Denitrification in recirculating systems: Theory and applications

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Abstract

Profitability of recirculating systems depends in part on the ability to manage nutrient wastes. Nitrogenous wastes in these systems can be eliminated through nitrifying and denitrifying biofilters. While nitrifying filters are incorporated in most recirculating systems according to well-established protocols, denitrifying filters are still under development. By means of denitrification, oxidized inorganic nitrogen compounds, such as nitrite and nitrate are reduced to elemental nitrogen (N_2). The process is conducted by facultative anaerobic microorganisms with electron donors derived from either organic (heterotrophic denitrification) or inorganic sources (autotrophic denitrification). In recirculating systems and traditional wastewater treatment plants, heterotrophic denitrification often is applied using external electron and carbon donors (e.g. carbohydrates, organic alcohols) or endogenous organic donors originating from the waste. In addition to nitrate removal, denitrifying organisms are associated with other processes relevant to water quality control in aquaculture systems. Denitrification raises the alkalinity and, hence, replenishes some of the inorganic carbon lost through nitrification. Organic carbon discharge from recirculating systems is reduced when endogenous carbon sources originating from the fish waste are used to fuel denitrification. In addition to the carbon cycle, denitrifiers also are associated with sulfur and phosphorus cycles in recirculating systems. Orthophosphate uptake by some denitrifiers takes place in excess of their metabolic requirements and may result in a considerable reduction of orthophosphate from the culture water. Finally, autotrophic denitrifiers may prevent the accumulation of toxic sulfide resulting from sulfate reduction in marine recirculating systems. Information on nitrate removal in recirculating systems is limited to studies with small-scale experimental systems. Packed bed reactors supplemented with external carbon sources are used most widely for nitrate removal in these systems. Although studies on the application of denitrification in freshwater and marine recirculating systems were initiated some thirty years ago, a unifying concept for the design and operation of denitrifying biofilters in recirculating systems is lacking.
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1. Introduction

In most recirculating systems, ammonia (referring to NH_3 and NH_4^+) removal by nitrification, sludge removal by sedimentation or mechanical filtration, and water exchange are the vital forms of water treatment (van Rijn, 1996). Often, 5–10% of the system volume is replaced each day with new water to prevent accumulation of nitrate and dissolved organic solids (Masser et al., 1999). When comparing the various biological processes important for water quality control in regular fishponds with those in recirculating systems, it can be concluded that biological water treatment in the latter systems is very limited. In regular, earthen fishponds, inorganic nitrogen levels in the water column are low, despite the input of protein-rich supplementary feed. Biological removal of ammonia in these ponds takes place by several biological processes: algal assimilation and bacterial decomposition of algae, ammonification, nitrification and denitrification (Shilo and Rimon, 1982; van Rijn et al., 1984; Diab and Shilo, 1986; Hargreaves, 1998). Denitrification in these ponds is confined to the sediments, where the presence of anoxic conditions as a result of degradation of organic matter and, in addition, the liberation of low molecular weight carbon compounds, provide suitable conditions for denitrification (Diab and Shilo, 1986; Avnimelech et al., 1992; Hopkins et al., 1994; Hargreaves, 1998; Gross et al., 2000). Mimicking these conditions in recirculating systems, by compartmentalization of each of the above nitrogen transformation processes, is essential for reducing water consumption and environmental impact of these systems.

Nitrate reaches high concentrations in recirculating systems where nitrifying biofilters are used for ammonia removal. Reported maximum values of nitrate in recirculating systems are as high as 400–500 mg NO3-N/l (Otte and Rosenthal, 1979; Honda et al., 1993). Maximum nitrate levels differ among recirculating systems and are dictated mainly by water exchange rates and the extent of nitrification and nitrate removal. Contrary to ammonia and nitrite, nitrate is relatively non-toxic to aquatic organisms. However, high nitrate concentrations can affect the growth of commercially cultured aquatic organisms, such as: eel (Kamstra and van der Heul, 1998), octopus (Hyrayama, 1966), trout (Berka et al., 1981) and shrimp (Muir et al., 1991). Increased efforts are now directed toward nitrate control in recirculating systems. Apart from the direct toxic effect on fish, nitrate removal is conducted for other reasons in recirculating systems: (1) environmental regulations associated with effluent discharge have permissible nitrate levels as low as 11.3 mg NO_3-N/l (European Council Directive, 1998); (2) prevention of high nitrite levels resulting from incomplete "passive" nitrate reduction; (3) stabilization of the buffering capacity; and (4) the concomitant elimination of organic carbon, orthophosphate and sulfide from the culture water during biological nitrate removal.

