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Effect of plant-based carbon sources on denitrifying microorganisms in a vertical flow constructed wetland

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HIGHLIGHTS

- Combined plant-based carbon source could improve the N removal efficiency of VFCWs.
- Effects of plant-based carbon sources on N removal genes were estimated.
- Partial nitrification-denitrification and classic denitrification found in VFCWs.

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ABSTRACT

The effects of supplementing plant-based carbon sources, fermented tissues of *Arundo donax* and *Pontederia cordata*, and a combination of the two plants, on the nitrogen removal efficiency and microbial composition in a vertical flow constructed wetland (VFCW) were examined. The results showed that the addition of the composite carbon source produced the highest removal efficiencies of NH_4^+-N 91.5%, NO_3^--N 94.5% and TN 92.8% in VFCW. The detected abundance of *amoA*, *nirS*, and *nxrA* genes indicated that ammonia oxidation bacteria and denitrifying bacteria were more abundant than the nitrite oxidation bacteria. Furthermore, the addition of the composite carbon source significantly promoted the growth of the denitrifying bacteria in VFCW. The results indicated that supplementing the system with plant-based carbon sources achieved partial nitrification and denitrification, as well as classic denitrification in VFCWs. The study suggested that multiple nitrogen removal pathways were required to feasibly and efficiently remove nitrogen.

1. Introduction

The design of constructed wetlands, a type of wastewater treatment technology, is based on ecological principles. This technology has several advantages, such as the low cost of investment, operations, and maintenance; good effluent quality; and minimal secondary pollution (Wu et al., 2015). Constructed wetlands are commonly used for tertiary sewage treatment in China, as well as in other countries (Vymazal, 2014). In Shenzhen City, a major city in Guangdong Province, China, several sewage treatment plants, such as Longhua, Binhe, and Shangyang, have been utilizing constructed wetlands for tertiary treatment. The combined treatment capacity of these wetlands has reached 120,000 m^3/d. However, the low C/N ratios in the effluent after the secondary treatment in the sewage treatment plants have been indicated as unfavorable for microbial denitrification, thereby reducing the nitrogen removal efficiency of the constructed wetlands. Therefore, a variety of carbon sources, such as glucose, sludge, and plant tissues have been added to constructed wetlands to enhance nitrogen removal (Lu et al., 2009). Among such carbon sources, the plant-based carbon sources have several advantages, such as low cost, renewability, and convenient collection (Wen et al., 2010). Moreover, the plant-based carbon sources can provide additional organic matter and establish more anaerobic zones for the growth of wetland microorganisms (Zhang et al., 2016). However, different plant materials produce carbon sources with varying properties and these differences still need to be explored (Zhang et al., 2014).

Microbes play a major role in nitrogen removal in constructed wetlands (Faulwetter et al., 2009). Classic microbial nitrogen metabolic pathways consist of ammonification, nitrification, and denitrification. However, other unconventional nitrogen removal pathways, such as partial nitrification and denitrification, have been discovered in recent years. In this pathway, nitrites

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(NO_2^--N) in the anaerobic environment will be fed directly into the denitrification pathway and finally converted to N_2. Therefore, compared with the traditional nitrogen removal pathways, partial nitrification and denitrification could reduce the oxygen required for nitrification and the carbon source required for denitrification (Wei et al., 2014). This process has been extensively applied in sequencing batch reactors (Guo et al., 2010; Ge et al., 2014), and recent studies have shown that partial nitrification and denitrification can be implemented in constructed wetlands (Zhang et al., 2010; Hu et al., 2014). However, relevant research on this topic is scarce, particularly because the correlation between the abundance and distribution of microorganisms and the water quality parameters in partial nitrification and denitrification has not been determined yet. Therefore, in the current research, a vertical flow constructed wetland (VFCW) system was created, in which partial nitrification and denitrification was achieved by regulating the internal wetland environment. The influence of different plant-based carbon sources on the removal of various forms of nitrogen in the system was investigated. Quantitative polymerase chain reaction technology (qPCR) was used to investigate the spatial distributions of the functional microbial genes involved in the nitrogen transformation. This was done to determine the changes in nitrogen transformation pathways in constructed wetlands after different plant-based carbon sources had been added.

2. Materials and methods

2.1. VFCW set-up

Small-scale VFCW systems were set up in the greenhouses (temperature 27 ± 2 °C) of the flower gardens at Shenzhen University. Each test system was constructed from a polyvinyl butyral column (diameter 30 cm and height 90 cm), with an effective volume of 20 L. The test system was filled with clean river sand (particle size 0–2 mm, porosity 0.36, and thickness 80 cm) (Fig. 1), and the top 10 cm of sand was mixed with 0.5% wetland substrates (Table 1) for microbial inoculation (Fu et al., 2015). These substrates were obtained from the Wenshan Lake constructed wetland at Shenzhen University. The test system was planted with *Cyperus alternifolius* (height 30–40 mm, depth 10–20 cm, and density 40 plants m^{-2}). Five sampling points were set at different depths, namely, 0, 20, 40, 60, and 80 cm from the top to the bottom section of the VFCWs (Fig. 1). Water from Wenshan Lake (Table 1), on the campus, was used to acclimatize the plants and the microbial communities for the first two months, after which the operating conditions in the test system were stabilized.

