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Nitrite reduction by a mixed culture under conditions relevant to shortcut biological nitrogen removal

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Abstract

Dissimilative reduction of nitrite by nitrite-acclimated cells was investigated in a batch reactor under various environmental conditions that can be encountered in shortcut biological nitrogen removal (SBNR: ammonia to nitrite and nitrite to nitrogen gas). The maximum specific nitrite reduction rate was as much as 4.3 times faster than the rate of nitrate reduction when individually tested, but the reaction was inhibited in the presence of nitrate when the initial nitrate concentration was greater than approximately 25 mg-N/l or the initial NO_3^-–N/NO_2^-–N ratio was larger than 0.5. Nitrite reduction was also inhibited by nitrite itself when the concentration was higher than that to which the cells had been acclimated. Therefore, it was desirable to avoid excessively high nitrite and nitrate concentrations in a denitrification reactor. Nitrite reduction, however, was not affected by an alkaline pH (in the range of 7–9) or a high concentration of FA (in the range of 16–39 mg/l), which can be common in SBNR processes. The chemical oxygen demand (COD) requirement for nitrite reduction was approximately 22–38% lower than that for nitrate reduction, demonstrating that the SBNR process can be economical. The specific consumption, measured as the ratio of COD consumed to nitrogen removed, was affected by the availability of COD and the physiological state of the cells. The ratio increased when the cells grew rapidly and were storing carbon and electrons.

Introduction

A shortcut biological nitrogen removal (SBNR) process that oxidizes ammonia to nitrite and reduces nitrite to nitrogen gas (Chung 2002; Turk & Mavinic 1989a; Hellinga et al. 1998; Abeling & Seyfried 1992) can be an innovative technology because it theoretically saves approximately 25% of electron acceptor and 40% of electron donor (Turk & Mavinc 1987; Chen et al. 1991; Abeling & Seyfried 1992). In addition, Turk & Mavinic (1987) reported that nitrite reduction enhanced the denitrification rate by 63% with a lower biomass yield per unit amount of nitrogen removed.

The most critical condition that is needed for the success of the SBNR process is to suppress nitrite oxidation without excessively retarding the ammonia-oxidation rate. One powerful tool to achieve this condition is biochemical selection by inhibition of nitrite oxidation. Researchers found that certain concentrations of unionized free ammonia (FA) (Bae et al. 2002; Anthonisen et al. 1976; Turk & Mavinic 1987; Sauter & Alleman 1984; Abeling & Seyfried 1992) or free hydroxylamine (Yang & Alleman 1992) inhibited nitrite oxidation. A high concentration of FA (0.1–10 mg/l) inhibited the nitrite oxidation effectively in the beginning, resulting in an accumulation of nitrite. But the nitrite oxidizers acclimated to FA to a concentration as high as 22 mg NH_3—N/l with time (Turk & Mavinic 1989b; Villaverde et al. 2000). Thus, a gradual increase in FA concentration was needed. However, an excessively high FA level, for instance above 10–150 mg/l (Anthonisen et al. 1976), will inhibit ammonia oxidation also, which results in process failure.

One practical way to overcome this problem may be maintaining the nitrite concentration in the reactor as low as possible by reducing it quickly. Since nitrite oxidizers and nitrite reducers compete for nitrite, we can minimize nitrite oxidation by stimulating nitrite reduction.

Nitrite denitrification has been investigated by several group of researchers and shown to be effectively achieved with a pure culture (Almeida et al. 1995b; Kučera et al. 1986) or a mixed culture (Beccari et al. 1983; Akunna et al. 1993; Wilderer et al. 1987). The transport and reduction rate of nitrite was faster (Beccari et al. 1983; Akunna et al. 1993) or slower (Carlson & Ingraham 1983; Almeida et al. 1995b) than that of nitrate, depending on the experimental conditions. From a biochemical point of view, nitrite appears to be the preferred electron acceptor, since the nitrite reductase is located closer to the environment (periplasmic space) than the nitrate reductase (cytoplasmic side on the membrane) (Hochstein & Tomlinson 1988; Stouthamer, 1988; Brittain et al. 1992; Schulthess et al. 1995; Moreno-Vivian et al. 1999), which means that nitrite has less transport resistance. On the other hand, the nitrite reductase might be subject to change in environmental conditions. Indeed, the nitrite reduction responded more sensitively than nitrate reduction to pH changes; the nitrite-reduction rate fell sharply at a pH lower than that which the cells had been acclimated, while the nitrate reduction rate remained unchanged (Beccari et al. 1983).

