The University of Manitoba

AN IN SITU STUDY OF DENITRIFICATION IN A FACULTATIVE WASTE STABILIZATION POND

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James K. Stibbard

# A Thesis

submitted to the Faculty of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Science

> Department of Civil Engineering Winnipeg, Manitoba

> > May, 1987 🕜

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ΒY

#### JAMES K. STIBBARD

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#### ABSTRACT

This report describes a study performed to determine if denitrification occurs in a facultative wastewater stabilization pond, at what rate and under what conditions. Literature indicated that denitrification occured in marsh and lake sediments but information for stabilization ponds appeared to be lacking.

Gas collectors were placed on the surface and bottom of an operating facultative pond and samples were collected approximately once per week for 2 months. Surface gas consisted of approximately 80 to 90 percent nitrogen and 10 to 20 percent oxygen. Methane was only collected in any quantity under anaerobic conditions. Benthic gas consisted of approximately 20 to 30 percent nitrogen and 70 to 80 percent methane. Temperature, dissolved oxygen, pH, ammonia and nitrate were monitored. Gas production showed a corelation to temperature, but changes in pH, ammonia and nitrate levels over the test period were too low to allow meaningful corelation. An attempt was made to calculate an ammonia balance but this could not be done due to unaccounted for variables.

A tentative conclusion was made that denitrification was occuring in the pond sediment and that the rate of nitrogen gas production varied linearily with temperature from 30 ml per square meter per day at  $5^{\circ}$ C. to 108 ml per square meter per day at  $15^{\circ}$ C.

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# AN IN SITU STUDY OF DENITRIFICATION IN A FACULTATIVE WASTE STABILIZATION POND

# CHAPTER 1 INTRODUCTION

#### 1.1. General

Wastes in various forms are an inevitable by-product of almost all human activities. A large portion of the liquid waste consists of a variety of organic matter which serves as a substrate for other living organisms, primarily bacteria. This is part of a natural recycling process which has gone on since the dawn of life and will occur whereever waste matter is present, whether we wish it to or not. If large quantities of organic wastes are allowed to accumulate in an uncontrolled manner, this process of recycling can cause environmental degradation and present a threat to human health. In order to reduce these undesirable effects of waste degradation, a number of processes have been developed to allow the stabilization of waste matter under controlled conditions.

The facultative stabilization pond is one of the processes used for treatment of liquid wastes. Approximately 25 percent of the municipal waste treatment works in the

United States consist of ponds, located primarily in small communities.  $(1)^*$  In Manitoba, 226 communities use ponds wholly or in part for treatment of their waste water. (2) In view of the widespread dependence upon ponds for waste water treatment and protection of the environment, a complete understanding of their operation and the ways by which they achieve treatment objectives is important.

Nitrogen, in various compounds, is an essential part of all living matter. Remarkably however, the reservoir pool of the element, atmospheric nitrogen gas, can only be utilized directly by a small number of specialized microorganisms. As a result, most living organisms must obtain their nitrogen from other sources, including the waste products from other organisms. This movement of nitrogen through various forms as it is utilized by living organisms is the basis of the nitrogen cycle. Ammonia and reduced nitrogen compounds including nitrite and nitrate, are major components of this cycle and as such are produced by some metabolic activities and utilized by others. As a waste, these products of nitrogen have the potential to cause problems if discharged to the environment under uncontrolled conditions. Ammonia in water can be toxic to fish and stabilization of it can exert a high demand for dissolved oxygen. It can also be used as a nutrient by aquatic plants

\* Numbers in brackets refer to references quoted in the text. References are listed in numerical order at the end of the text.

and algae and thereby contribute to the problem of cultural eutrophication. Nitrite and nitrate in water can also act as nutrients and contribute to eutrophication. In sufficient concentration, these compounds in drinking water can cause methemoglobinemia, a potentially fatal condition in infants. Research also indicates that ingested nitrate can be converted in the body to nitrosamine, a carcinogenic compound.

As far as is known, there is only one process by which the various nitrogen compounds can be returned to the reservoir pool in the atmosphere. This is the process of biological nitrification and denitrification whereby ammonia is first oxidized to nitrite and nitrate and then reduced to nitrogen gas by various genera of bacteria. As such, an understanding of the nitrification-denitrification process in ponds is important in order to allow predictions to be made with regard to the amount of nitrogen which may be expected to be removed from waste water in ponds as well as in what forms and under what conditions this removal will occur.

## 1.2. Purpose of the Study

The purpose of this study was to determine whether denitrification occurs in facultative waste water stabilization ponds and under what conditions. In this respect, nitrogen gas quantification, as well as the influence of environmental factors such as temperature, pH and substrate concentration were investigated. The results of such a

study would be valuable because they would provide information which would further contribute to an understanding of the dynamics of this important nutrient in a waste treatment process which is widely depended upon for protection of our health and environment.

#### CHAPTER 2

## THEORY AND LITERATURE REVIEW

#### 2.1. Microbial Metabolism

Living organisms have certain requirements to sustain life. These requirements may be broadly classified as a favourable environment and sufficient nutrition. In this context, nutrition refers to the manner in which an organism obtains the energy and chemical materials required for metabolism and cell growth. Nutrients may therefore be classified according to whether they provide chemical materials for cell synthesis or provide the energy required for that synthesis. This energy may be obtained from an oxidation-reduction reaction in which one chemical gives up electrons and is oxidized and another receives the electrons and is reduced. As electrons move from oxidized donor to reduced acceptor, microorganisms derive energy by a series of enzyme mediated metabolic reactions. Microoganisms may be classified according to the type of nutrients used for their cellular material or for their carbon and energy source. Figure 1. summarizes these nutrient sources and classifications.<sup>(1,3)</sup>

It may be seen from figure 1. that a number of potential combinations of nutrient sources and processes of use are available. In addition, many microorganisms have the capacity to change nutrient sources or processes of use depending upon environment and availability. This



Classification of Nutrient Requirements of Microorganisms

FIGURE 1.

has lead to development of the term "mixotrophs" to describe such modes of life.<sup>(3)</sup> Thus, although they are not always clearly delineated, the categories given in figure 1. are valuable for classifying microorganisms.

## 2.2. The Nitrification Process

2.2.1. Nitrogen in Living Processes

In life processes, nitrogen moves through a series of transformations which make up a biogeochemical cycle known as the nitrogen cycle. The reservoir pool of this cycle is atmospheric nitrogen gas and the exchange pool consists of a number of organic and inorganic steps. the primary production step is the conversion, or fixation, of nitrogen gas to ammonia which is performed by a few specialized microorganisms. Once formed, ammonia can be used directly by many organisms to synthesize aminoacids, proteins and nucleic acids, all of which are essential to the makeup of living matter, Nitrogen is required for formation of the peptide linkage, the chemical bond by which amino-acids are combined into proteins. The nitrogen content of protein is constant at approximately 16 percent by weight (4) and nitrogen is the third most abundant element in organic matter after carbon and oxygen.<sup>(3)</sup>

2.2.2. Mineralization of Organic Nitrogen

In Environmental Engineering, the various forms of organic nitrogen are usually not differentiated but measured collectively as "organic nitrogen" or "total Kjeldahl nitrogen" from the analysis procedure used to measure organic nitrogen. Nitrogen enters waste water in the form of a variety of proteinaceous metabolic wastes and urea. Waste protein matter is broken down to aminoacids by bacterial proteolytic enzymes and ammonia is

liberated from the amino-acids by the process illustrated in equation 1. (5)



The keto acid remaining from this reaction is subject to further breakdown.

Urea, a compound by which higher animals rid their bodies of ammonia, is hydrolyzed as shown in equation 2. (6)

$$NH_2$$
  
 $C = 0 + 2H_20 \xrightarrow{\text{urease}} (NH_4)_2CO_3 \cdots 2$   
 $NH_2 \xrightarrow{\text{enzyme}} 2$ 

The reactions represented by equations 1 and 2 are performed by a large number of saprophytic bacteria and proceed rapidly in waste water containing proteinaceous wastes. The processes are collectively called "mineralization" since the ammonia liberated is not considered an organic compound.

2.2.3. Conversion of Ammonia to Nitrate

Ammonia may be oxidized to release energy which is the basis of the biological process utilized by two groups of the family Nitrobacteraceae, Nitrosomonas and Nitrobacter, which oxidize ammonia to nitrite and nitrite

to nitrate respectively to obtain energy which is then used, with carbon dioxide as a carbon source, for cell growth. The energy reaction of ammonia to nitrite is represented by equation 3.<sup>(7)</sup>

 $NH_4^+$  + 1.50<sub>2</sub>  $\longrightarrow$  2H<sup>+</sup> + NO<sub>2</sub> + H<sub>2</sub>O .....3

Ammonia is also used as a source of nitrogen for synthesis of cellular matter. An overall reaction for conversion of ammonia to nitrite, with synthesis of cellular matter, may be represented by equation 4.<sup>(7)</sup>

 $55NH_4^+ + 760_2 + 109HCO_3^- \longrightarrow C_5H_7NO_2 + 54NO_2^- + 57H_2O_1 + 104H_2CO_3.4$ 

In equation 4,  $C_5H_7NO_2$  is a generalized formula for cell matter. The bacteria carrying out this reaction are primarily <u>Nitrosomonas europaea</u> and <u>N. monocella</u>, but <u>Nitrococcus</u>, <u>Nitrosotobulus multiformis</u> and <u>Nitrospira</u> <u>Briensis</u> can also perfrom it.<sup>(8)</sup> <u>N. europaea</u> is the species most frequently isolated from waste water so the ammonia to nitrite reaction is generally spoken of as being performed by "Nitrosomonas".

The oxidation of nitrite to nitrate may be represented by equation 5.(7)

 $NO_2^- + .5O_2 \longrightarrow NO_3^- .....5$ 

The combined reaction for energy release and cellular synthesis for this process can be represented by equation 6.(7)

 $400NO_{2}^{-} + NH_{4}^{+} + 4H_{2}CO_{3} + HCO_{3}^{-} + 195O_{2} \longrightarrow C_{5}H_{7}NO_{2} + 3H_{2}O + 400NO_{3}^{-} \dots 6$ 

This reaction is performed primarily by the genus Nitrobacter, specifically <u>N. agilis</u> and <u>N. winogradski</u> as well as <u>Nitrocystis</u>, <u>Nitrosococcus mobilis</u> and <u>Nitrospira gracilis</u>. <sup>(8)</sup> Since <u>N.agilis</u> appears to be the most common species in waste water, all nitrite oxidizers are usually referred to as "Nitrobacter" when speaking of sewage treatment.

2.2.4. Growth of Nitrifying Bacteria

Since they use an inorganic carbon source, derive energy from oxidation of inorganic ammonia and nitrite and use molecular oxygen as their terminal electron acceptor, both Nitrosomonas and Nitrobactor are referred to as autotrophic chemosynthetic aerobes. <sup>(1)</sup> Nitrifiers are ubiquitous organisms, found in soils, compost piles, sewage disposal systems, fresh and salt water habitats and in almost any other aerobic environment where organic decomposition is occuring. <sup>(9)</sup>

Compared to heterotrophic or organic carbon oxidizing bacteria, nitrifiers are slow growing, with low biomass yield per unit of substrate consumed. This is partly due to the large amount of energy they must use to reduce carbon dioxide to obtain carbon.<sup>(8)</sup> In microbial kinetics, the rate of growth per unit of biomass, or specific growth rate, represented by  $\mu$ , is a measure of the speed

at which a species of microorganisms utilizes its substrate, with higher numerical values indicating more rapid growth. Typical values of  $\mu$  for carbonaceous oxidation, nitrification and denitrification are given in table 1.<sup>(10)</sup>

Reaction µ days<sup>-1</sup> carbonaceous oxidation .5 - .2 nitrification .1 - .05 (overall reaction)

denitrification

1 - .5

## TABLE 1.

Specific growth rate for microbial reactions

It may be seen from equations 4 and 6 that large amounts of oxygen are required for the nitrification reactions. A stoichiometric calculation gives 4.19 milligrams of oxygen required per milligram of ammonia converted to nitrate.<sup>(7)</sup> This large oxygen requirement can exert a significant oxygen demand upon receiving waters into which ammonia is discharged. As far as is known, all nitrifying organisms are obligate aerobes, although absence of oxygen does not kill but merely inactivates them.<sup>(9)</sup> Required dissolved oxygen levels for nitrification to occur in water are the subject of controversy, with efficient nitrification reported at levels from 8 milligrams per litre (mg/l) to as low as .5 mg/l of dissolved oxygen.<sup>(7)</sup>

Like most microorganisms, nitrifiers are sensitive

to the pH of their environment, with optimum pH for both stages of nitrification appearing to be in the alkaline range from 7.5 to 8.5, although reports are available of growth at pH values ranging from 6 to 9.<sup>(8)</sup> It may be seen from equations 4 and 6 that nitrification consumes bicarbonate alkalinity and produces carbonic acid, which can cause a drop in pH. From this relationship, it can be calculated that for every mg/l of ammonia nitrified, 7.15 mg/l of alkalinity as CaCO<sub>3</sub> are consumed.<sup>(7)</sup> This can cause problems in treatment processes if sufficient alkalinity is not present to buffer the reaction.

