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EFFECT OF FOUR DIFFERENT PRETREATMENTS IN NITROGEN AND PHOSPHORUS FLOW AND MASS BALANCE IN EFFLUENTS OF A RECIRCULATING AQUACULTURE SYSTEM

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ABSTRACT. The effluents from intensive aquaculture operations such as recirculating aquaculture systems (RAS) have high concentrations of sludge that can become a source of pollution if they are not properly treated and disposed. Anaerobic digestion is commonly used for biological degradation of sludge. Pretreatments prior to anaerobic digestion can enhance sludge degradation and decrease nitrogen and phosphorus load through microbial activity. This study examines the effect of four different pretreatments (biological, chemical, mechanical and thermal) in the N and P fluxes and mass balance from a RAS effluent in a seven-month period at ambient temperature. Each month a 15-day experiment was performed. All pretreatments, except chemical, removed N (thermal 29.78%, biological 36.75%, control 42.25%, mechanical 49.46%, chemical -7.68%). All pretreatments produced phosphorus (chemical 1.96%, mechanical 16.07%, thermal 24.37%, biological 32.39%, control 58.50%). Our results showed that the mechanical pretreatment was the most effective in removing N. In contrast, none of the pretreatments reduced P content in the sludge.

Keywords: Sludge, pretreatments, mass balance, recirculating aquaculture systems

Efecto de cuatro pretratamientos en el flujo y balance del nitrógeno y el fósforo en efluentes de un sistema de recirculación acuícola

RESUMEN. Los efluentes de un tipo de cultivo intensivo como los Sistemas de Recirculación Acuícola (SRA) presentan altas concentraciones de lodos que pueden llegar a ser una fuente de contaminación si no son tratados y dispuestos apropiadamente. La digestión anaeróbica es usualmente empleada para llevar a cabo la degradación de los lodos. Los pretratamientos previos a la digestión anaeróbica pueden mejorar la degradación de los lodos, así como reducir la carga de nitrógeno y fósforo a través de la actividad microbiana. Este estudio examinó el efecto de cuatro pretratamientos (biológico, químico, mecánico y térmico) en el flujo y balance de masas de N y P de efluentes de un SRA durante un periodo de 7 meses a temperatura ambiente. En cada mes se llevó a cabo un experimento de 15 días. Todos los pretratamientos a excepción del químico, eliminaron nitrógeno (térmico 29.78%, biológico 36.75%, control 42.25%, mecánico 49.46%, químico -7.68%). Todos los pretratamientos produjeron fósforo (químico 1.96%, mecánico 16.07%, térmico 24.37%, biológico 32.39%, control 58.60%). Nuestros resultados indican que el pretratamiento mecánico fue el más efectivo para eliminar N. En contraste, ninguno de los pretratamientos redujo la concentración de fósforo.

Palabras clave: Lodos, pretratamientos, balance de masas, sistemas de recirculación acuícola.

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INTRODUCTION

The aquaculture industry is the fastest growing food production activity worldwide, with an annual increase of 8.8% over the last three decades (FAO, 2014). The global growth of aquaculture has brought an increase in some negative impacts through the discharge of substantial amounts of polluting effluents (Chávez-Crooker & Obreque-Contreras, 2010). The effluents produced by these increasing aquaculture activities are characterized by high concentrations of organic and inorganic waste such as ammonia, nitrate, nitrite, phosphorus, dissolved organic carbon, feces, and uneaten food (Sugiura *et al.,* 2006; Mirzoyan *et al.,* 2010; Liu *et al.,* 2016).

Recirculating aquaculture systems (RAS) are an alternative to traditional flow through aquaculture production systems (pond or raceway) due to economical, hydrological and environmental constraints. These systems have been characterized by low water consumption, strict control of water quality and effective solid removal efficiency (Timmons *et al.,* 2001; Hall *et al.,* 2002; Mirzoyan *et al.,* 2010). Two of the most important aspects involved in the operation of a RAS are water quality control and total suspended solid management, first within the system and later as effluent disposal. The RAS effluents are composed mainly of fish excretions and a small percentage of uneaten feed and biological floc that is shed from the biological filter, and it is characterized by a low total solid content (1.5 - 3%) compared to other animal production and industrial wastewater (Mirzoyan *et al.,* 2008; Sharrer *et al.,* 2010).

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In most RAS the effluents are removed in a concentrated sludge that is either treated on site, discharged to a receiving water body, a local sewer system, or disposed into a decentralized treatment unit (Timmons & Ebeling, 2007; van Rijn, 2013). The main onsite effluent stream treatment is sludge thickening. Once the sludge is concentrated it is usually degraded in aerobic or anaerobic digestion (AD). The objective of the overall treatment (either aerobic or anaerobic) is to reduce the volume and mass of degradable materials. However, AD is a favored stabilization method compared to aerobic digestion due to lower cost, lower energy footprint, and moderate performance, especially for stabilization (Novak *et al.,* 2003; Appels *et al.,* 2008; Zhang *et al.,* 2013).

Sludge disposal can become problematic due to the high retention time needed for AD (20-30 days), however the retention time can be shortened with a pretreatment whose main goal is to enhance the AD by altering sludge physical or chemical properties (Appels *et al.,* 2008; Carrere *et al.,* 2010; Park *et al.,* 2014). There are four different types of pretreatments that can be applied to the sludge: 1) biological (Ge *et al.,* 2010), 2) thermal (Neyens & Baeyens, 2003; Appels *et al.,* 2010), 3) mechanical (Bougrier *et al.,* 2006), and 4) chemical (Li *et al.,* 2008).

The sludge pretreatments may not be compared directly among them as they depend on the nature of the sludge (primary or activated), the pretreatment effectiveness, and the cost (particularly energy cost) (Eggeman & Elanderb, 2005; Carrere *et al.,* 2010; Carballa *et al.,* 2011). However, a nitrogen and phosphorus mass balance can be performed to compare the effect of different pretreatments on sludge degradability since the extracellular polymeric substances are degraded many organic, nitrogen, and phosphorus constituents will be dissolved, hydrolyzed and released in different concentrations (Wang *et al.,* 2010). Nitrogen and phosphorus loading is expected to increase and the amount released will differ among the different pretreatments. Therefore, the objective of this study was to determine the effect of four different pretreatments: biological, thermal, mechanical and chemical, in the nitrogen and phosphorus load of a RAS effluent through a mass balance, and assess the potential impact in the overall anaerobic digestion process at ambient temperature through seven months.

