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Influence of Ammonium on the Performance of a Denitrifying Culture Under Heterotrophic Conditions

Francisco Cervantes,* Oscar Monroy, and Jorge Gómez

Department of Biotechnology,
Universidad Autónoma Metropolitana-Iztapalapa, AP 55-535,
09340 Iztapalapa, D.F., Mexico, E-mail: dani@xanum.uam.mx

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Abstract

The effect of ammonium on a denitrifying reactor of the upflow anaerobic sludge blanket type was studied. At a constant nitrate loading rate (2500 mg NO_3^--N/[L · d]), using acetate as organic electron donor and at a C/NO_3^--N ratio of 1.23, an increase in the N_2 production rate was observed when the ammonium loading rate was increased (25, 250, and 500 mg NH_4^+-N/[L · d]). Dissimilatory nitrate reduction to ammonium (DNRA) was not observed, and the N_2 production efficiency was increased from 84 to 100% or higher. Since NH_4^+ in the output was lower than in the input, it was suggested that it was used for nitrate reduction. At constant NH_4^+-N/NO_3^--N and C/NO_3^--N ratios of 0.2 and 1.63, respectively, the molecular nitrogen production rate was increased at 300 and 500 mg NH_4^+-N/(L · d), whereas at 200 mg NH_4^+-N/(L · d) DNRA took place probably owing to culture conditions of low reductive power. Molecular nitrogen production was not observed under autotrophic conditions, and the addition of acetate to the culture recovered its high nitrogen removal rate. Experimental results and balances indicated that the consumed ammonium was used as an additional reductive source.

Index Entries: Nitrogen removal; denitrification; ammonium oxidation; dissimilatory nitrate reduction.

Introduction

High concentrations of ammonium can be present in wastewaters from the fishery or hog-raising industries, causing severe environmental problems such as bad odors and fish mortality when these effluents are discharged into water bodies *(1,2)*. Ammonium has also been widely reported

*Author to whom all correspondence and reprint requests should be addressed.

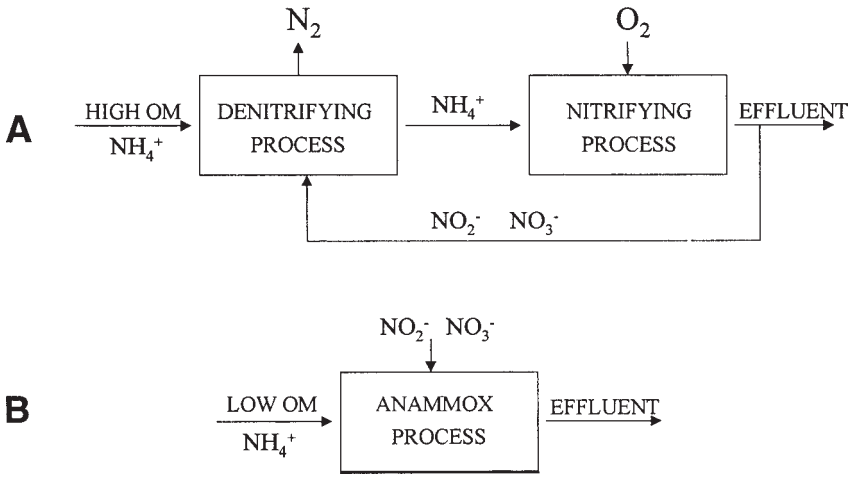

Fig. 1. Nitrogen removal processes. **(A)** Conventional nitrification-denitrification; **(B)** ANAMMOX. OM, organic matter.

as an inhibitor of anaerobic digestion of cattle and pig manure and of fish-processing effluents *(3–5)*. Hence, processes for the removal of nitrogen and organic matter must be applied in order to control pollution. Biological nitrification-denitrification processes are usually applied because they can be coupled with anaerobic and aerobic carbon removal processes. Nitrification is an aerobic, autotrophic process in which ammonium is oxidized to nitrite and nitrate *(6)*, which by denitrification are reduced to molecular nitrogen in the presence of an electron donor such as carbohydrates, volatile fatty acids, sulfur compounds, and hydrogen gas *(7)*. Ammonium can also be used as an electron donor to reduce nitrite to N_2 by the biologically mediated anaerobic ammonium oxidation (ANAMMOX) process *(8,9)*, for which the presence of nitrite and the absence of organic matter is essential *(10,11)*.

