# Tertiary denitrification of the secondary effluent in biofilters packed with composite carriers under different carbon to nitrogen ratios

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#### ABSTRACT

A new type of biofilter packed with composite carriers was designed for tertiary denitrification of the secondary effluent with removal of both oxidized nitrogen and suspended solids (SS). At the empty bed residence time of 15 min and organic carbon to nitrate nitrogen (C/NO<sub>3</sub>-N) ratios of 2, 1.5 and 1 g/g, the removal percentage of NO<sub>3</sub>-N was 67%, 58% and 36% in the ethanol biofilter, and was 61%, 43% and 26% in the acetate biofilter, respectively. The biofilters packed with composite carriers removed SS effectively, with the effluent turbidity in both biofilters of less than 3 NTU. During the operating cycle between the biofilter backwashings, the NO3-N removal percentage decreased initially after backwashing, and then gradually increased. Under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, the NO<sub>3</sub>-N reduction rate was 1.75, 1.04 and 0.68 g/m<sup>2</sup>/d in the ethanol biofilter, and was 1.56, 1.07 and 0.76 g/m<sup>2</sup>/d in the acetate biofilter, respectively. In addition, during denitrification, the ratio of the consumed chemical oxygen demand to the removed NO<sub>3</sub>-N was 5.06-8.23 g/g in the ethanol biofilter, and was 4.26-8.6 g/g in the acetate biofilter.

Keywords: Biofilter, Composite carriers, Denitrifying biokinetics, External organic carbon, Tertiary denitrification

#### 1. Introduction

With the stringent discharging standard for removing nitrogen from wastewater, besides conventional secondary biological nitrogen removal processes, tertiary denitrification of the secondary effluent in wastewater treatment plants has been received intensive attention so as to achieve the high discharging standard.

Due to its high treatment efficiency and simple operation, biofilm systems with suspended carriers have been widely used for advanced wastewater treatment since its development as moving bed biofilm reactor (MBBR) in the late 80s [1, 2]. In the Cole Pollution Control Plant, the MBBR system was shown to be able to reduce the influent oxidized nitrogen (NOx-N) of 7 mg/L to below 2 mg/L [3]. However, the main drawback of biofilters with suspended carriers was that the effluent contained a high concentration of suspended solids (SS). For example, by using MBBR systems, Motsch et al. [3] obtained the effluent SS was 14.5 mg/L with the influent SS of 7.3 mg/L, while Stinson et al. [4] obtained the effluent SS of 10.8 mg/L with the influent SS of 3 mg/L. Therefore, an additional process is required to remove SS after the MBBR treatment. For example, MBBR was used for tertiary denitrification in South Caboolture Water Reclamation Plant, and filtration was followed to remove SS [5]. On the other hand, when conventional sand filtration systems are used for tertiary denitrification, although a good SS removal efficiency can be achieved, a relatively low carrier filling proportion of around half reactor volume and a high head loss usually exist due to the high sand density. Based on the above situations, a new type of tertiary denitrifying biofilter with both suspended carriers (mainly used for denitrification) and sands (mainly used for the removal of SS) was proposed.

Denitrification requires electron donors such as organic carbon for the removal of oxidized nitrogen. There is limited amount of biodegradable organic matters in the secondary effluent, which cannot support adequate denitrification. Therefore, external organic carbon should be supplied for tertiary denitrification. Because carbon dosage is an important factor affecting the operating cost, several factors should be considered for choosing organic carbons, such as the denitrification rate, cost and degree of utilization [6]. Compared with methanol, denitrification with ethanol or acetate



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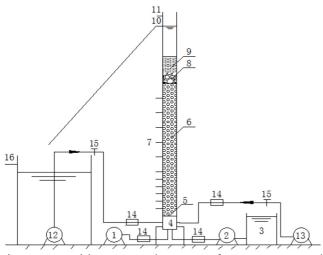
could be achieved more efficiently and was less affected by temperature [7, 8]. In addition, a high carbon dosage also increases the sludge yield, the effluent SS concentration leaving the MBBR system and the load on downstream solid-liquid separation processes [9]. Therefore, the dosage of the external organic carbon for tertiary denitrification should be carefully investigated.

In this study, under organic carbon to nitrate nitrogen ( $C/NO_3-N$ ) ratios of 2, 1.5 and 1 g/g, two tertiary denitrifying biofilters packed with composite carriers were operated at the empty bed retention time (EBRT) of 15 min. System performance and denitrifying biokinetics were examined so as to clarify their performance in denitrification and in the removal of SS with ethanol or acetate as the external organic carbon.

