The influence of the outlet position and substrate on the nitrogen purification of vertical flow constructed wetlands

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Abstract. To investigate the influence of outlet location on nitrogen removal in Vertical Flow Wetlands (VFWS), three VFWS, that were, wetland H (planting Pennisetum Hydridum), wetland X (planting Pennisetum purpureum Schum.) and wetland CK (no planting) were constructed. High, medium and low water outlets were set in each wetland, namely A, B and C. The main results were as followed: TN (total nitrogen) removal efficiency of A, B, C in H wetland was 61.3%, 55.4%, 53.2 %, and variance analysis was 100.2, 137.1, 177.8. A, B, C in X wetland was 58.3%, 56.6% , 50.2%, and variance analysis was 55.6, 68.8, 116.4. A, B, C in CK wetland was 53.1%, 51.5%, 50.2%, and variance analysis was 131.9, 188. 7, 275.4, respectively. The content of nitrogen and urease activity in the substrate decreased with increasing of substrate depth. These results indicated that planting Pennisetum Hydridum and Pennisetum purpureum Schum. In VFW and appropriately raising outlet position could help improve nitrogen removal efficiency and obtain relatively stable effluent quality, and the upper substrate (0-10 cm) was the main site for nitrogen interception and purification. It can provide theoretical basis for the scientific construction and management of VFW.

1. Introduction
Constructed Wetland (CW) is a new-type sewage treatment technology in recent years, because of low cost, simple management [1-4]. At present, it was used to treat industry sewage, lake pollution, swine farm wastewater, rural wastewater and storm runoff [5-10]. CWs are divided into Surface How Wetland (SFW), Horisontal Subsurface Flow Wetland (HSF) and Vertical Flow Wetland (VFW) depended on water distribution and water flow indirection [11]. VFW generally has higher aerobic treatment capacity, the advantages of small floor area and good treatment effect. In recent years, as the discharge of sewage increasing, the content of nitrogen and phosphorus in sewage has been increasing. Various secondary processes in municipal wastewater treatment plants are not effective in nitrogen removal. Therefore, VFW is an optimal choice for nitrogen removal. Nitrogen is removed by substrate through physical and chemical path way, such as absorption, adsorption, filtration, ion exchange [13], and it can also permanently have removed from sewage when plants are harvested [12]. However, the main mechanisms of nitrogen removal in wetlands are microbial nitrification and denitrification. Nitrification refers to the biological oxidation of ammonium ions to nitrite by autotrophic aerobic microorganism nitrification bacteria, and nitrite is the intermediate in this process. Denitrification refers to the reduction of nitrate to molecular nitrogen by heterotrophic microorganisms under anoxic conditions using carbon sources. It indicated that the oxygen environment determines the removal of nitrogen from wetlands [14, 15]. Study has found that the outlet location can effectively change the DO content of VFW [16]. Different inlet and outlet
modes will also affect the water flow path in the device, leading to the difference in the distribution size of "dead zone", which may be one of the important reasons affecting its hydraulic efficiency [17, 18]. According to the calculation of hydraulic efficiency, it was found that the distribution of outlet locations has a greater impact on small-size wetlands, and the hydraulic characteristics of wetlands could be optimized by changing their locations [19]. Therefore, the study of outlet location has practical guiding significance for the purification of constructed wetland pollutants. In this paper, we consider the type of vegetation, the outlet location and substrate as influencing factors on nitrogen removal in CW. It could provide the basic basis for improving the design of CW and improving the efficiency of nitrogen removal.

