

Effects of HRT on the efficiency of denitrification and carbon source release in constructed wetland filled with bark

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ABSTRACT

Constructed wetland is widely used to treat municipal sewage. However, lack of carbon source always constraints the application of constructed wetland in advanced tailwater treatment process. Bark was used as the filler and external carbon source of constructed wetland in the study, and the effects of hydraulic retention time (HRT) on NO_3^- -N removal efficiency and carbon release velocity were explored. Results showed that the NO_3^- -N removal process was steady in the constructed wetland filled with bark without additional carbon source. The NO_3^- -N removal efficiency and NO_3^- -N concentration presented a first-order reaction. The reaction rate constant k was 0.4 day^{-1} . The relationship between NO_3^- -N removal efficiency (η) and HRT (t) was $\eta = 1 - e^{-0.4t}$, and η was increased with increasing of HRT. η reached a maximum of 77% at HRT of 4.48 days. η obtained the minimum of 20% at HRT of 0.75 days. The relationship between the carbon source releasing velocity (v) by bark and HRT was $v = 0.53(1.62/t - 1/t^2) + 0.32$. v increased first and then decreased with HRT increasing. The maximum v was detected at $t = 1.12$ days.

Key words | bark, carbon-releasing, constructed wetland, denitrification

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INTRODUCTION

In wastewater treatment plants (WWTPs), constructed wetland is widely used because of its simple construction and low operating cost. However, insufficient carbon source always inhibits the denitrification due to low concentration of biodegradable organic matters in the tailwater from sewage plant. This condition usually occurs in constructed wetland used as an advanced denitrogenation treatment process in WWTPs. Many researchers have conducted related experiments to explore external carbon sources, such as methanol (Hareendran 2010), ethanol (Gomez *et al.* 2006), acetic acid (Rustige & Nolde 2007), glucose (Lu *et al.* 2009; Wu *et al.* 2014), fructose (Lin *et al.* 2002) and other similar liquid organic matters, plant straw (Liang *et al.* 2015; Chang *et al.* 2016), plant biomass (Chen *et al.* 2014), wood chips (Domingos *et al.* 2009; Greenan *et al.* 2009), cattail (Lu *et al.* 2015), cotton (Rocca *et al.* 2005; Ullah & Faulkner 2006), newspapers (Volkita *et al.* 1996; Fu *et al.* 2014), loofah, platanus acerifolia leaf (Zhang *et al.* 2014), corncob (Li *et al.* 2012) and other solid carbon materials containing cellulose substances.

Liquid carbon source was based on small molecule organic matters, and its microbial utilization rate is high.

However, the price of liquid carbon source is expensive, and the dosing equipment is needed. Some small molecule carbon sources, such as methanol, exhibit toxicity. By contrast, natural solid carbon material containing cellulose substances is widely utilized in recent years because of its convenience and low price. Among the abovementioned solid carbon sources, the plant straw releases a large amount of carbon source at the beginning of the reaction and leads to deterioration of effluent quality, while the released carbon source in later stage is insufficient, leading to unstable nitrogen removal. Wood chips always lead to a blockage of wetlands when directly used as external carbon source because of the small volume. Newspaper surface gradually becomes smooth and the adhesion of biomass becomes weaken when used as external carbon source after a long-run (Fu *et al.* 2014). Cotton can be utilized as a kind of external carbon source; however, the cotton fiber surface is smooth and is not suitable for microbial attachment and utilization. Besides, cotton should be fixed by gravel, and the replacement of cotton is inconvenient. Bark was acted as carbon source of the constructed wetland device in this

study because of its low cost, easily available materials, high carbon content, rough surface, and easy microbial adhesion. Bark is not only used as denitrification carbon source but also as a filler to carry biofilm (Warneke et al. 2011). In addition, the probability of collapse and block is smaller compared with other solid carbon sources. Bark is easy to be replaced when used as the filler of constructed wetland system. Many factors such as the quantity and activity of microorganism, wetland construction, temperature, pH, hydraulic retention time (HRT), nitrate load, and dissolved oxygen (DO), affect nitrogen removal of constructed wetland. Among which, HRT plays an important role.