In wastewater treatment processes for nitrogen and carbon removal, a denitrifying followed by a nitrifying reactor with partial effluent recycling to the first reactor (Fig. 1A) is a normal practice *(12)*. More recently ANAMMOX has been employed to treat wastewaters with a high ammonium and limited organic matter content (Fig. 1B) such as sludge digester effluents *(13–15)*. Likewise, the coupling between denitrification and ANAMMOX (Fig. 2) for nitrogen and carbon removal could be an option for the treatment of wastewaters with a low organic carbon-to-nitrate ratio in which nitrite would be used simultaneously by denitrification and ANAMMOX depending on the availability of the electron donors (ammonium and organic matter).

The objective of the present work was to study the effect of different ammonium loading rates on the N_2 production rate, under heterotrophic and anoxic conditions, in a denitrifying upflow anaerobic sludge blanket (UASB) reactor.

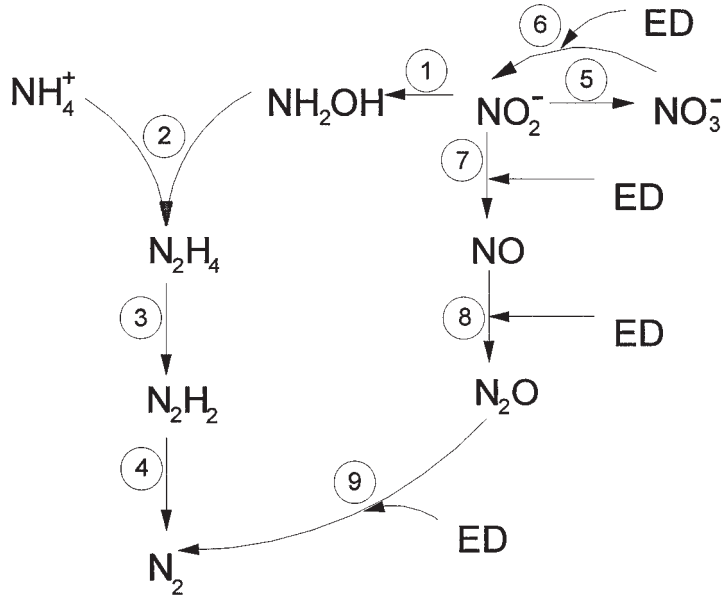

Fig. 2. Metabolic pathways for nitrate and ammonium removal. Steps 1–5 refer to ANAMMOX (according to van de Graf et al. *[11]*) and steps 6–9 to denitrification. ED, electron donors.

Materials and Methods

Analytical Procedures

Nitrite and nitrate were measured using a capillary electrophoresis ion analyzer (Millipore, Model 4000 [Bedford, MA]) as described by Gomez et al. *(16)*. Ammonium concentration was measured by an ammonia-specific electrode Phoenix® (Phoenix Electrode Co., Houston, TX). N_2, N_2O, and CO_2 were analyzed by gas chromatography (Gow-Mac, series 550 thermal conductivity detector [Bridgewater, NJ]) under the following conditions: column, detector, and injector temperatures were 140, 190, and 170°C, respectively; filament current was 120 mA, and sample volume was 100 µL. Chemical oxygen demand (COD), total suspended solids, volatile suspended solids (VSS), and fixed suspended solids were determined by standard methods *(17)*. Nitrogen content in VSS was determined in a CHNS/O analyzer (PE 2400 Series II, Perkin Elmer [Norwalk, CT]). In all the analytical methods, the coefficient of variation was <7%, as established by the regular introduction of standards.

