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Pilot-scale continuous flow granular reactor for the treatment of extremely low-strength recirculating aquaculture system wastewater

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ABSTRACT

To avoid toxic ammonium and nitrite concentrations in aquaculture systems is crucial to maintain the fish production. When recirculating aquaculture systems (RAS) operate in freshwater farms during the dry seasons, the concentrations of these pollutants increase. The objective of the present study is the evaluation of a Continuous Flow Granular Reactor (CFGR) for the treatment of freshwater RAS stream at pilot-scale during two consecutive dry seasons. The CFGR was fed with a extremely low-strength recirculation stream of a trout farm (0.12–1.84 mg NH_4^+-N/L and 2.2–8.14 mg C/L). Two different configurations were evaluated. The first configuration consisted on a CFGR fed from the bottom, being the up-flow velocity the only shear force to mix the biomass. The second configuration incorporated a mechanical stirrer and a sieve to improve the biomass mixing and retention. The CFGR was operated at short hydraulic retention times (HRT) which ranged from 11 to 68 min. The configuration with a mechanical stirrer and sieve was optimal in terms of biomass retention and nitrogen removal performance. Despite the low nitrogen and organic matter concentrations, granulation was achieved in 55 days, with an average granule diameter up to 0.47 mm. Ammonium and nitrite removal percentages up to 81% and 100% were achieved, respectively. The ammonium and nitrite production rate in the trout farm were lower than the removal achieved by the CFGR, which makes the implementation of this system appropriated to maintain the concentration of these compounds below toxic levels for rainbow trout.

1. Introduction

The exponential increase of human population involves the raising of aquaculture activities as an essential sector to provide a human food source in the future. While wild fish captures increased only 8% from 1990 to 2012, aquaculture production increased more than five times [1]. Nowadays, the aquaculture sector produces more than 50% of the fish consumed [2]. In addition, freshwater aquaculture activities require large areas and water supply that, in certain regions such as Mediterranean countries, is difficult to access.

Compared with other forms of production, recirculating aquaculture systems (RAS) reduce water usage and improve waste management and nutrient recycling boosting aquaculture production [3]. Trout production is one of the most extended freshwater aquaculture sectors in Europe. Thus, the application of RAS in intensive rainbow trout farms is been studied to satisfy the production demand [4–6]. However, the closed water flow in the RAS has associated an increase of nitrogen compounds, organic matter and phosphorous concentrations [7]. In RAS, ammonium and free ammonia (FA) are in an equilibrium which is influenced by pH and temperature [8]. Both species can be toxic to fish, however, FA is more harmful because it has higher lipid solubility and consequently it can diffuse through the biological membranes easier than the ammonium ions [9]. While ammonium concentrations over 1.25 mg NH_4^+-N/L are toxic for rainbow trout, concentrations over 0.021 mg NH_3-N/L are toxic as FA [10]. Regarding nitrite, in equilibrium with free nitrous acid (FNA), it is more toxic than ammonium. Russo et al. [11] demonstrated that concentrations between 0.06 and 0.12 mg NO_2-N/L caused 50% of lethality on rainbow trout. Their study also shows that FNA was toxic even at lower concentrations due to its liposolubility. Therefore, RAS are developed to remove the ammonium

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avoiding nitrite accumulation as a crucial aspect to ensure fish health.

To reduce these pollutants concentrations, the use of biofilters in RAS is widely applied [12] where heterotrophic and nitrifying bacteria are developed for this purpose [13]. With respect to their application Suhr and Pedersen [4] showed that biofilters operated in rainbow trout RAS require hydraulic retention times (HRT) between 85 and 150 min to oxidize all the ammonium. However, when the water flow demand is extremely high (such as in rainbow trout production) the hydraulic retention time (HRT) must be as short as possible, to avoid the use of enormous biological reactors that might limit this conversion.

Aerobic granular sludge (AGS) based technologies appear as an alternative to traditional attached biofilters in RAS, due to its biomass retention properties and potentially exposed to better mass transfer conditions. In AGS systems the microorganisms are self-immobilised in aggregates, with better settling properties and tolerance than conventional activated sludge to toxic compounds, such as pharmaceuticals [14]. Furthermore, in reactors operated in aerobic conditions the presence of anoxic (internal) and aerobic (external) layers allow the simultaneous occurrence of anoxic and aerobic processes inside the granules [15]. Besides, AGS technology requires a small implantation area which is crucial in freshwater aquaculture farms. AGS has been widely investigated for the treatment of urban and industrial wastewater and is one of the biotechnologies in expansion [16]. Up to now, AGS technologies have not been widely studied in aquaculture systems, where the very low pollutant concentrations (C and N) and high flows are a challenge.

There are some studies about the AGS start-up treating low-strength wastewater (between 35 and 320 mg COD/L and 10–55 mg N/L) at pilot scale [17–20]. However, the pollutant concentrations of freshwater aquaculture streams are much lower (5 mg COD/L and 0.30–0.81 mg N/L) [21,22]. Most of these studies at pilot scale were carried out in sequencing batch reactors (SBR). Only Sun et al. [19] succeeded to cultivate aerobic granules treating low-strength wastewater in a pilot-scale continuous flow granular reactor (CFGR). The continuous reactors have certain advantages over SBRs such as a robust operation and a larger treatment capacity [23] which is essential to treat the large aquaculture flows. We et al. [24] highlighted the interest of evaluating the aerobic granulation process in continuous systems (especially in terms of the nutrient transformations) as a conclusion of their critical review about AGS technologies for the treatment of low-strength wastewater.

In this context, this is the first research study that tackles the application of CFGR at pilot scale for the treatment of extremely low-strength wastewater in an aquaculture trout farm. Since treating large flows is mandatory to allow water recirculation inside the plant, the present research work is focused on biomass retention and granulation at short HRT. Moreover, nitrogen species transformations and removal are also followed to evaluate the viability of the system to produce effluents with enough quality to protect fish health and enhance its production.

2. Materials and methods

2.1. Trout farm performance

The experiments were developed with a pilot plant comprising a CFGR installed in an intensive rainbow trout farm located in the northwest of Spain (Grupo Tres Mares S.L.). The factory takes the water for the process from a near river at a flow in the range 1–3 m^3/s. This water flows through the fish ponds and it is discharged to the seaside containing negligible nitrogen, organic matter concentrations and suspended solids. During dry seasons the river flow decreases and, consequently, the availability of water for the farm does as well. Thus, the water stream is recirculated inside the plant (RAS system) to keep the flow constant allowing trout feeding and avoiding fish stress. However, with this recirculation, the ammonium and organic matter concentrations increase daily due to fish metabolism. Since the fish tanks are aerated part of the ammonium excreted is oxidised to nitrite and nitrate, therefore also the nitrogen oxides concentrations increase. The RAS of this farm comprises a treatment system based on six rotatory biofilters. However, facing the extremely high recirculation flows (up to 10,800 m^3/h) this treatment is only able to separate suspended solids, while the nitrogen compounds are not removed. If the recirculation is extended in time the ammonium (daily rise up to 0.1–0.2 mg N/L) and nitrite concentrations increase to toxic values causing fish mortality. Consequently, the water is more polluted and fish production is limited during these periods.

2.2. Reactor set-up and seeding sludge

Since the fish farm only recirculates water during dry seasons the pilot plant was operated in two independent experiments corresponding to two consecutive summers (years 2019 and 2020, respectively). In both experiments a pilot-scale CFGR, with an effective volume of 30 L, was evaluated for the treatment of the recirculation stream from the trout farm. The reactor dimensions were inner diameter of 22.4 cm and total height of 88.5 cm.

In the first experiment (Summer 2019), several reactor configurations and operational parameters were tested to find the optimal performance. Initially, the reactor was not provided with aeration and the feeding media was pumped through the bottom in a continuous mode. Thus, the feeding up-flow was the mechanism to expand the biomass bed inside the reactor (Fig. 1a). Throughout the operational period, a mechanical stirrer was added to improve the mixture, favor the mass transfer and increase the hydraulic shear forces. The mechanical stirrer performed at low rotational speed (40 rpm) and presented a cross vane near to the reactor bottom to avoid an excessive sludge bed expansion. Simultaneously, a sieve was integrated at the upper part of the reactor to prevent biomass wash out (Fig. 1b). The sieve screen was able to retain particles with diameters above 0.1 mm being a particle size granulation driving force. The effluent was discharged continuously from the top of the reactor by overflow. In the second experiment (Summer 2020), the reactor was operated during the whole period with the best configuration found in the first experiment (Fig. 1b).

Temperature and pH were measured but not controlled. The temperature of the recirculation water stream in the trout farm varied between 18.6 and 21.9 °C and 17.3–26.4 °C and the pH values ranged from 5.1 to 5.7 and 5.6–6.4 in the first and second experiments, respectively. The dissolved oxygen (DO) concentration was variable due to the trout tanks oxygenation and varied between 2.9 and 6.4 mg O_2/L and 4.7 and 9.6 mg O_2/L in the first and in the second experiments, respectively.

