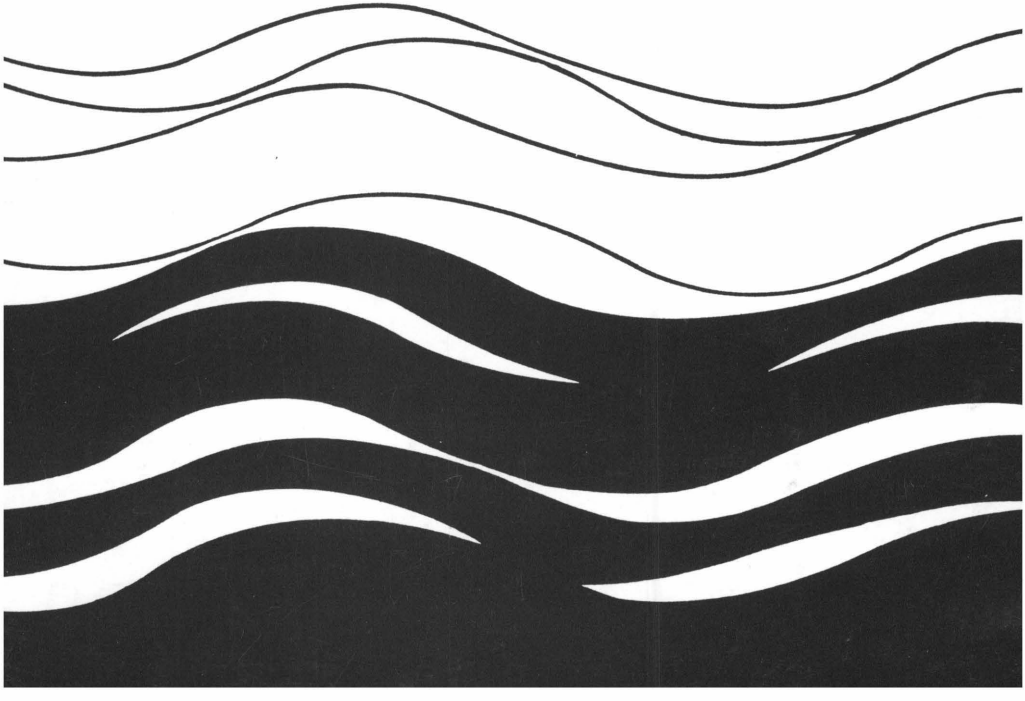
Virginia Water Resources Research Center **Bulletin 171**
Virginia Polytechnic Institute and State University

Denitrification in Onsite Wastewater Treatment and Disposal Systems

Marcia B. Degen, R.B. Reneau, Jr., Charles Hagedorn, D.C. Martens



Bulletin 171
November 1991

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VPI-VWRRC-BULL 171
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Virginia Water Resources Research Center
Virginia Polytechnic Institute and State University
Blacksburg • 1991

This bulletin is published with funds provided in part by the U.S. Geological Survey, Department of the Interior, as authorized by Public Law 101-397.

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Library of Congress Catalog Number:
90-66079

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Acknowledgments

The authors of this report gratefully acknowledge the financial support of the Virginia Water Resources Research Center and the Virginia Department of Health. Special thanks go to Dr. Diana Weigmann for her guidance, patience, and persistence in completion of this research and William R. Walker for his administration of the project. The authors also wish to thank Mike Saluda, Sean Clark, Dana Brown, Jody Compton, Norma Nelson, and Greg Monnett for their technical support. Without their help, this project would not have been completed. The Virginia Tech Statistical Department Consulting Center provided expertise in the data analysis.

The members of the Project Advisory Committee: Robert Schneider, Consulting Hydrogeologist; William Pully, Hatcher-Sayre, Inc.; David Effert, Department of Health, Richmond; Sarah Pugh, Dept. of Ag. and Consumer Services; Stu Wilson, Dept. of Conservation and Historic Resources; and Fred Holt, Virginia Water Control Board, deserve special credit and thanks for providing suggestions and comments during this research project. The successful completion of this investigation and the relevance of the Virginia Water Resources Research Center's total research program can be partially attributed to the guidance and participation of these knowledgeable and interested members of this committee.

Diana L. Weigmann
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Abstract

The effects of effluent type, effluent loading rate, dosing interval, and temperature on denitrification in onsite wastewater treatment and disposal systems (OSWTDSs) were evaluated in this study. The variables were soil horizon, effluent type, effluent loading rate, dosing interval, and temperature. Surface and subsurface soil cores were collected from a Groseclose silt loam soil (clayey, mixed, mesic Typic Hapludult) and subjected to the following treatments: aerobic and anaerobic effluent, loading rates of 0.5, 1.0, and 1.5 times the Virginia Department of Health (VDH)-recommended levels, 24-hour and 48-hour dosing rates, and summer and winter temperatures. The effects of the treatments on denitrification were evaluated based on analyses of leachate from the cores, soil chemical analyses, and microcosm studies to estimate actual denitrification activity. From the study, a model was developed that predicted the mean nitrous oxide (N_2O) production for each combination of the experimental treatments. The results of the study and the model indicate that denitrification can be enhanced in OSWTDSs by the application of anaerobic effluent at the VDH-recommended effluent loading rate to surface soil horizons using a 48-hour dosing interval.

A field study was conducted on a Lowell silt loam soil (fine, mixed, mesic Typic Hapludalf). Denitrification was measured at this site using acetylene blocking, and the results compared to those predicted by the denitrification model developed from the laboratory data. The field measurements of denitrification based on N_2O concentration in the soil atmosphere were almost three orders of magnitude higher than that predicted by the model.

Keywords: Onsite wastewater treatment and disposal system, denitrification, effluent loading rate, temperature, effluent type, acetylene blocking.

1. Introduction

Onsite wastewater treatment and disposal systems (OSWTDSs) are the primary method for domestic waste disposal in sparsely populated areas and in numerous urban counties. Currently, over one-fourth of the homes in the United States are served by OSWTDSs (Bureau of the Census 1983), which apply approximately 14×10^9 L of domestic wastewater to the soil each day. In Virginia, an estimated 650,000, or 34 percent, of the year-round housing units are served by OSWTDSs. The potential for degradation of groundwater and surface water is apparent, considering that 0.24×10^9 L of partially treated wastewater are applied to Virginia soils daily. OSWTDSs apply the largest volume of wastewater to soils overlying groundwater and are the most frequently reported source of groundwater contamination.

The design of most OSWTDSs allows for adequate treatment of all wastewater constituents but nitrogen (N). Due to the aerobic nature of a conventional gravity drainfield, most of the ammonium (NH_4^+)-N and organic N applied to the soil is converted to nitrate (NO_3^-) by nitrification (Preul and Schroepfer 1968; Bouma 1979). If NO_3^- should enter the groundwater or other drinking water supplies, potential health problems can arise.

Few mechanisms are available to remove NO_3^- from the soil subsurface environment. Generally, the placement of a subsurface OSWTDS is too deep for plant uptake to be significant, and the carbon/N ratio (C/N) of these soils is too low for microbial immobilization to occur. Nitrate can be held on soil anion exchange sites via weak electrostatic bonds, but this affects only a small portion of the applied NO_3^-. Most of the NO_3^- moves readily with the soil solution.

An economical process for the removal of N is currently unavailable for OSWTDSs. Denitrification, the sequential microbial reduction of NO_3^- to gaseous N forms under anaerobic conditions, is the largest biological leak in the N cycle and offers the best potential for reducing the quantities of NO_3^- leached to groundwater and surface water. Data on the actual amount of denitrification occurring in OSWTDSs is minimal. In aerobic subsurface absorption fields, denitrification is believed to be of little importance and limited to anaerobic microsites. However, many of the alternatives to the conventional gravity-flow OSWTDSs that would be used in conjunction with drainfields use an effluent dosing system that allows for a fluctuating aerobic/anaerobic environment that should encourage the nitrifying and subsequent denitrifying systems as well as encourage an accumulation of organic matter to fuel the denitrification process.

Groundwater beneath soils that are considered to be best suited for OSWTDSs is most subject to NO_3^- contamination. OSWTDSs normally are placed in subsurface soil horizons of well-drained, permeable soils that encourage nitrification but have limited potential for denitrification.

The addition of NO_3^- to groundwater and surface water from OSWTDSs has been documented in a number of areas (Gibbs 1977; Geraghty and Miller 1978; Hill 1982; Spruill 1983; Perkins 1984; Yates 1986). Gibbs (1977) estimated that a single septic system near a lake shore could add up to 30 kg of N per year to the lake. For groundwater, OSWTDSs have been identified as the most frequently reported cause of contamination (Pye et al. 1983).

2. Literature Review

2.1 Overview

Onsite sewage disposal has proven to be an economical alternative to full-scale wastewater treatment systems for homes in sparsely populated areas. If sewered by conventional methods, the cost would be two to four times more per household than in more densely populated areas (Kreissl 1977). Currently, more than 26 percent of the homes in the United States are served by OSWTDSs, about 85 percent of which are septic systems with soil absorption fields (Scalf et al. 1977). This translates to about 21 million housing units discharging approximately 14 x 10^9 L of wastewater into subsurface absorption fields annually (Bureau of the Census 1983). According to statistics recently released by the VDH, in the majority of the state's counties, more than 60% of the households are served by OSWTDSs, and approximately 40,000 applications for new OSWTDSs are received annually by the VDH. Not all OSWTDSs are located in rural areas; they are found in densely populated areas such as Nassau and Suffolk counties in New York, Dade County, Florida, and Los Angeles County, California. Each of these counties have over 100,000 housing units being served by OSWTDSs. An additional 23 counties have over 50,000 OSWTDSs in place (Geraghty and Miller 1978). As the density of OSWTDSs increases, the potential for groundwater contamination also increases.

The average flow from a home to an OSWTDS is 170 L/capita/day (45 gpcd) up to a maximum of 284 Lpcd (75 gpcd) (Clements and Otis 1980). The raw wastewater entering the septic tank can be characterized by its solids content, biochemical oxygen demand (BOD) and chemical oxygen demand (COD), N and phosphorus (P) content, and the bacterial population (see Table 1, Canter and Knox 1985).

In the septic tank, the raw wastewater is subjected to two main processes: solids separation (flotation and settling) and anaerobic decomposition. These two processes can remove up to 60 percent of the BOD and 70 percent of the total suspended solids (TSS) (Bouma 1979). The effluent produced typically has a BOD_5 of 300 mg L^{-1}, suspended solids of 75 mg L^{-1}, total N (TN) of 40 mg L^{-1}, and total P (TP) of 15 mg L^{-1} (Canter and Knox 1985). The N is 15-25% organic N and 75-85% NH_4^+-N. Coliform bacteria are reduced to 9 x 10^5/100 ml (Sauer 1976).

The septic tank effluent is treated in the soil absorption field by a combination of physical and chemical processes and aerobic and anaerobic biological processes. Bacterial contaminants are removed primarily by the filtering action of the soil and natural die-off, although other mechanisms such as sedimentation and adsorption are also active (Gerba et al. 1975). The formation of a clogging mat below the absorption lines, the soil properties, and the flow status of the wastewater are all contributing factors to the efficiency of the system (McCoy and Ziebell 1977).

Phosphorus removal in a soil system is rapid through adsorption, precipitation, chemisorption, and biological uptake. Up to 90% of the added P is removed by adsorption in the first 2-5 days. The adsorbed P is converted to insoluble forms via reactions with aluminum (Al), iron (Fe) and calcium (Ca) (Sawhney 1977). Reneau and Pettry (1976) observed that P transport away from a 15-year-old OSWTDS was minimal, and that long-term exposure of the soil to the septic tank effluent resulted in an increase of the Al- and Fe-P fractions. As adsorption sites are occupied, P could extend farther from the septic field and eventually reach an aquifer. However, because of the ongoing precipitation reactions, adsorption sites are regenerated and the P advance is halted (Sawhney 1977). In coarse-textured soils, Al and Fe oxides may not be adequate for the adsorption and precipitation of P, and the possibility of P traveling for longer distances increases. Soils with fluctuating water tables may encourage the movement of P into solution and into groundwater (Hill 1972). Even in shallow groundwaters, P is still subject to adsorption and precipitation, and, consequently, very little P enters surface waters (Sikora and Corey 1976).

The suspended solids remaining in the septic tank effluent are removed by the filtering action of the soil. The BOD and COD are used by the microbial population as energy and C sources, although the ability of the microbes to degrade this material depends on favorable environmental conditions and the complexity of the C-containing compound.

The design of most OSTWSs allows for adequate treatment of all wastewater constituents but N. Due to the aerobic nature of a conventional gravity drainfield, most of the NH_4^+-N and organic N applied to the soil is converted to NO_3^- by nitrification (Preul and Schroepfer 1968; Bouma 1979). NO_3^- is extremely mobile in the soil environment and moves with water as it percolates through the profile. If NO_3^- should enter the groundwater or other drinking water supplies, potential health problems can arise. In the body, NO_3^- is reduced to nitrite (NO_2^-) by microbial action. The NO_2^- then can oxidize the Fe of the hemoglobin molecule in blood so that it is no longer capable of carrying oxygen (O_2) (Baum 1982). This condition, methemoglobenemia, particularly affects human infants, poultry, and ruminants because the lower pH of their gastric juices favors microbe growth (Koren 1980). To prevent this condition, a standard of less than 10 mg L^{-1} NO_3^--N has been imposed on all drinking water supplies (Alexander 1977). In surface waters, NO_3^- can trigger algal blooms, leading to eutrophic conditions.

The addition of NO_3^- to groundwater and surface water from OSWTDSs has been documented in a number of areas (Gibbs 1977; Geraghty and Miller 1978; Hill 1982; Spruill 1983; Perkins 1984; Yates 1986). Gibbs (1977) estimated that a single OSWTDS near a lake shore could add up to 30 kg of N per year to the lake. For groundwater, OSWTDSs have been identified as the most frequently reported cause of contamination (Freeze and Cherry 1979; Pye et al. 1983).

Ironically, soils identified as acceptable for OSWTDSs generally are selected on the basis of permeability, or how well the hydraulic load can be dissipated. By specifying minimum separation distances between a drainfield and groundwater or surface water, treatment of pathogens, P, and other materials can be assumed, but the only treatment NO_3^- receives is dilution. Nitrate travels with the soil water so that, in actuality, every nonfailing, aerobic septic system is producing NO_3^- and has the potential to pollute water supplies. This potential increases as the pollutant loading to a given area increases.

2.2 Nitrogen Processes in Drainfields

Often, for the sake of simplicity in modeling, all N entering a drainfield is considered to be nitrified to NO_3^-. In reality, there are a number of processes that can affect the fate of N in a soil system (Keeney 1981):

- mineralization/immobilization
- ammonia (NH_3) volatilization
- nitrification
- denitrification
- chemical decomposition of NO_2^-
- uptake by plants

Figure 1 illustrates the relationship between these processes. The prevailing conditions in the soil absorption field dictate which of these processes are most active.

2.2.1 Mineralization/Immobilization

Mineralization is the conversion of organic N to an inorganic form by soil microorganisms (Alexander 1977). Immobilization is the conversion of inorganic N to the organic state (Jannson and Persson 1982). When organic molecules are used as C and energy sources, some N is retained in the cell (immobilized) for various synthesis reactions. Excess N is released (mineralized) as a waste product in an inorganic form, usually NH_4^+. If the C/N ratio of the system is low (less than 22), excess N is available in relation to the C available, and N is released from the biomass. When the C/N ratio exceeds 22, N is limiting and all of the available N is immobilized by the biomass (Black 1968; Lynch 1979; Keeney 1981). In septic tank effluent, however, C is usually limiting, so the C/N ratio rarely exceeds 10 and net mineralizing conditions result. (Clements and Otis 1980).

The rate of N mineralization is affected by several environmental factors. A neutral pH encourages mineralization, while acidification depresses the rate as seen by organic N accumulations in acid soils (Alexander 1977). Low temperatures also depress mineralization, with the optimum temperature at 40-60°C, but mineralization still occurs at reduced rates, down to 2°C (Alexander 1977). Both anaerobic and aerobic organisms can mineralize organic N so that the mineralization pro-

cess is still significant in submerged soils. As with all microbial processes, water potentials below -1.5 MPa impair the process (Alexander 1977).

The N mineralized from the small amount of organic N added to the soil absorption field is subject to the same fate as the rest of the NH_4^+-N initially applied. In more acid soils, NH_4^+ is adsorbed onto clay minerals. In alkaline soils, adsorption of NH_4^+ onto organic matter is more prevalent due to an increase in the pH-dependent charge (Lance 1975). These adsorption processes can be related to the cation exchange capacity (CEC) of the soil. The quantity of NH_4^+ occupying exchange sites depends primarily on the CEC of the soil, the affinity of the exchange sites, for NH_4^+, and the activity of NH_4^+ and competing ions in the soil solution. The amount of NH_4^+ available for adsorption also depends on the extent of nitrification, which is related to environmental conditions such as temperature, moisture status, and pH.

In soils surrounding OSWTDSs where NH_4^+ fluxes exceed removal, an equilibrium is reached between adsorbed NH_4^+ and soil solution NH_4^+. Leaching of NH_4^+ to groundwater and surface water then may occur. The velocity of the NH_4^+ front moving through the soil varies between soils as illustrated by Brown et al. (1984), who reported average vertical peak velocities of the NH_4^+ front of 25 cm/yr for both sandy clay and clay loam soils and 100 cm/yr for a sandy loam soil.

2.2.2 Ammonia Volatilization

The NH_4^+ present in the soil solution will volatilize when the equilibrium between NH_4^+ and NH_3 favors the NH_3 form (Freney et al. 1981).

$$NH_4^+ + OH \rightarrow NH_3 + H_2O$$

The primary influencing factor is pH (Court et al. 1964). The equilibrium pH for the equation is 9.5. At a pH of 5, 6, or 9, the NH_3/NH_4^+ would exist as 0.0036, 0.36, and 36% NH_3, respectively, in the soil solution (Nelson 1982). Once in solution, the rate of volatilization of the aqueous NH_3 depends on the content of NH_3 in the atmosphere above the solution and the NH_3 in solution. In solutions with large surface areas exposed to the atmosphere, the low NH_3 concentration in the atmosphere favors NH_3 volatilization. Increases in temperature up to 46°C also increase volatilization due to changes in the equilibrium constant and the rate of diffusion (Nelson 1982). In subsurface disposal systems, removal of N by volatilization would be hindered by the distance to the soil surface. The NH_3 must be transported to the soil surface before it can be lost to the atmosphere (Freney et al. 1981). If the NH_3 remains trapped in the soil atmosphere, it will increase in concentration until it retards further NH_3 volatilization. Ammonia can be transported to the surface in either the gaseous or aqueous phase. Diffusion in the gaseous phase is determined by the porosity and tortuosity of the soil and the concentration of NH_3 in solution (Nelson 1982). In the aqueous

phase, the upward movement of the solution depends on capillary action under unsaturated soil conditions. This movement to the surface also depends on a favorable pH to maintain the NH_3 form. If the pH should drop, the resulting NH_4^+ would be available for nitrification or assimilation.

