

Existing forms and changes of nitrogen inside of horizontal subsurface constructed wetlands

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Abstract Horizontal zeolite subsurface constructed wetland system (HZCWs) and horizontal limestone subsurface constructed wetland system (HLCWs) were applied to the removal of nitrogen in lightly polluted wastewater, and the existing forms, changes, and removal mechanism of nitrogen in the constructed wetlands were analyzed. The results indicated that compared with HLCWs, HZCWs exhibited better nitrogen removal effect, and the maximum removal rates of ammonia nitrogen and total nitrogen could reach 96.97 ± 5.29 and $93.12 \pm 3.35\%$, respectively. Besides, analysis of the removal effect on nitrogen in different existing forms on different substrate heights in the constructed wetlands showed that variation of nitrogen removal efficiency had certain regularities, which were related to the interior construction features of the wetland systems, and agreed with the regularities in the changes of the influential factors such as DO inside of the wetlands. In addition, degradation mechanism of pollutions was also analyzed, and the results indicated that the quantity of microorganisms and enzymes, including FDA, catalase, and urease, on the surface of the substrates had significant influence on the removal regularities and effects of the major pollutions in constructed wetlands.

Keywords Subsurface constructed wetland system · Nitrogen removal · Substrate height · Microorganism characteristics · Zeolite · Limestone

Introduction

At urban communities in China, most domestic wastewater is usually treated by conventional wastewater treatment facilities that combined primary and secondary treatment processes. Effluent from this kind of systems generally contains relatively high level of nitrogen (N), the discharge of which can contribute to the eutrophication of aquatic ecosystems (Wu et al. 2014; Wang et al. 2015). Therefore, advanced treatment facilities should be applied to meet more rigorous effluent discharge standard.

Wastewater from the sewage disposal plants in Tangshan, boreal China, is first treated by a conventional biochemical wastewater treatment facility. The system could decompose the major organic matter in the wastewater, but it is inadequate at removing nitrogen. The effluent of these sewage disposal plants becomes a major source of nutrients entering Douhe River, a belt water system with landscape purposes. In order to solve this problem and meet the new effluent discharge standard of China, the local government begins to take measures to reduce the nutrient loading from the sewage disposal plants.

In the last two decades, constructed wetlands have turned out to be an efficient ecological technology for the treatment of various kinds of polluted waters (Harrington and McInnes 2009; Larue et al. 2010; Maine et al. 2007; Tang et al. 2015; Verhoeven and Meuleman 1999). The advantages of constructed wetlands over other conventional treatment systems are their lower costs and easier operation and maintenance (Inamori et al. 2007; Khandare et al. 2014). Constructed

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wetland treatment technology has become a standard method of water treatment in many countries because of the requirement for low-carbon, environment-friendly technologies (Brix 1999; Vymazal 2007, 2009).

Because of the popularity of constructed wetland, more and more researches have been done on its pollutant removal effect (Gale et al. 1993; Maltais-Landry et al. 2009; Oon et al. 2016). In these researches, the change in concentration of major pollutants in effluent and influent was generally used to determine the removal effect of pollutants of the entire wetland system (Chang et al. 2012; Fibbi et al. 2012; Sehar et al. 2015). Constructed wetland was a “dark box” system, research on the process and mechanism of the degradation of various pollutions inside of the system was still rare, except for the researches on the growth characteristics of microorganism on the substrate surface, which reached the conclusion that the quantity of nitrifying bacteria and denitrifying bacteria had a significant impact on nitrogen removal effect (Coban et al. 2015; Hu et al. 2014). And there are few researches on the regularities of changes in nitrogen at different layers of substrates and its relationship with dissolved oxygen. In this research, under the condition of step intermittent feeding, numbers of sampling outlets were preinstalled at various substrate heights and flow areas, and variation of nitrogen concentration in effluent taken from different sampling outlets was continuously monitored, then existing forms and removal regularities of nitrogen inside of the constructed wetlands were discussed. Meanwhile, characteristics of the substrates taken from different sampling outlets were studied, and the impact of these characteristics on the nitrogen removal effects in subsurface constructed wetlands was analyzed.

Materials and methods

Subsurface wetland systems

The experiment was carried out in a laboratory with controlled environment in Tangshan, China (36°21'36" N, 118°6'36" E). The horizontal subsurface wetland systems consisted of two wetland mesocosms (1.6 × 0.6 × 0.6 m). Gravel, with a particle diameter of 15–25 mm, was laid at the bottom of the two systems, and the depth was 0.10 m. Zeolite and limestone were laid at the middle layers of the two wetlands, respectively, both with particle diameters of 6–10 mm and depth of 0.20 m. The upper beds consisted of lytag with a depth of 0.15 m and a particle diameter of 3–5 mm. The sieving soils were laid at the uppermost layers, the depth of which was 0.05 m. *Phragmites* sp. and cattail sp. were planted in the soils with the interplanting ratio of 1:1, and the plant density of them was 50 plants/m². The schematic diagram of the main apparatus is shown in Fig. 1.

Horizontally, the constructed wetland was divided into five zones: influent zone, influent buffer zone, main zone of the constructed wetland, effluent buffer zone, and drainage zone. In order to ensure even inlet and outlet, large-sized zeolites (with particle size of 10–15 mm) were filled in influent buffer zone and effluent buffer zone. Raw wastewater flowed into influent buffer zone from the top of it, through the main zone of the constructed wetland, out of the bottom of the effluent buffer zone, into the drainage zone. In addition, in the front of every constructed wetland system, nine wastewater sampling tubes were arranged in three rows and three columns. The designed heights of the three rows were 0.100, 0.300, and 0.450 m (to the bottom), respectively. And the designed widths of the three columns were 0.200, 0.500, and 0.800 m

