An innovative wood-chip-framework substrate used as slow-release carbon source to treat high-strength nitrogen wastewater

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ABSTRACT

Removal of nitrogen in wastewater before discharge into receiving water courses is an important consideration in treatment systems. However, nitrogen removal efficiency is usually limited due to the low carbon/nitrogen (C/N) ratio. A common solution is to add external carbon sources, but amount of liquid is difficult to determine. Therefore, a combined wood-chip-framework substrate (with wood, slag and gravel) as a slow-release carbon source was constructed in baffled subsurface-flow constructed wetlands to overcome the problem. Results show that the removal rate of ammonia nitrogen (NH4+–N), total nitrogen (TN) and chemical oxygen demand (COD) could reach 37.5%–85%, 57.4%–86%, 32.4%–78%, respectively, indicating the combined substrate could diffuse sufficient oxygen for the nitrification process (slag and gravel zone) and provide carbon source for denitrification process (wood-chip zone). The nitrification and denitrification were determined according to the location of slag/gravel and wood-chip, respectively. Nitrogen removal was efficient at the steady phase before a shock loading using slag-wood-gravel combined substrate because of nitrification–denitrification process, while nitrogen removal was efficient under a shock loading with wood-slag-gravel combined substrate because of ANAMMOX process. This study provides a new idea for wetland treatment of high-strength nitrogen wastewater.

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Introduction

Constructed wetland (CW) is a promising technique for removing pollutants from wastewaters due to their low energy consumption and cost-effective operation (Fountoulakis et al., 2009). Removal of nitrogen from wastewater before discharging into receiving water courses is an important consideration in treatment systems. Major removal processes for nitrogen in CW are nitrification–denitrification processes. And the sediment adsorption also plays an important role in nitrogen removal. However, field tests have shown insufficient oxygenation of the rhizosphere in horizontal-flow (HF) CWs, leading to incomplete nitrification, and becoming the major cause of limited nitrogen removal (Vymazal, 2007; Tunçsiper, 2009). In contrast, the vertical-flow (VF) CWs have a much greater oxygen transport capacity, which allows ammonia nitrogen (NH4+–N) to be successfully removed, but limited denitrification occurs in such system (Abou-Elela et al., 2013). Therefore, either HF or VF

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system alone cannot achieve high removal efficiency of nitrogen because of their inability to provide both aerobic and anaerobic conditions simultaneously. In fact, in the hybrid system, the variation in the oxidation and reduction conditions for the VF and HF systems can be fully exploited for nitrogen and chemical oxygen demand (COD) removal (Perfler et al., 1999; Li et al., 2009). However, the limitations of hybrid systems include the requirement of a relatively large land area and a recycling system. More recently, the implementation of horizontal baffled subsurface-flow CW (HBSFCW) (Tee et al., 2012) demonstrated enhanced pollutants removal performances, due to prolonged water pathway by forcing the wastewater to flow up and down, and effective use of aerobic-anaerobic-anaerobic regions of the system. Thereby nitrification and denitrification can coexist in the same CW. However, it is difficult to achieve efficient denitrification in CW when the influent has low C/N ratio or the major labile organic matters are eliminated efficiently denitrification in CW when the influent has low C/N ratio and BOD or the major labile organic matters are eliminated via microbial oxidation (Wen et al., 2010).

Thus, methanol (Huet et al., 2005), fructose (Lin et al., 2002) and glucose (Caselles-Osorio and García, 2006) were used as external carbon sources to enhance the denitrification efficiencies in CWs. Zhao et al. (2010) found that conventional VF obtained total nitrogen (TN) removal efficiency of 25%–62% when C/N ratios was 2.5 to 10, and highest TN removal rates were observed at C/N ratios of 2.5–5. However, Zhao et al. (2012) obtained better TN removal (62%–87%) in hybrid VF at C/N ratios of 5–10, although the influent TN concentration was almost the same in the two studies. While Ding et al. (2012) found that TN removal efficiency (21%–54%) increased with increasing C/N ratios (0–9). So it could be concluded that the addition volume of liquid carbon source was difficult to control in CWs for different structures and operating conditions.

To overcome these problems, a novel wood-chip-framework, including wood-chips, slag and gravel, was introduced as a slow-release carbon source for treating high-strength nitrogen wastewater in HBSFCW. The wood-chip-framework attached with pulverous soil was believed to be beneficial in developing a more complex microbial community. In addition, gravel and slag could help maintain pH and promote nitrification because of their oxygen diffusion ability. Also, nitrification-denitrification processes were adjusted by controlling the sequences of combined substrates to withstand shock loading of wastewater.

