Ammonia, nitrite and nitrate separation from sweet water by nanofiltration

Beatriz Cancino-Madariaga^{a,*}, Carlos Felipe Hurtado^b, Paula Pinto Villegas^a

^aINPROMEM (Research in Membrane Processes), Carrera 241, Villa Alemana, Chile, email: bcancinomadariaga@gmail.com, beatrizcancino-madariaga@inpromem.cl (B. Cancino-Madariaga), paula.pv.84@gmail.com, paula.pinto@inpromem.cl (P.P. Villegas) ^bSchool of Marine Sciences, Pontificia Universidad Católica de Valparaíso, Av, Altamirano 1480, Valparaíso Chile, Tel. 56322274264, Fax 56-32-2274264, email: felipe.hurtado@ucv.cl (C.F. Hurtado)

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ABSTRACT

The aim of this study is to show the use of nanofiltration to separate nitrogen compounds (ammonia, nitrate and nitrite) from water in recirculating aquaculture systems (RAS). In previous studies, we analysed the NF 270 membrane to separate nitrite, nitrate and ammonia from water separately. The best transmembrane pressure (TMP) was 15 bar. In the present study, the idea is to investigate the separation of all the N-compounds together, using the same membrane and TMP as before, but with a water hardness similar to that of RAS in Chile. The high concentrations of N-compounds used in the study were selected in accordance with dangerous limits for fish in RAS. Experiments using soft water with high concentrations of nitrite and nitrate (3.5 mg/L and 150 mg/L, respectively) and 1.5 mg/L of TAN (total ammonia nitrogen) showed there is no influence from nitrite and nitrate concentration on TAN retention, with a rejection percentage (%R) between 45 and 55% for all. Similar behavior is seen when TAN is 5 mg/L. However, when the nitrite and nitrate concentrations are low (0.5 mg/L and 5 mg/L, respectively), there is an important difference in TAN retention. In hard water, the retention of the membrane is different, and there are significant differences in the retention of the nitrogen compounds. With this data, a membrane process to separate the different nitrogen compounds using an NF membrane was proposed. In the proposed strategy, the separation must be focused on TAN retention. The %R for TAN is close to 80% when the nitrite and nitrate concentrations are low. In this case, the permeate of the membrane system can be returned to the fish tank to recycle the water.

Keywords: Ammonia; Aquaculture; Nanofiltration; Nitrate removal; Nitrite removal

1. Introduction

In recent years, clean freshwater has gone from being a plentiful and free resource to a scarce and expensive "ingredient" for different uses [1–3]. In industry, the trend is to reuse water after a treatment process chosen in accordance with the quality that is required. This is not any different in the aquaculture industry where water is the principal component. Local fishermen who once collected their products from the sea or from lakes have been encouraged to become "farmers", where the "fish crop" is seen as an industry governed by a common problem: water availability and its quality [4–6]. In this new scenario, Recirculating Aquaculture Systems (RAS) are increasingly common, with the purpose of reusing water. However, in a traditional RAS, only a small percentage of the water is treated: currently less than 1% [7,8]. To date, the most common treatment system uses nitrifying bacteria (biofilter) to control ammonium compounds that are the main pollutants in aquaculture produced by fish metabolism [9]. Biofilters are used in other industries with similar purposes, i.e., the treatment of N-molecules, such as in municipal wastewater plants [10,11], for drinking water purification [12], for drainage water [13] and in different wastewater treatments in industries [14–17].

When using RAS and biofilters some problems are caused by the normal behavior of microorganisms, producing a high degree of fluctuation in the efficiency of the nitrification process [18–21]. For example, increasing the stocking

^{*}Corresponding author.

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density in RAS implies more waste from fish metabolism which tends to increase the concentration of anions and cations involved in nitrogen and carbon cycles [22], as well as dissolved minerals [23]. The main compounds produced in fish metabolism are ammonium (cation), nitrite (anion) and nitrate (anion) [7,24]. These molecules are dangerous for the fish and produce different diseases when their concentration levels in an RAS are over a certain limit, depending on the species [18,19]. In general, ammonia and nitrite are toxic for fish at a level above 0.02 mg/L and 2.0 mg/L, respectively [25]. Nitrate is the final product in biofilters and the least toxic compound in the nitrification system. Nitrate concentrations in water between 200 and 500 mg/L are tolerable to fish [26-28]. However, levels above 100 mg/L produce growth problems and have a negative effect on feed conversion [25]. Nitrate levels in RAS are normally maintained by daily water renovation [29]. Changes in the temperature of biofilters also generate problems with process efficiency that are not easy to control [30]. If biofilters do not work properly, they can increase the level of ammonia and nitrite, thereby increasing fish mortality [8,31–34].