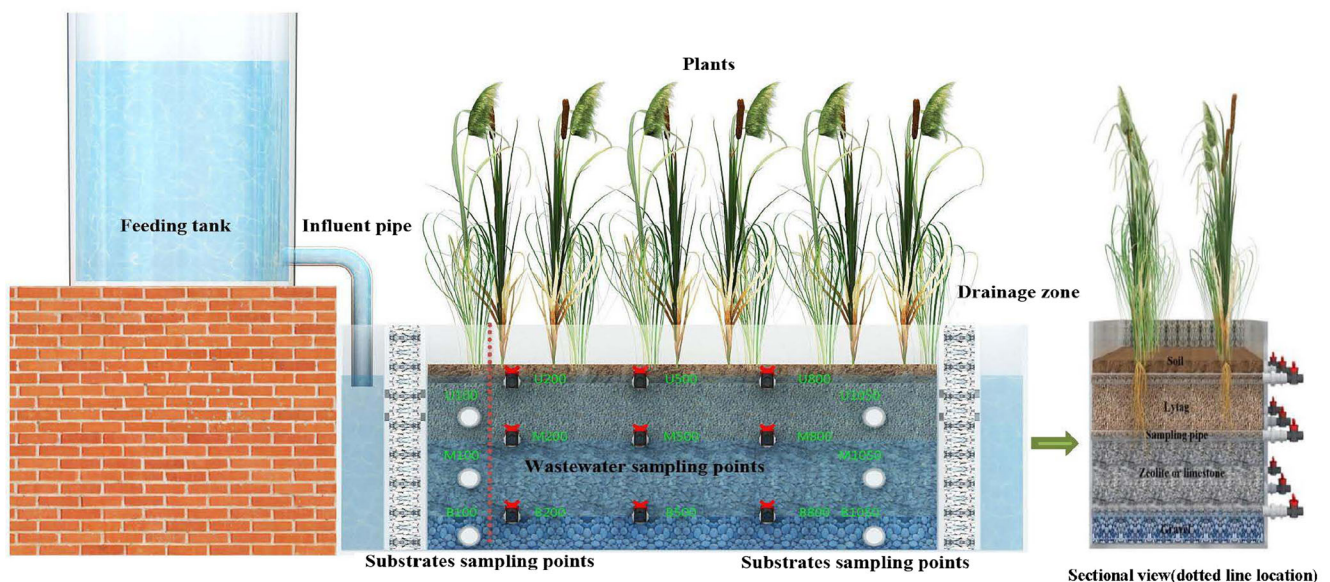


Fig. 1 Schematic diagram of the main apparatus

(to the left edge), respectively. The sampling tubes in the upper row were labeled as U200, U500, and U800, respectively; those in the middle row were labeled as M200, M500, and M800, respectively; and those in the bottom row were labeled as B200, B500, and B800, respectively. Besides, six substrate sampling holes were arranged in three rows and two columns. The designed heights of the three rows were 0.050, 0.200, and 0.375 m (to the bottom), respectively. And the designed widths of the two columns were 0.100 and 1.050 m (to the left edge), respectively. The sampling holes in the upper row were labeled as U100 and U1050, respectively; those in the middle row were labeled as M100 and M1050, respectively; and those in the bottom row were labeled as B100 and B1050, respectively.

Influent quality

The raw wastewater was collected from a wastewater treatment plant in Tangshan. The composition of the influent used in all experiments is shown in Table 1.

Water sampling and analysis

In the experiment, the quality of influent met the secondary emission standard of sewage treatment plant in China, and samples were collected with the intervals of 5d after the hydraulic load ($0.056 \text{ m}^3/(\text{m}^2 \cdot \text{day})$) was controlled intermittently. The nitrogen ($\text{NH}_3\text{-N}$, $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, and TN) concentrations in water samples were obtained according to standard methods (APHA 2005). Water temperature and dissolved oxygen (DO) were measured in situ by a DO meter (HQ 30d 53LED™ HACH, USA).

Enzyme and microbial analysis

At the early, intermediate, and terminal stages of the experiment, substrate samples were collected from sampling holes (U100, M100, B100, U1050, M1050, and B1050). Then all samples were transported to the laboratory immediately and stored at 4 °C. Fluorophotometry, phenol-sodium hypochlorite colorimetry, permanganimetric method, and most probable number method were employed in this research to

investigate FDA hydrolase activity, catalase activity, urease activity, and microbial community composition, respectively, in each horizontal subsurface constructed wetland.

Statistical analysis

All statistical analyses were performed through the statistical program SPSS 17.0 (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) was performed to compare the differences between HZCWs and HLCWs. And two-sample *t* tests were used to evaluate the significance of differences between means. In all tests, differences and correlations were considered statistically significant when $p < 0.05$.

Results and discussion

Nitrogen removal

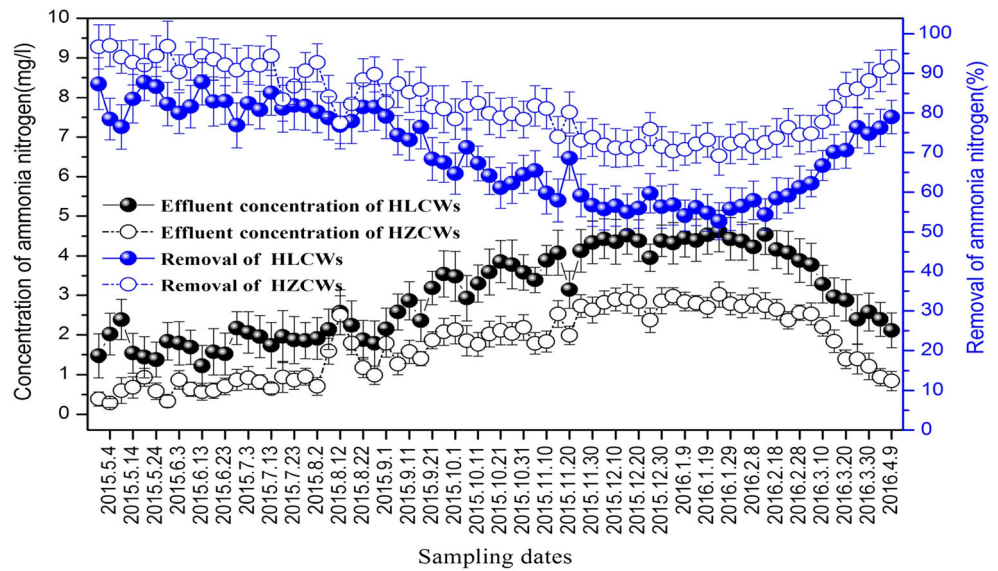
As is shown in Fig. 2, the two constructed wetlands exhibited favorable ammonia nitrogen removal effect on the secondary effluent of wastewater treatment plant under optimal hydraulic loading; the removal rates in the two constructed wetlands could be maintained above $52.71 \pm 4.66\%$, and the ammonia nitrogen concentration of effluent could be maintained under $4.63 \pm 0.52 \text{ mg/l}$ (HLCWs). In addition, HZCWs exhibited optimal ammonia nitrogen removal efficiency, with minimum effluent concentration of $0.28 \pm 0.11 \text{ mg/l}$ and maximum removal rate of $96.97 \pm 5.29\%$ (at early stage). The above data showed that HZCWs, with the zeolite as the main substrate, has satisfactory ammonia nitrogen removal efficiency for secondary effluent of wastewater treatment plant, which contained small quantity of ammonia nitrogen. The high efficiency was associated with characteristics of zeolite; it not only possessed favorable adsorption properties, but also was an efficacious ion-exchange material, and its surface was easy for nitrifying bacteria to adhere to (Zhou and Boyd 2014). Compared with HZCWs, HLCWs exhibited inferior ammonia nitrogen removal effect, with minimum effluent concentration of $1.22 \pm 0.51 \text{ mg/l}$ and maximum removal rate of $87.84 \pm 5.83\%$, which is related with the characteristics of limestone, including the smoother surface and stable chemical characteristic (Hussain et al. 2015).

