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Effects of plants and temperature on nitrogen removal and microbiology in a pilot-scale integrated vertical-flow wetland treating primary domestic wastewater

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ABSTRACT

Constructed wetlands are continuously being developed due to their cost effectiveness and ecological characteristics. A pilot-scale integrated vertical constructed wetlands (IVCWs) system was implemented for the treatment of primary domestic wastewater from student dorms in a university to investigate the nitrogen transformation properties. The removal efficiency of the total nitrogen of the pilot system ranged from 22.9% to 35.8%, i.e., there was insufficient denitrification in the wetland system. The maximum nitrification intensities of the media in the down-flow wetland and the up-flow wetland of the IVCW were 0.065 mg/(kg h) and 0.06 mg/(kg h), respectively. The nitrification intensity of the media in the rhizosphere of plants was significantly higher than that in the non-rhizosphere ($P<0.01$). Larger numbers of the ammonia-oxidizing bacteria and nitrite-oxidizing bacteria were observed in the rhizosphere than in the non-rhizosphere in all the wetland cells, while the numbers of denitrifying bacteria exhibited the profiles contrary to those of the ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. The nitrogen bacteria exhibited temperature-related patterns. The maximum oxygen concentrations at the surface of the roots of *Juncus effusus* could reach 106 μmol/l. The differences between the nitrification intensity, the numbers of bacteria in the rhizosphere and the numbers in the non-rhizosphere were attributed to the oxygen-secretion capacity of the plant roots.

1. Introduction

Constructed treatment wetlands, an economical and ecological method to treat wastewater, have been widely used in the world during the past decades. Constructed wetlands can effectively remove suspended solids, organic pollutants and nutrients from wastewater (Vymazal, 2007; Kadlec, 2008). Vertical flow constructed wetlands have undergone continuous development in recent years, and they can be divided into two types, down-flow and up-flow, according to the flow direction of the wastewater. Some researchers studied the integrated vertical constructed wetlands (IVCWs) by combining the two types of wetlands to improve the water quality (Perfler et al., 1999; Chang et al., 2012).

In the constructed wetlands, the purifying effects for domestic wastewater are mediated by the cooperation of the media, plants and microorganisms (Cheng et al., 2011), and knowledge of the nitrogen cycling in these wetlands is crucial for scientists and managers seeking to create long-term improvement of the water quality (Poe et al., 2003). Microorganisms are the principal component of the nitrogen cycling process, and estimates of the nitrogen removal via denitrification indicated that a significant portion of the wetland nitrogen cycling is microbially mediated (Poe et al., 2003). The bacteria of nitrification and denitrification are mainly distributed at the surface and in the middle of the ground media and in the rhizosphere of plants (Machate et al., 1997). Wetland plants can assimilate the nitrogen from wastewater and release oxygen to the rhizosphere via their roots, resulting in the higher oxygen content near the root zone. The process of the oxygen change was of great importance to the growth and reproduction of bacteria involved in the nitrogen cycling, especially the nitrification and denitrification (Coleman et al., 2001; Sindilariu et al., 2008; Shimp et al., 1993).

The distributions of nitrifying bacteria and denitrifying bacteria have a direct or indirect relationship with the oxygen conditions in the wetlands. Therefore, there most likely exist a difference of the number of nitrogen cycling bacteria between the rhizosphere and the non-rhizosphere. The oxygen production in the micro-environment of the plant roots may vary with the species of wetland plants, which probably affects the performance of the nitrogen removal in the constructed wetlands. Given the increasing

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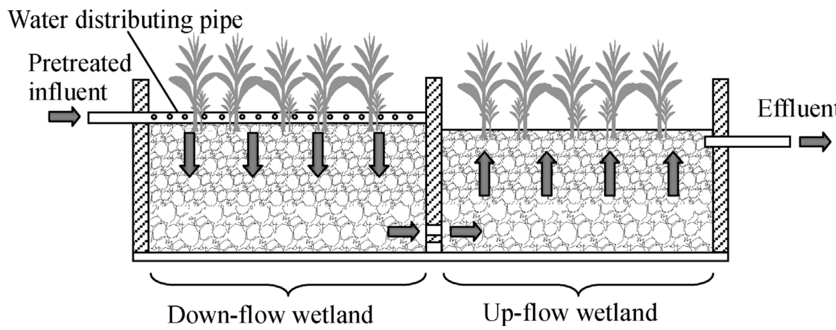

Fig. 1. Structure of the IVCWs. The gray arrows represent the flow direction of the wastewater in the systems.

application of constructed wetlands and the potential relationship between nitrogen transformation and the plant in the wetlands, the aim of this study with pilot systems of IVCWs was to investigate the nitrification and denitrification in the rhizosphere of the plants and the non-rhizosphere and to compare the nitrogen removal characteristics of the different plant types of *Juncus effusus* and *Canna*. The effect of temperature on the nitrification and denitrification was discussed as well.

