Journal of Health Science, **55**(5) 757–766 (2009)

Comparison of the Treatment Performances of High-strength Wastewater in Vertical Subsurface Flow Constructed Wetlands Planted with *Acorus calamus* and *Lythrum salicaria*

Yongjun Zhao,[a, b] Bo Liu,[a, c] Wenguang Zhang,[a] Weijing Kong,[a] Changwei Hu,[d] and Shuqing An*,[a, b]

[a]State Key Laboratory of Pollution Control and Resource Reuse, Nanjing University, 22Hankou Road, Nanjing 210093, China, [b]The Institute of Wetland Ecology, School of Life Science, Nanjing University, 22Hankou Road, Nanjing 210093, China, [c]Department of Environmental Engineering, College of Environmental Science, Nanjing University, 22Hankou Road, Nanjing 210093, China and [d]School of Life Science, Linyi Normal University, 18 Tongda Road, Linyi 276005, China

(Received May 25, 2009; Accepted July 16, 2009)

The ability of pilot-scale vertical subsurface flow constructed wetlands (VSCWs) to treat two types of high-strength [400 mg/l of COD or 80 mg/l total nitrogen (TN)] simulated wastewater under greenhouse conditions was studied during an 8-month period. Eighteen CWs units were used: six units were planted with *Acorus calamus* (*A. calamus*), another six units were planted with *Lythrum salicaria* (*L. salicaria*) and six units were unplanted. Each set of units was operated at hydraulic loading rates of 40 l/d. The treatment performance displayed that average removal rates of chemical oxygen demand (COD), TN, total phosphorous (TP) and total organic carbon (TOC) were 44–66%, 35–63%, 47–76% and 22–40%, respectively. Both species grew well under any high loading treatment. We noted that plant uptake and storage were both important factors responsible for nitrogen removal during the study period. Both planted wetlands improved pollutants removal compared with the unplanted control wetland. *L. salicaria* produced more shoots and biomass than the *A. calamus*, which can remove more P nutrients than *A. calamus* especially for high nitrogen (N) loading treatment. However, *A. calamus* was always more efficient species that improved nitrogen nutrients plant uptake because of their longer growth period. Our findings suggested that the vertical subsurface flow constructed wetlands could well treat the high-strength wastewater in greenhouse condition. If good capacity of all nutrients removal is considered, *A. calamus* is more appropriate than *L. salicaria* particularly under high N loading in the influent.

Key words——*Acorus calamus*, chemical oxygen demand removal, *Lythrum salicaria*, nitrogen removal, phosphorus removal, synthetic sewage

INTRODUCTION

At present, in China, the large amount of wastewater from the livestock farm is mostly discharged directly into canals and rivers without appropriate treatment, but these wastewaters contain highly concentrated pollutants, including high chemical oxygen demand (COD) and nitrogen (N) contents, and may deteriorate the quality of aquatic environments.[1, 2] There is therefore an urgent need to improve the conditions by introducing proper treatment of the wastewater prior to discharge. To lessen the impact, treatment of this type wastewater before discharge has been proposed and further practiced by employing constructed wetlands, for these systems have significant advantages of low implementation costs, and versatile removal mechanisms.[3–5] Constructed wetlands are known to be particularly efficient in removing COD, and have also successfully been used in temperate countries to treat wastewaters with high concentrations of N.[6, 7] Of constructed wetlands used for this wastewater treatment, most are surface flow systems, only a few belong to subsurface flow types.[8] With rising concerns of sustainable management of biore-

---

*To whom correspondence should be addressed: The Institute of Wetland Ecology, School of Life Science, Nanjing University, 22 Hankou Road, Nanjing 210093, China. Tel.: +86-25-83594560; Fax: +86-25-83594560; E-mail: anshq@nju.edu.cn source, and more stringent effluent standards, this type wastewater treatment by vertical subsurface flow constructed wetlands (VSCWs) can be a great potential and challenge. However, VSCW subjected to changing of loading level in the influent is poorly understood, especially when used to treat domestic animal breeding wastewater under heavy loads. In this treatment alternative an important issue arises in what and how the VSCW perform under heavy loads, to which available information is very limited. So, we need to design and operate at relative high organic and N loads for purpose of producing effluent that could meet discharged criteria. For improving the wetland treatment performance during a grow season, it becomes increasingly important to examine the response of the system to the different species and influent concentrations.

Different species of wetland plants, including species of *Typha angustifolia* and *Cyperus involucratus*, have been used in constructed wetland systems for treatment high-strength wastewater.[9] *Acorus calamus* (*A. calamus*) is also widely used and is known to be highly tolerant to various types of wastewater, and *Lythrum salicaria* (*L. salicaria*) is a species with aesthetic appearance that can grow very well in a subsurface flow constructed wetland system. Climate and other local conditions influence wastewater characteristics, plant growth and evaporation as well as the removal processes in the constructed wetland, particularly the microbial processes which are expected to be stimulated by the high temperatures.[2] Thus, there is a demand to gain more information about the performance of vertical flow constructed wetlands under high temperature conditions. The aims of this study were to investigate the ability of vertical flow constructed wetland systems to treat high COD or high N wastewater, and to evaluate the performance of systems planted with *A. calamus* and *L. salicaria* with unplanted systems. Furthermore, the effect of season on treatment performances was studied in order to get more insight into the capacity of vertical flow systems under greenhouse conditions.

