Pretreatment methods for aquatic plant biomass as carbon sources for potential use in treating eutrophic water in subsurface-flow constructed wetlands

Xiang-Feng Huang, Xin Liu, Jia-Jia Shang, Yi Feng, Jia Liu and Li-Jun Lu

ABSTRACT

Plant biomass is usually added to constructed wetlands (CW) to enhance denitrification. In this study, we investigated effects of different pretreatments on two common external plant carbon sources, cattail and reed litter. We determined the average ratio of chemical oxygen demand (COD) to total nitrogen (TN), designated as C/N, in water samples after addition of litter subjected to various pretreatments. The C/N in the water samples ranged from 4.8 to 6.4 after addition of NaOH-pretreated cattail litter, which was four to six times greater than that of water from the Yapu River and 3.84–39.15% higher than that of systems that received untreated cattail litter. The C/N of systems that received H2SO4-pretreated carbon sources varied from 1.7 to 3.6. These two methods resulted in TN and total phosphorus (TP) levels lower than those in river water. The C/N was 1.4–1.7 after addition of CH3COOH-pretreated reed litter, which was 34.87–53.83% higher than that of river water. The C/N was 2.5 in systems that received mild alkali/oxidation-pretreated reeds, which was 30.59% higher than that of systems that received non-pretreated reeds. The residue rates of cattail and reed litter subjected to various pretreatments were greater than 60%. Our results showed that NaOH, H2SO4, and mild alkali/oxidation pretreatments were useful to rapidly improve the C/N of river water and enhance denitrification.

Key words | constructed wetlands, denitrification, external plant carbon sources, pretreatment

INTRODUCTION

From the latter half of the last century, there has been increasing concern about the elevated nutrient status and eutrophication of rivers and lakes (Sierp et al. 2009). Almost 22% of lakes in China are eutrophic or hypereutrophic (Zhao et al. 2015). Lake Taihu is a hyper-eutrophic lake. Nitrogen (N) is one of the major factors influencing water eutrophication. Because conventional wastewater systems cannot decrease the amount of N, other measures should be taken to address high N levels and to lower the impacts of nutrient pollution (Wu et al. 2011). Ecological engineering for low-cost removal of pollutants is an emerging field in which the focus is to design and construct sustainable ecosystems that provide a balance between natural and human values (Mitsch et al. 2002). Constructed wetlands (CWs) are increasingly used to treat eutrophic waters worldwide, especially in developing countries.

CWs are biological wastewater treatment systems that were developed in Europe and the USA in the 1970s (IWA 2000). It is generally believed that CWs can effectively remove total nitrogen (TN) from eutrophic water (IWA 2000; Bulc et al. 2011); however, the reported removal efficiencies range from 30 to 90% (Fleming-Singer & Horne 2002). Denitrification is widely accepted as the dominant process in removing nitrate from water in CWs (Langergraber et al. 2009; Cooper et al. 2010; Wen et al. 2010). Nevertheless, many factors influence denitrification rates in CWs, including operation time, hydraulic retention time, pH, temperature, dissolved oxygen concentration, the microbial community, and carbon sources (Bolton & Greenway 1999; Albuquerque et al. 2009; Lu et al. 2009).

Horizontal subsurface-flow constructed wetlands (HSCW) provide an anoxic and anaerobic habitat in which the denitrification potential is considered to be
Accordingly, aquatic litter should be pretreated to improve the rate of lignocellulose hydrolysis to allow complete denitrification in CW. However, few studies have investigated the effects of pretreatments on aquatic biomass as external carbon sources in CW, and how this technique could be applied in CW engineering. Therefore, in this study, we investigated the effects of different pretreatments of cattail (Typha latifolia) and reed (Phragmites spp.) litter materials.

### METHODS

#### Materials

Cattail and reed litter were used as external carbon sources according to previous studies (Wen et al. 2010). The leaves of plants were collected near the Yapu River at the Village of Yapu in Changzhou, Jiangsu Province, China (31.78° N, 119.95° E) in February 2011. The leaves were cleaned, cut into 1–2 cm pieces, and air-dried to a constant mass. Leaf material was kept in a moisture-proof container at room temperature (20 °C) until use.