It has been found that a temperature relationship of the form shown in equation 7 describes the growth of nitrifiers up to approximately 30 degrees Centigrade.  $({}^{O}C.)$ . (8)

 $U_{T} = u_{15}e^{C}t^{(T-15)}$ where:  $u_{T} = \text{growth rate at temperature T, }^{O}C$ .  $u_{15} = \text{growth rate at 15 }^{O}C$ .  $C_{t} = \text{temperature constant}$ 

Research indicates that both genera of nitrifying orgainsms can be inhibited by large concentrations of their own energy substrates, ammonia for Nitrosomonas and nitrite for Nitrobacter respectively. However, these concentrations are generally much higher than are found in waste water treatment situations.<sup>(8)</sup>

## 2.3. The Denitrification Process

2.3.1. Nitrate as an Alternative Electron Acceptor

From figure 1, it may be seen that microorganisms may be classified as aerobic or anaerobic according to their electron acceptor. The purpose of the oxidation reduction reaction is to provide energy and as such, the reaction which provides the greatest energy output will be the most favourable. The largest energy yielding reaction is oxidation of organic matter combined with reduction of molecular oxygen as the electron acceptor. This is the energy reaction of chemoorganotrophic aerobes and, due to the high energy yield, will proceed rapidly as long as organic substrate and molecular oxygen are available.<sup>(11)</sup> Should free oxygen become limited or unavailable, other electron acceptors must be utilized. The next reaction to reduction of oxygen in terms of yield of energy is oxidation of organic matter coupled with dissimilatory reduction of nitrate. A simplified representation of the two reactions, with energy yields, are given in equations 8 and 9.(7)

 $C_6H_{12}O_6 + 6O_2 \xrightarrow{6CO_2 + 6H_2O} \dots 8$ mole glucose

 $^{C}6^{H}12^{0}6$  + 14KNO<sub>3</sub>  $\xrightarrow{30CO_2}$  + 18H<sub>2</sub>O + 14KOH + 12N<sub>2</sub>. 9 mole glucose The reduction of nitrate to nitrogen gas represented by equation 9 is termed dissimilatory denitrification and is an important biological pathway. It may be seen that organic matter serves as the electron donor for both reactions. The major difference is that the electron acceptor is nitrate rather than molecular oxygen. Thus, the process may be termed chemoorganotrophic anaerobic, although it is usually referred to as "anoxic" to distinguish it from fermentation reactions. The electron transport system for reactions 8 and 9 is identical except for one enzyme.<sup>(7)</sup> For this reason, a large number of aerobic organisms are able to move facultatively from one reaction to the other depending upon which electron acceptor is available.

The process of dissimilatory denitrification is more complex than represented in equation 9 and involves intermediate reactions which are not completely understood. One representation of the reaction is as shown in equation 10. <sup>(12)</sup>

redox state

Jeter<sup>(13)</sup> describes denitrification as a four step process consisting of 1) reduction of nitrate to nitrite, 2) reduction of nitrite to nitric oxide, 3) reduction of nitric oxide to nitrous oxide and 4) reduction of nitrous oxide to nitrogen gas. It is noted that each step of this process yields energy and that organisms have been isolated which are capable of performing only part of the pathway as well as the entire progression.

2.3.2. Denitrifying Microorganisms

As noted in section 2.3.1., a large number of genera of microorganisms are capable of dissimilatory nitrate reduction. Jeter<sup>(13)</sup> lists 73 genera considered capable of partial or complete denitrification. This is in contrast to the limited number of genera capable of nitrification. Metcalf & Eddy<sup>(10)</sup> list Pseudomonas, <u>Micrococcus</u>, <u>Archromobacter</u> and Bacillus as the principal genera of denitrifiers in waste water treatment and Jeter's list includes these as well as such strains as Salmonella, Clostridium, Esherichia, Vibrio, Shigella and others. It may be noted that bacteria capable of denitrifying of the genera Neisseria, Pseudomonas, Branhamella and Kingella have been clinically isolated in humans and animals.<sup>(13)</sup> Thus, denitrifying bacteria are ubiquitous and very numerous. Denitrification is significant in that it is the method by which most of the nitrogen gas in the atmosphere was formed and is a source of serious loss of nitrate fertilizers in soils. It is noted that heavily fertilized soils and sewage are particularily

richly populated with denitrifiers.<sup>(13)</sup>

Nedwell<sup>(12)</sup> enumerated organisms capable of denitrifying reactions in swamp sediments. Two tests were performed, one on sediments adjacent to a treated sewage outfall, the other approximately 2 kilometers downstream. Adjacent to the outfall, 38 percent of colonies isolated were organisma which reduced nitrate to nitrite, 11 percent reduced nitrate to nitrogen gas and 32 percent reduced nitrate to ammonia. Twenty percent did not denitrify in any way. From the downstream station, 25 percent did not denitrify, 43 percent reduced nitrate to nitrite, 2 percent reduced nitrate to nitrogen gas and 30 percent reduced nitrate to ammonia. It may be noted from these results that the proportion of organisms performing the nitrate to nitrogen gas reaction fell significantly from the outfall to the downstream station while the proportions of the other populations remained approximately constant.

2.3.3. Growth of Denitrifying Bacteria

The relationship of denitrification to temperature is usually expressed as an exponential equation. Equations 11 and 12 are examples.

of denitrification as shown in equation 12.

where: P = percent of denitrification growth rate at 20 °C.

 $T = temperature {}^{O}C.$ 

The optimum pH for denitrification is reported to be between 6.5 and 7.5.<sup>(7)</sup> It may be noted from equation 9 that hydroxyl ions are formed in the denitrification reaction. This can have an offsetting effect to the acid formation during nitrification. It can be calculated that approximately 3 milligrams of alkalinity are produced when the equivalent nitrate from 1 milligram of ammonia is denitrified.<sup>(8)</sup> Thus, denitrification can replace approximately half the alkalinity consumed during nitrification.

2.3.4. Carbon Requirements

It may be seen from equation 9 that, in order for nitrate to be used as an acceptor of electrons, a source of electrons or electron donor must be available. This electron donor is usually referred to as a carbon source, since it is generally an organic compound and in addition to providing hydrogen,or electrons for energy production, it also provides carbon for cellular synthesis. Simplified versions of the reactions for dissimilatory denitrification and cellular synthesis, using methanol as the electron source, are shown in equations 13 and 14.<sup>(7)</sup>

 $.833CH_{3}OH + NO_{3}^{-} \longrightarrow .5N_{2} + .833CO_{2} + 1.167H_{2}O + OH^{-} \dots 13$  $14CH_{3}OH + 3NO_{3}^{-} + 4H_{2}CO_{3} \longrightarrow 3C_{5}H_{7}O_{2}N + 2OH_{2}O + 3HCO_{3}^{-} \dots 14$ 

The reactions in equations 13 and 14 are essentially the reverse of the carbonaceous BOD<sub>5</sub> removal reaction, in that in carbonaceous BOD<sub>5</sub> removal, the organic matter is the pollutant which is removed by adding oxygen as an electron acceptor. In denitrification, the electron acceptor, the nitrate, is the pollutant which is removed by providing an electron donor.

Literature indicates that if methanol is used as a carbon sourcefor denitrification a ratio of between 2.5 and 3.0 weight units of methanol must be supplied for each weight unit of nitrate removed. This large carbonaceous substrate requirement has prompted a search for other sources of carbon to replace expensive methanol. A number of potential carbon sources such as industrial wastes, volatile acids and food processing wastes have been investigated.<sup>(7)</sup> Methane gas in solution can be used as a carbon source by denitrifiers.<sup>(8)</sup>

Early research indicated that most alternative carbon sources were not as easily degraded by denitrifying bacteria as methanol and that reaction rates were significantly reduced using alternative carbon sources.<sup>(7)</sup> However, in a more recent study, Skrinde<sup>(15)</sup> compared denitrification rates using yeast, corn waste, whey and spent sulphite liquor to those of methanol in a fluidized bed reactor and

found a similar rate and capacity for denitrification with all five carbon sources.

The waste organic matter present in waste water can be utilized as a carbon source and this is the basis of several alternating aerobic/anoxic processes for carbonaceous  $BOD_5$  removal combined with nitrification and denitrification. <sup>(16)</sup> Rittman<sup>(17)</sup> operated a nitrification and denitrification process in an oxidation ditch reactor in which he postulated that denitrification occured in anoxic microzones in the activated sludge floc. Organic matter in the waste would provide the carbon source in this process since no specific carbon source was added to the reactor. It was also noted that use of waste organic matter as a carbon source by denitrifiers under anoxic conditions can remove some of the BOD<sub>5</sub> which would otherwise have to be removed by aerobic metabolism and thereby reduce the requirement for oxygen and concomitantly reduce aeration requirements.

From the foregoing discussion, it would appear that the carbon in waste organic matter in waste water can supply the carbon requirement of denitrifying bacteria. As such, it would seem reasonable to assume that sufficient carbon would be available either in suspension in the water or in the benthic sediments in a stabilization pond, to satisfy the carbon requirements of the denitrifying organisms.

## 2.4. Waste Water Stabilization Ponds

2.4.1. Description and Definition

A stabilization pond is a form of waste water treatment reactor in which removal of pollutants is accomplished by a combination of physical and biological processes. The primary advantages of stabilization ponds over constructed treatment works are low capital cost, low operating cost and simplicity of operation. Drawbacks include large requirement for land area and inability to guarantee consistent effluent quality.

Stabilization ponds are generally classified into 5 categories: 1) aerobic ponds, 2) facultative ponds, 3) anaerobic ponds, 4) tertiary or polishing ponds and 5) aerated lagoons.<sup>(1)</sup> Only facultative ponds will be considered in the following discussion and a facultative pond shall be defined as: "a pond 3 to 8 feet (.9 to 2.4 meters) deep with an anaerobic lower zone, a facultative middle zone and an aerobic upper zone maintained by photosynthesis and surface reaeration."<sup>(1)</sup> No mechanical mixing or aeration is used in a facultaive pond. As noted in the definition, a facultative pond is considered to consist of three processes which occur in layers. The surface layer contains photosynthetic autotrophic algae which use sunlight and carbon dioxide to synthesize cellular matter and release oxygen. Heterotrophic aerobic bacteria use this oxygen to stabilize the organic waste matter from the waste water. Since phototrophs only metabolize when light is present, they

stop producing oxygen during night hours, although heterotrophs continue to metabolize. Thus, the dissolved oxygen in the pond undergoes a daily cycling in concentration. Some oxygen is also provided by surface aeration from the atmosphere, but photosynthesis is considered to be the primary source of dissolved oxygen in a facultative pond.<sup>(1)</sup> Photosynthetic algae use carbon dioxide as they metabolize and this removal of carbon dioxide can cause the pH of a pond to rise. Heterotrophs release carbon dioxide as they metabolize and this release and use by different types of organisms causes the pH of facultative ponds to undergo a diurnal variation. The relative depths of the aerobic and facultative regions of the pond expand and contract as the amount of dissolved oxygen provided by photosynthesis increases and decreases. The aerobic zone may extend from only a few centimeters below the water surface to the bottom of the pond at different times and under different conditions of activity of the algae and bacteria.

A layer of sediment composed of settled suspended solids collects on the bottom of a facultative pond. This layer is anaerobic and fermentation reactions take place, releasing gases including carbon dioxide and methane. A simplified illustration of the zones of a facultative pond is shown in figure 2.<sup>(1)</sup>

2.4.2. Design Procedures

A number of design procedures have been proposed for facultative ponds but, due to the complexity of the



a facultative waste water stabilization pond<sup>(1)</sup> Zones in

FIGURE 2.

reactions involved, a rigorous mathematical model has not been developed. For this reason, most design procedures rely upon empirical factors or loading rates based upon experience.

Benefield & Randall<sup>(1)</sup> presented a design procedure using influent BOD<sub>5</sub>, flow rate, temperature and empirical factors for algal toxicity and sulphides. Their design equation is:

 $V = CQS_0 \theta^{35-T} F_1 F_2$  ..... 15 where: V = pond volume Q = flow rate  $S_0 = influent BOD_5$   $\theta = temperature coefficient$   $F_1 = algal toxicity factor$   $F_2 = sulphide toxicity factor$ C = conversion factor

A design procedure by Rich<sup>(18)</sup> relates BOD<sub>5</sub> removal to methane production in the benthic sediment layer using empirical factors. He gives a relation by which sediment temperature may be estimated from air temperature as follows.

The WPCF<sup>(19)</sup> gives a design procedure which relates oxygen availablity to algal activity and gives detention times based upon surface and volumetric BOD<sub>5</sub> loading rates. Environment Canada<sup>(20)</sup> recommends facultative ponds

for northern regions be designed on the basis of 32 Kilograms  $BOD_5$  per hectare per day.

Finney<sup>(21)</sup> reviewed design models for facultative ponds and compared them to operational results. The models examined were an area loading method with meterological factors, the toxicity method proposed by Rich<sup>(18)</sup>, originally proposed by Gloyna and a set of design equations based upon assumptions of complete mixing and first order reaction kinetics. He concluded that none of the design models reviewed gave an adequate design, primarily due to lack of a way of accurately predicting hydraulic residence time.

It may be noted that none of the design methods described above include any terms for removal or conversion of forms of nitrogen. Although removal of nitrogen in ponds is known to occur, sufficient information upon which to predict removal rates consistently has not been available and estimates of nitrogen conversion efficiencies could not be made.<sup>(22)</sup>

Stone et al<sup>(23)</sup> studied nitrification in oxidation ponds. In a thorough study, they determined that nitrifying bacteria were present in the ponds and all environmental conditions were favourable for nitrification, yet it did not occur. They mixed the pond contents to assess the
effect of suspension of nitrifying organisms, but found no significant improvement in nitrification. They performed tests, using steel barrels, containing pond effluent, as reactors and found that nitrification and denitrification proceeded rapidly in the barrels and that ammonia removal exceeded theoretical predictions. They were unable to explain their findings other than to postulate that nitrification did not occur because the nitrifying bacteria were unable to attach themselves to the algal suspended solids in the ponds. They noted that nitrification could not occur on the bottom of the ponds due to anaerobic conditions.

