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Research Article

Nitrogenated compounds' biofiltration under alternative bacterium fixation substrates

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ABSTRACT. This study compares the behavior of nitrification (NH_4^+ , NO_2^- and NO_3^-), and performance, in terms of the surface TAN conversion rate (STR), volumetric TAN conversion rate (VTR) and removal percentage of TAN (PTR) among three fixation media of nitrifying bacteria (two alternatives (S_1 , S_2) and one commercial (C_o)). The experiment was performed in two tests of 42 days each. Three isolated biofiltration systems were built for the experience, to which were added media colonized by bacteria as a "seed" to start the process of nitrification. Ammonium chloride (NH_4Cl) was attached as source of ammonium in reconditioned freshwater, also gradually adding inorganic carbon (HCO_3^-) to maintain moderate water hardness. The average results for both tests indicate that the substrates S_1 and S_2 show a statistically similar behavior to the substrate C_o ($P > 0.05$) during the first 33 days (until steady state). For the second test in terms of performance, STR values were 0.40, 0.39, 0.39 $\text{g TAN m}^{-2} \text{d}^{-1}$ recorded for S_1 , S_2 and C_o , respectively; in terms of PRN, values were 92, 97 and 93% for S_1 , S_2 and C_o , respectively. Regarding VTR, values of 72.31, 114.94, and 39.02 $\text{g TAN m}^{-3} \text{d}^{-1}$ were recorded for S_1 , S_2 and C_o , respectively. Statistical analysis provided that for STR and PRN, no significant differences, were found. But for VTR, statistically significant differences between means were evaluated, registering for the S_2 media the highest value of VTR.

Keywords: biofiltration, nitrification, ammonium, biofilm, Chile.

Biofiltración de compuestos nitrogenados bajo medios de fijación bacteriana alternativos

RESUMEN. Se compara el comportamiento del proceso de nitrificación (NH_4^+ , NO_2^- y NO_3^-), y el rendimiento, en términos de la tasa superficial de conversión de NAT, tasa volumétrica de conversión de NAT y porcentaje de remoción de NAT (PRN) entre tres medios de fijación de bacterias nitrificantes, dos alternativos (S_1 , S_2) y uno comercial (C_o). La experiencia se realizó en dos pruebas de 42 días cada una. Se construyeron tres sistemas aislados para la experiencia, a los cuales se adicionaron medios colonizados por bacterias a modo de "semilla", para el inicio del proceso de nitrificación. Cloruro de amonio (NH_4Cl) fue adherido como fuente de amonio en agua dulce re acondicionada, adicionando gradualmente también carbón inorgánico (HCO_3^-) para mantener una dureza moderada del agua. Los resultados promedio para ambas pruebas, indican que los sustratos S_1 y S_2 muestran un comportamiento estadísticamente similar al sustrato C_o ($P > 0,05$) durante los primeros 33 días (hasta el estado estacionario). Para el segundo test, en términos de rendimiento de STR se registraron valores de 0,4, 0,39, y 0,39 $\text{g TAN m}^{-2} \text{d}^{-1}$ para S_1 , S_2 y C_o , respectivamente; en términos de PRN valores de 92, 97, 93% para S_1 , S_2 y C_o , respectivamente y en función de VTR se registraron valores de 72,31, 114,94, 39,02 $\text{g NAT m}^{-3} \text{d}^{-1}$, para S_1 , S_2 y C_o , respectivamente. Los análisis estadísticos aportaron que, para STR y PRN, no se encontraron diferencias significativas. En cambio, para VTR se identificaron diferencias estadísticamente significativas entre los medios evaluados, siendo el medio S_2 , el que presenta un mayor valor de VTR.

Palabras clave: biofiltración, nitrificación, biofilm, amonio, Chile.

INTRODUCTION

Aquaculture has undergone large changes during the last decades, increasing its productive volumes from small scale to commercial levels (Gutierrez-Wing & Malone, 2006). The contribution of aquaculture to the fish, crustacean, mollusks and other aquatic animal supply in the world has increased from 3.9% of the total weight production in 1970 to 36.0% in 2006 (FAO, 2008).

The increase in productivity has promoted continuous improvements in culture systems, which may be classified in different forms. Lekang (2007) and Coll (1991) have developed a classification based on the productive technology or method used, which is divided in "extensive", "semi-intensive" and "intensive" methods.

In an intensive system, larger human intervention is observed, thus obtaining productions per volume units that may reach 100 kg m^{-3} ; likewise, these systems have experienced a greater technological development (Coll, 1991) and consequently, the investment cost may be even greater (Lekang, 2007). The need for aquaculture production increases has led the industry to even more intensive practices. Some factors influencing such trend are: water quality and quantity restrictions, availability and cost of land, restrictions for wastewater discharge and environmental impacts (Gutierrez-Wing & Malone, 2006). Due to the latter, aquaculture recirculation technologies have become very popular in the world, mainly due to their ability to operate with high culture densities (Al-Hafedh *et al.*, 2003).

Water Recirculation Systems (WRSs) for Aquaculture represent a type of intensive culture system that has been constantly under development and improvement for more than 30 years (Timmons *et al.*, 2002). These systems re-use a vast percentage of the water of the system, thus drastically reducing the entry of fresh water. The quality of the water to be used decreases considerably due to the production and consumption of some chemical compounds, such as the increase of the Total Ammonia Nitrogen (TAN) concentration, a toxic compound for organisms being cultured ($\text{TAN} = \text{NH}_4^+ + \text{NH}_3$) (Crab *et al.*, 2007).

In order to maintain the water quality in a recirculation system, a large number of equipment and treatment systems are needed, which include oxygenating solid removal, nitrogen compound biofiltration and CO_2 degassing systems, among others (Parker, 2002).

