การเปลี่ยนแปลงของปริมาณแอมโมเนียไนโตรเจนรวมในระบบเลี้ยงปลาแบบความหนาแน่นสูงที่มีการหมุนเวียนน้ำและเชื่อมต่อกับระบบบำบัดชีวภาพแบบผสมผสานระหว่างบึงประดิษฐ์และไบโอฟิลเตอร์แบบไร้อากาศ

Total ammonia nitrogen (TAN) variation in flows of a recirculating intensive aquaculture system

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บทคัดย่อ

ระบบบำบัดชีวภาพแบบผสมผสานได้ถูกสร้างขึ้นโดยนำระบบบึงประดิษฐ์แบบไหลผ่านพื้นผิว (FWS : free water surface wetland) มาเชื่อมต่อกับระบบไบโอฟิลเตอร์แบบไร้อากาศ (SSF-NP : anaerobic biofilter with subsurface horizontal flow) เพื่อบำบัดน้ำจากบ่อเลี้ยงปลา ระบบนี้สามารถกำจัดไนเตรทได้อย่างมีประสิทธิภาพในขณะที่ประสิทธิภาพการกำจัดปริมาณแอมโมเนียรวม (TAN : total ammonia nitrogen) มีค่าต่ำ ผลการทดลองชี้ให้เห็นว่ากระบวนการ denitrification nitrate reduction to ammonia (DNRA) ในระบบ FWS และระยะเวลากักเก็บที่สั้นเกินไปของระบบ SSF-NP เป็นสาเหตุหลักที่ทำให้กำจัด TAN ได้ไม่ดี อย่างไรก็ตามระบบบำบัดชีวภาพแบบผสมผสานนี้สามารถรักษาระดับความเข้มข้นของ TAN ให้อยู่ในระดับ 0.3-2.0 มก./ล. ซึ่งถือว่ายอมรับได้สำหรับการเลี้ยงปลาดุก ประสิทธิภาพการกำจัด TAN ที่ระบบ SSF-NP ทำได้มีค่าสูงสุดเท่ากับ 25.2 % ที่ระยะเวลากักเก็บ 0.62 วัน

คำสำคัญ : ไบโอฟิลเตอร์ ปลาดุก บึงประดิษฐ์ ระบบหมุนเวียนน้ำ การกำจัดปริมาณแอมโมเนียไนโตรเจนรวม

Abstract

A biological hybrid configuration was constructed by combining a constructed free water surface wetland (FWS) to a facultative anaerobic biofilter with subsurface horizontal flow (SSF-NP) in order to treat aquaculture wastewater. It could efficiently remove nitrate while the TAN (total ammonia nitrogen) removal efficiency was low. Experimental results indicated that the denitrification nitrate reduction to ammonia (DNRA) mechanism in FWS units and the short hydraulic retention time (HRT) of SSF-NP units were the cause. However, the hybrid configuration could keep TAN concentration in the acceptable level (0.3-2.0 mg/ L) for catfish. SSF-NP units in this study could reduce TAN at the highest removal efficiency of 25.2% with 0.62 days HRT.

Keywords: biofilter, catfish, constructed wetland, recirculating system, TAN removal.

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Introduction

Total ammonia nitrogen, namely TAN is the combination of two forms, unionized ammonia (NH_3) and ammonium ion (NH_4^+). In aquatic environment of aquaculture systems, TAN always exists in two forms including unionized form of ammonia (NH_3) and ionized form of ammonium (NH_4^+). The acidity and temperature of aquatic environment will specify the content ratio of each form[1,2]. The unionized form (NH_3) is more dominant when the water is alkaline while the ionized form (NH_4^+) is predominant when the water is acidic[2]. Ammonia is toxic to fish[2] and aquatic animals[3]. High ammonia levels in water impair the excretion of ammonia from fish across its gills. This affects damages in blood pH, enzyme systems, and gills for fish. Therefore, TAN control in fishpond should be done not only to prevent phytoplankton bloom but also to diminish the unionized ammonia form which is toxic for fish. Constructed wetland technology has collected public attention in the field of wastewater treatment since the early 1970s[4,5]. Previous studies showed that constructed wetland could reduce TAN concentration in treated wastewater[6] but the removal of ammonia in a wetland depended on the configuration of the wetland and the availability of dissolved oxygen[7].