#### 2. Materials and Methods

#### 2.1. On-site Denitrifying Systems

The schematic diagram of the experimental system is shown in Fig. 1. Two tertiary denitrifying biofilters were made from plexiglass column with a diameter of 10 cm and a height of 150 cm. One biofilter was dosed with ethanol and the other with sodium acetate (for simplification, acetate was used instead of sodium acetate). The packed height of suspended carriers (specific surface area of  $500~\text{m}^2/\text{m}^3$ , SPR-1 type, Spring, China) was 90 cm. Above the suspended carriers, there were a support gravel stone layer of 5 cm and a quartz sand layer of 10 cm with sizes between 2-4 mm for the removal of SS. Sampling ports were set at different biofilter heights. The 10 cm biofilter bottom was the premixing zone to facilitate the mixing of the secondary effluent and the organic carbon. The biofilters were backwashed every 24 h for 15 min with combined air (a flow rate of 10 L/m²/s) and water (a flow rate of 2 L/m²/s).



**Fig. 1.** Diagram of the experimental system. 1: Influent pump; 2: External carbon dosing pump; 3: External carbon stock tank; 4: Premixing zone; 5: Perforated plate; 6: Suspended carriers; 7: Water sampling port; 8: Gravel stone layer; 9: Quartz sand layer; 10: Effluent port; 11: Backwashing effluent; 12: Backwashing water pump; 13: Backwashing air pump; 14: Controller; 15: Check valve; 16: Effluent.

The secondary effluent in the 7th wastewater treatment plant, Kunming, was used as the feeding. During the study period, the influent chemical oxygen demand (COD) concentration was 20 mg/L, total nitrogen (TN) was 15.78 mg/L, ammonium nitrogen (NH<sub>4</sub>-N) was 1.41 mg/L, NO<sub>3</sub>-N was 11.58 mg/L, nitrite nitrogen (NO<sub>2</sub>-N) was 0.06 mg/L, pH was 6.9, dissolved oxygen (DO) was 2.4 mg/L and turbidity was 9.9 NTU. Ethanol and acetate were dosed by peristaltic pumps from stock solutions. The flow direction of both the influent and backwashing were up-flow.

The two tertiary denitrifying biofilters were operated under the EBRT of 15 min and C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, respectively. During the start-up period, the C/NO<sub>3</sub>-N ratio was 2 g/g. From the 44th day on, the two biofilters were operated at the C/NO<sub>3</sub>-N ratio of 1 g/g. During days 56-70, the C/NO<sub>3</sub>-N ratio was 1.5 g/g.

#### 2.2. System Performance and Batch Experiments

During operation, parameters such as COD,  $NO_3$ -N,  $NO_2$ -N,  $NH_4$ -N, TN, pH, DO and NTU, were tested daily to examine system performance of both biofilters.

Under steady state at each  $C/NO_3$ -N ratio, the performance of denitrifying biofilters within an operating cycle between the biofilter backwashings was examined to evaluate the stability of the system. Samples were taken at intervals of hours 0.5, 1, 2, 4, 6, 8, 12 and 24, respectively.

Under steady state at each C/NO<sub>3</sub>-N ratio, samples were taken at 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 cm along the biofilter depth, and concentrations of typical parameters (NO<sub>3</sub>-N, NO<sub>2</sub>-N, COD, NTU, DO and pH ) were tested so as to investigate denitrifying biokinetics of both biofilters. Denitrification activities were obtained by fitting dynamics of NO<sub>3</sub>-N or NO<sub>2</sub>-N with first- or half-order linear equations. In addition, suspended carriers were taken from 0-30, 30-60 and 60-90 cm along the biofilter depth, and their biofilm biomass was tested.

At different stages of the experiment, backwashed water with detached biofilm biomass was used for examining their denitrifying activities. Adequate nitrate and ethanol or acetate were added to the solution to achieve the initial NO<sub>3</sub>-N concentration of 20 mg/L and the COD concentration of 200 mg/L. Samples were taken at 15 min intervals during the experiment and centrifuged at 12000 rpm for 2 min, and then the supernatant was stored at 4°C for further analysis of NO<sub>3</sub>-N, NO<sub>2</sub>-N and COD.

