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Phosphorus removal in a marine prototype, recirculating aquaculture system

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Abstract

Phosphorus dynamics were examined in a prototype, zero-discharge, marine-recirculating system. Operation of the system without discharge of water and sludge was enabled by recirculation of effluent water through two separate treatment loops. Surface water from the fish basin was pumped over a trickling filter in one loop, while bottom-water was recirculated through a sedimentation basin followed by a fluidized bed reactor in the other treatment loop. Ammonia oxidation to nitrate in the trickling filter and organic matter digestion together with nitrate reduction in the sedimentation basin and fluidized bed reactor were the main biological features of this treatment system. Orthophosphate concentrations did not exceed 15 mg PO_4–P/l in the culture water during more than 1 year of system operation. Much of the phosphorus was retained within the sedimentation basin and fluidized bed reactor. In these treatment stages, the phosphorus content of organic matter was as high as 17.5% and 19%, respectively. High concentrations of total phosphorus and low concentrations of soluble orthophosphate were measured in the initial stages of sedimentation under oxic and anoxic conditions, suggesting that most of the phosphorus was associated with organic matter. Depletion of oxygen and nitrate in the sludge layers of the sedimentation basin coincided with sulfate reduction to sulfide and a release of soluble orthophosphate. The observed phosphorus dynamics in this marine system supported findings from previous studies in which it was demonstrated that denitrifiers underlie phosphorus immobilization under these conditions.

Keywords: Recirculating system; Mariculture; Phosphate removal; Denitrification; Anaerobic water treatment

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1. Introduction

Eutrophication of water bodies is one of the greatest concerns associated with the discharge of effluents from municipal wastewater facilities, food processing, and agricultural point and nonpoint sources. Nitrogen and phosphorus, discharged with these effluents, cause the enhanced growth of organisms associated with eutrophication. In freshwater ecosystems (Reddy et al., 1993; Sharpley et al., 1994), as well as in some seawater environments (Krom et al., 1991), phosphorus is the limiting nutrient for algal growth and thus, it is this nutrient that needs to be removed.

Principal sources of aquaculture wastes are uneaten feeds and excreta. The bulk of this waste is in the particulate form. In semiclosed, recirculating systems, this waste is removed by gravitational or mechanical methods in a concentrated form (Chen et al., 1994). Dissolved organic and inorganic nutrients, which make up the remaining fraction of the total waste in these systems, are generally discharged with the effluent water. Because ammonia is toxic to fish, it is often converted to relatively harmless nitrate by bacterial nitrification. As a result, nitrate is present at high concentrations in the effluent of semiclosed, recirculating systems (Ingolfsson, 2001). Much less is known about phosphorus cycling in such systems than is known about nitrogen cycling. In general, phosphorus effluent concentrations are high because much of the phosphorus added with the feed is unutilized by the fish (Rodehutscord and Pfeffer, 1995). Moreover, appropriate methods for phosphorus removal in such systems have not been developed.

Traditional methods for phosphorus removal from wastewater are based on chemical precipitation of phosphorus with mainly iron and aluminum salts. However, due to the technical and economical constraints of these chemical processes, the use of alternative, biological treatment methods is steadily increasing. Enhanced biological phosphorus removal (EBPR) is the most common biological phosphorus-removal method. The method is based on enrichment of so-called "polyphosphate accumulating organisms" (PAOs) through sludge recycling between anaerobic and aerobic or anoxic (with nitrate) zones (Toerien et al., 1990). Under anaerobic conditions, these PAOs convert acetate or other low-molecular organic compounds to polyhydroxyalkanoates (PHA), with concomitant degradation of polyphosphate and glycogen and release of phosphate. Under aerobic or anoxic conditions, PHA is converted to glycogen, phosphate is assimilated, and polyphosphate is intracellularly produced. Under the latter conditions, bacterial growth and phosphate uptake are regulated by the energy released from the breakdown of PHA (Mino et al., 1998). A different mechanism of phosphate removal by denitrifiers was described by van Rijn and Barak (2000) and Barak and van Rijn (2000). It was found that denitrifiers are capable of phosphorus storage in excess of their metabolic requirements under either oxic or anoxic conditions. Unlike PAOs, phosphate uptake by these denitrifiers does not require switches between aerobic/anaerobic conditions and, rather than PHA, organic carbon serves as their energy and carbon source.

In the present study, results are presented on the phosphorus dynamics in a zero – discharge, marine-recirculating system.

