Biological Denitrification Using Upflow Biofiltration In Recirculating Aquaculture Systems: Pilot-Scale Experience and Implications For Full-Scale

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Introduction

Recirculating aquaculture systems (RASs) rear high densities of fish while the culture water is continuously recycled, thereby employing water conservation techniques. Since fresh water addition is minimized, the quality of the culture water can deteriorate quite rapidly from the accumulation of ammonia and particulate waste generated from the metabolism of feed. Aquaculturists employ common wastewater treatment techniques in RASs to yield an environment that is conducive to rearing aquatic organisms. Solids removal is typically achieved through clarification or filtration, while nitrification is employed to convert ammonia to nitrate, via nitrite, in order to prevent free ammonia toxicity (8). The combined implementation of the nitrification process and decreased water exchanges leads to the accumulation of nitrates over time in recirculating aquaculture systems (3). Chronic toxicity to certain fish species (6), as well as tightening water regulations with regard to nutrient discharge, have led to concern over the accumulation of nitrates in recirculating systems.

Biological denitrification can be used to remove nitrates from RAS waters. Denitrification is the dissimilative reduction of nitrate (NO_3^-) to nitrogen gas (N_2), through the production of nitrite (NO_2^-) and gaseous nitric oxide (NO) and nitrous oxide (N_2O) intermediates.

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO_{(g)} \longrightarrow N_2O_{(g)} \longrightarrow N_{2(g)}$$
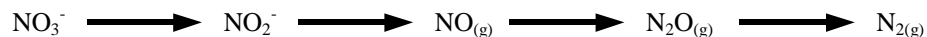
This process is performed by heterotrophic bacteria under anoxic conditions and uses nitrate as a terminal electron acceptor in the presence of a carbon and energy source.

An electron donor is required as a carbon and energy source to fuel the denitrification process. Dissolved organic carbon (DOC) compounds accumulate in RASs as a result of the introduction of feed, and the extent of accumulation is greatly affected by fish stocking densities and feeding rates (5). However, these systems typically possess relatively low concentrations of DOC (3). Wastewater treatment plants often add an exogenous carbon source, such as methanol or acetate, when a carbon deficiency exists (2, 11), though the associated cost does not make this an attractive option for aquaculturists. Growing

interest has been expressed for using biosolids as a carbon supplement in the denitrification process. Fermented municipal sludge and swine waste have been shown to be good electron donors, effecting enhanced denitrification rates over methanol and acetate alone (7). Fish waste and uneaten feed constitute a source of organic matter produced within the fish culture unit that can be used to generate a suitable carbon source for the denitrification process (1, 10). Since this organic matter is in the particulate form and not readily available for microbial use, hydrolysis and fermentation can be applied to convert these substances into volatile fatty acids (VFAs), which can be more easily consumed by denitrifying microorganisms (4, 7). The use of an organic substrate that is prevalent in the system is aimed towards the development of a self-sustaining treatment process. In addition, the amount of particulate waste requiring disposal is reduced by converting a fraction of the particulate matter into a soluble form that is consumed by the denitrification process.

Biofilters are an attached growth process in which a biofilm is generated from the propagation of microorganisms on an inert surface. Biofilters maintain a higher active fraction of biomass, as compared to suspended growth environments, which enables the use of a smaller reactor (9). The efficient operation and compact size makes biofilters an attractive treatment device for the aquaculture industry, as is illustrated by their wide scale use in the performance of nitrification. Complete nitrogen removal can be achieved in recirculating aquaculture systems through the implementation of a coupled biofiltration treatment scheme employing nitrification and denitrification.

This study was designed to investigate the removal of nitrates from recirculating aquaculture system waters using a denitrifying biofilter to reduce nitrate to nitrogen gas and a supplemental carbon source provided through the fermentation of fish food. Implications for full-scale operation are discussed.

