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Growth of *Nitrosomonas* and *Nitrobacter* in Sandy Soils Amended with Organic Constituents.

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ABSTRACT

Growth of *Nitrosomonas* in incubated soil samples amended with 170 ppm ammonium sulfate-N had apparently not been affected by dextrose incorporation. When an equivalent amount of nitrogen was applied as urea instead of ammonium sulfate, a tenfold suppression in growth was observed. A drastic effect for casein treatment was noted in both cases. Hence, counts slightly above 200 cells per gram soil were obtained after 2 days of incubation. Casein incorporation had apparently activated proteolytic groups of microorganisms whose synergistic effects were most pronounced by the alkaline soil condition imposed by substrate decomposition.

Although *Nitrobacter* growth in ammonium sulfate-treated soils was not affected by dextrose incorporation, a significant growth appreciation was observed in urea-treated soils. Thus, counts of more than 1×10^5 were obtained within 14 days of incubation. The activity of urea hydrolyzing bacteria whose counts exceeded 1×10^6 cells per gram soil in 4 days had apparently neutralized the synergistic effects of other groups of heterotrophic bacteria. Therefore, the partial inhibition of *Nitrobacter* growth in casein-treated soils is likely to have occurred because of the sensitivity of this bacterium to the unfavorable soil pH prevailing after 2 days of incubation.

INTRODUCTION

In most well-aerated soils, nitrate is the principal form of available nitrogen, and plants adapted to such soils grow well with nitrate as the sole source of nitrogen. Many such plants can also utilize ammonium, but suffer impairments when only ammonium ion furnish nitrogen (10). Some plants can use urea as the sole source of nitrogen (21), but several problems including damage to seedlings and young plants has been encountered with urea application due to rapid hydrolysis of urea in soils (23).

The chemoautrotrophs *Nitrosomonas* and *Nitrobacter* are responsible for the bulk of the nitrification in agricultural soils. The rate constants for oxidation of ammonium and nitrite are proportional to the numbers of corresponding *Nitrosomonas* and *Nitrobacter* species (5). Under field conditions, these constants were estimated as 2.5×10^3 ppm per hour cm^3 per *Nitrosomonas cell and* 0.6×10^3 ppm per cm^3 per *Nitrobacter* cell (6).

Nitrifying bacteria, however, are known to respond rapidly to various accelerating deleterous environmental conditions. Both *Nitrosomonas* and *Nitrobacter* are strict

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aerobes and get inhibited at an oxygen level of 0.5% in the gaseous phase (15). Apparently, they function well only in soils with pH's nearly neutral or slightly alkaline (4,11,17,24). If the soil is distinctly alkaline, *Nitrosomonas* activity outstrips *Nitrobacter* activity, and there may be an accumulation of nitrite (9). Moreover, nitrifying bacteria are more sensitive to salt stress than most soil microorganisms (13,16,25), and get inhibited by low concentrations of many compounds containing sulfhydryl groups (7,18,20). The stimulation reported for inorganic substances such as phosphorus (19), sulfates (1), and trace elements (4), likely not nutritional. The demand by species of both genera of these elements for the oxidation process is extremely small and can easily be satisfied without resource to additional source of supply.

No sugar, organic acid or other organic molecule seems to serve as a source of either carbon or energy for growth of these bacteria (4). Exogenously supplied B vitamins or amino acids have shown no beneficial effect upon either of the two important soil genera (9). There is no basis, however, for the conclusion that nitrification in nature is inhibited by organic matter per se especially when the reaction occurs in environments with considerable soluble carbonaceous materials (2).

Preliminary investigations indicated the existence of 200 to 5,400 cells of *Nitrosomonas* per gram in all sandy soil samples collected from cultivated areas extending from Misrata to Mirziq (unpublished data). *Nitrobacter*, however, was not detected except in four Misrata and one Sokana soil samples in a range of about 170 to 490 cells per gram soil. None of the investigated soil samples was found to contain more than 0.20% organic matter. Under the conditions of these soils, the response of the nitrifying bacteria to any treatment with organic constituents is still unknown.

The purpose of this investigation was to evaluate the effect of a polypeptide in the form of casein hydrolyzate and a simple carbohydrate as dextrose on the growth of *Nitrosomonas* and *Nitrobacter* in sandy soils extremely low in native organic matter. Nitrogen was added to soils either as ammonium sulfate or urea along with a blanket treatment of phosphorus and potassium.

