Removal of nitrogen and phosphorus from the secondary effluent in tertiary denitrifying biofilters combined with micro-coagulation

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ABSTRACT

Effective control of nitrogen and phosphorus in secondary effluent can reduce or avoid the eutrophication of receiving water bodies. Two denitrifying biofilters (DNBFs) packed with different sizes of quartz sands combined with micro-coagulation were operated for simultaneous removal of nitrogen and phosphorus from the secondary effluent. The quartz sand size in one DNBF was 2–4 mm (DNBFS), and in the other was 4–6 mm (DNBFL). In both DNBFs, methanol was used as the electron donor and different organic carbon to nitrogen (C/N) ratios were applied. Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the nitrate nitrogen (NO3- C0-N) removal percentages were 73%, 77%, and 50% in DNBFS, and 43%, 25%, and 21% in DNBFL; the effluent total phosphorus concentrations were 0.15, 0.14, and 0.18 mg/L in DNBFS, and 0.29, 0.35, and 0.24 mg/L in DNBFL. The performance of both biofilters was quite stable within a backwashing cycle. The NO3- C0-N reduction rates were 1.31, 1.10, and 0.48 mg/(L·min) in DNBFS, and 0.97, 0.27, and 0.10 mg/(L·min) in DNBFL. For biomass detached from both biofilters, their denitrifying activities were similar. Biofilm biomass in DNBFS was higher than that in DNBFL, inducing a high denitrification efficiency in DNBFS.

Key words | biofilm, micro-coagulation, secondary effluent, tertiary denitrifying biofilters

INTRODUCTION

Eutrophication is a serious environmental issue nowadays, and nitrogen and phosphorus are crucial contributors to the eutrophication of water bodies. In the later 1970s, phosphorus was considered to be the main cause of the eutrophication of water bodies, and nitrogen was considered as the other factor causing eutrophication in the mid-1980s (Schindler 1977; Hecky & Kilham 1988). Furthermore, a combined effect of nitrogen and phosphorus on eutrophication was proposed after 1990 (Elser et al. 1990; Paerl et al. 2011). Therefore, reducing only phosphorus or only nitrogen cannot control eutrophication effectively. For sensitive water bodies in the USA, a limit has been applied to remove total nitrogen (TN) below 3 mg/L and total phosphorus (TP) below 0.1 mg/L (de Barbadillo et al. 2006; Boltz et al. 2012). Nitrate nitrogen (NO3-N) is the major component of TN in secondary effluent. Therefore, denitrification is the main technology for tertiary nitrogen removal, and coagulation–filtration is used for TP removal. Combining denitrifying biofilters (DNBFs) with coagulation may have the potential for removing NO3-N and TP simultaneously. However, only limited studies have been reported in this field and further research is required.

For denitrifying secondary effluent, external organic carbon dosage has been usually applied (Capua et al. 2015; Shi et al. 2015). Methanol has been widely used due to its low cost and high efficiency (Wei et al. 2014). Ledwell et al. (2010) reported that the applied organic carbon to nitrogen (C/N) ratio was lowest when methanol was used for denitrification. The lower carbon dosage requirement means a lower operating cost since the carbon dosage cost is the major investment for denitrification (Sage et al. 2006). An insufficiently dosed carbon source would lead to a decrease in denitrification rate, while excessive dosage would lead to a rise in chemical oxygen demand (COD)
concentration in the effluent. Hence, appropriate C/N ratios should be controlled.

Phosphorus is an essential element for microbial growth, and a low phosphorus concentration may inhibit denitrification in tertiary denitrifying filters. When the orthophosphate (PO₄³⁻-P) was around 0.1 mg/L, the performance of the DNBF was not affected, while the performance was significantly affected when the PO₄³⁻-P was lower than 0.03 mg/L (Hultman et al. 1994; Jonsson et al. 1997). The PO₄³⁻-P/NO₃⁻-N threshold value affected the denitrification process, and de Barbadillo et al. (2006) and Boltz et al. (2012) obtained the value of 0.0086 through mathematical modeling. Therefore, it is necessary to further examine the effect of phosphorus on nitrogen removal in tertiary biofilters.

