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CATALYTIC EFFECT OF SOIL COMPONENTS ON THE NITRITE TRANSFORMATION IN BUFFER ACID SOLUTIONS

by

Laxman G. Kuratti

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Soil Science

UTAH STATE UNIVERSITY
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Laxman G. Kuratti.

Laxman G. Kuratti

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ABSTRACT

Catalytic Effect of Soil Components on the Nitrite Transformation in Buffer Acid Solutions

by

Laxman G. Kuratti, Doctor of Philosophy

Utah State University, 1969

Major Professor: Dr. R. L. Smith
Department: Soils and Meteorology

Nitrite decomposition in buffer solutions of pH 3, 4, and 5 was observed to be a first order reaction with rate constants (k) 6.39×10^{-3}, 1.15×10^{-3}, and 0.17×10^{-3}, respectively. When 10 grams of two different soils were introduced, the reaction was catalyzed in all three pH conditions studied. This effect, however, was more pronounced in pH 5.

When 10 grams of soil were introduced, all the added nitrite was not recovered. The deficit ranged from 17 to 30 parts per million when 150 parts per million nitrite nitrogen was added and allowed to react for 6 hours. The deficits seemed to increase when the incubated soil samples were used. The major portion of the deficit was suspected to be due to the formation of N_2 gas.

The effect of three amino acids was studied on the nitrite decomposition. Tryptophan and tyrosine increased the deficit whereas cystine did not have any effect.

(90 pages)

INTRODUCTION

An appreciable magnitude of nitrogen loss that cannot be attributed to leaching or crop removal occurs in soil. Several mechanisms have been proposed to account for these losses.

The process of denitrification was perhaps the best known of these mechanisms. Surprisingly enough, great deficits were noted in well aerated soils. In such soils the nitrite ion, an intermediate product of nitrification and denitrification, is now suspected to be the seat of a number of reactions. In view of the high reactivity of nitrite ion in acid conditions, many pathways have been proposed to suggest the possible losses of nitrogen. The reactivity of nitrite ion in the pH ranges found in the soil is now established beyond doubt. It can undergo rapid decomposition to various end products.

The end products of nitrous acid decomposition have been shown to be nitrate nitrogen, oxides of nitrogen, and elemental nitrogen. Several workers, including Reuss and Smith (1965), Mahendrappa, Smith, and Kuratti (1969), and Rashid (1966), determined quantitatively the amount of elemental nitrogen evolved and the nitrates formed during the nitrite decomposition in acid soils. In their studies the apparatus was designed to retain all the gases produced. The system used to trap the nitrogen oxides consisted of alkaline potassium permanganate solution in a vial within the reaction flask and gave, at best, only a qualitative test for the oxides of nitrogen since these were free to react continually with the soil constituents and possibly be converted to other forms of nitrogen. Kuratti and Smith (1969) developed a system where a quantitative determination of nitrogen oxides was possible. From their observations it was shown that these oxides of nitrogen ($NO + NO_2$) are produced in significant amounts and the constituents of soil affect the quantity and rate of production.

Rashid (1966) showed that decomposing organic matter had a significant influence on the amount of elemental nitrogen produced. In his study it was pointed out that the N_2 loss was catalyzed by the addition of organic matter.

Kuratti (1966) showed that the nitrite decomposition in soil and a sand plus bentonite system was much faster than in a solution system with comparable pH values.

In view of the above observations it was suspected that the various components of the soil must be responsible for the catalytic effect of nitrite transformation in soil. The work reported here was undertaken with the following objectives:

1. To study the kinetics of nitrite decomposition in buffered solutions of pH 3, 4, and 5 to which 10 g of three different soils were added.

2. To study the effects of various catalytic agents on the nitrite decomposition, nitrate formation, and nitrogen oxides produced during 6 hours reaction period in buffered solution of pH 3 and 5.

REVIEW OF LITERATURE

Even today, after several decades of intensive study, we do not completely understand the nitrogen transformations in soil. Our ignorance is greatest with regard to changes under acid conditions. The nitrite ion has long been known as an intermediate product in the processes of biological oxidation of ammonia (nitrification) and reduction of nitrate (denitrification). It is now suspected, and some of the experimental evidence substantiates this, that the nitrite ion is the operating point of a number of reactions and subsequent nitrogen transformations.

Factors Influencing Nitrite Accumulation

Nitrite has been found to accumulate in soils for varying lengths of time as a transitory intermediate in the biological processes of nitrification and denitrification. Considerable amounts of nitrite have been reported under field and laboratory conditions (Kelley, 1916; Martin et al., 1942; Chapman and Liebig, 1952; Broadbent et al., 1957; Stojanovic and Alexander, 1958; Justice and Smith, 1962). In view of the importance of nitrite in connection with possible nitrogen losses, it is considered necessary to determine under what conditions nitrite accumulation takes place in soils.

Soil pH

Soil reaction seems to influence nitrite accumulation directly and indirectly.

Direct effect. Nitrification is a two-phase process carried out by different organisms. The nitrite bacteria, Nitrosomanas, convert the ammonium ion to nitrite in the first stage

$$NH_4^+ + \frac{3}{2} O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad . \; . \; . \; . \; . \; . \; . \; . \quad [1]$$

and the nitrate bacteria, Nitrobacter, convert nitrite to nitrate

$$NO_2^- + \frac{1}{2} O_2 \rightarrow NO_3^- \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad [2]$$

Kelley (1916) and Martin et al. (1942) were of the opinion that alkaline conditions are not conducive to the activity of Nitrobacter, whereas they do not affect Nitrosomanas.

Indirect effect. Growth and metabolism of Nitrobacter were reported to be affected by high concentration of ammonium ion (Lees and Quastel, 1946). It was further pointed out that the degree of inhibition increases with higher pH. This results in blocking of the oxidation of nitrite to nitrate. Broadbent et al. (1957) and Stojanovic and Alexander (1958), while supporting this view, suggested that at high pH the amount of ammonia present is also high and that it acts as a selective inhibitor of Nitrobacter. Aleem and Alexander (1960) showed this selective inhibition on Nitrobacter agilis. Under comparable condition there was only a slight effect on Nitrosomanas.

Accumulation of nitrite is reported by many workers. The two above mentioned reasons could be equally important in inhibiting the oxidation of nitrite to nitrate or one may be more important than the other. But the net result is nitrite accumulation.

Kelley (1916) observed large amounts of nitrite when a small amount of sodium carbonate was added to the soil fertilized with dried blood and ammonium sulfate. He noted larger amounts of nitrite and suppression of nitrification when alkali salts were added. Robinson (1923) observed longer retention of nitrite when calcium carbonate was incorporated into an acid soil receiving sodium nitrite. Midgeley (1923), while studying the rate of nitrification in heavy and light limed soils, found 16 times more nitrite in soils receiving heavy lime applications. Fraps and Sterges (1939) and Albrecht and McCalla (1937) also came up with conclusions similar to those of Midgeley (1932).

Martin et al. (1942) studied the nitrification of ammonium sulfate and urea in some of the Arizona desert soils. They observed an accumulation of nitrite in well aerated alkaline soils and concluded that the alkaline pH hindered the nitrite oxidation phase of nitrification until other natural (biological) processes reduce the pH value to a point where nitrite oxidation could take place. In case the pH was not lowered, the formation of nitrates was greatly retarded. There are evidences, however, to show that nitrification occurs at much higher pH values though at slower rates, and the threshold pH of 7.7 ± 0.2 suggested by them is not of much importance.

With the application of urea, anhydrous ammonia, ammonium or ammonia-forming fertilizers to neutral or alkaline soils, Chapman and Liebig (1952) found substantial amounts of nitrite under both field and laboratory conditions. From this observation, they concluded that in neutral and alkaline soils of the arid and semiarid regions heavy applications of ammonia and ammonia-forming fertilizers are likely to lead to nitrite accumulation. Nitrite may persist or disappear, depending on the resistance of the soil to pH changes.

Wahhab and Uddin (1954) reported accumulation and persistence of large quantities of nitrite in soils of Punjab with pH 8.0 to 8.3 that had been fertilized with ammonium sulfate. Similar observations were made by Duisberg and Buehrer (1954), Martin and Cox (1956), Broadbent et al. (1958), and Stojanovic and Alexander (1958).

Nitrogen fertilizer

It is pointed out in the foregoing discussion that ammonia is probably toxic to Nitrobacter at the higher pH levels, and, as such, applications of ammonia or ammonia-yielding fertilizers give rise to large amounts of nitrite. Stojanovic and Alexander (1958) reported on the role of ammonia in nitrite accumulation.

Organic matter

Smith and Burns (1965) reported higher nitrite accumulation in regions of active organic matter decomposition.

Soil temperature

The Nitrobacter organisms are sensitive to soil temperature; their functioning seems to be inhibited at both the extremes. The Nitrosomanas, on the other hand, are less susceptible to temperature fluctuations. Chapman and Liebig (1952) stated that nitrites would likely persist for several months if the temperature is not conducive to the activity of the Nitrobacter. Tyler and Broadbent (1960) and Justice and Smith (1962) are in agreement with the above observation.

Photochemical processes

Rao and Dhar (1931) suggested that ammonia or ammonium salts are oxidized to nitrite in the presence of light and photosensitizers. They are of the opinion that oxygen (0_2) is converted into ozone (0_3), and that ozone reacts with ammonia as shown below:

$$NH_4OH + O_3 \rightarrow HNO_2 + 2H_2O \quad . . . . \quad [3]$$

Production of the Oxides of Nitrogen

Nitrite ion is seen to be stable and found to accumulate in alkaline media. In acid media, however, nitrite readily decomposes, due to its instability, giving rise to various oxides of nitrogen.

Dismutation of nitrous acid

Temple (1914) was the first one to identify nitrogen dioxide (NO_2) by smell and the formation of nitrate in the solution when he added sodium nitrite ($NaNO_2$) to either an acid soil or an acidic solution. He proposed the following scheme as the possible pathway of nitrite decomposition.

$$4HNO_2 \rightarrow 2\ NO + 2NO_2 + 2H_2O \quad . . . . \quad [4]$$

$$2NO_2 \rightarrow N_2O_4 \quad . . . . \quad [5]$$

$$N_2O_4 + H_2O \rightarrow HNO_3 + HNO_2 \quad . . . . \quad [6]$$

The sum of reactions [4] through [6] is equation [7].

$$3HNO_2 \rightarrow 2NO + HNO_3 + H_2O \quad . . . . \quad [7]$$

Robinson (1923), while investigating the usefulness of sodium nitrite as a source of nitrogen fertilizer, found that nitrite was lost in the form of various oxides of nitrogen. Such loss was considered to be greater in acid soils than in neutral or alkaline soils. Similar results were obtained by Olendskii (1931) when sodium nitrite was added to acidified soils.

Corbet (1934) was able to establish beyond doubt that nitrous acid decomposition is nonenzymatic by sterilizing the solutions he used in his studies. He indicated that the probable scheme would be the one shown in equation [7].

Wagner and Smith (1958) studied the various forms in which added nitrogen could be lost from acid soils. The oxides of nitrogen were found to be produced during the decomposition of nitrite.

Tyler and Broadbent (1960), using sterile and non sterile soils, found not more than trace amounts of the oxides of nitrogen when nitrite was added to acid soils. Thorne (1961) studied the decomposition of nitrous acid in acid solution media. Equation [7] was believed to explain most of his results.

Reuss and Smith (1965), while studying the reaction of nitrite in acid media, concluded that nitric oxide and nitrogen dioxide were the products of decomposition. They further concluded that the evolution of these oxides in the absence of adequate moisture may lead to considerable losses of added nitrogen.

Fraps and Sterges (1939) added 100 ppm of nitrite nitrogen to acidic soils that ranged in pH from 4.4 to 6.9. They observed a loss of 10 to 95 ppm. They were of the opinion that the loss had occurred through formation of nitrogen oxides according to equation [7].

Role of transition metals

Yost and Russel (1944) suggested that copper (Cu^+) catalyzed nitrite decomposition to nitric oxide according to the following equation.

$$Cu^+ + NO_2^- + 2H^+ \rightarrow Cu^{++} + NO + H_2O \quad . \; . \; . \; . \; . \; . \; . \; . \; . \quad [8]$$

Wullstein and Gilmour (1964) found iron (Fe^{++}) and manganese (Mn^{++}) to catalyze the decomposition of nitrite as shown in equation [8]. Chao and Bartholomew (1964) found aluminum (Al) and iron (Fe) to catalyze the same reaction.

Transformation of the Oxides of Nitrogen

To nitrates

The reactions of nitrite decomposition, as given by Sneed and Brasted (1956), indicate the importance of the oxides of nitrogen in the non-enzymatic nitrification:

$$2\ HNO_2 \longrightarrow NO + NO_2 + H_2O \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [9]$$

$$NO + 1/2\ O_2 \longrightarrow NO_2 \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [10]$$

$$2\ NO_2 \longrightarrow N_2O_4 \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [11]$$

$$N_2O_4 + H_2O \longrightarrow HNO_3 + HNO_2 \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [12]$$

The sum of these reactions is presented in equation [13].

$$HNO_2 + 1/2\ O_2 \longrightarrow HNO_3 \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [13]$$

In view of the importance attached to biological nitrification, Hall et al. (1907) and Stephenson (1920) were not able to recognize the chemical nitrification in acid soils where the conditions were not favorable for bacterial growth. They emphasized that nitrate that was found in small pockets in acid soils was the result of a situation where the soil reaction was basic enough to permit biological nitrification.

In the early 1930's, Barrit (1933) and Corbet (1934) independently obtained evidence for chemical nitrification. In bacterial cultures when the pH dropped to 5.5, the bacterial action stopped. However, they noticed the disappearance of nitrite and the formation of nitric acid which further reduced the pH of the medium to 4.5. They were of the opinion that the acidity of the medium was limiting for the bacterial action and so the only possibility of nitrate formation was through chemical reaction. They confirmed this by adding sodium nitrite to acidified solutions. Barrit and Corbet assumed the reaction probably proceeded as described in equation [13].

Allison and Doetsch (1951) suggested that nitric oxide produced during nitrite decomposition in moist and acid soils is readily oxidized to nitric acid. Recently Reuss and Smith (1965) observed non-enzymatic nitrification both in acid soil and in a resin system.

From the above review of literature, it is clear that non-enzymatic nitrification takes place in acid soils. However, there has been no attempt made to indicate that this reaction replaces biological nitrification which takes place simultaneously under favorable conditions.