#### 2.3. Analytical Methods

COD,  $NO_3$ -N,  $NO_2$ -N,  $NH_4$ -N, TN, SS and volatile suspended solids (VSS) were tested according to standard methods for the examination of water and wastewater [10]. The pH, DO and NTU were measured using probes of pH3110 (WTW, Germany), OXI315i (WTW, Germany) and 1900C (HACH, America), respectively.

To measure the biofilm biomass, four pieces of media with biofilm were taken from the biofilter. The biomass was washed off from the suspended carriers with distilled water. SS and VSS were measured to represent the biomass concentration.

Under different operating conditions, denitrification could be described by first, half or zero-order equations. Harremoës [11] proposed that due to substrate diffusion limitation in biofilm systems, denitrification of nitrate could be described by the half-order equation.

#### 3. Results and Discussion

#### 3.1. System Performance at Different C/NO<sub>3</sub>-N Ratios

Dynamics of nitrate in two biofilters during the 70 d operation is shown in Fig. 2. The two biofilters reached steady state after only 5 d operation. On Day 5, as to the influent NO<sub>3</sub>-N concentration of 11.55 mg/L, the effluent NO<sub>3</sub>-N concentration was 3.54 mg/L in the ethanol biofilter and was 3.71 mg/L in the acetate biofilter. Aspegren et al. [12] operated a MBBR system at 16°C and the acclimation time was 11 weeks with methanol as the external carbon source. By using synthetic wastewater as the influent, Bill et al. [13] obtained that the MBBR system reached steady state after 22 d operation with methanol or ethanol as the organic carbon, and the carbon dosage was 5.7 g [COD]/g [NO<sub>3</sub>-N] with methanol and 6.9 g [COD]/g [NO<sub>3</sub>-N] with ethanol. deBarbadillo et al. [14] obtained that with acetate as the organic carbon, the biofilter reached steady state after 3 d operation at 6.9-8.1 g [COD]/g [NOx-N]. Compared with previous studies, the starting up period was relatively short in the present study. The quick start up of both biofilters could be due to that (1) ethanol or acetate could support the quick growth of denitrifiers [15]; (2) the high carbon dosage of 8.0 g [COD]/g [NO<sub>3</sub>-N] for ethanol and 5.3 g [COD]/g [NO<sub>3</sub>-N] for acetate were adequate for denitrifiers growth, which was higher than the theoretical value of 5.4 g [COD]/g [NO<sub>3</sub>-N] for ethanol and 4.3 g [COD]/g [NO<sub>3</sub>-N] for acetate; and (3) denitrifiers in the secondary effluent seeded the biofilters constantly.

Under steady state, at C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, the performance of the two biofilters was summarized in Table 1. Under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, in the ethanol biofilter, the NO<sub>3</sub>-N removal percentage was 67%, 58% and 36%, while in the acetate biofilter, the NO<sub>3</sub>-N removal percentage was 61%, 43% and 26%, respectively. Therefore, with decreasing C/NO<sub>3</sub>-N ratios, the NO<sub>3</sub>-N removal percentage decreased. The performance of two biofilters was similar at the C/NO<sub>3</sub>-N ratio of 2 g/g, while under C/NO<sub>3</sub>-N ratios of 1.5 and 1 g/g, the effluent NO<sub>3</sub>-N concentration in the ethanol biofilter was lower than that of the acetate biofilter. At the same C/NO<sub>3</sub>-N ratio, the influent COD in the ethanol biofilter was much higher than that in the acetate biofilter, due to that the COD equivalent per unit carbon of ethanol (2.08 g/g) was higher than that of acetate (0.78 g/g). At the C/NO<sub>3</sub>-N ratio

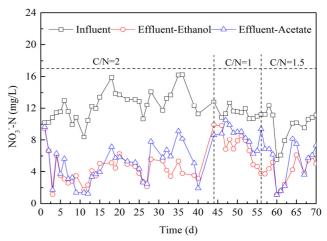


Fig. 2. Dynamics of influent and effluent NO<sub>3</sub>-N concentrations in both biofilters under different C/NO<sub>3</sub>-N ratios.

of 2 g/g, both biofilters were operated with adequate carbon sources, so their performance was similar. Under C/NO $_3$ -N ratios of 1.5 and 1 g/g, inadequate carbon source was the main reason causing poor NO $_3$ -N removal in the acetate biofilter.

