redin Revista Facultad de Ingeniería, Universidad de Antioquia, No.97, pp. 93-102, Oct-Dec 2020

Recirculating aquaculture system with three phase fluidized bed reactor: Carbon and nitrogen removal

Sistema de recirculación acuícola con reactor de lecho fluidizado trifásico: Remoción de carbono y nitrógeno

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ABSTRACT: The core objective of the study was to evaluate the organic matter and nitrogen removal efficiency in a recirculating aquaculture system for the intensive laboratory-bred rainbow trout. The treatment system consisted of an upflow reactor (UR), a pre-filtration unit, a three-phase airlift fluidized bed reactor (AFBR), a granular unit for the UR and the AFBR effluents filtration, and an ultraviolet (UV) unit for the final effluent disinfection. A plastic material was used as support media in the UR, and granular zeolite with an effective size of 1.30 mm in an 80 g/L constant concentration was used as a carrier for the AFBR. Average removal efficiencies of biochemical oxygen demand (BOD) chemical oxygen demand (COD), ammonium, nitrite, nitrate, and total nitrogen were 94.4, 91.7, 52.5, 13.4, 1.3 and 6.0% respectively. In the rainbow trout rearing tanks, there was a water volume of 125 L and water exchange rates of 125 and 250 L/h, there were no registered mortalities; the calculated daily weight gains were 1.55 and 1.51 g/day and the final stocking densities were respectively 20.87 and 20.58 kg/m^3. The results suggested that the system had the capability to develop a nitrification process for maintaining water quality characteristics within the recommended values for rainbow trout farming, but total nitrogen was not effectively removed due to the weak denitrification process, since there were modest values of nitrite and overall nitrogen removal.

RESUMEN: El estudio evaluó la eficiencia de eliminación de nitrógeno y materia orgánica en un sistema intensivo de recirculación acuícola de trucha arcoiris en laboratorio, que contó con un reactor de flujo ascendente con medio plástico (RFAMP), un prefiltro, un reactor de lecho fluidizado trifásico (RLFT), un lecho granular para filtrar conjuntamente los efluentes de RFAMP y RLFT y una unidad ultravioleta (UV) para la desinfección final del efluente. Se usó material plástico como medio soporte en el RFAMP y zeolita granular con tamaño efectivo de 1,30 mm en una concentración constante de 80 g/L como medio soporte en el RLFT. Las eficiencias medias de eliminación de demanda bioquímica de oxígeno (DBO), demanda química de oxígeno (DQO), amonio, nitrito, nitrato y nitrógeno total fueron 94,4, 91,7, 52,5, 13,4, 1,3 y 6,0% respectivamente. En los tanques de cultivo de trucha, que tuvieron un volumen de 125 L y tasas de recambio de 125 y 250 L/h, no registraron mortalidades; las ganancias de peso diarias fueron 1,55 y 1,51 g/día y las densidades finales de cultivo fueron 20,87 y 20,58 kg/m^3. Los resultados sugieren que el sistema desarrolló el proceso de nitrificación hasta valores de calidad del agua recomendados para trucha arcoiris, el nitrógeno total no se eliminó efectivamente debido al débil proceso de desnitrificación, pues se registraron bajos valores para nitritos y para la remoción global del nitrógeno.

CITE THIS ARTICLE AS:

G. L. Cárdenas and I. A. Sánchez. "Recirculating aquaculture system with three phase fluidized bed reactor: Carbon and nitrogen removal", *Revista Facultad de Ingeniería Universidad de Antioquia*, no. 97, pp. 93-102, Oct-Dec 2020. [Online]. Available: https://www.doi.org/10.17533/udea.redin.20200264

ARTICLE INFO:

Received: September 23, 2019
Accepted: February 07, 2020
Available online: February 07, 2020

KEYWORDS:

Aquaculture; organic matter; wastewater; water treatment

Agua residual; materia orgánica; acuacultura; tratamiento del agua

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ISSN 0120-6230
e-ISSN 2422-2844

1. Introduction

Fisheries and aquaculture remain important sources of food, nutrients, income, and livelihoods for hundreds of millions of people around the world. World per capita fish supply reached a new high record of 20 kg in 2014; due to vigorous growth in aquaculture [1]. Trout farming requires a high amount of water, since producing 1 kg in raceways regularly requires 210,000 L, while in contrast only 21,000 L are required to produce tilapia [2].

