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Evaluation of nutrient removal efficiency and microbial enzyme activity in a baffled subsurface-flow constructed wetland system

Lihua Cui [a,*], Ying Ouyang [b], Wenjie Gu [c], Weizhi Yang [a], Qiaoling Xu [a]

[a] *College of Natural Resource & Environment, South China Agricultural University, Guangzhou 510642, P.R. China*
[b] *USDA Forest Service, Center for Bottomland Hardwoods Research, 100 Stone Blvd., Thompson Hall, Room 309, Mississippi State, MS 39762, USA*
[c] *Institute of Soil and Fertilizer, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, P.R. China*

HIGHLIGHTS

- Baffled subsurface-flow constructed wetland (CW) is a new type CW.
- Very significant correlation exists between the activity of urease and the rate of N removal in this CW.
- Activity of urease in the CW is an important indicator for N removal from wastewaters.

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ABSTRACT

In this study, the enzyme activities and their relationships to domestic wastewater purification are investigated in four different types of subsurface-flow constructed wetlands (CWs), namely the traditional horizontal subsurface-flow, horizontal baffled subsurface-flow, vertical baffled subsurface-flow, and composite baffled subsurface-flow CWs. Results showed that the urease activity in the composite baffled subsurface-flow CW was significantly higher than in the other three CWs, while the phosphatase activity in the vertical baffled subsurface-flow CW were higher than in the other three CWs. There were significant and very significant correlations between the activities of urease and the removal rates of TN and NH_4^+—N for the horizontal baffled flow, horizontal subsurface flow, and composite baffled subsurface flow CWs. This study suggests that the activity of urease in the root zones of those three CWs is an important indicator for N purification from wastewaters.

1. Introduction

Constructed wetland (CW) is a promising technique for removing pollutants from wastewaters due to their low energy consumption and cost-effective operation (Kadlec and Knight, 1996). The major mechanism of CWs for purifying wastewater is the interactions among substrates, plants, and microorganisms through a series of physical, chemical and biological processes (Martin and Moshiri, 1994). Although the pollutant removal efficiency varies with different CW systems, the effects of microorganisms cannot be ignored (Gersberg et al., 1986; Wu et al., 2001). Microorganisms play a primary role in pollutant adsorption and degradation (Hoppe et al., 1988; Savin and Amador, 1998;). Degradation of organics, nitrification and denitrification, and transformation of nitrogen and phosphorus in CWs were mainly resulted from the activities of microorganisms in the root zone (Corbitt and Bowen, 1994; Bachand and Home, 2000; Stottmeister et al., 2003). The biochemical reaction processes such as synthesis and degradation of organic compounds, hydrolysis and transformation of plants and microbial residues, and oxydoreduction reaction are controlled by microbial enzyme activities.

Microbial enzymes include intracellular and extracellular enzymes. A variety of enzyme accumulations are due to activities of microorganisms, animal and plants (Zhou et al., 2005). Phosphatase include acidic, alkaline and neutral (three types) enzymes. They could promote the hydrolysis of the phosphonolipid and organophosphorous compounds to release phosphate at different pH conditions. The urease as a kind of soil enzyme is a hydrolase of linear amide C–N bond (not peptide). The urease could make organic N pollutants hydrolysis to improve wetland nitrogen removal and plays important roles in CW system. In the last decade, scientists have paid close attentions to the distribution and effect of microbial enzymes in CWs. Shackle and Freeman (2000) studied the regulation of carbon quantity and quality and improved the activity of extracellular enzyme in CW to achieve the best wastewater purification. Similar studies on enzyme activities in CW system can be found elsewhere (Koottatep and Polprascrt, 1997; Liu

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[*] Corresponding author at: College of Natural Resource & Environment, South China Agricultural University, Guangzhou 510642, P.R. China.
E-mail address: Lihcui@scau.edu.cn (L. Cui).

