

SHI-YANG ZHANG^{1,2}, GU LI¹, XIAOLI LI¹, LING TAO¹

FOUR STAGE HYBRID CONSTRUCTED WETLANDS TREATING LOW-STRENGTH AQUACULTURE WASTEWATER WITH AND WITHOUT ARTIFICIAL AERATION

Driven by the booming demands for healthy food, aquaculture industry has to deal with the problem of water pollution appropriately so as to achieve sustainable development. In this study, a combination of four stage CWs (three horizontal subsurface flows followed by one free water surface flow) was configured to treat low-strength aquaculture wastewater. For performance assessment, the wetlands were monitored over three years, during which artificial aeration was added to them. By the results, the organic matters and nutrients were mainly sequestered in the anterior subsurface flows, while the surface flow mainly contributed to DO improvement. These results probably implied no necessity of excessive subsurface flows connected in a staged manner. In addition, the artificial aeration improved the treatment performance on ammonium-N, TN and TP in the first-stage CW.

1. INTRODUCTION

As the world's largest consumer of fish and seafood, and the largest producer of aquaculture products, China contributed to the fraction of 67% of the global production in terms of quantity and 49% of the global value in 2006 [1]. Nevertheless, with the fast expansion of the aquaculture industry, China has also been facing many environmental issues. One of the typical problems is the serious water pollution triggered by the intensive agriculture or industrialization. Therefore, exploring appropriate solution to the problem is imperative. Constructed wetlands (CWs) are engineered systems that are designed to use natural processes among wetland vegetation, soils, and associated microbial communities for wastewater treatment [2]. CWs become increasingly

¹Key Laboratory of Freshwater Biodiversity Conservation, Ministry of Agriculture of China, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, PR China, corresponding author S. Zhang, e-mail: zhangshiyang7@126.com

²Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences, Wuxi 214081, PR China.

popular worldwide for removing organic matter, nutrients, pathogens, or other pollutants from various wastewaters. In the past, CWs were most designed for municipal wastewater treatment, but recently, an increasing number of CWs are employed for aquaculture wastewater treatment [3–5].

Historically, operational results indicate that the removal of organic substances is satisfactory [6]. However, the removal of inorganic nitrogen and phosphorus is frequently problematic [7]. In fact, various types of CW may be combined to achieve high purifying performance, for example, using the nitrification potential of vertical flow and the denitrification potential of horizontal flow. For aquaculture wastewater, horizontal plus surface flow may be the ideal combination due to their high volume and low-strength [8]. That is because horizontal flow can provide high removal of organics and suspended solids and a good condition for denitrification, while surface flow can be used for reoxygenation since dissolved oxygen (DO) in effluent from subsurface flow is too low to satisfy the requirement for aquaculture use.

Horizontal flow CWs are known to achieve only limited total nitrogen (TN) removal due to the lack of DO for the nitrification step [9]. In the past, a variety of approaches to physically promoting oxygen availability were proposed and applied in subsurface flow CWs [6, 10, 11]. As an alternative solution, artificial aeration in wetland matrix could achieve the alternation of aerobic and anaerobic environment, and create conditions for improved nutrient removal [12]. Nevertheless, for multistage or hybrid CW systems, DO is usually consumed fast by the anterior compartment of subsurface flow, and subsequently leads to a serious lack of DO for the following treatment. For most previous surveys, artificial aeration was usually connected to wetland matrix. Studies on the supplement of artificial aeration to the influent of multistage horizontal flows are rare.

In the work presented here, we utilized a narrow vacant field nearby intensive fish farming ponds to construct a hybrid of four stage CWs, which were then integrated into an outdoor recirculating aquaculture system to regulate the quality of rearing water. The primary objectives were the following: (1) to investigate the regulation effect of each CW on water quality and their differences among the four stage treatments, (2) to explore the impact of artificial aeration on treatment performance and their potential mechanisms.