It can also be seen in Fig. 2 that after the constructed wetlands steadily operated for 4 months, the ammonia nitrogen removal rates declined when autumn came. On one hand, this was probably because the reproduction speed of degradation microorganism decreased with the drop of the temperature. On the other hand, when clog condition started to appear in the wetland system, reoxygenation speed reduced; thus, nitrifying bacteria activity slowed down. When winter came, in the two constructed wetlands, the ammonia nitrogen removal rates reached the minimum value $69.14 \pm 4.86\%$ in HZCMs

Table 1 Characteristics of the wastewater sample used in the experiments

Parameter	Concentration, mgL^{-1}
pH	6.5~8.0
Chemical oxygen demand (COD)	31.6~81.1
Ammonia nitrogen ($\text{NH}_3\text{-N}$)	9.5~11.7
Total nitrogen (TN)	15.7~21.1
Total phosphorus (TP)	0.89~1.22

Fig. 2 Ammonia nitrogen removal effect in constructed wetlands under optimal hydraulic loading (removal rates and concentrations of $\text{NH}_3\text{-N}$ are shown as the means \pm s.d., $n = 3$)

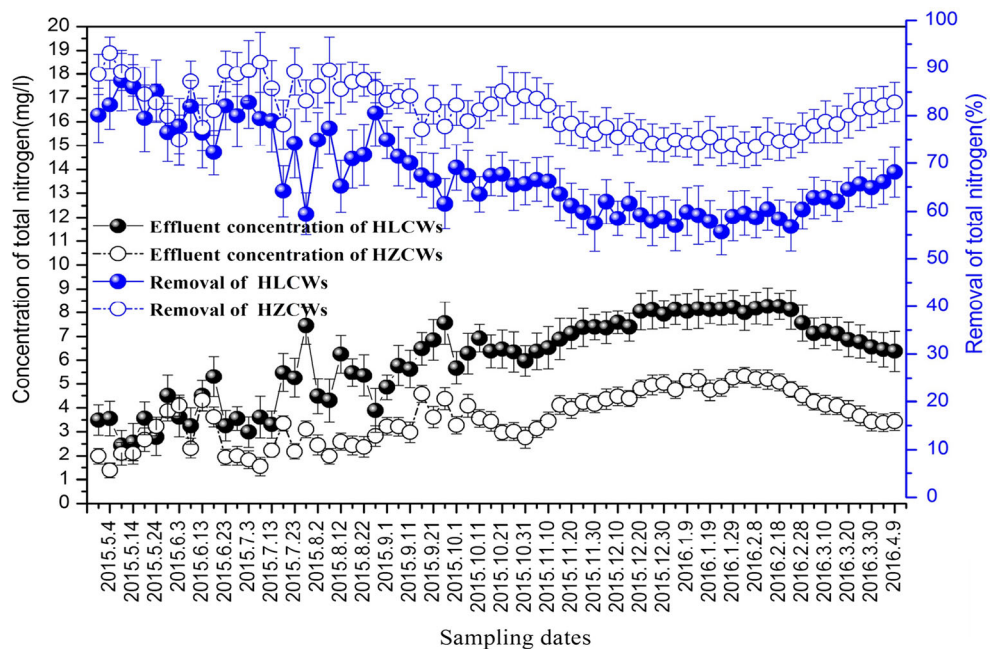


and $52.71 \pm 4.66\%$ in HLCMs. In the spring of 2015, as the temperature rose, the ammonia nitrogen removal effect in the two wetlands began to increase gradually and tended to become stable.

As is shown in Fig. 3, total nitrogen removal showed similar tendency with ammonia nitrogen removal in the two constructed wetlands. Continuous experiments were carried out under the condition of the same hydraulic loading, and two constructed wetlands exhibited favorable total nitrogen removal effect, with effluent concentration kept under 8.27 ± 0.56 mg/l, basically, and removal rates above $55.61 \pm 4.78\%$. In the initial stage of the experiment, the difference of total nitrogen removal effects in the two

constructed wetland was not significant, but the removal rates of HZCWs were still higher than HLCWs ($p < 0.05$). After the constructed wetland systems operated for 2 months, the total nitrogen removal effects in the two wetland systems began to show great differences, total nitrogen removal efficiency of HLCWs decreased, while HZCWs exhibited better removal effect, with the removal rates maintained at $77.08 \pm 3.20\sim 93.12 \pm 3.35\%$ before autumn, compared with $59.42 \pm 4.38\sim 87.40 \pm 6.38\%$ in HLCWs. The higher removal efficiency of HZCWs proved the preferable denitrification performance of zeolite compared with limestone. It also showed that HZCWs can provide better habitat for nitrifying bacteria and denitrifying bacteria, which also promoted the

Fig. 3 Total nitrogen removal effect in constructed wetlands under optimal hydraulic loading (removal rates and concentrations of TN are shown as the means \pm s.d., $n = 3$)



denitrification (Li et al. 2015). Similar to ammonia nitrogen removal, the total nitrogen removal efficiency in the two constructed wetlands decreased when autumn came. On one hand, this is probably because the reproduction and activity speed of the degradation microcosm dropped as a result of the lower temperature; on the other hand, denitrification course was probably obstructed by the insufficient carbon source in constructed wetlands (Shen et al. 2015; Waki et al. 2015). During the winter, the total nitrogen removal rates reached the minimum of 55.61 ± 4.78 and $72.87 \pm 3.77\%$ in the HLCWs and HZCWs, respectively. In the spring of 2015, as the temperature rose, the total nitrogen removal effect began to increase gradually and tended to become stable, which was similar to the ammonia nitrogen removal.

In summary, the two constructed wetlands both have noticeable nitrogen removal effect, while HZCWs have better performance; for ammonia nitrogen, the effluent concentrations and removal rates in HLCWs could be maintained at $1.22 \pm 0.51 \sim 4.63 \pm 0.52$ mg/l and $52.71 \pm 4.66 \sim 87.84 \pm 5.83\%$, respectively, compared with $0.28 \pm 0.11 \sim 3.02 \pm 0.33$ mg/l and $69.14 \pm 4.86 \sim 96.97 \pm 5.29\%$ in HZCWs; for total nitrogen, the effluent concentrations and removal rates in HLCWs could be maintained at $2.43 \pm 0.63 \sim 8.27 \pm 0.56$ mg/l and $56.76 \pm 5.13 \sim 87.40 \pm 6.38\%$, respectively, compared with $1.38 \pm 0.31 \sim 5.36 \pm 0.35$ mg/l and $72.87 \pm 3.77 \sim 93.12 \pm 3.35\%$ in HZCWs.

