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Original Research

Aerated Enhanced Treatment of Aquaculture Effluent by Three-stage, Subsurface-Flow Constructed Wetlands under a High Loading Rate

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Abstract

To obtain sustainable aquaculture, developing appropriate treatment processes for wastewater is essential. In this study, two three-stage hybrid wetland systems were configured to treat aquaculture effluent. The two systems added with or without artificial aeration were operated under a high HLR (8.0 m/day) with a short retention time (0.96 h). By the results, COD could be effectively removed by both the systems, and it had been significantly enhanced by continuous aeration (air:water ratio being 7.5:1). For nitrogen (N) compounds, NH_4^+-N concentration of outflow compared to the inflow was elevated in most cases under the non-aerated condition, but an opposite trend was observed for the aerated state, which indicated that dissolved oxygen (DO) required for nitrification in the natural bed was more insufficient at the high organic loading rate. TN mass removal was efficient without aeration, but it significantly declined after enhancement, possibly due to the resuspension of trapped organic N promoted by the strong airflow. The artificial aeration also significantly improved the treatment performance on phosphorus. By canonical correspondence analysis (CCA), the first-order removal rate constants of various pollutants were significantly correlated to the measured environment of the inflow. Under the high constant HLR accompanied by low DO, pollutant loading rate became the first dependent factor on removal rate for such a rapid filtration system.

Keywords: aquaculture, wetland, artificial aeration, treatment performance, pollutant loading rate

Introduction

Aquaculture activities are well-known as the major contributors to the increasing level of organic waste and toxic compounds in receiving waters. As the aquaculture industry intensively develops, its environmental impact increases; discharges from aquaculture deteriorate the receiving environment [1]. Thus, the need for developing economically viable approaches for aquaculture wastewater treatment has become a major concern in water environmental protection. Biotreatment of aquaculture wastewater for recirculation purposes is a sensible means to support the further growth of the industry. In recent years, rotating biological contactors, trickling filters, bead filters, and fluidized sand biofilters have been conventionally used in intensive aquaculture systems to remove waste from culture water [2].

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Another viable method to treat wastewater is constructed wetland (CW), which offers numerous advantages over the conventional methods; for example, CW just needs cheap investment and operation costs but with relatively high treatment efficiency and more ecosystem service functions [3]. As a result, CW is commonly considered a low-energy 'green' technique and has been applied in aquaculture, especially in tropical or sub-tropical developing countries [4-7].

A subsurface-flow CW generally consists of a bed of sand or gravel in which emergent wetland plants are planted. Then water flows either horizontally or vertically through the bed medium. During the process, pollutants are primarily decomposed from microbial activity, which is considered the main pathway for nitrogen (N) removal, through nitrification followed by denitrification. It is generally regarded that vertical-flow CWs are better locations for nitrification over horizontal-flow ones, while the latter present a dominant potential in denitrification [8]. Thereby different flow regimes are usually combined to achieve better performance.

Recently, the integration of CW into aquaculture for water quality control in recirculating aquaculture systems (RASs) has evoked researchers' interest [4, 6, 9-13]. However, most of the studies focus on a single flow regime at a high or low loading rate. Systematic investigations on a hybrid system for aquaculture effluent recirculation purposes operated under a high loading rate and the associated impact factors are rarely reported.

In the work presented here, two identical hybrid subsurface-flow CW systems (CW 1+2) were constructed to treat tilapia aquaculture effluent at a high loading rate. Due to the low nitrification rate revealed by previous surveys [14], artificial aeration was added to one of the two systems. Such an adjustment was to determine the influence of artificial aeration on treatment performance. To obtain the above aims, the present work was divided into three stages. During the first one, CW 1 without aeration was investigated, while in stage 2, CW 1 with aeration was investigated; in stage 3, both CW 1 with aeration and CW 2 without aeration were simultaneously investigated. All the investigations were carried out on alternate days with each stage lasting a month. Once the investigation was finished for the current stage, the wetland systems were switched into the running conditions of the next stage and lasted 2 months for microbial acclimation before data collection [15]. Such a sampling strategy allowed for a ready comparison between the aeration and non-aeration experiments, with considerations for both temporal and spatial heterogeneity.

Materials and Methods

Site Description

The study site was located in Jingzhou city (30º16' N, 112º18' E), Hubei Province (part of central China with a typical subtropical climate). Two parallel, identical hybrid wetland systems (CW 1+2), each with down, up and horizontal flow chambers (5.0 m length×4.0 m width×1.0 m depth for each chamber) (Fig. 1A), were constructed in the field. The different flow regimes were achieved by laying water distribution pipes (denoted as 1 in Fig. 1) on the surface of the down flow chamber, laying drainage pipes (4) on the bottom or the surface of the up flow chamber, and laying distribution (1) or drainage pipes (4) at the start or end of the horizontal flow chamber, respectively. All the perforated pipes were made of PVC, with diameters ranging from 75 to 160 mm. The frame of the chambers was braced with bricks, with the bottom fixed to a slope of

