



Characterization of dissolved organic matter and carbon release from wetland plants for enhanced nitrogen removal in constructed wetlands for low C–N wastewater treatment



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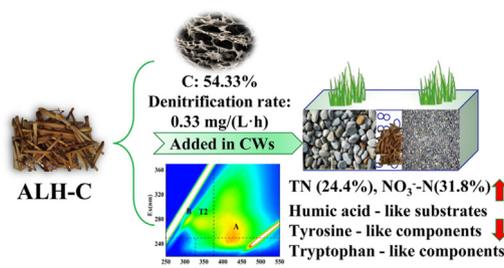
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HIGHLIGHTS

- Plants heated in NaOH solution showed better denitrification promotion ability.
- Alkaline-heated cattails improved the nitrogen removal in CWs significantly.
- DOM released by cattails was readily utilized by denitrification bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of pretreatment methods on the structure of functional groups and denitrification promotion capacity of solid carbon sources derived from reeds and cattails were elucidated. Alkaline treatment improved the relative content of carbon in the plant tissues, as well as prolonged the high denitrification rate of 0.40 mg/(L·h) from 6 days up to circa 28 days. Moreover, alkaline-heated cattails (ALH-C) showed high denitrification promotion capacity, and increased the removal rate of TN, NO₃-N and NH₄-N in the CW by 24.41%, 31.80% and 8.80%, respectively. Furthermore, the quantity, quality and migration of dissolved organic matter (DOM) released from ALH-C in CW analyzed via fluorescence excitation-emission matrix spectrophotometry showed mainly humic acid-like, tyrosine-like, and tryptophan-like components. These DOM components were highly bioavailable and had minimal effects on COD removal. These results provide insights into the preparation and environmental applications of plant carbon sources.

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1. Introduction

Constructed wetlands (CWs) not only have the advantages of sustainability, easy operation and low cost, but also can be widely used in the treatment of various wastewater (Wu et al., 2016). Thus,

CWs have attracted lots of attention in recent decades. The removal of nitrogen in CWs is mainly accomplished by microbial nitrification and denitrification (Peng et al., 2014). These processes are affected primarily by factors such as temperature, pH, substrate type, electron donor, dissolved oxygen (DO) and carbon source (Gao et al., 2017). The carbon source serves as a carrier for electron acceptors and denitrifying microorganisms, provides nutrients and electrons for denitrifying bacteria, and facilitates the conversion of nitrate into N_2 (Fu et al., 2017). Thus, the carbon source is indispensable in the process of denitrification in CWs.

However, the carbon/nitrogen (C/N) ratio of most urban wastewater is gradually decreasing in recent years (Zhou et al., 2017). This decrease has led to the lack of electron donors for the denitrification process, limiting the nitrogen removal in CWs. Therefore, extensive research has been conducted to improve the nitrogen removal efficiency of low-C/N wastewater treatment by adding external carbon sources to CWs. Methanol, ethanol, glucose, corn starch/polycaprolactone blends and alkaline-treated microalgal flour have all been used as additional carbon sources to improve nitrogen removal in CWs (Shen et al., 2015; Zhong et al., 2019). Although the addition of these liquid and powdered carbon sources improved the denitrification rates of CWs, the operating costs also increased significantly. Moreover, the rapid diffusion and release of carbon from these sources also cause sharp increases in the effluent concentration. Harvested wetland plants biomass, which is usually reused as feedstock candidates for organic fertilizers, biofuel production (Kaur et al., 2018), and soil conditioners (Maietta et al., 2020), could be also used as external carbon sources for wastewater treatment. More particularly, plant-based carbon sources have the advantages of slow release of organic matter, long service life and cost-effective (Jia et al., 2018; Yang et al., 2017). Therefore, harvested plants from CWs have good potential for use as an external carbon source after proper pretreatment. However, the mechanism for the use of common plant species in CWs as external carbon sources is still poorly studied.

Lignocellulose (cellulose and hemicellulose) in the plant tissues is easily decomposed and used by microorganisms. However, its tight bonding to lignin through covalent and hydrogen bonds limits the biodegradability of plant tissues, which reduces the utilization of plants as carbon sources. This phenomenon is more severe in low-temperature seasons (Hang et al., 2016). For example, Si et al. (2018) reported that the addition of newspaper and wheat straw as carbon sources to CWs could significantly improve the removal of NO_3^- -N and TN at 24.55 ± 2.35 °C. However, this effect was decreased at low temperatures (12.5 ± 4.0 °C). Therefore, pretreatment of plant materials with suitable methods to destroy the lignin structure, promote the release of cellulose and hemicellulose, and improve its utilization rate, especially in low-temperature seasons, have gradually become the focus of research in the application of plants as carbon sources. According to Li et al. (2018), corn stalks treated with alkaline had a better denitrification promotion effect on low C/N wastewater than the untreated one. However, current studies are mainly focused on the improvement of the nitrogen removal of CWs by different types of plant carbon sources with/without pretreatment. The organic matter release process and functional mechanisms of plant carbon sources in CWs are still poorly understood. In addition, changes in the structure, morphology and functional group composition of plant tissue affect its effectiveness as an additional carbon source in CWs directly. Therefore, the functional characteristics of plant materials as carbon source could be effectively evaluated via the analysis of the chemical composition, structural properties, organic matter release process and denitrification promotion ability.

Nevertheless, the plant materials added to the CWs as carbon sources also release some recalcitrant organic matter, colored

substances and nutrients (Tang et al., 2016). Therefore, it is imperative to identify the effective active ingredients of the released organic matter from the plant carbon source. Furthermore, the functional pathways of the active ingredients released by plant carbon source in CWs remain unclear, albeit there are few related studies. Consequently, it is particularly important to analyze the internal fluorescence characteristics of CWs with plant carbon sources and identify the distribution and migration of humic acids, amino acids, lignin and other new products (Weishaar et al., 2003). This article aims to address the research gaps as mentioned above with the specific objectives to: (1) analyze the changes in surface morphology and structure of the functional group of wetland plants under different pretreatment methods; (2) assess the carbon release and denitrification promotion abilities of the plants under different pretreatment methods; (3) evaluate the changes in the composition and properties of DOM released by plant carbon sources in CWs during low-temperature seasons.

