Performance of constructed wetlands with different substrates for the treated effluent from municipal sewage plants
Cao Shiwei, Jing Zhaoqian, Yuan Peng, Wang Yue and Wang Yin

ABSTRACT

Constructed wetlands (CWs) are effective as an advanced treatment process for the treated effluent of municipal wastewater plants. An appropriate substrate, suitable macrophytes, and proper operation are crucial for pollutant abatement. In this research, three subsurface flow CWs with various substrates were investigated. Pollutants abatement efficiency under various operational schemes were analyzed. The results showed that the satisfactory hydraulic loading rate was 0.25 m³/(m²·d). When the C/N ratio of influent was adjusted to 5.87 by adding a carbon source, the denitrification and deposphorization efficiency would be improved, with 7–8 mg/L for total nitrogen (TN) and 0.4 mg/L for total phosphorus (TP) in the effluent, which can achieve the Class 1A Discharge Standard for discharge to natural waterways in China. A greater depth of submersion for the substrate layer resulted in a more conducive environment for the abatement of nitrogen substances. However, a 40-cm depth of submersion in CWs results in better removal efficiency of TN and TP. A plastic ring substrate (PRS) contains biological enzyme promoter formula, which was conducive to nitrifying and denitrifying bacteria. The biofilm affinity and coordination with plants made the PRS more effective than the other two substrates, especially for NO₃-N and TN abatement efficiency.

Key words | advanced treatment for treated effluent, C/N ratio, hydraulic loading rate, submerged depth, substrates, subsurface flow CWs

INTRODUCTION

The treated effluent from a municipal sewage plant may contain pollutants that contribute to eutrophication of waterbodies when the effluent is directly discharged. Different countries have different requirements for treated effluent discharged into natural rivers, as shown in Table 1 (American Water Works Association 2009; Smith & Guo 2019).

The Pollutant Discharge Standard for municipal sewage treatment plants was further restricted to Class 1A in China, which may require advanced wastewater treatment to meet the standard (Discharge Standard of Water Pollutants DB11/307-2005 2005). Because of deficits of carbon sources in the treated effluent, denitrification may not be fully carried out. The main excess-standard pollutants in the treated effluent were ammonia nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃-N), total nitrogen (TN), and total phosphorus (TP) (Leverenz et al. 2010; Liang et al. 2017). Therefore, the denitrification and deposphorization performance were the key factors for the advanced treatment process (Wang et al. 2016). Compared with many other wastewater treatment technologies, constructed wetlands (CWs) can be easily constructed, which provides an advantage in denitrification and deposphorization performance. CWs are widely applied to...
multiple treatments, including sewage, leather wastewater, wastewaters containing hormones, milk factory wastewater, landscape water, and rainwater (Zhaoqian et al. 2015; Toro-Vélez et al. 2016; Carvalho et al. 2017).

The pollutant abatement efficiency of CWs depended on the function of substrates, macrophytes, and microbes within the CW (Huang et al. 2016; Ilyas & Masih 2017). Appropriate substrate, suitable macrophytes, and proper operations are crucial for CWs treatment efficiency. In this study, three planted CWs with various substrates were evaluated under different operational conditions. The corresponding pollutant abatement efficiencies were monitored and analyzed, aiming to optimize the process for the treated effluent of municipal plants.

**MATERIALS AND METHODS**

**Experimental apparatus**

Three planted subsurface flow CWs were evaluated, with each CW being 1.1 m long, 0.25 m wide, and 0.9 m high (including 0.2 m safe height). A hydrolysis acidification unit was set before the CWs to enhance the biodegradability of the water (Ayaz et al. 2015). Treated effluent flowed into the CW at the upper surface and infiltrated through the system before discharging at the bottom and then drained out through the pipe set outside of the CW. The flowrate of feed into the CWs was controlled by a flowmeter located on the inlet pipe. Different height drainage pipe could result in different submergence depth of the substrate inside the CWs. Water Hyacinths were planted on the surface of the CW with a density of 100 plants/m² to strengthen the pollutant abatement efficiency (experimental apparatus shown in Figure 1).

**Substrate selection**

The substrate was an important part of CWs, which could abate pollutants by physical filtration, chemical reaction, and biodegradation. Substrates had a great impact on the treatment effect because of its features (Herrera-Melián et al. 2018). Three substrates were selected to explore the influence for the pollutant abatement efficiency in CWs, including a combinatorial substrate.

