NITRIFYING BIOMASS AND KINETICS IN CARBON REMOVAL - NITRIFICATION SYSTEMS

by

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ABSTRACT

Although several dynamic models of the nitrification process have been developed in recent years, application of this work to full scale design and operation has been limited. This is primarily due to the difficulties associated with accurate estimation of the several kinetic parameters required in these models. An examination of the literature indicated that differences in system SRT (Solids Retention Time) and influent carbon to nitrogen ratio (C:N) contribute to the wide diversity in reported nitrifier kinetics. This dissertation examines the degree to which these effects may be quantified for a combined carbon removal - nitrification process.

A kinetic parameter, batch estimation technique was proposed which allows the measurement of nitrifier kinetics in the presence of heterotrophic microorganisms. The method utilizes a pure culture activity equivalent approach, and requires a specific nitrification inhibitor to differentiate between heterotrophic and autotrophic nitrogen dynamics measured _in situ_. This technique was found to give satisfactory results at several levels of nitrifier biomass fraction.

The parameter estimation technique was then applied to

laboratory scale extended aeration plants operated at a number of SRT and influent C:N levels. Parameter values determined under these conditions were summarized by empirical regression equations to quantify the SRT and C:N effects. Arrhenius temperature coefficients for pure nitrifiers at several SRTs were also determined.

The equations relating SRT, C:N and temperature effects were then incorporated into a dynamic mechanistic model of the suspended growth carbon removal-nitrification process. Following verification against steady state and transient data from independent sources, it was concluded that the model adequately predicts mixed culture nitrification rates without prior parameter calibration.

Several simulation runs were then utilized to illustrate that previously observed C:N effects were the result of altered nitrifier kinetics. However, reported SRT effects could be attributed to autotrophic-heterotrophic population dynamics.

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"The meaning of this degree is that the recipient of instruction is examined for the last time in his life, and is pronounced completely full. After this, no new ideas may be imparted to him."

Stephen Leacock

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1. INTRODUCTION

In recent years nitrogenous compounds have been of considerable interest to those concerned with environmental management. This has resulted from the recognition that nitrogen can have significant and deleterious consequences not only for the environment, but also for public health. Some of these undesirable effects are listed by the U.S. Environmental Protection Agency (1975) as:

(i) depletion of dissolved oxygen in receiving waters,

(ii) stimulation of eutrophication in receiving waters,

(iii) toxicity of ammonia to aquatic life,

(iv) increased chlorine requirements for adequate wastewater disinfection,

(v) association of nitrates in drinking water with infant methemoglobinemia, and

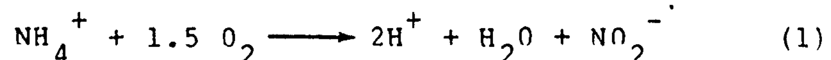
(vi) reduction in suitability of water for industrial reuse.

Of the known sources contributing nitrogen to the environment, the activities of man constitute a major proportion, primarily through wastewater discharge and run-off from urban and agricultural lands. Data reported

for the San Francisco Bay basin indicate that wastewater effluents, both municipal and industrial, contain 79% of the total nitrogen discharged (U.S. EPA, 1975). This illustrates that nitrogen control for wastewaters should be a priority consideration in any overall management strategy.

A variety of nitrogen removal processes are now available (Sutton, Murphy and Dawson, 1975). Physico-chemical treatment alternatives include breakpoint chlorination, ion exchange and air stripping. For biological treatment, complete nitrogen elimination can be effected through nitrification-denitrification. Biological nitrogen control is easily adapted to existing activated sludge plants which were originally designed solely for carbon removal. Therefore biological nitrification-denitrification has become the most popular treatment alternative for municipal and industrial wastewater.

Nitrification-denitrification consists of two distinct processes. The first, nitrification, is itself a two step reaction involving different genera of obligate autotrophic nitrifying bacteria. Nitrosomonas refers to that group capable of oxidizing ammonium to nitrite. Neglecting cell synthesis, the nitritification reaction is represented by:



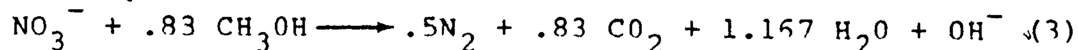
$$NH_4^+ + 1.5\ O_2 \longrightarrow 2H^+ + H_2O + NO_2^- \qquad (1)$$

Nitrobacter are able to oxidize nitrite further to nitrate:

$$NO_2^- + .5O_2 \longrightarrow NO_3^- \quad (2)$$

The nitratification reaction again neglects synthesis. Most of the nitrogen in a municipal wastewater is in the ammonium form. A smaller proportion exists as organic nitrogen. This can be metabolized by microorganisms to produce ammonium.

The second process, biological denitrification, is carried out under anaerobic conditions by a broad variety of facultative heterotrophic bacteria and results in the reduction of nitrate to nitrogen gas. For the particular case where methanol serves as the electron donor and neglecting synthesis the reaction is:



$$NO_3^- + .83\ CH_3OH \longrightarrow .5N_2 + .83\ CO_2 + 1.167\ H_2O + OH^- \quad (3)$$

The degree of nitrogen removal obtained by denitrification depends directly on the extent of nitrification achieved. As nitrifying bacteria have substantially lower growth rates than the heterotrophs (Lawrence and McCarty, 1970) and are more sensitive to environmental conditions, nitrification appears to be the limiting step in the overall process.

Sufficient information is now available to provide a

rational basis for the design of a nitrifying system (Focht and Chang, 1975; U.S. EPA, 1975; Sutton, Murphy and Jank, 1975; Adams and Eckenfelder, 1977). In addition several models for the nitrifying activated sludge process have been proposed (Poduska and Andrews, 1975; Gujer, 1977; Murphy, Sutton and Jank, 1977) to indicate the effects of changing influent conditions on the quality of the effluent.

As with any biochemical process, successful design and operation of a nitrification system requires adequate knowledge of the microbial kinetics involved. In a combined carbon removal - nitrification system the majority of the biomass is heterotrophic. At the present time there is no practical method to distinguish nitrifying bacteria from heterotrophs in a mixed culture. Therefore when kinetic studies are carried out, nitrifier concentrations must be measured indirectly by equating nitrification rates in mixed culture to a given concentration of autotrophs in pure culture. To date, this has been done for a narrow range of waste strengths and operating conditions. The resulting kinetic parameters are utilized in design models assuming that:

(i) parameter values remain constant under all conditions, and

(ii) there are no effects of heterotrophic growth on nitrifier activity in mixed culture.

Recently evidence has been reported which suggests that neither of these assumptions are valid.

Nitrifying activity is known to be dependent on dissolved oxygen (Nagel and Haworth, 1969), pH (Srinath, Prakasam and Loehr, 1976) and temperature (Knowles, Downing and Barrett, 1965). Various models have been proposed to predict this behaviour (U.S. EPA, 1975). More subtle variations were noted by Downing (1977), who speculated that nitrifier kinetics are altered by waste strength. On the question of heterotrophic-autotrophic interaction, Pan and Umbreit (1972) and Hockenbury, Daigger and Grady (1977) showed that nitrifier activity may be enhanced in mixed cultures. If effects of this type could be quantified and modelled it would permit the application of nitrification design and modelling to a wider variety of conditions.

This study has undertaken the development of a technique for the in situ estimation of nitrifier concentrations and kinetics in mixed culture. The method has been applied to a nitrifying extended aeration system operated at a number of different conditions. Empirical models have been constructed to predict the behaviour of the resulting kinetic parameters. Finally a mathematical model of the combined carbon removal - nitrification process has been proposed and utilized to investigate previously observed effects in process response.

2. BACKGROUND

2.1. Microbiology of Nitrification

2.1.1 Organisms and reactions

In a biological nitrogen removal system, autotrophic nitrification is a central component in the overall reaction network (Figure 1). The term autotrophic nitrification refers to the oxidation of ammonium to nitrate by organisms which derive their energy from this process. This is in contrast to heterotrophic metabolism in which organic compounds are oxidized for energy production. Both autotrophic and heterotrophic bacteria are thought to be capable of nitrification. However, it is generally held that autotrophic bacteria assume the major role in naturally occurring nitrification. For a more comprehensive examination of nitrification with emphasis on the aquatic environment, the reader is referred to reviews by Painter (1970), (1977) and Sharma and Ahlert (1977).

The list of autotrophic nitrifiers known to be associated with nitrification in natural environments is short. There are five genera of ammonium oxidizers and three genera of nitrite oxidizers (Table 1). Nitrification of sewage is thought to result primarily from the action of

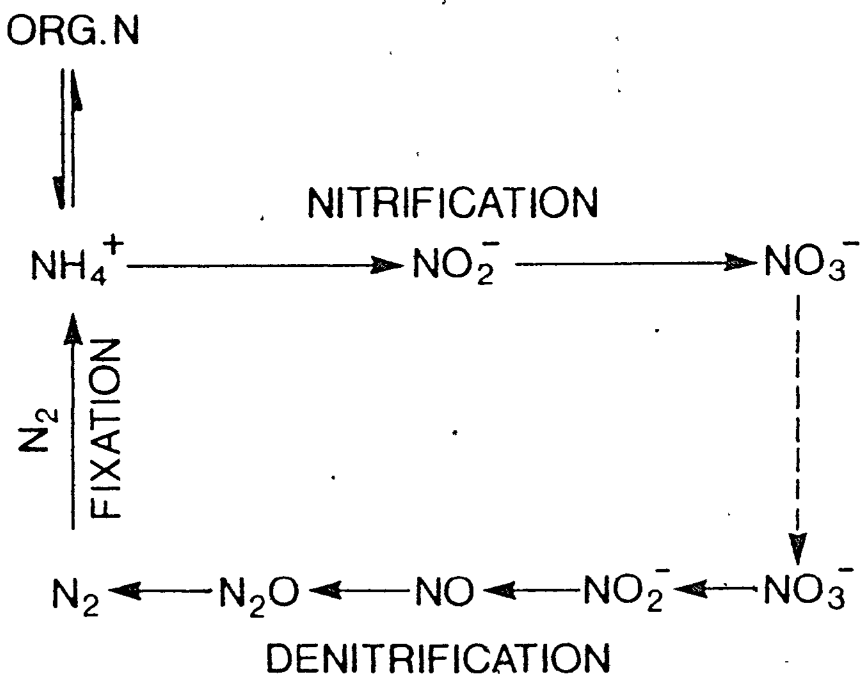


Figure 1. Pathways of Inorganic Nitrogen Metabolism

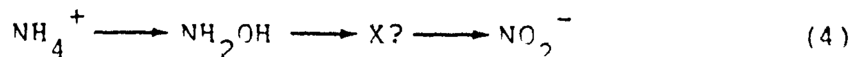
TABLE 1. Listing of Chemoautotrophic Nitrifiers from Schmidt (1978)

GENUS	SPECIES	HABITAT
	(Oxidize ammonia to nitrite)	
Nitrosomonas	europaea	soil,water,sewage
Nitrosospira	briensis	soil
Nitrosococcus	nitrosus	marine
	oceanus	marine
	mobilis	soil
Nitrosolobus	multiformis	soil
Nitrosovibrio	tenuis	soil
	(Oxidize nitrite to nitrate)	
Nitrobacter	winogradskyi*	soil
	(agilis)*	soil,water
Nitrospina	gracilis	marine
Nitrococcus	mobilis	marine

* N. winogradskyi is comprised of at least 2 serotypes, one of which has been traditionally (prior to the 8th edition of Bergey's Manual) referred to as N. agilis.

Nitrosomonas europaea, Nitrobacter winogradskyi and Nitrobacter agilis, although a restriction to these three species is uncertain (Painter, 1977). The use of generic names allows the simplified grouping of all nitrifiers into two classifications. The terms Nitrosomonas and Nitrobacter refer to those groups capable of oxidizing ammonium and nitrite respectively.

The mechanism of ammonium oxidation by Nitrosomonas is not completely understood. The overall reaction is thought to occur in three steps (Lees, 1954), with the first step being the formation of hydroxylamine (NH_2OH):

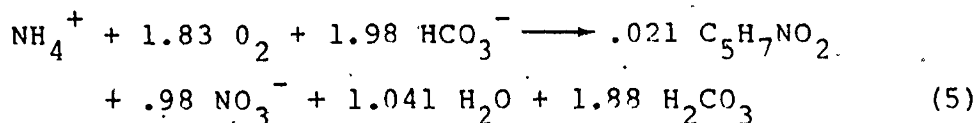


$$NH_4^+ \longrightarrow NH_2OH \longrightarrow X? \longrightarrow NO_2^- \quad (4)$$

Several researchers have presented convincing evidence that NH_2OH is an intermediate (Painter, 1977), but the identity of the second oxidation product (X?) is unknown. No intermediates have been found in the oxidation of nitrite to nitrate (Equation 2).

The free energy released by autotrophic nitrification is between 58 and 84 kcal·mole^{-1} for ammonium oxidation and between 15.4 and 20.9 kcal·mole^{-1} for nitrite oxidation. The energy liberated is then coupled to inorganic carbon assimilation or adenosine triphosphate (ATP) synthesis through an electron transport chain (Kiesow,

1972). Because Nitrosomonas is able to extract more energy per mole of nitrogen oxidized than is Nitrobacter, the mass of additional Nitrosomonas synthesized per mole of nitrogen oxidized is also larger than Nitrobacter. This fact is borne out in later discussion of cell yields. In autotrophic metabolism only inorganic carbon is used for cell synthesis. Assuming an empirical formulation of $C_5H_7NO_2$ for a bacterial cell, an overall synthesis-oxidation reaction for nitrification may be written as (U.S. EPA, 1975):



$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^- \longrightarrow .021\ C_5H_7NO_2 + .98\ NO_3^- + 1.041\ H_2O + 1.88\ H_2CO_3 \quad (5)$$

2.1.2 Nutritional and environmental requirements

Perhaps due to the limited quantities of energy released through autotrophic metabolism, nitrifying bacteria grow very slowly in comparison to heterotrophs. This, in addition to difficulties associated with the maintenance of nitrifiers in pure culture has prevented the establishment of their nutritional requirements in detail. Sharma and Ahlert (1977) have compiled an exhaustive list of substances that have been demonstrated to be necessary or stimulatory to nitrifier activity. The list includes macronutrients (nitrogen and phosphorus), trace metals, salts, vitamins and several organic compounds such as nucleosides, amino acids and glucose. There is some doubt about the necessity of

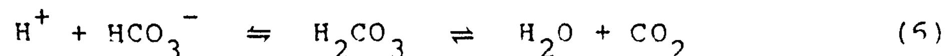
organics for nitrifer growth. However it is known that many organic molecules can permeate the cell wall of Nitrobacter, but none were observed to replace inorganic carbon in cellular synthesis (Ida and Alexander, 1965, cited by Painter, 1970).

For the oxidation of inorganic nitrogen, Nitrosomonas and Nitrobacter are obligate aerobes. As seen from Equations 1 & 2, the stoichiometric oxygen requirements are 3.43 mg $O_2 \cdot mg^{-1}$ N for NH_4 oxidation and 1.14 mg $O_2 \cdot mg^{-1}$ N for NO_2 oxidation. There appears also to be a minimum dissolved oxygen concentration required for nitrification (Boon and Laudelot, 1962; Downing, Painter and Knowles, 1964; Loveless and Painter, 1968; Stenstrom and Poduska, 1980). Activity ceases when the dissolved oxygen in the bulk liquid is somewhat less than 1 mg $\cdot L^{-1}$. For practical purposes it is usually assumed that if the dissolved oxygen is greater than 2 mg $\cdot L^{-1}$ no limitation should occur. However, Heukelekian (1942) has indicated that even with a bulk liquid concentration of 4 or 5 mg $\cdot L^{-1}$ the interior of individual flocs may still be anaerobic due to diffusion limitations.

Nitrifying bacteria are active over a wide pH range with an optimum between 7.0 and 9.0 (Engel and Alexander, 1958; Wild, Sawyer and McMahon, 1971). The diversity in reported pH optima has often been attributed to acclimation.

Haug and McCarty (1972) and Stankewich (1972) reported that nitrifying organisms grow preferentially at pH 7.0 to 8.0, but also that a ten day acclimation at pH 6.0 resulted in a return to optimal growth rates. It has been proposed that pH inhibition may depend upon the $NH_4^+ - NH_3$ and $NO_2^- - HNO_2$ equilibria (Anthonisen et al., 1976; Neufeld, Hill and Adekoya, 1980) which in turn are pH dependent. Above and below the optimal pH range, the formation of free ammonia (NH_3) and free nitrous acid (HNO_2) respectively may occur. The unionized forms are thought to permeate nitrifier cells readily; a property which may make them more toxic than the ions.

Hydrogen ions (H^+) are a product of ammonium oxidation (Equation 1). If continuous nitrification is desired, this acidity must be neutralized to maintain the pH above 6.0. Combination of H^+ with bicarbonate alkalinity in the wastewater (Equation 6) and subsequent stripping of CO_2 by aeration tends to maintain pH values near neutrality.



$$H^+ + HCO_3^- \leftrightharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2 \qquad (6)$$

From Equation 5 the stoichiometric alkalinity requirement is about 7.1 mg as $CaCO_3$ per mg of ammonium nitrogen oxidized. In situations where the available alkalinity is less than 8 to 10 times the NH_4-N undergoing nitrification, it may be

necessary to provide additional neutralization capacity.

The rate of any biologically mediated process is a strong function of temperature. For nitrification, pure culture studies have shown that the optimum growth temperature for _Nitrosomonas_ is in the range 30-36°C with lower and upper limits of 4°C and 45°C respectively (Buswell _et al._, 1954). The _Nitrobacter_ optimum is between 35-42°C (Painter, 1977). Temperatures in the optimum range for nitrifiers are seldom encountered in wastewater in temperate climates (Lin and Heinke, 1977). Consequently temperature is often a controlling factor for nitrification. Sharma and Ahlert (1977) reported that serious difficulties may arise during the operation of a biological nitrification system when wastewater temperatures are below 8.3°C.

Nitrifying bacteria are subject to inhibition by a variety of substances. Both _Nitrosomonas_ and _Nitrobacter_ are known to be sensitive to their own substrate and to that of the other organism (Anthonisen _et al._, 1976). Inhibiting concentrations are generally much higher than the levels associated with municipal sewage, but substrate inhibition may be significant in the treatment of highly nitrogenous industrial wastewater.

Organic matter generally is no longer thought to be inhibitory to nitrifying bacteria (Painter, 1977). Nevertheless inhibition has been linked to a large number of

specific organic compounds (Tomlinson, Boon and Trotman, 1966; Hockenbury and Grady, 1977). Some of these substances were reported to interfere with the electron transport chain; perhaps by chelation of an essential element like copper (Campbell and Aleem, 1965a). While a number of metals are known to be toxic to nitrifiers (Sharma and Ahlert, 1977), at lower concentrations many of them are also essential micronutrients.

2.2 Design and Modelling for Nitrification

2.2.1 Kinetics and models

The most popular model utilized to describe microbial sustrate removal is that of Monod (1949):

$$k = \frac{1}{X} \cdot \frac{dS}{dt} = k_{max} \cdot \frac{S}{K_s + S} \tag{7}$$

where:
- X is the microorganism concentration ($mg \cdot L^{-1}$),
- S is the substrate concentration ($mg \cdot L^{-1}$),
- k is the specific substrate removal rate ($time^{-1}$),
- k_{max} is the maximum specific removal rate ($time^{-1}$),
- K_s is the half-velocity coefficient for substrate ($mg \cdot L^{-1}$).

This expression has been applied to nitrogen oxidation by nitrifying bacteria by many authors (Downing, Painter and Knowles, 1964; Melamed, Saliternik and Wacks, 1970; Poduska and Andrews, 1975; Williamson and McCarty, 1975; Gujer, 1977; Marais

and Ekama, 1976; Ekama and Marais, 1977). The Monod expression provides for a continuous transition between first and zero order kinetics based on substrate concentration. Experimentally determined values of K_s for nitrification are much smaller than the nitrogen concentrations usually found in wastewater. Under these conditions the model reduces to a zero order expression:

$$k = -\frac{1}{X}\cdot\frac{dS}{dt} = k_{max} \tag{8}$$

Several researchers have confirmed that the rate of nitrification is independent of the mixing regime and the initial substrate concentration and hence is zero order as predicted by Equation 8.

Monod kinetics also may be used to describe microbial growth:

$$\mu = \frac{1}{X}\cdot\frac{dX}{dt} = \mu_{max}\cdot\frac{S}{K_s+S} \tag{9}$$

where: μ is the specific growth rate ($time^{-1}$),

μ_{max} is the maximum specific growth rate ($time^{-1}$), and

K_s is the half-velocity coefficient ($mg\cdot L^{-1}$).

Stratton and McCarty (1967) introduced a decay term (k_D) to represent the loss of viable organisms by endogenous metabolism when substrate concentrations are small:

$$\mu = \frac{1}{X}\cdot\frac{dX}{dt} = \mu_{max} \cdot \frac{S}{K_s+S} - k_D \tag{10}$$

where: k_D is the decay coefficient ($time^{-1}$)

Substrate removal (Equation 7) and organism growth (Equation 10) are related through:

$$\mu = \frac{Y}{X}\cdot\left(\frac{-dS}{dt}\right) - k_D \tag{11}$$

where Y = yield coefficient (mg cells · mg^{-1} substrate)

For steady state design of a carbon removal-nitrification system it is necessary that the overall growth rate of the biomass be maintained at a value which prevents washout of the slowly growing nitrifiers. This is done by adjusting the sludge age or solids retention time (SRT) of the process. Sludge age is the average retention time of microorganisms in the system and is defined by Lawrence and McCarty (1970) for steady state conditions as:

$$SRT(days) = \frac{\text{mass of solids carried in system}}{\text{mass of solids wasted per day}} \tag{12}$$

The relationship between the minimum required SRT (SRT_c) and nitrifier growth is under non-limiting substrate conditions given by:

$$SRT_c = \frac{1}{\mu_{max}} \tag{13}$$

where: μ_{max} is the maximum specific nitrifier growth rate under specified environmental conditions.

A number of dynamic mathematical models of the nitrification process have been proposed (Stratton and McCarty, 1967; Lijklema, 1973; Poduska and Andrews, 1975; Gujer and Jenkins, 1975; Gujer, 1977; Murphy, Sutton and Jank, 1977; Ekama and Marais, 1977). With one exception all of the models are mechanistic and are based upon Monod kinetics for substrate oxidation and nitrifier growth. Murphy, Sutton and Jank (1977) used linear transfer function models to describe time dependent behaviour, together with linear time series models to account for unexplained output behaviour. Agreement between model prediction and experimental observation appeared satisfactory for all of the models, but their application to nitrification design still is limited. To date these models have been developed and verified over a restricted range of conditions. Further, the accurate estimation of nitrifier kinetic parameters in mixed cultures of heterotrophs and autotrophs has proved difficult.

To provide a basis for more extensive study of the carbon removal-nitrification process, a dynamic mechanistic model of the system was developed (Figure 2). This model was adapted from that of Sutton (1976) who extended the work of Poduska and Andrews (1975) by including the effect of NH_4 uptake by heterotrophic bacteria. Details of model derivation and computer

$$\frac{dS_{1,2}}{dt} = \frac{1}{V_1}\cdot(Q_1\cdot S_{1,1} + Q_{15}\cdot S_{1,15} - Q_2\cdot S_{1,2}) - k^1_{max}\frac{S_{1,2}\cdot X_{1,2}}{K^1_s + S_{1,2}}$$

$$\frac{dS_{2,2}}{dt} = \frac{1}{V_1}\cdot(Q_1\cdot S_{2,1} + Q_{15}\cdot S_{2,15} - Q_2\cdot S_{2,2}) - k^2_{max}\frac{S_{2,2}\cdot X_{2,2}}{K^2_s + S_{2,2}} - f\cdot Y^1\cdot k^1_{max}\frac{S_{1,2}\cdot X_{1,2}}{K^1_s + S_{1,2}}$$

$$\frac{dS_{3,2}}{dt} = \frac{1}{V_1}\cdot(Q_{15}\cdot S_{3,15} - Q_2\cdot S_{3,2}) + k^2_{max}\frac{S_{2,2}\cdot X_{2,2}}{K^2_s + S_{2,2}} - k^3_{max}\frac{S_{3,2}\cdot X_{3,2}}{K^3_s + S_{3,2}}$$

$$\frac{dS_{4,2}}{dt} = \frac{1}{V_1}\cdot(Q_{15}\cdot S_{4,15} - Q_2\cdot S_{4,2}) + k^3_{max}\frac{S_{3,2}\cdot X_{3,2}}{K^3_s + S_{3,2}}$$

$$\frac{dX_{1,2}}{dt} = \frac{1}{V_1}\cdot(Q_1\cdot X_{1,1} + Q_{15}\cdot X_{1,15} - Q_2\cdot X_{1,2}) + \left(\frac{Y^1\cdot k^1_{max}\cdot S_{1,2}}{K^1_s + S_{1,2}} - k^1_D\right)\cdot X_{1,2}$$

$$\frac{dX_{2,2}}{dt} = \frac{1}{V_1}\cdot(Q_{15}\cdot X_{2,15} - Q_2\cdot X_{2,2}) + \left(\frac{Y^2\cdot k^2_{max}\cdot S_{2,2}}{K^2_s + S_{2,2}} - k^2_D\right)\cdot X_{2,2}$$

$$\frac{dX_{3,2}}{dt} = \frac{1}{V_1}\cdot(Q_{15}\cdot X_{3,15} - Q_2\cdot X_{3,2}) + \left(\frac{Y^3\cdot k^3_{max}\cdot S_{3,2}}{K^3_s + S_{3,2}} - k^3_D\right)\cdot X_{3,2}$$

Figure 2. Material Balance Equations for NITOX Aeration Tank Model.
(Complete model development and definition of symbols is given in Appendix C)

simulation are presented in Appendix C.

2.2.2 Model parameters

The utilization of the process model (Figure 2) in computer simulation requires prior estimation of the initial values for the differential equations and of all the kinetic parameters. The determination of most of the initial values is straightforward. Initial substrate concentrations can be measured directly by chemical techniques. The initial concentration of heterotrophic bacteria can be approximated by a suspended solids measurement. However no simple method exists for the measurement of nitrifier kinetic parameters in a mixed culture. Nitrifying bacteria are indistinct morphologically from heterotrophs and consequently autotrophic populations must be determined indirectly. Unfortunately, the methods used to estimate nitrifier concentrations require several simplifying assumptions which limit the usefulness of the resulting models.

Stratton and McCarty (1967) performed a batch kinetic run and simultaneously determined rate constants and initial nitrifier populations by a least squares analysis, resulting in highly correlated estimates. Poduska and Andrews (1975) performed short term simulations and assumed that nitrifier concentrations were constant for the duration of the run. Initial populations were established by applying an optimization routine to the results of a forcing test. Gujer (1977) defined a nitrifier mass balance in terms of specific nitrification rate

($\hat{q}$) which was considered to be a function only of temperature. A previously determined regression equation was used to calculate an initial value for $\hat{q}$ at a given system temperature.

The kinetic parameters required for computer simulation are those of the Monod expression (k_{max}, K_s, Y and k_D). The literature contains several sets of values for each parameter. Regression equations are also available for the prediction of k_{max} and K_s as functions of temperature (Knowles, Downing and Barrett, 1965). Poduska (1973) and Lijklema (1973) analyzed the sensitivity of their models to variation in the Monod constants and found that the maximum specific growth rate constant (μ_{max}) is by far the most critical parameter. Poduska also noted that the yield coefficient (Y) showed the same degree of sensitivity as μ_{max} for modelling substrate removal. This results from the relationship between substrate removal and growth rate constants, which under conditions of negligible endogenous respiration reduces to:

$$\mu_{max} = Y \cdot k_{max} \tag{14}$$

Table 2 indicates the wide range of values for μ_{max} and Y that have been reported in the literature for Nitrosomonas and Nitrobacter. The variability is probably caused by the differing culture conditions under which the measurements were made. Some of this variability is accounted for by temperature differences.

TABLE 2. Reported values of cell yield and maximum specific growth rate for nitrifying bacteria*

PARAMETER	NITROSOMONAS	NITROBACTER	HETEROTROPHS
Substrate	NH_4-N	NO_2-N	BOD_5
Y (mg VSS·mg^{-1} substrate)	.03 - .15	.02 - .08	.39 - .79
μ_{max} (day^{-1})	.45 - 2.2	.28 - 1.4	7.2 - 17.0
k_{max} (day^{-1})+	3.0 - 73.3	3.5 - 72.	9.1 - 46.

* from Sharma and Ahlert (1977); also includes results of Beccari, Marani and Ramadori (1979).

+ approximate values calculated from Equation 12 using ranges of Y and μ_{max} given above.

Knowles, Downing and Barrett (1965) found that the maximum specific growth rate of Nitrosomonas increased by 9.5 per cent for each degree celcius increase in temperature. For Nitrobacter, the increment was 5.9 per cent.

Knowledge of temperature alone is insufficient for accurate prediction of nitrification rate constants. The ratio of carbon to nitrogen (C:N) in the influent strongly influences mixed culture nitrification rates (Figure 3). At higher ratios of BOD_5/TKN the observed temperature sensitivity is increased. Sutton (1976) used the Arrhenius relationship to model temperature effects on nitrification:

$$K = Ae^{-E/RT} \tag{15}$$

where: K is the reaction rate constant (day^{-1}),

A is the frequency factor (day^{-1}),

E is the activation energy ($cal \cdot g\text{-}mole^{-1}$),

R is the universal gas constant ($cal \cdot g\text{-}mole^{-1} \cdot {}^{o}K^{-1}$), and

T is the temperature (${}^{o}K$).

At three different SRT's, separate Arrhenius models were found to adequately describe the effects of temperature (Figure 4). Temperature sensitivity was considerably greater at shorter SRT's. Sutton attributed the observations of Figure 4 to

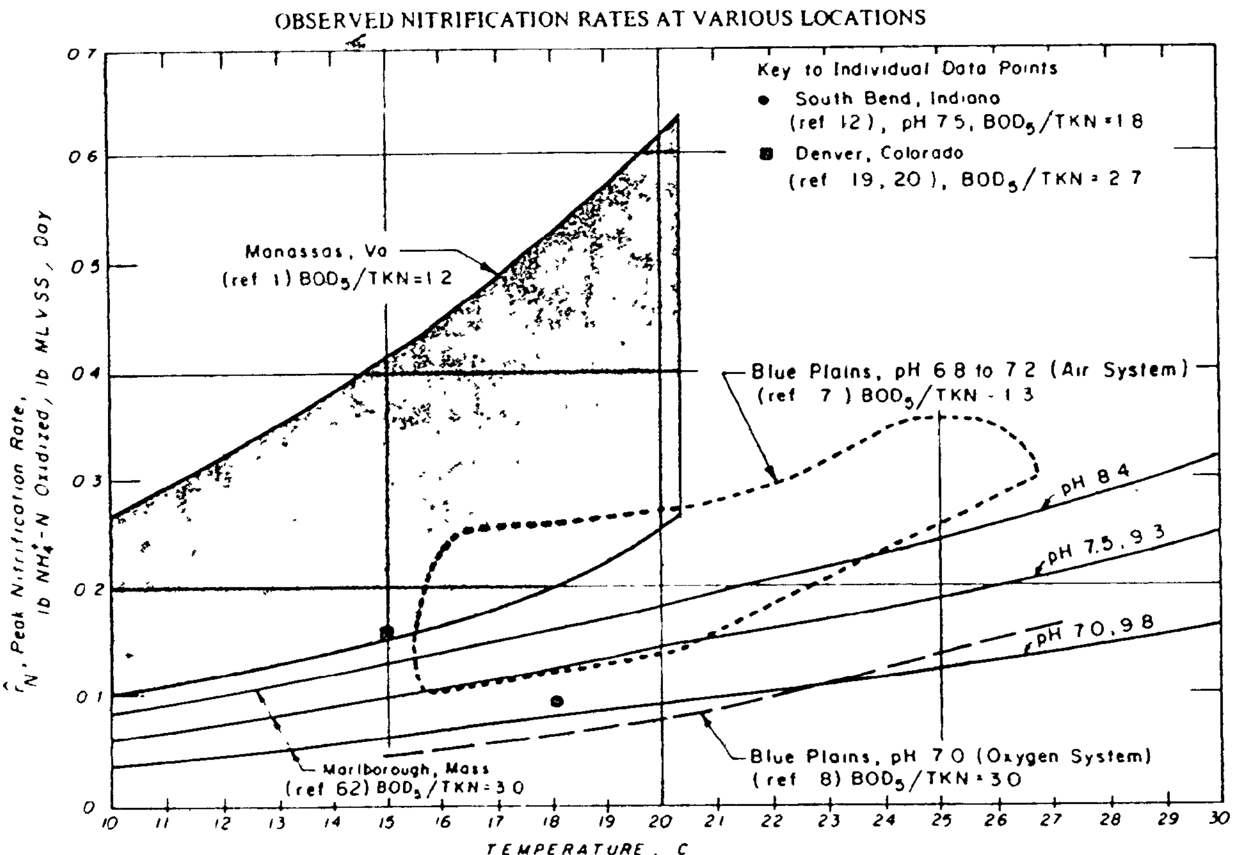


Figure 3. Observed Nitrification Rates at Various Locations (U.S. EPA, 1975)

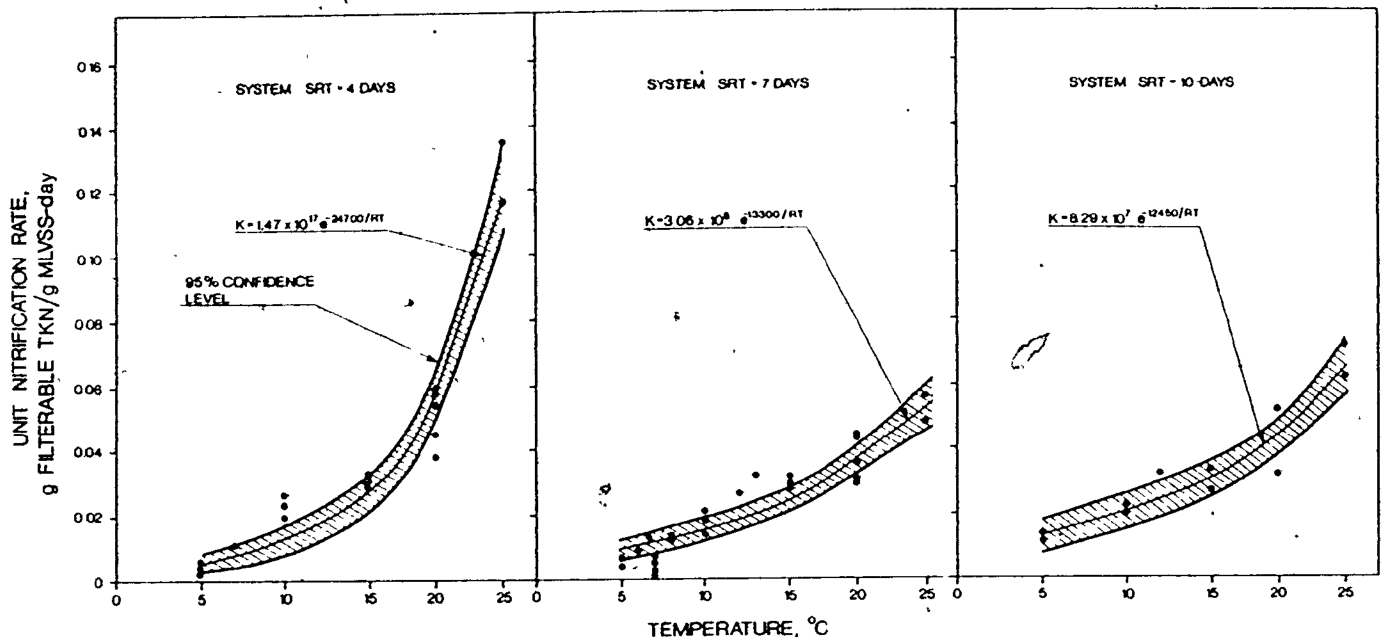


Figure 4. Unit Rate of Nitrification in a Combined Sludge System (Sutton, 1976)

different relative changes in the fraction of nitrifiers present for a given temperature change for systems at different sludge ages. The carbon to nitrogen ratio of the feed may influence temperature sensitivity in a similar manner.

It is apparent that modelling of nitrogen oxidation in a combined carbon removal-nitrification system requires the accurate estimation of nitrifier concentrations and rate constants. These parameters may be significantly affected by temperature, SRT and influent C:N ratio, but no satisfactory procedure is available for parameter estimation or measurement under these conditions. Furthermore, to measure specific growth rates or specific substrate removal rates first it is neccessary to know the fraction of microorganisms which are autotrophic in a mixed culture sample.

2.3 Measurement of Nitrifier Populations and Kinetics in Mixed Culture

2.3.1 Introduction

Several previous workers have presented techniques for nitrifier measurement based on:

(i) Cell counts or most probable number (MPN) (Matulewich, Strom and Finstein 1975; Ford, Churchwell and Kachtick, 1980).

(ii) Fluorescent antibody (FA) techniques (Fliermans, Bohlool and Schmidt, 1974; Schmidt, 1973, 1974).

(iii) ^{14}C-Bicarbonate incorporation (Billen, 1976; Somville, 1978).

(iv) Pure culture equivalent activities (Srinath, Prakasam and Loehr, 1976).

