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NITRATE REDUCTION IN THE VICINITY OF TILE DRAINS

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F I N A L R E P O R T

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ABSTRACT

NITRATE REDUCTION IN THE VICINITY OF TILE DRAINS

The fate of nitrates as they travel through a long porous column at a slow rate was observed in this study with temperature and substrate materials variable.

During a one month period of flow with pore velocities averaging up to 21 centimeters per day, losses as high as 89 percent were found for a methanol treatment at 24°C while for 13°C losses were reduced to 46 percent. A sawdust substrate material resulted in very little reduction of nitrate concentrations at 24°C and actual increases (presumably from mineralization) at 13°C.

Since methanol was found to be an effective means of removing nitrate from a slowly moving stream of water at temperatures as low as 13°C, it will be used as a standard in future field studies to evaluate less expensive substrate materials.

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NITRATE REDUCTION IN THE VICINITY OF TILE DRAINS

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I. INTRODUCTION

The threat of nitrate as a major pollutant of water resources in Illinois deserves serious consideration since agricultural fertilization has increased at a rapid rate. The extensive flat to moderately sloping and generally well-drained soils of northern and central Illinois, plus moderate amounts of precipitation, tend to increase the potential loss of applied fertilizers due to surface runoff and leaching.

Leaching of nitrogen can be expected to increase nitrate concentrations in the effluent of tile drains. Dawes, et al. (9) found that the peak nitrogen load of many of our streams is coincident with periods of high agricultural runoff. This may be caused primarily by leaching rather than fertilizer losses due to surface runoff.

Excessive nitrate concentrations can be responsible for two serious types of problems. Where nitrogen is a limiting nutrient factor nitrate inputs to surface waters can cause eutrophication to proceed rapidly, severely reducing the quality of the water for use by man and wildlife, as well as destroying the aesthetic beauty of lakes and waterways. High nitrate concentrations in drinking water can be fatal to children due to methemoglobinemia, which results from the conversion of nitrate to toxic nitrite in the digestive tract.

It appears likely that state and/or federal regulations to control nitrate concentrations due to agricultural runoff will be enacted. This will force farmers to utilize methods of

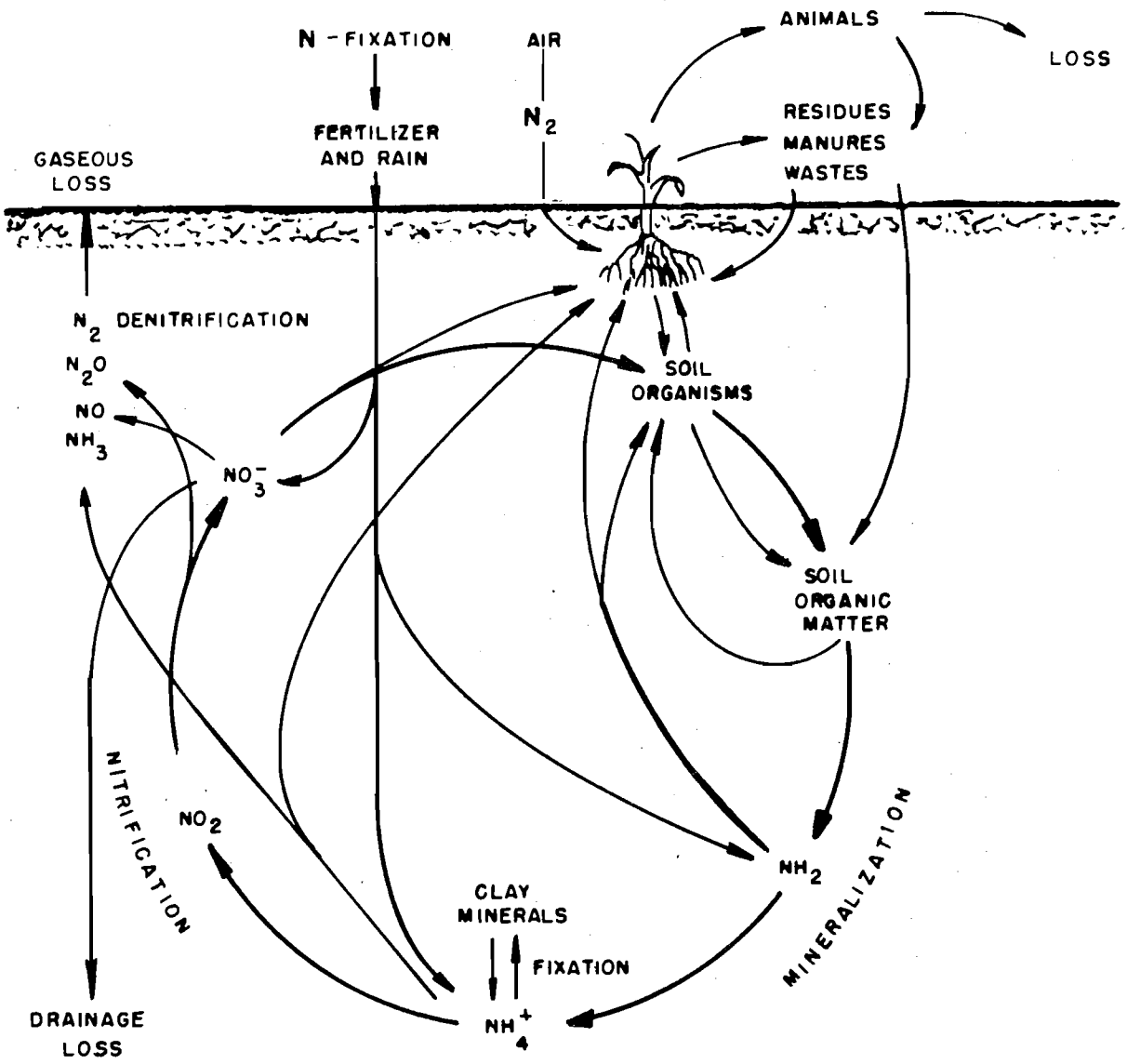
nitrogen control or to reduce rates of nitrogen fertilizer applications.

Since the major loss of nitrogen in the soil occurs from leaching in flat and gently sloping soils, denitrification is a possible means of reducing nitrogen in field tile drainage waters. Denitrification plays an important part in the nitrogen cycle (see Figure 1) by converting nitrate to nitrogen gas through microbial action. By improving the environment for the denitrifying bacteria, significant decreases of nitrate in ground water may result.

Recent field studies have been conducted in California to evaluate denitrification as a means of removing nitrogen from water en route to tile drains (24). Evidence that denitrification occurred on a large scale was not conclusive. The relatively humid climate and acid soils in Illinois, compared to California, present a unique environment for denitrification in the field.

FIGURE I

THE NITROGEN CYCLE



II. LITERATURE REVIEW

A. Denitrification in General

Denitrification is primarily a dissimilatory process in which facultative anaerobes reduce nitrate and nitrite to volatile gases, usually nitrous oxide and/or molecular nitrogen. Denitrifying bacteria grow under anaerobic or partially anaerobic conditions, provided a suitable oxidizable substrate, usually organic matter, is present in the soil. Denitrification is commonly considered a two or three step process whereby nitrate is first reduced to nitrite and then to nitrous oxide and nitrogen gas.

Although many common facultative bacteria are capable of denitrification, their ability to do so varies. Some species can reduce both nitrate and nitrite to molecular nitrogen; some can only reduce nitrate to nitrite; while others reduce nitrites to molecular nitrogen (15). Since heterogenous populations of these organisms are found in soils, denitrification can be basically considered a two step process. This process is represented stoichiometrically below, using methanol as a soluble carbon source (12). Note that nitrate is first reduced to nitrite and then to molecular nitrogen.

Step 1: $3NO_3^- + CH_3OH = 3NO_2^- + CO_2 + 2H_2O$

Step 2: $2NO_2^- + CH_3OH = N_2 + CO_2 + H_2O + 2OH^-$

Overall: $6NO_3^- + 5CH_3OH = 3N_2 + 5CO_2 + 7H_2O + 6OH^-$

The term denitrification has also been used to describe processes such as the assimilitory reduction of nitrate by higher

plants and organisms to satisfy their needs for organic nitrogen (3). Here it will refer only to the microbial process leading to the gaseous loss of nitrogen.

B. Factors Affecting Denitrification

Much of present knowledge about the denitrification process has been discovered in the laboratory due to the many experimental difficulties associated with a denitrification field study. The physical factors suitable for a denitrifying environment are complex and interrelated. Control of these factors in addition to identifying and measuring the inputs and products of denitrification has discouraged field investigations. However, the need for such research has become increasingly great.

1. Soil Oxygen

One of the primary conditions necessary for denitrification is a limited oxygen supply. Denitrification can be extensive in saturated or partially saturated soils where gaseous interchange with the atmosphere is low. The facultative denitrifers substitute nitrate for oxygen as a hydrogen acceptor as the oxygen in the soil is depleted in a reduced soil-water environment.

The effect of low oxygen levels on denitrification has been subject to extensive laboratory research. Broadbent and Stojanovic (6) found that the degree of denitrification is inversely related to the partial pressure of oxygen in the soil. Bremner and Shaw (4) showed that when waterlogged soils containing nitrate and glucose were mixed with oxygen in stoppered flasks, denitrification was completely inhibited. They also

found denitrification was reduced by 68% from incubated soil suspensions which were not aerated to those that were aerated continuously.

2. Soil Moisture

Soil moisture content has an indirect effect on denitrification as high moisture contents inhibit oxygen diffusion into the soil. Research conducted by Meek, et al. (16) indicated nitrate-nitrogen became unstable when the moisture was raised above 41% in a silty clay loam. Bremner and Shaw (4) stated that even when other conditions were very favorable for denitrification, little loss of nitrogen occurred if the moisture content was less than 60% of the water holding capacity of the soil. Mahendrappa and Smith (14) found that denitrification proceeded at faster rates at water contents near saturation.

Denitrification apparently increases with increasing moisture content beyond 100 percent moisture holding capacity by weight. Bremner and Shaw (4) found that by adding water to soil to form a soil slurry with a 450 percent moisture content, there resulted an increased loss of nitrogen. Studies by Nommik (18) and Jansson and Clark (11) confirm this effect.

In discussing the effect of soil moisture content or limited oxygen supply on denitrification, the microscopic as well as the macroscopic denitrifying environment must be considered. Broadbent and Stojanovic (6) and Allison, et al. (1), in working with aerated soils, both observed some degree of denitrification. In contrast, other researchers report little denitrification in well aerated systems. Wijler and Delwiche (25)

rate at which the organic matter in the soil can be decomposed and soluble carbon supplied to the denitrifying bacteria directly influences the rate of denitrification.

Many soils may contain sufficient organic matter to sustain high rates of denitrification without applying additional carbon. Jones (13) found that a loam soil contained sufficient energy material to support almost an identical denitrification rate as that induced by a controlled amount of sucrose added to the same soil.

Cellulose, an organic material which decomposes rapidly, generally results in higher denitrification rates than lignin, which decomposes much more slowly. Bremner and Shaw (4) found that 800 mg of wheat straw added to a soil sample gave the same rate of denitrification after twelve days as 5 mg of sucrose added to the same soil. Corey, et al. (8) showed that increasing sucrose from 50 to 200 ppm in a sandy soil increased the denitrification rate by 46%.

4. Temperature

Denitrifying bacteria are sensitive to temperature changes. Bremner and Shaw's (4) studies indicate denitrification is negligible below 2°C and increases rapidly as the temperature is raised to 25°C. The optimum temperature was found to be approximately 60°C. Nommik (18) found a comparable optimum temperature for denitrification of about 65°C.

Broadbent and Clark (5) state that the relative proportions of nitrous oxide and molecular nitrogen in the gaseous products of denitrification vary with temperature. Nitrous oxide

found that oxygen tensions of 2.0 cm of mercury suppressed denitrification rates to about one-twentieth of the anaerobic denitrification found in the same soil.

It is possible that these conflicting reports may be due to the development of microscopic volumes of anaerobic environment within systems thought to be well aerated. If a readily decomposable substrate is present in a merium suitable for denitrification, the biological oxygen demand may increase to a point where the rate of oxygen diffusion into the system is insufficient and scattered microzones may undergo considerable denitrification. This may occur in soils thought to be well aerated. This theory is substantiated by Nommik (18), who found that denitrification increased with decreasing size of soil aggregates. Presumably, this may be due to the formation of anaerobic zones within the small pores spaces, while larger pores would yet contain air voids.

From the previous discussion it is obvious that the rate of oxygen diffusion into and through the soil voids is more important than the presence of oxygen in the soil in predicting the extent of denitrification under field conditions (5).

3. Oxidizable Materials

The nature and content of an oxidizable substrate in the soil can have a marked effect on denitrification. An energy source is needed for the denitrification process to proceed since the free energy change in reducing nitrate to molecular nitrogen is positive. The organic matter in the soil serves as an energy source by supplying carbon as a hydrogen donor. The

is predominant at lower temperatures and molecular gas at higher temperatures. This suggests a high temperature coefficient for the reduction of nitrous oxide to molecular nitrogen.

5. pH

The rate of denitrification has been found to be very slow in acid soils and rapid in soils of high pH. Bremner and Shaw (4) showed that an increase in pH from 4.8 to 5.1 increased the denitrification rate from 15% to 80% while an increase in pH from 5.1 to 8.0 only increased the denitrification rate by 5%. To support these findings, Wijler and Delwiche (25) found little change in denitrification rates above pH 6.0.

Wijler and Delwiche (25) also found that nitrous oxide was predominant in the denitrification gas below pH 7 and further reduction of nitrous oxide to nitrogen gas was not significant until pH 7 had been exceeded.

6. Flow Velocity

Loss of added nitrate to experimental soil samples has been found to be lower when the soil water is moving than under static conditions. Although most laboratory denitrification studies have been conducted under static conditions, in actuality the soil water is always flowing in the field, especially near tile drains. Corey, et al. (8) found that immobilization and denitrification by microbial activity is directly related to the velocity of flow. At a velocity of 1.32 cm/hr through a soil column no added nitrate was lost, but at 0.11 cm/hr 21% of the added nitrate was either immobilized or denitrified, with no distinction made as to the fate of the nitrate.

7. Redox Potential

Redox potential is defined as the equivalent free energy per mole of electrons associated with a given reduction (22). Since many oxidation-reduction couples are present in the field, redox measurements are inadequate in characterizing a particular redox reaction such as nitrate reduction. Redox potentials can be valuable in the laboratory under conditions which allow the researcher to control a specific reaction.

Redox potential as a denitrification environmental parameter can be important in submerged or saturated soils where sufficient control of variables affecting denitrification is possible. Patrick and Mahapatra (20) cite a number of advantages of measuring redox potentials in saturated soils. The range of potential is relatively high in saturated soils (1000 mv) as compared to well-drained soils (300 mv). The higher concentrations of reduced elements under waterlogged soils contribute to better poising and thus to better reproducibility of redox potential. Also, redox potential is a convenient means of determining the oxygen status in submerged soils. Conventional methods of measuring oxygen content and diffusion rates cannot be used in waterlogged soils.

Results of several oxidation-reduction studies (19, 21) are in general agreement that oxygen disappears and nitrate reduction begins at a redox potential of approximately 320 millivolts at pH 7.

C. Field Denitrification Studies

As stated earlier, field studies on denitrification have been limited due to experimental difficulty in identifying and monitoring the inputs and products of the denitrification process. However, there have been several recent field investigations which have not only added to the general knowledge about denitrification but also have investigated the possibility of utilizing the denitrification process to remove nitrogen from drainage effluent.

Studies conducted by Meek, et al. (16) in the Imperial Valley of California on an irrigated field showed that nitrate concentrations were high near the soil surface but decreased at depths approaching the water table, indicating denitrification possibly occurred in the capillary fringe above the water table. Further studies by Willardson, et al. (24) in relation to submerged field tile drains in the San Joaquin Valley were inconclusive as to whether significant nitrogen loss due to denitrification occurred. No added soluble carbon source was placed in the soil.

Tusneem and Patrick (22) found large losses of total soil nitrogen can occur during alternate periods of wetting and drying of the soil which permits nitrification and subsequently denitrification. Losses up to 14% to 16% were recorded.

Beer and Koelliker (22), working with filtration through soil profiles to reduce ground water pollution due to lagoon effluent irrigation, attributed 40% to 80% nitrogen loss to denitrification. Their research also indicated soil organic

matter may be a limiting factor over a period of several years as the denitrification processes utilize the available carbon. This suggests addition of a soluble carbon source, such as methanol, would be necessary to maintain high denitrification rates over an extended time period.

III. OBJECTIVES

The objectives of this study were:

1. To conduct a laboratory investigation to determine if significant quantities of nitrate nitrogen could be reduced to the gaseous state in a porous medium.
2. To compare methanol and sawdust as carbon substrates for denitrification.
3. To determine the effect of temperature as a variable in the denitrification process.
4. To introduce flow velocity as a factor in the denitrification process.

IV. APPARATUS

A temperature control chamber with dimensions of 2.44 m. by 2.44 m. by 5.09 m. was constructed in which the temperature could be controlled from 13°C (56°F) to approximately 27°C (90°F). The chamber and general layout of the apparatus are shown in Figures 3a and 3b.

The denitrification flow apparatus consisted of 152 and 182 cm. lengths of plexiglass tubes having a 11.4 cm. outer diameter and a .32 cm. wall thickness. Pairs of these tubes were connected by plexiglass flanges to form 6 tubes of either 304 cm. or 364 cm. in length. These cylinders were filled with sand compacted by hand to approximate field density and laid horizontally across a supporting table.

All tubes were filled with a brown sand except Tube 1 which was filled with a washed white silica sand of smaller particle size. A sieve analysis was conducted to determine the particle size distribution for both sands. The results are listed in Table 1.

Three carbon treatments were replicated in the six sand columns. As shown in Figure 2, Tubes 1 and 5 served as checks, Tubes 2 and 6 were filled with a sand-sawdust mixture containing 7% sawdust by dry weight, and Tubes 3 and 4 were treated with a continuous flow of 1000 ppm methanol which was added to the demineralized water supply reservoir.

A diagram of the denitrification flow apparatus contained within the controlled temperature chamber is shown in Figure 4.

FIGURE 2

TUBE TREATMENTS — ADDED CARBON

FRONT VIEW

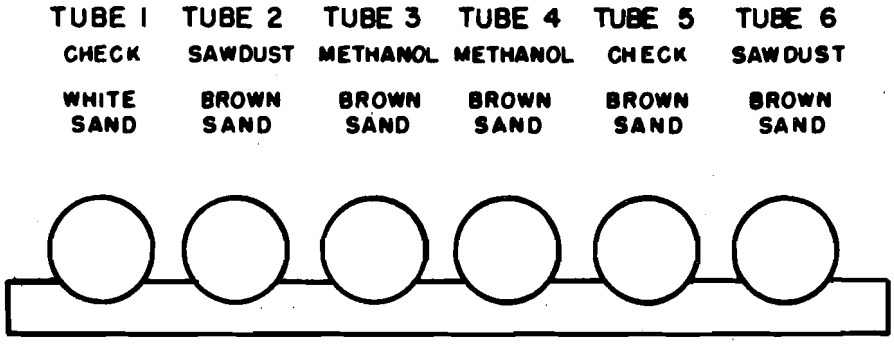


TABLE 1

SIEVE ANALYSIS

BROWN SAND		WHITE SAND	
SIZE MICRONS	% BY WEIGHT	SIZE MICRON	% BY WEIGHT
0 - 149	3.8	0 — 70	25.2
149 - 210	13.0	70 — 100	43.0
210 - 250	12.7	100 — 120	12.3
250 - 420	50.4	120 — 200	17.7
420 - 500	9.6	200 — 230	0.8
>500	10.5	>230	1.0

