Comparison of denitrification performances using PLA/starch with different mass ratios as carbon source
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ABSTRACT
A suitable carbon source is significant for biological nitrate removal from groundwater. In this study, slow-release carbon sources containing polylactic acid (PLA) and starch at 8:2, 7:3, 6:4, 5:5, 4:6, and 3:7 ratios were prepared using a blending and fusing technique. The PLA/starch blend was then used as a solid carbon source for biological nitrate removal. The carbon release rate of PLA/starch was found to increase with increased starch content in leaching experiments. PLA/starch at 5:5 mass ratio was found to have the highest denitrification performance and organic carbon consumption efficiency in semi-continuous denitrification experiments, and was also revealed to support complete denitrification at 50 mg-N/L influent nitrate concentration in continuous experiments. The effluent nitrate concentration was <2 mg NO₃⁻-N/L, which met the national standard (GB 14848-93) for groundwater. Scanning electron microscopy results further showed that the surface roughness of PLA/starch increased with prolonged experimental time, which may be conducive to microorganism attachment. Therefore, PLA/starch was a suitable carbon source and biofilm carrier for groundwater remediation.

Key words | denitrification, groundwater, polylactic acid, solid carbon source, starch

INTRODUCTION
Excessive fertilizer used in intensive agriculture, and nitrogen-containing wastewaters discharged from municipal and industrial wastewater treatment plants cause nitrate pollution in lakes and groundwater. Direct use of groundwater, containing high concentrations of nitrate, for drinking water may result in methemoglobinemia in infants and potentially cause some cancers (Aslan & Cakici 2007). The limit of NO₃⁻-N in groundwater set by the Ministry of Land and Resources of the People’s Republic of China for a drinking water source is 20.0 mg/L (GB 14848-93). Among various available methods for nitrate removal from groundwater, heterotrophic biological denitrification is considered the best option because of its practicability, moderate cost, and environmentally sound nature (Van Rijn et al. 2006; Khanitchaidecha & Kazama 2012; Saeed & Sun 2012; Zhang et al. 2012). In denitrification, heterotrophic denitrifying bacteria require sufficient organic carbon as an electron donor for nitrate removal (Shen & Wang 2011). However, contaminated groundwater tends to contain high concentrations of nitrate but relatively insufficient organic carbon to support denitrification (Srinandan et al. 2012). Liquid carbon sources, such as methanol, glucose, and acetic acid, have been used to stimulate microbial denitrification in organic carbon-limited wastewater. However, apart from its sophisticated and costly process, liquid carbon sources may pose a risk of overdose, resulting in deterioration of the quality of the effluent (Zhang et al. 2012).

To avoid these problems, denitrifying biofilters and denitrification walls have been developed for nitrate removal from groundwater. In these systems, solid carbon sources, such as wood, barley straw, and rice husks, are used as carbon sources to facilitate a sustainable denitrification reaction (Hashemi et al. 2011). In addition, denitrification has been reported to improve when applied with solid carbons of sawdust, kenaf, or corncob (Chen et al. 2009; Li et al. 2012). Although natural materials are much cheaper, they may result in high dissolved organic carbon (DOC), color problems, and unsustainability over long periods of time because of the depletion of readily available carbon.
Preparation of the blends

Ten-gram blends with different mass ratios of PLA and starch were added to seven 250-mL Erlenmeyer flasks. Each flask was filled with nitrogen gas to maintain anaerobic conditions. The solution pH was adjusted to ∼7.5 with 1 M NaOH/HCl solution. Each flask was then sealed and placed in a shaking incubator at 70 rpm and 25 ± 1 °C. The liquid samples (2.5 mL/time) were taken every 2 days and the chemical oxygen demand (COD) concentration was analyzed to investigate the carbon-releasing behavior.

Semi-continuous denitrification experiments

Ten-gram blends with different mass ratios of PLA and starch were added to seven 250-mL Erlenmeyer flasks. Each flask was filled with certain amounts of domestic activated sludge, KNO₃, KH₂PO₄, and distilled water. Each flask's liquid volume was 150 mL. The mixed liquor suspended solid, nitrate, and phosphorus concentrations were 800 mg/L, 50 mg/L, NO₃⁻, and 10 mg/L, respectively. The solution pH was adjusted to ∼7.5 with 1 M NaOH/HCl solution. The tanks were filled with nitrogen gas to maintain anaerobic conditions. Each flask was then sealed and placed in a shaking incubator at 70 rpm and 25 ± 1 °C. The batch reaction time (BRT) in semi-continuous experiments was 12 h. After standing for 15 min, 10 mL liquid samples were collected at the end of the experiments to analyze water quality (i.e., NO₂⁻, NO₃⁻, COD, and pH). Fresh synthetic wastewater was then used to replace the 120 mL solution. The pH, nitrate, and phosphorus concentrations of the solution were set to the desired condition as previously mentioned.

