AQUACULTURE - A BIOTECHNOLOGY IN PROGRESS. 1989
N.De Pauw, E. Jaspers, H. Ackefors, N. Wilkins (Eds)
European Aquaculture Society, Bredene, Belgium

Practical basis of nitrification in aquaculture waste-water

P. Poquillon and J. Petit

Laboratoire de Physiologie des Poissons, INRA
Campus de Beaulieu, F-35042 Rennes Cédex, France

Abstract

Nitrification is an important process in the chain of water treatment procedures required to operate a recycling unit for fish culture. Experiments were performed with reconstituted fish breeding effluent on an experimental submerged fixed-bed reactor under pressure. The filter medium consisted of a calcinid clay (Biogrog, 1.2-1.5mm) with a specific surface area ($10^6 m^2.m^{-3}$). The treatment efficiency reached 9.6kgN-$NH_4.m^{-3}.d^{-1}$ at 20°C and 4.9kgN-$NH_4.m^{-3}.d^{-1}$ at 13°C for hydraulic load of $644 m^3.m^{-2}.d^{-1}$. Reduction of the ammonia amount ceased when the dissolved oxygen concentration was equal or inferior to 3mg$0_2.l^{-1}$. The nitrification's natural starting up phase lasted 42 days. This process could be reduced to 24 days by injection of commercial lyophilized bacteria into the filter.

KEYWORDS: Nitrification, Wastewater, Water quality, Purification.

Introduction

Fish culture in recycled water can considerably reduce water requirements and possibly calories. It is especially suited for accelerating the hatching of eggs and the growth of fry, breeding for warm-water fish (*Anguilla*, tilapia, etc.), and for stocking or breeding fish in towns, to replace natural spawning.

Nitrification is an important component of the water treatment process of a recycling unit for fish breeding. Through nitrification, toxic ammonia is oxidized to nitrate which is less toxic for fish. Bacterial nitrification is a biological phenomenon of which the kinetics depend on many factors and particularly on available substrates. A number of authors (Roques et al., 1976; Grasmick et al., 1979; Roques et al., 1982; Gonenc and Harremoes, 1985) have proposed models of its kinetics. We find that Monod model (Sharma and Ahlert, 1977) is a special case of such models. (Fig. 1). On the basis of this formula, Faup et al. (1982) found that the ammonia concentration of the effluent had a much altered K_s value (1.5mgN-$NH_4.l^{-1}$). This was true with industrial waste water. But in aquaculture waste water the amount of ammonia was not very much altered. Many authors (Csavas and Varadi, 1981; Kujal, 1981; Scott and Allard, 1984; Wickins, 1985) studied bacterial nitrification in aquaculture situations but some did not make allowance for all the parameters which modify nitrification kinetics, in

$$-\frac{dS_n}{dt} = Y_n \times \mu_{max} \times x_n \times \frac{S_n}{K_s + S_n}$$

Fig. 1. Monod equation. (S) Substrate concentration, mass/volume; (t) time; (Y) yield coefficient, mass of organism grown/mass of substrate utilized; (μ_{max}) maximum specific growth rate, $time^{-1}$; (X) concentration of microorganisms, mass/volume; (K_s) Monod half velocity constant, mass/volume; (n) a specific type of microorganism. (Sharma and Ahlert, 1977).

particular the amount of dissolved oxygen (Sharma and Ahlert, 1977; Stenstrom and Poduska, 1980), pH (Martin, 1979; Quinlan, 1984), temperature, BOD (Paolini and Variali, 1982) and phosphate levels.

Our approach consisted in studying biological depuration in conditions as near as possible to aquaculture. A purification technique, then a filter medium were chosen, and experiments were carried out to determine the treatment efficiency of the filter medium, the oxygen requirement and the means of commencing nitrification in an environment comparable to the exploitation environment.

Materials and methods

Choice of a purification technique

Among different techniques available, and which are utilized in fish-farm waste-water treatment, the pressurized and submerged fixed bed reactor is, for a given nitrification efficiency, more compact than trickling filters or a rotating disc system. Its disadvantage is that the oxygen necessary for nitrification is brought on by water and not by air as in the other process. We chose the technique of pressurized and submerged fixed bed reactor in our studies.