Ammonia volatilization is self limiting. As the NH_3 volatizes, it leaves behind an excess H^+ from the NH_4^+ form. If there is not sufficient buffering capacity in the system, the pH will drop and the equilibrium will shift back toward the NH_4^+ form. In agricultural soils, NH_3 volatilization can continue unchecked, aggravated by the additional buffering capacity of the soil due to liming, and result in large losses of N fertilizer. (Mills et al. 1974; Fenn and Kissel 1975).

2.2.3 Nitrification

Nitrification is the biological formation of NO_2 and/or NO_3^- from reduced N (Alexander 1977). The dominant organisms involved are obligate chemolithotrophic bacteria. These bacteria use the inorganic N compounds for their energy needs. Carbon compounds can be used for cellular synthesis reactions, but not energy-producing reactions. Most of these organisms are capable of using carbon dioxide (CO_2) as their sole C source (Hamilton 1979). Heterotrophic nitrification by some bacteria, actinomycetes, and fungi also may occur, but it is not considered to be a significant contributor to the nitrification process (Campbell and Lees 1967).

Most nitrification in soil can be attributed to two genera of chemolithotrophic bacteria, *Nitrosomonas* and *Nitrobacter*. These two bacteria are able to use the energy obtained from the oxidation of NH_4^+ or NO_2 to drive the reduction of C for cellular synthesis. The following formulas describe the reactions and the subsequent energy evolved (Gilmour et al. 1977).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O + 66\ \text{Kcal}$$
$$NO_2^- + 1.5\ O_2 \rightarrow NO_3^- + 17.5\ \text{Kcal}$$

Nitrosomonas is associated with the oxidation of NH_4^+ to NO_2^-, and *Nitrobacter* completes the oxidation of NO_2^- to NO_3^-.

In natural soil environments, the breakdown of organic matter and subsequent release of NH_4^+ generally is considered the rate-controlling step for nitrification, given that the other environmental conditions are favorable (Black 1968). In an OSWTDS soil absorption field, the N enters the soil system as 75-85% NH_4^+-N and only 15-25% organic N (Otis et al. 1975; Lance 1972; 1975). The effect of the natural rate-limiting step of organic N to NH_4^+ is low because of the low amount of organic N applied. Carbon generally is not limiting, as nitrifiers are primarily chemolithotrophic and can use CO_2 as a sole C source. Sev-

eral other factors that also influence the nitrification rate, including pH, temperature, and O_2, or soil moisture, are discussed in the following section.

The nitrification process is sensitive to acidic conditions. The rate declines below a pH of 6 and is negligible below a pH of 5 (Dancer et al. 1973; Alexander 1977; Schmidt 1982). The optimum pH for nitrification in pure culture is 7.8-8.8 (Martin and Focht 1977); but, in soils, a range of 6.6-8.0 is a more realistic optimum (Alexander 1977). A pH above 8.5 may cause inhibition of nitrification, especially of *Nitrobacter*, due to NH_3 toxicity (Campbell and Lees 1967; Schmidt 1982). The nitrification process consumes alkalinity by the release of H^+ ions and may, in time, acidify its immediate environment (Andreoli et al. 1979). Stoichiometrically, 7.14 mg of alkalinity are consumed for every mg of NH_4^+-N converted to NO_3^--N. There are some strains of nitrifiers that are acid-adapted to a pH as low as 4.5 or 4.0. It is possible that heterotrophic nitrifiers, especially fungi, are dominant at lower pHs if an oxidizable C source is available.

The overall temperature range is believed to be 4-50°C, with much lower nitrification rates above and below the optimum (Barnes and Bliss 1983). The optimal temperature is 30-35°C (Black 1968). In soil absorption fields, nitrification may be slightly depressed in the winter, but the process should still be significant.

The most important factor controlling nitrification rates is the availability of O_2 to the nitrifiers in the soil. Nitrifiers are obligate aerobes that use O_2 as a terminal electron acceptor, so they are most efficient in aerobic, well-drained soils (Martin and Focht 1977). Preul and Schroepfer (1968) noted that in a well-aerated OSWTDS, most of the N was nitrified within 0.3-0.6 m (1-2 feet) of the influent surface. As the O_2 level drops due to respiration or water saturation, nitrification slows and stops completely below 3 micromoles of O_2 (Black 1968). The dependence of nitrification on O_2 has been demonstrated many times (Pilot and Patrick 1972; Andreoli et al. 1979; Gilmour 1984).

Conditions in a conventional aerobic OSWTDS are conducive to nitrification (Bouma 1979). Walker et al. (1973) reported nitrification in the subcrust portion (<10 cm) of the trench bottom. Redox potential and N distribution data (Simon et al. 1986) indicate that nitrification is not limited in clayey soils prior to ponding. As effluent ponds, conditions become anoxic below the trench, reducing by at least one-third the area available for O_2 exchange and nitrification. This observation is supported by the prediction that nitrification would be limited below OSWTDSs in fine-textured soils (Sikora and Corey 1976). However, in OSWTDSs that were not ponded, high redox potential (E_H) values and predominance of NO_3^- indicate active nitrification (Simon et al. 1986).

Once formed, NO_3^- is very mobile. It can be held loosely on soil colloids, but that reaction occurs only at pHs less than 6 (Preul and Schroepfer 1968). In general, unless the NO_3^- is used by biological organisms, it is free to travel in the soil solution.

2.2.4 Biological Denitrification

Biological denitrification is the reduction of N oxides to a gaseous form of N. Facultative anaerobic bacteria use the N oxides as terminal electron acceptors in the absence of O_2 (Black 1968; Alexander 1977; Firestone 1982). For denitrification to occur, the following must be present (Firestone 1982):

- bacteria possessing the metabolic capacity to denitrify
- suitable electron donors such as organic C, H_2, or reduced sulfur
- anaerobic conditions or restricted O_2 availability
- nitrogen oxides, such as NO_3^-, NO_2^-, nitrogen oxide (NO), or nitrous oxide (N_2O), to serve as electron acceptors

There are several bacteria that can use NO_3^- as a terminal electron acceptor, but not all can reduce the NO_3^- to a gaseous end product (Payne 1973). There are a limited number of genera (see Table 2) known to denitrify (Fillery 1983). Nitrous oxide also can be produced by a number of other organisms, and not necessarily in an anaerobic environment (Bollag and Tung 1972; Bleakley and Tiedje 1982). Nitrous oxide also has been shown to be a by-product of nitrification (Yoshida and Alexander 1970; Bremner and Blackmer 1978; 1980).

Denitrification occurs under anaerobic conditions, but it frequently occurs in well-aerated soils. This may be due to anaerobic microsites that develop when respiration rates are greater than the diffusion of O_2 to the microsite (Cady and Bartholomew 1961; Greenland 1962; Gray and Williams 1971; Martin and Focht 1977). Freney et al. (1979) observed measurable amounts of N_2O emitted from air-dried soils for 50 days. As water was added to the soil, up to 62% saturation, the rate of N_2O increased markedly. There are several theories as to why denitrification increases with increasing moisture content. Myers and McGarity (1972) concluded that there is a direct effect of moisture increasing microbial activity and an indirect effect of impaired O_2 diffusion. Mahendrappa and Smith (1967) postulated that soil moisture may affect the distribution of N compounds so that the probability of contact with the organisms was increased. Other research varying actual O_2 concentrations in the soil environment reports that, as the proportion of O_2 increased, the total denitrification activity decreased (Firestone et al. 1980). The prevailing thought on the effect of moisture content in soils on denitrification is that the rate of O_2 diffusion in saturated soils is not adequate to meet the requirements of the soil microorganisms, so they use NO_3^- or other N oxides as a terminal electron acceptor (Bremner and Shaw 1958; Greenwood 1962; Focht and Verstraete 1977).

Perhaps a better measure than O_2 diffusion or water content is the redox potential of the system. The upper limit of the redox potential at which denitrification will occur is approximately 421 mv, which corresponds to the critical E_H for the NO_3^-/NO_2^- couple at pH 7 and 25°C (Focht 1978). The E_H for the reduction of N_2O to dinitrogen gas (N_2) is approximately 250 mv at pH 7 and 25°C.

Denitrification occurs over a wide range of temperatures with an exponential increase in emissions at 15-30°C and an optimum above 25-65°C (Alexander 1977; Stanford et al. 1975a). Substantial denitrification can occur at cool temperatures, however, and studies have shown significant N_2O release during spring and autumn, up to 18-20% of the yearly emissions (Bremner et al. 1980; Keeney et al. 1979).

The optimal pH for denitrification is 8-8.6, but, in some soils, the reaction still can be rapid at a pH of 4.7 (Bremner and Shaw 1958; Russell 1973; Alexander 1977). The idea of an optimum pH, however, has come under attack in recent years (Cooper and Smith 1963; Fillery 1979). It is postulated that an alkaline environment solubilizes organic material and increases the amount of available C, and it is the increased C that affects the rate positively (Fillery 1983). Regardless of the effect of pH on the overall efficiency of the process, pH does affect the ratio of N_2O produced to N_2. At a pH less than 6-6.5 N_2O is the dominant gas released; above pH 6.5, N_2 predominates. It is suggested that the acidity inhibits the nitrous oxide reductase (Alexander 1977). Bremner and Blackmer (1978) suggested that the effect was due to the intervention of NO_3^-, which was stated to be enhanced at lower pHs. The accumulation of NO_2^- at lower pHs is another possible explanation (Fillery 1979). At this time, no one theory has been generally accepted to explain the effect of pH on the end products of denitrification.

The rate of denitrification also depends on sufficient NO_3^- and soluble C levels (Bremner and Shaw 1958; Myers and McGarity 1972; Burford and Bremner 1975; Firestone et al. 1979; Firestone et al. 1980; Koskinen and Keeney 1982). In general, denitrification follows first-order kinetics with respect to NO_3^- when the oxidizable substrate is not limiting and NO_3^- concentration is less than 40 mg L^{-1} (Stanford et al. 1975b). When the oxidizable substrate is limiting and NO_3^- concentrations are greater than 40 mg L^{-1}, the reaction follows zero-order kinetics.

Denitrification is believed to be of little importance in aerobic OSWTDSs, and would be limited to anaerobic microsites (Bouma 1979). However, as many as one-half of all OSWTDSs are not operating satisfactorily and may have anaerobic conditions developing (Scalf et al. 1977). Laak (1981) reported enhanced denitrification after modification of a conventional OSWTDS. In this system, the black and grey water are separated. Toilet wastes are considered black water, and wastewater

from other household sources, such as sinks and washing machines, is termed grey water. The N present in the black water is nitrified, and the grey water is used as a C source for denitrification in a media filter. Wert and Paeth (1985) applied effluent from a recirculating sand filter to an OSWTDS and observed NO_3^- reductions of 71-97%. Reneau (1977) suggested the possibility of denitrification occurring in systems with fluctuating water tables. Denitrification also may be significant in soils with restricted drainage if the effluent can be nitrified first (Bouma 1975; Otis and Boyle 1976).

2.2.5 Chemical Decomposition of Nitrite

Given that NO_2^- has accumulated in a soil, it can decompose abiotically through a reaction with organic matter to form N_2, nitrogen dioxide (NO_2), or N_2O (Alexander 1977). Nitrite accumulation in soil is usually due either to inhibition of *Nitrobacter* so that the conversion of NO_2 to NO_3^- is delayed, or buildup of NO_2^- during biological denitrification.

Normally in nitrification, the conversion of NO_2^- to NO_3^- proceeds at a much faster rate than NH_4^+ to NO_2^- so that very little NO_2^- ever accumulates (Chalk and Smith 1983). However, increases in pH above 9.0 inhibit *Nitrobacter* before *Nitrosomonas* so that NO_2^- accumulates. This inhibition is related to the toxicity of free NH_3 at higher pHs. During denitrification, NO_2^- can accumulate if NO_3^- concentrations are high. The microorganisms will use the NO_3^- first, producing NO_2^-, and then switch to NO_2^- as the NO_3^- is limited (Alexander 1977). Nitrite decomposition is believed to involve nitrous acid, so that decomposition would be favored under increasingly acid conditions. The reaction may occur at the clay mineral or organic matter surface where the pH could be lower than the measured soil pH (Nelson and Bremner 1970).

Chemical decomposition of NO_2^-, with the subsequent release of gaseous N, is probably negligible in an OSWTDS because nitrification is essentially completed a short distance from the system (Simon et al. 1986). If denitrification is active, however, the large NO_3^- source may result in an accumulation of NO_2^-, which then could decompose.

2.2.6 Plant Uptake

Plants can assimilate N if the OSWTDS is located high enough in the soil profile to be in the plant root zone. Brown and Thomas (1978) constructed prototype systems with the top of the gravel layer 30 cm below the surface and extended to 60 cm below the surface. They observed an inverse relationship between N uptake by Common Bermudagrass (*Cynodon dactylon L.*) and the amount of N applied to a given trench. Uptake was limited laterally to 60 cm on either side of the trench. The highest uptake was observed in a slowly permeable soil ($<$0.3 cm/hr), where they obtained 46% removal of the 1735 kg/ha/yr of applied N. It is possible that ponding occurred, which kept the N in the root zone. This phenomenon also may occur in areas with fluctuating water tables.

2.3 Nitrogen Movement in OSWTDSs

2.3.1 Groundwater Contamination

OSWTDSs normally are placed in subsurface soil horizons of well-drained, permeable soils that can transmit high hydraulic loads but have limited potential for denitrification. Hence, groundwater beneath soils considered to be best suited for OSWTDSs is the most subject to potential NO_3^- contamination. Nitrate contamination of groundwater was attributed to OSWTDSs by Quan et al. (1974) and Miller (1975) based on NO_3^- concentrations in drainage and well waters. Quan et al. (1974) reported that 30280-37850 m^3 day^{-1} of effluent is introduced into OSWTDSs in a 78 km^2 area in East Portland, Oregon. They reported NO_3^--N levels of 5-12 mg L^{-1} in shallow groundwater that eventually reached a surface drain. Nitrate levels in deeper aquifers and upgradient shallow groundwater were <1 mg L^{-1}. Miller (1975) observed increased NO_3^--N concentrations in Delaware Coastal Plain groundwater where OSWTDSs and home water wells were located on the same site. In an area comprised of well-drained soils with a water table at 4.5-7.5 m, samples collected ranged from 5 to 30 mg NO_3^--N L^{-1}. In a second area characterized by soils with varying permeability and normally high seasonal fluctuating water tables, NO_3^--N concentrations ranged from 0.01 to 11.3 mg NO_3^--N L^{-1}. Even though population density and well depth varied between the two areas, this study implies increased NO_3^- accumulation in groundwater underlying well-drained soils used for OSWTDSs.

Walker et al. (1973b) reported NO_3^--N concentrations as high as 40 mg L^{-1} in the upper 30 cm of the aquifer adjacent to an OSWTDS. Whelan and Barrow (1984a) observed NO_3^--N concentrations in soil solution as high as 224 mg L^{-1} at a depth of 5.5 m for a black-water soak well in a Karrakatta sand (Inceptisol) of the Swan Coastal Plain in Australia, although soak wells do have a very high fluid loading rate that would enhance saturated flow and the extent of any contamination. These data indicate that in well-drained soils where nitrification occurs immediately below the OSWTDS, denitrification does not adequately remove NO_3^-, and the most probable mechanism for reducing the N concentration in groundwater is by dilution. Walker et al. (1973b) estimated that 0.2 ha down gradient was needed for NO_3^--N concentrations in the top layer of the groundwater to be diluted to <10 mg L^{-1}. Miller (1972) recommended that lot size in Delaware be increased from 0.2 to 0.8 ha to reduce NO_3^--N concentrations in groundwater. Perkins (1984) has an excellent review on lot size and pollution of water table aquifers. Based on computer simulation, he suggests a lot size of 0.3-0.4 ha.

2.3.2 Evidence of Field Denitrification

2.3.2.1 Conventional OSWTDSs. Conventional gravity-fed OSWTDSs have been used successfully for the treatment of most contaminants in septic tank effluent (STE) when the system is installed in a deep soil

with adequate permeability and following accepted setback distances from wells, streams, etc. Denitrification is believed to be of little importance in aerobic OSWTDSs, and would be limited to anaerobic microsites (Bouma 1979). Ritter and Eastburn (1988) reported values of 0-35% N removal by denitrification in conventional OSWTDSs. Laak (1981) reported enhanced denitrification after modification of a conventional OSWTDS. Wert and Paeth (1985) applied effluent from a recirculating sand filter to an OSWTDS and observed NO_3^- reductions of 71-97%. Reneau (1977) suggested the possibility of denitrification occurring in systems with fluctuating water tables. Denitrification also may be significant in soils with restricted drainage if the effluent can be nitrified first (Bouma 1975; Otis and Boyle 1976).

2.3.2.2 Alternative OSWTDSs. A critical factor in the hydraulic success of an OSWTDS is the uniformity in the distribution of the STE throughout the soil absorption system (SAS). This becomes most critical in marginal soils with limited hydraulic capacity. With gravity distribution, localized overloading at the lowest elevations of conventional OSWTDSs often will lead to system failure, especially in a marginal soil. The most common alternative OSWTDSs that use the soil as a final treatment medium generally dose the soil with effluent periodically using a pump or dosing siphon.

Low-Pressure Distribution: Low-pressure distribution (LPD) systems use small-diameter perforated pipe to uniformly distribute effluent to a series of gravel-filled trenches. The household effluent first is treated in a conventional septic tank. The STE then flows into a holding tank, or pump tank, which contains a submersible effluent pump. The pump is preset, using mercury switches or their equivalent, to deliver a specified volume of effluent to the drainfield. An LPD OSWTDS differs from a gravity-fed OSWTDS in that: 1) in an LPD system, effluent is uniformly applied to all trenches with a pressurized dosing system; 2) each lateral or line in an LPD system is level across the lateral length to ensure even distribution; and 3) a smaller-diameter pipe is used in an LPD system.

