ENVIRONMENTS OF DENITRIFICATION IN A BARRIERED LANDSCAPE WATER RENOVATION SYSTEM

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY
Loren James Moshier

1974

K-317

A012-4-054

ABSTRACT

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IN A
BARRIERED LANDSCAPE WATER RENOVATION SYSTEM

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Existence of environments of denitrification in a Barriered Landscape Water Renovation System (BLWRS) was verified by applying a solution of nitrate as $Ca(NO_3)_2$ and chloride as $CaCl_2$ onto the BLWRS surface. Swine wastes had been applied for extensive periods of time before experimental solution application and continued to be applied afterwards to maintain, as closely as possible, natural conditions. Ratios of the concentration of nitrate-nitrogen to concentration of chloride was monitored as the solution front moved through the BLWRS soil profile. Denitrification was presumed to have occurred in a specific environment if reduction of nitrate-N/chloride ratio occurred as the solution passed through that environment. Environments in the BLWRS which were found to be conducive to denitrification were the surface horizon and the saturated zone. Additions of organic carbon as cracked corn or molasses stimulated denitrification in the saturated zone. Denitrification was also found to have

occurred in the zone between the surface horizon and the saturated zone but to a lesser degree.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Crop and Soil Sciences

1974

To My Wife

ACKNOWLEDGMENTS

The author wishes to gratefully acknowledge his sincere appreciation to Dr. A. E. Erickson for his guidance and patience at all times during this study and in the thesis preparation.

Gratitude is expressed to Dr. B. G. Ellis, Dr. E. H. Kidder, Dr. J. M. Tiedje and Dr. A. R. Wolcott who served on my guidance committee.

Appreciation is extended to Mrs. Elizabeth Shields for her assistance in laboratory analysis and to my wife, Deanna, for typing the manuscript.

Appreciation is also extended to the Agricultural Experiment Station for financial assistance.

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INTRODUCTION

Renovation of wastewaters from agricultural and municipal sources is a serious problem that faces present day society. The trend toward geographical concentration of human and domesticated animal populations has created and magnified the problem. Plant products are grown on large land acreages, transported for considerable distances in most cases and consumed by large populations concentrated on small land areas. In the past, waste by products were usually absorbed and renovated by large volumes of soil and water resources located at or near locations of plant nutrient removal. In recent years, however, a considerable fraction of our water resources have become polluted due to discharge of wastes in the forms of treatment plant effluents, sewer tile effluent, runoff and seepage from feedlot manures. The failure to return nutrients present in these wastes to the soil and pollution of water resources have disrupted the ecosystems of both of these environments. The result has been eutrophication of surface waters, aquifer contamination, and an increase in dependency on limited fertilizer resources.

Irrigation of raw and treated wastes on well managed soils has been presented as a solution to the waste renovation problem. The soil can be used as a physical,

chemical and biological filter in this renovative process. Nitrogen, the nutrient of concern in this thesis, is removed from applied waste waters by the combined action of all three components of the soil filter. The soil surface acts as a physical filter by sieving out nitrogen containing suspended particulate solids present in wastes (Erickson et al, 1971; Thomas, 1973). Chemically, the soil "filters out" organic nitrogen compounds and ammonium cations via absorption on particle surfaces (Ellis, 1973). The biological component of the soil filter, consisting of both plants and soil microorganisms, then transforms the various nitrogen compounds that have entered the soil environment into various innocuous forms (Miller, 1973). Nitrate, a potential pollutant, which enters the soil solution directly as a wastewater constituent or as a product of various soil microbial processes can be removed by this "living filter". Thus, applications on land of agricultural and municipal wastewater can be used to reclaim and fertilize land and to return water in a purified state to underground supplies.

This investigation was designed to examine the role that denitrification, the microbial transformation of nitrate to nitrogen gas, plays in a soil system designed to renovate water carrying suspended animal waste. The objectives of this study were: 1) to determine the amount of denitrification, 2) to determine the locations of

denitrification in this system, and 3) to determine the environmental conditions of these locations.

LITERATURE REVIEW

Nitrogen Cycle

Several authors (Campbell and Lees, 1967; Delwiche, 1970; Jansson, 1958; Stevenson, 1965; Wolcott, 1972) have described the nitrogen cycle in the biosphere. A flow diagram, presented in a recent study (Wolcott, 1972) includes the introduction of treated and untreated wastes with low C/N ratios into the nitrogen cycle. This diagram (Figure 1) illustrates the importance of plant uptake and microbial transformations in the removal of nitrogen from wastewaters. Plant uptake of nitrate (NO_3^-) referred to as plant assimilatory nitrate reduction (Arrow 1, Figure 1) can be significant if crops such as corn or reed canary grass are grown and harvested on waste disposal sites (Sopper and Kardos, 1973). The microbial reactions involved in nitrogen removal are mineralization, immobilization, nitrification and denitrification.

Mineralization is the microbial conversion of organic nitrogen to ammonium-nitrogen (Arrow 2). Immobilization (Arrow 3) which includes both ammonium assimilation and assimilatory nitrate reduction is the microbial conversion of inorganic nitrogen to organic nitrogen.

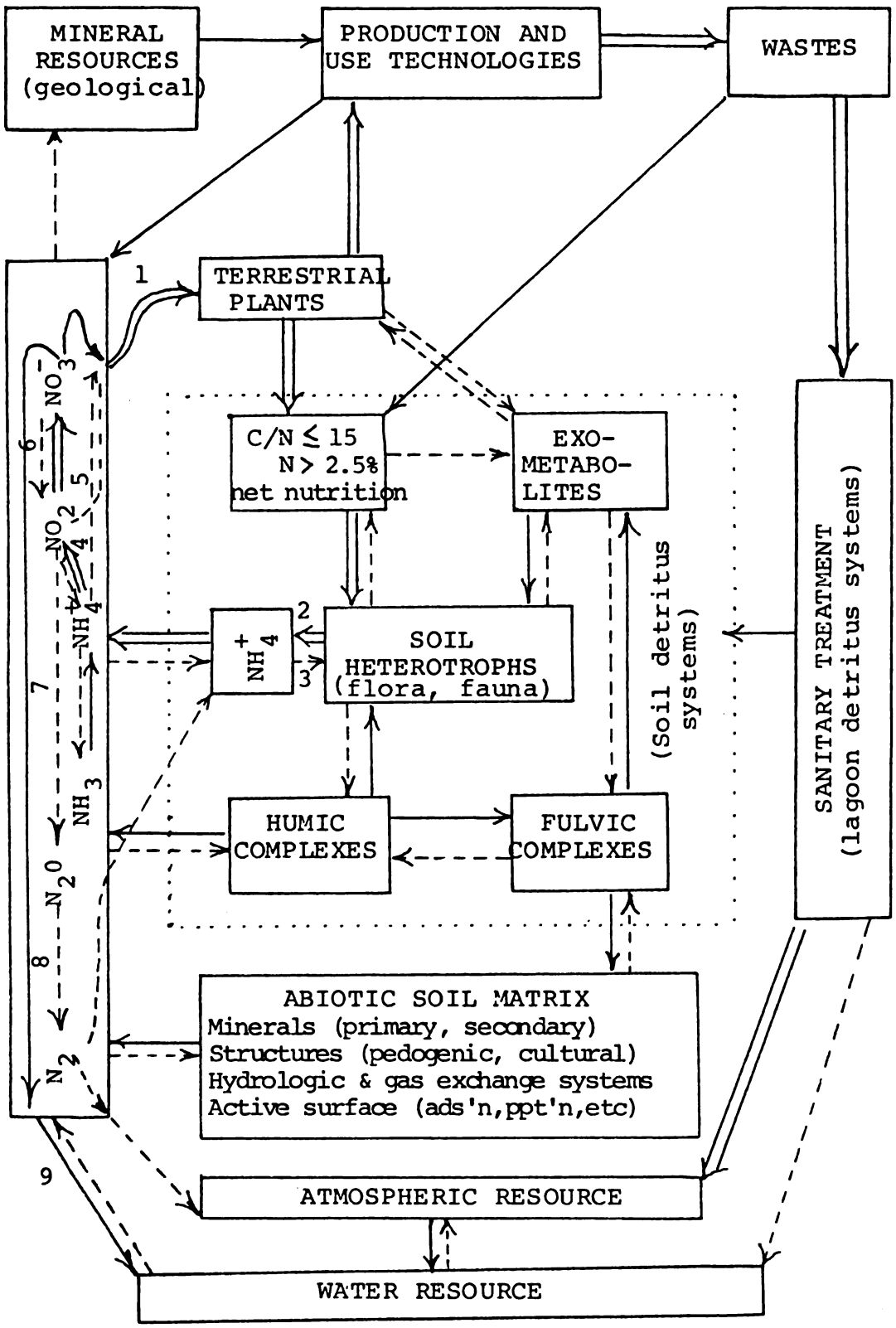


Figure 1. Nitrogen flows in a land waste disposal system.

⟹ Primary flow

⟶ Intermediate flow, or net flow

⇢ Restricted flow, or intermittent flow

(Adapted from Wolcott, 1972)

Mineralization and immobilization are essentially reverse reactions with the net flow of nitrogen being dependent on the ratio of carbon to nitrogen present in the soil environment. If the C/N ratio of wastewaters being applied is less than or equal to 10, the average C/N ratio in living cells, then net mineralization will occur in the soil (Miller, 1973). C/N ratios in swine wastes, which were used in this investigation, were calculated using biological oxygen demand (BOD) values and total nitrogen values reported in the literature. Concentrations of carbon present in the wastes were estimated by using the following formula: $BOD \times \frac{12\ (a.w.-C)}{32\ (m.w.-O_2)} \times 0.75 = C$. The use of this equation assumes that carbon in the presence of oxygen is transformed to CO_2 and that oxidation of fatty acids and inorganic nitrogen are accounted for by the factor 0.75 (Wolcott, 1974). The calculated C/N ratios were 0.33 (Erickson et al, 1974), 0.95 (O'Callaghan, Dodd and Pollock, 1973), 1.43 (Ngoddy et al, 1971), 1.75 (Hart and Turner, 1965), and 1.89 (Taiganides et al, 1964). This low energy/N ratios in wastes must be considered when flows of nitrogen in the soil environment are outlined (Wolcott, 1972) as shown in Figure 1.

