

Remediation of nitrate-contaminated wastewater using denitrification biofilters with straws of ornamental flowers added as carbon source

Junjun Chang, Luyao Ma, Yuanyang Zhou, Shenghua Zhang and Weilu Wang

ABSTRACT

Straws of four ornamental flowers (carnation, rose, lily, and violet) were added into denitrification biofilters using gravel as matrix through vertically installed perforated polyvinylchloride pipes to provide organic carbon for the treatment of nitrate-contaminated wastewater operating in batch mode. Removal efficiencies of nitrate and phosphate, as well as temporal variations of nitrogen and carbon during batches 10 and 19, were investigated and assessed. Nitrate removal was efficiently enhanced by the addition of flower straws, but decreased gradually as the organic substances were consumed. Phosphate removal was also improved, although this very limited. High nitrate removal rates were achieved during the initial 12 h in the two batches each lasting for 3 days, along with the depletion of influent dissolved oxygen due to aerobic degradation of the organic compounds. NO_2^- -N of 0.01–2.83 mg/L and NH_4^+ -N of 0.02–1.69 mg/L were formed and both positively correlated to the nitrate reduced. Inorganic carbon (IC) concentrations increased during the batches and varied conversely with the nitrate contents, and could be indicative of nitrate removal due to the highly significant positive correlation between NO_3^- -N removed and IC concentration ($r^2 = 0.881$, $p < 0.0001$). It is feasible and economical to use the denitrification biofilter to treat nitrate-contaminated wastewater, although further optimization of carbon source addition is still required.

Key words | denitrification biofilters, nitrate removal, phosphate removal, solid carbon source, temporal variations

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INTRODUCTION

Nitrate-nitrogen (NO_3^- -N) can range from 5.0 to 325 mg/L in secondary effluent from wastewater treatment plants and septic tanks, agricultural runoff, greenhouse outflow, etc., and can contribute to eutrophication and algal blooms in the receiving waters (McIsaac *et al.* 2001; Park *et al.* 2009; Schipper *et al.* 2010). Furthermore, shallow groundwater is vulnerable to this nitrate pollution, which could cause methemoglobinemia in infants and alimentary canal cancers to the drinkers (Gulis *et al.* 2002). Thus, effective methods for nitrate pollution remediation need to be developed.

Biological heterotrophic denitrification, converting NO_3^- -N to gaseous nitrogen by denitrifying bacteria under a reducing environment using organic carbon as electron donor and energy source, is an efficient, economical, environmentally friendly and thus a promising process for NO_3^- -N

removal (Shrimali & Singh 2001; Schipper *et al.* 2010). Furthermore, some heterotrophic denitrifiers can accumulate excess phosphorus, another critical pollutant responsible for eutrophication, beyond their metabolic requirements, by the synthesis of poly-phosphate (poly-P) using organic carbon as energy source (Barak & van Rijn 2000). However, these processes are usually restrained by the low availability of organic carbon source in nitrate-contaminated wastewaters, thus the supplement of exogenous organic matter is needed to achieve the effective removal of pollutants.

In comparison to liquid carbon sources usually applied such as methanol, ethanol, and acetate (Gómez *et al.* 2000), solid organic material of natural origin is a preferable alternative owing to its low/zero cost and simple dosing and process control (Ashok & Hait 2015). Various natural

organic solids, such as wheat or rice straw (Zhang *et al.* 2012; Yang *et al.* 2015), maize cobs (Warneke *et al.* 2011), woodchips (Schipper *et al.* 2010), vegetable litters (Park *et al.* 2009), and cattail litters (Wen *et al.* 2010), have been successfully employed to promote denitrification in recent years. It is termed as 'solid-phase denitrification' with the solid organic matter serving not only as constant power to fuel denitrification but also carrier for microbial films development (Wang & Wang 2013).

Some denitrifying bioreactors with microbial attached rather than suspended growth processes, including denitrification wall/bed/filter and constructed wetland, are applicable for nitrate pollution mitigation, especially in agricultural and rural areas due to their cost-effective and simple construction and operation (Vymazal 2009; Schipper *et al.* 2010; Wen *et al.* 2010). In these systems, organic solids are usually employed as the matrix directly or mixed with inert materials such as gravel, sand, etc. to provide carriers for microbial proliferation and carbon source for denitrification. However, from a practical point of view, maintaining the structural stability and permeability of the installation using solid organic material as bed media may be a challenge (Della Rocca *et al.* 2005; Cameron & Schipper 2010). Furthermore, the numbers of microorganisms attached on the same volume of solid organic substrate are orders of magnitude lower than those on an inert matrix (Chen *et al.* 2014), which may limit the nitrate removal rate. In fact, appropriate dosage and frequency of supplement and replacement of the solid carbon source in denitrification systems are crucial to keep effective nitrate removal and reduce the generation of secondary pollutants, including N₂O, which is a relevant greenhouse gas, caused by excessive leachings of carbon and nutrient (Park *et al.* 2009; Wen *et al.* 2010; Wunderlin *et al.* 2012; Hernandez-Paniagua *et al.* 2014). Some efforts have been made in this field. Woodchips are usually chosen as common denitrification bed media due to their slow decomposition and mild releases of carbon and nitrogen which allow them to work for a longer lifespan and generate low secondary pollution (Cameron & Schipper 2010; Warneke *et al.* 2011). Park *et al.* (2009) designed a hybrid denitrification filter by combining a solid organic material digester zone and another denitrification filter zone filled with gravel to treat hydroponic wastewater, achieving high nitrate removal (>95%) with the vegetable litters replaced every 2 days and the effluent recirculated at a rate of 100% of the influent flow. Efficient phosphate removal (76.6–93.2%) was also achieved, probably due to excess P accumulation by heterotrophic denitrifying

