

52 (2014) 744–752 January



# Nitrogen budget and effluent nitrogen components in aquaponics recirculation system

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Received 21 January 2013; Accepted 28 February 2013

## ABSTRACT

In this study, the dynamics of nitrogen through aquaponics recirculation system was examined by developing a nitrogen budget. The model evaluated total ammonia nitrogen (TAN) production and removal in biofilters, identifying and quantifying the fate of nitrate nitrogen ( $NO_3^-$ -N) and determining the system maximum carrying capacity. Of the nitrogen input into the culture tank via feed, 83.8% was recovered from different pool: 39.4% as fish flesh (harvested), 2.1% as mortalities, 34.7% as dissolved inorganic forms of nitrogen and 7.6% as total organic nitrogen. The remaining 16.2% of nitrogen unaccounted for likely was lost as nitrogen gas due to passive denitrification and as volatization of ammonia. Average TAN in the culture tanks was 2.08 mg/L. Under current condition, system loading with fish biomass at average of 68.5% of the maximum predicted. The hydroponic troughs removal efficiency averaged 60.4% TAN per pass. From TAN production, 88% was removed in hydroponic troughs, 11% by passive nitrification and 1% by water exchange. Under conditions of reusing treated effluent with residual TAN, the hydroponic troughs work normally, while TAN in the systems did not increase noticeably.

*Keywords:* Aquaponics recirculation system; Mass balance; Nitrogen budget; Passive denitrification; Passive nitrification

# 1. Introduction

The intensive development of the aquaculture industry has been accompanied by an increase in environmental impacts. The production process generates substantial amounts of polluted effluent, containing uneaten feed and feces [1]. Discharges from aquaculture into the aquatic environment contain nutrients, various organic and inorganic compounds, such as ammonium, phosphorus, dissolved organic carbon and organic matter [2,3]. The nutrient discharge from a fish farm (nutrient load) can be described by a mass balance equation, in its most simple form as the difference between feed supply and fish utilization [4]. An important principle of intensive aquaculture is to provide large quantities of high-nutrient feed to cultured animals. However, only

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Presented at the Fifth Annual International Conference on "Challenges in Environmental Science & Engineering—CESE 2012" Melbourne, Australia, 9–13 September 2012

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30% of nitrogen added through feed is removed through fish harvest in an intensive fish farming [5]. The remaining amount of the dissolved nitrogen which is released to the surrounding environment depends on the species, culture systems, feed quality, and feeding management [6]. Nitrogen is associated with protein, the most expensive component of feeds, and feeds constitute over half of the variable costs of production. Hence, the performance and efficiency of an aquaculture system can be evaluated through analysis of the conversion of nitrogen to fish biomass [7]. During biological degradation, the organic nitrogen is transformed to ammonia. In operating a recirculating fish culture system, care must be taken to prevent ammonia concentration from reaching toxic levels within the culture tanks.

Therefore, it is important to estimate the total ammonia loading on the water treatment system to ensure that ammonia removal units and water exchange rates will remove ammonia at a rate such that concentration design goals are met. By estimating total nitrogen budgets for the particular species cultured and culture conditions, it should be possible to determine the amount and nature of the dissolved nutrient load both within the aquaculture facility and downstream, to institute appropriate treatment action, and to prevent or at least mitigate the effects of pollutants downstream. A nitrogen budget is also necessary to determine how fish at various stocking densities utilize nitrogen [8,9], to identify and to quantify the major processes affecting water quality, and to understand the role of each nitrogenous compound, such as the amount and nature of the nutrient release into the water column by the dissolved and particulate excretion of pellet-fed fish [10].

This study investigates a nitrogen budget for production system and estimating of system carrying capacity with respect to total ammonia nitrogen (TAN) by fish cultured with water spinach in aquaponics system. These evaluations are important to provide crucial information for the design and the optimization of recirculation, feeding strategies, and water and effluent treatment technologies.

The objectives of this study were to quantify a nitrogen flow and nitrogen budget by using mass balance equation and to evaluate ammonia production, loading, and removal efficiency of hydroponic bed as well as to determine the aquaponics recirculation system (ARS) maximum carrying capacity when African catfish are integrated with water spinach in a closed ARS. Studies on integrated multi-trophic aquaculture systems have been reported in the culture of other species, such as rainbow trout [11], barramundi [12], tilapia [13,14], and shrimp [15]; however, no study has

been reported on nitrogen budget on integrated of African catfish and water spinach in ARS.

