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Evaluation of Organic Matter Removal Efficiency and Microbial Enzyme Activity in Vertical-Flow Constructed Wetland Systems

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Abstract: In this study, enzyme activities and their relationships to organics purification were investigated in three different vertical flow constructed wetlands, namely system A (planting *Pennisetum sinese Roxb*), system B (planting *Pennisetum purpureum Schum.*), and system C (no plant). These three wetland systems were fed with simulation domestic sewage at an influent flow rate of 20 cm/day. The results showed that the final removal efficiency of Chemical Oxygen Demand (COD) in these three systems was 87%, 85% and 63%, respectively. Planting *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* could improve the amount of adsorption and interception for organic matter in the substrate, and the amount of interception of organic matter in planting the *Pennisetum sinese Roxb* system was higher than that in planting the *Pennisetum purpureum Schum.* system. The activities of enzymes (urease, phosphatase and cellulase) in systems A and B were higher than those in system C, and these enzyme activities in the top layer (0–30 cm) were significantly higher than in the other layers. The correlations between the activities of urease, phosphatase, cellulase and the COD removal rates were $R = 0.815$, 0.961 and 0.973, respectively. It suggests that using *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* as wetland plants could promote organics removal, and the activities of urease, phosphatase and cellulase in those three systems were important indicators for COD purification from wastewater. In addition, 0–30 cm was the main function layer. This study could provide a theoretical basis for COD removal in the wetland system and supply new plant materials for selection.

Keywords: vertical-flow constructed wetland; organics; *Pennisetum sinese Roxb*; *Pennisetum purpureum Schum.*; enzyme activity

1. Introduction

As a kind of wastewater treatment technology, constructed wetlands (CWs) have become a popular method for domestic wastewater treatment with low cost, simple operation, and low energy consumption. Wetlands rely on natural microbial, biological, physical and chemical processes to remove organic matter and nutrients [1]. CWs can be divided into surface (horizontal flow constructed wetland, HFCW) and subsurface flow (horizontal subsurface flow constructed wetland, HSCW, and vertical flow constructed wetland, VFCW) systems. Horizontal flow constructed wetlands (HFCW) can provide a reliable treatment for Biochemical Oxygen Demand (BOD)and Total Suspended Solids (TSS) [2], but frequently are less effective for nitrogen removal, unless a longer hydraulic retention time and enough oxygenation are provided [3]. Recently, research works were directed toward the vertical flow constructed wetland (VFCW) system since it was more effective not only for the mineralization of biodegradable organic matter but also for nitrification even at a high loading rate [4,5]. The VFCW system possesses an unsaturated flow and it has greater oxygen transport ability than the horizontal system; it also has the advantages of high hydraulic load rates and good removal of nutrients during any season and their small size, although pore clogging could be a problem [6]. The wetland plant is one of the components used for wastewater treatment. Plants play both direct and indirect roles in wetlands. There are some disputes on the function of wetland plants. Many studies have reported the importance of the presence of vegetation in subsurface flow-constructed wetlands, which might affect the development of clogging [7,8]. However, some studies have suggested that plants could benefit the Sub-surface Flow Constructed Wetlands (SSFCWs) by making the substrate more porous to increase the hydraulic conductivity [9,10]. Moreover, some research also indicated that the diversity of macrophytes could change the microbial community structure and enhance the microbial activities in CW systems [11]. Thus, it can be seen there were some disputes on the roles of plants in constructed wetlands. There are also very few species of wetland plants presently, with only some conventional plants, such as reed, Canna and windmill grass, water hyacinth, etc. In our previous study, we investigated *Cyperus alternifolius* and *Canna indica* L. used as wetland vegetation and evaluated their ability for removing nutrients from wastewater [1,12,13]. In this study, we selected two new energy plant species, *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.*, as CW bioenergy plants, to investigate the performance of these plants on the removal efficiency of organic matter. *Pennisetum sinese Roxb*, also called grain bamboo grass, is an efficiently economic energy crop, whereas *Pennisetum purpureum Schum.*, also named elephant grass, is one of the good forage plants in tropical and subtropical regions. The purification of CW was based on combined action between the microbes and substrate, which might be complemented by wetland plants. The organic matter (removal efficiency of COD was used as an evaluation criteria) removal in wetlands was mainly due to biodegradation, and was secondarily due to the uptake of plants, so microorganisms played a primary role in the adsorption and degradation of pollutants [14–17]. The degradation of organics, nitrification and denitrification, and the transformation of nitrogen and phosphorus in CWs mainly resulted from the activities of microorganisms in the root zone [18–20]. However, it is known that the removal of organic matter is mostly driven by microorganisms, and a limited number of studies have focused on microbes and microbial enzymes. In addition, we used newly bioenergy plants *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* as CW plants, which provided new materials for the selection of wetland plants. The aims of this study were to: (1) examine the microbial enzyme activities in CWs (i.e., systems A, B, C); (2) investigate *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* used as wetland vegetation and evaluate their abilities for removing organic matter from wastewater; and (3) obtain the relationship between enzyme activity and organics purification. This study could provide a theoretic basis and reference to reveal the mechanism of organics removal and the changes of enzyme activities in three different vertical flow constructed wetlands.