The pilot CFGR was seeded with secondary sludge (Fig. 2a) collected from the biological treatment of a wastewater treatment plant (WWTP) (near to Santiago de Compostela). The achieved initial biomass concentration was of 1.22 and 4.94 g TSS/L in the first and second experiments, respectively. This seeding sludge was characterized before the reactor start-up in terms of sludge volume index (SVI). The maximum heterotrophic, ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) activities of the sludge were also determined.

2.3. Operational conditions

2.3.1. Operational strategy

Since trout farming needs the use of technologies able to treat its recycling water as fast as possible due to the high water flow requirements, short HRT values were imposed during the reactor operation (between 11.2 and 68.2 min). With this stress conditions, only the biomass able to aggregate in flocks and granules could remain inside the reactor.

2.3.2. First experiment

The CFGR operation in the first experiment was divided into three stages depending on the nitrogen conversion performance, (Table 1).

During Stage I (day 1–15) the ammonium and organic matter

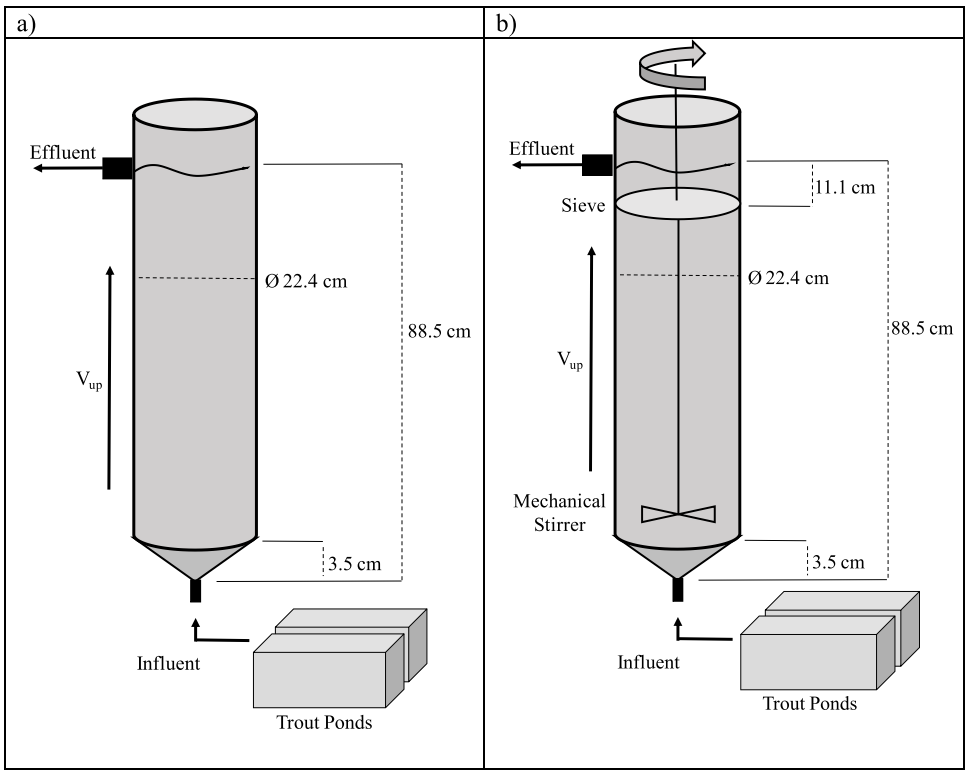

Fig. 1. Layouts of both CFGR configurations: (a) without sieve and mechanical stirrer; (b) with sieve and mechanical stirrer.

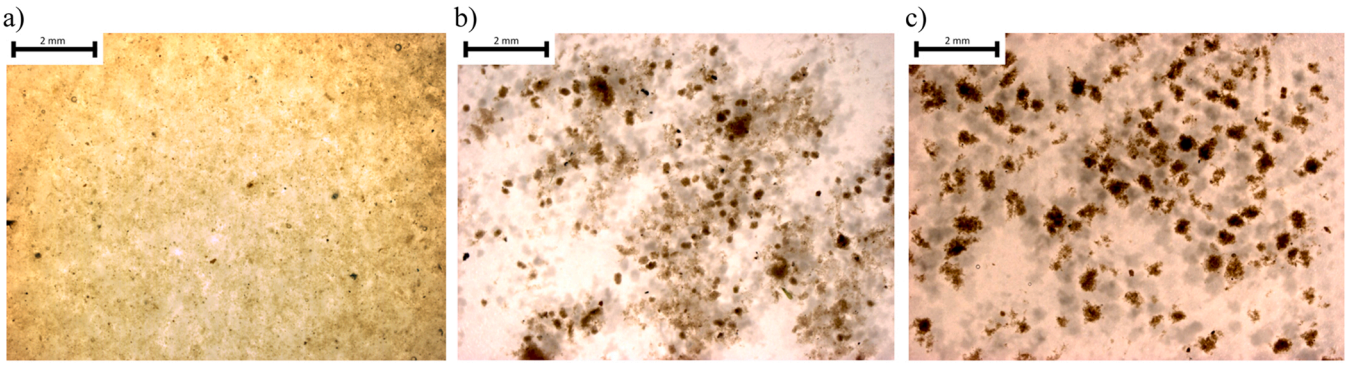

Fig. 2. Images of the CFGR biomass: (a) seeding sludge, (b) on day 79 of the first experiment and (c) on day 85 of the second experiment. The size bar indicates 2 mm.

concentrations in the feeding remained relatively constant. At the beginning the imposed feed flow was fixed at 45.6 L/h, which resulted in an HRT of 39.5 min and an up-flow velocity (V_{up}) of 1.1 m/h. Then, to keep the sludge bed expanded, the feeding flow was increased progressively and on day 14 it was of 160 L/h (HRT of 11 min and a V_{up} of 3.8 m/h).

During Stage II (day 16–55) the reactor suffered salinity and starvation shocks. The salinity shocks occurred when the trout farming plant used seawater to keep the water levels of the trout tanks stable, due to the scarcity of freshwater from the river in dry season periods. The CFGR experienced the salinity shocks during two episodes (days 16–18 and days 25–28 of operation). Consequently the Na^+ concentration increased from 9 (usual freshwater concentration) to 780 mg Na^+/L. On the other hand, the starvation shocks occurred during sporadic rainy days, when the trout farm turned off the recirculation and took the water back from the river (days 35–40 and days 48 – 49 of operation). Therefore, the concentration of ammonium and organic matter in the water fed to the reactor decreased rapidly. To improve the biomass retention, the HRT was increased to 14.2 min and consequently, the V_{up} diminished to 3 m/h.

In Stage III (day 56–91), a mechanical stirrer was placed inside the reactor to expand the sludge bed and a sieve was fixed at the top to retain the biomass aggregates. Moreover, on day 56 the reactor was re-inoculated with 90% (in volume) of new seeding sludge from the same WWTP origin of the inoculum, corresponding to the remaining 10% (in volume) the biomass enriched in the previous stages. At the beginning of Stage III the HRT was 32.3 min to reduce the V_{up} allowing the adaptation of the new inoculated biomass to the system conditions. Then the feeding flow was increased gradually up to 108.6 L/h and consequently the HRT was of 16 min

2.3.3. *Second experiment*

This second experiment was performed with the CFGR configuration which provided the best results in the previous experiment: using the mechanical stirrer to expand the biomass bed and the sieve at the top to improve the biomass retention (tested in Stage III). In this operational

Table 1
Operational conditions and influent characteristics of the CFGR in both experiments.

Experiment	First			Second
Operational Stage	Stage I	Stage II	Stage III	Single-stage
Main feature	Start-up	Salinity and starvation shocks	Reinoculation. Mechanical stirrer and sieve at the top of the reactor.	Mechanical stirrer and sieve at the top of the reactor.
Days	0–15	16–55	56–91	1–99
Temperature (°C)*	20.0–22.1	18.8–23.0	18.4–22.7	16.4–26.4
Dissolved oxygen (mg O_2/L)*	5.1–6.1	1.7–6.2	3.0–6.4	4.7–9.6
pH*	5.8–6.1	5.7–6.2	5.8–6.2	5.7–6.5
TOC (mg C/L)*	2.2–3.3	3.2–7.9	4.13–8.14	2.8–7.7
IC (mg C/L)*	1.3–2.4	0.5–2.5	0.6–2.5	0.4–2.4
NH_4^+ (mg N/L)*	0.52–0.76	0.12–1.00	0.14–1.84	0.32–1.44
NO_2^- (mg N/L)*	0.01–0.05	0.01–0.22	0.01–0.13	0.04–0.34
NO_3^- (mg N/L)*	0.78–1.01	0.66–1.23	0.81–1.24	2.20–2.95
Feeding flow (L/h)	46–72 (days 0–3)	160 (days 16–18)	56–120 (days 56–68)	26–54 (days 1–65)
	120–160 (days 4–15)	126 (days 19–55)	77–109 (days 69–91)	27–40 (days 66–91)
HRT (min)	39.5–25.0	11.2	32.3–14.9	68.2–33.3
	15.0–11.2	14.2	23.4–16.6	45.5–66.7
Up-flow velocity (m/h)	1.1–1.7	3.8	1.3–2.9	0.6–1.3
	2.9–3.8	3.0	1.8–2.6	0.6–0.9

HRT: hydraulic retention time; TOC: total organic carbon.
*Influent values.

period (summer 2020) occurred less rainy periods than in the first experiment (summer 2019) and, consequently the CFGR suffered fewer starvation shocks. The HRT was between 68.2 and 33.3 min and consequently the Vup was lower (0.6–1.3 m/h) than in the first experiment (Table 1). In this second experiment the pilot plant operated for 99 days without the necessity of biomass reinoculation.