2. Materials and methods

2.1. Preparation of the solid carbon source

The aboveground parts of *Phragmites* spp. (reeds) and *Typha* spp. (cattails) from a CW in Xi'an, China were harvested and washed with deionized water. The washed plants were cut into 2–3 cm pieces, oven dried at 45 °C to a constant weight, and mixed uniformly. Four subsamples of reeds with equal weights were taken, one of which was the control group without any treatment, and labelled as untreated reeds (UN-R). Another one was soaked in 2% H_2SO_4 solution at 25 °C for 24 h, and labelled as acid-soaked reeds (ACS-R). A third subsample was soaked in 2% NaOH solution at 25 °C for 24 h, and labelled as alkali-soaked reeds (ALS-R). The fourth subsample was soaked in 2% NaOH solution at 90 °C for 1 h, and labelled as alkaline-heated reeds (ALH-R). The cattails were treated using the same method as the reeds, and labelled as untreated cattails (UN-C), acid-soaked cattails (ACS-C), alkali-soaked cattails (ALS-C), and alkali-heated cattails (ALH-C), respectively. All treated plant carbon source samples were washed to neutrality with deionized water, dried at 45 °C to a constant weight, and stored in a dryer at room temperature.

2.2. Static release ability

2 g of each prelabelled subsample was weighed into 250 mL Erlenmeyer flasks in triplicate, to each of which 250 mL of deionized water was added. All the Erlenmeyer flasks were then placed into an incubator at 100 r/min and 30 °C. During the thirty days' experiment, the concentrations of total nitrogen (TN), nitrate (NO_3^- -N), ammonium (NH_4^+ -N), nitrite (NO_2^- -N), total phosphorus (TP) and chemical oxygen demand (COD) in the original soaking solution were analyzed every 48 h, and then the deionized water was replaced. TN, NO_3^- -N, NH_4^+ -N, NO_2^- -N, TP and COD were analyzed with an ultraviolet spectrophotometer (Principles TU-1901), according to the standard method of water quality monitoring (Zhang et al., 2018).

According to the pollutant concentration in the soaking solution, the total release and average release amounts of different pollutants in each group within 30 days were calculated as follows:

$$TR = AC30 \times V/m \quad (1)$$

$$AR = AC30 \times V/(m \times 30) \quad (2)$$

where TR represents the total amount of release (mg/g), AR represents the daily average amount of release (mg/g), $AC30$ represents

the cumulative release concentration of each pollutant in 30 days (mg/L), V is the total volume of the solution, take 0.25 L, m is the mass of the plants added in the solution, take 2 g.

2.3. Denitrification rate

2 g of each prelabelled subsamples was weighed into 250 mL Erlenmeyer flasks, to each of which 200 mL of artificial wastewater and 50 mL of washed activated sludge (taken from the anoxic tank of a wastewater treatment plant in Xi'an) were added. The concentration of nitrate-nitrogen in the artificial wastewater was 20 mg/L and N:P was 5:1. High-pressure nitrogen was used to ensure anoxic conditions in all Erlenmeyer flasks. Each group was setup in triplicate. The Erlenmeyer flasks were sealed and incubated at 100 r/min and 30 °C, and the concentrations of NO_3^- -N, NO_2^- -N and COD in the water were determined every 24 h. The denitrification rate of each group was calculated as follows:

$$V_{DN} = [\Delta p (\text{NO}_3^- - \text{N}) + 0.6 \times \Delta p (\text{NO}_2^- - \text{N})] / \Delta t \quad (3)$$

where V_{DN} represents the denitrification rate, $\Delta p (\text{NO}_3^- - \text{N})$ represents the change of nitrate concentration during the culture period; $\Delta p (\text{NO}_2^- - \text{N})$ represents the change of nitrite concentration during the culture period; Δt represents the culture period, which is 24 h.

2.4. Carbon source addition experiment

2.4.1. Constructed wetlands setup and operation

Two CWs (CW1 and CW2) with the same configuration and size were constructed using PVC. Both CWs were with a length of 1.5 m, width of 0.5 m and height of 0.7 m (Fig. 1). The substrate was gravel, the size of the gravel in the first half of the CW was 5–30 mm, and that in the last half was 5–15 mm. Common reeds were planted at a density of 13 plants/m². Artificial wastewater was used and the average concentrations of TN, NO_3^- -N, NH_4^+ -N, NO_2^- -N, TP and COD in the influent were 29.8 ± 0.5 mg/L, 20.3 ± 0.5 mg/L, and 8.9 ± 0.03 mg/L, 0.01 ± 0.01 mg/L, 1.9 ± 0.1 mg/L, 66.5 ± 0.1 mg/L, respectively. The average hydraulic loading of the two CWs were 58 mm/d, which correspond to an average hydraulic retention time of about 4 days. After an acclimation period of three months, continuous operation of the CWs began at the designed values. The experimental period lasted from October 2018 to January 2019, and was divided into two phases. The first phase was from October to December 2018, where 900 g ALH-C was added into the middle of CW1 (C/N was 5:1), while CW2 was without carbon source added. The second phase was from December 2018 to January 2019, where

the carbon source in CW1 was replaced with another 900 g ALH-C, and CW2 still had no carbon source added.

2.4.2. Samples collection and analysis

During the experiment period, water samples were collected from the influent, 1/4, 3/4 and effluent of the two CWs every week. TN, NO_3^- -N, NH_4^+ -N, COD, TP, NO_2^- -N concentrations were analyzed. After the water samples were filtered through a 0.45 μm polyethersulfone filter, the fluorescence characteristics of the DOM were analyzed by a fluorescence spectrophotometer (Hitachi F7000). The mass percentages of C, N, O, P, Ca, and Zr in the unused and used plants added in CW1 were measured using an SEM coupled with EDX spectroscopy (JSM-IT 500 LA). 100 g of the plant carbon sources (UN-R, ACS-R, ALS-R, ALH-R, UN-C, ACS-C, ALS-C, and ALH-C) was weighed, pulverized and passed through a 50-mesh sieve, respectively. Field emission scanning electron microscope (Quanta FEG 250) was used to observe the cross-sectional shape of different plant samples. Fourier transform infrared imaging spectrometer (Nicolet Nexus 410) was used to analyze the functional groups of the different plant samples.

