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Influence of filter medium type, temperature and ammonia production on nitrifying trickling filters performance

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ABSTRACT

This work focuses on the achieving of optimal design and modelling of nitrifying trickling filters for closed circuit aquaculture turbot *(Psetta maxima)* farms. Several process parameters influential in nitrifying filtration were established on experimental biofilters and their efficiency was tested, based on the removal of nitrogen contained in total ammonia nitrogen (N-TAN) in a fixed time (24 hours). Those process parameters were filter media types (Type A Biofill®, BactoBalls® and MECHpro® rings), temperatures (24.3ºC, 19.0ºC, 15.3ºC) and production of TAN (1.5, 3.0 and 4.5 g per day) while other process parameters values remained constant. TAN production was simulated with the addition of ammonium chloride (NH_4Cl) in the recirculation system. Constant measuring of the total ammonia nitrogen concentration in the biofilter effluent was required to perform a model of N-TAN fluctuation based on a specific feeding regime and to ascertain performance differences between biofilters.

At the end of the experiment, notable differences were observed in the ammonia removal rates depending on different process parameters. The BactoBalls® filter medium led to the highest mean N-TAN removal rates (0.24 g N-TAN removed m^{-2} day^{-1}). The N-TAN removal rate generally increased with higher temperatures, the trials with the highest mean temperature (24.3ºC) led to the highest mean N-TAN removal rate (0.26 g N-TAN removed m^{-2} day^{-1}). Similarly, the N-TAN removal rate increased with high TAN production. The trials in which production was 4.5 g per day showed the highest N-TAN mean removal rate (0.27 g N-TAN removed m^{-2} day^{-1}).

KEYWORDS: Nitrification; Trickling filters, RAS; Aquaculture

1. Introduction

Aquaculture farms in land in which the culture water is recycled are gradually increasing during the course of the current century. In those farms, water is constantly moved by pumps in closed circuits called recirculating aquaculture systems (RAS), thus assuring that the water is completely recycled expect the minimum losses caused by evaporation or management [1]. Recirculation of water in aquaculture presents an important alternative to traditional production methods, because it means independency of natural water resources and allows manipulating water characteristics [2], the temperature being the most important, because fishes have an optimal temperaure on

which their growth is remarkably better, as observed for different species, such as gilthead sea bream *(Sparus aurata)* [3], European sea bass *(Dicentrarchus labrax)* [4], and white grouper *(Epinephelus aeneus)* [5]. Another important feature of the use of recycled water for in-land aquaculture farms is that it allows to increase the culture water volume, thus allowing to increase the fish production [6]. Turbot *(Psetta maxima)* is an excellent candidate for production in those systems due to its benthonic nature and the capability of living under relatively high stocking densities [7]. Those characteristics properly allow to culture turbot in tanks with low water volume. In fact, stocking densities in commercial turbot aquaculture, cultured in tanks or in sea water cages, have reached 25 to 30 kg m^{-3} [7]. It is also a fast-growing aquatic species of great economic value [8].

A well designed RAS allows keeping water quality optimal, because all parameters are controllable and manageable by the producer. Parameters that are influential with respect to the correct operation of an RAS are oxygen (O_2) and carbon dioxide (CO_2), organic matter, pH, suspended solids (SS), alkalinity, hardness, nitrite, nitrate or total ammonia nitrogen (TAN), plus the presence of opportunistic pathogens, as they determine the survival and optimal growth of the fishes [9]. All the above mentioned water quality parameters unavoidably deteriorate with every recirculation cycle. Oxygen is rapidly consumed by the fishes and, depending of the feed intake and the feed properties, organic matter (both dissolved and suspended) and ammonia are produced and accumulated in the bulk water. The latter is the most threatening product [10] and the increment of a concentration beyond certain limits can be really detrimental for fish health and welfare [11] as it lowers the ammonia excretion and produces an accumulation of ammonia levels in blood, causing nervous system problems and death [12].

Fixed-film biological filters are usually installed in aquaculture facilities with the main goal of TAN removal [13]. They consist in a series of diverse solid surfaces where a population of nitrifying bacteria attaches and grows with excreted extra-cellular polymers [14]. Four kinds of biofilters are commonly used: rotating biological contactors, trickling filters, bead filters and fluidised sand biofilters [15]. Rotating biological contractors have been reported as the most efficient in TAN removal, although their cost is quite high. Among the other possibilities, trickling filters display relatively higher removal rates [16]. Besides, they have considerable advantages: low price, the oxygen transfer is provided as water cascades directly over the media [17] and degasification of

CO_2 and simplicity of design, construction, operation and management [13]. Therefore, the trickling filter was used for this study.

TAN production is directly correlated to the fish production plan of aquaculture facilities and usually determines the volume of biofilters installed and the pumping requirements. Nevertheless, a wide range of process parameters apart from biofilter size affects the speed of TAN removal (and therefore the achievement of the targets in TAN concentration), meaning that the proper design and evaluation of performance is essential to avoid the construction of larger trickling filters than needed and the related expenses. A large number of process parameters affects the nitrification rate, for example influent concentration of TAN and oxygen, organic matter, nitrite, temperature, alkalinity, pH and hydraulic loading [13]. Although models have been constructed to approximate the influence of process parameters of the biofilm on TAN removal efficiency by applying the nitrification kinetics theory [18–20], the influence of several process parameters on the rates at which nitrification reactions take place still have to be assessed empirically. For example, Nijhof [19] included in his model “a” and “b” parameters depending on external factors, or internal proprieties, and presented an equation relating the value of “a” with several hydraulic loadings. Kamstra et al. [21], validated Nijhof’s model and observed several variations of predicted TAN removal rates depending on the filter medium type.

Three of these process parameters were tested in this article. One of them is the influent TAN concentration, which acts as a limiting substrate. Kinetics of the nitrification reaction are described by the Monod-type expression [22–24]. Besides, experimental procedures that demonstrate a relation between the TAN concentration in the influent and biofilter performance [16,21,25,26] have also been performed. Yet Bovendeur et al. [18], noticed that the nitrification reaction is sometimes independent of the substrate when oxygen acts a limiting factor, which is not desirable for the culture. Greiner and Timmons [16] also reported 0-order reactions in their research at high TAN concentrations (above 2.5 mg L^{-1}). Nonetheless, these articles often analyse the impact of a steady TAN loading rather than analyse the effect of a fluctuating TAN concentration produced by the shifting excretion rate during the day occurring after the feeding, observed in turbot [27] as well as in other teleost species [28]. In the present research, three TAN productions are tested, and the N-TAN concentration is monitored during 24 hours.

The filter medium type was another of the process parameters tested in this study. The influence of different filter media types on the TAN removal rate of trickling filters has been studied in several articles [21,29], although the influence of the filter medium type is often discussed when analysing the performance of all kind of biofilters [15,16,30]**.** Characteristics of the filter media types considered to affect the performance of the nitrifying trickling filter include void ratio (volume filled with air/total filter volume when not in operation), specific surface area (biofilter surface/biofilter volume) and the type of flow that the shape of the filter media allows across the biofilter (vertical flow, random flow or cross flow) [21]**.**

Temperature was the last factor selected for its great influence on the speed of chemical reactions (based on the Van't Hoff-Arrhenius equation) and bacterial growth and therefore the huge influence on biofilter performance. Some examples of papers analysing the impact of temperature on the TAN removal in trickling filters include the experiments of Zhu and Chen [31] and Lyssenko and Wheaton [32]. Zhu and Chen [31] discovered that the influence of temperature on nitrification speed was lower than predicted by the Van't Hoff-Arrhenius equation, but still had a considerable influence.