To elemental nitrogen

Thorne (1961) proposed the formation of elemental nitrogen and nitrate produced according to the reaction:

$$5HNO_2 \rightarrow N_2 + 3H^+ + 3NO_3^- + H_2O \quad . \quad . \quad . \quad . \quad . \qquad [14]$$

He was of the opinion that hyponitric acid ($H_2N_2O_3$) was an intermediate compound. The reaction between hyponitrous acid and hyponitric acid is also supposed to give rise to elemental nitrogen (N_2), as proposed by Thorne (1961).

$$H_2N_2O_3 + H_2N_2O_2 \rightarrow N_2 + HNO_3 + HNO_2 + H_2O \quad . \quad . \quad . \quad . \quad . \qquad [15]$$

MATERIALS AND METHODS

The procedure for this study consisted of adding sodium nitrite ($NaNO_2$) to the reaction medium and then removing all the oxides of nitrogen produced, by passing air through the system. The oxides of nitrogen were trapped in alkaline potassium permanganate solution and the nitrite nitrogen left over in the media and the nitrate nitrogen formed were determined. It was intended to remove all the nitrogen oxides produced before they could be oxidized to nitrate according to the equation [10]. Preliminary experiments showed that nitrate was formed in the solution system to which nitrite was added when an air flow less than 100 ml per minute was used to sweep out the oxides. Therefore, in all the experiments a flow rate of 100 ml per minute was used.

Preparation of the Media

Preparation of buffer solution used

Buffer solutions of pH 3, 4, and 5 were prepared by the method of Vogel (1958) by mixing different proportions of 0.2$\underline{M}$ sodium phosphate (Na_2HPO_4) and 0.1$\underline{M}$ citric acid [$HOOCCH_2$ $C(OH)COOH)CH_2COOH$] as indicated in Table 1.

Soils used

Three acid soils, namely Miami silt loam, Olympic silty clay loam, and Dayton silty clay loam, were used in this study. Some of the properties of the soils used are given in Table 2.

Table 1. Quantities of sodium phosphate and a citric acid used to make 100 ml of pH 3, 4, and 5 buffer solution (Vogel, 1958)

0.2 M Na_2HPO_4	0.1 M $(CH_2COOH)_2C(OH)COOH$	pH[a]
ml	ml	
20.55	79.45	3
38.55	61.45	4
51.50	48.50	5

[a] A sample of the stock solution was tested before using after adjusting the pH meter against standard Beckman pH solution.

Table 2. Some of the physical and chemical characteristics of the soils used in this investigation

			Metals[b]				Moisture content	
Soils[a]	pH	CEC	Fe	Cu	Mn	Organic matter	0.3 bar	Air dry
	paste	meq/100g		ppm			percent	
Olympic silty clay loam	4.6	31.4	525	50	180	6.98	34.0	3.12
Dayton silty clay loam	4.9	11.5	1459	25	153	3.76	30.2	2.0
Miami silt loam	5.2	11.2	525	50	153	2.0	24.2	1.24

[a] The Olympic silty clay loam and Dayton silty clay loam came from Oregon. The two soils were supplied by Dr. T. Jackson, Department of Soils, Oregon State University. The Miami silt loam came from Ohio and was supplied by Dr. R. B. Clark, Ohio State Experiment Station, Wooster.

[b] The metals were extracted by 1% EDTA and determined on Perkin Elmer Atomic Absorption Spectrophotometer Model 303.

Preparation of soil

Soils were dug out from surface to a depth of 6 inches, air dried, ground, passed through a 200 mesh sieve, and stored in air tight bins.

In order to study the effect of activated organic matter complex of these soils, 10 g soil samples were brought to 1/3 bar tension and incubated at 30 C in a humid atmosphere chamber. The flasks were covered with polyethylene film, which permits free exchange of gases like carbon dioxide and oxygen and restricts the movement of water vapor.

Fractionation of soil components

Ten g oven dry soil samples were put in 50 ml centrifuge tubes. To this was added 20 ml of "Purex" (NaOCl) of pH 9.5. The tubes were placed in a boiling water bath for 15 minutes and then centrifuged for 5 to 10 minutes at 800 to 1000 rpm. The supernatant solution was decanted off and the procedure repeated for a total of five times.

About 30 ml of 2% sodium carbonate and sodium bicarbonate solution was added, stirred, and heated in boiling water bath for 10 minutes. This was again centrifuged for 5 to 10 minutes at 800 to 1000 rpm and the solution decanted. This treatment was repeated two more times. At the end of the last treatment, the final solution was centrifuged at 2500 rpm for 10 minutes.

The liquid was drained as thoroughly as possible from the tube. Ten ml of the dispersing agent (35.7 g sodium hexametaphosphate and 7.94 g sodium carbonate in a liter) and 10 ml water were added, stirred to break up the aggregates, and the soil plus solution transferred to a nursing bottle. The contents in the nursing bottles were shaken overnight.

The dispersed sample was washed through a 300 mesh sieve into a 1-liter cylinder. All particles less than 20 mμ were in the cylinder by now. The sieve containing sand was placed in an aluminum dish. The sample was dried at 110 C, and the sand fraction was determined by weighing. The suspension was stirred for 6 minutes with an electric stirrer and then with hand stirrer for 30 to 60 seconds with an up and down movement. The suspension in the cylinder was made to 1 liter and covered with a watch glass. Thermal jackets were placed around the cylinders.

A sample was siphoned out from the 9 cm depth at the time determined and placed into a bottle. This suspension was centrifuged at 2500 rpm for 15 to 20 minutes and the supernatant liquid decanted. The suspension was dried at 110 C and collected as the clay fraction. The suspension below the 9-cm depth was similarly dried and collected as the silt fraction.

Apparatus Used

Air from the line was controlled by a one-stage pressure regulator and measured in a rotometer. A safety valve system was introduced in order to permit the excess air to escape. The safety valve consisted of the vertical arm of the "T" tapering down dipped into water contained in 1000 ml cylinder (Figure 1). The air, regulated at 100 ml per minute, was saturated with moisture by bubbling it through water contained in an Erlenmeyer flask. The moisture-saturated air was then directed to the reaction flask.

The reaction vessel consists of a 300 ml Erlenmeyer flask. Forty-five ml of the buffer solution was put in this flask. A plastic vial

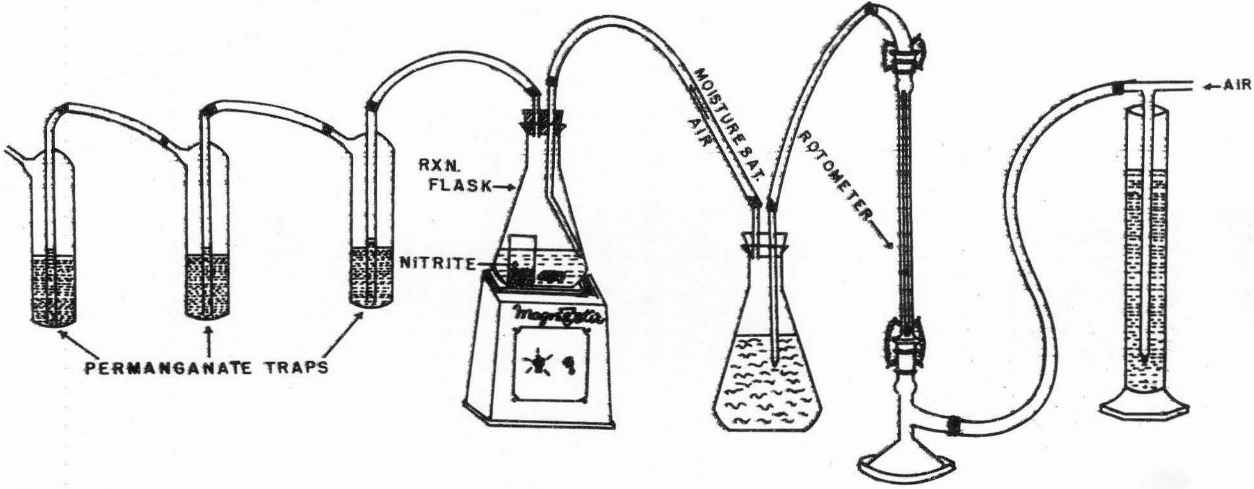


Figure 1. Apparatus and set-up for experiments where NO_2^--N was allowed to decompose and the oxides were trapped.

containing 5 ml sodium nitrite solution to provide 150 ppm nitrite-nitrogen was also placed in it. A stirring bar was put in the flask to stir the reaction medium by placing the flask on a magnetic stirrer. Care was taken to prevent the build-up of heat from the magnetic stirrer by placing an asbestos sheet and a block of wood between the vessel and the stirrer.

Three, four, or five alkaline permanganate tubes were connected in succession and joined to the reaction vessel. The air removing the oxides was forced through the alkaline permanganate tubes.

After ascertaining that all the connections were air tight, the vial containing the sodium nitrite solution was tipped over and the reaction begun.

Analysis of the Different Forms of Nitrogen

Nitrate and nitrite

Pure buffer solution. A sample (2 to 5 ml) was pipetted into a 250 or 500 ml volumetric flask about one-half full of filtered saturated calcium hydroxide solution. The volume was made up to the mark. The pH was recorded on the remaining reaction medium in the reaction flask.

An aliquot (5 to 10 ml) of the above solution was taken to determine the nitrite nitrogen by the method described by Shinn (1941), employing a diazo coupling of sulfanilic acid and N-(1-naphthyl)-ethylene diamine dihydrochloride as a coupling reagent. A standard phenol disulfonic acid method, similar to that of Jackson (1958), was used to determine nitrate after destroying nitrite with sulfamic acid. The complete analytical procedures are included in the appendix.

Soil or soil components in buffer solution. The pH was determined immediately after removing the reaction flask from the air streams. About 50 to 60 ml of saturated calcium hydroxide solution and two scoops of characoal were added to the reaction flask. The flask was stoppered and shaken for 15 minutes and the suspension allowed to settle for 10 minutes. The solution was filtered by using suction and the volume was made up to 500 ml to ensure complete extraction of nitrite. From here on the procedure was the same as used with the solution system.

Oxides of nitrogen

The alkaline permanganate solution (20 ml) held in each of the tubes was used to trap the nitric oxide and nitrogen dioxide gases swept out of the system during the course of reaction. The permanganate solutions were analyzed at the end of each reaction period. The solution was washed into beakers and the excess potassium permanganate solution destroyed by adding about 20 ml oxalic acid (0.6 M $Na_2C_2O_4$ in 7 N H_2SO_4). The solution was made alkaline by adding 5 N sodium hydroxide and then digested on a hot plate for 30 minutes. After filtering, the manganous hydroxide precipitate on the filter paper was washed several times with hot distilled water and the volume made up to 250 ml. Aliquots from this filtrate were analyzed for nitrate by the phenol disulfonic acid method. The method was calibrated by adding known amounts of potassium nitrate to the alkaline permanganate trapping solution. Calibration curves, using either sodium nitrite or potassium nitrate, were identical. The complete procedure is included in the appendix.

Experiments

Kinetic studies

Nitrite decomposition kinetics were worked out under a variety of conditions, namely

1. A pure buffer solution system, and
2. A buffer solution system plus 10 g of two different soils.

Nitrite nitrogen (150 ppm) was added as sodium nitrite, and the nitrite nitrogen that disappeared was followed for a total of 9 hours with many samplings in the initial stages. Nitrate nitrogen and the nitrogen oxides were not determined in this study since the objective was to determine the kinetics of nitrite decomposition.

Catalytic effect on nitrite decomposition and creation of deficit

Using the results obtained in buffer solutions as a reference, the various soil factors expected to catalyze the reaction/s responsible for nitrite decomposition were tested. Depending on the speed of nitrite decomposition, the experiments were conducted for a period of 3 or 6 hours reaction time. In these experiments, the nitrogen oxides collected and the nitrate formed were determined.

Effect of soils. Three different soils were used in this set of experiments. Ten g soil was mixed with the 45 ml buffer solution and then the 150 ppm nitrite nitrogen was added.

Effect of soil fraction. The sand, silt, or clay fractions that were obtained from the 10 g soil samples were added to the 45 ml buffer solution to study the effect of various soil components in the catalysis of nitrite decomposition and also the deficits noticed when the soils were used.

Effect of activated organic complex. Ten g soil samples were incubated at 30 C to activate the organic matter. These samples were added to the buffer solution and the disappearance of nitrite nitrogen followed.

Effect of amino acids. Various amino acids were tested to see if they have any catalytic effect.

EXPERIMENTAL RESULTS

Kinetic Studies

Solution systems

The kinetics of nitrite decomposition in acid buffer solutions of pH 3, 4, and 5 were carried out. The amount of nitrite remaining after various time intervals was determined after a standard amount of nitrite (150 ppm) had been added. The time intervals chosen were 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 300, 420, and 540 minutes.

Solution system at pH 3. The nitrite nitrogen recovered from the solution system of pH 3, after various time intervals, is given in Table 3. These data are plotted in Figure 2. A plot was made of the logarithms of the concentration versus time (Figure 3), and from this the rate constant, k, was found to be 6.39×10^{-3} per minute.

Solution system at pH 4. The nitrite nitrogen recovered at various time intervals from the solution system of pH 4 is given in Table 4. These data are plotted in Figure 4 and the logarithms of concentration versus time are plotted in Figure 5. From this figure the rate constant, k, was found to be 1.15×10^{-3}.

Solution system at pH 5. The nitrite nitrogen recovered at various time intervals is given in Table 5. These data are plotted in Figure 6 and the logarithms of concentration versus time are plotted in figure 7. From this figure the rate constant, k, was calculated to be 0.172×10^{-3}.