Materials and Methods

Biofilter. A pilot-scale biological denitrification system comprised of an upflow, fixed film column and two fermentation units (Figure 1) was operated at the Virginia Tech Aquaculture Center (Blacksburg, VA). Low (1.13 kg NO_3 -N/m^3/day) and high (2.52 kg NO_3 -N/m^3/day) nitrate loading conditions were studied at a hydraulic loading rate of 3.0 m^3/m^2/hr. A fixed film biological denitrifying column (4.0 m x 15.2 cm) was constructed with schedule 40 clear PVC pipe connected by schedule 80 PVC couplings. The column was packed with 0.044 m^3 of 2-3 mm floating, polystyrene media (Biostyr®, Krüger, Cary, North Carolina) possessing a specific surface area of 1000 m^2/m^3. A steel screen retained the media within the column. The column was seeded with activated sludge obtained from a denitrification basin at a local municipal wastewater treatment plant (Blacksburg, VA).
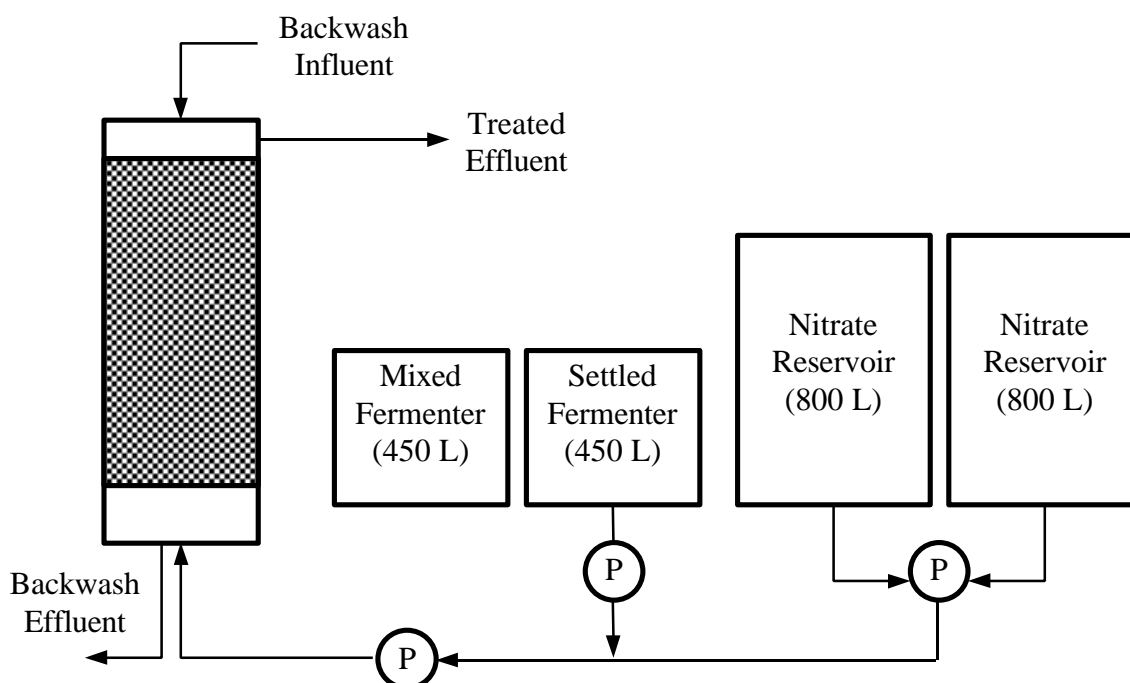
Figure 1.   Pilot-scale system comprised of an upflow, denitrifying biofilter, two fermentation units, and two nitrate stock reservoirs. P represents pumps.

Fermentation. Commercial fish food (Southern States Cooperative, Inc., Richmond, VA) was provided as the fermentation source. The feed was ground prior to addition in order to facilitate hydrolysis. Sodium bicarbonate (NaHCO$_3$) was added as a buffer to maintain a neutral pH. Mixing was achieved in each fermenter with one submersible pump (1/6 hp). Pilot-scale fermentation was conducted under two operational regimes during this study using 450 L tanks. From days 1 – 101 fermentation was conducted in one tank, operated as a sequencing batch reactor (SBR) with a solids retention time (SRT) and hydraulic retention time (HRT) equaling 3 days. The daily wastage from the fermenter was collected, settled in buckets, and the supernatant was transferred to a 380 L storage container. Fermented supernatant was then pumped from the storage container into the denitrifying column. On day 102, fermenter operation was modified to allow for the continuous pumping of supernatant directly from the fermentation tank into the column during the SBR decant phase. This was accomplished by increasing the complete fermentation cycle to a 48 hour period and adding a second fermenter so that operations for each fermenter were offset from each other by 24 hours. During this second operational regime, the two fermenters were operated as modified SBRs on a 6 day SRT and 3.5 day HRT.

System Operation. A synthetic nitrate wastewater was prepared daily in two tanks (800 L each) using industrial grade sodium nitrate (NaNO$_3$) (Chilean Nitrate Corporation, Norfolk, VA) and tap water. The nitrate feed and fermented supernatant were pumped and mixed in-line prior to entering the upflow column. The system was operated on a 24

hour cycle, with maintenance occurring in the last hour. After 23 hours of operation, the column was shutdown and backwashed to remove excess biomass. In order to effect media bed turnover for efficient biomass sloughing, backwashing was achieved by draining the column and then adding pressurized tap water in a downflow manner. At the same time, the fermenters were either settled or wasted and fed.

Results and Conclusions

Fixed film denitrification was investigated under two different nitrate loading conditions. During the high nitrate loading period, the flowrate was decreased from 3.0 $m^3/m^2/hr$ to 1.5 $m^3/m^2/hr$ from day 283 through 306 in order to address high nitrite concentrations detected in the column effluent. Table 1 outlines the loading conditions for each phase of this study.

Table 1. Nitrate and hydraulic loading conditions for the denitrification system. Average values are provided ± standard error.

Parameter	Low nitrate loading	High nitrate loading	
Period of study (days)	1 - 200	201 - 282, 307 – 346	283 - 306
Influent concentration* (mg NO_3-N/L)	38.5 ± 1.0	85.3 ± 1.7	64.8 ± 6.4
(mg NO_2-N/L)	0.65 ± 0.31	2.94 ± 0.46	14.6 ± 5.6
Mass loading (kg NO_3-N/m^3/day)	1.13	2.52	0.96
(kg NO_2-N/m^3/day)	0.02	0.09	0.22
Mass removal (kg NO_x-N/m^3/day)	0.81	2.21	1.08
Hydraulic loading (m^3/m^2/hr)	3.0	3.0	1.5

*Measurable influent nitrite concentrations were detected only during the high nitrate loading phase under the low hydraulic loading.