The effect of various inorganic sulfur compounds on the growth and activity of these chemoautotrophs in sandy soils is currently under investigation.

MATERIALS AND METHODS

A composite sample from 2 to 20 cm deep virgin soil was collected from the experimental farm of the Faculty of Agriculture. This soil sample was air-dried, passed through 2 mm sieve, and its water holding capacity was determined. Replicate 150 gram soil portions were placed into each of 250 ml capacity cotton-plugged Erlenmeyer flasks. Nitrogen, 170 ppm, in the form of either ammonium sulfate or urea aqueous solution was pipetted on the surface of the soil sample contained into each flask. Similarly, 100 ppm of triple superphosphate and 50 ppm of potassium sulfate were added. The soil samples were then brought to 60% water holding capacity with sterile distilled water. Two other groups of soil portions were mixed with 1.0% (w/w) dextrose or casein hydrolyzate, prior to the forementioned treatments, and brought to 60 percent water holding capacity in the same manner. All soil samples were incubated at 28°C at about 80% relative humidity for the required periods of time. Loss of moisture through evaporation was corrected with sterile distilled water periodically.

The soil pH was determined for each soil sample by WTW-390 glass electrode pH-meter on a 1:1 soil-water ratio.

Enumeration of heterotrophic soil bacteria was carried out using the plate-dilution

frequency technique described by Harris and Sommers (12) on yeast extract nutrient agar. The plates were incubated at 28°C for 48 hours.

Nitrosomonas and *Nitrobacter* numbers were determined according to Alexander's most probable number technique (3) in ammonium mineral- and nitrite mineral-carbonate. Five inoculated replications were performed for each ten-fold serial dilution, and the 95% confidence interval was determined accordingly.

Soil urease activity was determined according to Broadbent's modified method (8). The optical densities were measured at 660 mu and 1.0 nm spectral bandwidth using Perkin-Elmer double beam spectrophotometer, Coleman 124.

Urease-production tests were carried out on urea-agar (22). The ability of bacteria to hydrolyze urea causing a rise in pH indicated by the indicator colour change in 48 hours at 28°C was considered positive.

RESULTS AND DISCUSSION

Effect of Dextrose and Casein on the Growth of Chemoautotrophic Nitrifying Bacteria in Soils Amended with Ammonium Sulfate

This experiment was initiated to investigate the relative response of *Nitrosomonas* and *Nitrobacter* to organic constituents which are readily decomposable by indigeneous heterotrophic bacteria in a sandy soil containing less than 0.14% native organic carbon.

Apparently, 1.0% dextrose had no significant effect on *Nitrosomonas* growth during the initial 4 weeks of incubation. Maximum growth was attained during the 14 to 28 day

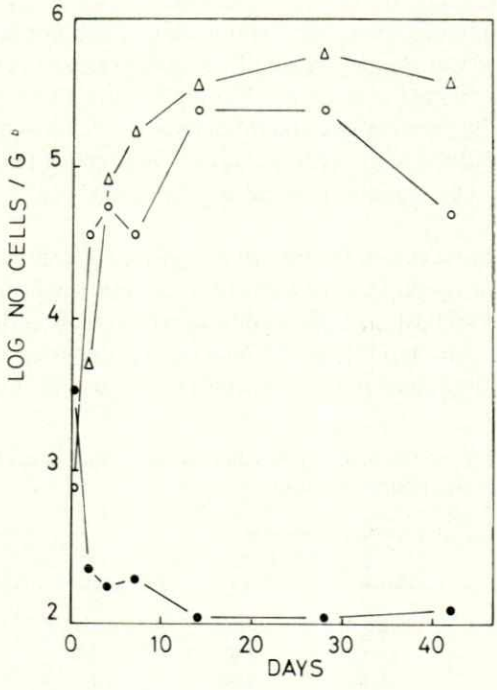

Fig. 1. Growth of *Nitrosomonas* spp. in incubated soils treated with ammonium sulphate alone (△) or in combination with dextrose (○) or casein (●). (b) 95% confidence interval was D/3.30 to 3.30 D where D is the number of bacteria.