Usually, the constructed wetland includes 2 types, Free Water Surface (FWS) and Subsurface Flow (SSF)[8,9,10]. However, a hybrid wetland system could be constructed based on combining water flow regimes and types of plants in order to exploit specific advantages of the different systems[9,10]. FWS units consist of shallow basins partially filled with soil, peat or any other media that will support plant roots. FWS generally have a soil bottom, emergent vegetation, and a water surface above the substrate. Water moves slowly through the wetland above the substrate. The near-surface layer of water is aerobic while the deeper waters and the substrate are usually anaerobic[8]. In terms of SSFs, the water level is designed to be kept below the wet land surface. In contrast to the FWSs, coarse sand contributes to the treatment processes by providing a surface area for microbial growth and supporting adsorption and filtration processes. This effect results in a lower area demand and generally higher treatment performance per area than FWS wetland[10]. Tran[5] has found that in anaerobic or facultative anaerobic condition, SSF constructed wetland units and biofilter units had similar nitrogenous removal mechanisms including denitrification and anammox. Furthermore, biofilter units could be constructed using many kinds of various size media to increase microbial adhering surface area. Consequently, hydraulic retention time (HRT) required for biofilter units was shorter than that of SSF units. By these fundamental advantages, a new model of biological hybrid configuration consisting of a constructed free water surface wetland (FWS) and a facultative anaerobic biofilter with subsurface horizontal flow (SSF-NP) was built by Tran and his co-researchers[5]. Using shells and construction lime-stones as the biofilter media, the results demonstrated that this hybrid configuration could reduce certain levels of turbidity (75.2%), Chemical Oxygen Demand (COD 48%), and nitrate (88.2%) in experimental recirculation aquaculture systems. In this study, TAN removal ability of Tran's hybrid configuration was focused. TAN variation in 3 recirculating intensive aquaculture systems was discussed due to 3 different HRTs. Based on the result, suggested HRT and other conditions for TAN control in aquaculture system was proposed.

Materials and Methods

Recirculating aquaculture systems

The experimental recirculating aquaculture systems were designed as shown in Figure 1. Their sizes are described in Table 1. Three fish ponds were dug and laid with plastic sheets. Water and catfish (*Clarias sp*) were released into each pond with the density of 47.2 fishes/ m^3. An aeration compressor was installed in each pond. Water in the ponds was continuously pumped by controlled pumps to the FWS units and consequently passed through the SSF-NP units which were separated into 2 tanks.

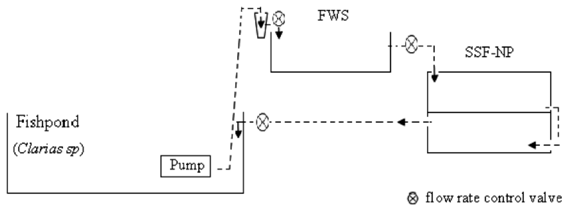


Figure 1 Recirculating aquaculture system layout

Table 1 Designed sizes of fish ponds and treatment

Designed sizes	Fish ponds	Treatment units	
		FWS	SSF-NP
Length (L) m	4	1.5	1.5
$Width_{min}$ (W_{min}) m	1.0	0.8	0.85
$Width_{max}$ (W_{max}) m	1.5	0.8	0.85
Depth (d) m	1.0	0.5	0.9
Nominal porosity (ε)	1	0.864	0.478
Area (A=LW) m^2	6	1.2	1.275

Table 2 Designed parameters for recirculating treatment systems operation

No.	Water recirculation (%/day)	Flow rate (m^3/day)	HRT (day)	
			FWS	SSF-NP
1	20	0.72	0.39	0.62
2	40	1.44	0.19	0.31
3	60	2.16	0.13	0.21

The treated effluent of these biological hybrid configurations was then discharged back to the fishponds by gravity. The flow rate of each recirculating aquaculture system was controlled by gate valves to ensure designed requirements as shown in Table 2. Each FWS unit was filled with 0.28 m^3 of water. The thickness of the soil layer was 0.2 m with a silty clay loam to sand ratio of 3:1[11]. The type of plant in FWS was water hyacinth with the density of 15 plants/ m^2 [12, 13]. Each SSW-NP unit was filled with lime stones and shells in a ratio of 3:1. The top of the SSF-NP units was covered by polyethylene (PE) plastic sheets to produce an anaerobic condition.