bacteria in the filter, although this might not be sustainable over a long operational period (Park *et al.* 2009).

In this study, biofilters using common gravel as the matrix for denitrifying bacteria colonization were set up to treat nitrate-contaminated wastewater and the straws from four common ornamental flowers: carnation, rose, lily, and violet, were added respectively through vertically installed perforated polyvinylchloride (PVC) pipes as carbon source. Flower straw was employed because the cultivation of ornamental flowers and plants is an important industry in the Kunming, Yunnan province of China, with substantial plant litters as well as nursery runoff containing high concentrations of nitrate and phosphate threatening the adjacent environment, especially Dianchi Lake. This was a preliminary study without the straws replaced or replenished when the nitrate removal efficiency obviously declined, and the main purposes were to investigate and evaluate the nitrate and phosphate removals, and the variations of nitrogen and carbon in the biofilters to offer some useful information for the design and operation of denitrification biofilters treating nitrate-contaminated wastewater.

MATERIALS AND METHODS

Solid carbon source

Straws of four common ornamental flowers which were composed of stems and a small part of leaves: carnation (*Dianthus caryophyllus*), rose (*Rosa rugosa*), lily (*Lilium brownii*), and violet (*Matthiola incana*) were collected from a local ornamental plants trading market and used as carbon source in this study. The straws were taken back to the laboratory, cleaned with tap water, cut into sections of about 5 cm and dried in an oven at 70 °C to a constant weight before being preserved in vacuum containers.

Experimental set-up of the denitrification biofilters

Five identical laboratory-scale denitrification biofilters were set up using polyethylene tanks each with dimensions of 0.7 m in length, 0.45 m in width and 0.45 m in depth. Each biofilter was divided into influent (0.05 m × 0.45 m × 0.45 m), treatment (0.60 m × 0.45 m × 0.45 m), and effluent (0.05 m × 0.45 m × 0.45 m) zones, with gravel of grain size of 15–25 mm filled in influent and effluent zones to facilitate the distribution and collection of wastewater and gravel of 3–6 mm in the treatment zone except that for organic solid addition. The depth of the substrate was 0.40 m, and

the schematic diagrams of the biofilter are shown in Figure 1.

With regard to the addition of carbon source, a perforated PVC pipe of diameter 110 mm was put vertically into the biofilter at the length of 0.2 m ahead of gravel filling (Figure 1). The flower straw was put into a nylon bag and then added into the pipe. Fresh organic material can be easily replenished or replaced at this assembling, which is significant in field application as mentioned above.

Additionally, another PVC pipe with diameter of 20 mm was installed in the center of each biofilter at a depth of 20 cm for temporal water sampling (Figure 1). The effective working volume of each biofilter was 38.0 L. No inoculation with external microorganisms was conducted, and a stabilization period of about 4 months was allowed for the development of microbial consortia in the biofilters before the formal experiment began.

Influent and operations of the biofilters

The biofilters were operated in batch mode, with the wastewater fed at the beginning of each batch within 30 min, then drained with gravity after a retention time of 3 days (72 h). Nitrate-contaminated wastewater was artificially prepared in five feeding tanks before each batch by adding NaNO_3 and K_2HPO_4 into tap water to form a NO_3^- -N concentration of 57.0 ± 0.4 mg/L and PO_4^{3-} -P concentration of 6.0 ± 0.1 mg/L. After 12 operation batches, the pollutant concentrations were approximately reduced by 50% due to the low nitrate removal efficiency.

When the formal experiment started, 320 g dried flower straw was added, respectively, into the carbon source addition pipe in each biofilter apart from the control system. The dosage of the straw was referred to the

amount of plant biomass added by Wen *et al.* (2010) in spite of the different modes for carbon source addition.

