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Preliminary research on the anammox process and control of nitrogen compounds in a recirculating aquaculture system

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Abstract. Using anammox process in biological filtration of technological water within recirculating aquaculture system is an alternative to nitrification / denitrification process which can reduce production costs, especially energy by replacing aerobic biological processes that require high oxygen consumption, provided through the addition for optimal function of biofilter (conversion of $N\text{-}NH_4$ and $N\text{-}NO_2$). Aim of this paper is to highlight the first steps in the control of nitrogen compounds in recirculating aquaculture systems using anammox process. Data shown are obtained after 78 researching days (research still ongoing), the results being encouraging, registering an ammonium removal efficiency of 77% in SC1 and of 32% in SC2. Nitrites registered an increase in SC1 and a decrease in SC2 and there are all prerequisites showing that in SC2 anammox activity is more advanced than in SC1. The average of oxygen consumption rate was 0.73 mgL^{-1} in SC1 smaller than 0.99 mgL^{-1} from SC2, which reinforces the idea that there are anammox process in SC2.
Key Words: aquaculture, reicrculating system, intensive, anammox.

Riassunto. Utilizzando anammox processo a processo di filtrazione biologica dell`aqua di un sistema di ricircolo di acquacoltura rappresenta una alternativa al processo di nitrificazione / denitrificazione che puo ridurre i costi di produzione, in particolare energia, sostituendo aerobici processi biologici che richiedono un elevato consumo di ossigeno, erogata attraverso l`ulteriore aggiunta ottimale di biofiltro (decomposizione $N\text{-}NH_4$ i $N\text{-}NO_2$). Lo scopo di questo lavoro e quello di evidenziare i primi passi nel controllo dei composti azotati in sistemi di ricircolo di acquacoltura che utilizzano processi anammox. I dati riportati sono ottenuti dopo 78 giorni di ricerca (esperimento ancora in corso), i risultati sono incoraggianti, registrando un efficienza di rimozione di ammonio del 77% al 32% in SC1 e SC2.nel frattempo, e aumentato in nitriti SC1 e una diminuzione in SC2, dimonstrando che ci sono tutti i presupposti per processo di lavoro anammox in SC2 e piu avanyato che in SC1. Tasso medio di consumo di ossigeno era di 0,73 mgL^{-1} SC1 meno di 0,99 mgL^{-1} in SC2, che rafforza l`idea che ci processo anammox in SC2.
Parole Chiave: acquacoltura, sistema di ricircolo, intensivo, anammox.

Rezumat. Utilizarea procesului anammox la filtrarea biologică a apei tehnologice din cadrul unui sistem recirculant de acvacultură reprezintă o alternativă a procesului de nitrificare/denitrificare care poate determina reducerea costurilor de producţie, în special cele energetice, prin înlocuirea proceselor biologice aerobe, care necesită consumuri mari de oxigen, asigurate prin intermediul adiţiei suplimentare, pentru funcţionarea optimă a biofiltrului (descompunerea $N\text{-}NH_4$ şi $N\text{-}NO_2$). Scopul acestei lucrări este de a pune în evidenţa primii paşi în ceea ce priveşte controlul compuşilor de azot din cadrul sistemelor recirculante de acvacultură cu ajutorul procesului anammox. Datele prezentate sunt obţinute după o perioadă de 78 de zile de cercetare (experimentul continuă încă), rezultatele fiind încurajatoare, înregistrându-se o eficienţă a îndepărtării amoniului de 77% în SC1 şi de 32% în SC2. În acelaşi timp nitriţii au înregistrat o creştere în cadrul SC1 şi o scădere în SC2, existând toate premizele care arată că în SC2 activitatea procesului anammox să fie mai înaintată decât în SC1. Media ratei consumului de oxigen a fost de 0,73 mgL^{-1} în SC1 mai mică decât 0,99 mgL^{-1} din SC2, lucru ce întăreşte ideea existenţei procesului anammox în SC2.
Cuvinte cheie: acvacultură, sistem recirculant, intensiv, anammox.

