

Partial nitrification of non-ammonium-rich wastewater within biofilm filters under ambient temperature

Hongyu Wang, Jiajie He and Kai Yang

ABSTRACT

This study evaluated the partial nitrification performances of two biofilm filters over a synthetic non-ammonium-rich wastewater at a 20°C room temperature under both limited DO (~2.0 mg/L) and unlimited DO (~4.0 mg/L) conditions. The two filters were each of 80 cm long and used different biofilm carriers: activated carbon and ceramic granule. Results showed that partial nitrification was accomplished for both filters under the limited DO condition. However, the effluent NO₂-N was higher in the ceramic granule filter than in the activated carbon filter, and was less susceptible to the influent COD/N changes. Further investigation into the water phase COD and NH₄-N depth profiles and bacteria population within the two filters showed that by putting upper filter layer (upstream) to confront relatively higher influent COD/N ratios, the filtration process naturally put lower filter layers (downstream) relatively more favorable for nitrifying bacteria (ammonia oxidizing bacteria in this study) to prosper, making the filter depth left for nitrification a crucial factor for the effectiveness of nitrification with a filter. The potentially different porous flow velocities of the two filters might be the reason to cause their different partial nitrification performances, with a lower porous flow velocity (the ceramic granule filter) favoring partial nitrification more. In summation, DO, filter depth, and filtration speed should be played together to successfully operate a biofilm filter for partial nitrification.

Key words | activated carbon, biofilm, ceramic granule, filtration, partial nitrification

Hongyu Wang (corresponding author)

Kai Yang

School of Civil Engineering,
Wuhan University,
430072 Wuhan,
China

E-mail: hywangwhu@yahoo.com;
yangkaiz@163.com

Jiajie He

Biosystems Engineering Department,
Auburn University,
36849 Auburn,
Alabama,
USA

E-mail: hejjaji@auburn.edu

INTRODUCTION

It is anticipated that 40–50% energy in nitrification and carbon source consumption can be saved if biological nitrification and denitrification can be controlled via NO₂-N instead of NO₃-N (Turk & Mavinic 1986; Abeling & Seyfried 1992; Yoo *et al.* 1999; Fux *et al.* 2002). Controlling biological nitrification to stop at NO₂-N (partial nitrification) was often accomplished by exploiting the differences between ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in their sensitivities to environment changes such as temperature (Jetten *et al.* 1997) and DO (Hanaki *et al.* 1990). It has been observed that temperature raise can exert inhibitory effect on NOB, but not on AOB (Jetten *et al.* 1997; Wu *et al.* 2007). It normally

requires the reactor temperature to be elevated above 30°C in order to attain an obvious selection between AOB and NOB (Jetten *et al.* 1997; Hellinga *et al.* 1998). It has also been consistently observed that AOB can sustain under broader DO levels than NOB, and AOB can sustain better than NOB under limited DO conditions (Hanaki *et al.* 1990; Wiesmann 1994; Wu *et al.* 2007). Nevertheless, due to economic considerations, water temperature is not practically to be modified and controlled in a full-scale reactor from the engineering point of view. Therefore, biological partial nitrification was more often economically realized by limited aeration under room temperatures (Peng *et al.* 2007).

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There also exists a natural competition for ammonium nitrogen ($\text{NH}_4\text{-N}$) between nitrifying bacteria and heterotrophic bacteria (Verhagen & Laanbroek 1991; Strauss & Lamberti 2000). Abundant carbon supply can stimulate heterotrophic bacteria growth and thus putting nitrifying bacteria out of compete, while limited carbon supply can make heterotrophic bacteria not ammonium-limited and let nitrifying bacteria to compete successfully for nitrification to occur (Verhagen & Laanbroek 1991; Strauss & Lamberti 2000). Therefore, it is normally more feasible to achieve biological partial nitrification over ammonium-rich wastewater (Khin & Annachhatre 2004; Mosquera-Corral *et al.* 2005; Ahn & Choi 2006). Nevertheless, the restrictions on biological partial nitrification from the competition between nitrifying bacteria and heterotrophic bacteria can potentially be overcome through biofilm technology by spatially separating them within the same reactor (Nicolella *et al.* 2000).