Trout farms had a significant impact on dissolved oxygen (DO), biological oxygen demand (BOD_5), nitrite, nitrate and total phosphorus concentrations in streams [3]. Making the most of a large water resource is crucial, water is often serially reused as it flows downhill from raceway to raceway that is on stair-stepped or terraced hillside; however, water quality deteriorates as it moves from one raceway to the next.

In a mass balance study made to examine the loading of particulate and dissolved N waste components deriving from juvenile rainbow trout fed three different rations: 1.3, 1.5 or 1.7% of the biomass per day, the authors founded that total dissolved nitrogen (N) and total ammonia nitrogen (TAN) wastes contributed respectively with 81.0–82.3% and 62.6–64.4% of the total N waste recovered [4].

Particulate wastes from trout accumulate along raceway bottoms and within the quiescent zones at the end of each raceway [5]; according to the studies of Lam *et al.*, the total suspended solid measured as fish feces represented 9 to 13% of the daily feeding rate and a daily production rate of 0.94 to 2.00 mg/L of total suspended solid expressed as 8.43 to 13.22 mg/L of BOD_5 [6].

A partial water reuse system uses recirculated water flow to control the accumulation of ammonium [7]. Some of the reasons for recirculating aquaculture are the increasing shortage of water resources, the management of environmental pollution, and food safety [8]. A recirculating aquaculture system (RAS) can be defined as a system that incorporates the treatment and reuse of water with less than 10% of total water volume replaced per day, and its concept is to reuse a volume of water through continual treatment and delivery to the organisms being cultured [9]. Increasing costs for make-up water, wastewater discharge, temperature control and separation of waste streams are driving RAS production more and more towards intensification of water reuse [10].

A perfect biofilter would remove all of the ammonium entering the unit, produce no nitrites, would support dense microbial growth on an inexpensive support material that does not capture solids, require little or no water pressure or maintenance, and leave a small footprint. Unfortunately, no biofilter type can meet all these objectives, but each biofilter type has their own advantages and limitations [11]. According to Areerachakul, some of the most commonly used biofilters in recirculating aquaculture are submerged filter, trickling filters, rotating filter, bead filter, and fluidized bed filters [12]. Under aerobic conditions, biological filtration includes autotrophic ammonium and nitrite removal and heterotrophic degradation of dissolved and particulate organic matter [13].

RAS technology for fattening farms have several advantages such as reduced dependency on antibiotics and therapeutants, reduction of direct operating costs associated with feed, predator control, parasites, potentially eliminate release of parasites to recipient waters, risk reduction due to climatic factors, enabling production of a broad range of species irrespective of temperature requirements and enabling secure production of non-endemic species [14].

Developing efficient, productive, biologically secure, and disease-free RAS requires a thorough understanding of all life support processes from mechanical (oxygen, temperature, ozonation, UV, pH, and salinity) to the biological filtration systems. While mechanical processes can be monitored and controlled, biological filtration systems rely on the interaction of microbial communities with each other and their environment as a consequence of nutrient input (fish waste output) and therefore, are not easy to control [15].

The main objective of this research project was to evaluate the performance in terms of the efficiency of removal of organic matter and nitrogen compounds by a RAS for rainbow trout culture with an upflow reactor, filtration units and an aerobic three phase airlift fluidized bed reactor.

2. Material and methods

The experiment was carried out in the Hydraulic Laboratory of the Mariana University, Alvernia Campus in San Juan de Pasto municipality (Nariño, Colombia), at the altitude of 2,527 m above sea level with an annual average temperature of 12 °C. The evaluated RAS was contained in two plastic tanks (water volume 125 L); the water exchange rate in the T1 tank was 125 L/h, in the T2 tank was 250 L/h. Each rearing tank had 40 rainbow trout fingerlings with approximately 30 g initial average weight, a starting biomass density up to 10.0 kg/m^3, an expected final average weight of 60 g and a final density of 20.0 kg/m^3.

The T1 and T2 rearing tanks had two outlets; in order to guarantee 85% of the effluent flow proceeded from the surface, the effluent was collected through a circular wire that was 0.05 cm in diameter and 0.42 m in height, and the 15% remaining flow proceeded from the bottom of the tanks through a 0.025 m diameter orifice. In both cases, the flow was controlled through plastic polyvinyl chloride (PVC) valves. Inside each tank, two diffuser bars were utilized for oxygen transfer to the water through the air injection from a 373 W blower with 1.69 m^3/h air flow rate capacity.