MATERIALS AND METHODS

Characterization of the Pilot-scale Wetlands——The set-up of the pilot-scale wetlands consisted of three types of VSCWs, with *A. calamus*, *L. salicaria* and the other unplanted, respectively (Fig. 1). The wetland frame, 1.00 m (length) × 0.60 m (width) × 0.80 m (height) were made of reinforced cement, and were filled with gravel (nominal mean diameter of 1.20 cm) up to a depth of 0.20 m in the lower layer, and finally with sieved blast furnace granulated slag (0.25 m of nominal mean diameter of 1.50 cm) in the upper layer, which was provided from CHANGQING Iron and Steel Company, Nanjing, China. The gravel and slag had been previously sterilized with a solution of 5% sodium hypochlorite, to ensure that the new microbial colonization occurred during the operation stages. In this study, the slag has been cleaned for many times in order to prevent the creation of high (alkaline) pH values, because this would be incompatible with microorganism growth in the substrate, as well as with the growth of commonly applied *A. calamus* and *L. salicaria*. Three types of experimental wetlands were run in parallel under identical conditions for 240 days.

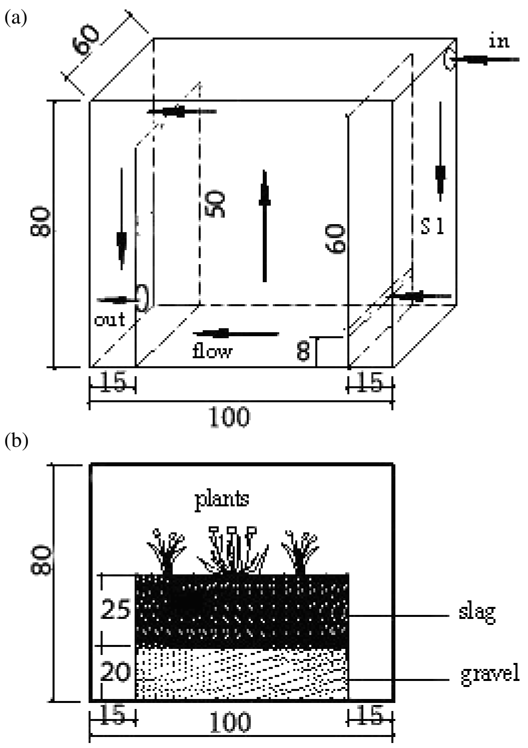

Fig. 1. Diagram of the Pilot-scale Wetlands Used in This Study
(a) Construction chart, (b) wetland stuffs and plants.

At April 5, 2008, six units were planted with *A. calamus* (16.37 ± 1.59 cm tall), and six with *L. salicaria* (20.68 ± 2.34 cm tall), using 8–12 stems per unit. Before planting, most soil was carefully removed from the roots to avoid clogging of the system. After planting, they were kept flooded for one month with tap water, and then the influent wa-

Table 1. Mean Concentrations ± S.D. and Pollutants Removal Efficiencies for COD, TN, TP, TOC and Physico-chemical Water Parameters of Water Temperature (T), DO, Eh and pH in the Influent and the Effluent Waters of the Three VSCWs under High Organic Loading with Influent

Parameters	Influent	Effluent		
		A. calamus	*L. salicaria*	The unplanted wetlands
COD				
Concentration (mg/l)	$403.21^{a} \pm 5.26$	$135.31^{c} \pm 88.98$	$176.49^{b} \pm 84.04$	$207.65^{b} \pm 61.49$
Removal (%)		$66.44^{a} \pm 22.07$	$56.23^{b} \pm 20.84$	$48.51^{b} \pm 15.25$
Removal capacity (mg/m^3·d)		$850.47^{a} \pm 282.53$	$719.75^{b} \pm 266.80$	$620.82^{b} \pm 195.23$
TN				
Concentration (mg/l)	$40.09^{a} \pm 2.28$	$23.49^{bc} \pm 5.11$	$23.14^{c} \pm 7.61$	$26.04^{b} \pm 3.76$
Removal (%)		$41.41^{a} \pm 12.75$	$42.28^{a} \pm 18.99$	$35.06^{b} \pm 9.37$
Removal capacity (mg/m^3·d)		$44.62^{a} \pm 11.93$	$52.70^{a} \pm 16.23$	$53.81^{b} \pm 24.17$
TP				
Concentration (mg/l)	$5.22^{a} \pm 0.18$	$1.74^{c} \pm 0.62$	$1.38^{c} \pm 0.45$	$2.20^{b} \pm 0.66$
Removal (%)		$66.60^{ab} \pm 11.86$	$73.51^{a} \pm 8.10$	$57.79^{b} \pm 12.64$
Removal capacity (mg/m^3·d)		$11.04^{ab} \pm 1.97$	$12.18^{a} \pm 1.34$	$9.58^{b} \pm 2.09$
TOC				
Concentration (mg/l)	$183.29^{a} \pm 3.75$	$122.91^{b} \pm 29.06$	$137.68^{b} \pm 21.57$	$115.72^{b} \pm 16.94$
Removal (%)		$36.86^{a} \pm 9.24$	$24.88^{a} \pm 11.77$	$39.95^{a} \pm 15.85$
Removal capacity (mg/m^3·d)		$191.70^{a} \pm 92.25$	$144.78^{a} \pm 68.46$	$214.50^{a} \pm 53.79$
T (°C)	$26.7^{a} \pm 9.6$	$26.3^{a} \pm 9.5$	$26.3^{a} \pm 9.5$	$26.5^{a} \pm 9.4$
DO (mg/l)	$3.38^{a} \pm 1.32$	$1.81^{b} \pm 0.87$	$1.78^{b} \pm 0.93$	$1.88^{b} \pm 1.02$
Eh (mv)	$47.31^{b} \pm 1.09$	$29.39^{a} \pm 11.98$	$28.41^{a} \pm 12.74$	$63.25^{a} \pm 52.69$
pH	$7.54^{a} \pm 0.15$	$7.29^{c} \pm 0.22$	$7.21^{c} \pm 0.17$	$7.39^{b} \pm 0.13$