Sampling and analysis

The overall influent and effluent of each biofilter were sampled for each batch with wastewater fed and then drained after a retention time of 3 days. Moreover, water sampling campaigns were conducted at times equivalent to different retention times of the wastewater during batches 10 and 19 using a 50 ml syringe at the sampling point (Figure 1) to determine the temporal variations of water quality characteristics in the biofilters. Dissolved oxygen (DO) concentration was measured *in situ* by a portable DO meter (HANNA HI-9146, Italy). Water samples were then taken into the laboratory and analyzed immediately. NO_3^- -N, NO_2^- -N, NH_4^+ -N and PO_4^{3-} -P were determined according to the standard colorimetric methods after proper pretreatment (SEPA 2002). The total organic carbon (TOC) and inorganic carbon (IC) contents were analyzed by a TOC analyzer (GE Sievers InnovOx, USA). The correlation analysis between parameters was performed by Origin 7.0 software.

RESULTS AND DISCUSSION

Nitrate and phosphate removal efficiencies in the denitrification biofilters

NO_3^- -N and PO_4^{3-} -P concentrations in the influent and effluent of the denitrification biofilters are presented in Figure 2.

Almost no removal of nitrate occurred in the control biofilter without flower straw addition. However, effective nitrate removal was achieved in the systems with added

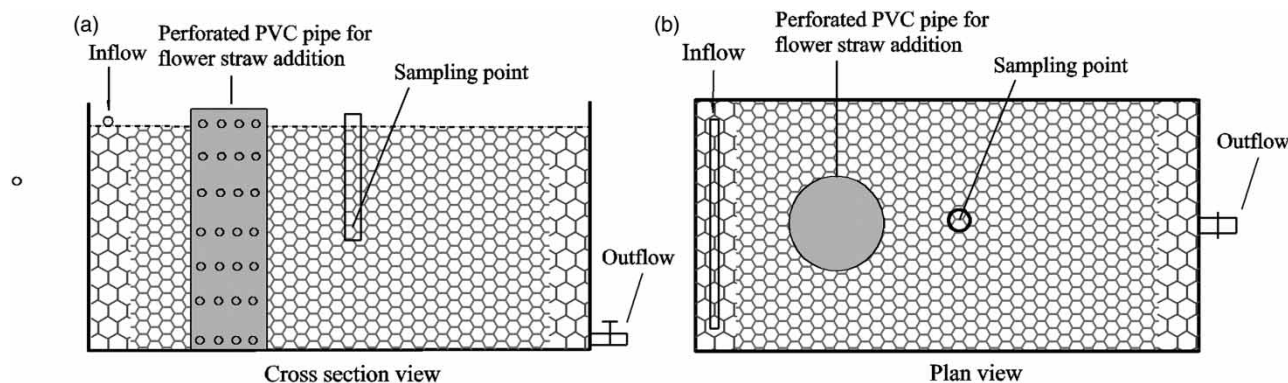


Figure 1 | Schematic diagrams of the experimental denitrification biofilters.

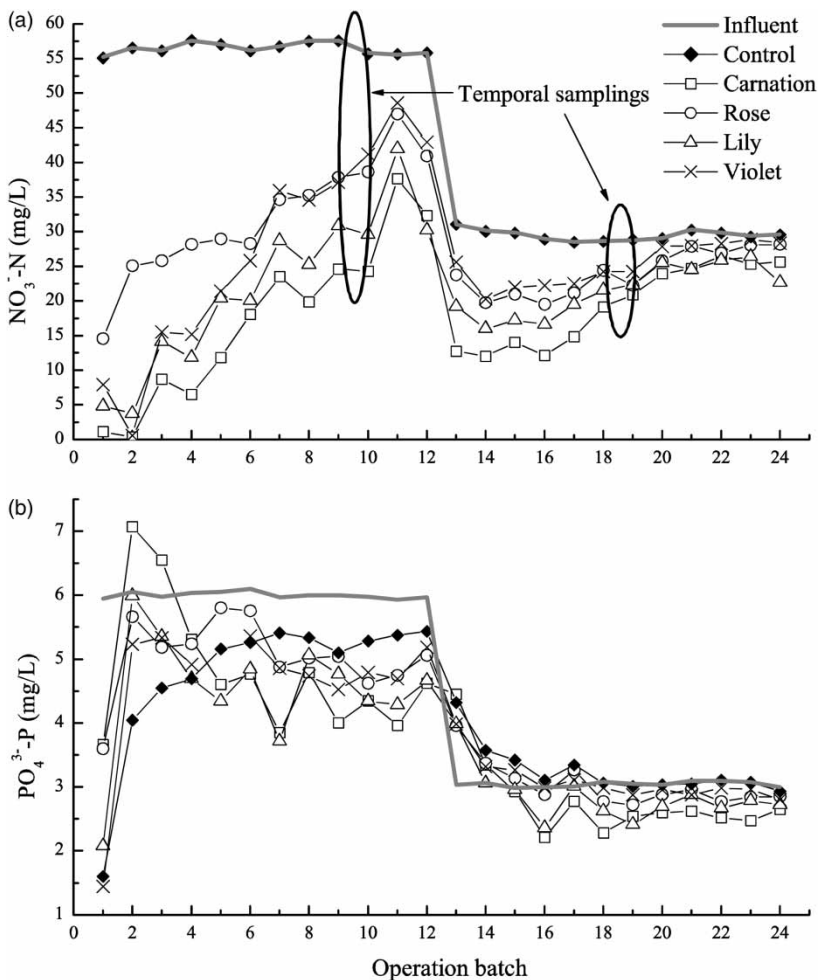


Figure 2 | Influent and effluent NO_3^- -N (a) and PO_4^{3-} -P (b) concentrations of the denitrification biofilters.