With a purpose to achieve partial nitrification of low ammonium level wastewater through biofilm filtration process under ambient room temperatures, this study evaluated the partial nitrification performances of two 80 cm long biofilm filters over a synthetic non-ammonium-rich wastewater under a 20°C room temperature. The two biofilm filters were made of different carriers: activated carbon and ceramic granule. The experiment was carried under both limited DO (~ 2.0 mg/L) and unlimited DO (~ 4.0 mg/L) conditions with the purpose to evaluate the effectiveness of DO control in favoring partial nitrification. To better recognize the partial nitrification progress during the filtration process, the depth profiles of both filters were sampled during the limited DO testing period for the water phase COD and nitrogen ($\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$) and the biofilm bacteria numbers (AOB, NOB, and heterotrophic bacteria).

MATERIALS AND METHODS

Experiment apparatus

The experiment apparatus and specifics are shown in Figure 1. Two identical polymethyl methacrylate columns of 120 cm height and 60 mm inside diameter were used

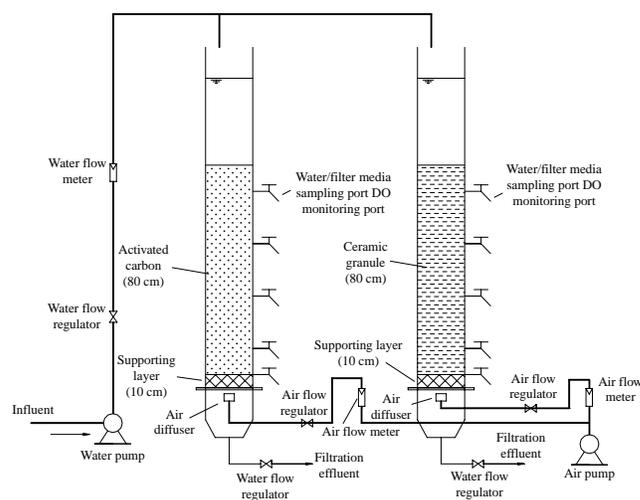


Figure 1 | Experimental apparatus and specifics (not to scale).

as filtration columns. An 80 cm depth of filter media (biofilm carrier) was made in both columns, using ceramic granule and active carbon respectively. The ceramic granule had a particle size of 4.0–6.0 mm, a specific area of 500–800 m^2/m^3 , and a porosity of 45–63%. The activated carbon had a particle size of 2.0–6.0 mm, a specific area of 900–1,200 m^2/m^3 , and a porosity of 30–43%.

Synthetic wastewater was designed to feed into the two filtration columns from the top. Filtration effluent was collected at the bottom of each column and the filtration speed of each column was controllable by a flow valve on the discharge line. Aeration was created by pumping air into each column from the bottom and was controllable by an in-line air flow regulator. Five sampling ports with both liquid and filter media sampling capabilities were created on each column at the locations of 10, 30, 50, 70, and 80 cm from the top of the 80 cm filter media as indicated in Figure 1.

Biofilm inoculation, startup, and system operation

Biofilm inoculation was carried by repeating five times of: filling the filters (filtration column) with a *nitrosomonas* solution (8.65×10^6 cfu/ml, containing ingredients were (g/L): NH_4Cl , 1.0; KH_2PO_4 , 0.7; NaCl, 25; NaHCO_3 , 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5; pH 7.2) and a cultural medium at a 1:5 volumetric ratio, followed by a 48-hour aeration. The purpose of adding the *nitrosomonas* solution

was to speed up nitrification establishment within the filters, so as to shorten the system startup period. Then, fresh cultural medium was let consistently filtrated through the two filters at a filtration speed of 6 m/h for five days. The cultural medium contained the following ingredients (g/L): $(\text{NH}_4)_2\text{SO}_4$, 2.0; Na_2HPO_4 , 0.25; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.01; K_2HPO_4 , 0.75; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.03; CaCO_3 , 1.0; distilled water 1,000 mL; pH 7.2. Trace element solution was added at 1 ml/L with the following ingredients (g/L): EDTA, 57.1; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 3.9; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 7.0; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 5.1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 1.1; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.6; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1.6. Aeration was controlled at a volumetric air: water flow (filtration) ratio of 3:1, making the measured DO at the five sampling ports on both filters ranged around 2.0 mg/L. The DO was monitored on the water samples from the five sampling ports as indicated on [Figure 1](#) three times a day by a YSI DO meter (Model Pro 20).