The aim of this study is to select the best set of these process parameters for achieving the best possible performance of nitrifying trickling filters, but also to provide information on the performance of the efficiency of biofilters under a wide range of conditions, depending on fish production plans. The three TAN productions simulated (1.5, 3.0 and 4.5 g per day) are in accordance with TAN productions estimated for turbot aquaculture facilities depending on growth state (based on the study of Dosdat et al. [27]) and in which density is 7.5, 11 and 22.5 kg m^{-3}. The three temperature values (15ºC, 19ºC and 24ºC) set were in accordance with water temperatures established in recirculating aquaculture systems to produce several species, and to mean temperatures reached in the sea at certain time periods of the year for a sea-cage aquaculture facility. With regard to the filter media, trickling filters were traditionally constructed using rocks, but today most filters use plastic media, because of their low weight, high specific surface area and high void ratio (>90%). In the present experiment, three plastic materials were selected for their positive characteristics such as availability, easy manipulation (low weight) and price. Hitherto, to our knowledge no study has been made to determine the influence of these filter media on the efficiency of trickling filters.

In summary, this paper presents a tri-factorial study where the influence of three process parameters (temperature, filter media and TAN production) on the performance of trickling filters is assessed. Influence of each one of them are determined, but also the influence of the combination of process parameters on the achievement of certain N-TAN removal rates.

2. Material and methods

2.1. Tanks and biofilters

The system was composed by six 500 L tanks connected to six trickling filters. The water flows from the drainpipe of these tanks to the top of the filter by a peristaltic pump (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. At the top of the filter the water was dispersed by a series of several holes in the pipe, to ensure the soaking of the entire surface area of the filter medium, contained in a home-made cube. The base of this home-made cube had several holes, from which water was returned to the tank. Biofilters were designed to establish an equal hydraulic surface loading rate of 12 m^3 m^{-2} $hour^{-1}$ in all of them. The water flow provided by the pump was 2400 L h^{-1}, minus friction and elevation losses.

The three different filter media used in this paper are BactoBalls® (Aqua-Medic®, Bissendorf, Germany), MECHpro® rings (Eheim®, Deizisau, Germany) and type A Biofill® (Bioscience, Inc., Allentown, USA) Two biofilters each contain the same type of filter medium, in different amounts, leading to six biofilters with duplicate filter media, although with a different biofiltration area. The characteristics of the biofilters are shown in Table 1, and were not modified until the end of the study. The reason for adding different amounts of filter medium to two different biofilters containing the same type of filter medium was to observe the differences in the performance. Nevertheless, when expressing the N-TAN removal rate the results are standardized by the biofiltration area.

Table 1. Characteristics of biofilters.

	FILTER MEDIA TYPE	SPECIFIC SURFACE (m^2m^{-3})	FILTER MEDIA VOLUME (m^3)	FILTER AREA (m^2)
FILTER 1	*BactoBalls®*	*300*	*0.017*	*5.1*
FILTER 2	*MECHpro®*	*1150*	*0.009*	*10.35*
FILTER 3	*Type A Biofill®*	*180*	*0.025*	*4.5*
FILTER 4	*BactoBalls®*	*300*	*0.050*	*15.0*
FILTER 5	*MECHpro®*	*1150*	*0.050*	*57.5*
FILTER 6	*Type A Biofill®*	*180*	*0.050*	*9.0*

The water in the tanks was manufactured in the laboratory, adding the corresponding amount of salt (Sea salt for human consumption, Salinera Española S.A., San Pedro del Pinatar, Murcia, Spain), 18.5 kg, to 500 L of tap water, which was kept in one large water reservoir during at least one day to remove chlorine. In that way, it is ensured that no organic matter or microorganisms are added to the bulk water, assuring the continuous biofilter performance.

There was also no supplemental aeration: the constant falling of the water from the cube to the tank was enough to achieve an approximate constant oxygen concentration. No action was taken to adjust or compensate pH variations, above all, because those variations were very low all over the trials and neither was supplementary carbon added to the tanks.

2.2. Measurements

2.2.1. Trickling filter development

Filter development took up to four weeks until it was considered that the full-grown status had been reached. In this period, 1.5 g of NH_4Cl was added daily to each tank, and the TAN concentration was measured at 9:00 a.m. The objective was to reduce the TAN concentration to 0 mg L^{-1} after 24 hours had passed since its addition. When the measured TAN was 0 mg L^{-1} at 9:00 a.m. along several days, the trial began.

2.2.2. Trial measurements

TAN and oxygen concentrations, pH and temperature of the effluent water of the six biofilters were measured in duplicated tanks every two hours along a 24-hour period. 6 trials of 24 hours were performed in a period, simulating three TAN productions (1.5, 3.0, and 4.5 g per day, one

for each week). Three periods were selected according to room temperature (one period in summer, one in autumn and one in winter). In summary, 18 trials were conducted. For every week, two trials were conducted, and an identical protocol was designed:

- Each Monday and Tuesday the bulk water of all tanks was manufactured to assure that the TAN was 0 at the beginning of the trials.
- On Wednesdays at 8:00 a.m. the first samples were taken, and after that the corresponding TAN dose was added, distributed for a few hours to simulate a long feeding period.
- On Thursdays at 8:00 a.m., after taking the corresponding samples from the first trial, the second trial began with the addition of a new equal dose to the tanks, and the second trial was conducted until the end of it on Fridays at 8:00 a.m.

A detailed protocol of the experiment can be seen in Table 2.

Table 2. Detailed experimental procedure

	Addition-depending protocol		
Hour	**1.5 g TAN/day**	**3.0 g TAN/day**	**4.5 g TAN/day**
8:00	Sampling followed by 0.9 g NH_4Cl addition	Sampling followed by 1.8 g NH_4Cl addition	Sampling followed by 2.7 g NH_4Cl addition
8:15	TAN measurement	TAN measurement	TAN measurement
10:00	Sampling followed by 2.25 g NH_4Cl addition	Sampling followed by 4.5 g NH_4Cl addition	Sampling followed by 6.75 g NH_4Cl addition
10:15	TAN measurement	TAN measurement	TAN measurement
12:00	Sampling followed by 1.35 g NH_4Cl addition	Sampling followed by 2.7 g NH_4Cl addition	Sampling followed by 4.05 g NH_4Cl addition
12:15	TAN measurement	TAN measurement	TAN measurement
14:00	Sampling every two hours from this moment until 8:00 of the following day and repeat	Sampling every two hours from this moment until 8:00 of the following day and repeat	Sampling every two hours from this moment until 8:00 of the following day and repeat

Mean temperature of the summer period (July-August) was established as 24.3ºC, mean temperature of the autumn period (September-October) was established as 19.0ºC, and mean temperature of the winter period (January-February) was established as 15.3ºC.

2.3. Equipment and chemical products

The TAN concentration and pH measurements were carried out by Orion® 4-Star Plus probe (ThermoScientific®, Waltham, Massachusetts, USA) together with ammonia and pH specific electrodes. Measurements of the TAN concentration were performed as described in García-García et al. [33]. After the sample was taken, 100 µL of hydrochloric acid (J.T.Baker®, Avantor™, Central Valley, U.S.A.) were added and 1 mL of sodium hydroxide (Scharlau, Scharlab, Barcelona, España) was also added just before the ammonia ion selective electrode was used.

Temperature and oxygen concentration measurements were carried out with a Handy Polaris® oximeter (OxyGuard®, Farum, Denmark). The probe package contained chemical products used for their preservation.