Nitrification (Arrows 4 and 5) is the microbial oxidation of ammonium to nitrate by the combined action of the chemoautotrophs Nitrosomonas and Nitrobacter. Denitrification, sometimes referred to as dissimilatory nitrate reduction (Arrows 6, 7 and 8), is the microbial

reduction of nitrate to nitrous oxide or nitrogen gas. In summary, nitrate enters the soil solution as a wastewater constituent or as a product of the mineralization-nitrification reaction sequence and is removed by assimilatory nitrate reduction by plants and microorganisms and by denitrification. Nitrate not removed from the percolation stream by immobilization or denitrification during passage through soil environments influenced significantly by plant roots or microorganisms is transported to the aquifer in the leachate (Arrow 9).

Summaries and reviews have been published on the various processes occurring within the nitrogen cycle. Processes which have been studied in detail are: nitrification (Alexander, 1965), microbial assimilatory nitrate reduction (Payne, 1973), plant uptake of nitrate and ammonium (Viets and Hageman, 1971), nitrogen fixation (Postgate, 1971), humification (Schnitzer and Khan, 1972) and nitrate leaching (Viets and Hageman, 1971). In addition, the thermodynamics and kinetics of energy transfer which closely parallel cycles of carbon and nitrogen in natural systems have been extensively formalized (Bolin, 1970; Delwiche, 1967, 1970).

Role of Denitrification

The presence of nitrate in the aquifer is a pollution hazard to both humans and animals. Federal health standards (U. S. Department Health, Education, and Welfare,

1962) call for less than 10 ppm nitrate-nitrogen in water used for human consumption. Nitrate upon reduction yields nitrite (NO_2^-) which is commonly regarded as ten times as toxic as nitrate when present in drinking water, food and feeds (Viets and Hageman, 1971). Nitrite has been shown to have deleterious effects on red blood cells (Gruener and Shuval, 1970). Nitrite can overoxidize the ferrous (Fe^{+2}) iron of hemoglobin to ferric (Fe^{+3}) iron. The resulting methehemoglobin in red blood cells is incapable of carrying oxygen. Nitrate can also combine irreversibly with hemoglobin to form nitrohemoglobin which like carbon monoxyhemoglobin cannot carry oxygen. Nitrosoamines formed by reaction of nitrite with secondary amines in an acidic environment are carcinogenic, teratogenic (tendency to produce monstrous growths or fetuses) or mutagenic (Lijinsky and Epstein, 1970). Nitrite can also combine with myosin, a filamentous protein component of skeletal muscle (Gruener and Shuval, 1970).

Aquatic forms of life are also endangered when nitrate enters a lake or stream via seepage of polluted underground water. The process of enrichment of aquatic systems with nutrients, especially nitrogen and phosphorous, has been termed eutrophication. Undesirable features of eutrophication are a change in the type of algae to a blue-green algae form which produces obnoxious blooms and an excessive growth of macrophytes. This change in lake flora upsets the base of the normal food chain and results in the

creation of new food chains that lead to far less desirable species of fish (Provasoli, 1970). Provasoli (1970) also states that algae can accumulate in eutrophic lakes because of reduced grazing by the aquatic herbivores. As a result, large quantities of organic matter are produced in eutrophic lakes. Rapid degradation of this organic matter results in depletion of the lake's dissolved oxygen supply. Depletion of oxygen then results in the death of fish and other desirable forms of aquatic life.

Presence of nitrate in soil systems designed to renovate wastewaters is a dangerous pollutant unless it is removed in a harmless manner. Denitrification plays this very important role in renovation of wastewaters applied on soils.

Biochemistry of Denitrification

Denitrification can be defined biochemically as a series of anaerobic respiratory processes which reduce nitrate to elemental nitrogen. Various facultatively anaerobic organisms called denitrifiers utilize denitrification as an energy yielding type of respiration to generate adenosine triphosphate (Payne, 1973). Mixed cultures of denitrifying microbial populations found in sewage sludge have been shown to make use of glycolysis, the pentose phosphate shunt and the tricarboxylic acid cycle (DuToit, Toerien, and Davies, 1970). Genera of bacteria, reported to be capable of denitrification include: Achromobacter, Alcaligenes, Bacillus,

Chromobacterium, Corynebacterium, Halobacterium, Hypomicrobium, Micrococcus, Moraxella, Nitrosomonas, Propionibacterium, Pseudomonas, Spirillum, Thiobacillus, and Xanthromonas (Payne, 1973). In addition, a compilation of thirty three other genera of bacteria known to be nitrate respiring ($NO_3^- \longrightarrow NO_2^-$) has been done although some of these may actually be denitrifiers in which further reduction has not been recognized (Payne, 1973).

Denitrifiers can utilize either oxygen or nitrate as an electron acceptor and therefore are called facultative anaerobic heterotrophs. The mean energy yield for the transfer of a molar equivalent of electrons from an organic compound to oxygen has been determined as 26.5 Kilocalories (Payne, 1970) whereas the energy yield of an identical transfer of electrons to nitrate is approximately 18 Kilocalories (McCarty, 1972). It is obvious then that denitrifying organisms will be utilizing aerobic respiration whenever possible.

In recent years much information has been obtained through research on the effects of aerobiosis on denitrifying organisms. An experiment was conducted (Collins, 1955) which revealed that even in presumably aerated cultures, the ability of Pseudomonas aeruginosa to gain the capacity for nitrate respiration was directly related to the shape of the culture flask employed. Growth in shaken flasks with gradually more confining structures yielded cells that approached the control (cells respiring anaerobically) in

nitrate-reducing ability. Oxygen was found to inhibit the onset of synthesis of the nitrate reductase complex used for denitrification by Enterobacter aerogenes (Pichinoty) and D'Ornano, 1961). Production of nitrate reductase in cultures of Bacillus stearothermophilus is inversely related to the quantity of oxygen present (Downey, 1966). Studies done on Escherichia coli indicate that nitrate reductase, once formed, is repressed by oxygen functioning as an electron acceptor rather than oxygen per se (Simoni and Shullenberger, 1972). Anoxia has been found to derepress biosynthesis of nitrate reductase in several bacteria (Showe and DeMoss, 1968; DeGroot and Stouthamer, 1970).

Indications are that a variance exists among denitrifiers in their response to oxygen levels in their immediate environment. Studies conducted with Spirillum, Pseudomonas and Xanthomonas showed that some require anoxia for initiation and continuation of denitrification whereas other organisms were denitrifying at gaseous phase oxygen tensions as high as 153 mm (Von Mechsner and Wahrmann, 1963). In cultures of Haemophilus parainfluenzae, nitrate reductase synthesis occurs when the quantity of oxygen is lowered to approximately 100μm (Sinclair and White, 1970).

The effect of nitrate concentrations on nitrate reductase synthesis and activity has been found to vary among denitrifying organisms. Production of nitrate reductase in Bacillus stearothermophilus is directly related

to the concentration of nitrate (Downey, 1966). Several denitrifying soil isolates show optimal growth when the nitrate concentrations in the media are 0.1% to 0.5% (Bollag, Orcutt and Bollag, 1970). On the other hand, Haemophilus parainfluenzae, Bacillus licheniformis and some species of Enteriobacteriaceae are able to synthesize nitrate reductase in either the presence or absence of nitrate (Sinclair and White, 1970; Schulp and Stouthamer, 1970). The conflicting observations may be resolved by considering the nitrogen status of the organism undergoing change in environmental aeration status. Nitrogen depleted Bacillus stearothermophilus cells synthesize nitrate reductase more rapidly when transferred to anaerobic nitrate-containing media than do fully nourished cells that are grown aerobically before transfer (Downey and Nuner, 1967).

Because denitrification is a respiratory process, an organic substrate is required as an electron donor. An array of carbohydrates, alcohols, organic acids, or organic components of complex culture media can support denitrifiers. The rapidly growing Mycobacterium species are able to utilize seventeen different carbohydrates (Bonicke and Kazda, 1970). Methanol is known to support growth of a Hypomicrobium species (Sperl and Hoare, 1970). Short chained fatty acids and related organic acids increase nitrate reductase activity of Mycobacterium tubercolosis and lactic acid is used as an electron donor in

Mycobacterium scrofulaceum species (Bonicke, Juhasc and Diener, 1970). Enumeration of denitrifiers is usually accomplished on Difco nitrate broth containing a beef extract, in addition to supplementary amino acids, and potassium nitrate as a growth medium (Focht and Joseph, 1973). The metabolism of the well known chemolithotrophic bacterium Thiobacillus denitrificans is unique since an oxidizable inorganic sulfur compound is used as electron donor.

Initial events in electron transport which result in dissimilatory nitrate reduction occur via the use of pyridine nucleotides, flavines, and quinones in a manner similar to those which occur in aerobic respiration. Transport of electrons beyond the quinone stage then occurs via a branch at the cytochrome b level (Payne, 1973). A schematic diagram summarizing the electron transport system as found in several denitrifying bacteria is presented in Figure 2.