In this review, biological pathways of nitrate removal are discussed as well as links between denitrifying organisms and carbon, phosphate and sulfur cycles in recirculating systems. Applications of biological nitrate removal in recirculating systems are reviewed. Finally, the anammox process, an alternative pathway for ammonia and nitrate removal, is discussed.

2. Biological nitrate removal

Biological nitrate removal is conducted by a wide variety of organisms by either assimilatory or dissimilatory pathways (Table 1). Organisms capable of assimilatory nitrate reduction use nitrate, rather

Table 1
Biological nitrate reduction[a]

Process	Regulator(s)	Organisms
Assimilatory nitrate reduction ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$)	NH_4^+	Plants, fungi, algae, bacteria
Dissimilatory nitrate reduction		
Dissimilatory 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

[a] From van Rijn and Barak (1998), adapted from Tiedje (1990).

than ammonia, as a biosynthetic nitrogen source. In most organisms, this process occurs in the absence of more reduced inorganic nitrogen species (e.g. ammonia). Assimilatory nitrate reduction takes place under aerobic as well as anaerobic conditions. No net removal of inorganic nitrogen is accomplished by this process, since inorganic nitrogen is converted to organic nitrogen.

Dissimilatory nitrate removal refers to the reduction of nitrate to more reduced inorganic nitrogen species with the concomitant release of energy. The dissimilatory pathway is employed mainly by two groups of prokaryotic organisms. Nitrate is reduced to either nitrite or ammonia by one group, and the other group reduces nitrate via nitrite to gaseous nitrogen forms with elemental nitrogen (N_2) as the end product. The former process, dissimilatory nitrate reduction to ammonia (DNRA), is conducted by fermentative bacteria using nitrate as a final electron acceptor when, for bioenergetic reasons, reduction of organic matter (fermentation) is not possible (Tiedje, 1990). Denitrifiers represent the second group of dissimilatory nitrate reducers and comprise a wide array of prokaryotic organisms. Most of these organisms are facultative anaerobes and use nitrate as a final electron acceptor in the absence of oxygen. Elemental nitrogen is the end product of this process, but intermediate accumulation of nitrite, nitric oxide and nitrous oxide may take place under certain conditions. Heterotrophic denitrifiers, using organic carbon compounds as a source of biosynthetic carbon and electrons, are the most common denitrifiers in nature. In some reduced environments low in dissolved carbon, autotrophic denitrifiers are the prevalent denitrifiers using reduced inorganic compounds, such as Mn^{2+}, Fe^{2+}, sulfur and H_2 as electron sources and inorganic carbon as a biosynthetic carbon source (Korom, 1992).

Environmental factors, in particular the availability and type of organic carbon compounds and the oxidation/reduction state of the aquatic environment, dictate to a large extent the occurrence dissimilatory nitrate reducers. High C/N ratios (Tiedje, 1990) and high sulfide concentrations (Brunet and Garcia-Gil, 1996) in the environment are thought to favor DNRA organisms over denitrifiers. Among the denitrifiers, the type and quantity of organic carbon compounds influences the accumulation of intermediate products, such as nitrite and inorganic nitrogen gases (Nishi-

mura et al., 1979; Nishimura et al., 1980; van Rijn and Sich, 1992; Blaszczyk, 1993; van Rijn et al., 1996). Oxygen is an important regulator of denitrification. Although aerobic denitrification has been reported (Robertson and Kuenen, 1984), most denitrifiers are facultative anaerobes and reduce nitrate in the absence of oxygen. Incomplete reduction of nitrate to intermediate products occurs at low oxygen concentrations due to differential repression of oxygen on enzymes involved in the nitrate reduction pathway (Betlach and Tiedje, 1981). Oxygen repression often is accompanied by nitrite accumulation in the aquatic medium (van Rijn and Rivera, 1990). Other environmental factors that repress denitrification activity and cause nitrite accumulation are: sub-optimal pH values (Beccari et al., 1983; Thomsen et al., 1994; Almeida et al., 1995) and high light intensities (Barak et al., 1998).

