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Effect of carbon source on the denitrification in constructed wetlands

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Abstract

The ability of constructed wetlands with different plants in nitrate removal were investigated. The factors promoting the rates of denitrification including organic carbon, nitrate load, plants in wetlands, pH and water temperature in field were systematically investigated. The results showed that the additional carbon source (glucose) can remarkably improve the nitrate removal ability of the constructed wetland. It demonstrated that the nitrate removal rate can increase from 20% to more than 50% in summer and from 10% to 30% in winter, when the nitrate concentration was 30–40 mg/L, the retention time was 24 h and 25 mg/L dissolved organic carbon (DOC) was ploughed into the constructed wetland. However, the nitrite in the constructed wetland accumulated a little with the supply of the additional carbon source in summer and winter, and it increased from 0.15 to 2 mg/L in the effluent. It was also found that the abilities of plant in adjusting pH and temperature can result in an increase of denitrification in wetlands. The seasonal change may also impact the denitrification.

Key words: constructed wetland; carbon source; denitrification; nitrate; nitrite

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Introduction

Constructed wetlands have become increasingly favored in the remediation of agricultural runoff and municipal wastewater treatment due to their low capital cost, very low operating cost, and environmental friendliness (Kadlec, 1995; Bezbaruah and Zhang, 2003; Huett *et al*., 2005). Agricultural runoffs frequently contain high concentrations of nitrate which makes up the majority of the nitrogen in wastewater (Lin *et al*., 2003; Huett *et al*., 2005). Treated municipal wastewaters still contain relatively high level of nitrate, which would enter water environments such as rivers, lakes, and sea. The high nitrate load in water stimulates algal blooms which could degrade water quality and reduce biological diversity. This eutrophic condition is the major cause of the hypoxia in water, which has an adverse effect on its ecosystem and economy (Sirivedhin and Gray, 2006). Furthermore, it has been reported that high nitrate concentration in drinking water can cause both methemoglobinemia and bladder cancer (Weyer *et al*., 2001). Therefore, nitrate removal from agricultural runoffs and effluent of treated wastewater has become more and more important (Bezbaruah and Zhang, 2003).

Nitrogen in wetlands is removed from the water mainly by plant uptake, soil adsorption, and microbe catabolism, and permanent and dominating nitrogen removal occurs via denitrification by denitrifying bacteria (Clement *et al*., 2002; Poe *et al*., 2003). As the main mechanism of removing nitrate in constructed wetlands, denitrification is an anaerobic dissimilative pathway in which nitrate is used as an electron acceptor for anaerobic respiration to generate energy (IWA, 2000). Denitrification is affected by many factors including oxygen availability, temperature, concentration of nitrite, organic carbon supply, and the species of the wetlands plants (Beauchamp *et al*., 1989; Ingersoll and Baker, 1998; Sirivedhin and Gray, 2006). Because denitrification is an anaerobic dissimilative pathway in which the synthesis of the enzyme involved in each denitrification step and the corresponding denitrification rates are greatly repressed by the presence of O_2 (IWA, 2000; Sirivedhin and Gray, 2006). The process of denitrification is strictly anoxic process and is sensitive to the oxygen levels presented in constructed wetlands systems. However, denitrification activities have been observed in wetland systems that have measurable dissolved oxygen concentrations in their surface waters (Phipps and Crumpton, 1994; Sirivedhin and Gray, 2006). The concentration of nitrate also has great impacts on the denitrification in constructed wetlands. Many researchers have found that high nitrate concentrations improve the denitrification rates in wetlands (Willems *et al*., 1997; Sartoris *et al*., 2000). In addition, carbon source is a controlling factor in the process of denitrification. The carbon source in the system of constructed wetlands usually comes from wastewater, soil, and the root exudates of plants. The addition of various carbon sources such as glucose, sodium acetate,

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methanol, starch, cellulose, plant materials, and wheat straw (Ragab *et al*., 1994) can also enhance the denitrification rate in wetlands (Ingersoll and Baker, 1998; Davidsson and Stahl, 2000; Robins et al., 2000; Sirivedhin and Gray, 2006). Temperature can not only influence the activity of denitrifying bacteria but also impact the growth of plants in wetland. Herkowitz (1986) found denitrifying bacteria in wetland sediments to be more abundant in spring and summer compared to fall and winter. Subsequently, Stober *et al*. (1997) demonstrated that the overall nitrate removal rate was significantly higher in summer than in winter.