2.5. Data analysis

Analysis of the experimental data was performed using SPSS 20.0 software (SPSS Inc., Chicago, USA). The differences in the removal efficiencies and denitrification abilities were evaluated by One-way analysis of variance (ANOVA). In all statistical tests, the significance level of P was taken as 0.05.

3. Results and discussion

3.1. Morphology and composition of plant-based carbon source

SEM images of the reeds and cattails under different pretreatment methods are shown in Fig. 2. The UN-R and UN-C showed uniformly arranged bundle structures under scanning electron microscopy, but the structures of reeds were denser and more uniform than that of cattails (Fig. 2a, e). Pretreatment with an acid or alkali solution damaged the network structures of cellulose-lignin-hemicellulose in the reeds and cattails to expose the internal structure (Kirupa Sankar et al., 2018). Consequently, both plants showed many irregular, rough and loosened fibrous networks in the pith, microporous and surface cracks (Fig. 2b, c, f, g). Acid treatment reduced the molecular weight of lignin by breaking the aryl ether bonds and facilitating condensation reactions. Moreover, acid treatment could also improve the enzymatic hydrolysis efficiency of cellulase through hydrolysis of the polysaccharides in cellulose and adsorption of more cellulase on the surface of cellulose (Silverstein et al., 2007). In contrast, alkali treatment could cause the swelling of cellulose and reduce the crystallinity of cellulose, leading to the separation of the structural chains between lignin and carbohydrates. Furthermore, alkali treatment could also remove components that hinder the accessibility of cellulose, such as acetyl groups, lignin, and furfural acid substitutes. Therefore, alkali treatment could destroy the structure of lignin and facilitate the hydrolysis of cellulase. Also, it could promote the formation of more irregular polygonal fiber cross sections and increase the specific surface area and porosity of the plants. All these effects are beneficial to the release and utilization of organic matter in the use of plants as carbon source (Fig. 2d, h). In addition, the structure of cattails was much more easily destroyed by both acid and alkali than reeds, which was conducive for the release of cellulose and hemicellulose. The relative content of carbon in cattails was higher than that of reeds (51.89%, 46.11%). All the pretreatment methods increased the relative content of carbon in the plant tissues of

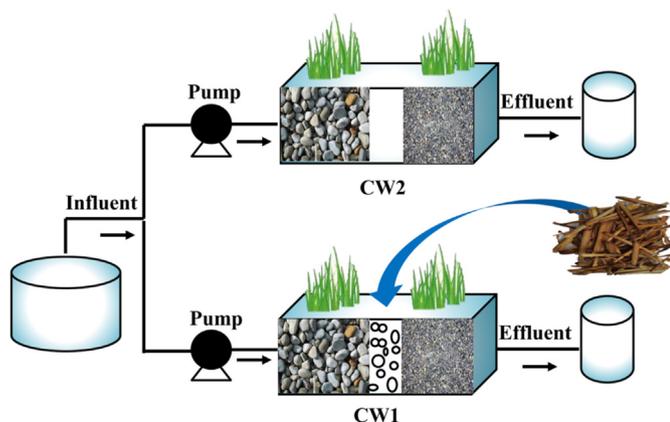


Fig. 1. The schematic diagram of the CWs.

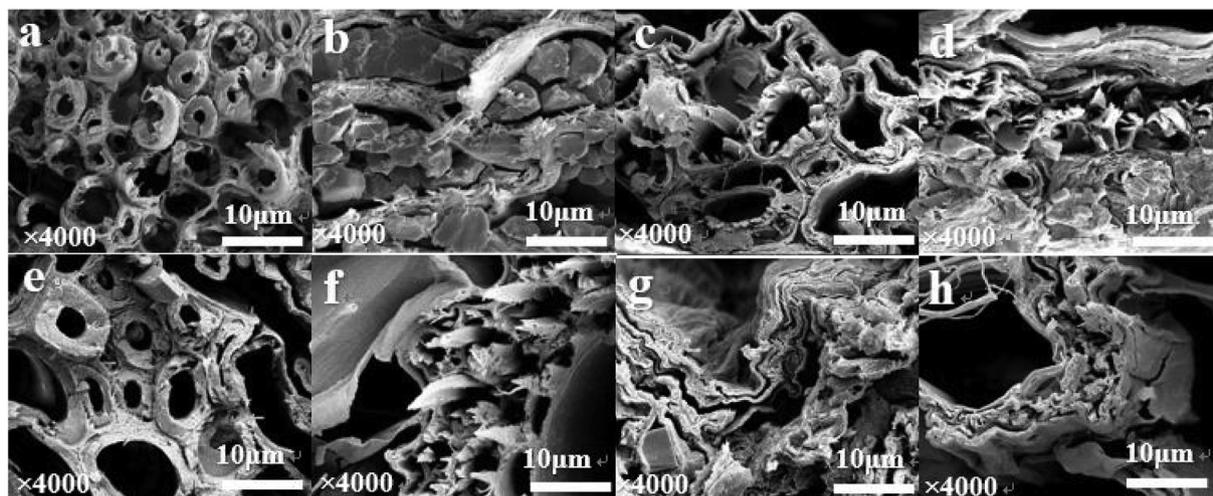


Fig. 2. SEM images of reeds and cattails under different pretreatment methods, (a) UN-R, (b) ACS-R, (c) ALS-R, (d) ALH-R, (e) UN-C, (f) ACS-C, (g) ALS-C, (h) ALH-C.

cattails and reeds. However, the relative carbon content in the plants treated with alkali was the highest, favoring its use as a carbon source.