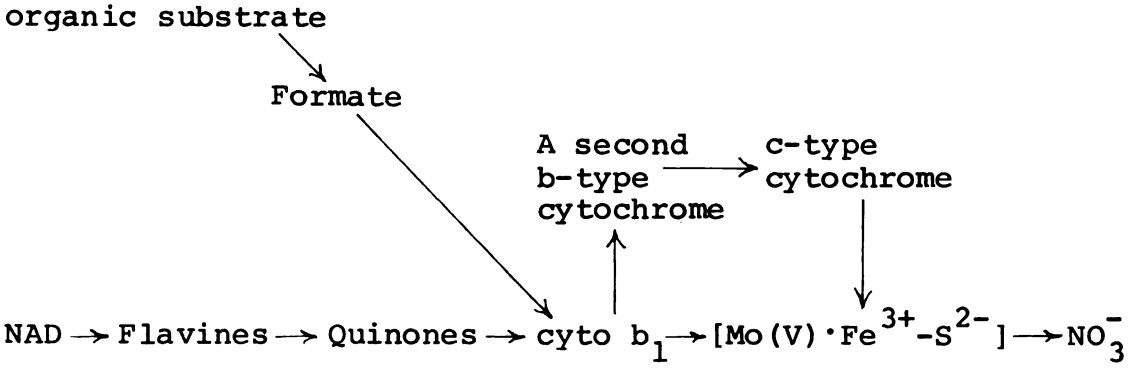


Figure 2. Schematic diagram summarizing the electron transport as found in several denitrifying bacteria (Payne, 1973).

Studies have shown that an increase in c-type cytochrome synthesis occurs accompanied by a decrease in a-type cytochromes when several organisms begin reducing nitrate (Downey and Kiszkiss, 1969; Schulp and Stouthamer, 1970; Sinclair and White, 1970). Membrane-associated and soluble cytochromes have been found to assist in transfer of electrons beyond nitrate in the following manner: $NO_3^- \xrightarrow{e} NO_2^- \xrightarrow{e} NO \xrightarrow{e} N_2O \xrightarrow{e} N_2$ (Cox, Payne and Dervartian, 1971). These cytochromes may be considered as cofactors of the various reducing enzymes which include the nitrate, nitrite, nitric oxide and nitrous oxide reductases.

Because a complex of enzymes are involved in transport of electrons from one nitrogenous oxide to the next, roles of N_2 production are dependent on temperature and pH of the surrounding environment. Various optimum temperatures for N_2 production have been reported, depending on the species involved. A psychrophilic pseudomonad has been found with an optimum temperature less than 20^oC (Konishi, 1969) while certain thermophilic denitrifiers show optimum temperatures as high as 55^oC (Downey and Kiszkiss, 1969). Incubation temperatures near 30^oC were found to favor denitrification by several soil isolates (Bollag, et al, 1970). In this same study, pH values slightly above neutrality were found to favor denitrification.

In summary, the biochemical requirements for denitrification are: 1) denitrifying populations, 2) anaerobic conditions, 3) nitrate as an electron acceptor, 4) readily

available source of carbonaceous compounds as electron donors, 5) moderate temperatures, and 6) a slightly alkaline pH.

Ecological Studies on Denitrification

As discussed previously, anaerobic conditions are essential if denitrification is to occur. Anaerobiosis exists in the field when rate of oxygen utilization exceeds rate of diffusion to a particular site or when rate of diffusion is insignificant to a particular site. Anaerobic sites are possible whenever respiratory consumption of oxygen by plant roots and microorganisms is taking place (Stefanson, 1972; Woldendorp, Dilz and Kolenbrander, 1966) and include microenvironments, macropores or entire layers within aerated soils (Broadbent, 1973). The presence of water in an environment also allows that environment to become anaerobic since oxygen which has been displaced diffuses very slowly in water. Anaerobic microenvironments may exist where films of water surround soil particles. The presence of anaerobic microenvironments allows plant uptake, organic material decomposition, nitrification and denitrification to occur in the same soil macroenvironment. This allows renovation of wastewaters containing various forms of nitrogen to occur when applied to an aerated soil. Saturated soils represent anaerobic macroenvironments. These include soils above the water table if rates of water application are very high, and soils at or below the water

table. Redox potentials equal to or less than a positive 200 milli-volts indicates that an environment is sufficiently anaerobic for denitrification to occur (Miller, 1973).

During the past several years, an extensive study has been conducted concerning renovation of water from livestock liquid wastes by modified soil system (Erickson et al, 1971; 1972; 1974). This modified soil system, called a Barriered Landscape Water Renovation System (BLWRS) was designed to remove nitrogen as well as phosphate and carbonaceous materials present in animal wastes by allowing various soil reactions to occur. The soil reactions important in this renovative process are mineralization (decomposition of organic matter), nitrification, denitrification and phosphate fixation. A schematic diagram of the BLWRS with accompanying soil reactions is shown in Figure 3.

Nitrate not denitrified in anaerobic microenvironments in the aerobic zone enters the saturated soil (anaerobic) zone created by installation of a water barrier. As the nitrate is forced to move laterally through the anaerobic soil, it is denitrified to nitrogen which diffuses to the soil surface. In a pilot system constructed in May, 1970, and operated during the following summer months, 99.5 per cent of the nitrogen was removed from the original applied swine waste (Erickson et al, 1971). Swine and dairy liquid wastes were applied for 17 months and 15 months respectively on larger BLWRS constructed in spring, 1971. In these

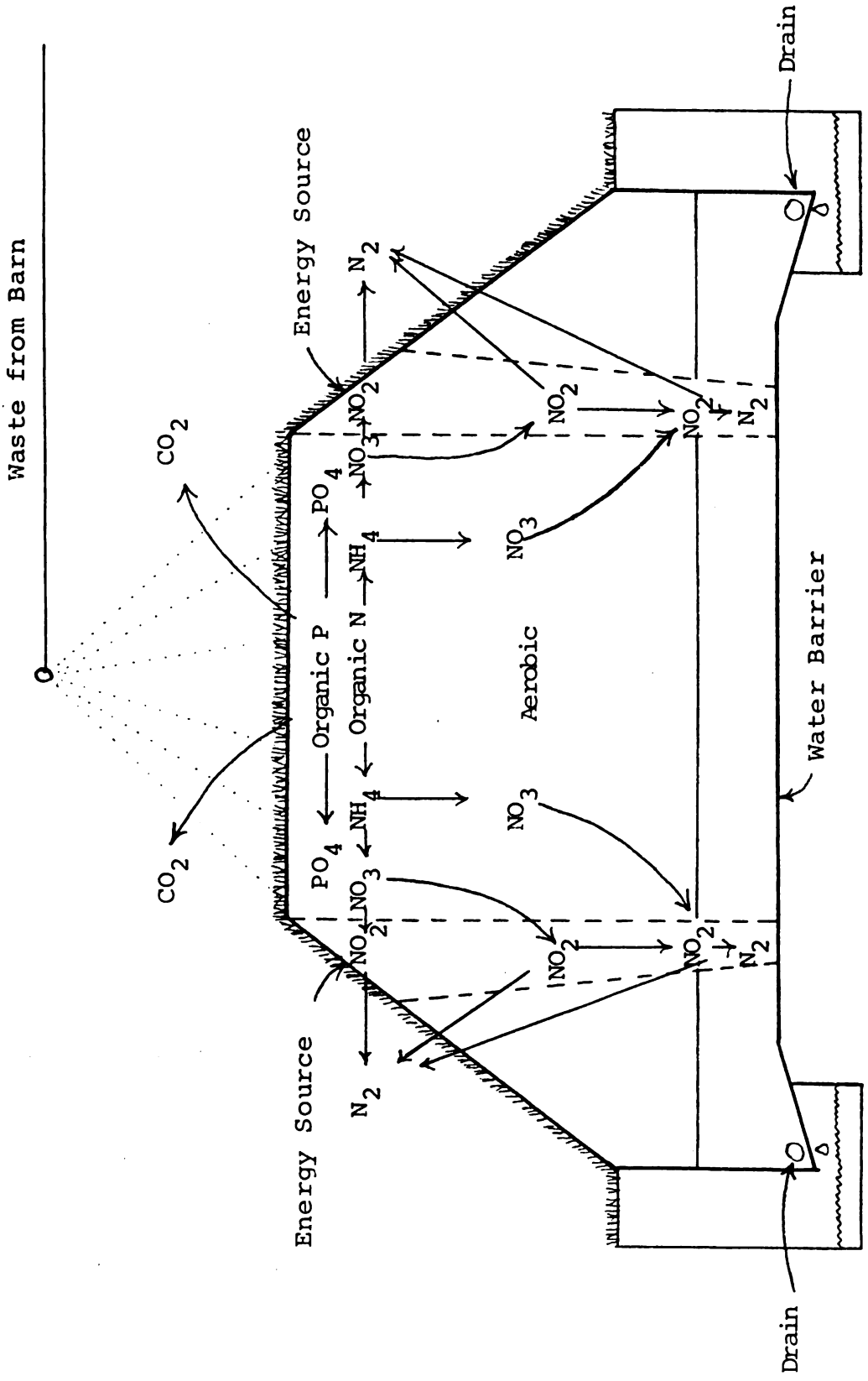


Figure 3. Schematic diagram of BLWRS and accompanying soil reactions.

studies, 80 per cent and 97 per cent respectively of the nitrogen was removed (Erickson et al, 1974).

In the BLWRS, intermittent applications of wastes was found to be effective in promoting the nitrification-denitrification sequence. Other investigators have also determined that intermittent applications of water on soils results in considerable nitrogen removal (Bouwer, 1973; Broadbent, 1973; International Rice Research Institute, 1969; Viets and Hageman, 1971). In the Flushing Meadows Project (Bouwer, 1973) a series of basins were flooded intermittently with municipal wastewater which was allowed to percolate through underlying soil layers. During flooding an anaerobic environment was formed which allowed nitrate to be reduced to N_2 by denitrification. At the same time, nitrogen in the ammonium form was being adsorbed by the clay and organic matter. During the following dry-up period the ammonium was then nitrified to nitrate and became subject to denitrification when the next flooding occurred. Thirty per cent of the nitrogen applied was estimated to have been removed. In a laboratory study conducted on an intermittent-flooded column of Tulare sandy loam, 83 per cent of the nitrogen added was removed and presumed to be denitrified (Broadbent, 1973).

