THE INFLUENCE OF COMBINATIONS OF OPERATION PARAMETERS ON SEWAGE TREATMENT IN VERTICAL-FLOW CONSTRUCTED WETLANDS

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Abstract. This paper aims to research the relationship between design parameters and purification effect. An Orthogonal experiment was conducted on the aeration mode, hydraulic load rate and organic load, to study the influence of operation parameters on the pollutant removal rate and substrate enzyme activities of vertical-flow constructed wetlands. These results revealed that the best treatment under the conditions of 20 cm/d hydraulic, 60 g/($m^2 \cdot d$) organic load and intermittent aeration has outstanding decontamination performance and the removal efficiency of total nitrogen, total phosphorous and chemical oxygen demand reached 61%, 31% and 92%, respectively. It was higher than those of other treatments significantly ($p < 0.05$), indicating that proper improvement of organic load, reduction of hydraulic load and auxiliary intermittent aeration were beneficial to the improvement of sewage removal capacity of vertical flow constructed wetland. By analyzing the correlation of pollutant removal efficiency and enzyme activity, urease and catalase appear to have significant correlation with total nitrogen removal efficiency ($p < 0.01$), while catalase appear to have significant correlation to chemical oxygen demand removal efficiency ($p < 0.01$). The results for the use of enzymes as evaluation indices for the purification effect and the improvement of the decontamination capability in vertical-flow constructed wetland provides a theoretical basis.
Keywords: *vertical-flow constructed wetland, orthogonal experiment, operation parameters, enzyme, removal efficiency*

Introduction

Constructed Wetlands (CW) are used extensively for the removal of contaminants from sewage, improving the environment in the world. Compared with traditional sewage treatment technologies, CW has the advantage of being simple, low cost and high efficiency, which makes it very suitable for application in developing countries. CW can remove nitrogen, phosphorus, organic and inorganic pollutants therefore, it could prevent the spread of germs in water (Kivaisi, 2001). It can generally be divided into three types: surface-flow constructed wetland (SFCW), horizontal subsurface-flow constructed wetland (HSFCW) and vertical-flow constructed wetland (VFCW) according to the difference of bed fabric water method and flow mode (Cui et al., 2009). Vertical-Flow constructed wetland (VFCW) has been widely used because of its small area and high processing efficiency. Moreover, recent studies have shown that VFCW not only performs well in the treatment of BOD (biological oxygen demand) and TSS (total suspended solids), but also exhibits strong nitrification at low temperature and high load conditions (Cooper, 2005; Prochaska et al., 2007). Hydraulic load and organic load could affect the treatment efficiency of COD (chemical oxygen demand) and orthophosphate in VFCW (Sani et al., 2013). There are many studies on the purification effect of wetland plants, microorganisms and enzymes on wetland pollutants (Martens et al., 1992; Freeman et al., 1997; Kang et al., 1998; Shackle et al., 2000; Cheng et al., 2002). Among them, the enzyme activity of wetland soil directly affects the rate of material transformation and circulation in the environment, which plays a crucial role in maintaining the balance of the wetland ecosystem (Hill et al., 2006; Sun et al., 2018). Shackle (2006) demonstrated that extracellular enzymes can improve the biodegradation process. Soil enzymes together with microorganisms promote the conversion of substances. There are many factors influencing soil enzyme activity, including biological factors, soil factors and environmental factors (Duarte et al., 2008; Reboreda et al., 2008). In the study of soil enzymes, it was found that phosphatase can promote the hydrolysis of organophosphates. Urease is the hydrolase of C-N and catalase can convert hydrogen peroxide in the organism and matrix into water and oxygen. Sun (2018) studied the soil of reed community in Huishan karst wetland in Guilin, and found that the content of TOC (total organic carbon), TN (total nitrogen) and TP (total phosphorous) were significantly positively correlated with the activities of acid phosphatase, catalase, cellulase and other enzymes, which to some extent represented the changes in soil quality of reed community. Li (2015) proved that there was a significantly positive correlation between catalase activity and organic matter, total nitrogen, and alkali-hydrolyzed nitrogen in decanting wetlands in Baiguishan reservoir area, indicating that catalase activity was consistent with soil fertility changes. The above studies have proved that enzymes are necessarily related to nutrient cycling in soil. Therefore, it is necessary to study the relations among the substrate enzymes, pollutant removal and operating parameters to improve the research on wetland decontamination.