Nitrification produced in a biofiltration system is the most widely used technique to remove the toxic compounds of nitrogen from a recirculation system (Labomascu & Robinson, 1988; Crab *et al.*, 2007). A process that is carried out by autotrophic bacteria (independent from organic carbon) in two different stages (Willoughby, 1999). The first group of bacteria is the Ammonia Oxidizing Bacteria (AOB), species from the genus *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira* and *Nitrosolobus*, that oxidize ammonium (NH_4^+) to nitrite (NO_2^-). Subsequently, the second group of bacteria acts, the Nitrite Oxidizing Bacteria (NOB), which belong to the genus *Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospina*, and oxidizes nitrite to nitrate (NO_3^-) (Willoughby, 1999; Parker, 2002; Timmons *et al.*, 2002, APHA, 2005; Chen *et al.*, 2006, Gutiérrez, 2008; Emparanza, 2009). Oxidation of these compounds in a sequence is regarded as the typical performance of a biofilter. Both AOB and NOB may grow either attached to a surface or suspended in the water column (Wheaton, 1982; Timmons *et al.*, 2002; Chen *et al.*, 2006). Almost all biofilters used in recirculation systems involve a fixed film, where nitrifying bacteria grow over the surface of a media of bacteria growth (or support media) (Timmons & Ebeling, 2007). The removal capacity of ammonia of biofilters is widely dependant on the total available surface for the development of nitrifying bacteria (Timmons & Ebeling, 2007). Media generally used in biofilters for aquaculture are sand, ground rock, some form of plastic or ceramic materials forming small granules or big spheres (Timmons *et al.*, 1994; Parker, 2002; Timmons & Ebeling, 2007; Lekang, 2007). The plastic media are sold in specific companies, reducing their availability and cost increases. This limits the design criteria for biofilters in the market and other economic opportunities. Because of this, it is proposed, in this research, the use of alternative plastic substrates for biofiltration of nitrogen compounds for this purpose. We selected two polypropylene parts currently used in the field of construction, which appears to have similarities with traditional biofiltration.

As alternative means, it may be considered necessary to see first if fixed nitrifying bacteria grows over them, because despite being of the same material, it is unknown whether changes in manufacturing process could affect bacterial attachment. Therefore, in this investigation comparing two alternative means of biofiltration we will call " S_1 and S_2 ", and commercial media of biofiltration " C_0 ". First, identify the behaviors of different nitrogen compounds until the activation of the biofilters. Subsequently

calculated performance indicators VTR (Volumetric Conversion Rate), STR (Surface Conversion Rate) and PRN (Percentage of TAN removal).

MATERIALS AND METHODS

Biofiltration station

In this research a biofiltration station involving three isolated systems test was used. Each system is formed by two stock tanks with a usable volume of 0.40 m^3 and a central tank with a usable volume of 0.2 m^3 used as a biofilter. In order to make water circulate through tanks, three centrifuge pumps Pedrollo (mod. AK60) of 40 L m^{-1} were used. The biofiltration station includes identical conditions for all three systems (Fig. 1) in terms of *fitting* and piping, all tanks of 0.40 m^3 are continuously aerated to comply with the oxygen requirements of nitrifiers.

The experiment was conducted in two tests; the first was conducted in October and November, the second in December and January. It was divided because the experimental setup did not count with sufficient number of biofiltration systems to be done in one single time, not having a temperature's control system for the different seasons of the year.

The pumps are activated through a level sensor located in the first tank (in effluent tank of system 1), at intervals of 3 min of functioning per 5 min of settlement. During this period (8 min), an average of 34 L min^{-1} of water flow is observed, creating a hydraulic retention time (HRT) at the biofilter, of approximately 6 min.

Bacteria fixation media

Three different types of plastic pieces were used as media for nitrifying bacteria growth. The first involves the control media, which is currently used in commercial bio filtration equipment. In this study, this media will be referred to as " C_o ".

"Alternative" media mainly consist of polypropylene pieces (pp) used for purposes different to bio filtration. One alternative media (S_1) is used in construction works and has a wheel shape (Fig. 2a). The other alternative media (S_2) is cross-shaped and currently used as ceramic spacer in the construction field (Fig. 2b), both manufacture by DVP (www.dvp.cl).

According to the manufacturer data, the C_o media has a specific surface of $144 \text{ m}^2 \text{ m}^{-3}$, 77 cm^2 of surface area available per unit, cylindrical shape, manufactured in pp and commercially known as "bio-barrel" (Fig. 2c). The alternative media do not include information on the specific area or unit surface, thus the specific surface area was assessed through digitalization using the design software Rhinoceros ® (Fig. 3).

The number of media per cubic meter was estimated using a container of known volumetric capacity and extrapolating this result to one cubic meter. This allows, together with available surface of each media, to calculate the specific surface area for each alternative media.

Finally the void percentage described as the air volume remaining in a filter after it is filled with the media (Timmons *et al.*, 2002; Piedrahita, 2005); was calculated as follows, for all bacteria fixation media.

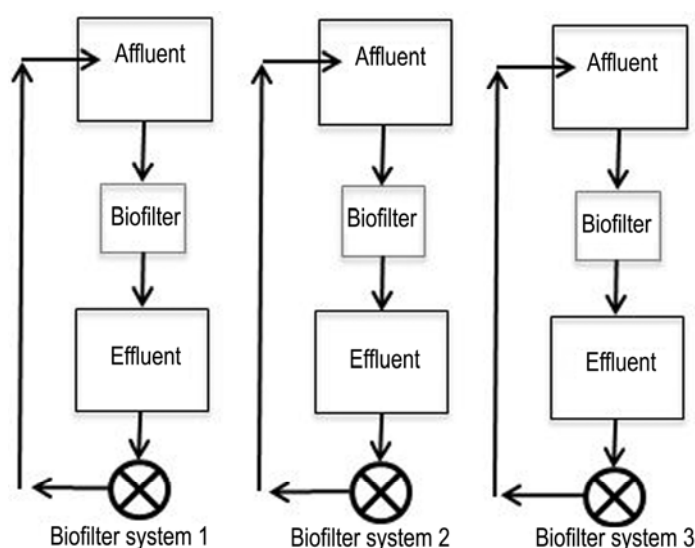


Figure 1. Biofiltration station.