The treatment system was comprised of a strictly aerobic treatment line that includes air injection, for the treatment of the surface tanks effluents; and a treatment of the bottom effluents respectively, without air injection.

The bottom effluents treatment unit was included with the aim to produce conditions for the denitrification process. This unit was an upflow with plastic media reactor (UPMR) made with 0.20 m external diameter plastic PVC tube, 1.16 m total height and 1.05 m effective height operated at HRT of 18 and 36 min.

The aerobic line had an up flow pre-filtration unit made with 0.20 m external diameter plastic PVC tube, which had a total height of 1.0 m and operated at HRT of 4.9 and 9.8 min. The tube was located in front of a three-phase aerobic airlift fluidized bed reactor (AAFBR); the AAFBR was made with concentric tubes of 0.20 m and 0.10 m for the external and internal diameters of plastic PVC tubes, which were 1.8 m wide and 1.6 m in height, respectively which operated at HRT of 5 and 11 min. The assembly of the treatment system was based on the reports by [16, 17].

In this experiment, the treatment system had an up flow post filtration unit for the UPMR and the AAFBR reactors effluents. The effluent filtration was made with 0.20 m external diameter plastic PVC tube, 1.0 m of total height, operated with 5.8 and 11.6 min as HRT; a UV unit for the final effluent disinfection with a flow treatment capacity of 750 L/h. At the end of the process, two 373 W pumps released the disinfected water from an 80 L tank to an acrylic tank. The water level remained constant from the tank that distributed the liquid for the recirculation to the rearing tanks.

Commonly used commercial plastic curlers filled with small nylon mesh were used as support media in the UPMR which occupied 84.3% the volume of the reactor. A granular zeolite with an effective size of 1.30 mm at an 80 g/L constant concentration was used as a carrier for the AAFBR. A pre and post filtration units were filled with five layers of granular material according to the following range of sizes: 19-25, 13-19, 6-13, 3-6 and 1.6-3 mm with a layer height of 0.25, 0.20, 0.15, 0.15 and 0.10 m, respectively.

A schematic diagram of the different units and components of the RAS and the different lines of the treatment system are presented in Figure 1. Additional details about the fluidized bed reactor geometry and operation were described by [17].

In the AAFBR, the pressurized air flow from a 1.55 kN/m^2 compressor was injected at the central bottom part through a device made of PVC with 25 mm diameter tube with holes of 1.0 mm in diameter. At the upper part, the reactor had a settling unit made of an acrylic sheet for the carrier retention. The fish in the tanks were fed 6 times a day with commercial fish food with 45% protein. The daily amounts of food were calculated according to the recommendation made by [18].

The granulometric characteristics of three possible carriers for the AAFBR and the filtration units were determined based on the Colombian standard methods of granulometric analysis and the number of samples, with the NTC 1522 [19] and NTC 77 [20] methods respectively. The grain size analysis offers the effective size of D_{10}, D_{30}, and D_{60} values. These represent the diameter in the particle-size distribution curve corresponding to 10, 30 and 60% finer respectively for determining the uniformity coefficient (UC, Equation 1) and the coefficient of gradation (CC, Equation 2). Those are the main characteristics of granular materials for filtration of drinking water [21] and wastewater [22]. The carrier was added progressively at the top of the reactor by addition of a mass quantities equivalent to 10 g/L concentration of anthracite until it reached the concentration of evaluation of the system (80 g/L).

$$UC = \frac{D_{60}}{D_{10}} \quad (1)$$

$$CC = \frac{{D_{30}}^2}{D_{60} * D_{10}} \quad (2)$$

The inoculation of the UPMR and the AAFBR reactors was made using the liquid and the settled solids accumulated in three rainbow trout culture tanks, which were respectively disposed every day from the reactors for 3 weeks. Following the inoculation process, and to allow the maturation of the reactors as a startup condition, the whole RAS were operated during a six-week period using 48 juvenile rainbow trout in the three tanks. After this period, the culture units were emptied, disinfected and dried. Tanks T1 and T2 were then filled with fresh water and 40 fish per tank.