2. Materials and methods

2.1. Construction of VFW
Three VFWs were used in this experiment, and each wetland was structured at 200 cm x 100 cm x 50 cm (length x wide x height). The substrate from bottom to top was 10 cm gravel, 30 cm mixed substrate, and 10 cm water distribution area. The experimental wetlands were planting *Pennisetum Hydridum* Wetland, planting *Pennisetum purpureum Schum.* wetland and blank control wetland (represented by H, X and CK respectively). The vegetation planting density in the wetland was 10 plants /m², and these three wetlands were used to treat simulated domestic sewage. At present, soil, river sand and gravel are often used as the substrate in constructed wetlands, but this single substrate was easy to absorb and saturate, and has low efficiency of nitrogen and phosphorus removal [13]. In this paper, the mixed substrate (coarse sand + vermiculite) was selected as the substrate filler, hoping to achieve the ideal sewage treatment effect through a better combination. In order to reduce the influence of external environment on the experimental results, the test device was built within the greenhouse facility. The trial operation was hanging membrane for a month, and began formal operation in April. The water inflow time was 8 hours per day, and the hydraulic load was 0.1 m /d. The water quality index was first time monitored on April 11, and sampling monitoring was conducted weekly. The wetland substrate was collected at the end of the experiment, and the wetland vegetation were harvested after the experiment. The test device was shown in Figure 1.

![Figure 1. Profile map of VFW](image)

2.2. Sample collection
Substrate collection: The sample of substrate collected from various depth which were 0-10 cm, 10-20 cm, 20-30 cm respectively, to measure nitrogen and enzyme activities in substrate. Water collection: Collect water sample every 7 day to measure the temperature (T) and dissolve oxygen (DO), and these indexes test can be done at spot while TN, NH3-N and NO3-N were tested at lab.
2.3. Sample analysis
DO and T were tested daily by using portable hand-held dissolved oxygen meter at spot; water samples were analyzed for TN, NH$_3$-N and NO$_3$-N. Concentration of TN, NH$_3$-N and NO$_3$- were tested according to standard methods [20]; after the experiment, the content of total nitrogen was measured by the Kjeldahl method [21], and urease activity in substrate was monitored by a buffered method [22].

2.4. Statistical method
Excel 2007 and SPSS 22 were used for data processing and drawing.

3. Result and analysis

3.1. The influence of outlet position on DO concentration
The three water outlet (A, B, C) in these three VFWs have various levels of dissolve oxygen. As can be seen from figure 2, there was significant difference between water outlet A and outlet C. The level of DO was lowered as the outlet position decreasing. It demonstrated that the outlet position could have influence on DO content [23]. In this experiment, through correlation analysis, it was found that the dissolved oxygen was extremely significant correlated with the outlet location ($r=-.293^{**}$) (Table 2), that was, with the raising of outlet location, the dissolved oxygen content increased. The DO content in X system (planting *Pennisetum purpureum* Schum.) lower than H (planting *Pennisetum Hydridum*) and CK (no plant). It’s due to *Pennisetum purpureum* Schum. has a denser root system, more respiration and more oxygen consumption. In the experiment, the aboveground biomass of *Pennisetum purpureum* Schum. was larger than that of *Pennisetum Hydridum*, and the atmospheric reoxygenation was reduced due to plant sheltering. Some scholars have shown that in constructed wetlands with an oxygen charge of 5.86 g/m$^2$/d, direct atmospheric re-oxygenation accounts for about 3.76 g, and the oxygen delivered by plants to roots is about 2.1 g. However, 2.08 g of the 2.1 g is used for root respiration, leaving only 0.02 g [24]. So atmospheric oxygen is the main source of oxygen to the wetland [25]. Under natural conditions, the oxygen in the air rare changed, so the water temperature become the main factors affecting the DO level. By monitoring the water temperature at different outlet locations in different wetlands, it was found that within a certain temperature range, the lower the water temperature, the higher the dissolved oxygen. There was a significant correlation between water temperature and dissolved oxygen ($r=-.462^{**}$) (Table 2). Therefore, planting wetland vegetation to control its planting density, timely harvest, to ensure the wetland atmosphere re-oxygenation.

