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TREATMENT OF WASTEWATER FROM INTENSIVE STRIPED CATFISH FARMING USING HYMENACHNE ACUTIGLUMA IN BATCH SURFACE-CONSTRUCTED WETLAND

Intensive striped catfish (*Pangasianodon hypophthalmus*) farming has recently expanded owing to its economic benefits but it has led to severe aquatic pollution. In this study, a batch surface-constructed wetland system cultivated with *Hymenachne acutigluma* grass was designed to treat wastewater collected from catfish ponds. The planted wetland system showed effective operation, as 81.7% of NO_3^-N ,

63.6% of NH_4^+ -N, and 77.5% of PO_4^{3-} -P (w/w) in the wastewater were removed over four months. Besides,

the wetland significantly decreased the chemical oxygen demand and total suspended solids from the wastewater. The absorption by the grass resulted in the removal of 20.3 and 22.2% of the total nitrogen (N) and phosphorus (P) from wastewater, respectively. The determination of the nutrient mass balance in the planted system showed that phosphorus in the wastewater was mainly removed through sediment deposition, while a large amount of nitrogen was not lost because of the sediment deposition and plant uptake. Moreover, *H. acutigluma* cultured in the system can serve as food for cattle. This study shows an eco-friendly approach for the effective remediation of wastewater obtained from the farming of intensive striped catfish.

1. INTRODUCTION

Food production from aquaculture has significantly increased, and aquaculture has become a global source of aquatic food. Among commercial fish varieties, Pangasius is widely cultured in some countries, such as Vietnam, India, Thailand, Indonesia, Bangladesh, and China. P. hypophthalmus is relatively raised and reproduced easily. Thus, catfish farming has become one of the most important aquaculture sectors in some countries. Currently, Vietnam is the largest catfish exporting country in the world, with more

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than 90% of its products exported to over 100 countries, and the related activities provide opportunities for economic growth [1, 2]. The fish farming economy is developing, and catfish ponds are projected to reach 13 000 ha by 2020 [3].

Intensive striped catfish farming has been reported to cause aquatic pollution in the Mekong Delta [2-5] and India [6]. A large amount of nitrogen and phosphorus compounds exists in the wastewater from aquatic ponds [2-5], but the physical and chemical properties of the wastewater from the ponds have not been investigated in detail. Most of the wastewater was directly discharged into the main river (63%) and primary canals (19%), while only a small proportion of the effluent was screened (7.8%) and treated with chlorine and lime (11.2%) [1]. Therefore, the effluent should be adequately treated before being discharged into the environment. However, to the best of our knowledge, no paper has described the extensive remediation of the wastewater obtained from intensive striped catfish farming.

Da et al. [3] reported that the wastewater from striped catfish ponds might be used as nutrient sources for rice and vegetable farming. In their study, approximately 50% of nitrogen and phosphorus compounds were removed from wastewater [3]. However, catfish ponds in the Mekong Delta are mainly sited near main rivers and/or far from rice fields and vegetable farms. Moreover, water in striped catfish ponds is usually replaced at four-day intervals, which is suitable for batch treatment.

Wetlands with aquatic plants have been applied to remove nitrogen and phosphorus compounds from aquaculture effluents [7–9]. Moreover, planted wetlands have been widely used for treating effluents generated from aquaculture systems as well as polluted rivers and ponds. Wetlands and phytoremediation are cost-effective, easily monitored, eco-friendly methods with low energy requirements for remediating pollutant-contaminated environments [9, 10].

The helophyte *Hymenachne acutigluma* (Steud.) is widely distributed in the floodplains and sometimes floats on freshwater bodies. It grows quickly even on low fertile, acidic, and waterlogged soils, and can adapt to the natural conditions in the Mekong Delta. This grass is also a vital source of food for cattle in some countries. Furthermore, it effectively absorbs nitrogen and phosphorus compounds present in wastewater from intensive striped catfish ponds under net house laboratory conditions [11].

In this study, batch surface-constructed wetland cultivated with *H. acutigluma* was investigated to treat wastewater from intensive striped catfish ponds. Nitrogen and phosphorus mass balances in the system were also determined. Additionally, the nutrient components of *H. acutigluma* for cattle feeding were analyzed.