Plant is the most important composition in constructed wetlands. It can not only take up the nitrogen or phosphate as nutrition, but also provide a convenient condition for nitrification and denitrification. Wetland plants can simultaneously provide strictly anaerobic and aerobic habitats in the rhizosphere, which provides convenient conditions for nitrogen removal (Nikolausz *et al*., 2008). Wetland systems supporting with plant communities tend to remove nitrate more effectively than non-vegetated systems (Zhu and Sikora, 1995). Belmont and Metcalfe (2003) reported that plants influenced nitrogen removal rates significantly. Nitrate can be effectively removed without the addition of carbon source as long as macrophytes present in wetlands (Lin *et al*., 2003; Bastviken *et al*., 2005).

Several studies have investigated how these factors affect denitrification in riparian buffer zones (Willems *et al*., 1997; Martin *et al*., 1999; Clement *et al*., 2002; Matheson *et al*., 2003; Rotkin-Ellman *et al*., 2004), and some have investigated the factors affecting the denitrification in creating wetlands receiving non-point source pollution or river flood water (Poe *et al*., 2003; Sidle and Goodrich, 2003; Srivedhin and Gray, 2006). To date, there is still very limited study aiming at the performance of the constructed wetland in nitrate removal and denitrification when receiving wastewater with high concentration of nitrate.

The purpose of this research was to investigate systematically the ability of constructed wetlands with different plants in nitrate removal with the artificial influent containing high level of nitrate, to determine the effect of carbon source on the denitrification, and to evaluate the nitrite accumulation and the factors impacting the nitrate removal in the constructed wetland.

1 Materials and methods

1.1 Constructed wetlands

The constructed wetlands studied were operated during February 2006 to December 2006 in Tsinghua University, Beijing, China (39.92°N, 116.46°E). Three parallel constructed wetlands were undercurrent constructed wetlands, and area of each wetland was 3 m^2 (with length of 4 m, width 0.75 m, depth 0.7 m, and gradient of 1%). The wetlands were built with concrete and contained a 50-cm layer of slag at bottom and a 10-cm of local soil above the soil layer. The wetlands included 30 cm of river gravel (nominal diameter 10–20 mm) in the entrance of influent, following 40 cm of subsurface water flow within the gravel layer. Three wetlands were planted with *Canna*, *Zizania caduciflora* and *Lythrum salicari*, respectively, with a space of 30 cm between each plant. A lateral perforated pipe was installed at the inlet of each wetland for the distribution of inflow. To control the water depth, a lateral trough-shaped collector for drainage was installed at the upper wall of the distal end of the wetlands, and a lateral perforated pipe served as a collection drain was installed at the bottom of distal end of the wetlands.

1.2 Materials and water samples

From the early February 2006 to the middle July 2006, the sodium nitrate concentration in wetlands influent was 30–40 mg/L NO_3^--N and without supplement of organic carbon. From the middle July 2006 to the November 2006, the influent has sodium nitrate concentration of 30–40 mg/L NO_3^--N with glucose as additional carbon source, and the proportion of glucose to NO_3^--N was 3:1. The influent was prepared according to water consumption, stored in a tank and can flow continuously via gate valves and distribution pipes into the wetlands by a peristaltic pump. During this study, the inflow rates of the wetlands were maintained constant by adjusting the gate.

1.3 Water quality analysis

Water samples of influent and effluent were collected once a week from the three wetlands. Because the three wetlands received the same contaminated groundwater, their influent samples were taken from the influent port identically. The effluent samples of each wetland were taken from the effluent port separately. Water samples were analyzed for NO_2^--N, and NO_3^--N concentration by ultraviolet spectrophotometer (UV-2401 PC, Shimadzu, Japan) according to standard method (American Public Health Association, 1995). Dissolved organic carbon (DOC) was determined using a total organic carbon (TOC) analyzer (TOC 5000A, Shimadzu, Japan). Dissolved oxygen content (DO), pH and oxidation-reduction potential (ORP) were measured *in situ* when sampling the water sample.