2.4. Batch activity tests

To follow the biomass specific activity, several assays were performed in batch mode throughout the reactors operation. Respirometric assays were performed to follow the specific aerobic heterotrophic activity (HET_{act}) and the specific ammonium (AOB_{act}) and nitrite (NOB_{act}) oxidizing activities [25]. To avoid the nitrifying activity, during the HET_{act} test, 0.01 mmol/L of allylthiourea was added. Also, in the AOB_{act} tests 24 μmol/L of sodium azide was added to inhibit the NOB activity. All these batch activity tests were conducted at 20 °C and in triplicate.

2.5. Analytical methods

Influent and effluent streams of the CFGR were sampled 2–3 days a week to follow the reactor performance. Liquid samples were filtered using a cellulose-ester filter (0.45 μm pore size) to remove suspended solids. A spectrophotometric method was applied to determine ammonium, nitrite and nitrate concentrations. Volatile suspended solids (VSS), total suspended solids (TSS) and SVI were determined according to the Standard Methods [26]. Total organic carbon (TOC) and inorganic carbon (IC) concentrations were measured by a Shimadzu analyzer (TOC-L, automatic sample injector Shimadzu ASI-L). The respirometric assays were conducted using a biological 152 oxygen monitor (BOM, Ysi Inc. model 5300) equipped with oxygen selective probes (YSI 5331). The granule density was calculated as the mass of the granule per granule volume applying the blue dextran method [27]. Settling velocity was measured in a 100 mL cylinder (internal diameter of 25 mm) by following height of the water-biomass interface with the time. The average diameter and size distribution of the granules were determined using a stereomicroscope (Stemi 2000-C, Zeiss) incorporating a digital camera (Coolsnap, Roper Scientific Photometrics), these images were processed using the Image ProPlus® software.

2.6. Calculations

The solids retention time (SRT) was calculated according to [28] (Eq. (1)):

$$SRT = \frac{TSS * V_r}{TSS_{eff} * \quad Q_{eff} + TSS_w * \quad Q_w} \tag{1}$$

Where TSS is the total suspended solids concentration inside the reactor (g TSS/L); V_r is the reactor volume (L); TSS_{eff} is the TSS concentration in the effluent (g TSS/L); Q_{eff} is the effluent flow rate (L/d); TSS_w is the TSS concentration in the withdrawn sludge (g TSS/L); and Q_w is the withdrawn sludge flow rate (L/d).

The FA (Eq. (2)) and FNA (Eq. (4)) concentrations were calculated theoretically according to Anthonisen et al. [29]:

$$FA = \frac{\left[N - NH_4^+\right] * 10^{pH}}{\left(\frac{kb}{kw}\right) + 10^{pH}}, \tag{2}$$

$$\frac{kb}{kw} = e^{\frac{6344}{273+T}}, \tag{3}$$

$$FNA = \frac{\left[N - NO_2^-\right]}{(ka) * 10^{pH}}, \tag{4}$$

$$ka = \quad e^{\frac{2300}{273+T}}, \tag{5}$$

Where $[NH_4^+$-N] and $[NO_2^-$-N] are the ammonium and nitrite nitrogen concentrations, respectively. The Eqs. (3) and (5) were used to calculate the kinetic parameters kb/kw and ka that depend on T which is the temperature in Celsius degrees.

3. Results and discussion

3.1. Granulation process and biomass retention

3.1.1. Granule formation

Considering the high flows of freshwater used in aquaculture farms to apply a HRT value as low as possible is needed to be able to operate a moderate volume reactor. In the present research work the applied HRT was between 11 and 68 min. This short HRT value was required also to achieve the biomass granulation process with such a low-strength wastewater as it helped to increase the organic (OLR) and nitrogen loading rates (NLR). Short HRT values are commonly applied in the treatment of low-strength wastewater with granular sludge [30–32], although the values used, in the range of 5.6–8 h, are still too long for aquaculture effluents treatment. Besides, large feeding flows involve a fast V_{up} which favours the formation of aggregates with better settleability at low-strength conditions [18]. The initial up-flow velocity was

fixed at 1.1 m/h as this value was assumed to impose the appropriate hydrodynamic conditions to promote the granulation process [33].

During the first experiment, aggregates of flocculent biomass appeared in Stage I at day 10. These aggregates remained in the reactor throughout the duration of Stages I and II. Nevertheless, the granulation process was not achieved taking into account the poor biomass settling properties (SVI_{10}/SVI_{30} ratio under 0.8) and the small size of the aggregates (below 0.2 mm) [34]. Thus, during more than 55 days mature granules were not observed probably due to the low organic matter and nutrient concentrations that difficulted the granulation process. In addition, in the first experiment the initial concentration of the seeding sludge was of 1.02 g VSS/L, probably too low to facilitate the achievement of a stable granulation. Inoculation with high sludge concentration (20 g VSS/L) was reported to accelerate the granulation process treating low-strength wastewaters [35].

Moreover, the biomass washing episodes, occurred for example in rainy days 24 and 41, contributed to the delay of the granulation process. In this sense, Pronk et al. [34] proposes to operate granular reactors with a flexible HRT to control biomass washing episodes associated to rainy periods. This flexible strategy is crucial at fast V_{up}, otherwise severe biomass washout episodes would take place [18]. Therefore, in the present study the HRT was lengthened in rainy periods and shortened in dry ones. For example, on day 68 of the first experiment it rained and the HRT was increased from 14.9 to 23.4 min with the consequent V_{up} reduction. However, despite this reduction, the solids concentration in the reactor diminished from 1.94 to 1.38 g VSS/L. This biomass washing can be attributed to the OLR and NLR decrease due to dilution of the used water with rainwater. Between days 63 and 73 the OLR and NLR values were reduced around 50% and 33%, respectively.

In Stage III, after the reinoculation of a 90% of the reactor volume (with a biomass concentration of 2.64 mg TSS/L) and the sieve incorporation, the flocculent biomass started to be more compact as a result of the biomass retention improvement. Afterwards, the SRT was around 4.9 d, which means that it is possible to achieve nitrifying bacteria activity [36]. Wagner and da Costa [37] reported that ammonium oxidisation efficiency increased from 15% to 70% when the SRT augmented from 3 to 7 days in a SBR treating low-strength domestic wastewater.

Independent granules appeared on day 72 in the first experiment indicating that with the operational change (mechanical stirrer and sieve at the top) the formation of the granules was promoted, needing only 14 days after the change. An average granules diameter value of 0.31 ± 0.11 mm was measured on day 79 (Fig. 2b).

In the second experiment, despite the application of the optimized configuration including mechanical stirrer and sieve at the top, the granulation process was slower. Aggregation of flocculent sludge started before day 7. However, 37 days were necessary to observe the first aggregates and 55 days to observe the first independent granules. Although the ammonium and nitrite removal performances were much higher in this experiment than in the first one, and taking into account the larger concentration of the seeding sludge (4.25 g VSS/L), the time to granulate the biomass was longer (55 days) than in previous Stage III (14 days). This fact can be directly related to the longer HRT applied which caused a lower V_{up} (0.6–1.3 in front of 1.1–3.8 m/h). Besides, the SRT fluctuated between 3.2 and 14.5 d due to the variable biomass concentrations in the effluent. The average granules diameter value was of 0.47 ± 0.18 mm on day 85 (Fig. 2c).

