The biofilter consisted of a plexiglass column with a height of 3000 mm and an inner diameter of 250 mm. A mixing chamber used to mix the incoming waters was set at the top of the biofilter, and a perforated plate used to support the media and collect the treated water was located at the bottom. 3 media sampling ports at 0, 400 and 800 mm and 17 water sampling ports at 100 mm intervals were distributed along the height of the column from media top to bottom. Tanks 1 (volume 1000 L) and 2 (volume 50 L) were used to collect raw groundwater, which was obtained from the GWTP. The raw groundwater was sprayed to tank 1, and then aerated to achieve the concentration of DO at about 8 mg/L; while DO in tank 2 was about 0.2 mg/L.

Two kinds of support materials were packed in the biofilter, and the upper part of the media (300 mm) was columnar anthracite with a height of 5 mm and a mean diameter of 1 mm, while the lower part (1200 mm) was manganese sand with a mean diameter of 0.8–1 mm. Down-flow pattern was

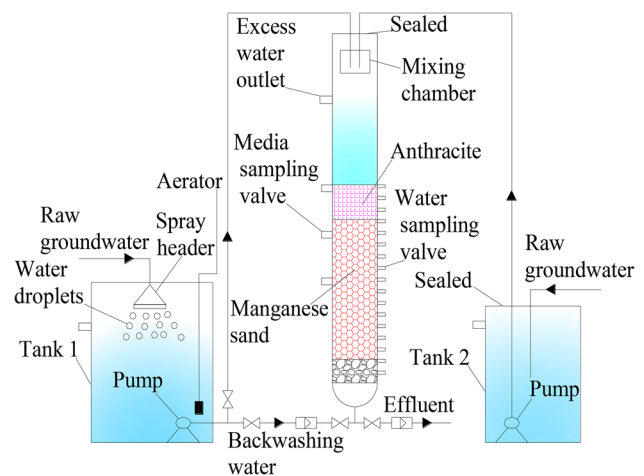


Fig. 1 Schematic drawing of the pilot-scale biofilter system

adopted, and the amount of flow was controlled at the entry point (Cheng 2016). In addition, the biofilter was operated about 8 months before this experiment. In this experiment, real groundwater extracted from the wells with a depth of 40–50 m, in Harbin city, P.R. China was used. The concentration of ammonia, total iron and manganese in raw groundwater was about 0.9–1.4, 6–14 and 0.9–1.4 mg/L, respectively, and the temperature was 7.8 °C.

Operation of the biofilter system

Water in tank 1 was pumped to the biofilter to investigate the removal efficiency of ammonia, iron and manganese at sufficient DO condition. About a month later, the waters in tanks 1 and 2 were both pumped into the biofilter with a suitable proportion to ensure DO in the influent was about 6 and 4 mg/L, respectively. The flow rate of the water in tank 2 was almost constant, because DO was very low and few ferrous irons were oxidized to iron hydroxides, which could reduce the pipe diameter and flow rate. Therefore, the flow rate of the water in tank 1 was regulated to ensure the proportion of the waters in tanks 1 and 2 was suitable. The biofilter was operated about 2 months when DO in the influent was about 6 or 4 mg/L. The flow rate of the biofilter was fixed at 4 m/h in this experiment because of high iron concentration in the influent.

Sampling and analytical procedure

Analysis of DO and pH were conducted using a DO meter (Oxi 315i-WTW) and a pH meter (pH 315i-WTW), respectively. Ammonia, iron and manganese concentration was measured by photometric method according to Standard Methods for the Examination of Water and Wastewater (Rittmann and Snoeyink 1984). The filter media (manganese

sand or anthracite) were collected at 0, 400 and 800 mm of the filter bed, and stored at -80°C for further analysis. Furthermore, the microorganisms in mature media from the biofilter (day 156) was examined by scanning electron microscopy (SEM, JSM-6480LV) according to previous studies (Tang et al. 2016).

Microbial diversity analysis

DNA extraction and polymerase chain reaction (PCR) amplification

Media samples from 0, 400 and 800 mm of the filter layer were explored to deeply understand the microbial community structure evolution at different DO concentrations. These samples are analyzed by 454 HTP at days 35 (DO8-0, DO8-0.4 and DO8-0.8), 95 (DO6-0, DO6-0.4 and DO6-0.8) and 156 (DO4-0, DO4-0.4 and DO4-0.8). DNA was extracted from the media samples by Powersoil DNA Isolation Kit (MoBio Laboratories Inc, USA). The quality of the DNA was examined by 1% (w/v) agarose gel electrophoresis and the concentration was measured with a UV-Vis spectrophotometer (NanoDrop 2000, USA) (Li et al. 2013). The V3-V4 region of the 16S rRNA gene was amplified using bacterial primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3'). PCR amplifications were carried out in a GeneAmp 9700 thermocycler (ABI, USA) according to previous research (Cheng et al. 2017b).