2.4. Data processing

TAN concentration results (mgTAN L^{-1}) presented were transformed into mass units (mgTAN), multiplying concentration by tank volume (500 L), and subsequently transformed into mg N-TAN (which is the quantity of nitrogen contained in the TAN molecule). That second transformation was made with the following equation:

$$\text{N-TAN (mass units)} = \text{TAN (mass units)} * \text{MW N (mass units mol}^{-1}) / \text{MW } NH_4 \text{ (mass units mol}^{-1})$$

where MW N is the molecular weight of nitrogen, and WM NH_4 is the molecular weight of NH_4

The objective of measuring the concentration every two hours was to detect patterns in N-TAN concentration variation along a standard day of operation in an aquaculture farm, based in a specific feeding regime, as well as the maximum N-TAN concentration values and the time of maximum N-TAN removal. All results for each trial described a function that was estimated and quantified. A third-degree polynomial regression thus performed for the series of results of the 24-hours trials. This model follows the typical pattern of a TAN or N-TAN daily variation in an aquaculture tank in which feeding is distributed for a relatively large period on the same time every

day, and it has been described in literature [13], and can be used to estimate the approximate N-TAN value at a specific type provided that the process parameters are similar to the determinate model. To determinate if our results fitted to that model, replicates were averaged out, the regressions were made with these results and squared Rs were observed. Statgraphics also providad information concerning quality the of the model. To detect the period up to maximum N-TAN removal a derivative of the models was established. The minimum of that derivative indicated maximum N-TAN removal, which was added to the results.

To calculate N-TAN removal in one day (expressed in g of N-TAN removed divided by biofiltration area), the difference between the N-TAN (g) added to the tank and the N-TAN (g) value at t=24 h was calculated and divided by the biofiltration area, for each one of the six biofilters, 24-hour trials, temperature and TAN additions tested. When there were significant differences in the achievement of N-TAN removal rates depending on the value of an isolated process parameter (temperature and TAN production), simple regressions were performed with the objective of estimate these relations quantitatively. The type of simple regression (lineal for TAN production and exponential for temperature) was selected by observation of the data and literature review in case [16,21,31,34].

2.5. Statistics

All statistical analyses were made by Statgraphics® Centurion XVI for Windows®. One variable ANOVAS were made to evaluate the influence of each one of the process parameters on the biofilter performance. The combination of the influence of two process parameters on the biofilter performance were tested by multivariable ANOVAS. In both cases, the multiple range test was carried out by the Student-Newman-Keuls test. Third-degree polynomial regressions for the modelation of the N-TAN daily variation and lineal and exponential regressions (both simple and multiple) for the evaluation of the influence of process parameters on N-TAN removal rate were performed with Statgraphics®. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. pH and oxygen

pH and oxygen concentration remained quite constant throughout the entire experiment. No important changes along the 24 hour trials when the bacterial activity was at maximum point. Mean pH calculated was 8.26 ± 0.05 and mean oxygen concentration 6.22 ± 0.18 mg L^{-1}.

3.2. N-TAN daily variation

In Figure 1, Figure 2 and Figure 3 are presented the N-TAN evolution along the day gathered by filter media, dose and period. The two series of data corresponding to the two biofilters that contain the same type of filter media in different biofiltration area are presented on the same chart, being *a1* the biofilters associated to tanks 1, 2, and 3 and *a2* the biofilters associated to tanks 4, 5 and 6. The equations of the individual third-degree polynomial regressions for each series of data are presented in Table 3, as well as the time of maximum N-TAN removal.

Squared Rs ranged from 0.50 to 0.90 and every series of N-TAN concentration values fitted to a third degree polynomial regression according to Statgraphics®. In every measurement, the N-TAN concentration shows a peek between 7 and 10 hours and after that it diminishes gradually until reaching a minimum around 20 hours. In several cases, the N-TAN concentration reached 0 at that point.

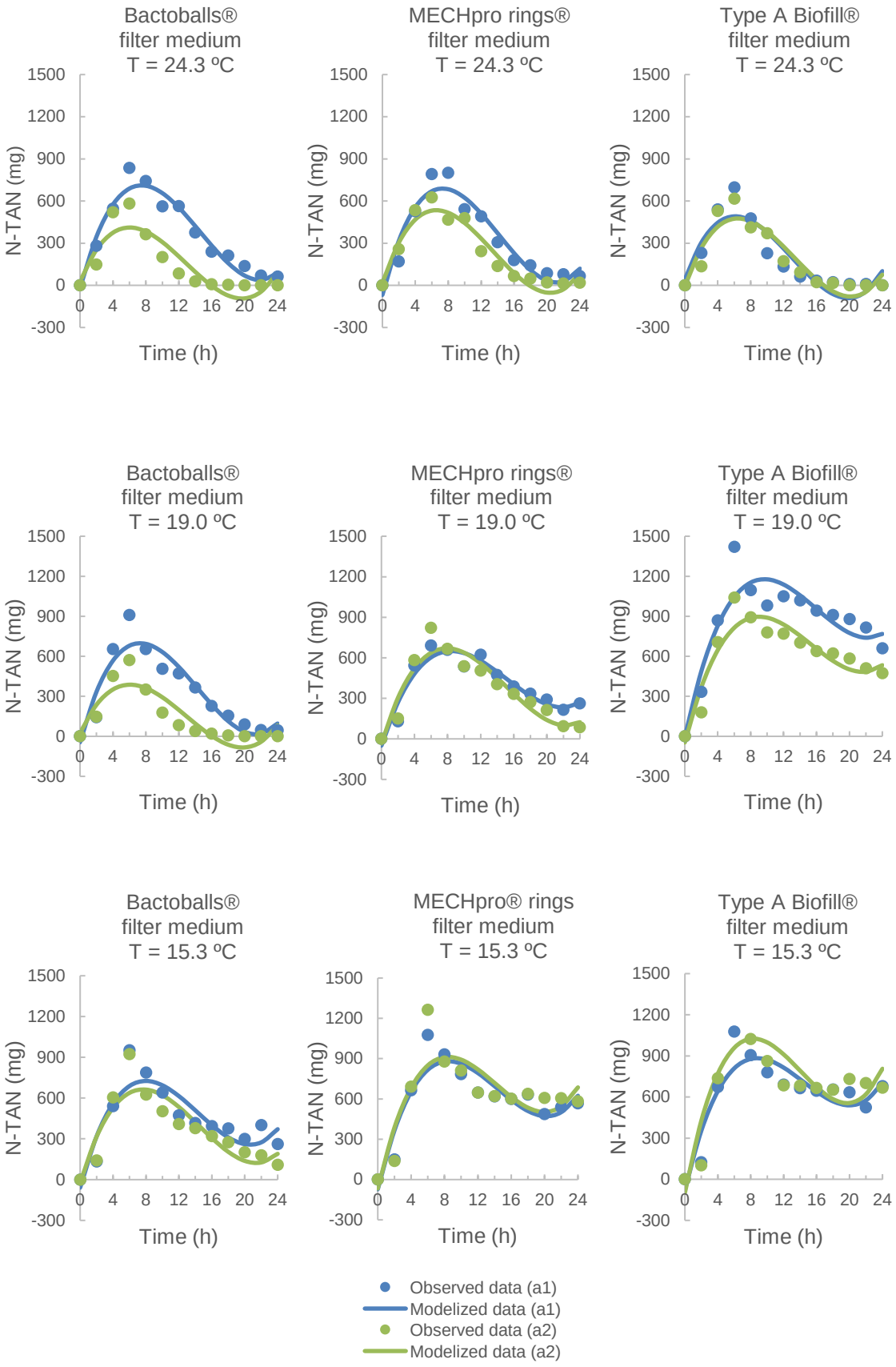

Figure 1. Summary of the daily N-TAN variation for every trial whose addition was 1.5 g TAN / day (a1 and a2 stand for the two different biofiltration areas of the two biofilters containing the same filter media).