Bansel<sup>(24)</sup> found that nitrification in natural streams could be related to turbulent mixing and derived a function incorporating Reynolds and Froude numbers in an equation to predict nitrification rates.

Barnes & Bliss<sup>(8)</sup> noted that attachment of nitrifying organisms is not essential to their growth but stated that they "tend to attach themselves to surfaces if available."

Watson<sup>(9)</sup> observed that high concentrations of nitrifiers are found at the sediment-water interface in rivers and streams and attached to the sides of aeration tanks in sewage disposal plants. This could explain Stone's results as described above, that nitrification proceeded rapidly in barrels but not in the ponds. The sides of a barrel would provide a much larger surface area, relative to volume, than a pond.

2.4.3. Nitrogen Balance in Ponds

In recent years, several researchers have examined the problem of nitrogen balancing in stabilization ponds.

Pano<sup>(25)</sup> noted that ammonia may be removed from a pond by 3 mechanisms: 1) volatilization to the atmosphere, 2) assimilation in algal biomass and 3) biological nitrification and denitrification. Ammonia in aqueous solution exists either as unionized ammonia or ammonium ion with the equilibrium depending primarily upon pH. At high pH, ammonia predominates, at low pH, ammonium ion. Unionized ammonia establishes an equilibrium in water in accordance with Henry's law, which states that the concentration of a gas in solution in a liquid is proportional to the partial pressure of that gas above the liquid. (26) Since the partial pressure of ammonia in air is essentially zero, unionized ammonia could be expected to escape rapidly to the atmosphere. Ammonium ion however, cannot escape from water. Pano derived an equation to measure ammonia volatilization as shown in equation 17

 $\frac{C_e}{C_o} = \frac{1}{1 + \frac{A}{Q} (.0038 + .000134T) e^{((1.041 + .044T)(pH - 6.6))}} .17$ where:  $C_e$  = final concentration of ammonia  $C_o$  = influent concentration of ammonia A = pond area, square meters Q = flow rate, cubic meters per day

 $T = temperature, ^{O}C.$ 

pH = the pH of the water

The reader is referred to Pano's work for the details of derivation of equation 17. The equation was calibrated on operating facultative ponds and found to give "excellent" agreement between calculated and measured ammonia reduction.

Gordin<sup>(27)</sup>et al studied removal of total mineral nitrogen, defined as the sum of ammonia, nitrite and nitrate, by algal biomass in treatment ponds in the U.S.S.R. They found that mineral nitrogen removal could be related to algal growth using a sunusoidal function to model algal growth and nitrogen removal over the summer season. Their equation was in the form shown in equation 18.

 $\frac{dS}{dt} = K(t)c^{m}s^{n} \qquad .... 18$ 

where: K(t) = rate constant
C<sup>m</sup> = concentration of nitrogen
S<sup>n</sup> = concentration of algal cells

The rate constant K(t) has the form shown in equation 19.

where:  $A_k$  = kinetic constant  $T_k$  = growth conditions factor  $\Upsilon_k$  = time segment of season for algal growth A computerized solution to the equations was used and good agreement obtained between the model and measured data.

Ferrara<sup>(28)</sup> studied nitrogen dynamics in stabilization ponds using a set of mass balance differential equations to model effects of sedimentation, volatilization, biological growth and nitrification and denitrification. He compared his model to results from 4 sets of ponds and concluded that sedimentation, primarily of organic nitrogen forms, was the predominant vehicle for nitrogen removal in ponds and volatilization and nitrification/denitrification accounted for only a small part of the nitrogen removal. He also noted that denitrification would be limited in facultative ponds due to low nitrification rates.

Reed<sup>(22)</sup> derived a first order kinetic equation to predict total nitrogen removal in facultative ponds. He noted that no corelation appeared to exist between algal growth and nitrogen removal, which would seem to contradict Gordin's findings as noted above. Reed did not attempt to differentiate between the various forms of nitrogen or determine the various removal mechanisms or transformations of nitrogen in his work, rather, he only wanted to predict the difference between influent and effluent total nitrogen. His equation is shown in equation 20.

$$\frac{N_{e}}{N_{o}} = e^{-K_{t}(t=60.6(pH-6.6))} \dots 20$$

where: N<sub>e</sub> and N<sub>o</sub> = efflent and influent nitrogen concentrations  $K_t = k_{20} \theta^{T-20}$  $k_{20} = .0064$  $\theta = 1.039$ 

This model showed good agreement with measured data. He noted that volatilization appears overall to be the major factor in ammonia removal, which would appear to contradict Ferrara's conclusions.

Reddy<sup>(29)</sup> studied nitrogen transformations in waste water ponds using <sup>15</sup>N labeled ammonia and nitrate. He found that macrophytic plants could remove between 34 and 40 percent of added ammonia and nitrate although the plants prefered ammonia. From 45 to 52 percent of the added nitrogen could not be accounted for. In a reservoir with no large plants, algae removed approximately 5 percent of added nitrogen and again between 40 and 50 percent of the nitrogen was not recovered in any form. It was assumed lost by nitrification and denitrification although no attempt was made to test this hypothesis. It was also observed that the role of sediments is often ignored in calculations of nutrient removal processes in ponds.

Bouldin et al<sup>(30)</sup> studied nitrogen loses from small, freshwater ponds by adding ammonia and nitrate. They found that loss of ammonia by biological uptake was small

and that, while loss due to volatilization varied widely, it was the predominent vehicle for removal of ammonia from the pond. They found that denitrification in the bottom sediments was the primary sink of the added nitrate.

2.2.4. The Nitrogen Cycle in a Pond

It was noted in section 2.2.1. that the transformations which nitrogen undergoes as it moves through living processes make up the nitrogen cycle. From the preceeding discussions, it is possible to construct a nitrogen cycle which would depict the forms and movement of nitrogen in a waste stabilization pond. The cycle would consist of four major forms of nitrogen: organic nitrogen, ammonia, the reduced forms, nitrite and nitrate and nitrogen gas. Attempts to calculate a mass balance for this cycle would be complicated by the fact that all four of the major forms of nitrogen could move into and out of the cycle in ways not directly related to each other. A schematic diagram of the nitrogen cycle as it might apply to a waste stabilization pond is given in figure 3.



### FIGURE 3.

The nitrogen cycle in a waste stabilization pond

#### 2.5. Nitrogen Transformations in Sediments

2.5.1. Focus of Research Activities

As described in section 2.4.3., the reduction of nitrogen concentrations between the influent and effluent of facultative ponds is primarily attributed to 3 mechanisms: sedimentation of organic nitrogen, volatilization of ammonia and biological nitrification-denitrification. Of these mechanisms, sedimentation of organic nitrogen and volatilization of ammonia have been studied and, while nitrification has been examined, research reports attempting to quantify denitrification in waste water stabilization ponds are lacking. As noted in section 2.3.1., denitrification is an alternate pathway to aerobic respiration and many genera of soil and water microorganisms can readily alternate between oxygen and nitrate as terminal electron acceptor. Therefore, it would seem reasonable to expect denitrification to occur in the anoxic bottom sediments of ponds and other bodies of water. Limnologists and oceanographers have studied nitrification and denitrification in sediments and a resource of information is available with respect to the transformations of nitrogen within the sediments of lakes, marshes and the oceans. The purpose of this research is to understand the role of sediments in the cycling of nitrogen in the aquatic environment.

The nitrogen cycle was described in section 2.4.4. All forms of nitrogen may be found in sediment and water systems and all transformation of the cycle may proceed simultaneously.

2.5.2. Origin of Nitrogen Gas

A factor complicating the study of denitrification is the fact that the end product of the reaction, nitrogen gas, is a major constituent of the atmosphere and may be expected to be present in any water exposed to the air. There are several ways of overcoming this problem. The amount of dissolved nitrogen at equilibrium in water exposed to air can be calculated for a given temperature and pressure using Henry's law. A significant difference between calculated and measured levels of nitrogen gas in a situation where conditions conducive to denitrification exist may be considered evidence that denitrification is occuring. Isotopically labeled nitrogen combined as nitrate or ammonia can be inoculated into a culture and any transformations traced accordingly. This method is widely used by agricultural researchers to examine the movement of fertilizer nitrogen in soils. Air consists of .94 percent  $argon^{(4)}$  which forms solution equilibrium in water. Argon is not known to participate in any biological reaction (31), so biogenic nitrogen gas would be expected to contain no argon. The small difference in argon between atmospheric and biogenic nitrogen can be measured with sufficiently sensitive instruments. It has been found that acetylene will selectively inhibit nitrous oxide reductase and thereby stop the final reaction of the denitrification sequence, the reduction of nitrous oxide to nitrogen gas.<sup>(13)</sup> Since nitrous oxide is not

known to be produced by any other biological process, the accumulation of nitrous oxide may be considered evidence that denitrification is occuring.

2.5.3. The Sediment-Water System

A model of the sediment-water system as the interaction of three seperate layers is presented in figure 4. (32) The layers are the overlying water, an aerobic sediment layer adjacent to the water and an anaerobic lower sediment layer. Diffusion due to chemical concentration gradients is an important driving force in this model. Diffusion of oxygen from the overlying water creates a thin, aerobic sediment surface layer in which nitrifying organisms oxidize ammonia to nitrate. This creates a concentration gradient which causes nitrate to diffuse downward into the anaerobic sediment layer where denitrifying organisms reduce it to nitrogen qas. Simultaneously, the loss of ammonia from the aerobic zone causes a concentration gradient of ammonia, thereby causing ammonia to move from the lower, anaerobic layer toward the aerobic layer. It is noted that the denitrification of nitrate in the anaerobic layer occurs rapidly and that little nitrate is found in either the aerobic or anaerobic layer due to this rapid diffusion and denitrification.<sup>(32)</sup> The aerobic layer is thin, estimates of its thickness range from 2 centimeters to as little as .4 millimeter. (12,30,32)

Sedimentation of organic matter from the overlying water is estimated to be the source of approximately half



FIGURE 4.

The 3 layers of the sediment-water system<sup>(32)</sup>

of the organic nitrogen and ammonia which is nitrified in the aerobic sediment layer. This has been demonstrated in lakes and could be expected to be even more significant in a waste water stabilization pond. The remaining fifty percent of ammonia in the aerobic layer was supplied by diffusion from below.<sup>(32)</sup> Anaerobic bacterial action in the underlying sediments would contribute to this ammonia. <u>Clostridium pasteurianum</u> has been shown to fix nitrogen gas and excrete large quantities of ammonia.<sup>(33)</sup> This would provide a pathway for the recycling of nitrogen gas back to ammonia, where it could again be made available to nitrifiers.

2.5.4. Nitrogen Cycling in Sediments

2.5.4.1. Nitrogen Tracing

Goering<sup>(34)</sup> studied denitrification in lake sediments in Alaska. He used isotopically labeled nitrate added to water above samples of lake sediment. Isotopically labeled nitrogen gas was detected after incubation. The rate of production of nitrogen gas was calculated to be approximately 90micrograms per liter per day at  $5^{\circ}$ C. An "insignificant" amount of labeled ammonia was also found. Only labeled nitrogen gas was found as the product of denitrification. No intermediate products such as nitric or nitrous oxides were detected.

Richards<sup>(35)</sup> used the nitrogen, argon ratio technique to study nitrogen in ocean water. A nitrogen, argon ratio larger than that expected from atmospheric nitrogen was

found in anaerobic zones. The possibility of direct oxidation of amino nitrogen to nitrogen gas with sulphate ion as an oxidant was examined and it was concluded that this reaction was not possible, thereby tending to confirm that the biological nitrification/denitrification process is the only pathway by which ammonia can be converted to nitrogen gas.

In a study of stream bed sediments, Sian et al<sup>(36)</sup> incubated samples from a stream receiving nitrate inputs. They observed steady loss of nitrate from the sediment-water systems and tentatively concluded that this was due to denitification. They noted that the denitrification appeared to occur in the top 5 centimeters of the sediment since increasing the sediment depth beyond 5 centimeters made no difference to the reaction rate.

Mixing of sediments can have an effect upon the equilibrium described above. Chen et al<sup>(37)</sup> investigated nitrification in quiescent versus stirred sediments, using isotopically labeled ammonia. Nitrification was found to occur rapidly and completely in stirred sediments but slowly in quiescent sediment. Addition of N-Serve (2-chloro-6-trichloromethyl pyridine), a compound which selectively inhibits <u>Nitrosomonas</u>, to the stirred sediment stopped nitrification completely, thereby demonstrating that conversion of ammonia to nitrate in the sediment was biological. The workers concluded that the rate of diffusion of oxygen into the quiescent sediment was too low to provide for significant nitrification, a finding which would support the

contention that the aerobic layer of sediment is quite thin under normal conditions. Mixing of sediments can be caused by benthic organisms, bubbling of fermentation gases, wind currents, rough fish and boating currents. (33) The last two would not be significant in a waste stabilization pond, but the first three factors could be expected to contribute to movement of sediments in ponds. Diurnal overturn due to formation and dissipation of thermal layers is cited as a factor in mixing of ponds as as a vehicle for bringing oxygenated water from the surface of the pond to the bottom. (18) This would result in a daily cycle of aerobic and anoxic conditions at the sediment interface, thereby setting up conditions for a cycle of nitrification and denitrification.

