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Improved performance of an intensive rotifer culture system by using a nitrifying inoculum (ABIL)

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Abstract

A dense nitrifying culture (ABIL) has been examined for its capacity to stimulate rotifer growth in a labscale culture system. The nitrifiers were applied in different ways. When ABIL was added directly to rotifer batch cultures, it gave rise to significantly higher population densities (factor 1.5–2.5 higher, $P < 0.05$). The nitrifiers were subsequently examined for their capacity to enhance the start-up of bioreactors, commonly installed in aquaculture rearing tanks. Of the different carrier materials used in these bioreactors, i.e. $CaCO_3$, gravel and a PVC matrix (Bionet), $CaCO_3$ gave by far the best results. In a third set of experiments, effectively nitrifying bioreactor systems were connected to rotifer culture tanks and operated over a period of up to 10 days. It was demonstrated that the ABIL inoculated $CaCO_3$-based bioreactor allowed excellent rotifer growth reaching rotifer densities up to 5500 rotifers per mL. Moreover, a new system in which the ABIL culture was recirculated through hollow fibres was developed and demonstrated to be effective for supporting rotifer growth up to 3500 rotifers per mL. Overall, the use of the dense nitrifying culture either in seed batch cultures, conventional bioreactors or hollow fibre bioreactor systems in support of rotifer cultures was demonstrated to be effective for improving the water quality and the rotifer growth.

Keywords: ABIL, *Brachionus plicatilis*, intensive, nitrifying inoculum, rotifer

Introduction

In aquaculture production systems which are not equipped with a proper water treatment system, the accumulation of nitrogen compounds is inevitable. The principal nitrogenous waste product, i.e. total ammoniacal nitrogen (TAN) can easily reach inhibitory or even toxic concentrations. Filtration systems in closed aquaculture units usually have a solid substratum to promote the growth of nitrifying bacteria and thus remove the toxic ammonia from the water. Nitrifying micro-organisms are slow-growing bacteria with doubling times from 12 to 32 h (Hovanec, Taylor, Blakis & Delong 1998). Therefore, before the start up of the culture, a pre-establishment phase, in which the nitrifying bacteria are allowed to develop, is often programmed. To decrease the length of time for establishment of an active nitrifying consortium, commercial products of nitrifying organisms adapted to such environments can be used to seed the bioreactors.

Use of recirculation technology for the production of rotifers in high-density systems has been shown to be advantageous (Suantika, Dhert, Nurhudah & Sorgeloos 2000). Efficient nitrification is essential for maintaining good water quality and thus a prerequisite for good rotifer growth in these systems.

In this study, the effect of the addition of a dense nitrifying culture, ABIL (Avecom, Germany), on the performance of a labscale intensive rotifer culture system, has been evaluated. The seeding procedure and the length of the start-up phase of the biological water treatment system (referred to later as bioreactor) have been studied for different biomass carrier materials.

Materials and methods

Nitrifying culture, ABIL (Avecom, Belgium)

The nitrifying suspension, called ABIL, is cultivated in a 500-L reactor in fed-batch mode. The nitrifying culture was obtained by gradual enrichment starting from natural surface water. The culture received a daily load of 88 g N (58.7 $g\,d^{-1}$ TAN as ammonium chloride and 29.3 $g\,d^{-1}$ NO_2-N as sodium nitrite) to support the growth of the nitrifying bacteria. The feed also contained calcium carbonate as a carrier matrix, buffer and carbon source. The pH of the reactor was controlled at 7.0 by addition of NaOH. The temperature was kept at 22–24 °C. Compressed air was used to aerate the culture at a dissolved oxygen level of 6.0 $mg\,L^{-1}$ O_2. The harvested cell suspension can be concentrated to desirable cell densities by sedimentation or centrifugation. A bacterial suspension (1 $g\,L^{-1}$ volatile suspended solids (VSS)) of this product was used as seed. The VSS content of ABIL was determined by subtracting the ash content from the total suspended solids (TSS) content. Ash content and TSS content were determined gravimetrically (APHA *et al.* 1992).

ABIL dosed to a batch rotifer culture (Fig. 1a)

In a series of separate batch tests in which rotifers were grown in 1-L vessels, the impact of ABIL given as a probiotic was tested. One day before the rotifers were introduced in the culture water, the seawater (25 $g\,L^{-1}$ salinity) was amended with a carbon source (0.08 $g\,L^{-1}$of gamma-irradiated Culture Selco) to allow bacterial activity to become established. This culture medium was incubated for 1 day at 28 °C. To test the effect of a nitrifying culture, 1.0 g of cotton (dry weight) was submerged for one day in a nitrifying culture that contained 1 $g\,L^{-1}$ VSS. After 24 h, the cotton was rinsed with tap water and suspended in the culture vessels. The culture vessel of the control treatment did not receive the ABIL inoculum on the cotton filter but was otherwise treated in the same way. Four replicates were used per treatment and the experiment was repeated four times.