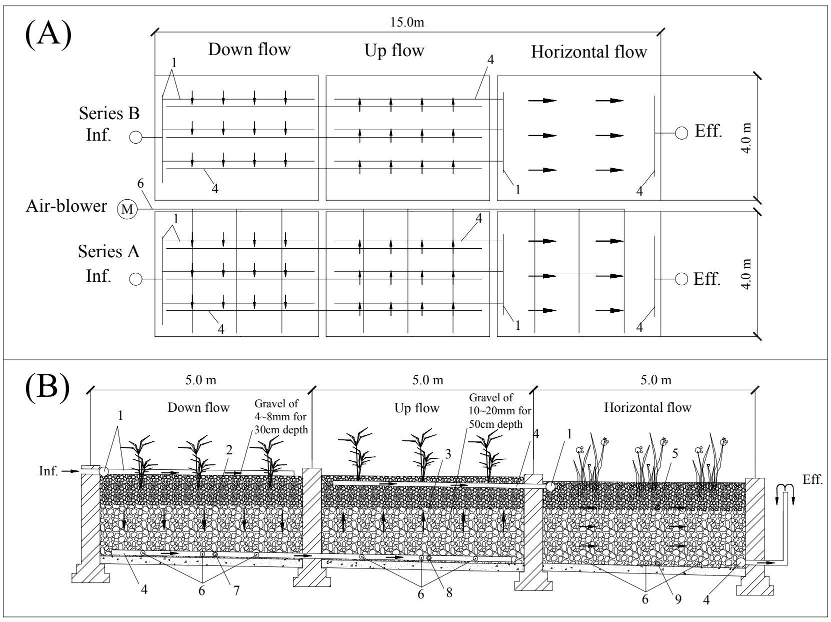

Fig. 1. (A) The plane drawing of the two three-stage hybrid wetland systems; (B) The section drawing of the hybrid system for CW 1. The denotation of numbers was listed as follows: 1 – water distribution pipe, 2 – "1-up" sampling site, 3 – "2-up" sampling site, 4 – drainage pipe, 5 – "3-up" sampling site, 6 – aeration pipe, 7 – "1-down" sampling site, 8 – "2-down" sampling site, and 9 – "3-down" sampling site. A perforated PVC pipe (diameter 25 mm) was set up in the center (the same laying direction as aeration, pipes denoted in Fig. 1A) of each chamber at a depth of 30 cm for the up sampling sites (2, 3, and 5) and 80 cm depth for the down locations (7, 8, and 9).

0.3%. Gravel of 10-20 mm diameter in size was added to a depth of 50 cm for each chamber followed by a 30-cm-thick layer of 4-8 mm diameter gravel (Fig. 1B). The porosity of the substrate was determined as 0.40.

Three species of emergent wetland plants were used in this study. *P. cordata* (*Pontederia* L.), *A. donax* (*Arundo* L.), and *I. tectorum* (*Iris* L.) were transplanted to the down, up, and horizontal flow chambers, respectively, at a density of 9 plants/m^2. In CW 1, a bottom of diffused-air system was added to the wetland bed. In detail, three PVC pipes of 75 mm diameter in size and 3.8 m in length were installed in each chamber (Fig. 1A). Four rows of holes (5 mm diameter in size and 50 mm in distance) were drilled in each of the pipes, which were connected to an air-blower (power: 1.6 kW; air output rate: 150 m^3/h; pressure difference: 28,000 Pa; brand and model: 2DG420-H36; supplied by Pengduxing Co. Ltd., Shenzhen, China) via inserted microporous tubes. The two CW systems had been in use since January 2010 (more than 2 years old) with well-established, thick plant beds before the study.

The two CW systems were induced continuously with effluent (40 m^3/h) from Nile tilapia (*Oreochromis niloticus*) farming ponds in an RAS. The fish was fed to satiation with a compound feed (30% crude protein, 3% fat, 7% fiber, 12% ash, and 0.6% phosphorus). The feed ration was maintained between 2-5% of standing biomass and adjusted slightly according to the daily feeding conditions. The effluent fed the two CW systems for the entire day, yielding a hydraulic loading rate (HLR) of 8.0 m/day. The theoretical hydraulic retention time (HRT) was 0.96 h.

Samples and Analysis

During stages 1 and 2, a physicochemical investigation was conducted on CW 1 from the sites of the inlet, "up," "down," and outlet (Fig. 1B) of each chamber, while in stage 3, sampling was conducted on both CW 1 and 2, but only at the inlet and outlet of each system. On each site, water samples were taken by means of automatic sampling devices (American Sigma 900, American Sigma Inc, Medina NY) over 24 h by an interval of 30 min. Pooled grab samples were collected into 1000 mL plastic recipients and taken to the lab for constituent analysis within 24 h. Temperature, pH, DO, electrical conductivity (EC), and redox potential (ORP) were measured *in situ* with a YSI 6600 V2 multiparametric sonde (Yellow Spring Instruments, USA). Chemical oxygen demand (COD), ammonium N (NH_4^+-N), nitrate N (NO_3^--N), nitrite N (NO_2^--N), total N (TN), and total phosphorus (TP) were analyzed following the standard procedures [16].

Removal Rate Constant

The first-order plug flow kinetic model was used to describe the pollutant removal in the two CW systems [17]:

$$k = Q(\ln C_i - \ln C_e)/(h_w A_w \varepsilon)$$

...where k is the first-order removal-rate constant (day^{-1}), Q is the flow rate of wastewater through the wetlands (m^3/day), C_i and C_e are the influent and effluent concentrations (mg/L), respectively, h_w is the wetland depth (m), A_w is the total surface area of the wetlands (m^2), and ε is the substrate porosity (averaging 0.40 for the present case).