2. Materials and Methods

2.1. Wetland Design

Three vertical wetlands were established and maintained in the South China Agriculture University (SCAU) greenhouse facility. The experimental wetlands units were made from cement, with dimension of 2 m × 1 m × 1.3 m, (L × W × D). From bottom to top, the wetland filled with gravels (size) of 10–30 mm, forming a depth 20 cm layer, river sand of 1–2.5 mm, forming a depth 90 cm layer, the top left 20 cm space was water distribution area. Several types of PVC (Polyvinyl chloride) pipes were used to distribute the wastewater flow into wetland system (Figure 1). The wastewater was pumped into wetlands by peristaltic pump.

Three systems were separately system A (planting *Pennisetum sinese Roxb*), system B (planting *Pennisetum purpureum Schum.*) and system C (no plant). *Pennisetum sinese Roxb* and *Pennisetum* *purpureum Schum.* Were gotten from the farm of SCAU, Guangzhou City, and selected the stout and no diseases, stem cuttings for breeding. The stems of *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* were transplanted into systems A and B, respectively, with a density of 10 plant/m^2.

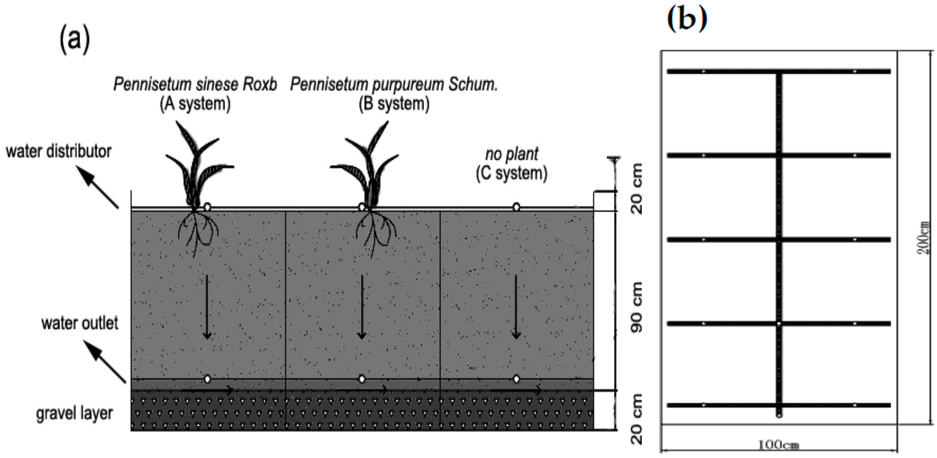


Figure 1. Schematic diagram of the simulate vertical flow constructed wetland (**a**) and plane figure (**b**).

2.2. Operation and Monitoring

Wastewater was pumped into CWs by peristaltic pump. Hydraulic loading adjusted to 20 cm/day for each system, Irrigating 400 L each system. Hydraulic retention time was 1.4 day. Monitor water quality started on April 11, once every seven days. Wetlands were conducted from April 2014 to September 2014. The wastewater used for this study was simulation domestic sewage, water quality parameters were shown in Table 1.

Table 1. The quality of wastewater.

Parameters	pH	TN (mg/L)	TP (mg/L)	COD (mg/L)	DO (mg/L)
Variation range	6.39–6.7	30–50	3–5	235–359	4–6
Average value	/	40	4	330	/

Note: TN-Total nitrogen; TP-Total phosphorus; COD-Chemical oxygen demand; DO-Dissolved oxygen.

2.3. Water Sampling and Chemical Analysis

From 11 April 2014 to 26 September 2014, influent and effluent water of three systems were sampled every week. Then the chemical oxygen demand (COD) of water samples and the organic matter (OM) of substrate samples were analyzed [21,22].

