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Environmentally sustainable land-based marine aquaculture

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ABSTRACT

Reduced fishery harvests and increased consumer demand for seafood have precipitated an increase in intensive fish farming, predominantly in coastal and open ocean net-pens. However, as currently practiced, aquaculture is widely viewed as detrimental to the environment and typical operations are vulnerable to environmental influences, including pollution and endemic diseases. Here we report the development of a land-based, marine recirculating aquaculture system that is fully contained, with virtually no environmental impact as a result of highly efficient biological waste treatment and water recycling. Over 99% of the water volume was recycled daily by integrating aerobic nitrification to eliminate toxic ammonia and, for the first time, simultaneous, anaerobic denitrification and anaerobic ammonium oxidation, to convert ammonia and nitrate to nitrogen gas. Hydrogen sulfide generated by the separated endogenous organic solids was used as an electron source for nitrate reduction via autotrophic denitrification and the remaining organic solids were converted to methane and carbon dioxide. System viability was validated by growing gilthead seabream (*Sparus aurata*) from 61 g to 412 g for a total of 1.7 tons in a record 131 days with 99% fish survival. Ammonia nitrite and nitrate did not exceed an average daily concentration of 0.8 mg/l, 0.2 mg/l and 150 mg/l, respectively. Food conversion values were 16% lower than recorded levels for net-pen aquaculture and saltwater usage of less than 16 l/every kg of fish produced. The system is site-independent, biosecure, devoid of environmental contaminants and is not restricted to a single species.

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1. Introduction

Anthropogenic activities such as intensive fishing, environmental pollution and marine and coastal habitat destruction, together with global climate change, have been adversely linked to a significant decrease in marine biodiversity (Dulvy et al., 2003; Halpern et al., 2008; Jackson et al., 2001; Lotze et al., 2006; Pandolfi et al., 2003; Worm et al., 2005). Human-driven erosion of marine biodiversity has recently been projected to lead to the collapse of all currently fished taxa by 2048 (Worm et al., 2006). Reversing this trend, restoring marine biodiversity, and meeting the ever-increasing global demand for seafood will require the integration of fisheries management (Pauly et al., 2002), pollution reduction (Lotze et al., 2006), habitat restoration (Lotze et al., 2006; Worm et al., 2006) and, to a large extent, development of environmentally sustainable marine aquaculture. Responding to the continuous decline in fishery harvests and in an effort to meet seafood consumption, aquaculture has become the world's fastest growing sector of food production, increasing nearly 60-fold during the last five decades (FAO, 2007). Currently, however, farmed marine species account for only 36% (3.2% for finfish) of the global shellfish and finfish aquaculture production (FAO, 2006) and provide only 11.5% (1.1% for finfish) of all seafood products, inclusive of fisheries and aquaculture. It is clear that in order to ease fishing pressures on marine stocks, the production of marine species (especially finfish) through aquaculture must be accelerated. Two primary obstacles impede the ecologically sustainable growth of marine aquaculture: its interaction with the environment, which is addressed in this study, and the use of fish proteins and oils as aquafeed components (Gatlin et al., 2007; Gyllenhammar and Hakanson, 2005; Naylor et al., 1998, 2000). Coastal net-pen and pond aquaculture facilities emit nutrients and chemicals into the marine environment (Gyllenhammar and Hakanson, 2005; Naylor et al., 2000) and impact wild stocks through interbreeding with escaped animals, ultimately leading to reduced fitness (McGinnity et al., 2003; Naylor et al., 2005). Additionally, these aquaculture practices pose the risk of feral stock establishment (Soto et al., 2001; Volpe et al., 2000), competition for resources between escaped and indigenous fish populations (Fleming et al., 2000; Soto et al., 2001; Volpe et al., 2001) and disease transmission from farmed to wild animals (Krkosek et al., 2005; Naylor et al., 2000). The future expansion of marine fish production through aquaculture largely depends on our ability to reduce the risk of these environmental interactions and impacts. Recirculating aquaculture systems (RAS) are one of the future platforms that offer a sustainable method for farming

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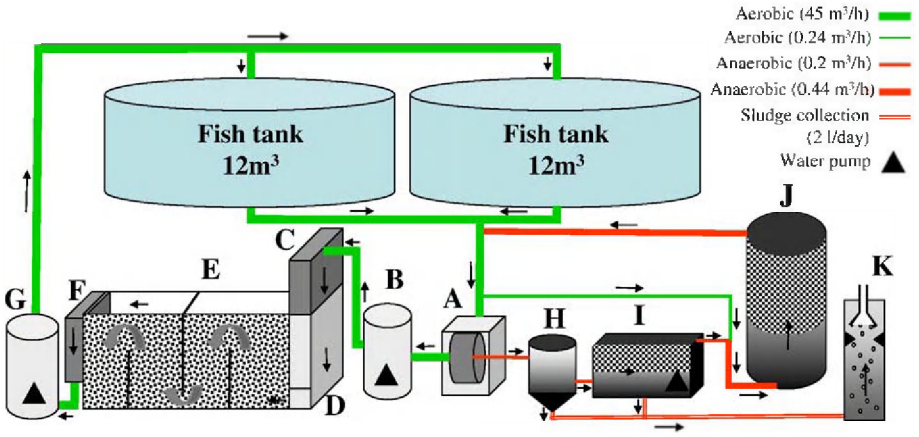

