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THE EFFECT OF THERMAL DISCHARGE ON OXYGEN DEMANDS IN THE ASSINIBOINE RIVER

by

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The effects of the Brandon Generating Station's cooling water discharge into the Assiniboine River were investigated from January 10 through March 14 of 1975. The biological reaction rates of the carbonaceous and nitrogenous stages of deoxygenation at various river temperatures were evaluated and confirmed by a simulated laboratory study. The thermal discharge does not appear to have any adverse effects on biological reaction rates. The biochemical oxygen demand in the river during the winter months is low.

The simulation laboratory study concluded that if sufficient substrate (ammonia) is available, nitrification can account for a higher oxygen demand than the carbonaceous stages of deoxygenation. The simulation study also indicated that nitrification may occur at temperatures below $5^{o}C$.

CHAPTER I INTRODUCTION

1.1. Statement of Problem

Rivers and streams have been subjected to a role of accepting the waste of man's various activities since the beginning of civilization. These wastes include domestic sewage, industrial wastes of innumerable types and the most recent type of concern, thermal pollution. An article by Krenkel, Thackston and Parker (1) quotes Senator Muskie and the American Senate's subcommittee on air and water pollution as follows, "Excessive heat is as much a pollutant as municipal wastes or industrial wastes." Specialists[1-6] involved in environmental protection and pollution control agree with the senate subcommittee and predict increasing volumes of thermal discharge from the production of electric energy either by fossil fuel or nuclear power plants. Novotny and Krenkel (2) predict that by the year 2000 about nine times as much electric energy will be produced as in 1970. They further state that this will increase the volume of thermal discharge proportionately, resulting in increased receiving water temperatures which may have harmful effects on the ecosystems of the receiving streams.

Many articles[3,4,5,6] discuss the requirements to be met in order to preserve established aquatic ecosystems. A summary of these requirements is as follows:

a) Thermal effluents should not alter significantly the productive characteristics of indigenous organisms;

b) Temperatures should not exceed the maximum or minimum levels tolerated by indigenous organisms;

c) High temperature acclimation of aquatic organisms can cause low temperature stress. High temperature acclimation should not be permitted;

d) Seasonal cycles of reproduction and other activities should not be varied due to thermal discharges;

e) Species diversity should be maintained;

f) Temperature increases should not interfere with downstream drift of bacteria, insects or fish;

g) Temperature increases should not cause undue depletion of dissolved oxygen due to increased bacterial action.

The above requirements can be considered applicable to all rivers regardless of natural temperature patterns prior to the introduction of a thermal source. The Assiniboine River in Manitoba is one such stream since it receives cooling water from a fossil fuel electric generating station located at Brandon.

1.2. Study Objectives

The objective of this study was to determine the effects of thermal discharge on the reaction rate of the nitrogenous stage of the biochemical oxygen demand in the Assiniboine River. The study was a sequence to previous

studies done on the Assiniboine River to help determine why a fish kill occurred downstream of the Hydro plant's cooling water discharge. The report deals with winter conditions because the generating station operates only during peak consumption periods which occur during the winter. Consequently, the objective was achieved through the correlation of nitrogenous reaction rates, ammonia and nitrate concentrations and temperatures on samples collected during January, February and March of 1975.

CHAPTER II LITERATURE REVIEW

2.1. Introduction

The ecosystem of natural bodies of water is easily disturbed and modern society has in the past treated streams, rivers and lakes as collectors of wastes without concern for these ecosystems. Fortunately, a few far-visioned individuals considered streams as something special and worth protecting. Metcalfe and Eddy, Inc. (7) quote the late Earle E. Phelps as follows, "A stream is something more than a geographic feature, a line on a map, a part of the fixed permanent terrain. It cannot be adequately portrayed in terms of topography and geology. A stream is a living thing, a thing of energy, of movement, of change."

The responsibility of ecologists and environmental engineers is to ensure that the "change" is not a detrimental one due to the discharge of society's pollutants. Water is used widely throughout all industry including power generation. According to Gerald E. Arnold (5) approximately 80 per cent of all water withdrawn by industry in the U.S. is used for cooling. W.R. Donald (8) quotes a figure of 90 per cent. The ever increasing industrial growth along with the possible rapid growth of fossil fuel and nuclear generating plants could make thermal discharges the single most important pollutant to be dealt with in the next few decades.

2.2. Physical Effects of Thermal Discharge

The obvious physical effect of thermal discharge on a receiving stream is an increase in the water temperature of the stream. Donald (8) reports the occurrence of a definite temperature profile consisting of a peak immediately adjacent to the point of discharge, followed by a logarithmic shaped temperature decay curve. The decay curve is influenced by turbulence and atmospheric conditions. Velz and Gannon (9) discuss the effect of atmospheric conditions in some detail including convection loss, radiation loss, solar radiation gain and other meteorologic factors including air temperature, wind velocity and vapor pressure.

The two main factors influencing downstream temperature profiles is the heat content of the thermal discharge and volume of flow in the receiving stream. Velz and Gannon (9) state that the critical condition with respect to temperature rise of stream water from heat load occurs during drought stream flow conditions combined with meteorological conditions which are unfavourable to heat dissipation. In North America this combination usually occurs during the summer months on a calm clear day and the above mentioned article (9) shows several seasonal temperature patterns to support this supposition. These graphs adequately predict the critical temperature conditions that must be considered when planning the use of a stream for receipt of thermal discharge.

Pommen (10) mentions that literature on the effects of thermal discharges on cold water streams or streams under severe winter conditions is nearly non-existant. According to Pommen (10) other researchers have warned against extrapolating the results of investigations conducted in the United States to the Canadian scene. He further states that the effects of thermal discharges in Canada may not be as severe as those in the much studied milder climate of the United States.

2.2.1. Dissolved Oxygen

Many investigators including Velz (11) discuss the effect of temperature on the dissolved oxygen content of streams. Theoretically, a higher temperature decreases the solubility of oxygen in water (6), increases the rate of reaeration and the rate of biological deoxygenation causing a net decrease in dissolved oxygen content. Pommen (10) refers to several investigators who report that no decrease in dissolved oxygen concentration was found downstream from a thermal discharge. Donald (8) also concluded that little or no change in dissolved oxygen occurs as a result of the passage of cooling water through the condensers of stream generating stations.

Donald (8) and Pommen (10) both conclude that no adverse affects occur in levels of dissolved oxygen due to thermal discharge during winter periods.

2.3. Biological Effects of Thermal Pollution

2.3.1. Introduction

Thermal pollution can be defined as a substantial change in water temperature that adversely affects the water ecology and the resulting detrimental effect on the aquatic organisms which depend on the ecology (12). Aquatic life may show immediate adverse affects or the changes may be slow.

2.3.2. General Effect on Aquatic Life

Velz and Gannon (9) note that a moderate temperature rise may not be injurious but if the rise is beyond the tolerance range of any particular species that species may die or at the least decrease in numbers. Species are rarely eliminated en mass but as the temperature changes the more sensitive species are reduced in numbers. Cairns (6) states that most aquatic organisms can only withstand the effects of heat within narrow limits. This can cause interruption in migratory practises and reproduction schedules.

The more tolerant species will out-perform the more sensitive organisms and eventually cause a qualitative shift in the kinds of species present. This is particularly evident in algae where warmer water temperatures causes the more noxious blue-green algae to predominate (6). Bacteria are also affected by increasing water temperatures. Canadian rivers are usually in the 0-20^{o} Celsius

range (8,10). In this range increasing temperatures would result in increased activity as discussed in the following sections.

2.4. Thermal Effects on Carbonaceous Biochemical Oxygen Demand

2.4.1. Introduction

"The understanding of bio-oxidation kinetics is of fundamental importance for environmental engineers." This statement by Varma and Nepal (13) serves as a good opening statement for a study of temperature effects on biochemical oxygen demand, hereafter designated as BOD. Organic matter is assimilated by heterogeneous micro-organisms using molecular oxygen dissolved in water for their metabolic activities.

The basic monomolecular reactions which are normally associated with BOD are well documented throughout the literature and will only be discussed in general terms for the purpose of this paper. Monomolecular reaction rates are observed whenever the amount of a given reaction occurring in unit time is directly proportional to the amount of reactant present (13).

2.4.2. Classification of Bacteria According to Temperature

Busch (14) states that individual species of

bacteria do not grow over the entire temperature range. Each species has an optimum temperature for growth which usually occurs near the maximum temperature for that species. Busch (14) classifies bacteria as follows:

Bacteria having optimum growth rates below 20°C are psychrophilic, bacteria having optimum growth rates between 20°C and 50°C are mesophilic and bacteria having optimum growth rates above 50°C are thermophilic. McKinney (15) and Downing (16) agree generally with this classification of bacteria according to temperature. The psychrophilic bacteria are of the greatest importance in connection with stream pollution and thermal pollution in Canadian rivers. McKinney (15) mentions that the optimum temperature for the psychrophilic bacteria is near 20°C and that the rate of growth and metabolish are comparatively slow.

2.4.3. Kinetics of Carbonaceous BOD

A literature examination conducted by Gaudy, Bhatla, Follett and Abel-Niaaj (17) shows the concept most prevalently used is the first-order continually-decreasing rate type of kinetics which prevailed in studies done by early researchers such as Theriault. This concept only requires two constants to describe the BOD curve. They are: L, ultimate demand and k_1, the rate constant. Schroepfer, Robins and Susag, (18) reviewed the work of

Streeter and Phelps who stated that the k_1 reaction rate was constant at 0.1. The classic work of Streeter and Phelps has been recorded in nearly all articles and texts dealing with biological oxidation and stream pollution (11-18). Velz (11) quotes Phelps' generalized expression which covers the rational method of stream analysis as follows: "the rate of biochemical oxidation of organic matter is proportional to the remaining concentration of unoxidized substance, measured in terms of oxidizability." The derivation of the classic formulation of deoxygenation due to biological degradation is as follows:

Differentially: $\frac{-dL}{dt} = k_1 L$

which may be integrated: $\text{Log}_e \frac{L_t}{L} = -k_1 t$

or $L_t = L(10^{-k_1 t})$

where L and k_1 are as defined previously and L_t is the amount of oxidizable matter remaining at any time t.

Landine (19) explains the derivation of the most common form of the equation which is an equation expressing the oxygen already consumed. This equation is expressed as follows:

$$Y_t = L(1-10^{-k_1 t})$$

where Y_t represents BOD already exerted. The terms Y_t and t are determined experimentally. A plot of Log L_t versus t yields a straight line whose slope is $-k_1$.

2.4.3.1. Factors Complicating k_1 Determination

Landine (19) states that k_1 is normally associated with the standard five day BOD (BOD_5) test and actually represents an average of those five days. The reaction rate k_1 is dependent upon the nature of the substrate and the ability of the microorganisms to utilize it. Dissolved organic matter is more readily available than suspended matter and can cause high k_1 rates during the first few hours of degradation. Jank and Drynan (20) state that the only phase of bacterial growth with a constant growth rate occurs during the exponential growth rate which normally takes place the first 24 hours of incubation.

One other important factor which affects the reaction rate of bacterial oxidation, k_1, is temperature. The temperature factor was widely discussed in the literature and several textbooks including Metcalfe and Eddy (7) and Sawyer and McCarty (27). The Van't Hoff-Arrhennius rule serves as the basis of an equation which approximates the temperature effect. It is written as follows:

$$k_{1t} = k_{120}\ \theta^{(T-20^{o})}$$

where k_{1t} = reaction rate at any temperature T.

θ = a constant developed empirically.

The value of θ has been found to vary from 1.056 in the temperature range of 20^{o} to 30^{o}C to 1.135 in the temperature ranges of 4^{o} to 20^{o}C. The most quoted value in the literature is 1.047 but this value does not apply at cold temperatures.

Schroepfer, Robins and Susag (18) studied twelve hundred BOD determinations between 4°C and 30°C and concluded that the calculated ultimate demands for those samples whose deoxygenations are described by the monomolecular law were approximately constant. The variation of k_1 with temperature can be described by the Arrhenius relation using a value of $\theta = 1.05$ for converting from 20°C to 30°C and of 1.135 for converting from 20°C to 4°C.

2.4.4. Methods of Calculating k_1 and L

Marske and Polkowski (25) concluded that the first order model adequately describes BOD data over a k_1 range up to 0.20. There are several methods which can be used to calculate k_1 and L. All the methods require BOD data obtained following the procedures set down in Standard Methods for the Examination of Water and Wastewater (22), hereafter referred to as Standard Methods. The necessary BOD data is obtained and k_1 and L can be determined using one of the common methods outlined below.

(1) The Reed-Theriault method is discussed by Landine (19) and Carroll (23) as "The standard method of computing k_1 and L values". A trial value for k_1 is assumed and successive approximations are repeated until the trial value equals the calculated value. However, Ludzack, Moore and Ruchcroft (24) state that the necessary calculations involve a great deal of time. This view is shared by

Marske and Polkowski (25) who note that the researcher should have access to a digital computer.

(2) The Thomas slope method is the simplest of the least squares methods according to Landine (19). Thomas (26) who developed the method states that the results are in good agreement with those obtained by more elaborate least square methods. The procedure in determining the BOD constants consist of the following steps:(26)

(i) using the experimental data Y_t and t calculate the value of $(t/Y_t)^{1/3}$ for each day;

(ii) plot $(t/Y_t)^{1/3}$ versus t on arithmetic graph paper and draw a line of best fit;

(iii) from the plot measure the intercept A and the slope B;

(iv) k_1 and L can be calculated using the equations

$$k_1 = \frac{2.61B}{A}$$

and

$$L = \frac{1}{2.3k_1A^3}$$

The method employs the rate of change of Y_t in the equation $\frac{dY_t}{dt} = k_1(L-Y_t)$ and is illustrated graphically in Figure I. The Thomas method is fast and simple but some researchers (25) state that the Thomas method consistently underestimates the k_1 constant and consequently overestimates the ultimate BOD.