2.4. Statistical Analysis

Statistical analyses of experimental data were performed using software SPSS17.0. A one-way analysis of variance was conducted for constructed wetland: system A (planting *Pennisetum sinese Roxb*), system B (planting *Pennisetum purpureum Schum.*) and system C (no plant), to be detected the statistical significance of differences ($p < 0.05$) between means of treatments, and the Duncan test was performed.

3. Results and Discussion

3.1. Removal Efficiency of COD in Vertical Flow Constructed Wetlands

The removal efficiency of COD in the three systems is shown in Figure 2. The results indicated that the removal efficiency of COD in the three systems was satisfied, and all reached up to 90% before

12 September. The average removal efficiency of COD was 90% in these three systems, which was 20% to 31% higher than that reported by Zhang [23]. The high removal efficiency of COD in this study might be ascribed to the high temperature in summer, which was beneficial to the decomposition of organic compounds. Temperature and season indeed had great effects on the performance of CWs [24]. As the test was run, the COD removal efficiency of each system (systems A, B, C) in the final stages of the experiment was, respectively, 87%, 85% and 63%. Obviously, there was a significant difference between A, B and C. System A (planting *Pennisetum sinese Roxb*) and system B (planting *Pennisetum purpureum Schum.*) had a high removal efficiency, while the removal efficiency of COD in system C only reached 63%, and there was a significant difference ($p < 0.05$) between the other two plant systems. This finding was contrary to some views. Fan et al. found that plants did not play an important role in the removal of organic carbon [24]. However, Tanner and Sukias compared accumulation rates of organic matter in planted versus unplanted treatment beds, and found that the accumulation rates were higher in the planted systems [7].

In this study, at the beginning of the experiment, the COD removal of the different systems (A, B, C) was similar. However, at the later stage, system C had a lower efficiency than the other two planting systems. This is because, as the test progressed, system C (no plant) had clogging, but there was no clogging in systems A and B. Thus, the plants showed their superiority in the final experiment. As the plants grew, the roots were expanding and that might increase the porosity of the matrix and avoid premature clogging. Wang et al. found an increase in hydraulic conductivity, crediting this to the fact that roots could open up the clogged soil to increase the porosity of the substrate [10]. Torrens et al. also found that a fraction of the water from the first batches flowed through the planted beds more quickly than through unplanted filters in vertical flow constructed wetlands (VFCWs) [25]. So, this result also implied that the effect of plants on wetlands should be separately evaluated at different stage. In the early stage of the experiment, the substrate had a larger porosity and strong adsorption, and the effect of plants was not obvious; however, in the later stage, as the porosity of the substrate became smaller and was blocked, at this time the plants could increase the porosity of the substrate and avoid premature clogging, which prolonged the life of the wetland.

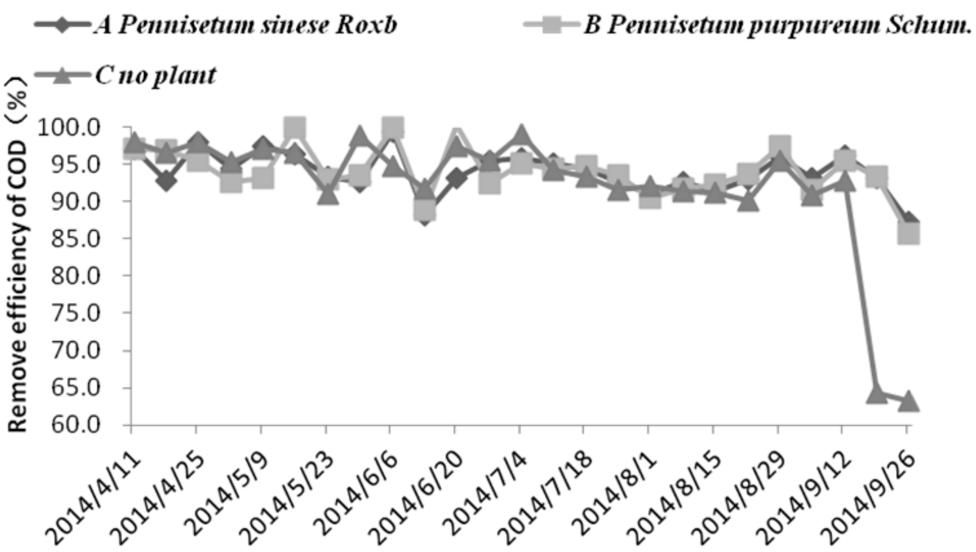


Figure 2. Removal efficiency of COD in three systems.

