

# Nitrogen Dynamics and Removal by Algal Turf Scrubber under High Ammonia and Organic Matter Loading in a Recirculating Aquaculture System

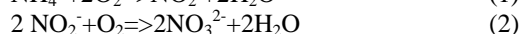
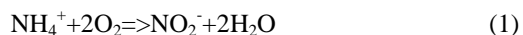
Joshua S. Valeta, and Marc C. Verdegem

**Abstract**—A study was undertaken to assess the potential of an Algal Turf Scrubber to remove nitrogen from aquaculture effluent to reduce environmental pollution. High total ammonia nitrogen concentrations were introduced to an Algal Turf Scrubber developed under varying hydraulic surface loading rates of African catfish (*Clarius gariepinus*) effluent in a recirculating aquaculture system. Nutrient removal rates were not affected at total suspended solids concentration of up to 0.04g TSS/l ( $P > 0.05$ ). Nitrogen removal rates 0.93-0.99g TAN/m<sup>2</sup>/d were recorded at very high loading rates 3.76-3.81 g TAN/m<sup>2</sup>/d. Total ammonia removal showed ½ order kinetics between 1.6 to 2.3mg/l Total Ammonia Nitrogen concentrations. Nitrogen removal increased with its loading, which increased with hydraulic surface loading rate. Total Ammonia Nitrogen removal by Algal turf scrubber was higher than reported values for fluidized bed filters and trickling filters. The algal turf scrubber also effectively removed nitrate thereby reducing the need for water exchange.

**Keywords**—Algal turf, loading rate, nitrogen, organic matter, removal rate.

## I. INTRODUCTION

HIGH concentration of nitrogen (N) and phosphorus (P) in effluent discharge is one of the major water quality problem of environmental concern reported from aquaculture [1]. Periphyton has demonstrated ability to improve water quality in a Recirculation Aquaculture System (RAS). A periphyton reactor can replace a trickling filter and a sedimentation unit in a regular RAS processing a feed load of up to 32g/m<sup>2</sup>/day [2]. The primary goal of biofiltration is to remove ammonia (NH<sub>4</sub><sup>+</sup>-N and NH<sub>3</sub>-N) and nitrite. NH<sub>3</sub>-N is highly toxic un-ionized form [3]. Nitrate (NO<sub>3</sub>) is the least harmful form of inorganic N [4]. In RAS, at sites with very low oxygen levels, NO<sub>3</sub> can be denitrified to give elemental (gaseous) nitrogen [5]. The first step in nitrification is performed by *Nitrosomonas sp.* (Refer to (1)), while the second is by *Nitrobacter sp.*, (Refer to (2)).



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Ammonia uptake rates can be measured under light and dark conditions. Under dark conditions, irregular patterns of uptake have been observed for reasons not well understood. Reference [3] also observed higher removal rates, under dark conditions, at low TAN concentrations than at high concentrations, which was unlikely. This was the case probably because the nitrifying bacteria were present in the biofilm in a dormant state, and once exposed to ammonia (when the algae do not work) become active. It takes a few hours for bacteria to become activated, as it takes some time for ammonia to diffuse to the nitrifying bacteria. On the contrary, under light conditions, the ammonia uptake rate showed 1/2-order kinetics up to 1.6-2.8mg/l TAN and 0-order kinetics beyond that concentration. Increasing loading generally increases nutrient content. In waste water treatment, reported nutrient removal capacity of periphyton either by itself [6] or in combination with emergent floating or submerged macrophytes [7]-[8]-[9] varied, probably declining with extremely increased areal loading rate, decreasing periphyton surface to water volume ratio and increasing depth. This study aimed at estimating potential TAN removal in an ATS grown under different loading conditions and subjected to high initial ammonia concentrations. The effluent used came from African catfish (*Clarias gariepinus*) RAS.

## II. MATERIALS AND METHODS

The study was conducted in the hatchery “De Haar Vissen” at Wageningen University, The Netherlands. The periphyton RAS generally consists of fish tank, periphyton reactor and a sump (Fig. 1). In this experiment, effluent water (predetermined Q) from catfish tanks was diverted into a collector tank where it was homogenized by stirring before passing to the ATS. The ATS was illuminated by two halide lamps.

The ATS was operated under different flow rates to change the organic matter load to the ATS. The experiment lasted 5 weeks. On the seventh day of each week, the sump was emptied. This is when a short circuit was introduced in the system. The periphyton reactor was disconnected from the RAS, and operated in short circuit reusing sump water for a maximum 12hrs under light condition. The overflow pipes from the collecting bucket to sump and from sump to the main effluent pipe were disconnected. At the start of the circuit, the water in the sump was treated with dissolved Ammonium Chloride (NH<sub>4</sub>Cl) to achieve 3.1mg/l, 5.13mg/l or 8mg NH<sub>4</sub>-N mg/l, respectively.