According to [23] in biofilm systems, treatment performance is primarily dependent on the availability of biofilm growth on the surface area in the reactor. In order to have as much surface area as possible in the anoxic

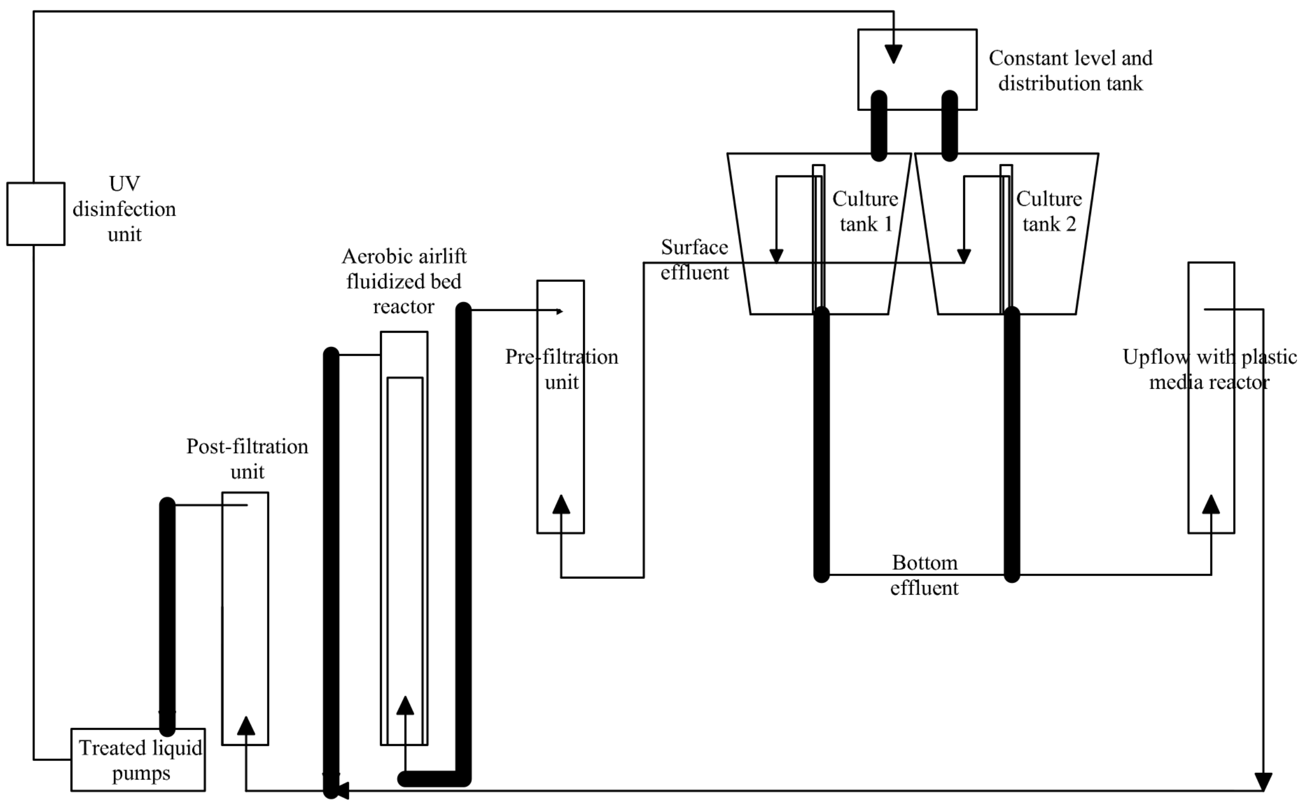

Figure 1 Schematic view of the RAS components and water flow

reactor and consequently promote the microorganism adherence, the hair rollers were filled with plastic mesh. The surface area was determined by measuring the roller and mesh components geometry of five items by using a Vernier scale; the volume of the objects was determined with the Archimedes' principle of flotation.

The RAS monitoring was done twice a week for five weeks. The samples collected and the parameters measured in situ were done at the inlet and outlet of the UPMR, the AAFBR, and the pre and post -filtration units.

The parameters were measured twice a week following the recommendations of APHA, AWWA & WEF, using the following Standard Methods for the Examination of Water and Wastewater [24]: 5,220 C (COD), 5,210 B (BOD), 4,500 N_C (total nitrogen –TN), 4,500 NO2_B (nitrite), 4,500 NO3_B (nitrate) and 4,500 NH3_F (ammonium) with a Hach DR2,500® spectrophotometer.

