AMMONIA AND NITRITE REMOVAL RATES IN A CLOSED RECIRCULATING-WATER SYSTEM, UNDER THREE LOAD RATES OF RAINBOW TROUT *Oncorhynchus mykiss*

TASAS DE REMOCIÓN DE AMONIACO Y NITRITO EN UN SISTEMA CERRADO DE RECIRCULACIÓN DE AGUA, BAJO TRES CARGAS DE TRUCHA ARCO IRIS *Oncorhynchus mykiss*

J. L. Arredondo-Figueroa [1*], G. Ingle de la Mora[2], I. Guerrero-Legarreta[3], J. T. Ponce-Palafox[4] and I. de los A. Barriga-Sosa[1]

[1,3] *Planta Experimental de Producción Acuícola, Departamento de Hidrobiología, y Departamento de Biotecnología, CBS, Universidad Autónoma Metropolitana-Iztapalapa, Mexico.*
Av. Michoacán y La Purísima s/n, Col. Vicentina, Iztapalapa. Apartado Postal 55-535, México 09340 D.F.
[2] *Instituto Nacional de la Pesca, Secretaría de Agricultura, Recursos Hidráulicos, Pesca y Alimentación (SEMARNAP), México, D.F. Pitágoras 1320, Colonia Santa Cruz Atoyac, Mexico 03310, D.F., Mexico.*
[4] *Laboratorio de Bioingeniería Acuícola, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Apartado Postal 584, Ciudad Universitaria, Cuernavaca 62001, Cuernavaca Morelos, Mexico.*

Received 9th February 2007; Accepted 20th November 2007

Abstract

Nitrification and denitrification rates of inorganic nitrogen were studied in a closed recirculating-water system, comparing three load rates of rainbow trout *Oncorhynchus mykiss* (89, 156 and 194 kg in each tank with two repetitions). Six self-cleaning water circular fish tanks with a volume of 4.3 m^3 were used, maintaining a 3.94 m^3/day of average flow rate and constant aeration. A total of 371 rainbow trout, 524 ± 8 g initial wet weight were introduced in the system and fed with a commercial feed that contained 38% of protein. A total study time of 44 days was divided into three phases of 14, 17 and 13 days according to the load fish rate. Temperature, dissolved oxygen, pH, total ammonia nitrogen (TAN), un-ionized ammonia, nitrite and nitrate were daily evaluated at four monitoring sites: fish tank (FT), settling tank (ST), biofilter (B) and reconditioning tank (RT). Water physicochemical characteristics and their fluctuations played an important role in treatment efficiency. Water temperature varied between 18 °C and 20.5 °C and dissolved oxygen from 4.6 to 7.7 mg/l. The lowest values of these two variables were registered in the ST where all wastes accumulate. No significant differences ($p \leq 0.05$) were observed in pH values (8.3-8.6). These conditions allowed good nitrification and denitrification rates. TAN varied from 0.2 to 1.96 mg/l; however, this value was 80% lower in the outlet (RT) as compared to the inlet (ST). The load fish rate caused a significant difference ($p \leq 0.05$) in TAN and non-ionized ammonia in the FT with the lowest value for 89 kg load density as compared to 156 and 194 kg respectively. Conversely, nitrite concentration did not show a significant difference ($p \geq 0.05$) among load fish rate. Nitrate concentration had an accumulative tendency at 156 kg load rate batch up to 30 days with a further decrease. The results showed that a reduction of load rate did not change apparently the equilibrium of bacteria population. Therefore, it is possible to control variables such as TAN, non-ionized ammonia and nitrite concentration, hence maintaining an adequate water quality for rainbow trout.

Keywords: closed recirculating system, denitrification, load density, nitrification rate, rainbow trout.

Resumen

Se estudiaron las tasas de nitrificación y desnitrificación del nitrógeno inorgánico, en un sistema cerrado de recirculación de agua, comparando tres cargas de biomasa de trucha arco iris *Oncorhynchus mykiss* (89, 156 y 194 kg por estanque con dos repeticiones). Se utilizaron seis estanques circulares de autolimpieza con volumen de 4.3 m^3 de volumen, con un flujo promedio diario total de agua de 10.93 m^3 y aireación constante. Un total de 371 truchas arco iris con peso inicial de 524 ± 8 g fueron introducidas en el sistema y alimentadas con alimento balanceado, que contenía 38% de proteína. El estudio duró 44 días continuos, divididos en tres fases de 14, 17 y 13 días respectivamente, de acuerdo con la carga de biomasa de peces. La temperatura, oxígeno disuelto, pH, nitrógeno amoniacal total (NAT), amoniaco, nitrito y nitrato fueron evaluados diariamente en cuatro sitios de monitoreo: estanque de peces (EP), estanque de sedimentación (ES), biofiltro I (BI) y estanque de