Continuous experiment

The optimal mass ratio of the blends obtained in leaching experiments was applied in the continuous denitrification experiment. Experiments were conducted in a Plexiglas bioreactor with 10 cm internal diameter and 45 cm effective height. The effective working volume was 1 L. Before being transferred into the bioreactor for the continuous experiment, 300 g of blends were immersed in the domestic active sludge for 15 days. The bioreactor was operated in upflow mode and supplied with synthetic wastewater having the same nitrogen and phosphorus concentrations as in those of semi-continuous experiments. The synthetic wastewater’s influent rate was controlled using a peristaltic pump and set to 5.6 mL/min, which resulted in a 5 h hydraulic retention time (HRT). Continuous denitrification
Denitrification rate

Water samples were filtered through a 0.45 μm membrane filter before analysis. The concentrations of \( \text{NO}_3^-\cdot\text{N} \), \( \text{NH}_4^+\cdot\text{N} \), \( \text{NH}_2\cdot\text{N} \), and COD in filtered liquid were assayed according to the Water and Wastewater Monitoring Analysis Method (SEPA 2002). The pH value was determined by pH meter. Moreover, surface changes of the blends in different experimental stages were observed by scanning electron microscope (ZEISS, Jena, Germany). The denitrification rate (mg L\(^{-1}\) h\(^{-1}\)) was described by Equation (1):

\[
\text{Denitrification rate} = \frac{[\text{NO}_3^-\text{(inf)} + \text{NO}_2\text{(inf)}] - \text{NO}_3\text{eff}}{- \text{NO}_2\text{eff}}} / \text{BRT}
\]

where \( \text{NO}_3^-\text{inf} \) and \( \text{NO}_2\text{inf} \) are the initial nitrate and nitrite concentrations (mg L\(^{-1}\)); \( \text{NO}_3\text{eff} \) and \( \text{NO}_2\text{eff} \) are the nitrate and nitrite concentrations of the drainage (mg L\(^{-1}\)); BRT is BRT (h) in semi-continuous experiments.

RESULTS

Carbon release performance of blends with different mass ratios

As shown in Figure 1, the total carbon release amounts (i.e., COD release amounts) of the blends after 27 days of leaching experiments were 27.0 ± 1.3, 25.9 ± 1.9, 36.9 ± 3.2, 59.2 ± 4.5, 99.0 ± 3.1, and 131.4 ± 4.3 mg for P:S ratios of 8:2, 7:3, 6:4, 5:5, 4:6, and 3:7, respectively, thereby indicating that a high PLA content corresponded to a low carbon release amount. The carbon release rate of the different blends was divided into the rapid release (0–1 day) and the slow-release stages (1–27 days). The total carbon release amounts of the blends in the first day were 10.7 ± 2.1, 10.3 ± 1.3, 16.5 ± 0.4, 23.3 ± 3.4, 22.5 ± 4.9, and 51.6 ± 2.8 mg for P:S ratios of 8:2, 7:3, 6:4, 5:5, 4:6, and 3:7, respectively. The rapid release of carbon from the blends was attributed to the release of the attached starch in the blends’ surface. The carbon release amount increased over time in the slow-release stage when the P:S ratio of the blends was ≤5:5. Conversely, the organic carbon was barely released from the blends when the ratio >5:5. This might be because the carbon release from the blends in distilled water was attributed to dissolution. The starch contained a large amount of the hydrophilic hydroxyl group, and can therefore be more rapidly released into water. Thus, the mass ratio of PLA to starch was a key parameter in determining the blend’s carbon release rate (Nampoothiri et al. 2010).