454 high-throughput 16S rRNA gene pyrosequencing

Sequencing of the DNA samples was carried out using the same method as previous research (Cheng et al. 2017b). Raw sequence data of this study was deposited to the NCBI Sequence Read Archive with accession No. SRP095713.

Biodiversity analysis and phylogenetic classification

Low quality reads (ambiguous nucleotides and quality value < 20) were removed from the raw sequence data as described in (Caporaso et al. 2011). The paired-end reads from the samples were overlapped to assembly V3-V4 tags of 16S rRNA gene using SeqPrep (<https://github.com/jstjohn/SeqPrep>), and chimera sequences from the tags were removed by usearch6. Eventually, the numbers of high quality sequences were 11, 430 (DO8-0), 11, 691 (DO8-0.4), 11, 123 (DO8-0.8), 15, 512 (DO6-0), 14, 181 (DO6-0.4), 22, 134 (DO6-0.8), 12, 793 (DO4-0), 5, 427 (DO4-0.4) and 16, 520 (DO4-0.8) with an average length of 291 bp. The sequences were processed using the same method as previous research (Cheng et al. 2017b).

When DO in the influent was sufficient (about 8 mg/L), ammonia, total iron and manganese in the effluent was lower than 0.1, 0.1 and 0.05 mg/L (Table 1), and the corresponding removal efficiencies were 97.2%, 98.7% and 99.6%, respectively. When DO in the influent was reduced to about 6 and 4 mg/L, ammonia in the effluent obviously increased to 0.2 and 0.6 mg/L, respectively, which exceeded the MCL. However, removal efficiency of iron and manganese was not affected by decreased DO concentrations.

Results

Overall performance of the biofilter and SEM images

When DO in the influent was sufficient (about 8 mg/L), ammonia, total iron and manganese in the effluent was lower than 0.1, 0.1 and 0.05 mg/L (Table 1), and the corresponding removal efficiencies were 97.2%, 98.7% and 99.6%, respectively. When DO in the influent was reduced to about 6 and 4 mg/L, ammonia in the effluent obviously increased to 0.2 and 0.6 mg/L, respectively, which exceeded the MCL. However, removal efficiency of iron and manganese was not affected by decreased DO concentrations.

Iron and ammonia were removed from the top of the filter depth, and total iron and ammonia decreased to 0.13 and 0.11 mg/L in 0.2 and 0.8 m of the filter depth (Table 2), respectively. While manganese was removed from 0.2 m of the filter depth, and decreased to 0.024 mg/L in 1.2 m. When DO was reduced to about 6 mg/L, iron and manganese were mainly removed in 0–0.2 and 0.2–0.8 m, respectively. Ammonia was quickly oxidized in 0–0.2 m, oxidized in 0.2–0.8 m, and slowly oxidized in 0.8–1.5 m, since DO was only 0.43 mg/L in 0.8 m. When DO was reduced to about 4 mg/L, the pollutants along the filter depth was varied in the same way.

SEM images of the mature media were examined to verify the existence and growth of bacteria in the biofilter. Plenty of microorganisms were observed in the micrograph, and the surfaces of these bacteria were covered by large amounts of iron and manganese oxides (Fig. S1). Two species of microorganisms, which had a very typical structure of twisted stalk and a rod-shaped, were observed.

Table 1 Effect of biofilter on total iron, manganese and ammonia removal for the DO in influent of about 8, 6 and 4 mg/L, respectively

Pollutants	Influent (mg/L)	Effluent (mg/L)			Removal efficiencies (%)		
		DO 8	DO 6	DO 4	DO 8	DO 6	DO 4
Total Fe	6.48–13.38	0.0046–0.097	0.0046–0.082	0.0046–0.10	98.7	98.4	98.9
Mn ²⁺	0.93–1.31	0.0012–0.024	0.0012–0.046	0.0012–0.046	99.6	99.6	99.4
NH ₄ ⁺ -N	0.94–1.38	0.0044–0.075	0.16–0.29	0.58–0.67	97.2	78.3	47.9

Table 2 Total iron, manganese, ammonia and DO concentration profiles along the filter depth at different DO conditions (about 8, 6 and 4 mg/L, respectively)