The nitrogen assimilation rate (VSS-N_c) was calculated as follows:

$$\text{VSS-N}_c = \text{N}_{\text{vss}} \cdot \{[(\delta V_s \cdot X + V_{ss} \cdot X)/(T \cdot V)] + (Q \cdot X_{\text{out}}/V)\}$$

where N_{vss} = nitrogen content in VSS (g/g); δV_s = increase in sludge volume (L); X = VSS concentration in the sludge blanket (g/L); V_{ss} = sampled sludge volume for analysis (L); Q = flow (L/d); X_{out} = VSS concentration in the output (g/L); T = sampling period (d); and V = reactor volume (L).

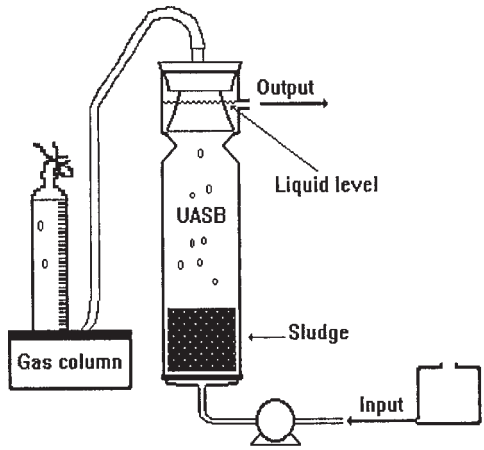

Fig. 3. Schematic diagram of the experimental denitrifying UASB reactor.

Culture Medium

The mineral medium contained 300 mg/L KH_2PO_4; 61.3 mg/L $MgSO_4 \cdot 7H_2O$; 17.2 mg/L $FeSO_4 \cdot 7H_2O$; 75 mg/L $CaCl_2 \cdot 2H_2O$; and 1 mL/L of a trace element solution containing 0.5 g/L $MnCl_2 \cdot 4H_2O$; 0.05 g/L H_3BO_3; 0.05 g/L $ZnCl_2$; 0.15 g/L $CuCl_2$; 0.01 g/L $Na_2MoO_4 \cdot 2H_2O$; 0.5 g/L $CoCl_2 \cdot 6H_2O$; 0.01 g/L $NiCl_2 \cdot 6H_2O$; and 0.01 g/L Na_2SeO_3. KNO_3 and $NaCH_3COO$ were used as nitrogen and carbon sources, respectively. When the ammonium effect was evaluated, NH_4NO_3 and KNO_3 were added to adjust the ammonium and nitrate concentrations. The pH of the culture medium was adjusted to 7.0.

Denitrifying UASB Reactor

A 1.5-L UASB glass reactor (Fig. 3) was operated at 35°C with a hydraulic residence time of 1 d throughout all the experimental work. The reactor was inoculated with 500 mL of denitrifying sludge (VSS = 10.6 $g/L_{Reactor}$) coming from a bench scale UASB denitrifying reactor. The produced gas was collected and measured by a liquid displacement device.

Results and Discussion

During an initial period of 18 d, the denitrifying UASB reactor was fed at 2500 mg NO_3^--N/(L · d), with a C/NO_3^--N ratio of 1.23 and reached a semi–steady state in which a molecular nitrogen production rate (γ_N) of 84 ± 5% of the input nitrate loading rate (NO_3^--N_{in}) and a COD removal efficiency of 91 ± 2% (Table 1) occurred. The ammonium formation, owing to dissimilatory nitrate reduction to ammonium (DNRA), was negligible. At this regime of high nitrate loading rate, there was a production of nitrous oxide (254 ± 62, N_2O-N mg/[L · d]). This result could be explained because it has been observed that the N_2O reduction to N_2 is the slowest step in the heterotrophic denitrifying process *(18,19)*.

