Effects of Plants and Plant Fermentation Broth on the Removal and Characteristics of Dissolved Organic Matter in Self-Supplying Carbon Source Constructed Wetlands Treating Secondary Effluent

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Abstract. In this study, four constructed wetlands (CWs) were developed. The effects of plants and plant fermentation broth on the removal and characteristics of dissolved organic matter (DOM) were evaluated. As the ratio of added COD to influent nitrate (COD Add/NO3-N ratio) increased from 0 to 4, the DOC removal rate increased from 0.48 to 5.41 g m⁻³ d⁻¹ in the unplanted microcosms, and from 0.75 to 5.73 g m⁻³ d⁻¹ in the planted ones. The presence of plants could increase the DOC removal rate from 0.48–5.41 g m⁻³ d⁻¹ to 0.75–5.73 g m⁻³ d⁻¹. This indicated that both the addition of plant fermentation broth and planting could greatly improve the DOC removal during the CW treatment. Moreover, adding plant fermentation broth introduced highly aromatic compounds into the microcosms, and thus increased the UV₂₅₄ and SUVA values of the effluent. The plants could lower the effluent UV₂₅₄ value through improving the removal of aromatic compounds, but it increased the SUVA value by increasing the proportion of aromatic compounds in total DOMs.

1. Introduction
Dissolved organic matters (DOMs) are frequently found in the effluent of wastewater treatment plants (WWTPs), which pose potential threats to human health and ecological environment. Chlorine disinfection is a worldwide technology for sewage disinfection [1]. However, in the process of chlorine disinfection, DOMs in the water are prone to react with chlorine-containing disinfectants to generate various disinfection by-products (DBPs) with the characteristics of toxicity, carcinogenicity and mutagenicity [2-4], which can cause potential health risks when discharged into surface water [5]. The characteristics of DOM, i.e., composition and concentration, have significant effects on the formation of DBPs [6-7].

As an ecological wastewater treatment technology, CWs are widely used to treat the secondary effluent of WWTPs [8]. The “self-supplying carbon source CW” was demonstrated to greatly improve nitrate removal in our previous study [9]. In this type of CW, the plants are harvested and fermented, and the plant fermentation broth is finally added as carbon sources to the CW, to enhance denitrification. However, the plant fermentation broth commonly contains aromatic substances which are known as important DBPs precursors [10-11]. Its addition quantity, indicated as COD Add/NO3-N
ratio, is likely to have significant effects on the DOM characteristics in CWs, and consequently affect the formation potential of DBPs in the subsequent chlorine disinfection process. Moreover, as an indispensable part of CWs, plants can also affect the DOM characteristics through the release and/or uptake of organic carbon [12], and therefore, affect the subsequent DBPs formation process. However, to date, the aforementioned effects of addition quantity of plant fermentation broth (i.e., COD$_{Add}$/NO$_3$-N ratio) and plants on the DOM characteristics have not been sufficiently investigated, which limits the practical application of the “self-supplying carbon source CW”, and thus, relevant studies are needed.

On the basis of this, the objectives of this study were to investigate the effects of COD$_{Add}$/NO$_3$-N ratio and plants on the removal and characteristics of DOM in “self-supplying carbon source CW”, in order to provide technical guidance for the application of this type of CW.

2. Materials and methods

2.1. Source of plant litter and culture media
Cattail (Typha latifolia) litter used in this study was purchased from an aquatic plant cultivation center in Xiaoshan District, Hangzhou City, Zhejiang Province, China, and was harvested at the end of 2017. After collection, the cattail litter was cleaned, cut into lengths between 1.0 and 1.5 cm, milled into powders with an average diameter of 0.15 mm, dried at 40 °C to a constant mass, and finally preserved in a moisture free container at room temperature (20 °C). The inoculated sludge was taken from the recirculating sludge pump house of a municipal WWTP (anaerobic-aerobic process) in Shanghai, China.

