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TITLE

Influence of temperature, ammonia load and hydraulic loading on the performance of nitrifying trickling filters for recirculating aquaculture systems

ABSTRACT

In recirculating aquaculture systems, performance of nitrifying biofilters for total ammonia nitrogen (TAN) removal from the culture water and thus minimizing eutrophication depends on numerous elements of design. In this article the combined effect of three of these process parameters (temperature, hydraulic loading and TAN load) is evaluated. Ammonia removal rates (N-TAN divided by biofiltration area and day) were measured for every combination of five different temperatures, three different hydraulic loadings and three different ammonia loads. Every one of the process parameters were influential on nitrification rates and the lowest process parameters values corresponded with significantly lower N-TAN removal rates. A significantly higher mean N-TAN removal rate (0.241 gN-TAN removed m^{-2} day^{-1}) was found for the combination of the highest water temperature (27 ºC), the highest hydraulic loading (11

m^3 m^{-2} h^{-1}) and the highest TAN load (9 gTAN m^{-3} day^{-1}), suggesting a positive synergy of the three process parameters on the achievement of greater biofilter performances.

KEYWORDS: Recirculating aquaculture; trickling filters; nitrification kinetics; ammonia nitrogen

1 Introduction

Intensive aquaculture requires a meticulous waste treatment planning, especially in indoor recirculating aquaculture systems (RAS) [1,2]. Wastes of the aquaculture industry include fecal matter, uneaten food, and dissolved carbonous, nitrogenous and phosphorous compounds that accumulate rapidly in the production units, in particular in RAS with high hydraulic retention time [3], and filtration and purification systems must be implement to prevent fish health and welfare issues. Additionally, it is imperative this treatment before water is discharged to prevent environmental impacts [1].

Ammonia is one of the key water quality parameters to be controlled in recirculating aquaculture. It is generated and excreted as end product of the deamination of free amino acids taken in the fish diet with the purpose of generating energy [4]. Fish diets in aquaculture usually contain a high protein content. As a result, excretion of ammonia is quite high and is one of the chemical compounds that most quickly accumulates in water after the feeding period [5–8]. Ammonia is toxic to fishes, most notably in unionized form, causing nervous system-related malfunctions [9,10]. Several researchers have conducted toxicity experiments that demonstrate negative effects of low concentrations of NH_3 in both survival and growth rate for several species [11–13]. Because of that the guidelines for good aquaculture practices include recommendations on levels of unionized ammonia as low as 0.0125 mg L^{-1} [14].

Nitrifying biofilters are installed in recirculating aquaculture systems to reduce this ammonia concentration and transform it into the less toxic nitrate in two phases: (1) conversion of ammonia to nitrite and (2) conversion of nitrite to nitrate. Both conversions have been thoroughly studied [15,16]. The theoretical rate at which ammonia is eliminated follows a Monod-type equation directly dependent on the ammonia concentration if enough oxygen and $CaCO_3$ (limiting factors) are provided up until a certain limit [17]. However, the exact nitrification rate depends on multiple process parameters (besides the dissolved oxygen and $CaCO_3$ concentration described above), which and include filter media, TAN concentration in the biofilter influent, temperature, hydraulic loading, organic matter and pH [14,18].

Some of these process parameters require a compromise between maximizing biofilter performance and improving fish yield. For instance, an adequate temperature is crucial for maximizing the latter [19,20], but certain species that require cold water for survival and optimal growth such as turbot *(Psetta maxima),* which leads to problems in biofilter performance because nitrification is inhibited at low temperatures [21]. The TAN load (defined by the amount of dissolved ammonia-nitrogenous compounds excreted by fish and carried out to the biofilter for removal) is a result of fish density [22], fish size [23] or protein intake [24], different for every farming system. Hydraulic loading, which is defined as water flow divided by cross-sectional biofilter area [18], is an element of design of biofilters that can be increased via increasing water flow or shaping the biofilter increasing its height and reducing its diameter. Nijhof [25] first suggested a positive effect of the hydraulic loading on ammonia removal rate explained by an improved wetting of the filter medium and a higher TAN load rate. On the other hand, increasing water flow or the height of the biofilter would result in an increase of the energetic costs [26]. Therefore, the establishment of the optimal hydraulic loading is very important.

