

## Effect of supplying a carbon extracting solution on denitrification in horizontal subsurface flow constructed wetlands

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(Received 4 April 2012 • accepted 22 August 2012)

**Abstract**—Denitrification strongly depends on the availability of carbon source in constructed wetlands (CWs). In this study, several relevant carbon source extracting solutions made from hydrolyzate of selected wetland litters were added to CWs for nitrogen removal enhancement. The feasibility of supplying a carbon extracting solution to improve potential denitrification rate in horizontal subsurface flow constructed wetland was deeply investigated. Combinations of different hydraulic retention time (HRT, especially for 2-day and 4-day) with different influent COD/N ratios were designed to prove the enhancement on denitrification by carbon source supplement. In addition, specific denitrification rate (SDNR) was calculated for the comparison of the nitrogen removal at different COD/N ratios. The sequential operation results on total nitrogen (TN) and nitrate ( $\text{NO}_3^-$ -N) removal efficiencies were obtained in CW system with an influent COD/N ratio of 4.0. The accumulation of nitrite ( $\text{NO}_2^-$ -N) was found to be closely related to the removal of  $\text{NO}_3^-$ -N. Meanwhile, no obvious accumulation of  $\text{NO}_2^-$ -N was found when the removal of  $\text{NO}_3^-$ -N was relatively high.

**Key words:** Carbon Extracting Solution, COD/N Ratio, Denitrification, Horizontal Subsurface Flow Constructed Wetland, Nitrogen Removal

### INTRODUCTION

Nitrogen is one of the main causes of eutrophication in water bodies, especially for lakes and rivers. The removal of nitrogen in wastewater before discharging into adjacent aquatic systems is a priority in constructed wetland treatment [1]. Horizontal subsurface-flow constructed wetlands (HSSFCWs) for wastewater purification are characterized by the advantages of moderate economic costs, lower energy consumption and maintenance requirements than other systems [2,3]. According to documents, the usage of HSSFCWs is demonstrated especially to be suitable for the removal of suspended solids, organic matter and nitrogen [4,5]. The primary nitrogen removal processes in HSSFCWs are nitrification and denitrification. Associated processes such as volatilization, adsorption and plant uptake play a secondary role for nitrogen removal in HSSFCWs [6,7]. It is known that nitrification is indirectly attributed to nitrogen removal in effluent; however, providing essential nitrate for denitrification transforming nitrate into  $\text{N}_2$  or  $\text{N}_2\text{O}$  in wetlands.

Denitrification can be affected by many factors, including temperature, nitrate concentration and especially the organic carbon source [8-10]. Carbon source in CW systems usually comes from wastewater. However, the majority of the organic matters are eliminated in the first stage via microbial oxidation from the influent, causing difficulties in achieving efficient denitrification rate. As a result, nitrate removal rate is lowered in most of HSSFCWs by limited supplement of carbon source. As the carbon source is a key restricting factor for denitrification, organic materials like glucose, fruc-

tose, wheat straw, cellulose and plant litters can be used to enhance the denitrification efficiency in organic carbon-limited CWs [11-13]. Among the various carbon sources, plant materials have been used as an alternative carbon source due to the low cost, non-toxicity, and wide availability. It was reported that the plant litter can be accumulated with productivity of  $500\text{--}2,000\text{ g cm}^{-2}\text{ y}^{-2}$  in a matured wetland [14]. Therefore, it is reasonable to prepare carbon source from plant litter as an efficient and economic approach. In this study, we comprehensively analyzed the processes and addition pattern to improve the degradability of plant materials. The influence of the carbon extracting solutions from the degradation of plant materials on the nitrogen removal was demonstrated.