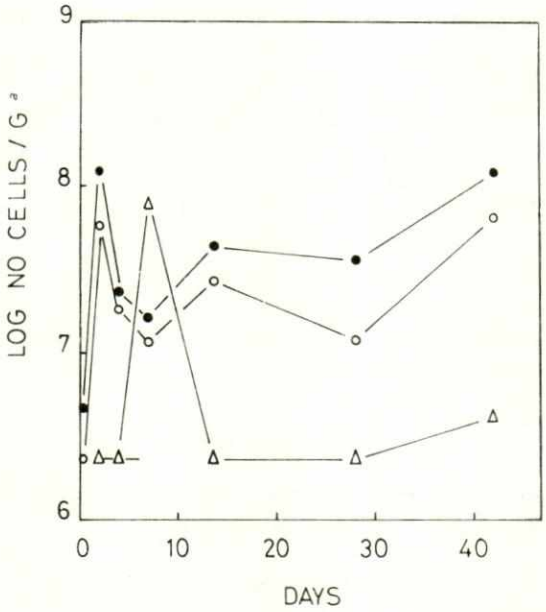

Fig. 2. Numbers of soil bacteria in incubated nonamended (△) and dextrose- (○) or casein-amended (●) soils treated with ammonium sulfate. (a) 95% confidence interval was D/2.47 to 2.47 D where D is the number of bacteria.

period, reaching about 2.3×10^5 cells per gram soil. After 28 days, however, the tendency of decline in numbers was about 7 times that of the control treatment (Fig. 1). This could be attributed to the depletion of the inorganic nitrogen supply by the flora requiring inorganic nutrients for dextrose decomposition. The growth of heterotrophic bacteria was so rapid due to dextrose amendment that counts of more than 7×10^7 cells per gram soil were obtained after only 2 days of incubation (Fig. 2). The growth was fluctuating thereafter, but was maintained at about the same level after 42 days of incubation.

Casein treatment, however, resulted in a rapid and continuous decrease in the numbers of *Nitrosomonas* (Fig. 1). An eleven-fold reduction in numbers was approached after only 2 days of incubation, and counts of about 200 cells per gram soil were obtained after 2 weeks. The inhibition of *Nitrosomonas* in this case can not be attributed to inorganic nitrogen depletion only. The sudden increase in soil pH (Table 1) caused

Table 1 Effect of incubation on the pH of soils amended with either ammonium sulfate or urea alone or in combination with dextrose or casein.

Incubation time, days	Ammonium sulfate treatments			Urea treatments		
	Control	Dextrose	Casein	Control	Dextrose	Casein
¼	8.50	8.40	7.70	8.40	8.40	7.60
2	8.30	7.80	8.90	8.70	7.60	8.90
4	8.20	7.80	9.00	8.60	7.90	9.00
7	8.00	7.90	9.20	8.20	7.40	9.20
14	8.10	8.30	9.20	7.00	7.80	9.30
28	7.80	7.90	9.00	7.10	7.60	9.00
42	7.50	8.00	8.80	7.30	7.70	9.00

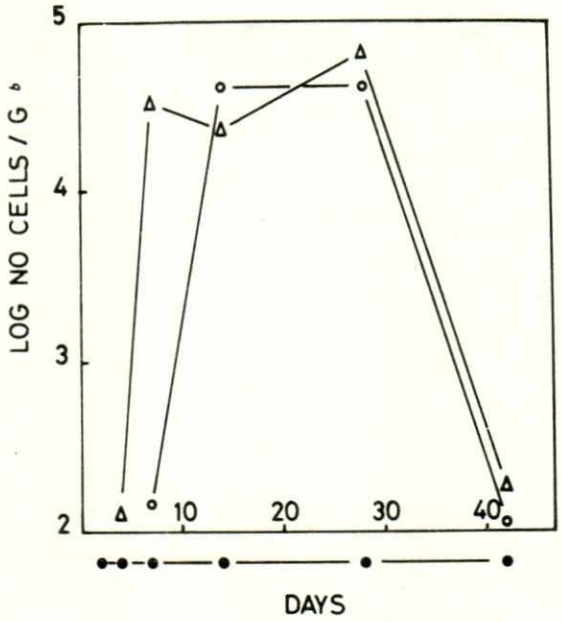

Fig. 3. Growth of *Nitrobacter* spp. in incubated soils treated with ammonium sulfate alone (△) or in combination with dextrose (○) or casein (●). (b) 95% confidence interval was D/3.30 to 3.30 D where D is the number of bacteria.

by casein hydrolysis, and the metabolic by-products of proteolytic bacteria, whose activities were enhanced by casein (Fig. 2), were undoubtedly more important factors behind the growth repression of *Nitrosomonas*.