After the effluent $\text{NH}_4\text{-N}$ of the two filters stabilized (data not shown), the two filters were tested by filtering a synthetic wastewater for 23 days under the same DO condition (limited DO condition) as in the biofilm startup process. The synthetic wastewater used ammonium chloride (NH_4Cl) as nitrogen source and sodium acetate (NaAc) as carbon source. Other contained major ingredients (mg/L) were: CaCl_2 , 10; KH_2PO_4 , 22; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50; NaHCO_3 , 3–4 g/g $\text{NH}_4\text{-N}$. The pH of the synthetic wastewater was adjusted to approximately 7.0. The filtration speed was still controlled at 6.0 m/h for both filters. The room temperature during the experiment was maintained constant at 20°C. A backwash was applied to each filter after every 48 hours filtration.

During the 23-day limited DO testing period, the two filters were daily monitored for their influent (synthetic wastewater) and effluent COD, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$. The influent COD was gradually increased from 106 mg/L to 316 mg/L with the influent $\text{NH}_4\text{-N}$ being maintained around 50–70 mg/L. At the 16th day during the 23-day experiment, both filters were sampled at the depths of 0, 10, 30, 50, 70, and 80 cm for the water phase COD, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$. COD was measured by closed reflux method as given in *Standard Methods for Examination of Water and Wastewater* (1998). $\text{NH}_4\text{-N}$ was measured by Nesslerization. $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ were

measured by an ion chromatography (DIONEX-100). 10 g filter media (including the carrier and biofilm) was also sampled in both filters at the depths of 10, 30, 50, and 70 cm for AOB, NOB, and heterotrophic bacteria enumeration. AOB and NOB were enumerated by the most probably number (MPN) procedure (Oblinger & Koburger 1975; Schmidt & Belser 1982; Donaldson & Henderson 1989; Verhagen & Laanbroek 1991). Heterotrophic bacteria were enumerated by the plate counting procedure (Harris & Sommers 1968; Verhagen & Laanbroek 1991).

After the 23-day limited DO testing period, the two filters were tested under a relatively unlimited DO condition for 21 days, during which the volumetric air: water flow ratio was controlled at 5:1, making the measured DO at the five sampling ports on both filters ranged around 4.0 mg/L. The room temperature was still maintained at 20°C. Influent COD was gradually decreased from 141 mg/L to 35 mg/L and influent $\text{NH}_4\text{-N}$ was maintained around 11–41 mg/L. Again, the two filters were daily monitored for their influent and effluent COD, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$. However, no depth profile observation was carried during this period.

RESULTS AND DISCUSSION

Partial nitrification performance

The influent and effluent of the two filters during the 23-day limited DO testing period are illustrated in [Figure 2](#) for their COD (A), $\text{NH}_4\text{-N}$ (B), $\text{NO}_2\text{-N}$ (C), and $\text{NO}_3\text{-N}$ (D). It was observed that there was no obvious difference between the two filters in their effluent COD as the influent COD being gradually increased ([Figure 2A](#)). However, the ceramic granule filter showed a lower $\text{NH}_4\text{-N}$ ([Figure 2B](#)) and higher $\text{NO}_2\text{-N}$ ([Figure 2C](#)) in its effluent than that of the activated carbon filter ([Figure 2B](#)). Nevertheless, effluent $\text{NO}_3\text{-N}$ was in general significantly lower in both filters ([Figure 2D](#)) than their effluent $\text{NO}_2\text{-N}$ ([Figure 2C](#)), indicating an environment favoring partial nitrification within the two filters under the testing conditions (DO, temperature, and filtration speed).