A soil OSWTDS with alternating aerobic/anaerobic cycles may produce optimum conditions for denitrification. The STE first is nitrified during the aerobic cycle, and then the NO_3^- is denitrified to a gaseous form in the anaerobic stage. Smith and Patrick (1981) reported higher rates of N_2O evolution from soils under fluctuating moisture conditions than from soils that were continuously well aerated. The higher rates are linked to the N transformations, but also to the increased decomposition of organic matter, which would then supply the soluble C source needed for denitfication.

In STE, the high amount of N relative to the available C generally is considered to be the limiting factor to extensive denitrification in drainfields, even in LPD-SAS. Much of Virginia's Coastal Plain is subjected to high seasonally fluctuating water tables. These poorly drained soils

have a high organic matter content in the surface horizon that may suffice as a C source for denitrification. Reneau (1977 and 1979) reported that, in soils with a high seasonally fluctuating water table, NO_3^- concentrations decreased rapidly with lateral distance from the SAS. In a subsequent study, Stewart and Reneau (1988) used a shallow-placed LPD system in a Typic Orchraquult and noted that NO_3^--N/Cl^- ratios averaged 0.70 in the drainfield and dropped to 0.015 at 8.4 m laterally from the drainfield. In these OSWTDSs, the NO_3^- that had accumulated during low water table periods was transported upward through the soil profile with the rising water table. It was hypothesized that denitrification occurred as the water approached the surface horizon. The C source may be a combination of soil organic matter and fresh C sources supplied by the grass cover growing over the shallow-placed OSWTDS. Cogger and Carlile (1984) evaluated 15 conventional and alternative systems in wet soils, and they also suggested denitrification as an explanation for low NO_3^--N/Cl^- at a distance from some of the drainfields.

Most studies concerning denitrification and OSWTDSs have investigated the use of alternative C sources such as methanol (Sikora et al. 1977), grey water (Laak 1981), and a histic-epipedon (Stewart et al. 1979). The dosing cycle in an LPD system may provide the energy source and the aerobic/anaerobic condition needed for denitrification. The extent to which denitrification occurs in these systems has not been determined.

Elevated Sand Mound Systems: A mound system is an OSWTDS that is elevated above the natural soil surface in a suitable fill material (usually sand). Mound systems are used when soil and site conditions limit the use of a conventional gravity system or an LPD system. Common limitations include slowly permeable soils, sandy soils, soils with high water tables, and other restrictions close to the surface that limit the available soil depth. Mounds commonly are constructed on level sites, although slopes up to 10% can be used (Cogger et al. 1982).

The design and construction of mounds in Virginia follow the *Design and Construction Manual for Wisconsin Mounds,* prepared by the Agricultural Engineering Department of the University of Wisconsin - Madison, dated September 1978. The construction of a mound begins with the preparation of the soil beneath the mound. The soil first is plowed to ensure good contact between the sand layer and the original soil layer. The sand layer is placed over the plowed soil. Care is taken throughout construction to minimize the use of heavy equipment in and around the mound to avoid compaction of the soil. A gravel layer is placed on the sand layer, with the distribution lines placed in the gravel layer—low pressure distribution is preferred to ensure uniform distribution of effluent across the sand layer. A cover of building paper, straw, or woven fiber cloth is placed over the gravel, and the entire mound is capped with topsoil.

Because there is usually a restriction to water movement at or near the original soil surface, conditions are favorable for denitrification. Nitrification occurs as the effluent moves through the sand layer. The NO_3^- then denitrifies when it reaches the restricted, anaerobic zone. Magdoff et al. (1974) demonstrated this in sand columns; however, a lack of energy was blamed for only 32% denitrification. A later study of 33 operating mound systems in Wisconsin found an average of 44% denitrification of influent N (Harkin et al. 1979). Denitrification was higher (up to 86%) in systems that maintained aerobic/anaerobic zones.

Mass Drainfields: Soil absorption systems that treat more than the flow from a single-family home can be termed mass drainfields. These drainfields serve clusters of homes, small communities, and small businesses. In most cases, a septic tank or series of tanks is used for pretreatment. The effluent is distributed via low-pressure distribution or enhanced flow, based on VDH regulations. The LPD systems are similar to those described for single-family homes, but are much larger. Enhanced-flow systems use a pump to deliver the specified volume of effluent to the drainfield. The effluent is distributed essentially by gravity, however, as the drainfield is designed as a conventional gravity system with distribution boxes and 10-cm (4-inch) drain tiles.

The processes by which effluent in these larger soil absorption systems is treated are identical to those found in smaller systems. The contamination of groundwater and surface water by NO_3^- is still of major concern, especially because the probability for contamination increases when a large quantity of effluent is applied to an area and sufficient dilution areas are not maintained. The problem of localized overloading, often found in small conventional OSWTDSs, is compounded in gravity-distributed mass drainfields. The high volume of waste entering the drainfield may overload the soil system, and rapid movement of NO_3^- to the nearest groundwater or surface water may occur. If these systems are placed on lake shores or near other surface waters, the potential for pollution is high. In Virginia, the setback distance for surface waters is only 15.2 m (50 feet) from the shoreline.

LPD and enhanced-flow mass drainfield systems should be subject to alternating aerobic/anaerobic cycles with the potential for denitrification. Little to no research has been performed on these systems.

2.4 Analytical Methods to Evaluate Denitrification

Due to the gaseous end products of denitrification, early measurements of the process used a N mass balance approach, often using ^{13}N and ^{15}N. Any N that could not be accounted for in the soil-crop system was assumed to be denitrified. With the advent of gas chromatography and the development of better detectors, direct measurement of N_2O and N_2 is possible. However, the measurement of minute fluctuations of N_2 in an atmosphere is extremely difficult due to the abundance of N_2 in the natural atmosphere. This problem is overcome with the addition of ace-

tylene (C_2H_2) to the system, which has been found to be effective at blocking the final reduction of N_2O to N_2 (Balderston et al. 1976). The evolution of N_2O in the presence of C_2H_2 then can be measured using a gas chromatograph to quantify denitrification.

The C_2H_2 blocking method has some drawbacks. Nitrification is inhibited by C_2H_2 at levels as low as 0.01 KPa (Berg et al. 1982). This inhibition is reversible, but it may take eight to ten days for the nitrification rate to recover fully (Walter et al. 1979). This may lead to an underestimation of denitrification if additional NO_3^- is not formed during aerobic periods as would normally occur. A general reduction in respiration rate due to the inhibited nitrifiers also would occur, leading to a decrease in anaerobic microsite development (Greenwood 1962). This also may lead to an underestimation on the denitrification activity. Metabolism of C_2H_2 by some soil organisms has been reported, but only after extended continued use of C_2H_2 (Haider et al. 1983). Given these limitations, it is suggested that the use of C_2H_2 blocking for quantifying denitrification be limited to short study periods of one to three days (Rolston 1986).

The C_2H_2 blocking technique has been adapted to both field and laboratory experiments. Field studies involve first inserting diffusion tubes for C_2H_2 into a selected soil at regular intervals so that C_2H_2 can be pumped into the soil and diffused uniformly through the soil pores. Gas samples then can be extracted from gas sampling probes placed below the surface of the soil and collected from soil covers placed on the surface. Ryden et al. (1979b) successfully used this method to measure N_2O loss from an irrigated Haploxeroll. No net increase in N_2 in areas treated with C_2H_2 was observed, indicating that the reduction of N_2O to N_2 was blocked.

Currently, there are two designs in use for soil covers: open cover and closed cover. In both cases, a box is placed over the soil. In the closed-cover method, gas is allowed to accumulate in the box and increases in concentration with time (Focht 1978; Rolston et al. 1978; Matthias et al. 1980; Hutchinson and Mosier 1981). With the open-cover method, the gas is swept from the box as it evolves and is trapped, thus keeping the concentration within the box low (Ryden et al. 1979b; Denmead 1979; Ryden and Lund 1980).

The main objection to the closed-cover method is that the buildup of gas under the cover causes a back pressure, which forces lateral movement of the gas and may decrease the flux of gas from the soil to the box by as much as 55% (Matthias et al. 1980). Jury et al. (1982) compared the two methods using a simulation model and concluded that both methods were valid under certain conditions. They note that a steady state must be reached in the entire soil atmosphere before a quantitative relationship between N_2O production rate and surface flux is assumed. For wet soils, steady state may require a longer time period while, in drier soils, steady state may be reached rather rapidly. They

suggest that the cover, whether open or closed, not be allowed to let gas concentration build up too high. This would involve frequent flushing of a closed-cover system and frequent replacement of the trap material in an open-cover system. The sampling period also must cover the entire denitrification event to ensure accurate accounting.

Laboratory methods use incubation studies, soil columns, or soil cores. Incubation studies involve placing a soil sample into a sealed container, adding excess NO_3^- and C, and replacing the container atmosphere with an inert gas such as helium or argon. Acetylene may be added. The gaseous products then are measured in the container atmosphere with time (Bailey and Beauchamp 1973; Yeomans and Beauchamp 1978; Smith et al. 1978; Firestone et al. 1979; Yoshinari et al. 1977; Ryden et al. 1979a). Due to the addition of excess C and N, the denitrification rate obtained by this method can be considered a maximum. The applicability of denitrification rates achieved by this method to field rates has been questioned due to the loss of soil structure and high C and N.

Soil columns are more realistic than incubation studies in simulating field conditions. Cylinders are packed with air-dried, sieved soils to a realistic bulk density (Lance and Whisler 1972; Rolston et al. 1976; Pilot and Patrick 1972), or intact columns may be taken directly from the field (Guthrie and Duxbury 1978). A porous ceramic plate or other device is placed in the bottom of the column to maintain a constant tension. Often, various sampling and monitoring devices are inserted along the column's length (Rolston et al. 1976). Denitrification is quantified by NO_3^- disappearance (Pilot and Patrick 1972) or by analysis of the atmosphere above the column using a soil cover technique (Guthrie and Duxbury 1978).

Parkin et al. (1984) described a gas-flow soil core method to measure field denitrification rates. Soil cores are obtained intact and sealed off immediately and fitted with gas-tight fittings. The cores then are connected to a gas source, which recirculates an inert gas through the cores with 20% (v/v) C_2H_2. Samples of the recirculating gas are analyzed periodically on a gas chromatograph for N_2O. This system overcomes the problems of changes in soil structure, moisture content, and N and C concentrations found in the previously described methods, and provides a better estimate of field denitrification rates. It does, however, involve a complex laboratory setup that allows for automatic analysis of N_2O by the gas chromatograph.

3. Materials and Methods

3.1 Laboratory Study

3.1.1 Overview

A laboratory soil column study was performed to determine whether the amount of denitrification occurring in an LPD-OSWTDS could be increased by manipulating five operational and environmental factors. Two trench bottom depths, three effluent loading rates, two dosing intervals, two temperatures, and two effluent types were examined. The two trench depths were the minimum depth of 46 cm required by current VDH Sewerage Regulations (1989) and the surface horizon. The surface horizon was examined to determine whether the additional organic matter present in that horizon might enhance denitrification. In the VDH regulations, effluent loading rates are based on the estimated percolation rate of the soil, and are calculated on a daily dosing interval. The three loading rates examined represent multiples of the normal loading rates for the soil. Dosing intervals of 24 and 48 hours were used. Dosing intervals of less than 24 hours are prohibitive due to the pump and piping requirements for an average three-bedroom house situated on a slowly permeable soil. Such soils require extensive piping networks, and it would be difficult to deliver a small volume of effluent and still meet the dosing requirements of 7-10 pipe volumes required by VDH regulations. The loading rate and dosing interval interacted so that, on a 24-hour dosing interval, the soil received the specified daily loading rate. On a 48-hour dosing interval, the soil received twice the daily dose every other day, but received the same total amount of effluent throughout the study. The two effluent types were anaerobic effluent (inorganic N present as NH_4^+), which is the predominant form in septic tank effluent, and aerobic effluent (inorganic N present as NO_3^-), which would predominate in a package wastewater treatment plant or sand filter effluent. Temperatures of 20°C and 10°C were chosen to simulate summer and winter soil conditions, respectively. The study first was performed at 20°C, and then the temperature was reduced to 10°C and repeated so that no replication of temperature occurred.

3.1.2 Soil Description

The laboratory study was conducted using a Groseclose silt loam soil (clayey, mixed, mesic Typic Hapludult) collected at Blacksburg, Virginia. This soil was formed in residuum of limestone, shale, siltstone, and sandstone on uplands, and is deep and well drained. Depth to bedrock is greater than 120 cm. This soil would be considered marginally suited for OSWTDSs due to a restriction in hydraulic conductivity. The percolation rate for this soil is estimated at 11.8 min cm^{-1} (30 min $inch^{-1}$) for the surface horizon and 47.2 min cm^{-1} (120 min $inch^{-1}$) for the subsurface soil. Table 3 contains a description of the soil and Table 4 lists the physical properties of the soil.

3.1.3 Description of Soil Columns

A total of 78 soil cores, 5 cm in diameter and 15 cm deep, were obtained from two soil depths of 0-15 cm from the Ap horizon and 45-60 cm from the Bt1 horizon (39 cores per depth). Six cores were used for control columns, and 72 cores received experimental treatments. The cores were collected randomly from an area approximately 3 m^2. To obtain the cores, schedule 40 Polyvinyl Chloride (PVC) pipe (5 cm I.D.) was prepared by cutting columns to 28-cm lengths and then grinding one end of each column to produce a beveled edge. Two 0.64-cm holes were placed within 1-2 cm of the opposite end of the column and 180° from each other. The soil surface was prepared by removing vegetation down to the soil without disturbing the subsurface roots. The columns first were pushed into the ground, beveled edge down, to a depth of 18 cm using a tractor-mounted hydraulic coring machine. The columns, with the cores intact, were retrieved from the ground by placing a metal rod through the predrilled hole at the top of the column and pulling them out with the coring machine. Entering the same hole again, soil was removed, using the coring machine, to a depth of 45 cm. A second column was pushed into the ground an additional 18 cm, and the column and core were retrieved as described previously. This process was repeated for all 78 columns.

The columns were returned to the laboratory and prepared for the study by removing the bottom 2-3 cm of soil to allow room for the installation of fibre floss to prevent downward migration of soil particles and a rubber stopper with a glass tube for drainage. The top of the column was trimmed so that the total length was 25 cm. The final length of each soil core was 15 cm. See Figure 2.

3.1.4 Experimental Procedure

3.1.4.1 Leachate Study. Loading rates were based on the VDH-recommended rate, 0.5 times that rate, and 1.5 times that rate. For the surface cores, the application rates were 1.25, 2.5, and 3.75 cm/day. The subsurface rates were 0.45, 0.9, and 1.35 cm/day. The effluent was applied either every 24 hours or every 48 hours. The cores receiving effluent daily had effluent applied at the above rates; those cores receiving effluent every 48 hours were dosed with twice that amount every other day. Three surface soil cores and three subsurface cores were used for controls. The controls received distilled water at the 2.5 and 0.9 cm/day loading on a daily basis for the surface and subsurface soil, respectively. The two temperatures selected, 20° and 10°C, simulate natural soil conditions for the summer and winter seasons, respectively.

The aerobic effluent for the study was obtained from a pilot treatment plant operated by the Environmental Engineering Department at Virginia Tech. The pilot plant treated wastewater directly from the Blacksburg municipal sewer and was operated to encourage nitrification. The

anaerobic effluent was essentially primary-treated municipal wastewater; effluent characteristics are listed in Table 5. The main criteria for choosing an effluent source were N forms and representative C content. The small packaged extended-aeration plants used to treat wastewater from single-family homes are required to meet discharge limits of at least 30 mg L^{-1} BOD and 30 mg L^{-1} TSS, and most nitrify to some degree. The aerobic effluent then needed to have the majority of the N in the NO_3^- form and a low C content. For the anaerobic effluent, it was necessary that the majority of the N be in the NH_4^+ form and that the C content be high, which is typical of septic tank anaerobic effluent. The anaerobic effluent used met these criteria, but was slightly weaker than typical septic tank effluent, with a total organic carbon (TOC) of 73 mg L^{-1} as compared to a TOC of 100 to 200 for septic tank effluent (Mitchell et al. 1982).

The experimental design was a completely randomized design replicated three times. This design was used for both the 20° and 10°C experiments. The study first was performed at 20°C, and then the temperature was reduced to 10°C and repeated. This was done because of the sensitivity of the nitrifying bacterial populations to low temperatures. The concern was that, if the experiment were started at 10°C, the nitrifiers would never become established and the results would be skewed due to a lack of nitrification.

The soil cores were equilibrated to 20°C for three weeks before sampling began. Effluent was applied during this time, the dosing procedure differing for the surface and subsurface columns. A 30 kPa tension was applied to the bottom of the surface soil cores for approximately 10 minutes before dosing to simulate field tension. Leachate was collected and the volume recorded. Effluent then was applied and the columns were allowed to gravity-drain into collection bottles until the next dosing event. The amount of leachate collected by gravity was recorded and the dosing procedure repeated. This short application period of tension to the subsurface columns was insufficient to induce movement of effluent through the column. The procedure was altered slightly for the subsurface columns and consisted of first applying 30 kPa tension to the columns for a minimum of one hour, and any volume of leachate that collected in the flasks was recorded. Effluent then was added and the tension reapplied to the columns a minimum of three times in a 24-hour period. Again, the leachate was collected and the volume recorded.

After the 3-week equilibration period, 5 sets of leachate samples were collected from the columns over a 4-week period for a total of 390 samples. Each sample was analyzed for NO_3^-, NH_4^+, Cl^-, and pH. Sets 1, 3, and 5 were analyzed for TOC. Because the organic N in the effluent applied should readily transform to an inorganic form, a a total Kjeldahl N (TKN) analysis was performed on randomly selected samples only to verify this assumption. Experimental results were analyzed using the

Statistical Analysis System (SAS) (Ray 1982) available on Virginia Tech's mainframe computer system. An analysis of variance (ANOVA) and Duncan's multiple-range test were performed to determine the statistically significant treatments with respect to the parameters measured. The temperature then was reduced to 10°C and the same process of dosing and sampling used at the higher temperature was repeated.

At the end of the 10°C study, the columns were dismantled and subjected to chemical and microbial analyses. Concerns over maintaining active and representative microbial populations led to dosing the soil cores with effluent until the day before they were dismantled. The wet weight and length of each core were recorded, followed by removal of the entire core from the column. Each core was mixed to produce a homogenous sample. Dry weight was determined by heating approximately 5 g of the mixed soil in an oven overnight at 104°C. Care was taken not to cross-contaminate samples. Subsamples from each core were immediately placed into sterile "Whirlpak" bags for storage. Samples for microbial analysis were held at 4°C until analyzed. The samples for chemical analysis were split into two subsamples; one subsample was allowed to air dry and the second subsample was frozen.