Table 1
Denitrifying Reactor Performance
During Start-Up Period[a]

COD removal (%)	91 ± 2
$\gamma_N/NO_3^-N_{in}$ (%)	84 ± 5
$NO_3^-N_{in}$ (mg/[L · d])	2559 ± 131
$NO_3^-N_{out}$ (mg/[L · d])	ND[b]
NO_2^-N (mg/[L · d])	ND[b]
N_2O-N (mg/[L · d])	254 ± 62
γ_N (mg/[L · d])	2152 ± 24
pH_{out}	10 ± 0.1

[a]Without ammonium feed.
[b]ND, not detected.

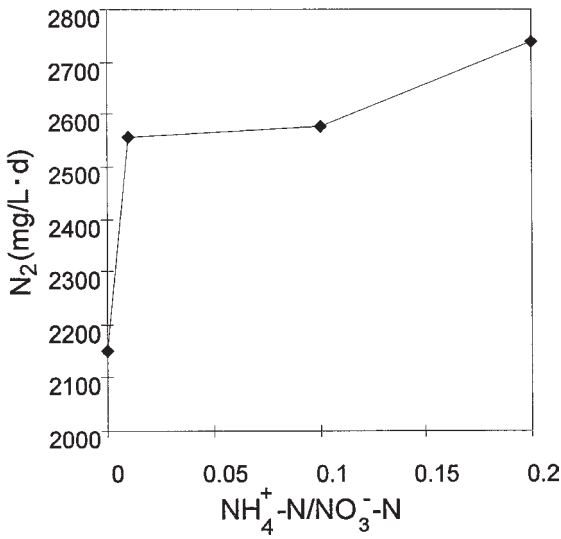

Fig. 4. N_2 production rate at different NH_4^+-N/NO_3^--N ratios with a constant C/NO_3^--N ratio of 1.23.

After the initial period, the C/NO_3^--N ratio was kept constant while ammonium feeding was increased from 25 to 500 mg of NH_4^+-N/(L · d) to establish the NH_4^+-N/NO_3^--N ratio at 0.01, 0.1, and 0.2. After feeding NH_4^+, N_2O was no longer detected, and, as a consequence, the γ_N increased, as shown in Fig. 4. However, the γ_N was always higher than the $NO_3^-N_{in}$ at each NH_4^+-N/NO_3^--N ratio. This indicated that a fraction of N_2 could have been formed from ammonium since it was consumed ($NH_4^+N_c$) at a significant rate, as can be seen in Table 2.

To eliminate the possibility that ammonium was stripped off the reactor owing to the high pH value (9.78 ± 0.12), a control was set up with a 500 mg NH_4^+-N/L solution that was kept for 3 d at the same pH, temperature, and gas flow rate (0.002 L/[L · min]) as in the experimental culture.

Table 2
Performance of the UASB Reactor at Each Ammonium/Nitrate Ratio[a]

	Ratio of NH_4^+-N/NO_3^--N		
	0.01	0.1	0.2
NO_3^--N_{in} (mg/[L · d])	2500	2500	2500
NH_4^+-N_{in} (mg/[L · d])	25	250	500
NH_4^+-N_{out} (mg/[L · d])	3 ± 0.76	155 ± 24	219 ± 9
NH_4^+-N_c (mg/[L · d])	23 ± 1	95 ± 22	282 ± 5
VSS-N_c (mg/[L · d])	9 ± 0.3	22 ± 1	56 ± 0.5
VSS-N_c/NH_4^+-N_c	0.4 ± 0.15	0.23 ± 0.13	0.2 ± 0.1
γ_N/NO_3^--N_{in} (%)	100	103	109

[a]With a C/NO_3^--N ratio of 1.23.

Table 3
Nitrogen Balance at Different Ammonium/Nitrate Ratios[a]

	Ratio of NH_4^+-N/NO_3^--N		
	0.01	0.1	0.2
NH_4^+-N_c (mg/[L · d])	22	94	280
VSS-N_c + (γ_N - NO_3^--N_{in}) (mg/[L · d])	21	98	296

[a]With a C/NO_3^--N ratio of 1.23.