1. Plastic ring substrate (PRS) in CW No.1: PRS is a kind of biofilm carrier that is widely applied in biological Table 1 | Pollutant Discharge Standard for treated effluent of several countries

<table>
<thead>
<tr>
<th>Parameter</th>
<th>China (mg/L)</th>
<th>Germany (mg/L)</th>
<th>United States (mg/L)</th>
<th>Japan (mg/L)</th>
<th>European (mg/L)</th>
<th>Russia (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical oxygen demand (COD)</td>
<td>Class 1A 50</td>
<td>Class 1B 60</td>
<td>75 n/a</td>
<td>120</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Biological oxygen demand (BOD₂)</td>
<td>10</td>
<td>20</td>
<td>15 30</td>
<td>120</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Suspended solids (SS)</td>
<td>10</td>
<td>20</td>
<td>n/a 30</td>
<td>150</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Ammonium-nitrogen (NH₄-N)</td>
<td>5</td>
<td>8</td>
<td>10 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Total nitrogen (TN)</td>
<td>15</td>
<td>20</td>
<td>13 n/a</td>
<td>60</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Total phosphorus (TP)</td>
<td>0.5</td>
<td>1</td>
<td>1 n/a</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1 | Experimental apparatus.
contact oxidation processes for wastewater (Zhao et al. 2017). PRS is made of polymer materials with stable properties, large specific surface area, and excellent hydrophilicity, which was beneficial for colonization of a biofilm. As shown in Figure 2(a), the single size of the unit was 11 mm × 7 mm (diameter × height) with an 845 m²/m³ specific surface area and a specific gravity of 150 kg/m³. The nitrophobic microbial enzyme was added in the PRS during the production process to improve the affinity between the substrate, nitrifying and denitrifying bacteria, for a substantial increase in denitrification performance. The PRS used in this research was purchased from Yixing Dieal Technology Co., Ltd, Jiangsu Province, China, which is often applied as a suspended filler in a Moving Bed Biofilm Reactor (MBBR) to treat sewage.

(2) Combinatorial substrate in CW No. 2: A combinatorial layer (FLAZS) was applied in the second CW. In addition to the 300 mm high fly ash ceramsite and 200 mm high zeolite, a 100 mm high gravel layer was set at the bottom as a support and a drainage layer, and a 100 mm fine sand layer was set at the top to fix the plants. Fly ash, a waste generated by coal-fired power plants, was used to make ceramsite by combining with clay and admixture at high temperature. The ceramsite had many active points of Si and Al, and a large specific surface area and a high adsorption capacity (Zhulai et al. 2017). Fly ash from different sources had a variety of chemical compositions and dissimilar internal structures. So, the ceramsite made from different sources of fly ash will subsequently have different adsorption capacities and reactions with chemical substances in CWs. The fly ash ceramsite had an average pore diameter of 20.4 nm and a pore area of 23.325 m²/g. The fly ash ceramsite in this CW had a slightly higher calcium content, as shown in Figure 2(b). The total calcium content was 23.16%, which was proven to improve the abatement of phosphorus from the water (Andreo-Martínez et al. 2017; Shiwei et al. 2019). The substrate with good adsorption capacity can also effectively improve the efficiency of CWs (Zheng et al. 2018). Zeolite, a porous silicate mineral shown in Figure 2(b), is a ‘giant molecule’ composed of silicon oxygen tetrahedrons and aluminum oxygen tetrahedrons that share the oxygen atoms at the tetrahedron vertices. In alumina tetrahedron, trivalent aluminum could not balance the oxygen atom valence electrons, so alkali metal or alkaline earth metal cations were needed. The K⁺ in the zeolite combined with silicon aluminate is weak and might be easily exchanged with other cations, while the replacement does not destroy the structure of zeolite so the zeolite has a high ion exchange capacity. There were pores and channels inside the zeolite because of the different connection modes of silicon oxycetetrahedron and aluminum oxytetrahedron so the specific surface area of zeolite was very large (Mery et al. 2012), which is in the range of 500–1,000 m²/g. In this research, zeolite and ceramsite were the main biofilm carriers in CW No. 2.