Due to the particular importance of these three process parameters being temperature, TAN loading and hydraulic loading, the combination of them have been tested in this paper, selecting five temperatures (16 ºC, 18 ºC, 21 ºC, 24 ºC and 27 ºC) three TAN loading rates (3 gTAN m^{-3} day^{-1}, 6 gTAN m^{-3} day^{-1} and 9 gTAN m^{-3} day^{-1}) and three hydraulic loadings (11 m^3 m^{-2} h^{-1}, 8 m^3 m^{-2} h^{-1} and 4 m^3 m^{-2} h^{-1}) and designing a three-factorial assay in which a range of N-TAN removal rates will be presented depending on potential fish production plans of aquaculture facilities.

Another important relevance of this research is the biofilter performance measurements in saltwater, whose literature is exceedingly low compared to freshwater systems, maybe on the grounds that most typical RAS facilities are employed for the culture of freshwater species or freshwater cycle of Atlantic salmons [27].

2 Materials and methods

2.1 Experimental design

The experimental system was composed by independent six 330 L tanks connected to six independent trickling filters. Water was recirculated by peristaltic pumps (Oceanrunner® OR3500, Aqua-Medic®, Bissendorf, Germany). The height from the bottom of the drainpipe to the top of the filter was close to 2.5 m. Water was dispersed through the top area of the biofilters by means of “rain effect” assuring the whole surface was wet. Water flow provided by the pump was equal for the six subsystems (1.08 m^3 h^{-1}). A diagram of the experimental system is presented in Figure 1.

Figure 1

The pilot-scale trickling filters, composed by plastic nets with cylindrical form and covered by plastic, were constructed in a way that three different hydraulic loadings could be evaluated without altering water flow. Two identical biofilters per hydraulic loading were constructed. All six biofilters were filled with an equal volume (0.02 m^3) of Bactoballs® (plastic filter media, specific surface area 300 m^2 m^{-3}, Aqua-Medic®, Bissendorf, Germany). Detailed characteristics of biofilters are summarized in Table 1. Experiments were carried out in the Aquaculture Laboratory of the Polytechnic University of Valencia.

Table 1

Water flowing in the tanks was artificial sea water manufactured in the laboratory composed of tap water with the addition of 12 Kg of sea salt (Sea salt for human consumption, Salinera Española S.A., San Pedro del Pinatar, Murcia, Spain) for each 330 L of water thus establishing a constant 37‰ salinity. Tap water was kept in a large reservoir for 24 hours to ensure the removal of chlorine. Water temperature was adjusted by internal heaters (EHEIM thermocontrol 300, Eheim GmbH, Deizisau, Germany), depending on the trial. Water was completely removed from every tank at the end of each trial, and replaced with ammonia-free (as well as other substances) water in which the next trials were carried out. This was done to ensure the least possible amount of interferences, as well as keeping pH and alkalinity identical for each biofilter performance measurement.

During the development of the microbial communities, 1.5 g of ammonium chloride was added daily to each tank. Alkalinity was measured every two days and sodium bicarbonate was added when needed to compensate the CO_3^- consumed by the bacteria. This procedure was followed up to six weeks, when the biofilter acquired full-grown status. TAN concentration was measured at 9:00 A.M. every day until it was 0 mg L^{-1} for several consecutive days. After that, performance measurements were initiated.

2.2 Performance measurements

An equal amount of ammonium chloride was added to each one of the six tanks following an identical protocol summarized in Table 2 which simulated a wide feeding period. Prior to the ammonia addition and every 2 hours until 24 hours since the addition samples were collected. The ammonia removal rates were calculated by difference between the ammonia concentration at the beginning (t=0) plus the ammonia that was added to the tanks minus the ammonia concentration at the end of periods of 24 hours, divided by biofiltration area. This protocol was performed twice in every tank (12 total) for each one of the three TAN load rates tested at a certain temperature. After that the temperature was increased to the next value and the protocol was carried out in the same manner.