3.2. Variation of Dissolved Oxygen (DO) in Vertical Flow Constructed Wetlands

The DO values of the effluent in the three systems were further supported by Figure 3. The monitoring of DO began on 16 June. As can be observed, the trends of the DO values in the three systems were steadily inclined until 12 September. The DO value of the effluent in system C (no plant) was obviously lower than that in the other systems, which was due to two reasons: first, system C was suffering clogging at that time. Someone found CWs clogging might lead to the oxygen content and the pollutant removal efficiency being reduced [26]. Secondly, the roots systems of *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* could secrete oxygen to improve the oxygen level in the wetlands, but system C had no plants. The previous studies had found that the ability of roots to secrete oxygen could activate surrounding microbes, which improved the degradation and adsorption of organic matter, and increased the oxygen concentration of the wetland at the same time [27–29].

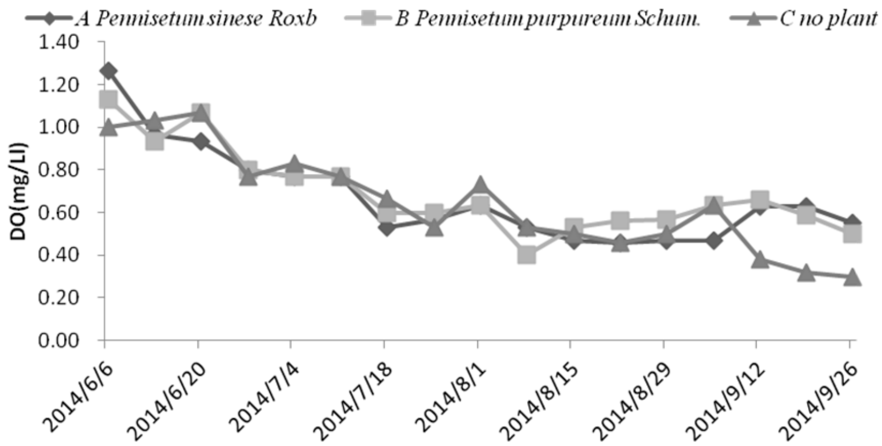


Figure 3. DO of the effluent in three systems.

3.3. The Changes of Organic Matter in Wetland Systems

Figure 4 shows the content of the accumulated organic matter (OM) of each layer in the three systems. The content of OM in the upper layer (0–30 cm) was the highest. System A (planting *Pennisetum sinese Roxb*) and system B (planting *Pennisetum purpureum Schum.*) had higher values than system C (no plant), which improved the roots system of the plants and was advantageous to the adhesion of organic matter. Finally, it could improve the removal of OM. In addition, the content of OM in the *Pennisetum sinese Roxb* system was higher than that in the *Pennisetum purpureum Schum.* system. This suggested that choosing wetland plants was important and necessary.

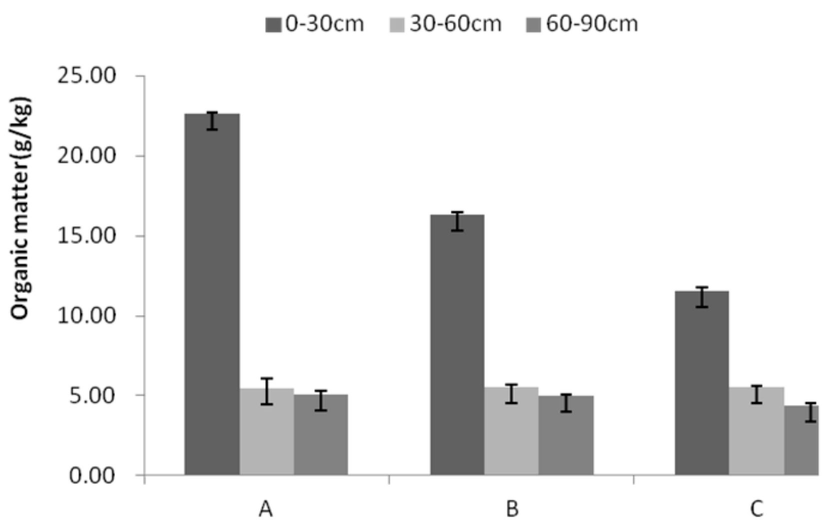
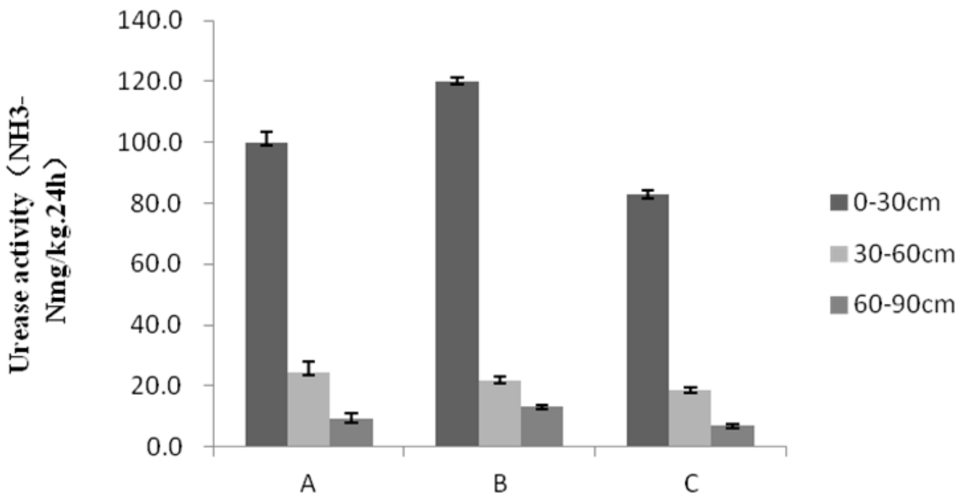