The usefulness of MPN and FA methods is limited by practical considerations. Immunofluorescence studies require sophisticated techniques and are more suited to qualitative than quantitative estimation. To obtain the maximum MPN of nitrifying bacteria, incubation periods of up to 113 days are required (Matulewich, Strom and Finstein, 1975). Even if nitrifier populations are determined with these methods, no information on their specific activities is provided.

The ^{14}C-bicarbonate assimilation technique has short incubation times and will evaluate the activity of nitrifying bacteria _in situ_. Using a specific nitrification inhibitor (N-Serve) Billen (1976) and Somville (1978) contended that nitrifier bicarbonate uptake may be differentiated from both heterotrophic uptake and incorporation by other autotrophic microorganisms. Although the inhibition of _Nitrosomonas_ at low N-Serve levels ($<10\ mg \cdot L^{-1}$) is well documented (Shattuck and Alexander, 1963; Campbell and Aleem, 1965a; Young, 1969) it is doubtful that significant reductions of _Nitrobacter_ activity would be obtained (Campbell and Aleem, 1965b; Young, 1969). This suggests that the N-Serve sensitive ^{14}C-bicarbonate incorporation measured by Billen and Somville is in fact an indication only of

the activity of Nitrosomonas. This carbon uptake rate was used to approximate nitrogen oxidation rates by assuming a constant C uptake/oxidized N factor (Billen, 1976). However inorganic carbon assimilation by nitrifiers has been demonstrated to be affected by oxygen (Schön, 1965; Gundersen, Carlucci and Boström, 1966). In wastewater treatment plants where not only dissolved oxygen, but ammonia concentrations and cell age can vary over wide ranges, the relationship between carbon incorporation and nitrogen oxidation will be variable.

With wastewater, the technique of Srinath et al., (1976) offers distinct advantages. It encompasses the simultaneous determination of Nitrosomonas and Nitrobacter activity in mixed and pure cultures under optimum environmental conditions. The pure culture specific rates are compared to mixed culture rates to provide an estimate of nitrifier populations. Only moderate incubation times (4-6 hrs.), are required and the nitrogen oxidation rates for both Nitrosomonas and Nitrobacter are measured directly. Unfortunately it has several drawbacks. By washing the mixed and pure culture cells and subsequently optimizing conditions for nitrification, any heterotrophic-autotrophic interaction is disregarded. Specific nitrifier growth rates may be altered by the presence of heterotrophs (Knowles, Downing and Barrett, 1965; Pan and Umbreit, 1972) or by the addition of mixed liquor filtrates from an activated sludge reactor (Hockenbury, Daigger and Grady,

1977). Further no consideration is given to the nitrogen uptake by heterotrophic organisms. Under conditions of variable carbon to nitrogen ratios (C:N) in the feed to a treatment plant, the metabolism of carbon removing bacteria will significantly alter the nitrogen pool available for nitrification. This could lead to erroneous predictions of effluent oxidized nitrogen concentrations.

Using an equivalent activity approach derived from the work of Srinath _et al._, (1976), a method for the estimation of nitrifier populations and kinetics _in situ_, has been developed (Hall and Murphy, 1980). The proposed technique allows the determination of nitrifying mass and maximum specific substrate removal rates in mixed culture and incorporates the effects of heterotrophic activity upon the measured nitrification rates.

2.3.2 Theoretical development

If the maximum specific substrate removal rate is known the concentration of nitrifiers (X) can be evaluated from the results of a batch kinetic assay under non-limiting substrate conditions using Equation 8. Unfortunately, a wide range of values for k_{max} has been reported for both _Nitrosomonas_ and _Nitrobacter_ (Sharma and Ahlert, 1977). Although this variation is most likely caused by differing culture conditions, no relationship is available to allow the prediction of k_{max} at specified levels of temperature, pH, SRT, C:N, etc. Therefore to be useful for nitrification modelling any nitrifier measurement

technique must encompass an *in situ* determination of k_{max} as well as the nitrifying mass, X.

The approach taken was to perform a three part assay procedure. First, batch kinetic runs were performed on the wastewater sample following the addition of either NH_4-N or NO_2-N. This provided the indigenous activity (dS/dt) of *Nitrosomonas* and *Nitrobacter* in the mixed culture.

To determine k_{max} *in situ*, this assay was repeated. but to each batch flask a known quantity of either *Nitrosomonas* or *Nitrobacter* was added. These nitrifiers had previously been maintained in pure culture under environmental conditions (pH, temp, etc) similar to those of the wastewater sample. The increment in activity resulting from this known addition can then be used to calculate the prevailing k_{max}. An estimate of the nitrifier population in the wastewater is then obtained with Equation 8.

The effect of heterotrophic metabolism on the NH_4-N pool was examined by inhibiting *Nitrosomonas* activity. N-Serve^R (2-chloro-6-trichloromethyl pyridine) has been shown to be a potent inhibitor of *Nitrosomonas* at concentrations as low as 5 $mg \cdot L^{-1}$ (Campbell and Aleem, 1965a; Young, 1969; Billen, 1976). At these levels it has no effect on other organisms, including *Nitrobacter* (Campbell and Aleem, 1965b; Young, 1969). When an additional *Nitrosomonas* assay was carried out in the presence of N-Serve, any changes in ammonia concentration could be attributed

to heterotrophic effects.

The major assumption involved in this estimation technique is that the activity of the pure culture nitrifiers is representative of the mixed culture. Watson (1974) has listed the autotrophic bacteria associated with naturally occuring nitrification. Of the five genera of ammonium oxidizers given, only Nitrosomonas europaea has been linked to nitrification in sewage.

Similarly, although three genera of nitrite oxidizers are reported, Fliermans, Bohlool and Schmidt, (1974) found that when 15 such organisms were isolated from several different types of habitats, all proved to be Nitrobacter of either the winodgradskyi or agilis type. The specific activity of these organisms as measured by Srinath et al., (1976) were identical. For these reasons it was felt that Nitrosomonas europaea and Nitrobacter agilis would provide pure cultures of ammonium and nitrite oxidizing bacteria with a high degree of relevance to the natural environment.

3. EXPERIMENTAL MATERIALS AND METHODS

3.1 Maintenance of Biological Cultures

3.1.1 Pure cultures

Nitrosomonas europaea and *Nitrobacter agilis* were obtained from American Type Culture Collection, Maryland. Separate populations were maintained in batch fed Virtis fermentation vessels of 3.5 liter capacities, utilizing the growth media indicated in Table 3. Each vessel was equipped with a magnetically coupled stirrer, a sterile air supply and a peristaltic pump for the addition of sterile feed. For *Nitrosomonas* growth, reactor pH was maintained between 7.0 and 8.0 by the addition of sterile, 50% K_2CO_3. Sufficient mixing and aeration were provided to each culture to ensure bulk liquid dissolved oxygen concentrations near saturation values. Figures 5 & 6 show the equipment used for the growth of a single nitrifying bacterium. The entire apparatus was placed in a dark, controlled temperature chamber which maintained reactor temperature within 0.5^{o}C of a setpoint.

Nitrifier cultures were fed daily by withdrawing a measured amount of culture broth from the reactor and replacing it with fresh feed. This technique allowed the adjustment of the system hydraulic and solids retention time

TABLE 3. Composition of Media used in Pure Culture Studies

SOLUTION	SUBSTANCE	QUANTITY	
Medium A	Distilled water	1	liter
	K_2HPO_4	5	g
	NaCl	3	g
	Seq-138-Fe	1.7	mg
	$MgSO_4$	10	mg
	Ca Cl_2.	10	mg
	$MnSO_4 \cdot H_2O$	.07	mg
	$CuSO_4$	.004	mg
	$Na_2MoO_4 \cdot 2H_2O$	.002	mg
	$ZnSO_4 \cdot 7H_2O$	.07	mg
	1.7 M KH_2PO_4	1.2	ml
	pH	7.8	
Nitrosomonas			
Growth Medium	Medium A	1	liter
	$(NH_4)_2SO_4$	4.8	g
	Cresol Red	1	mg
Nitrobacter			
Growth Medium	Medium A	1	liter
	$NaNO_2$	12	g

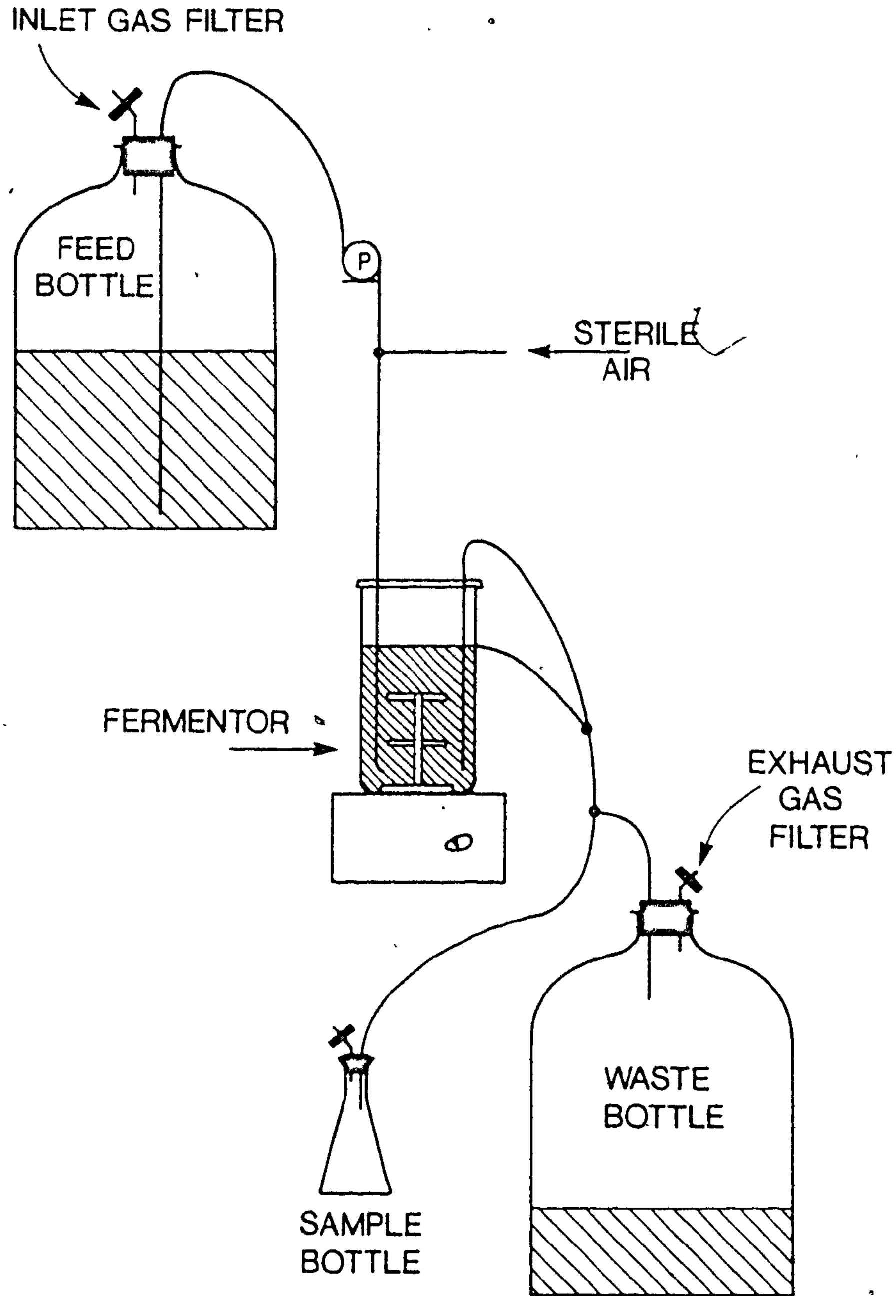


Figure 5. Apparatus Used for Maintenance of Pure Nitrifier Cultures.

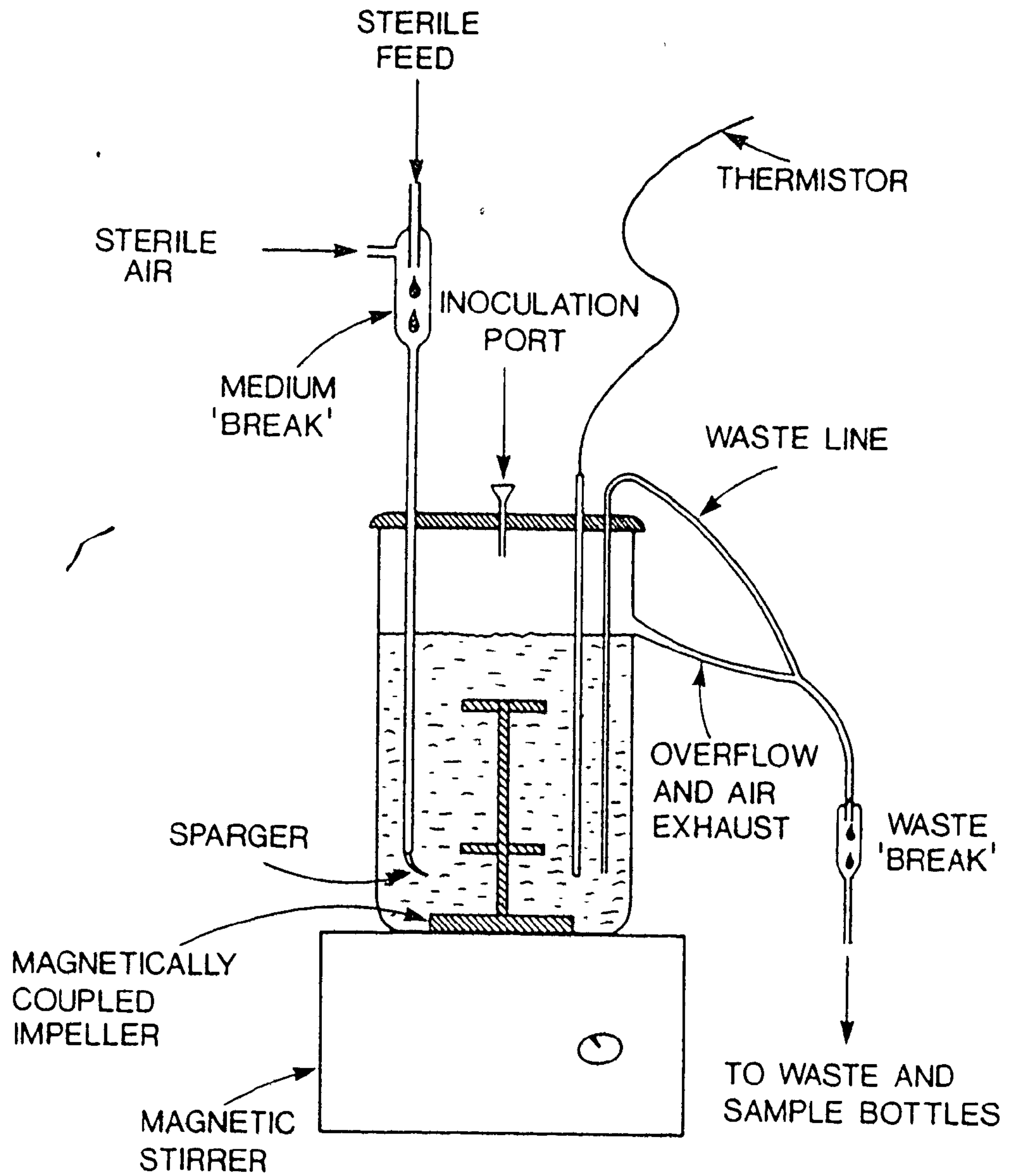


Figure 6. Details of Fermentation Vessel Used for Maintenance of Pure Nitrifier Cultures.

to any desired value. If necessary, constant substrate loading could be achieved by altering the feed concentration.

Periodically, the cultures were examined for contamination by heterotrophic microorganisms. A 100 microliter aliquot of nitrifier broth was spread on the surface of nutrient agar and incubated at 30°C for at least seven days. The appearance of colonies under these conditions was indicative of heterotrophic contamination and nitrifier cultures were restarted from stock cultures.

Nitrifying bacteria were recovered by centrifugation of culture broth at 4500 x g for 5 minutes. The pellets were rinsed once and then resuspended in Medium A to a final volume of 30-50 ml. The bacterial mass in each suspension was measured as Total Suspended Solids (TSS) or as nonfilterable Total Kjeldahl Nitrogen (TKN).

3.1.2 Enrichment cultures

For some experiments, Nitrosomonas and Nitrobacter were maintained as enriched cultures. Ten liter glass carboys served as the culture vessel with aeration and mixing provided by a coarse gas diffuser connected to a pressurized air source. Culture broth removal and feed addition was performed manually. No attempt was made to maintain pure stocks by sterilization of the feed or air supplies, however care was taken to minimize any

contamination of the individual cultures. All other aspects of enrichment culturing were identical to those described for pure cultures.

3.1.3 Mixed cultures

To provide a continuous supply of mixed cultures of heterotrophic and autotrophic microorganisms, two laboratory scale extended aeration plants were constructed. Each plant consisted of a 27 liter aeration tank which overflowed into a circular clarifier. The aeration tank was sized so that even at the longest sludge age under study, removal of a mixed liquor sample for an assay would still allow the SRT to be maintained on a day to day basis. At a feed rate of 33 $L \cdot day^{-1}$ the aeration tank hydraulic residence time (HRT) was 20 hours. Each reactor was equipped with an automatic control system which kept the mixed liquor pH near 7.6 by the addition of 25% $KHCO_3$ as required. The bulk liquid dissolved oxygen concentration was always between 6-8 $mg \cdot L^{-1}$ and the mixed liquor temperature was 18°C.

The 25 cm diameter clarifier provided a surface overflow rate of .03 $m \cdot hr^{-1}$ (25 $gpd \cdot ft^{-2}$). Underflow was returned to the aeration tank at 350-400 per cent of the feed rate to prevent any accumulation of biomass in the clarifier. This approach allowed simplified SRT control and reduced the risk of denitrification in the clarifier. Solids retention time was adjusted by wasting an appropriate

amount of mixed liquor directly from the aeration tank.

The extended aeration plants were seeded with screened return sludge from the Dundas Wastewater Treatment Plant. Primary effluent from Dundas served as the basic feed. Feed TKN and chemical oxygen demand (COD) concentrations were adjusted either by dilution with tap water or by the addition of stock solutions of NH_4Cl (25.5%) and/or 2.5% glucose-7.5% soluble starch. All feed supplies were stored at $4^{o}C$.

In some experiments, mixed cultures were also grown in a Cole-Parmer Biooxidation System. This apparatus had a total volume of six liters and included a small internal clarifier for solids recycling. Sufficient potassium bicarbonate was added to the feed to prevent the reactor pH from dropping below 7.0. All other operating conditions were identical to those used for the larger systems.

3.2 Nitrification Assay Procedure

Mixed culture nitrifying bacterial populations and kinetics were estimated *in situ* by an extension of the method of Srinath, Prakasam and Loehr (1976). Four batch flasks were prepared as indicated in Figure 7 and placed on a rotary shaker which provided moderate aeration and mixing. Dissolved oxygen and pH were measured periodically to ensure that optimal conditions were maintained. At 50-60 minute

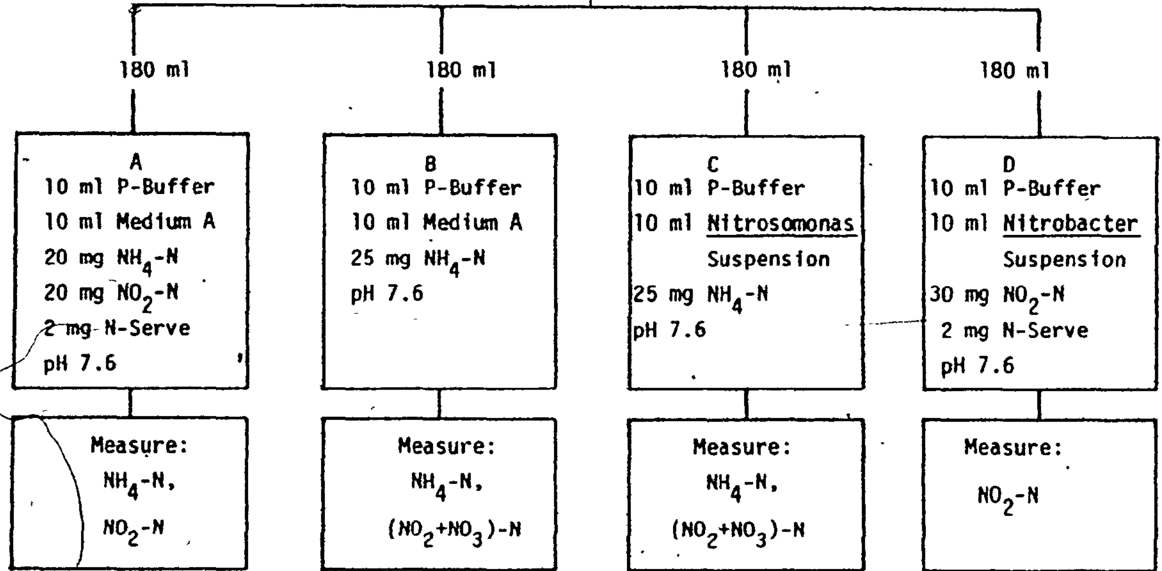


Figure 7. Composition of Batch Flasks Used for Nitrification Assay.

intervals, 10 ml samples were withdrawn, the solids were removed by centrifugation and the supernatants were acidified and stored at 4°C prior to analysis. Normally, the duration of an assay was 5-6 hours, but this period was often extended up to 24 hours in cases were nitrification activity was small.

Pure culture nitrifiers were assayed in a similar manner. In these experiments mixed liquor was replaced by Medium A and usually no N-Serve was used.

3.3 Sampling and Reagents

Samples of sewage and mixed liquor were analyzed for COD and TKN following acidification to 0.2% H_2SO_4. Filterable constituents (TOC, COD, TKN, NH_4-N, NO_x-N) were measured after passage of the sample through glass fiber filters. All samples were stored at 4°C.

Solutions and reagents used in this study were prepared in distilled-deionized water. Sequestrine - 138 - Fe containing 6 per cent iron was obtained from Ciba-Geigy, Toronto. N-Serve (2-chloro-6-trichloromethyl pyridine) was made available by Dow Chemical of Canada, Sarnia, Ontario.

3.4 Experimental Design

The experimental program was carried out in four phases, beginning in September, 1977.

3.4.1 Phases I & II

In Phase I, the extended aeration plants and pure culture fermenters were designed and constructed. In addition, analytical methodologies were established for the determination of suspended solids, COD, TKN, NH_4-N, NO_2-N, (NO_2 + NO_3)-N and TOC.

Phase II began with the start-up of the mixed and pure culture facilities. Preliminary experiments were undertaken in which both extended aeration systems 1 & 2 were operated at identical conditions. Following this, the adequacy of design settings for Phase III were examined. Since Phase III involved a variety of SRTs and COD and TKN loadings, it was necessary to ascertain first that the mixed culture systems would be operable over the entire experimental design. Therefore they were operated at those experimental points (Table 4) which were thought most likely to lead to process failure. At each point the system was examined for the establishment of nitrification and for the production of a settleable biomass.

Also during Phase II, continuous culturing of pure nitrifiers was initiated and the methodology for assaying nitrifying bacterial kinetics in mixed culture was developed. Phase II was completed in April, 1978.

3.4.2 Phase III

To assess the effects of SRT and COD and TKN loadings

TABLE 4. Phase II Experimental Design

RUN	EXTENDED AERATION SYSTEM	VARIABLE SETTINGS SRT	COD	TKN
II -1	1	–	+	+
-2	2	–	+	+
-3	1	–	–	+
-4	2	+	–	+
-5	1	+	–	–
-6	2	–	+	–
-7	1	–	+	0
-8	2	+	+	+
-9	1	–	–	–
-10	2	+	+	–
-11	1	+	–	0
-12	2	–	–	0

Design Settings:

SRT (days)	5	–
	17.5	0
	30	+
COD ($mg \cdot L^{-1}$)	200	–
	550	0
	900	+
TKN ($mg \cdot L^{-1}$)	20	–
	155	0
	300	+

on the kinetics of nitrifiers in mixed culture, a partial 3^3 factorial design was prepared as described by Box and Behnken (1960). A complete design allowing the estimation of main, interaction and quadratic effects requires 15 experiments. To this design were added another two center point replicates and three further replicates at outlying points to improve variance estimation. The complete design is shown in Table 5. Variable settings were selected by two criteria. First, the TKN and COD loadings approximated both low and high strength wastes and together covered a wide range of C:N ratios. Levels of SRT reflected those commonly used in full scale units and were long enough to allow complete nitrification. Secondly, the entire range of variable settings permitted successful operation as judged by the Phase II results. Nitrogen loadings were selected to result in reactor concentrations that would not be inhibitory at a pH of 7.6. Phase III experiments were completed in April, 1979.

3.4.3 Phase IV

Between May and October, 1979, studies were carried out to determine the effects of temperature and SRT on the kinetics of nitrifiers. The three levels of SRT shown previously were retained and were combined with three levels of temperature (Table 6). Following a change in SRT, a minimum time of one SRT was allowed for system acclimation.

TABLE 5. Phase III Experimental Design

RUN	EXTENDED AERATION SYSTEM	VARIABLE SETTINGS SRT	COD	TKN
III -16	2	0	+	+
-10	2	0	+	−
-11	1	0	−	+
-6	2	0	−	−
-4	2	+	+	0
-13	1	−	+	0
-17	1	+	−	0
-15	1	−	−	0
-5	1	+	0	+
-12	2	−	0	+
-3	1	+	0	−
-7	1	−	0	−
-1	1	0	0	0
-8	2	0	0	0
-14	2	0	0	0
Repeats				
III -19	1	0	0	0
-20	2	0	0	0
-9	1	0	+	+
-2	2	+	−	0
-18	2	0	−	+

Design Settings:

SRT (days)	5	−
	17.5	0
	30	+
COD ($mg \cdot L^{-1}$)	200	−
	550	0
	900	+
TKN ($mg \cdot L^{-1}$)	20	−
	155	0
	300	+

For changes in temperature a minimum equilibration period of seven days was provided.

TABLE 6. Phase IV Experimental Design

Run	Variable Settings Temp.	SRT
IV -1	0	-
-2	-	-
-3	0	-
-4	+	-
-5	+	+
-6	0	+
-7	-	+
-8	0	+
-9	0	0

Design Settings

Temperature (°C)	11	-
	18.5	0
	26	+
SRT (days)	5	-
	17.5	0
	30	+

4. RESULTS AND DISCUSSION

4.1 Nitrification Assay Procedures

4.1.1 Pure culture studies

Before the proposed assay technique was utilized in the measurement of nitrifier activities in mixed culture, the effects of assay conditions on the activitiy of pure nitrifiers was examined. Axenic cultures of *Nitrosomonas europaea* and *Nitrobacter agilis* were grown as described above and maintained at a solids retention time of 4 days. The results of typical assays on both species are shown in Figures 8 and 9. Environmental conditions used in all runs were optimized for pH and dissolved oxygen following the criteria of Srinath *et al*. (1976).

For the *Nitrosomonas* assay of Figure 8, the time course of nitrification was followed by measurement of both substrate (NH_4-N) and product (NO_2-N). Flasks A and B contained equal quantities of *Nitrosomonas* measured as non-filterable TKN, but the former also contained 2 mg of N-Serve. It can be seen that in the presence of this quantity of inhibitor no ammonia removal or nitrite accumulation occurred. It should be noted that N-Serve is very insoluble in water and although 2 mg of solid inhibitor was added to the assay flask a large proportion remained

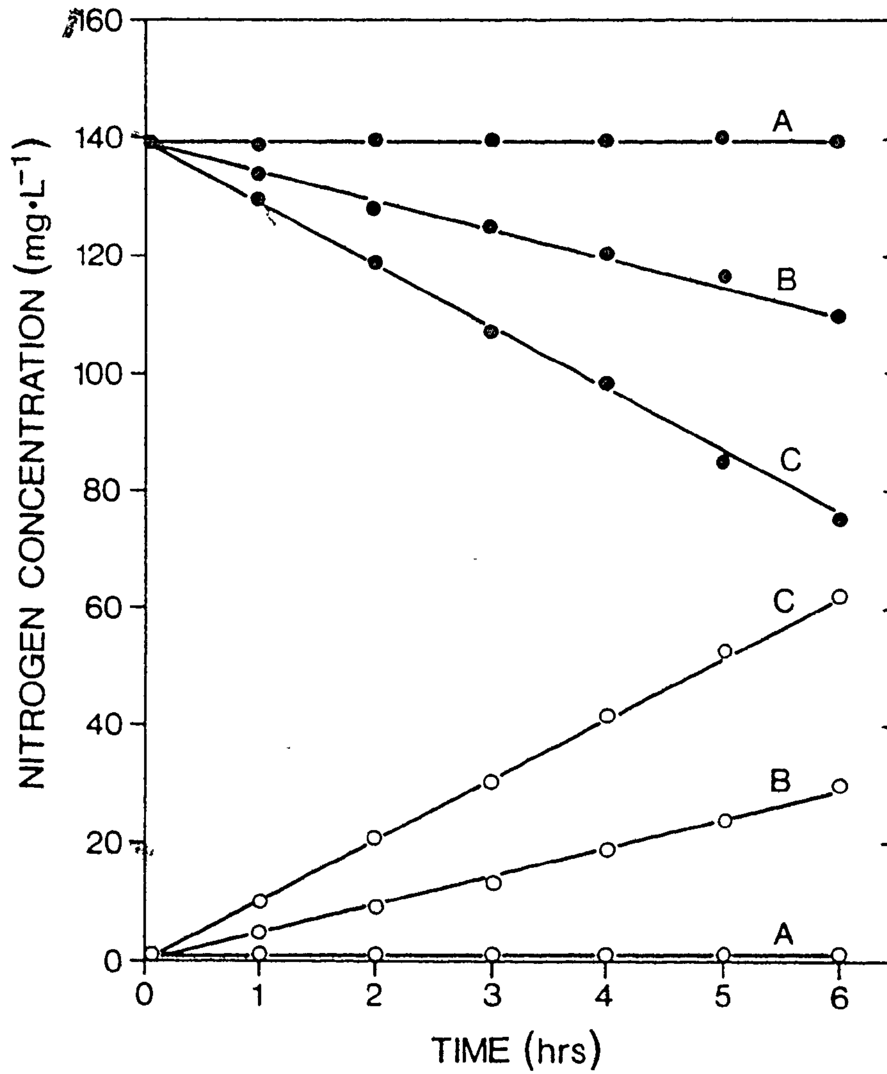


Figure 8. Effect of Assay Conditions on Pure Cultures of Nitrosomonas.
(●——● NH_4-N ; ○——○ NO_2-N ; A contains 0.7 mg cellular TKN + 2 mg N-Serve ; B contains 0.7 mg cellular TKN ; C contains 1.4 mg cellular TKN)

undissolved. Although the estimation of the dissolved N-Serve concentration is not possible, the quantity present was sufficient for complete inhibition. A comparison of the results obtained from Flasks B and C indicated that measured activity was first order with respect to Nitrosomonas concentration. Similarly, all plots remained linear with respect to substrate concentration indicating that the reaction is zero order for substrate. Additional assays have indicated that linearity will persist to substrate concentrations of less than 10 $mg \cdot L^{-1}$.

Figure 9 shows the results of a similar assay for Nitrobacter agilis. With the exception of substrate type, the assay procedure was identical to that for Nitrosomonas. The data (Flasks A & B) demonstrate that nitrite oxidizing activity is insensitive to the presence of N-Serve. Campbell and Aleem (1965b) were unable to find any Nitrobacter inhibition at N-Serve concentrations of 50 $mg \cdot L^{-1}$, although a 50 to 75 per cent reduction in activity was obtained at a level of 175 $mg \cdot L^{-1}$. In this study the maximum possible inhibitor concentration was 10 $mg \cdot L^{-1}$, well below the threshold established by Campbell and Aleem. The Nitrobacter response was zero order for substrate and first order with respect to bacterial concentration.

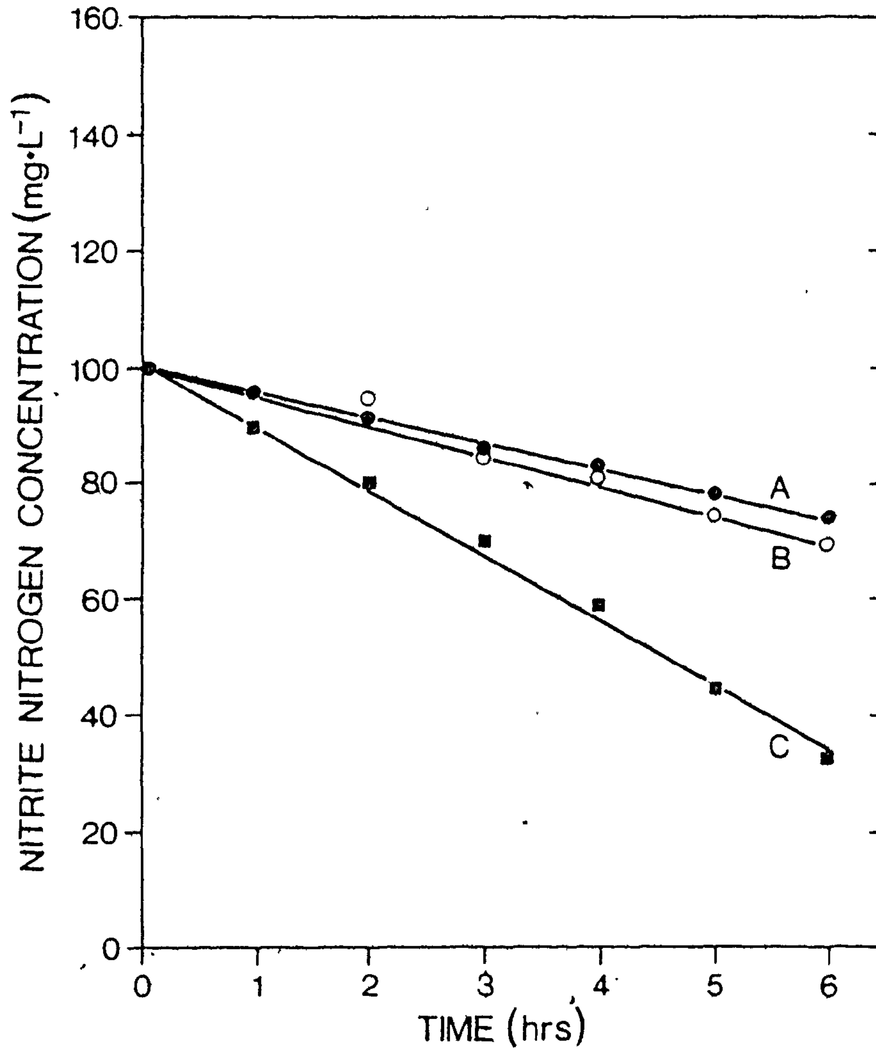


Figure 9. Effect of Assay Conditions on Pure Cultures of Nitrobacter.
(A contains 0.2 mg cellular TKN ; B contains 0.2 mg cellular TKN + 2 mg N-Serve ; C contains 0.4 mg cellular TKN)

4.1.2 Mixed culture studies

The effects of N-Serve on nitrification activity in mixed culture are presented in Figures 10 and 11. Samples of nitrifying mixed liquor were taken from a laboratory scale extended aeration plant, treating primary effluent from the Dundas Wastewater Treatment Plant spiked with NH_4Cl to a TKN concentration of 150 $mg \cdot L^{-1}$. In Figure 10, Nitrosomonas activity is indicated by a decrease in NH_4-N and an increase in (NO_2+NO_3)-N. In the presence of N-Serve this response is not observed and in fact NH_4-N increased slightly over the course of the assay. This phenomenon was always observed in the presence of N-Serve. The rate of increase in NH_4-N concentration was found subsequently to be independent of the initial substrate concentration, but strongly dependent on the MLVSS concentration in the batch flask. This increase may be due to endogenous release of NH_4-N by heterotrophic organisms which would depend on the microorganism concentration. Since the occurrence of endogenous NH_4-N production can have a significant effect on Nitrosomonas rates measured as dNH_4-N/dt, better activity estimates can be obtained either by measuring (NO_2+NO_3)-N, or by correcting the ammonia removal rate obtained without inhibitor by subtraction of the rate determined with N-Serve. Over the course of numerous experiments, a mass balance was obtained from:

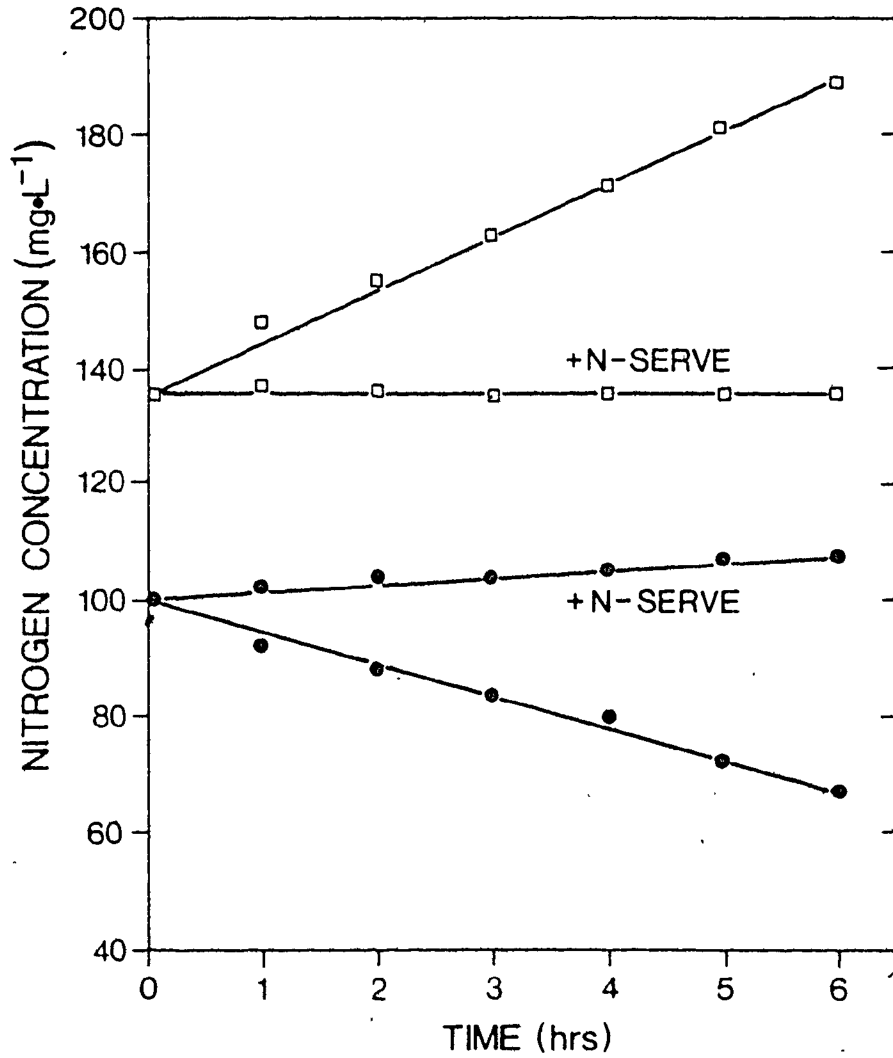


Figure 10. Effect of Assay Conditions on Nitrosomonas Activity in Mixed Culture. (●—● NH_4-N ; □—□ (NO_2+ NO_3)-N)

$$-\frac{d(NO_2+NO_3)-N}{dt} = \frac{d(NH_4)-N}{dt} - \frac{d(NH_4)-N}{dt}\ \text{N-Serve} \qquad (16)$$

Figure 11 shows the effect of N-Serve on _Nitrobacter_ in mixed culture. Preliminary experiments appeared to indicate that N-Serve had a slight stimulatory effect on _Nitrobacter_ (Figure 11). As NO_2 is an intermediate in the overall nitrification reaction, a measurement of dNO_2-N/dt reflects the activity of both _Nitrosomonas_ and _Nitrobacter_. Although no NH_4-N was thought to be available for oxidation, it was demonstrated that NH_4-N can be produced endogenously. Without N-Serve this NH_4-N will normally undergo oxidation and decrease the rate of disappearance of NO_2. Only when _Nitrosomonas_ is inhibited by N-Serve will a true dNO_2-N/dt estimate of _Nitrobacter_ activity be obtained.