* *Corresponding author: E-mail: afjl@xanum.uam.mx*
Phone (55) 58046585. Fax: (55) 58044737

Publicado por la Academia Mexicana de Investigación y Docencia en Ingeniería Química A.C.

reacondicionamiento (ER). Las características fisicoquímicas del agua y la fluctuación de los parámetros jugaron un importante papel en la eficiencia del tratamiento. La temperatura del agua varió de 18 °C a 20.5 °C y el oxígeno disuelto de 4.6 a 7.7 mg/l. Los valores más bajos de estas dos variables fueron registrados en el ST donde los desechos se acumulan. No se observaron diferencias significativas ($p \leq 0.05$) en los valores de pH (8.3-8.6). Estas condiciones permitieron una buena tasa de nitrificación y desnitrificación. El NAT varió de 0.2 a 1.96 mg/l, sin embargo, este valor fue 80% más bajo en la salida (ET) comparada con la entrada al sistema (ES). La carga de biomasa de peces causó una diferencia significativa ($p \leq 0.05$) en los valores de NAT y amoniaco en el EP, con los valores más bajos para 89 kg, comparado con 156 y 194 kg respectivamente. Por su parte la concentración del nitrito no mostró diferencias significativas ($p \geq 0.05$) entre las diferentes cargas. Las concentraciones de nitrato tuvieron una tendencia acumulativa a 156 kg hasta los 30 días con un rápido decremento. Los resultados mostraron que la reducción de la carga de biomasa de peces, no cambia aparentemente el equilibrio de la población bacteriana del biofiltro. Además, es posible controlar las variables como el NAT, el amoniaco y la concentración de nitrito, manteniendo una adecuada calidad del agua para la trucha arco iris.

Palabras clave: sistema de recirculación, desnitrificación, carga de peces, tasa de nitrificación, trucha arco iris.

1. Introduction

The most important factors to be monitored in semi-closed or closed recirculating-water systems for semi-intensive or intensive aquaculture are the non-ionized ammonia (NH_3) and nitrite (NO_2^-) (Burrows, 1964; Liao and Mayo, 1974; Leitritz and Lewis, 1976; Spotte, 1979; Crab *et al*., 2007). These toxic forms of nitrogen can be considered as limiting factors for fish survival and growth. The main pollution source in an intensive fish culture system is undoubtedly protein-rich feeds supplied to the fish. Avnimelech and Lacher (1979) found that 80% of nitrogen in feeds is released into the water and at the pond bottom. Piedrahita (2003) and Gutierrez-Wing and Malon (2006) reported that around 75% of the feed N and P are unutilized and remain as waste in the water.

Toxic nitrogen forms must be removed from aquaculture systems since those high concentrations of nitrite and non-ionized ammonia can drastically reduce the growth rate, due to damage in gills and other internal organs. These compounds also predispose fish to diseases (Burrows, 1964; Colt and Armstrong, 1981). Three potential remotion methods for total nitrogen ammonia (TAN) in water reuse systems are generally applied: (1) air-stripping, (2) ion exchange and (3) biofiltration (Marking and Bills, 1982; Franco-Nava *et al*., 2004).

Biological nitrification is the commonest method used to eliminate toxic metabolites in high density semi-closed and closed aquaculture systems. However, ammonia oxidation to relatively harmless nitrate forms by biological oxidation must be closely monitored in the system (Lucchetti and Gray, 1988).

Biofilters used in aquaculture activities are designed to facilitate ammonia oxidation to nitrite and nitrate by nitrifying bacteria too. The advantages and requirements of these devises, have been reported by DeWitt and Saloa (1960); McCrimmon and Berst (1966); Burrows and Comb (1968); Scott and Gillespie (1972); Scherer *et al*. (1977); Scott and Allard (1983); Heinsbroek and Kamstra (1990); Craig *et al*. (1990) and Millamena *et al*. (1991); Ling and Chen (2005) and Malone and Pfeiffer, (2006). These authors coincide in the importance of this approach, based in two facts: a constant increase in water cost and a reducing supply of water of suitable quality for aquaculture.

Mechanisms such as air-stripping, agitation and aeration can remove ammonia in gaseous form from high pH water. Eighty to 90% efficiency of ammonia removal by air-stripping of effluents can be obtained in domestic and industrial wastewater treatment plants (O´Farrel *et al*. 1972; Yang, 1997).

Several forms of toxic nitrogen can seriously affect rainbow trout growth. Smith and Piper (1975) reported that six months of continuous fish exposure to 0.021 mg/l of non-ionized ammonia could promote pathological damages on gills tissues. Smart *et al.* (1978) indicated that the exposition to sub lethal levels produced a 3-fold increase in oxygen consumption. Campbell (1973) suggested that excessive hyperplasia in gills caused several types of bacterial diseases. Burkhalter and Kaya (1977) found that 0.05 mg/l of non-ionized ammonia had a significant effect on the growth rate.