In a biofilter, two different species of bacteria are used in two consecutive stages. Nitrosomonas convert ammonium (NH_4^+) to nitrite (NO_2^-) and Nitrobacter convert nitrite to nitrate (NO_3^-) [18,35,36]. Nitrification is the oxidation of ammonia to nitrite and nitrite is converted to nitrate by autotrophic bacteria [37]. This fixed-film biological process occurs in biofilters [38,39]. In fact, an RAS can be expected to contain all three N-molecules, ammonium, nitrite and nitrate, where their concentration in the water is the parameter to be controlled.

In addition to fish production, N-molecules appear in groundwater mainly as a result of the intensive application of fertilisers [9,40] causing health problems to humans.

Therefore, the separation of N-molecules is an important issue for freshwater aquaculture and for drinking water production. In a previous paper, our group studied the separation of different N-molecules using nanofiltration membranes separately, i.e., ammonium separately from nitrite and nitrate. The purpose was to identify the mechanisms of this separation and the range of pressure, pH, retention percentage and membrane types that would optimise separation. In the present study, we analyse the separation of the three N-molecules together, giving the rejection percentage for each one, and proposing a separation strategy based on previous research together with the new results. Thus, the aim of this study is to propose a nanofiltration process to separate ammonium, nitrite and nitrate from freshwater using different process conditions.

2. Materials and methods

Following the same protocol and equipment used in previous research [41], the tests were carried out using three stainless steel cells in parallel connection (Fig. 1), developed previously by INPROMEM (Research in Membrane Processes). Each cell can resist 100 bar and contains a removable flat disc membrane of 5 cm diameter ($1.65 \times 10^{-3} \text{ m}^2$ of membrane area). The membrane used was NF 270 (Dow Liquid Separations, USA), a commercial nanofiltration membrane. The equipment also includes a high-pressure pump, (Speck



Fig. 1. Schematic representation of the membrane system arrange that was the same than in Hurtado et al. (2016). C(i) is the Concentration of the species in the feed of the cell arrange. C(c) is the Concentration of the species in the retentate and C(p) is the concentration of the species in the permeate. (1) High pressure cell, (2) high pressure pump, (3) manometers, and (4) valve.

GmbH pump, Germany) working between 0 and 140 bar, and a Resun model C-1500 chiller to control temperature [41,42]. The pump allows the permeate to return to the feed tank, which is useful for evaluation of membrane resistance, or it can discharge the permeate to evaluate the other conditions of interest in this study. To determine the permeate flow, the equipment includes an electronic scale to record mass flow (Radwag 220, Poland). Data acquisition was carried out using software developed by INPROMEM. The total volume in the feed tank is 5 L.

Each membrane was washed and conditioned according to the protocol described in Cancino-Madariaga et al. [42].

The permeate from each cell was analysed to confirm the reproducibility of the experiment. Parameters were evaluated in the permeate and concentrate in accordance with the protocol developed by Hurtado et al. [41]. The TMP was set at 15 bar.

Ammonium (NH₄⁺) was prepared using ammonium salt (NH₄⁺Cl⁻, Merck Germany). Anions of NO₂⁻ and NO₃⁻ were obtained from NaNO₂ and NaNO₃, which were in crystalizing salt form. Two kinds of water were tested, with varying levels of hardness [41]:

- a. Standard freshwater with low concentrations of salts, called "soft water", (see Table 1 for the composition), and
- b. Standard freshwater with high concentrations of salts, called "hard water" (see Table 1 for the composition).

The initial volume used for each experiment was 5 L and the conductivity of the deionised water used in the experiments was lower than $5 \,\mu$ S/cm.

For each set of measurements described below, samples of 200–300 mL were taken. The permeate volume was accumulated during the process time until this sample volume

was attained. Each set of measurements considered the analysis described below, in accordance with Hurtado et al. [41], except for the ammonium, which was taken from Cancino-Madariaga et al. [42].

