Desalination and Water Treatment
www.deswater.com

24 (2010) 308–320
December

1944-3994/1944-3986
doi: 10.5004/dwt.2010.1456

The influence of aeration on nitrification and the nitrifier distribution in an upflow biological aerated filter for tertiary treatment of municipal sewage

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Received 18 December 2009; accepted 3 May 2010

ABSTRACT

A series of two pilot-scale aerobic and anaerobic upflow biofilters were constructed and operated for tertiary treatment of municipal sewage. The effects of aeration on the nitrification performance and nitrifier distribution between attached and suspended biomass of the aerobic upflow biofilter (UBAF) were studied. Process operating results revealed that, for the secondary effluent with volumetric loads of 0.31–0.65 kgCOD/m^3 d and 0.14–0.34 kgNH_4-N/m^3 d, the UBAF showed an unstable ammonia removal rate of 60.6–72.2% with an average bulk DO concentration of 2.18 mg/L at an aeration rate of 0.6 m^3/h. Partial nitrification was observed at the first 1.2 m of the filter (DO < 1.42 mg/L), and more than 38.4% nitrite accumulation ratio was detected. When the aeration rate was increased to 1.0 m^3/h, the nitrification efficiency of UBAF was stabilized at the range of 90.1–93.5% with an average bulk DO concentration of 3.82 mg/L. No significant nitrite accumulation took place in the whole filter. Specific activity tests of attached and suspended biomass of the filter demonstrated that the buildup of nitrification efficiency at an aeration rate of 1.0 m^3/h mainly arose from the improvement of the nitrifying activity of the attached biofilm. The increase of nitrifying activity of attached biofilm increased the stability of nitrification performance of the UBAF. Most probable number (MPN) emuneration showed that about 60.8% of ammonia oxidizing bacteria (AOB) and 90.8% of nitrite oxidizing bacteria (NOB) were present in attached biofilm at aeration rate of 0.6 m^3/h. The increase of aeration rate gave rise to 38.9% and 66.7% increase of AOB and NOB of attached biofilm, but only 10.6% and 23.1% increase of AOB and NOB of suspended biosolid. The increase of aeration rate showed little effect on the growth of AOB and NOB of suspended biosolid. NOB was more inclined to grow in attached biofilm than AOB at two aeration rates.

Keywords: Nitrification; Nitrifier distribution; Suspended biosolid; Attached biofilm; Nitrifying activity

1. Introduction

Water shortage and environmental pollution are always serious problems in China. One of the effective ways to resolve these problems is water reuse. In 2008,

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the amount of municipal sewage discharged was 33 billion tons, which accounted for 57.7% of all wastewater [1]. However, the reuse ratio of municipal sewage was less than 10%. This was much lower than the 80% reuse ratio of industrial wastewater [1]. Water pollutants such as nitrogen and phosphorous are important factors limiting the reuse of municipal sewage. Thus, improving the quality and quantity of wastewater reuse is an important and necessary task for the municipal sewage treatment industry

A biological aerated filter (BAF) is a small footprint alternative to the conventional biological treatment processes used for industrial and domestic wastewater [2]. A submerged packed bed of granular media provides a large surface area per unit volume for heterotrophic and autotrophic microorganisms to grow and form a biofilm. The immobilization of biomass makes it possible to maintain optimal conditions for the relevant microorganisms independently of hydraulic retention times, and therefore the process has achieved high levels of nitrification, denitrification and phosphate uptake [3]. As an important operating parameter of the process, aeration rate has a great influence on nitrification performance of BAF because it not only controls the concentration of bulk dissolved oxygen, but also affects oxygen transfer, flow conditions and shear [4,5].

Biological nitrification is the process by which ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) and then nitrite is subsequently oxidized to nitrate by nitrite-oxidizing bacteria (NOB). In biofilm systems, the maximum volumetric conversion of ammonia is usually limited by the liquid-biofilm or the gas-liquid oxygen mass transfer rate [6]. Though AOB and NOB are all aerobic microbes, AOB show much stronger oxygen-affinity than NOB [7]. Many experiments have shown that ammonia conversion in biofilm systems can be effectively achieved by limiting the activity of NOB with low dissolved oxygen (DO) conditions to achieve shortcut nitrification [6,8–11]. However, this method is usually operated in combination with the control of some other factors, such as pH, temperature, etc. The sole control of DO to achieve nitrite accumulation may decrease the overall nitrification rate [8]. Horn [12] and Garrido [6] studied the models of oxygen mass transfer in biofilm systems. Their work demonstrated that the increase of bulk oxygen concentration and air velocity can improve the mass transfer rate of oxygen at the bulk/biofilm interface and in the biofilm. The effects of aeration on the nitrification performance of the BAF process have been investigated by many researchers [11–17]. However, most of their work has centered on synthetic wastewater. Only a few studies have used actual wastewater. Compared with synthetic wastewater, real wastewater is usually unstable. In addition, the influent stream of real wastewater is always characterized by a certain content of suspended solids and particulates. This is prone to cause periodic clogging of the filter bed and increase backwash frequency [18,19]. The published results indicated that an increase of aeration rate is favorable for the conversion of ammonia. But the effect of aeration is moderated by concentration of influent organic carbon and ammonia loads. A continuous increase of DO concentration will result in high power consumption and is not useful for improving ammonia removal efficiency when the DO concentrations in the BAF satisfy nitrification needs [17]. Therefore, for the tertiary treatment of municipal sewage in the BAF process, we need to optimize the aeration rate in connection with the wastewater characteristics. A high aeration rate also causes the increase of operating cost.

In a BAF, besides the attached-growth biofilm, there are some suspended biosolids retained in the interstices between the media particles. The suspended biosolids consist of sloughy biofilm and intercepting matter. Villaverde [20] compared the nitrifying activity of attached biofilm and suspended biosolids, and found that suspended biosolids showed high ammonia oxidizing activity while the attached biofilm showed higher nitrite oxidizing activity. They also reported significant differences in the spatial distribution of AOB and NOB between the two compartments. The spatial distribution of nitrifiers within the two kinds of biomass mainly arises from the competition between different microbe groups for space, oxygen and substrate. Since oxygen affects the growth and decay rates of nitrifiers [7,21,22], oxygen mass transfer is vital to maintain the activity and distribution of the nitrifiers in both attached biofilm and suspended biosolids. The effects of aeration and oxygen on the activity and distribution of nitrifiers in a BAF have been investigated [23–25]. However, all the previous works looked only at attached biomass. There are no reports about the influence of aeration on the changes of activity and distribution of nitrifiers in suspended biomass, nor a comparison between attached and suspended biomass. Unlike attached biofilm, suspended biosolids are susceptible to backwashing. Investigating the effects of aeration on the changes of activity and spatial distribution of nitrifiers between attached biofilm and suspended biosolids is essential for the optimization of BAF performance. In addition, although published results have demonstrated that an increased of aeration rate is beneficial for the mass transport of oxygen and substrate, for wastewater with low organic carbon and

