



# A novel approach to denitrification processes in a zero-discharge recirculating system for small-scale urban aquaculture

Alon Singer<sup>a</sup>, Shmuel Parnes<sup>b</sup>, Amit Gross<sup>c</sup>, Amir Sagi<sup>b</sup>, Asher Brenner<sup>a,\*</sup>

<sup>a</sup> Department of Biotechnology Engineering and Unit of Environmental Engineering, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel

<sup>b</sup> Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel

<sup>c</sup> Department of Environmental Hydrology & Microbiology, Zuckerberg Institute for Water Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, Sede Boqer 84990, Israel

## ARTICLE INFO

### Article history:

Received 6 December 2007

Accepted 28 July 2008

### Keywords:

Urban  
Backyard  
Aquaculture  
Sustainability  
Zero-discharge  
Recirculation  
Denitrification  
Nitrate  
Bioreactor  
Cotton wool  
Degassing

## ABSTRACT

This paper presents an innovative process to solve the nitrate build-up problem in recirculating aquaculture systems (RAS). The novel aspects of the process lie in a denitrification bioreactor system that uses solid cotton wool as the primary carbon source and a unique degassing chamber. In the latter, the water is physically stripped of dissolved gaseous O<sub>2</sub> (by means of a Venturi vacuum tube), and the subsequent denitrification becomes more efficient due to elimination of the problems of oxygen inhibition of denitrification and aerobic consumption of cotton wool. The cotton wool medium also serves as a physical barrier that traps organic particles, which, in turn, act as an additional carbon source for denitrification. Operation in the proposed system gives an extremely low C/N ratio of 0.82 g of cotton wool/g of nitrate N, which contributes to a significant reduction of biofilter volume. The additional advantage of using solid cotton wool as the carbon source is that it does not release organic residuals into the liquid to be recycled. Operation of the system over a long period consistently produced effluents with low nitrate levels (below 10 mg N/l), and there was only a very small need to replace system water. The overall treatment scheme, also incorporating an aerobic nitrification biofilter and a granular filtration device, produced water of excellent quality, i.e., with near-zero levels of nitrite and ammonia, a sufficiently high pH for aquaculture, and low turbidity. The proposed system thus provides a solution for sustainable small-scale, urban aquaculture operation with a very high recovery of water (over 99%) and minimal waste disposal.

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## 1. Introduction

### 1.1. Recirculating aquaculture systems (RAS)

The aquaculture industry that began developing in the late 1960s has exploded into a major global industry of 60 million tons a year, with huge annual revenues in excess of US\$ 70 billion (FAO, 2006). With the current increase in environmental awareness and the consequent stringency in environmental legislation, a new approach to dealing with the ecological problems associated with aquaculture has been developed—recirculating aquaculture systems. This approach was originally developed to provide a solution to the environmental problems generated by the traditional pond and flow-through aquaculture systems, since it enables the treatment of polluted water within a closed loop, offers improved

control of effluent discharge, and allows complete environmental control (van Gorder, 1994; Shnel et al., 2002). Moreover, RAS confers ecological and economic advantages in that it facilitates a reduction in the amounts of water and energy required and reduces land use. In addition, it provides growers with the geographical freedom to set up aquaculture systems in “nontraditional” farming areas (Shnel et al., 2002); for example, small RAS, such as the one presented in this study, producing lucrative seafood species might be suitable for small-scale aquaculture for food production in urban areas (Zohar et al., 2005). Thus, RAS could contribute to meeting the demand for protein foods in highly populated urban centers.

### 1.2. Biological nitrogen removal processes in aquaculture systems

Maintaining acceptable water quality currently constitutes the main bottleneck in RAS (van Rijn, 1996; Menasveta et al., 2001). The water quality parameters of greatest relevance in such systems are ammonia, nitrite and nitrate. Although nitrate is considered the

\* Corresponding author. Tel.: +972 8 6479029; fax: +972 8 6479030.  
E-mail address: [brenner@bgu.ac.il](mailto:brenner@bgu.ac.il) (A. Brenner).