2.2. Acquisition of plant materials and fermentation experiments

Two common types of plants in Shenzhen constructed wetlands, namely, *Arundo donax* and *Pontederia cordata*, were collected for use as the plant-based carbon source. After washing and drying, the plant stems and leaves were cut into 1–2 cm pieces and dried to a constant weight. Three fermentation tanks, with an effective volume of 2 L were used, to which 50 g of dried plant tissue with 2 L tap water was added, after which the tanks were placed in an incubator at 30 °C for 7 d of anaerobic fermentation. Subsequently, the plant carbon fermentation broths were collected from the supernatant and stored at 4 °C. The broths were subsequently divided into three groups, namely, a single plant fermentation broth of *A. donax* tissue, a single plant fermentation broth of *P. cordata* tissue, and the mixed fermentation broth containing equivalent amounts of *P. cordata* and *A. donax* tissues.

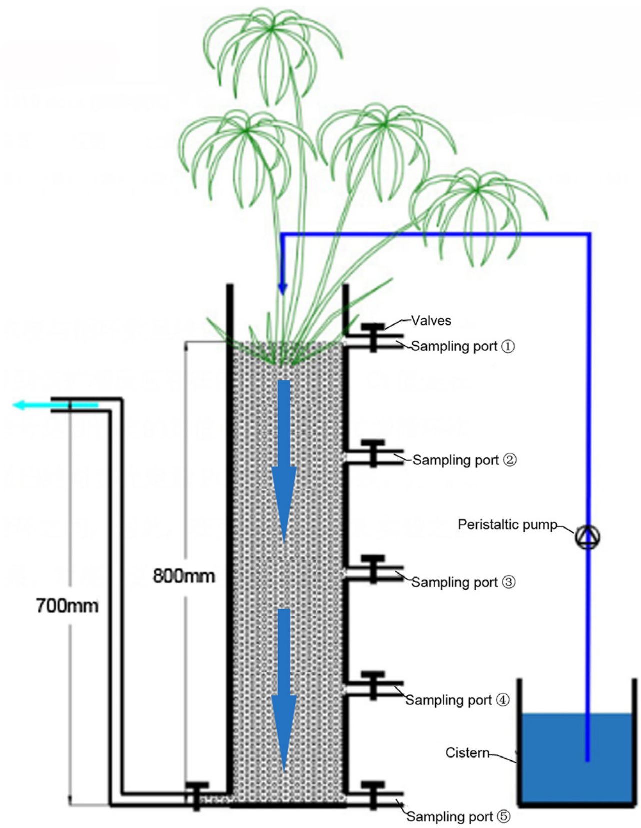

Fig. 1. The schematic diagram for small-scale plot of VFCW.

2.3. Determination of denitrification rate

To determine the rate of denitrification, 10 g of wetland substrate was obtained and placed in a conical flask, to which 40 mL of artificial effluent was added. In the artificial effluent, the concentrations of nitrate (NO_3^--N), nitrite (NO_2^--N), and total nitrogen (TN) were 38 ± 1 mg/L, 6.5 mg/L, and 45 ± 1 mg/L, respectively. Three types of anaerobic fermentation broth made of different plant materials were added to the flask, respectively, and the COD_{Cr} (chemical oxygen demand)/TN was adjusted to 6. The flask was evacuated, sealed, and placed in an incubator at 37 °C for 4 d. The concentrations of nitrate (NO_3^--N), nitrite (NO_2^--N), and total nitrogen (TN) were measured by a CleverChem 380 discontinuous water quality analyzer (Dechem-Tech, Germany), based on the standard methods (GHZB1-1999, China), before and after incubation.

The denitrification efficiency was calculated by using formula (1) (Ding et al., 2013).

$$\text{Specific denitrification rate (SDNR)} = [\Delta\beta(NO_3^-\text{-N}) + 0.6\Delta\beta(NO_2^-\text{-}N)]/\Delta t \quad (1)$$

where $\triangle\beta(NO_3^-\text{-N})$ (mg/L) is the difference in nitrate concentration before and after incubation, $\triangle\beta(NO_2^-\text{-N})$ (mg/L) is the difference in nitrite concentration before and after incubation, and $\triangle t$ (h) is the incubation time.

2.4. Operation strategy

Artificial sewage was used as the influent of the VFCW system (Table 1), and the water quality was determined with reference to the effluent quality of the sewage treatment plant (Xiong et al., 2011). The plant fermentation broth was added to the influ-