The pH increased through the denitrification column from 7.08 ± 0.07 to 7.87 ± 0.12 under low nitrate loadings, and from 7.33 ± 0.03 to 8.59 ± 0.05 under high nitrate loadings. The pH in the fermentation process averaged 6.31 ± 0.06 and 7.41 ± 0.02 during the low and high nitrate loadings, respectively.

COD to NO_3-N Ratio. In order to prevent the reduction of sulfate (SO_4^{-2}) to hydrogen sulfide (H_2S), the denitrification column was originally run under carbon limiting conditions during the low nitrate loading phase. Available carbon limiting conditions

prevailed for influent soluble chemical oxygen demand (sCOD) to NO_3-N ratios less than 5 and resulted in incomplete nitrate removal (Figure 2a) and measurable effluent nitrite concentrations as high as 12 mg NO_2^-N/L (Figure 2b). For influent sCOD to NO_3-N ratios above 5, high total nitrogen removals greater than 95% were consistently achieved, except in one case where the influent pH fell below 7.0. This influent ratio corresponded with a consumption ratio of 4.62 ± 0.28 mg sCOD/mg NO_x-N for complete nitrogen removal. COD was detected in all effluent samples (Figure 2c), with an average concentration of 22.5 ± 3.02 mg sCOD/L even when NO_x removal was incomplete.

During the high nitrate loading phase nitrate removal efficiencies greater than 99% were regularly seen (Figure 3a). However, effluent nitrite concentrations were greater than those measured during the lower nitrate loading, reaching values as high as 34 mg NO_2^-N/L (Figure 3b). The presence of measurable COD in the effluent suggested that carbon limitation was not the problem (Figure 3c). In order to remedy the high effluent nitrite problem, the hydraulic loading was decreased by 50%, thereby doubling the column retention time. After observing several days of complete nitrogen removal during the low hydraulic loading period, operation was returned to the original flowrate. At this point, nitrate removal efficiencies greater than 95% were achieved and effluent nitrite remained at or below 1 mg NO_2 -N /L for the final 2 months of the study (data not shown). During the low nitrate loading phase, visual inspection revealed that biomass growth was concentrated at the bottom of the column where a majority of the nitrogen removal occurred, while sparse microbial colonization prevailed at the top. The improved efficiency that resulted from a decreased hydraulic loading was attributed to an acclimation period during which biomass growth occurred throughout the entire column as a result of increased contact time with substrates. It was thought that this increase in biofilm density enabled the removal of higher nitrate concentrations. Complete nitrogen removal was achieved for a COD to NO_x -N consumption ratio of 3.07 ± 0.58 mg sCOD/mg NO_x-N during the high nitrate loading, but did not correspond to a distinct influent sCOD to NO_3-N ratio and exhibited great variability. When the nitrate loading was increased, the amount of fish feed added to the fermenters was elevated to raise the soluble COD production. Even though the fermenters were settled prior to supernatant removal, a greater amount of suspended solids were visually detected in the carbon source line leading to the column. It is possible that a portion of these fermentation solids were retained within the column and as a result, the hydrolysis of influent fermentation solids may have occurred to generate a COD source not measured by filtered samples.