Choice of the filter medium

In the literature, the best media (measured by $kgN\text{-}NH_4\text{+}NH_3$ epurated.m^{-3}.d^{-1}) for nitrification on submerged filters were biolite (Faup et al., 1982), then pouzzolane (Richard et al., 1979), marble (Kowalski and Lewandowski, 1983), gravel (Kowalski and Lewandowski, 1983; Wickins, 1985), foam polystyrene (Kujal, 1981), and plastic (Boller and Gujer, 1986; Hall, 1986).

We chose Biogrog, manufactured by the Société Argiles et Minéraux AGS (Clerac, 17270 Montguyon, France). Biogrog is a calcined clay. It is made by cooking and milling kaolinite. It is very resistant to abrasion and does not disaggregate after filter washing. It does not need to be replaced or regenerated.

Its physical characteristics are:

granulometry:1.2-1.5mm
apparent density:1.5t.m^{-3}
porosity:0.3-0.4m^3.m^{-3}
specific area:$10^6 m^2.m^{-3}$

Biogrog grains (Photo 1) are variable. Their area is uneven and there are many holes on the surface; this explains the importance of the specific area. Nitrification bacteria (Photo 2) settled in the holes and fracture area of the Biogrog grains.

Experiments

Pilot unit

Experiments were carried out on a pilot unit (Fig. 2) consisting of a biological filter (height 2m, diameter 160mm) with seven sampling points, an oxygenator, a thermoregulation unit and a 250 l tank. The height of the Biogrog filterbed was 1.5m. The flow in the biofilter can be ascending or descending.

Photo 1. Biogrog grain, 1mm;(x32).

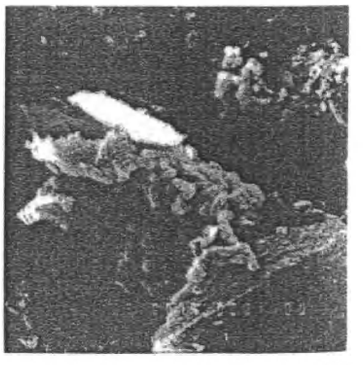

Photo 2. Nitrification bacteria, 1µm;(x10 000).

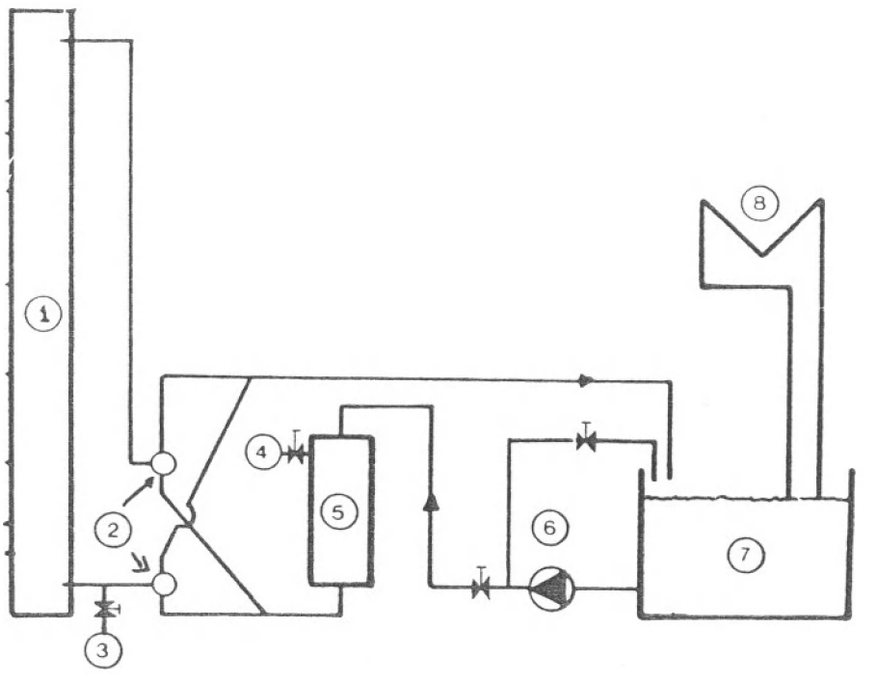

Fig. 2. Diagram of pilot unit. (1) Biological filter (2m height, 100mm diameter); (2) three-ways valve; (3) washing water and air inlet; (4) oxygen inlet; (5) oxygenator; (6) pump and bypass; (7) 250 l tank; (8) thermoregulation unit.