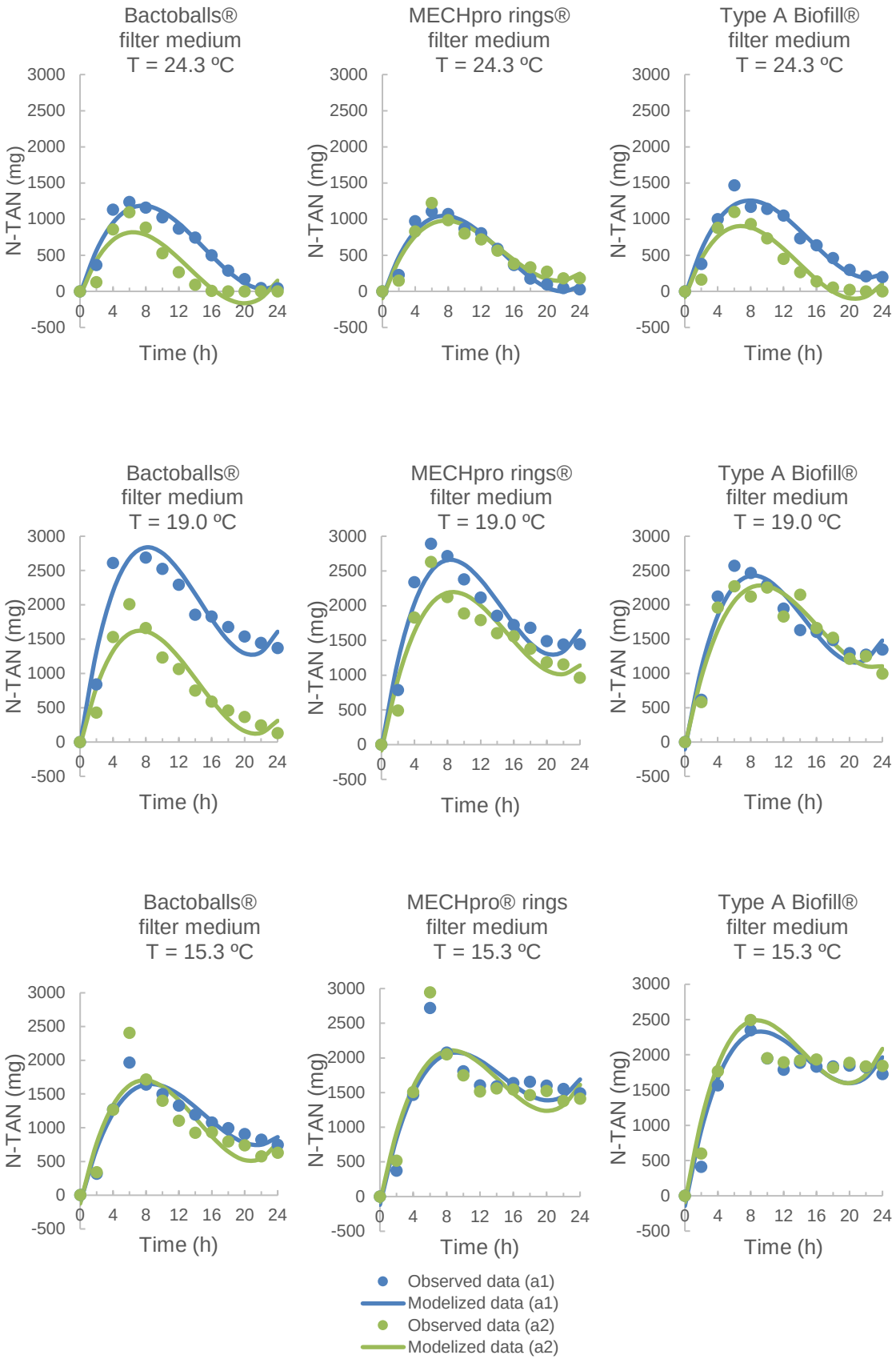

Figure 2. Summary of the daily N-TAN variation for every trial whose addition was 3.0 g TAN / day (a1 and a2 stand for the two different biofiltration areas of the two biofilters containing the same filter media).

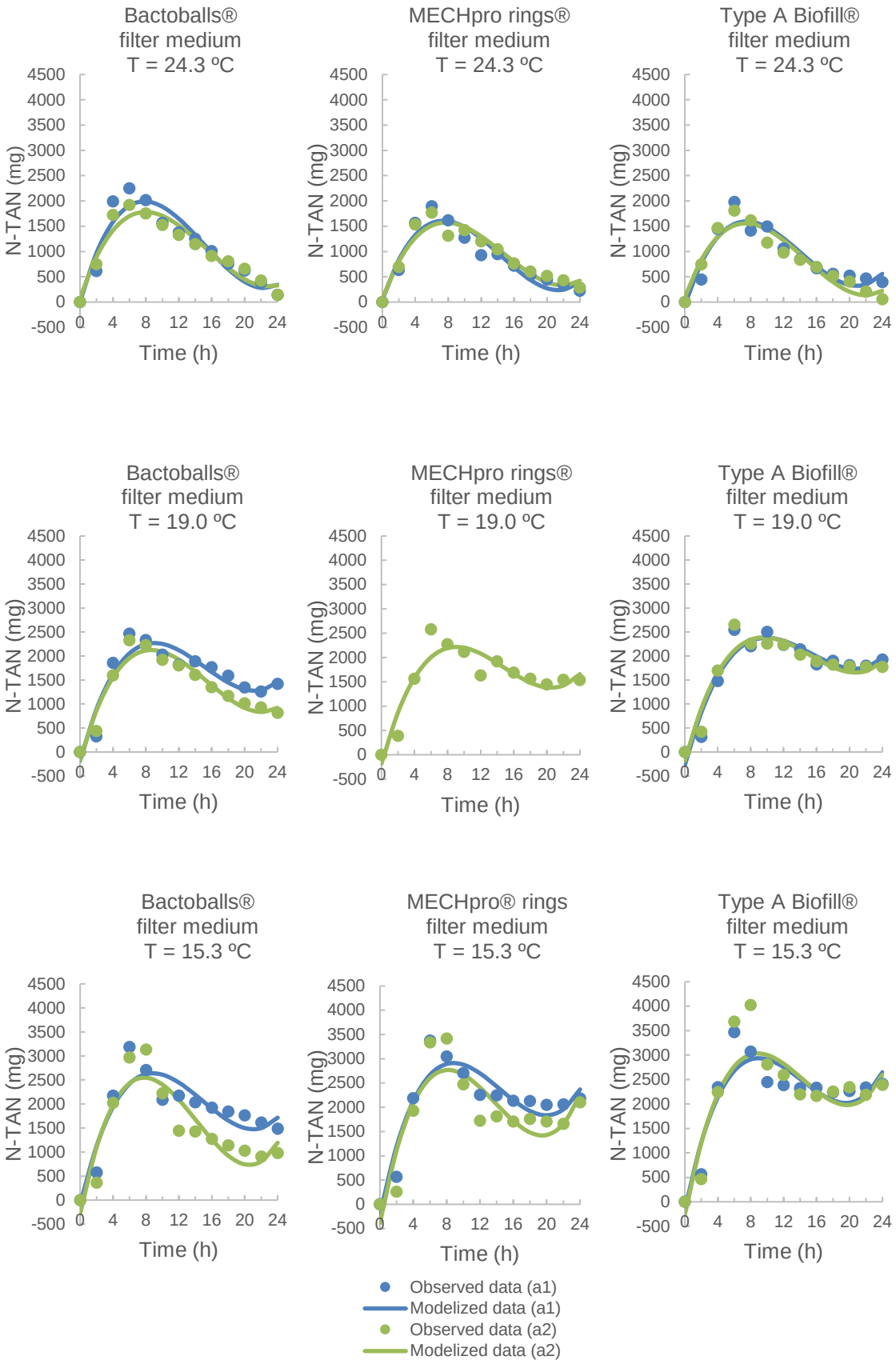

Figure 3. Summary of the daily N-TAN variation for every trial whose addition was 4.5 g TAN / day (a1 and a2 stand for the two different biofiltration areas of the two biofilters containing the same filter media).