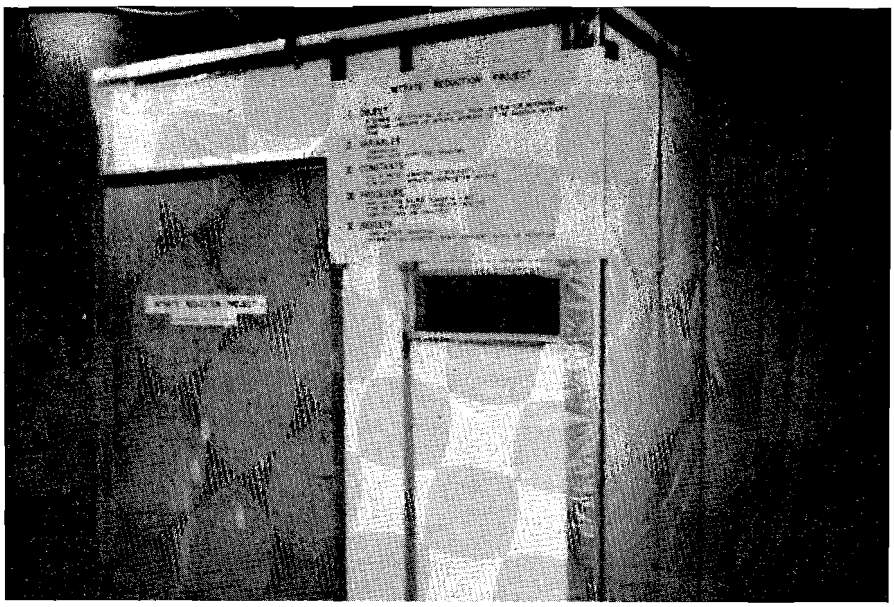


FIGURE 3a. TEMPERATURE CONTROL CHAMBER

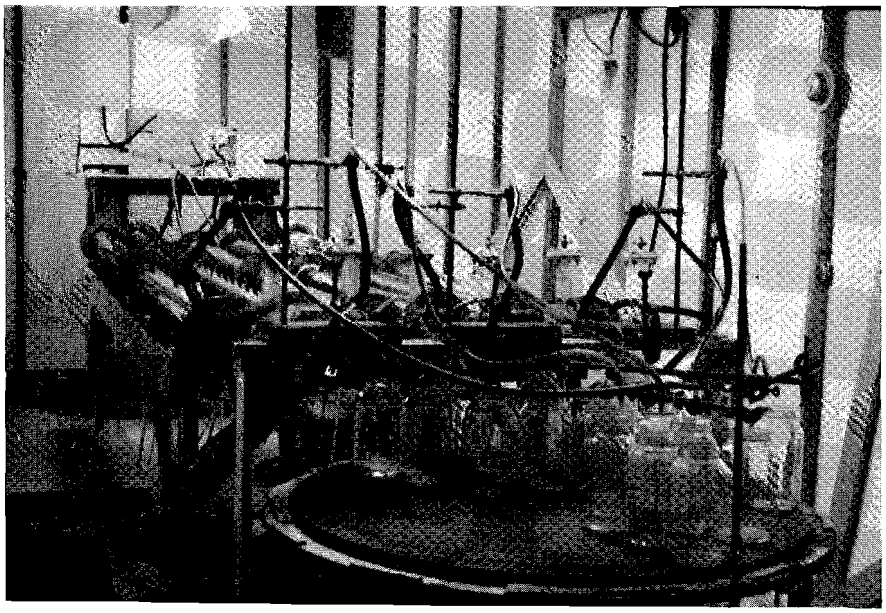
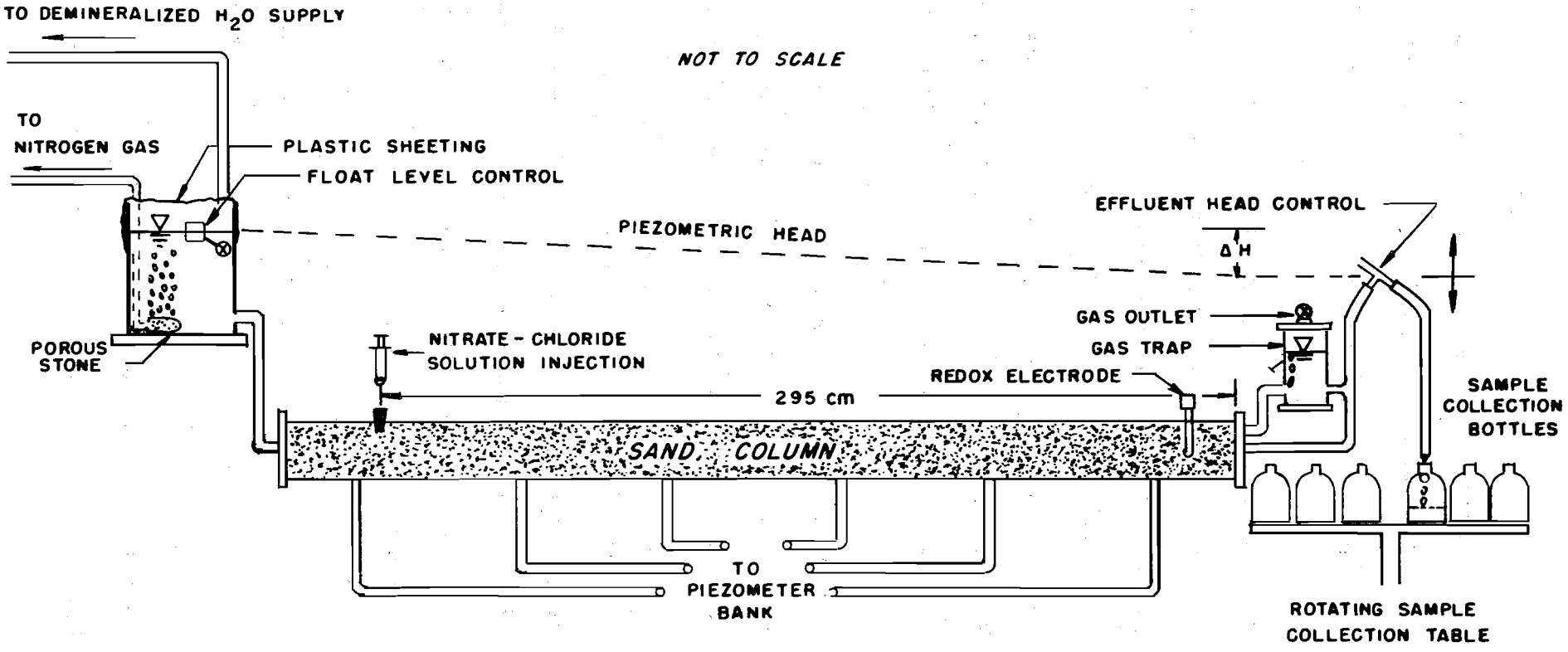


FIGURE 3b. GENERAL LAYOUT OF APPARATUS

FIGURE 4

DENITRIFICATION FLOW APPARATUS

SIDE VIEW



Demineralized water entered the sand columns at the left of the diagram through rubber tubing connected to the center of the end of each plexiglass cylinder. Two cylindrical plexiglass containers were used as water supply reservoirs in which the water level was maintained at a constant level by float valves. The supply reservoirs are shown in Figure 5a. The water supply was demineralized with a Barnstead mixed bed cartridge demineralizer before entering the water supply reservoirs.

One of the water supply reservoirs was connected to the two check sand columns and the two columns containing the sand-sawdust mixture. A 1000 ppm methanol solution was added to the demineralized water entering the other supply reservoir, which was connected to the two remaining sand columns. Both water supply reservoirs contained ceramic stones through which high purity nitrogen gas entered the water under a constant pressure. The nitrogen gas was used to saturate the water supply, thus stripping oxygen from the demineralized water. Each of the supply reservoirs was covered with polyethylene sheeting. Random testing of the water supply reservoirs with a Yellow Springs oxygen meter indicated that the oxygen content of the water was reduced to approximately 4 ppm.

Effluent tubes were connected to the center of the downstream end of each column as shown in Figure 5b. The effluent tubes were connected by adjustable clamps to ringstands so that their elevation in relation to the water storage reservoir levels could be controlled. The outlets of the effluent tubes were clamped in fixed position over a rotating table (Figure 3b)

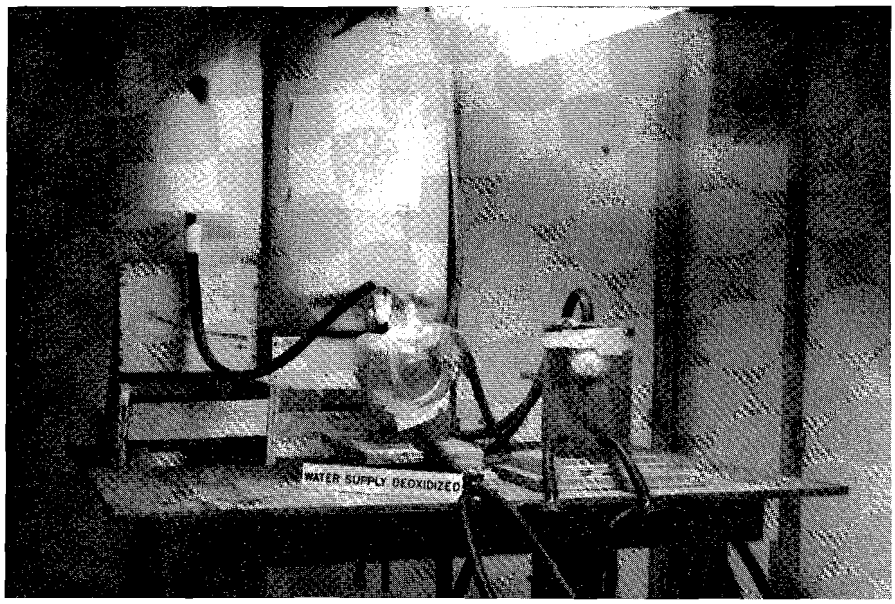


FIGURE 5a. DEMINERALIZED WATER SUPPLY AND RESERVOIRS

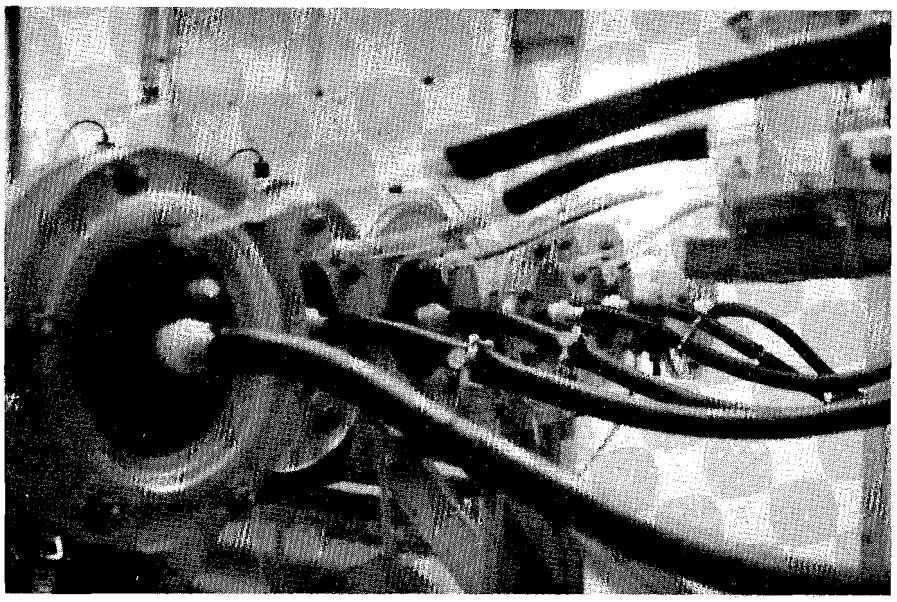


FIGURE 5b. EFFLUENT TUBING CONNECTIONS

containing flint glass sampling bottles of one liter capacity. A timer and microswitch system was set so that an electric motor rotated the table to a new set of sample bottles at twelve hour intervals.

Gas collection devices, shown on the right in Figure 4, were constructed to trap gas collecting within the upper downstream portion of the sand columns. This was accomplished by closing the valve for direct flow through the lower effluent tubing so that flow was directed through the gas collection devices. These devices were made of plexiglass cylinders and flanges and had a maximum volume of 100 cubic centimeters. Stopcocks at the top of each gas collection device were used to allow entrapped gas to escape and also to obtain samples for analysis.

Six glass tubing ports were positioned equidistant along the bottom side of each sand column and connected to a piezometer bank. The black arrows of Figure 6a indicate supply reservoir and effluent tube elevations. Plastic mesh was rolled and placed in each stud to act as filters and prevent sand from entering the piezometer tubing.

FIGURE 6a. PIEZOMETER BANK

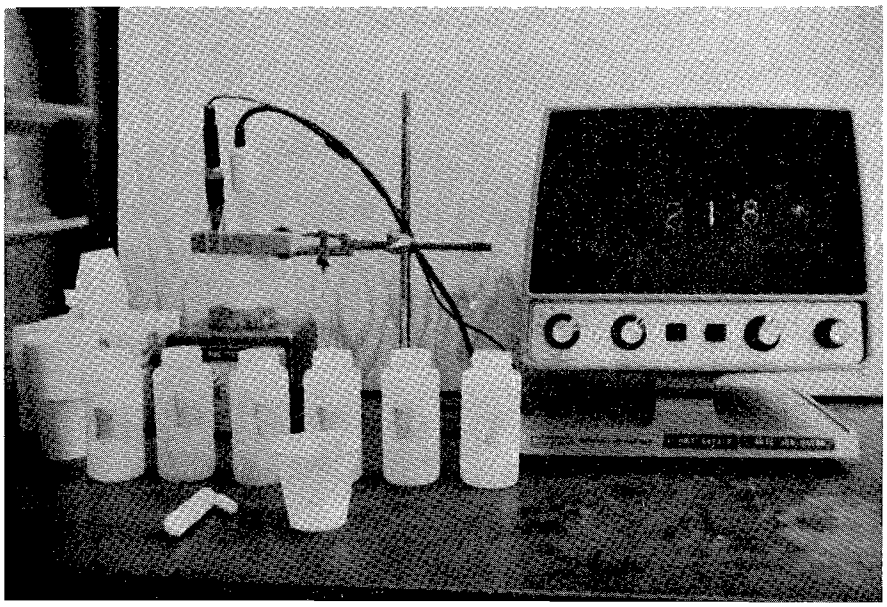


FIGURE 6b. NITRATE ANALYSIS APPARATUS

V. PROCEDURE

Nitrate losses were measured by using the technique developed by Corey, et al. (8), in which "breakthrough curves" of nitrate were compared with chloride by plotting effluent concentrations of both ions versus effluent volume increments for a soil column flow apparatus.

For this study, a nitrate/chloride solution containing equal concentration of both ions was prepared by dissolving sodium nitrate and potassium chloride in one liter of double distilled water. This solution was used as a nitrate/chloride source for each test and contained 50,000 ppm of nitrate and 50,000 ppm of chloride.

The sand columns were pre-saturated before the tests were started. One-half inch diameter holes were drilled at one foot intervals in the top of the plexiglass cylinders containing the sand columns. As the saturation front advanced along the sand columns the holes were sealed with rubber stoppers. After the columns were saturated, the effluent tubes were adjusted in relation to the water supply reservoirs to obtain an average flow rate of approximately 500 milliliters per day. Differences in elevation were measured with a small level.

Each piezometer was checked for two way flow by back pressuring the piezometer connections through a manifold. Any piezometer not exhibiting two way flow was checked for filter clogging. Testing was not begun until the total head loss along each sand column was equally distributed between the piezometers

for that column, as shown in Figure 6a. This procedure helped assure uniform saturation of the sand columns.

A constant volume of 50 milliliters of the nitrate/chloride solution was added to each column for each test. The solution was injected with a 50 milliliter capacity hypodermic syringe through a rubber stopper located 295 centimeters from the downstream end of each column, thus introducing 2500 milligrams of nitrate and 2500 milligrams of chloride at one point. The needle of the syringe extended approximately two centimeters into the upper half of the column cross-section.

From the time of each nitrate/chloride injection, flow was measured volumetrically at twelve hour intervals using the rotating sampling table. Samples were taken from each effluent volume, measured, stored in 4 ounce plastic bottles, and analyzed daily for nitrate and chloride concentrations. Samples not analyzed the same day collected were refrigerated until analysis. Head loss and piezometer elevations were recorded daily for each column.

Nitrate concentrations were measured using the specific ion method. An Orion nitrate electrode model 92-07 in combination with a Beckman 39400 reference electrode were connected to a Beckman expanded scale digital pH meter as shown in Figure 6b. The nitrate electrode was calibrated before and after each use with a set of standards ranging in concentration from 5 to 2500 ppm. A typical calibration curve plotted on semilogarithmic paper is shown in Figure B-1, Appendix B. Sample sizes of 20 to 50 milliliters were tested with the electrode. Corrections were

made for chloride interference of the nitrate electrode when the nitrate concentration was found to be two orders of magnitude less than the chloride concentration. This correction for chloride ion interference is discussed in detail under Section 4, Part G, of Results and Discussion.

Chloride concentrations were determined by using the argentometric technique described in Standard Methods for the Examination of Water and Wastewater, 1971. Samples were diluted 1:3 or 1:4 with distilled water to 30 milliliters and titrated with silver nitrate using potassium chromate as an indicator. The titration apparatus is shown in Figure 7a.

Beckman Model 39186 platinum reference combination electrodes for measurement of redox potential were installed halfway into each sand column through holes in the top of each plexiglass cylinder. These were drilled approximately 15 centimeters from the effluent end of the column (Figure 7b). Before installation all redox electrodes were checked in a quinhydrone standard. The millivolt readings for this standard are listed below:

TABLE 2

STANDARDIZATION OF REDOX ELECTRODES

Redox Electrode	Redox Potential (mv)
Tube 1	222
Tube 2	218
Tube 3	212
Tube 4	221
Tube 5	215
Tube 6	218

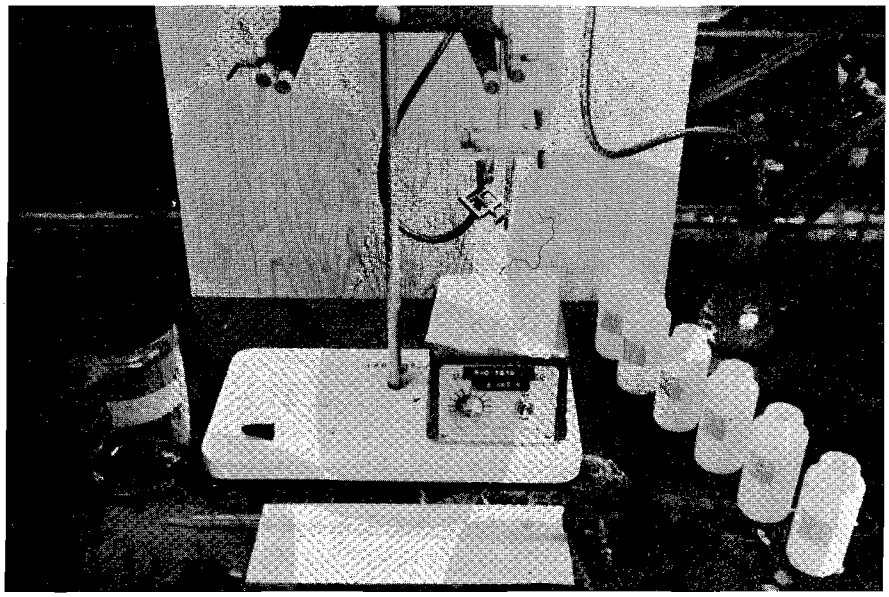


FIGURE 7a. CHLORIDE ANALYSIS APPARATUS

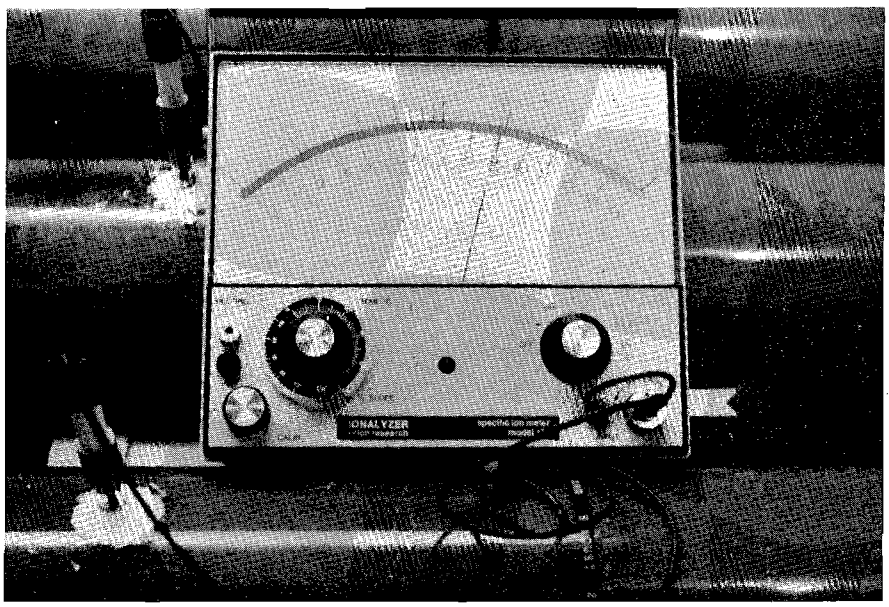


FIGURE 7b. REDOX ELECTRODES

An Orion Model 401 specific ion meter was used with the redox electrodes to obtain daily redox potentials, in millivolts, at point locations in the sand columns.

When the effluent analysis indicated the nitrate/chloride applications were leaving the methanol treated sand columns during the 24°C and 13°C tests, effluent samples were kept and acidified with sulfuric acid until analyzed for total organic carbon. Samples were also taken from the methanol treated water supply reservoir to determine if the 1000 ppm methanol level was maintained. All samples were tested with a Beckman Model 915 Total Carbon Analyzer to determine if soluble carbon levels actually decreased during flow intervals where nitrate losses due to denitrification were expected. Only the total organic carbon furnace of the organic carbon analyzer was used. The analyzer was calibrated before and after each use using diluted samples of a 1000 ppm organic carbon standard. Sample volumes of 20 microliters were injected into the furnace by a spring release syringe.

Two gas samples were extracted from the gas collection device of Tube 3 near the completion of the 18°C test. These samples were analyzed with a single/double focusing mass spectrometer on line to a small dedicated computer operated by the Burnsides Research Center, College of Agriculture, University of Illinois at Urbana, Illinois. Only the qualitative structure of the gas samples was determined using this equipment. Before analysis, the samples were cleaned of moisture with a dry ice trap.

VI. RESULTS AND DISCUSSION

A. Loss of Added Nitrate

Before discussing the results, some general terminology should first be defined. Losses of nitrate added to the sand columns (hereafter simply referred to as columns) are expressed as the percent of added nitrate mass not recovered, rather than nitrate reduction. Column refers only to the sand within the plexiglass tube unit. Nitrate reduction is the decrease in oxidation number of nitrogen associated with denitrification or assimilation. In this study, since the gases evolved within the columns during test intervals were not positively identified or measured volumetrically, denitrification or nitrate reduction were only inferred from other measurements and observations.

A general summary of recovery of added nitrate is given in Table 3. The percent loss of added nitrate, or the percent mass of added nitrate not recovered, was calculated based on the total mass of chloride recovered since chloride was used as a reference ion in this study. Nitrate and chloride concentrations determined for each volume increment of effluent were multiplied times their respective volume increments to find increment masses. These mass increments were then totaled for each test to determine the total chloride and nitrate recovered. The total mass of nitrate recovered was corrected for base nitrate concentrations in the effluent of each column observed between test intervals. These base nitrate concentrations ranged from 3 to 8 milligrams per liter.

