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Operational parameters required for the start-up process of a biofilter to remove Fe, Mn, and NH_3-N from low-temperature groundwater

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ABSTRACT

Start-up experiments were carried out using a constructed a pilot-scale biofilter in a water supply plant to purify iron (Fe), manganese (Mn), and ammonia (NH_3-N) from low-temperature groundwater (3–4˚C throughout the year). The results of the biofilter operation indicated that the simultaneous removal of these contaminants could be obtained with a one-stage biofilter, while the operational parameters such as aeration and backwashing strength (BWS) should be optimized during the start-up operation period. Removal of Fe was most likely dominated by chemical oxygen oxidation since the biofilter exhibited high removal efficiency at the initial running period and the start-up time was not required. Higher levels of aeration (that corresponds to higher dissolved oxygen concentration) helped to transform the water redox environment and increase Mn removal. Nitrification effects were irrelevant with the extra aeration. A weaker BWS was helpful in avoiding biomass loss during the initial operation of the biofilter; however, the BWS should be improved to eliminate excess amounts of metal oxide accumulation after a long operation time, since it could enhance the overall removal. Using high-throughput sequencing technology, the functional bacteria genera were identified. It was demonstrated that the corresponding functional oxidizing bacteria could be acclimated sufficiently in low-temperature water.

Keywords: Biological filter; Fe and Mn removal; Low-temperature groundwater; Nitrification

1. Introduction

Fe, Mn, and NH_3-N usually exist in the groundwater together in their dissolvable reduced state due to the natural chemical environment of the earth; however, the presence of NH_3-N may be also caused by human activities. In drinking water resources, the contained Fe and Mn usually cause an undesired color, a bad taste, clogging of pipelines, and some other operational problems [1,2]. The NH_3-N will cause algae growth or consume some agents in the chlorination process.

Generally, Fe and Mn removal can be achieved by oxidation and subsequent precipitation or filtration. NH_3-N is converted to nitrates or nitrogen gas. The

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removal can be achieved by abiotic or biotic methods. One of the chemical methods is the successful application of potassium permanganate or chlorine as strong oxidizing agents to remove Fe and Mn [3–5]. However, there are some disadvantages, such as the appearance of a higher total Mn concentration and a pink color in the produced water that is caused by a slight overdose in potassium permanganate. Additionally, there is a significant consumption of chlorine when NH_3-N is present in the water; it is especially problematic as it can also produce disinfection by-products. Recently, the biological removal process has drawn much attention since there is no need to add extra chemical agents, and many researchers have demonstrated that biological technology for drinking water purification is successful [2,6–9], especially for the simultaneous removal of these contaminants with a one-stage filter [10–12]. It is well known that the unavoidable start-up period of the biofilter is the main drawback for its application. Therefore, backwashing sludge off the biofilter and matured sand materials are usually used as effective methods to inoculate the biofilter and to shorten the start-up period [13]. The water temperature should also be considered, since it also affects the start-up period significantly.

In the Hailar district of China where the groundwater remains at a constantly low temperature of 3–4℃ throughout the year and contains the three contaminants mentioned above, chlorine contact oxidation has been used for many years during drinking water treatment. Using the HeiYangZhan water supply plant as an example, the simultaneous removal of contaminants could be achieved with a one-stage filter, but an additional problem is encountered in addition to the ones already mentioned, the pipes and valves corrode over the long operating duration of the filters. This problem could be potentially resolved by adopting the biotechnology in the filters instead of adding chemical agents. There are few studies however, that describe the application of biotechnology with water at these cold temperatures. In addition, the model constructed by researchers described the relationship between temperature and the growth rate of bacteria, based on the growth rate of the *Pseudomonas* group 1 strain 16L16, and found a decrease in growth rate of about 80% from 15 to 3℃ [14]. Additionally, the modified model indicated it could be applied to the growth of a wide range of bacteria [15]. Obviously, such low-temperature water conditions will challenge the growth rate of the functional oxidizing bacteria (which corresponds to the oxidizing activity),in removing the three contaminants.