Table 3. Summary of the third degree polynomial regressions with R^2 and time of maximum N-TAN removal (g N-TAN removed m^{-2} day^{-1}), classified by biofilter, mean temperature and TAN production

TAN production = 1.5 g TAN per day	**Summer period (Mean temperature = 24.3 ºC)**			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -18.61 + 220.63*t - 19.82*t^2 + 0.45*t^3	94.38	up to 14.6 h
	4) Bactoballs® a2	N-TAN (t) = 28.68 + 141.42*t - 15.32*t^2 + 0.40*t^3	77.31	up to 12.9 h
	2) MECHpro® a1	N-TAN (t) = -68.15 + 234.08*t - 21.55*t^2 + 0.50*t^3	91.55	up to14.3 h
	5) MECHpro® a2	N-TAN (t) = 19.20 + 176.50*t - 17.82*t^2 + 0.44*t^3	92.45	up to 13.8 h
	3) Type A Biofill® a1	N-TAN (t) = 41.87 + 163.70*t - 17.51*t^2 + 0.45*t^3	80.22	up to 13.0 h
	6) Type A Biofill® a2	N-TAN (t) = -5.80 + 167.43*t - 17.16*t^2 + 0.43*t^3	85.79	up to 13.3 h
	Autumn period (Mean temperature = 19.0 ºC)			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -44.56 + 229.50*t - 21.09*t^2 + 0.49*t^3	87.26	up to 14.3 h
	4) Bactoballs® a2	N-TAN (t) = 25.70 + 132.60*t - 14.28*t^2 + 0.37*t^3	77.26	up to 12.9 h
	2) MECHpro® a1	N-TAN (t) = -50.24 + 190.10*t - 15.58*t^2 + 0.34*t^3	91.18	up to 15.2 h
	5) MECHpro® a2	N-TAN (t) = -33.92 + 201.72*t - 17.25*t^2 + 0.38*t^3	88.59	up to 15.1 h
	3) Type A Biofill® a1	N-TAN (t) = -4.56 + 286.09*t - 21.23*t^2 + 0.44*t^3	83.48	up to 16.0 h
	6) Type A Biofill® a2	N-TAN (t) = -46.09 + 242.61*t - 19.05*t^2 + 0.41*t^3	86.34	up to 15.4 h
	Winter period (Mean temperature = 15.3 ºC)			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -59.07 + 226.05*t - 19.65*t^2 + 0.46*t^3	77.40	up to 14.2 h
	4) Bactoballs® a2	N-TAN (t) = -23.13 + 204.14*t - 18.15*t^2 + 0.42*t^3	78.15	up to 14.4 h
	2) MECHpro® a1	N-TAN (t) = -72.40 + 260.74*t - 21.78*t^2 + 0.50*t^3	81.70	up to 14.5 h
	5) MECHpro® a2	N-TAN (t) = -66.34 + 269.93*t - 22.73*t^2 + 0.53*t^3	71.93	up to 14.3 h
	3) Type A Biofill® a1	N-TAN (t) = -76.58 + 256.10*t - 20.99*t^2 + 0.49*t^3	81.38	up to 14.3 h
	6) Type A Biofill® a2	N-TAN (t) = -97.28 + 313.87*t - 26.80*t^2 + 0.64*t^3	65.40	up to 14.0 h
TAN production = 3.0 g TAN per day	**Summer period (Mean temperature = 24.3 ºC)**			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -39.57 + 363.65*t - 32.05*t^2 + 0.71*t^3	96.11	up to 14.9 h
	4) Bactoballs® a2	N-TAN (t) = -61.01 + 305.82*t - 31.40*t^2 + 0.79*t^3	81.96	up to 13.2 h
	2) MECHpro® a1	N-TAN (t) = -87.60 + 341.57*t - 30.59*t^2 + 0.69*t^3	94.73	up to 14.7 h
	5) MECHpro® a2	N-TAN (t) = -95.15 + 317.26*t - 28.04*t^2 + 0.64*t^3	87.16	up to 14.5 h
	3) Type A Biofill® a1	N-TAN (t) = -65.79 + 379.95*t - 32.60*t^2 + 0.72*t^3	94.30	up to 15.1 h
	6) Type A Biofill® a2	N-TAN (t) = -81.06 + 323.34*t - 31.44*t^2 + 0.76*t^3	89.34	up to 13.8 h
	Autumn period (Mean temperature = 19.0 ºC)			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -23.83 + 800.62*t - 67.80*t^2 + 1.55*t^3	87.47	up to 14.5 h