MATERIALS AND METHODS

All experiments were carried out using the sludge collected from the backwash of a propeller bead filter (0.28 m^3/10 ft^3 of media) (PBF10) from a tilapia intensive recirculating aquaculture pilot operation. The pilot operation consisted of six RAS where the tilapia were cultured at an average density of 80 kg/m^3 under an average temperature of 28°C. In all six RAS systems, a propeller bead filter (PBF10) was used. The effluents from the backwash of the PBF10 were collected in three connected sumps for equalization and further distribution to the experimental anaerobic digesters. Simultaneous pretreatments were applied to the sludge treatments prior to AD.

Anaerobic digester set up and operation

Circular anaerobic digesters (20.3 cm diameter x 18.5 cm high) were used in the experiment. Anaerobic batch digestion was operated for 15 days each month during seven-month period from March to September 2013 at ambient temperature, without pH control. Prior to each experiment, digesters were filled with a sludge/effluent at 1:4 ratio (Rustian *et al.,* 1997; Sumico *et al.,* 2006), and each digester was seeded with 180 ml of activated sludge from a well-established digester. For all digesters, anaerobic conditions were present throughout all experiments.

Analytical methods

Temperature (ºC) and pH were measured daily using an YSI 56 multiparameter probe. Alkalinity was measured by the colorimetric method using the phenolphthalein indicator and a mixed indicator (Adams, 1990). All nitrogen and phosphorus forms were measured daily during the 15-day experimental trial performed every month during a seven-month period using a 3 ml sample volume by triplicate from each digester. Total nitrogen (TN) was determined by the potassium persulfate method (Solórzano & Sharp, 1980; Pitts & Adams, 1987). Total ammonia nitrogen (TAN) was measured by the indophenol method (Solórzano, 1969). Nitrite (NO_2-N) was measured by the diazotization method (Boltz, 1958). Nitrate (NO_3-N) was measured by the ultraviolet spectrophotometric screening method at two different wavelengths: 220nm to measure nitrate and organic matter and 275nm to measure organic matter concentration (Nydahl, 1976). Phosphates (PO_4-P) were determined by the ascorbic acid method (Sletten & Bach, 1961). Total phosphorus (TP) was measured by ammonium persulfate method (APHA, 1989). All analyses were performed using nine replicates per treatment for each 15-day experimental period through the seven months of the experiment.

Experimental design

A complete randomized design was used. Sludge collected from the sumps was distributed in nine digesters per treatment, and each set of replicates was exposed to one of the four different pretreatments: 1) biological, only activated sludge was added; 2) chemical, adding NaOH to a final concentration of 0.08 M; 3) mechanical, stirring the sludge at 5000 rpm for 30 min; 4) thermal, sludge exposed to 100˚C for 30 min; and 5) a control with no pretreatment. After pretreatments 10% of activated sludge, except for the control, were added up to a total volume of 800 ml to achieving the same initial conditions, and monitored to determine nitrogen and phosphorus fluxes. Nine replicates per treatment were used. TN, TAN, NO_2-N, NO_3-N, organic nitrogen, TP, PO_4-P and organic phosphate produced (positive sign) or removed (negative sign) concentrations were obtained by subtracting the initial (day 0) from the final concentration (day 15).

Nitrogen and Phosphorus mass balance

Nitrogen and phosphorus mass balance in the digesters for each pretreatment was determined with the following general equations:

$$N_{in} = N_f + N_g - N_{ll} \qquad (1)$$

Where, for equation 1, N_{in} (mg/l) is the total N initial sludge concentration in the digesters after each pretreatment, N_f (mg/l) in the total N final pretreated sludge (PS) concentration, N_g (mg/l) in the N gained in the PS throughout the 15 days of each experiment due to ammonification, N_l (mg/l) is the nitrogen in the PS lost throughout the experimental period due to denitrification, volatilization, sedimentation and biomass loss, leading to equation 2:

$$N_{in} = N_f + (NAT) - (NO_2\text{-}N + NO_3\text{-}N + N_{org}) \qquad (2)$$

Where NAT, NO_2-N and NO_3-N are the result of ammonification and denitrification, and the N_{org} are the N organic compounds contained in the sludge and microbial biomass.

Phosphorus mass balance in the digester for each pretreatment was determined by the following equation

$$P_{in} = P_f + P_g - P_l \qquad (3)$$

For equation 3, P_{in} (mg/l) is the P initial sludge concentration in the digesters, P_f (mg/l) is the P final sludge concentration, P_g is P released from the pretreated sludge throughout the experimental period, P_l (mg/l) is the P lost in the sludge throughout assimilation in the experimental period, which will lead to the following equation

$$P_{in} = P_f + (PO_4) - (P_{org}) \qquad (4)$$

Where PO_4-P is the phosphate concentration released by mineralization and P_{org} are the P organic compounds contained in the sludge and microbial biomass. The premise of the N and P mass balance is that it should be possible to account for all the N and P entering the process of AD via initial influent concentration of RAS sludge. Total nitrogen and total phosphorus fluxes (TAN, NO_2, NO_3, N_{org}, PO_4^-, P_{org}) due to denitrification, ammonification, assimilation and mineralization were elaborated for the four pretreatments and the control by subtracting the N or P initial concentration forms from their final concentration forms.

Data analysis

A Shapiro-Wilks test and Levene test were used to for testing assumptions of normality and homogeneity of variance. Temperature of each 15-day experimental trial over the seven months of the experiment was analyzed with a two way ANOVA. A repeated measure analysis was used to determine the NAT, NO_2, NO_3 and PO_4 dynamics in each 15-day experimental trial in each of the 7 months of experiments. A two-way ANOVA was performed to determine differences in total nitrogen (TN), total phosphorus (TP), NAT, NO_2, NO_3 and PO_4 among treatments and time (months). A Tukey pairwise comparison test was used when significant differences were detected. A value $p < 0.05$ was chosen as level of significance. Statistical analyses were performed using SAS 9.2 for Windows (SAS Institute, 2002).