At present, most researchers improve the decontamination efficiency through aeration, parameter optimization, wetland plant selection and other methods (Green et al., 1998; Ouellet-Plamondon et al., 2006; Nivala et al., 2012; Abou-Elela et al., 2012; Guo et al.,2014; Ma et al., 2019; Pu et al., 2019; Kang et al., 2019). The research studies mainly concentrated on the aeration rate, aeration location, load size, the plant collocation on pollutants removal, but regarding the hydraulic load, organic load and combinatorial optimization in VFCW, and relationship between the pollutants removal and substrate enzyme activity of VFCW there are fewer investigations. Therefore, by carrying the different operation parameters of orthogonal test and monitoring the change of phosphatase, urease and catalase among the removal efficiency of TN, TP and COD (chemical oxygen demand), the effect of operation parameters combination optimization of VFCW on N, P, COD removal and the correlation between enzyme activity and pollutant removal was studied. This could provide theoretical basis for improving the decontamination of VFCW and using enzyme activity as the index to evaluate the purification effect of wetland.

Materials and methods

Construction of the vertical-flow constructed wetlands

In this experiment, PVC column was used to simulate the vertical-flow constructed wetland, with the following specifications: diameter of 30 cm × height of 45 cm. Two Hybrid Giant Napier were planted in each column. From bottom to top, the packing was

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5 cm gravel layer and 35 cm mixed substrate (river sand + yellow soil). The experimental equipment was built in southwest China and shown in *Figure 1*.

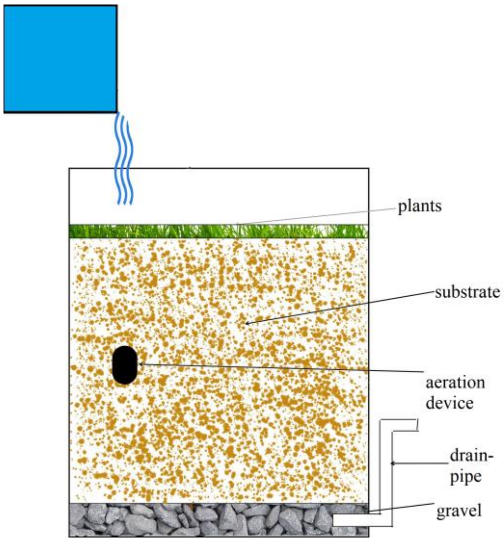

Figure 1. *Simulation diagram of experimental equipment*

Experimental process

The test settings were built outdoors in May 20th, and began to run in early June, and ended in September 20th. Quantitative peristaltic pump was used for continuous irrigation for 12 h. Three factors (1: hydraulic load, 2: organic load, 3: aeration mode) were set for the test. Hydraulic load was set at 3 levels and the corresponding hydraulic load levels were 20, 40 and 60 cm/d respectively. In addition, the level of organic load was set at 3 levels as follows: 20 g/(m^2·d), 40 g/(m^2·d) and 60 g/(m^2·d) (see *Table 1* for details). At the same time, aeration mode was also set at 3 levels: continuous aeration, no aeration and intermittent aeration. The aeration device was installed with a timer to control the aeration time and the aeration mode, the specific operations were as follows: the continuous aeration in this experiment refers to the continuous aeration for 12 h during the irrigation period. The aeration volume was set to 1 L/min, and the total amount of aeration was 720 L/d. Intermittent aeration refers to aeration of 1 h every 3 h during irrigation, and the total aeration time was 3 h. The aeration volume was set to 4 L/min, and the total amount of aeration was 720 L/d. Orthogonal design of three factors three levels, a total of 9 treatments, 3 repetitions, were presented in *Table 1*.