Filter Depth (m)	Effluent (mg/L)											
	Day 35				Day 95				Day 156			
	Total Fe	Mn ²⁺	NH ₄ ⁺ -N	DO	Total Fe	Mn ²⁺	NH ₄ ⁺ -N	DO	Total Fe	Mn ²⁺	NH ₄ ⁺ -N	DO
0	10.68	1.15	1.18	8.32	10.79	1.12	1.20	6.42	10.74	1.16	1.17	4.38
0.2	0.13	1.13	0.41	2.12	0.12	1.08	0.52	1.72	0.14	1.13	0.86	1.42
0.4	0.093	0.33	0.15	1.29	0.10	0.31	0.36	0.71	0.12	0.32	0.75	0.56
0.8	0.039	0.16	0.11	0.99	0.043	0.15	0.31	0.43	0.063	0.14	0.69	0.21
1.2	0.019	0.024	0.075	0.72	0.029	0.046	0.29	0.26	0.021	0.024	0.68	0.16
1.5	0.0095	0.0012	0.037	0.51	0.014	0.0012	0.28	0.16	0.010	0.012	0.67	0.13

Table 3 Richness and diversity estimators of the bacteria phylotypes in the biofilter

Sample ID	0.03 distance			
	OTU	Chao1 richness estimation	Shannon diversity index	Good's coverage
DO8-0	1021	1929	7.05	0.94
DO8-0.4	1118	2554	7.15	0.93
DO8-0.8	1150	2079	7.16	0.94
DO6-0	972	1715	6.96	0.96
DO6-0.4	984	1921	7.05	0.96
DO6-0.8	1674	2411	8.54	0.97
DO4-0	1037	1945	6.58	0.95
DO4-0.4	613	1775	6.04	0.91
DO4-0.8	1487	2351	8.29	0.95

Richness and diversity of microbial community

The parameters related to the alpha diversity of microbial community for the nine samples at distance cutoff level of 0.03 were shown in Table 3. 1, 021 (DO8-0), 1, 118 (DO8-0.4), 1, 150 (DO8-0.8), 972 (DO6-0), 984 (DO6-0.4), 1, 674 (DO6-0.8), 1, 037 (DO4-0), 613 (DO4-0.4) and 1, 487 (DO4-0.8) OTUs were clustered at a 3% distance. The total number of OTUs estimated by Chao1 estimator were 1, 929 (DO8-0), 2, 554 (DO8-0.4), 2, 079 (DO8-0.8), 1, 715 (DO6-0), 1, 921 (DO6-0.4), 2, 411 (DO6-0.8), 1, 945 (DO4-0), 1, 775 (DO4-0.4) and 2, 351 (DO4-0.8) with infinite sampling, indicating that DO8-0.4 had the greatest richness and DO6-0 had the lowest richness. The pyrosequencing of the nine samples revealed that the bacterial communities were obviously different along the filter depths at different phases. The Shannon diversity index provides not only the simply species richness, but also how the abundance of each species is distributed among all the species in the community. DO6-0.8 had the highest

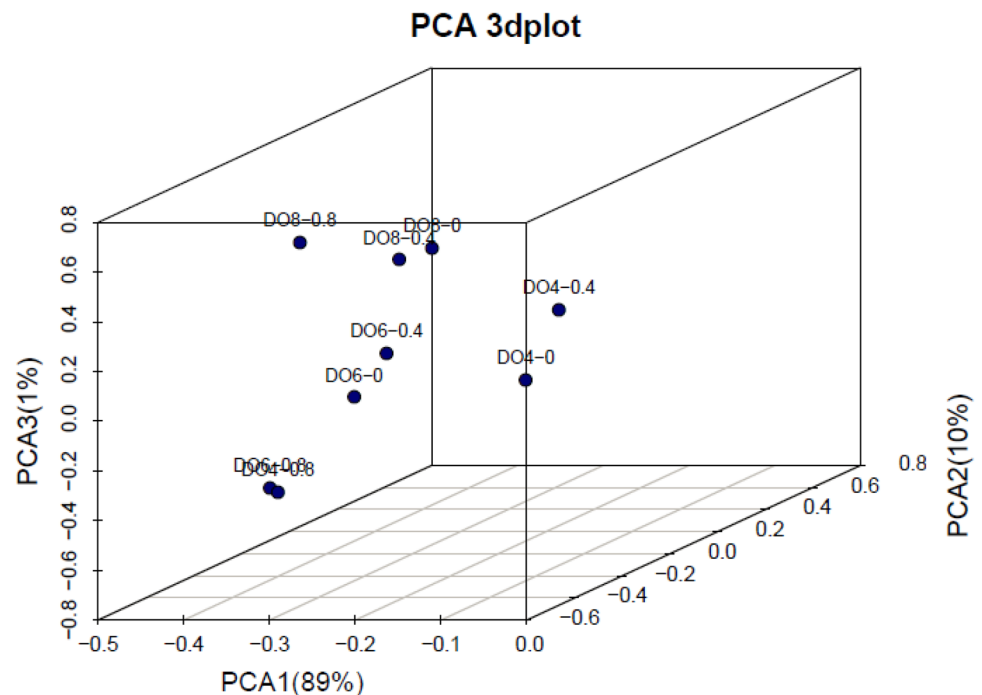
diversity (Shannon = 8.54) among the nine communities, while DO4-0.4 had the lowest diversity (Shannon = 6.04).