least toxic of the different inorganic nitrogen forms, various fishes indigenous to soft water habitats and some commonly farmed invertebrate species are susceptible to elevated nitrate levels (Tal et al., 2003). While aerobic nitrification of ammonia and nitrite has become a general standard in RAS, the resulting nitrate accumulation has not attracted sufficient attention, especially since the build-up of nitrate necessitates the exchange of 10–20% of the water each day to maintain adequate water quality. Currently used designs to remove excess nitrate – although relatively effective – are cumbersome, difficult to maintain, and hence expensive. Two major problems characterize these systems: first, it is difficult to control the exact amounts of soluble carbon compounds (such as methanol) that have to be added to support bacterial growth (due to fluctuations of water quantity and quality) and organic residuals from these soluble carbon compounds may leach into and contaminate the system water. Second, high levels of oxygen in the process inflow (close to saturation due to intensive aeration of the grow-out tanks) inhibit denitrification and cause excessive consumption of the organic carbon applied, due to aerobic activity.

### 1.3. RAS denitrification biofilters

The problem of nitrate accumulation in RAS is not trivial, as discussed above, and there is a need for currently used solutions to be improved. The very few studies that have been conducted on this problem have shown that there are a number of issues that should be addressed. Asano et al. (2003) showed that it is possible to decrease the amount of water used in the system simply by providing the most basic biofilters. Menasveta et al. (2001) employed methanol and ethanol as carbon sources and physical oxygen removal from the anaerobic biofilter via gaseous  $N_2$ . Suzuki et al. (2003) did not employ any means to deoxygenate the water and found that such technologies had to use an extremely large denitrification biofilter. Vidal et al. (2002) drew attention to the necessity to develop methods to reduce the dissolved oxygen on the premise that they could be cost effective for achieving active denitrification and higher removal efficiencies.

To date, cheap soluble substances, such as methanol, ethanol or glucose, have usually been the materials of choice as the external carbon source (Sauthier et al., 1998), but some studies have shown certain residual concentrations of carbonaceous compounds in the effluent, a finding that could be problematic for certain aquaculture species (Gómez et al., 2000; Shnel et al., 2002). A number of alternative materials have thus been tested. Soares et al. (2000), for example, used solid cotton wool as the sole carbon source for the treatment of well-water contaminated with high levels of nitrate. Although almost total denitrification was obtained, the process suffered operational problems, mainly due to clogging. The use of an intrinsic source of carbon to support denitrification has been considered in several studies. Abufayed and Schroeder (1986) for example, used primary sludge from domestic wastewater as a feedstock for separate stage denitrification. Arbiv and van Rijn (1995) used organic debris accumulating in a culture unit to support denitrification in a fluidized bed reactor. A similar approach was tested by Klas et al. (2006), who used organic solid waste of a typical RAS as an electron donor in a single-sludge denitrification process for treating system effluents.

To solve some of the problems described above, we set out to design a system based on an insoluble carbon source and a degassing technique. The rationale for the design was that an insoluble carbon source would prevent “leakage” of organics into the water of the grow-out tank, while the degassing technique would eliminate the problems of oxygen inhibition of denitrification and of the aerobic consumption of organic carbon. In the present study, we thus tested whether a denitrification system

based on the combined technology of degassing followed by passage through a cotton wool biofilter would lead to a reduction in reactor volume and an improvement in overall efficiency and sustainability.