#### Aquatic plant biomass pretreatments

As shown in Table 1, we varied concentrations of NaOH and the duration of boiling, as described by Wen et al. (2010). Briefly, 100 g cattail litter was placed in a stainless steel pot, soaked in analytically pure NaOH at a solid: liquid ratio of 1:4 and boiled for various times (Table 1). The pH was then adjusted to neutral and the cattail litter was filtered, washed, and air-dried to constant mass. The treated litter was stored in a moisture-proof container at room temperature until use.

The appropriate concentration of sulfuric acid to pretreat materials is 0.5–1.0% under mild conditions (Zhao 2007). We selected the concentrations of H$_2$SO$_4$ (analytically pure) and duration of boiling based on the results of previous studies and practical conditions. The procedure

### Table 1

<table>
<thead>
<tr>
<th>Pretreatment methods for aquatic plant biomass as carbon sources</th>
<th>NaOH Pretreatment</th>
<th>H$_2$SO$_4$ Pretreatment</th>
<th>CH$_3$COOH Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A$_1$</td>
<td>A$_2$</td>
<td>A$_3$</td>
</tr>
<tr>
<td>Concentration</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Duration of treatment (h)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
was the same as that used for the alkaline pretreatment (Table 1).

Reed biomass can contain up to 66.30% cellulose and hemicellulose. These substances are easily dissolved by acetic acid at room temperature (Zhao et al. 2005). Therefore, we used 10, 20, and 40% (vol/vol) acetic acid (analytically pure) to pretreat reed litter based on a study conducted by Hang & Cui (2005). The procedure for acetic acid pretreatment of reeds was the same as that used for the alkaline pretreatment. The details are shown in Table 1.

Reed biomass contains 22.67% lignin (Zhao et al. 2005). Mild alkali/oxidation pretreatment causes H₂O₂ to produce oxygen root ions. Although the oxidation ability of these ions is weaker than that of H₂O₂, they have a more reactive electrophilic center, enabling more effective dissolution of lignin (Zhao 2007). In the D1# treatment, reed litter was soaked for 24 h in 1% NaOH, and then soaked in a mixture of 1% (w/v) NaOH and 0.3% (v/v) H₂O₂. In the D2# treatment, reed litter was soaked for 24 h in a mixture of 1.5% (w/v) NaOH and 0.6% (v/v) H₂O₂. Both the D1# and D2# treatments were conducted at room temperature (solid/liquid ratio was 1:4). The pH was then adjusted to neutral and the reed litter was filtered, washed, and air-dried to constant mass. The dried litter was kept in a moisture-proof container at room temperature until use.

**Experimental operation**

We used eutrophic water from the Yapu River collected at Yapu Village, Changzhou, China. The river is a branch of the river around Wujin Port that flows into Lake Taihu. Because of the subtropical monsoon climate, the average temperature in the study area is 4 °C in winter and 27.5 °C in summer. The chemical oxygen demand based on the permanganate index (CODₘₙ) was less than 6 mg/L during the cold period (November to April), but the TN was greater than 5 mg/L. Almost 65% of TN was NO₃-N. Therefore, the Yapu River water was eutrophic with a low C/N, and the main pollutant was TN. The annual average concentrations of TN, total phosphorus (TP), CODₘₙ, and chlorophyll a concentration (Chl a) are approximately 6.5, 0.3, 6.9 mg/L, and 3.24 mg/m³, respectively.

We added 5 L river water to each of 23 plastic drums. Each drum had a total volume of 7.5 L. In April 2011, we added 50 g pretreated cattail or reed litter to 20 of the drums, untreated cattail litter and untreated reed litter to two drums, and nothing to the final drum (control). Each sample was replicated twice, giving a total of 46 drums. The experiment was conducted for 36 days, during which time water samples were collected (in triplicate) almost every 3 days and river water was changed daily at 9:00 am to simulate ordinary CW systems.