During the experiment period, the effluent water samples were collected every 10 day. After the experiment, soil samples were collected in each VFCW system and mixed to store.

Influent quality

The test sewage was synthetic simulated domestic sewage, the concentrations of TN and TP were 30-50 mg/L and 3-5 mg/L, respectively. COD concentrations of the 9 treatments were 100 mg/L, 150 mg/L, 66 mg/L, 200 mg/L, 50 mg/L, 100 mg/L, 300 mg/L, 100 mg/L, 33 mg/L, and correspondingly the organic load were 20 g/(m^2·d),

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60g/(m^2·d), 40 g/(m^2·d), 40 g/(m^2·d), 20 g/(m^2·d), 60 g/(m^2·d), 60 g/(m^2·d), 40 g/(m^2·d), 20 g/(m^2·d).

Table 1. *Orthogonal design of three factors at three levels*

Test number	Hydraulic load (cm/d)	Organic load (g/(m^2·d))	Aeration mode
A ($H_1O_1A_1$)	20	20	Continuous aeration
B ($H_2O_3A_1$)	40	60	Continuous aeration
C ($H_3O_2A_1$)	60	40	Continuous aeration
D ($H_1O_2A_2$)	20	40	No aeration
E ($H_2O_1A_2$)	40	20	No aeration
F ($H_3O_3A_2$)	60	60	No aeration
G ($H_1O_3A_3$)	20	60	Intermittent aeration
H ($H_2O_2A_3$)	40	40	Intermittent aeration
I ($H_3O_1A_3$)	60	20	Intermittent aeration

Orthogonal design of three factors three at levels: the three levels of hydraulic load were H1, H2 and H3 successively, namely: 20 cm/d, 40 cm/d and 60 cm/d; The three levels of organic load were O1, O2 and O3, namely: 20 g/(m^2·d), 40 g/(m^2·d), 60 g/(m^2·d); The three levels of aeration level were A1, A2 and A3 successively, that was, continuous aeration, non-aeration and intermittent aeration

Analytical methods

For COD, TN and TP methods prescribed by national standards (State Environmental Protection Bureau, 1989) were adopted. Phosphatase, urease and catalase were tested by phenyl disodium phosphate, naismith colorimetry, and potassium permanganate titration methods, respectively (Guan, 1986).

Statistical method

The software of SPSS 17.0 and Excel 2007 were used to analyse the variance and calculate the mean and standard deviation of the correlation analysis. The main factors were analyzed by visual analysis (Li and Hu, 2009).

Results and discussion

Removal efficiency of TN

The removal efficiency of TN in nine VFCW systems have rapidly declined in different degree in the early days (*Fig. 2*). The main reason for this result is that the microorganisms are in the adaptive period and the overall activity is unstable. After the 12^{th} July, the removal effect of TN in 9 systems became stable, and the effect continued until the end of the experiment. It was also proved that the microorganisms meet the stable period in these systems. It is generally believed that the main mechanism of nitrogen removal is nitrification and denitrification by microorganisms (Gao, 2017). The removal rate of TN were G > A > B > C > D > H > E > I > F. At the end of the experiment, there was a significant difference between G ($H_1O_3A_3$) and other treatments ($p < 0.05$). It could be seen that increasing the organic load of inlet water and assisting intermittent artificial aeration are helpful for TN removal. The removal rate of TN in the

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system G was above 60%. It was higher than in previous studies on the removal rate of TN (Vymazal, 2002; Arias et al., 2005; Liu et al., 2005).