## 2. Materials and methods

### 2.1. Experimental system

The experimental set-up included two replicate systems (designated 1 and 2) placed in an aquaculture greenhouse on the Bergmann Campus of Ben-Gurion University of the Negev, Beer-Sheva, Israel. Each system comprised an aquaculture tank and a water treatment facility (Fig. 1). The aquaculture tanks were located inside a dark area (6 m × 12 m) that occupied half of the greenhouse. Water from the aquaculture tank was allowed to flow out of the dark room into the water treatment equipment that was placed in the light in the other half of the greenhouse. Each water treatment facility comprised an aerobic nitrification biofilter, a deep-bed sand filter (Astral 750, Astralpool, Spain), and a denitrification biofilter that was connected in parallel to the main water flow. A small aquarium pump fed the water from the aquaculture tank to the denitrification system, and the outlet water flowed to the aerobic biofilter (Fig. 1). The aerobic biofilter comprised a polyethylene container (~100 l) filled with plastic beads (surface area 860 m<sup>2</sup> and 160 kg per cubic meter, Aridal Bio-Balls, Israel).  $CaCO_3$  in the form of quarry gravel was added to the aerobic biofilter before the start of the experiment to compensate for any extreme drop in alkalinity due to the nitrification. Each aquaculture tank was filled with 13 m<sup>3</sup> of synthetic brackish water and was maintained at  $29 \pm 1$  °C. The synthetic brackish water was prepared by adding Red Sea salt to local tap water to raise the salinity to 4 ppt. The aquaculture tanks were stocked with a prawn (*Macrobrachium rosenbergii*) broodstock at a biomass density of 590 g/m<sup>3</sup>. Dry feed was supplied three times a week at a rate of approximately 2.5–3.5% of the total biomass per day. Before the start of the experiment, ammonium chloride was added to both systems to ensure proper initial function of the aerobic biofilter, and thereafter ammonia and nitrite levels were monitored routinely.

### 2.2. Denitrification biofilter

The denitrification system is based on a novel two-stage approach. The first stage comprises a small (10 l) plastic degassing

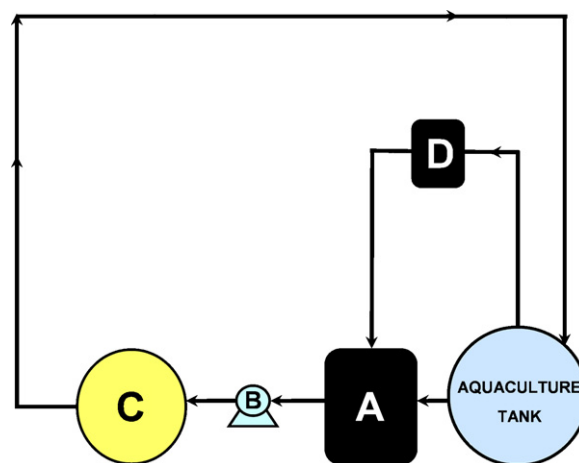
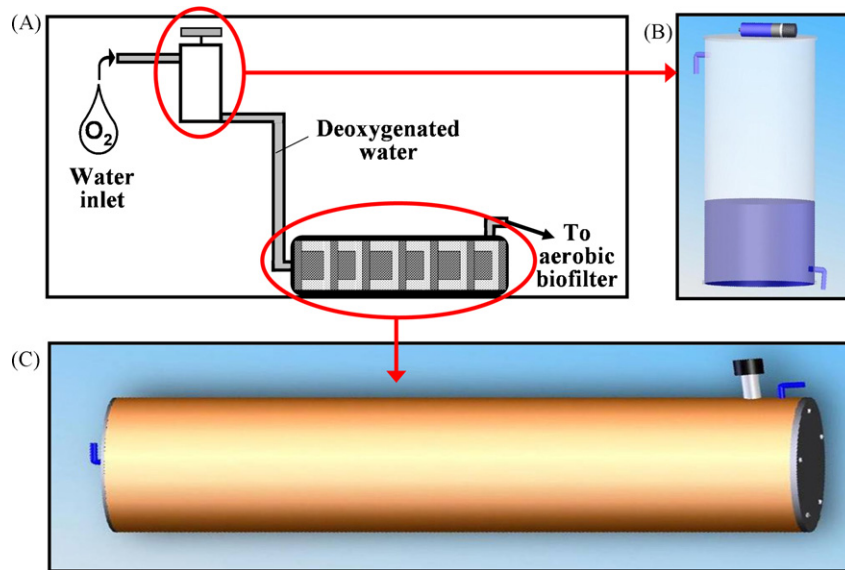


Fig. 1. Schematic representation of the system layout. (A) Aerobic nitrification biofilter, (B) system pump (centrifugal), (C) sand filter, and (D) denitrification system.