Fig. 1. Schematic configuration of the novel marine RAS. System components include: (A) 0.3 m^3 microscreen drum filter, (B) 0.4 m^3 pump reservoir, (C) 0.9 m^3 CO_2 stripper, (D) 1.5 m^3 protein skimmer, (E) 8 m^3 nitrifying moving bed bioreactor (MBB), (F) 1 m^3 low head oxygenator, (G) 0.6 m^3 pump reservoir, (H) 0.15 m^3 conical sludge collection tank, (I) 0.5 m^3 sludge digestion tank, (J) 3 m^3 denammox fixed-bed up-flow biofilter, (K) 0.02 m^3 biogas reactor with gas collection. Tank water was used to backwash organic solids from the microscreen drum filter (A).

marine and fresh water fish. RAS ability to effectively manage, collect and treat nutrient wastes that accumulate during the fish growth, is a key factor in their future development as mainstream environmentally sound fish production systems (Piedrahita, 2003; van Rijn, 1996).

Here we report the development of a viable, fully contained, land-based, marine RAS. The system discharges only a negligible amount of wastewater, approaching near-zero discharge. Moreover, it is biosecure and devoid of contaminants. It integrates aerobic and anaerobic microbial processes, including a novel combination of denitrification, anaerobic ammonium oxidation (anammox), and methanogenesis, to eliminate toxic inorganic nitrogen compounds and organic solids. As proof of system viability, a commercial species, gilthead seabream (*Sparus aurata*) has been produced at growth rates, food conversion values and production output beyond previously reported levels in commercial net-pen systems.

2. Materials and methods

2.1. System configuration

A schematic of the marine RAS used in this study and some of its characteristics are shown in Fig. 1 and Table 1. The system consists of two 12 m^3 fish tanks connected to an integrated modular filtration (IMF) module (Water Management Technologies, Baton Rouge, LA, USA) as the primary water treatment component. The IMF module integrates the life support components including a CO_2 stripper, protein skimmer, nitrifying moving bed bioreactor (MBB) and low head oxygen generator (LHO) for oxygen delivery (Fig. 1C–F, respectively). The nitrifying MBB was filled with 4 m^3 of polyethylene beads, 1cm in diameter and surface to volume ratio of 500 m^2/m^3. Ozone produced by an ozone generator (Pacific Ozone Technology, CA, USA) was injected directly into the protein skimmer at a rate of 10–20 l/min to disinfect the water and improve water quality parameters, including water turbidity. Water flow through the IMF module was set at a rate of 45 m^3/h, allowing approximately 2 cycles of tank water through the IMF module every hour.

The main aerobic water stream was connected to the anaerobic side loop, which was composed of two sludge tanks, an up-flow fixed-bed denammox biofilter and a biogas reactor (Fig. 1H–K, respectively). Sludge was collected with a microscreen drum filter (60 μm screen mesh; Hydrotech, Model 801, Vellinge, Sweden) and backwash system that used tank water (Fig. 1A). Sludge and backwash water were collected in the first sludge tank (Fig. 1H) and overflow water in the second (Fig. 1I). The second sludge tank contained 0.3 m^3 polyethylene beads (as described above) to promote solids retention, and serve as a substrate for bacterial colonization. Water retention time in the two sludge tanks was dependent on the frequencies of backwashing the drum screen filter, but averaged 2.5 h. Water overflowing from the second sludge tank was pumped simultaneously with tank water to the up-flow fixed-bed denammox biofilter. This configuration provided the denammox biofilter with nitrate-rich water from the main water loop, as well as sulfide, ammonia and dissolved organic compounds from the sludge collection and digestion tanks. The denammox biofilter contained 1.5 m^3 polyethylene beads (as described above) for solids retention and bacterial colonization. The flow rate in the denammox filter was 0.44 m^3/h for an average water retention of 6.8 h, or one volume of tank water recycled every 2 days. Liquid oxygen was dissolved in a low head oxygenator (Fig. 1F) and saturated water was mixed with water flowing back to the fish tanks. Oxygen concentrations in the system were continually monitored with a dissolved oxygen analyzer (Model 9100, Royce Technologies, NC, USA) and maintained at a minimum value of 5 mg/l. Redox potential (ORP) was monitored in the fish tanks by an online ORP analyzer (Model 5000, Royce Technologies, NC, USA) and ozone was automatically supplied to the protein skimmer at ORP values below 350 mV. System pH was maintained at 7.2±0.3 by automated addition of 20% (v/v) solution of sodium hydroxide (Black Stone pH Controller

Table 1
Characteristics of different components of the marine RAS

Compartment	Volume (m^3)[a]	Flow rate (m^3/h)	Retention time (h)
Fish tanks[b]	24	45	0.53
Nitrifying moving bed biofilter[c]	8	45	0.17
Denammox fixed-bed up-flow biofilter[d]	3	0.44	6.8
Sludge digestion tanks[e]	0.65	0.2	2.5
Pilot UASB reactor[f]	0.02	0.00008	250
Microscreen drum filter (60 μm)[g]	0.3	45	N.D
CO_2 stripper chamber[h]	0.9	45	0.02
Protein skimmer[i]	1.5	45	0.03
Low head oxygenator[j]	1	45	0.02

