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Competitive Growth of *Nitrosomonas* and *Nitrobacter* in Inorganic Sulfur Containing Sandy Soils

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ABSTRACT

Growth of *Nitrosomonas* was competitively inhibited by zymogenously growing *T. thioparus* and other sulfur bacteria in soils containing 640 ppm thiosulfate-S. *Nitrosomonas* was, therefore, recovered in numbers as low as 100 cells/g soil within 16 days of incubation. Growth of *Nitrobacter*, however, was significantly enhanced by this treatment, and counts of about 9.1×10^5 cells/g were thus obtained within 8 days of incubation.

Incorporation of an equivalent amount of sulfur in the form of either elemental sulfur or diluted sulfuric acid, though did not show any effect on the activity of *Nitrosomonas*, both of these treatments had apparently stimulated the growth of *Nitrobacter* in soil.

INTRODUCTION

Naturally occurring inorganic forms of sulfur must be either oxidized to sulfate or reduced to sulfide before they are available for biosynthetic reactions (12). They are oxidized in soil in a fashion analogous to the nitrification of ammonium and nitrite, and the conditions necessary for the reduction of sulfate are essentially identical to those for nitrate (1). The bacteria utilizing inorganic sulfur compounds for energy are physiologically and morphologically diverse. The primary physiological groups of aerobic autotrophic bacteria that specifically utilize sulfides, thiosulfate or elemental sulfur as electron donors in their respiratory metabolism appear to be restricted to three species of the genus *Thiobacillus* (8). The chemoautotrophic sulfur oxidation process was found to be catalyzed by a glutathione-dependent, nonheme iron-containing enzyme synthesized by *T. thioparus* and probably other thiobacilli (16). The mechanism of this oxidation process still remains obscure due to the great reactivity and chemical instability of many of the proposed intermediates (13).

Nitrification which involves reduction of oxygen and fixation of carbon dioxide by the chemoautotrophic *Nitrosomonas* and *Nitrobacter* (17) has been shown to be inhibited by various volatile sulfure metabolites produced in soil (4,5,7,14). Little is known about the direct effect of thiobacilli, whose activities are greatly enhanced by inorganic sulfur treatments (3), on the performance of autotrophic nitrifying bacteria in soil. The

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equations (11) describining competition between species for the same nutrient and space seem to provide only a limited application under conditions likely to be encountered in soil treated with reduced inorganic nitrogen and sulfur compounds simultaneously.

This paper evaluates the effect of elemental sulfur, sodium thiosulfate, or diluted sulfuric acid treatments at the rate of 640 ppm sulfur and the subsequent proliferation of sulfur bacteria on the survival and competitive growth of *Nitrosomonas* and *Nitrobacter* in sandy soils treated with 100 ppm ammonium sulfate-N;

MATERIALS AND METHODS

A representative 2 to 20 cm deep soil sample was collected from a recently reclaimed uncultivated strip at the experimental farm of the Faculty of Agriculture. This sample was air-dried sieved through 2 mm mesh and its water holding capacity was determined. Replicated 100.0 g soil portions were placed in 250 ml capacity cotton-plugged Erlenmeyer flasks. An aqueous solution of ammonium sulfate was pippetted on the surface of each soil portion to yield 100 ppm nitrogen. Similarly, 50 ppm triple superphosphate-P and 50 ppm potassium sulfate-K were added. Three other groups of soil portions were evenly mixed with 640 ppm S as powdered elemental sulfur, sodium thiosulfate, or diluted sulfuric acid prior to the forementioned treatments. All soil portions were then brought to 60% water holding capacity with sterile distilled water and incubated at 28°C and about 80% relative humidity for the required periods of time. Loss of moisture through evaporation was corrected with sterile distilled water periodically.

Numbers of *Nitrosomonas*, *Nitrobacter* and heterotrophic soil bacteria were estimated as described previously (15).

Enumerations of *Thiobacillus thiopprus* and total sulfur bacteria capable of growth on Ibid's thiosulfate agar described by Allen (2) were carried out using the plate-dilution frequency technique (9). The agar used in this medium was pretreated by refluxing with 1N sulfuric acid in 25 × 80 mm Whatman cellulose extraction thimbles for 6 hours or until brownish discoloration was evident. The agar pretreatment provided an additional distinctive property for the colonies of the sulfur bacteria. Visible clear zones around the colonies were developed as the medium-thiosulfate was oxidized to sulfuric acid. The final medium pH was adjusted to 7.2.