Thus, the aim of this work was to investigate the biological possibility of removing Fe, Mn, and NH_3-N simultaneously with a one-stage filter from low-temperature groundwater (3–4℃ throughout the year) and provide supporting information for innovating the operation of the filters in the plant. During the start-up period, the details about conventional operation parameters such as dissolved oxygen (DO), backwashing strength (BWS), and filtration rate (FR) were also collected. Meanwhile, molecular techniques were also applied to identity the functional bacteria in the filter.

2. Materials and methods

2.1. Groundwater and pilot device

The pilot-scale biofilter was built in the HeiYangZhan water supply plant located in the Hailar district of China, where the groundwater has a constant temperature of 3–4℃. The characteristics of the water were shown in Table 1, and Fe and Mn were in their reduced state due to the anaerobic environment of the groundwater. The guideline values of Fe, Mn, and NH_3-N in drinking water in China is 0.3, 0.1 and 0.5 mg/L [16], respectively.

The biofilter was made of plexiglass, with a height of 2.5 m and an inner diameter of 185 mm. The cobblestone support is 30 cm high and has a particle size of 1–3 cm. The new Mn sand filter bed was 120 cm high with a particle size of 0.6–1.2 mm. Furthermore, the matured Mn sand, which was selected from a biological Fe, Mn, and NH_3-N removal plant that operated at an approximate 10℃ water temperature, was inoculated to the upper part of the filter bed when the operation began, and thus, the total height of the filter bed was 130 cm. Additionally, the biofilters in that plant have been operated for several years and the start-up period was about 2–3 months [17]. The raw water was pumped from the secondary cascaded-aeration tank of the water supply plant, and used as

Table 1
The groundwater characteristics in the water supply plant

Parameters	Units	Raw water
Temperature	℃	3–4
Chromaticity	degree	25
Turbidity	NTU	<2
pH		7.5
Fe	mg/L	3.04
Mn	mg/L	0.52
Total hardness	mgCaCO$_3$/L	170
NH_4^+-N	mg/L	1.11
NO_3^--N	mg/L	<0.01
NO_2^--N	mg/L	<0.001

inflow and backwashing water. The experimental equipment of the biofilter is shown in Fig. 1.

2.2. Analytical methods

During the experiments, Fe, Mn, NH_3-N, COD_{Mn}, turbidity, nitrite, and temperature were detected by spectrophotometry according to standard methods [16]. The oxygen measurements were made using an electrode (pH/oxi340i, WTW). The demands of DO, FR, and BWS of the biofilter were calculated based on the following equations:

DO demands:

$$4Fe^{2+} + O_2 \rightarrow 4Fe^{3+} + 2O^{2-} \quad (1)$$

$$2Mn^{2+} + O_2 \rightarrow 2Mn^{4+} + 2O^{2-} \quad (2)$$

$$NH_4^+\text{-N} + 2O_2 \rightarrow NO_3^-\text{-N} + 2H^+ + H_2O \quad (3)$$

In which about 0.14, 0.29, and 4.57 mg DO were needed per mg of Fe, Mn, and NH_3-N, respectively.

FR calculation:

$$FR = Q_1/A \quad (4)$$

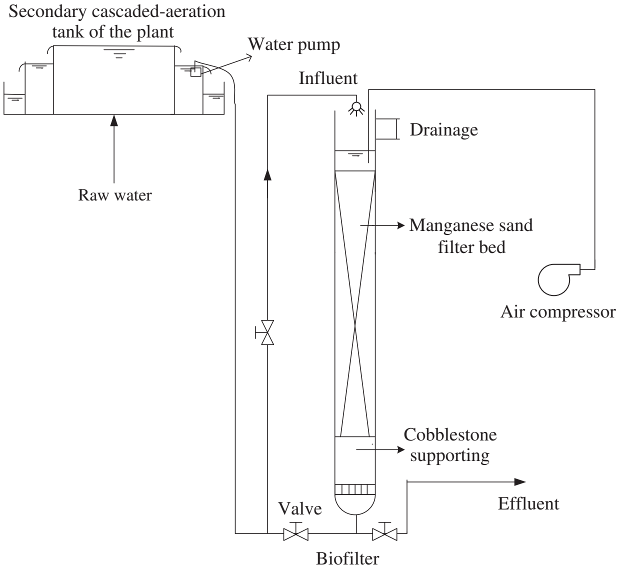

Fig. 1. Schematic diagram of the biofilter reactor in this study.