So far, reduction of nitrite under conditions needed for shortcut biological nitrogen removal has not been studied sufficiently. Thus, the main purpose of this study is to investigate whether denitrification is achieved effectively under SBNR conditions by a mixed culture. We investigated how the rate of nitrite reduction depends on the concentration of nitrate, the pH, the concentration of FA, and different carbon sources.

Material and methods

Preparation of culture

A mixed culture of denitrifiers, whose inoculum was obtained from a SBNR reactor (Chung 2002), was grown and maintained in a denitrifying sequencing batch reactor (SBR). The SBR was fed with glucose as the sole electron-donor source and sodium nitrite as the sole electron-acceptor source. The composition of the medium was (in mg/l): $NaNO_2$ as N 100, glucose as chemical oxygen demand (COD) 400, K_2HPO_4 84, $NaHCO_3$ 100, $MgSO_4 \cdot 7H_2O$ 50, $FeSO_4 \cdot 7H_2O$ 2, $CaCl_2$ 4, $MnSO_4 \cdot H_2O$ 5, and KCl 7. The operation mode of the SBR was: fill-0.1 h, react-3.3 h, settle-0.3 h, and draw-0.3 h, making the overall cycle 4-hours long. In each cycle, 50% of the reactor medium was replaced with a fresh medium, giving an initial NO_2^-—N concentration of 50 mg/l. The reactor was operated at 25 °C with pH 8.0–8.7.

Batch experiments

The batch experiments for denitrification were performed with a 1-L Erlenmeyer flask that was tightly sealed and continuously purged with nitrogen gas to maintain anoxic conditions. Required amounts of substrates were added to the reactor that already contained 500 ml of cell suspension of approximately 4,803,800 mg/l as volatile suspended solids. The cells were taken from the SBR at the end of each 'react' period. The initial pH of the medium was adjusted to 7.5 unless mentioned otherwise. The batch reactor was agitated, keeping the temperature at 25 °C.

Sampling and analyses

A fraction of reaction medium (50-ml) was removed from the reactor using a sampling tube at appropriate intervals and immediately filtered through a 1-μm glass-fiber filter paper. The NO_2^-–N, NO_3^-–N, COD, and volatile suspended solids (VSS) were determined following the Standard Methods (APHA 1998).

Results and discussion

Maximum specific denitrification rates of nitrite and nitrate

A set of results of nitrite and nitrate reduction with the concomitant consumption of COD are shown in Figure 1. For the nitrite reduction experiment, the 40 mg/l of initial NO_2^-–N was completely consumed in 40 minutes (Figure 1a), while a similar concentration of NO_3^-–N was depleted in 120 minutes (Figure 1b). In both experiments, the carbon source was not the limiting source, because the remaining COD concentration was above 200 mg/l at the end of the experiments. The initial specific removal rate of nitrite and nitrate, apparently the maximum specific utilization rate, was estimated from four repeated runs and found in the range of 0.76 to 1.59 (1.22 ± 0.33) mgNO_2^-–N/mgVSS·d for nitrite reduction and 0.29 to 0.37 (0.33