2. Materials and methods

2.1. General description of the experimental system

The pilot plant, situated at the Faculty of Agriculture, Rehovot, Israel, consisted of a fish culture tank, a trickling filter, a foam fractionator, a sedimentation basin, and a fluidized bed reactor. The system was operated in a complete, closed mode, i.e. neither water nor sludge was discharged. Tap water compensated for water losses resulting from evaporation and leakage. Red tilapia hybrids (*Oreochromis niloticus* × *Oreochromis aureus*) were cultured for the first 167 days of operation and were replaced by gilthead seabream (*Sparus aurata* L.). The latter marine fish was cultured for an additional 225 days. The system was initially filled with tap water. During the tilapia culture period, salinity was gradually increased by the addition of sea salt (Red Sea pHarm, Israel). Salinity levels of the culture water were: 0 ppt (until day 90), 5 ppt (days 90–105), 10 ppt (days 105–125), 15 ppt (days 125–135), and 20 ppt (from day 135 onward). Salinity was kept at 20 ± 2 ppt during culture of gilthead seabream. General performance parameters with respect to water quality and fish growth of the system during the reported experimental period have been recently submitted (Gelfand et al., submitted for publication).

2.2. System configuration

A schematic presentation of the system is depicted in Fig. 1. The main components of the system are: (i) a polyethylene, round fish-culture basin with a sloping bottom (upper diameter: 2.1 m; volume: 2.3 m^3; Aquatic Eco – Systems, USA; catalogue number: TP650), (ii) a polyethylene sedimentation basin (dimensions: 1.8 m length × 0.8 m width × 0.8 m depth; working volume: 0.4–0.8 m^3), (iii) a trickling filter and (iv) a

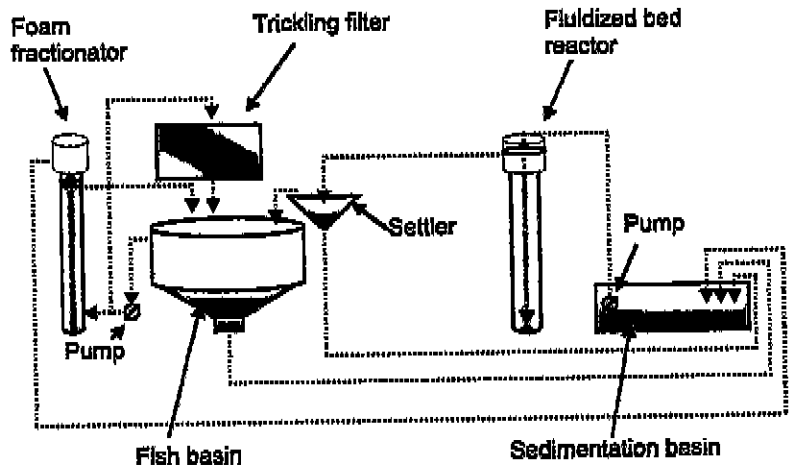

Fig. 1. Schematic presentation of the zero-discharge mariculture system at Rehovot (not to scale).

fluidized bed reactor (FBR). The trickling filter (dimensions: 1.2 m length × 0.6 m width × 1.4 m height; volume: 1 m^3) contained PVC cross – flow medium with a specific surface area of 240 m^2/m^3 (Jerushalmi, Israel). The FBR (total height: 198 cm) consisted of a Perspex column that was widened at the top (column diameter: 6.1 cm; diameter, upper 25 cm of the column: 23 cm). The total working volume of the reactor was 6.25 l. The reactor was filled with 1.5 kg of sand (>97% SiO_2), serving as bacterial carrier material, with a diameter ranging from 0.8 to 1.5 mm (at most 15% of grains >1.4 mm and 10% of grains <0.85 mm). Water from the upper layers in the fish basin was pumped over the trickling filter at a rate of ± 30 l/min (hydraulic loading: 60.0 m/day). Water was continuously withdrawn into the sedimentation basin by gravitation through a standpipe from the bottom – center of the fish culture basin fitted with a double drain (Aquatic Eco – Systems; catalogue number: D2). Water from the upper layers of the sedimentation basin was pumped at a rate of 5–7 l/min into the FBR (hydraulic loadings of FBR: 107–150 m/h), through a vertical pipe extending from the top to ± 3 cm above the base of the reactor. From the FBR, water was returned by gravitation to the fish basin after passing a rectangular settling deck (0.96 × 0.51 m; Aquatic Eco – Systems; catalogue number: FFPT) for removal of particulate matter.