Bioreactor set-up (Fig. 1b)

A series of rectangular shaped bioreactors (total volume of one tank 10 L) were used for the experiments. The bottom of each tank was equipped with a cover punched with holes that retained the carrier material in the upper part of the bioreactor. Through the cover, two airlifts were installed which provided the reactor with oxygen and allowed the aqueous phase to recirculate. The reactors were filled with 4 dm^3 of carrier material and 4 L of artificial seawater (Instant Ocean®, Aquarium System Sarrebourg, France) at 25 $g\,L^{-1}$ salinity. Three replicates were used for each treatment. All experiments were conducted at 28 °C in a temperature-controlled room. Ammonia stripping was quantified using allylthioureum (ATU; 2.5 $mg\,L^{-1}$) as the inhibitor of the nitrification process (Blum & Speece 1991). Water samples were taken daily and all analyses were carried out according to standard methods (APHA 1992). The concentrations of TAN and ($NO_3^- + NO_2^-$)-N (total oxidized nitrogen, TON) were determined by the Kjeldahl distillation method (APHA 1992). NO_2-N was measured colorimetrically according to Montgommery & Dumock (1961). The pH was determined potentiometrically with a digital portable pH meter (Knick, portamess 751). The DO was measured with a digital portable DO meter (Endress & Hauser, Belgium).

Different materials were used as biomass carriers. Calcium carbonate stones (2.0 ± 0.7 mm), gravel (16 ± 2 mm), a mixture of both types of stones and an artificial carrier material (Bionet) were tested with respect to the start-up period of the aquaculture systems. For the rotifer production periods, only the crushed calcium carbonate stones were used as biomass carrier.

During the phase prior to the introduction of the rotifers (the so-called start-up phase), tap water enriched with ammonium chloride was used in all experiments. On days 0, 3 and 7 of the experiment in which different carrier materials were tested, the bioreactors were supplemented with 20, 40 and 80 mg TAN L^{-1} respectively. Ammonia stripping was quantified during the start-up period by adding 20 and 40 mg TAN L^{-1} on day 0 and 4 respectively. Each reactor was inoculated with 25 mg VSS (volatile suspended solids) of nitrifying culture per L of bioreactor.

Integration of the bioreactor in a recirculating rotifer culture system

Bioreactor with calcium carbonate as biomass carrier material (Fig. 1b)

After the start-up period of 11 days, the water of the different bioreactors was drained out of the system