flower straws because the straws could be utilized by denitrifying bacteria on the substrate after being dissolved, hydrolyzed and diffused. The highest efficiency was achieved by carnation straw while rose straw was the least efficient (Figure 2(a)), with an average value of 51.85% and 31.09% respectively. This might be due to higher contents of cellulose and hemicellulose in carnation straw while the higher lignin content in rose straw could affect the carbon assimilation of the microbial consortia (Wen *et al.* 2010; Pulou *et al.* 2012). Effluent NO_3^- -N concentrations of all experimental biofilters increased gradually with the operation time, from 1.1–14.6 mg/L in batch 1 to 37.7–48.6 mg/L in batch 11 as a result of the decline in the availability of carbon source, and the effects of straws were insignificant after 20 operational batches. Similar trends were also recorded in previous studies (Park *et al.* 2009; Wen *et al.* 2010) due to the unsteady release rate of carbon from the natural solid materials, which could result in

high content of organic matter discharged during the initial operational stage followed by insufficiency of carbon availability for denitrification. It was noted that the declines in nitrate removal were not influenced when the nitrate influent concentration was halved. Thus, the optimization of dosage and frequency of flower straw supplement in the denitrification biofilters is necessary, which is feasible under this operational regime. However, it was expected that the flower straws might be able to work for a longer time than the experimental period of this study with the insoluble organic carbon decomposed gradually at a slow rate, and the subsequent supplement frequency could be reduced.

PO_4^{3-} -P concentration increased sharply from 1.45–3.66 mg/L in batch 1 to 4.05–7.08 mg/L in batch 2, indicating the inefficiency of the denitrification biofilters for P removal, probably due to the rapid reduction of adsorption capacity of the gravel (Brix *et al.* 2001; Ayaz *et al.* 2012).

Fast adsorption followed by slower precipitation is the main pathway for P removal in the biofilters (Vymazal 2009), with a very limited portion of P eliminated from batch 2. Higher effluent phosphate content than that in influent was observed after reducing by half the influent concentration, due to desorption and release of some P retained within the matrix. Saturation of P retention capacity might have occurred in the control biofilter since batch 18, without phosphate removed. This was consistent with some previous reports (Ayaz et al. 2012; Chang et al. 2015) stating that the P removal efficiency in gravel-based biofilters was low and varied greatly, especially after long-term operation.

Regarding the effect of flower straw addition on phosphate removal, higher outflow contents were determined in the experimental biofilters than in the control during the initial four to six operational batches (Figure 2(b)), caused by the leaching and transformation of P from the organic solids. Nevertheless, after that, lower effluent concentrations were observed in the experimental systems, especially that with carnation straw added, with a mean removal efficiency of 5.5–15.2% higher than that of the

control, suggesting that the addition of flower straws could promote the phosphate removal of the denitrification biofilters although the release of organic P could not be ignored. This might be a result from heterotrophic denitrifying P-removal bacteria (DPB), which can store excess phosphorus through the synthesis of poly-P under anoxic conditions and obtain energy by oxidation of the organic carbon (Barak & van Rijn 2000), were presented in the biofilters and their performances were enhanced by the addition of flower straws. Park et al. (2009) treated hydropenic wastewater using a hybrid denitrification filter and obtained significantly higher phosphate removal than in our study. This might be due to the much higher capacity of DPB for P accumulation in that system.

Temporal variations of water quality characteristics in the biofilters

The variations in water quality characteristics in the denitrification biofilters with retention time during batch 10 and 19 are depicted in Figures 3 and 4, respectively.