Note: The data showed in the table were means ± S.D. Values with different superscript letters in the same row indicate a significant difference at $p < 0.05$ according to the Duncan's multiple range tests.

ter was administered to the wetlands. In operation stage, the hydraulic loading rate was 40 l/d. The entire batch volume of the synthetic sewage was applied in a single batch through a round distribution polyvinyl chloride (PVC) pipe of 5 cm internal diameter, which was perforated with holes of 1.5 mm. The PVC pipe was placed on the right of the wetlands surface and in 35 cm distance from the top layer of wetlands. The substrate of the wetlands held 60 l of water (according to the substrate materials net void capacity); therefore the batch volumes of 40 l applied to each wetland with a constant flow rate of 15 l/min were achieving an overall hydraulic retention time (HRT) of 1.5 days. The wetlands were not fed every day, but one time every two weeks. All the treatment including the control was triplicate and totally 18 wetlands were used for this study. The operation and monitoring of the wetlands were conducted between May and December 2008.

For health and safety reasons, as well as for comparison of the parallel experiments, the wetlands were fed with synthetic wastewater, simulating high COD or high N loading and medium strength [total phosphorus (TP)] rural domestic sewage. It was prepared prior to each (batch) feeding by mixing (in tap water) the following different components (g/m^3): glucose 400, carbamide 80, NaH_2PO_4 15, KH_2PO_4 1.5, $CaCl_2$ 4, $MgSO_4$ 2 (high COD loading treatment) The following different components for the high N loading treatment (g/m^3): glucose 200, carbamide 160, NaH_2PO_4 15, KH_2PO_4 1.5, $CaCl_2$ 4, $MgSO_4$ 2. The experimental influent conditions applied to the unplanted systems are same as the planted systems (Tables 1 and 2).

Water Sampling and Chemical Analysis——From May, 2008 to December, 2008, influent and effluent water of the pilot-scale VSCWs were sampled approximately every two weeks under normal conditions to evaluate their treatment performances. The sampling time is fixed during the operational periods, and simulated wastewater continuously flowed about 1.5 day in the wetland. Water samples were analyzed for COD, total organic carbon (TOC), total nitrogen (TN) and TP. COD was determined by titrimetric method. Determination of TP were performed using a segmented flow analysis (Automated Chemistry An-

Table 2. Mean Concentrations ± S.D. and Pollutants Removal Efficiencies for COD, TN, TP, TOC and Physico-chemical Water Parameters of Water Temperature (T), DO, Eh and pH in the Influent and the Effluent Waters of the Three VSCWs under High N Loading with Influent

Parameters	Influent	Effluent		
		A. calamus	*L. salicaria*	The unplanted wetlands
COD				
Concentration (mg/l)	203.35^{a} ± 3.41	79.18^{c} ± 34.88	97.21^{c} ± 42.14	114.72^{b} ± 53.05
Removal (%)		61.06^{a} ± 17.15	52.20^{a} ± 20.72	43.58^{b} ± 26.09
Removal capacity (mg/m^{3}·d)		394.18^{a} ± 110.73	336.95^{a} ± 133.79	281.36^{b} ± 168.40
TN				
Concentration (mg/l)	81.32^{a} ± 2.95	29.88^{c} ± 14.01	41.38^{b} ± 8.54	41.57^{b} ± 13.76
Removal (%)		63.25^{a} ± 12.22	49.11^{b} ± 10.49	48.86^{b} ± 16.92
Removal capacity (mg/m^{3}·d)		163.29^{a} ± 44.46	126.77^{b} ± 27.10	126.18^{b} ± 43.69
TP				
Concentration (mg/l)	5.14^{a} ± 0.47	1.84^{c} ± 0.73	1.25^{c} ± 0.49	2.75^{b} ± 0.55
Removal (%)		64.14^{a} ± 14.29	75.66^{a} ± 9.71	46.62^{b} ± 10.66
Removal capacity (mg/m^{3}·d)		10.47^{a} ± 2.33	12.35^{a} ± 1.58	7.57^{b} ± 1.74
TOC				
Concentration (mg/l)	92.88^{a} ± 3.12	68.57^{b} ± 10.55	72.80^{b} ± 12.07	65.67^{b} ± 10.59
Removal (%)		26.18^{a} ± 11.36	21.62^{a} ± 12.99	29.31^{a} ± 11.41
Removal capacity (mg/m^{3}·d)		86.38^{a} ± 33.65	63.75^{a} ± 38.32	77.18^{a} ± 33.49
T°C	26.7^{a} ± 9.6	26.3^{a} ± 9.5	26.3^{a} ± 9.5	26.5^{a} ± 9.4
DO (mg/l)	6.22^{a} ± 1.36	3.08^{b} ± 0.98	3.47^{b} ± 1.12	3.51^{b} ± 1.26
Eh (mv)	67.52^{a} ± 5.82	52.43^{b} ± 14.82	41.99^{b} ± 9.33	58.77^{b} ± 57.11
pH	7.51^{a} ± 0.17	7.13^{c} ± 0.11	7.19^{c} ± 0.15	7.35^{b} ± 0.21