Data Analysis

A paired t-test (inflow sampling corresponded to outflow each time) was performed to detect any significant differences in physicochemical parameters between the inflow and outflow over the three stages. Meanwhile, an analysis of covariance (outflow covaried with inflow) was used to detect any significant differences between the aeration and non-aeration experiments. These analyses were completed in the SPSS software (SPSS Inc., Chicago, IL, USA; Version 17.0) with a value of $P < 0.05$ defined as an indication of significant difference. Furthermore, the relationship between the first-order removal rate constants and the associated environment was explored using canonical correspondence analysis (CCA), which was completed in Canoco 4.5 for Windows.

Results

The mean inflow/outflow concentrations of various pollutants and their removal efficiencies are shown in Table 1. Similarly, the water constituents along the flow direction inside the three-stage wetland system are depicted in Fig. 2. By the statistical results, there was a significant decrease in temperature, pH, DO, ORP, nitrite, and TN concentrations, and a significant increase in EC and NH_4^+-N concentrations in the outflow without artificial aeration. But for the wetland with diffused-air enhancement, there was a significant decrease in COD and NH_4^+-N concentrations after filtration. Further, the aeration significantly increased the levels of DO, ORP, nitrite, and TN, while significantly decreasing the levels of EC, COD, NH_4^+-N, and TP concentrations in the outflow compared to the non-aeration treatment (Table 1).

COD Removal

In CW 1, mean mass removals of 5.6 and 24.8 g/(m^2·day), with mean percentage reductions of 4.2% and 17.0%, were achieved for the non-aerated (stage 1) and aerated (stage 2) conditions, respectively, under the high organic loading rates (132.0 and 145.6 g COD/(m^2·day) for stage 1 and 2, respectively). Similarly, in stage 3, mean mass removals of 23.2 and 15.2 g/(m^2·day), with mean percentage reductions of 25.9% and 17.0%, were obtained for CW 1 and 2, respectively (Table 1).

N Removal and Transformation

NO_2^--N

During stage 1, very little nitrite was detected in the outflow, but its amount increased dramatically in stage 2 cor-

Table 1. Characteristics of the inflow/outflow pollutant concentration, mass loading rate (g/(m^2·day)), mass removal (g/(m^2·day)), and percentage reduction (%). Significant differences on physicochemical parameters between the inflow and outflow are marked with triangles (Δ). Similarly, significant differences between the aeration and non-aeration experiments are marked with asterisks (*)[a].

Parameter	Stage 1		Stage 2		Stage 3		
	CW 1 (non-aerated)		CW 1 (aerated)		CW 1+2	CW 1 (aerated)	CW 2 (non-aerated)
	Inflow	Outflow	Inflow	Outflow	Inflow	Outflow	Outflow
Temperature (ºC)	30.9±0.5	30.7±0.5Δ	31.7±1.0	31.6±1.2	23.4±2.2	23.5±2.2	23.4±1.9
pH	7.55±0.15	7.43±0.08	7.47±0.11	7.44±0.11	7.38±0.07	7.35±0.08	7.32±0.06Δ
DO (mg/L)[b]	2.16±0.24	0.13±0.02ΔΔΔ	1.46±0.69	1.55±0.74**	4.32±2.13	1.17±1.59	0.13±0.06Δ,*
Conductivity (μS/cm)	986.8±25.1	989.6±26.5Δ	1070.0±50.8	1069.8±57.7*	693.8±22.6	688.0±20.0	690.3±19.6
ORP (mV)	71.9±93.1	-222.3±13.9ΔΔ	69.5±96.2	136.0±22.5**	110.8±9.8	122.2±8.5	-119.3±13.8ΔΔ
COD (mg/L)	16.5±1.2	15.8±1.7	18.2±1.0	15.1±0.4Δ*	11.2±3.2	8.3±2.5	9.3±1.5*
Mass loading rate (g/(m^2·day))[c]	132.0±9.6		145.6±8.0		89.6±25.6		
Mass removal[c]/ Percentage reduction[d]		5.6/4.2		24.8/17.0		23.2/25.9	15.2/17.0
NO_2^--N (mg/L)	0.15±0.02	0ΔΔΔ	0.36±0.27	0.36±0.26*	0.25±0.11	0.18±0.08	0.13±0.14Δ
Mass loading rate (g/(m^2·day))	1.20±0.16		2.88±2.16		2.00±0.88		
Mass removal/Percentage reduction		1.2/100		0/0		0.6/28.0	1.0/48.0
NO_3^--N (mg/L)	0.19±0.02	0.14±0.06	0.43±0.38	0.50±0.46	3.07±2.09	3.97±1.60	2.79±2.55
Mass loading rate (g/(m^2·day))	1.52±0.16		3.44±3.04		24.56±16.72		
Mass removal/Percentage reduction		0.4/26.3		-0.6/-16.3		-7.2/-29.3	2.2/9.1
NH_4^+-N (mg/L)	4.78±1.21	5.64±1.29ΔΔ	8.56±2.61	7.95±2.70Δ,**	2.84±1.81	1.90±1.52	2.87±1.01*
Mass loading rate (g/(m^2·day))	38.24±9.68		68.48±20.88		22.72±14.48		
Mass removal/Percentage reduction		-6.9/-18.0		4.9/7.1		7.5/33.1	-0.2/-1.1
TN (mg/L)	7.16±1.48	6.47±1.41Δ	10.45±2.47	10.37±2.06*	7.73±0.65	7.24±1.04	6.57±0.99Δ,*
Mass loading rate (g/(m^2·day))	57.28±11.84		83.60±19.76		61.84±5.20		
Mass removal/Percentage reduction		5.5/9.6		0.6/0.8		3.9/6.3	9.3/15.0
TP (mg/L)	2.74±0.15	2.78±0.12	4.44±3.73	2.94±1.24*	5.65±0.40	4.80±0.38	5.60±0.68*
Mass loading rate (g/(m^2·day))	21.92±1.20		35.52±29.84		45.20±3.20		
Mass removal/Percentage reduction		-0.3/-1.5		12.0/33.8		6.8/15.0	0.4/0.9