Although previous studies have focused on the effect of carbon source on the denitrification in CWs, there still appears limited knowledge on the nitrate removal efficiency by supplying carbon extracting solution to denitrification. The influent COD/N ratio probably affects aerobic and anaerobic conditions in CWs. Consequently, it would be one of the crucial parameters for the nitrogen removal. The main objectives of this paper were focused on the following. (i) to test the COD content of several wetland litters under different hydrolysis conditions, and to determine the optimal hydrolysis condition, (ii) to evaluate the effect of COD/N ratio on nitrate removal efficiency, revealing the impacts of nitrite accumulation on the nitrate removal rate in HSSFCW.

### MATERIALS AND METHODS

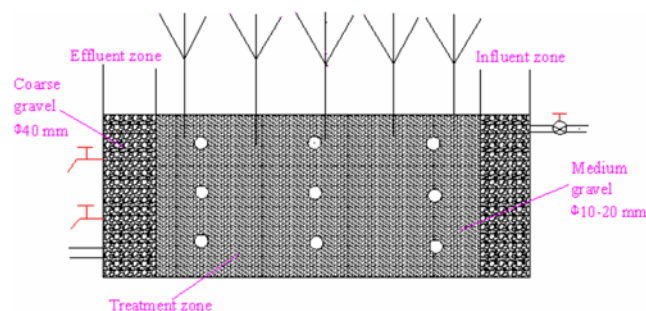
#### 1. Experimental Setup

A lab-scale horizontal subsurface flow CW (HSSFCW) unit planted with cannas was employed in the nitrogen removal experiment. After

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**Table 1. Components of nutrient solution used to cultivate micro-organisms**

Trace element		Buffer solution	
Content	mg/L	Content	g/L
MgCl <sub>2</sub> ·6H <sub>2</sub> O	70.00	KH <sub>2</sub> PO <sub>4</sub>	10.7
CaCl <sub>2</sub> ·2H <sub>2</sub> O	21.50	K <sub>2</sub> HPO <sub>4</sub>	10.7
CoCl <sub>2</sub> ·6H <sub>2</sub> O	3.00	Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O	7.8
MnCl <sub>2</sub> ·6H <sub>2</sub> O	1.25	NH <sub>4</sub> Cl	1.5
CuCl <sub>2</sub> ·2H <sub>2</sub> O	0.15	EDTA	0.05
ZnCl <sub>2</sub>	0.12		
KCl	56.7		
H <sub>3</sub> BO <sub>3</sub>	0.27		

**Fig. 1. Schematic drawing of the experimental HSSF CW.**

plant acclimation, the microcosm was incubated with artificial sewage including nutrient solution (Table 1) for a month. The dimension of the microcosm was 2 m in length, 0.5 m in width and 0.7 m in depth. The microcosm was divided into three zones: the influent zone (0.2 m×0.5 m×0.7 m), treatment zone (1.6 m×0.5 m×0.7 m), and effluent (0.2 m×0.5 m×0.7 m) zone (Fig. 1), each with two different gravel types. The influent and effluent zones were both filled with gravel (φ40 mm) to facilitate the distribution and collection of the wastewater. The middle treatment zone consisted of a 60 cm layer of medium gravel (φ10-20 mm). A lateral perforated pipe was attached at the inlet for the distribution of inflow, and the other lateral perforated pipe served as a collection drain was installed at the bottom of distal end of the wetlands.

## 2. Influent for Experiment

Influent wastewater was prepared artificially in the lab. To begin with, the influent was kept in a container. Then the solution was pumped continuously at a stable inflow rate. Inflow velocity of the wetland was maintained constantly through a controllable water pump. The composition of the influents for each experimental opera-

tion is listed in Table 2.

## 3. Preparation of a Carbon Extracting Solution

Cattail (*Typha latifolia*) litter, canna (*Canna indica*) litter and rice straw were collected for the preparation of carbon extracting solution. All of raw materials were initially cleaned by deionized water and cut into sections in the length of 1-2 cm. Then the mass was air-dried to a constant weight before finally preserved in a moisture-proof container at room temperature (25 °C). The mixture of each plant material (1 g) hydrolyzate (800 ml, deionized water, 2% H<sub>2</sub>SO<sub>4</sub> or 5% H<sub>2</sub>SO<sub>4</sub>) was kept in a 1,000 ml beaker. The beaker was heated by electromagnetic oven, and the reaction time ranged from 0 to 60 min.