Two tertiary DNBFs were operated under different C/N ratios. Long-term performance of the two DNBFs, nutrient removal along the biofilter depth, and denitrifying activities were investigated for tertiary nitrogen and phosphorus removal.

MATERIALS AND METHODS

Experimental systems

Two tertiary DNBFs were made from a plexiglass column with a diameter of 10 cm and a height of 125 cm (for the schematic diagram refer to Wei et al. 2014). One biofilter was packed with quartz sand with sizes between 2 and 4 mm (DNBFs), and the other with sizes of 4–6 mm (DNBFs). The quartz sand size was chosen based on its wide application (Horn & Telgmann 2000; Moore et al. 2001). The packed depth of quartz sand was 50 cm with a support gravel stone layer of 10 cm at the bottom. The biofilters were backwashed every 24 h for 15 min with combined air and water. During backwashing, the water flow rate was 5 L/min and the air flow rate was 15 L/min.

The micro-coagulated secondary effluent in the seventh wastewater treatment plant, Kunming, was used as the feed and methanol was dosed as the external organic carbon with empty bed retention time (EBRT) of 15 min. During the study period, the influent concentration of COD was 30 mg/L, ammonium nitrogen (NH₄⁺-N) was 1.3 mg/L, NO₃⁻-N was 14.7 mg/L, nitrite nitrogen (NO₂⁻-N) was 0.05 mg/L, pH was 7.1, and the water temperature was 10–15 °C.

System performance and batch experiments

The two tertiary DNBFs were operated sequentially under C/N ratios of 1.5, 1.25, and 0.75 g/g, respectively. During the long-term operation, parameters such as NO₃⁻-N, TP, and NO₂⁻-N were tested daily to examine nutrient removal in both biofilters.

Stability of DNBFs within a backwashing cycle was examined under each C/N ratio at steady state. Samples were taken at intervals (hours 0, 0.5, 1, 2, 4, 6, 8, 12, and 24) starting from the end of each backwashing to the beginning of the next backwashing, and parameters of NO₃⁻-N, NO₂⁻-N, COD, pH, and dissolved oxygen (DO) were tested.

Under steady state at each C/N ratio, samples were taken every 10 cm along the biofilter depth, and concentrations of typical parameters (NO₃⁻-N, NO₂⁻-N, PO₄³⁻-P, pH, and DO) were tested so as to investigate denitrifying biokinetics of each biofilter.

Under steady state at each C/N ratio, quartz sands were taken from biofilters at depths of 10, 30, and 50 cm, and biofilm biomass with its extracellular carbohydrate and protein content was tested. In addition, backwashed biofilm biomass was taken under steady state for the examination of its denitrifying biokinetics. During the batch experiment, nitrate and methanol were added and then concentrations of NO₃⁻-N, NO₂⁻-N, and COD were tested every 10 min.

Analytical methods

COD, NO₃⁻-N, NO₂⁻-N, TP, PO₄³⁻-P, NH₄⁺-N, suspended solids (SS), volatile suspended solids (VSS), and NTU were determined according to Standard Methods (APHA/AWWA/WEF 1998). The pH and DO were measured using pH3110 and OXI315i probes (WTW, Munich, Germany), respectively.

Biofilm biomass was tested by weight analysis. Thirty grams of sands taken from biofilters were heated at 105 °C and then heated at 600 °C to determine SS and VSS, respectively. During testing, the weight of inorganic components in the biofilms could be ignored because it was very low compared to the weight of quartz sand. The extracellular carbohydrate and protein in biofilm biomass were extracted by heating in a water bath at 80 °C for 40 min. The protein was determined by the Lowry method (Lowry et al. 1951) and the carbohydrate was determined by the phenol–sulfuric acid method (Dubois et al. 1956).

Wastewater flow along the biofilter depth could be considered as a plug flow (Capua et al. 2015), and
denitrification was described with a half-order reaction as proposed by Harremoës (1976).

RESULTS AND DISCUSSION

Long-term system performance and operational stability

Both biofilters were started at the EBRT of 15 min and the C/N ratio of 1.5 g/g, and the two biofilters reached steady state after 15 days’ operation. After a period of stable operation, the C/N ratio was then decreased to 1.25 and 0.75 g/g sequentially. The performance of the two biofilters during the long-term operation at C/N ratios of 1.5, 1.25, and 0.75 g/g is summarized in Table 1.

