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Biodenitrification of Aquaculture Wastewater at Different Drying Times in Water Reuse System

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ABSTRACT

Introduction of denitrification in water recirculating system to remove nitrate-nitrogen which is produced by nitrification and tend to be toxic at high concentration has been investigated by some researchers but mostly for temperate aquaculture and information on the time for readiness of the system is virtually not available. Therefore this study examined the biodenitrification of aquaculture wastewater from a tropical fish species at different drying times. Aquaculture effluent from monoculture of *Clarias gariepinus* was passed through nitrifying followed by denitrifying biofilter at three drying times (24, 72 and 144 hours). Water quality parameters; Temperature, Dissolved oxygen (DO), pH, Total ammonia-nitrogen (TAN), Nitrite-nitrogen (NO_2-N) and Nitrate-nitrogen (NO_3-N) were measured in the filtrates of the nitrification and denitrification column to assess the change in water quality. Denitrification efficiency of the biofilter was determined using Percentage nitrate-nitrogen removed (PNR) and Volumetric nitrate-nitrogen conversion rate (VNR). The result showed that all the water quality parameters were within the range for fish culture but the nitrate-nitrogen in the nitrification is higher than the recommended level for discharge into the environment. The PNR recorded were 33.33±57.74%, 47.87±4.19 % and 59.09±7.87% while the VNR recorded were 3116.88±599.60 mgNO_3-N/m^3d, 14125.26±746.55, 58775.51±7068.43 mgNO_3-N/m^3d for the 24, 72 and 144 hours drying time respectively. The study revealed that biodenitrification increases with drying time and prevented NO_3-N accumulation in the system. The incorporation of denitrification column in recirculating system will enhance sustainable aquaculture production and save the aquatic environment from eutrophication.
KEYWORDS: Aquaculture effluents, Nitrate removal, Drying time, Total ammonia nitrogen, Recirculating system.

INTRODUCTION

The trend in fish culture has been to move from conventional open systems to high density and highly productive land-based systems in which recirculation aquaculture system (RAS) is one of them. The use of recirculating system has been tested both at experimental and pilot scale and it is now growing in number in commercial scale. The use of recirculation system is growing in the fast developing urban and peri-urban aquaculture in Nigeria [2]. Recirculating aquaculture systems (RAS) are used to rear high densities of fish while employing water conservation techniques by continuously recycling the culture water. The percentage of the total system volume that is recycled varies with system operation, but for completely closed systems water replacement compensates mainly for evaporation and splash out losses [19]. In most recirculating systems, total ammonia (referring to NH_3 and NH_4^+) removal by nitrification, sludge removal by sedimentation or mechanical filtration, and water exchange are the vital forms of water treatment [20]. High pollutant load results in the water drained from the inland aquaculture systems [15], and may be due to nitrate and phosphate in RAS. The removal of toxic ammonia from fish water is carried out mainly by biofilters, the less toxic nitrate resulted from conversion of ammonia by biofilters may accumulates over time to the extent of becoming a limiting factor to fish production. High nitrate concentration in the recirculating system can be toxic to fish species and for tropical fresh water fish species less than 250mg/L is recommended [21]. Increased efforts are now directed toward nitrate control in recirculating systems which apart from the direct toxic effect on fish at high concentrations can also lead to environmental degradation. Nitrates can be reduced in RAS if the conventional nitrification is followed by denitrification [17]. Biological denitrification can be used to remove nitrates from recirculating aquaculture system waters and this is done in anoxic condition by heterotophic bacteria and nitrates is used as terminal electron acceptor in the presence of carbon and energy source [13] . Biodenitrification has been experimented by some researchers but mainly for temperate fish species, therefore this study investigated biodenitrification of wastewater from *Clarias gariepinus*

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(an important cultured fish in tropical region) at different drying times in order to examine biodenitrification in warm water fish culture and effect of drying times on the performance of the system.

MATERIALS AND METHODS

The experiment was carried out in the University of Ibadan, Department of Aquaculture and Fisheries Management laboratory.

