Stimulating Denitrification in a Marine Recirculating Aquaculture System Biofilter Using Granular Starch as a Carbon Source

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ABSTRACT

Maintaining superior water quality in intensive recirculating aquaculture systems (RAS) by controlling levels of inorganic nitrogenous waste—ammonia, nitrate and nitrite—derived from uneaten food and fecal excretion is often a challenge. In most systems, solids are removed mechanically and ammonia is oxidized to nitrate by nitrifying biological filtration; nitrate is subsequently eliminated through numerous water exchanges. Alternatively, nitrate removal is achieved using a bacterial-mediated denitrification component that reduces nitrate to nitrogen gas under anoxic conditions, a process that depends on the application of external or endogenous electron and carbon donors, e.g. carbohydrates or organic alcohols. In this study, we compared the capacity of acetate, glucose, soluble starch, and granular starches to promote the denitrifying activity of heterotrophic bacteria in biofilm-coated polyethylene beads

from a marine RAS moving bed bioreactor (MBB) under anaerobic conditions. Granular starches (corn, wheat, and rice) were as effective as glucose in supporting denitrification, and were 7.6 and 9.8 times more effective in removing nitrate when compared to soluble starch and acetate, respectively. Furthermore, granular starches retained their denitrification potential for longer time periods than soluble starch or acetate. The low cost, ease of use, and non-toxic nature of granular starches make them an ideal exogenous carbon source for promoting denitrification in RAS bioreactors.

INTRODUCTION

Recirculating aquaculture systems (RAS) have become an attractive approach for farming fish due to their advantage in providing high yields of fish stock, as well as their capacity to be both biosecure and environmentally sustainable (Tal *et al.* 2009, Zohar *et al.* 2005). Because the success of commercial aquaculture depends on creating an environment optimized for rapid growth, one of the benefits of using a semi-closed culture unit is the manageability of water parameters that influence fish health and growth rate densities (Cytryn *et al.* 2003, Timmons *et al.* 2002, Zohar *et al.* 2005). At the same time, a major drawback of RAS stems from significant loading of organic matter derived from uneaten food and fecal excretion, which leads to oxygen depletion and the accumulation of toxic nitrogen compounds such as ammonia and nitrite (Prinsloo *et al.* 1999, van Rijn 1996). As part of the remedy for these problems, solid wastes are usually removed by mechanical filtration or sedimentation. In addition, RAS incorporate biological filtration that includes nitrification to remove toxic inorganic nitrogen. This microbe-driven process oxidizes ammonia to nitrite and, subsequently, nitrate, under aerobic conditions (Timmons *et al.* 2002, van Rijn and Rivera 1990). To alleviate the threat of low oxygen levels, oxygen is pumped directly into the culture chamber and heavy aeration is employed to ensure nitrifying bacteria receive ample oxygen to support their oxidizing activity (Timmons *et al.* 2002).

A major challenge faced by industry is the need for a mechanism to manage the accumulation of nitrate that occurs in recirculating systems as water exchange rates are reduced (van Rijn *et al.* 2006, Zohar *et al.* 2005). However, studies focusing on nitrate removal have been few,

primarily because high nitrate concentrations have not been considered to directly impact most cultured organisms. Still, control of nitrate levels is warranted, since fish have been shown to suffer from nitrate stress (Burgess 1995, Grguric *et al*. 2000, Hrubec *et al*. 1996). Additionally, waste management and disposal have become increasingly important due to increases in the stringency of environmental regulations (Costa-Pierce and Desbonnet 2005, White *et al*. 2004).

Successful removal of nitrate from wastewater has been achieved by the inclusion of biological denitrification, an anaerobic process that reduces nitrate to nitrogen gas. The process requires a suitable electron donor to fuel the heterotrophic activity as a carbon and energy source (Gomez *et al*. 2000, Grguric *et al*. 2000, Lee and Welaner 1996, van Rijn *et al*. 2006). Denitrification of industrial wastewater containing high nitrate (>1000 mg-N/l influent) has been accomplished using an activated sludge process (Glass and Silverstein 1999, Labelle *et al*. 2005, Mycielski *et al*. 1983) and van Rijn and Rivera (1990) attempted a similar nitrate reduction treatment by moving organic matter derived from uneaten feed and fish waste from an intensive RAS tank through a denitrifying fluidized bed reactor. Performance of this system, however, suggested that carbon limitation was a likely factor underlying the low denitrification rates (Arbiv and van Rijn 1995).