Nitrate applied as fertilizer has been found to disappear as the soil solution approaches the water table (Kolenbrander, 1972; Meek, Grass and MacKensie, 1969). In one of these studies (Meek et al, 1969) nitrate

concentrations and redox potential values were measured as a function of depth in a cotton field of high water table in the Imperial Valley, California, after application of 280 kilograms anhydrous ammonia per hectare. Reduction in nitrate concentrations and a drop in redox potential were found as the soil solution approached the water table. An experiment conducted on one-meter columns of Venice peat revealed that a striking contrast exists in amount of denitrification occurring in a partly saturated soil compared to an unsaturated soil (Broadbent, 1973). Venice peat is a very permeable soil and contains forty per cent organic carbon. One column was free draining; the other had the water table maintained at 70 cm from the surface. Recovery of tagged nitrate in effluents obtained at the bottom of the column revealed that 21 per cent of the added nitrate was recovered from the unsaturated column whereas less than one per cent of the added nitrate was recovered from the column with the 30 cm saturated layer.

The importance of a readily available source of organic compounds has been realized in systems designed to renovate nitrate-contaminated waters. Sucrose, lactose and methanol have been employed as electron donors in purification of waters (Klotter, 1970; McCarty, Beck, and St. Amant, 1969). Use of methanol has proven feasible since methanol when metabolized, yields nitrogen gas, carbon dioxide, water, and a small crop of cells (McCarty et al, 1969). A 2:1 to 3:1 ratio of methanol to nitrate is indicated by model

experiments since greater ratios may leave residues that contribute to unwanted increases in biological oxygen demand (BOD) of treated waters (Dholakia, Stone and Burchfield, 1970).

In soil systems, there is evidence that vegetation has a beneficial effect on denitrification (Bouwer, 1973; Broadbent, 1973; Woldendorp et al, 1966). As already mentioned, plant roots consume oxygen and therefore create anaerobic pockets within the rhizosphere. However, the vegetation also may have a beneficial effect on denitrification by providing energy to the denitrifiers in the form of organic carbon exuded by live roots and that returned to the soil by decaying plant debris (Woldendorp et al, 1966). In one study (Woldendorp et al, 1966) it was noted that fifteen to twenty per cent of the nitrate passing through the rhizosphere may be denitrified.

When wastewaters are applied to vegetated areas, removal of additional nitrate occurs due to incorporation into microbial cells supported by organic carbon present in wastes and in vegetative exudates and detritus (Miller, 1973). To ensure greater efficiency of nitrate removal in soils, however, it may be necessary to add oxidizable organic materials into the soil environment, especially at lower depths. Saturated soil environments containing molasses or corn were found to be more effective in removing nitrate when compared to the soil environment containing no additional energy source (Erickson et al, 1974). The

experiment conducted on the partly saturated Venice peat soil containing 40 per cent organic carbon (Broadbent, 1973), mentioned earlier, revealed that essentially all nitrate can be removed from the leachate when sufficient organic substrate is available.

Soil temperature also has been found to be an important parameter in waste disposal on land. In the BLWRS, temperature of the soil environment was found to effect the concentration of nitrate in the effluents (Erickson et al, 1974). By November, soil temperatures in the anaerobic zone reached $10^{o}C$ or lower and at the same time the concentration of nitrate in the effluents increased greatly. Reduced efficiency of denitrifiers at this temperature or lower seemed to be responsible.

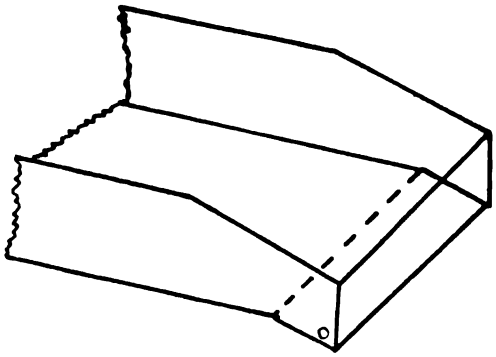
MATERIALS AND METHODS

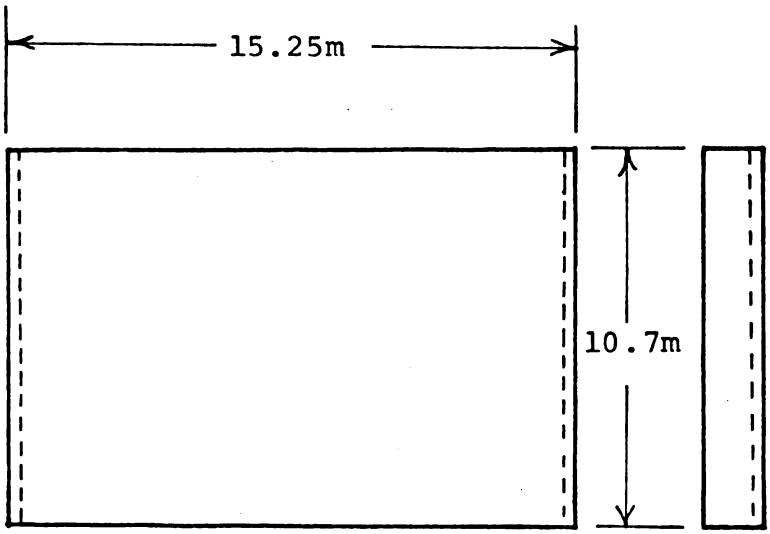
BLWRS Description and Operation

The Barriered Landscape Water Renovation System (BLWRS), a modified soil system designed to renovate animal wastes, was used in this investigation.

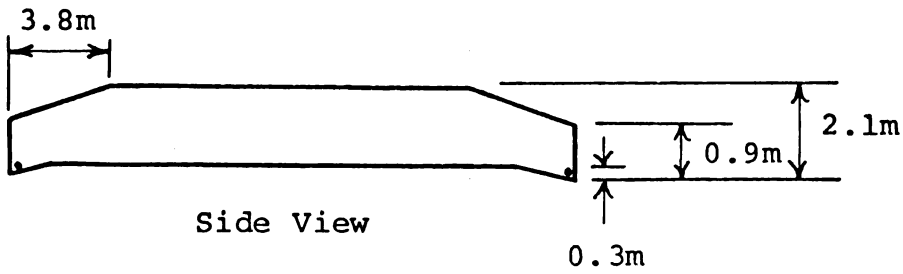
A pair of BLWRS, referred to as North and South Swine BLWRS, were constructed of fine sand in the Spring of 1971 at the Swine Research Facility at Michigan State University. Each BLWRS was 10.7 meters wide and 15.25 meters long with an anaerobic zone (saturated zone) 30.5 cm deep in the middle and 61 cm deep in the ends overlain by an aerobic zone (unsaturated zone) 1.5 meters deep within the waste application area. A uniform slope began 3.8 meters from each end and continued down to the end of the BLWRS. These slopes were designed to promote better aeration within the upper layers of the BLWRS. The dimensions of the BLWRS are illustrated in a three-view diagram (Figure 4).

The barrier was made of 30 mil polyvinyl sheeting which was turned up at the edges and sealed in the corners to form a complete basin. The only outlet was the tile opening which was reduced to 1.25 cm, the inner diameter of tygon tubing which was inserted into a tile plug. This tygon tubing loop was approximately one meter in length and was





Top View End View



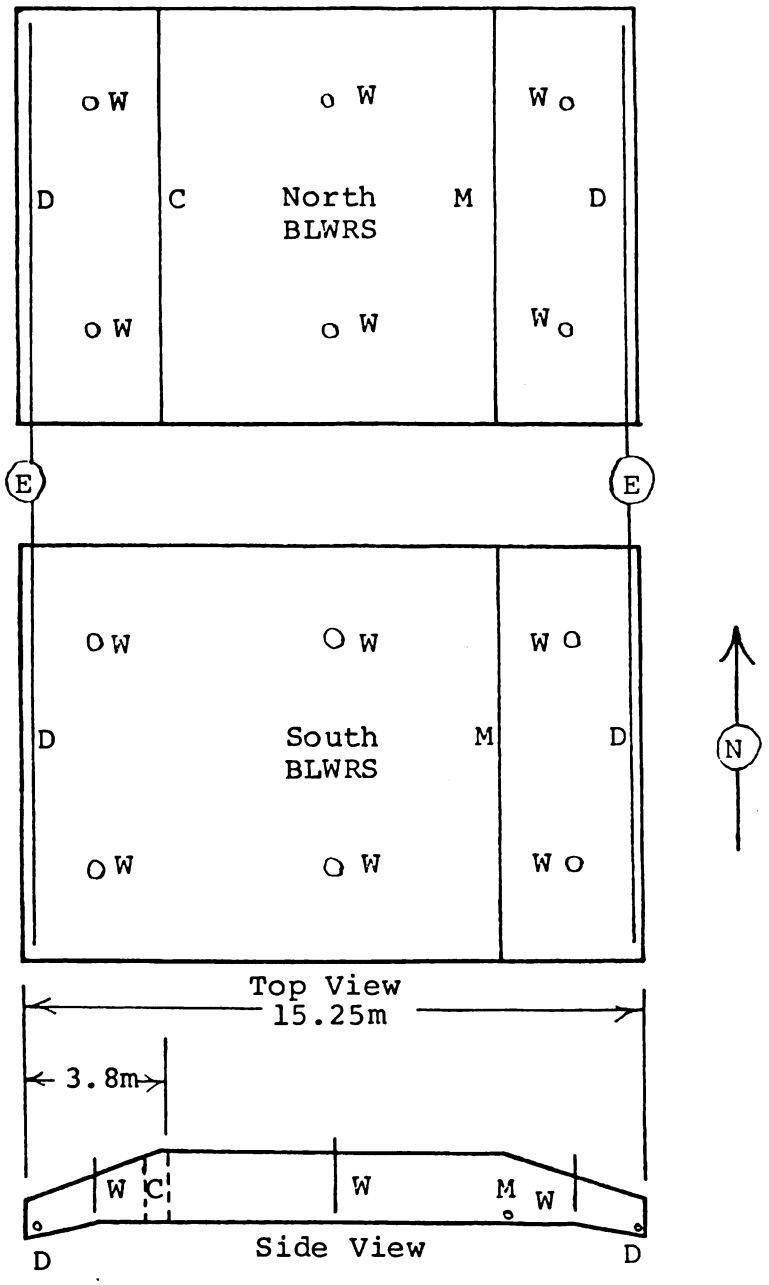
Side View

Figure 4. Drawings for the BLWRS, showing dimensions of polyvinyl barrier.

raised to maintain the level of the saturated zone at 30.5 cm from the bottom of the BLWRS.