In this test, constructed wetland model was filled with bark. HRT was gradually changed when denitrification effect became stable. The effects of HRT on NO_3^- -N removal efficiency and carbon source release process with bark filled in the constructed wetland system were investigated. Current studies about the effects of HRT on plant carbon sources' release are always conducted through the statistical tests with single reaction. Shao (Shao et al. 2011) put carbon materials and deionized water into a flask and measured chemical oxygen demand (COD) concentration of different times. In Shao's study, carbon materials and deionized water were placed into a flask and COD concentration was measured at different time points. In addition, carbon release was considered a single reaction process, and the influences of denitrification reaction and constructed wetland environment were not considered. The present study was conducted during the NO_3^- -N removal process in a constructed wetland filled with bark to analyze the carbon release process, including not only a single carbon-releasing process but also denitrification. This work also provided an experiment on the dynamic release of carbon from bark.

EXPERIMENTAL DEVICE AND METHODS

Constructed wetland system model

The constructed wetland system model is shown in Figure 1. Constructed wetland device is cylindrical and made of Plexiglas production. The total height is 40 cm. The outlet height is 30 cm. The filled bark height is 28 cm, and the inner diameter of the device is 11 cm. The loading volume of the bark was $2,660 \text{ cm}^3$, with 212.2 g dry weight and 0.08 g cm^{-3} bulk density. In the bottom, a perforated plate is located for water distribution. The distance between the perforated plate and the bottom of model is 2 cm.

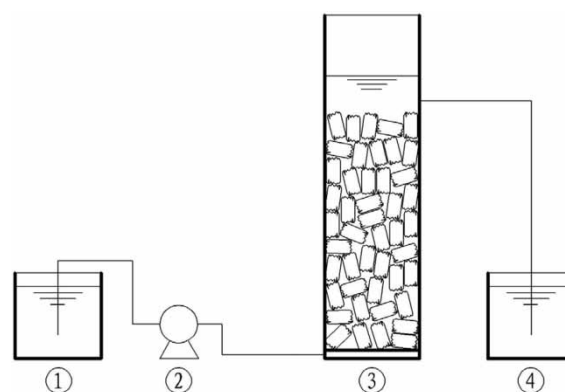


Figure 1 | Constructed wetland system diagram 1. Raw water container 2. Peristaltic pump 3. Constructed wetland device 4. Effluent container.

Methods

First, biofilm formation was conducted. During the biofilm formation period, bark was cut into approximately $1 \text{ cm} \times 2 \text{ cm}$ squares and placed in a container similar to the biofilm wetland device. Chinese fir bark collected from a timber market in Wuhan, China was used in this study. The bark contains cellulose, lignin, hemicellulose, pectin and inorganic contents. The proportion of each component is 38.87%, 30.69%, 23.27%, 6.21% and 0.96%, respectively, based on the Van Soest fiber-washing method. The specific surface area of bark is $0.73 \text{ m}^2/\text{g}$. Meanwhile, the returned sludge from a WWTP was added as seed sludge. Artificial water acted as the influent, containing 200 mg/L NaAc, 70 mg/L NaNO_3 , 20 mg/L KH_2PO_4 , and sufficient amounts of trace element nutrients. The trace elements consist of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (1.5 g/L), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.03 g/L), KI (0.18 g/L), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.12 g/L), H_3BO_3 (0.15 g/L), $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ (0.15 g/L), $\text{MnCl}_4 \cdot 4\text{H}_2\text{O}$ (0.12 g/L) and EDTA (10 g/L), in which each 1 L of artificial water contains 1 mL of nutrient solution. The flow was controlled by a peristaltic pump. After an 8-day biofilm formation, bark was becoming black. Then, the bark was removed into the wetland device and microbe acclimation was conducted. The HRT of microbe acclimation was 1.12 days. The initial composition of the influent was the same as the raw water of biofilm formation period. Then, the content of NaAc in raw water was gradually reduced to 0 mg/L. The NO_3^- -N concentration of effluent was stable after a month of acclimation.