[a] n = 15. The data are presented as mean value ± standard deviation for both Table 1 and Fig. 2.
[b] Δ $P < 0.05$, ΔΔ $P < 0.01$, ΔΔΔ $P < 0.001$; * $P < 0.05$, ** $P < 0.01$.
[c] Mass loading rate was calculated by C_i×HLR, and mass removal by $(C_i – C_e)$×HLR with HLR in m/day.
[d] Percentage reduction was calculated by $(C_i – C_e)/C_i$×100; C_i and C_e were the inflow and outflow concentrations in g/m^3.

responding to zero or negative removals. In stage 3, mean mass removals of 0.6 and 1.0 g/(m^2·day) with mean percentage reductions of 28.0% and 48.0% were observed for CW 1 and 2, respectively. Nitrite was primarily reduced in the first two chambers after filtration (Table 1 and Fig. 2).

NO_3^--N

During the whole experiment, outflow from the aerated condition presented a higher level of nitrate than the inflow. However, for the non-aerated condition, mean mass removals of 0.4 and 2.2 g/(m²·day) with mean percentage reductions of 26.3% and 9.1% were achieved for stage 1 and 3, respectively. Nitrate reduction mainly occurred in the first two chambers under the aerated condition (Table 1 and Fig. 2).

NH_4^+-N

In contrast to nitrate, ammonium N concentrations of outflow were higher than those of inflow under the non-aerated condition. For the aerated condition, mean mass removals of 4.9 and 7.5 g/(m²·day), with mean percentage reductions of 7.1% and 33.1%, were achieved for stage 2 and 3, respectively (Table 1).

TN

In CW 1, the mean mass removals of 5.5 and 0.6 g/(m²·day) with mean percentage reductions of 9.6% and 0.8% were achieved for stages 1 and 2, respectively. Similarly, in stage 3 the mean mass removals of 3.9 and 9.3 g/(m²·day) with mean percentage reductions of 6.3% and 15.0% were obtained for CW 1 and 2, respectively (Table 1).

TP Removal

During stage 1, TP concentrations in the outflow were higher than those in the inflow. However, in stage 2 the mean mass removal and percentage reduction were 12.0 g/(m²·day) and 33.8%, respectively. In stage 3, mean mass removals of 6.8 and 0.4 g/(m²·day) with mean percentage reductions of 15.0% and 0.9% were achieved for CW 1 and 2, respectively. TP was sequestered primarily in the first chamber under the aerated condition (Table 1 and Fig. 2).

Discussion

Evaluation of Treatment Performance on COD

In a RAS, COD is an important competitor for DO against a rearing object. Reduction of COD can not only alleviate the ambient pollution load but also help to meet the requirement of fish in the water. In the current study, the three-stage hybrid wetland systems were effective in the removal of COD, despite the high organic loading rate (89.6-145.6 g/(m²·day)). The mean COD mass removal (5.6-24.8 g/(m²·day)) and percentage reduction (4.2-25.9%) by the two wetland systems were lower than the values reported by Schulz et al. [18] and Sindilariu et al. [19, 20], but were higher than those given by Zachritz et al. [9] and Konnerup et al. [4] under similar operating conditions (Tables 1-3). As we know, a major part of COD in fish farm effluent is particle bound, while subsurface-flow CWs usually perform well as water treatment filters since sedimentation and filtration are the primary approaches for particle removal. In the present study, filtration was still an important pathway for COD removal.

The removal rate constant of COD (k_{COD}) varied greatly from 1.08 to 7.49 day^{-1} over the entire investigation but still located in the broad range as documented in the literature (Table 3). It is generally regarded that organic matter is primarily removed by biological aerobic degradation in the filter bed of a CW, which is highly dependent on DO concentration [21, 22], hydraulic and pollutant loading rate [23], and seasonal variations [24]. In the present study, the COD concentration of outflow under the aerated condition was significantly lower than that of the non-aerated state,