2.2. Characterization of fermentation experiments
There were 5 sets of fermentation tank with an effective volume of 10 L, each of which is equipped with a stirring paddle and an automatic temperature control. The fermentation mixtures in each tank contained 120 g (dry weight) of cattail litter and 7g (dry weight of volatile suspend solids) of inoculated sludge with distilled water added to a final volume of 10 L, along with equal trace elements (Fe$^{2+}$, Mo$^{4+}$, etc.), mineral salts (Ca$^{2+}$, K$^+$, Mg$^{2+}$, etc.), and vitamins based upon a low ionic strength (0.03 M) modification of standard methanogenic culture media [13]. The fermentation tanks were then incubated at 35 ± 0.5 °C while shaking at 150 rpm (rotations per minute). The pH of the fermentation broth was adjusted to 7.0 ± 0.1 (once every 12 h) with 2 M HCl and 2 M NaOH. Incubations lasted for 20 days, then the suspensions were centrifuged at 11000 rpm for 10 min, after which the supernatant was collected in 2L serum bottles and stored at 4 °C.

2.3. Experimental design and operation
Four continuous-flow subsurface CW microcosms, each with a volume of 0.24 m$^3$ (length: 1.2 m, width: 0.4 m, height: 0.5 m) and a pore volume of 40 L, were located in a controlled greenhouse environment, on Tongji University campus, Shanghai, China. They are designated as follows: unplanted control (W1), planted microcosms (W2), carbon-added microcosms (W3), and planted plus carbon-added microcosms (W4). All the four microcosms were established in a temperature-controlled greenhouse (25 ± 2 °C), filled with gravel (Φ8‒13 mm, porosity = 0.4) to create a bed depth of 0.50 m, and two of them (i.e., W2 and W4) were planted with cattail (Typha latifolia, 20 plants m$^{-2}$).

Prior to the start of the experiment, the four microcosms were fed, in batches, with a modified secondary effluent for 3 months in order to establish the plant shoots and microorganisms. The secondary effluent was collected from a WWTP and was spiked with KNO$_3$ (100 g L$^{-1}$) to obtain a final concentration of 15 ± 0.5 mg N L$^{-1}$. The synthetic wastewater was introduced continuously into the microcosms from two 100 L plastic feeding tanks using peristaltic pumps at flow rates set to achieve a 4-day hydraulic retention time. Plant fermentation broth was added into W3 and W4 to achieve target COD$_{Add}$/NO$_3$-N ratios (i.e., 1, 2, 3, and 4). The operation duration of each microcosm
was 120 d, and was divided into four 30-day stages. In each stage of W3 and W4, a successively higher $\text{COD}_{\text{Add}}/\text{NO}_3$-N ratio (i.e., 1, 2, 3, and 4) was set.

2.4. Sampling and analysis

Before the cattail litters were fermented, they were cleaned, oven dried at 40 °C to a constant mass, and milled to pass a 60-mesh screen. The hemicellulose, cellulose and lignin contents were determined with resulting powders. After fermentation, the suspensions were centrifuged at 11000 rpm for 10 min and then tested for reducing sugar, protein as well as volatile fatty acids (VFAs). The analyses of the parameters mentioned above were the same as described in the previous publication [14].

Water samples were collected from each microcosm at the inlet and outlet every two days after the effluent quality of each batch is stable. Samples were filtered using 0.45-μm membrane filters. The analyses of COD and dissolved organic carbon (DOC) were conducted in accordance with standard methods [15]. In addition, water samples were collected from each microcosm at five points (inlet, outlet, the length of 1/4, 1/2 and 3/4) on the 9th, 19th, and 29th days of each stage to determine the DOC profiles. Ultraviolet at 254 nm (UV$_{254}$, m$^{-1}$) measurements and specific UVA (SUVA, L m$^{-1}$ mg$^{-1}$) values calculation were the same as described in the previous publication [16].