Figure 4. The contents of organic matter (OM) in the three systems. System A (planting *Pennisetum sinese Roxb*) and system B (planting *Pennisetum purpureum Schum.*) had higher values than system C (no plant).

3.4. Enzyme Activity in Constructed Wetland Systems

For the three systems, there were significant differences in the activity of urease, phosphatase and cellulase (Figure 5a–c) between the top layer and the middle layer, as well as between the top layer and the low layer ($p = 0.000 < 0.01$), whereas no significant differences in the activity of urease, phosphatase and cellulase were found between the middle layer and the low layer. In general, the activity of urease, phosphatase and cellulase tended to decrease with the wetland depth. A stronger activity of urease, phosphatase and cellulase in the top layer (0–30 cm) occurred because of higher activities of rhizospheric microorganisms, animals with rich nutrients and better aeration in this layer. Differences in the activity of urease, phosphatase and cellulase for the three layers were significant. This result suggests that the top substrate plays a key role in pollutant removal in wetlands. Overall, the activities of urease, phosphatase and cellulase were higher in the plant energy systems (A and B). The reason might be that during the operation period, the rhizosphere effects promoted the growth of microorganisms, and promoted the secretion of enzymes in the CWs. This was consistent with our previous study, where the more roots grew, the higher the enzyme activities were [30].

(a)



(b)

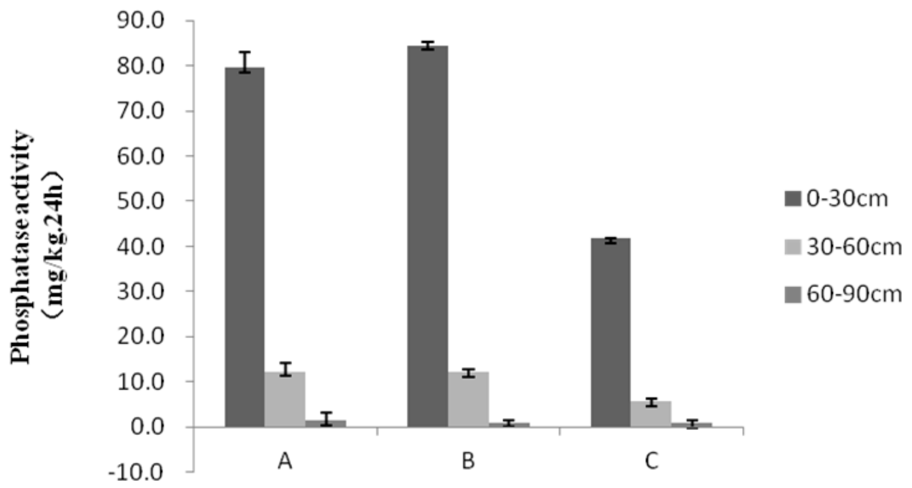


Figure 5. *Cont.*

(c)