3.1.4.2 Soil Chemical Analyses. The air-dried soil subsample was delivered to the Virginia Tech Soil Testing Laboratory for routine analysis, which included pH, K, P, Mg, Mn, Ca, Zn, and organic matter. A subsample of the air-dried soil was retained for TKN analysis, while the frozen samples were analyzed for NH_4^+, NO_3^-, and Cl^-. Ammonium and NO_3^- were extracted with nine parts of 2M KCl solution shaken with one part sample for one hour. TKN was determined for selected soil samples after digestion with 18M H_2SO_4 at 400°C following the Kjeldahl method (Bremner and Mulvaney 1982). TKN, NH_4^+, and NO_3^- were determined colorimetrically using an automated analyzer (Scientific Instruments Corporation Model CFA-200). Ammonium N was determined by the indophenol blue method, and NO_3^--N by the sulfanilamide method after reduction to NO_2^--N in a cadmium-copper column (Keeney and Nelson 1982). The results of the analyses were analyzed using the SAS. An ANOVA and Duncan's new multiple-range test were performed to determine the statistically significant treatments with respect to the parameters measured.

3.1.4.3 Microbial Studies. The chemical analyses are indirect measures of denitrification. The microbial analyses, however, examine direct byproducts of denitrification such as N_2O. The microbial analyses included an estimate of denitrifying activity, an estimate of denitrifier numbers, and identification of the denitrifying bacteria to the genus level. All tests were performed on subsamples of thoroughly mixed, composite soil samples from each soil column. A total of 1170 incubations were performed and 2106 gas samples were analyzed for N_2O.

Denitrifying Activity: Microcosm incubation studies were performed to assess the amount of denitrification occurring in each soil column and to determine whether the denitrification process was limited by C. These studies were initiated within 24 hours after dismantling the columns. To assess the amount of denitrification occurring, approximately 10 g of soil (wet weight) from the composited soil sample for each soil column was added to a 125-ml Erlenmeyer flask for each incubation. Six incubations were set up from each soil column. The appropriate effluent was added to the flask in an amount equivalent to a dosing event. That amount was determined by developing a proportion between the wet weight of the intact soil core and the amount of effluent that had been added to the core during a dosing event. Each flask then was sealed with a rubber septum, and 10 ml of the gas in the headspace was replaced with C_2H_2. Acetylene blocks the final transformation of N_2O to N_2 in the denitrification process so that evolution of the end product (N_2O) can be recorded with a gas chromatograph. The samples then were incubated at 10°C and 20°C for 48 hours. Three incubations were performed for each soil column at each temperature, for a total of six incubations per soil column. Gas samples of the headspace were analyzed for N_2O after 48 hours of incubation.

A second set of incubations (in triplicate) was performed to determine whether the denitrification process was limited by C. As before, each 10-g wet-weight soil sample was placed in a 125-ml Erlenmeyer flask and excess glucose was added to each flask. The flasks were sealed with a rubber septum and the atmosphere in the flask was replaced with helium to create an anaerobic environment for optimum conditions for denitrification. A 10-ml aliquot of the headspace was replaced with C_2H_2. The flasks then were incubated at 20°C for 48 hours. Gas samples of the headspace were sampled at 48 hours for N_2O.

Gas samples were analyzed on a Varian 3700 gas chromatograph equipped with an electron capture detector. A Porapak-Q column (2 m long, 2 mm inside diameter, 6 mm outside diameter, and 80/100 mesh size) was used. The gas chromatograph was operated at an inlet temperature of 60°C, a column/oven temperature of 50°C, and a detector temperature of 350°C. Good separation of the N_2O peak has been observed under these conditions with a retention time of approximately 1.3 minutes. One-half ml gas samples were injected directly into the gas chromatograph, and the amount of N_2O produced was assumed to be directly related to the activity of denitrifiers under the various experimental conditions. All samples were corrected for N_2O solubility in water (Tiedje 1982).

A normalizing function of $y^{0.2}$ was used to reduce the variation in the experimental results. A Duncan's multiple-range test was used to analyze for significant differences within treatments on the normalized variable only. Because all of the 10°C incubations were performed in one incubator and the 20°C incubations were performed in a separate incu-

bator, temperature could not be considered as a treatment, but rather as a constant for each of the studies. As a result, the data were analyzed separately for the two temperatures.

Enumeration of the Denitrifying Populations: The populations were enumerated using a most probable number (MPN) technique (Tiedje 1982). For each soil column, a 10-g wet-weight soil subsample was added to a 90-ml blank of nutrient broth containing 0.5 g L^{-1} KNO_3. Serial dilutions were made of the dispersed soil sample so that a dilution series of 10^{-1} to 10^{-6} resulted. The cultures were incubated for 14 days at 30°C. At the end of the incubation period, the atmosphere in the headspace of the test tube was analyzed by gas chromatography for N_2O as described previously. All samples were corrected for N_2O solubility in water. Positive samples were identified based on minimum values of N_2O in the headspace (i.e., 20% of added NO_3^- converted). Population numbers then were estimated based on an MPN technique.

Identification of Denitrifiers: Samples of the cultures that tested positive for denitrifiers in the enumeration study were transferred to trypticase soy agar media. The plates were incubated at 30°C for 24-48 hours. The colonies that developed were isolated and transferred to individual general-growth agar media plates. These isolated colonies then were regrown at 30°C for 24-48 hours. A sample of each purified colony type was transferred to a tube of nutrient medium with KNO_3 added and incubated as in the MPN procedure. The headspace was analyzed for N_2O as described previously. The production of N_2O was used to verify the sample as a denitrifying isolate. Once isolated and verified, the bacteria were subjected to diagnostic tests to determine gram-stain reaction, morphology, and biochemical reactions to identify the bacteria to the genus level. Biochemical tests included an oxidase test for gram-negative bacteria. Oxidase-positive samples were inoculated onto an Oxi/ferm tube (TM Hoffmann-LaRoche, Inc.), which is a prepared, sterile multimedia tube for the rapid identification of oxidative-fermentative gram-negative rods. The oxidase-negative samples were inoculated onto Enterotube II (TM Hoffman-Roche, Inc.), a prepared multimedia tube for the rapid identification of members of the Enterobacteriaceae family. The results from the tests on these prepared identification kits were interpreted with a coded system supplied by the manufacturer of the media. Samples that were not completely identified by this system, that is, the test narrowed the identification to several genera, were further classified based on morphological characteristics.

3.2 Field Study

3.2.1 Overview

Experiments using laboratory soil columns can be faulted for being conducted under artificial environmental conditions. However, the construction of full-scale LPD systems for each of the experimental designs

used in the laboratory study would be prohibitive both in cost and space. Also, the variability found in soils over relatively short distances encourages the use of small, closely placed systems. In the field study, soil columns placed in the field were used to simulate isolated OSWTDSs. The confined nature of a column allows for quantification of input and output and, with the addition of monitoring equipment, conditions in the column can be recorded. As a result of the large number of laboratory experimental designs, the field study considered only three loading rates, one effluent source, and one dosing interval.

3.2.2 Soil Description

The soil used in this study is a variant of the Lowell series (fine, mixed, Typic Hapludalf). This soil was formed in the weathered products of limestone and shale and is relatively deep, with more than 100 cm of soil to bedrock. No seasonal high water table is evident. The experimental site was on a nose position of a gently sloping interfluve and surrounded by karst topography. This soil would be considered suitable for LPD based on current VDH regulations. Table 6 contains a description of the soil, and Table 7 presents physical properties. It was not possible to construct the field columns at the Groseclose soil site that was used in the laboratory study; however, these two soils are closely related, are developed from the same parent materials, and have similar characteristics. The Lowell is typically better drained, coarser textured, and has a higher base saturation than the Groseclose soil.

3.2.3 Column Description

The columns were constructed of 20-cm diameter PVC piping cut to 76-cm lengths. To simulate drainfield conditions, a trench was dug 46 cm deep and approximately 30 cm wide. The columns were pushed into the trench bottom to an additional depth of 30 cm with a Giddings coring machine. Enough soil was removed around the columns to allow for installation of redox electrodes, thermocouples, tensiometers, and solution samples (see Figure 3).

Once the monitoring equipment was in place, a gas diffusion tube was installed through the center of the column for later additions of C_2H_2 to the system for N_2O determinations. The diffusion tube is made of rigid acrylic tubing, 0.64 cm O.D. and 0.32 cm I.D., with 2-cm holes placed every 5 cm along the length of the tube. Each hole was rotated 90° on the axis of the tube from the above hole. The bottom of the tube was sealed with epoxy.

Approximately 23 cm of No. 8 gravel was placed inside the column on top of the undisturbed soil. A 2.54-cm diameter well was placed through the gravel layer and extended to the surface. A 1.3-cm influent line was placed approximately 3 cm below the top of the gravel. A layer of geofabric was placed on top of the gravel layer to prevent an influx of soil from the above soil layer, which might clog the gravel system. The

columns were backfilled using sieved Ap and Bt1 soil to within 7.5 cm of the column top.

Dosing chambers, designed to apply the appropriate quantity of effluent, were constructed from 10-cm PVC pipe and capped top and bottom with standard PVC caps. Effluent entered the dosing chamber through a 1.3-cm PVC pipe, and the volume was controlled by an overflow pipe of the same diameter. After filling with effluent, the soil columns were dosed once daily via a solenoid valve regulated by a timer. The dosing tanks and tensiometer columns were insulated to prevent freezing by encasing the dosing tanks in 5-cm polystyrene boxes with wood bases. A 40-Watt light bulb inside each box provided additional heat during the winter. The tensiometer columns were protected with standard 7.6-cm fiberglass insulation and covered with plastic.

3.2.4 Experimental Procedure

3.2.4.1 General. The columns were placed in a randomized complete block design. Each block contained four treatments with effluent dosing rates of 0.9, 1.8, and 3.6 cm/day as well as a control. The loading rates were chosen based on an estimated percolation rate of 47.2 min cm^{-1} (120 min in^{-1}) and multiples of one, two, and three times that rate. There were 4 blocks in all, for a total of 16 columns. There was no field verification for the 15-cm depth, the 48-hour dosing interval, or the aerated effluent in this soil.

The columns were dosed daily with STE for a period of 24 months. Flow was unsaturated and, subsequently, no leachate samples were collected from the base of the columns. The septic tank effluent characteristics were monitored and are reported in Table 8.

3.2.4.2 Field Denitrification Study. Acetylene gas was introduced into the columns through the diffusion tubes at a rate of 1 L min^{-1} for 10 minutes before sampling. At this rate, the C_2H_2 concentration was kept at a minimum of 10% vol/vol throughout the sampling period. Tygon tubing was used to connect the diffusion tube of each column to a five-line manifold and then to a tank of N_2O gas. One-quarter-inch brass pin valves were used to control the gas flow rate to each line. Gas flow rates were measured using a ball-type flowmeter, and the valves across the manifold were adjusted until each of the five lines was delivering the desired rate of 1 L min^{-1}. The delivery rate was calculated based on an estimated pore space of 40% and a total soil volume of 9.9 L. Given these assumptions, the C_2H_2 flow rate replaced the pore volume 2.5 times. Therefore, the C_2H_2 concentration was sufficient to infiltrate all soil pores. Gas samples, taken from the soil cores just after the C_2H_2 was applied, indicated a concentration in excess of 10%.

Gas samples were obtained from the soil atmosphere just before effluent dosing and at 2, 4, 6, and 8 hours after dosing. Gas samples were obtained from the gravel layer by placing a rubber septum over the 2.5-

cm well that extended into the gravel layer. A 60-ml syringe was used to mix the atmosphere in the tube before sampling. Three-ml gas samples were taken from each well at each sampling interval and injected into 3-ml Vacutainer tubes until the samples could be analyzed for N_2O.

The N_2O-N content of the gas samples was determined by the same procedure described in section 3.1.4.3, Microbial Studies. All samples were corrected for N_2O solubility in water. A normalizing function of y raised to the 0.2 power was used to reduce the variation in the data, and a Duncan's multiple-range test was used to analyze for significant differences between treatments for the normalized data. The N_2O produced was assumed to be directly related to the activity of the denitrifiers under the various experimental conditions.

Cumulative amounts of N_2O were estimated by plotting concentration with time and determining the area under the curve. That value then was adjusted to reflect the total amount of pore space in the columns. Because N_2O measurements were made for 8 hours after dosing, an estimate of N_2O production over a 24-hour dosing interval was made by assuming that the 0-hour production concentration represented a background concentration and by extending each graph from the 8-hour concentration to the background concentration at 24 hours.

The field denitrification study was performed three times on each column, with at least one week between events to allow the nitrifiying populations to recover and produce NO_3^-. No soil water samples were present in the solution samplers. Once the field denitrification studies were complete, the columns continued to be dosed for three weeks to allow the microbial populations to recover before the columns were dismantled.

3.2.4.3 Soil Analyses.

Column Sampling: The system received effluent for three weeks after the field denitrification study was completed. Effluent had ponded on the surface of some of the columns, making soil sampling difficult. As a result, the system was turned off to allow the ponded columns to dry slightly before sampling.

Each column was sampled identically. The surface soil above the gravel layer was composited and subsamples removed for chemical and biological studies. A similar procedure was used for the gravel layer. Below the gravel layer, soil samples were taken in duplicate from 0-15, 15-30, 30-45, and 45-60 cm for chemical analysis. Two 30-cm (length) by 2.5-cm (diameter) cores were aseptically obtained from each column to be used in microbial studies.

Soil Chemical Analyses: Each soil sample for chemical analysis was split into three subsamples. One subsample was frozen for NO_3^--N, NH_4^+-N, and Cl^-. A second sample was air dried and ground to be used for

TKN. A third subsample was air dried and delivered to the Virginia Tech Soil Laboratory for the following analyses: organic matter, pH, P, K, Ca, Mg, Zn, and Mn. All analytical procedures were identical to those used in the laboratory study.

Enumeration of the Denitrifying Populations: The procedures used to enumerate the denitrifiers in the laboratory study were followed in this study.

Identification of Denitrifiers: The same identification procedure that was used for the laboratory study was followed for the field study.

3.3 Model Development

One of the objectives of this study was to develop a predictive equation for denitrification based on the parameters examined in the laboratory study. This equation then would be applied to the data from the field study to evaluate and verify the accuracy of the model. Due to the design of the experiment, most of the experimental variables were considered class variables and only had two levels, so that standard regression techniques could not be applied. Instead, ANOVA and standard error of prediction were used to produce a series of predictions of denitrification for all combinations of the experimental treatments. As the denitrifying activity experiment conducted on the laboratory soil columns measured N_2O directly, it was considered the best estimate of denitrification. The total production of N_2O over the 48-hour period was examined.

An ANOVA first was performed on the whole data set, separated by temperature, using a model that incorporated all main effects and interactions. The results were analyzed as to the significant effects and interactions. The insignificant effects and interactions were deleted for each temperature, the refined model for each temperature was again subjected to an ANOVA, and the refined model evaluated as to the significance of the model parameters. This process was continued until the model was refined, using the R_2 values as a guide to model accuracy. Using the refined model, the predicted mean value with confidence interval was calculated.

4. Results and Discussion

4.1 Laboratory Study

4.1.1 Leachate Study

Leachate from the soil columns was analyzed for NO_3^-, NH_4^+, Cl, and TOC (Tables 9 to 13). The NO_3^-, NH_4^+, and TOC data were examined first for each temperature (Tables 9 and 10). The control columns, which received distilled water at a medium loading rate on a 24-hour dosing interval, provide a reference as to what can be leached from the soils just by applying a fluid flux. At both 10° and 20°C, the control columns show that approximately 1 mg L^{-1} of N can be leached from this soil without application of wastewater. At 20°C, the TOC content in the control column leachate was not significantly different from the aerobic effluent treatment leachate, but, at 10°C, the TOC content was much higher than in either of the effluent-treated columns. It is probable that the low temperature and lack of added N retarded any microbial uptake of C in those control samples. It also seems to indicate that the soil has a high content of native C available for denitrification.

Although the 10° and 20°C data cannot be directly compared due to the experimental design, some observations can be made. Nitrate-N and NH_4^+-N were generally higher at 20°C, but the relative differences between the treatments remained the same except that NH_4^+ was higher at the 48-hour interval for 20°C as opposed to the 24-hour interval at 10°C. The TOC content was noticeably higher during the 10°C study. The 20°C study was performed first so that readily leached C would have already been removed. It is suspected that the lower temperature reduced the biological activity to such an extent that C uptake was inhibited and, as a result, higher amounts of TOC were leached through the columns.

Further examination of the data in Tables 9 and 10 shows soil horizon (surface vs. subsurface) to be the most significant treatment factor. Surface soils produced 2.7 and 4.1 times as much NO_3^--N as the subsurface soils at 10° and 20°C, respectively. This was anticipated because the higher permeability of the surface soils would reduce saturated soil conditions and encourage nitrification. Ammonium-N was also higher in the surface soils, however, which is not consistent with the above statement concerning increased nitrification in these soils. Even though the conditions, i.e., permeability, allow for nitrification, the rapid movement of fluid through surface soils may not allow adequate contact time for all of the NH_4^+-N in the effluent to nitrify, thus leading to some of the NH_4^+ leaching from the surface horizon. Overall, the subsurface soils produced less total inorganic N—4.16 mg L^{-1} as compared to 14.9 mg L^{-1} for the surface soils at 10°C and 3.2 mg L^{-1} as compared to 17.5 mg L^{-1} at 20°C.

The aerobic effluent produced the most NO_3^--N and the least NH_4^+-N in the leachate. At 10°C, the NO_3^--N was 10.2 mg L^{-1} and NH_4^+-N was 0.43 mg L^{-1}. The 20°C data were similar at 10.2 mg L^{-1} for NO_3^--N and 0.32 mg L^{-1} for NH_4^+. Given that the aerobic effluent contained 15.4 mg L^{-1} of inorganic N, approximately 30% of the inorganic N was removed by the soil at 10° and 20°C. For the soils amended with anaerobic effluent at 10°C, a total of 8.44 mg L^{-1} of inorganic N was recovered out of 18.2 mg L^{-1} inorganic N applied, or 50% of the inorganic N was removed or retained in the soil core. At 20°C, the amount of N removed or retained by the soil was 40%. It was anticipated that the aerobic effluent would encourage denitrification due to the presence of NO_3^-. These data, however, indicate that the higher C content of the anaerobic effluent is the controlling factor in determining the amount of denitrification occurring in OSWTDSs.

