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Ammonium removal pathways and microbial community in GAC-sand dual media filter in drinking water treatment

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Abstract

A GAC-sand dual media filter (GSF) was devised as an alternative solution for drinking water treatment plant to tackle the raw water polluted by ammonium in place of expensive ozone-GAC processes or bio-pretreatments. The ammonium removal pathways and microbial community in the GSFs were investigated. The concentrations of ammonium, nitrite and nitrate nitrogen were monitored along the filter. Total inorganic nitrogen (TIN) loss occurred during the filtration. For 1 mg ammonium removal, the TIN loss was as high as 0.35 mg, DO consumption was 3.06 mg, and alkalinity consumption was 5.55 mg. It was assumed that both nitrification and denitrification processes occur in the filters to fit the TIN loss and low DO consumption. During the filtration, nitritation, nitrification and nitritation-anaerobic ammonium oxidation processes probably occur, while traditional nitrification and denitrification and simultaneous nitrification and denitrification processes may occur. In the GSFs, *Nitrosomonas* and *Nitrospira* are likely to be involved in nitrification processes, while *Novosphingobium, Comamonadaceae* and *Oxalobacteraceae* may be involved in denitrification processes.

Key words: nitritation; nitrification; nitritation-anaerobic ammonium oxidation; nitrification and denitrification;

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Introduction

More than 40% of the surface water in China cannot meet the national standard for drinking water sources, being mainly contaminated by organic matter and ammonium, according to monitoring data from recent years. At the same time, more than 95% of the water treatment plants (WTPs) in China were still using the conventional water treatment process as of 2010, and limited removal of organic matter and ammonium could be achieved. Biological treatment processes such as O_3-BAC or bio-pretreatment are ideal choices to remove those contaminants, however, many WTPs in China have limited space and investment available to realize those processes. Therefore the reconstruction and optimization of rapid filters deserves more attention.

Changing existing sand filters into GAC-sand dual media filters (GSF) represents an ideal choice, because there is little difference in turbidity removal between a GSF and a sand filter, while much better organic matter and ammonium removal can be achieved by a GSF (Andersson et al., 2001; Kim and Kang, 2008). Adsorption and biodegradation are the main organic matter removal pathways in GAC filters, and studies have been done to differentiate the two functions (Liang and Chiang, 2007; Wang et al., 2007). Ammonium removal in the filters was attributed to biodegradation, and work has been done on the effects of operating factors on ammonium removal efficiencies (Andersson et al., 2001; Niquette et al., 1998), and a method was developed to estimate the active nitrifying biomass in drinking water filters (Tränckner et al., 2008). Little work had been done on ammonium removal pathways until recent years. Both nitrification and denitrification were proposed to take place in bio-active filters treating drinking water (Yu et al., 2007; Murphy et al., 2010), and the theories were taken from wastewater treatment.

To cut down the cost of ammonium removal in wastewater treatment, a great deal of work has been done on ammonium treatment processes and removal pathways. Traditional nitrification and denitrification theory had been widely accepted for years, with two reactors needed for the two processes separately. New nitrogen removal processes showed better nitrogen removal efficiencies with lower cost, which made them more attractive for wastewater treatment plants (WWTPs). The simultaneous nitrification and denitrification (SND) process saved 25% in the consumption of dissolved oxygen (DO) and 40% of the consumption of chemical oxygen demand

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(COD) compared with the Traditional Nitrification and Denitrification process (Turk and Mavinic, 1987). A single reactor for high-activity ammonium removal over nitrite (SHARON) was developed to realize the SND in wastewater treatment (Hellinga et al., 1998; Zhao et al., 1999; Walters et al., 2009). Nitritation-anaerobic ammonium oxidation (ANAMMOX) showed even more benefits to WWTPs, because 62.5% consumption of DO and 100% consumption of COD could be saved during this process (Van de Graaf et al., 1995). Oxygen-limited autotrophic nitrification and denitrification (OLAND) and completely autotrophic nitrogen removal over nitrite (CANON) were developed to realize nitritation-ANAMMOX in wastewater treatment (Kuai and Verstraete, 1998; Strous et al., 1997; Sliekers et al., 2002; Vlaeminck et al., 2010). Aerobic deammonification could also be realized in wastewater treatment (Hippen et al., 1997). Both nitrification and denitrification processes were sensitive to dissolved oxygen (DO) (Yoo et al., 1999; Gómeeza et al., 2002; He et al., 2009; Yuan and Gao, 2010), therefore the ideal reactor for those processes was the sequencing batch reactor (SBR), for which the operational parameters such as DO could be easily controlled (Joss et al., 2009; Gao et al., 2011). Some of the well-accepted ammonium removal pathways are listed below:

