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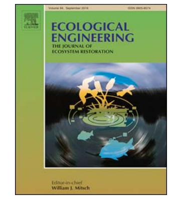

Effects of plants and temperature on nitrogen removal and microbiology in pilot-scale horizontal subsurface flow constructed wetlands treating domestic wastewater

Yumei Hua[a,*], Lian Peng[a], Shaohui Zhang[b], Kate V. Heal[c], Jianwei Zhao[a], Duanwei Zhu[a]

[a] College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, PR China
[b] School of Civil Engineering and Architecture, Wuhan University of Technology, Wuhan, 430070, PR China
[c] School of GeoSciences, University of Edinburgh, Edinburgh EH9 3FF, UK

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ABSTRACT

Two pilot-scale intermittently operated horizontal subsurface-flow constructed wetlands (HSSFCWs), one planted with *Acorus calamus* L. and one with *Phragmites australis* (Cav.) Trin. ex Steud., were implemented for the polishing treatment of domestic wastewater from Huazhong Agricultural University dormitories, Hubei Province, China. The characteristics of nitrogen removal between the plant rhizosphere and non-rhizosphere were assessed from 19 September to 12 December 2012 to address the effect of plants. The mean removal efficiency of total nitrogen (TN) was 45.2% for the two HSSFCWs with a hydraulic loading rate of $0.15\ m^3\ m^{-2}\ d^{-1}$. In both pilot-scale HSSFCWs, the nitrification intensity and numbers of nitrite-oxidizing bacteria and ammonia-oxidizing bacteria in the rhizosphere were significantly higher than in the non-rhizosphere, associated with oxygen release from plant roots. In contrast, the denitrification intensity and number of denitrifying bacteria were higher in the non-rhizosphere. Although significantly higher dissolved oxygen concentration at the root surface and greater numbers of nitrogen-processing bacteria in the rhizosphere occurred for *Acorus calamus* compared to *Phragmites australis*, very similar nitrogen removal efficiencies were observed in the two HSSFCWs, probably due to the relatively low N concentration of the wastewater treated in the wetlands. In addition, the removal efficiencies of NH_4^+-N and NO_3^--N were significantly positively correlated with water temperature in both HSSFCWs.

1. Introduction

Excessive nitrogen input to unimpacted water bodies increases the potential risk of eutrophication, making it essential to protect ecosystems by reducing nitrogen loading prior to entering surface waters (Maltais-Landry et al., 2009). Constructed wetlands, an ecological technology for wastewater treatment, can be very useful as they are cost-effective and easily operated and maintained (Mietto and Borin, 2013). Hence, they have been used worldwide during the past decades, providing a natural means for ecological and simple wastewater treatment (Tunçsiper et al., 2009; Knowles et al., 2010).

Horizontal subsurface-flow constructed wetlands (HSSFCWs) are systems used for wastewater treatment that are particularly suitable for small communities (Pedescoll et al., 2011), and are generally used as a polishing process for the effluent from various biological wastewater treatment systems. However, although HSSFCWs are efficient in solids and organic matter removal, they are less efficient in nitrogen removal (Vymazal, 2009; Ngo et al., 2010; da Costa et al., 2013). Optimising the operation of HSSFCWs aims to improve the removal ability for nitrogen by HSSFCWs. Hence, several studies have evaluated the effect of the operation mode on removal efficiency and found that intermittent operation promotes better performance in ammonium removal than continuous operation (Stein et al., 2003; Caselles-Osorio and García, 2007; Pedescoll et al., 2011; Zhang et al., 2012).

The degradation of wastewater pollutants within HSSFCW systems occurs through various biological, physical and chemical processes simultaneously, and is related to the interactions between media, plants and microorganisms (Mburu et al., 2013). The removal of nitrogen by constructed wetlands is carried out through different mechanisms: plant absorption (ammonium and nitrate), ammonification of organic nitrogen, nitrification, denitrification, and volatilization as ammonium (Camacho et al., 2010). Biological nitrification, which is realized by nitrifying bacteria, is the major pathway for ammonium removal in HSSFCWs (Gersberg et al., 1985; Mayo and Bigambo, 2005).

* Corresponding author.
E-mail address: ymhua@mail.hzau.edu.cn (Y. Hua).

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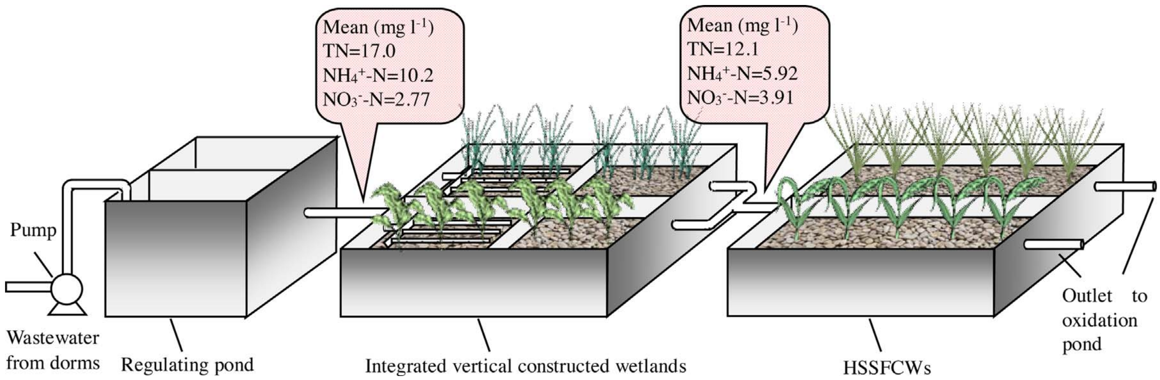

Fig. 1. Structure of the constructed wetland system. Values are the mean of 6 sampling times from 19 September to 12 December 2012. *Canna* and *Juncus effuses* were planted in the integrated vertical wetlands.