RESULTS

Sludge temperature and pH

Significant differences (p=0.0001) in temperature were detected among the seven months of the experiment. The highest temperature was detected in August (22.73 ± 1.73°C), and the lower in March (15.96 ± 1.15°C) (Fig. 1).

From the 1st to the 15th day pH decreased in average from 8.4 to 7.7. Significant differences (p=0.0001) in pH were detected among pretreatments within each 15-day experimental run. For each month, pH in the chemical pretreatment was significantly higher (p<0.0001) than in the other pretreatments and the control. Among the rest of the pretreatments and the control no differences were detected. The chemical pretreatment consistently showed the highest pH during all months (Fig. 1). Significant differences (p=0.0001) among pretreatments were detected in alkalinity values; the alkaline treatment being different from the others pretreatments and the control. No differences among the other pretreatments and control were observed during the seven months that the experiment lasted. Within each experimental 15-day run, the alkalinity increased over time in average 83.44% and for the other pretreatments and control 52.98 ± 1.97%.

Effect of pretreatments in nitrogen forms, production and removal efficiency

The TN produced or removed, expressed in percent for all pretreatments in the seven months of the experiment, are presented in Table 1. In April, the chemical pretreatment produced the highest percent of TN (133.81%). The thermal pretreatment in March showed an 84.57% removal rate, which was the highest of all pretreatments. The TN flux is the result that the TAN, NO_2, NO_3 and organic nitrogen is either produced or removed after each 15-day experiment in all seven months of the experiment (Table 1).

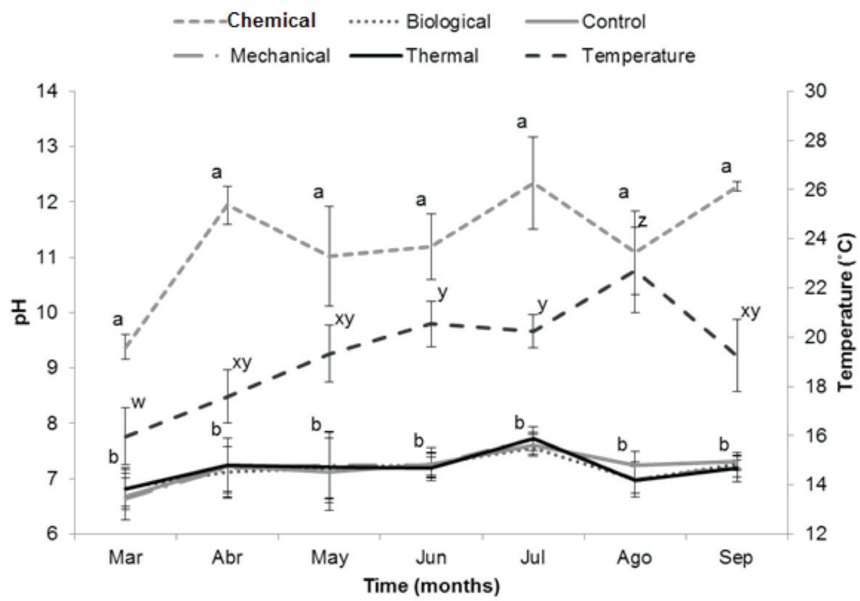

Figure 1. Average temperature and pH in the digesters of the four pretreatments (biological, chemical, mechanical, thermal) and control during the seven months of the experiment. Superscripts a>b mean significant differences between treatments in pH and superscripts z>y>x>w indicate significant differences between months in temperature.

TN, TAN, NO_2-N, NO_3-N and N_{org} fluxes

All pretreatments produced TAN. Thermal pretreatment in all months produced the highest concentration of TAN betweeen all pretreatments and control (49.25 mg/L). The highest TAN concentration was detected in March and the lowest in September. Significant differences (p<0.0001) in TAN were detected betweeen the seven months and pretreatments. Within each 15-day experimental run, thermal pretreatment consistently released a significant (p=0.0001) and higher concentration of TAN with the highest production in day 12, and the chemical pretreatment with the lowest (-6.40 mg/L) in September. No differences in TAN among the other pretreatments and control were observed (Fig. 2A).

All the pretreatments and the control removed NO_2–N. In contrast, the chemical pretreatment always produced NO_2. The highest concentration of NO_2 was produced in June and July, but it was removed during all other months. Significant differences (p<0.0001) in NO_2–N among months and pretreatments were recorded. Within each experimental 15-day run, the alkaline pretreatment produced a higher and significantly different NO_2–N concentration than the other pretreatments and control. The highest concentration of NO_2–N was found at day 5 and the lowest of day 9 (Fig. 2B).

In all months NO_3–N was removed except for the chemical pretreatment. Significant differences (p<0.0001) were detected in NO_3–N among months and pretreatments. The highest NO_3 removal was in May with the mechanical pretreatment and the lowest by the thermal pretreatment in September. Within each experimental 15-day run the chemical pretreatment released the highest NO_3–N concentration and was significantly higher than the rest of pretreatments and control. For TAN, NO_2–N and NO_3–N within each of the 7 experiments 15-day run, the variation in concentration was due to effect of the pretreatments (Fig. 2C).

April was the month where the highest concentration of organic nitrogen was produced, and March where the lowest amount was removed. Organic nitrogen was also produced in August and July, and in all remaining months (May, June, September and March) it was removed (Table 1). Significant differences (p<0.0001) in organic nitrogen betweeen months and pretreatments were detected. The chemical pretreatment produced organic nitrogen and the thermal > mechanical > biological removed it.

The highest TN production was detected in April by the chemical pretreatment. In March, the highest concentration of TN was removed by the mechanical pretreatment. Significant differences (p<0.0001) were found in TN betweeen months and pre-treatments.

Effect of pretreatments in phosphorus forms and removal efficiency

The total phosphorus produced and removed expressed in percent for all pretreatments in the seven months of the experiment are presented in Table 2. Significant differences were detected (p<0.0001) in the percentage of total phosphorus produced among months and pretreatments. The biological pretreatment produced the highest percentage of total phosphorus and the chemical pretreatment, the lowest.