Analysis of removal effects on major pollutants in different substrate heights and flow areas

In the middle of August, 2015, when the operation of the constructed wetlands was in the favorable stage, influent was controlled intermittently, namely, wastewater was sampled when hydraulic loadings were 0.056, 0.070, 0.093, 0.139, and 0.278 m/day. Then nitrogen removal effects, existing forms, and changes of nitrogen inside of HZCWs and HLCWs were investigated, respectively. In addition, according to different substrate heights and flow areas, each constructed wetland was divided into three parts: horizontally: fore part (including U200, M200, and B200), middle part (including U500, M500, and B500), and rear part (including U800, M800, and B800); and three layers vertically: upper layer (including U200, U500, and U800), middle layer (including M200, M500, and M800), and bottom layer (including B200, B500, and B800). Moreover, variations of nitrogen concentration in the effluent taken from sampling outlets were analyzed. Variations of nitrogen concentration inside of HZCWs under different hydraulic loadings were illustrated by graphs Fig. 4a, Fig. 4b, Fig. 4c, Fig. 4d, and Fig. 4e, respectively; those inside HLCWs were illustrated by graphs Fig. 5a, Fig. 5b, Fig. 5c, Fig. 5d, and Fig. 5e.

Nitrogen removal effects inside of HZCWs under different hydraulic loadings

As is shown in Fig. 4, under different hydraulic loadings, the changes of DO and nitrogen concentrations inside of HZCWs showed that when the hydraulic loadings were 0.070, 0.093, and 0.139 m/day, the removal rates of TN achieved a favorable level in different sampling outlets, and the lowest concentration of TN was 1.02 ± 0.09 mg/l (HZCWs 0.070). In the vertical direction, when the hydraulic loadings were not higher than 0.093, the TN concentrations were generally low in effluent from the sampling outlets of the bottom layer (B200, B500, B800). In contrast, when the hydraulic loadings were higher than 0.093, the TN concentrations were lower in effluent from the sampling outlets of the middle layer (M200, M500, M800). The possible reason for this was when the hydraulic loading was lower, it was easier to form an anoxic environment at bottom layer; at the same time, the carbon source was relatively abundant, which could promote the denitrification reaction, so the total nitrogen concentrations were lower at the bottom layer. However, when the hydraulic loading was higher, the hydraulic retention time was shorter, which made the carbon source inadequate for the bottom layer; at the same time, due to the less oxygen consumption factors as well as the relatively higher DO concentrations, the anoxic condition was not easy to form, so the total nitrogen concentrations were higher at the bottom layer of the wetland system than at the middle layer. In the horizontal direction, when the hydraulic loadings were not higher than 0.093 m/day, the total nitrogen concentrations in the effluent at the sampling outlets in the rear part (U800, M800, B800) of the wetland were higher than those in the fore part (U200, M200, B200) and the middle part (U500, M500, B500). However, when the hydraulic loadings were higher than 0.093 m/day, the TN concentrations in the effluent from the fore part, middle part, and rear part of the wetland showed only slight differences. The possible reason for this was when the hydraulic loadings were lower, there were enough time and carbon source in the fore part and middle part of HZCWs for the degradation of pollutants; however, in the rear part, the degradation effect on TN decreased due to the lack of carbon source. Nevertheless, when the hydraulic loadings were higher, there were little differences in the reaction conditions (including time and carbon source) in the fore part, middle part, and rear part of the wetland; thus, the TN concentration showed slight difference.

Meanwhile, under different hydraulic loadings, the changes of nitrate nitrogen concentrations showed certain regularities, namely, when the hydraulic loading was 0.056 m/day, the concentration of nitrate nitrogen was the lowest, reaching 0.11 ± 0.02 mg/l. This was probably because the reoxygenation speed lowered down as the hydraulic loading decreased, which made the internal nitrification speed decrease and the denitrification speed increase. In addition, in the vertical direction, compared with the bottom layer, the nitrate nitrogen

