Promoting effects of corn straw and exceed sludge as carbon sources on denitrification of constructed wetlands

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Abstract. As the low C/N ratio of effluent from wastewater treatment plant, the advanced treatment of constructed wetlands (CWs) on the effluent is often unsatisfactory, especially for the removal of N. In order to increase the N removal efficiency, this paper tested three kinds of carbon sources acid and alkali treated straw, alkali treated straw and exceed sludge for promoting the denitrification of CWs. The results showed that alkali treated straw performed the strongest carbon release ability (0.43mgꞏg⁻¹ꞏh⁻¹) and the maximum C/N ratio (27.3). While acid and alkali treated straw’s carbon release ability and C/N ratio were 0.37 mgꞏg⁻¹ꞏh⁻¹ and 12.8, exceed sludge’s carbon release ability and C/N ratio were 0.37 mgꞏg⁻¹ꞏh⁻¹ and 8.4. In order to improve the C/N ratio in CWs to 5/1, 450g alkali treated straw and exceed sludge were added into tide flow wetlands (TFCWs) and subsurface flow wetlands (SSFWs) respectively to test the improvement of denitrification. Compared with the control groups, the N removal of CWs with carbon sources were significantly improved. CWs with alkali treated straw showed better removal effects of N than CWs exceed sludge. The analysis results of the first-order kinetic process suggested that the denitrification rate constants of TFCW control group, TFCW with exceed sludge and TFCW with alkali treated straw were 0.126, 0.262 and 0.345, respectively. The denitrification rate of TFCW with alkali treated straw was 2.74 times of TFCW control group, and 1.32 times of TFCW with excess sludge. This study has contributed to understand the possibility of straw and exceed sludge as the carbon sources to strengthen the denitrification in CWs.

1. Introduction

Constructed wetlands (CWs) are designed to treat wastewater depending on natural processes within a controlled environment[1-2]. CWs are often used as advanced treatment of effluent from wastewater treatment plant (WWTPs). Due to the low C/N ratio of effluent water, the N treatment of constructed wetland is often unsatisfactory, resulting in the increase of N concentration and eutrophication of receiving waterbody[3]. According to extensive studies and applications, adding carbon source can effectively improve the C/N ratio and denitrification activity of CWs[4].

As an important limiting factor of denitrification, carbon source mainly affect the denitrification of CWs by affecting the activity of denitrifying bacteria[5]. As reported, various carbon sources can be used by denitrifying bacteria, they usually can be divided into three categories: carbon source contained in sewage, endogenous carbon source and external carbon source. The carbon source contained in the wastewater refers to the COD existing in polluted water. The endogenous carbon refers to the organic carbon released from dry branches, fallen leaves and the microbial death. The
external carbon source means the additional needs when the carbon source in the biochemical system is insufficient. External carbon source usually can be divided into two categories: one is the traditional liquid carbon source mainly composed of glucose, methanol, acetic acid and the organic chemical liquid (such as primary sludge hydrolysate and landfill leachate)[6-7]. The others are some low-cost solid organics, including natural plants containing cellulose, some biodegradable polymers and so on[8-9]. Among these sources, agriculture wastes and excess sludge are considered to have widely available features due to their low cost and readily available. In recent years, there are many studies on straw as carbon source of CWs, while a few studies on excess sludge as carbon source of CWs had been reported. For example, Zhang et al. promoted nitrogen removal in surface flow CWs through adding rice straw[10]. Si et al. supplemented wheat straw, cotton, poly (butylene succinate), and newspaper as the carbon source to compare the denitrification efficiency and bacterial communities in CWs[11]. However, in most studies, agriculture wastes were added directly into CWs for N removal intensification without pretreatment. In order to obtain more and rapid biodegradable carbon release from agricultural wastes, alkali and acid are two potential pretreatment methods, because they help breaking down lignocellulose, destroying the crystalline structure of cellulose and dissolving hemicellulose[12]. Thus, the excess sludge, alkali and acid treated straws as carbon sources for denitrification of CWs should be investigated.

Here, three tidal flow wetlands (TFCWs) and three subsurface flow wetlands (SFFWs) were built to analyse the effect of acid and alkali treated straw, alkali treated straw and excess sludge as carbon sources on denitrification of CWs. The C and N removal efficiency of CWs were measured to compare the promoting effects of different carbon sources. The first order dynamic equation was utilized to study the denitrification kinetics of TFCWs with three types of carbon source. Our results contribute to the understanding of the promoting effect of straw and sludge carbon sources on denitrification of CWs, have important sense to the reduction and recycling of solid wastes.