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

Nitritation would consume 3.43 mg DO and 7.14 mg alkalinity when 1 mg NH_4^+-N was removed, as illustrated in Reaction (1).

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+ \quad (2)$$

Nitrification would consume 4.57 mg DO and 7.14 mg alkalinity when 1 mg NH_4^+-N was removed, as illustrated in Reaction (2).

$$2NH_4^+ + 4O_2 + 10[H] \longrightarrow N_2\uparrow + 2H^+ + 8H_2O \quad (3)$$

Traditional nitrification and denitrification would consume 4.57 mg DO and 3.57 mg alkalinity when 1 mg NH_4^+-N was removed, as illustrated in Reaction (3).

$$2NH_4^+ + 3O_2 + 6[H] \longrightarrow N_2\uparrow + 2H^+ + 6H_2O \quad (4)$$

SND would consume 3.43 mg DO and 3.57 mg alkalinity when 1 mg NH_4^+-N was removed, as illustrated in Reaction (4).

$$2NH_4^+ + 1.5O_2 \longrightarrow N_2\uparrow + 2H^+ + 3H_2O \quad (5)$$

Nitritation-ANAMMOX would consume 1.71 mg DO and 3.57 mg alkalinity when 1 mg NH_4^+-N was removed, as illustrated in Reaction (5).

In this study, two sets of GSFs were used as rapid filters to treat surface water with more than 0.5 mg/L ammonium. Ammonium removal pathways were hypothesized according to the DO consumption, alkalinity consumption and total inorganic nitrogen (TIN) loss monitored in the filter. A clone library was constructed to study the microbial community structure in a GSF and identify the microorganism responsible for this process.

1 Materials and methods

1.1 Water characteristics

The raw water of a WTP was pumped from the Dongjiang River in Guangdong Province (China) which is contaminated by a connected and heavily polluted urban canal during frequent storms from June to September. Once the water level in the canal becomes elevated to a certain height by storms, the gate in the canal must be opened to release water into the Dongjiang River to protect Dongguan City, which causes the raw water quality to deteriorate. From May, 2010 to May, 2011, the average ammonium concentration in the raw water was 1.67 mg/L and the maximum concentration was 3.89 mg/L from June to September, while the average ammonium concentration in raw water was 0.58 mg/L and the maximum concentration was 1.45 mg/L during other times. Since coagulation and sedimentation had little effect on ammonium removal, rapid filters had to deal with the high ammonium concentration, and the residual ammonium in the filter effluent would result in extra chlorine consumption in the disinfection tank, which could ruin the disinfection efficiency.

1.2 Bench-scale instruments

Two bench-scale GSF instruments were set up in the WTP and operated continuously. These instruments were made of Lucite. The outside of the filters was covered with shade cloth so that photosynthetic bacteria could not survive, and no DO could be supplemented during the filtration. The inner diameter of the filters was 56 mm and the height was 2500 mm. The filters were filled with 400 mm height of sand (diameter 0.5–1.0 mm), 1000 mm height of GAC (size 8×30 mesh), and 200 mm height of graded gravel.

The two filters were operated in parallel with a flow velocity of 8 m/hr, while the empty bed contact time (EBCT) was 7.5 min in the GAC media and 3 min in the sand media. The flux was 20 L/hr for each filter, and the influent was the settling tank effluent from the WTP. Ammonium chloride was added to the influent by a metering pump to simulate the required ammonium concentration. Filters were backwashed every 48 hr with a 30% expansion rate. The backwash water was the non-chlorine-containing effluent of the sand filters in the WTP.