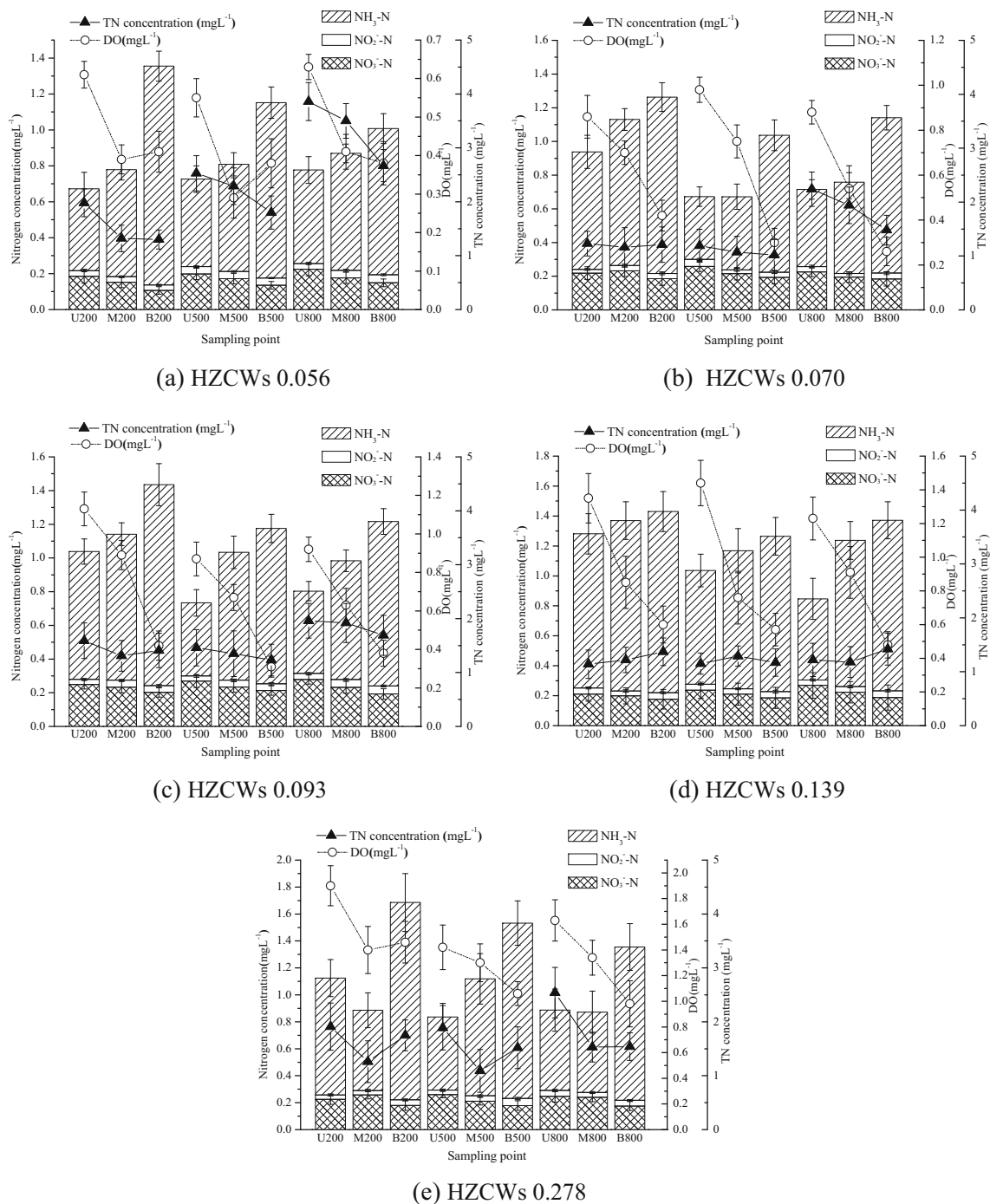


Fig. 4 Removal effect on nitrogenous compounds inside of HZCWs under different hydraulic loadings (concentrations of nitrogen, TN, and DO are shown as the means \pm s.d., $n = 3$)

concentrations in the effluent at the sampling outlets of the upper and middle layers were generally higher, and the maximum concentration reached 0.28 ± 0.03 mg/l (Fig. 4b). This was probably because the DO concentrations were higher at the sampling outlets of the upper and middle layers, which made the nitrification more thoroughly accomplished; on the contrary, an anoxic environment was easy to form in the bottom layer due to the lower DO concentration, which promoted

the denitrification. Besides, in the horizontal direction, compared with the fore part and middle part, the nitrate nitrogen concentrations were generally higher in the rear part.

Different from nitrate nitrogen, the concentrations of nitrite nitrogen at the sampling outlets were lower and did not exhibit obvious regularity. On the other hand, the changes of the ammonia nitrogen concentrations in the effluence from the sampling outlets showed a tendency opposite to that of nitrate nitrogen,

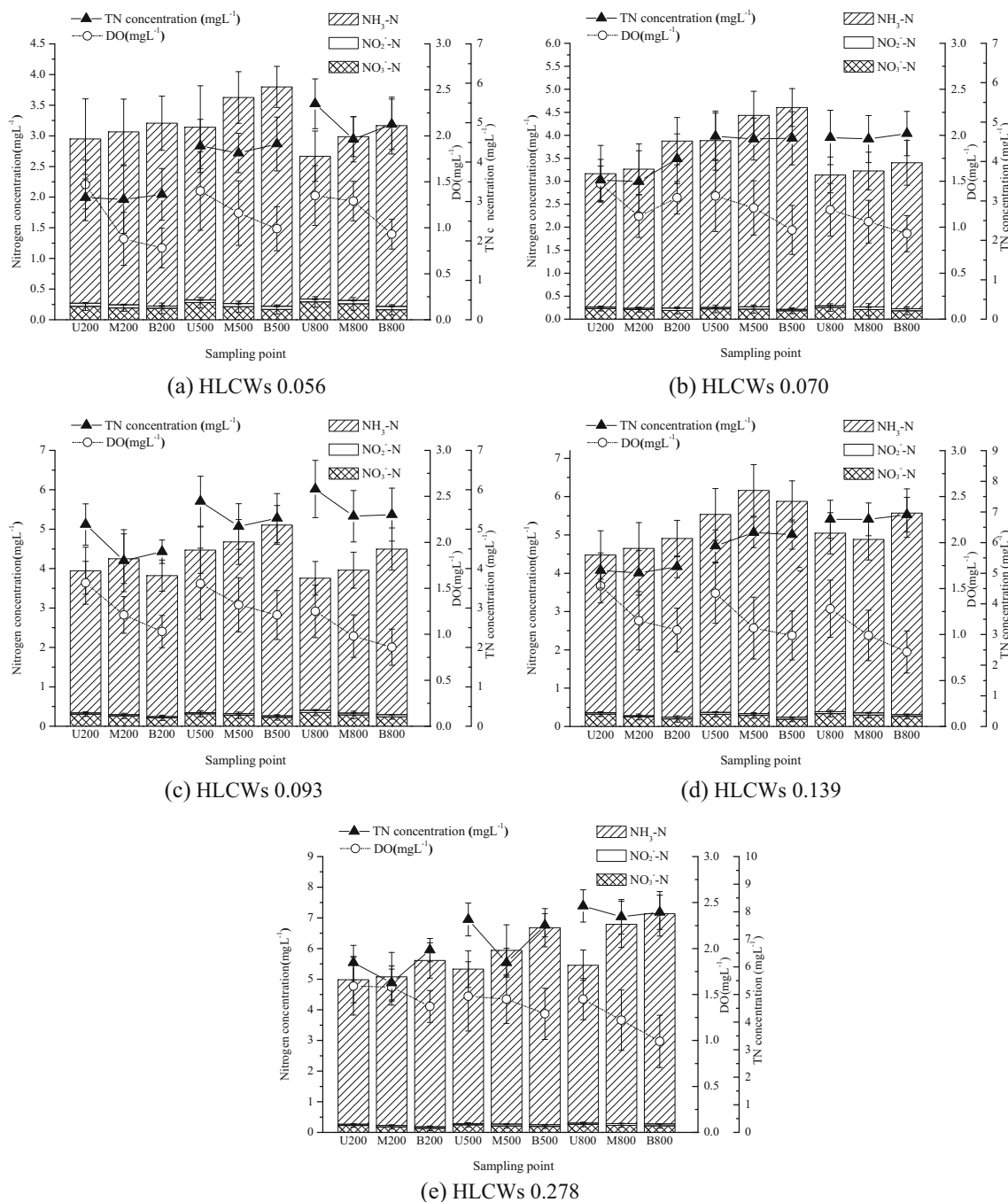


Fig. 5 Removal effect on nitrogenous compounds inside of HLCWs under different hydraulic loadings (concentrations of nitrogen, TN, and DO are shown as the means \pm s.d., $n = 3$)

namely, under different hydraulic loadings, the ammonia nitrogen concentrations were generally lower at the sampling outlets of the upper and middle layers, with the lowest concentration of $0.37 \pm 0.06 \text{ mg/l}$ (Fig. 4b), probably due to the higher DO concentration there, which made the nitrification more thoroughly accomplished. In the horizontal direction, compared with the fore part and middle part, ammonia nitrogen concentrations were generally higher at the rear part. In summary, the distribution of DO inside of the wetland systems was the key factor for the

nitrogen removal. Inside of HLCWs, the DO values exhibited a tendency to diminish vertically, while horizontally, DO concentrations at upper and middle layers did not change obviously, and those at the bottom layer diminished. This was probably because most of the plant roots gathered at the upper and middle layers (Vymazal and Kröpfelová 2015). The oxygen delivered by the roots mainly supplemented the DO of the upper and middle layers; meanwhile, atmosphere reoxygenation also raised the DO concentrations at the upper and middle layer.