Dosing interval had no statistically significant effect on the N forms. Nitrate-N concentration in the leachate at the low and medium loading rates was the same at either temperature, but was higher than the NO_3^--N present in the leachate from the highest loading rate, which again reflects the difference in moisture status with three rates of effluent application. At the highest rate, the soil was saturated and nitrification was inhibited when compared to the lower application rates. Loading rate had no significant effect on NH_4^+-N concentration. Ammonia-N was higher from the 20°C study, and increased with temperature instead of the expected decrease in the leachate. This may be due to a higher mineralization rate of the native organic N at the higher temperature.

An attempt was made to examine N/Cl ratios to estimate N removal due to denitrification. Nitrate and Cl move through soil at similar rates, except that NO_3^- is subject to biochemical processes such as denitrification, and Cl is considered a conservative ion and is not affected by biochemical processes. By comparing the ratios in the leachate to original ratios in the effluent applied, the loss of N to some mechanism, i.e., denitrification, can be estimated. The aerobic effluent contained ratios of 0.27 and 0.344 for NO_3^--N/Cl and N/Cl, respectively, while the ratios in the anaerobic effluent were 0.048 and 0.309, respectively. Only the NO_3^--N/Cl ratio normally is considered, however; because the majority of the NH4+ was nitrified either before application or within the soil core, the inorganic N/Cl is considered more applicable to this data. The data were separated by effluent type and are presented in Tables 13 and 14.

In all cases, the NO_3^--N/Cl increased in the leachate when compared to the effluent. This is to be expected, especially with the anaerobic effluent as NO_3^- is formed in the soil via nitrification. However, the inorganic-N/Cl also produced some data that suggest that an amount of organic N was mineralized in the columns so that the inorganic-N/Cl ratio was higher in the treatments than in the effluent. For those data showing losses of N, the maximum N lost for the aerobic effluent treatment was

22% for the 10°C subsurface soil treatment. For the anaerobic effluent, the highest loss of N was found for the subsurface treatment, 76%. For this data set then, NO_3^--N/Cl and total inorganic N/Cl do not appear to be adequate indicators of denitrification.

4.1.2 Soil Chemical Analyses

The soil in each laboratory column was subjected to the following chemical analyses: NO_3^--N, NH_4^+-N, TKN, Cl, P, K, Ca, Mg, Mn, Zn, and organic matter. The P, K, Ca, Mg, Mn, and Zn data, although interesting, are not directly applicable to this project, but are presented for review in Appendix I. The remainder of the data is presented in Table 15.

Soil horizon was highly significant for all parameters measured. With the exception of the inorganic N forms, however, the differences are related to the innate differences between surface and subsurface soils and not to any affect of the experimental treatments. As a result, the data were separated according to soil horizon and analyzed as two separate files. The results of these analyses are presented in Tables 16 and 17.

The surface soil results (Table 16) show that TKN and organic matter were not influenced by any of the treatments. Chloride was higher in soils receiving anaerobic effluent and in those soils receiving the low effluent loading rate. The high Cl content in the anaerobic effluent-amended soils is due to a slightly higher Cl concentration in the anaerobic effluent (58.8 vs. 44.8 mg L^{-1}). For the low loading rate columns, the higher Cl concentration is probably related to the slower rate of fluid movement through the columns. This would result in larger quantities of Cl diffusing into the smaller pores.

Nitrate was higher in the anaerobic effluent treatments (20.2 mg kg^{-1}) than in the aerobic effluent treatments (11.5 mg kg^{-1}). No difference in NO_3^--N was observed with dosing interval and loading rate. Similarly, TKN was not affected by any of the treatments. Soil TKN is naturally high, especially in surface soils, and the minor TKN additions by the effluent did little to affect the overall amount of TKN. There was no significant difference in NH_4^+ based on effluent type or loading rate, but the 24-hour dosing interval produced a significantly higher concentration (18.4 mg kg^{-1}) as compared to the 48-hour dosing interval (9.73 mg L^{-1}). It is likely that the shorter time period between doses did not allow for adequate reaeration and nitrification, which would have allowed the NH_4^+ to accumulate.

Table 17 shows the same soil data for the subsurface horizon. The treatment that produced the widest variation in NO_3^--N concentration was the effluent type. The aerobic effluent treatment contained 12.4 mg kg^{-1} of NO_3^--N as opposed to 1.85 mg kg^{-1} for the anaerobic effluent

treatment. These data suggest that limited nitrification was occurring in the subsurface soil columns, which was probably due to saturated conditions present at the loading rates used. Ammonia N was influenced by effluent type, dosing interval, and loading rate. As expected, NH_4^+-N was highest in the anaerobic effluent treatments at 54.3 mg kg^{-1} as compared to an average of 5.22 mg kg^{-1} for the aerobic effluent treatments. The 48-hour dosing interval produced a significantly higher concentration of 38.9 mg kg^{-1} NH_4^+-N versus 22.5 mg kg^{-1} for the 24-hour dosing interval. There was no significant difference between the loading rates, although the low loading rate resulted in the lowest NH_4^+-N. Dosing interval did not produce anticipated results. It was expected that the 48-hour dosing interval would allow for a longer reaeration period and greater nitrification.

4.1.3 Microbial Studies

4.1.3.1 Denitrifier Activity.

Estimate of Denitrifying Activity: The incubated microcosms amended with a proportionate amount of effluent were used to simulate the denitrifying activity that occurred in the soil columns. The amount of N_2O-N produced in the microcosm was adjusted to account for soluble N_2O (Tiedje 1982) and is presented on a mg N_2O-N produced per mg of soil (dry weight) basis.

Results from the effluent-amended incubation study are given in Tables 18 and 19. In the 10°C studies (Table 18), N_2O-N production was influenced by each of the treatments studied except effluent type. Surface soils produced 10 times more N_2O-N than the subsurface soils. This is to be expected, as surface soils generally have a naturally higher microbial population than subsurface soils, and the organic matter in the soil would provide a needed C source to encourage denitrification. The samples receiving effluent produced 12-28 times more N_2O-N than the distilled water control application. The application of anaerobic effluent resulted in twice as much N_2O-N evolution as the aerobic effluent application, but was not significantly higher. It was anticipated that the aerobic effluent, with N in the NO_3^- form, would encourage more denitrification because nitrification would not be a limiting factor. The data in this study seem to suggest that another factor is responsible for the apparent increase in denitrification with the anaerobic effluent. Referring to Table 5, which describes the properties of the two effluents applied to the soils, TOC is significantly higher in the anaerobic effluent than in the aerobic effluent due to the differences in the degree of treatment between the two effluents. The anaerobic effluent was taken directly from a sewer collection line so that little degradation/treatment had occurred. The aerobic effluent, on the other hand, had been processed in a pilot wastewater treatment plant designed for nitrification. For the nitrification process to occur, the C in the effluent must be reduced first; thus, the C available as an energy source for denitrifiers is extremely low in the aerobic effluent. It would seem logical to suggest

that the added C in the anaerobic effluent promoted denitrification to a greater degree than did the NO_3^- in the aerobic effluent.

The N_2O-N produced under a 48-hour dosing interval was significantly higher than the 24-hour interval. The 48-hour interval may allow more time for reaeration and subsequent nitrification of NH_4^+. This would provide a sufficient NO_3^- source to denitrify at the next anaerobic phase, i.e., the next dosing cycle.

The highest loading rate for both surface soils and subsurface soils produced the most N_2O-N, with no significant difference between the low and medium rates and the medium and high rates. This may be related to an increase in the length of the anaerobic cycle, or to N and C amounts present with the higher dosing level.

At 20°C, N_2O-N production increased approximately three fold for all treatments as compared to the soils incubated at 10°C (Table 19). This increase in microbial activity at higher temperatures is well documented (Alexander 1977). Surface soil production of N_2O-N was 26 times greater than subsurface soil, which is related to the superior ability of the surface soil to nitrify the effluent and to support microbial growth. The highest loading rate again resulted in the highest N_2O-N production, as did the anaerobic effluent treatment. The N_2O-N evolution from the soils receiving anaerobic effluent was again not significantly greater than from the soils receiving aerobic effluent.

An ANOVA indicated that soil horizon was responsible for most of the variation in the samples ($p>F=0.0001$), so the data set was subdivided by temperature and soil horizon and analyzed again (Tables 20 and 21). For the surface soils, the application of anaerobic effluent produced a higher N_2O-N concentration than the aerobic effluent (Table 20). As before, it is suggested that the anaerobic effluent treatment resulted in more N_2O-N than the aerobic treatment due to the added C in that effluent, which would promote denitrification. The 48-hour dosing interval also significantly increased N_2O-N evolution, which is to be expected as this would allow for more complete nitrification between dosing cycles. The high loading rate produced the highest N_2O-N, which was significantly different from the lower application rates. Nitrous oxide-N concentration increased two to five times with the increase from 10° to 20°C.

For the subsurface soil (Table 21), temperature had little effect on the N_2O-N evolution. In this less-permeable soil, the aerobic effluent produced more N_2O-N at 20°C than the anaerobic effluent, indicating that nitrification of the anaerobic effluent was limited. Thus, even though additional C was present in the anaerobic effluent, N_2O-N emission was limited by the quantity of NO_3^--N present. At 10°C, this effect was not evident due to overall reduced biological activity. There was, however, a trend toward higher N_2O-N emission with the addition of aerobic effluent (Table 21).

The 48-hour interval between doses appears to be necessary in these slowly permeable subsurface soils to allow for reaeration and nitrification. The emission of N_2O-N increased where 48-hour intervals were compared to the 24-hour dosing interval for both temperatures. The N_2O-N production at the low and medium loading rates was the same, but was lower than N_2O-N produced from the high loading rate. This appears to be related to the total amount of N available for denitrification, as nitrification of the anaerobic effluent was limited.

If the assumption is correct that the amount of N_2O-N evolved can be directly related to denitrification, it becomes meaningful to examine N_2O-N production on a trench bottom area basis. The data was converted to mg of N_2O-N evolved per m^2 and ft^2 per dosing cycle, and is presented in Tables 22 and 23. It should be noted that these are conservative estimates of denitrification, as they are based on a 15-cm soil depth only. In field situations, the soil around the trench and below the 15-cm depth also would be involved in treatment and, presumably, denitrification, so that the N_2O-N evolved on a trench bottom basis would include approximately three times the volume of soil found in a 15-cm depth directly under the trench.

The relative amounts of N_2O-N produced for each treatment are, of course, the same as those shown in Tables 20 and 21 as those data were used to produce Tables 22 and 23. The most N_2O-N is produced with a surface soil horizon, anaerobic effluent, 48-hour dosing interval, and a high loading rate, which is consistent with the discussion in the previous section.

Denitrification Potential: Effluent is most often considered to be deficient in C with respect to promoting denitrification. In sewage treatment plants, methanol has been added to provide sufficient C for the denitrification process. An incubation study was performed to see whether the addition of C in the form of glucose would increase denitrification in the subject soil.

The N_2O-N produced in the glucose trials was compared to the N_2O-N produced from the effluent-amended incubation study at the 48-hour time period and the 20°C incubations only (Table 24). The glucose addition significantly increased N_2O-N evolved from an average of 1.662×10^{-6} mg N_2O-N for the effluent-amended treatments to 7.13×10^{-6} mg N_2O-N for the glucose-amended soils. Based on this test, it would appear that the soils are deficient with respect to C. The addition of an outside C source would almost triple the amount of N denitrified.

The influence of added C on N_2O-N is further examined in Table 25, which compares the results of the glucose-amended samples to the effluent-amended samples. With glucose, only soil type produced a significant difference in N_2O-N concentration, with the surface soil N_2O-N concentration (12.06×10^{-6}) being six times the amount generated by the subsurface soil samples (2.2×10^{-6}). The addition of C to the subsur-

face soil was not expected to increase the rate of N_2O-N emission to the levels measured in the surface soil since NO_3^- was limited in this horizon.

4.1.3.2 Enumeration of Denitrifiers. The number of denitrifiers was estimated using an MPN technique that allowed for six ten-fold dilutions with duplicate samples. The results are presented in Table 26 as number of denitrifiers per g of dry soil.

Effluent loading rate did not effect the number of denitrifiers; however, soil horizon, effluent type, and dosing interval did alter the microbial populations. The surface soil treatment had the highest denitrifier population, which is consistent with the results of the incubation study. The soils that were dosed on a 48-hour interval also had higher denitrifier populations, which again is consistent with the incubation results. Denitrifier numbers were higher in the soils amended with aerobic effluent, but the most N_2O-N was produced by soils receiving anaerobic effluent in the incubation study. This would suggest that, although there was a higher population of denitrifiers in the aerobic-effluent amended soils, the anaerobic-effluent amended soils experienced conditions that encouraged denitrification. The additional C in the anaerobic effluent is the most logical reason for the increased denitrification rate. This is confirmed by the increased N_2O-N produced when additional C was added to the soil samples. Additionally, O_2 diffusion was limited in the subsurface horizon both by water-filled pores and by consumption of O_2 for decomposition of readily degradable organic compounds.

4.1.3.3 Identification of Denitrifiers. An identification of denitrifier organisms to the genus level was performed. Although a subsample from each soil column was examined, the study was conducted only to identify which genera of denitrifier bacteria exist in soils amended with effluent. No attempt was made to estimate relative numbers of each genus or to maintain a database that could be analyzed statistically according to the experimental treatments.

The genera identified are listed in Table 27. The four genera, *Pseudomonas, Flavobacterium, Acinetobacter,* and *Bacillus,* are common soil bacteria that have species that are known to denitrify. Based on the number of times that the genus was isolated, *Pseudomonas* and *Bacillus* were the predominant organisms. This does not mean that these genera were responsible for most of the denitrification occurring, only that they were isolated from a larger number of soil samples.

4.2 Field Study

4.2.1 Soil Chemical Analyses

The field study soil was analyzed for TKN, NO_3^--N, NH_4^+-N, organic matter, Cl, P, Ca, Mg, Mn, and Zn according to methods described pre-

viously. The P, Ca, Mg, Mn, and Zn values are reported in Appendix II. The remaining data is presented in Table 28. Except for TKN and Cl, all parameters listed were altered by loading rate.

Nitrate-N and NH_4^+-N varied with loading rate. Nitrate-N increased at the 1.8 and 3.6 cm application rate. These NO_3^--N concentrations were not different from the control, which had 1.38 mg kg^{-1} of NO_3^--N. The lowest NO_3^--N concentration of 0.22 mg kg^{-1} was present at the 0.9 cm/day loading rate and was not different from the 0 effluent application treatment. The NH_4^+-N levels demonstrated a similar trend, with the highest concentration of 32.5 and 24.7 mg kg^{-1} present at the 1.8 and 3.6 cm/day loading rate, respectively. The control and 0.9 cm/day loading rate averaged of 7.59 and 6.41 mg kg^{-1}, respectively.

These data suggest that 0.9 cm/day, which is the VDH-recommended loading rate, produces the lowest NO_3^--N and NH_4^+-N values. For NO_3^-, the 0.9 cm/day loading rate produced an even lower concentration than the controls. The low NH_4^+-N concentration suggests that nitrification was not hindered; however, the extremely low NO_3^--N concentration does not appear to support that theory unless the NO_3^- is being removed or transformed, i.e., denitrified. The higher loading rates, 1.8 and 3.6 cm/day, have limited nitrification occurring as indicated by the high NH_4^+-N and low NO_3^--N concentration present in the soil. This lack of nitrification is to be expected, given the saturated and ponded conditions of many the columns receiving the higher effluent loading rates.

Table 28 also considers the analyzed parameters by depth. The S depth is the surface soil above the effluent addition point. The remaining depths, 0 through 45 cm, indicate the depth below the gravel layer, or trench bottom. TKN and organic matter are significantly higher in the surface layer only, and do not show difference with depth below the trench bottom. Chloride increases with depth below the trench bottom. There is no significant difference in NO_3^--N with depth, but NH_4^+-N decreases with depth.

Because the zero loading rate, or the control, did not receive application of any wastewater, the data were analyzed again with the control values removed (Table 29). The analysis by loading rate was not affected, and resulted in the same means and significant results as would be expected. The data by depth, however, did change slightly with the removal of the control data. In general, the means increased slightly, but, with few exceptions, the overall trends did not change. The differences in the means for Cl were reduced so that there was no significant difference in Cl with depth. The surface TKN value decreased slightly, but was still the highest value of the five depths. The most change in means was observed for NH_4^+-N with the 0- and 15-cm depth increasing from 34.3 to 41.3 mg kg^{-1} and 28.5 to 34.3 mg kg^{-1}, respectively. These changes do not affect the interpretation of the data.

To assess the effect of loading rate on N forms with depth, the data were analyzed by loading rate and depth (Table 30). Nitrate was low, less than 5 mg kg^{-1} in all cases. There was no significant depth or interaction response. The 0.9 cm/day rate produced the lowest NO_3^--N levels, with concentrations decreasing with depth from 0.65 mg kg^{-1} to nondetectable levels at 45 cm. The NO_3^--N levels in the soils receiving effluent appeared to be less than the control samples at most depths. The control samples may reflect residual N in the soil from previous farming activities. The effluent-amended soil may have sufficient C added to denitrify the residual N as well as the added N. The low NO_3^- in the soils receiving the higher effluent loading rate is probably due conditions that limit O_2 diffusion, which inhibits nitrification as explained earlier.

Ammonium-N changed with depth and loading rate, although there was no interaction between the two treatments. In the 1.8 and 3.6 cm/day treatments, the NH_4^+-N is highest just below the gravel layer, and then decreases with depth. As the soils had a higher moisture content just below the gravel layer, there was insufficient O_2 to allow for nitrification, so the NH_4^+ would have accumulated at this depth. As the wastewater travels deeper into the soil column, the decrease in NH_4^+ would be due to either nitrification as the wastewater entered more unsaturated zones, or adsorption onto the exchange sites in the shallower soil layers. Both the control and 0.9 cm/day treatments are highest in NH_4^+-N in the surface soil. Below the trench, the control soils decrease in NH_4^+-N with depth until the 45-cm depth, where the concentration increases to 10.5 mg kg^{-1}. The increase in NH_4^+ at the 45-cm depth may be associated with increased adsorption of NH_4^+ as a result of an increase in clay-sized particles. The lack of this increased NH_4^+ at the 45-cm depth in any of the soils receiving effluent suggests that the naturally occurring NH_4^+ has been removed from the soil, either by leaching or denitrification. The soils receiving the lowest loading rate, 0.9 cm/day, exhibit a decrease in NH_4^+ with depth with all values less than 5 mg kg^{-1} below the trench bottom.