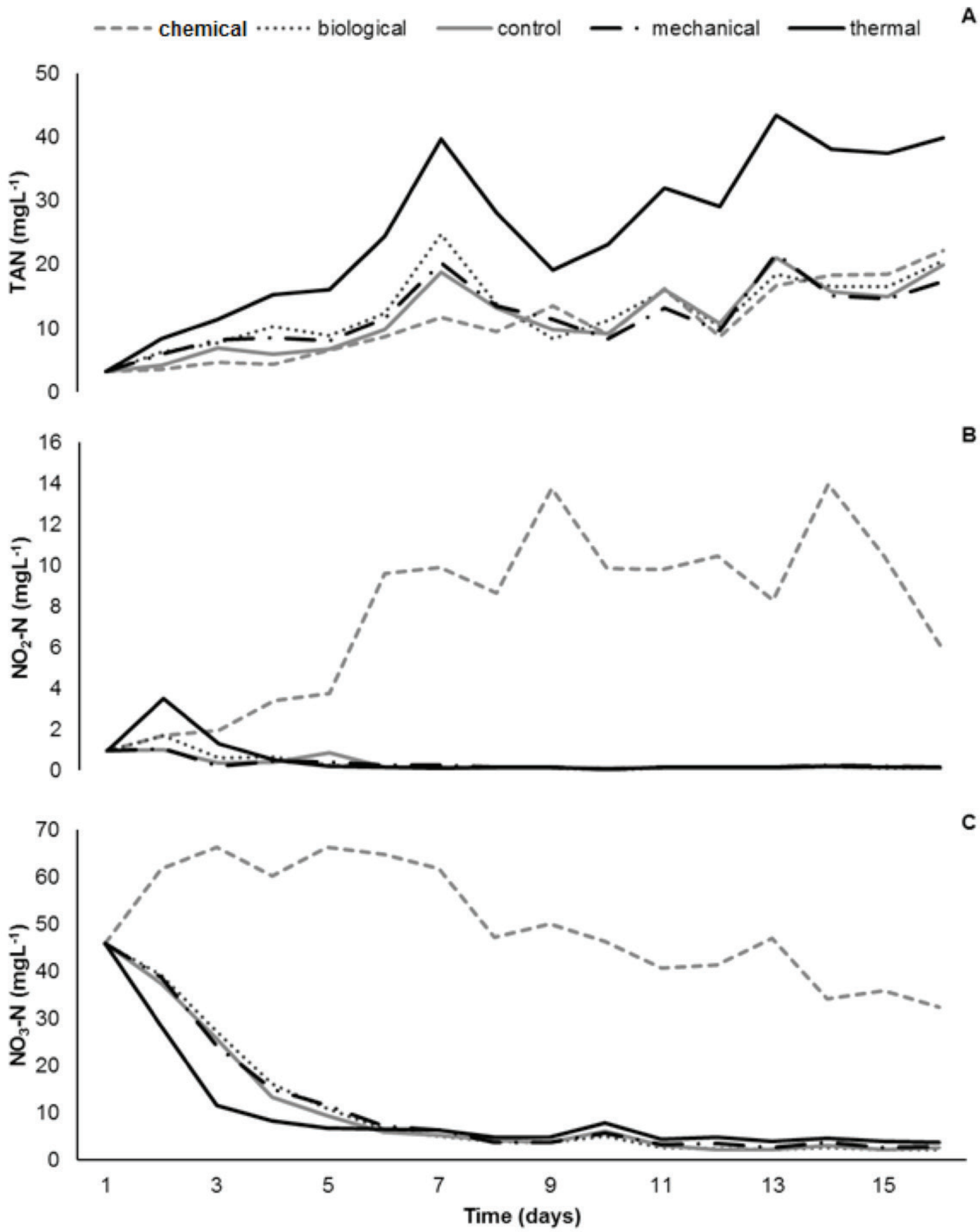

Figure 2. Average A) TAN, B) NO_2-N and C) NO_3-N concentration in the digesters for each pretreatment (biological, chemical, mechanical and thermal) and control for each one of the 15-day experimental run.

In all months except for August (-10.76% removed) total phosphorus was produced. The TP presented in Table 2 is the result of PO_4^- and organic phosphorus produced or removed among their initial and final concentration after each 15-day experiment in all seven months of the experiment.

TP, PO_4^-P and P_{org} fluxes

Significant differences (p<0.0001) were detected in TP betweeen the seven months and pretreatments. The biological pretreatment showed the highest concentration of TP and chemical pretreatment

Table 1. Total nitrogen (mgL^{-1}) fluxes (TAN, NO_2-N, NO_3-N, N_{org}) (%) produced (+) or removed (-) by the four pretreatments (biological, chemical, mechanical, thermal) and control during the seven months of the experiment. Superscripts a>b>c>d>e>f indicate significant differences in TN between months and superscripts z>y>x>w>v indicate significant differences in TN between pretreatments.