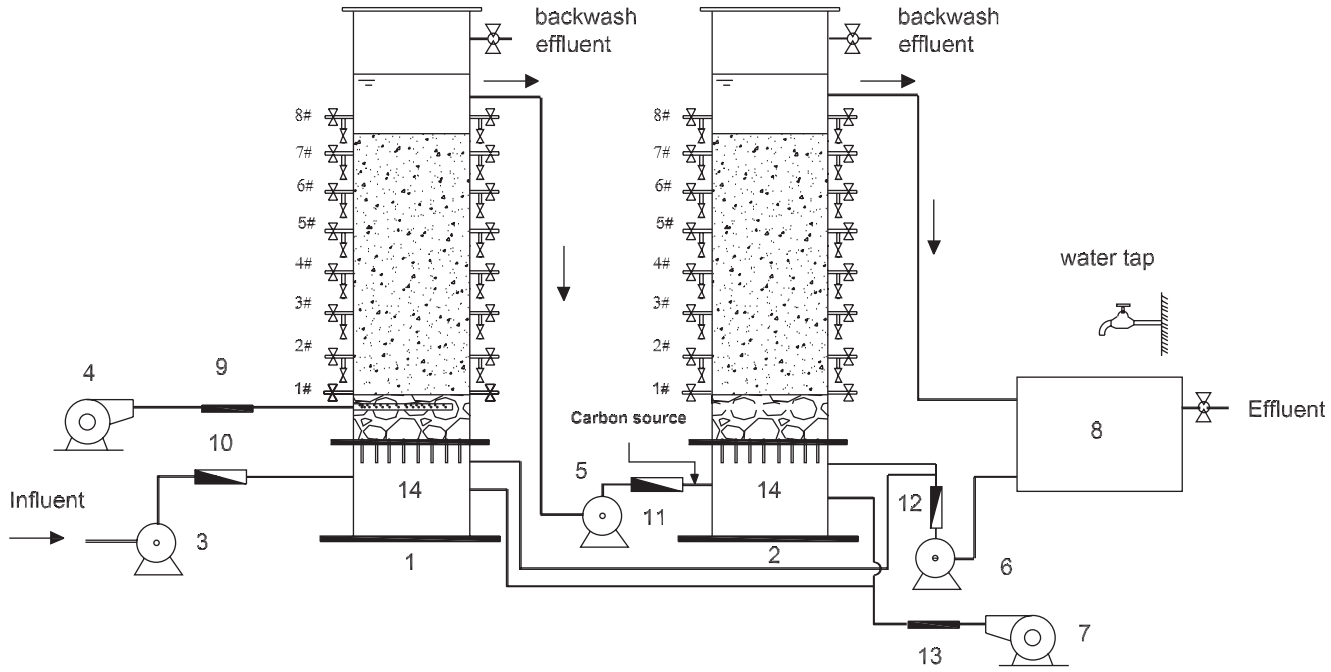

Fig. 1. Schematic diagram of the biofilm system (1.NB, nitrification biofilter, 2. DB, denitrification biofilter, 3. influent pump, 4. air blower, 5. pressurize pump, 6. backwashing pump, 7. backwashing air blower, 8. Effluent storage tank (backwashing water tank), 9. air flow meter, 10, 11. water flow meter, 12. water flow meter (backwash), 13. air flow meter (backwash), 14. filter head.

ammonia loads, the effect of aeration needs further study because when ammonia is limiting, oxygen radicals are formed and reducing equivalents are needed to remove them [26].This might give rise to the buildup of nitrifier decay rate and thus affect process performance. In this study, to achieve a better understanding of the influences of aeration on the nitrification performance of BAF for tertiary treatment of municipal sewage, the changes of activity and distribution of nitrifiers between attached and suspended biomass under different aeration rates were analyzed.

Before this study, a series of two pilot-scale upflow biofilters had already been operated for about three months at the Jinan municipal wastewater treatment plant for tertiary treatment of secondary effluent. The first biofilter is aerated and designed for the removal of organic carbon and nitrification, and the second for denitrification without aeration. This paper mainly focuses on the nitrification performance of the first biofilter. The secondary effluent was fed to the upflow biofilters continuously and the nitrification performance of the first biofilter under different aeration rate conditions was observed. A respiratory experiment was applied to determine the specific activity of attached and suspended biomass. Bacterial cell counting was used to assess the distribution of AOB and NOB on the attached biofilm and suspended biosolids. The main purposes were (1) to investigate the effects of aeration rate on the nitrification efficiency of UBAF for tertiary treatment of municipal sewage; (2) to study the influence of aeration on the nitrification performance of UBAF in connection with the nitrifying activity of attached and suspended biomass; (3) to evaluate the influence of aeration on the distribution of nitrifiers between attached biofilm and suspended biosolids in the UBAF.

2. Materials and methods

2.1. Experiment setup

A schematic diagram of the experimental system is shown in Fig. 1. The overall biofilm system consisted of two filters in series, named NB (nitrification biofilter) and DB (denitrification biofilter). Both NB and DB had the same dimensions – height 6.5 m, diameter 1.0 m and 4.55 m^3 of effective volume. Fig. 2 is the on-site photograph of the pilot-scale experimental apparatus. About 60% of the reactor volume of each filter was filled with granular ceramic particles which had been provided by Sifon Environmental Protection and Bioenergy Ltd, China. The loading heights and packing density of the media in the filter were 4 m and 1.03×10^3 kg/m^3. The media has an effective average diameter of 3–5 mm, density of 2.17 kg/m^3, porosity

Fig. 2. On site photograph of the pilot-scale experimental apparatus.

Table 1
Characteristics of the test wastewater

Parameter	Range	Mean (SD)
pH	6.9–7.2	7.1(0.1)
Temperature (°C)	7.9–26.2	19.4(4.9)
SS (mg/L)	6.2–13.6	8.4(1.9)
COD (mg/L)	20.3–50.4	25.8(7.2)
BOD_5 (mg/L)	4.2–8.7	5.5(1.3)
NH_4^+-N (mg/L)	7.0–25.4	16.9(4.5)
NO_2^--N (mg/L)	0.5–1.4	0.9(0.3)
NO_3^--N (mg/L)	3.8–15.7	7.9(3.4)
TN (mg/L)	14.5–29.8	22.9(5.1)
TP (mg/L)	0.9–1.4	1.1(0.2)

Note: SD, standard deviation.

of 0.53 and specific surface areas of 6.39 m^2/g. Each filter was provided with eight sampling ports located at heights of 1.2, 1.8, 2.4, 3.0, 3.6, 4.2, 4.8 and 5.4 m, allowing for liquid and substratum sampling during filter operation. Under the media layer, there was a pebble layer of about 30 cm depth. The diameter range of the pebbles was 5–10 cm. For NB, air was introduced into the reactor with an annular perforated pipe (pore size, 0.5 mm) fixed in the pebble layer. The aeration rate was controlled with an air flow meter. The wastewater was pumped into NB and flowed upward through the media layer. In order to ensure the fully contact of substrate and microorganism, filter head was assembled to improve the uniformity of water distribution. Both the wastewater and air flowed through the NB in a co-current flow. The effluent of NB with nitrogen oxide (NO_x) was pumped into DB for denitrification. The final effluent of DB was collected in a storage tank to provide backwash water. Both NB and DB were backwashed every 72 h. The backwash sequence included air scour (5 min), followed by combined air scour and water backwash (10 min). The water and air backwash intensity were set at 5 and 12 $L/m^{-2}\ s^{-1}$, respectively.