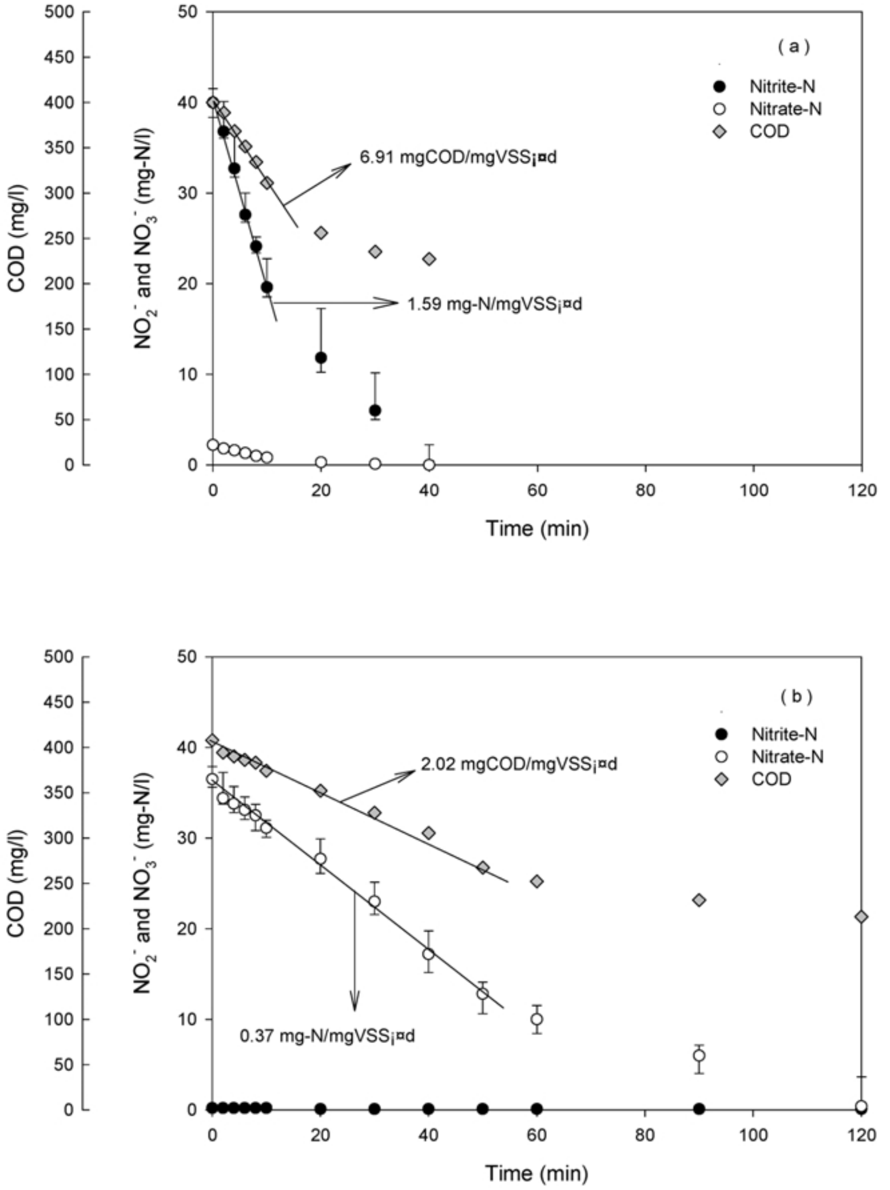

Figure 1. Denitrification of nitrite (a) and nitrate (b). The solid lines represent the initial reaction rates found through a linear regression of the data between time zero to 10-min in (a) and time zero to 50-min in (b). Error bars represent the range of one standard deviation of the NO_x^-–N concentration for each data point.

± 0.04) $mgNO_3^-$–N/mgVSS·d for nitrate reduction. The range of one standard deviation of the NO_x^-–N concentration for each data point is shown with an error bar in the figure. The maximum specific denitrification rate for nitrite was as much as 4.3 times greater than that for nitrate. These results can be compared with the results obtained in mixed culture experiments by Beccari et al. (1983), in which the maximum specific denitrification rate for nitrite and nitrate were 0.52 and 0.32 mg-N/mgVSS·d, respectively, and by Turk & Mavinic (1987), in which the nitrite reduction rate (1.17 $mgNO_2^-$–N/mgVSS·d) was 63% faster than nitrate reduction rate (0.73 $mgNO_3^-$–N/mgVSS·d). Both sets of published results showed that the rate of nitrite reduction was significantly faster, although their cells were adapted to nitrate. Our results show a much larger difference between the two reduction rates. The relatively large difference in our experiments may reflect a gradual adaptation of the cells to the nitrite-reducing environment, since the rate gradually increased with time (data not shown).

The results of our experiments, however, were different from the pure culture results obtained by Almeida et al. (1995b). They reported that the nitrite reduction rate was substantially slower than the nitrate reduction rate and, therefore, limited the overall denitrification reaction. Their cells were not specifically acclimated to nitrite in advance. The relatively poor ability for nitrite reduction seen by Almeida et al. (1995b) probably was caused by a lack of selection of microbial species well adapted to nitrite reduction.

In our nitrate denitrification experiment, little nitrite accumulated in the medium (Figure 1b), unlike the results obtained by Almeida et al. (1995a), who used a pure culture of *Pseudomonas fluorescens* (in that, almost a stoichiometric amount of nitrite was released into the medium, and then consumed after the nitrate was depleted). Probably the majority of species in our mixed culture had utilized nitrate (fed) and nitrite (produced) simultaneously, and the faster kinetics for nitrite reduction prevented it from accumulating.

