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Biomimetic and Microbial Reduction of Nitric Oxide

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ABSTRACT

The biomimetic reduction of nitric oxide (NO) to nitrous oxide (N_2O) by dithiothreitol in the presence of cyanocobalamin and cobalt-centered porphyrins has been investigated. Reactions were monitored directly using Fourier Transform Infrared (FTIR) Spectroscopy vapor-phase spectra. Reaction rates were twofold faster for the corrin than for the cobalt-centered porphyrins. The stoichiometry showed the loss of two molecules of NO per molecule of N_2O produced.

We have also demonstrated that the facultative anaerobe and chemoautotroph, *Thiobacillus denitrificans*, can be cultured anoxically in batch reactors using NO as a terminal electron acceptor with reduction to elemental nitrogen (N_2). We have proposed that the concentrated stream of NO_x, as obtained from certain regenerable processes for the gas desulfurization and NO_x removal, could be converted to N_2 for disposal by contact with a culture of *T. denitrificans*. Four heterotrophic bacteria have also been identified that may be grown in batch cultures with succinate, yeast extract, or heat and alkali pretreated sewage sludge as carbon and energy sources and NO as a terminal electron acceptor. These are *Paracoccus dentrificans, Pseudomonas denitrificans, Alcaligens denitrificans,* and *Thiophaera pantotropha.*

Index Entries: Nitric oxide; denitrifying bacteria; porphyrins; corrins; biomimetic.

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INTRODUCTION

Several promising technologies for flue gas desulfurization are under development that combine sulfur dioxide (SO_2) and NO_x removal. These include dry, regenerable scrubbing processes, like the NOXSO process (*1*). Dry, regenerable scrubbing processes offer considerable advantage over the use of throwaway adsorbents. Primary among these are reduced costs for chemical make-up and the simultaneous removal of SO_2 and NO_x. In the NOXSO process, the sorbent consists of sodium aluminate ($NaAlO_2$) on γ-alumina, which chemisorbs both SO_2 and NO_x. During regeneration, heating the sorbent produces a concentrated stream of NO_x (primarily NO). Subsequent treatment of the sorbent with a reducing gas produces a mixture of SO_2, hydrogen sulfide (H_2S), and elemental sulfur. We have previously proposed a microbial/chemical process for disposal of and/or byproduct recovery from the concentrated SO_2-containing gas stream produced during sorbent regeneration in the NOXSO and other dry, regenerable scrubbing processes (*2,3*). However, a need also exists for new technology for disposal of the concentrated NO_x streams obtained from certain of these processes, such as the NOXSO process, and the removal and disposal of NO_x from more dilute gas streams produced by nitric acid plants.

In this article, we address two mechanisms, one microbial and one biomimetic, by which nitric oxide can be chemically reduced to facilitate disposal. Five denitrifying bacteria (*Thiobacillus denitrificans, Paracoccus denitrificans, Pseudomonas denitrificans, Alcaligens dentrificans,* and *Thiophaera pantotropha*) have been cultured anoxically in batch reactors using NO as a terminal electron acceptor with reduction to elemental nitrogen. Carbon and energy sources consisted of $CO_2/S_2O_3^{-2}$ (*T. denitrificans*), succinate (*P. denitrificans*), yeast extract (*Ps. denitrificans, A. denitrificans,* and *T. pantotropha*) and heat- and alkali-pretreated sewage sludge (all heterotrophs).

The NO reduction chemistry in these organisms is not well characterized, but could well involve enzymes with porphyrin or porphyrin-like prosthetic groups. Porphyrins are planar, tetrapyrrole ring systems that are metal chelating. Porphyrins are often found in the active sites of enzymes involved in oxidation-reduction reactions. We have previously reported that cobalt-centered porphyrins will catalyze the reduction of nitroaromatics to corresponding amines (*4*). We present here the results of a preliminary investigation of the reduction of NO catalyzed by cobalt-centered porphyrins and cyanocobalamin (vitamin B12).

MATERIALS AND METHODS

Biomimetic Reduction of NO

Co^{3+}-centered hematoporphyrin, Co^{3+}-centered protoporphyrin IX, and cyanocobalamin were purchased from Porphyrin Products Inc. (Logan,

UT). Dithiothreitol (DTT) was obtained from Sigma Chemical Co. (St. Louis, MO). Aqueous solutions (0.5 mL) were prepared containing 1.8 μmol Co-porphyrin or cyanocobalamin and 265 μmol DTT in either Tris or phosphate buffer (50 m*M*, pH 8).