Once a day, organic matter, captured in the settling deck, was diverted back into the sedimentation basin. Air was added by means of a 0.55-kW air blower (model: R4110 – 2; Gast, USA) and led into the fish basin by means of three airlifts distributed at intervals along the perimeter of the fish basin. At day 315 into the experimental period, pure oxygen was added to the fish basin by means of a 0.15-kW oxygen generator (model: 1498; SeQual, USA). Dissolved and suspended particles were removed from the water in the fish basin by means of a foam fractionator (model: TF8AZ; Top Fathom, USA) in which inlet water was derived from the upper water layers in the fish basin at a rate of 30 l/min. Effluent water was led back into the fish basin, while foam produced in the fractionator was diverted into the sedimentation basin. Water velocity in the fish basin (approximately 20 cm/s in the upper water layer close to the basin wall) was generated by: (i) diverting some of the trickling filter inlet stream directly into the fish basin, perpendicular to the basin's radius and (ii) similarly directing the outflows from the airlifts and foam fractionator.

2.3. Nutrient profiles in sedimentation basin

Profiles of oxygen, nitrate, sulfate, sulfide, phosphate and total phosphorus were determined in the sedimentation basin (Fig. 2) in three stations (in, middle and out), at three defined depths (top—14 cm from bottom; middle—7 cm from bottom; and bottom—1 cm from bottom). The various stations were sampled for three consecutive days towards the end of the experimental period (days 385, 386 and 387) by means of a peristaltic pump with a silicon tube connected to a metal bar with fixed markings displaying the sampling depths.

2.4. Quantitative and qualitative phosphorus analyses

Triplicate samples from the fluidized bed reactor (5 g biofilm – coated sand), the trickling filter (6 cm^2 of biofilm – coated PVC), the sedimentation basin (3 ml sludge), and water in the system (2 l) were examined for dry weight and total phosphorus content. Total

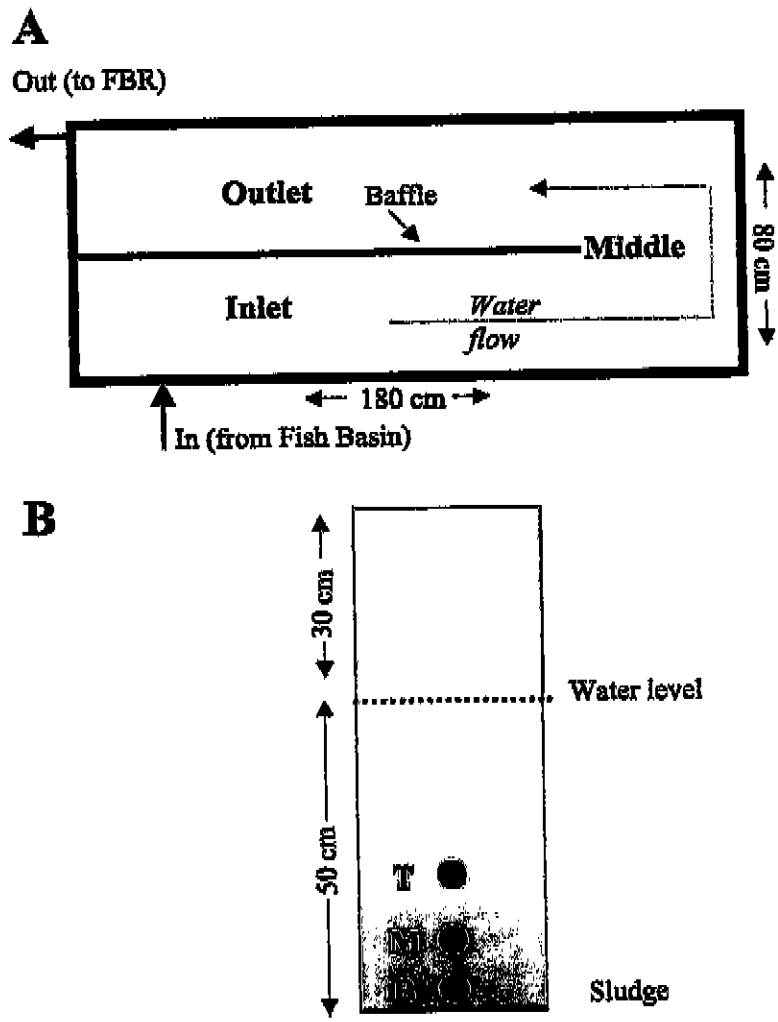

Fig. 2. (A) Top view and (B) side view of sedimentation basin indicating dimensions and horizontal (inlet, middle and outlet) and vertical positions (T = top; M = middle; B = bottom) of sampling points.