The effect of HRT on denitrification process was investigated after completion of bark filler biofilm formation and microbe acclimation. The experiment was conducted from June to August 2016. The water temperature in the

reactor was approximately 22 ± 2 °C. The experiment was divided into five stages. The first three stages lasted 7 days, whereas the last two stages lasted 10 days. The water flow was 3.56, 2.96, 2.37, 1.19, and 0.59 L/day at a corresponding HRT of 0.75, 0.90, 1.12, 2.24, and 4.48 days, respectively. Artificial water was prepared as the influent, containing 70 mg/L NaNO_3 , 20 mg/L KH_2PO_4 and sufficient amount of other trace elements with the same composition during the biofilm formation period. At each stage of the test, the influent and effluent of constructed wetland were analyzed every day. The constructed wetland was considered stable when the effluent quality slightly changed. Stable water samples were selected once a day for analysis. Six water samples were collected at the third stage and four water samples at the other stages. NO_3^- -N, NO_2^- -N, COD and DO were measured according to Chinese NEPA standards (Chinese NEPA 2002). SPSS 20.0 and Origin 8.0 were used for variance analysis and data fitting, respectively.

RESULTS AND DISCUSSION

Effects of HRT on NO_3^- -N removal efficiency

The effect of denitrification became stable when the change of NO_3^- -N removal efficiency was negligible. Concentration of influent and effluent NO_3^- -N and NO_3^- -N removal efficiency of different stages is shown in Figure 2. Figure 2 shows that the concentration of influent NO_3^- -N was in the range of 27.05–28.54 mg/L. The concentration of effluent NO_3^- -N decreased and the NO_3^- -N removal efficiency gradually increased with the increasing of HRT. At the first stage, HRT was 0.75 days and the NO_3^- -N

removal efficiency was only approximately 20%. At the third stage, HRT was 1.12 days and the NO_3^- -N removal efficiency increased to 43%. At the fifth stage, the HRT was 4.48 days and NO_3^- -N removal efficiency reached 77%, which achieved the highest removal efficiency in this experiment. The results indicated that the longer HRT facilitated the higher NO_3^- -N removal efficiency. This change trend was attributed to the following reasons. A longer HRT means more carbon sources released by the bark, thereby supplying more energy for the denitrifying bacteria. Moreover, a longer HRT results in extended contact time between the water and the biofilm attached to the bark, which is favorable for NO_3^- -N entering the biofilm for microorganism consumption. Accordingly, the removal efficiency of NO_3^- -N increased along with HRT due to the sufficient contact time between the denitrifying bacteria and wastewater (Jiang *et al.* 2016). Furthermore, a longer HRT means a better effect of hypoxia in constructed wetlands, resulting in adequate denitrification effect.

Denitrification reaction rate is assumed to satisfy a first-order reaction formula:

$$-\frac{dC}{dt} = kC \quad (1)$$

where C is concentration of NO_3^- -N, mg/L; t is HRT, hydraulic retention time, days; k is first-order reaction rate constant, days⁻¹.

After integration, Equation (1) is substituted into Equation (2):

$$C_e = C_0 e^{-kt} \quad (2)$$

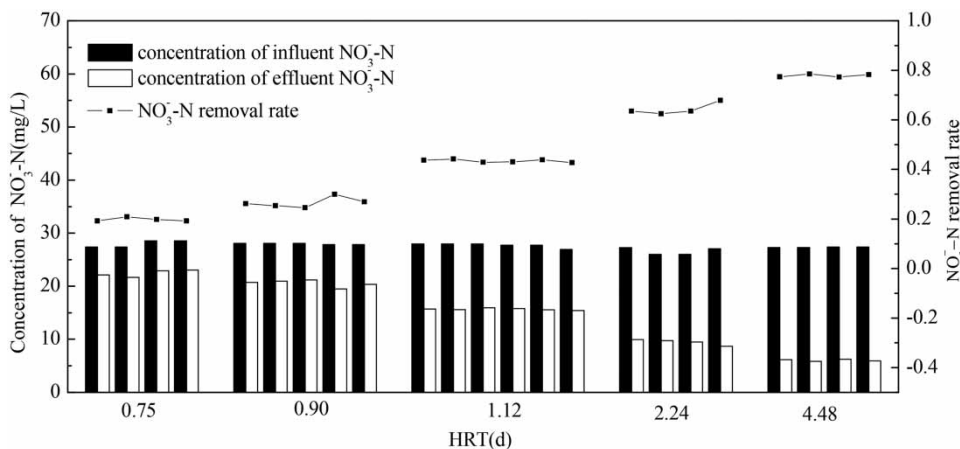


Figure 2 | Concentration of influent and effluent NO_3^- -N and NO_3^- -N removal rate in different stages.