Introduction. Nitrogen management and its cycle in water play an important role for many social, pro-environmental, agricultural or industrial activities (Szabo et al 2010; Vincze et al 2011), such as recirculating aquaculture.

A high removal efficiency of nitrogen compounds, and in particular total amount of ammonia nitrogen, from recirculating aquaculture systems causes optimizing and developing fish intensive rearing technology expressed by practicing a intensive high degree when is reared. In most intensive fish farms, removal of production limiting factor, total ammonia nitrogen (or rather nitrogen compounds), is achieved through a biofilter based on nitrification and denitrification processes (Losordo et al 1992; Losordo et al 1994; Kamstra et al 1998; Gilmore et al 1999). However, these aerobic processes require large amounts of oxygen which translate into higher operating costs, especially energy.

With the discovery of a new innovative technological process, anaerobic ammonium oxidation (anammox) used for biological filtration of wastewater (Strous et al 1997; Strous et al 1998; Strous et al 1999; van Dongen et al 2001), this process can be implemented in intensive system for fish rearing, in the biological filtration of technological water to ensure two major advantages: efficient removal of nitrogen compounds and reducing operating expenses of recirculating aquaculture systems (Tal et al 2004; Tal et al 2006).

Anammox activity was highlighted in a variety of biological filters, e.g. rotating biological contactor (Tal et al 2004), sequential biological reactor (Strous et al 1997), which suggests that anammox bacteria can achieve reasonable populations to make this process working. In recirculating aquaculture systems an opportunity to complete nitrogen cycle without achieving denitrification is integration of anaerobic ammonium oxidation (anammox) process (Tal et al 2006). An important aspect, which can be a limitation of this process type is the long stabilization period of biological filter (to form biofilm) for anaerobic ammonium oxidation, some few months after inoculation such bacteria.

Aim of this paper is to highlight the fist steps in the control of nitrogen compounds through anammox process in recirculating aquaculture systems.

Material and Method. Research take place at the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture from Galați (experiment is still ongoing), the data presented in this paper were obtained from 14 August to 31 October 2012.

The research focused on two different rearins systems SC1 (rearing system 1 – see Figure 1) and SC2 (rearing system 2 – see Figure 2). They started in august 2012, at wich time biological filters from both systems were inoculated with anammox bacteria. The two rearing systems functioning for a period of 20 days, without fish, to allow formation of bacterial population in the filter. After the 20 days of operation, the two rearing systems were populated with tilapia one age old, at a density 21 kgm^{-3} in SC1 (51 pieces with 221 g average weight) and 12 kgm^{-3} in SC2 (8 pieces with 196 g average weight).

The two rearing systems consist of:

Rearing system 1 (SC1): rearing tank - Ewos type (1.4 x 1.4 x 0.4m) with a volume of 530L, Nocchi MCX 120/60m pump and biological filter (who has and the role of mechanical filter) submerged, cylindrical (Φ = 54 cm; h = 74 cm), with a volume of 170L and a 100L volume of bactoballs. Water circulation in the filter is upward and flow of the rearing system is 327 Lh^{-1}.

Rearing system 2 (SC2): rearing tank – aquarium type (0,8 x 0,4 x 0,65m) with 130L water volume and a filter system Tetra EX 700 type formed by mechanical filter – sponge, chemical filter – charcoal and biological filter – ceramic rings and bioballs. Volume of filter material is 1,3L. water circulation in the filter is also upward with a flow of 700 Lh^{-1}.

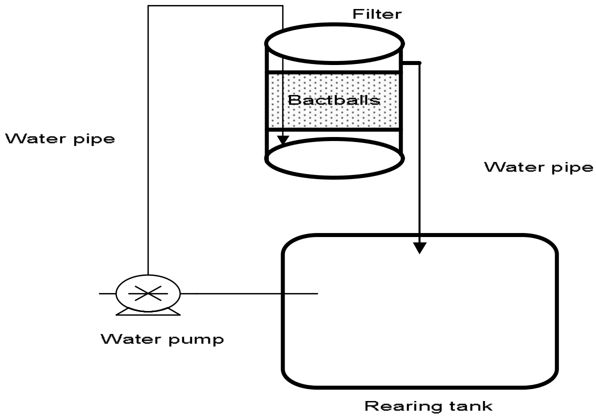

Figure 1. Rearing system 1 (SC1).