The culture time and hydraulic conditions

The fish cultivation started on June 15th 2011 and was harvested on September 20th. During this study period, no water discharge or displacement was done in the three fishponds except in cases of water replacement, which was occasionally done when water was lost from evaporation, and an unexpected problem that occurred. At the end of the experiment, fishes were sampled and measured for their weights. Table 2 illustrates the hydraulic conditions for the recirculating systems.

Sampling and analysis

Water samples from effluents at the edge of the fish ponds were collected as the influents of FWS units, namely P_1 for system no.1, P_2 for system no.2, and P_3 for system no.3. FWS and SSF-NP units in system no.1, 2, and 3 were defined as FWS_1, SSF-NP_1, FWS_2, SSF-NP_2, FWS_3, SSF-NP_3, respectively. Effluents of FWS_1, FWS_2, FWS_3 which were the influent of SSF-NP_1, SSF-NP_2, SSF-NP_3 were collected as W_1, W_2, and W_3. Similarly, effluents of SSF-NP_1, SSF-NP_2, SSF-NP_3 were collected as B_1, B_2, and B_3 samples, respectively. All samples were collected around 10 a.m. each time. The sampling was scheduled for three times a week for pH, temperature, dissolved oxygen (DO); and once a week for chemical oxygen demand (COD), nitrate (NO_3-N), total ammonia nitrogen (TAN), and orthophosphate (PO_4-P). Temperature, pH, and DO of the water in the sampling locations were measured by hand-held equipment, a DO and temperature incorporated meter (YSI 5000,USA), and a pH meter (Mettler Toledo AG, Switzerland). COD, NO_3-N, TAN, and PO_4-P were measured using methods described in Water and Wastewater Analysis[14].

Tests for significant differences in water quality between influents and effluents of the treatment units were determined by t-test: two samples, paired t-tests and F-tests at a significant level of 0.05 for each set of data.

Results and discussions

Water quality in fishponds

The water quality factors in fishponds are not only directly affecting to fish living and growth but also acting on fluctuation and transformation processes of other factors in aquatic environment. The water quality (mean $\overline{X}$ and standard deviation ε) in the three experimental fishponds are described as shown in Table 3. The results showed that average values of pH and temperature were in the recommended range (6.5-9.0 of pH, and 26-32^{0}C of temperature) for the catfish[2]. Although, nitrate is relatively non-toxic to aquatic organisms[15] and none of nitrate limitation force to catfish, however the average value of nitrate is below 50 mg/L which is a safe nitrate level in fresh water for common fishponds[16]. The suitable range of TAN for catfish is not excess 0.3-2 mg/ L according to the recommendation of USAID[2]. COD was higher than 60 mg/ L of the Vietnamese national standard for wastewater in aquaculture[17]. DO concentrations in fishponds were less than 3 mg/ L which were lower than the value recommended by FAO[18], 3 mg/ L for *Clarias gariepinus*. USAID[2] supported that DO should be from 4 mg/ L to saturation for catfish eggs, larvae, fry and fingerlings. However, *Clarias gariepinus* can live at DO level of 2 mg/ L [19], and hybrid catfish can even withstand DO level from 1 to 2 mg/L[20]. The main reason for this low DO value should be the extremely high density of catfish in the experimental ponds (47.2 fishes/ m^3). The excess of COD value against the standard is clear evidence.

Table 3 Water quality in fishponds

		pH	DO (mg/L)	Temp (0C)	COD (mg/L)	N-NO_3 (mg/L)	P-PO_4 (mg/L)	TAN (mg/L)
P1		7.20	2.12	27.79	74	15.33	1.27	0.16
	$\overline{X}$	0.22	0.40	0.88	26.17	6.30	0.95	0.21
P2		7.27	2.18	28.07	68.5	15.50	1.49	0.13
	$\overline{X}$	0.20	0.54	0.98	23.09	6.42	0.87	0.15
P3		7.28	1.92	28.17	65.75	12.57	1.33	0.15
	$\overline{X}$	0.23	0.62	1.20	20.86	4.8	0.85	0.18

Due to a survival ratio of greater than 95% and normal fish growth observed at the end of the experiment, DO as well as the other parameters were considered acceptable for this experimental hybrid catfish. According to t-test results, no significant difference was detected between P_1, P_2, and P_3 for all measured parameters.