The characteristic absorption frequencies of specific functional groups and the corresponding organic matter in the plant samples were analyzed via Fourier transform infrared spectroscopy. Fig. 3 shows that the absorption peaks of both cattails and reeds occurred at the same wavenumber. However, the absorbance of each functional group of cattails was stronger than that of reeds, and much more easily affected by the pretreatment methods than reeds. These differences were mainly because the content of hemicellulose, cellulose and lignin in these two plants species were different. Moreover, the wavenumbers at 3341.76 cm^{-1} and 3407.56 cm^{-1} indicated stretching vibrations of N–H. N–H is related to the presence of proteins, which have high biological activity (Singh et al., 2010). The absorbance of N–H of cattails was higher than that of reeds, and the N–H absorbance of reeds was less affected by pretreatment (absorbance value 0.43). Conversely, the N–H absorbance in cattails was significantly reduced after pretreatment (untreated 0.76 and alkali heat 0.57) (Fig. 3a and b). C–H stretching vibrations of saturated hydrocarbons occurred at the wavenumbers of 2919.75 cm^{-1} and 2924.50 cm^{-1} . These absorption bands correspond to organic compounds such as hydrocarbons and lipids (Kania et al., 2018). The absorbance of cattails at C–H was also higher and easily affected by pretreatment than that of reeds, which decreased from 0.29 to 0.19 after acid or alkali treatment (Fig. 3a and b). The wavenumbers at 1739.51 cm^{-1} and 1743.30 cm^{-1} were the absorption bands of the esters C=O, such as

carboxylic acids and aromatic esters. The wavenumbers at 1426.13 cm^{-1} and 1430.00 cm^{-1} were the bending vibrations of alkanes C–H, which was mainly the deformation of C–H in lignin (Fig. 3a and b). The absorbance of C=O and C–H of cattails were both higher than that of reeds, and the UN-C (0.27 and 0.28) had the highest absorbance followed by ALH-C (0.21, 0.24). The absorbance of reeds was less affected by pretreatment (0.16, 0.16). The wavenumbers at 1234.24 cm^{-1} and 1245.81 cm^{-1} were C–O telescopic (Ar–O telescopic), and those at 1050.07 cm^{-1} and 1038.50 cm^{-1} were C–O telescopic vibration (R–O telescopic). Both of them were half stretching vibrations of cellulose (Bekiaris et al., 2015). Although the absorbance of C–O of cattails was higher than that of reeds, the absorbances of both plants were both affected by pretreatment. For reeds, the effects follow the order of ACS-R (0.23) > UN-R (0.2) > ALS-R (0.16) > ALH-R (0.12). For cattails, the order was UN-C (0.33) > ACS-C (0.25) > ALH-C (0.17) > ALS-C (0.15). The out-of-plane bending of O–H was at wave the numbers of 662.44 cm^{-1} and 664.37 cm^{-1} . Nevertheless, the absorbance of cattails (0.15) was still higher than that of reeds (0.06), albeit the absorbances of both plants were less affected by pretreatment. Overall, the absorbances of cattails were higher than that of reeds, but it was affected by the pretreatment method significantly. This finding indicated that acid and alkali treatment could degrade complex compounds into simpler compounds such as alkanes, alcohols, and phenols (Kataki et al., 2017). Alkali treatment could destroy the ester and ether linkages between lignin and hemicellulose, the ester and carbon-carbon bonds between lignin molecules, as well as weaken the hydrogen bonding between

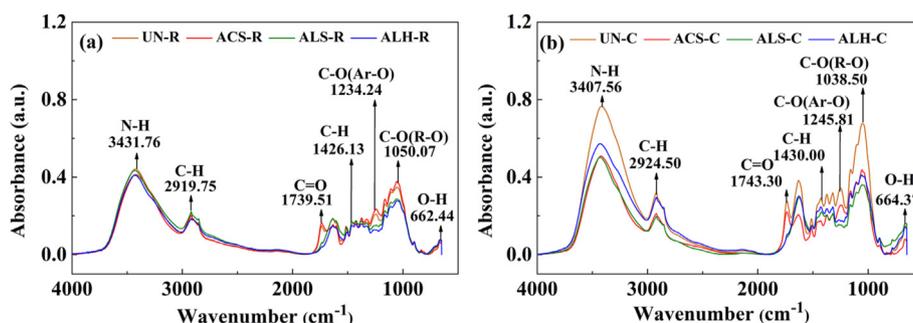


Fig. 3. Fourier transform infrared spectroscopy scans of reeds (a) and cattails (b) with different pretreatments.

hemicellulose and cellulose. Consequently, alkali treatment could remove lots of lignin and hemicellulose from the plant tissues, and increase the accessibility of enzymes and microorganisms to cellulose (Hu et al., 2017). Additionally, alkali treatment could also cut off the intermolecular connections between hemicellulose and other molecular components, and break down complex carbohydrates into glucose and xylose, which are easily used by microorganisms. Therefore, alkali treatment was more suitable as a pretreatment method for the preparation of the plant carbon source than acid treatment.

3.2. Carbon release and denitrification potential

The wetland plants treated with alkali showed more promise for use as a carbon source. Thus, the carbon release and denitrification promotion ability of both reeds and cattails under alkali treatment were further evaluated. The release of COD from untreated and alkali-treated reeds and cattails reached the maximum on the first day (about 250 mg/L). The release rapidly decreased to about 50 mg/L within four days and tended to stabilize. The reason for the higher release of COD at the beginning of the experiment was due to the release of soluble carbon from the plants (Liu et al., 2018). The amount of COD released from reeds and cattails with different pretreatment methods decreased in the following order: ALH-R > ALH-C > ALS-C > UN-C > ALS-R > UN-R. The order of release corresponded to daily averages of 2.33 mg/g, 2.12 mg/g, 2.00 mg/g, 1.92 mg/g, 1.90 mg/g and 1.85 mg/g, respectively (Table 1). The release of COD from ALH-R and ALH-C were significantly higher than that of others ($P < 0.05$). However, there was no significant difference between ALH-R and ALH-C ($P > 0.05$). This finding indicated that alkali-heat treatment could significantly improve the COD release capacity of plants. The release of COD from cattails was higher than that of reeds, but this difference was not significant. Nevertheless, the effect of pretreatment on the release of COD from reeds was more evident ($P < 0.05$).