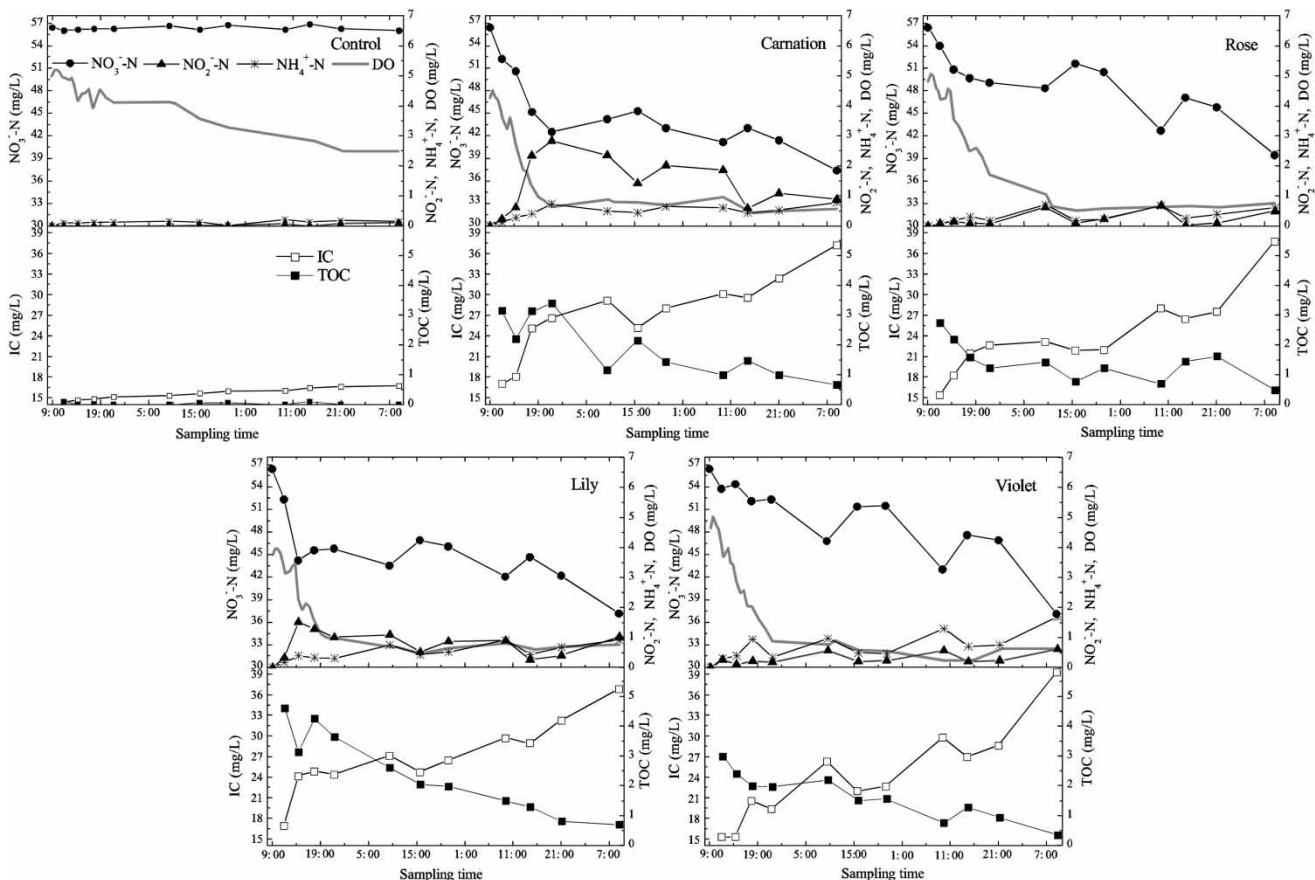


Figure 3 | Changes in water quality characteristics vs. hydraulic retention time in the denitrification biofilters during batch 10.