Under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, the effluent NO<sub>2</sub>-N concentrations were 0.58, 0.51 and 1.25 mg/L with ethanol as the organic carbon, and were 1.15, 1.16 and 1.42 mg/L with acetate as the organic carbon, respectively. Compared with C/NO<sub>3</sub>-N ratios of 2 and 1.5 g/g, the effluent NO<sub>2</sub>-N concentration was significantly higher at the C/NO<sub>3</sub>-N ratio of 1 g/g. In addition, the NO<sub>2</sub>-N concentration was higher in the acetate biofilter than that in the ethanol biofilter.

Under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, the effluent turbidity was  $2.7 \pm 2.0$ ,  $2.1 \pm 0.5$  and  $1.8 \pm 0.3$  NTU in the ethanol biofilter and was  $1.6 \pm 0.4$ ,  $1.9 \pm 0.5$  and  $1.7 \pm 0.3$  NTU in the acetate biofilter. Therefore, biofilters packed with composite carriers reduced SS effectively and a very low turbidity in the effluent was obtained.

#### 3.2. Performance of Biofilters within an Operating Cycle

Under different C/NO<sub>3</sub>-N ratios, the performance of two biofilters within the operating cycle between the biofilter backwashings is shown in Fig. 3.

Table 1. Performance of Two Biofilters with the Dosage of Ethanol or Acetate during the System Operation

		Ethanol			Acetate			
		$C/NO_3-N = 2 g/g$	$C/NO_3-N = 1.5 g/g$	$C/NO_3-N = 1 g/g$	$C/NO_3-N = 2 g/g$	$C/NO_3-N = 1.5 g/g$	$C/NO_3-N = 1 g/g$	
COD* (mg/L)	Influent	$122 \pm 26$	$82 \pm 13$	$65 \pm 17$	$97 \pm 21$	$63 \pm 7$	$48 \pm 10$	
	Effluent	$69 \pm 20$	$35 \pm 12$	$29 \pm 9$	$43 \pm 18$	$28 \pm 10$	$27 \pm 7$	
(IIIg/L)	Removal	43%	57%	55%	56%	56%	44%	
NO <sub>3</sub> -N (mg/L)	Influent	$12.4 \pm 1.8$	$9.8 \pm 2.0$	$11.5~\pm~0.7$	$12.3 \pm 1.8$	$9.8 \pm 2.0$	$11.5 \pm 0.7$	
	Effluent	$4.1 \pm 1.7$	$4.1 \pm 1.5$	$7.4 \pm 1.6$	$4.8 \pm 2.3$	$5.6 \pm 2.5$	$8.5 \pm 1.2$	
(IIIg/L)	Removal	67%	58%	36%	61%	43%	26%	
NO <sub>2</sub> -N	Influent	$0.06 \pm 0.05$	$0.06 \pm 0.03$	$0.05 \pm 0.03$	$0.06 \pm 0.05$	$0.06 \pm 0.03$	$0.05 \pm 0.03$	
(mg/L)	Effluent	$0.58 \pm 0.58$	$0.51 \pm 0.25$	$1.25 \pm 0.49$	$1.15 \pm 0.94$	$1.16 \pm 0.63$	$1.42 \pm 0.65$	
Turbidity (NTU)	Influent	$5.2 \pm 2.5$	$4.0 \pm 0.6$	$4.2 \pm 0.7$	$5.2 \pm 1.7$	$3.9 \pm 0.6$	$4.2 \pm 0.7$	
	Effluent	$2.7 \pm 2.0$	$2.1 \pm 0.5$	$1.8 \pm 0.3$	$1.6 \pm 0.4$	$1.9 \pm 0.5$	$1.7 \pm 0.3$	

<sup>\*</sup>The influent COD value included the COD in the secondary effluent and the COD from the dosed external organic carbon.

During the operating cycle, the removal percentage of nitrate was stable in the two biofilters. At the initial 2 h, the removal rate of nitrate was slightly low. This was mainly due to that some suspended and biofilm biomasses were washed out from the biofilter. After 4 h of backwashing, both biofilters maintained a high nitrate removal efficiency, indicating that backwashing did not cause an excessive detachment of biofilm. Farabegoli et al. [16] obtained that the removal of nitrate in the initial 3 h was lower than that in the last 3 h during the operating cycle in an up-flow filter. The decrease in denitrification efficiency during the initial stage after backwashing was due to the loss of biofilm during backwashing [17].