phosphorus in each of the treatment components was extrapolated based on the following information: total sand, dry weight in the fluidized bed reactor: 1.5 kg; total surface area of PVC in the trickling filter: 240 m^2; total sludge, dry weight in the sedimentation basin: 20 kg; and total water volume in the system: 2.7 m^3.

2.5. Phosphate-release test

Samples (100 ml sludge) were derived from three depths at three different sampling stations within the sedimentation basin (see Fig. 2 for position of sampling station). Sludge

was washed twice with filtered water (GF/C, Whatman, UK) derived from the fish basin. Sludge was resuspended in this water, supplemented with trace metals (Visniac and Santer, 1975) and incubated for 96 h in BOD bottles under anaerobic conditions at 30 °C. During the incubation period, phosphate and nitrate were determined at 24-h intervals. The test was conducted on triplicate samples from each sampling point. Differences between the mean values were determined by the Student's *t*-test.

2.6. Analytical procedures

Oxygen and temperature were measured with a YSI (model 57) temperature/oxygen probe (Yellow Springs Instruments, USA). Nitrate and sulfate were measured using a Quick Chem Ion Analyzer (Lachat Instruments, Milwaukee, USA). Sulfide was analyzed by the methylene blue method according to Cline (1969). Inorganic orthophosphate (phosphate throughout the text) in filtered samples was determined with the ascorbic acid method described by Golterman et al. (1978). Total phosphorus (organic, particulate, and inorganic orthophosphate) was determined by the same method after conversion to inorganic phosphate, by digestion of unfiltered samples with a mixture of sulphuric acid and potassium persulphate. Dry weight of particulate material was determined after overnight drying at 105 °C.

3. Results

3.1. Phosphorus changes in the system over the experimental period

Despite the closed mode of operation, dissolved phosphate concentrations in the fish basin were stable and only fluctuated between 7 and 15 mg PO_4–P/l (average concentration: 8.2 ± 1.8 mg PO_4–P/l, $n=91$) over the experimental period (Fig. 3). Total phosphorus in the various treatment compartments showed that phosphorus did not accumulate in the trickling filter and fluidized bed reactor (Fig. 4). In contrast, phosphorus accumulated with time in the sedimentation basin. It was estimated from the data presented in Fig. 4 that, by the end of the sampling period, particulate-associated phosphorus in the sedimentation basin accounted for 76% of the total phosphorus in the system. An additional 13% of the total phosphorus in the system was present in the fluidized bed reactor. It should be noted, however, that the total phosphorus contents are mere estimations as they were based on subsamples derived from each of the compartments. Especially in the sedimentation basin, where phosphorus content was determined in sludge samples derived from regions close to the outlet of the basin, large spatial variations were found in the phosphorus content of sludge (see below).

3.2. Phosphorus dynamics in the sedimentation basin

More detailed information on phosphorus dynamics within the sedimentation basin was obtained by measurements of phosphate and total phosphorus concentrations, as well as those of the main electron acceptors (oxygen, nitrate and sulfate), during three consecutive

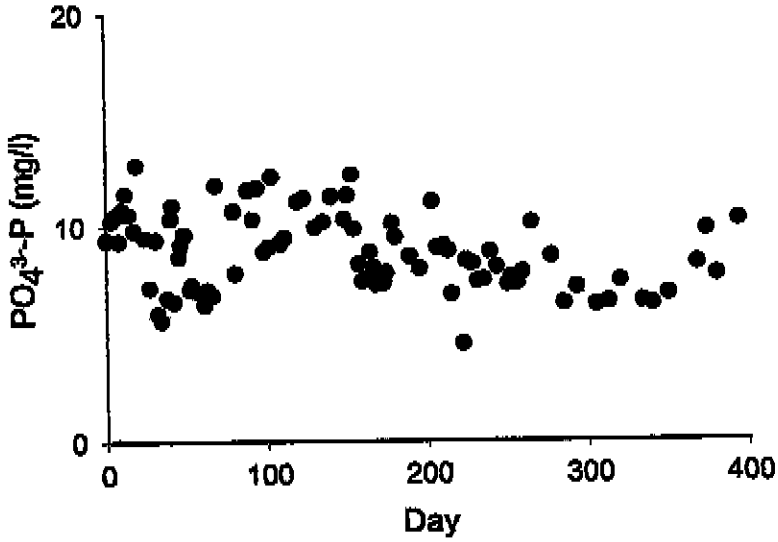

Fig. 3. Orthophosphate concentrations in the fish basin over the experimental period.