The biofilm formation in the GSFs was completed with 0.72 mg/L ammonium in the influent on average at around 20°C. Then the filters were operated to study the ammonium removal capacities and pathways. The ammonium concentration fluctuated between 1.12 and 5.26 mg/L in the influent, with an average concentration of 2.99 mg/L and standard deviation of 1.28 mg/L. The experiments

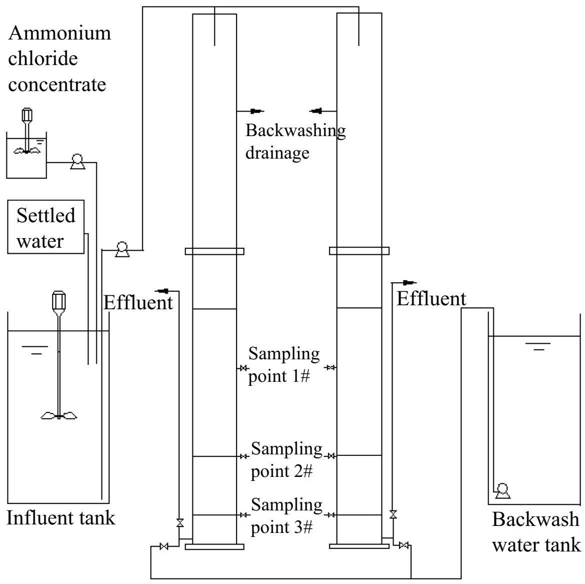

Fig. 1 Schematic of bench-scale instruments.

lasted half a year, and the temperature fluctuated between 10 and 30°C.

1.3 Water quality analysis

Water samples of the influent at point 1# (400 mm GAC in depth), point 2# (bottom of GAC layer), and point 3# (effluent of the filter) were taken during the experiments. The concentrations of ammonium, nitrite, nitrate, DO and alkalinity were analyzed in order to calculate the stoichiometric relationship and deduce the ammonium removal pathways.

Ammonium, nitrite and nitrate were analyzed by Nessler's reagent spectrophotometric method, N-(1-naphthyl)-ethylenediamine spectrophotometric method and thymol spectrophotometric method respectively, according to the Chinese National Standard Method (GB5749-2006). Alkalinity was analyzed by titration according to the method introduced in Monitoring and Assays for Water and Wastewater (2002). DO and temperature of the water samples were measured by a HACH HQ30d with a LDO probe. pH was measured with a HACH HQ11d with a glass electrode probe.

1.4 Microbial community analysis

A clone library was constructed after the GSFs had been in stable operation for 45 days. GAC samples (400 mm in the upper layer of GAC) were collected in a sterilized bottle. About 200 mL sterilized PBS (0.03 mol/L, pH 7.2) buffer was added into the bottle and then ultrasonication (15 kHz) was carried out for 20 min. The process was repeated five times for each sample. The liquid was collected and centrifuged for 5 min (4°C, 5000 r/min) and the supernatant was abandoned. DNA was extracted using the UltraClean DNA extraction kit. 16S rRNA genes were amplified using bacterial primers 27F (5′-GAGTTTGATCMT GGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR conditions were as follows: 94°C (5 min); 94°C (30 sec); 55°C (30 sec); 72°C (1.5 min) (30 cycles); 72°C (5 min). The PCR products were purified with QIA quick PCR purification kit and cloned into pGEM-T-easy Vector. The white colonies were verified by PCR with primers M13 F (5′-TGTAAAACGACGGCCAGT-3′) and M13 R (5′-AACAGCTATGACCATG-3′). The clone library was constructed with 120 randomly selected clones.

2 Results and discussion

2.1 Inorganic nitrogen transformation in GSFs

The influence of DO, temperature, filtration velocity and backwashing on ammonium transformation was studied (not discussed in this article), and DO was recognized as the dominating factor: on one hand a good linear relationship existed between ammonium removal capacity and influent DO; on the other hand the transformation of nitrite was mainly determined by available DO.

The concentrations of ammonium, nitrite and nitrate were analyzed along the filter depth. It was found that the majority of ammonium removal was accomplished within the upper 400 mm of the GAC media and as was the TIN loss, while the nitrite concentration increased in this part. In the lower part of the GAC layer, some of the nitrite could be oxidized into nitrate if sufficient DO was available. There was little inorganic nitrogen transformation in the sand layer. When more than 2 mg/L DO was available in the filters, there was little nitrite in the effluent, while with less DO, nitrite accumulated in the effluent. Two examples are listed in Fig. 2a and b.