TAN variation in fishponds

The results in Figure 2 showed that TAN content tendentiously rose at the first period time of experiment, and then remained in a steady range until systems met leakage and overflow problems on August 21st, 2011. After the problems were solved, 70% of water in all fishponds was changed to ensure the same condition. From August 22nd, 2011 to the end of the experiment, TAN stayed at low concentration. Water refilling was slightly done during the time since evaporation and leakage occurred at all fishponds.

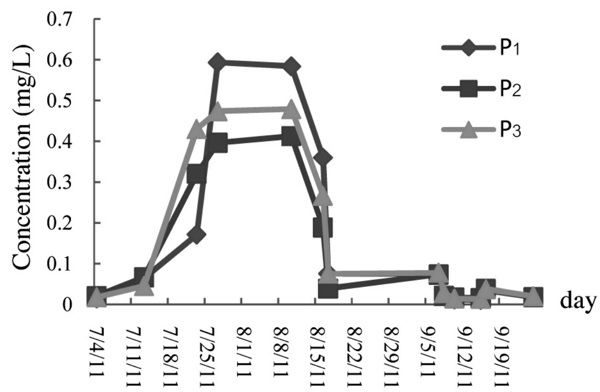
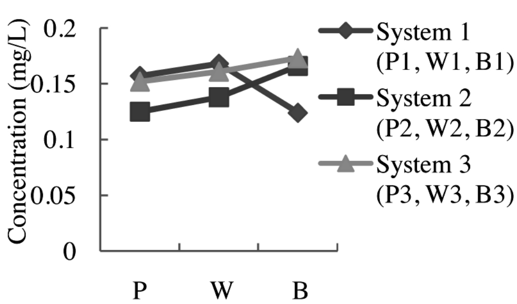


Figure 2 TAN variations in fishponds

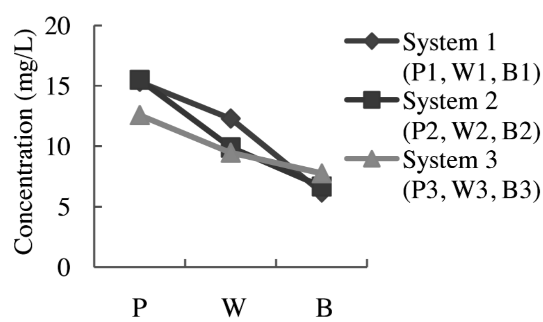
In fishponds, ammonia is the by-product from protein metabolism excreted by fish and bacterial decomposition of organic matters. All nitrogen forms including nitrogen gas (N_2) are biochemically interconvertible and are components of the nitrogen cycle[7]. There are 3 stages in a normal biological nitrogen cycle. Mineralization is the first step in the biochemical decomposition and the end products are ammonia (NH_3) and organic acids. Nitrification is a second stage which the ammonia (NH_3) and ammonium (NH_4^+) molecules are oxidized into nitrite (NO_2^-) and nitrate (NO_3^-), respectively. Denitrification is the stage which the end products are dinitrogen oxide (N_2O) and nitrogen (N_2) gases. These two gases escape through the water surface. Similarly, in this study, TAN in fishponds were directly produced from fish excreta and first step of the biochemical organic matters decomposition process. Usually, TAN in fishponds increases along with the growth of fish because of excreta, residue and redundant food accumulation. TAN reduction occurs through natural mechanisms themselves (nitrification) with the presence of oxygen, and attached treatment systems. Although the TAN increased at the first period, the maximum value did not reach the standard value for catfish 0.3-2 mg/ L[2]. This fluctuation proclaimed lower treatment ability of the systems at starting up period that corresponding to the result of Lin et al.[4]. Low DO and high COD in fishponds also considered as the reasons inhibiting nitrification which leaded to the restriction of TAN removal. EPA[7] reported that nitrification required 4.3 mg/L O_2 per mg N oxidized. Consequently, increasing the DO or lessening the fish density is the solution to reduce TAN content in these fishponds.