**Chemical and data analysis**

Samples were filtered through qualitative filter paper and analyzed for TN, TP, and CODₘₙ. TN was measured by alkaline potassium persulfate oxidation ultraspectrophotometry and TP was measured by potassium persulfate oxidation–molybdenum colorimetry. COD was determined by the potassium permanganate index according to the standard method (APHA 1998). Statistical analyses were carried out using SPSS version 16.0 software. All data were tested to ensure that they were normally distributed. Mean values are presented in figures and tables.

**RESULTS AND DISCUSSION**

**Effects of NaOH pretreatments**

As the lignocellulose content of cattail biomass is greater than 53.9%, simple sugars and other nutrients hydrolyzed from the lignocellulose could be used as carbon sources for denitrification (Liu et al. 2010). Wen et al. (2010) demonstrated that NaOH pretreatment of biomass resulted in easy removal of lignin and a decrease in the crystallinity of cellulose. These structural changes benefit the growth of filamentous fungi and make it easier to separate lignin and carbohydrate (Zhao 2007). Figure 1(a) shows the CODₘₙ concentrations after NaOH pretreatments of various durations. The CODₘₙ concentration was always lowest in natural river water. Our results showed that addition of cattail litter significantly increased the CODₘₙ. The CODₘₙ value increased initially after addition of litter, and then decreased. The highest concentration was 20.99 mg/L when the retention time was 6 d; this value was almost 3.5 times that of natural water (6.02 mg/L). Subsequently, the concentration decreased gradually and stabilized on day 13.

The CODₘₙ values of groups containing NaOH-pretreated materials were markedly lower than those of the blank controls. The decrease may be because some carbon sources from NaOH-treated plant litter were consumed during denitrification. As shown in Figure 1(a), the CODₘₙ of water samples decreased as the NaOH concentration increased. After pretreatment with 1% NaOH and 1 h heating, the CODₘₙ was higher than those of the other alkali-pretreated groups. The highest CODₘₙ was...
19.67 mg/L after 1 h of heating, while the lowest was 7.77 mg/L, which was more than 1.5 times higher than that of river water containing non-pretreated cattail litter after 13 d. The maximum CODMn concentration after 2 h heating was 12.74 mg/L after 6 d in the 1% NaOH pretreatment group, which was only 64.97% of the maximum obtained after 1 h of heating. On day 13, the CODMn decreased to 6.97 mg/L. Overall, these findings indicate that heating time was inversely proportional to the amount of organic compounds released.

As shown in Figure 1(b), the TN of the water decreased after addition of cattail litter, regardless of whether the materials were pretreated. From day 3 to 6, the TN concentrations in the NaOH-pretreated groups were the same as those of the control, possibly because of an absorption effect of the litter. After 10 days, the TN concentrations of the NaOH-pretreated groups were much lower than those of the untreated groups. This may have been because addition of plant litter increased carbon concentrations and enhanced denitrification, resulting in a decrease in TN concentration. The lowest TN concentration was 0.87 mg/L (17.27% of that of the river water) after 13 days of incubation with 1% NaOH-pretreated materials. There was an increase of TN in water samples containing NaOH-pretreated materials after 24 days, which may reflect a large amount of organic nitrogen released by proteolysis (Zhao et al. 2009). These results indicate that addition of cattail litter did not positively increase TN, whereas NaOH concentration and heating time slightly affected TN.

TP concentrations were significantly lower in the pretreated groups than in un-pretreated groups, but were similar to those of river water. NaOH concentrations and heating time had little effect on TP. The lowest TP value was 0.045 mg/L on day 23 after 4% NaOH pretreatment and 2 h heating; this value was 27.75% of that of river water and 28.57% of that of the control.