Ammonium concentration was measured using a multi-parameter photometer (Hanna Instruments, series HI-83000) with a resolution of 0.01 mg/L and a typical EMC deviation of ± 0.02 .

Nitrite concentration was determined in the form of NO₂⁻ using a Hach model DR3900 spectrophotometer, with the program 371N and Hach Method 8507 diazotisation.

Nitrate concentration was determined in the form of NO₂, this anion was determined using ion selective probe nitrate, model ISENO318101, Hach.

Sulphate concentration was determined in the form of SO₄²⁻ using a Hach model DR3900 spectrophotometer, with the program 680 Sulfate and Hach Sulfaver 4 turbidimetric method.

Sodium concentration was determined using cation selective probe sodium ion Na⁺, ISENa38101 model, Hach.

Total hardness and Calcium Hardness were determined in the form of CaCO₃ using the titration method, included in the HA-DT mark of the Hach kit. The methods 8213 and 8204 for calcium hardness were used for total hardness, both using EDTA solution.

pH and Temperature were determined using a pH meter and temperature probe, connected to desktop Spercientific 860,031.

Conductivity was determined using a conductivity probe connected to the meter desktop Spercientific 860,031.

2.1. Description of the experiments

The study was separated into two phases (Table 2) with different feed solutions formulated to analyse the behavior using the NF 270 membrane at 15 bar of TMP. This TMP was selected based on the rejection percentage (R%) shown in Hurtado et al. [41] for Nitrite and Nitrate, as well as non-published data for NH₄⁺. For TMP higher than 15 bar, the improvement in R% is not statistically significant using the Bonferroni method (multiple range test, using a 95% confidence level with Bonferroni).

Phase I tests involved NH_4^+ , NO_2^- and NO_3^- dissolved in the soft water. The concentrations of the N-compounds were selected to represent two extreme concentrations named Low (L) and High (H).

The NH_4^+ concentrations were 1.5 and 5.0 mg/L based on the maximum NH₄⁺ concentration permitted in RAS (1.5 mg/L), and the expected concentration in a membrane process with recirculation of the concentrate (5 mg/L) based on previous experiments [18].

The high and low concentrations of NO₂⁻ and NO₂⁻ were based on a compilation of the main field RAS currently in operation in Chile [41]. The low and high concentrations of NO_2^- were 0.5 and 3.5 mg/L, respectively and for NO_3^- they were 5.0 and 150 mg/L, respectively.

Phase II involved NH_4^+ , NO_2^- and NO_3^- dissolved in the hard water (see Table 2).

The differences between Phase I and II is the hardness of the water in which the N-molecules are dissolved, as shown in Table 1.

Temperature and pH were 15 ± 1°C and 7.0 respectively, since these parameters are the most common in RAS operation in Chile [35,41].

The rejection percentage (%R) for NH₄⁺, NO₃⁻ or NO₂⁻ was calculated using Eq. (1).

$$\%R = \left[1 - \left(\frac{C_p}{C_f}\right)\right] \times 100\tag{1}$$

where C_n and C_{ℓ} represent the N-molecule concentrations (mg/L) in the permeate and concentrate, respectively.

Table 2 Description of the experimental phases, concentration of NO₂and NO,

Phase	Water type	Concentration (mg/L) in solution		Concentration level of nitrite	
		NH_4^+	NO_2^{-}	NO_3^-	and nitrate *
Ι	Soft	1.5	0.5	5.0	L
		5.0	0.5	5.0	
		1.5	3.5	150.0	Н
		5.0	3.5	150.0	
Π	Hard	1.5	0.5	5.0	L
		5.0	0.5	5.0	
		1.5	3.5	150.0	Н
		5.0	3.5	150.0	

* L: Low concentration, H: High concentration.

Table 1

Composition and hardness of the water used as hard water and soft water [41]						
Salts and quality parameters	Soft water mg/L	Hard water mg/L	Hard water mg/L			
NaHCO ₃	48	192				
$CaSO_4 \cdot 2H_2O$	30	120				
MgSO ₄	30	120				
KCl	2	8				
pH	7.2–7.6	7.6-8.0				
Total hardness	40–48	160–180				
Alkalinity expressed in mg CaCO ₃ /L	30–35	110–120				

The chemical analyses were carried out in triplicate and their results were processed statistically using the commercial software Centurion Statgraphics and using ANOVA and Fisher's LSD at 95% of confidence, according to the distribution of the data [43].