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 available carbon source in the fermentation broth accounts for 92.7%. VFAs (86.2%) were the main available carbon source in plant fermentation broth, and acetate was the dominant (62.3%). Besides, the fermentation broth also contains sugar (2.2%), protein (4.3%), and non-biodegradable aromatic substances (7.3%). Previous studies have shown that VFAs are a kind of labile carbon source, and acetate is the optimal electron donor for denitrification [17]. In addition, sugar and protein can also be converted to readily biodegradable organics in the CWs [14]. Thus, the plant fermentation broth added into the CWs can significantly increase the quantity of labile carbon sources, and consequently improve the denitrification efficiency of CWs. However, the non-biodegradable aromatic substances in the fermentation broth will flow out with the effluent and become an important source of DBPs precursors.

3.2. DOC removal rate

DOMs could be input into W1 and W3 through the influent. Plant release was another DOM input pathway for W2 and W4 [12]. While for W3 and W4, the sources of DOMs also included the plant fermentation broth.

As shown in Table 1, the average DOC removal rate in W1 and W2 during the experiment was only 0.48 and 0.75 g m$^{-3}$ d$^{-1}$, respectively. The DOC removal rates in W3 were 1.67, 3.05, 4.16 and 5.41 g m$^{-3}$ d$^{-1}$ at the $\text{COD}_{\text{Add}}/\text{NO}_3$-N ratios of 1–4, respectively, which were 3.5–11.3 times those in W1. The DOC removal rates in W4 were 1.94, 3.30, 4.47 and 5.73 g m$^{-3}$ d$^{-1}$ at the $\text{COD}_{\text{Add}}/\text{NO}_3$-N ratios of 1–4, respectively, which were 2.6–7.6 times those in W2. These results showed that the increase in $\text{COD}_{\text{Add}}/\text{NO}_3$-N ratio could greatly improve the DOC removal rate ($p < 0.05$), which further confirmed the easy biodegradability of plant fermentation broth. Moreover, the effluent DOC concentrations of W1 and W3 had no significant difference ($p > 0.05$), so did those of W2 and W4. This meant that the externally added organic carbon was exhausted by the heterotrophic microorganisms in the CWs, and thus, the increase in $\text{COD}_{\text{Add}}/\text{NO}_3$-N ratio from 0 to 4 did not cause significant increase in the effluent DOC concentrations.

Previous studies have reported the functions of plants for removing DOMs in CWs, including plant uptake, providing a micro-aerobic environment via root oxygen release and providing root surface for microbial growth [18]. Simultaneously, plants can increase organic compounds in water by leaching.
root exudates [6, 12]. As shown in Table 1, the DOC removal rate in W2 was 0.75 g m\(^{-3}\) d\(^{-1}\), which was 1.6 times that in W1. Furthermore, the DOC removal rate in W4 was and 1.1–1.2 times those in W3 at the COD\(_{Add}\)/NO\(_3\)-N ratios of 1 to 4, respectively. These results indicated that the presence of plants significantly improved the removal of DOM (p < 0.05), which meant that the DOM removal effect of plants is stronger than the generation effect in the microcosms of this study.

Table 1. Characteristics of the inflow and outflow (mean ± SD), as well as removal rate of DOC in four microcosms.