Such a cycle was found to occur in a lake in Wisconsin. Ismirimah et al<sup>(38)</sup> studied nitrification and denitrification in stirred and unstirred sediment samples and found that stirring resulted in rapid nitrification. They found that approximately 40 percent of isotopically labeled nitrate added to sediments was recovered either as ammonia or organic nitrogen. The remainder was presumed lost to denitrification. They concluded that stirring of loose sediments by wind generated waves promoted rapid nitrification in the lake and that calm conditions allowed "significant" nitrate loss by denitrification. The average depth of the lake was 3 meters.

2.4.5.2. Oxidation Reduction Potential

The oxidation-reduction, or redox potential of a biological system can be used as an indication of the type of activity occuring. The disappearance of oxygen occurs at a potential of approximately 300 to 350 millivolts (mV) and the reduction of nitrate, the first anoxic reaction, is indicated by a potential of 100 to 200 mV. The second or obligate anaerobic reaction begins at approximately -150 mV. (33)

Whisler et al<sup>(39)</sup> studied changes in redox potentials in flooded soil columns. They measured redox potentials in the 200 to 300 mV range while gas containing 98 percent nitrogen was released from the columns. As nitrate disappeared from the columns, the redox potential fell into the negative range and nitrogen gas release stopped. They concluded that denitrification was occuring and that it occured close to the soil surface primarily due to limited carbon substrate at greater depths.

Engler et al<sup>(40)</sup> studied the effects of dissolved oxygen and nitrate on redox potentials in marsh soils. Their work supported the thin layer theory of sediments outlined in section 2.5.3. in that they found that nitrate disappeared rapidly from water in contact with sediment but that no change occured in the water when separated from the sediments. Up to 90 mg/l of nitrate was removed from soil, sediment systems over 4 day test periods. Significantly, the presence of largeamounts of dissolved oxygen, up to 16 mg/l.

in the water did not appear to inhibit denitrification. This was attributed to rapid depletion of the oxygen in the sediment. They found that initial redox potentials between 300 and 500 mV dropped rapidly to between 50 and 200 mV as oxygen disappeared and remained at that level as long as nitrate was present.

2.5.5. Analysis of Sediment Gases

Several researchers have collected gases in situ from sediments in an attempt to gain understanding of the processes occuring therein.

Reeburgh<sup>(31)</sup> studied gases collected from sediments in Chesapeake Bay using the nitrogen, argon technique. He found decreasing levels of nitrogen and argon with depth in the sediments to depths of approximately 100 centimeters. He noted that methane was being formed and that methane ebullition could remove other gases such as nitrogen and argon by a gas stripping process. However, he did not find significant quantities of methane in the water overlying the sediments and attributed this to use of the methane as an energy substrate underaerobic conditions. He concluded that any changes in nitrogen concentration due to biological activity were masked by methane stripping.

Kuznetsov<sup>(41)</sup> summarized the results of sediment gas studies in the U.S.S.R. Volume composition of gases from several lakes and reservoirs was reported. Gas compositions of 10.7 percent nitrogen, 80.3 percent methane and 5 percent hydrogen and carbon dioxide were found in Lake Beloya while

gas from the lake sediment contained 25.4 percent nitrogen, 55.2 percent methane and 19.7 percent hydrogen and carbon dioxide. Sediment gas from the Rybinsk reservoir contained 28.8 percent nitrogen, 62.8 percent methane and 9.4 percent hydrogen and carbon dioxide. It was not reported whether the "reservoir" was a natural lake of a man made structure.

Chen et al<sup>(42)</sup> collected sediment gases in situ from a lake in Wisconsin, using SCUBA divers and a cone apparatus. They found 24 percent nitrogen and 75 percent methane in one sample and 50 percent nitrogen and 45 percent methane in another. Denitrifying organisms were identified in the sediments. Sediment samples were incubated under controlled conditions and found to release nitrogen and methane together, thereby indicating that denitrification and anaerobic fermentation can occur simultaneously. Sediment samples were inoculated with <sup>15</sup>N labelled nitrate and <sup>15</sup>N labelled nitrogen gas was subsequently recovered, thereby proving that denitrification was taking place. Nitrous oxide was identified as an intermediate in the reaction but nitric oxide was not found.

The results of these gas collection studies are summarized in table 2.

2.5.6. Rates of Denitrification in Sediments

A number of researchers have attempted to measure the rate of denitrification in sediments on an areal basis. This can provide useful comparative information as well as providing figures for estimating nitrogen transformations

				-
Reference	Location	Nitrogen	Gas analysı ethane H	s, percent ydrogen & Carbon dioxide
Kuznetsov <sup>(41)</sup>	water, Lake Beloya	10.7	80.3	: م
	sediment Lake Beloya	25.4	55.2	19.7
	sediment Rybinsk reservoir	28.8	62.8	9.4
Chen <sup>(42)</sup>	lake sediment Wisconsin			
·	location 1	24	75	not reported
	location 2	50	45	not reported
		TABLE 2.		
	Water and sediment ga	s analysis by	other resear	chers.
			(1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	

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for mass balance calculations. Most research activity appears to have been focused on shore line or marine estuary sediments.

Sorenson<sup>(43)</sup> studied denitrification in Fjord sediments in Denmark using the acetylene inhibition technique. His findings indicated that denitrification was occuring in the upper 8 centimeters of the sediment but that it essentially stopped below 8 centimeters. He also found nitrification occuring in the upper sediment. He calculated denitrification to be occuring at the rate of 13.8 milligrams nitrogen as N per square meter per day at a temperature of  $2.5^{\circ}$ C. He noted that nitrification and denitrification are "apparently mutually exclusive." This assumption would be based upon the microbiological theory discussed in sections 2.2. and 2.3. that nitrification is an obligately aerobic process while denitrification occurs under anoxic conditions. Other work indicates however, that the two processes can proceed simultaneously. Rittman<sup>(17)</sup> operated a one reactor oxidation ditch sewage treatment plant which achieved nitrification and denitrification simultaneously by maintaining a dissolved oxygen level of between .1 and .9 mg/l. He postulated that denitrification occured in anoxic microzones within the activated sludge floc while nitrification was occuring on the aerobic surface of the particles. He noted that operation of such a system was "simple", with dissolved oxygen concentration and oxygen transfer rate being the control parameters. The distance between nitrifying and denitrifying zones in the floc was not given, but

it could be expected to be small, probably in the order of microns. When Rittmen's results are considered, the phenomonon observed by Sorenson becomes understandable.

Oren<sup>(44)</sup> estimated denitrification rates in Fjord sediments using isotopic nitrogen in laboratory samples and related this to in situ nitrate concentrations. He found redox potentials fell in proportion to nitrate concentrations in the sediments, indicating a relation between redox potential and denitrification similar to that found by other workers described in section 2.4.5.2. The rate of denitrification was calculated, using Michaelis-Menton kinetics, to be 2.3 milligrams N per square meter per day at  $12^{\circ}$ C. As with Sorenson's work, denitrification was observed to occur in the presence of dissolved oxygen. The author noted however, that this phenomenon is not uncommon and has been observed by other workers.

Chan<sup>(45)</sup>used the acetylene inhibition technique to estimate in situ denitrification rates in ponds around Toronto. A rate of 2.3 milligrams N per square meter per day was deduced from their findings. Temperature was not reported, but it was noted that the experiments were performed during July and August.

Kasper<sup>(46)</sup> studied denitrification in intertidal mud flats. Cores were incubated in the laboratory and acetylene inhibition was used to measure denitrification. The greatest potential for denitrification was found to be in the top 3 centimeters with, from theoretical considerations,

approximately 67 percent of denitrification occuring in this zone. He also found variations in denitrification rates on an areal basis in adjacent areas with some areas giving higher rates than others. The denitrification rate in the sediments was estimated at between 1.4 and 16.4 milligrams N per square meter per day. Sources of error in the measurements were discussed. These included disturbance of the sediment during sample collection. changes in parameters such as temperature and the effects of acetylene on microbial metabolism. It was concluded that an error range of from -170 percent to +95 percent could be attributed to the results. The experiments were performed at 22<sup>o</sup>C.

Smith<sup>(47)</sup> examined estuarine sediments from the Louisiana Gulf Coast. He used isotopically labeled nitrate and acetylene inhibition to measure denitrification in fresh and marine sediments. Inoculation of nitrate into sediment samples inhibited with acetylene caused a corresponding accumulation of nitrous oxide, thereby indicating denitrification was occuring. Addition of <sup>15</sup>N labeled ammonia also resulted in production of  ${}^{15}N$  labeled nitrous oxide, indicating that both nitrification and denitrification were taking place in the sediment. Approximately 50 percent of the added ammonia was recovered from the sediments as organic nitrogen. The average denitrification rate for the sediment was estimated to be 3.8 milligrams N per square meter per day. The tests were performed at  $25^{\circ}$ C.

The areal denitrification rates reported by these researchers are summarized in table 3.

2.5.7. Effect of Temperature on Denitrification

The effect of temperature upon denitrifying bacteria was discussed in section 2.3.3.

Terry<sup>(48)</sup> studied denitrification in lakes and found the rate of denitrification increased with temperature over a range of 5 to  $23^{\circ}$ C. but that significant denitrification occured at  $5^{\circ}$ C. This was noted to be in contrast to the findings of other researchers who reported no denitrification at that temperature.

It would appear that different populations of denitrifyers may be active at different temperatures. Kaplan et al<sup>(49)</sup> studied denitrification in salt marshes and found evidence that temperature variation caused a selection between at least two distinct populations with temperature optima in the 5 to  $10^{\circ}$ C. and greater than  $10^{\circ}$ C. ranges respectively. It was observed that 10 to  $12^{\circ}$ C. appeared to be the "cross over" temperature between obligate psychrophilic and mesophilic denitrifiers. The researchers compared sediment temperature to unit nitrogen gas production and found a linear relationship with a good statistical corelation. Their finding is reproduced in figure 5. 2.6. Summary

From the evidence in literature noted in sections 2.5.3. through 2.5.7., there would seem little doubt biological denitrification occurs in bottom sediments of water

le ference	type of sediment	rate mgs N/m <sup>2</sup> /dav	temperature o <sub>C</sub> .	notes
Sorenson <sup>(43)</sup>	Fjord estuary	13.8	2.5	
0ren <sup>(44)</sup>	Kysing Fjord	2.3	12	
Chan <sup>(45)</sup>	drainage pond and small lake	2.3	not reported	
Kaspar(46)	intertidal mud flat	1.4 - 16.4	22	estimated error -170% to +95%
Smith <sup>(47)</sup>	fresh and saline estruary	о. С	25	
		TABLE 3.		
	Denitrification rate	s by area from c	other researchers	



FIGURE 5.

Unit nitrogen gas production versus sediment temperature in salt marsh muds<sup>(49)</sup>

bodies. The conversion of ammonia to nitrate to nitrogen gas in sediments has been traced in the laboratory using isotopic nitrogen and inhibition techniques. Nitrogen gas, along with methane, has been collected in sediments which were shown to contain nitrifying organisms. Several researchers have measured the rate of denitrification in sediments at various locations and under different conditions. The wide variation in estimated reaction rates would indicate that the reaction is complicated by a number of factors and probably a situation specific interaction of these factors.

However, as noted in section 2.4.3., it would appear that, while attempts have been made to determine denitrification rates in marsh and lake sediments, no comparable work has been performed on waste stabilization ponds. Therefore, research into this aspect of the nitrogen cycle in ponds could provide further information toward a more complete understanding of this process.

#### CHAPTER 3

### EXPERIMENTAL PROCEDURE AND RESULTS

3.1. Objective

The objective of the project was to attempt to determine if denitrification occurs in waste stabilization ponds and under what conditions. It was decided to concentrate upon collection of evidence for denitrification from an operating pond, rather that attempt a laboratory simulation. To this end, a field study was undertaken to gather data upon which to test the hypothesis that denitrification does occur in a stabilization pond.

The field study had 2 objectives: 1) to collect samples of gases evolved in a waste water stabilization pond and to analyze the composition of these gases to gain information as to their origin. The primary gas sought was nitrogen, but a total composition analysis was planned to provide information as to the various gases collected and the relative proportions. 2) to record the conditions under which the gas samples were obtained and collect water samples upon which to perform tests to measure parameters effecting the rate and production of gases in the pond.

Specifically, the gases analysed were nitrogen, methane, oxygen and carbon dioxide. Water temperature and dissolved oxygen were determined at the time of

sample collection and tests for pH, ammonia and nitrate were performed upon water samples taken simultaneously with collection of the gas samples.

#### 3.2. Apparatus

Floating and benthic gas collectors were constructed in the Environmental Engineering laboratory at the University of Manitoba. The floating collector (Fig.6) consisted of a plastic tub mounted in a wooden framed styrofoam float such that the tub was held inverted in the water. A gas removal spigot was installed in the tub. The benthic collector (Fig.7), consisted of a galvanized sheet steel cone with riveted and soldered joints. A metal tube was mounted at the apex of the cone and a section of Tygon tubing of sufficient length to reach the water surface was secured to the tube with clamps and silicone sealant. Spigots were sealed by folding over and securing with laboratory screw clamps.