Denitrification performances of blends in semi-continuous experiments

Denitrification performances of the blends with different mass ratios are shown in Figure 2. The COD concentrations of the drainage in the experiments with PLA/starch ratios of 6:4, 5:5, 4:6, and 3:7 were higher than the others for the first 5 days (Figure 2(a)). This result was attributed to the rapid release of carbon from the surface of the blends. DOC then subsequently dropped and became almost constant, which indicated that the biofilm on the surface of the blends was mature. Except for the blends with mass ratios of 4:6 and 3:7, the COD concentrations of the drainage were maintained at a low level from 6 to 27 days for all groups. Figure 2(c) and 2(d) show \( \text{NO}_3^-\cdot\text{N} \) and \( \text{NO}_2\cdot\text{N} \) concentrations in the drainage. The nitrate concentrations sharply decreased in the drainage when the P:S ratios of the blends was ≤6:4 for the first 2 days. Moreover, nitrate removal efficiency reached ~98.0% after 6 days except for the blend with 6:4 mass ratio, which reached ~98.0% on day 12. No nitrite accumulation was observed in the blends with <0.1 mg \( \text{NO}_2^-\cdot\text{N} \)/L, suggesting complete denitrification (Yang et al. 2012). In contrast, nitrate and nitrite concentrations increased from the day 4 and reached around 50 and 6 mg/L, respectively, when the P:S ratios of the blends were >6:4. Notably, nitrate or nitrite accumulated in the drainage in the primary stage (i.e., Day 1 to Day 6) when the P:S ratios of the blends were ≤4:6. This phenomenon might be due to the sharp decline in pH (Figure 2(b)).
rapid release of organic carbon from the surface of the blends in the primary stage was due to the surplus of the denitrification reaction (Figure 2(a)). Excessive organic carbon was converted into volatile fatty acid, causing the reduction in pH. Little or no accumulation of nitrate and nitrite was observed during the period of 6–16 days, probably because of the relatively stable release rate of organic carbon, pH, and adaptation of the denitrifier.

**Denitrification performance of blends in continuous experiments**

To prevent the increase of NO$_X$-N in the effluent (i.e., Day 12 of Figure 2(c) and 2(d)), and given the insufficiency of the carbon source and secondary pollution caused by excessive organic carbon release from the blends with low P:S ratios (i.e., 4:6 and 3:7), the blend with the 5:5 mass ratio was selected for continuous experiments to evaluate its ability as carbon source for biologic denitrification. The continuous experiment was divided into two stages (Figure 3). The first stage (0–15 days) was the initiating stage. COD and nitrate concentrations of the effluent were high, and pH varied from 7 to 7.5 in this period. In the second stage (stable stage: 15–37 days), the COD concentration of effluent remained at ca. 40–60 mg/L and decreased later. Nitrate and nitrite concentrations were <2.0 and 0.2 mg/L. A high denitrification efficiency, which was up to 96.9%, was obtained. These results indicated that the blend with the P:S ratio of 5:5 was sufficient for use as a carbon source for nitrate-contaminated groundwater remediation. In this experiment, the COD concentration of the effluent was higher than that reported by Shen & Wang (2011), who employed an HRT of 2 h. However, the present experiment obtained higher nitrate removal efficiency. The COD of the effluent could be further reduced when a short HRT of 2 h was applied, as demonstrated by Shen & Wang (2011). In this case, the nitrate concentration of the effluent might increase. Regardless, the limit of nitrate concentration for drinking water is around 10 mg/L (GB 5749-2006; National Standards of the People’s Republic of China 2006). Future studies clarifying the relationship between the COD in effluent, nitrate loading, and composition of the blends are needed.

**DISCUSSION**

**Carbon release characteristics of the blends**

Related experimental data were converted into mass COD with the mg-O unit to identify the dissolution and
enzymolysis contribution to carbon released from the blends. The concrete method was as follows: NO$_3^-$-N into NO$_2^-$-N was assumed to serve as electron acceptor under anoxic conditions. Oxygen served as the electron acceptor in the presence of oxygen. The reduction of 1 mg NO$_3^-$-N into NO$_2^-$-N and N$_2$ consumed 1.15 and 2.85 mg-O, respectively. The enzymolysis effect was also assumed to slightly influence the carbon release rate contributed by the dissolution effect. Therefore, COD released in semi-continuous experiments was calculated using Equations (2)–(6):

\[ \text{COD}_{dT} = (\text{COD}_{DN} + \text{COD}_{DO} + \text{COD}_{Eff}) - \text{COD}_{Inf} \]  

(2)

\[ \text{COD}_{DN} = \left( C_{0,NO_3} - C_{t,NO_2} - C_{t,NO_3} \right) \times V \times 2.86 + C_{t,NO_2} \times V \times 1.15 \]  

(3)

\[ \text{COD}_{DO} = \left( C_{0,DO} - C_{t,DO} \right) \times V \times 2.86 \]  