In which Q_1 is the filtration flow that can be measured using a measuring cylinder in m^3/h. A is the sectional area of the filter bed in m^2.

BWS calculation:

$$BWS = Q_2/A \quad (5)$$

In which Q_2 is the backwashing flow that also can be measured using a measuring cylinder in L/s. A is the sectional area of the filter bed in m^2.

2.3. Operational parameters

During the start-up periods, the operational parameter values in each period were presented in Table 2. The biofilter was operated as a continuous flow reactor, and the water head above the filter bed was kept at a relatively constant value of 40 cm by adjusting the valve frequently during each working cycle. Then, the procedure of backwashing was carried out when the flow rate of each level decreased by about 0.5 m/h. According to the water characteristics and equations above, the DO concentration during the start-up periods always met the calculated theoretical DO concentration of about 5.6 mg/L. The previous operating parameter's values were consulted and referenced in period A when the filter began operation and were adjusted in other periods according to the removal.

2.4. Analysis of bacterial 16S rDNA

Bacterial genomic DNA was extracted from a wet sand sample of 5 g by a Bacterial Genomic DNA Isolation Kit (Sangon, Shanghai), in accordance with the manufacturer's protocol. For analyzing of the functional bacteria, the DNA products were then submitted to Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China) to perform a high-throughput sequencing. And 23,514 sequences were obtained after the processing step with Roche 454 platform; these sequences could be divided into 95 genera. Then, the functional genera were investigated by comparing with those well-known Fe/Mn oxidizers and nitrifier.

3. Results and discussion

3.1. Fe removal

During the start-up periods, the performance of the Fe removal was exhibited in Fig. 2. It is well known that Fe can be oxidized through simple aeration at a neutral pH compared with Mn [18], thus

Table 2
The operational parameters during the start-up periods

Period	Days	DO mg/L	BWS L/(s m^2)	Backwashing time min	FR m/h
A	1–180	6.5–7.5	7	4	5
B	180–210	6.5–7.5	7	4	3
C	210–240	9.3–10.7	7	4	3
D	240–298	9.3–10.7	13	4	from 3 to 6

oxygen can be used as an effective oxidizing agent in addition to biological action in the Fe removal process. The difference between total Fe and Fe^{2+} in the influent from Fig. 2 indicated that the chemical DO oxidation contributed substantially to the Fe oxidation process before the aerated water entered the filter bed. The residual Fe^{2+} then continued to be oxidized in the filter bed, resulting in a lower Fe level in the effluent. The low temperature seemed to have no significant effect on Fe removal, and the start-up time was not required. That means the Fe oxidation was not due to bacterial activity, and thus, it seemed more likely that Fe oxidation in the filter was caused chemically, by oxygen supplied during the aeration step [13].

Researchers had demonstrated that chemical oxidation dominated the Fe removal process [19], while the biological oxidation process only contributed about 6% using a matured biofilter [1]. Therefore, the proportion of biological action would be less than 6% before the biofilter became mature. Previous work suggested that the theory of Fe removal using a filter is by chemical oxidation in the filter bed, using a continuous contact oxidation process, in which the oxidized products of oxyhydroxide (FeOOH) coated the sand surface continually and acted as catalysts for the adsorption and oxidation of Fe^{2+}. This would dramatically improve the oxidation efficiency, and as a result, a high oxidation rate of Fe with oxygen in the filter was reported [20,21]. This could explain the high removal efficiency in Fig. 2 even when the filter operated using extreme low temperatures, especially during the operation of an immature biofilter. Since the aim of this work was to remove Fe, the contribution rate of the biological oxidation was not analyzed here and needs to be studied in the future.