2 Results and discussion

2.1 Dissolved organic carbon removal ability of the constructed wetlands

The results in Fig. 1 show that DOC in influent and effluent of the three constructed wetlands was about 2 mg/L without additional carbon source from February to July. Three constructed wetlands with different plants showed no obvious difference during long time performance. It is widely recognized that root-deposited photosynthate serves as an important carbon source for microorganisms in the vicinity of growing roots, and previous studies showed that 28%–59% of the photosynthate was transferred to the underground, 4%–70% of which entered into the soil (Lynch and Whipps, 1990). In turn, plants rely on the microbially mediated decomposition of organic materials for their supply of available nutrients. Several

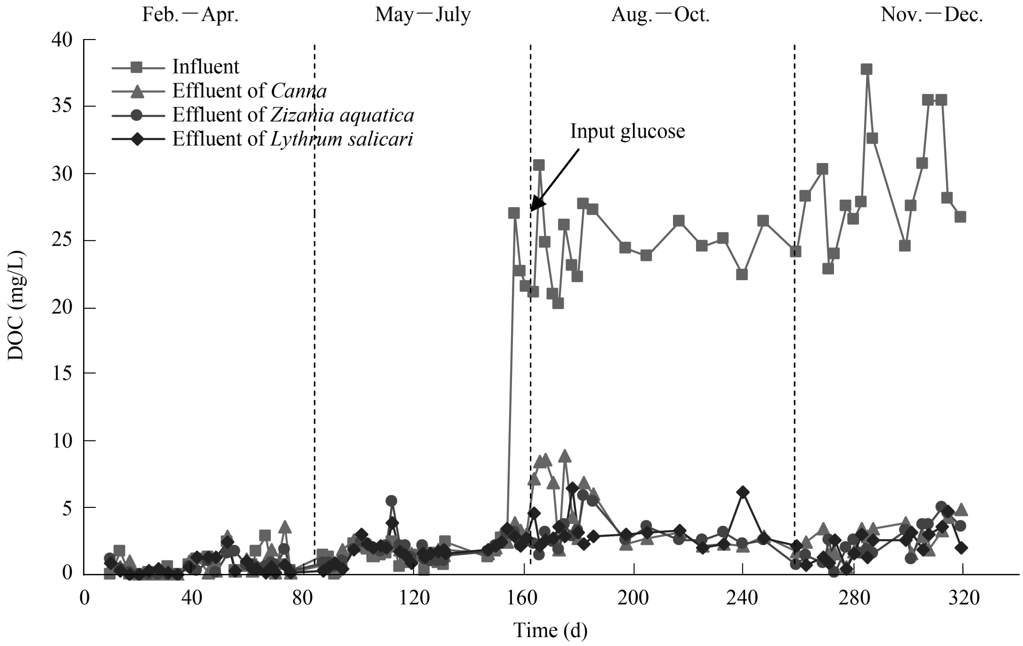

Fig. 1 Dissolved organic carbon (DOC) concentrations of influent and effluent in the three constructed wetlands.

studies have examined the partitioning of photosynthate throughout the plant-soil system and few of them have monitored the incorporation of this photosynthate into the soil microbial biomass (Kuzyakov *et al*., 2002; Butler *et al*., 2003). The results indicated that all root-deposited photosynthate and decomposition of organic materials in the soil can be utilized by the microorganisms in the constructed wetlands.

Most of the carbon required to fuel denitrification comes from the plants growing in the wetlands (Bachand and Horne, 2000) and additional carbon source (Huett *et al*., 2005). In this study, 25 mg/L glucose were added to the influent since the end of July, however, DOC in the effluent of three constructed wetlands were still low (3 mg/L) (Fig. 1). The results indicated that the microorganisms in wetlands can consume the carbon inner the wetlands and additional carbon simultaneously. The carbon source was a controlling factor in the process of denitrification in wetlands, which was in agreement with the previous research (Starr and Gillham, 1993; Davidsson and Stahl, 2000).