![Figure 2. DO concentration changes at different water outlets in three wetlands](image-url)
3.2. The influence of outlet location on NH$_3$-N removal

The figure 3 showed that from April 11 to June 13, ammonia nitrogen removal rate of total average was 45.9%, 44.9%, 44.3% in H, X and CK respectively. The average removal rate of ammonia nitrogen at different outlets varied from 40% to 51.7%, and the basic performance was A > B > C. Investigate its reason, ammonia nitrogen removal mainly by nitrification, and dissolved oxygen concentration is the main factor influencing the nitrification. In this experiment, the outlet position A have a higher level of DO, more conducive to form more aerobic area, these areas are advantageous to the nitrifying bacteria to ammonia nitrogen into nitrate nitrogen, thus improve the ammonia nitrogen removal rate [26]. Studies have found that the use of aeration to increase DO concentration could improve the nitrification rate of ammonia nitrogen [27]. In this test, DO concentration has extremely correlation with ammonia nitrogen removal (r=.423**) (Table 2).

![Figure 3. NH$_3$-N removal changes at different water outlets in three wetlands](image)

3.3. The influence of outlet location on NO$_3$-N removal

It can be seen from figure 4 that the nitrate effluent concentration of the three wetlands was higher than the influent concentration, indicating that the denitrification of this system was not complete. A study on the treatment of rural grey water by composite vertical flow constructed wetland found that the nitrification and denitrification in the wetland were not sufficient due to the restriction of oxygen supply in vertical flow wetland [28]. The denitrification of microorganisms is a strict anaerobic process. When the concentration of dissolved oxygen in the system exceeds 0.2 mg/L, denitrification is not complete. This was the main reason that nitrate nitrogen removal rate was negative and the effluent concentration was high. It can be seen from the figure 4 that the effluent concentrations of H and X systems are similar and lower than that of CK, indicated that the root exudates of wetland plants could increase carbon source supplement required for denitrification in wetland. Therefore, increasing the substrate thickness in the later improvement experiment could create a suitable anaerobic environment for the denitrification of VFW.
3.4. The influence of outlet location on TN removal

TN (Total nitrogen) removal rate of H, X and CK (Table 1) was decreasing along with the outlet location lowering. TN removal rate was A > B > C, but the variance was A < B < C. TN removal rate of outlet A was highest with the smallest fluctuation range, was also the most stable. The experimental results showed that the location of the outlet was at a certain height of the substrate layer, which was beneficial to improve the total nitrogen removal rate in VFW and was expected to obtain stable effluent quality. On the contrary, the outlet height was set too low, which was not conducive to obtaining a higher total nitrogen removal rate and stable water quality. Studies have suggested that when the wetland is in a saturated water state, the hydraulic retention time is prolonged, which is more conducive to the absorption of plants and microorganisms to remove total nitrogen [29]. In this experiment, the hydraulic retention time of outlet A was longer than that of B and C, and the flow velocity was relatively smaller, which was conducive to the removal of total nitrogen. The variance of CK wetland without planting plants was higher than that of H and X wetlands with planting vegetation, indicating that planting vegetation could reduce the outflow fluctuation and help the outflow water quality of nitrogen stabilize.

Table 1. Mean value and variance of total nitrogen removal rates of each wetland

| wetland | Outlet position | Mean value | Variance |
|---------|-----------------|------------|----------|
| H       | A               | 61.3       | 100.2    |
|         | B               | 55.4       | 137.1    |
|         | C               | 53.2       | 177.8    |
|         | A               | 58.3       | 55.6     |
| X       | B               | 56.6       | 68.8     |
|         | C               | 50.2       | 116.4    |
|         | A               | 53.1       | 131.9    |
| CK      | B               | 51.5       | 188.7    |
|         | C               | 50.2       | 275.4    |
Table 2. The correlation analysis between the outlet location and various factors

|                | DO     | T       | TN%    | NH3-N%  | NO3-N%  | Outlet location |
|----------------|--------|---------|--------|---------|---------|-----------------|
| DO pearson correlation significance (double tails) | 1      |         |        |         |         |                 |
| T pearson correlation significance (double tails)  | -0.462** | 1       |        |         |         |                 |
| TN% pearson correlation significance (double tails) | 0.098  | 0.317** | 1      |         |         |                 |
| NH3-N% pearson correlation significance (double tails) | 0.423** | -0.362** | 0.277** | 0.382** | 1       |                 |
| NO3-N% pearson correlation significance (double tails) | 0.314** | -0.295** | 0.361** | 0.382** | 1       |                 |
| Outlet location pearson correlation significance (double tails) | -0.293** | 0.071  | -0.161 | -0.132  | 0.136  | 1               |