The daily controlled parameters were measured three times a day by a YSI Inc. 550A® probe according to the methods: 4,500_OG (dissolved oxygen), 4,500H+B (pH: concentration of hydrogen ions) and 2,550 B (temperature). Water flow in the tanks was measured with a volumetric method; also, the water levels and air pressure were controlled and regulated. The carrier concentration was controlled by a volumetric method on a weekly basis. The physical-chemical parameter measurements were done at the Sanitary and Environmental Engineering Laboratories of the Mariana University.

For the determination of the global removal efficiencies of the parameters associated with organic matter and nitrogen, the calculation considered the influent and effluent loads of the analyzed parameter; the determination of the influent load was based on the wastewater flow from the bottom and the surface of the culture tanks, and the effluent load was calculated based on the outlet post-filtration unit. After studying the fish, the survival percentage and the growth measurements were calculated. The daily weight gain (DWG) was then computed as grams of wet weight gain for each day (Equation 3), and the specific growth rate (SGR) was calculated as % of wet weight gain day-1. Over an experimental interval where "t" is the time, in days, the SGR was calculated from an individual fish's wet weight gain–W_f: final weight and W_0: initial weight- in grams according to [25] based on the Equation 4:

$$DWF = \frac{W_f - W_0}{t} \quad (3)$$

$$SGR = \frac{\ln W_f - \ln W_0}{t} * 100 \quad (4)$$

When comparing the average values of the removal efficiencies in the experimental design it is important to use comparisons according to [26]; for data with the normality of the Student's t-distribution- was completed and for not normalized data, the W Mann-Whitney non-parametric test was applied with the α set at 0.05 (significance at P $<$ 0.05).

In order to compare the averaged values of the removal efficiencies, an experimental design of simple comparisons was used to analyze the data with the normality of the Student's t-distribution [26]. For data that was not normalized, the W Mann-Whitney non-parametric test was applied with the α set at 0.05 (significance at P $<$ 0.05). The software Statgraphics Centurion XVI.II (2012) was used for the complete statistical data analysis collected during the research.

3. Results

3.1 Granulometric characteristics of granular materials

Based on the particle size analysis values for the AAFBR, possible carriers i.e. D_{10}, D_{30} and D_{60}, the uniformity coefficient and the coefficient of gradation values were calculated. The main results for the pumice stone, the fine and coarse zeolite are presented in Table 1.

3.2 General monitored parameters

Table 2 shows the average values and standard deviations of the measured parameters for the different points of the treatment system.

3.3 Nitrogen and organic matter removal parameters monitoring

Table 3 shows the average values and standard deviations in mg/L of the measured parameters related to the nitrogen removal during the research period.

3.4 Fish survival and specific growth rate

In the two culture tanks, there was 100% survival registered as the result of the good performance of the treatment system, which maintained the water quality parameters between the values recommended for rainbow trout [27]. The initial average weight of the fishes in the tanks was of 31.0 $\pm$ 0.27 g, and the final weights were 63.6 $\pm$ 1.37 and 62.7 $\pm$ 0.67 g, showing no statistic differences between the final values; based on these weights and the time of the study -21 days, the values of the SGR were 3.42 $\pm$ 0.11 and 3.36 $\pm$ 0.08 %/d.

4. Discussion

4.1 Granulometric characteristics of granular materials

Based on the values of the UC and CC, the fine zeolite was chosen as the carrier, because it has the best values in terms of uniformity close to 1.00 for uniformity coefficient and coefficient of the gradation [28, 29]. These characteristics of the Zeolite guaranteed the predominance of one size and promoted a good performance of the fluidized bed biofilter.

The specific surface area of the materials used as carriers in the upflow reactor was: 208.15 cm^2 for the hair rollers and 203.23 cm^2 for the plastic net, making a total surface area of 208.15 cm^2, and a total volume of 8.80 cubic centimeters, representing 4,674.88 m^2/m^3.

4.2 General monitored parameters

The lower values of the DO concentration registered in the bottom effluent of the rearing tanks and the UPMR were calculated based on the number of organic solids in the bottom part of the tanks and the consumption of oxygen for the stabilization of the organic matter. Concentrations above 0.0 mg/L of oxygenated water in the UPMR effluent were due to the low HRT of the treatment unit which probably limited the growth of the anaerobic bacterial community and calculates the values for BOD and COD that will be discussed.

