**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 ($\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio) increased from 0 to 3, the nitrate removal rate attributed to the plants increased from 0.09 to 0.29 $\text{g N m}^{-3} \text{ d}^{-1}$, 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 $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ 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 $\text{g N m}^{-2} \text{ d}^{-1}$ in self-supplying carbon source CWs.

Keywords

Constructed wetlands (CWs); *Typha latifolia*; carbon source; nitrate removal

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 (Kuschik 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 yr⁻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 fermented. 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 (*Typha 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 11,000g 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 *Typha 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 ($\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ 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 $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ 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 11,000g 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](#)).

Influent and effluent flux and grab samples were regularly collected every five days from each wetland microcosm. In addition, grab samples were collected at the established locations along the length of each wetland on days of 35, 40 and 45 in each stage to determine the COD and nitrate profiles. Analyses for COD, ammonia (NH₄-N), nitrate (NO₃-N) and total nitrogen (TN) were analyzed according to standard methods ([APHA, 1998](#)).

2.5 Data analysis

The accumulation of reducing sugar, soluble protein and VFAs was expressed as mg COD L⁻¹, using COD conversion factors for reducing sugar, soluble protein, acetic and propionic of 1.070, 1.500, 1.066 and 1.512, respectively ([Grady et al., 1999](#)). Both the volumetric removal rate (g m⁻³ d⁻¹) and areal removal rate (g m⁻² d⁻¹) were used to evaluate the COD and nitrate removal efficiencies at different COD_{Add} / NO₃-N ratios.

Statistical analysis was carried out using SPSS version 19.0 software. All variables were tested prior to analysis to ensure that they were normally distributed. One-way ANOVA procedures were used to evaluate the effects of plants and plant fermentation

broth on COD and nitrate removal rates, and statistically significant differences were set with p value < 0.05 .

3 Results and discussion

3.1 Characterization of plant fermentation broth

Cellulose (28.6%), hemicellulose (13.7%) and lignin (9.3%) were the main constituents of plant litter. The lignocellulose can be hydrolyzed to reducing sugars, after which the monosaccharides are converted to VFAs through the anaerobic microbial metabolism ([Rittmann and McCarty, 2001](#)). In fermentation experiments, 10 g DW L⁻¹ cattail litter was added in each fermentation tank. After fermentation, the average COD, NH₄-N and TN concentrations in the fermentation broth were 2,467, 6.4 and 15.6 mg L⁻¹, respectively. Accordingly, the calculated COD conversion factor of plant fermentation broth was about 0.25 g COD g⁻¹ DW plant litter.

Chemical analyses showed that VFAs (86.2%), of which acetic was dominant (62.3%), were the main available carbon source in the plant fermentation broth. Besides, reducing sugar (2.2%) and soluble protein (4.3%), which the fermentation broth contains, can also be converted to readily biodegradable organisms in the wetland microcosms ([Wen et al., 2010](#)). Previous studies have shown that VFAs are a kind of easy biodegradable carbon source which the denitrifying bacteria favored, and acetic is not only the preferred VFA species, but also the fastest electron donor for denitrification ([Elefsiniotis and Wareham, 2007](#)). In addition, the calculated average COD / TN ratio in the fermentation broth was 158. These results indicated that using plant fermentation broth as a self-supplied carbon source could greatly improve the quality and quantity of available carbon in W3 and W4 microcosms.