The temperature was controlled, and O_2, pH, NH_4, NO_2 were measured.

Experimental conditions were as follows:

- temperature 18±1°C

- pH 7-7.2 UpH

- dissolved oxygen >5mgO_2.l^{-1}

Reconstituted fish breeding effluent

In order to limit the parameters which might affect the experiments, we worked with a reconstituted effluent having a constant composition. de Kinkelin et al. (1986) reported that trout nitrogenous excreta consist of 80% ammonia and 20% faeces and urea. Therefore, the reconstituted effluent was composed of potable water and nitrogen (80% in ammonia form and 20% in artificial faeces form). Ammonia was injected as $(NH_4)_2SO_4$. Artificial faeces were specially made by the experimental food factory of INRA according to Luquet (pers. comm.).

Start-up of nitrification

Natural start-up and start-up by injection of commercial lyophilized bacteria (Bioarium manufactured by Lobial SA, 53000 Laval, France) were studied.

The pilot unit was washed with a solution of hydrochloric acid, then the filter was filled with Biogrog up to 1.5m, and the whole unit was rinsed with potable water for 7 days. The pollutant [0.8g $(NH_4)_2SO_4$ and 8.4g reconstituted faeces] and, if required, Bioarium were injected, then ammonia and nitrite concentrations in the tank were recorded each day. When these parameters were stable the filter was seeded. Samples (5g) of Biogrog were collected at the top, the middle, and the bottom of the filter on days 1, 28, and 40 of natural nitrification starting up.

Sampling of the filter medium were kept at -20°C in a mixture of 50% water and 50% glycerol. Observation of bacteria on the medium was made, after fixation, with a scanning electron microscope. Samples were fixed according to Lafrance et al. (1983). Then they were dried in a critical point desiccator Balzer, covered with gold and three grains of each sample were observed with a scanning electron microscope Jeol JSM35 of the Faculté des Sciences de Rennes. The magnification was up to x 20 000. Observations were qualitative only.

Treatment efficiency

Biogrog efficiency was determined when nitrification was started. The flow rate in a filter descended but oxygen and pH were not limiting. Retention time of the treated medium was from 200s to 250s. Pollutant was injected once a day and efficiency was calculated from the amount of ammonia oxidized between the entry and the exit of the filter. Efficiency was expressed in kg $N\text{-}NH_4$ oxidized per m^3 Biogrog per day.

Influence of dissolved oxygen

In the experiments we wished to determine the dissolved oxygen available for nitrification under aquacultural conditions. Oxygen injection was stopped after injection of the pollutant and the evolution of dissolved oxygen, ammonia and nitrite in the tank of the pilot unit was recorded.

Results and discussion

Start-up of nitrification

Natural nitrification start-up was slow (Koiller and Avtailion, 1985). It lasted 42 days (Fig. 3). Injection of Bioarium reduced it to 24 days (Fig. 4). The effect of Bioarium was fast. Twenty-four h after injection we observed the appearance of nitrites and a decrease of ammonia. But 3 days after injection ammonia concentration increased. This nitrogen production could have resulted from bacterial lysis. Bernard and Chabot (1986) have shown, for example, that lyophilized bacteria cannot attach themselves to the filter and die. However when injection of Bioarium was stopped, ammonia decreased 5 days after the last injection. Previous studies (Lésel and Leffemberg, 1977; Bower and Turner, 1984; Bernard and Chabot, 1986) did not report acceleration of the process with injection of lyophilized bacteria into the filter. Sutterlin et al. (1984) and Scott and Allard (1984) used lyophilized bacteria to reduce nitrification starting up.