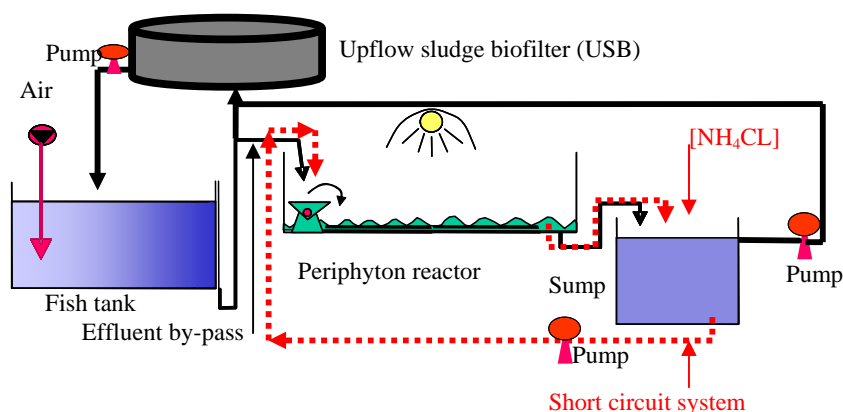


Fig. 1 Schematic overview of the Algal Turf Scrubber experimental set-up

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The first water sample was collected at the inlet immediately after dissolving the  $\text{NH}_4\text{Cl}$  into the sump. Thirty (30) minutes were allowed for stabilizing the system after which samples were collected every 20-30 minutes. Samples were collected in 10ml tubes and were analyzed the same day for  $\text{NH}_4$ ,  $\text{NO}_3$ , and  $\text{NO}_2$  using a SAN auto-analyzer (Skalar, The Netherlands). Water samples were also collected in 500-ml bottles prior to the short circuit, which were treated with sulphuric acid to lower pH below 2 and were kept under  $4^\circ\text{C}$ . They were later analyzed for total solids (total suspended solids (TSS) and dry matter (DM), in accordance with [10].

Dissolved oxygen (DO) (WTW, Oxi 340i, Retsch, The Netherlands), temperature (Testo 110, Retsch, The Netherlands), pH (WTW, pH340, Retsch, The Netherlands) and conductivity (WTW, LF318, Retsch, The Netherlands) were also measured throughout each experiment. After each experiment, the periphyton screens were partially (50%) harvested and sludge was collected from the periphyton tank to give the biofilm vigor. Data were analyzed in S-Plus 6.1.

### III. RESULTS AND DISCUSSION

Temperature ranged between  $25.7$  and  $28.6^\circ\text{C}$ , which was appropriate for the performance of the ATS. In all the five

#### A. Nitrogen Dynamics and Removal Rates

##### 1. TAN Removal

experiments, DO, and pH slightly increased with time in the ATS. pH increased from 7.14 to 8.49 following nitrification process, but it was still within the recommended range for the performance of the bacteria. Water volume in the periphyton tank was maintained at  $0.028\text{ m}^3$ , while in the sump it was at  $0.060\text{ m}^3$ . Water flow rate (Q) was  $4.55\text{ m}^3/\text{d}$ . TSS concentration was almost similar throughout the experiment implying that the system's maximum TSS ( $0.04\text{ g/l}$ ) was reached (Table I) [11]. Similarly, sludge dry matter did not differ significantly ( $p>0.05$ ) across the experiments.

TABLE I  
INITIAL  $\text{NH}_4\text{-N}$  CONCENTRATIONS FOR CORRESPONDING WEEKLY HYDRAULIC SURFACE LOADING RATES, TOTAL SUSPENDED SOLIDS AND SLUDGE DRY MATTER

$[\text{NH}_4\text{-N}]$ $\text{g/m}^3$	HSL for week ( $\text{m}^3/\text{m}^2/\text{d}$ )	TSS ( $\text{g/l}$ )	Sludge dry matter ( $\text{g/week}$ )
3.11	0.62	0.04	40.29
3.11	2.08	0.04	58.09
5.13	2.17	0.03	63.49
8.00	0.66	0.04	61.81
8.00	1.96	0.04	58.40

TABLE II  
INITIAL HSL, TAN CONCENTRATIONS, REMOVAL RATES AND TIME TAKEN TO REACH ZERO ORDER REMOVAL RATES

$[\text{NH}_4\text{-N}]$ ( $\text{g/m}^3$ )	Max [TAN] ( $\text{g/m}^3$ )	Min [TAN] ( $\text{g/m}^3$ )	Average ${}^t\text{TAN}$ ( $\text{g/m}^2/\text{d}$ ) at inflection point <sup>1</sup>	$[\text{TAN}]$ ( $\text{g/m}^3$ or ${}^t\text{TAN}$ at inflection of ${}^t\text{TAN}$	Time to constant ${}^t\text{TAN}$ (minutes)	Experiment Time (minutes)	HSL for the week
3.11	3.02	0.33	0.68	2.00	120	675	0.62
3.11	3.55	0.70	0.40	1.60	190	430	2.08
5.13	4.34	0.52	0.70	2.05	180	610	2.17
8.00	9.49	0.27	0.90	2.30	410	670	0.66
8.00	9.37	0.41	0.72	1.90	500	725	1.96

<sup>1</sup> Average  ${}^t\text{TAN}$  and corresponding inflection points were estimated from graphs (Fig. 3, 5, 7, 9 and 11).