This estimation procedure was utilized extensively for _in situ_ determinations of the nitrifying biomass in combined carbon removal - nitrification systems. A complete assay of the same lab scale treatment unit is shown in Figure 12. The unit was operated with an SRT of 17.5 days, and feed COD and TKN of 550 and 155 $mg \cdot L^{-1}$ respectively. Figure 12 presents the analysis of the four batch flasks required. Rates of change of the various inorganic nitrogen species are labelled r_1 through r_7. The calculation sequence leading to estimates of X_a and μ_{max} for _Nitrosomonas_ and _Nitrobacter_ is indicated in Table 7. The

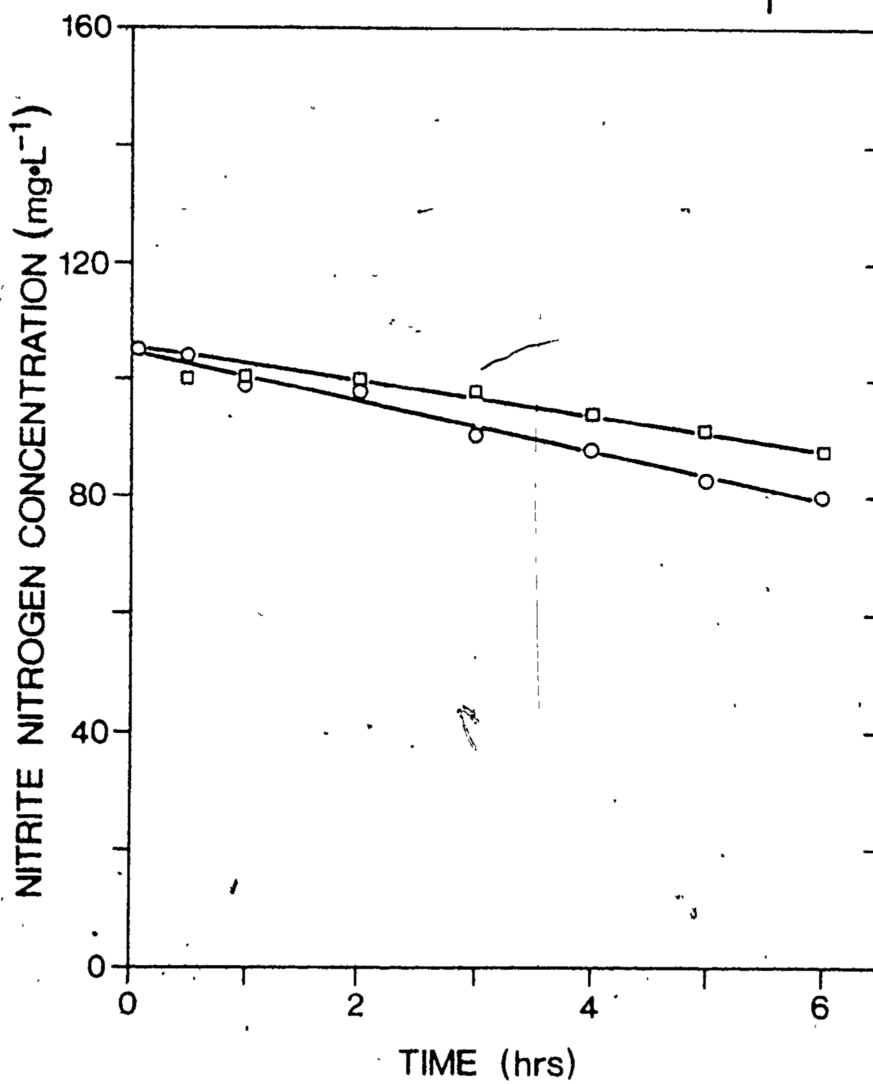


Figure 11. Effect of Assay Conditions on Mixed Culture *Nitrobacter* Activity.
(□——□ no N-serve ; ○——○ with N-serve)

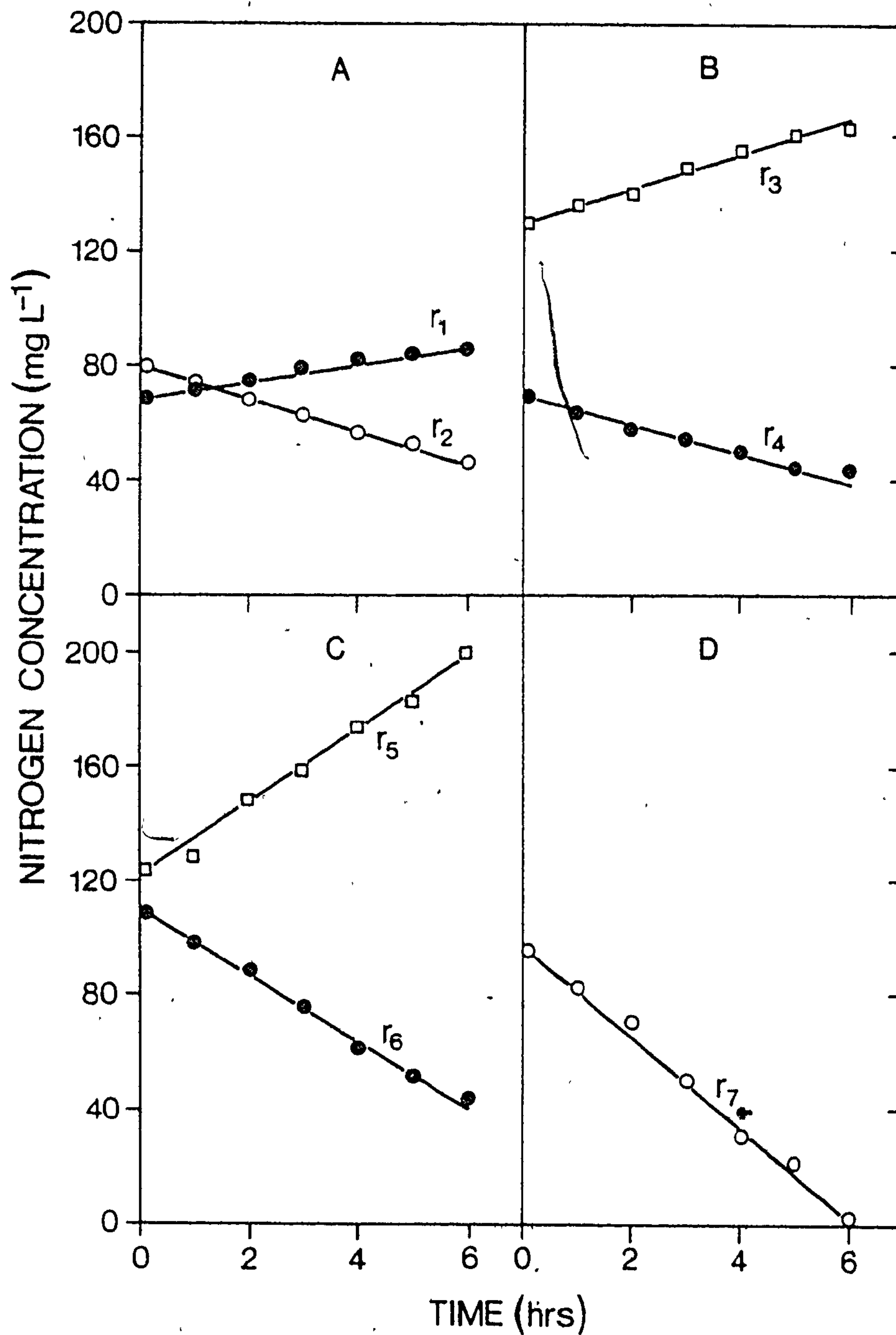


Figure 12. Results of a Complete Nitrification Assay.
(●—● NH_4-N ; ○—○ NO_2-N ; □—□ (NO_2+NO_3)-N ;
Refer to Figure 7 for Composition of Individual Flasks)

TABLE 7. Calculation Sequence for Nitrification Assay

PARAMETER	ACTIVITY $mgN \cdot L^{-1} \cdot hr^{-1}$	k_{max} $\frac{mgN}{mgTSS\text{-}hr}$	k_{max} $\frac{mgN}{mgTKN\text{-}hr}$	MIXED CULTURE NITRIFIERS X_a $mgTSS \cdot L^{-1}$	MIXED CULTURE NITRIFIERS X_a $mgTKN \cdot L^{-1}$
(i) Intrinsic Activities					
(a) Nitrosomonas					
$\Delta NH_4\text{-}N = r_4 - r_1$	- 7.66				
$\Delta(NO_2+NO_3)\text{-}N = r_3$	6.50				
(b) Nitrobacter					
$\Delta NO_2\text{-}N = r_2$	- 5.83				
(ii) Determination of k_{max}					
(a) Nitrosomonas					
$k_{max}(NH_4) = (r_6 - r_4)/X_b$	- 6.17	- .149	- 1.08		
$k_{max}(NO_2+NO_3) = (r_5 - r_3)/X_b$	6.0	.141	1.02		
(b) Nitrobacter					
$k_{max}(NO_2) = (r_7 - r_2)/X_b$	-10.1	- .523	- 3.89		
(iii) Mixed Culture Nitrifier Conc's					
(a) Nitrosomonas					
$\Delta NH_4\text{-}N/k_{max}(NH_4)$				51	7.1
$\Delta(NO_2+NO_3)\text{-}N/k_{max}(NO_2+NO_3)$				46	6.4
(b) Nitrobacter					
$\Delta NO_2\text{-}N/k_{max}(NO_2)$				11.1	1.5

formulas in brackets indicate which nitrogen measurement was used in the parameter calculation.

Additional experiments examined the range of kinetic parameters obtained by this method under a variety of TKN loadings. The extended aeration system was fed a constant carbon loading and influent TKN concentrations of 20,155, and 300 $mg \cdot L^{-1}$. Nitrification assays were performed at each loading level (Table 8). As expected, the resulting rates and estimated nitrifier populations increase significantly with increased TKN loadings. At a constant carbon loading nitrifier concentrations appear to be strongly dependent on the feed nitrogen. Large changes were also noted in the maximum unit removal rates (k_{max}). The cause of these changes are unknown at this time, but their occurrence is in agreement with the findings of others (Knowles *et al.*, 1965; Pan & Umbreit, 1972; Hockenbury *et al.*, 1977). Experiments have found that normal and added *Nitrobacter* activity is completely inhibited under some growth conditions in which *Nitrosomonas* seem to thrive.

The proposed estimation method is applicable to any aqueous system which contains suspended nitrifying organisms. However, as activity is determined directly by the measurement of changes in inorganic nitrogen, the technique is most suited to wastewater, where changes will

TABLE 8. Variation of Nitrifier Kinetic Parameters with Changing Nitrogen Loadings

PARAMETER		INFLUENT TKN ($mg \cdot L^{-1}$) 20	155	300
Nitrosomonas				
ΔNH_4-N	$mg\ N \cdot L^{-1} \cdot hr^{-1}$	.58	8.5	13.5
$\Delta(NO_2+NO_3)$-N	$mg\ N \cdot L^{-1} \cdot hr^{-1}$	.46	6.8	11.3
$k_{max}(NH_4)$	$mg\ N \cdot mgTSS^{-1} \cdot hr^{-1}$	.32	.16	.13
$k_{max}(NO_2+NO_3)$	$mg\ N \cdot mgTSS^{-1} \cdot hr^{-1}$	.31	.13	.13
$k_{max}(NH_4)$	$mg\ N \cdot mgTKN^{-1} \cdot hr^{-1}$	2.6	1.2	1.1
$k_{max}(NO_2+NO_3)$	$mg\ N \cdot mgTKN^{-1} \cdot hr^{-1}$	2.5	1.0	1.1
$X_a(NH_4)$	$mgTSS \cdot L^{-1}$	1.7	54	104
$X_a(NO_2+NO_3)$	$mgTSS \cdot L^{-1}$	1.6	52	87
$X_a(NH_4)$	$mgTKN \cdot L^{-1}$	.22	7.1	12.3
$X_a(NO_2+NO_3)$	$mgTKN \cdot L^{-1}$	.19	6.9	10.3
Nitrobacter				
ΔNO_2	$mg\ N \cdot L^{-1} \cdot hr^{-1}$	.46	5.3	9.2
$k_{max}(NO_2)$	$mg\ N \cdot mgTSS^{-1} \cdot hr^{-1}$	.68	.55	.63
$k_{max}(NO_2)$	$mg\ N \cdot mgTKN^{-1} \cdot hr^{-1}$	6.2	4.5	6.5
$X_a(NO_2)$	$mgTSS \cdot L^{-1}$	.67	9.6	14.6
$X_a(NO_2)$	$mgTKN \cdot L^{-1}$	.08	1.2	1.9

be appreciable. In cases where either very small or very large nitrifier activities are anticipated, the sensitivity of the method can be adjusted by increasing or decreasing the assay period. Oxidation rates from .08 to 38 mg $N \cdot L^{-1} \cdot hr^{-1}$ have been measured in nitrifying activated sludge samples.

4.2 Kinetic Studies on Pure and Enriched Nitrifier Cultures

4.2.1 Specific substrate removal rate

The results of several pure culture assays are summarized in Table 9. For this set of determinations, nitrifier cultures were grown at a 4 day SRT and a temperature of 24°C. Reaction rates for *Nitrosomonas* determined as either dNH_4-N/dt or dNO_2-N/dt were found to be statistically equivalent. This allowed the pooling of data and the subsequent expression of activity as dN/dt. Specific activities or unit substrate removal rates were determined using both TSS and TKN as an index of bacterial cell concentration. Generally, volatile solids are considered a better indicator of biomass than TSS, but it was found that nitrifiers maintained in pure and enriched culture were not flocculent, and could not be retained on any filter with pore sizes above the 0.45 micron level. Therefore VSS determination with glass fiber filters was not possible. The relationships between TKN and TSS (for both

bacteria) are indicated in Table 9. When compared to the TKN/TSS ratios presented by Srinath, Prakasam and Loehr (1976), these values are seen to be significantly higher.

Similarly, the specific activities obtained for Nitrosomonas and Nitrobacter are about three times larger than the equivalent values observed by Srinath *et al*. Some of this variation can be accounted for by temperature discrepancies between the two studies. However, the maximum temperature difference is only 4°C and thus temperature alone cannot explain the observations.

There is some indication that the properties of the nitrifying cultures in the two studies were not identical. It was reported by Srinath *et al*. that nitrifiers were flocculent and could be settled and recycled internally. In this study it was found that pure cultures and enriched cultures derived from pure cultures were not flocculent, but remained dispersed even at SRTs of 30 days. Poor bioflocculation of the nitrifying biomass has also been reported by others (Hutton and La Rocca, 1975; Ford, Churchwell and Kachtick, 1980). The more flocculent nature of the biomass, together with the significantly lower specific activities described by Srinath *et al*. could be associated with significant contamination of the nitrifiers by heterotrophic bacteria. Unfortunately, the nitrifying cultures maintained by Srinath *et al*. were not examined for

TABLE 9. Summary of Pure Culture Assays Data

PARAMETER	SAMPLE SIZE	MEAN	STD. DEV.	COEFF. OF VAR. %	REFERENCE
(a) <u>Nitrosomonas europaea</u>					
% TKN/TSS	30	11.6	1.0	8.6	this study
% TKN/VSS		7.6			Srinath <u>et</u>. <u>al</u>.
Sp. Act. mg N·mg TSS^{-1}·hr^{-1}	16	.21	.05	22	this study
Sp. Act. mg N·mg TKN^{-1}·hr^{-1}	16	1.97	.45	23	this study
Sp. Act. mg N·mg TKN^{-1}·hr^{-1}		.58			Srinath <u>et</u> <u>al</u>.
(b) <u>Nitrobacter agilis</u>					
% TKN/TSS	28	11.1	1.14	10	this study
% TKN/VSS		6.6			Srinath <u>et</u> <u>al</u>.
Sp. Act. mg N·mg TSS^{-1}·hr^{-1}	8	.60	.13	21	this study
Sp. Act. mg N·mg TKN^{-1}·hr^{-1}	8	5.06	.57	11	this study
Sp. Act. mg N·mg TKN^{-1}·hr^{-1}		1.7			Srinath <u>et</u> <u>al</u>.

the presence of heterotrophs.

4.2.2 Temperature-SRT effects in enriched cultures

As shown in Figures 3 and 4, the temperature response of nitrifying bacteria in an activated sludge process appears to be influenced by the system SRT and the C:N ratio of the feed. SRT and C:N ratio effects were examined in one experimental design (Table 5) with a mixed culture of heterotrophs and nitrifiers. From the results it would then be possible to predict the concentrations and activities of *Nitrosomonas* and *Nitrobacter* at one temperature for changing SRT and C:N conditions. Modelling the effect of a fourth independent variable, temperature, would have necessitated replicating the entire Phase III design at varying temperature settings. To minimize the required experimental work it was assumed that sufficient data would result from Phase III to quantify SRT-C:N effects on nitrifiers, and that a similar relationship would hold at other temperatures. Then to gauge the effect of temperature, a single experimental design was devised (Table 6) utilizing enriched cultures of nitrifying bacteria.

The results of plating checks confirmed that although enriched cultures were not consistently pure, the amount of heterotrophic contamination, if detectable at all, was not significant. When heterotrophic colonies were found they generally occurred in numbers marginally greater than those

obtained with tap water. In the absence of significant contamination, enriched cultures required a simpler apparatus for growth than did pure cultures and it was therefore possible to maintain three systems each of Nitrosomonas and Nitrobacter. The variables chosen for study in Phase IV were limited to temperature and SRT. In this case C:N ratio has no meaning since enriched nitrifiers were grown without organic carbon in the feed.

To examine the effects of temperature and SRT on nitrifying bacteria, cultures were maintained at SRTs of 5, 17.5 and 30 days and specific activity was measured following temperature equilibration for 7-14 days. The vessels used were chemostats and consequently sludge age was controlled hydraulically. To ensure that sufficient bacteria were available for assay in the volume of effluent removed from each reactor daily, mass loadings of substrate were maintained approximately equal in all systems irregardless of the hydraulic loading. For Nitrosomonas, this posed no problem as long as adequate amounts of K_2CO_3 were added to buffer the H^+ produced. However, cultures of Nitrobacter could not be sustained at the 17.5 and 30 day SRTs. Contrary to the findings of Anthonisen et al. (1976), the Nitrobacter appeared to be subject to product inhibition of the type described by Gould and Lees (1960). Due to the variation in hydraulic retention times, the longer SRT

cultures eventually reached much higher NO_3 concentrations than the 5 day system. Nitrification proceeded satisfactorily in all Nitrobacter cultures until NO_3-N levels approached 2500 $mg \cdot L^{-1}$. Above this value nitrite oxidation slowly decreased. In the 5 day SRT system NO_3-N concentrations never exceeded 1800-2000 $mg \cdot L^{-1}$. Because Nitrobacter could not be gravity settled, hydraulic control of SRT and high nitrate concentrations were considered unavoidable in the 17.5 and 30 day cultures. Consequently, the temperature dependency of nitrite oxidation was examined only at a 5 day SRT.

The effect of temperature on the specific substrate removal rate of Nitrosomonas is indicated by Figure 13. A visual inspection of the results revealed that temperature dependency was slightly greater at the longer SRTs. To quantify any differences in temperature effects between the three cultures, an Arrhenius expression (Equation 15) was fitted to each data set using a non-linear least squares method (Appendix D). The correlation between model parameters was minimized by reparameterization of the equation to the form:

$$k = k_1 \, e^{-E/R\left(\frac{1}{T} - \frac{1}{T_o}\right)} \qquad (17)$$

where: $k_1 = Ae^{-E/RT_o}$

k is the specific substrate removal rate (hr^{-1}),

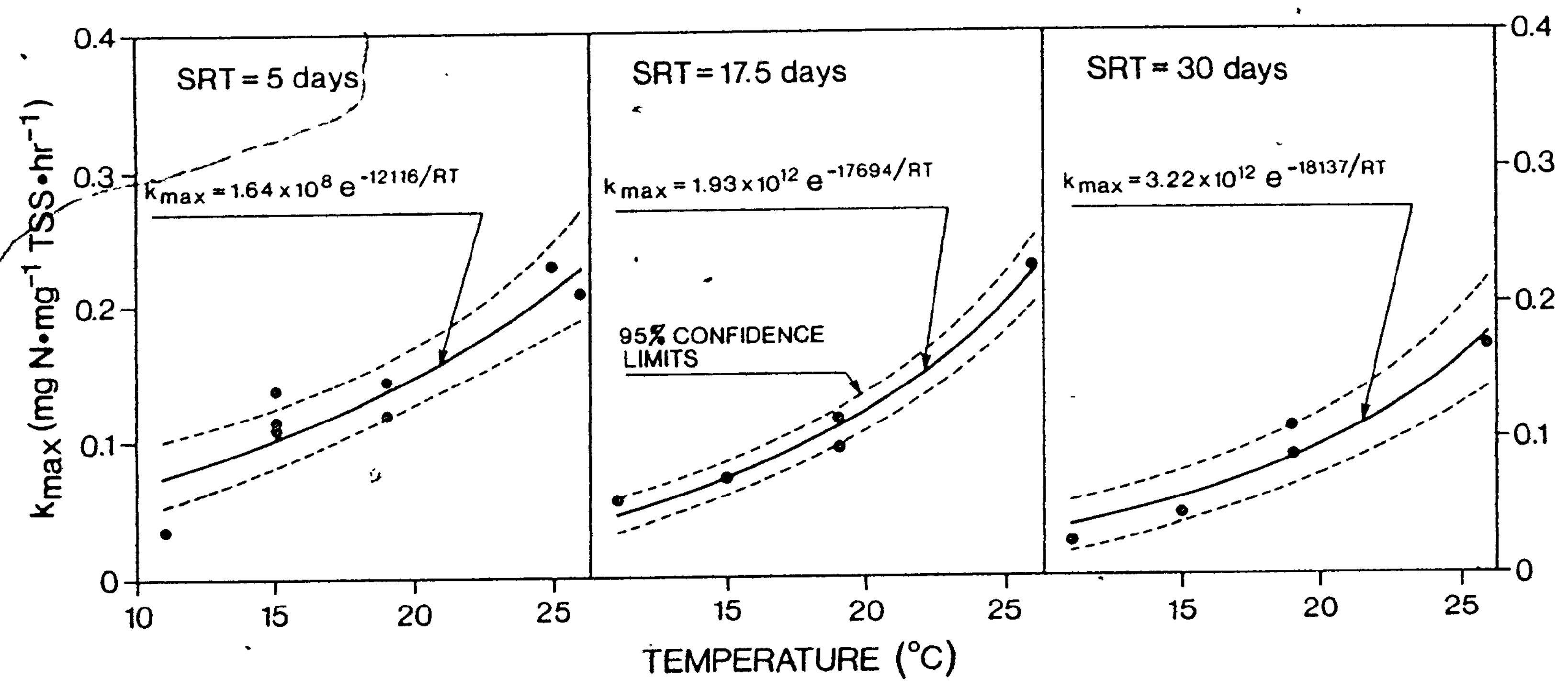


Figure 13. Effects of Temperature and SRT on *Nitrosomonas* Activity.

A is the frequency factor (hr^{-1}),

E is the activation energy ($cal \cdot g\text{-}mole^{-1}$),

R is the universal gas constant ($cal \cdot g\text{-}mole^{-1} \cdot {}^{o}K^{-1}$),

T is the temperature (^{o}K), and

T_o is the mean of the temperature range (^{o}K).

An analysis of variance (ANOVA) showed no lack of fit when either replicate data or the previously estimated standard deviation were used as a measure of the residual mean squares due to pure error (Table 10).

As expected, the increase in activation energy (E) with SRT implied a slight increase in temperature dependency at longer sludge ages. However, the confidence intervals for each computed activation energy exhibited considerable overlap and therefore cast doubt on the validity of the separate models. This was examined further by pooling all the Nitrosomonas data to estimate parameters for a single model (Figure 14). When the analysis of variance again indicated no lack of fit (Table 10), it was concluded that the single model adequately described the temperature effects and that no significant SRT effect was detectable.

The results for Nitrobacter (Figure 15) indicated a relationship similar to that found for Nitrosomonas. A paucity of data and the lack of replicates made an assessment of model adequacy difficult. The model was

TABLE 10. ANOVA Table for Arrhenius - Nitrification Models

SYSTEM	MODEL PARAMETERS E cal·g-mole^{-1}	A hr^{-1}	MSLOF/MSPE	$F_{.05}$
Nitrosomonas				
5 day SRT	12,100	$1.64x10^{8}$	4.12	9.28
17.5 day SRT	17,700	$1.93x10^{12}$	3.52	18.5
30 day SRT	18,100	$3.22x10^{12}$	.525	18.5
Pooled Data	14,900	$1.67x10^{10}$	1.29	3.26

Nitrobacter

Insufficient data for ANOVA

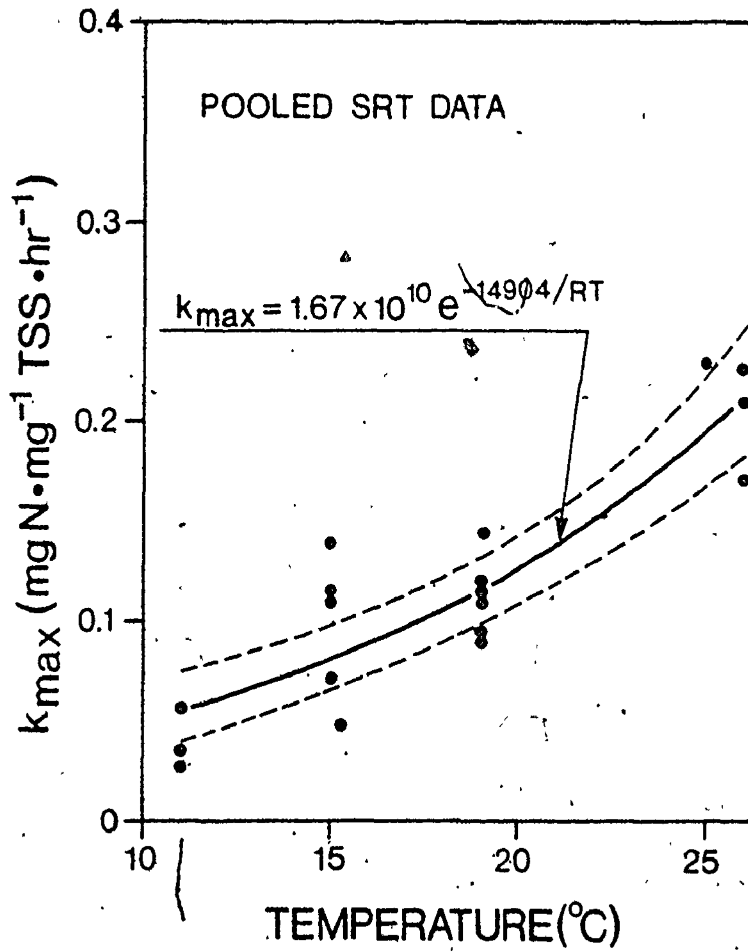


Figure 14. Effect of Temperature on Nitrosomonas Activity Using Pooled Data.

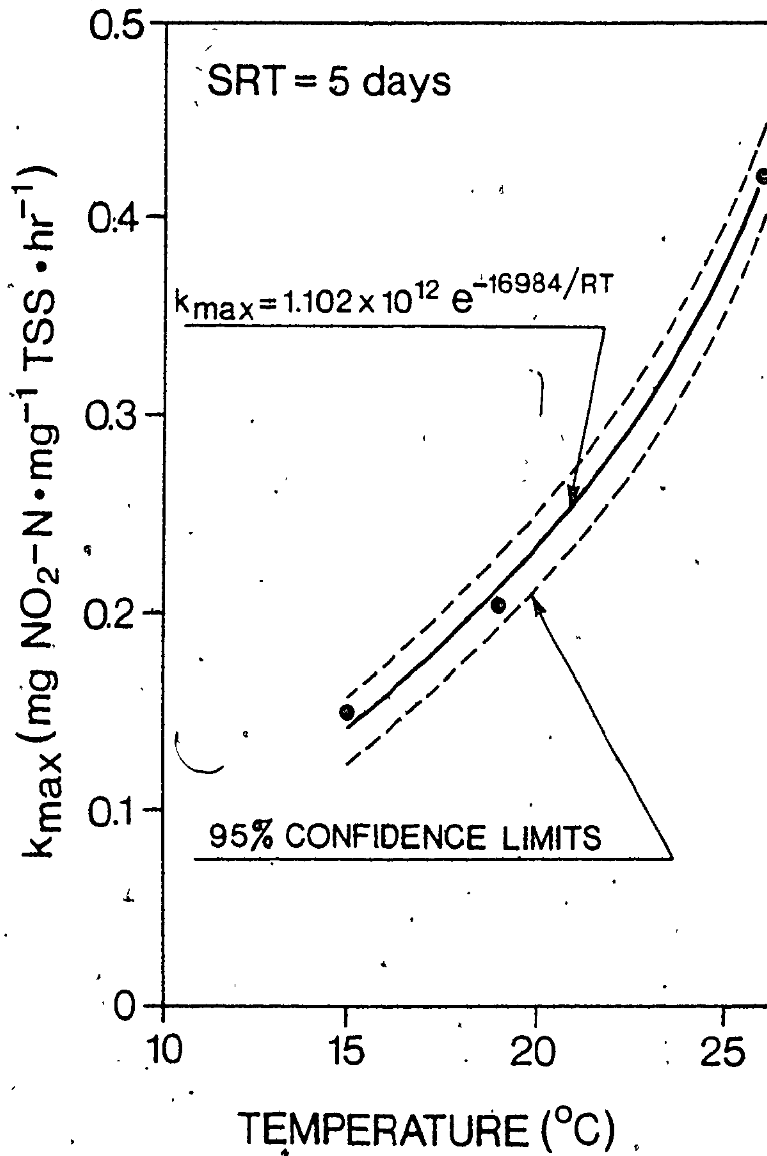


Figure 15. Effect of Temperature on Nitrobacter Activity.

judged satisfactory by using the coefficient of variation from Table 9 to estimate pure error. In the absence of data for the 17.5 and 30 day cultures, it was assumed that Nitrobacter would not exhibit a significant SRT effect and that the 5 day SRT model would adequately predict specific nitrite oxidation rates.

Several previous studies have reported that nitrification substrate oxidation rates follow an Arrhenius-type temperature dependency (Table 11). The activation energy obtained for ammonium oxidation, 14,900 $cal \cdot g\text{-}mole^{-1}$, is in good agreement with other values measured either for overall nitrification rate or for Nitrosomonas activity alone. For nitrite oxidation the value of 16,700 $cal \cdot g\text{-}mole^{-1}$ is also comparable to reported overall rate data, but is considerably larger than the figure given by Wong-Chong and Loehr (1975).

4.3 Effect of Carbohydrate and Acclimation Time on Mixed Culture Nitrification Assay

The measurement of nitrifier kinetics at different carbon loadings required that supplementary COD be added to the relatively low strength Dundas primary effluent which was the basic feed to the extended aeration plants. The additional organic carbon source was a 100 $g \cdot L^{-1}$ stock COD solution containing glucose and soluble starch. A

TABLE 11. Reported Values of Arrhenius Activation Energy for Nitrogen Oxidation Rate

REACTION SYSTEM	ACTIVATION ENERGY $cal \cdot g\text{-}mole^{-1}$	REFERENCE
Nitrification, single sludge		Sutton et al. (1975)
4 day SRT	25,100	
7 day SRT	21,250	
10 day SRT	11,900	
Nitrification, single sludge	12,350	Keenan et al. (1979)
Nitrosomonas, enriched pH 7.5	16,000	Wong-Chong and Loehr (1975)
Nitrosomonas, enriched pH 7.8	14,900	This study
Nitrobacter, enriched pH 7.3	6,600	Wong-Chong and Loehr (1975)
Nitrobacter, enriched pH 7.5	16,700	This study

carbohydrate mixture is not a satisfactory representation of the organic content of a typical municipal wastewater. In fact, a carbohydrate waste can seriously destabilize a treatment plant (Bruner, Smith & Schroeder, 1978). To avoid this problem it has been a common practice to use a more heterogeneous carbon source in laboratory scale studies, such as nutrient broth or yeast extract (Gulas, Bond and Benefield, 1979). These mixtures were unsuitable in this study because both contained significant amounts of TKN and therefore did not allow the independent setting of both COD and TKN loading levels. The same argument applied to the use of other carbon sources such as amino acids and proteins. Consequently a carbohydrate mixture composed of a readily adsorbable component and a polymeric component which requires extracellular hydrolysis prior to adsorption was selected as the supplemental source of COD.

Another variable deemed important in the mixed culture studies was the time allowed for the system of heterotrophs and autotrophs to reach equilibrium under given operating conditions. A sufficient period of time must be provided to establish both the equilibrium population of nitrifiers and any microbial interactions which may affect their specific substrate removal rates. Sutton (1976) utilized an equilibration period of one or more SRTs prior to the measurement of nitrification kinetics. At short

sludge ages this approach is satisfactory, but for longer SRTs the acclimation periods required are excessive.

To study the effects of carbohydrate COD and acclimation time on measured nitrification rates, two mixed cultures were established in 6 liter Cole-Parmer Biooxidation reactors. Both systems were started close to equilibrium conditions by seeding with an appropriate quantity of return sludge from Dundas. The expected steady state solids concentration was calculated for an SRT of 18 days by assuming a yield coefficient of 0.23 mg VSS$\cdot$mg^{-1} COD removed. System A was fed Dundas primary effluent. For system B, the primary effluent was diluted 1:1 with tap water and the COD level was then readjusted to its original value by the addition of the carbohydrate mixture. No attempt was made to fix the daily COD concentrations at any particular value. Instead a pseudo-steady state was maintained in which feed COD fluctuated with the daily variation in Dundas primary effluent. However, both feed TKNs were adjusted to 80 mg$\cdot$L^{-1} with NH_4Cl. After equilibration periods of 9, 18 and 36 days, which corresponded to 1/2, 1 and 2 SRTs respectively, mixed liquor samples were taken from each reactor and assayed as described previously.