Table 3. Amounts of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 3 when 150 ppm NO_2^- -N was added as $NaNO_2$

	NO_2^- -N found			
Reaction time	Run 1	Run 2	Average	Log of average
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	124.8	125.2	125.0	2.0969
30	115.7	114.9	115.3	2.0618
45	94.9	95.1	95.0	1.9777
60	80.6	81.2	80.9	1.9079
90	68.9	69.5	69.2	1.8401
120	46.8	45.3	46.05	1.6633
150	46.8	46.7	46.75	1.6696
180	39.0	37.0	38.0	1.5798
210	29.6	30.1	29.8	1.4742
300	19.5	18.2	18.8	1.2742
420	13.0	12.6	12.8	1.1072
540	6.5	6.6	6.55	0.8162

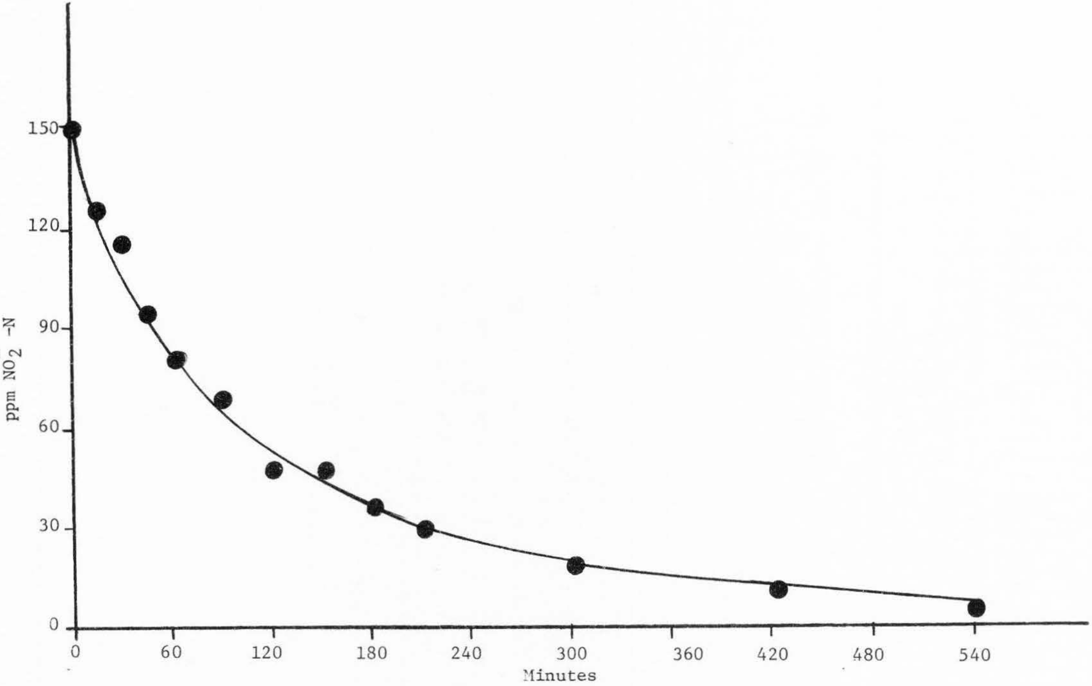


Figure 2. Amount of NO_2^- -N recovered at various intervals from the solution system of pH 3 to which 150 ppm NO_2^- -N was added as $NaNO_2$.

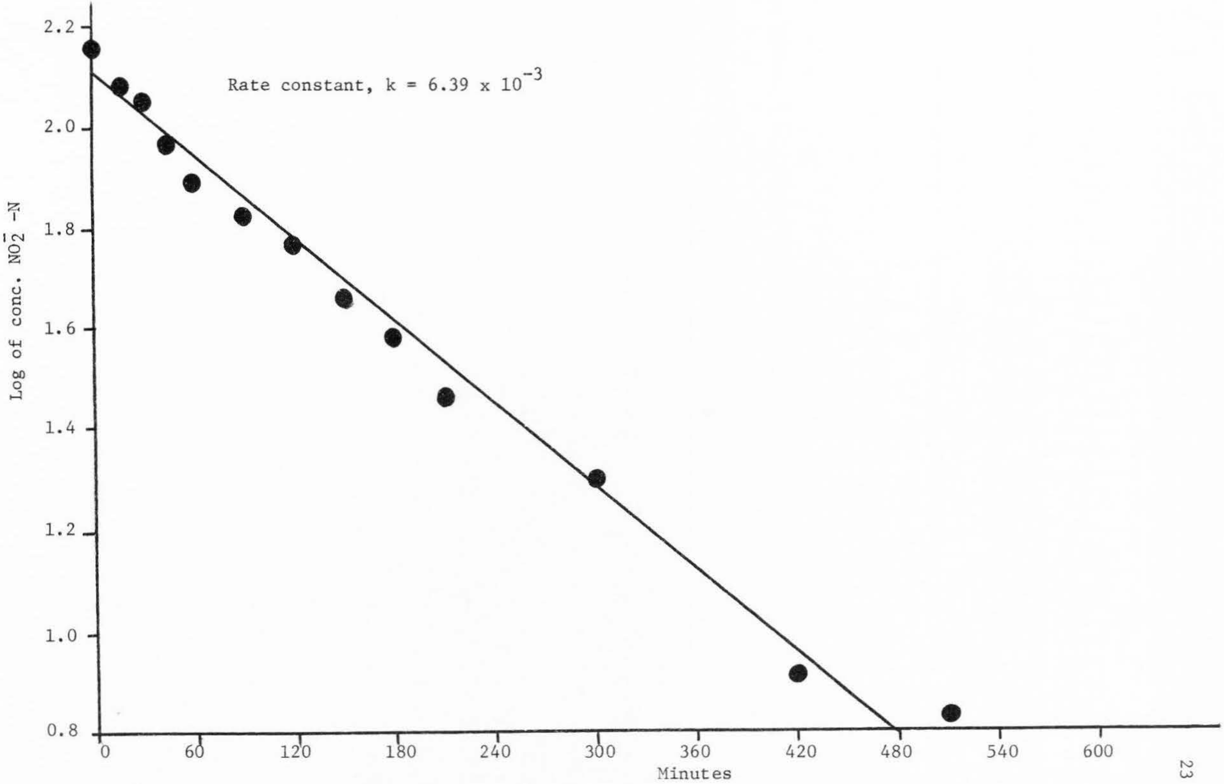


Figure 3. Logarithm of concentration of NO_2^- -N recovered versus time in the solution system of pH 3 to which 150 ppm NO_2^- -N was added as $NaNO_2$.

Table 4. Amounts of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 4 when 150 ppm NO_2^- -N was added as $NaNO_2$

Reaction time	NO_2^- -N found Run 1	Run 2	Average	Log of average
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	143.0	142.1	142.55	2.1538
34	140.0	141.0	140.5	2.1476
48	139.1	137.0	138.05	2.1399
60	137.8	138.0	137.9	2.1396
100	130.0	129.5	129.75	2.1132
120	126.1	125.2	125.65	2.0990
160	122.1	122.5	122.3	2.0871
180	111.8	110.5	111.2	2.0461
210	109.2	107.5	108.3	2.0346
300	98.8	99.5	99.2	1.9965
420	93.0	94.0	93.5	1.9708
540	92.5	94.5	93.0	1.9685

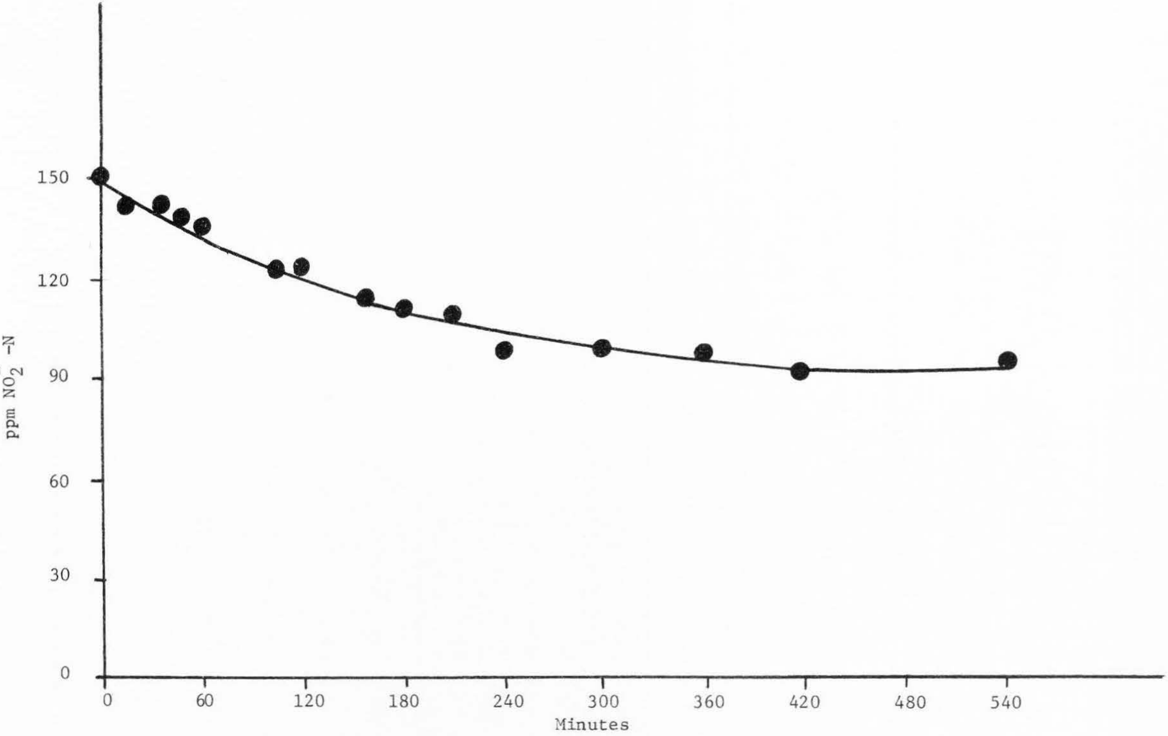


Figure 4. Amount of NO_2^- -N recovered at various intervals from the solution system of pH 4 to which 150 ppm NO_2^- -N was added as $NaNO_2$.

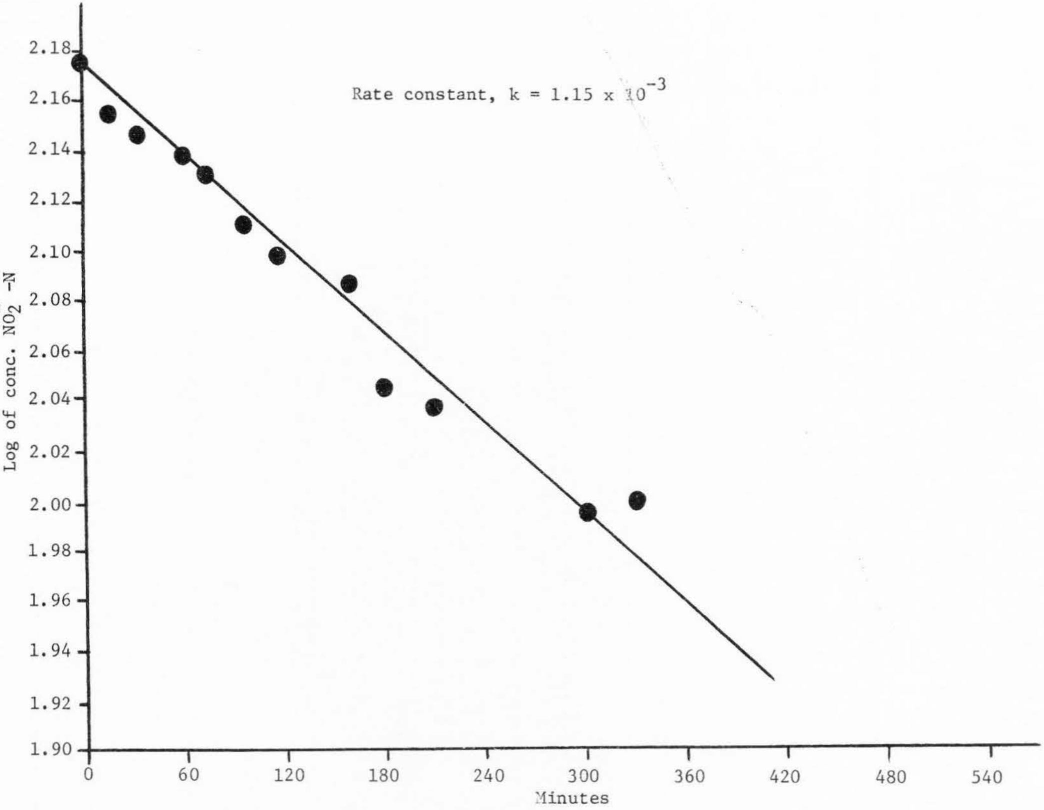


Figure 5. Logarithm of concentration of NO_2^- -N recovered versus time in the solution system of pH 4 to which 150 ppm NO_2^- -N was added as $NaNO_2$.

Table 5. Amounts of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 5 when 150 ppm NO_2^- -N was added as $NaNO_2$

Reaction time	NO_2^- -N found Run 1	 Run 2	 Average	Log of average
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	147.5	149.5	148.5	2.1718
35	149.5	146.5	148.0	2.1703
48	149.5	147.5	148.5	2.1718
60	146.3	145.3	145.8	2.1638
90	146.9	146.3	146.6	2.1662
120	147.5	146.8	147.1	2.1676
150	144.3	145.2	144.7	2.1605
180	143.0	142.8	142.9	2.1550
210	143.0	141.4	142.7	2.1544
300	143.0	142.5	142.25	2.1530
420	140.0	141.0	140.5	2.1476
510	140.0	139.0	139.5	2.1446

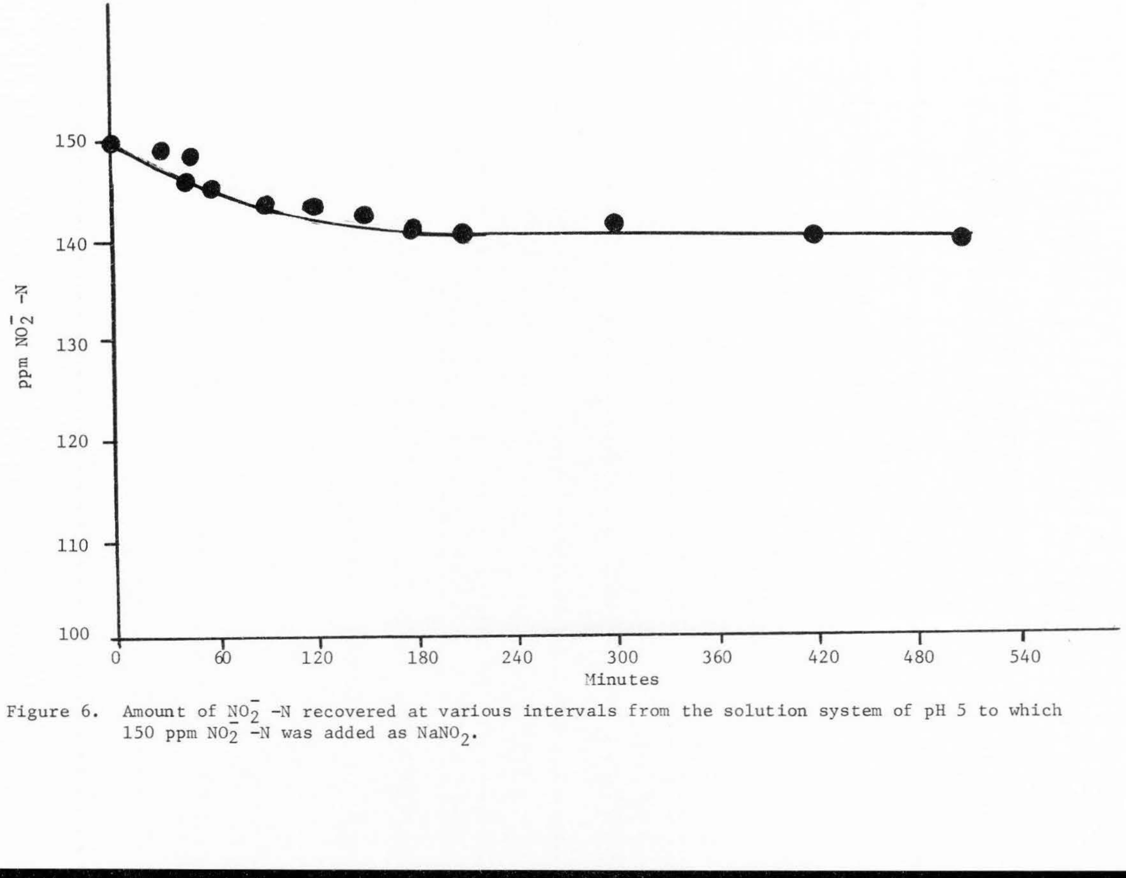


Figure 6. Amount of NO_2^- -N recovered at various intervals from the solution system of pH 5 to which 150 ppm NO_2^- -N was added as $NaNO_2$.