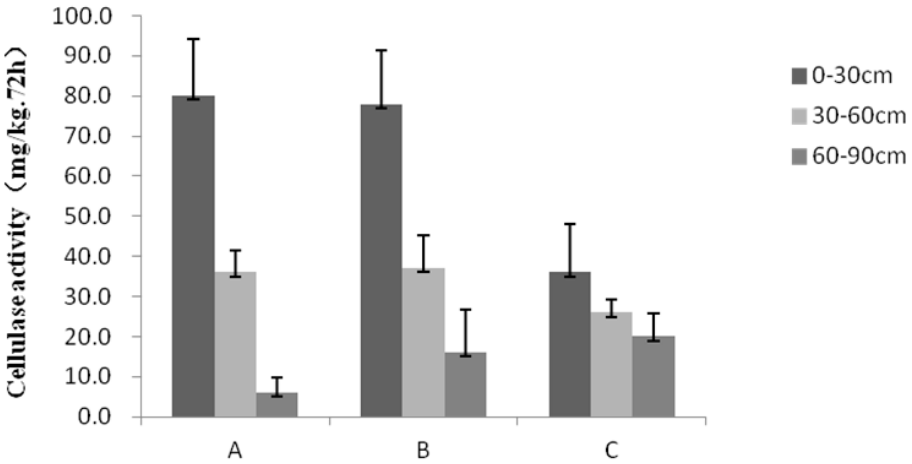


Figure 5. Enzyme activity in the three constructed wetland system. System A (planting *Pennisetum sinese Roxb*) and system B (planting *Pennisetum purpureum Schum.*) had higher values than system C (no plant). Activity of urease (**a**); phosphatase (**b**) and cellulose (**c**).

3.5. The Relationship between Enzyme Activity and COD Removal

In this study, the wastewater quality was monitored and the enzyme activities were obtained at a hydraulic loading rate (HLR) of 20 cm/day. Results showed that the activities of urease, phosphatase and cellulase had a strong positive correlation with COD removal. Shackle found that adding an organic carbon source enables the modification of extracellular enzyme activities [31]. In this paper, COD was not only a pollutant component, but also as a carbon source of microorganisms. The removal efficiency of COD could be linked to the enzyme activities. Table 2 showed the correlations between the activities of urease, phosphatase, cellulase and the COD removal rates. The COD removal rates was strongly correlated to the urease activity (R = 0.815 **, at the 0.01 level), strongly positively correlated to the phosphatase activity (R = 0.961 **, at the 0.01 level), and strongly positively correlated to the cellulase activity (R = 0.973 **, at the 0.01 level). The results of this study were similar to that of the following view where Sangave and Pandit demonstrated that enzyme cellulose could increase the removal rate of COD [32]. In these three systems, there were positive correlations among these three enzymes. It suggests that there was a mutually cooperative relationship among the related microbes.

Table 2. Pearson correlation with COD removal efficiency and enzyme activity.

	Urease	Phosphatase	Cellulase	COD
Urease (NH3-N mg/kg·24 h)	1	0.824 **	0.762 *.	0.815 **
Phosphatase (mg/kg·24 h)	0.824 **	1	0.968 **	0.961 **
Cellulose (mg/kg·72 h)	0.762 *	0.968 **	1	0.973 **
COD (%)	0.815 **	0.961 **	0.973 **	1

Note: * represents a significant difference; ** represents a very significant difference.

4. Conclusions

(1) The results of this study demonstrated that the effect of plants on wetlands should be separately evaluated at different stages. At the end of the experiment, plants in wetlands could avoid premature clogging and prolong the life of the wetland.

(2) Plants in the wetlands were beneficial to absorbing and intercepting organic matter. The amounts of adsorption and interception of OM were different, and the *Pennisetum sinese Roxb* system had higher values than the *Pennisetum purpureum Schum.* system. It suggests that choosing

wetland plants is important and necessary. *Pennisetum sinese Roxb* and *Pennisetum purpureum Schum.* had a good influence on COD removal in the wetlands.

(3) Stronger activities of urease, phosphatase and cellulase occurred at the top layer of the three systems and decreased with the CW substrate depth. Among the three systems, the activities of urease, phosphatase and cellulase in the plant energy systems (A and B) were significantly higher than those of system C. The activities of urease, phosphatase, cellulase and the COD removal rates were strongly positively correlated. This study proved that the activities of urease, phosphatase and cellulase in the root zones could be an important indicator for COD purification from wastewater. The removal of organic matter mainly occurred in the top (0–30 cm) substrate of the vertical flow wetland.

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Author Contributions: Qiaoling Xu analyzed the data and wrote the manuscript; Xiaomao Wang took part in the experiments; Lihua Cui, Shuona Chen, and Zhujian Huang revised this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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