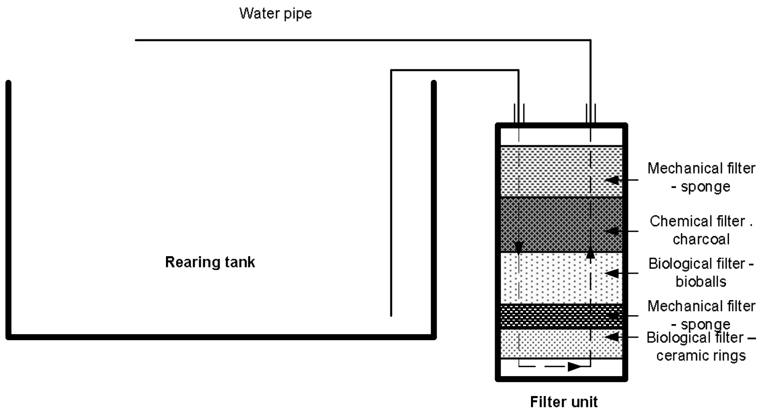

Figure 2. Rearing system 2 (SC2).

Physical-chemical parameters monitored were temperature and dissolved oxygen from water using Hach-Lange HQ30D device, pH, and nitrites, nitrates, ammonia, ammonium and total nitrogen using the Hach-Lange DR2800 spectrophotometer and LCK 138, LCK 304, LCK 339, LCK 341 kits. To assess the performance of both filtration system, in terms of control of nitrogen compounds, samples were taken both water inlet and outlet in and from filtration system. Filtration systems efficiency for the nitrogen compounds control and highlight the presence of anammox process (growth of inoculated bacteria) is determined primarily by ammonium and nitrite removal efficiency. Also, was evaluated total nitrogen removal rate, oxygen consumption rate to see if nitrification take place or not specially that .

The hydraulic retention time of the two filter was 0.5 h in case of SC1 and 0.03 h in case of SC2.

Results and Discussion. The presence of anammox bacteria in aquaculture biofilters can be very beneficial because can simultaneously remove both ammonium and nitrites with reduced oxygen consumption. After analyzing the data obtained until now in the research, distinguishes the differences between the two rearing systems, SC1 and SC2, the only parameters registering similar values being dissolved oxygen and pH of the water (see Table 1). Water pH registered an appropriate average in the two systems, approx. 7.8 upH (Table 1), registering a low decrease between effluent and influent water in filter system. These values fall into optimal range necessary for anammox bacteria development, these being inhibited at a pH less 6.5 and greater than 8.5 upH (Strous et al 1998; Egli et al 2001).

Table 1

Average values of water physical-chemical parameters from the two rearing systems, both water influent and effluent from filter system

Parameter	SC1			SC2		
	Average in	*Average out*	*Removal rate (%)*	*Average in*	*Average out*	*Removal rate (%)*
pH (upH)	7.89±0.1*	7.85±0.1	-	7.87±0.1	7.83±0.1	-
DO (mgL^{-1})	2.61±0.9	1.88±0.8	-	3.20±1.5	2.88±1.9	-
$N\text{-}NH_4$ (mgL^{-1})	5.10±2.4	0.85±0.3	77	8.26±12.8	7.58±12.2	32
$N\text{-}NH_3$ (mgL^{-1})	0.36±0.1	0.29±0.1	15	1.00±0.2	0.93±0.2	6
$N\text{-}NO_2$ (mgL^{-1})	0.33±0.1	0.37±0.1	-	0.84±1.4	0.75±1.2	6
$N\text{-}NO_3$ (mgL^{-1})	3.48±1.8	3.91±1.0	-	17.1±9.6	16.1±8.7	3
Nt (mgL^{-1})	8.87±3.6	7.01±1.7	14	24.84±4.4	24.07±4.1	3

DO – disolved oxygen; * - standard deviation.