The 0.5-mL solution was placed directly in a 10-cm pathlength mini-gas cell containing water-insensitive CaF_2 windows (Willmad, NJ). The gas cell was sealed using white-rubber septa and evacuated for 5 min. The cell was then flushed with helium to atmospheric pressure.

Spectra were recorded using a Nicolet 510P FTIR spectrophotometer (32 scans, 1 cm^{-1} resolution). The background spectra consisted of the helium-flushed cell. At time zero, NO (10% in He, 4.08 m*M*, 45 cm^3 flush) was injected anoxically using a three-way valve, and the inlet and exit lines of the cell were sealed.

NO and N_2O concentration were determined from the integrated areas from 1880 to 1960 cm^{-1} and 2150 to 2280 cm^{-1} absorptions, respectively. These integration limits were chosen to avoid any contribution from water vapor bands. Both NO and N_2O spectra correlated precisely with reference spectra. No evidence of NO_2 was observed.

Microbial Reduction of NO

Thiobacillus denitrificans

T. denitrificans (ATCC 23642) was obtained from the American Type. Culture Collection (Rockville, MD). The organism was cultured anaerobically for stocks in an autotrophic medium with thiosulfate as the sole energy source, as described previously (*3*). This medium uses nitrate as the terminal electron acceptor, bicarbonate as a carbon source, and ammonium ion as a source of reduced nitrogen.

T. denitrificans was cultured anoxically in NO(g) as the terminal electron acceptor in a B. Braun Biostate M fermenter (culture volume of 1.44 L). In a typical batch experiment, *T. denitrificans* was grown in the thiosulfate medium described above at 30°C and pH 7.0 to an optical density at 460 nm of about 1.0, corresponding to approx 5×10^8 cells/mL (*5*). At this time, cells were harvested aseptically by centrifugation at 5000*g* for 10 min and resuspended in the same medium without nitrate. A gas feed of 0.48% (4800 ppm) NO, 5% CO_2, and the balance nitrogen was then initiated at 10.5–16.2 L/h, corresponding to a molar NO feed rate of 2.0–3.1 mmol/h. An agitation rate of 500–900 rpm was used. Cumulative gas flow was measured with a Precision Wet Test meter. The culture medium and outlet gas were sampled periodically as the culture was maintained on an NO feed for up to 7 d.

Paracoccus denitrificans

P. denitrificans (ATCC 13543) was obtained from the American Type Culture Collection (Rockville, MD). The organism was cultured aerobically

Table 1
Effect of Heat-Alkali Treatment
on Suspensions of Sewage Sludge[a]

	Before treatment	After treatment
MLSS (mg/L)	5800	4370
Soluble COD (mg/L)[b]	70	4400
Soluble protein (mg/L)[b]	24	550

[a]100 g/L wet-packed sludge suspended in mineral salts medium, pH adjusted to 12.0 with 10*N* NaOH, autoclaved at 120°C for 30 min then pH adjusted back to 7.0 with 6*M* H_3PO_4.
[b]Concentration in supernatant after centrifugation at 5000*g* and 4°C for 15 min.

for stocks at 30°C on nutrient agar slants. Stocks were stored at 4°C until used with transfers every 30 d.

P. denitrificans were cultured anoxically on NO(g) as the terminal electron acceptor, in either a succinate-supplemented mineral salts medium or the same mineral salts medium supplemented with heat- and alkali-pretreated municipal sewage sludge. The basic mineral salts medium used for growth of both *P. denitrificans* and *Ps. denitrificans* consisted of (in m*M* unless otherwise indicated): KH_2PO_4 (14.7); $NaHCO_3$ (11.9); NH_4Cl (9.3); $MgSO_4$ (6.7); and trace element solution (2.0 mL/L). The trace element solution has been described previously (*6*).

P. denitrificans was cultured anoxically on succinate as a carbon and energy source, and NO(g) as the terminal electron acceptor, in a Marubishi MD 300 fermenter (culture volume 2 L). In a typical batch experiment, *P.denitrificans* was grown in the mineral salts medium supplemented with 37 m*M* succinate and 50 m*M* KNO_3 at 30°C and pH 7.0 to an optical density at 520 nm of about 0.6. At this time, cells were harvested aseptically by centrifugation at 5000*g* and 25°C for 10 min and resuspended in the same medium without nitrate. A gas feed of 0.50% NO, 5% CO_2, and the balance nitrogen was then initiated at 30 mL/min. The agitation rate was 450 rpm. The culture medium and outlet gas were sampled periodically as the culture was maintained on an NO feed for up to 8 d.