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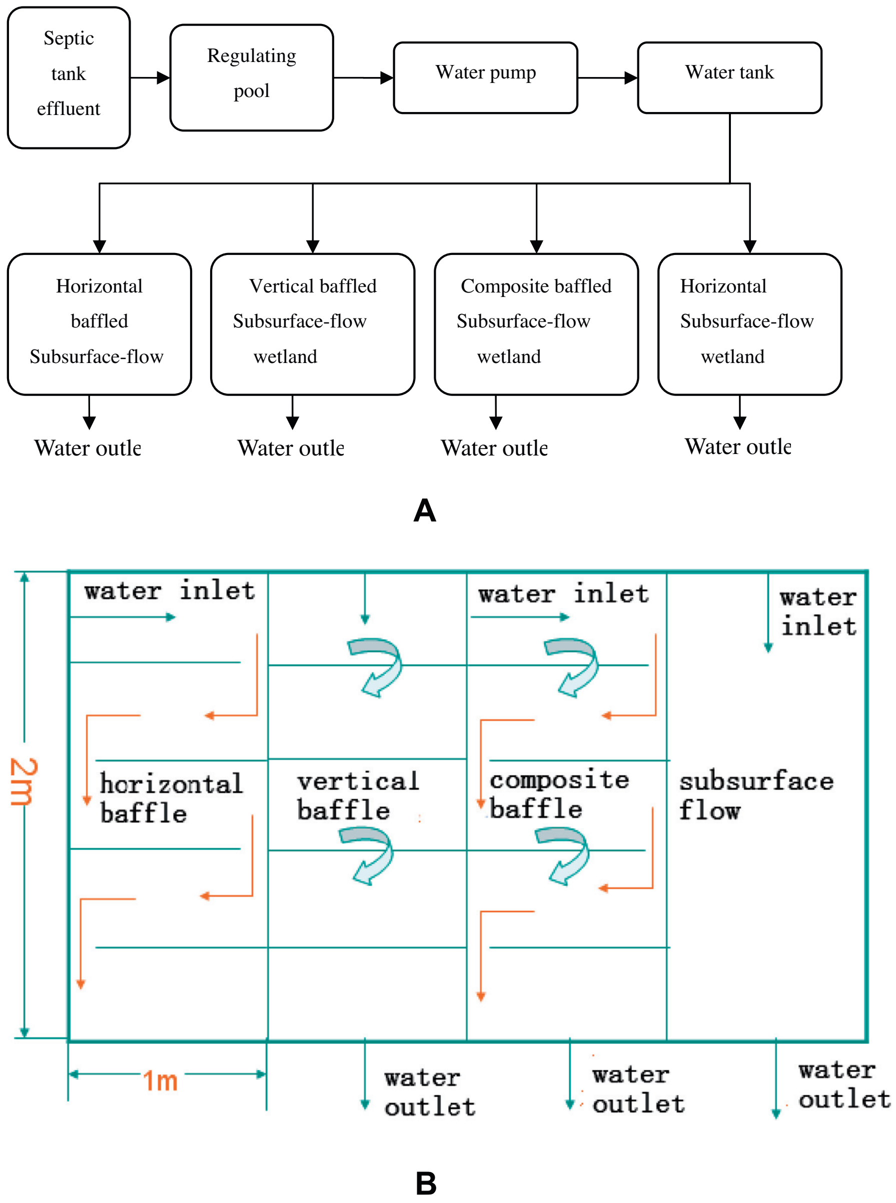

Fig. 1. Schematic diagram of four different types of constructed wetlands (A) and their plain views (B).

and Cao, 2001; Ebersberger et al., 2003). By measuring the wetland root zone phosphatase and urease activities, Wu et al., 2001 found the relationship of urease activity to total nitrogen (TN) removal was apparent and visible, while the relationship of phosphatase activity to total phosphorus (TP) removal was not significant. A similar result also was obtained by Cui et al., (2011). The removal of phosphorus in CWs is through the matrix adsorption and precipitation, plant and microorganism uptake, while the removal of nitrogen is mainly due to plant uptake, ammonization, nitrification, denitrification, and volatilization of ammonia (Fan et al., 2013). Under normal circumstances, organic nitrogen was decomposed by microorganisms and transformation. Urease is used urea as a substrate for the hydrolysis of urea to form ammonia and carbon dioxide. So urease can be used as an important indicator of wetland treatment. Currently, no literature reports are found on investigating enzyme activity in the baffle-type CWs.