However, the two filters showed an obvious difference in their effluent $\text{NO}_2\text{-N}$ changes in response to the changing influent COD/N ([Figure 2E](#)). It was observed that

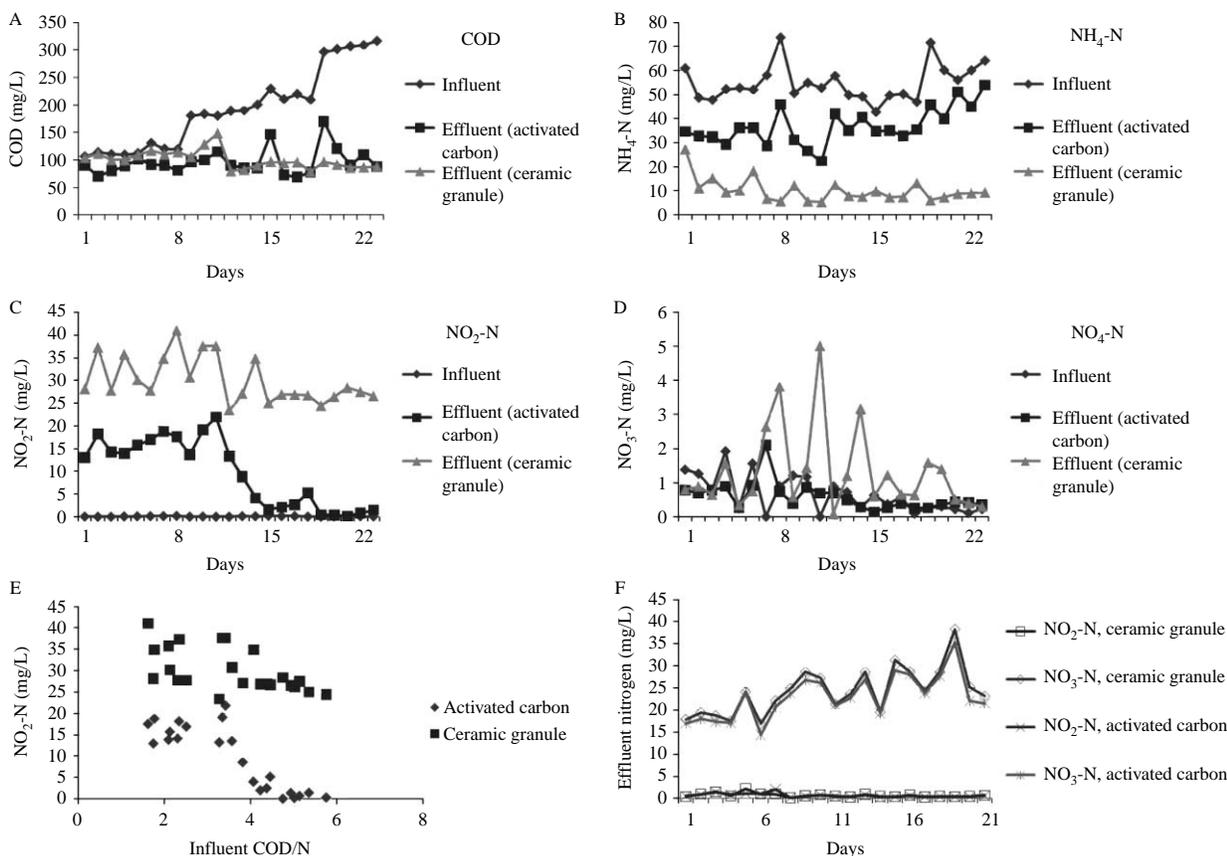


Figure 2 | The influent and effluent COD (A), $\text{NH}_4\text{-N}$ (B), $\text{NO}_2\text{-N}$ (C), and $\text{NO}_3\text{-N}$ (D) of the two biofilm filters during the 23-days limited DO testing period, their effluent $\text{NO}_2\text{-N}$ changes in response to the changing influent COD/N ratios during the 23-days limited DO testing period (E), and the effluent $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ of the two biofilm filters during the 21-day unlimited DO testing period (F).

the effluent $\text{NO}_2\text{-N}$ of the activated carbon filter had a significant decrease as the influent COD/N being increased to above 3.5. Meanwhile, the effluent $\text{NO}_2\text{-N}$ of the ceramic granule filter only showed a much gentle decreasing trend over the entire influent COD/N increasing period. Although the decreasing trend of effluent $\text{NO}_2\text{-N}$ conforms to the theory that a higher COD/N favors heterotrophic bacteria and inhibits nitrifying bacteria (Verhagen & Laanbroek 1991; Strauss & Lamberti 2000), the observed different effluent $\text{NO}_2\text{-N}$ decreasing trends between the two filters indicated that the competition between nitrifying bacteria and heterotrophic bacteria within the two filters might be of different extent under the same testing conditions.