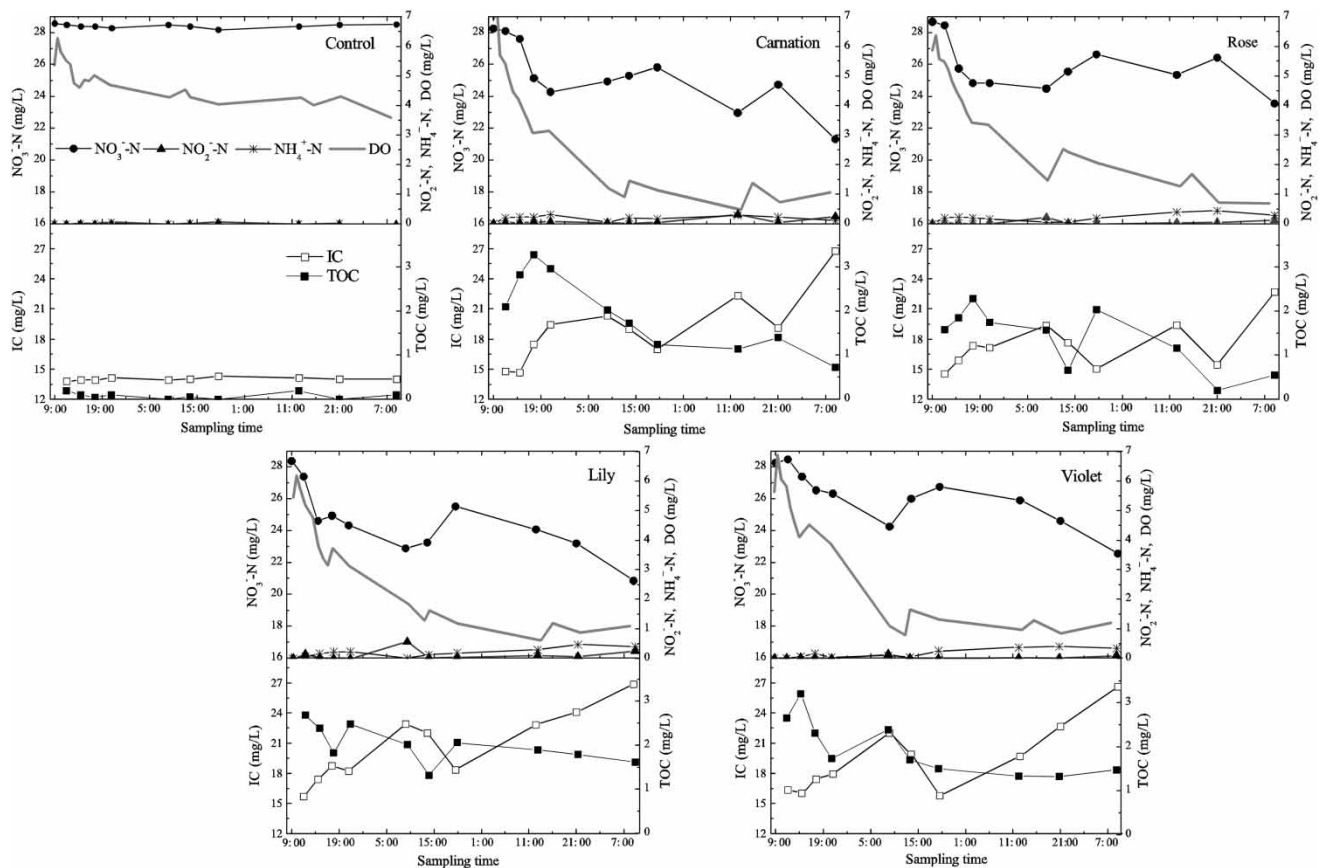


Figure 4 | Changes in water quality characteristics vs. hydraulic retention time in the denitrification biofilters during batch 19.

Very little change in the water quality took place in the control system. In the experimental biofilters, only a portion of the influent NO_3^- -N was reduced within 3 days, ranging from 30.1–34.3% in batch 10 and 17.1–26.6% in batch 19, due to the lack of usable organic carbon which could be reflected by the quite low TOC content in all cases. The nitrate content decreased at relatively fast rates during the initial 12 h, along with the rapid depletion of DO primarily due to aerobic degradation of the organic matter in the biofilters, indicating the important role of flower straws in the creation of a reducing environment beneficial to denitrification. After fresh wastewater was fed in, the carbon source was washed out and diffused greatly, with nitrate reduced despite the fact that the DO depletion was still proceeding, suggesting that the presence of DO did not inhibit the denitrification in the biofilters (Hernandez-Paniagua *et al.* 2014). This was consistent with the findings of Huang *et al.* (2015), and might be due to the presence of anoxic microsites within the biofilters. The shortage of carbon source was more severe in batch 19, corresponding to the lower amount nitrate reduced and notably longer time needed for DO consumption (Figures 3 and 4).

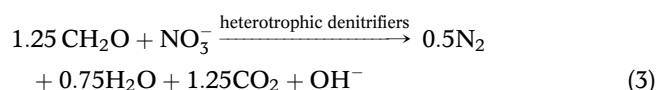
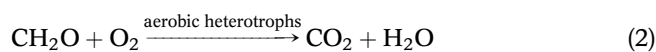
It was observed that nitrite, a toxic intermediate product in the denitrification process, was generated with the reduction of nitrate, especially during batch 10, probably due to the low availability of carbon source and the presence of DO, which restricted the denitrification (Yang *et al.* 2012; Chang *et al.* 2013). Ammonium was also formed, primarily derived from the dissimilatory nitrate reduction to ammonium process. Indeed, significant negative correlations between NO_3^- -N and NO_2^- -N/ NH_4^+ -N concentrations were detected in both batches ($p < 0.01$), showing the transformation of nitrogen in the biofilters. Similar to the nitrate removal efficiency, the production of nitrite and ammonium was also related to the species of carbon sources added. Despite the fact that the NO_3^- -N removed was significantly more than the sum of NO_2^- -N and NH_4^+ -N generated, minimization of the production of nitrite and ammonium in such denitrification biofilters is the object of a future study. In addition to nitrogen conversions, the increases in nitrate concentrations during a batch could be a result of its diffusion around the sampling point due to the nitrate content in the biofilter being very variable (Zhong *et al.* 2014) especially

adding the carbon source in such a 'point' mode rather than dosing it uniformly into the biofilter in this study. In fact, the nitrate concentration determined at the sampling point at the end of a batch was different from that of the overall out-flow (Figures 2–4). In addition, it was observed in all cases that apparent nitrate reduction occurred during the night, probably due to the slower diffusion and absence of photosynthesis of the microalgae in the biofilter, which was favorable for denitrification.