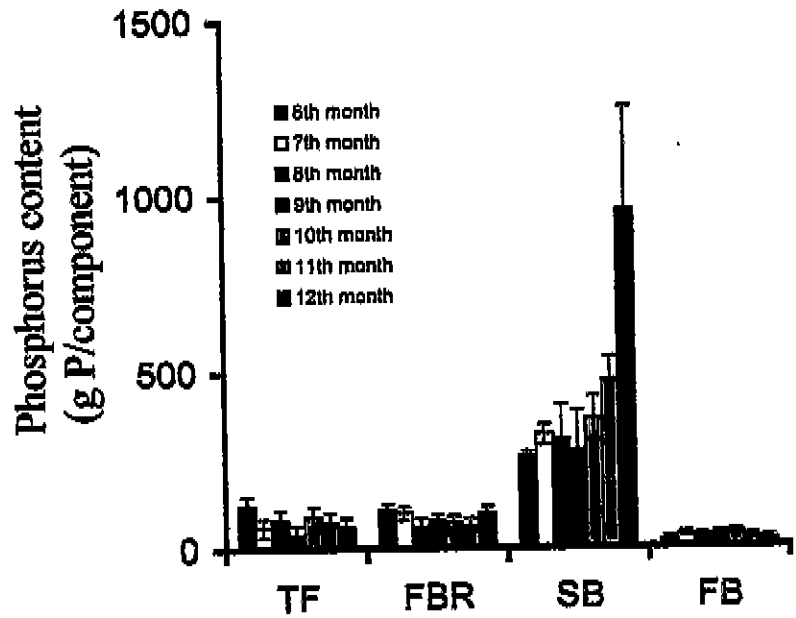

Fig. 4. Total phosphorus content in different components of the fish culture system at selected days. The seven columns for each treatment compartment represent seven consecutive monthly samplings, extending from 6 months after start-up until the end of the experimental period. Error bars represent standard deviation between means. (TF: trickling filter; FBR: fluidized bed reactor; SB: sedimentation basin; FB: fish basin.)