Note: The data showed in the table were means ± S.D. Values with different superscript letters in the same row indicate a significant difference at $p < 0.05$ according to the Duncan's multiple range tests.

alyzer, Brighton, U.K.) and TOC and TN using liquor TOC II (Munich, Germany). All the parameters mentioned above were determined according to the method as described in the standard method for Examination of Water and Wastewater. The physico-chemical water parameters, such as water temperature, redox-potential (Eh), pH, and dissolved oxygen (DO) were measured *in situ*. DO was assayed using an Orion Dissolved Oxygen Probe (Model 862Aplus, Thermo Orion, Granville, Michigan, U.S.A.). Water Eh was recorded with an Orion 250Aplus ORP Field Kit, and water pH with a water pH with an Orion Portable pH Meter (Model 250Aplus, Thermo Orion, Granville, Michigan, U.S.A.).

Plant Growth and Uptake—— At the end of the experiment, the number of plants in each unit was counted and five representative *A. calamus* and three *L. salicaria* plants from each unit harvested to estimate biomass. The plants were fractionated into belowground (roots and rhizomes) and aboveground (leaves and stems) tissues, and their dry weight measured. Subsamples of the dried plant tissue were homogenized and the contents of N analyzed by a standard Kjeldahl method (National Institute of Agricultural Sciences, 1977). The uptake of N was calculated from the total biomass and the N concentration in the tissues.

Statistical Analysis—— The treatment efficiency was calculated as the percent removal R for each parameter, which was calculated by $R = (1 - C_e/C_i) \times 100$, where C_i and C_e are the influent and effluent concentrations in mg/l. Mean effluent values of every batch sampling in a month were used to calculated removal rates of monthly average as mass removal rate for each parameter. All statistical analysis were performed using the SPSS software package (SPSS, 2003), including analysis of variance (ANOVA), Bartlett's and Levine's test for homogeneity of variance and normality, and Duncan's multiple range test for differences between means.

RESULTS

Effluent Concentrations and Mass Removal Rates of Pollutants

At high organic loading in the influent, the effluent concentration of COD was significant lower in *A. calamus* than in *L. salicaria* and the unplanted systems (Table 1). However, there were no significant difference between *L. salicaria* and the unplanted systems ($p > 0.05$). Mass removal rates of COD were high in all wetlands at high organic loading treatments, the differences in removal efficiencies of COD between the two planted systems were highly significant ($p < 0.05$). At high N loading, furthermore, COD concentrations of effluent were independent on species (Table 2). But significant difference was observed in the effluent concentrations of COD between the planted and the unplanted system ($p < 0.05$). Meanwhile, the mass removal rates of COD were significantly higher in systems planted with *A. calamus* compared to the unplanted systems (Tables 1 and 2). Effluent concentrations of TN were generally high, and highest in the unplanted systems at high N loading (Table 2). Species affected TN effluent concentration at high N loading, whereas no effect was observed in the planted systems at high organic loading in the influent ($p > 0.05$). In systems planted with *A. calamus* generally had significantly higher TN mass removal than the unplanted system and the difference was more obvious at the high N loading treatments. However, no significant difference was detected between in beds with *L. salicaria* and the unplanted system at high N loading treatments ($p > 0.05$). For TP, the effluent concentration was significantly higher in planted than in the unplanted systems ($p < 0.05$). There was no significant effect of species on effluent TP concentrations under any high-strength wastewater in the influent. Multiple comparisons detected significantly higher TP removal rates in the two planted than the unplanted system at high N loading. But no significant difference was observed in removal rates of TP between in beds with *A. calamus* and the unplanted system at high organic loading ($p > 0.05$). Effluent concentrations of TOC did not differ between planted and unplanted systems, but concentrations in the planted system were generally higher than that of the unplanted systems with high organic or N loading in the influent (Tables 1 and 2). The mass removal of TOC showed a similar pattern to that of TP with weak correlations between species (including the unplanted treatment) and with higher average removal rates in the unplanted systems. But no significant difference was observed among three VSCWs ($p > 0.05$).

Physico-chemical Variables of VSCWs

Changes in values of the situ measured physicochemical variables of water temperature, pH, Eh and DO are presented in Table 1 and Table 2. Mean temperature in the influent and within the effluents among the three VSCWs was not significantly different. In contrast, pH values were considerably lower in the outlet water of the three VSCWs than in the inlet water. Outlet pH was significantly affected by plant present or not, and there was a significant difference between the unplanted and the planted wetlands for two types treatments ($p < 0.05$). Furthermore, DO in effluent was significantly lower in the influent than that of in the effluent at high organic loading treatment ($p < 0.05$). However, no significant difference in effluent for DO was detected among in the three VSCWs ($p > 0.05$), whereas at high N loading treatments the same result was observed (Table 2). Eh values in effluent were independent on species (including the unplanted treatment), and no effect was observed among the three VSCWs at high organic or N loading treatments ($p > 0.05$).