Due to the dosed methanol in the system, the effluent COD increased slightly. At C/N ratios of 1.5, 1.25, and 0.75 g/g, with respect to the influent NO$_3$-N concentrations of 17.39, 11.97, and 14.65 mg/L, its removal percentages were 73%, 77%, and 50% in DNBFS and 43%, 25%, and 21% in DNBFL, respectively. Therefore, with decreasing C/N ratios, the NO$_3$-N removal percentage decreased. The removal percentage in DNBFL was much lower than that in DNBFS, indicating that the small sand size DNBF possessed a high denitrification potential with the same packed volume compared to the large one. For the C/N ratios of 1.5 and 1.25 g/g in DNBFS, the performance in the removal of NO$_3$-N was similar, whereas when this ratio was lowered to 0.75 g/g, the denitrification efficiency decreased significantly. At the C/N ratio of 0.75 g/g, the NO$_2$-N concentration in both biofilters was much higher than that at the other two ratios, indicating that the dosed methanol was insufficient for denitrification. Foglar & Briški (2003) reported that the denitrifying process with methanol as the organic carbon proceeded completely when the C/N ratio was higher than 0.93. In the study of Her & Huang (1995), with methanol as the organic carbon, the denitrifying process proceeded incompletely with the accumulation of NO$_2$-N when the C/N ratio was lower than 0.9.

The effluent concentrations of TP and turbidity in DNBFS were lower than those in DNBFL, which might be due to the fact that the packed sand in DNBFS possessed a better particle removal efficiency with a low porosity (Moore et al. 2001). Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the removed PO$_4^{3-}$-P per gram NO$_3$-N removed was 0.012, 0.013, and 0.009 g in DNBFS, and 0.013, 0.021, and 0.019 g in DNBFL, respectively. Therefore, the PO$_4^{3-}$-P requirement for denitrification in DNBFS was around 0.01 g PO$_4^{3-}$-P/g NO$_3$-N, which was similar to that obtained in previous studies (de Barbadillo et al. 2006; Boltz et al. 2012). Neethling et al. (2010) considered that the demand for PO$_4^{3-}$-P was around 0.02–0.03 g PO$_4^{3-}$-P/g NO$_3$-N when PO$_4^{3-}$-P was

| Performance of the two biofilters during the long-term operation |
|------------------|------------------|------------------|
| **DNBFS**        | **DNBFL**        |
|                  | C/N – 1.5 | C/N – 1.25 | C/N – 0.75 | C/N – 1.5 | C/N – 1.25 | C/N – 0.75 |
| COD              |           |           |           |           |           |           |
| Influent         | 40        | 23        | 24        | 40        | 23        | 24        |
| Effluent         | 50        | 29        | 30        | 81        | 42        | 55        |
| NO$_3$-N         |           |           |           |           |           |           |
| Influent         | 17.39     | 11.97     | 14.65     | 17.39     | 11.97     | 14.65     |
| Effluent         | 4.59      | 2.79      | 7.27      | 9.88      | 9.05      | 11.47     |
| NO$_2$-N         |           |           |           |           |           |           |
| Influent         | 0.04      | 0.04      | 0.03      | 0.04      | 0.04      | 0.03      |
| Effluent         | 0.82      | 0.54      | 2.39      | 0.86      | 0.92      | 1.41      |
| TN               |           |           |           |           |           |           |
| Effluent         | 6.01      | 5.22      | 11.11     | 11.97     | 9.59      | 16.22     |
| TP               |           |           |           |           |           |           |
| Influent         | 0.65      | 0.57      | 0.43      | 0.65      | 0.57      | 0.43      |
| Effluent         | 0.15      | 0.14      | 0.18      | 0.29      | 0.35      | 0.22      |
| PO$_4^{3-}$-P    |           |           |           |           |           |           |
| Influent         | 0.24      | 0.23      | 0.18      | 0.24      | 0.23      | 0.19      |
| Effluent         | 0.09      | 0.11      | 0.11      | 0.14      | 0.17      | 0.13      |
| NTU              |           |           |           |           |           |           |
| Influent         | 6.72      | 7.90      | 7.05      | 6.72      | 7.90      | 7.05      |
| Effluent         | 1.97      | 1.84      | 1.66      | 2.22      | 2.23      | 2.11      |
| DO               |           |           |           |           |           |           |
| Influent         | 2.28      | 2.51      | 2.31      | 2.28      | 2.51      | 2.31      |
| Effluent         | 1.56      | 1.95      | 1.82      | 1.68      | 2.20      | 1.76      |
sufficient, and was 0.01 g PO$_4^3-$P/g NO$_3^-$N or lower when PO$_4^3-$P was insufficient. Under C/N ratios of 1.25 and 0.75 g/g, the PO$_4^3-$P in DNBFL could be considered sufficient due to the low denitrification efficiency (only 2.65 and 3.18 mg/L NO$_3^-$N was removed), inducing a higher PO$_4^3-$P demand.