The responses of each system are shown in Figures 16 through 19. The arrows indicate points in time at which

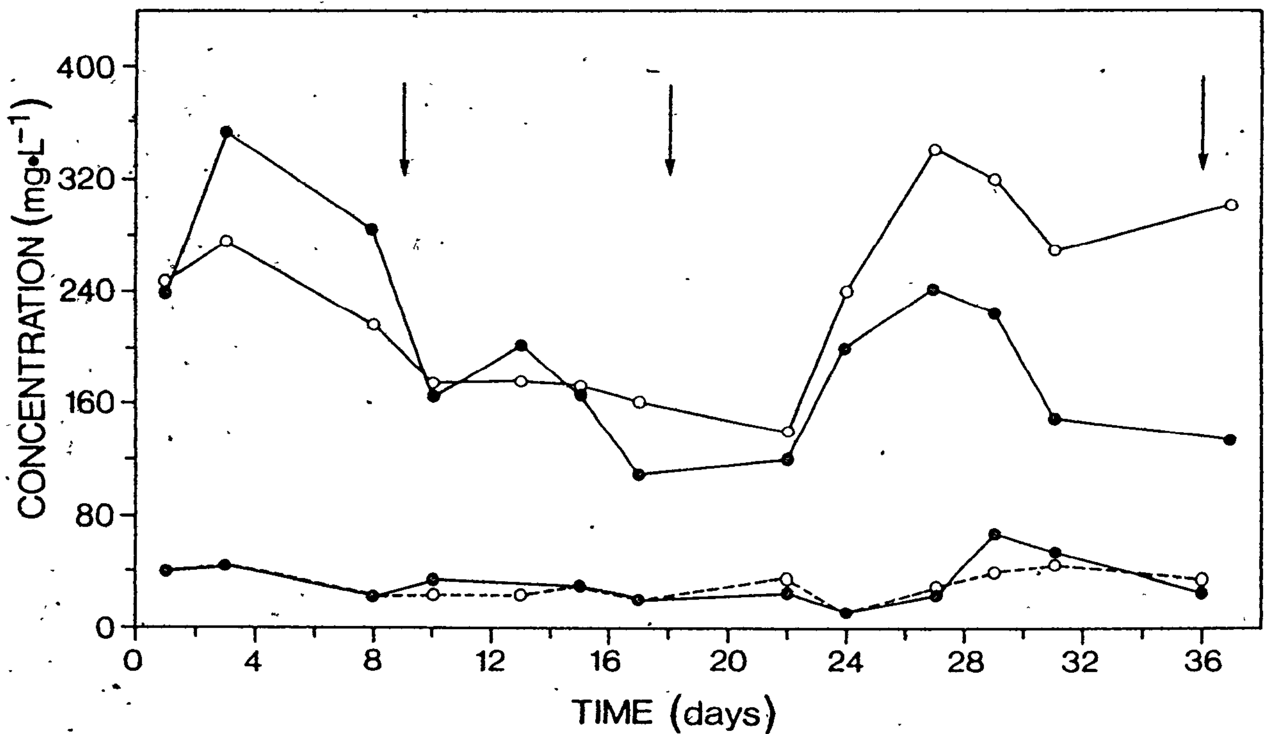


Figure 16. Influent and Effluent COD Measurements. (● System A; o System B; —— Influent COD; ---- Effluent Filterable COD).

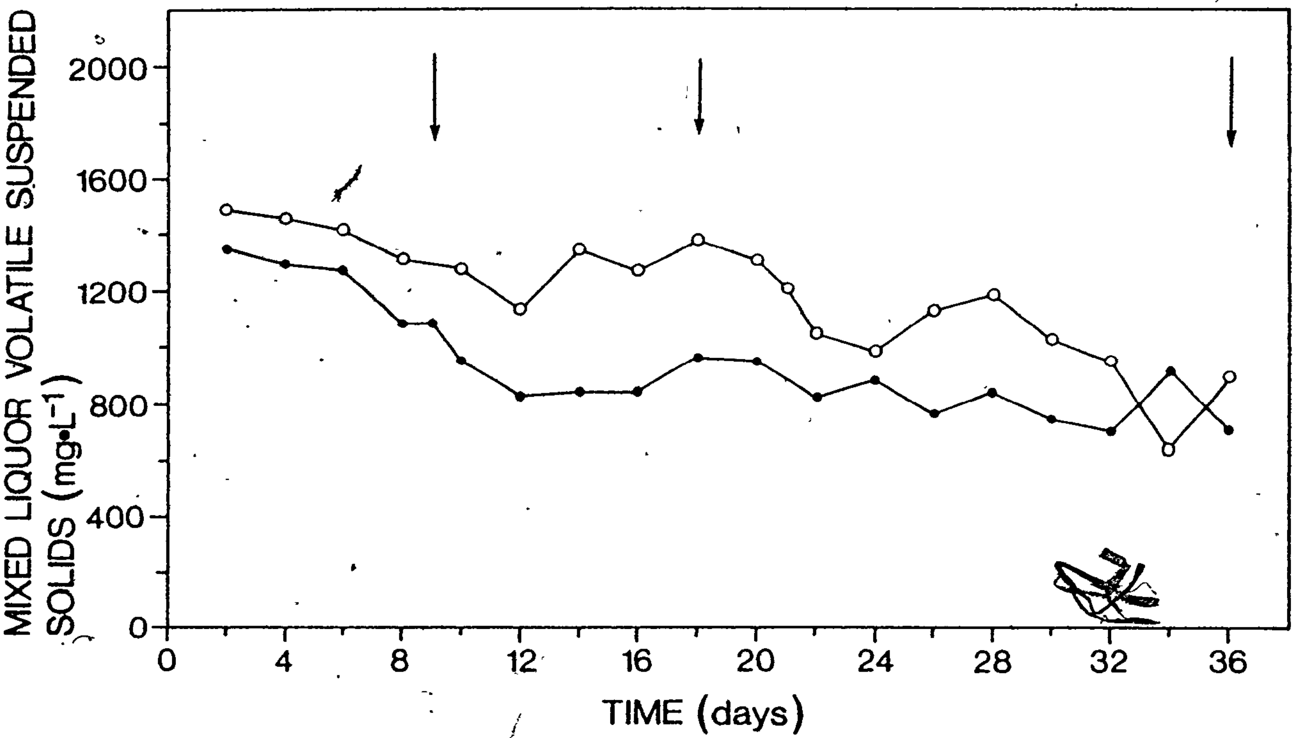


Figure 17. Mixed Liquor Volatile Suspended Solids. (● System A; o System B).

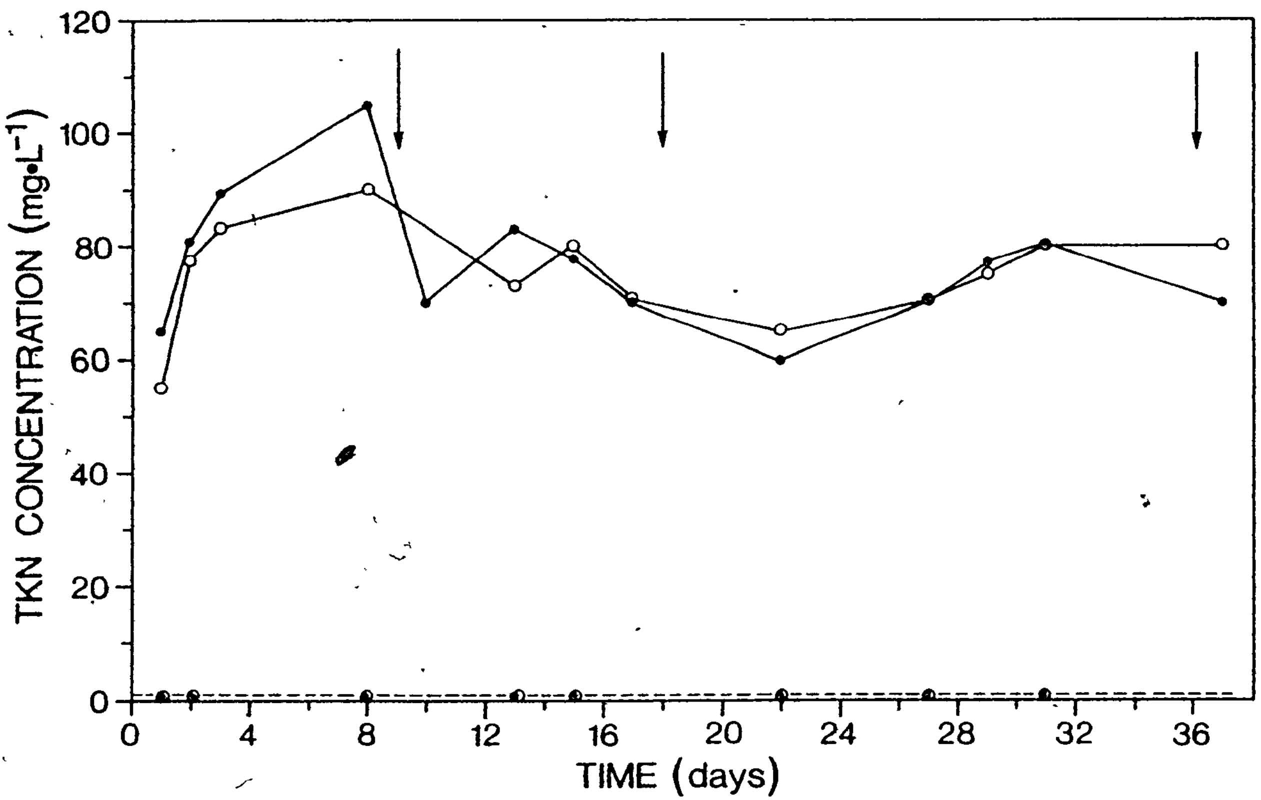


Figure 18. Influent and Effluent TKN Measurements (● System A; o System B; —— Influent TKN; ---- Effluent Filterable TKN).

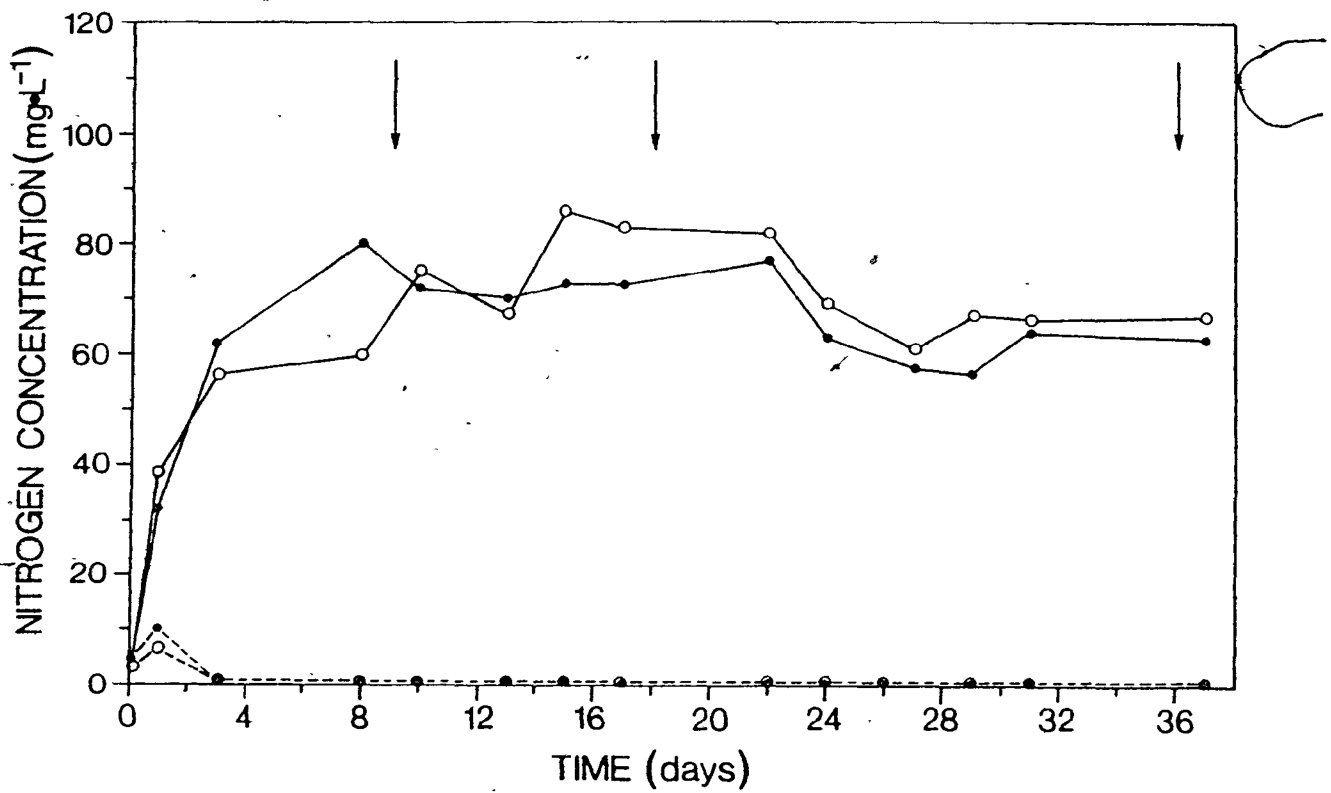


Figure 19. Effluent Nitrite and Nitrate Nitrogen Measurements. (● System A; o System B; --- NO_2-N; ——— NO_3-N).

assays were performed. From Figures 18 and 19 it is evident that complete nitrification is reached between days 4 and 8, and is maintained even though COD loadings and suspended solids changed significantly (Figures 16 and 17).

Nitrification kinetic parameters obtained from the three assays are compared in Table 12. No major chronological effects are apparent, upon either the indigenous mixed culture nitrification rates or the activities of the known addition of pure nitrifiers (k_{max}).

A comparison of the effects of alternative carbon sources is shown in Table 13. Although the number of data points was small, a t-test was constructed to compare systems A and B. Equilibration time was eliminated as a variable and it was assumed that the same measurements had equal variances in both systems. At a significance level of 95%, sample populations with statistically equivalent means will have a t statistic less than the tabulated $t_{.05}$ value. This is true for all responses except the mixed culture Nitrosomonas activity, which is not significant at the 99% level. It was therefore concluded that there were no significant effects of the alternative carbon sources on measured nitrifier kinetics.

Subsequent studies were carried out in such a way that equilibration time was minimized. All runs were started as close as possible to the expected equilibrium

TABLE 12 . Nitrification Assay Results for Carbon and Equilibration Time Study

PARAMETER	SYSTEM A			SYSTEM B		
	Day 9	Day 18	Day 36	Day 9	Day 18	Day 36
Mixed culture activities						
(a) Nitrosomonas						
ΔN $mgN \cdot L^{-1} \cdot hr^{-1}$	2.1	1.7	1.9	2.6	2.9	3.0
(b) Nitrobacter						
ΔN $mgN \cdot L^{-1} \cdot hr^{-1}$	2.7	2.4	1.5	1.6	2.5	1.8
Added nitrifiers: k_{max}						
(a) Nitrosomonas						
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TSS$	.28	.24	.23	.28	.28	.24
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TKN$	2.0	2.1	2.0	2.4	1.9	2.0
(b) Nitrobacter						
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TSS$	.42	.59	.57	.66	.58	.49
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TKN$	3.7	5.3	5.8	5.5	4.6	4.5

TABLE 13 . Paired t-test for Comparison of Carbon Sources

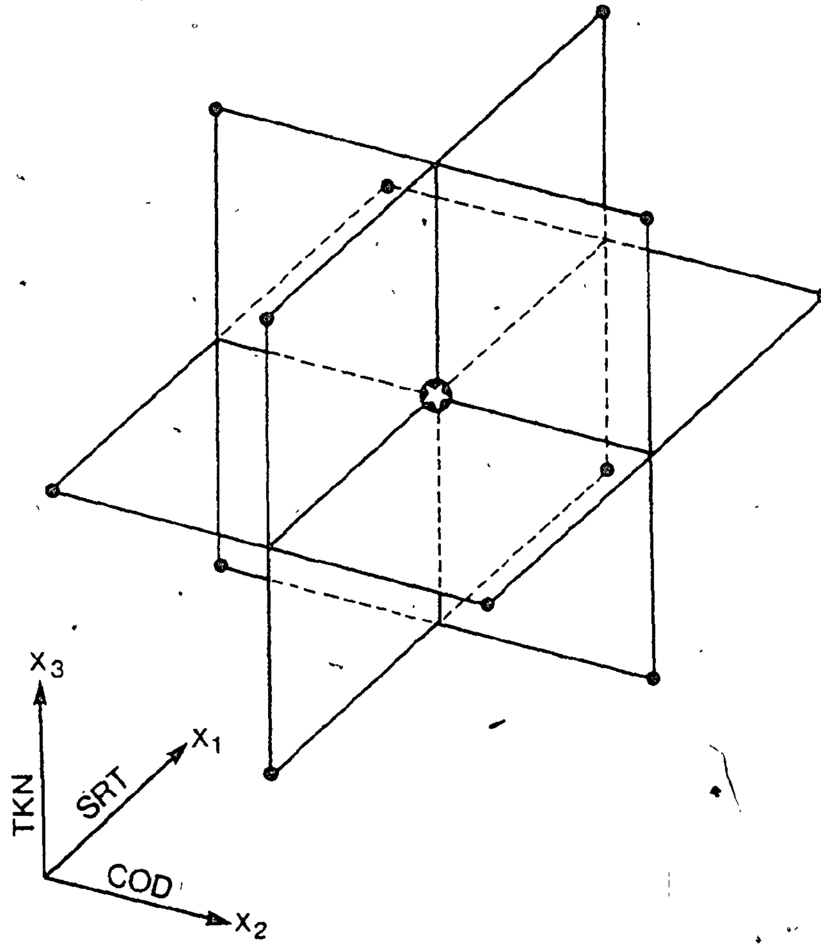
PARAMETER	$\bar{y}_A - \bar{y}_B$	S^2_{AB}	t~	$t_{.05}$	$t_{.01}$
Mixed culture activities					
(a) Nitrosomonas					
ΔN $mgN \cdot L^{-1} \cdot hr^{-1}$	.9	.107	3.44	2.13	3.75
(b) Nitrobacter					
ΔN $mgN \cdot L^{-1} \cdot hr^{-1}$	.2	.31	.51	2.13	3.75
Added nitrifiers : k_{max}					
(a) Nitrosomonas					
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TSS$	.02	.0006	.95	2.13	3.75
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TKN$	.06	.03	.45	2.13	3.75
(b) Nitrobacter					
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TSS$	.05	.008	.67	2.13	3.75
k_{max} $mgN \cdot hr^{-1} \cdot mg^{-1}TKN$	.07	.79	0.08	2.13	3.75

conditions and nitrification was allowed to develop spontaneously. After the establishment of complete nitrification an additional 3-4 days were provided prior to making kinetic measurements. This approach ensured a minimum acclimation period of 10 days, irrespective of the SRT of the system.

4.4 Kinetic Studies of Mixed Culture Nitrifiers

To examine the effects of SRT and COD and TKN loadings on nitrification kinetic parameters, a partial factorial experimental design (Table 5) was applied to the lab scale extended aeration plants. A schematic representation of the design is illustrated in Figure 20 along with the general linear model utilized for data regression. At each experimental point one mixed culture system was operated under steady state conditions and nine system responses were measured using the nitrification assay technique. Periodic operating data and chemical analyses for this set of experiments are contained in Appendix B.

In a suspended growth system, a significant portion of the mixed liquor solids will consist of inactive cells, biological debris and inorganic particulates. Consequently, the solids mass tends to reflect the amount and type of solids in the influent, in addition to biological growth. Since kinetic parameters would have more universal



$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_1^2 + \beta_5 x_2^2 + \beta_6 x_3^2 + \beta_1 x_1 x_2 + \beta_8 x_1 x_3 + \beta_9 x_2 x_3$$

Figure 20. Three Dimensional Representation of the Phase III Experimental Design with the General Regression Equation.

application if they could be related to the active biomass rather than the total solids mass, the nitrifying activity found in a mixed culture sample was attributed to fully active cells. Therefore pure nitrifier cultures, which provided the activity equivalent, were maintained in an active state of growth by operation at an SRT close to the critical minimum value. It is generally accepted that the SRT_c for nitrifiers is in the range 2-5 days for growth at 20-25^oC (Poduska, 1973). The sludge age of 4 days used in this study provided pure cultures of autotrophs which were growing at close to their maximum rates.

4.4.1 Experimentally measured responses

The independent variable settings for COD and TKN indicated in Tables 14 and 15 are slightly different from the scaled settings of the original experimental design. The design settings were in fact used as target operating values, but the loadings actually attained under those conditions provided a more meaningful basis for analysis. By expressing COD and TKN as daily mass removals per unit reactor volume ($g \cdot L^{-1} \cdot d^{-1}$), they can be applied to any carbon removal-nitrification system with feed concentrations and HRTs which are different from this study. Substrate removal was taken to be the difference between total influent concentration and filterable effluent concentration.

TABLE 14. Measured Responses for Ammonium Oxidation in Mixed Culture Studies

RUN	INDEPENDENT VARIABLES**			MEASURED RESPONSES*					
	SRT	COD	TKN	ΔNH_4-N	ΔNO_x-N	k_{max} (NH_4,TSS)	k_{max} (NH_4,TKN)	k_{max} (NO_x,TSS)	k_{max} (NO_x,TKN)
III-1	17.5	.63	.19	6.93	8.66	.22	1.98	.21	1.84
-8	"	"	"	8.49	6.82	.16	1.23	.13	1.0
-14	"	"	"	16.0	15.5	.14	1.46	.15	1.62
-19	"	"	"	8.6	8.8	.17	1.54	.10	.89
-20	"	"	"	6.2	8.4	.27	2.18	.27	2.18
-2	30	.23	.19	-	-	.17	1.32	.22	1.62
-17	"	"	"	7.2	6.92	.19	1.63	.16	1.40
-9	17.5	1.02	.37	17.0	12.8	.11	.97	.06	.48
-16	"	"	"	29.0	19.9	.17	1.52	.14	1.24
-3	30	.57	.03	3.33	2.08	.33	2.77	.20	1.73
-4	30	1.02	.20	8.0	8.0	.29	2.33	.29	2.33
-5	30	.58	.37	13.5	11.3	.13	1.08	.13	1.08
-6	17.5	.20	.03	.58	.46	.32	2.63	.31	2.52
-7	5	.66	.03	.10	.15	.32	2.80	.26	2.25
-10	17.5	1.12	.03	0.0	.08	.37	3.15	.35	2.93
-11	17.5	.16	.37	18.2	16.5	.04	.48	.12	1.49
-12	5	.43	.37	18.5	19.5	.13	1.09	.13	1.09
-13	5	1.03	.19	10.3	6.33	.16	1.55	.18	1.68
-15	5	.18	.19	17.0	13.7	.16	1.59	.16	1.50
-18	17.5	.31	.38	38.0	33.6	.35	2.74	.35	2.74

* Units as indicated in Table 12.

** SRT in days ; COD and TKN in grams removed · L^{-1} reactor volume · day^{-1}

The measured kinetic parameters associated with ammonium oxidation are presented in Table 14. As expected, mixed culture nitrifying activity (ΔNH_4-N, ΔNO_x-N) tends to increase with increased nitrogen loadings. Conversely, it appears that maximum specific substrate removal rate for Nitrosomonas decreases with increased nitrogen loadings.

Results for nitrite oxidation (Table 15) indicate that Nitrobacter are more sensitive to environmental conditions than Nitrosomonas. In runs III-10, -12 and -15, no mixed culture nitrite removal was measureable by the assay procedure. Operating data for the same runs (Appendix B) showed that Nitrobacter activity had been slowly lost from the system and that nitrite accumulation had continued to a steady state value. In some runs (III-7, -10) the known additions of pure culture Nitrobacter were also completely inhibited. Settings of high COD and lower sludge ages appear most likely to result in inhibition.

A similar observation was reported by Stover et al. (1976) for a completely mixed carbon removal-nitrification system treating synthetic wastewater. When that system was subjected to a two-fold increase in influent COD, nitrite oxidation was suppressed for two days before recovering. The authors have suggested that the inhibition of Nitrobacter was associated with the rapid cell growth accompanying an organic step loading; perhaps through the

TABLE 15 . Measured Responses for Nitrite Oxidation in Mixed Culture Studies

INDEPENDENT VARIABLES**				RESPONSES*		
RUN	SRT	COD	TKN	ΔNO_2-N	k_{max} (NO_2,TSS)	k_{max} (NO_2, TKN)
III-1	17.5	.63	.19	-	.32	3.1
-8	"	"	"	5.32	.52	4.17
-14	"	"	"	5.59	.42	5.48
-19	"	"	"	4.20	.59	5.0
-20	"	"	"	4.50	.71	5.42
-2	30	.23	.19	3.3	.75	6.34
-17	"	"	"	4.79	.71	6.62
-9	17.5	1.02	.37	9.83	.21	1.79
-16	"	"	"	8.17	.53	4.53
-3	30	.59	.03	0.5	.49	4.75
-4	30	1.02	.20	3.75	.47	4.10
-5	30	.58	.37	9.17	.63	6.46
-6	17.5	.20	.03	0.5	.68	6.19
-7	5	.66	.03	.12	0	0
-10	17.5	1.12	.03	0	0	0
-11	17.5	.16	.37	7.5	1.07	7.87
-12	5	.43	.37	0	.91	7.32
-13	5	1.03	.19	6.33	.57	5.63
-15	5	.18	.19	0	.68	7.20
-18	17.5	.31	.38	7.6	.53	4.39

* Units as indicated in Table 12

** SRT in days ; COD and TKN in grams removed · L^{-1} reactor volume · day^{-1}

increased production of toxic metabolic byproducts. Observations of this type have also been attributed to a dissolved oxygen effect. Kiff (1972) and Downing, Painter and Knowles (1964) speculated that under conditions of high rate growth, the interior of individual floc particles may become anaerobic. This could occur even though the bulk liquid oxygen concentration is maintained at a high level.

It is interesting to note from Table 15 that inhibition of both pure and mixed culture *Nitrobacter* was not always observed under the same conditions. In cases where mixed culture activity was lost and pure culture activity was normal (III-12, -15), the variation is probably not due to acclimation, but to some fundamental difference in the nature of nitrite oxidation in pure and mixed culture.

4.4.2 Response surface modelling

Mechanistic modelling of nitrogen oxidation requires that nitrifier kinetic constants be known. Unfortunately, most biological kinetic parameters are not constants, but are functions of the current growth conditions and the culture history. Often the behaviour of these "constants" may be described by an additional mechanistic model derived from a theoretical consideration of the variables which are known to affect it. When the mechanism of a particular effect is not known, an empirical equation must be

constructed to allow the prediction of the necessary parameters.

An approach of this type was used to quantify the effects of SRT, COD and TKN upon the nitrification parameters of Tables 14 and 15. For each of the nine responses a polynomial model was derived, using appropriate terms of the general regression equation (Figure 20). By selecting among main effects, interaction and quadratic terms, even a response surface which exhibits considerable curvature can be adequately modelled.

Starting with the general ten term model, a "best" regression equation was chosen for each response using a stepwise linear regression method (Draper and Smith, 1966). This procedure involved a test of significance for each model term and a lack of fit test for the overall equation using replicated data points. Once a "best" regression equation had been chosen for a specific response, an examination was made of the residuals for any obvious lack of fit not identified by the statistical procedures. The results of this analysis are eight equations describing the response of mixed culture nitrification activity and nitrifer k_{max}'s under conditions of variable SRT and COD and TKN loadings (Table 16). For the equations which can be portrayed in 2 or 3 dimensions, Figures 21 through 25 provide a visual representation of the response surfaces.

TABLE 16. Regression Equations Describing Nitrifier Kinetics in Mixed Culture*

A. Indigenous Mixed Culture Activities

(i) Ammonium oxidation

$\Delta NH_4\text{-}N = -.05 + 61.9(TKN) - .77(SRT)(TKN)$ ...(18)

(ii) Nitrite oxidation

$\Delta NO_2\text{-}N = .19 + .67(SRT)(TKN) + 11.9(COD)(TKN)$ ...(19)

B. Nitrifier Maximum Specific Substrate Removal Rates

(i) <u>Nitrosomonas</u>

$k_{max}(N,TSS) = .28 - .55(TKN) + .003(SRT)(COD)$ ...(20)

$k_{max}(N,TKN) = 2.42 - 4.42(TKN) + .36(COD)^2$ ...(21)

(ii) <u>Nitrobacter</u> (all data)

$k_{max}(NO_2,TSS) = .66 - .52(COD) + .87(TKN)$ ...(22)

$k_{max}(NO_2,TKN) = 7.47 - 6.4(COD) + 9.26(TKN)(COD)$ ...(23)

(iii) <u>Nitrobacter</u> (eliminating zero points)

$k_{max}(NO_2,TSS) = .84 - .42(COD)$ ...(24)

$k_{max}(NO_2,TKN) = 7.26 - 3.39(COD)$ ...(25)

*SRT: days

COD: $g \cdot L^{-1} \cdot d^{-1}$

TKN: $g \cdot L^{-1} \cdot d^{-1}$

other units as in Table 8

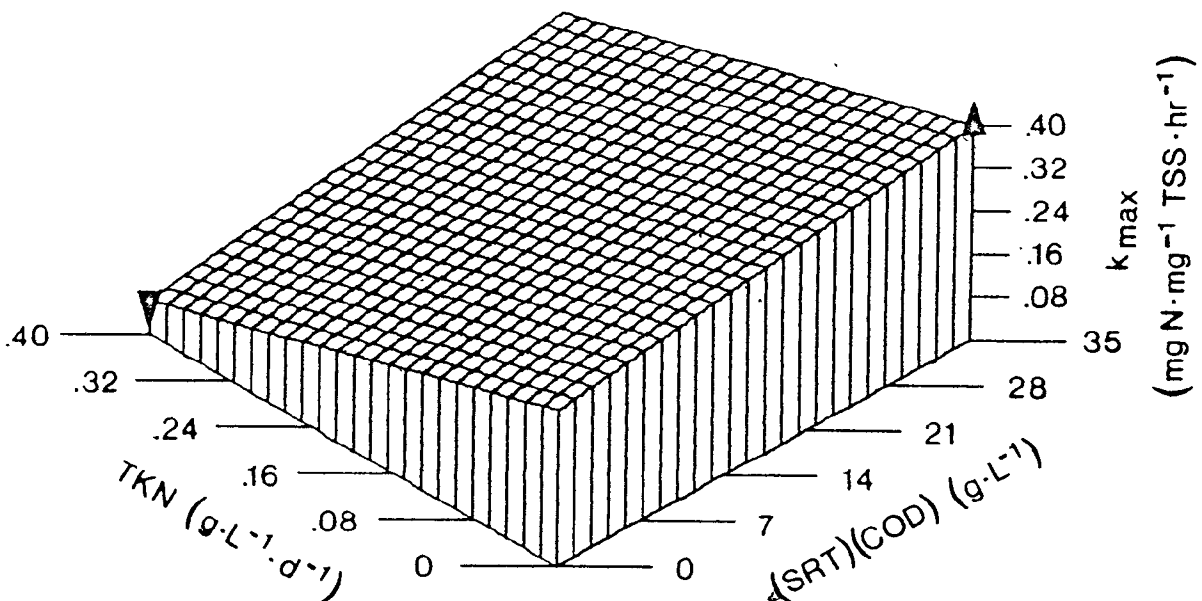


Figure 21. Response Surface for _Nitrosomonas_ k_{max} Based on TSS Measurements.

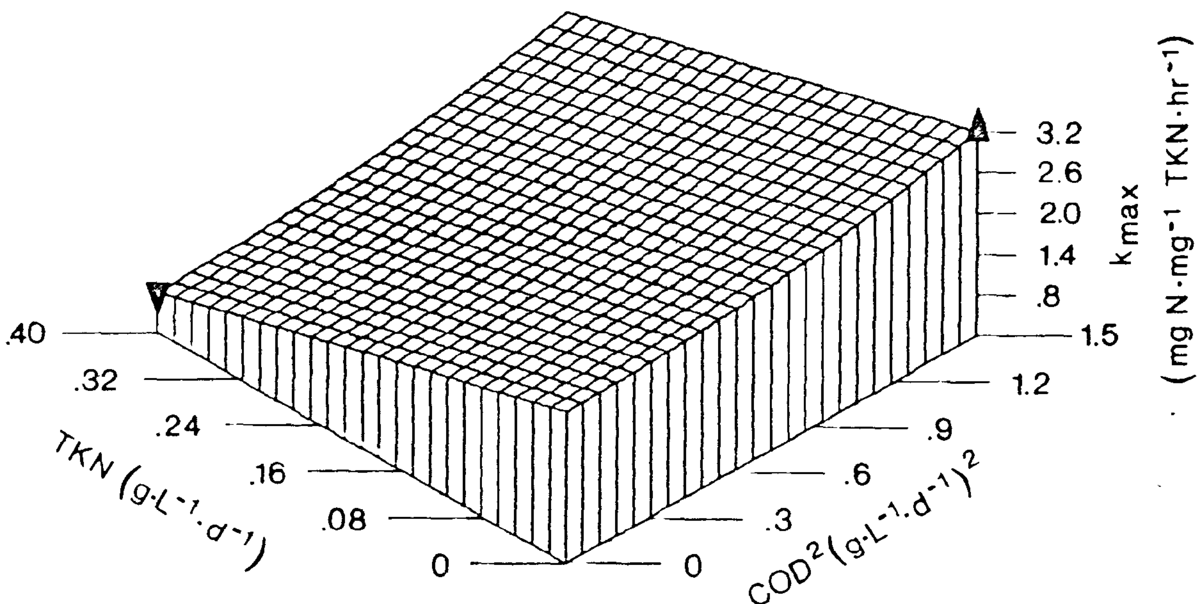


Figure 22. Response Surface for Nitrosomonas k_{max} Based on TKN Measurements.

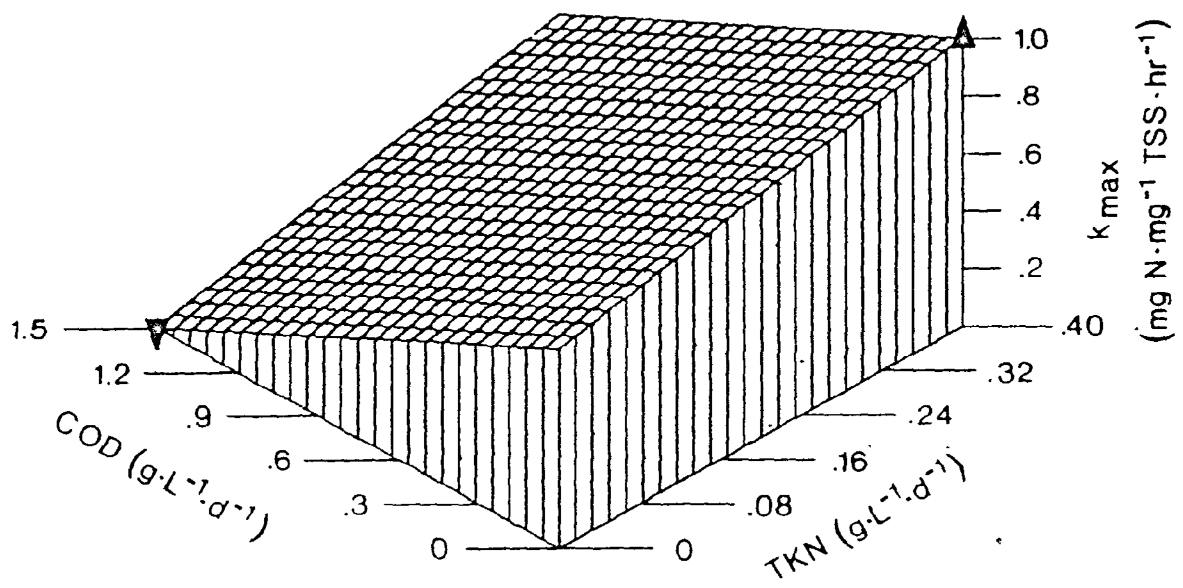


Figure 23. Response Surface for Nitrobacter k_{max} Based on TSS Measurements and Incorporating All Results.

$$k_{max}(NO_2, TKN) = 7.47 - 6.4(COD) + 9.26\,(TKN)(COD)$$

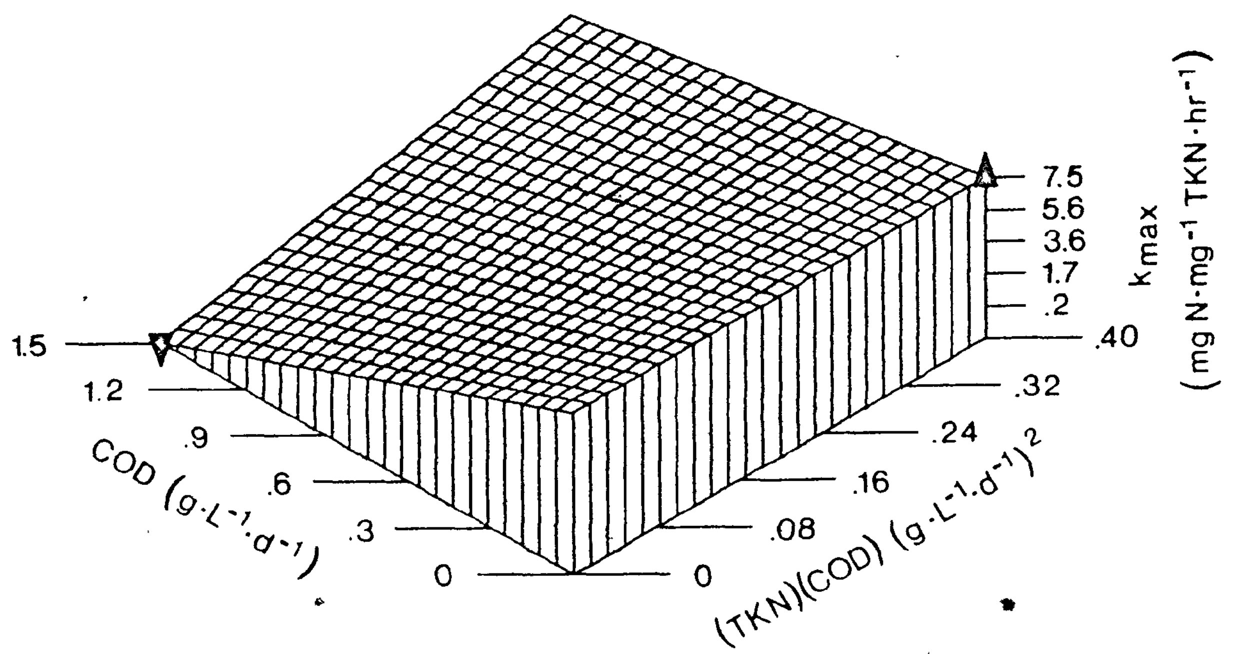


Figure 24. Response Surface for Nitrobacter k_{max} Based on TKN Measurements and Incorporating All Results.