[a] Actual water volume.
[b] Dimensions: diameter 3.65 m, height 1.7 m.
[c] Two identical chambers with dimensions of: width 1.2 m, length 1.8 m, height 1.9 m.
[d] Dimensions: diameter 1.2 m, length 2.75 m.
[e] Two tanks were used: (1) diameter 0.5 m, height 0.8 m (2) width 0.85 m, length 1.2 m, height 0.5 m.
[f] Dimensions: diameter 0.15 m, height 1.15 m.
[g] Dimensions: width 0.6 m, length 0.8 m, height 0.5 m.
[h] Dimensions: width 0.4 m, length 1.3 m, height 1.8 m.
[i] Dimensions: width 0.4 m, length 1.3 m, height 2.1 m.
[j] Dimensions: width 0.45 m, length 1.7 m, height 1.3 m.

Table 2
Major microbial processes and location in the RAS anaerobic water treatment loop

Microbial processes	Process equation	Location
Sulfate reduction	$SO_4^{2-}+10H^++8e^-\rightarrow H_2S+4H_2O$	Sludge tank (Fig. 1I)
Autotrophic denitrification	$5H_2S+8NO_3^-\rightarrow 5SO_4^{2-}+4N_2+4H_2O+2H^+$	Denammox (Fig. 1J)
Heterotrophic denitrification	$5CH_3COO^-+8NO_3^-+3H^+\rightarrow 10HCO_3^-+4N_2+4H_2O$	Denammox Sludge tanks (Fig. 1H–J)
Anammox	$NH_4^++NO_2^-\rightarrow N_2+2H_2O$	Denammox (Fig. 1J)
Methanogenesis	$4H_2+H^++HCO_3^-\rightarrow CH_4+3H_2O$	Biogas reactor (Fig. 1K)

and Pump, Hanna Instruments, MA, USA). The system temperature was maintained at 26±1 °C. The system was filled with artificial seawater prepared by mixing the required salts from individual ingredients according to a formulation developed by scientists from the Center of Marine Biotechnology (Zohar et al., 2005); salinity was maintained between 15 and 17 g/l. Water lost to evaporation was replaced with freshwater.

2.2. System stocking and fish growth

5000 seabream fingerlings with an average weight of 0.5 g were shipped via air freight from Magan-Michael Hatchery, Israel. After growth to an average weight of 61 g in a nursery system, 4230 fish were transferred to the marine RAS and divided equally between the two 12 m^3 tanks. Fish growth was determined by averaging the weight of 50 fish from each tank every 2 weeks during the course of the experiment. Fish were fed with seabream diet produced by EWOS, Canada (www.ewos.com), containing 45% protein, 18% fat and 1.8% phosphorus. Pellet size was gradually increased according to fish size, starting with crumble feed for fingerlings and ending with 7 mm pellets for the final growth period. Feeding rate was gradually increased using timer-controlled shaking feeders (Sweeney, TX, USA) in distinct pre-set intervals from 6:00 AM to 4:00 PM. System photoperiod was set at 16 h daylight.

2.3. Chemical and physical analyses

Oxygen and temperature were monitored using a YSI temperature/oxygen probe (Model 57, Yellow Springs Instruments, Yellow Springs, OH, USA). Salinity was monitored via refractometer (Model S-10E, Atago, Japan). Total ammonia (TAN — NH_3 and NH_4^+) was determined by the hypochlorite oxidation reaction (Scheiner, 1976). Nitrite, sulfide and phosphorus were determined according to Strickland and Parsons (Strickland and Parsons, 1972). Sulfide concentration in the gas phase of the methane reactor was measured by dissolving 1 ml gas collected from the reactor gas trap in 10 ml of anaerobic distilled water in a 10 ml sealed glass tube. Nitrate was measured by ion exchange chromatography using a Dionex DX600 IC system with UV detection (Dionex UVD170). Total nitrogen, total phosphorus, and total suspended solids in the organic sludge were determined by Martel Laboratories, Inc. (Baltimore, MD) according to EPA methods 351.3, 365.2, and 160.3 respectively. Alkalinity was measured by titration with hydrochloric acid (Strickland and Parsons, 1972). Total ammonia and nitrite were measured daily. Sulfide, nitrate and alkalinity were measured weekly. Methane analysis was performed by gas chromatography (Hewlett Packard 5890A) using a flame ionization detector (Sowers and Ferry, 1983). All other gas analyses were performed by gas chromatography using a thermal conductivity detector in series with a methanizer (Model 510, SRI Instruments, Inc., Las Vegas, NV) and flame ionization detector. The column was 0.32 by 457 cm stainless steel that contained Carboxen 1000 (60/80 mesh; Supelco). The column oven was operated at 130 °C and N_2 was the carrier gas. All measurements were carried out in duplicate or triplicate with a maximum average deviation of 5%.