Figura 1. Estación de biofiltración.

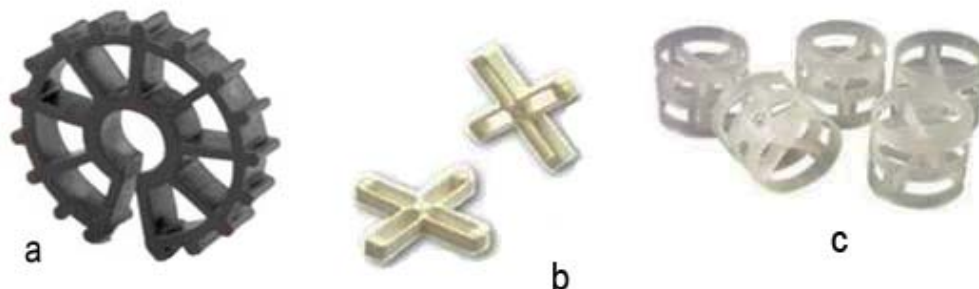


Figure 2. Bio filtration media. a) Alternative media “S₂”, b) alternative media “S₁”, c) commercial media “C_o”.

Figura 2. Medios de biofiltración. a) Medio alternativo “S₂”, b) medio alternativo “S₁”, c) medio comercial “C_o”.

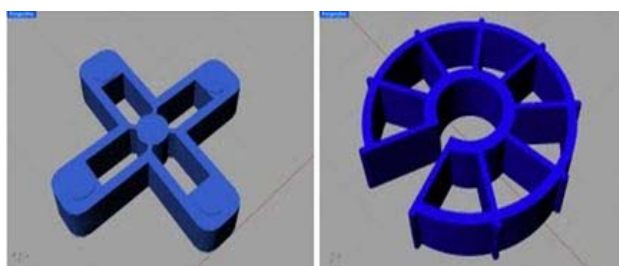


Figure 3. Media for bacteria fixation digitalized through the software Rhinoceros®. a) Digitalized alternative media S₂, b) digitalized alternative media S₁.

Figura 3. Medios de fijación bacteriana digitalizados a través del programa computacional Rhinoceros®. a) Digitalización de medio alternativo S₂, b) digitalización de medio alternativo S₁.

All possible media were placed in a known volume container. Water was then added until the container was filled. The final water volume introduced in the container shows the void rate. The result is expressed as a percentage, using the following equation:

$$\text{Void percentage} = (\text{Vinw}/\text{Vw}) 100 \quad (1)$$

where:

Vinw = volume of introduced water.

Vw = volume of water of the container.

Constitution of re-conditioned freshwater

The first stage involved the elaboration of adequate water; thus, it was decided to re-condition tap water to better suit defined chemical conditions, thus obtaining a control on different variability sources.

Chlorine from tap water was removed through aeration (Galli, 2007), for 48 h, corroborating the chloride degassing (under $0.2 \pm 0.1 \text{ mg L}^{-1}$). Water quality parameters of interest were then characterized

(ammonia, nitrite, nitrate, phosphorous, alkalinity, pH, oxygen, and temperature).

According to the analysis, the water was then conditioned adding the necessary doses of alkalinity in order to achieve a type of water defined as “moderately hard” (Table 1), in accordance to Standard Methods (APHA, 1999). In this solution, phosphate and ammonia concentrations suggested as feeding media for nitrifiers (Kim *et al.*, 2000; Seo *et al.*, 2000; Gutiérrez, 2008) were leveled off. Subsequently, the water was maintained at 15°C until use.

Determination of physical and chemical parameters

Table 2 shows the equipment used for the measurement of parameters during this study. It is necessary to point out that each measurement involved the use of clean and independent supplies for each system.

Starting-up nitrifying biofilters

Labomascus & Robinson (1988) and Piedrahita (2005) suggested that a fast way to activate a biofilter is through an inoculation generation process or bacterial seed. Therefore, an active- immobilized nitrifying inoculator was generated inside a 10 L continuous air-lift bioreactor (Seo *et al.*, 2001; Gutierrez, 2008). Same quantity of available surface area of each media. Nitrifiers for the start-up of the air-lift bioreactor were obtained from colonies incubated in agar plates according to methodology described by Gutierrez (2008). Then, after 54 days of incubation at $14.0 \pm 0.9^\circ\text{C}$, each activated media with nitrifiers immobilized was transferred to each biofiltration station (Fig. 1) to begin the nitrification performance evaluations.

Table 1. Composition of the feed medium (FM) and nitrifying water quality.**Tabla 1.** Composición de medio de alimentación (MA) y calidad de agua para nitrificantes.

Water quality (mg L ⁻¹)			Feeding media (mg L ⁻¹)	
Water type	Alkalinity	pH	NH ₄ ⁺	PO ₄ ⁻³
Moderately hard	60-70	7.4-7.8	15.5	4.1

Source: APHA (1999); Seo *et al.* (2000); Kim *et al.* (2001); Gutierrez (2008).

Table 2. Instruments and methods used.**Tabla 2.** Instrumentos y métodos utilizados.

	Brand	Model	Method	Range (mg L ⁻¹)
TAN	Hanna ins.	HI83000	Nessler	0-14
NO ₂ ⁻	Hanna ins.	HI83000	Chlorometric test	0-3.50
TAN	La motte	Smart 2	Zinc reduction	0-60
CaCO ₃	La motte	Smart 2	Chlorometric test	0-200
PO ₄ ⁻³	La motte	Smart 2	Vanodomolybd phosphoric acid	0-70
T°	Hatch	HQ40d	LDO	
pH	Hatch	HQ40d	Gel electrolytes	0-14

Determination of efficiency parameter

Surface Conversion Rate (STR)

The first is the STR, which is defined as the TAN grams converted to nitrate per unit of surface per day (Timmons *et al.*, 2002; Colt *et al.*, 2006; Drennan II *et al.*, 2006). The STR was calculated using the following formula:

$$\text{STR} = (M_{\text{TANA}} - M_{\text{TANE}}) / \text{Sup}$$

where:

M_{TANA} = Ammonium mass in affluent per day (g of TAN per day)

M_{TANE} = Ammonium mass in effluent per day (g of TAN per day)

Sup = Surface of the media in m²

It must be remembered that the mass of NH₄⁺ entered through the ammonium chloride is represented as TAN (NH₄⁺ + NH₃). When the NH₄Cl molecules are dissociated in the water, one part of the theoretical NH₄⁺ will transform to NH₃ depending on the pH of the media at that particular time.