Month	Treatment				
	Control	Biological	Chemical	Mechanical	Thermal
March					
TAN	24.65±2.41	14.49±1.69	37.61±4.69	26.87±0.42	31.64±2.53
NO_2	-0.35±0.18	-0.38±0.12	-2.20±0.66	-0.57±0.05	-12.71±0.34
NO_3	-31.54±1.29	-36.13±2.63	-59.82±2.91	-26.79±3.55	-44.95±1.94
N_{org}	-35.01±9.89	2.09±5.87	-29.11±10.29	-124.59±17.73	-210.91±2.94
TN	-42.25±7.90^{x,a}	-19.93±5.51^{w,e}	-53.52±5.09^{x,b}	-125.08±16.71^{y,a}	-236.94±4.08^{z,a}
April					
TAN	8.23±0.57	19.26±4.54	1.17±0.89	12.23±0.04	34.37±1.65
NO_2	-1.85±0.12	-4.71±0.12	-1.73±0.14	-2.66±0.05	-3.61±0.34
NO_3	-48.71±3.06	-42.12+1.40	15.42±6.17	-53.29±1.37	-41.50±2.97
N_{org}	15.03±7.01	1.43±3.56	108.44±5.14	65.0±6.27	64.91±4.49
TN	-27.24±0.89^{z,b}	-26.14±1.97^{z,d}	124.14±0.24^{w,a}	20.71±0.05^{y,a}	52.80±0.05^{x,a}
May					
TAN	16.59±0.90	8.59±0.90	26.47±3.23	8.30±2.36	21.90±0.93
NO_2	-0.24±0.12	-0.06±0.03	1.26±0.01	-0.24±0.07	-3.30±0.33
NO_3	-46.40±2.58	-48.21±1.91	-82.48±4.89	-40.05±9.56	-14.64±7.39
N_{org}	-48.46±9.94	-3.84±4.22	-1.83±0.76	-5.71±17.39	-44.86±2.33
TN	-78.51±7.73^{z,d}	-43.52±3.90^{x,b}	-56.59±1.45^{y,e}	-37.70±10.07^{x,bc}	-40.90±4.14^{xy,e}
Jun					
TAN	26.36±4.83	24.66±4.55	41.79±4.77	12.33±3.60	37.30±6.47
NO_2	-0.57±0.12	-0.36±0.05	8.64±0.18	-0.41±0.03	-3.11±0.02
NO_3	-19.39±1.94	-20.99±1.59	-41.96±1.03	-15.18±1.97	-5.38±0.99
N_{org}	-56.43±6.12	-26.65±2.66	-29.07±1.62	-61.93±2.50	-31.24±11.30
TN	-50.04±0.45^{y,c}	-23.33±0.43^{x,d}	-20.60±5.30^{x,d}	-65.19±1.49^{z,d}	-2.42±6.14^{w,d}
July					
TAN	22.11±0.68	16.80±0.42	8.42±0.81	14.64±1.90	39.05±0.52
NO_2	-1.73±0.06	-2.08±0.02	9.90±2.36	-1.01±0.03	-1.12±0.04
NO_3	-29.88±0.73	-38.43±0.59	5.93±2.99	-46.16±0.79	-23.18±1.46
N_{org}	-4.91±3.94	-7.98±4.33	43.98±7.42	-21.14±6.46	+19.9±4.56
TN	-14.41±5.14^{x,ab}	-31.69±3.53^{c,y}	68.23±3.24^{v,b}	-53.67±5.13^{z,cd}	34.66±5.84^{w,b}
August					
TAN	15.34±0.21	-1.52±2.26	5.77±0.88	8.84±0.83	49.25±3.30
NO_2	-0.84±0.47	-2.50±0.32	-0.22±0.15	0.22±0.17	0.03±0.30
NO_3	-27.45±1.06	-29.09±0.62	-43.55±0.35	-28.44±1.46	-34.40±0.93
N_{org}	12.23±3.79	14.14±4.66	75.42±7.30	-0.08±2.48	0.84±5.07
TN	-0.69±4.13^{y,a}	-18.98±2.82^{e,z}	39.20±8.77^{w,c}	-19.02±3.38^{z,b}	16.07±4.09^{x,c}
September					
TAN	-0.12±2.66	-9.21±0.58	-6.40±0.48	-4.17±0.53	13.58±4.77
NO_2	-0.72±0.03	-1.16±0.04	-1.41±0.46	-1.65±0.05	-0.71±0.14
NO_3	-40.75±1.02	-44.38±2.09	10.51±1.76	-40.96±2.82	-9.54±1.71
N_{org}	-52.22±3.69	-32.51±2.71	29.45±6.90	-57.47±4.68	-99.24±3.00
TN	-98.81±3.07^{z,e}	-87.27±4.41^{y,a}	32.16±6.98^{x,c}	-104.25±5.25^{z,e}	-95.96±1.27^{z,f}

Table 2. Total phosphorus (mgL^{-1}) fluxes (PO_4-P, P_{org}) produced (+) or removed (-) by the four pretreatments (biological, chemical, mechanical, thermal) and control during the seven months of the experiment. Superscripts a>b>c>d indicate significant differences in TP between months and superscripts z>y>x>w>v indicate significant differences in TP between pretreatments.

Month	Treatment				
March	Control	Biological	Chemical	Mechanical	Thermal
PO_4	1.10±0.40	0.24±0.30	1.16±0.46	0.23±0.18	0.83±0.20
P_{org}	1.45±0.28	1.42±0.29	-0.97±0.78	1.59±0.20	0.73±0.24
TP	2.55±0.61^{y,b}	1.66±0.41^{y,ab}	0.19±0.49^{z,bc}	1.82±0.07^{y,a}	1.56±0.17^{y,bc}
April					
PO_4	0.50±0.09	0.68±0.24	-0.10±0.07	0.71±0.13	-0.01±0.71
P_{org}	0.81±0.10	0.25±0.41	-0.62±0.69	0.20±0.14	-0.17±0.85
TP	1.31±0.18^{y,d}	0.93±0.27^{y,b}	-0.72±0.64^{z,c}	0.91±0.20^{y,b}	-0.18±0.30^{z,d}
May					
PO_4	0.07±0.20	-0.03±0.06	-0.20±0.12	0.01±0.03	-0.53±0.37
P_{org}	2.12±0.45	2.17±0.31	1.76±0.18	1.66±0.05	1.30±0.19
TP	2.19±0.26^{x,bc}	2.14±0.28^{x,a}	1.56±0.07^{y,a}	1.67±0.05^{y,a}	-0.77±0.17^{z,c}
Jun					
PO_4	0.35±0.62	0.30±0.27	0.30±0.14	-0.08±0.05	0.40±0.06
P_{org}	1.17±0.74	1.00±0.57	0.38±0.39	-1.82±0.28	2.69±0.08
TP	1.53±0.28^{x,cd}	1.30±0.34^{x,b}	0.68±0.31^{y,ab}	-1.91±0.22^{z,d}	3.09±0.04^{w,a}
July					
PO_4	0.28±0.20	0.52±0.61	0.16±0.03	0.98±0.41	0.58±0.23
P_{org}	3.45±0.22	1.57±0.90	0.57±0.39	0.78±0.49	1.15±0.61
TP	3.74±0.22^{w,a}	2.09±0.52^{x,ab}	0.73±0.40^{z,ab}	1.71±0.26^{y,a}	1.73±0.65^{yz,b}
August					
PO_4	1.23±0.04	0.92±0.02	0.74±0.03	-0.10±0.10	1.69±0.05
P_{org}	-0.97±0.22	-1.18±0.09	-2.69±030	-0.13±0.02	-2.09±0.12
TP	0.26±0.18^{x,e}	-0.26±0.11^{xy,c}	-1.95±0.27^{z,d}	-0.23±0.10^{xy,c}	-0.04±0.14^{y,d}
September					
PO_4	2.10±0.44	1.92±0.11	0.70±0.16	0.93±0.30	2.14±0.82
P_{org}	0.39±0.34	-0.88±0.09	-0.63±0.37	-0.17±0.36	-1.23±0.61
TP	2.49±0.14^{v,b}	1.04±0.10^{w,b}	0.07±0.24^{y,bc}	0.76±0.07^{x,b}	-0.92±0.22^{z,bc}