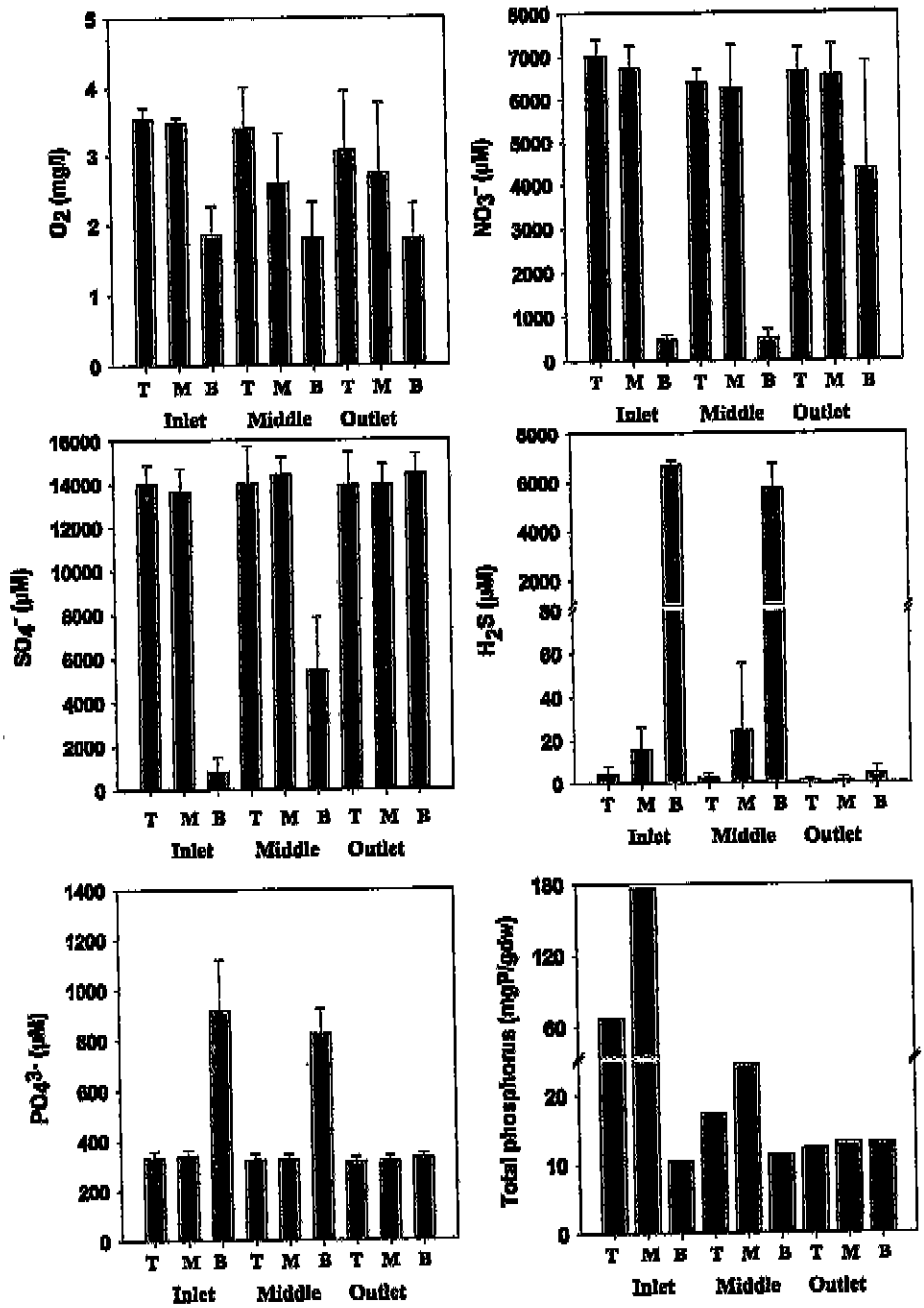

Fig. 5. Fluctuations in nutrients within the sedimentation basin at different sampling points (see Fig. 2). Each column represents the mean value (± standard deviation) of samples derived from each sampling point during three consecutive sampling days (days 385, 386 and 387).

days at three different sampling stations and at three different depths (see Fig. 2 for position of sampling stations). Lowest oxygen, nitrate and sulfate concentrations were found in the sludge layer at the inlet and middle sampling stations (Fig. 5). At these stations, sulfide and dissolved phosphate concentrations were high. The total phosphorus content of organic matter in the sedimentation basin was measured once and was found to be highest in samples derived from the upper layers of the sampling station closest to the basin inlet (6.6% and 17.5% P of sludge dry weight at stations inlet-top and inlet-middle, respectively). The lowest phosphorus and highest phosphate concentrations were found at sampling points where anaerobic respiration was high (inlet-bottom and middle-bottom).