**Fig. 2.** Two-stage denitrification system (A). In stage 1, water enters a degassing chamber (B) where the dissolved oxygen is removed. Thereafter, it flows into a denitrification biofilter (C), where cotton wool serves as the primary carbon source. The dark region in the cotton-bead packing regime (inside lower oval) depicts the region occupied by the porous plastic beads. The lighter region represents the area filled with cotton.

chamber, where the dissolved oxygen is removed by means of a Venturi vacuum tube (Vaccon JD-100M-STAA4). The influent pipe of the degassing chamber terminates in a nozzle, containing a number of small holes, thereby increasing the surface area to volume ratio of the water to be deoxygenated. The second stage, the denitrification biofilter, is placed below the degassing chamber. The biofilter comprises a PVC pipe (0.3 m diameter, ~45 l in volume) filled with commercial cotton wool (the kind used in first-aid kits) and plastic beads packed in the manner shown in Fig. 2. The beads in the column occupied approximately 26 l, and the total cotton wool content was approximately 1.1 kg.

### 2.3. Sampling regime and analytical procedures

Samples from the aquaculture tanks and from the denitrification unit outlet were collected over a 4-month period and analyzed for the following parameters according to the APHA (1998), unless otherwise specified, at the following time intervals: turbidity, pH and temperature were analyzed three times a week; ammonia, nitrite and alkalinity, once a week; and nitrate, twice a week. Total suspended solids (TSS), volatile suspended solids (VSS), total dissolved solids (TDS) and total organic carbon (TOC) were analyzed periodically. Ammonia was determined by the Nesslerization method, nitrite by the azo dye colorimetric method, and nitrate by the Szechrome NAS reagent (diphenylamine sulfonic acid chromogene) method according to Gross and Boyd (1998). TOC was determined with a Tekmar Dohrmann–Apollo 9000 analyzer (Cincinnati, OH, USA). Temperature and pH were

measured with a standard thermometer and a Cyberscan 510 meter (Euteoh Instruments, Singapore), respectively. Turbidity was measured by Hach 2100P turbidometer (Hach Company, Loveland, CO, USA).

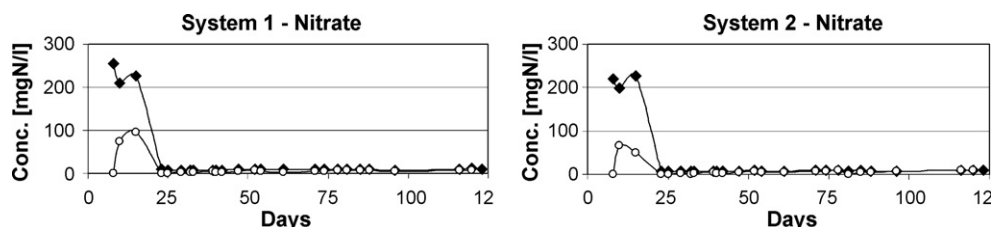
### 2.4. Sludge management

The sludge that accumulated in the sand filter was flushed out by backwashing the filter once or twice a week, with the system's water, into a separate sedimentation basin. The backwash suspension (500 l) was allowed to settle for 30 min, the sludge sediment was removed, and the remaining water was pumped back into the system. This procedure meant, in practice, that very little water was lost. Tap water was added to compensate for losses due to evaporation.