The gas sample extraction apparatus consisted of a Nalgene 6130 hand held vacuum pump, liquid trap flask, Fisher 134-190 Septum Port Gas Sampling Tube and Tygon tubing and spring clamps (Fig.8). Use of this apparatus was as follows. Initially, the sampling tube and tail tubes were completely filled with water by placing the sampling tail tube in a beaker of water and drawing water through the apparatus to the trap flask with the vacuum pump. The trap flask was required to avoid drawing water into the vacuum pump. Care was taken to remove all air bubbles when filling the gas sampling tube and the tail tubes. Once full of water,



### FIGURE 6.

Floating gas collection apparatus dimensions in millimeters (not to scale)



plan





Pond benthic gas sample collection apparatus Dimensions in millimeters (not to scale)



## FIGURE 8.

Gas sample extraction apparatus

(not to scale) 56 the stop cocks on the gas sampling tube were closed and spring clamps placed on the ends of the tail tubes to retain the water in the tubes. To obtain a gas sample, the collecting tail tube was connected to the spigot on the pond gas collector, a small vacuum applied to the apparatus with the pump and the clamps removed. The stopcocks were opened while the gas sampling tube was held upright with the withdrawl tube down. The water in the apparatus was drawn out by pressure differential and displaced with the gas sample. In order to prevent contamination of the sample with air from the trap flask, the lower stopcock was closed just prior to all water being withdrawn from the gas sampling tube. A new rubber septum was placed on each sampling tube before gas samples were collected.

The following automated analysis apparatus was used.

For gas analysis: Gow-Mac Series 550 Thermal Conductivity Gas Chromatograph with Gow Model 70-700 strip chart recorder. The column for determination of nitrogen, oxygen and methane was an SA 60/80 mesh molecular sieve, the column for carbon dioxide contained Poropak Q, 80/100 mesh.

For ammonia analysis: Tecator Kjeltec System 1002 distilling unit and Baush and Lomb Spectronic 21 spectrophotometer.

For nitrate analysis: Scientific Instrument Corp. autoanalyser consisting of sampler, proportioning pump unit and colorimetric analytical cartridge and Fisher

Recordall Series 5000 strip chart recorder.

For pH measurment: Fisher Accumet Model 230 pH/ion meter.

#### 3.3. Procedure and Results

3.3.1. Test Site and Location of Collectors

Gas collectors, as described in section 3.2. were placed in the number 2 cell of the City of Winnipeg West End Water Pollution Control Centre (WEWPCC) in June of 1986. This cell is a facultative waste stabilization pond with a surface area of approximately 18.2 hectares and a water depth of approximately 0.9 meters. The pond was put in service in 1965 and a layer of compression settling sediment has accumulated on the bottom, making exact determination of the water depth difficult. The pond has no mechanical mixing or aeration.

Three collectors, 2 surface and 1 benthic, were placed in the pond at the locations shown in figure 9. The surface collectors were anchored with sufficient slack to allow free movement with any change in water level. The benthic collector was placed adjacent to one of the surface collectors. This collector will hencefroth be called "S1". The other surface collector will be called "S2" and the benthic collector will be called "B0". The arrangement of collectors S1 and B0 in the pond is illustrated in figure 10. Collector S2 was anchored similarily except that no benthic collector was adjacent to it.

The WEWPCC consists of an activated sludge treatment plant and 5 ponds, numbered 1,2 and 3 primary and 1 and 2 secondary. The 3 primary cells are the same size and dimensions. During August, September and October of 1986,



of gas sample collectors. scale 1:3333 (metric)



# FIGURE 10.

Surface and benthic gas collecting apparatus as arranged in #2 pond. (not to scale)
waste water flows up to approximately 22 million litres per day were directed into the treatment plant and all flow in excess of this was diverted to number 2 primary cell and thence by overflow into 1 and 3 primary cells.<sup>(50)</sup> The WEWPCC receives flows from the west and south west sectors of Winnipeg. The waste water is primarily from domestic and light commercial sources with little industrial waste water contribution. A characterization of the influent is given in Table 4.<sup>(51)</sup>

Characteristic Average value ( mg/1 ) BOD<sub>5</sub> 249 Suspended solids 178 TKN 36 Ammonia 27 Nitrate 0.04 Total phosphorus 7.6 pН 7.5

TABLE 4.

Raw waste water influent characterization-West End Treatment Plant, Sept.-Oct., 1986<sup>(51)</sup>

3.3.2. Collection of Samples

On September 4, a sample of gas was extracted from collector S2 using the collection technique described in section 3.2. Previous attempts to collect samples had not

used this technique and the integrity of the gas samples collected cannot be guaranteed. Therefore, no results from samples taken prior to September 4 will be reported.

On September 8 and approximately once per week thereafteruntil October 30, gas samples were collected from the three collectors. These sampling intervals are designated as periods 1 through 9 and identified accordingly in subsequent discussion. After each sample was taken, the collectors were purged of all remaining gas and left filled with water. At each sampling, the three gas samples were collected within a period of approximately 40 minutes between the hours of 9:30 and 11:00 o'clock in the morning. The same gas sampling tube was used to take gas from each collector respectively for the duration of the test period. At the time of collection, the approximate volume of gas collected was noted, based upon an observed fraction of the gas sampling tube filled. The tubes were not calibrated, so a more accurate determination of gas volume was not possible. A sample of less than approximately one eighth of the tube was considered too small to be reliable and was noted as such. In some cases, more than a full tube of gas had collected in the pond collector. This was noted as one full tube plus and no determination could be made of the excess volume.

Gas composition analysis of the three samples from each collection was performed within 4 hours of collection of the samples. Gases analysed were oxygen, nitrogen and

methane, analysis being performed in the gas chromatograph described in section 3.2 above. The procedure was as given in Standard Methods, section 511B.<sup>(52)</sup> Oxygen, nitrogen and methane were analyzed on the molecular sieve. Difficulties with the Poropak prevented analysis of carbon dioxide until October 22. Carbon dioxide was measured in thebenthic samples collected on October 22 and 30.

At the time of collection of each set of gas samples, water samples were taken adjacent to collector S1 for analysis of ammonia, dissolved oxygen, nitrate and pH. Surface and bottom water temperatures were recorded at the time of sample collection. Samples for ammonia analysis were preserved with 0.8 ml of sulphuric acid per liter, as required in section 417 of Standard Methods<sup>(52)</sup> Samples for nitrate analysis were preserved with 1 drop of chloroform per 100 ml , as recommended by the manufacturer of the autoanalyzer.(53) Samples for pH measurment were not preserved, but pH determination was made within 4 hours of sample collection. Reagents were added to the dissolved oxygen samples immediately upon collection and titration was performed within 4 hours of collection. Dissolved oxygen testing was performed as detailed in Standard Methods,<sup>(52)</sup> method 421B, azide modification. Ammonia analysis was performed by steam distillation, method 417A, followed by Nesslerization, method 417B with use of a spectrophotometer for color comparison. Nitrate analysis was performed according to method 418F.

automated cadmium reduction. Samples were filtered through Whatman 934-AH paper before analysis for color and turbidity removal. pH measurment was by method 423. Gas analysis was by method 511B.

On October 30, the pond temperature having fallen to  $1^{\circ}$ C., the collectors were removed from the pond and the field study terminated. Ice had begun to form around the shore of the pond.

3.3.3. Verification of Collection Apparatus

The gas collectors were returned to the University, cleaned and tested for integrity. The procedure was as follows. An open top tank in the hydraulics laboratory was filled with tap water and allowed to come to room temperature. Once the water temperature remained constant for 3 successive days, one of the collectors was placed in the tank and evacuated of air using the procedure described in section 3.2. The collector was then observed each day for seven days. This procedure was repeated with each of the three gas collectors. At the end of each seven day period, no gas bubbles could be detected in any of the three collectors. From this, it can be concluded that the three collectors were gas tight and no atmospheric contamination occured during accumulation of the samples.

The integrity of the three gas sampling tubes was tested by filling them with a mixture of nitrogen and methane from the laboratory standard gas cylinders. Gas composition was measured immediately after filling and at 3 and 5 hours

after filling. No detectable change in the composition of the gases occured in any of the three tubes at 3 and 5 hours. From this, it may be concluded that no changes occured in the samples collected in the ponds between collection and analysis since, as noted in section 3.3.2., all gas analyses were performed within 4 hours of sample collection.

After the collectors were determined to be gas tight, an experiment was performed to test the ability of Henry's law to predict gas dissolution from water due to an increase in temperature. The test tank was filled with tap water and the temperature and dissolved oxygen immediately recorded. A collector was placed in the tank and all air removed. The water was allowed to reach room temperature and the temperature and dissolved oxygen again recorded. Dissolved oxygen saturation values for the initial and final water temperatures were obtained from Sawyer.<sup>(6)</sup> The gas volume coming out of solution was measured and compared to the volume of air predicted to come out of solution by Henry's law. The gas composition was determined with the gas chromatograph. Three trials of this test were performed, one with each of the gas collectors. The results of this experiment are given in tables 5a and 5b.

3.3.4. Analytical Results

Results of analysis of the samples obtained in the pond during the field study are given in tables 6 through 10 as follows:

Table 6: Gas composition analysis, collector S1.
Table 7: Gas composition analysis, collector S2.
Table 8: Gas composition analysis, benthic collector.
Table 9: Dissolved oxygen, temperature and pH results.
Table10: Ammonia and nitrate analysis results.

[ria]	Water	temp.		Dissolved	oxygen mg/	•	Theoretical	Actual gas	Ratio
	start	tinish	sta	rt	fini	sh	gas evolved	collected	
	) )	· · )	measured	saturation	measured	saturation	( L m )	( L m )	
	വ	19	12.2	12.8	9.3	9.4	371	330	89
2	9	19	11.9	12.5	9.1	9.4	264	239	т. С
ŝ	വ	18	12.0	12.8	9.1	<b>9</b> • £	338	303	.905
	Initia	land	final diss	olved oxyge	TABLE 5a. en, tempera	ature and v	olumes of ga	is collected	
			Trial		U	as analysi:	s, percent		
					I N	trogen	Oxygen	Total	
			1			77	20	67	
			2	-		80	21	101	
			т			80	19	66	
				Compositi	TABLE 5b. ion of gas	collected			
	2	о f t с	+ + 5 8 9 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	משון כא משון כא	TABLE 5.	ייד ייריי יריי	יין המין היי דים ליים ליי	עם דיים גייוס גייוס	٤
-	2000		טר רכ בתגנ	מו מי אי אי איי	מות בטוויים		ייש מיכייע מי	וטו ומט אמים	

Sampling	Date	Gas an	alysis, percer	nt	Total
period		Nitrogen	Methane	0×ygen	percent
-	Sept. 8	80	0	14	94
5	Sept. 11	85	trace	ထ	93
т	Sept. 17	95	• • •	4	100
4	Sept. 26	92	trace	4	96
പ	0ct. 1	68	30	2	100
Q	0ct. 8	insut	fficient sampl	e	1
7	Oct. 16	84	trace	16	100
ω	0ct. 22	82		16	6 6
σ	0ct. 30	63	ç	19	85

Gas composition analyses for surface collector S1

TABLE 6.

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and the second se

Total	percent	101	67	67	66	ı	98.5	ł	·	100	101	
دىر	0×ygen	4	17	12	4	0	15			19	21	
nalysis, percen	Methane	4	0	2	12	ufficient sampl	1.5	ufficient sample	ufficient sampl€	7	2	
Gas A	Nitrogen	93	80	83	83	insı	82	insı	insu	79	78	
		4	ω	11	17	26	1	ω	16	22	30	
Date		Sept.	Sept.	Sept. 1	Sept. 1	Sept. 2	Oct.	Oct.	0ct. 1	0ct. 2	Oct.	
Sampling	period	I	1	7	ო	4	വ	Q	7	ω	თ	

Gas composition analyses for surface collector S2

TABLE 7.

Sampling	Date			Gas Anal	ysis, per	cent	Total
period			Nitrogen	Methane	Oxygen	Carbon dioxide	percent
	Sept.	ω	7.2.	с. 		not tested	96
2	Sept.	11	26	76	trace	Ξ	102
ო	Sept.	17	23	73		Ξ	67
4	Sept.	26	24	68	4	=	96
വ	Oct.		15	82		Ξ	98
Q	Oct.	œ	27	73	1.5	=	101.5
7	Oct.	16	22	79	<del>~~~</del> f	Ξ	102
ω	Oct.	22	19	74	ى •	4.5	8
б	Oct.	30	32	67	2.5	7	101.5

Gas composition analyses for benthic collector

TABLE 8.

Sampling period	Date	Dissolved (mg/	d Oxygen 1 )	<u>Water Ten</u> ( <sup>C</sup>	perature 'C.)	рН
		Surface	Bottom	Surface	Bottom	
-	Sept. 4	1.5	2.3	15	15	7.2
1	Sept, 8	2.8	1.2	13	12	7.4
2	Sept. 11	4.4	0.1	16	14	7.2
3	Sept. 17	0.2	0	11	11	7.5
4	Sept. 26	0.9	0.9	15	15	7.3
5	Oct. 1	0	0.2	12	12	7.6
6	0ct. 8	1.6	1.5	7	7	7.8
7	Oct. 16	3.9	2.9	5	5	7.7
8	0ct. 22	3.1	2.8	10	10	7.8
9	0ct. 30	4.9	4.2	1	1	7.7

# TABLE 9.

Dissolved oxygen, water temperature and

pH values for number 2 cell

Sampling	Date	Ammo	nia(mg/l)	Nitr	ate(mg/1)
period		Adjacent	Outfall	Adjacent	Outfall
		to S1		to Si	
-	Sept. 4	20.8	-	.06	-
i	Sept. 8	20.0	-	.10	-
2	Sept. 11	21.6	-	.08	-
3	Sept. 17	21.6	-	.07	-
4	Sept. 26	24.6	-	.05	-
5	Oct. 1	24.8	22.5	trace	.05
6	0ct. 8	25.6	23.6	.05	.06
7	Oct. 16	31.2	28.4	.08	.08
8	0ct. 22	28.8	27.2	.08	.06
9	0ct. 30	26.4	22.4	.06	.06

### TABLE 10.

Ammonia and nitrate concentration adjacent to collector S1 and at outfall, Cell 2,

#### CHAPTER 4

#### DISCUSSION

#### 4.1. Gas Analysis Results

The percentage composition of the gas samples collected for each sample period are given in tables 6,7 and 8. It may be seen that the totals of nitrogen, methane and oxygen do not always add to 100 percent. This may be due to the presence of other gases or to randon errors in the apparatus or injection of the gas samples. A statistical analysis was performed to attempt to determine the random error of the apparatus and a 95% confidence interval of  $\frac{+}{-}$  1.88 percentage units was calculated. Therefore, any value of total gas composition between 98.12% and 101.88% can be considered to be within the random error of the apparatus and procedure. Details of this calculation are given in appendix A. Considering these limits first with respect to the results in table 6, only one result, October 30, is significantly below the lower confidence limit. However, the ratios of nitrogen, methane and oxygen in this sample are similar to the other samples. This low value is probably an error in use of the injection syringe. The totals in tables 7 and 8 are all within or close to the 95% confidence limits of the apparatus.