(3) A third method of estimating k_1 and L values was developed by Moore, Thomas and Snow (27) to eliminate the extensive computations involved in the previously developed

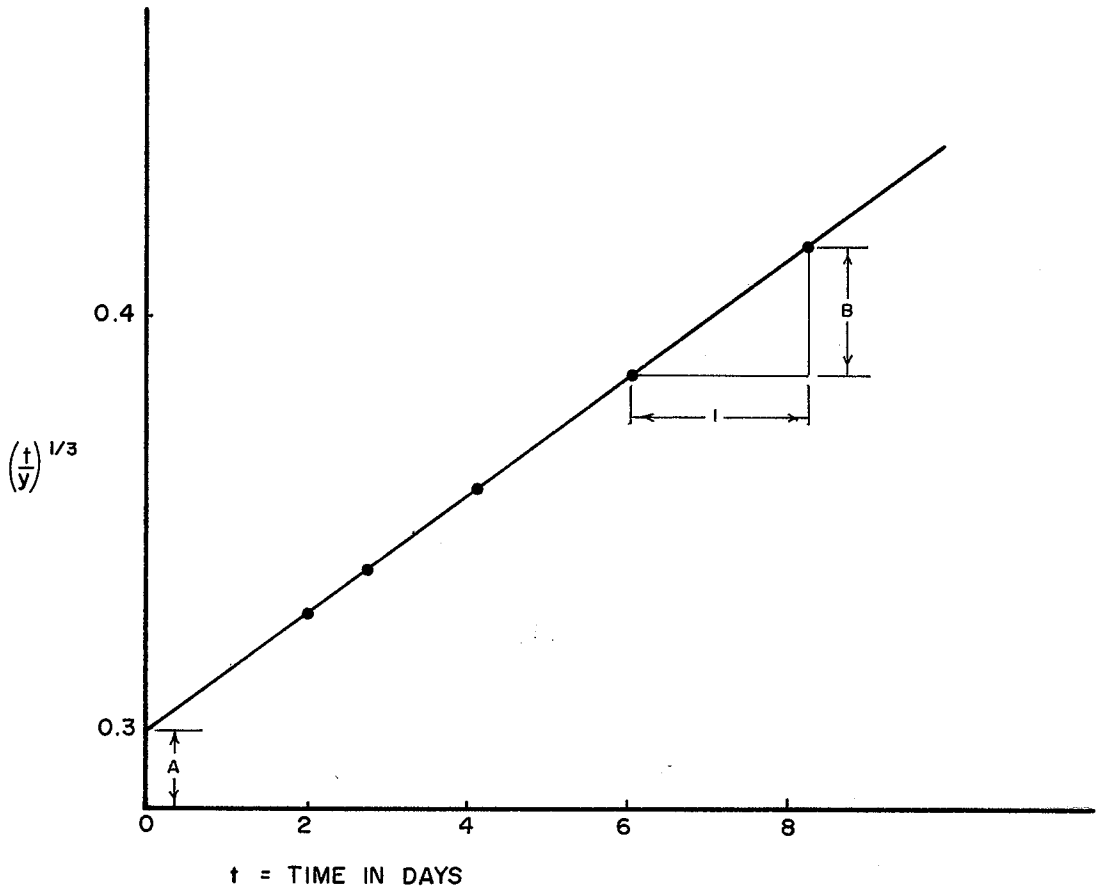

FIGURE 1: Thomas Method - Graphical Representation.

methods. Moore, et al (27) believe that if a simple method of developing k_1 and L was available more researchers will foresake the BOD_5 test and measure the strength of sewage in terms of total oxygen demand and the rate of exertion.

The method of moments is based on no lag phase and as Landine (19) states this method assumes all points have equal accuracy and a curve of best fit is drawn for which the sum of the squares of the deviations is a minimum. The no lag phase method of moments assumes that the oxygen demand proceeds at its maximum rate immediately. Moore, et al (27), also developed a method of moments for determining k_1 and L when evidence of a lag phase in the first part of the curve occurs. The lag phase may be due to the absence of adequate numbers of organisms or perhaps due to a difficulty in acclimation to the substrate. In any case such a BOD curve cannot be formulated by the standard equation $Y_t = L(1-10^{-k_1 t})$ but can be written as $Y_t = L(1-10^{-k_1(t-t_o)})$ in which t_o is the lag period (27). Due to the addition of this extra variable the amount of computation increases.

The moment method for determining k_1 and L is considered to be the one in best agreement with the standard Reed-Theriault method (18, 24, 27).

(4) The daily difference method of evaluating k_1 and L is briefly discussed by Gannon (28) as a comparison

against other methods of determining k_1 values. The method as described by Sparling (29) involves the following steps:

(i) the five day BOD data is obtained in the usual manner and the daily difference is evaluated;

(ii) the daily difference values are plotted versus time on a semi-log scale resulting in a straight line if first order kinetics is the case;

(iii) the slope of the line is equivalent to k_1.

The daily-difference method of obtaining k_1 was shown by Gannon (18) to be within the range of values obtained by other methods.

2.4.5. Comparison of BOD rates in the Laboratory Versus Actual Stream Conditions

Gannon (28) reports that for some rivers the monomolecular equation developed by Streeter and Phelps expresses the river BOD accurately with a k_1 rate having a statistical average approximating the generally accepted laboratory value. More recent investigators have found marked differences between actual river BOD rate and that normally expected from laboratory work.

Gannon (28) proposed a river BOD rate of k_r as opposed to the laboratory rate k_1 and studied two different rivers to observe any differences. There was a marked increase in the k_r of a slow moving river as compared to normally expected k_1 rates. This increase was explained by Gannon (28) as being the result of contact with sludge

deposits, heavy vegetation obstructing flow and abundant aquatic growth. He also concluded that the difference in BOD reaction rates were most noticeable between mixed and non-mixed categories.

Ali and Beutra (30) support the theory that the BOD values obtained under stirred conditions are always higher than the BOD obtained in the standard procedure without mixing. These findings are clarified, however, by the statement that the effect of stirred versus non-sitrred BOD values was less pronounced in final effluents.

Schroepfer, et al (18) allude to the fact that the different k_1 rates from river to laboratory evaluations may be due to a change from normal river temperature to the standard laboratory incubation temperatures of $20^{o}C$. They further concluded that a 24 hour delay from sampling to laboratory set-up caused little or no effect on BOD kinetics.

Nejedly (31) attributes the discrepancy between laboratory k_1 rates and stream k_1 rates to longitudinal dispersion by the stream. He concluded that the deoxygenation coefficient, k_1, of streams is consistently higher than those found under standard laboratory tests at the same temperature. The ratio between the two values can amount to as much as 30:1 and is directly proportional to the longitudinal dispersion. Unfortunately, these conclusions are only tentative since they were based on limited evidence.

2.4.6. Typical k_1 Values

The k_1 values of typical stream samples evaluated under standard laboratory conditions of $20^{o}C$ is 0.1 (11,24). Schroepfer, et al (18) found k_1 values in streams ranging from 0.003 to 0.146 at $20^{o}C$ with a mean value of 0.065. They further found that the k_1 values peaked for the 12-24 hour time period and then dissipated with time. Landine (19) had an explanation for this phenomenon as discussed in section 2.4.3.1. Gannon (28) found that the k_1 value varies with the method used to evaluate it and also on the strength of the river BOD. The k_1 of one set of samples with a river BOD_5 averaging 2.5 mg/l varied from 0.0002 to 0.0232 while a set of samples with a river BOD_5 of 18 mg/l varied from 0.0975 to 0.1600.

2.5. Nitrification in Streams

2.5.1. Introduction

The compounds of nitrogen are of great interest to environmental engineers because nitrogen is an integral part of the life processes of all plants and animals (21). The nitrogen cycle in nature is as shown in Figure II. An important aspect of stream sanitation is the oxygen demand caused by the nitrogenous compounds which are oxidized by autotrophic bacteria (7).

The nitrogenous stage of BOD does not normally begin until after the seventh day of incubation and is thus

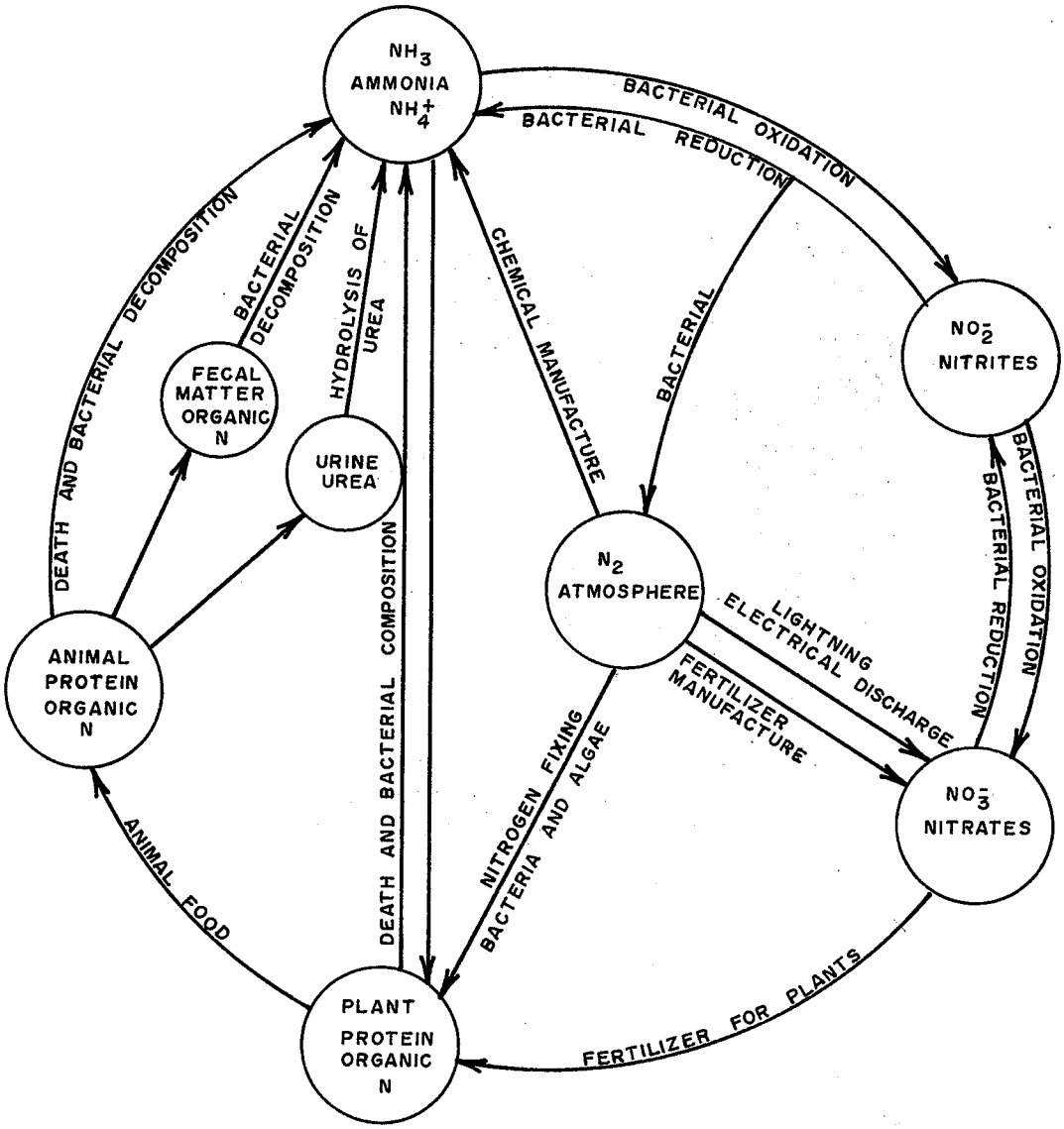

FIGURE II: Nitrogen Cycle.

excluded from normal five-day BOD tests (32). Nitrification, however, has caused serious error in BOD measurements of secondary effluents.

Courchaine (33) states that in stream studies oxidation of nitrogenous BOD can be a highly significant factor accounting for over seventy-five percent of the total demand excerted. Wild, Sawyer and McMahon (34) mention that although the nitrogenous oxygen demand (NOD) was well understood by engineers in 1940 it was dismissed as inconsequential until the late nineteen sixties.

The three premises for dismissal were:

(i) nitrification is caused by special organisms which are of minimal population in surface waters;

(ii) the reaction constant for nitrogenous oxidation is small in relation to the constant for carbonaceous matter;

(iii) oxidation of ammonia to nitrates simply converts dissolved oxygen to a chemical form which is still available to prevent development of anaerobic conditions.

The work of Courchaine (33) and other investigators (21, 33) have proved the above assumptions incorrect and as Zanoni (47) states, knowledge of the kinetics of nitrogenous assimilation is necessary for establishing the assimilation capacity of the receiving body of water.

Prasad and Jones (35) report that little work has been done on the biological activity of the micro-

organisms inhabiting waste treatment plants and streams at low temperatures, but W.E. Zernak (36) and Gannon (28) demonstrated that the impact of nitrification on stream oxygen is important enough that it be considered in oxygen balance equations. Some work has been done by several investigators on nitrification kinetics and is reported by Novak (37).

2.5.2. Biological Aspects of Nitrifiers

The cytology of nitrifiers is important in understanding the biological activities of the nitrifying organisms. Meiklejohn (38) reports that nitrifying bacteria were first isolated in pure culture by Winogradsky in 1890. Winogradsky determined that nitrifiers are autotrophic organisms meaning they do not use organic compounds as primary sources of energy. The autotrophs are able to synthesize organic compounds needed for respiratory requirements from carbon dioxide and water, using either light energy (photosynthesis) or the energy released from exothermic organic chemical reactions (chemo synthesis) (39).

The bacteria traditionally studied in relation to sewage treatment are heterotrophic and require organic compounds as their primary source of energy and are dependent directly or indirectly, upon autotrophic organisms for such food.

Meiklejohn (38) reports that Warrington in 1891 discovered two separate species of nitrifiers; one which oxidized ammonia to nitrite he named Nitrosomonas spp. while Nitrobacter spp. completed the reaction by oxidizing nitrite to nitrate. Meiklejohn (38) described the Nitrosomonas spp. and Nitrobacter spp. as being small oval cells usually single but occasionally two were formed end to end. The bacteria are gram-negative and non-motile organisms.

There are other organisms which will convert ammonia to nitrite to nitrate but Nitrosomonas and Nitrobacter are the most important. These other organisms include some heterotrophs and fungi.

Lees (40) states that microorganisms of the genus Nitrosomonas spp. are autotrophs which oxidize ammonia to nitrite and no cell growth or proliferation can take place without ammonia oxidation. He also states that Nelson, in 1931, showed that ammonia oxidation could take place in the absence of carbon dioxide, the energy released being dissipated rather than being used for carbon dioxide assimilation. This means that while a compound may inhibit proliferation it may not inhibit ammonia oxidation. Optimal pH for nitrite formation by Nitrosomonas spp. is 8.5. Lees (40) determined that nitrite does not interfere with nitrate determinations at nitrite concentrations less than 700 micrograms per milliliter of sample.

Lees and Simpson (41) in 1955 reported that Nitrobacter spp. are autotrophic microorganisms which obtains their entire energy supply by oxidizing nitrite to

nitrate. In a follow-up article in 1957, Lees and Simpson (42) state that Nitrobacter spp. will oxidize nitrite to nitrate in 21 days at 28°C. The reaction rate is constant until all nitrite is gone unless interfered with by chlorate (ClO_3^-) ions.