	4) Bactoballs® a2	N-TAN (t) = -54.44 + 516.50*t - 47.30*t^2 + 1.10*t^3	87.90	up to 14.3 h
	2) MECHpro® a1	N-TAN (t) = -51.32 + 750.50*t - 63.07*t^2 + 1.45*t^3	90.22	up to 14.5 h
	5) MECHpro® a2	N-TAN (t) = -84.62 + 606.86*t - 48.97*t^2 + 1.08*t^3	85.18	up to 15.2 h
	3) Type A Biofill® a1	N-TAN (t) = -106.80 + 700.91*t - 58.87*t^2 + 1.35*t^3	91.08	up to 14.5 h
	6) Type A Biofill® a2	N-TAN (t) = -61.78 + 584.78*t - 44.26*t^2 + 0.91*t^3	91.52	up to 16.2 h
	Winter period (Mean temperature = 15.3 ºC)			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = = -114.29 + 472.19*t - 38.39*t^2 + 0.85*t^3	86.21	up to 15.1 h
	4) Bactoballs® a2	N-TAN (t) = -125.14 + 535.96*t - 47.28*t^2+ 1.10*t^3	76.27	up to 14.3 h
	2) MECHpro® a1	N-TAN (t) = -118.97 + 565.77*t - 44.95*t^2+ 1.02*t^3	76.08	up to 14.7 h
	5) MECHpro® a2	N-TAN (t) = -76.17 + 593.72*t - 49.39*t^2+ 1.15*t^3	71.77	up to 14.3 h
	3) Type A Biofill® a1	N-TAN (t) = -144.08 + 635.76*t - 50.47*t^2+ 1.15*t^3	73.92	up to 14.6 h
	6) Type A Biofill® a2	N-TAN (t) = -90.65 + 690.27*t - 56.81*t^2+ 1.33*t^3	73.92	up to 14.3 h
TAN production = 4.5 g TAN per day	**Summer period (Mean temperature = 24.3 ºC)**			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = 11.73 + 574.22*t - 49.63*t^2 + 1.10*t^3	89.24	up to 15.2 h
	4) Bactoballs® a2	N-TAN (t) = 83.86 + 482.61*t - 40.87*t^2 + 0.88*t^3	92.23	up to 14.1 h
	2) MECHpro® a1	N-TAN (t) = 46.23 + 477.2*t - 43.43*t^2 + 1.01*t^3	89.00	up to 14.4 h
	5) MECHpro® a2	N-TAN (t) = 70.17 + 441.23*t - 38.68*t^2 + 0.87*t^3	91.80	up to 14.8 h
	3) Type A Biofill® a1	N-TAN (t) = -55.47 + 498.85*t - 45.14*t^2 + 1.06*t^3	87.13	up to 14.2 h
	6) Type A Biofill® a2	N-TAN (t) = 93.02 + 445.57*t - 40.25*t^2 + 0.91*t^3	91.09	up to 14.7 h
	Autumn period (Mean temperature = 19.0 ºC)			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -181.58 + 632.37*t - 49.90*t^2+ 1.10*t^3	87.37	up to 15.2 h
	4) Bactoballs® a2	N-TAN (t) = -166.51 + 608.98*t - 48.96*t^2+ 1.06*t^3	92.20	up to 15.4 h
	5) MECHpro® a2	N-TAN (t) = -184.92 + 621.65*t - 49.44*t^2+ 1.11*t^3	85.41	up to 14.9 h
	3) Type A Biofill® a1	N-TAN (t) = -272.47 + 636.84*t - 47.52*t^2 + 1.04*t^3	90.77	up to 15.4 h
	6) Type A Biofill® a2	N-TAN (t) = = -181.12 + 633.43*t - 48.28*t^2+ 1.06*t^3	90.19	up to 15.2 h
	Winter period (Mean temperature = 15.3 ºC)			
	Biofilter	N-TAN (mg) variation model	R^2 (%)	Max rN-TAN (g N-TAN removed m^{-2} day^{-1})
	1) Bactoballs® a1	N-TAN (t) = -88.08 + 717.11*t - 57.58*t^2 + 1.28*t^3	81.27	up to 15.0 h
	4) Bactoballs® a2	N-TAN (t) = -280.72 + 832.01*t - 73.70*t^2 + 1.73*t^3	80.79	up to 15.2 h
	2) MECHpro® a1	N-TAN (t) = -204.00 + 827.84*t - 67.62*t^2 + 1.57*t^3	85.96	up to 14.4 h
	5) MECHpro® a2	N-TAN (t) = -384.02 + 897.28*t - 77.96*t^2 + 1.88*t^3	76.57	up to 13.8 h
	3) Type A Biofill® a1	N-TAN (t) = -154.92 + 813.87*t - 66.21*t^2 + 1.55*t^3	83.19	up to 14.3 h
	6) Type A Biofill® a2	N-TAN (t) = -258.59 + 871.40*t - 71.08*t^2 + 1.66*t^3	81.92	up to 14.3 h

Relation between TAN production and mainly N-TAN mass measured in the tanks throughout the trials is evident. The higher the TAN production was, the higher are the peaks of N-TAN mass. In addition, in Figure 1, Figure 2 and Figure 3 is shown a relation between temperature and N-TAN

mass. It is observed that in the addition of 3.0 g and of 4.5 g TAN per day and for type A Biofill® over all, that, when the addition is the same, the combination of trials performed in the summer period (mean temperature = 24.3ºC) led to lower peaks of N-TAN than in the autumn period (mean temperature = 19.0ºC) and in the winter period (mean temperature = 15.3ºC).

3.3. Influence of individual process parameters

3.3.1. Influence of the filter media type on the N-TAN removal rate

Table 4. Mean (replicates indicated by n) and standard error of the N-TAN removal rates in relation to the filter media type. Different superscripts (a, b, c) in the same column indicate statistical differences (P<0.05).

Filter media type	n	Mean rN-TAN (g N-TAN removed m^{-2} day^{-1})	Standard error
MECHpro®	34	0.078^{b}	0.024
Type A Biofill®	36	0.223^{a}	0.024
BactoBalls®	36	0.235^{a}	0.024

Table 4 shows the mean of N-TAN removal rates achieved in relation to the filter media type, for every trial in every period. Bactoballs® was the filter medium type that let the biofilters which contained them achieve the highest mean efficiency in N-TAN removal (rN-TAN = 0.24 g N-TAN removed m^{-2} day^{-1}), although there were no significant differences with the mean N-TAN removal rate achieved by biofilters containing type A Biofill® (rN-TAN = 0.22 g N-TAN removed m^{-2} day^{-1}).

3.3.2. Influence of the temperature on the N-TAN removal rate

Table 5. Mean (replicates indicated by n) and standard error of the N-TAN removal rates in relation to the mean temperature. Different superscripts (a, b, c) in the same column indicate statistical differences (P<0.05).

Temperature (ºC)	n	Mean rN-TAN (g N-TAN removed m^{-2} day^{-1})	Standard error
15.3	36	0.126^{b}	0.024
19.0	34	0.151^{b}	0.025
24.3	36	0.263^{a}	0.024

Table 5 shows the mean of N-TAN removal rates achieved in relation to the temperature of the water. The highest mean N-TAN removal rate, 0.26 g N-TAN removed m^{-2} day^{-1}, was achieved in the summer period (24.3ºC). However, there were no significant differences between the mean of N-TAN removal rates achieved in the autumn period and in the winter period.

3.3.3. Influence of the TAN production on the N-TAN removal rate

Table 6. Mean (replicates indicated by n) and standard error of the N-TAN removal rates in relation to TAN added to tank water. Different superscripts (a, b, c) in the same column indicate statistical differences (P<0.05).

TAN production (g/day)	n	Mean rN-TAN (g N-TAN removed m^{-2} day^{-1})	Standard error
1.5	36	0.105^{c}	0.023
3.0	34	0.174^{b}	0.023
4.5	36	0.269^{a}	0.024

Table 6 shows the mean of N-TAN removal rates achieved in relation to the TAN production. Every increase on addition produced a significantly higher N-TAN removal rate. The highest mean N-TAN removal rate was 0.27 g N-TAN removed m^{-2} day^{-1}, at an TAN production of 4.5 g per day.

3.4. Influence of combination of process parameters

In Figure 4 mean N-TAN removal rates achieved for each possible combination of process parameters are presented, with the standard error of the multivariable ANOVA. In Table 7 is displayed a summary of the significance of the relation of values of a certain process parameter and N-TAN removal rates, when the remaining two process parameters were fixed. In the case of significant differences, a simple regression (linear or exponential) is performed and the regression equation and squared-R is presented.