Volumetric Conversion Rate (VTR)

The second indicator is the VTR, which is defined as the grams of TAN converted to nitrate per unit volume per day (Colt *et al.*, 2006; Drennan II *et al.*, 2006; Malone & Pfeiffer, 2006; Timmons & Ebeling, 2007;

Kirk & Timur, 2009; Guerdat *et al.*, 2010). For the estimation of VTR the following formula was used:

$$\text{VTR} = (M_{\text{TANA}} - M_{\text{TANE}}) / \text{Vol}$$

where:

M_{TANA} = ammonium mass in affluent per day (g of TAN per day)

M_{TANE} = ammonium mass in effluent per day (g of TAN per day)

Vol = volume of the media (m³)

Percentage of TAN removal (PRN)

Finally, the PRN (Colt *et al.*, 2006) was used as an indicator of the media performance. PRN is defined as the percentage of TAN transformed into nitrite per day, and it was estimated as follows:

$$\text{PRN} = ((M_{\text{TANA}} - M_{\text{TANE}}) / M_{\text{TANA}}) \times 100$$

where:

PRN = percentage of TAN removal

M_{TANA} = ammonium mass in affluent per day (g of TAN per day)

M_{TANE} = ammonium mass in effluent per day (g of TAN per day)

The procedure to estimate the TAN mass entered daily, was exposed by Chang (2002) and Rosenberg & Epstein (1998), which uses the molecular weight to

obtain the NH_4^+ grams entered through the ammonium chloride (NH_4Cl).

Statistical analysis

The statistical analysis of the data involved an analysis of variance (ANOVA) using the statistical software SPSS version 17. The criterion used was: if $FC > FT$ then there is no statistically significant differences (Montgomery & Runger, 1998; Montgomery, 2002; Pérez, 2009).

P -value criterion was: if value- p is lower than α then no statistically significant differences among the media would exist (Montgomery, 2002, Pérez, 2009; Montgomery & Runger, 1996). For estimation, a value of α of 5% was used. After the ANOVA, a Tukey analysis was carried out to detect which media had differences. This analysis was developed using the same statistical software.

RESULTS

Secondary parameters were monitored daily and the results can be seen in Table 3, the temperature ranged between 14 and 18°C, oxygen levels did not drop below the 6 mg L^{-1} degrees of concentration, the pH remained near 7.6.

Biofiltration station

Table 4 shows the significant data for this study; there, the hydraulic performance of biofiltration and the different qualities of the studied biofiltration media can be observed.

Activation of biofilters

The first nitrogen compound detected was the total ammonia nitrogen (TAN) (Fig. 4). It is observed that during test 1, it showed a peak of 4.5 mg L^{-1} and an abrupt decrease of concentration at day 29. Likewise, test 2 showed a peak of 2.5 mg L^{-1} , with a considerable reduction of the concentration at day 19. In general, both tests showed a low concentration of TAN from day 33.

Figure 5 shows the performance developed by nitrite for both tests. Test one showed a peak of concentration at day 33. Likewise, test 2 showed a peak at day 21. In both cases after the peak a reduction in the concentration is produced, which in both tests showed values below 0.5 mg L^{-1} .

The performance recorded for nitrate for both tests may be observed in Figure 6. This showed different peaks and subsequent reductions. Test 1 showed its first peak at day 33, with a subsequent decrease in its concentration. Then, an increase was observed until it

reached concentrations close to 60 mg L^{-1} . Test two showed a peak early in day 23, with a subsequent reduction in its concentration. Finally, test two showed an increase of concentration which reached 100 mg L^{-1} at the end of the experience.

Performance test of bacterial fixation media

Table 5 shows the performance results for the first test. There, it may be observed that the average values of STR for all studied media oscillated between 0.39 and 0.42 $\text{g TAN}/(\text{m}^2 \text{d}^{-1})$. The maximum VTR value was obtained for media S_2 , recording 82.95 $\text{g TAN}/(\text{m}^3 \text{d}^{-1})$. Values of PRN exceeding 90% were obtained for all media.

Table 6 shows the average performance and standard deviation for each indicator. There, the similarities in the average STR between the evaluated media can be observed, with values from 0.39 to 0.41 $\text{g TAN m}^{-2} \text{d}^{-1}$. VTR showed a maximum value for media S_1 with 114.94 $\text{g of TAN m}^{-3} \text{d}^{-1}$. Regarding PRN, all media showed values exceeding the 92%.

Statistical analysis

Since the research was divided into two tests at different times, temperature differences between these two tests results were analyzed. According to the analyses carried out, there are no statistically significant differences between the alternative media (S_1 and S_2) and the commercial media (C_o) for the TAN ($P = 0.961$ and 0.625 for the first and second test, respectively). Regarding the nitrite and nitrate, no significant differences were observed for the first or second test respectively.

The analysis between tests for these parameters and studied media showed that there are no statistically significant differences for nitrite and nitrate, with p -values from 0.056 to 0.912. On the other hand, for TAN the statistical analysis showed P -values of 0.008, 0.015 and 0.041 for media C_o , S_2 and S_1 respectively, obtaining statistically significant differences between tests.