Butt and Lees (43) report that inorganic phosphates (orthophosphate) are utilized by Nitrobacter spp. Butt and Lees (44) determined that high concentrations of nitrite will inhibit nitrite oxidation by Nitrobacter spp. The optimum nitrite concentration, the concentration at which oxidation is maximal is dependent upon the oxygen tension; the oxygen to nitrogen ratio in the substrate. The optimal nitrite concentration decreases with decreasing oxygen tension. Once a culture of Nitrobacter spp. has completely oxidized a given concentration of nitrite and another batch of nitrite is added, at a concentration equal to or lower than the original given concentration, it will be oxidized without lag. A higher concentration would exhibit lag.

Knowles, Downing and Barrett, (45) determined that nitrifiers are mainly mesophilic with optimum growth temperatures at around 30°C. They also stated that Monod's first order kinetics applied to nitrification. This has been substantiated by Wild, et al. (34). Knowles, et al, (45) also found that the rate of nitrification increased with temperature throughout the range of 5°C to 30°C and

that up to five times the retention time may be needed to accomplish complete nitrification in the colder season since the kinetics are in agreement with the Van't Hoff-Arrhenius rate. The time required for nitrification is also proportional to the number of nitrifiers present in the system.

2.5.3. Chemistry for Nitrification

Goering (46) feels that the universal presence of nitrogen in all living matter explains the intimate association of nitrogen's environmental chemistry with biological systems. He further states that the biological transformations of nitrogen in aquatic ecosystems is well understood, but a thorough understanding of the rates and mechanisms controlling these reactions in streams and lakes are lacking.

Nitrogen has five valence electrons (46) and can exist in seven states of valence (21). The bonds are almost exclusively covalent in character (45). The N-N triple bond is one of the strongest bonds known. The most abundant form of nitrogen in unpolluted aquatic systems is molecular nitrogen with an oxidation state of zero and exceeds other nitrogen compounds by about twenty times. Ammonia (-3) or ammonium ion and organic nitrogen (NH_2(-2) and NH(-1)) are the most plentiful forms of reduced nitrogen. Nitrate is normally the most abundant form of oxidized nitrogen and at times nitrite is also present.

The primary sources of nitrogen compounds in domestic sewage are the end products of nitrogen metabolism in man (36, 46). Ammonia and urea constitute approximately 85% of the total nitrogen in sewage. The ammonia is oxidized to nitrite by Nitrosomonas spp. and the nitrite to nitrate by Nitrobacter spp. according to the following equations (35):

$$NH_4^+ + 1.5\ O_2 \longrightarrow 2H^+ + NO_2^- + H_2O$$

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^-$$

A more common method of writing these equations is as follows (21, 46, 47):

$$2NH_3 + 3O_2 \xrightarrow{(\text{Nitrosomonas spp.})} 2NO_2^- + 2H^+ + 2H_2O$$

$$2NO_2^- + O_2 \xrightarrow{(\text{Nitrobacter spp.})} 2NO_3^-$$

2.5.3.1. Oxygen and Nitrification

According to Lees (51) nitrifying organisms consume more than one hundred parts of oxygen per one part of carbon. This means that under conditions of low dissolved oxygen or poor aeration the heterotrophic organisms with a low oxygen demand proliferate much faster than nitrifiers with high oxygen demand.

Young (48) states that if the nitrogenous reactions were carried to completion, theoretically 3.43 g

of molecular oxygen would be used per gram of ammonia nitrogen oxidized to nitrite; and 1.14 g of oxygen would be consumed for each gram of nitrite nitrogen converted to nitrate. Some of the reduced nitrogen is assimilated as cell material in the carbon dioxide fixation reaction in the following manner:

$$5CO_2 + NH_3 \longrightarrow C_5H_7NO_2^{*} + 5O_2$$

* Empirical Formula for cell composition

Some of the ammonia is assimilated as cell material and is not converted to nitrite or nitrate thus not consuming oxygen. To obtain an accurate oxygen balance the oxygen equivalent of the assimilated ammonia nitrogen must be substracted from that theoretically needed for nitrification.

Young (48) and other researchers (23, 36) state that the theoretical nitrogenous oxygen demand -NOD is accurately given as follows:

$$NOD = 3.22\ (NH_3 - N \longrightarrow NO_2^- - N)$$

$$+1.11\ (NO_2^- - N \longrightarrow NO_3^- - N)$$

2.5.4. Nitrification Kinetics

Zanoni (47), like other researchers, feels that the deoxygenation progression dating back to the classic works of Streeter and Phelps expressed as the monomolecular or first order BOD, is valid for expressing both the

carbonaceous and nitrogenous stages of deoxygenation. The integrated form of the equation is as follows:

$$Y = L_c\ (1-10^{-k_c t}) + L_n\ (1-10^{-k_n (t-t_n)}).$$

where Y = BOD in mg/l;

L_c and L_n = ultimate carbonaceous and nitrogenous demands, respectively, in mg/l;

k_c and k_n = carbonaceous and nitrogenous velocity constants or reaction rates, respectively, per day, and

t_n = time lag to the onset of nitrogenous deoxygenation, days.

Figure III presents an "idealized" sketch of the monomolecular deoxygenation curve showing the two separate stages. It is sometimes difficult to discern clearly when the first stage ends and the second stage begins. Depending upon such factors as substrate characteristics and the quantity, viability, and type-distribution of the microbial population it is possible that the two stages may occur simultaneously. Velz (11) states that during the first or carbonaceous oxidation stage, about 70 to 80 percent of the organic carbon is oxidized. During the second or nitrification stage biochemical oxidation of ammonia nitrogen occurs sometimes simultaneously and the remaining 20 to 30 percent of the organic carbon is utilized by the bacteria for growth.

Courchaine (33) determined that at 20°C nitrification became active at about the ninth or tenth day and

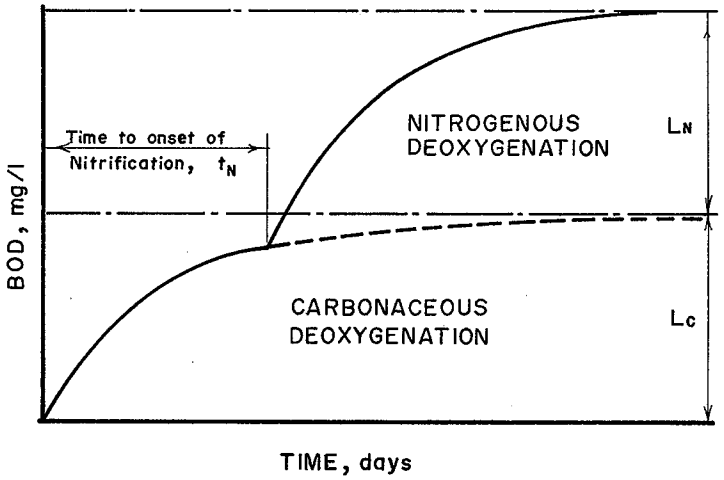

FIGURE III: Idealized Monomolecular Deoxygenation.

was complete ten days later. However, during summer conditions when the temperature was 25 to 30^{o}C nitrification, once begun, was complete within a period of hours. This is dependent upon the original number of nitrifying bacteria present (33).

Velz (11) obtained BOD curves which indicated that nitrification started within the first day of incubation and became very active about the fourth day and after six days the BOD exerted through at a very rapid rate thereafter and was virtually completed by the twelfth day. Landine (19) quotes several researchers, all with their own data indicating that nitrification begins in ten to twenty days, some in five to seven days and some suggest that all polluted river waters show nitrification in the BOD_5 test. The reason for the erratic period of time elapsed before nitrification begins is mainly dependent upon the number of nitrifying organisms present at the beginning of the test. Several researchers (11, 33, 47) agree to this fact since they were able to show that very little lag occurred, when an adequate innoculum of nitrifying organisms was used. Nitrification was complete in five to six days.

A publication (52) of the British Department of the Environment states that recent research has shown that nitrifying bacteria multiply slowly. This slow growth of Nitrosomonas, coupled with the fact that the population in sewage is small, could account for the erroneous statements

sometimes made that nitrification does not begin until carbonaceous oxidation is actually complete. The doubling time of nitrite (and hence, presumably, of Nitrosomonas spp.) was found to be not less than one day even under favourable conditions. Consequently the number of generations possible in five days is small and the amount of ammonia oxidized in the first five days depends critically on the concentration of Nitrosomonas spp. cells initially present in the sample.

The paper also states that the rate of nitrification for a given concentration of Nitrosomonas spp. is virtually independent of the ammonia content of the sample provided that it is not less than about 3 mg/l. As the ammonia content falls below 3 mg/l, the rate also falls and is about one half the maximum at 0.5 mg/l of ammonia. The rate is only slightly affected by the concentration of dissolved oxygen at levels above 2 mg/l.

Montgomery and Borne (53) concluded that the doubling time of Nitrosomonas spp. averaged about 1.7 day although the doubling time of pure cultures was 0.7 days. Generally, the formation of nitrite was roughly exponential when ammonia was not limiting. A true lag phase was not evident.

Statton and McCarty (54) developed a generally applicable method of estimating the amount of oxygen required to oxidize ammonia nitrogen. The method was based on the principles of biological kinetics and an estimation

of the concentration of viable nitrifying organisms. They determined that the rate parameters for ammonia and nitrite nitrogen oxidation are functions of environmental conditions including temperature, pH and the chemical composition of the water. Temperature was considered to be the main factor.

Anthonison (55) considers the sequential oxidation of ammonia to nitrate to be a series of consecutive first order reactions. If it is assumed that nitrification follows first order kinetics the nitrogen transformations can be shown as in Figure IV. It was assumed that the concentration of NO_2^- and NO_3^- were zero at time - t = 0 and the oxidation rate follows first order kinetics and that the ammonia and nitrite removal rates were directly proportional to existing concentrations. Anthonison (55) described this method and assumed that the reaction rate for Nitrosomonas spp. (k_{n1}) was equal to twice the reaction rate for Nitrobacter spp. (k_{n2}). Another approach known as the bacterial-growth-kinetics theory by Knowles, et al, (45) is based on the concept that the rates of oxidation of Nitrosomonas spp. and Nitrobacter, spp. respectively, and that these growth rates are each proportional to the concentration of bacteria. Figure V presents this theory based on incubated samples of estuary water and the curves were calculated assuming that the system obeyed bacterial growth kinetics. Anthonison (55) considers the nitrification reactions to occur as shown in Figure VI.

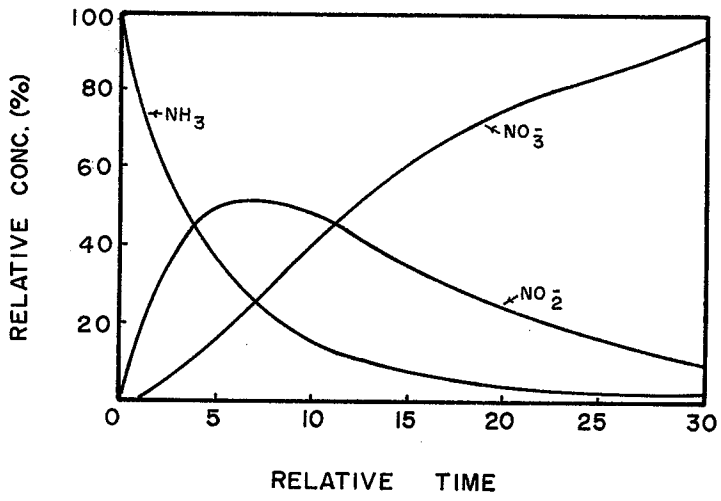

FIGURE IV: Nitrogen Changes First Order Reaction.

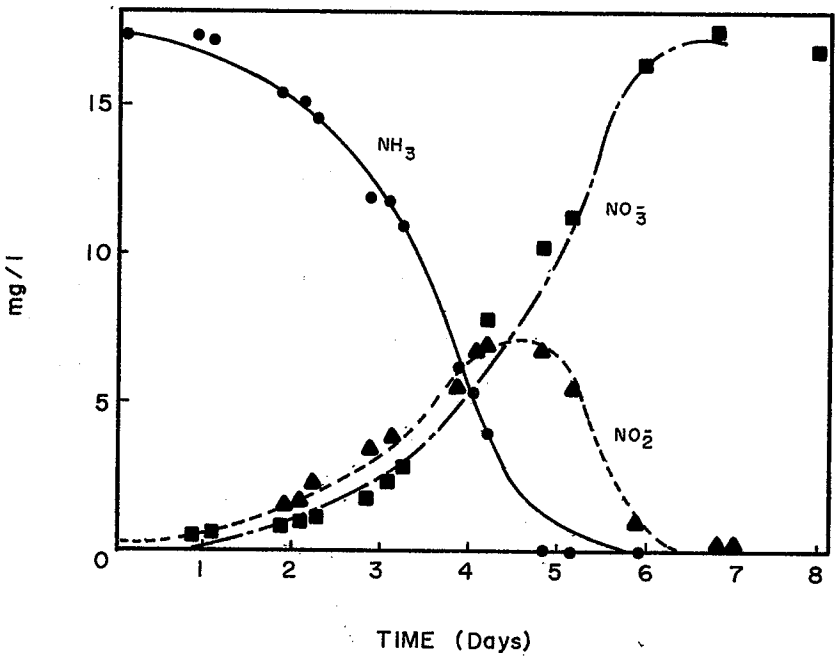

FIGURE V: Nitrogen Changes - Bacterial Growth Kinetics.