Summary and Recommendations

The results of this study demonstrated the feasibility of using a fermentation generated carbon source in the denitrification of high nitrate recirculating aquaculture system waters. The fermentation of both fish waste and fish food generated volatile fatty acids that were assimilated by denitrifying organisms. In addition, limiting the amount of available carbon supplied to the denitrification system resulted in an increase in effluent nitrite and incomplete nitrate removal, while influent COD to NO_3-N ratios greater than 5
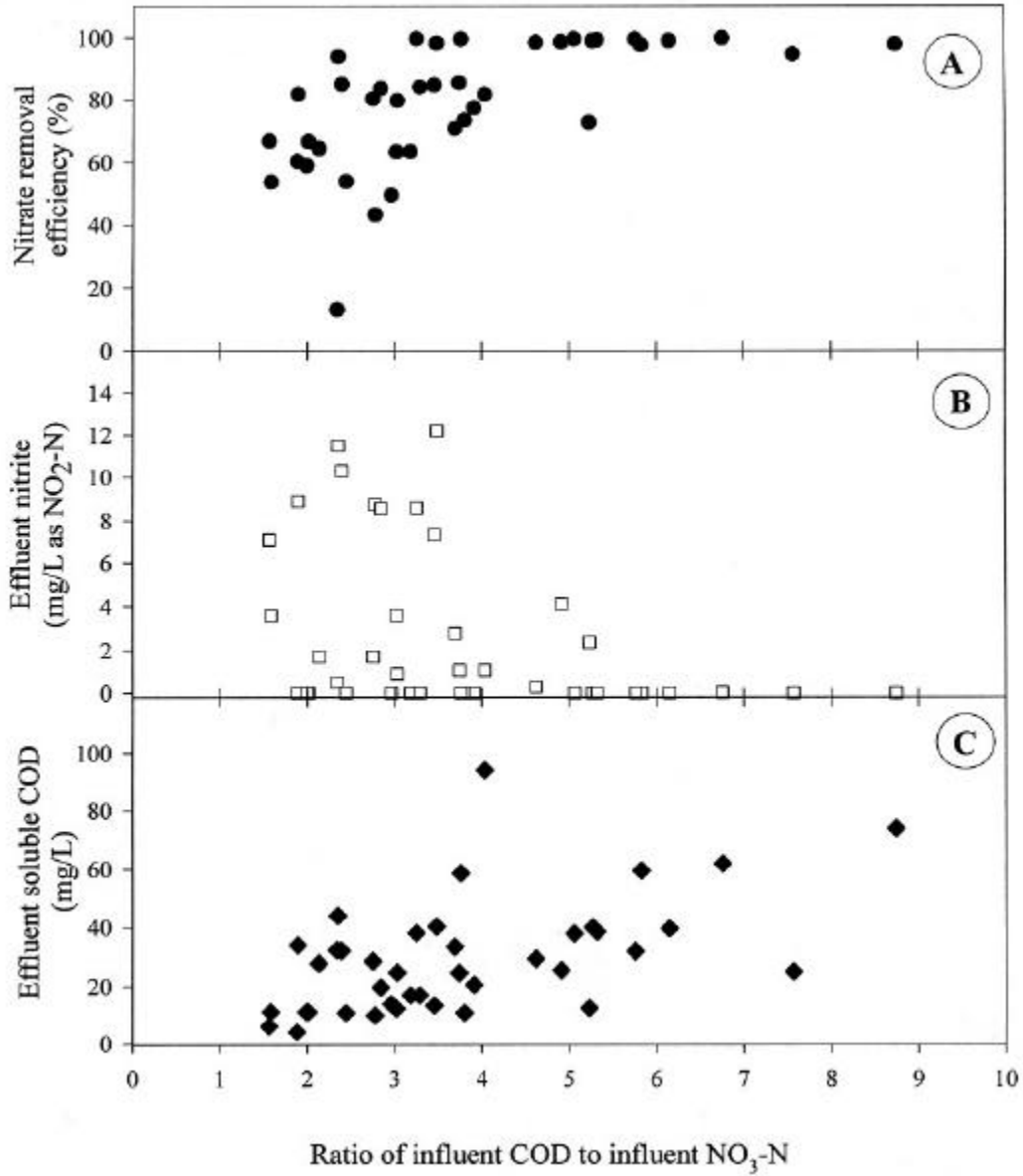
Figure 2. Denitrification column performance during the low nitrate loading phase with respect to (A) nitrate removal, (B) effluent nitrite concentration, and (C) effluent soluble COD concentration, as a function of the influent COD to influent NO_3-N ratio.

Figure 3. Denitrification column performance during the high nitrate loading phase with respect to (A) nitrate removal, (B) effluent nitrite concentration, and (C) effluent soluble COD concentration, as a function of the influent COD to influent NO3-N ratio. Data represent days 201 to 282, prior to lowering the flowrate.
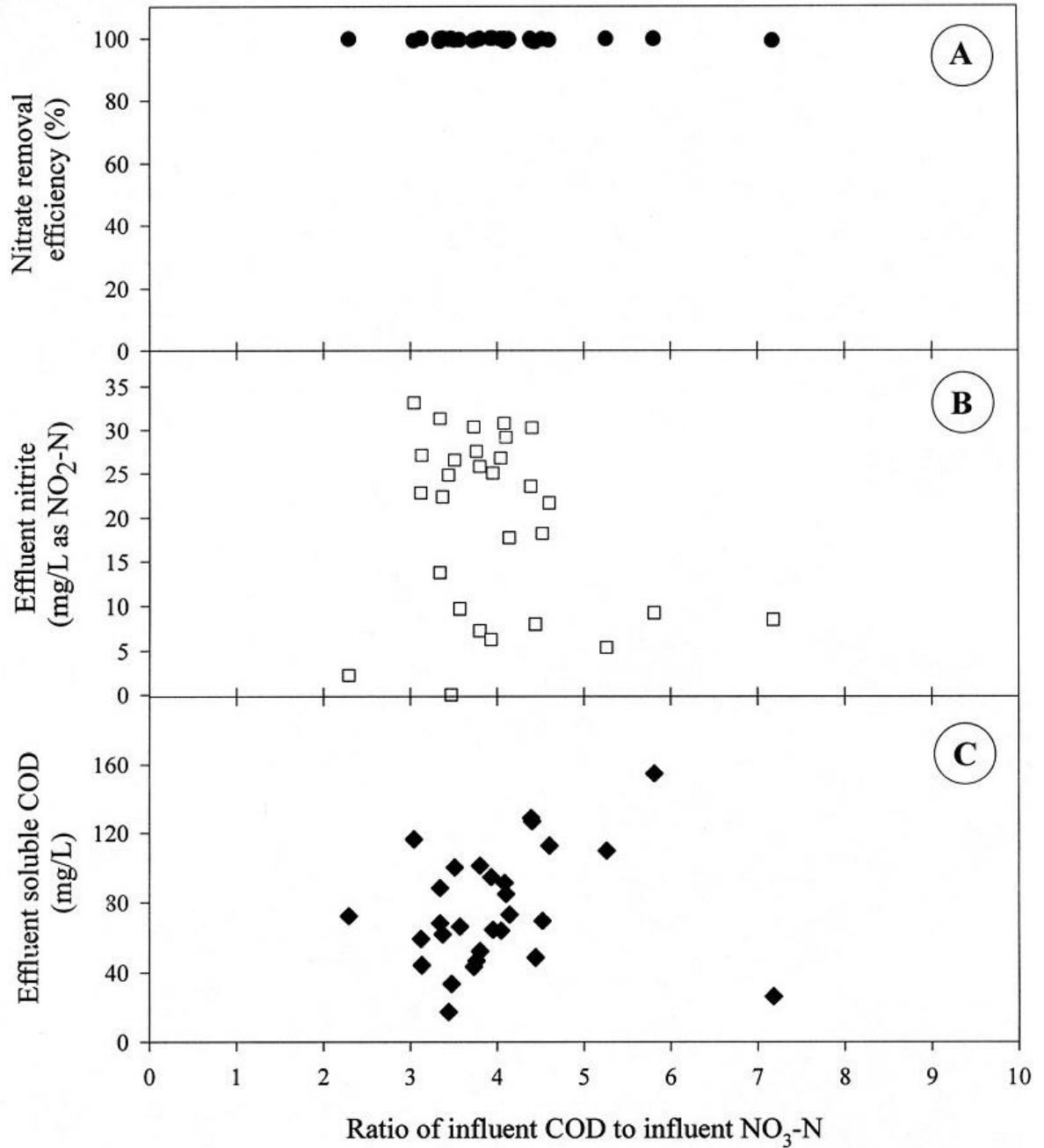
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typically achieved high total nitrogen removals greater than 95%. To prevent nitrite accumulation as well as the discharge of significant amounts of carbon that would return to the fish culture tank, a denitrification system using fermented fish waste or food as the carbon source to produce volatile fatty acids should be operated close to this ratio.