3. Heterotrophic versus autotrophic denitrification

3.1. Heterotrophic denitrification

Heterotrophic denitrifiers derive electrons and protons required for nitrate reduction to elemental nitrogen from organic carbon compounds. Such compounds include carbohydrates, organic alcohols, amino acids and fatty acids. For example, utilization of acetate as a carbon source for denitrification proceeds as follows:

$$5CH_3COO^- + 8NO_3^- + 3H^+$$
$$\rightarrow 10HCO_3^- + 4N_2(g) + 4H_2O \qquad (1)$$
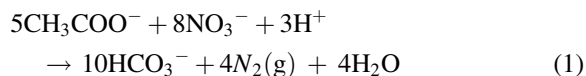
The C/N ratio required for complete nitrate reduction to nitrogen gas by denitrifying bacteria depends on the nature of the carbon source and the bacterial species (Payne, 1973). For most readily available organic carbon sources, a COD/NO_3^--N (w/w) ratio from 3.0 to 6.0 enables complete nitrate reduction to elemental nitrogen (Montieth et al., 1979; Narcis et al., 1979; Skinde and Bhagat, 1982), where COD stands for chemical oxidation demand and is expressed as mgO_2/l. As noted above, carbon limitation will result in the accumulation of intermediate products, such as NO_2 and N_2O, while excess

carbon will promote dissimilatory nitrate reduction to ammonia. In addition, denitrification rates depend on the type of carbon source. In anaerobic reactors, for example, denitrification was faster with acetate than glucose or ethanol (Tam et al., 1992). Differences in denitrification rates were found when denitrifying isolates from a fluidized bed reactor in a recirculating system were incubated with different short-chain volatile fatty acids (Aboutboul et al., 1995). In wastewater treatment plants and aquaculture systems, exogenous carbon substrates often are used to drive denitrification, with methanol most often used (Payne, 1973). However, endogenous carbon compounds liberated from organic sludge digestion may be used for this purpose in recirculating systems (Aboutboul et al., 1995).

3.2. Autotrophic denitrification

In addition to organic carbon, some denitrifying bacteria may use inorganic compounds, such as hydrogen and reduced sulfur, manganese and iron species as electron donors. Few studies have demonstrated the use of these processes to remove nitrate from contaminated water, but a sulfur-limestone reactor was used to promote autotrophic denitrification from wastewater (Flere and Zhang, 1998; Zhang and Lampe, 1999). The feasibility of denitrification at low COD/N ratios was demonstrated by taking advantage of the symbiotic relationship between sulfur denitrifying bacteria and sulfate reducing bacteria (Kim and Son, 2000). Some advantages of autotrophic denitrification over heterotrophic denitrification include: (1) low biomass buildup (biofouling) and reduction of reactor clogging and (2) avoidance of organic carbon contamination of treated water.

4. Denitrifiers and phosphate removal

Enhanced biological phosphorus removal (EBPR) from domestic wastewater in activated sludge plants is accomplished by alternate stages, where sludge is subjected to anaerobic and aerobic conditions. Phosphorus is released from bacterial biomass in the anaerobic stage and is assimilated by these bacteria in excess as polyphosphate (poly-P) during the aerobic stage. Phosphorus is removed from the process stream

by harvesting a fraction of the phosphorus-rich bacterial biomass (Toerien et al., 1990). Some of these polyphosphate accumulating organisms (PAO) are also capable of poly-P accumulation under denitrifying conditions, i.e. with nitrate instead of oxygen serving as the terminal electron acceptor (Barker and Dold, 1996; Mino et al., 1998). Studies on poly-P accumulating organisms have revealed the involvement of specific metabolic properties under anaerobic, aerobic and anoxic conditions (Mino et al., 1998). Under anaerobic conditions, acetate or other low molecular weight organic compounds are converted to polyhydroxyalkanoates (PHA), poly-P and glycogen are degraded and phosphate is released. Under aerobic and anoxic conditions, PHA is converted to glycogen, phosphate is taken up and poly-P is synthesized intracellularly. Under the latter conditions, growth and phosphate uptake is regulated by the energy released from the breakdown of PHA.

Some heterotrophic denitrifiers exhibit phosphorus storage in excess of their metabolic requirements through poly-P synthesis under either aerobic or anoxic conditions, without the need for alternating anaerobic/aerobic switches (Barak and van Rijn, 2000a). Unlike PAO, these denitrifiers were unable to use PHA as an energy source for poly-P synthesis and derived energy from oxidation of external carbon sources. The feasibility of this type of phosphate removal was demonstrated for freshwater as well as marine recirculating systems (Barak and van Rijn, 2000b; Shnel et al., 2002; Barak et al., 2003; Gelfand et al., 2003). In the culture water of these systems, stable orthophosphate concentrations were found throughout the culture period. Phosphorus immobilization took place in the anoxic treatment stages of the system where it accumulated to up to 19% of the sludge dry weight.