The levels of dissolved oxygen corresponding to

the percentages of oxygen in the gas samples from collector S1 were calculated using Henry's law constants from Perry.<sup>(54)</sup> The results, with the corresponding levels of dissolved oxygen measured in the water adjacent to S1 are presented in table 11. The predicted and measured values are shown in time line graphical form in figure 11. It may be seen that the predicted dissolved oxygen levels are, with one exception, higher than those determined by Winkler test. While there may be some corelation of predicted to measured dissolved oxygen values, figure 11 would indicate that the proportions of gases in the samples cannot be used to predict accurately corresponding levels of dissolved gases in the water below. This is understandable, considering that Henry's law applies only to equilibrium conditions.

With two exceptions, methane was not present in the surface samples or present only in small quantities. The exceptions were period 3 and period 5 when 12 and 30 percent methane were found in S2 and S1 respectively. It may be noted from tables 6 and 7 that in the former case, 4 percent oxygen was present and in the latter case, 2 percent. When samples were collected, it was not always possible to prevent some small air bubbles from becoming entrained in the sampling tubes when connecting the tubes to the gas collectors. Thus, these oxygen values may be due to atmospheric contamination. It may be noted from table 9 that on October 1, no dissolved

Date	Calculated dissolved oxygen concentration from O <sub>2</sub> in collector S1 (mg/l)	Measured dissolved oxygen concentration adjacent to collector S1 (mg/l)
Sept. 8	7.3	2.8
11	3.9	4.4
17	2.1	0.2
26	2.0	0.8
Oct. i	1.0	0
8	no sample	1.6
16	9.8	3.9
22	8.7	3.1
<b>3</b> 0 ····	12.8	4.9

TABLE 11.

Calculated versus measured dissolved oxygen levels

at collector Si





Calculated versus measured dissolved oxygen levels collector S1, September and October, 1986 77 oxygen was found at the surface and .2 mg/l at the bottom adjacent to S1. The pond was anaerobic on this occasion when 30 percent methane was collected. On the other occasion when the pond was anaerobic, September 17, 12 percent methane was collected in S2.

It may be noted from tables 6 and 7 that a note "insufficient sample" appears on four occasions. The volume of the gas sampling tube is 300 ml and when a gas sample collected was less than approximately one eighth of the tube or approximately 40 mls, it was considered too small for a reliable analysis due to the fact that atmospheric contamination could not be completely avoided in collecting the samples. Collector S2 showed an inadequate sample three times and S1 once. Possible reasons for this will be discussed in a following section.

The results of gas analysis for the benthic collector are given in table 8. It may be noted that an adequate sample volume was obtained in each case and that the percentage composition adds to within or close to the 95% confidence limits of the apparatus. The samples consisted predominantly of methane and nitrogen with small quantities of oxygen. As noted above, the oxygen may be due to atmospheric contamination. As explained in section 3.3, carbon dioxide was determined only for the samples from periods 8 and 9 but in these, was found to be a small percentage. Since the other samples all add to close to or within the confidence limits, it may

be assumed that carbon dioxide was present only in small percentages in the other samples. Thebenthic sample taken in period 1 may be seen to be quite different in composition to the other smaples. The evacuation of the benthic collector prior to collection of this sample cannot be relied upon and this sample may have been contaminated with air. For this reason, this result will not be considered further. As may be seen, the other results show a fairly consistent ratio of approximately 20 to 25 percent nitrogen and 75 to 80 percent methane. 4.2. Origin of the Nitrogen Gas Collected.

The important question with respect to these results is the origin of the gases collected. Methane is not an atmospheric gas and the methane collected may be assumed to have originated from biological activity. Nitrogen, however, may originate from dissolution of atmospheric nitrogen dissolved in the water. The use of isotopic nitrogen tracing or nitrogen-argon analysis, as described in section 2.5.2was beyond the scope of this project, however, an expression may be derived, using Henry's law, to estimate the amount of nitrogen gas which might be liberated from solution due to changes in water temperature or atmospheric pressure over a sampling period. Such an expression would be derived by combining Henry's law and the ideal gas law and would have the form shown in equation 21.

$$V = \left( \frac{(x_{m1} - x_{m2}) \times 55.56 \times .082 \times T}{P} \right) \times 10^3 \text{ ml /litre .. 21}$$

where: V = theoretical volume of gas liberated, in

ml of gas per liter of water.  $x_{mi}$  = initial mole fraction of gas in solution.  $x_{m2}$  = final mole fraction of gas in solution. T = gas collection temperature, <sup>O</sup>K. P = collection pressure, atmospheres.

Derivation of equation 21 is given in detail in appendix B. Equation 21 was used to calculate the theoretical volumes of air expected to be liberated due to increases in temperature shown in table 5 and, as may be seem, the calculated values consistently gave a result approximately 10 percent above the measured values in 3 trials. The 10 percent difference is probably due to the difference between actual and theoretical saturation values as well as inability to collect all of the evolved air. However, the results in table 5 show that equation 21 may be used to obtain an estimate of the maximum amount of gas which would theoretically be evolved from a water sample due to changes in temperature and pressure.

Maximum and minimum daily air temperatures and barometric pressures for each day of September and October, 1986 were obtained from Environment Canada (55) and the maximum and minimum pressures, converted to standard atmospheres, were determined for each sampling period. These were used in equation 21, along with initial and final water temperatures for the sampling periods, to calculate the maximum theoretical volume of nitrogen which could be evolved due to changes in pressure and temperature during each sampling period. Where the water temperature at the end of the sampling period was higher than at the beginning, the difference was accounted for by using Henry's law constants for the initial and final temperatures. (54) For a period over which the pond tem-

perature fell from beginning to end, the lower temperature was used for both calculations on the assumption that gas movement inside the collectors was essentially a one way process.<sup>(56)</sup> Volume of the collected samples was calculated using the water temperature and atmospheric pressure on the day the sample was collected. The vapor pressure of water, at the temperature in question, was subtracted from total pressure in each calculation, as noted by Crockford.<sup>(26)</sup> The theoretical volumes of nitrogen calculated, as ml per litre, were then multiplied by the volume of the water column under each collector to obtain a total volume of nitrogen gas which theoretically could have been evolved due to changes in pressure and temperature over each sampling period. These theoretical maximum volumes are given in column IV of tables 12, 13 and 14. An example of the calculation is given in detail in appendix B. The actual volumes of nitrogen collected are given in column III of tables 12, 13 and 14.

It may be seen from tables 12, 13 and 14 that the volume of nitrogen collected was always larger than the theoretical volume. It should be emphasized that the theoretical volumes given in the tables are the maximum amount which could be evolved, not necessarily the amount which was evolved in any sample period. The results given in table 5 indicate that this calculation tends to overestimate the actual gas produced by approximately

	Ι	ΙI	ΙΙΙ	ΙV
Period	Total gas	Proportion of	Volume of	Theoretical
	collected	Nitrogen	Nitrogen	volume of
	(ml)	(percent)	(m])	Nitrogen(ml)
1	100	80	80	27
2	200	85	170	130
3	200	95	190	105
4	290	92	267	179
5	100	68	68	12
6	(insuffic	ient sample volu	me)	
7	113	84	95	42
8	290+	82	238+	200
9	150	63	95	40

### TABLE 12.

Theoretical and actual volumes of nitrogen gas collected in collector S1 in each period

	Ι	II	III	ΙV
Period	Total gas	Proportion	of Volume of	Theoretical
	collected	Nitrogen	Nitrogen	volume of
	( ml )	(percent)	(m1)	Nitrogen(ml)
1	75	80	60	22
_				
2	150	83	125	106
3	100	83	83	27
				27
4	(insuff <b>i</b> c	cient sample	volume)	
۲,	75	0.2	6.2	11.0
	<b>73</b> .	02	02	11.0
6	(insuffic	ient sample	volume)	
7		-		
/	(insuffic	ient sample	volume)	
8	225	79	178	162
	, ,		1,0	102
9	100	78	78	40
		1	and the second	

TABLE 13.

Theoretical and actual volumes of nitrogen gas collected in collector S2 in each period

	Ι	ΙI	III	ΙV
Period	Total gas	Proportion of	Volume of	Theoretical
	collected	Nitrogen	Nitrogen	volume of
	(ml)	(percent)	(m])	Nitrogen(ml)
i	- ;	-	-	-
2	75	26	20	1.7
3	200	23	45	.59
4	290+	24	70+	3.2
5	200	15	30	.24
6	150	27	4 1	.62
7	100	22	22	.80
8	150	19	29	3.3
9	100	32	32	1.4

## TABLE 14.

Theoretical and actual volumes of nitrogen gas collected in benthic collector, Bo, in each period

10 percent. Due to the large water column under each surface collector, the theoretical volumes shown in tables 12 and 13 were large, although in every case the volume of nitrogen collected was larger than the theoretical volume.

One possibility which may be considered for this variation is solar heating of the surface layer of the pond, thereby causing larger volumes of nitrogen to be released. This possibility cannot be discounted entirely since a continuous record of the surface water temperature is not available. However, the possibility may be examined by observing from table 9 that the surface and bottom temperatures recorded for each sample collected did not vary by more than 2 degrees and for 8 out of the 10 periods, the temperatures were identical. A graph of the maximum and minimum daily temperatures and measured water temperatures is given in figure 12. It may be seen that water temperature approximately follows the air temperature. It appears unlikely that significant changes in surface water temperature could have gone undetected over the entire test period, so any error due to warming of surface water was probably minimal. Another possibility for error is stripping of atmospheric nitrogen by methane as described in section 2.5.5. However, as noted in section 4.1, on only two occasions were significant quantities of methane detected in the surface samplers so stripping by methane would not appear to be an important factor.



Without data on initial concentrations of dissolved nitrogen gas in the raw influent, these possibilities cannot be discussed further.

Table 14 gives the theoretical volumes of nitrogen which could have been evolved in the benthic collector due to changes in temperature as calculated by equation 21. Ιt may be seen that these values are small compared to the actual volumes of nitrogen gas collected. As noted in section 2.5.5., Kuznetsov<sup>(41)</sup> and Chen<sup>(42)</sup> reported sediment gas compositions similar to those found in this study. Chen<sup>(42)</sup>, using a collection apparatus similar to that used here, obtained results similar to those obtained here, as may be seen by comparing tables 2 and 8. As noted, Chen proved that the nitrogen gas he collected was produced from denitrification. Given the results from these researchers, plus the point as described above, that the theoretical volumes of nitrogen gas evolved due to temperature and pressure changes in the benthic collector were small compared to the total volumes collected, it would seem reasonable to make a tentative conclusion that some of the nitrogen gas collected in this study was of biological origin. A more definitive conclusion with respect to that portion of the nitrogen which resulted from denitrification was beyond the scope of this investigation.

### 4.3. Rate of Nitrogen Production

Estimates of the rate of nitrogen gas production on an area and time basis may be obtained from the results of these tests by dividing the sample volume by the number of days in the test period and by the bottom area covered by the collector. The results of this calculation for the benthic collector are given in table 15. The theoretical nitrogen contribution was not subtracted from the total nitrogen collected in this calculation due to its small size relative to the size of the sample. A similar calculation was performed using the results from the surface collectors but 90 percent of the theoretical nitrogen evolved was subtracted from the actual nitrogen collected before the unit nitrogen production was calculated. This was done in an attempt to correct for the significant size of the theoretical nitrogen quantities. The unit nitrogen gas production figures for the surface collectors are given in tables 16 and 17. Gas volumes, after subtraction of theoretical volumes, were corrected to  $0^{\circ}$ C. Unit gas production rates are plotted on a time line graph of the test periods in figure 13a. The pond water temperature is plotted over the test period in figure 13b. The average water temperature over each test period is plotted as well as the beginning and ending temperatures. It may be seen from figure 13a that the unit nitrogen collection rates were more eratic for the surface collectors than the benthic collector but that a pattern is discernable

Period	Tem	peratu	re e	Nitrogen volume	Davs in period	llnit nitroden
	start	end a (°C.)	verage	collected ml corrected to 0 <sup>0</sup> C.		ml $N_2/m^2$ of bottom area
-	ł	ı	ł	I	·	I
2	12	14	13	67	٣	92
ო	14	11	13	43	7	87
4	+1 +(	15	13	67	σ	106
വ	15	12	14	29	വ	82
9	12	7	10	40	7	81
7	7	ъ	Q	22	ω	40
ω	ъ	10	7	28	Q	65
6	10	<b>*</b> 4	9	31	ω	56

Period temperatures and unit nitrogen gas production per period forbenthic collector

TABLE 15.

eriod	Теш	perat	ure	Nitrogen	.9 times	V9T	davs in	Unit volume
	start	end (°C.	average )	collected ml (V <sub>n</sub> )	theoretical volume (.9T)	corrected to 0°C.	period	of nitrogen ml N <sub>2</sub> /m <sup>2</sup> of bottom area
+4	15	13	14	80	24	53	4	127
N	13	16	15	170	117	50	m	167
ო	16	11	13	190	95	06	7	127
4	***	15	13	267	161	101	თ	110
2	15	12	14	68	11	54	വ	110
9	12	7	10	0	ı	0	7	0
7	7	വ	9	95	38	56	7	68
ω	വ	10	7	238	180	56	Q	88
б	10		9	95	36	20	ω	71

Period temperatures and unit nitrogen gas production per period for collector S1

TABLE 16.