Methanol requirement correlated with the removed oxidized nitrogen and DO could be estimated by the following equation (McCarthy et al. 1989):

$$C_{\text{Methanol}} = 2.47\text{NO}_3^-\text{-N} + 1.53\text{NO}_2^-\text{-N} + 0.87\text{O}_2$$

Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the theoretical required C/N ratios calculated were 0.94, 0.95, and 0.96 g/g in DNBFS, and 0.95, 0.96, and 0.98 g/g in DNBFL, respectively. The observed C/N ratios in the two biofilters were 1.33, 1.17, and 1.08 g/g in DNBFS, and 1.16, 1.70, and 1.70 g/g in DNBFL. The higher observed C/N ratios were due to the fact that partially consumed methanol might be degraded by processes other than denitrification. De Barbadillo et al. (2008) concluded that the C/N ratio was in the range of 1.19–1.3 g/g with methanol as the electron donor, which was similar to that in DNBFS. The observed ratios in DNBFL under C/N ratios of 1.25 and 0.75 were both 1.70 g/g, which was higher than those of 1.19–1.3 g/g. Under these two ratios, only 2.65 mg/L and 3.18 mg/L NO$_3^-$N were removed in DNBFL, indicating that denitrifying activities were low and more methanol was utilized by other pathways.

Samples were taken at intervals starting from the end of the backwashing to the beginning of the next backwashing, and an example of NO$_3^-$N dynamics is shown in Figure 1. The performance of both biofilters recovered within 1 hour after backwashing. During the backwashing cycle, the performance of both biofilters was stable, with the effluent NO$_3^-$N concentration fluctuating within 1 mg/L and TP concentration fluctuating within 0.05 mg/L in most cases.

### Nutrient removal along the biofilter depth

Concentrations of NO$_3^-$N and NO$_2^-$N along the biofilter depth are shown in Figure 2. The NO$_3^-$N concentration decreased with the increasing filter depth while the NO$_2^-$N concentration followed the contrary trend.

The concentrations within different biofilter depths were then converted into their dynamics with time and linearly regressed, with results shown in Table 2. As shown in Table 2, the DNBFS had a high denitrifying rate and the rate in both biofilters decreased with decreasing C/N ratios. In addition, the NO$_2^-$N accumulation rate increased significantly under the C/N ratio of 0.75 in DNBFS, indicating that insufficient methanol caused significant NO$_2^-$N accumulation. Foglar & Briški (2003) also found that a significant amount of NO$_2^-$N (higher than 1 mg/L) accumulated in a denitrifying process with methanol as carbon source when the C/N ratios were 0.75 and 0.55.

Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the half-order coefficients were 0.40, 0.38, and 0.14 (mg/L)$^{1/2}$/min in DNBFS, and 0.26, 0.08, and 0.02 (mg/L)$^{1/2}$/min in DNBFL, respectively. The half-order coefficient decreased with decreasing C/N ratios, with lower values in DNBFL. Janning et al. (1995) found that the half-order coefficient decreased with decreasing initial NO$_3^-$N concentrations in a denitrification filter. Additionally, it could be concluded from this study that the half-order coefficient increased with increasing C/N ratios or with decreasing sand sizes.
Biofilm biomass and denitrification activities

Dynamics of oxidized nitrogen during denitrification for biomass taken from the backwashing samples from both biofilters is shown in Figure 3. Due to the high NO₂⁻N accumulation in the experiments, the NO₂⁻N concentration was taken into account for examining the denitrification activities of the biomass. Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the nitrate reduction rates were 0.53, 0.40, and 0.17 mg/(g·min) in DNBFS, and 0.46, 0.36, and 0.14 mg/(g·min) in DNBFL, respectively. The denitrification activities of the biomass were similar in the two biofilters and decreased with decreasing C/N ratios. Foglar & Briški (2003) investigated denitrification with methanol as the organic carbon, and demonstrated that the denitrification rate was high and stable when the C/N ratio was 1.1–1.7, then slightly decreased when the C/N ratio was around 0.93, and decreased significantly when the C/N ratio was 0.75 and 0.55.