3. Results and discussion

The results of the rejection percentage of the N-Molecules in the soft and hard water are shown in Fig. 2.

It can be seen in Fig. 2a, that for the soft water and TAN rejection (see number 1 and 2) there are significant differences when the nitrite and nitrate concentrations are high. For low concentration, the %R is similar for both concentrations of TAN, 1.5 and 5 mg/L.

For NO₂⁻, the % R is different only when all the concentrations are low, i.e., number 3 in Fig. 2a. However, for NO₂⁻, the % R is close to 55% in all other situations (number 1, 2 and 4).

In the case of NO_3^- , the %*R* does not depend on L or H. However, in the separation of the different N-molecules, it can be seen that for the low concentration of ammonium (TAN) and the high concentrations of nitrite and nitrate, there are no differences between the results, which are between 45 and 55%. Similar behavior is observed at 5 mg/L TAN. However, when the concentration of nitrite and nitrate are reduced to the low concentration, the difference between the % R values is remarkable. This means that separation in soft water depends mainly on the nitrite and nitrate concentrations and not on the TAN concentration.

Fig. 2b shows the results for the hard water. The differences in the % R values for TAN increase for the soft water using high and low concentrations of nitrite and nitrate. For NO₂⁻ rejection, there are no differences between H and L, though there are differences for NO₃⁻.

The rejection percentage for ammonium in the hard and soft water is more or less in the same range as the differences mentioned before. However, for $NO_{2'}$ the % R is lower in the case of the hard water (40% approximately) in comparison with the soft water (between 45 to 55%) except for the case of low concentrations of all N compounds and soft water (Fig. 2a), for which it is close to 20 % R.

For NO_3^- , there are differences between the soft and hard water for all cases.

The results for the flux during the experiment time are shown in Fig. 3. For the soft water, the highest flux is when the solution contains low TAN (1.5 mg/L) and the concentration for nitrite and nitrate is high. The same behavior is observed for the hard water. The statistical analysis between the different experiments in Fig. 3 shows significant differences in flux between them using the LSD fisher analysis



Fig. 2. Rejection percentage for the N-molecules at Low (L) and High (H) concentration in (a) Soft Water and (b) Hard water at 15 bar transmembrane pressure.



Fig. 3. Permeate Flux during the time in (a) Soft water solutions, with N-molecules at different concentration with 15 bar. (b) in Hard water solutions, with N-molecules at different concentration with 15 bar.

with a 95% of confidence. However for each experiments, the flux is constant over time as proved by the p-value > 0.05 from the ANOVA test.

Fig. 4 shows the %*R* of the salts depending on different concentrations of the N-compounds. CaCO₃, Ca⁺², SO₄⁻², and Mg⁺² present %*R* values over 70%, depending on the water hardness. In Fig. 4, carbonate was measured using the total hardness method described in the Materials and Methods section. This method was preferred over the Alkalinity method, because the limit of detection is 10 mg/L compared with 25 mg/L for the Alkalinity method. Different methods produce a variation with the results shown in Hurtado et al. [41], where the CaCO₃ was measured using the Alkalinity method.

In the aquaculture industry, it is desirable to maintain carbonate within certain limits in the system because it is used in fish metabolism. To do that it is necessary that the concentrate of the membrane systems is returned to the aquaculture tank. This situation is not convenient, because the N-compounds also present high %*R* values. If the carbonate is returned to the tank, the N-compounds will also increase in the tank. To solve the problem of decreasing carbonate concentration, salt can be added to the water at the adequate level.

Fig. 5 shows the traditional and the proposed membrane process. Here, water is cleaned using biofilters or



Fig. 4. Rejection percentage for the ions concentration in (a) Soft Water and (b) Hard water at 15 bar transmembrane pressure.

membranes or both, depending of the efficiency that must be tested in a prototype with fish. Fig. 6 shows the concentration operation in a membrane system where the concentration is increased over time and the permeate is returned to the tank to recover the highest quantity of water. The final mass balance must be obtained with a real fish tank, however, some situations depending on water hardness can be analysed to shed some light on the proposed membrane system. Therefore, depending on the water hardness, as mentioned before, one of the following situations, Case A or Case B, may appear.