Principal component analysis (PCA) is one of the most commonly used tools in the analysis of ecological data. This method reduces the effective dimensionality of a multivariate data set by producing linear combinations of the original variables (i.e., components) that summarize the predominant patterns in the data. Therefore, PCA was used to analyze the relationship between the DO concentrations and microbial community structure, the results indicated that DO obviously affected the microbial community structure (Fig. 2). The bacterial communities in DO8-0.8 were obviously different from the others, but the bacterial communities in DO4-0.8 and DO6-0.8 were similar.

Taxonomic complexity of the bacterial community

The phylogenetic diversity of bacterial communities from the nine samples at phylum level is shown in Fig. 3a, demonstrating a very high diversity, reflected in the fact that 32 (DO8-0), 31 (DO8-0.4), 29 (DO8-0.8), 31 (DO6-0), 29 (DO6-0.4), 38 (DO6-0.8), 30 (DO4-0), 27 (DO4-0.4) and 39 (DO4-0.8) identified bacterial phyla were detected. 43 identified bacterial phyla and 3 identified archaea phyla in total were observed. Even so, 1.31% (DO8-0), 1.18% (DO8-0.4), 2.91% (DO8-0.8), 1.51% (DO6-0), 2.84% (DO6-0.4), 3.78% (DO6-0.8), 1.79% (DO4-0), 1.45% (DO4-0.4) and 2.92% (DO4-0.8) of the total reads in each sample were not classified, indicating these bacteria are unknown. *Proteobacteria* which existed widely in biofilters treated groundwater containing iron and manganese (Katsoyiannis and Zouboulis 2004), was the dominant bacterial phyla, and accounted for 76.32% (DO8-0), 78.20% (DO8-0.4), 67.99% (DO8-0.8), 84.59% (DO6-0), 79.64% (DO6-0.4), 49.16% (DO6-0.8), 80.46% (DO4-0), 82.74% (DO4-0.4) and 54.85% (DO4-0.8) of the total reads. *Nitrospirae* which involved in nitrification (Lu et al. 2012), existed in the nine communities, and was highest in abundance in DO4-0.8 (1.86%).

Fig. 2 PCA of relationship between the DO concentrations and microbial community structure based on OTU in the nine samples



The class level identification of the bacterial communities in the nine samples was illustrated in Fig. 3b. 108 bacterial classes and 5 archaea classes were detected in the nine communities, and 71, 65, 71, 71, 72, 94, 72, 54 and 90 bacterial classes were detected in DO8-0, DO8-0.4, DO8-0.8, DO6-0, DO6-0.4, DO6-0.8, DO4-0, DO4-0.4 and DO4-0.8, respectively. *Betaproteobacteria* and *Gammaproteobacteria* were the dominant bacterial community in all nine communities, and the sum of these two classes accounted for 66.61% (DO8-0), 68.05% (DO8-0.4), 59.37% (DO8-0.8), 69.25% (DO6-0), 65.25% (DO6-0.4), 38.43% (DO6-0.8), 73.05% (DO4-0), 75.91% (DO4-0.4) and 44.07% (DO4-0.8) of the total reads.

At the genus level, 6.19% (DO8-0), 5.82% (DO8-0.4), 9.34% (DO8-0.8), 7.22% (DO6-0), 5.05% (DO6-0.4), 8.15% (DO6-0.8), 5.50% (DO4-0), 4.46% (DO4-0.4) and 9.79% (DO4-0.8) of the total reads in each sample were not classified (Fig. 3c). The dominant population in DO8-0 (30.45%), DO8-0.4 (29.60%), DO6-0 (30.21%), DO6-0.4 (30.76%), DO6-0.8 (18.50%), DO4-0 (31.83%), DO4-0.4 (37.24%) and DO4-0.8 (20.30%) was *Crenothrix*, which could oxidize not only iron but also manganese; while the dominant population in DO8-0.8 (18.14%) was *Achromobacter*, which could oxidize organic matter (Lasík et al. 1993). Five kinds of IOB were found in the biofilter, including *Crenothrix*, *Gallionella*, *Leptospirillum*, *Bacillus* and *Pseudomonas*, and six kinds of MnOB were found, including *Crenothrix*, *Bacillus*, *Gallionella*, *Pseudomonas*, *Arthrobacter* and *Hyphomicrobium*. The appearance of AOB (*Nitrosomonas*, *Nitrosococcus* and *Nitrosovibrio*) and NOB (*Nitrospira*) demonstrated

that nitrification and nitrification process occurred in the biofilter to remove ammonia and nitrite, respectively (Lu et al. 2012; Li et al. 2017). In addition, several kinds of other genera were present in the biofilter, for example, *Methylotenera* and *Methylomonas* may oxidize methane, *Paludibacter*, *Novosphingobium*, *Flavobacterium* and *Burkholderia* may be organic matter oxidizers, and *Sulfuritalea* may be sulfur oxidizing bacterium. This is because the chemical composition in the real groundwater was complex.