Aquaculture Wastewater

Aquaculture effluents were obtained from a commercial fish farm within Ibadan metropolis, operating monoculture system and using concrete tank. The wastewater was collected between 0800 to 0900 hours of the day using four pieces of twenty five litres plastic containers for each batch and transported to the laboratory for the experiment. The tank at the time of collecting the wastewater contains 1,100 individual *Clarias gariepinus* of 24 weeks old in 11.4 m^3 of water. The fish were fed with floating pellet of 42% crude protein twice daily.

The Biofilter Units

The biofilter units have a nitrification and denitrification column. Each column has essentially the biofilter housing and the media. The biofilter housing was made of a pair of 150mm diameter, 3mm thick, and 1,200mm high Polyvinylchloride (PVC) pipe, in each pair. One of the filter had its top opened while the other one had its top covered with black polythene. The one with opened top was used as the nitrification column while the covered top was used as the denitrification column, because denitrification occurs in anaerobic condition [1]. The bottom end of these filter housing was made of solid PVC end plug piece drilled with 20mm holes. A plastic funnel was attached to the bottom end of the housing to collect the filtrate into a plastic container placed under the funnel. A wooden frame was constructed to hold the filter housing. Three biofilter units were used because the experiment was carried out in triplicates. The biofilter media is the commonly used polypropylene (PP) bioblock, the bioblock comes in $1m^3$ and was cut to fit into the biofilter housing unit with diameter 150mm and each of them were made to have height of 600mm. Six biofilter media was used in all, one for the nitrification column and one for the denitrification column with three replicates each.

Experimental Procedure

The filter housings were prepared in line with [4]. Thereafter the PP block of 60cm height was inserted to fill up the filter housing to 75cm height. The biofilter media was inoculated using wastewater from the aquaculture system before applying for treatment after 24 hours of inoculation. The nitrifying filter column was inoculated with the aquaculture wastewater, while the filtrate was quickly analysed for the amount of Nitrate-nitrogen (mg/L) in it and methanol (99.5% minimum assay) was added at the ratio of 3:1 Methanol to Nitrate-nitrogen in mg/l in line with [1]. The filtrate with the methanol added was used for the inoculation of denitrification column and the housing cover was replaced immediately. The system was left for 24 hours for the bacteria to build up before the commencement of the experiment.

The wastewater samples were poured into an open container (bowl) to allow more dissolution of oxygen at the water-air interface in order to increase the dissolved oxygen content so as to enhance the performance of the aerobic bacteria. Five litres of the wastewater was measured out and poured into the nitrification filter column. The filtrate was collected in plastic container, carefully observed and timed (using stop watch) until water hardly drops from the column. This was done in order to determine the residence time (Res time) and biofiltration rate of the wastewater that pass through the filter unit. After the wastewater had drained completely, 60cl of the filtrate was collected for the analysis of the selected water quality parameters. The nitrate-nitrogen level was determined on the spot and methanol (99.5%) was added to the remaining filtrates at ratio of 3:1 of methanol to nitrate-nitrogen (mg/L) in line with [1], before the filtrate is gently poured into the denitrification column. The filter column was also observed and timed until water hardly drops from it. After the water had drained completely through the filter, the filtrate was collected for analysis. The system was backwashed after each phase and allow to dry for two days before the commencement of another phase and the whole process was repeated for three drying times; 24 hours, 72 hours and 144 hours to assess the change in performance of the biofilter with days. The experiment was conducted in triplicates and average of each selected water quality parameter was determined at the end of the experiment.

Water Quality Analysis

The wastewater influents and filtrates were examined for the following parameters; Dissolved oxygen (DO), Total ammonia nitrogen (TAN), pH, Temperature, Nitrate-nitrogen (NO_3-N) and Nitrite-nitrogen(NO_2-N).