5. Alkalinity control by denitrification

In recirculating systems, intensive nitrification leads to an alkalinity loss and a resulting pH decline of the culture water. Acidic conditions negatively impact the biofilter performance and alkalinity supplements, such as sodium bicarbonate are routinely administered to stabilize pH and alkalinity. Heterotrophic denitrification results in an alkalinity gain and by incorporating

this process in the treatment scheme of a recirculating system one might be able to eliminate or reduce the use of alkalinity supplements (van Rijn, 1996).

The amount of acid required to titrate the bases in water is a measure of the alkalinity of water. A chemical reaction producing acid will lower the alkalinity of the water, while the opposite holds for a reaction in which acid is consumed or hydroxyl ions are produced. During nitrification, alkalinity decreases by approximately 7 mg $CaCO_3$ for each mg of ammonia-N oxidized to nitrate according to the following simplified stoichiometry:

$$NH_4^+ + 2O_2 = NO_3^- + 2H^+ + H_2O \qquad (2)$$

(Alkalinity loss = 2 meq of alkalinity per mole NH_4^+ or 7.14 mg $CaCO_3$/mg NH_4^+-N)

Some of this alkalinity loss is regained when, in addition to nitrification, denitrification is used as a water treatment stage. Heterotrophic denitrification causes a release of hydroxyl ions and raises alkalinity. Each mg of nitrate-N reduced to N2 causes an alkalinity increase of 3.57 mg $CaCO_3$ according to the following stoichiometry:

$$2NO_3^- + 12H^+ + 10e^- = N_2 + 6H_2O \qquad (3)$$
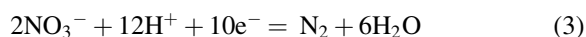
(Alkalinity gain = 1 meq of alkalinity per mole NO_3 or 3.57 mg $CaCO_3$/mg NO_3^--N)

Autotrophic denitrification on reduced sulfur compounds may generate or consume alkalinity depending on the reduced sulfur species oxidized (Oh et al., 2001; Kleerebezem and Mendez, 2002). In marine systems, reduced sulfur species are often produced by reduction of sulfate, an alkalinity-generating process (Eq. (4)). Sulfate reduction in combination with oxidation of reduced sulfur compounds will cause an overall increase in alkalinity as illustrated by the reduction of sulfate to sulfide and its subsequent reoxidation to sulfate (Eqs. (4) and (5)).

$$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O \qquad (4)$$
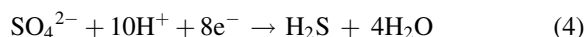
(Alkalinity gain = 2 meq of alkalinity per mole SO_4^{2-} or 100 mg $CaCO_3$/mole SO_4^{2-})

$$5H_2S + 8NO_3^- \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O + 2H^+ \qquad (5)$$
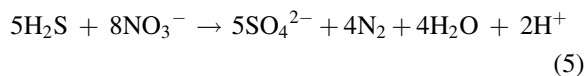
(Alkalinity loss = 2 meq per 5 moles H_2S or 20 mg $CaCO_3$/mole H_2S)

Sulfate reduction to sulfide generates an alkalinity of 100 mg $CaCO_3$ per mole SO_4^{2-} (Eq. (4)), and sulfide driven nitrate reduction to N_2 consumes an alkalinity of 20 mg $CaCO_3$/mole H_2S (Eq. (5)). Like heterotrophic denitrification, the coupled process of sulfate reduction, sulfide oxidation and nitrate reduction results in a net alkalinity generation of 400 mg $CaCO_3$ per 8 moles of NO_3 reduced or 3.57 mg $CaCO_3$ per mg NO_3-N reduced.

6. Denitrification in recirculating aquaculture systems

In the following section, a distinction is made between passive and induced denitrification. Freshwater and marine recirculating systems are discussed separately as are polymer-based, denitrification reactors used in aquariums. A summary of applications of denitrification reactors in recirculating systems is presented in Table 2 and denitrification rates by some of these reactors, discussed in the last part of this section, are presented in Table 3.