The nitrate loadings examined in this study were lower than the maximum nitrate concentrations observed in nitrifying closed recirculating aquaculture systems not employing denitrification. However, nitrate concentrations in the fish rearing tanks increase gradually over the span of a growth period and it may be possible to maintain concentrations at manageable levels by applying denitrification as a sidestream process so that extreme concentrations do not result. In order to evaluate the efficiency and self-sustainability of this denitrification system at increased nitrate concentrations, additional studies are recommended. It is anticipated that a full-scale recirculating aquaculture facility would generally have several culture tanks containing fish at all stages of growth and would be able to provide a more consistent source of fish waste for the fermentation process. However, this aspect of the treatment system must be evaluated further to determine if complete self-sustainability is possible, or whether an external carbon source must be partially supplemented.

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Denitrification in Recirculating Aquaculture Systems: From Biochemistry to Biofilters

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Introduction

Relatively little efforts have been made to remove nitrate in recirculating aquaculture systems. This is partially due to the fact that, unlike other forms of inorganic nitrogen (ammonia and nitrite), nitrate does not pose a direct threat to organisms cultured in aquaculture facilities. Most cultured fish species are able to grow in nitrate-rich waters although recent evidence has pointed to nitrate stress in hybrid striped bass (Hrubec et al., 1996). A more common problem associated with high nitrate concentrations in fish culture systems is the formation of nitrite due to an incomplete reduction of nitrate in oxygen-poor zones. As most culture systems harbor such zones (e.g. pipes and low-flow areas where organic matter accumulates), relatively high nitrite concentrations can often be detected in nitrate-rich fish culture systems. Discharge of effluent water is an additional problem related to nitrate-rich fish culture systems. When discharged to surface or groundwaters, nitrate may lead to eutrophication in the former and drinking-water contamination in the latter. These environmental and public health considerations have lead to stringent regulations on nitrate discharge in many countries and permissible nitrate levels in effluent water are now as low as 11.6 mg NO_3-N/l (European Community Directive).

Studies on nitrate removal in fish culture systems were reviewed by van Rijn (1996). In all these studies nitrate removal was accomplished by denitrification, a process carried out by facultative anaerobic bacteria which, in the absence of oxygen and presence of metabolizable organic matter, are capable of reducing nitrate, nitrite, nitric oxide or nitrous oxide to N_2. The present contribution summarizes some of the main environmental factors regulating denitrification and, in addition, summarizes our current research on denitrifying organisms and nitrate removal in recirculating aquaculture systems.

Nitrate removal by microorganisms

A wide array of microorganisms is capable of reducing nitrate to more reduced inorganic nitrogen compounds. Most of these organisms have a heterotrophic mode of metabolism, i.e. the requirement for an organic carbon source for growth. Microbial nitrate reduction is either carried out for assimilatory or dissimilatory purposes (Table 1). Assimilatory nitrate reduction, a process in which nitrate is reduced to ammonia followed by ammonia assimilation into cell biomass, takes place in the absence of reduced inorganic nitrogen compounds. The dissimilatory pathway, in which nitrogen oxides serve as electron

acceptors for energy generation by the cells, primarily takes place in the absence of oxygen and is a process carried out by two distinct bacterial groups. One group reduces nitrate to ammonia while the other reduces it to nitrogen gas. Bacteria belonging to the latter group are called denitrifiers. The ratio of available organic carbon to nitrate in the environment is the main determinant for which of these two latter processes takes place. At high ratios, nitrate is reduced to ammonia whereas denitrification is the preferred pathway at low ratios (Tiedje, 1990).