Heat-alkali-pretreated sludge medium for *P. denitrificans* was prepared as follows: 100 g of wet-packed sewage sludge was suspended in 1 L of the mineral salts medium described above. The suspension was adjusted to pH 12 with 10*N* NaOH, and autoclaved at 121°C for 30 min. The cooled suspension was then adjusted to pH 7.0 with 6*N* H_3PO_4, and diluted to 1.5 L with additional mineral salts medium. Table 1 shows the mixed-liquid suspended solids (MLSS), soluble chemical oxygen demand (COD), and protein concentrations before and after treatment of sludge suspensions. Both heat and alkali treatments were required to produce significant solubilization of sewage solids.

When *P. denitrificans* was to be cultured on heat-alkali-pretreated sewage sludge as a carbon and energy source, and NO(g) as the terminal electron acceptor, the organism was first grown on succinate-nitrate as described above. Following harvesting by centrifugation at 5000*g* and 25°C, the cells were resuspended in 1.5 L of the sludge-mineral salts medium in a Marubishi MD 300 fermenter. A gas feed of 0.50% NO, 5% CO_2, and balance nitrogen was then initiated at 30 mL/min. All other operating conditions were identical to those described above for growth on succinate.

Pseudomonas denitrificans

P. denitrificans (ATCC 13867) was also obtained from the American Type Culture Collection (Rockville, MD). The organism was cultured aerobically for stocks at 30°C on nutrient agar slants. Stocks were stored at 4°C until used and transferred every 30 d.

Ps. denitrificans was cultured anoxically on NO(g) as a terminal electron acceptor in the mineral salts medium (without nitrate), supplemented with either 3 g/L yeast extract or heat-alkali-pretreated sewage sludge. When yeast extract served as the carbon and energy source, the organism was first grown in mineral salts-yeast extract medium supplemented with 50 m*M* KNO_3 (at pH 7.0 and 30°C) to an optical density (520 nm) of about 0.6. Cells were then harvested aseptically as described above and resuspended in the same medium without nitrate. The yeast extract used was shown to be nitrate-free as determined by the cadmium reduction method (*7*). A gas feed of 0.50% NO, 5% CO_2, balance N_2 was then initiated at 30 mL/min. All other operating conditions were identical to those described above for growth of *P. denitrificans* on succinate-NO.

When pretreated sewage sludge served as the carbon and energy source, *Ps. denitrificans* was initially grown on yeast extract-nitrate as described above. Following harvesting by centrifugation, the cells were resuspended in the sludge-mineral salts medium. A gas feed of 0.50% NO, 5% CO_2, balance N_2 was then initiated at 30 mL/min. All other operating conditions were identical to those described above.

Alcaligens denitrificans

A. denitrificans (ATCC 31040) was also obtained from the American Type Culture Collection. The organism was cultured for stocks at 30°C on nutrient agar slants. Stocks were stored at 4°C until used and transferred every 30 d.

A. denitrificans was cultured anoxically on NO(g) as a terminal electron acceptor in mineral salts medium (without nitrate) supplemented with either 3 g/L yeast extract or heat-alkali-pretreated sewage sludge. These cultures were developed and operated in a manner identical to that described above for similar cultures of *Ps. denitrificans*.

Thiopohaera pantotropha

T. pantotropha (ATCC 35512) was also obtained from the American Type Culture Collection. The organism was cultured for stocks at 30°C on nutrient agar slants. Stocks were stored at 4°C until used, and transferred every 30 d.

T. pantotropha was cultured anoxically on NO(g) as a terminal electron acceptor in mineral salts medium (without nitrate) supplemented with either 3 g/L yeast extract or heat-alkali-pretreated sewage sludge. These cultures were developed and operated in a manner identical to that described above for similar cultures of *Ps. denitrificans*, except that *T. pantotropha* cultures were maintained at pH 8.0–8.2.

Relative Rates of Microbial NO Reduction

The specific activities of *P. denitrificans*, *Ps. denitrificans*, *A. denitrificans*, and *T. denitrificans* for NO reduction were compared under defined reaction conditions. For each case, the organism was grown anoxically under optimum growth conditions in a yeast extract/mineral salts medium (*P. denitrificans*, *Ps. denitrificans*, and *A. denitrificans*) or thiosulfate mineral medium (*T. denitrificans*) to an optical density (460 nm) of 0.8. In each case, nitrate served as the terminal electron acceptor. Cells were then harvested by centrifugation at 5000*g* and 25°C, and resuspended in the same media without nitrate. Cell suspension (25.0 mL) were then transferred to 125-mL serum bottles and gassed with 0.50% NO, 5% CO_2, balance N_2. Bottles were shaken together in an environmental shaker at 30°C. The gas volume was sampled periodically and analyzed for NO by gas chromatography. Controls containing 25 mL of medium without nitrate or cells were treated and analyzed in an identical manner. At the end of the experiment, each suspension containing cells was analyzed for total biomass protein.