Temperature was measured with mercury in glass thermometer, the thermometer was inserted into the water sample and left for two minutes before the reading was taken. DO was determined using winklers method in line with [12], pH was measured using a HANNA probe pH meter. Total ammonia nitrogen (TAN) was determine colourimetrcally using Merck (2011) test kit, nitrite-nitrogen (NO_2-N) was determined using Merck (2010) test kit. Nitrate-nitrogen (NO_3-N) was determined colourimetrically using HAGEN (2008), test kit. Procedures for analysis was done according to standard methods [6]. The change in these selected water quality parameters from influent wastewater (collected from farm) and filtrates (from the biofilters) were used to evaluate the performance of the biofilter media. Water residence time and biofilter flow, the residence time is the time which the wastewater used in each biofilter column and it was determined using a stopwatch. This was done to determine the time used for treating the water in each column. The duration was taken from the time when the wastewater is poured into filter column until water hardly dropped. The biofilter flow (Q) in m^3/s was calculated as follows;

$$\frac{\text{Quantity of water that moved through the filter } (m^3)}{\text{Residence time (seconds)}}$$

Performance Assessment of the Biofilter Media

The degree of change in selected water quality parameters was used to evaluate performance of the system while the denitrification efficiency of the biofilter was determined using the formulae below:

Denitrification efficiency

It was evaluated using Percentage nitrate-nitrogen removed (PNR) and Volumetric nitrate-nitrogen conversion rate (VNR), the formulae were adapted from that of nitrification efficiency stated by [7]

$$\text{Percent } NO_3\text{-N removed (PNR)} = \frac{(NO_3\text{-}N_{in} - NO_3\text{-}N_{out}) \times 100}{NO_3\text{-}N_{in}}$$

$$\text{Volumetric Nitrate-nitrogen conversion rate } (mgNO_3\text{-}N/m^3d)\ VNR = \frac{86{,}400Q\,(NO_3\text{-}N_{in} - NO_3\text{-}N_{out})}{V}$$

Where:

NO_3-N_{in} is the Nitrate-nitrogen in the nitrification filtrate

NO_3-N_{out} is the Nitrate-nitrogen in the denitrification filtrate

V is the volume (m^3) of the media,

Q is the biofilter flow (m^3/day),

8,640 is a conversion factor (60x60x24).

Statistical / Data Analysis

Mean and standard deviation of the selected water quality parameters, change in water quality parameters, residence time and biofiltration rate were determined using descriptive statistics. The volumetric Nitrate-nitrogen conversion rate (VNR) and Percentage Nitrate-nitrogen removed (PNR) were calculated using the means of the Nitrate-nitrogen. One way analysis of variance (ANOVA) was used to determine if there was significant difference in Residence times, biofilter flow, PNR and VNR among the three drying time for denitrification column. Duncan multiple range test was used to determine the exact pairs of the drying times that were significantly different at $P < 0.05$. The statistical analysis was done using IBM SPSS version 20.

RESULTS AND DISCUSSION

Temperature, pH and Dissolved Oxygen

The temperature recorded in the nitrification filtrates ranged between 27.33±0.58^{o}C and 28.67±0.29^{o}C while in the denitrification filtrates, it ranged between 28.00±0.50^{o}C and 28.83±0.28^{o}C, with temperature increasing slightly with drying times (Table 1). The change in temperature between the influents and the effluents for the nitrification filtrates was highest at 24 hours drying (0.67±0.58^{o}C (2.39%)) while for the denitrification filtrates, 144 hours drying time had the highest change (-0.17±0.29 (-0.59%)), there was no change at 72 hours drying time (Table 2). The temperature readings in both the nitrification and denitrification filtrates were suitable for warm water fish culture, they were within the recommended level of 20 – 30^{o}C [3] and also safe for discharge into the environment [8]. The negative values in change in temperature in some of the drying times indicated that the filtrates temperature was higher than that of the influents and this may be attributed to the metabolic activities of the nitrifying and denitrifying bacteria. The low percentage change in values of temperature between the influents and the filtrates for

all the drying times showed that temperature in the systems did not experience wide variation and hence the culture organism will not experience sudden change in temperature and shock that may arise from it [11].