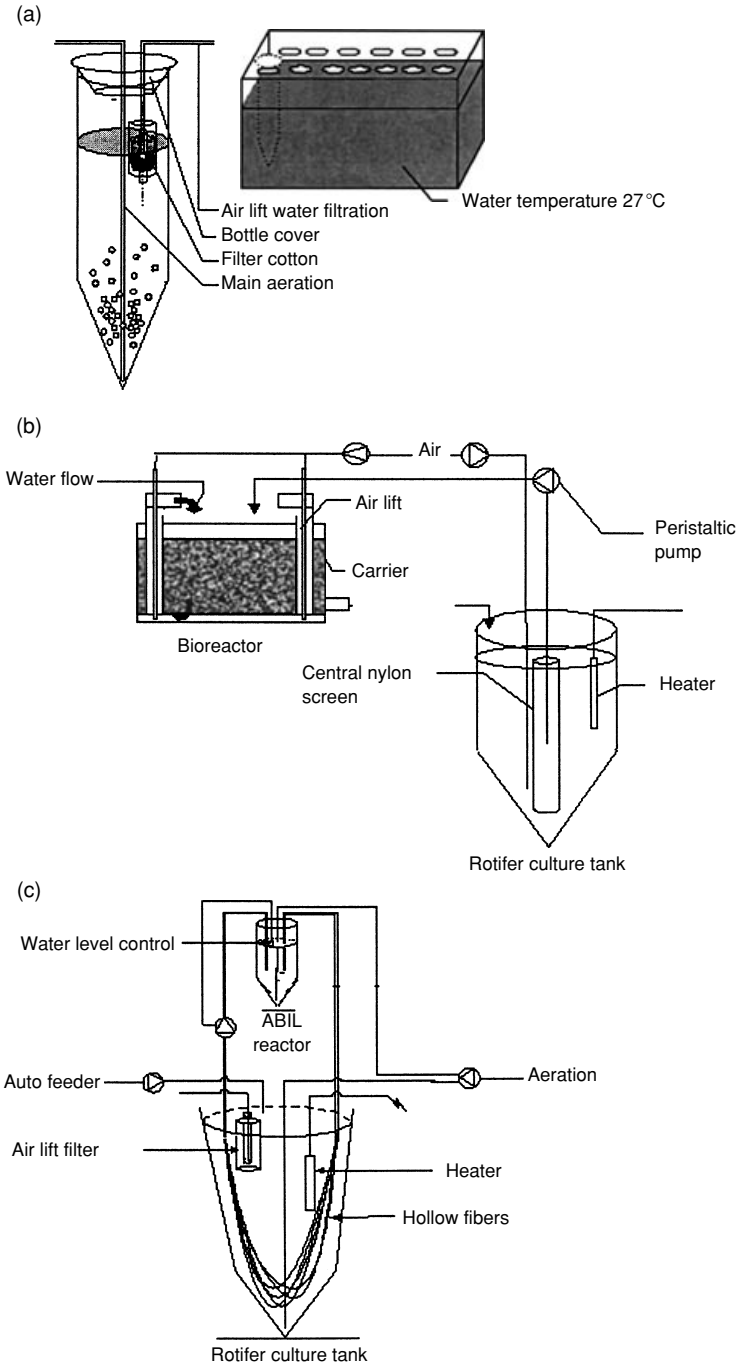

Figure 1 Scheme of the experimental set-ups used in these experiments. (a) Batch system to test ABIL for rotifers. (b) Bioreactor set-ups with four different carrier materials. (c) Integration of an ABIL reactor with hollow fibres to the rotifer culture system.

and replaced by new diluted seawater ($25\,g\,L^{-1}$ salinity). Except for the system with Bionet as the carrier material, the bioreactors were connected to a cylindro-conical culture tank of 10 L. Aeration was provided at the bottom of the tank to ensure good oxygenation and a uniform distribution. The culture-rearing tank was filled with diluted seawater ($25\,g\,L^{-1}$) and maintained at a constant temperature ($25 \pm 1\,^{\circ}C$). A central nylon screen (mesh size 33 μm) sieve was constructed in the centre of each tank to retain the rotifers in the system and to enable pumping of the culture water by means of

a peristaltic pump to the bioreactors. The water returned by gravity to the rotifer culture tanks.

Hollow fibre experiment (Fig. 1c)

Conical PVC tanks were used to culture the rotifers and were filled with 10 L of artificial seawater (25 g L^{-1} salinity). Hydrophilic hollow fibres (Accurel® type pp s6/2, produced by Membrana Accurel Systems,Obernburg, Germany) (nominal pore size 0.2 μm; wall thickness 450 μm; inner diameter 1800 μm), adjusted to pieces of 1 m and 10 fibres (total surface area = 0.078 m^2), were installed in each culture tank. The ends of the hollow fibres were connected to the ABIL reactor (1 g L^{-1} VSS). Inside the ABIL reactor, filter cotton was placed at the bottom to collect the large particles of debris. The water and the bacteria from the ABIL reactor were pumped through the hollow fibres by means of a peristaltic pump. The pump direction was controlled by a water level controller. The rotifer feed was stored in an icebox to keep the temperature below 10 °C and was dosed (15 min h^{-1}) by a peristaltic pump. The rotifer culture tanks were continuously aerated at the bottom of each culture tank. The temperature was controlled by a heater at 28 °C. At day 0, rotifers were inoculated at a density of approximately 200 rotifers per mL. Before the rotifers were inoculated, they were cleaned by rinsing them with seawater on a 50-μm sieve.

Rotifer growth

All experiments were performed with *Brachionus plicatilis* (L-strain with lorica length 180 ± 15 μm). Before the start of the experiment, the rotifer strain was kept in culture at the Laboratory of Aquaculture and Artemia Reference Center (Ghent University, Belgium), following the culture procedure described by Sorgeloos & Lavens (1996).

To monitor the culture, three samples of 500 μL were taken from the rotifer cultures using an automatic micropipette. The rotifers in each sample were killed by adding 3 drops of lugol, and counted. Empty and transparent lorica belonging to dead rotifers were not counted.

The rotifer diet consisted out of an experimental diet Culture Selco High, CSH (INVE N.V., Belgium). The suspension containing exactly the daily food ratio was kept in suspension by aeration in a 10-L food container at ambient temperature (20 ± 1 °C) for 24 h. The rotifers were fed following a standard feeding regime (Suantika, Dhert, Nurhudah & Sorgeloos 2000).

Statistical analysis

The average of each treatment was compared statistically with the corresponding control treatment of the same experimental run using the Duncan's multiple range test ($P < 0.05$). The statistical comparison was performed with SPSS for Windows release 7.5.2 (SPSS, USA).

Results

ABIL dosed to batch rotifer cultures

Four independent trials were performed in which the effect of ABIL on the rotifer population growth was tested. The addition of a nitrifying culture (ABIL) had a significantly positive effect ($P < 0.05$) on the rotifer population density. The rotifer density in the ABIL-treated culture tanks was a factor 1.3–2.6 higher than in the culture tanks of the control treatment. Figure 2 illustrates the effect of the best trial.