Only in a few research works managed to cultivate granular sludge with low-strength wastewater successfully at pilot scale. However, in none of them succeeded with the granulation at these extremely low concentrations (2.8–7.7 mg C/L and 0.32–1.44 mg NH_4^+-N/L). Ni et al. [17] treated municipal wastewater with concentrations of 35–120 mg COD/L and 10–40 mg NH_4^+-N/L, and observed the first granules after 80 days of operation with a similar diameter as those from the present study (0.3 mm). They also reported that at day 120 the average granule diameter increased to 0.4 mm. Their results suggest that longer operational periods allow to form bigger granules as it could happen when water recirculation in the farm is used in longer dry seasons. Zou et al. [38] treating municipal wastewater containing organic matter and nitrogen concentrations of 125 ± 36 mg COD/L and 19–4 mg NH_4^+-N/L, respectively, obtained the first granules after 61 days of operation in a continuous flow reactor. Nevertheless, they added sludge-based micropowder (20 μm) to accelerate the granulation. In another study Santorio et al. [39] achieved granules with a diameter of 1.9 cm in a CFGR treating extremely low-strength wastewater (simulating an aquaculture effluent). Nevertheless, their research work was performed with a laboratory-scale reactor fed with synthetic wastewater with concentrations of 15–37.3 mg COD/L and 2.5–2.9 mg NH_4^+-N/L, higher than in the present study. Therefore, the strategy evaluated in the present study was suitable to achieve granules, taking into account the challenge of treating extremely low-strength aquaculture effluents in the same farm plant, where it varies in composition and experiencing shock episodes (rain and salinity) that cannot be controlled as at laboratory scale.

3.1.2. Biomass retention

The main challenge in treating aquaculture streams with these high flows and extremely low-strength composition through a CFGR is the biomass retention. With the up-flow velocity as the only mechanism to stir the biomass and keep the bed expanded (Stages I and II of first experiment) to avoid biomass wash out was difficult. Thus, during Stages I and II, influent changes, like salinity increase or dilution by rain, caused biomass wash out in several operational days. This fact affected the biomass adaptation to these stream conditions in a negative way.

On day 3 of the first experiment, the sludge remained in the reactor lower part. Therefore, the feeding flow was increased to 72 L/h which resulted in an HRT of 25 min and a V_{up} of 1.7 m/h. The sludge settleability continued improving and the feeding flow was augmented consequently. After 7 days from the inoculation, the biomass sedimentation velocity was 0.19 m/h and 63 days later was 0.26 m/h (Fig. 3a).

With the shocks occurrence (of salinity and starvation), the alternation of biomass wash out episodes and biomass accumulation at the bottom of the reactor was usual during Stage II, reducing the removal capacities of the system. These problems caused notable biomass loss inside the reactor. While during the saline shocks the biomass retention was worse due to the increase of the water density, during starvation periods (by rainy episodes) it was worse due to cellular decay. To face these problems, on day 19 the HRT was lengthened to 14.2 min to reduce de Vup to 3 m/h and to maintain the biomass inside the reactor. Even with the V_{up} reduction, approximately 90% of the biomass was lost at the end of Stage II. These results showed that imposed operational changes were necessary to increase biomass retention and mass transference.

In Stage III with the sieve incorporation the biomass aggregates retention improved. Thus, a sedimentation velocity of 2.92 m/h was achieved on day 85. Therefore, although the biomass settling improvement took place in all operational stages, during stage III this increase was more marked (Fig. 3a).

In the second experiment the biomass settling improvement was faster. The seeding sludge presented a sedimentation velocity of 0.02 m/h and on day 30 it increased to 0.26 m/h, indicating the benefits of the mechanical stirrer and the sieve. The sedimentation velocity continued improving up to 3.39 m/h on day 84 (Fig. 3b).

This upgrade was similar for the SVI. In the first experiment, the inoculum presented a SVI_{30} of 343 mL/g TSS which is high to keep the biomass inside the CFGR. However, on day 7 it went down to 244 mL/g TSS showing a fast adaptation to the CFGR conditions. Moreover, the SVI_{30} improved on day 83 achieving a value of 108 mL/g TSS (Fig. 4a). The second experiment presented the same trend. While the inoculum had a SVI_{30} of 461 mL/g TSS on day 36 it was of 153 mL/g TSS. This SVI_{30} improvement continued, being 79 mL/g TSS on day 84 (Fig. 4b).

This amelioration is related to a more stable operation after the mechanical stirrer and sieve incorporation. Derlon et al. [18] reported that stable granulation during the treatment of low-strength wastewater

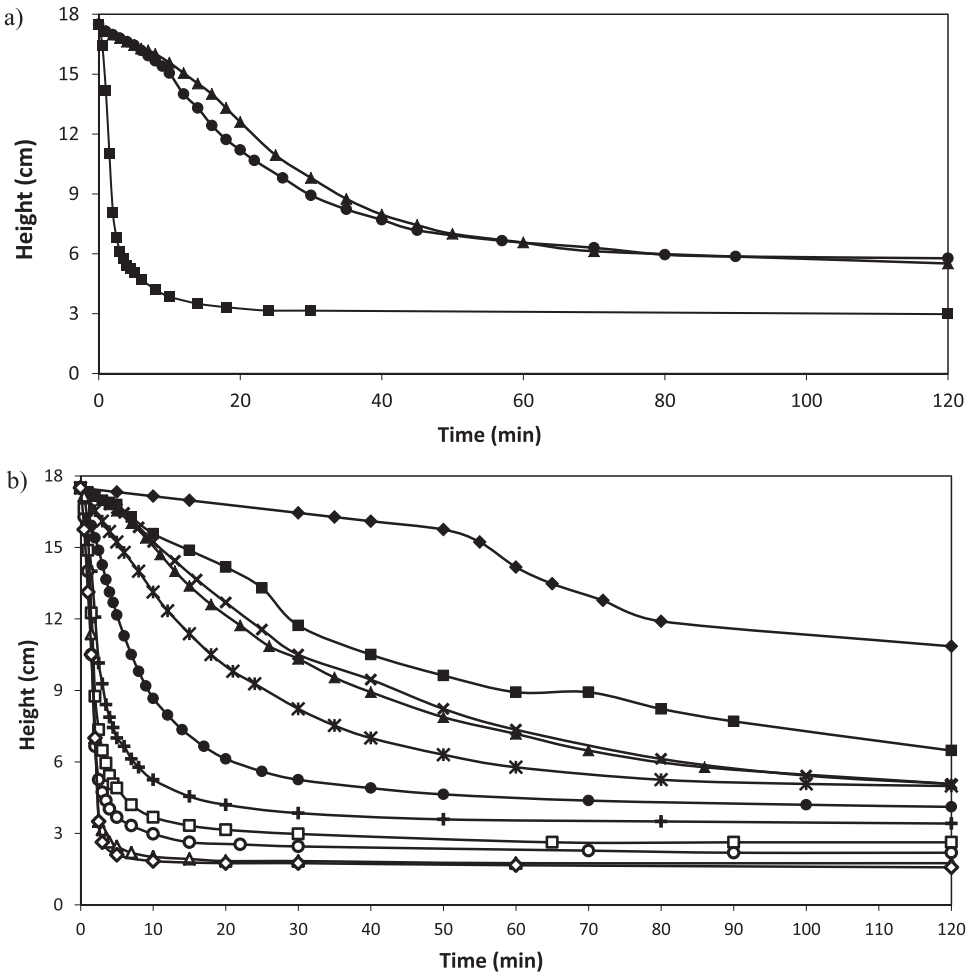

Fig. 3. Biomass sedimentation velocity curves: a) First experiment day 7 (▲), day 63 (●), day 85 (■); b) Second experiment day 1(◆), day 8 (■), day 15 (▲), day 21 (*), day 30 (Ж), day 36 (●), day 48 (+), day 55 (○), day 65 (□), day 78 (Δ), day 84 (◊).

was only observed applying a low hydrodynamic shear. Thus, this stirrer operated at the minimum velocity (40 rpm), which was enough to expand the sludge bed minimizing the shear stress.

These results are similar to the SVI_{30} reported by Guimarães et al. [20] (109 mL/g TSS) in a granular sludge reactor for nutrient removal from low-strength domestic wastewater. Besides, it is important to highlight that the SVI_{30} to SVI_{10} ratio which indicates the granule formation status and the biomass settling properties. While in the inoculum this ratio was 0.53, for the first experiment it increased to 0.82 on day 83 (Fig. 4a). This SVI_{30}/SVI_{10} ratio was even higher in the second experiment (0.95). These results match the faster sedimentation velocity achieved in these periods. Therefore, results showed the capability of the CFGR system to improve aggregates settling properties treating freshwater aquaculture streams at a very low HRT. In addition, this configuration showed the fast adaptability of the biomass to be retained in the reactor with the V_{up} increase.

Another reason for the biomass wash out episodes might be the mass transfer limitations associated to the low pollutant concentrations. At this respect it is important to point out that considering the half-saturation constant of AOB (k_{NH4}) and NOB (k_{NO2}) determined by Manser et al. [40], of 0.14 mg NH_4^+-N/L and 0.17–0.28 mg NO_2^--N/L, respectively, during Stage II the ammonium and nitrite influent concentrations were lower than the k_{NH4} and k_{NO2} indicating a poor mass transfer and a limitation in the growth yield. Thus, these conditions could cause biomass washing episodes. Stage III was the most stable in terms of nitrogen conversions, probably due to the biomass retention improvement, showing that the introduction of the stirrer and the sieve improved the reactor performance as well.