Table 1 | NO_3^- -N average removal efficiency in different stages

HRT (d)	C_0 (mg/L)	Standard deviation of C_0	C_e (mg/L)	Standard deviation of C_e	η (%)
0.75	27.80	0.65	22.24	0.65	20
0.90	27.80		20.02	0.67	28
1.12	27.80		15.85	0.19	43
2.24	27.80		10.29	0.55	63
4.48	27.80		6.39	0.19	77

The expression for NO_3^- -N removal efficiency is:

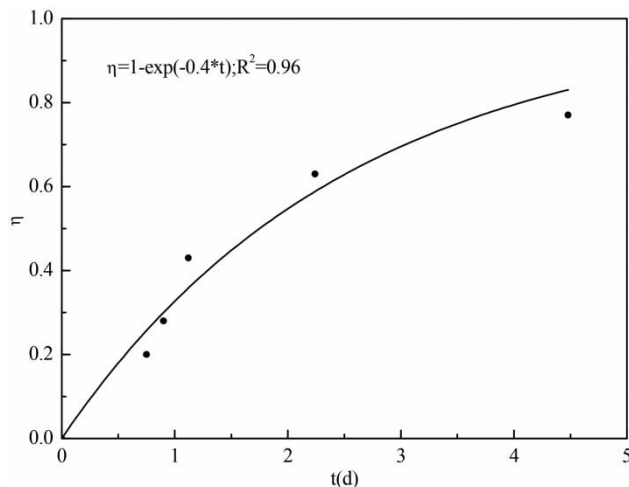
$$\eta = \frac{C_0 - C_e}{C_0} = 1 - e^{-kt} \quad (3)$$

where C_0 is influent concentration of NO_3^- -N, mg/L; C_e is effluent concentration of NO_3^- -N, mg/L; η is NO_3^- -N removal efficiency.

For convenience, the influent and effluent NO_3^- -N concentrations and NO_3^- -N removal efficiency of each stage were averaged when analyzing the impact of HRT on denitrification process. The statistical values are shown in Table 1. Software origin and data in Table 1 were applied to fit Equation (3). The result is shown in Figure 3.

The fitting result was $k = 0.40$, and this result indicated that the NO_3^- -N removal process followed the first-order reaction.

Effluent and influent NO_3^- -N concentration, and t satisfied the relationship of $C_e = C_0 e^{-0.4t}$. The expression of NO_3^- -N removal efficiency was $\eta = 1 - e^{-0.4t}$, which

**Figure 3** | Fitting curve of relationship between η and t .

illustrated that NO_3^- -N removal efficiency increased with the increasing of HRT.

Effect of HRT on the release velocity of carbon source from bark

The released organic carbon could be divided into three parts, which were those utilized by the denitrification process, consumed by dissolved oxygen and resided in water (Li et al. 2013). Meanwhile, microbial biomass net growth was less in the constructed wetland used in this experiment, so carbon consumed by cell synthesis was neglected. Carbon concentration was expressed as COD. Based on electron balances, the carbon source consumed in denitrification, S_{C1} , was calculated as Equation (4) (Fernandez-Nava et al. 2010):

$$S_{C1} = 2.86\Delta\text{NO}_3^- - \text{N} + 1.71\Delta\text{NO}_2^- - \text{N} \quad (4)$$

where $\Delta\text{NO}_3^- - \text{N}$ - the difference between influent and effluent NO_3^- -N concentrations, mg/L; $\Delta\text{NO}_2^- - \text{N}$ - the difference between influent and effluent NO_2^- -N concentrations, mg/L.

Since the influent and effluent NO_2^- -N concentrations of the constructed wetland model were both almost zero, Equation (4) can be abbreviated as Equation (5).

$$S_{C1} = 2.86\Delta\text{NO}_3^- - \text{N} \quad (5)$$

The carbon source consumed by dissolved oxygen was S_{C2} , and it equaled the influent dissolved oxygen, of which the average value was 8.55 mg/L. The carbon source residing in water, S_{C3} , was calculated as Equation (6)

$$S_{C3} = \Delta\text{COD} \quad (6)$$

where ΔCOD is the difference between influent and effluent COD concentrations.