Nitrogen removal effect inside of HLCWs under different hydraulic loadings

As is shown in Fig. 5, under different hydraulic loadings, the changes of compounds containing nitrogen inside of HLCWs exhibited certain regularities. Compared with HZCWs, the total nitrogen removal efficiency was lower in HLCWs. When the hydraulic loadings were 0.070 and 0.056 m/day, the removal of TN from the wastewater was most efficient, with the lowest TN concentration at the sampling outlet reaching 3.05 ± 0.54 mg/l (Fig. 5a). Furthermore, similar to HZCWs, in the vertical direction, the TN concentrations in the effluent from the sampling outlets of the middle layer were generally lower, probably because the anoxic environment was easy to form in the middle layer, which could promote the denitrification reaction. In the horizontal direction, the TN concentrations in the effluent from the sampling outlets in the rear part of the system were generally higher than those in the fore part and middle part.

Under different hydraulic loadings, the changes in the NO_3^- -N concentrations also showed certain regularity, namely, in the vertical direction, the NO_3^- -N concentrations in the effluent from the sampling outlets of the upper and middle layers of the system were generally higher, with the highest concentration reaching 0.35 ± 0.01 mg/l (Fig. 5c). The reason for this was the higher DO concentration in the effluent from these layers made the nitrification thorough. In the horizontal direction, the effluent from the sampling outlets in the rear part of the wetland had higher NO_3^- -N concentrations than that from the fore part and middle part, which is probably because of the deposition of NO_3^- -N in the rear part after its accumulation and flow in the fore part.

Different from the concentrations of NO_3^- -N, the concentrations of NO_2^- -N in the effluent from the sampling outlets were low and did not show obvious regularity. On the other hand, the NH_3 -N concentrations in the effluent from the sampling outlets exhibited a tendency opposite to that of NO_3^- -N, namely, under different hydraulic loadings; in the vertical direction, the NH_3 -N concentrations in the effluent from the upper and middle layers were generally lower, with the lowest concentration of 2.33 ± 0.33 mg/l (Fig. 5a), which is because of the higher DO concentrations in the effluent from these layers made the nitrification thorough. In the horizontal direction, the effluent from the sampling outlets of the rear part of the wetland had lower NH_3 -N concentrations than that from the fore part and middle part, probably because the NH_3 -N was partly removed by the other parts of the wetland, and some of the NH_3 -N was already transformed to NO_2^- -N and NO_3^- -N before the effluent arrived at the rear part. Compared with HZCWs, the NH_3 -N removal efficiency was lower in HLCWs.

In summary, the distribution of DO inside of the wetland systems was the key factor for the nitrogen removal. Inside of

HLCWs, the DO values exhibited a tendency to diminish vertically, while horizontally, DO concentrations at upper and middle layers did not change obviously, and those at the bottom layer diminished. This was probably because most of the plant roots gathered at the upper and middle layers. The oxygen delivered by the roots mainly supplemented the DO of the upper and middle layers; meanwhile, atmosphere reoxygenation also raised the DO concentrations at the upper and middle layer.

To sum up, according to the design features of the wetland, the changes in the forms and concentrations of nitrogen inside of the HZCWs and HLCWs both displayed certain regularities, which agreed with the regularities in the changes of the influential factors such as DO inside of the wetlands. In addition, we found statistically significant differences ($p < 0.05$) between different nitrogen removal efficiencies in vertical direction and horizontal direction.

Analysis on the characteristics of enzyme and microbiology in the wetland systems

In the complicated wetland systems, the accumulation of different enzymes results from the activities of soil microorganisms, wetland fauna, and the roots of plants. Enzymes participate in the decomposition and composition of the humus, the hydrolysis of organic compounds, and the wrack of higher plants and microorganisms, as well as the redox reactions (Chang et al. 2015). They also function in the biological cyclic process of elements such as nitrogen, carbon, sulfur, and phosphorus. Especially in the initial stage in the decomposition of the wrack of organisms and the transformation of some inorganic compounds, and under conditions unfavorable for the reproduction of microorganisms, the enzymes and microorganisms on the surface of substrate together promoted the transformation of substance (Meng et al. 2014). In addition, the organic matter in the wetland system is removed first through the physical actions of separation and retention, then through the degradation by microorganisms; and the removal of nitrogen mainly relies on the nitrification and denitrification in the aerobic and anaerobic areas around the roots of plants (Sochacki et al. 2014a, b). To sum up, the microorganisms in the wetland system play an important role in the degradation of the major pollutants.

Analysis on the characteristics of enzyme on the substrate of the wetland systems

In this experiment, the activity of FDA hydrolase, catalase, and urease was tested, the characteristics of each index were analyzed, and the pollutant removal mechanism in the wetland system was discussed.

When the operation of the wetland systems entered the stable stage, on Sep. 1, 2015 and Jan. 7, 2016, the activity of

enzymes on the substrate was tested, respectively. The results are shown in Table 2.

Among the characteristics of all the enzymes on the substrates, FDA hydrolysis involved different kinds of enzymes, which reflected the change in the activity of total microorganism in the soil; catalase released the living organisms from the toxicity of hydrogen peroxide; and the urease activity was in proportion to the removal of nitrogen. As is shown in Table 2, with the passing of time, the FDA activity, catalase, and urease all increased, which showed because of the high temperature in summer, the activity of living organisms was higher. This also contributed to the high nitrogen removal efficiency.

In HZCWs, the activity of FDA and catalase showed a rising tendency from the fore part to the rear part horizontally, which indicated that the content of hydrogen peroxide was higher in the rear part of the system than in the fore part, but the living organisms were not poisoned because of the activity of catalase, for the activity of total microorganism was higher in the rear part than in the fore part of the wetland. At the same time, the activity of FDA and catalase displayed a falling tendency from the upper layer to the bottom layer vertically, which indicated that as the depth of substrate grew, the activity of total microorganism decreased gradually, and the nitrogen removal rate also decreased. In addition, the activity of urease showed a falling tendency both from fore part to rear part and from upper layer to bottom layer, which indicated that the change of nitrogen removal rate in HZCWs also had the same tendency, which agreed with the regularities of nitrogen removal efficiency discussed in the former parts. Moreover, when summer came, the activity of the three enzymes changed obviously, which also suggested that the pollutant removal

effect in the wetland was better when the temperature was higher.