2.2. Raw wastewater

The test wastewater was collected from the outlet of the secondary sedimentation tank in the Jinan wastewater treatment plant, Shandong province. The main characteristics of the wastewater are summarized in Table 1.

2.3. Operating conditions

The nitrification performance of NB was studied under low and high aeration rate conditions. The selection of aeration rate was mainly based on published empirical parameters of dissolved oxygen. According to Sutton et al. [27], an average DO concentration in the effluent liquid of at least 3 mg/L should enable the nitrifying biofilm activity. Published results about BAFs treating synthetic and actual wastewater demonstrated that satisfactory nitrification can be achieved if the average bulk DO concentration in the reactor is maintained at approximately 4 mg/L [15,17,19]. On this basis, we adjusted the aeration rate and conducted oxygen tests. The results indicated that when the aeration rate was controlled at 0.6 and 1.0 m^3/h, respectively, the requirement of low and high oxygen concentration mentioned above could be met in the reactor. So, in this study, we selected 0.6 and 1.0 m^3/h as low and high aeration rates for comparison.

The experiment was divided into two stages. Stages one and two were lasted 90 and 99 d, respectively. The aeration rate of NB in stage one was controlled at 0.6 m^3/h, and that in stage two was controlled at 1.0 m^3/h. During each stage, the feed flow rate was kept identical and controlled in the range of $2.0 \pm 0.1\ m^3/h$. The corresponding hydraulic load and hydraulic retention time (HRT) were 4.5 (m^3/m^2 h) and 2.3 h. The water temperature in stage one varied from 18.2 to 25.4°C, and that in stage two varied from 16.9 to 26.2°C throughout the experiment. There was no significant difference in the temperature of the two stages.

2.4. Chemical and physical analysis

Ammonia (NH_4^+-N), nitrate (NO_3^--N), nitrite (NO_2^--N), chemical oxygen demand (COD), suspended solid (SS), total nitrogen (TN) and total phosphorus (TP) were analyzed according to standard methods [28]. DO was measured by chemical iodometry method (GB/T 2489-1987, China). The temperature and pH were monitored with a PHS-3C pH meter

(Shanghai prcecison & scientific Instrument Co., Ltd, China). Turbidity was detected by a SGZ-1A Turbidity-meter (Shanghai Yuefeng Instrument & Meters Co., Ltd, China). Microscopic examination of biofacies was performed using an OLYMPUS-CX31 microscope (OLYMPUS Corporation, Japan).

The concentration of biomass was determined and differentiated into two forms: attached and suspended biomass. According to Villaverde [20], the fraction of solids that can be sloughed from the substratum particles by gentle rinsing with distilled water is considered the suspended biomass, and the rest of the biomass remaining on the particles is considered the fraction of attached biomass. The volatile biomass of the two forms of biosolid was determined from the difference between the masses of total dry matter (dried at 105°C) and the mass of the residual of dry matter after heating to 550°C.

2.5. Activity tests

The specific activities of nitrifying and heterotrophic bacteria in terms of oxygen uptake rates (OUR), were determined by closed respirometry. The respirometer consists of a 500 ml plastic container with inlets for inserting oxygen electrode (YSI-50B, Yellow Springs Instrument Co. Inc, USA) and pH probe, and introducing the sample and substrates. The oxygen was supplied through a porous aerator. A magnetic stirrer was used to provide mixing conditions inside the reactor.

The experiment was carried out at room temperature. The specific OUR test method was that of Giudada [29] and Villaverde [20]. The DO concentration in the reactor was controlled in the range of 4–8 mg/L. The endogenous OUR of the sample was measured firstly for 40 min without substrate. The oxygen concentration was recorded at intervals of 2 mins. Glucose, ammonium sulphate and sodium nitrite were used as the specific substrates for determining the activity of heterotrophs, ammonia and nitrite oxidizers, respectively. The injection sequence of the substrate was as follows: the first injection of sodium nitrite was followed by ammonium sulphate and finally the injection of glucose. According to the kinetics principles provided by Cech [30] and Chudoba [31], the initial substrate concentrations of NH_4^+-N, NO_2^--N and COD in the reactor were controlled at 10, 5 and 30 mg/L. The measurements of OUR of heterotrophs, ammonia and nitrite oxidizers with substrate all lasted for 40 minutes. All the batch experiments were performed with duplicate samples. The final results for the specific activities are expressed as milligrams of O_2 consumed per gram of volatile solids per hour (mg O_2·g VS^{-1} h^{-1}).

2.6. Extraction and counting of nitrifying bacteria

To obtain a sample of nitrifying bacteria for cell counting, 20 g of fresh ceramic media was weighed and gently rinsed with distilled water. The liquid containing suspended biosolids was collected and poured into a 300 mL of conical beaker. Several sterile beads were added. The liquid was sealed and kept vibrating on a shaker at 4°C for 2 h (125 cycles/min). A bacteria suspension of suspended biosolid was prepared. For the attached biofilm, a method of extracting bacteria [32] was applied to extract the bacteria from the biofilm. Rinsed media with attached biomass was put into a 500 mL of beaker and 200 mL of 50 mmol/L sodium pyrophosphate solution was added. After 10 min of stabilization, the solution was agitated by a mechanical agitator at the maximum speed for 10 min in an ice bath. The mixed liquid with stripped biofilm was then separated. The further preparation of a bacteria suspension of attached biofilm was the same as for the suspended biosolids. All the containers, tools and solutions were sterilized in an autoclave.

AOB and NOB were counted by most probable number (MPN)-Griess method [33]. First, 1 mL of sample was taken from the bacteria suspension. Serial 10-fold dilutions of the sample were prepared in sterile distilled water and 1 ml portions were transferred to MPN tubes containing 9 ml of the enumeration medium. Five replicate tubes were prepared per dilution for enumeration. The composition of enumeration media for AOB and NOB are the same as in Li [33]. Inoculated MPN tubes were incubated for 5 weeks at 28°C. The presence and disappearance of nitrite in each tube were checked by colour determination after adding zinc powder and Griess reagent.