2.4. Anammox and denitrification activity assays

Anammox activity of the bacterial community in the denammox biofilter, was assayed by inoculating polyethylene beads (n=200) from the biofilter, into 500 ml glass conical flasks with 400 ml of autoclaved 15 ppt seawater. The flasks were supplied with ammonia, and nitrate at final concentrations of 15 mg/l and 35 mg/l respectively, sealed under nitrogen gas with gas-tight butyl rubber stoppers, and incubated in the dark with shaking (150 rpm) at 37 °C. Samples (5 ml) were periodically collected and analyzed for ammonia, nitrite and nitrate. Total moles of nitrate reduced by autotrophic denitrification were estimated from the total daily amount of hydrogen sulfide removed by the denammox biofilter using the stoichiometric equation (Table 2). This estimate was subtracted from the total moles of nitrate removed to estimate daily nitrate removal by the heterotrophic pathway.

3. Results and discussion

3.1. System design

The anaerobic loop (Fig. 1H–K; Table 1) that is part of the marine RAS described herein integrated complementary microbial processes (Table 2) that provided highly efficient treatment of toxic

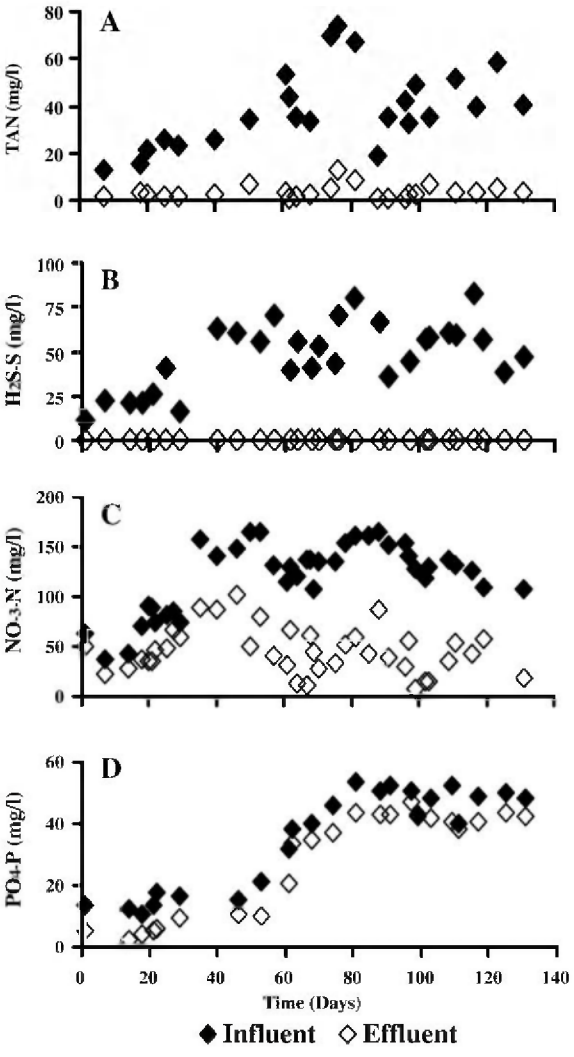

Fig. 2. Influent (♦) and effluent (◊) concentration of TAN (A), sulfide (B), nitrate (C), and phosphate (D) in the denammox biofilter.

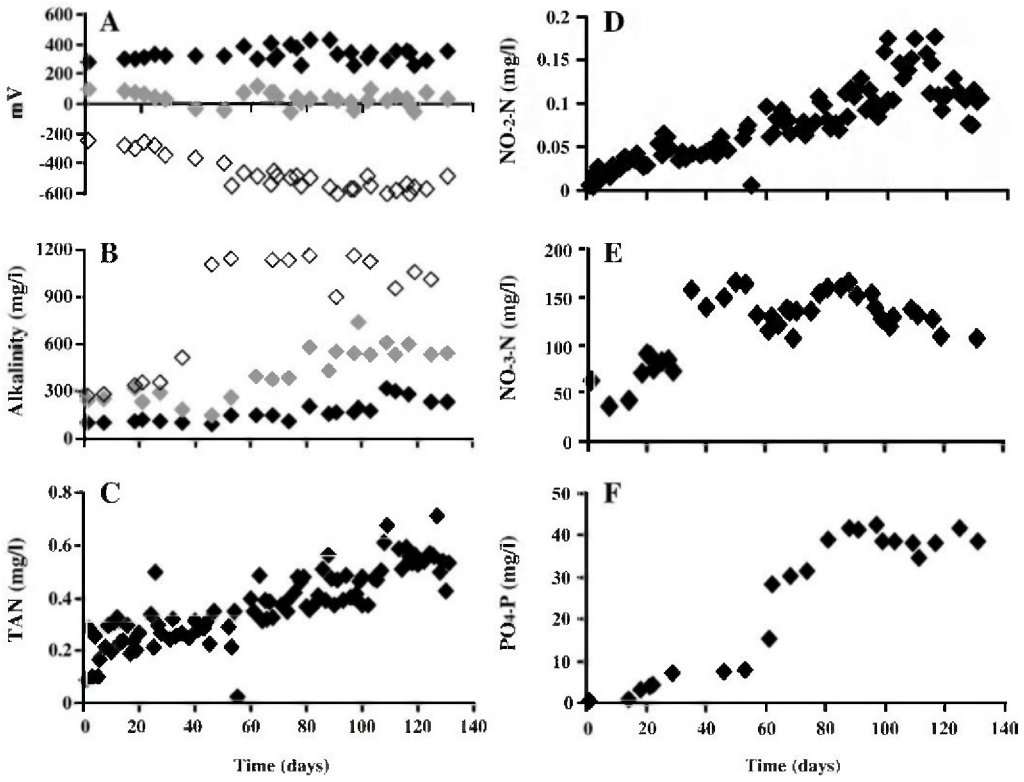

Fig. 3. Redox potentials (A) and alkalinity values (B) in fish tanks (♦), denammox biofilter (◆) and sludge tanks (◊) as well as concentrations of TAN (C), nitrite (D), nitrate (E) and phosphate (F) in the fish rearing water. Latter figures extracted from Fig. 2C and D, respectively.