The activity of enzymes in HLCWs had the similar tendency with that in HZCWs, namely, the two wetland systems' pollutant removal rates had similar tendency. On the other hand, the above data also showed that the activity of FDA, catalase, and urease was higher in the middle layer of zeolites in reactor HZCWs than that in the middle layer of limestone in HLCWs, which suggested that zeolite was more capable than limestone in the removal of major pollutants. This also showed a good fit to the above discussed experimental results in pollutant removal. Moreover, temperature had great influence on the three enzymes: during spring, summer, and winter, the general tendency of their contents was first rising then falling, which also fitted the tendency in the degradation of pollutants illustrated by Fig. 2 and Fig. 3.

Analysis on the characteristics of microorganisms in the wetland systems

In this experiment, the counts of bacteria, fungi, and actinomycetes were calculated, and the pollutant removal mechanism in the wetland system was further studied. When the operation of the wetland systems entered the stable stage, on May 19, 2015, Sep. 1, 2015, and Jan. 7, 2016, the microorganisms on the substrate were tested, respectively. The results are shown in Table 3.

Among the characteristics of all the microorganisms, the bacterial count was related to the transformation efficiency and speed of nitrate nitrogen, while the count of fungi and actinomycetes was related to the removal effect on the major

Table 2 Activity of enzymes on the substrates

Samples	FDA activity / (μg/100 g)			Catalase activity / (ml/100 g)			Urease activity / (mg/100 g)		
	2015.5.19	2015.9.1	2016.1.7	2015.5.19	2015.9.1	2016.1.7	2015.5.19	2015.9.1	2016.1.7
HZCWs-U100	13.78 ± 2.67	35.29 ± 3.79	12.36 ± 1.32	48.52 ± 4.56	67.96 ± 8.77	37.63 ± 5.29	2.97 ± 0.78	10.04 ± 2.15	2.56 ± 0.57
HZCWs-M100	10.56 ± 1.93	21.36 ± 1.45	10.78 ± 1.68	20.10 ± 2.18	28.64 ± 4.31	17.08 ± 1.83	2.59 ± 0.41	5.74 ± 0.83	2.03 ± 0.55
HZCWs-B100	7.53 ± 1.42	15.97 ± 2.78	5.36 ± 1.05	7.50 ± 1.54	15.06 ± 2.56	8.25 ± 1.32	1.25 ± 0.22	2.97 ± 0.35	1.07 ± 0.31
HZCWs-U1050	31.49 ± 4.03	51.30 ± 3.22	35.36 ± 2.65	23.01 ± 2.79	38.48 ± 5.12	17.78 ± 2.52	2.69 ± 0.67	8.45 ± 1.21	2.53 ± 0.58
HZCWs-M1050	15.34 ± 2.35	44.16 ± 2.15	13.23 ± 1.67	17.20 ± 2.13	6.39 ± 1.56	13.18 ± 1.92	2.22 ± 0.43	7.23 ± 0.98	1.98 ± 0.35
HZCWs-B1050	9.35 ± 1.38	10.47 ± 1.76	10.22 ± 1.07	6.50 ± 1.35	1.14 ± 0.32	3.50 ± 0.86	1.41 ± 0.19	1.88 ± 0.43	1.17 ± 0.24
HLCWs-U100	30.62 ± 5.32	32.24 ± 5.75	23.85 ± 4.41	10.80 ± 1.63	10.87 ± 1.87	8.74 ± 1.21	2.06 ± 0.47	11.67 ± 2.93	1.96 ± 0.52
HLCWs-M100	1.93 ± 0.36	3.92 ± 0.78	2.06 ± 0.55	1.30 ± 0.29	6.12 ± 0.95	2.09 ± 0.73	0.86 ± 0.25	4.13 ± 1.01	1.23 ± 0.68
HLCWs -B100	0.44 ± 0.12	2.59 ± 0.23	0.78 ± 0.25	0.20 ± 0.11	2.18 ± 0.28	0.65 ± 0.16	0.44 ± 0.21	3.98 ± 0.85	0.56 ± 0.17
HLCWs-U1050	34.77 ± 3.73	40.10 ± 5.32	28.65 ± 2.87	13.58 ± 3.53	14.89 ± 2.93	10.26 ± 2.89	2.31 ± 0.73	8.29 ± 1.77	2.22 ± 0.62
HLCWs-M1050	2.28 ± 0.58	21.41 ± 1.88	5.76 ± 1.12	2.41 ± 0.34	1.86 ± 0.75	1.26 ± 0.56	0.95 ± 0.31	4.10 ± 0.99	0.69 ± 0.25
HLCWs-B1050	0.67 ± 0.09	5.49 ± 0.61	2.32 ± 0.23	0.31 ± 0.07	1.73 ± 0.48	0.73 ± 0.19	0.52 ± 0.19	3.94 ± 0.76	0.37 ± 0.11

(1) The FDA activity was represented by the weight of fluorescein (μg) in 100 g of dry substrate after cultivated for 30 min. (2) The catalase activity was represented by the volume of 0.1 mol/l KMnO₄ (ml) consumed by 100 g of dry substrate. (3) The urease activity was represented by the weight of NH₃-N (mg) in 100 g of dry substrate after cultivated for 24 h