The acceptable nitrite level in a recirculating-water system is around 0.55 mg/l (Jaffe, 1964). When this concentration is higher, it can promote methemoglobinemia, drastically reducing oxygen transportation capacity (Jaffe, 1964). Nitrate toxicity is only a minor problem in closed recirculating-water systems due to high tolerance in most fishes.

The objective of this study was to investigate the effect of air-stripping or biological nitrification mechanism on ammonia and nitrite remotion rate in a closed recirculating-water system under three different load rates of rainbow trout culture.

2. Materials and methods.

The study was conducted in the Planta Experimental de Produccion Acuicola of the Universidad Autonoma Metropolitana Iztapalapa. Arredondo *et al*. (1996) give a description of the recirculating-water system. The experiment involved three rainbow trout, *Oncorhynchus mykiss* load rates

(89, 156 and 194 kg) with two replicates in six 4.3 m^3 self-cleaning circular tanks. Volume and daily water turnover in each part of the system is shown in Table 1. Air was supplied to the recirculating-water system by means of a high-volume blower of 10 HP.

Table 1. Volume and daily water turnover, in the different parts of the recirculating-water system utilized in this experiment.

Parts	Volume (m^3)	Average turnover water per day
Fish tanks (FT)	24.0*	3.94
Settling tank (ST)	2.64	4.19
Biofilter I (BI)	3.20	2.84
Biofilter II (BII)	5.80	1.62
Reconditioning tank	4.80	1.80

* Six fish tanks of 4.0 m^3.

Three hundred seventy one "kamloop" rainbow trout, 524 ± 9 g initial wet weight previously conditioned during 15 days were placed at random into the tanks. A total study time of 44 days was divided into three phases: 14, 17 and 13 days. Load rates were fixed at 194, 156 and 89 kg with respect to phase duration, the number of fishes for each load densities were 371, 261 and 129 respectively.

A commercial 38% protein trout feed was used (Almazan Silver Cup, Toluca, Mexico) at 2% rate of fish body weight. Fishes were fed twice a day with equal portions.

Variable measured every 24 hours were: water temperature; dissolved oxygen using a dissolved oxygen-temperature meter (YSI model 57, Yellow Springs Instruments, Ohio, USA); pH with a pH meter (Beckman 50, Fullerton, CA, USA); total ammonia nitrogen (TAN), nitrite and nitrate were also measured daily with a Hach kit model DR/2000 (Hach Chemical Company, Ames, Iowa, USA).

Water samples were taken at four sites in the closed recirculating-water system: Fish tanks (FT); Settling tank (ST); Biofilter I tank (B I) and reconditioning tank (RT) (Fig. 1). Non-ionized ammonia fraction was calculated using the method and tables reported by Emerson *et al*. (1975) (Fig. 1).

The experiment consisted in a completely randomized design, with three treatments (load rates) and two replicates. Data obtained were analysed for analysis of variance and Turkey's test for unequal populations by Statistica package, version 4.5 (Statsoft Inc., Tulsa, USA).

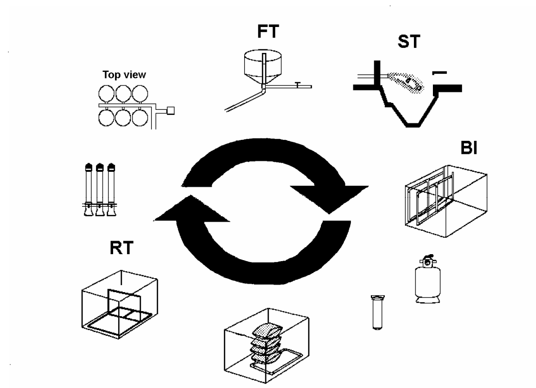

Fig. 1. Flow diagram of the recirculating-water system, utilized in this study. Not scale. The sampled sites were: FT, ST, BI and RT.

3. Results

Survival rate was 100% and no adverse effects on growth rate of rainbow trout were detected as consequence of a bad fish management neither a bad water quality in the closed system. Table 2 shows the results of load fish rates, biomass, number of fishes, average wet weight and amount of feed consumed in each phase.

The results of the physicochemical parameters and nitrogenous compounds registered during the experiment are shown in the Table 3.