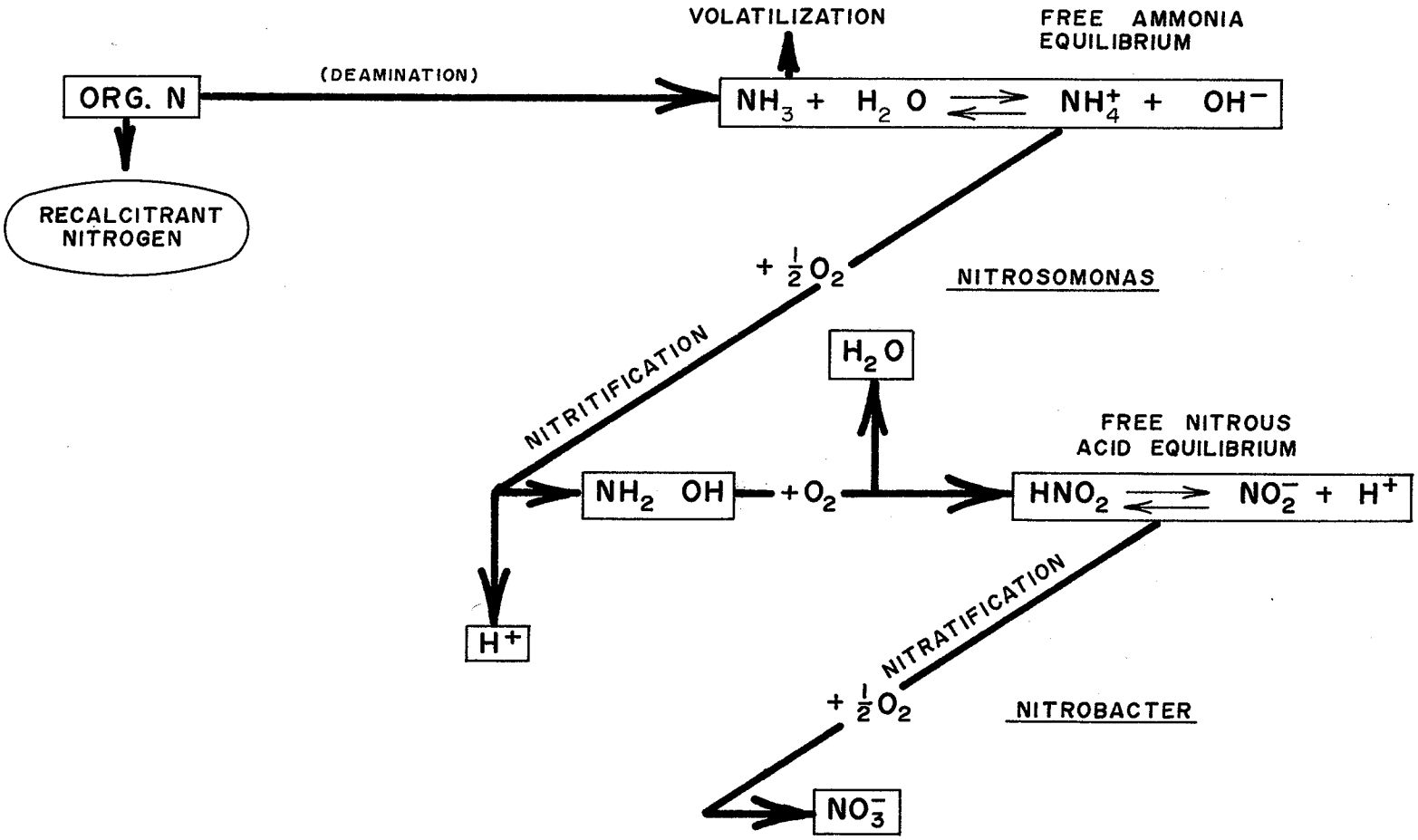

FIGURE VI: Nitrification Reactions.

Buswell, Mueller and Van Meter (56) in opposition to the previously mentioned researchers believe that the monomolecular reaction is invalid for determining nitrogenous BOD. They do agree that the rate of nitrification is directly related to the multiplication of Nitrosomonas spp. and is independent of substrate concentration. They give an equation which demonstrates the usual growth curve for the logarithmic phase as follows:

$$r = \frac{k}{2.3} = \frac{1}{t} \log\left(\frac{N}{N_o}\right)$$

where r = rate constant for common logarithms;

k = rate constant when expressed in natural logarithms;

t = time interval

N_o = bacterial population at beginning of time t;

N = bacterial population at end of time interval t.

2.5.5. Methods of Determining Nitrification Rate Constants

Courchaine (33) describes a method of determining the nitrogenous stage by using the standard carbonaceous BOD integration and adding to this the oxygen required to produce the observed increase in stream nitrates. This will yield a total BOD which should equal the actual twenty day BOD of the sample. The actual kinetics of

nitrification were not determined since the calculated BOD was compared to DO of the stream. Nemerov (57) agrees that at least a twenty day BOD test should be run to determine total BOD due to nitrification - NOD. He states that it is difficult to separate nitrogenous BOD and carbonaceous BOD in the standard five day test.

Some researchers such as Wild, et al. (34) and Downing, et al, (45) determined nitrification rates by defining it as the weight ratio of ammonia nitrogen oxidized per day to the mixed liquor volatile suspended solids - MLVSS. At 20°C the rate varies from a maximum of 0.185 grams NH_3 - N nitrified/day/g MLVSS at pH of 6.0. This method can be evaluated using a mixed natural culture or a pure culture of Nitrosomonas spp. and Nitrobacter spp. and is similar to the method proposed by Buswell, et al. (56) where the rate of nitrification was dependent on the initial number of nitrifying organisms.

Velz (11) discusses a dual incubation with and without a nitrification inhibitor, such that the difference between the two values gives the nitrogenous fraction. Inhibition was also used by Montgomery and Borne (53) to determine the NOD of samples of sewage. Carroll (23) did extensive work on nitrification inhibition but actual rate constants were not determined. Velz (11) did determine that a two-stage nitrification occurs with the growth coefficient of the first stage being 1.12 extending to the

thirteenth day when the growth coefficient increased to 2.52. He felt that this differentiated between the Nitrosomonas spp. and the Nitrobacter spp.

Zanoni (47) did some thorough testing on nitrification kinetics. He ran BOD tests on the samples in the standard manner in conjunction with nitrite and nitrate-nitrogen tests. Both BOD and nitrogen analyses were made at greater frequency during the transition phase from the carbonaceous to the nitrogenous stages. A larger number of nitrite-nitrogen determinations were made in the earlier stages with more numerous nitrate-nitrogen analyses being made in the later stages. An optimum testing program was developed by running several test series prior to the actual test. All analyses were conducted in accordance with Standard Methods (22).

Zanoni (47) felt that based on past experience the "moments" method of Moore, Thomas and Snow (27) was the most suitable. An important step was to accurately delineate the two stages of deoxygenation. This was accomplished by using numerous nitrogen analyses and assuming that nitrification proceeded quantitatively according to the standard nitrogenous equations shown in Figure IV. The nitrite nitrogen concentration multiplied by 3.43 is equal to the total BOD involved in the oxidation of ammonia and the nitrite nitrogen converted to nitrate nitrogen would be multiplied by 1.14 for a total BOD conversion of 4.57. The first step was to convert the nitrogen

values to equivalent BOD and then subtract these demands from the total BODs as determined from the standard BOD test. The resulting points on the BOD graph should represent the progression for the carbonaceous demand. The carbonaceous velocity constant, k_1, and the ultimate demand L, were determined by the moments method. The theoretical carbonaceous curve was determined and by subtracting these calculated points from the observed points deoxygenation progression resulted assumed to be solely for the nitrogenous phase. The curve of best fit was then established and the nitrogenous velocity constants and ultimate demand determined.

The exact time of nitrification onset is important since it has a significant influence on the value of the nitrogenous rate constant. Zanoni (47) used the term "active" nitrification to signify the portion of the curve which was actually included in the evaluation of deoxygenation constants. The "moments" method was then used to establish the constants and the theoretical nitrogenous demand curve plotted. If this plot did not fit the actual curve a new time for nitrification onset was selected and the process repeated until a good fit was obtained. The first evaluation was usually sufficient.

2.5.6. Effect of Temperature on Nitrification Kinetics

Zanoni (47) states that the effect of temperature on the nitrification stage of deoxygenation has been

almost totally ignored and to his knowledge no θ values have been reported for nitrification. The term θ originated from the temperature equation of Streeter and Phelps $\frac{k_1}{k_r} = \theta^{(t-2o)}$. It was found to vary from 1.097 in the temperature range of 10°C to 22°C to 0.877 in the temperature range of 22°C to 30°C. In the range from 5°C to 10°C it was found that θ ranged from 1.092 to 1.184 which is a little higher than the traditional value of 1.047 from Streeter and Phelps.

Benedict and Carlson (61) agree that the Monod formulation adequately describes the kinetics of substrate utilization for bio-oxidation systems over the temperature range of 10°C to 20°C. Kinetic response to temperature differentials follows the Van't Hoff equation and its approximation the Streeter-Phelps expression. Temperature inhibition does not occur in the temperature range 10°C to 20°C.

Zanoni (47) based his nitrification kinetics study on 33 test runs including 700 BOD determinations, 1250 nitrite-nitrogen determinations, 500 nitrate-nitrogen determinations and 70 ammonia determinations. He found that the rate constant-k_n for the nitrogenous stage was 0.1149 at 20°C, 0.034 at 5°C, 0.052 at 10°C and 0.08 at 15°C. The optimum temperature for the nitrogenous stage is often quoted as being 30°C but most of the studies were done on pure cultures and do not necessarily corres-

pond with below 20°C there is a longer nitrification lag and a more obvious transitional change could be detected.

Zanoni (47) also concluded that the ultimate nitrogenous demand did not vary significantly with temperature. The time lag before "active" nitrification commenced was greatly dependent on temperature and at 5°C the time lag was six times that at 10°C.

Velz (11) reports a maximum nitrification rate at 28°C. Wild, et al. (34) reports that nitrification occurs at all temperatures between 5°C and 30°C. The rate of nitrification increased with temperature throughout the whole range in reasonable agreement with the Van't Hoff Arrhenius law. Statton and McCarty (54) report that in the temperature range of 15°C to 25°C the rate for ammonia oxidation increased by seven to nine percent per degree centigrade while for nitrite oxidation a six percent increase per degree was found. However, they also state that nitrification rate parameters reported in the literature indicates a three hundred percent variation in rate constants found by different workers studying ammonia oxidation at corresponding temperatures. Knowles, et al. (45) found that Nitrosomonas spp. rate constants increased by 9.5 percent per degree centigrade in the range from 8 to 23°C while Nitrobacter spp. growth rates increased by six percent per degree centigrade, thus more or less agreeing with other workers (11, 34, 54).

Courchaine (33) states that the optimum ranges for nitrifiers is 25°C to 30°C. Nitrosomonas spp. bacteria have an optimum temperature of 25°C while Nitrobacter spp. have an optimum temperature at 28°C. The resulting generation time was about thirty-one hours. He concluded that a thermal discharge raising the river water temperature by 5°C resulted in a high degree of nitrification causing the nitrogenous demand to be seventy five percent of the total BOD.

Landine (19) found no nitrification at 0.4°C and he reports that other researchers found no nitrification below 4°C. The lag for nitrification at 10°C was thirty days. This concurs with Zanoni (47) who reported a lag of twenty days at 10°C and forty days at 5°C.

Prasad and Jones (35) found that physiological activity of psychrophiles in the presence of various organic nitrogenous compounds clearly indicates that temperature exerts an important influence on the rate of metabolism since in all cases the activity was higher at 20°C than at 5°C.

Beckman, Avendt, Mulligan and Kehrberger (58) concluded that temperature is a controlling factor in all biological reactions and has a pronounced effect on the growth rate of nitrifying organisms. The growth rate of nitrifying bacteria roughly doubles for a 7°C to 10°C increase in temperatures between 6°C and 25°C with an

optimum growth rate at 30°C. Reeves (59) concurs with these findings as described in a 1972 article.

Buck and Rankin (60) found that the number of mesophilic bacteria including nitrifiers in a stream was quite stable over a temperature range of 0°C to 30°C. The number of bacteria only varied by one order of magnitude from the 0°C winter conditions to the 30°C summer conditions. Psychrophilic bacteria varied by five orders of magnitude over the same temperature range, the low counts occurring during the warm summer months.

A number of researchers (11, 19, 33, 47, 54, 57, 61) into nitrification kinetics agree that little or no nitrification occurs below 4°C, the optimum temperature for nitrification is 22°C to 30°C, nitrification rates increase with temperatures and nitrification can be an important aspect of stream BOD if the temperature is raised into the optimum range due to thermal discharges.

CHAPTER III BACKGROUND INFORMATION

3.1. Introduction

The effect of thermal discharge on biological reaction rates of nitrifying organisms was studied in two different parts. The first part of the investigation was carried out on Assiniboine River samples taken near Brandon during the months of January, February and March of 1975. Several test runs were made during October, November and December of 1974 to determine the method of sampling, the type of analysis to be performed and the range of results to expect.

The second part of the study was carried out on laboratory samples which were heated to simulate actual river conditions at Brandon in terms of temperature. Red River water was used since it was assumed that a basic population of nitrifyers was present, due to the discharge from the city of Winnipeg's south end sewage treatment plant. The samples were spiked (innoculated) with approximately two milligrams per liter of ammonia to ensure adequate substrate concentrations for the nitrifiers. Two milligrams were used to ensure that the samples were within the realm of reality.

3.2. Sampling Station Locations

3.2.1. Assiniboine River Samples

Three stations were sampled for each set of

analyses. Station number 1 was located on the Assiniboine River about two hundred feet upstream of the cooling water discharge from Manitoba Hydro's stream generating station. Station number 2 was located approximately one thousand feet (300 meters) downstream of the cooling water discharge while station number 3 was located approximately four thousand feet (1200 meters) downstream of the discharge culvert. Station Number 5 was at Lost Island farm and was of interest since a temperature recorder was situated there. The stations are shown on Figure VII.

3.2.2. Simulated Laboratory Samples

The samples used in the laboratory simulation study were obtained from the Red River at Crescent Drive Park in Fort Garry, Winnipeg. All the samples were taken from the same station.

3.3. Sampling Conditions

3.3.1. Assiniboine River

The generating station did not begin full time operation until early in January. The river had been ice-covered up until this period and sampling was not possible until the thermal discharge kept the river free of ice beyond station 3. The river at station 1 was open due to the dam at the Hydro's intake structure.