On incubation at 26°C for 10 days, all colonies which appeared as small (1 to 2 mm in diameter), circular, whitish yellow which turned pink to brown on aging were considered *T. thioparus* (6), unless otherwise proven differently.

To differentiate between *T. thioparus* and *T. neopolitanus*, suspected isolates were tested according to Hutchinson (10). These tests included thiocyanate oxidation, anaerobic growth, and tolerance to 6% thiosulfate, 4% phosphate, 5% sodium chloride, 1% sodium glutamate, and 100 ppm phenol.

All other colonies developed on Ibid's thiosulfate agar which precipitated sulfur and including *T. thioparus* were referred to as total sulfur bacteria.

RESULTS AND DISCUSSION

Growth of *Nitrosomonas* in soils treated with sulfur, thiosulfate, or sulfuric acid

Neither elemental sulfur nor sulfuric acid had significantly affected the growth of *Nitrosomonas*. After 16 days of incubation, counts of 7.1×10^4 and 2.4×10^4 cells/g were obtained for sulfur- and sulfuric acid-treated soils respectively (Fig. 1). A steady state of growth was generally accomplished in both treatments after 32 days of incubation.

Thiosulfate incorporation inhibited the growth of *Nitrosomonas* almost instantaneously. Counts of 170 cells/g were obtained after 4 days of incubation (Fig. 1). Although a partial recovery was observed after 8 days, the numbers dropped to 100 cells/g or lower thereafter.

T. thioparus, however, responded quite rapidly to thiosulfate treatment. Counts of 8.1×10^5 cells/g were recorded after 8 days of incubation (Fig. 2). Within the same period of incubation time, the growth of total sulfur bacteria was generally activated by this treatment to attain 2.7×10^6 cells/g (Fig. 3).

In view of these results and the knowledge available about the nutritional requirements of both *Nitrosomonas* and thiobacilli (1,12), it is concluded that *T. thioparus* had competitively inhibited the growth of *Nitrosomonas* in thiosulfate-treated soils. The limiting factor in this case was the ammonium ion, which represented the sole source of energy for the biosynthetic activities of *Nitrosomonas*.

The results shown in Table 1 suggest no detrimental effect of associated heterotrophic bacteria on *Nitrosomonas*. A significant increase in numbers of these heterotrophs was noted in the thiosulfate-treated soils only. The four-fold increase in their

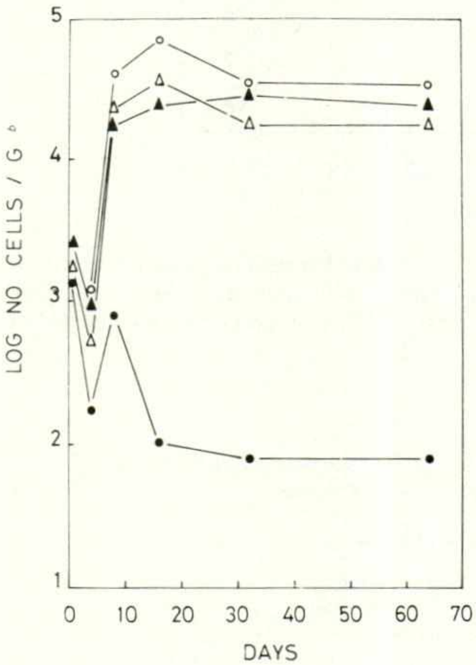

Fig. 1. Growth of *Nitrosomonas* spp. in incubated soils treated with ammonium sulfate alone (△), or in combination with sulfur (○), sodium thiosulfate (●) or diluted sulfuric acid (▲). (b) 95% confidence interval was D/3.30 to 3.30D where D is the number of bacteria.

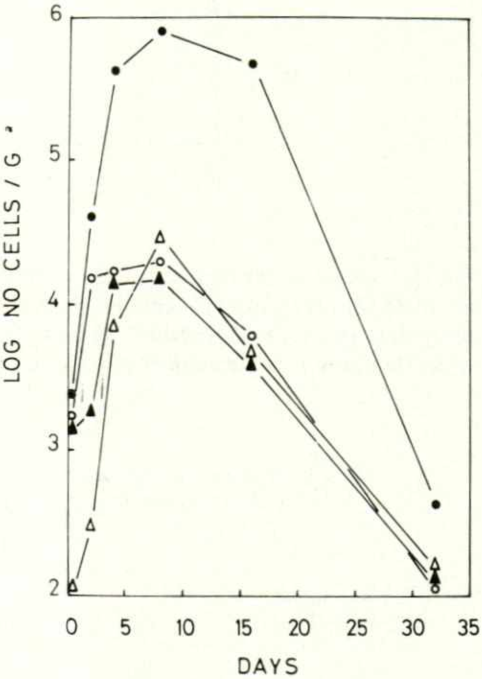

Fig. 2. Effect of incubation on the numbers of *T. thioparus* in soils containing ammonium sulfate alone (△) or with sulfur (○), sodium thiosulfate (●) or diluted sulfuric acid (▲) treatments. (a) 95% confidence interval was D/2.47 to 2.47D where D is the number of bacteria.