Because the concentration of available carbon sources in RAS may be insufficient to sustain nitrate removal, an external source must be supplied (Isaacs *et al*. 1994, Phillips and Love 1998). Chemostat experiments demonstrated that activated sludge could yield comparatively high denitrification rates (from 26-76 mg NO_3-N/g TSS/h) when fed with carbon sources including hydrolyzed starch, methanol, acetic acid and crude syrup (Lee and Welaner 1996). Chen *et al*. (1991) supplied a combined nitrification loop and denitrifying submerged bioreactor with excess methanol in long-term continuous cultivation to completely reduce 200-1000 mg NO_2-N/l. Similarly, in a study using a submerged filter to remove nitrate from groundwater, ethanol and methanol were found to be more effective than sucrose when added to treat groundwater containing 100 mg NO_3-N/l (Gomez *et al*. 2000).

In previous studies we observed that nitrifying biofilters from marine RAS filter systems harboring different organic loads exhibited differing potential for carrying out nitrogenous transformations. We found that

filters with high organic load levels demonstrated a denitrification capacity in the absence of an external carbon source, while adding acetate could stimulate denitrifying activity for the low organic load (Tal *et al*. 2003). In the present study, we evaluated the effectiveness of soluble and granular carbon sources in stimulating denitrification activity of heterotrophic bacteria associated with both intensive (high load) and relatively low intensive (low load) marine RAS biofilters. Carbon sources were selected based on their complexity and molecular weight, as it has been shown that simple carbon compounds favor biological nitrogen removal over those with more complex molecular structures (Gomez *et al*. 2000, Hallin and Pell 1998, Peng *et al*. 2007), and that the molecular weight of a carbon source significantly influenced denitrification efficiency (Her and Huang 1995). We found that granular starches (corn, wheat, and rice) were as effective in supporting denitrification as glucose and acetate, and furthermore, maintained significant potential for nitrate removal long after acetate and soluble starch.

MATERIALS AND METHODS

Laboratory-Scale Experiments

Batch experiments were performed using polyethylene beads removed from "high" (8-10 mg dry organic matter/bead; initial O_2 consumption rate of 0.9 mg O_2/l/min/bead) and "low" organic load (2-4 mg dry organic matter/bead; initial O_2 consumption rate of 0.4 mg O_2/l/min/bead) marine recirculating nitrifying moving-bed biofilter (MBB) systems (Tal *et al*. 2003). High-load filter beads were obtained from a 2 m^3 aerobic nitrifying MBB filled with a bead volume of 1 m^3. The aerobic MBB linked two 4.2 m^3 tanks containing 10-20 kg/m^3 of gilthead seabream, *Sparus aurata*, and a 0.3 m^3 anaerobic cylindrical denitrification tank, densely packed with 0.2 m^3 of polyethylene beads having a specific surface area of 500 m^2/m^3. The system was maintained with a salinity of 17 ppt and was operated as described previously (Tal *et al*. 2003). Low organic load filter beads were collected from a separate nitrification MBB unit that was connected to a large 10.5 m^3 culture tank holding a variety of marine fish at stock density of approximately 5 kg/m^3.

Under anaerobic conditions and in the presence of 200 mg NO_3^--N/ml, beads were incubated at room temperature until they were no longer capable of reducing additional nitrate applications, indicating that all

endogenous organic sources were depleted. At this point, corn, wheat, and rice starches, soluble starch, glucose, and (potassium) acetate were added to evaluate their ability to independently stimulate denitrification. Biofilter beads (170) from the low organic load biofilter system were partitioned into 200 ml capped roller tubes containing synthetic saltwater media (Tal *et al*. 2003) at pH 7.0 - 7.5 and supplemented with a carbon source at a final concentration of 2.7 mg carbon source/ml with 150 mg NO_3-N/l. Under these conditions, carbon to nitrogen (C:N) ratios at the start of each experiment were 8:1 for each carbon source. The high organic load bead samples were treated in the same manner and each condition was done in duplicate. All sample solutions were flushed with nitrogen gas and tubes were immediately incubated at room temperature and rotated continuously at 10 rev/min in an Amersham Hybridization Oven/Shaker (GE Healthcare, Amersham Biosciences, Pittsburgh, PA, USA).