6.1. Passive denitrification in recirculating systems

Denitrification occurs in anoxic environments in the presence of oxidized carbon and inorganic nitrogen compounds. Given these requirements, it might be assumed that such conditions, confined to specific microsites, exist in most recirculating aquaculture systems. In a study on trickling filter biofilms, denitrification activity was observed in distinct zones of the biofilm (Dalsgaard and Revsbech, 1992). By means of microsensors, denitrification activity was measured at a depth of 0.2–0.3 mm below the biofilm surface. Oxygen levels and organic matter availability dictated the depth of the denitrifying zone. Ammonia lowered nitrate assimilation rates and increased nitrate availability for denitrification. Few studies have quantified passive denitrifying activity in recirculating systems. Passive denitrification, estimated by mass and isotopic balances of major nitrogen pools (Thoman et al., 2001), accounted for a

Table 2

Denitrification reactors in recirculating systems

Denitrifying reactor	Organism(s) cultured	Carbon/electron donor	Reference
Freshwater systems			
Activated sludge	Carp	Endogenous	Meske (1976)
Activated sludge	Tilapia, eel	Glucose/methanol	Otte and Rosenthal (1979)
Activated sludge	Trout	Hydrolyzed corn starch	Kaiser and Schmitz (1988)
Digestion basin and fluidized bed reactor	Tilapia	Endogenous	van Rijn and Rivera (1990), Arbiv and van Rijn (1995), Shnel et al. (2002)
Activated sludge	Eel	Endogenous	Knosche (1994)
Packed bed reactor	?	Methanol	Abeysinghe et al. (1996)
Packed bed reactor	?	Endogenous	Phillips and Love (1998)
Polymers	Ornamental carp	Endogenous	Nagadomi et al. (1999)
Polymers	Ornamental fish	Biodegradable polymers	Boley et al. (2000)
Packed bed reactor	Eel	Methanol	Suzuki et al. (2003)
Marine systems			
Packed bed reactor	Atlantic and Chinook salmon	Methanol	Balderston and Sieburth (1976)
Packed bed reactor	Japanese Flounder	Glucose	Honda et al. (1993)
Packed bed reactor	Squids	Methanol	Whitson et al. (1993)
Packed bed reactor	?	Ethanol	Sauthier et al. (1998)
Fluidized bed reactor	Ornamental fish	Methanol	Grguric and Coston (1998)
Polymers	?	Glucose	Park et al. (2001)
Packed bed reactor	Ornamental fish	Methanol	Grguric et al. (2000a,b)
Packed bed reactor	Shrimp	Ethanol/methanol	Menasveta et al. (2001)
Polymers	Ornamental fish	Starch	Tal et al. (2003a)
Digestion basin and fluidized bed reactor	Gilthead seabream	Endogenous	Gelfand et al. (2003)
Moving bed bioreactor	Gilthead seabream	Starch	Morrison et al. (2004)

Table 3

Volumetric denitrification rates by some denitrifying reactors

Denitrifying reactor	Medium	Carbon source	Nitrate removal rate (mg NO$_3$-N/l/h)	Reference
Freshwater systems				
Fluidized bed	Sand	Endogenous	35.8	Arbiv and van Rijn (1995)
Packed bed	Biodegradable polymers	PHB $(C_4H_6O_2)_n$	7–41	Boley et al. (2000)
Packed bed	Biodegradable polymers	PCL $(C_6H_{10}O_2)_n$	21–166	Boley et al. (2000)
Packed bed	Biodegradable polymers	Bionolle $(C_6H_8O_4)_n$	1.5–77	Boley et al. (2000)
Packed bed	Polyethylene	Methanol	1.8[a]	Suzuki et al. (2003)
Digestion basin	Sludge	Endogenous	5.9	Shnel et al. (2002)
Fluidized bed	Sand	Endogenous	55.4	Shnel et al. (2002)
Packed bed	Freeze-dried alginate beads	Starch	26.0	Tal et al. (2003)
Digestion basin	Sludge	Endogenous	1.5	Gelfand et al. (2003)
Fluidized bed	Sand	Endogenous	43.3	Gelfand et al. (2003)
Marine systems				
Packed bed	Plastic medium	Glucose	1.7	Honda et al. (1993)
Packed bed	Brick granules	Ethanol	100	Sauthier et al. (1998)
Packed bed	Porous medium	Methanol	7.3–8.4[a]	Grguric et al. (2000a, b)
Packed bed	Polyvinyl alcohol	Glucose	1.4	Park et al. (2001)
Packed bed	Plastic balls/crushed oyster shells	Ethanol/methanol	6.6[a]	Menasveta et al. (2001)
Packed bed	Freeze-dried alginate beads	Starch	2.6	Tal et al. (2003)
Digestion basin	Sludge	Endogenous	2.5	Gelfand et al. (2003)
Fluidized bed	Sand	Endogenous	72.6	Gelfand et al. (2003)
Moving bed reactor	Plastic medium	Endogenous	24.0	Tal and Schreier (2004)