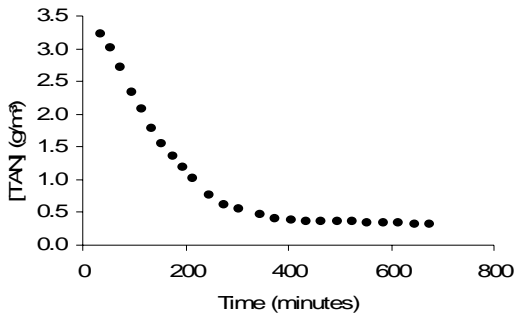


Fig. 2 Change in [TAN] over time for initial load 3.11g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 0.62m<sup>3</sup>/m<sup>2</sup>/d

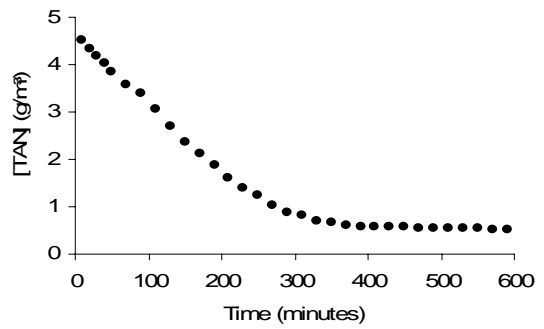


Fig. 6 Change in [TAN] over time for initial load 5.13g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 2.17 m<sup>3</sup>/m<sup>2</sup>/d

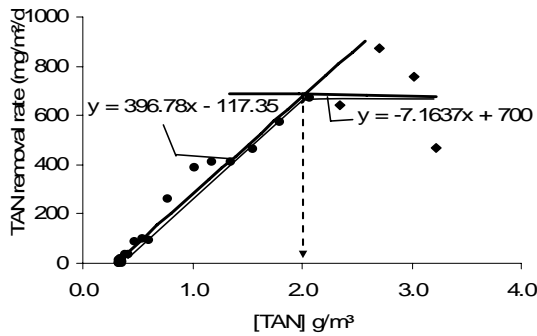


Fig. 3 Change in <sup>r</sup>TAN over time for initial load 3.11g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 0.62m<sup>3</sup>/m<sup>2</sup>/d

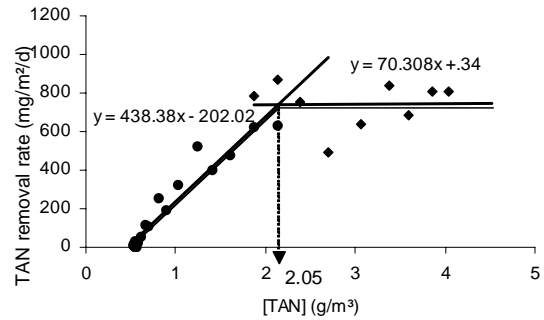


Fig. 7 Change in <sup>r</sup>TAN for initial load 5.13g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 2.17 m<sup>3</sup>/m<sup>2</sup>/d

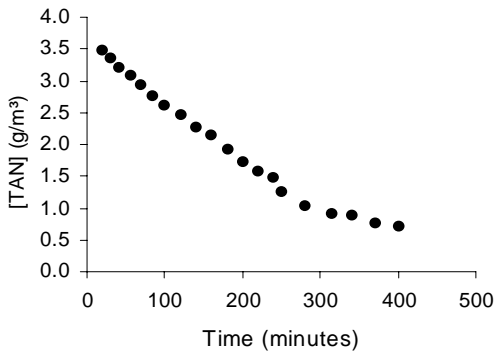


Fig. 4 Change in [TAN] over time for initial load 3.11g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 2.08 m<sup>3</sup>/m<sup>2</sup>/d

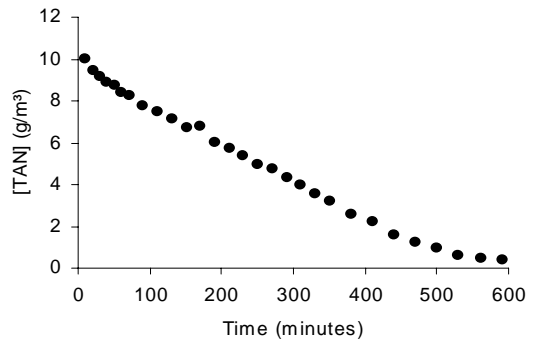


Fig. 8 Change in [TAN] over time for initial load 8.00g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 0.66 m<sup>3</sup>/m<sup>2</sup>/d

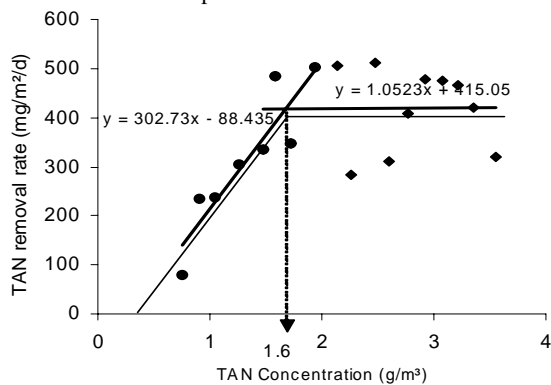


Fig. 5 Change in <sup>r</sup>TAN over time for initial load 3.11g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 2.08 m<sup>3</sup>/m<sup>2</sup>/d

