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Reduction of Nitric Oxide to Elemental Nitrogen by *Thiobacillus Denitrificans*

Scientific Note

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ABSTRACT

A need exists for new technology for the disposal of concentrated NO_x streams obtained from certain regenerable, dry scrubbing processes, such as the NOXSO process, and the removal and disposal of NO_x from more dilute gas streams produced by nitric acid plants. It has been demonstrated that the facultative anaerobe and autotroph, *Thiobacillus denitrificans,* may be cultured anaerobically in batch reactors using NO (g) as a terminal electron acceptor. Thiosulfate served as an energy source, CO_2 (g) as a carbon source, and ammonium ion as a source of reduced nitrogen. The growth of *T. denitrificans* was indicated by depletion of thiosulfate and ammonium ion and the accumulation of biomass. The feed gas consisted of 5000 ppmv NO, 5%, CO_2, and balance nitrogen. The NO concentration in the outlet gas was typically 200 ppmv.

Index Entries: NO_x; CO_2; thiosulfate; *T. denitrificans.*

INTRODUCTION

Thiobacillus denitrificans is a strict autotroph and facultative anaerobe first described in detail by Baalsrud and Baalsrud (*1*). Under anaerobic conditions, nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen. Thiosulfate, elemental sulfur, and sulfide

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may be used as energy sources with oxidation to sulfate. Nitric oxide has been shown to be an intermediate in the reduction of nitrate to elemental nitrogen in *T. denitrificans* (2–4). Ishaque and Aleem (3) and Baldensperger and Garcia (4) have demonstrated that whole cells of *T. denitrificans* will catalyze the reduction of nitric oxide to elemental nitrogen, with a concomitant oxidation of thiosulfate (electron donor). However, these experiments utilized "resting cells"; that is, the cells were not actively growing and reproducing.

The purpose of this study was to determine whether nitric oxide would support the anaerobic growth of *T. denitrificans* as a terminal electron acceptor. Thiosulfate was used as an energy source.

MATERIALS AND METHODS

Organism and Culture

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 (5). This medium uses nitrate as a terminal electron acceptor, bicarbonate as a carbon source, and ammonium ion as a source of reduced nitrogen.

T. denitrificans was cultured anaerobically on NO (g) as a terminal electron acceptor in a B. Braun Biostat 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 approximately 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 balanced 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 (Sargent-Welch). The culture medium and outlet gas were sampled periodically as the culture was maintained on a NO feed for up to 7 d.

Analytical

Analytical methods for quantitating thiosulfate, biomass protein, ammonium ion, and nitrite have been described previously (5). Nitric oxide in the outlet gas was determined by Gastec Analyzer tubes (Gastec Corp., Yokohama, Japan). These tubes had a range of 0–200 ppm NO and an accuracy, as given by the manufacturer, of ±25%. This was considered acceptable for a preliminary study while gas chromatography methods are being developed.

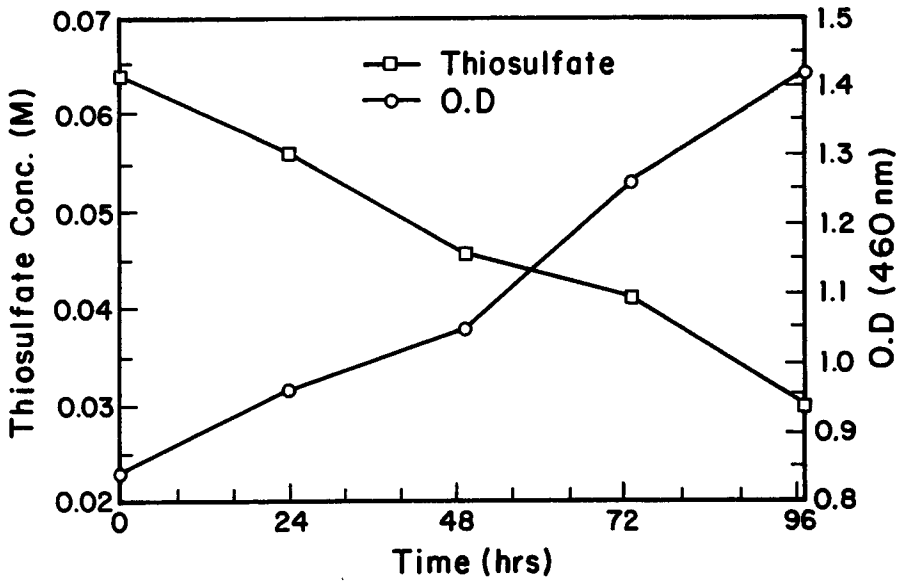

Fig. 1. Optical density and thiosulfate ($S_2O_3^{-2}$) concentration in a *T. denitrificans* reactor receiving a nitric oxide (NO) feed.