Average water temperature was 24°C in both rearing systems, ranging between 19.8 and 29.3°C in SC1 and between 19.8 and 28.5°C in SC2. According to the literature (Egli et al 2001; Dapena-Mora et al 2004; Hao et al 2002) anammox bacteria registered an activity at 11°C that represent 24% of the maximum recorded at 37°C. Most authors say that in the temperature gap between 5 and 40°C is observed an activity of anammox bacteria.

The total nitrogen registered ranged from 18.4 to 30 mgL^{-1} in influent of filter system and between 18 si 28.2 mgL^{-1} in effluent (Table 1), in case of SC2, values significantly higher that those recorded in SC1, were ranged between 4.01 si 15 mgL^{-1}, in influent of filter system and, and between 4.58 si 9 mgL^{-1}, in effluent (Table 1). Practical all values of nitrogen compounds were higher in SC2 than SC1. Meanwhile, following water filtration has been observed an increase in nitrites and nitrates in SC1, and a decrease in SC2. This increase in SC1 is due to both an incomplete nitrification and higher development of heterotrophic bacteria that have occurred because of high organic waste from water coupled with lack of mechanical filtration prior biofiltration.

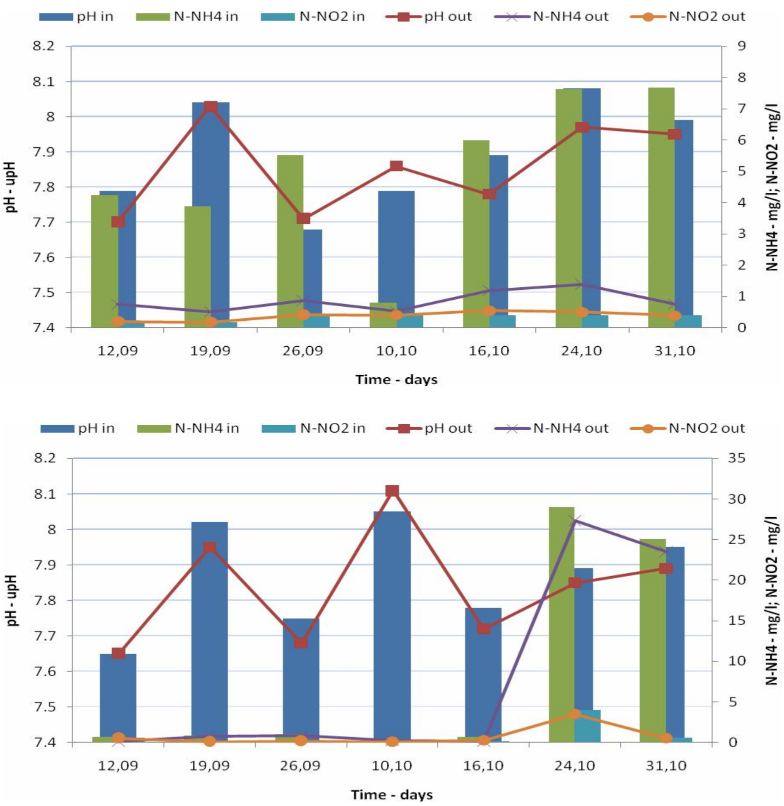

Figure 3. Ammonium, nitrites and pH variation in the two rearing systems, both in influent and effluent from filter system.

Dissolved oxygen values registered in SC1 were lower than those registered in SC2 (Table 1) where, most values were higher then 2 mgL^{-1}, threshold value sustained by several authors (Dapena-Mora et al 2004; Van de Graaf et al 1996), over which anammox process is inhibited. However, it is known that in the biofilters, especially for those submerged, exist anoxic zones where are formed anaerobic bacteria population. On the other hand, many authors (Dapena-Mora et al 2004) believe that to obtain better results after biological filtration of wastewater, before anammox process must achieve a partial nitrification, obtaining NO_2, because the nitrites is the substrate for anammox bacteria growth. Thus, this aspect must be considered as the nitrification process is inhibited when dissolved oxygen drops below 1.5 mgL^{-1}.