(3) Fly ash ceramsite substrate (FACS) in CW No. 3: The substrate of the third CW device was like the second one, a multiple layer with a gravel layer at the bottom and fine sand layer at the top. The difference was that another fly ash ceramsite with a slightly higher carbon content was set in the middle, as shown in Figure 2(c). This fly ash ceramsite’s pore area reached 79.605 m²/g because including a combinatorial substrate.

Figure 2 | Selected substrates. (a) Plastic ring substrate (PRS). (b) Combinatorial substrate including zeolite substrate and fly ash ceramsite with more calcium element. (c) Fly ash ceramsite with more carbon element.
of 8.67% carbon elements that remained after firing and had an internal aperture of 22.0 nm. Zeolite was no longer used as an auxiliary because of its huge pore area.

**Pollutant index of influent**

An artificial influent was made to emulate the treated effluent of the municipal sewage plant. Activated sludge from the secondary sedimentation tank of Nanjing Qiaobei Municipal Sewage Treatment Plant, Jiangsu Province was used as the inoculation fluid for the influent. \( \text{NH}_4\text{Cl}, \text{NaNO}_3, \text{KH}_2\text{PO}_4, \) and low molecular organic carbohydrates were applied to simulate the pollutants of the water. The pollutants index is shown in Table 2.

The abatement of nitrogen and phosphorus nutrient pollutants was the goal of the treated effluent. So, TN, TP, \( \text{NH}_4^+\text{-N}, \) and \( \text{NO}_3^-\text{-N} \) were the indexes that were monitored and compared to the ‘Water and Wastewater Monitoring and Analysis Methods’ (the Fourth Edition) (State Environmental Protection Administration 2002). According to the effluent discharge standard, the CW with a proper substrate and corresponding operational procedures will be demonstrated.

**Research methods**

According to the factors that may influence the pollutant abatement efficiency, the parameters investigated in the research were as follows:

**Hydraulic loading rate**

Hydraulic loading rate (HLR) was an important parameter to characterize the unit area treatment efficiency of CW (Çakir et al. 2015). When CW was filled with different substrates, there were various physical and chemical reactions and biodegradation inside the CW which resulted in different pollutant abatement efficiencies.

<table>
<thead>
<tr>
<th>Pollutants index</th>
<th>COD</th>
<th>( \text{NH}_4\text{-N} )</th>
<th>( \text{NO}_3^-\text{-N} )</th>
<th>TN</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (mg/L)</td>
<td>50–100</td>
<td>8–10</td>
<td>8–10</td>
<td>16–25</td>
<td>1</td>
</tr>
</tbody>
</table>

**Carbon–nitrogen ratio of influent**

Insufficient carbon source inhibited denitrification because of low concentrations of biodegradable organic matter in the treated effluent from sewage plants (Jiang et al. 2017). Thus, it was necessary to test the carbon–nitrogen (C/N) ratio of the influent and assess whether it could satisfy the denitrification and dephosphorization process before further treatment through the CWs. If C/N was too low, a carbon source was usually added to improve the C/N ratio and ensure denitrification and dephosphorization efficiency. However, the appropriate C/N ratio adjusted is crucial for the efficiency of the removal process.

**Submerged depth of substrate**

The design of the submerged layer followed the non-submerged layer along the height of CWs, ensuring the anaerobic condition followed the aerobic condition sequence (Payne et al. 2014), and thus the requirements of biological nitrogen removal could be satisfied. However, anaerobic conditions that followed the aerobic condition were not conducive to biological dephosphorization. Therefore, the appropriate proportion of non-submerged with submerged layers should be determined to not only enhance the biological nitrogen removal effect but also ensure certain biological dephosphorization removal efficiency.

**RESULTS AND DISCUSSION**

**Pollutant abatement efficiency in CWs under different HLR**

Influent with the same pollutant concentrations was fed into three devices. Four HLR levels with 0.1 m\(^3\)/(m\(^2\)-d), 0.15 m\(^3\)/(m\(^2\)-d), 0.25 m\(^3\)/(m\(^2\)-d), and 0.4 m\(^3\)/(m\(^2\)-d) were controlled by the flowmeter. The TN concentration of influent was about 15 mg/L, while its nitrogen ratio (C/N) was 7. The submergence depth of the substrate layer was maintained at 40 cm high. The pollutants abatement efficiency of each device was monitored and compared after the running was stable. The optimal HLR corresponding to each system was determined.
The HLR should match with the capacity of the substrate. Overload hydraulic would result in insufficient retention times, which might inhibit the denitrification. However, because lower HLR corresponded to enough retention time for the bacteria, the dissolved oxygen in the system would be reduced. The deficit of dissolved oxygen was not conducive to the biodegradation of ammonia nitrogen (Zheng et al. 2016; Bonner et al. 2017).