TAN variations after pass through FWS units

Water from fishponds was drained to FWS treatment units. TAN concentrations in the effluents of FWS units were tendentiously higher than the influents (Figure 3.a, P and W). However this tendency was not significant according to t-Test: Paired Two Sample for Means (p=0.05). The TAN increasing tendency was considered as a result of TAN producing and losing processes that occurred in FWS units. Ammonification produced ammonia concomitant with organic acid as the end products in mineralization stage. This resulted in pH decreasing tendency from P to W units as shown in Figure 3.c. Dissimilation nitrate reduction to ammonia (DNRA) is the process which causes nitrate decreasing and ammonia increasing in the condition of low DO and high organic carbon15. Values of DO and COD in Table 3 and fluctuation tendency of ammonia and nitrate in Figure 3.a, b were the proof of the DNRA process in FWS units. Low DO also encouraged phosphorus decomposition process that increased orthophosphate concentration as shown in Figure 3.f. TAN could be reduced by the processes of nitrification, assimilation, plants uptake, and anammox. However, these mechanisms were not supported because of the increasing of TAN in FWS units as shown in Figure 3.a, W. Significant nitrate reduction in FWS units (Figure 3.b) could be performed by denitrification, assimilation, plants uptake and DNRA processes. However, assimilation and plants uptake processes needs the absence of reduced inorganic nitrogen species such as ammonia. Further, denitrification would concomitantly raise pH and synthesize polyphosphate15 that contrasted to the inhibition of Figure 3.c, f. Therefore, DNRA should be the main process in this study that reduced nitrate which concomitantly increased ammonia. Besides, TAN reduction in this study did not result in the increasing of nitrate concentration. Consequently, FWS treatment units used in this study could not reduce TAN. The nitrate removal was considered as an indirect factor which caused TAN increasing. To eliminate TAN in these units, DO have to be raised and the fish density should be reduced