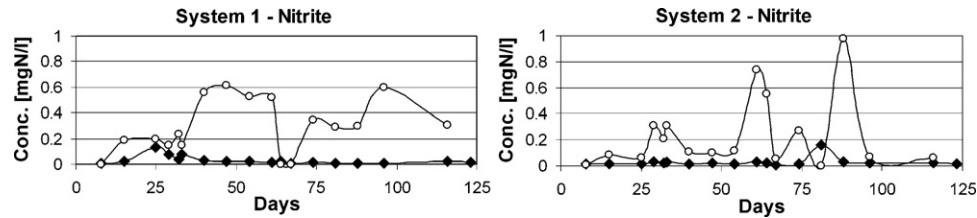
## 3. Results and discussion

### 3.1. General water quality parameters

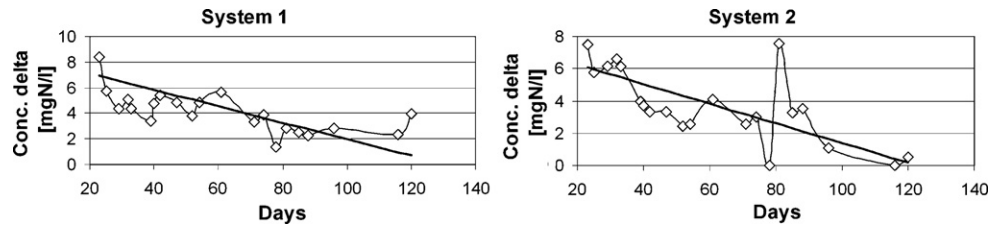
The results indicate that the two experimental systems behaved almost identically, as demonstrated in Figs. 3–5, and Tables 1–3. Throughout the entire study, ammonia and nitrite concentrations in the aquaculture tanks remained at low levels suitable for most aquatic life (Table 1). The pH in the system fluctuated between 7.5 and 8, a range that is considered by some as suitable for aquaculture growth (Boyd and Tucker, 1998). This pH range is also commonly cited as optimal for nitrifying biofilters (Hagopian



**Fig. 3.** Nitrate concentrations of systems 1 and 2 for the entire duration of the experiment. Black diamonds represent aquaculture tank (inlet) values, and open circles represent the denitrification biofilter outlet values.



**Fig. 4.** Nitrite concentrations of systems 1 and 2 for the entire duration of the experiment. Black diamonds represent aquaculture tank (inlet) values, and open circles represent the denitrification biofilter outlet values.



**Fig. 5.** Nitrate delta concentration values (influent less effluent) for systems 1 and 2 for the entire duration of the experiment. Linear regression line depicts the nitrate delta function (NDF).

**Table 1**

Summary of water quality parameters in the aquaculture tanks

Parameter	Tank 1	Tank 2
Temperature ( $^{\circ}\text{C}$ )	$27.2 \pm 2.1$	$27.2 \pm 2.1$
pH	$7.79 \pm 0.14$	$7.74 \pm 0.09$
Alkalinity (mg/l as $\text{CaCO}_3$ )	$120.6 \pm 16.9$	$111.1 \pm 19.4$
Turbidity (NTU)	$1.4 \pm 1$	$1.1 \pm 0.5$
Ammonia (mgN/l)	$0.13 \pm 0.11$	$0.13 \pm 0.11$
Nitrate (mgN/l)	$8.6 \pm 1.1$	$7.9 \pm 1$
Nitrite (mgN/l)	$0.03 \pm 0.03$	$0.03 \pm 0.03$

and Riley, 1998). However, many studies have noted that the pH acceptable for aquatic animals varies, depending on type of animal and the salinity of the water, and that the pH is liable to drop rapidly unless a buffering agent is used (Menasveta et al., 2001; Shnel et al., 2002; Akunna et al., 1993; Skjølstrup et al., 1998; Vidal et al., 2002). These studies usually applied a batch-wise approach to solving the pH problem by periodically adding small amounts of chemicals, such as sodium bicarbonate. Another option for controlling the pH is the addition of lime, which is a common practice to buffer natural acidity or the acidity that is incurred by

nitritification in earthen ponds for fish aquaculture or in RAS (Boyd and Tucker, 1998). This option was applied here by incorporating a small amount of  $\text{CaCO}_3$ , in the form of quarry gravel, into the aerobic biofilter. Water alkalinity in the tanks remained stable throughout the experiment (Table 1), since the denitrification in the anoxic biofilter increased the alkalinity (Table 2), as was to have been expected (van Rijn, 1996).

### 3.2. Denitrification efficiency

In addition to the pilot trials in the greenhouse, laboratory batch experiments were carried out with undigested cotton wool and nitrate solution to evaluate the consumption of cotton wool in the denitrification process. To this end, four Erlenmeyer flasks containing various quantities of pure cotton wool (0, 0.25, 0.5, and 1.0 g) and tap water supplemented with nitrate to a concentration of 400 mg/l were placed in an anaerobic hood (COY Laboratory Products Inc., MI, USA). After 10 days, the remaining cotton wool was washed free of salts, and the nitrate concentrations in the tested solutions were measured. The cotton wool was dried at  $105^{\circ}\text{C}$  and weighed. The C/N ratio obtained was  $1.83 \pm 0.52$  g of cotton wool/g of nitrate N.