nitrogen waste products and organic carbon waste produced during the fish growth cycle. It includes compartments for the anaerobic digestion of organic solids collected from the fish tanks, coupled to sulfate reduction (Fig. 1H, I), heterotrophic and autotrophic denitrification using organic compounds and sulfide as electron donors for nitrate reduction, as well as the anammox process (Fig. 1). Coupling anammox and denitrification processes in the same reactor resulted in the simultaneous uptake of sulfide, ammonia and nitrate (Fig. 2A–C) under anaerobic conditions, creating a process termed denammox (Pathak and Kazama, 2007; van der Star et al., 2007). Moreover, the organic sludge generated by the system was consumed partially as an endogenous carbon source for denitrification and the remaining sludge was converted to biogas in a methanogenic bioreactor (Fig. 1K). The combined processes ultimately reduced the total volume of discharged organic solids by more than 96% and resulted in the production of methane that may be harvested and used directly as an energy source. Since salt-laden sludge generated by the RAS cannot be used as fertilizer or landfill, these system components are essential for reducing the solid waste generated by a marine RAS. The unique configuration of the water treatment system promotes the formation of three distinct redox zones that support the complementary microbial processes necessary for complete water recovery (Fig. 3A); the aerated central fish culture tanks and nitrification biofilter were at the highest redox potential (400±100 mV) while the sludge digestion tanks were at the lowest (−500±100 mV), generating a redox gradient within the water treatment system of almost 1V.

The design of the marine RAS also provided autonomous maintenance of optimal water alkalinity and pH. Aerobic nitrification and fish respiration reduced alkalinity (as bicarbonate) and pH, whereas the anaerobic solids digestion and denitrification, increased alkalinity, ultimately balancing the system pH (Fig. 3B). This phenomenon has been described previously (van Rijn et al., 2006) and emphasizes the self-sustaining nature of the system.

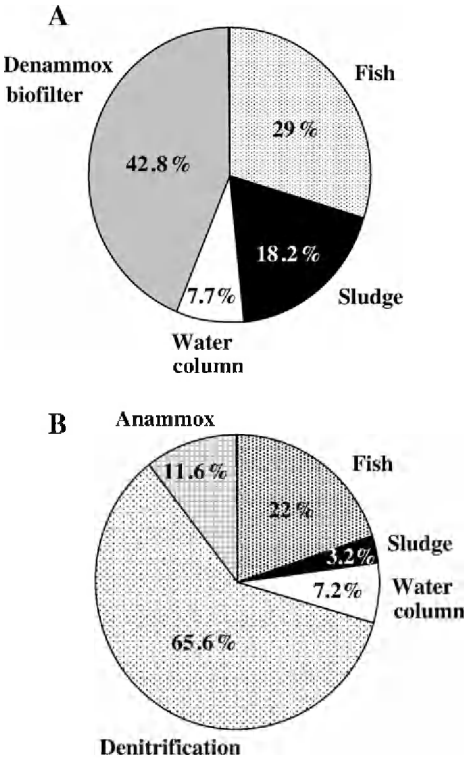

Fig. 4. Total phosphate (A) and nitrogen (B) mass balance during the complete fish growth trial (131 days). The total amount of P and N in the feed (32.5 kg and 130.1 kg respectively) that was used during the growout trial was considered as 100% of each nutrient. 97.7% of the total P and slightly over 100% of the total N could be accounted for via the different compartments and microbial processes.

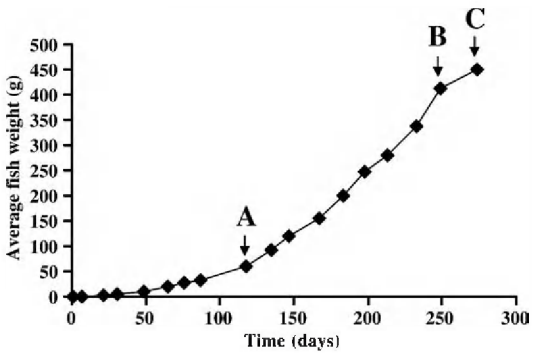

Fig. 5. Fish growth curve. The growout experiment in the marine RAS started at day 118 (A) with an average post-nursery fish weight of 61 g and extended for 131 days to day 249 (B) with an average weight of 412 g. Harvesting extended from day 249 to day 274 (C) of fish growth.

In addition to the levels of organic and inorganic nitrogenous waste in the system, accumulation of phosphate introduced through fish feed was also investigated. Although it is a major environmental pollutant, phosphate toxicity to fish is minimal even in high concentrations (Iwama, 1991). It has been reported that only 29% of total phosphate in fish feed is assimilated by fish (Lupatsch and Kissel, 1998). In the current study only 7.7% of the introduced phosphate accumulated in the water (Fig. 3F), with the remainder sequestered in organic solids that were collected in the sludge digestion tanks (18.2%) and the denammox biofilter (42.8%) (Figs. 2, and 4A). Thus, periodic removal of the inorganic solids that accumulate in the denammox biofilter, followed by full digestion of sludge in the biogas reactor, allowed for the collection and safe disposal of phosphate as part of the negligible amount of inorganic solids that leave the system.