1. Materials and methods

1.1. Synthetic high-strength nitrogen wastewater

The synthetic wastewater compositions were COD, 100–200 mg/L; NH₄-N, 15–40 mg/L; NO₃-N, 15–50 mg/L; TN, 30–90 mg/L; total phosphate (TP), 10 mg/L; pH, 7.2–8.0; and trace element solution (in g/L) 0.2 mL/L (50.0 EDTA-N₂₃₂·2.2 ZnSO₄·5H₂O, 5.5 CaCl₂·2H₂O, 0.56 MnCl₂·4H₂O, 5.0 FeSO₄·7H₂O, 1.1 (NH₄)₆Mo₇O₂₄·4H₂O, 1.57 CuSO₄·5H₂O, 1.61 CoCl₂·6H₂O, pH = 7.0) was prepared according to the literature (Ozeki et al., 2001).

1.2. Design and operation of lab-scale HBSFCW systems

Two lab-scale HBSFCWs with the same configurations 1.0 × 0.5 × 0.5 m (length × width × height), namely systems A and B, were built in greenhouse, and were planted with the combined Canna and Lythrum at a density of 26 rhizomes/m² (Fig. 1). For both HBSFCWs, five vertical baffles were placed lengthwise to facilitate up-flow and down-flow conditions sequentially in different segments of the units. The support media which were packed to a depth of 0.4 m consisting of a combination of slag, wood-chips and gravel in a volume ratio of 1:1:1 (0.3 m), with slag occupying the first one third and wood-chips for the second one third for system A. Tops were covered with gravel (0.1 m). On the contrary, the support media with wood-chips occupying the first one third and slag for the second one third for system B. For system A, taps were placed at a height of 0 and 0.2 m from the bottom corresponding to the positions of A01–A06 and A201–A206, respectively (Fig. 1a). Taps were the same in the system B (B01–B06 and B201–B206) (Fig. 1b). Briefly, the wood-chips were 3–4 cm long, 2–3 cm wide and 0.4–0.6 cm thick, and were soaked in tap water for 2 weeks before being packed in the HBSFCWs. Then the soaked wood-chips were rolled over the soil. The bulk volume ratio of wood-chips to soil was about 3:1.

Both systems were initially fed with the synthetic wastewater at a fixed influent rate of 30 mL/min, and the operational period was 100 days. To startup both HBSFCWs, wastewater with 100–200 mg/L COD, 15–40 mg/L NH₄-N, and 15–50 mg/L NO₃-N was used. After the startup stage (i.e. the first 40 days after installation), another two shock loading stages were carried out in the two systems. The controlling conditions of the both HBSFCWs are illustrated in Table 1.

1.3. Sampling and analysis

The sampling frequency was once a day, and the collected samples were stored at −4 °C before testing. COD, NH₄-N, NO₃-N, and TN were analyzed according to the “Standard Methods for the Examination of Water and Wastewater” (APHA, 1998). The oxidation-reduction potential (ORP) and pH values of samples were measured using an ORP/pH meter (Eutech Model pH 1100).

2. Results and discussion

2.1. Effect of combined substrates on nitrogen removal

Both HBSFCWs were started up with a hydraulic retention time (HRT) of 2 days, and the organic volume loading rate (OLR), NH₄-N and NO₃-N volume loading rates (NLR) in stage 1 were approximately 78.7 g-COD/(m³·day), 9.3 g-NH₄-N/(m³·day) and 17.1 g-NO₃-N/(m³·day), respectively (Table 1). Greenhouse temperature was 21.2 ± 0.5 °C, and the temperature of wastewater was 20.3 ± 0.4 °C for system A and 19.7 ± 0.5 °C for system B (Fig. 2), which was suitable for the metabolism of microorganism and could ensure the HBSFCWs to obtain a good efficiency of COD and nitrogen removal (Chang et al., 2013; Huang et al., 2013). During the startup period, NH₄-N concentration first decreased and then increased in both systems (Fig. 3). The results were in accordance with previous publication (Buelna et al., 2008), in which the absorption and ion exchange contributed a significant importance to NH₄-N removal in
the beginning. When the saturation sorption was achieved on day 5, NH₄⁺-N removal rate decreased over time, and then increased quickly (23 days) until reaching stability on day 34, indicating that ammonia oxidizing bacteria was gradually enriched in the system. NO₃⁻-N removal rate reached around 99% after a little decreasing at the first few days for both systems, indicating that carbon source for denitrification was sufficient in both systems. Average TN removal rates of 86.8% and 81.3% were obtained for systems A and B, respectively, at the startup stage, indicating that nitrification and denitrification were achieved simultaneously in the systems.