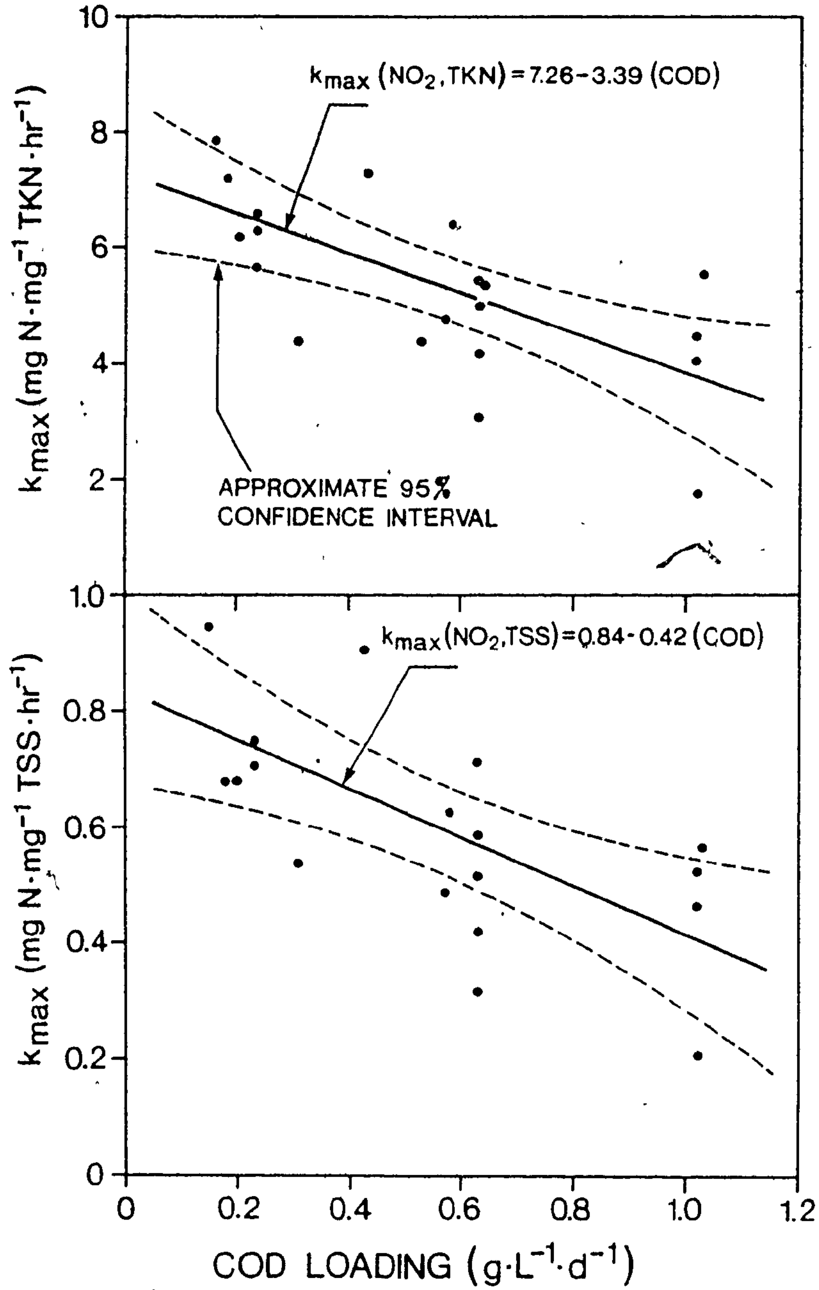


Figure 25. Response Surface for *Nitrobacter* k_{max} Eliminating Zero Point Results.

With the exception of Equation 19, none of the regression models exhibited lack of fit at the 95% significance level. Equation 19 was considered to be a borderline case because although lack of fit was indicated at the 95% level, the model appeared satisfactory at the 99% level. Originally, individual models for ammonium oxidation and Nitrosomonas activity were fitted for both substrate (NH_4-N) and product (NO_x-N) measurements. When it was found that the individual models for NH_4-N and NO_x-N had the same terms and statistically equivalent variances, the data was subsequently pooled and an overall model derived. Therefore models for ammonium oxidation and Nitrosomonas k_{max} are expressed in terms of nitrogen transformation rates and adequately describe rates measured as NH_4-N or NO_x-N.

The response surfaces for maximum nitrification rates measured in mixed culture samples are modelled by Equations 18 and 19. As expected, the main factor affecting ammonium oxidation rates is the TKN loading (Equation 18). However the inclusion of an interaction term indicates that the TKN effect is moderated by varying levels of SRT. Nitrite oxidation (Equation 19) exhibits a more complex behaviour. The absence of a definitive main effect and the borderline significance of the equation are probably due to the intermittent inhibition noted for this response. In order that both zero and non-zero responses may be depicted, a

regression equation assumes a continuous transition between them. This can have the effect of artificially distorting the surface in the vicinity of the zero point when a discontinuity might be a more accurate representation of the response. Experimental evidence of this behaviour was reported by Stover et al. (1976) who noted that nitrification could be inhibited when organic loadings to a mixed culture system exceeded a threshold value. From the data of Table 15, there is no suggestion of an inhibition threshold. Observed responses were measured over the entire range between 0 and 9.83 mg $N \cdot L^{-1} \cdot hr^{-1}$, and at least some of the zeros are partially attributable to low TKN loadings. Therefore all data was utilized in the fitting of Equation 19.

The activities of nitrifying bacteria under varying growth conditions are described by the equations for maximum specific substrate removal rates (Equations 20 to 15). For Nitrosomonas, the major factor affecting activity is TKN loading. As TKN removal was increased, the k_{max} of added Nitrosomonas was observed to decrease (Equations 20 and 21). A small positive effect of COD loading was also noted.

The negative relationship between Nitrosomonas activity and TKN loading is interesting. The effect can not be explained by changes in culture pH, temperature or dissolved oxygen, as no significant variation in these

parameters occurred. Although larger quantities of bicarbonate buffer were required to maintain reactor pH at the high TKN loadings, there was no correlation between Nitrosomonas k_{max} and inorganic carbon concentration. As TKN removal was increased during the experimental run, one additional variable that changed in direct proportion to TKN loading was the reactor NO_3-N concentration. Studies by Anthonisen et al. (1976) indicated that product inhibition of Nitrosomonas may occur, but is most likely related to NO_2-N, not NO_3-N. Downing (1977) also pointed out that nitrifier growth rates obtained with low strength Zürich wastewater were considerably higher than the same parameters measured in more concentrated waste in England and the U.S.A.

The wide range of growth rates reported for Nitrosomonas has been attributed to differing culture conditions (Sharma and Ahlert, 1977). Nutritional requirements of nitrifying bacteria are quite complex and Painter (1970) has suggested that variation in growth rates is due to limitation by one or more essential nutrients or to inhibition by toxic contaminants. Trace nutrient limitation would be consistent with the responses of Equations 20 and 21. At high substrate loadings it is possible that an additional nutrient may become limiting. As long as sufficient hydraulic residence time is provided

for complete nitrification, the reduced activity due to nutrient limitation might not be evident in terms of TKN removal.

The results obtained for Nitrobacter were correlated in two ways. For Equations 22 and 23 all available data was used, including the two experiments in which added Nitrobacter showed no activity. These regressions indicate that COD and TKN have negative and positive effects respectively on Nitrobacter specific activity. Equations 24 and 25 were derived by a regression of data excluding the inhibition points. An examination of the measured responses (Table 15) did not reveal any consistent pattern which would explain this inhibition. In addition, these two results appear to be isolated from the bulk of the observations. If they are in fact representative of a real discontinuity in the response surface, the remaining surface will be more accurately modelled by omitting these values. With this approach, the significant TKN effect on Nitrobacter activity was removed (Equations 24 and 25), leaving only a negative effect of COD loading.

It should be emphasized that empirical modelling of the type described here does not substantiate a cause and effect relationship between the independent and dependent variables. The limitations of this approach are amply illustrated by the fact that several of the equations in

Table 16 would erroneously predict k_{max} to be zero if substrate loadings were outside the ranges examined in this study. Nevertheless, this technique can provide a useful screening procedure to establish the relative importance of several variables on a single response. In the case of nitrifier substrate removal rates, the major effects observed in this study have also been cited by other researchers. When these effects are quantified by an equation whose terms have been properly identified the resulting model can be a useful tool for forecasting purposes.

4.5 Dynamic Modelling of the Combined Carbon Removal-Nitrification Process (NITOX).

4.5.1 Model development and parameter selection.

In Section 4.4 empirical models were developed which related nitrifier k_{max}'s in mixed culture to carbon and nitrogen process loadings and to the system solids retention time. In themselves, these equations provide limited insight into the behaviour of nitrifying bacteria in an activated sludge environment. To be useful in a broader engineering sense, information of this type must be applied in conjunction with a more general predictive model for process performance. This would allow individual microbial population dynamics to be integrated into a network which

predicts total system response to changing process inputs.

A general process model must provide reliable predictive capability under both steady state and dynamic conditions. The steady state approach generates information which is best suited to process design and to the identification of long term process operational strategies. However, most wastewater treatment facilities are subject to short term fluctuations which are beyond the operator's control and which are of sufficient magnitude that effluent quality may be affected. In this situation a dynamic model could provide the forecasting required for feed-forward control of the process to maintain effluent quality. Over the longer term, the same dynamic model could provide insight into operational changes needed to accommodate seasonal fluctuations in wastewater temperature, flow, and strength. For examples of the application of process modelling in the engineering of nitrification systems, the reader is referred to Poduska (1973), Sutton (1976) and Gujer (1977).

Figure 2 illustrates the general form of a dynamic mechanistic model (NITOX) which was derived for a mixed culture carbon removal-nitrification system. Details of the model development are given in Appendix C. Although the model includes material balance equations for COD and heterotrophs, it is not intended that NITOX be a complete

representation of a nitrifying activated sludge process. In cases where a more accurate accounting of stored, metabolized and refractory constituents is required, a general, structured model of the type described by Ekama and Marais (1977) should be considered. The purpose of NITOX is simply to model the conversion of ammonium to nitrate in the presence of heterotrophic activity. For wastes in which a large proportion of the influent nitrogen is organic, it will be necessary to consider the transformation of organic nitrogen to ammonium prior to nitrification.

An examination of Figure 2 indicates that five model parameters must be specified before the system of ordinary differential equations can be integrated. Four of the parameters, k_{max}, K_S, k_D and Y must be evaluated for the heterotrophic population as well as Nitrosomonas and Nitrobacter. The fifth parameter, f, is the fraction of newly synthesized heterotrophic biomass which is composed of nitrogen. The inclusion of this factor allows the simulation of nitrogen uptake by heterotrophic bacteria for cell synthesis. The factor, f, was evaluated by averaging the mixed liquor non-filterable TKN measurements from all the experimental runs of Phase III (Appendix B).

The relative importance of k_{max}, K_S, k_D and Y for nitrifiers was examined in an analysis of NITOX sensitivity to changes in these parameter values. Figures 26 through 28

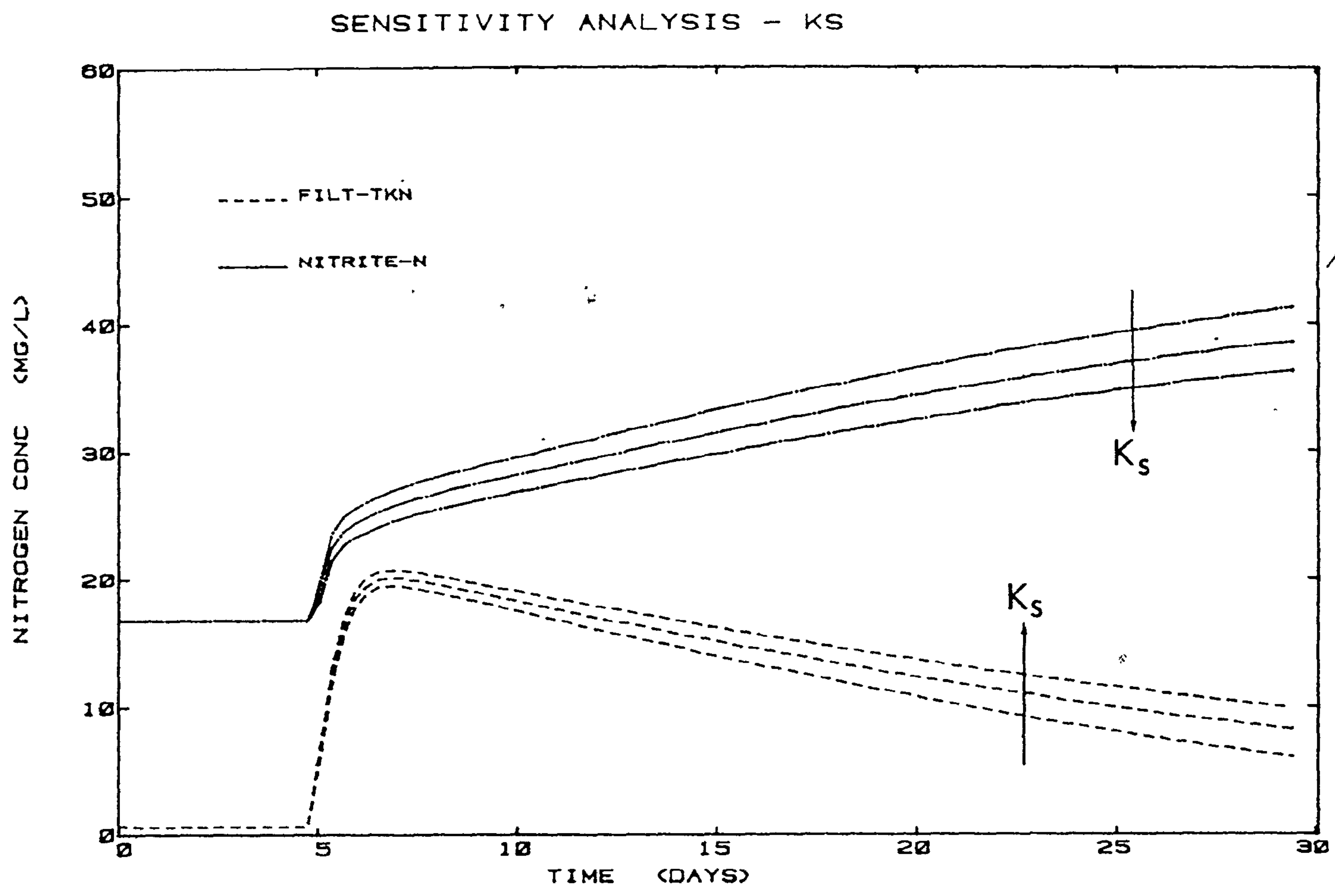


Figure 26. NITOX Model Sensitivity Analysis - K_s Parameter (K_s = 0.5, 1.0, 1.5 $mg \cdot L^{-1}$).

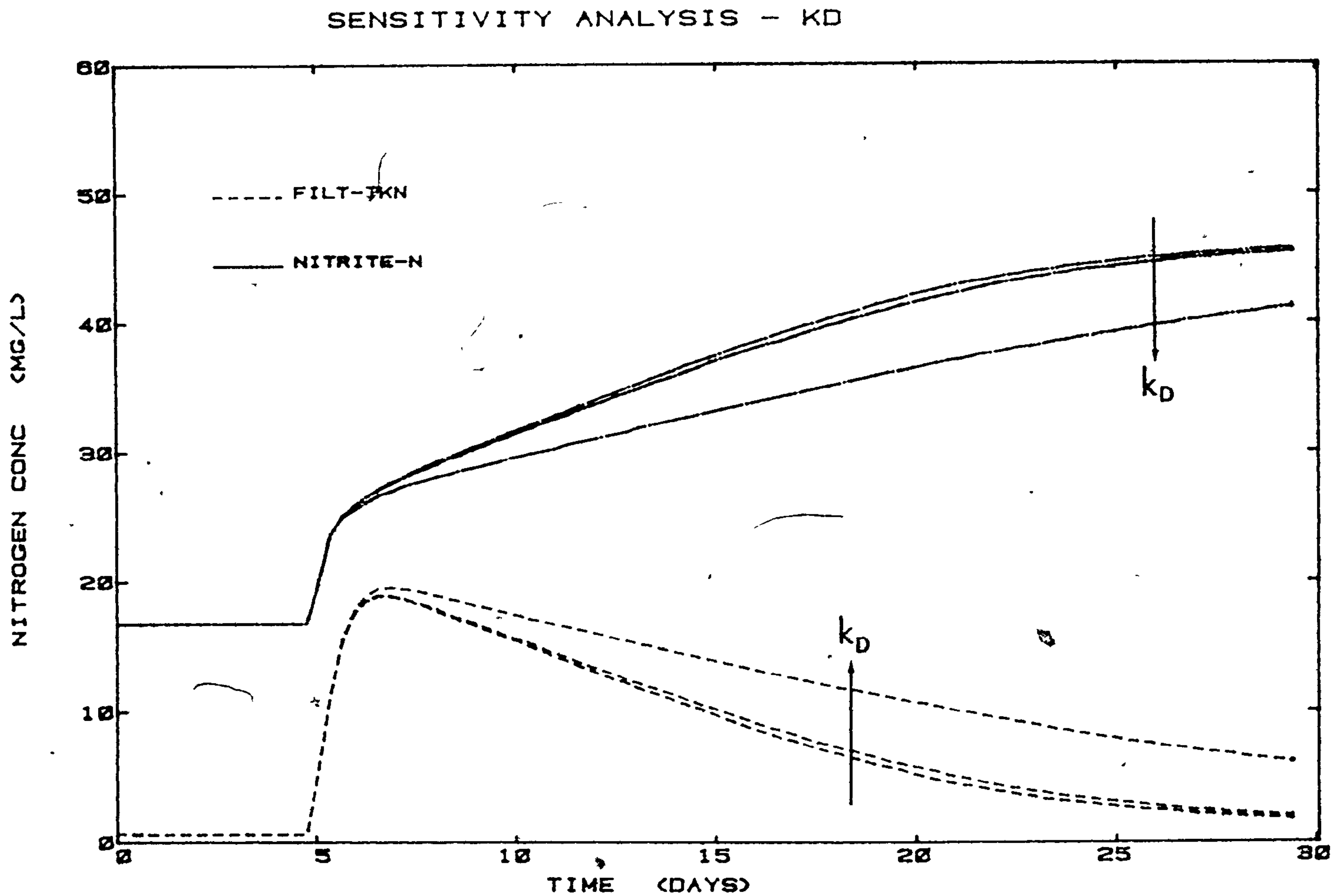


Figure 27. NITOX Model Sensitivity Analysis - k_D Parameter (k_D = 0.05, 0.1, 0.5 $days^{-1}$).

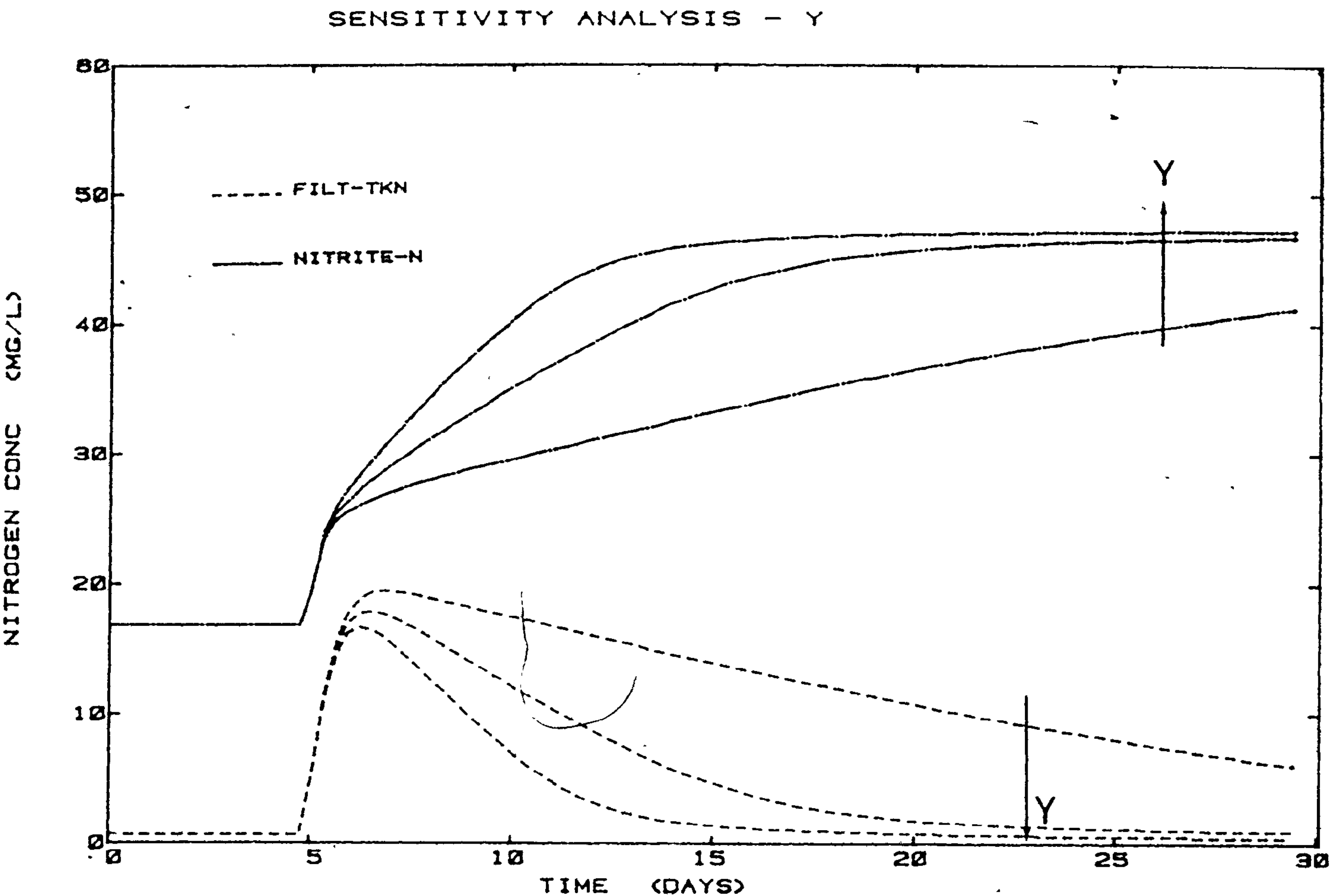


Figure 28. NITOX Model Sensitivity Analysis - Y Parameter (Y = 0.05, 0.075, 0.10 mg VSS·mg^{-1}N).

TABLE 17. Summary of Simulation Conditions for NITOX Sensitivity Analysis

PARAMETER	VALUE
HRT (hours)	6.4
SRT (days)	10.0
Recycle/Feed Ratio	1.25
Temperature (^{o}C)	20
Influent COD ($mg.L^{-1}$)	300
Heterotroph Kinetics	
k_{max} ($days^{-1}$)	4.8
Y ($mg\ VSS \cdot mg^{-1}\ COD$)	0.45
K_s ($mg \cdot L^{-1}$)	22.0
k_D ($days^{-1}$)	0.03

show the simulated response to a step change in feed ammonium nitrogen from 25 to 50 mg.L^{-1} after 5 days for the nitritification step. Other simulation conditions for the sensitivity analysis are given in Table 17. The nitratification behaviour was essentially identical.

The NITOX response to a step change in substrate concentration confirms the findings of Poduska (1973). The parameters K_S and k_D are of relatively less importance to model response than the nitrifier maximum specific growth rate, μ_{max}. For the NITOX sensitivity analysis, changes in μ_{max} are governed by the relationship of Equation 14:

$$\mu_{max} = Y \cdot k_{max} \tag{14}$$

Therefore any variation in Y has the effect of altering μ_{max}. The k_{max} parameter was not specifically examined in this procedure since its value will be fixed by the empirical relationships of Table 16. In addition, from Equation 14, it can be seen that changes in Y have the same effect as those in k_{max}.

The values of nitrifier kinetic parameters are known to vary with environmental conditions. Knowles, Downing and Barrett (1965) determined regression equations which describe the effect of temperature on nitrifier half-saturation coefficients, K_S:

Nitrosomonas : $K_s = 0.405 \exp(.118(T-15))$ (26)

Nitrobacter : $K_s = 0.625 \exp(.146(T-15))$ (27)

where T is the temperature in ^{o}C.

However, because the sensitivity analysis did not indicate that the half-saturation coefficient played a major role in modelling results, K_s values were assumed to be constant with temperature. The various kinetic parameters used in subsequent NITOX simulations are given in Table 18. Heterotroph parameters were estimated from Lawrence and McCarty (1970). Nitrifier parameters were evaluated as representing approximately the mid-points of the ranges of reported values summarized by Poduska (1973) and Sharma and Ahlert (1977).

TABLE 18. Summary of Kinetic Parameter Values Used in NITOX

PARAMETER	HETEROTROPHS	NITROSOMONAS	NITROBACTER
Y $(mg \cdot mg^{-1})$*	.45	.05	.03
K_s $(mg \cdot L^{-1})$	22.0	0.5	0.5
k_D $(days^{-1})$	0.03	0.05	0.05

*mg VSS mg^{-1} COD for heterotrophs
*mg VSS mg^{-1} N for nitrifiers

Several authors have proposed modifications to the Monod equation to account for environmental effects on observed nitrification rates. Stenstrom and Poduska (1980) reviewed the use of a double Monod function to explain dual substrate rate control when both ammonia and dissolved oxygen concentrations are limiting. The effects of pH on nitrifying bacteria can be estimated by relationships of the type presented by Downing and Knowles (1966) or Laudelot, Lambert and Pham (1976). Edwards (1970) and Neufeld, Hill and Adekoya (1980) have derived inhibition functions to describe the effects of high substrate concentrations on nitrification rates. Relationships of these types have not been included in NITOX. Model simulations assume non-limiting dissolved oxygen and pH levels, and do not consider substrate inhibition effects.

4.5.2 Model verification

The adequacy of the proposed simulation routine was assessed by comparison of model outputs to data derived from external sources. For accurate prediction of system response, NITOX requires a complete input list detailing the plant operating conditions and wastewater characteristics (Table 17). Such detailed information was provided by Sutton (1976) who carried out pilot scale studies on several process configurations for carbon removal - nitrification.

In Figure 29 the results of several steady state

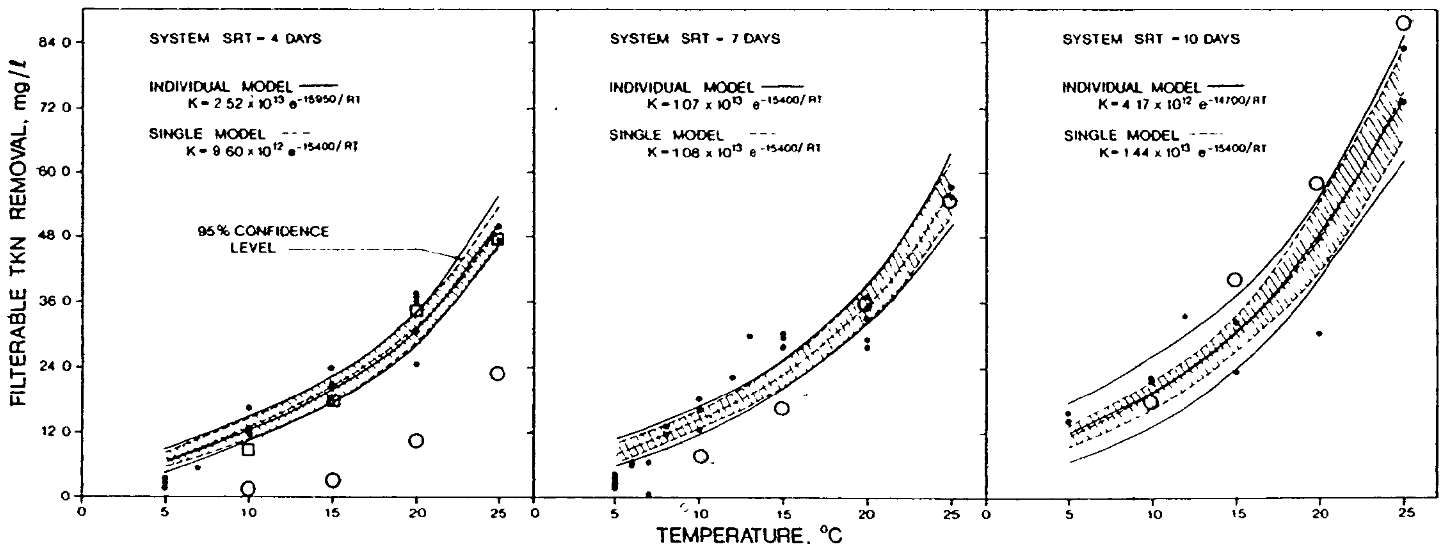


Figure 29. Comparison of Predicted Steady State Filterable TKN Removal to Results of Sutton (1976). (Predicted results ; ⊙ Y = 0.05 ; □ Y = 0.10)

simulations of ammonium nitrogen removal are superimposed upon the results measured experimentally under the same conditions by Sutton for a combined sludge system. To ensure an adequate nitrogen residual during nitrification rate determinations, an ammonium salt was added to the primary effluent used for process feed. This fact, plus the nature of the Burlington Skyway wastewater, resulted in ammonium nitrogen concentrations that were almost identical to the filterable TKN. Therefore, even though NITOX considers only inorganic nitrogen transformations, the results can be safely compared to Sutton's data.

Figure 29 indicates that agreement between predicted and observed results is very close over a range of temperatures and SRTs. However, at an SRT of 4 days, the model consistently under-estimates nitrogen removal. Better agreement can be obtained by assuming a larger yield coefficient for the Nitrosomonas population.

It is not surprising that a discrepancy exists at the lower SRT. As indicated by Poduska (1973) the critical SRT for nitrification lies in the range between 2 and 5 days (Figure 30). In this range, a small difference in nitrifier growth rate could translate into a large difference in the percent nitrogen removal achieved (Figure 30). Thus NITOX may not be adequate for exact prediction near the critical SRT. A larger yield coefficient was assumed at the 4 day

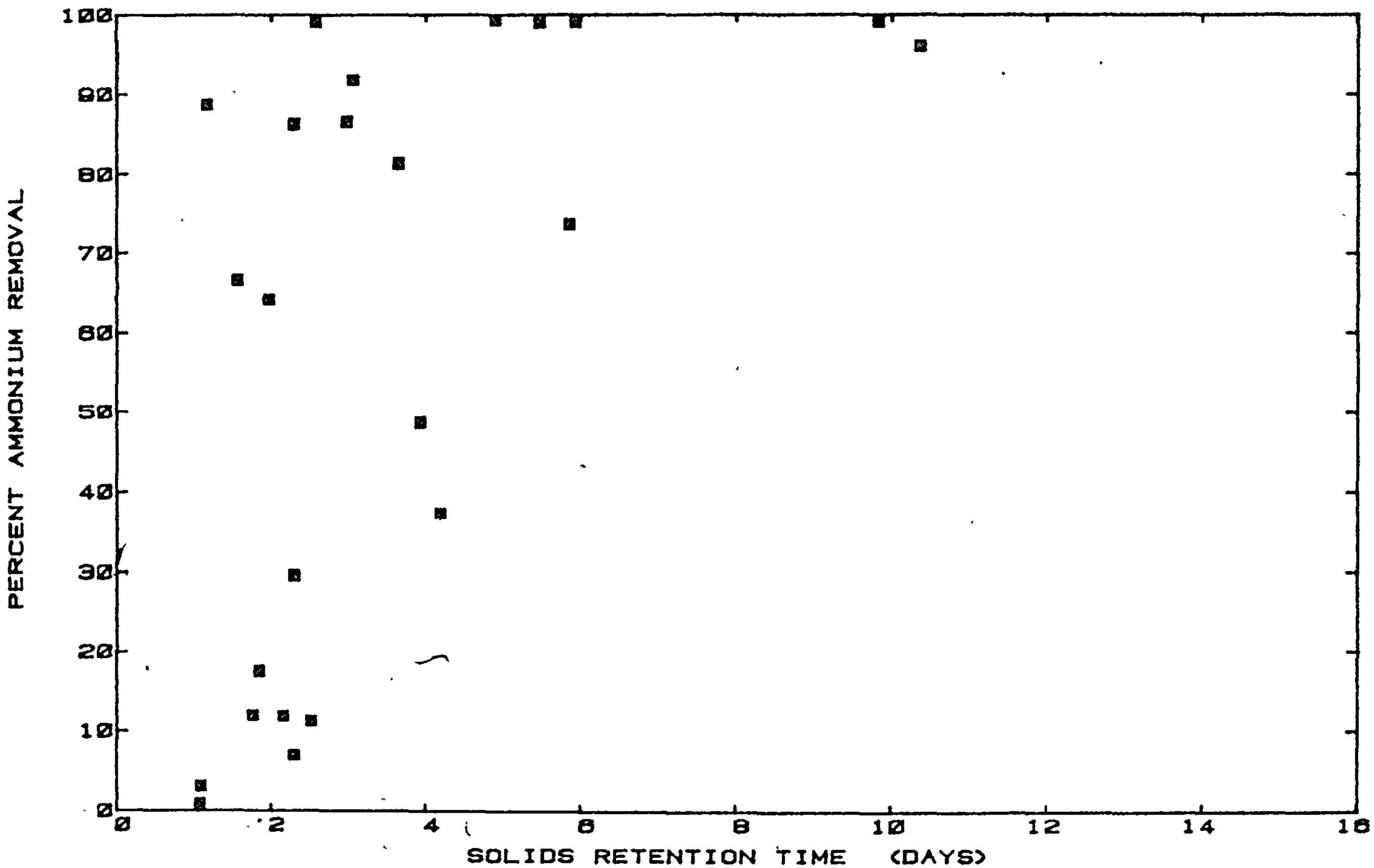


Figure 30. Summary of Nitrification Efficiencies Reported at 20 - 23°C. (Poduska, 1973).

SRT to facilitate further comparisons.

Figure 29 also shows an effect of including wastewater C:N ratio and system SRT in the model calculations. Without these terms, the predicted temperature response would approximate more closely the smooth Arrhenius function illustrated by Sutton. By incorporating other effects in the rate estimation some deviation from the Arrhenius curve is produced, but agreement with measured rate data still is evident.