TAN, nitrite, nitrate and oxygen concentrations, pH and temperature of the biofilter effluent of the six biofilters were measured every two hours along a 24-hour period. Alkalinity was also measured every six hours, beginning at 8:00 A.M. Protocol followed in every 24-hour sampling was identical, except for the change in the dosage of ammonium chloride.

Table 2

2.3 Analytical methods

Temperature and oxygen were measured by a Handy Polaris® oximeter (OxyGuard®, Farum, Denmark). pH was measured by Orion® 4-Star Plus probe (ThermoScientific®, Waltham, Massachusetts, USA) and its associated pH electrode. No further data processing was necessary, and values obtained are presented directly in the paper.

TAN and nitrite concentration were measured by spectrophotometry. The apparatus used in both determinations was a T60V UV-VIS spectrophotometer (PG Instruments, Leicester, UK). TAN concentration was determined with the indophenol method. Nitrite was measured by the Griess determination involving sulfanilamide and N-(1-naphthyl)ethylendiamine dihydrochloride. For the measuring of nitrate, a novel determination was carried out based on the use of vanadium(III) chloride as a reducing agent, first described in the paper of Miranda *et al.* (2001) and developed by Schnetger and Lehners (2014) for microplate readers. TAN and nitrite measurements were directly carried out as the samples were collected, and nitrate was measured for samples that were filtrated and then frozen at -18ºC up until the measurement day, which was performed with a Victor 1420 microplate reader (Perkin Elmer, formerly Wallac Oy, Massachussets, USA).

2.3. Statistics

Ammonia removal rates were calculated according to the following equation:

$$Ammonia\ removal\ rate = \frac{Tank\ volume\ (m^3)\ (N\text{-}TAN_{t=0} + N\text{-}TAN_{added} - \ N\text{-}TAN_{t=24})}{Biofiltration\ area\ (m^2)}$$

where N-TAN(t=0) is the N-TAN concentration of the samples taken before the corresponding dosage of ammonium chloride and N-TAN(t=24) is the N-TAN concentration of the same taken 24 hours after. Ammonia removal rates, maximum and minimum concentrations are presented as mean ± standard deviation of their respective groups, minimum of four measurements (trial performed twice per two identical biofilters).

A multifactorial ANOVA was performed to evaluate the significance of the three process parameters on the achievement of mean N-TAN removal rates. Multiple range tests (Tukey HSD) were performed to obtain significant differences on N-TAN removal rates depending on individual parameters and a multiple regression was performed to quantify the relationship between the parameters and the nitrification rate. Additionally, the mean maximum N-TAN concentration (among the values measured) value is indicated as well as the minimum N-TAN concentration (value of the 24th hour if ammonia remained in water), being these two factors relevant to fish welfare and survival.

All ANOVAS and regressions were performed by Statgraphics® Centurion XVII for Microsoft Windows®.

3 Results

3.1 N-TAN, nitrite and nitrate hourly variation

Figure 2, Figure 3 and Figure 4 show the variation of N-TAN, N-NO_2^- and N-NO_3^- concentrations throughout the biofilter performance measurements, which were obtained every two hours for every combination of water temperature and hydraulic loading. N-TAN rapidly ascended after the TAN addition (which simulated fish feeding period) and in every case, rose until it reached a peak approximately after 6 hours, and then started to descend. The variation pattern was similar irrespective of hydraulic loading and temperature, although maximum and minimum N-TAN concentrations, as well as N-TAN decrease rate varied with each combination of process parameters.

Figure 2

Figure 3

Figure 4

N-NO_2^- showed a small variation for all trials, the concentration levels rose and decreased shortly after. The rise of NO_2^- concentration was notably bigger for the combination of water temperature of 27ºC and hydraulic loading of 11 and 8 m^3 m^{-2} h^{-1}.

N-NO_3^- concentrations increased throughout the 24-hour period for every combination of temperature and hydraulic loading, the concentrations being dependent mainly on N-TAN concentrations. Nitrification was therefore achieved for every combination of process parameters.