4. Discussion

Physicochemical parameters of water and their fluctuations play a decisive role in treatment efficiency. Some environmental factors could affect ammonia and nitrite oxidizers such as substrate, dissolved oxygen concentration, organic matter, temperature, pH, alkalinity, salinity, turbulence level, products inhibition and light intensity (Rijn and Rivera, 1990; Sato *et al*., 2000: Chen *et al*., 2006). Oxidation of total ammonia nitrogen (TAN) to nitrate utilizes dissolved oxygen can occur only if oxygen levels are such that prevent development of anaerobic conditions (Kruner and Rosenthal, 1987). In the present study, all factors remained within ranges that exclude the possibility of nitrite accumulation.

Table 2. Average load fish rate, number of fishes, total wet weight (± SD) and feed consumption in the three phases of the study.

Phase and duration (days)	Load rate (kg)	Number of fishes	Average wet weight (g)	Total feed consumed (kg)
I-14	194	371	524 (± 9)	26.4
II-17	156	261	596 (± 7)	24.6
III-13	89	129	694 (± 8)	14.7

Table 3. Average values (standard deviation) of physicochemical parameters and nitrogenous compounds registered during the experiment.

Load rate (kg)	Sample points	pH	T (°C)	D.O (mg/l)	TAN (mg/l)	NH_3 (mg/l)	NO_2^- (mg/l)	NO_3^- (mg/l)
194	FT	8.5[a]	20.5[a]	6.4[a]	1.96[a]	0.11[a]	0.44[a]	25.9[a]
		(0.17)	(1.0)	(1.8)	(0.69)	(0.04)	(0.22)	(4.5)
	ST	8.5[a]	20.5[a]	4.6[b]	1.03[b]	0.07[a]	0.64[b]	24.5[a]
		(0.13)	(1.2)	(1.7)	(0.28)	(0.02)	(0.26)	(2.6)
	BI	8.4[a]	20.4[a]	5.8[a]	0.36[c]	0.01[b]	0.41[a]	24.9[a]
		(0.08)	(1.0)	(1.8)	(0.09)	(0.01)	(0.22)	(3.4)
	RT	8.5[a]	19.6[a]	7.2[a]	0.26[c]	0.02[b]	0.24[c]	23.5[a]
		(0.07)	(1.0)	(2.6)	(0.06)	(0.01)	(0.15)	(3.1)
156	FT	8.5[a]	19.1[a]	7.7[a]	1.73[a]	0.08[a]	0.41[a]	28.6[a]
		(0.17)	(1.0)	(1.8)	(0.33)	(0.02)	(0.10)	(3.4)
	ST	8.4[a]	19.0[a]	5.9[b]	0.91[b]	0.06[a]	0.61[b]	27.5[a]
		(0.13)	(1.2)	(1.7)	(0.19)	(0.02)	(0.14)	(2.8)
	BI	8.3[a]	19.0[a]	6.1[a]	0.37[c]	0.01[b]	0.40[a]	27.2[a]
		(0.08)	(1.0)	(1.8)	(0.14)	(0.01)	(0.10)	(2.2)
	RT	8.5[a]	19.0[a]	7.0[a]	0.22[c]	0.01[b]	0.25[c]	27.0[a]
		(0.07)	(1.0)	(2.6)	(0.08)	(0.01)	(0.12)	(3.6)
89	FT	8.6[a]	18.9[a]	7.0[a]	1.27[a]	0.07[a]	0.44[a]	26.9[a]
		(0.17)	(1.1)	(1.8)	(0.42)	(0.02)	(0.10)	(4.6)
	ST	8.5[a]	19.6[a]	5.4[b]	0.64[b]	0.05[a]	0.59[b]	22.7[b]
		(0.13)	(1.2)	(1.7)	(0.08)	(0.01)	(0.19)	(9.0)
	BI	8.3[a]	18.9[a]	6.3[a]	0.25[c]	0.01[b]	0.32[c]	23.3[b]
		(0.08)	(1.0)	(1.8)	(0.05)	(0.01)	(0.15)	(8.3)
	RT	8.6[a]	18.0[a]	7.4[a]	0.22[c]	0.01[b]	0.28[c]	25.5[b]
		(0.07)	(1.0)	(2.6)	(0.04)	(0.01)	(0.13)	(3.5)

[a,b,c] Different superscript letters in columns, mean significant differences ($p \leq 0.05$)
FT = Fish tank; ST = Settling tank; BI = Biofilter; RT = Reconditioning tank.

Preliminary tests results showed that all requirement for a good nitrification rate in a well-aerated culture system were fulfilled in this study; presence of ammonia, dissolved oxygen, trace nutrients, and a relative low level of organic carbon in the biofilter. During the experimental period, average water temperature varied between 18.0 and 20.5 °C. The highest values were registered during the first phase of the experiment with no significant differences ($p \geq 0.05$) among sampling sites and phases (Table 3). However, average temperature at the reconditioning tank (RT) in the first phase was slightly lower with respect to other sampling sites.