The dissolved gas registered levels above the minimum of 2.0 mg/L recommended for culture units in RAS [30] confirms that the injected air inside the tanks was enough for promoting the growth of the rainbow trout and guarantee the recommended values for farm-raised rainbow trout [18, 31]. The high value of DO at the AAFBR and the post-filtered effluent indicates that during the experiment the liquid had enough dissolved gas and that aerobic processes were developed inside that treatment units.

The water temperature had low variability and was between the optimum values from 10 to 18°C for farm-raised rainbow trout [2, 32, 33]. The measured pH at the different points of the RAS stable with low oscillations from 7.5 to 8.5, which are suitable values for farm-raised rainbow trout [18], and this promotes a lower percentage of ammonium in the water.

4.3 BOD and COD

The BOD concentrations of the bottom and surface effluent of the farm-raised rainbow trout units were similar due to

Table 1 Resume of the granulometric results for the granular materials

Material	D_{10}	D_{30}	D_{60}	UC	CC
Pumice Stone	0.04mm	0.58mm	2.10mm	52.50	4.00
Coarse zeolite	1.30mm	2.05mm	2.30mm	1.77	1.41
Fine zeolite	1.50mm	1.65mm	2.00mm	1.33	0.91

D_{10}, D_{30}, D_{60}: Diameters in the particle-size distribution curve corresponding to 10, 30 and 60% finer.
UC : Uniformity coefficient
CC : Coefficient of gradation

Table 2 Average values of water quality parameters DO, Temperature and pH

Parameter	Monitored liquid or point						
	BE	SE	EUPMR	EPrF	EAAFBR	EPoF	WCLT
DO (mg/L)	2.60±1.06	4.41±0.58	3.00±0.98	4.19±0.86	4.98±0.43	4.52±0.77	4.74±0.60
Temp. (oC)	17.01±0.81	16.56±0.81	16.74±0.81	16.47±0.69	16.49±0.66	16.51±0.73	16.56±0.70
pH	7.40±0.63	7.65±0.17	7.56±0.32	7.61±0.20	7.70±0.25	7.63±0.24	7.62±0.22

BE : Bottom Effluent of the culture units
SE : Surface Effluent of the culture units
EUPMR : Effluent of the Upflow with Plastic Media Reactor
EPrF : Effluent of the Pre-Filtration unit
EAAFBR : Effluent of the Three Phase Fluidized Bed Reactor
EPoF : Effluent of the Post-Filtration unit
WCLT : Water Constant Level Tank
DO : Dissolved Oxygen
Temp. : Temperature

Table 3 Average values of water quality parameters BOD, COD and Nitrogen

Parameter	Monitored liquid or point					
	BE	SE	EUPMR	EPrF	EAAFBR	EPoF
BOD	37.55 ± 6.33	44.28 ± 7.33	26.84 ± 11.85	5.06 ± 2.52	3.62 ± 1.88	2.49 ± 1.58
COD	154.64 ± 95.19	128.22 ± 127.26	52.78 ± 30.43	41.96 ± 45.00	27.08 ± 39.41	10.80 ± 9.87
Ammonium	2.60 ± 3.11	0.75 ± 0.91	1.06 ± 1.22	0.56 ± 0.65	0.46 ± 0.48	0.42 ± 0.47
Nitrite	0.97 ± 0.98	0.60 ± 0.52	0.47 ± 0.40	0.59 ± 0.49	0.59 ± 0.49	0.53 ± 0.40
Nitrate	6.99 ± 7.0	1.14 ± 1.14	12.70 ± 12.54	6.07 ± 6.06	11.16 ± 11.05	5.80 ± 5.77
TN	23.23 ± 13.26	20.10 ± 8.70	22.44 ± 11.6	19.67 ± 8.35	19.39 ± 8.42	19.16 ± 8.29

BE : Bottom Effluent of the culture units
SE : Surface Effluent of the culture units
EUPMR : Effluent of the Upflow with Plastic Media Reactor
EPrF : Effluent of the Pre-Filtration unit
EAAFBR : Effluent of the Three Phase Fluidized Bed Reactor
EPoF : Effluent of the Post-Filtration unit
BOD : Biochemical Oxygen Demand
COD : Chemical Oxygen Demand
TN : Total Nitrogen

the re-suspension of a fraction of the settled concentration of organic matter. This was because of the ascension of air bubbles injected inside the culture units. But the levels of COD at the bottom effluent were higher than at surface effluent.