TABLE 3

SUMMARY OF NITRATE RECOVERY

Chamber Temperature 24°C (75°F)

Tube	Carbon treatment	Total NO_3^- recovered (mg)	Total Cl^- recovered (mg)	Loss of added NO_3^- (%)
1	check	1530	2000	28.5
5	check	1953	2388	18.2
2	sawdust	2080	2285	9.0
6	sawdust	2215	2321	4.5
3	methanol	319	2281	86.0
4	methanol	253	2261	88.8

Chamber Temperature 18°C (65°F)

Tube	Carbon treatment	Total NO_3^- recovered (mg)	Total Cl^- recovered (mg)	Loss of added NO_3^- (%)
1	check	2246	2456	12.0
5	check	2761	2535	- 8.9
2	sawdust	2635	2365	-11.4
6	sawdust	2291	2343	2.2
3	methanol	717	2353	70.0
4	methanol	421	2268	81.0

Chamber Temperature 13°C (56°F)

Tube	Carbon treatment	Total NO_3^- recovered (mg)	Total Cl^- recovered (mg)	Loss of added NO_3^- (%)
1	check	2360	2392	1.3
5	check	3667	2561	-43.1
2	sawdust	3441	2470	-39.3
6	sawdust	2783	2487	-11.9
3	methanol	1340	2473	46.0
4	methanol	540	2468	78.0

Since in each test, 2,500 milligrams of nitrate and 2,500 milligrams of chloride were applied to each column, and since microbial action does not affect the chloride ion, the same mass of chloride initially applied would be expected to be recovered in the effluent. As shown in Table 3, less than 2,500 milligrams of chloride was generally recovered in each column. This was probably due to either error in analysis or isolation of chloride in pore volumes not displaced by water flowing through the column. Losses of nitrate were based on the amount of nitrate mass recovered relative to the total chloride mass recovered according to the following relationship:

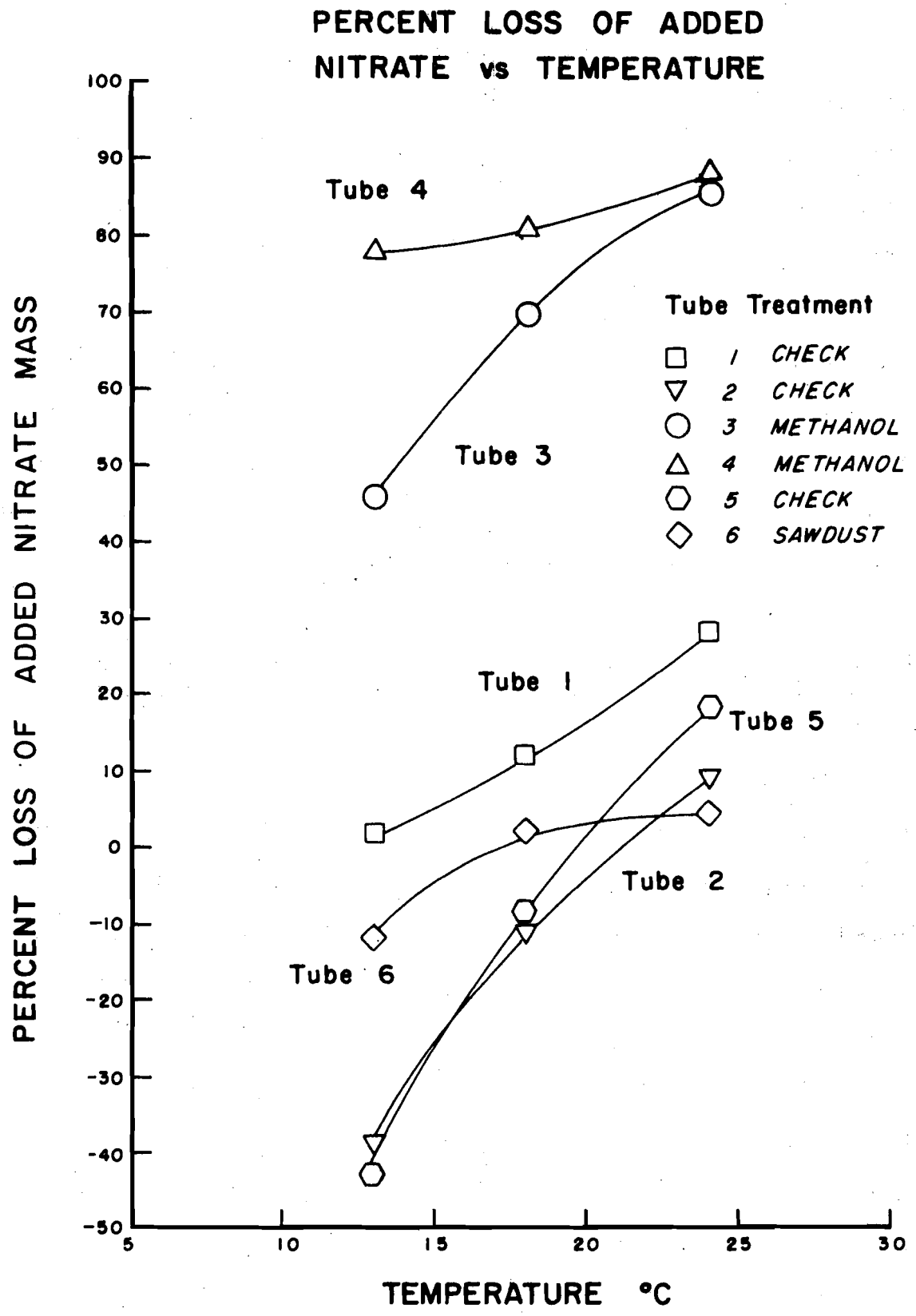
$$\% \ NO_3^- \text{ lost} = \frac{\text{total } Cl^- \text{ mass recovered} - \text{adjusted } NO_3^- \text{ mass recovered}}{\text{total } Cl^- \text{ mass recovered}}$$

Table 3 indicates that less nitrate was recovered from each column as the temperature increased. This general observation is consistent with results of other studies noted in the literature review. Figure 8 shows nitrate losses plotted versus temperature. The rate of decrease of nitrate loss with temperature was not consistent either between or among carbon treatments.

Tubes 3 and 4, which were treated with 1,000 ppm methanol, showed much greater losses of nitrates than the other carbon treatments. Based on the nitrate mass recovered relative to the chloride mass recovered, the range of nitrate mass lost varied from almost 90% at the 24°C test to 46% in the 13°C test.

Tubes 2 and 6 contained added carbon in the form of sawdust mixed 7% by weight with the sand in these columns. These

FIGURE 8.


PERCENT LOSS OF ADDED NITRATE vs TEMPERATURE
PERCENT LOSS OF ADDED NITRATE MASS
TEMPERATURE °C
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
5
10
15
20
25
30
Tube 4
Tube 3
Tube 1
Tube 5
Tube 2
Tube 6
Tube Treatment
1 CHECK
2 CHECK
3 METHANOL
4 METHANOL
5 CHECK
6 SAWDUST

columns exhibited little nitrate loss, even at the 24°C test. However, at 13°C and 18°C, actual increases of nitrate mass were recovered.

The two remaining columns, Tubes 1 and 5, served as treatment checks with no carbon added to the sand. Nitrate losses were higher in the check columns than in the columns treated with sawdust at the 24°C test. Percent of nitrate loss reached 28.5% and 18.2% at the 24°C test but was not significant in Tube 1 and was actually negative in Tube 5 at the lower temperatures. The washed white silica sand in Tube 1 may have resulted in the difference in temperature effect. Although Tube 1 showed some loss of nitrate in each test, Tube 5 was similar to the sawdust columns in that it exhibited gains of nitrate for the 13°C and 18°C tests. This phenomena will be discussed in detail in a later section (p. 77).

The preceeding discussion based on total outflow shows the relative importance of a readily available carbon source, such as methanol, to maintain high losses of nitrate as the temperature is lowered. However, to gain a closer study of individual column behavior, "breakthrough curves" of nitrate and chloride were plotted for each column at each temperature tested. A presentation of these curves and their characteristics are made in section C.

B. Flow Conditions

General flow data is shown in Table 4. The headings of each vertical column are defined and discussed in the following paragraphs.

TABLE 4

SUMMARY OF HEAD LOSS GRADIENT, EFFECTIVE PORE SPACE AND FLOW VELOCITY

Chamber Temperature 24°C

Tube	Carbon treatment	Total flow (liters)	Flow to peak (liters)	Effective pore space (%)	Time to peak (days)	Pore vel. (cm/day)	Head loss gradient $x10^3$
1	check	21.5	13.4	44.3	24	12.3	6.1
5	check	16.5	11.0	36.4	17	17.4	6.1
2	sawdust	13.5	9.9	32.7	16	18.4	40.7
6	sawdust	12.5	9.5	31.4	18	16.4	50.8
3	methanol	9.9	6.0	19.8	17	17.4	20.3
4	methanol	10.6	7.9	26.1	14	21.1	16.9

Chamber Temperature 18°C

Tube	Carbon treatment	Total flow (liters)	Flow to peak (liters)	Effective pore space (%)	Time to peak (days)	Pore vel. (cm/day)	Head loss gradient $x10^3$
1	check	16.7	9.8	32.4	17	17.4	7.4
5	check	15.9	10.3	34.1	20	14.7	7.4
2	sawdust	13.0	9.9	32.7	20	14.7	35.6
6	sawdust	13.0	10.1	33.4	21	14.0	40.0
3	methanol	10.7	7.0	23.2	19	15.5	27.1
4	methanol	10.9	6.6	21.8	17	17.4	23.0

Chamber Temperature 13°C

Tube	Carbon treatment	Total flow (liters)	Flow to peak (liters)	Effective pore space (%)	Time to peak (days)	Pore vel. (cm/day)	Head loss gradient $x10^3$
1	check	14.9	9.7	32.1	19	15.5	8.1
5	check	15.1	9.7	32.1	20	14.7	6.1
2	sawdust	13.1	9.7	32.1	21	14.0	39.3
6	sawdust	13.5	10.2	33.7	23	12.8	49.2
3	methanol	11.0	7.3	24.1	18	16.4	25.4
4	methanol	10.4	6.6	21.8	20	14.7	25.4

Total flow was the flow through each tube from the date of nitrate/chloride application until the date that the original chloride mass had been determined to be recovered in the tube effluent. A definite trend for total flow between carbon treatments is found in Table 4. Less total flow was required for the methanol treated columns than the other treatments and less total flow was required in the sawdust treated columns than the check columns. This trend is directly related to the amount of gas collected in each column.

Flow to peak is the flow required from the date of nitrate/chloride application until the date the peak chloride concentration was detected in the effluent. Although little difference in flow to peak is indicated in Table 4 between the check and sawdust treated columns, less flow to peak was required in the methanol treatment columns. Time to peak is the days required from date of application until the date of peak chloride concentration detection. Variations in time to peak are randomly distributed between treatments and replications.

Effective pore space is the flow to peak divided by the total column volume along the 295 centimeter flow length, consequently trends for effective pore space are identical to those of flow to peak.

Pore velocity is the flow to peak divided by the quantity, column cross-section times day to peak. No trends are indicated in Table 4 for pore velocity.

Head loss gradient is the head loss for each column length divided by the total column length of 295 cm. To maintain

the desired flow rate, highest head loss gradients were needed for the sawdust treatment columns, while the check columns required the lowest head loss gradients.

C. Breakthrough Curves

1. Theoretical Breakthrough Curves

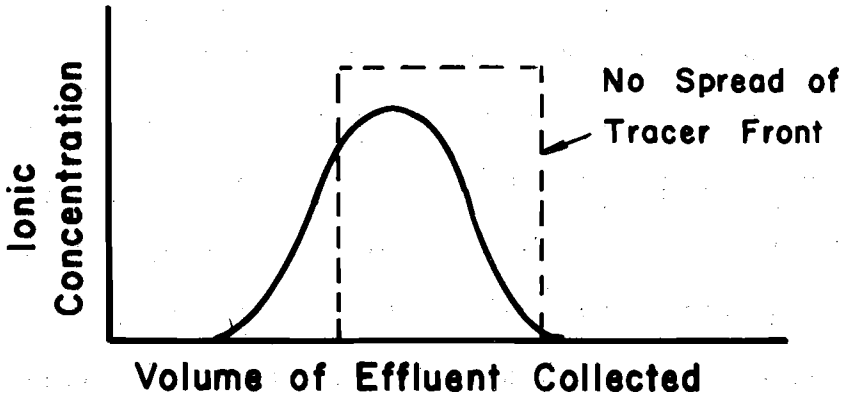
Studies of miscible displacement using chloride as a tracer have shown that flow velocity distributions through porous media, such as soils, disperse ionic concentrations due to the varied number of flow paths available through the soil pores. This diffusional effect in soils has been experimentally demonstrated by Nielsen and Bigger (17) and a typical illustration of their results is shown in Figure 9 through the use of a "breakthrough curve." This curve was obtained by plotting the tracer ion concentration of small volume increments of effluent versus volume of effluent collected from a soil column. The breakthrough curve is extended at either end due to dispersion and diffusion, rather than the piston type displacement represented by the dotted vertical lines in which no spreading of the tracer front occurs. The height of the tracer ion peak with respect to the original concentration varies with flow rate, length of column, and soil type.

Convection, or the mass flow of the soil solution, determines the location of the bulk of the breakthrough curve. The spreading of the tracer front at either end is due to diffusion resulting from concentration gradients within the solution and also dispersion due to pore velocity distributions. The net effect of velocity tends to increase the diffusion process on

the downstream side of the peak and decrease it on the upstream side, thus shifting the peak of the curve to the left in Figure 9 (10).

FIGURE 9

TYPICAL BREAKTHROUGH CURVE OF CHLORIDE ION IN A SOIL COLUMN



As stated earlier in the experimental procedure, a fixed volume of solution containing equal nitrate and chloride ion concentrations was applied at the same point in each column as shown in Figure 4. Measurements of effluent volume, nitrate concentration, and chloride concentration were made at twelve hour increments to construct the breakthrough curves. Approximately one month was required for test completion at each temperature.

In this study it was assumed that chloride and nitrate behave in an identical manner when displaced through soil. Therefore, at points along respective breakthrough curves, denitrification, immobilization or mineralization may have caused the nitrate curve to fall below or above the chloride curve within the limits of experimental error at a particular flow increment.

2. Tube 1 - No Added Carbon Source

The breakthrough curves of this column are shown in Figures 10, 11, and 12 in the order of decreasing temperature. The white silica sand used in this column consisted of finer particle sizes than the brown sand used in the other columns (see Table 1). The relatively small pores in this column were responsible for the extensive dispersion of the nitrate/chloride application, thus causing greater spreading on the curves and relatively low nitrate and chloride peaks as compared to the other columns. The small pore sizes greatly increased the tortuosity of the available flow paths (17), requiring a large flow volume to leach the nitrate and chloride application through the column. The greater flow required for Tube 1 can be seen in Table 4.

The extensive dispersion caused slow recovery rates of nitrate and chloride resulting in relatively high concentrations of both ions at the tails of the breakthrough curves of this column and also a lack of sufficient time in this study to completely leach each application through the column. To insure recovery of sufficient chloride mass comparable to that applied, the chloride peak for each test was bracketed by sufficient flow so that the concentration of chloride in the tails of the curves was approximately the same. This amount depended on the initial chloride concentration at the start of the breakthrough curve. Nitrate losses were based on the total masses recovered within this flow volume.

In this column and all other columns except those treated with methanol, nitrate losses occurred primarily in the flow

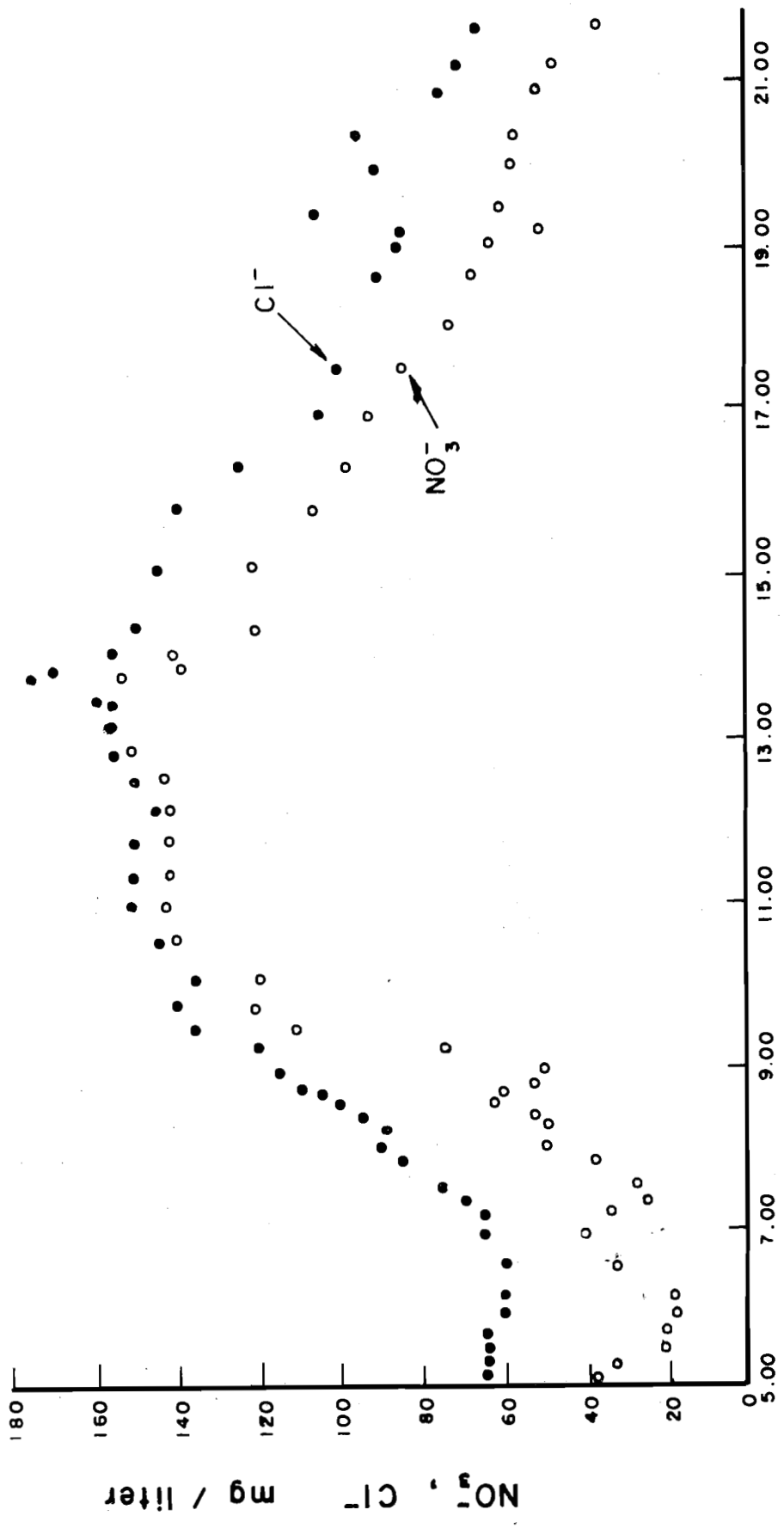


FIGURE 10. BREAKTHROUGH CURVES FOR TUBE 1, CHECK, AT 24°C WITH AN AVERAGE FLOW OF 630 ml/DAY AND 28.5 PERCENT LOSS OF ADDED NITRATE

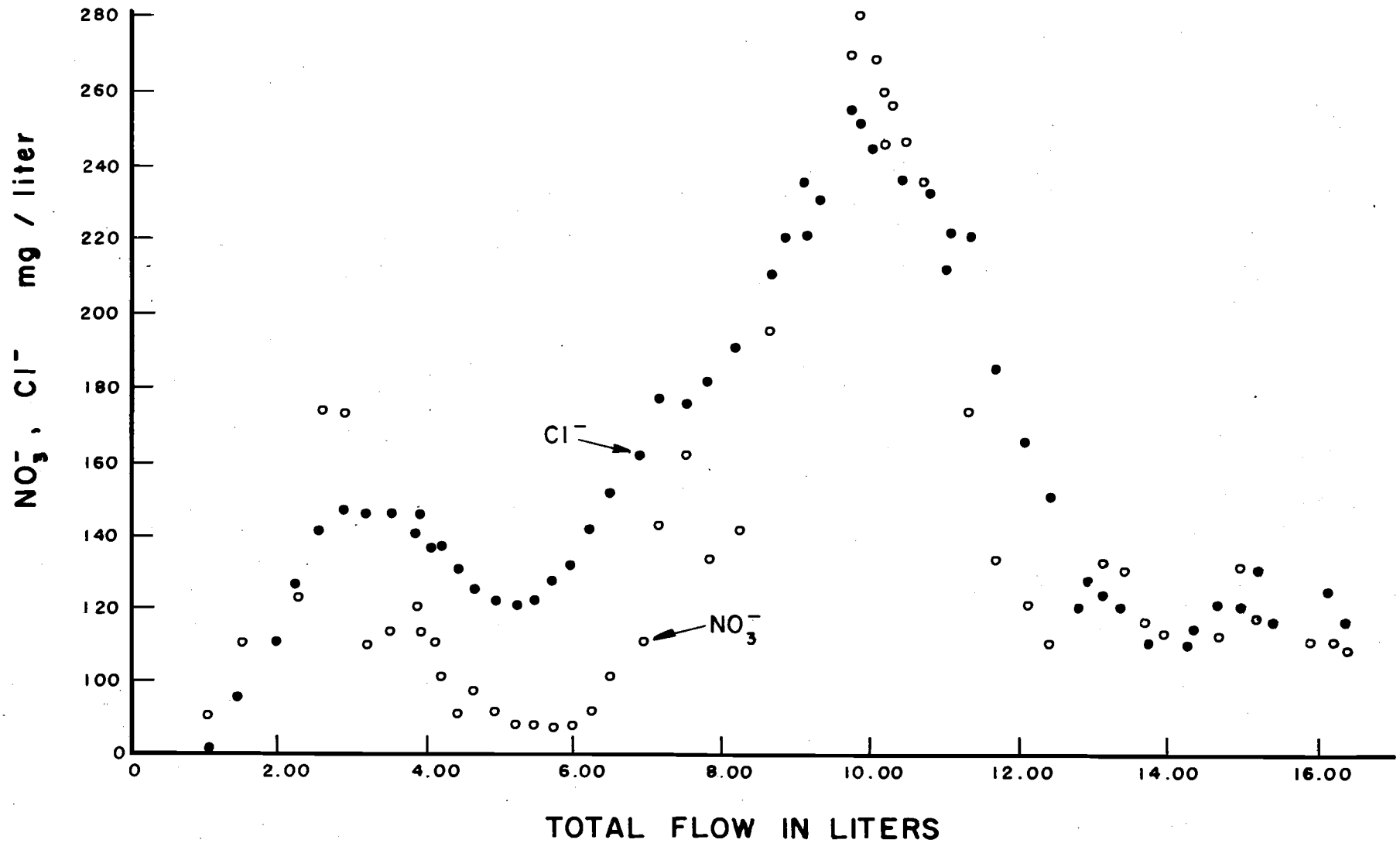


FIGURE 11. BREAKTHROUGH CURVES FOR TUBE 1, CHECK, AT 18°C WITH AN AVERAGE FLOW OF 565 ml/DAY AND 12 PERCENT LOSS OF ADDED NITRATE

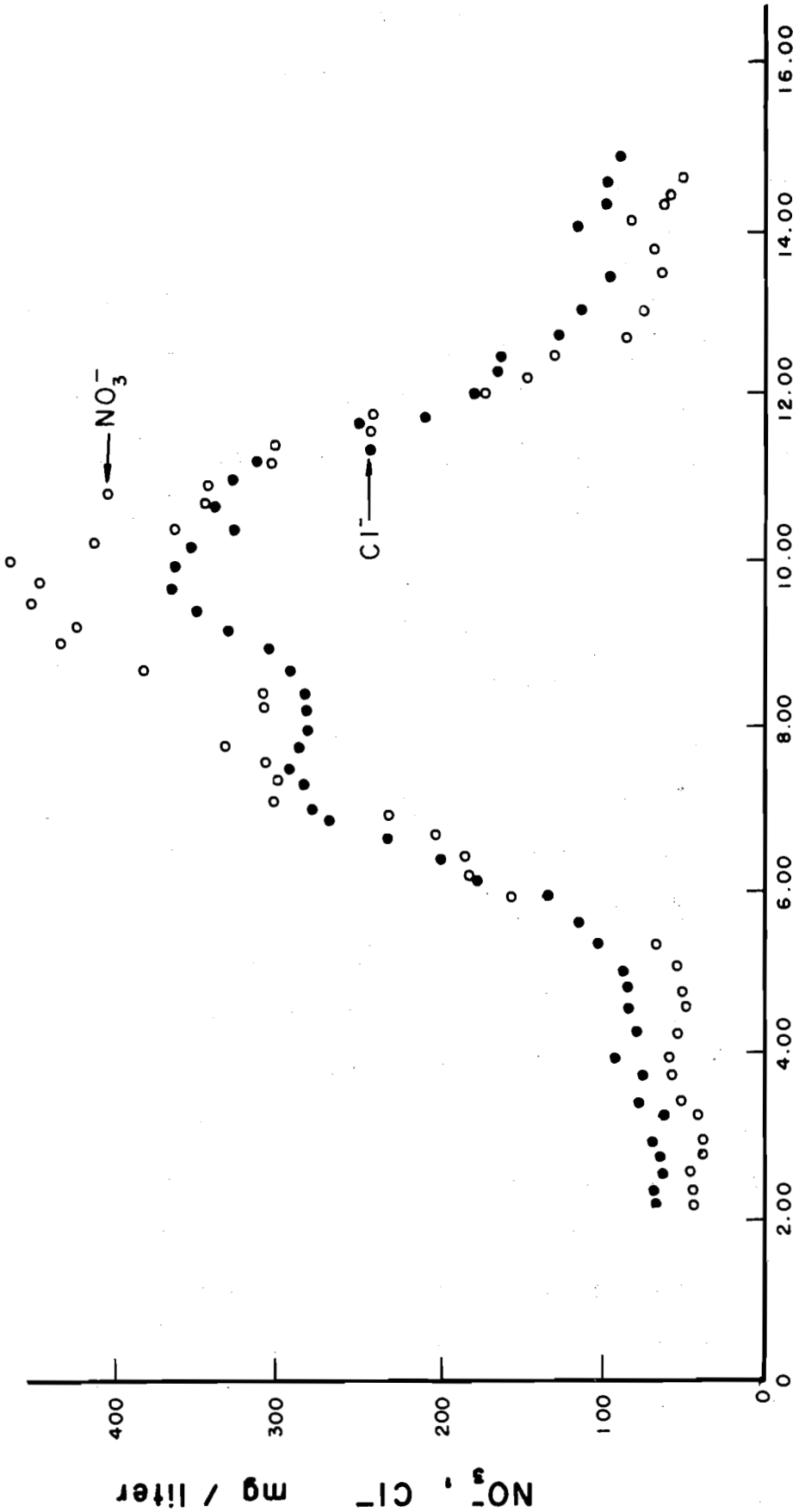


FIGURE 12. BREAKTHROUGH CURVES FOR TUBE 1, CHECK, AT 13°C WITH AN AVERAGE FLOW OF 500 ml/DAY AND 1.3 PERCENT LOSS OF ADDED NITRATE

increments located at the tails of the breakthrough curves. As ionic concentrations increased in all tubes except the methanol columns, nitrate quickly rose above chloride concentration for individual flow increments. The nitrate peak exceeded the chloride peak in all of these columns except in the case of Tube 1 at the 24°C test. The tendency of the nitrate peak to exceed the chloride peak was greater at the 13°C test than the 18°C test of Tube 1, although in neither case was the total mass of nitrate recovered greater than the total mass of chloride recovered.