The concentrations of TN released from both reeds and cattails also reached the highest on the first day (about 10 mg/L). Subsequently, the release decreased to about 1 mg/L on the sixth day and remained stable. The release of TN from cattails (3.37 mg/g) was higher than that of reeds (2.86 mg/g). Alkali treatment could reduce the release of TN from both plants to similar extents (Table 1, $P > 0.05$). Moreover, the concentrations of $\text{NH}_4^+\text{-N}$ released from both reeds and cattails under different pretreatment methods were about 3 mg/L on the first day. The concentrations decreased to about 1 mg/L on the fourth day and remained stable. The amount of $\text{NH}_4^+\text{-N}$ released from reeds (1.37 ± 0.20 mg/g) was slightly higher than that of cattails (1.12 ± 0.07 mg/g), but the effect of alkali treatment on the $\text{NH}_4^+\text{-N}$ release by plants was also not evident

(Table 1, $P > 0.05$). Under different pretreatment methods, the release of $\text{NO}_3^-\text{-N}$ from both reeds and cattails reached the highest concentrations on the first day (1 mg/L) and fluctuated in the range of 0.2–0.6 mg/L afterwards. The amount of $\text{NO}_3^-\text{-N}$ released from plants with different pretreatment methods decreased in the order of UN-C > UN-R > ALS-C > ALS-R > ALH-C > ALH-R. This order corresponded to average daily releases of 0.033 mg/g, 0.029 mg/g, 0.026 mg/g, 0.024 mg/g, 0.023 mg/g, 0.019 mg/g, respectively (Table 1). The amount of release from cattails was higher than that of reeds under the same pretreatment method. Additionally, alkali treatment, especially alkali-heat treatment, could significantly reduce the release of $\text{NO}_3^-\text{-N}$ from plants ($P < 0.05$). The total amount of $\text{NO}_2^-\text{-N}$ released from both reeds and cattails were minimal at about 0.01 mg/g (Table 1). Overall, the difference in nitrogen release from reeds and cattails was not significant. Thus, the plant species did not affect the nitrogen release significantly. However, alkali-heat treatment could inhibit the release of $\text{NO}_3^-\text{-N}$ from plants effectively. The concentrations of TP released from both reeds and cattails under different pretreatment methods were about 0.8 mg/L at first, which decreased to about 0.04 mg/L on the fourth day and stabilized. The TP release from cattails was higher than that of reeds ($P < 0.05$), but the effect of alkali treatment on TP release was not evident (Table 1).

A high denitrification rate was recorded for each group immediately after the plant carbon source was added (Fig. 4, about 0.37 mg/(L·h)). The high denitrification rate of UN-R and UN-C was

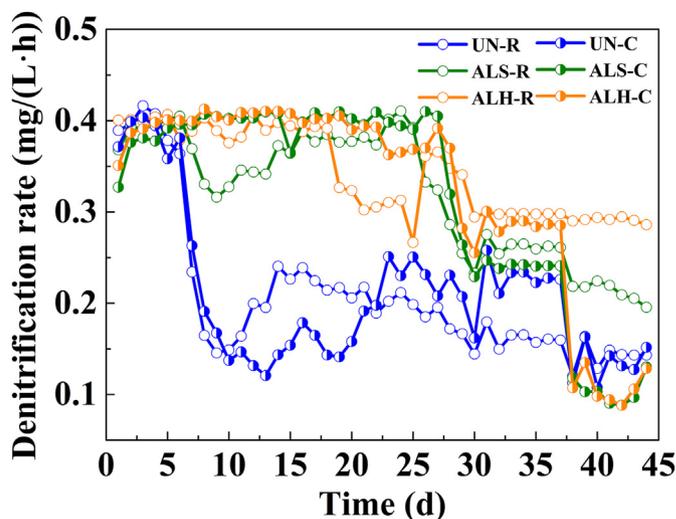


Fig. 4. Denitrification rates of reeds and cattails under different pretreatments.

Table 1
The releases of various pollutants of reeds and cattails under different pretreatments.

Parameter	Release	UN-R	ALS-R	ALH-R	UN-C	ALS-C	ALH-C
COD	Total release (mg/g)	55.59 ± 1.64	57.64 ± 0.64	69.78 ± 0.91	57.75 ± 1.38	59.98 ± 0.55	63.55 ± 1.03
	Average release (mg/g)	1.85 ± 0.007	1.90 ± 0.003	2.33 ± 0.009	1.92 ± 0.005	2.00 ± 0.002	2.12 ± 0.010
TN	Total release (mg/g)	3.23 ± 0.28	2.89 ± 0.18	2.47 ± 0.25	3.58 ± 0.14	3.29 ± 0.22	3.24 ± 0.19
	Average release (mg/g)	0.10 ± 0.001	0.09 ± 0.001	0.07 ± 0.001	0.11 ± 0.001	0.10 ± 0.001	0.10 ± 0.001
$\text{NH}_4^+\text{-N}$	Total release (mg/g)	1.37 ± 0.20	1.08 ± 0.05	0.91 ± 0.06	1.71 ± 0.03	1.28 ± 0.05	1.12 ± 0.07
	Average release (mg/g)	0.07 ± 0.0012	0.05 ± 0.0003	0.05 ± 0.0004	0.09 ± 0.0001	0.06 ± 0.0003	0.06 ± 0.0005
$\text{NO}_3^-\text{-N}$	Total release (mg/g)	0.55 ± 0.01	0.48 ± 0.01	0.38 ± 0.02	0.66 ± 0.01	0.51 ± 0.01	0.46 ± 0.02
	Average release (mg/g)	0.029 ± 0.0003	0.024 ± 0.0005	0.019 ± 0.0002	0.033 ± 0.0002	0.026 ± 0.0003	0.023 ± 0.0002
$\text{NO}_2^-\text{-N}$	Total release (mg/g)	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.0001	0.01 ± 0.006	0.01 ± 0.001	0.01 ± 0.002
	Average release (mg/g)	0.00079 ± 0.00002	0.00055 ± 0.00003	0.00053 ± 0.00001	0.00059 ± 0.00001	0.00068 ± 0.00002	0.00058 ± 0.00003
TP	Total release (mg/g)	0.23 ± 0.08	0.19 ± 0.06	0.21 ± 0.08	0.44 ± 0.06	0.49 ± 0.04	0.58 ± 0.04
	Average release (mg/g)	0.0072 ± 0.0003	0.0058 ± 0.0002	0.0064 ± 0.0003	0.0138 ± 0.0002	0.0154 ± 0.0001	0.0180 ± 0.0001

UN-R: untreated reeds, ALS-R: alkali-soaked reeds, ALH-R: alkaline-heated reeds, UN-C: untreated cattails, ALS-C: alkali-soaked cattails, ALH-C: alkali-heated.