The growth patterns of *Nitrobacter* under similar conditions were relatively different. Although a lag of 7 days was needed for growth initiation, maximum growth of *Nitrobacter* was not significantly affected by dextrose treatment. A value of about 4×10^4 *Nitrobacter* cells per gram soil was obtained in two weeks (Fig. 3). A sudden decline in numbers occurred after 28 days and counts of about 200 cells per gram soil were observed after 42 days.

In casein-amended soils, *Nitrobacter* counts were consistently below the detection limits of the procedure used in this experiment. This indicates even a greater sensitivity of this bacterium to the synergistic effect of heterotrophic bacteria, especially the proteolytic groups, under the prevailing unfavourable alkaline soil condition.

Effect of dextrose and casein on the growth of *Nitrosomonas* and *Nitrobacter* in soils amended with urea

The urease activity of the soils under investigation was found to be relatively high. Inspite of the unfavourable conditions associated with these soils, a value of about 55.8 ppm urea hydrolyzed per day was recorded. The urease activity is known to be a function of not only the microorganisms that produce the enzyme but also the soil pH, organic matter and carbonate contents (14). Thus, the rate of ammonium release is subject to change with incubation time according to the type of organic matter application. Therefore, beyond the initial 7 days of incubation, *Nitrosomonas* growth equilibrated at about 5.5×10^5 cells per gram soil in both urea and ammonium sulfate treatments when dextrose and casein were not involved. During the first week of in-

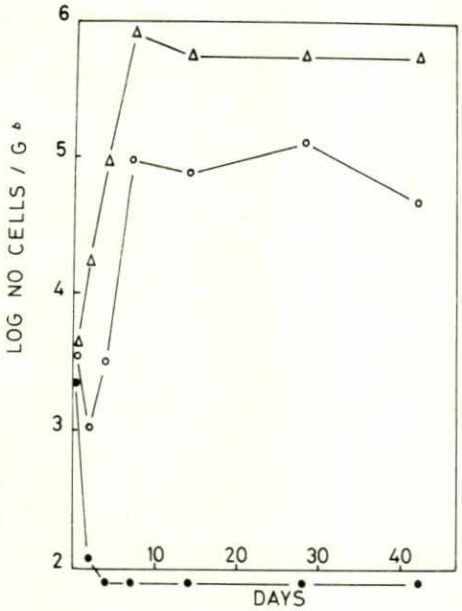

Fig. 4. Growth of *Nitrosomonas* spp. in incubated soils treated with urea alone (△) or in combination with dextrose (○) or casein (●). (b) 95% confidence interval was D/3.30 to 3.30 D where D is the number of bacteria.

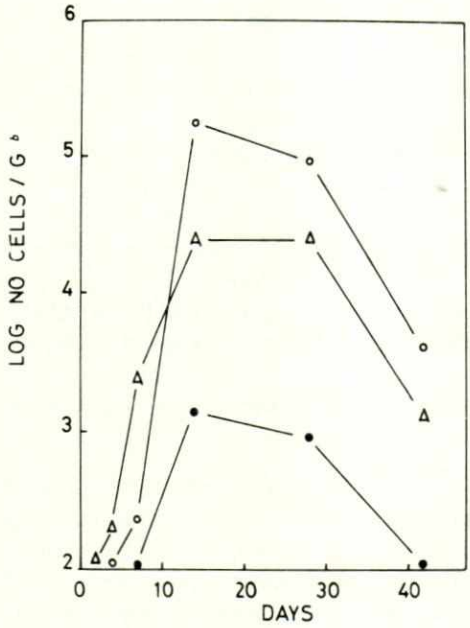

Fig. 5. Growth of *Nitrobacter* spp. in incubated soils treated with urea alone (△) or in combination with dextrose (○) or casein (●). (b) 95% confidence interval was D/3.30 to 3.30 D where D is the number of bacteria.

cubation, however, counts of 8.0×10^5 and 2.0×10^5 cells of *Nitrosomonas* per gram soil were recorded for urea and ammonium sulfate treatments respectively. In dextrose-amended soils, *Nitrosomonas* counts were close to 1×10^5 cells per gram soil after 7 days of incubation (Fig. 4), an eight-fold decrease over the control treatment. As with ammonium sulfate treatment, *Nitrosomonas* growth was significantly inhibited by casein treatment, and counts lower than 200 cells per gram soil were obtained after only 4 days of incubation.