The effluent $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ of the two filters during the 21-day unlimited DO testing period were illustrated in Figure 2F. The influent COD/N was maintained generally

below 3.5 (data not shown on Figure 2F), above which a quick drop of effluent $\text{NO}_2\text{-N}$ was observed for the activated carbon during the limited DO testing period. Therefore, the influent COD/N during this unlimited DO testing period potentially won't exert inhibitory effect on nitrification, at least in $\text{NO}_2\text{-N}$ production. However, it was observed that conversely to the 23-day limited DO testing period when $\text{NO}_2\text{-N}$ was dominant in the filtration effluent, $\text{NO}_3\text{-N}$ was instead dominant in the filtration effluents of both filters under the relatively unlimited DO condition (Figure 2F).

This apparent shift from $\text{NO}_2\text{-N}$ dominant effluent to $\text{NO}_3\text{-N}$ dominant effluent is indicating the effectiveness of DO control (limited DO) to favor the production of $\text{NO}_2\text{-N}$ over $\text{NO}_3\text{-N}$ under the 20°C room temperature. This observation can be explained by the different DO affinities by AOB and NOB, which has been consistently observed

that AOB can sustain better than NOB under lower water dissolved oxygen (DO) conditions (Peng *et al.* 2007; Wu *et al.* 2007; Brockmann & Morgenroth 2010). This experimental result also conforms to the concept that limiting DO is an effective approach to achieve partial nitrification (Peng *et al.* 2007).

Depth profile snapshot

The depth profiles of the two filters in their water phase COD, NH₄-N, NO₂-N, and NO₃-N at the 16th day during the 23-day limited DO testing period were illustrated in Figure 3A for the activated carbon filter and in Figure 3B for the ceramic granule filter.

It was observed that the COD and NH₄-N depth drop were quicker in the ceramic granule filter than that in the activated carbon filter. The COD at the 30 cm depth into

the ceramic granule filter was found comparable to that at the 50 cm depth into the activated carbon filter. The NH₄-N only dropped from around 50 mg/L to around 40 mg/L over the 80 cm activated carbon filter, but was decreased to around 10 mg/L at the 80 cm depth of the ceramic granule filter. The NO₂-N depth profile of the ceramic granule filter showed an increasing trend which was higher than that of the activated carbon filter. The NO₂-N increasing trend went even higher between the depths of 50 cm and 80 cm in the ceramic granule filter, where the NO₂-N depth profile of the activated carbon filter conversely showed a decreasing trend. Sampled NO₃-N were generally lower than NO₂-N and showed no obvious change along the entire 80 cm depth in both filters. These observations showed that NO₃-N production was effectively limited in both filters and partial nitrification was gradually favored as the sampling depth went deeper within