Start-up of marine bioreactors

Experiments were set up to determine the ability of the nitrifying inoculum to minimize the length of the start-up period for a marine nitrifying bioreactor to be used in rotifer systems. Four types of carrier materials were tested at an initial TAN concentration of

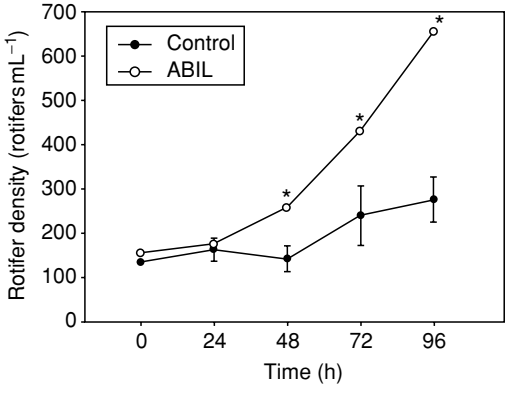

Figure 2 Effect of ABIL on the rotifer population growth in batch tests ($n = 4$; mean ± SE). *, mean significantly different from control at $P < 0.05$ (Duncan's multiple range test).

20 mg L^{-1} (Table 1). Subsequently, increasing TAN concentrations were added to the system from the moment at which the added TAN was depleted. The start-up of the bioreactors can be divided into three different periods spread over 264 h. During the first 72 h, the TAN concentrations decreased immediately after supplementation with TAN in the bioreactors with $CaCO_3$, gravel and $CaCO_3$/gravel as carrier material with similar removal rates. However, the TAN concentration in the bioreactor with the plastic carrier material (Bionet) decreased only slightly and after 72 h there was still approximately 17 L^{-1} mg TAN present in that system. Subsequently, 40 mg L^{-1} TAN was dosed and its removal monitored during the second period (72–143 h). During this period, the removal rate in all systems increased significantly. The removal rate in the bioreactors with $CaCO_3$ as biomass carrier was significantly higher ($P < 0.05$) than all the others. During the third period, the removal rate in the bioreactors with gravel as carrier material did not increase significantly. However, a significantly increased removal rate (factor 1.5 higher; $P < 0.05$) was observed in all other systems (Table 1). During this period, the highest removal rate was measured in the bioreactors with only $CaCO_3$ as biomass carrier (Table 1).

During the first 144 h of the experiment, complete oxidation of TAN occurred. Gradually the TON-level increased, while the NO_2-N concentration in the bioreactor water remained low (< 4 mg L^{-1}). Subsequently, the NO_2-N levels started to increase considerably until all TON levels measured were present in the form of NO_2-N. Clearly under the conditions imposed, the start-up procedure was not able to sustain complete nitrification.

Table 1 The effect of different carrier materials on the removal rate of TAN (Total Ammoniacal Nitrogen) in marine bioreactor systems inoculated with a nitrifying inoculum (mean ± SE)

	Removal rate (mg TAN (L day)$^{-1}$)		
Carrier	**First period***	**Second period†**	**Third period‡**
$CaCO_3$	5.61 ± 0.14[a]	12.67 ± 0.24[a]	19.78 ± 1.56[a]
$CaCO_3$/Gravel	5.16 ± 0.29[a]	10.68 ± 0.24[b]	14.98 ± 1.65[b]
Gravel	5.50 ± 0.19[a]	10.68 ± 0.50[b]	12.10 ± 1.34[b]
Bionet	1.51 ± 0.14[b]	6.00 ± 0.22[c]	13.73 ± 0.74[b]

*From 0 to 71 h.
†From 72 to 143 h.
‡From 144 to 240 h.
Different letters indicate the subsets of significantly different groups per period.

The physicochemical parameters determined on the system did not differ among the various tanks. The pH in all the tanks decreased slightly from 8.2 at start to 7.8 after 264 h. During the different periods, the oxygen level was more or less stable and ranged between 6 and 7 mg L^{-1} O_2.

Stripping of NH_3 from the aqueous phase of a bioreactor system is related to the pH and temperature of the system. Experiments were conducted to quantify this effect in the absence of active nitrifying organisms (Fig. 3). Due to the high nitrifying activity in the systems inoculated with ABIL, an extra amount of TAN (80 mg L^{-1} TAN) was added only to these bioreactors on day 9. ATU (allylthioureum, 2.5 mg L^{-1}) was added to one series of three bioreactors to inhibit the ammonia oxidizers and to stop the total nitrification process (Blum & Speece 1991). The data in Fig. 3 illustrate that almost one-third of the initial TAN (60 mg L^{-1} TAN) was removed after 168 h in the containers without active nitrification.