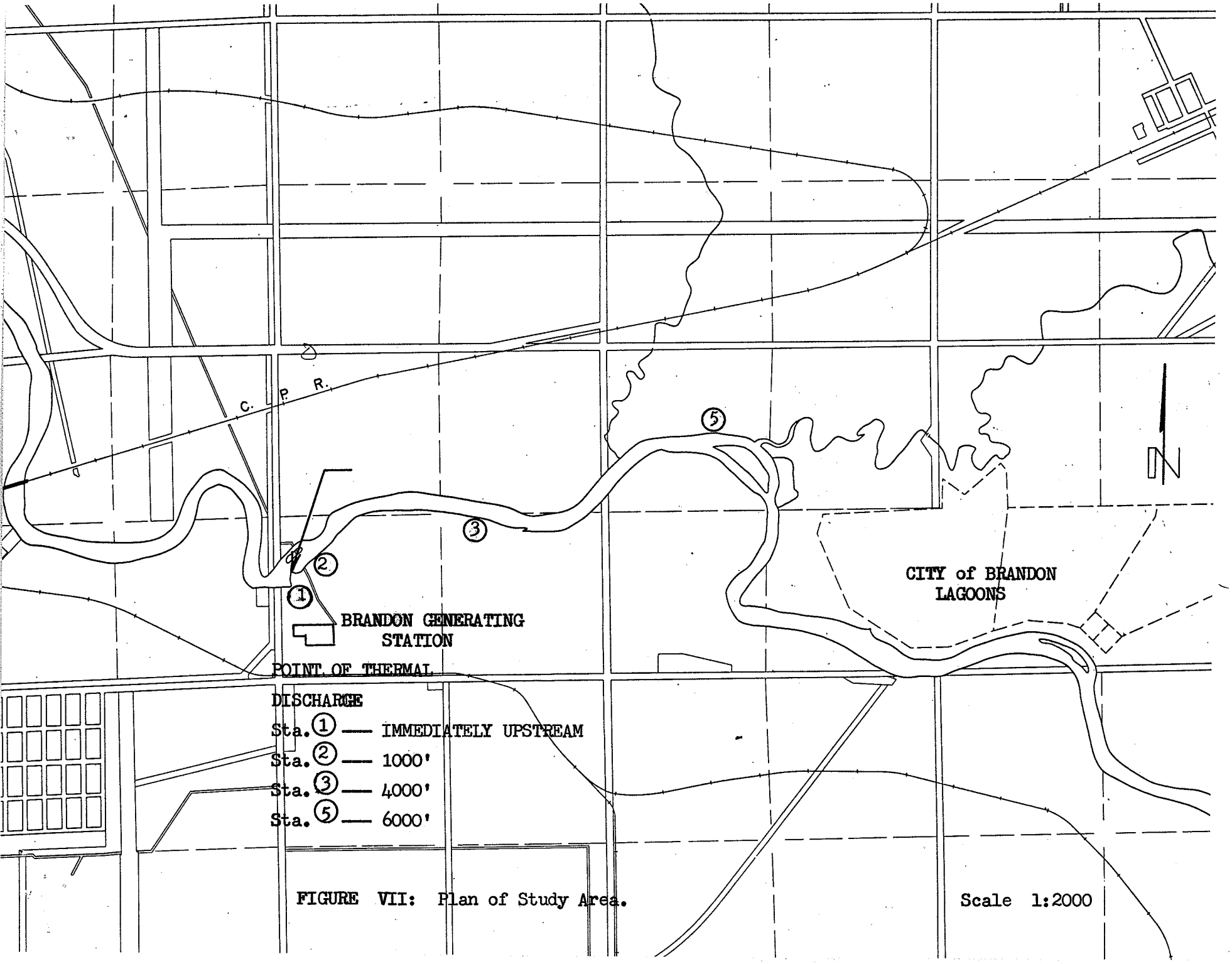

FIGURE VII: Plan of Study Area.

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Sampling was done from a boat launched at station 1 and then proceeding to stations 2 and 3. Due to the extremely cold ambient air temperatures during most of the sampling period obtaining samples for BOD analyses was impossible using the standard A.P.H.A. sampler and only one sample was taken for dissolved oxygen from each station in this manner. The remaining 20 samples from each station were obtained by filling a five gallon container, keeping it at river temperatures while carrying them back to the laboratory, warming them to 20^{o}C in a water bath, bubbling out the excess dissolved oxygen by using air and then siphoning the sample into the standard BOD bottles for incubation at 20^{o}C.

3.3.2. Simulated Laboratory Samples

The samples for the simulated laboratory study were obtained from the Red River during flood stage in late April. The water temperature was close to 0^{o}C; similar to the Assiniboine River temperature upstream of the thermal water discharge. Eight samples were taken about fifteen minutes apart by filling a five gallon container from the river bank.

The eight samples were innoculated with approximately 2 mg/l of ammonia as ammonium chloride upon return to the laboratory. Two gallons of each five gallon sample were heated to about 17^{o}C over a period of about twenty

minutes to simulate heating of the cooling water through the steam generating plant. The other 3 gallons of sample were kept at $0^{o}C$ in the incubator. The $17^{o}C$ samples were then mixed into the $0^{o}C$ samples until the combined water temperature was approximately $5^{o}C$. This simulated the flow of cooling water into the Assiniboine river heating the river water at station 2 to a maximum of $5^{o}C$ under the flow conditions during the study.

The simulated river sample was then siphoned into BOD bottles for incubation at $20^{o}C$ and at $5^{o}C$. Samples for ammonia and nitrate analysis were also incubated at those temperatures.

3.4. Laboratory Analysis

3.4.1. Physical Parameters

The main physical parameter checked on all the Brandon samples was the temperature. Water temperature was monitored at stations 1, 2 and 5. One thermocouple was installed on the river bottom at the water intake of the cooling water pumphouse to monitor upstream water temperatures. Two thermocouples on the river bottom at station 2 monitored the water temperature downstream of the thermal discharge.

A Yellow Springs Instrument Company (Y.S.I.) model 401 thermister with a Y.S.I. model 80A recorder measured the river water temperature at station 5. The temperatures at station 3 were obtained by extrapolating

the temperature recordings from stations 2 and 5. An illustration of the temperature charts obtained is shown in Figure VIII.

Surface water temperatures were also measured for each set of samples at each station. The measurements, made with a standard Celsius thermometer, served as a check on the river temperatures.

The temperature was also measured on the Red River samples for laboratory incubation. These measurements were made with a standard Celsius thermometer.

The only other physical parameter checked on the Brandon samples was the flow which was obtained from monthly flow data provided by the Provincial Water Resources Branch. The flow over the three month study period was stable. Variations in temperatures downstream of the thermal discharge was solely due to the temperature and volume of cooling water. Any fluctuations or chemical analyses results would be due to natural conditions and not influenced by different dilutions.

The flow in the Red River which was in flood stage at the time of sampling was approximately 40,000 c.f.s. as reported by the Federal Government.

3.4.2. Chemical Parameters

The Assiniboine River samples and the Red River samples were all analysed for the same chemical parameters. The samples were analysed for the following:

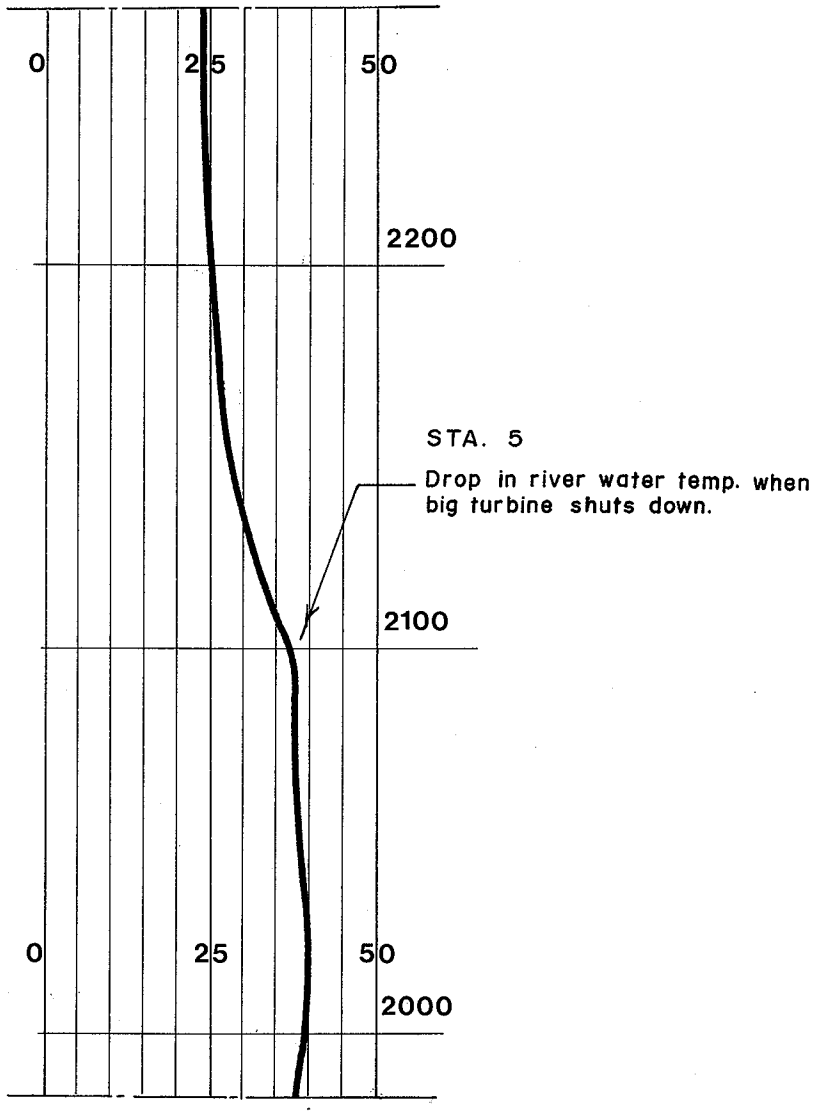

FIGURE VIII: Example of Temperature Graphs.

1. dissolved oxygen concentration (DO)
2. biochemical oxygen demand (BOD)
3. ammonia concentration (NH_4)
4. nitrate concentration (NO_3)

The BOD samples were incubated for 20 days at 20°C. The dissolved oxygen concentrations were determined using the azide modification at the iodometric method described in section 218B of Standard Methods (22). The simulated samples were handled in the same manner except an additional set of BOD samples were run at 5°C.

The Assiniboine River samples were not diluted since it had been predetermined that the natural river water BOD was low. The BOD samples were analysed for one, two, three, four and five day progressions for carbonaceous demand and also seven, nine, eleven, fifteen and twenty day BODs were determined to evaluate the nitrogenous demand.

The ammonia analyses were done according to section 132A and B of Standard Methods (22). The samples were analyzed for ammonia on the day of sampling, to obtain the actual amounts in the river water, and, every three to four days thereafter up until 20 days to determine the amount of depletion.

The nitrate analyses were done on the same days as the ammonia analysis in order to measure the increase due to nitrification of ammonia. The analyses were done exactly as described in section 213C of Standard Methods (22).

CHAPTER IV. RESULTS AND OBSERVATIONS

4.1. Assiniboine River Study

4.1.1. Water Temperature

The water temperature data collected from stations 1, 2, 3 and 5 during the three month study period is summarized in Table I. The results for station 3 were obtained by interpolating the temperature data from stations 2 and 5. The temperatures at each station reflect the average temperature of the water during the days the sampling was done. These temperatures were considered valid since the total retention time in the section of the river being sampled was less than two hours thus ensuring that the bacteria had not been exposed to higher or lower temperatures than the one indicated on the recording charts.

Station 1 was under ice cover during the whole study period and therefore the temperature was always zero degrees Celsius. The samples were obtained under the ice in order to obtain a true dissolved oxygen content.

4.1.2. Flow in the Assiniboine River

The flows in the Assiniboine River, at Brandon, during the study period were as shown in Tables II, III and IV. The flow data reported was obtained from the mean

Date	Station 1 (under ice)	Station 2	Station 3	Station 5
Jan. 10	0°	2.0°	1.5°	1.5°
18	0°	3.5°	2.5°	2.0°
24	0°	2.5°	1.5°	1.5°
31	0°	4.0°	3.0°	2.0°
Feb. 6	0°	5.0°	3.0°	2.0°
14	0°	3.0°	1.5°	1.5°
21	0°	2.0°	1.0°	0.5°
Mar. 1	0°	1.0°	0.5°	0°
14	0°	0.5°	0°	0°
Average	0°	2.6°	1.6°	1.3°

TABLE I: Average River Water Temperature* at Time of Sampling (Degrees Celsius).

* Temperature recorder

Date	Temp (^{o}C)	Flow (CFS)	BOD_5 (mg/l)	BOD_{20} (mg/l)
Jan. 10	0^{o}	440	1.3	3.1
18	0^{o}	436	1.4	2.9
24	0^{o}	436	0.8	1.9
31	0^{o}	437	1.0	2.2
Feb. 6	0^{o}	437	1.9	2.6
14	0^{o}	437	1.0	2.0
21	0^{o}	437	0.9	1.6
Mar. 1	0^{o}	450	0.4	1.1
14	0^{o}	475	0.4	0.8
Average	0^{o}	440	1.01	2.02

TABLE II: River Water Temperature, BOD and River Flows (incubated at 20^{o}C) - Station 1.

Date	Temp (^{o}C)	Flow (CFS)	BOD_5 (mg/l)	BOD_{20} (mg/l)
Jan. 10	2.0^{o}	440	1.2	2.8
18	3.5^{o}	436	0.8	2.3
24	2.5^{o}	436	1.0	2.4
31	4.0^{o}	437	0.9	1.8
Feb. 6	5.0^{o}	437	1.7	2.8
14	3.0^{o}	437	0.7	1.5
21	2.0^{o}	437	0.5	3.2
Mar. 1	1.0^{o}	450	0.5	1.7
14	0.5^{o}	475	1.1	1.7
Average	2.61	440	0.93	2.24

TABLE III: River Water Temperature, BOD and River Flows (incubated at 20^{o}C) - Station 2.

Date	Temp (^{o}C)	Flow (CFS)	BOD_5 (mg/l)	BOD_{20} (mg/l)
Jan. 10	1.5^{o}	440	0.7	3.0
18	2.0^{o}	436	1.0	2.4
24	1.5^{o}	436	0.8	2.3
31	3.0^{o}	437	1.1	1.9
Feb. 6	3.0^{o}	437	1.2	2.4
14	1.5^{o}	437	0.6	1.3
21	1^{o}	437	0.6	1.2
Mar. 1	0.5^{o}	450	0.5	1.3
14	0^{o}	475	0.5	1.5
Average	1.56	440	0.78	1.92

TABLE IV: River Water Temperature, BOD and River Flows (incubated at 20^{o}C) - Station 3.

weekly stream flow data as reported by the Water Resources Branch of the Manitoba Department of Mines, Resources and Environmental Management. The flow did not vary appreciably from January to early March when it began to increase. The flow during the last week of sampling was less than ten percent above that of the first week. (January, 440 cfs, middle of March, 475 cfs).

4.1.3. Biochemical Oxygen Demand Analysis

Water samples were collected for analysis from stations 1, 2 and 3 on a weekly basis from January through March 1 of 1975. The last set of samples was collected on March 14, 1975. The five day and twenty day BOD results from each station were as shown in Tables II, III and IV.

4.1.3.1. Carbonaceous k_1 Rates

The one, two, three, four and five day BOD analyses were used to draw BOD progression curves which in turn were used to calculate the carbonaceous reaction rate ($-k_1$). The k_1 rates are temperature dependent and the rates from each station were compared with the temperature. The k_1 rates at each temperature are shown in Table V.

The reaction rate can also be dependent upon substrate concentration. Table VI shows the k_1 rates from

Sample	Station 1		Station 2		Station 3	
Date	Temp (°C)	k_1 rate	Temp (°C)	k_1 rate	Temp (°C)	k_1 rate
Jan. 10	0	0.1	2.0	0.1	1.5	0.1
18	0	0.14	3.5	0.09	2.0	0.05
24	0	0.03	2.5	0.04	1.5	0.03
31	0	0.11	4.0	0.11	3.0	0.13
Feb. 6	0	0.16	5.0	0.15	3.0	0.12
14	0	0.14	3.0	0.13	1.5	0.13
21	0	0.14	2.0	0.11	1.0	0.13
Mar. 1	0	0.05	1.0	0.08	0.5	0.08
14	0	0.10	0.5	0.13	0	0.11
Average	0	0.108	2.61	0.104	1.56	0.098
Std. Deviation		0.04		0.03		0.04

TABLE V: Carbonaceous k_1 Rates and River Water Temperature (incubated at 20°C).