[a] Extrapolated (rates were not provided by authors).

nitrogen loss of 9–21% in a closed recirculating mariculture system for culture of red drum (*Sciaenops ocellatus*). These findings were supported by a study on a marine recirculating shrimp production system (McCarthy and Gardner, 2003) where, using membrane inlet mass spectrometry, significant nitrate removal was detected in media from a nitrifying filter and sediment derived from the system. Additional evidence for the denitrification potential of nitrifying media was recently provided in a study on a moving bed bioreactor in a recirculating facility for culture of gilthead seabream (*Sparus aurata*) by Tal et al. (2003).

6.2. Induced denitrification in freshwater recirculating systems

Studies on these reactors were initiated in Germany by Meske (1976) by incorporating an activated sludge tank in an experimental recirculating culture system for common carp (*Cyprinus carpio*). Similar experimental systems with or without addition of external carbon sources were subsequently operated by a number of investigators with different freshwater fish (Otte and Rosenthal, 1979; Gabel, 1984; Kaiser and Schmitz, 1988; Schmitz-Schlang and Moskwa, 1992; Knosche, 1994). Denitrifying activity in packed bed columns was studied by Abeysinghe et al. (1996) and Suzuki et al. (2003) with methanol as an external carbon source. Denitrification on endogenous carbon sources was studied in a closed freshwater recirculating culture system for tilapia (Arbiv and van Rijn, 1995; van Rijn and Barak, 1998; Shnel et al., 2002). In these studies, carbon compounds, released from the breakdown of endogenous carbon, were used to fuel denitrification in an anoxic treatment step consisting of a digestion basin and a fluidized bed reactor. The feasibility of using endogenous fermentation generated carbon sources for denitrification in recirculating aquaculture systems also was described by Phillips and Love (1998).

6.3. Induced denitrification in marine recirculating systems

Pioneer work on marine closed systems for the culture of salmonids was conducted by Meade and coworkers (Meade, 1973; Meade, 1974; Meade and Kenworthy, 1974). Nitrate removal in these systems was examined by Balderston and Sieburth (1976)

using experimental packed columns fed with methanol. A spin-off system is successfully used in a recirculating marine culture system for cephalopods (Whitson et al., 1993; Lee et al., 2000). Packed bed reactors fed with different external carbon sources were used in a number of other studies with different marine organisms (Honda et al., 1993; Sauthier et al., 1998; Menasveta et al., 2001). Starch-supplemented moving bed bioreactors were used for denitrification in a gilthead seabream (*Sparus aurata*) recirculating system (Morrison et al., 2004). Large denitrification units for treatment of public aquarium water at the New Jersey State Aquarium (total aquarium volume: 2.9 million l) and the Living Seas at EPCOT Center, Florida (total aquarium volume: 23 million l) have been employed successfully in recent years. Denitrification is induced in these systems using submerged and fluidized bed reactors with addition of methanol (Grguric and Coston, 1998; Grguric et al., 2000a,b). The feasibility of denitrification in a marine recirculating system for culture of gilthead seabream with endogenous carbon as the sole carbon source was demonstrated in a closed system comprising an anoxic digestion basin and fluidized bed reactor (Gelfand et al., 2003). Nitrate removal in this system was mediated by both heterotrophic and autotrophic denitrification. Chemical analyses of the sulfur transformations and microbiological analyses of the bacterial populations in this treatment system revealed that sulfide, produced by sulfate reduction in the anaerobic parts of the digestion basin, was reoxidized by autotrophic denitrifiers (Cytryn et al., 2003). It is interesting to note that alkalinity lost in the nitrifying treatment stage was fully regained in the anoxic treatment stage (Gelfand et al., 2003). A recirculating system for culture of gilthead seabream with nitrate removal by autotropic denitrifiers on reduced sulfur compounds was recently reported by Tal and Schreier (2004).