No significant change was observed in the ammonium concentration. In addition, a nitrogen balance in the continuous culture corroborated that the increase in γ_N might be owing to the NH_4^+-N_c. Table 3 shows that the N_2 produced in excess (γ_N – NO_3^--N_{in}) plus the assimilated nitrogen (VSS-N_c) was very close to the ammonium consumption rate (NH_4^+-N_c). Furthermore, the balance did not indicate any important ammonium loss owing to stripping off the reactor. Thus, the balance agreed with the control, in which no loss of ammonium was observed. The absence of ammonium stripping could be explained as a function of the very low gas flow rate (0.002 L/[L · min]) through the UASB reactor.

To eliminate the possibility of an endogenous source of ammonium, its feeding was stopped for 5 d, and as a result, γ_N decreased to its initial value. When ammonium was fed again, there was an enhancement of γ_N, indicating that ammonium could have contributed to the N_2 formation.

Table 2 shows that the nitrogen assimilation rate (VSS-N_c) was lower than the ammonium consumption rate (NH_4^+-N_c), and that increasing NH_4^+-N/NO_3^--N ratios decreased nitrogen yields (VSS-N_c/NH_4^+-N_c). These data suggest that the ammonium consumption could be related to a different biological nitrate reductive process in which ammonium was used for N_2 production, such as the ANAMMOX process, and this might be related to the disappearance of N_2O observed after feeding ammonium. In fact, the

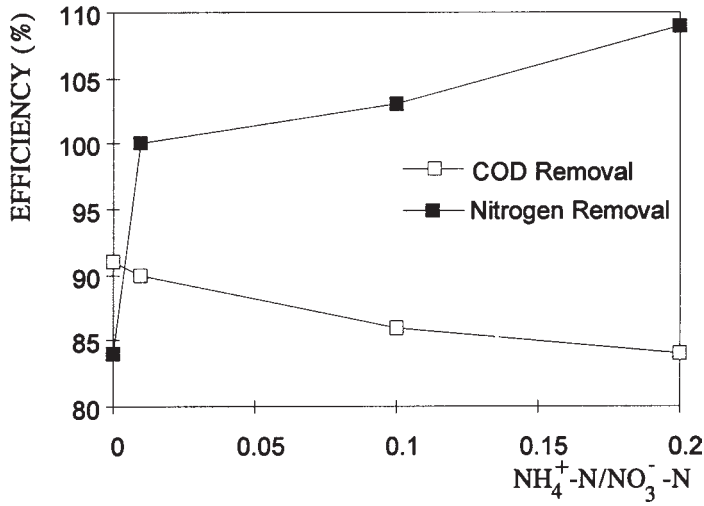

Fig. 5. Nitrogen (nitrate and ammonium in milligrams/liter) and COD removal efficiencies at each NH_4^+-N/NO_3^--N with a C/NO_3^--N ratio of 1.23. (—□—), COD removal; (—■—), nitrogen removal.

competition for nitrite by the two different electron donors (acetate and ammonium) could have decreased the inhibition of nitrate loading rate on nitrous oxide reduction *(19)*, thereby allowing denitrification without N_2O accumulation.

While increasing the nitrogen removal efficiency (γ_N/NO_3^--N_{in}), a concomitant decrease in the COD removal efficiency occurred (Fig. 5). This might be owing to the coupling between denitrification and a possible ANAMMOX (Fig. 2), in which nitrite is the electron acceptor in both processes *(7,8)*, causing a lower consumption of acetate and increasing the N_2 production. A fraction of acetate could have been replaced by ammonium as an electron donor, accounting for the increase in the output COD. Although nitrite reduction by ANAMMOX is carried out under lithoautotrophic conditions *(10,11)*, the residual acetate concentration (680, 756, 1058, and 1209 mg/L for each ammonium concentration tested) did not prevent the nitrogen removal process; thus, heterotrophic conditions did not hinder nitrate removal.