Baffle subsurface-flow CW is based on the traditional horizontal subsurface-flow CW with increasing baffle through the horizontal and vertical directions to make wastewater repeatedly flow through the CWs. Thus, the pollutants removal efficiency is improved (Tee et al., 2012). The aims of this study were to: (1) examine the microbial enzyme activities in four types of CWs (i.e., traditional horizontal subsurface-flow, horizontal baffled subsurface-flow, vertical baffled subsurface-flow, and composite baffled subsurface-flow), and (2) obtain the relationship between enzyme activity and water purification. This study could provide a theoret-

Table 1
The contents of water quality constituents in original wastewater (mg/L).

Index	Variation range	Average
TN	52.46–234.12	145.20 (6.27)[a]
TP	3.31–50.05	12.72 (1.12)
BOD_5	11.12–214.32	84.78 (7.05)
COD	65.89–793.70	233.59 (18.78)
NH_4^+–N	34.65–240.66	137.93 (6.73)
pH	6.64–8.20	7.68 (0.071)

[a] Values in the parentheses were standard errors (S.E.).

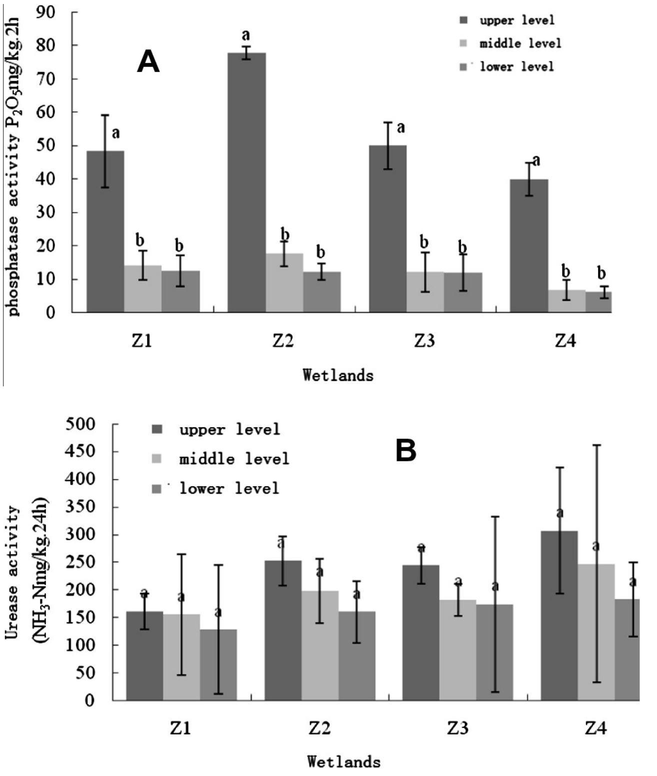

Fig. 2. Activities of phosphatase (A) and urease (B) in four different types of CWs.

ical basis and reference for nutrient removal and enzyme activity in the baffled subsurface-flow CW system.

2. Methods

2.1. CW system

Four different types of CWs were developed in this study (Fig. 1). Each CW was built with cement at 2 m length × 1 m wide - × 0.75 m height. These four CWs are the horizontal baffled subsurface-flow CW (Z1), vertical baffled subsurface-flow CW (Z2), composite baffled subsurface-flow CW (Z3) and horizontal subsurface-flow CW (Z4, as control). Each of the first three CWs (i.e., Z1, Z2, and Z3) was further divided into five compartments (Fig. 1B).