Analytical

NO in the reactor feed gas and reactor outlet gas was determined by gas chromatography using an HP 5840 gas chromatograph with a thermal conductivity detector. The column used was 76.2 × 0.3 cm id, stainless steel with 100/200 mesh HayeSep DB (Hayes Separation). The carrier gas was He at 30 mL/min. Operating temperature were column oven, 25°C; injection port, 25°C; and detector, 140°C. Pure NO served as a standard.

Biomass protein was determined by sonication of cell suspensions followed by analysis for total protein by either the micro-Folin method (*8,9*) or the Bradford method (*10*). Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was used as a standard.

MLSS were determined by filtering known volumes of culture medium samples through tared Whatman GF/C glass-fiber filters (*7*). COD was determined using Hach Chemical Co. (Loveland, CO) premeasured reagent vials. "Soluble" COD was obtained from the COD of the MLSS filtrate,

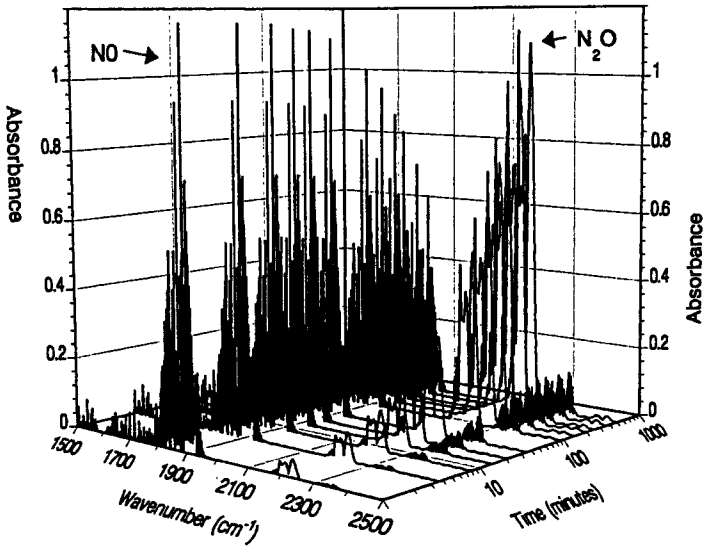

Fig. 1. Infrared absorption spectra for NO reduction. The reaction time series for NO reduction with N_2O production are shown over 478 min (log scale). Initial conditions: NO 102 μmol; DTT 265 μmol; cobalamin 1.8 μmol; 50 m*M* phosphate buffer, pH 8; fluid volume 0.5 mL; total volume 25 cm^3.

or supernatant, after centrifugation at 5000*g* and 4°C for 15 min. Ammonium ion was determined by the Nessler method (*7*).

Volatile fatty acids and succinate in culture medium samples were determined by gas chromatography using a Hewlett Packard 5840 gas chromatograph with a flame ionization detector. The column was a 2 m × 1.8 mm id (glass) packed with 80/120 Carbowax B-DA/4% Carbowax 20M (Supelco, Bellefonte, PA). The carrier gas was helium at 24 mL/min. The column oven, injection port, and detector temperatures were 185, 205, and 205°C, respectively.

RESULTS AND DISCUSSION

Biomimetic Reduction of NO

The infrared spectra showing the time-dependent decrease in the concentration of NO in the presence of DTT and cobalamin are presented in Fig. 1. The decrease in NO ($\upsilon_{N\text{-}O} \sim 1840\ cm^{-1}$) was matched by a concurrent increase in N_2O (prominent absorption bands near 2200 cm^{-1}). Other spectral features include minor absorption bands of N_2O above 2300 cm^{-1} and water vapor bands (between 1800 and 1500 cm^{-1}) corresponding to a slight mismatch between sample and background water vapor concentration.