Inhibition of nitrite removal in the presence of nitrate

When nitrite and nitrate were present in the reactor together, nitrite removal was inhibited, while the reduction of nitrate was unaffected. As shown in Figure 2, there was no removal, but rather a slight increase, of nitrite until X·t became approximately 30 mgVSS/l·d. The time delay varied from 90 min (Batch 1) to 20 min (in Batch 2) due to the difference in the cell concentration. Then, nitrite began to decrease rapidly after approximately half of the initial nitrate was consumed. The concentrations of nitrate at that point were 28.9 mg-N/l for Batch 1 and 17.5 mg-N/l for Batch 2. When the nitrate concentration was above these levels, the rate of nitrite removal was slightly less than the rate of nitrate reduction to nitrite. The rate of nitrate removal was little different from the rate observed in the single substrate experiment (Figure 1b), showing 0.35–0.37 mgNO_3^-–N/mgVSS·d as given with regression lines in Figure 2. The rate of nitrite reduction was slightly less than the nitrate reduction rate in the beginning and, therefore, substantially less than in Figure 1. The rate of nitrite removal recovered to the level in the single nitrite reduction when the inhibition was relieved. As shown in Figure 2, the maximum rate of nitrite removal consistently reached 0.61 mg-N/mgVSS·d. This gives an overall nitrite reduction rate (sum of the nitrite and nitrate removal rates) of approximately 1.0 mg-N/mgVSS·d, which falls in the range of the maximum specific nitrite-alone reduction rate of 0.76–1.59 mg-N/mgVSS·d.

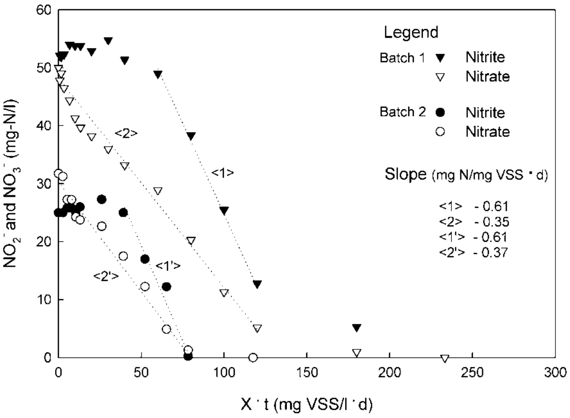

Figure 2. Simultaneous reduction of nitrite and nitrate by mixed culture adapted to 50 mg-N/l of nitrite. Each set of symbols (circle or triangle) represents a batch test.

To see the effect of the initial concentration of nitrate (or ratio of NO_3^-/NO_2^-) on nitrite removal, gradually decreasing initial concentrations (50 to 10 mg-N/l) of nitrate were tested at a fixed initial concentration of nitrite (50 mg-N/l). Figure 3 shows the results. Unlike the case when NO_3^- concentration was 50 mg-N/l (or, the NO_3^-–N/NO_2^-–N ratio was 1.0), there was little lag in nitrite removal when the NO_3^- concentration (or the ratio) was not greater than 25 mg-N/l (or 0.5) as shown in Figure 3a. The maximum specific removal rate of nitrite increased with the decrease of initial NO_3^- concentration. The overall nitrite reduction rate in the cell could be found by summing up the removal rates of nitrite and nitrate, as shown in Figure 3b. The slope of each data set in Figure 3b represents the overall nitrite reduction rate in that experiment. Compared to the nitrite removal rate when $NO_3^- = 0$ mg/l (which was transcribed from Figure 1a), the initial inhibition on nitrite reduction in the presence of nitrate was almost completely relieved when the initial NO_3^-–N/NO_2^-–N ratio was less than 0.5.

Inhibition by nitrite

Figure 4 shows the nitrite and nitrate reductions in the presence of a high concentration of nitrite. The initial nitrite concentration was varied from 50 mg/l to 300 mg/l, while the nitrate concentration was fixed at 50 mg/l. In all cases, nitrate began to decrease imme-