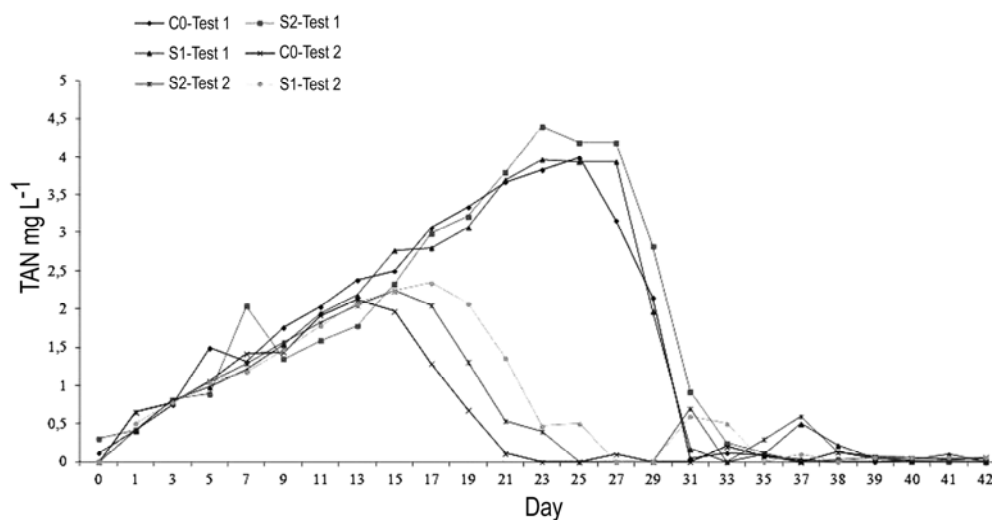
No statistically significant differences were observed for performance indicators STR and ATR, as their P -values were greater than 0.05, thus accepting the null hypothesis of equal means. On the other hand, for the VTR indicator the statistical analysis contributed with P -values of 0.027 and 0.008 for tests one and two respectively. These values show statistically significant differences between the studied media. No significant differences were recorded for the ANOVAS carried out between the tests for the VTR indicator. In the Tukey's test the P -value was 0.3 for S_2 on C_o , and 0.864 for S_2 on S_1 .

Table 3. Secondary parameters.**Tabla 3.** Parámetros secundarios.

	Temperature		Oxygen		pH	
	Average	σ	Average	σ	Average	σ
C _o	17.2	0.4	9.45	0.23	7.62	0.32
S ₁	17.3	0.4	9.43	0.21	7.61	0.31
S ₂	17.2	0.4	9.49	0.21	7.64	0.32

Table 4. Characteristics of the studied media and systems used.**Tabla 4.** Características del medio y sistema utilizado.

	C _o	S ₂	S ₁
Hydraulic load rate (m ³ m ⁻² día ⁻¹)	48.96	48.96	48.96
Cross-sectional area (m ²)	0.25	0.25	0.25
Rate of exchange per hour	2.04	2.04	2.04
Volume of water in the system (m ³)	1	1	1
Nº of entered pieces	310	571	2,744
Entered surface (m ²)	2,387	2,387	2,387
Specific surface of the media (m ² m ⁻³)	144	293	480
Unitary surface (cm ²)	77.0	41.8	8.7
Volume of the entered media (m ³)	0.0244	0.0132	0.0113
Void space (%)	93.8	75	82
Pump power (HP)	0.5	0.5	0.5

**Figure 4.** Performance of the total ammonia nitrogen (TAN) for both tests.**Figura 4.** Comportamiento del nitrógeno amoniacal total (TAN) en ambas pruebas.

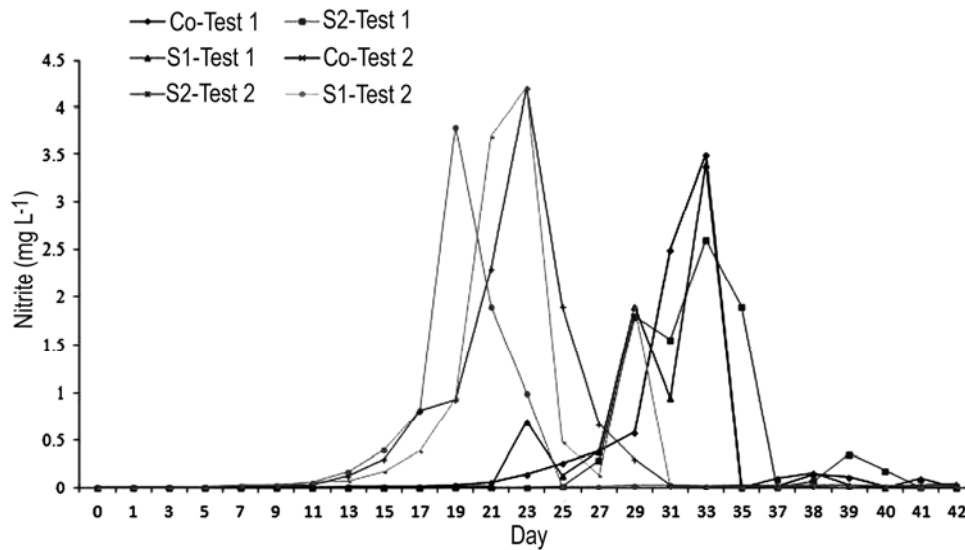


Figure 5. Performance of nitrite in both tests.

Figura 5. Comportamiento del nitrito en ambas pruebas.