The Swine BLWRS, which had been in operation from the Summer of 1971 until the Fall of 1972 was again put into operation in June 1973 in order to conduct a study on denitrification. Swine liquid wastes collected from a slotted floor, flush type, eighty-sow gestation barn were automatically sprayed on the BLWRS surface with an automatic boom spray apparatus which passed over the BLWRS every 30 minutes. Each BLWRS was sprayed for seven days and then allowed to dry out for seven days; this type of intermittent spraying was used in order to promote the nitrification-denitrification reaction sequence and to promote decomposition of slimes which had been filtered out on the surface.

A vegetative cover (quack grass, rye grass and various weeds) was maintained in order to increase infiltration and to provide an environment (rhizosphere) conducive to denitrifiers. Also energy in several forms was provided for denitrifiers. In the Summer of 1971, a trench was dug on the west end of the North Swine BLWRS; this trench was filled with approximately 1000 pounds of cracked corn. During construction, a perforated pipe 1.9 cm in diameter was laid along the edges and bottom of the east end of each BLWRS; in July 1973 these pipes were each filled with three liters of molasses. No energy was added to the west end of the South Swine BLWRS; this end served as the control. The location of the energy zones can be seen in Figure 5.



M = molasses energy source
C = corn energy source
E = effluent catchment
D = drain
W = wellpoint

Figure 5. Location of energy sources, effluent catchments and wellpoints in Swine BLWRS.

Swine waste was applied on the Swine BLWRS intermittently as previously described for a period of two months. During this period, it was felt that a type of equilibrium would be established among the various soil reactions occurring in each Swine BLWRS. During this period, 44,034 liters in 30 days and 53,025 liters of waste in 40 days were applied on the South and North Swine BLWRS respectively. Averages of waste applied were 1,468 liters and 1,326 liters of 1.80 cm and 1.63 cm per day respectively.

Application of Experimental Solution

On August 14, 1973, 78.5 liters of a solution containing 10.19% nitrogen as $Ca(NO_3)_2$ and 4.57% chloride as $CaCl_2$ was applied on the South Swine BLWRS. This sytem had undergone a seven day aeration period followed by a five day waste application period before the solution was applied. The solution was made up in tap water and sprayed onto the soil surface in the same manner that waste was applied. On August 30, 1973, 82.3 liters of a solution containing 12.24% nitrogen and 4.99% chloride was applied on the North Swine BLWRS. This system had just undergone a seven day aeration period before the solution was applied. Swine wastes were applied intermittently on the Swine BLWRS after application of the solutions until the experiment was terminated in November.

A concentrated chloride solution was used in order that a chloride peak could be followed down through the soil

profile of the BLWRS. Chloride was used as a tracer since it is a mobile anion in soils and undergoes essentially no microbial transformations. A tracer was required so that dilution of the experimental solution in the soil environment would be accounted for. Nitrate, an anion of similar mobility, would be used as a microbial substrate (electron donor) in the denitrification process. Ratios of concentration of nitrate-nitrogen to concentration chloride in the samples containing the chloride peak were then calculated and changes in these ratios observed to determine the extent to which nitrate was being used as a substrate by denitrifiers. In further discussions, these ratios shall be referred to as nitrate-N/chloride ratios.

Sampling of Wastes, Soils, and Water

Waste samples were collected in narrow mouth 250 ml polyethylene bottles periodically throughout July from a sampling port which had been installed into the plumbing system of the spray boom apparatus. A total nitrogen and chloride analysis was made on these samples in order that a nitrogen/chloride ratio could be determined. The experimental solutions were made up such that the ratio of nitrogen to chloride was similar to that present in the wastes.

The soil profile of each BLWRS was sampled within the spray area just prior to application of experimental solutions and then at intervals until the chloride peak had moved into the saturated zone. Both north and south sides

of each BLWRS were sampled; therefore the experiment was conducted in duplicate. Soil samples were taken at the following depths: 0-5, 5-10, 10-15, 15-30.5, 30.5-46, 46-61, 61-76, 76-91.5, 91.5-107, 107-122, 122-137, and 137-152 cm. A soil sampling probe (cutting edge of 1.9 cm diameter) was used to take samples from 0 to 30.5 cm. Three to six different soil samples were taken and then mixed and placed into sealed moisture proof plastic bags. Samples in the remaining portion of the profile were taken with a soil auger (2.5 cm diameter) in duplicate, mixed and placed in similar bags.

Well points were placed in the BLWRS in order that water samples could be taken from the saturated zone. These well points were made from thin-walled aluminum tubing 2.5 cm in diameter which were perforated throughout a 25 cm portion on the end. The well points with plastic plugs inserted in the ends were placed in the BLWRS in holes previously dug with the soil auger. Two well points were placed in the center within the spray area of each BLWRS and two well points were placed on each end. Figure 5 shows the location of the various well points. When the ends of the tubes were 25 cm below the interface between the saturated and unsaturated zone, the plastic plugs were then removed.

Water samples were taken from the well points by an evacuation apparatus consisting of a vacuum tank, an erlenmeyer flask as a trap, and vacuum hose. The first sample was discarded due to the higher aeration status within the

well point compared to the soil environment. The well points were allowed to recharge and then water samples were collected in narrow mouth 250 ml polyethylene bottles. Two samples had to be taken from each well point since the volume of the tubing within the saturated zone was approximately 125 ml.

Water samples were taken from the saturated zone after waste had been applied for several days and continued to be taken until the chloride peak appeared in the tile effluent. Water samples were also taken from the tile outlet. Figure 5 shows the locations of the tile outlets in the North and South Swine BLWRS respectively.

All wastes, soil and water samples were stored at $4^{o}C$ in a walk-in cooler and were analyzed as soon as possible for chloride, nitrate, and in some cases other forms of nitrogen.

Methods of Analysis

Waste samples were analyzed for ammonium, Kjeldahl, organic, nitrate and nitrite-nitrogen and chloride. Ammonium nitrogen was analyzed by steam distillation procedure. Two ml of waste were pipetted into a micro distillation flask and the flask then attached to a micro steam distillation apparatus. Ten ml of 0.1 N NaOH were added to the sample via a tube connecting a reservoir to the flask. The solution was steam distilled as rapidly as possible and the distillate was collected in a 50 ml erlenmeyer flask

containing 5 ml of a 2 per cent boric acid solution and 1 drop of Fleisher methyl-purple indicator.[1] The ammonium present was then titrated with H_2SO_4 solution which had been standardized by use of THAM-tris (hydroxymethyl) amino methane[2] as the primary acidimetric standard. Concentrations of ammonium-nitrogen in the samples were then calculated.

Kjeldahl nitrogen (organic nitrogen plus ammonium nitrogen) was analyzed by the semi-micro Kjeldahl method (Bremner, 1965a). Two ml of waste were pipetted into a micro distillation flask and two ml of concentrated H_2SO_4 were then added. Approximately one-third gram of a mixture containing K_2SO_4, $CuSO_4$ and Selenium was added to the solution. Potassium sulfate (K_2SO_4) was used to raise the boiling point of H_2SO_4 and thus the temperature at which digestion could occur whereas $CuSO_4$ and Se were used as catalysts to promote oxidation of organic matter (Bremner, 1965a; Skoog and West, 1969). The sample then was placed on micro digestion plates and digested for several hours. Samples were assumed to be completely digested after clearing of H_2SO_4 fumes had occurred. After allowing the sample to cool, 10 ml of distilled water were added. The sample then was steam distilled using the same procedure discussed

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[1]Can be obtained from Fleisher Chemical Company, Washington, D. C.

[2]Can be obtained from Fisher Scientific Company, Chicago, Illinois.

above except that 10N NaOH was used as the alkali. Concentrations of Kjeldahl nitrogen were then calculated.

Since Kjeldahl nitrogen represents organic nitrogen plus ammonium nitrogen, organic nitrogen than can be calculated by subtracting concentrations of ammonium nitrogen as determined by the steam distillation procedure from Kjeldahl nitrogen as determined by the semi-micro Kjeldahl method.

Nitrate-nitrogen was determined by an electrode procedure. The apparatus used consisted of the Orion Nitrate Specific Ion Electrode, Model 92-07; a calomel reference electrode, and a Model 801 digital pH/mv meter. Standard solutions containing 5, 10, 50, 100, 200 ppm nitrogen as KNO_3 in distilled water were prepared and a standard curve of concentration versus millivolts was made. Nitrate-nitrogen in both wastes and standard solutions was analyzed by introducing approximately 30 milliliters of the solution into a 50 ml beaker, stirring the solution with a magnetic stirrer and immersing the electrodes into the solution. Concentrations of nitrate-nitrogen in the wastes was then determined by use of the standard curve.