Nitrite increased in the upper 400 mm of the GAC layer, and nitrite accumulated in the effluent if DO was insufficient, therefore nitritation should occur in the filters. Nitrate concentration increased along the filter depth, therefore nitrification should occur in the filters.

Denitrification might be the reason for the TIN loss observed. It has been well accepted that biofilms on porous filter media could create regional anoxic or anaerobic niches. So there were opportunities for bacteria related to denitrification processes to exist in the GSFs and play a role in inorganic nitrogen transformation, which could cause the TIN loss. A study speculated that aerobic denitrification or SND might be the reason for 10% TIN loss (Mekinia et al., 2009). In this study, an average of 11.2% TIN loss occurred, while the TIN loss accounted for 35.2% of the ammonium removal amount on average, based on 86 groups of data.

2.2 DO consumption in GSFs

The DO consumption could be used as one clue for better understanding the pathway of TIN loss. In this study, two assumptions were made to simplify the DO consumption analysis:

(1) DO consumed by heterotrophic bacteria could be neglected. The TOC of the influent was 1–2 mg/L and the GSF could remove about 50% of the TOC, so less than 1 mg/L TOC was removed during filtration. The GAC in the filters was just put into use which meant that adsorption could be the dominating pathway for organic

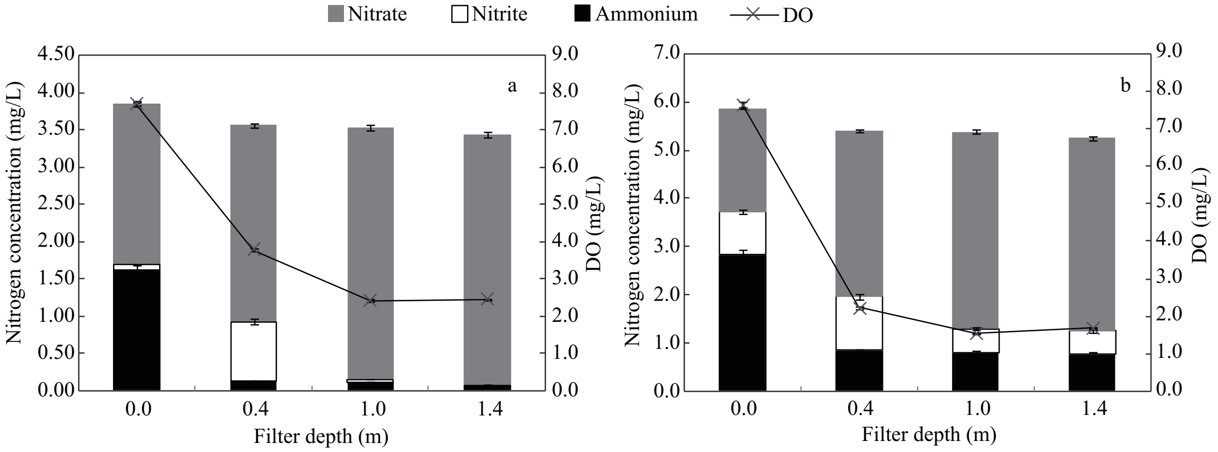

Fig. 2 Inorganic nitrogen transformation with sufficient DO (a) and limited DO (b). The filter was backwashed every two days and the water samples were taken under the steady operation condition one day after backwashing.

matter removal (Kim and Kang, 2008), thus the organic matter utilized by the heterotrophic bacteria was limited. The heterotrophic bacteria activity should be low and DO consumed should be limited as a result.

(2) DO consumed by nitrite already existing in the influent could be neglected. The average nitrite concentration in the influent was 0.26 mg/L during the half year, and 0.37 mg/L DO would be consumed if all of the nitrite in the influent was oxidized into nitrate. This consumption was much less than the DO consumption during the filtration process. Since some of the nitrite might be involved in the denitrification processes, DO consumed by nitrite in the influent would be even less.