3. Results

3.1. The distribution of DO in NB

The bulk DO profiles along the column of NB for the two aeration rates (Fig. 3) increased along the height of the column and the highest bulk DO concentration was achieved at the top of the filter. The height of 1.2 m ($1^{\#}$ sampling port) is the position of the bottom of the media layer, which is located at 10 cm above the aeration point. As the aeration rate in stage one was controlled at 0.6 m^3/h, the average DO concentrations varied from 0.82 to 3.05 mg/L along the media bottom to the height of 5.4 m ($8^{\#}$ sampling port). In stage two, the average concentrations of $DO_{(1.2m)}$ and $DO_{(5.4m)}$ were increased to 1.62 and 4.88 mg/L, respectively resulting from the aeration rate being raised to 1.0 m^3/h.

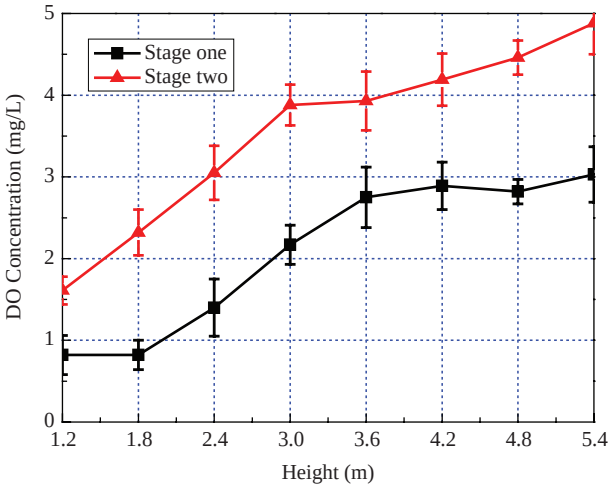

Fig. 3. The distribution profiles of bulk DO concentration in NB under two aeration conditions (Stage one, aeration rate = 0.6 m^3/h; Stage two, aeration rate = 1.0 m^3/h).

3.2. Nitrification performance

The ammonia and NO_3^--N of influent and effluent in NB during the two stages are presented in Fig. 4.

During the 90 d of stage one, the influent ammonia varied from 10.3 to 20.4 mg/L. The corresponding influent ammonia loads varied within the range of 0.16–0.31 kgNH_4^+-N/m^3·d (average 0.22 kgNH_4^+-N/m^3·d). NB showed 60.6–72.2% (average 64.4%) ammonia removal rate at aeration rate of 0.6 m^3/h. The ammonia in the effluent was in the range of 3.5–7.9 mg/L (average 5.4 mg/L). The nitrification efficiency of the process was relatively low and showed a certain fluctuation with the influent ammonia loads. Most of the oxidized ammonia in the effluent was in the form of NO_3^--N while little NO_2^--N was present. The average concentrations of NO_2^--N and NO_3^--N in the effluent were about 0.4 and 16.7 mg/L, respectively.

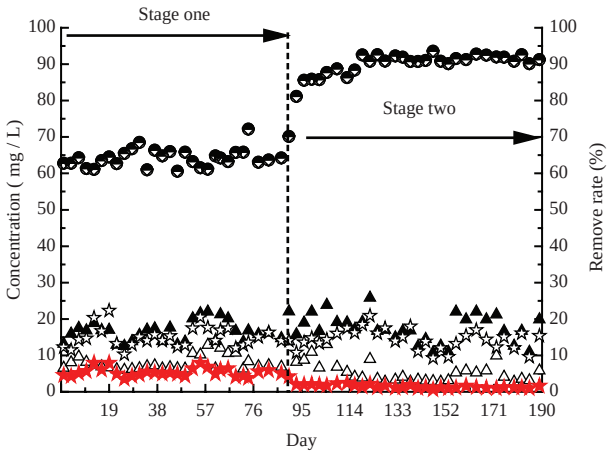

Fig. 4. The profiles of ammonia oxidization under two different aeration conditions in NB (Stage one, aeration rate = 0.6 m^3/h; Stage two, aeration rate = 1.0 m^3/h. The symbols of (★) and (★) represent the concentrations of influent and effluent NH_4^+ –N, (△) and (▲) represent the concentrations of influent and effluent NO_3^- –N, (◓) represents the remove rate of NH_4^+ –N, respectively)

During the 99 d of stage two, the aeration rate was adjusted to 1.0 m^3/h to investigate the effect of an increase of aeration rate on the nitrification efficiency of NB. At this stage, the influent ammonia load showed no significant change and varied within the range of 0.14–0.34 kgNH_4^+-N/m^3·d. At the initial 28 d, the ammonia removal rate kept increasing. After 28 d, the efficiency was over 90% and remained stable. The total nitrification efficiency was in the range of 90.1–93.5% (average 91.6%) during the steady state of stage two. Compared with stage one, NB showed a strong adaptability to the shock of ammonia load, and the effluent ammonia remained almost stable in the range of 0.6–1.9 mg/L (average 1.3 mg/L). Just as in stage one, the majority of the ammonia removed was converted to NO_3^--N. However, due to the fluctuation of influent ammonia, the effluent NO_3^--N was unstable. We found the NO_3^--N in the effluent was in the range of 10.8–25.8 mg/L. The average concentration of NO_2^--N in the effluent was below 0.2 mg/L.

The profiles of ammonia removal and NO_2^--N accumulation along the column are shown in Fig. 4. The nitrite accumulation ratio was calculated as follows:

$$\text{Nitrite accumulation ratio (NAR) \%} = \frac{NO_2^- - N}{NO_2^- - N + NO_3^- - N} \times 100\%. \quad (1)$$

In stage one (Fig. 5a), as the influent ammonia loads were in the range of 0.16–0.31 kgNH_4^+-N/m^3 d, up to 37.4% of ammonia was removed in the first 1.2 m corresponding to a 64.4% nitrification efficiency of the whole filter. In this case, incomplete nitrification was achieved and the highest nitrite accumulation ratio of 38.4% (average) was observed at the height of 1.8 m corresponding to the bulk DO concentration of 0.82 mg/L. Above the height of 2.4 m, as the average bulk DO concentration was over 1.42 mg/L, the nitrite accumulation ratio decreased to 2.9–3.3%. In stage two (Fig. 5b), for almost the same influent ammonia loads as stage one, more than 91.6% nitrification efficiency was shown in NB and the ammonia removal rate in the first 1.2 m was increased up to 52.7%. Compared with stage one, the average bulk DO concentration range in the first 1.2 m of the filter increased from 0.82–1.42 to

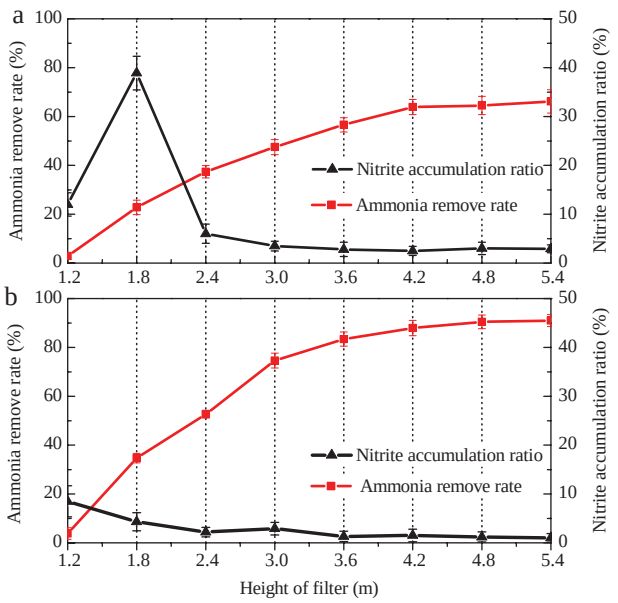

Fig. 5. Profiles of ammonia removal and nitrite accumulation along the column in the filter under two aeration conditions (a, stage one, aeration rate = 0.6 m^3/h; b, stage two, aeration rate = 1.0 m^3/h).