3.2. NH_3-N removal

Because of the limited amount of organic carbon in the drinking water source, NH_3-N only participated in the nitrification process [22]. NH_3-N is firstly oxidized to nitrite by NH_3-N oxidizing bacteria, then as substrates of the nitrite-oxidizing bacteria (NOB), nitrite continues to be converted to nitrate. The results of NH_3-N and nitrite removal over the nitrification process were shown in Figs. 3 and 4, respectively.

In period A, NH_3-N was removed with a removal efficiency of about 30–45% in the first 15 d after inoculation (Fig. 3); simultaneously, nitrite appeared in the effluent in Fig. 4, which is the result of ammonia-oxidizing bacteria (AOB) activity. The NH_3-N concentration in the effluent increased in the subsequent operation days along with a decreasing removal efficiency. Then, the NH_3-N removal entered into a steady state with a removal efficiency of about 20% up until 30 d, and the nitrite concentration decreased to a lower level of about 0.2 mg/L in Fig. 4. This revealed the temperature effect on AOB activity and was in agreement with the operation results of the matured nitrification biofilter that the NH_3-N removal efficiency was below 30% at a low temperature (below 4), after

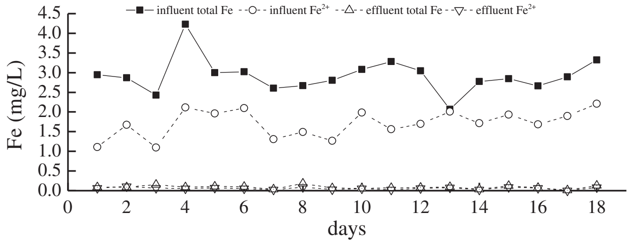

Fig. 2. Performance of Fe removal from low-temperature groundwater using a biofilter.

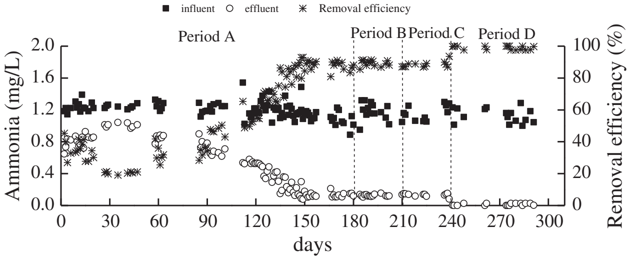

Fig. 3. Performance of NH_3-N removal from low-temperature groundwater using a biofilter.

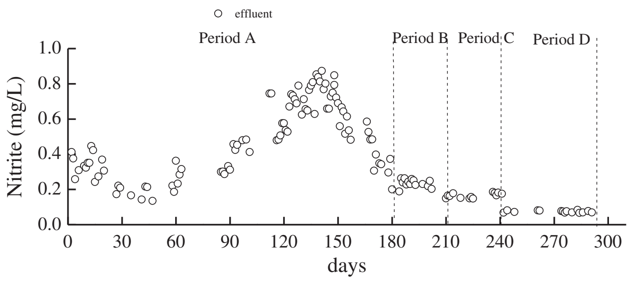

Fig. 4. Performance of nitrite removal from low-temperature groundwater using a biofilter.

several weeks of operation due to the seasonal changes [23]. Although the low water temperature affected AOB significantly, the oxidizing ability was demonstrated at least, suggesting that the acclimation and growth of AOB became important in the nitrification process. After the adaptation time of about 45 d, the variation trend of the effluent NH_3-N concentration, the removal efficiency, and the effluent nitrite in Figs. 3 and 4 indicated that AOB could adapt to a low temperature and grow considerably. However, the long start-up period of 155 d to obtain the effluent NH_3-N concentration of 0.1–0.2 mg/L with a 90% removal efficiency in Fig. 3 revealed the negative impact of low temperature on the AOB further. It was still a slow process since the shorter start-up period of the biofilter that operated at a higher water temperature was obtained [13]. In addition, the decreasing nitrite concentration after reaching its maximum value of about 0.9 mg/L in Fig. 4 also indicated the enhancement of NOB activity. However, the nitrite oxidation lagged behind NH_3-N oxidation, apparently as a result of the different growth rates of AOB and NOB, with a low-temperature groundwater environment. Thus, the nitrite (as an intermediate product of the NH_3-N oxidation) oxidation process determines that complete nitrification would extend the start-up period to remove NH_3-N.

