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Enhanced nitrate removal in self-supplying carbon source constructed wetlands treating secondary effluent: The roles of plants and plant fermentation broth

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ABSTRACT

In this study, a self-supplying carbon source constructed wetland (CW) was developed and evaluated. Both the effects of plants (*Typha latifolia*) and plant fermentation broth on nitrate removal were measured. The results showed that the addition of plant fermentation broth greatly improved the nitrate removal rate. As the ratio of added chemical oxygen demand to influent nitrate (COD_{Add}/NO_3-N ratio) increased from 0 to 3, the nitrate removal rate attributed to the plants increased from 0.09 to 0.29 g N m^{-3} d^{-1}, but the proportion of total nitrate removal decreased from 27.3% to 10.7%, and denitrification was always the dominant nitrate removal mechanism. Furthermore, there were strong positive correlations between the COD_{Add}/NO_3-N ratio and the nitrate removal rate, both in unplanted (R^2 = 0.977) and planted (R^2 = 0.996) microcosms. Plant biomass could potentially support a nitrate removal rate of 0.05–0.54 g N m^{-2} d^{-1} in self-supplying carbon source CWs.

1. Introduction

Compared to conventional tertiary treatment technologies, the use of constructed wetlands (CWs) as a cost-effective, extensive and efficient wastewater treatment technology has been increasing over recent decades (Vymazal, 2005). In horizontal subsurface flow constructed wetlands (HSSF CWs), the denitrification potential is considered to be high, as HSSF CWs can provide anoxic or anaerobic conditions (IWA, 2000; Kadlec and Wallace, 2008). However, the influent carbon is mostly oxidized in the aeration processes (Leverenz et al., 2010) and the internal carbon from the rhizosphere is insufficient for denitrification (Kuschk et al., 2003). Therefore, an additional carbon source is needed in CWs to enhance denitrification.

Various carbon sources have been used to improve the denitrification performance in carbon-limited wetlands, including glucose, fructose, soils and plant biomass (Davidsson and Ståhl, 2000; Lin et al., 2002; Lu et al., 2009). Among the various carbon sources, plant biomass is an attractive alternative carbon source because of its low cost, renewability and wide availability (Wen et al., 2010). The investigation of Zhao et al. (2009) showed that cattail (*Typha latifolia*) is one of the aquatic plants that is widely used in CWs, and can utilize solar energy effectively and grow quickly. Following plant harvest, the large amount of plant biomass produced, which has a net aboveground primary productivity of 322–3560 g (dry weight, DW) m^{-2} $year^{-1}$ in mature CWs (Vymazal and Kröpfelová, 2008), can be returned directly to the wetland or used in other ways.

However, adding plant biomass into CWs directly has some disadvantages. Firstly, plant biomass additions will cause an unstable carbon supply in the CW, presenting the characteristics of a carbon source that is excessive in the initial stage of the wetland but insufficient in the terminal stage (Wen et al., 2010). Secondly, plant biomass additions will cause a low effective utilization of the carbon source for denitrification (Chen et al., 2014a). Thirdly, additions of plant biomass in CWs also tend to increase the effluent chemical oxygen demand (COD) concentration, which may exceed effluent standards.

To overcome those disadvantages, a "self-supplying carbon source constructed wetland" was developed in this study. In this CW, a synthetic carbon cycle was established through the following steps. To begin the cycle, the plants were harvested in the mature season. After collection, the plant biomass was pretreated and fer-

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mented. Finally, to enhance denitrification, the plant fermentation broth was added as a self-supplied carbon source to the CW from which it was derived. That is to say, the synthetic carbon cycle established the spatial transformation (i.e., from aboveground to underground) of the seasonally fixed plant carbon that exists in CWs. In addition, the production of fermentation broth allowed the quantity and quality of the added carbon to be controlled. As such, the technology could overcome the three key disadvantages associated with CWs. However, as a CW technology for nitrate removal, it is very important to establish the relationship between the nitrate removal rate and the quantity of plant fermentation broth in self-supplying carbon source CWs. Furthermore, the renewability of plant biomass as a carbon source must be estimated to facilitate engineering design of these CWs.

Plants are considered to be an indispensable component in CWs (Kadlec and Wallace, 2008). Previous studies have reported the functions of plants for nitrate removal in CWs, including uptake of nitrate, supplying organic carbon available for denitrification and providing attachment sites for denitrifying bacteria (Bais et al., 2006; Zhao et al., 2010; Kadlec, 2012). Hodge et al. (1996) further indicated that the qualitative and quantitative composition of root exudates is affected by oxygen status, nutrient availability and microorganisms in the rhizosphere. Therefore, the addition of plant fermentation broth could lead to both an increase in the oxygen-deficiency and an increase in the quantity of nutrients available in the CWs. So, the effects of plants on nitrate removal in the self-supplying carbon source CW and in a conventional CW should be different. However, this difference remains unclear and must be quantified.

Thus, the objectives of this study were: (1) to investigate the effects of plants and plant fermentation broth on nitrate removal in self-supplying carbon source CWs treating secondary effluent; (2) to establish the relationship between the rate of nitrate removal and the quantity of plant fermentation broth, and (3) to estimate the renewability of a self-supplied carbon source (plant biomass) on nitrate removal in the CWs.