ď.

Period	Temperature <sup>O</sup> C.(from S1) average	Nitrogen collected ml (V <sub>n</sub> )	.9 times theoretical volume (.9T)	V <sub>n</sub> 9T corrected to 0 <sup>0</sup> C.	Days in period	Unit volume of nitrogen ml N <sub>2</sub> /m <sup>2</sup> of bottom area
+1	14	60	20	38	4	93
2	15	125		28	m	86
т	13	83	24	56	7	78
4	13	0	ı	0	סז	0
പ	14	62	10.4	50	ъ	98
Q	10	0	ı	0	7	0
7	9	0	ı	0	ω	0
ω	7	178	146	31	ى	4 9
თ	Q	78	36	41	IJ	49
			TABLE 17.			

Period temperatures and unit nitrogen gas production per period for collector S2

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FIGURE 13.

Unit nitrogen gas production and temperature versus time

for the three collectors. The unit nitrogen production rate, particularily for the benthic collector, tends to follow the average water temperature profile. The unit nitrogen production rates and corresponding average period temperatures for the three collectors are summarized in table 18 and plotted in figure 14. A least squares calculation to fit a line to these data points gave a y intercept of 1.5, a slope of 6.85 and a corelation coefficient of .56. Using the equation of the least sugares fitted line for this data, a relationship between temperature and nitrogen production can be expressed in the form shown in equation 22.

where: U = unit nitrogen gas production rate, mls per square meter of bottom per day. T = water temperature  ${}^{O}C$ .

Twenty six data points,or degrees of freedom, were available to construct the line in figure 14. Fround<sup>(57)</sup> states that a corelation coefficient of .423 or higher is required for a statistically valid relationship to exist with 26 degrees of freedom. Therefore, a statistical relationship can be seen to exist between temperature and nitrogen gas production rates in these tests.

As described in section 2.5.7, Kaplan<sup>(49)</sup> compared

Benthic collector average water unit nitrogen temp.( $^{O}$ C.) production (ml $N_{2}/m^{2}/d$ )	1	13 92	13 87	13 106	14 82	10 81	6 40	7 65	56
tor S2 unit nitrogen production ( ml N <sub>2</sub> /m <sup>2</sup> /d )	6 3	98	78	0	86	0	0	49	49
Collect average water temp.( <sup>o</sup> C.)	14	15	13	13	14	10	Q	7	Q
or S1 unit nitrogen production [m] N <sub>2</sub> /m <sup>2</sup> /d )	127	167	127	110	110	0	68	88	71
Collect verage water temp.( <sup>o</sup> C.)	14	15	13	13	14	10	, 0 0 1 0		
Period	<del></del> 1	2	т	4	ഗ 5	Q	7	ω	თ

temperature and unit nitrogen gas production for the three collectors Water

**Weeks** 

TABLE 18.



### FIGURE 14.

Unit nitrogen gas production versus average pond water temperature

sediment gas production to temperature and prepared a curve of his findings, reproduced in figure 5. It may be seen by comparing this and figure 14 that a similar relationship was found in the series of tests described herein. Exact comparison cannot be made since Kaplan's data is based on nitrogen production per unit volume of sediment while figure 14 is based upon production per unit area of bottom, but the shape of the curves is significant. In both cases, a straight line relationship with a small ordinate was developed. A greater statistical corelation was found by Kaplan but his experiments were performed under laboratory conditions where greater control of variables is possible.

As noted previously, figure 13a illustrates that nitrogen production fell essentially to zero in the surface collectors over period 6 and that S1 recovered in period 7 but S2 did not resume production until period 8. Collector S2 also recorded no production in period 4. It may be seen from figure 13b that the average pond temperature fell over periods 6 and 7 from a range of between 11 and 15  $^{\circ}$ C. to a range of between 5 and 7 $^{\circ}$ C. As described in section 2.5.7, Kaplan<sup>(49)</sup> observed a temperature selection between psychrophylic and mesophylic denitrifyers with approximately 10 $^{\circ}$ C. being the cross over temperature. This offers a possible explanation for the loss of gas production in the surface collectors in periods 6 and 7. The drop in range of average water temperature corresponds
closely to Kaplan's findings. If this were true, mesophylic denitrifiers were operating during periods 1 through 5 and the drop in water temperature over periods 6 and 7 inhibited these organisms. By period 7, psychrophylic denitrifiers had started to operate and nitrogen gas production resumed. This leaves the question of why the benthic collector, while it showed a drop in nitrogen production over periods 6 and 7, did not suffer a complete cessation of gas production. The equation proposed by Rich $^{(18)}$ . discussed in section 2.4.2., may be used to estimate the sediment temperature. Using this equation, the estimated sediment temperatures for September and October can be calculated as  $12.1^{\circ}$ C. and  $10.1^{\circ}$ C. respectively. Presumably the more constant temperature is due to heat liberated by microbial activity in the sediment. It may be noted that the sediment temperatures estimated by this method do not vary as widely as the water temperature and remain above the 10 to 12°C. cross over temperature noted previously. Thus, it is possible that the benthic collector provided some insulation against changes in water temperature and allowed mesophylic denitrifiers to continue producing gas despite changes in water temperature. This theory must be proposed with caution but it does provide a plausible explanation of the observations. The drop in nitrogen production at S2 in period 4 may have been due to a more pronounced change in temperature at this location due to

the shallowness of the water. Temperature readings were not taken at collector S2, so this postulate can only be given without further comment.

Rates of denitrification from other researchers, expressed as mgs N per square meter of sediment per day. are summarized in table 3. Nitrogen gas as mls. can be converted to mgs N by noting that at O<sup>O</sup>C. and 1 atmosphere pressure, 1 ml of nitrogen gas contains  $4.47 \times 10^{-5}$ moles of nitrogen. This is equivalent to 1.25 mgs of nitrogen or .63 mgs of monomolecular nitrogen or N. Taking a nitrogen gas production rate of 70 mls per square meter per day of nitrogen gas at 10°C. from figure 14, this calculation yields a nitrogen production rate of 44 mgs N per square meter per day. The calculations to arrive at this figure are given in appendix C. It may be noted from table 3 that 44 mgs N per square meter per day is higher than that reported by the other workers. Comparison is difficult however, since these researchers used lake bottom or estuarine sediments which may not be comparable to waste stabilization pond sediments in terms of organic matter or bacterial population. Even among the research summarized, a considerable variation in recorded denitrification rates was reported.

The result reported by Sorenson<sup>(43)</sup> of 13.8 mgs N per square meter per day at  $2.5^{\circ}$ C. is notable. The nitrogen gas production rate at this temperature from figure 14 is 19 mgs nitrogen gas per square meter per day. Corrected</sup>

to 1 atmosphere pressure and converted to mgs N per square meter per day, this equals 11.7 mgs N per square meter per day, which is close to Sorenson's figure. A comparison such as this must be made with caution due to the limited information about the sites and sampling conditions, but it is noteworthy. As noted in section 2.5.1, no reports could be found in readily available literature of denitrification rates in waste water pond sediments. Therefore, beyond the foregoing comments, further comparison of the rates given in figures 13 and 14 will have to await the results of further research.

# 4.4. Ammonia Nitrogen Balance

Influent ammonia nitrogen levels for the SEWPCC were obtained for September and October, 1986.<sup>(51)</sup> Ammonia levels were measured at collector S1 for each sampling period and pond effluent ammonia levels were measured for sampling periods 5 through 9. Results from S1 and the effluent tests are presented in table 10 and these, along with the influent values, are illustrated in figure 15.

Figure 15 illustrates that a net removal of ammonia occured in the pond during October. The average influent ammonia level was 27.9 mg/l and the average effluent level was 24.8 mg/l, giving an average reduction of 3.1 mg/l. The average flow rate through the pond during October was 7.6 million litres per day.<sup>(51)</sup> This flow gives an average mass loss of 23.6 Kgs of ammonia per day. The average pond water temperature during October was  $8.5^{\circ}$ C. From figure 14, this corresponds to a unit nitrogen gas production rate of 60 mls nitrogen gas per square meter per day. The area of pond cell number 2 is approximately 170,000 square meters. If a uniform nitrogen gas production rate is assumed, this gives 9,935 litres of nitrogen gas at STP, per day. This is stoichiometrically equivilant to 15.09 Kgs of ammonia lost per day. As noted in section 2.4.3, Pano<sup>(25)</sup> and Ferrara<sup>(28)</sup> list loss to sediment, uptake of ammonia and nitrate and nitrification and denitrification as the potential



processes for loss of ammonia in ponds. Equation 17 (Pano's equation) may be used to obtain an estimate of the ammonia lost to volatilization during October since the influent ammonia level, temperature and pH are known. This equation gives an expected effluent ammonia concentration of 26.5 mg/l of ammonia. Expected ammonia loss due to volatilization is thus 10.64 Kgs per day for a total loss due to volatilization and nitrification and denitrification of 25.73 Kgs of ammonia per day. This is more that the 23.6 Kgs daily mass loss calculated from influent and effluent ammonia levels. However, these calculations are based on limited data and a number of factors could exert an influence upon the availability of ammonia in the pond. The pond influent contained approximately 9 mg/l of organic nitrogen (51) which would furnish a ready source of additional ammonia in the pond. Considerable quantities of nitrogen in all forms might be accumulated in the pond sediments and these could be mobilized at a rate which cannot be accounted for in the above calculations. The assumption of a uniform and constant rate of nitrogen gas production over the entire pond is open to question and, as may be noted from figure 15, the influent and effluent ammonia levels in the pond are not uniform over the month of October. Calculations performed with averages from such fluctuating data give results which must be interpreted with caution.

It is nevertheless interesting to observe that even

at the relatively low temperature and pH levels over October, volatilization accounted for approximately half the ammonia lost. This is in contrast to the work of Ferrara<sup>(28)</sup>who, as noted in section 2.4.3, asserted that volatilization played virtually no part in ammonia loss from ponds. Attempts to obtain a balance of ammonia in a waste stabilization pond would be complicated by the fact that organisms in the pond excrete as well as utilize ammonia. It was noted in section 2.5 that <u>Clostridia</u> excrete large amounts of ammonia. There are probably other organisms with similar metabolism.

Tests performed by Ismirimah<sup>(38)</sup> indicated that lake sediments released ammonia at the rate of approximately 6.7 mgs ammonia N per square meter of bottom per day. A release rate such as this would account for the excess ammonia in the above calculations.

Without more quantitive information as to the contribution of these factors to the ammonia levels in the pond, a calculation such as the one presented above can only be used as a first estimate of the gain and loss of the compound in the pond.

## 4.5. Results of pH Determination

The pH of the pond was monitored in each sampling period and the results are shown in table 9. The pH of the pond rose over the test period but the total rise of .6 pH units was too small to allow any corelation to be made between pH and nitrogen gas production. The influent pH fluctuated between 7.3 and  $7.7^{(51)}$  and the maximum difference in pH between the pond and influent values during the entire test period was .5 pH units.

#### 4.6. Nitrate and Denitrification

Nitrate levels were determined in the pond water adjacent to collector S1 over the entire test period and in the effluent for periods 5 through 9. These are given in table 10. Influent nitrate levels did not exceed .04 mg/l at any time during the 2 month test period.<sup>(51)</sup> A nitrate test was run on City tap water, using the same apparatus and reagents used for the pond tests. No observable variation occured in the base line of the plotting paper from the autoanalyzer. From this, it may be assumed that the background level of nitrate in City water was too low to effect these readings.

A test was performed to determine statistical parameters for the autoanalyzer. Ten replicates of .025 mg/l nitrate standard, all from the same standard preparation, were run consecutively using the same reagents and without adjusting the apparatus. The variation between any of the 10 peaks obtained was less than the thickness of the pen trace. From this it may be concluded that any errors due to apparatus in this determination are too small to be accurately measured. Average nitrate levels for October, in the pond and in the effluent were .06 mg/l. The low nitrate readings, along with the relatively high levels of ammonia, might indicate that nitrification was either not occuring or occuring very slowly. This would be in keeping with considerations discussed in section 2.4.2 that limited nitrification occurs in facultative

ponds. However, it is also possible that nitrification was occuring but the nitrate formed was lost almost as rapidly as it was formed. The work of several of the researchers discussed in section 2.5.4 indicates that nitrate disappears rapidly from sediments under anoxic conditions and that nitrate accumulation does not occur. Another possibility which can only be put forward as an hypothesis, is that the nitrification reaction is not allowed to proceed to completion. As shown in equations 3 and 5, nitrite is the intermediate product of both the nitrification and denitrification reactions. The obvious question is whether the denitrifying bacteria use the nitrite produced by Nitrosomonas directly and the reduction to nitrate and subsequent oxidation back to nitrite is bypassed. Without additional evidence, this question can only be put forward without further discussion.