During the operating cycle, the effluent turbidity of both biofilters was stable. Under  $C/NO_3$ -N ratios of 2, 1.5 and 1 g/g, the influent turbidity was 3.9, 3.9 and 4.4 NTU 0.5 h after backwashing and 4.1, 4.0 and 5.2 NTU 24 h after backwashing. In the ethanol biofilter, the effluent turbidity was 3.2, 2.8 and 2.3 NTU 0.5 h after the backwashing and 3.6, 2.3 and 1.8 NTU 24 h after the backwashing; in the acetate biofilter, the effluent turbidity was 2.2, 2.0 and 2.3 NTU 0.5 h after the backwashing and 1.5, 1.6 and 1.7 NTU 24 h after the backwashing. The reason for the slightly high effluent turbidity 0.5 h after backwashing might be due to

that the detached biofilm was washed out from the biofilter. With ethanol as the organic carbon, the effluent turbidity varied significantly and was higher than that of the acetate biofilter.

## 3.3. Biokinetics of Biofilm Biomass and Backwashed Biofilm Biomass

Under different C/NO<sub>3</sub>-N ratios, dynamics of nitrite and nitrate along the biofilter depth are shown in Fig. 4.

Under different C/NO<sub>3</sub>-N ratios, denitrification occurred within all biofilter depth in both biofilters. At C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, in the ethanol biofilter, the COD removal rate was 12.18, 12.66 and 5.60 g/m²/d; in the acetate biofilter, the COD removal rate was 10.15, 9.13 and 5.36 g/m²/d, respectively. At C/NO<sub>3</sub>-N ratios of 2 and 1.5 g/g, the COD removal rate was similar in the two biofilters, which was significantly higher than that at the C/NO<sub>3</sub>-N ratio of 1 g/g. At C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, in the ethanol biofilter, the NO<sub>3</sub>-N reduction rate was 1.75, 1.04 and 0.68 g/m²/d, respectively. In the acetate biofilter, the NO<sub>3</sub>-N reduction rate was 1.56, 1.07 and 0.76 g/m²/d, respectively. The NO<sub>3</sub>-N reduction rate decreased with decreasing C/NO<sub>3</sub>-N ratios. At the C/NO<sub>3</sub>-N ratio of 2 g/g, the NO<sub>3</sub>-N reduction rate

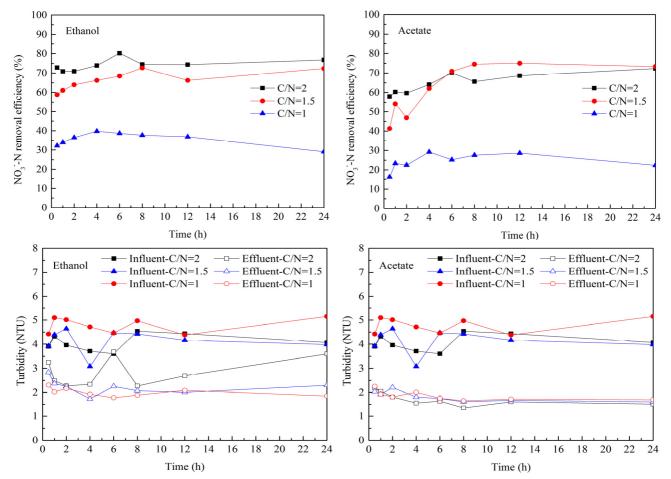


Fig. 3. Dynamics of NO<sub>3</sub>-N and turbidity in both biofilters with the dosage of ethanol or acetate during an operating cycle between the biofilter backwashings.

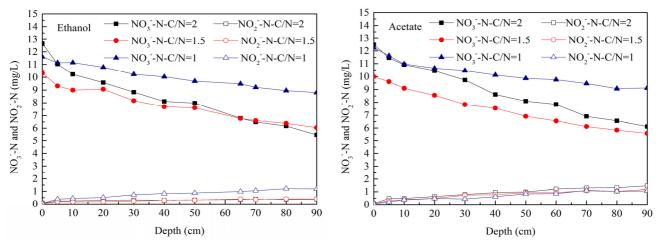


Fig. 4. Dynamics of NO<sub>3</sub>-N and NO<sub>2</sub>-N concentrations along biofilter depth.