The oxygen concentration in constructed wetlands is of great importance to the activities and reproduction of bacteria involved in the processes of nitrification and denitrification (Coleman et al., 2001; Sindilariu et al., 2008). Nitrification occurs preferentially in aerobic conditions, whilst anoxic conditions favor denitrification. Constructed wetlands can provide an effective zone for the degradation of contaminants due to enhanced microbial activity in the rhizosphere of plants (Stottmeister et al., 2003). Using microelectrodes in experimental wetlands, it has been shown that the redox potential at the root surface of *Scirpus validus* is higher than in the bulk water (Bezbaruah and Zhang, 2004), which is related to the presence of oxygen released by the plant roots (Peng et al., 2014).

In most cases the vegetation has a positive effect on the removal of nitrogen in constructed wetlands, i.e. it leads to higher treatment efficiency, and *Phragmites australis* is the wetland plant most frequently used worldwide (Vymazal, 2011). The plants used in constructed wetlands play an important role partly due to their rhizosphere creating a beneficial environment for removal processes. The rhizosphere provides media for bacterial growth and for solid filtration. The aerenchymatous oxygen supply to the rhizosphere enhances the decomposition of organic matter, further promoting degradation of nutrients by aerobic microbes (Schulz et al., 2003). Oxygen production in the micro-environment of plant roots may vary between species of wetland plants (Lai et al., 2011; Peng et al., 2014), suggesting that plant choice may influence nitrogen removal in HSSFCWs.

Although previous studies have investigated nitrogen transformation in HSSFCWs (Mayo and Bigambo, 2005; Ding et al., 2014; Coban et al., 2015), there appears to be no published research comparing nitrification and denitrification between the rhizosphere and non-rhizosphere in HSSFCWs operated intermittently. The main purpose of the current study was to investigate the effect of plants on nitrogen removal, including the numbers of nitrifying and denitrifying bacteria, the nitrification and denitrification abilities of the medium, and the oxygen concentrations at the root surface of plants in pilot HSSFCW systems.

2. Materials and methods

2.1. Description of the constructed wetlands

The pilot HSSFCWs were set up at Huazhong Agricultural University, Wuhan, Hubei Province, China (30°28′43″ N, 114°21′12″ E). Two pilot HSSFCWs, one planted with *Acorus calamus* L. (HSSFCW-A) and one planted with *Phragmites australis* (Cav.) Trin. ex Steud. (HSSFCW-P), were monitored from 19 September to 12 December 2012. Both HSSFCWs consisted of concrete basins (8 m length × 3 m width) with a bottom slope of 1%. The depth of the medium layer was 0.6 m, including a lower layer of 0.2 m depth (gravel size 30–50 mm), and an upper layer of 0.4 m depth (gravel size 10–40 mm). The mean porosity of the bulk medium was 42%, and the main chemical composition of gravel was SiO_2 and Al_2O_3. Pipes (diameters 80 mm and 50 mm for main and branch pipes, respectively) were used for wastewater inflow and outflow from the subsurface wetlands. The water level in the HSSFCWs was kept 0.05 m below the gravel surface, to give a water depth of 0.55 m after inflow, by adjusting the height of the flexible outlet pipe.

The constructed wetlands were planted on 27 May 2011 with shoots from plants purchased from Wuhan Botanic Garden, Chinese Academy of Sciences. Shoots were planted in clusters (four shoots for *Acorus calamus* and five shoots for *Phragmites australis*), spaced 20 and 25 cm apart, respectively, to give the same shoot density of 80 shoots per m^2 for both HSSFCWs.

The inlet wastewater for the HSSFCWs was domestic wastewater from the student dormitory of Huazhong Agricultural University after treatment by a regulating tank and integrated vertical constructed wetlands (Peng et al., 2014). The water entering the HSSFCWs was a mixture of the effluents from the two integrated vertical constructed wetlands (Fig. 1), and the HSSFCW effluent entered a oxidation pond used for aquatic plant cultivation. The main aim of the HSSFCWs was to reduce the N concentration in the effluent from the integrated vertical constructed wetlands which is typically $> 10\ mg\ l^{-1}$ total nitrogen (TN). The inlet pH value ranged from 7.54 to 7.63.

The HSSFCWs were operated in intermittent mode with a hydraulic loading rate of $0.15\ m^3\ m^{-2}\ d^{-1}$ since 2 December 2011. The inlet and outlet valves were closed after inflow to attain the designed value of hydraulic loading rate and the hydraulic retention time was 2.5 d. The hydraulic parameters were designed based on a BOD_5 loading rate of approximately $5\ g\ m^{-2}\ d^{-1}$ (Akratos and Tsihrintzis, 2007; Pedescoll et al., 2011). The mean surface NH_4^+-N load was $0.88\ g\ m^{-2}\ d^{-1}$.

2.2. Sampling

Inlet and outlet wastewater samples from each HSSFCW were collected on six occasions (19 September, 5 October, 21 October, 4 November, 21 November and 6 December 2012). The influent temperature was measured in situ during sampling. The samples were stored at 4 °C immediately after collection and then the pH, TN, NH_4^+-N, NO_3^--N and NO_2^--N concentrations were determined. On the same days as the water sampling, three samples in each situation of the medium in the rhizosphere and non-rhizosphere of the HSSFCWs were collected to determine the nitrification/denitrification intensity as well as the numbers of bacteria relevant to nitrogen cycling. Media samples from the rhizosphere were collected within a diameter of 10 cm around the plant main root at a depth of approximately 10 cm, and media samples from the non-rhizosphere were collected at the same depth but in the middle between two plant clusters. Both water and media samples were characterized in triplicate.