Sampling Procedure and Analysis

During the course of incubation, 1 ml samples were removed from each tube, centrifuged at 12,000 x g for 5 minutes and concentrations of nitrite, nitrate, total carbon (TC), and total available carbon (TAC) were determined in supernatant fractions. Periodic adjustments were made with additions of NaOH to maintain pH within a range of 7.0 - 7.5. All measurements were done in duplicate within 24 hours of collection, or when not analyzed immediately, after storage at 4°C. Nitrite and nitrate concentrations were determined as described by Tal *et al*. (2003). TAC and TC measurements of starch solutions were determined using the anthrone reagent as described previously (Tal *et al*. 1999). Statistical analyses for nitrate consumption rates were determined within the linear portions of the graphs (correlation coefficient >0.9) using the LINEST least squares method in Excel X for Mac (Microsoft, Redmond, WA, USA).
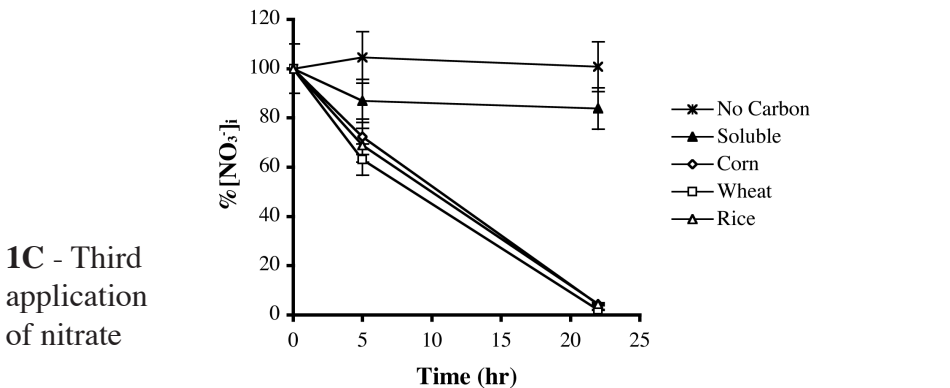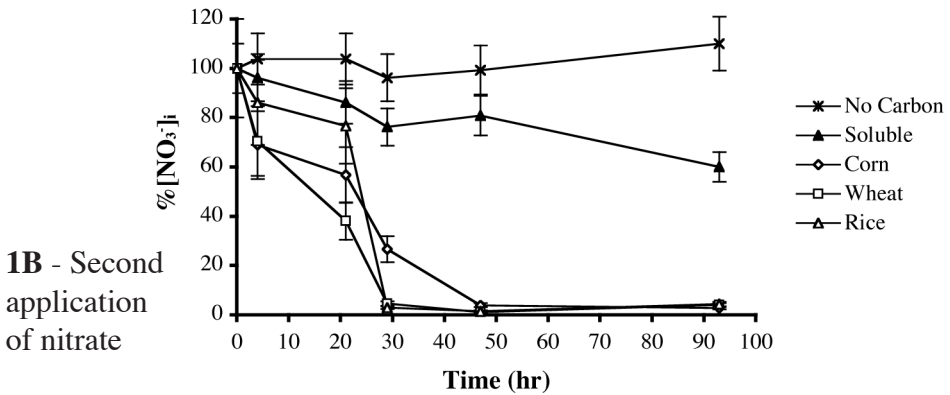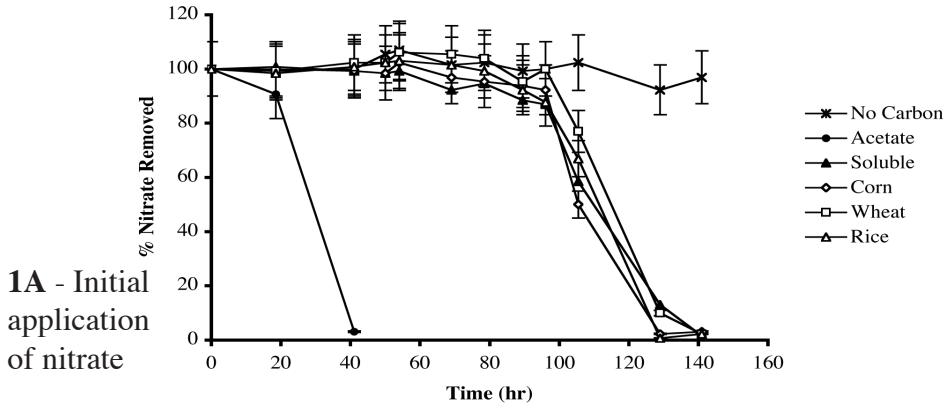
RESULTS

Effect of Carbon Source on Denitrification Potential of Low-Load Beads

To examine the ability of individual carbon sources to stimulate denitrification, low-load beads were incubated under anoxic conditions in the presence of nitrate (150 mg/l) to promote complete utilization of

Figure 1. Nitrate removal activity of low-load beads in the presence of various carbon sources. The initial nitrate and carbon source concentrations were 150 mg NO$_3$-N/l and 2.7 mg/ml, respectively. Nitrate utilization is expressed as the percentage of initial nitrate concentration (% [NO$_3$-]$_i$) that remained at each time point sampled.