Sample	Station 1		Station 2		Station 3	
Date	k_1 rate	mg/l	k_1 rate	mg/l	k_1 rate	mg/l
Jan. 10	0.10	2.9	0.10	1.9	0.10	2.0
18	0.14	2.0	0.09	1.4	0.05	2.1
24	0.03	3.1	0.04	3.1	0.03	3.1
31	0.11	1.3	0.11	1.3	0.13	3.1
Feb. 6	0.16	2.5	0.15	2.2	0.12	1.6
14	0.14	1.4	0.13	1.0	0.13	0.9
21	0.14	1.1	0.11	0.8	0.13	0.9
Mar. 1	0.05	1.0	0.08	0.7	0.08	0.7
14	0.10	1.2	0.13	1.5	0.11	1.0
Average	0.108	1.68	0.104	1.51	0.098	1.49
Std. Deviation	0.04		0.03		0.04	

TABLE VI: Reaction Rates (k_1) and Ultimate Carbonaceous Substrate Concentration (incubated at 20°C).

each station compared to the theoretical ultimate carbonaceous demand.

The k_1 rate can be calculated using several recognized methods. Some methods have a better correlation with the actual situation than others, depending on BOD progression data. The Thomas method was used to determine the k_1 rates shown in Tables V and VI. A comparison of five methods for determining k_1 and the ultimate carbonaceous demand is shown in Table VII.

4.1.3.2. Nitrogenous Demand - NOD

The ultimate nitrogenous demands were calculated in three different ways. The observed NOD was obtained by subtracting the theoretical carbonaceous curve from the total BOD curve. The calculated NOD was determined by calculating an ultimate BOD based on the progression curves and the theoretical NOD was calculated by multiplying the observed ammonia depletion for each sample by the theoretical oxygen molecular equivalent. The nitrate increase should yield similar results when multiplied by the molecular equivalent. These results are shown in Tables VIII, IX and X.

The lag time before nitrification sets in is dependent on the number of nitrifying organisms originally present in the sample and the temperature. The lag time and temperatures have also been shown in Tables VIII, IX and X.

Method	k_1 value	L value (mg/l)	Comments Theoretical vs. Observed
Thomas	0.086	3.3	Close to observed
Moments (no lag)	0.086	3.1	Close to observed
Slope	0.078	3.6	High
Daily Difference	0.075	2.9	Low
Moments (lag)	0.082	4.0	High
Std. Deviation	0.005	0.43	

TABLE VII: Comparison of Various Methods of Determining k_1 Values (data from January 10, 1975).

Date	Temp (°C)	Time Lag (Days)	NOD (mg/l) observed	NOD calculated	NOD Theoretical NH_4 Dep.	NO_3^- Build-up
Jan. 10	0	7	0.17	0.17	0	0
18	0	8	0.75	0.58	0.26	0
24	0	-	0	-	0	0.09
31	0	4	0.95	0.80	0.35	0.09
Feb. 6	0	-	0	-	0.69	0.26
14	0	10	0.75	0.65	0	0.09
21	0	2	0.47	0.30	0	0.04
Mar. 1	0	14	0.30	0.30	0	0.09
14	0	-	0	-	0	0.04
Average	0	7.5	0.565	.467	0.12	0.05
Std. Deviation		4.3				

TABLE VIII: Ultimate Nitrogenous Demand*, Time Lag and River Water Temperature (incubated at 20°C) - Station 1.

* Data only used if there was some observed NOD.

Date	Temp (°C)	Time Lag (Days)	NOD (mg/l) observed	NOD calculated	NOD Theoretical NH_4 Dep.	NOD Theoretical NO_3^- build-up
Jan. 10	2.0	-	0.	-	0	0
18	3.5	9	0.70	0.72	0	0.04
24	4.5	-	0	-	0.04	0.17
31	7.0	7	0.56	0.56	0.69	0.13
Feb. 6	5.0	7	0.55	0.78	0.09	0.52
14	3.0	9	0.55	0.50	0	0.13
21	2.0	4	2.65	2.20	0.13	0.09
Mar. 1	1.5	11	0.80	0.65	0.09	0.04
10	0.5	6	0.17	0.17	0	0.04
Average	2.61	7.5	0.86	0.80	0.14	0.14
Std. Deviation		2.3				

TABLE IX: Ultimate Nitrogenous Demand*, Time Lag and River Water Temperature (incubated at 20°C) - Station 2

* Data only used if there was some observed NOD.

DATE	Temp (°C)	Time Lag (Days)	NOD (mg/l) observed	NOD calculated	NOD Theoretical NH_4 Dep.	NOD Theoretical NO_3^- Build-up
Jan. 10	1.5	9	0.15	0.16	0.39	0.22
18	2.0	12	0.20	0.25	0	0.09
24	1.5	-	0	-	0	0.09
31	3.0	-	0	-	0.18	0.30
Feb. 6	3.0	4	0.85	0.77	0.69	0.52
14	1.5	10	0.50	0.50	0.35	0.09
21	1	5	0.30	0.35	0.13	0.04
Mar. 1	0.5	12	0.52	0.53	0.17	0.04
10	0	7	0.55	0.65	0	0
Average	1.56	8.5	0.44	0.44	0.25	0.14
Std. Deviation		3.2				

TABLE X: Ultimate Nitrogenous Demand*, Time Lag and River Water Temperature (incubated at 20°C) - Station 3

* Data only used if there was some observed NOD.

4.1.3.3. Nitrogenous Reaction Rates

The nitrogenous reaction rates can be calculated similarly to the carbonaceous reaction rates provided that an assumption is made that nitrogenous reactions are first order reactions. If the reactions are first order the nitrogenous reaction rate would be temperature dependent. The nitrogenous reaction rates for each station from each sampling period are listed with the temperature and lag times in Tables XI, XII and XIII.

The nitrogenous reaction rates, assuming they are first order, can be evaluated using the same methods as those used for carbonaceous reactions. There are two methods which work out quite close to the actual observed results. The reaction rates determined using both methods are also shown in Tables XI, XII and XIII. A general comparison of all five methods is shown in Table XIV.

4.2. Simulated Laboratory Study at $20^{o}C$

The BOD and ammonia levels in the Assiniboine River at Brandon were low during the study period. These low results made it difficult to determine if the data was valid due to the inherent inaccuracy of the BOD test. A simulation study with spiked samples to obtain more reliable results was conducted to confirm the Assiniboine River data.

Sample Date	River Temp (°C)	Time Lag (Days)	Reaction Rate (Method) Thomas	Moments
Jan. 10	0	7	0.15	0.19
18	0	8	0.08	0.32
24	0	-	-	-
31	0	4	0.09	0.1
Feb. 6	0	-	-	-
14	0	10	0.20	0.21
21	0	2	0.24	0.11
Mar. 1	0	14	0.08	0.12
14	0	-	-	-
Average	0	7.5	0.14	0.175
Std. Deviation		4.3	0.07	0.08

TABLE XI: Nitrogenous Reaction Rates, Time Lag and River Water Temperatures - Station 1.

Sample	River Temp (°C)	Time Lag (Days)	Reaction Rates (Methods) Thomas	Moments
Jan. 10	2.0	-	-	-
18	3.5	9	0.08	0.09
24	2.5	-	-	-
31	4.0	7	0.11	0.11
Feb. 6	5.0	7	0.02	0.02
14	3.0	9	0.19	0.24
21	2.0	4	0.125	0.05
Mar. 1	1.0	11	0.125	0.12
14	0.5	6	0.22	0.14
Average	2.61	7.5	0.125	0.11
Std. Deviation		2.3	0.07	0.07

TABLE XII: Nitrogenous Reaction Rates, Time Lag and River Water Temperatures - Station 2.

Sample	River Temp (°C)	Time Lag (Days)	Reaction Rates (Methods) Thomas	Moments
Jan. 10	1.5	9	0.15	0.19
18	2.0	12	0.06	0.05
24	1.5	-	-	-
31	3.0	-	-	-
Feb. 6	3.0	4	0.08	0.08
14	1.5	10	0.18	0.20
21	1	5	0.04	0.04
Mar. 1	0.5	1.2	0.085	0.03
14	0	7	0.075	0.075
Average	1.56	8.5	0.095	0.095
Std. Deviation		3.2	0.05	0.07

TABLE XIII: Nitrogenous Reaction Rates, Time Lag and River Water Temperatures - Station 3.

Method	Reaction Rate	Ultimate NOD	Comment Theoretical vs. Observed
Thomas	0.15	0.2	Close Fit
Moments (no lag)	0.19	0.17	Close Fit
Slope	0.06	-	-
Daily Difference	-	-	-
Moments (lag)	0.17	0.14	Low
Std. Deviation	0.06	0.03	

TABLE XIV: Comparison of Various Methods of Determining Nitrogenous Reaction Rates (k_n). (Data from January 10, 1975).

4.2.1. Carbonaceous BOD and Reaction Rates - k_1

A total of eight different samples were obtained from the Red River to simulate a thermal discharge effect on the Assiniboine River. The BOD's and the k_1 rates for each sample are shown in Table XV. The BOD data was obtained at 17°C instead of 20°C due to the fact that the incubator's temperature was 3°C below the setting of the control gauge. A comparison of five different methods of determining the k_1 rates and the ultimate carbonaceous demand is shown in Table XVI.

4.2.2. Nitrogenous Demand

The nitrogenous demand was evaluated using four different determinations as discussed in section 4.1.3.2. The results for the first four samples are shown in Table XVII. The observed and calculated NODs were based on incubation at 17°C due to the incubator temperature control problem. Samples 1-4 for ammonia and nitrate analysis were stored in another incubator due to lack of space. The actual temperature of the samples in the second incubator was 20°C. This resulted in the theoretical NOD values being calculated at 20°C as indicated in Table XVII. The remaining four samples were all incubated at 17°C and the results were as shown in Table XVIII.

Sample	BOD_5 (17^{o}) (mg/l)	k_1 (17^{o})	k_1 (20^{o})
1	2.8	0.040	0.045
2	2.1	0.052	0.060
3	1.8	0.08	0.09
4	1.8	0.08	0.09
5	2.3	0.07	0.08
6	2.3	0.07	0.08
7	2.3	0.07	0.08
8	2.3	0.07	0.08
Average	2.2	0.067	0.076
Std. Deviation		0.01	0.02

TABLE XV: Laboratory Simulation - Carbonaceous k_1 Rates (Thomas Method)*(incubated at $17^{o}C$).

* $\theta = 1.047$ used to convert from $17^{o}C$ to $20^{o}C$.

Simulation using various methods at 20°C (May, 1975).

Method	k_1 value	L value	Comments Theoretical vs. Observed
Thomas	0.08	3.5	Close
Moments (no lag)	0.07	3.7	Close
Slope	0.09	4.3	High
Daily Difference	0.10	3.9	High
Moments (lag)	0.08	3.9	High
Std. Deviation	0.01	0.3	

TABLE XVI: Comparison of Various Methods of Determining k_1 Values (data from May, 1975).

Sample	Observed (17°C) (Total-Carbonaceous)	NOD (mg/l) calculated $y = L(1-10^{-k_{nt}})$	at 20°C Theoretical NH_4 Depletion	NO_3 Incr
1	4.7	4.2	7.2	7.3
2	5.1	6.2	6.7	6.1
3	5.2	6.1	6.2	7.3
4	5.5	6.5	6.3	4.8
Average	5.1	5.6	6.6	6.4
Std. Deviation	0.33	1.05	0.45	1.19

TABLE XVII: Comparison of NOD Values Obtained by four Different Means (incubated for 10 days at 20°C).

Sample	Observed (Total-Carbonaceous)	Calculated $y = L\ (1-10^{-k}nt)$	Theoretical NH_4 depletion	Theoretical NO_3 inc
5	4.5	5.3	2.3	4.8
6	5.8	7.5	1.7	4.3
7	4.7	5.2	2.4	4.3
8	5.1	5.8	3.9	3.5
Average	5.0	6.0	2.6	4.2
Std. Deviation	.57	1.07	0.94	0.54

TABLE XVIII: Comparison of NOD Values Obtained by four Different Means (incubated for 10 days at 17°C).

4.2.2.1. Nitrogenous Reaction Rates

The nitrogenous reaction rates were calculated as previously described in section 4.1.3.3. The reaction rates obtained using the most accurate two methods are shown in Table XIX along with the lag time until nitrification. The samples were incubated at 17°C but the reaction rates shown were converted to 20°C for ease of comparison to other researchers.

The simulated samples were evaluated using five methods. The reaction rates and ultimate NODs obtained using each method are shown in Table XX.

4.3. Simulated Laboratory Study at 5°C

4.3.1. Carbonaceous BOD and Reaction Rates

One set of eight simulated samples was incubated at 5°C while another set was incubated at 20°C. Two different incubators were necessary to store all the samples. The first four samples were incubated at 5°C while the remaining four were incubated at 3°C.

The BODs, k_1 rates and ultimate BOD on the first four samples were calculated using the moments method. The results are shown in Table XXI.

The last four samples were treated identically except for the temperature and the carbonaceous BODs and k_1 rates are shown in Table XXII.

Sample	Lag Time (Days)	NOD Reaction Rates (Methods Thomas	Moments)
1	4	0.14	0.13
2	5	0.09	0.09
3	4	0.11	0.10
4	4	0.11	0.09
5	3	0.125	0.12
6	3	0.09	0.085
7	3	0.125	0.16
8	3	0.13	0.13
Average	3.6	0.115	0.114
Std. Deviation	0.74	0.02	0.03

TABLE XIX: Nitrogenous Reaction Rates and Lag Time (incubated at 17°C converted to 20°C).

Method	Reaction Rate	Ultimate NOD	Comments
Sample I			
Thomas	0.14	4.4	Low
Moments (no lag)	0.13	5.3	Close
Slope	0.10	6.1	High
Daily Difference	0.19	6.1	High
Moments (lag)	0.08	6.9	High
Std. Deviation	0.04	0.95	
Sample VIII			
Thomas	0.09	7.1	Close
Moments (no lag)	0.09	7.8	Close
Slope	0.075	8.7	High
Daily Difference	0.13	8.8	High
Moments (lag)	0.075	7.1	Close
Std. Deviation	0.02	0.83	

TABLE XX: Comparison of Various Methods of Determining Various Nitrogenous Reaction Rates (data from samples I and VIII of the simulated study).

Sample	BOD_5	$BOD_{Ultimate}$	k_1
1	1.2	2.95	0.05
2	1.25	4.00	0.02
3	0.90	2.90	0.02
4	0.50	2.50	0.005
Average	0.96	3.10	0.024
Std. Deviation	0.34	0.64	0.02

TABLE XXI: Laboratory Simulation Carbonaceous k_1 Rates (incubated at $5^{o}C$).