3.2 Alkalinity

Alkalinity (measured as mg$CaCO_3$ L^{-1}) decreased unceasingly during the 24 hour trials. The reduction was directly related to the amount of TAN consumed by the bacteria as it can be seen in Figure 5. A linear regression is relatively well adjusted to the data (R=57.39%) although the random factor in quite high as well. Due to the constant replacement of tank water prior to biofilter performance measurements, alkalinity was similar for all tanks at time 0 (average=168.96 mg$CaCO_3$ L^{-1}±1.72, relatively high due to the nature of Valencian freshwater) and never was completely emptied after 24 hours (average=140.20 mg$CaCO_3$ L^{-1}±4.55).

Figure 5

3.3 Influence of process parameters on N-TAN removal rate

Table 3

Results of the multivariate ANOVA (Table 3) indicate that the three factors had a significant effect on the mean ammonia removal rate, as well as the interaction of TAN load with both temperature and hydraulic loading (p-value<0.05). Multiple range tests were made for each individual process parameter to analyze the sole influence of a particular process parameter on nitrification rates irrespective of the value of the remaining ones.

In the case of temperature (Table 4), mean water temperatures of 24ºC and 27ºC led to the achievement of the highest mean N-TAN removal rates (0.146 and 0.157 gN-TAN m^{-2} day^{-1}, respectively), without significant differences between them. There were no significant differences of N-TAN removal rates between the remaining temperatures. On the other hand, there were no significant differences on maximum or minimum TAN concentrations observed at any temperature.

Table 4

According to the hydraulic loading of the biofilters (Table 5), a hydraulic loading of 4 m^3 m^{-2} h^{-1} led to the lowest mean N-TAN removal rate. There were no significant differences between the mean N-TAN removal rate of biofilters whose hydraulic loading were 8 and 11 m^3 m^{-2} h^{-1} (0.137 and 0.143 gN-TAN m^{-2} day^{-1}). No significant differences were found on the maximum TAN concentrations for any hydraulic loading but mean minimum TAN concentration was found to be significantly higher (3.236±0.275 gN-TAN m^{-2} day^{-1}) for the hydraulic loading of 4 m^3 m^{-2} h^{-1}.

Table 5

The influence of TAN load proved to be also significant on nitrification rate (Table 6). Maximum mean N-TAN removal rates were obtained when 6 and 9 gTAN m^{-3} day^{-1} were added (0.141 and 0.154 gN-TAN m^{-2} day^{-1}, respectively). There were no significant differences between these two N-TAN removal rates. Maximum and minimum TAN concentrations augmented significantly with every increase of TAN load.

Table 6

A model generated with the equation of a multiple regression based on the data is presented in Figure 6. The influence of temperature and TAN load on nitrification rate was similar for every hydraulic loading tested, but the performance of biofilters with hydraulic loading of 4 m^3 m^{-2} h^{-1} showed considerably lower performance that the biofilters with other hydraulic loadings.

Figure 6

3.4 Influence of the combination of process parameters on N-TAN removal rates, maximum and minimum N-TAN concentrations

Differences on mean N-TAN removal rates, maximum and minimum N-TAN concentrations obtained on the trials in which process parameters' values were constant were analyzed with several one-way ANOVAS and multiple range tests (Tukey HSD). Results also provided information on the relative strength of the individual process parameters, based on the information gathered with the multivariate ANOVA as well as the one-way ANOVAS made for the singular parameters.

With regard to N-TAN removal rates (Table 7), it is observed that different hydraulic loadings at constant TAN loads and temperatures, were not particularly influential on nitrification rates at lower temperatures. However, as temperatures increased, hydraulic loading started to influence nitrification rates in the same matter as showed in Table 5, being the biofilters with hydraulic loading of 4 m^3 m^{-2} h^{-1} which significantly removed less ammonia from the rearing water. At higher temperatures (24ºC and 27ºC) and low TAN load (3 gTAN m^{-3} day^{-1}) nitrification rates were very similar for every biofilter due to the entire amount of ammonia being removed from the rearing water.

Similarly, N-TAN removal rates generally increased along with the temperature, particularly on biofilters with a hydraulic loading of 11 m^3 m^{-2} h^{-1}.