2. MATERIALS AND METHODS

Batch surface-constructed wetland system. The wetland system was constructed in the Tam Nong District, Dong Thap Province, southern Vietnam (10°40'07.6" N, 105°33'45.6" E), where several intensive striped catfish ponds have been in use for years. The system had an area of 20 m² and was designed using the following parameters: length × width × depth of $10 \times 2 \times 0.8$ m [12] (Fig. 1). An impermeable plastic liner was placed at the bottom of the system to prevent water leakage. Sediments (0–20 cm deep) were collected from a nearby intensive striped catfish pond. Large pieces of debris were removed, and the sediments were transformed into a wetland. The dry sediment sample contained 2.48% of organic carbon, 0.33% of total nitrogen, and 0.23% of total phosphorus on a dry weight basis. The grain sizes were analyzed, as described by Hossain et al. [13]. The silt, clay, and sand contents in the sediments were 65.0, 21.4, and 13.6 wt. %, respectively, and pH of the sediments was 6.6.



Fig. 1. Horizontal (a) and vertical (b) diagram of batch surface wetland system planted with *H. acutigluma*

Grass shoots of *H. acutigluma*, having an average length of 73.9 ± 9.7 cm were harvested from the surrounding natural area, cleaned with the site water, and cultivated to a density of 40 shoots/m², which corresponds to 327.0 ± 7.6 g/m² of fresh biomass. After several days, the wetland was flooded with wastewater up to a depth of 40 cm. This wastewater was collected from nearby intensive catfish ponds with a stocking density

of 50 fish/m². Nitrogen and phosphorus concentrations in the wastewater were low at the young fish stage, so the experimental tests were carried out after the catfish had been cultured for 2.5 months. The wastewater was stored for 93 h (3.9 days) and then replaced with a new batch. The replacement process took 2 h (from 7 to 9 a.m.). A control system, without planting *H. acutigluma*, was also constructed in parallel for comparison. The experiments were carried out in triplicate, from July to November 2017, until the fish were harvested. The temperature during this period was relatively stable at 28–30 °C.

Water, sediment, and plant sampling. The water samples and sediments were collected from the sample sites (Fig. 1a). Water was collected at 16-day intervals from the sites, whereas the sediments were collected at the end of the period. The water temperature and pH were measured with a pH meter (HI 8314, Hanna, Romania), and DO (dissolved oxygen, mg/dm³) was determined using a DO meter (HI 9146, Hanna, Romania). During each collection process, 1.0 dm³ of water was taken at a depth of 0.15 m below the surface at the collection site. The water samples were stored at 4 °C and transported to the laboratory within the same day for analysis. For the collection of sediments, 1.0 kg samples were collected at each sampling site (0–20 cm deep) and transported to the laboratory. Leakage water was removed, and the sediment components were determined.

The experiment was divided into two phases. The first phase lasted for 64 days while the second lasted for the remaining period. At the end of the first phase, the harvested shoots were cut off at 5–7 cm above the sediment level to determine the shoot height, shoot numbers, and chemical composition. After 128 days, all the grass plants with the accompanying leaves, shoots, rhizomes, and roots were harvested for biomass and chemical analysis. The shoot numbers of the plants grown at the sampling sites were counted before the plants were cut, and the shoot height was measured after the plants were harvested. For the shoot height measurements, 30 shoots were randomly collected at the sites for measurement.

Mass balance calculations. The mass balances of nitrogen and phosphorus in the constructed wetland for four months were calculated based on the amounts of input and output, amounts assimilated by the plants, and amounts stored (e.g., via adsorption and precipitation) in the sediments. Other losses involving the uptake by microorganisms, dead leaves, and nitrification-denitrification were calculated as follows:

Other losses = wastewater influent - water effluent - plant uptake - sediment storage

Chemical analysis. The harvested grass was dried in an oven (Ecocell-LIS-B2V /EC55, Germany) at 60 °C until it had a constant weight before its components were analyzed. The amounts of total nitrogen and phosphorus in the grass, sediments, and liquids were analyzed according to the protocols described in the American Public

Health Association (APHA) method [14]. The chemical oxygen demand (COD) and total suspended solids (TSS) were also determined during the treatment process using the APHA method [14]. Other grass components such as the total mineral, acid-detergent fiber (ADF), and neutral detergent fiber (NDF), were measured, as described by Van Soest et al. [15].