Table 2. Denitrifying Kinetics of Backwashed Biofilm Biomass and Biofilter Biofilm (mg/g [VSS]/h)

	, 0			,	50		
		Backwashed b	iofilm biomass	Biofilter biofilm			
	C/NO <sub>3</sub> -N (g/g)	rNO <sub>3</sub> -N	rNO <sub>2</sub> -N	rnet	rNO <sub>3</sub> -N	rNO <sub>2</sub> -N	rnet
Ethanol	2	26.2	9.3	16.9	14.4	0.6	13.8
	1.5	14.5	6.8	7.7	9.5	0.8	8.7
	1	9.5	7.0	2.5	5.7	2.3	3.4
Acetate	2	22.0	7.3	14.7	17.7	3.8	13.9
	1.5	17.6	14.3	3.3	8.5	2.2	6.3
	1	14.3	7.4	6.9	11.8	3.7	8.1

Note:  $rNO_3-N$  means the denitrifying rate of  $NO_3-N$ ,  $rNO_2-N$  means the production rate of  $NO_2-N$ , and rnet means the denitrifying rate of  $NO_3-N$  (equals to  $rNO_3-N-rNO_2-N$ ).

was high in the ethanol biofilter, which could be due to that the dosed COD in the ethanol biofilter was high. While under C/NO<sub>3</sub>-N ratios of 1.5 and 1 g/g, the NO<sub>3</sub>-N reduction rate was higher in the acetate biofilter than that in the ethanol biofilter. Constantin and Fick [18] concluded that ethanol must be converted into acetate firstly for further utilization, while acetate could be utilized directly by microorganisms, so its denitrifying rate was higher than that of ethanol.

During denitrification, NO2-N accumulation has been received intensive attentions in lots of studies [19]. The accumulation of NO<sub>2</sub>-N could be due to that: (1) the NO<sub>3</sub>-N denitrifying rate was higher than that of NO<sub>2</sub>-N [20], (2) some denitrifiers only denitrify NO<sub>3</sub>-N to NO<sub>2</sub>-N [21], and (3) low temperature results in a decreased activity of denitrifiers [16]. NO2-N accumulation occurred in both biofilters under all C/NO<sub>3</sub>-N ratios during denitrification. In the ethanol biofilter, under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, the highest NO2-N concentration was 0.37, 0.40 and 1.20 mg/L and its accumulating rate was 0.068, 0.087 and 0.275 g/m<sup>2</sup>/d, respectively. The concentration and rate of NO2-N accumulation increased with decreasing C/NO<sub>3</sub>-N ratios. Compared with C/NO<sub>3</sub>-N ratios of 2 and 1.5 g/g, the concentration and rate of NO<sub>2</sub>-N accumulation was high under the C/NO<sub>3</sub>-N ratio of 1 g/g. Therefore, when ethanol was used as the organic carbon, the dosed organic carbon affected NO<sub>2</sub>-N accumulation significantly. In the acetate biofilter, under different C/NO<sub>3</sub>-N ratios, the highest NO<sub>2</sub>-N concentration was 1.46, 1.19 and 1.07 mg/L and the accumulating rate was 1.56, 1.07 and 0.76 g/m²/d, respectively. The concentration and rate of NO<sub>2</sub>-N accumulation decreased with decreasing C/NO<sub>3</sub>-N ratios. Compared with ethanol, obvious NO<sub>2</sub>-N accumulation occurred with acetate as the organic carbon. The reason for high NO<sub>2</sub>-N accumulation with acetate as the organic carbon could be due to that the utilization of acetate could induce nitrite accumulation easily [22].

Under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, the consumed COD to the removed NO<sub>3</sub>-N ratios were 5.06, 6.55 and 8.23 g/g in the ethanol biofilter, and were 4.26, 4.57 and 8.60 g/g in the acetate biofilter, respectively. ÆsØy et al. [6] obtained a value of 4.5 g [COD]/g [NO<sub>3</sub>-N] with ethanol as the organic carbon for denitrification in a denitrifying biofilter. By operating a MBBR system at 12.4-15.3°C, Choi et al. [23] obtained a value of 4.11 g [COD]/g [NO<sub>3</sub>-N] with acetate as the organic carbon. deBarbadillo et al. [14] obtained that the carbon requirement was 5.2 g [COD]/g [NO<sub>3</sub>-N] with ethanol as the organic carbon in biofilm systems. Generally, carbon requirement increased with decreasing C/NO<sub>3</sub>-N ratios. Under low C/NO<sub>3</sub>-N ratios, the organic carbon might be consumed by other pathways rather than denitrification [6], such as the proportion consumed by DO might be increased.