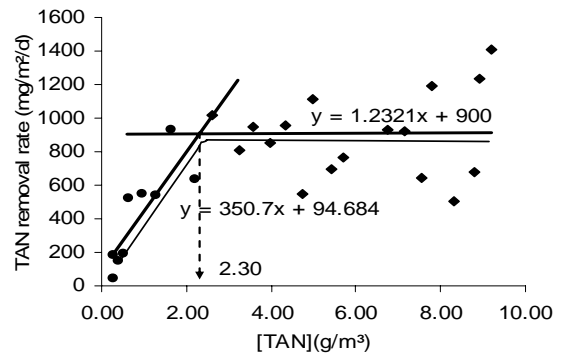


Fig. 9 Change in <sup>r</sup>TAN for initial load 8.00gNH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 0.66 m<sup>3</sup>/m<sup>2</sup>/d

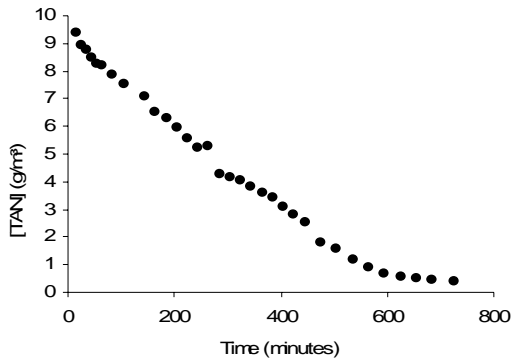


Fig. 10 Change in [TAN] over time for initial load 8.00 gNH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 1.96 m<sup>3</sup>/m<sup>2</sup>/d

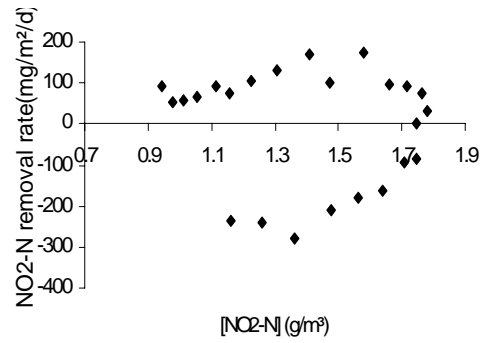


Fig. 13 Change in net NO<sub>2</sub>-N removal rate for initial load 3.11 g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 0.62 m<sup>3</sup>/m<sup>2</sup>/d

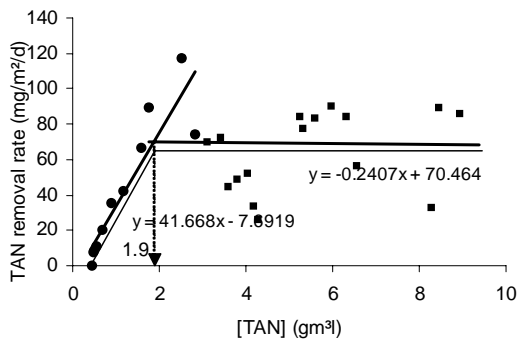


Fig. 11 Change in TAN for initial load 8.00gNH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 1.96 m<sup>3</sup>/m<sup>2</sup>/d

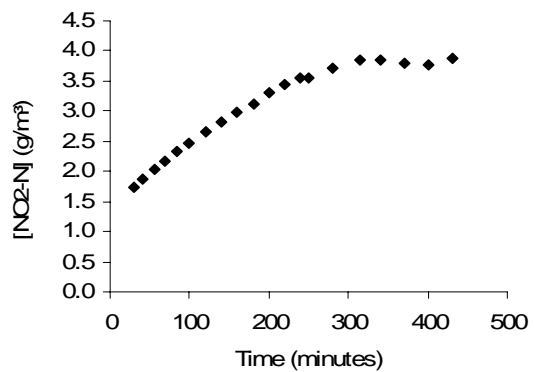


Fig. 14 Change in [NO<sub>2</sub>-N] over time for initial load 3.11 g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 2.08 m<sup>3</sup>/m<sup>2</sup>/d

TAN removal started immediately after ammonia was dissolved into the system. Depending on prior loading conditions and the initial ammonia concentrations, TAN removal took different patterns (Table II and Fig. 2-11). At higher initial NH<sub>4</sub>-N loading, it took longer for removal rate to assume zero order rates (comparing almost similar HSL; 0.62 and 0.66; and HSL=2.08 and 2.17). N removal rates increased with NH<sub>4</sub>-N loading (Fig. 3, 5, 7, 9, and 11).

## 2. Nitrite Nitrogen (NO<sub>2</sub>-N) Removal

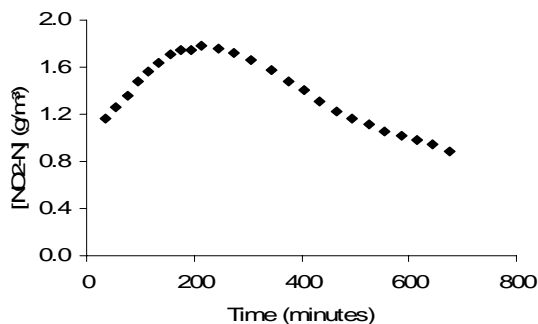


Fig. 12 Change in [NO<sub>2</sub>-N] over time for initial load 3.11 g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 0.62 m<sup>3</sup>/m<sup>2</sup>/d

