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Research article

Implementation of a constructed wetland for the sustainable treatment of inland shrimp farming water

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ABSTRACT

In the Mekong delta, inland-based shrimp breeding requires significant inflow of high-quality freshwater. In turn, discharge of substantial loads of poor-quality effluents negatively impacts adjacent water bodies and favors disease outbreaks. This project describes the implementation of a laboratory-based continuous closed recirculation aquaculture system composed of a constructed wetland (CW) with horizontal subsurface flow as a water treatment filter for mesohaline conditions, functioning under high loading rate (HLR = 1.54 m/d with HRT = 1.31 h). This CW was equipped of successive compartment dedicated to the successive elimination of the contaminants of interests. CW performance was measured over a complete growth cycle of the White-leg shrimps (*Litopenaeus vannamei*). Results showed that the designed system was pertinent, improving water quality of the shrimp culture substantially. Complete removal of nitrite was attained, with a concomitant reduction of respectively 78% and 76% of nitrate and COD. Bacteria enumeration tests showed that *Vibrio* sp. cells were fully removed, and that a 3 Log reduction was reached in total aerobic bacteria.

1. Introduction

Over the last 30 years, world production of cultivated aquatic food has increased rapidly and has driven aquaculture to be one of the fastest-growing animals-food-producing sectors (Letchumanan et al., 2015). Aquaculture production is expected to reach 140 million tons by 2050, about the double of the production achieved in 2010. In Viet Nam, shrimp farming (so-called "red gold farming") is a profitable activity for the local population. While providing large commercial benefits, shrimp farming causes severe environmental problems and affects sustainable use of natural resources (Nguyen et al., 2016). Environmental impacts of shrimp farming include the destruction of wetland habitats, such as mangroves, the dispersion of chemicals and nutrients into the environment; the depletion and the biological contamination of wild fish and shrimp populations, as well as the decrease of the diversity of wild aquatic species (Henares et al., 2019).

Lan (2013) reported that 53% of the households cultivated ca. 0.5 ha basins, from which 16% were engaged in semi-intensive and intensive cultures, requiring running water production mode as well as constant and careful water quality monitoring. Nutrient assimilation is poor in these culture conditions, and a relatively low percentage (lower than 20%) of the nitrogen, phosphorus and organic carbon are recovered in the biomass (Henares et al., 2019). To keep the water quality in conditions that allow animal growth, common practices involve the renewal of up to 30–40% of the pond water daily with constant and costly aeration. Furthermore, pond water and sediments remaining after one round of shrimp culturing are released into natural water bodies without any treatment, inducing strong eutrophication and salinization processes (Lan, 2013). These culturing systems rely massively on pesticides and antibiotics (ABs) to avoid shrimp disease outbreak (Rico and Van der Brick, 2014). As mentioned in Pham et al. (2018), each Vietnamese farm used three different ABs on average, with 10% of the farms using up to six different products. It was estimated that ca. 700 g of ABs is used per ton of cultured fish, seven times more than other countries, resulting in a high prevalence of residual concentrations in aquaculture products and waste (Uchida et al., 2016). The resulting dispersion of antibiotic resistance genes among pathogenic bacteria already had a significant effect on shrimp cultures worldwide, linked to massive losses in

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productivity (Letchumanan et al., 2015).

Negative impacts accompanying intensive culturing could be reduced easily and efficiently with the implementation of recirculating aquaculture systems (RAS). These systems are considered nowadays as the future standard of aquaculture industry and defines the continuous treatment of effluent culture water through a series of consecutive processes such as filtration, disinfection and oxygenation before the reinjection of the treated water into the culture pond (Takeuchi, 2017). As microbial load in the effluent is strongly reduced, these systems also provide better control over pathogenic bacteria and viruses, decreasing in turn the need for AB usage (Martins et al., 2010). RAS have been implemented successfully in Viet Nam for the breeding of Pangasius fish, with a concomitant increase in yields and profits (Ngoc et al., 2016). However, and contrariwise to the Pangasius industry, 90% of the shrimp breeding basins are operated by small holders, reaching up to 65% of the country production. A full RAS including a growing pond, with an added bio-filter and a septic tank for denitrification still requires an investment of ca. 280,000 USD per hectare, which is beyond the reach of most small producers (Ngoc et al., 2016).