At the bottom of the first compartment (inlet), there was a 10 cm-thick limestone and a 4 cm-thick gravel above the limestone. The rest of compartment was filled with 25% cinder, 25% stone and 50% blast furnace slag. The corner of baffle was filled with cinder to prevent jam. The remaining compartments were filled with blast furnace slag. A 3 cm thick sand shop was placed on the top of each compartment.

Filler materials used for the CW systems were as follows: (1) limestone: a kind of rock that is widely distributed in nature. The main ingredient of the limestone was calcium carbonate ($CaCO_3$), which could be used as the building material and many important industrial raw materials. The limestone contains calcium magnesium carbonate or calcium carbonate and magnesium carbonate mixture; (2) cinder residue slag: a kind of industry solid waste, coal waste residue from thermal power plant, civil and industrial boilers. The main chemical compositions of cinder residue slag were 40–50% of SiO_2, 30–35% of Al_2O_3, 4–20% of alumina, iron oxide, calcium oxide and 1–5% of CaO and a small amount of magnesium, sulfur, carbon and other. This slag has loose porous structure with large surface area; and (3) blast furnace slag: blast furnace slag is solid waste produced by the iron making process. Its chemical compositions were mainly made of CaO, SiO_2, MgO, FeO, Al_2O_3 and a small amount of manganese, titanium, sodium, potassium, phosphorus oxide. The calcium oxide and silica contents generally account for more than 70%.

Canna was used in this study and is a perennial herbaceous flower, up to 1 m high. *Canna* known as Saka siri, Indian shot or *Canna* lily was a beautiful plant that made an excellent centerpiece for a sunny, sheltered border, great for every garden and a perfect choice for the exotic garden. The canna was planted and the average cultivation density of each compartment was 3 strains. The wastewater was obtained from septic tank effluent from Building 5, College of Natural Resources and Environment, South China Agricultural University and the contents of TN, TP, BOD_5, COD, NH_4^+—N, and pH in the wastewater were given in Table 1.

2.2. CW operation and sample analysis

Each CW system was run for 24 months from March 2005 to March 2007 with different hydraulic retention times (HRTs), which were set at three levels (i.e., 1, 2, and 3 days). The system was dried and adjusted as appropriate with a slow inlet and fast outlet discharge mode. The plants were harvested each quarter. For the period from June to November 2006, the HRT was set up to 2 days. During this period, each CW system (or pool) was punched at 0, 50, 100, 150, and 200 cm from the water inlet to outlet with siphon mode for monitoring the system pollutant changes.

At the end of the experiment, the samples are collected from upper layer (0–10 cm), middle layer (30–40 cm) and lower layer (60–70 cm) of each CW with three samples for each layer. These three samples were mixed to obtain one representative sample for each layer. The activities of phosphatase and urease were measured, respectively, with nesslerization and colorimetry (Xu and Zheng, 1986). The contents of nitrogen and phosphorus species