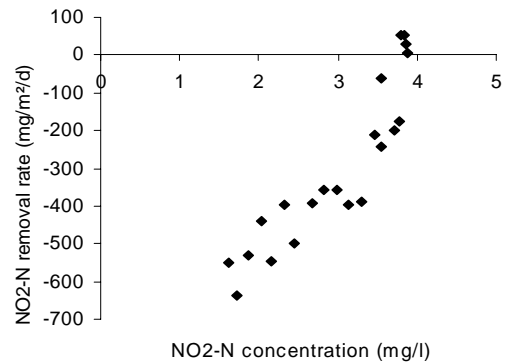


Fig. 15 Change in net NO<sub>2</sub>-N removal rate for initial load 3.11g NH<sub>4</sub>-N/m<sup>3</sup> in ATS developed under HSL 2.08 m<sup>3</sup>/m<sup>2</sup>/d

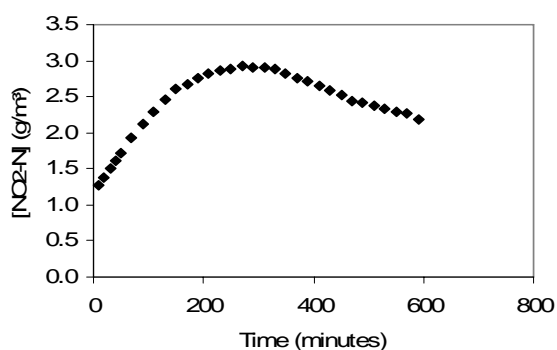


Fig. 16 Change in  $[NO_2-N]$  over time for initial load  $5.13g NH_4-N/m^3$  in ATS developed under HSL  $2.17 m^3/m^2/d$

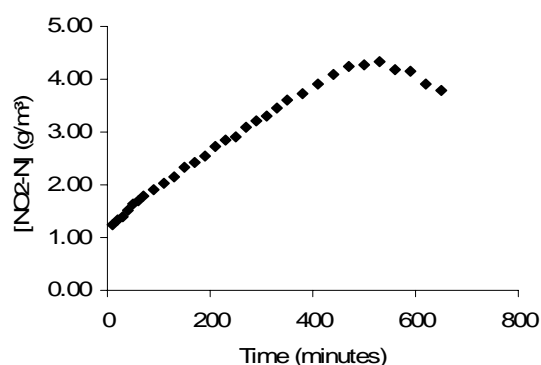


Fig. 18 Change in  $[NO_2-N]$  over time for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $0.66 m^3/m^2/d$

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TAN removal showed  $\frac{1}{2}$  order kinetics between 1.6 to  $2.3mg/l$  TAN concentrations, which agrees with results from [3] who reported  $1.6-2.8mg/l$ . However, the  $\frac{1}{2}$  order TAN removal rates ( $r_{TAN}$ ) estimated in this study were higher than reported in many aquaculture and waste water treatment research findings. For example, the ATS  $r_{TAN}$  (esp.  $0.68, 0.70, 0.72$  and  $0.90g TAN/m^2/d$ ; Table III) were significantly superior to those reported for other types of biofilters. For example, [12] reported removal rates  $0.27$  and  $0.21g TAN/m^2/d$  for two fluidized beds when the inlet concentration was  $2.2 mg TAN/l$ , whereas [13] found approximately  $0.3g TAN/m^2/d$  for trickling filter. Reference [14] reported  $r_{TAN}$   $0.43g TAN m^2/d$  for  $2 mg TAN/l$  inlet concentrations. Reference [15] reported slightly higher values:  $5.7g TAN/m^2/d$  for  $10mg TAN/l$  inlet concentrations and this was because of the high initial TAN concentration used.

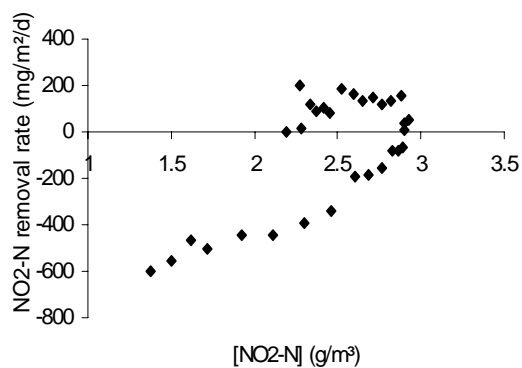


Fig. 17 Change in net  $NO_2-N$  removal rate for initial load  $5.13g NH_4-N/m^3$  in ATS developed under HSL  $2.17 m^3/m^2/d$

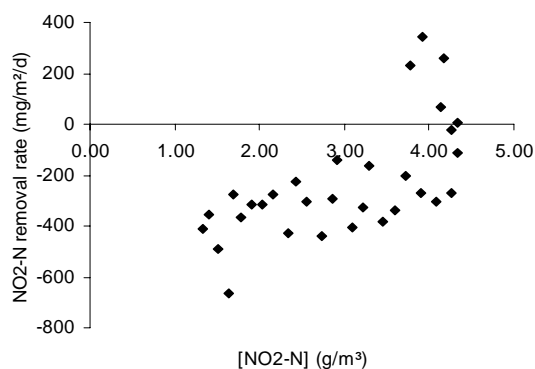


Fig. 19 Change in net  $NO_2-N$  removal rate over time for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $0.66 m^3/m^2/d$