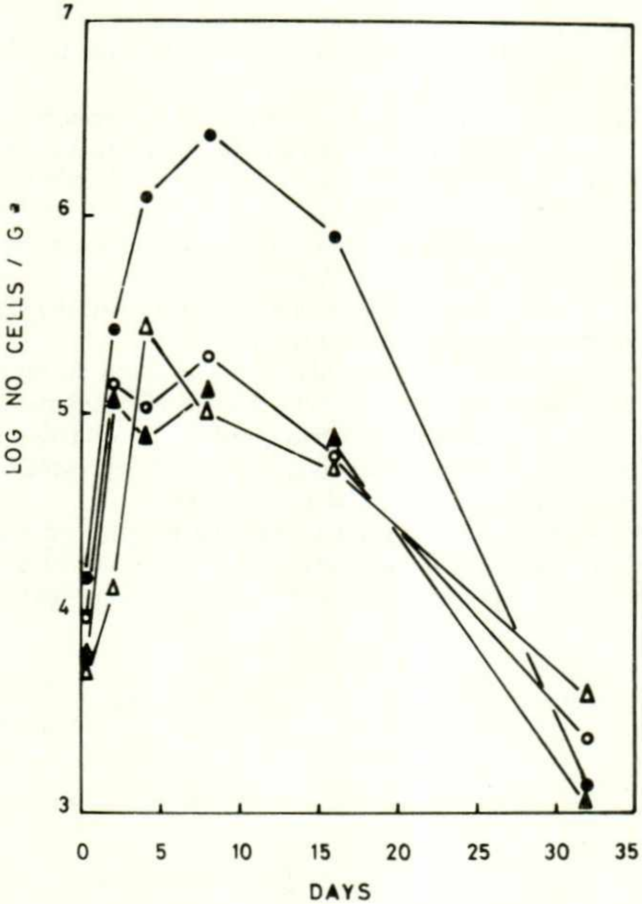

Fig. 3. Effect of incubation on the numbers of soil sulfur bacteria, capable of growth on modified Ibid agar, in soils containing ammonium sulfate alone (△) or with sulfur (○), sodium thiosulfate (●) or diluted sulfuric acid (▲) treatments. (a) 95% confidence interval was D/2.47 to 2.47D where D is the number of bacteria.

Table 1 Numbers of heterotrophic soil bacteria in incubated nonamended and sulfur-, sodium thiosulfate-, or sulfuric acid-treated soils.

Incubation time, days	Bacteria, thousands/g[a]			
	Control	Sulfur	Sodium thiosulfate	Sulfuric acid
¼	590	581	581	1730
2	306	1020	695	1370
4	780	796	1330	1020
8	581	581	2280	780
16	422	428	780	245
32	306	336	590	428
64	228	422	428	180

[a]95% confidence interval was D/2.47 to 2.47 D where D is the number of bacteria.

Table 2 Effect of incubation on the pH of soils amended with ammonium sulfate alone or in combination with sulfur, sodium thiosulfate, or diluted sulfuric acid.

Incubation time, days	Control	Sulfur	Sodium thiosulfate	Sulfuric acid
¼	8.20	8.10	8.15	7.56
2	8.00	8.10	8.12	7.75
4	7.60	7.95	8.09	7.70
8	7.90	7.85	7.95	7.58
16	7.75	7.60	7.65	7.85
32	7.35	7.00	6.90	7.25
64	7.25	6.55	6.45	7.15