## 4. Sampling and Analysis

The initial and final compositions of the rice straw were measured to determine the organic matter loss percentage during the experimental period. The cellulose, hemicellulose and lignin contents were determined based on Van Soest's method [15].

Water variables were measured six times per week sampled from the influent and effluent zone. Total nitrogen (TN), nitrite nitrogen (NO<sub>2</sub>-N) and nitrate nitrogen (NO<sub>3</sub>-N) concentration were analyzed by gas-phase absorption spectrometer (GMA3202). Chemical oxygen demand (COD) was measured by using COD testing instrument (HH-6). The temperature in the laboratory was kept constant at 25.0±3.5 °C.

## 5. Data Analysis

Removal efficiency calculations were based on the following mass balance:

$$\text{Concentration-based removal efficiency (\%)} = \frac{\text{Influent concentration} - \text{Wetland effluent concentration}}{\text{Influent concentration}} \times 100\%$$

The specific denitrification rate (SDNR) was calculated as follows:

$$\text{SDNR (mg/L/h)} = (\Delta\text{NO}_3^- - \text{N} + 0.6\Delta\text{NO}_2^- - \text{N}) / \Delta t$$

where NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N are the added required electron donors in the denitrification process, ΔNO<sub>3</sub><sup>-</sup>-N is the NO<sub>3</sub><sup>-</sup>-N concentration difference between the influent and effluent, and ΔNO<sub>2</sub><sup>-</sup>-N is the NO<sub>2</sub><sup>-</sup>-N concentration difference between the influent and effluent, and Δt is hydraulic retention time.

Statistical procedures and data analysis were evaluated using the built-in statistical functions of Origin 8.0 graphics and analysis software (OriginLab Corp., MA, USA). To investigate the difference between mean removal efficiencies, the t-Student confidence interval, for 98% probability, was calculated. Statistical significance was defined as p<0.01. Mean overall removals were computed in CW for all operational periods.

**Table 2. Composition of influents (Mean±SD, n≥3)**

Parameter	COD/N ratio				
	0 : 1	1 : 1	2 : 1	3 : 1	4 : 1
COD (mg/L)	4.37±3.58	52.26±7.58	101.2±8.52	135.7±7.53	193.5±9.12
NO <sub>3</sub> <sup>-</sup> -N (mg/L)	48.57±1.58	46.23±1.22	48.02±2.51	45.95±2.84	47.52±1.46
TN (mg/L)	48.90±2.27	47.90±1.43	48.80±2.52	47.26±2.46	48.66±1.37
pH	6.95±0.20	6.90±0.15	7.12±0.11	6.98±0.17	7.08±0.15
DO (mg/L)	4.31±0.40	4.12±0.34	4.28±0.30	3.98±0.45	3.89±0.30