Effects of H2SO4 pretreatments

Acid can destroy the crystalline structure of cellulose, break connections between cellulose and lignin, and dissolve hemicellulose (Yang et al. 2008). As shown in Figure 1(c), the H2SO4 concentration inversely affected the CODMn concentration in water samples. On day 3, the CODMn values were 15.11 and 17.99 mg/L in the groups pretreated with 1 and 2% H2SO4 with 1 h heating, respectively; these values were 23.30 and 46.86% higher than those of the blank control, respectively. After day 3, the CODMn decreased to less than that of the control. For identical H2SO4 concentrations, longer heating time was associated with lower CODMn concentration.
The TN concentrations in the H₂SO₄-pretreated groups were higher than those of the river water and peaked on day 3, decreasing thereafter. The TN levels in the group pre-treated with 1% H₂SO₄ with 1 h heating showed the greatest decrease (Figure 1(d)), with the lowest value (1.73 mg/L) on day 13. This value was 34.22% of the TN concentration of river water and 40.81% of that of the blank control. Heating time did not significantly affect the TN in the water samples.

The TP levels of the H₂SO₄-pretreated groups were generally similar (Figure 2). According to Hang & Cui (2005), cellulose would be hydrolyzed quickly within the first hour if treated with 20% CH₃COOH (mass ratio) at 40 °C. When the soaking time was extended to 6 or 12 h, reactions that completely degrade glucose in the samples via cellulose hydrolysis may occur. Accordingly, most biodegradable organic materials in reed litter could have been lost before it was added to the plastic drums. As shown in Figure 2, CH₃COOH concentrations and soaking time did not affect the CODₘₙ concentration of the samples.

Table 2 shows the average CODₘₙ, TN, and TP concentrations of different groups. The average TN concentrations in water samples containing CH₃COOH-pretreated materials were higher than those of the blank control. For example, in the 40% CH₃COOH pretreatment group after 12 h soaking, the TN was 33.82% higher than that of the blank control. The TN values of pretreated groups were generally similar (Figure 2). According to Hang & Cui (2005), cellulose would be hydrolyzed quickly within the first hour if treated with 20% CH₃COOH (mass ratio) at 40 °C. When the soaking time was extended to 6 or 12 h, reactions that completely degrade glucose in the samples via cellulose hydrolysis may occur. Accordingly, most biodegradable organic materials in reed litter could have been lost before it was added to the plastic drums. As shown in Figure 2, CH₃COOH concentrations and soaking time did not affect the CODₘₙ concentration of the samples.

### Effects of CH₃COOH pretreatments

The CODₘₙ levels in samples pretreated with acetic acid were lower than those of the blank control and the trends were generally similar (Figure 2). According to Hang & Cui (2005), cellulose would be hydrolyzed quickly within the first hour if treated with 20% CH₃COOH (mass ratio) at 40 °C. When the soaking time was extended to 6 or 12 h, reactions that completely degrade glucose in the samples via cellulose hydrolysis may occur. Accordingly, most biodegradable organic materials in reed litter could have been lost before it was added to the plastic drums. As shown in Figure 2, CH₃COOH concentrations and soaking time did not affect the CODₘₙ concentration of the samples.