Nitrite-nitrogen in the wastes was analyzed by the Modified Griess-Ilosvay Method (Bremner, 1965b). One-third ml of sample was pipetted into small plastic tubes, then 2 ml of 1 per cent sulfanalamide solution and 2 ml of 0.2 per cent N-1-Napthylethylene dihydrochloride were added. The color was then allowed to develop for 30 minutes and transmittance measured on a Bausch and Lomb Spectronic 20 at a wavelength

of 540 millimicrons. Standard solutions of 0, 0.5, 1.0, 1.5, 2.0 ppm nitrogen as $NaNO_2$ were prepared and a standard curve then was graphed on semi-log graph paper. Concentrations of nitrite-nitrogen in the wastes were calculated by use of the standard curve.

Chloride was analyzed by a $AgNO_3$ titration procedure. Five ml of waste were diluted with 20 ml distilled water and this solution then placed in a 50 ml flask. One drop of KOH solution was added to adjust pH from 7.0 to 10.0. Indicator solution (0.5 ml of K_2CrO_4) was added and the solution was titrated to a pinkish-yellow endpoint with $AgNO_3$ solution which had been standardized against solutions of known NaCl concentrations. Concentrations of chloride in the wastes then were calculated.

Kjeldahl nitrogen, nitrate-nitrogen and chloride analysis were conducted on wet soil samples which had been stored in moisture-proof plastic bags. Air drying of soil samples was not done since it was feared that significant loss of ammonium and nitrate-nitrogen could occur. Chloride analyses were conducted on wet soils because chloride and nitrate were analyzed in the same soil extract.

Kjeldahl nitrogen was analyzed in soil samples taken on the day that experimental solutions were applied. Two grams of wet soil were placed in a dry micro distillation flask. Two ml of H_2O were then added and the sample was allowed to stand for 30 minutes. Approximately 1.1 grams of K_2SO_4 catalyst mixture and 2 ml of concentrated H_2SO_4 were

added to the flasks which were then placed on micro digestion plates for approximately four hours. Twenty to thirty ml of deionized water were added after the flasks had cooled and the samples then were steam distilled using the same procedure as discussed for waste analysis.

Nitrate in the soil samples was analyzed by using the electrode procedure. The same apparatus was used as discussed in waste analysis. Twenty grams of wet soil was weighed and placed in 125 ml erlenmeyer flasks. Fifty ml of saturated $CaSO_4$ solution were added to the samples and the samples then were shaken on a rotary shaker at 200 rpm for 30 minutes. The nitrate in the supernatant portion of the sample then was analyzed by electrode. Standard solutions of 5, 10, 50, 100, 200 ppm nitrogen as KNO_3 were made up in saturated $CaSO_4$ solution. A standard curve was then developed which was used for calculation of the nitrate concentration of the soil samples.

Chloride in the same soil extracts also was analyzed by an electrode procedure immediately after nitrate analysis had been completed. The apparatus used in the analysis consisted of Orion Chloride Specific Ion Electrode 94-17; a calomel reference electrode, and Sargent pH meter, Model DR S-30000. Standard solutions of 1, 5, 10, 50, 100, 200 ppm Cl^- as KCl were used to develop a standard curve. Concentrations then were calculated by use of the standard curve.

Nitrate and chloride in water samples taken from the saturated zone and tile effluent were also analyzed by electrode procedure. The water samples were allowed to come to room temperature and nitrate-nitrogen and chloride was analyzed directly by use of analytical equipment previously mentioned.

Ratios of weight of wet soil sample to weight of solution in sample were also determined. Weight of solution was determined by subtracting weight of oven dried sample from weight of wet sample. Nitrate and chloride concentrations as determined by electrode analysis on wet samples then were multiplied by these ratios in order to obtain concentrations on a solution basis. An example of these calculations are shown below:

weight of wet soil sample = 20.00 gm

weight of O.D.S. = 18.77 gm

weight of water = 1.23 gm

$$\frac{\text{weight of wet soil}}{\text{weight of water}} = \frac{20.00}{1.23} = 16.26$$

nitrate concentration in wet soil sample = 81.84 ppm

nitrate concentration in soil solution = 81.84 ppm x 16.26 = 1331 ppm

RESULTS AND DISCUSSION

Waste Characteristics

Data obtained from analysis of swine waste samples are presented in Table 1. The average concentration of total nitrogen was found to be 372.2 mg/liter. The ratio of average nitrogen concentration to average chloride concentration in the wastes equalled 2.36. The experimental solutions were prepared such that nitrogen to chloride ratios were similar to this value.

Table 1. Concentrations of nitrogen and chloride in swine waste applied before application of experimental solutions.

Date of Sampling	NH_4^+-N mg/liter	Org N mg/liter	NO_3-N mg/liter	NO_2-N mg/liter	Total N mg/liter	Cl^- mg/liter
July 12	326.5	32.5	17.0	0.18	376.2	91.0
July 19	354.9	34.0	8.2	0.11	397.2	170.0
July 26	300.8	35.5	15.0	0.24	351.5	160.0
August 2	324.0	27.3	12.0	0.30	363.5	210.0
Average	326.6	32.3	13.1	0.21	372.1	158.0

Movement of Experimental Solution

An experimental solution containing 12.24% (122,400 ppm) nitrogen as $Ca(NO_3)_2$ and 4.99% (49,900 ppm) chloride as $CaCl_2$ was applied on the North Swine BLWRS whereas a similar solution was applied on the South Swine BLWRS. The use of chloride as a tracer proved to be successful in most cases. The chloride peak was usually easily detected in the profile after sampling and analysis had been completed. A direct relationship existed between depth at which the chloride and nitrate peak occurred and length of time waste had been applied. Tables 2 and 3 illustrate this relationship. Similar movements of chloride and nitrate were observed in the other sampling sites. Movement of the chloride peak laterally along the saturated zone also could easily be detected and was dependent on length of time that had passed since solution was applied (Table 4). Similar movements of chloride in the saturated zone were observed in the saturated zones of the other sampling sites. A comparison between Tables 2 and 3 reveals that nitrate concentrations usually peaked at the same location in the profile as did chloride. This indicated that the mobility of the nitrate anion in the soil was very similar to the mobility of the chloride anion.

Table 2. Chloride concentrations in soil samples taken from north replication site on North BLWRS after experimental solution had been applied.

Depth (cm)	Chloride concentrations - ppm solution basis										
	Sample 1 (1-1)[a]	Sample 2 (2-2)	Sample 3 3-3)	Sample 4 (4-4)	Sample 5 (5-5)	Sample 6 (6-6)	Sample 7 (7-7)	Sample 8 (8-7)	Sample 9 (9-7)	Sample 10 (11-7)	Sample 11 (13-7)
0-5	440.7	275.2									
5-10	3123.0	419.9									
10-15	2213.0	1212.8									
15-30.5	556.0	2793.5	510.9	491.9							
30.5-46	1196.4	863.3	936.9	648.6							
46-61		415.8	1522.1	1671.2	460.7	724.7					
61-76			516.4	1702.8	504.6	482.1					
76-91.5				630.0	1518.6	1754.6	292.3				
91.5-107				609.0	507.0	1145.1	349.7	815.9	434.6	349.8	
107-122					433.0	312.9	482.9	1002.1	884.1	402.2	180.1
122-137							431.5	712.7	1327.6	326.3	311.4
137-152							362.0	326.9	409.9	176.4	872.1

[a](x-y) where x is number of days after application of experimental solution and y is number of days of actual waste application.

Table 3. Nitrate-nitrogen concentrations in soil samples taken from north replication site on North BLWRS after experimental solution had been applied.

Depth (cm)	Nitrate concentrations - ppm solution basis										
	Sample 1 (1-1)[a]	Sample 2 (2-2)	Sample 3 (3-3)	Sample 4 (4-4)	Sample 5 (5-5)	Sample 6 (6-6)	Sample 7 (7-7)	Sample 8 (8-7)	Sample 9 (9-7)	Sample 10 (11-7)	Sample 11 (13-7)
0-5	2610.6	235.0									
5-10	4258.2	442.9									
10-15	2406.3	3318.2									
15-30.5	232.9	7510.5	583.8	199.1							
30.5-46	932.9	1693.5	1483.9	245.4							
46-61		438.6	2485.5	1126.1	297.2	559.9					
61-76			614.0	2376.1	461.0	459.8					
76-91.5				529.9	2687.5	3472.4	36.47				
91.5-107				421.4	646.3	1761.1	73.9	1420.6	2548.4	271.4	
107-122					387.9	226.1	379.7	1874.9	1414.9	432.1	176.7
122-137							511.2	1236.6	2426.0	363.3	495.3
137-152							428.1	440.5	508.4	135.0	1931.9

[a](x-y) where x is number of days after application of experimental solution and y is number of days of actual waste application.

Table 4. Chloride concentrations in mg/liter in water samples taken from saturated zone from south replication site on North BLWRS after experimental solution had been applied.

SW Tile	SW Slope	S Top	SE Slope	SE Tile
321 (54-29)[a]	271 (42-22)	654 (26-15)	418 (56-29)	351 (56-29)
367 (56-29)	232 (44-24)	654 (28-15)	437 (58-31)	307 (58-31)
367 (58-31)	292 (47-27)	1180 (30-17)	458 (61-34)	402 (61-34)
309 (61-34)	198 (49-29)	654 (33-20)	391 (63-36)	331 (63-36)
318 (63-36)	182 (51-29)	739 (35-22)	353 (65-36)	272 (65-36)

[a](x-y) where x is number of days after application of experimental solution and y is number of days of actual waste application.

Status of BLWRS Before Experiment

Nitrate-N/chloride ratios were calculated after analysis had been completed on the initial soil and water (tile) samples. These data are presented in Tables 5 and 6. The nitrate-N/chloride ratios in the North BLWRS samples are higher than the corresponding ratios in the South BLWRS samples. This difference is due to higher concentrations of nitrate which were found in the North BLWRS samples. The North BLWRS had undergone a seven day aeration period before samples were taken whereas the South BLWRS had undergone a seven day aeration period which was then followed by a five day waste application period. Wastes were continually being applied to the South BLWRS when these samples were taken. The improved aeration status in the North BLWRS would have favored nitrification and increased stability of the nitrate produced.