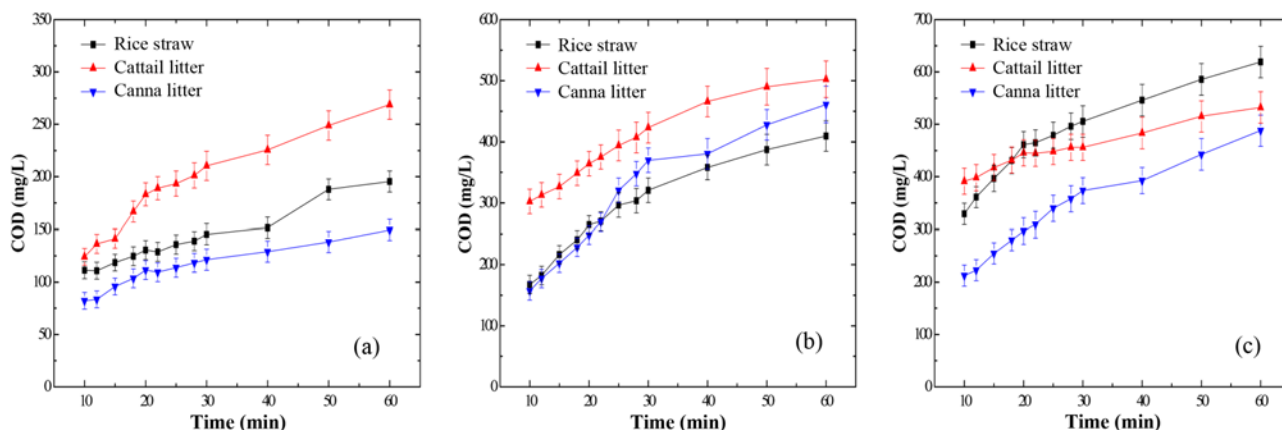


Fig. 2. Release of carbon source under different hydrolysis conditions, deionized water (a), 2% $\text{H}_2\text{SO}_4$  (b), 5% $\text{H}_2\text{SO}_4$  (c).

## RESULTS AND DISCUSSION

### 1. Selection of Optimal Carbon Source

Cellulose, hemicellulose and lignin are the main compositions of wetland litters serving as carbon source for denitrifying bacteria. Common wetland litters differ only slightly in carbon content. However, different percentage of lignin, hemicellulose and cellulose content strongly influences the degradation rates of wetland litter. This indicated that denitrification process activity in the wetlands was affected by the carbon source quality (e.g., COD/N ratio, lignin, carbohydrate) extracted from the different plant litters [14]. As shown in Fig. 2, COD content had good agreement with hydrolyzing time and dilute acid concentration. The main reason was that dilute acid pretreatment contributed to breaking the lignin seal and disrupting the crystalline structure of cellulose of the wetland litters, thus improving biodegradability of cellulosic biomass [14,16]. According to COD content, the optimal reaction condition was obtained as: rice straw material hydrolyzed in 5%  $\text{H}_2\text{SO}_4$  aqueous solution would

yield highest COD content over 60 minutes hydrolysis. Changes of main compositions of rice straw extracting solution are shown in Table 3. As shown in Fig. 2 and Table 3, the COD contents were higher due to solubilization of the organic material in acid solution; cellulose and hemicellulose contents increased, while lignin content decreased with acidic condition. For 5%  $\text{H}_2\text{SO}_4$  extraction, the content of cellulose and hemicellulose could be increased by 23.0% and 29.6%, respectively, compared to a reduction of lignin by 39.6% in raw materials. The increased content of carbohydrate (cellulose and hemicellulose) would improve the bioavailability and contribute to the increase of denitrification capability in CWs [17]. Therefore, 5%  $\text{H}_2\text{SO}_4$  aqueous solution was optimal to extract rice straw for denitrification.

### 2. Nitrogen Removal

Comparison of specific denitrification rate (SDNR) and bCOD (biodegradable COD) removal efficiency under different COD/N ratios is given in Table 4. Obviously, the SDNR was closely related to the influent COD/N ratio. SDNR increased with COD/N ratio. The SDNR at a COD/N of 2.0 was two-times greater than that at a COD/N ratio of 1.0 and 0.0. As shown in Table 4, higher removal efficiencies of  $\text{NO}_3^-$ -N and TN could be obtained when the influent COD/N ratio was greater than 2.0. In addition, the bCOD was detected in the effluent when the influent COD/N ratio was greater than 2.0, which indicated that the carbon source for denitrification was sufficient. Appropriate control of COD/N ratio in the influent was crucial to achieve the optimal nitrogen removal in the denitrification process.