In the second experiment, although the inoculated concentration was higher (4.94 g TSS/L) it decreased rapidly during the first two days presenting relatively high solid concentrations in the effluent up to 69 mg TSS/L (Fig. 5). Afterwards, the reactor biomass concentration remained stable at approximately 2.0 g TSS/L for 50 days (Fig. 5). Afterwards, the biomass concentration decreased ranging between 1.52 and 0.72 g TSS/L. These results match with the influent ammonium concentration decrease taking place after day 50 (Fig. 6). The range of TSS concentrations reported by other studies operating granular pilot plants treating low-strength wastewater streams was 8–10 g TSS/L [17, 30]. Nevertheless, the organic matter and nitrogen influent concentrations in these studies were more than twenty times higher. Thus, the CFGR biomass was well retained in both experiments and was directly related to the ammonium influent concentration being the mechanical stirrer and sieve crucial for this purpose.

3.2. Nitrogen conversions

3.2.1. First experiment

Ammonium concentrations in the feeding were variable throughout the operation of the first experiment (Fig. 6). This behaviour was caused by changing weather, with more rainy periods than which are usual in the summer season. Therefore, on some days like 48 and 86 the ammonium concentration was below 0.15 mg NH_4^+-N/L. However, during the longest dry period (day 49–80) the ammonium concentration increased from 0.14 to 1.84 mg NH_4^+-N/L. The introduction of seawater

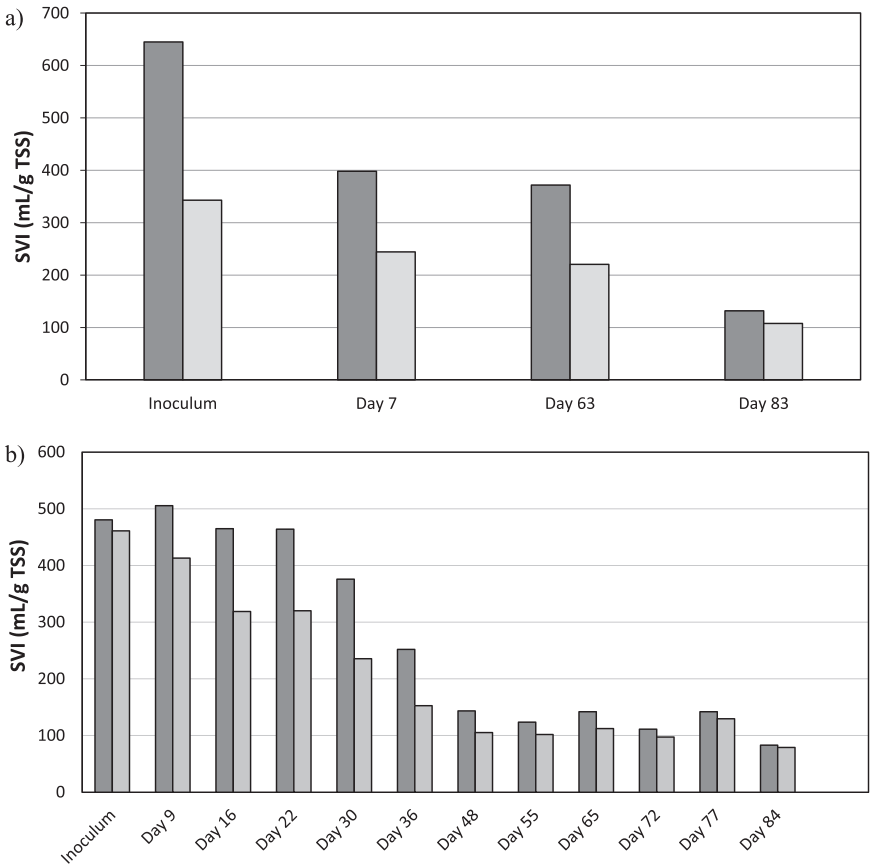

Fig. 4. Evolution of the biomass sludge volumetric index values at minute 10 (■) and minute 30 (■) during the reactor operation. (a) first experiment and (b) second experiment.

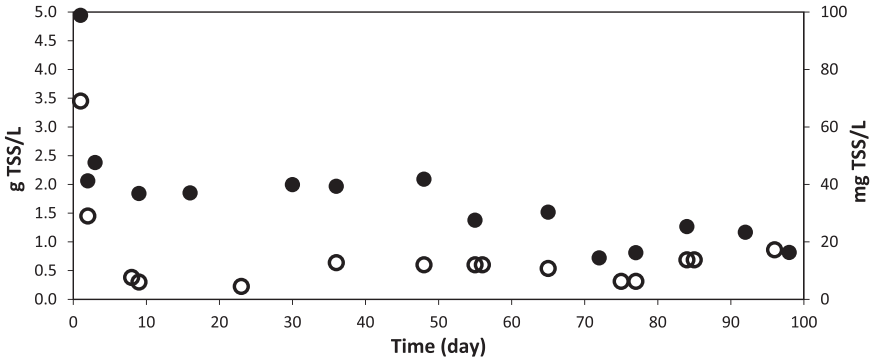

Fig. 5. Total suspended solids concentration in the CFGR second experiment (•) and effluent solids concentration (○).

in the trout farm caused a similar dilution effect and a significant salt increase. After the first and second salinity shocks (days 15 and 24, respectively) the ammonium concentration diminished from 0.77 to 0.68 and from 1.00 to 0.45 mg NH_4^+-N/L, respectively.

Nitrite present in the influent to the CFGR is produced by the oxidation of ammonium inside the fish plant (tanks and pipes), but its accumulation is relevant only when the recirculation is applied. In the first operational days, the nitrite concentration in the influent was almost zero. However, as the recirculation went by, it started to increase and was detectable from day 10 onwards. Nevertheless, when rainy periods started, ammonium and nitrite concentrations decreased. For example on day 48 the nitrite concentration was of 0.01 mg N/L (Fig. 6). It is important to consider that nitrite is extremely toxic for fishes even at low concentrations. Russo et al. [41] reported that nitrite concentrations between 0.06 and 0.12 mg NO_2^--N/L caused a 50% of mortality in rainbow trout.

The nitrate concentration of the water used in the farm is similar to that in the near river or a little bit higher due to the nitrification inside the fish ponds. Thus, the nitrate influent concentration was stable, being between 0.81 and 1.25 mg NO_3^--N/L throughout all the performance period. Despite the influent fluctuations in composition and the salinity shocks, the CFGR developed nitrification activity. Throughout the CFGR operation, nitrate concentrations increased in the effluent showing the presence of ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Fig. 6).

Mass balances calculated between influent and effluent indicate that all ammonium and nitrite removed fits with the nitrate produced. Thus, the denitrification process did not occur in the reactor and the ammonium assimilated to heterotrophic growth was very low. The lack of organic matter the electron donor was responsible for the absence of

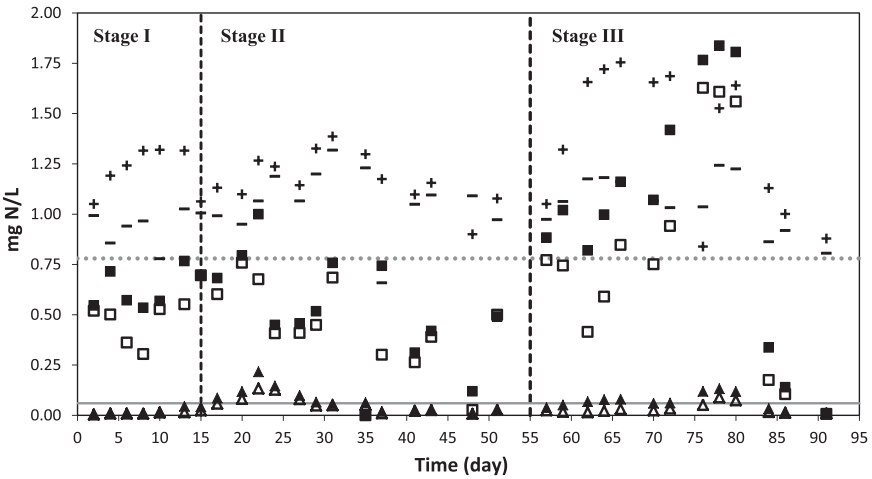

Fig. 6. Ammonium influent (■) and effluent (□) concentrations, nitrite influent (▲) and effluent (Δ) concentrations and nitrate influent (-) and effluent (+) concentrations in first CFGR experiment. Inhibitory ammonium concentration for fishes is 0.78 mg N/L (···), inhibitory nitrite concentration is 0.06 mg N/L (▬).

denitrification [20]. Moreover, the high oxygen concentration near to saturation most of the operational time and the small diameter of the granules prevented the existence of anoxic layers deep enough inside the granules where the denitrification could take place. De Kreuk et al. [42] reported the same performance in granules operated in an SBR where nitrogen removal took place via denitrification. Considering the nitrification as the only process of nitrogen transformation, it caused a pH decreasing during operation due to IC consumption. During almost all stage III the pH values decreased approximately 0.15 units in the effluent respect to the influent. Besides, the inorganic carbon was consumed in the CFGR indicating the occurrence of the autotrophic processes. These results indicated that pH and oxygen profiles can be used to monitor the CFGR performance.