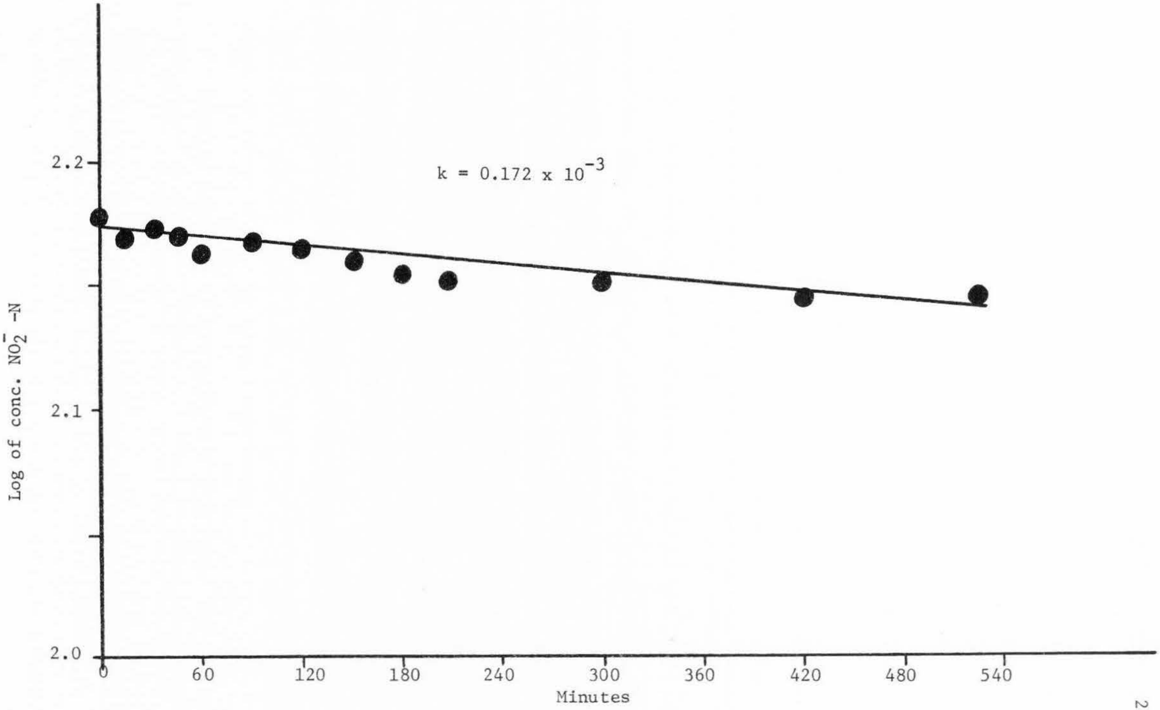


Figure 7. Logarithm of concentration of NO_2^- -N recovered versus time in the solution system of pH 5 to which 150 ppm NO_2^- -N was added as $NaNO_2$.

Solution system treated with soils

Two soils, namely the Dayton silty clay loam and the Miami silt loam, were introduced as contaminants in the solution systems at pH 3, 4, and 5, and the kinetics of nitrite decomposition of the 150 ppm added NO_2^- -N were studied.

Solution system of pH 3 treated with 10 g soil. The quantities of nitrite nitrogen recovered after various time intervals from the solution system of pH 3 treated with 10 g of the two soils mentioned above and receiving 150 ppm NO_2^- -N are given in Tables 6 and 7. The logarithms of concentration of NO_2^- -N versus time are presented in Figures 8 and 9. Rate constants were calculated to be 8.13 and 7.89 x 10^{-3} per minute when the solution of pH 3 was treated with Miami silt loam and Dayton silty clay loam, respectively.

Solution system of pH 4 treated with 10 g soil. The quantities of nitrite nitrogen recovered after various reaction periods from the solution systems of pH 4 treated with the same two soils mentioned earlier and receiving 150 ppm NO_2^- -N are given in Tables 8 and 9. The logarithms of concentration of NO_2^- -N versus time are presented in Figures 10 and 11. Rate constants were calculated to be 3.84 and 3.22 x 10^{-3} per minute when the solution was treated with Miami silt loam and Dayton silty clay loam, respectively.

Solution system of pH 5 treated with 10 g soil. The quantities of nitrite nitrogen recovered after the various reaction periods from the solution systems of pH 5 treated with 10 g of the two soils mentioned earlier are given in Tables 10 and 11. The logarithms of concentration of NO_2^- -N versus time for these two soils are presented in Figures 12 and 13. The rate constants are 0.639 and 0.643 x 10^{-3} per minute, respectively.

Table 6. Amount of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 3 treated with 10 g Miami silt loam and receiving 150 ppm NO_2^- -N

	NO_2^- -N found			Log of
Reaction time	Run 1	Run 2	Average	average
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
20	79.0	77.0	78.0	1.8921
30	65.0	63.4	64.2	1.8075
45	60.5	57.3	58.9	1.7701
60	54.2	53.2	53.7	1.7300
90	42.0	44.6	43.3	1.6365
120	27.5	31.5	29.5	1.4698
150	26.3	25.3	25.8	1.4116
180	19.2	17.2	18.2	1.2601
210	19.0	16.0	17.5	1.2430
300	8.5	7.3	7.9	0.8976
420	2.5	2.7	2.6	0.4150
540	2.5	1.5	2.0	0.3010

Table 7. Amount of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 3 treated with 10 g Dayton silty clay loam and receiving 150 ppm NO_2^- -N

Reaction time	NO_2^- -N found			Log of average
	Run 1	Run 2	Average	
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	75.6	76.2	75.9	1.8802
30	73.0	72.0	72.5	1.8603
45	57.0	58.2	57.6	1.7604
60	59.0	61.0	60.0	1.7782
90	44.2	42.6	43.4	1.6375
120	39.0	37.0	38.0	1.5798
150	25.1	27.5	26.3	1.4200
180	17.2	16.0	16.6	1.2201
210	18.0	18.4	18.2	1.2601
300	9.2	6.8	8.0	0.9031
420	3.0	3.4	3.2	0.5051
540	2.8	1.2	2.0	0.3010

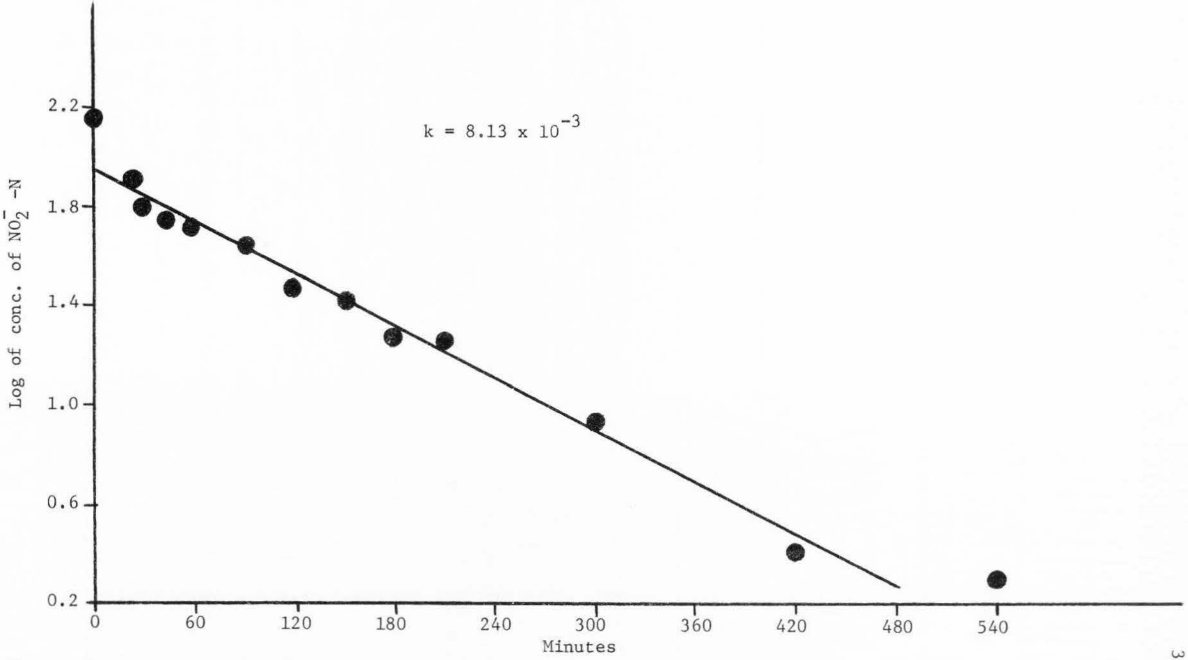


Figure 8. Logarithm of NO$_2^-$ -N found in the solution system of pH 3 treated with 10 g Miami silt loam and receiving 150 ppm NO$_2^-$ -N added as NaNO$_2$.

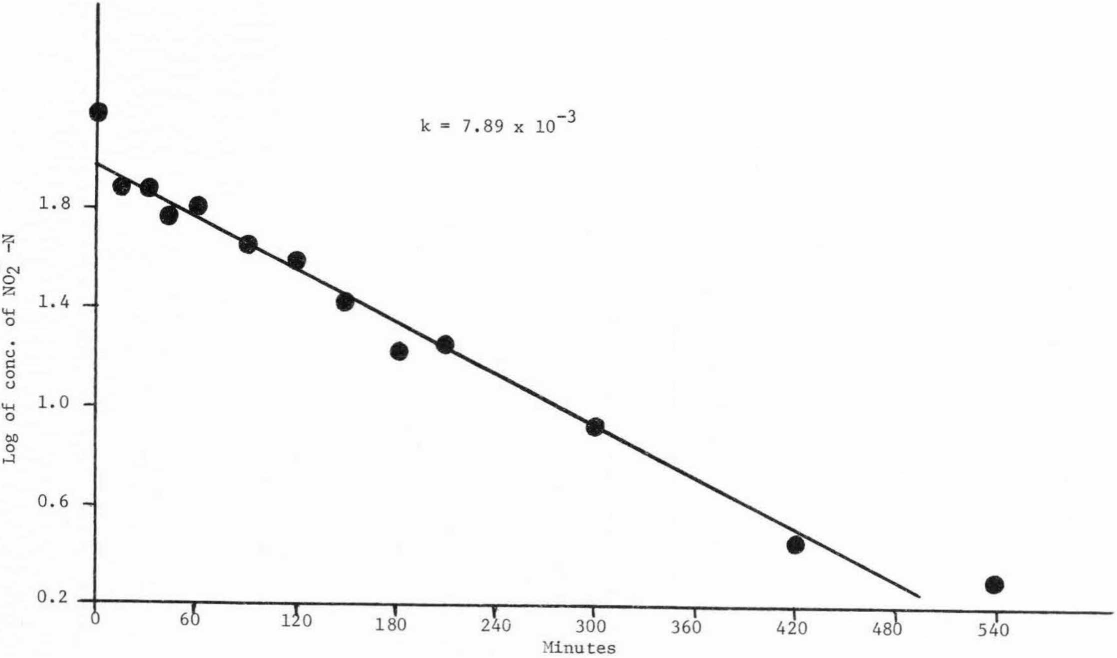


Figure 9. Logarithm of concentration of NO_2^- -N recovered from the solution system of pH 3 treated with 10 g of Dayton silty clay loam and receiving 150 ppm NO_2^- -N added as $NaNO_2$.