Plant Uptake of N

The concentration of N in the plant varied between 2.3 and 2.9% of the dry weight for *A. calamus* and 1.9 and 2.2% of the dry weight for *L. salicaria*. There was no significant difference between the N concentrations between the two species at different high loading treatments. The average plant uptakes of N throughout the study period were 1.39 ± 0.28 g/m^2·d for *A. calamus* and 0.14 ± 0.03 g/m^2·d for *L. salicaria* (mean ± S.D.). Comparing the plant uptake with the average mass removal rates of N in the treatment systems, the uptake of N by *A. calamus* constituted between 6.3% of the mass N removal at high organic loading to 14.4% at high N loading. Because of the short growth period and N content in the tissues of *L. salicaria*, the plant uptake only constituted 0.3–5.1% of the mass N removal rates in the *L. salicaria* planted systems.

Time Course of Removal of Pollutants

It was found that the nutrient removal rates fluctuated in each unit during the wetlands operational period (beginning of May up to the end of December) (Figs. 2–5). Though *A. calamus* and *L. salicaria* showed similarly higher trend COD removal than the unplanted system (Fig. 2a, 2b), no statistical significant seasonal differences were detected ($p > 0.05$). Likewise, high fluctuations

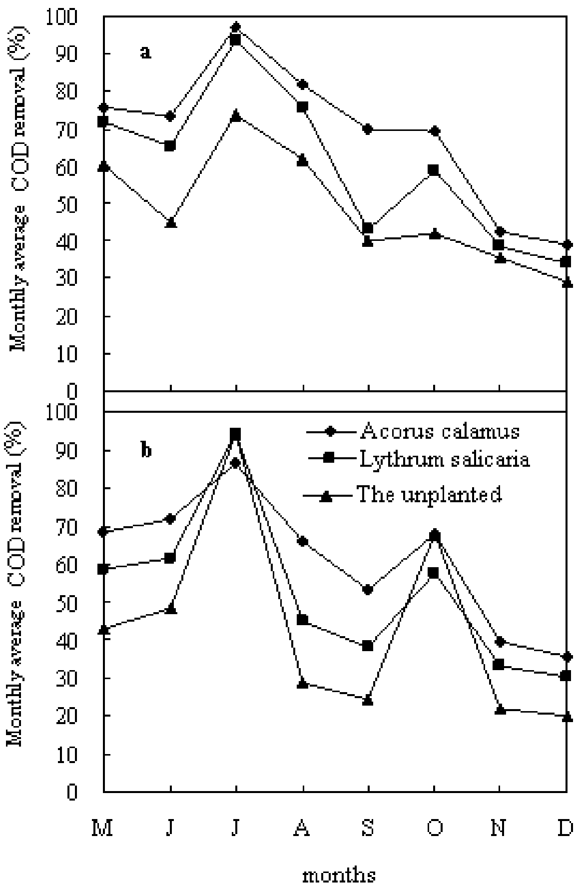

Fig. 2. Temporal Course of Removal Rates of COD in the Three VSCWs During the Wetlands Operational Period
a: High organic loading treatment; b: high N loading treatment.

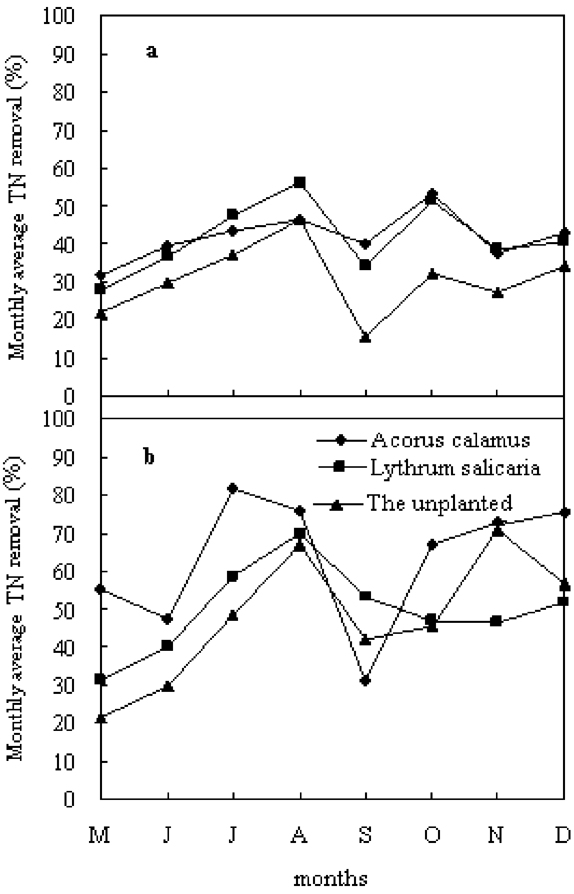

Fig. 3. Temporal Course of Removal Rates of TN in the Three VSCWs During the Wetlands Operational Period
a: High organic loading treatment; b: high N loading treatment.

were observed for TN (Fig. 3a, 3b) removal in the three VSCWs, high TN removal effects appeared in summer (July–August) and autumn (October–November) in three VSCWs. However, under any high loading treatments, in winter (December) and in May and June (beginning of study period), the removal rates of TN remained relatively the lowest in all VSCWs. Despite apparently lower TN removal efficiencies in the unplanted system at high organic loading treatments, no statistical significant seasonal variation was detected among three VSCWs ($p > 0.05$) (Fig. 2a). Rather, the removal rate of TP was higher in June and July than those in May in the three VSCWs, and then decreased and/or fluctuated over the rest experimental period (Fig. 4a, 4b). Meanwhile, higher TOC removal effects appeared in August and October in the planted system (Fig. 5a, 5b). However, the removal rates of TOC remained relatively lower in other months. It was decreased from November to December in all wetlands. Additionally, at high organic loading in the influent, the unplanted system usually showed higher trend TOC removal than those in the planted wetlands during the operational period (Fig. 5a).