In the experimental biofilters of the pilot system, nitrate levels were initially very high (more than 200 mg N/l), probably due to the addition of ammonium chloride before the start of the experiment, as explained above. However, approximately 2 weeks after the beginning of operation of the denitrification biofilter, nitrate concentrations had fallen to very low levels (less than 10 mg N/l), as shown in Fig. 3. These findings were probably due to the time required for biomass build-up in the denitrification biofilter. While nitrate levels were consistently reduced in the

**Table 2**

Operation and performance parameters of the denitrification process

Parameter	System 1	System 2
Volume of denitrification biofilter (l)	45	45
Flow rate (l/h)	~20	~20
Days operated	115	115
Total water volume passing through biofilter ( $\text{m}^3$ )	55.2	55.2
Inlet alkalinity (mg/l as $\text{CaCO}_3$ )	$120.6 \pm 16.9$	$111.1 \pm 19.4$
Outlet alkalinity (mg/l as $\text{CaCO}_3$ )	$137.0 \pm 37.2$	$129.7 \pm 31.8$
Apparent <sup>a,b</sup> C/N ratio; based on the NDF <sup>c</sup> (g cotton applied)/(g nitrate N removed)	0.839	0.782
Apparent <sup>a,b</sup> C/V ratio; based on the NDF <sup>c</sup> (g cotton applied)/( $\text{m}^3$ water treated)	17.18	18.59
Calculated C/N ratio; based on the RFC <sup>d</sup> (g cotton applied)/(g nitrate N removed)	0.836	0.802

<sup>a</sup> Based on weighing (cotton completely degraded).

<sup>b</sup> Due to simultaneous oxidation of waste organic particles trapped in the biofilter medium.

<sup>c</sup> Nitrate delta function.

<sup>d</sup> Relative feed contribution.

**Table 3**

Sludge balance and quality parameters

Parameter	System 1	System 2
Total sludge extracted (l)	183.25	173.50
Sludge per extraction (l)	$8.33 \pm 2.33$	$8.26 \pm 2.3$
Sludge TSS (g/l)	$13.33 \pm 3.33$	$12.57 \pm 5.39$
Sludge VSS (g/l)	$7.28 \pm 2.12$	$7.48 \pm 2.93$
(g TSS extracted)/(kg animal feed)	86.35	77.09



denitrification unit, nitrite production was evident in the process, as shown in Fig. 4. These residual levels of nitrite, a known intermediate product of denitrification (van Rijn and Rivera, 1990), were subsequently removed in the nitrification aerobic biofilter. Thus, the unique design approach of the overall water treatment system ensured dependable water quality with regard to ammonia, nitrite and nitrate.

While both systems 1 and 2 reached a quasi-steady state with low levels of nitrate (Table 1 and Fig. 3), the nitrate delta (inlet concentration less outlet concentration) showed a steady decline (Fig. 5). Linearization of the difference as a function of time (nitrate delta function, NDF) enabled us to ascertain when, approximately, the denitrification system became ineffective, probably due to the limitation of organic carbon. This approximation was based on the assumption that denitrification can be modeled as a zero-order reaction with respect to nitrate concentrations down to very low levels (Vidal et al., 2002). The NDF obtained from Fig. 5 can give us a clearer definition of the anoxic biofilter capacity with regard to the ultimate end point (point of near-zero effectiveness). NDF was found to be  $-0.0641 \times t + 8.38$  and  $-0.0607 \times t + 7.47$  for systems 1 and 2, respectively, where  $t$  is the time in days. Calculations based on the NDF figures showed that the anoxic biofilter would become ineffective (NDF = 0) on day 130 for system 1 and on day 123 for system 2. It was believed that the anoxic biofilter would remain effective until those dates, even though system nitrate levels would continue to increase slowly. On the basis of this assumption, it was therefore possible to calculate the mass of cotton wool required to treat a certain load of nitrate (Table 2). Our calculations gave an average actual C/N value of 0.81 g of cotton wool/g of nitrate N for the two systems. This extremely low ratio is below the theoretical ratio (1.36) reported for cellulose (Rocca et al., 2005) and below that obtained for pure undigested cotton wool in the batch experiment described above (1.83). This discrepancy stems from the simultaneous oxidation of waste organic particles flowing out of the aquaculture tank and trapped in the cotton wool filter medium. The suitability of such waste particles to serve as a carbon source for denitrification has been shown elsewhere (Abufayed and Schroeder, 1986; Arbiv and van Rijn, 1995; Klas et al., 2006).