Table 8. Amount of NO_2^--N found at various reaction periods in phosphate buffer solution of pH 4 treated with 10 g Miami silt loam and receiving 150 ppm NO_2^--N

Reaction time	NO_2^--N found Run 1	Run 2	Average	Log of average
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	103.9	103.0	103.45	2.0146
30	-	96.5	96.5	1.9845
45	93.5	94.0	93.25	1.9696
60	90.8	91.8	91.3	1.9605
90	89.2	87.0	88.6	1.9474
120	84.1	85.1	84.6	1.9274
150	71.5	70.0	70.75	1.8497
180	70.8	69.2	70.0	1.8451
210	63.1	65.0	64.05	1.8065
300	47.9	49.9	48.9	1.6893
420	26.90	25.0	25.95	1.4143
540	20.0	21.0	20.5	1.3118

Table 9. Amount of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 4 treated with 10 g Dayton silty clay loam and receiving 150 ppm NO_2^- -N

Reaction time	NO_2^- -N found Run 1	Run 2	Average	Log of average
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	103.2	105.2	104.2	2.0178
30	96.0	97.0	96.5	1.9845
45	93.9	92.7	93.2	1.9694
60	90.0	88.0	89.0	1.9494
90	85.7	14.5	85.2	1.9304
120	78.5	80.5	79.5	1.9004
150	72.0	72.8	72.4	1.8597
180	68.1	72.1	70.1	1.8457
210	63.5	62.5	63.0	1.7993
300	45.9	44.7	45.3	1.6561
420	27.0	28.6	27.8	1.4440
540	22.4	22.0	22.2	1.3464

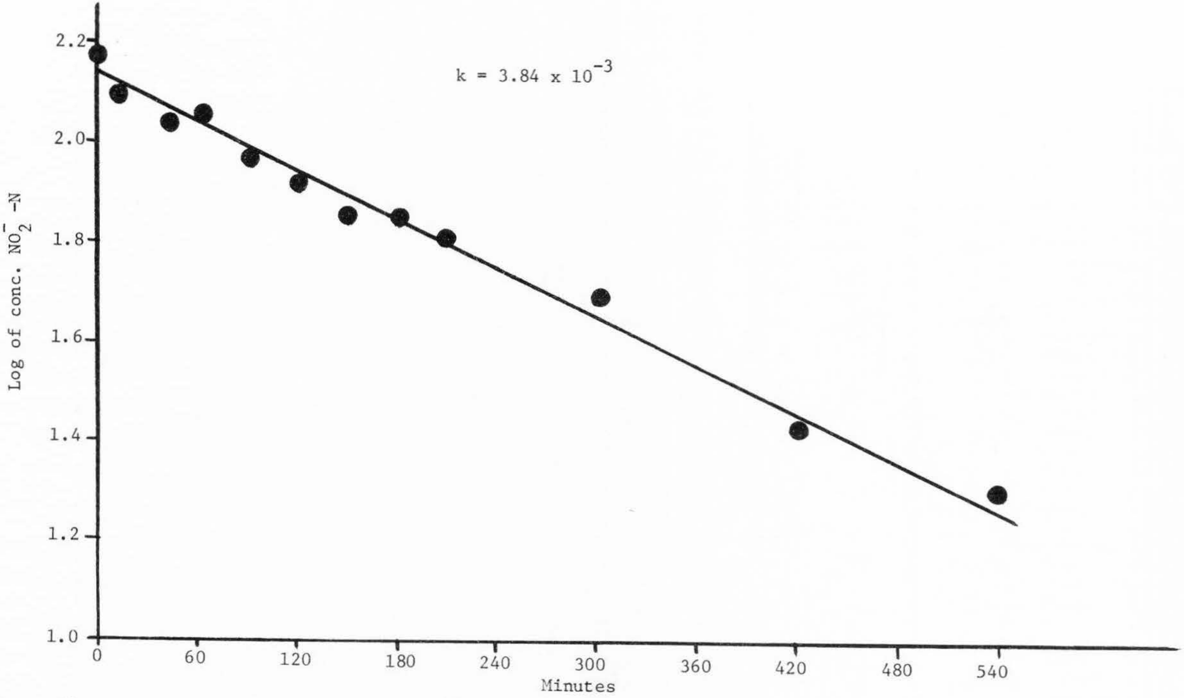


Figure 10. Logarithm of concentration of NO_2^- -N recovered from the solution system of pH 4 treated with Miami silt loam and receiving 150 ppm NO_2^- -N added as $NaNO_2$.

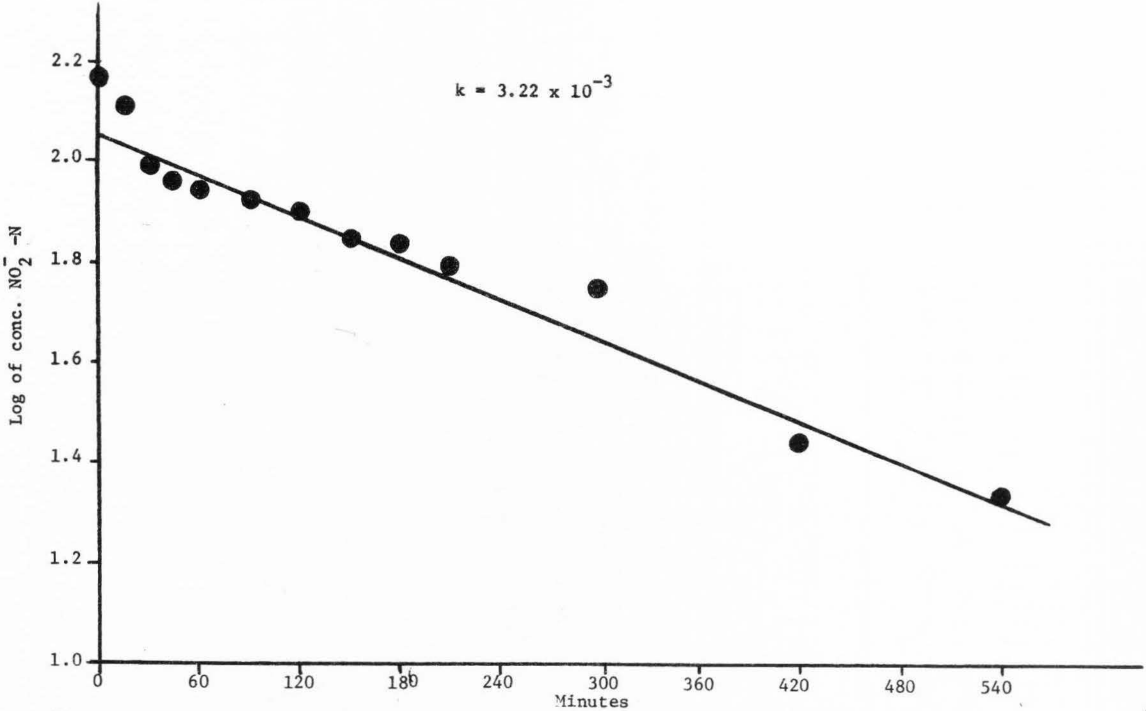


Figure 11. Logarithm of concentration of NO_2^- -N recovered from the solution system of pH 4 treated with 10 g Dayton silty clay loam and receiving 150 ppm NO_2^- -N added as $NaNO_2$.

Table 10. Amount of NO_2^- -N found at various reaction periods in phosphate buffer solution of pH 5 treated with 10 g Miami silt loam soil and receiving 150 ppm NO_2^- -N

Reaction time	NO_2^- -N found			Log of average
	Run 1	Run 2	Average	
minutes	ppm	ppm	ppm	
0	150.0	150.0	150.0	2.1761
15	127.9	128.5	128.2	2.1079
30	126.9	126.5	126.7	2.1028
45	125.8	121.7	123.7	2.0923
60	125.8	122.7	124.2	2.0941
90	118.6	120.6	119.6	2.0778
120	-	119.6	119.6	2.0778
180	115.9	116.5	116.2	2.0653
240	113.8	113.6	113.7	2.0557
270	110.6	-	110.6	2.0437
360	107.0	106.0	106.5	2.0274
540	94.6	93.0	93.8	1.9722

Table 11. Amount of NO_2^--N found at various reaction periods in phosphate buffer solution of pH 5 treated with 10 g Dayton silty clay loam and receiving 150 ppm NO_2^--N

Reaction time	NO_2^--N found Run 1	Run 2	Average	Log of average
minutes	ppm	ppm	ppm	
0			150.0	2.1761
15	128.9	129.4	129.6	2.1127
30	126.9	127.9	127.4	2.1052
45	120.6	119.6	120.1	2.0795
60	119.6	120.6	120.1	2.0795
90	118.5	-	118.5	2.0738
120	118.5	118.5	118.5	2.0738
150	117.5	118.1	117.8	2.0712
180	116.5	116.9	116.7	2.0671
210	112.3	114.4	113.3	2.0542
240	110.2	111.3	110.7	2.0440
360	109.0	109.8	109.4	2.0391
540	93.6	95.7	94.7	1.9763

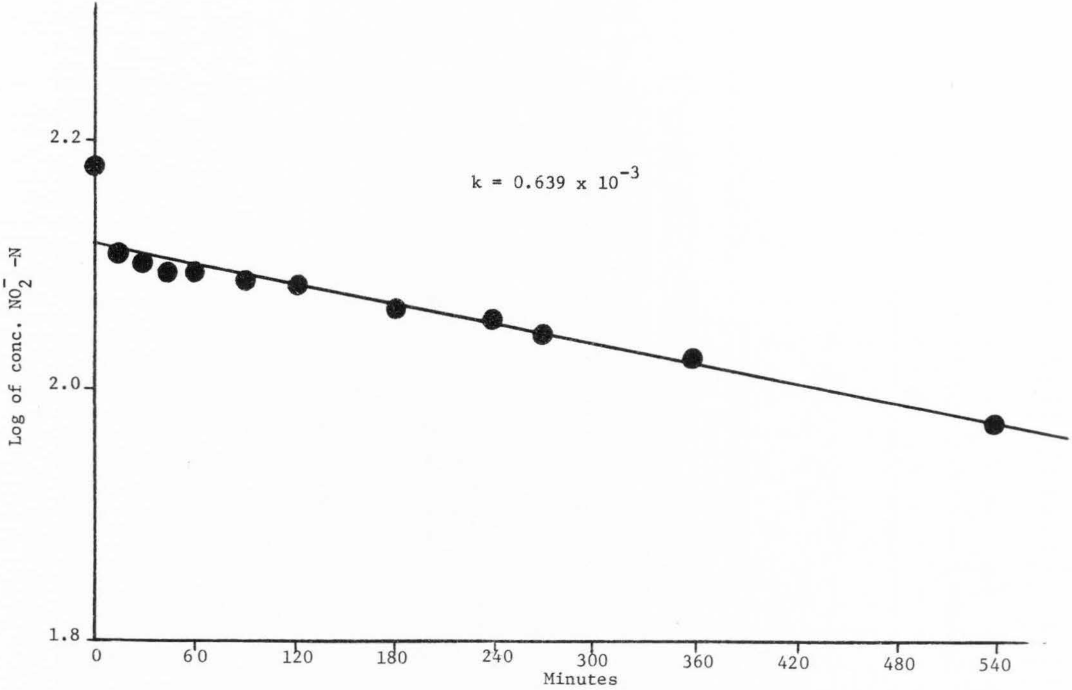


Figure 12. Logarithm of concentration of NO_2^- -N found in the solution system of pH 5 treated with 10 g Miami silt loam and receiving 150 ppm NO_2^- -N added as $NaNO_2$.

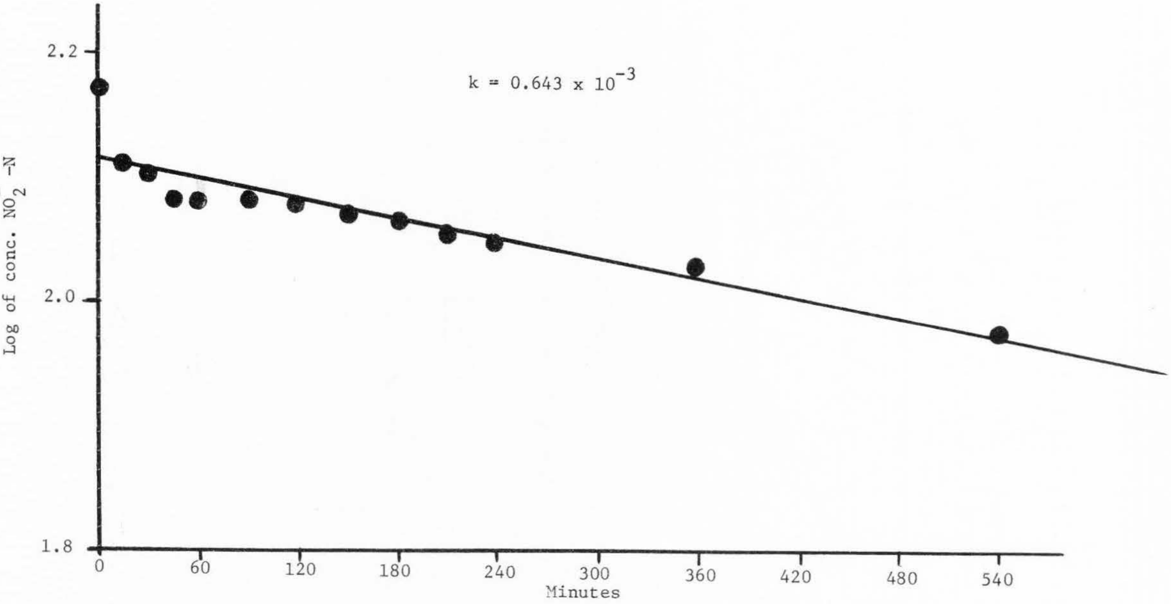


Figure 13. Logarithm of concentration of NO_2^- -N found in the solution system of pH 5 treated with 10 g Dayton silty clay loam and receiving 150 ppm NO_2^- -N added as $NaNO_2$.

Catalytic Effect on Nitrite Decomposition and Creation of Deficit

Effect of soil

Three different soils, namely Dayton silty clay loam, Miami silt loam, and Olympic silty clay loam, were added to the phosphate buffer solutions of pH 3 and 5. The NO_2^- -N (150 ppm) was added as sodium nitrite. The amount of NO_2^- -N remaining, the NO_3^- -N formed, and the oxides ($NO + NO_2$) produced and trapped in the alkaline permanganate solutions are given in Tables 12 and 13. Graphical representation of the data is given in Figures 14 and 15.

When the balance sheets showing the various nitrogen components of the systems were made up, there was no deficit noted in the pure solution systems. There were, however, varying amounts of deficit with the addition of the different soils in the solutions. The deficit seemed to increase with the lowering of the pH except in the case of Miami silt loam. The magnitudes of these deficits are presented in Figure 16.

Effect of soil fractions

The purpose of these experiments was to determine the contribution that the various components of soil may have in producing the nitrogen deficits reported in Figure 16.