The amount of carbon source released by bark was S_C , and $S_C = S_{C1} + S_{C2} + S_{C3}$. S_C was calculated as Equation (7) and the calculation results of S_C in different stages was shown in Figure 4.

$$S_C = 2.86\Delta\text{NO}_3^- - \text{N} + \Delta\text{COD} + 8.55 \quad (7)$$

Figure 4 indicates that S_C increases with HRT. Shao (Shao et al. 2011) investigated single carbon release of

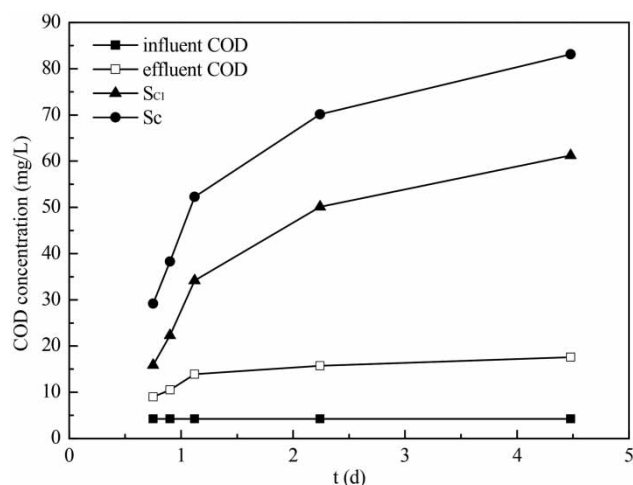


Figure 4 | Carbon releasing quantity of bark at different NO₃-N controlled loading.

corn-cob, rice hull, rice straw, and sawdust, and found the same change trend.

The carbon release average velocity by bark could be expressed as Equation (8)

$$v = \frac{QS_C}{m} \quad (8)$$

where v is carbon release average velocity by bark, mgCOD/(g·d); Q is influent flow of different stages, L/d; m is the dry weight of bark, g.

The equilibrium concentration of COD released by bark at the bottom of wetland model was zero, and the released COD equilibrium concentration was S_{C3} at the top of wetland model. Therefore, the average equilibrium concentration of COD released by bark in the wetland device at time t was S , $S = S_{C3}/2$. The calculation results of v and S are shown in Table 2.

Table 2 shows that all the maximum of v in different stages was 0.58 mgCOD/(g·L), which was much smaller than Sun's study where rotten wood was used as carbon

source for nitrate-nitrogen and COD release velocity of rotten wood was 2.43 mgCOD/(g·L) (Sun et al. 2010). Compared with rotten wood, bark had characteristics of compact structure and strong fibers, which made bark a kind of good slow-release carbon source and a longer life cycle. According to Table 3, the relationship between the S and HRT fitted by Origin software was shown in Equation (9):

$$S = \frac{7.57 - 3.63}{t} \quad R^2 = 0.96. \quad (9)$$

Li (Li et al. 2013) applied starch/polyvinyl alcohol blended materials as solid carbon source for tertiary denitrification of secondary effluent, and the abiotic and biological release of organic carbon was investigated. Their study showed that biological hydrolysis, which originated from denitrifying bacteria, dominates the organic carbon release process rather than abiotic dissolution. The chemical reaction velocity theory was used to analyze carbon release velocity of bark. The carbon molecules released by the bark could not only be used in denitrifying bacteria but also in producing the chemical composition of the bark in reverse reaction. So, the reaction of carbon released from bark was considered as reversible, and the carbon release velocity by bark (v) could be expressed as: $v = v_f - v_r$ (v_f was the forward reaction rate, and v_r was the reverse rate). v is the carbon content released by the bark in a time gradient and represents the efficiency of carbon release reaction. The concentration of reactant was approximately constant as the amount of bark was adequate, so v_f was approximately constant. The v_r can be represented by one of the three forms: power function, hyperbolic, and series types. Power function type is often used for homogeneous and single reaction; hyperbolic type is mostly applied in gas-solid catalytic reaction; and series type is adopted as a numerical regression model (Liao et al. 2008). Carbon release of bark is a hydrolysis reaction occurring in water and irrelevant to adsorption of gas-solid catalytic reaction. Thus, the reverse reaction