The observations with the scanning electron microscope showed the evolution of the organic material on the Biogrog surface. Early in nitrification start up (day 1), organisms were scarce and located in sheltered areas. We observed bacteria and fungal spores (Photo 3). Later (day 28) the number of bacteria seemed to increase, amoebae (Photo 4) and eucaryotic fungi appeared. When the filter was seeded (day 40) ;there were mainly only two species of bacteria present (Photo 2 and 5). But we could not identify them because their morphology depends on their environment (Johnson and Sieburth, 1976).

In fish breeding, filters must be disinfected after each breeding cycle and the nitrification restart is expensive in terms of time and energy. The use of Bioarium reduces the duration and the cost of this operation.

Two other techniques to accelerate nitrification start-up will be tested in our next experiment. These are doping with metal supply (iron and copper) and preseeding the Biogrog.

Treatment efficiency

Biogrog efficiency was more than 9kg $N\text{-}NH_4.m^{-3}.d^{-1}$ (Table I). We obtained 4.9kg $N\text{-}NH_4.m^{-3}.d^{-1}$ when these results were extrapolated to 13°C (according to the formula reported by Martin, 1979). These results were better than those observed previously by Faup et al. (1982) on biolite ($1.1kgN\text{-}NH_4m^{-3}.d^{-1}$). This difference could be explained by the greatest specific area of Biogrog and by the experimental conditions. These results were obtained with sequential injection of pollutant. Biogrog efficiency will be tested with a continuous injection of pollutant and with a weaker hydraulic burden. Indeed the hydraulic burden utilized in our experimentations could be employed in fish breeding only if there was a very efficient decantor.

Influence of dissolved oxygen

Fig. 5 shows that if the injection of oxygen is stopped, the reduction of total ammonia

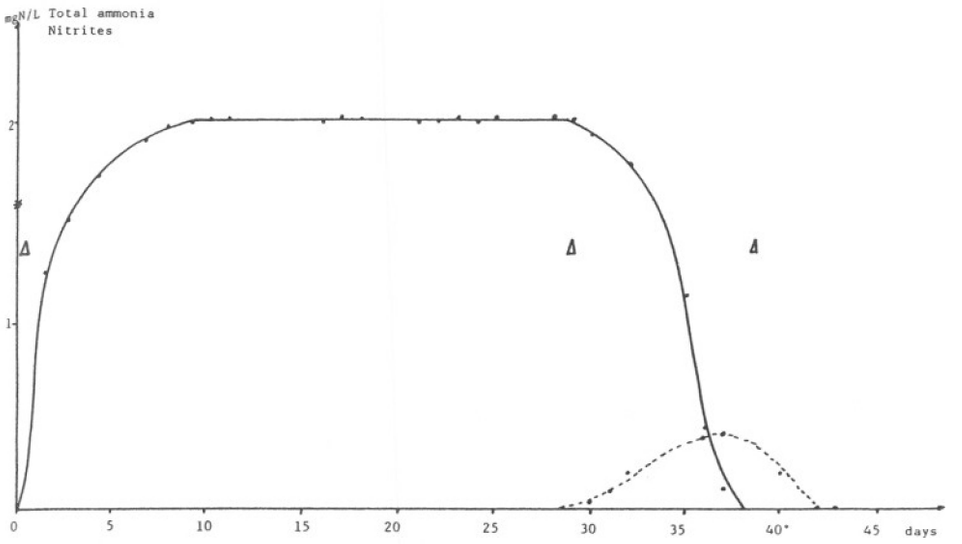

Fig. 3. Natural start-up of nitrification. (*) Pollutant injection; (Δ) Biogrog sampling; (——) total ammonia in tank; (....) nitrites in tank.