When DO in the influent was sufficient (day 35), IOB-*Crenothrix* (30.45%) and *Gallionella* (9.77%) were found in 0 m of the filter depth with a high abundance, which was agreed with the previous result that iron was mainly removed in 0–0.2 m. In the biofilter, *Crenothrix* and *Gallionella* not only oxidized iron but also manganese, and manganese removal was occurred after ferrous iron was completely oxidized (Cheng 2016); therefore, *Crenothrix* and *Gallionella* oxidized manganese in 0.4–0.8 m of the filter depth (the concentration of ferrous iron in 0.4 m could not be detected). The abundance of *Crenothrix* and *Gallionella* decreased from 29.60% and 8.16% (0.4 m) to 16.87% and 3.28% (0.8 m), which was consistent with the concentration of manganese that decreased from 0.33 mg/L (0.4 m) to 0.16 mg/L (0.8 m). The abundance of *Nitrosococcus* increased from 1.51% (DO8-0) to 2.46% (DO8-0.4) and 8.61% (DO8-0.8) along the filter depth, while ammonia decreased from 1.18 mg/L (0 m) to 0.15 (0.4 m) and 0.11 mg/L (0.8 m).

When DO in the influent decreased to about 6 mg/L (day 95), DO was insufficient to completely oxidize ammonia,