2. MATERIALS AND METHODS

Construction of the system. The study site was located in Jingzhou City (30°16' N, 112°18' E), Hubei Province (part of central China with a typical subtropical climate). The hybrid CW systems comprised four wetland units (CW_1 to CW_4), i.e. three horizontal subsurface flows followed by one free water surface flow (Fig. 1). The design parameters were as follows: 30 m long, 3.1 m wide, and 1 m deep for CW_1,

CW_2, and 30 m long, 2.4 m wide and 1 m deep for CW_3, CW_4. Wastewater from nearby fish farming ponds was first induced into a storage tank (1 in Fig. 1a), then flowed automatically through the four CWs in turn by head drop, and finally flowed back into the fish ponds.

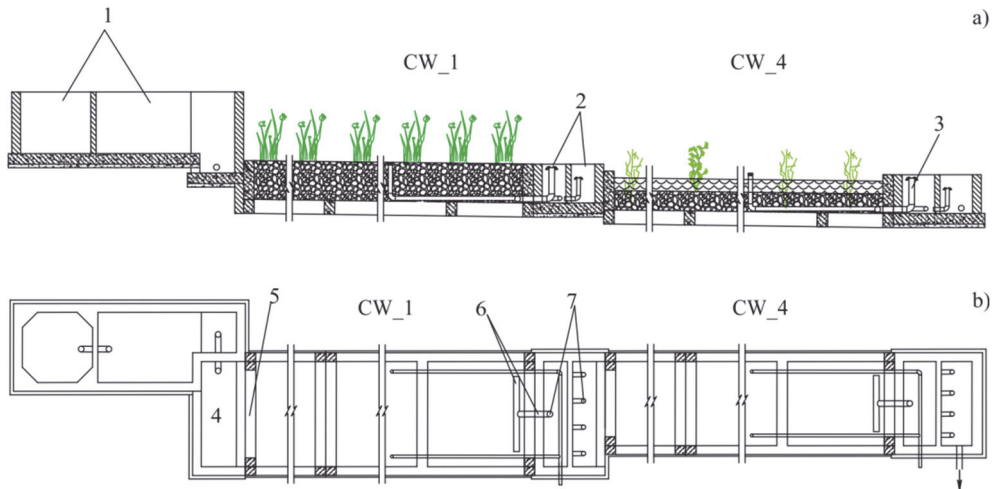


Fig. 1. Storage tank, the inlet, CW_1, CW_4 and their outlets:
a) section drawing, b) plane drawing CW_2 and CW_3, possessing identical configuration, have been omitted in the picture (for details, see the text)

In the bottom of each wetland near the outlet, a system of perforated $\text{Ø}200$ mm PVC pipes (6 in Fig. 1b) was fixed to secure efficient drainage. The frame of all the CWs was built using bricks and mortar, while the bottom got geotextile as an impermeable liner fixed to a slope of 0.33% for CW_1 and 0.17% for CW_2, CW_4. The first three units (CW_1, CW_3) were filled with ceramisite (nominal diameter: 80–150 mm) to the depth of 80 cm. In CW_4, an $\text{Ø}160$ mm PVC pipe (3, connected to the drainage pipe) was erected at the outlet with the tail end 40 cm higher than the surface level of the media to store water. After impounding, *Myriophyllum spicatum* L. and a minor quantity of *Nymphaea alba* L. were planted in April 2010 with a plant distance of 80–100 cm for *M. spicatum* and 5–7 m for *N. alba*.

At the inlet of CW_1, a storing cistern (4) was constructed with a serrated overflow weir (5) on one side, which facilitated horizontal water flow. At the end of each wetland, a set of two-stage storing cisterns (2) was designed for passive aeration. In detail, a PVC pipe (7, $\text{Ø}160$ mm, connected to the drainage pipe) was erected at the outlet with the tail end slightly lower than the surface level of the media. Then, water upwelled from the standing pipe. For the first three CWs, *Thalia dealbata*, *Arundo donax f. versicolor* and *Phragmites australis* were planted in order with a plant distance of 30–40 cm for each. These aquatic plants, as well as those in CW_4, propagated

rapidly and soon covered the entire surface of each wetland within a growing season. After construction and planting, the wetlands were allowed to acclimatize for 2 months in order to let the plants and microorganisms develop. During this period, the CWs were loaded with water from the culture pond at loading rates varying in the range of 0.4–0.6 m \cdot day $^{-1}$ on average.