#### 2. Materials and methods

# 2.1. ARS and culture conditions

The experiment was carried out in a greenhouse to provide uniform conditions throughout the growth phase. Three experimental units, each consists of three fiber glass rearing tanks, three hydroponics troughs, sump, and water holding tank were used to conduct the study. Pipelines made of polyvinyl chloride were installed to connect each component in the system for the purpose of water recirculation. Water level in each culture tank was kept at 0.80 m deep to maintain the water volume at 3,000 L. Water lost through evaporation, transpiration, and sludge removal was replenished with water in the pre-aeration tank. The schematic experimental setup is shown in Fig. 1.

Water drained out and flowed from the culture tank was sprinkled over the vegetables in the hydroponics trough and outflow trickled down to the sump for denitrification process. The components were installed such that the water flowed by gravity, by placing components at appropriate elevation relative to one another. The water was then pumped vertically to water storage tank and was continuously flowed under gravitational force to fish culture tank through the water spreader bar.

Samples of African catfish (*Clarias gariepinus*) fingerlings with an initial body weight in the range of 30–40 g were obtained from a local catfish producer. The fish were hand fed with a commercial floating pellet manually in the range of 2–4% of fish body weight/day; feeding occurred twice a day between 08.30 and 18.00 h. Feeding rates began with 4.0% body weight/day and gradually decreased to 2% body



Fig. 1. Top view of layout of ARS. (A): Culture tank, (B): hydroponic trough (planted bed), (C): hydroponic trough (control bed), (D): filter, (E): sprinkler, (F): sump, (G): submersible pump, (H): rapid sand filter, (I): water storage tank, (J): air blower, and (K): valves.

weight/day towards the end of experiment. With this regime, fish were expected to reach a market size of 220–250 g in eleven weeks. Feed rates were adjusted weekly based on an estimated growth rate. No water discharge or displacement took place except for replacing water lost through evaporation, transpiration, and sludge removal of less than 5%.

#### 2.1. Inputs, outputs, and nitrogen pools

ARS has a single, measurable flow stream that provides the water input for all subsystems. It originates from pre-aerated water reservoir. No measurable amounts of dissolved inorganic nitrogen (i.e. TAN, nitrite–N, and nitrate–N) were identified in the replacement water. Hence, the feed provided to the fish was the sole nitrogen source for each subsystem in the form of organic nitrogen [N<sub>feed</sub>, (gkg<sup>-1</sup> of feed)], which was calculated as in Eq. (1):

$$N_{feed} = \sum (FA \times PC \times 0.16) \tag{1}$$

where FA = amount of feed (kg); PC = protein content of feed (decimal fraction).

The multiplication of  $N_{feed}$  by the total amount of feed provided the mass of total nitrogen input (TNI). Two different types of feed with 28 and 32% protein content were used for determination of  $N_{feed}$ . The removal of nitrogen was accounted for in a variety of known pools as follows:

- Nitrogen fixed in fish biomass as organic nitrogen [N<sub>fish</sub>, (g kg<sup>-1</sup> fish produced)];
- Nitrogen fixed in dead fish biomass as organic nitrogen [N<sub>mort</sub>, (g kg<sup>-1</sup> fish removed)];
- (3) Dissolved inorganic nitrogen [N<sub>DIN</sub>, (g L<sup>-1</sup>)], including TAN, NO<sub>2</sub>, and NO<sub>3</sub>;
- (4) Total organic nitrogen in the effluent [N<sub>TON</sub>, (g L<sup>-1</sup>)];
- (5) Nitrogen gas removed from the system by passive denitrification  $[N_{denit}, (gL^{-1})]$  and by ammonia volatilization  $[N_{NH3 \ vol}, (gL^{-1})]$ .