2. Materials and methods

2.1. Preparation of carbon source

2.1.1. Straw carbon source
Corn straws as a kind of agricultural waste are largely produced in China. In this study, corn straws were recycled as carbon source to promoting the denitrification of CWs. They were washed with Milli-Q water, dried at 108°C in oven to a constant weight and cut into 2 cm pieces. Two pretreatment methods were used to improving the carbon release efficiency of straws. The first approach was that the cut corn straws were added into 1% NaOH solution with solid to liquid ratio 1:30, and heated for 1 hour at 90 °C in water-bath. The second approach consists of two steps. The first step the cut corn straws were added into 20%HCL with solid to liquid ratio 1:30, and heated for 1 hour at 90 °C. The second step straws continued to be added into NaOH solution and heating, the operation process was same to approach one.

2.1.2. Excess sludge carbon source
There is high content of organic matter in excess sludge. The organic components include oils, fats, proteins, polysaccharides, hemicellulose, cellulose and lignin. The first four kinds of substances are easy to be decomposed and utilized by microorganisms, and the last three kinds of substances are not easy to be decomposed directly by microorganisms as nutrients. In this report, the excess sludge was mixed with rice husks to reduce the moisture content to 60%. After 22 days of aerobic biological drying process, the moisture content of excess sludge reduced to 40%. This kind of excess sludge came from Zhenxing Sludge Disposal Plant in Shenyang city, China.

2.2. Release behaviors of carbon sources
The above three carbon sources were dried to constant weight and weighed 1g respectively into three
250 ml conical flask, 200 ml Milli-Q soaking water were added. In order to prevent dust from entering, the bottle mouths were sealed with preservative film. Water samples were taken at 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 7 h, 10 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, respectively. After centrifugation, the supernatant were kept for determination of water quality indexes. Soaking water was replaced after each sampling and the water quality and temperature remained constant.

2.3. Specifications of CWs and experimental design
The study was undertaken from 2nd November 2016 using six CWs in Shenyang City, China. CW1: SSFW blank control; CW2: SSFW with excess sludge; CW3: SSFW with alkali treated straw; CW4: TFCW blank control; CW5: TFCW with excess sludge; CW6: TFCW with alkali treated straw. As shown in Fig. 1, all CWs were composed of plexiglass cylinder with 30 cm in diameter, 80 cm in height, 42 L volume. The substrates consisted of gravel with particle size 25–50 cm (15cm in height), 8–12 cm (20cm in height) and 3–5 cm (30cm in height). 5cm high soil covering the surface of CWs and planted with reeds. Each TFCW was controlled by PCL time controller.

The experiment parameters of TFCW were determined to be: rapid feed water - reaction (3 h), rapid out water - idle time (3 h), each cycle for 20 L treatment of 6 h, four cycles per day[13]. The parameters of SSFW were determined to be: continuous water supply and drainage, 46 ml/min of the flow rate, 6 hours for 20L treatment. The six CWs were stably operated for 1 months at 80 L/day, and the hydraulic retention time was approximately 12.6 h. Four sampling points (A, B, C, D) were set up in gravel layer for analysis the dynamics of TFCW. The laboratory temperature was 11-15℃. The simulated sewage was made up of glucose, ammonium chloride, potassium dihydrogen phosphate, sodium nitrate and sodium nitrite. Trace elements CaCl₂•2H₂O, MgSO₄•7H₂O and FeSO₄•7H₂O were also added.

2.4. Sampling and monitoring
In the sampling period, five wastewater samples (influent, Point A, Point B, Point C, effluent) were collected every two days, and Point A, Point B and Point C were collected ever half hour in TFCW reaction time (3 h). Each water sample was collected 200 mL one time and save in 500-mL glass jars. To prevent microbial growth, approximately 25 mL of methanol was added to each water sample and the samples were adjusted to pH = 3 using 4 M H₂SO₄. The samples were stored in the dark at 4 ℃ in laboratory, where they prior to extraction (within 24 h) and analysis. Wastewater quality parameters
included pH, chemical oxygen demand (COD), total nitrogen (TN), ammonium nitrogen (NH₃-N) and Nitrate nitrogen (NO₃⁻). pH was measured by a pH Meter (720APLUS Benchtop, Thermo Orion Co., USA). COD was determined by UV spectrophotometer (UV-6100H, Shanghai Metash Instruments Co., China) with standard methods (GB 11914-89). TN, NH₃-N and NO₃⁻ were determined using the alkaline potassium persulfate digestion UV spectrophotometric method (HJ 636–2012), Nessler's reagent spectrophotometric method (HJ 535–2009) and UV spectrophotometric method (HJ/T 346–2007) respectively.