Sample	BOD_5 mg/l	$BOD_{Ultimate}$	k_1
5	1.40	4.25	0.015
6	0.70	2.25	0.030
7	0.95	3.30	0.005
8	0.90	3.10	0.015
Average	1.0	3.2	0.016
Std. Deviation	0.29	0.82	0.01

TABLE XXII: Laboratory Simulation - Carbonaceous k_1 Rates (incubated at 3°C).

4.3.2. Nitrogenous Demand at 5°C and 3°C

The nitrogenous demand was calculated by the moments method described in section 4.1.3.3. The results of the four samples incubated at 5°C are shown in Table XXIII. The results of the four samples incubated at 3°C are shown in Table XIV.

4.3.2.1. Nitrogenous Reaction Rate

The nitrogenous reaction rates were evaluated using the best fitting of the five different methods previously compared in Table XX of the report.

The lag time, the observed NOD and the resulting reaction rate are listed in Tables XXV and XXVI, respectively, for the samples at 5°C and 3°C.

4.4. Nitrate Results

The laboratory at the Brandon generating station conducted weekly nitrate analyses on the Assiniboine River upstream and downstream of the thermal discharge point. Their results were compared to those obtained by the author of this study in Table XXVII. This comparison acts as a check on the nitrate values obtained during the study.

Sample	NOD observed (total BOD-carbonaceous BOD) (mg/l)	NOD calculated $Y=L(1-10^{-k}nt)$ (mg/l)	NOD theoretical NH_4 depl.	NO_3 inc.
1	0.6	0.65	0.65	0.45
2	0	0	0	0
3	1.3	1.90	0.90	1.9
4	1.8	1.20	0.25	1.3
Average	1.33	1.35	0.60	1.22
Std. Deviation	0.79	0.81	0.40	0.85

TABLE XXIII: Ultimate NOD Values (incubated at $5^{o}C$).

Sample	NOD observed (total BOD-carbonaceous BOD) (mg/l)	NOD calculated $Y=L(1-10^{-k}nt)$ (mg/l)	NOD theoretical NH_4 depl.	NO_3 inc.
5	0.7	1.30	1.50	0.90
6	0.45	0.35	1.4	0.90
7	0.25	0.65	1.3	1.3
8	0.40	0.90	0.9	2.0
Average	0.45	0.80	1.3	1.3
Std. Deviation	0.19	0.40	0.26	0.52

TABLE XXIV: Ultimate NOD Values at $3^{o}C$.

Sample	Time Lag (Days)	NOD (observed) (mg/l)	Reaction Rate
1	12	0.60	0.05
2	13	0	-
3	10	1.30	0.03
4	5	1.80	0.06
Average	9	1.33	0.047
Std. Deviation	3.6	0.79	0.02

TABLE XXV: Observed NOD, Time Lag and Reaction Rates (incubated for 20 days at 5°C).

Sample	Time Lag (Days)	NOD (observed) (mg/l)	Reaction Rate
5	16	0.70	0.03
6	10	0.45	0.01
7	18	0.25	0.03
8	18	0.40	0.08
Average	15.5	0.45	0.038
Std. Deviation	3.8	0.19	0.03

TABLE XXVI: Observed NOD, Time Lag and Reaction Rates (incubated for 20 days at $3^{o}C$).

Date	NO_3 Level Thesis Study		Manitoba Hydro	
	Station 1	Station 3	Station 1	Station 3
Jan. 10	0.28	0.35	0.28	0.28
18	0.26	0.26	0.90	0.30
24	0.32	0.32	1.10	0.40
31	0.40	0.38	0.45	0.40
Feb. 6	0.34	0.32	0.4	0.42
14	0.38	0.40	0.8	2.92
21	0.42	0.40	0.4	0.42
Mar. 1	0.44	0.43	0.48	0.50
14	0.52	0.52	0.45	0.45

TABLE XXVII: Thesis Study Nitrate Levels and Manitoba Hydro's Nitrate Levels in the Assiniboine River during January, February, and March of 1975.

CHAPTER V. DISCUSSION OF RESULTS

5.1. The Assiniboine River Study

5.1.1. Physical Effect on the Assiniboine River

The average river water temperature upstream of the thermal discharge was 0°C throughout the study period. This would be expected since during the months of January, February and March the Assiniboine River upstream of the thermal discharge was ice-covered. The ambient air temperatures were well below the freezing point, during the whole study.

The temperatures at station 2 ranged from 0.5°C to 5.0°C during the course of the study. The flow in the river was stable at around 440 c.f.s. during the three month period so that any temperature fluctuations would be due to the thermal discharge. The cold ambient temperatures would cause the temperature of the river water to decrease from the thermal outlet to station 3.

The maximum temperature of the cooling water as it discharged into the river was 18-19°C. This temperature was only reached when the generating station had been operating at full capacity for several hours.

The river flow in the approximately one thousand foot reach from the point of thermal discharge to station 2 was turbulent. One narrow channel carried most of the

flow due to ice formation in the shallow areas. The river water temperature at station 2 was assumed to be homogeneous and not affected by thermal plumes.

The river water temperatures at station 3 varied from $0^{o}C$ to $3^{o}C$ depending on the temperature and volume of the thermal discharge. The temperatures at station 3 were determined by extrapolating the temperatures at stations 2 and 5 and the accuracy was double checked by manually checking the temperature at station 3 for each set of samples. The correlation was reasonably close and the temperatures listed in Table I are assumed to be valid.

The river was open from station 1 right past station 5 during the January 10 through February 6 sampling periods but became partially ice-covered downstream of station 3 during the last three weeks of sampling.

5.1.1.1. Simulated Laboratory Study

The sample temperature was controlled throughout the simulated laboratory study. Several variations did occur due to the necessity of having to use more than one incubator. One incubator when set at $20^{o}C$ actually ran at a temperature of $17^{o}C$ causing four samples to be incubated at $17^{o}C$ and four at $20^{o}C$. A similar problem occurred at the low temperature incubation since one incubator ran at $5^{o}C$ and the other at $3^{o}C$. However, the main objective of the study was not disrupted but a wider range of data did result.

5.1.2. Effect on Dissolved Oxygen

The dissolved oxygen content of water is inversely proportional to the temperature. Velz (11), among others, states that the higher the temperature the lower the dissolved oxygen content. The dissolved oxygen content, under ice, in the Assiniboine River averaged 6.5 mg/l during the study period. A temperature rise from $0^{o}C$ to $5^{o}C$ was not enough to depress the dissolved oxygen content but rather caused an increase due to the reaeration which became possible with removal of the ice cover. The average dissolved oxygen content at station 2 was 8.0 mg/l and at station 3 it averaged 10 mg/l, reflecting the longer reaeration period.

5.1.3. Biochemical Oxygen Demand

The determination of the one, two, three, four, and five day BODs is necessary in order to draw the progression curves required to determine the reaction rate, k_1. The DO's for the study were determined according to section 218B of Standard Methods (22). The average BOD_5 at station 1 was one mg/l. The average BOD_{20} at station 1 was two mg/l.

The average BOD_5 and BOD_{20} at station 2 were 0.9 mg/l and 2.2 mg/l, respectively, while at station 3 the BOD_5 and BOD_{20} values averaged 0.8 and 1.9 mg/l, respectively.

The values are quite low and according to some researchers may be meaningless. However, the results are consistent for the several hundred BOD tests which were completed beginning on day one and done periodically until day twenty. On each sample the BOD progression curves (Figure IX) drawn using this data resemble the idealized monomolecular BOD curves as described by Zanoni (47) and shown in Figure III. The results although questionable, considering the accuracy range of the BOD test, were assumed to be a reasonable representation of the BOD in the Assiniboine River at Brandon during the study period.

The average BOD_5 decreases from station 1 to station 2 and to station 3 indicating that higher river water temperatures do not adversely affect the BOD_5 of the river. Donald (8) found that the thermal discharge has no effect on the BOD_5 of the Assiniboine River.

5.1.4. Carbonaceous Reaction Rates - k_1

The carbonaceous reaction rate - k_1 was determined using the method described by Thomas (26). The moments method described by Moore, et al. (27) is considered the easiest and best method of determining k_1 values (18, 24, 27). However, for the samples analyzed during the Assiniboine River study the Thomas (26) method appeared to agree more closely with observed values than other methods as indicated in Table VII.

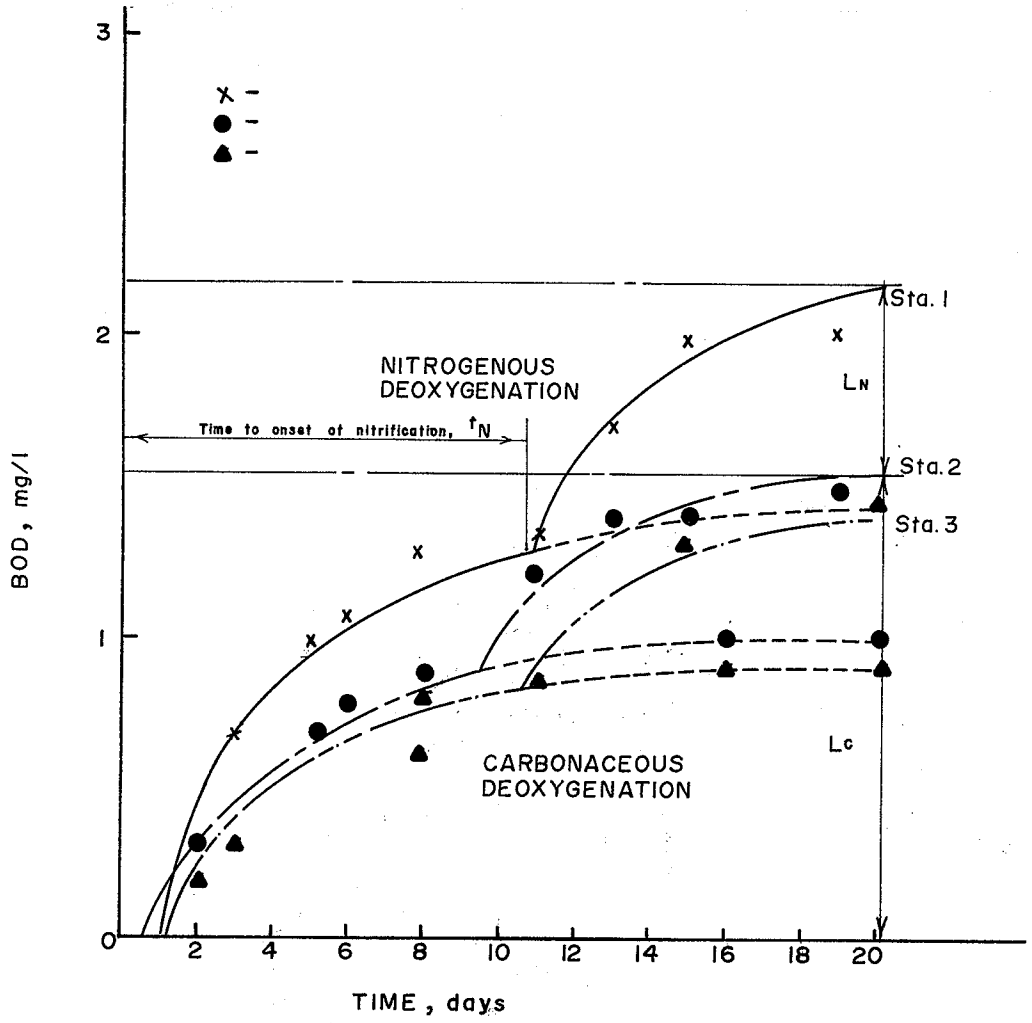

FIGURE IX: Example of Assiniboine River Data.

The k_1 values from station 1 averaged 0.108 with values ranging from 0.03 to 0.16 at an incubation temperature of 20°C. Stations 2 and 3 had average k_1 values of 0.104 and 0.098 respectively. These values are typical for river water as confirmed by Velz (11) and Ludzack, et al. (24) who state that the average k_1 value for river water incubated at 20°C is 0.1. Schroepfer, et al. (18) found k_1 values ranging from 0.003 to 0.146 with a mean value of 0.065 when incubated at 20°C.

According to Gannon (28) the actual k_1 values in a stream may be higher than those obtained under laboratory conditions because mixing takes place in a stream. The Assiniboine River at station 1, 2 and 3 in Brandon is turbulent and there are no sludge deposits or heavy vegetation in this stretch of the river. Therefore, good mixing takes place and the k_1 values obtained are probably lower than those actually existing in the river at Brandon.

The single most important factor affecting k_1 rates is temperature. Metcalfe and Eddy (7) state that the k_1 reaction rate is temperature dependent according to the Van't Hoff-Arrhenius rule which states that k_1 increases with temperature. The data given in Table V for stations 2 and 3 indicate that the k_1 rate is lower at lower river temperatures. However, the k_1 and river temperature data from station 1 do not conform to the rule. The effect of the varying river water temperature on the k_1 value is probably negated by the fact that all the

samples were incubated at 20°C.

Some researchers including Gannon (28) discuss the effect of substrate concentration on k_1 rates. The general thought being that k_1 rates increase with increasing substrate concentrations. The data presented in Table VI indicates that a higher substrate concentration does cause a higher k_1 rate. However, the differences in substrate concentration are slight and the decreasing k_1 rates may be coincidental.

5.1.5. Nitrogenous Demand

The Assiniboine River at Brandon has a slight nitrogenous demand. The NOD on incubated samples was always less than one mg/l during the winter study period. The NOD was determined in three different ways. The observed NOD was obtained by subtracting the theoretical carbonaceous demand from the total demand measured by the standard twenty day BOD test. The NOD was also calculated by using the nitrogenous part of the standard equation of Streeter and Phelps as described by Zanoni (47) in section 2.5.3.1. This requires a value for the nitrogenous reaction rate and an ultimate nitrogenous demand which are discussed in the next section.

The other way of determining the NOD is by measuring the ammonia depletion and/or the nitrate increase in the samples and then multiplying those results by the

theoretical oxygen demand required for conversion of ammonia to nitrite and then to nitrate. The chemistry of this nitrifying process is discussed in section 2.5.3.

Carroll (23), Wezernak and Gannon (35) and Young (48) agree that the theoretical NOD of one gram of ammonia completely converted to nitrate is 4.33 grams of oxygen. The results from the various ways of determining the NOD of the Assiniboine River samples are shown in Tables VIII, IX and X. The observed NOD and the calculated NOD show good correlation but the two theoretically calculated NODs do not correlate with each other or the practically determined NODs. One possible reason for the poor correlation is that the ammonia concentrations were so low that it was difficult to obtain accurate readings. Similarly due to the low ammonia concentrations the increase in nitrate was so slight that it was probably less than the accuracy of the test much of the time. New standards were run with each set of samples but some inaccuracy could have occurred due to the colour comparison type of test.