2.2 Nitrate removal ability of the three constructed wetlands

The nitrate concentrations in influent and effluent and nitrate removal efficiencies of the three constructed wetlands are presented in Fig. 2. As shown in Fig. 2a, there was a little difference in nitrate removal efficiency among the three constructed wetlands during the performance period. The nitrate removal efficiencies of the three wetlands were varied along with seasonal change and plants growth. Nitrate removal efficiencies were slightly increased from Feb. to July, which was in accordance with growth periods of wetlands plants. The possible reason for this phenomenon may be that a part of nitrate was assimilated by plants in wetlands and the wetland plants can provide increasing organic carbon source with plants growth, which can improve the ability of denitrification. However, the removal do not perform excellent even in July when plants in exuberant growth, which can only attain 20% in three wetlands.

Various additional carbon sources, including methanol (Huett *et al*., 2005), glucose (Davidsson and Stahl, 2000), and starch (Robins *et al*., 2000), have been tentatively added into carbon-limited wastewaters to enhance the heterotrophic denitrification rate in constructed wetlands. In this study, glucose was used as additional organic carbon source and the proportion of DOC and nitrate was about 1:1 in influent. When glucose was added as organic carbon source from day 150 to 160, nitrate removal efficiency was improved rapidly from 20% to 40% and the following largest removal efficiency was almost 60%, which indicated that organic carbon source improved the removal of nitrate in the wetlands. Additional carbon source contributed to only 10 mg/L of nitrate removal comparing the removal efficiency between day 140 to 170 (Fig. 2). Less than 40% of additional glucose was used by denitrification process and a majority of additional carbon source was assimilated by other microorganism in wetlands, which was similar to previous study which employed C:nitrate-N ratios of 1.13:1 (Skrinde and Bhagat, 1982).

The nitrate was removed most effectively during the last ten days, in August (around 190 d, Fig. 2b), which accorded with the growth cycle of plants. Plants grow best during August and September, and become contabescence from October. Seasonal changes affect the assimilation of nitrate and supply organic carbon source for denitrification. Although efficient nitrate removal in constructed wetlands, e.g., 68% or higher, has been frequently reported by other researchers, comparative studies also revealed a poor nitrate removal performance, e.g., < 25%, or even an increase in nitrate concentration from influent to effluent