NITOX output during unsteady state simulation was examined by comparison to the results obtained by Sutton for a series of step changes in influent strength and flow rate (Sutton Run D5). The programmed flow changes (Figure 31 (a)) posed a severe test of model adequacy under dynamic conditions. Initial conditions for this simulation were determined by generating steady state values for a period preceding the dynamic portion, under the conditions described by Sutton. Both the steady and dynamic experimental periods were conducted at an SRT of 4 days with a mixed liquor temperature of 14-15°C. Influent ammonium levels measured during the dynamic run were approximated for NITOX by the horizontal bars shown superimposed over Sutton's measured concentrations in Figure 31(b). By averaging several closely spaced data points, the complexity

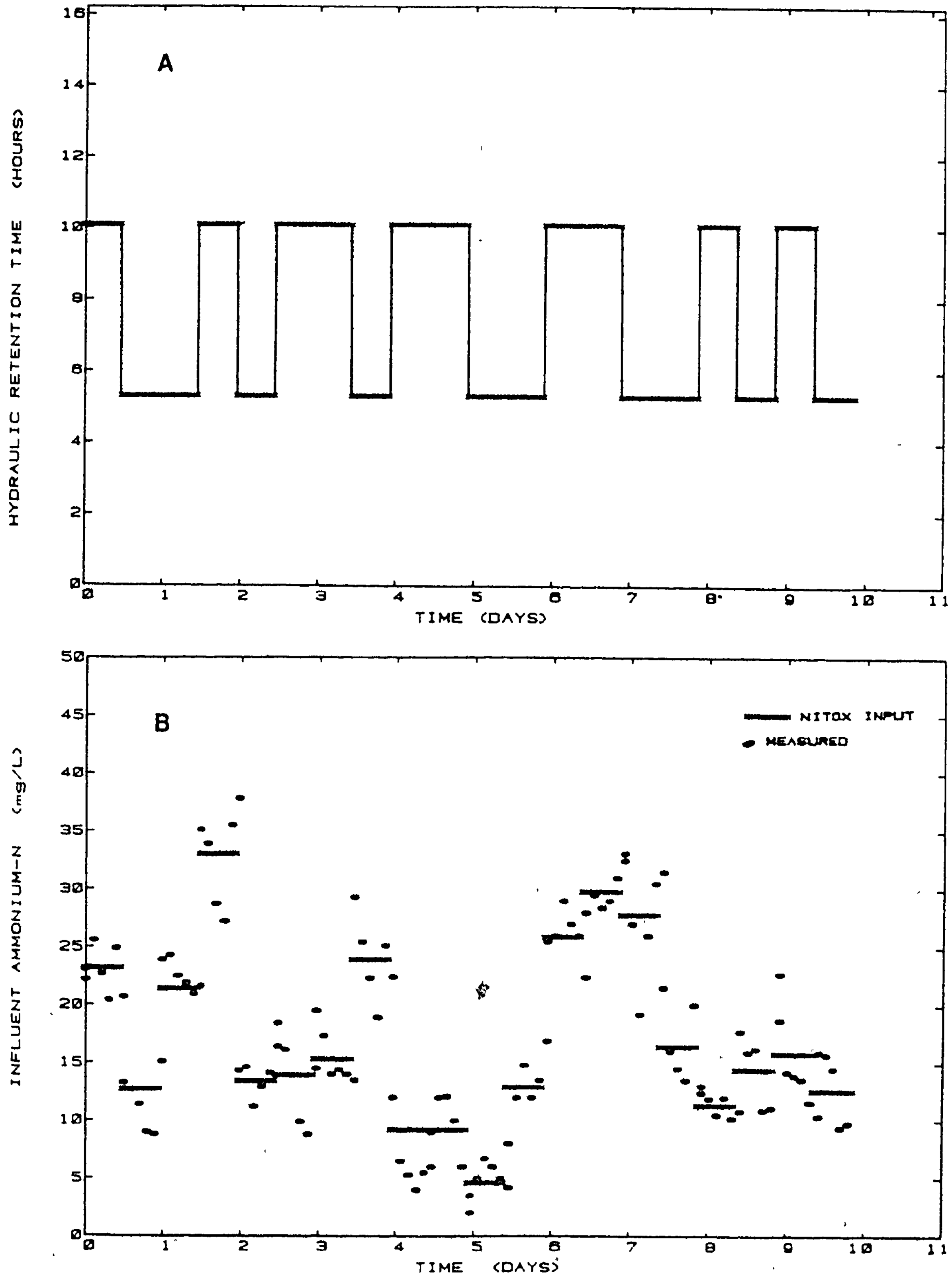


Figure 31. Approximation of Dynamic Conditions for Simulation of Sutton D5 Run.

of the model input function was reduced to more manageable proportions.

The effluent concentration predicted by NITOX is compared to Sutton's measured data in Figure 32. the agreement between simulated and observed results is very close. Most of the discrepancy between the two data sets can be traced to the averaging technique used to simulate feed strength. Unless every measured influent value is entered into NITOX separately, the simulation result can not be expected to match the observed data exactly.

The major variation between predicted and measured results occurs between days 2 and 3 of the 10 day run. During this period the actual effluent concentrations peaked at about 7-8 $mg \cdot L^{-1}$, while the model output indicates no such response. An examination of the operating conditions for this period (Figure 31) did not suggest any reason for the observed increase in ammonium concentration and therefore the discrepancy remains unexplained.

A further verification run under dynamic loading conditions is illustrated by Figure 33. Here the conditions reported by Gujer (1977) for pilot scale treatment of Zürich wastewater were simulated using the flow and concentration inputs reported by that author. As previously, initial values were established by generating steady state solutions for the average wastewater characteristics and operating

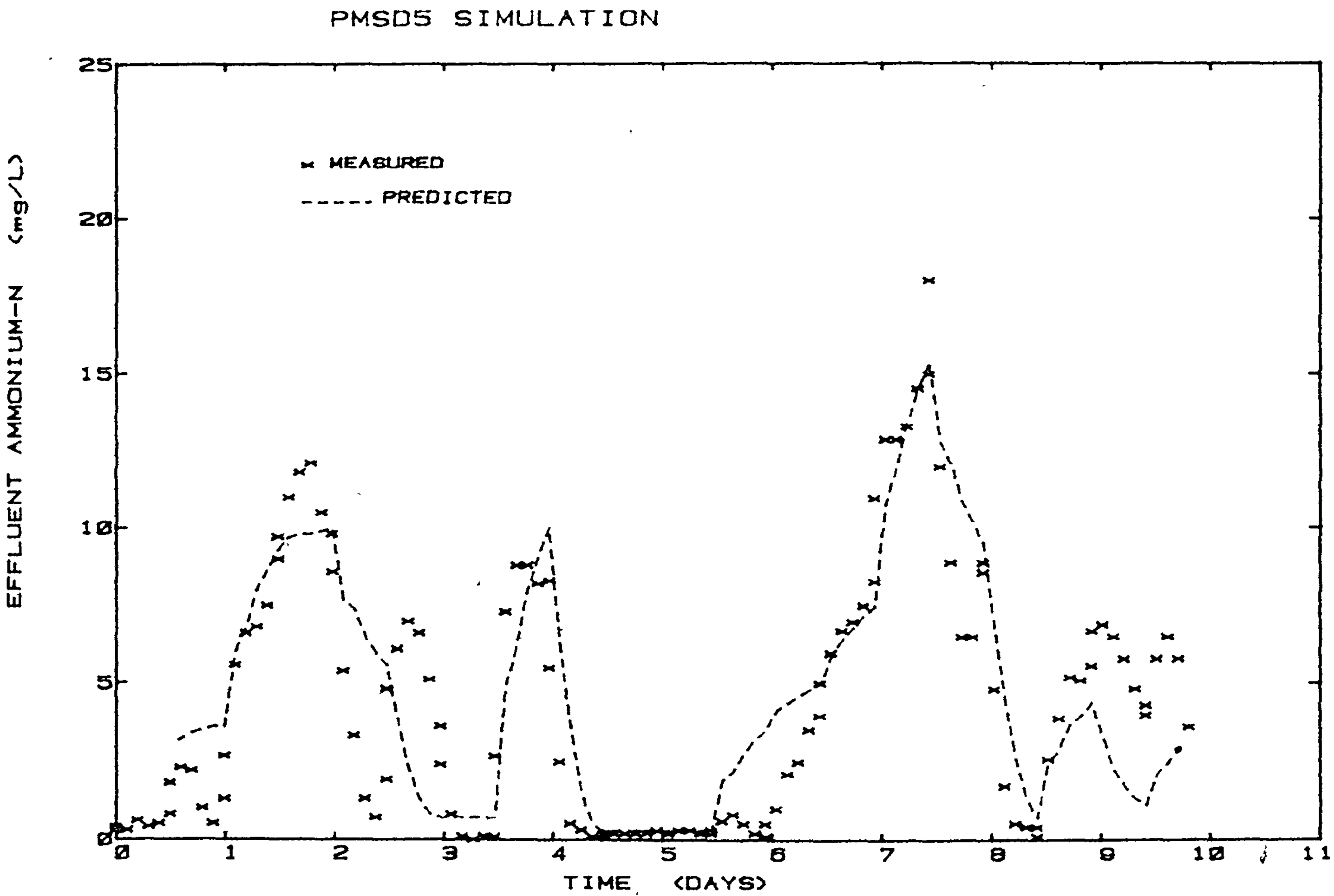


Figure 32. NITOX Simulation Results and Measured Data for Sutton D5 Run.

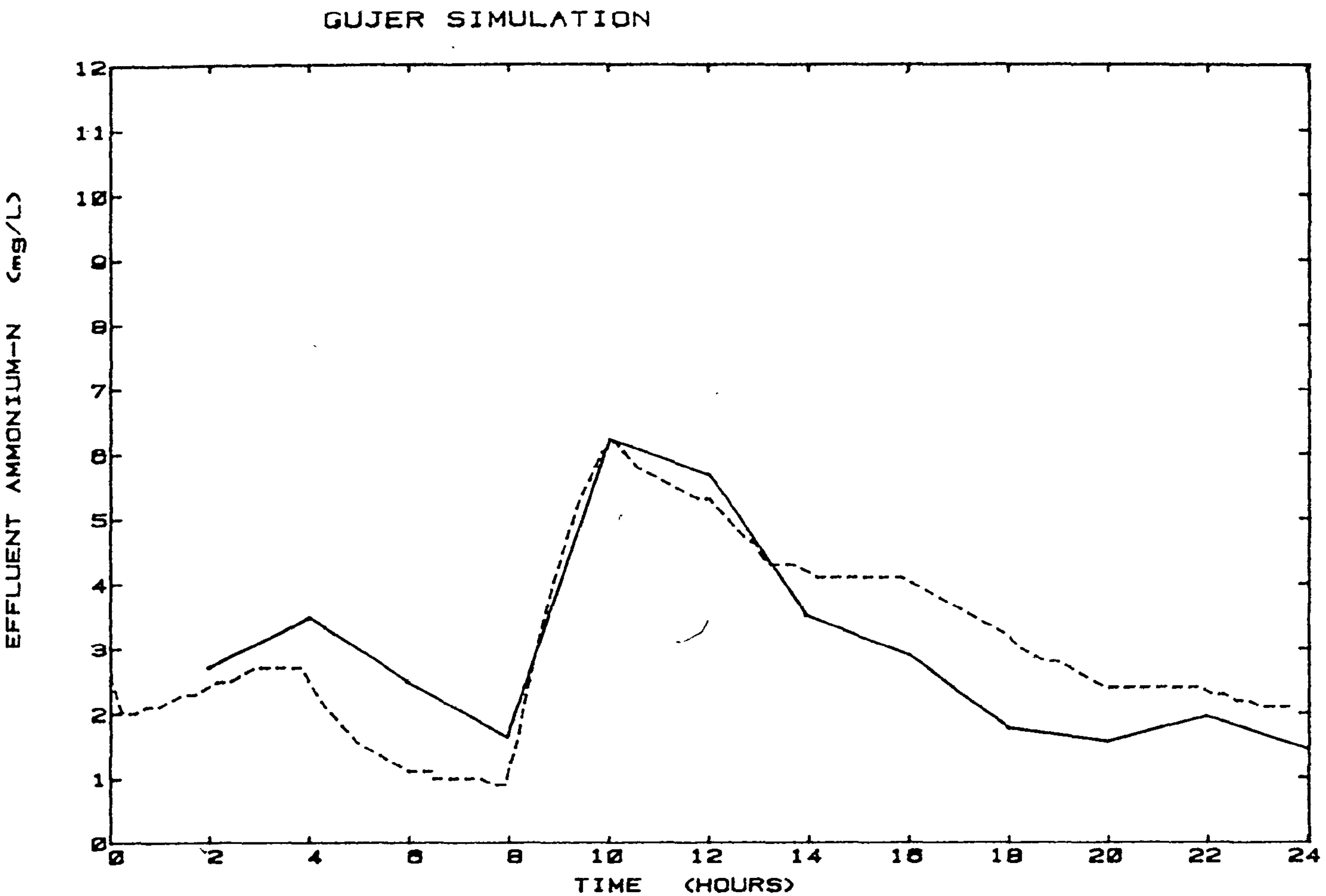


Figure 33. Comparison of NITOX and Gujer Simulation Results for Zürich Wastewater. (NITOX ---- ;Gujer ——)

conditions of the complete pilot plant study. The dynamic portion of the simulation indicated by Figure 33 shows the effluent ammonium nitrogen levels predicted by NITOX under varying diurnal loading conditions. The results attributable to Gujer in this figure are actually calculated values produced by a simulation model which had been calibrated to Zürich wastewater. Measured results for this run were not identified in the paper. Consequently direct comparison between real and simulated conditions was not possible, however, the author demonstrated that his model produced excellent agreement with other measured results. The comparison between NITOX and Gujer's model indicates essentially similar results. The degree of similarity is remarkable considering that NITOX did not require parameter calibration prior to application.

Mechanistic models such as NITOX appear to provide satisfactory predictive capability under a variety of steady state and dynamic conditions. However, there are some situations in which model response does not accurately reflect the behaviour of a real biological process. Figure 34 shows the measured influent ammonium nitrogen concentrations reported by Sutton (1976) during a steady flow experiment. The simulated feed values for NITOX are also shown. After 24 hours of operation at 15°C and an SRT

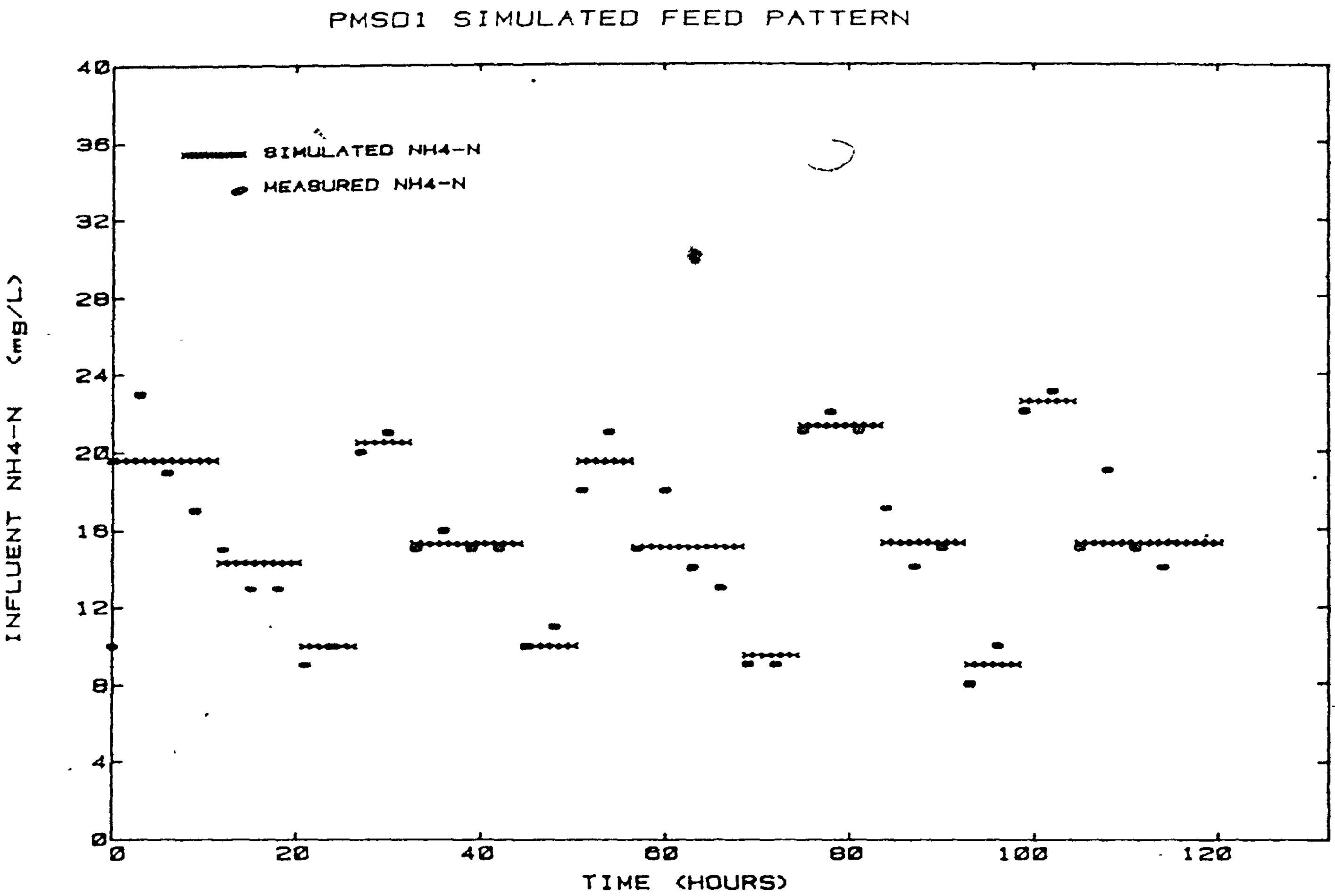


Figure 34. Approximation of Feed Conditions for Simulation of Sutton D1 Run.

of 4 days, the temperature was stepped down to 10°C.

The observed effluent ammonium nitrogen levels during the temperature step down run are shown in Figure 35 along with two output curves generated by NITOX. The measured data indicates that the extent of nitrification decreased rapidly following the temperature change and continued to decrease over the remaining 4 day period. Curve A is the predicted effluent concentration generated by NITOX using the conditions described by Sutton. Curve B is the simulation result assuming a temperature step down to 0°C rather than to 10°C.

The comparison illustrates that NITOX may not accurately predict the short term transient effects of a temperature step down. For Sutton's D1 run, the shock effect which takes place over a period of hours, is accommodated only by forcing the nitrifier k_{max}'s to zero by decreasing the simulation temperature to 0°C. A more detailed examination of Sutton's data indicated that measured nitrate concentrations were beginning to increase over the last day of the run. TKN removals would probably have continued to increase with time to a level predicted by curve A. The adequacy of NITOX for prediction of long term temperature effects was established by comparison to Sutton's pseudo-steady state results. Simulation PMSD1 suggests that under critical nitrification conditions, NITOX

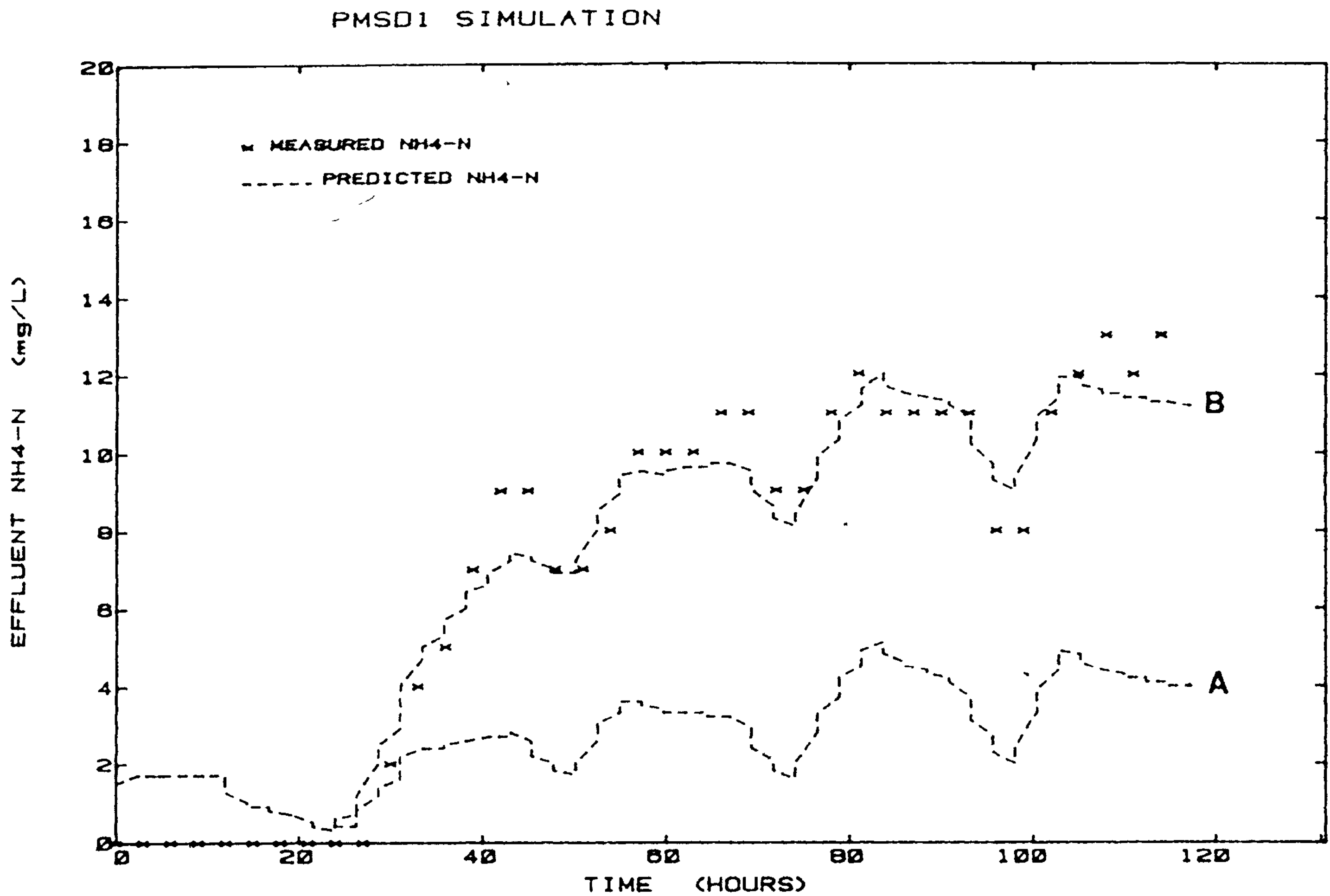


Figure 35. NITOX Simulation Results and Measured Data for Sutton D1 Run. (A Step down to 10°C ; B Step down to 0°C)

response to a temperature shock may deviate from actual results for a period of at least several days.

4.5.3 Simulation of C:N, SRT, and temperature effects

An objective of this work was to use experimental results and mathematical modelling techniques to examine the effects of solids retention time and influent carbon to nitrogen ratios on the temperature sensitivity of nitrification in mixed culture systems. The data in Figures 3 and 4 suggest that both SRT and C:N might be major causes of the diversity in reported nitrification rates.

As discussed in Section 2.2.2, the differing Arrhenius parameters (Figure 4) determined at three levels of SRT were attributed to differences in the changing fraction of nitrifiers at the various sludge ages. In Figure 3 as the C:N ratio decreased, the fraction of nitrifiers in a combined sludge system increased. This has the effect of increasing the overall unit nitrification rate expressed as mg $N \cdot mg^{-1}$ $MLVSS \cdot day^{-1}$. Unfortunately, the ranges of reported rates were wide, and the operating conditions associated with these measurements were not described completely by the authors. It is difficult to assess whether any additional effect of C:N ratio on nitrifier kinetics exists, since such an effect is completely masked by the changing fraction of nitrifiers.

NITOX was used to examine SRT effects on nitrification temperature sensitivity; again using the pseudo-steady results of Sutton (1976). Figure 36 indicates that the predicted rates are in agreement with the experimentally determined values if a Nitrosomonas yield coefficient of 0.10 mg VSS·mg^{-1}N is employed at the 4 day SRT prediction. With this assumption, the model simulates observed temperature effects satisfactorily.

The fractions of Nitrosomonas predicted by NITOX under the conditions of Figure 36 are reported in Table 19. As expected, the quantity of nitrifying bacteria present decreases with temperature. This observation can be linked to the lower filterable TKN removal observed at lower temperatures (Figure 29). However, although variation was definitely observed, there appears to be no readily apparent

TABLE 19. Predicted Biomass Fractions of Nitrosomonas at Different Levels of SRT and Temperature

TEMPERATURE (°C)	SOLIDS RETENTION TIME 4 days	7 days	10 days
25	.028	.016	.027
20	.022	.012	.022
15	.012	.006	.014
10	.004	.002	.007

relationship between SRT and nitrifier fraction. The actual size of the nitrifying mass must be a more complex function of C:N ratio, hydraulic residence time and temperature. In

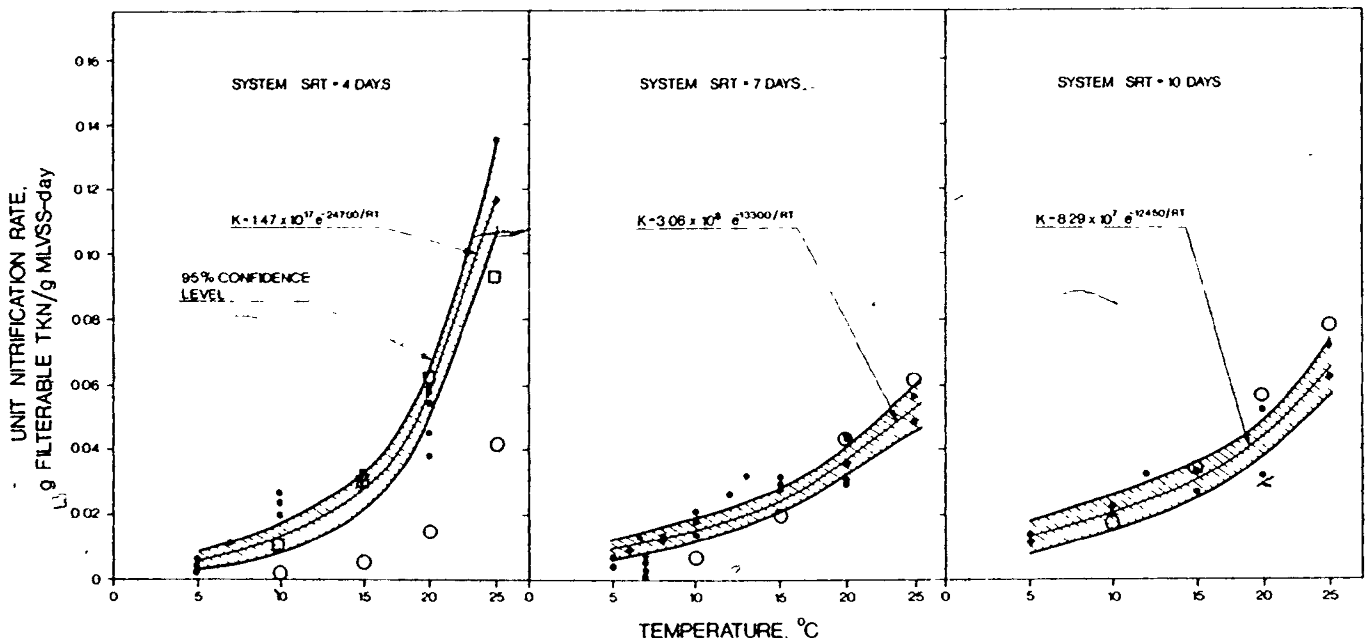


Figure 36. Comparison of Predicted Unit Nitrification Rates to Results of Sutton (1976). (NITOX Results ; ○ Y = 0.05 ; □ Y = 0.10)

fact, contrary to the hypothesis of Sutton, Table 19 does not confirm any consistent differences in the relative changes in nitrifier populations for a given temperature change at different sludge ages. The experimental results of this study, together with the predictive capability of NITOX, suggest that a common temperature relationship for nitrifiers can be applied at SRTs of 4, 7 and 10 days to predict mixed culture nitrification rates with acceptable accuracy.

To illustrate the interactions between C:N, SRT and temperature more clearly, NITOX was used to generate Arrhenius-type curves for nitrifier k_{max}'s under different conditions. An examination of the behaviour of k_{max} provides kinetic information that is difficult to observe in mixed culture. The plots shown in Figures 37 to 41 were computed by varying either carbon loading, nitrogen loading or SRT alone, while the other variables were maintained constant at the midpoints of their ranges.

For Nitrosomonas, carbon removal and SRT (Figures 37 and 38) do not have a large effect on the temperature response. Nitrogen removal (Figure 39) does alter the calculated temperature response. At higher removals the sensitivity to temperature change is reduced. As illustrated in Figure 29, Sutton found greater TKN removals at longer sludge ages - a fact which may have contributed to

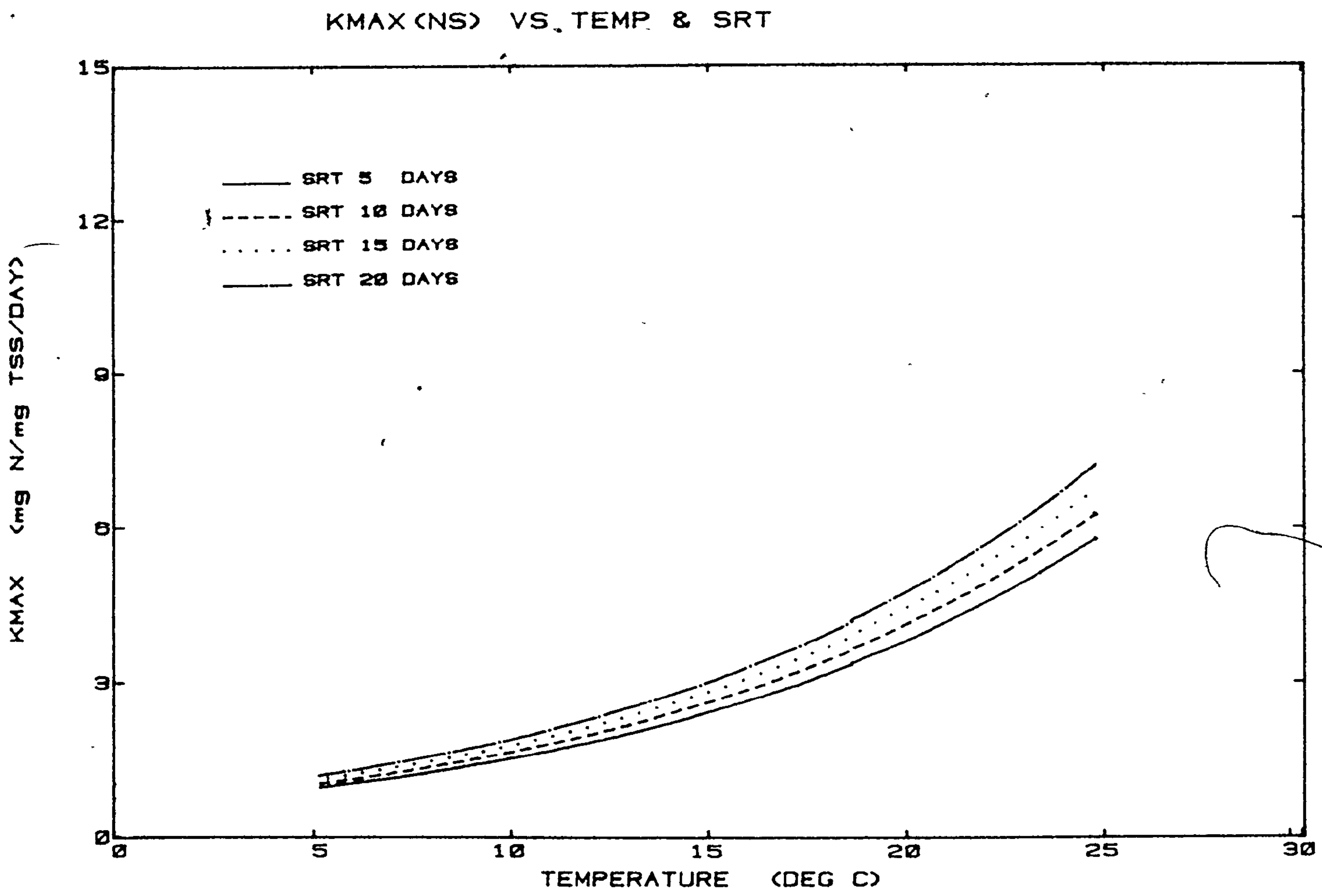


Figure 37. The Effect of SRT on the Temperature Sensitivity of <u>Nitrosomonas</u> k_{max}.

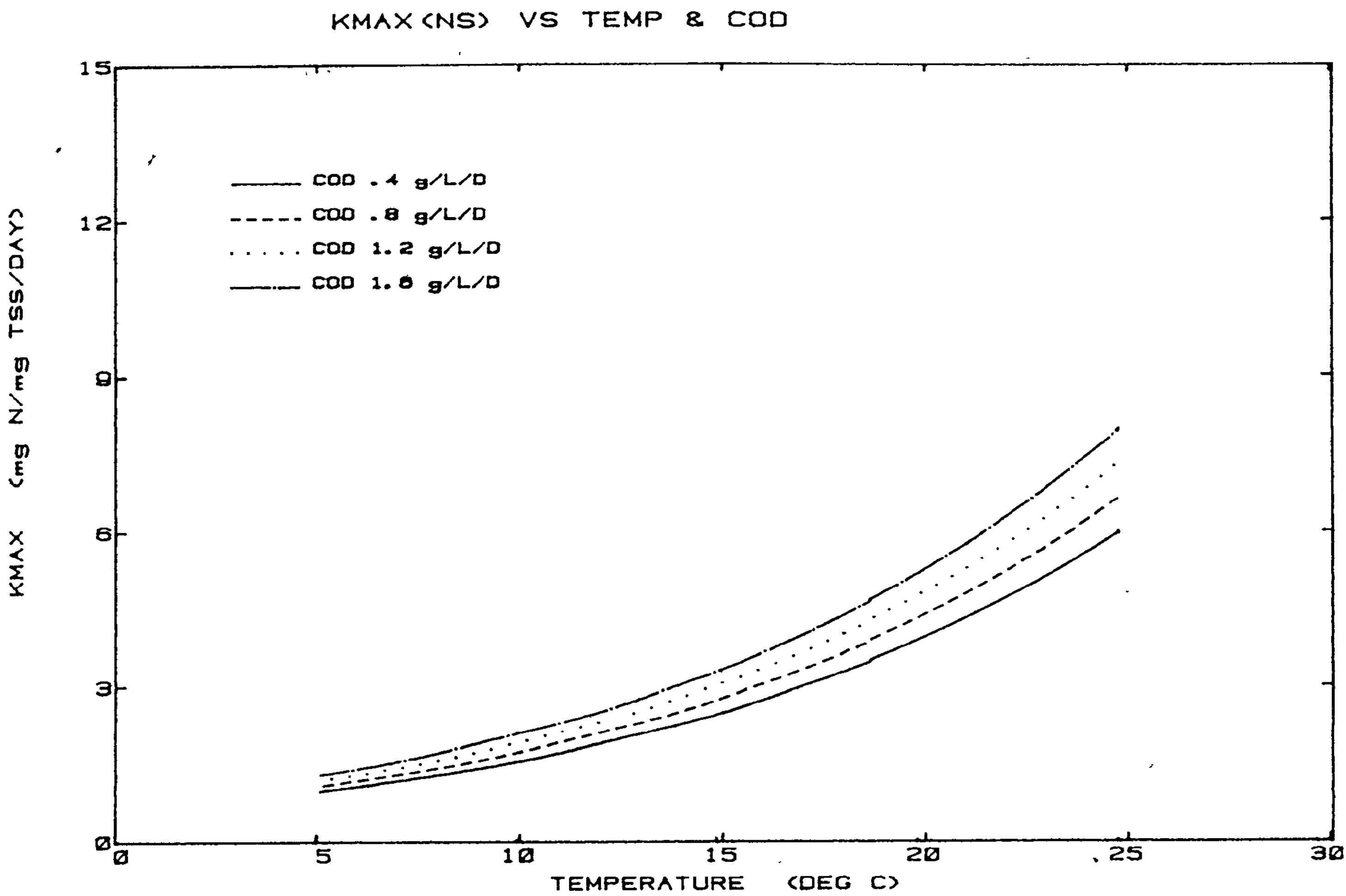


Figure 38. The Effect of COD Removal on the Temperature Sensitivity of _Nitrosomonas_ k_{max}.

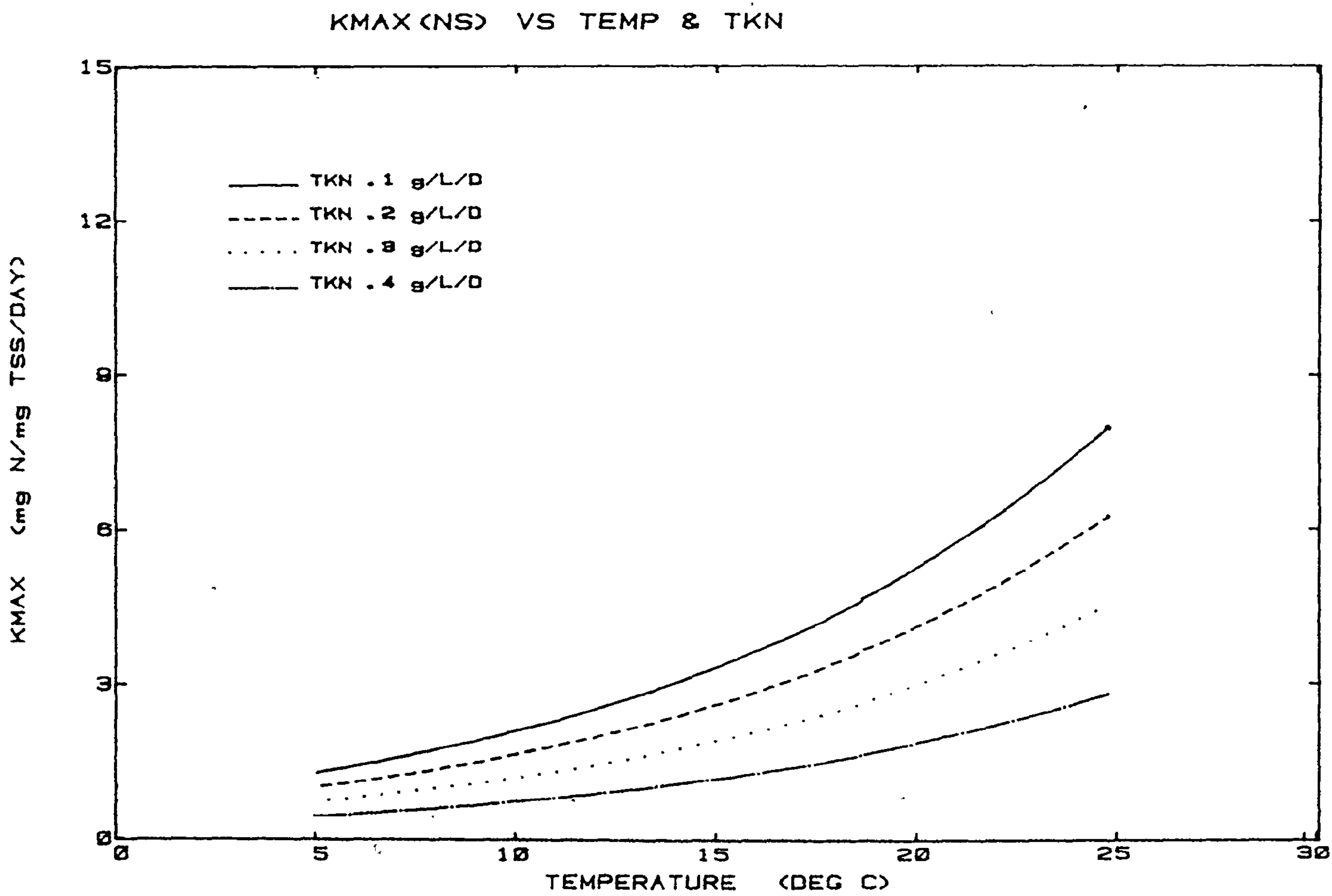


Figure 39. The Effect of TKN Removal on the Temperature Sensitivity of <u>Nitrosomonas</u> k_{max}.