| Wetland types | Inflow DOC (mg L\(^{-1}\)) | Inflow UV\(_{254}\) (m\(^{-1}\)) | Inflow SUVA (L m\(^{-1}\) mg\(^{-1}\)) | Outflow DOC (mg L\(^{-1}\)) | Outflow UV\(_{254}\) (m\(^{-1}\)) | Outflow SUVA (L m\(^{-1}\) mg\(^{-1}\)) | DOC removal rate (g m\(^{-3}\) d\(^{-1}\)) |
|---------------|-----------------------------|------------------------------|-------------------------------|-----------------------------|------------------------------|-------------------------------|-------------------------------|
| W1 (unplanted)| - 5.39 ± 0.32\(^a\)          | 11.68 ± 0.46\(^a\)          | 2.18 ± 0.15\(^a\)            | 3.47 ± 0.44\(^a\)           | 8.28 ± 0.34\(^a\)           | 3.24 ± 0.40\(^a\)            | 0.48                          |
| W2 (planted)  | - 5.31 ± 0.27\(^a\)          | 11.67 ± 0.48\(^a\)          | 2.20 ± 0.14\(^a\)            | 3.21 ± 0.45\(^a\)           | 8.26 ± 0.53\(^a\)           | 3.58 ± 0.81\(^a\)            | 0.75                          |
| W3 (unplanted)| COD\(_{Add}\)/NO\(_3\)-N = 1 | 10.21 ± 0.55\(^b\)          | 14.95 ± 0.47\(^b\)           | 1.47 ± 0.08\(^b\)           | 3.54 ± 0.72\(^b\)           | 10.03 ± 0.42\(^b\)           | 2.83 ± 0.56\(^b\)            | 1.67                          |
|               | COD\(_{Add}\)/NO\(_3\)-N = 2 | 15.74 ± 0.97\(^b\)          | 16.25 ± 0.64\(^b\)           | 1.02 ± 0.07\(^b\)           | 3.55 ± 0.45\(^b\)           | 10.75 ± 0.80\(^b\)           | 3.03 ± 0.30\(^b\)            | 3.05                          |
|               | COD\(_{Add}\)/NO\(_3\)-N = 3 | 20.23 ± 0.88\(^b\)          | 18.69 ± 1.18\(^b\)           | 0.92 ± 0.07\(^b\)           | 3.59 ± 0.25\(^b\)           | 12.85 ± 1.00\(^b\)           | 3.58 ± 0.25\(^b\)            | 4.16                          |
|               | COD\(_{Add}\)/NO\(_3\)-N = 4 | 25.24 ± 1.21\(^b\)          | 23.29 ± 0.96\(^b\)           | 0.93 ± 0.06\(^b\)           | 3.61 ± 0.28\(^b\)           | 14.69 ± 0.76\(^b\)           | 4.07 ± 0.44\(^b\)            | 5.41                          |
| W4 (planted)  | COD\(_{Add}\)/NO\(_3\)-N = 1 | 10.12 ± 0.87\(^b\)          | 15.07 ± 0.53\(^b\)           | 1.50 ± 0.11\(^b\)           | 2.37 ± 0.52\(^b\)           | 8.82 ± 0.52\(^b\)            | 3.72 ± 0.67\(^b\)            | 1.94                          |
|               | COD\(_{Add}\)/NO\(_3\)-N = 2 | 15.62 ± 0.41\(^b\)          | 17.43 ± 0.86\(^b\)           | 1.03 ± 0.06\(^b\)           | 2.40 ± 0.49\(^b\)           | 10.10 ± 0.30\(^b\)           | 4.21 ± 0.78\(^b\)            | 3.30                          |
|               | COD\(_{Add}\)/NO\(_3\)-N = 3 | 20.30 ± 0.65\(^b\)          | 19.28 ± 0.95\(^b\)           | 0.95 ± 0.06\(^b\)           | 2.41 ± 0.21\(^b\)           | 11.17 ± 0.85\(^b\)           | 4.65 ± 0.58\(^b\)            | 4.47                          |
|               | COD\(_{Add}\)/NO\(_3\)-N = 4 | 25.33 ± 1.28\(^b\)          | 23.56 ± 1.11\(^b\)           | 0.93 ± 0.07\(^b\)           | 2.40 ± 0.44\(^b\)           | 12.20 ± 0.79\(^b\)           | 5.08 ± 0.63\(^b\)            | 5.73                          |

\(^a\) Values are given as mean ± SD (n = 60).
\(^b\) Values are given as mean ± SD (n = 15).

3.3. DOC profiles

DOC profiles along the length of unplanted and planted microcosms at different COD\(_{Add}\)/NO\(_3\)-N ratios are presented in Figure 1. As shown in Figure 1, the DOC profiles presented similar variations under all COD\(_{Add}\)/NO\(_3\)-N ratios. The DOC concentration decreased rapidly in the first quarter-length of each microcosm, and stabilized in the remaining length of the wetlands. The fast degradation of organics might be attributed to the easy biodegradability of plant fermentation broth, which contained VFAs, sugar, and protein.
3.4. DOM characteristics

UV$_{254}$ and SUVA values in the influent and effluent of each microcosm are shown in Table 1. UV$_{254}$, indicating the quantity of aromatic DOMs, is usually used to characterize the quantity of DBPs precursors in water [19-20]. As a surrogate for aromatic carbon proportion in total DOMs, SUVA is significantly correlated with the formation potential of DBPs [21-22].