Marine bioreactors connected to rotifer cultures

The bioreactors described in Fig. 1b, except for those with Bionet as carrier, were connected to a small-scale rotifer recirculation system according to the set-up described by Suantika *et al.* (2000). The starting values of the overall water quality can be seen in Fig. 4.

Rotifers were cultured and the physicochemical parameters of the system were measured and analysed. The TAN concentration in the systems remained quite stable during the first 6 days (< 5 mg L^{-1} TAN), but increased gradually afterwards. On the last day of culture, the TAN concentration in all the systems was higher than 10 mg L^{-1} TAN. No differences in terms of the nitrogen compounds could be observed between the different treatments. However the nitrogen load in the different systems was not equal because the feeding rate depended on the rotifer density. During the first day of culture, nitrite concentrations increased gradually but were smaller than the total oxidized nitrogen concentrations, indicating that nitrite oxidation occurred.

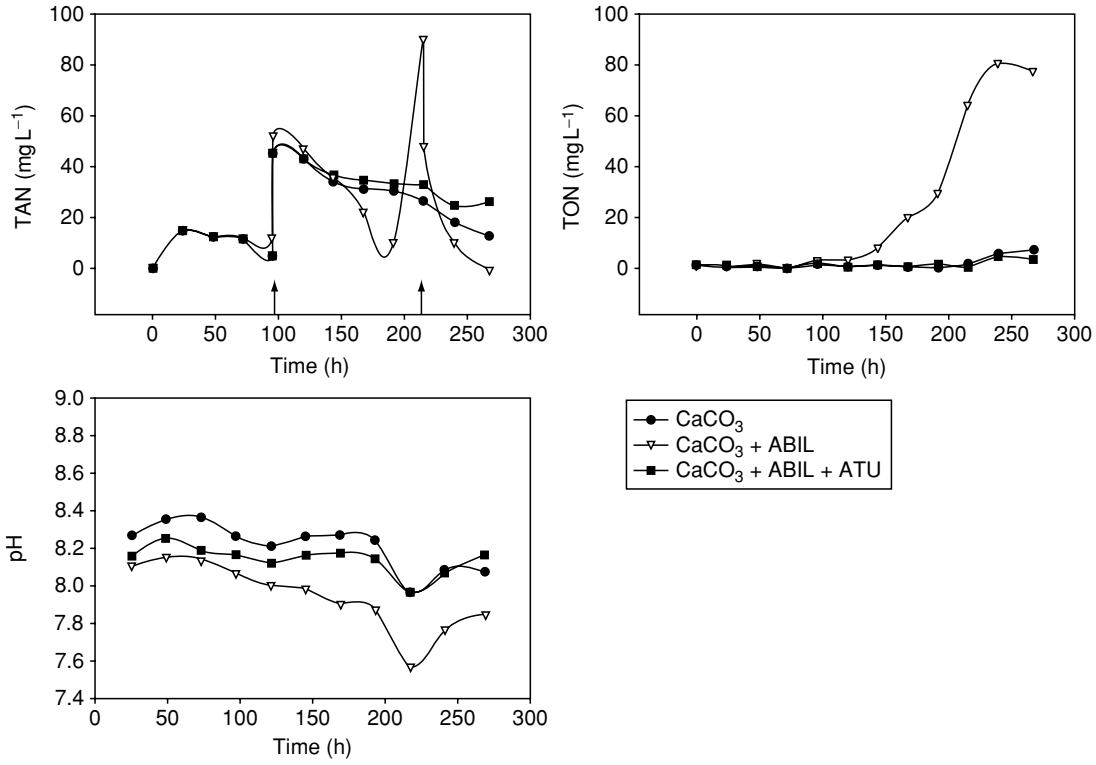

Figure 3 The effect of allylthioureum (ATU) on the evolution of TAN, TON, and NO_2-N in marine bioreactors with $CaCO_3$ as biomass carrier ($n = 3$). The arrows indicate the moment at which NH_4Cl (20, 40 and 80 mg TAN L^{-1} respectively) was added. Only the bioreactors inoculated with ABIL received a dose of 80 mg TAN L^{-1}.

After 144 h, the nitrite nitrogen concentration represented the total oxidized nitrogen concentration, indicating that the micro-organisms responsible for the nitrite oxidation were not performing adequately. After 240 h, the nitrite concentration reached on average 30 mg L^{-1} NO_2-N in all the culture tanks. The dissolved oxygen concentration in the different tanks was also measured and decreased slightly from 7 to 4 mg $L^{-1}O_2$. The pH in all tanks was more or less stable.

All culture tanks were inoculated with 250 rotifers mL^{-1} and the density was recorded daily (Fig. 4). The rotifer density in the culture tanks connected to the bioreactors with $CaCO_3$ as the only biomass carrier increased fastest. During the whole experimental period, the average rotifer density was higher than that in the other treatments. After 200 h, a plateau was reached in all tanks. Subsequently the culture was harvested.