Among the four-level HLR, the removal rate (Figure 3) was highest when the HLR was 0.25 m³/(m²·d). Under this condition, the concentration of NH₄⁺-N, NO₃⁻-N, TN, TP, and COD in the effluent was 1–4, 2–3, 5–7, 0.2–0.4, and 3–12.1 mg/L, respectively. All the indexes could reach the Class 1A Discharge Standard for waterways in China.

In this study, three substrates were carriers of microorganisms and resulted in COD abatement efficiency of 95%. The efficiency of the third device with FLAS was slightly higher than that of the other two for the abatement of NH₄⁺-N, NO₃⁻-N, and TN. For the nitrogen in the water, the removal process was relatively complex. The organic nitrogen in the water must be transformed into NH₄⁺-N in the aerobic condition by ammoniating bacteria, and then NH₄⁺-N could be oxidized into NO₂⁻-N by nitrous acid bacteria, followed by further oxidation to NO₃⁻-N by nitrobacteria. Finally, the NO₃⁻-N was transformed into N₂ by denitrifying bacteria in an anoxic environment (Chyan et al. 2016; Uggetti et al. 2016), so the removal rate of TN depends on the removal rate of NH₄⁺-N and NO₃⁻-N. Although the CW with FACZS was better than the other two in terms of NH₄⁺-N abatement, the CW with PRS was superior in the NO₃⁻-N, so the final TN removal was greatly determined by NO₃⁻-N. Therefore, the TN
removal in the CW with PRS was optimal with 5.03 mg/L in the effluent. Compared with other inorganic substrates, the PRS had biological enzyme promoter formula in its ingredient, which might improve the performance of the biological carrier and result in a more efficient bio-denitrification, especially NO$_3^-$N abatement (Lu et al. 2017). The results suggest that the substrate with nitrogen and microbial enzyme promoter formula could enhance the bio-denitrification process.

There were four functions during the phosphorus abatement of the water including physical adsorption, chemical reaction, biological dephosphorization, and assimilation by macrophytes (Bus & Karczmarcy 2017). Physical adsorption and chemical reaction were relatively simple, mainly determined by the features of the substrates. The biological dephosphorization process was rather complicated, which required the assimilation and release of polyphosphoric accumulating bacteria (PAOs) surviving in different dissolved oxygen environments inside the CWs. In this research, the zeolite and fly ash ceramsite applied into the CW had developed pores inside and calcium content. However, because of its single ingredient, there was no chemical reaction and the physical adsorption was also weak so biological dephosphorization was the main function. However, the CW with PRS had a better TP removal ability than the other two, as shown in Figure 3(d). The TP concentration in the effluent reached 0.2 mg/L with a 72.17% removal rate, which was higher than the other two inorganic substrates. Although the second CW was filled with fly ash ceramsite that had a measurable calcium content and was supposed to assume a promising phosphorus adsorption capacity. Results fell short of expectations, possibly because of limited adsorption capacity or coordination of macrophytes with the ceramsite. Plants transfer oxygen from the air to the inside of CW by means of its roots system to achieve an aerobic environment around its roots, which was different from the anaerobic or anoxic low dissolved oxygen environment far from the roots. Various environments with differing dissolved oxygen concentrations facilitated biological dephosphorization in CWs (Abou-Elela & Hellal 2012). Pollutant abatement efficiency was affected by the growth of plants and the robustness of the system to secrete oxygen from the roots. Water hyacinth was the macrophyte selected for this study because of the oxygen-secreting capacity of the zeolite + ceramsite substrate, even though they had a larger area for the microbial growth. PRS made of organic polymer might prompt the macrophytes with good coordination. The environment inside the CW was suitable for PAOs under dual action of the substrate with a huge specific area and plants with robust roots oxygen-secreting capacity, which resulted in better phosphorus abatement.

The CW with PRS had better biological denitrification and dephosphorization performance than that with FLAS and FLA. Their optimal HLR was 0.25 m$^3/(m^2\cdot d)$.