The data in Tables 29 and 30 agree that the 0.9 cm/day effluent loading rate produced the least amount of residual inorganic soil N.

4.2.2 Field Denitrification

The N_2O-N concentration in the soil atmosphere is presented in Table 31. The analysis did not detect any differences in the means based on loading rate, but there was a trend in the data observed that suggests that the concentration increased with increasing effluent application rate. This increase may be related to a higher total amount of N available for denitrification at the highest loading rate.

Because of the variability in the moisture content of the soil, the data were adjusted to reflect N_2O-N concentration per mg of dry soil (Table 32). Again, no significant difference in the means was detected with

loading rate, but the trend is clear: N_2O production increases with increased loading rate.

Table 32 also contains N_2O-N production with time after dosing. The function appears to peak after 6 hours and then returns to a baseline, or background, concentration. These data represent concentrations at points in time after dosing only, and do not reflect total amounts of N_2O produced per dosing event. Cumulative amounts of N_2O-N produced per 24-hour dosing cycle per mg of soil (dry weight) contained in the field column also are included in Table 32. These values are necessary to evaluate the data on the same basis and with the same units as the laboratory study. Cumulative amounts of N_2O on a trench bottom area basis are presented in Table 33. These data all show an increase in N_2O production with increasing loading rate. Interestingly, the background N_2O level in the control columns was higher than the low loading rate columns.

4.2.3 Enumeration of Denitrifiers

The number of denitrifiers were examined with depth in each of the field columns (Table 34). There was no difference in any denitrifier numbers for any of the loading rates except at the deepest depth (30-45 cm). At the 30-45-cm depth, the control soils had higher denitrifier populations than any of the soils amended with effluent.

Although not statistically significant, some trends can be seen by examining Table 34. The highest loading rate, 3.6 cm/day, consistently produced the lowest number of denitrifiers, except in the gravel layer. Many of these high-rate columns had standing effluent for at least some time after dosing and, thus, had highly anaerobic subsoils at all times. Because denitrifiers are primarily facultative aerobes and require NO_3^- to survive under anaerobic conditions, these ponded conditions would not have allowed much, if any, nitrification to occur and, thus, denitrifiers would not be favored.

For the soil beneath the trench bottom, the 1.8 cm/day loading rate allowed for slightly higher denitrifier growth than the 3.6 cm/day loading rate, while the columns receiving the 0.9 cm/day loading rate contained the highest denitrifier population. The highest populations were found at the gravel/soil interface, where the daily dosing would set up the desired aerobic/anaerobic cycle. As a result the denitrifiers would tend to accumulate at that point.

In the gravel layer, the 0.9 cm/day loading rate had the lowest population of denitrifiers, while the two higher loading rates had similar values at 2850 organism/g for the 1.8 loading and 2860 organisms/g for the 3.6 cm/day loading rate. This is probably related to the saturated conditions in the soils of the higher-rate columns, which resulted in ponding of the effluent into the gravel layer. This ponding in the gravel layer set up the desired aerobic/anaerobic cycle in the gravel layer instead of in the soil.

4.2.4 Identification of Denitrifiers

As in the laboratory study, an identification of denitrifier organisms to the genus level was performed. No attempt was made to estimate relative numbers of each genus or to maintain a database that would be analyzed statistically.

The same organisms were found in the field study as in the laboratory study (Table 35). Again, *Bacillus* and *Pseudomonas* were the predominant genera identified.

4.3 Model Development

The predicted values for N_2O-N production based on the experimental treatments are listed in Table 36 for 10°C and 20°C. At 20°C, N_2O-N production increases approximately 10 fold over the 10°C production. The application of anaerobic effluent to a surface soil produced the most denitrification. Specifically, anaerobic effluent at the medium loading rate, or the VDH-recommended rate, applied to surface soils at 48-hour intervals would result in the greatest evolution of N_2O-N via denitrification.

The results of the model are expressed only in the amount of N_2O-N evolved. Of more importance is the amount of N removed via denitrification as compared to the amount of N applied. The higher the percent of N removed, the lower the concentration of N available to leaching. Using the amount of N applied based on effluent type, dosing interval, and loading rate, and the amount of N removed by denitrification as estimated by the model, the percent of N removed by denitrification was calculated and is presented in Table 37. These data identify the same combination of treatment variables as optimizing N removal, except for loading rate. The low loading rate, which is one-half the VDH-recommended rate, removed 40% of the N applied at 10°C and 175% of the N applied at 20°C, while the medium loading rate removed only 22.8% at 10°C and 128% at 20°C. The denitrification of residual N in the surface soils may account for the removal percentages exceeding 100%.

Based on this model, the anaerobic effluent applied at a medium loading rate at 48-hour intervals to a surface soil produced the highest amounts of N_2O-N via denitrification. For maximum percent removal of applied N, however, the anaerobic effluent applied to a surface soil every 48 hours at a low loading rate should be used.

To evaluate the applicability of this model to field conditions, the results of the field study were examined. Because the application rates used in the field study were one, two, and three times the VDH-recommended loading rate, only the lowest loading rate, the VDH-recommended rate, was used for comparison. The field study then conforms to the following model parameters: a subsurface soil, an anaerobic effluent, a 24-

hour dosing interval, and medium loading rate. The model predicts that 0.019×10^{-6} mg N_2O-N per mg of soil should be evolved at 10°C, and 0.007×10^{-6} mg N_2O per mg soil at 20°C. The field denitrification study suggests that the actual rate is higher at 6.59×10^{-6} mg N_2O-N per mg of soil. This suggests that the model does not predict accurately the expected field denitrification rates. Given the innate difficulties associated with transferring laboratory data to field data, this result was not unexpected. However, the model does allow for a qualitative evaluation of the experimental treatments and their effects on denitrification.

4.4 Discussion

The model developed from the laboratory incubation study predicted that an anaerobic effluent applied to a surface soil at 48-hour intervals would produce the highest level of denitrification at a medium loading rate and the highest percent removal of applied N at the low loading rate. It was anticipated that an aerobic effluent applied to a surface soil would result in the most denitrification because: 1) nitrification would not occur in the soil and, thus, would not be limiting, and 2) the excess organic matter in the soil would provide a C source to fuel the denitrification process. This study suggests that the high permeability of the surface horizon allows adequate nitrification of anaerobic effluent to occur. The laboratory leachate study confirms this statement, although some NH_4^+-N was collected in the leachate from the surface horizons, which was probably due to inadequate retention time within the soil column. The higher C in the anaerobic effluent apparently fueled the denitrification process. If the soil C had been sufficient, the aerobic effluent would have produced the same or similar amounts of denitrification as similar amounts of N, especially inorganic N, were applied in both effluents. Stewart and Reneau (1988) postulated that the energy source for elevated denitrification levels in a shallow-placed LPD system was probably a combination of soil organic matter, C present in effluent, and fresh C sources supplied by grass growing over the shallow-placed system. However, Stewart et al. (1979) concluded from laboratory column studies using a mixture of a histic epipedon and sand that residual soil organic matter is probably not a satisfactory long-term energy source for denitrification. Thus, continued denitrification over long periods of time may depend more on energy that is added to the system. As in this study, such C sources might be the anaerobic effluent and materials associated with root growth.

The model developed from laboratory data significantly underestimated the amount of denitrification observed in the field study. The scale of the study, i.e., the effect of the different column sizes on the O_2 diffusion rate and retention time, and field conditions vs. controlled laboratory conditions, all potentially contributed to the discrepancy. The two soils, although similar, did differ to some degree in texture and structure. The laboratory soil was finer textured and more weakly structured that the field soil. The field soils also were exposed to a constant tension so that effluent was drawn through the column on a continual

basis. Therefore, effluent movement through the laboratory subsurface cores was more limited when compared to the field soils, which possibly resulted in more extended anaerobic periods. Where anaerobic effluent containing large quantities of NH_4^+ and minimal quantities of NO_3^- is applied to a system, there must be a combination of aerobic followed by anaerobic conditions, or a combination of aerobic and anaerobic zones present in the soil, for the NH_4^+ to be nitrified and the NO_3^- to be denitrified. It would appear that the field soil, due to its better structure and reaeration abilities, was able to fluctuate between aerobic and anaerobic cycles more successfully than the laboratory subsurface soil and, thus, encouraged denitrification to a greater degree.

The variables used in this study were chosen because they are elements that can be readily incorporated into an OSWTDS design with little or no additional cost over a traditional design, with the exception of the aerobic effluent. The treatment variables that resulted in the highest percent of N removed—anaerobic effluent, 48-hour dosing interval, surface soil, and a low loading rate—would require only slight alterations to conventional OSWTDS design. The 48-hour dosing interval would require that slightly larger volumes of effluent be pumped each dosing cycle than with the normal 24-hour dosing interval. This would require only that the pumps would run slightly longer every other day, but the overall run time would be the same as in a conventional design. The traditional problem with applying effluent to surface horizons is the probability of freezing in the winter. This can be overcome by use of at-grade systems that include a soil cover of sufficient depth to eliminate the possibility of freezing. An at-grade system designed to apply effluent to the surface soil could have the distribution lines placed in either a gravel layer or inside of an infiltrator system (this system is currently being evaluated by the authors). The loading rate that produced the most N_2O-N emission was one-half the VDH-recommended loading rate, which will result in soil absorption fields that are twice the current size. Given that the medium loading rate, i.e., the VDH-recommended rate, removed over 100% of the added N in some instances, and that the model appears to underestimate denitrification, it is suggested that the VDH-recommended loading rate be considered instead of a lower loading rate because the increased construction costs do not appear to be cost effective for a slight increase in denitrification. The denifrification rates measured in this study are compared with rates reported in the literature for OSWTDSs in Table 38.

Some of the limits of this study already have been discussed. The results of this study should not be construed as applicable to all soils in Virginia. The extent to which the results can be extrapolated to other soils has not been determined. There is some question as to the appropriateness of the effluents used, although they simulated OSWTDS aerobic and anaerobic effluent well. This study, however, provides direction for future research with other soils and full-scale field sites.

5. Conclusions

The following conclusions were drawn from this study.

- The application of anaerobic effluent to surface soil horizons rather than subsurface horizons resulted in a 10- to 26-fold increase in N_2O emission (denitrification).
- Carbon was the limiting factor for denitrification in OSWTDSs. Thus, the higher C content present in anaerobic effluent is more important in promoting denitrification than the higher NO_3^- in aerobic effluent.
- Effluent applied once every 48 hours approximately doubled denitrification as compared to effluent application every 24 hours.
- Effluent application equal to the VDH-recommended rate produced the highest levels of denitrification.
- The combination of treatments that resulted in the most denitrification was the application of anaerobic effluent to a surface horizon at a 48-hour dosing interval at the VDH-recommended effluent loading rate.
- The combination of treatments that resulted in the highest percent of applied N removed was the application of an anaerobic effluent to a surface horizon at a 48-hour dosing interval at one-half the VDH-recommended effluent loading rate.
- Although the low loading rate (0.5 VDH-recommended rate) resulted in the highest percent of applied N removed, given a surface horizon and 48-hour dosing interval, the cost of doubling the size of a soil absorption field to allow for the lower loading rate does not seem warranted. The VDH-recommended rate removed 23-128% of the N applied as compared to 40-175% of the N applied at one-half that loading rate.
- The model developed to predict denitrification was not verified by field measurements of denitrification. Field measurements of denitrification were almost three orders of magnitude higher than laboratory measurements. This difference probably reflects a more favorable ratio between aerobic and anaerobic conditions in the field that enhanced denitrification, as compared to limited fluctuations that occurred in the subsurface laboratory columns.
- Field measurements of denitrification showed that denitrification increased with effluent loading rate, even at loading rates above the VDH-recommended loading rate.
- The predominant denitrifying organisms isolated in both the laboratory and field studies were of the following genera: *Pseudomonas, Bacillus, Flavobacterium,* and *Acinetobacter*.

- This study was performed on Groseclose and Lowell silt loam soils. The applicability of this study to other soils has not been determined; however, the information derived from this study should be applicable to soil surface horizons in general, and soils with finer-textured B horizons.
- Recommendations for future studies should include a similar study on coarser-textured soils, and the application of the results of this study to a full-scale experimental system.

Based on the information obtained in this study, it is suggested that N removal from wastewater via denitrification in low pressure distribution systems can be enhanced by applying septic tank effluent (anaerobic effluent) to surface soil horizons at the VDH-recommended effluent loading rate at a 48-hour dosing interval.

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Appendix I

Chemical Analyses of Soil Cores Used in the Laboratory Study

This section contains the results of chemical analyses performed on samples of the soil cores used in the laboratory study. These analyses were not included in their entirety in the main body of the report; they are presented here to supplement the other available information.

Results of all laboratory soil chemical analyses. All chemical results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	P	K	Ca	Mg	Mn	Zn	OM
Soil											
Surface	15.2a	14.5b	1918a	131.3a	36.8a	102a	845a	152b	44.7a	2.47a	2.72a
Subsurface	6.75b	28.5a	277b	36.0b	2.68b	106a	472b	189a	2.46b	1.48b	0.82b
Effluent											
Aerobic	11.9a	8.03b	1187a	79.2a	23.4a	115a	697a	178a	25.7a	1.91b	1.86a
Anaerobic	11.0a	35.9a	1163a	97.4a	15.6a	94.6b	639a	165a	20.7a	1.89b	1.72ab
Distilled	5.55b	12.7b	648b	38.1b	22.8a	95.4b	539b	156a	28.4a	2.85a	1.5b
Interval											
24 hour	9.8b	19.3a	1117a	79.9a	18.8a	104a	662a	175a	23.2a	1.89a	1.79a
48 hour	12.5a	23.9a	1150a	89.5a	20.9a	104a	654a	165a	24.0a	2.07a	1.74a
Loading Rate, cm day^{-1}											
Low	13.4a	16.9b	1190a	90.1a	21.2a	102a	649a	167a	26.2a	2.0a	1.80a
Medium	9.9b	18.8b	1056a	71.4a	19.7a	105a	661a	171a	23.4a	2.13a	1.73a
High	10.0b	29.0a	1168a	93.6a	18.4a	104a	664a	173a	21.3a	1.74a	1.78a

*Letter represents significantly different values at 0.05 level

Results of all laboratory surface soil chemical analyses. All values in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	P	K	Ca	Mg	Mn	Zn	OM
Effluent											
Aerobic	11.5b	10.7a	1933a	30.7b	42.4a	118a	877a	160a	48.2a	2.44a	2.81a
Anaerobic	20.2a	17.4a	2063a	45.4a	30.1b	85.7b	844a	147a	39.7a	2.47a	2.74a
Interval											
24 hour	14.3a	18.4a	2084a	38.5a	34.3a	103a	876a	163a	43.1a	2.36a	2.89a
48 hour	17.4a	9.73b	1902a	36.8a	38.2a	100a	845a	144a	44.9a	2.55a	2.66b
Loading Rate, cm day^{-1}											
Low	17.4a	10.6a	2061a	45.0a	40.7a	105a	893a	153a	51.1a	2.64a	2.85a
Medium	14.2a	12.5a	1923a	27.8b	33.2a	104a	878a	162a	39.8a	2.45a	2.67a
High	15.9a	19.1a	2010a	38.6ab	34.8a	96.2a	811a	146a	41.a	2.27a	2.80a

*Letter represents significantly different values at 0.05 level

Results of all laboratory subsurface soil chemical analyses. All values in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	P	K	Ca	Mg	Mn	Zn	OM
Effluent											
Aerobic	12.4a	5.22b	292a	128a	4.44a	111a	517a	196a	3.13a	1.38a	0.91a
Anaerobic	1.85b	54.3a	264a	149a	1.08b	103a	435a	183a	1.69a	1.32a	0.71a
Interval											
24 hour	6.69a	22.5b	258a	133a	1.90a	107a	489a	193a	3.25a	1.11b	0.79a
48 hour	7.29a	38.9a	297a	142a	3.62a	108a	463a	186a	1.57a	1.59a	0.83a
Loading Rate, cm day^{-1}											
Low	9.47a	23.3a	319a	144a	1.33a	99.5	426a	181a	1.96a	1.3a	0.75a
Medium	7.45a	29.0a	261ab	121a	2.23a	116a	445a	191a	1.35a	1.4a	0.91a
High	4.05b	38.9a	249b	149a	4.72a	107a	557a	197a	3.91a	1.35a	0.77a

*Letter represents significantly different values at the 0.05 level

Appendix II

Chemical Analyses of Soil Cores Used in the Field Study

This section contains the results of chemical analyses performed on samples of the soil cores used in the field column study. These analyses were not included in their entirety in the main body of the report; they are presented here to supplement the other available information.

Results of all field study soil chemical analyses. All chemical results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	P	K	Ca	Mg	Mn	Zn	OM
Loading Rate, cm											
0	1.38ab*	7.59b	554.6a	29.9a	1.82c	29.5a	399c	189a	10.2b	0.42b	0.81a
0.9	0.22b	6.41b	691.7a	29.9a	3.61bc	23.7b	496a	193a	14.9b	0.54ab	0.82a
1.8	2.02a	32.5a	564.4a	26.1a	5.04b	30.8a	453b	162b	21.1a	0.58a	0.86a
3.6	2.55a	24.65a	510.0a	30.6a	9.12a	30.0a	447b	165b	20.3a	0.59a	0.86a
Depth, cm											
S**	1.75a	24.5abc	1581a	32.3ab	13.1a	51.2a	693a	140c	34.0a	1.48a	2.24a
0	1.24a	34.3a	422.1b	20.6b	5.76b	30.2b	453b	138c	25.7b	0.54b	0.68b
15	0.99a	28.5ab	347.1b	30.1ab	3.99bc	27.9bc	427b	160b	15.8c	0.42b	0.65b
30	2.83a	12.1bc	533.3b	26.2ab	4.32b	23.4c	445b	201a	11.4cd	0.38b	0.65b
45	1.43a	6.1c	454.0b	36.1a	1.45c	22.9c	342c	214a	8.85d	0.36b	0.62b

*Letter represent significantly different values at the 0.05 level.
**S = surface soil above gravel layer. Remaining depths indicate depth below the gravel layer.