(a) TAN variations



(b) Nitrate variations

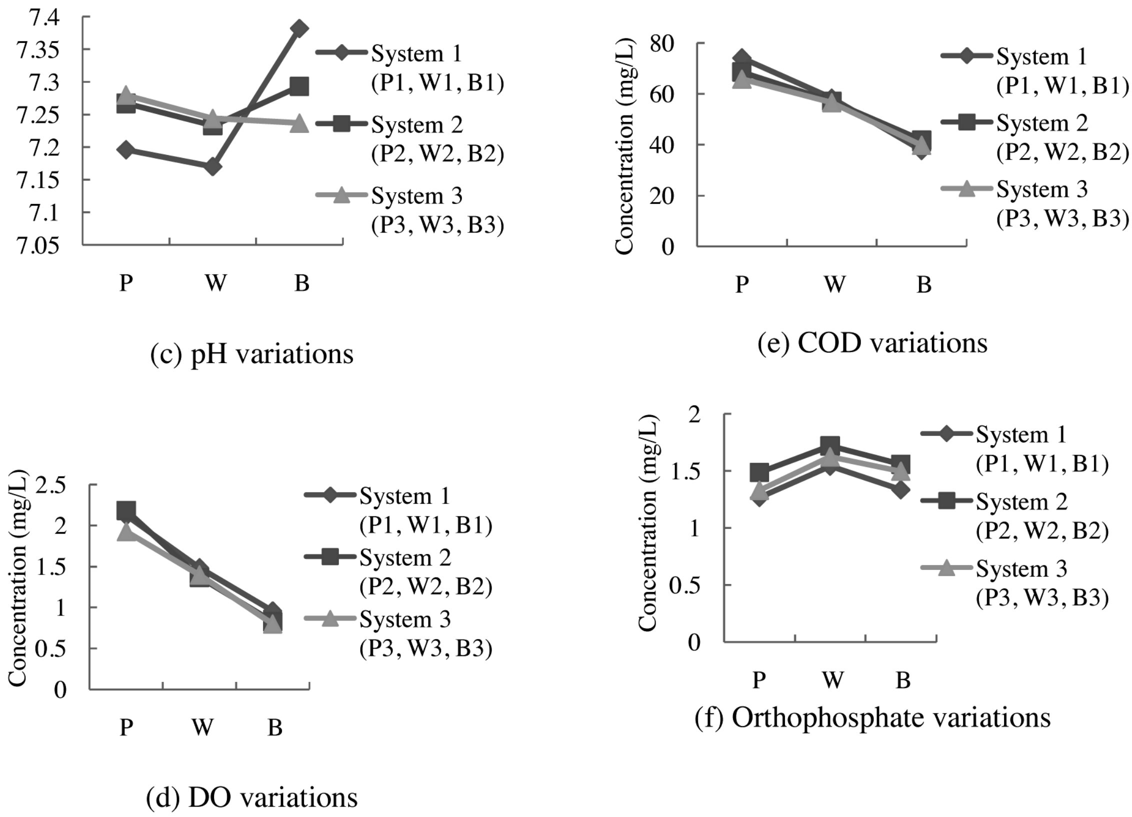


Figure 3 Water quality variations after passing through treatment units

TAN variation after pass through SSF-NP units

Subsurface flow wetlands without plant treatment (SSF-NP) units were designed to promote denitrification and anammox processes. Lime-stones and shells in SSF-NP were aimed to function as the pH buffer. In SSF-NP treatment units, denitrification directly reduced nitrate in recirculating water [5] as shown in Figure 3.b. Anammox which enables complete ammonia removal was considered to occur in SSF-NP treatment units via autotrophic pathways without the requirement of organic carbon. Ammonia and nitrite were combined together to produce nitrogen gas as shown in following equations[15, 21].

Partial nitrification:

$$2NH_4^+ + 1.5O_2 \rightarrow NH_4^+ + NO_2^- + H_2O + 2H^+$$

Anammox:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

Total: $$2\,NH_4^+ + 1.5O_2 \rightarrow N_2 + 3H_2O + 2H^+$$

However, only SSF-NP_1 could remove TAN at the confidence level of 92% ($p \leq 0.08$). Increasing tendency of TAN was expressed at SSF-NP_2 and SSF-NP_3 treatment units. This increasing trend might originate from DNRA. Further, Lin et al.[4] reported that subsurface water constructed wetland could efficiently remove TAN after start-up period with the HTR values from 0.6 to 4.4 days which were longer than this experiment with HTR values from 0.21 to 0.62 days. Consequently, DNRA and HRT were thought as the main factors for low TAN removal ability in the 2 treatment units.

TAN removal efficiency of treatment systems

TAN removal efficiency of treatment units (E_u) and the systems (E_s) could be calculated following *Eq.*1 and *Eq.*2 and the results were describing as shown in Table 4.

$$E_u(\%) = \frac{(C_i - C_e)}{C_i} * 100 \qquad Eq.1$$

$$E_s(\%) = [(E_{FWS} + E_{SSF-NP}) - (E_{FWS} * E_{SSF-NP})] * 100 \qquad Eq.2$$

Where

C_i = TAN concentration at influent point

C_e = TAN concentration at effluent point

E_{FWS} = TAN removal efficiency of FWS treatment unit

E_{SSF-NP} = TAN removal efficiency of SSF-NP treatment unit

Table 4 TAN removal efficie

No.	FWS unit's efficiency (%)	SSF-NP unit's efficiency (%)	Total efficiency (%)
1	ND	25.2	8.37
2	ND	ND	ND
3	ND	14.8	0.32

ND : No determination

According to the result, TAN removal efficiencies in this experiment were extremely low comparing to the results of Lin et al.[22] which reached 64%-66%. However, TAN concentration in the fishponds was at a low level (Table 3) which is far from the standard value for catfish as described in section 3.1. Significant nitrate reduction of these systems through the paths of DNRA as well as dissimilatory denitrification[5] was supposed to cause these low TAN concentration and low TAN removal efficiency. FWS units in this experiment could not remove TAN because of DNRA process which happened at low average DO and high COD values (Figure 3.d, e). They were different from FWS units in the model of Lin et al.[22] which could remove TAN based on nitrification process at high levels of DO (6.9-10 mg/ L). Therefore, the encouragement of the nitrification process by increasing DO and the prevention of DNRA process by reducing organic matters should enhance the TAN removal ability of the units.

FWS unit was designed with highest HRT but lowest TAN removal efficiency was derived. The appearance of freshwater crabs (unexpected problem) destroyed water hyacinths in FWS_1 unit. As the result, lowest TAN removal efficiency was observed in FWS_1. This suggested that the amount of water hyacinth played an important role in TAN removal of the FWS units.

Conclusion

Treatment units used in this study could not accomplish high TAN removal, but they could keep TAN concentration in acceptable level for catfish. TAN increasing was found in FWS units due to the DNRA process. To inhibit this process, DO in fishponds have to be intensified and organic matters should be reduced from the unit- influent. Draining wastewater into SSW-NP units before delivering to the FWS units is the possible solution. SSF-NP treatment units could reduce TAN but the efficiency was low. In this experiment, the SSF-NP unit with HRT of 0.62 day should be chosen for the TAN removal. However, longer HRT in SSF-NP units should bring to higher TAN reduction. Further study to determine relationship between HRT and TAN removal efficiency is necessary.

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