RESULTS AND DISCUSSION

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 ion were reduced in a culture medium, with a corresponding increase in optical density and biomass protein (Figs. 1 and 2). Growth of *T. denitrificans* on thiosulfate as an energy source and NO as a terminal electron acceptor is 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. Nitrite accumulated in the absence of biomass; however, little or no nitrite was detected in the culture medium in the presence of *T. denitrificans*.

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 $NO/S_2O_3^{-2}$ ratios for four duplicate experiments are given in Table 1. The average ratio is 4:1. The purely chemical reduction of NO by $S_2O_3^{-2}$ would be given by

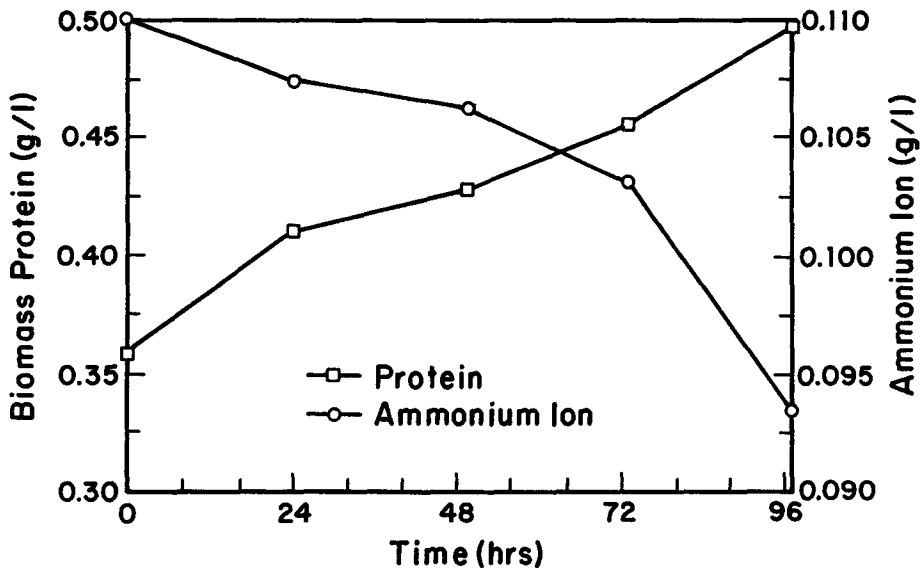

Fig. 2. Biomass protein concentration and ammonium ion (NH_4^+) concentration in a *T. denitrificans* reactor receiving a nitric oxide (NO) feed.

$$S_2O_3^{-2} + 4NO \rightarrow 2\,SO_4^{-2} + 2\,N_2$$

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, a $NO/S_2O_3^{-2}$ ratio of less than 4 would be expected since some electrons derived from $S_2O_3^{-2}$ would be used as reducing equivalents to support biosynthesis (growth). The discrepancy between this analysis and the data presented in Table 1 is likely owing to errors in gas analysis for NO.

CONCLUSION

It has been demonstrated that nitric oxide will support the growth of *T. denitrificans* as a terminal electron acceptor, with thiosulfate as the energy source (electron donor). The low solubility of NO in water resulted in incomplete removal of NO from the feed gas in one contacting stage. However, up to 96% removal of NO was observed. Microbial reduction of NO (and NO_2) merits further study as a potential means of disposal of oxides of nitrogen (NO_x).

ACKNOWLEDGMENT

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Table 1
Stoichiometry of NO Reduction by *T. denitrificans* with Thiosulfate as Electron Donor

Exp. no.	$NO/S_2O_3^{-2}$
5NA	3.6
6NA	4.2
7A	4.4
9A	4.2
	4.1 average

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