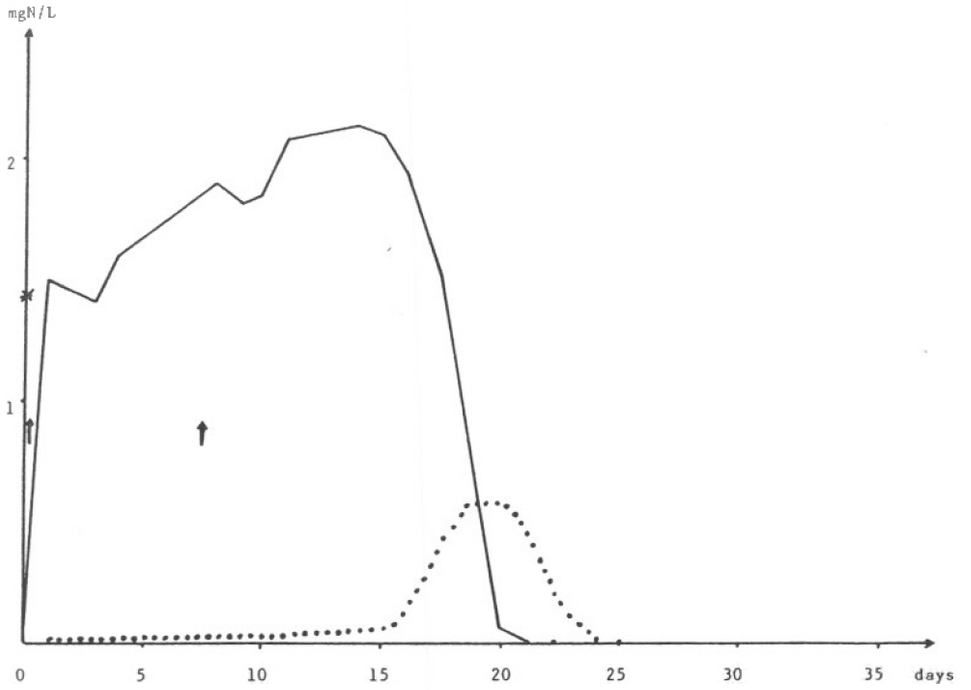

Fig. 4. Start-up with injection of Bioarium. (*) Pollutant injection; (↑) Bioarium injection; (–) total ammonia in tank; (. . .) nitrites in tank.

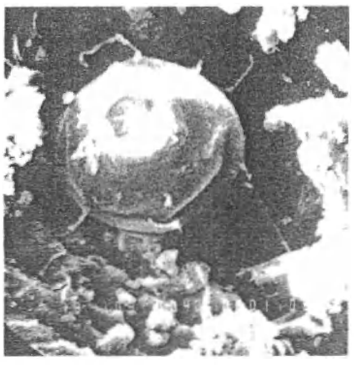

Photo 3. Mushroom spore, 1µm;(x10 000).

Photo 4. Amoeba, 1µm;(x6 000).

concentration decreases when the amount of dissolved oxygen is less than $4mgO_2.l^{-1}$, and stops when it is less than $3mgO_2.l^{-1}$. As soon as oxygen was injected the process of nitrification

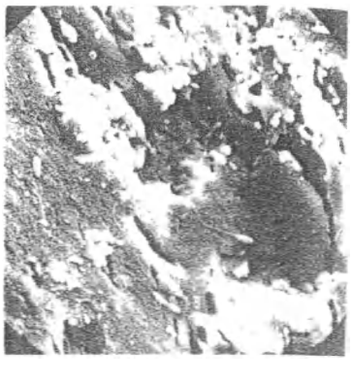

Photo 5. Nitrification bacteria, 1µm;(x6 000).

restarted and recovered its maximal efficiency after 48h. Oxidizing of 1g ammonia required 4.5g oxygen. So, nitrification depended on available oxygen. According to Sharma and Ahlert (1977) there was a threshold value of dissolved oxygen below which there was no bacterial growth. However according to Quinlan (1984) and Stenstrom and Poduska (1980) the influence of dissolved oxygen on nitrification followed a Michaelis law.

The most probable explanation of our results was that when dissolved oxygen decreased below $4mgO_2.l^{-1}$, nitrification was very reduced but not stopped. On the other hand, ammonification appeared and resulted in a stagnation of ammonia and dissolved oxygen in the unit.

So the oxygen quantity available for bacterial epuration of aquaculture waste water is the

Table I. Efficiency of Biogrog

N_o[a] ($mg.l^{-1}$)	Temp. (°C)	Hydraulic load ($m^3.m^{-2}.d^{-1}$)	Retention time	Efficiency ($kgN\text{-}NH_4.m^{-3}.d^{-1}$)
1.5-2.5	20±1	515	4min 10s	9.18±2.44
0.3-0.5	20±1	644	3min 20s	9.60±2.14

[a]N_o: amount of total ammonia in filter entry.
Experimental filter: height 2m, diameter 160mm.
Biogrog: height 1.5m.