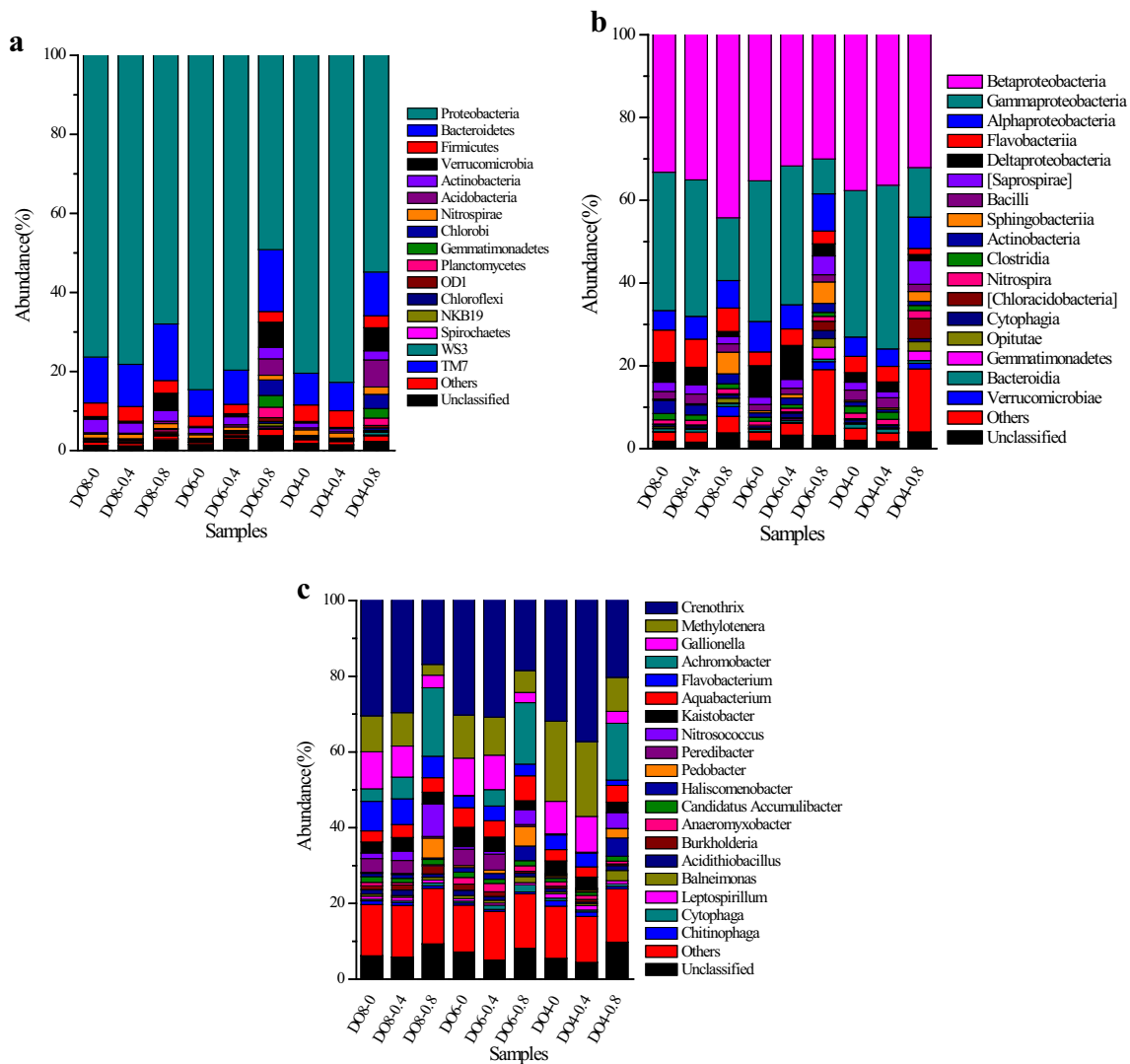


Fig. 3 Taxonomic classification of pyrosequences from bacterial communities of the nine samples at the phylum (a), class (b) and genus (c) levels. Phyla, classes and genera with relative low abundance in all nine libraries were classified as “others”

iron and manganese. The abundance of IOB (*Crenothrix* and *Gallionella*) in 0 m of the filter depth was hardly changed with decreased DO concentration. The abundance of MnOB-*Crenothrix* and *Gallionella* slightly increased in 0.4 m of the filter depth, but the concentration of manganese was hardly varied. And the abundance of *Crenothrix* in 0.8 m increased from 16.87% (DO8-0.8) to 18.50% (DO6-0.8). AOB-*Nitrosococcus* was affected significantly by DO, and the abundance of *Nitrosococcus* along the filter depth obviously decreased from 1.51% (DO8-0), 2.46% (DO8-0.4) and 8.61% (DO8-0.8) to 0.69% (DO6-0), 0.72% (DO6-0.4) and 3.94% (DO6-0.8), respectively; correspondingly the concentration of ammonia in 0.4 and 0.8 m of the filter depth increased from 0.15 to 0.11 mg/L to 0.36 and 0.31 mg/L, respectively.

When DO in the influent decreased to about 4 mg/L (day 156), iron was also completely removed, and the

abundance of AOB-*Crenothrix* and *Gallionella* in 0 m of the filter depth was hardly varied, too. Manganese in 0.4 and 0.8 m of the filter depth was slightly changed, but the abundance of MnOB-*Crenothrix* in 0.4 and 0.8 m obviously increased from 30.76% (DO6-0.4) and 18.50% (DO6-0.8) to 37.24% (DO4-0.4) and 20.30% (DO4-0.8), respectively. The abundance of *Nitrosococcus* in 0 and 0.4 m decreased from 0.69% (DO6-0) and 0.72% (DO6-0.4) to 0.22% (DO4-0) and 0.20% (DO4-0.4), respectively, and ammonia in 0.4 m increased from 0.36 to 0.75 mg/L accordingly. Surprisingly, the abundance of *Nitrosococcus* in 0.8 m increased from 3.94% (DO6-0.8) to 4.21% (DO4-0.8), although ammonia obviously increased from 0.31 to 0.69 mg/L.

Composition of the archaea community

The numbers of reads of the archaea 16S rRNA gene accounted for 0.33% (DO8-0), 0.34% (DO8-0.4), 0.29% (DO8-0.8), 0.61% (DO6-0), 1.19% (DO6-0.4), 1.18% (DO6-0.8), 0.76% (DO4-0), 0.47% (DO4-0.4) and 0.54% (DO4-0.8) of the total 16S rRNA genes. 3 archaea phyla [*Parvarchaeota*], *Crenarchaeota* and *Euryarchaeota*, and 5 archaea classes-*Thaumarchaeota*, *MCG*, *Thermoplasmata*, *Methanomicrobia* and *Methanobacteria* were detected in all nine communities (Fig. 4). The dominant phylum in the nine communities was *Euryarchaeota*, while the dominant class was *Thermoplasmata*. And the dominant genera were *Ferroplasma* (40.00%, 46.88%, 32.14%, 38.46%, 19.23%, 20.75%, 40.98%, 47.06%, and 27.27%) and *Thermogymnomonas* (30.00%, 31.25%, 25.00%, 34.62%, 46.15%, 33.96%, 39.34%, 17.65%, and 33.77%) (Fig. 5). In addition, *Ferroplasma* is iron oxidizing archaea (IOA) (Rawlings 2005), *Candidatus Nitrososphaera* is ammonia oxidizing archaea (AOA) (Oishi et al. 2012), and *Methanosarcina*, *Methanospirillum*, *Methanobacterium*, *Methanosaeta*, *Methanoculleus* and *Candidatus Methanoregula*, were possible related to methane oxidizing (Bharathi and Chellapandi 2017; Rocheleau et al. 1999).