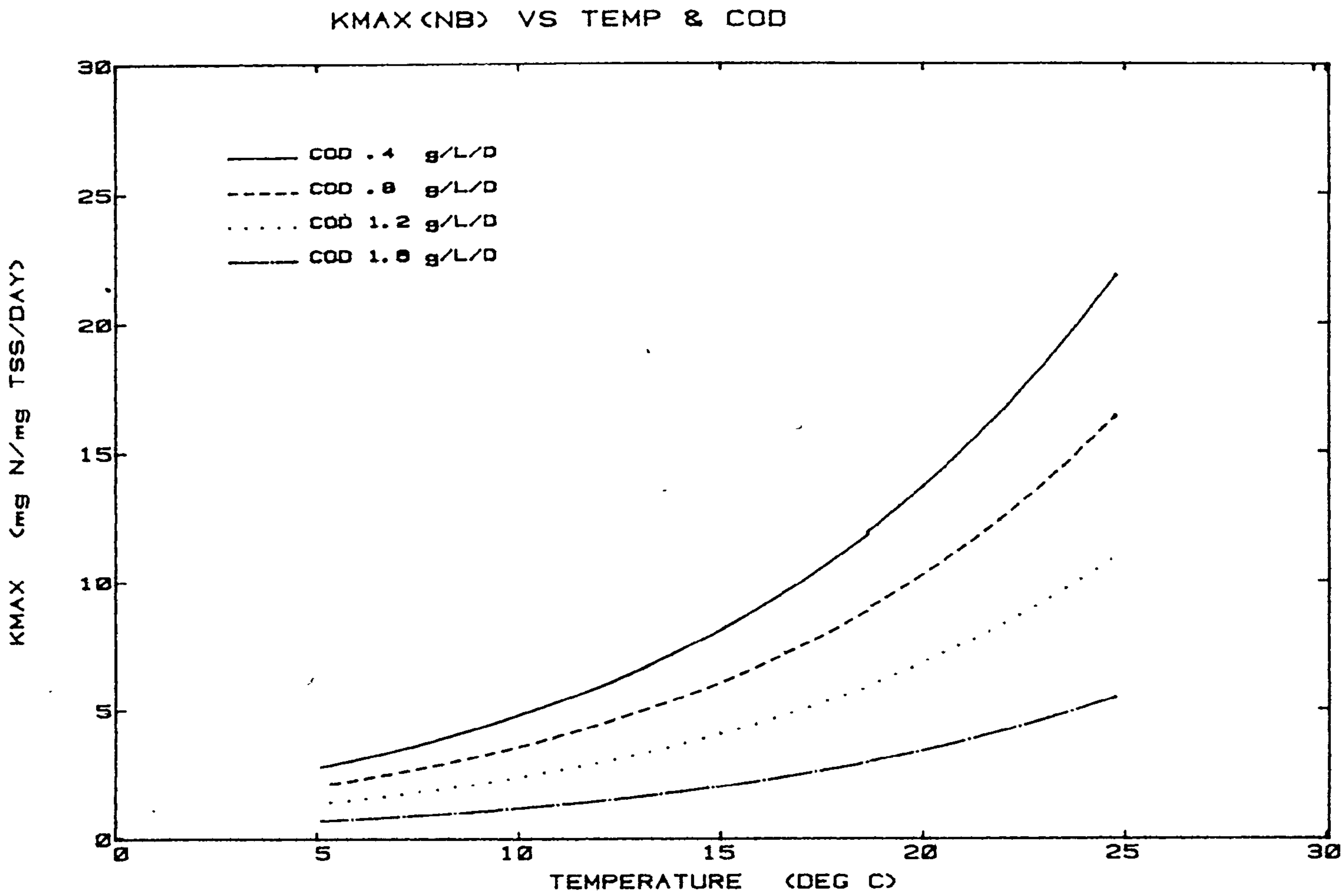


Figure 40. The Effect of COD Removal on the Temperature Sensitivity of *Nitrobacter* k_{max}.

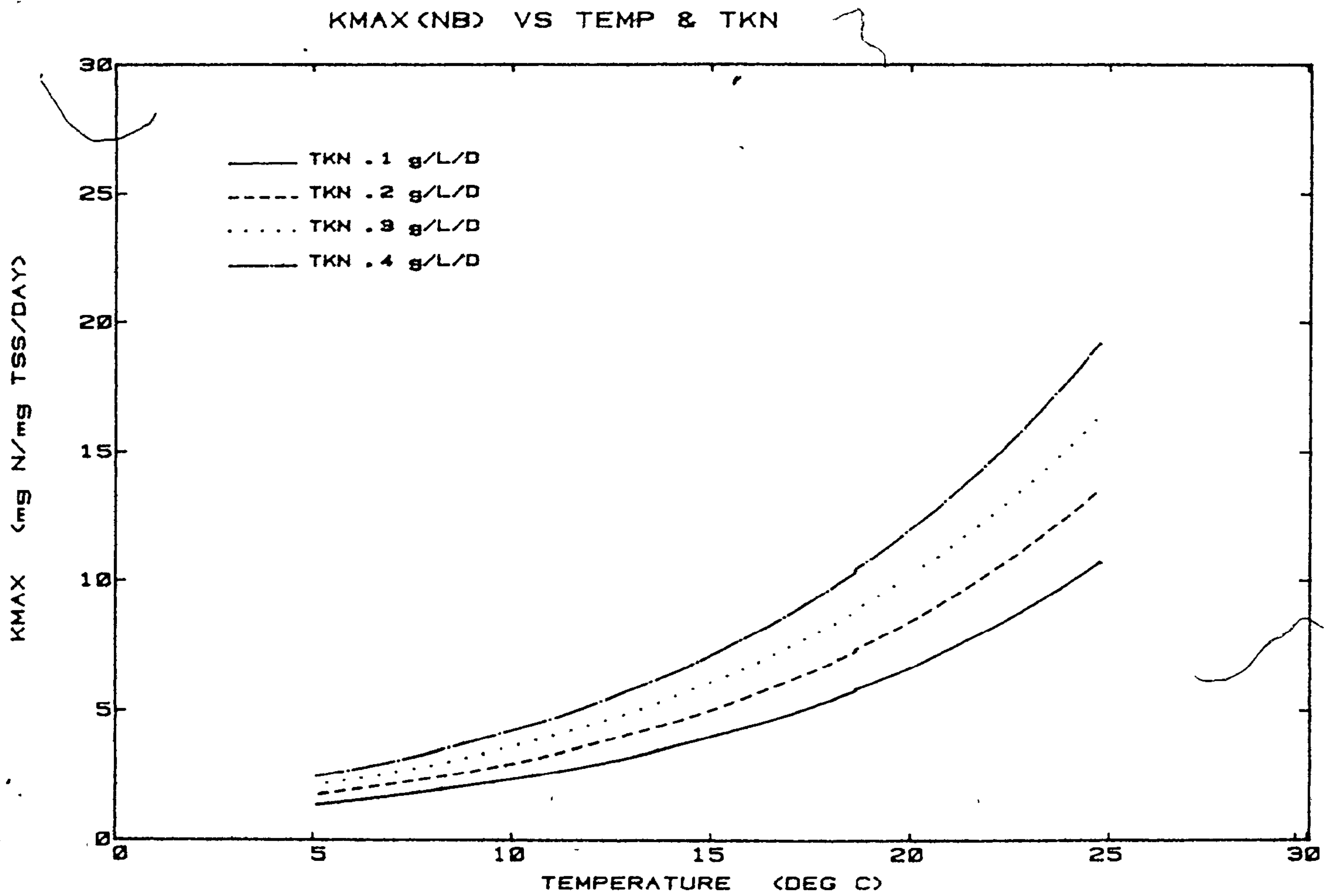


Figure 41. The Effect of TKN Removal on the Temperature Sensitivity of *Nitrobacter* k_{max}.

the observed decrease in activation energy with SRT. Since the conversion of ammonium to nitrite is usually the rate limiting step in nitrification, the same observation would hold for the overall process.

For Nitrobacter, there is no effect of SRT (Equations 22 and 24) on the thermal sensitivity of k_{max}. Carbon and nitrogen removal, however, are important. Figures 40 and 41 indicate that increasing carbon and nitrogen removals have the opposite effect on Nitrobacter activity. Increased COD removal tends to reduce the observed activation energy while greater nitrogen removal appears to increase temperature sensitivity. Normally, effects of this type would not be readily apparent because when expressed on a MLVSS basis, Nitrobacter rates are heavily masked by autotrophic - heterotrophic population dynamics and by the overall rate control of the nitritification step.

5. CONCLUSIONS

5.1 The method described provides a technique for in situ measurement of nitrogen oxidation rates in wastewaters. These rate determinations can subsequently be used to estimate nitrifying populations and their maximum unit rates in the presence of heterotrophs.

5.2 The effects of SRT and C:N ratio on nitrification temperature sensitivity reported from mixed culture systems can be satisfactorily explained using relationships derived from pure cultures of nitrifying bacteria. Previously reported SRT effects were shown to be the result of changing autotrophic-heterotrophic population dynamics.

5.3 Estimated nitrifier populations in mixed liquor increase significantly with TKN loading.

5.4 The temperature sensitivity of enriched cultures of *Nitrosomonas* is independent of solids retention time.

5.5 Once complete nitrification is established, a carbohydrate organic source and a longer equilibration period do not have a significant effect on nitrifier kinetics estimated in mixed culture.

5.6 Of the process variables SRT, COD loading, and TKN loading, COD and TKN have the major effect on nitrifier kinetics measured in mixed culture.

5.7 Using the <u>in situ</u> measurement technique, nitrifier kinetic parameters estimated from pure, enriched, and mixed culture systems can be utilized in a dynamic, mechanistic model to predict previously reported nitrification rates under steady and non-steady conditions without prior calibration. However, this model has limited predictive capabilities near the critical SRT and under conditions of temperature shock.

6. RECOMMENDATIONS FOR FURTHER WORK

6.1 Extend the use of the proposed estimation technique to real nitrification systems for varying process configurations. An extended data base encompassing different waste types and operational approaches would allow the refinement of the relationships derived in this study which were based on a laboratory extended aeration system.

6.2 Apply the proposed estimation technique to a nitrification-denitrification system to assess the effect on nitrifying bacteria of exposure to alternating aerobic-anoxic periods.

6.3 Investigate the short term transient effects of temperature on nitrifying bacteria.

6.4 Study the mechanism of the COD and TKN loading effects reported in this study and attempt to identify the source and mechanism of *Nitrobacter* inhibition observed.

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APPENDIX A

Analytical Procedures

A.1. Total Kjeldahl Nitrogen (TKN)

Samples for TKN analysis were first manually digested to NH_4-N using 100 ml Kjeldahl flasks and following the procedure described in Standard Methods (1971). After digestion, the samples were diluted to a volume of 100 ml with distilled water and analyzed for NH_4-N content. The concentrations of unknown samples were determined by comparison to an NH_4-N calibration curve prepared by carrying a set of NH_4Cl standards through the digestion and NH_4-N measurement steps.

A.2. Ammonium nitrogen (NH_4-N)

Ammonium nitrogen was determined using a Technicon Auto-Analyzer with Industrial Method 19-69 W. This technique utilizes a colourimetric procedure in which ammonium is converted to a blue indophenol by the addition of alkaline phenol and sodium hypochlorite.

A.3. Nitrite nitrogen (NO_2-N)

Technicon Auto-analyzer Industrial Method 35-69 W was utilized for NO_2-N determination. This produces a diazo

compound by reacting nitrite with sulfanilamide under acidic conditions. The diazo compound is then measured colourimetrically by coupling with N-1-naphthylethylenediamine to form a soluble dye.

A.4. Nitrite plus nitrate nitrogen $(NO_2 + NO_3)$-N)

For the determination of $(NO_2 + NO_3)$-N, nitrate was first reduced to nitrite by an alkaline solution of hydrazine sulfate containing a copper catalyst as described in Technicon Industrial Method 33-69 W. Nitrite nitrogen was then estimated as described previously.

A.5. Chemical oxygen demand (COD)

COD was measured using the culture tube method of Knechtel (1978). Samples were digested in a closed tube for 2 hours at 150°C. The tubes were then cooled and the increase in Cr^{3+} was measured directly by using the tubes as cuvettes for a Spectronic 20 at a wavelength of 600 nm.

A.6. Total organic carbon (TOC)

Samples were filtered through 0.45 micron filter papers and were then analyzed for TOC by injection of a 20 microliter aliquot into a Beckman Infrared Carbon Analyzer.

A.7. Total suspended solids (TSS)

Gelman 0.45 micron glass fibre filters were placed in a muffle furnace for 15 minutes at 550°C. They were then cooled in a dessicator and weighed. A minimum volume of 10 ml of sample was passed through a filter, which was then dried overnight at 103°C. After cooling again in a dessicator the increase in filter paper weight was used to calculate the suspended solids of the sample.

A.8. Volatile suspended solids (VSS)

Following weighing for TSS as described previously the filter paper and solids were placed in a muffle furnace at 550°C for 15 minutes. The filter was then cooled in a dessicator and reweighed. The weight lost during this procedure was used for a VSS calculation.

A.9. Dissolved oxygen

A YSI Dissolved oxygen meter calibrated with air saturated water was used for dissolved oxygen measurements.

A.10. pH

An Orion Specific Ion Meter with a six-channel selector, set-point controller and printer was used for pH monitoring and control. The probes were gel-filled and were cleaned and standardized twice per week.

APPENDIX B

Mixed Culture Reactor Operating Results

A listing of the routine analytical results and several calculated and operating parameters for the laboratory extended aeration plants appears on the following pages.

B.1. Suspended solids data

(a) This section identifies suspended solids data (Table B1) and illustrates the wasting calculations for SRT control. The procedure for SRT calculation and wasting was as follows.

(i) After determination of mixed liquor volatile suspended solids (MLVSS), the total system solids (TVSS) mass was calculated using an average MLVSS over the 24 hour period since the last wasting.

(ii) The total daily solids mass lost in the clarifer overflow was then calculated, again using an effluent VSS concentration averaged over the wasting period.

(iii) The required net solids wasting was taken as

$$\frac{TVSS}{SRT} - 24 \text{ hr effluent VSS} = \text{net waste}$$

(iv) An appropriate volume of mixed liquor was then wasted to maintain the set point SRT.

(b) Columns headed by Kinetic Run indicate the date on which a particular mixed culture nitrification assay was performed.

(c) Units of the measurements are $mg.L^{-1}$, except for the TVSS and net waste values which are in milligrams. Abbreviations and symbols used in the data listing (Table B1) are interpreted in Appendix E.

B.2. <u>Chemical analysis results</u>

Units of all measurements are $mg.L^{-1}$. The abbreviations used in the data listing (Table B2) are explained in Appendix E.

TABLE B1 . Suspended Solids Data and Wasting Calculations for Extended Aeration Systems

RUN III- 1

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
8/10/78	1	5140	2680					
9/10/78	2	5530	3070	28	924	3500		
10/10/78	3	5070	2750	26	860	3520		
11/10/78	4	5160	2820	26	860	3329		
12/10/78	5	5130	2830	26	860	3396		
13/10/78	6	4860	2710	37	1220	2959		
14/10/78	7	4860	2760	27	891	3240		
15/10/78	8	4800	2770	17	561	3596		
16/10/78	9	4660	2720	13	429	3684		
17/10/78	10	4570	2690	9	297	3749		
18/10/78	11	4490	2740	11	363	3698	1	
19/10/78	12	4280	2650	11	363	3669		
20/10/78	13	3980	2490	11	363			

RUN III- 2

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
8/10/78	1	3080	1590			1100		
9/10/78	2	3110	1660	19	630	780		
10/10/78	3	3360	1850	16	528	1000		
11/10/78	4	3380	1870	13	430	1195		
12/10/78	5	3310	1830	19	627	979		
13/10/78	6	3500	2030	16	528	1150		
14/10/78	7	3420	2000	17	561	1190		
15/10/78	8	3410	2010	23	602	1139		
16/10/78	9	3290	1910	18	594	1108		
17/10/78	10	3250	1900	17	561	1093		
18/10/78	11	3250	1940	12	396	1269		
19/10/78	12	3380	2110	12	396	1358	2	
20/10/78	13	3490	2150	12	396		3	

RUN III- 3

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
24/10/78	1	7170	4180	27	702	3032		
25/10/78	2	7120	4240	16	528	3191		
26/10/78	3	6960	4170	16	528	3184		
27/10/78	4	6890	4110	30	990	2671		
28/10/78	5			27	891			
29/10/78	6	6630	3990	21	792	2794		
30/10/78	7	6340	3770	29	825	2608		
31/10/78	8	6320	3720	33	1023	2296		
1/11/78	9	6080	3420	33	1023	2144		
2/11/78	10	6180	3580	16	808	2296	4	277
3/11/78	11	5930	3470	11	363	1729		
4/11/78	12	6060	3600	11	363	2736	6	

RUN III- 4

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
24/10/78	1	9150	5360	18	468			
25/10/78	2	10040	5980	20	660	4562		
26/10/78	3	10240	6140	20	660	4567		
27/10/78	4	10090	6090	25	825	4453		
28/10/78	5			28	924			
29/10/78	6	9960	6070	32	990	4262		
30/10/78	7	9450	5710	56	1452	3645		
31/10/78	8	9350	5600	64	1980	2930		
1/11/78	9	9040	5370	78	2343	2437		
2/11/78	10	8910	5220	66	2376	2247		
3/11/78	11	8910	5250	42	1782	2782	5	450
4/11/78	12	9240	5470	20	1023	3628		

RUN III- 5

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
7/11/78	1	6160	3700	31	651	2654		
8/11/78	2	6290	3790	32	1056	2269		
9/11/78	3	6440	3880	30	1023	2382		
10/11/78	4	6570	3930	27	891	2572		
12/11/78	6	6710	4160	24	1650	5546		
13/11/78	7	6630	4080	17	693	2906		
14/11/78	8	6640	4290	25	693	3008		
15/11/78	9	6540	4160	15	660	3075		
16/11/78	10	6610	4200	15	660	3033		
17/11/78	11	6680	4260	15	432	3303	9	412

RUN III- 6

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
7/11/78	1	1460	930	13	273	1119		
8/11/78	2	1480	930	9	363	1088		
9/11/78	3	1410	860	15	396	926		
10/11/78	4	1430	870	8	363	916		
12/11/78	6	1450	830	6	462	2057		
13/11/78	7	1320	810	7	231			
14/11/78	8	1420	950	8	264	1053		
15/11/78	9	1330	900	6	231	1130	7	
16/11/78	10	1350	950	7	231	1122	8	
17/11/78	11	1310	880	7	224			80

RUN III- 7

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
21/11/78	1	1712	980	23	276	4763		
22/11/78	2	1692	1024	40	882	4100		
23/11/78	3	1628	1024	27	938	4234		
24/11/78	4	1612	1028	30	840	4319		
26/11/78	6	1840	1256	11	308	10789		
27/11/78	7	1376	964					
28/11/78	8	2036	1332	10	280	5901		
29/11/78	9	1948	1272	17	364	6060		
30/11/78	10	1872	1220	20	518	5006	10	150

RUN III- 8

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
21/11/78	1	5030	2910	25	360	3870		
22/11/78	2	5090	2920	26	800	3476		
23/11/78	3	5110	2980	17	688	3648		
24/11/78	4	5160	3080	17	544	3904		
26/11/78	6	5410	3290	17	1088	8288		
27/11/78	7	5120	3110		544	4025		
28/11/78	8	5240	3060	8	256	4255		
29/11/78	9	4996	3120		256	4255		
30/11/78	10	4988	3116		256	4297		
1/12/78	11	4972	3208		256	4354	11	
2/12/78	12	4944	3188				12	358

RUN III- 9

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
5/12/78	1	8520	4970	34	1088	6585		
6/12/78	2	8310	4840	27	976	6375		
7/12/78	3	8280	4800	11	608	6608		
8/12/78	4	8470	5010	11	352	6981		
9/12/78	5	8350	4700	11	352	6895		
11/12/78	7	8580	5230	9	288	14259		
12/12/78	8	8200	5090	17	300	7263		
13/12/78	9	7900	5110		544	7076		
14/12/78	10	8130	5210		544	7171		
15/12/78	11	8040	5190		544	7230	14	569
16/12/78	12	8040	5220				15	

RUN III-10

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
5/12/78	1	8380	4790	21	672	6537		
6/12/78	2	8390	4840	17	608	6431		
7/12/78	3	8050	4510	11	448	6380		
8/12/78	4	8130	4740	11	352	6400		
9/12/78	5	8180	4710	11	352	6549		
11/12/78	7	8340	4900	11	704	13349		
12/12/78	8	7970	4870		750	6203		
13/12/78	9	8280	4950	13	416	6769		
14/12/78	10	7910	4750		416	6663	13	
15/12/78	11	7990	4820		416			438

RUN III-11

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
19/12/78	1	1450	920	10	244	1150		
20/12/78	2	1445	900		320	1049		
21/12/78	3	1450	920	8	256	1115		
22/12/78	4	1450	920		256	1128		
23/12/78	5	1460	945			1147		
24/12/78	6	1510	990		256	1200		
25/12/78	7	1504	1016	12	384	1128		
26/12/78	8	1548	1036		384	1165		
27/12/78	9	1560	1040		384	1183		
28/12/78	10	1692	1148	26	608	1050		
29/12/78	11	1708	1164	8	512	1242		
30/12/78	12	1745	1200	14	352	1432		
31/12/78	13	1665	1110	27	656	1090		

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
1/ 1/79	14	1660	1130	32	928	782		
2/ 1/79	15	1872	1165		864	895		
3/ 1/79	16	1692	1184	22	704	1089		
4/ 1/79	17	1732	1196	32	704	1107		
5/ 1/79	18	1800	1208	21	672	1187	16	
6/ 1/79	19	1920	1260		650	1224		
7/ 1/79	20	1888	1312	25	800	1157	17	
8/ 1/79	21	1952	1348					110

RUN III-12

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
19/12/78	1	1660	1090	18	418	5210		
20/12/78	2	1604	1084		576	4656		
21/12/78	3	1570	1120	6	192	5136		
22/12/78	4	1500	1090		168	5125		
23/12/78	5	1480	1120		168	5126		
24/12/78	6	1600	1140		168	5157		
25/12/78	7	1484	1112	8	224	5249		
26/12/78	8	1436	1072		224	4995		
27/12/78	9	1412	1040		224	4832		
28/12/78	10	1636	1104	53	854	4365		
29/12/78	11	1508	1064	33	1204	4159		
30/12/78	12	1465	1064	17	700	4529		
31/12/78	13	1416	992	12	406	4568		
1/ 1/79	14	1170	880	38	700	3818		
2/ 1/79	15	1290	890	35	980	3373		
3/ 1/79	16	1210	980	56	1344	3352		
4/ 1/79	17	1310	940	22	1500	3345		
5/ 1/79	18	1280	870	60	1590	2976		
6/ 1/79	19	1356	996		1500	3241		
7/ 1/79	20	1365	1025	35	1120	3962		
8/ 1/79	21	1470	1040	26	854	4245		96
9/ 1/79	22	1450	1050		854	4281		
10/ 1/79	23	1400	1030	10	280	4769	18	
11/ 1/79	24	1430	1020				19	

RUN III-13

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
9/ 1/79	1	2440	1580	15	210	7790		
10/ 1/79	2	2590	1720	9	252	7838		
11/ 1/79	3	2740	1860		252	8584		
12/ 1/79	4	2780	1950		224	9150		
13/ 1/79	5	2810	2050		224	9603		
14/ 1/79	6	2930	2130	12	336	9811		
15/10/79	7	2740	2020		336	9710		
16/ 1/79	8	2490	1880		336	9178		
17/ 1/79	9	2710	2030		336	9136		
18/ 1/79	10	2430	1760	25	700	8491	20	
19/ 1/79	11	1680	1290					

RUN III-14

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
12/ 1/79	1	4970	3010	22	506	4000		
13/ 1/79	2	4980	3010		506	3900		
14/ 1/79	3	4930	2930	17	561	3790		
15/ 1/79	4	4910	2980		561	3770		
16/ 1/79	5	4910	2950		561	3786		
17/ 1/79	6	5040	3080		561	3921		
18/ 1/79	7	5140	3110	29	957	3591		
19/ 1/79	8	4990	3170	19	792	3829		
20/ 1/79	9	5250	3350	29	825	4027		
21/ 1/79	10	5140	3220		660	4219		
22/ 1/79	11	5060	3220	11	352	4358	21	
23/ 1/79	12		3270				22	280

RUN III-15

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
23/ 1/79	1	472	304	8	240	1389		
24/ 1/79	2	456	292	8	224	1256		
25/ 1/79	3	788	316		224	1302		
26/ 1/79	4	472	320		224	1372		
27/ 1/79	5	500	356	12	280	1422		
28/ 1/79	6	472	362		280	1449		
29/ 1/79	7	424	316		280	1339		
30/ 1/79	8	484	340	14	392	1271		
1/ 2/79	10	650	460	15	840	3361		

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
2/ 2/79	11	512	384		400	1565		
3/ 2/79	12	508	380	11	308	1613		
5/ 2/79	14	420	324	16	448	1322		
6/ 2/79	15	412	316	18	476	1154		
7/ 2/79	16	504	388		450	1366		
8/ 2/79	17	544	424	9	252	1812	26	
9/ 2/79	18	604	468				27	47

RUN III-16

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
25/ 1/79	1	7840	5320	16	352	7448		
26/ 1/79	2	8630	5310		384	7371		
27/ 1/79	3	8900	5520		390	7517		
28/ 1/79	4	8550	5260		390	7475		
29/ 1/79	5	8220	5150		390	7200		
30/ 1/79	6	8340	5200		390	7163		
1/ 2/79	8	8510	5370	23	1280	14208		
2/ 2/79	9	7750	4970		350	7000		
3/ 2/79	10	7800	4960		350	6893		
5/ 2/79	12	7450	4810	21	672	6463		
6/ 2/79	13	7300	4730	28	784	6195	24	
7/ 2/79	14	7290	4680				25	555

RUN III-17

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
21/ 2/79	1	3320	1970	13	286	1473		
22/ 2/79	2	3340	2020		429	1335		
23/ 2/79	3	3300	2040	12	396	1401		
25/ 2/79	5	3320	2020	46	957	849		
26/ 2/79	6	3200	1920	20	1089	674		
27/ 2/79	7	3200	1880		1000	702		
28/ 2/79	8	3280	2000	28	320	1486		
1/ 3/79	9	3140	1910		660	1074		
2/ 3/79	10	3170	1960	28	924	802		
4/ 3/79	12	3320	2000	15	1419	2139		
5/ 3/79	13	3040	1810		495	1179	28	
6/ 3/79	14	3360	2020	13	563	1334		
7/ 3/79	15	3290	2020		448	1341	29	
8/ 3/79	16	3510	2160				30	197

RUN III-18

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
21/ 2/79	1	2050	1260	14	308	1578		
22/ 2/79	2	1950	1210		448	1368		
23/ 2/79	3	2020	1220	15	480	1346		
25/ 2/79	5	1940	1250		320	1499		
26/ 2/79	6	1920	1190	24	544	1255		
27/ 2/79	7	1870	1180		500	1252		
28/ 2/79	8	1950	1210	15	748	1026		
1/ 3/79	9	1930	1200		624	1168		
2/ 3/79	10	1920	1190	21	528	1242		
4/ 3/79	12	2040	1230	41	1984	1681		
5/ 3/79	13	1910	1140		500	522		
6/ 3/79	14	2130	1350	26	1614	252		
7/ 3/79	15	2130	1370		832	1220		
8/ 3/79	16	2270	1470	17	544	1562		
9/ 3/79	17	2420	1540				31	158

RUN III-19

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
11/ 4/79	1	5500	3190			4857		
12/ 4/79	2	5630	3190	8	264	4490		
13/ 4/79	3	5730	3400		264	4661		
14/ 4/79	4	5700	3350		264	4778		
15/ 4/79	5	5500	3210		264	4630		
16/ 4/79	6	5800	3390		264	4664		
17/ 4/79	7	5950	3440		275	4829	32	

RUN III-20

DATE	RUN DAY	MLSS	MLVSS	EFFLUENT VSS	24 HR EFFLUENT VSS	NET WASTE	KINETIC RUN	NON-FILTERED TKN
11/ 4/79	1	5320	3050			4537		
12/ 4/79	2	5410	3070	8	264	4195		
13/ 4/79	3	5690	3330		264	4399		
14/ 4/79	4	5620	3280		264	4565		
15/ 4/79	5	5510	3180		264	4448		
16/ 4/79	6	5800	3360		396	4410		
17/ 4/79	7	5970	3430		300	4665	33	

TABLE B1. Chemical Analysis Data for Extended Aeration Systems

RUN III- 1

DATE		FEED							EFFLUENT					
DY/MO/YR	RUN DAY	COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC
9/10/78	2	550	505	180	165	165			115	38	0.0	1.3	100	
11/10/78	4	665	475	190	190	175			105	36	0.0	1.5	135	
13/10/78	6	640	545		160	150	0.0		90		0.0	1.3	145	
15/10/78	8	545	485											
16/10/78	9	555	495	135	143	150	0.0	.2	45	22	0.0	2.5	133	77
18/10/78	11	655	530	145	155	145	0.0	.3	50	8	0.0	1.3	144	83
20/10/78	13	575	510	155	163	155	0.0	.2	55	11	0.0	2.3	153	87

RUN III- 2

DATE		FEED							EFFLUENT					
DY/MO/YR	RUN DAY	COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC
9/10/78	2	215	150	55	165	163			55	18	0.0	2.5	93	
11/10/78	4	370	155	48	185	175			90	26	0.0	2.3	133	
13/10/78	6	280	195		160	150			75		0.0	1.5	145	
15/10/78	8	175	140											
16/10/78	9	160	130	43	148	148	0.0	.2	40	9	0.0	.6	129	55
18/10/78	11	310	185	65	140	135	0.0	.4	35	4	0.0	.9	149	77
20/10/78	13	240	170	52	155	150	0.0	.3	55	5	0.0	1.6	153	77

RUN III- 3

DATE		*	FEED						*	EFFLUENT					*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT	
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC	
25/10/78	2	550	525	128	25	23	0.0	.1	60	27	1.5	.1	17		
27/10/78	4	525	510		26	22	0.0	.2	85		3.1	.1	12		
30/10/78	7	490	470	185	18	21	0.0	.2	70	30	2.1	.3	13	44	
31/10/78	8	450													
1/11/78	9	500	420	170	21	19	0.0	.2	60	31	.6	.4	22	86	
3/11/78	11	520	445	116	28	20	0.0	.1	40	14	1.0	.4	12	55	

RUN III- 4

DATE		*	FEED						*	EFFLUENT					*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT	
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC	
25/10/78	2	930	870		155	145	0.0	.2	95	35	0.0	.4	137		
27/10/78	4	840	800		178	143	0.0	.2	70		0.0	.4	120		
30/10/78	7	820	760	266	143	138	0.0	.2	100	34	0.0	.5	132	63	
31/10/78	8	930													
1/11/78	9	940	690	268	148	143	0.0	.2	110	35	0.0	.4	125	118	
3/11/78	11	880	710	204			0.0	.2	85	27	0.0	.6	124	116	

RUN III-5

DATE	RUN	*	FEED						*	EFFLUENT				*
DY/MO/YR	DAY	COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC
8/11/78	2	515	440		340	350	0.0	.4	125		29.0	13.8	111	
10/11/78	4	580	480	207	310	260	0.0	.4	125	37	15.0		222	
13/11/78	7	580	440		308	290	0.0	.4	75		0.0	3.0	235	
15/11/78	9	550	440	193	330	310			65	14	0.0	1.3	267	119
17/11/78	11	510	415		295	285		.1	55		0.0		300	

RUN III-6

DATE	RUN	*	FEED						*	EFFLUENT				*
DY/MO/YR	DAY	COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC
8/11/78	2	205	140		23	23	0.0	.2	40		.8	.2	12	
10/11/78	4	185	145	52	22	13	0.0	.3	45	20	0.0	.2	14	
13/11/78	7	220	145		21	17	0.0	.3						
15/11/78	9	205	150	64	23	21			35	11	1.5	.2	14	37
17/11/78	11	170	130		21	18		.2	20		1.0		16	

RUN III-7

DATE	RUN	*	FEED						*	EFFLUENT				*
DY/MO/YR	DAY	COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC
22/11/78	2	580	510	216	23	19			145	68	3.5	0.0	3	
24/11/78	4	590	470						85			.1	2	
27/11/78	7	560	495		20	16								
29/11/78	9	580	485	178	24	20			35	16	.3	.1	2	74
1/12/78	11	565	510		25	20			40		3.5	0.0	3	

RUN III- 8

DATE		*			FEED				*		EFFLUENT			*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
22/11/78	2	570	460	208	140	130			100	42	100.0	0.0	12	
24/11/78	4	615	425						55			1.5	22	
27/11/78	7	560	445		143	136			45		5.0	11.0	109	
29/11/78	9	590	420	143	170	163			60	16	0.0	.8	134	94
1/12/78	11	550	430		163	148			55		0.0	.2	127	

RUN III- 9

DATE		*			FEED				*		EFFLUENT			*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
6/12/78	2	1024	980		300	300			102		173.0	1.0	87	
8/12/78	4	950	840		305	290	.1	1.2	140		80.0	1.2	126	
11/12/78	7	870	750		280	280	0.0	0.0	75		2.5	26.0	248	
13/12/78	9	940	860	362	315	305	0.0	.4	110	24	0.0	.4	238	0
15/12/78	11	960	840	352	313	305			130	17	0.0	.4	257	100

RUN III-10

DATE		*			FEED				*		EFFLUENT			*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
6/12/78	2	830	880		27	20			102		2.1	0.0	2	
8/12/78	4	900	810		24	19	.1	.5	80		1.5	0.0	0	
11/12/78	7	870	750		27	20	0.0	0.0	50		1.5	0.0	0	
13/12/78	9	940	970	378	27	18	0.0	.1	65	24	1.5	0.0	0	79
15/12/78	11	960	940	368	27	23			45	16	1.3	0.0	1	72

RUN III-11

DATE		* FEED							* EFFLUENT					*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
20/12/78	2	205	135	30	290	285	.1	1.0	105	20	203.0	9.8	57	
22/12/78	4	220	140		345	326	.1	.5	110		168.0	42.5	80	
24/12/78	6	325	178		287	276			120		102.0	67.0	111	
26/12/78	8	265	165		276	276			98		6.0	75.0	147	
28/12/78	10	250	175		395	390	0.0	.4	110		43.0	79.0	144	
29/12/78	11	195			312									
30/12/78	12	275			312				97		.5	94.0	186	
2/ 1/79	15	233	195		340	330	.1	.6	120		1.3	40.0	251	
4/ 1/79	17	240	100		285	285	.2	4.3	92		0.0	22.0	264	
6/ 1/79	19	225			292				75		0.0	4.0	296	
8/ 1/79	21	180	90	16	310	295	.1	1.3	75	6	0.0	3.0	291	82

RUN III-12

DATE		* FEED							* EFFLUENT					*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
20/12/78	2	560	475	287	287	270	.1	.4	100	23	201.0	7.9	46	
22/12/78	4	530	455		335	315	.8	1.2	135		218.0	27.5	53	
24/12/78	6	595	455		292	270			120		175.0	41.0	47	
26/12/78	8	540	435		295	280			120		156.0	54.0	46	
28/12/78	10	600	490		390	380	0.0	.4	140		210.0	49.0	11	
29/12/78	11				312									
30/12/78	12	565			300				115		130.0	77.0	40	
2/ 1/79	15	610	545		335	320	0.0	.6	185		108.0	109.0	67	
4/ 1/79	17	567	420		292	287	.3	3.6	160		97.0	163.0	15	
6/ 1/79	19	552			313		.1	1.0	200		88.0	178.0	20	
8/ 1/79	21	475	350	130	304	295			185	17	35.0	225.0	8	156
10/ 1/79	23	595			310	295	.1	.7	220		6.0	235.0	10	
11/ 1/79	24								560		7.0	245.0	15	

DATE DY/MO/YR	RUN DAY	* FEED COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	* EFFLUENT FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC *
10/ 1/79	2	920	760		165	155	.1	.4	65		8.0	15.5	105	
12/ 1/79	4	950	780		158	150	.1	.4	80		0.0	5.0	115	
15/ 1/79	7	900	790		158	158			70		0.0	6.0	114	
17/ 1/79	9	950	780		158	150			85		0.0	22.0	97	
19/ 1/79	11	904	720		160	148			85		0.0	8.0	112	