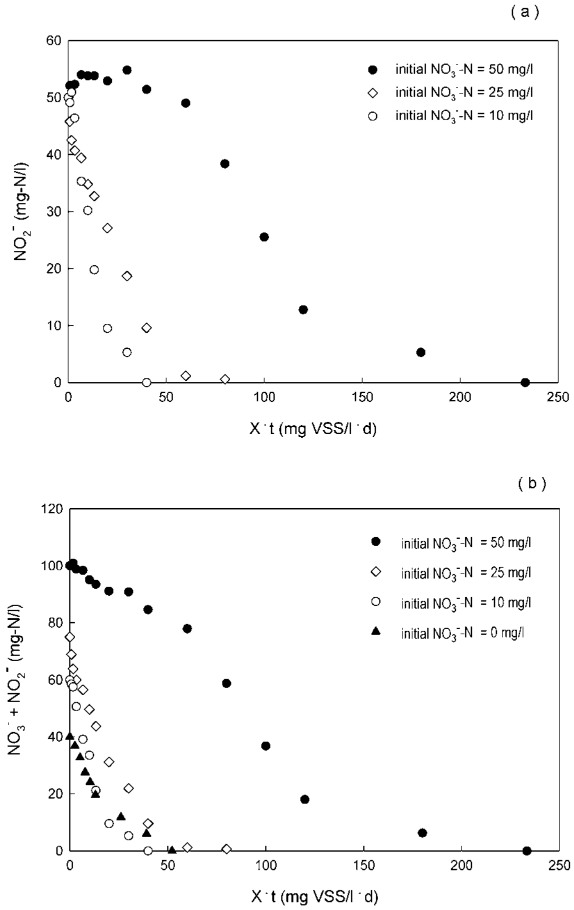

Figure 3. Simultaneous removal of nitrite and nitrate under various initial nitrate concentrations. (a) Nitrite profiles; (b) sums of reduction of nitrite and nitrate, which represent the overall nitrite reduction rates.

diately after the experiment started (Figure 4b). The initial removal rates were identical (around 0.35 mg-N/mgVSS·d), but nitrate reduction from 20 mg-N/l to zero was slowed when the initial NO_2^- concentration was higher. The nitrite concentration (Figure 4a) increased slightly in the beginning and began to decrease when nitrate reduction was approximately 50% of the initial concentration. The specific rate of removal after the initial lag was 0.61 mg NO_2^-–N/mgVSS·d when the initial NO_2^- concentration was 50 mg-N/l (slope <1> in Figure 2).

The maximum rate of nitrite reduction decreased with higher initial nitrite concentration, although complete removal occurred eventually. The smallest maximum specific nitrite removal rate was approximately 0.09 mg-N/mgVSS·d when the initial NO_2^- was 200 mg-N/l or higher, and the lag period to reach that

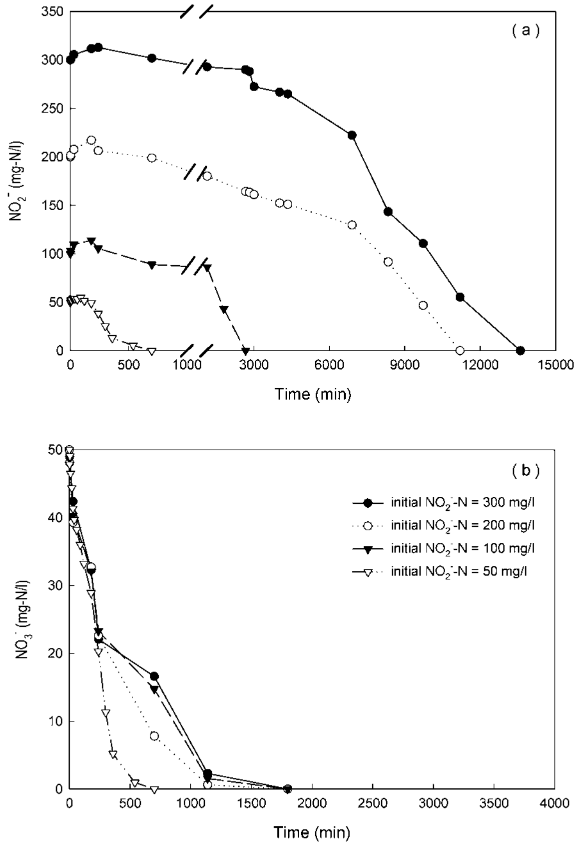

Figure 4. Simultaneous reduction of nitrite (a) and nitrate (b) under various, high nitrite concentrations. Each symbol represents a run.

maximum rate extended to about 7,000 min. Beccari et al. (1983) observed a similar nitrite self-inhibition at an initial concentration higher than that the cells had been acclimated. They speculated that the inhibition was probably caused by the presence of free nitric acid (FNA), because the nitrite reduction rate dropped sharply with a slight decrease of pH in their experiment.

Based on the observations in our experiment, the nitrite concentration in the reactor should remain below 50 mg-N/l or the level to which the cells were fully acclimated.

Effects of pH and free ammonia

Since nitrite accumulation by incomplete nitrification is achieved by FA at alkaline pH, a series of nitrite-reduction experiments were performed under various pH values and FA concentrations. When the initial pH was set at 7, 8, or 9, there was no observable difference in the nitrite reduction (data not shown). This observation was inconsistent with the results obtained by Almeida et al. (1995a) who observed that the reaction was significantly inhibited at pH 7.8. This difference probably was caused by the difference in cell selection or adaptation. The cells in our experiment were cultivated in a SBR reactor whose pH varied between 8.0–8.7, while the cells used by Almeida et al. (1995a) had been grown at pH 7.0. FA did not affect nitrite reduction either. The nitrite reduction rate was the same with 16–39 mgNH3/l or without any FA in the reactor (data not shown). Since the pH value or the FA concentration in SBNR reactions seldom exceeds 9 or 39 mg/l, respectively, our results suggest that the nitrite reduction will not be adversely affected by pH or FA in most SBNR processes.