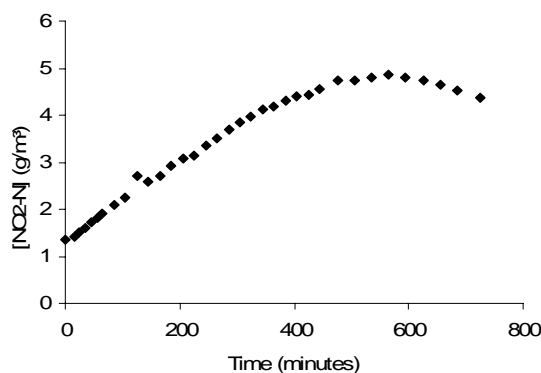


Fig. 20 Change in  $[NO_2-N]$  over time for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $1.96 m^3/m^2/d$

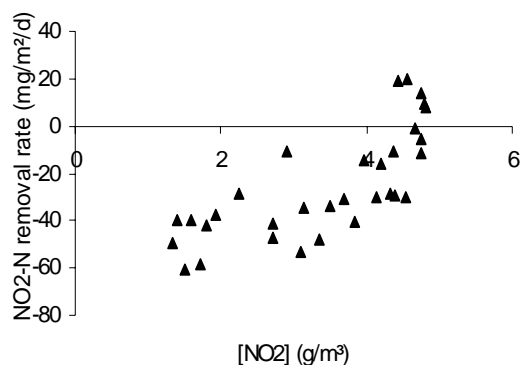


Fig. 21 Change in net  $\text{NO}_2\text{-N}$  removal rate for initial load  $8.00\text{g NH}_4\text{-N/m}^3$  in ATS developed under HSL  $1.96\text{ m}^3/\text{m}^2/\text{d}$

The minimum TAN concentrations ( $0.27\text{mg/l}$  -  $0.7\text{mg/l}$ ) achieved in the 7-12 hour period of the experiments were higher than values reported from a 24hour experiment with a periphyton reactor, trickling filter and a combination of the two (below  $0.01\text{mg/l}$ ) [2]. Lower values would be attained with experimental time much longer than 12 hours as in [2]. Nevertheless, the minimum concentrations achieved in this study were still lower than those found in other types of systems e.g.  $0.6\text{-}0.7\text{mgTAN/l}$  reported by [16] Leclercq and Hopkins (1985) for an airlift recycling tank.

Higher zero-order removal rates were observed for higher ammonia loads. Reference [14], [6] and [15], also reported that N removal rates increased with increasing ammonia loading rate and biomass.

Nitrite accumulated in the ATS before removal took place in all cases (Fig. 12 - 21). However, the time of accumulation to the onset of removal varied with loading conditions. Nitrite accumulated due to initial suboptimal balance between *Nitrosomonas* and *Nitrobacter* like organisms. When nitrification rate approached a constant rate (2-4 hours later) (Table II), accumulation of  $\text{NO}_2\text{-N}$  stopped (Figs. 13, 15, 17, 19, and 21). After this point, oxidation rate of nitrite exceeded its production rate giving a net reduction in concentration in the water. Both negative and positive removal rates recorded between  $0.8\text{mg/l}$  and  $1.75\text{mg/l}$   $\text{NO}_2\text{-N}$  concentrations are indicative of removal and accumulation, respectively. The minimum concentration of  $0.8\text{mg/l}$  from the study is already lower than threshold for certain cultured organisms (e.g. Tilapia) [17]. However, this minimum was only achieved in a biofilm developed under low HSL ( $0.62\text{m}^3/\text{m}^2/\text{d}$ ). The case of a low initial TAN ( $3.11\text{mg/l}$ ) gave a clear trend with a moderately steep graph tail (Fig. 13) suggesting that more time would probably be required to reach true minimum  $\text{NO}_2\text{-N}$  concentration in the effluent.

It should be noted that at high HSL (HSL  $1.96$ ,  $2.08$  and  $2.17\text{m}^3/\text{m}^2/\text{d}$ ) the peak  $\text{NO}_2\text{-N}$  concentrations were much higher (Fig. 15, 17 and 21) due to the  $\text{NO}_2\text{-N}$  already present prior to the experiment. Furthermore, at such high initial  $\text{NH}_4\text{-N}$  loading the  $\text{NO}_2\text{-N}$  accumulated for longer period (7-9hours) before declining, as a consequence of increased nitrification.

Net nitrite removal rate was difficult to interpret because it only measured the difference between accumulation rate and the true removal rate, and these fractions were not estimated.

The high (peak) nitrite concentrations pose a potential problem for culture organisms in RAS using ATS. As such, at high nutrient loading rates it would be necessary to remove sludge thrice every two weeks to reduce the cumulative effect. Furthermore, complete harvesting of the periphyton mat would increase nitrite removal potential since young periphyton exhibits higher nutrient removal capacity [18]. As (re)colonization takes place, the imbalance between *Nitrosomonas* and *Nitrobacter* can be minimized as *Nitrobacter* grows faster. Since nitrite is the growth-limiting substrate for *Nitrobacter*, according to [19], high nitrite levels present in the water at harvest will enhance development of *Nitrobacter*. At high nutrient concentrations, low flow rates may also be used to control nutrient loading rate and increase effluent residence time in the ATS.

### 3. Nitrate Nitrogen ( $\text{NO}_3\text{-N}$ ) Removal

Nitrate removal started immediately following denitrification in some cases (Fig. 424, 26 and 30) while in others it accumulated for some time before removal took place (Fig. 22 and 28). Nitrate removal rates took no definite pattern (Fig. 23, 25, 27, 28 and 31).