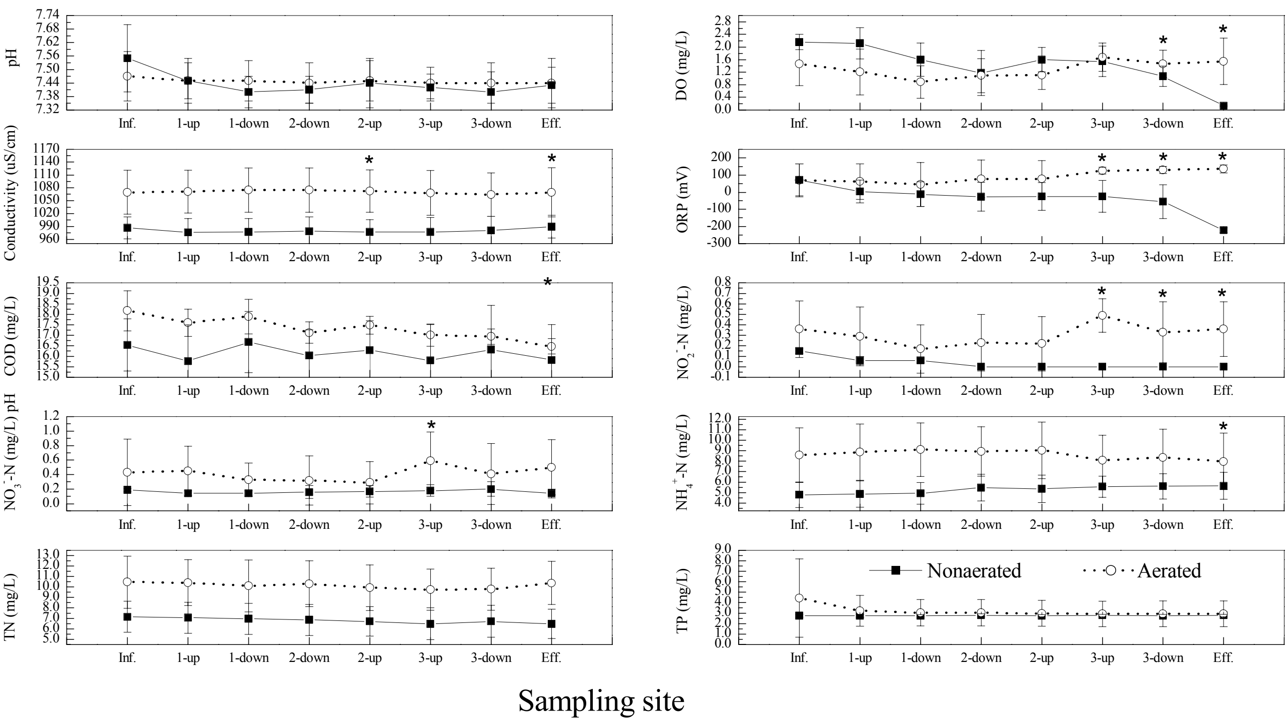

Fig. 2. Constituents of the water along the flow direction inside the three-stage wetland system. Significant differences on physicochemical parameters between the aeration and non-aeration experiments are marked with asterisks for each sampling site.

Table 2. Removal efficiencies of various subsurface-flow CWs in treated aquaculture wastewaters under similar operating conditions [a)].

Location	System characteristics	HLR (m/day)	Percentage reduction (%)			Mass removal (g/(m²·day))			Reference
			COD	TN	TP	COD	TN	TP	
Germany	HF planted with P*hragmites× australis* [b)]	5.14	70.1	23.5	57.7	127.1	2.65	1.07	Schulz et al. [18][c)]
Germany	HF dominated by *Phragmites× communis* and *Phalaris× arundinacea*	12.1	38.3	1.6	46.4	51.5	1.51	0.58	Sindilariu et al. [19]
Germany	HF dominated by *Phragmites× communis* and *Phalaris× arundinacea*	8.0	61.5	7.5	42.7	66.3	3.23	0.87	Sindilariu et al. [20]
Vietnam	HF planted with *Canna× generalis*	3.0	-0.8	-2.4	-8.7	-3.0	-0.57	-0.60	Konnerup et al. [4]

[a)] Calculated according to the values or extracted from the graphs in the reference by the software of GetData.Graph.Digitizer.v.2.24.
[b)] HF: horizontal subsurface-flow CW.
[c)] The maximum value.

Table 3. A summary of area-based removal rate constants (day^{-1}) from various subsurface-flow CW studies for treated aquaculture wastewaters under broad HLRs (from 0.0375 to 14.5 m/day)[a)].