Effects of electron-donor sources

Four different electron-donor sources – glucose, methanol, ethanol, and bacto-peptone – were tested for nitrite reduction. Figure 5 shows that glucose gave the fastest nitrite reduction, ethanol and bacto-peptone the next, and methanol the slowest. Glucose probably gave a relatively faster reaction rate, since the cells had been grown on glucose and thus were well adapted to it. The slow rate with methanol surely was caused by a lack of specialized one-carbon heterotrophs in our SBR biomass (Hallin et al. 1996; Timmermans & Van Haute 1983; Van Verseveld & Stouthamer 1978). Blaszczyk (1983) also reported that methanol was the least effective carbon source in nitrite denitrification among glucose, ethanol and methanol with his packed-bed reactor continuously supplied with two kinds of the substrate (nitrite or nitrate). The nitrite reduction rate in his reactor was (in mg-N/l·h) 43, 20 or 9 with a carbon source of glucose, ethanol or methanol, respectively. Blaszczyk (1983) also tested nitrate with methanol. Interestingly, the nitrate reduction rate was 507 mg-N/l·h, much faster than nitrite reduction rate, and the fastest among the carbon sources tested. In his experiment, a different source of electron donor or electron acceptor selected a different species, which appeared to be the main reason for the different reaction rates.

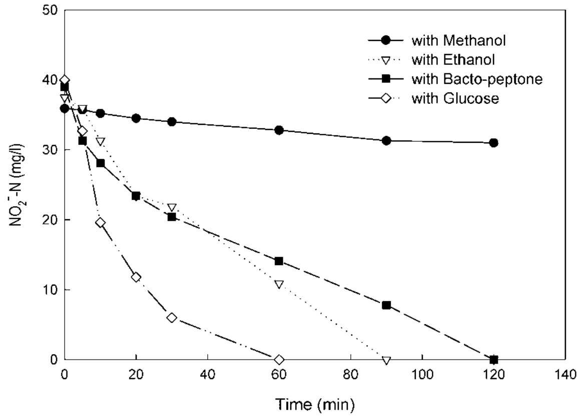

Figure 5. Nitrite reduction with different electron-donor sources by cells grown on glucose.

Carbon-source requirements for various electron acceptors and donors

The specific consumption of COD was compared between nitrite reduction and nitrate reduction. The COD consumption for nitrite reduction was 22.2–37.7% lower than that for nitrate reduction as shown in Table 1. In theory, the savings should be 40%, because nitrite is reduced by 3 electron equivalents per mol N, while nitrate is reduced by 5 electron equivalents per mole N. The ratio of $\Delta COD/\Delta NO_2^-$–N was in the range of 3.8–4.3 and that of $\Delta COD/\Delta NO_3^-$–N was in the range of 5.3–6.3. The theoretical ratio depends on the biomass growth rate and must be at least 3.4 g-COD/g-NO_2^-–N and 5.7 g-COD/g-NO_3^-–N (Rittmann & McCarty 2001), based on 50% of the donor electrons used for net biomass synthesis. Thus, our experimental results are reasonable. Akunna et al. (1993) observed a 13% savings in the carbon source with glucose and 46% savings with acetate. Our results, bolstered by others (Akunna et al. 1993), document that the carbon source indeed was saved with nitrite denitrification, although the COD savings was smaller than theoretically expected.

Figure 6 compares the ratios of the COD consumed to NO_2^-–N removed along the course of experiments for abundant COD (the data in Figure 1a), simultaneous depletion of nitrite and COD (for minimal COD), and starved-cell experiment with abundant COD. In the starved-cell experiment, the cells were rinsed with distilled water and, thus, deprived of an electron donor for 12 hours. The total consumption ratios differed by electron-donor availability. When a minimal amount of COD was fed (initial COD/NO_2^-–N = 3.0), the final ratio was only 2.8, while when an abundant amount of COD was fed (initial COD/NO_2^-–N = 10.0), the final ratio was 4.3. When starved cells were used (initial COD/ NO_2^-–N = 19.6), the $\Sigma\Delta COD/\Sigma\Delta N$ ratio became even higher, giving a final ratio of 9.2. These results show that cells in a rapid-growth mode take up and store more electron donor and carbon source per

Table 1. The respective ratio of COD consumption to nitrite or nitrate reduction through repeated tests