Hollow fibre bioreactor connected to a rotifer culture

The experiment was carried out in 10-L tanks ($n = 3$) and was operated for 264 h (11 days). Figure 5 shows the results of the rotifer population growth in the rotifer culture tanks equipped with the hollow fibres and in the control culture tanks. The rotifer density in the culture tanks treated with ABIL and equipped with hollow fibres was higher from 72 h of culture onwards. The rotifer density in the culture tanks treated with ABIL increased up to more than 3500 rotifers mL^{-1} after 240 h of culture. In contrast, the rotifer density in the control tanks did not increase any more after 9 days of culture and finally resulted in a lower rotifer density (2000 rotifers mL^{-1}) compared with the rotifer density obtained in the culture tanks treated with ABIL.

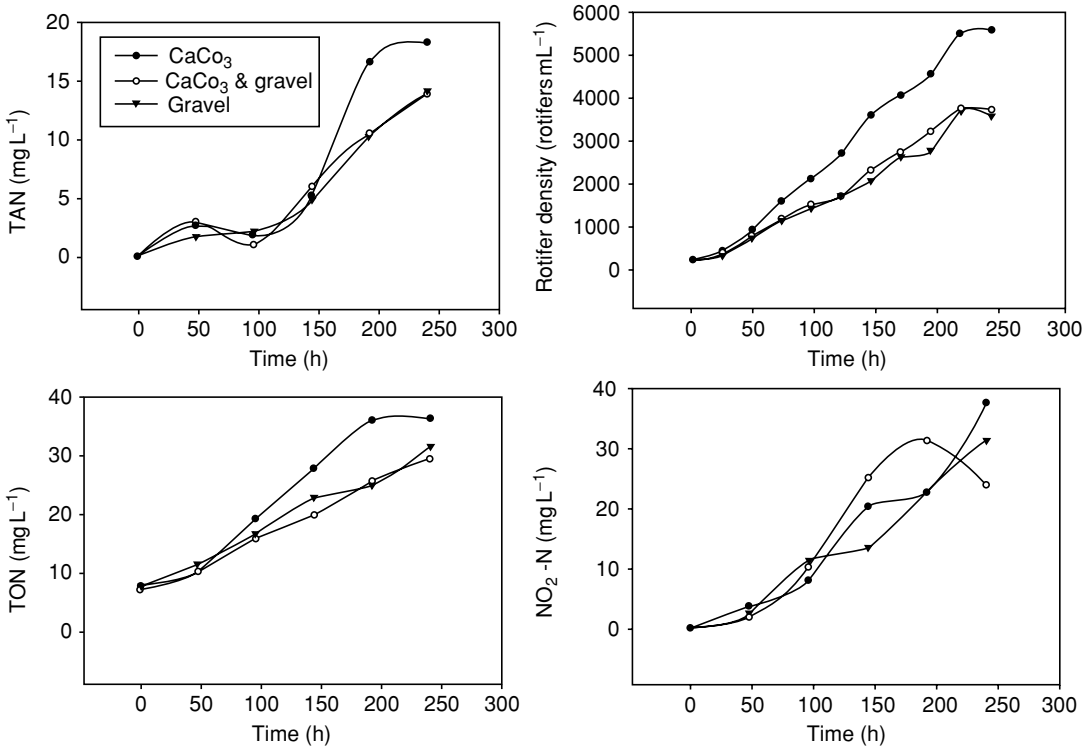

Figure 4 Different parameters measured in the rotifer culture systems connected to the matured bioreactors with different carrier materials in the presence of rotifers ($n = 3$).

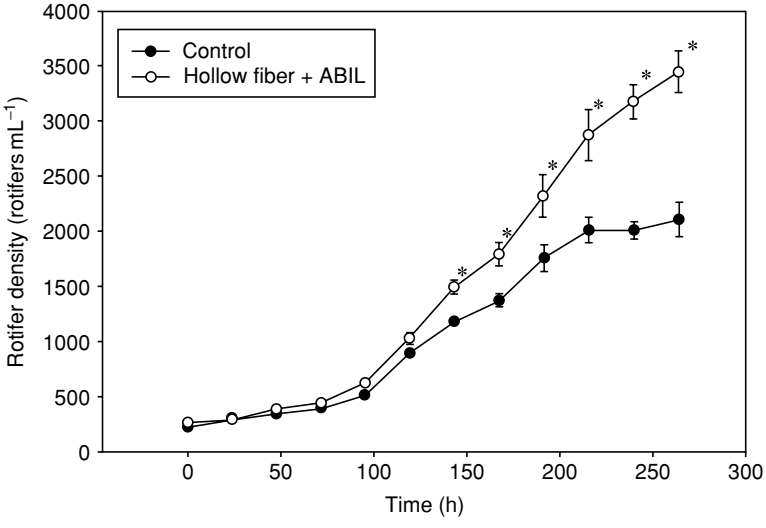

Figure 5 Effect of ABIL and hollow fibres bioreactor on the population growth of rotifers. Mean rotifer density ± SE is plotted ($n = 3$). *, significant difference at $P < 0.05$ (Duncan's multiple range test).