Organic carbon in the biofilters was gradually consumed, with the TOC content gradually decreasing during the batch experiments, and the IC concentration increased and varied conversely with NO_3^- -N concentration exactly. Statistical analysis revealed that the NO_3^- -N removed (mg/L) was positively correlated with the IC concentration (mg/L) highly significantly as in Equation (1):

$$\text{NO}_3^- - \text{N}_{\text{removed}} = 0.789 \times \text{IC} - 10.99 (r^2 = 0.881, p < 0.0001, n = 84) \quad (1)$$

This agrees with Yamashita *et al.* (2011) because the IC could be produced during the aerobic decomposition of organic compounds and heterotrophic denitrification as described by Equations (2) and (3) (Della Rocca *et al.* 2005; Yamashita *et al.* 2011):



where CH_2O represents a generic organic compound.

The temporary declines in IC were probably due to the diffusion around the sampling point mentioned above, and the high value of correlation coefficient suggested that the IC concentration could be a considerably good indicator for the nitrate removal in the denitrification biofilter, with the less nitrate reduced, the closer to the blank value the IC concentration being.

CONCLUSIONS

Based on the findings of this study, the main conclusions are as follows:

1. Nitrogen in nitrate-contaminated wastewater was effectively removed by gravel-based denitrification biofilters

applying straws of ornamental flowers as exogenous carbon source, which were added through vertically installed perforated PVC pipes. Without intentional replenishment or replacement of the straws, nitrate removal rates decreased gradually as organic substances were consumed. Phosphate removal was also enhanced by the supplement of flower straws although this was very limited.

2. During operational batches 10 and 19, each lasting for 3 days, high nitrate removal rates were achieved in the initial 12 h, along with the consumption of DO in the wastewater due to aerobic degradation of the organic compounds. NO_2^- -N and NH_4^+ -N were generated and both were positively related to the nitrate reduced, although their sum was substantially lower than the NO_3^- -N removed.
3. TOC contents were low and decreased gradually during the two batches, and the IC increased and varied conversely with the nitrate contents, which could be a good indicator for nitrate removal due to its highly significant positive correlation with NO_3^- -N removed ($r^2 = 0.881$, $p < 0.0001$).
4. It is feasible and economical to use the denitrification biofilter to treat nitrate-contaminated wastewater, especially nursery runoff, although further study on optimization of the carbon source addition is still needed.

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REFERENCES

- Ashok, V. & Hait, S. 2015 *Remediation of nitrate-contaminated water by solid-phase denitrification process—a review. Environmental Science and Pollution Research* **22** (11), 8075–8093.
- Ayaz, S. Ç., Aktaş, Ö., Findik, N. & Akça, L. 2012 *Phosphorus removal and effect of adsorbent type in a constructed wetland system. Desalination and Water Treatment* **37** (1–3), 152–159.