3.2 Nitrate removal and profile

3.2.1 Effect of plant fermentation broth on nitrate removal

As shown in [Table 1](#), the average nitrate removal rates during the experiment in W1 and W2 microcosms were only $0.24 \text{ g N m}^{-3} \text{ d}^{-1}$ and $0.33 \text{ g N m}^{-3} \text{ d}^{-1}$, respectively. The nitrate removal rates in the W3 microcosm during stages 1–4 were 0.95, 1.71, 2.42 and $3.72 \text{ g N m}^{-3} \text{ d}^{-1}$, respectively, at $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios of 1, 2, 3 and 4, respectively. These nitrate removal rates were 4.0–15.5 times those measured in the W1 microcosm. The nitrate removal rates in the W4 microcosm were 1.17, 1.98, 2.71 and $3.74 \text{ g N m}^{-3} \text{ d}^{-1}$ during stages 1–4, respectively, while the corresponding $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios were 1, 2, 3 and 4, respectively. These nitrate removal rates were 3.5–11.3 times those measured in the W2 microcosm. These results showed that using plant fermentation broth as a self-supplied carbon source could greatly improve the nitrate removal rate in CWs ($p < 0.05$). Further, the nitrate removal rate increased as the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio increased. Previous studies showed that most denitrifying bacteria are chemoheterotrophs, and the quality and quantity of available carbon sources can be used as indices of carbon bioavailability to evaluate the influence of carbon on nitrate removal ([Hume et al., 2002](#); [Wen et al., 2010](#)). Given that the reducing sugar (2.2%), soluble protein (4.3%) and VFAs (86.2%) were the main components of the added carbon source, denitrifying bacteria could quickly use these organic compounds for denitrification. In addition, the higher $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios in W3 and W4 microcosms might have favored the denitrifying bacteria in other ways. For example, the DO concentrations and oxidation-reduction potential (ORP) conditions in CWs are lowered by high heterotrophic activity and limited diffusion ([Bernet et al., 2001](#); [Seo and DeLaune, 2010](#)).

Wen et al. (2010) reported that the adaptation period for denitrification and nitrate removal in CWs could be shortened by adding plant biomass. However, such additions were an unstable carbon supply because carbon availability was excessive in the initial stage but insufficient in the terminal stage. Chen et al. (2014a) further found that the relative contribution of denitrifying bacteria to COD consumption was 8.4–9.2%, suggesting ineffective utilization of carbon source for denitrification, due to the excessive plant carbon supply in the initial stage; instead, most of the carbon source was consumed by sulfate-reducing bacteria (20.6–26.0%) and methane-producing bacteria (47.3–61.0%). Obviously, using plant fermentation broth as a self-supplied carbon source successfully solved the problem of an unstable carbon supply, thus achieving a stable and substantial nitrate removal rate (Table 1). Furthermore, the availability of the fermentation broth carbon source for denitrification can also be improved by adjusting the influent $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio.

3.2.2 Effect of plants on nitrate removal

Compared with W1, the nitrate removal rate was $0.09 \text{ g N m}^{-3} \text{ d}^{-1}$ greater in W2. Accordingly, $0.09 \text{ g N m}^{-3} \text{ d}^{-1}$ of nitrate removal could be attributed to the plants, accounting for 27.3% of the nitrate removal in this study. Suggesting that *Typha latifolia* could improve the removal of nitrate in CWs ($p < 0.05$).

Compared with W3, the nitrate removal rate was $0.22\text{--}0.29 \text{ g N m}^{-3} \text{ d}^{-1}$ greater in W4, as the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios were adjusted between 1 and 3. Accordingly, $0.22\text{--}0.29 \text{ g N m}^{-3} \text{ d}^{-1}$ of nitrate removal could be attributed to the plants in microcosms amended with fermentation broth. These nitrate removal rates were 2.4–3.2 times those measured in the W2 microcosm. These results showed that the nitrate removal rate attributed to the plants was much higher in the wetland microcosm to which

fermentation broth was added than in the non-amended system. The improved nitrate removal occurred because the fermentation broth enhanced the denitrification rate, which reduced the nitrate concentrations in wetland microcosms. In addition, the reduced nitrate concentrations in the HSSF CWs stimulated plant roots to increase the amount of carbon they released (Hodge et al., 1996; Wu et al., 2012), which further enhanced the denitrification process. As a result, the nitrate removal rate attributed to the plants increased as the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio increased (from 1 to 3).

However, as a proportion of all nitrate removed in W4, plants accounted for 18.8%, 13.6% and 10.7% when the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios were 1, 2 and 3, respectively. Thus, the proportion of nitrate removal attributed to the plants was relatively low and decreased as the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio increased (from 1 to 3). Although the use of fermentation broth can enhance root secretion of carbon and thereby stimulate denitrification, this indirect enhancement of denitrification was much lower than the direct enhancement prompted by carbon in the broth itself, as indicated by the lower indirect enhancement rate compared to the direct enhancement rate. Nevertheless, microbial denitrification was always the dominant nitrate removal mechanism in CWs amended with plant fermentation broth. These results were consistent with the findings of previous studies that used plant biomass as an additional carbon source in subsurface-batch CWs (Wen et al., 2010; Chen et al., 2014b).

However, there was no significant difference ($p > 0.05$) in nitrate removal efficiency between W3 (98.6%) and W4 (98.8%) microcosms when the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio reached to 4. This lack of difference might be a function of the amount of carbon supplied in the fermentation broth at the highest $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio was sufficient for denitrification, and cover up the similar effects of plant.