Data analysis. The removal efficiency (*RE*) of the chemicals was calculated using the following equation:

$$RE = \frac{C_2 - C_1}{C_1} \times 100\%$$

where C_1 and C_2 are the concentrations (mg/dm³) before and after the treatment.

Hemicellulose = NDF - ADF [15]

3. RESULTS AND DISCUSSION

3.1. PHYSICAL AND CHEMICAL PROPERTIES OF WASTEWATER DURING THE TREATMENT

The physical and chemical properties of the inlets and outlets of the batch surfaceconstructed wetland were analyzed. The total nitrogen and total phosphorus concentrations and the COD levels in the wastewater collected from the fish ponds increased from the first to the third month when the fish were cultured from 2.5 months to 5.5 months. The increase in these components occurred because the older fish were fed with higher amounts of aqua feed (ranging from 8.0 metric tons per 1000 m² in the first month to 12.0 metric tons per 1000 m² in the third month of the study). However, the NO_2^--N and NO₃⁻-N concentrations in the influent were high at the early measurement periods and reached up to 0.82 and 1.65 mg/dm³, respectively (Fig. 2). Nevertheless, NH⁺₄-N and total nitrogen were low before 64 days but the concentrations increased during the remaining period. Similarly, the PO_4^{3-} -P and total phosphorus concentrations in the wastewater were low before 64 days (Fig. 3) with average values of 0.46 and 0.92 mg/dm³, respectively. These concentrations increased during the later period by 4.16 and 5.10 mg/dm³, respectively, on average. Most of the measured parameters in the wastewater exceeded the criteria specified in the Vietnamese national standards for surface water quality; for example, the maximum levels of NO_2^--N , $PO_4^{3-}-P$, and TSS should be limited to 0.05, 0.3, and 50.0 mg/dm^3 , respectively [16].



Fig. 2. Concentrations of a) NO₂⁻-N, b) NO₃⁻-N, c) NH₄⁺-N, and d) total nitrogen in the inlets (□) and outlets of unplanted (⊠) and planted (⊠) wetland systems



Fig. 3. Concentrations of a) PO₄^{3−}-P and b) total P in inlets (□) and outlets of unplanted (⊠) and planted (⊠) wetland systems

During the storage period of the wastewater in the constructed system, the pH levels of the wastewater slightly increased, while the DO levels varied from approximately 3.0

to 6.0 mg O_2/dm^3 (Fig. 4). The increase in the pH occurred probably due to the photosynthesis of macrophytes and algae [12].



Fig. 4. pH (a), DO (b), TSS (c), and COD (d) in inlets (□) and outlets of unplanted (⊠) and planted (⊠) wetland systems

The DO was significantly low because of the organic decomposition and nitrification that occurred in the constructed system [17]. However, the TSS and COD levels significantly decreased when passing through the wetland, especially in the planted treatment (p < 0.05). Similarly, the DO, TSS, and COD levels of the catfish wastewater in the rice plots also decreased [2]. Coarse sand and gravel are generally used as filters in wastewater treatment [18]; however, abundant sediments produced from fishpond systems in the Mekong Delta can be cultivated with *H. acutigluma*. *H. acutigluma* also grows well in swampy areas, where sediments containing silt are abundant [19]. The removal of pollutants in wetland systems depends on factors, such as the macrophytes, substrate, surface loading rate, influents, microorganisms, retention time [10]. The system with 20 cm thick sediments used in this study is suitable for the batch surface treatment of wastewater generated from the fish ponds.