Nitrate removal from aquaculture systems is based on creating optimal conditions for growth of denitrifying organisms as only these organisms cause a net removal of inorganic nitrogen from the water. From the above it follows that in order to create such conditions several factors have to be taken into account: a) reduced inorganic nitrogen compounds should be present, b) oxygen should be absent, and c) the C/N ratio should be low enough to exclude the growth of organisms which reduce nitrate to ammonia.

Table 1. Biological nitrate reduction*

Process	Regulator(s):	Organisms
Assimilatory nitrate reduction ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$)	NH_4^+	plants, fungi, algae, bacteria
Dissimilatory nitrate reduction *Dissimilary nitrate reduction to ammonia* ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$)	O_2, C/N	anaerobic and facultative anaerobic bacteria
Denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$)	O_2, C/N	facultative anaerobic bacteria

* adapted from Tiedje (1990)

Denitrifying bacteria

Many different species are capable of denitrification. Especially the genera *Pseudomonas, Alcaligenes, Paracoccus* and *Bacillus* comprise many denitrifiers (Knowles, 1982). In the natural environment, a complex interaction of physical, chemical and biological conditions governs the predominance of a particular denitrifying species. In denitrifying reactors, species composition is influenced by the nature of the added carbon source (Grabinska-Loniewska, 1991). Addition of complex organic carbon sources will give rise to a higher species diversity than when simple organic carbon sources are used. In this context, denitrifying reactors are often operated with methanol as this carbon source causes the development of a distinct microbial population and, hence, a predictable performance of the reactor. It should be noted, however, that even with addition of simple organic compounds to denitrifying reactors the resulting denitrifying population is often far from predictable. Other forms of organic matter present in the reactor such as those liberated by the decay of microorganisms or present in the water to be treated may give rise to a diverse microbial population. A highly versatile bacterial

composition and dynamic bacterial colonization pattern was found on sand particles derived from fluidized bed reactors used for nitrate removal from fish culture water (Sich and van Rijn, 1997).

In view of the large diversity of denitrifiers, denitrification takes place at a wide range of environmental conditions (temperature, salinity, etc.). Unlike nitrification, where the species diversity is narrow, single environmental determinants do not have a measurable effect on denitrification.

Nitrite accumulation by denitrifiers

Denitrification often coincides with an accumulation of intermediates in the denitrification pathway. One of these intermediates, nitrite, is extremely toxic to fish and as such it is important to understand the factors underlying nitrite accumulation during this process. In denitrifying bacteria exposed to low levels of oxygen, nitrite accumulation resulted from differential repression of nitrite reductase synthesis and activity, as compared to nitrate reductase (Coyne and Tiedje, 1990; Korner and Zumft, 1989). Shifts in the pH values of the medium were also found to affect nitrite accumulation (Almeida et al., 1995; Beccari et al., 1983; Thomsen et al., 1994). Competition between nitrate and nitrite reductases for common electron donors is an additional factor causing nitrite accumulation by some denitrifiers (Betlach and Tiedje, 1981; Kucera et al., 1983; Thomsen et al., 1994). The choice of carbon source was shown to affect the level of nitrite accumulation in denitrifying reactors (McCarthy et al., 1969). The carbon source may lead to specific enrichment of nitrite-accumulating bacteria, as was found in a study on denitrifying reactors where addition of fermentable substrates enhanced the growth of such bacteria (Wilderer et al., 1987). Alternatively, individual denitrifiers may accumulate nitrite when grown on different carbon sources (Blaszczyk, 1993; Nishimura et al., 1979; 1980; van Rijn et al. 1996). Temporary carbon starvation may lead to nitrite accumulation in denitrifying bacteria with constitutive nitrate reductases and inducible nitrite reductases (Barak, 1997, Table 2). Finally, in a

Table 2. Maximum nitrate and nitrite reduction rates during carbon starvation of *Pseudomonas* sp. Strain JR12 isolated from a fluidized bed reactor*.

Starvation period (h)	$V_{max} NO_3^-$ (mM NO_3^--N/g protein/min)	$V_{max} NO_2^-$ (mM NO_2^--N/g protein/min)
0	0.298 ± 0.00	0.353 ± 0.03[a]
12	0.300 ± 0.01	0.253 ± 0.05[b]
24	0.321 ± 0.03	0.204 ± 0.03[b]
36	0.285 ± 0.04	0.185 ± 0.01[c]

*different superscripts indicate significant differences within maximum nitrite reduction values (students T-test, $p<0.05$)

recent study on a denitrifying bacterium isolated from a denitrifying fluidized bed reactor used for nitrate removal in a recirculating aquaculture facility, nitrite accumulation

resulted from light exposure. It was shown that at light intensities as low as 5% of full sunlight intensity, nitrite accumulated as a result of light inhibition of nitrite but not of nitrate reduction (Barak et al., 1998).

Application of denitrification in recirculating aquaculture systems

Relatively few studies have been conducted on nitrate removal in recirculating systems. As most recirculating systems are not closed but are operated in a semi-closed mode, nitrate levels are controlled by means of a daily discharge of nitrate with the effluent water. Passive denitrification in low-oxygen areas of the recirculating system, furthermore, reduces the nitrate concentrations in these systems.