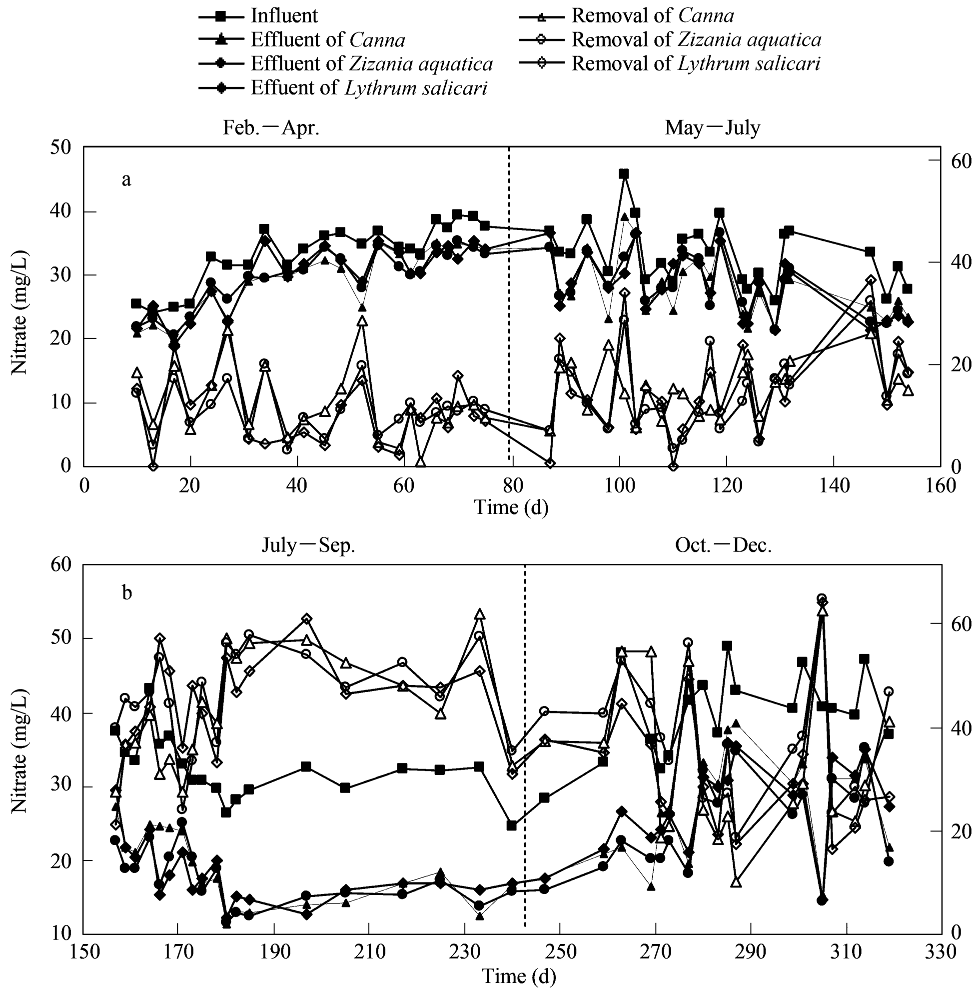

Fig. 2 Nitrate concentrations of the influent and effluent and nitrate removal efficiencies of the three constructed wetlands from Feb to July (a), from July to Dec (b).

in constructed wetlands (Yang *et al.*, 2008). The difference in nitrate removal performance was often contributed to the available carbon source. The additional carbon source can obviously improve the ability of nitrate removal.

The difference of nitrate removal efficiency between August and December was about 25%–30% (Fig. 2b) when plants grow best and almost cease in wetlands. Comparing the results in Fig. 2, the difference of nitrate removal efficiency between day 160 and day 170 was about 20%, which was contributed by the additional glucose to denitrification. In conclusion, additional carbon source caused about 10% nitrate removal, which was according to nitrate removal efficiency during February and March. Moreover, for these three wetlands, nitrate was removed 35% in summer and 10% in winter without additional carbon source. The addition of plants will increase the ability of buffer action and the denitrification in the wetland is slightly impacted by temperature and pH because the nitrate removal efficiency is relatively high in the winter when additional carbon source was added (Fig. 2b).

Organic carbon sources for denitrification include additional carbon source, root-deposited of plants, and organic materials from soil (Butler *et al.*, 2003). Plants assimilation and plant roots decomposition accorded with the plant growth cycle. The contribution of additional carbon source to nitrate removal was impacted slightly by seasonal change without considering the activity of microorganism impacted by temperature. Thereby, it is important to consider carbon sources in designing a wetland to treat nursery runoff, which has a low DOC concentration. Plants may be required to enhance nutrient removal. The addition of plants will increase water loss through evapotranspiration and then less water will be available for recycling (Huett *et al.*, 2005). The additional carbon source will be added to the influent and substance with rich organic carbon source will be filled into the soil of constructed wetlands.

2.3 Nitrite accumulation in the three constructed wetlands

Nitrite concentrations of influent and effluent and nitrate removal efficiencies of the constructed wetlands during Feb. to Dec. are presented in Fig. 3. Nitrite would be accumulated during denitrification. Nitrite have a significant impact on ecosystem functioning and microbial populations, since it is toxic to both plants and soil microorganisms (Gelfand and Yakir, 2008).