Table 5. Nitrate-N/chloride ratios in soil and water samples taken from North Swine BLWRS before application of experimental solution. System had undergone a 7-day aeration period before sampling.

Depth	North Top	SE Tile	South Top	SW Tile
Surface	0.08		0.08	
0-5 cm.	3.27		5.08	
5-10	1.34		2.30	
10-15	0.80		1.39	
15-30.5	2.52		1.41	
30.5-46	0.68		1.17	
46-61	0.75		0.70	
61-76	0.88		0.59	
76-91.5	1.10		0.84	
91.5-107	1.19		0.59	
107-122	1.29		0.76	
122-137	1.26		0.82	
137-152	0.73		1.32	
Sat. Zone		0.36 ↑ Molasses Treatment		↑ Cracked Corn Treatment 0.16

Table 6. Nitrate-N/chloride ratios in soil and water samples taken from South Swine BLWRS before application of experimental solution. System had undergone a 7-day aeration period followed by a 5-day waste application period before sampling.

Depth	NE Tile	North Top	NW Tile	South Top
Surface		0.08		0.08
0-5		0.47		0.49
5-10		0.53		0.48
10-15		0.67		0.58
15-30.5		0.85		0.47
30.5-46		0.59		0.58
46-61		0.57		0.70
61-76		0.49		0.86
76-91.5		0.52		0.79
91.5-107		0.42		0.47
107-122		0.36		0.32
122-137		0.55		0.34
137-152		0.64		0.67
Sat. Zone	0.20 ↑ Molasses Treatment		↑ Control 1.08	

Nitrogen analysis by the Kjeldahl method was done on these samples; this data is presented in Table 7. These data shows that considerable nitrogen substrate was available in both BLWRS for the mineralization and nitrification; however the tendency for values to be lower in the upper profile of the North BLWRS compared to the South BLWRS indicates that greater net mineralization has occurred in the former. Moisture content data of the two systems, presented in Table 8, verifies the difference in aeration status. Only at depths near the saturated zone did the moisture content of the North BLWRS approach that of the South BLWRS; therefore, more oxygen was present in the soil profile of the North BLWRS at the time of sampling.

Decrease in aeration status as indicated by moisture content data (Table 8), promoted denitrification in the South BLWRS at the time of sampling. This also explains the lower nitrate-N/chloride ratios in the South BLWRS samples. Enough organic carbon was being added as a waste constituent and as plant root exudates and debris to allow some denitrification to occur. Data reported by Erickson et al (1974), indicated that an accumulation of carbon had occurred in the soil profile of the Swine BLWRS during the previous 17 months of operation.

Variations in nitrate-N/chloride ratios in the tile effluent from both North and South Swine BLWRS illustrate the importance of the presence of organic carbon at lower depths in the soil profile. Nitrate-N/chloride ratios of

Table 7. Concentrations of Kjeldahl-nitrogen expressed in μg/gram in soil samples taken from North and South Swine BLWRS before application of experimental solutions.

Depth (cm)	North BLWRS		South BLWRS	
	North Replication	South Replication	North Replication	South Replication
0-5	856.6	1289.6	1193.0	1622.4
5-10	148.7	195.4	249.5	275.3
10-15	142.4	107.8	133.7	186.4
15-30.5	172.2	107.8	103.3	115.3
30.5-46	104.4	109.2	95.5	114.0
46-61	86.1	84.9	80.7	80.7
61-76	54.1	80.0	73.9	78.5
76-91.5	55.5	46.2	131.7	80.9
91.5-107	61.2	77.2	100.6	88.4
107-122	55.0	75.1	147.9	71.1
122-137	83.3	96.4	154.3	67.0
137-152	55.2	117.8	182.8	86.3

Table 8. Moisture content (dry weight basis) of initial soil samples from North and South Swine BLWRS respectively.

Depth (cm.)	North BLWRS		South BLWRS	
	North Top	South Top	North Top	South Top
0-5	6.6%	9.7%	24.1%	33.5%
5-10	6.6	4.0	12.0	12.2
10-15	4.9	3.6	10.5	11.0
15-30.5	4.5	5.4	10.4	11.0
30.5-46	5.8	5.2	9.8	10.7
46-61	6.8	6.0	10.0	12.2
61-76	7.0	5.8	11.0	11.4
76-91.5	7.8	6.6	12.4	11.6
91.5-107	6.8	7.4	10.6	9.1
107-122	9.2	10.0	11.4	11.0
122-137	13.9	11.8	14.0	14.6
137-152	18.0	14.1	18.0	18.2

water samples are given in Tables 5 and 6. All three organic carbon treatment sites proved to be more effective than the control site in removing nitrate from water passing through these sites.

Evaluation of Amount and Environments of Denitrification

After the experimental solutions were applied, soil and water samples were taken at various depths corresponding to the location of the chloride peak in the BLWRS. The nitrate-N/chloride ratios of these samples are shown in Tables 9-12. The ratio at the surface represents the nitrate-N/chloride ratio of the experimental solution applied to the BLWRS surface. These ratios, 2.41 and 2.32 for the North and South Swine BLWRS experimental solutions respectively, were close to the ratio of the swine waste (2.36).

Ratios from the data in Tables 9-12 were selected from specific locations and changes in these ratios were evaluated. Table 13 presents this data.

Data in Table 13 shows that a 44 and 47 per cent decrease in nitrate-N/chloride ratios occurred as the experimental solution passed through the upper ten centimeters of the South and North BLWRS profiles respectively. This is evidence that denitrification is occurring in the surface horizon of the BLWRS. Apparently, sufficient organic carbon from the swine wastes and from root exudates was available to the denitrifiers. In addition, anaerobic environments were present even in the previously well-aerated soil to

Table 9. Nitrate-N/Cl ratios determined from analysis of soil and water samples taken from North Swine BLWRS - north replication site after application of experimental solution. System had undergone a 7-day aeration period before application of experimental solution.

Depth (cm)	NE Slope	North Top	NW Slope
Surface		2.41 (0-0)[a]	
0-5		- - -	
5-10		1.36 (1-1)	
10-15		- - -	
15-30.5		2.69 (2-2)	
30.5-46		- - -	
46-61		1.63 (3-3)	
61-76		1.40 (4-4)	
76-91.5		1.77 (5-5), 1.98 (6-6)	
91.5-107		- - -	
107-122		0.79 (7-7), 1.87 (8-7)	
		1.07 (11-7), 2.09 (15-9), 1.07 (20-14)	
122-137		1.83 (9-7)	
137-152		2.22 (13-7), 1.76 (16-10)	
		1.25 (18-12), 1.36 (19-13)	
Sat. Zone	1.44 (35-22)	0.48 (21-15)	0.33 (47-27)
	↑ Molasses Treatment	↑ Cracked Corn Treatment	

[a](x-y) where x is number of days after application of experimental solution and y is number of days of waste application required to move Cl^- peak to designated depth.

Table 10. Nitrate-N/Cl^- ratios determined from analysis of soil and water samples taken from North Swine BLWRS - south replication site after application of experimental solution. System had undergone a 7-day aeration period before application of experimental solution.

Depth (cm)	SE Tile	SE Slope	South Top	SW Slope	SW Tile
Surface			2.41 (0-0)		
0-5			- - -		
5-10			1.18 (1-1)		
10-15			3.00 (2-2)		
15-30.5			2.48 (3-3), 1.68 (4-4)		
30.5-46			- - -		
46-61			1.86 (5-5), 1.82 (6-6)		
61-76			1.74 (8-7)		
76-91.5			1.96 (7-7), 1.94 (11-7), 2.73 (13-7)		
91.5-107			2.02 (16-10)		
107-122			1.85 (9-7), 1.97 (15-9), 2.21 (18-12)		
			2.18 (19-13), 1.59 (23-15), 1.53 (26-15)		
122-137			1.61 (20-14)		
137-152			1.40 (21-15), 1.39 (28-15), 1.25 (30-17)		
Sat. Zone	1.41 (61-34)	1.52 (61-34)	0.46 (30-17)	0.72 (47-27)	1.21 (58-31)
			↑ Molasses Treatment	↑ Cracked Corn Treatment	

Table 11. Nitrate-N/Cl ratios determined from analysis of soil and water samples taken from South Swine BLWRS - north replication site after application of experimental solution. System had undergone a 7-day aeration period followed by 5-day waste application before application of experimental solution.

Depth (cm)	NE Tile	NE Slope	North Top	NW Slope	NW Tile
Surface			2.32 (0-0)		
0-5			- - -		
5-10			1.36 (1-1)		
10-15			1.97 (2-2)		
15-30.5			1.70 (4-2)		
30.5-46			2.09 (8-2), 1.47 (10-3)		
46-61			1.63 (6-2)		
61-76			1.66 (11-4)		
76-91.5			- - -		
91.5-107			1.64 (13-6), 1.75 (14-7)		
			2.11 (15-8), 1.88 (16-9)		
107-122			1.92 (17-9)		
122-137			1.45 (21-9), 2.29 (23-9)		
			1.77 (25-11), 1.74 (27-13)		
137-152			2.21 (19-9), 1.71 (28-14)		
			1.29 (29-15), 1.53 (30-16)		
Sat. Zone	0.49 (63-30)	0.95 (60-30)	0.59 (31-16)	1.47 (46-23)	1.51 (53-25)

Molasses Treatment

Control

Table 12. Nitrate-N/Cl ratios determined from analysis of soil and water samples taken from South Swine BLWRS - south replication site after application of experimental solution. System had undergone a 7-day aeration period followed by 5-day waste application before application of experimental solution.