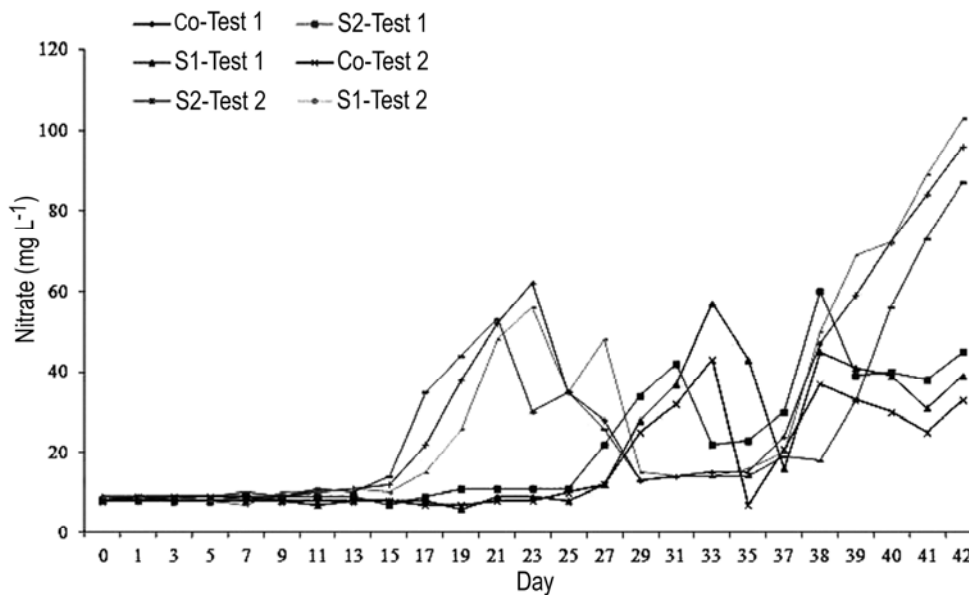


Figure 6. Performance of nitrate for both tests.

Figura 6. Comportamiento del nitrato en ambas pruebas.

DISCUSSION

Performance of nitrogen compounds

Different authors describe almost in the same way how nitrogen compounds behave when a biofilter is activated (Wheaton, 1982; Timmons *et al.*, 2002; Piedrahita, 2005; Chen *et al.*, 2006). They all agree that, in a first stage, an accumulation or increase of the TAN concentration is observed, until a maximum or *peak* of concentration is reached, with a subsequent

reduction that shows the establishment of a healthy population of the AOBs (Wheaton, 1982).

Labomascus & Robinson (1988) obtained maximum concentrations of 2.0 mg L^{-1} of TAN. Timmons *et al.* (2002) recorded concentrations close to 4 mg L^{-1} of TAN at day 14 of their experience and Wolters *et al.* (2009) 2.3 mg L^{-1} of TAN at day 29 of their study. After the recorded maximums, all authors recorded that TAN concentration did not exceed 0.6 mg L^{-1} .

Table 5. Performance indicators for the studied media, test one.**Tabla 5.** Indicadores de desempeño del medio, test uno.

Performance Media	STR		VTR		PRN	
	Average	σ	Average	σ	Average	σ
C _o	0.42	± 0.12	41.47	± 11.03	99.80	± 0.01
S ₁	0.42	± 0.12	75.64	± 21.94	97.68	± 0.03
S ₂	0.39	± 0.14	82.94	± 29.62	90.42	± 0.13

Table 6. Performance indicators for the studied media, test two.**Tabla 6.** Indicadores de desempeño del medio, test dos.

Performance Media	STR		VTR		PRN	
	Average	σ	Average	σ	Average	σ
C _o	0.39	± 0.12	39.02	± 12.09	92.79	± 0.07
S ₂	0.39	± 0.13	72.31	± 23.29	92.54	± 0.07
S ₁	0.41	± 0.12	114.94	± 23.18	97.08	± 0.03

During this study, it was possible to observe, for all treatments in both tests, the performance described by Wheaton (1982), Timmons *et al.* (2002), Piedrahita (2005), Chen *et al.* (2006) y Wolters *et al.* (2009) regarding the TAN relationship. TAN was accumulated up to concentrations of 4 mg L⁻¹ for the first test and up to 2.5 mg L⁻¹ for the second test. Likewise, the maximum *peak* observed was recorded at day 23 and 17 for the first and second test respectively.

The periods for the first cycle and the recorded concentrations showed a wide similarity with those described in the literature and, at the same time, it has been established that the treatments behave similarly for both tests (Fig. 4), a situation confirmed according to the statistical results, which demonstrate that there are no statistically significant differences between treatments regarding the TAN variability (or performance) between the performed tests.

This may be mainly due to the fact that both tests were done at different periods of the year. The first test was done in October and November, and the second in summer between January and February, where average temperatures of $15.64 \pm 0.66^\circ\text{C}$ and $18.71 \pm 0.55^\circ\text{C}$ were recorded for the first and second tests respectively. A decrease of 10°C of the operational temperature results in a 50% reduction of the removal rate. Chen *et al.* (2006) have suggested that the effect of temperature does not only influence the metabolism of bacteria, but it also affects the mass transference between the water column and the

bacteria, as well as the diffusion and transport of gases present in the water column. Further, Zhu & Chen (2002) demonstrated that, above a temperature of 20°C , the nitrification specific rate increases between 1.1 and 4.2% for each $^\circ\text{C}$. The difference in temperature of 3°C would explain the statistical difference found by the ANOVA carried out for the analysis between tests.

The nitrite also exhibits the performance described in the literature. As previously mentioned, a low concentration of ammonia, followed by an increase of the nitrite concentration, shows that AOB have settled in the media used in a biofilter.

The maximum concentrations recorded by the literature vary widely among authors. Labomascus & Robinson (1988) report concentrations of 2.75 mg L⁻¹ at day 8 of their experiment; Timmons *et al.* (2002) recorded a maximum concentration close to 7 mg L⁻¹ at day 28. Finally, Wolters *et al.* (2009) observed a maximum concentration close to 3 mg L⁻¹ at day 71.

According to what has been observed during this study, the nitrite showed a maximum concentration of 3.5 and 4 mg L⁻¹ at days 33 and 23 for the first and second test, respectively (Fig. 5). No statistical differences were observed for variability (or performance) of nitrite between treatments and tests.

After the nitrite concentration increase, like ammonia, nitrite starts to decrease in concentration to remain at low concentrations in time. Wheaton (1982) describes such process developed by the second Group or NOB family, which oxidizes nitrite to nitrate, thus

suggesting the settlement of such bacterial group on the support media.