The C/NO_3^--N ratio was increased 32% (from 1.23 to 1.63) in order to test the respiratory behavior of the denitrifying sludge under increased heterotrophic conditions. To keep the NH_4^+-N/NO_3^--N ratio constant at 0.2, ammonium and nitrate loading rates were changed to 200, 300, and 500 mg of NH_4^+-N/(L · d) and 1000, 1500, and 2500 mg of NO_3^--N/(L · d), respectively. Denitrification intermediates such as nitrite and N_2O were not detected at any loading rate. However, at 200 mg of NH_4^+-N/(L · d), NH_4^+-N_{out} was higher than NH_4^+-N_{in}, indicating the presence of the DNRA, which led to a decrease in γ_N (Table 4). However, at higher ammonium loading rates (300 and 500 mg of NH_4^+-N/[L · d]), there was no DNRA but an impor-

Table 4
Performance of the UASB Reactor at Different Ammonium Loading Rates[a]

	NH_4^+-N_{in} (mg/[L · d])		
	200	300	500
NH_4^+-N_{out} (mg/[L · d])	348 ± 8	265 ± 8.4	264 ± 22
γ_N (mg/[L · d])	855 ± 38	1541 ± 26.5	2672 ± 104
VSS-N_c (mg/[L · d])	6.4 ± 0.5	12.5 ± 1.5	69 ± 1.6
γ_N/NO_3^--N_{in} (%)	87 ± 3.5	102 ± 0.55	107 ± 1

[a]C/NO_3^--N and NH_4^+-N/NO_3^--N ratios of 1.63 and 0.2, respectively.

tant NH_4^+-N_c was observed (Table 4). The increase of NH_4^+-N_{out} by the DNRA could be explained in terms of the low availability of the reductive power, and in this specific case (200 mg of NH_4^+-N/[L · d] and 1630 mg of C-acetate/[L · d]) the reductive power was lower. Because this result was contrary to that reported elsewhere *(20)*, it is necessary to do further work. Thus, different ammonium loading rates affected the respiration of the denitrifying sludge regulating the nitrate reduction pathway to denitrification, DNRA, and probably ANAMMOX. As a consequence, these respiratory changes caused different nitrogen removal efficiencies (Table 4). No significant difference was observed in the γ_N obtained with different C/NO_3^--N ratios (1.23 and 1.63) at the same ammonium and nitrate loading rates (500 mg of NH_4^+-N/[L · d] and 2500 mg of NO_3^--N/[L · d]) (*see* Tables 2 and 4). Hence, nitrogen removal was affected by the changes in ammonium loading rate rather than in the C/NO_3^--N ratio.

Bicarbonate was added for 4 d instead of the organic carbon source (acetate) in the medium at the same C/NO_3^--N ratio (1.63) in order to verify whether nitrogen removal was heterotrophic. During this period, neither ammonium nor nitrate consumption was observed, and the γ_N decreased up to a value close to zero. When bicarbonate was replaced by acetate, γ_N returned to its previous level. This result indicated that acetate was needed for consumption of nitrate and ammonium. In fact, the absence of organic carbon source did not allow nitrate reduction by denitrification, and, as a consequence, neither N_2 nor the denitrification intermediates such as nitrite and N_2O were obtained. In the same way, the absence of nitrite in the culture could have stopped the ammonium removal since nitrite is essential for ANAMMOX activity *(10)*.

Conclusion

The addition of ammonium to a denitrifying culture allowed an N_2 production higher than the nitrate addition alone. An important ammonium consumption was observed, indicating that this could have contributed to a higher N_2 formation. At the same time, an increase in the COD (as acetate) output was observed, suggesting that a fraction of acetate was

replaced by ammonium as electron donor for nitrate reduction. Namely, a different nitrate reductive pathway could have formed a fraction of N_2 from ammonium under heterotrophic conditions.

At a higher C/NO_3^--N ratio (1.63), it was observed that different ammonium loading rates caused changes in the nitrate reductive pathway to denitrification in which ammonium was employed as electron donor in the presence of organic matter (acetate). These changes had different effects on the nitrate and ammonium removal efficiencies.

The results of this work indicated that an organic carbon source (acetate) was needed in order to achieve simultaneous nitrate and ammonium removal although this was not possible under autotrophic conditions.

Acknowledgments

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