In the following periods of B, C, and D (Figs. 3 and 4), the operational parameters were adjusted as described in Table 2 according to the profiles of the effluent Mn concentration (which will be described in the Mn removal section below), particularly for the lower FR in periods B and C and the improvement of BWS when the biofilter operation entered into period D. This is of interest because despite the slight nitrite decrease in periods B and C (Fig. 4), the effluent NH_3-N concentration did not continue to decrease even with the lower inflow loading (Fig. 3). Conversely, an enhanced nitrification effect was obtained after improving the BWS in period D to obtain almost 100% NH_3-N removal efficiency, and with a low steady nitrite concentration of 0.05 mg/L even FR was improved to 6 m/h. The long start-up period of nitrification obtained from Figs. 3 and 4 revealed the low growth rate of the nitrifier, and it is well known that a higher BWS will significantly wash out more nitrifier from the filter bed, which theoretically, will degrade the nitrification performance during the biofilter operation, especially for the immature biofilter. While the enhanced nitrification performance obtained in period D of Figs. 3 and 4 may be sufficiently explained by the biofilter operation mechanism of excess biomass or other substances accumulating within the filter bed often resulting in clogging, water stream channels form and consequently there is a performance deterioration [24,25]. Therefore, improving the hydraulic BWS was an effective way to avoid the excess accumulation and maintain a stable biofilter performance. This also could explain why the NH_3-N removal was not improved further in periods B and C (Fig. 3). Given the low growth rate of bacteria here, it was impossible that biomass accumulation occurred. It is more likely that metal oxides accumulated within the filter bed. The insufficient BWS was unable to wash out enough oxides to maintain a clean filter bed, resulting in a gradual metal oxide accumulation after a long period of operation. More details about the importance of improving the BWS were also described in the following section.

Obviously, the application of biotechnology for NH_3-N removal from low-temperature water was feasible, but a longer start-up period was required, during which the BWS operation parameter played an important role.

3.3. Mn removal

The Mn removal performance was shown in Fig. 5.

In period A, the new Mn sand showed a strong adsorption capacity for Mn in the first few weeks and the effluent Mn concentration was below 0.1 mg/L; therefore, it was difficult to confirm the Mn oxidizing bacteria (MOB) activity. Subsequently, the effluent Mn concentration increased gradually in addition to the adsorption capacity becoming saturated and reaching a steady level of 0.28 mg/L after about 90 d. This resulted in a demonstration of the MOB activity, by subtracting the Mn concentration in the effluent from the influent, since Mn could only be oxidized through bacteria at neutral pH [18]. Thus, MOB decreased the effluent Mn in the subsequent operation days; however, it was surprising that a new steady level of effluent Mn concentration of 0.2 mg/L was obtained

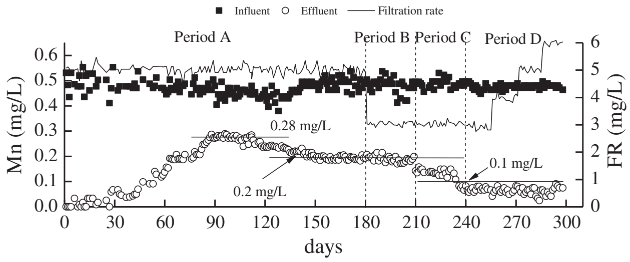

Fig. 5. Mn removal performance from low-temperature groundwater using a biofilter.