Many irregularities are apparent in the breakthrough curves of Tube 1 as shown in Figures 10 through 12. Generally, peaks and valleys simultaneously occurred between the nitrate and chloride curves. This behavior may be caused by buildup and release of gases within pore spaces in the column, thus delaying portions of the nitrate/chloride application and causing small peaks and valleys on the breakthrough curves. Occurrences of these irregularities were also evident in the breakthrough curves of other columns, but to a lesser degree.

Tube 1 contained no added carbon but showed the greatest losses of added nitrate of any of the columns except those treated with methanol. Formation of anaerobic microzones favorable for high denitrification rates may have occurred in the relatively smaller pore spaces of Tube 1. Similar results with soils of small particle size were previously noted by Nommik (18).

3. Tube 5 - No Added Carbon Source

Tube 5 was a replication of Tube 1 but contained the same brown sand used in all columns except for the white sand used in Tube 1. More uniform breakthrough curves were exhibited in this check column, shown in Figures 13, 14, and 15, than those of Tube 1. This effect may have been associated with the larger pores of the brown sand.

The total chloride mass recovered was within 5% of the 2,500 mg of chloride applied for each temperature tested. Nitrate mass recovered increased as the temperature decreased, and actually exceeded total chloride mass recovered for the 18°C and 13°C tests. The nitrate peak exceeded the chloride peak in all tests for this column; the difference between the two peaks increasing as the temperature decreased. Either experimental error or release of nitrogen as nitrate from the column must be responsible for the recovery of more nitrate mass than was applied for a particular test. A more complete discussion of this phenomenon is included in Section 5 of Part G on page 77.

Tube 5 showed a loss of added nitrate of 18.5% for the 24°C test, which was the only positive loss found for this column. Figure 13 shows that nitrate losses occurring in the effluent volume increments located at the tails of the breakthrough curves exceeded nitrate gains found at the nitrate/chloride peak for the 24°C test. At the lower temperatures, nitrate gains at the peaks shown in Figures 14 and 15 greatly exceeded nitrate losses at the tails of the breakthrough curves, thus causing nitrate masses to exceed the original nitrate mass introduced to the column.

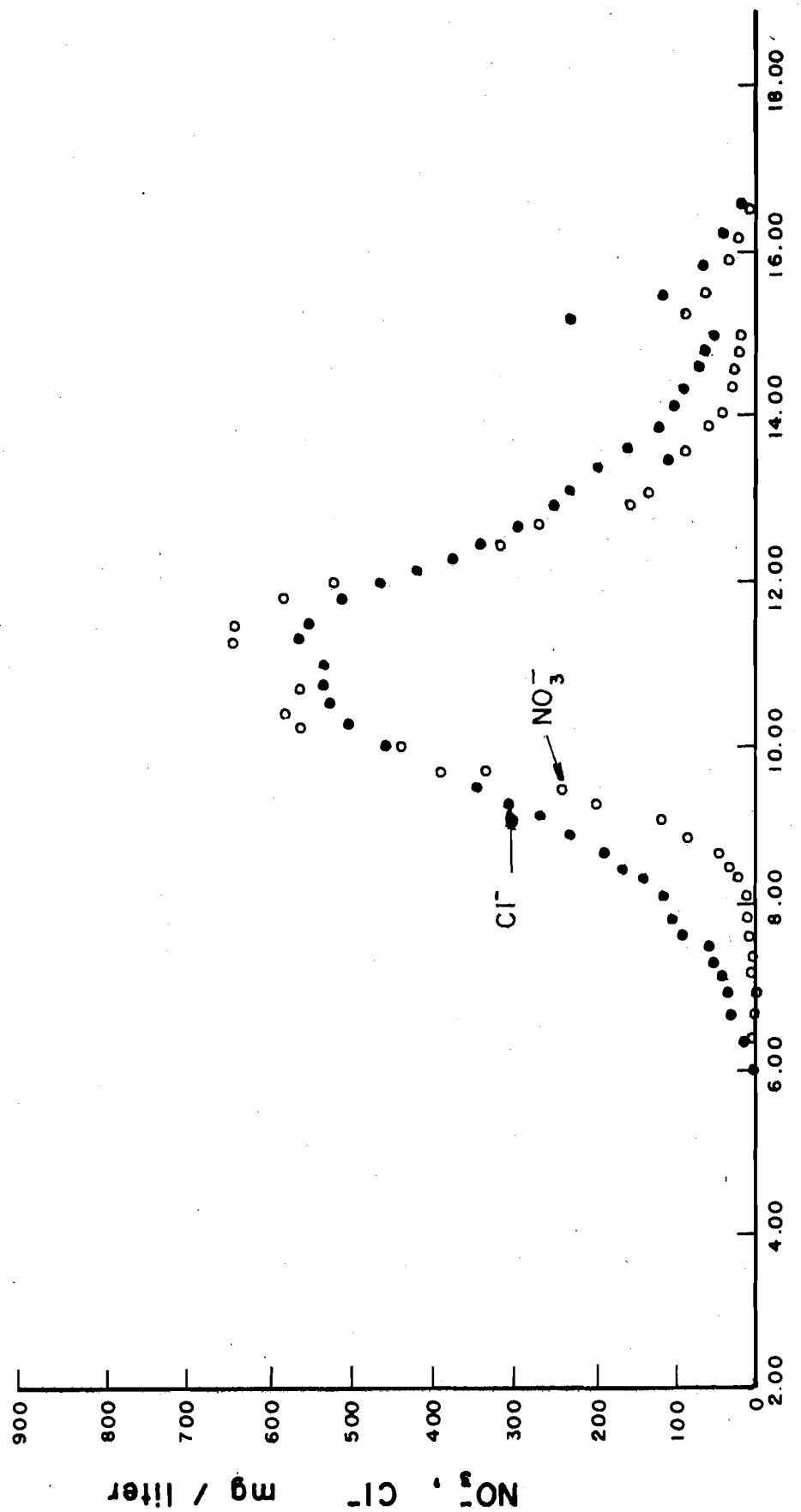


FIGURE 13. BREAKTHROUGH CURVES FOR TUBE 5, CHECK, AT 24°C WITH AN AVERAGE FLOW OF 495 ml/DAY AND 18 PERCENT LOSS OF ADDED NITRATE

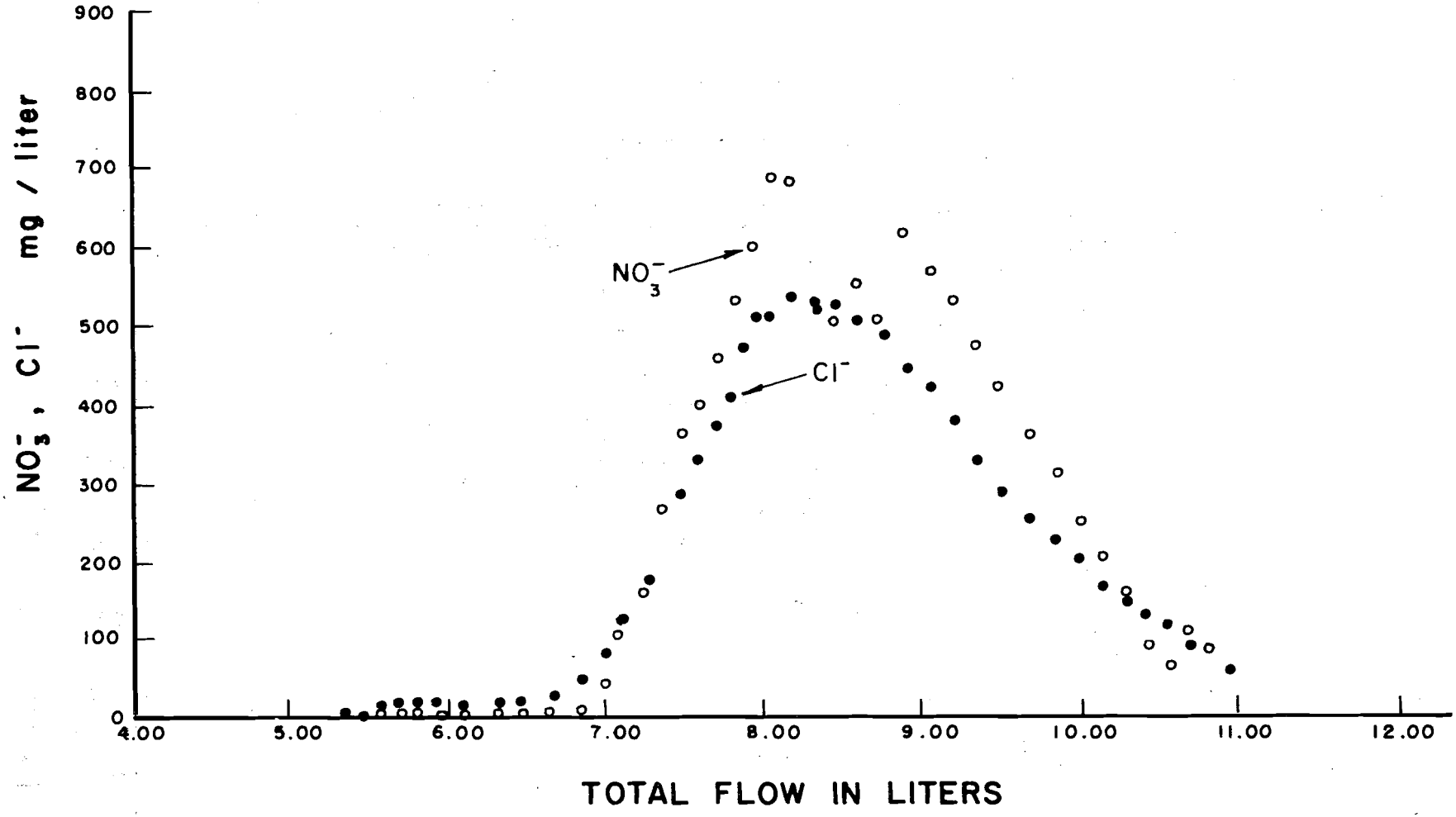


FIGURE 14. BREAKTHROUGH CURVES FOR TUBE 5, CHECK, AT 18°C WITH AN AVERAGE FLOW OF 540 ml/DAY AND 8.9 PERCENT LOSS OF ADDED NITRATE

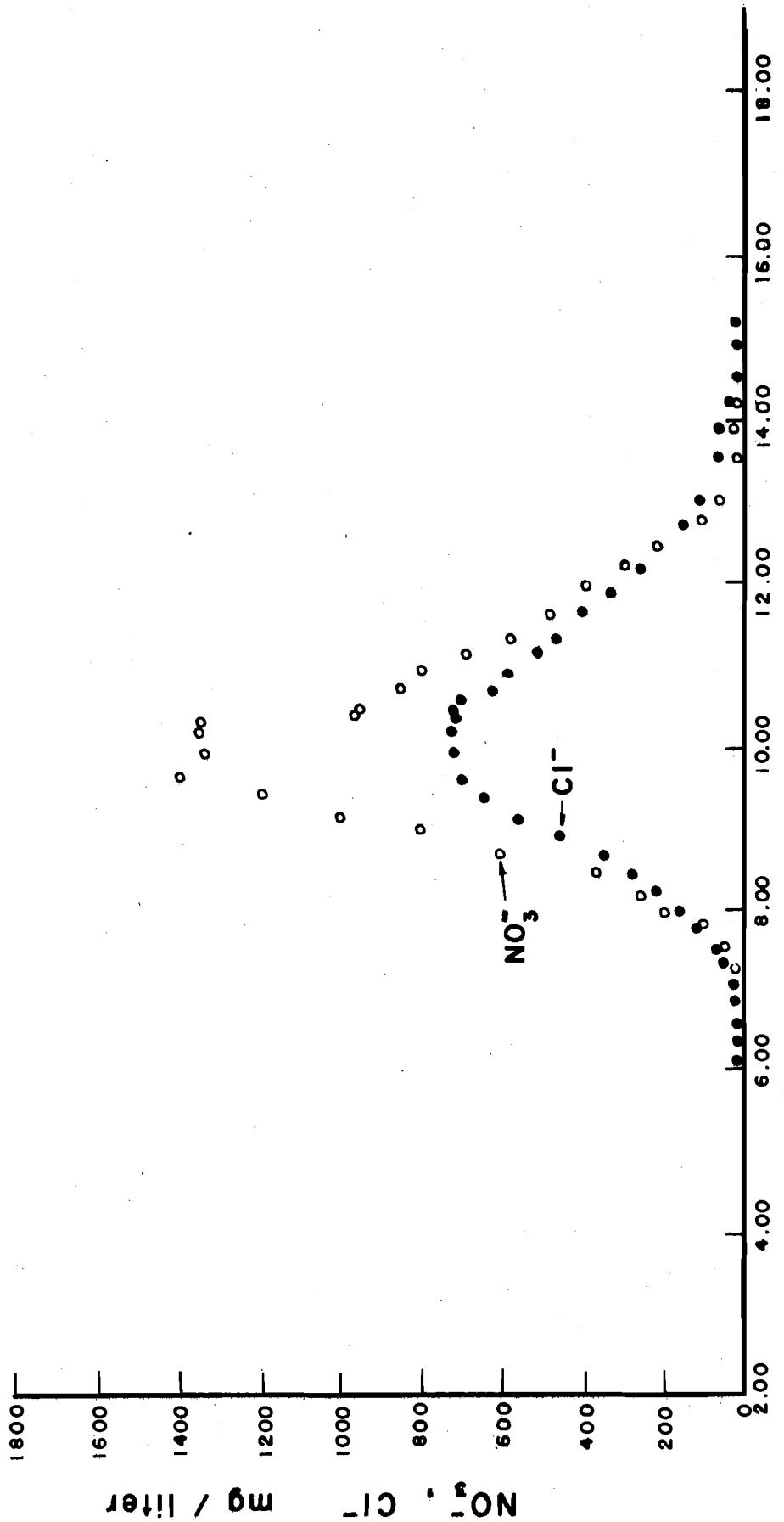


FIGURE 15. BREAKTHROUGH CURVES FOR TUBE 5, CHECK, at 13°C WITH AN AVERAGE FLOW OF 505 ml/DAY AND A 43.1 PERCENT GAIN OF ADDED NITRATE

Although the chloride curve showed a constant rise and decline in concentration as the effluent volume was recovered, irregularities were present in the nitrate breakthrough curve at the 24°C and 18°C tests. The sharp increase in nitrate shown at the tail of the curve in Figure 13 was accompanied by a corresponding increase in chloride. This behavior was also noted for Tube 1. However, as shown in Figures 13 and 14, the nitrate breakthrough curve separates into two peaks at both the 24°C test and 18°C test. These peak separations were not accompanied by chloride peak irregularities. The cause of this behavior is unknown.

4. Tubes 2 and 6 - Sawdust Added

Sawdust was added 7% by dry weight to each column. These columns exhibited the lowest added nitrate losses of the two carbon treatments. At the 24°C test, nitrate losses were 9.0% for Tube 2 and only 4.5% for Tube 6 based on the total chloride mass recovered. Tube 6 showed 2.2% loss of added nitrate at 18°C, and a gain at 13°C while Tube 2 showed a gain for both lower temperatures. Total chloride masses recovered in these columns ranged from 2321 milligrams to 2487 milligrams as shown in Table 3, compared to the 2500 milligram chloride mass introduced for each test.

The breakthrough curves of Tube 2 are shown in Figures 16, 17, and 18 and those of Tube 6 in Figures 19, 20 and 21. The chloride peak for each breakthrough curve of Tube 2 was approximately 1200 ppm in each test. However, the nitrate peak constantly increased from 1250 ppm at 24°C to 2200 ppm at 13°C.

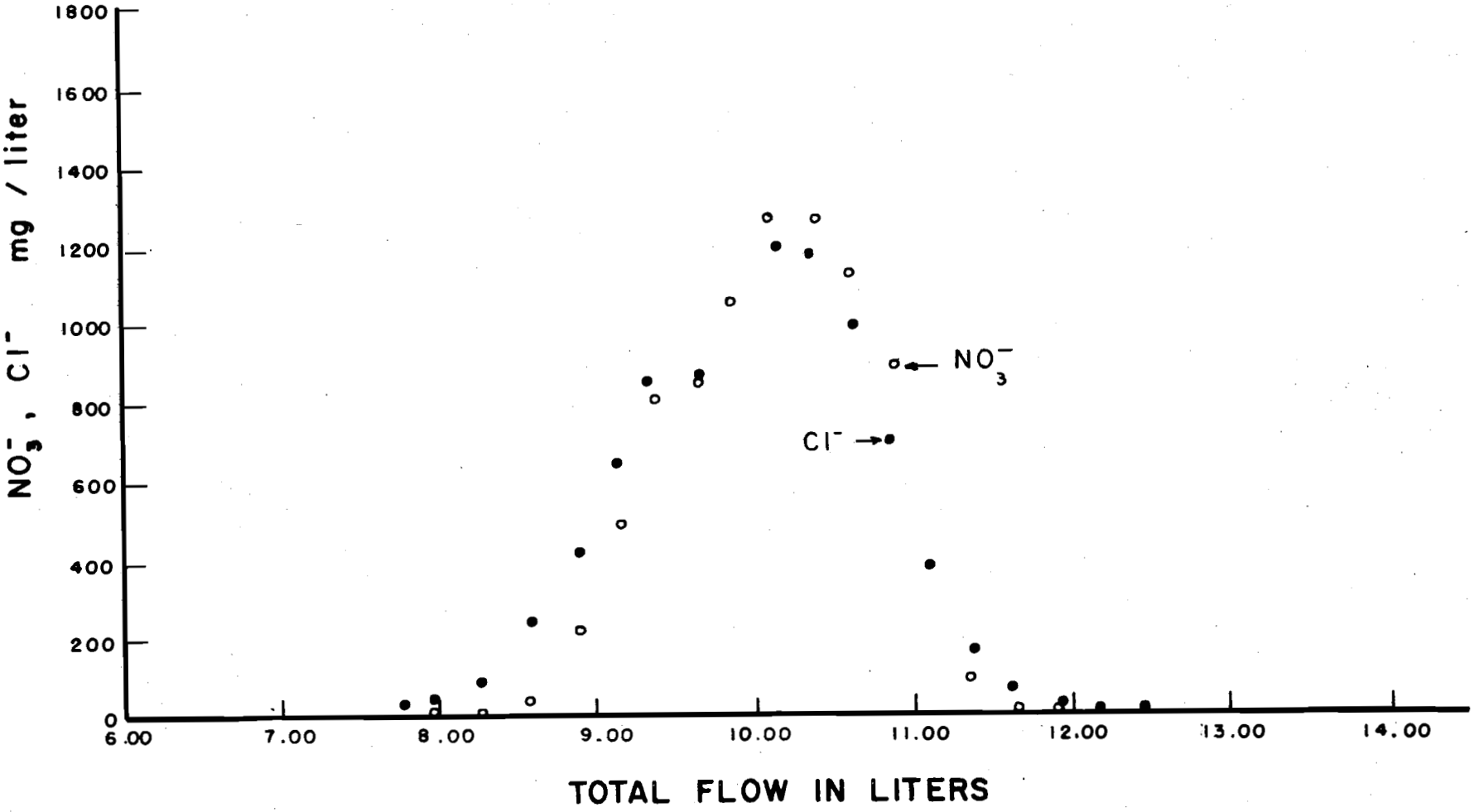


FIGURE 16. BREAKTHROUGH CURVES FOR TUBE 2, SAWDUST, AT 24°C WITH AN AVERAGE FLOW OF 550 ml/DAY AND A 9 PERCENT LOSS OF ADDED NITRATE