2. Methods

2.1. Source of plant litter and culture media

The cattail (*T. latifolia*) litter used in this study was collected along the watercourses in the vicinity of our laboratory (121°29′ E, 31°14′ N, subtropical monsoon climate) in December, 2012. After collection, the plant litter was cleaned and dried, milled into powders with an average diameter of 0.15 mm, and further air-dried to a constant mass before being preserved in a container free from moisture and stored at fermentation. The inoculated sludge was taken from the anaerobic digestion tank of a WWTP (anaerobic–aerobic process) in Shanghai, China.

2.2. Characterization of fermentation experiments

Five fermentation tanks, each with an effective volume of 10 L, were constructed in the laboratory. The fermentation mixtures in each tank contained 100 g (dry weight, DW) of raw plant litter, 7 g (dry weight of volatile suspend solids) of inoculated sludge, and distilled water added to bring the mixture to the working volume. To ensure that plant carbon was the only limiting resource for fermentation, trace metals (Fe^{2+}, Mo^{4+}, etc.), mineral salts (Ca^{2+}, K^{+}, Mg^{2+}, etc.) and vitamins (Kit V-1, Sigma Chemical Co.) were also added based upon a low ionic strength (0.03 M) modification of standard methanogenic culture media (Fleming-Singer and Horne, 2002).

The fermentation tanks were capped with perforated screw tops and then sealed with silicon rubbers to ensure that the tanks were airtight. To achieve the optimum performance, the fermentation tanks were incubated at 35 ± 0.5 °C and the pH values were adjusted to 7.0 ± 0.1 by 5 M NaOH and 5 M HCl every 12 h while mixing the contents at 150 rpm for 20 days. Then, the suspensions were centrifuged at 11,000 × *g* for 10 min, after which the supernatant was collected in 2 L serum bottles that were then sealed and stored at 4 °C, to prevent the volatilization of VFAs.

2.3. Experimental design and operation

The experimental HSSF CW microcosms (length: 1.5 m, width: 0.4 m, height: 0.6 m) were located in a temperature-controlled greenhouse (25 ± 2 °C) on the Tongji University campus, Shanghai, China. Each CW microcosm included an inlet section (0.15 m), working section (1.2 m), and outlet section (0.15 m). All the microcosms were filled with gravel (ϕ 8–13 mm, porosity = 0.4, height: 0.5 m), and planted with *T. latifolia* at a density of approximately 20 plants m^{-2} in each planted microcosm. Secondary effluent flowed into or out of the wetland microcosms through water distribution areas filled with gravel (ϕ 10–20 mm). Sampling points were established at the inlet and outlet of each experimental wetland and at one-quarter, one-half and three-quarters the length.

Four continuous-flow HSSF CW microcosms were used in the study, designated as follows: unplanted unit without added carbon (W1); planted unit without added carbon (W2); unplanted unit with added carbon (i.e., fermentation broth) (W3); and planted unit with added carbon (W4). With this design, the effects of plants and plant fermentation broth on nitrate removal in CWs could be assessed. The effects of broth alone could be seen by comparing results from W1 and W3, and the effect of broth in the presence of plants could be seen by comparing results from W2 and W4. Likewise, the effect of plants alone could be identified by comparing results from W1 and W2, and the effect of plants in the presence of broth could be examined by comparing results from W3 and W4.

The secondary effluent used in the CWs was collected from a neighboring WWTP (in Shanghai, China) and was introduced continuously into the microcosms from two, 100 L plastic feeding tanks using peristaltic pumps at flow rates set to achieve a target 4-day hydraulic retention time in each wetland. As secondary effluent was being delivered, stock KNO_3 solution was added in sufficient quantity to yield a wastewater nitrate concentration of 15 mg N L^{-1}. In units W3 and W4, the fermentation supernatant (diluted to 1500 mg L^{-1}), which was used as the electron donor and self-supplied carbon source, was introduced together with the flow of wastewater using a positive displacement pump at a predetermined flow rate that was calculated to achieve target ratios of added COD to influent nitrate (COD_{Add}/NO_3-N ratios) between 1 and 4.

The formal experiments began after 3 months of stable plant growth, during which time the bacterial communities adapted to the wetlands environment. Each experiment involved operating the CWs for 180 days divided into four 45-day stages. In each stage, a successively higher COD_{Add}/NO_3-N ratio (i.e., 1, 2, 3, and 4, respectively) was examined in the W3 and W4 microcosms. The composition of the influent to each wetland microcosm is shown in Table 1.

2.4. Sampling and analysis

Before the plant litters were fermented, they were cleaned, oven-dried at 40 °C to a constant mass, and milled to pass a 60-mesh screen. The resulting powders were tested for hemicellulose, cellulose and lignin contents. After fermentation, the suspensions were centrifuged at 11,000 × *g* for 10 min and then tested for COD, reducing sugar, soluble protein as well as VFAs. The analyses of the parameters mentioned above were the same as described in the previous publication (Wen et al., 2010).