Dissolved oxygen fluctuated from 4.6 to 7.7 mg/l, the lowest values were observed in the settling tank (ST), due that all wastes were accumulated and concentrated in this site. Significant differences ($p \leq 0.05$) were observed among sampling sites particularly in the ST, and it can be attributed to an increase in water temperature. Oversaturated values were registered in the fish tank (FT) and reaconditioning tank (RT), where a vigorous aeration was maintained throughout the experiment. However, average oxygen concentration was 6.4 mg/l, a larger value than that required for complete oxidation. Therefore, it can be assumed that it is necessary to maintain an optimum growth and survival rate of the rainbow trout (Klontz, 1991).

Results in Table 3 agreed with those reported by Kruner and Rosenthal (1987) and Michaud *et al*., (2006) for fish culture systems. pH values were almost constant throughout the experiment with minor variations. These values had no significantly differences ($p \geq 0.05$) between sampling sites and phases. Nitrification depends on the release of inorganic nitrogen compounds from organic matter usually degraded by heterotrophs. Normally, nitrification rates are strongly influenced by pH. Srna and Baggaley (1975) observed that the establishment of nitrifying bacteria and conditions in which they grow determine their response to pH changes. Optimum pH for complete nitrification is *circa* 7.45, effective nitrification is achieved within a pH range of 7.0 to 8.2. Wild *et al*. (1971) reported that the optimum pH for nitrification in freshwater was 8.4, which coincides with the value found in our study.

Total ammonia nitrogen (TAN) range was between 0.22 and 1.96 mg/l. The higher load fish rate (194 kg) coincides with higher TAN value. Significant differences ($p \leq 0.05$) were observed among load fish rates, with highly significant differences in fish and settling tanks. Total ammonia oxidation in the three load rates was similar throughout the study meaning that metabolism of nitrifying microorganisms were near their maximum (Table 3). No adverse effects were observed on the metabolic activity of bacteria due to changes in nitrogen concentration neither in the substrate nor in load fish rates. Similarly, no inhibitory effect was detected on the nitrifying rate at pH 8.4, in agreement with the result reported by Wild *et al*. (1971).

Rjin *et al*. (1986) studied the concentrations of ammonia nitrogen and nitrite generally found in wastewater from fish culture. The values reported by these authors (0.0 to 5.0 mg/l and 0.0 to 2.0 mg/l of of TAN and nitrite, respectively) did not reach inhibitory levels for nitrifiers. Therefore, it was assumed that the bacteria nitrifying rate in the biofilter was enough to maintain ammonia and nitrate concentrations within necessary limits for nitrification, keeping water quality suitable for rainbow trout growth.

The response to a high ammonia concentration was the expected one. TAN concentrations significantly decrease as a function of load rate. The system reacted to this variation, with an 80% decrease in ammonia concentration in the outlet (RT) as compared to inlet (FT). When the load fish rate was 194 kg the percentage reduction was 96.7%, 87.3% with 156 kg load fish rate and 82.7% with 89 kg.

The TAN concentration difference between fish and settling tanks, where all effluents were collected, was around 50%. These differences can be attributed to an air-stripping effect due to the strong airflow generated on the fish tanks bottom. Chiang and Lee (1986) and Körner *et al*. (2001) demonstrated that TAN consisted of ammonium ion (NH_4^+) and unionized ammonia (NH_3). Although both may be toxic to fish, unionized ammonia is the more toxic form attributable to the fact that is uncharged and lipid soluble and consequently traverses biological membranes more readily than the charged and hydrated NH_4^+ ions. These chemical forms vary their relative concentrations according with the medium pH. Under conditions of high airflow, temperature and pH, it is possible to shift the relative concentration almost completely to NH_3. In the air-stripping process, agitation and aeration remove ammonia in gaseous form from water under conditions of high pH. Ammonia removal efficiencies of 80 to 90% have been achieved in domestic and industrial effluents by this method using wastewater treatment plants (Yang, 1997). It was estimated that most of the ammonia in our study system was removed by an air-stripping effect in the fish tank, the rest was eliminated by biofilter nitrifiers as well as in reconditioning tank (outlet) remaining only a small proportion of this gas (between 12.7 and 17.3% of the TAN). However, under our experimental conditions, this level (0.23 mg/l) can be considered permissible and did not affect the growth and survival rates of rainbow trout (Arredondo *et al*., 1996).