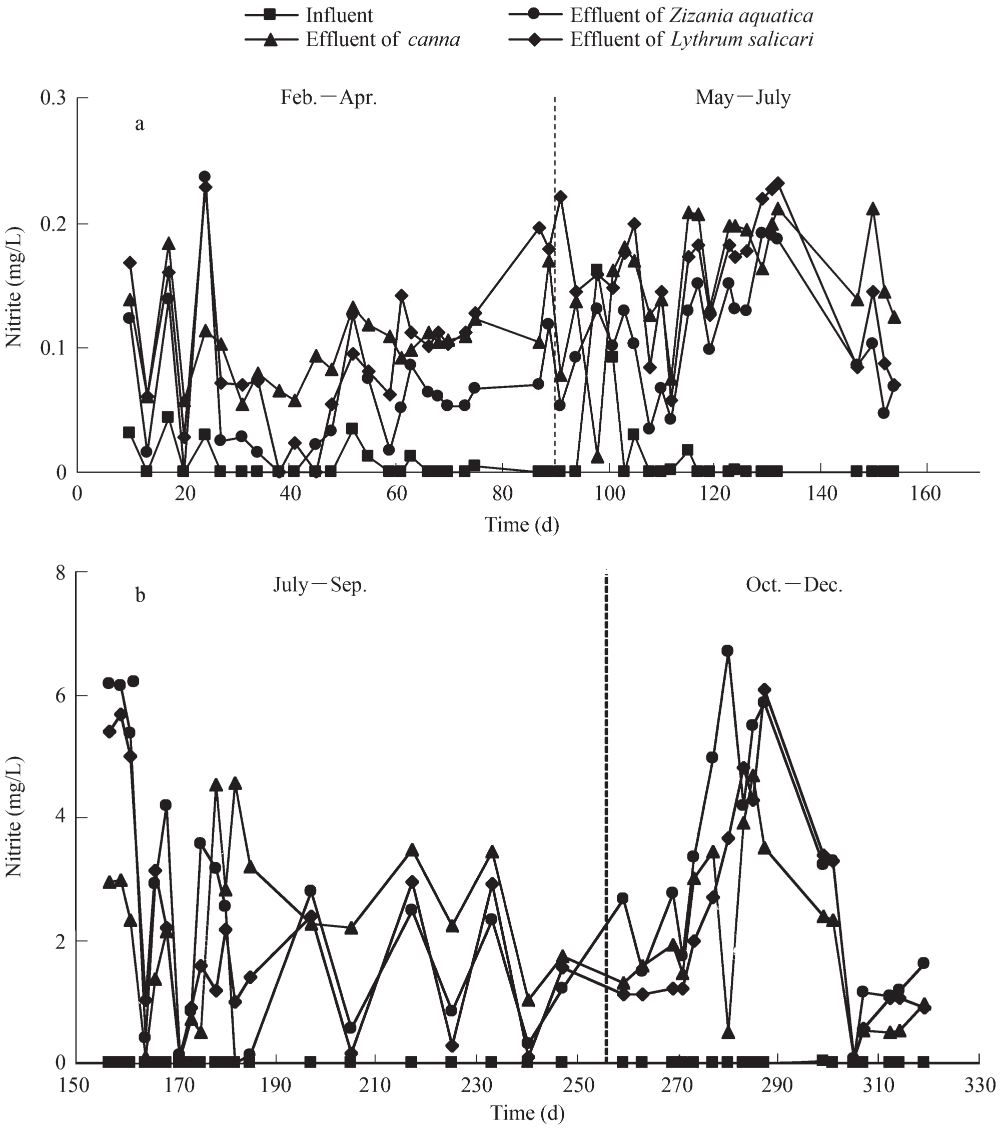

Fig. 3 Nitrite concentrations of influent and effluent and nitrate removal efficiencies of three constructed wetlands from Feb. to July (a) and from Aug. to Dec. (b).

It was obvious that the nitrite concentration was below 0.15 mg/L and the peak concentration was less than 0.25 mg/L in effluent without additional carbon source (Fig. 3a). When nitrate concentration in influent was more than 30 mg/L and nitrate removal efficiency was above 10%, the nitrite produced during the removal process in wetlands accounted for less than 5% of the removed nitrate. These results illuminated that there was no nitrite accumulation during denitrification in the wetlands without additional organic carbon source. The results are in agreement with previous research by Rittmann and McCarty (2001), who indicated that nitrite accumulation was rarely observed in the environment due to a low substrate concentration capable of supporting biomass.