Alternatively, the applied C/N ratio can be theoretically calculated on the basis of the amount of feed and the amount of nitrogen that was added to the system throughout the experiment. In such a calculation, the initial and final nitrate levels in the aquaculture tank must be taken into account. It is commonly held that the total nitrate mass (mg N) contributed by the animal feed and subsequently removed by the nitrification–denitrification processes may be expressed as  $F \times D \times Pr \times Np \times Ns$ , where  $F$  is the total amount [in mg] of feed added into the system for the entire duration of the experiment,  $D$  is the dry weight of the feed [estimated at 88%],  $Pr$  is the total protein content of the feed [indicated at 45% by the manufacturer],  $Np$  is the average percentage of nitrogen in the protein [estimated to be 16% by the FAO, 2006], and  $Ns$  is the percentage of nitrogen secreted into the system [estimated at 78% by Lupatsch and Kissil, 1998]. Using this approach and taking into account the aquaculture tank water volume and the difference between final and initial nitrate concentrations, we calculated the average C/N ratio to be 0.82 g of cotton wool/g of nitrate N (Table 2). Thus, it was found that the actual measured C/N ratio and the theoretical calculation methods, i.e., the NDF and the relative feed contribution (RFC) – although employing different assumptions – all gave very similar values of approximately 0.82 g of cotton wool/g of nitrate N.

A number of studies have been conducted to determine the C/N ratio required for effective denitrification with different carbon sources. The experiments of Akunna et al. (1993) showed that for a batch type reactor, the C/N ratio was 5.4, 4.8, 4.8, 5.0 and 3.7 for

glucose, glycerol, acetic acid, lactic acid and methanol, respectively. In an attempt to reduce nitrate levels by using freeze-dried starch as both the carbon source and the matrix for the denitrifying bacteria, Tal et al. (2003) found a C/N ratio of approximately 3.2. The studies of Sauthier et al. (1998), with ethanol as the carbon source, showed that an optimal C/N ratio of 1 was needed to maintain low nitrate levels. Gómez et al., 2000 found C/N ratios of 1.08 for ethanol, 1.1 for methanol, and 2.5 for sucrose. Menasveta et al. (2001) showed that C/N ratios of 0.4 and 0.92 for ethanol and methanol, respectively, were adequate to maintain low nitrate concentrations. Rocca et al. (2005) found a ratio of 2.9 g of cotton/g N in treatment of nitrate-rich drinking water, a value well above the ratio obtained in the present study for the same carbon source.

Denitrification processes in RAS using soluble carbon sources require sophisticated process controls and continuous monitoring to prevent any spill into the recirculated water of residual organics that might harm the animals. Gómez et al., 2000 found, for example, that even when using a C/N ratio well below the ratio needed to effectively remove system nitrate, remaining concentrations of the soluble carbon source could be found in the effluent. A system based on an insoluble solid carbon source would therefore seem to be a good solution to these problems. We believe cotton wool, being completely insoluble in water, to be the material of choice, since it can serve both as a carbon source and as a biomass growth bed. Its low cost, availability, and low toxicity are added advantages. In addition, it can act as a physical barrier that traps particles, some of which can serve as an additional carbon source for denitrification (Abufayed and Schroeder, 1986; Arbiv and van Rijn, 1995; Klas et al., 2006). In our system, an attempt was made to reduce the overall compressibility of the cotton wool by using plastic beads (identical to those used in the aerobic biofilter) as spacers, and by arranging the biofilter in a horizontal flow regime. This set-up increased the active zone (zone where the denitrification takes place) and prevented the clogging and channeling problems reported elsewhere (Sauthier et al., 1998; Soares et al., 2000). Periodic monitoring showed no increase of TOC in the denitrification unit (data not shown).