1A - Initial application of nitrate

1B - Second application of nitrate

1C - Third application of nitrate

endogenous carbon sources. Under these conditions, nitrate removal by denitrifying heterotrophs established within the nitrifying community was stimulated after several days of incubation under anaerobic conditions (Tal *et al*. 2003). Once the ability to metabolize nitrate could no longer be detected (as determined by the absence of measurable nitrate-removing activity), beads were distributed equally into roller tubes and nitrate removal was measured after addition of acetate, soluble starch, and granular corn, wheat and rice starches. Compared to control (no carbon source addition), all carbon sources were found to support nitrate utilization (Figure 1A). With the exception of acetate, which stimulated nitrate utilization within approximately 20 hours after its addition, significant decreases in nitrate concentrations were detected 90 to 100 hours after carbon source addition. The delay in nitrate-removing activity likely reflected a difference in the availability of simple and complex soluble and insoluble carbohydrates for use as reducing agents. Once nitrate utilization occurred, nitrate removal rates were found to vary between 2.5 and 5.4 mg NO_3-N/l/hr, with acetate providing the greatest activity (Table 1).

Table 1. Nitrate removal rates for low- and high-load beads in the presence of the various carbon sources[a].

Carbon source	Low-Load Beads NO_3- removal (mg NO_3-N/l/hr)			High-Load Beads NO_3- removal (mg NO_3-N/l/hr)		
	1st NO_3- Addition	1st NO_3- Addition	1st NO_3- Addition	1st NO_3- Addition	1st NO_3- Addition	1st NO_3- Addition
Acetate	5.4 ± 0.1	ND[b]	ND	8.5 ± 1.7	13.6 ± 3.2	1.1 ± 0.2
Glucose	ND	ND	ND	10.2 ± 2.0	9.5 ± 1.5	10.9 ± 2.2
Soluble Starch	2.5 ± 0.2	0.61 ± 0.03	0.84 ± 0.21	ND	ND	ND
Wheat Starch	3.5 ± 0.4	3.8 ± 0.8	6.4 ± 1.1	5.6 ± 0.8	10.7 ± 3.1	9.1 ± 1.8
Rice Starch	3.6 ± 0.4	4.4 ± 0.7	6.3 ± 0.7	3.6 ± 0.7	10.7 ± 2.1	10.6 ± 2.1
Starch	3.7 ± 0.5	2.8 ± 0.5	6.4 ± 0.4	4.2 ± 0.9	11.1 ± 3.0	10.8 ± 2.0

[a]*Nitrate removal rates were calculated using the linear portions (displaying a correlation coefficient >0.9) of the graphs from Figures 1 (low-load beads) and 3 (high-load beads).*