DISCUSSION

In this study, it clearly showed that vertical subsurface flow constructed wetland systems have a good capacity to treat high organic or N loading wastewater in greenhouse condition. Many studies from temperate areas have documented that vertical subsurface flow constructed wetland systems are very efficient in removing biodegradable organic matter as well as able to nitrify ammonium,5,10,11) the temperature of the effluent wastewater ranged between 21 and 30.8°C during the experiment in their studies. In the present study, we confirmed that this is also true in greenhouse condition. Both species of aquatic macrophyte tested in this study grew well in the gravel-based and slag-based VSCW units when loaded with high-strength

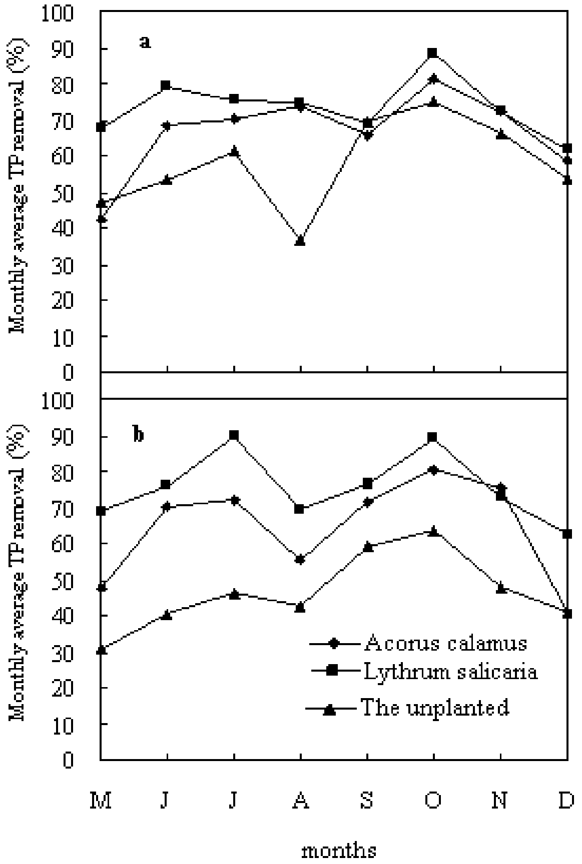

Fig. 4. Temporal Course of Removal Rates of TP in the Three VSCWs During the Wetlands Operational Period

a: High organic loading treatment; b: high N loading treatment.

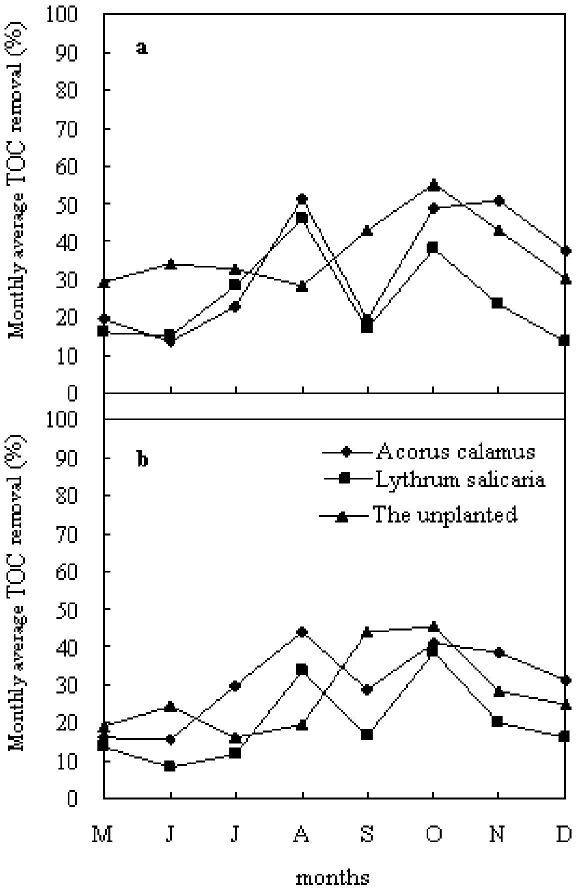

Fig. 5. Temporal Course of Removal Rates of TOC in the Three VSCWs During the Wetlands Operational Period

a: High organic loading treatment; b: high N loading treatment.

simulated domestic wastewater. Especially *Lythrum* seems to grow well in the domestic wastewater, but *Acorus* is generally considered a more valuable species than *Lythrum* for wastewater treatment. At the end of the growing season, *Acorus* forms short over-wintering leaves that start to re-grow early in the spring. Moreover, *Acorus* displays efficient use of nitrogen (N) by their uptake and internal cycling.[12)]

The removal of COD was relative efficient at high organic or N loading treatment and there was no effect of plant species under high N loading treatment. Whereas at high organic loading treatment, the beds with *Acorus* removed more organic matter than the beds with *Lythrum*, and as expected this species effect was more pronounced at high organic loading where plants play a greater relative role in the removal of organic matter. The wastewater used in the present study had relatively low concentrations of COD which probably partially because of the higher temperature in greenhouse condition that possess high degradation rates in the wastewater treatment. Although the influent wastewater of high-loading were applied, the removal of COD was still efficient in the VSCWs. Other studies comparing planted and unplanted beds have shown no or only little difference in removals of COD between the beds.[13, 14)] We also found significant higher removal of COD in beds with *Acorus* compared to unplanted beds, whereas beds planted with *Lythrum* did not show a higher removal than unplanted beds at high organic loading treatment. These results suggested that plants can play a significant role in the removal of organic matter but also that the plant effect is usually small and depends on the species.[5)]