the lowest. July was the month with the highest concentration. TP was produced in all months except for August, where it was removed. Biological pretreatment showed the highest PO_4-P concentration and the mechanical the lowest. September was the month with the highest concentration, in all months PO_4 was produced except for May where it was removed. Significant differences (p<0.0001) in PO_4-P were detected betweeen months and pre-treatments. Within each 15-day experimental trial, no differences between biological, mechanical, thermal and control were detected. The chemical pretreatment released the lowest PO_4–P concentration and was significantly different from the rest of pretreatments and control (Fig. 3).

The biological pretreatment produced the highest concentration and chemical was the only pretreatment where organic phosphorus was removed. The highest concentration of organic phosphorus was registered in May, the only two months where it was removed were September and August. Significant differences (p<0.0001) in organic phosphorus were detected among months of the experiment and pretreatments.

N and P mass balance

Nitrogen and phosphorus mass balances were developed based on the initial and final total nitrogen entering the anaerobic digester and a general mass balance for the fate of all nitrogen and phosphorus nutrients produced or removed by the pre-

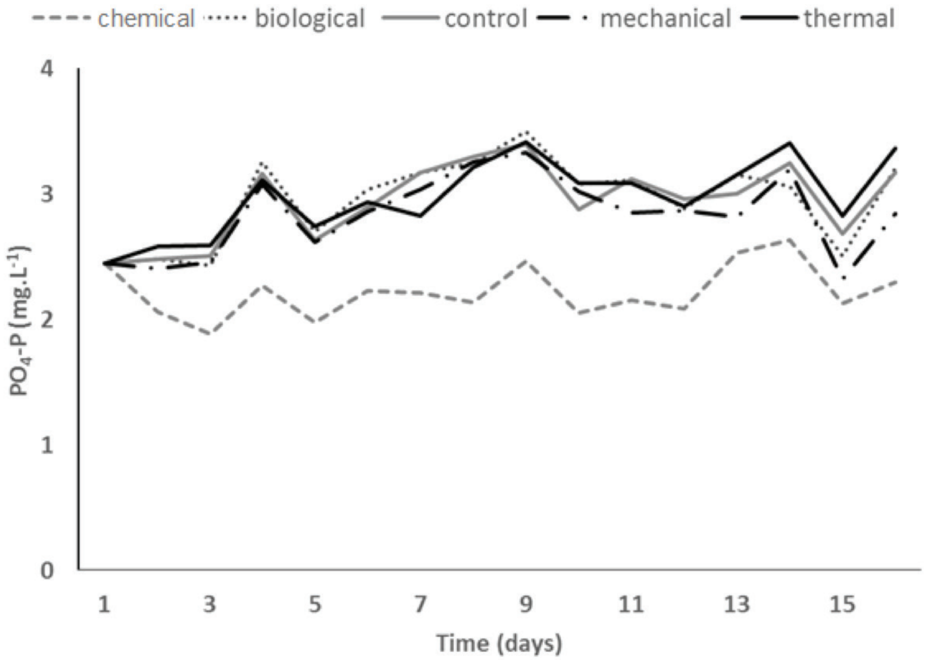

Figure 3. Average PO_4-P concentration in the digesters for each pretreatment (biological, chemical, mechanical and thermal) and control for each one of the 15-day experimental run.

treatments was developed based on the results obtained from each 15-day experiment through the seven months. In all pretreatments nitrogen was removed by denitrification (p=0.1867). The thermal pretreatment released a higher N concentration than the rest of the pretreatments by ammonification (p<0.0001). The organic nitrogen was produced by the chemical pretreatment and removed by the others (p=0.0005) where the thermal pretreatment removed the highest concentration (-42.94 mg/L / -32.21%) (Fig. 4). The final nitrogen concentration account for the denitrification, ammonification, and organic nitrogen either produced (chemical pretreatment in April) or removed (thermal pretreatment in March). The mechanical pretreatment gave better results removing TN in a 15-day sludge retention time, in contrast the chemical pretreatment produced TN (Fig. 4). The final phosphorus concentration account for mineralization that was similar betweeen all pretreatments (p=0.1542) and organic P either removed (chemical) or produced (all other pretreatments) by bacterial process in the digester (p=0.0112). The thermal pretreatment produced the highest final P concentration and the chemical the lowest (p=0.0007) (Fig. 5).

DISCUSSION

Anaerobic digestion is one of the main processes used for sludge stabilization (Gavala *et al.,* 2003; Mirzoyan *et al.,* 2010; Ennoun *et al.,* 2016). It is a natural process where biological degradation of organic matter by bacteria takes place. However, AD of aquaculture sludge is a fairly new concept, because in the traditional methods of aquaculture in ponds, flow-through systems and net pens sludge is not collected (Mirzoyan *et al.,* 2010).

Temperature and pH

Sludge produced by aquaculture activities is usually treated at ambient temperature. Temperature affects AD performance by modulating microbial community composition and diversity, altering their biochemical conversion pathways and thermodynamic equilibrium of the biochemical reactions (Wilson *et al.,* 2008). Ambient temperature for AD after pretreatments in our experiment ranged among 15.73 and 22.73°C for each 15-day experiment in the seven months that lasted the experimental trial. For this experiment, temperature often imposed some limitations for AD, decreasing growth rate, metabolism and population dynamics of bacteria in the anaerobic reactor (Appels, 2008). However, nitrification proceeded at acceptable rates for practical purposes and denitrification was not affected (Obaja *et al.,* 2003).