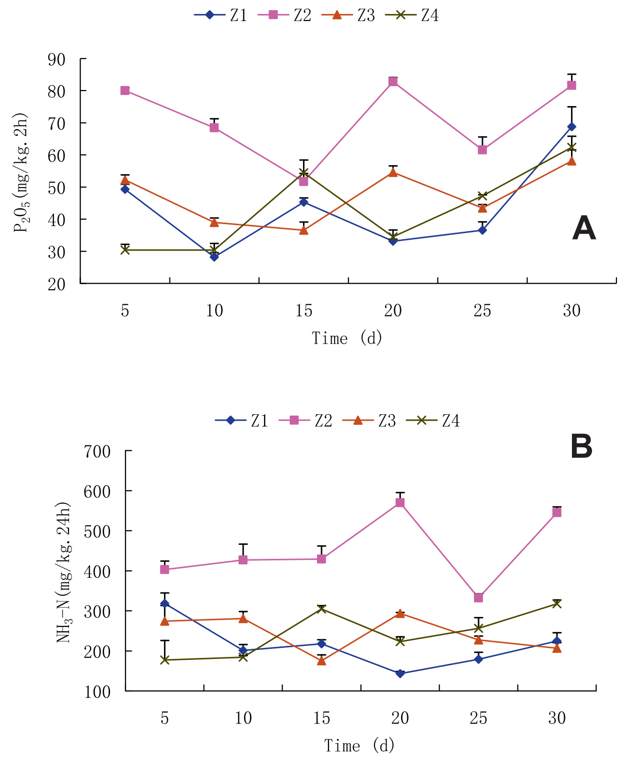

Fig. 3. Variations of phosphatase (A) and urease activities (B) as a function of time.

Table 2
Phosphatase activity and removal rate of TP, soluble TP and soluble IP in baffled subsurface-flow CWs.

Times	Phosphatase (P_2O_5 mg/kg/2 h)	TP removal rate (%)	Phosphatase (P_2O_5mg/kg/2 h)	Soluble TP removal rate (%)	Soluble IP removal rate (%)
Horizontal baffled subsurface-flow wetland					
1	92.13	92.90	49.32	100.00	99.94
2	82.82	89.47	30.06	99.94	100.00
3	89.43	91.18	47.60	99.06	100.00
4	138.94	98.74	41.75	99.44	99.98
5	69.74	99.91	56.54	99.41	99.88
Correlation coefficient R^2		0.2753		−0.4110	−0.7719
Probability *P*		0.6540		0.4918	0.1262
Vertical baffled subsurface-flow wetland					
1	73.66	93.97	80.04	44.73	36.68
2	92.60	94.09	64.10	29.40	29.92
3	73.40	61.86	56.19	25.02	23.71
4	61.71	73.91	68.57	33.71	30.94
5	69.33	87.49	75.45	23.83	23.09
Correlation coefficient R^2		0.4788		0.5963	0.5166
Probability *P*		0.4146		0.2885	0.3728
Composite baffled subsurface-flow wetland					
1	91.46	97.00	52.24	99.28	98.97
2	78.56	96.96	40.37	99.00	99.17
3	80.64	86.39	35.22	97.83	99.13
4	65.05	99.35	56.19	99.60	99.35
5	54.78	98.76	41.41	99.22	99.37
Correlation coefficient R^2		−0.4109		0.8104	0.0398
Probability *P*		0.4920		0.0962	0.9494
Horizontal subsurface-flow wetland					
1	86.09	89.33	32.12	99.75	99.94
2	87.05	91.86	31.09	99.61	99.95
3	34.84	95.74	46.91	99.38	99.92
4	40.68	99.59	32.81	99.80	99.97
5	43.15	99.51	29.95	98.96	99.82
Correlation coefficient R^2		−0.8732		−0.05496	0.1228
Probability *P*		0.0532		0.9301	0.8441

in the samples were measured following the Standard Methods (APHA, 1998). Excel 2003 and SAS 8.1 software packages were used for statistical analysis. The correlation and multiple comparisons were estimated with the DUNCAN method.