maintained only for 6 days (0.39 mg/(L·h)). The denitrification rate then gradually decreased to 0.15 mg/(L·h), corresponding to an average of about 0.21 mg/(L·h). There was no significant difference between UN-R and UN-C, which recorded similar denitrification potentials of 0.07 mg/(L·h) and 0.08 mg/(L·h) ($P > 0.05$). However, both reeds and cattails treated with alkali were able to maintain high denitrification rates (0.40 mg/(L·h)) for 25 days and 28 days, respectively (Fig. 4). The denitrification rates of the alkali treated reeds and cattails were significantly higher than that of UN-R and UN-C during the whole period ($P < 0.05$). The average denitrification rate of ALS-R, ALH-R, ALS-C and ALH-C reached 0.28 mg/(L·h), 0.29 mg/(L·h), 0.32 mg/(L·h) and 0.33 mg/(L·h), respectively (Fig. 4). Alkali-treated cattails sustained longer periods of high denitrification rate than reeds. Thus, alkali-treated cattails had a better ability to maintain high denitrification rates (Fig. 4, $P < 0.05$) (Mengyu et al., 2018). Moreover, the average denitrification potentials of ALS-R, ALH-R, ALS-C and ALH-C was 0.12 mg/(L·h), 0.15 mg/(L·h), 0.14 mg/(L·h) and 0.30 mg/(L·h), respectively (Fig. 4). These values indicated that the alkali-treated cattails also had the best denitrification promotion ability ($P < 0.05$). In addition, the C/N for all groups that recorded high denitrification rates was higher than 5, and pH was about 7.5 ± 0.5 throughout the experiment. Therefore, ALH-C showed the best ability to promote denitrification and, thus, more suitable for use as an additional carbon source in CWs.

3.3. Effects of carbon source on the performance of CW

The average influent concentrations of TN, NO_3^- -N, NH_4^+ -N, NO_2^- -N, TP and COD of the two CWs were 29.8 ± 0.5 mg/L, 20.3 ± 0.5 mg/L, and 8.9 ± 0.03 mg/L, 0.01 ± 0.01 mg/L, 1.9 ± 0.1 mg/L, 66.5 ± 0.1 mg/L, respectively. In the acclimation period where no carbon source was added to the two CWs, the pollutant removal efficiencies were similar.

ALH-C was added to CW1 as a carbon source at the beginning of the first and second phases of the experiment, while no carbon source was added to CW2. Fluctuations in the TN and NO_3^- -N concentrations in the effluents of the two CWs were similar. However, the average concentrations of TN and NO_3^- -N in the CW1 (16.8 mg/L and 11.5 mg/L) were significantly lower than that of CW2 (24.1 mg/L and 17.9 mg/L) ($P < 0.05$, Fig. 5a and b). The carbon released from the ALH-C effectively improved the denitrification capacity of the wetland. In turn, the removal of TN and NO_3^- -N during low temperature seasons improved significantly. The average removal efficiencies of TN in CW1 and CW2 were 43.51% and 19.10%, and those of NO_3^- -N were 43.34% and 11.54%, respectively. Similar findings were reported by Si et al. (2018) and Zhang et al. (2019). Si et al. (2018) found the average TN removal efficiency of constructed wetlands with wheat straw and cotton to be 37.82% and 40.79% significantly higher than that of wetlands without a solid carbon source added ($14.07 \pm 3.46\%$) at around 12.5 ± 4.0 °C, respectively. The values recorded in this study were found to be slightly lower than those reported in the previous study, because the temperature (about 5 °C) was considerably lower. Zhang et al. (2019) also indicated that the rice straw application led to an average of 10.7% increase in the potential denitrification rate of constructed wetlands. In addition, although the removal of nitrogen was mainly completed within 1/4 of the distance along the length of the two CWs, different processes occurred along the wetlands (Figs. S1a and b). Whereas only nitrification occurred and there was basically no denitrification in the latter part of CW2, both denitrification and nitrification in CW1 were enhanced due to the presence of ALH-C. Carbon source was the limiting factor for denitrification in the two CWs, especially within the last 3/4 of its

length. Removal of NO_3^- -N in the CW was achieved via the following reaction: NO_3^- -N + Organic C \rightarrow N_2 (NO and N_2O) + CO_2 + H_2O . Plants carbon source not only provided sufficient electron donors for the denitrification process (Fu et al., 2017), but also improved the nitrogen removal efficiency of the CW by increasing the number and activity of denitrifying bacteria (Chen et al., 2019). The average NH_4^+ -N removal rates in the two CWs were 52.75% and 43.95%, respectively (Fig. 5c). This finding indicated that the presence of plant carbon sources in CW1 did not reduce the nitrification rate, but it played a critical role in intercepting and adsorbing NH_4^+ -N (Hu et al., 2016). Fig. 5d shows that the concentration of NO_2^- -N in the effluent of the two CWs both were slightly higher than that in the influent, which intermittently increased to slightly exceed 1 mg/L. Although the concentration of NO_2^- -N in CW1 was higher than CW2, the difference was not significant ($P > 0.05$).