(4)

\[ \text{COD}_{Eff} = C_{t,COD} \times V \]  

(5)

\[ \text{COD}_{Inf} = C_{0,COD} \times V \]  

(6)

where COD$_{dT}$ (mg-O) is the total COD released in one batch of the semi-continuous experiments (i.e., 12 h); COD$_{DN}$ (mg-O) is COD consumption by denitrification; COD$_{DO}$ (mg-O) is COD reduction caused by the dissolved oxygen utilization; COD$_{Eff}$ (mg-O) and COD$_{Inf}$ (mg-O) are the CODs of effluent and influent; C$_{0,NO_3}$ (mg-N/L) and C$_{t,NO_3}$ (mg-N/L) are the initial and final nitrate concentrations, respectively; C$_{t,NO_2}$ (mg-N/L) is the final nitrite concentration; V (L) is the solution volume (i.e., 150 mL); C$_{0,DO}$ (mg-O/L) and C$_{t,DO}$ (mg-O/L) are the initial and final dissolved oxygen concentrations; C$_{0,COD}$ (mg-O/L) and C$_{t,COD}$ (mg-O/L) are the initial and final COD concentrations, respectively.

The COD release behaviors of the blends with different mass ratios are shown in Figure 4. The COD release amount increased with an increased starch proportion in the presence of microorganisms (Figure 4(I)). The enzymolysis effect’s contribution to carbon release was much higher than the dissolution effect. Moreover, the blends’ COD release rate in the initial stage was faster than that in the later stage, except for PLA.

The COD$_{DN}$, COD$_{DO}$, and COD$_{Eff}$–COD$_{Inf}$ proportions in COD$_{dT}$ of the blends with different mass ratios during denitrification experiments are shown in Figure 4(II). The COD$_{DN}$ proportions of the blends with different mass ratios in COD$_{dT}$ were different. Carbon released in the initial stage satisfied the carbon’s requirement for denitrification when the P:S ratio of the blends was >6:4; COD$_{DN}$ occupied a large COD$_{dT}$ fraction. However, the released carbon did not maintain microorganism activities when the carbon release rate became steady. The proportion of COD$_{DN}$ to COD$_{dT}$ was below 50% in the later stage. In contrast, effective denitrification was maintained when P:S ratios of the blends was ≤5:5 (Figure 2). However, a large
amount of carbon accumulated under this condition. This carbon accumulation resulted in the wastage of more than half of the carbon (Figure 5(e) and 5(f)). The average denitrification rate of 16.3 mg L⁻¹ h⁻¹ was obtained when P:S ratios of the blends ranged from 6:4 to 5:5. This rate was lower than those (i.e., 26.9 and 25.4 mg L⁻¹ h⁻¹, respectively) observed by Shen & Wang (2011) and Shen et al. (2013). It could be attributed to the longer BRT we employed, and the higher nitrate removal rate we desired in the experiments. The CODDN occupied a large CODIT fraction under this condition, thereby avoiding the wastage of carbon and secondary pollution. The application of a blend with a 5:5 mass ratio for nitrate-contaminated groundwater was feasible.

Change of the blends’ surface properties

The surfaces of blends with a 5:5 mass ratio in different experimental stages were scanned and the results are shown in Figure 5. The raw blend’s surface was smooth (Figure 5(a)). Pores and asperities appeared after the 28-day leaching experiment (Figure 5(b)). Moreover, cavities appeared when the blend was used for denitrification experiments (Figure 5(c) and 5(d)). The blend’s surface roughness was magnified after starch utilization. Irregular hollows that developed on the blend’s surface provided large surface areas for bacterial growth. These hollows were beneficial for magnifying the effective contact area between substrate and enzyme. Therefore, the PLA/starch blend was a suitable carbon source and biofilm carrier for bacteria, especially for heterotrophic denitrifying bacteria.

CONCLUSIONS

The effects of PLA/starch blends with different mass ratios, as a slow-release carbon source, on denitrification performance were investigated. The blends’ carbon release rate was
controlled by adjusting the mass ratio of PLA to starch. The PLA/starch blend with a 5:5 mass ratio provided sufficient carbon for denitrification, as demonstrated by the high denitrification efficiency obtained (96.9–98%). SEM observation revealed that the blend can be used as a carrier for denitrifying bacteria. Therefore, the PLA/starch blends developed in this study can be applied in nitrate-polluted groundwater remediation.

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REFERENCES


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