Table 2 | Calculation of v and S in different stages

HRT (d)	v mgCOD/(g·d)	S (mg/L)	Standard deviation of S
0.75	0.49	2.37	0.48
0.90	0.53	3.72	0.24
1.12	0.58	4.78	0.51
2.24	0.39	5.74	0.16
4.48	0.23	6.72	0.53

Table 3 | The fitting results of v with power function type

α	v	R^2
1	$v = 0.75 - 0.067 \cdot (7.57 - 3.63/t)$	0.54
1.5	$v = 0.67 - 0.021 \cdot (7.57 - 3.63/t)^{1.5}$	0.61
2	$v = 0.62 - 0.007 \cdot (7.57 - 3.63/t)^2$	0.67

velocity can be fitted separately by a power function or series function.

Fitting with power function type

The reverse rate v_r was described by $v_r = k_1 S^\alpha$, where k_1 was the rate constant; S was the average concentration of COD released in constructed wetland, mg/L; and α was the reaction order. The reaction orders must be determined by experiments. Equation (9) and $v_r = k_1 S^\alpha$ were applied to express v . The result is shown as Equation (10).

$$v = v_f - k_1 S^\alpha = v_f - k_1 \left(\frac{7.57 - 3.63}{t} \right)^\alpha \quad (10)$$

Origin software was applied to fit Equation (10). The fitting results are shown in Table 3, which indicates that the effects of reverse reaction velocity fitted by power function were unsatisfied in different reaction order values. Carbon released by bark was a complex reaction, and not as a simple form of $A \rightarrow B$ reaction. The bark did not only release one kind of carbon source.

Fitting with series type

In series type, v_r was expressed by Equation (11). Equations (9) and (11) were used to obtain the expression of v . The result was expressed as Equation (12). Origin software was applied to fit Equation (12) and the results are shown in Figure 5.

$$v_r = a_0 + a_1 S^1 + \dots + a_k S^k, (k \geq 1) \quad (11)$$

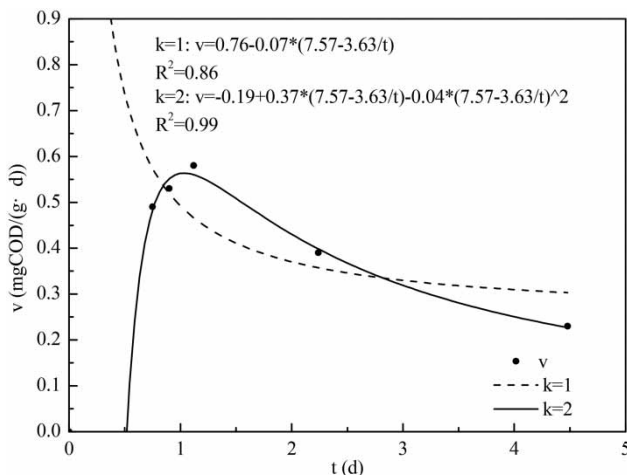


Figure 5 | Fitting curve of relationship between v and t in series type.

$$v = v_f - v_r = a - a_1 S^1 - \dots - a_k S^k \\ = v_f - a - a_1 \left(\frac{7.57 - 3.63}{t} \right) - \dots - a_k \left(\frac{7.57 - 3.63}{t} \right)^k \quad (12)$$

$k = 1, 2$, respectively.

From Figure 5, it can be seen that there was a strong correlation between t and v at $k = 2$, where $R^2 = 0.99$. Thus, the relationship between v and HRT can be expressed as Equation (13).

$$v = 0.53 \left(\frac{1.62}{t} - \frac{1}{t^2} \right) + 0.32 \quad (13)$$

Taking the derivative of t in Equation (13), the result was:

$$v' = 0.53 \frac{(2/t - 1.62)}{t^2} \quad (14)$$

when $v' = 0$, $t = 1.12$ days. When $0 < t < 1.12$ days, $v' > 0$, v was increased with the increasing of HRT. When $t = 1.12$ days, v reached the maximum value. When $t > 1.12$ days, v was decreased with the increasing of HRT.