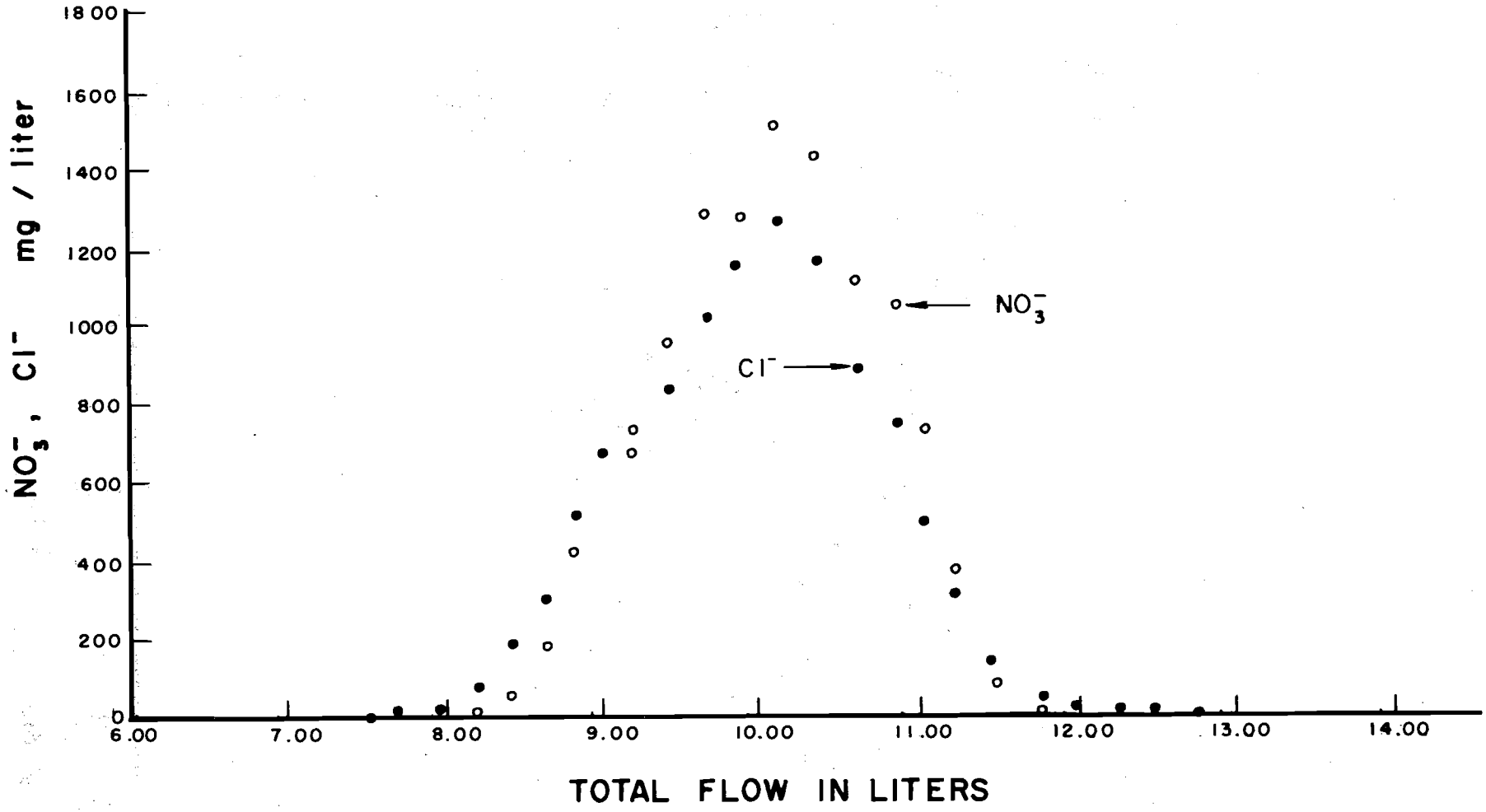


FIGURE 17. BREAKTHROUGH CURVES FOR TUBE 2, SAWDUST, AT 18°C WITH AN AVERAGE FLOW OF 500 ml/DAY AND A 11.4 PERCENT GAIN OF ADDED NITRATE

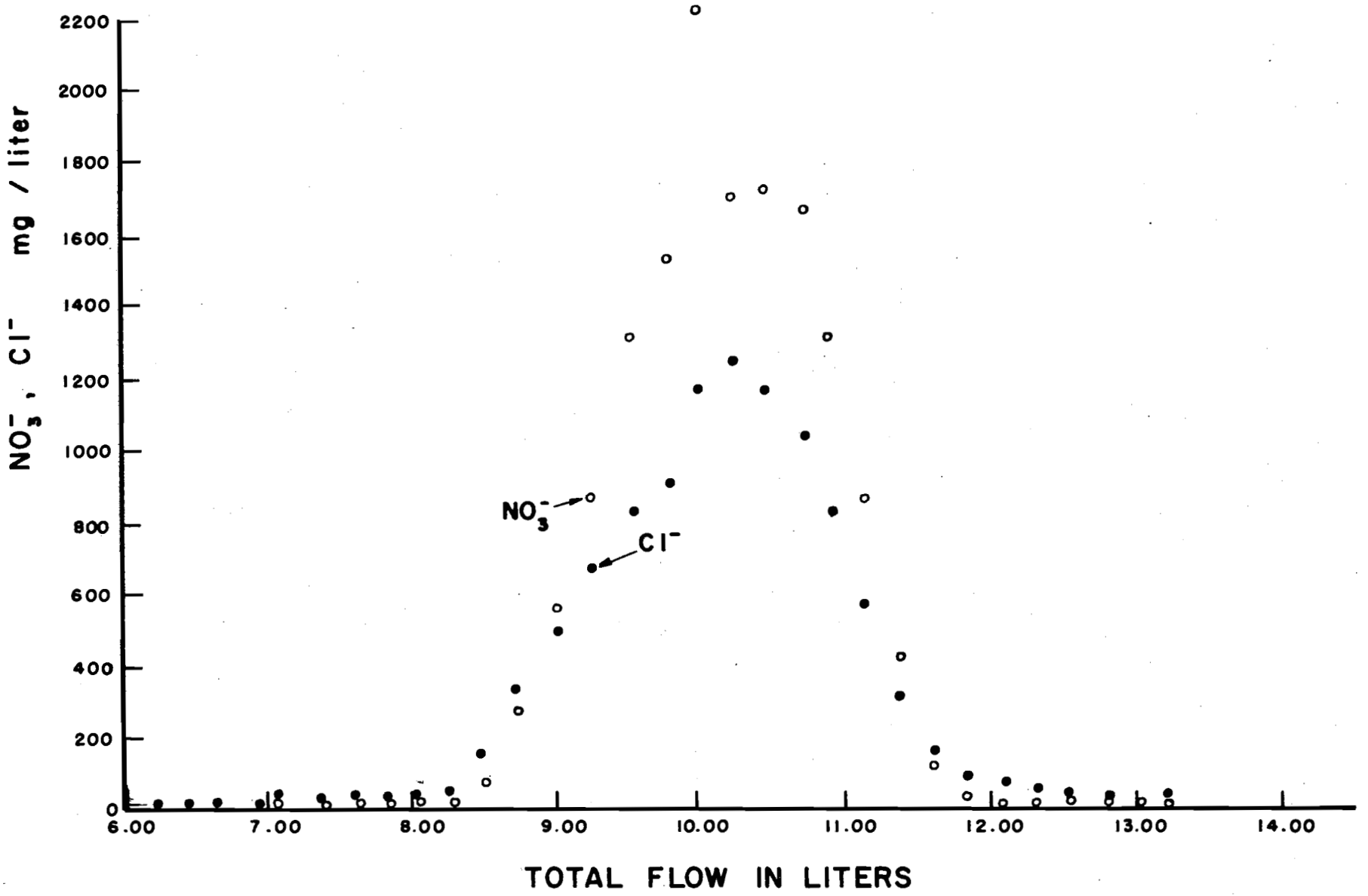


FIGURE 18. BREAKTHROUGH CURVES FOR TUBE 2, SAWDUST, AT 13°C WITH AN AVERAGE FLOW OF 480 ml/DAY AND A 39.3 PERCENT GAIN OF ADDED NITRATE

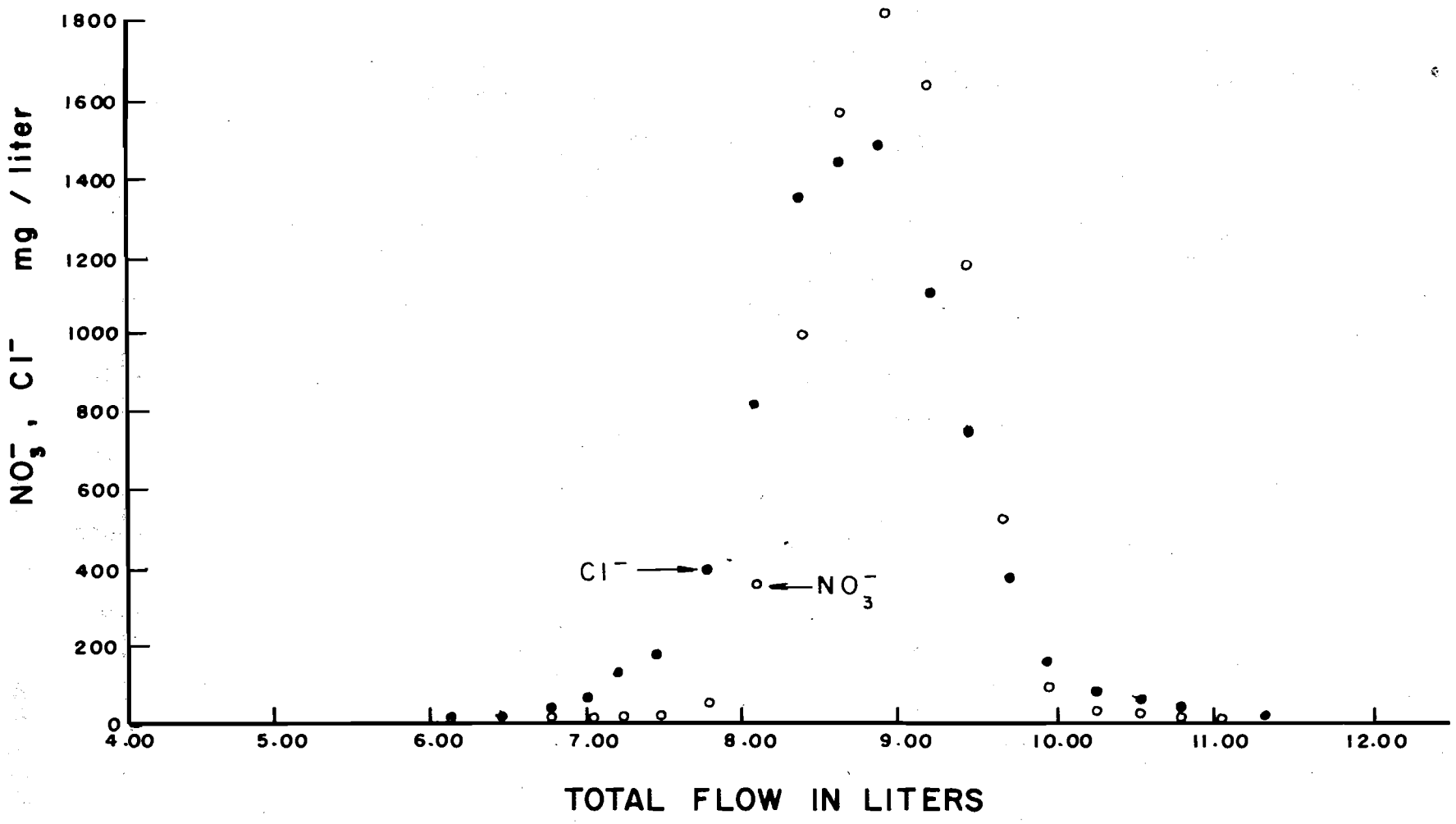


FIGURE 19. BREAKTHROUGH CURVES FOR TUBE 6, SAWDUST, AT 24°C WITH AN AVERAGE FLOW OF 520 ml/DAY AND A 4.5% LOSS OF ADDED NITRATE

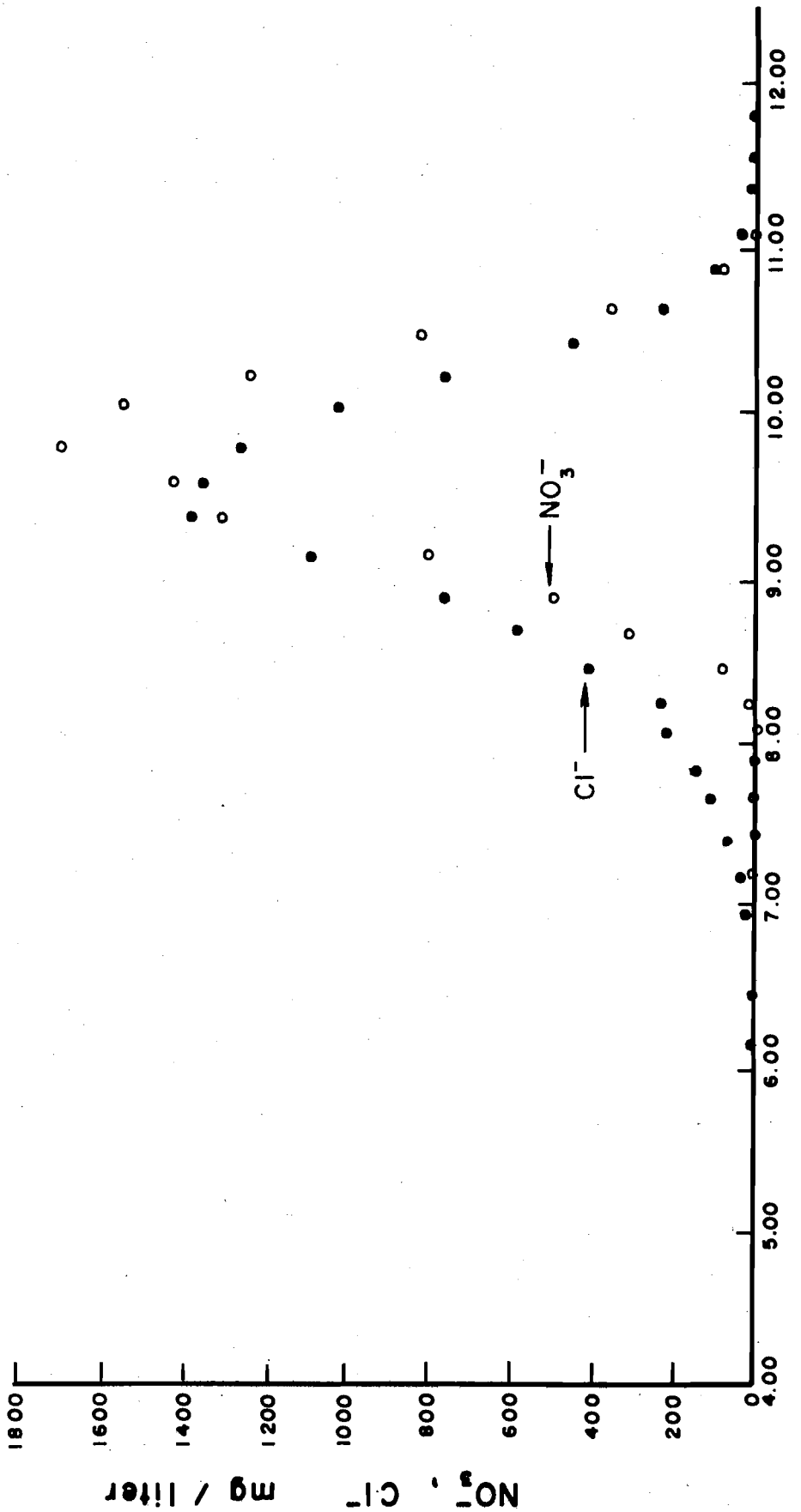


FIGURE 20. BREAKTHROUGH CURVES FOR TUBE 6, SAWDUST, AT 18°C WITH AN AVERAGE FLOW OF 480 ml/DAY AND A 2.2 PERCENT LOSS OF ADDED NITRATE

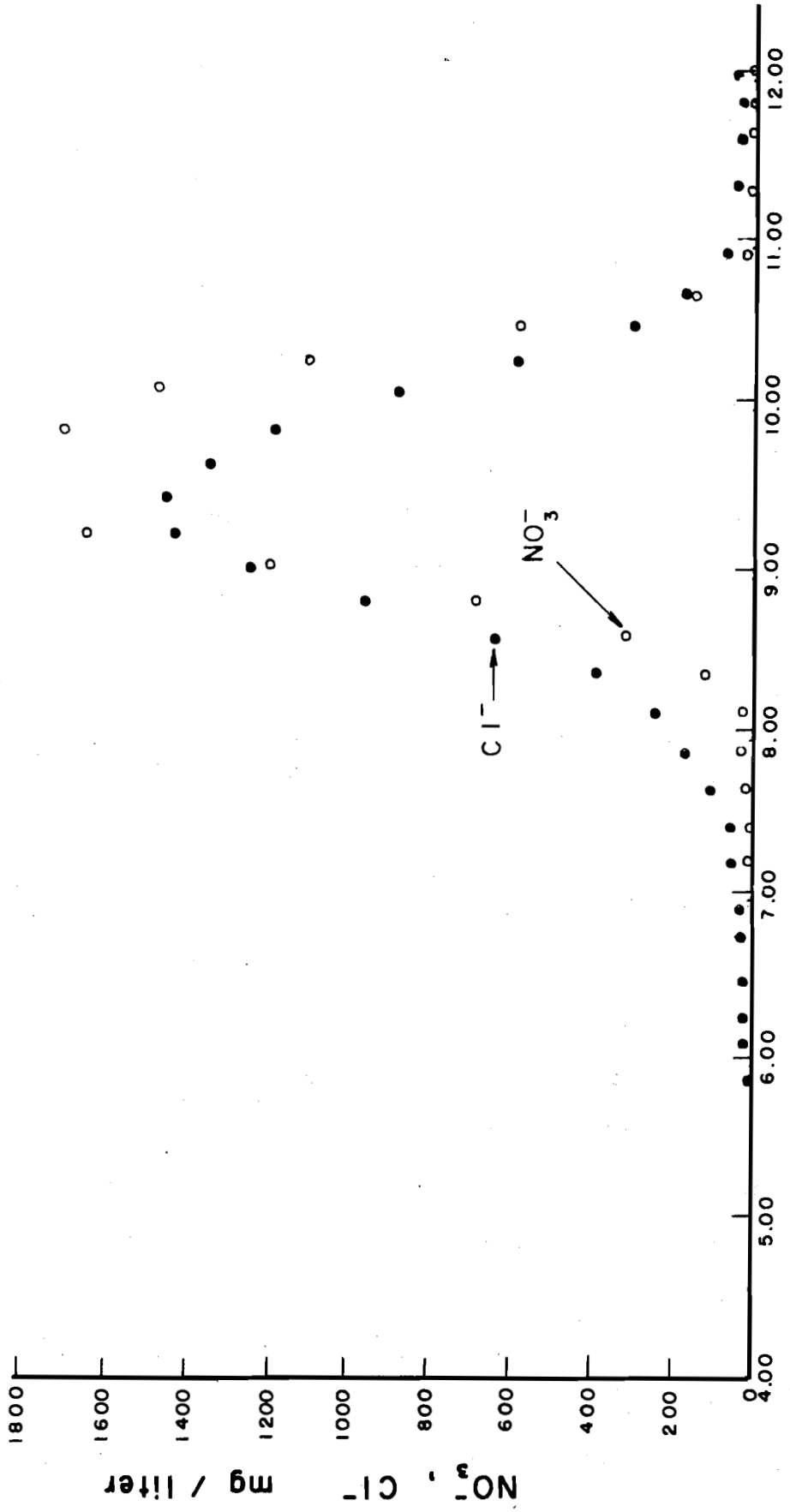


FIGURE 21. BREAKTHROUGH CURVES FOR TUBE 6, SAWDUST, AT 13°C WITH AN AVERAGE FLOW OF 450 ml/DAY AND AN 11.9 PERCENT GAIN OF ADDED NITRATE

The only major irregularity of these curves was the very high nitrate peak at 13°C, which was not accompanied by a corresponding chloride peak. In Tube 6, the peak chloride concentration was approximately 1450 ppm in each test but peak nitrate concentration did not appear temperature related. In each test, the nitrate peak did exceed the peak chloride concentration, the highest chloride peak reaching 2000 ppm at 13°C. No irregularities occurred in the breakthrough curves of Tube 6.

As in Tube 5, the check replicate, the sawdust treatments showed nitrate gains in flow increments at the simultaneous nitrate/chloride peaks compared to relatively small nitrate losses occurring at the tails of the breakthrough curves. As indicated in Table 3, total masses of nitrate recovered for Tube 2 were 2635 and 3441 milligrams at 18°C and 13°C, respectively, and 2783 milligrams for Tube 6 at 13°C. In each of these cases total chloride mass recovered was slightly less than 2500 milligrams.

5. Tubes 3 and 4 - Methanol Added

In contrast to the other carbon treatments, the columns treated with 1000 ppm methanol showed low recovery of nitrates at all three temperatures tested. The breakthrough curves of Tube 3 are shown in Figures 22, 23, and 24, and those of Tube 4 in Figures 25, 26, and 27.