COD removal rate dropped dramatically and fluctuated between 5th and 14th days for both systems, most likely due to the washout of the organic matter from the media. Then COD removal rate increased quickly and reached a plateau. Higher organic removal rates were mainly derived from two aspects. Firstly, the design of the baffled units could provide a longer treatment pathway thus allowing more duration of exposure to microorganisms (Garcia et al., 2005; Huang et al., 2005; Noorvee et al., 2007). Secondly, oxygen leakage from macrophytes roots could support aerobic degradation at top layer of the systems, and anaerobic degradation at the bottom confirmed by the lower redox potential values (Fig. 2) was also contributed to COD removal.

The interference of COD removal on nitrification was demonstrated in Fig. 4. Effluent NH₄⁺-N concentrations were raised with increasing of influent COD/NH₄⁺-N ratios, further supporting the interference of COD removal on nitrification process, indicating the competition for DO (Sun et al., 2006). However, nitrification was not diminished drastically despite higher organic removal rate, which does not coincide with the previous studies (Luanaigh et al., 2010; Wu et al., 2011), indicating simultaneous nitrification and higher organic removal and sufficient oxygen availability in both systems.

### Table 1 – Stages and operating parameters of both horizontal baffled subsurface-flow constructed wetlands (HBSFCWs).

<table>
<thead>
<tr>
<th>Stages</th>
<th>Day</th>
<th>HRT (day)</th>
<th>COD volume loading (g/(m³-day))</th>
<th>NH₄⁺-N/NO₃⁻-N volume loading (g/(m³-day))</th>
<th>Wastewater COD (mg/L)</th>
<th>NH₄⁺-N (mg/L)</th>
<th>NO₃⁻-N (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-40</td>
<td>2</td>
<td>78.7 ± 19.4</td>
<td>9.3 ± 1.5/17.1 ± 6.6</td>
<td>157.3 ± 38.7</td>
<td>18.5 ± 3.0</td>
<td>34.1 ± 13.2</td>
</tr>
<tr>
<td>2</td>
<td>41-73</td>
<td>2</td>
<td>53.7 ± 6.0</td>
<td>7.9 ± 0.1/9.6 ± 1.4</td>
<td>107.4 ± 12.0</td>
<td>15.9 ± 0.2</td>
<td>19.1 ± 2.7</td>
</tr>
<tr>
<td>3</td>
<td>74-100</td>
<td>2</td>
<td>91.0 ± 7.3</td>
<td>18.2 ± 1.1/22.1 ± 1.0</td>
<td>182.1 ± 14.6</td>
<td>36.3 ± 2.1</td>
<td>44.1 ± 1.9</td>
</tr>
</tbody>
</table>

HRT: hydraulic retention time.
COD: chemical oxygen demand.
This is because a good channel of oxygen diffusion was formed by slag and gravel, and thus promoting diffusion oxygen of plant roots secreting.

The NO$_3^-$-N removal was generally accomplished by denitrification (Bachand and Horne, 2000), and carbon source was critical to the process. Substantial organic removal often decreased carbon source availability in wastewater, and thus restricting denitrification process (Luanaigh et al., 2010; Lavrova and Koumanova, 2010). Simultaneous denitrification and higher organic removal performances in both systems indicated that, carbon source requirement (for denitrification) was not supported by the input COD quantity in wastewater. This was also demonstrated in Fig. 4, where the effluent NO$_3^-$-N concentrations did not reduce obviously with increasing COD/NO$_3^-$-N ratios. These findings indicated that the employed wood-chip media could have provided required carbon source to support denitrification.