The pH in our experiment in all pretreatments, with the exception of chemical treatment, was close to neutral for all seven months of the experiment. Within each 15-day experiment, pH decreased in average from 8.4 ± 1.91 to 7.8 ± 1.57. The optimal pH for most anaerobic organisms is around 7.0-7.5 (García *et al.,* 2000; Mirzoyan *et al.,* 2008; Estuardo *et al.,* 2008), which suggests optimal conditions for AD. A pH lower than 6 or higher than 9 can inhi-

DENITRIFICATION	mgL^{-1}	%
Control	-35.77	-34.45
Biological	-38.66	-39.65
Chemical	-26.68	-20.83
Mechanical	-36.74	-33.12
Thermal	-28.30	-21.72

N in	mgL^{-1}	%
Control	103.83	100
Biological	97.50	100
Chemical	128.09	100
Mechanical	110.92	100
Thermal	133.30	100

AMMONIFICATION	mgL^{-1}	%
Control	16.16	15.56
Biological	10.44	10.71
Chemical	16.88	13.18
Mechanical	11.29	10.18
Thermal	32.44	24.89

ORGANIC N	mgL^{-1}	%
Control	-24.25	-23.35
Biological	-7.62	-7.81
Chemical	23.34	18.22
Mechanical	-29.42	-26.52
Thermal	-42.94	-32.95

N out	mgL^{-1}	%
Control	59.97	57.75
Biological	61.66	63.25
Chemical	137.93	107.68
Mechanical	56.05	50.54
Thermal	94.50	70.22

Figure 4. General nitrogen mass balance of four pretreatments (biological, chemical, mechanical and thermal) and control expressed in concentration (mgL^{-1}) and percent after a seven-month experimental trial.

bit both, the acetogenic stage and the denitrification process (Estuardo *et al.,* 2008). High concentration of protein in the sludge has an effect in the pH, as 1 mol of alkalinity is produced in the generation of 1 mol NH_4^+, therefore neutralizing the acidity process associated with the production of volatile fatty acids produced by hydrolysis which lowers the pH. This effect was present in all the seven-month experimental trial in our experiment (Conroy & Couturier, 2010; Zhang *et al.,* 2013).

Total N and P fluxes

In the digesters, sludge residence time (SRT) is a major factor dictating the extent of sludge digestion (van Rijn, 2013). Pretreatments were used to accelerate sludge hydrolysis and enhance anaerobic sludge digestion (Kim *et al.,* 2015), and at the same time decrease SRT and increase the release of P, N, and organic compounds that could be more susceptible for bacterial degradation. The chemical pretreatment produced the highest TN concentration because of their effective sludge dissolution. However, high concentrations of Na caused a subsequent inhibition of AD (Mouneimme *et al.,* 2003; Appels *et al.,* 2008). In contrast, the mechanical pretreatment removed the highest concentration of TN because it was able to accelerate AD by mechanically disrupting the cell structure and floc matrix, which increased the surface area providing a better contact among substrate and anaerobic bacteria (Nah *et al.,* 2000; Carrere *et al.,* 2010; Ariunbaatar *et al.,* 2014).

In our experiment the thermal pretreatment consistently produced the highest concentration of TAN throughout the months of experimentation. The highest concentration of TAN released by the thermal pretreatment occurred in August, where the maximum temperature in the trial was registered. The thermal pretreatment improved hydrolysis of sludge particles and macromolecular organic substances enhancing disintegration of cell membranes and conversion of particulate organic matter into soluble organic matter that was easily to be assimilated by the active biomass in anaerobic conditions (Neyens & Baeyens, 2003; Ferrer *et al.,* 2008; Audrey *et al.,* 2011; Liao *et al.,* 2016). Li and Noike (1992) found that the optimum conditions for pretreatments of waste activated sludge are a temperature of 170ºC for ~30-60 min with a hydraulic retention time (HRT) of 5-10 days. In our experiment, we activated the sludge at 100ºC and extended the HTR to 15 days, increasing the sludge hydrolysis and degradability (Hiraoka *et al.,* 1985; Appels *et al.,* 2010; Audrey *et al.,* 2011). In AD at low temperatures, pretreatment plays a more dominant role than temperature. The lowest concentration of TAN was produced by the mechanical pretreatment, due to a combined effect of two different factors: 1) time and intensity of the pretreatment, and 2) impellent size and digester size which led to a partial dissolution and degradation of organic matter in the sludge (Appels, 2008). At the end of each experiment, due to the pretreatments, TAN concentrations were found to increase by ammonification of nitrogenous organic matter released by the sludge dissolution (Stewart *et al.,* 2006; Conroy & Couturier, 2010).

NO_2-N and NO_3-N in sludge are closely related to the denitrification process. In our experiment both NO_2-N and NO_3-N were removed. In general, in our experiment a 90% reduction was detected within a 5-day HRT, except for the chemical pretreatment, where nitrification and denitrification were inhibited. Denitrification was also favored by the pH throughout the monthly pretreatment experiments because it remains fairly constant. At the end of each 15-day experiment, a reduction of more than 90% of NO_2-N and NO_3-N for the chemical, thermal, and biological pretreatments was observed. Similar results were obtained by Fontenot *et al.* (2007) where a 100% and 90% removal rate for NO_2-N and NO_3-N were observed when effluent temperature was registered at 22ºC, well within the range of the temperature registered in our experiment. NO_2-N may be denitrified to elemental nitrogen at appropriate hydraulic retention times, but may indirectly,