3.2.3 Nitrate profiles

Nitrate profiles along the length of unplanted and planted CWs under different $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios are presented in Fig. 1. As shown in Fig. 1, the nitrate profiles presented similar variations under all $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios. Specifically, the nitrate concentration decreased rapidly in the first quarter-length of each reactor, and stabilized in the second half-length. As the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio increased, the amount of nitrate that was removed in the first quarter-length of each microcosm also increased. As discussed in Section 3.1, the fermentation broth used in this study is a readily biodegradable carbon source, which the denitrifying bacteria favored. Therefore, the nitrate concentration decreased rapidly in the first quarter-length of the wetlands. However, the available carbon source was exhausted as flow distance increased (Fig. 2), which caused the declining activity of denitrifying microorganisms along the length of each wetland until the supply and demand for carbon stabilized in the second half-length of the wetlands.

However, under the condition of $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$, the nitrate concentration increased in the first quarter-length of W1, which can be explained by the insufficient quantity of carbon for denitrification, yet the nitrification of ammonia could proceed and contributed to the accumulation of nitrate. In contrast to W1, this phenomenon was not observed in W2 mainly because ammonia can be removed by plant uptake as a preferred nitrogen source (Kadlec, 2012). Besides, the supply of organic carbon for denitrification and provision of attachment sites for denitrifying bacteria by plants could be other explanations for the difference (Bais et al., 2006; Zhao et al., 2010).

3.3 Effluent TN

As shown in Table 1, nitrate accounted for most of the influent TN, whereas

ammonia nitrogen fluctuated during the experiment. Both nitrate and ammonia nitrogen in W1 and W2 microcosms originated solely from the influent, while in W3 and W4 microcosms, the nitrate originated from the influent, but the ammonia nitrogen originated from a combination of the influent and the fermentation broth. However, it is worth noting that the ammonia concentration attributed to the plant fermentation broth was very low ($0.04\text{--}0.16\text{ mg L}^{-1}$) and could be ignored compared with the influent ammonia concentration ($0.5\text{--}6.0\text{ mg L}^{-1}$).

The processes by which ammonia is removed in CWs are manifold and include volatilization, matrix adsorption, plant uptake, nitrification-denitrification and anaerobic ammonia oxidation (ANAMMOX) (Vymazal and Kröpfelová, 2008). In this study, although the influent ammonia concentration ranged from 0.5 to 6.0 mg L^{-1} , the effluent ammonia concentration was stable at $0.21 \pm 0.06\text{ mg L}^{-1}$, indicating that the influent ammonia can be removed consistently and efficiently in self-supplying carbon source CWs treating secondary effluent.

Due to a great shortage of carbon for denitrification, the average TN removal efficiencies in W1 and W2 microcosms over the period of this study were only $13.6 \pm 6.2\%$ and $16.2 \pm 6.4\%$, respectively, which were at the lower end of values ($11\text{--}90.5\%$) reported in the literature (Kusch et al., 2003; Søvik and Mørkved, 2008). However, in both W3 and W4 microcosms, the TN removal efficiencies increased as the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio increased, and the highest TN removal efficiencies were 95.1% and 97.1% , respectively, at the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio of 4. This suggested that larger additions of fermentation broth to HSSF CWs results in higher TN removal efficiencies when treating nitrate-contaminated wastewater. That is to say, in this study, as long as the ammonia was efficiently nitrified in the nitrification processes at the WWTP, and the

addition of fermentation broth was sufficient for denitrification in the CWs, the effluent TN concentrations were low enough to meet strict discharge standards.

3.4 COD removal and profile

3.4.1 Effect of plant fermentation broth on COD removal

As the influent wastewater carbon was mostly oxidized during the aeration processes (Leverenz et al., 2010), the average COD removal rates in W1 and W2 microcosms over the duration of the experiment were only $1.59 \pm 0.78 \text{ g COD m}^{-3} \text{ d}^{-1}$ and $1.65 \pm 0.69 \text{ g COD m}^{-3} \text{ d}^{-1}$, respectively. As shown in Table 1, the COD removal rates in W3 during stages 1–4 were 4.35, 7.73, 10.57 and $13.64 \text{ g COD m}^{-3} \text{ d}^{-1}$, respectively, at $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios of 1, 2, 3 and 4, respectively. These removal rates were 2.7–8.6 times those measured in the W1 microcosm. The COD removal rates in W4 during stages 1–4 were 4.50, 7.96, 10.94 and $14.23 \text{ g COD m}^{-3} \text{ d}^{-1}$, respectively, at $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios of 1, 2, 3 and 4, respectively. These removal rates were 2.7–8.6 folds of those measured in the W2 microcosm. These results showed that the COD removal rate increased as the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio increased ($p < 0.05$), which further confirmed the easy biodegradability of plant fermentation broth.