**Pollutant abatement efficiency in CWs with different C/N ratio of the influent**

The C/N ratio of the treated effluent from municipal wastewater plants ranged from 1 to 3 if no additional carbon source was added. So, the experiments were carried on with eight C/N ratios: in the range of 1.53–6.83, which were adjusted by changing COD concentration while TN concentration was maintained stable at 15 mg/L. An appropriate C/N ratio with good pollutant abatement efficiency would be determined for each CW.

The C/N ratio of influent mainly affected the dominant species of microorganisms that play a key role in the treatment process. An appropriate C/N ratio could maintain the presence of aerobic microorganisms, nitrifying and denitrifying bacteria, phosphorus removal, and accumulating bacteria in different parts of the CWs (Liu et al. 2017). Because the CWs performed well with the HLR of 0.2 m$^3/(m^2\cdot d)$ as previously discussed, the research about the C/N ratio was carried on with this hydraulic load (Figure 4).

According to Figure 4(a) – 4(c), with a relatively low C/N ratio without extra carbon source, the TN of effluent remained around 10 mg/L, which could not meet the discharge standard. Therefore, it was necessary to add a carbon source in the influent. But the NH$_4^+\cdot$N concentration of effluent increased as the C/N ratio increased. This may result from the carbon source which can consume dissolved oxygen in CWs and inhibit the nitration process. The NO$_3^-$N and TN concentration decreases with the increase of C/N, resulting in an inverse correlation with the C/N ratio. The additional carbon source enhanced the reproduction of denitrifying bacteria and
improved NO$_3^-$-N abatement. TN was mainly constituted of NH$_4^+$-N and NO$_3^-$-N, and the removal efficiency of TN gradually increased with the changes of NO$_3^-$-N. The proper C/N ratio should not only ensure the NO$_3^-$-N removal but also the NH$_4^+$-N removal. An excessively high C/N ratio could not increase the abatement efficiency because the consumption of carbon source and the influence of NH$_4^+$-N abatement would be adversely affected. So, based on the nature of the denitrification and effluent requirements for TN, a C/N ratio of influent was better controlled at 5.87, resulting in 55% TN removal, and the effluent concentration was 7–8 mg/L.

According to Figure 4(d), the TP concentration of effluent and the removal rate of TP were relatively stable at a lower C/N ratio without adding carbon source, but with the increase of C/N ratio by the additional carbon source, the TP abatement increased and its concentration of effluent decreased to less than 0.4 mg/L. The growth of PAOs also required an appropriate carbon source (de Rozari et al. 2016). The increase of the carbon source was conducive to the growth of PAO and had a positive impact on the removal of TP.

CW with PRS for the treatment of NH$_4^+$-N was slightly worse than the other two treatments in terms of nitrogen abatement, but for the treatment effect of NO$_3^-$-N it was slightly better than the other two and overall advantageous for TN abatement. More denitrifying bacteria produced higher removal efficiency for TN.

The CW with FLAS performed the worst for pollutant abatement efficiency. Even when the C/N ratio was adjusted...
to 8, the TN removal rate could only reach around 55%. For the indicator TP removal, the trend of the three with the change of C/N ratio was similar, but the actual removal rate showed a larger difference. CW with PRS performed best, followed by the FLAS. The CW with FLAZS had the worst performance because the phosphorus adsorption capacity was quickly exhausted during the initial period. The result was consistent with previous research on HLR. The pollutant abatement of wastewater depended on the physical, chemical, and biological processes. Although there was only a biological dephosphorization process in the CW with PRS, the efficiency was best, indicating that biological dephosphorization was the most sustainable and reliable. Biological dephosphorization efficiency of the CW with FLAZS and FLAS was weaker than that of PRS and might be because of the feather of the substrate itself, or the influence of macrophytes and the coordination between them, which needs to be studied by changing plant species in subsequent studies.

**Pollutant abatement efficiency in CWs with different depth of substrate submergence**

The height of the discharge pipe outside the CWs could be adjusted in the range of 20–60 cm and resulted in corresponding submerged depths of the substrates. A lab test was carried out under the same conditions, with a HLR of 0.2 m³/(m²·d) and an influent C/N ratio of 5, with results shown in Figure 5.