With data shown in Fig. 4, good linear relationships between the half-order  $NO_3$ -N concentration and time were obtained in both biofilters under different  $C/NO_3$ -N ratios. Under  $C/NO_3$ -N

ratios of 2, 1.5 and 1 g/g, the half-order rate constants were 0.14, 0.092 and 0.057 (mg  $[NO_3-N]/L)1/2/min$  for the ethanol biofilter and were 0.13, 0.11 and 0.057 (mg  $[NO_3-N]/L)1/2/min$  for the acetate biofilter. With decreasing  $C/NO_3-N$  ratios, the half-order rate constant decreased. In addition to the effect of  $C/NO_3-N$  ratios, hydraulic conditions in the biofilter might also affect the half-order rate constant of denitrification. For example, by operating a fluidized bed biofilm system, Harremoës [11] obtained that the half-order rate constant could be as high as 12 (mg  $[NO_3-N]/L)1/2/min$ .

Kinetics of backwashed biofilm biomass and biofilter biofim are given in Table 2. Compared with biofilter biofilm, the NO<sub>3</sub>-N reduction rate and the NO2-N accumulation rate of the backwashed biofilm biomass was much higher. This confirmed that diffusion limitation occurred within biofilm systems, inducing a low microbial activity. Usually, denitrification could be divided into the step of denitrifying NO<sub>3</sub>-N to NO<sub>2</sub>-N and the step from NO<sub>2</sub>-N to N<sub>2</sub>. During denitrification, the denitrifying NO<sub>2</sub>-N rate was obtained by subtracting the NO2-N accumulating rate from the denitrifying NO<sub>3</sub>-N rate. In both biofilters, at the C/NO<sub>3</sub>-N ratio of 2 g/g, the denitrifying NO<sub>2</sub>-N rate of the backwashed biofilm biomass was high; while under C/NO<sub>3</sub>-N ratios of 1.5 and 1 g/g, the denitrification NO<sub>2</sub>-N rate of biofilter biofilm was high. Therefore, for the backwashed biofilm biomass, the rate of denitrifying NO<sub>3</sub>-N to NO<sub>2</sub>-N was enhanced higher than that of denitrifying NO<sub>2</sub>-N to N<sub>2</sub>, especially under high C/NO<sub>3</sub>-N ratio conditions, resulting in a high NO<sub>2</sub>-N accumulation potential. However, in biofilm systems, due to the limitation, the step of denitrifying NO<sub>3</sub>-N to NO<sub>2</sub>-N was lowered, especially under low C/NO<sub>3</sub>-N ratio conditions, inducing a low NO<sub>2</sub>-N accumulation potential. This should be received a high attention during the operation of tertiary biofilters.

#### 4. Conclusions

- (1) The two denitrifying biofilters reached steady state after only 5 days operation. At  $C/NO_3$ -N ratios of 2, 1.5 and 1 g/g, the  $NO_3$ -N removal percentage was 67%, 58% and 36% in the ethanol biofilter, and was 61%, 43% and 26% in the acetate biofilter.
- (2) The biofilters packed with composite carriers removed SS effectively. Under  $C/NO_3$ -N ratios of 2, 1.5 and 1 g/g, 24 h after the backwashing, the effluent turbidity was 3.6, 2.3 and 1.8 NTU in the ethanol biofilter, and was 1.5, 1.6 and 1.7 NTU in the acetate biofilter, respectively.
- (3) Under C/NO<sub>3</sub>-N ratios of 2, 1.5 and 1 g/g, in the ethanol biofilter, the NO<sub>3</sub>-N reduction rate was 1.75, 1.04 and 0.68 g/m²/d and the consumed COD to the removed NO<sub>3</sub>-N ratios was 5.06, 6.55 and 8.23 g/g, respectively, while in the acetate biofilter, the NO<sub>3</sub>-N reduction rate was 1.56, 1.07 and 0.76 g/m²/d and the consumed COD to the removed NO<sub>3</sub>-N ratios was 4.26, 4.57 and 8.60 g/g, respectively.