It is therefore necessary to address sustainability issues of inland shrimp cultures with the implementation of simpler but equally effective RAS-based technical alternatives such as constructed wetlands (CWs). CWs have been confirmed to be a robust ecological technique for treating various types of wastewater and have been successfully used for the treatment of intensive aquaculture basins in developing countries (Stefanakis, 2018; Zhang et al., 2014). They have become efficient alternative treatment systems in rural areas due to their simple design, low costs and energy consumption, ease of operation and maintenance (International Water Association, 2000; Chen et al., 2016; Tepe and Temel, 2018). CWs effectively remove organic matter, suspended matter and nutrients with various processes like sedimentation, filtration, assimilation, plant uptake as well as biological and microbiological activities, reducing the need for water exchange and heavy aeration.

The current project aims at solving the issues of poor-quality water of shrimp breeding ponds and alleviate the continuous discharge of substantial loads of effluents into adjacent surface water courses. Although appliance of constructed wetlands is not new per se, application of this technology to inland shrimp ponds has not been scientifically investigated in detail in Viet Nam. The major inherent difficulty encountered in this study is the need to use a minor fraction only of the surface of the shrimp breeding ponds to carry out the water purification process, as breeders are reluctant to sacrifice large areas for a water cleaning system. The novelty of this project lies in the design of a small and modular subsurface flow horizontal (HSSF) CW, in which successive compartments are dedicated to the sequential elimination of the contaminants of interest. Because of its small size, the efficiency of the CW would be insured by a high loading rate, an infrequent feature in CW technology. In the present study, the efficacy of this modular design was tested using a laboratory-based CW connected to a shrimp-breeding tank in closed continuous recirculation mode. Our objectives were reached by testing the water cleaning capacity of the CW in laboratory conditions during an intensive culture cycle of White-leg shrimps (*Litopenaeus vannamei*), allowing to evaluate the performance and to identify key mechanisms involved in this experimental system.

2. Material and methods

2.1. Constructed wetland

The laboratory-based CW with horizontal subsurface flow (HSSF) was built using 8 mm thick glass plates, which were assembled manually with silicone adhesive. The CW had a total length of 125 cm, a width of 20 cm (0.25 m^2) and a height of 50 cm, providing a practical volume of ca. 50 L with a 20 cm water height (Fig. 1). In addition, a swirl filter (capacity ca. 8 L) was added at the inlet of the system to remove suspended solids (fecal and food pellets).

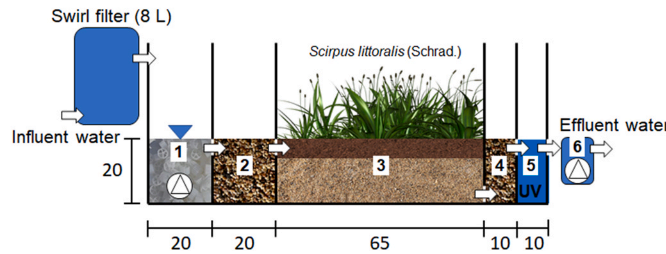

Fig. 1. Schematic representation of the constructed wetland with the successive compartments (1–6, see text for details). Two aquarium air pumps provided aeration within compartments 1 and 6. Measures are in cm.