1.62–3.05 mg/L and there was no significant NO_2^--N accumulation. Moreover, with the rise of aeration rate, a 12.9% (average) increase of nitrification efficiency was observed at the middle and top regions (2.4–4.8 m) of the filter.

The ratios between NO_x^--N (NO_2^--N+NO_3^--N) production and ammonia removal along the height of the filter are shown in Fig. 6. In stage one, about 98.6% of removed ammonia was oxidized into NO_2^--N

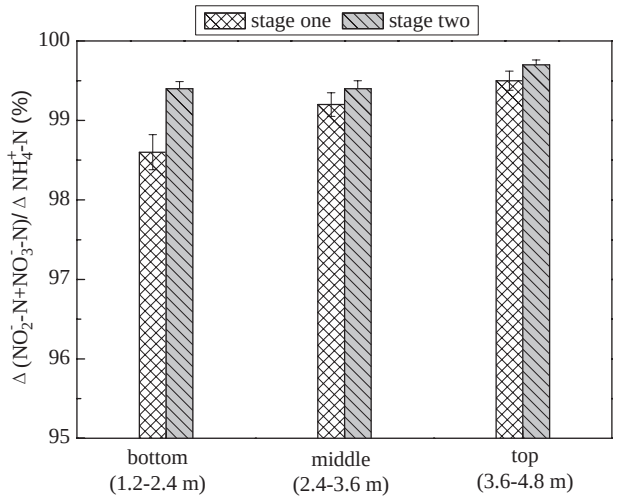

Fig. 6. Ratios between NO_x^--N (NO_2^--N+NO_3^--N) production and ammonia removal along the height of filter in the two test stages.

and NO_3^--N at the bottom region (1.2–2.4 m) of the filter. However, at the middle and top region (2.4–4.8 m), the production of NO_2^--N and NO_3^--N accounted for 99.2–99.5% of the removed ammonia. In stage two, more than 99.4–99.7% of removed ammonia was oxidized into NO_2^--N and NO_3^--N in the whole reactor. According to the results of Figs. 5 and 6, it can be observed that a relatively high loss of nitrogen occurred at the bottom region (1.2–2.4 m) of the filter in stage one, corresponding to the higher nitrite accumulation ratio.

3.3. Removal of chemical oxygen demond (COD)

During both test stages, the influent COD varied within the range of 19.6–42.2 mg/L (average, 23.5 mg/L), and the corresponding volumetric loads were in the range of 0.31–0.65 kg COD/m^3·day (average 0.36 kg COD/m^3·day). For the aeration rates of 0.6 and 1.0 m^3/h, NB showed 25.2% and 31.6% COD removal, respectively.

3.4. The detection of microorganism activity

The microbial activity tests were carried out during the steady state of stage one (from the 65th day to the 79th day) and stage two (from the 164th day to the 176th day). The profiles of volatile biomass and specific activities of AOB, NOB and heterotrophic bacteria of the two forms of biosolid along the height of the filter at two different aeration rates (0.6 and 1.0 m^3/h) are summarized in Tables 2 and 3.

According to Table 2, the suspended biosolids showed higher ammonia oxidizing activity but lower nitrite oxidizing activity than the attached biofilm at the aeration rate of 0.6 m^3/h. Higher specific activity of AOB was observed at the bottom region of the filter (from 1.2 to 2.4 m). This was consistent with the high ammonia removal performance of the bottom region shown by Fig. 4. At heights above 2.4 m, the activities of ammonia oxidization and nitrite oxidization of the two forms of biosolid all declined along the column because of the gradual decrease of substrate. In addition, Table 2 also shows that the specific activity of NOB was lower in the first 0.6 m of the filter at aeration rate of 0.6 m^3/h. Higher specific activity of AOB and lower specific activity of NOB accounted for the nitrite accumulation of the bottom region in stage one (Fig. 4).Table 3 shows that, as the aeration rate was raised to 1.0 m^3/h, the activities of ammonia oxidization and nitrite oxidization of attached biofilm at the bottom region of the filter (1.2–2.4 m) increased by 2.3 and 3.7 times (average) respectively, while those of suspended biosolid only increased by 3.3% and

Table 2
Profiles of biomass and specific activity of attached biofilm and suspended biosolid in the UBAF at an aeration rate of 0.6 m^3/h

	Attached biofilm					Suspended biosolids				
		Specific activity (mg $O_2 \cdot gVS^{-1}h^{-1}$)					Specific activity (mg $O_2 \cdot gVS^{-1}h^{-1}$)			
Height (m)	Biomass (VS) (mg /g_{media})	Endogenous	Ammonia oxidization	Nitrite oxidization	Heterotrophy activity	Biomass (VS) (mg/g_{media})	Endogenous	Ammonia oxidization	Nitrite oxidization	Heterotrophy Activity
1.2	6.61[a] (0.35[b])	1.09 (0.22)	1.21 (0.18)	0.29 (0.15)	0.31 (0.11)	1.63 (0.32)	2.58 (0.36)	4.89 (0.52)	0.26 (0.13)	2.01 (0.16)
1.8	6.38 (0.44)	1.01 (0.29)	1.14 (0.22)	0.34 (0.13)	0.28 (0.09)	1.13 (0.55)	2.71 (0.46)	4.83 (0.44)	0.22 (0.10)	2.07 (0.22)
2.4	5.89 (0.57)	1.42 (0.38)	1.95 (0.31)	0.95 (0.22)	0.36 (0.12)	0.96 (0.38)	2.86 (0.38)	4.95 (0.32)	0.38 (0.14)	1.65 (0.31)
3.0	4.45 (0.36)	1.35 (0.22)	1.79 (0.29)	0.92 (0.19)	0.32 (0.15)	0.53 (0.18)	3.42 (0.28)	3.93 (0.37)	0.29 (0.09)	1.33 (0.24)
3.6	3.61 (0.35)	1.63 (0.41)	1.62 (0.21)	0.78 (0.25)	0.36 (0.15)	0.39 (0.14)	3.57 (0.24)	2.82 (0.41)	0.25 (0.11)	1.42 (0.28)
4.2	3.01 (0.25)	1.77 (0.35)	1.11 (0.22)	0.59 (0.19)	0.25 (0.12)	0.33 (0.23)	4.12 (0.31)	2.29 (0.33)	0.19 (0.10)	1.14 (0.18)
4.8	2.78 (0.23)	1.74 (0.29)	0.92 (0.19)	0.51 (0.28)	0.26 (0.14)	0.26 (0.09)	3.95 (0.19)	2.05 (0.31)	0.21 (0.14)	0.98 (0.20)

Note: Investigating period was from the 65th to 79th day and the sample time was at 2 h before backwash. a, average; b, standard deviation ($n = 4$).