The DO of the filtrates from nitrification column was between 2.50±0.78 mg/L and 3.13±0.06 mg/L while that of denitrification filtrates ranged between 2.40±0.35 mg/L and 3.43±0.38 mg/L (Table 1). The highest change in DO between the influents and the effluents in the nitrification column was recorded at 144 hour drying time (0.20±0.65 mg/L (6.45%)) while 72 hours drying time had the highest in the denitrification column (0.40±0.92 mg/L (12.78%)) (Table 2). Though the dissolved oxygen values in both nitrification and denitrification filtrates were below the >4mg/L recommended for warm water fish culture [3], they were still within the safe range (>2mg/L) for discharge into surface water [8]. The DO can still support the metabolic activities of the nitrifying bacteria and this is in line with [14], who stated that, DO below 2mg/L will limit the activities of Nitrobacters and Nitrosomonas in the biological filters. The denitrification column is expected to be anaerobic, the DO present may constitute nuisance and affect the performance of the system. The negative values recorded in change in DO in some nitrification filtrates indicated that the filtrates DO is higher than that of the influents. This is contrary to the observation of [4], who recorded lower values for the filtrates compared to the influents. This can be attributed to the higher void ratio of Polypropylene media compared to that of sand used as biofilter media by [4]. The denitrification column is an anaerobic column and oxygen is not expected to be consumed, but the positive values reflected that filtrates had lesser values than influents. This may be attributed to presence of DO in the influents that encouraged some nitrifying bacteria to still thrive in the columns and that may likely reduce the denitrification efficiency.

The pH in the nitrification filtrates ranged between 7.37±0.06 and 7.47±0.32 while for the dentrification filtrates, it was between 7.47±0.23 and 7.57±0.25 (Table 1). The highest change in pH of 0.03±0.32 (0.40%) was recorded at 144 hours drying time in the nitrification filtrates while -0.07±0.06 (-0.94) was recorded at 24 hours drying time as the highest in the denitrification filtrates (Table 2). All the pH values were within the recommended range (6.5-8.5) for warm water fish culture [4] and range recommended (6.0 – 9.0) for discharge to the environment [8]. pH in the nitrification filtrates fell within the range of 7.0 – 8.0 regarded as optimum for nitrifying bacteria [17]. The percent change was very small for all the drying time and this indicated a stable system. The pH for all the denitrification filtrates was also within optimum range for denitrifying bacteria. A pH below 7.0 will lead to decrease in denitrification rate in the system [9,10].

Table 1: Mean±SD of the investigated water quality parameters

Parameter	Aquaculture effluent	Nitrification filtrate	Denitrification filtrate
		24 HRS	
Temperature (^oC)	28.00±0.00	27.33±0.58	28.00±0.50
Dissolved Oxygen (mg/L)	2.10±0.00	2.50±0.78	2.40±0.35
pH	7.20±0.00	7.47±0.06	7.53±.057
NH_4-N (mg/L)	5.40±0.00	3.90±0.00	2.73±2.02
NO_2-N (mg/L)	0.00±0.00	0.01±0.00	0.04±0.04
NO_3-N (mg/L)	5.00±0.00	8.33±2.87	5.00±5.00
		72 HRS	
Temperature (^oC)	26.00±0.00	28.17±0.29	28.17±0.29
Dissolved Oxygen (mg/L)	2.40±0.00	3.13±0.06	2.73±0.96
pH	7.30±0.00	7.37±0.06	7.47±0.23
NH_4-N (mg/L)	3.90±0.00	2.60±1.18	3.10±2.02
NO_2-N (mg/L)	0.01±0.00	0.13±0.03	0.14±2.17
NO_3-N (mg/L)	0.00±0.00	73.33±63.51	38.23±53.51
		144HRS	
Temperature (^oC)	28.50±0.00	28.67±0.29	28.83±0.28
Dissolved Oxygen (mg/L)	3.10±0.00	2.90±0.66	3.43±0.38
pH	7.50±0.00	7.47±0.32	7.57±0.25
NH_4-N (mg/L)	2.30±0.00	1.60±0.00	1.33±0.46
NO_2-N (mg/L)	0.00±0.00	0.15±0.00	0.15±0.00
NO_3-N (mg/L)	0.00±0.00	110.00±0.00	45.00±8.66