[b]*ND; not determined.*

2A -
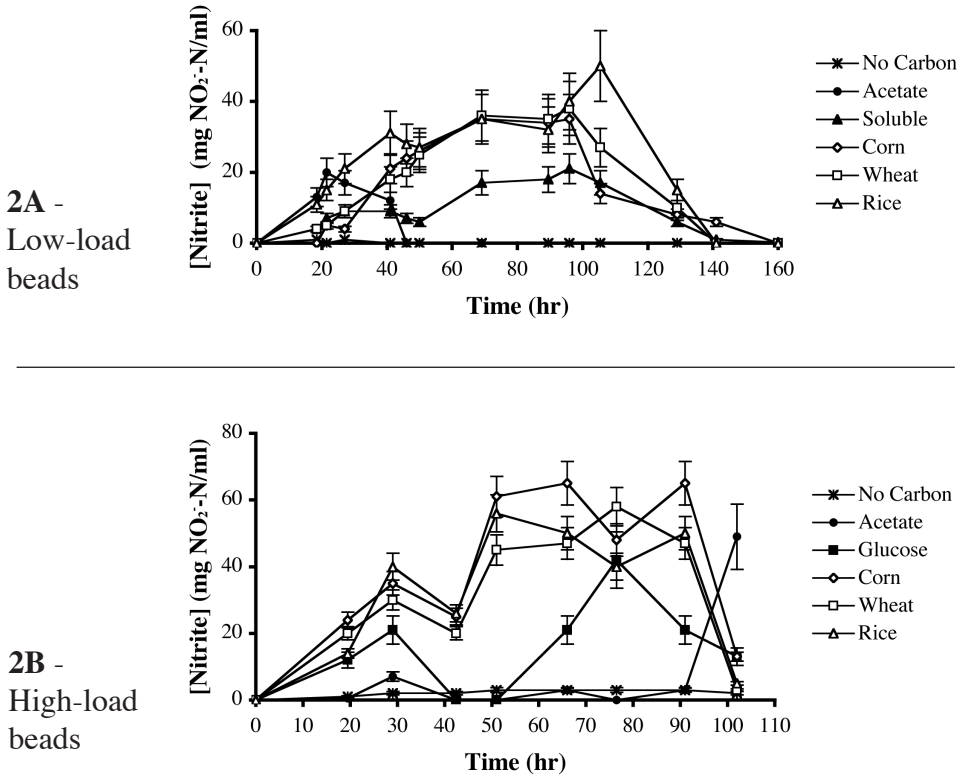Low-load
beads

2B -
High-load
beads

Figure 2. Nitrite removal activity of beads in the presence of various carbon sources. Low-load (A) and high-load (B) beads were treated with an initial nitrate concentration of 150 mg NO_3-N /l. Carbon sources were added at a final concentration of 2.7 mg/ml.

For all treatments (except control), nitrite levels were found to gradually accumulate—rising to their highest levels near the time that nitrate consumption could be detected—and then decreasing to undetectable levels at a time that was nearly coincidental with complete nitrate utilization (Figure 2A). For acetate, nitrite levels were highest (20 mg NO_2-N/l) 21 hours after acetate addition and decreased to undetectable levels after nitrate was completely utilized. Similarly, 96 hours after addition of the other carbon sources, nitrite levels peaked between 35 and 50 mg NO_2-N/l in the presence of granular corn, wheat, and rice starches and 21 mg NO_2-N/l for soluble starch (Figure 2A). At 106 hours, or near the time when nitrate concentrations began to decrease (Figure 1A), nitrite levels also declined and completely disappeared after all nitrate

was utilized (Figure 2A). Nitrite could not be detected at any time in tubes that were not supplemented with carbon source (control).

After nitrate levels decreased below detection limits, TAC measurements of incubations supplemented with soluble and granular starches indicated the absence of measurable soluble carbohydrates (data not shown). To assess remaining denitrification potential in the absence of additional carbon source application, a second dose of nitrate (150 mg NO_3-N/l) was placed into each tube and nitrate levels were monitored. As shown in Figure 1B, nitrate consumption occurred shortly after the second nitrate treatment in beads supplemented with the granular starches at rates between 2.8 to 4.4 mg NO_3-N/l/hr (Table 1), which were similar to those observed for the first dose of nitrate, and nearly complete nitrate removal was observed between 30 to 50 hours post-application (Figure 1B). Beads supplemented with soluble starch, on the other hand, exhibited a 4.2-fold decrease in nitrate utilization activity compared to the first dose, and approximately 60% of the second dose of nitrate remained 90 hours post-addition (Figure 1B). Consumption of nitrate by the acetate-supplemented tubes could not be detected (data not shown). A third treatment of nitrate (150 mg NO_3-N/l) resulted in nitrate removal patterns for granular starches that were similar to those obtained for the second dose (Figure 1C) although consumption rates were nearly two times greater than those obtained for the first nitrate application (Table 1). Nitrate-removing activity for the soluble starch-supplemented beads in the third nitrate treatment was minimal (Figure 1C and Table 1) and similar to the activity observed after the second addition (Figure 1B).

Denitrification Potential of High-Load Beads with Added Carbon Source

The denitrification activity of beads from a high-load filtration system were examined in the same manner as the low-load beads (Figure 3). As was observed for the low-load beads, all carbon source supplements were capable of stimulating nitrate removal. After an approximate 20-30 hr delay, acetate and glucose yielded nearly 100% removal approximately 40 hours after their addition, at rates of 8.5 ± 1.7 and 10.2 ± 2.0 mg NO_3-N/l/hr, respectively. In the presence of the granular starches, nitrate levels started to decrease between 40 to 75 hours after carbon source addition—25 to 60 hours earlier than observed for the low-load beads (compare Figures 1A and 3A), - with maximum removal rates between