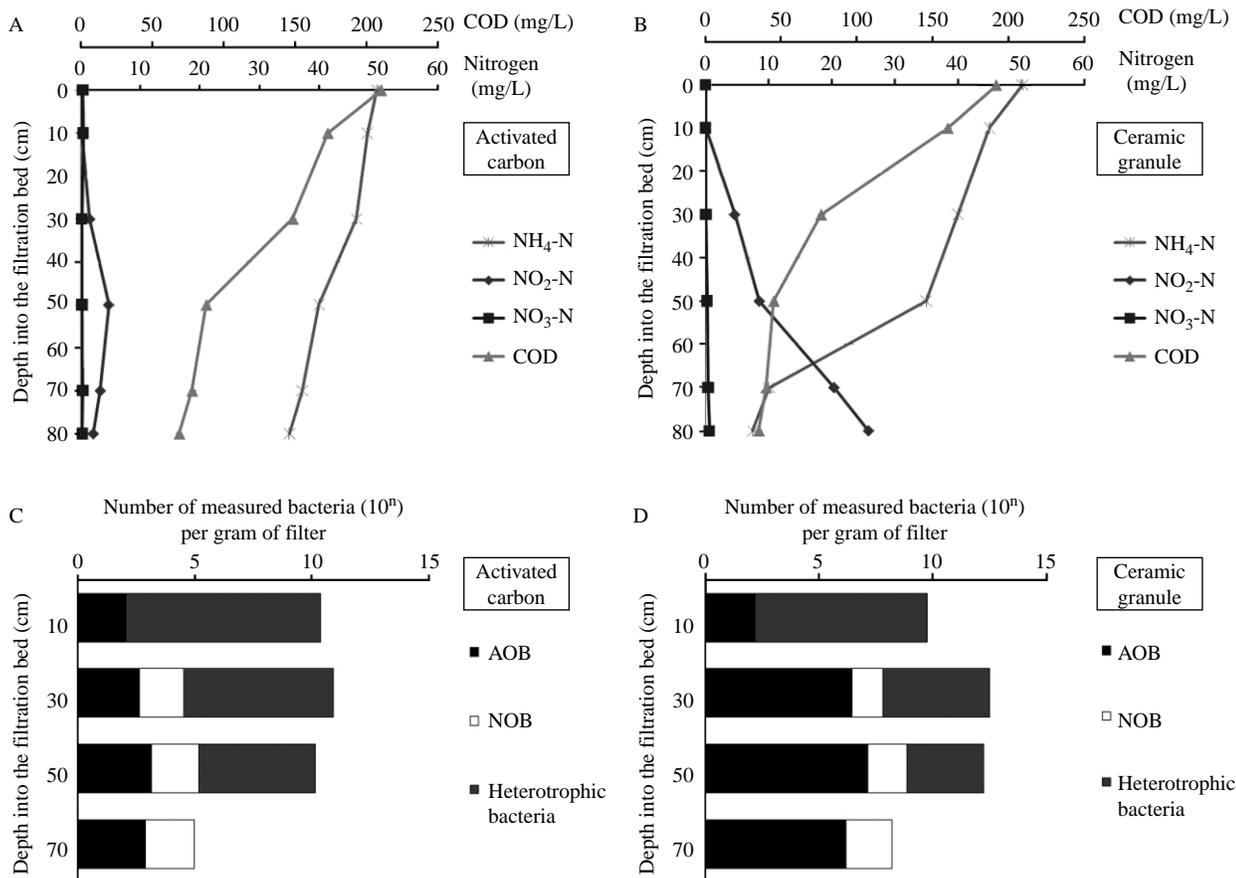


Figure 3 | COD and nitrogen depth profiles of the two biofilm filters: activated carbon (A), ceramic granule (B) at the 16th day of the 23-day limited DO testing period, and the corresponding depth profiles of biofilm AOB, NOB, and heterotrophic bacteria numbers of the two biofilm filters: activated carbon (C), ceramic granule (D). (Water temperature was maintained at 20°C, DO was maintained at approximately 2.0 mg/L, pH was between 7.1–7.8).

the ceramic granule filter, but partial nitrification was maintained at a relatively much lower level in the activated carbon filter.

The depth profiles of AOB, NOB, and heterotrophic bacteria numbers on the biofilms were illustrated in Figure 3C for the activated carbon filter and in Figure 3D for the ceramic granule filter. In terms of their relative numbers, it was observed that heterotrophic bacteria were dominant in both filters at the 10 cm depth. However, as the sampling location went deeper, heterotrophic bacteria quickly lost its dominant place to AOB in the ceramic granule filter starting from the 30 cm depth, whereas heterotrophic bacteria was dominant in the activated carbon filter until the depth of 70 cm. NOB were observed in both filters at the depths of 30, 50, and 70 cm. The measured NOB were at comparable levels to that of AOB in the activated carbon filter, but were at significantly lower levels to AOB in the ceramic granule filter. These observations correspond to the observed relatively limited $\text{NO}_3\text{-N}$ production in both filters and a higher partial nitrification process in the ceramic granule filter than that in the activated carbon filter.