The nitrate is the third nitrogen compound participating in the activation of a biofilter. This compound is released by the NOB when the nitrite is oxidized to nitrate. According to Timmons *et al.* (2002), a constant and continuous accumulation of such compound occurs in time. In the current experience, the authors indicate that such process starts after day 21.

Labomascus & Robinson (1988) obtained a gradual accumulation of nitrate, with increases and decreases of concentration showing different *peaks* along the study. Thus, day 14 showed values of 85.8 mg L⁻¹ of nitrate, to subsequently decrease to 48.4 mg L⁻¹ the next day. The largest *peak* recorded by these authors was at day 21 of the experience, with concentrations of 152 mg L⁻¹ while the next day the concentrations were 50 mg L⁻¹.

Al-Hafedh *et al.* (2003) in his work described a similar performance to that described by Labomascus & Robinson (1988), although with different nitrate concentrations. Al-Hafedh *et al.* (2003) obtained a maximum *peak* in the nitrate concentration at day 29 with values close to 35 mg L⁻¹ and subsequently recording values below 25 mg L⁻¹.

The research of Wolters *et al.* (2009), inform a similar performance, although showing four *peaks* in nitrate concentration, with values close to 11.5 mg L⁻¹ at days 90, 110, 130 and 200 approximately (the authors do not describe the exact days of the facts).

The first test shows a performance similar to that described by Labomascus & Robinson (1988), Al-Hafedh *et al.* (2003) and Wolters *et al.* (2009), with two *peaks* in the nitrate concentration for all treatments, with a subsequent tendency to an increase of concentration. The observed *peaks* showed concentrations between 35 and 60 mg L⁻¹, almost at the same time, at days 31 and 38 of the experience.

The second test shows a performance similar to that described by Timmons *et al.* (2002) in the last days of their experience, with a marked increase in the concentration of linear form until the end of the research. In the mean stages along the development of the experience, test two shows a similar performance to that described by test one, with two *peaks* at days 23 and 27, of nearly 60 mg L⁻¹ of concentration.

The reduction may be attributed to the entrance of new water to the system, a common practice for the nitrate control in RAS (Timmons *et al.*, 2002). The literature previously mentioned does not specify whether new water entered the system or not, but this is assumed as a common procedure for productive

systems. During the experience, reconditioned freshwater and different pattern solution entry was done at the time when fluctuations were recorded.

On the other hand, all nitrogen compounds showed an homogeneous performance between treatments, as no statistically significant differences were observed at the 5% level (for all treatments in both tests value-*P* > 0.05 and FT > FC). Likewise, such performance agrees with that described in the literature, obtaining concentrations of the nitrogen compound inside the ranges suggested by different authors.

Biofilter activation and steady state

The literature suggests a biofilter is ready to be used for aquatic organisms or fish farming in SRA when the steady state is reached. Colt *et al.* (2006), suggest that the steady state is generally reached when the TAN level in the effluent stabilizes and a straight horizontal line may be observed in a time graph. Colt *et al.* (2006) suggestion was observed for all tests and treatments and the inoculation experience. During the inoculation it was not possible to see a low concentration of nitrite, although it was considered that the media were active when the steady state was observed.

Huguenin & Colt (1989), suggest that normally 1-3 months are needed to achieve the "steady state". Lekang (2007) indicates that a biofilter is ready to be used in a period of 20-40 days, depending on the entry of ammonia and factors such as temperature and pH. Timmons *et al.* (2002) obtained a steady state at day 39 during their experience.

Here, the steady state was achieved for all tests and treatments at days 37 or 38 for the first test, and 27 and 29 for the second test, thus observing linearity in the TAN time and the nitrite. Therefore, from that period on, it is considered that the biofilters, and consequently the treatments, have the necessary conditions to estimate the performance of each bacterial support media under study.

Although the literature indicates that there are many ranges considered as optimal, regarding the growth temperature for nitrifying bacteria (Timmons & Losordo, 1994; Chen *et al.*, 2006), it is suggested that the higher the temperature, the higher the metabolic development of the individuals and, therefore, bacteria are able to achieve a steady state in less time.

Regarding the performed experience, it may be observed that there is a 10-day difference between the first and the second test in terms of the steady state achievement. The first test was done at a temperature of 15.2°C, while the second was carried out at an

average temperature of 18.7°C. Such difference (3.5°C) may have affected the time in which both achieved the steady state.

Performances

The biofilters used in this research may be classified as submerged-media biofilters. Timmons & Ebeling (2007) indicate that these systems consist of a support media where nitrifying bacteria develop, through which waste water still goes through, either in an ascendant or a descendant flow. In these filters, floating media are used with a density smaller than water (Piedrahita, 2005).

Performance in terms of STR for these biofilters has been established by different authors. Atland *et al.* (2009) have suggested these systems may transform 0.2-1.0 g TAN m⁻² d⁻¹, with the use of plastic elements at temperatures between 10-20°C. On the other hand, Willoughby (1999) has established that a medium with a surface area of 150 m² m⁻³ has a performance of 0.3 to 1.0 g TAN m⁻² d⁻¹, with temperatures between 5 to 25°C; however, at temperatures of 15°C the observed performance was 0.7 g TAN m⁻² d⁻¹. According to Piedrahita (2005) this type of filter has a removal rate that reaches values of 0.1 to 0.5 TAN m⁻² d⁻¹.

Regarding the observations in the performed experiences, we have observed, for all treatments and both tests, a performance statistically homogenous as a function of the STR. The average values were similar between tests and among treatments. The observed concentrations are between the ranges observed in the literature; therefore, we observed a maximum performance with values between 0.54 and 0.56 g TAN m⁻² d⁻¹. These values show that the alternative media have a performance in terms of the STR that results acceptable to be used as bacterial growth media in nitrifying biofilters.