Operation and management of the system. The wetland systems were loaded with effluent (24 m 3 \cdot h $^{-1}$) from fish farming ponds during 8:00 a.m.–6:00 p.m., resulting in a hydraulic loading rate (HLR) of 2.58 m \cdot day $^{-1}$ for the first two and 3.33 m \cdot day $^{-1}$ for the third one, corresponding to a hydraulic retention time (HRT) of 3.0 h for the first two and 2.3 h for the third one. In 2011, the fish farming ponds were mainly stocked with juvenile crucian carp (*Carassius auratus gibelio*) or grass carp (*Ctenopharyngodon idella*), at the stocking density of 2.42 \times 10 4 ind. \cdot ha $^{-1}$; while in 2011 and 2012, the main culture species was changed to juvenile yellow catfish (*Pelteobagrus fulvidraco*), at the stocking density of 1.82 \times 10 5 ind. \cdot ha $^{-1}$. The fish were fed to satiation twice daily with a commercial diet supplied by the Shashi Tongwei Feedstuff Co., Ltd., Jingzhou, Hubei Province, China. The feed ratio was maintained between 2% and 5% of standing biomass and adjusted slightly according to the daily feeding conditions.

To explore the impact of artificial aeration on treatment performance, diffused air system was added to the inlet of each wetland in 2012. The aeration was provided from an air-blower (power 1.6 kW, air output rate 150 m 3 \cdot h $^{-1}$, pressure difference 28 kPa, brand and model 2DG420-H36 supplied by Pengduxing Co., Ltd., Shenzhen, China through 2 diffuser discs placed in the bottom of each cistern. The diffuser discs, with one brick as a sinker, were configured by twisting a microporous tube (the total length of 5 m, inner diameter 8 mm, outside diameter 16 mm) around a cross-shaped frame made of bamboo. After the installation, the diffused-air system and the water pump operated simultaneously for the aeration experiment.

Methods for sampling and analysis. Sampling was conducted fortnightly from June to October each year (2010–2012) that usually corresponded to the main rearing period. Given the extreme daytime temperatures in the region, water samples were obtained by mixing an equal volume of water that was sampled three times every ten minutes between 10:00 a.m. and 11:00 a.m. Samples were saved in 2.5 dm 3 polyethylene bottle and taken to lab for analysis within 24 h. Temperature, electrical conductivity (EC), total dissolved solids (TDS), redox potential (ORP), pH and DO were measured in situ with a YSI 6600 V2 multiparametric sonde (Yellow Spring Instruments, USA). Chemical oxygen demand (COD), total ammonium nitrogen NH $_4^+$ -N, NO $_3^-$ -N, NO $_2^-$ -N, TN and total phosphorus (TP) were analyzed following the standard procedures [13].

Removal rate constant. The pollutant removal in CW can be described by the first order plug flow kinetic model. The removal rate constant used for system design was calculated by rearranging the first order kinetic equation [3]:

$$K = \frac{Q(\ln C_i - \ln C_e)}{h_w A_w \varepsilon} \quad (1)$$

where K is the first order removal rate constant (day^{-1}), Q is the flow rate of wastewater through the wetlands ($240 \text{ m}^3 \cdot \text{day}^{-1}$), C_i is the inflow pollutant concentration ($\text{mg} \cdot \text{dm}^{-3}$), C_e is the outflow pollutant concentration ($\text{mg} \cdot \text{dm}^{-3}$), h_w is the average water depth of the wetlands (m), A_w is the total surface area of the wetlands (m^2), and ε is the porosity of the wetlands (averaging 0.40).