Case A. This case is for RAS with soft water. This case aims to identify what happens with membrane rejection when using the system proposed in Fig. 6, where the water is processed by the membrane system, separately from the fish tank, and the permeate is then returned to the fish tank.







Fig. 5. Traditional (A) and Proposed (B) membrane process for the separation of the N-compounds in a RAS.



Fig. 6. The concentration operation in a membrane system.

In the first days of the RAS operation, the water quality is in accordance with situation 3. In Fig. 2, i.e., there is low concentration of TAN (produced by fish metabolism, feeding and other microorganisms in the water), low concentration of nitrite and nitrate (produced by nitrification). Later, if the membrane process is ON, the %R for TAN is high (i.e., it is maintained in the concentrate, see Fig 6), most of the NO,⁻ is in the permeate and approximately 60% of the NO₃⁻ is in the permeate (see Fig. 2). Over time, the conditions in the water move to situation 4) in Fig. 2, as the TAN is accumulated in the concentrate, and NO_3^- and NO_2^- are mostly in the permeate. This situation is not desirable, since the current is returned to the fish tank. At this point, the %*R* for TAN is similar to before, but the situation for NO2⁻ and NO3⁻ changes, increasing the concentration in the concentrate, with their %R values at approximately 55%. With more time, this produces situation 2 (see Fig. 2), where the concentration for all the N compounds in the water is high, and rejection is similar for all three molecule, i.e., close to 60% for all N compounds, with no significant differences in the separation with this membrane. Over time, the system cannot return to a low concentration in the concentrate, though the water in the permeate can be used for the RAS within certain limits that can be obtained in a real situation.

Case B. This case is for the hard water. Similarly to the previous case, the initial conditions of the N compounds in the RAS are in line with situation number 3. Here the %R of TAN is similar to that of case A, where most of the TAN remains in the concentrate (ca. 80%). NO₂⁻ and NO₂⁻ present low %*R*, meaning most remains in the permeate, which is not desirable due to their toxicity. Some time after the beginning of operation, the situation moves to number 4. Here the % R for TAN is high (ca. 80%), as it accumulates over time due to the high rejection of the membrane, and NO₂⁻ remains low while the same occurs with NO₃⁻ although the % R for NO₃⁻ is higher than in situation 3. This situation is maintained, since the rejection percentage of all the N-compounds does not increase in the concentrate. Whether the system should be stopped and a part of the water changed will depend on the TAN, and as with case A, it will depend on the fish species, the number of fish in the RAS and the feeding.

In this scenario it is necessary to formulate some water to add to the RAS, because the retention of salts with NF is high in the permeate and the carbonate will be lost from the tank.

From a biologist's point of view, the NO_2^- and NO_3^- concentration depends strongly on the TAN concentration, since they are products of the nitrification process, as mentioned in the introduction. Therefore, the membrane process should maintain the TAN at low levels, meaning that high concentrations of NO_2^- and NO_3^- should not be produced in the real process when TAN is low. Following this analysis, the best process to propose would focus on high TAN retention, using the permeate of the membrane process for the fish tank. This situation is seen in Fig. 2a and 2b in number 3 and 4 for TAN rejection. The rejection percentage for TAN is over 80% and in this situation and without a biofilter in which the nitrification process is produced, the permeate can be used in the RAS. The area of the membrane to process this flow must be determined in a real fish tank.

4. Conclusions

The rejection percentages for all the N-compounds found in RAS were tested with a NF 270 membrane at 15 bar and 15°C in freshwater with two types of hardness, named soft and hard. The influence between ammonium, nitrite and nitrate at low and high concentrations was observed. In soft and hard water, the TAN rejection percentage depends proportionally on the nitrite and nitrate concentration, increasing at high concentrations.

The salt rejection percentage for the different salts was obtained, concluding that most of the salt is in the concentrate of the NF membrane, reaching levels over 50%. For CaCO₃, the %R value is close to 80% irrespective of the water hardness. It was concluded that the proposed membrane system needs to formulate the water to maintain the salt concentration required by the fish, as the permeate of the membrane system is the current that returns to the fish tank.

It can be concluded that the TAN concentration is the most important factor to be taken into account in membrane processes, since the other N-compounds are normally produced in the system when a biofilter is present. The % R of TAN is close to 80% for hard and soft water, meaning the permeate can be returned to the fish tank. The limits of water use and recycling must be determined in a real test with fish with the membrane processes under the conditions proposed in this study.

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