3.2. System performance

A growout trial with gilthead seabream (*S. aurata*) was conducted using the above land-based marine RAS to test its ability to produce marine fish in a sustainable and efficient manner. A popular high-value marine fish species with declining landings (FAO, 2006), seabream is commonly produced in net-pen farms across the Mediterranean Sea. In North America, seabream is a non-native species that can be grown only in biosecure systems such as the RAS. As a result of this restriction, there is no commercial production of seabream currently in the US. Two 12 m^3 culture tanks were set to an optimal temperature of 26±1 °C, salinity of 15±1 ppt and oxygen concentration of ≥5 mg/l. The tanks were stocked with 4230 post-nursery fish with an average weight of 61 g. Harvesting began after 131 days at an average weight of 412 g, maximum fish density of 73 kg/m^3 and fish survival rate of 99%. The fish reached an average market size of 450 g after 153 days and the overall fish growth cycle from 0.45 to 450 g, including the nursery phase, was 274 days (Fig. 5, Table 3). These growth rates are up to 85% faster than the 14–17 months necessary, depending on location and strain, to reach a similar size in net-pen systems that are considered the industry standard (Theodorou, 2002). During the 131-day growth trial period in the marine RAS system, the average food conversion ratio (FCR) was 1.2 (Table 3). FCR reflects the relative efficiency at which fish assimilate feed (a weight unit of feed required to produce a weight unit of fish). In comparison, the accepted FCR value for seabream grown in coastal net-pens is 1.8 (Theodorou, 2002). The high growth rate and low FCR values of the seabream in this study reflect the fact that water parameters including inorganic nitrogen, oxygen, temperature, and salinity were tailored and controlled to meet optimal requirements of seabream throughout the growth cycle. Moreover, the continuous long day photoperiod regimen (16 h light) implemented during the growth cycle is known to delay the onset of sexual maturation and gonadal growth, resulting in greater fish yields (Kissel et al., 2001; Norberg et al., 2001). As much as 1000 kg of the produced fish was distributed to elite seafood restaurants in the Baltimore-Washington D.C. area. Feedback on fish quality parameters including texture and flavor was positive from all participating restaurants without exception. Polychlorinated biphenyls (PCBs) and mercury analyses (Lancaster Laboratories, PA) showed no detectable accumulation of mercury (<0.05 mg/kg) or PCBs (<8.4 μg/kg) in the fish tissues. Despite the high biomass of fish (73 kg/m^3), over 99% of the culture water volume of 40 m^3 was recycled per day. A total of 22 m^3 of salt water was lost due to system leaks and fish handling (0.36% daily), which may be reduced further with system improvements, and 2.35 m^3 was lost due to sludge removal (0.05% daily). The 26.2 m^3 freshwater lost due to evaporation (0.5% daily), which does not pose an environmental hazard, was replaced by municipal (fresh) water, thus adding only negligible cost to the operation. Despite the high degree of water re-use and the high fish biomass, water quality parameters throughout the fish growth cycle were well below concentrations considered stressful to marine fish. Ammonia and nitrite did not exceed an average daily concentration of 0.8 mg/l and 0.2 mg/l, respectively, throughout the growout period (Fig. 3C,D).

Table 4
Nitrogen removal parameters for the different water treatment compartments

Compartment	Areal removal rate (g/m^2/day)	Hydraulic loading rate (m^3/m^2/day)	Daily removal rate (g/day)
Nitrifying moving bed biofilter	0.6±0.05 (TAN)	316	1200±100 (TAN)
Denammox fixed-bed up-flow biofilter	0.7±0.2 (NO_3^-–N) 0.25±0.1 (TAN)	9.3	530±130 (NO_3^-–N) 200±60 (TAN)
Sludge digestion tanks	−4.1±0.8 (NO_3^-–N)	2.1	620±110 (NO_3^-–N)

Table 3
Fish performance parameters during 131-day growth trial in the marine RAS

Day	Total fish number	Average weight (g)	Total fish weight (kg)	Average fish density (kg/m^3)	Daily feed intake (kg)	% of daily feed from fish body weight	[a]GR g/day	[b]SGR %/day^{-1}	[c]FCR
1	4230	61	258	10.8	5	2.0			
14	4230	93	393.4	16.4	8	2.0			
26	4230	120	507.6	21.2	11	2.2			
46	4230	154.5	653.5	27.2	12	1.8	2.03	2	1.05
62	4230	199.9	845.6	35.2	15	1.8			
77	4230	247.1	1045.2	43.6	17	1.6			
92	4230	280.5	1186.5	49.4	18	1.5	2.74	1.3	1.20
112	4230	338.4	1431.4	59.6	20	1.4			
131	4230	412	1742.8	72.6	22	1.3	3.37	1	1.35

[a] GR — Growth Rate.
[b] SGR — Specific Growth Rate.
[c] FCR — Food Conversion Ratio.