The initial forms of output nitrogen undergo partial physical, chemical, and biochemical transformation through the nitrogen cycle, moving within and among the pools. Processes affecting  $N_{TON}$  pool included solubilization of organic fecal components in water, assimilation of ammonia into bacterial cells as  $N_{TON}$ , ammonia release following the bacterial lyses and decay, and uptake of nitrogenous species by phytoplankton. Transformation affecting  $N_{DIN}$  included nitrification of ammonia in biofilters, and

loss of nitrogen due to dissimilatory nitrate reduction, passive denitrification, and volatilization of ammonia. Nitrogen uptake by phytoplankton also affects N<sub>DIN</sub>. Dynamics of these processes depend on numerous factors, such as system design, mode of operation, management strategy, size and biomass of fish, type and ration of feed, and exchange rate of water. The large number of variables makes it impossible to identify the magnitude of an individual transformation throughout a subsystem. For this reason, all transformations were assumed to be in a dynamic equilibrium over a definite period of time, which allowed determination of the forms of nitrogen for each pool. Consequently, the mass balances presented the status quo of each pool under steady-state conditions for the case of production system. The mass fractions of nitrogen from the Pools 1 to 4 (i.e. measurable pools) were accounted for as the total nitrogen recovered (TNR), while the difference between TNI and TNR constituted the mass fraction of total nitrogen unaccounted for (TNUA, Pool 5).

#### 2.2. Water sampling and analyses

Water samples were taken from each culture tank, influent, and effluent of the hydroponics and inflow of culture tank, once a week for chemical analyses. The TAN, NO<sub>2</sub>-N, and NO<sub>3</sub>-N measurements were analyzed by using HACH DR4000 spectrophotometer according to Nessler, diazotization, and cadmium reduction methods, respectively. Total kjeldahl nitrogen (TKN) was determined using macro Kjeldahl [16]. Analyses of fish and feed for protein content were carried out according to Thiex et al. [17], who indicated that by dry weight, 16% of protein is nitrogen. Dissolved oxygen, pH, and temperature in the sampling locations were also monitored.

#### 2.3. Estimating of nitrogen budget

To estimate fish utilization of nitrogen ( $N_{\rm fish}$ ), samples of muscle tissue from fish from three size classes were analyzed for protein content (in triplicate). The proportions of fish in each size class were estimated as 10% juveniles (i.e. newly introduced to the system), 60% intermediate, and 30% marketable size. Data on protein content of each fish size class allowed determination of  $N_{\rm fish}$  as a composite, using the Eq. (2) as follows:

$$N_{fish} = \sum (FB \times FP \times 0.16) \tag{2}$$

where FB = biomass of fish (kg); FP = protein content of the fish (decimal fraction).

About 2.1% of the fish production (by number) was lost as mortalities. N<sub>mort</sub> was assumed to have the same nitrogen content as N<sub>fish</sub>. In order to determine the weight biomass of N<sub>mort</sub>, dead fish was collected daily from the culture tank. These data were used to determine N<sub>mort</sub> using Eq. (2). All nitrogen from the N<sub>feed</sub> which was not accounted for as N<sub>fish</sub> or N<sub>mort</sub> was quantified as nitrogen load to the water, which was entered the water column in dissolved form (N<sub>DIN</sub> pool) as ammonia, and as organic nitrogen bound in feces (N<sub>TON</sub> pool). N<sub>TON</sub> was determined as the difference between TKN and TAN from the effluent. All N<sub>feed</sub> that was not recovered as N<sub>fish</sub> N<sub>mort</sub> or N<sub>TON</sub> represented the dissolved inorganic fraction that entered the water as TAN. Hence, it was possible to determine ammonia production (P<sub>TAN</sub>,  $gNkg^{-1}$  feed) using the equation:

$$P_{TAN} = N_{feed} - (N_{fish} + N_{mort} + N_{TON})/FA$$
(3)

The sum of TAN, NO<sub>2</sub>–N, and NO<sub>3</sub>–N found in the effluent represented the recovered fraction of N<sub>DIN</sub>. The summation of this fraction, N<sub>fish</sub>, N<sub>mort</sub>, and N<sub>TON</sub> provided the value for total nitrogen recovered (TNR). Expressed as a percentage of TNI (considered 100%), % TNR was determined by using the equation:

$$\% \text{TNR} = \% \text{ N}_{\text{fish}} + \% \text{ N}_{\text{mort}} + \% \text{ N}_{\text{DIN}} + \% \text{ N}_{\text{TON}}$$
(4)

The TNUA then was determined using the equation:

$$\% \text{TNUA} = 100 - \% \text{TNR}$$
 (5)