In this experiment, when t was shorter than 1.12 days, the hydraulic loading was relatively large. As a result, the water adhered on to bark surface was updated quickly, and the released COD on bark surface was lower, which caused a small v_r and promoted carbon source release reaction into forward direction. As t was increased, the hydraulic loading was decreased and the released COD on bark surface was increased. However, the equilibrium concentration of released COD was still lower and it could not inhibit forward reaction. v kept increasing and reached the maximum value when $t = 1.12$ days, and average released COD was 4.78 mg/L. When t was longer than 1.12 days, the hydraulic loading was relatively small. Released COD was accumulated in the reactor and the equilibrium concentration of released COD on bark surface was higher. Thereby, forward reaction was inhibited and v started to decrease. As t was increasing, more and more released COD was accumulated on bark surface, and v decreased continually.

Power function and series types were used to fit the carbon release velocity from bark, and the former fitting result was much worse than the latter. The reason was that bark is mainly broken into several kinds of small molecule organic matters, such as glucose and oligosaccharides under the action of cellulose decomposing bacteria, which applied carbon source to denitrification reaction. Furthermore, the

oligosaccharides could be broken into glucose by microorganism. In conclusion, carbon source release reaction by bark was a complex reaction, which could produce a variety of products during the reaction.

CONCLUSION

- (1) In the constructed wetland model, the bark could be used as filler and could provide external carbon source, achieving enhanced nitrogen removal effect.
- (2) HRT had a great impact on denitrification in constructed wetland. The NO_3^- -N removal efficiency was increased with the increasing HRT and achieved the maximum value of 77% when HRT was 4.48 days. Denitrification process could be described according to the first-order reaction, that is $-dC/dt = kC$, $k = 0.4$. The relationship between nitrogen removal efficiency and HRT was $\eta = 1 - e^{-0.4t}$.
- (3) HRT had an effect on carbon source release in constructed wetland. The carbon release velocity (v) increased firstly and then decreased with HRT increasing. When $0 < \text{HRT} < 1.12$ days, v was increased with the increase of HRT. When $\text{HRT} = 1.12$ days, v achieved the maximum value of 0.58 mgCOD/(g·d). When $\text{HRT} > 1.12$ days, v was decreased with the increasing HRT. The relationship between carbon release velocity by bark and HRT was $v = 0.53(1.62/t - 1/t^2) + 0.32$. According to this equation, the carbon release velocity can be predicted by bark with a known HRT.