3. Results and discussion

3.1. Enzyme activity in constructed wetland system

The activities of phosphatase and urease among the four different types of CWs are showed in Fig. 2. For the horizontal baffled subsurface-flow CW (Z1), significant differences in the activity of phosphatase were observed (Fig. 2A) between the top layer and the middle layer as well as between the top layer and the low layer ($P = 0.0403 < 0.05$), whereas no significant difference in the activity of phosphatase was found between the middle layer and the low layer although the former was stronger than the latter. Similar results were obtained for the vertical baffled subsurface-flow (Z2), composite baffled subsurface-flow (Z3), and horizontal subsurface-flow (Z4 or control) CWs. That is, very significant differences in the activity of phosphatase were observed between the top layer and the middle layer as well as between the top layer and the low layer ($P = 0.0001$ for Z2, $P = 0.0070$ for Z3, and $P = 0.0024$ for Z4). No significant difference ($P > 0.05$) in the activity of phosphatase was found between the middle layers and the low layers for those three CWs although the former was stronger than the latter. In general, the activity of phosphatase tended to decrease with the CW depth although this decrease was not statistically significant. A stronger activity of phosphatase in the top layer occurred because of higher activities of rhizospheric microorganisms, animals and plants with rich nutrients and better aeration in this layer. This finding was consistent with the results from Wu et al., (2003).

Differences in the activity of urease for the three layers were not significant (Fig. 2B) among the four CWs ($P = 0.9681$ for Z1, $P = 0.5069$ for Z2, $P = 0.7836$ for Z3, and $P = 0.8827$ for Z4) although the activity of urease decreased gradually with the CW depth. The stronger activity of urease in the top layer occurred due to the same reason as for the case of phosphatase.

Fig. 3 shows the activity of phosphatase changed in a "W" shape with time among the four different types of CWs, while the activity of urease varied in an "N" shape for CW Z2. Among the four CWs, the activity of urease in the vertical baffled subsurface-flow CW (Z2) were significantly different from the other three CWs ($P < 0.01$) (Fig. 3B), while the activity of phosphatase in the same CW was not significant from the other three CWs ($P > 0.05$). Overall, the activities of urease and phosphates were higher in vertical baffled subsurface-flow CW (Z2). The reason might be that during the 2-year operation period, the aboveground canna biomass in CW Z2 was about two times larger than the other CWs. A higher biomass production indicated a well development of plant roots. The more the roots grew, the higher the enzyme activities were. The "W" and "N" shapes in the activities of phosphatase and urease with time indicated a complicate biological process and is warranted for further study.

3.2. Enzyme activity vs. nutrient

In this study, the wastewater quality was monitored and the enzyme activities were obtained at hydraulic retention time (HRT) = 2 d. Results showed that the activity of phosphatase was related to the concentrations of TP, soluble TP and soluble inorganic phosphorus (IP) in the wastewater, whereas the activity of urease was related to the concentrations of NH_4^+—N and TN. However, the phosphatase activity and TP removal rate was not signif-

Table 3
The activity of urease and removal rate of TN, NH_4^+—N in four horizontal subsurface-flow wetlands.

Times	Urease (NH_3-N mg/kg/24 h)	TN removal rate (%)	Times	Urease (NH_3-N mg/kg/24 h)	NH_4^+—N removal rate (%)
Horizontal baffled subsurface-flow wetland					
1	145.55	44.14	1	110.78	91.32
2	197.53	66.31	2	142.04	92.82
3	193.88	62.10	3	188.28	99.07
4	297.86	82.58	4	297.86	99.76
5	172.04	59.09	5	237.65	99.70
			6	213.98	99.20
Correlation coefficient R^2		0.9538			0.8725
Probability P		0.0119		0.0233	
Vertical baffled subsurface-flow wetland					
1	193.55	68.51	1	111.58	91.05
2	167.34	41.64	2	167.34	94.10
3	181.32	72.48	3	179.11	95.28
4	124.00	13.92	4	218.73	26.02
5	178.95	27.43	5	124.00	55.45
			6	360.77	22.77
Correlation coefficient R^2		0.7848			−0.6926
Probability P		0.1159		0.1272	
Composite baffled subsurface-flow wetland					
1	298.39	76.52	1	213.72	73.21
2	348.59	86.14	2	259.79	80.15
3	312.74	84.51	3	363.53	90.99
4	289.74	69.09	4	304.02	89.09
5	217.36	53.33	5	348.59	99.76
			6	312.74	98.86
Correlation coefficient R^2		0.9628			0.8551
Probability P		0.0086		0.0300	
Horizontal subsurface-flow wetland					
1	133.52	30.50	1	133.52	84.74
2	320.03	51.02	2	224.97	91.23
3	400.46	64.97	3	305.66	95.40
4	240.14	53.20	4	240.14	97.42
5	296.50	62.63	5	103.69	78.31
			6	198.85	93.57
Correlation coefficient R^2		0.8867			0.8883
Probability P		0.0450		0.0180	