Literature source	CW type [b)]	HLR	*k* for COD	*k* for NO_3^--N	*k* for NO_2^--N	*k* for NH_4^+-N	*k* for TN	*k* for TP
This study (Stage 1 CW 1)	VF+HF	8.0	1.08	7.63		-4.14	2.53	-0.36
This study (Stage 2 CW 1)	VF+HF	8.0	4.67	-3.77		1.85	0.19	10.31
This study (Stage 3 CW 1)	VF+HF	8.0	7.49	-6.43	8.21	10.05	1.64	4.08
This study (Stage 3 CW 2)	VF+HF	8.0	4.65	2.39	16.35	-0.26	4.06	0.22
Schulz et al. [18]	HF	1.03	3.80	-1.08		5.52	1.73	3.76
Schulz et al. [18]	HF	3.09	11.72	-5.93		17.02	3.08	8.06
Schulz et al. [18]	HF	5.14	19.71	-10.70		29.10	4.37	14.04
Lin et al. [27]	HF	1.54		-0.34	7.99	3.70		
Lin et al. [27]	HF	1.95		-0.21	8.90	4.19		
Sindilariu et al. [19]	HF	10.6	8.19	-0.53	12.93	59.76	-0.57	14.14
Sindilariu et al. [19]	HF	13.6	27.90	1.72	20.30	25.48	1.96	29.89
Zachritz et al. [9]	HF	3.03	-0.54	6.79	14.20	0.30		-0.26
Sindilariu et al. [10]	HF	3.3		-0.79	-1.40	17.94	1.16	8.10
Sindilariu et al. [10]	HF	6.9		-1.91	-4.71	36.26	1.66	16.52
Sindilariu et al. [10]	HF	14.5		-1.97	-19.40	22.31	2.43	31.50
Konnerup et al. [4]	HF	0.75	3.20	-8.52	-1.87	-6.39	1.94	2.35
Konnerup et al. [4]	HF	1.5	2.85	-6.93	-12.99	-2.53	1.46	0.76
Konnerup et al. [4]	HF	3.0	-0.16	8.11	11.19	-13.27	-0.47	-1.67
Konnerup et al. [4]	VF	0.75	0.86	-3.65	-1.39	-0.26	0.07	0.59
Konnerup et al. [4]	VF	1.5	1.52	-12.27	-8.13	1.49	1.56	-0.08
Konnerup et al. [4]	VF	3.0	3.16	-26.94	-1.39	3.92	1.61	0.83
Shi et al. [12]	VF+HF	0.862	0.89	2.48	5.82	3.43	3.09	0.81
Idris et al. [13]	HF	0.0375				1.43	0.91	0.81

[a)] Calculated according to the values or extracted from the graphs in the references by the software of GetData.Graph.Digitizer.v.2.24.
[b)] HF: horizontal flow CW; VF: vertical flow CW.

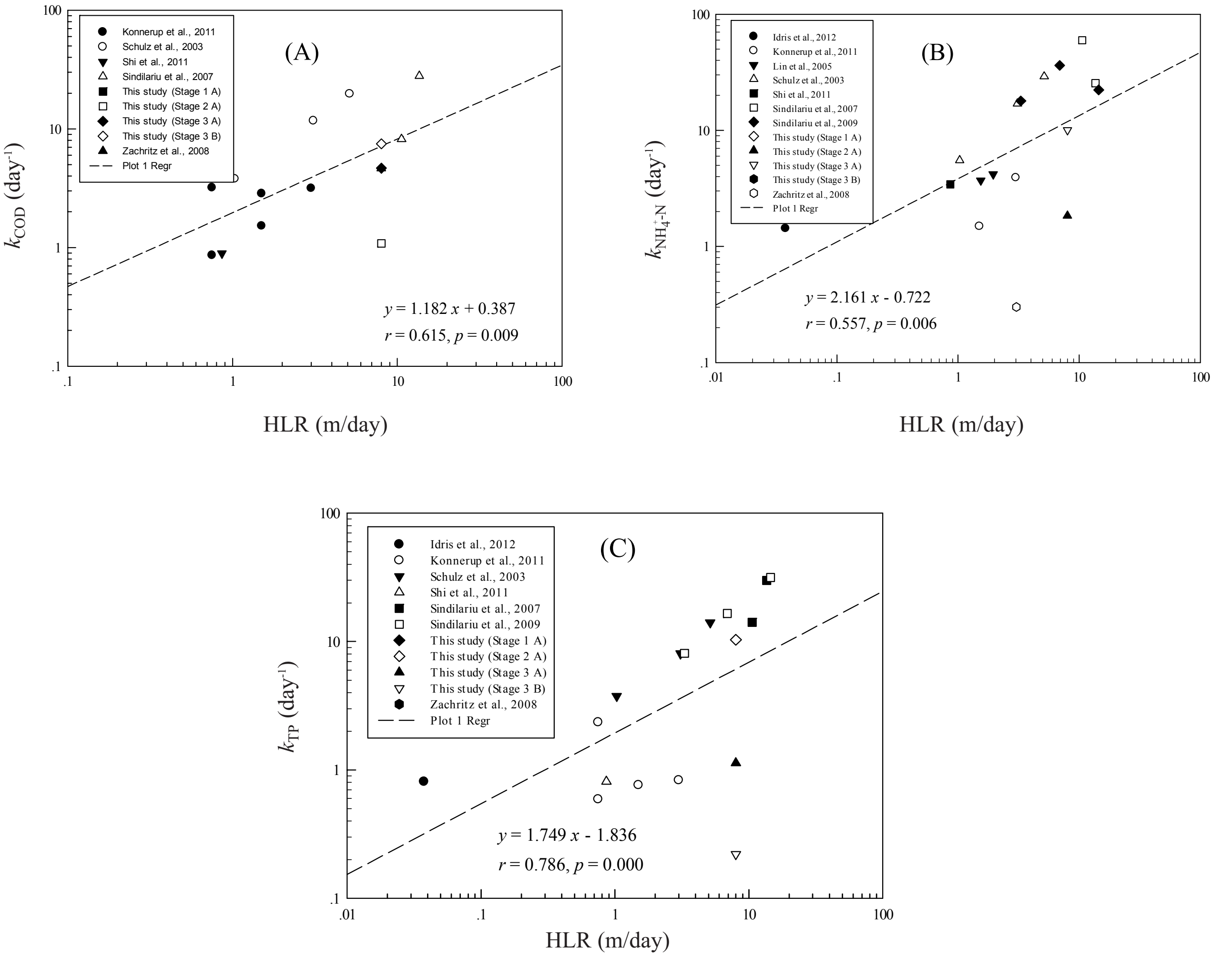

Fig. 3. The linear relationships between hydraulic loading rate (HLR) and the first-order removal rate constants of COD, NH_4^+-N, and TP listed in Table 3.

indicating that COD removal in the wetland system was highly dependent on DO, which had been well illustrated by the results of Tao et al. [22] and Chang et al. [25].