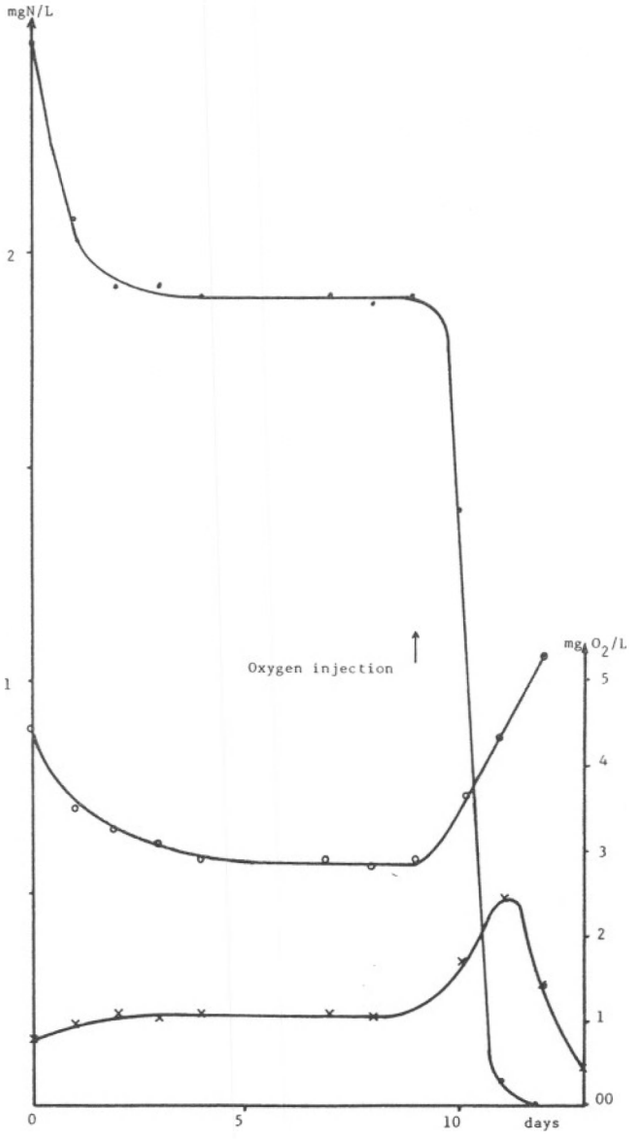

Fig. 5. Influence of dissolved oxygen on nitrification. (o) Dissolved oxygen in tank; (•) total ammonia in tank; (*) nitrites in tank.

dissolved oxygen quantity int he water less $4mgO_2.l^{-1}$.

Conclusion

Biogrog appeared to be an excellent medium for nitrification in a submerged filter. Its high specific area increased its efficiency therefore reducing filter litter. These experiments were performed with a single dose pollutant loading. In our next experiments we will test biogrog with continuous injection of pollutant (ammonia and reconstituted faeces) and with variations of loading.

In the present experiments, the oxygen quantity available for nitrification was found to be the dissolved oxygen quantity in the water, less $4mgO_2.l^{-1}$. this means that, if the amount of dissolved oxygen is $15mgO_2.l^{-1}$, the oxygen quantity available for nitrification is $11mgO_2.l^{-1}$.

The start-up of nitrification could be shortened to 24 days by the injection of Bioarium. This reduces filter starting costs and could reduce the effects of an accidental pollution of the biological filter.

References

Bernard S.C. and M. Chabot. 1986. Les activateurs biologiques et leur utilisations dans les eaux résiduaires. L'eau, l'industrie, les nuisances 101:47-48.

Boller M. and W. Gujer. 1986. Nitrification in tertiary trickling filters followed by deep-bed filters. Water Res. 20(11):1363-1373.