The proliferation of *Nitrobacter* in soils supplemented with urea followed entirely different pattern. In this case, no growth suppression due to dextrose amendment was observed. On the contrary, counts of about 1.8×10^5 cells per gram soil (Fig. 5) were observed after an incubation period of two weeks, an eight-fold increase over the control treatment. Unlike ammonium sulfate, urea treatment with casein resulted in only a partial growth repression of *Nitrobacter* and counts of more than 1000 cells per gram soil were obtained after two weeks.

Among the heterotrophic growth shown in Fig. 6, urea hydrolyzing bacteria were found to make 1.6×10^6 and 2.1×10^6 cells per gram soil in dextrose and casein

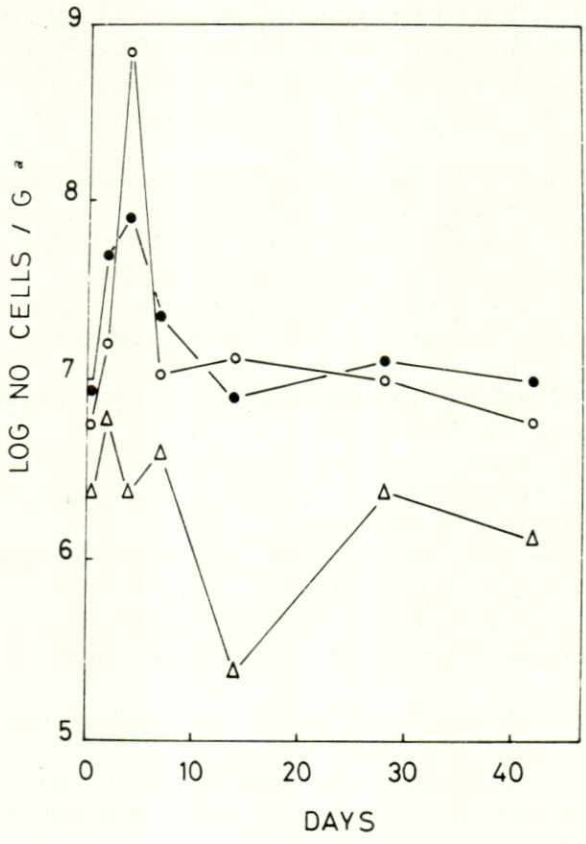

Fig. 6. Numbers of soil bacteria in incubated nonamended (△) and dextrose- (○) or casein-amended (●) soils treated with urea. (a) 95% confidence interval was D/2.47 to 2.47 D where D is the number of bacteria.

amended soils respectively. The morphological examination of these urea hydrolyzing bacteria revealed two predominant groups, spore-forming bacilli and streptomycetes.

Apparently, the activity of the bacteria under discussion had at least partly buffered the deleterious effects of the other associated heterotrophic microorganisms on the growth of *Nitrobacter*. Thus, the latter bacteria were able to perform in urea-treated soils relatively better than in soils treated with ammonium sulfate.

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نمو النيتروسوموناس والنيتروباكتر فى ترب رملية معاملة بمكونات عضـــوية

صالح محسن صالح ، عبد العـــزيز عزوز

المستخلـــــص

لم يتأثر نمو النيتروسوموناس باضافة الدكستروز الرمليـة المحتوية على ١٧٠ جزء بالمليون نيتروجين بشكل كبريتات الامونيوم · بينما انخفض عدد هذه البكتـرية الى عشر المقدار عندما استعيض عن كبريتات الامونيوم بمقدار مماثل من النيتروجين بشكل يوريا · وقد انخفض العدد فى كلا الحالتين عند اضافة الكاسئين الى التـربة · ويدل ذلك على تأثير البكترية المحللة للبروتين التى ازداد نشاطها بســـبب اضـــافة الكاسئين وخاصة فى الظروف القلوية التى الت اليها التربة ·

وبالرغم من عدم النيتروباكتر باضافة الدكتستروز للتـــرب المعاملة بكبريتات الامونيوم ، لوحظ زيادة فى عدد هذه البكترية فى الترب المعامـــلة باليوريا · لذلك ارتفع عددها الى حوالى ١٠٠ر٠٠٠ خلية / جرام بعد اسبوعين مـن المعاملة وقد ادت اضافة الكاسئين الى لتربة الى انخفاض عدد النيتروباكتر لنفس الاسباب السابقة ·