The nitrate curve was lower than the chloride curve throughout each test in both replications, thus losses of added nitrate occurred at each flow increment over the entire curve. Also, the greatest nitrate losses in individual increments of

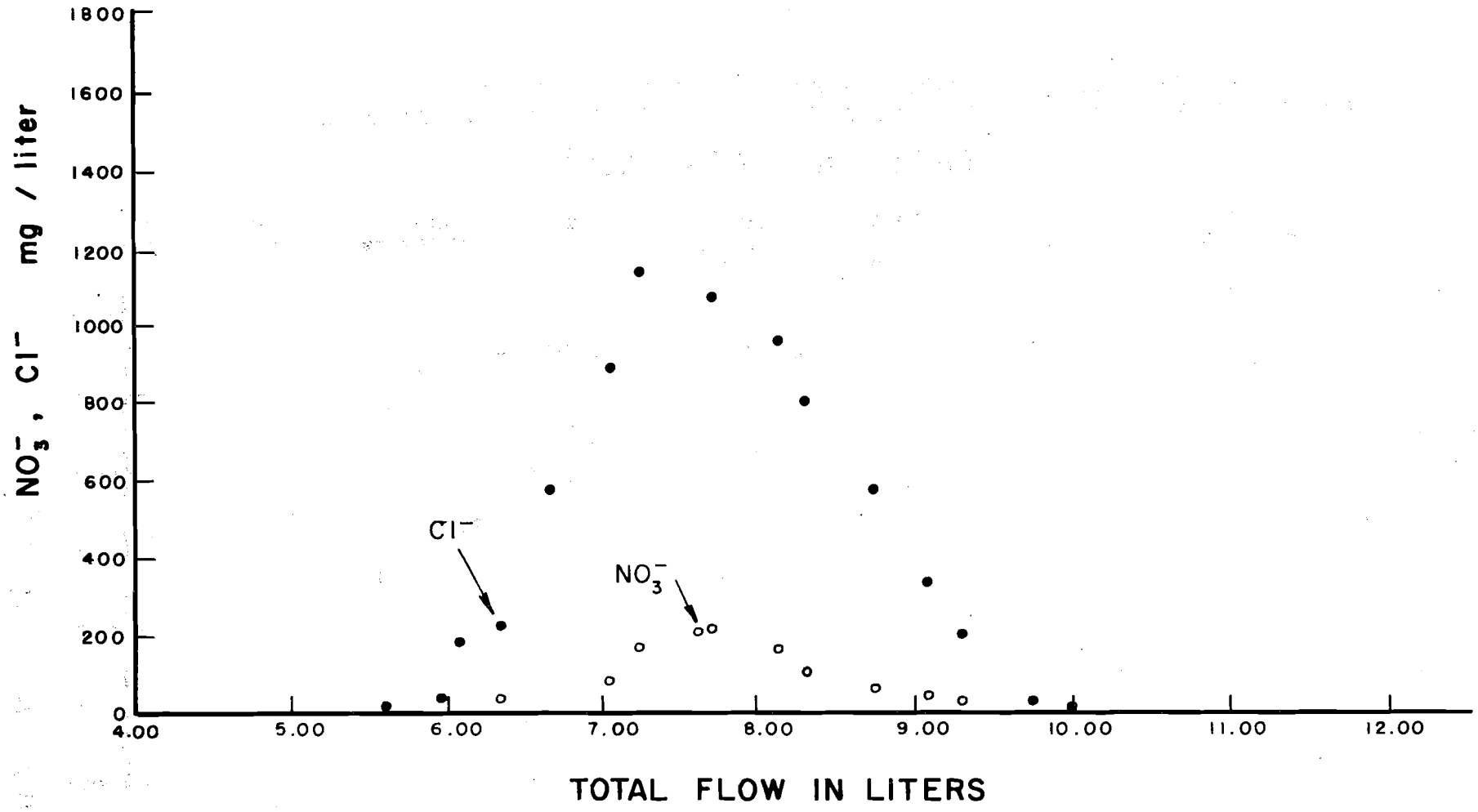


FIGURE 22. BREAKTHROUGH CURVES FOR TUBE 3, METHANOL, AT 24°C WITH AN AVERAGE FLOW OF 425 ml/DAY AND AN 86 PERCENT LOSS OF ADDED NITRATE

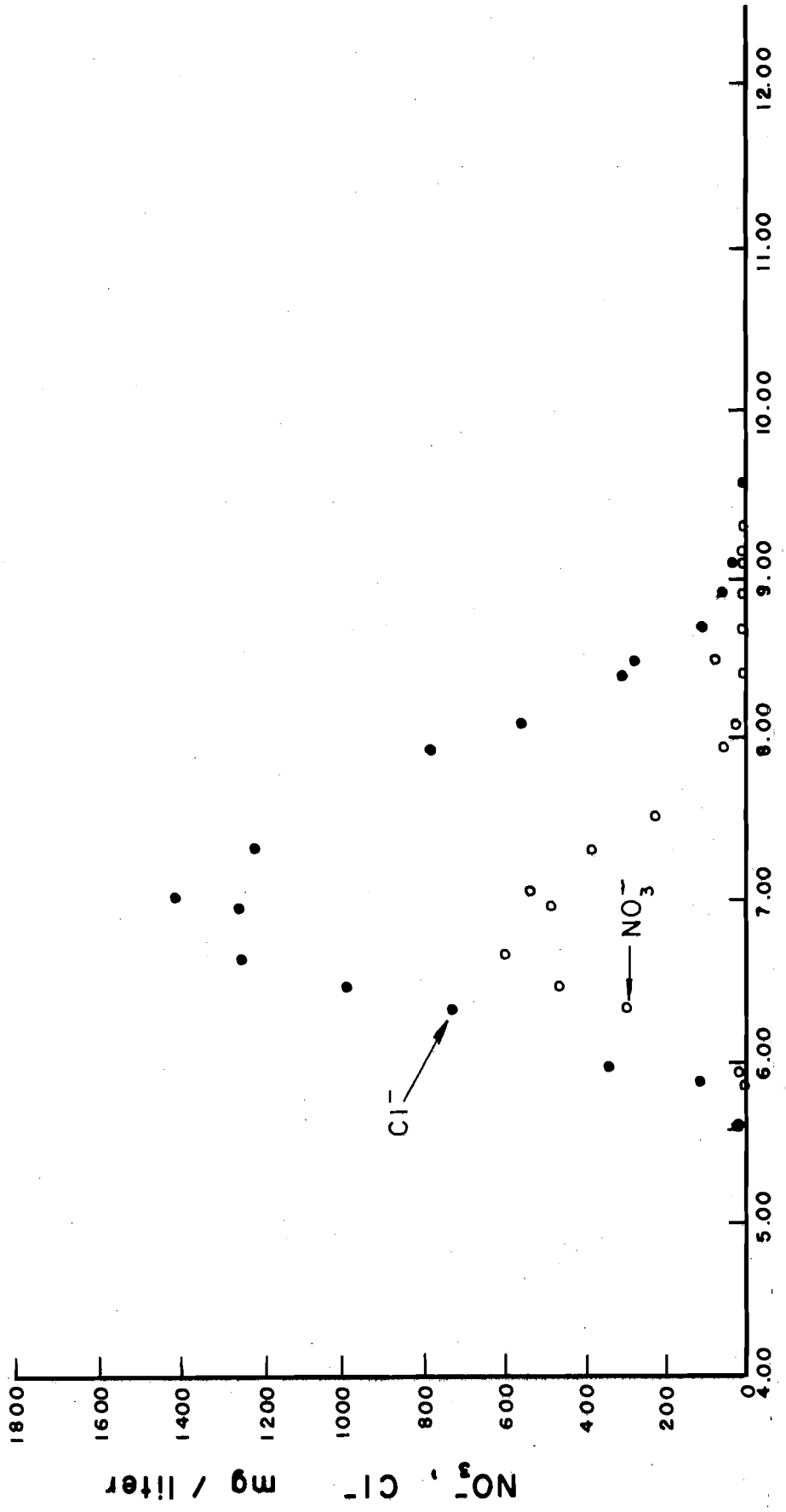


FIGURE 23. BREAKTHROUGH CURVES FOR TUBE 3, METHANOL, AT 18°C WITH AN AVERAGE FLOW OF 400 ml/DAY AND A 70 PERCENT LOSS OF ADDED NITRATE

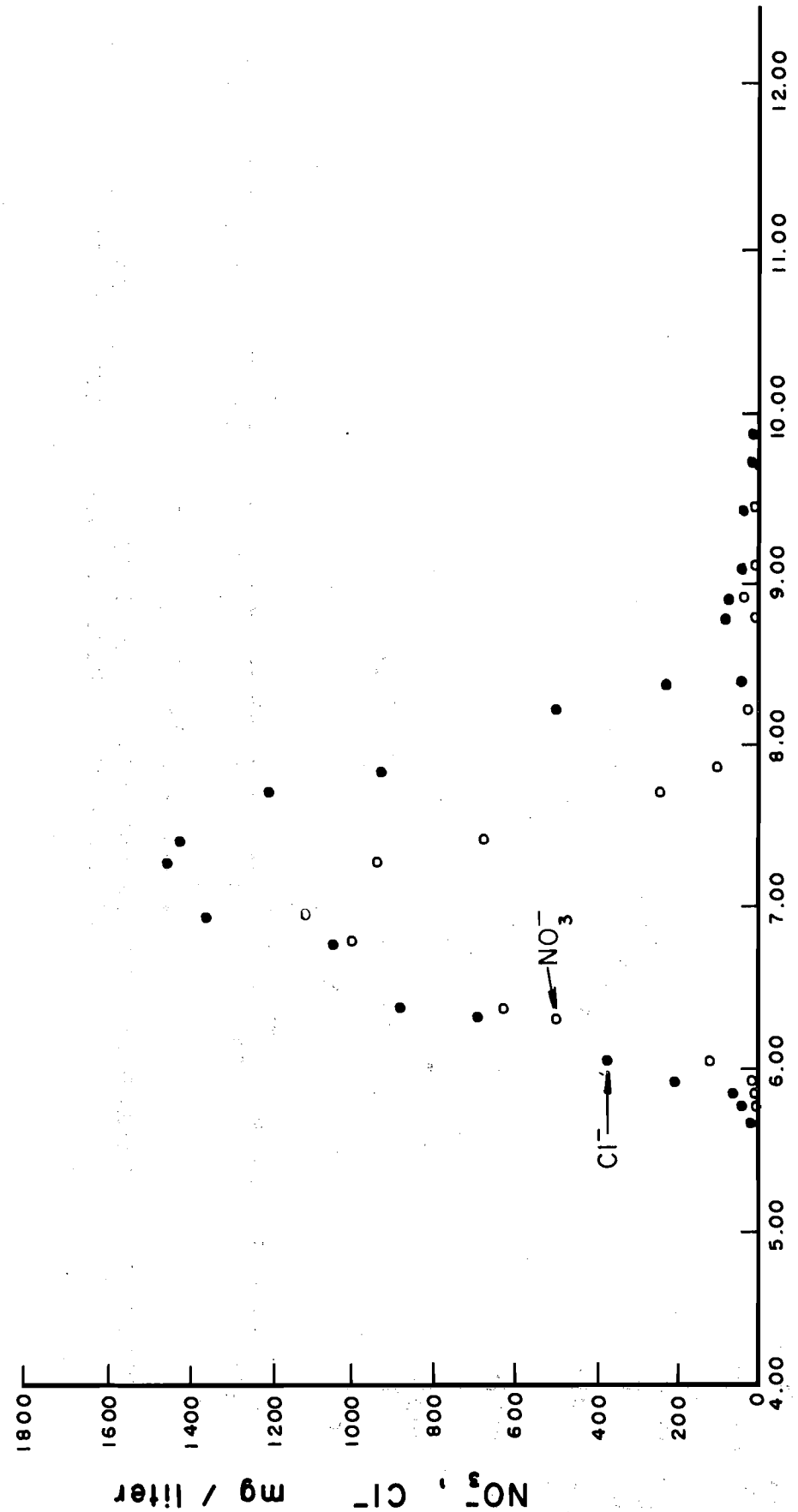


FIGURE 24. BREAKTHROUGH CURVES FOR TUBE 3, METHANOL, AT 13°C WITH AN AVERAGE FLOW OF 410 ml/DAY AND A 46 PERCENT LOSS OF ADDED NITRATE

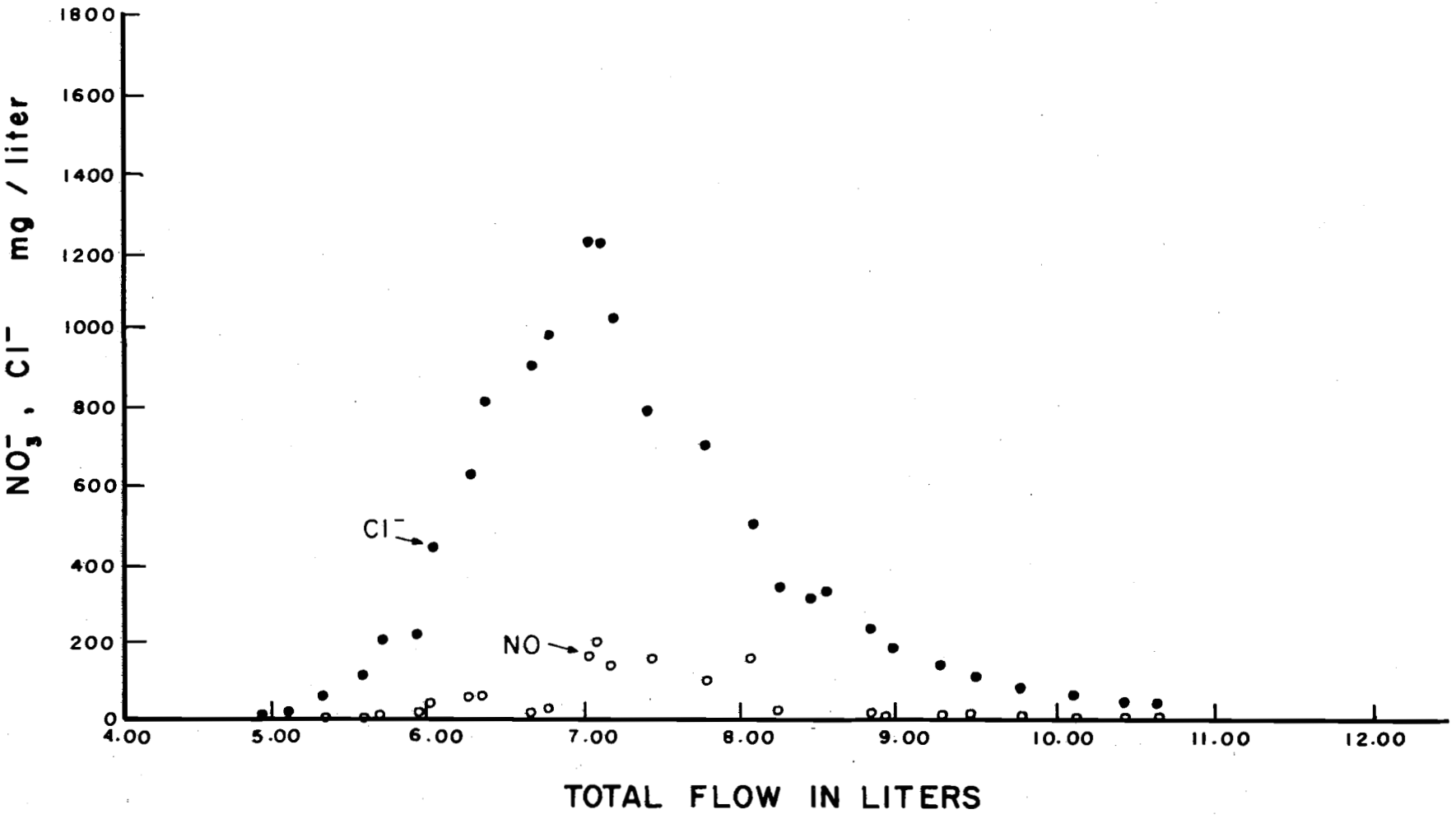


FIGURE 25. BREAKTHROUGH CURVES FOR TUBE 4, METHANOL, AT 24°C WITH AN AVERAGE FLOW OF 445 ml/DAY AND AN 89 PERCENT LOSS OF ADDED NITRATE

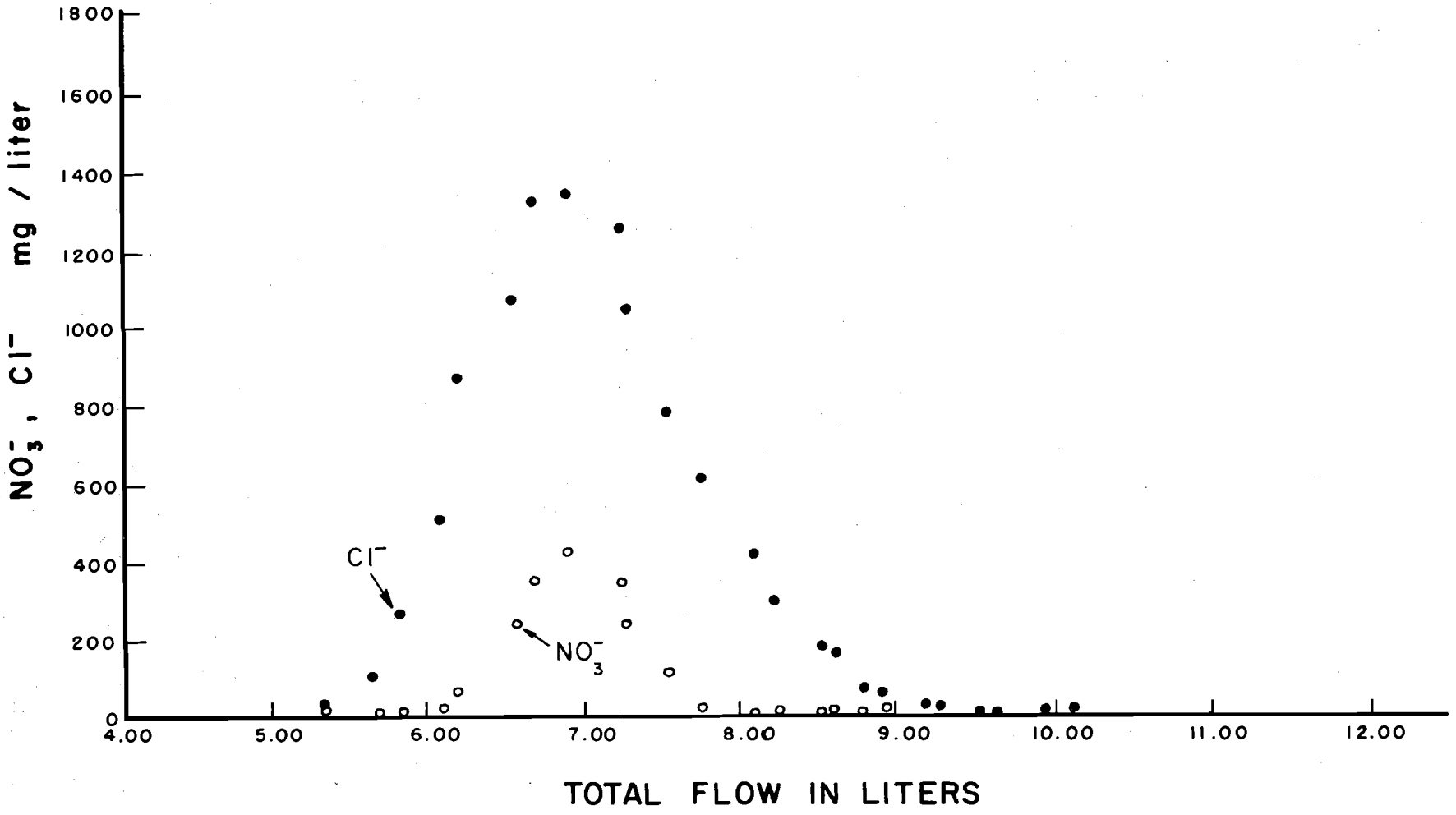


FIGURE 26. BREAKTHROUGH CURVES FOR TUBE 4, METHANOL, AT 18°C WITH AN AVERAGE FLOW OF 400 ml/DAY AND AN 81 PERCENT LOSS OF ADDED NITRATE

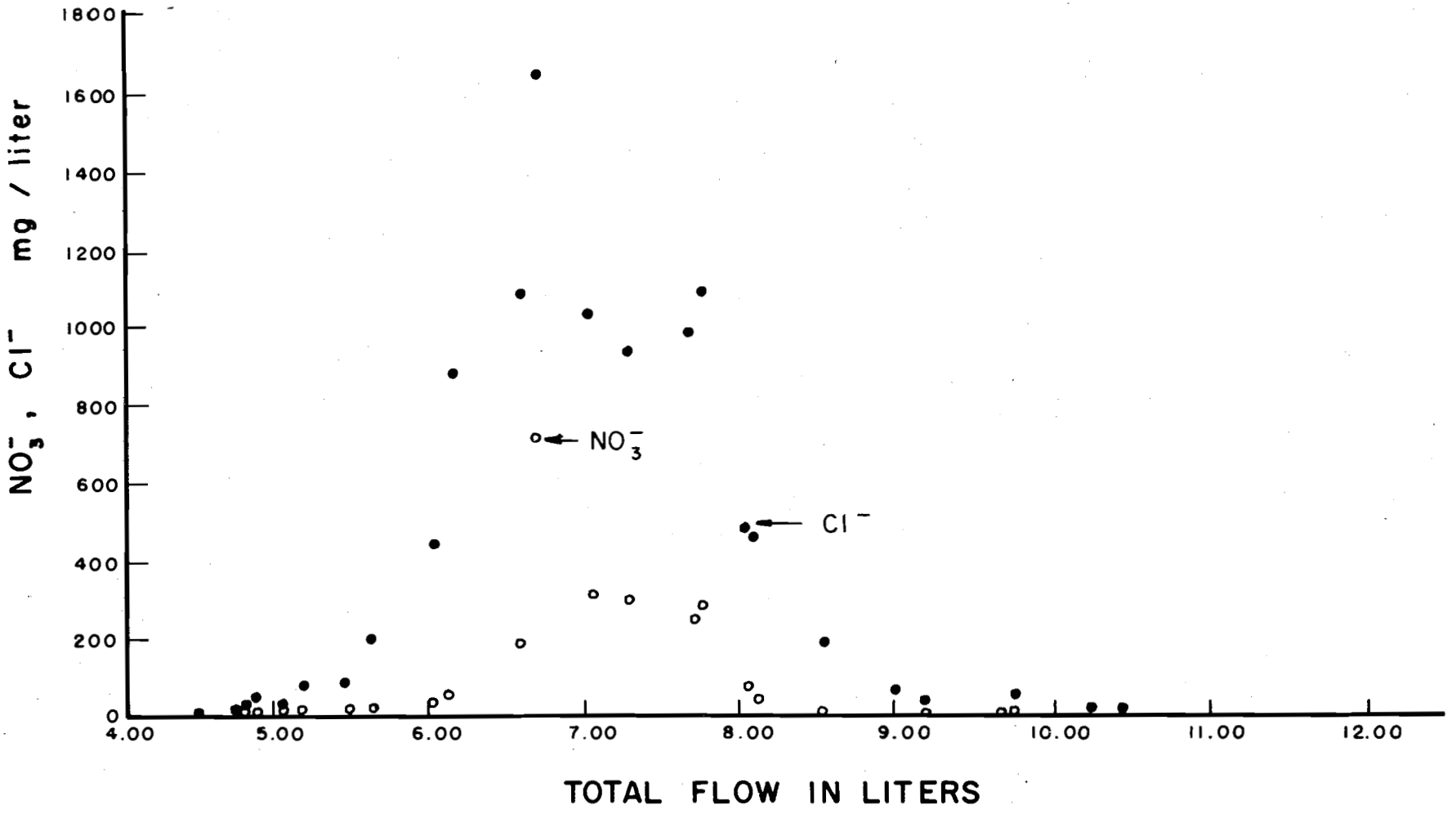


FIGURE 27. BREAKTHROUGH CURVES FOR TUBE 4, METHANOL, AT 13°C WITH AN AVERAGE FLOW OF 390 ml/DAY AND A 78 PERCENT LOSS OF ADDED NITRATE

flow occurred at or near the peaks of nitrate and chloride concentration rather than at the tails of the breakthrough curves as noted in the other columns. During 12 hour flow periods, nitrate losses of approximately 1000 ppm were found at the nitrate/chloride peak of both Tubes 3 and 4 during the 24°C test.