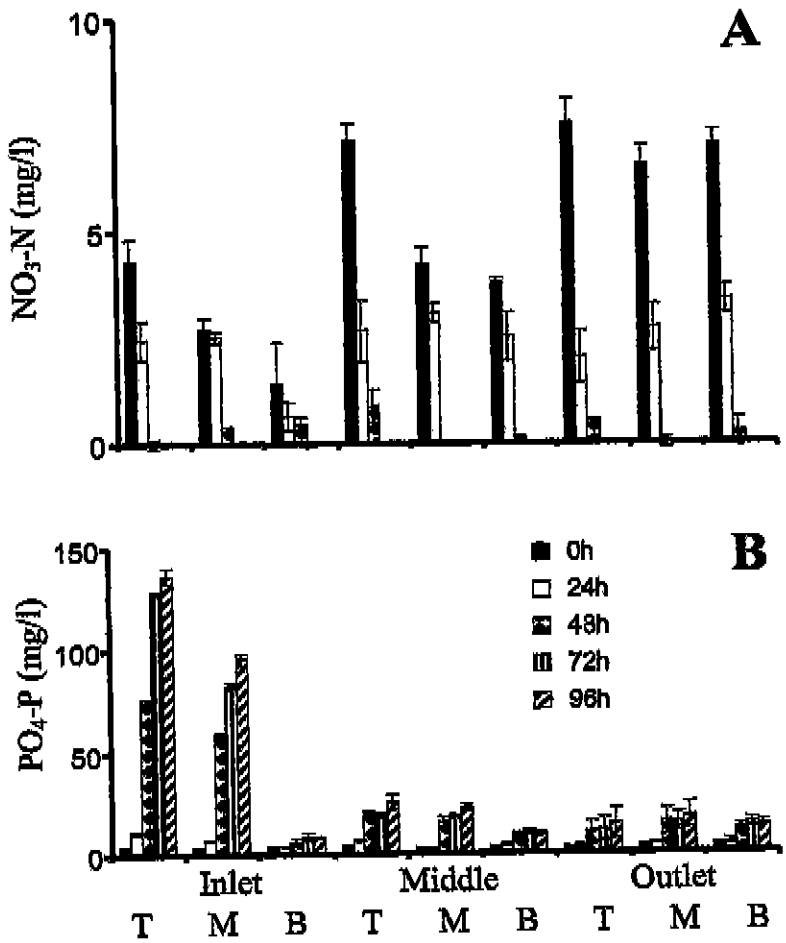

Fig. 6. Changes in (A) nitrate and (B) phosphate concentrations during laboratory incubation of sludge collected from different sampling points (see Fig. 2) in the sedimentation basin. Total initial phosphorus content of sludge is shown in Fig. 5. The five columns for each of the different samples represent nitrate and phosphate concentrations after 0, 24, 48, 72, and 96 h of incubation. Error bars represent standard deviation between means.

Sludge samples from the various sampling points with phosphorus contents as depicted in Fig. 5 were incubated under laboratory conditions for 96 h. Nitrate and phosphate concentrations in the medium were determined at 24-h intervals. With time, nitrate reached undetectable concentrations in all samples (Fig. 6A). Nitrate depletion coincided with a release of soluble phosphate during incubation of all samples (Fig. 6B).

3.3. Total phosphorus in the fluidized bed reactor

Biofilm development on the sand grains, in the fluidized bed reactor, followed a predictable pattern. Thin biofilms were found on sand grains situated in the bottom regions of the column, and thick biofilms were found in sand grains situated at the top layers in the column. Significant differences ($p < 0.05$, Student's t-test) in phosphorus contents were found in biofilms derived from different positions within the fluidized bed reactor. Biofilms derived from the upper column layers had a relatively high phosphorus content of 190 ± 4 mg PO_4–P/g biofilm dry weight; biofilms from the middle part of the reactor contained 122 ± 8 mg PO_4–P/g biofilm dry weight, and small biofilms, derived from the bottom part of the column, contained 74 ± 5 mg PO_4–P/g biofilm dry weight.

4. Discussion

Previous studies on phosphorus dynamics in freshwater aquaculture systems have shown that a relatively large fraction of the feed phosphorus (80–90%) is released within fish culture systems due to low phosphorus uptake by fish (Avnimelech and Lacher, 1979; Boyd, 1985; Schroeder et al., 1991). Modern diets, however, combine lower phosphorus content and improved bioavailability to reduce phosphorus losses (Bergheim and Aasgaard, 1996). In conventional, earthen-bottom fish culture systems, much of the phosphorus is retained within the sediment (Boyd, 1995). In semiclosed, recirculating systems, phosphorus is discharged, either with the sludge or with effluent water (Bodvin et al., 1996). Sludge from these systems contains most of the phosphorus (Summerfelt, 1998). Treatment of such aquaculture sludge includes: composting (Shelton et al., 1998), anaerobic digestion (Chen et al., 1996), treatment by wetlands (Adler et al., 1996; Massingill et al., 1998), or direct land application for fertilization purposes (Willet and Jacobson, 1986; Bergheim et al., 1993). Most commercial, semiclosed, recirculating systems, however, use none of these treatment forms and phosphorus pollution by such systems is common and of serious environmental concern.