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### Table 2

<table>
<thead>
<tr>
<th>Water samples</th>
<th>CODₘₙ (mg/L)</th>
<th>TN (mg/L)</th>
<th>TP (mg/L)</th>
<th>C/N</th>
<th>Residue ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water</td>
<td>5.0 ± 0.7</td>
<td>5.2 ± 0.9</td>
<td>0.13 ± 0.05</td>
<td>1.1 ± 0.3</td>
<td>/</td>
</tr>
<tr>
<td>A0</td>
<td>13.4 ± 4.4</td>
<td>3.0 ± 0.9</td>
<td>0.16 ± 0.08</td>
<td>4.6 ± 2.4</td>
<td>77.3</td>
</tr>
<tr>
<td>A1</td>
<td>10.4 ± 4.2</td>
<td>1.6 ± 0.7</td>
<td>0.09 ± 0.02</td>
<td>6.4 ± 2.0</td>
<td>61.3</td>
</tr>
<tr>
<td>A2</td>
<td>9.9 ± 2.7</td>
<td>2.0 ± 1.2</td>
<td>0.11 ± 0.04</td>
<td>5.2 ± 1.7</td>
<td>82.3</td>
</tr>
<tr>
<td>A3</td>
<td>9.5 ± 2.3</td>
<td>1.6 ± 0.4</td>
<td>0.11 ± 0.02</td>
<td>5.7 ± 1.5</td>
<td>69.0</td>
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<tr>
<td>A4</td>
<td>9.2 ± 2.9</td>
<td>1.9 ± 1.0</td>
<td>0.11 ± 0.05</td>
<td>5.3 ± 1.5</td>
<td>84.3</td>
</tr>
<tr>
<td>A5</td>
<td>9.3 ± 3.0</td>
<td>1.7 ± 0.7</td>
<td>0.11 ± 0.04</td>
<td>5.5 ± 1.4</td>
<td>80.7</td>
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<tr>
<td>A6</td>
<td>7.7 ± 2.1</td>
<td>1.7 ± 1.0</td>
<td>0.09 ± 0.03</td>
<td>4.8 ± 1.5</td>
<td>89.3</td>
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<tr>
<td>B1</td>
<td>6.6 ± 1.8</td>
<td>4.1 ± 1.5</td>
<td>0.09 ± 0.03</td>
<td>1.8 ± 0.6</td>
<td>66.7</td>
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<tr>
<td>B2</td>
<td>6.0 ± 1.5</td>
<td>4.1 ± 1.4</td>
<td>0.11 ± 0.06</td>
<td>1.7 ± 0.7</td>
<td>74.3</td>
</tr>
<tr>
<td>B3</td>
<td>9.5 ± 3.1</td>
<td>2.8 ± 1.5</td>
<td>0.09 ± 0.03</td>
<td>3.6 ± 0.7</td>
<td>87.7</td>
</tr>
<tr>
<td>B4</td>
<td>9.5 ± 3.9</td>
<td>3.1 ± 1.9</td>
<td>0.09 ± 0.06</td>
<td>3.4 ± 1.0</td>
<td>85.3</td>
</tr>
<tr>
<td>B5</td>
<td>10.0 ± 4.2</td>
<td>3.2 ± 1.3</td>
<td>0.10 ± 0.05</td>
<td>3.0 ± 0.9</td>
<td>85.7</td>
</tr>
<tr>
<td>B6</td>
<td>7.8 ± 2.9</td>
<td>3.8 ± 0.7</td>
<td>0.09 ± 0.03</td>
<td>2.1 ± 0.5</td>
<td>74.3</td>
</tr>
<tr>
<td>C0</td>
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<td>4.6 ± 2.4</td>
<td>0.14 ± 0.39</td>
<td>1.9 ± 0.4</td>
<td>91.5</td>
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<tr>
<td>C1</td>
<td>7.4 ± 3.1</td>
<td>5.9 ± 3.5</td>
<td>0.15 ± 0.32</td>
<td>1.6 ± 0.7</td>
<td>84.8</td>
</tr>
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<td>7.1 ± 3.0</td>
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<td>0.17 ± 0.66</td>
<td>1.5 ± 0.6</td>
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<td>C3</td>
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<td>1.7 ± 0.5</td>
<td>93.2</td>
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<td>7.2 ± 3.0</td>
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<td>0.14 ± 0.48</td>
<td>1.5 ± 0.6</td>
<td>93.8</td>
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<tr>
<td>C5</td>
<td>6.9 ± 2.9</td>
<td>5.9 ± 3.1</td>
<td>0.14 ± 0.53</td>
<td>1.5 ± 0.6</td>
<td>74.8</td>
</tr>
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<td>6.8 ± 2.9</td>
<td>6.2 ± 3.5</td>
<td>0.14 ± 0.40</td>
<td>1.4 ± 0.5</td>
<td>77.0</td>
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<td>D1</td>
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<td>2.5 ± 0.8</td>
<td>76.0</td>
</tr>
<tr>
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<td>9.8 ± 4.2</td>
<td>4.6 ± 2.3</td>
<td>0.12 ± 0.36</td>
<td>2.5 ± 0.7</td>
<td>75.5</td>
</tr>
</tbody>
</table>
average TP concentrations after CH₃COOH pretreatment were similar to those of the control and higher than those of river water, which differed from the samples that received pretreated cattail litter.