Nitrogen

The TAN recorded during the study period was between 3.90±0.00 mg/L and 1.60±0.00 mg/L in the nitrification filtrates while in the denitrification filtrates, TAN ranged between 3.10±2.02 mg/L and 1.33±0.46 mg/L

(Table 1). The highest change in TAN for the nitrification filtrates (1.30±1.18 mg/l (33.33%)) was observed at 72 hours drying time while in the denitrification filtrates, 1.17±2.02 mg/L (30.00%) was obtained as the highest change in TAN (Table 2). All the TAN reported were still within the recommended level (< 8.8 mg/L) for warm water fish culture [4]. Although the TAN was higher than what was reported by[5,18] but it was still within the range with observation of [4], who reported a value of 2.98 mg/L as a low TAN, The positive values at 24 and 144 hours drying time for the change in TAN in the denitrification column indicated that there was further nitrification in the system and this tends to affect the denitrification performance of the column, the systems were able to nitrify further probably because of the DO that was above 2mg/L [14].

The NO_2-N in the nitrification filtrates ranged between 0.01±0.00 mg/L and 0.15±0.00 mg/L while in the denitrification filtrates, it was between 0.04±0.04 mg/L and 0.15±0.00 mg/L (Table 1). The change in NO_2-N for the nitrification filtrates was highest at 72 hours (-0.12±0.03 mg/L (-1200%)) while -0.03±0.04 mg/L (-300%) recorded at 24 hours was the highest in the denitrification filtrates (Table 2). All the nitrite-nitrogen values were within the level recommended (<0.25 mg/L) for warm water fish culture [3]. The negative values in change in NO_2-N in the nitrification filtrates indicated that the values of NO_2-N in the filtrate were higher than that of the influents.

The NO_3-N recorded during the study period ranged between 8.33±2.87 mg/L and 110.00±0.00 mg/L, for the nitrification filtrates and between 5.00±5.00 mg/L and 73.33±63.51 mg/L for the denitrification filtrates (Table 1). The highest change in the NO_3-N between the influents and the filtrates (-110.00±0.00 (NA) mg/L) was recorded at 144 hours for the nitrification column and 65.00±8.66 mg/L (59.09%) also at 144 hours for the denitrification filtrates (Table 2). The NO_3-N concentration in the nitrification column was increasing with the drying time. This can be attributed to probable increase in the bacteria population with time, though no bacteria count was done to confirm. The observation established that nitrification did occur in all the systems. The values of the NO_3-N in all the filtrates were still within the recommended level (50 - 200 mg/L) for fish culture [3] but some were above the level recommended for discharge into the environment (11.3 mg/L). The denitrification can still be regarded to be low in all the systems compared to 100% NO_3-N removal achieved by [1] though at 144 hours drying time, it was better than 26% reported by [19].