Most of denitrification reactors employed in (experimental) aquaculture facilities are based on immobilization of denitrifiers on suitable media (plastic, sand). Within these reactors, anoxic conditions are created by flushing the reactor with N_2 gas (Whitson et al., 1993) or by operating the reactor at retention times low enough to secure a complete, biologically-mediated, oxygen depletion within the reactor. As denitrification is primarily a heterotrophic process, the inlet stream should be supplied with a suitable carbon source. Alcohols and sugars are often used for this purpose (Balderston and Sieburth, 1976; Otte and Rosenthal, 1979; Whitson et al., 1993; Honda et al., 1993; Abeysinghe et al., 1996).

Another, cheaper carbon source for denitrification is the organic carbon produced in the fish culture units (fish feces and unutilized feed). Arbiv and van Rijn (1995) demonstrated the potential use of this latter carbon source for nitrate removal in recirculating aquaculture systems. In this study, organic matter was periodically withdrawn from a fish culture unit and led into a sedimentation basin where high redox potentials were maintained by a constant flow-through of oxygen and nitrate-rich water from the culture unit. Under these conditions it was found that degradation of sludge resulted in the release of short-chain volatile fatty acids. Before being returned to the culture unit, water withdrawn from the sedimentation basin, devoid of oxygen but rich in nitrate and volatile fatty acids, was led through a fluidized bed reactor where denitrifying bacteria reduced nitrate to N_2 using volatile fatty acids as a carbon source (Aboutboul et al., 1995). The latter method not only caused a considerable reduction of nitrate from the culture systems but also proved to be an efficient means for decomposing the organic sludge produced in the fish culture unit (van Rijn et al., 1995; van Rijn and Nussinovitch, 1997). By means of this treatment system water loss was extremely small since neither sludge nor water were discharged. It should be noted, however, that since all the nitrogen added with the feed is retained within the system, a considerable larger nitrification unit is required when using this treatment scheme than in systems where sludge is discharged.

Recently, a new type of denitrifying reactor was developed (Nussinovitch et al., 1996; Tal et al., 1997) in which instead of immobilization, the denitrifying bacteria are entrapped within polymeric beads. These freeze-dried polymeric beads, co-entrapping bacteria and a suitable carbon source, provide the advantage that denitrification is initiated immediately upon introducing the beads in nitrate-rich water. Whereas the product has been successfully tested in marine and freshwater aquariums, its full-scale use in aquaculture facilities remains to be examined.

Denitrification and phosphorus removal

Reduction of phosphorus concentrations in intensive fish culture systems is mainly accomplished by increasing the dietary phosphorus availability. Once released into the culture systems, phosphorus is generally left untreated and is discharged with the effluent water either in the organic or inorganic form. Although intensive fish culture systems are a considerable source of phosphorus pollution, relatively few studies have been conducted on phosphorus removal in these systems (Adler et al., 1995; Abeysinghe et al., 1996). In Europe as well as the USA, legislative measures to reduce this pollution source have been implemented and the expectations are that discharge restrictions/fees will become more severe in the future.

Treatment of phosphorus by standard chemical and physical methods to very low levels is expensive and increases in complexity as the required effluent concentration decreases. Enhanced biological phosphorus removal from domestic wastewaters in activated sludge plants is accomplished by alternate stages in which the sludge is subjected to anaerobic and aerobic conditions, respectively. Under these conditions, phosphorus after being released from the bacterial biomass in the anaerobic stage, is assimilated in excess (luxury phosphorus uptake) by these bacteria during the aerobic stage. Phosphorus is subsequently removed from the process stream by harvesting a fraction of the phosphorus-rich bacterial biomass (Toerien et al., 1990). Recently, evidence was provided for single-stage phosphorus removal by denitrifying bacteria (Barker and Dold, 1996). Under anoxic conditions, the latter bacteria were shown to be capable of luxury phosphorus uptake with nitrate serving as electron acceptor.