3A - Initial application of nitrate
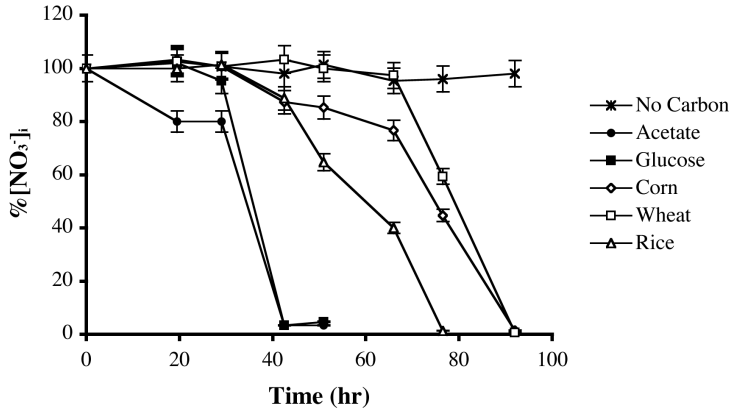
3B - Second application of nitrate
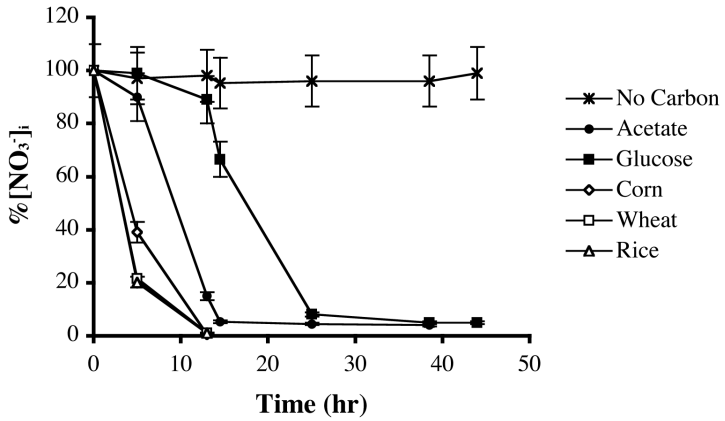
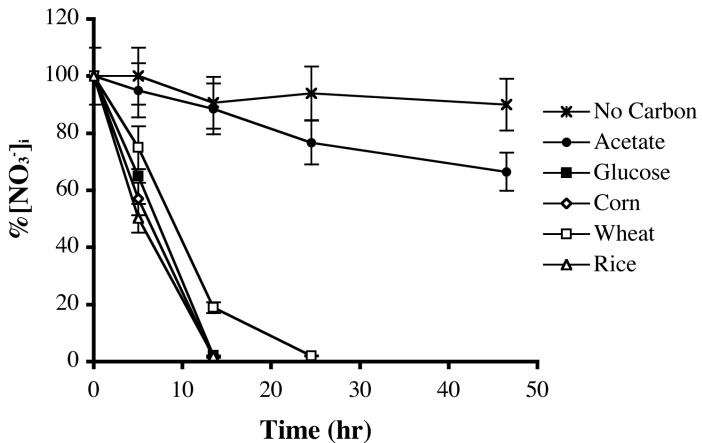**3C** - Third application of nitrate

Figure 3. Nitrate removal activity of high-load beads in the presence of various carbon sources. The initial nitrate and carbon source concentrations were 150 mg NO_3-N/l and 2.7 mg/ml, respectively. Nitrate utilization is expressed as the percentage of initial nitrate concentration (% [NO_3^-]$_i$) that remained at each time point sampled.

approximately 3.6 and 5.6 mg NO_3-N/l/hr, which were similar to those observed for the low-load beads (Table 1).

Nitrite levels for high-load beads supplemented with granular starch exhibited patterns very similar to those found for the low-load bead experiments (Figure 2B). Levels gradually increased to as much as 65 mg NO_2-N/l from 50 to 70 hours post nitrate addition for the granular starches and only 7 and 23 mg NO_2-N/l for acetate and glucose, respectively, 29 hours after nitrate addition. Within 10-15 hours after reaching their maximal levels, nitrite concentrations in the presence of each carbon source decreased to their lowest levels (Figure 2B), in parallel with nitrate consumption. Nitrite could not be detected at any time in tubes with beads lacking a carbon source (Figure 3A).