- Barak, Y. & van Rijn, J. 2000 [Atypical polyphosphate accumulation by the denitrifying bacterium *Paracoccus denitrificans*](#). *Applied and Environmental Microbiology* **66**, 1209–1212.
- Brix, H., Arias, C. A. & Del Bubba, M. 2001 [Media selection for sustainable phosphorus removal in subsurface flow constructed wetlands](#). *Water Science and Technology* **44** (11–12), 47–54.
- Cameron, S. G. & Schipper, L. A. 2010 [Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds](#). *Ecological Engineering* **36** (11), 1588–1595.
- Chang, J., Wu, S., Dai, Y., Liang, W. & Wu, Z. 2013 [Nitrogen removal from nitrate-laden wastewater by integrated vertical-flow constructed wetland systems](#). *Ecological Engineering* **58**, 192–201.
- Chang, J., Wu, S., Zhang, S., Zhang, S. & Liang, W. 2015 [Comparative evaluation of total phosphorus removal performances for treatment of domestic and secondary wastewater using integrated vertical-flow constructed wetlands: two years' experience](#). *Desalination and Water Treatment* **56** (5), 1379–1388.
- Chen, Y., Wen, Y., Zhou, J., Tang, Z., Li, L., Zhou, Q. & Vymazal, J. 2014 [Effects of cattail biomass on sulfate removal and carbon sources competition in subsurface-flow constructed wetlands treating secondary effluent](#). *Water Research* **59**, 1–10.
- Della Rocca, C., Belgiorno, V. & Meric, S. 2005 [Cotton-supported heterotrophic denitrification of nitrate-rich drinking water with a sand filtration post-treatment](#). *Water SA* **31**, 229–236.
- Gómez, M. A., González-López, J. & Hontoria-García, E. 2000 [Influence of carbon source on nitrate removal of contaminated groundwater in a denitrifying submerged filter](#). *Journal of Hazardous Materials* **80** (1), 69–80.
- Gulis, G., Czompolyova, M. & Cerhan, J. R. 2002 [An ecologic study of nitrate in municipal drinking water and cancer incidence in Trnava District, Slovakia](#). *Environmental Research* **88** (3), 182–187.
- Hernandez-Paniagua, I. Y., Ramirez-Vargas, R., Ramos-Gomez, M. S., Dendooven, L., Avelar-Gonzalez, F. J. & Thalasso, F. 2014 [Greenhouse gas emissions from stabilization ponds in subtropical climate](#). *Environmental Technology* **35** (6), 727–734.
- Huang, G., Huang, Y., Hu, H., Liu, F., Zhang, Y. & Deng, R. 2015 [Remediation of nitrate-nitrogen contaminated groundwater using a pilot-scale two-layer heterotrophic-autotrophic denitrification permeable reactive barrier with spongy iron/pine bark](#). *Chemosphere* **130**, 8–16.
- McIsaac, G. F., David, M. B., Gertner, G. Z. & Goolsby, D. A. 2001 [Eutrophication: nitrate flux in the Mississippi River](#). *Nature* **414** (6860), 166–167.
- Park, J. B. K., Craggs, R. J. & Sukias, J. P. S. 2009 [Removal of nitrate and phosphorus from hydroponic wastewater using a hybrid denitrification filter \(HDF\)](#). *Bioresource Technology* **100** (13), 3175–3179.
- Pulou, J., Tournebize, J., Chaumont, C., Haury, J. & Laverman, A. M. 2012 [Carbon availability limits potential denitrification in watercress farm sediment](#). *Ecological Engineering* **49**, 212–220.
- Schipper, L. A., Robertson, W., Gold, A. J., Jaynes, D. B. & Cameron, S. C. 2010 [Denitrifying bioreactors—an approach for reducing nitrate loads to receiving waters](#). *Ecological Engineering* **36** (11), 1532–1543.
- Shrimali, M. & Singh, K. P. 2001 [New methods of nitrate removal from water](#). *Environmental Pollution* **112** (3), 351–359.
- State Environmental Protection Administration (SEPA) 2002 *Monitoring and Analysis Methods of Water and Wastewater*. 4th edn. China Environmental Science Press, Beijing.
- Vymazal, J. 2009 [The use of constructed wetlands with horizontal subsurface flow for various types of wastewater](#). *Ecological Engineering* **35** (1), 1–17.
- Wang, X. M. & Wang, J. L. 2013 [Nitrate removal from groundwater using solid-phase denitrification process without inoculating with external microorganisms](#). *International Journal of Environmental Science and Technology* **10** (5), 955–960.
- Warneke, S., Schipper, L. A., Matiassek, M. G., Scow, K. M., Cameron, S., Bruesewitz, D. A. & McDonald, I. R. 2011 [Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrification beds](#). *Water Research* **45** (17), 5463–5475.
- Wen, Y., Chen, Y., Zheng, N., Yang, D. & Zhou, Q. 2010 [Effects of plant biomass on nitrate removal and transformation of carbon sources in subsurface-flow constructed wetlands](#). *Bioresource Technology* **101** (19), 7286–7292.
- Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L. & Siegrist, H. 2012 [Mechanisms of N₂O production in biological wastewater treatment under nitrifying and denitrifying conditions](#). *Water Research* **46** (4), 1027–1037.
- Yamashita, T., Yamamoto-Ikemoto, R. & Zhu, J. 2011 [Sulfate-reducing bacteria in a denitrification reactor packed with wood as a carbon source](#). *Bioresource Technology* **102** (3), 2235–2241.
- Yang, X., Wang, S. & Zhou, L. 2012 [Effect of carbon source, C/N ratio, nitrate and dissolved oxygen concentration on nitrite and ammonium production from denitrification process by *Pseudomonas stutzeri* D6](#). *Bioresource Technology* **104**, 65–72.
- Yang, X. L., Jiang, Q., Song, H. L., Gu, T. T. & Xia, M. Q. 2015 [Selection and application of agricultural wastes as solid carbon sources and biofilm carriers in MBR](#). *Journal of Hazardous Materials* **283**, 186–192.
- Zhang, J., Feng, C., Hong, S., Hao, H. & Yang, Y. 2012 [Behavior of solid carbon sources for biological denitrification in groundwater remediation](#). *Water Science and Technology* **65** (9), 1696–1704.
- Zhong, F., Wu, J., Dai, Y., Cheng, S., Zhang, Z. & Ji, H. 2014 [Effects of front aeration on the purification process in horizontal subsurface flow constructed wetlands shown with 2D contour plots](#). *Ecological Engineering* **73**, 699–704.

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