Bower C.E. and D.T. Turner. 1984. Evaluation of two commercial nitrification accelerators in closed seawater culture systems. Aquaculture 41:155-159.

Csavas I. and L. Varadi. 1981. Design and operation of a large scale experimental recycling system heated with geothermal energy at the fish culture research institute, Szarvas, Hungary. FAO-EIFAC Symp. Stavanger 2:445-454.

de Kinkelin P., C. Michel, and P. Guittino, 1986. Précis de pathologie des poissons. INRA-OIE, Paris. 348 p.

Faup G.M., A. Leprince, and M. Pannier. 1982. Biological nitrification in a up flow fixed bed reactor (UFBR) Water Sci. Tech. 14:795-810.

Gonenc E. and P. Harremoes. 1985. Nitrification in rotating dis systems. I. Criteria for transition from oxygen to ammonia rate limitation. Water Res. 19(9)1119-1127.

Grasmick A., S. Elmaleh, and R. Ben Aim. 1979. Théorie de l'épuration par filtration biologique immergée. Water Res. 13:1134-1137.

Hall D.J. 1986. Nitrification options evaluated using pilot filters. Water Pollut. Contr. 431-439.

Johnson P.W. and J.McSieburth. 1976. In situ morphology on nitrifying-like bacteria in aquaculture systems. Appl. Environ. Microbiol. 31(3):423-432.

Koiller M. and R.R. Avtalion. 1985. A laboratory scale recycling water unit for tilapia breeding. Aquacult. Eng. 4(4):235-246.

Kowalski E. and Z. Lewandowski. 1983. Nitrification process in a packed bed reactor with chemically active bed. Water Res. 17:157-160.

Kujal B. 1981. Pre-treatment of water for intensive aquaculture by filters with invented water flow. FAO-EIFAC Symp. Stavanger 1:383-394.

Lafrance P., M. Mazet and D. Villessot. 1983. Croissance bactérienne sur charbon actif granulé, investigation au microscope à balayage. Water Res. 17(10):1467-1470.

Lésel R. and F. Leffemberg. 1977. Etude de la nitrification dans les circuits d'eau recyclée. I. Oxydation des nitrites. Bull. Cent. Etud. Rech. Sci. Biarritz 11(4):441-459.

Martin G. 1979. Le problème de l'azote dans l'eau. Technique et Documentation Lavoisier, Paris. 279 p.

Paolini A.E. and G. Variali. 1982. Kinetic consideration on the performance of activated sludge reactors and rotating biological contactors. Water Res. 16(2):155-160.

Quinlan A.V. 1984. Prediction of the optimum pH for ammonia N oxidation by *Nitrosomonas europaea* in well aerated natural and domestic waste water. Water Res. 18(5):561-566.

Richard Y., L. Brener, and G. Martin. 1979. Nitrification des eaux souterraines et des eaux clarfiées. TSM L'eau 74(3):157-163.

Roques H., L. Masbermat, and J. Tsacoyannis. 1976. Essais de mise au point d'un modèle général de croissance des microorganismes englobant les phénomènes de nitrification et la métabolisation des matières hydrocarbonées. Water Res. 10(3):265-270.

Roques H., S. Yue, S. Saipanich, and B. Capdeville. 1982. Faut-il abandonner le formalisme de MONOD pour la modélisation des processur par voie biologique. Water Res. 16:839-847.

Scott K.R. and L. Allard. 1984. A four-tank water recirculation system with a hydrocyclone prefilter and a single water reconditioning unit. Prog. Fish-Cult. 64(4):254-261.

Sharma B. and R.C. Ahlert. 1977. Nitrification and nitrogen removal. Water Res. 11:897-925.

Stenstrom M.K. and R.A. Poduska. 1980. The effect

of dissolved oxygen concentration on nitrification. Water Res. 14:643-649.

Stutterlin A.M., C.Y. Couturier, and T. Devereaux. 1984. A recirculation system using ozone for the culture of Atlantic salmon. Prog. Fish - Cult. 46(4):239-244.

Wickins J.F. 1985. Ammonia production and oxidation during the culture of marine prawns and lobsters in laboratory recirculation systems. Aquacult. Eng. 4(3):155-174.