3.4.2 Effect of plants on COD removal

Compared with W1, the COD removal rate was $0.06 \text{ g COD m}^{-3} \text{ d}^{-1}$ greater in W2. Compared with W3, the COD removal rate was $0.14\text{--}0.59 \text{ g COD m}^{-3} \text{ d}^{-1}$ greater in W4. Accordingly, the COD removal rate attributed to the plants increased from 0.06 to $0.59 \text{ g COD m}^{-3} \text{ d}^{-1}$ while the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio was successively increased from 0 to 4.

Previous studies have reported the functions of plants for removing organic compounds in CWs, including plant uptake, providing a micro-aerobic environment via root oxygen release and providing root surface for microbial growth (IWA, 2000; Ong

et al., 2010). This study further demonstrated that the COD removal rate attributed to plants increased as the influent loading of COD increased (i.e., at $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios of 0–4). However, one-way ANOVA analysis showed that there was no significant difference ($p > 0.05$) in COD removal rates between the planted and unplanted microcosms, either with or without an added carbon source (the fermentation broth). These findings indicated that although plants enhanced organic matter removal in the CWs, their effect was marginal.

3.4.3 COD profiles and effluent COD

COD profiles along the length of unplanted and planted CWs at different $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios are presented in Fig. 2. As shown in Fig. 2, the COD profiles showed similar variations at all $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios. Specifically, as observed for nitrate concentration, the COD concentration dropped rapidly in the first quarter-length of the wetlands and stabilized along the remaining length of the wetlands. The fast degradation of organics might be attributed to the easy biodegradability of the fermentation broth and the relatively high temperature (25 ± 2 °C) at which the wetlands were operated. Li et al. (2014) also found that COD was effectively reduced in the initial length of CWs and then stabilized in the remainder of the wetland.

Although the increase of the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio (from 0 to 4) led to both an increase in organic loading and an increase in the oxygen-deficiency in the CWs, the organics resistant to biodegradation accounted for only a tiny fraction of the plant fermentation broth (< 7.3%), and the hydrophobic fraction of these probably sank in the CWs due to matrix adsorption (IWA, 2000). As a result, even though the effluent COD concentrations in the W3 and W4 microcosms were 3.3–10.0 mg L⁻¹ greater than those from W1 and W2 microcosms, the effluent COD concentrations from W3 and W4

microcosms were always less than 30 mg L⁻¹.

In summary, the addition of plant fermentation broth and the presence of plants improved nitrate removal in the self-supplying carbon source HSSF CWs, which produced effluent COD concentrations that were less than 30 mg L⁻¹ while the COD_{Add} / NO₃-N ratios were adjusted between 0 and 4. Further, the plant biomass used in this study is low cost, renewable and widely available (Wen et al., 2010). Therefore, using self-supplying carbon source constructed wetland treating secondary effluent is an appropriate wastewater treatment technology by which to improve the treatment performance of the CWs.

3.5 The relationship between available carbon sources and nitrate removal

Relationships between the COD_{Add} / NO₃-N ratio and nitrate removal rate in unplanted and planted microcosms were examined via simple linear regression analyses. Fig. 3 shows that there were strong positive correlations between the COD_{Add} / NO₃-N ratio and nitrate removal rate both in unplanted ($R^2 = 0.977$) and in planted ($R^2 = 0.996$) microcosms. This suggested that a high quantity of carbon in CWs may contribute to a high effective nitrate removal rate regardless of the type of carbon supply (root-derived or biomass-derived). The reliability of the results is confirmed by other studies. Wen et al. (2010) found that there were strong positive correlations between available carbon sources and nitrate removal rate ($R^2 = 0.93$), and between the labile carbon ratio and nitrate removal rate ($R^2 = 0.99$). Li et al. (2014) found that low effluent TN concentration coincided with high values of COD / NO₃-N and the negative correlation coefficient (0.78) between TN concentration and COD / NO₃-N ratio strongly confirmed that the COD / NO₃-N ratio affected denitrification. Furthermore, the similar slopes and intercepts of the two fitting regressions indicated that the nitrate removal attributed to

the plant was very low in microcosms to which fermentation broth was added.