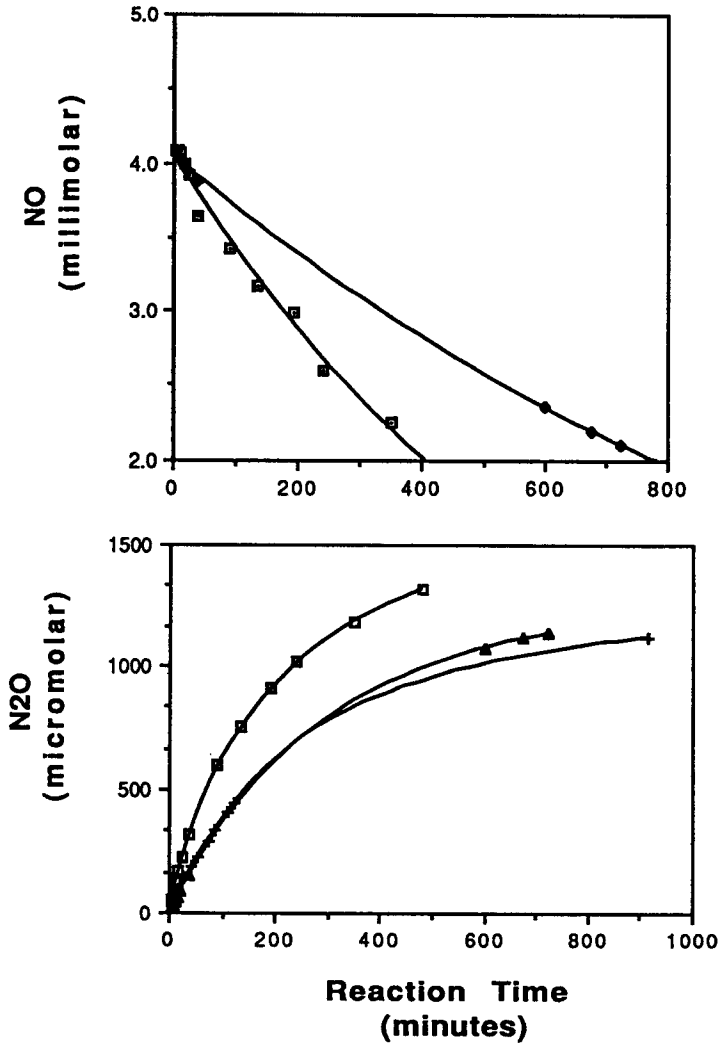

Fig. 2. NO reduction (top: □, cyanocobalamin; ◆Co protoporphyrin 1X) and N_2O production (bottom: □, cyanocobalamin; ▲, Co protoporphyrin 1X; +, Co hematoporphyrin). Results for Co-centered porphyrin and cobalamin are shown. Initial conditions: NO, 102 μmol; Co macrocycle, 1.8 μmol; DTT, 265 μmol; 50 m*M* phosphate, pH 8, 0.5 mL.

The reaction time-course was determined by integration of the prominent spectral regions for NO and N_2O for each of the cobalt-centered porphyrin or corrin used (Fig. 2). The reaction rates were distinctly nonlinear over longer reaction times; however, initial rates (for times <30 min) were relatively linear except for the cyanocobalamin derivative (Fig. 3). Absolute concentrations of NO and N_2O were determined from standard curves taken within the gas cell containing only water (0.5 mL) to correct for any differences in gas solubility.