In spite of the high TAN values recorded in the fish tank (1.96 mg/l highest value), the main factors determining non-ionized ammonia concentration are water temperature and pH, 19°C average water temperature and pH 8.4 were recorded in our study system. Taking into account these values, it is possible to obtain 9 to 11% of unionized ammonia (NH_3). However, the effect of this relatively high concentration on rainbow trout was probably reduced by the presence of factors such as high chlorine, sodium and potassium salts concentration as well as high levels of dissolved oxygen. The concentration of both, salts and oxygen had a compensatory effect on the additional demand of rainbow trout and ammonia loss by air-stripping. It is important to note that high concentrations of chloride (> 300 mg), potassium (57 mg/l) and sodium ions (1.5 g/l) have been consistently reported in epicontinental bodies of water at Central Mexican Plateau. High concentrations of these ions have a direct effect on some physiological processes because they reduce ammonia and nitrite toxicity, and reduce pH fluctuations and osmorregulatory problems on fish (Parker and Davis, 1981; Arredondo and Lozano, 1994).

Not all-available ammonia was eliminated by air-stripping and biological nitrification due that the fish tanks were cleaned twice a day, and faeces and pieces of uneaten foods were removed from the bottom. Other debris were eliminated in other ways such as pressure washing of the sand filter and periodical elimination of solids in the settling tank.

Unionized ammonia (NH_3) concentration varied from 0.01 to 0.11 mg/l. Higher values were observed in FT and ST with significant differences ($p \leq 0.05$) with respect to the other sample sites. Also, was observed a direct relationship with load fish rates. Unionized ammonia was significantly different in 156 and 194 kg treatments with respect to lower load rate (Table 3). Occasionally, average non-ionized ammonia in the FT and ST with different densities exceeded levels reported as acceptable for rainbow trout (Smith and Piper, 1975; Smart, 1976; Alabaster *et al*., 1979; Klontz, 1991; Neori *et al*., 2004; Colt, 2006) where an optimal range falls between 0.01 and 0.5 mg/l. If higher values are kept, for extended periods of time, gill hyperplasia as well as reduction in growth rate and increased in oxygen consumption can be observed in rainbow trout culture. There was also evidence, that high ammonia level could cause weakening and fish predisposition to parasite infestations, hence reducing their stamina index (Liao and Mayo, 1974). Even though, no adverse effects were observed in the trout subjected to this experiment.

Removal of ammonia at higher rates could probably be achieved at higher concentrations of ambient ammonia. Ammonia removal by the biofilter increased linearly with load rate. These results agreed with those reported by Rjin and Rivera (1990).

The nitrite (NO_2^-) averages were between 0.24 and 0.64 mg/l with the highest values registered in the ST (Table 3). These values remained within the recommended range for rainbow trout culture (Klontz, 1991). Nitrite concentration decreased as fish density increased, although there was significant differences ($p \leq 0.05$) among fish load rate with respect to nitrite concentration. Hence, it can be concluded that the population of nitrifying bacteria was rapidly adjusted as a response of high ammonia concentration in similar manner as in a stabilized system. Some authors (Saeki, 1963; Kawai *et al*., 1965; Liao and Mayo, 1974) reported similar patterns, although studied ammonia and nitrite concentrations were different. Similarly, as TAN the nitrite toxicity can be reduced or blocked by chlorine ions, usually 5 to 6 parts of chlorine protect fish from 1 part of nitrite (Masser *et al*., 1992).

Adjustment of nitrifying bacteria population can be detected, when nitrate behavior is analysed. This parameter increased regardless of rainbow trout load rate reduction with a noticeable further decrease in the third phase of the experiment. This fact can occur as a result of nitrifying bacteria adaptation due to a decrease in ammonia concentration and adjustment to new conditions.

Average nitrate (NO_3^-) values fluctuated from 23.3 to 28.6 mg/l. Non-significant differences ($p \geq 0.05$) were observed between sample sites, except in the low load rate (89 kg) where FT presented significantly differences ($p \leq 0.05$) with the other sites (Table 3). Nitrate is relatively non-toxic except at very high concentrations (over 300 mg/l) but usually does not reach these values. In many recirculating-water systems, some denitrification seems to occur within the system keeping nitrate concentration below toxic levels (Masser *et al*., 1992).

References

Alabaster, J. S., Shurben, D. G: and Mallett, M. J. (1979). The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon. *Salmo salar* L. *Journal of Fish Biology 15*, 705-712.

Arredondo-Figueroa, J. L. and Lozano-Gracia, S. D. (1994). Water quality and yields in a polyculture of non-native cyprinids in Mexico. *Hidrobiológica* 4(1-2), 1-8.

Arredondo-Figueroa, J. L., Valdivia-Soto, R. H., Hernández-Lastiri, L. y Campos-Verduzco, R. (1996). Evaluación del crecimiento, factor de conversión de alimento y calidad del agua del cultivo de trucha arco iris (*Oncorhynchus mykiss*) en un sistema cerrado. *Hidrobiológica 6*(*1-2*), 59-65.