As shown in Table 1, a $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio of 4 is needed for complete denitrification. The COD equivalent of nitrate in denitrification to N_2 is $2.86 \text{ g COD g}^{-1} \text{ N}$. Typically, the heterotrophs yield (Y_{H}) is $0.67 \text{ g COD g}^{-1} \text{ COD}$, and 80% of the formed biomass will be lost via the endogenous respiration process in system without biomass wastage (Henze et al., 2008). As a result, for every 1 g nitrate denitrified to N_2 gas in denitrification systems without biomass wastage, approximately $(2.86 / (1 - (1 - 0.8) \cdot Y_{\text{H}})) = 3.3 \text{ g COD}$ is utilized. Considering the microbial loss due to incomplete interception ($\sim 2 \text{ mg L}^{-1}$ of effluent suspended solids) and the COD consumption by other microbes (e.g., aerobic heterotrophs, sulfate reducers, methanogens, etc.) (Chen et al., 2014a), a higher $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio is needed for complete denitrification in these CWs.

3.6 Engineering application

As shown in Fig. 3, a nitrate removal rate of $0.84 \text{ g N m}^{-3} \text{ d}^{-1}$ ($0.38 \text{ g N m}^{-2} \text{ d}^{-1}$) can be increased by increasing the $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio. 1 g (DW) of raw plant litter resulted in the removal of 0.055 g N nitrate. This is an important relationship for use in the engineering application of self-supplying carbon source CWs. Vymazal and Kröpfelová (2008) reported that the net aboveground primary productivity of *Typha latifolia* is about $322\text{--}3560 \text{ g DW m}^{-2} \text{ yr}^{-1}$ in mature CWs. Accordingly, as a self-supplied carbon source in CWs, plant biomass may potentially support a nitrate removal rate of $0.05\text{--}0.54 \text{ g N m}^{-2} \text{ d}^{-1}$. Furthermore, if the net aboveground primary productivity of *Typha latifolia* and/or the COD release rate of plant biomass can be improved, the nitrate removal rate can be improved further.

Undoubtedly, the operation of the synthetic carbon cycle process will generate

some cost to the operation of a self-supplying carbon source CW. However, this operation cost should be calculated in a comprehensive context in comparison with the operation of conventional CWs with external carbon resource addition. Firstly, plant biomass harvest is usually needed in all CWs. Therefore, compared to the conventional CWs, harvesting of plants in self-supplying carbon source CWs will not generate additional cost. Secondly, pretreatment and fermentation in self-supplying carbon source CWs replace plant biomass treatment and commercial carbon source addition in the conventional CWs. In fact, the combination of pretreatment and fermentation is a common method of plant biomass treatment and should not be considered an added cost that is particular to self-supplying carbon source CWs. Obviously, the purchase cost of commercial carbon sources can be saved for self-supplying carbon source CWs. Thus, in most cases, this self-supplying carbon source CW has economic advantages. Additionally, the fermentation residues are excellent soil amendments (Teglia et al., 2011), which can further improve the economy of the technology.

4 Conclusions

A self-supplying carbon source constructed wetland was developed and evaluated. The main conclusions from this investigation are outlined below.

(1) The addition of fermentation broth greatly improves the nitrate removal rate. The presence of plants has diverse effects on nitrate removal at different $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios. Denitrification is always the dominant nitrate removal mechanism.

(2) Based on the results obtained, 0.055 g N nitrate can be removed by 1 g (DW) raw plant litter.

(3) For typical productivity rates, as a self-supplying carbon source plant biomass can potentially support a nitrate removal rate of $0.05\text{--}0.54 \text{ g N m}^{-2} \text{ d}^{-1}$ in the CWs.

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Figure captions

Figure 1: Nitrate profiles along the length of the wetland microcosms under different $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios. (A) In unplanted wetland microcosms; (B) In planted wetland microcosms. Values are the average of duplicate samples from triplicate treatments ($\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$: $n = 12$; $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 1\text{--}4$: $n = 3$).

W1: unplanted control, $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$; W2: planted with *Typha latifolia*, 20 plants/ m^2 , $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$; W3: fermentation broth applied, $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 1\text{--}4$; W4: 20 plants/ m^2 + fermentation broth, $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 1\text{--}4$.