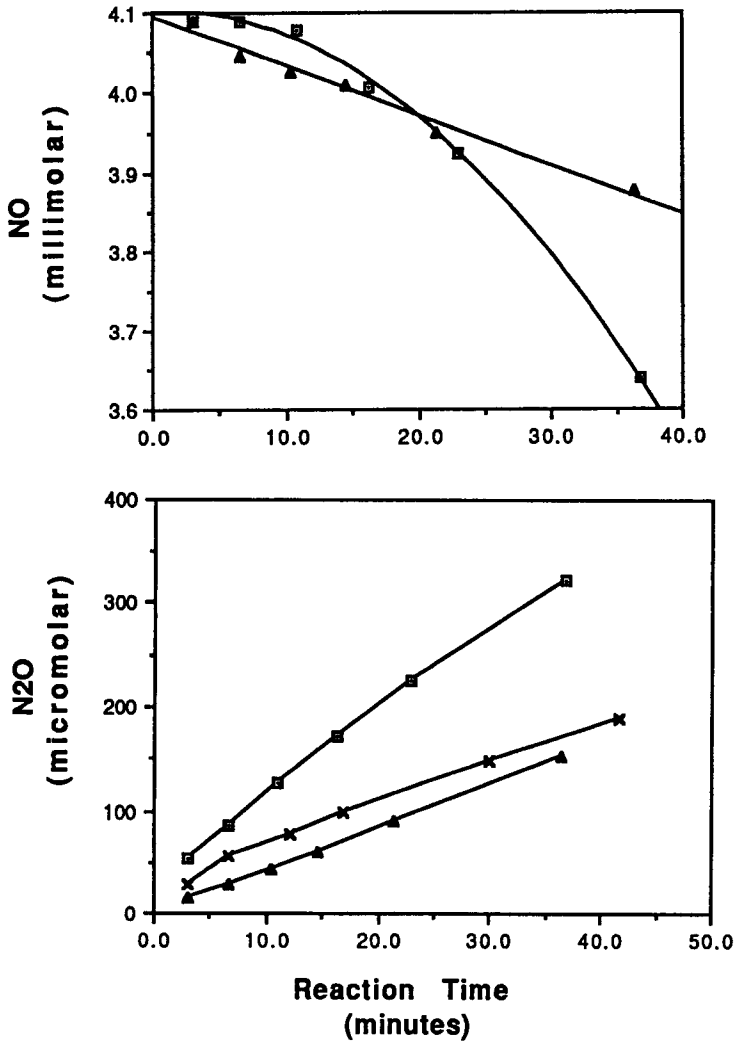

Fig. 3. Initial NO reduction (top: □, cyanocobalamin; ▲ Co protoporphyrin 1X) and N_2O production (bottom: □, cyanocobalamin; X, Co hematoporphyrin; ▲, Co protoporphyrin IX). Conditions are as described in Fig. 2.

The initial reaction rate for N_2O production and NO decrease was nearly twofold faster for cobalamin than either cobalt-centered hematoporphyrin or protoporphyrin IX (Fig. 3). The rate for NO disappearance was twice as fast as N_2O formation for either macrocyclic system.

The direct FTIR observations support the stoichiometry of 2 NO/N_2O produced. A reaction consistent with our observations is as follows:

$$2NO + 2RSH \text{-------} > N_2O + RSSR + H_2O \quad (1)$$

Microbial Reduction of NO

Thiobacillus dentrificans

When NO was introduced into *T. denitrificans* cultures previously grown on thiosulfate with nitrate as the terminal electron acceptor, the NO content of the feed gas was typically reduced to 100–200 ppm in the outlet gas, and remained at this level throughout the course of the experiment. In general, higher feed rates resulted in higher concentrations of NO in the outlet gas (>1000 ppm at 16 L/h, for example). As NO was removed from the feed gas, the concentrations of thiosulfate and ammonium were reduced in a culture medium, with a corresponding increase in optical density and biomass protein concentration. Growth of *T. denitrificans* on thiosulfate as an energy source, and NO as a terminal electron acceptor, was clearly indicated. In control experiments without biomass, NO broke through almost immediately at concentrations comparable to the feed gas, and no oxidation of thiosulfate was observed.

In a typical experiment, the oxidation of 45.8 mmol thiosulfate was accompanied by the reduction of 190.1 mmol NO, the utilization of 4.7 mmol of NH_4^+, and the production of 188 mg of biomass protein. The NO–$S_2O_3^{-2}$ ratio was determined in four duplicate experiments. The average ratio was 4.1. The purely chemical reduction of NO by $S_2O_3^{-2}$ would be given by:

$$H_2O + S_2O_3^{-2} + 4NO \text{ -------> } 2SO_4^{-2} + 2N_2 + 2H^+ \quad (2)$$

Therefore, the chemical reduction of NO by $S_2O_3^{-2}$ has a stoichiometry of 4 ($NO/S_2O_3^{-2}$). Given that NO supports the growth of *T. denitrificans* as a terminal electron acceptor, an $NO/S_2O_3^{-2}$ ratio of <4 would be expected, since some electrons derived from $S_2O_3^{-2}$ would be used as reducing equivalents to support biosynthesis (growth).

Paracoccus denitrificans

When NO was introduced into suspensions of *P. denitrificans* in mineral salts medium supplemented with succinate, complete removal of NO from the feed gas (30 mL/min of 0.50% NO) was observed. As NO was removed from the feed gas, there was a corresponding decrease in the succinate and ammonium ion concentrations, and an increase in the biomass protein concentration. Growth of *P. denitrificans* on succinate as a carbon and energy source and NO as a terminal electron acceptor was clearly indicated. In control experiments without biomass, NO broke through rapidly at concentrations comparable to the feed gas.