Avnimelech, Y. and Lacher, M. (1979). A tentative nutrient balance for intensive fish ponds. *Bamidgeh 31*, 3-8.

Burrows, R. E. (1964). *Effects of accumulated excretory products on hatchery-reared salmonids*. U.S. Bur. Sport Fish. Wildl. Res. Rep. 66.

Burrows, R. E. and Combs, B. D. (1968). Controlled environment for salmon propagation. *Progressive Fish-Culturist* 30, 123-136.

Burkhalter D. F., and Kaya, C. M. (1977). Effects of prolonged exposure trout. *Transaction of the American Fishery Society 106*(*5*), 470-475

Campbell J. W., (1973). Nitrogen excretion. In: *Comparative animal physiology*. (U. L. Prosset, ed.), Pp. 279-346. W B Saunders, Philadelphia. USA.

Chen, S., Ling, J., and Blancheton, J. P. (2006). Nitrification kinetics of biofilm as affected by water quality factors. *Aquaculture Engineering 34*, 179-197.

Chiang, H. C. and Lee, J. C. (1986). Study of treatment and reuse of aquacultural waster in Taiwan. *Aquacultural Engineering 5*, 301-312.

Colt, J. E. and Amstrong, D. A. (1981). Nitrogen toxicity to fish, crustaceans and mollusks. In: *Proceedings of the bioengineering symposium for fish culture.* (American Fisheries Society, Fish Culture Section, ed.), Pp. 39-41. Bethesda, Maryland, USA.

Colt, J. (2006). Water quality requirements for reuse systems. *Aquaculture Engineering* 34, 143-156.

Crab, R., Avinimelech, Y., Defoirdt, T., Bossier, P., and Verstraete, W. (2007). Nitrogen removal techniques in aquaculture for the sustainable production. *Aquaculture 270*, 1-14.

Craig, S. R., Hatch, S. J. and Holt, G. J. (1990). Biological filter for conical tanks. *Progressive Fish-Culturist 52*, 61-62.

DeWitt, J. W., and Saloa, E. A. (1960). An experiment with the complete recirculation of water. *Progressive Fish-Culturist 22*, 3-6.

Emerson, K., Russo, C. R., Lund, E. R. and Thurston, V. R. (1975). Aqueous ammonia equilibrium calculations: effects of pH and temperature. *Journal of Fisheries Research Board of Canada 32*, 2379-2383.

Franco-Nava, M. A., Blancheton, J. P., Deviller, G., Charrier, A., and Le-Gall, J. Y. (2004). Effect of fish size and hydraulic regime on particulate organic matter dynamics in a recirculating aquaculture system: elemental carbon and nitrogen approach. *Aquaculture* 239, 179-198.

Gutierrez-Wing, M. T., and Malone, R.F. (2006). Biological filters in aquaculture: trends and research directions for freshwater and marine applications. *Aquaculture Engineering 34*, 163-171

Heinsbroek, L. T. and Kamstra, N. A. (1990). Design and Performance of Water Recirculation

Systems for Culture. *Aquacultural Engineering 9*, 187-207.

Jaffe, E. R. (1964). Metabolic processes involved in the formation and reduction of methemoglobin in human erythrocytes. In: *The red blood cell*, (C. Bishop and D. Surgenor, eds.), Pp. 397-422. Academic Press, New York.

Kawai, A. Yoshida, Y. and Kimata, M. (1965). Biochemical studies on the bacteria in aquarium with circulating system-II. Nitrifying activity of the filter-sand. *Bulletin of the Japan Society of Science Fisheries 31*, 65-71.

Klontz, W. G. (1991). *Fish for the Future: Concepts and Methods of Intensive Aquaculture*. Text Number 5. The Idaho Forest, Wild-Life and Range Experiment Station, Collage of Forestry, Wildlife and Range Sciences, University of Idaho, Moscow, Idaho.

Körner, S., Das, S. K., Veenstra, S., and Vermaat, J. E. (2001). The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to Lemna gibba. *Aquatic Botany 71*, 71-78.

Kruner, G. and Rosenthal, H. (1987). Circadian periodicity of biological oxidation under three different operational conditions. *Aquaculture Engineering*, 79-95.

Liao, P. B., and Mayo, R. D. (1974). Intensified fish culture combining water reconditioning with pollution abatement. *Aquaculture* 3; 61-85.

Ling, J., and Chen, S. (2005). Impact of organic carbon on nitrification performance of different biofilters. *Aquaculture Engineering 33*, 150-162.

Leitritz, E. and Lewis, R. C. (1976). *Trout and salmon culture: hatchery methods*. California Department of Fish and Game. Fish Bulletin 164.

Lucchetti, G. L. and Gray, G. A. (1988). Prototype water reuse system. *Progressive Fish-Culturist 50*, 46-49.