Ten g of the three soils was fractionated into sand, silt, and clay. These were in turn introduced into the solution systems of pH 3 and 5 that had received 150 ppm NO_2^- -N. The nitrite nitrogen remaining, nitrate formed, and the oxides of nitrogen trapped at the end of 6 hours reaction period were determined. The deficit was then calculated for

Table 12. Amounts of various forms of nitrogen found after a 6-hour reaction period in different media of pH 3 which received 150 ppm NO_2^- -N as $NaNO_2$

Media	Forms of N			Total	Deficit
	NO_2^- -N	NO_3^- -N	NO + NO_2		
	ppm	ppm	ppm	ppm	ppm
1. Solution system	12.4	--	138.0	150.4	--
2. Solution system + 10 g Dayton sicl	4.2	31.0	91.5	126.7	23.3
3. Solution system + 10 g Miami sil	7.9	31.0	93.6	132.5	17.5
4. Solution system + 10 g Olympic sicl	7.9	36.5	73.8	118.2	31.8

Table 13. Amounts of various forms of nitrogen found after a 6-hour reaction period in different media of pH 5 which received 150 ppm NO_2^- -N as $NaNO_2$

Media	Forms of N			Total	Deficit
	NO_2^- -N	NO_3^- -N	NO + NO_2		
	ppm	ppm	ppm	ppm	ppm
1. Solution system	145.6	--	4.0	149.6	0.4
2. Solution system + 10 g Dayton sicl	108.3	11.8	11.0	131.1	18.9
3. Solution system + 10 g Miami sil	110.6	11.0	9.5	131.1	18.9
4. Solution system + 10 g Olympic sicl	108.3	11.0	8.0	127.3	22.7

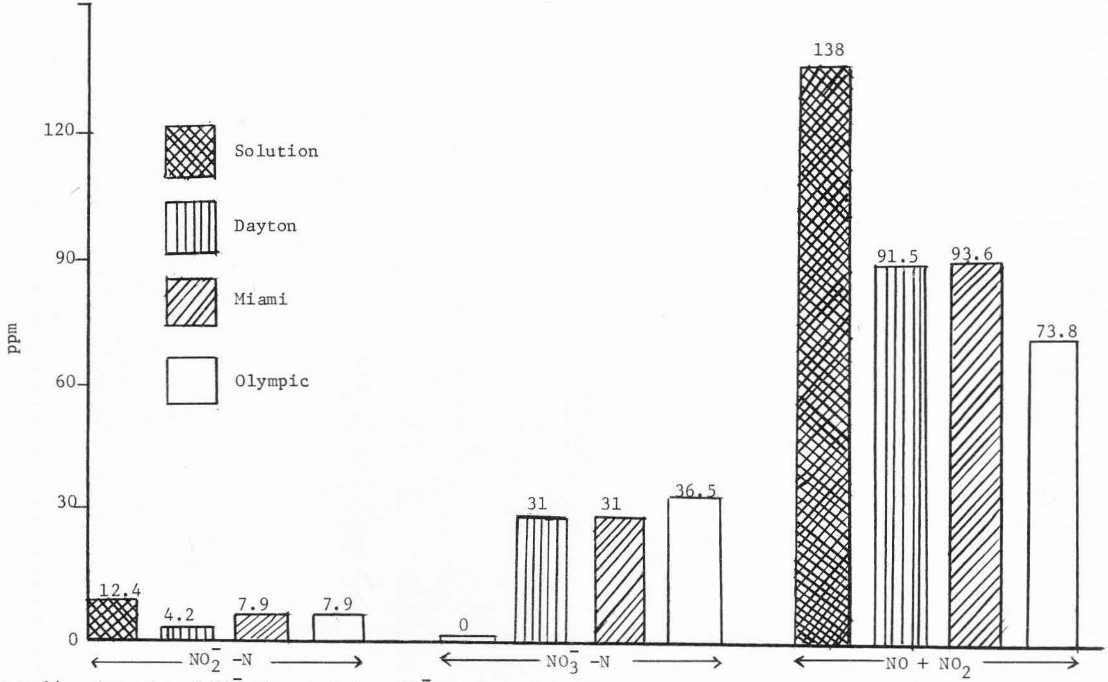


Figure 14. Amounts of NO_2^- -N remaining, NO_3^- -N formed in the reaction media, and oxides (NO + NO_2) trapped at the end of 6 hours reaction period when 150 ppm NO_2^- -N was added to solution system and the solution system treated with 10 g of three different soils whose pH was adjust to 3.

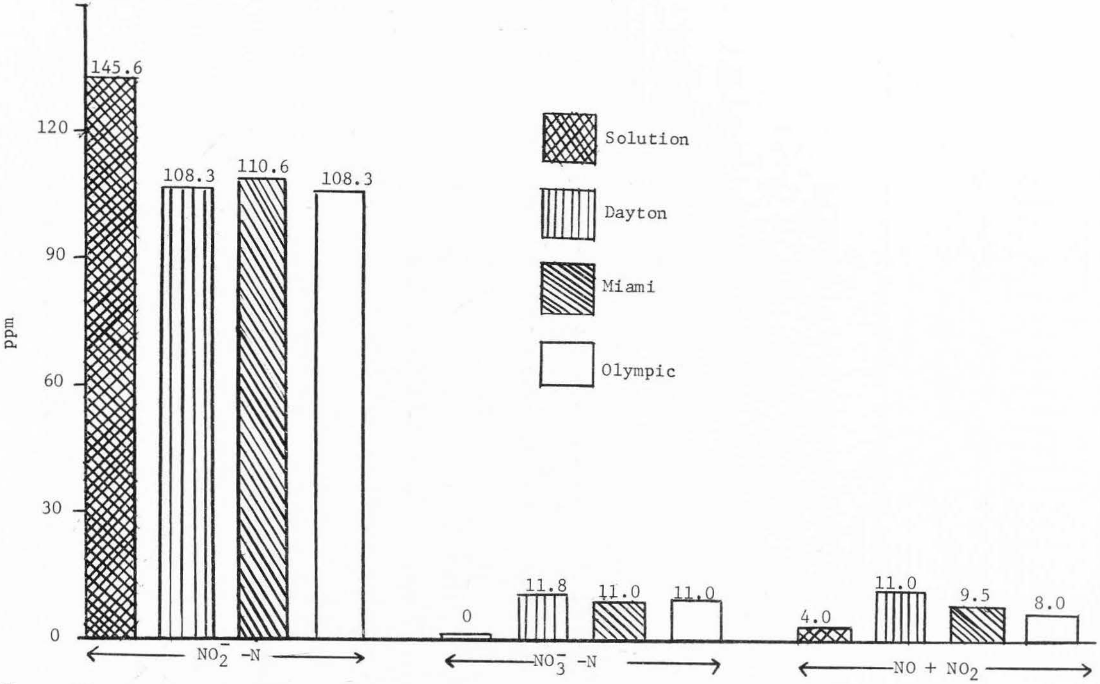


Figure 15. Amounts of NO_2^- -N remaining, NO_3^- -N formed in the reaction media, and oxides ($NO + NO_2$) trapped at the end of 6 hours reaction period when 150 ppm NO_2^- -N was added to solution system and the solution system treated with 10 g of three different soils whose pH was adjusted to 5.

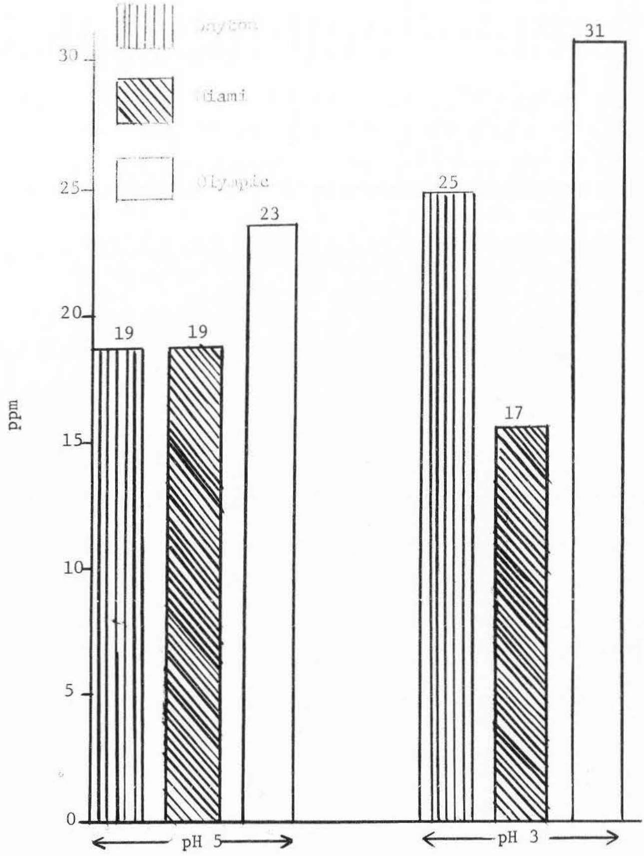


Figure 16. Amount of nitrogen not recovered (deficit) as NO_2^- -N, NO_3^- -N, or oxides at the end of 6 hours reaction period from solution system treated with 10 g of three different soils receiving 150 ppm NO_2^- -N.

each soil fraction and this is reported as percent of the whole soil (Figures 17 and 18).

Effect of activated organic complex. The amounts of NO_2^- -N remaining, nitrate formed, and the oxides produced during a 6-hour reaction period when 10 g of three different soils were added to the buffered solution systems of pH 3 and 5 receiving 150 ppm NO_2^- -N are reported in Tables 14 and 15. The deficits are presented in Figure 19.

Effect of amino acids. Three different amino acids, tyrosine, tryptophan, and cystine, were used in this study. One ml solution was added to solution systems of pH 3 and 5 treated with 10 g Miami silt loam to which 150 ppm NO_2^- -N was added. At the end of a 6-hour reaction period, the NO_2^- -N remaining, NO_3^- -N formed, and the oxides produced were determined and are given in Table 16.

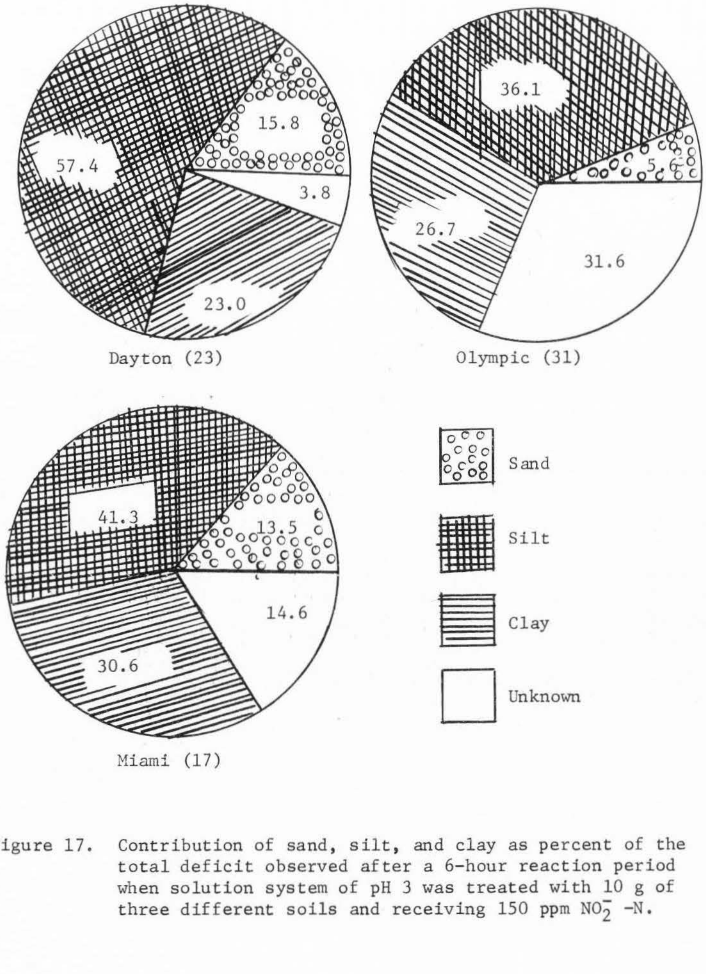


Figure 17. Contribution of sand, silt, and clay as percent of the total deficit observed after a 6-hour reaction period when solution system of pH 3 was treated with 10 g of three different soils and receiving 150 ppm NO_2^- -N.

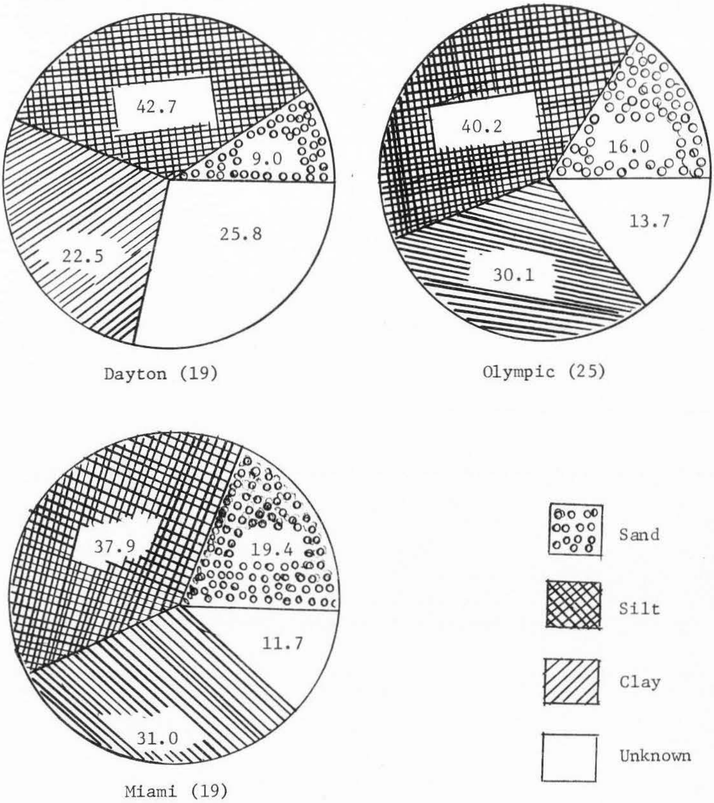


Figure 18. Contribution of sand, silt, and clay as percent of the total deficit observed after a 6-hour reaction period when solution system of pH 5 was treated with 10 g of three different soils and receiving 150 ppm NO_2^- -N.

Table 14. The amounts of NO_2^- -N remaining, NO_3^- -N formed, and the oxides ($NO + NO_2$) trapped after a 6-hour reaction period in solution system of pH 3 treated with 10 g of three different incubated soils (at 30 C and 1/3 bar moisture for 15 days) and receiving 150 ppm NO_2^- -N

Systems	Forms of N[a]			Total	Deficit
	NO_2^- -N	NO_3^- -N	$NO + NO_2$		
	ppm	ppm	ppm	ppm	ppm
1. Solution + Dayton silty clay loam	4.0	28.5	85.6	118.1	31.9
2. Solution + Miami silt loam	6.9	26.4	86.2	119.5	30.5
3. Solution + Olympic silty clay loam	6.4	34.2	75.4	116.0	34.0

[a] Average of 2 replications.