We tested the phosphorus removal capacity of denitrifying sludge obtained from a fluidized bed reactor operated on fish culture effluent. A concomitant nitrate and phosphorus removal was found (Fig. 1A). As assimilatory phosphorus requirements are related to assimilatory nitrogen requirements, it could be estimated by determination of the disappearance of both nitrogen and phosphorus from the medium that phosphorus uptake was in excess of biosynthetic phosphorus requirements by the bacteria present in the sludge. Pure bacterial cultures were obtained after enrichment of the sludge with phosphorus, nitrate and acetate. As was observed with crude sludge, also these isolates were capable of combined nitrate removal and luxury phosphorus uptake (Fig. 1B). The observation that phosphorus concentrations increased upon depletion of nitrate from the medium may serve as an additional indication for the fact that these bacteria were indeed capable of luxury phosphorus uptake (Fig. 1B). An electron microscopic examination of bacteria incubated under denitrifying conditions in the presence of phosphorus clearly revealed the presence of considerable concentrations of polyphosphate inclusion-bodies (not shown). Once stored within the denitrifying biomass, the phosphorus can be removed from the culture system by means of a periodical, partial harvest (wasting) of the denitrifying biomass. To what extent this process can be adapted to recirculating fish culture systems is currently under investigation.
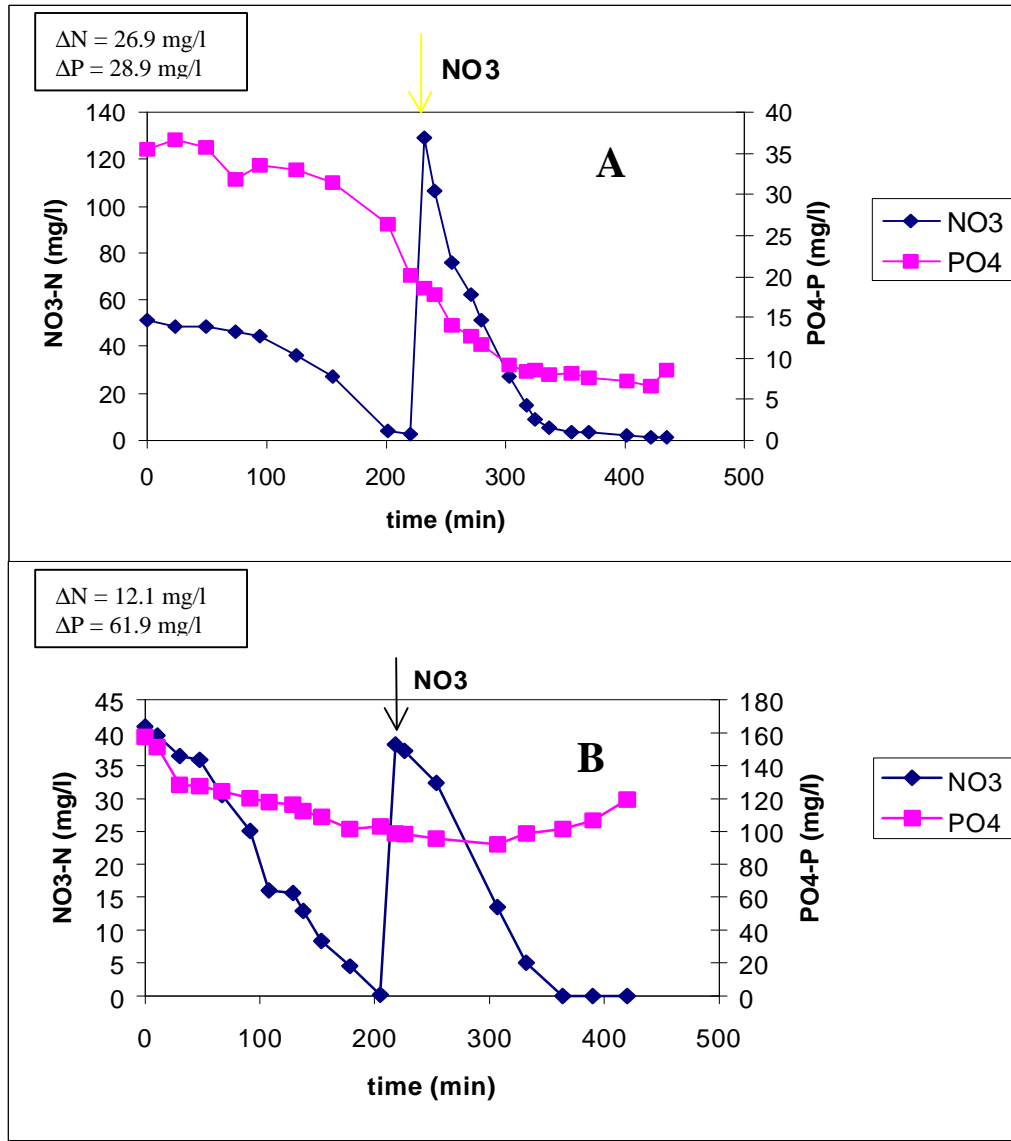
Figure 1. Combined nitrate and phosphate removal by (A) denitrifying sludge from a fluidized bed reactor and (B) a denitrifying strain *(Pseudomonas* sp.) isolated from the sludge of a fluidized bed reactor. Values of ammonia and phosphate removal during both experiments are inserted. After depletion, nitrate was added at times indicated by arrows.

Conclusions

Biological water quality control in recirculating aquaculture systems has so far focused mainly on the prevention of accumulation of ammonia through the induction and management of nitrification. Accumulation of other inorganic nutrients such as nitrate and phosphorus has received little attention since high concentrations of these nutrients do not impose a direct threat to most cultured organisms. Reduction of environmental pollution by using recirculating technology is considered an important advantage over other fish culture technologies. Present recirculation technology reduces pollution by

more than 50% in comparison to traditional flow-through systems but still are a considerable source of pollution by discharge of organic matter, nitrate and phosphorus. Although proven to be feasible, full-scale commercial use of denitrification systems has thus far not been initiated. It is expected, however, that with increasingly stringent discharge regulations, methods for nitrate removal will be incorporated either within the treatment-process stream or in the effluent stream of recirculating aquaculture systems. The finding that, in addition to nitrate removal, denitrifiers are capable of luxury phosphorus uptake provides the interesting possibility to remove both pollutants in a single treatment step.

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Nitrite Accumulation in Denitrification Systems – The Role of Dissolved Oxygen and Substrate Limitation

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