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#### References

- McGettigan J, Stinson B, Wilson T. Tertiary denitrifying MBBRs: The right choice. In: Proceedings of the Water Environment Federation. Florida; 2009. p. 446-457.
- Täljemark K, Aspegren H, Gruvberger C, Hanner N, Nyberg U, Andersson B. 10 Years of experiences of an MBBR process for post-denitrication. In: Proceedings of the Water Environment Federation. Louisiana; 2004. p. 355-366.
- Motsch S, Fetherolf D, Guhse G, McGettigan J, Wilson T. MBBR and IFAS pilot program for denitrification at Fairfax County's Noman Cole Pollution Control Plant. Water Practice 2007;1:1-11.
- Stinson B, Peric M, Neupane D, et al. Design and operating considerations for a post denitrification MBBR to achieve limit of technology effluent NOx 1 mg/L and effluent TP 0.18 mg/L. In: Proceedings of the Water Environment Federation, Nutrient Removal. Florida; 2009. p. 1225-1254.
- Wilson T, Toth Z, Anderson G, McSweeny R, McGettign J. Using MBBRs to meet ENR nitrogen levels for over 8 years. In: Proceedings of the Water Environment Federation. Chicago; 2008. p. 3622-3630.
- ÆsØy A, Ødegaard H, Bach K, Pujol R, Hamon M. Denitrification in a packed bed biofilm reactor (BIOFOR)—Experiments with different carbon sources. Water Res. 1998;32:1463-1470.
- Mokhayeri Y, Nichols A, Murthy S, Riffat R, Dold P. Examining the influence of substrates and temperature on maximum specific growth rate of denitrifiers. Water Sci. Technol. 2006;54:155-162.
- 8. Van Rijn J, Tal Y, Barak Y. Influence of volatile fatty acids on nitrite accumulation by a *Pseudomonas stutzeri* strain isolated from a denitrifying fluidized bed reactor. *Appl. Environ. Microbiol.* 1996;62:2615-2620.
- Shrestha A, Riffat R, Bott C, et al. Denitrification stoichiometry and kinetics of moving bed biofilm reactor. In: Proceedings of the Water Environment Federation, Nutrient Removal. Washington; 2009. p. 153-165.
- American Public Health Association/American Water Works Association/Water Environment Federation. Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington DC, USA; 1998.
- Harremoës P. The significance of pore diffusion to filter denitrification. J. Water Pollut. Control Fed. 1976;48:377-388.
- 12. Aspegren H, Nyberg U, Andersson B, Gotthardsson S, Jansen J. Post denitrification in a moving bed biofilm reactor process. *Water Sci. Technol.* 1998;38:31-38.
- Bill KA, Bott CB, Murthy SN. Evaluation of alternative electron donors for denitrifying moving bed biofilm reactors (MBBRs). Water Sci. Technol. 2009;60:2647-2657.
- 14. deBarbadillo C, Miller P, Ledwell S. A comparison of operating issues and dosing requirements for alternative carbon sources in denitrification filters. In: Proceedings of the Water Environment Federation. Chicago; 2008. p. 6603-6617.
- Nyberg U, Andersson B, Aspegren H. Long-term experiences with external carbon sources for nitrogen removal. Water Sci.

- Technol. 1996;33:109-116.
- Farabegoli G, Gavasci R, Lombardi F, Romani F. Denitrification in tertiary filtration: application of an up-flow filter. *J. Environ.* Sci. Health A 2003;38:2169-2177.
- 17. Koch G, Siegrist H. Denitrification with methanol in tertiary filtration at wastewater treatment plant Zürich-Werdhoölzli. *Water Sci. Technol.* 1997;36:165-172.
- Constantin H, Fick M. Influence of C-sources on the denitrification rate of a high-nitrate concentrated industrial wastewater. Water Res. 1997;31:583-589.
- 19. Dos Santos SG, Varesche MBA, Zaiat M, Foresti E. Comparison of methanol, ethanol, and methane as electron donors for denitrification. *Environ. Eng. Sci.* 2004;21:313-320.

- Betlach MR, Tiedje JM. Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. Appl. Environ. Microbiol. 1981;42:1074-1084.
- Martienssen M, Schops R. Population dynamics of denitrifying bacteria in a model biocommunity. Water Res. 1999;33:639-646.
- 22. Yin FF, Wang SY, Ang XY, Peng YZ, Wang W. Effects of carbon source types on denitrification performance at low temperature. *Environ. Sci.* 2009;30:108-113.
- Choi HJ, Lee AH, Lee SM. Comparison between a moving bed bioreactor and a fixed bed bioreactor for biological phosphate removal and denitrification. Water Sci. Technol. 2012;65: 1834-183.