Table 3
Profiles of biomass and specific activity of attached biofilm and suspended biosolid in the UBAF at an aeration rate of 1.0 m^3/h

	Attached biofilm					Suspended biosolids				
		Specific activity (mg $O_2 \cdot gVS^{-1}h^{-1}$)					Specific activity (mg $O_2 \cdot gVS^{-1}h^{-1}$)			
Height (m)	Biomass (VS) (mg /g_{media})	Endogenous	Ammonia oxidization	Nitrite oxidization	Heterotrophy activity	Biomass (VS) (mg/g_{media})	Endogenous	Ammonia oxidization	Nitrite oxidization	Heterotrophy activity
1.2	6.78[a] (0.25[b])	1.38 (0.35)	2.78 (0.14)	1.51 (0.24)	0.36 (0.14)	1.79 (0.24)	2.47 (0.22)	4.77 (0.28)	0.29 (0.11)	1.95 (0.27)
1.8	6.53 (0.39)	1.57 (0.64)	3.04 (0.17)	1.44 (0.18)	0.44 (0.22)	1.26 (0.25)	3.01 (0.35)	5.06 (0.38)	0.35 (0.13)	2.24 (0.19)
2.4	6.02 (0.41)	2.27 (0.48)	3.44 (0.22)	1.56 (0.22)	0.47 (0.16)	0.95 (0.32)	3.23 (0.29)	5.04 (0.29)	0.39(0.21)	1.58 (0.11)
3.0	4.78 (0.35)	2.54 (0.36)	1.87 (0.29)	0.98 (0.25)	0.33 (0.20)	0.47 (0.15)	3.78 (0.39)	3.75 (0.24)	0.41 (0.17)	1.42 (0.20)
3.6	4.02 (0.30)	3.17 (0.22)	1.69 (0.24)	0.69 (0.14)	0.44 (0.19)	0.43 (0.12)	5.02 (0.40)	2.83 (0.33)	0.29 (0.19)	1.29 (0.24)
4.2	3.49 (0.21)	3.19 (0.32)	1.39 (0.22)	0.73 (0.30)	0.39 (0.22)	0.39 (0.23)	4.35 (0.24)	2.36 (0.19)	0.19 (0.11)	1.23 (0.21)
4.8	2.94 (0.25)	3.28 (0.24)	1.26 (0.33)	0.62 (0.25)	0.34 (0.17)	0.35 (0.11)	4.76 (0.35)	2.09 (0.19)	0.22 (0.08)	0.79 (0.15)

Note: Investigating period was from the 164th to 176th day and the sample time was at 2 h before backwash. a, average; b,standard deviation ($n = 4$).

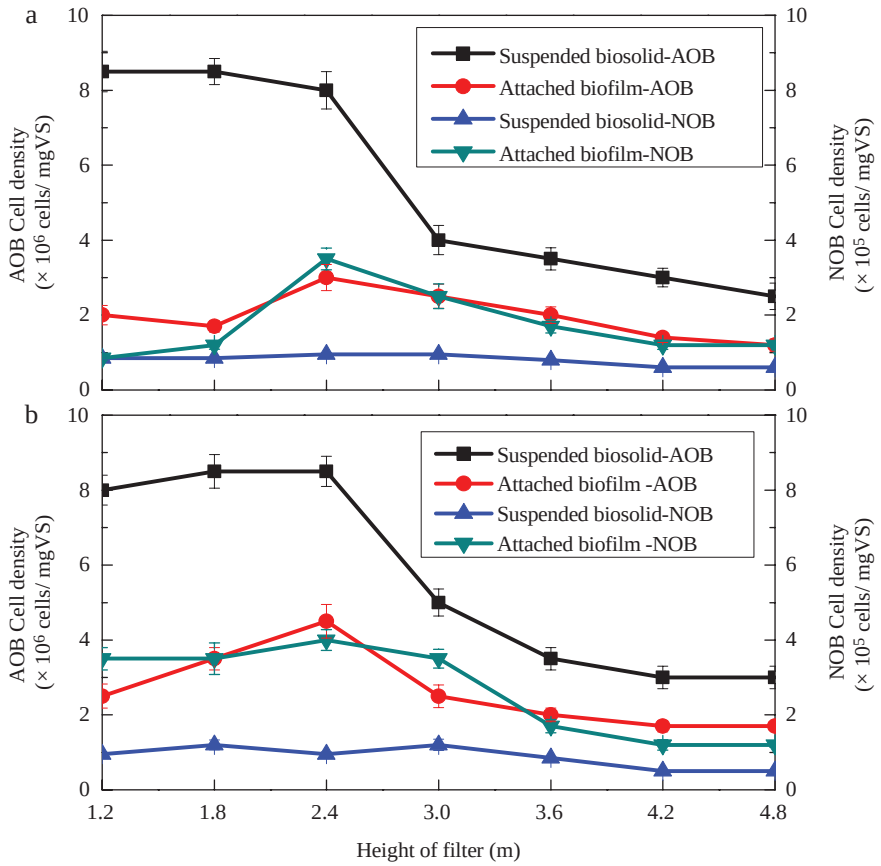

Fig. 7. Bacteria cell counts at the steady state of both test stages (a, stage one, aeration rate = 0.6 m^3/h; b, stage two, aeration rate = 1.0 m^3/h).

35.2% (average). The biomass measurements in Tables 2 and 3 reveal that attached biomass was more than five times higher than suspended biomass at the bottom region in the two test stages. So, it can be predicted that the buildup of nitrification efficiency of the bottom region in stage two (Fig. 3) was mainly from the improvement of the nitrifying activity of attached biofilm. For the middle and top region of the filter, the buildup of nitrification efficiency was also mainly achieved by the nitrifiers on the attached biofilm.

It also can be observed in Table 2 that the suspended biosolids showed higher heterotroph and endogenous activity than the attached biofilm at the low aeration rate. As the aeration rate was raised to 1.0 m^3/h, the average heterotrophy and endogenous activities on the suspended biosolids increased by 1.6% and 14.5%, while those on the attached biofilm increased by 29.3% and 74.1%, respectively (Tables 2 and 3). Just as for the ammonia oxidization activity, the heterotroph activity of the suspended biosolids also showed no significant variation in stage two.