Based on those assumptions, DO was mainly consumed by the bacteria involved in ammonium transformation. A stoichiometric relationship between the ammonium removal and DO consumption in the GSFs was fitted based on 103 groups of data in Fig. 3, and 3.06 mg DO was found to be consumed when 1 mg ammonium was removed. Based on the stoichiometric relationships, the consumption was not enough for the nitritation process (3.43 mg DO/mg ammonium), let alone the nitrification process (4.57 mg DO/mg ammonium), so both aforementioned processes were not the correct way to explain the ammonium removal in the filters. If DO consumed by heterotrophic bacteria and the oxidation of nitrite in the influent was considered, the DO consumption for ammonium removal would be even less.

Nitritation-ANAMMOX probably occurred in the GSFs since it was the only low-DO-consumption pathway to fit the final DO consumption of 3.06 mg when 1 mg ammonium was removed. As mentioned before, with 1 mg ammonium removal, 4.57 mg DO would be consumed by nitrification or traditional nitrification and denitrification, and 3.43 mg DO would be consumed by nitritation or SND, while 1.71 mg DO would be consumed by nitritation-ANAMMOX. If there was no nitritation-ANAMMOX process occurring, the fitted DO consumption would be between 3.43 mg and 4.57 mg.

A similar study found that DO consumption was 30% lower than the theoretical DO demand, if nitrification was regarded as the only pathway to remove ammonium (Yu et al., 2007), and nitritation-ANAMMOX was proposed as the explanation. Nitritation could be readily obtained under oxygen limitation (Pollice et al., 2002), therefore low DO in the filter could promote nitritation-ANAMMOX to occur. A study found that nitritation-ANAMMOX could take place with DO below 1 mg/L (Joss et al., 2009), which could be realized in the inner layer of the biofilm in this study. The affinity constants for the substrates of ammonium and nitrite were each less than 0.1 mg/L in ANAMMOX (Strous et al., 1999), so concentrations of the substrate in GSFs were high enough for this process to take place.

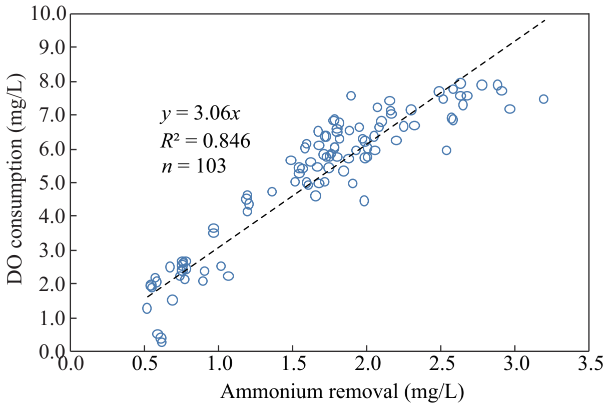

Fig. 3 Relationship between ammonium removal and DO consumption.

2.3 Alkalinity consumption in GSFs

The stoichiometric relationship between the ammonium removal and alkalinity consumption in the GSFs was fitted based on 57 groups of data in Fig. 4, finding that 5.55 mg alkalinity was consumed when 1 mg ammonium was removed.

During nitritation or nitrification processes, 7.14 mg alkalinity would be consumed when 1 mg ammonium was removed, and X was used to present the ammonium removal ratio through these two pathways. During traditional nitrification and denitrification, SND or nitritation-ANAMMOX processes, 3.57 mg alkalinity would be consumed when 1 mg ammonium was removed, and Y was used to present the ammonium removal ratio through these three pathways. Thus two equations could

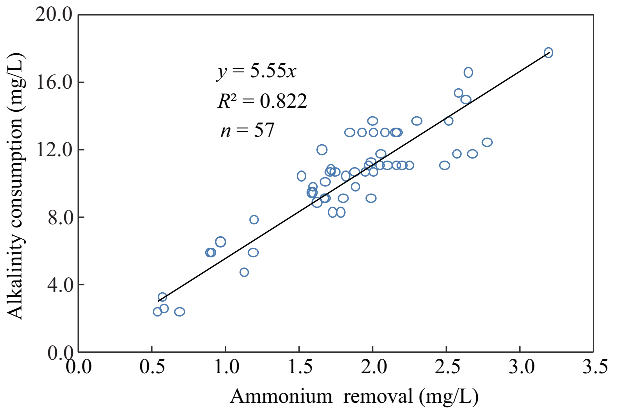

Fig. 4 Relationship between ammonium removal and alkalinity consumption.