The ammonium removal was relatively stable during Stage I. The system was able to remove up to 43% of ammonium in this stage, achieving an ammonium removal rate (ARR) of 22 mg NH_4^+-N/(L·d). The CFGR was capable to reduce the ammonium concentration to an average value of 0.21 mg NH_4^+-N/L during this stage (Fig. 6). These results show that the reactor biomass was adapted rapidly to the extreme conditions of low ammonium concentration.

At the end of Stage I the nitrite began to appear in the feeding and during the first days of stage II it increased rapidly achieving a value of 0.22 mg NO_2^--N/L (day 22) with a removal efficiency of 38% in the system. The ammonium removal performance during stage II was low at percentages between 9% and 0%. Therefore, the ARR was lower too with values between 10 and 0 mg NH_4^+-N/(L·d). Nevertheless, when the reactor was not affected by a salinity shock or starvation period, for example on day 37, was able to remove 60% of ammonium with an ARR of 45 mg NH_4^+-N/(L·d).

After the configuration change, the ammonium removal was stabilized in stage III with ARR of 39 mg NH_4^+-N/(L·d) and removal percentages near to 50% were achieved. Since the feeding concentration of ammonium increased gradually during this stage the removal percentages decreased with values between 7% and 13% (days 76–80). However, the ARR was approximately 20 mg NH_4^+-N/(L·d). Thus, the improvement of biomass retention (Fig. 3a and 4a) led to a better performance in ammonium oxidation.

At the beginning of Stage III, the nitrite concentration in the influent increased again up to 0.22 mg NO_2^--N/L. During this stage, the reactor was able to remove up to 78% of nitrite.

Despite the short HRT values, the results show that there was no nitrite accumulation during the operation. This behaviour could be related to the DO concentration and the granular size. The shorter the granule diameter, the higher the specific surface; consequently, the oxygen flux towards the biofilm surfaces is improved and facilitates the complete nitrification. Thus, with the high DO value (close to saturation) and the small granule diameter of 0.31 mm a high O_2 flux is ensured. Similarly, Bartrolí et al. [43] reported nitrite accumulation at high DO concentration in nitrifying granules. They attributed this nitrite production to a DO/ammonium ratio below 0.35 g O_2/g NH_4^+-N. However, in the present study, the DO/ammonium ratio ranged from 1.4 to 51.6 indicating that the complete ammonium oxidation to nitrate is favoured.

Summarizing, on day 4 (Stage I) nitrification appeared inside the reactor, showing the fast adaptation of the seeding sludge to the farm water composition. The nitrification remained constant until the beginning of Stage II, then the salinity shock occurred which diminished the nitrate conversion. This result indicates that salinity shocks affect AOB and NOB activity severely. With the stirrer incorporation (Stage III) the mass transfer inside the reactor was improved. Consequently, the ammonium nitrification to nitrate was enhanced in terms of stability achieving a nitrate concentration increase up to 0.6 mg NO_3^--N/L.

As it was mentioned before, on day 76 the nitrate concentration in the effluent was much lower than in the influent which indicates that on this day denitrification activity took place inside the reactor. This result matches with the oxygen concentration which was close to 0 mg/L, providing the anoxic conditions required to denitrify. Therefore, denitrifiers were present too in the reactor. However, there was no apparent denitrifying activity during most of the reactor performance due to the existing operational conditions.

3.2.2. *Second experiment*

The second experiment resulted in a better performance in terms of ammonium and nitrite removal (Fig. 7). The occurrence of salinity shocks and rainy events (starvation) was less more frequent than in the previous summer. This fact resulted in a less variable ammonium influent concentration. Besides, the configuration with the sieve and mechanical stirrer allowed to maintain a good performance despite these events. Again, the main process developed in CFGR was nitrification showing that this process is favourable facing freshwater aquaculture conditions. Moreover, mass balances confirm that the sum of ammonium and nitrite consumed matched with the nitrate produced. Therefore, the ammonium consumed for cellular growth was very low. This result corresponds with the slow growth of the biomass inside the reactor (Fig. 5). Besides, pH and inorganic carbon concentration decreased in the effluent (in comparison with the influent) like in the previous experiment.

The reactor biomass rapidly adapted to freshwater aquaculture conditions achieving ammonium removal percentages up to 80% and ARR of 20.8 mg NH_4^+-N/(L·d) during the first 16 days. From day 16 to day 48, the ammonium removal fluctuated between 11% and 51%. This

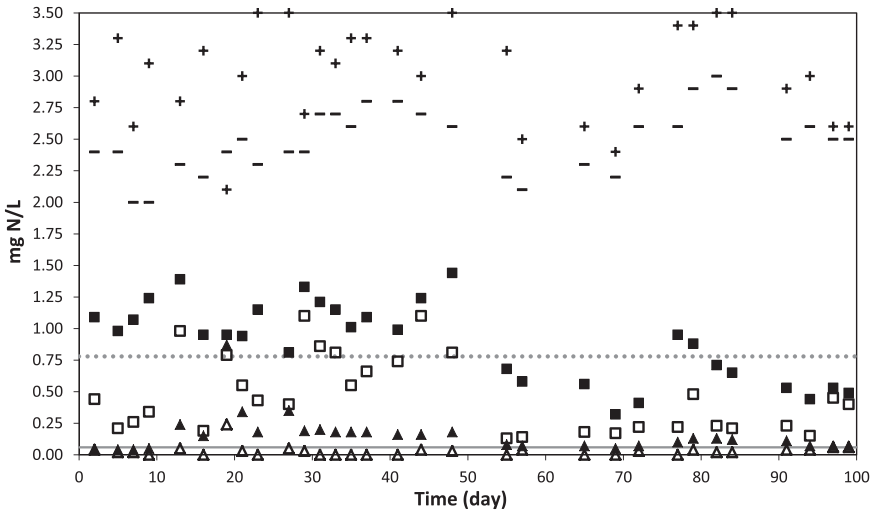

Fig. 7. Ammonium influent (■) and effluent (□) concentrations, nitrite influent (▲) and effluent (Δ) concentrations and nitrate influent (-) and effluent (+) concentrations in the second CFGR experiment. Inhibitory ammonium concentration for fishes is 0.78 mg N/L (···), inhibitory nitrite concentration is 0.06 mg N/L (▬).

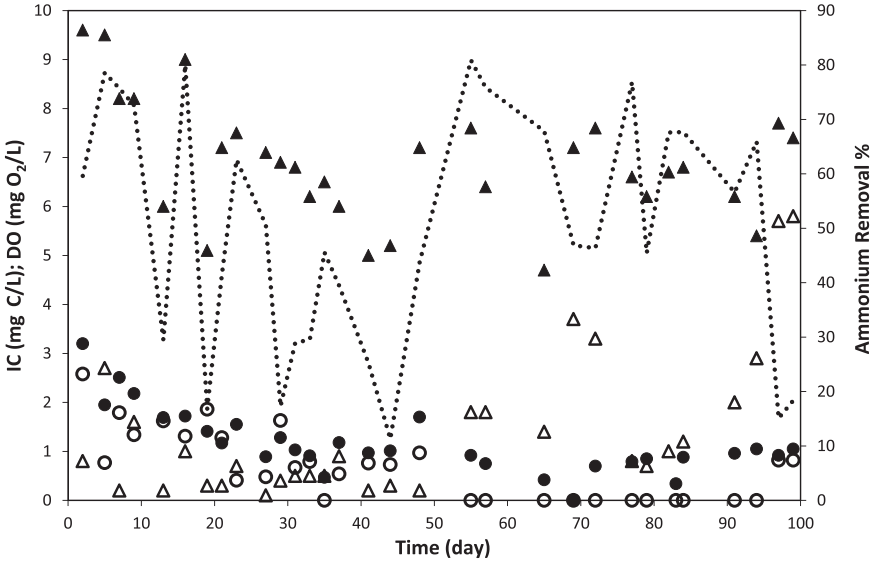

Fig. 8. Dissolved oxygen influent (▲) and effluent (Δ) concentrations, inorganic carbon influent (●) and effluent (○) concentrations and ammonium removal percentage (···) in second CFGR experiment.

decrease could be related to lower DO and IC influent concentrations (Fig. 8) since both are substrates nedded for the nitrification process. From day 48 until the end of the operation the ammonium removal increased up to 81%. This increase can be associated with better biomass settling properties (Fig. 3b and 4b) and lower ammonium influent concentrations (0.32–0.95 mg N/L) being the maximum ARR achieved of 16.4 mg NH_4^+-N/(L·d).