6.4. Denitrification by means of immobilized systems

Nitrate removal by means of immobilized denitrifiers has been studied since the 1980s (Nilson et al., 1980). Entrapment of denitrifiers is accomplished with non-synthetic materials, such as agar, κ-carrageenan, chitosan and alginate, or synthetic polymers, such as PVC—polyvinylchloride, PP—polypropylene and

PS—polystyrene with or without addition of a degradable carbon source (Tal et al., 2001). Biodegradable polymers, serving both as matrix and carbon source, are also used for this purpose (Biedermann et al., 1992). Nitrate removal by immobilized complexes has been studied only on an experimental scale in aquariums. Nagadomi et al. (1999) performed tests on nitrate removal in aquariums stocked with ornamental carp by means of the photosynthetic bacterium, *Rhodobacter spaeroides* S, immobilized in alginate and polyvinyl alcohol (PVA) gel beads. PVA gels were also used by Park et al. (2001) with immobilized denitrifiers derived from activated sludge in a study on nitrate removal in marine recirculating aquarium systems. Tal et al. (2003a,b) used a freeze-dried, alginate-starch matrix as an entrapping agent for heterotrophic denitrifiers (*Pseudomonas* spp.) in the removal of nitrate from freshwater and marine aquariums. A different approach was used in a study by Boley et al. (2000) where several types of biodegradable polymers were used a substrate for endemic denitrifiers in a freshwater aquarium system.

6.5. Denitrification rates

Oxidation of an organic carbon and electron donor and subsequent reduction of nitrate to elemental nitrogen yields around 70% of the energy gained with oxygen as the final electron acceptor (Payne, 1970). High nitrate removal rates can be accomplished with this energy efficient process under suitable conditions. As stated earlier, information on denitrification in recirculating systems is scarce and nitrate removal rates by denitrification reactors are reported in only few studies. In some studies, sufficient information is provided to allow calculation of these rates, while in others this information is lacking. Volumetric nitrate removal rates by different denitrifying reactors used in aquaculture facilities and in aquariums are summarized in Table 3. The wide range (1–166 mgNO$_3$-N/l/h) in rates is most likely due to differences in operational parameters, such as system configuration, types of electron donor, reduction states of the reactors, and the ambient nitrate concentrations at which the various reactors were operated. No clear differences in denitrification rates are found between systems 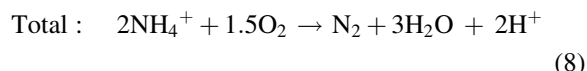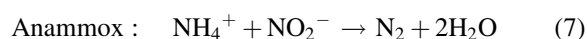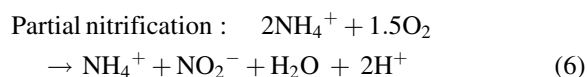in which external carbon sources are used to fuel denitrification and systems that are operated with endogenous carbon sources. Also, no distinct differences are found between denitrification reactors operated in freshwater and marine systems. It should be noted, however, that due to differences in operational parameters of these systems, such comparisons are extremely difficult.

The reported volumetric nitrate removal rates do provide an indication for the size of denitrification reactors relative to that of nitrification reactors Volumetric ammonia removal rates in commonly used nitrification filters, such as bead filters and trickling towers are 1.4–15 mg TAN/l/h and 3–4 mg TAN/l/h, respectively (calculated from Timmons et al., 2001). These values are often lower than reported nitrate-nitrogen removal rates (Table 1), implying that nitrate removal can be accomplished in smaller reactors than ammonia removal. This finding might be explained by the different requirements of both processes. Nitrifying filters are characterized by a relatively large void volume in order to prevent organic matter accumulation and optimal oxygen penetration into the nitrifying biofilm. This is in contrast to denitrifying reactors, which can be designed in a more compact manner due to their anaerobic mode of operation. In addition to size, daily water flow through nitrification and denitrification reactors differs significantly due to differences in allowable ammonia and nitrate concentrations in the culture systems. The need for low ambient ammonia concentrations requires a rapid water exchange between fish tanks and nitrification reactors, coinciding with relatively low ammonia removal rates per single filter pass. System operation at relatively high ambient nitrate concentrations supports relatively high nitrate removal rates per single reactor pass and allows a much smaller water exchange between fish tanks and denitrification reactors.