2.2. Effect of substrates sequence on nitrification and denitrification process

Spatial distribution of pH, ORP and nitrogenous compounds illustrated nitrification–denitrification potential in different locations of HBSFCW as shown in Fig. 5. pH values in both systems were ranged from 6.8 to 7.8, which were beneficial for nitrifying and denitrifying bacteria (Al-Omari and Fayyad, 2003; Tanveer and Sun, 2012). pH first increased at top layer from A$_{20}$ 1 to A$_{20}$ 2 for system A, because of the introduced alkalinity of slag. There was obviously a decreasing trend in pH from A$_{20}$ 3 to A$_{20}$ 6, indicating that nitrification process was dominant because of plants secreting oxygen. pH at the bottom layer from A$_{0}$ 1 to A$_{0}$ 6 had the same trend with that of top layer, but was slightly higher at A$_{0}$ 4, indicating the denitrification in wood-chip zone at the bottom. pH of B$_{20}$ 1–B$_{20}$ 6 and B$_{0}$ 1–B$_{0}$ 6 showed the same downward trend, suggesting the nitrification in slag and gravel zones, which could result in a drop of pH (Gray, 2004; Vymazal, 1999). Results revealed that the sequence of nitrogen removal was nitrification (slag zone)–denitrification (wood-chip zone)–nitrification (gravel zone) in system A, while the sequence in system B was denitrification (wood-chip zone)–nitrification (slag zone)–nitrification (gravel zone).

Redox potentials greater than 100 mV indicate an aerobic environment, whereas ones less than −100 mV indicate an anaerobic environment (Suthersan, 2002). As shown in Fig. 5, ORP in systems of A and B were ranged from −50 mV to −350 mV, which suggested the anoxic and anaerobic condition (Faulwetter et al., 2009). In addition, ORP values decreased first
and then increased at top layer of A\textsubscript{20} 1–A\textsubscript{20} 6 and bottom layer of A\textsubscript{0} 1–A\textsubscript{0} 6 for system A, indicating anoxic condition in slag and gravel zones, and anaerobic conditions in middle position (wood ship zone), which was in accordance with pH value. ORP at top layer of B\textsubscript{20} 1–B\textsubscript{20} 6 and bottom layer of B\textsubscript{0} 1–B\textsubscript{0} 6 showed the same upward trend, ranging from \(-300\) to \(-50\) mV. The lowest ORP (<\(-300\) mV) was situated on fore-end (wood ship zone, B\textsubscript{20} 1a and B\textsubscript{0} 1) for system B which favored for denitrification process; and the highest ORP was lain in rear-end (slag and gravel zone, B\textsubscript{20} 6a and B\textsubscript{0} 6), which was benefit for nitrification process. Results of ORP and pH could confirm each other in the systems. That is, the wood-chip could form denitrification zone because of the slow-release carbon source, and the slag and gravel could form nitrification zones because of oxygen diffusion channel.

Comparison of NH\textsubscript{4}\textsuperscript{+}-N, NO\textsubscript{3}\textsuperscript{-}-N and total nitrogen (TN) removal in different locations of HBSFCWs is presented in Fig. 5. NH\textsubscript{4}\textsuperscript{+}-N concentrations in the fore-end were higher than those in the rear-end. The reason was that oxidation of organic matter restrained reaction of nitrification by competition for DO in the fore-end of system (Ding et al., 2014). Consequently, the rear-end (gravel zone) was the main zone for NH\textsubscript{4}\textsuperscript{+}-N degradation, which was consistent with the results of pH. The average NH\textsubscript{4}\textsuperscript{+}-N removal in the rear-end for system A reached 88.7% while those in the fore-end (slag zone) was 73.1%. Although soil infiltration had a favorable ability of nitrification in the
fore-end zone where oxygen was sufficient (van Cuyk and Siegrist, 2007), NH$_4$-N removal revealed a first decreased and then increased trend in system B, because of the inhibition of nitrification in fore-end (wood-chips zone). Spatial variation in NO$_3$-N showed that the mean effluent concentrations of NO$_3$-N were ranged from 0 to 0.8 mg/L (Fig. 5). The NO$_3$-N removal was relatively good and stable in the whole process, because of anaerobic environment and wood-chips as internal carbon source in both systems. The sufficient carbon resource caused comparatively efficient denitrification.

Generally, both systems reached stable after 40 days. Removal efficiency of NH$_4$-N, TN and COD were better for system A than that for system B, while NO$_3$-N removal efficiency was consistent in both systems. Average removal rates of NH$_4$-N, NO$_3$-N, TN and COD for system A were 85%, 98.4%, 86% and 78% respectively, while 72%, 98.7%, 82% and 66% for system B. So, nitrogen removal was efficient at a steady phase using slag–wood–gravel combined substrate due to the nitrification–denitrification process.