The subsequent nitrogen mass balance was:

$$TNI = TNR + TNUA \tag{6}$$

Alternatively, the mass balance was determined based on the mass fraction nitrogen composition of all measured budget elements relative to the feed input to the system:

$$N_{\text{feed}} = \% N_{\text{fish}} + \% N_{\text{mort}} + \% N_{\text{DIN}} + \% N_{\text{TON}} + \% N_{\text{denit}} + \% N_{\text{NH3}} \text{voln}$$
(7)

# 2.4. Estimating system carrying capacity with respect to TAN

To determine the maximum system carrying capacity of ARS from the production subsystem, a

simplified version of a model with respect to TAN was used as proposed by Losordo and Timmons [18]. All three units of ARS were chosen for tests. Total fish biomass, fish size, feeding rate, type of feed (crude protein content), daily percent body weight fed, flow rate through the system, and daily rate of exchange were known for each unit.

Determination of the maximum system carrying capacity with respect to TAN was determined as follows:

Calculation of the maximum allowable TAN concentration  $(A_{TAN}, gm^{-3})$ :

$$A_{\rm TAN} = A_{\rm NH3-N}/a \tag{8}$$

where  $A_{NH3-N}$  = concentration of unionized ammonia nitrogen (g m<sup>-3</sup>); *a* = mole fraction of unionized ammonia nitrogen (decimal fraction).

The value of *a* was selected from the mole fraction of unionized ammonia nitrogen based on the pH and temperature values during the experiments. The maximum allowable unionized ammonia  $(A_{NH3-N})$  value is assumed 0.104 g m<sup>-3</sup> [19].

Maximum feed rate (FR<sub>max</sub>TAN, kg feed  $d^{-1}$ ) was calculated based on the assumption that the TAN concentration of a fish tank equals A<sub>TAN</sub>, using the Eq. (9) as follows:

$$FR_{max} TAN = [A_{TAN} \times Q_r \times Ea +Q(C_{TAN} - C_{TANi})]/(0.092 \times PC)$$
(9)

where  $Q_r$  = recirculating flow rate, or flow rate to the hydroponic troughs (L h<sup>-1</sup>); Q = flow rate through system (L h<sup>-1</sup>); Ea = hydroponic trough removal efficiency (%); C<sub>TAN</sub> = TAN concentration of fish tank (g m<sup>-3</sup>); C<sub>TANi</sub> = TAN concentration of new water (g m<sup>-3</sup>); 0.092 = model constant coefficient; PC = protein content of feed (decimal fraction).

The maximum biomass that could be sustained within the system (SBM<sub>max</sub>TAN, kg fish) was determined using Eq. (10):

$$SBM_{max}TAN = FR_{max}TAN / \%BW$$
(10)

where % BW = time unit rate of fish feeding, expressed as a percent of body weight.

Ea was determined using Eq. (11):

$$Ea = [(C_{TAN} - C_{TANe})/C_{TAN}] \times 100$$
(11)

where  $C_{TANe}$  = TAN concentration in the effluent from the hydroponic trough (g m<sup>-3</sup>).

The production rate of TAN ( $P_{TAN}$ ,  $gm^{-3}$ ) refers to the rate of production of TAN in the system as a result of the metabolism of the fish and the microbial degradation of uneaten feed.  $P_{TAN}$  was estimated as a function of the feed rate and the percentage of protein in feed such that:

$$P_{\text{TAN}} = (\text{FA} \times \text{PC} \times 0.102)/t \tag{12}$$

where FA = amount fed (kg); PC = protein content of the feed (decimal fraction); t = period of time from the onset of feeding to the next feeding (h).

The equation is based on the following assumptions:

- (a) 16% of feed protein is nitrogen,
- (b) 80% of the nitrogen is assimilated,
- (c) unassimilated nitrogen in fecal matter is removed rapidly from the tank,
- (d) 80% of assimilated nitrogen is excreted, and
- (e) all of the TAN is excreted during *t* hours.

The numeric coefficient 0.102 represents the product of values suggested by assumptions (a) through (d) (as decimal fractions) in the estimation of the TAN produced from the metabolic activity of fish (i.e.  $0.16 \times 0.8 \times 0.8 = 0.102$ ).