Data analysis. The data were expressed as mean \pm standard deviation. After checking for a normal distribution (the Shapiro–Wilks test), differences among the four stage treatments were tested by one-way analysis of variance (ANOVA), followed by multiple comparison with least squared difference (LSD) for equal variance and Games–Howell for unequal variance. When ANOVA could not be applied, the data were transformed by ranking cases first, and then tested by ANOVA [14]. The differences between the aeration and non-aeration treatments were detected by analysis of covariance (outflow covaried with inflow). These analyses were completed in the SPSS software (SPSS Inc., Chicago, IL, USA; Version 17.0). Furthermore, the relationship between the first order removal rate constants and the measured environment was explored using redundancy analysis (RDA), which was completed in Canoco 4.5 for Windows.

3. RESULTS AND DISCUSSION

3.1. COMPARISON OF THE INFLUENT AND EFFLUENTS

For subsurface flow CWs, the DO of effluent is considerably low due to their huge consumption for organic decomposition and nitrification [3]. In the present study, the DO of the four CW effluents without artificial aeration were all significantly lower than the influent, not exceeding $1 \text{ mg} \cdot \text{dm}^{-3}$ for the first three effluents but soon amounted to a relatively higher level ($3.15 \text{ mg} \cdot \text{dm}^{-3}$) for the final one. The fast increase for the final effluent was attributable to the physical re-aeration and photosynthetic oxygenation by submerged plants and algae (mainly comprised of *Spirogyra*) in CW_4. But with the treatment of artificial aeration, the DO levels of the four effluents were all elevated to a much higher level (5.63 – $9.68 \text{ mg} \cdot \text{dm}^{-3}$, Fig. 2).