The CW was designed as a successive series of compartments dedicated each to specific water cleaning activities. The first compartment (20 cm, capacity 8 L) was filled with polypropylene plastic pellets used for microbial biomass fixation and was intended for organic matter oxidation and nitrification. This compartment was continuously aerated using a submerged aquarium pump (Europet-Bernina Hi-Tech Air 1400 cc) at a rate of 80 L/h. The second compartment (20 cm, 8 L) was designed for the reduction of dissolved oxygen, particle sedimentation and reduction of preferential flow paths. It was filled with coarse centimetric gravel (porosity 60%). The third compartment (65 cm, 26 L) composed the HSSF per se. It was filled with a bottom layer of 14 cm of millimeter-size silica gravel (porosity 50%) on the top of which was laid a layer of 6 cm of fine sediment (porosity 20%) originating from soil material excavated during the building of a shrimp basin in the Mekong delta river. This HSSF compartment was intended for anaerobic processes, such as denitrification, organic matter and phosphorus removal by microbial activities, plant uptake and passive adsorption. This compartment was planted with a halophyte, *Scirpus littoralis* (Schrad.), which was selected due to the facility with which it grows around artificial shrimp basins, tolerating medium concentrations of salty water (Trang et al., 2018). Small plants (ca. 30–40 cm in height) were retrieved from natural sites with their root system and adapted to laboratory conditions for a few days before being planted in the CW. The fourth compartment (10 cm, 4 L volume) was filled with centimetric coarse gravel (porosity 60%) and was set to stabilize water flow and retaining possible particulates at the outlet of the precedent compartment before entering the fifth compartment (10 cm, 4 L), free from any solid, and equipped with a 20 Watts 254 nm UV LED-type lamp in continuous mode for a disinfection stage. Finally, the sixth compartment was composed of a 5 L plastic bucket in which the effluent water was oxygenated (Europet-Bernina Hi-Tech Air 1400 cc) at a rate of 80 L/h before being conducted by gravity in the shrimp breeding tank.

The whole system was run for five consecutive days in closed loop for stabilization before the connection to the shrimp tank, followed by fifty days of continuous operation. A second aquarium with an identical volume was set as a control in order to compare the evolution with time of the water quality. This second tank was managed in an identical way, except that there was no recirculation of the water through the CW.

2.2. Hydraulic management

The CW was connected to a 400 L (0.7 m^2) aquarium used for shrimp breeding with a submerged pump (Eheim CompactOn 300 pump) at a flow rate of 18.1 L/h in average. The recirculating ratio R is defined as the ratio of the daily volume of recirculation water to the total water volume present within the shrimp tank and was $R = 1.09$. This flow rate induced a mean hydraulic loading rate (HLR) and hydraulic retention time (HRT) of respectively 1.536 m/d and 1.31 h (0.054 d). Water level in both shrimp aquarium was not exchanged during the experiment, but water level was corrected with the addition of water volumes to replace water loss due to evapotranspiration, leaks, and sampling.

2.3. Shrimp culturing

Fifty days-old White-leg shrimps (*Litopenaeus vannamei*) were collected from a breeding farm located in the Can Gio district (Ho Chi Minh City, Viet Nam). Each aquarium received 70 animals, corresponding to an animal density typical for intensive breeding systems (ca 100 animals per m^2). Animals dying during the experiment were removed immediately. At the end of the experiment, all animals were weighed, their length was measured. Growing conditions were adjusted to the ones observed in the farm culture basin: pH: 7.8, salinity: 1.5% NaCl, alkalinity: 100 mg/L and dissolved oxygen: 6.0 mg/L using submerged pumps. Shrimps were fed with 7 g of indissoluble organic pellets four times a day (total of 28 g per day) using an automated food dispenser. Typical shrimp food found on the local market is composed of ca. 43% crude protein, between 4 and 6% total fat and 4% crude fiber, with the addition of 1.2% methionine and cysteine, 1.8% lysine, 1–2.3% Ca, 1–2% P, 150 ppm ethoxyquin (a quinoline-based antioxidant), with a metabolizable energy of 2600 kcal/kg. Water salinity and hardness of the aquarium water was adjusted on a weekly basis to maintain growing conditions identical to those prevailing in culture farms by the addition of 30 g/m^3 (each) of three additives (Charoen Pokphand, Malaysia). In details, "Soda-Mix" contained 52–56% $CaSO_4$, 9–13% $MgSO_4$ and 31–39% NaCl. "Hardness" contained 72–77% $CaCO_3$ with NaCl as carrier. Finally, "Ka-Max" was composed of 17–21% MgO and 11–14% K_2O with NaCl as carrier.