Figure 2: COD profiles along the length of the wetland microcosms under different $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios. (A) In unplanted wetland microcosms; (B) In planted wetland microcosms. Values are the average of duplicate samples from triplicate treatments

(COD_{Add} / NO₃-N = 0: n = 12; COD_{Add} / NO₃-N = 1–4: n = 3).

W1: unplanted control, COD_{Add} / NO₃-N = 0; W2: planted with *Typha latifolia*, 20 plants/m², COD_{Add} / NO₃-N = 0; W3: fermentation broth applied, COD_{Add} / NO₃-N = 1–4; W4: 20 plants/m² + fermentation broth, COD_{Add} / NO₃-N = 1–4.

Figure 3: Relationships between the COD_{Add} / NO₃-N ratio and nitrate removal rate in wetland microcosms. (A) In unplanted wetland microcosms; (B) In planted wetland microcosms.

Table captions

Table 1: Characteristics of the influent and effluent (mean ± SD), as well as the removal rates of COD and NO₃-N in four wetland microcosms.

Figure 1

Fig. 1. Nitrate profiles along the length of the wetland microcosms under different $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratios. (A) In unplanted wetland microcosms; (B) In planted wetland microcosms. Values are the average of duplicate samples from triplicate treatments ($\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$: $n = 12$; $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 1\text{--}4$: $n = 3$).

W1: unplanted control, $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$; W2: planted with *Typha latifolia*, 20 plants/ m^2 , $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 0$; W3: fermentation broth applied, $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 1\text{--}4$; W4: 20 plants/ m^2 + fermentation broth, $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N} = 1\text{--}4$.

Figure 2

Fig. 2. COD profiles along the length of the wetland microcosms under different COD_{Add} / NO₃-N ratios. (A) In unplanted wetland microcosms; (B) In planted wetland microcosms. Values are the average of duplicate samples from triplicate treatments (COD_{Add} / NO₃-N = 0: n = 12; COD_{Add} / NO₃-N = 1–4: n = 3).
W1: unplanted control, COD_{Add} / NO₃-N = 0; W2: planted with *Typha latifolia*, 20 plants/m², COD_{Add} / NO₃-N = 0; W3: fermentation broth applied, COD_{Add} / NO₃-N = 1–4; W4: 20 plants/m² + fermentation broth, COD_{Add} / NO₃-N = 1–4.

Figure 3

Fig. 3. Relationships between $\text{COD}_{\text{Add}} / \text{NO}_3\text{-N}$ ratio and nitrate removal rate in wetland microcosms. (A) In unplanted wetland microcosms; (B) In planted wetland microcosms.

Table 1
Characteristics of the influent and effluent (mean ± SD), as well as the removal rates of COD and NO₃-N in four wetland microcosms.