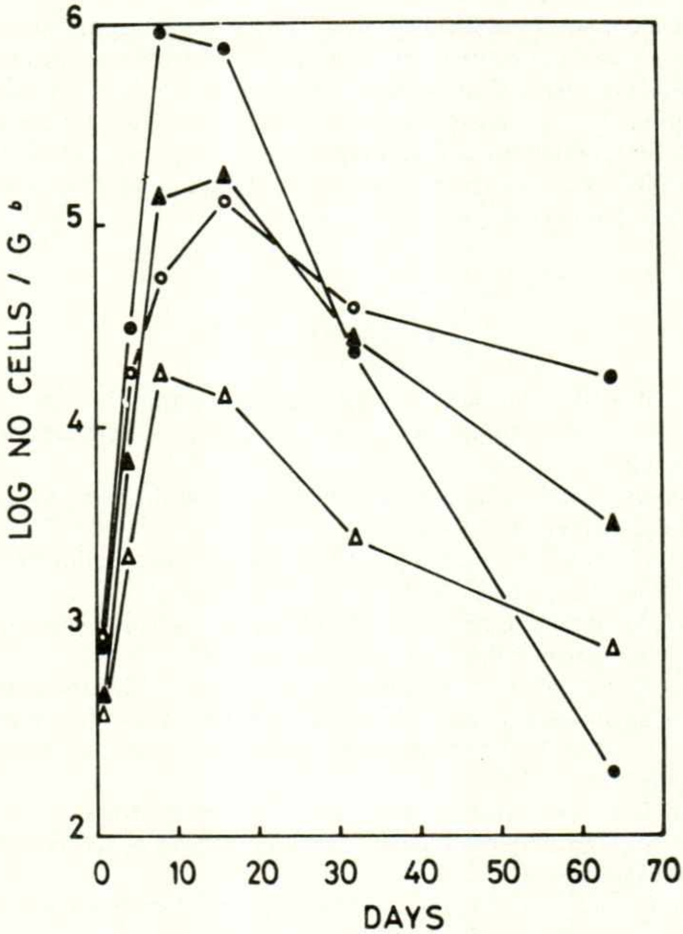

Fig. 4. Growth of *Nitrobacter* spp. in incubated soils treated with ammonium sulfate alone (△), or in combination with sulfur (○), sodium thiosulfate (●), or diluted sulfuric acid (▲). (b) 95% confidence interval was D/3.30 to 3.30D where D is the number of bacteria.

numbers, though had no apparent effect on *T. thioparus*, it probably had contributed to growth retardation of *Nitrosomonas*.

The gradual decline in soil pH (Table 2) caused by substrate oxidation had evidently no significant effect on *Nitrosomonas* growth. Whence, the pH values recorded at 8 days of incubation were practically the same for all treatments.

Growth response of *Nitrobacter* to sulfur, thiosulfate, or diluted sulfuric acid treatment

Unlike *Nitrosomonas*, growth of *Nitrobacter* was significantly promoted by all treatments during the initial 8 days of incubation. The rate of growth varied, however, with the treatments. While a maximum growth of 9.1×10^5 cells/g was noted for the thiosulfate treatment, sulfuric acid and elemental sulfur resulted in 1.4×10^5 and 5.8×10^4 *Nitrobacter* cells/g respectively (Fig. 4). All counts were shown to decrease rapidly after 16 days of incubation. The rate of decline in thiosulfate-treated soils, however, was so fast that almost 200 cells/g were detected after 64 days of incubation.

The sudden increase in numbers of *Nitrobacter* particularly in thiosulfate-treated soils indicated a better competitive ability of this bacterium for nitrite anions. Growth of thiobacilli was obviously enhanced by the added ammonium and the nitrate produced through oxidation. The proliferation of *Nitrobacter* and the concomitant nitrite oxidation was, therefore, enhanced due to endproduct removal by thiobacilli.

A commensalism, thus, had been accomplished between *Nitrobacter* and associated thiobacilli during that period.

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التنافس فى نمو النيتروسوموناس والنيتروباكتر فى الترب الرملية المحتوية على الكبــريت

صالح محـسن صالح

المستخلـــــص

تاثر نمو النيتروسوموناس فى الترب المحتوية على ٦٤٠ جزء بالمليون كبريـــت بشكل ثايو سلفات الصوديوم وذلك لنشاط بكتــرية الكبريـــت وخاصة ثايوباسلس ثايوبارس ٠ وعليه فقد انخفض عدد النيتروسوموناس الى حوالى ١٠٠ خليــة / جرام بعد ١٦ يوم من معاملة لتربة ٠ وعلى العكس من ذلك فقد ارتفع عدد النيتروباكتر امى حوالى ٩١٠ر٠٠٠ خلية / جرام خلال ٨ أيام فقط ٠

ولم تؤثر الاشكال الاخرى من الكبريت كحامـض الكبريتيـــك المخفف او زهر الكبريت على نشاط النيتروسوموناس بينما لوحظت استجابة نســبية للنيتروباكتر لهذه المعاملات ٠