5.1.6. Nitrification Kinetics

5.1.6.1. Lag Time

There has been much questionning by various researchers as to exactly when nitrification begins. Knowles, et al. (45) and other researchers (52) state that the lag time is proportional to the number of nitrifying

organisms initially present in the sample. Zanoni (48) and Velz (11) state that the ammonia oxidation can occur simultaneously with the carbonaceous demand. The lag time before nitrification begins varied from two to fourteen days at station 1 and averaged 7.5 days. Station 2 results indicate a lag time of four to eleven days with an average of 7.5 days, while at station 3 the time lag varied from four to twelve days averaging 8.5 days.

It can be assumed that the number of nitrifyers present at stations 1, 2 and 3 would be the same. Although the water temperature was highest at station 2 the retention time was not enough to have any effect on the rate of multiplication of the organisms. The fact that nitrifyers are mesophilic (45) and follow the Van't Hoff Arrhenius rule means that the optimum temperatures are in the 25-30^{o}C range. Their activity at 5^{o}C and less would be low or non-existant. Courchaine (33) and Velz (11) agree that nitrification could begin at anytime after incubation but due to a low growth rate (doubling time greater than one day) it would not show in the BOD test until after five to twenty days and usually become apparent on the ninth or tenth day. The lag time for this study was much shorter than that indicated by the literature.

5.1.6.2. Nitrogenous Reaction Rates - k_n

The nitrogenous stage of deoxygenation can be

expressed as a monomolecular or first order reaction (47). Based on this assumption the nitrogenous reaction rate can be determined in a similar manner as the carbonaceous k_1 rate. The nitrogenous reaction rates for stations 1, 2 and 3 are shown along with the river temperature at the time of sampling and the lag time in Tables XI, XII and XIII. The k_n rates average 0.14, 0.125 and 0.095, respectively, for stations 1, 2 and 3. As discussed in the previous section, the length of exposure to the warmer temperatures downstream of the thermal discharge is not enough to cause greater numbers of nitrifying organisms and since they are all incubated at 20°C the reaction rates should be similar for each station. The results seem to indicate that the samples from the warmer station 2 has a higher k_n rate than the other two stations but station 3 is lower than stations 1 and 2.

A publication of the British Department of the Environment (52) states that the rate of nitrification is independent of the ammonia content unless it is below 3 mg/l. It also states that at 0.5 mg/l the reaction rate is one-half of the maximum. Since the ammonia concentration in the samples were always well below this level the reaction rates calculated would be less than one-half of the maximum possible in a stream with ammonia content greater than 3 mg/l.

5.1.6.3. Method of Determining the Nitrogenous Reaction Rate

The method used to evaluate the nitrogenous reaction rate in the study was similar to the one described by Zanoni (47). He felt that the moments method by Moore, et al. (27) was best suited. The moments method was compared to several other methods and checked for accuracy against the observed values. The comparison is shown in Table XIV and indicates that either the moments method (27) or the Thomas Method (26) give good results.

5.2. Simulated Laboratory Samples at 20°C

5.2.1. Carbonaceous k_1 Rates

The BOD progression curves were developed in the same manner as described previously in section 5.1.3. The calculated k_1 values from the simulation study are shown in Table XV. The temperature control on the incubator was reading incorrectly and the samples were incubated at 17°C instead of 20°C. This may explain the fact that the simulated k_1 values are lower than the k_1 values from the Assiniboine River. The different chemical properties of the Red River water could also cause different results. The k_1 values of the simulated samples average 0.08 at 20°C. The Van't Hoff Arrhenius rule was applied using $\theta = 1.047$. According to Benedict and Carlson (61) and Zanoni (47) θ may vary from 1.097 to 0.877 in the temper-

ature range of 10°C to 22°C. A θ = 1.047 was used in this study because it is the most quoted value for temperature around 20°C.

The Thomas method (26) and method of moments (27) were both used to calculate the reaction rate constants since they both give good correlation with actual observed conditions. A comparison between various methods is shown in Table XVI.

5.2.2. Nitrogenous Demand

The nitrogenous demand on the simulated sample were obtained in the manner described in section 5.1.5. The observed values were based on incubation at 17°C. The calculated values are higher than the observed perhaps indicating that the nitrogenous stage of deoxygenation does not conform to the Monod first order reaction.

The correlation between the theoretical NOD (ammonia and nitrate) and the calculated NOD using the first order equation is quite good. The samples were spiked with 2 mg/l of ammonia. The initial ammonia content was approximately 2 mg/l since very little was present in the river water samples. This level is well below the 3 mg/l concentration required for optimum nitrification rates as described by the British Department of the Environment (52). This could account for the discrepancies between the actual observed NOD and the

theoretical as shown in Table XVII.

A second set of samples was incubated at 17°C and analyzed for BOD, nitrate and ammonia. The results were different in that the observed NOD values are higher than the theoretical values although the theoretical values obtained based on nitrate increase are only slightly lower. The theoretical values obtained based on ammonia depletion are about one-half the observed values. This is probably due to the nature of the ammonia test where the results are inaccurate below 0.5 mg/l due to the limitations of the spectrophotometer as described in section 132B of Standard Methods (22).

The theoretical NOD values obtained at 20°C incubation are about one hundred and fifty percent higher than those at 17°C. This concurs with the findings of Stratton and McCarty (54) and Knowles, et al. (45) who report an approximate ammonia oxidation rate increase of 9.5% per degree centigrade in the range from 10 to 25°C. Zanoni (47) concluded that the ultimate NOD does not vary significantly with temperature and the simulated samples of this study concur with his findings.

The simulated laboratory sample analyses indicates that the nitrogenous demand can exceed the carbonaceous demand if adequate ammonia is available. Table XV indicates five day BODs in the range of 2 mg/l while TABLES XVII and XVIII indicate ten day BODs in excess of

5 mg/l or two hundred and fifty percent greater at 17°C, indicating that NOD can be a serious problem in a polluted river at higher temperatures.

5.2.2.1. Nitrogenous Reaction Rate

The nitrogenous reaction rates at 20°C average 0.115. This corresponds with the findings of other researchers (11, 34, 45, 47, 54) who report average nitrogenous reaction rates of 0.115 at 20°C. The lag time is also shown in Table XIX and averages approximately four days confirming the supposition that nitrification can occur simultaneously with carbonaceous deoxygenation. The probable reason for a lower lag time in the laboratory samples than the Assiniboine River samples is due to a higher population of nitrifyers in the Red River than in the Assiniboine River. This is expected since the sampling point on the Red River was downstream of the effluent discharge from the south end plant. In addition, the different chemical properties of the water could cause some variation in results.

5.2.2.2. Method of Determining NOD Reaction Rate

The reaction rate can be determined in a similar manner as the carbonaceous k_1 rate if it is assumed that the nitrogenous reaction follows Monods first order equation. There are several researchers who agree with Zanoni

(47) that this is the case. However, others like Busnell, et al. (56) disagree. The method based on the first order assumption was therefore continually checked to ensure that the values obtained for k_n and L_n agreed with actual observations. Table XX indicates that the moments method (27) consistently achieves a close correlation with actual conditions. This consistent correlation indicates that the first order equation can be used to adequately formulate the nitrogenous stage of deoxygenation as it occurred in the Assiniboine River during this study.

5.3. Simulated Laboratory Samples at Cold Temperatures

5.3.1. Carbonaceous BOD (5^oC and 3^oC)

The original intent was to incubate the cold temperature samples at 5^oC to simulate the maximum warming conditions caused by the thermal discharge. Due to a lack of space two incubators had to be used and the first four samples were incubated at 5^oC while the second four samples were incubated at 3^oC. The carbonaceous BODs at 5^oC averaged less than forty percent of the carbonaceous BODs at 20^oC. This is consistent with the Van't Hoff Arrhenius rule which states that the reaction rates is halved for a 10^oC drop in temperature from 10^oC. The ultimate BOD was not appreciably different which correlates with the findings of Zanoni (47) and others who state that the ultimate BOD is not temperature dependent.

5.3.1.1. Carbonaceous k_1 Rates ($5^{o}C$ and $3^{o}C$)

The carbonaceous k_1 rates average less than 0.02 as shown in Tables XXI and XXII. These values are in the expected range based on the work of Zanoni (47), Velz (11) and others. There is little difference between the k_1 rates found at $5^{o}C$ and $3^{o}C$.

5.3.2. Nitrogenous Demand ($5^{o}C$ and $3^{o}C$)

The nitrogenous demand was determined in the manner previously discussed in sections 5.1.5. and 5.2.2. The results tabulated in Tables XXIII and XXIV seem to indicate that some noticeable nitrification takes place below $5^{o}C$. The observed NOD values at $3^{o}C$ are less than half of the levels found at $5^{o}C$. There is poor agreement between the observed NOD and the theoretical NOD probably due to low ammonia content resulting in inaccurate results.

The optimum temperature range for nitrifyers is $25^{o}C$ to $30^{o}C$ (33, 35, 54). Zanoni (47) found the optimum temperature to be $27^{o}C$. The discrepancies are due to the fact that some researchers work with pure cultures of nitrifyers while others did tests on mixed cultures. However, many researchers (11, 33, 34, 47, 54, 55, 59, 61) agree that little or no nitrification occurs below $5^{o}C$.

The results of the Brandon study seem to indicate that some nitrification did occur at temperatures below 5°C. Landine (19) found no nitrification at 0.4°C which could be interpreted to indicate that he feels nitrification may occur right down to 0.4°C. Most of the research was done in a milder winter climate than western Canada during January, February and March and as Zanoni (47) states; virtually no work has been done on nitrification in streams at low temperatures. This may account for his assumption that little or no nitrification occurs at low temperatures.

5.3.2.1. Nitrogenous Reaction Rates (5°C and 3°C)

The nitrogenous reaction rates for incubation at 5°C and 3°C are shown in Tables XXV and XXVI, respectively. The average nitrification reaction rates are 0.047 and 0.038, respectively, at 5°C and 3°C. These rates are higher than those of Zanoni (47). He found the nitrification velocity constants at 5°C to be 0.034. The reaction rates at 20°C averaged 0.115 which is about a two hundred and fifty percent increase over a temperature increase of 15°C. This corresponds with the nine to ten percent increase per 1°C discovered by Stratton and McCarty (54), Knowles, et al. (45) and other workers (11, 34, 47). The findings of this study compared to other research falls well within the variation in rate constants found by various workers studying ammonia oxidation as

reported by Stratton and McCarty (54). The data from the simulated laboratory study seems to have a relatively close correlation with previous workers such as Zanoni (47) and Landine (19).

5.3.2.2. Lag Time

The lag time before nitrification became obvious averaged twelve days and seventeen days, respectively, at $5^{o}C$ and $3^{o}C$. This lag time is below that reported by Landine (19) who found a lag time of thirty days at $10^{o}C$. Zanoni (47) reports a lag time of twenty days at $10^{o}C$ and forty days at $5^{o}C$. The lag time at $5^{o}C$ was three to four times as long as at $20^{o}C$. Zanoni (47) reports the lag time at $5^{o}C$ to be six times that at $20^{o}C$.

The findings of the Assiniboine River study does agree with those of other researchers (58, 59, 60) in that temperature is an important controlling factor in all biological reactions and has a pronounced effect on the growth rate of nitrifying organisms.

5.4. Nitrate and Ammonia Levels in the Assiniboine River

The Manitoba Hydro Laboratory personnel (62) at the Brandon Generating Station checked weekly samples for nitrates and their results are tabulated in Table XXVII along with the results of this study. Generally,

the nitrate concentrations are very similar. They used a different method than the Brucine method and some discrepancy would be normal.

The Environmental Control Branch (63) also ran monthly nitrate and ammonia levels in the Assiniboine River at Brandon downstream of the Hydro plant. Their tests indicate nitrate levels in the same range as found in this study and ammonia levels averaging less than 0.02 mg/l. The fact that three independent groups obtained similar results lends authenticity to the findings of the study and the results are assumed to be valid.

CHAPTER VI. CONCLUSIONS

1. The thermal discharge from the Manitoba Hydro steam generating station at Brandon had no adverse downstream effect on the Assiniboine River during the study period of January, February and March of 1975.

2. The dissolved oxygen content, in the stretch of the Assiniboine River kept ice free by the thermal discharge, increased by an average of four milligrams per liter.

3. The five day carbonaceous biochemical oxygen demand at stations 1, 2 and 3 averaged 1.0, 0.9 and 0.8 mg/l respectively when the river water samples were incubated at $20^{o}C$.

4. The carbonaceous reaction rates at stations 1, 2 and 3, averaged 0.106, 0.104 and 0.098 respectively at $20^{o}C$ incubation.

5. The nitrogenous oxygen demand of the samples studied averaged 0.45 mg/l, 0.41 mg/l and 0.34 mg/l at station 1, 2 and 3, respectively, at $20^{o}C$ incubation.

6. The nitrogenous reaction rates averaged 0.133, 0.145 and 0.105 respectively, at stations 1, 2 and 3 at $20^{o}C$ incubation with an average lag time of eight days.

7. The carbonaceous reaction rates from the simulated laboratory study averaged approximately 0.08 at $20^{o}C$ incubation.

8. The nitrogenous reaction rates of the simulated samples averaged 0.115 at 20^{o}C with a lag time of about four days.

9. The carbonaceous reaction rates of the simulated samples at 5^{o}C and 3^{o}C averaged 0.024 and 0.016, respectively.

10. Nitrification appeared to occur at 5^{o}C and 3^{o}C in the simulation study. The nitrogenous reaction rates at 5^{o}C averaged 0.045 while at 3^{o}C the reaction rates averaged 0.035.

11. The lag time of the simulated samples incubated at 5^{o}C appeared to average twelve days while at 3^{o}C it averaged seventeen days.

CHAPTER VII. RECOMMENDATIONS FOR FUTURE WORK

1. The effect of cooling water discharge into the Assiniboine River should be studied under summer conditions to determine the effect of higher temperatures on biological oxidation, specifically nitrification.

2. A detailed study on nitrification (low temperatures) in the range from $5^{o}C$ to $0^{o}C$ should be carried out on samples containing more than three mg/l of ammonia.

3. Study the effect of open river conditions at Brandon on the quality of water reaching downstream users, specifically Portage la Prairie. It may be adviseable to by-pass cooling towers during winter conditions in northern climate.

4. A computer model should be built to simulate the effect of thermal discharge on a "cold climate" receiving stream. This could be accomplished quite easily since the temperature of the cooling water is known, the stream flow is usually available, the chemical and biological nature of the stream is usually available from control agencies and the reaction rates can be obtained from the literature. Such a model would have wide application in the future since various electrical power generating agencies are seriously looking at nuclear plants which require large volumes of cooling water.

5. A more meaningful and accurate test than the BOD test should be developed for this type of work.

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