Table 7

DISCUSSION

Results of the multivariate ANOVA demonstrated that the three parameters studied (hydraulic loading, TAN load and water temperature) affect the performance of nitrifying trickling filters. A multiple linear regression was able to quantify well (R^2=0.88) the influence of the three process parameters within the ranges studied on biofilter performance, which cover a large range of temperatures and daily TAN productions. Figure 6 represents the range of possible nitrification rates based on the combination of process parameters. The efforts in the constant replacement of tank water as well as homogenization of other parameters critical to nitrification rates (such as $CaCO_3$ concentration) further reinforces the absence of interferences. Both initial and final alkalinity levels reached in the trails were apparently far from the levels required to reduce nitrification [30]. Oxygen concentration was constant (6.499 ± 0.025 mg L^{-1}) during the 24 hour measurements and did not become a limiting factor for any combination of variables. No significant drops of pH were observed as well (mean pH calculated was 8.363 ± 0.008.

In our experiment, TAN load had the most prominent influence on N-TAN removal rates. At the lowest TAN load (3 gTAN m^{-3} day^{-1}) mean N-TAN removal rate was significantly lower (0.092 gN-TAN m^{-2} day^{-1}) that at higher TAN loads, although there were no significant differences between the remaining TAN loads (6 gTAN m^{-3} day^{-1} and 9 gTAN m^{-3} day^{-1}) on nitrification rates. Usually it is also reported elsewhere [31,32] a direct relationship between

TAN load and nitrification rate up until a transition concentration, therefore our results are in concordance with literature. In these papers, lower biofilm capacity at low influent ammonia concentrations is usually explained by the lack of mass ammonia availability rather than diffusion limitations. In this paper, at lower TAN loads, all ammonia is removed from the bulk water provided temperature and hydraulic loading are favorable (Figures 2, 3 and 4). N-TAN concentration reaches 0 before the 24-hour period in some cases, which means that full biofiltration potential is not reached. However, increasing TAN load from 6 to 9 gTAN m^{-3} day^{-1} did not lead to an increasing nitrification rate which also shows as well the limitation of increasing ammonia influent concentration on improving biofilter performance. Nevertheless, N-TAN concentrations (both maximum and minimum), were significantly lower for the 6 gTAN m^{-3} day^{-1} TAN load, which may shift aquaculture management towards this particular process parameter value. They were as well significantly lower for the lowest TAN load tested (3 gTAN m^{-3} day^{-1}), therefore they might be a convenient tradeoff for achieving lower nitrification rates. As shown in Figure 5, final alkalinity concentrations were in average lower as well the bigger the nitrification rate was, although the decrease of pH through the trials were not significant. Therefore, no drawbacks are to be expected if $CaCO_3$ are kept to a minimum adding base to bulk water almost once a day [33].

Biofilters with the lowest hydraulic loading tested (4 m^3 m^{-2} h^{-1}) showed a significantly lower performance compared with the biofilters with higher hydraulic loadings. This relationship between hydraulic loading and nitrification rate has been previously suggested by various reserarchers [26,34], being the ammonia availability the basis of this relationship as well, being that at equal influent ammonia concentrations, an increase of the water flow results in a higher TAN load. They also stated that there is a limit on the positive influence of hydraulic loading on achieving higher TAN removal, which has been also demonstrated in this paper. Nitrification rates were not significantly higher for the highest hydraulic loading (11 m^3 m^{-2} h^{-1}) for any combination of TAN load and temperature compared to the second highest (8 m^3 m^{-2} h^{-1}). Peng *et al.*,[35] tested similar hydraulic loadings used in our study, found comparable relationship between hydraulic loading rate and nitrification rates. They found no significant differences between nitrification rates for biofilter with 8.3 and 12.5 m3 m^{-2} h^{-1}our, but an increasing positive effect for lower hydraulic loading. Greiner and Timmons [36] concluded that hydraulic loading did not showed to be influential to nitrification rates, however the hydraulic loadings studied by them were far superior to the hydraulic loadings tested in this research (19.54 m^3 m^{-2} h^{-1} being their lowest and 11 m^3 m^{-2} h^{-1} being our highest).