after about 150 d, and even when the operation time was extended to 180 d. The MOB activity seemed to be inhibited to continue further Mn oxidation. As is well known, biological Mn oxidation relies on a higher water redox potential [8,18], and nitrification participates in the evolution of the water redox for Mn removal [18]. Simultaneous Mn and NH_3-N removal with nitrite accumulation is based on this and was reported during the start-up period of the biofilter [2,13,26]. The variation trends of the effluent concentrations of Mn, NH_3-N, and nitrite from 90 to 150 d are also based on the redox potential (Figs. 3–5). Although the inhibitory effects of nitrite on Mn removal was also demonstrated at the same time [27], the decreasing nitrite amount (from 150 to 180 d, Fig. 4) did not result in Mn removal, as the second steady level of effluent Mn concentration showed, thus it was assumed that the NH_3-N oxidation significantly contributed to the evolution of the redox environment for Mn removal, more than nitrite oxidation.

In period B, the effluent Mn concentration had no obvious variation even when FR was decreased, which indicated that inflow loading was not the significant limiting factor for Mn removal and measures must be taken to create the suitable water environment for MOB growth. Besides nitrification and temperature, aeration maybe the only effective way among the conventional operational parameters to change the water redox environment, since some other reducing material in the water can be oxidized or stripped. Most important of all, the corresponding higher pH value will reduce the redox field of biological Mn oxidation [8]. Subsequently, the effluent Mn concentration decreased to 0.15 mg/L immediately, as DO was improved to 9.3–10.7 mg/L in period C. Enhanced Mn removal was not obtained again even when DO continued to be improved to above 11.5 mg/L in the subsequent several days, during which the effluent Mn was at a steady level of 0.15 mg/L. This indicated the indirect effect of aeration on Mn removal. Therefore, DO concentration was optimized over a range of 9.3–10.7 mg/L. Regrettably, redox values were not detected during the start-up period of the biofilter, and some corresponding conclusions were drawn according to well-known studies and removal effects, while the evolution of redox value by aeration was observed in the complementary tests after start-up of the biofilter.

Meanwhile, the influence of backwashing on Mn removal was investigated, to more fully understand the removal performance. After backwashing, profiles of Mn removal as the biofilter operation were studied in period C first, as shown in Fig. 6.

The BWS in period C was about 7 L/(s m^2), and it could be seen from the results of Mn removal in the selected working cycle that the effluent Mn concentration dropped to a level of below 0.1 mg/L within 1 h and met the required value of 0.1 mg/L in the first few hours, followed by an increasing trend. The stable operation of the biofilter for about 8 h after backwashing was completed prior to analysis of Mn in the first day of the working cycle, so that the Mn concentration was not detected below 0.1 mg/L. It was abnormal, and the rebound phenomenon of the Mn concentration should not appear as the operation time is extended. Although the oxides could provide a space for bacteria to attach to, considering the negative influence of metal oxide accumulation on biofilter operation as mentioned above, an improvement of BWS is necessary to eliminate oxide accumulation for maintaining the removal. Besides the enhanced performance of nitrification, the effluent Mn concentration was also below 0.1 mg/L, immediately after the BWS was improved in period D (Fig. 5). A different Mn removal amount in the selected working cycle of

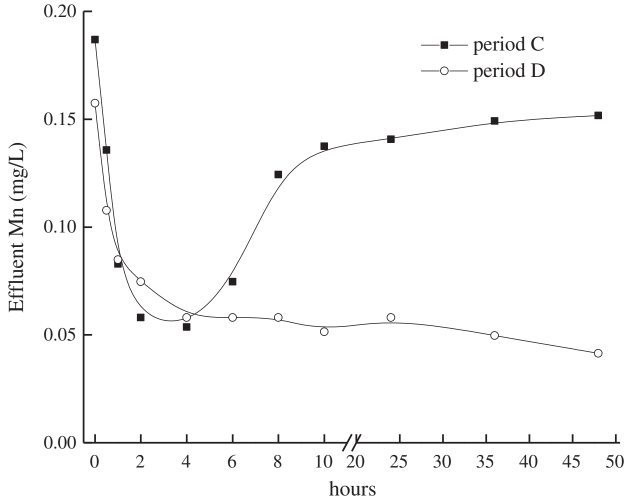

Fig. 6. Mn removal performance after backwashing in period C and period D, respectively; BWS of period C: 7 L/(s m^2), and BWS of period D: 13 L/(s m^2).