The sampled water phase COD and $\text{NH}_4\text{-N}$ depth profiles of the two filters (Figure 3A,B) visibly demonstrated that their dropping trend physically put lower filter layers (downstream) at relatively lower water phase COD/N levels than that on the upper filter layers (upstream). It has been confirmed that heterotrophic bacteria compete favorably over nitrifying bacteria for $\text{NH}_4\text{-N}$ in the presence of abundant carbon source, resulting an inhibited nitrification process (Verhagen & Laanbroek 1991; Strauss & Lamberti 2000). Consequently, the lower filter layers were confronted with relatively lower COD/N levels and were potentially more favorable for nitrifying bacteria while upper filter layers were relatively more favorable for heterotrophic bacteria (Figure 3C,D). By naturally creating heterotrophic bacteria and nitrifying bacteria favoring zones in tandem, the physical process of filtration potentially circumvented the nitrifying bacteria and heterotrophic bacteria competition incurred limitation on partial nitrification over non-ammonium-rich wastewater.

The observed different partial nitrification performances of the two filters might be ascribed to their different porous flow velocities. Assuming the biofilm thickness on each

biofilm carrier was of a much lower influence on the porosity of the whole filtration column than the actual physical size of the biofilm carrier did, then under the same filtration speed, the porous flow velocity within the ceramic granule filter might be lower than that within the activated carbon filter since the ceramic granule was of a higher porosity. A lower porous flow velocity can give more time for its carrying substrates to be uptake by local bacteria, thus potentially leaving less substrates for those downstream bacteria. The observed quicker depth drop of the water phase COD and $\text{NH}_4\text{-N}$ and the shallower starting depth of AOB domination in the ceramic granule filter (Figure 3B) seemed conform to this hypothesis. Also, the observed earlier drop of effluent $\text{NO}_2\text{-N}$ of the activated carbon filter in response to the influent COD/N increase (Figure 2C) might be ascribed to the quicker exhaustion of its filter depth for COD/N reduction before downstream nitrifying bacteria could prosper.

It should be noted that there are also recognitions that porous flow velocity plays a vital role in the mass transfer rate between liquid and biofilms, with a higher porous flow velocity provides a more efficient mass transfer (Zhu & Chen 2001). Therefore, a better partial nitrification result might be expected under a lower porous flow velocity as a result of limited oxygen supply into the biofilm that essentially favors AOB over NOB due to their different oxygen affinities (Peng *et al.* 2007; Wu *et al.* 2007; Brockmann & Morgenroth 2010).

This proposed porous flow velocity hypothesis can be tested by evaluating the two filters at different filtration velocities and see if the depth profiles of the biofilm bacteria and the water phase COD and nitrogen will change accordingly. It is anticipated that a higher influent COD/N ratio or a higher filtration speed will require more top filter depths to be dominated by heterotrophic bacteria, thus driving the starting depth favoring nitrifying bacteria even deeper. For this particular experiment, if the increase on influent COD/N had been continued, a quick drop of effluent $\text{NO}_2\text{-N}$ might also be observed for the ceramic granule filter.

CONCLUSIONS

By controlling DO at relatively limited levels (~ 2.0 mg/L), this study successfully realized partial nitrification over

a synthetic non-ammonium rich wastewater within two biofilm filters (activated carbon and ceramic granule) under an ambient room temperature (20°C). This success was attributed to: 1) a limited DO condition that favors AOB over NOB; and 2) a spatially separated heterotrophic bacteria and nitrifying bacteria dominating zones naturally created by the filtration process that allows nitrification to occur.

Nevertheless, the two biofilm filters showed different partial nitrification performances, with the effluent NO₂-N was higher in the ceramic granule filter than in the activated carbon filter, and was less susceptible to the influent COD/N changes. This was potentially caused by their different porous flow velocities, with a lower porous flow velocity potentially provides a better partial nitrification performance.

The experimental results of this study indicate that DO, filter depth, and filtration speed (directly related to porous flow velocity) should be played together to achieve a successful partial nitrification. DO control should be used to favor AOB over NOB. Sufficient filter depth should be maintained to allow nitrification to occur and be used as a buffer to the influent COD/N fluctuations. Filtration speed, although needs further confirmation, determines: 1) the starting depth within a filter that favors nitrifying bacteria; 2) whether partial nitrification can be thoroughly completed within a filter. In summation, a biofilm filter intended to be operated for partial nitrification should have an enough depth to provide not only adequate reductions of the incoming COD/N to favor AOB prosper, but also enough hydraulic residence time for the downstream AOB to provide adequate nitrification over the NH₄-N leftovers from the upstream.

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