2. Materials and methods

2.1. Description of the constructed wetlands

The down-flow wetlands and the up-flow wetlands are operated in series. There were walls with some holes at the bottom between the down-flow wetland and the up-flow wetland. The wastewater flowed to the up-flow wetlands following the treatment by the down-flow wetlands. On the surface of the down-flow wetlands, there were pipes with $\Phi = 50$ mm used for the distribution of wastewater. Fig. 1 shows the basic structure of the IVCWs. Both the down-flow and the up-flow wetlands have a bottom slope of 0.5%.

The constructed wetlands were filled with gravel as the media. For the down-flow wetlands of the IVCW, the height of the media layer was 0.8 m, including two layers, a lower layer of height of 0.3 m, with gravel of 30–50 mm in diameter, and an upper layer of height of 0.5 m, with gravel of 10–40 mm in diameter. The up-flow wetlands of the IVCW had the same structure as the down-flow wetlands, except for the upper layer with gravel of 0.45-m height; the 5 cm of height reduction could lead to the wastewater from the former to flow the latter without consuming any external energy.

Two IVCWs with different plant types were set. One was planted with *Juncus effusus*, and the other was planted with *Canna*. Each IVCW included a down-flow cell and a up-flow cell. The size for each cell was 3 m × 8 m, i.e., an area of 24 m^2. The study period was from 19 September to 7 December 2012, during which time the constructed wetlands was in a stable running period. The temperature was gradually reduced from 28 °C to 12 °C.

The wastewater of relatively stable quality was domestic wastewater from the student dormitory of the Huazhong Agricultural University, and it was lifted into a regulating pond for the primary treatment before flowing into the constructed wetlands. Each cell of the down-flow constructed wetland was fed with the primary effluent at a nitrogen level similar to that of the secondary effluent from the municipal wastewater treatment plant. The hydraulic loading and hydraulic retention time of the IVCW were 0.24 $m^3/(m^2\ d)$ and 1 d, respectively.

2.2. Sampling

The influent temperature was measured *in situ* during sampling. The inflow and outflow water of each IVCW was collected, followed by the determination of the concentrations of ammonia, nitrate, nitrite and total nitrogen (State Environmental Protection Administration of the People's Republic of China, 2002). The rhizosphere and non-rhizosphere media were sampled from the IVCW to determine the nitrification intensity as well as the bacteria numbers of the nitrogen cycling. For each wetland cell, the samples were collected at random. The media samples of the rhizosphere were collected around the plant main root with a radius of 10 cm at a depth of approximately 10 cm, and samples of the non-rhizosphere were collected at the same depth. Both the media and the water samples were characterized in triplicate.

2.3. Analytical methods

A 50-ml volume of NH_4^+ medium with a concentration of 25 mg/l was added into a 150-ml triangular flask. A 20-g media sample was added to each flask, which was subsequently sealed by plastic wrap with 10 small holes. The flasks were placed onto the oscillating machine for 24 h at a temperature of 25 °C, and then, the suspension was removed and filtered, followed by the measurement of the NO_3^--N concentration in the supernatant. The nitrification intensity, in units of mgN/(kg media h), was calculated by the abatement of the NO_3^--N content after culturing (Wang and Zhang, 2006).

The media sample, each with a weight of 20 g, from the rhizosphere or non-rhizosphere was added into a conical flask with a volume of 150 ml, along with a volume of 50 ml of normal saline, followed by agitation of the mixture on the vortex mixer for 30 min. The samples were used to count the number of ammonia-oxidizing bacteria, nitrite-oxidizing bacteria and denitrifying bacteria (Xu and Zheng, 1986).

Table 1
Nitrogen concentrations in the influent and effluent of IVCWs.

	TN (mg/l)	NH_4^+-N (mg/l)	NO_3^--N (mg/l)	NO_2^--N (mg/l)	pH
Influent	15.5–18.4 (17.0)	9.06–10.8 (10.2)	2.64–3.03 (2.77)	0.25–0.32 (0.28)	7.34–7.67 (7.52)
Effluent; IVCW 1	10.9–13.0 (12.0)	4.90–6.97 (5.94)	3.36–4.37 (3.92)	0.12–0.22 (0.16)	7.45–7.64 (7.57)
Effluent; IVCW 2	11.5–12.9 (12.2)	4.76–6.76 (5.89)	3.40–4.25 (3.90)	0.10–0.22 (0.15)	7.58–7.77 (7.67)

Note: the mean is shown in parentheses.