2.5. First-order kinetic model
First-order kinetic model is a kind of mathematical model which has been widely studied and applied in CWs[14]. The expression of the first-order kinetic model is as follow:

\[ \frac{dC}{dt} = -k_tC \]  

\( dC/dt \) represent the removal rate of contaminants (mg/L·d); \( k_T \) is the volume removal rate constant corrected by temperature; \( C \) is the concentration of contaminants (mg/L).

After integral calculation, the formula could be transformed as follow:

\[ \ln \left( \frac{C_{out}}{C_{in}} \right) = -k_T t \]  

\( C_{out} \) represent the concentration of effluent; \( C_{in} \) represent the concentration of influent; \( t \) is the hydraulic retention time of CWs (d).

Eq. 2 is often used in the correlation analysis of denitrification kinetics. By plotting \( t \) to \( -\ln(C_{out}/C_{in}) \), the first-order kinetic constant \( k_T \) of denitrification can be obtained according to the slope of the regression line.

3. Results and discussion
3.1. Release of carbon and nitrogen form carbon sources
The C cumulative releases of three carbon sources were showing in Fig. 2a. The C releases of corn straws under two different treatments showed different variation tendencies. Alkali treated straw and exceed sludge reached the maximum C release rates at 0.5h with 135 mg·g⁻¹·h⁻¹ and 54 mg·g⁻¹·h⁻¹ respectively. The acid and alkali treated straw reached the maximum C release rate at 1.5h with 52 mg·g⁻¹·h⁻¹. The release rates of these three solid carbon sources were faster in the early stage and reached equilibrium about 10 hours later. In stable period, the C release rate of alkali treated straw was stable at 0.43mg·g⁻¹·h⁻¹, the other two carbon sources were both stable at 0.37 mg·g⁻¹·h⁻¹. The accumulated C release of alkali treatment straw was obvious higher than the acid and alkali treated straw and exceed sludge. At 168 h, the accumulated C release of alkali treatment straw reached 296.06 mg·g⁻¹, while the acid and alkali treated straw and exceed sludge were 151.50 mg·g⁻¹ and 135.61 mg·g⁻¹ respectively. The results indicated that alkali treatment was better than acid and alkali treatment in the release of straw lignocellulose. That may because in acid treatment straw adsorbed a small amount of acid, and then in alkali treatment the neutralization between acid and alkalis affected the treatment efficiency. It was also possible that the bond-forming reaction between hydroxyl ion and broken nucleoside bond occurred after alkali addition, which reduced the release and degradation of cellulose.

Inorganic N is the main nutrient needed for the growth of plants and microorganisms. Here, the release of N was calculated as the sum of released NH₄⁺-N, NO₃⁻-N and NO₂⁻-N. The N cumulative releases of three carbon sources were shown in Fig. 2b. The release rates of N of three carbon sources were faster in the early stage, and tend to be stable after 10 hours. The cumulative release of N from exceed sludge was significantly higher than that from the two straw carbon sources. At 168 h, the accumulated N release of exceed sludge reached 14.40 mg·g⁻¹, while the acid and alkali treated straw and alkali treatment straw were 10.32 mg·g⁻¹ and 10.19 mg·g⁻¹ respectively. In stable period, the N
release rate of acid and alkali treated straw, alkali treated straw and exceed sludge were 0.20 mg·g⁻¹·h⁻¹, 0.20 mg·g⁻¹·h⁻¹ and 0.21 mg·g⁻¹·h⁻¹ respectively, showed similar variations.

The C/N ratios of cumulative releases from the three carbon sources were showing in Fig.2c. The C/N ratios decreased in first 5 h, increased rapidly during 5-10 h, and tend to be stable during 10-168 h. The C/N ratios of acid and alkali treated straw, alkali treated straw and sludge were 12.8, 27.3 and 8.4 in stable period. The results indicated that the three carbon sources have different carbon and nitrogen release characteristics. Alkali treated straw seemed to be a satisfactory carbon source with released more carbon and less nitrogen. Although the releases of carbon and nitrogen from exceed sludge were not ideal, the C/N ratio was 8.4, which was slightly higher than the threshold value 5 of suitable C/N ratio for denitrification in CWs. Considering the purpose of recycling, reducing and harmlessness of excess sludge, it also have wide application prospects as denitrification carbon source.