Table 2: Mean±SD (%) of Change in investigated water quality parameters

Parameter	Nitrification filtrate (%)	Denitrification filtrate (%)
	24 HRS	
Temperature (^oC)	0.67±0.58 (2.39)	-0.67±0.29 (-2.45)
Dissolved oxygen (mg/L)	-0.40±0.78 (-19.00)	0.10±1.13 (4.00)
pH	-0.27±0.06 (-3.75)	-0.07±0.06 (-0.94)
NH_4-N (mg/L)	1.50±0.00 (27.78)	1.17±2.02 (30.00)
NO_2-N (mg/L)	-0.01±0.00 (NA)	-0.03±0.04 (-300.00)
NO_3-N (mg/L)	-3.33±2.89 (-66.60)	3.33±5.77 (39.98)
	72 HRS	
Temperature (^oC)	-2.17±0.29 (-8.35)	0.00±0.00 (0.00)
Dissolved oxygen (mg/L)	-0.73±0.06 (-30.4)	0.40±0.92 (12.78)
pH	-0.07±0.06 (-0.96)	-0.10±0.84 (-1.36)
NH_4-N (mg/L)	1.30±1.18 (33.33)	-0.50±0.87 (-19.23)
NO_2-N (mg/L)	-0.12±0.03 (-1200.00)	-0.01±2.20 (-7.69)
NO_3-N (mg/L)	-73.33±63.51 (NA)	35.10±10.00 (47.87)
	144 HRS	
Temperature (^oC)	-0.50±0.00 (-0.60)	-0.17±0.29 (-0.59)
Dissolved oxygen (mg/L)	0.20±0.65 (6.45)	-0.53±0.31 (-18.28)
pH	0.03±0.32 (0.40)	-0.10±0.10 (-1.34)
NH_4-N (mg/L)	0.70±0.00 (30.43)	0.27±0.46 (16.88)
NO_2-N (mg/L)	-0.15±0.00 (NA)	0.00±0.00 (0.00)
NO_3-N (mg/L)	-110.00±0.00 (NA)	65.00±8.66 (59.09)

(NA) means % change is Not applicable.

Residence time and biofilter flow

The residence time for 24, 72 and 144 hours drying time in the denitrification column were 39.37±1.20 s, 45.81±12.60 s and 31.34±1.15 s respectively and the biofilter flow were 10.14±0.13 $x10^{-5}$ m^3/s, 10.09±1.55 $x10^{-5}$ m^3/s and 9.68±0.27 $x10^{-5}$ m^3/s at the three drying time respectively, ANOVA showed no significant difference among the three drying times for both residence time and biofilter flow.

Table 3: Denitrification efficiency of the biofilters

DRYING TIMES (HOURS)	PNR (%)	VNR ($mgNO_3$-N/m^3d)
24 Hrs	33.33±57.74[a]	3116.88±599.60 [a]
72Hrs	47.87±4.19 [a]	14125.26±746.55[a]
144 Hrs	59.09±7.87[a]	58775.51±7068.43[b]

Values (means±SD) in the same column with different letters as superscripts are significantly different at $P < 0.05$.

Denitrification efficiency of the biofilters

The PNR for the filtrates were 33.33±57.74%, 47.87±4.19% and 59.09±7.87% for 24, 72 and 144 hours drying times respectively while the VNR were 3116.88±599.60 $mgNO_3$-N/m^3d, 14125.26±746.55 $mgNO_3$-N/m^3d and 58775.51±7068.43 $mgNO_3$-N/m^3d respectively (Table 3). ANOVA did not show any significant difference among the three drying times for the PNR while for the VNR there was significant difference between 24 and 144 hours and between 72 and 144 hours drying times at $P< 0.05$. The polypropylene media was able to reduce NO_3-N from above 100mg/L to a of 45mg/L (at 144 hours), this is similar to the findings of [19] and increase in both PNR and VNR with drying time showed that 24 hours is not the optimum drying time for the denitrification system. Therefore it can be established that biodenitrification is achievable if the proper conditions (pH, temperature and DO) are put in place, also that drying time has significant effect on the optimum performance of the biofilter system.

CONCLUSION

The study revealed that removal of NO_3-N in tropical aquaculture water reuse system can be achieved by incorporating denitrification column in addition to the usual nitrification column. It also showed that denitrification column in warm water fish culture may not be at optimum performance within 24 hours of starting the system and that running the system for up to 144 hours before loading with the wastewater in recirculating system will enhance building of denitrifying bacteria and hence assured better performance especially where the system is used for grow-out (table size) fish and high metabolic waste is expected. It is also worthy of note that dissolved oxygen and pH are extremely important water quality parameters for optimum performance of denitrification system and therefore should be maintained within the recommended range for the system. Furthermore, it can be stated that zero discharge aquaculture system can be achieved if denitrification column is incorporated into the components of recirculating aquaculture systems. This will enhance sustainable aquaculture production and save the aquatic environment from deterioration due to aquaculture effluents.

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