N removal in VSCWs is accomplished primarily by physical settlement, denitrification and plant/microbial uptake.[15, 16)] In our study, TN removal efficiency differed significantly between the planted and the unplanted system. Both *Acorus* and *Lythrum* showed higher removal rates than the unplanted system at high organic loading treatment. Plant uptake did contribute to the N removal, particularly at the relatively low influent TN concentrations of high organic loading treatment.[16] The N mass removal rates recorded of approx 49% and 46% for *Acorus* and *Lythrum*, respectively, are, therefore, probably close to the maximum capacity for N removal in this type of VSCW system. Whereas at high N loading treatment, an average TN removal of 63% in bed with *Acorus* was significantly higher than 48–49% in bed with *Lythrum* and the unplanted system. Our results showed that the growth reactions of *Acorus* depend on the N dose applied. Under field conditions, *Acorus* benefits and displays vigorous growth in nutrient-rich wetland habitats, while this species does not occur in the nutrient poor habitats.[17] Comparing the plant uptake with the average mass removal rates of N at high N loading treatment, the uptake of N by *Acorus* usually had higher than that of *Lythrum*. This could be because of higher roots biomass in *Acorus* or it could indicate that the *Acorus* roots provide better conditions for nitrification/denitrification processes. So, the beds with *Acorus* removed more N than the beds with *Lythrum*, and as expected this species effect was more pronounced at high N loading where plants play a greater relative role in the removal of nutrients. N processing in the three wetlands was high, but not complete. If a significantly higher removal of N is desired, a solution could be to use a vertical flow system with recirculation which has been shown to give good performance regarding N removal.[5, 18]

In the present study we did find statistically significant effects of plants, particularly on N removal, but plants also affected P removal. The higher removal rate of P in the planted systems, particularly the systems planted with *Lythrum*, affected the effluent P concentrations by growth of plant. Some removal of P occurred in the experiments with higher removals in beds with *Lythrum* than in beds with *Acorus* and the greatest difference at high organic loading treatment. Thus, *Lythrum* play a greater role in the removal of P especially for high organic concentration in the influent. Other studies also show that this species had good capacity for remove P compared to other wetland plant.[19] Chemical adsorption is usually considered the main mechanism for P removal in CWs.[20] We did not quantify the amount of P removed by plant uptake in this study, but we assume that a large fraction of the P that was removed was through binding to the bed substrate. During the monitoring period, the effluent P concentrations fluctuated reflecting the changes in seasons, pH and binding capacity of media. Generally, the effluent phosphorus concentrations of three VSCWs were almost constant and relative low only at the beginning of the operation period (independent of the organic or N influent concentrations). It might also be explained mainly by the higher adsorption capacity, where the slag substrate was fresh and the adsorption sites were free of phosphorus. However, the effluent phosphorus concentrations rose with the operation time. If an efficient and sustainable removal of P is to be achieved in a VSCW system, the best solution is probably to install a separate filter unit with a high P binding capacity material, which can be changed when saturated with P, or to add Al or Fe-based precipitation chemicals to the wastewater.[21, 22] In this study, the bottom layer of the gravel bed could not be described as a wetland cell with noticeable P-adsorption capacity. So, further investigations should be performed about the P-adsorption capacities of slag with other material combination.

In this study, plants did not exert a strong effect on the removal rate of total organic carbon in the VSCWs. The results showed that the unplanted system removed carbon more efficiently than the planted wetlands and it was more obvious at high organic loading treatment. Baptista *et al.*[23] also suggested that wetlands without plants showed higher total organic carbon removal than those with plants because of the certain functional groups difference between the planted and the unplanted system. Based on mass balance calculations and stoichiometric relationships, indirect observations allowed to assume that influent C/N ratio and organic and N concentration had a slightly effect on the removal of organic carbon matter. However, the influence mechanism is complex, and needed to be studied further.

In conclusions, the wetland species *Acorus* and *Lythrum* grow well in gravel and slag based VSCWs fed with high-strength simulated domestic sewage, and thus can potentially be used to enhance the aesthetic appearance and hence the public acceptance of wastewater treatment systems. The treatment systems have efficient removals of COD, TN and TP under any high loading treatment. However, removals of TOC are generally lower in this kind of system implying that vertical subsurface flow CW systems have limited use if organic carbon removal is required. Both planted wetlands improved pollutants removal compared with the unplanted control wetland. The performances in terms of average COD, TN, and TP removal rates obtained in the planted wetlands under different influent condition were in the ranges 52–66%, 41–63% and 64–76% respectively. *L. salicaria*, which exhibited greater growth, can remove more P nutrients than *A. calamus*. However, *A. calamus* always more efficient species that improved N nutrients plant uptake by their longer growth period. On the whole, they are all probably the preferred species based on good performance for high-strength wastewater treatment. In addition, further research is still needed for improving the nutrients removal by the optimal bed design in order to exploit the specific advantages of the two species.

Acknowledgements We thank the colleagues and students from Nanjing University for maintaining the treatment wetland systems during the study period. This study is financially supported by the National Water Special Project of China (No. 2008ZX07010-001-004 and 2008ZX07526-002-003).