Table 15. The amounts of NO_2^- -N remaining, NO_3^- -N formed, and the oxides ($NO + NO_2$) trapped after a 6-hour reaction period in solution system of pH 5 treated with 10 g of three different incubated soils (at 30 C and 1/3 bar moisture for 15 days) and receiving 150 ppm NO_2^- -N

Systems	Forms of N[a]			Total	Deficit
	NO_2^- -N	NO_3^- -N	$NO + NO_2$		
	ppm	ppm	ppm	ppm	ppm
1. Solution + Dayton silty clay loam	101.2	10.4	9.1	120.7	29.3
2. Solution + Miami silt loam	105.3	10.1	6.9	122.3	27.7
3. Solution + Olympic silty clay loam	100.7	10.2	6.0	122.9	33.1

[a] Average of 2 replications.

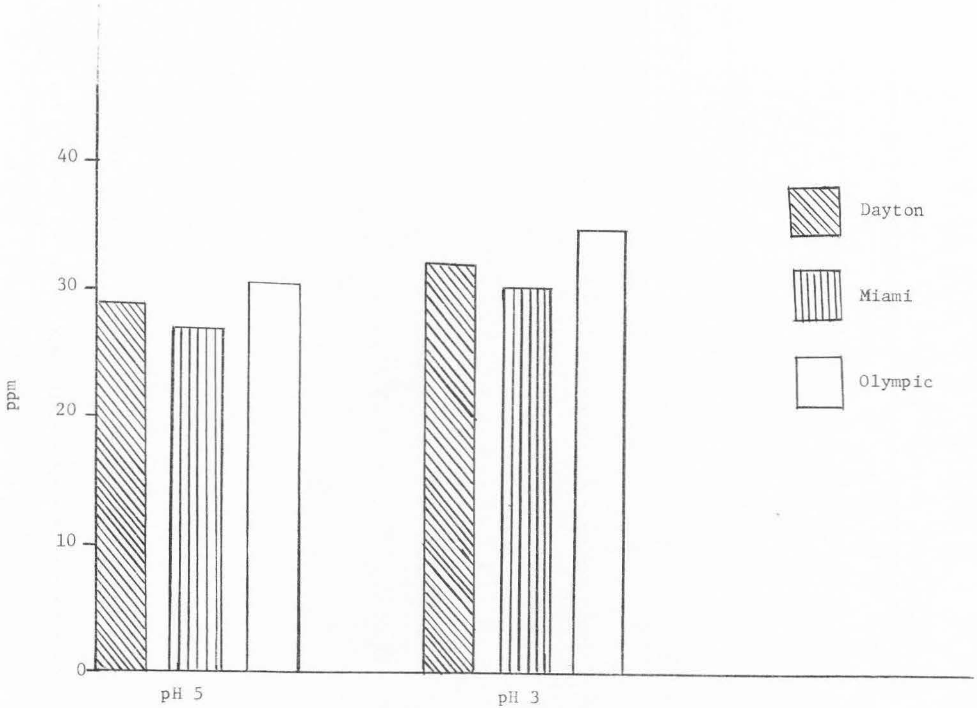


Figure 19. Amount of nitrogen not recovered (deficit) as NO_2^- -N, NO_3^- -N, or as oxides at the end of a 6-hour reaction period from solution system treated with 10 g of three different incubated soils receiving 150 ppm NO_2^- -N.

Table 16. Amounts of NO_2^- -N remaining, NO_3^- -N formed, and oxides ($NO + NO_2$) trapped in solution systems of pH 3 and 5 treated with 10 g of Miami silt loam and three different amino acids

Systems	Forms of N			Total	Deficit
	NO_2^- -N	NO_3^- -N	$NO + NO_2$		
	ppm	ppm	ppm	ppm	ppm
pH 3					
1. Solution + Miami sil + cystine	8.0	30.8	94.0	132.8	17.2
2. Solution + Miami sil + tyrosine	7.5	28.1	84.3	119.9	30.1
3. Solution + Miami sil + tryprophan	7.9	29.5	83.2	120.6	29.4
pH 5					
1. Solution + Miami sil + cystine	111.1	10.7	9.8	131.6	18.4
2. Solution + Miami sil + tyrosine	108.1	10.5	7.1	125.7	24.3
3. Solution + Miami sil + tryprophan	107.5	10.2	8.9	126.6	23.4

DISCUSSION

Kinetic Studies

Solution systems

The nitrite decomposition studied at the three pH conditions seems to follow the first order reaction law, as the logarithm of concentration versus time gives a straight line relationship.

Rate constant, k, was calculated by the equation

$$k = \frac{2.303}{t_2 - t_1} \log \frac{C_1}{C_2} \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [16]$$

Where:

k = Rate constant per minute.

t_1 and t_2 = Initial time and final time in minutes when NO_2^--N concentrations were determined.

C_1 and C_2 = Concentrations of NO_2^--N at t_1 and t_2, respectively.

The rate constants for solutions at pH 3, 4, and 5 for NO_2^--N decomposition are 6.39×10^{-3}, 1.15×10^{-3}, and 0.172×10^{-3}, respectively.

From the rate constants and also from the data given in Tables 3, 4, and 5, it is evident that the nitrite decomposition is pH dependent.

$$H^+ + NO_2^- \longrightarrow HNO_2 \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \ . \quad [17]$$

This means it is the proportion of nitrous acid present that is important rather than the amount of nitrite added. The equilibrium constant, k, for the above reaction is 6.0×10^{-4} (Sneed and Brasted, 1956). Reuss and Smith (1965) calculated the amount of nitrous acid present at pH 3, 4, and 5 to be 63, 14, and 1.6%, respectively, of the nitrite added.

The products of nitrite decomposition were not determined since the purpose of this set of experiments was to study only the kinetics of NO_2^- -N decomposition. This could be done by following the NO_2^- -N disappearance.

Solution systems treated with soil

The comparative statement of rate constants given in Table 17 indicates that nitrite decomposition is faster in the presence of soil. The catalytic effect seems to be more pronounced at pH 5 where the reaction rate was increased about four times in the presence of soil. At pH 4, however, it was about three times faster. The catalytic effect in pH 3 was not very much at all. From these observations, we can theorize that the nitrite reaction is mainly pH dependent at pH 3, but it could be considerably influenced in solution systems of pH 5 by the presence of soil. The effect of the soil in a system of pH 4 is intermediate. Similar findings were reported by Kuratti and Smith (1966), using different systems.

Table 17. The comparison of rate constants of NO_2^- -N decomposition in different media

Media	pH 3	pH 4	pH 5
1. Solution system	6.39×10^{-3}	1.15×10^{-3}	0.172×10^{-3}
2. Solution system + Dayton silty clay loam	8.13×10^{-3}	3.84×10^{-3}	0.643×10^{-3}
3. Solution system + Miami silt loam	7.89×10^{-3}	3.22×10^{-3}	0.639×10^{-3}

It is possible that the presence of the soil in the solution may actually increase the proportion of nitrous acid present at any given time by acting as a source of protons (H^+) required to generate nitrous acid.

It is surprising that the first order reaction fits so well even though it is conceivable that there is more than one reaction taking place in the system. It is just possible that there is one reaction that is more important than the others.

Catalytic effect of nitrite decomposition and creation of deficit

Effect of soils. It is clear from Figures 14 and 15 that nitrite decomposition is catalyzed in the presence of soil material at the two pH conditions studied. This was pointed out in the kinetic results discussed earlier.

Another thing that stands out very prominently is the formation of nitrate in systems treated with soil, whereas nitrate did not form in the pure solution system. It was purposely avoided by adjusting the air flow to the 100 ml per minute rate to remove all the oxides produced.

The possible explanation for such behavior in solutions treated with soil could be:

1. As pointed out in the earlier section, soil, by acting as a source of protons, may increase the proportion of nitrous acid in the system. If this is so, the 100 ml per minute air flow, which was set up to remove all the oxides from the solution system, may be inadequate to handle all the oxides produced. The oxides seem to stay in the system and oxidize to nitrate according to equations

$$2HNO_2 \rightarrow NO + NO_2 + H_2O \quad [9]$$

$$NO + 1/2\ O_2 \rightarrow NO_2 \quad [10]$$

$$2NO_2 \rightarrow N_2O_4 \quad [11]$$

$$N_2O_4 + H_2O \rightarrow NO_3^- + H^+ + HNO_2 \quad [12]$$

2. It is possible that the energy of activation for the conversion of HNO_2 to nitrate may be much less in the presence of soil.

3. There may be a totally different pathway available for the formation of nitrate in soil and in the solution system.

The nitrates produced were in higher amounts at pH 3 as compared to those at pH 5.

The oxides of nitrogen were less when the systems were treated with soil at pH 3. But in systems of pH 5, the opposite was found. The increase in the oxides of nitrogen obtained is so small that it may not be significant.

When all the forms of nitrogen recovered were added up, a deficit was noted in the systems treated with soil. This deficit seemed to be greatest at the lower pH values with the exception of the Miami silt loam.

Effect of soil fractions. The fractions from the three different soils behaved somewhat similar in general to the whole soil in their ability to catalyze nitrite decomposition and the production of nitrate and of nitrogen oxides. The deficit noted in the presence of these individual components did not add up to the total deficit found when the whole soil was used (Figures 17 and 18). This may be due to several factors or interactions.

The organic matter contribution could not be determined as a separate soil fraction because it was destroyed during the mechanical separation of sand, silt, and clay. Rashid (1966) found an increased N_2 production when 1% dry alfalfa was added to the soil prior to receiving 200 ppm NO_2^- -N. It is conceivable that the organic matter in the whole soil could have been responsible for the kind of effect Rashid (1966) noted by adding organic matter. In recent years, Stevenson and Swaby (1963), Stevenson and Kirkman (1964), and Stevenson and Swaby (1964), in their studies observed the reaction of nitrous acid (HNO_2) with humic acid, fulvic acid, lignin, lignin-building units, and polyphenols resulting in an evolution of N_2, N_2O, CO_2, and CH_3NO_2 (Methyl nitrite). In the present studies it is surmized that the N_2 or N_2O that could not be measured with flowing gas system could have been the additional deficits noted when the whole soil was used.

Many of the soluble chemical species were removed during mechanical separation of the soil. It is possible that among these chemical species there might have been iron, copper, or manganese. These might have been in the reduced forms which are actually favored at the low pH conditions. These transition metals, in the whole soil, might have stimulated conversion of the oxides of nitrogen to N_2 in accordance with the equation:

$$4\ Cu^{+} + 2NO + 4H^{+} \rightarrow 4\ Cu^{+2} + N_2 + 2H_2O \quad . \quad . \quad . \quad . \qquad [18]$$

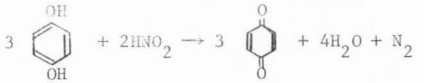
Instead of an additive effect of all these factors, there might be an interaction. An interaction effect is noticed in many crop response trials with regard to fertilizers. It is hard to speculate or extrapolate these ideas here because of the totally different situation.

Effect of activated organic complex. A comparison of deficits presented in Figures 16 and 19 and Table 18 shows that there is a greater deficit when incubated soil is introduced into the buffered solution system than when the soil (unincubated) was used.

More than circumstantial evidence is available today to show that the deficit is due to the production of N_2 gas (Reuss, 1963; Patil, 1963; Rashid, 1966; Mahendrappa et al., 1969). It has also been shown that N_2 is produced due to reactions of nitrous acid with humic acid, fulvic acid, aminoacids, lignin, and polyphenols (Stevenson and Swaby, 1963; Stevenson and Kirkman, 1964; Stevenson and Swaby, 1964).

When the soil is incubated for 15 days under optimum temperature and moisture conditions, there will be an initial microbial population explosion. The microbial population utilizes the available carbon as the source of energy and nitrogen for body building. More complex substances eventually are released in the form or forms like humic acid, fulvic acid, and polyphenols, which are capable of producing N_2 or complexing nitrite (Fuhr and Bremner, 1964).

The production of N_2 due to a reaction between nitrous acid and polyphenolic compounds is cited by Rashid (1966).

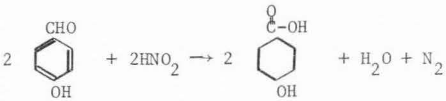


. . . . . . [19]

Hydroquinone Quinone

In this reaction hydroquinone is oxidized to quinone and nitrite is reduced to N_2.

Vanillin may react with nitrous acid to produce N_2.



. . . . . [20]

The degree of reactivity of the various phenolic compounds depends on the chemical nature and position of substitution.

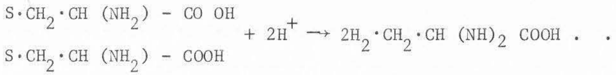
Dubach and Mehta (1963) have reported the presence of over 30 phenolic compounds in humic and fulvic acids. Coffin and DeLong (1960) have reported that 30% of the humic fraction from the B horizon of podzol was phenols and they were able to isolate 19 different phenolic substances.

Table 18. The comparison of deficits noted at the end of a 6-hour reaction period in the solution systems treated with 10 g incubated and non-incubated soils receiving 150 ppm NO_2^- -N

System	Incubated	Non-incubated
	ppm	ppm
pH 3		
Solution + Dayton silty clay loam	37.9	31.0
Solution + Miami silt loam	30.5	17.0
Solution + Olympic silty clay loam	35.0	25.0
pH 5		
Solution + Dayton silty clay loam	29.3	19.0
Solution + Miami silt loam	27.7	19.0
Solution + Olympic silty clay loam	29.0	23.0

Effect of amino acids. It is important and interesting to note that greater deficits are noticed when the systems received tyrosine and tryptophan, while the presence of cystine did not make much difference.

Cystine is reduced to cysteine.



$$\begin{array}{l} S\cdot CH_2\cdot CH\ (NH_2)\ -\ CO\ OH \\ S\cdot CH_2\cdot CH\ (NH_2)\ -\ COOH \end{array} + 2H^+ \rightarrow 2H_2\cdot CH_2\cdot CH\ (NH)_2\ COOH\ .\ . \qquad [21]$$

Cysteine is active in oxidation-reduction reactions (Rashid, 1966).

It is possible in the time period with which we were concerned, the cystine is not converted to cysteine and, as such, the presence of cystine does not make any difference in nitrite decomposition.

Tryptophan and tyrosine have produced greater deficits. This may very well be due to the production of N_2 according to a Van Slyke-type reaction.

$$RNH_2 + HNO_2 \longrightarrow ROH + N_2 + H_2O \quad . \; . \; . \; . \; . \; . \; . \; . \; . \qquad [22]$$

Barrit (1933) has reported loss of N_2 as a result of the interaction of nitrite and glycine at pH 5.8. Wilson (1943) and Gerretsen and DeHoop (1957) have strongly favored nitrite-amino acid reactions. Patil (1963) has suggested the possibility of reactions of amino acids with nitrite. The study reported here and others are in conflict with those reported by Allison and Sterling (1948), Allison and Doetsch (1951), Allison, Doetsch, and Sterling (1952), Jones (1951), and Smith and Clark (1960).