The mass flow rate of TAN to trough, or ammonia loading ( $L_{TAN}$ , g m<sup>-3</sup>) was determined from known data ( $Q_r$ ) and experimentally determined ( $C_{TANf}$ ) using Eq. (13):

$$L_{TAN} = Q_r \times C_{TANf} \tag{13}$$

where  $C_{TANf}$  = TAN concentration of trough influent.

The ammonia removal rate  $(R_{TAN}, gh^{-1})$  was determined using Eq. (14):

$$\mathbf{R}_{\mathrm{TAN}} = (\mathbf{C}_{\mathrm{TANf}} - \mathbf{C}_{\mathrm{TANe}}) \times \mathbf{Q}_r \tag{14}$$

Finally, the mass balance expressing the partitioning of  $P_{TAN}$  removal was expressed using the equation:

$$P_{TAN} = TAN_{pass+vol} + TAN_{hydronitri} + TAN_{exchange}.$$
 (15)

where  $TAN_{pass+vol} = TAN$  removed by passive nitrification into system and ammonia volatilization (g m<sup>-3</sup>),  $TAN_{hydro nitri} = TAN$  removed by nitrification into hydroponic trough (g m<sup>-3</sup>),  $TAN_{exchange} = TAN$  removed with the exchange water (g m<sup>-3</sup>).

 $TAN_{nitrification}$  and  $TAN_{exchange}$  were determined experimentally, and  $TAN_{pass+vol}$  were determined by subtracting the summation of the other two values from  $P_{TAN}$ .

## 3. Results

#### 3.1. Nitrogen budget for production system

The daily nitrogen budget derived over each unit based on the mass fraction nitrogen composition of all measured budget elements is shown in Table 1.

For the production of 1,120 kg of fish biomass, ARS administers 940 kg of feeds. These amounts correspond to 12.21 kg feed consumed d<sup>-1</sup> and 14.54 kg fish gain d<sup>-1</sup>. Of the feed utilized, 80% (752 kg) and 20% (188 kg) were nominally 28 and 32% protein content, respectively. The estimated percentages of feed types and the laboratory-determined protein concentrations were used in Eq. (1) for determining  $N_{feed} = 46.08 \text{ g N kg}^{-1}$  feed. By extrapolating  $N_{feed}$  to daily feed input, a TNI = 562.64 g N day<sup>-1</sup> was determined.

Table 1 Daily nitrogen budget derived for systems based on the mass fraction nitrogen composition of all measured budget elements

N pool (units)	Units				
	1	2	3	Average	
TNI (g)	$558.24 \pm 10.22$	$563.30 \pm 8.24$	$566.40 \pm 8.82$	$562.65 \pm 9.24$	
N <sub>fish</sub> (g)	$233.12 \pm 4.22$	$233.95 \pm 4.26$	$234.15 \pm 4.20$	$233.74 \pm 4.18$	
N <sub>mort</sub> (g)	$4.88 \pm 0.24$	$4.93 \pm 0.18$	$4.96 \pm 0.16$	$4.92\pm0.20$	
N <sub>TAN</sub> (g)	$9.28 \pm 0.78$	$9.34 \pm 0.62$	$9.46 \pm 0.65$	$9.36\pm0.58$	
N <sub>NO2</sub> (g)	$3.18 \pm 0.18$	$3.24 \pm 0.16$	$3.30 \pm 0.18$	$3.24\pm0.15$	
N <sub>NO3</sub> (g)	$181.24 \pm 2.45$	$182.12 \pm 2.50$	$183.40 \pm 2.48$	$182.25 \pm 2.22$	
N <sub>TON</sub> (g)	$42.38 \pm 2.02$	$42.45 \pm 1.98$	$43.42 \pm 1.92$	$42.75 \pm 1,95$	
TNUA (g)	$84.16 \pm 1.94$	$87.27 \pm 2.12$	$87.71 \pm 2.32$	$86.38 \pm 1.86$	

Laboratory analyses showed that the three classes of fish (order by size from small to large, i.e. 30, 150, and 250 g) had  $20.08 \pm 0.14$ ,  $22.52 \pm 0.12$ , and  $24.42 \pm 0.74\%$  protein content, respectively. From these data,  $N_{\rm fish} = 16.12 \, {\rm gN \, kg^{-1}}$  fish produced was determined (Eq. (2)). Extrapolating to the daily biomass of fish produced, the total nitrogen assimilated in fish was  $233.74 \, {\rm g \, N \, d^{-1}}$ .