3.5. The accumulation of total nitrogen and urease activity in the substrate

TN accumulation of H wetland was 1.487 mg/kg, that of X wetland was 1.471 mg/kg, and CK wetland was 0.97 mg/kg. The matrix cumulative nitrogen of H wetland and X wetland was close to and significantly higher than that of CK wetland, indicating that planting plants can help improve the capacity of substrate to retain and absorb nitrogen. The distribution of nitrogen content and urease activity in matrix layer decreased with the increasing of matrix depth, that was: 20-30 cm < 10-20 cm < 0-10 cm. The nitrogen content of H in the 0-10 cm layer was 6 times that in the 10-20 cm layer and 10.5 times that in the 20-30 cm layer; the nitrogen content of X in the 0-10 cm matrix layer was 5.8 times that in the 10-20cm layer, and 10.5 times that in the 20-30cm layer; the content of nitrogen in the 0-10cm matrix layer in CK wetland was 4.11 times that in the 10-20 cm layer, and 6.9 times that in the 20-30 cm layer. The main reason for this result may be that aerobic microorganisms and related enzyme in the upper substrate have relatively active activities (Table 3). Our previous studies have found that enzyme activity is closely related to the accumulation and migration of nitrogen pollutants [30].

Table 3. Nitrogen accumulation (mg/kg) and urease activity in substrate at different depths in wetland

| Wetland | Depth   | Accumulation (mg/kg) | Urease activity(NH3-N mg/kg·24h) |
|---------|---------|----------------------|----------------------------------|
| H       | 0-10 cm | 1.179                | 100.91±18.7a                     |
|         | 10-20 cm| 0.196                | 21.62±2.77b                      |
|         | 20-30 cm| 0.112                | 9.87±3.10b                       |
| X       | 0-10 cm | 1.161                | 72.89±18.8a                      |
|         | 10-20 cm| 0.200                | 12.91±8.39b                      |
|        | Nitrogen Content | Urease Activity |
|--------|-----------------|-----------------|
| 20-30 cm | 0.110           | 7.57±1.07b      |
| 0-10 cm  | 0.699           | 63.82±20.96a    |
| 10-20 cm | 0.170           | 16.93±6.80b     |
| 20-30 cm | 0.101           | 8.75±2.49b      |

Note: The nitrogen content and urease activity in the matrix is the substrate sample collected after the end of the experiment, and the cumulative amount is the average value of the absolute repetition of the experiment sample.

4. Conclusion

(1) The outlet location has great influence on dissolved oxygen in VFW. The dissolved oxygen concentration, ammonia nitrogen and total nitrogen removal rate all showed the same rule A > B > C; the variance analysis of the total nitrogen removal rate showed that A < B < C. The above results showed that adjusting the outlet location in the VFW could regulate the nitrogen removal efficiency, and appropriately raising the outlet location was conducive to improve the nitrogen removal efficiency and obtain more stable water quality.

(2) The nitrogen removal efficiency and the total amount of nitrogen retention in H and X wetland planted with vegetation were all higher than those in CK wetland planted with no vegetation, and the fluctuation range of nitrogen removal in H and X was relatively small, which proved that the planting of *Pennisetum Hyridium* and *Pennisetum purpureum Schum.* could help VFW obtain relatively stable effluent quality. The accumulation of nitrogen and urease enzyme activity in the substrate was mainly concentrated in the upper substrate, which could provide nitrogen source for the growth of microorganisms and vegetation in the upper substrate.

In short, in order to improve the nitrogen removal efficiency and obtain stable effluent of the VFW, the outlet location could be appropriately raised, and suitable wetland vegetation can be selected for planting. The upper substrate (0-10 cm) was the main site for nitrogen accumulation and degradation. Therefore, when the wetland system is blocked, simply replacing the upper substrate can quickly restore the system's decontamination function, so as to reduce the cost of wetland restoration.

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