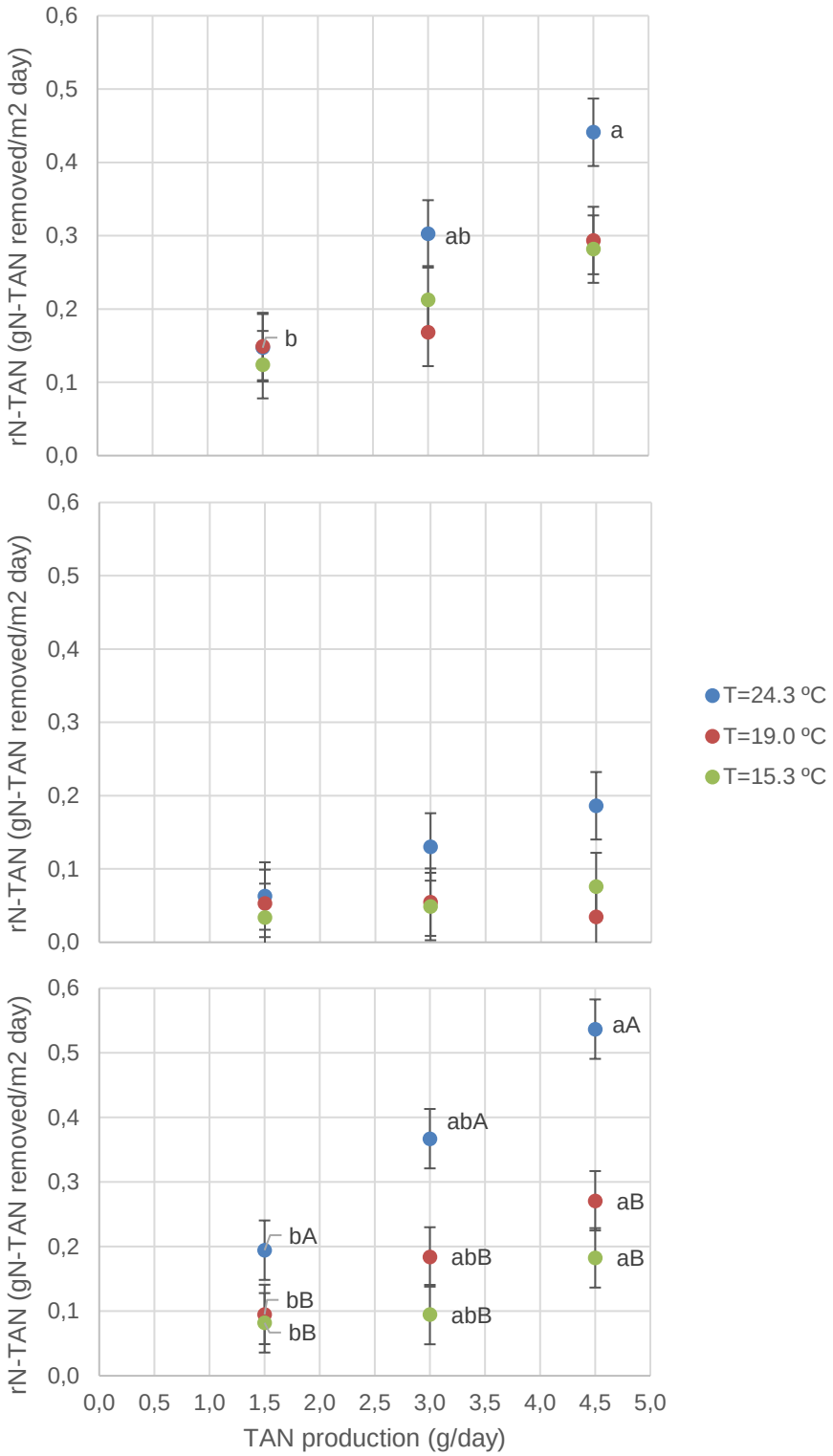

Figure 4. Mean N-TAN removal rates (±SEM: pooled standard error of the mean) achieved by biofilters classified by filter medium under fixed mean temperature and N-TAN influent concentration conditions. Small letters indicate statistical differences ($p<0.05$) on the N-TAN removal rates achieved depending on the N-TAN influent concentration whilst capital letters indicate significant differences on N-TAN removal rates achieved depending on the mean temperature.

Table 7. Significance of the differences of N-TAN removal rates achieved depending on the value of a certain process parameter. Differences were considered significant at $P<0.05$.

Filter medium	Fixed temperature (ºC)	Significant differences depending on TAN production	Regression / Squared-R
Bactoballs®	24.3	Yes	$y=0.098x+0.003$ $R^2=0.9989$
	19.0	No	
	15.3	No	
	Fixed TAN production (mg/L)	Significant differences depending on temperature	Regression / Squared-R
	1.5	No	
	3.0	No	
	4.5	No	
	Fixed temperature (ºC)	Significant differences depending on TAN production	Regression / Squared-R
MECHpro® rings	24.3	No	
	19.0	No	
	15.3	No	
	Fixed TAN production (mg/L)	Significant differences depending on temperature	Regression / Squared-R
	1.5	No	
	3.0	No	
	4.5	No	
	Fixed temperature (ºC)	Significant differences depending on TAN production	Regression / Squared-R
Type A Biofill®	24.3	Yes	$y=0.1141x+0.0239$ $R^2=1$
	19.0	Yes	$y=0.0587x+0.0073$ $R^2=1$
	15.3	Yes	$y=0.0335x+0.0192$ $R^2=0.8433$
	Fixed TAN production (mg/L)	Significant differences depending on temperature	Regression / Squared-R
	1.5	Yes	$y=0.0167*e^{0.0987x}$ $R^2=0.933$
	3.0	Yes	$y=0.0101*e^{0.1491x}$ $R^2=0.9918$
	4.5	Yes	$y=0.0284*e^{0.1204x}$ $R^2=0.9974$

These results combined with the simple ANOVAS results, suggest a poor performance of biofilters containing MECHpro® rings filter medium. No combination of temperature and TAN production led to a mean N-TAN removal rate comparable to mean N-TAN removal rates achieved by Bactoballs® and Type A Biofill® biofilters. In MECHpro® rings biofilters there were not any

significant differences in mean N-TAN removal rates when an isolated process parameter (TAN production or temperature) was modified.

For Bactoballs®, although the global mean N-TAN removal rate (0.24 g N-TAN removed m^{-2} day^{-1}) is slightly higher than the global mean N-TAN removal rate for Type A Biofill® (0.22 g N-TAN removed m^{-2} day^{-1}), the maximum N-TAN removal rate (0.54 g N-TAN removed m^{-2} day^{-1}) was achieved by the biofilters that contained Type A Biofill® when operating at a mean temperature of 24.3ºC and a TAN production of 4.5 g per day. In the same conditions, mean N-TAN removal rate achieved by biofilters that contained Bactoballs® was 0.44 g N-TAN removed m^{-2} day^{-1}, which was nevertheless the maximum N-TAN removal rate achieved by these biofilters. Temperature did not show a correlation with rN-TAN for Bactoballs® biofilters in any TAN production. In the case of Bactoballs®, TAN production was only correlated with rN-TAN in the highest temperature. However, when Type A Biofill® was used, temperature (in exponential fitting) and also TAN production (with linear fitting) showed high correlation with N-TAN removal rates.

4. Discussion

TAN concentration along the trial followed an identical pattern for every tank/biofilter tested. This pattern was easily identified with the ammonia concentration variation during a day in an aquaculture farm as simulated by the trails. This N-TAN concentration is easily portrayed as a third degree polynomial model: N-TAN rapidly inscreases as the excretion of NH_4 by the fishes takes place (reaching a peak at 6-8 hours) and starts diminishing right after the end of the feeding period because no more NH_4 is produced and the biofilter slowly start to eliminate ammonia in an increasing efficiency up to a maximum removal around 13-14 hours. Finally ammonia reaches its lowel level (ideally 0) until it increases rapidly again with the next feeding period. Similar polynomial models (second degree or fourth degree) didn't fit adequately with N-TAN concentration values obtained, either because squared Rs were too low or because p-values of the third degree terms were higher than 0.05, and thus statistically not significant.

The values of N-TAN concentration are however related to the different TAN productions (1.5, 3.0 and 4.5 g per day). Other differences between biofilters performances (based on filter media type or mean temperature) were noticed in the amount of TAN concentration. However, the stages of high TAN concentration lasted for a similar time. The mean N-TAN removal rate of all the

performed trials was 0.18 g N-TAN removed m^{-2} day^{-1}, equivalent to 0.23 g TAN removed m^{-2} day^{-1}, although mean N-TAN removal rates ranged from 0.04 g TAN removed m^{-2} day^{-1} to 0.69 g TAN removed m^{-2} day^{-1}. These values are consistent with the range of TAN removal rates reported by other authors and reviewed by Crab et al. [15], which range from 0.16 and 1.1 g TAN removed m^{-2} day^{-1}.