**Effects of alkali/oxidation pretreatments**

The CODₘₚₙ levels were higher in the pretreated groups than in the blank controls and the trends were similar to those observed in river water. The highest CODₘₚₙ concentrations in samples containing pretreated reed litter were 13.19 and 13.42 mg/L on day 3, which were 38.35% and 40.78% higher than the bank control, respectively. The average values of the treated groups were 9.93 and 9.74 mg/L, which were 88.26% and 84.69% higher than those of river water, respectively. Although the CODₘₚₙ was slightly lower in the D1 pretreatment group than in the D2 pretreatment group, it remained at a higher level than that of the controls, especially after 17 d. The CODₘₚₙ of water samples containing D2-pretreated materials fluctuated, and the value was lower than that of the blank control at 20 d. Accordingly, the D1 pretreatment was more suitable for engineering applications than the D2 pretreatment.

The TN values of the alkali/oxidation-pretreated groups were similar to those of the blank control, but those of the D2 group varied. The TP values of the treated groups were slightly lower than those of river water and decreased initially, then increased.

**Effects of C/N**

In general, external carbon sources should be added to CW systems when the C/N is below 3–5 to facilitate denitrification (Liu et al. 2010). The C/N increased after addition of cattail litter pretreated using six different treatments (Table 2). The C/N of the water sample containing cattail litter treated with 1% NaOH for 1 h had the highest C/N value (6.4), which was six times greater than that of river water and 39.15% higher than that of blank controls. However, the C/N of the water sample containing 0.5% NaOH-pretreated cattails for 2 h was only 1.7 (the lowest C/N), which was 56.51% higher than that of river water and 26.61% of the maximum. Overall, the C/N values of NaOH-pretreated groups were higher than those of H₂SO₄-pretreated groups. As shown in Table 2, after adding reed litter pretreated with CH₃COOH, the C/N values were lower than those of the blank controls. In addition, the C/N ratio of the water sample subjected to 40% CH₃COOH pretreated reeds for 12 h hours decreased to the lowest value (1.4), which was 76.46% of the blank control and only 34.87% higher than that of the river water. After the two alkali/oxidation pretreatments, the C/N ratio was 2.5, or 30.59% higher than that of the blank control.

**Effects of residues**

The plant litter was collected and weighed to analyze the decomposition after different pretreatments. Table 2 shows the residues of cattail and reed litter. The decomposition rate of cattail litter subjected to NaOH pretreatment increased with increasing NaOH concentration, even when the same heating time was used. When the H₂SO₄ concentrations were too high or low, the amounts of residues decreased. The greatest amount of residue was in the reed litter pretreated with a low concentration of CH₃COOH (93.8% after the 12 h pretreatment). This was 53.08% higher than the lowest value of 61.3%.

**Suggestions for engineering applications**

The service time of carbon resources used in CWs may determine whether alkali- or sulfuric acid-pretreated cattail litter should be used. Addition of cattail litter pretreated with 1% NaOH with 1 h heating could increase the nitrogen removal efficiency so rapidly that it could be used to improve water quality when urgently needed. However, it may be better to add un-treated cattail, because this can enable the release of bioremediating organisms that persist for almost 1 month. Mild alkali/oxidation pretreatments can effectively improve the C/N and function at room temperature. Therefore, mild alkali/oxidation pretreatments with 1% NaOH and 0.3% H₂O₂ are suitable for engineering applications.
CONCLUSION

In this study, the COD$_{Mn}$ concentrations in water samples containing materials pretreated with NaOH, H$_2$SO$_4$ and CH$_3$COOH were lower than those of the control blank. The COD$_{Mn}$ concentration markedly increased after addition of reed litter subjected to mild alkali/oxidation pretreatment. The TN of water samples in the NaOH, H$_2$SO$_4$, and mild alkali/oxidation pretreatment groups did not increase, while the TP of the NaOH and H$_2$SO$_4$ pretreatment groups decreased significantly. Our results indicate that NaOH, H$_2$SO$_4$, and mild alkali/oxidation pretreatments are suitable for engineering applications.

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