The effluent concentration of COD in CW2 was stable at about 11.54 mg/L during the two phases, corresponding to a removal rate of 82.64% (Fig. 5e). The concentration of COD in CW1 was somewhat higher than CW2. The average concentrations during the first and second phases were 34.29 mg/L and 31.03 mg/L, respectively (Fig. 5e). This difference in COD may be because the release of organic matter seems to have activated rapidly after the addition of ALH-C into the CW1. However, the heterotrophic microorganisms in the wetland had not adapted to the carbon release conditions at the same time. Also, the low concentration of DO (about 0.08 mg/L) in the wetland was not conducive for organic matter degradation by aerobic microorganisms (Martínez et al., 2018). A sharp increase in the COD concentration in CW1 was recorded at the end of the first phase. This COD increase was due to gradual increase in the proportion of refractory biodegradable components in the released organic matter as the plant tissue of ALH-C decayed after 40 days of its addition. Energy dispersive spectrum analysis showed the organic matter in plants was used efficiently as the C mass proportion of the ALH-C decreased from $51.91 \pm 0.19\%$ to $23.17 \pm 0.16\%$ after its use in CW1 (Table S1). The Ca mass proportion also increased from $16.95 \pm 0.15\%$ to $29.71 \pm 0.25\%$ (Table S1). Biopolymers contain ionizable groups such as the carboxyl group (COO^-) and Ca^{2+} may precipitate these negative ions. These findings indicate the degradation of ALH-C and the high utilization efficiency of the carbon source by microbes. Moreover, stoichiometric relationships indicate that 1.00 mg/L of NO_3^- -N is used for consuming 3.705 mg/L of COD in complete denitrification (Zhan et al., 2020). Table S2 shows that only 38.00% of the COD removed in CW2 could be used in nitrogen transformation. Assuming that this value of the COD removed in CW1 could be utilized for nitrogen removal, the amount of COD in the influent only accounts for 27.02% of the required amount in the nitrogen cycle. Therefore, the COD released from ALH-C accounts for 72.98% of the nitrogen cycle in CW1 (Table S2), as well as the only carbon source for denitrification processes in the distal part of the wetland. Additionally, the organic matter released from ALH-C could diffuse from the point of addition to the surrounding area. However, it was mainly concentrated and used in the distal part of the wetland along the direction of water flow (Fig. S1c). Consequently, the shortage and the utilization rate of carbon were improved significantly.

The average removal rates of TP in CW1 and CW2 during the first phase were -0.39% and 16.78%, respectively (Fig. 5f, $P < 0.05$). The removal of phosphorus in CWs is mainly achieved through sedimentation, plant uptake and substrate adsorption (Jing et al., 2015). During the experiment period, both plants uptake and substrate adsorption were affected by lower temperature (about 4 °C). The addition of ALH-C in CW1 also released some phosphorus, thus, increasing the effluent TP concentration. However, as the wetland

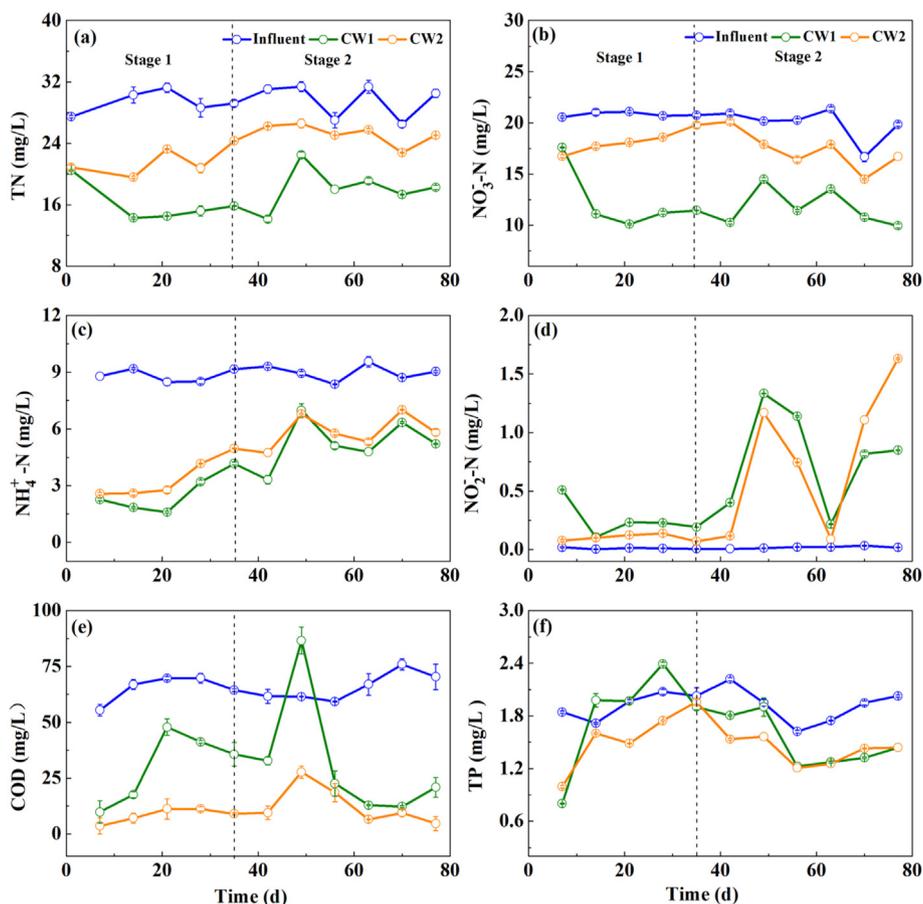


Fig. 5. Influent and effluent pollutants concentrations of the two CWs, (a) TN, (b) NO₃-N, (c) NH₄⁺-N, (d) NO₂-N, (e) COD, (f) TP.

adapted to the conditions of the external plant carbon source during the second phase, the difference in TP removal between the two CWs was no longer significant, which stabilized at 22.95% and 25.86%, respectively (Fig. 5e, $P > 0.05$).

3.4. Distribution of DOM in CWs

Fig. 6 shows the excitation-emission matrix spectroscopy (EEMs) of the two CWs. The peaks appearing in the two CWs were