2.4. Water sampling and analyses

Water samples were taken twice a week both from the influent and the effluent of the CW and from the control aquarium. These water samples were filtered (0.45 μm PES filters, Millipore) when necessary for the analysis of ammonia, nitrate and nitrite. Other analyses included total phosphorus, total nitrogen, chemical oxygen demand (COD). All elements were analyzed in triplicate using dedicated colorimetric tests on a DR 1900 Portable Spectrophotometer (Hach, Germany). The error on the measurement was estimated at 5% based on data provided by the maufacturer. NH_4^+, temperature, dissolved oxygen, conductivity and pH were monitored on-line (ProDSS Multiparameter Water Quality Meter, YSI, USA). Numeration of total aerobic bacteria and *Vibrio* sp. was carried out twice during the experiment using respectively Nutrient Agar and TCBS media (Merck, USA).

3. Results and discussion

3.1. CW design and operation

Pollutant removal in CWs can be described using a first-order plug flow kinetic model equation, from which an estimate of the required wetland surface for efficient water treatment can be derived (Lin et al., 2005). Classically, CWs require a low HLR and a long HRT to achieve efficient pollutant removal. Suggested HRT and HLR are 1–15 days and 0.014–0.135 m/day, respectively for optimal operation when organic matter concentration was relatively low (Zhang et al., 2014). However, these values resulted in a wetland size being 0.7–2.7 times the size of the culture pond area (Turcios and Papenbrock, 2014; Shi et al., 2011). These sizes are not applicable when production is a priority for land use in rural areas. In the present study, a compromise was established between an estimate of the potential benefits (such as increased water quality and reduced mortality) and possible disadvantages associated with loss of productive surface area. The determining parameter is the surface occupied by the CW as well as the water volume ratio between the CW and culture tank. The relatively modest surface of the CW naturally imposes a high HLR, which is conceivable in the observed trophic conditions present in semi-intensive cultures, in which high concentrations of organic matter are observed. This is particularly relevant as large quantities of small-size suspended particles are produced from food scraps and fecal pellets. In the present study, the experimental CW had a practical volume of 50 L, matching ca. 12.5% of the total volume of the culture tank and an estimated 21 L of water passing through the different compartments (5.25%), coming from the 400 L culture tank. Accordingly, HRT and HLR values were set at respectively 1.31 h (0.054 day) and 1.536 m/day. In this way, the culture aquarium volume could be exchanged fully in ca. 24 h (R = 1.09). Comparatively, Lin et al. (2005) reached excellent contaminant removal rates using high HLR (1.57–1.95 m/day) with a ratio of CW to culture tank of 0.43.

3.2. Monitoring running parameters

Small variations of temperature within both tanks were measured over time as laboratory conditions could be controlled partially only. Average temperature values were 31.5 ± 0.3 °C, 32.3 ± 0.6 °C, 31.3 ± 0.4 °C in the CW influent and effluent, as well as in the control tank respectively. Presence of a submerged UV lamp within the last compartment of the CW induced a Joule effect that accounted for the difference observed between the inlet and the outlet of the CW.

Average pH values recorded within the CW influent and effluent as well as the control tank were respectively 7.74 ± 0.35, 7.96 ± 0.28 and 7.69 ± 0.53. However, values measured in the CW influent showed large variations with time, ranging from 6.8 to 8.3. Interestingly, pH values recorded in the effluent were systematically higher, ranging from 7.4 to 8.4, with a difference of 0.22 ± 0.15 on average. The variability of these values measure in the effluent were equally smaller, possibly indicating that the CW played an important role in the buffering of pH values. Higher values may have been induced by denitrification activities producing alkalinity. Furthermore, these recorded values agreed with the pH = 8 proposed by Lee et al. (2009) for an optimal rate of denitrification. Finally, highest variations were measured in the control tank, with pH values ranging from 6.5 to 8.4.

At start of the experiment, dissolved oxygen (DO) values were identical in both tank with 6 mg/L. Martínez-Córdova et al. (2011) reported that this value is generally recommended for the growth of white-leg shrimp *Litopenaeus vannamei*. Data collected during the experiment showed that constant aeration was effective at keeping DO values in the range of 5.6–6.6 mg/L, with an average of 6.23 mg/L ± 0.32 in the CW influent, 4.49 mg/L ± 0.24 in the CW effluent and 5.98 mg/L ± 0.32 in the control tank. Interestingly, and from Day 22, DO values were systematically higher in the CW influent. Differences in DO values between the CW inlet and outlet was in average 1.73 mg/L ± 0.44. Oxygen consumption within the CW was expected as the result of aerobic respiration mechanisms involved in the microbial heterotrophic feeding mode of the microbial communities colonizing the CW.