The  $\text{NO}_3^-$ -N and TN concentrations in both effluent and influent are presented in Figs. 3 and 4. As is indicated, the  $\text{NO}_3^-$ -N and TN

Table 3. Changes of main compositions under different hydrolysis conditions (%)<sup>a</sup>

Rice straw	Cellulose	Hemicellulose	Lignin
Raw material	34.08±3.12	26.19±2.98	15.80±2.18
Water extracted	37.20±3.26	29.38±3.05	13.12±1.89
2% $\text{H}_2\text{SO}_4$ extracted	38.87±3.35	31.92±3.33	11.02±1.67
5% $\text{H}_2\text{SO}_4$ extracted	41.81±3.89	33.95±3.78	9.53±1.53

<sup>a</sup>Values are the means±SD (n≥3)

Table 4. Comparison of TN,  $\text{NO}_3^-$ -N, bCOD removal and SDNR in HSSFCW<sup>a</sup>

Item	2-day HRT					4-day HRT				
	C/N=0.0	C/N=1.0	C/N=2.0	C/N=3.0	C/N=4.0	C/N=0.0	C/N=1.0	C/N=2.0	C/N=3.0	C/N=4.0
$\text{NO}_3^-$ -N removal efficiency (%)	19.1±1.0	24.0±1.2	53.2±2.1	64.3±2.4	70.0±2.3	25.0±1.4	36.0±1.7	82.2±2.0	88.4±0.8	97.1±1.7
TN removal efficiency (%)	19.0±0.8	21.3±1.0	38.0±1.8	43.2±1.9	46.0±2.0	23.0±1.2	30.0±1.8	73.2±1.9	77.6±0.6	94.4±2.4
bCOD removal efficiency (%)	99.9±0.1	99.9±0.1	74.3±2.9	73.6±3.4	77.2±2.8	99.9±0.1	99.9±0.1	97.8±2.2	91.8±3.4	89.6±3.5
SDNR (mg/L/h)	0.19±0.05	0.23±0.05	0.53±0.10	0.61±0.20	0.69±0.20	0.16±0.05	0.20±0.05	0.44±0.10	0.46±0.10	0.48±0.10

<sup>a</sup>Values are the means±SD (n≥3)

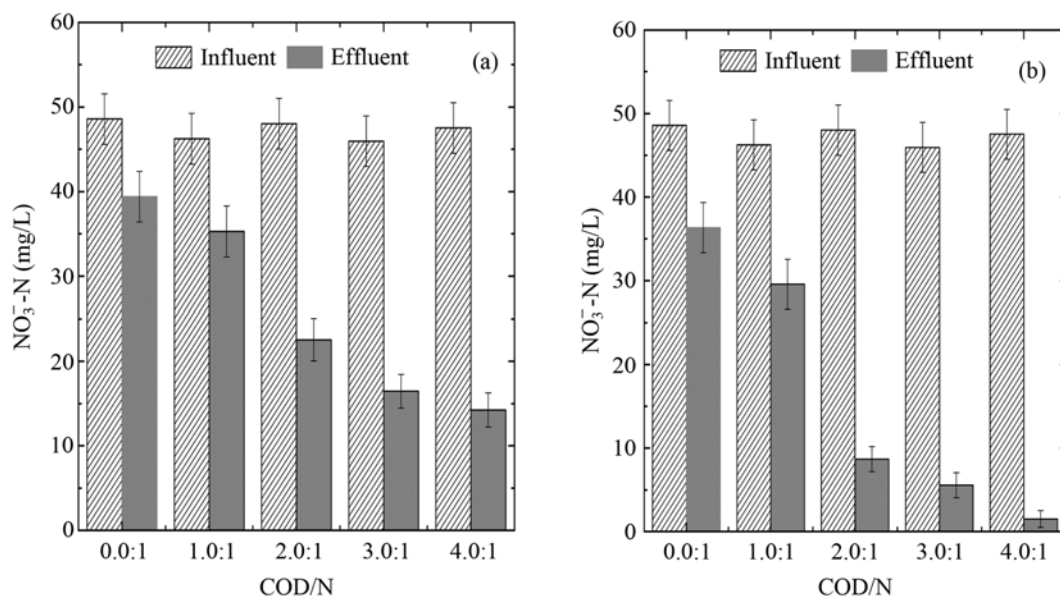


Fig. 3.  $\text{NO}_3\text{-N}$  concentrations in effluent and influent and influence of COD/N ratio on  $\text{NO}_3\text{-N}$  removal, (a) 2-day HRT, (b) 4-day HRT.