7. Anammox as an alternative to denitrification

Anaerobic ammonia oxidation (anammox) is a microbially-mediated process (Mulder et al., 1995) identified in engineered systems as well as in natural environments, and has been applied to wastewater treatment systems (Schmidt et al., 2003). Carried out by bacteria of the order *Planctomycetales*, anammox eliminates nitrogen by combining ammonia and nitrite

to produce nitrogen gas (van de Graaf et al., 1995), thereby providing an alternative approach to nitrogen removal via denitrification. Application of anammox in treating recirculating system water is desirable as it has the potential of providing significant oxygen and energy savings due the oxidation of only half of the ammonia produced in the system (Eqs. (6)–(8)). Moreover, anammox enables complete ammonia removal via autotrophic pathways without the requirement of organic carbon.

$$\text{Partial nitrification}: \quad 2NH_4^+ + 1.5O_2$$
$$\rightarrow NH_4^+ + NO_2^- + H_2O + 2H^+ \tag{6}$$

$$\text{Anammox}: \quad NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \tag{7}$$

$$\text{Total}: \quad 2NH_4^+ + 1.5O_2 \rightarrow N_2 + 3H_2O + 2H^+ \tag{8}$$

In studies designed to characterize the microbial consortium of aerobic and anaerobic biofilters, Tal et al. (2003b, 2004) obtained evidence for the presence of anammox-related microorganisms in aquaculture recirculating systems. Using molecular identification methods based on 16S-rRNA gene sequences, anammox bacterial 16S rRNA sequences were amplified from the microbial consortia of the filters from both marine and freshwater recirculated aquaculture systems (Tal et al., 2004). Anammox activity was demonstrated in lab-scale experiments by incubating microbial consortia under anaerobic conditions in the presence of ammonia and nitrite. While the actual portion of nitrogen released via anammox is difficult to assess, it is reasonable to consider that some of the "passive denitrification" or nitrogen loss observed in recirculating systems could be explained by anammox. Whether anammox could be applied to recirculating systems as a means to control nitrogen load in lieu of conventional denitrification approaches remains to be determined. A major limitation of the anammox process is the slow growth rate for these bacteria. With doubling times of around 11 days (Strous et al., 1999a,b), it seems unlikely that these organisms can be enriched in biofilter systems. Nevertheless, recent reports on the successful application of anammox in wastewater treatment plants (Strous et al., 1998; Schmidt et al., 2003) are

encouraging and justify studies on the exploitation of this process in aquaculture systems.

8. Future directions concerned with denitrification in recirculating systems

Research on denitrification in recirculating systems has been conducted for a considerable time. Often, these studies were performed on laboratory simulation systems or small, experimental facilities. These systems can only partially simulate conditions in commercial recirculating systems, and a need exists for information on the performance of denitrifying reactors in whole systems. Even before the implementation of a denitrification treatment step, basic studies on nutrient budgets, such as those by Thoman et al. (2001) and McCarthy and Gardner (2003) for recirculating systems and, more extensively, for pond systems (e.g. Krom et al., 1985; Schroeder, 1987; Krom et al., 1995), should be conducted. Proper design of a denitrification reactor should be based on comprehensive understanding of the dynamics of nitrogen, carbon and other inorganic nutrients in a particular recirculating system. Internal versus external carbon and electron donors for induction of denitrification as well as induction of heterotrophic or autotrophic denitrifiers should be based on rational rather than arbitrary information. Denitrification combined with organic matter digestion enables a virtually closed operation of freshwater and marine recirculating systems. Enabling the culture of marine species away from the coast will direct the aquaculture industry to new, unexplored avenues (Krom et al., 2001).

At present, application of denitrification in commercial recirculating systems is conducted at a limited scale. Based on the experimental systems reviewed in this paper, it seems that full scale implementation of denitrification is feasible. However, the lack of studies on large-scale recirculating systems, as mentioned above, has limited commercial application of denitrification in recirculating systems. Moreover, incentives to implement denitrification in commercial recirculating systems are still lacking. Economic incentives related to savings on water usage, pH control and environmental discharge fees are still of inadequate significance in the total operation costs to necessitate

nitrate removal in these systems. Illustrative of this point is the fact that large scale denitrification, applied in public aquariums (Grguric and Coston, 1998; Grguric et al., 2000a,b), probably is based less on financial considerations than system performance and environmental impact. The fact that little or no documentation exists on the performance of denitrification reactors in the few commercial systems using this technology is another drawback for full-scale application of denitrification. Finally, like any new technology, information transfer from experimental facilities to commercial applications is time consuming and requires cooperation to enable exchange of information on benefits of the new technology.

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