3.2. NITROGEN AND PHOSPHORUS REMOVAL USING BATCH SURFACE-CONSTRUCTED WETLAND

Figures 2 and 3 show the nitrogen and phosphorus reductions, respectively, in the planted system compared with the unplanted system. After nearly four days, all the chemical components in the wastewater decreased in both systems and satisfied the water quality requirements [16]. The removal of NH_4^+ -N, PO_4^{3-} -P and the total phosphorus in the planted system were more significant than those of the unplanted system during several analyses. Although the plants regenerated, sprouted, and bloomed in the second phase, the wetland still showed effective removal of the total nitrogen and phosphorus (Figs. 2, 3).

Table 1

System	Input			Output				
	Influent	Plant	Sediment	Effluent	Plant	Sediment	Other	
		biomass			biomass			
	Nitrogen							
Unplanted	4539.3±384.5	-	6217.2±49.3	2298.6±40.4	_	6738.5±162.6	1719.5±589.6	
Planted	4550.9±287.9	10.5 ± 0.4	6134.1±112.5	1597.5 ± 81.1	937.3±201.4	6912.8±399.7	1247.9±260.9	
	Phosphorus							
Unplanted	$1101.9{\pm}112.8$	-	4479.9±439.9	490.3±38.5	_	5049.5 ± 397.5	42.0±21.5	
Planted	1108.6 ± 73.8	2.2 ± 0.3	4404.3 ± 451.6	298.7±17.8	247.1±50.6	4912.7±524.1	56.5±32.5	

Nitrogen and phosphorus mass balance influenced by nitrogen and phosphorus treatments in constructed wetland over four months [g]

Over the four months, the average removal efficiencies of NO_2^--N , NO_3^--N , and NH_4^+-N , from the planted system were 85.4, 81.7, and 63.6%, respectively, while those from the unplanted system were 82.4, 67.4, and 47.5%, respectively. The absorption of NH_4^+-N and NO_3^--N by the planted system accounted for a higher decrease in these components. However, the removal of nitrogen from the two systems was not statistically different for some analysis periods (Fig. 2). The nitrogen and phosphorus losses from both the planted and unplanted systems were not induced by the absorption into the plants or by the sediment deposition (Table 1). These losses occurred probably because of the functioning of algae and other microorganisms. Some previous studies showed that nitrification, denitrification, the formation of organic films by microorganisms, and the absorption by plants were the main factors responsible for the nitrogen removal from the wetlands [8, 10]. Furthermore, the pH levels obtained in this study were suitable for the nitrification and denitrification processes, as described by Hoffmann et al. [20].

For the phosphorus removal, the total phosphorus concentrations did not decrease in both wetlands, which was attributed to low concentrations in the wastewater during the first phase. Within four months, the removal efficiencies of PO_4^{3-} -P in the planted and

unplanted systems were 77.5 and 73.1%, respectively. The removal of nutrients by the wetland plants depends on the concentrations of the wastewater and aquatic plants [8].

3.3. MASS BALANCE OF NITROGEN AND PHOSPHORUS IN THE CONSTRUCTED WETLAND

Table 1 shows that the total nitrogen and total phosphorus removal rates in the planted system were 64.9 and 73.1% on average respectively, which exceeded the removal rates in the unplanted system by 15.5 and 17.6%, respectively. Approximately 20.3 and 22.2% of the total nitrogen and total phosphorus, respectively, in the wastewater, were assimilated into the plant biomass after four months. Other natural losses mainly induced nitrogen removal, and the sediment storage contributed the most to the removal of phosphorus from the wastewater. The ratio of phosphorus deposited in the sediments was higher than that of nitrogen, which was consistent with the results obtained in a previous study [21]. The amounts of the removed phosphorus and the sediment storage in the unplanted and planted wetlands were similar. Other removed phosphorus amounts were lower, mainly owing to the release from the decomposition of litter and microbial biomass [21]. The settling of the total phosphorus in the sediments was mainly attributed to its removal, which also corresponded to published findings [22].

Even though the reductions in NO_2^-N and $PO_4^{3-}P$ in the planted and unplanted wetlands were similar, the removal of the total nitrogen and total phosphorus were more significant in the planted wetlands (p < 0.05). In a previous study, the removal of nitrogen by constructed wetlands was relatively low [10]. The phosphorus removal rate is also typically low but may increase using special media with a high sorption capacity [10]. Another study showed that nutrient removal in constructed wetlands is mainly carried out by microorganisms, and only a small amount of nitrogen and no more than 5% of phosphorus in wastewater are absorbed by plants [23]. The findings from these previous studies were not reproduced in this study. Harvesting has been reported to improve nitrogen and phosphorus concentrations through plants [18, 19]. Moreover, the plants can serve as media and create suitable conditions for enhancing microbial activities. In this study, both the plants and microorganisms played vital roles in the bioremediation process.