Literature suggests that insufficient wetting of the media is responsible for the reduced nitrification rates shown by low hydraulic loading biofilters. Van den Akker *et al.*, [31] investigated the effect of increasing water flow (thus increasing hydraulic loading) on the overall ammonia removal rate and concluded that biofilter performance was higher due to an active filtration overall biofilter area. Moreover, an excessive hydraulic loading results on either sloughing of the biofilm by high water flows or bacterial stratification in taller biofilters. Bacterial stratification inside biofilters has been observed by other authors [31,37], and it would had possibly occurred in our experiment, due to the fact that the highest hydraulic loading biofilm was shaped as a tall cylinder in contrast to the second highest.

Hydraulic loading did not affect the maximum TAN concentration observed in the rearing water. However, significant differences were found in the minimum TAN concentration according to nitrification rate. Biofilters that showed higher nitrification rates removed more ammonia from the water and as a result after 24 hours the ammonia concentration was lower, but it did not prevent achieving similar peaks of TAN concentrations as with the other biofilters.

Temperature also affected nitrification rates. In particular, mean water temperatures of 24ºC and 27ºC led to the achievement of the highest mean N-TAN removal rates (0.146 and 0.157 gN-TAN m^{-2} day^{-1}, respectively). This direct relationship has been published on other articles [38,39], and according to the results found in literature as well as the results presented on this paper, there might be a biofilter performance increase at even higher temperatures, but these are going to be unlikely fit for the culture of marketable species. On the other hand, no significant differences were found on maximum or minimum N-TAN concentration between every temperature.

The combination of temperature and other process parameters had an interesting effect on biofilter performance (Table 7). For instance, only at high temperatures (21 ºC, 24 ºC and 27ºC) significant differences on N-TAN removal rates between hydraulic loadings were observed. This suggests that if nitrifiers are inactivated by low temperature, their relative abundance and/or nitrification potential may not be perceived enough to establish a difference between several biofilter configurations. Considering only biofilters with a set hydraulic loading, it is also observed that different temperatures led to significantly different ammonia removal rates particularly at the lowest TAN load, as well as among the biofilters with hydraulic loading of 11 m^3 m^{-2} h^{-1} in the other TAN loads tested. The latter data may suggest a synergetic effect between temperature and hydraulic loading. The highest mean N-TAN removal rate (0.241 gN-TAN removed m^{-2} day^{-1}) was observed on the trials with biofilters with a hydraulic loading of 11 m^3 m^{-2} h^{-1}, water temperature of 27 ºC and TAN load of 9 gTAN m^{-3} day^{-1}.

In conclusion, this paper addresses the importance of the three process parameters studied, as well as their interactions with one another. Figure 3 and well as Table 7 provide a summary on the mean nitrification rates obtained by a large set of combinations of process parameters values expressed by N-TAN removed by day and biofiltration area. For the volume of the pilot scale biofilters used in this experiment, ammonia was not completely removed from the rearing water during the day for the majority of the process parameter combinations (Figure 2, Figure 3 and Figure 4), hence TAN is expected to be accumulated with every feeding. The quantity of ammonia removed from the bulk water could increase if larger biofilters (provided the hydraulic loading remains constant) are installed, although additional RAS engineering is perfectly suitable for improving N-TAN removal such as divide the effluent of the water tanks and pump it into two identical biofilters instead of one or even heating of the biofilter influent water or the biofilter itself with microwave heating as some authors have suggested [40], although probably not economically convenient.

Another important thing to consider is that operational hydraulic loadings are dependent on filter media [18], thus the nitrification rates obtained in the paper respective of hydraulic loading may be interesting for the design and construction of trickling filter with Bactoballs® (Aqua-Medic®, Bissendorf, Germany) or plastic filter media with similar characteristics.

CONCLUSIONS

TAN load, temperature and hydraulic loading proved to be influential on the achieving of particular ammonia removal rates, being the TAN load the most determinant. The relationship between TAN load and ammonia removal rate is, however, not linear, and increasing TAN load may only lead to increase the environmental ammonia in rearing water, harmful to fish welfare. Very low temperatures and hydraulic loadings inactivate biofilms and lead to this influence being much less noticeable.

CONFLICT OF INTEREST

Declarations of interest: none

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