Nitrite removal percentages of 80–100% were achieved during most of the CFGR operation. Since nitrite is one of the most dangerous compounds to rainbow trout, this result enhances the potential of this technology for the treatment of freshwater aquaculture streams. Nitrite influent concentration ranged between 0.08 and 0.35 mg N/L and the effluent concentration was under toxic value (0.06 mg N/L) throughout all the operation except on day 19.

3.3. Free ammonia and free nitrous acid

Since FA and FNA are more harmful to the fishes and the nitrifying populations than ammonium and nitrite themselves, it is important to avoid their accumulation in the plant. Temperature and pH have a crucial role in the equilibrium between the ionized and unionized forms of both compounds [29]. Thus, FA and FNA concentrations were evaluated in this study (Fig. 9).

The water in the trout plant has low pH (5.1–6.4) due to the low IC concentration of river water. This condition moves the chemical balance to the formation of the acid species like FNA [29]. Therefore, FA concentrations were much lower than FNA ones, despite the ammonium concentrations were higher than nitrite ones throughout all operation (Fig. 6). During the first experiment, FA concentrations were around 0.5–0.25 μg NH_3-N/L in the influent, being the concentration in the effluent up to 60% lower (Fig. 9a). Since the nitrification took place in the CFGR during both experiments, the pH values of the effluent were around 0.1–0.4 units lower than in the influent. Thus, the FA removal was higher than the ammonium removal due to the pH decrease in the CFGR. However, when the pH increased this effect was lower, even the FA increased in the effluent like in day 76 of the first experiment

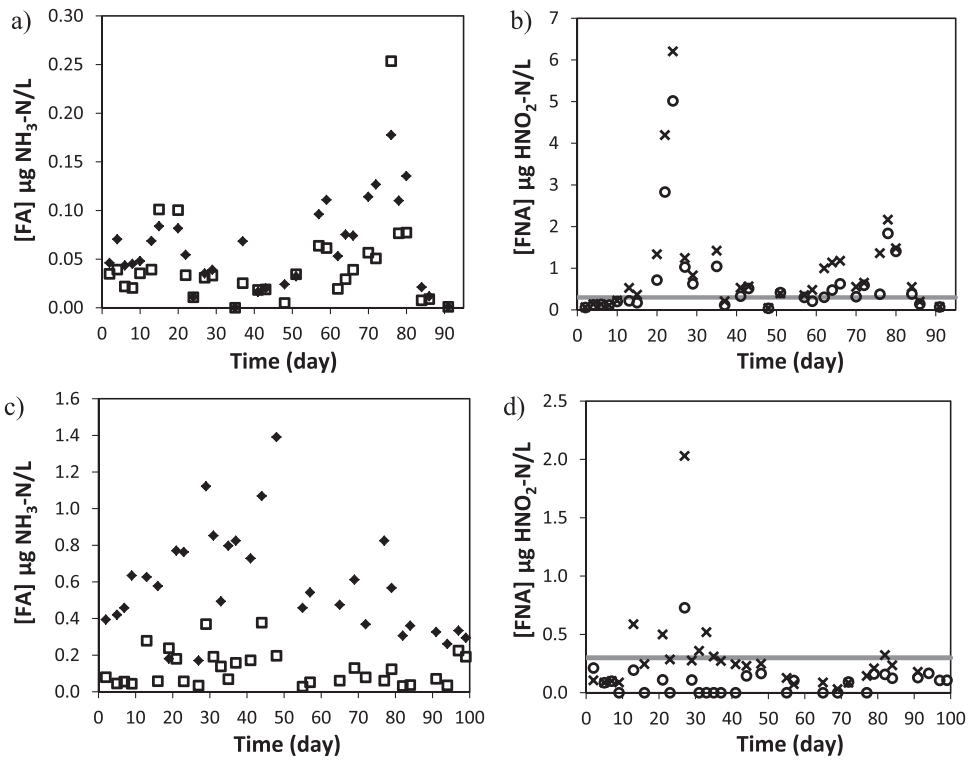

Fig. 9. Influent (◆), effluent (□) FA concentrations in CFGR, a) first experiment, c) second experiment; Influent (x), effluent (○) FNA concentrations in CFGR, b) first experiment, d) second experiment. FNA concentrations inhibitory for fishes (▬).

(Fig. 9a). On the other hand, the FA concentrations were higher in the second experiment due to the higher pH (Fig. 9c). Nevertheless, the FA concentrations were between 56% and 94% lower in the effluent due to the high ammonium removal. These FA concentrations have no toxic effects on rainbow trout. Thurston et al. [44] reported that it was necessary 96 µg NH_3-N/L to cause toxicity and negative effects on motility. Therefore, in both experiments, the FA concentration in the effluent does not represent a threat to rainbow trout if recirculation is maintained, mainly due to the low pH of operation.

FNA concentrations were approximately of 0.06–2.2 µg HNO_2-N/L in the influent of the first experiment, achieving values up to 6.2 µg NH_3-N/L (Fig. 9b). The FNA concentration decreased in a range of 15–100% in the effluent, being lower than nitrite removal (80–100%) due to the pH decrease between influent and effluent (0.2–0.4 pH units). Nevertheless, in the second experiment, the FNA concentration decreased to almost zero in the effluent due to the high nitrite removal (Fig. 9b). Russo et al. [11] reported that the exposition to 0.3 µg HNO_2-N/L for 96 h caused toxicity to the rainbow trout. Despite the CFGR reactor nitrite removal, during the first experiment FNA concentrations were higher than the toxic value several days during the operation. However, this problem was solved on the second experiment operating with a longer HRT, and after the implementation of the mechanical stirrer and a sieve. Only on day 27, the FNA effluent concentration was over toxic value for rainbow trout.

Regarding the inhibitory effect on the nitrifying populations of FA and FNA, Vadivelu et al. [45] reported inhibitory values of 16 mg NH_3-N/L and 0.4 mg HNO_2-N/L on *Nitrosomonas sp.* culture; and 0.1 mg NH_3-N/L and 24 µg HNO_2-N/L on *Nitrobacter sp.* culture. In addition, Blackburne et al. [46] reported inhibitory values of 40 µg NH_3-N/L and 30 µg HNO_2-N/L on *Nitrospira sp.* culture. Therefore, even in the worst scenario of this research work (0.38 µg NH_3-N/L and 5.02 µg HNO_2-N/L), the FA and FNA concentrations during the operation were low enough to avoid AOB and NOB inhibition.

3.4. Activities of the CFGR biomass

The conversions of nitrogen species during the operation indicated the occurrence of nitrifying inside the CFGR. This oxidizing activity was promoted when the stirrer was incorporated apparently by a mass transfer improvement. Furthermore, when DO concentration was low, some denitrifying activity was detected inside the CFGR.

Since fish farms need high DO concentration in the water, it is expected that inside the reactor this concentration is high too. However, during a few days this concentration was lower due to the oxygen consumption by the sludge inside the tubing system of the pilot plant. Nevertheless, this effect is not expected at full scale with high diameter pipes. Therefore, the characteristics of this freshwater aquaculture stream seem to promote the AOB and NOB bacteria activities when is treated with the CFGR configuration proposed, confirmed by batch activity tests (Table 2).

Table 2 shows that the seeding sludge had good heterotrophic activity and also presented AOB activity. Nevertheless, no activity was detected regarding NOB. This behaviour could be attributed to a poor performance of the sludge in the STP. The AOB activity increased from 38.3 ± 0.4 to 50.2 ± 3.4 mg NH_4^+-N/(g VSS·d) on day 73 matching with the reactor operation, which had a high nitrate production on that days (Fig. 6). On day 85 AOB activity decreased to 27.9 ± 1.6 mg NH_4^+-N/(g VSS·d), this result also fitted the reactor operation that these days

Table 2
Summary of respirometric assays activities of the CFGR first experiment.

Day	Heterotrophic activity mg COD/ (g VSS d)	AOB activity mg NH_4^+-N/ (g VSS d)	NOB activity mg NO_2^--N/ (g VSS d)	Suspended Solids* g VSS/L
0	129.3 ± 13.8	38.3 ± 0.4	No activity	0.93 ± 0.04
72	39.6 ± 11.1	50.2 ± 3.4	No activity	0.66 ± 0.03
85	No activity	27.9 ± 1.6	35.6 ± 3.6	1.31 ± 0.18

* Suspended solids concentration in the assay.

presented a low nitrate production due to ammonium concentration decrease in the influent (Fig. 6).

On the other hand, the CFGR did not present NOB activity by respirometric assays on day 72. This result does not match with the nitrate production during the operation. This could be due to the low suspended solids concentration of the batch assay (0.66 mg VSS/L). Previous experiments showed that at low VSS concentrations to measure the NOB activity is difficult with the respirometric method [47]. Nevertheless, the assay of day 85 showed a NOB activity of 35.6 ± 3.6 mg NO_2^--N/(g VSS·d), therefore with a higher VSS concentration (1.31 g VSS/L) in the vial the activity is measurable.