During the course of a typical experiment, the reduction of 32.3 mmol of NO gas was accompanied by the oxidation of 5.0 mmol of succinate, the utilization of 0.74 mmol of NH_4^+, and the production of 99.3 mg of biomass protein. The average NO–succinate stoichiometric ratio observed in these experiments was 6.0. The purely chemical oxidation of succinate (to CO_2) would require 7.0 mol of NO/mol of succinate. The difference

between the biochemical reaction and a purely chemical reaction can be attributed to diversion of reducing equivalents for biosynthesis in the microorganism.

In the specific experiment referenced above, the initial specific NO feed rate was 4.3 mmol NO/h/g of biomass protein. However, subsequent experiments showed that specific NO feed rates of up to 23.6 mmol NO/h/g biomass protein (140 mL/min of 0.50% NO at a biomass protein concentration of 70 mg/L) could be tolerated without breakthrough of NO.

A similar series of experiments was conducted to determine whether *P. denitrificans* could use heat-alkali-pretreated sewage sludge as a carbon and energy source, with NO as a terminal electron acceptor. When NO was introduced into a suspension of *P. denitrificans* in mineral salts medium, supplemented with heat-alkali-pretreated sewage sludge, complete removal of NO from the feed gas (30 mL/min of 0.50% NO) was observed. As NO was removed from the feed gas, there was a corresponding decrease in the concentration of soluble COD. These data indicate that *P. denitrificans* was utilizing biomolecules solubilized from the sewage sludge, as sources of carbon and energy, and NO as a terminal electron acceptor. The ammonium ion concentration was seen to increase as NO was removed from the feed gas. This has been attributed to liberation of NH_4^+ during metabolism of N-containing compounds from the sewage sludge. In control experiments with no biomass, NO broke through rapidly at concentrations comparable to the feed gas.

In order to demonstrate clearly that the utilization of soluble COD in these cultures was directly linked to the utilization of NO as a terminal electron acceptor, experiments were conducted in *P. denitrificans* cultures grown on a pretreated sewage sludge and an NO gas feed as described above. When utilization of soluble COD in the culture medium was clearly established, the NO gas feed was stopped. As seen in Fig. 4, when NO was no longer available as a terminal electron acceptor, the soluble COD concentration remained stable. When the NO feed was restarted about 72 h later, the soluble COD concentration again began to decline.

One additional observation is worthy to note. *P. denitrificans* cultures growing on pretreated sewage sludge were occasionally subject to NO breakthrough even when soluble COD was still available in the medium. However, addition of more pretreated sludge resulted once again in complete NO removal. These observations indicate that *P. denitrificans* could use only certain components of the soluble COD as carbon and energy sources, or that some other essential nutrient had become limiting.

Pseudomonas denitrificans

When *Ps. denitrificans* cells were suspended in mineral salts medium supplemented with yeast extract, the suspensions initially received a feed of elemental nitrogen only. The COD concentration, the optical density, and biomass protein concentration remained relatively constant during this time (40 h). However, when NO feed was initiated (30 mL/min of

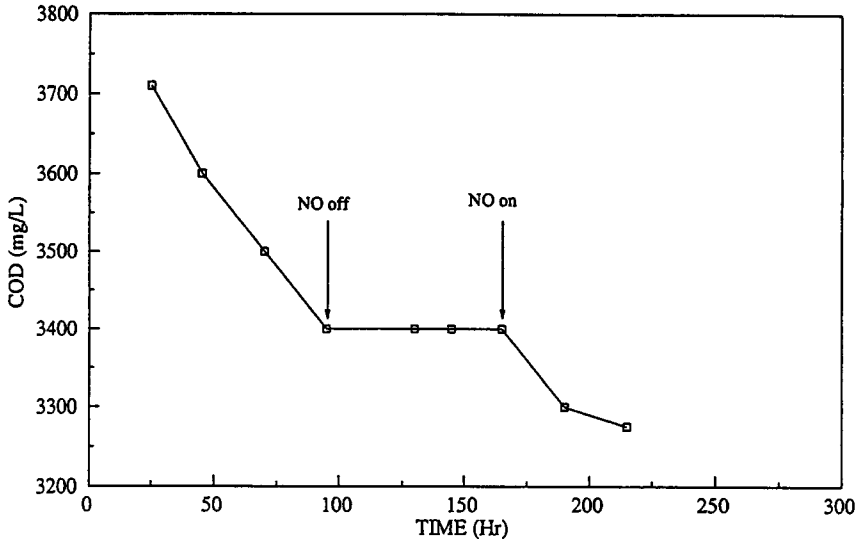

Fig. 4. Soluble COD concentration in a *P. denitrificans* culture with heat-alkali-pretreated sewage sludge as carbon and energy source receiving an intermittent NO feed.