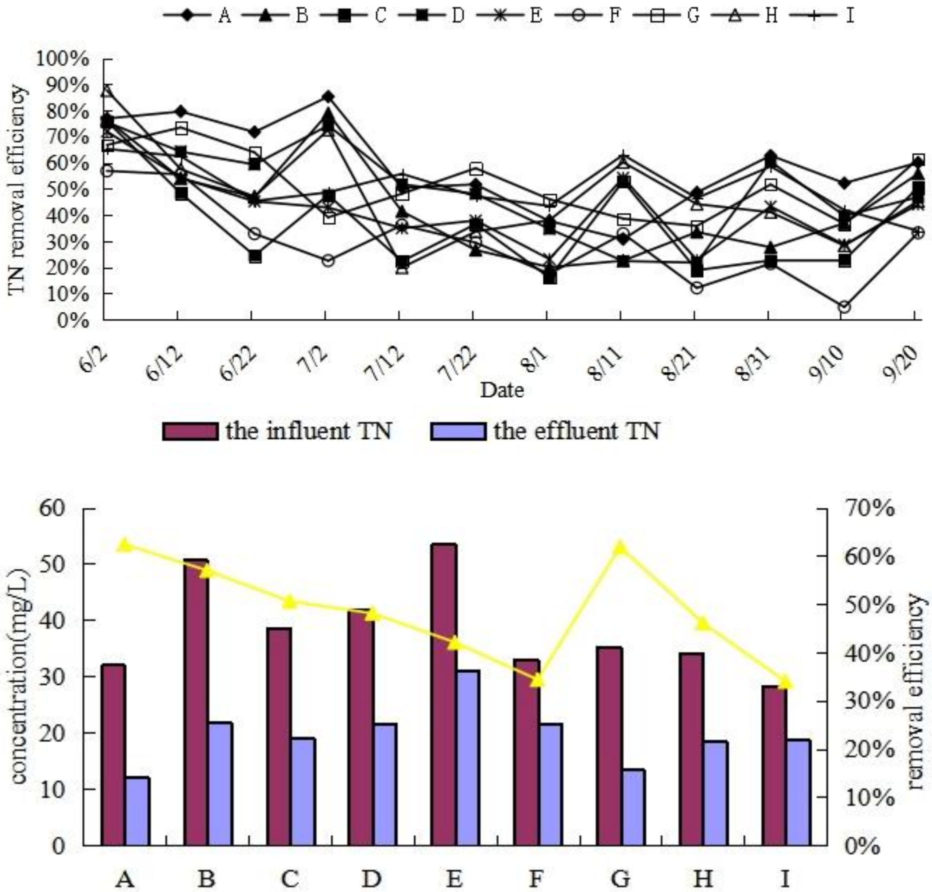

Figure 2. *Removal efficiency of TN in systems*

Removal efficiency of TP

As seen from *Figure3*, in the first week of the experiment, the removal rate of TP was high, ranging from 55% to 91%. After one week, the TP removal rate dropped sharply, and after the 2nd July, the TP removal rate was below 30% except for G. This influence continued to the end of the test, the removal rate of TP in the 9 treatments was G > A > D > H > F > E > I > B > C, and the first two systems (G and A) were significantly different from other systems ($p < 0.05$). Brix and Arias (2005) found that the average removal rate of TP in constructed wetlands was about 20%-30%. The removal efficiency of TP was relatively low in this test, it could be due to more rain during the experiment or a slightly thinner substrate layer.

Removal efficiency of COD

The COD removal efficiency of these 9 treatments were excellent (*Fig. 4*). the removal rate of COD in these systems reached up to 60%~90%, which were significantly higher than in other studies (Chazarenc et al., 2009; Tao et al., 2010; Li et al., 2011; Liu et al., 2011). The reason for this may be that the experiment was in summer, and the temperature was suitable for the growth of wetland plants and

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microorganisms, creating a favorable environment for COD removal. At the end of the experiment, D and G were as high as 90%, this is due to the higher concentrations of influent in D and G. Liao (2002) studied the effect of constructed wetlands on organic matter treatment of pig farm wastewater, and found that the removal effect of COD and BOD in wetlands was significantly improved under the operation condition that the concentration of influent water was gradually increased. Because maintaining a high organic load can provide sufficient carbon source for wetland microorganisms, intermittent aeration could moderately increase DO, which is conducive to microbial growth and pollutant removal in the system.