In Figure 4 we can see that in SC1 oxygen consumption rate ranged between 0.07 and 1.97 mgL^{-1}, and in SC2 ranged between 0.64 and 1.7 mgL^{-1}, values quite small compared to demand of 4.57 grams of oxygen for the oxidation of one gram of total ammonia nitrogen (USEPA 1975). This confirm that, besides nitrification process in the two rearing systems begin to take place and anammox process, its activity being higher in SC2 that in SC1. Low activity of the process is based on lengthy growth of these bacteria and temperature decrease that himself causes a slow growth rate of these bacteria (Egli et al 2001; Dapena-Mora et al 2004).

Specific growth rate in the number of bacteria on the substrate, according to the literature, is about 0.0012/h – 0.0027/h (Hao et al 2002) and 0.016/h (Isaka et al 2006). These growth rates correspond to a doubling of the number of bacteria in a period of 25, 11 and 1.8 days.

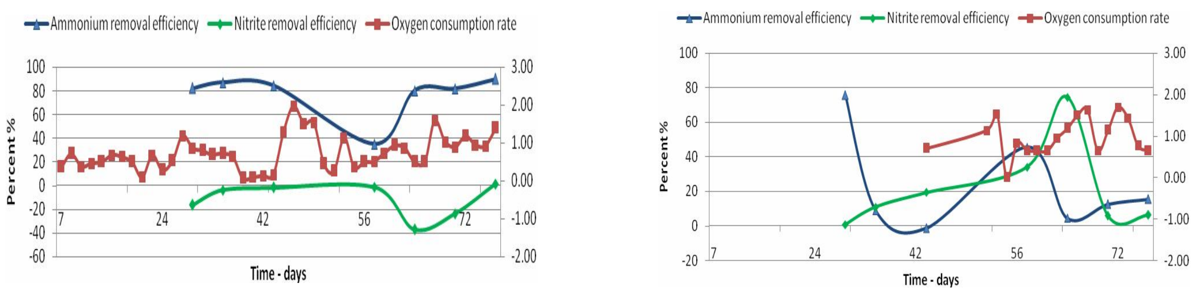

Figure 4. Efficiency removal of ammonium and nitrites and oxygen consumption rate registered in the two rearing systems.

Ammonium removal efficiency from SC1 was 77%, registering encouraging values between 80 and 90%, except a value of 35% perhaps obtained in the moment of extinction of bacteria population and the formation of a new one. Instead nitrites were up from 0.33 to 0.37 mgL^{-1} (Table 1), which shows that in biofilter still prevails nitrification process. In SC2 ammonium removal efficiency was 32%, significantly lower that SC1, and nitrites registered a decrease from 0.84 to 0.75 mgL^{-1} (Table 1). This in conjunction with oxygen consumption and nitrates conversion shows that in this biofilters begin activity of anammox process.

Strous et al (1997), after 150 days of researching two fluidized bed reactors inoculated with anammox bacteria, have achieved an ammonium removal efficiency of 82%, and nitrites by 99%. Tal et al (2006) has obtained a reduction of nitrogen compounds from technological water, 65% through denitrification process and 12% through anammox process. His conclusion was that integration of aerobic and anaerobic microbial processes, one after the other, determines possibility of applying high density rearing and achieving higher production.

Conclusions. Although performance on the control of nitrogen compounds are higher in SC1 than SC2, anammox activity being at the beginning, stands a plus for SC2, while in SC1 is more visible nitrification process.

We can conclude that drastically limiting the amount of dissolved oxygen entering in the filtration system, under 1.5 – 2 mgL^{-1}, makes that nitrites no longer be produced and thus no longer exist the most important substrate for anammox development.

Another important point obtained from research until now is the need of a mechanical filtration of water before biological filtration, to prevent especially suspended solids to cover bactoballs and cause higher growth of heterotrophic bacteria which shows very high growth rate and who lead to high loading of biofilter (clogging the biofilter) and an inhibition of nitrification and anammmox process.

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