SUMMARY AND CONCLUSIONS

Experiments were conducted to study the kinetics of nitrite decomposition in acid solutions of pH 3, 4, and 5 with and without 10 g soil. In the kinetic experiments, only the nitrite remaining was analyzed and other forms of nitrogen were not determined. In another study, the solution systems of pH 3 and 5 were treated with soil, soil components, incubated soil, and amino acids. The NO_2^--N (150 ppm) was allowed to react for 6 hours. The various forms of nitrogen--NO_2^--N remaining, NO_3^--N formed, and the nitrogen oxides ($NO + NO_2$)--produced and trapped in an alkaline permanganate system were analyzed and the deficits calculated.

From the results of these experiments the following conclusions are made.

1. Nitrite decomposition in all the systems and for the duration of time studied here seems to be a first order rate reaction.

2. The rate constant (k) depends on the proportion of nitrous acid present (HNO_2) and not on the total nitrite; in other words, it is pH dependent.

3. The rate of nitrite decomposition increased in the presence of added soil. There is a greater increase in rate constant in the presence of soil when compared to pure solution at higher pH.

4. When soil or its components were added to the buffered solution system, all the added nitrogen was not recovered. This deficit increased with the pH of the buffered solution.

5. There were greater deficits of added nitrogen when incubated soils rather than nonincubated soils were added to the solutions.

6. The addition of amino acids to the buffered solutions increased the nitrogen deficit over control. It was theorized that the amino acids reacted with nitrous acid to produce N_2.

IMPROVEMENTS AND FUTURE LINE OF WORK

This is the first effort made to study kinetics of nitrite nitrogen decomposed in relation to soil systems. The author is aware of the nature of temperature dependency of the reaction. The reaction should be studied in constant temperature conditions to measure kinetic changes.

In the study of the effect of soil components, instead of using sand, silt, and clay coming from 10 g soil, it would be better to use 10 g of each fraction.

An effort should be made to establish the correlation between the nitrogen deficits noted in the presence of the different soils with various factors like organic matter, heavy metals, surface area, and mineralogical make-up.

A better method of analysis of the oxides of nitrogen needs to be developed to work out the kinetics of oxides of nitrogen in this study.

A system, where N_2 liberated and oxides ($NO + NO_2$) determination is possible, needs to be worked out to describe completely the nitrite transformation.

Attention should be given to the identification of intermediate products. This would possibly lead to a positive presentation of the mechanism and a chain of reactions involved in nitrite transformation in acid media.

The reaction needs to be run for longer duration in order to study whether the order of the reaction changes.

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APPENDIX

Table 19. The amounts of various forms of nitrogen recovered from solution systems of pH 3 treated with different fractions of three soils and receiving 150 ppm NO_2^- -N at the end of a 6-hour reaction period

System	Forms of N			Total	Deficit
	NO_2^- -N	NO_3^- -N	NO + NO_2		
	ppm	ppm	ppm	ppm	ppm
Dayton silty clay loam					
Solution + sand	12.0	28.0	106.6	146.6	3.4
Solution + silt	9.5	29.0	98.3	136.8	13.2
Solution + clay	10.1	28.5	106.0	144.6	5.4
Miami silt loam					
Solution + sand	13.1	29.3	105.3	147.7	2.3
Solution + silt	10.0	26.5	104.0	140.5	9.5
Solution + clay	12.5	28.2	104.1	144.8	5.2
Olympic silty clay loam					
Solution + sand	14.0	30.2	104.1	148.3	1.7
Solution + silt	11.9	33.2	93.7	138.8	11.2
Solution + clay	11.3	31.0	99.4	141.7	8.3

Nitrate Nitrogen Determination

Extraction of soil for nitrite and nitrate

1. Weigh out 10 or 20 g soil into 250 ml erlenmeyer flask.
2. Add 100 ml $Ca(OH)_2$ solution.
3. Place flasks on mechanical shaker and shake for 5 minutes.
4. Let stand for 20 minutes.
5. Filter into numbered 125 ml erlenmeyer flasks, using small funnels. Mix the last portion of soil and extract by swirling the flask quickly and pour the soil and liquid onto the filter paper. Let stand until the extract has stopped dripping from the funnels.
6. Retain filtrate for NO_2 and NO_3 determination.

Reagents:

Phenoldisulfonic acid ($C_6H_cOH(HSO_3)_2$).--Dissolve 200 g pure white phenol in 1200 ml concentrated H_2SO_4. Add 600 ml fuming sulfuric acid (15% free SO_3); stir well, heat for 2 hours on hot water bath.

Acetic acid solution.--One part glacial acetic acid and 3 parts distilled water.

Ammonium sulfamate.--0.5% solution in water. Dissolve 5 g ammonium sulfamate in distilled water and dilute to 1 liter.

Ammonium hydroxide.--One part concentrated NH_4OH to one part distilled water.

Standard KNO_3 solution.

Stock.--Dissolve 0.722 g anhydrous KNO_3 and dilute to 1 liter with distilled water. Contains 0.1 mg N/ml.

Standard.--Evaporate 50 ml stock KNO_3 solution to dryness; dissolve residue by rubbing with 2 ml phenoldisulfonic acid, dilute to 500 ml with distilled water. 1 ml = 0.01 mg N.

For preparation of standard curve use 0, 0.5, 1.0, 2.0, 3.5, 8.0, 10.0, 15.0, 20.0 ml of standard KNO_3 solution (1 ml = 0.01 mg N).

Treat and evaporate as outlined in "Procedure."

Procedure

1. (a) Pipette 5 ml of the filtered $Ca(OH)_2$ extract into 100 ml tall beakers.

   (b) Pipette into extra beaker 5 ml of the $Ca(OH)_2$ extracting solution.

2. Add 5 drops of acetic acid; swirl to mix.

3. Add 0.5 ml of ammonium sulfamate solution; swirl to mix. (Add 1 ml to those samples having high NO_2^- content.)

4. Place on hot plate.

5. When warmed to 60 to 80 C, add 5 drops of 10% NaOH solution; swirl to mix.

6. Let contents evaporate to dryness.

7. Remove beakers from hot plate.

8. Add (from pipette) 2 ml of phenoldisulfonic acid to the beakers in such a way that the residue will be moistened quickly and thoroughly.

9. Rub sides of beakers with a stirring rod to bring the phenoldisulfonic acid in contact with any of the nitrates that may have spattered onto the wall on drying.

10. Let stand about 20 minutes (or longer) or until the residue is dissolved.

11. Add 34 ml of distilled water to beaker.

12. Add NH_4OH solution (1:1) to beaker until a permanent yellow color remains (14 ml). Perform this step under the fume hood.

13. Let cool. Make further dilution if color is too intense. Record final volume.

14. Mix contents by stirring with stirring rod or swirling carefully and read on electrophotometer after balancing the bridge and setting null adjustment with a blank ($Ca(OH)_2$ solution).

Nitrite Nitrogen Determination

Reagents:

Sulfanilamide.--0.2% solution in water. Dissolve 4 g p-aminobenzenesulfanilamide in distilled water in 2000 ml volumetric flask. Dilute to mark, then transfer to storage bottle. Keep tightly stoppered in refrigerator. Prepare a new solution one or two days before required, or at least every month.

Hydrochloric acid.--1 to 1 dilution of the concentrated acid with distilled water.

Coupling reagent.--Dissolve 1 g N-(1-Naphthyl)-Ethylenediamine dihydrochloride in 1 liter distilled water. Store in dark bottle.

Sodium nitrite standard

1. Employing assayed $NaNO_2$ prepare an accurately known solution containing about 0.005 mg of N per ml (Solution A).
2. Dilute 50 ml of above solution to 100 ml in volumetric flask (Solution B).
3. With a volumetric pipette measure 0.5, 1.0, 1.5, 1.8 ml of A, and 1, 2, 4 ml of B into 50 ml volumetric flasks.
4. Add 20 ml of $Ca(OH)_2$ extracting solution (filtered) to each.
5. Treat as outline in steps 2 to 7 of "Procedure."
6. Prepare graph.
7. Keep standard sodium nitrite solution tightly stoppered, in refrigerator.

Procedure:

1. (a) Pipette 20 ml of the filtered $Ca(OH)_2$ soil extract into 50 ml volumetric flasks. Use smaller aliquot (2 to 5 ml) if NO_2^- content of sample is high. Make up to 20 ml with $Ca(OH)_2$ solution.
   (b) Pipette 20 ml of the $Ca(OH)_2$ extracting solution into an extra 50 ml volumetric flask.
   (c) Fill burettes with 50% HCl, 0.2% sulfanilamide and coupling reagent.

2. From a burette add 1 ml of 50% HCl.

3. From a burette add 5 ml of 0.2% sulfanilamide solution.

4. Wait 3 minutes.

   Note: Steps 2 and 3 can be performed on 10 samples within the 3 minutes as follows: Add the HCl and the sulfanilamide to the first sample, then set the time clock for 3 minutes.

Then quickly add the HCl and sulfanilamide to the other 9 samples in succession.

5. At the end of the 3-minute interval, begin adding 1 ml of the coupling reagent to each sample, timing the additions so that each sample will have remained at 3 minutes after the addition of sulfanilamide.

6. Make up to volume with distilled water.

7. If the color is too dark, take smaller quantities of aliquot and develop color.

8. Read on electrophotometer, using the blank to set the null point. Use 525-B filter or read at 550 mμ wavelength.

Sodium Nitrite

Tests for identity:

Dry about 1 g sodium nitrite to constant weight over sulfuric acid, weigh accurately in a stoppered weighing-bottle, dissolve the salt in a volumetric flask with sufficient distilled water to make 100 ml and add 10 ml of this solution, from a pipette, to a mixture of 40 ml of tenth-normal potassium permanganate, 100 ml of distilled water, and 5 ml of sulfuric acid. When adding the sodium nitrite solution, immerse the tip of the pipette beneath the surface of the permanganate mixture. Warm the liquid to 40 C, allow it to stand for 5 minutes, and add 25 ml of tenth-normal oxalic acid or sodium oxalate. Heat the mixture to about 80 C and titrate with tenth-normal potassium permanganate. Each ml of tenth-normal potassium permanganate is equivalent to 0.003450 g $NaNO_2$.

Storage:

Preserve sodium nitrite in air-tight containers in refrigerator.

Analysis of Alkaline Permanganate Traps

for Oxides of Nitrogen

The oxides of nitrogen that dissolve in the traps will be oxidized to nitrate by the $KMnO_4$. Thus this procedure is designed to destroy the color of the $KMnO_4$ and permit colorimetric nitrate determination.

Reagents:

1. Trapping solution of alkaline permanganate 0.2 $\underline{M}$ $KMnO_4$ in 2 $\underline{N}$ KOH

   Dissolve 112.2 g reagent grade KOH in about 800 ml of distilled water. Add 31.7 g $KMnO_4$, warm and stir till solution is complete. Transfer to 1 liter volumetric flask and make up to volume. Store in brown bottle away from direct light.

2. Oxalate sulfuric acid solution 0.6 $\underline{M}$ $Na_2C_2O_4$ in 7 $\underline{N}$ H_2SO_4

   Place about 1200 ml distilled water in a 2 liter beaker. Add carefully 390 ml concentrated H_2SO_4. Weigh out and add 160.6 g $Na_2C_2O_4$. Stir until oxalate is completely dissolved, transfer to 2 liter volumetric flask and make up to volume. Store in brown plastic bottle; the oxalate will attack glass.

3. 5 $\underline{N}$ NaOH

   Weigh out 200 g reagent grade NaOH and dissolve in about 800 ml distilled water. Allow to cool; transfer to 1 liter volumetric flask and make to volume.

Procedure:

Wash the entire contents (20 ml) of the trap into a 125 ml beaker. Avoid using excess water. A blank of unused trap solution should be included. Add 20 ml of reagent 2 (acid and oxalate). In a few minutes the reaction should be complete and the material should be colorless. Warming on the hot plate will facilitate reaction. Incomplete reaction may be the result of insufficient acidity or insufficient oxalate.

After slight warming add 30 ml of reagent 3 (5 $\underline{N}$ NaOH) to each beaker. Precipitation will occur at this point and the base must be in sufficient quantities to make the solution alkaline. Upon standing a slight brown scum of MnO_2 should form on the surface. Failure of this to form may be due to insufficient base.

The precipitate should be digested with gentle warming for at least 0.5 hour. The material is then filtered into 250 ml volumetric flask. This filtration must be quantitative and the residue washed several times with small portions of warm water. The volume is then made to 250 ml and an aliquot (5 ml) taken for the standard phenoldisulphonic acid nitrate determination. The addition of ammonium sulfamate to destroy nitrites is unnecessary

The blank carried through the entire procedure is used to set the zero on the spectrophotometer.

Standard curve and calculations:

KNO_3 standard--200 μg N/ml

1.44 g KNO_3 made up to 1 liter volume.

Add 0, 1, 2, 3, . . . 8 ml of KNO_3 standard to a series of beakers containing 5 ml $KMnO_4$ trap solution. Carry through the procedure as outlined taking a 5 ml aliquot for nitrate determination. Carry through the standard nitrate procedure making up the colored solution to 50 ml for reading on the spectrophotometer. There will now be 0, 10, 20, . . . 80 μg N per 50 ml. Read on spectrophotometer and plot curve.

Determine from the curve the ratio μg N/optical density (inverse of slope if optical density is plotted on the y axis and μg on the x).

Ratio x opt. den. x 50 = ppm N

Ratio determined by Reuss (1963) was 128.

Notes

Do not use excess water to wash the trap solution into the beaker or for washing the precipitate as the total volume must be less than 1000 ml. The destruction of $KMnO_4$ by $C_2O_4^{=}$ requires high acidity, and incomplete reaction at this point is often the result of insufficient acidity. If the traps are to be exposed to large amounts of CO_2 evolution, it may be necessary to use 4 N KOH and/or 10 ml trapping solution per trap. If more trapping solution is used the amounts or the strengths of reagents No. 2 and 3 may be increased accordingly. If 4 N KOH is used in the trap solution, the acidity of reagent 2 should be increased to 9 N.

After the residue from the filtration is exposed to the air, it should assume a dark brown color. If it remains gray add a ml or two of NaOH to the filtrate. If any precipitate occurs, refilter.

VITA

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