Loss of fish was about 2% of the total production by number, which was equivalent of 305 g fish  $d^{-1}$  or 4.92 g total  $N_{mort} d^{-1}$  and represented 2.10% of the total nitrogen assimilated. Hence, 41.54% of nitrogen from feed was assimilated in fish flesh (39.44% harvested and 2.10% removed with mortalities), and 58.46% was unassimilated or excreted in different forms. In this, latter term was included nitrogen in uneaten feed that was accounted for in the overall budget as  $N_{TON}$ .

Analyses of the effluent wastewater (estimated  $4.5 \text{ m}^3 \text{ d}^{-1}$ ) indicated that it contained (on average)  $2.08 \text{ mg L}^{-1}$  TAN,  $0.72 \text{ mg L}^{-1}$  NO<sub>2</sub>-N,  $40.50 \text{ mg L}^{-1}$  NO<sub>3</sub>-N, and  $9.50 \text{ mg L}^{-1}$  TON. Extrapolated to the entire volume, the overall flows were  $9.36 \text{ g N}_{TAN} \text{ d}^{-1}$  (1.66% TNI),  $3.24 \text{ g N}_{NO2} \text{ d}^{-1}$  (0.58% TNI), 182.30 g N<sub>NO3</sub> d<sup>-1</sup> (32.40% TNI), and 42.75 g N<sub>TON</sub> d<sup>-1</sup> (7.60% TNI). The recovered fraction of N<sub>DIN</sub> resulted from the summation:

 $1.66\%\,N_{TAN} + 0.58\%\,N_{NO2} + 32.40\%\,N_{NO3} = 34.64\%$ 

TNR was determined as a percentage of TNI from Eq. (4):

83.78% TNR = 39.44% N<sub>fish</sub> + 2.10% N<sub>mort</sub> + 1.66% N<sub>TAN</sub> + 0.58% N<sub>NO2</sub> + 32.40% N<sub>NO3</sub> + 7.60% N<sub>TON</sub>. The value of TNUA then was estimated to represent 16.22% of TNI (Eq. (5)). Hence, the subsequent nitrogen mass balance in the production system was (Eq. (6)):

562.64 g TNI 
$$d^{-1} = 471.38$$
 g TNR  $d^{-1}$   
+ 91.26 g TNUA  $d^{-1}$ 

The relatively low percentage of TNUA was probably due to nitrogen lost as  $N_{denit}$  and as  $N_{TAN \ voln}$ . However, passive denitrification was likely the primary cause, considering that the water was passing through the sump numerous times. In this unit, the bioballs created conditions favorable for denitrification. Fig. 2 show the nitrogen mass balance based on the mass fraction nitrogen composition of all measured budget elements relative to nitrogen in the feed input into the culture tanks.



Fig. 2. Nitrogen mass balance for the production system based on the mass fraction nitrogen composition relative to feed input to the culture tank.

3.2. Estimating of system carrying capacity with respect to TAN

The assumptions and the pertinent informations for the development of the design criteria in this system are summarized and presented in Table 2. The use of the model indicated that an ARS could support transformation of a maximum concentration of 4.5 mg  $TANL^{-1}$  (Eq. (8)). This value was corresponded to  $0.104 \,\mathrm{g}\,\mathrm{m}^{-3}$  maximum allowable unionized ammonia (A<sub>NH3</sub>) under condition of pH  $\approx$  7.8 and temperature  $\approx 28^{\circ}$ C [19]—the average values of these parameters determined over the three tested ARSs were pH 7.5 and temperature 29°C. ARS able to receive an average of 1.96 kg feed/d (FR<sub>max</sub> TAN, determined using Eq. (9)), at this maximum allowable concentration of TAN, which supports an average fish biomass (SBM<sub>max</sub> TAN) of  $\hat{61.33}$  kg fish system<sup>-1</sup> (Eq. (10)). An average of system loadings was 68.5% of the maximum estimated (Table 2).