Experimental set	Reaction	Substrate	Concentration (mg/l)		ΔCOD/Δ NO_x^-–N
			Beginning	End	
# 1	Nitrite	COD	403.5	255.2	4.0
	reduction	Nitrite	39.5	2.4	
	Nitrate	COD	401.9	213.3	5.3
	reduction	Nitrate	37.2	1.5	
# 2	Nitrite	COD	391.3	234.4	4.3
	reduction	Nitrite	38.2	1.6	
	Nitrate	COD	385.8	175.8	6.3
	reduction	Nitrate	38	4.5	
# 3	Nitrite	COD	412.1	255.8	3.8
	reduction	Nitrite	42	1.1	
	Nitrate	COD	404.6	194.2	6.1
	reduction	Nitrate	35.3	0.7	
# 4	Nitrite	COD	400	235.2	4.2
	reduction	Nitrite	40	0.3	
	Nitrate	COD	408	213.2	5.4
	reduction	Nitrate	36.5	0.4	

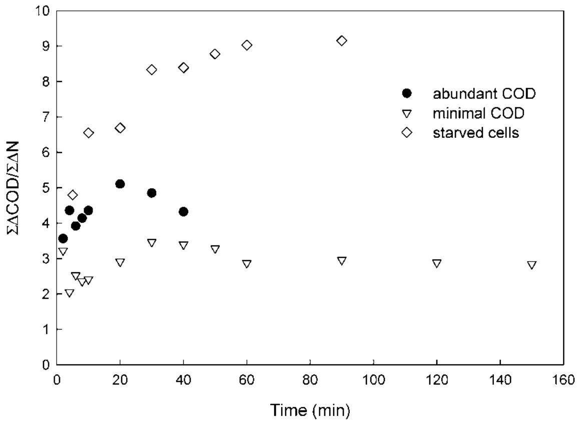

Figure 6. Ratios of COD consumed to NO_2^-–N removed. The COD and nitrogen data were from Figure 1a (abundant COD), another test for simultaneous depletion of nitrite and COD (minimal COD), and a third test performed with starved cells, providing sufficient amount of COD.

unit of N reduced. For a steady-state SBNR process, the biomass is likely to behave more like the minimal-COD experiment, with a ratio near 2.8 g-COD/g-NO_2^-–N. Because the stoicheometric requirement for electron-donor is likely to fluctuate in a continuous process, some excess COD may leave in the effluent. Therefore, it will be necessary to have post-treatment to remove the residual COD.

Conclusions

The maximum specific denitrification rate of nitrite was as much as 4.3 times faster than that of nitrate when nitrite-acclimated cells were used. However, the nitrite reduction rate was significantly retarded when the initial nitrate concentration in the batch reactor was greater than approximately 25 mg-N/l or the initial NO_3^--N/NO_2^-–N ratio was larger than 0.5. Also, when the nitrite was present above the concentration to which the cells had been acclimated, the nitrite denitrification was self-inhibited. Therefore, a shortcut biological nitrogen removal (SBNR) process should avoid excessive accumulation of nitrite and nitrate in the anoxic reactor. Nitrite reduction was not affected by alkaline pH in the range of 7–9 or by free ammonia in the range of 16–39 mg/l, the common operating conditions of a SBNR process. The COD requirement for nitrite reduction was approximately 22–38% lower than that for nitrate reduction, demonstrating that the SBNR process can be economical and the COD consumption was approximately equal to that expected from stoichiometry that includes biomass net synthesis. The ratio of ΔCOD/ΔNO_2^-–N increased when the cells were in rapid-growth, but the ratio for typical continuous-treatment conditions was around 2.8 g-COD/g-NO_2^-–N.

References

Abeling U & Seyfried CF (1992) Anaerobic-aerobic treatment of high strength ammonium wastewater nitrogen removal via nitrite. Water Sci. Tech. 26: 1007–1015

Akunna JC, Bizeau C & Moletta R (1993) Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol. Water Res. 27: 1303–1312

Almeida JS, Reis MAM & Carrondo MJT (1995a) Competition between nitrate and nitrite reduction in denitrification by *Pseudomonas fluorescens*. Biotechnol. Bioengng 46: 476–484

Almeida JS, Julio SM, Reis MAM & Carrondo MJT (1995b) Nitrite inhibition of denitrification by *Pseudomonas fluorescens*. Biotechnol. Bioengng 46: 194–201

Anthonisen AC, Loehr RC, Prakasam TBS & Srinath EG (1976) Inhibition of nitrification by ammonia and nitrous acid. J. Water Pollut. Control. Fed. 48: 835–852

APHA, AWWA & WEF (1998) Standard Methods for the Examination of Water and Wastewater. 19 edn. Washington, DC