After nitrate concentrations decreased below the level of detection, nitrate (150 mg NO_3-N/l) was added to each tube to assess residual denitrification potential of the high-load beads. As can be seen in Figure 3B, nitrate removal in incubations supplemented with granular starches occurred without significant delay, with all nitrate utilized within 12 hours post-addition at a rate of approximately 11 mg NO_3-N/l/hr (Table 1) - almost 3 times faster than their activities during the initial nitrate addition. On the other hand, nitrate removal for acetate and glucose-supplemented tubes was delayed almost 20 and 40 hours, respectively, and maximum removal rates were similar to those observed after the first nitrate addition (Table 1). After 70 hours—the point at which nitrate concentrations were no longer detectable (Figure 3B)—each tube received a third application of nitrate (150 mg NO_3-N/l). Beads treated with glucose and the three granular starches continued to show nitrate removal activity that was comparable to the second nitrate application in both appearance and removal rates (Figure 3C and Table 1). Acetate-supplemented beads, on the other hand, exhibited a marked decrease in their ability to support significant denitrification activity, with a nitrate removal rate that was 12.4-fold lower than that observed for the second dose, suggesting that acetate was no longer available as a carbon source to support this process (Figure 3C).

Unlike the pattern of activity observed with the first nitrate addition, nitrite levels examined in the granular starch-supplemented tubes remained very low after the second dose of nitrate and did not rise to levels greater than 15 mg NO_2-N/l (not shown). On the other hand,

the decreased ability of the glucose-supplemented filter to support denitrification was reflected in the accumulation of nitrite levels that exceeded 40 mg NO_2-N/l, which declined to near undetectable levels only after complete exhaustion of nitrate at 70 hours (not shown).

DISCUSSION

Wastewater treatment systems that rely on activated sludge or submerged filters often require addition of suitable carbon sources such as ethanol, methanol, or acetate in order to enhance nitrate removal (Lee and Welaner 1996, Stief 2001). Using these carbon sources to stimulate denitrification, however, requires their frequent application, since they are consumed by denitrifying bacteria at a rapid rate and are favored for nitrogen removal over more complex ones (Gomez *et al*. 2000, Hallin and Pell 1998, Peng *et al*. 2007). This is consistent with our finding that the microbial communities of low- and high-load beads obtained from RAS MBBs actively reduced nitrate in the presence of acetate and glucose at rates of between 1.5 to 2.8 times faster than those obtained for soluble or granular starches (Table 1). For the granular starches, comparable nitrate removal rates were initially obtained for both low- and high-load systems, although appearance of nitrate utilization in the high-load system in the presence of the granular starches occurred between 25 and 60 hours earlier than for the low-load beads (compare Figures 1A and 3A). The difference in timing is likely due to a variation in the composition and abundance of the bacterial communities in the two systems; the high-load biofilter beads originated from a RAS that included an active denitrification loop, providing a pool of bacterial denitrifiers (Tal *et al*. 2003, Tal *et al*. 2006). Enrichment of bacterial denitrifiers and/or stimulation of their activities after the first application of nitrate for both low- and high-load beads would also explain the appearance of nitrate removal shortly after addition of both the second and third nitrate doses for both soluble and granular starches.

Once nitrate consumption began, nitrate removal rates in the presence of granular starches were comparable for both low- and high-load beads (Table 1), and were similar to the rates observed for soluble starch in the low-load system (Table 1). After complete consumption of the initial nitrate dose by the low-load beads, reapplication of nitrate demonstrated that beads supplemented with granular starch maintained

their denitrification potential, whereas those provided with soluble starch lacked this additional activity. Interestingly, while the C:N ratio of the soluble starch incubations in the low-load system was approximately similar to that of the granular starches, soluble starch was only capable of supporting a third of the total nitrate reduction measured (by mass) for the granular starches. This difference might be explained by the contribution of non-denitrifying, starch-hydrolyzing *Bacillus* spp. (Morrison, Tal and Schreier, unpublished; Tal *et al*. 2003) present within the microbial consortia of the biofilter system. As TAC measurements were not able to detect any significant carbohydrate levels after the initial consumption of nitrate, it is conceivable that the ability to utilize subsequent additions of nitrate was due to either slow solubilization and/or hydrolysis of carbohydrates from granular starch particles trapped within beads, or immediate metabolism by the bacteria coating starch particles, thereby enhancing the efficiency of starch utilization for denitrification. The varying particle sizes for the three granular starches used in this study may influence their ability to be metabolized by heterotrophic bacteria; corn, rice, and wheat starches have diameters of 5-20 μm, 2-6 μm, and a mixture of 5-10 μm and 20-50 μm, respectively (International Starch Institute, Aarhus, Denmark). Whether starch utilization could take place in the absence of nitrate was not examined.