3.4. The variation of nitrifier quantity

Bacteria counts were made during the same steady-state periods of investigation as above. Fig. 7a shows that the initial cell densities (average) of AOB and NOB of suspended biosolid at the height of 1.2 m were 8.48×10^6 and 8.03×10^4 cells/mgVS, and those of attached biofilm were 2.04×10^6 and 8.52×10^4 cells/mgVS, respectively. From the height of 1.2 to 2.4 m, the average cell densities of AOB and NOB of attached biofilm increased by 1.5 and 4.2 times while those of suspended biosolid showed no significant variations. At heights above 2.4 m, the average cell densities of AOB and NOB in the two forms of biosolid all declined along the column. The total amount of AOB and NOB on the whole retained biomass could be evaluated by the following equation

$$TNB = \frac{M}{n} \cdot \sum_{i=1}^{n} (D_S i \cdot VS_i + D_{Ai} \cdot VA_i) \quad (2)$$

where TNB is the total amount of bacteria, *cells*; i stands for sampling cross-section number; $D_S i$ and $D_A i$ are the

Table 4
Calculation results of total amount of AOB and NOB in the whole retained biomass for stage one (aeration rate = 0.6 m^3/h) and stage two (aeration rate =1.0 m^3/h)

	AOB (cells, $\times 10^{13}$)			NOB (cells, $\times 10^{12}$)		
	Suspended	Attached	Total	Suspended	Attached	Total
Stage one	1.69[a] (0.53[b])	2.62 (0.49)	4.31 (0.78)	0.39 (0.12)	3.86 (0.57)	4.25 (0.58)
Stage two	1.87 (0.48)	3.64 (0.55)	5.51 (0.84)	0.48 (0.23)	6.47 (0.68)	6.95 (0.79)

Note: a, average, b, standard deviation (SD), $n = 4$.

cell density of nitrifier for suspended and attached biomass at the different sampling cross-section, cells/mgVSS; VS_i and VA_i are the volatile biomass of suspended biosolids and attached biofilm per mass of media, mgVSS/$g_{\text{-media}}$; and M is the total mass of media, kg ($M = \rho \cdot \pi \cdot r^2 \cdot h, \rho = 1.03\times10^3$ kg/m^3).

The calculation results summarized in Table 4 show that the amount of AOB of suspended biosolids and attached biofilm in stage one accounted for approximately 39.2% and 60.8% of the total AOB on the whole retained biomass. Corresponding to its higher ammonia oxidizing activity, suspended biosolid showed higher AOB density. But the total amount of AOB on the suspended biosolids was still lower than that of the attached biofilm because of the smaller biomass. As the aeration rate was increased to 1.0 m^3/h, the total amount of AOB in the attached biofilm increased by 38.9%, while that of suspended biosolids only increased by 10.6%. In stage two, the proportion of AOB on the suspended biosolids dropped from 39.2% to 33.8%, while that of the attached biofilm increased from 60.8% to 66.2%. The calculation results of NOB showed that only about 9.2% of total NOB was in the suspended biosolids at an aeration rate of 0.6 m^3/h. With the rise of aeration rate, the NOB amount of suspended biosolids increased by 23.1%, while that of attached biofilm increased by 67.7%. In stage two, more than 93.1% of the total NOB was present in the attached biofilm. It can be seen that the growth rate of AOB and NOB in the attached biofilm was much greater than that of the suspended biosolids as the aeration rate was raised from 0.6 to 1.0 m^3/h. The variation of nitrifier amount of the two forms of biosolid was basically consistent with the variation of their nitrifying activities.

4. Discussion

4.1. The influence of aeration on the nitrification of NB

In stage one, for the aeration rate of 0.6 m^3/h, NB showed a 60.6–72.2% (average 64.4%) nitrification efficiency with the bulk DO concentration of 0.82–3.05 mg/L (average 2.18 mg/L). In this case, shortcut nitrification and relatively high loss of nitrogen were observed in the first 1.2 m of the filter (Figs. 5 and 6). Published results demonstrated that oxygen level for nitrite accumulation is in the range of 0.5–1.5 mg/L for suspended culture [9,34,35], and 0.8–2.0 mg/L for the biofilm system [6,11]. Therefore, the low bulk DO concentration (0.82–1.42 mg/L) at the bottom region of the filter (1.2–2.4 m) was expected to be the main factor limiting the oxidization of nitrite in the reactor. Tallec [36] reported that when the bulk DO concentration in the biofilter was in the range of 0.5–1.0 mg/L, nitrifier denitrification would take place and nitrous oxide (N_2O) could be produced. The bulk DO concentration level (0.82–1.42 mg/L) of the bottom region in stage one is favorable for autotrophic bacteria to carry out nitrifier denitrification and reduce nitrite into N_2O in the reactor. So, it can be speculated that the relatively high loss of nitrogen occurring at the bottom region of the filter in stage one might be a consequence of nitrifier denitrification resulting from low oxygen concentrations. As the aeration rate was raised to 1.0 m^3/h, the total nitrification efficiency was increased to 91.6% (average) and no significant nitrite accumulation was deteced in the whole reactor. This means the oxidization of nitrite was not inhibited in the reactor at an aeration rate of 1.0 m^3/h. As a co-substrate of nitrification, the bulk concentration of DO influences the reaction rates of both ammonia oxidization and nitrite oxidization [38–40]. Sufficient concentration of DO is essential to maintain an effective nitrifying biofilm reactor [37,41]. Chen [14] reported the nitrification performance of a nitrifying biofilm reactor with high ammonia loads in synthetic wastewater and suggested the bulk concentration of DO to be maintained in the range of 4–5 mg/L. Chui [15] studied the nitrification performance of an upflow fixed bed filter with volumetric loading rate of 1.7 kgCOD/m^3.day and 0.33 kgN/m^3.day, and found that nitrification was inhibited as the average bulk DO was below 3 mg/L. This study demonstrated, for secondary effluent with an average

volumetric loading rate of 0.36 kgCOD/m^3·day and 0.22 kgNH_4-N/m^3·day, sufficient bulk DO concentration (average 3.82 mg/L) is also necessary for the UBAF process to achieve a satisfactory nitrification efficiency (average 91.6%).