In Tube 4, the nitrate peak and general breakthrough curve corresponded well with the chloride peak and curve characteristics. The irregularities of the curves at 24°C and 18°C may have been due to gas blockage of the sand pores, since the gas collection device for this column indicated significant gas quantities were being produced.

The breakthrough curves of Tube 3 were relatively smooth. The nitrate peak, however, did not occur at the same flow increment in which the chloride peak occurred. At the 24°C test, the nitrate peak was slightly behind the chloride peak and at both colder temperatures the nitrate peak was slightly in front of the chloride peak. Again, gas production by denitrifying bacteria may have altered the pattern of the breakthrough curves for this column. Further evidence of gas blockage was the steep hydraulic gradient and the lower total flow required to move the nitrate/chloride application through both methanol columns as shown in Table 3.

As shown in Figure 5, Tube 3 exhibited a greater temperature effect on nitrate losses than Tube 4. However, results of the total organic carbon analysis and trends in redox potentials recorded did not show that the denitrification environment was more favorable in Tube 4.

D. Redox Potentials

Redox potentials were recorded daily for each column at a point 15 centimeters from the effluent end of the columns. A summary of these potentials including corresponding incremental nitrate and chloride concentration are presented in Appendix A. Tables A-1 through A-3 include the 24°C test; Tables A-4 through A-6 include the 18°C test; and Tables A-7 through A-9 include the redox potential readings recorded during the 13°C test. Carbon treatment replications are paired for comparison in each table.

All of the redox potentials recorded for the columns were within the moderately reduced to highly reduced range of redox potentials usually encountered in water-logged soils, as shown in Figure 28. As referred to previously (23), nitrate becomes unstable at a redox potential of approximately +320 millivolts at a pH of 7.

FIGURE 28
RANGE OF REDOX POTENTIAL IN WATERLOGGED SOILS
(millivolts)

Waterlogged Soil							Aerated Soil	
Highly Reduced			Moderately Reduced					Oxidized
-300	-200	-100	0	+100	+200	+300	+400	+500

The treatment checks were mutually different in oxidation-reduction characteristics. Redox potential in Tube 1 at all three temperatures ranged from -200 to -100 millivolts and slight increases in redox potential were exhibited as the nitrate/chloride application passed out of the column for the 18°C and 13°C tests. Redox potential in this check replicate

remained fairly constant at -200 to -190 millivolts during the 24°C test. Conversely, the other check replicate, Tube 5, showed a sharp increase in redox potential from approximately -500 to -75 millivolts as the nitrate/chloride application passed through the column at each temperature tested. The redox potential decreased to -500 millivolts as each test neared completion.

The sawdust treated columns, Tubes 2 and 6, also showed dissimilar behavior with respect to redox potential. Tube 2 showed an increase in redox potential during each test from about 180 to 230 millivolts as the nitrate/chloride application moved through the column, returning to 180 millivolts after each test. The second sawdust replication, Tube 6, also showed a slight increase of 40 to 80 millivolts and subsequent decrease in redox potential, although the redox potential did not return to the same level after passage of the nitrate/chloride front. Redox potential ranged from -90 to -10 millivolts at 24°C, +70 to +140 millivolts at 18°C, and +140 to +180 millivolts at 13°C.

The increase in redox potential accompanying the passage of the nitrate/chloride application in the sawdust treated columns was accompanied by a yellow to light orange color in the effluent water. Particulates of reduced organic matter were also evident in the effluent at these times, suggesting that increases in redox potential resulted in some mobilization. The color of the effluent water was not observed until contact with the atmosphere was made in the effluent tubes.

Tubes 3 and 4 were replications of the 1000 ppm methanol treatment. Both columns were highly reduced, with redox

potential returning to approximately -520 millivolts between tests. Tube 3 experienced an increase of 40 and 70 millivolts at 24°C and 18°C, respectively, but an increase of approximately 500 millivolts at 13°C. Redox potential in Tube 4 increased to -40 millivolts at 24°C, -90 millivolts at 18°C and -140 millivolts at 13°C from a stable level of -520 millivolts exhibited before each test. The redox potential returned to approximately -520 millivolts at the completion of each test.

All sand columns except Tube 1 exhibited some increase in redox potential as the nitrate/chloride application was recovered from the column. Since nitrate acts as an electron acceptor in the denitrification process, a deficiency of electrons may occur due to microbial action accompanying high nitrate concentrations.

E. Total Organic Carbon Analysis

1. Stoichiometric Requirements for Denitrification

As previously discussed, denitrification is basically considered a two step process accomplished by facultative anaerobes in which nitrate is first reduced to nitrite and then to nitrogen gas. The process is shown in the following chemical reactions, using methanol as a soluble carbon source. These reactions are revised slightly from those referred to earlier by McCarty, et al. (15).

First step: $NO_3^- + 1/3CH_3OH = NO_2^- + 1/3CO_2 + 2/3H_2O$

Second step: $NO_2^- + \frac{1}{2}CH_3OH = \frac{1}{2}N_2 + \frac{1}{2}CO_2 + \frac{1}{2}H_2O + OH^-$

Overall: $NO_3^- + 5/6CH_3OH = \frac{1}{2}N_2 + 5/6CO_2 + 7/6H_2O + OH^-$

According to the stoichiometry of these equations, reduction of one mole of nitrate to nitrogen gas requires 5/6 mole of methanol. However, additional methanol must also be provided for bacterial growth. To account for this extra carbon, McCarty, et al. (15) define a consumptive ratio, which is the ratio of the total quantity of an organic chemical used during denitrification to the stoichiometric organic chemical requirement for denitrification and deoxygenation alone. This ratio is estimated to be 1.3 for methanol, thus increasing the amount of methanol required for complete denitrification, including bacterial assimilation, to 1.08 moles per mole of nitrate.

2. Experimental Results

The results of the total organic carbon analysis for the 24°C and 13°C tests are listed in Table 5. Data for the 18°C test was not included due to breakdown of the Beckman Total Carbon Analyzer. Samples for carbon analysis were taken from the same samples previously analyzed for nitrate and chloride. Nitrate and chloride concentrations measured at the same time are also included in Table 5.

Samples analyzed were taken from the demineralized water supply reservoir treated with 1000 ppm of methanol, and also from the effluent of the methanol treated columns. The supply reservoir samples are referred to as methanol inlet samples in Table 5. The carbon check columns and columns treated with sawdust were not included in this analysis due to the lack of knowledge of the amount of soluble carbon available for denitrification and the relatively small extent of added nitrate losses within these columns.

TABLE 5

TOTAL ORGANIC CARBON RESULTS

24°C

Date	T. O. C. (mg/l)	NO_3^- (mg/l)	Cl^- (mg/l)
	TUBE #3		
9/19/72	165	6	0
9/21/72	110	4	0
9/24/72	50	39	220
9/25/72	15	72	880
9/26/72	80	200	1090*
9/27/72	45	172	940
9/28/72	40	65	570
9/30/72	45	9	35
	TUBE #4		
9/19/72	45	30	225
9/21/72	35	41	920
9/24/72	45	110	810*
9/25/72	40	42	510
9/26/72	50	32	325
9/27/72	40	28	245
9/28/72	20	22	155
9/30/72	65	10	55
*Chloride Peak			
Tube 1, 10/3/72	20	138	170
Tube 2, 10/3/72	20	-	-
Tube 5, 10/3/72	5	320	340
Tube 6, 10/3/72	45	-	-
Methanol Inlet 9/19/72	440		
Methanol Inlet 9/21/72	440		
Methanol Inlet 9/26/72	395		
Methanol Inlet 9/28/72	380		

TABLE 5 (Cont'd)

TOTAL ORGANIC CARBON RESULTS

$13^{o}C$

Date	T. O. C. (mg/l)	NO_3^- (mg/l)	Cl^- (mg/l)
	TUBE #3		
11/22/72	90	4	0
11/24/72	100	6	45
11/26/72	95	500	695
11/28/72	90	1100	1360
11/30/72	70	120	940*
12/ 2/72	85	60	90
12/ 4/72	80	21	50
12/ 6/72	55	8	40
	TUBE #4		
11/22/72	75	3	10
11/24/72	100	9	35
11/26/72	135	9	35
11/28/72	85	38	200
11/30/72	95	750	1650*
12/ 2/72	60	295	1100
12/ 4/72	100	17	200
12/ 6/72	90	8	60

*Chloride Peak

Methanol Inlet 11/27/72	425
Methanol Inlet 11/29/72	420
Methanol Inlet 12/ 4/72	425
Methanol Inlet 12/11/72	360

An average of 410 ppm of total organic carbon was present in the methanol treated water supply reservoir over the two test periods analyzed. Converting this figure to methanol resulted in 1090 ppm, compared to the desired level of 1000 ppm. The difference is probably due to experimental error.

More methanol should have been consumed in the 24°C test than the 13°C test as greater losses of added nitrate occurred. The peak chloride concentration for the 24°C test for both columns was 1090 ppm as indicated in Table 5. Since chloride served as a basis for determining added nitrate losses, 1090 ppm of nitrate was assumed to be the nitrate concentration at the chloride peak, if the nitrate had not been transformed or held within the column. 1090 ppm of nitrate converts to 0.0175 moles per liter. To satisfy complete requirements for denitrification and microbial assimilation for 0.0175 moles per liter of nitrate, multiplication by 1.08 mole of methanol required per mole of nitrate yields 0.020 moles per liter of methanol required. Conversion of 1090 ppm of methanol available in the water supply results in 0.0340 moles per liter of methanol available. Thus, methanol available to the denitrifying organisms was more than adequate for complete conversion of up to 1090 ppm of nitrate, yet Table 3 indicates the maximum loss of added nitrate during the 24°C test was only 85% based on the total chloride mass recovered. Table 5 shows that concentrations of total organic carbon in the effluent samples ranged from 15 to 165 ppm in the 24°C test and 55 to 135 ppm in the 13°C test. No trends in

carbon concentration were apparent between tests, although it appears less carbon was used in the 13°C test.

Since tests for total organic carbon were only taken after the nitrate/chloride peak was first detected, the effluent carbon concentrations shown in Table 5 cannot be averaged over the entire test flow period. Tests were only made on samples included in approximately the last half of the test flow periods. Thus, no attempt was made to calculate total amount of methanol used. While consumptive ratio would be expected to increase with temperature as nitrate losses due to denitrification increase, insufficient samples were analyzed to indicate this trend.

F. Gas Analysis

The effluent gas collection devices were effective in collecting gases accumulated at the top surface of each column, although facilities to analyze the gases collected were not available until the tests were over half completed. Gases were released during the 24°C test, which exhibited the most gas production. Approximately four days after the nitrate/chloride peak had been detected in the effluent of Tube 3 during the 18°C test, two identical gas samples were extracted from the collection device, dried in a dry ice moisture trap, and analyzed on the mass spectrograph as described in Procedure. Attempts were made only to identify qualitatively the unit mass peaks of the spectrometer results, and to compare the relative peak magnitudes. Unit masses of 0 to 18 and 19 to 44 were found. Both gas samples analyzed gave the same results.

The gaseous products expected in Tube 3, which was a methanol treatment, were nitrogen gas, carbon dioxide, and water vapor, according to the nitrate-methanol denitrification reaction discussed previously. Nitrous oxide was also expected as an intermediate gas product in the formation of molecular nitrogen.

Several major peaks were indicated, with each peak possibly representing more than one compound. One large peak at unit mass 28 indicated nitrogen gas, while a peak at 44 represented either carbon dioxide or nitrous oxide. Other peaks occurring at unit masses 12 through 18 probably indicated hydrocarbon compounds expected under the anaerobic conditions of the denitrification environment. Relatively small peaks at 32 and 40 were oxygen gas and argon gas respectively, indicating some contamination of the gas sample may have occurred.

The relative magnitudes of the peaks indicated gaseous products expected from the denitrification process were predominant, and provided some assurance that denitrification may have been a major factor in the extensive nitrate losses that occurred in the methanol treated columns. However, nitrogen gas was used to saturate the water supply and did affect these results.

G. Problems Encountered in Experimental Procedure

1. Flow Rate

Daily flow rates were adjusted by controlling the elevation of the effluent tubes with respect to the demineralized water supply reservoirs. Due to the small daily flows and

relatively long columns, small changes of one or two centimeters in head loss resulted in changes of approximately 100 milliliters in daily flow. Since the desired flow rate was 500 milliliters per day, frequent maintenance of head loss was required. This problem was critical with the check treatment columns, in which a head loss of only two centimeters was needed to obtain the desired daily flow.

Adjustment of head loss was most frequently required for the methanol treated columns which showed the greatest losses of added nitrates and also the most gas accumulation within the columns. Gas accumulation at the entrance to the methanol treated columns was sufficient to completely block flow into the columns. Small holes were drilled in the top of the plexiglass cylinders near the entrance to the columns to release the accumulated gases. Gas buildup in other portions of the methanol treated columns required lowering of the effluent tubes to maintain daily flow rates. According to Darcy's flow equation for saturated soils,

$$Q = AK \frac{h}{L}$$

where Q is flow, A is cross-sectional flow area, h is head loss, and L is the flow length, if h, L and K remain constant, flow will decrease as gas accumulation reduces the cross-sectional flow area. To maintain constant flow, h is increased as A decreases. Pore velocity should also increase as the total pore volume decreases.

Head loss adjustments were infrequently needed in the check treatment and sawdust treatment columns. Added nitrate

losses were relatively small and gas accumulation was not as serious as with the methanol treatment.

Some of the flow adjustment problems may have been related to the horizontal alignment and length of the columns. While buildup of gases within the long horizontal columns did induce small pressures on the denitrifying environment, and the length of the columns allowed considerable time for bacterial action to occur, this is a comparable situation to what might be found in the field near a tile drain.

2. Piezometer Readings

Initially, the piezometers were intended for use as indicators of pressures caused by gas accumulation within the columns due to the denitrification process. However, the piezometer readings were also found to be valuable as indicators of variations in head loss distribution along the column and were useful in making flow rate adjustments.

Although the piezometers were connected to the bottom of the sand columns, gas accumulation in the vicinity of the connections was often sufficient to cause gas bubbles to enter the piezometer tubing. Removal of these bubbles could be accomplished by adding water to the manometer to back pressure the piezometer so that the gases were forced back into the column. The frequent occurrences requiring this type of maintenance rendered regular and precise piezometer readings impossible. Piezometer trends indicated increases in water pressure head of 7 to 10 centimeters for particular piezometers in the methanol

treatment columns as the nitrate/chloride application moved through with associated gas development.

3. Detection of the Applied Nitrate/Chloride Front

Although the nitrate/chloride application had to follow the same horizontal distance through each sand column, a definite trend was noted between carbon treatments and the flow required to move the application through the column. Less flow was required for the nitrate/chloride application to move completely through the methanol treatment columns than the sawdust treatment columns, and less for the sawdust than the check treatment columns. This phenomenon was apparent at each temperature tested and can be seen in the total flow per column, effective pore space and flow to peak in Table 4.

These flow patterns may be due to desaturation of the voids and lower effective pore space within the sand columns due to gas accumulation, especially in the methanol treatment columns. Although nitrate losses were slightly higher in the check than the sawdust treatment columns, biological activity was greater in the sawdust treatments as more gaseous buildup was observed.

Desaturation of the columns was observed to require less total flow to transfer the nitrate/chloride application through the column. Corey, et al. (8) also observed this trend in working with denitrification breakthrough curves in unsaturated soil columns. Their results indicated less flow was required to transfer nitrates as lower soil moisture contents were tested. The investigators offered two possible explanations for this

behavior: (1) there was less water ahead of the advancing ionic solution; and (2) some water was isolated in parts of the soil column and was not displaced by the ionic solution. In consideration of these flow interference effects on ionic concentrations, no denitrification study can be conducted at constant soil moisture content, for production of nitrogenous gases decreases the effective pore space for water. To have a steady state condition, a system must be provided to constantly remove the gas produced and replace it with water. This procedure was not done in this study.

Gaseous denitrification products replacing water as the nitrate/chloride solution advances may explain the relatively narrow breakthrough curves associated with the methanol treatment columns. Less diffusion and dispersion would occur if less water was present in the column, thus producing a narrower breakthrough curve with a higher peak ionic concentration. Increased pore velocities due to desaturation would allow less time for diffusion.

The temperature tests were conducted with the highest temperature first ($24^{o}C$) and progressed to the coldest temperature ($13^{o}C$). Although periods of five to ten days were allowed between tests to permit flow stabilization within the columns, accumulated gas may have been retained between tests. Furthermore, the columns were used for preliminary tests which also may have been a source of gas accumulation.

Flow rates were averaged over twenty to thirty day test periods, but variation in daily flow ranged from 0 to over 1000

milliliters. Attempts were made to evenly distribute large flow variations over the test periods. To keep a uniform time to peaks, the average daily flow rate for the methanol treatment columns was approximately 1000 milliliters less than most of the other columns, while Tube 1 was run at higher flow rates than the average. This was done to keep the detention time of the applied nitrate reasonably the same between the columns so that time of the applied nitrate within columns would not be a significant variable in this study.

4. Chloride Ion Interference in Nitrate Analysis

The fact that other ions introduce errors in determining nitrate concentration by the specific ion method was recognized. Since chloride was applied to the columns at an equal concentrations with nitrate and the chloride ion was not removed from effluent samples before nitrate analysis, a study was made to determine the magnitude of possible chloride ion interference.

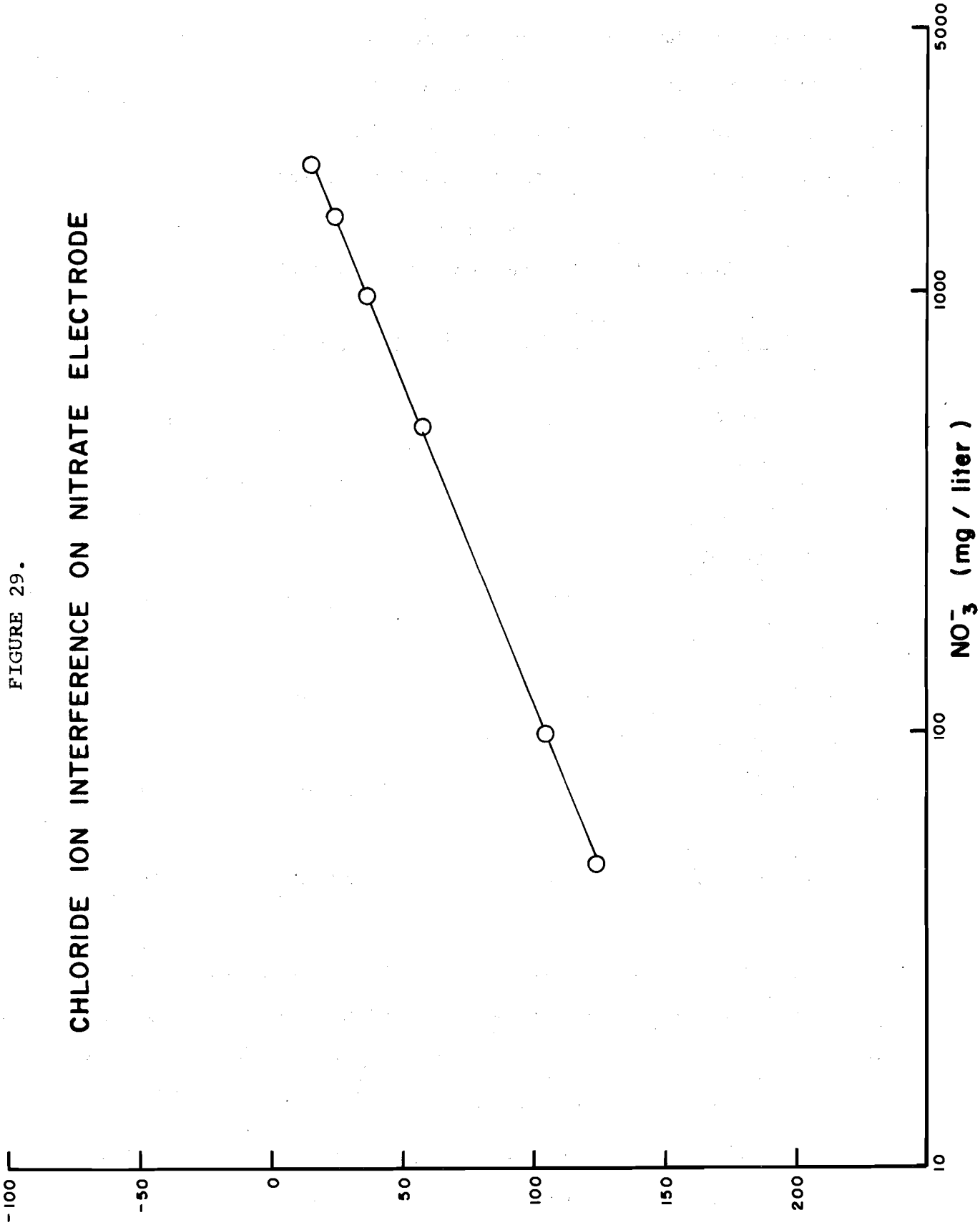
The presence of anions other than nitrate in solutions tested using a nitrate sensitive electrode lowers the measured potential readout for the solution. Plotting the potential readout on a semilogarithmic calibration curve for the nitrate electrode results in a higher nitrate concentration for the solution than is actually present. The magnitude of error due to interfering ions depends on the interference factor for the additional anion in solution, as reflected in the following equation for calculating percentage error:

$$\% \text{ Error} = \frac{(100\%)(A_x)(K_x)}{(A_{NO_3^-}}$$

where A_x and $A_{NO_3^-}$ are the activities of the interfering and nitrate ions, respectively, and K_x is the interference factor for the interfering ion. The interference factor for chloride is 4×10^{-3}. According to the percentage error equation, the chloride ion activity would have to be two orders of magnitude greater than the nitrate ion activity in solution to have a significant effect. For instance, a chloride activity ten times greater than nitrate activity would cause an error of only 4%. Only in the methanol treatment columns were there cases where the chloride activity was ont order of magnitude greater than the nitrate activity. Errors of this magnitude may have been accompanied by electrode calibration errors. Small corrections (less than 10%) were made for effluent nitrate concentrations in the methanol treatment columns.

To aid in determining the effect of equal nitrate and chloride concentrations in solution at different levels of concentration, a 50,000 ppm nitrate and 50,000 ppm chloride solution similar to that used as a nitrate source in this study, was diluted to 2000, 1500, 1000, 500, 100, and 50 ppm. A similar solution containing 50,000 ppm of nitrate only was diluted to the same concentrations. Samples of both sets of dilutions each having the same nitrate concentrations were then tested using the nitrate electrode. The millivolt readings for the 50,000 ppm nitrate dilutions were used to plot a calibration curve on semilogarithmic paper shown in Figure 29. Millivolt readings for both sets of dilutions are contained in Table 6. Comparison of these values reveals that the chloride ion had little effect

FIGURE 29.