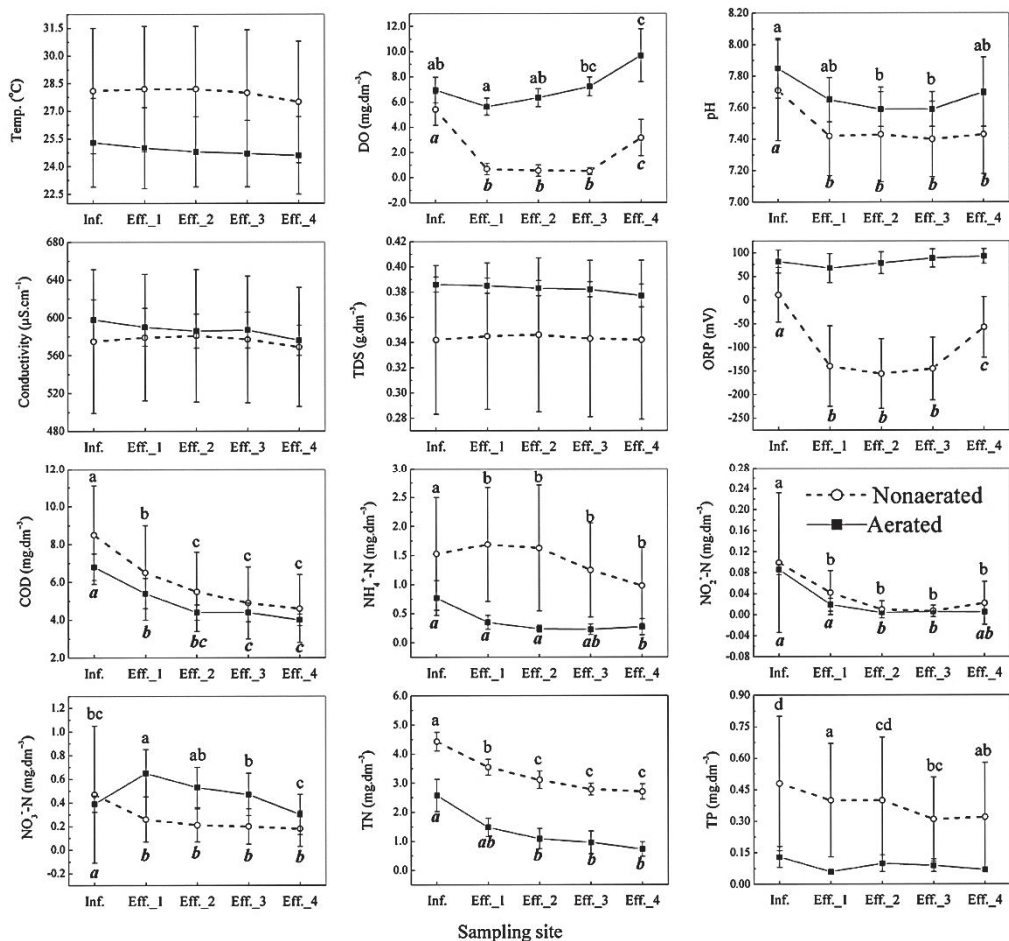


Fig. 2. Mean composition of the influent and effluents with ($n = 10$) and without ($n = 20$) artificial aeration for each wetland unit. Error bars indicate one standard deviation. Different letters between columns indicate significant differences; the non-aerated comparison was marked with bold italics below, and the aerated comparison was marked with letters above

For the conditions without artificial aeration, there were significant differences in ORP between the influent and effluents. But for the aerated conditions, there was no significant difference among them, and the values of ORP were all increased to a much higher level (67.4–92.6 mV). This could be associated with the concentration of DO (Pearson correlation between DO and ORP = 0.963, $P = 0.000$), which was increased distinctively after the artificial aeration.

Generally, nitrification produces hydrogen ions that can decrease alkalinity, while uptake of dissolved CO_2 by photosynthesis of wetland macrophytes and algae increases pH [2]. In the present study, significant differences in the values of pH between the

influent and effluents were observed regardless of artificial aeration. The basic trend was that the pH of the influent was obviously higher than that of the effluents. The obvious decrease of pH after the first treatment (Fig. 2) indicated that the role of nitrification in accounting for pH decrease went beyond the photosynthesis in the wetland system.

For organic matters, a decreasing level of concentration among the four stage effluents was observed. Regardless of artificial aeration, the four stage CWs generally reduced these substances to a significantly lower level compared to the influent. Similarly, the levels of NO_2^- -N, TN and TP also showed a decreasing trend among the four stage effluents and were significantly lower compared to the influent. For the index of NH_4^+ -N without artificial aeration, it generally increased after the first two treatments and then decreased, while for the aerated conditions, it decreased gradually along the four stage treatments; the variation of NO_3^- -N showed the opposite trend against NH_4^+ -N (Fig. 2).

3.2. AERATION IMPACT ON POLLUTANT REMOVAL

By the mean value of percent reduction or mass removal, the COD was mainly reduced by CW_1–CW_2. The ammonium-N without artificial aeration was mainly reduced by CW_3–CW_4; while for the aerated conditions, the ammonium-N was mainly reduced by CW_1 (Figs. 3 and 4). This was due to that the removal of ammonium-N without the artificial aeration might mainly comprise plant uptake, ammonia volatilization, partial nitrification and anaerobic ammonium oxidation [15, 16]. While for the aerated conditions, the ammonium-N was mainly reduced by nitrification due to the more sufficient supply of DO.