be derived:

$$X + Y = 1 \tag{6}$$

$$7.14X + 3.57Y = 5.55 \tag{7}$$

The result was calculated as: X = 55.4% and Y = 44.6%. The high value of Y confirmed the existence of nitrogen-loss processes. When ammonium is removed by traditional nitrification and denitrification, SND or nitritation-ANAMMOX processes, it is transformed into nitrogen gas, which meant TIN loss would occur. In this study, based on the calculation of alkalinity consumption, TIN loss should account for 44.6% of the ammonium removal, which was close to the actual TIN loss of 35.2%. The alkalinity consumption thus helps to demonstrate the existence of denitrification processes.

2.4 16S rRNA clone library results

As far as the authors know, the microbial community structure had never been determined for rapid filters treating surface water with high ammonium concentration. Since most of the ammonium removal and TIN loss occurred on the upper filter (400 mm GAC in depth), a filter media sample in this section was collected and a clone library was constructed.

In this study, most of the bacteria were identified to genera or families, because the information obtained from 16s rRNA was not enough to further identify the species of the bacteria. Therefore the characteristics of the genera or families related to nitrification and denitrification processes were introduced.

Genus *Geothrix* were the dominant species in the GAC surface and accounted for 35.50% in the system (Table 1). They were chemo-organotrophic heterotrophs, and were Gram-negative, anaerobic, and rod-shaped, and obtained energy by anaerobic respiration or by fermentation (Brenner et al., 2005). The filters had been operated with high ammonium concentration in the influent for 45 days before the GAC sample was removed, and DO in the filters kept low during the period. The prevalence of *Geothrix* demonstrated the existence of anaerobic niches in the filters, where denitrification processes could occur.

Genus *Nitrospira* accounted for 17.34% in the system, which were Gram-negative, lithoautotrophic nitrite oxidizers. They use nitrite as a sole source of energy and CO_2 as the main source of carbon. *Nitrospira* instead of *Nitrobacter* were recognized as the dominant nitrite-oxidizing bacteria (NOB) in freshwater aquaria, biofilms and activated sludge in recent years (Brenner et al., 2005; Hovanec et al., 1998), and members belonging to the genus *Nitrospira* were the only NOB found in another study (De Vet et al., 2009). The biomass of NOB was sufficient when the filters were in stable operation, confirning the conclusion that the accumulation of nitrite in the effluent should be attributed to limited DO being available.

Class *Alphaproteobacteria* accounted for 14.01% in the system, and the main genus identified was *Novosphingobium,* which accounted for 10.73% in the system. Members of genus *Novosphingobium* were Gram-negative and chemo-organotrophic bacteria, widespread in soil, marine and fresh water. They are best known for their ability to degrade a wide variety of aromatic hydrocarbons, and are important in the biogeochemical cycles of carbon, nitrogen or chlorine in their surrounding environments (Liu et al., 2005). All strains belonging to this genus could reduce nitrate into nitrite (Takeuchi et al., 2001), while they were strictly aerobic bacteria. Therefore, it is speculated that *Novosphingobium* spread on the surface of the biofilm where the DO concentration was relatively higher. Nitrate could be reduced into nitrite and then the nitrite could be further reduced in the inner layer of the biofilm by denitrifiers.

Class *Betaproteobacteria* accounted for 23.87% in the system, and included the families *Comamonadaceae, Oxalobacteraceae* and *Nitrosomonadales.* Family *Comamonadaceae* accounted for 5.77% in the system. A study found that eight strains belonging to family *Comamonadaceae* showed potential for SND under aerobic conditions (Khardenavis et al., 2007), and some studies suggested that the members of family *Comamonadaceae* played a major role in denitrification processes in the presence of acetate (Ginige et al., 2005). A SBR could effectively convert nitrite to nitrogen gas, with the families *Comamonadaceae* and *Nitrosomonadaceae* as predominant members (Adav et al., 2010). *Oxalobacteraceae* accounted for 16.46% in the system, which is a family with metabolically diverse members, including strict anaerobes, strict aerobes, and nitrogen-fixing organisms (Brenner et al., 2005). Some species in this family were connected with the inorganic nitrogen transformation. For example, some species included in genus *Herbaspirillum* could use ammonium as nitrogen source, and some species included in genus *Herbaspirillum*, *Janthinobacterium* and *Telluria* could reduce nitrate and nitrite. If those species were in the GSF, nitrogen loss and DO consumption during ammonium removal would be affected.