Biofilm biomass and its extracellular protein and carbohydrate contents at different biofilter depths were tested under different C/N ratios, with results shown in Table 3. Most biomass was located at the filter depth of 30 cm.

Table 2 | Denitrifying biokinetics in the two biofilters obtained from the depth distribution of oxidized nitrogen

<table>
<thead>
<tr>
<th>C/N ratio</th>
<th>DNBFS</th>
<th>DNBFL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reaction rate</td>
<td></td>
</tr>
<tr>
<td>NO₃⁻N reduction rate (mg/L·min)</td>
<td>1.31</td>
<td>1.10</td>
</tr>
<tr>
<td>NO₂⁻N accumulation rate (mg/L·min)</td>
<td>0.13</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Figure 2 | Dynamics of NO₃⁻N and NO₂⁻N concentrations in both biofilters along the filtration depth.

Figure 3 | Dynamics of NO₃⁻N and NO₂⁻N concentrations for biomass taken from the backwashing samples in both biofilters.
higher amount of biomass adhered to per grams sands was obtained in DNBFS than in DNBFL. In addition, the proportion of extracellular protein and carbohydrate in biomass under high C/N ratios with high denitrifying rates was lower than under low C/N ratios with low denitrifying rates. This might be because more extracellular protein and carbohydrate were produced under organic limited conditions to protect microorganisms from unfavorable conditions (Zhang & Bishop 2005).

Owing to similar denitrifying activities of biofilm in the two biofilters and a higher amount of biofilm biomass existing in DNBFS, the difference in denitrification performance of the two biofilters was mainly due to the different amount of biofilm biomass. Therefore, for biofilm-based systems, it would be better to increase the biofilm biomass to enhance the system performance, such as using small-size carriers. In addition, besides the biofilm biomass, diffusion limitation should also be considered by controlling biofilm thickness for enhancing biofilm activities (Picioreanu et al. 1997).

### CONCLUSIONS

1. Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the NO$_3^-$-N removal efficiency was 73%, 77%, and 50% and the effluent TP concentration was 0.15, 0.14, and 0.18 mg/L in DNBFS; the NO$_3^-$-N removal efficiency was 43%, 25%, and 21% and the effluent TP concentration was 0.29, 0.35, and 0.24 mg/L in DNBFL. The NO$_3^-$-N removal efficiency decreased with decreasing C/N ratios while the NO$_2^-$-N accumulation followed a contrary trend.
2. Under C/N ratios of 1.5, 1.25, and 0.75 g/g, the NO$_3^-$-N reduction rates were 1.31, 1.10, and 0.48 mg/(L·min) and the half-order coefficients were 0.40, 0.38, and 0.14 (mg/L)$^{1/2}$/min in DNBFS; the NO$_3^-$-N reduction rates were 0.97, 0.27, and 0.10 mg/(L·min) and the half-order coefficients were 0.26, 0.08, and 0.02 (mg/L)$^{1/2}$/min in DNBFL, respectively. The reduction rates and the half-order coefficients in DNBFS were higher than those in DNBFL and decreased with decreasing C/N ratios in both biofilters.
3. Under the C/N ratios of 1.5, 1.25, and 0.75 g/g, the consumed PO$_4^{3-}$-P to NO$_3^-$-N ratios in DNBFS were 0.012, 0.013, and 0.009 g/g, and were 0.013, 0.021, and 0.019 g/g in DNBFL. Thus, DNBFL had a higher requirement of PO$_4^{3-}$-P for denitrification.
4. Different denitrification performance in the two biofilters was due to different amounts of biofilm biomass rather than denitrifying activities.

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## REFERENCES