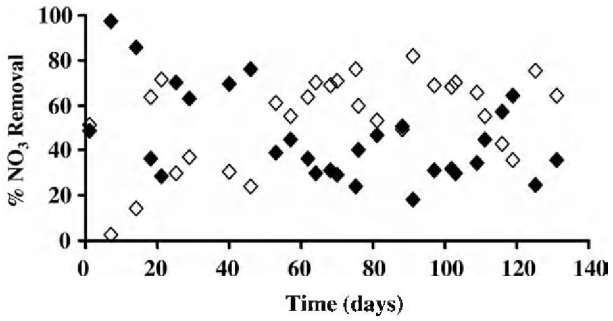

Fig. 6. Nitrate removal through autotrophic (♦) and heterotrophic (◊) denitrification in the denammox biofilter during the 131-day experiment (Fig. 4). Autotrophic nitrate removal was calculated according to the stoichiometric denitrification equation with hydrogen sulfide as an electron donor (see Materials and methods).

Although threshold limits may be species- or stage-specific and may vary due to tank system influences, marine species such as seabream can safely tolerate ammonia levels as high as 1.7–2.5 mg/l unionized ammonia (39–57 mg/l TAN; Person-Le Ruyet et al., 1995) and nitrite levels up to 200 mg/l (200 ppm; Parra and Yufera, 1999) if environmental conditions are otherwise optimal. Nitrate concentrations increased during the first 50 days and stabilized at an average value of 150 mg/l for the remainder of the experimental period (Fig. 3E). Elimination of nitrate occurred in the sludge tank and denammox biofilter (Table 4). Production of sulfide by sulfate-reducing bacteria in the sludge digester tank (Fig. 1I) was mitigated by stimulating autotrophic denitrification activity using the sulfide as an electron donor for nitrate reduction (Fig. 2B). The sulfide-dependent autotrophic denitrification in the denammox reactor dominated the heterotrophic denitrification during the low feeding rate period from day 1 to day 50, reducing a daily average of nearly 60% of the total nitrate produced. In contrast, during the high feeding rate period from day 50 to day 130, the organic carbon-dependent heterotrophic denitrification dominated the autotrophic pathway (Fig. 6). This pattern demonstrated the complementary nature of the two processes, which together stabilized the system nitrate concentration associated with increased feeding rates during fish growth. Ammonia removal processes occurred simultaneously within the aerobic moving bed biofilter via nitrification and within the anaerobic denammox biofilter via the anammox reaction (Table 4). We previously identified anammox bacteria as part of the microbial community associated with the marine denitrification biofilter (Tal et al., 2006) and demonstrated integration and activity of anammox as an important pathway for nitrogen removal in the marine RAS. Incorporating the anammox process into the anaerobic water loop enables the treatment of ammonia produced during the degradation of organic sludge without the need for increasing the capacity of the more energy-consuming aerobic nitrification biofilter. Lab-scale short-term incubations of microbial consortia taken from the denammox biofilter

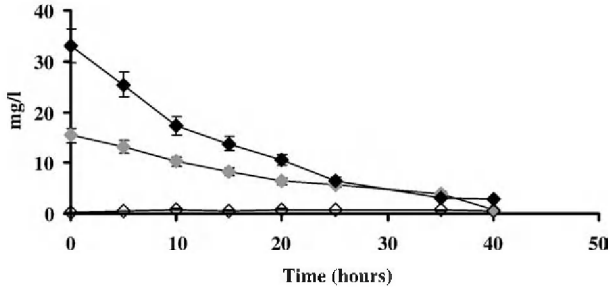

Fig. 7. Anaerobic incubation of 100 biofilm-covered polyurethane beads removed from the denammox biofilter, demonstrating anammox activity by simultaneous ammonia (●) (TAN) and nitrate (♦) removal with no nitrite (◊) accumulation. Control incubation with only ammonia showed no ammonia uptake (data not shown).

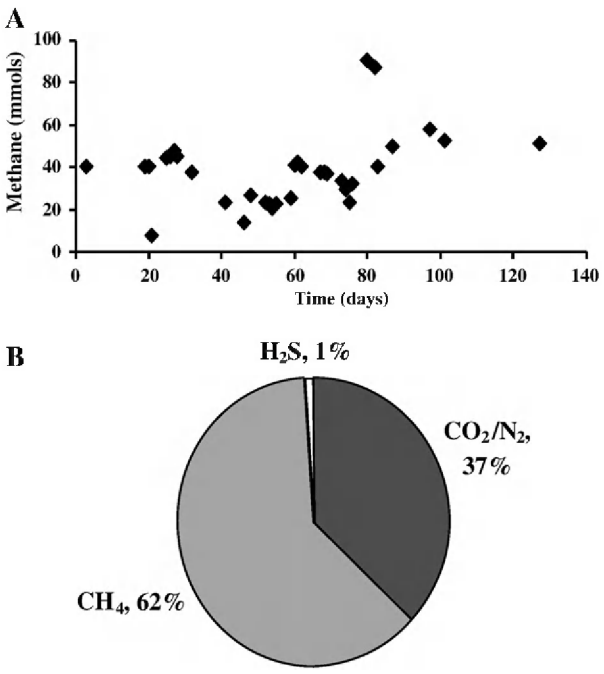

Fig. 8. Methane production by the pilot scale biogas reactor throughout the growth trial (A) and an analysis (B) of the biogases collected from the head space of the reactor.