Table 4
The regression equations and correlation coefficients between the phosphatase activity and the removal rates of TP, soluble TP, and soluble IP.

Relationship	Regression equation	R^2	P value
Horizontal baffled subsurface-flow wetland			
TP (y) vs. Phosphatase (x)	$y1 = 0.04858x + 89.84331$	0.27484	0.6545
Soluble TP (y) vs. Phosphatase (x)	$y2 = -0.01636x + 100.30724$	−0.41018	0.4928
Soluble IP (y) vs. Phosphatase (x)	$y3 = -0.00400x + 100.14008$	−0.77634	0.1226
Vertical baffled subsurface-flow wetland			
TP (y) vs. Phosphatase(x)	$y1 = 0.59033x + 38.49730$	0.47847	0.4149
Soluble TP (y) vs. Phosphatase (x)	$y2 = 0.53692x - 5.63991$	0.59643	0.2884
Soluble IP (y) vs. Phosphatase (x)	$y3 = 0.30994x + 7.52212$	0.51696	0.3724
Composite baffled subsurface-flow wetland			
TP (y) vs. Phosphatase (x)	$y1 = -0.15241x + 106.98506$	−0.41101	0.4918
Soluble TP (y) vs. Phosphatase (x)	$y2 = 0.06308x + 96.14220$	0.81225	0.0949
Soluble IP (y) vs. Phosphatase (x)	$y3 = 0.06489x + 95.87220$	0.03907	0.9503
Horizontal subsurface-flow wetland			
TP (y) vs. Phosphatase (x)	$y1 = -0.15413x + 104.20105$	−0.87334	0.0531
Soluble TP (y) vs. Phosphatase (x)	$y2 = -0.00261x + 99.59038$	−0.05319	0.9323
Soluble IP (y) vs. Phosphatase (x)	$y3 = 0.00113x + 99.88085$	0.13454	0.8292

Table 5
The regression equations and correlation coefficients between the urease activity and the removal rates of TN, NH_4^+—N.

Relationship	Regression equation	R^2	P value
Horizontal baffled subsurface-flow wetland			
TN (y) vs. Urease (x)	$y1 = 0.22840x + 16.85143$	0.95374*	0.0119
NH_4^+—N (y) vs. Urease (x)	$y2 = 0.04980x + 87.09623$	0.88181*	0.0479
Vertical baffled subsurface-flow wetland			
TN (y) – Urease (x)	$y1 = 0.74451x - 81.05016$	0.78471	0.116
NH_4^+—N (y) vs. Urease (x)	$y2 = -0.26101x + 114.64045$	−0.45424	0.4422
Composite baffled subsurface-flow wetland			
TN (y) – Urease (x)	$y1 = 0.26791x - 4.67611$	0.96276**	0.0086
NH_4^+—N (y) vs. Urease (x)	$y2 = 0.15952x + 40.75664$	0.92035*	0.0267
Horizontal subsurface-flow wetland			
TN (y) vs. Urease (x)	$y1 = 0.12190x + 18.56055$	0.88674*	0.045
NH_4^+—N (y) vs. Urease (x)	$y2 = 0.08749x + 72.51474$	0.91741*	0.0281

* Significant.
** Very significant.

icantly correlated (Table 2), which indicated that phosphorus removal may be accomplished by the CW matrix. Matrix played a major role in removal of phosphorus. When the wastewater went through the CWs, some physical and chemical processes such as

Table 6
Multiple comparisons of the activities of urease and phosphatase in four different types of CWs.