In several trials biofilter performance surpassed initial estimation and all TAN added to the corresponding tank was totally consumed earlier than the end of the trials, in biofilters whose biofiltration area was elevated over all. In other cases, the monitoring of two biofilters with identical filter medium presented no differences in N-TAN concentration values although the amount of filter medium (and therefore a higher biofiltration area) was higher in one of them. Both cases indicate that a fraction of filter medium was not necessary to fill the nitrification requirements and the efficiency of those biofilters was not optimal. This is reflected on mean rN-TAN removal rates being slightly lower than expected. The best example is that the biofilter which presented the highest N-TAN removal rate (0.89 g TAN removed m^{-2} day^{-1}) was biofilter 3, which contained 4.5 m^2 of Type A Biofill® filter medium and was operated under a mean temperature of 24.3ºC and a TAN production of 4.5 g per day; whilst biofilter 6, which contained 9 m^2 of filter media, presented under the same operating conditions an N-TAN removal rate of 0.49 g TAN removed m^{-2} day^{-1}.

Results presented in Table 3, Table 4 and Table 5 and Figure 4 prove that the values of the three operational parameters selected influence on the achievement of a wide range of N-TAN rates, although an isolated process parameter may not have an effect under certain circumstances. The effect of TAN production and temperature on the achievement of N-TAN removal rates, when observed, can be estimated quantitatively. Results pointed out that, when present, correlation of TAN production and N-TAN removal rate was lineal, while correlation of N-TAN removal rate and temperature was exponential. This is also similar to what is found in literature [34]. In this study every increase of TAN production lead to higher N-TAN removal rates, which was expected with high biofiltration areas as the ones that had the biofilters in this paper. Effect of temperature and TAN production is combined, and therefore the highest mean temperature and the highest TAN productions were the conditions where maximum N-TAN removal rate was achieved for almost every biofilter.

Figure 5 shows a modelling of N-TAN removal rates achieved according to the combination of the three process parameters. The modelling was prepared based on the quantification of the effect of temperature and N-TAN influent concentration (by a multiple regression) for every filter media.

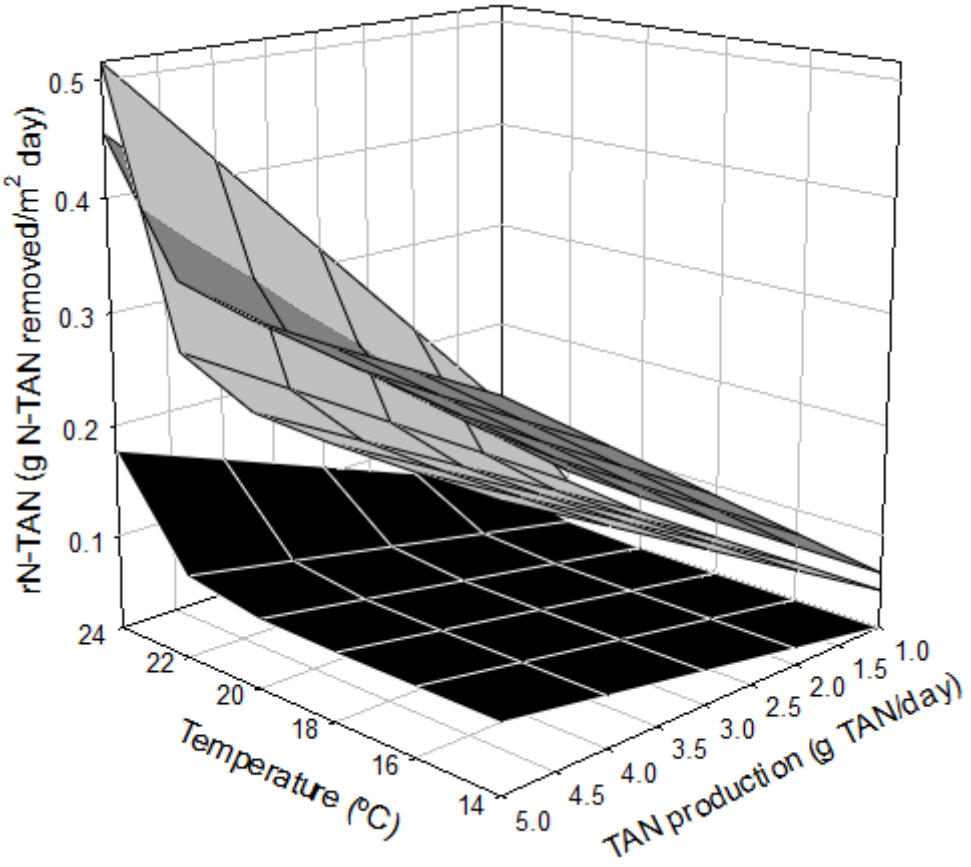

Figure 5. Three dimensional model showing quantitatively relations between N-TAN removal rates and the process parameters ammonia production and mean temperature for each one of the three filter media tested.

Results point out that MECHpro® rings, among the filter media tested in this study, led to the lowest N-TAN removal rates, and therefore it is not suggested in the construction of biofilters. The convenience of the usage of the other two filter media types largely depends on the operating conditions of the aquaculture plant. If a high mean water temperature and N-TAN concentration is allowed, Type A Biofill® presents the highest efficiency, but if the aquaculture plant requires low temperature BactoBalls® presents better performance.

The poor performance of MECHpro® rings could be explained by the small void ratio and by the water distribution through the biofilter rather than by the specific surface area, which is the highest among the filter media tested. Besides, very little differences are observed (in Figure 1, Figure 2

and Figure 3) between the N-TAN (mg) measured in the biofilters effluent from the two biofilters containing the same filter media, despite the fact that one biofilter had approximately 5 times more filter media than the other, indicating that the nitrification reaction is not taking place in the entire surface area. The other filter media types showed no significant difference in the biofilter performance in general, but under the best conditions one filter medium type (Type A Biofill®) led to a higher mean N-TAN removal rate.

It is shown that biofilters in which the TAN load and temperature was high present higher N-TAN removal rates. There is therefore needed to set the water or at least the biofilter water influent to a certain conditions that are not always desirable. A high TAN load may suppose a higher risk of illness and death to the fishes, as N-TAN will certainly be present in culture water at high concentrations, as showed in Figure 1, Figure 2 and Figure 3. Besides, it is not always possible to maintain a high fish density or a constantly elevated feeding rate. Temperature is also another problem for the turbot culture, considering that its optimal temperature range is lower than the 24ºC presented as the best temperature for maximizing biofilter performance. Results of N-TAN removal rates at lower TAN load rates and lower mean temperatures are presented for those cases, in which the modification of biofilters intrinsic properties (size, hydraulic loading, seriation…) may be the only solution.

5. Conclusions

N-TAN values followed an identical pattern for all biofilters set-ups, but at a certain TAN production differences in N-TAN maximum values are observed depending on temperature and filter media type. N-TAN removal rate (as a biofilter efficiency measure) is dependent on all three (filter medium type, TAN production and temperature) process parameters. The effect of a single process parameter when the others remain constant can be sometimes estimated quantitatively, linearly in case of the TAN production and exponentially in case of the mean temperature. The combination of the optimal values of these process parameters can lead to higher N-TAN removal rates in certain occasions, while in other cases the filter media type is the most determining factor and optimal values of the other process parameters doesn't imply a higher efficiency. In the same way less optimal values of temperature or TAN production didn't suppose a significant reduction in biofilters efficiency when Bactoballs® were their filter media. On the other hand, conditions for

maximizing biofilter performance can be compromised with fish welfare as higher TAN loads are required.

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