Malone, R. F., and Pfeiffer, T. J. (2006). Relating fixed film nitrifying biofilters used in recirculating aquaculture systems. *Aquaculture Engineering 34*, 389-402.

Marking, L. L. and Bills, T. D. (1982). Factors affecting the efficiency of clinoptilolite for removing ammonia. *The Progressive Fish-Culturist 44*, 187-189.

Masser, P. M., Rakocy, J. and Losordo, M. T. (1992). Recirculating aquaculture tank production system. Management of recirculating systems. *SRAC Publications* No. 452: 12 p.

McCrimmon, H. R., and Berst, A. H. (1966). A water recirculation unit for use in fishery laboratories. *Progressive Fish-Culturist 28*, 165-170.

Michaud, L., Blancheton, J. P., Bruni, V., and Piedrahita, R. (2006). Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquaculture Engineering 34*, 224-233.

Millamena, O. M., Casalmir, C. M. and Subosa, P. F. (1991). Performance of recirculating systems for prawn hatchery and broodstock maturation tanks. *Aquacultural Engineering* 10, 161- 171.

Neori, A., Chopin, T., Troell, M., Bushmann, A. H., Kraemer, G. P., Halling, C., Shpigel, M., and Yarish, C. (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modem mariculture. *Aquaculture 231*, 361-391.

O´Farrel, T. P., Frauson, F. P., Cassel, A. F. and Bishop, D. F. (1972). Nitrogen removal by ammonia stripping. *Journal of Water Pollution Control Federation 44*, 1527-1535.

Parker, C. N. and Davis, B. K. (1981). Requirements of warmwater fish. In: *Bio-Engineering Symposium for fish culture*, (American Fisheries Society, Fish Culture Section, ed.), Pp. 21-28. Betesdha, Maryland, USA.

Piedrahita, R.H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture 226*, 35-44.

Rjin, J., Stutz, S., Diab, S. and Shilo, M. (1986). Chemical, physical and biological parameters of superintensive concrete fish ponds. *Bamidgeh 38*(*2*), 35-43.

Rjin, J. and Rivera, G. (1990). Aerobic and anaerobic biofiltration in an aquaculture unit- nitrite accumulation as a result of nitrification and denitrification. *Aquacultural Engineering 9*, 217-234.

Saeki, A. (1963). The composition and some chemical control of the sea water of the closed circulation aquarium. *Bulletin of the Marine Biology Station. Asamushi, Tohoku Uni*versity *11*, 99-104.

Sato, H., Okabe, N., and Watanabe, Y. (2000). Significance of substrate C/N ratio on structure and activity of nitrifying biofilm determined by in situ hybridization and the use of microelectrodes. *Water Science Technology 41*, 317-321.

Scherer, E., Scott, K. R., and Nowak, S. H. (1977). *A modular large scale laboratory system to acclimate and test aquatic organisms*. Canadian Fisheries Marine Services Technical Report 728.

Scott, K. R., and Allard, L. (1983). High-flow rate water recirculation system incorporating a hydrocyclone prefilter for the rearing fish. *Progressive Fish- Culturist 45*,148-153.

Scott, K. R., and Gillespie, D. C. (1972). A compact recirculation unit for rearing and maintenance of fish. *Journal Fishery Research Board of Canada 29*, 1071-1074.

Smart, G. R. (1976). The effect of ammonia exposure on the gill structure of the rainbow trout (*Salmo gairdneri* R.) *Journal of Fish Biology 8*(*6*), 474-475.

Smart, G. R., Knox, D., Harrison, J. G., Ralph, J. A., Richards, R. H. and Cowey, C. B. (1978). Nephrocalcinosis in rainbow trout *Salmo gairdneri* Richardson; the effect of exposure to elevated CO_2 concentrations. *Journal of Fish Diseases 2*, 279-289.

Smith, C. E., and Piper, R. G. (1975). *Effects of metabolic products on the quality of rainbow trout*. Bozeman Information Leaflet No. 4, Fish Cultural Development Center, Bozeman, M. T. 10 pp.

Spotte, S. (1979). *Fish and invertebrate culture: water management in closed system*. 2nd. Edition, Wiley Interscience, New York.

Srna, R. F. and Baggaley, A. (1975). Kinetic response of perturbed marine nitrification systems. *Journal of Water Pollution Control Federation 47*, 427-486.

Wild, H. E., Sawyer, C. N. and McMahan, T. C. (1971). Factors affecting nitrification kinetics. *Journal of Water Pollution Control Federation 43*, 1845-1854.

Yang, L. (1997). Investigation of nitrification by co-immobilized nitrifying bacteria and zeolite in a batchwise fluidized bed. *Water Science and Technology 35*, 169-175.