The different treatment units demonstrated a reasonable performance with final concentrations close to 2.5

mgBOD/L and 11.0 mgCOD/L. Similar values to other research in RAS involving the use of three-phase airlift fluidized bed reactors were obtained as reported by Sánchez and Matsumoto of 2.7 mgBOD/L and 6.3 mgCOD/L [17] and Maigual of 2.6 mgBOD/L and 10.1 mgCOD/L [34].

The filtration units assumed an important role in organic matter removal, especially through the particulate carbonaceous matter retention which gradually reduced the water flux due to the obstruction of the pores and defined the need to make the backwash every day in order to clean the filters.

The combination of fixed bed and moving bed biofilters can improve the organic matter removal efficiency, because in a comparative study between fixed bed biofilters and mobile bed biofilters, Fernandes *et al*. reported that: (a) the fixed bed biofilters remove a higher amount of filtered BOD than moving bed biofilters (b) the moving bed biofilters remove more particulate BOD than the fixed bed, presumably due to disintegration of particles in moving bed reactors [35].

Based on the BOD and COD concentrations registered during the research, the global average removal efficiencies of the system were 94.4 and 91.7% respectively for those parameters. The average performance of the system was higher than those reported by the literature in similar studies of 47.4% for BOD and 77.3% for COD [17], and 48.0% for BOD and 64.9% for COD [34].

In this research data, the treatment system included filtration units and the two cited sources only had sedimentation units after the AAFBR. Based on the calculated values, the treatment system configuration could be suitable for other kinds of aquaculture systems. It is expected to achieve higher efficiencies for warm water species because as stated by von Sperling, the biological reactions, within certain ranges, increase with higher temperatures [36].

In this research, the upflow and the AAFBR reactors showed similar performance in terms of BOD removal, with 30.2% of the influent organic matter by the UPMR and 27.5% by the AAFBR. One of the advantages of including carriers in upflow reactors is that depending on the material used, this could lead to the efficient adherence of the biofilm allowing a high solid retention time for the loss of HRT, especially when the carriers have high surface area [37, 38]; similar condition was observed in a research for the evaluation of seashell, synthetic material, vitrified material, and river gravel as carrier for anaerobic upflow reactors [39]. The best performance of these carriers registered by the seashell, with 1,210 m^2/m^3 and the synthetic material 2,027 m^2/m^3. The hydraulic surface loading rates applied in this research for the UPMR were of 41.6 and 83.3 $m^3/m^2/d$, 4 to 8 times higher than the values recommended, from 6 to 15 $m^3/m^2/d$ to anaerobic reactors as UASB [40].

4.4 Nitrogen

Based on the average values of pH and temperature registered at the effluent of the culture tanks, the unionized ammonium fraction in the water was close to 1.16%, representing 0.03 mgNH_3/L, which guarantees the welfare of the fishes because it was below of lethality levels for salmonids of 0.54 mg/L for 96 h exposure and 2.85 mg/L for 24 h [41]. Below the range of LC50 values for 96 h exposure of 0.16 to 1.10 mg/L [42]; the lethal values of 0.62 mgNH_3/L [43]; or the maximum exposure levels recommended [32].

The global removal efficiency of the ionized ammonium (NH_4^+) was of 52.5% higher than the values reported in similar treatment systems, of 31.0% [34] and of 27.1% [17]. The main difference between those studies and this research was the presence of filtration units that improved the removal of the pollutants, mainly in the particulate form. The low removal efficiency of the AAFBR, with less than the 7%, had two main reasons: the experiment had some troubles with energy instability that lead to stopping the recirculation, affecting the microbial community at the carrier because temporal lack of oxygen, the second reason was the loss of the carrier due the re-expansion of the three-phase system because the restart of the circulation. The performance of the AAFBR was lower than the reported by other authors [18] and [11] with ammonium removal from 8 to 11% using sand grains with D_{10} between 0.45 and 0.80 mm in fluidized sand biofilters; Davidson *et al*. using D_{10} grains between 0.11 and 0.19 mm obtained removal efficiencies of ammonium of 88% and 86% respectively [44].

The nitrite and nitrate concentrations measured in the effluent of the biological reactors were higher than the influent levels; those values indicated nitrification processes inside the UPMR and the AAFBR, especially the related to nitrate the end product of the nitrification. An opposite situation happened with the total nitrogen concentrations, with slightly lower values in the effluent of the reactors when compared with their influents; that phenomena suggest denitrification processes, maybe in anoxic-anaerobic layers due the growth of biofilm in the carrier [45].