CHLORIDE ION INTERFERENCE ON NITRATE ELECTRODE
-100
-50
0
50
100
150
200
10
100
1000
5000
NO_3^- (mg / liter)

in detection of the actual nitrate concentration in solution where both ions are equal over a wide range of concentrations. The range of concentration used is similar to those encountered in this study for all but the methanol treatments.

TABLE 6

CHLORIDE ION INTERFERENCE ON NITRATE ELECTRODE

Concentration	Millivolt reading	Concentration	Millivolt reading
50 ppm NO_3^-			
50 ppm Cl^-	124	50 ppm NO_3^-	122
100 ppm NO_3^-			
100 ppm Cl^-	105	100 ppm NO_3^-	104
500 ppm NO_3^-			
500 ppm Cl^-	58	500 ppm NO_3^-	58
1000 ppm NO_3^-			
1000 ppm Cl^-	37	1000 ppm NO_3^-	40
1500 ppm NO_3^-			
1500 ppm Cl^-	25	1500 ppm NO_3^-	27
2000 ppm NO_3^-			
2000 ppm Cl^-	17	2000 ppm NO_3^-	18

Table 7 contains chloride concentrations for the set of dilutions from the 50,000 ppm nitrate-50,000 ppm chloride solution. Chloride concentration was determined using the argentometric method described in the procedure.

The nitrite ion also interfers with the nitrate sensitive electrode. Nitrite errors were not considered, as nitrite

in the column effluent would have oxidized to nitrate as the effluent came in contact with the atmosphere as it was collected.

TABLE 7

CHLORIDE ANALYSIS OF 50,000 ppm DILUTIONS

Assumed chloride concentration	Actual chloride concentration
50 ppm	70 ppm
100 ppm	130 ppm
500 ppm	510 ppm
1000 ppm	990 ppm
1500 ppm	1450 ppm
2000 ppm	1930 ppm

5. Excess Nitrate Mass Recovery

As noted in previous discussion, the nitrate peak exceeded the chloride peak at each temperature tested for both the check and sawdust treatments except for Tube 1 at 24°C. This trend was accompanied by recovery of total nitrate mass that exceeded total chloride mass recovered in Tubes 2 and 5 at 18°C and 13°C and in Tube 6 at 13°C. Total mass applied in a single test was 2500 milligrams of nitrate and 2500 milligrams of chloride. Table 3 shows the excess nitrate mass recovered based on the total chloride mass recovered. These are expressed as a negative loss.

The excess nitrate mass recovered may be due to four possible sources:

(1) release of nitrate fixed by bacterial assimilation during previous tests conducted at higher temperatures;

(two 24°C tests with similar procedure were conducted before this study).

(2) release of nitrate fixed by ion exchange combined with differences in the effective diffusion of nitrate compared to chloride.

(3) release of nitrate accumulated in unsaturated pore spaces or pore volumes not displaced during flow.

(4) chloride ion interference of the nitrate electrode used to determine nitrate concentration in the effluent samples.

Higher nitrate than chloride concentrations were coincident with the center of the breakthrough curves. This could have resulted either from the increased microbial activity accompanying the nitrate/chloride front releasing nitrate assimilated by denitrifying bacteria during previous tests, or a difference in the effective diffusion of nitrate as compared to chloride.

Errors in nitrate concentration due to chloride ion interference of the nitrate electrode could not be responsible alone for the magnitude of excess nitrate concentrations recovered. As discussed previously, chloride activity must be at least one order of magnitude greater than nitrate activity to cause significant errors.

Note that in the columns discussed, the total chloride mass recovered, upon which excess nitrate recoveries were based, closely approximated the original chloride mass applied of 2500 milligrams. Chloride mass recovery ranged from 2300 to 2550 milligrams in all the columns under discussion.

VII. RECOMMENDATIONS FOR FURTHER STUDY

A. Laboratory Investigations

Two major problems were associated with this study: erratic flow due to gas accumulation within the columns and recovery of excess nitrate in the column effluent.

To minimize the flow problem, pumps are available that are capable of maintaining very small constant flow rates such as those desired in this study. Installation of these pumps would perhaps force any gas produced through the column to be trapped by the gas collection devices and analyzed or released. Differences in total flow required to transfer the nitrate/chloride application through the columns might also be reduced. Control of the oxygen content of the influent water would also be simplified.

The recovery of excess nitrate in some of the columns was unexpected but not unknown to other researchers. Corey, et al. (8) noted that nitrate gained through nitrification and mineralization during flow studies on unsaturated soil columns exceeded any nitrate losses due to immobilization and denitrification. The investigators attributed the gain in nitrate to oxygen diffusion through openings along the column sides which would increase nitrification. Although soil columns were not used during this study, redox potentials indicated the conditions near the downstream end of the sand columns were moderately to highly reduced, thus making the presence of oxygen very unlikely. Increases in redox potential observed in many of the columns

indicated the possible presence of oxygen and could explain the release of nitrate during the test through mineralization.

Bremner and Shaw (4) also conducted studies showing evidence that excess nitrate was released when wheat straw was added to soil-water mixtures to increase denitrification, but they gave this phenomenon only passing notice. Their results showed addition of wheat straw above 4% by weight to 5000 milligrams of waterlogged soil increased the rate of denitrification, but this was accompanied by a release of nitrate at 25°C after a period of eight to ten days. They also noted that denitrification was followed by nitrogen fixation when large amounts of straw were used.

Excess nitrate recovery was noted only at the 18°C and 13°C tests in this study. Tests were conducted in the order 24°C, 18°C, and 13°C. Further tests should be conducted in the reverse order to determine the effect of nitrate immobilization.

With the type of denitrification flow apparatus used in this study, pH and oxygen content could be regularly monitored at many points along each column. A check of effluent pH between the 24°C and the 18°C tests showed pH of all columns to be between 7.0 and 7.5.

Daily effluent samples could have been analyzed for total organic carbon for this study. This information could be used to determine the total amount of carbon utilized by microbial activity for the methanol treatment columns. Consumptive ratios could then be calculated.

Frequent analysis of gases collected from the sand columns would be valuable in determining the gaseous components and their relative magnitudes. Mass spectrometer results in this study are suspect as nitrogen gas was used to saturate the demineralized water supply for oxygen removal. Use of N^{15} as a tracer to determine the fate of applied nitrate is also a possibility, especially to determine microbial fixation.

The sand within the columns was not removed between tests. Available carbon probably decreased, especially in the sawdust treatment columns, in all columns except those treated with methanol as the tests progressed. Filling the columns with fresh sand between tests would also prevent nitrates fixed in previous tests from appearing in subsequent tests.

As mentioned earlier, previous research has not positively found that nitrate and chloride behave identically under flow situations. To determine if these two ions have the same effective diffusivity in the sand columns used in this study, phenol could be applied to the columns to destroy the bacteria population. Lowering pH of the columns to reduce denitrifer activity is also a possibility.

B. Field Research

Although many experimental problems yet exist with laboratory denitrification experiments, field studies are needed. The results of this study have shown that removal of nitrate can be accomplished by addition of methanol to a denitrifying environment over a moderate range of soil temperatures.

Methanol, while it is expensive at $0.70 a gallon, possesses several advantages over other carbon sources in denitrification studies and should probably be used as a standard substrate in field studies. It is readily available to denitrifying bacteria and soluble in water. Dilution to meet denitrification requirements for nitrate concentrations in tile drainage in Illinois would reduce methanol concentrations well below toxic limits. Periodic addition of methanol to a field system would overcome soluble carbon demands by denitrifying microorganisms as the natural carbon supply is reduced in the soil.

Application of methanol in the field to irrigation or tile drainage systems with controlled water tables could be accomplished through the irrigation water supply, or through shallow wells so that methanol could be directly applied to the water table.

Several soil types in Illinois are suitable for denitrification field studies. Sandy soils cover almost one and a half million acres in the state, and would be excellent for comparing substrate materials for inducing denitrification. The fertility of these highly permeable soils is low, requiring continuous levels of high fertilization. Irrigation and drainage are often needed. Natural organic carbon content is generally low.

Difficulty in measuring nitrogen inputs and monitoring denitrification products require the scale of field studies to be small. Plots of less than an acre would be suitable. An effective means of uniformly distributing an added carbon source to the subsurface profile should also be investigated.

Flanagan and Drummer soil types are very productive dark prairie soils often underlain by sand or coarse particulates at shallow depths. These soils contain a natural supply of carbon, generally require tile drainage and would be suitable for water table control studies on denitrification. The development of practical techniques for denitrification as a means of removing nitrates from soil water should follow field studies.

Summary

The fate of nitrates as they travel through various filter materials at a slow rate toward a tile drain was observed in this study using a porous column to simulate the region near the drain. Temperature and substrate materials were variable.

For readily available substrates, a substantial decrease in nitrate was observed with an accompanying production of gas, discoloration of effluent and an increase in redox potential.

Temperature was a factor in the disappearance of nitrate. For the one month time of these tests, and for low temperatures, there were increases of nitrate for less available substrates. For example, nitrate gains as high as 43 percent were measured in sawdust and check treatments with a temperature of 13°C. On the other hand with pore velocities up to 21 centimeters per day nitrate losses approaching 89 percent were found for a methanol treatment at 24°C while for 13°C, the methanol treatment resulted in nitrate losses as low as 46%.

Where little or no reduction occurred, chloride breakthrough curves were found to be generally lower and wider than those for nitrate. This may have been due to a difference in the diffusion of chlorides as compared to nitrates.

The desaturation of a porous material as a result of gas produced in the denitrification process was found to be a problem in maintaining flow rates in this study.

In general, methanol was found to be an effective means of removing nitrate from a slowly moving stream of water in porous material at temperatures as low as 13°C. Since methanol is expensive, future efforts should be directed toward developing a field technique for studying the effect of various less expensive substrates on nitrogen removal using methanol as a standard substrate material.

In this study it was found that filter material in the region near a tile drain does have potential to reduce nitrates to the gaseous nitrogen form at temperatures as low as 13°C. Future studies will be in the field where this will be accomplished using a tile drain system to raise the water table to create an anaerobic zone where a readily available substrate material can be added to the region near a drain.

LIST OF REFERENCES

1. Allison, F. E., Carter, J. N. and Sterling, L. D. The effect of partial pressure of oxygen on denitrification in soil. S.S.S.A. Proc. 24:283-285. 1963.

2. Beer, C. E. and Koelliker, J. K. Using soil filtration to reduce pollution potential of lagoon effluent entering ground water system. Completion report to Iowa State Water Resources Research Institute. Iowa State University, Ames, Iowa. August, 1971.

3. Bremner, J. M. and Shaw, K. Denitrification in soil I. Methods of investigation. Jour. of Agr. Science. 51:22-39. 1958.

4. Bremner, J. M. and Shaw, K. Denitrification in soil II. Factors affecting denitrification. Jour. of Agr. Science. 51:40-52. 1958.

5. Broadbent, F. E. and Clark, F. E. Denitrification in Soil Nitrogen. Agronomy Monograph 10. 9:344-359. 1965.

6. Broadbent, F. E. and Stojanovic, B. F. The effect of partial pressure of oxygen on some soil nitrogen transformations. S.S.S.A. Proc. 16:359-363. 1952.

7. Corey, J. C., Kirkham, D. and Nielson, D. R. The movement of chloride and nitrate through certain Iowa soils. Journal of the Iowa Academy of Science. 74:130-141. 1967.

8. Corey, J. C., Nielson, D. R. and Kirkham, D. Miscible displacement of nitrate through sand columns. S.S.S.A. Proc. 31:497-501. 1967.

9. Dawes, J. H., Larson, T. E. and Harmeson, R. H. Nitrate pollution of water. Paper presented at the Soil Cons. Soc. of Amer. Annual Meeting. S.C.S.A., Ankeny, Iowa. August, 1969.

10. Hillel, D. Soil and water: Physical principles and processes. Academic Press, New York. 1971.

11. Jansson, S. L. and Clark, F. E. Losses of nitrogen during decomposition of plant material in the presence of inorganic nitrogen. S.S.S.A. Proc. 16:330-334. 1952.

12. Johnson, W. K. Process kinetics for denitrification. Proc. of the American Society of Civil Engineers. Jour. of the Sanitary Engineering Division. SA 98:623-634. August, 1972.

13. Jones, E. J. Loss of elemental nitrogen from soils under anaerobic conditions. Soil Science. 71:193-196. 1951.

14. Mahendrappa, M. K. and Smith, R. L. Some effects of moisture on denitrification in acid and alkaline soils. S.S.S.A. Proc. 31:212-215. 1967.

15. McCarty, P. L., Beck, L. A. and St. Amant, P. P. Biological denitrification of wastewaters by addition of organic materials. Proc. 24th Industrial Waste Conference, Purdue University, Lafayette, Indiana. May, 1969.

16. Meek, B. D., Grass, L. B. and MacKenzie, A. J. Applied nitrogen losses in relation to oxygen status of soils. S.S.S.A. Proc. 33:575-578. 1969.

17. Nielson, D. R. and Biggar, J. W. Miscible displacement in soils: I. Experimental information. S.S.S.A. Proc. 25: 1-4. 1961.

18. Nommik, H. Investigations of denitrification in soil. Acta. Agric. Scand. VI. 2:195-228. 1956.

19. Patrick, W. H. Nitrate reduction rates in a submerged soil as affected by redox potential. 7th Inter. Congress of Soil Science, Madison, Wisconsin. 3:494-500. 1960.

20. Patrick, W. H. and Mahapatra, I. C. Transformation and availability of nitrogen and phosphorus in waterlogged soils. Advances in Agronomy 20:323-339. 1968.

21. Pearsall, W. H. and Martimer, C. H. Oxidation-reduction potentials in waterlogged soils, natural waters, and muds. J. Ecology. 27:483-501. 1939.

22. Stumm, W. Redox potential as an environmental parameter; conceptual significance and operational limitation. 3rd Inter. Conference on Water Pollution Research. Sec. 1, Paper no. 13. 1966.

23. Tusneem, M. E. and Patrick, W. H. Nitrogen transformations in waterlogged soils. Louisiana Agricultural Experiment Station, Bulletin No. 657. June, 1971.

24. Willardson, L. S., Meek, B. D., Grass, L. B., Dickey, G. L. and Bailey, J. W. Drain installation for nitrate reduction. Paper no. 69-734 presented at Amer. Soc. of Agric. Engr. Meeting at Chicago, Ill. A.S.A.E., St. Joseph, Michigan. December, 1969.

25. Wijler, J. and Delwiche, C. C. Investigations on the denitrifying process in soil. Plant and Soil. 5:155-169. 1954.

APPENDIX A

REDOX POTENTIALS

TABLE A-1

REDOX POTENTIALS - 24°C

Days from start of test	Tube 1 - Check			Tube 5 - Check		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
9	38	65	-200	4	0	-460
10	22	65	-200	5	0	-460
11	19	60	-190	5	0	-460
12	33	60	-190	6	20	-460
13	35	65	-200	6	40	-470
14	28	75	-190	5	55	-440
15	50	90	-200	14	90	-240
16	53	95	*	19	115	*
17	60	105	-190	34	165	-110
18	50	115	-190	84	230	-100
19	110	135	-190	190	305	-90
20	120	135	-190	335	390	-80
21	142	150	-190	560	500	-80
22	142	150	-200	560	530	-80
23	142	150	-200	640	565	-80
24	155	155	*	580	510	*
25	160	160	-190	415	415	-80
26	138	170	-190	320	340	-90
27	120	150	-180	160	250	-110
28	116	140	-190	112	195	-410
29	92	115	-160	63	120	-450
30	72	95	*	36	90	*
31	62	85	*	27	65	*
32	60	105	-190	92	230	-480
33	57	90	*	38	75	*

*Redox potential not recorded

TABLE A-1 (Cont'd)
REDOX POTENTIALS - 24°C

Days from start of test	Tube 2 - Sawdust			Tube 6 - Sawdust		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	DO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
10	6	0	+180	11	0	-90
11	6	0	+180	11	0	-80
12	6	0	+180	9	0	-80
13	5	20	+190	7	0	-50
14	8	80	+190	4	0	-80
15	210	415	+200	7	10	-80
16	800	850	*	13	60	*
17	1010	1030	+230	20	185	-70
18	1250	1180	+220	355	805	-40
19	880	700	+2--	1550	1430	-10
20	77	170	+180	1610	1100	-10
21	13	25	+170	510	360	-20
22	13	25	+160	20	80	-30
23	12	35	+170	17	35	-40
24	11	20	+180	19	20	-50

*Redox potential not recorded

TABLE A-1 (Cont'd)
REDOX POTENTIALS - 24°C

Days from start of test	Tube 3 - Methanol			Tube 4 - Methanol		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
10	6	0	-530	9	35	-280
11	6	0	-530	18	125	-280
12	6	0	-530	30	225	-230
13	6	0	-530	62	645	-140
14	4	0	-530	41	920	-110
15	16	40	-510	178	1230	-80
16	-	-	*	155	1030	*
17	39	220	-510	110	810	-40
18	72	880	-510	42	510	-60
19	200	1090	-490	32	325	-70
20	172	940	-490	28	245	-40
21	65	570	-490	22	155	-20
22	23	190	-490	13	85	-230
23	9	35	-490	10	55	-370
24	6	20	-490	9	50	-420

*Redox potential not recorded

TABLE A-2

REDOX POTENTIALS - 18°C

Days from start of test	Tube 1 - Check			Tube 5 - Check		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
9	96	120	-210	2	10	-500
10	87	120	-210	2	15	-500
11	87	130	-210	2	20	-190
12	100	150	-210	2	20	-160
13	142	175	-200	2	25	-130
14	132	180	-180	14	50	-90
15	195	210	-170	110	125	-80
16	220	235	-160	265	270	-80
17	268	255	-110	400	330	-70
18	268	245	-130	530	410	-70
19	245	255	-130	690	510	-70
20	232	235	-130	530	540	-70
21	210	220	-130	550	530	-70
22	132	165	-125	620	490	-70
23	110	120	-130	530	420	-80
24	132	120	-120	420	330	-80
25	116	110	-120	316	255	-80
26	112	120	-120	205	200	-90
27	130	130	-130	98	150	-90
28	70	115	-120	108	115	-100
29	110	125	-120	70	85	-290

TABLE A-2 (Cont'd)
REDOX POTENTIALS - 18°C

Days from start of test	Tube 2 - Sawdust			Tube 6 - Sawdust		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
12	4	10	+190			
13	4	0	+200	3	0	+70
14	5	5	+190	3	15	+80
15	7	10	+190	7	10	+80
16	11	85	+190	9	45	+90
17	180	305	+220	14	115	+90
18	600	660	+220	18	215	+120
19	960	830	+230	118	405	+130
20	1280	1150	+235	500	860	+130
21	1420	1170	+230	1310	1380	+140
22	1050	750	+220	1700	1260	+140
23	370	310	+210	1240	770	+130
24	11	50	+210	360	235	+130
25	8	20	+200	14	45	+120
26	7	15	+200	19	25	+100
27	4	0	+200	8	10	+110

TABLE A-2 (Cont'd)
REDOX POTENTIALS - 18°C

Days from start of test	Tube 3 - Methanol			Tube 4 - Methanol		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
11	2	0	-520	2	0	-480
12	2	0	-520	2	0	-330
13	2	0	-520	2	0	-220
14	2	0	-520	3	25	-190
15	4	25	-520	12	265	-250
16	28	350	-500	55	870	-320
17	470	1000	-480	370	1310	-350
18	-	-	-450	-	-	-160
19	600	1420	-470	250	1040	-160
20	650	1130	-470	35	610	-160
21	44	565	-470	11	290	-170
22	83	275	-470	18	160	-160
23	18	65	-480	10	75	-140
24	7	30	-480	5	25	-120
25	-	-	-480	5	20	-110
26	11	10	-480	8	25	-100
27	3	10	-480	3	25	-90

TABLE A-3

REDOX POTENTIALS - 13°C

Days from start of test	Tube 1 - Check			Tube 5 - Check		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
8	58	90	-110			
9	48	80	-110			
10	54	85	-110			
11	112	115	-110			
12	178	170	-110	3	10	-500
13	200	230	*	3	15	*
14	300	275	*	7	35	*
15	305	290	-120	53	80	-100
16	285	280	*	195	165	*
17	305	280	-130	360	280	-90
18	430	300	-140	810	465	-90
19	450	340	-150	1200	650	-80
20	460	360	-160	1350	730	-90
21	360	325	-170	1350	720	-90
22	400	350	*	950	710	*
23	300	310	-130	800	600	-90
24	240	245	-120	570	470	-100
25	170	180	-110	390	335	-110
26	128	160	-100	195	205	-120
27	76	115	-95	68	115	-330
28	70	110	*	25	65	*
29	61	100	-90	6	35	-460
30	53	90	-90	3	30	-490

*Redox potential not recorded

TABLE A-3 (Cont'd)
REDOX POTENTIALS - 13°C

Days from start of test	Tube 2 - Sawdust			Tube 6 - Sawdust		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
12	5	10	+150	7	10	+140
13	7	15	*	6	10	*
14	7	15	*	5	10	*
15	7	20	+190	5	10	+140
16	8	20	*	7	15	*
17	17	30	+220	8	20	+140
18	66	155	+230	15	25	+140
19	540	495	+235	18	60	+150
20	1300	830	+235	29	160	+160
21	2200	1160	+240	128	390	+160
22	1700	1170	+235	690	960	*
23	1300	830	+235	1650	1430	+170
24	410	315	+235	2000	1340	+180
25	36	90	+180	1460	890	+180
26	13	55	+220	580	310	+170
27	7	30	+220	36	85	+160

*Redox potential not recorded

TABLE A-3 (Cont'd)

REDOX POTENTIALS - 13°C

Days from start of test	Tube 3 - Methanol			Tube 4 - Methanol		
	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)	NO_3^- (mg/l)	Cl^- (mg/l)	E_H (mv)
12	4	0	-480	3	10	-460
13	5	0	*	-	-	*
14	6	45	*	-	-	*
15	29	210	-320	9	35	-455
16	500	695	*	26	80	*
17	640	890	-30	28	95	-440
18	1100	1360	-140	38	200	-430
19	680	1430	-320	58	895	-415
20	120	940	-360	750	1650	-190
21	52	240	-410	300	940	-180
22	60	90	*	295	1100	*
23	-	-	-430	70	470	-140
24	21	50	-440	17	200	-150
25	9	20	-440	13	55	-140
26	8	40	-450	8	60	-140
27	3	30	-450	3	30	-170

*Redox potential not recorded

APPENDIX B

TYPICAL NITRATE ELECTRODE CALIBRATION

FIGURE B-1

TYPICAL NITRATE ELECTRODE CALIBRATION