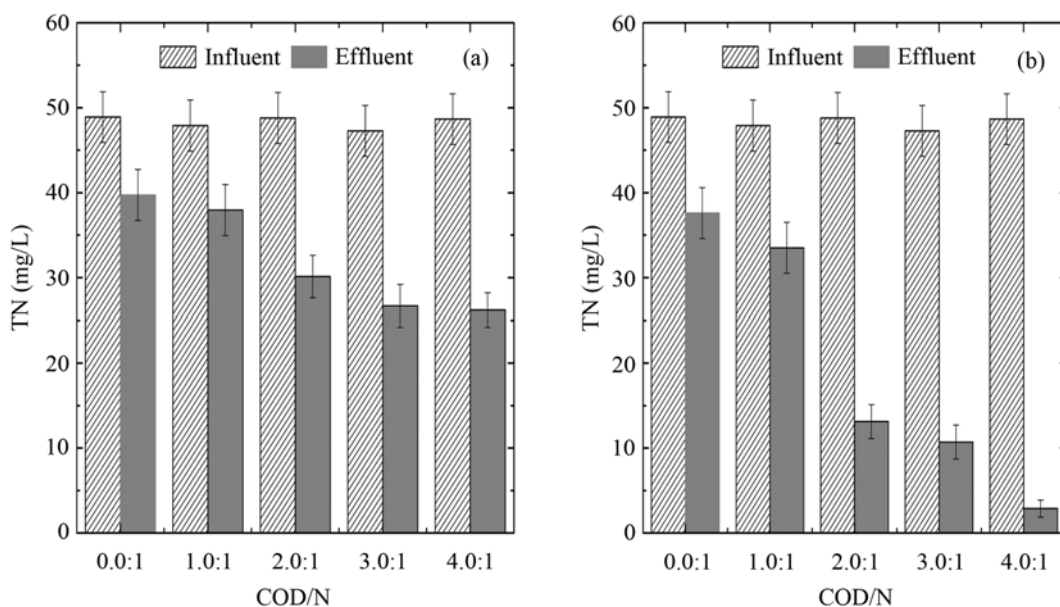


Fig. 4. TN concentrations in effluent and influent and influence of COD/N ratio on TN removal, (a) 2-day HRT, (b) 4-day HRT.

removal was strongly influenced by COD/N ratio and hydraulic retention time (HRT). TN and  $\text{NO}_3\text{-N}$  removal efficiencies in HSS-FCW are given in Table 4. As is indicated in Table 4,  $\text{NO}_3\text{-N}$  and TN removal efficiency was higher in the condition of 4-day HRT than 2-day HRT in HSSFCW. When comes to carbon source shortage in the influent, a part of  $\text{NO}_3\text{-N}$  was assimilated by plants in CWs [11]. However, the removal did not perform well; average removal efficiency for  $\text{NO}_3\text{-N}$  only reached 25.0% and the average removal efficiency for TN was 23.0%, which was significantly lower than other cases ( $p < 0.01$ ). With influent COD/N ratio of 1.0, average removal efficiency for  $\text{NO}_3\text{-N}$  was improved from 25.0% to 36.0%, while average removal efficiency for TN was improved from 23.0% to 30.0%. The accumulation of  $\text{NO}_3\text{-N}$  resulted from de-

pleted carbon source eventually had an influence on the TN removal efficiency. When COD/N ratio increased to 2.0, average removal efficiency for  $\text{NO}_3\text{-N}$  exceeded 82.2%, followed by the slightly increase for the COD/N ratio. In addition, TN removal efficiency was significantly improved as a result of high nitrate removal. It was indicated that carbon extracting solution acted as an electron donor played an important role in nitrate reduction. The best TN removal performance was observed in the case with the influent COD/N ratio of 4.0. TN removal efficiency exceeded 94.4%, and constant final effluent concentrations were obtained ( $\text{TN} < 3.0 \text{ mg/L}$ ). Adding carbon extracting solution not only supplied the nutrition for denitrifying bacterium, but also supported the metabolism and reproduction. The denitrification reaction took place more easily, and