Wetland types	Influent				Effluent				Removal rate ^a	
	COD (mg L ⁻¹)	NH ₄ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	TN (mg L ⁻¹)	COD (mg L ⁻¹)	NH ₄ -N (mg L ⁻¹)	NO ₃ -N (mg L ⁻¹)	TN (mg L ⁻¹)	COD (g COD m ⁻³ d ⁻¹)	NO ₃ -N (g N m ⁻³ d ⁻¹)
W1 (unplanted)										
-	20.8 ± 3.6 ^c	1.09 ± 1.18 ^c	15.2 ± 0.2 ^c	16.9 ± 1.6 ^c	14.5 ± 4.0 ^c	0.20 ± 0.05 ^c	14.2 ± 0.2 ^c	14.5 ± 0.2 ^c	1.59 (0.71) ^b	0.24 (0.11) ^b
W2 (planted)										
-	20.8 ± 3.5 ^c	1.04 ± 1.13 ^c	15.2 ± 0.1 ^c	17.0 ± 1.5 ^c	14.2 ± 3.5 ^c	0.18 ± 0.05 ^c	13.9 ± 0.2 ^c	14.1 ± 0.2 ^c	1.65 (0.74) ^b	0.33 (0.15) ^b
W3 (unplanted)										
COD _{Add} / NO ₃ -N = 1	35.9 ± 2.1 ^d	0.57 ± 0.34 ^d	15.2 ± 0.3 ^d	16.3 ± 0.2 ^d	18.5 ± 3.4 ^d	0.22 ± 0.02 ^d	11.3 ± 0.2 ^d	11.8 ± 0.2 ^d	4.36 (1.96) ^b	0.95 (0.43) ^b
COD _{Add} / NO ₃ -N = 2	51.1 ± 2.1 ^d	1.98 ± 2.24 ^d	15.2 ± 0.2 ^d	18.2 ± 2.8 ^d	20.2 ± 2.9 ^d	0.25 ± 0.04 ^d	8.3 ± 0.2 ^d	9.1 ± 0.6 ^d	7.73 (3.48) ^b	1.71 (0.77) ^b
COD _{Add} / NO ₃ -N = 3	64.6 ± 2.5 ^d	1.29 ± 0.71 ^d	15.2 ± 0.1 ^d	17.1 ± 0.6 ^d	22.3 ± 5.6 ^d	0.26 ± 0.03 ^d	5.5 ± 0.3 ^d	6.5 ± 0.3 ^d	10.57 (4.76) ^b	2.42 (1.09) ^b
COD _{Add} / NO ₃ -N = 4	79.0 ± 2.1 ^d	0.88 ± 0.37 ^d	15.1 ± 0.2 ^d	16.5 ± 0.4 ^d	24.5 ± 3.8 ^d	0.27 ± 0.04 ^d	0.2 ± 0.1 ^d	0.8 ± 0.1 ^d	13.64 (6.14) ^b	3.72 (1.76) ^b
W4 (planted)										
COD _{Add} / NO ₃ -N = 1	35.5 ± 2.2 ^d	0.60 ± 0.34 ^d	15.1 ± 0.3 ^d	16.3 ± 0.2 ^d	17.5 ± 4.0 ^d	0.18 ± 0.03 ^d	10.5 ± 0.2 ^d	10.8 ± 0.2 ^d	4.50 (2.02) ^b	1.17 (0.53) ^b
COD _{Add} / NO ₃ -N = 2	50.8 ± 1.6 ^d	1.90 ± 2.01 ^d	15.2 ± 0.2 ^d	18.3 ± 2.9 ^d	19.0 ± 3.0 ^d	0.20 ± 0.04 ^d	7.2 ± 0.3 ^d	7.7 ± 0.7 ^d	7.96 (3.58) ^b	1.98 (0.89) ^b
COD _{Add} / NO ₃ -N = 3	65.1 ± 1.8 ^d	1.26 ± 0.63 ^d	15.2 ± 0.2 ^d	17.0 ± 0.6 ^d	21.3 ± 5.5 ^d	0.21 ± 0.02 ^d	4.4 ± 0.2 ^d	4.7 ± 0.2 ^d	10.94 (4.92) ^b	2.71 (1.22) ^b
COD _{Add} / NO ₃ -N = 4	79.9 ± 3.7 ^d	0.90 ± 0.38 ^d	15.1 ± 0.2 ^d	16.4 ± 0.4 ^d	22.8 ± 3.2 ^d	0.25 ± 0.03 ^d	0.2 ± 0.1 ^d	0.5 ± 0.1 ^d	14.23 (6.40) ^b	3.74 (1.68) ^b

^a The removal rate was calculated as follows: $R_{i,V} = V^{-1} \cdot (Q_{in} \cdot C_{i,in} - Q_e \cdot C_{i,e})$, where $R_{i,V}$ —volumetric removal rate (g m⁻³ d⁻¹) of parameter i [i = COD and NO₃-N]; Q_{in} , Q_e —influent and effluent flux (m³ d⁻¹); $C_{i,in}$, $C_{i,e}$ —influent and effluent concentration (mg L⁻¹) of parameter i; V —the working volumn of the wetland microcosms (m³), $V = 0.216$ m³.

^b Areal removal rate, calculated as follows: $R_{i,A} = A^{-1} \cdot (Q_{in} \cdot C_{i,in} - Q_e \cdot C_{i,e})$, where $R_{i,A}$ —area removal rate (g m⁻² d⁻¹) of parameter i [i = COD and NO₃-N]; Q_{in} , Q_e —influent and effluent flux (m³ d⁻¹); $C_{i,in}$, $C_{i,e}$ —influent and effluent concentration (mg L⁻¹) of parameter i; A —the working area of the wetland microcosms (m²), $A = 0.48$ m².

^c Values are given as mean ± SD (n = 24).

^d Values are given as mean ± SD (n = 6).

Graphical Abstract