Depth (cm)	SE Slope	South Top	SW Slope
Surface		2.32 (0-0)	
0-5		- - -	
5-10		- - -	
10-15		1.23 (1-1)	
15-30.5		1.86 (2-2)	
30.5-46		1.90 (4-2), 2.04 (10-3)	
46-61		1.77 (6-2)	
61-76		1.61 (8-2)	
76-91.5		1.35 (11-4), 1.22 (13-6), 1.97 (15-8)	
		1.99 (16-9), 2.07 (17-9)	
91.5-107		1.73 (13-6), 2.21 (19-9, 2.28 (25-11)	
107-122		1.29 (14-7), 1.73 (21-9)	
		2.36 (23-9), 1.39 (27-13)	
122-137		2.02 (29-15)	
137-152		2.23 (28-14), 1.96 (30-16)	
		1.59 (31-16), 2.03 (34-16)	
Sat. Zone	0.93 (58-30)	0.62 (39-18)	1.39 (49-23)
	↑ Molasses Treatment		↑ Control

promote denitrification. Application of wastewater to the surface would exclude oxygen from this environment; oxygen also would be depleted because of active root respiration and microbial decomposition of the organic matter added to the BLWRS surface.

Table 13. Selected ratios of experimental solution and per cent changes between these ratios as solution passes through various locations in the North and South BLWRS.

Location	North BLWRS[a] Ratio	%Change[b]	South BLWRS[a] Ratio	%Change[b]
Surface	2.41	---	2.32	---
Center, 0-10 cm	1.27	47(-)	1.30	44(-)
Center, 10-30 cm	2.85	124(+)	1.94	49(+)
Center, 30-152 cm[c]	1.73	39(-)	1.77	9(-)
Sat. zone, control trtmt.	---	---	1.43	19(-)
Sat. zone, molasses trtmt.	1.48	14(-)	0.94	47(-)
Sat. zone, corn trtmt.	0.53	69(-)	---	---
Tile, beyond control trtmt.	---	---	1.51	6(+)
Tile, beyond molasses trtmt.	1.41	5(-)	0.49	48(-)
Tile, beyond corn trtmt.	1.21	128(+)	---	---

[a]Values for center, and saturated zone were averages of 2 replications; other values represent single determinations.

[b]Negative sign indicates decrease in percent, positive sign indicates increase in per cent.

[c]Median ratio was selected from this location.

Nitrate-N/chloride ratios increased 49 and 124 per cent as the experimental solution in the profiles moved from the 0-10 cm increment to the 10-30 cm increment of the South and North BLWRS respectively. This indicates that nitrate had entered the experimental solution percolate.

Nitrification within these increments was occurring at a rate greater than the rate of downward movement of the experimental solution front and/or greater than rate of denitrification. Aeration status may have been such that nitrification was promoted while denitrification was slowed. Data obtained by Erickson et al (1974), show that oxidation-reduction potentials were higher in the South BLWRS at the 30 cm depth compared to redox potentials at the 15 cm depth (Table 14). Also, organic carbon content of the soils below the surface would be lower; this would help explain a decrease in denitrification. The occurrence of nitrification is indicated by the observance that ratios increased more in the North BLWRS than in the South BLWRS which was well aerated before experimental solution was applied.

Table 14. Oxidation-reduction potential measurements made in the Swine South BLWRS during the Summer of 1972 in millivolts (Erickson et al, 1974).

Date	6/28	7/5	7/28	8/1
Depth				
15 cm	185	160	130	210
	218	180	140	180
30 cm	180	190	240	250
	260	215		
120 cm	20	120	165	230
	160	170	180	230
150 cm	-150	-140	- 20	-120
	-170	-180	- 40	- 40
180 cm	-160	-170	-105	- 80
	-100	-130	- 60	- 40

After peaking near the 30 cm depth, the nitrate-N/chloride ratios decreased 9 and 39 per cent as the solution passed through the 30-152 cm increment of the South and North BLWRS respectively. This indicates that denitrification was occurring even at lower depths in the BLWRS. As mentioned earlier, organic carbon seems to be accumulating in the profile even at lower depths; therefore an organic substrate is present to allow some denitrification to occur.

Ratios in the South BLWRS then decreased 19 per cent as the solution passed from the 30-152 cm increment of the profile into the saturated zone at the control site. This indicates that the saturated zone by being anaerobic provided an environment conducive to denitrification. Additional sources of carbon were found to increase amount of denitrification since the ratio decreased 47 per cent as the solution moved through the molasses treatment compared to 19 per cent in the control treatment. Decreases in ratios also occurred as the experimental solution passed through the molasses and cracked corn treatment sites in the North BLWRS.

The nitrate-N/chloride ratios of the tile effluents also reveal the importance of additions of carbon in the saturated zone. The ratio of tile effluent beyond the control site was 1.51 as compared to 1.41, 0.49 and 1.21, which were ratios of the experimental solution after the solution had passed through molasses, molasses, and corn treatment sites respectively.

Nitrate-N/chloride ratios in water samples taken from tile effluent (R_T) were used in computing percentage of nitrate removed by the BLWRS. The nitrate-N/chloride ratio of the experimental solution (R_E) was assigned a value of 100 per cent and then multiplied by the fraction $(\frac{R_E - R_T}{R_E})$. For example, per cent nitrate removed by the South BLWRS at the control treatment end equalled $100\% \times \frac{2.32 - 1.51}{2.32} = 35\%$. Other percentages of nitrate removed were 41 per cent, 50 per cent, and 78 per cent by the North molasses, North cracked corn and South molasses treatment ends respectively. An explanation for the disparity between the two values for molasses treatments may be that molasses was added to the North BLWRS on the side farthest away from the tile effluent whereas the molasses was added to the South BLWRS on the side closest to the tile outlet. This means that in the South BLWRS a higher concentration of organic carbon most likely was present in the zone through which a major part of the solution was passing through.

Data concerning the movement of the chloride peak in the BLWRS are also shown in Tables 9-12. These data show that chloride movement depended on the number of days waste had been applied after application of the experimental solution.

The effect of various chloride concentrations on nitrate analysis by electrode has been studied (Balasubramanian and Kanehiro, 1974). In this study, errors less than or equal to 14 per cent were reported when nitrate-N/chloride

ratios were between 0.10 and 1.00. Concentrations of nitrate-N used in this study were 10 ppm or greater as was true for most of the BLWRS samples. This error would affect analysis of BLWRS samples whose ratios were in the reported range. Analysis of samples, whose ratios were greater than 1.00 would have been less affected because chloride interference is much less when its concentration is less than nitrate-nitrogen.

Population counts of total anaerobes and denitrifiers were conducted on the Swine BLWRS in November 1972 (Erickson et al, 1974). Data from this study (Table 15) show that the largest populations of both anaerobes and denitrifiers were in the surface soil. This is evidence that considerable denitrification was occurring in the microenvironments of the surface soil. Denitrifier populations were one to two orders lower in the anaerobic zone, yet apparently this was sufficient biomass to allow nitrate reduction to occur. Furthermore, the populations within the anaerobic zone where carbon was added, were not assayed. Populations in these environments could be expected to be higher.

Some of the reduction of nitrate in the BLWRS can be attributed to assimilatory nitrate reduction. In studies conducted on the rich organic sediments in fresh water lakes (Keeney et al, 1971), it was determined that approximately one-third of the nitrate-nitrogen passing through these sediments was assimilated whereas two-thirds of the nitrate-

Table 15. Numbers of anaerobes and denitrifiers found in North Swine BLWRS soil collected in November 1972. (Erickson et al, 1974).

Sample Site and Depth	Microorganisms/g oven dry soil x 10^{-6}		
	Anaerobes	Denitrifiers	% of Anaerobes as Denitrifiers
Center, 0-5 cm.	340	100 (190)[a]	30
Center, 10-15	36	7.2	19
Center, near barrier[b]	42	11	26
West slope, near barrier[b]	36	3.6	11
East slope, near barrier[b]	3	1.1	33

[a]Population of microorganisms that reduced nitrate only to nitrite resulting in nitrite accumulation.

[b]Samples collected from anaerobic zone.

nitrogen was denitrified. In light of this observation, denitrification still assumes the major role in nitrate removal in the BLWRS.

CONCLUSIONS

An experiment was conducted to determine amounts, locations and environmental conditions of denitrification in a Barriered Landscape Water Renovation System (BLWRS). Solutions of nitrate and chloride were added to the BLWRS surface and the ratio of concentration of nitrate-nitrogen to concentration of chloride was monitored as the solution front moved through the BLWRS soil profile and out of the BLWRS as tile effluent. Reduction in nitrate-N/chloride ratio of the experimental solution as it passed through a particular location was interpreted as evidence that denitrification had occurred. Amounts of reduction in nitrate-N/chloride ratio between various locations within the North and South BLWRS are listed below.

1) Nitrate-N/chloride ratios decreased 44 and 47 per cent as the solution passed through the upper 10 centimeters of the soil profiles of the South and North BLWRS respectively.

2) Nitrate-N/chloride ratios decreased 9 and 39 per cent as the solution passed from the 10-30 cm increment to the 30-152 cm increment of the profiles of the South and North BLWRS respectively.

3) Nitrate-N/chloride ratios in the South BLWRS decreased 19 per cent at the control site in the saturated zone whereas the ratio decreased 47 per cent at the molasses treatment site in the saturated zone. In the North BLWRS, ratios of the experimental solution also decreased at the molasses and corn treatment sites.

These data indicate that a significant amount of denitrification occurred in the surface horizon of the BLWRS. Denitrification also can be assumed to have occurred in the lower portion of the aeration zone. A significant amount of denitrification also was occurring in the saturated zone. Increases in amount of denitrification occurred in the saturated zone where organic carbon in the form of either cracked corn or molasses had been added.

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