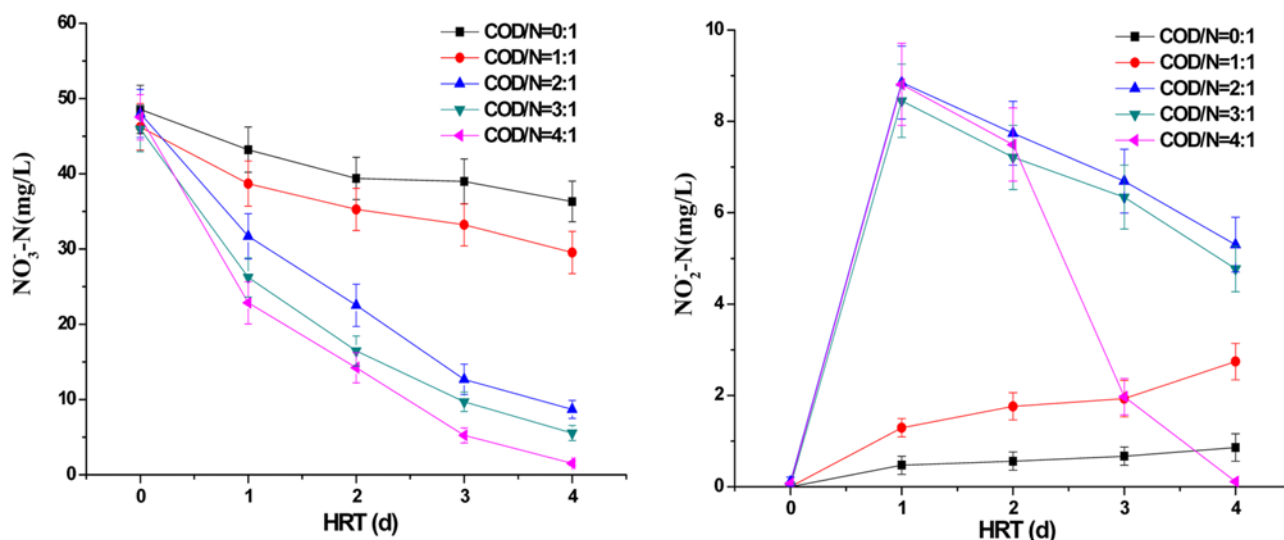


Fig. 5. Variations of NO<sub>3</sub>-N and NO<sub>2</sub>-N concentrations at different COD/N ratios.

consequently the efficiency of nitrogen removal increased.

### 3. The Relation between Nitrite Accumulation and Nitrate Removal

According to the mechanism of denitrification, denitrification processes involved the initial NO<sub>3</sub>-N reduction to NO<sub>2</sub>-N, followed by further reduction to NO, N<sub>2</sub>O and finally to N<sub>2</sub> [18]. Each half-reaction process was described as follows:



As shown in Fig. 5, with influent COD/N ratios of 0.0 and 1.0, NO<sub>3</sub>-N was accumulated significantly for the lack of carbon source. Accumulated NO<sub>2</sub>-N concentration gradually increased from 0 mg/L in the influent to the maximum, respectively, at 0.86 mg/L and 1.73 mg/L in the effluent. Low influent COD/N ratio was unfavorable to the progress of heterotrophic denitrification in CWs and caused the accumulation of NO<sub>2</sub>-N. A peak of NO<sub>2</sub>-N concentration was detected in 1-day HRT in the system with the influent COD/N ratios of 2.0, 3.0 and 4.0. The NO<sub>2</sub>-N concentration gradually decreased to 5.3 mg/L, 4.77 mg/L and 0.11 mg/L in the following day. Meanwhile, a sharp decrease of NO<sub>3</sub>-N concentration was detected. At a COD/N ratio of 4.0, the NO<sub>3</sub>-N concentration was gradually decreased to 2.87 mg/L, and the NO<sub>3</sub>-N removal efficiency was significantly higher than other cases ( $p < 0.01$ ).