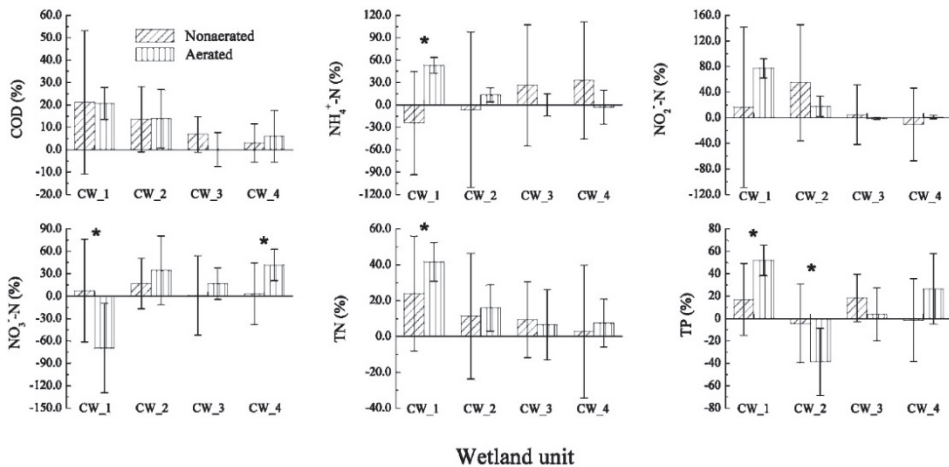


Fig. 3. Mean percent reduction of main pollutants for each wetland unit. Error bars indicate one standard deviation ($n = 10$ for aerated, and $n = 20$ for non-aerated). Significant differences between the aeration and non-aeration treatments were marked with asterisks above the column

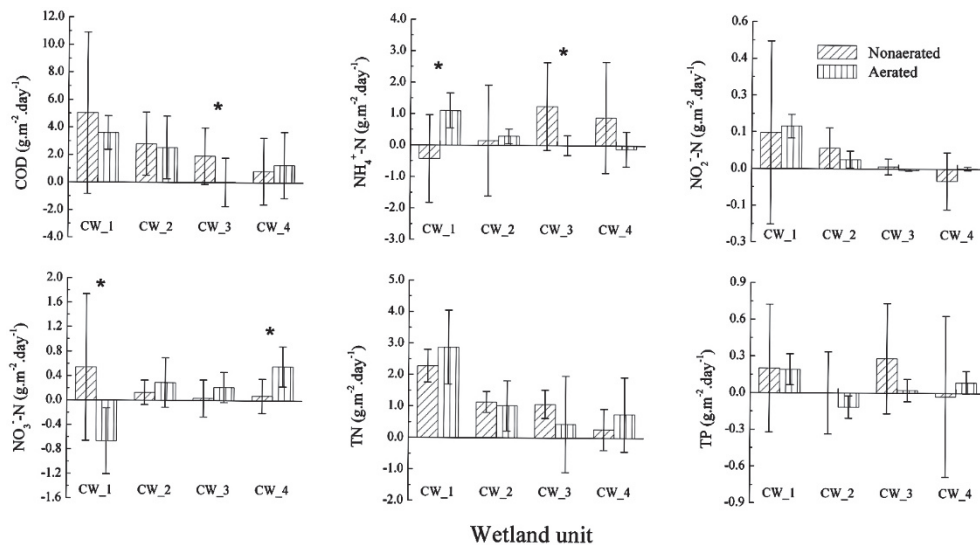


Fig. 4. Mean areal removal rate of main pollutants for each wetland unit. Error bars indicate one standard deviation ($n = 10$ for aerated, and $n = 20$ for non-aerated). Significant differences between the aeration and non-aeration treatments were marked with asterisks above the column

The nitrites were mainly reduced by CW_1–CW_2 regardless of artificial aeration. Similarly, the nitrates were mainly reduced by CW_1 –CW_2 under the non-aerated conditions. Under the aerated conditions, the nitrates were mainly removed by CW_4 (Figs. 3 and 4). This was probably due to that, under the aerated conditions, more ammonium-N had been nitrified into nitrate leading to a minus reduction.

The percent reduction or mass removal of TN showed a decreasing trend among the four stage treatments and mainly occurred in CW_1 (Figs. 3 and 4). This was due to that the TN in the current water or wastewater was mainly composed of organic particles, while subsurface flow CW usually performed well as water treatment filters since sedimentation and filtration were the primary approaches for TSS removal within them [17, 18]. In addition, the significantly higher percent reduction of TN in the aerated conditions compared to the non-aerated state indicated that the artificial aeration improved the treatment performance on TN.