Whereas, nitrite concentration was more than 2 mg/L in the effluent when additional carbon source was added (Fig. 3b). The nitrite produced during removal processes in the wetlands occupied more than 15% of the removed nitrate with additional carbon source. It was determinate that nitrite was accumulated under this condition. The nitrite concentration in effluent varies randomly (Fig. 3b), which may be due to the variation of nitrate and DOC concentration in influent. Comparing DOC concentration in the influent, DOC may be the leading factor for nitrite in the effluent. The phenomena of nitrite accumulation were also observed in other studies, and there were many special conditions that can conduce to produce nitrite, such as at low DO concentrations, high temperatures and inhibitory nitrite-oxidization environment (Alleman, 1985; Kim *et al.*, 2008). The results are in agreement with the hypothesis that the oxidation rate of nitrate is faster than that of nitrite, leading to the conversion of nitrate to nitrite prior to conversion of nitrate to gaseous nitrogen. The same phenomena were also observed in the flask experiments of denitrification. As shown in Fig. 3, nitrite accumulation became more evident with the increasing temperature, which is in agreement with the faster increase of bacteria activity converting nitrate to nitrite. This result is in line with that of Kim *et al*. (2008).

2.4 Factors affecting the ability of nitrate removal in constructed wetlands

Constructed wetland is a complex system and there are many factors would influence nitrate removal. In addition to such controlling factors as readily available carbon source, temperature, and pH, high rates of denitrification depend upon anaerobic circumstance in denitrification constructed wetlands. The rhizosphere theory indicated that there are intersectional aerobic and anaerobic reactions. The denitrification constructed wetlands are in favor of the plants which have slightly ability of oxygen transmission. The influences of pH and temperature in constructed wetlands were also investigated in this study.

As shown in Fig. 4a, the pH in the influent and effluent were in the range of 7–8, mostly above 7.5, which was propitious to denitrification. Although denitrification process would produce alkalinity, the root secretion and putrefaction of dead plants can consume alkalinity. As a result, the constructed wetlands system has a strong ability of maintaining suitable pH for denitrification.

It is well known that most suitable temperature range for denitrifying bacteria was from 20 to 40°C. Sirivedhin and Gray (2006) indicated that denitrification slow down below 15°C and almost ceases below 5°C. As shown in Fig. 4b, temperatures in influent were little higher than in effluent, and were above 15°C except November and December. As a result, the temperature may impact the denitrification in winter. Sirivedhin and Gray (2006) also found that the overall nitrate removal rate was significantly higher in summer than that in winter. However, the effect of temperature on denitrification in constructed wetland was little compare with that of carbon source.

Nutrient removal and transformation processes in constructed wetlands include microbial conversion, decomposition, plant uptake, sedimentation, volatilization and adsorption-fixation reactions (Tchobanoglous, 1993).