Fig. 4 illustrates that, in stage one, up to 37.4% of ammonia was removed at the bottom region of the filter (1.2–2.4 m) corresponding to a 64.4% nitrification efficiency of the whole reactor. The plentiful ammonia of the influent and the high oxygen affinity caused the ammonia oxidizing bacteria at the bottom region of the filter to play high-efficiency roles in the conversion of ammonia even though the average bulk concentration of DO was less than 1.42 mg/L. In this case, more than 40.2% (average) of ammonia oxidization efficiency was achieved by the bottom suspended biosolid whose biomass only accounted for 16.3% (average) of the total bottom biomass (Table 2). It can be seen that suspended biosolids made great contributions to the nitrification of UBAF under the low oxygen conditions. With the rise of aeration rate, the efficiency and stability of nitrification of UBAF improved significantly (Figs. 3 and 4). The increase of bulk DO concentration and stronger turbulence accompanying with the rise of aeration rate could increase volumetric nitrification rates by improving the oxygen diffusion and advective transport in the granular media [13]. In spite of this, the effect of aeration on the nitrification mainly relied on the function of nitrifier activity. The specific activities presented in Tables 2 and 3 reveal that the buildup of nitrification efficiency in stage two was mainly from the improvement of the nitrifying activity of attached biofilm. Suspended biosolids showed little increase of nitrifying activity as the aeration rate was raised from 0.6 to 1.0 m^3/h. The nitrifying activitiy of both attached biofilm and suspended biosolids was mainly dependent on their active biomass. Compared with attached biofilm, suspended biosolids were more susceptible to backwashing and showed better wash-out effects. This might cause more loss of active biomass and limit the growth of nitrifying bacteria on the suspended biosolids. The increase of nitrifying activity of the attached biofilm increases the shock resistance capacity of NB, and thus maintains the stability of the nitrification performance in stage two.

In a BAF process, heterotrophic bacteria are the main competitors with nitrifiers for oxygen. However, in this study, because of the low organic loading of the influent, the total activity level of heterotrophic bacteria observed in the filter was relatively low (Tables 2 and 3). As for nitrifying activity, the improvement of oxygen conditions showed little effect on the heterotroph activity of the suspended biosolids. The role of backwashing is also suggested to be the main factor limiting the growth of heterotrophs on suspended biosolids. In contrast, the heterotroph activity on the attached biofilm increased more as the aeration rate was raised from 0.6 to 1.0 m^3/h. It was analyzed that the buildup of heterotroph activity on the attached biofilm may be arisen from two aspects. Firstly, the increase of oxygen concentration and diffusion efficiency could strengthen the utilization of influent organic substrate by heterotrophic bacteria on the attached biofilm. Secondly, it has been reported that heterotrophic bacteria in an autotrophic nitrifying biofilm could use soluble microbe products (SMP) produced or released by nitrifiers as growth substrate [42,43]. Therefore, the improvement of nitrifying activity on the attached biofilm might simultaneously stimulate the growth of heterotrophic bacteria via production of more SMP.

4.2. The influence of aeration on the distribution of nitrifying bacteria

The results of AOB enumeration (Table 4) demonstrated that attached biofilm showed a higher proportion of AOB on the whole retained biomass than that of the suspended biosolids in the two test stages. The growth rate of AOB in the attached biofilm was obviously greater than that of the suspended biosolids with the improvement of oxygen conditions.

From the viewpoint of mass transfer, the growth of AOB of the suspended biosolids is dominant over that of the attached biofilm because better transport conditions exist in the liquid phase than those encountered within the biofilm. Nevertheless, a better wash-out effect makes the growth of AOB of suspended biosolids more depends on the retention time of the suspended biosolids in the filter. With regards to the distribution of AOB in UBAF, Villaverde [20] reported that as much as 78% of ammonia was removed by the suspended biosolid and most of the ammonia oxidizers were suggested to be suspended instead of attached to the substratum particles. In the work of Villaverde, backwash was conducted weekly, and the biomass ratio between suspended biosolids and attached biofilm was in the range of 0.5–1.6. This was much greater than the biomass ratio of 0.11–0.26 of our study. So, the relatively smaller proportion and lower growth rate of AOB amount of suspended biosolid in the filter might be a consequence of frequent backwash (3 d) of this study. The limitation of the growth of AOB on the suspended biosolids provides more opportunities to the growth of AOB on the attached biofilm. With the improvement of mass transfer efficiency of oxygen and substrate within the biofilm, the growth of AOB of the attached biofilm gradually becomes dominant because immobilization

is advantageous. The influence of backwashing on the growth and distribution of AOB needs to be further investigated.

The results of NOB enumeration (Table 4) indicated that majority of NOB (90.2–93.1%) was distributed on the attached biofilm in the two test stages. The increase of aeration rate showed little effect on the growth of NOB on the suspended biosolids.

Just as AOB, the growth of NOB in the suspended biosolids might be also limited by the frequent backwashing in this study. In addition, a much lower proportion of NOB of suspended biosolid indicated that NOB is more inclined to grow in the attached biofilm than is AOB at the two aeration conditions. This distribution of NOB is consistent with the report of Villaverde [20]. With regard to the reasons for the high accumulation of NOB in the attached biofilm, Villaverde proposed that the growth conditions that NOB encounters within the biofilm including the presence of nitrite, oxygen and absence of free ammonia are better than in the liquid phase. Compared with AOB, NOB showed relatively lower oxygen affinity [7]. Published results showed that most of AOB were present throughout the biofilms, while the nitrite oxidizing bacteria were restricted to the inner parts of the biofilms [44,45]. This means more effective transport of oxygen is vital to maintain the growth of NOB. Considering the mass transport conditions in liquid phase are better than those within the biofilm [20], the mass transfer efficiency of oxygen in suspended biosolid is suggested to be higher than that in the attached biofilm. By investigating the effects of DO on the distribution of NOB in the filter, it can be predicted that oxygen is not the main reason limiting the growth of NOB on the suspended biosolids at the aeration rate of 1.0 m^3/h. We believe that the concentrations of nitrite and free ammonia in liquid phase might be the other important factor limiting the growth of NOB on the suspended biosolid at the higher aeration condition.

5. Conclusions

Based on the results of this study, the following conclusions may be drawn:

1. It has been demonstrated that the increase of aeration rate not only increased the ammonia removal rate, but also improved the stability of nitrification performance of the UBAF. For the secondary effluent of municipal sewage with the volumetric loading rate of 0.31–0.65 kgCOD/m^3·day and 0.14–0.34 kgNH_4-N/m^3·day, more than 91.6% nitrification efficiency can be achieved at an aeration rate of 1.0 m^3/h with average bulk DO concentration of 3.82 mg/L.
2. The improvement of nitrification performance of the UBAF with the rise of aeration rate was mainly from the buildup of nitrifying activity of the attached biofilm.
3. NOB was more inclined to grow on the attached biofilm than AOB at the two aeration rates. The increase of aeration rate from 0.6 to 1.0 m^3/h greatly stimulated the growth of AOB and NOB of attached biofilm, but showed little effects on the growth of AOB and NOB of the suspended biosolid. We suggest that the growth of the AOB and NOB on the suspended biosolids might be limited by the frequent backwashing employed in this study. In addition, the concentrations of nitrite and free ammonia in liquid phase are also considered to be an important limiting factor for the growth of NOB on the suspended biosolids.

Acknowledgements

This research was financially supported by the National Major Fundamental Research Program of China (Grant No.2007CB416600); the Australia-China Special Fund (2006DFA93110); Key Research Foundation of Shandong Provincial Environmental Protection Bureau (hcyf0602) and Project of Shandong Provincial Environmental Protection Bureau (2006034). The authors thank Dr. Pamela Holt for revising the manuscript.

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