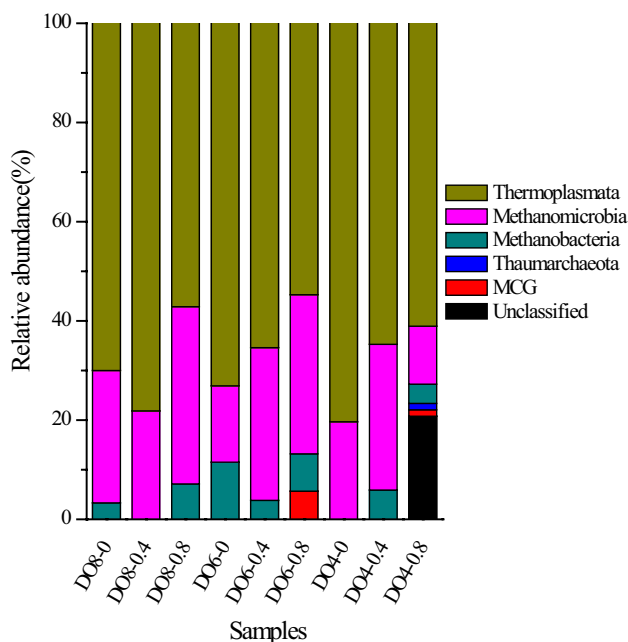


Fig. 4 Taxonomic classification of pyrosequences from archaeal communities of the nine samples at the class levels. Relative abundance was defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample

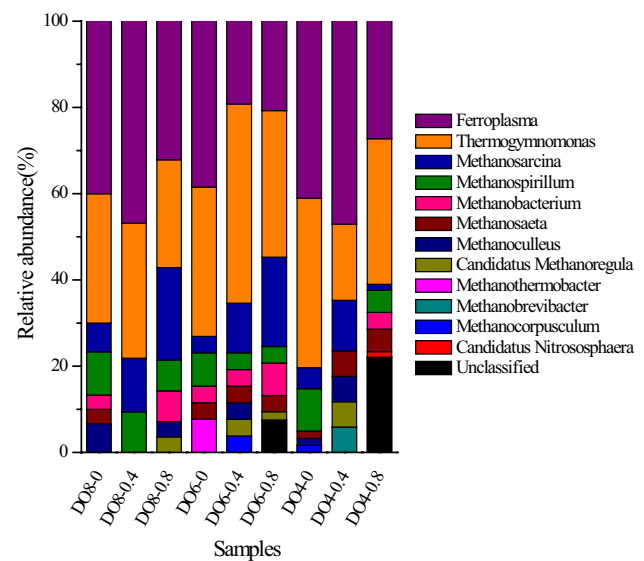


Fig. 5 Taxonomic classification of pyrosequences from archaeal communities of the nine samples at the genus levels. Relative abundance was defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample

Discussion

In this study, the removal performance of ammonia, iron and manganese in the biofilter at different DO conditions was investigated. An excellent removal efficiency for ammonia, iron and manganese was achieved in sufficient DO condition. This due to plenty of AOB, NOB, IOB and MnOB attached on the media of biofilter, which could oxidize the pollutants quickly. When DO in the influent was reduced to about 6 and 4 from 8 mg/L, ammonia in the effluent obviously increased, but removal efficiency of total iron and manganese were not affected. The reasons could be explained as followed: (1) Iron was removed at the top of the filter depth, where DO was sufficient. (2) MnOB could utilize DO preferentially to oxidize manganese when DO was insufficient (Cheng 2016). The variation of ammonia, iron and manganese along the filter depth indicated that iron and ammonia were simultaneously removed from the top of filter media. Since manganese removal only took place after ferrous iron was oxidized completely due to ferrous iron could react with manganese oxides, iron was mainly removed in 0–0.2 m, while ammonia and manganese were simultaneously removed in 0.2–0.8 m. The concentration of iron and manganese along the filter depth was not affected by DO, while ammonia increased with the decreasing of DO concentrations. The biofilter could be operated at relatively low DO condition to save energy consumption and reduce operating cost, since when DO in the influent was about 6 mg/L, ammonia, iron and manganese were all below the MCLs in this experiment.

Large amounts of iron and manganese oxides, which covered on the surfaces of the bacteria in SEM images, evidenced that microorganisms acted as catalytic role in iron and manganese removal from groundwater (Yang et al. 2014). A type of microorganisms which presented typical structure of twisted stalk may be recognized as the most common IOB and MnOB (*Gallionella*), and it is very common microorganisms in groundwater treatment plants for iron and manganese removal (Li et al. 2013; Hallbeck and Pedersen 1990; Katsoyiannis and Zouboulis 2004). Another type of microorganisms presented rod-shaped characteristic may be recognized as *bacillus*, which is an endospore-forming bacterium commonly found in water sources and soil (Kunst et al. 1995). This bacterium has the ability to catalyze manganese oxidation (Yang et al. 2014).