Linear regression analysis was performed using the data listed in Table 3. As a result, significantly positive correlations were detected between k_{COD}, k_{TAN}, k_{TP}, and HLR (Fig. 3), implying that HLR was a key limiting factor for COD, ammonium N, and TP removal in such subsurface-flow CW systems. Furthermore, CCA analysis coupled with linear regression revealed that k_{COD} was negatively correlated with COD concentration and temperature in the inflow, but not closely associated with DO (Fig. 4). Previous surveys have proven that higher COD mass load beyond the removal capability of CW is constrained by the available oxygen in the filter bed [26]. The extremely low level of DO in the outflow under the non-aerated conditions (Table 1 and Fig. 2) implied that the wetland bed was in serious lack of DO. The outflow organic matter could be further reduced if the wetland systems were fed with low organic matter loads [23]. Additionally, higher temperatures promoted the decomposition of trapped organic solids, which also led to a lower percentage reduction.

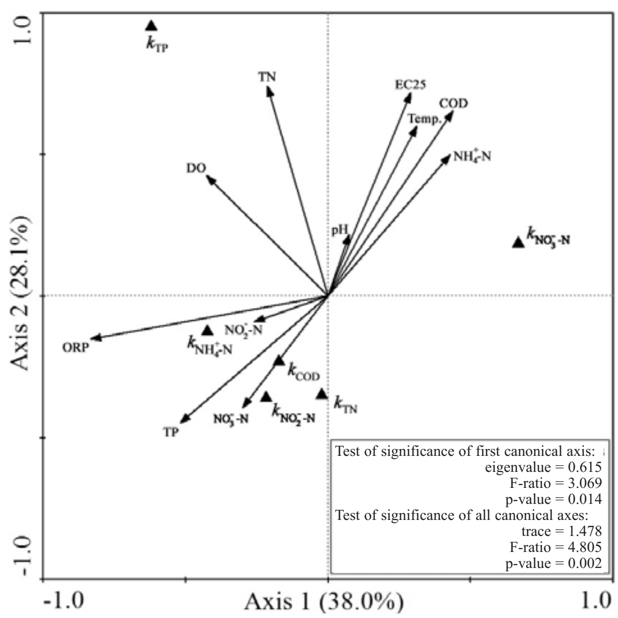

Fig. 4. CCA ordination plot based upon the first-order removal rate constants and the measured environment of the inflow.

Evaluation of Treatment Performance on N and P

With regard to the removal of TN, its percentage reduction under non-aerated conditions (9.6-15.0%) was higher than those reported by Sindilariu et al. [19-20] and Konnerup et al. [4], and was lower than the values given by Schulz et al. [18]. Nevertheless, the CW systems without diffused-air enhancement were superior in TN mass removal (mean values ranged from 5.5 to 9.3 g/(m^2·day)) compared to the above cases when operated at similar HLRs or pollutant loading rates (Tables 1 and 2). Using accumulative analysis, sedimentation of organic N was determined to be the main pathway for TN removal under non-aerated conditions, while ammonium N removal contributed to a dominant fraction in TN removal under the aerated state (Fig. 5). It is easily comprehensible that sedimentation of organic N or particles in aquaculture effluent usually correspond to considerable reductions of TN, TP, and COD for a subsurface flow CW [9, 27].

It is generally accepted that microbial processes are the dominant mechanisms for TN removal in a CW. Additionally, NH_3 volatilization and the adsorption of granular N and NH_4^+-N in a bed medium, as well as in plant uptake, can contribute to TN removal [8]. In the current case, TN removal through NH_3 volatilization might be negligible due to the low pH of the wetland bed (≤7.6), (Table 1 and Fig. 2) [28]. Granular N and NH_4^+-N adsorption of the substrate could also be neglected, as the systems had been operated for more than two years, which might have led to saturated adsorption sites and the reversible nature of the adsorption processes [8]. It is reported that plant uptake only constitutes 2-10% of TN removal, even at low loading rates [29, 30]. As a result, it was less important quantitatively compared with other removal mechanisms, especially at high loading rates [31]. Thus, it could be concluded that the microbial process was an important pathway (inferior to sedimentation) for TN removal in the current case.