Over the three selected tanks, TAN removal efficiency per pass (Ea) was on average 60.4% (Eq. (11)). The rate of TAN production ( $P_{TAN}$ ) was determined on a daily basis using Eq. (12). P<sub>TAN</sub> per kg of feed consumed was then determined by dividing these values by the daily amount of feed introduced into a system, i.e. 9.52 g. The mass flow rate of TAN to hydroponic trough (L<sub>TAN</sub>) had an average of 9.89 g/day (Eq. (13)), which was removed at an average rate  $(R_{TAN})$  of 8.80 g/day (Eq. (14)). Details (i.e. per-system values) are presented in Table 2. The ratio between  $R_{TAN}$  and  $P_{TAN}$  (Eq. (15)) showed that hydroponic trough removed an average of 88.05% of P<sub>TAN</sub> from the selected systems. From the difference, 1.19% was recovered from the exchanged water and 10.76% remained unaccounted for, probably being transformed in NO<sub>2</sub>-N and NO<sub>3</sub>-N by passive nitrification, or being lost by ammonia volatilization.

750 Table 2

Experimentally determined and predicted parameters with regard to TAN removal for tested ARS

Parameters	Tested ARS		
	1	2	3
Max. feed rate $(FR_{max})$ $(kg/d)$	$1.96 \pm 0.15$	$2.00 \pm 0.25$	$1.93 \pm 0.12$
Max. system biomass (SBM <sub>max</sub> ) (kg)	$61.32 \pm 2.76$	$62.45 \pm 2.89$	$60.23 \pm 2.70$
Actual BM as % from SBM <sub>max</sub> (%)	$68.49 \pm 3.25$	$67.25 \pm 5.25$	$69.73 \pm 3.55$
$C_{TAN}$ in fish tank (mg/L)	$4.30\pm0.28$	$4.18 \pm 0.32$	$4.40\pm0.25$
$C_{TAN}$ in influent trough (mg/L)	$4.30\pm0.30$	$4.18 \pm 0.31$	$4.40\pm0.28$
$C_{TAN}$ in effluent trough (mg/L)	$1.70\pm0.18$	$1.60 \pm 0.22$	$1.80\pm0.15$
$C_{TAN}$ in influent culture tank (mg/L)	$0.50\pm0.05$	$0.36 \pm 0.08$	$0.56\pm0.05$
TAN % removal per pass (Ea) (%)	$60.47 \pm 1.54$	$61.72 \pm 2.05$	$59.09 \pm 2.65$
Feed rate $(kg/d)$	$1.05\pm0.08$	$1.05 \pm 0.09$	$1.05\pm0.08$
$P_{TAN}/kg$ feed (g)	$9.52 \pm 1.35$	$9.52 \pm 1.38$	$9.52 \pm 1.28$
Daily TAN production $(P_{TAN})$ $(g/d)$	$10.00 \pm 1.25$	$10.00 \pm 1.90$	$10.00 \pm 1.85$
Ammonia loading $(L_{TAN})$ $(g/d)$	$9.91 \pm 1.70$	$9.63 \pm 1.90$	$10.14 \pm 2.12$
Ammonia removal rate $(R_{TAN})$ $(g/d)$	$8.76 \pm 1.47$	$8.80 \pm 1.34$	$8.85 \pm 1.52$
Mass TAN introduced by exchange $(g/d)$	$0.13 \pm 0.03$	$0.09 \pm 0.02$	$0.14 \pm 0.06$
TAN removal trough (nitrification) (%)	$87.59 \pm 2.35$	$88.05 \pm 2.06$	$88.51 \pm 2.15$
TAN removed by water exchange (%)	$1.26\pm0.02$	$0.91 \pm 0.02$	$1.41 \pm 0.04$
TAN removed by passive nitrification (%)	$11.15 \pm 1.32$	$11.02 \pm 1.25$	$10.18 \pm 1.03$