Nitrosomonas belonged to *Nitrosomonadales* and accounted for 0.82% in the system. *Nitrosomonas* is a genus of ammonium-oxidizing bacteria (AOB) and plays an important role in wastewater treatment. Cells of the obligately lithotrophic species *Nitrosomonas europaea* and *Nitrosomonas eutropha* were found able to nitrify and

Table 1 Data of bacterial 16S rRNA clone library in a GSF

OTU species	Percentage (%)
Bacteria	3.29
Bacteria, Acidobacteria, Holophagae, Holophagales, Holophagaceae, Geothrix	35.50
Bacteria, Bacteroidetes, Sphingobacteria, Sphingobacteriales, Chitinophagaceae, Sediminibacterium	0.82
Bacteria, Nitrospira, Nitrospira, Nitrospirales, Nitrospiraceae, Nitrospira	17.34
Bacteria, Proteobacteria, Alphaproteobacteria	0.82
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae	0.82
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae, Agromonas	0.82
Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae	0.82
Bacteria, Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae, Novosphingobium	10.73
Bacteria, Proteobacteria, Betaproteobacteria	0.82
Bacteria, Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae	1.64
Bacteria, Proteobacteria, Betaproteobacteria, Burkholderiales, Comamonadaceae, Curvibacter	4.13
Bacteria, Proteobacteria, Betaproteobacteria, Burkholderiales, Oxalobacteraceae	13.16
Bacteria, Proteobacteria, Betaproteobacteria, Burkholderiales, Oxalobacteraceae, Massilia	3.30
Bacteria, Proteobacteria, Betaproteobacteria, Nitrosomonadales, Nitrosomonadaceae, Nitrosomonas	0.82
Bacteria, Proteobacteria, Gammaproteobacteria	1.65
Bacteria, Proteobacteria, Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, Pseudomonas	3.30

denitrify at the same time when grown under oxygen limitation (Bock et al., 1995), so Nitritation-ANAMMOX could be realized with those species. *Nitrosomonas* must consume large amounts of ammonia before cell division can occur, and ammonium used for anabolism accounted for only 2% of the ammonium removed by *Nitrosomonas*, so the GSF could remove quantities of ammonium with limited biomass of *Nitrosomona*s. This explained the large biomass difference between the *Nitrospira* and *Nitrosomonas* in the filters.

Pseudomonas accounted for 3.30% in the system. *Pseudomonas* cells could produce exopolysaccharides, which was of benefit to the biofilm formation on the filter media surface, so that the bacteria in the biofilm would not be easily washed out under frequent filter backwashing (every 36–48 hr). Therefore, *Pseudomonas* played an important role in keeping the filters in stable operation.

From the results, it could be concluded that there existed many bacteria which could be involved in the nitrification and denitrification processes.

3 Conclusions

Ammonium removal pathways and the microbial community in rapid filters treating drinking water were studied, and the conclusions are as follows: (1) TIN loss was detected in the GSF filters when treating the high-ammonia-contaminated water. TIN loss was 11.2% on average, and it accounted for 35.2% of the ammonium removal amount on average. (2) The pathways of nitrogen transformation could be hypothesized according to the dynamic of ammonium, nitrite, nitrate, DO and alkalinity. 3.06 mg and 5.55 mg DO and alkalinity were consumed respectively, when 1 mg ammonium was removed in the GSF filter. Nitritation-ANAMMOX probably occurred in the GSFs since it was the only low-DO-consumption pathway able to fit the final DO consumption. Traditional nitrification and denitrification, and SND processes also possibly occurred in the GSF filters. (3) *Nitrosomonas* and *Nitrospira* were the dominant AOB and NOB in the GSFs from the 16S rRNA clone library results, and should be involved in nitrification processes. *Novosphingobium, Comamonadaceae* and *Oxalobacteraceae* may be involved in the denitrification processes.

Acknowledgments

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