period D after backwashing was obtained in Fig. 6, due to the disappearance of the rebounded Mn concentration. Meanwhile, biological Mn removal always exhibited an enhanced performance in period D as FR was increased to 6 m/h, which is similar to the FR value of the mid-speed biofilter in practical engineering, thus the Mn removal from low-temperature water can also be completed by a biological method.

Additionally, according to the results obtained above, there is a possibility that the start-up period of the biofilter can be shortened by optimizing the operational parameters without delay. Since backwashing will lead to biomass loss, a weaker BWS accompanied by a higher DO concentration is useful when the biofilter begins to operate. Then, the rebound phenomenon of Mn removal in the working cycle after backwashing, as Fig. 6 shows, can be referenced as the sign of improving BWS. Moreover, the thickness of the filter bed, which in a fluidization condition when the BWS was 7 L/(s m^2), was 30 cm; while the thickness continued to be increased to 80 cm as the improvement of the BWS to 13 L/(s m^2).

3.4. The variation of turbidity, COD$_{Mn}$, and temperature during the start-up period

During the start-up period of the biofilter, the variation of turbidity, COD$_{Mn}$, and temperature of the influent and effluent was exhibited in Fig. 7.

It could be observed from Fig. 7(a) that the turbidity of the raw water pumped from the deep well was below 2 NTU. The generated ferric iron after the aeration led to a higher turbidity in the influent, while the turbidity continued to be decreased to a lower level of below 1 NTU after the filtration. The variations in the water quality index of COD$_{Mn}$ reflect the removal of reducing materials, for example, some organic matter. In Fig. 7(b), the COD$_{Mn}$ between influent and effluent had no significant change until after about 90 d of operation. And the COD$_{Mn}$ concentration in the effluent was stable at about 2 mg/L after the operational period of about 210 d, which also indicated the slow growth rate of bacteria. Moreover, the remaining COD$_{Mn}$ in the effluent may be ascribed to some reduced materials which were difficult to be biologically oxidized. Generally, the start-up period of the biofilter for the simultaneous removal of Fe, Mn, and NH_3-N lasts for several weeks to months. The reason why the long start-up period was needed in this study could be explained by the low water temperature, as it could be seen from Fig. 7(c) that the water temperature of influent and effluent was at a relative constant value of 3–4℃.

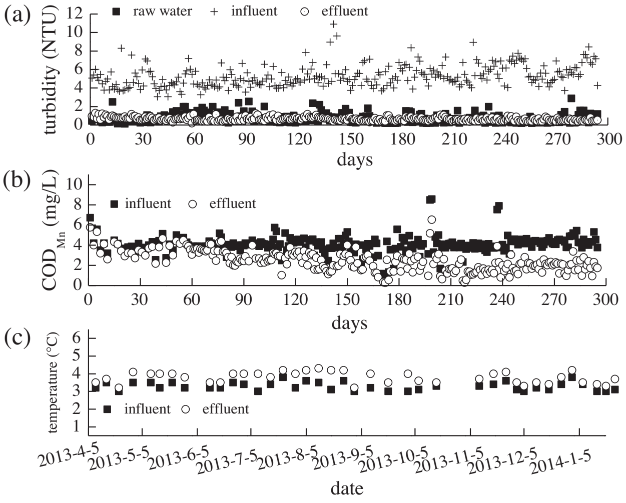

Fig. 7. The profiles of turbidity, COD$_{Mn}$, and temperature of the influent and effluent during the start-up period. (a) Profiles of turbidity between the raw water, influent, and effluent; (b) Profiles of COD$_{Mn}$ between the influent and effluent; (c) Profiles of water temperature between the influent and effluent.