REFERENCES

1) Stone, K. C., Hunt, P. G., Humenik, F. J. and Johnson, M. H. (1998) Impact of swine waste application on ground and stream water quality in an eastern coastal plain watershed. *Trans. ASAE*, **41**, 1665–1670.
2) Kantawanichkul, S., Kladprasert, S. and Brix, H. (2009) Treatment of high-strength wastewater in tropical vertical flow constructed wetlands planted with *Typha angustifolia* and *Cyperus involucratus*. *Ecol. Eng.*, **35**, 238–247.
3) Kadlec, R. and Knight, R. L. (1996) *Treatment Wetlands*, Lewis Publishers, Boca Raton, FL.
4) Humenik, F. J., Szögi, A. A., Hunt, P. G., Broome, S. and Rice, M. (1999) Wastewater utilization: a place for managed wetlands—Review. Asian-Australian *J. Anim. Sci.*, **12**, 629–632.
5) Konnerup, D., Thammarat Koottatep, T. and Brix, H. (2009) Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with Canna and Heliconia. *Ecol. Eng.*, **35**, 248–257.
6) Gottschall, N., Boutin, C., Crolla, A., Kinsley, C. and Champagne, P. (2007) The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. *Ecol. Eng.*, **29**, 154–163.
7) Poach, M. E., Hunt, P. G., Reddy, G. B., Stone, K. C., Johnson, M. H. and Grubbs, A. (2007) Effect of intermittent drainage on swine wastewater treatment by marsh-pond-marsh constructed wetlands. *Ecol. Eng.*, **30**, 43–50.
8) Hunt, P. G. and Poach, M. E. (2001) State of the art for animal wastewater treatment in constructed wetlands. *Water Sci. Technol.*, **44**, 19–25.
9) Koottatep, T., Polprasert, C., Oanh, N. T. K., Heinss, U., Montangero, A. and Strauss, M. (2001) Septage dewatering in vertical-flow constructed wetlands located in the tropics. *Water Sci. Technol.*, **44**, 181–188.
10) Brix, H., Arias, C. A. and Johansen, N. H. (2002) BOD and nitrogen removal from municipal wastewater in an experimental two-stage vertical flow constructed wetland system with recycling, *Proceedings of the 8th International Conference on Wetland Systems for Water Pollution Control*, vol. 1, Arusha, Dar es Salam, Tanzania, September 16–19, 2002, pp. 400–410.
11) Weedon, C. M. (2003) Compact vertical flow constructed wetland systems—first two years' performance. *Water Sci. Technol.*, **48**, 15–23.
12) Weber, M. and Brändle, R. (1996) Some aspects of the extreme anoxia tolerance of the sweet flag, *Acorus calamus* L. *Folia Geobotanica and Phytotaxonomica*, **31**, 37–46.
13) Coleman, J., Hench, K., Garbutt, K., Sexstone, A., Bissonnette, G. and Skousen, J. (2001) Treatment of domestic wastewater by three plant species in constructed wetlands. *Water Air Soil Pollut.*, **128**, 283–295.
14) Belmont, M. A. and Metcalfe, C. D. (2003) Feasibility of using ornamental plants (*Zantedeschia aethiopica*) in subsurface flow treatment wetlands to remove nitrogen, chemical oxygen demand and nonylphenol ethoxylate surfactants—a laboratory-scale study. *Ecol. Eng.*, **21**, 233–247.
15) Healy, M. G., Rodgers, M. and Mulqueen, J. (2006) Treatment of dairy wastewater using constructed wetlands. *Bioresour. Technol.*, doi:10.1016/j.biortech.2006.07.036.
16) Li, L. F., Li, Y. H., Biswas, D. K., Nian, Y. G. and Jiang, G. (2008) Potential of constructed wetlands in treating the eutrophic water: Evidence from Taihu Lake of China. *Bioresour. Technol.*, **99**, 1656–1663.
17) Vojtíšková, L., Edita Munzarová, E., Votrubová, O., Řihová, A. and Barbora Juřicová, B. (2004) Growth and biomass allocation of sweet flag (*Acorus calamus* L.) under different nutrient conditions. *Hydrobiologia*, **518**, 9–22.
18) Arias, C. A., Brix, H. and Marti, E. (2005) Recycling of treated effluents enhances removal of total nitrogen in vertical flow constructed wetlands. *J. En-*

viron. Sci. Health Part A: Tox. Hazard Subst. Environ. Eng., **40**, 1431–1443.

19) Camacho, J. V., Martinez, A. D. L., Gomez, R. G. and Sanz, J. M. (2007) A comparative study of five horizontal subsurface flow constructed wetlands using different plant species for domestic wastewater treatment. *Environ. Technol.*, **28**, 1333–1343.
20) Arias, C. A., Del Bubba, M. and Brix, H. (2001) Phosphorus removal by sands for use as media in subsurface flow constructed reed beds. *Water Res.*, **35**, 1159–1168.
21) Brix, H., Arias, C. A. and Del Bubba, M. (2001) Media selection for sustainable phosphorus removal in subsurface flow constructed wetlands. *Water Sci. Technol.*, **44**, 47–54.
22) Vymazal, J. (2002) The use of sub-surface constructed wetlands for wastewater treatment in the Czech Republic: 10 years experience. *Ecol. Eng.*, **18**, 633–646.
23) Baptista, J. D. C., Donelly, T., Rayne, D. and Davenport, R. J. (2003) Microbial mechanisms of carbon removal in subsurface flow wetlands. *Water Sci. Technol.*, **48**, 127–134.