Although vacuum degassing – like nitrogen stripping – has its own intrinsic drawbacks (such as initial capital investment and long-term maintenance costs), both methods are relatively convenient and simple. Nitrogen stripping is probably more cost effective in the short term, because vacuum degassing requires a slightly greater initial capital investment. However, in the long term, vacuum degassing should be more economical. Vacuum degassing presents some unique engineering challenges, but once a suitable system has been developed, it can produce an almost inexhaustible supply of degassed water (Landman and van den Heuvel, 2003). This concept may have an additional advantage in intensive aquaculture systems for which the accumulation of carbon dioxide has been reported (Summerfelt et al., 2000), since the carbon dioxide can be stripped simultaneously with oxygen by the same simple method.

### 3.3. Sludge balance

Turbidity levels remained low, at less than 2 NTU, and quite constant throughout the entire experiment in both systems 1 and 2 (Table 1), suggesting that the sand filter was effective in removing most of the suspended solids produced in the aquaculture tanks. These suspended solids were filtered out and formed the sludge that was then removed from the system. Generally, the tested sludge parameters in the two systems proved to be similar (Table 3). Overall, the total amount of sludge extracted over the duration of the study was approximately 180 l for each system.

Thus, the total amount of water that would have to be added to each system to compensate for sludge loss was negligible. These figures work out to more than 99.99% overall system recovery for the duration of the study with regard to water reuse.

#### 4. Summary and conclusions

Environmentally friendly RAS, which conform to strict environmental legislation, are needed for small-scale, urban aquaculture. For these purposes, the most important water quality parameters are ammonia, nitrite and nitrate. While aerobic nitrification of ammonia and nitrite has become a general standard in such systems, the resulting nitrate accumulation has not received the necessary attention. To address this oversight, we have designed and tested an innovative denitrification bioreactor system based on solid cotton wool as the primary carbon source and a unique degassing device, which further increases the biofilter's efficiency and effectiveness. This design concept and the above-described sludge management practice resulted in a very high recovery of water and a negligible amount of waste for disposal. Our results show that the process offers a number of advantages: it provides highly efficient and controlled denitrification, it uses a cheap and renewable carbon source, and it has a low maintenance, compact configuration.

The results of the two pilot systems, which proved to be almost identical in most of the monitored parameters, showed that effective operational capability of the denitrification biofilter could be easily evaluated. Two alternative calculation methods, the NDF and the RFC, yielded the same apparent C/N ratio for the two experimental systems, averaging 0.82 g of cotton wool/g of nitrate N. This extremely low ratio may be attributed to the simultaneous oxidation of waste organic particles flowing out of the aquaculture tanks and trapped in the cotton wool filter medium. Since the solid cotton wool does not release organic residuals into the recirculating water, the new system is superior to systems that use a liquid carbon source. The degassing chamber that physically stripped the water of dissolved gaseous O<sub>2</sub> by means of a Venturi vacuum tube, prior to its passage through the denitrification biofilter, contributed to more efficient denitrification (no oxygen inhibition of denitrification and no aerobic consumption of cotton) and to the reduction of biofilter volume due to the increased process efficiency. Since denitrification could result in the formation of intermediate nitrite, the configuration places nitrification after denitrification, to ensure a reliable water quality for aquaculture systems. This design was shown to produce consistently good results over a long period with hardly any need to compensate for the loss of system water (except for evaporation losses), and with effluents having low nitrate levels (below 10 mg N/l). The overall treatment scheme, which also incorporates an aerobic nitrification biofilter and a granular filtration device, produces excellent water quality, having near-zero levels of ammonia and nitrite, low turbidity and a suitable pH.

#### Acknowledgements

This study was supported in part by research grant award MB-8707-04 from BARD, The US–Israel Binational Agricultural Research and Development fund. The authors wish to express their thanks to

Ms. Larissa Shemtov for her expert technical support and to Mr. Liron Friedman for husbandry and technical assistance.

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