Electrical conductivity showed different evolution with time in both tanks. Starting at 2.92 mS/cm, electrical conductivity reached respectively 3.26 and 3.54 mS/cm in the tank connected to the CW and in the control tank respectively.

3.3. Temporal evolution of the chemical contaminants

3.3.1. Nitrogen and phosphorus removal

The chemical composition of the shrimp food may vary substantially between providers. Shrimp food contains in general between 35% and 42% of proteins (dry weight), providing the main source of ammonia within the culture tanks. In our experiment, a linear increase of ammonia concentrations was observed in the control tank with the addition of food pellets at a rate of 28 g per day (Fig. 2). In contrast, concentrations of NH_4^+ and NH_3 remained stable throughout the experiment with the addition of the CW, with a constant removal rate of 10% ± 5.6 in average. The CW allowed the stabilization of NH_4^+ and NH_3 concentrations with time, reaching at the end respectively 27.3 and 1.4 mg/L in the CW influent. Nair et al. (2019) showed that ammonia-oxidizing communities could dominate microbial

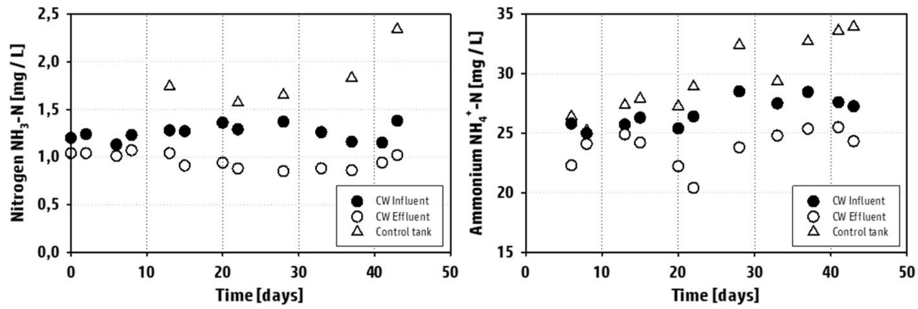

Fig. 2. Evolution of the concentration of NH_3 and NH_4^+ during the fifty days of the experiment.

communities in zero water exchange aquaculture systems when a careful environmental management of the cleaning system. Both ammonia-oxidizing bacteria (AOB) and Archaea (AOA) were found in marine aquaculture systems (Srithep et al., 2015), in which they were considered as main actor in ammonia removal. In our experiment, the apparent low rate of the nitrification process was unable to achieve the full removal of ammonia, possibly due to the short period of activity of the system. The autotrophic microbes involved in this process require long start-up and have relatively long multiplication rates.

In recirculating systems, facultative anaerobic microorganisms conduct denitrification with electron donors derived from organic sources (Van Rijn et al., 2006). In this experiment, presence of the CW had a clear impact on both nitrate and nitrite removal (Fig. 3). Impact of the denitrification processes could be observed after ca. 10 days of constant water recirculation. Nitrate concentrations diminished gradually in the CW effluent, reaching 1.1 mg/L after 50 days. At the same time, full removal of nitrite was attained. These values are in accordance with those presented by Lin et al. (2002), with >99% reduction of NO_2^- and 82%–99% reduction of NO_3^-.Conversely, and in the control tank, nitrite concentrations increased steadily with time and reached 0.6 mg/L by the end of the experiment. As denitrification processes occur in anoxic conditions only, it was assumed that it arose preferentially in the main compartment of the CW. Higher rates of nitrate reduction could be expected possibly with finer mineral material or with a corresponding higher HRT over a longer period.