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REFERENCES

- Chang, J. J., Ma, L. Y., Zhou, Y. Y., Zhang, S. H. & Wang, W. L. 2016 Remediation of nitrate-contaminated wastewater using denitrification biofilters with straws of ornamental flowers added as carbon source. *Water Science & Technology* **74** (2), 416–423.
- Chen, Y., Wen, Y., Zhou, Q. & Vymazal, J. 2014 Effects of plant biomass on denitrifying genes in subsurface-flow constructed wetlands. *Bioresource Technology* **157** (2), 341–345.
- Chinese NEPA 2002 *Water and Wastewater Monitoring Methods*, 4th edn. Chinese Environmental Science Publishing House, Beijing, China.
- Domingos, S., Boehler, K., Felstead, S., Dallas, S. & Ho, G. 2009 Effect of external carbon sources on nitrate removal in constructed wetlands treating industrial wastewater: woodchips and ethanol addition. In: Nair, J., Furedy, C., Hoysala, C. & Doelle, H. (eds). *Technologies and Management for Sustainable Biosystems*. Nova Science Publishers, New York, NY, USA, pp. 157–167.
- Fernandez-Nava, Y., Maranon, E., Soons, J. & Castrillon, L. 2010 Denitrification of high nitrate concentration wastewater using alternative carbon sources. *Journal of Hazardous Materials* **173** (1–3), 682–688.
- Fu, H., Ma, Q., Li, J., Du, Y., Dong, Z. & Wang, K. 2014 Study on removal of nitrate from water by using newspaper as solid carbon source. *Acta Agriculturae Jiangxi* **26** (7), 92–95 (in Chinese).
- Gomez, M. A., Gonzalez-Lopez, J. & Hontoria-Garcia, E. 2006 Influence of carbon source on nitrate removal of contaminated groundwater in a denitrifying submerged filter. *Journal of Hazardous Materials* **80**, 69–80.
- Greenan, C. M., Moorman, T. B., Parkin, T. B., Kaspar, T. C. & Jaynes, D. B. 2009 Denitrification in wood chip bioreactors at different water flows. *Journal of Environmental Quality* **38** (4), 1664–1671.
- Hareendran, R. 2010 *A Study of Denitrification Kinetics at Low Temperatures Using Methanol as the External Carbon Sources*. Thesis, George Washington University, Washington, DC, USA.
- Jiang, Y., Wang, H. Y., Shang, Y. & Yang, K. 2016 Simultaneous removal of aniline, nitrogen and phosphorus in aniline-containing wastewater treatment by using sequencing batch reactor. *Bioresource Technology* **207**, 422–429.
- Li, G. C., Chen, J., Yang, T., Sun, J. Q. & Yu, S. L. 2012 Denitrification with corncob as carbon source and biofilm carriers. *Water Science & Technology* **65** (7), 1238–1243.
- Li, P., Zuo, J., Xing, W., Tang, L., Ye, X., Li, Z., Yuan, L., Wang, K. & Zhang, H. 2013 Starch/polyvinyl alcohol blended materials used as solid carbon source for tertiary denitrification of secondary effluent. *Journal of Environmental Sciences* **25** (10), 1972–1979.
- Liang, X. Q., Lin, L. M., Ye, Y. S., Gu, J. T., Wang, Z. B., Xu, L. X., Jin, Y., Ru, Q. K. & Tian, G. M. 2015 Nutrient removal efficiency in a rice-straw denitrifying bioreactor. *Bioresource Technology* **198**, 746–754.
- Liao, C., Ren, X. & Wang, C. 2008 *The Reaction Process and Equipment*. China Petrochemical Press, Beijing, China (in Chinese).
- Lin, Y., Jing, S., Wang, T. & Li, D. 2002 Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. *Environmental Pollution* **119**, 413–420.
- Lu, S., Hu, H., Sun, Y. & Yang, J. 2009 Effect of carbon source on the denitrification in constructed wetlands. *Journal of Environmental Sciences* **21**, 1036–1043.

- Lu, L. J., Huang, X. F., Liu, X., Shang, J. J. & Liu, J. 2015 Performance of experimental horizontal subsurface-flow-constructed wetlands treating river water: effect of substrate, configuration, hydraulic retention time, temperature and external carbon source. *Desalination and Water Treatment* **56** (9), 2395–2401.
- Rocca, C. D., Belgiorno, B. & Meri, S. 2005 Cotton-supported heterotrophic denitrification of nitrate-rich drinking water with a sand filtration post-treatment. *Water SA*, 2005 **31** (2), 229–236.
- Rustige, H. & Nolde, E. 2007 Nitrogen elimination from landfill leachates using an extra carbon source in subsurface flow constructed wetlands. *Water Science & Technology* **56** (3), 125–133.
- Shao, L., Xu, Z., Wang, S., Jin, W. & Yin, H. 2011 Performance of new solid carbon source materials for denitrification. *Journal of Environmental Sciences-China* **32** (8), 2323–2327.
- Sun, Y. L., Zhang, G. C., Yan, Z., Li, X. J. & Wang, K. J. 2010 Removing nitrate-nitrogen from wastewater using rotten wood as carbon source. *Chinese Journal of Environmental Science* **31** (6), 1494–1498.
- Ullah, S. & Faulkner, S. P. 2006 Use of cotton gin trash to enhance denitrification in restored forested wetlands. *Forest Ecology and Management* **237**, 557–563.
- Volokita, M., Belkin, S., Abeliovich, A. & Soares, M. M. 1996 Biological denitrification of drinking water using newspaper. *Water Research* **30** (4), 965–971.
- Warneke, S., Schipper, L. A., Matiasek, 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.
- Wu, S., Kuschik, P., Brix, H., Vymazal, J. & Dong, R. 2014 Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. *Water Research* **57** (5), 40–55.
- Zhang, M., Zhao, L., Mei, C., Yi, L. & Hua, G. 2014 Effects of plant material as carbon sources on TN removal efficiency and N₂O flux in vertical-flow-constructed wetlands. *Water Air & Soil Pollution* **225** (11), 1–11.

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