Type of CW	Urease activity		Phosphatase activity	
	NH_4^+—N	TN	TP	STP, SIP
Horizontal baffled subsurface-flow wetland	198.43 ± 27.47B	201.37 ± 25.84BC	94.61 ± 11.74A	45.05 ± 4.43B
Vertical baffled subsurface-flow wetland	193.59 ± 36.99B	169.03 ± 12.00C	74.14 ± 5.10AB	68.87 ± 4.19A
Composite baffled subsurface-flow wetland	300.40 ± 22.83A	293.36 ± 21.49A	74.10 ± 6.40AB	45.09 ± 3.92B
Horizontal subsurface-flow wetland	201.14 ± 30.05B	278.13 ± 44.39AB	58.36 ± 11.60B	34.58 ± 3.12B

Note: (1) The numbers in the table are averaged values followed by standard errors. Five samples for each nutrient species are used for analysis except for NH_4^+—N, in which six samples are used for analysis.
(2) Duncan's multiply comparisons are used in this analysis and the same letters for each column mean no statistical difference at $P = 0.05$.

sorption, filtration, ion exchange, and complexation occurred in the matrix, which removed phosphorus from the wastewater (Dinges, 1982; Shalla, 2000). There was a positively correlation between the activity of urease and the removal of TN and NH_4^+—N with better R^2 as shown in Table 3. It is apparent that the activity of urease in the root zone from CWs can be used as one of the major indicators for estimating the removal of nitrogen-containing pollutants in wastewater.

The correlations along with equations between the phosphatase activity and the removal rates of TP, soluble TP and soluble inorganic phosphorus (IP) are given in Tables 2 and 4. There was no significant correlation between the phosphatase activity and these phosphorus species. Therefore, phosphatase activity was not a key factor for phosphorus purification. De-phosphorization was mainly by adsorption and precipitation. Phosphatase could promote hydrolysis of organic phosphide. Our results indicated that there was not much organic phosphide in the wastewater or the pH condition was not suitable for the enzymatic reaction in the CWs.

Table 3 shows the correlations between the urease activity and the TN and NH_4^+—N removal rates, while Table 5 gives the correlation equations and correlation coefficients between the activity of urease and the removal rates of TN and NH_4^+—N. In the horizontal baffled subsurface-flow and horizontal subsurface-flow CWs, the activity of urease and the removal rates of TN and NH_4^+—N had a significant positive correlation. In the composite baffled subsurface-flow CW, the urease activity was very significant positively correlated to the TN removal rate ($R = 0.96276$, $P = 0.0086 < 0.01$) and significant positively correlated to the NH_4^+—N removal rate ($R = 0.92035$, $P = 0.0267 < 0.05$). In the vertical baffled subsurface-flow CW, the urease activity was poorly correlated to the TN and NH_4^+—N removal rates ($R = 0.78471$, $P = 0.116 > 0.05$). In this CW, the urease activity was lowest among the four CWs and was dramatically different from the other three CW systems (Tables 3 and 6).

Among the four CWs, the urease activity was one of the major factors controlling the nitrogen purification from wastewater except for vertical baffled subsurface-flow CW. Nitrogen removal was mainly through biological degradation by urease. Thus, urease activity could be a key indicator of nitrogen purification, which is worthy of further study.

4. Conclusions

Stronger activities of phosphatase and urease occurred at the top layer of CWs and decreased with the CW depth. Among the four CWs, the activity of urease in the vertical baffled subsurface-flow CW (Z2) were significantly different from the other three CWs, while the activity of phosphatase for the same conditions was not significant. The activity of phosphatase and TP removal rate was not significantly correlated, which indicated that phosphorus removal may be accomplished by the CW matrix. This study proves that the activity of urease in the root zones could be an important indicator for N purification from wastewaters.

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