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

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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. NO2⁻/CO3⁻-N of 0.01–2.83 mg/L and NH4⁺-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 NO3⁻-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

INTRODUCTION

Nitrate-nitrogen (NO3⁻-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 NO3⁻-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 NO3⁻-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 2015).

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 hypodromic 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 NaNO₃ and K₂HPO₄ into tap water to form a NO₃⁻/C⁰⁻-N concentration of 57.0 ± 0.4 mg/L and PO₄³⁻/C⁰⁻-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. NO₃⁻-N, NO₂⁻-N, NH₄⁺-N and PO₄³⁻-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

NO₃⁻-N and PO₄³⁻-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](https://iwaponline.com/wst/article-pdf/74/2/416/460627/wst074020416.pdf) | Schematic diagrams of the experimental 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 hydroponic 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.
Very little change in the water quality took place in the control system. In the experimental biofilters, only a portion of the influent NO\textsubscript{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\textsubscript{3}-N and NO\textsubscript{2}-N/\textsubscript{NH}_\textsubscript{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\textsubscript{3}-N removed was significantly more than the sum of NO\textsubscript{2}-N and \textsubscript{NH}_\textsubscript{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, prob-
elably 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 NO3-N concentration exactly. Statistical
analysis revealed that the NO3-N removed (mg/L) was posi-
tively correlated with the IC concentration (mg/L) highly
significantly as in Equation (1):

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

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):

\[
\text{CH}_2\text{O} + O_2 \xrightarrow{\text{aerobic heterotrophs}} \text{CO}_2 + \text{H}_2\text{O} \tag{2}
\]

\[
1.25 \text{CH}_2\text{O} + \text{NO}_3^- \xrightarrow{\text{heterotrophic denitifiers}} 0.5\text{N}_2 + 0.75\text{H}_2\text{O} + 1.25\text{CO}_2 + \text{OH}^- \tag{3}
\]

where CH2O represents a generic organic compound.

The temporary declines in IC were probably due to the diffu-
sion around the sampling point mentioned above, and the high
value of correlation coefficient suggested that the IC concen-
tration 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 effec-
tively 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. NO2-N and NH4-N were generated and
both were positively related to the nitrate reduced,
although their sum was substantially lower than the
NO3-N removed.

3. TOC contents were low and decreased gradually during
the two batches, and the IC increased and varied con-
versely with the nitrate contents, which could be a good
indicator for nitrate removal due to its highly significant
positive correlation with NO3-N removed ($r^2 = 0.881,\n\ p < 0.0001$).

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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