3.4. GROWTH AND NUTRIENT VALUES OF H. ACUTIGLUMA

The grass grew, and most of the grass trunks and leaves grew above the water level. The shoot height and shoot numbers for the first phase increased by 1.8 and 8.3 times, respectively, compared with those observed at the beginning of the experiment. The fresh aboveground grass biomass increased by approximately 27 and 16 times during the first and second phases, respectively. The biomass was lower in the second phase compared to the first phase, possibly because the grass bloomed in November. The dry aboveground biomass accounted for 78.6% of the grass cultivated at 25 t/ha, which was

significantly higher than the biomass cultured on natural wetlands at only 2.0–3.5 t/ha in the wet season [24]. The suitable water level and nutrient conditions were the main factors accounting for the growth of *H. acutigluma*, which was consistent with the results of a previous study [11].

Table 2

Parameters	First phase	Second phase			
	Above ground	Above ground	Roots	Rhizome	
Shoot height, m	$1.4{\pm}0.3$	$1.4{\pm}0.2$	_	-	
Number of shoots, shoots/m ²	336.1±53.1	326.6±109.8	_	_	
Biomass, kg fresh weight/m ²	8.8±1.4	6.0±1.6	1.6±0.5	5.2±1.1	
Biomass, kg dry weight/m ²	$1.6{\pm}0.4$	$0.9{\pm}0.2$	$0.1{\pm}0.0$	0.6±0.2	
Nitrogen content, % of dry weight	$1.8{\pm}0.1$	$1.6{\pm}0.2$	1.1±0.1	1.1±0.1	
Phosphorus content, % of dry weight	$0.4{\pm}0.0$	$0.4{\pm}0.0$	0.3±0.0	$0.4{\pm}0.0$	
Nitrogen accumulation, g/m ²	28.7±6.3	13.8±4.0	$0.7{\pm}0.2$	6.8±2.6	
Nitrogen accumulation, g/m ²	7.0±1.9	3.3±0.5	0.2 ± 0.1	2.6±0.8	

Biomass and accumulation of nitrogen and phosphorus in H. acutigluma

Table 3

Nutrient contents of shoots and leaves of *H. acutigluma* 4 months cultured in the artificial wetland system [%]

Phase	Crude protein	NDF	ADF	Hemicellulose	Total mineral
1	11.3±0.6	61.1±2.3	33.4±0.7	27.7±1.6	$9.4{\pm}0.8$
2	9.8±1.1	61.9±0.3	33.1±2.2	28.8±2.4	9.1±0.7

The nitrogen and phosphorus contents in the aboveground grass biomass (shoots and leaves) during the first and second phases were not statistically different and were higher than in the rhizomes and roots, while the phosphorus contents in these parts were similar (Table 2). The crude protein, total minerals, NDF, ADF, and hemicellulose in the grass tissues are required for ruminants. These contents in the two growth phases were similar (Table 3), and components, such as the NDF and ADF, were not statistically different from the components of other common herbage used for cattle diets found in a previous study [25].

4. CONCLUSION

The effluent of intensive striped catfish farming contains high amounts of nitrogen and phosphorus compounds. A batch surface wetland system was constructed to treat the wastewater using a batch culture suitable for the catfish farming method. The constructed system showed effective nutrient removal dynamics, and the planted *H. acutigluma* grass in the system not only facilitated the removal of nitrogen and phosphorus compounds but also generated biomass that can be used as food for cattle. Additionally, absorption by the planted grass, sediment accumulation, and other losses accounted for the decrease in the nitrogen and phosphorus amount in the wastewater. This study demonstrates that the batch surface-constructed wetland planted with *H. acutigluma* is a potential approach for effectively treating effluent from the intensive striped catfish ponds.

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