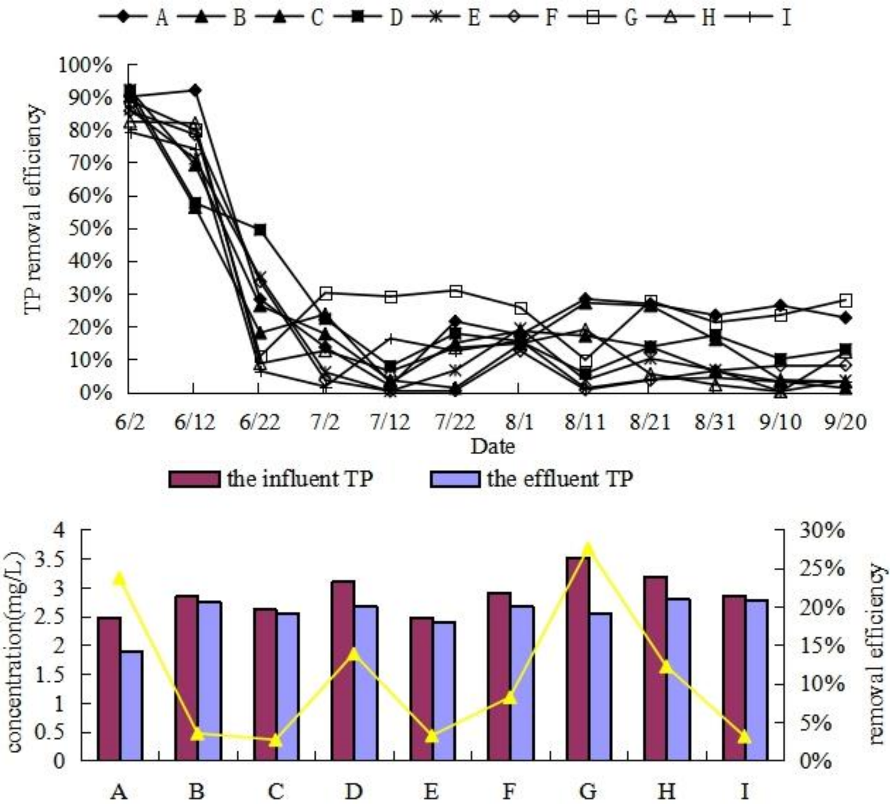

Figure 3. *Removal efficiency of TP in systems*

Analysis of influence factor range of orthogonal test

The main purpose of optimizing orthogonal experiment was to find the best values of the significant factors. Through the direct analysis of the orthogonal experiment of TN removal rate (*Table 2*), it can be seen that the influence size of the influence factor was: organic load > aeration level > hydraulic load, the optimal combination was $H_1O_3A_1$; Through the direct analysis of the orthogonal test of TP removal rate (*Table 3*), it can be seen that the influence size of the influence factor was: aeration level > organic load > hydraulic load, the optimal combination is $H_1O_3A_3$; Through the direct analysis of the COD removal rate orthogonal experiment (*Table 4*), it can be seen that the influence size of the influence factor was the organic load > aeration level > hydraulic load, and the optimal combination is $H_1O_3A_1$. Combined with the orthogonal experiment of nitrogen, phosphorus and COD removal, the comprehensive and intuitive

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analysis showed that under the conditions of low hydraulic load, high organic load and aeration, the pollutant removal performance was outstanding. Taking economic factors into consideration, we chose low hydraulic load of 20 cm/d, high organic load of 60 g/m^2·d, and intermittent aeration, as the optimal scheme, which was G ($H_1O_3A_3$). It was suggested that the design of low hydraulic load, proper increase of organic load and auxiliary aeration could improve the decontamination capacity in vertical-flow constructed wetlands.