Table 3 Quantity of bacteria, fungi, and actinomycetes on the substrates

Samples	Bacterial count (cfu/g)			Fungi count (cfu/g)			Actinomycetes count (cfu/g)		
	2015.5.19	2015.9.1	2016.1.7	2015.5.19	2015.9.1	2016.1.7	2015.5.19	2015.9.1	2016.1.7
HZCWs-U100	$(7.87 \pm 1.35) \times 10^6$	$(8.38 \pm 1.21) \times 10^7$	$(5.35 \pm 1.14) \times 10^6$	$(6.75 \pm 0.87) \times 10^4$	$(1.21 \pm 0.52) \times 10^5$	$(3.27 \pm 0.56) \times 10^4$	$(7.81 \pm 1.37) \times 10^4$	$(3.78 \pm 0.26) \times 10^5$	$(5.34 \pm 1.15) \times 10^4$
HZCWs-M100	$(4.31 \pm 0.97) \times 10^6$	$(1.56 \pm 0.67) \times 10^7$	$(2.16 \pm 0.68) \times 10^6$	$(8.46 \pm 0.72) \times 10^3$	$(8.53 \pm 1.15) \times 10^4$	$(5.37 \pm 0.86) \times 10^3$	$(7.31 \pm 1.06) \times 10^3$	$(6.29 \pm 0.79) \times 10^4$	$(5.26 \pm 0.83) \times 10^3$
HZCWs-B100	$(2.56 \pm 0.27) \times 10^5$	$(6.18 \pm 0.77) \times 10^6$	$(1.79 \pm 0.22) \times 10^5$	$(6.83 \pm 0.43) \times 10^3$	$(3.87 \pm 0.53) \times 10^4$	$(3.67 \pm 0.57) \times 10^3$	$(5.26 \pm 0.89) \times 10^3$	$(2.53 \pm 0.31) \times 10^4$	$(2.86 \pm 0.56) \times 10^3$
HZCWs-U1050	$(5.06 \pm 1.12) \times 10^6$	$(5.15 \pm 0.90) \times 10^7$	$(3.25 \pm 0.93) \times 10^6$	$(6.62 \pm 0.87) \times 10^3$	$(8.75 \pm 0.56) \times 10^4$	$(4.21 \pm 0.54) \times 10^3$	$(5.67 \pm 0.64) \times 10^4$	$(2.26 \pm 0.25) \times 10^5$	$(3.98 \pm 0.57) \times 10^4$
HZCWs-M1050	$(3.13 \pm 0.25) \times 10^6$	$(3.88 \pm 0.63) \times 10^7$	$(2.35 \pm 0.46) \times 10^6$	$(8.07 \pm 1.25) \times 10^3$	$(4.66 \pm 0.72) \times 10^4$	$(5.13 \pm 1.09) \times 10^3$	$(3.72 \pm 0.23) \times 10^3$	$(5.48 \pm 0.64) \times 10^4$	$(2.16 \pm 0.36) \times 10^3$
HZCWs-B1050	$(4.67 \pm 0.63) \times 10^5$	$(5.39 \pm 0.85) \times 10^6$	$(3.13 \pm 0.35) \times 10^5$	$(4.35 \pm 0.63) \times 10^3$	$(7.19 \pm 0.25) \times 10^4$	$(3.15 \pm 0.52) \times 10^3$	$(8.13 \pm 1.35) \times 10^2$	$(2.79 \pm 0.28) \times 10^4$	$(5.24 \pm 1.02) \times 10^2$
HLCWs-U100	$(7.23 \pm 1.87) \times 10^6$	$(6.46 \pm 1.25) \times 10^7$	$(5.12 \pm 1.31) \times 10^6$	$(6.45 \pm 0.54) \times 10^3$	$(7.62 \pm 1.11) \times 10^4$	$(4.46 \pm 0.63) \times 10^3$	$(7.45 \pm 1.12) \times 10^3$	$(1.13 \pm 0.25) \times 10^5$	$(5.26 \pm 1.09) \times 10^4$
HLCWs-M100	$(4.34 \pm 0.85) \times 10^6$	$(7.58 \pm 0.82) \times 10^6$	$(4.57 \pm 0.65) \times 10^6$	$(7.35 \pm 1.37) \times 10^3$	$(4.26 \pm 0.82) \times 10^4$	$(5.13 \pm 1.07) \times 10^3$	$(8.23 \pm 0.85) \times 10^3$	$(3.15 \pm 0.93) \times 10^4$	$(7.65 \pm 0.98) \times 10^3$
HLCWs-B100	$(5.69 \pm 1.04) \times 10^5$	$(4.55 \pm 1.16) \times 10^6$	$(3.98 \pm 0.57) \times 10^5$	$(5.46 \pm 0.89) \times 10^3$	$(1.08 \pm 0.21) \times 10^4$	$(3.95 \pm 0.58) \times 10^3$	$(6.64 \pm 0.62) \times 10^3$	$(1.81 \pm 1.27) \times 10^4$	$(5.32 \pm 0.43) \times 10^3$
HLCWs-U1050	$(4.15 \pm 0.73) \times 10^6$	$(3.47 \pm 0.58) \times 10^7$	$(2.45 \pm 0.38) \times 10^6$	$(5.38 \pm 0.69) \times 10^3$	$(5.79 \pm 0.63) \times 10^4$	$(4.13 \pm 0.53) \times 10^3$	$(7.56 \pm 1.01) \times 10^3$	$(6.30 \pm 0.75) \times 10^4$	$(6.12 \pm 1.26) \times 10^3$
HLCWs-M1050	$(1.38 \pm 0.36) \times 10^6$	$(1.62 \pm 0.23) \times 10^7$	$(1.12 \pm 0.26) \times 10^6$	$(4.12 \pm 0.61) \times 10^3$	$(1.83 \pm 0.26) \times 10^4$	$(2.67 \pm 0.72) \times 10^3$	$(4.87 \pm 0.77) \times 10^3$	$(2.74 \pm 0.32) \times 10^4$	$(1.95 \pm 0.53) \times 10^3$
HLCWs-B1050	$(1.57 \pm 0.41) \times 10^5$	$(3.17 \pm 1.75) \times 10^6$	$(1.19 \pm 0.32) \times 10^5$	$(1.86 \pm 1.02) \times 10^3$	$(2.13 \pm 0.22) \times 10^3$	$(1.38 \pm 0.97) \times 10^3$	$(1.95 \pm 0.26) \times 10^3$	$(1.86 \pm 0.12) \times 10^4$	$(1.26 \pm 0.37) \times 10^3$

pollutants in the wastewater. As is shown in Table 3, the count of bacteria, fungi, and actinomycetes on the substrate of the two wetland systems showed a falling tendency both vertically and horizontally, and the amount of the three microorganisms was kept in the order of magnitude of $10^5 \sim 10^7$, $10^3 \sim 10^5$, and $10^3 \sim 10^5$ cfu/g, respectively. This suggested that the fore part and upper layer of the wetland systems had better effect on the removal of major pollutants, which fitted the results of nitrogen removal discussed in the former parts.

In addition, the count of microorganisms on the substrate in HZCWs was obviously larger than that on the corresponding layer of substrate in the HLCWs, which showed that the HZCWs was more suitable for the growth of microorganisms, and its micro-biological degradation effect of the major pollutants in the wastewater was better than HLCWs', which fitted the experimental results (Song et al. 2011; Song et al. 2010). Moreover, after the wetland systems operated stably for several months, under the same conditions, the microorganisms on the substrate of the two wetland systems grew faster when the temperature was higher, and the number of microorganisms obviously increased. In contrast, when the temperature fell, the count of microorganisms obviously decreased. This was similar with the activity of enzymes and agreed with the nitrogen removal effect discussed in the former parts.

Conclusion

Existing forms and removal mechanism of nitrogen inside of the two constructed wetlands were studied. And the results showed that HZCWs had generally better nitrogen removal effect. The analysis on the removal effect on nitrogen in different forms inside of the subsurface wetland showed that the changes in nitrogen removal effect inside of the HLCWs and HZCWs were in accordance with the laying characteristics of the wetland systems. In addition, the quantity of enzymes and microorganisms on the substrate of HZCWs was generally larger than that of HLCWs, which greatly influenced the nitrogen removal effect in the wetland systems.

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