Results of all field study soil chemical analyses without control samples (mg kg^{-1} except organic matter (% OM)

Treatment	NO_3-N	NH_4-N	TKN	Cl	P	K	Ca	Mg	Mn	Zn	OM
Loading Rate, cm											
0.9	0.22b	6.41b	691.7a	29.9a	3.61b	23.7b	496a	193a	14.9b	0.54ab	0.82a
1.8	2.02a	32.5a	564.4a	26.1a	5.04b	30.8a	453b	162b	21.1a	0.58a	0.86a
3.6	2.55a	24.65a	510.0a	30.6a	9.12a	30.0a	447b	165b	20.3a	0.59a	0.86a
Depth, cm											
S**	1.85a	26.7abc	1471a	32.7a	14.3a	47.0a	706a	142bc	37.1a	1.54a	2.29a
0	1.48a	41.0a	49.0ab	2.4ab	6.95b	.	753b	118c	9.67b	0604b	0.68b
15	1.24a	34.3ab	360.3a	30.5a	4.77b	29.2bc	459b	154b	17.8c	0.42bc	0.66b
30	2.42a	14.3bc	565.9a	23.7ab	5.09b	23.2cd	441b	198a	12.5bc	0.4bc	0.65b
45	1.62a	4.9c	484.8b	34.9a	1.62c	22.1d	343c	210a	8.95d	0.37c	0.62b

*Letter represent significantly different values at the 0.05 level.
**S = surface soil above gravel layer. Remaining depths indicate depth below the gravel layer.
*extractable N
**S = surface soil, remaining depths (0, 15, 30, and 45) indicate depth below trench.

Tables

Table 1.
Septic tank influent characteristics (Canter and Knox 1985).

Parameter	Concentration
Total Solids	781 mg L^{-1}
BOD	300 mg L^{-1}
COD	750 mg L^{-1}
TOC	200 mg L^{-1}
Total N	50 mg L^{-1}
Organic-N	38 mg L^{-1}
Ammonia-N	12 mg L^{-1}
Nitrate-N	0.6 mg L^{-1}
Total P	25 mg L^{-1}
Total Coliform	2 X 10^6/100 ml

Table 2.
Reported genera of denitrifying bacteria (Fillery 1983).

Acinetobacter	Alcaligenes	Halobacterium
Gluconobacter	Bacillus	Hyphomicrobium
Micrococcus	Moraxella	Paracoccus
Pseudomonas	Rhodopseudomonas	Azospirillum
Spirillum	Thiobacillus	Xanthomonas
Cytophaga	Flavobacterium	Vibrio
Propionobacterium	Rhizobium	

Table 3.
Profile description of soil used in laboratory study.

The soil was collected on a gently sloping (2% slope) Groseclose silt loam soil near Blacksburg, VA.

Ap	0-25 cm. Brown (10YR 5/3) silt loam; moderate fine granular structure; friable, slightly sticky, slightly plastic; abrupt smooth boundary.
Bt1	25-75 cm. Yellowish brown (10YR 5/6) clay; moderate very fine and fine subangular blocky structure; friable, sticky, plastic; continuous clay films and few black coatings on ped faces; clear smooth boundary.
Bt2	75-105 cm. Mottled strong brown (7.5YR 5/8) and yellowish red (5YR 5/8) clay; moderate medium and coarse subangular blocky structure; friable, sticky, plastic; common slickensides; many thick patchy clay films and few black coatings on faces of peds; clear wavy boundary.
C1	105-135 cm. Mottled brown (7.5YR 5/8) and yellowish red (5YR 5/6) clay; massive; friable, sticky, slightly plastic; common slickensides; thick discontinuous clay flows; brownish yellow (10YR 6/6) saprolite that crushes easily.
C2	135-200 cm. Mottled reddish yellow (5YR 6/6) and yellowish red (5YR 5/8) clay loam; massive; friable, sticky, slightly plastic; common slickensides; discontinuous clay flows; brownish yellow (10 YR 6/6) saprolite that crushes easily.

Table 4.
Chemical and physical properties of soil used in laboratory study (Menelik et al. 1990).

Horizon	Depth (cm)	pH	sand	silt	clay
			----------percent----------		
Ap	0-25	5.3	26	61	14
Bt1	23-75	5.3	13	24	63
Bt2	75-105	5.0	23	31	47
C1	105-135	5.1	25	31	45
C2	135-200	5.0	22	43	36

Table 5.
Effluent characteristics for laboratory soil column study.

Parameter	Anaerobic	Aerobic
pH	7.0	7.2
Cl^- (mg L^{-1})	58.9	44.8
NH_4^+-N (mg L^{-1})	15.4	3.3
NO_3^--N (mg L^{-1})	2.8	12.1
TKN (mg L^{-1})	29.1	15.5
TOC (mg L^{-1})	73.7	15.4
NO_3^--N/Cl	0.048	0.270
Total N/Cl	0.542	0.616
$NO_3^- + NH_4^+$/Cl	0.309	0.344

Table 6.
Profile description for field study soil (Simon et al. 1986).

The soil was collected on a gently sloping nose position of an interfluve of Lowell silt loam near Fairlawn, Va.

Ap	0-25 cm. Brown (10YR 4/3) silt loam; moderate fine granular structure; friable (moist); clear, smooth boundary.
Bt1	25-26 cm. Brownish yellow (10YR 6/6) silty clay loam; many reddish yellow (7.5YR 6/8) and strong brown (7.5YR 5/8) mottles; strong fine subangular blocky structure; very friable (moist); moderately thick clay films; gradual smooth boundary.
Bt2	56-90 cm. Brownish yellow (10YR 6/6) and strong brown (7.5YR 5/8) clay; color patterns are arranged more in layers than as mottles; strong medium subangular blocky structure; friable (moist); 10 to 60 percent weathered shale in lower part of horizon; thick clay films noted on both ped and shale faces; gradual wavy boundary.
BC	90-130 cm. Brownish yellow (10YR 6/6) silt loam to silty clay loam with strong brown (7.5YR 5/8) layers of weathered shale which makes up 30 to 60 percent of horizon; friable (moist); some clay films evident on shale faces; clear wavy boundary.
C	130-150+ cm. Yellow (10YR 7/8) silt loam with strong brown (7.5YR 5/8) layers common; moderate medium to coarse structure, probably of parent material origin; 10 to 20 percent shale fragment in parts of the horizon.

NOTE: This unit is developed in limestone interbedded with shale. The abundance of clay films on both peds and shale faces in both the B and BC horizons, indicates that water moves freely through the profile. At the edge of the site, limestone outcrops at the surface.

Table 7.
Chemical and physical properties of field soil (Simon et al. 1986).

Horizon	Depth (cm)	pH	CEC*	base sat	sand	silt	clay
					----------percent----------		
Ap	0-23	5.6	13.0	51.0	14	60	26
Bt1	23-51	6.2	8.9	53.7	09	54	37
Bt2	63-86	6.1	14.9	56.3	10	40	50
BC	86-105	6.0	15.4	74.4	21	38	41
C	105-124	6.2	8.8	70.8	16	58	26

*cmol(+)kg^{-1}

Table 8.
Characteristics of septic tank effluent used in field column study.

Parameter	Mean Value
pH	7.3
Cl^- (mg L^{-1})	49.8
TKN (mg L^{-1})	62.3
NH_4^+-N (mg L^{-1})	55.0
NO_3^--N (mg L^{-1})	0.16
COD (mg L^{-1})	407
ortho-P (mg L^{-1})	17.1

Table 9.
Analysis of leachate from laboratory soil columns for 10°C; all results in mg L^{-1}.

Treatment	NO_3^--N*	NH_4^+-N	TOC
Soil			
Surface	10.1a	4.79a	44.4a
Subsurface	3.75b	0.41b	49.2a
Effluent			
Aerobic	10.2a	0.43b	41.8b
Anaerobic	3.47b	4.77a	50.1a
Dosing Interval			
24 hour	6.43a	2.82a	59.7a
48 hour	7.33a	2.18a	32.0b
Loading Rate			
Low	7.60a	2.28a	64.9a
Medium	7.62a	2.61a	44.0b
High	5.33b	2.63a	34.2c
Control	0.62	0.22	63.15

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 10.
Analysis of leachate from laboratory soil columns for 20°C; all results in mg L^{-1}

Treatment	NO_3^--N*	NH_4^+-N	TOC
Soil			
Surface	11.5a	6.01a	30.0b
Subsurface	2.78b	0.42b	46.2a
Effluent			
Aerobic	10.2a	0.32b	30.1b
Anaerobic	4.94b	6.08a	38.0a
Dosing Interval			
24 hour	7.11a	3.01a	37.4a
48 hour	7.73a	3.79a	32.5a
Loading Rate			
Low	7.63a	3.07a	48.5a
Medium	9.14a	3.46a	31.4b
High	5.47b	3.61a	26.6b
Control	0.55	0.65	31.1

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 11.
Analysis of leachate from laboratory surface soil columns; all results in mg L^{-1}.

10°C Study

Treatment	NO_3^--N*	NH_4^+-N	TOC
Effluent			
Aerobic	13.9a	0.64b	38.2b
Anaerobic	6.13b	9.74a	50.0a
Dosing Interval			
24 hour	9.30b	5.33a	54.0a
48 hour	10.9a	4.18a	31.1b
Loading Rate			
Low	11.8a	4.35a	63.3a
Medium	9.67b	4.87a	38.2b
High	8.58b	5.18a	34.3b

20°C Study

Treatment	NO_3^--N*	NH_4^+-N	TOC
Effluent			
Aerobic	14.68a	0.23b	26.8a
Anaerobic	8.63b	11.3a	32.6a
Dosing Interval			
24 hour	11.0a	5.35a	32.2a
48 hour	12.2a	6.72a	28.2a
Loading Rate			
Low	12.8a	5.39a	43.4a
Medium	12.4a	6.08a	27.1b
High	9.54b	6.57a	23.5b

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 12.
Analysis of leachate from laboratory subsurface soil columns; all results in mg L^{-1}.

10°C Study			
Treatment	NO_3^--N*	NH_4^+-N	TOC
Effluent			
Aerobic	6.54a	0.23b	48.3a
Anaerobic	0.87b	0.60a	50.2a
Dosing Interval			
24 hour	3.46a	0.31b	78.9a
48 hour	4.03a	0.51a	33.3b
Loading Rate			
Low	3.32b	0.35a	70.9a
Medium	5.68a	0.50a	51.9b
High	2.20b	0.38a	34.1c

20°C Study			
Treatment	NO_3^--N*	NH_4^+-N	TOC
Effluent			
Aerobic	4.90a	0.42a	39.9a
Anaerobic	0.98b	0.43a	50.0a
Dosing Interval			
24 hour	2.80a	0.40a	53.2a
48 hour	2.76a	0.45a	41.9a
Loading Rate			
Low	2.06b	0.48a	57.2a
Medium	5.49a	0.49a	42.7a
High	0.75b	0.30a	36.0a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 13.
Analysis of NO_3-N/Cl and total inorganic N/Cl in leachate from laboratory soil columns receiving aerobic effluent.

10°C Study		
Treatment	NO_3^--N/Cl	Inorganic-N/Cl
Effluent	0.27	0.344
Soil		
Surface	0.333a	0.347a
Subsurface	0.254a	0.267a
Dosing Interval		
24 hour	0.33a	0.342a
48 hour	0.275a	0.290a
Loading Rate		
Low	0.304a	0.325a
Medium	0.283a	0.294a
High	0.314a	0.322a

20°C Study		
Treatment	NO_3^--N/Cl	Inorganic-N/Cl
Effluent	0.27	0.344
Soil		
Surface	0.324a	0.328a
Subsurface	0.316a	0.371a
Dosing Interval		
24 hour	0.322a	0.349a
48 hour	0.320a	0.336a
Loading Rate		
Low	0.292a	0.315a
Medium	0.370a	0.394a
High	0.290a	0.307a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 14.
Analysis of NO_3-N/Cl and total inorganic N/Cl in leachate from laboratory soil columns receiving anaerobic effluent.

10°C Study		
Treatment	NO_3^--N/Cl	Inorganic-N/Cl
Effluent	0.048	0.309
Soil		
Surface	0.129a	0.354a
Subsurface	0.044b	0.073b
Dosing Interval		
24 hour	0.084a	0.260a
48 hour	0.100a	0.190b
Loading Rate		
Low	0.159a	0.260a
Medium	0.067b	0.196b
High	0.045b	0.200b

20°C Study		
Treatment	NO_3^--N/Cl	Inorganic-N/Cl
Effluent	0.048	0.309
Soil		
Surface	0.181a	0.432a
Subsurface	0.081b	0.111b
Dosing Interval		
24 hour	0.145a	0.317a
48 hour	0.144a	0.314a
Loading Rate		
Low	0.199a	0.351a
Medium	0.134b	0.324a
High	0.104b	0.275a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 15.
Results of laboratory soil chemical analyses; all results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	OM
Soil					
Surface	15.2a	14.5b	1918a	131.3a	2.72a
Subsurface	6.75b	28.5a	277b	36.0b	0.82b
Effluent					
Aerobic	11.9a	8.03b	1187a	79.2a	1.86a
Anaerobic	11.0a	35.9a	1163a	97.4a	1.72ab
Distilled	5.55b	12.7b	648b	38.1b	1.50b
Interval					
24 hour	9.8b	19.3a	1117a	79.9a	1.79a
48 hour	12.5a	23.9a	1150a	89.5a	1.74a
Loading Rate, cm day^{-1}					
Low	13.4a	16.9b	1190a	90.1a	1.80a
Medium	9.90b	18.8b	1056a	91.4a	1.73a
High	10.0b	29.0a	1168a	93.6a	1.78a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 16.
Results of laboratory surface soil chemical analyses; all results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	OM
Effluent					
Aerobic	11.5b	10.7a	1933a	30.7b	2.81a
Anaerobic	20.2a	17.4a	2063a	45.4a	2.74a
Interval					
24 hour	14.3a	18.4a	2084a	38.5a	2.89a
48 hour	17.4a	9.73b	1902a	36.8a	2.66b
Loading Rate, cm day^{-1}					
Low	17.4a	10.6a	2061a	45.0a	2.85a
Medium	14.2a	12.5a	1923a	27.8b	2.67a
High	15.9a	19.1a	2010a	38.6ab	2.80a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 17.
Results of laboratory subsurface soil chemical analyses; all results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	OM
Effluent					
Aerobic	12.4a	5.22b	292a	128a	0.91a
Anaerobic	1.85b	54.3a	264a	149a	0.71a
Interval					
24 hour	6.69a	22.5b	258a	133a	0.79a
48 hour	7.29a	38.9a	297a	142a	0.83a
Loading Rate, cm day^{-1}					
Low	9.47a	23.3a	319a	144a	0.75a
Medium	7.45a	29.0a	261ab	121a	0.91a
High	4.05b	38.9a	249b	149a	0.77a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 18.
N_2O-N production after 48 hours for soil amended with effluent at 10°C for laboratory study.

Treatment	mg N_2O-N/mg soil *
Soil	
Surface	1.274×10^{-6}a
Subsurface	0.126×10^{-6}b
Effluent	
Aerobic	0.438×10^{-6}a
Anaerobic	0.970×10^{-6}a
Dosing Interval	
24 hour	0.530×10^{-6}b
48 hour	0.881×10^{-6}a
Loading Rate	
Low	0.375×10^{-6}b
Medium	0.590×10^{-6}ab
High	1.164×10^{-6}a
Control	0.034×10^{-6}

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 19.
N_2O-N production for soil amended with effluent at 20°C for laboratory study.

Treatment	mg N_2O-N/mg soil*
Soil	
Surface	5.027×10^{-6}a
Subsurface	0.190×10^{-6}b
Effluent	
Aerobic	1.259×10^{-6}a
Anaerobic	3.959×10^{-6}a
Dosing Interval	
24 hour	1.667×10^{-6}b
48 hour	3.551×10^{-6}a
Loading Rate	
Low	2.269×10^{-6}b
Medium	2.500×10^{-6}a
High	3.057×10^{-6}a
Control	0.082×10^{-6}

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 20.
N_2O-N production after 48 hours for surface soil amended with effluent for laboratory study.

Treatment	mg N_2O-N/mg soil*	
	10°C	20°C
Effluent		
Aerobic	0.639×10^{-6}b	2.232×10^{-6}b
Anaerobic	1.907×10^{-6}a	7.820×10^{-6}a
Dosing Interval		
24 hour	0.996×10^{-6}b	3.264×10^{-6}b
48 hour	1.551×10^{-6}a	6.784×10^{-6}a
Loading Rate		
Low	0.641×10^{-6}b	4.486×10^{-6}b
Medium	0.973×10^{-6}b	4.768×10^{-6}ab
High	2.207×10^{-6}a	5.818×10^{-6}a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 21.
N_2O-N production after 48 hours for subsurface soil amended with effluent for laboratory study.

Treatment	mg N_2O-N/mg soil*	
	10°C	20°C
Effluent		
Aerobic	0.225×10^{-6}a	0.285×10^{-6}a
Anaerobic	0.033×10^{-6}a	0.095×10^{-6}b
Dosing Interval		
24 hour	0.036×10^{-6}b	0.072×10^{-6}b
48 hour	0.210×10^{-6}a	0.308×10^{-6}a
Loading Rate		
Low	0.085×10^{-6}b	0.055×10^{-6}b
Medium	0.169×10^{-6}b	0.220×10^{-6}b
High	0.120×10^{-6}a	0.296×10^{-6}a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 22.
N_2O-N production per dosing cycle on a trench bottom area basis at 10°C for laboratory study.

Treatment	mg N_2O-N/m^2*	mg N_2O/ft^2*
Soil		
Surface	0.266	0.025
Subsurface	0.024	0.003
Effluent		
Aerobic	0.091	0.008
Anaerobic	0.201	0.018
Dosing Interval		
24 hour	0.108	0.010
48 hour	0.185	0.017
Loading Rate		
Low	0.077	0.007
Medium	0.121	0.012
High	0.239	0.022

Table 23.
N_2O-N production per dosing cycle on a trench bottom basis at 20°C for laboratory study.

Treatment	mg N_2O-N/m^2*	mg N_2O-N/ft^2*
Soil		
Surface	1.048	0.097
Subsurface	0.037	0.003
Effluent		
Aerobic	0.263	0.024
Anaerobic	0.821	0.076
Dosing Interval		
24 hour	0.342	0.032
48 hour	0.742	0.070
Loading Rate		
Low	0.465	0.043
Medium	0.533	0.049
High	0.630	0.058

Table 24.
Comparison of N_2O-N production for soil amended with effluent to soil amended with excess glucose.

Amendment	mg N_2O-N/mg soil*
Glucose	7.130×10^{-6}a
Effluent	1.664×10^{-6}b

*Means with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 25.
Comparison of N_2O-N production for soil amended with effluent to soil amended with excess glucose for each treatment.