Total phosphorus (P) ranged from 0.014 to 0.028 mg/L, with a mean of 0.020 mg/L ± 0.003 in the CW influent. In the CW effluent, the range decreased by 15.2% ± 7.1, with values varying from 0.013 to 0.019 mg/L (Fig. 4). In the control tank, P had an average concentration of 0.029 mg/L ± 0.002, showing no accumulation with time. The removal rate obtained after fifty days of continuous operation reached 31%. Total phosphorus measured in the food pellet using ICP was 14.77 mg/g (data not shown). With 28 g of food per day, the total increase of phosphorus in both tanks was 1.034 mg P/L and per day. However, much of the food was immobilized as food pellets, and 84% of all phosphorus was shown to be associated with this solid material, accumulating at the bottom of both tanks (Turcios and Papenbrock, 2014). Adsorption is one of the long-term controlling processes for phosphorus sequestration in

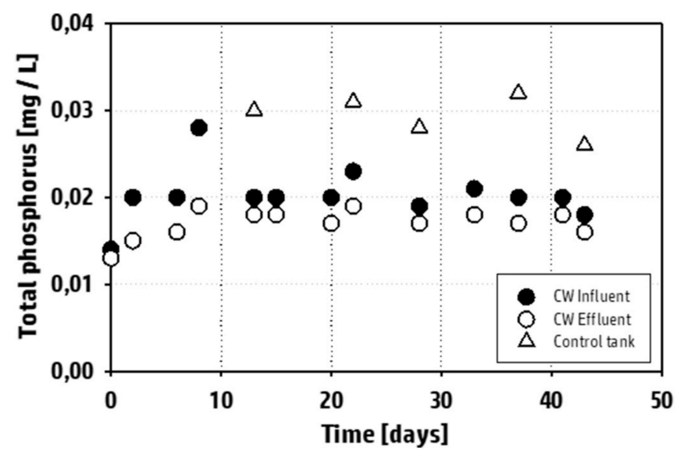

Fig. 4. Evolution of the concentration of total phosphorus during the fifty days of the experiment.

wetlands and is primarily responsible for phosphorus removal in SSF wetland (Vymazal, 2009). Furthermore, use of mixed geological substrates have been reported to be more effective in the removal of phosphate (Wu et al., 2015). A significant correlation was found between P removal and CaO and Ca content present within the filter material (Vohla et al., 2011). In our experiment, a supplementary compartment filled with calcareous sand could offer a solution for better P removal.

3.3.2. Chemical oxygen demand (COD)

COD measurement was carried out for the follow-up of the organic matter concentration within both culture tanks (Fig. 5). As a result, the set-up of the CW had a very positive impact on the reduction of COD. Average values measured in the CW influent were 324 mg/L ± 41.8, whereas the average COD value within the effluent dropped to 90.5 ± 36.4 mg/L, showing an efficiency of 72% ± 11 on average. Sedimentation and filtration were the primary mechanisms by which suspended solids were removed in the CW. An immediate COD reduction on Day 1

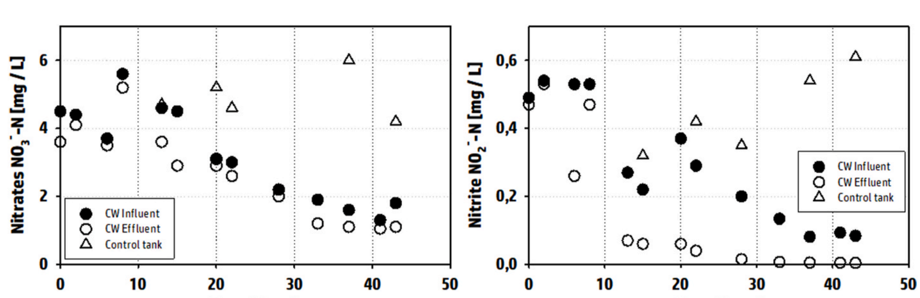

Fig. 3. Evolution of the concentration of nitrate and nitrite during the fifty days of the experiment.