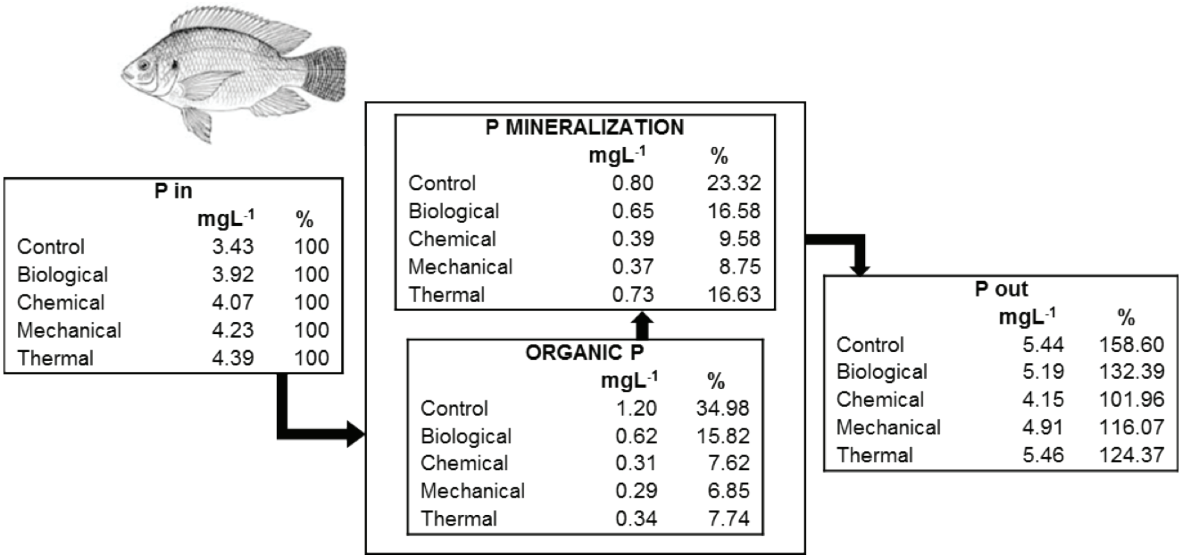

Figure 5. General phosphorus mass balance of four pretreatments (biological, chemical, mechanical and thermal) and control expressed in concentration (mgL^{-1}), and percent after a seven-month experimental trial.

through reduction to NO_3-N, serve as an electron acceptor so ammonia and NO_2-N can be converted to elemental nitrogen gas in anaerobic condition (Lahav *et al.,* 2009; Frison *et al.,* 2016).

Even when we detected that 42.09% of TP was removed in the mechanical pretreatment in June, or 118.88% of TP was produced by biological pretreatment in July, a small concentration range was either removed or produced by the pretreatments (-2.22 to 3.94 mg/l), as in those reported by Puigagut *et al.* (2011) at a temperature of 20^{o}C, within the range of temperature tested. In our experiment, the initial pH ranged among 7.14 and 8.16, except for the chemical pretreatment (12.81-10.88) but never dropped below 6.28 at the end of any of the 15 day experiments. Conroy and Couturier (2010), showed that the dissolution of phosphorus during hydrolysis of aquaculture waste sludge is simply a function of pH. In our experiment, the sludge degradation by effect of the pretreatments did not caused the phosphorus to solubilize because PO_4-P is released when an acidic pH (3-5) is achieved (Wu *et al.,* 2009) and the pH range in our experiment was significantly higher.

N and P mass balance

Chemical fluxes are indispensable to estimate nutrient budgets and modeling the dynamics of materials released by the different pretreatments and the posterior AD processes. The main potential sources or error associated with mass balance calculations are: (a) estimation of quantifying fluxes of input and output effluents, (b) nutrient variability of the effluents as a function of fish growth, feed conversion ratio and nutrient digestibility, (c) effect of hydrolysis magnitude as affected by the nature of each pretreatment, (d) denitrification rates, (e) volatilization, (f) nitrogen fixation, (g) presence or absence of phosphate accumulating organism biomass, (h) sludge retention time, (i) hydraulic retention time, (j) alkalinity and pH, and temperature betweeen other factors (Lee *et al.,* 2007; Carrere *et al.,* 2010; Zuthi *et al.,* 2013; Mariscal-Lagarda & Páez-Osuna, 2014).

The nitrogen and phosphorus balance was estimated for seven months in 15-day experimental runs (one per month). The expected effect of an enhanced hydrolysis from each pretreatment was to alter physical or chemical properties of the sludge that will potentially increase the release of nitrogen and phosphorus that could more effectively be used by anaerobic bacteria (Carrere *et al.,* 2010). Thermal pretreatment showed the highest N concentration after the pretreatment and in the final N concentration was mainly NAT and organic nitrogen, while the mechanical pretreatment produced the lowest final N concentration. Temperature and time of the pretreatment were effective in increasing sludge dissolution; in contrast for the mechanical pretreatment, a combination of volume of the digester, time of the pretreatment and the impeller weren't as effective in disrupting the cell structure and floc matrix (Carrere *et al.,* 2010). In general, N was removed by denitrification in all pretreatments (21.23-39.65%) in the seven months of the experiment except for the chemical pretreatment, where a high pH effectively disrupted any active anaerobic biomass and therefore, lowered removal rates (Jin *et al.,* 2008; Li *et al.,* 2008). In all pretreatments except for chemical pretreatment, organic nitrogen was consumed and NAT was produced by ammonification. In contrast with all other, chemical pretreatment produced organic nitrogen as it was released from the sludge but it was not reduced by bacteria (ammonification), which led to a higher final nitrogen concentration than all other pretreatments.

All pretreatments released more P than the control. P was produced as organic P and mineralization showing a minimal effect of the pretreatments by biological removal mechanisms. However, the highest final TP concentration was detected in the control. We suspect that: a) a low biomass of phosphorus accumulating organisms was present in the sludge (Seviour *et al.,* 2012), b) pretreatments did not lead to an effective destruction of the floc matrix and posterior hydrolysis for P to be released (Ferrer *et al.,* 2008; Audrey *et al.,* 2011), c) pH was not low enough to enhance hydrolysis (Conroy & Couturier, 2010), d) denitrification (NO_3-N) can inhibit P removal efficiency (Zuthi *et al.,* 2013).

CONCLUSIONS

This is the first study that evaluates the effect of different pretreatments in sludge effluents from a RAS. It focused on the N and P fluxes either produced or removed due the effect of different pretreatments applied to RAS effluents in a seven-month period. Results showed that NAT the N form with the highest concentration at the end of each the 15 day experiments except for the chemical pretreatment where NO_2-N and NO_3-N were the N fractions with the highest concentrations. Bases on the N mass balance denitrification accounts among 20.39% (chemical) – 39.65% (biological) of the net nitrogen removed and ammonification for 10.18% (mechanical) – 24.33% (thermal) of the N produced. In the TP final concentration were higher than initial which indicates that P was produced by mineralization and dissolution of organic P, 101.96% (chemical) – control (158.60%). All pretreatments were less effective in releasing TP at the end of each 15-day experiments than control; NO_3-N and pH were the limiting factors. As the water resource becomes scarcer, effluent recycling will become the most feasible alternative for inland aquaculture in RAS, and pretreatments or a combination of pretreatments that decrease the N and P load with low HRT will be a common, effective and sustainable practice to produce fish with a water budget.

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