![Fig. 2. (a) Carbon, (b) nitrogen and (c) C/N ratios of cumulative releases of alkali treated straw, acid and alkali treated straw and exceed sludge](image)

### 3.2. Removal of pollutants in CWs with carbon source

In order to increase the C/N ratios of the CWs to 5:1, 450g alkali treated straws and exceed sludge were added into TFCWs and SSFWs respectively. After the CWs were running stable, monitoring the COD, TN, NH₄⁺-N and NO₃⁻-N of the influent and effluent. Under the average COD concentrations of the influent were 77.86 mg·L⁻¹, the removal of COD of each CW was shown in Fig. 3a. The results showed that the COD removal rates of CW1, CW2, CW3, CW4, CW5, CW6 was 56.47%, 40.80%, 37.87%, 78.46%, 53.59%, 49.41%, respectively. The COD removals of CWs with carbon sources were obvious lower than that of the CWs without carbon sources. Compare to SSFW control group,
the COD removal of SSFW with alkali treated straw and SSFW with exceed sludge reduced 18.80% and 15.67% respectively. Compare to TFCW control group, the COD removal of TFCW with alkali treated straw and TFCW with exceed sludge reduced 29.05% and 24.87% respectively. Our results also founded that TFCWs showed significant advantages in COD removal. That because TFCW can greatly improve oxygen supply by a “tidal” operation strategy that repeats cycle of “saturated” and “unsaturated” periods. Consequently, nutrient removal was intensified[15].

The removal of TN of each CW was shown in Fig. 3b. With the average TN concentrations of the influent were 25.54 mg·L⁻¹, the TN removal rates of CW1, CW2, CW3, CW4, CW5, CW6 were 51.86%, 57.36%, 74.53%, 47.66%, 54.10% and 62.42%, respectively. Adding carbon sources effectively improved TN removal efficiencies of CWs. Alkali treated straw showed better promoting effect than exceed sludge. Comparison of two wetland types, SSFsWs performed better removal efficiencies than TFCWs. That because the substrate of SSFW is constant submerged, led to a long-term anoxic state of wetland environment, which is very conducive to denitrification[16].

The removal of NH₄⁺-N and NO₃⁻-N of CWs showed similar tendency with TN (Fig. 3c and Fig. 3d). CWs with alkali treatment straw showed better NH₄⁺-N and NO₃⁻-N removal effects than CWs with exceed sludge. However, the difference were that TFCWs performed better removal efficiencies on NH₄⁺-N and SSFWs performed better removal efficiencies on NO₃⁻-N.

Fig. 3. Pollutants (a) COD, (b) TN, (c) NH₄⁺-N and (d) NO₃⁻-N average removal (%) of CWs with carbon sources. (CW1: SSFW blank control; CW2: SSFW with excess sludge; CW3: SSFW with alkali treatment straw; CW4: TFCW blank control; CW5: TFCW with excess sludge; CW6: TFCW with alkali treatment straw)
3.3. Denitrification dynamics of TFCWs with carbon source

Through first-order kinetic process, this report analyzed the influence of the carbon sources alkali treated straw and exceed sludge on denitrification in TFCWs. The NO\textsubscript{3}\textsuperscript{-}-N concentrations of Point A, Point B, Point C and Point D were measured every half hour in reaction time of TFCWs (3 h). The variation of the average NO\textsubscript{3}\textsuperscript{-}-N concentrations in reaction time were showing in Fig.4a. The results showed that the removal of NO\textsubscript{3}\textsuperscript{-} had been significantly improved and the denitrification was obviously strengthened. Compared to TFCW control group, the NO\textsubscript{3}\textsuperscript{-}-N concentrations of the effluents of TFCW with alkali treated straw and TFCW with exceed sludge decreased 56.07% and 35.14%, respectively.

The analysis results of the first-order kinetic equation were shown in Fig. 4b. The denitrification rates constant $k_T$ of CW4, CW5 and CW6 were 0.126, 0.262 and 0.345, respectively. The $k_T$ of TFCW with alkali treated straw was 2.74 times of TFCW control group, and 1.32 times of TFCW with excess sludge. Alkali treated straw was proved had better character to promote the denitrification than exceed sludge in TFCWs.

4. Conclusion

In the present study, three kinds of carbon sources acid and alkali treated straw, alkali treated straw and exceed sludge were tested for promoting the denitrification of CWs. It demonstrated that adding straw and exceed sludge carbon source could significantly improve N removal efficiency of CWs. Alkali treated straw performed the strongest carbon release ability and the maximum C/N ratio, showed the most advanced effect on the removal of N in CWs, which is the best choice of three carbon sources. In addition, it was proved that TFCWs had better removal efficiency on COD and NH\textsubscript{4}\textsuperscript{+}-N, while the SSFWs had better removal efficiency on TN and NO\textsubscript{3}\textsuperscript{-}-N. Further studies on micro-structural changes of alkali treated straw, substrate enzyme activity and microbial community variation would significantly improve our understanding of the biochemistry processes taking place in CWs.

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