Bae W, Baek SC, Chung JW & Lee YW (2002) Nitrite accumulation in batch reactor under various operational conditions. Biodegradation 12: 359–366

Beccari M, Passino R, Ramadori R & Tandoi V (1983) Kinetics of dissimilatory nitrate and nitrite reduction in suspended growth culture. J. Water Pollut. Control. Fed. 55: 58–64

Blaszczyk M (1983) Effect of various sources organic carbon and high nitrite and nitrate concentrations on the selection of denitrifying bacteria. II. Continuous cultures in packed bed reactors. Acta Microbiologica Polonica 32: 65–71

Brittain T, Blackmore R, Greenwood C & Thomson AJ (1992) Bacterial nitrite-reducing enzymes. J. Biochem. 209: 793–802

Carlson CA & Ingraham JL (1983) Comparison of denitrification by *Pseudomonas stutzeri, Pseudomonas aeruginosa*, and *Paracoccus denitrificans*. Appl. Environ. Microbiol. 45: 1247–1253

Chen SK, Juaw CK & Cheng SS (1991) Nitrification and denitrification of high-strength ammonium and nitrite wastewater with biofilm reactor. Water Sci. Tech. 23: 1417–1425

Chung JW (2002) Factors affecting nitrite accumulation and process optimization in a shortcut biological nitrogen removal hybrid reactor. Ph.D. thesis. Hanyang University

Carlson CA & Ingraham JCL (1983) Comparison of denitrification by *Pseudomonas stutzeri, Pseodomonas aeruginosa*, and *Paracoccus denitrificans*. Appl. Environ. Microbiol. 45: 1247–1253

Hallin S, Rothman M & Pell M (1996) Adaptation of denitrifying bacteria to acetate and methanol in activated sludge. Water Res. 30: 1445–1450

Hellinga C, Schellen AAJC, Mulder JW, Loosdrecht MCM & Heijnen JJ (1998) The SHARON process: an innovative method for nitrogen removal from ammonium-rich wastewater. Water Sci. Tech. 37: 135–142

Hochstein LI & Tomlinson GA (1988) The enzymes associated with denitrification. A. Rev. Microbiol. 42: 231–261

Kučera I, Matyášek R & Dadák V (1986) The influence of pH on kinetics of dissimilatory nitrite reduction in *Paracoccus denitrificans*. Biochimica et Biophysica Acta 848: 1–7

Moreno-Vivian C, Cabello P, Martinez-Luque M, Blasco R & Castillo F (1999) Prokarytic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. J. Bacteriol. 181: 6573–6584

Rittmann BE & McCarty PL (2001) Environmental Biotechnology: Principles and Applications. McGraw-Hill, New York

Sauter LJ & Alleman JE (1981) A streamlined approach to biological nitrogen removal. Proc. ASCE Specialty Cong. Environ. Engr. (pp 296–306). New York

Schulthess RV, Kühni M & Gujer W (1995) Release of nitric and nitrous oxides from denitrifying activated sludge. Water Res. 29: 215–226

Stouhamer AH (1988) Dissimilatory reduction of oxidized nitrogen compounds. In: Zehnder AJB (Ed) Biology of Anaerobic Microorganisms (pp 242–303). John Wiley, New York

Timmermans P & Van Haute A (1983) Denitrification with methanol. Fundamental study of the growth and denitrification capacity of *Hyphomicrobium* sp. Water Res. 17: 1249–1225

Turk O & Mavinic DS (1987) Selective inhibition: a novel concept for removing nitrogen from highly nitrogeneous wastes. Env. Technol. Lett. 8: 419–426

Turk O & Mavinic DS (1989a) Stability of nitrite build-up in an activated sludge system. J. Water Pollut. Control. Fed. 6: 1440–1448

Turk O & Mavinic DS (1989b) Maintaining nitrite build-up in a system acclimated to free ammonia. Water Res. 23: 1383–1388

Van Verseveld HW & Stouthamer AH (1978) Electron-transport chain and coupled oxidative phosphorylation in methanol-grown *Paracoccus denitrificans*. Arch. Microbiol. 118: 13–20

Villaverde S, Fdz-Polanco F & Garcia PA (2000) Nitrifying biofilm acclimation to free ammonia in submerged biofilters. Start-up influence. Water Res. 34: 602–610

Wilderer PA, Jones WL & Dau U (1987) Competition in denitrification systems affecting reduction rate and accumulation of nitrite. Water Res. 21: 239–245

Yang L & Alleman JE (1992) Investigation of batchwise nitrite build-up by enriched nitrification culture. Water Sci. Tech. 26: 997–1005