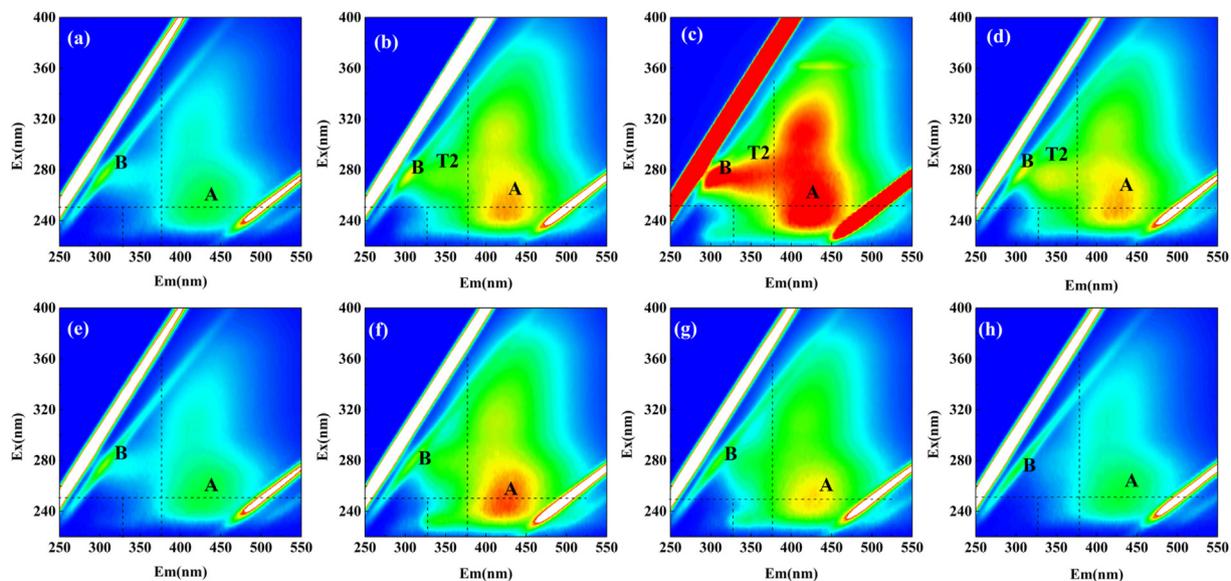


Fig. 6. Excitation emission matrixes inside the two CWs, (a) influent of CW1, (b) 1/4 of CW1, (c) 3/4 of CW1, (d) effluent of CW1, (e) influent of CW2, (f) 1/4 of CW2, (g) 3/4 of CW2, (h) effluent of CW2.

peak A (Ex: 230–260 nm, Em: 400–500 nm) and peak B (Ex: 260–290 nm, Em: 300–330 nm). The peak T2 (Ex: 260–280 nm, Em: 330–370 nm) only appeared in CW1 (Fig. 6). The corresponding peaks of A and B in the influent were 3691 ± 421 and 1738 ± 416 , respectively (Fig. 6). Although the fluorescence intensity of the peak A at 3/4 the length of CW1 increased significantly, the values in the effluent of the two CWs were not significantly different ($P > 0.05$, 4004, 4057). Additionally, the value at 1/4 the length of CW1 (3747) was lower than that of CW2 (5865) (Fig. 6). Peak A is a low-molecular-weight humic acid-like component, which is considered to be transformed by microorganisms and has high biological activity (Williams et al., 2010). Therefore, the humic acid-like components released from ALH-C had good bioavailability and could be fully utilized for biological processes in CWs. Thus, the addition of ALH-C not only strengthened the denitrification function at the distal end of CW1, but also improved the microbial activity inside the wetland. Also, it further enhanced the carbon source utilization efficiency at the proximal end of the wetland. However, the humic acid-like components contained in the two wetlands themselves were more difficult to degrade because they were rich in aliphatic and aromatic groups (carboxyl, phenolic, and amino). Furthermore, the peak B represented tyrosine-like components, and the peak T2 represented tryptophan-like components. The aromatic amino acids tyrosine and tryptophan are also microbial products, which represent complete proteins or peptide matter. The fluorescence intensity of peak B in CW1 was higher than that in CW2 (5804 vs 2010), but the difference in effluent was not significant (2057 vs 1725, $P > 0.05$) (Fig. 6). This finding indicated that the tyrosine-like components released from the ALH-C could also be used by microorganisms. Nonetheless, the ability to use tyrosine-like components is weaker than humic acid-like components (Wu et al., 2018). In addition, the fluorescence intensity of tryptophan-like components in CW1 was about 5748, and that in the effluent was reduced to 1948. This difference indicated that the microorganisms were least able to use tryptophan-like components (Du et al., 2014). The dissolved organic matter released from ALH-C were mainly related to the catabolism of plants and microorganisms in the CWs. Also, the high fluorescence index (about 5) indicated that the released organic compounds were weak in aromaticity, had few benzene rings, and easily degraded and used by microorganisms. In addition, the hydroxyl and carboxyl groups contained in the DOM released from ALH-C could bind to pollutants through electrostatic adsorption, complexation and ion exchange, thereby promoting the removal of pollutants (Zhou et al., 2019). Therefore, ALH-C addition could improve the nitrogen removal efficiency in the low-temperature seasons without increasing the organic matter concentration in the effluents of CWs.

3.5. Implications for practice

The addition of ALH-C could improve the nitrogen removal in constructed wetlands significantly. However, the organic matter released by ALH-C also contained some large molecular degradation-resistant matter. These matters may increase the degree of humification inside the wetland and the average molecular weight of organic matter, as well as cause undesirable color problems in the effluent. Additionally, the risk of greater N_2O flux feedback to global warming caused by excessive organic C release should also be mentioned (Zhang et al., 2019). Nevertheless, there is no doubt that the utilization of plant carbon sources still has significant advantages. The application of plant carbon sources in CWs replaces plant biomass treatment and commercial carbon source addition, which reduces the operational cost of constructed wetlands. Moreover, the residues could be used as feedstock candidates

for soil amendments and fermentation, which can further improve the economy of the technology.

For the application of plant carbon source, a separate unit or area may be set for the addition of plant materials in the constructed wetlands to avoid clogging of the substrate by plants decomposition. In addition, since the plants treated with alkali could sustain high denitrification rates for about 30 days, it is necessary to replace the plant materials according to the nitrogen removal efficiency. The results of this study demonstrate that the amount of the plant materials added in the CWs needs to be controlled carefully to avoid excessive use or lack thereof. Thus, the amount of plant materials addition based on a carbon and nitrogen mass balance should be considered during the design and operation stage.

4. Conclusions

Alkali treatment was demonstrated to effectively destroy the bonds between lignocellulose and other components, as well as increase the proportion of carbon in plant tissues. Alkali-heated cattails (ALH-C) showed the highest average denitrification rate (0.33 mg/(L·h)) and average denitrification potential (0.30 mg/(L·h)). Also, ALH-C improved the removal rates of TN, NO_3^- -N and NH_4^+ -N in CWs by 24.41%, 31.80% and 8.80%, respectively. Furthermore, ALH-C released DOM comprised of mainly humic acid-like, tyrosine-like, and tryptophan-like components, which were highly bioavailable and had minimal effects on COD removal in CWs. Overall, ALH-C could improve the nitrogen removal efficiency significantly during low-temperature seasons to overcome the limitation of CWs during such periods.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2021.129630>.

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