Treatment	Glucose mg N_2O-N/mg soil	Effluent mg N_2O-N/mg soil
Soil		
Surface	12.06×10^{-6}a	3.150×10^{-6}a
Subsurface	2.200×10^{-6}b	0.157×10^{-6}b
Effluent		
Aerobic	5.860×10^{-6}a	0.843×10^{-6}a
Anaerobic	8.403×10^{-6}a	3.851×10^{-6}a
Dosing Interval		
24 hour	5.076×10^{-6}a	1.091×10^{-6}b
48 hour	9.184×10^{-6}a	2.216×10^{-6}a
Loading Rate		
Low	8.154×10^{-6}a	1.315×10^{-6}b
Medium	7.488×10^{-6}a	1.535×10^{-6}a
High	5.748×10^{-6}a	2.111×10^{-6}a

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 26.
Estimate of the number of denitrifiers for laboratory study using a most probable number technique.

	Organisms/g†
Soil	
Surface	6350a*
Subsurface	2522b
Effluent	
Aerobic	5814a
Anaerobic	3451ab
Distilled	2077b
Dosing Interval	
24 hour	3685b
48 hour	5312a
Loading Rate	
Low	4179a
Medium	3723a
High	5584a

†Soil on dry weight basis.
*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 27.
Denitrifiers by genus identified in laboratory soil.

Gram negative rods
Pseudomonas
Flavobacterium
Acinetobacter
Gram positive cocci
Bacillus

Table 28.
Results of field study soil chemical analyses; all chemical results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	OM
Loading Rate, cm					
0	1.38ab*	7.59b	555a	29.9a	0.81a
0.9	0.22b	6.41b	692a	29.9a	0.82a
1.8	2.02a	32.5a	564a	26.1a	0.86a
3.6	2.55a	24.7a	510a	30.6a	0.86a
Depth, cm					
S†	1.75a	24.5abc	1580a	32.3ab	2.24a
0	1.24a	34.3a	422b	20.6b	0.68b
15	0.99a	28.5ab	347b	30.1ab	0.65b
30	2.83a	12.1bc	533b	26.2ab	0.65b
45	1.43a	6.1c	454b	36.1a	0.62b

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.
†S = surface soil above gravel layer. Remaining depths indicate depth below the gravel layer.

Table 29.
Results of field study soil chemical analyses without control samples; all chemical results in mg kg^{-1} except organic matter (% OM).

Treatment	NO_3-N	NH_4-N	TKN	Cl	OM
Loading Rate, cm					
0.9	0.22b*	6.41b	692a	29.9a	0.82a
1.8	2.02a	32.5a	564a	26.1a	0.86a
3.6	2.55a	24.7a	510a	30.6a	0.86a
Depth, cm					
S†	1.85a	26.7abc	1470a	32.7a	2.29a
0	1.48a	41.0a	49.0ab	2.4ab	0.68b
15	1.24a	34.3ab	360a	30.5a	0.66b
30	2.42a	14.3bc	566a	23.7ab	0.65b
45	1.62a	4.9c	485b	34.9a	0.62b

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.
†S = surface soil, remaining depths (0, 15, 30, and 45) indicate depth below trench.

Table 30.
Soil NO_3^--N and NH_4^+-N concentration (mg kg^{-1}) with depth below trench bottom by loading rate.

Loading Rate cm day^{-1}	Depth cm	NO_3^--N	NH_4^+-N	TKN
0.0	S	1.35	16.1	1980
0.0	0	0.31	6.91	112
0.0	15	ND	5.68	296
0.0	30	4.5	2.99	416
0.0	45	0.72	10.5	362
0.9	S	0.65	23.7	1440
0.9	0	0.25	4.38	426
0.9	15	0.38	3.34	290
0.9	30	0.25	4.47	707
0.9	45	ND	4.15	700
1.8	S	2.99	36.4	1460
1.8	0	2.29	57.5	423
1.8	15	1.42	50.4	429
1.8	30	2.58	17.2	567
1.8	45	1.38	4.51	480
3.6	S	1.06	12.7	1580
3.6	0	1.41	48.0	522
3.6	15	1.64	29.4	282
3.6	30	4.26	18.3	327
3.6	45	3.79	6.63	454

ND - Not detectable

Table 31.
N_2O-N concentration in gravel atmosphere in Radford field denitrification study.

Loading Rate, cm day^{-1}	N_2O-N, mg L^{-1}*
0.0	1.5×10^{-3}a
0.9	4.9×10^{-3}a
1.8	40.6×10^{-3}a
3.6	71.7×10^{-3}a

*Means with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 32.
N_2O production in mg N_2O-N per mg dry soil for field study; data presented as instantaneous and cumulative rate.

Treatment	Instantaneous Rate mg N_2O-N/mg dry soil*	Cumulative Rate mg N_2O-N/mg dry so
Loading Rate, cm day^{-1}		
0.0	0.896×10^{-6}a	12.08×10^{-6}
0.9	3.104×10^{-6}a	6.59×10^{-6}
1.8	25.5×10^{-6}a	38.32×10^{-6}
3.6	44.1×10^{-6}a	61.76×10^{-6}
Time after dosing, hours		
0	0.608×10^{-6}b	
2	31.27×10^{-6}b	
4	21.66×10^{-6}ab	
6	32.36×10^{-6}a	
8	47.42×10^{-6}ab	

*Means within a column and treatment with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 33.
N_2O-N production on trench bottom area basis for field study.

Loading Rate cm day^{-1}	mg N_2O-N/m^2 per 8 hr	mg N_2O-N/m^2 per day	mg N_2O-N/ft^2 per 8 hr	mg N_2O-N/ft^2 per day
0	0.066×10^{-3}	0.203×10^{-3}	0.712×10^{-3}	2.187×10^{-3}
0.9	0.050×10^{-3}	0.111×10^{-3}	0.536×10^{-3}	1.190×10^{-3}
1.8	0.511×10^{-3}	0.645×10^{-3}	5.50×10^{-3}	6.94×10^{-3}
3.6	0.889×10^{-3}	1.038×10^{-3}	9.57×10^{-3}	11.17×10^{-3}

Table 34.
Estimates of denitrifier populations by MPN technique (organisms/g soil dry weight).

Depth below trench bottom (cm)	Loading Rate (cm day^{-1})* 0.0	0.9	1.8	3.6
0-15	3474a	5299a	2702a	23a
15-30	3088a	3307a	2896a	245a
30-45	10661a	2784b	132b	2b
gravel	5404a	465a	2847a	2863a

*Means within a column and depth with differing lower case letters are different at the 0.05 probability level with the Duncan's New Multiple Range Test.

Table 35.
Identification of denitrifiers by genus in field soils.

Gram negative rods
Pseudomonas
Flavobacterium
Acinetobacter
Gram positive cocci
Bacillus

Table 36.
Prediction of N_2O-N production for experimental treatments.

Surface Soil				
Effluent	Dosing Interval	Loading Rate	mg N_2O-N per mg soil 10°C	20°C
Aerobic	24 h	Low	0.013×10^{-6}	0.263×10^{-6}
Aerobic	24 h	Medium	0.012×10^{-6}	0.986×10^{-6}
Aerobic	24 h	High	0.401×10^{-6}	3.20×10^{-6}
Aerobic	48 h	Low	0.200×10^{-6}	0.922×10^{-6}
Aerobic	48 h	Medium	0.246×10^{-6}	1.82×10^{-6}
Aerobic	48 h	High	0.205×10^{-6}	1.24×10^{-6}
Anaerobic	24 h	Low	0.048×10^{-6}	0.811×10^{-6}
Anaerobic	24 h	Medium	0.042×10^{-6}	2.15×10^{-6}
Anaerobic	24 h	High	0.794×10^{-6}	5.11×10^{-6}
Anaerobic	48 h	Low	1.58×10^{-6}	6.97×10^{-6}
Anaerobic	48 h	Medium	1.81×10^{-6}	10.2×10^{-6}
Anaerobic	48 h	High	1.60×10^{-6}	6.74×10^{-6}
Subsurface Soil				
Effluent	Dosing Interval	Loading Rate	mg N_2O-N per mg soil 10°C	20°C
Aerobic	24 h	Low	0.0001×10^{-6}	0.001×10^{-6}
Aerobic	24 h	Medium	0.012×10^{-6}	0.013×10^{-6}
Aerobic	24 h	High	0.004×10^{-6}	0.013×10^{-6}
Aerobic	48 h	Low	0.042×10^{-6}	0.017×10^{-6}
Aerobic	48 h	Medium	0.051×10^{-6}	0.189×10^{-6}
Aerobic	48 h	High	0.121×10^{-6}	0.545×10^{-6}
Anaerobic	24 h	Low	0.0006×10^{-6}	0.0006×10^{-6}
Anaerobic	24 h	Medium	0.019×10^{-6}	0.007×10^{-6}
Anaerobic	24 h	High	0.006×10^{-6}	0.003×10^{-6}
Anaerobic	48 h	Low	0.003×10^{-6}	0.0006×10^{-6}
Anaerobic	48 h	Medium	0.004×10^{-6}	0.018×10^{-6}
Anaerobic	48 h	High	0.017×10^{-6}	0.061×10^{-6}

Table 37.
Percent of N removed by denitrification.

Surface Soil				
Effluent	Dosing Interval	Loading Rate	Percent of N lost 10°C	20°C
Aerobic	24 h	Low	0.75	15.3
Aerobic	24 h	Medium	0.35	28.7
Aerobic	24 h	High	7.78	62.1
Aerobic	48 h	Low	5.82	26.83
Aerobic	48 h	Medium	3.58	26.48
Aerobic	48 h	High	1.99	12.03
Anaerobic	24 h	Low	2.41	40.71
Anaerobic	24 h	Medium	1.06	53.96
Anaerobic	24 h	High	13.34	85.86
Anaerobic	48 h	Low	39.66	174.9
Anaerobic	48 h	Medium	22.79	128.4
Anaerobic	48 h	High	13.42	56.51

Subsurface Soil				
Effluent	Dosing Interval	Loading Rate	Percent N lost 10°C	20°C
Aerobic	24 h	Low	0.02	0.18
Aerobic	24 h	Medium	1.12	1.2
Aerobic	24 h	High	0.25	0.8
Aerobic	48 h	Low	3.9	1.6
Aerobic	48 h	Medium	2.36	8.76
Aerobic	48 h	High	3.77	16.95
Anaerobic	24 h	Low	0.097	0.097
Anaerobic	24 h	Medium	1.54	0.56
Anaerobic	24 h	High	0.33	0.16
Anaerobic	48 h	Low	0.24	0.05
Anaerobic	48 h	Medium	0.16	0.72
Anaerobic	48 h	High	0.46	1.65

Table 38.
Denitrification rates as reported in the literature.

OSWTDS System	Source	Percent N removed
Conventional	Ritter & Eastburn 1988	0-35%
Sand filter	Wert & Paeth 1985	71-97%
LPD at grade	Stewart & Reneau 1984	98%
Mound	Harkin et al. 1979	44-86%
LPD shallow	Brown & Thomas 1978	46%

Figures

Figure 1.
The nitrogen cycle (Freeze and Cherry, 1979).

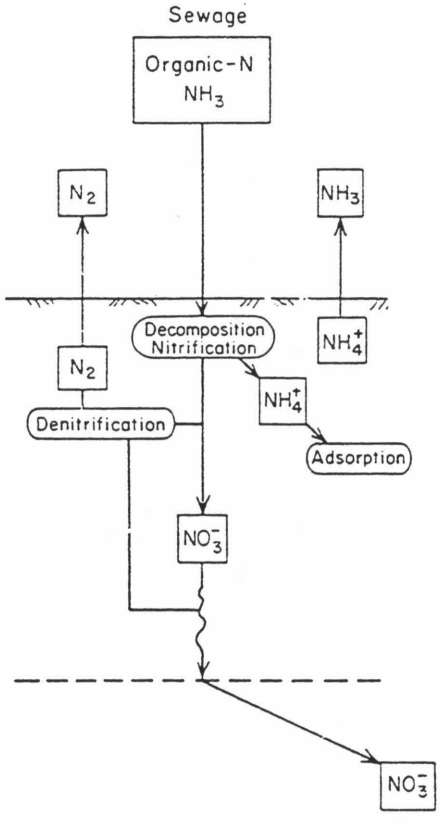


Figure 2.
Laboratory column construction.

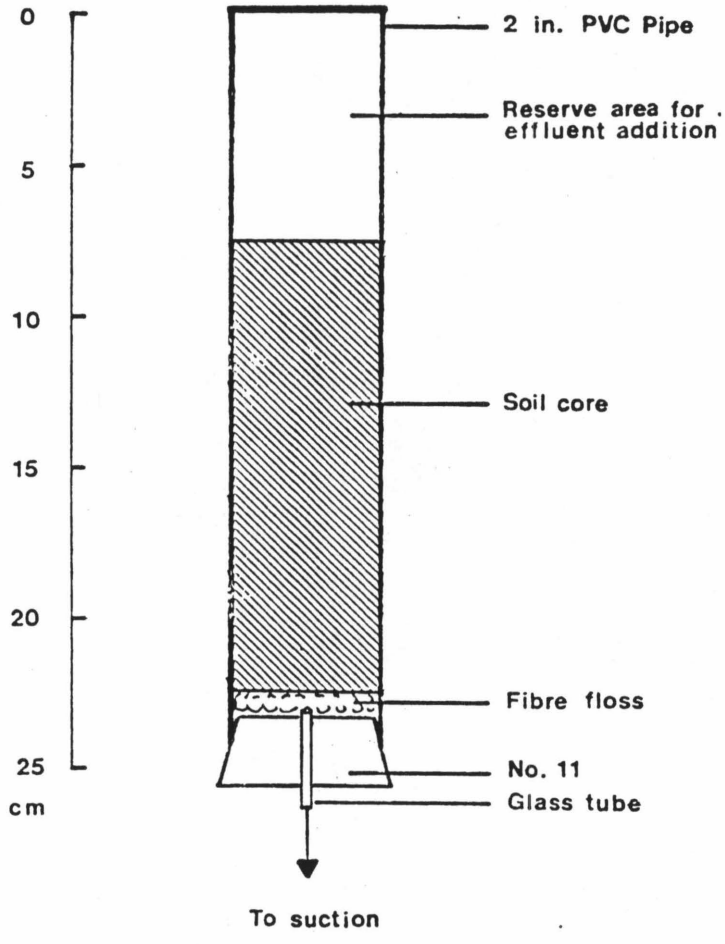
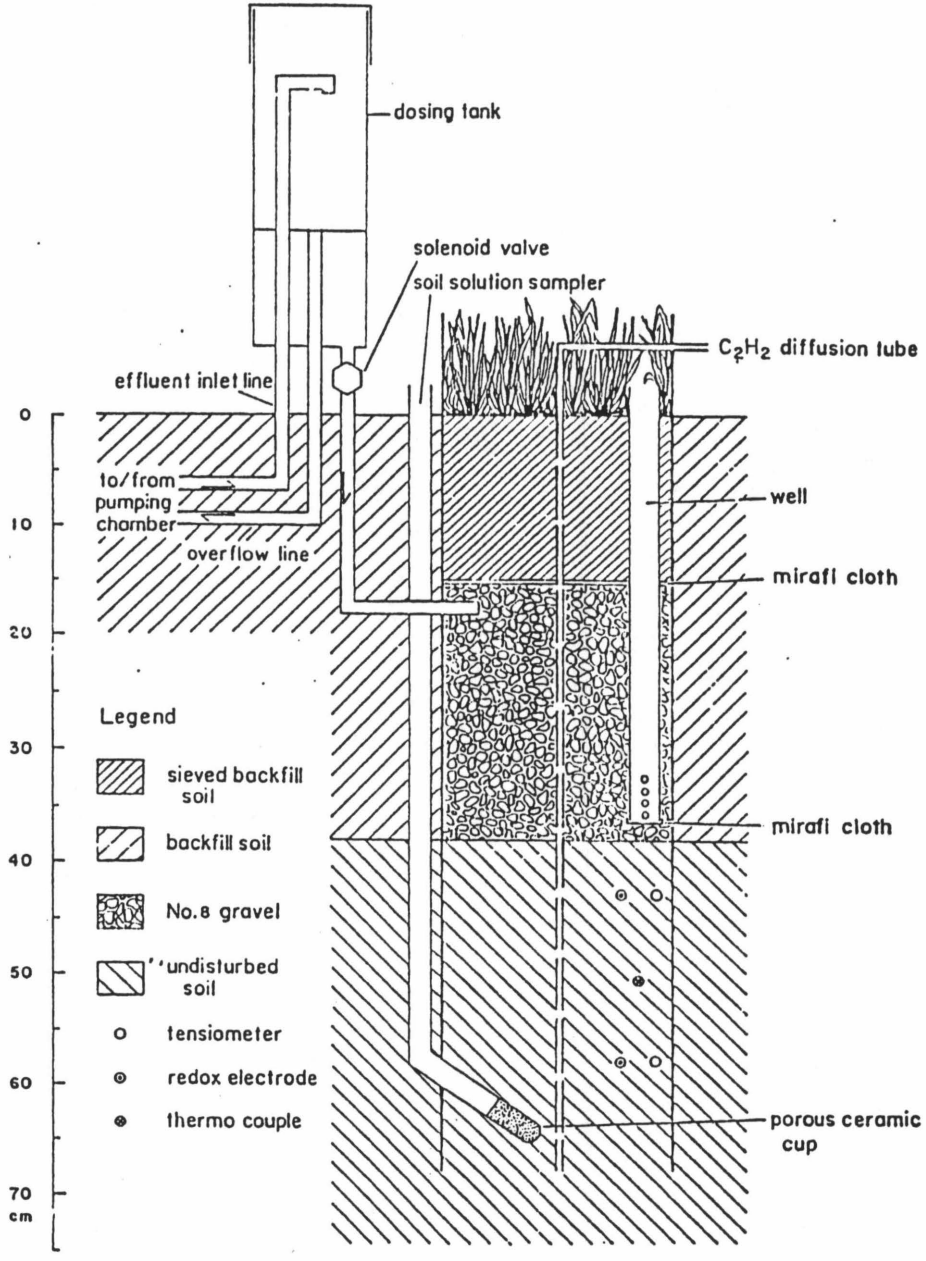


Figure 3.
Field column construction.



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- establish and administer research agreements with all universities in Virginia;
- facilitate and stimulate research that concerns policy issues facing the General Assembly, supports water resource agencies, and provides organizations with tools to increase effectiveness of water management;
- disseminate new information and facilitate application of new technology;
- serve as a liaison between Virginia and federal research funding agencies as an advocate for Virginia's water research needs; and
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