The water quality parameters (TAN, TON, salinity, pH, DO and temperature) were measured. The results are given in Table 2. Low $NH_4 \pm N$ levels were observed at the beginning of the culture period in both systems. For the control, the TAN concentration increased up to 8 mg TAN L^{-1} after 96 h of culture and continued to increase up to 26 mg L^{-1} after 216 h. In the rotifer culture tanks equipped

Table 2 TAN (Total Ammoniacal Nitrogen), TON (NO_2-N, NO_3-N), salinity, pH, DO and temperature measured in the culture water of the rotifer culture tanks ($n = 3$, mean ± SD)

	TAN (mg L^{-1})		TON (mg L^{-1})		DO (mg L^{-1})		pH		Salinity (g L^{-1})		T (°C)	
Hour	Control	ABIL	Control	ABIL	Control	ABIL	Control	ABIL	Control	ABIL	Control	ABIL
0	0.0 ± 0.0	0.3 ± 0.4	0.3 ± 0.3	13.4 ± 0.5	7.05 ± 0.09	7.02 ± 0.06	7.91 ± 0.02	7.80 ± 0.13	25.0 ± 0.0	24.8 ± 0.3	26.8 ± 0.9	26.8 ± 0.9
24	2.9 ± 1.3	0.9 ± 0.3	0.0 ± 0.0	27.1 ± 2.1	6.32 ± 0.19	6.53 ± 0.17	7.83 ± 0.02	7.76 ± 0.07	25.1 ± 0.1	25.0 ± 0.1	28.1 ± 0.7	28.2 ± 0.6
48	3.4 ± 1.1	1.4 ± 0.2	1.1 ± 0.9	15.2 ± 2.8	6.24 ± 0.33	6.52 ± 0.47	7.76 ± 0.03	7.70 ± 0.06	25.0 ± 0.1	25.0 ± 0.1	27.3 ± 0.2	27.6 ± 0.9
72	4.6 ± 0.2	1.6 ± 0.5	0.2 ± 0.5	6.9 ± 0.9	6.21 ± 0.12	6.07 ± 0.22	7.86 ± 0.01	7.73 ± 0.05	25.1 ± 0.0	25.1 ± 0.1	28.1 ± 0.7	28.8 ± 0.6
96	8.0 ± 0.1	1.4 ± 0.1	0.1 ± 0.2	8.9 ± 2.6	6.01 ± 0.29	5.43 ± 0.22	7.78 ± 0.05	7.56 ± 0.02	25.2 ± 0.3	26.3 ± 0.3	27.7 ± 0.6	29.0 ± 1.1
120	12.7 ± 0.5	3.1 ± 0.5	0.9 ± 1.6	15.9 ± 2.3	6.14 ± 0.01	6.57 ± 0.24	7.89 ± 0.02	7.77 ± 0.04	29.7 ± 0.6	29.8 ± 0.3	28.2 ± 0.3	28.2 ± 1.6
144	18.3 ± 0.4	4.1 ± 3.0	0.0 ± 0.0	15.1 ± 4.7	5.50 ± 0.05	6.10 ± 0.10	7.84 ± 0.01	7.60 ± 0.06	30.0 ± 0.0	29.0 ± 0.9	28.3 ± 1.0	27.4 ± 0.1
168	17.1 ± 1.7	3.4 ± 0.4	0.1 ± 0.1	17.5 ± 3.8	5.39 ± 0.40	6.11 ± 0.28	7.82 ± 0.01	7.53 ± 0.06	29.0 ± 0.0	30.0 ± 0.0	28.3 ± 1.0	27.4 ± 0.7
192	23.4 ± 5.6	5.6 ± 0.1	0.6 ± 0.9	28.1 ± 0.9	5.48 ± 0.07	5.76 ± 0.12	7.88 ± 0.07	7.54 ± 0.01	27.5 ± 0.0	29.5 ± 0.5	28.5 ± 0.9	27.7 ± 0.5
216	26.6 ± 1.1	8.1 ± 2.5	0.4 ± 0.7	23.6 ± 3.5	5.70 ± 0.16	5.93 ± 0.16	7.91 ± 0.05	7.45 ± 0.02	28.6 ± 0.3	30.0 ± 0.0	28.6 ± 0.3	27.4 ± 0.4

with hollow fibres containing ABIL, the TAN concentration slightly increased at the end of the culture period. An acceptable level of 5.6 mg L^{-1} TAN was observed on day 8 at high rotifer density (2300 individuals per mL). Low levels of TON concentration were observed in the control tanks, but not in the culture tanks treated with ABIL (Table 2). Interestingly, low levels of nitrite (< 1 mg L^{-1} N) were measured at all times in the tanks treated with the hollow fibres with ABIL. The pH at the beginning of the culture (day 0–day 1) decreased during the experiment. The pH level in the culture tanks ranged from 8.18 to 7.71 (during the first 5 days of experiment 1) and 7.80 to 7.45 (during the first 9 days of experiment 2). During the culture period the aeration was adjusted to a minimum dissolved oxygen concentration of 5.5 mg L^{-1}.