2.3. Effect of loading rate on performance of HBSFCWs

In order to evaluate the performance of the HBSFCWs at a shock loading, another two stages were carried out at different loading rates. The average COD removals in stage 2 with an OLR and NLR of 53.7 g-COD/(m$^3$.day), 7.9 g-NH$_4$-N/(m$^3$.day) and 9.6 g-NO$_3$-N/(m$^3$.day) reached 78.5% and 74.7% in system A and system B, respectively (Fig. 2). A satisfactory ability of nitrification was observed in both systems (Fig. 3). The average NH$_4$-N removal of 63.7% and 68.4% was obtained for system A and system B in stage 2, with an effluent NO$_3$-N 0.1 and 0.2 mg/L, respectively, while TN removals were 75.9% and 78.4%, respectively.

In system B, the wood-chips was located at the fore-end, so the three sections of hypoxia environment were formed due to the depletion of oxygen when wastewater was discharged into the system. While in system A, because wood-chips were located at the middle, when wastewater was discharged from the fore-end, aerobic–anoxic–anaerobic environment was formed. Therefore, denitrification coupled with anaerobic ammonium oxidation (ANAMMOX) process which was an anaerobic process in system B was more likely to happen, so nitrogen removal was higher in system B than that of system A. This phenomenon coincided with the study of Takekawa et al. (2014). During this anoxic process, NO$_3$-N was reduced to NO$_2$-N by denitrifying bacteria, and then NH$_4$-N was used as the electron donor while NO$_2$-N as electron acceptor, and then they were transferred to N$_2$ (Poth and Focht, 1985).

When the OLR and NLR increased to 91.0 g-COD/(m$^3$.day), 18.2 g-NH$_4$-N/(m$^3$.day) and 22.1 g-NO$_3$-N/(m$^3$.day) in stage 3, both systems were affected significantly, and strong fluctuation was observed in TN removal until day 90 (Fig. 3). The TN removal of system B was 62.6%, which was better than that of system A (57.4%). The result coincided with Hu et al. (2011), who reported that the nitrogen removal was ranged from 42% to 60% at a sudden loading of the submerged filters. Although the organic degrading microbes were more versatile to sudden loading changes, the COD removals were also significantly affected for both systems (Fig. 2), which was caused by plants withered and oxygen deficit. But COD removal of system B was better than that of system A, which was also caused by denitrification coupled with ANAMMOX process in anaerobic environment.

Reduction of nitrification rates after shock loading in both systems might be attributed to metabolic disruption of the sensitive nitrifiers (Grady et al., 1999). However, the denitrification process was not affected significantly, and NO$_3$-N removals were maintained at more than 99% for both systems (Fig. 3). Although the higher influent concentration (NH$_4$-N and NO$_3$-N) enhanced denitrification coupled ANAMMOX, NH$_4$-N removal decreased significantly for both systems, which was because the anaerobic nitrogen removal efficiency of NH$_4$-N was lower than that of aerobic. The average NH$_4$-N removals were 35.7% and 41.3% for system A and system B, respectively, which were much lower than that in stage 2. When both HBSFCWs performed stable in 91 days, the COD removal was 32.4% for system A and 42.1% for system B, respectively, which was obviously lower than that of stage 2. Therefore, removal efficiency of NH$_4$-N, TN and COD were better for system B than that of system A. That is to say, substrates subsequence of system B, i.e., wood–slag–gravel combined substrates, was more resistant to the shock loading.
Fig. 5 – Spatial variation of pH, oxidation-reduction potential (ORP), ammonia and nitrate in both HBSFCWs.
3. Conclusions

A combined wood-chip-framework substrate used in baffled subsurface-flow CWs was constructed to treat high-strength nitrogen wastewater. The combined substrate could provide sufficient oxygen for the nitrification process (slag and gravel zone) and slow-release carbon source for denitrification process (wood-chip zone), and thus could overcome the problem of controlling liquid carbon adding quantity. The position of denitrification was determined according to location of wood-chip, while the position of nitrification was determined on locations of slag and gravel. As slag–wood–gravel combined substrate, nitrogen removal was efficient at steady phase before a shock loading, because of nitrification-denitrification process. While as wood–slag–gravel combined substrate, nitrogen removal was efficient under a shock loading, because of ANAMMOX process. This study provides a new idea for wetland treatment of high-strength nitrogen wastewater.

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