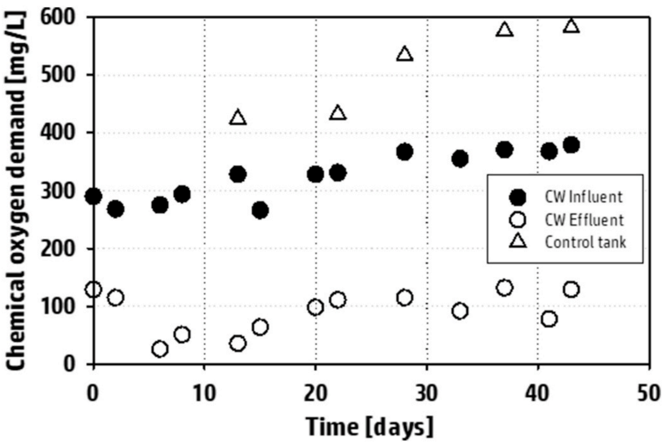

Fig. 5. Evolution of the concentration of chemical oxygen demand (COD) during the fifty days of the experiment.

of 161 mg/L (69.9 g COD day^{-1}), corresponding to a reduction of 55.6%, was attributed to the filtration capacity of the system to retain suspended solids, confirming data presented by Lin et al. (2005). Visual observation confirmed the nominal functioning of the swirl filter in the removal of suspended solids, preventing particulates from entering the CW. Clogging problem is the most determinant factor for successful long-term operation and application of HSSF CW in a recirculating aquaculture system. However, and in the present study, no clogging occurred throughout the operating period.

After 50 days of continuous activity, difference between COD values measured in the CW influent and effluent reached 270 mg/L (117.3 g COD day^{-1}), an average computed on values measured for the last five days of the experiment. When considering the filtration capacity of the CW to remove suspended solids as a constant process, the oxidative capacity of the system developed during this experiment would have reached 109 mg/L (47.3 g COD day^{-1}). Therefore, and from Day 28 on, COD values registered for the CW influent were shown constant. In comparison, COD values measured in the control tank increased steadily with time, reaching 582 mg/L by the end of the experiment.

Previous studies carried out using HSSF CW achieved similar COD removal rates (Lin et al., 2002; Vymazal, 2009; Zhang et al., 2014). Phan and Dinh (2017), using similar water composition (ca 570 mg/L COD) reached 89.4% COD removal with a lower HLR and larger CW size. In another study, removal rate was 26.7% after suspended solid removal when treating effluents from intensive shrimp cultures (Shi et al., 2011).

3.3.3. Bacteria enumeration

Two sets of microbiological analysis were carried out after 18 and 50 days of continuous operation (Table 1). Analysis involved the enumeration of total aerobic bacteria and *Vibrio* sp., including classical opportunistic shrimp pathogens *Vibrio parahaemolyticus* and *Vibrio vulnificus* (Global Aquaculture Alliance, 2016). Despite high standard deviations (up to 83%), results obtained for Day 18 showed a reduction of 99.8% of the total aerobic bacteria between the CW influent and effluent. At the same time, complete removal of the *Vibrio* sp. was attained. Results obtained by the end of the experiment (Day 50) showed a similar pattern, with a 99.9% reduction in total aerobic bacteria and complete removal of culturable *Vibrio* sp. Interestingly, a reduction of ca. 16% was observed at Day 50 in the CFUs of the total aerobic bacteria within the CW inlet. This reduction was stronger for *Vibrio* sp. with ca. 87%.

The two CFU values observed at Day 18 and Day 50 for the total aerobic bacteria were always higher in the basin connected to the CW (inlet) than in the control tank. Despite the strong CFU reduction, the microbial community populating the CW-associated tank was apparently developing more significantly. No significant difference could be observed neither between *Vibrio* sp. CFUs present in the CW inlet and in the control tank at Day 18. A noticeable difference was observed at Day 50 only, indicating a long-term reduction effect of the system. It was however difficult to estimate the respective role played by the filtration process and the UV illumination within the last CW compartment. UV lamps emitting at 254 nm (UV–C photons) are very effective for water disinfection (Lakeh et al., 2013). Recent development of LED technology rendered these lamps more effective, with low energy requirements. In turn, filtration capacities of CWs resulted in high removal rates (up to 99%) of bacterial cells and viruses when wastewater was treated (Wu et al., 2016).