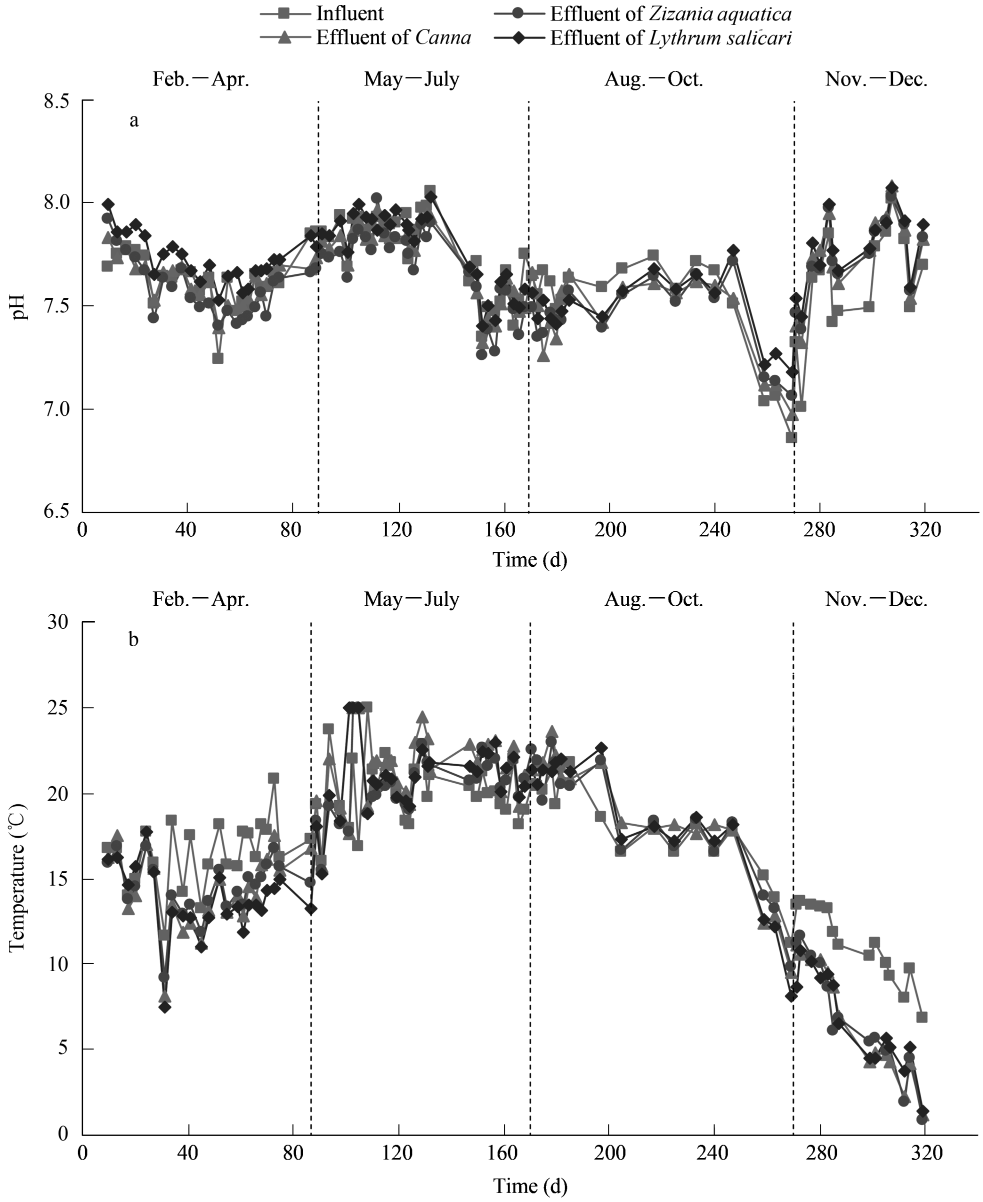

Fig. 4 Variation curves of pH (a) and temperature (b) in the constructed wetlands.

Plants uptake and denitrification are the main processes of nitrate removal in constructed wetlands considering nitrogen conservation. The factors impacting plants uptake and denitrification significantly affect the nitrate removal in wetlands. Wetland plants enhance nutrient removal through biomass accumulation, fixation of inorganic and organic particulates and the creation of an oxidized and anaerobic rhizosphere which are controlling factors for nitrification and denitrification (Burgoon *et al*., 1995). The contribution of plants in removing nitrate varies with the species of plants and the characteristics of the effluent. However, there was no distinct difference among three constructed wetlands planted with *Canna*, *Zizania caduciflora* or *Lythrum salicari* during almost one year performance.

3 Conclusions

In conclusion, the constructed wetland can not provide enough available organic carbon sources for nitrate removal when influent are rich in nitrate and poor in organic chemistry contamination. The additional carbon source (glucose) can remarkably improve the nitrate remove ability of the constructed wetland. However, nitrite in the constructed wetland accumulated a little when the additional carbon source was supplied. The wetland plants will increase the ability of buffer action which will result in that the denitrification in wetland is slightly impacted by temperature and pH. Three constructed wetlands planted with *Canna*, *Zizania caduciflora* or *Lythrum salicari* showed no obvious difference during almost one year performance because those three plants have similar ability of nitrogen assimilation. In the design of constructed wetland to treat wastewater with high nitrate and low carbon source, the plants, carbon source and substance should all be considered. Further studies about how to enhance the removal of nitrate by plants, carbon source and filling material are recommended.

Acknowledgments

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