For the present case, the TP under the non-aerated conditions was mainly reduced by CW_1 and CW_3. While for the aerated conditions, the TP was mainly reduced by CW_1 and CW_4. A minus removal of TP was observed in CW_2 that had been significantly enhanced by the artificial aeration (Figs. 3 and 4). The significantly higher percent reduction of TP in the aerated conditions compared to the non-aerated state indicated that the artificial aeration also improved the treatment performance on TP. This was probably due to that phosphorus was mainly absorbed or precipitated in the filter media [19]. However, oxidation conditions could influence phosphorus removal.

In anaerobic conditions, the phosphorus bound with iron (Fe) compounds due to the reduction of Fe^{3+} to Fe^{2+} might release as phosphates (PO_4^{3-}) and Fe^{2+} leading to a minus removal [20].

3.3. RELATIONSHIPS BETWEEN REMOVAL RATE AND ENVIRONMENT

The average values of the first order removal rate constant (K) for the main pollutants monitored over the three years are listed in Table 1.

Table 1

Mean first-order removal rate constant (K , day^{-1}) for various parameters in each CW (mean \pm SD; $n = 30$) and the results of one-way ANOVA statistics (P -value)

Parameter	CW 1	CW 2	CW 3	CW 4	P -value
COD_{Mn}	2.46 \pm 3.31	1.39 \pm 1.16	0.90 \pm 1.19	1.71 \pm 3.57	0.125 ^{NS}
$\text{NH}_4^+\text{-N}$	0.94 \pm 4.37	1.33 \pm 2.74	1.95 \pm 4.07	3.68 \pm 10.42	0.327 ^{NS}
$\text{NO}_2^-\text{-N}$	9.43 \pm 10.82 ^a	13.25 \pm 10.22 ^a	0.99 \pm 9.66 ^b	-11.03 \pm 28.76 ^b	0.000 ^{***}
$\text{NO}_3^-\text{-N}$	1.00 \pm 6.11 ^a	1.46 \pm 2.54 ^a	1.41 \pm 4.69 ^a	5.97 \pm 9.78 ^b	0.008 ^{**}
TN	2.98 \pm 2.14	1.83 \pm 1.77	2.02 \pm 3.46	2.75 \pm 8.07	0.726 ^{NS}
TP	3.15 \pm 3.34 ^a	-1.32 \pm 3.81 ^b	1.95 \pm 2.79 ^a	3.57 \pm 9.49 ^a	0.004 ^{**}

NS – not significant, ** – $P < 0.01$, *** – $P < 0.001$, superscripts a, b indicate significant differences.

There were significant differences in the K values for the indices of $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, and TP. Further multiple comparison analysis revealed that, the K values for $\text{NO}_2^-\text{-N}$ were significantly higher in the first two treatments (i.e. CW_1–CW_2) than in the remaining two implying the removal of $\text{NO}_2^-\text{-N}$ mainly occurred in the first two treatments (mainly due to denitrification) over the three year monitoring. As such, the K values for $\text{NO}_3^-\text{-N}$ in the first three CWs were significantly lower than that in the last one indicating that the removal of $\text{NO}_3^-\text{-N}$ was mainly affected by the last CW (mainly due to plant absorption). Likewise, the K value for TP in CW_2 was negative and significantly lower than the remaining three implying the phosphorus was released from the substrate that had been stimulated by the artificial aeration (Figs. 3 and 4).

To further explore the relationships between the first order removal rate of various pollutants and the measured environment, RDA was performed for each CW. As a result, only a significant correlation was detected in CW_1 (Table 2). Based on the RDA ordination plot, it was observed that the removal rates of ammonium-N and TP were positively correlated with DO implying that the artificial aeration was beneficial for the removal of these two pollutants.