0.50% NO), the COD concentration began to decline, and the optical density and biomass protein concentration began to increase, indicating growth of the organism on components of the yeast extract as carbon and energy sources and NO as a terminal electron acceptor. Complete removal of NO from the feed gas was observed. After about 100 h of operation with the NO feed, the NO feed gas was again replaced with N_2. The result was a cessation in growth and utilization of COD. When the NO feed was resumed after about 48 h on N_2, growth resumed with further utilization of COD and increase in optical density and biomass protein concentration. In control cultures in which NO was fed at the same rate to yeast extract-mineral salts medium without biomass, NO broke through rapidly at concentrations comparable to the feed gas.

When NO was introduced into suspensions of *Ps. denitrificans* in mineral salts medium supplemented with heat-alkali-pretreated sewage sludge, very similar results were obtained compared to *P. denitrificans* in the same medium. Again, complete removal of NO from the feed gas (30 mL/min of 0.50% NO) was observed. A corresponding decrease in soluble COD concentration indicates growth of *Ps. denitrificans* on biomolecules derived from the sewage sludge as carbon and energy sources and NO as terminal electron acceptor. When the NO feed was shut off, at about 120 h into the experiment, the soluble COD concentration leveled off, but resumed its decline when the NO feed was restarted. The ammonium ion concentration in *Ps. denitrificans* cultures also increased as NO was removed from the feed gas. However, the ammonium ion concentration leveled off, whereas the NO feed was replaced by pure N_2.

Table 2
Specific Activity of Denitrifying Bacteria for NO Reduction[a]

Organism	Total biomass protein, mg	NO depletion rate μmol/h	Specific activity, mmol NO/h/g protein
A. denitrificans	14.2	7.9	0.56
	14.1	9.8	0.70
P. denitrificans	13.0	15.2	1.17
	12.3	13.9	1.13
Ps. denitrificans	12.5	12.7	1.02
	12.5	11.3	0.90
T. denitrificans	10.6	8.8	0.83
	9.6	6.5	0.68

[a]25 mL of cell suspension in 125-mL serum bottle gassed with 0.50% NO, 5% CO_2, balance N_2.

Alcaligens denitrificans and Thiophaera pantotropha

A. denitrificans and *T. pantotropha* both produced results comparable to those observed with *Ps. denitrificans*. Complete removal of NO from the feed gas was observed with either yeast extract or heat-alkali-pretreated sewage sludge as carbon and energy sources. With both carbon sources, soluble COD in culture media decreased only when NO was available as a terminal electron acceptor. Both of these organisms are clearly capable of utilizing biomolecules solubilized from the sewage sludge as sources of carbon and energy and NO as a terminal electron acceptor.

Relative Rates of Microbial NO Reduction

NO disappeared from the gas space of each septum bottle containing a denitrifying bacterium (100% removal in < 200 min). There was no significant decrease in the NO concentration in control bottles (10–15% in 300 min). Initial rates of NO depletion were determined relative to the controls. From these depletion rates and the biomass protein concentrations, the specific activity of each organism for NO reduction under these conditions was calculated (Table 2). *P. denitrificans* and *Ps. denitrificans* exhibited the highest specific rates of NO reduction under these test conditions.

CONCLUSION

It has been demonstrated that NO will support the growth of *T. denitrificans* as the terminal electron acceptor with thiosulfate as the energy source (electron donor). It has also been shown that NO will support the growth of four heterotrophic denitrifying bacteria, *P. denitrificans, P. denitrificans, A. denitrificans,* and *T. pantotropha,* as a terminal electron acceptor

with succinate, yeast extract, and heat-alkali-pretreated sewage sludge as carbon and energy sources. These results suggest that growth on NO as a terminal electron acceptor may be a common property of denitrifying bacteria. The use of a potentially inexpensive carbon and energy source, pretreated sewage sludge, to support NO reduction indicates that a microbial process to dispose of NO_x may be economically viable.

It has also been demonstrated that NO can be reduced to N_2O in the presence of DTT with catalytic amounts of cyanocobalamin or Co-centered porphyrin. The corrin ring appears to enhance the reductive potential of the system. The reaction rates observed for this gas cell system may be subject to limitations in gas/liquid partitioning. Further solution-phase experiments to address this problem and to quantitate both the redox dependence for DTT and the Co-macrocycles are in progress. If N_2 can be obtained as a product of biomimetic reduction of NO, then the chemical process can offer distinct advantages over the microbial progress, since viable microbial cultures will not need to be maintained.

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