The wetland systems did not perform well in ammonium N removal at the high loading rates. Under the non-aerated condition, NH_4^+-N concentrations in the outflow were generally higher than those in the inflow, while an opposite trend was observed for the aerated state (Table 1 and Fig. 2). These implied that oxygen-dependent nitrification was the limiting step for ammonium N removal. It could be further demonstrated by the decreased percent reductions of nitrate after the aeration enhancement (more ammonium nitrified into nitrate, Table 1). By CCA and linear regression, a significantly negative correlation was detected between k_{TAN} and NH_4^+-N concentrations in the inflow (Fig. 4, r = -0.824, P = 0.012). Meanwhile, a significantly positive correlation was found between temperature and NH_4^+-N concentrations in the effluent (r = 0.774, P = 0.000), indicating that the temperature and pollutant load were the two main dependent factors for ammonium N removal in the oxygen-deficiency wetland bed.

During stage 1, notably little nitrite was detected in the outflow (Table 1), which suggested that the intermediate product of nitrification and denitrification was not accumulated in the system. While in stages 2 and 3, low nitrite concentrations were detected in the outflow, demonstrating that the denitrification process was impeded to some extent. In traditional N treatment, nitrification is performed in two sequential oxidative stages:

1) Ammonia to nitrite (ammonia oxidation)
2) Nitrite to nitrate (nitrite oxidation)

During stage 2, a significantly positive correlation was detected between DO and nitrite (Fig. 2, r = 0.962, P = 0.000), as well as between DO and nitrate (Fig. 2, r = 0.861, P = 0.006). Additionally, a significantly positive correlation was detected between nitrite and nitrate (Fig. 2, r = 0.927, P = 0.001). These correlations likely implied that under the aerated condition, more ammonium N could be nitrified to nitrite and then to nitrate, which led to decreased ammonium N concentrations or increased nitrate amount. Furthermore, the higher nitrate concentrations in the outflow or lower percentage reductions in stage 3 compared to stage 1 and 2 suggested that the denitrification process was also restrained with the short HRT (0.96 h), even though the carbon source was enough for denitrification [32]. That was

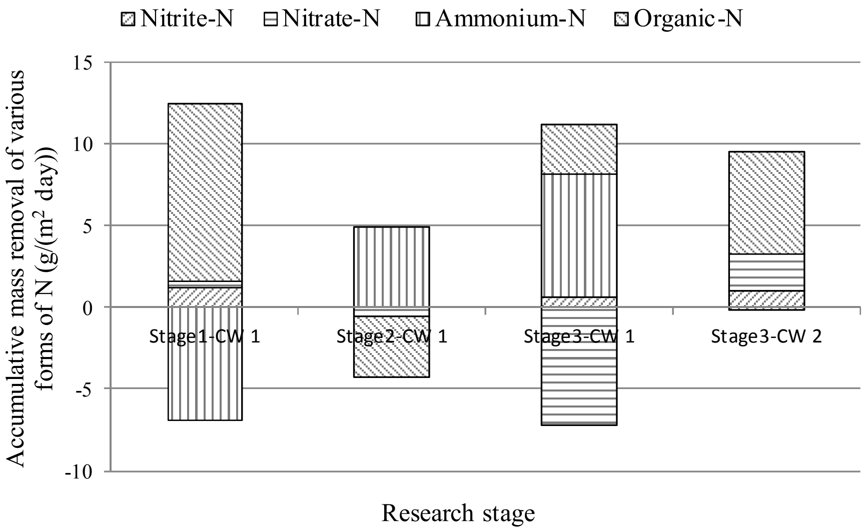

Fig. 5. The accumulative mass removal of various forms of N over the three stages.

because organic N made a major constituent in the aquaculture effluent and the high organic loading rate promised a sufficient carbon source. As we know, higher HRT implies lower loading and more contact time, which results in higher efficiency [27, 33]. The short HRT was hence becoming an important limiting factor on denitrification in stage 3.

The removal mechanisms of P from wastewater in a CW are mainly through substrate adsorption, iron exchange, and plant uptake [28]. In the current study, the mean percentage reductions of TP (-1.5-33.8%) were lower than those reported by Schulz et al. [18] and Sindilariu et al. [19, 20], but were higher than those given by Konnerup et al. [4]. The mass removals of TP under aerated conditions were obviously higher than those of the above case studies (Tables 1 and 2). The higher percentage reductions or mass removals under the aerated condition compared to the non-aerated state indicated that the diffused-air enhancement improved the treatment performance on P. This could possibly be due to increased sedimentation. That is because particulate P, which is the highest P fraction in aquaculture effluent, will not dissolve as long as enough oxygen is available. In anaerobic conditions, the P that is bound with iron (Fe) compounds due to the reduction of Fe^{3+} to Fe^{2+} may release as phosphates (PO_4^{3+}) and Fe^{2+}, leading to the no-treatment effect [34].

Conclusions

In the present study, two identical three-stage subsurface-flow CW systems (a combination of down, up, and horizontal flow) were constructed to treat tilapia effluent under a high loading rate (8.0 m/day) with a short retention time (0.96 h). Based on the investigation, the mass removals of COD and N were considerably higher: 5.6-24.8 g/(m^2·day) for COD, 0.6-9.3 g/(m^2·day) for TN. Artificial aeration enhanced the treatment performance on COD, NH_4^+-N, and P but not on TN. The higher NH_4^+-N concentrations in the final effluent under the non-aerated conditions indicated that DO required for nitrification in the natural bed was more insufficient at the high loading rates. Accompanied by the low level of DO, pollutant loading rates became the first dependent factor on removal rates for such a rapid filtration system.

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