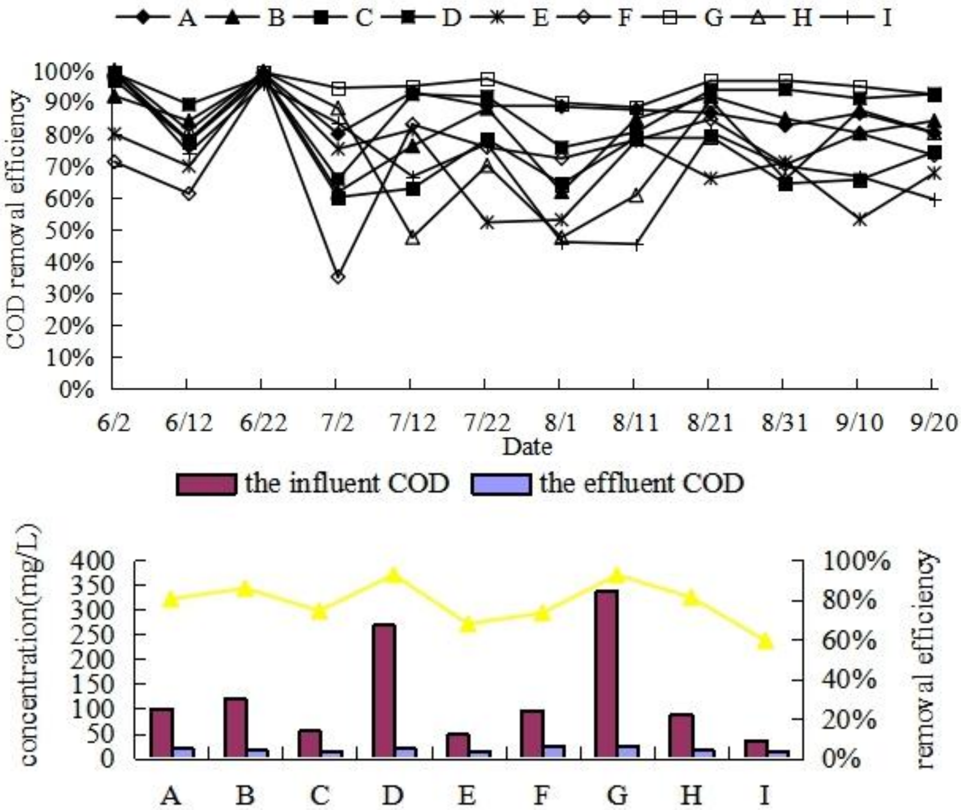

Figure 4. *Removal efficiency of COD in systems*

Table 2. *Orthogonal experiment visual analysis of TN removal rates*

	Hydraulic load	Organic load	Aeration level
K1	1.68	1.38	1.66
K2	1.45	1.43	1.24
K3	1.18	1.50	1.41
R	1.18	1.63	1.41

Table 3. *Orthogonal experiment visual analysis of TP removal rates*

	Hydraulic load	Organic load	Aeration level
K1	0.64	0.29	0.27
K2	0.19	0.26	0.25
K3	0.13	0.39	0.43
R	0.13	0.49	0.60

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Table 4. *Orthogonal experiment visual analysis of COD removal rates*

	Hydraulic load	Organic load	Aeration level
K1	2.65	2.07	2.39
K2	2.32	2.47	2.34
K3	2.07	2.50	2.32
R	2.07	2.92	2.32

K1, K2 and K3 represent the average of the different factors in the same level results, the level of the factor influence size; R value is the difference between the maximum and minimum values and it represents the influence of this factor on the index. The greater the difference, the greater the impact

The change of pH and ORP

From *Figure 5* it can be seen that at the end of the test, the pH value of inlet and outlet water of these treatments did not change much, but the ORP value changed significantly. Artificially assisted aeration helped to maintain a relatively high ORP value. Among these systems, G had the maximum ORP value at the end of the test. It indicated that intermittent aeration was more conducive to the reoxygenation in VFCW.