Similar to previous studies on a freshwater system (van Rijn and Barak, 2000) and with a denitrifying bacterium isolated from this system (Barak and van Rijn, 2000), results from the present study point to the essential role of denitrifiers in the phosphorus dynamics in this marine system. Evidence is provided for phosphate uptake by heterotrophic denitrifiers, under either oxic or anoxic (with nitrate) conditions, in the sedimentation basin and fluidized bed reactor and their phosphorus release under anaerobic conditions, in the absence of oxygen and nitrate. The relatively large spatial difference in phosphorus concentrations in the sedimentation basin, where most of the phosphate was found to

accumulate, can be explained by considering under which conditions phosphate is either released or removed by denitrifiers. Close to the inlet of the sedimentation basin, one might expect a high denitrifying activity due to the abundance of labile carbon required for heterotrophic denitrification, in addition to suitable electron acceptors such as oxygen or nitrate. Indeed, sludge samples derived from these stations were rich in total phosphorus. Deeper down in the sludge layers of these stations, nitrate concentrations were low and sulfide had accumulated. The latter process, dissimilatory sulfate reduction, occurs only in regions depleted of energetically favorable electron acceptors such as oxygen and nitrate. Under such anaerobic conditions, it is expected that denitrifiers would release phosphate. As predicted, total phosphorus at these stations was lower than elsewhere, while dissolved phosphate was higher. It should be noted that despite the presence of oxygen in these sludge layers, nitrate and sulfate concentrations were relatively low, while sulfide concentrations were relatively high. For thermodynamic reasons, oxygen, nitrate and sulfate respiration occur in separate redox zones. We assume that in these layers, respiration on the different electron acceptors is carried out in distinct microenvironments within the sludge aggregates. Presence of such zones has been confirmed in previous studies performed in marine and wastewater biofilms by using methods that enable a high spatial resolution of both chemical and microbial community gradients (Okabe et al., 1999; Ramsing et al., 1993).

Further evidence for phosphate release by denitrifiers was provided by laboratory incubation of sludge derived from the various stations in the sedimentation basin. It was found that the extent of phosphate release was a function of initial phosphorus content and the degree of nitrate depletion during incubation. Most phosphate was released once nitrate was completely depleted. The finding that, despite its lower phosphorus content, sludge derived from sampling station inlet-top released more phosphate than sludge derived from inlet-middle (Fig. 6) is probably due to the fact that nitrate depletion occurred more rapidly in the former sludge sample.

Phosphate was also removed in the fluidized bed reactor. In this reactor, sand particles with the thickest biofilms were situated in the upper layers of the column. The high phosphorus content of these latter particles were up to 19% of the dry weight, which corresponds to the maximum phosphate content found in denitrifying isolates (Barak and van Rijn, 2000). Differences in phosphate content of biofilms derived from different heights in the reactor may be related to the growth phase of the denitrifiers comprising these biofilms. In batch cultures of denitrifiers, it was found that phosphorus content increased with time and reached maximum values during the stationary phase of growth (Barak, 2002). It is possible, therefore, that as opposed to "growing biofilms" in lower parts of the fluidized bed reactor, larger biofilms, found in the upper parts of the reactor, were comprised of a denitrifying consortium in stationary phase of growth.

The findings presented in this study may have wider implications as to the understanding of phosphorus dynamics in aquatic sediments. Phosphorus immobilization in sediments is often attributed to chemical precipitation at suitable redox potentials (Boyd, 1995). However, it can easily be envisioned that phosphorus immobilization by denitrifiers is likely to occur in organic-rich sediments exposed to nitrate. An example of such an environment is the sediment–water interface in earthen-bottom fishponds. In

these ponds, much of the phosphorus is trapped in nitrate-rich sediment layers. In this respect, it is interesting to refer to a study by Masuda and Boyd (1994) who found that phosphate release in soils derived from aquaculture ponds ceased upon addition of nitrate and acetate. Near-shore marine sediments, rich in organic carbon, are another example of environments where phosphate trapping by denitrifiers could be of significance. The phosphorus content of marine sediments, like that of freshwater sediments, is often correlated to chemical precipitation or release (Krom and Berner, 1981). Since denitrification takes place in some of these organic-rich marine sediments (Cermelj et al., 2000), it seems possible that in here, denitrifiers play a role in phosphorus immobilization and subsequent partial release.

5. Conclusion

In the present study, it is demonstrated that phosphate can be effectively removed from the water of a recirculating marine fish culture system by incorporation of an anoxic treatment stage. Most of the phosphorus in the system accumulated in the sedimentation basin where phosphorus content of sludge was as high as 17.5%. Chen et al. (1996) estimated that the phosphorus content of sludge discharged from semiclosed recirculating aquaculture systems is 1.3%. Given the high phosphate content of the sludge by means of the presented anoxic treatment stage, it is envisioned that this form of treatment could also be applied as an effluent treatment stage in commonly used semiclosed recirculating systems.

Acknowledgements

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