3.5. Functional bacteria analysis

After the long running time, the functional bacteria had adapted to oxidize considerably in a low-temperature environment. The oxidizing bacteria in the filter bed were identified and presented in Fig. 8.

Nitrosomonas and *Nitrosospira* are the well-known AOB genera. There are four NOBs in the genus: *Nitrobacter*, *Nitrospina*, *Nitrospira*, and *Nitrococcus*. Additionally, Candidatus *Nitrotoga arctica* was also reported as a new NOB species [28]. The two AOB genera were detectable in the biofilter, as is shown in Fig. 8; however, there is only one commonly reported NOB genus of *Nitrospina* in addition to the novel cold-adapted nitrite oxidizer of Candidatus *Nitrotoga* [29,30]. These nitrifiers showed a significant amount of sequences, and thus, it provided supporting information for biological nitrification from the low-temperature groundwater.

Genera of *Crenothrix*, *Leptothrix*, *Hyphomicrobium*, *Siderocapsa*, *Siderocystis*, and *Metallogenium* were considered to be Fe/Mn oxidizers [8]. The removal of Fe/Mn from the low-temperature groundwater was related to *Crenothrix*, *Leptothrix*, and *Hyphomicrobium*. However, this removal process seemed to be dominated by *Crenothrix*, as the amount of *Leptothrix* or *Hyphomicrobium* was in a lower level. The *Gallionella*, which is considered to be with the ability of oxidizing Fe only, was not found from the total sequences. This

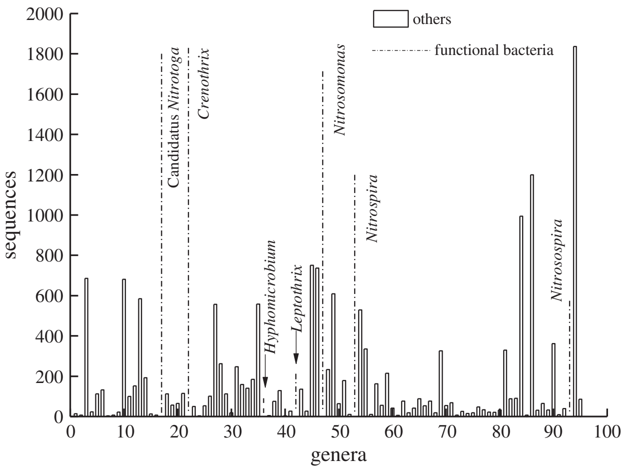

Fig. 8. The amount of sequences in each genus.

may be due to the slow growth rate of bacteria, and chemical DO oxidation dominated the Fe removal during the start-up period. Therefore, it was demonstrated that the corresponding functional oxidizing bacteria could be acclimated sufficiently in low-temperature water.

4. Conclusions

According to the removal of Fe, Mn, and NH_3-N from low-temperature groundwater (3–4℃), it is concluded that the simultaneous removal of these contaminants can be achieved with a one-stage biofilter. However, it still requires a long start-up period to acclimate the corresponding bacteria.

(1) The Fe removal process was more likely dominated by chemical oxygen oxidation, and not affected by the low water temperature. Thus, the biofilter exhibited a high removal efficiency at the initial running period and the start-up time was not required.

(2) The removal of NH_3-N and Mn could be achieved using a biological method, but a long start-up period was needed. Meanwhile, nitrite appeared throughout the whole start-up period.

(3) Among the operational parameters, DO and BWS played an important role in the biological removal process and should be optimized. A high strength aeration corresponding to a higher DO concentration was useful in further transforming the water redox environment for Mn removal. Nitrification was irrelevant with extra aeration. A weaker BWS was helpful to avoid biomass at the initial operation of the biofilter; however, the BWS should be improved to eliminate excess metal oxide accumulation after a long operation time, since it could enhance the overall removal.

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