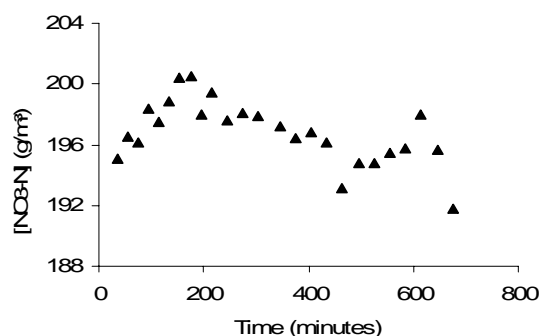


Fig. 22 Change in  $[\text{NO}_3\text{-N}]$  over time for initial load  $3.11\text{g NH}_4\text{-N/m}^3$  in ATS developed under HSL  $0.62\text{ m}^3/\text{m}^2/\text{d}$

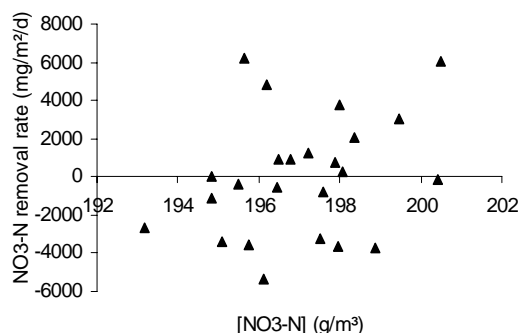


Fig. 23 Change in net  $\text{NO}_3\text{-N}$  removal rate for initial load  $3.11\text{g NH}_4\text{-N/m}^3$  in ATS developed under HSL  $0.62\text{ m}^3/\text{m}^2/\text{d}$

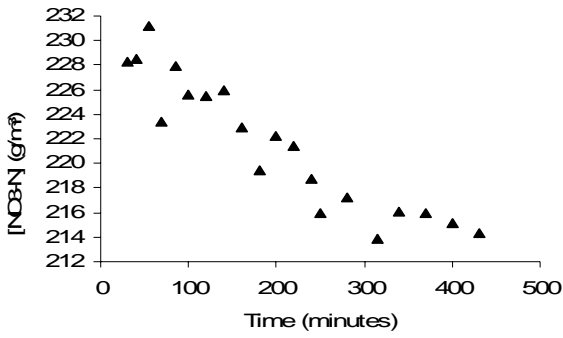


Fig. 24 Change in  $[NO_3-N]$  over time for initial load  $3.11g NH_4-N/m^3$  in ATS developed under HSL  $2.08 m^3/m^2/d$

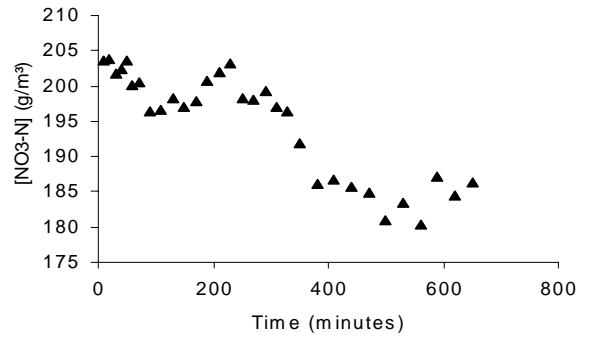


Fig. 28 Change in  $[NO_3-N]$  over time for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $0.66 m^3/m^2/d$

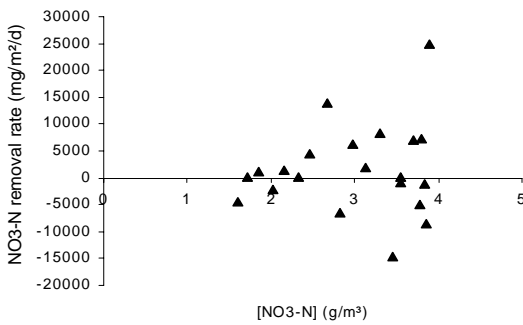


Fig. 25 Change in net  $NO_3-N$  removal rate for initial load  $3.11g NH_4-N/m^3$  in ATS developed under HSL  $2.08 m^3/m^2/d$

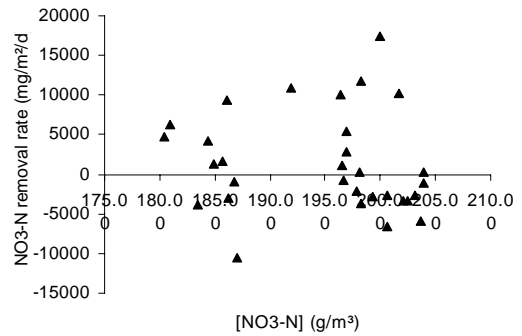


Fig. 29 Change in net  $NO_3-N$  removal rate for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $0.66 m^3/m^2/d$