# 4. Discussion

The results showed that the proportion of TNI assimilated by fish in this subsystem (39.44%) indicates excellent utilization of nitrogen for purpose of supporting fish growth relative to rates reported by other authors. For example, by using feed with 34% crude protein content, Siddiqui and Al-Harbi [8] reported 21.4% nitrogen assimilated by red tilapia. Suresh and Kwei [9] found that less than 20% of nitrogen was utilized by tilapia, using feed with 22% crude protein content and much lower densities of fish than those in this system. In a pond-based marine system growing Sparusaurata and Mugil sp., Krom et al. [10] found nitrogen assimilation of 20-40% from feeds with various nitrogen contents. The larger percent of nitrogen assimilation found in this study could be due to the configurations and management of the system and better quality of feeds, i.e. to higher protein concentration and better balance of the amino acids. Also, most of the studies cited reported greater mortalities, which could diminish the total nitrogen accumulated in fish. According to Piedrahita [20], losses of nitrogen and carbon within the system differ widely among the different recirculation aquaculture system and the accuracy of these determinations increase with the degree of the control over these systems [21]. The small amounts of nitrogen recovered as TAN (1.66%) and NO<sub>2</sub>-N (0.58%) was likely due to the nitrification

process, which oxidized them to NO<sub>3</sub>-N. Nevertheless, this is a general characteristic of ARS that include online aerobic biofiltration, which also explains the large amount of nitrogen recovered as NO<sub>3</sub>-N in this study. Most of the nitrogen recovered as N<sub>TON</sub> (7.60%) was probably due to feces, taking into account the observation that the feed was consumed by fish almost instantly at distribution, and only dust could escape as wasted feed. Assuming that some of the organic nitrogen bounded in feces dissolved upon contact with water, the results from this study, which took into account the nitrogen from the entire organic pool, are better with those of Thoman et al. [7] who recovered 14% nitrogen from the suspended solids.

From the nitrogen lost as  $T_{NUA}$  (16.22%), removal of N<sub>2</sub> gas through passive denitrification is the most reasonable explanation. Although this may appear surprising, the conditions for denitrification can occur in the sump of this system. Brandes and Devol [22] indicated that development of anoxic microsites in the sediment produces likely sites for denitrification in recirculating aquaculture systems. Additional evidence for the denitrification potential of nitrifying media was recently provided in a study on a moving bed bioreactor in a recirculating facility for culture of gilthead seabream (*Sparus aurata*) by Tal et al. [23]. Denitrifying activity in packed bed columns was studied by Suzuki et al. [24] with methanol as an external carbon source. In this study, these microsites could be zones in the sump where particles may have accumulated in the bioballs. Thoman et al. [7] determined that ammonia volatilization did not represent more than 0.25% of the unaccounted nitrogen and that the vast majority of the unaccounted nitrogen from this study could be lost by passive denitrification.

Overall, the nitrogen budgets supplied information allows a better estimation of nitrogen flow through the systems, identifying and quantifying each nitrogen pool throughout the facility. The estimation of nitrogen utilization by fish in this study showed that use of ARS is not only an attractive idea, but also worthwhile even when employed with fish having lower requirements for feed quality, such as African catfish.

This study also estimated how much existing ARS could support additional biomass with respect to TAN, and how these systems would handle the additional inorganic nitrogenous compounds generated under such conditions. The results indicated that the systems are not, in general, used at their maximum carrying capacity. Results showed that an average of 68.5% of the ARS productive potential is utilized in this system. Hence, there exists a much lower degree of occupancy in the systems holding fish of smaller size for long periods of time. This inference suggests that by improving management practices, net production could be increased in existing system. This possibility is supported by the excellent average removal efficiency found for hydroponic trough (60.4%) at a recirculation rate of almost one pass per hour which maintains an average TAN concentration of  $2.08 \text{ mg L}^{-1}$ . Additionally, simple calculations indicated that under conditions of returning water treated by a treatment 1.19% of the  $P_{TAN}\xspace$  will be reintroduced to the RAS. Hence, this additional loading will be easily removed without significant increase in TAN parameter throughout the systems. Consequently, the return of this residual TAN should not pose treat to the fish in the culture tank.

# 5. Conclusion

The study on the nitrogen budget indicated that the current practice of feeding catfish is worthwhile, with fish assimilating 39.44% of the nitrogen. Although this also implies higher amounts of ammonia excreted, the existing biofilters appear able to remove it, even if the systems are operated at their maximum carrying capacity. It was estimated that the current average systems' occupancy is only around 68.50%. Hence, better management, such as synchronizing shipping with repopulation and frequent grading could increase the production in the existing infrastructure. Of TAN production, 88.05% was removed by biofilters and the balance by passive nitrification throughout the systems.

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