Regarding heterotrophic activity, the inoculum presented a high potential with 129.3 ± 13.8 mg COD/(g VSS·d). However, this activity decreased to 36.9 ± 11.1 mg COD/(g VSS·d) on day 73 and completely disappeared at day 85. This behaviour matches with the TOC removal of the reactor which was frequently insignificant (Fig. 10). Therefore, the CFGR promotes the AOB and NOB populations activity when treating this type of freshwater aquaculture streams over the heterotrophic populations.

3.5. Organic matter removal

The organic matter concentration measured in the freshwater aquaculture stream of the farm was extremely low (2.2–8.1 mg TOC/L) being not toxic for fishes. Nevertheless, the organic matter can be oxidized consuming the DO present in the ponds. This effect increases the aeration costs of the farm. Moreover, the lack of electron donors can affect the microbiological processes developed in the reactor performance. Thus, the TOC concentration was measured during both CFGR experiments.

The organic matter removal performance fluctuated a lot in both experiments. In several days the concentration in the effluent was higher than in the influent of the CFGR, indicating biomass wash up episodes. Since the TOC influent concentration is extremely low this effect is more evident. This effect was observed frequently during both experiments indicating poor heterotrophic removal performance.

In the first experiment (Fig. 10a), the organic matter concentration in the feeding remained stable (approximately of 3–4 mg C/L) from the start-up to day 27. Afterwards, the TOC concentration began to increase and became more variable with values from 3.9 to 8.1 mg C/L. In addition, the TOC removal percentage fluctuated during all operation alternating values from 55% to 0%. When the removal percentage was zero (in Fig. 10) indicated that TOC was produced by cellular decay. This behaviour is typical during reactor start-ups due to the absence of adapted biomass wash out. In the CFGR this fact was observed during the start-up and occurred in several operational days. The respirometric assays confirm the hypothesis of a poor heterotrophic performance showing that the heterotrophic activity disappeared from the inoculum to day 85 (Table 2).

However, in the second experiment (Fig. 10b) the cellular decay process did not occur during the start-up. The TOC removal was approximately 33% in the first days. This better start-up can be attributed to the different operational conditions (HRT, stirring, sieve) and slightly higher concentrations of ammonium and TOC in the influent. Afterwards, the TOC removal fluctuated between 0% and 50%. From day 55 onwards, the removal was very low indicating again that this system does not promote heterotrophic processes facing extremely low strength freshwater aquaculture wastewater.

Peyong et al. [48] studied the inhibition by FA and FNA over the heterotrophic activity of AGS for the treatment of low-strength wastewater. The study reported inhibitory values of 40 mg NH_3-N/L and 15 μg HNO_2-N/L. Thus, the FA and FNA could not cause heterotrophic inhibition (Fig. 9). Besides, the half-saturation coefficient for heterotrophic biomass (k_s) ranged from 2.5 to 4 mg COD/L according to Kappeler and Gujer [49]. Therefore, the low pH and the lack of organic matter should be factors that caused this activity decay.

3.6. Technical viability

The CFGR was able to reduce the ammonium concentration in 0.20–0.70 mg NH_4^+-N/L during the second experiment, while the daily ammonium concentration increased with the recirculation progress was

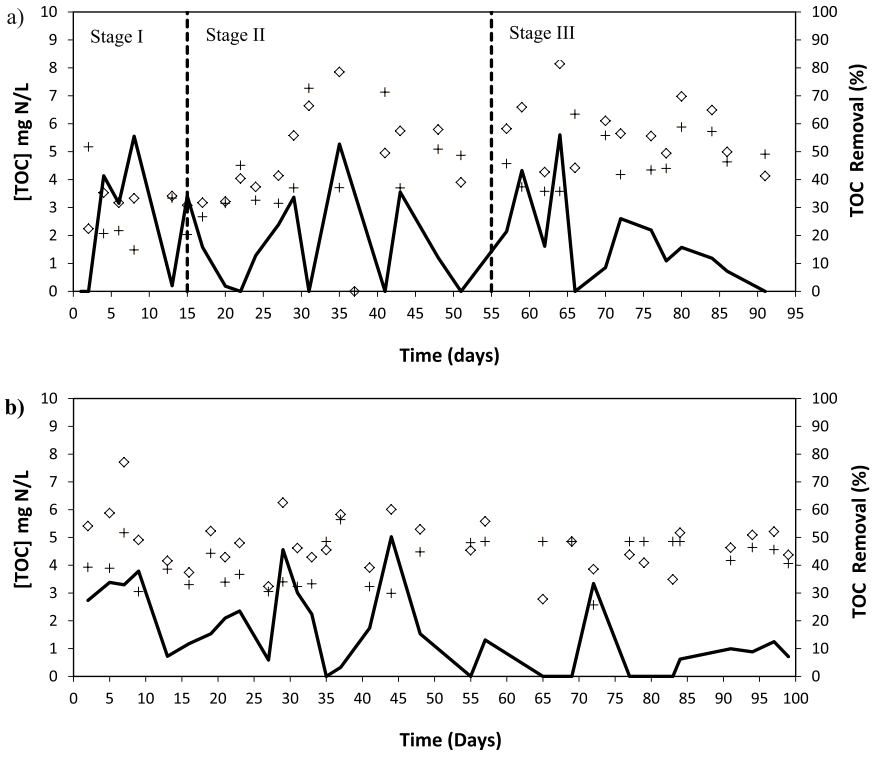

Fig. 10. Influent (◊), effluent (+) TOC concentrations and TOC removal percentage (▬) of the CFGR. a) First experiment; b) Second experiment.

approximately 0.05–0.20 mg NH_4^+-N/L (Fig. 7). Besides, Tahar et al. (2018) monitored the ammonium concentration in a flow-through trout farm, reporting an increase of 0.13 NH_4^+-N/L between the beginning and the end of the farm sampling points. Therefore, a full-scale CFGR would provide an effluent with an ammonium concentration below 0.78 mg NH_4^+-N/L to be recirculated to the fish tanks, which is the maximum toxic concentration acceptable in a long exposition for rainbow trout [10].

Moreover, the CFGR operational conditions are appropriated to avoid nitrite accumulation as well as to reduce its concentration, which is crucial to maintain fish production avoiding fish toxicity during all the second experiment and Stage III of the first experiment (Figs. 6 and 7). This indicates that the operation with a sieve and a mechanical stirrer was crucial to avoid toxic levels.

The most widely treatment systems applied in RAS are the rotating biological contactors (RBC), the trickling filters (TF) and the fluidized bed reactor (FBR). Miller and Libey [50] studied the performances of these systems in the RAS of a freshwater farm. They achieved and ARR between 2 and 40 and 9–38 mg NH_4^+-N/(L·d) in TF and RBC, respectively. The ammonium removal in FBR were in the range of 8–32%, 74–82% in RBC and 23–51% in TF. Thus, the CFGR achieved ammonium removal percentages up to 81%, higher than those reported for these conventional systems (FBR and TF), and similar ARRs of 16–45 mg NH_4^+-N/(L·d). Therefore, the CFGR appears as a competitive alternative to the conventional treatment systems applied in freshwater RAS.

4. Conclusions

A pilot-scale CFGR was used to treat extremely low-strength RAS stream (0.1–1.8 mg NH_4^+-N/L; 0–0.4 mg NO_2^--N/L; 3–8 mg TOC/L) at very short HRT values (16–40 min). The experiments performed indicate that the configuration with a mechanical stirrer and sieve (at the top) was the optimal for improving the biomass retention inside the CFGR and, consequently, the ammonium and nitrite removal performance.

Despite the low concentrations, granulation was achieved in 55 days, with an average granule diameter of 0.47 ± 0.18 mm using the optimized configuration (second experiment). The biomass retention properties improved in both experiments achieving SVI_{30} of 79 mL/g TSS with SVI_{30}/SVI_{10} ratio of 0.95 and a sludge settling velocity of 3.39 m/h on the second one.

Ammonium and nitrite removal percentages up to 81% and 100% were achieved, respectively. The CFGR performance promoted AOB and NOB populations over heterotrophs. This result was confirmed by respirometric assays. The denitrification process did not occur during almost all operation due to the high DO concentration. Daily ammonium and nitrite concentrations increase in the trout farm were lower than the removal of these compounds by the CFGR. Thus, the recirculation would be sustainable, avoiding toxic levels, when using a full-scale reactor.

CRediT authorship contribution statement

S. Santorio: Formal analysis, Investigation, Writing – original draft. **A. Val del Rio:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization. **C.L. Amorim:** Visualization, Conceptualization, Validation. **L. Arregui:** Conceptualization, Supervision, Project administration, Funding acquisition. **P.L.M. Castro:** Validation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **A. Mosquera-Corral:** Writing – review & editing, Validation, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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