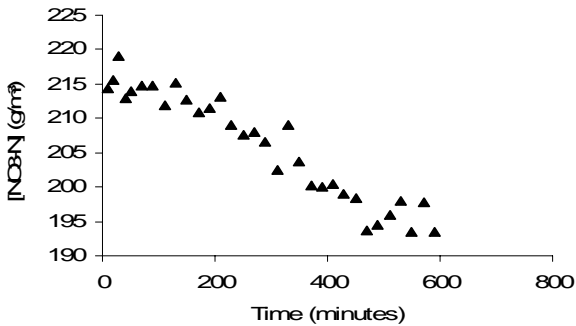


Fig. 26 Change in  $[NO_3-N]$  over time for initial load  $5.13g NH_4-N/m^3$  in ATS developed under HSL  $2.17 m^3/m^2/d$

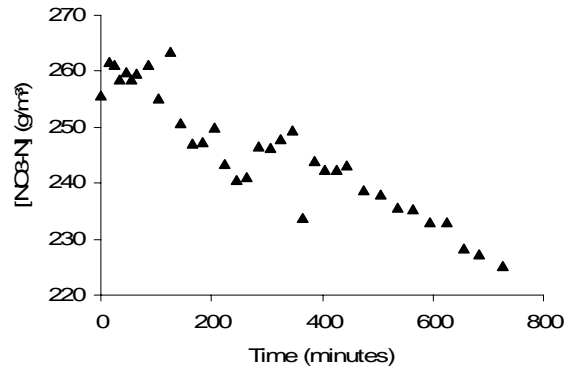


Fig. 30 Change in  $[NO_3-N]$  over time for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $1.96 m^3/m^2/d$

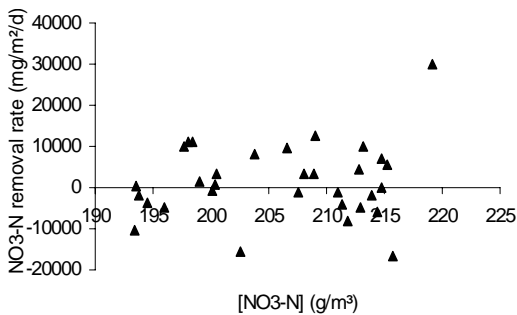


Fig. 27 Change in net  $NO_3-N$  removal rate for initial load  $5.13g NH_4-N/m^3$  in ATS developed under HSL  $2.17 m^3/m^2/d$

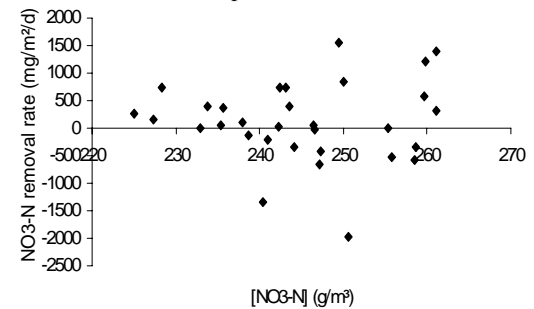


Fig. 31 Change in net  $NO_3-N$  removal rate for initial load  $8.00g NH_4-N/m^3$  in ATS developed under HSL  $1.96 m^3/m^2/d$

The rise in nitrate concentration reflected the immediate decline in nitrite at low HSL ( $0.62\text{m}^3/\text{m}^2/\text{d}$ ). Nitrate only started decreasing about 3 hours later (Fig. 23 and 29). Contrariwise, the higher HSL's ( $1.96$ ,  $2.08$  and  $2.17\text{m}^3/\text{m}^2/\text{d}$ ) resulted in immediate decline in nitrate at the start of the experiment (Fig. 25, 27 and 31) due to higher denitrification capacity arising from anoxic conditions created by high sludge accumulation. The steep slope present in all cases suggests that it would require more time to remove more  $\text{NO}_3\text{-N}$ . It was difficult to interpret removal rate data because what was measured was the difference between accumulation rate and the actual removal rate. There was no definite pattern in the evolution of net removal with change in  $\text{NO}_3\text{-N}$  concentration (Fig. 24, 26, 28, 30 and 32). Since  $\text{NO}_3\text{-N}$  uptake is inhibited by algae in the presence of ammonia [20] (Syrett, 1981) removal rates may not directly depend on the  $\text{NO}_3\text{-N}$  concentrations in the effluent although the system was closed. The general decrease in nitrate concentration with time makes ATS superior to trickling filters with regards to nitrate removal. The decrease in nitrate with time offers a possibility to reduce necessity of water exchange in RAS using ATS with the goal of reducing nitrate levels. Since removal rate is higher in young biofilm than in a mature one [18], complete harvesting of periphyton mat would further reduce the need for water exchange.

#### IV. CONCLUSION

The ATS ably removed TAN at removal rates as high as  $9.49\text{ TAN}/\text{m}^2/\text{d}$  under high initial TAN concentration of  $8\text{g}/\text{m}^3$ . TAN removal showed  $\frac{1}{2}$  order kinetics between  $1.6$  to  $2.3\text{mg}/\text{l}$  TAN concentrations. The maximum half-order TAN removal ( $0.90\text{g}/\text{m}^2/\text{d}$ ) was higher than rates recorded for other types of filters including fluidized bed filters and trickling filters. The ATS reduced TAN concentrations beyond thresholds for aquaculture species such as Tilapia. Regardless of increase in weekly sludge biomass accumulation in the ATS prior to ammonia loads, TAN removal rate increased with nutrient loading. The ATS is potentially also ideal for removal of nitrate which in turn would reduce need for water exchange in RAS. Future research should explore the maximum technical and economic potential for commercial application.

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