Removal rates of NH₄⁺-N and TN of the three CWs gradually increased with an increased depth of submergence. Compared with a 20-cm depth, the removal rate of NH₄⁺-N was 10% greater than removal with a 60-cm depth. The NH₄⁺-N concentration of the effluent was also stable at about 4 mg/L. NH₄⁺-N substance was transformed in an aerobic environment. Although the submerged depth would result in an anaerobic or anoxic environment inside the CW, which was not conducive to the survival of ammonia nitrogen bacteria, the NH₄⁺-N abatement efficiency increased (Nakamura et al. 2017). There was still enough oxygen for the ammonia nitrogen bacteria existing in the upper part of the CWs because of an increase in contact time and the plant root-secreting oxygen. A greater depth of submergence resulted in a more anaerobic and anoxic environment at

Figure 5 | Removal rate of pollutants in CWs at different depths.
the bottom layer inside the CWs, which might promote the denitrification process with the TN removal rate of 53.69% and 8.914 mg/L TN remaining in the effluent.

TP, however, had the opposite results. In general, removal efficiency decreased with an increase in depth of submergence. When the depth of submergence was 20 cm, the TP removal rate could reach 58.81% and corresponding concentration in the effluent was 0.367 mg/L, while the removal rate decreased to 49% when the submergence depth was 60 cm. Due to the change in the condition aggravating the release of the phosphorus, anaerobic conditions following aerobic conditions were unfavorable for microbially facilitated dephosphorization (Bus & Karczmarczyk 2017). Although the substrate had phosphorus adsorption capacity, it would not be helpful when the substrate reached saturation.

The CW with FLAZS was superior to the other two approaches for NH$_4$-N abatement. When the depth of submergence was 60 cm, the removal rate was 56.32%, and the NH$_4$-N concentration of the effluent water was 3.986 mg/L. Aerobic ammoniation process and adsorption by zeolite in the CW were contributing factors (Wua et al. 2015). The CW with PRS were superior to the other two in terms of TN removal including NH$_4$-N and NO$_3$-N. Though the NH$_4$-N abatement of the CW with PRS was inferior to the other two, it performed well for NO$_3$-N abatement. The biological enzyme promoter formula of the PRS still functioned and contributed to the survival of denitrifying bacteria when the substrate was submerged and enhanced the NO$_3$-N abatement process. The CW with FLAS performed worst for nitrogen removal, with the highest removal rates of NH$_4$-N reaching 47.5% with the effluent concentration of 4.791 mg/L. The CW with FLAZS also performed worst for the TP abatement. The removal rate was up to 51.63% with a corresponding effluent concentration of 4.791 mg/L when the submerged depth of substrate was 20 cm. Maybe the main reason was the saturation of the adsorption capacity of ceramsite (Li et al. 2008).

In conclusion, the denitrogen efficiency trend and the dephosphorization efficiency contradicted each other in terms of the submerged depth of substrate in CWs. In practice, a submerged depth of 40 cm in height ensured high removal efficiency for both nitrogen and phosphorus.

CONCLUSIONS

Three CWs with PRS, FLAZS and FLAS as substrates were investigated separately to explore the performance of the treating effluent from municipal plants. The pollutants abatement efficiency under different HLR and various C/N ratios of the influent and diverse submerged depth of substrate were discussed to determine the optimal substrate and corresponding operational conditions. The results showed that

1. Three CWs achieved a better pollutant efficiency when the HLR was 0.25 m$^3$/(m$^2$·d).
2. When the C/N ratio of influent was adjusted to 5.87 by additional carbon source, the denitrification and dephosphorization efficiency would improve, with 7–8 mg/L for TN and 0.4 mg/L for TP in the effluent, which can satisfy the Class 1A Discharge Standard for natural waterways in China.
3. A greater depth of submergence for a substrate layer in CWs resulted in the greater abatement of nitrogen substances. However, removal efficiency of phosphorus was optimal with submerging depth of 40 cm.
4. Because of the biological enzyme promoter formula contained in PRS, it was the substrate that better promoted habitats for bacteria that participated in the pollutant abatement processes. Biofilm affinity of PRS and coordination with plants was better than the other two substrates, especially for the abatement efficiency of NO$_3$N and TN.

The results of this research are useful for designing CWs for the abatement of nitrogen substances and phosphorus from waste streams. More studies for the PRS in CWs and the corresponding running model are expected.

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