It comes to a general agreement that sufficient carbon source and electronic donors can effectively promote the occurrence of half-reaction resulting in the reduction of NO<sub>2</sub>-N accumulation. Meanwhile, NO<sub>3</sub>-N removal efficiency remained comparatively high. Limited carbon supply could not provide enough electron donors to support the entire half-reaction processes, which resulted in the accumulation of NO<sub>2</sub>-N. Consequently, NO<sub>3</sub>-N removal efficiency was reasonably lowered.

## CONCLUSIONS

Insufficient organic carbon source supplement results in low NO<sub>3</sub>-N removal rate in the horizontal subsurface flow constructed wetlands for wastewater treatment. In this study, rice straw extracting solution was applied to the CW system. The NO<sub>3</sub>-N removal rate for wastewater was highly dependent on the influent COD/N ratio, which was one of the most significant factors in the denitrification process. When the influent COD/N ratio exceeded 2.0, the carbon source for denitrification was sufficient and better removal efficiencies of NO<sub>3</sub>-N and TN could be obtained. The result implied that the additional carbon extracting solution was an effective way for the removal of nitrate from CW. However, NO<sub>2</sub>-N accumulated slightly when the additional carbon extracting solution was supplied.

## ACKNOWLEDGEMENT

This work was supported by the National Natural Science Foundation of China (No. 51079028), the National Basic Research Program (973) of China (No. 2010CB951102) and the Natural Science Foundation of Shanghai (No. 10ZR1400300).

## REFERENCES

1. F. X. Ye and Y. Li, *Ecol. Eng.*, **35**, 1043 (2009).
2. J. Vymazal, *Ecol. Eng.*, **18**, 633 (2002).
3. A. I. Stefanakis and V. A. Tsihrintzis, *Desalination*, **248**, 961 (2009).
4. J. Vymazal, *Ecol. Eng.*, **35**, 1 (2009).
5. R. H. Kadlec, *Ecol. Eng.*, **33**, 126 (2008).
6. J. Vymazal, *Ecol. Eng.*, **25**, 478 (2005).
7. T. G. Bulc, *Ecol. Eng.*, **26**, 365 (2006).
8. Y. F. Lin, S. R. Jing, T.-W. Wang and D.-Y. Lee, *Environ. Pollut.*, **119**, 413 (2002).
9. T. Sirivedhin and K. A. Gray, *Ecol. Eng.*, **26**, 167 (2006).
10. C. C. Tanner and R. H. Kadlec, *Water Sci. Technol.*, **48**, 191 (2003).
11. T. L. Ingersoll and L. A. Baker, *Water Res.*, **32**, 677 (1998).
12. Y. Wen, Y. Chen, N. Zheng, D. H. Yang and Q. Zhou, *Bioresour.*

- Technol.*, **101**, 7286 (2010).
13. S. Y. Gebremariam and M. W. Beutel, *Ecol. Eng.*, **34**, 1 (2008).
  14. N. P. Hume, M. S. Fleming and A. J. Horne, *Water Res.*, **36**, 577 (2002).
  15. P. J. Vansoest, J. B. Robertson and B. A. Lewis, *J. Dairy Sci.*, **74**, 3583 (1991).
  16. N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzaple and M. Ladisch, *Bioresour. Technol.*, **96**, 673 (2005).
  17. N. P. Hume, M. S. Fleming and A. J. Horne, *Soil Sci. Soc. Am. J.*, **66**, 1706 (2002).
  18. M. D. Wallenstein, D. D. Myrold, M. Firestone and M. Voytek, *Ecol. Appl.*, **16**, 2143 (2006).