While the effect of soluble starch on nitrate removal was not examined using the high-load beads, we found that glucose stimulated nitrate removal activity at the same rate and at approximately the same time as acetate through the first and second doses of nitrate. However, in the presence of acetate, nitrate removal rates decreased 12.4-fold (Table 1) after the third application of nitrate, presumably due to rapid exhaustion of the three-carbon acetate in a manner that was physiologically different from the six-carbon glucose. Glucose, on the other hand, continued to stimulate nitrate removal after the second and third nitrate applications at rates similar to the initial loading of nitrate. Since glucose is a primary product of starch hydrolysis, it follows that nitrate removal rates for the granular starch incubations may be similar to those observed for the glucose-supplemented tubes, provided that the level of glucose released upon granular starch solubilization and hydrolysis was not limiting. Indeed, aside from the first nitrate application, removal rates for all three granular starches by the high-load beads were identical to those obtained for glucose (Table 1). For the initial nitrate application, the delay and slow

removal rates in the presence of the granular starches (1.8- to 2.8-fold lower compared to glucose) could be explained by low starch-degrading and solubilizing activities necessary for supplying an electron source to promote nitrate reduction; once the effective glucose concentration released by starch solubilization and hydrolysis increased, nitrate removal rates approached those obtained for glucose.

For both low- and high-load bead experiments, nitrite levels were found to gradually accumulate after nitrate addition, reaching their highest levels shortly after nitrate removal was detected; these levels began to decrease coincidental to, or shortly after, the corresponding drop in nitrate levels. Nitrite accumulation often occurs as a consequence of both incomplete reduction of nitrate to nitrogen gas and competition between various nitrate and nitrite reductases for common electron donors. The carbon source used for growth are likely occurring within the complex microbial communities associated with the biofilms in these bead systems (Blaszczyk 1993, Wilderer *et al*. 1987). Furthermore, accumulation of nitrite can also be attributed to localized variations in pH across the biofilm in individual beads or between sectors of beads (Almeida *et al*. 1995), or the presence of oxygen (Betlach and Tiedje 1981, van Rijn and Rivera 1990) that may have been introduced during sample handling. While our studies were not able to address the precise effects of nitrite and other denitrification intermediates (NO and N_2O) on nitrate reduction in detail, both nitrate and nitrite were effectively removed by the low- and high-load bead systems, with glucose and granular starches providing a continued source of reducing power. As these carbon sources were provided in excess (as evidenced by complete removal of nitrite) they were available for use by the bead microbial communities, suggesting that their ability to promote nitrate reduction proceeds towards the production of elemental nitrogen, i.e. via the denitrification route. Since ammonia accumulation was not measured, we are not able to rule out the possibility that dissimilatory nitrate reduction may be playing some role under these conditions (van Rijn *et al*. 2006).

In summary, our results indicated that granular starches were effective in fueling biological denitrification and appear to behave as "slow-release" carbon sources. The process of applying granular starch in an up-flow fixed-bed biofilter using polyethylene beads as a substrate for bacterial growth, and employing a retaining mechanism for the granular starch,

is simple and cost-effective. Many studies on wastewater treatment have focused on using mixed liquor or even introducing a granular polymer such as polyhydroxybutyrate, a constituent of sludge, as an external carbon source to stimulate denitrification (Anderson and Dawes 1990, Dionisi *et al.* 2004, Qin *et al.* 2005). As these alternatives are costly and require an additional dosing mechanism, we suggest that there is both an economical and practical advantage to using granular starch to stimulate denitrification. Furthermore, the characteristic low solubility of granular starch may contribute to a predictable and stable denitrifying environment and should prove beneficial in selecting the most appropriate form of starch for a RAS. We have found that supplementing a denitrification unit attached to a large-scale intensive closed marine RAS with granular starch was an effective approach for stimulating denitrification activity and maintaining minimal nitrate accumulation within the culture tank (Tal and Schreier, unpublished). Addition of granular starch to an up-flow fixed-bed biofilter in a ratio of 1:20 (starch to fish feed) every seven days successfully promoted nitrate reduction in filter water and overall low nitrate concentrations in the fish tanks with maximum fish densities of 50-60 kg/m^3 (Tal and Schreier, unpublished). The low cost, ease of usability, and non-toxic nature of granular starch, therefore, make it an ideal exogenous carbon source to stimulate nitrate waste removal in RAS denitrification bioreactors.

ACKNOWLEDGEMENTS

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