In terms of the VTR, the literature does not make any reference to this performance indicator for this particular type of biofilter. However, a series of VTR values has been reported for different media with specific areas similar to those used in this study. Ridha & Cruz (2001) obtained an average VTR of 9.3 ± 4.03 g TAN m⁻³ d⁻¹ in two plastic media of 200 m² m⁻³ of specific area. Timmons *et al.* (2002) report values of VTR of 6-7 g TAN m⁻³ d⁻¹ for granular and sand media that exceed the 500 m² m⁻³. Guerdat *et al.* (2010) presents a VTR of 267 ± 123 g TAN m⁻³ d⁻¹ for media of specific surface of 850 m² m⁻³.

Once again, if we compare the VTR value obtained for each media, it is clearly shown that these are close to those suggested by the cited literature. Likewise, it is observed that there is a statistically significant

difference between the recorded VTR for all treatments and tests performed. The differences recorded are due to the fact that each studied media has specific areas between the recorded VTR, a situation observed in the present study. The difference between the VTR may become a relevant factor when choosing a media for biofilters, as more ammonia will be oxidized per m³ if VTR is higher; this situation involves less media volume needed and less space for the construction of a biofilter.

The removal percentage of the TAN (PRN) is another indicator of performance generally used. Gutierrez (2008) obtained a PRN of 96% from day 35 of the experience in a continuous air-lift bioreactor, under continuous feeding, with a defined feeding media. Timmons *et al.* (2002) established a PRN of 50-90% for fluidized-sand biofilters in cold and moderately cold waters.

The PRN varies depending on the oxidizing capacity of the system and the entering concentration of TAN to the biofilter. In the present study, the PRN remained over 90% after achieving the steady state, which indicates that the biofilters could have successfully oxidized almost all the TAN entered through the ammonium chloride to the system. No statistical differences in the PRN were observed among the three studied media, or between the performed tests.

The procedure of applying the same quantity of surface area to each biofilter allowed delivering the same quantity of available surface for the immobilization and growth of the biofilm at each system of biofiltration. This has been shown when observing the performance of the media and the nitrification PRN of each biofilter.

A clear difference between the studied media was the void rate. The largest void rate corresponds to the C₀ media, which, because it was created for biofiltration, has a rate close to that recommended in the literature. Timmons *et al.* (2002) suggest that for this type of biofilter, a void rate above 95% is recommended and, considering the calculations done in this study, the void rate for this media was 93.8%. Likewise, the alternative media show void rates ranging between 75 and 82%. Timmons *et al.* (2002) have suggested that low void rates in this type of biofilters may lead to obstruction problems caused by the accumulation of solids and low concentration of dissolved oxygen.

In the experiences carried out, no low concentration of dissolved oxygen was observed, which remain above the minimal recommended concentrations for all studied media (exceeding 8 mg

L^{-1}), as well as their tests. It is worth mentioning the interpretation done by Timmons *et al.* (2002) for a productive system, where the farmed species constantly generates solids which, if not filtered correctly, may accumulate in the biofilter. In our research, no fish or other individuals outside the nitrifying bacteria generating solids were used, and no particle removal systems were involved. Therefore, the differences generated by the void rate among the studied media were not assessed. However, it is important to consider that the lower void rates of the alternative media could generate certain operational problems leading to functional difficulties of the biofilters when faced to an inadequate solid filtration. Therefore, the corrective and cleaning measures must be considered by those interested in the use of these media for aquaculture biofiltration; future evaluations of such alternative media in productive systems must be carried out.

Economic and logistic advantages

Traditional biofiltration media are commercialized by international specialized companies in Chile, making the availability of the media dependable to the stock of these companies, whose factories are located abroad. This fact significantly limits the availability of biofiltration media in the domestic market and, at the same time, increases its price.

Alternative media S_1 and S_2 are manufactured by Chilean companies and have stock along the country, the availability of which is not a constraint due to their use in the construction field. A good example of how availability could restraint studies or production was noted by Ridha & Cruz (2001), where the authors make a comparison of the water quality and the performance of the biofiltration process obtained in two commercial media in the culture of tilapia (*Oreochromis niloticus*) in Kuwait, and in order to carry out their study, the authors were required to seek for a supply company in Scotland to get the biofiltration media.

Availability is one of the attractive characteristics of the media identified in this study, as they may be found practically worldwide. In terms of price, the studied media have values oscillating, in the Chilean market, between US\$ 1.583, for S_2 media and US\$ 3.794 for S_1 media, per cubic meter of each media. The cost of C_o is over US\$ 4.000 per meter cubic of media. The low cost and high availability may transform the S_1 and S_2 media into an interesting and convenient alternative when designing or projecting the construction of a biofilter.

CONCLUSION

Based on the results obtained in this study and the subsequent analysis of the data and the objectives, we may conclude the following:

- Although the activation of biofilters showed similarities to the cited literature, this study documents faster activation periods (also known as maturation time) at day 27 and 37 for tests one and two, respectively, through nitrifying bacteria inoculation previously fixed in a support media to be used in biofiltration systems.
- Values of STR from 0.39 to 0.42 g TAN $m^{-2} d^{-1}$ were obtained, showing similarities with the ranges reported by the literature, where no statistically significant differences were found for the media and tests.
- Maximum values of VTR of 0.39 to 0.42 g TAN $m^{-2} d^{-1}$ were recorded for a specific surface of 480 $m^2 m^{-3}$ at a temperature of $18 \pm 0.55^\circ C$. Considering the data analysis, statistically significant differences were found among the studied media. The alternative media showed higher performance in terms of the TAN conversion volumetric rate under the parameters and conditions of the study. Media S_1 in particular showed a performance (as a function of the VTR) 277% higher than the commercial media (C_o). In order to biofiltrate the same quantity of ammonia almost three times less volume is required with the media S_1 when compared to the media C_o .
- Finally, it may be indicated that the alternative media (S_1 and S_2) have shown a performance adequate to be considered as support media for biofilters in RASs, showing a performance statistically similar, equal or higher to the commercial media analyzed (C_o). The consideration of such media (S_1 and S_2) may result in a real alternative for the design of a biofilter at a commercial scale.

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