As shown in Table 1, as the influent COD$_{Add}$/NO$_3$-N ratios increased from 0 to 4, the removal rate of aromatic organics in the unplanted CW microcosms (i.e., W1 and W3) did not significantly change ($p > 0.05$), which was related to the fact that the aromatic substances in the plant fermentation broth are mostly non-biodegradable. In W2, 29.2% of the UV$_{254}$ in the influent can be removed, while in W4, the UV$_{254}$ removal efficiency was 41.5%–48.2%. The UV$_{254}$ removal efficiencies in these two planted CWs were significantly higher than those in the unplanted CW microcosms. This indicated that plants can played an important role in removing the aromatic substances contained in the plant fermentation broth.

Different from UV$_{254}$, the SUVA values in the effluent of all the CW microcosms became higher compared to those in the influent, which meant that the DBPs formation potential in the water became greater after the treatment by CWs. This was because the labile organic matters were depleted in the CWs, increasing the proportion of aromatic compounds in total DOMs. As shown in Table 1, as the COD$_{Add}$/NO$_3$-N ratio increased from 1 to 4, the effluent SUVA values increased from 2.34 to 4.07 L m$^{-1}$ mg$^{-1}$ and from 3.58 to 5.08 L m$^{-1}$ mg$^{-1}$ in unplanted and planted microcosms, respectively. This was because the increase in the dosage of plant fermentation broth led to an increase in the input of aromatic carbon, which was mostly nonbiodegradable, and finally discharged with the effluent. Furthermore, in spite of the little difference between the influent SUVA values of the planted and unplanted microcosms, the effluent SUVA values of the former were significantly higher than those of the latter ($p < 0.05$). The effluent SUVA value of W2 was 1.4 times that of W1, while the values of W4 were 1.3–1.4 times those of W3. This should be due to that the plants improved the removal of labile organic compounds, and consequently increased the proportion of aromatic compounds in total DOMs in the effluent.
As shown in Figure 2, the UV 254 profiles presented similar variations under all COD Add/NO3-N ratios. Specifically, the UV 254 values decreased rapidly in the first quarter-length of each microcosms, and 16.3%–24.0% and 15.9%–31.8% of the influent UV 254 value was decreased during this part, respectively in unplanted and planted microcosms. The rapid removal of combined effects of microbial degradation and matrix adsorption should be the main reason [23]. In the remaining part of the microcosms, due to the depletion of labile organic compounds, the microbial degradation effect was weakened, and the UV 254 values became gradually stabilized. The SUVA profiles also presented similar variations under all COD Add/NO3-N ratios. The SUVA values increased rapidly in the first quarter-length of each microcosms, and in the remaining length of the unplanted microcosms, the SUVA values became relatively stabilized. In the planted microcosms, the plants released organic matters with high SUVA values [23]. Therefore, the SUVA values maintained continuously increase trend in the last three quarters.

4. Conclusion
As the ratio of COD Add/NO3-N ratio increased from 0 to 4, the DOC removal rate increased from 0.48 to 5.41 g m⁻³ d⁻¹ in the unplanted microcosms, and from 0.75 to 5.73 g m⁻³ d⁻¹ in the planted ones. Additionally, the presence of plants improved the DOC removal rate from 0.48–5.41 g m⁻³ d⁻¹ to 0.75–5.73 g m⁻³ d⁻¹. This indicated that both of adding plant fermentation broth and planting could improve the DOC removal.

The addition of plant fermentation broth introduced aromatic substances into the systems, which increased the UV 254 and SUVA values in the effluent. Planting lowered the effluent UV 254 value by enhancing the aromatic substances removal, but it increased the proportion of aromatic compounds in total DOMs, and thus increased the SUVA value in the effluent.

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