Table 2

Summary of Monte Carlo test for the four CW units based on RDA

Wetland unit	Item	Test of significance of first canonical axis	Test of significance of all canonical axes
CW_1	eigenvalue	0.293	0.740
	<i>F</i> -ratio	7.031	4.031
	<i>P</i> -value	0.002	0.002
CW_2	eigenvalue	0.188	0.510
	<i>F</i> -ratio	3.935	1.472
	<i>P</i> -value	0.204	0.044
CW_3	eigenvalue	0.155	0.514
	<i>F</i> -ratio	3.123	1.500
	<i>P</i> -value	0.266	0.032
CW_4	eigenvalue	0.194	0.561
	<i>F</i> -ratio	4.102	1.814
	<i>P</i> -value	0.228	0.006

Similarly, the *K* value of TN positively correlated with EC and TDS, and the *K* value of COD positively correlated with TN or TP (Fig. 5). This was partially due to that the nitrites/nitrates were the main constituents that accounted for EC (or TDS), and organic matters also made a main component in TN or TP for the current wastewater.

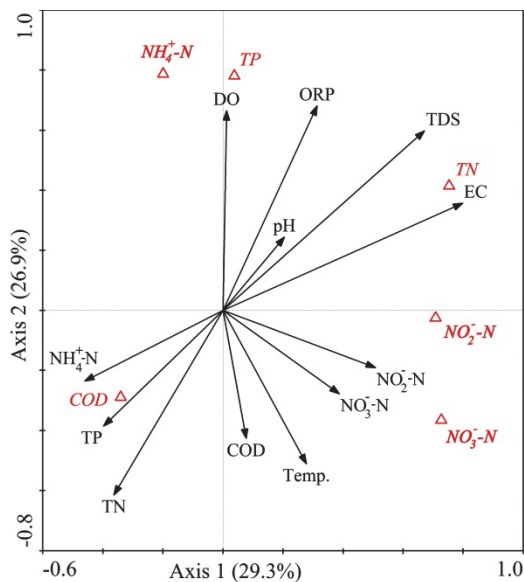


Fig. 5. RDA ordination plot based on the first order removal rate constants and the measured environment in CW_1

Generally, the first-order removal rate constants for nitrogen and phosphorus increased with the increase of standardized pollutant loading rate [2]. This was demonstrated in the present case, too (the coefficient between the K value and the index was 0.485 for NO_2^- -N and 0.473 for NO_3^- -N). Nevertheless, a significantly negative correlation was observed between the index of TN or TP and the K value for TN or TP (Fig. 5). As mentioned above, the TN was mainly comprised of organic matters. Hence, a high level of TN corresponded to a high level of organic matters leading to a high consumption of DO. This was adverse to the removal of TN and TP partially due to reduced microbial activities induced by the fast decline of DO.

Additionally, the only detected significant correlation between the first order removal rate and their environment in CW_1 was probably attributed to that the various pollutants were mainly sequestered in CW_1. This also implied that the first CW played a more important role on purification compared to the remaining three. As mentioned above, the NO_3^- -N in the last CW was mainly reduced by the absorption of aquatic plants. This could be further demonstrated by the significantly positive correlation between DO and the K value for NO_3^- -N ($r = 0.366$, $P < 0.05$) in CW_4. The high biomass of these plants emitted a considerable quantity of oxygen via the photosynthetic process during the daytime.

4. CONCLUSIONS

A combination of four stage CWs (three horizontal subsurface flows followed by one free water surface flow) was constructed to treat low-strength aquaculture wastewater. By the results, the organic matters and nutrients were mainly sequestered in the anterior subsurface flows, while the free water surface flow mainly contributed to DO improvement, as well as to the reduction of nitrates. These results probably implied no necessity of excessive subsurface flows connected in a staged manner. Meanwhile, the artificial aeration improved the treatment performance on ammonium-N, TN and TP in the first CW. Under the aerated conditions, more ammonium-N could be nitrified into nitrates, which then be absorbed by aquatic plants in the latter surface flow. Thereby, subsurface flow plus free water surface flow CW coupled with artificial aeration is possibly a readily means for aquaculture wastewater treatment.

ACKNOWLEDGMENT

This work was supported by grants from the National Natural Science Foundation of China (31202034), the Director Fund of the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, and the Special Scientific Research Fund of Public Welfare Profession of China.

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