Discussion

To test the effect of the nitrifying culture ABIL on the population growth rate of rotifers, this enrichment culture was first applied as a probiotic in small-scale rotifer batch cultures. Submerged filters, which are present in such systems to collect the larger particles in the water, were seeded with the nitrifying bacterial population and installed in the rotifer batch culture. Figure 2 demonstrates that this simple supplementation brought about improved culture performance.

In terms of water quality, two nitrogen species are of particular concern i.e. the highly toxic ammonia and the inhibitory nitrite. The separate tests demonstrated that TAN was mainly removed by active nitrification, but also to some extend by NH_3-stripping (Fig. 3). Removal of NH_3 by volatilization was possible as the systems were continuously aerated (100 L h^{-1}) with an air pump and operated between pH 8.3 and 8.1. At this operating pH, 8–10% of the total amount of TAN can be estimated to be present in the form of ammonia (pKa (NH_3/NH_4^+) = 9.25). Hence, although the activity of the nitrifiers is often judged on the basis of the decrease in TAN (Table 1), it is better to evaluate it by the increase in TON.

According to Catalan-Sakairi, Wang & Matsumura (1997), different factors can affect the nitrification process in seawater. Important parameters are the inorganic carbon concentration, the trace-element concentration and the physicochemical properties of the carrier.

Sufficient bicarbonate must be present in the system to provide carbon for growth while maintaining the system at an optimum pH range. A convenient chemical to increase pH and/or buffer capacity is $CaCO_3$. Chalk dissolution is known to be dependent on the physical characteristics of the chalk and the hydraulic flow characteristics (Green, Ruskol, Lahav & Tarre 2001). Table 1 reveals that the highest ammonium removal rates were found in the bioreactor with chalk as the only biomass carrier, followed by the bioreactor filled with the mixture of chalk and gravel stones (1/1). These observations confirm the results of Green *et al.* (2001) who used chalk as carrier and sole buffer agent in a fluidized bed reactor for nitrification.

Under certain conditions, the accumulation of the intermediate nitrite ion was observed (Fig. 4). The tolerance of the rotifer *Brachionus plicatilis* to nitrite has been investigated by Ostrensky (1993). The concentrations, in $mg\,L^{-1}$ NO_2-N, which decrease the population growth by 50% (EC50) ranged from $609\,mg\,L^{-1}$ in 24 h to $40\,mg\,L^{-1}$ NO_2-N in 96 h, and the median lethal concentrations (LC50) ranged from 732 to $168\,mg\,L^{-1}$ in the same period. The maximum concentration that causes no significant mortality, after 96 h of continuous exposure, was $55\,mg\,L^{-1}$ NO_2-N. These results demonstrate that *Brachionus plicatilis* is amongst the most nitrite-resistant aquatic organisms recorded (Ostrensky 1993). Therefore, the nitrite problem that occurred in our test runs can be considered to play a minor role in the performance of the rotifers cultured in the small-scale experiments (Fig. 4).

Of particular interest was the effect of the submerged membranes (in the form of hollow fibres connected to an ABIL bioreactor) in the treatment of rotifer culture water. One of the major functions of the applied system was to convert TAN to TON. Our results showed that there was a higher TAN concentration present in the control batch-culture system than in the hollow fibre recirculating system (Table 2). Moreover, under these conditions both the ammonium and the nitrite oxidizing activity were equally functional as no nitrite accumulated. This could be because the nitrifiers were not directly exposed to the rotifer environment. The improved water quality obtained with the hollow fibre recirculation system resulted in much higher rotifer densities compared with the normal batch culture (Fig. 5).

The development of cost-effective, integrated, nitrogen removal systems including both the nitrification and denitrification processes is essential to the advent of truly closed, recirculating commercial aquaculture systems. A long-term goal is to combine nitrification and denitrification processes into an integrated system, thereby decreasing the floor space needed for biological filtration (Lee 1995).

In contrast to other probiotic mixtures, which often have a complicated mode of action (Verschuere, Rombaut, Sorgeloos & Verstraete 2000), the use of nitrifying cultures in rotifer cultures enhances the rotifer culture performance considerably. This study has shown that the application of ABIL in rotifer cultures, as a starter for batch cultures or in use in a bioreactor system, had a positive effect on the growth of rotifers.

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