## 4.7. Methane and Loss of Carbon

The quantities of methane gas collected during these tests are worthy of further comment. As described above, methane was found in significant quantities in the benthic collector in all test periods but was only found in any quantity in the surface collectors when the pond was anaerobic. Several researchers have asserted that the escape of methane gas from benthic sediments is a pathway by which carbon may be removed from ponds without incurring oxygen demand. (18,42) However, as described in section 2.5, Reeburgh<sup>(31)</sup> found no methane in water over sediments and a similar situation would appear to have occured here. It was also noted that methane can be used as an energy source under aerobic conditions. These findings would indicate that methane formed in benthic sediments does not escape readily to the atmosphere and may not provide as large a carbon sink as is believed.

### 4.8. Nitrogen Transformations

Alternating periods of aerobic and anoxic conditions are the basis of advanced wastewater treatment processes for waste water treatment and nutrient removal. (10) In these processes, a period of aerobic growth of nitrifiers for conversion of ammonia to nitrate is followed by an anoxic period for denitrification of nitrates to nitrogen gas. In engineered treatment processes, a carbon source usually is required to provide carbon for the heterotrophic denitrifiers. Methanol is often used for this source, although considerable research has been devoted to finding alternate carbon sources. (1,10,15,16,17)

In section 2.5.4, it was noted that facultative ponds undergo alternating aerobic and anaerobic periods on a diurnal basis<sup>(18)</sup> and due to windy and calm conditions.<sup>(38)</sup> It can hardly be expected that organic carbon would be limited in a waste stabilization pond receiving domestic sewage. Thus, it is possible that a form, while crude and uncontrolled, of the aerobic/anoxic nutrient removal process is operating in facultative stabilization ponds.

#### CHAPTER 5

#### CONCLUSIONS

The following conclusions may be drawn from the results of the tests and analysis performed in this study.

Nitrogen gas was being produced in the pond during the study period. The large excess of nitrogen found over theoretical quantities released due to temperature and pressure changes is evidence that nitrogen was being evolved from another source.

Test results from this study were similar in terms of gas composition and gas production - temperature relationships to the results from other researchers who have proven that denitrification was occuring. On the basis of this plus the excess nitrogen found here, a tentative conclusion may be drawn that biological denitrification was the source of at least some of the excess nitrogen gas collected.

The fact that the volumes of nitrogen gas collected in the surface and benthic collectors were within the same order of magnitude on a unit area basis and not on a volume of water under the collector basis would indicate that the nitrogen gas collected is related to bottom area and not to water volume. Therefore, the benthic sediment appears to be the source of the excess nitrogen collected.

The production of nitrogen gas in the pond appears to be related to average water temperature by the relationship:

U = 6.85T + 1.5 ..... 22

where: U = unit nitrogen gas production, ml  $N_2/m^2/day$ T = temperature <sup>o</sup>C.

The changes in other parameters monitored over the test period: pH, ammonia and nitrate were too small to allow any conclusions as to the effect of these factors upon nitrogen production.

The collection of methane in thebenthic collector proved that anaerobic bacterial action was occuring in the bottom sediment. The fact that methane was only collected in the surface collectors under anaerobic conditions would indicate that under aerobic conditions, large quantities of the gas do not reach the surface and escape to the atmosphere. This, together with a similar finding by another researcher would indicate that the theory that methane formation is a major carbon sink in stabilization ponds should be reexamined.

The results of this study provide, while not absolute proof, at least strong evidence that denitrification was occuring in the pond studied. They also provide a relation which may be used, with caution, to predict the rate at which the reaction occurs dependent upon

temperature. It was noted in the literature review that information as to this process and its dynamics in ponds has not been readily available heretofore. If, by assembling this information, this study has provided another step toward a greater inderstanding of a system for protection of our environment, it will have been worth the time and effort expended.

### CHAPTER 6

# RECOMMENDATIONS FOR FURTHER WORK

The results of the work described in this report raise a number of points which could benefit from further research. A brief summary of these is as follows.

This work strongly indicates that biogenic nitrogen gas is produced in facultative ponds but there is still some question as to the proportions of biogenic and atmospheric nitrogen in the samples collected. The best estimate possible under the circumstances was made in these tests but decisive proof of the origin of the nitrogen was not possible. Further studies, using such techniques as isotopic nitrogen tracing, nitrogen-argon analysis or acetylene inhibition to seperate and quantify the origin of the nitrogen gas would help to resolve this question and provide more accurate data upon which to quantify the process.

It is clear from both the literature review and the test results that our knowledge of the nitrogen cycle in waste stabilization ponds is not complete. Further work is needed to elucidate the mechanisms of transformation of all forms of nitrogen, particularly the inorganic forms, ammonia, nitrite and other intermediates. The mechanism of nitrification in ponds needs more study, in particular the rate and conditions under which it

does or does not occur.

Further work on the dynamics of sediments in ponds would be helpful. Characterization of stabilization pond sediments and lake, river and marine sediments would allow a more meaningful comparison of the results of tests such as those described herein and the work of other researchers in other disciplines.

The mixing of facultative ponds and their benthic sediments due to stratification and wave turbulance requires more study. It appears from the available information that these forces play a significant role in nutrient cycling in natural waters and a better understanding of these phenomena in stabilization ponds would help to solve a problem which, as noted, has impeded the development of reliable design procedures for these ponds.

Further work on the evolution of other gases is needed. Information on the origin and fate of methane would be particularly helpful, in view of the fact that it is presently used as a parameter in the design of stabilization ponds. Research to quantify the rate of production and, equally important, the rate of loss to the atmosphere of methane, would be a significant contribution to our understanding of the stabilization process in facultative ponds.

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## APPENDIX A.

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Calculation of 95% confidence limits for sum of gas analysis in gas chromatograph.

Ten samples were injected consecutively from a sampling tube filled with nitrogen and methane from laboratory gas cylinders. Results as follows:

Injection	Analy	Analysis %		
	Nitrogen	Methane	total	
1	52	4 5	97	
2	52	45	97	
3	52	45	97	
4	53	45	98	
5	56	48	104	
6	51	44	95	
7	54	46	100	
8 .	54	46	100	
9	55	46	101	
10	53	44	97	
			$\bar{x} = 98.6$	
			s = 2.63	

Test of whether this approximates a normal distribution. Plot on normal probability graph paper.

A 1

Range	no. range	total n	umber accumulated
		of tri	als percent
1	94	0	0
2	96	1	10
3	98	5	50
4	100	6	60
5	102	8	80
6	104	9	90
7	106	10	100

A plot of ranges 2 through 7 is shown in figure A1. An approximate straight line can be drawn, with the 50 percentile point passing through 98.6. For a population of 10 samples, use a Student distribution. (Fround, p.204)<sup>\*</sup>

 $u = \overline{x} \pm t.025 \frac{S}{\sqrt{n}}$  where n = 10

for n = 10 - 1 = 9 degrees of freedom,  $t_{.025} = 2.262$ (Fround, p.306)

$$t.025 \frac{S}{\sqrt{n}} = 2.262 \frac{2.63}{\sqrt{10}}$$
  
= 1.88

So, 1.88 percentage points is the 95% confidence limit for the apparatus and procedure.

\* Fround, J.E. <u>Statistics, A First Course</u> Prentice Hall, 1970



21 4045 13

A 3

## APPENDIX B.

Derivation of an expression to estimate the volume of gas to come out of solution due to changes in temperature and pressure.

1.) Henry's Law:

P\_q = H x\_m
where: P\_q = partial pressure of gas, atmospheres
H = Henry's law constant
x\_m = equilibrium mole fraction of gas in
solution

Rearanging:

 $x_m = \frac{P_q}{H}$ 

Partial pressure of a gas in a mixture is equal to the total pressure times the percent composition of that gas: Dalton's law. (Sawyer, p.21)<sup>(1)</sup>A change in total pressure, assuming the percentage composition of the gas mixture does not change, therefore, brings about a proportinal change in partial pressure.

Henry's law constant, H, is dependent upon temperature. Tables of Henry's law constants are available. (Perry)<sup>(2)</sup>

Thus, for a change in pressure and temperature:

 $x_{m1} = \frac{P_{q1}}{H_{T1}}$ 

and:

B 1

$$x_{m2} = P_{q2}$$

H<sub>T2</sub>

where: x<sub>m1</sub> = initial mole fraction of dissolved gas.

- $x_{m2}$  = final mole fraction of dissolved gas.  $P_{q1}$  = initial partial pressure or % of gas
  - times initial total pressure.
- P<sub>q2</sub> = final partial pressure or % of gas times final total pressure.

The difference in initial and final equilibrium mole fractions in solution would be:

 $n = x_{m1} - x_{m2}$ 

where: n = change in moles of gas due to changes in temperature and pressure.

One litre of water contains;  $\frac{1000}{18}$  = 55.56 g-moles. So:  $(x_{m1} - x_{m2})$  55.56 = difference, in moles per litre, of gas in solution due to changes in pressure and temperature. (Metcalf & Eddy p.275)<sup>(3)</sup>

The ideal gas law:

PV = nRT

where: P = pressure in atmospheres.

V = volume of gas, in litres. n = number of moles of gas. R = .082 L-atm./mol-<sup>O</sup>K. T = temperature, <sup>O</sup>K. (<sup>O</sup>C. + 273)

Rearranging:

$$V = \underline{nRT}$$

substituting n for change in number of moles of gas per litre (above):

$$V_1 = ((x_{m1} - x_{m2}) 55.56) .082 T$$

where:  $V_1$  = volume of gas released due to changes

in temperature and pressure, litres.

Multiply by  $10^3$  to convert litres to milliliters.  $V = \left(\frac{(x_{m1} - x_{m2})}{P}\right) \times 10^3 \text{ mls/litre} \dots 21$ 

Equation 21 appears on page 80.

An example of a calculation using Equation 19. For period 2 (Sept. 8-11), collector S1. Proportion of nitrogen: 85% (table 6.) Initial temperature:  $13^{\circ}C$ . (table 9.) final temperature:  $16^{\circ}$ C. Maximum pressure during period: .975 atm.<sup>(4)</sup> Minimum pressure during period: .966 atm. At  $13^{\circ}C.$ , Vapor pressure of water  $V_{pH_20} = .014$  atm. At 16°C., Vapor pressure of water  $V_{pH_20} = .019$  atm. At 13<sup>0</sup>C., Henry's law constant for Nitrogen, H<sup>N</sup>2 is: 69,900 atm/mole fraction.<sup>(2)</sup> At  $16^{\circ}C$ .  $H_{16}^{N_2} = 75,120$  atm/mole fraction So: for Nitrogen: P<sub>q1</sub> = .85 (.975 - .014) = .817 atm.  $P_{q2} = .85 (.966 - .019) = .805 atm.$  $x_{m2} = \frac{.805}{.75100} = 1.072 \times 10^{-5}$  moles  $x_{m1} - x_{m2} = 1.022 \times 10^{-6}$  moles

Average pressure on Sept. 11: .968 atm.<sup>(4)</sup> Assume gas saturated when collected.

> So: collection pressure: .968 - .019 = .949 atm. temperature:  $16^{\circ}C. = 289^{\circ}$  K.

So: nitrogen gas off is:

 $V = \frac{1.022 \times 10^{-6} \times 55.56 \times .082 \times 289 \times 10^{3}}{.949}$ = 1.418 mls./liter of nitrogen The area under the collector S1 is .1018 m.<sup>2</sup> The water depth is approximately .9 m. So: volume of water column is : .9 x .1018 = .0916 m.<sup>3</sup> ,0916 m.<sup>3</sup> = 91.6 litres, round to 92 litres. So: theoretical volume of gas off is: 92 x 1.418 = 130.46 mls., round to 130 mls. of nitrogen The value 130 mls. appears in row 2, column IV of

table 12. (page 81)

References:

- 1 Sawyer C.N. & McCarty P.L. <u>Chemistry for Environmental</u> <u>Engineering</u>, Third Edition McGraw Hill 1978
- 2 Perry J.H. <u>Chemical Engineers Handbook</u>, 4th Edition McGraw Hill 1963
- 3 Metcalf & Eddy Inc. Wastewater Engineering: Treatment, Disposal, Reuse, 2nd Edition McGraw Hill 1979
- 4 Winnipeg Meteorological Monthy Summaries, Sept. and Oct. 1986, Environment Canada, Atmospheric Environment Service

# APPENDIX C.

Conversion of nitrogen gas to monomolecular nitrogen. 1 ml. N $_2$  (or any gas) at 273 $^{\rm O}$ K. and 1 atm. pressure: PV = nRTn = PVRT = 1 x .001 .082 x 273  $= 4.467 \times 10^{-5}$  moles 1 mole of  $N_2 = 28$  grams. so: 1 ml.  $N_2 = 4.467 \times 10^{-5} \times 28 = 1.251 \times 10^{-3}$  grams or 1.25 milligrams N<sub>2</sub>. The molecular weight ratio of nitrogen gas  $(N_2)$ to monomolecular nitrogen is  $\underline{28}$  or .5, therefore, 1.25 mgs.  $\frac{1.25}{2}$  = .625 mgs. N equals For a nitrogen gas production rate of 70  $mls/m^2$ : 70 mls  $N_2 = 1.25 \times 70 = 87.5 \text{ mgs } N_2$ 87.5 mgs N<sub>2</sub> =  $\frac{87.5}{2}$  = 43.7 or 44 mgs N/m<sup>2</sup>/day