3.3.4. Plant management

Vymazal (2011) showed that planted wetlands promote heavy metals immobilization, denitrification and nutrient uptake. In a mesohaline environment, the economic attractiveness of a CW could be upgraded using salt-tolerant species with a commercial value (Turcios and Papenbrock, 2014). Buhmann and Papenbrock (2013) showed that recirculating aquaculture water through constructed mangrove wetlands had a positive impact on shrimp growth. In this experiment, the main compartment of the constructed CW was planted with *Scirpus littoralis* (Schrad.). In the Mekong delta, this sedge is classically cultivated in extensive shrimp ponds as a source organic feed material (Trang et al., 2018). In the present experiment, the sedge adapted well to the laboratory conditions, keeping green shots with slow apparent growth. However, and due to the short period, the plants were expected to play a minor role only in nutrient uptake and oxygen supply to the main compartment of the CW.

3.3.5. Shrimp breeding

Animals used at the start of the experiment were already 50 days old. Mortality rates were identical in both tanks and reached 25% indicating that the CW had no negative impact on shrimp breeding. No significant difference was found in the development of the animals bred in both tanks. Average weight of the shrimps cultivated in the tank connected to the CW were 12.61 ± 1.6 g. In the control tank, the animals showed on average a weight of 11.96 ± 1.2 g. According to (Hung et al., 2013), no appreciable regional differences in the size of the animals were recorded in South Viet Nam after a period of 80–100 days of cultivation. About 60% of the animals reached a weight varying from 10 to 12 g. Shrimps present in both tanks reached their weight fully, indicating that laboratory conditions imposed in this experiment corresponded well to the conditions imposed in outdoor breeding.

4. Conclusions

In the artificial CW developed for this study, water flow was imposed horizontally and CW performance was measured over a complete growth cycle of White-leg shrimps (*Litopenaeus vannamei*). The CW was built using simple material and required low maintenance. The original

Table 1
Total aerobic bacteria and *Vibrio* sp. measured in the influent, effluent and control tank (in colony forming units).

	Day 18			Day 50		
	Influent	Effluent	Control	Influent	Effluent	Control
Total aerobic bacteria	7,9E6 ± 6,5E6	1,5E4 ± 6,6E3	1,6E6 ± 1,1E6	6,6E6 ± 1,8E6	4,9E3 ± 1,63	1,7E6 ± 1,1E6
Vibrio sp.	1.7E3 ± 3,5E2	0	7,5E2 ± 5,3E2	2,2E2 ± 1,2E2	0	1,0E3 ± 6,3E2

modular design was composed of a succession of compartments devoted to specific physical and microbial activities. This succession of compartments imposed a gradual decrease of the water oxygen content, promoting initially the oxidation of ammonia and organic matter, followed by the reduction of nitrite and nitrate. Integration of this CW had a positive impact on the reduction of water contaminants.

Despite the relatively short duration of the experiment, the CW showed excellent capacities to eliminate components that could disrupt animal development. As is standard for all CWs, the system had potentially not yet reached its full capacity at the end of this first culture cycle. It could be expected that the performances would improve over time, following the formation of soil bacterial community and fully grown *Scirpus littoralis* plants. In addition, this modular system is likely to receive modifications allowing optimization of microbial processes. This was particularly the case for the elimination of ammonium, which was still low, possibly due to the limited development of the corresponding bacterial guild members. The development of these microorganisms is relatively slow and is inhibited by high concentrations of dissolved organic matter. The addition of a compartment specifically dedicated to ammonium oxidation downstream of the main compartment could promote better performance in the future.

Finally, this study evidently demonstrated that a HSSF CW functioning with high HLR in mesohaline conditions can be implemented in a simple way and substantially improve the water quality of a semi-intensive shrimp culture. Implementation of CWs in shrimp culture farming systems would offer an advantageous alternative for achieving environmental sustainability.

Author contributions

Thi Thu Hang Pham, Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. Vincent Cochevelou, Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing – original draft. Hoang Dang Khoa Dinh, Methodology, Supervision, Resources, Writing – review & editing. Florian Breider, Supervision, Writing – review & editing. Pierre Rossi, Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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