confirmed the concurrent consumption of ammonia and nitrate (Fig. 7). Recent studies have demonstrated the simultaneous activity of anammox and denitrifying bacteria in natural or man-made environments (Trimmer et al., 2005). These studies suggest that some of the intermediary compounds in the denitrification pathway may "leak" out from the cell into the surrounding environment and provide a substrate, i.e. nitrite, for the anammox process. Thus, the parallel uptake of ammonia and nitrate in the denammox biofilter strongly supports this concept and demonstrates the dependence and coexistence of the two processes in the anaerobic environment. The nitrogen budget for the entire growout experiment (Fig. 4B) indicated that 11.6% of nitrogen introduced to the system through fish feed was removed by the anammox process while the majority was removed by denitrification processes.

The 1807 kg of fish feed used throughout the experiment consisted of 130.1 kg nitrogen (7.2% N) and 32.5 kg phosphorus (1.8% P). Of the total nitrogen in the feed, an estimated 28.6 kg was retained in fish (Lupatsch and Kissel, 1998), 4.2 kg was recovered in the sludge and 9.3 kg accumulated in the water column. Approximately 85.4 kg of the nitrogen was removed by denitrification processes and 15.1 kg by the anammox process (Fig. 3B). Of the total phosphorus in the feed, 9.4 kg was retained in the fish, 5.9 kg in the sludge, 2.5 kg in the water column and 13.9 kg accumulated in the denammox biofilter sequestered in organic particles and bacterial biomass (Fig. 3A). The total recovered nitrogen exceeded the estimated nitrogen in the feed (142.6 kg/109.6%) and the total recovered phosphorus was slightly less than the estimated total (31.7 kg/97.7%).

3.3. Utilization of organic solids

The collection and digestion of organic solids (uneaten feed and fish feces) as part of the water treatment system is one of the unique characteristics of this marine RAS. This feature not only provides an endogenous carbon source for denitrification, but also excess organic carbon remaining after the denitrification process is converted to methane gas as the final product of the sludge digestion process. The pilot biogas reactor, fed with partially

digested material from the two sludge tanks, maintained relatively constant (Fig. 8A) and efficient production of biogas, achieving greater than 60% (v/v) conversion to methane throughout the fish growout phase (Fig. 8B). Moreover, greater than 80% (v/v) of the solid waste introduced into the biogas reactor was digested to gaseous product (predominantly methane and carbon dioxide), significantly reducing the volume of sludge from the system. Although methanogenic waste digestion has been well characterized in non-marine systems such as freshwater sediments, municipal digestors and ruminants, there are fewer reports on marine methanogenesis. This is largely due to the general misconception that methanogenesis does not have a significant role in seawater due to competition from faster growing sulfate-reducing bacteria, which have a lower K_s for the same electron donors. However, marine methanogens compete successfully in habitats possessing excess substrate, i.e. an organic carbon source (Sowers and Ferry, 2002). It is generally presumed that a COD/SO_4^{2-} ratio higher than 10 will eliminate the inhibitory effect of sulfate-reducing bacteria on methanogenesis (Hulshoff Pol et al., 1998; Pind et al., 2003). In the current study, the COD/S_4^{2-} ratios ranged from 25–55, which is much higher than the reported threshold level for inhibition. As a result, the RAS bioreactor yielded efficient production of methane from the saline sludge. Throughout the entire experiment, 2.61 m^3 of sludge containing 3% solids (w/v) accumulated in the two sludge tanks. The 20-liter bench-scale biogas reactor digested only 0.26 m^3 of the total sludge generated by the system and produced an average of 40 mmol methane/day (Fig. 8A). Based on our data, a full-scale operation that will allow full treatment of the net accumulating sludge after denitrification removal will require a 100-liter biogas reactor generating 26 mol of methane gas for every 1000 kg of fish produced. Based on the technology described, only 10.5 kg of dry organic solids will need to be removed from the system for every 1000 kg of fish produced. This is only 2.8% of the estimated 375 kg of dry organic solids generated from the same amount of fish produced in a RAS using currently available technology, based on FCR of 1.5 and 75% feed uptake by the fish (Timmons et al., 2002). In addition to its low organic output, the system also minimizes saltwater usage. During 131 days of fish growth, only 16.4 l of 15 ppt saltwater was lost for every kilogram of fish produced. These numbers are extremely low compared to commercial or even lab-scale RASs that discharge hundreds and thousands of liters for every kilogram of fish produced (Piedrahita, 2003; Suzuki et al., 2003).

4. Conclusions

Integrating an array of aerobic and anaerobic microbial-mediated processes within a commercially available platform provided efficient production of high densities of marine fish in a system that is biosecure, with negligible input to the environment. This system is also generic, contaminant- and pathogen-free. Moreover, because the system does not rely on the availability of a natural saltwater source, its location can instead be determined by economic considerations such as proximity to markets, further reducing the carbon footprint of the operation. Considered together, the described system's superior performance and multiple benefits will drive its economic feasibility as a viable alternative for the production of high quality seafood with minimal environmental impact. This advancement in the development of a fully contained land-based marine production system provides an attractive alternative to current aquaculture practices that is both biosecure and ecologically sustainable.

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