The distribution and genetic diversity of the microorganisms in the biofilter at different DO conditions was also investigated. Under sufficient DO condition, iron was mainly oxidized by IOB-*Crenothrix* and *Gallionella* in 0 m of the filter depth, while MnOB-*Crenothrix* and *Gallionella* oxidized manganese in 0.4–0.8 m, since manganese removal could be occurred after ferrous iron was completely oxidized (Cheng 2016). The abundance of Nitrosococcus (AOB) increased from 0 to 0.4 and 0.8 m, but removal amount of ammonia decreased. The reason could be explained as the quantity of microbe in DO8-0 was much higher than that in DO8-0.4 and DO8-0.8, and although the quantity of *Nitrosococcus* obviously decreased from DO8-0 to DO8-0.4 and DO8-0.8, the abundance of *Nitrosococcus* increased. The main functional microbes for ammonia and manganese removal were *Nitrosomonas* and *Crenothrix*, respectively, which was mainly distributed at 0.8 and 0.8 m of the filter bed with a corresponding abundance of 8.61% and 16.87% under sufficient DO condition, respectively; while iron was mainly removed by *Crenothrix* and *Gallionella* in 0 m with a corresponding abundance of 30.45% and 9.77%.

Under insufficient DO condition, the abundance of IOB (*Crenothrix* and *Gallionella*) in 0 m of the filter depth was hardly changed, since iron was mainly removed at the top of the filter depth where DO was sufficient, and the activity of IOB was not affected. When DO decreased to about 6 and 4 mg/L, the abundance of MnOB-*Crenothrix* increased in 0.4 and 0.8 m, and MnOB-*Gallionella* slowly increased in 0.4 m, and slightly varied in 0.8 m; while the concentration of manganese was hardly varied. The reasons may be as follows: (1) kinds of bacteria cannot adapt the low DO condition, resulting in the amount of total bacteria decreased with the decreasing of DO concentration. (2) The activity of *Crenothrix* and *Gallionella* decreased with the decreasing of DO concentration, and larger number of *Crenothrix* and *Gallionella* was needed to oxidize manganese. (3) Adaptive ability of *Crenothrix* was stronger than *Gallionella* at low DO condition. MnOB

have an advantage over AOB in utilizing DO at low DO condition (DO < 0.5 mg/L) (Cheng 2016), leading to the increase of ammonia in 0.4 and 0.8 m in insufficient DO condition. With the decreasing of DO concentration, the abundance of AOB-*Nitrosococcus* decreased in 0 and 0.4 m, agreed with the increase of ammonia in 0.4. But *Nitrosococcus* first decreased and then increased in 0.8 m. The reason may be that the total amount of microorganism in 0.8 m largely decreased as DO decreased to 0.21 mg/L, while the AOB slightly decreased, so the abundance of *Nitrosococcus* increased.

The concentration of ammonia and manganese in raw groundwater was about 0.9–1.4 and 0.9–1.4 mg/L, respectively, but the abundance of AOB-*Nitrosococcus* was obviously lower than MnOB in the nine samples, which was agreed with our previous research (Cheng et al. 2017a, b). The reason may be that AOB-*Nitrosococcus* could oxidize ammonia with much higher efficiency compared with MnOB.

Although the proportion of archaea was very low compared with bacteria in the biofilter, the archaea played a significant role in ammonia and iron removal, such as IOA-*Ferroplasma* and AOA-*Nitrososphaera* were found in the biofilter. Leininger et al. (2006) demonstrated that archaea are far more important than bacteria for nitrification in different soils and suggested that they are the major ammonia oxidizing microorganisms in soil ecosystems around the world (Leininger et al. 2006). It is necessary to investigate the distribution and the role of archaea in the biofilters in the near future.

When DO in the influent decreased from about 8 to 4 mg/L, iron and manganese were still completely removed, while ammonia exceeded the MCL when DO was about 4 mg/L, indicating that the biofilter could be operated at relatively low DO condition (6 mg/L) to save energy consumption and reduce operating cost. AOB, NOB, AOA, IOB, IOA, MnOB which were related to ammonia, iron and manganese removal were found in the biofilter treating real groundwater, respectively. IOB-*Crenothrix* and *Gallionella* oxidized ferrous iron at the top of the filter depth, therefore, IOB were not affected by DO. The abundance of MnOB-*Crenothrix* increased in 0.4 and 0.8 m since the activity of the MnOB decreased with the decrease of DO concentration. The amount of AOB-*Nitrosococcus* in 0.4 and 0.8 m obviously decreased with the increase of ammonia. The microbial community structure was obviously changed as DO decreasing from about 8 to 4 mg/L, proving that DO was an important operational parameter.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Research involving human and animal rights This research did not involve Human Participants and Animals.

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