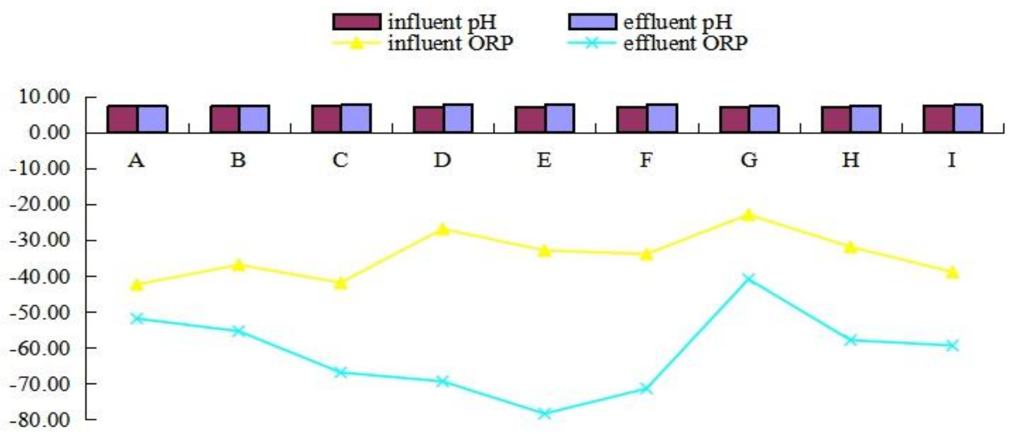

Figure 5. *Changes in pH and ORP*

Correlation analysis of substrate enzyme and sewage purification efficiency

Correlation analysis of matrix enzyme and pollutant removal is showed in *Table 5*. It was showed that there was no significant correlation between phosphatase and TP removal rate, because the removal of phosphorus mainly depended on the adsorption of matrix, while microorganisms and plants only played a certain role (Dinges, 1982; Wu et al., 2001; Yan et al., 2007). The urease and the removal rate of TN have a significant correlation ($p < 0.01$) (Wu et al., 2001; Huang et al., 2008). Catalase activity was also significantly correlated with TN removal rate ($p < 0.01$). It indicated that there may be some relationship between catalase and nitrogen degradation. In addition, catalase and COD removal rate also showed a very significant correlation ($p < 0.01$). It may be associated with the characteristics of catalase, which was the enzyme that breaks down hydrogen peroxide in living organisms and substrates, converting it into water and oxygen. It could directly result in changes of ORP of matrix, and TN removal was mainly done by nitrification and denitrification, oxygen produced for the degradation of

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COD removal provided a good environment. In the future, advantages of high molecular biology methods can help an in-depth study on substrate enzyme contact with the inner mechanism of the pollutants degradation, and establish an efficient fast measurement of wetland pollutant removal ability.

Table 5. *Correlation analysis of matrix enzyme and pollutant removal*

		TN (%)	TP (%)	COD (%)	Urease	Phosphatase	Catalase
Urease	Pearson correlation	.536**	-.202	.308	1	.071	-.025
	Significance (bilateral)	.004	.312	.350		.723	.902
Phosphatase	Pearson correlation	.154	.286	.327	.071	1	.306
	Significance (bilateral)	.443	.147	.026	.723		.121
Catalase	Pearson correlation	.791**	.223	.523**	-.025	.306	1
	Significance (bilateral)	.000	.263	.005	.902	.121	

*Significantly correlated at the level of 0.05 (bilateral). **Significantly correlated at the level of 0.01 (bilateral)

Conclusion

(1) In the VFCW, the order of influence on the removal effect of TN and COD were as follows: organic load > aeration method > hydraulic load; Based on the comprehensive analysis, it was concluded that for the VFCW system, maintaining a reasonable concentration of organic matter inflow, relatively low hydraulic load and auxiliary aeration are not only achieve the best removal effect, but also extend the life of wetland. It could provide theoretical support for the construction and management of VFCW in the future.

(2) Urease and catalase showed a very significant correlation with TN removal. Catalase may provide a good degradation environment for COD removal and it provide basic data for further study on the relationship between matrix enzyme and pollutant removal.

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