The complete nitrite removal was of 13.4%, but negative values, near -5.3% were registered in the pre-filter unit and in the AAFBR effluents. The complete nitrate removal was 1.3%; the highest efficiencies were reached at the UPMR with values up to 11.6% and slightly negative values which had an average percentage of -0.2%, and the average TN removal of 6.0% with the highest values registered at the post-filtration unit.

The calculated efficiency percentages suggested that the nitrification and denitrification processes inside the biological reactors and the removal of particulate material with a certain content of nitrogen at the filtration unit were better with the biofiltration system.

Maybe, the RAS operation for more extended time can lead to a stabilization of the microorganism communities and to a more uniform treatment performance. Based on studies with submerged biofilters, some researches reported the process of ammonium oxidation suggested functional resiliency in the face of changing environmental conditions through time for fish production, mainly due to the coexistence in biofilters of a diversity of ammonium-oxidizing bacteria and especially archaea [46].

Bartelme *et al.* in a commercial-scale freshwater RAS raising *Perca flavescens* studied a fluidized sand biofilter that has been in operation for more than 15 years [47]. The authors concluded that the bacterial community shifted around a stable nitrifying consortium of Ammonium-Oxidizing Archaea and completed ammonium-oxidizing Nitrospira with relatively equivalent and stable abundances.

The addition of the chemical substrate can provide the fastest biofilter startup and lead to a better performance on nitrification performance in RAS biofilters. In a research using a combination of sodium nitrite and ammonium chloride was observed that nitrification started one week before using only ammonium chloride or a clean start with rainbow trout (*Oncorhynchus mykiss*) [48].

The treatment system maintained the levels of nitrate below the 10 $mgNO_3/L$ which was the maximum recommended concentration for rainbow trout culture [32, 33]. The average final effluent TN concentration measured after the post-filtration process was similar to the one registered in a RAS system with AAFBR for farm-raised tilapia [34].

Higher nitrogen removal efficiencies are expected with an internal source of organic carbon in order to promote the denitrification by controlling the C/N ratio [49]. Also, an increase of nitrogen and organic matter removal efficiencies are expected in recirculating systems for warm water fish farming due to the increase of biological reactions velocity at higher temperatures.

4.5 Fish survival and specific growth rate

Zero mortality rates were registered in other investigations in similar recirculating aquaculture systems as reported by [31, 50]. The calculated daily weight gain was 1.55 ± 0.06 and 1.51 ± 0.04 g/d, which had a concurrence with the 1.5 g/d reported in other studies for rainbow trout culture in RAS [31, 51]. The DWG was higher than the values calculated in similar studies for authors as García *et al.* who reported a 1.13 g/d [52] and Arredondo *et al.* with 1.21 g/d [53], and the SGR was higher than the 2.59%/d calculated by Dalsgaard *et al.* for rainbow trout juvenile [4].

Based on the initial fish weights and considering the water volume of the farm-raised fish tanks of 125 L, the starting stocking density that was calculated in the T1 and T2 was of 10.17 ± 0.09 kg/m^3, and according to the final average weights, the final stocking densities were respectively 20.87 ± 0.45 and 20.58 ± 0.22 kg/m^3; those values were similar to the reported pilot scale by [54–56]. More research with different amounts of fish biomass is recommended in order to calculate the highest RAS capacity to maintain the water quality characteristics for the farm-raised species because it could make this water treatment option more profitable.

5. Conclusions

The use of the upflow with plastic media reactor, the aerobic airlift fluidized bed reactor, and the filtration units in the RAS guaranteed the maintenance of water quality parameters in the recommended values for rainbow trout farming. The organic matter removal, in the form of BOD and COD, was higher than 90% but were made mainly through the filtration units because of the retention of particulate matter. The wastewater treatment system transformed the ammonium into less toxic nitrogen forms as nitrite and nitrate via nitrification and suggested total nitrogen removal through denitrification processes. The performance of the evaluated treatment units suggested them as an option to water reuse on RAS, capable to keep the water quality characteristics at recommended values for rainbow trout farming in closed systems.

6. Declaration of competing interest

None declared under financial, profesional and personal competing interests.

7. Acknowledgments

The authors are grateful to the Mariana University and declared that it is the unique funding source for the research described in this work.

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