RUN III-14

DATE DY/MO/YR	RUN DAY	* FEED COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	* EFFLUENT FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC *
12/ 1/79	1	580	430		155	148	0.0	.5	76		103.0	22.0	49	
15/ 1/79	4	630	390		160	145			80		0.0	45.0	94	
17/ 1/79	6	550	380		150	138			55		0.0	1.0	135.	
19/ 1/79	8	530	370		160	148			60		0.0	.3	115	
22/ 1/79	11	510	380		135	128	0.0	.3	50		0.0	.1	140	

RUN III-15

DATE DY/MO/YR	RUN DAY	* FEED COD	FILT COD	FILT TOC	TKN	FILT TKN	NO2-N	NO3-N	* EFFLUENT FILT COD	FILT TOC	FILT TKN	NO2-N	NO3-N	FILT TIC *
24/ 1/79	2	170	80		175	167			70		120.0	4.0	7	
26/ 1/79	4	200	115		245	238			70		193.0	14.0	22	
29/ 1/79	7	260	110		143	133			75		82.0	40.0	18	
1/ 2/79	10	265	125		178	178			100		48.0	95.0	8	
5/ 2/79	14	285	90		150	145			100		19.0	123.0	3	
7/ 2/79	16	180	55		165	155			55		12.0	148.0	0	
9/ 2/79	18								105		8.8	95.8	0	

RUN III-16

DATE		*			FEED				*		EFFLUENT			*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
26/ 1/79	2	840	770		465	355			100		144.0	25.0	123	
29/ 1/79	5	1000	950		305	295			105		0.0	62.0	167	
1/ 2/79	8	930	760		335	330			80		0.0	0.0	256	
5/ 2/79	12	880	760		300	294			75		0.0	1.0	243	
7/ 2/79	14	880	750		310	305			50		0.0	.4	270	

RUN III-17

DATE		*			FEED				*		EFFLUENT			*
	RUN		FILT	FILT		FILT			FILT	FILT	FILT			FILT
DY/MO/YR	DAY	COD	COD	TOC	TKN	TKN	NO2-N	NO3-N	COD	TOC	TKN	NO2-N	NO3-N	TIC
21/ 2/79	1	355	185		162	148			35		62.0	15.0	4	
23/ 2/79	3					*			50		32.0	22.0	71	
26/ 2/79	6	210	110		160	147			15		0.0	1.0	179	
28/ 2/79	8	185	80		165	155			40		0.0	.5	95	
2/ 3/79	10	140	50		120	110			70		0.0	.1	170	
5/ 3/79	13	330	90		165	145			55		0.0	.4	150	
7/ 3/79	15	220			151				35		0.0	.2	153	

RUN III-18

DATE DY/MO/YR	RUN DAY	FEED COD	FEED FILT COD	FEED FILT TOC	FEED TKN	FEED FILT TKN	FEED NO2-N	FEED NO3-N	EFFLUENT FILT COD	EFFLUENT FILT TOC	EFFLUENT FILT TKN	EFFLUENT NO2-N	EFFLUENT NO3-N	EFFLUENT FILT TIC
21/ 2/79	1	390	220		305	290			75		156.0	17.0	0	
23/ 2/79	3								70		217.0	11.0	25	
26/ 2/79	6	310	280		295	290			55		167.0	15.0	110	
28/ 2/79	8	195	110		335	320			90		158.0	18.0	100	
2/ 3/79	10	150	100		256	246			105		37.0	65.0	145	
5/ 3/79	13	320	115		310	285			105		17.0	65.0	180	
7/ 3/79	15	260			310				70		0.0	15.0	271	
9/ 3/79	17										1.6	7.0	286	

RUN III-19

DATE DY/MO/YR	RUN DAY	FEED COD	FEED FILT COD	FEED FILT TOC	FEED TKN	FEED FILT TKN	FEED NO2-N	FEED NO3-N	EFFLUENT FILT COD	EFFLUENT FILT TOC	EFFLUENT FILT TKN	EFFLUENT NO2-N	EFFLUENT NO3-N	EFFLUENT FILT TIC
11/ 4/79	1	585	370		192	190	0.0		40		31.0	.6	49	
13/ 4/79	3	470	355		170	168			40		.7	5.0	119	
16/ 4/79	6	530	290		138	130			40		0.0	.2	122	
18/ 4/79	8	590			145				35		0.0	1.0	133	

RUN III-20

DATE DY/MO/YR	RUN DAY	FEED COD	FEED FILT COD	FEED FILT TOC	FEED TKN	FEED FILT TKN	FEED NO2-N	FEED NO3-N	EFFLUENT FILT COD	EFFLUENT FILT TOC	EFFLUENT FILT TKN	EFFLUENT NO2-N	EFFLUENT NO3-N	EFFLUENT FILT TIC
11/ 4/79	1	620	370		197	185			50		41.0	.3	45	
13/ 4/79	3	460	350		178	173			40		.7	6.0	122	
16/ 4/79	6	520	310		141	130			35		0.0	.3	117	
18/ 4/79	8	530			105				40		0.0	1.0	133	

APPENDIX C

Development of Model for Combined Carbon Removal - Nitrification System (NITOX)

The model used for simulation of the carbon removal-nitrification process was developed by adaptation and extension of the dynamic model originally proposed by Poduska and Andrews (1975). In Figure 42 is shown a schematic of the model system which may consist of as many as ten continuous stirred tank reactors (CSTR's) followed by a clarifier for solid-liquid separation. Sludge from the clarifier underflow is returned to aeration tank 1 and sludge wasting is carried out continuously to maintain a desired SRT. The numbers in circles identify the various process streams.

C.1. Aeration tank model

Model development was based on the following assumptions:

(i) The various input and output streams flow through zero volume networks and unless otherwise noted, were assumed to have no associated time delays or changes in substrate or microorganism concentrations.

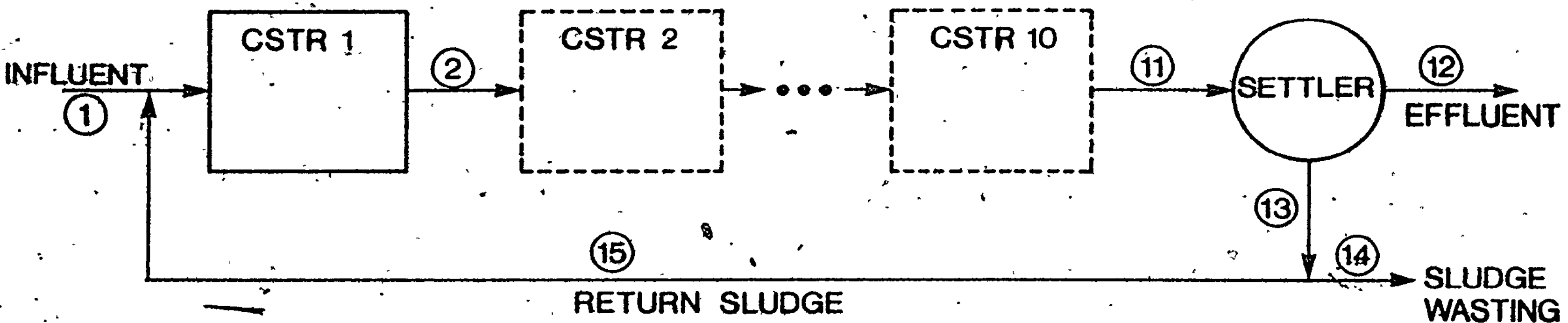

Figure 42. Schematic of Process Components Described by NITOX.

(ii) Bacterial populations remain intimately mixed and evenly distributed at all points in the process.

(iii) No bacterial growth or substrate removal occurs in the settler.

(iv) The aeration tank may be represented as a CSTR with no mass transfer limitations. In cases where this approximation is not adequate, a variable number of CSTR's in series could be used to simulate dispersed plug flow systems.

The general material balance which was applied to the aerator is given by:

$$\begin{bmatrix}\text{rate of mass}\\ \text{accumulation}\end{bmatrix} = \begin{bmatrix}\text{rate of mass flow}\\ \text{into aerator}\end{bmatrix} - \begin{bmatrix}\text{rate of mass flow}\\ \text{out of aerator}\end{bmatrix} \begin{matrix}\text{rate of appearance or}\\ \text{disappearance of mass}\\ \text{due to reaction}\end{matrix}$$

In the model equations:

$S_{i,j}$ = substrate concentration $(M \cdot L^{-3})$. The first subscript refers to substrate type, the second to a specific process stream.

$X_{i,j}$ = microorganism concentration $(M \cdot L^{-3})$. The subscripts indicate bacterial type and process stream.

V_i = volume of process component i (L^3).

Q_i = flow rate of process stream i ($L^3 \cdot T^{-1}$).

k_i = specific substrate removal rate for microorganism i ($M \cdot M^{-1} \cdot T^{-1}$).

C.1.1 Substrate Balances

Organic carbon (COD)

$$V_1 \frac{dS_1}{dt} = Q_1 S_{1,1} + Q_{15} S_{1,15} - Q_2 S_{1,2} - k_1 X_1 V_1 \tag{26}$$

dividing by the aeration tank volume gives:

$$\frac{dS_1}{dt} = \frac{1}{V_1} (Q_1 S_{1,1} + Q_{15} S_{1,15} - Q_2 S_{1,2}) - k_1 X_1 \tag{27}$$

Ammonium nitrogen

$$\frac{dS_2}{dt} = \frac{1}{V_1} (Q_1 S_{2,1} + Q_{15} S_{2,15} - Q_2 S_{2,2}) - k_2 X_2 - f Y_1 k_1 X_1 \tag{28}$$

The final term in Equation 28 represents the uptake of inorganic nitrogen for heterotrophic growth in stoichimetric proportion (f) to COD removal.

Nitrite nitrogen

$$\frac{dS_3}{dt} = \frac{1}{V_1} (Q_{15} S_{3,15} - Q_2 S_{3,2}) + k_2 X_2 - k_3 X_3 \tag{29}$$

In this equation, the reaction terms indicate that nitrite is formed by Nitrosomonas activity and is removed by Nitrobacter. It was also assumed that treatment plant influent contains no nitrite.

Nitrate nitrogen

$$\frac{dS_4}{dt} = \frac{1}{V_1}(Q_{15}\ S_{4,15} - Q_2\ S_{4,2}) + k_3X_3 \qquad (30)$$

where $S_{4,1}$ is assumed to be zero.

Substrate removal rates

To describe reaction rate terms for substrate removal, the Monod (1949) equation was used in which:

$$k = \frac{-1}{X}\cdot\frac{dS}{dt} = k_{max}\cdot\frac{S}{K_s+S} \qquad (7)$$

where: k_{max} = maximum substrate removal rate (T^{-1})

K_s = half velocity coefficient ($M{\cdot}L^{-3}$)

C.1.2 Microorganism balances

With the exception of specific subscripts, the mass balances for heterotrophs, Nitrosomonas and Nitrobacter were written in an identical manner. For heterotrophic bacteria the initial balance is:

$$\frac{dX_1}{dt} = \frac{1}{V_1}(Q_1\ X_{1,1} + Q_{15}\ X_{1,15} - Q_2\ X_{1,2}) + \mu_1X_1 \qquad (31)$$

If it is assumed that the microbial concentration in the feed is negligible ($X_{i,1} \approx 0$), then for Nitrosomonas the

balance becomes:

$$\frac{dX_2}{dt} = \frac{1}{V_1}(Q_{15}\,X_{2,15} - Q_2\,X_{2,2}) + \mu_2 X_2 \qquad (32)$$

A similar equation for <u>Nitrobacter</u> is:

$$\frac{dX_3}{dt} = \frac{1}{V_1}(Q_{15}\,X_{3,15} - Q_2\,X_{3,2}) + \mu_3 X_3 \qquad (33)$$

A shown previously in Equation (11)

$$\mu = \frac{Y}{X} \cdot \left(-\frac{dS}{dt}\right) - k_D \qquad (11)$$

where: Y = yield coefficient ($M \cdot M^{-1}$)

k_D = organism decay coefficient (T^{-1})

Substituting Equation (7) for $\frac{dS}{dt}$ gives:

$$\mu = \frac{Y \cdot k_{max} \cdot S}{K_s + S} - k_D \qquad (34)$$

C.2. <u>Clarifier model</u>

The simplified clarifier model illustrated in Figure 43 was used to represent secondary settler operation. In this model, the settler was assumed to be a zero volume device such that the feed stream was instantaneously separated into an overflow and underflow component. A solids balance about the settler at steady state is given by:

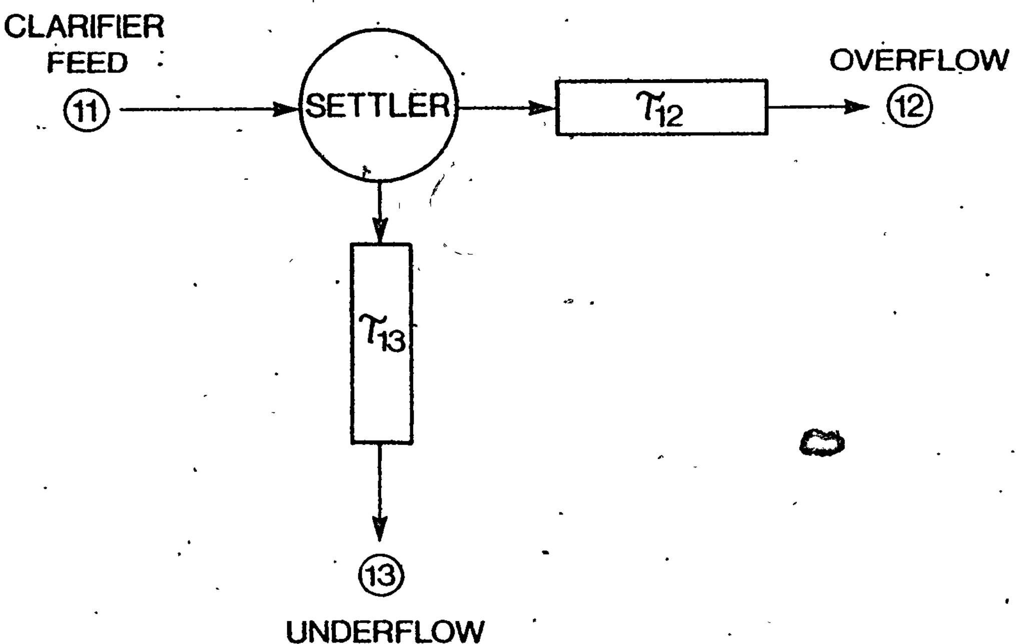

Figure 43. Schematic of Clarifier Model Structure Used in NITOX.

$$X_{i,11}\,Q_{11} = X_{i,13}\,Q_{13} + X_{i,12}\,Q_{12} \tag{35}$$

which can be rearranged to:

$$X_{i,13} = \frac{X_{i,11}\,Q_{11} - X_{i,12}\,Q_{12}}{Q_{13}} \tag{36}$$

To accommodate hold-up time in the clarifier, time delay terms were introduced to retard the appearance of the instantaneously settled stream at either the overflow or underflow. Therefore, at time t:

$$X_{i,13} = X_{i,13}\,(t-\tau_{13}) \tag{37}$$

$$S_{i,13} = S_{i,13}\,(t-\tau_{13}) \tag{38}$$

$$X_{i,12} = X_{i,12}\,(t-\tau_{12}) \tag{39}$$

$$S_{i,12} = S_{i,12}\,(t-\tau_{12}) \tag{40}$$

Terms τ_{12} and τ_{13} are pure time delays for streams 12 and 13 respectively, computed from the flowrates and volumes of the overflow and underflow compartments.

Representation of clarifier operation by this approach has two drawbacks. First, for dynamic operation, the model does not allow for storage of solids in the settler sludge blanket and therefore predicted distributions of solids in the aeration tank and the clarifier may not be completely realistic.

The other difficulty is that Equation (36) was developed to predict the biomass concentration in the

underflow, not the overflow. Therefore to solve Equation 36, values for the effluent suspended solids concentrations and the underflow pumping rate must be specified as model parameters. This does not pose a major dilemma since most stable activated sludge systems have relatively low and constant solids levels in the effluent and the underflow rate is usually known. In situations where these criteria are not met, or in which significant solids hold-up occurs in the sludge blanket, it may be necessary to provide an alternative model for the secondary clarifier.

To provide a facility for simulating system response at various levels of SRT, the settler model also included calculations for continuous solids wasting. The wasting rate (Q_{14}) was adjustable at specified increments in time, Δt, and its calculation was based upon the assumption that the entire system solids mass is resident only in the aeration tank.

The sequence of calculations leading to the estimation of Q_{15} was carried out in four steps as follows.

(i) The suspended solids concentration in the aeration tank and the clarifier overflow was averaged over the period since the last adjustment of Q_{14}.

$$X_{A,j} = \sum_{i=1}^{3} \left[\frac{X_{i,j}(t) + X_{i,j}(t-\Delta t)}{2}\right] \qquad (41)$$

where $X_{A,j}$ is the average solids concentration in

process component j ($mg \cdot L^{-1}$)

$X_{i,j}(t)$ is the concentration of microorganism i, at time t, in process component j ($mg \cdot L^{-1}$)

(ii) The required mass wasting rate (M_w) between t and t+Δt was calculated.

$$M_w = \frac{V_1 \ X_{A,1}}{SRT} - Q_{12} \ X_{A,12} \tag{42}$$

(iii) The waste sludge flowrate is given by:

$$Q_{14} = \frac{M_w}{\sum_{i=1}^{3} X_{i,13}} \tag{43}$$

(iv) The net return sludge ~~flow~~ (Q_{15}) is obtained by completion of the mass balance around the clarifier.

$$Q_{15} = Q_{13} - Q_{14} \tag{44}$$

C.3 Maximum specific substrate removal rates (kmax)

Maximum specific substrate removal rates were updated during computer simulations at time increments of 0.01 days. The update was carried out in two steps.

(a) Values of k_{max} for heterotrophs, Nitrosomonas and Nitrobacter were specified for a temperature of 22°C. For the nitrifying microorganisms, Equations 20 and 22 were used to calculate k^{22}_{max}. During steady state runs, the TKN and COD loadings required as input parameters were determined

simply by subtracting effluent from influent concentrations and multiplying by the ratio Q_1/V_1.

For dynamic simulations, the problem of time varying influent characteristics required that a series of step changes in concentration and flow be used to approximate actual dynamic feed patterns. Under these conditions, the steady state approach would result in large, instantaneous changes in the calculated COD loadings and thus also in values of k^{22}_{max}. To avoid this problem, substrate loadings used in k^{22}_{max} calculations were computed by subtracting the aeration tank effluent concentrations from theoretical values which approximated the aerator concentrations for non-reactive substrates. Following a step change in influent TKN, COD or flowrate, the theoretical feed value used for loading calculations was allowed to increase or decrease with a time constant equal to the hydraulic residence time of the completely mixed aeration tank (Equation 45). A similar approach was used to introduce flow

$$S = S_1 \, (1 - \exp(-\Delta t/\tau)) + S_o \, \exp(-\Delta t/\tau) \qquad (45)$$

where S = theoretical CSTR concentration ($mg \cdot L^{-1}$)

S_1 = actual feed concentration after step change ($mg \cdot L^{-1}$)

S_o = theoretical CSTR concentration at the time of the step change ($mg \cdot L^{-1}$)

Δt = elapsed time since step change (days)

τ = residence time of the aeration tank (days)

rate changes into the calculation of k^{22}_{max}.

For heterotrophs a constant k^{22}_{max} of 5.6 $days^{-1}$ was assumed.

(b) The effect of temperature on the k^{22}_{max} coefficients was then calculated from the relationship:

$$k^{T}_{max} = k^{22}_{max} \exp \left[\frac{E}{R} \left(\frac{1}{T} - \frac{1}{295}\right)\right] \quad (46)$$

where T = temperature (^oK)

E = activation energy ($cal \cdot g\text{-}mole^{-1}$)

R = gas constant ($cal \cdot g\text{-}mole^{-1} \cdot {}^oK^{-1}$)

The activation energies used for nitrifiers were those shown in Figures 14 and 15. For heterotrophs a value of 12,500 $cal \cdot g\text{-}mole^{-1}$ was used.

C.4. Integration method

The system of simultaneous ordinary differential equations was solved at time steps of 0.001 days by using Hamming's modified predictor-corrector method for the solution of linear initial value problems (Ralston and Wilf, 1960). Starting values for the Hamming method are provided

by applying a fourth-order Runge-Kutta procedure to the initial integration step.

Simulations were performed using double precision arithmetic on either a CYBER 171 computer or a Hewlett-Packard HP 1000 minicomputer.

APPENDIX D

Computer Programs

D1. Non-linear least squares regression program

```
0001 FTN4,L
0002       PROGRAM REGRS
0003       DIMENSION Y(22),TH(10),DIFF(10),SCRAT(1000),SIGNS(10),T(22)
0004       DIMENSION IP(5)
0005       COMMON X(22)
0006       EXTERNAL MODEL
0007
0008
0009       CALL RMPAR(IP)
0010       LU = IP(1)
0011       LL = IP(2)
0012
0013 C     ...READ NUMBER OF RESPONSES TO BE MODELLED
0014       WRITE(LU,601)
0015       READ(LU,*) NPROB
0016       WRITE(LU,602)
0017       READ(LU,*) NP
0018
0019 C     ...TYPE IN ARGUMENTS FOR UWHAS
0020       WRITE(LU,603)
0021       READ(LU,*) MIT,EPS2,EPS1,FNU,FLAM
0022
0023 C     ...READ NUMBER OF OBSERVATIONS
0024       WRITE(LU,604)
0025       READ(LU,*) NOB
0026
0027 C     ...READ X AND Y VALUES
0028       DO 50 I=1,NOB
0029       WRITE(LU,605)
0030       READ(LU,*) X(I),Y(I)
0031    50 CONTINUE
0032
0033 C     ...TYPE IN INITIAL GUESSES OF MODEL PARAMETERS
0034       WRITE(LU,606)
0035       READ(LU,*)(TH(I),I=1,NP)
0036
0037 C     ...SET PARAMETERS FOR UWHAS
0038       DO 60 L=1,NP
0039       DIFF(L) = .01
0040       SIGNS(L) = 0.1
0041    60 CONTINUE
0042
0043       CALL UWHAS(NPROB,MODEL,NOB,Y,NP,TH,DIFF,SIGNS,EPS1,EPS2,MIT
0044      (,FLAM,FNU,SCRAT,LL)
```

```
0045
0046    601 FORMAT(1X,"TYPE IN PROBLEM NUMBER")
0047    602 FORMAT(1X,"TYPE IN NUMBER OF MODEL PARAMETERS")
0048    603 FORMAT(1X,"TYPE IN MIT,EPS2,EPS1,FNU,FLAM")
0049    604 FORMAT(1X,"TYPE IN NUMBER OF OBSERVATIONS"")
0050    605 FORMAT(1X,"TYPE IN X,Y PAIR")
0051    606 FORMAT(1X,"TYPE INITIAL PARAMETER GUESSES")
0052        STOP
0053        END
```

```
0054        SUBROUTINE MODEL (NPROB,TH,F,NOB,NP)
0055        DIMENSION TH(10),F(22)
0056        COMMON X(22)
0057        DO 10 I=1,NOB
0058     10 F(I) = TH(2)*EXP(TH(1)*X(I))
0059        RETURN
0060        END
```

```
0002        SUBROUTINE UWHAS(NPROB,MODEL,NOB,Y,NP,TH,DIFF,SIGNS,EPS1,EPS2,
0003       1 MIT, FLAM, FNU, SCRAT,LL)
0004        DIMENSION SCRAT(1)
0005        IA=1
0006        IB=IA+NP
0007        IC=IB+NP
0008        ID=IC+NP
0009        IE=ID+NP
0010        IF=IE+NP
0011        IG=IF+NOB
0012        IH=IG+NOB
0013        II = IH + NP * NOB
0014        IJ = IH
0015            CALL  HAUS9(NPROB,MODEL,NOB,Y,NP,TH,DIFF,SIGNS,EPS1,EPS2,MIT
0016       1  ,FLAM,FNU,SCRAT(IA), SCRAT(IB), SCRAT(IC), SCRAT(ID),
0017       2  SCRAT(IE), SCRAT(IF), SCRAT(IG), SCRAT(IH), SCRAT(II),
0018       3  SCRAT(IJ),LL)
0019        RETURN
0020        END
0021        SUBROUTINE HAUS9(NPRBO, KODEL, NBO, Y,NQ,TH,DIFZ,SIGNS,EP1S,EP2S,
0022       1MIT,FLAM,FNU,   Q,P,E,PHI,TB,F,R,A,D,DELZ,LL)
0023  C
0024  C
0025  C
0026  C     DIMENSION TH(NQ), DIFZ(NQ), SIGNS(NQ), Y(NBO)
0027  C     DIMENSION Q(NQ), P(NQ), E(NQ), PHI(NQ), TB(NQ)
```

```
0028 C     DIMENSION F(NBO), R(NBO)
0029 C     DIMENSION A(NQ,NQ), D(NQ,NQ), DELZ(NBO,NQ)
0030 C
0031 C
0032 C
0033 C
0034       DIMENSION TH(1), DIFZ(1), SIGNS(1), Y(1), Q(1), P(1), E(1),
0035      1   PHI(1), TB(1), F(1), R(1), A(1), D(1), DELZ(1)
0036       ACOS(X) = ATAN(SQRT(1.0/X**2 - 1.0))
0037       NP = NQ
0038       NPROB = NPRBO
0039       NOB = NBO
0040       EPS1 = EP1S
0041       EPS2 = EP2S
0042       NPSQ = NP * NP
0043       NSCRAC = 5*NP+NPSQ +2*NOB+NP*NOB
0044       WRITE(LL,1000) NPROB, NOB, NP, NSCRAC
0045       WRITE(LL,1001)
0046       CALL GASS0(1, NP, TH, TEMP, TMEP,LL)
0047       WRITE(LL,1002)
0048       CALL GASS0(1, NP, DIFZ, TEMP, TEMP,LL)
0049       IF(MIN0(NP-1,50-NP,NOB-NP,MIT-1,999-MIT))99,15,15
0050  15   IF(FNU-1.0)99, 99, 16
0051  16   CONTINUE
0052       DO 19 I=1,NP
0053       TEMP = ABS(DIFZ(I))
0054       IF(AMIN1(1.0-TEMP, ABS(TH(I))))99, 99, 19
0055  19   CONTINUE
0056       GA = FLAM
0057       NIT = 1
0058       LAOS = 0
0059       IF(EPS1) 5,70,70
0060  5    EPS1 = 0
0061    70 SSQ = 0
0062       CALL MODEL(NPROB, TH, F, NOB, NP)
0063       DO 90 I = 1, NOB
0064       R(I) = Y(I) - F(I)
0065    90 SSQ=SSQ+R(I)*R(I)
0066       WRITE(LL,1003)SSQ
0067 C
0068 C                                          BEGIN ITERATION
0069 C
0070  100  GA = GA / FNU
0071       INTCNT = 0
0072       WRITE(LL,1004)NIT
0073  101  JS = 1 - NOB
0074       DO 130 J=1,NP
0075       TEMP = TH(J)
0076       P(J)=DIFZ(J)*TH(J)
0077       TH(J)= TH(J)+P(J)
0078       Q(J)=0
```

```
0079        JS = JS + NOB
0080        CALL MODEL(NPROB, TH, DELZ(JS), NOB, NP)
0081        IJ = JS-1
0082        DO 120 I = 1, NOB
0083        IJ = IJ + 1
0084        DELZ(IJ) = DELZ(IJ) - F(I)
0085   120  Q(J) = Q(J) + DELZ(IJ) * R(I)
0086        Q(J)= Q(J)/P(J)
0087  C                                       Q=XT*R (STEEPEST DESCENT)
0088   130  TH(J) = TEMP
0089        IF(LAOS) 131,131,414
0090   131  DO 150 I = 1, NP
0091        DO 151 J=1,I
0092        SUM = 0
0093        KJ = NOB*(J-1)
0094        KI = NOB*(I-1)
0095        DO 160 K = 1, NOB
0096        KI = KI + 1
0097        KJ = KJ + 1
0098   160  SUM = SUM + DELZ(KI) * DELZ(KJ)
0099        TEMP= SUM/(P(I)*P(J))
0100        JI = J + NP*(I-1)
0101        D(JI) = TEMP
0102        IJ = I + NP*(J-1)
0103   151  D(IJ) = TEMP
0104   150  E(I) = SQRT(D(JI))
0105   666  CONTINUE
0106        DO 153  I = 1, NP
0107        IJ = I-NP
0108        DO 153 J=1,I
0109        IJ = IJ + NP
0110        A(IJ) = D(IJ) / (E(I)*E(J))
0111        JI = J + NP*(I-1)
0112   153  A(JI) = A(IJ)
0113  C                                       A= SCALED MOMENT MATRIX
0114        II = - NP
0115        DO 155 I=1,NP
0116        P(I)=Q(I)/E(I)
0117        PHI(I)=P(I)
0118        II = NP + 1 + II
0119   155  A(II) = A(II) + GA
0120  C
0121        I=1
0122         CALL MATIN(A, NP, P, I, DET)
0123  C                                       P/E = CORRECTION VECTOR
0124         STEP=1.0
0125        SUM1=0.
0126        SUM2=0.
0127        SUM3=0.
0128        DO 231 I=1,NP
0129        SUM1=P(I)*PHI(I)+SUM1
```

```
0130        SUM2=P(I)*P(I)+SUM2
0131        SUM3= PHI(I) * PHI(I) + SUM3
0132   231  PHI(I) = P(I)
0133         TEMP = SUM1/SQRT(SUM2*SUM3)
0134        TEMP = AMIN1(TEMP, 1.0)
0135        TEMP = 57.295*ACOS(TEMP)
0136        WRITE(LL,1041) DET, TEMP
0137   170  DO 220 I = 1, NP
0138        P(I) = PHI(I) *STEP / E(I)
0139        TB(I) = TH(I) + P(I)
0140   220  CONTINUE
0141        WRITE(LL,7000)
0142   7000 FORMAT(30H0TEST POINT PARAMETER VALUES      )
0143        WRITE(LL,2006) (TB(I), I = 1, NP)
0144        DO 221 I = 1, NP
0145        IF(SIGNS(I)) 221, 221, 222
0146   222  IF(SIGN(1.0,TH(I))*SIGN(1.0,TB(I))) 663, 221, 221
0147   221  CONTINUE
0148        SUMB=0
0149        CALL MODEL(NPROB, TB, F, NOB, NP)
0150        DO 230 I=1,NOB
0151        R(I)=Y(I)-F(I)
0152  230   SUMB=SUMB+R(I)*R(I)
0153        WRITE(LL,1043)SUMB
0154        IF(SUMB - (1.0+EPS1)*SSQ) 662, 662, 663
0155   663  IF( AMIN1(TEMP-30.0, GA)) 665, 665, 664
0156  665   STEP=STEP/2.0
0157        INTCNT = INTCNT + 1
0158        IF(INTCNT - 36) 170, 2700, 2700
0159    664 GA=GA*FNU
0160        INTCNT = INTCNT + 1
0161        IF(INTCNT - 36) 666, 2700, 2700
0162   662  WRITE(LL,1007)
0163        DO 669 I=1,NP
0164  669   TH(I)=TB(I)
0165         CALL GASS0(1, NP, TH, TEMP, TEMP,LL)
0166        WRITE(LL,1040) GA, SUMB
0167        IF(EPS2) 229,229,225
0168    229 IF(EPS1) 270,270,265
0169   225  DO 240 I = 1, NP
0170        IF(ABS(P(I))/(1.E-20+ABS(TH(I)))-EPS2) 240, 240, 241
0171    241 IF(EPS1) 270,270,265
0172    240 CONTINUE
0173        WRITE(LL,1009)EPS2
0174        GO TO 280
0175   265  IF(ABS(SUMB - SSQ) - EPS1*SSQ) 266, 266, 270
0176   266  WRITE(LL,1010) EPS1
0177        GO TO 280
0178  270   SSQ=SUMB
```

```
0179        NIT=NIT+1
0180        IF(NIT - MIT) 100, 100, 280
0181   2700 WRITE(LL,2710)
0182   2710 FORMAT(//115H0**** THE SUM OF SQUARES CANNOT BE REDUCED TO THE SUM
0183       1OF SQUARES AT THE END OF THE LAST ITERATION - ITERATING STOPS   /)
0184  C
0185  C                                               END ITERATION
0186  C
0187   280  WRITE(LL,1011)
0188        WRITE(LL,2001) (F(I), I = 1, NOB)
0189        WRITE(LL,1012)
0190        WRITE(LL,2001) (R(I), I = 1, NOB)
0191        WRITE(LL,1017)
0192   1017 FORMAT(////," X PRIME-X MATRIX")
0193        CALL GASS0(4,NP,TEMP,TEMP,D,LL)
0194        SSQ=SUMB
0195        IDF=NOB-NP
0196        WRITE(LL,1015)
0197        I=0
0198         CALL MATIN(D, NP, P, I, DET)
0199        DO 7692 I=1,NP
0200        II = I + NP*(I-1)
0201   7692 E(I) = SQRT(D(II))
0202        DO 340 I=1,NP
0203        JI = I + NP*(I-1) - 1
0204        IJ = I + NP*(I-2)
0205        DO 340 J = I, NP
0206        JI = JI + 1
0207        A(JI) = D(JI)/ (E(I)*E(J))
0208        IJ = IJ + NP
0209   340   A(IJ) = A(JI)
0210         CALL GASS0(3, NP, TEMP, TEMP, A,LL)
0211        WRITE(LL,1016)
0212         CALL GASS0(1, NP, E, TEMP, TEMP,LL)
0213        IF(IDF) 341, 410, 341
0214   341  SDEV = SSQ / IDF
0215        WRITE(LL,1014) SDEV, IDF
0216         SDEV = SQRT(SDEV)
0217        DO 391 I=1,NP
0218        P(I)=TH(I)+2.0*E(I)*SDEV
0219   391  TB(I)=TH(I)-2.0*E(I)*SDEV
0220        WRITE(LL,1039)
0221         CALL GASS0(2, NP, TB, P, TEMP,LL)
0222        LAOS = 1
0223        GO TO 101
0224   414  DO 415 K = 1, NOB
0225        TEMP = 0
0226        DO 420 I=1,NP
0227        DO 420 J=1,NP
0228        ISUB = K+NOB*(I-1)
0229        DEBUG1 = DELZ(ISUB)
```

```
0230 C      DEBUG1 = DELZ(K + NOB*(I-1))
0231        ISUB = K+NOB*(J-1)
0232        DEBUG2 = DELZ(ISUB)
0233 C      DEBUG2 = DELZ(K + NOB*(J-1))
0234        IJ = I + NP*(J-1)
0235        DEBUG3 = D(IJ)/(DIFZ(I)*TH(I)*DIFZ(J)*TH(J))
0236   420  TEMP = TEMP + DEBUG1 * DEBUG2 * DEBUG3
0237        TEMP = 2.0*SQRT(TEMP)*SDEV
0238        R(K)=F(K)+TEMP
0239   415  F(K)=F(K)-TEMP
0240        WRITE(LL,1008)
0241        IE=0
0242        DO 425 I=1,NOB,5
0243        IE=IE+5
0244        IF(NOB-IE) 430,435,435
0245   430  IE=NOB
0246   435  WRITE(LL,2001) (R(J), J = I, IE)
0247   425  WRITE(LL,2006) (F(J), J = I, IE)
0248   410  WRITE(LL,1033) NPROB
0249        RETURN
0250   99   WRITE(LL,1034)
0251        GO TO 410
0252   1000 FORMAT(38H1NON-LINEAR ESTIMATION, PROBLEM NUMBER   I3,// I5,
0253       1  14H OBSERVATIONS, I5, 11H PARAMETERS  I7, 17H SCRATCH REQUIRED)
0254   1001 FORMAT(/25H0INITIAL PARAMETER VALUES  )
0255   1002 FORMAT(/54H0PROPORTIONS USED IN CALCULATING DIFFERENCE QUOTIENTS )
0256   1003 FORMAT(/25H0INITIAL SUM OF SQUARES =   E12.4)
0257   1004 FORMAT(/////45X,13HITERATION NO.  I4)
0258   1007 FORMAT(/32H0PARAMETER VALUES VIA REGRESSION  )
0259   1008 FORMAT(////54H0APPROXIMATE CONFIDENCE LIMITS FOR EACH FUNCTION VAL
0260       1UE    )
0261   1009 FORMAT(/62H0ITERATION STOPS - RELATIVE CHANGE IN EACH PARAMETER LE
0262       1SS THAN   E12.4)
0263   1010 FORMAT(/62H0ITERATION STOPS - RELATIVE CHANGE IN SUM OF SQUARES LE
0264       1SS THAN   E12.4)
0265   1011 FORMAT(22H1FINAL FUNCTION VALUES   )
0266   1012 FORMAT(////10H0RESIDUALS  )
0267   1014 FORMAT(//24H0VARIANCE OF RESIDUALS =    ,E12.4,1H,I4,
0268       120H DEGREES OF FREEDOM   )
0269   1015 FORMAT(////19H0CORRELATION MATRIX   )
0270   1016 FORMAT(////21H0NORMALIZING ELEMENTS   )
0271   1033 FORMAT(//19H0END OF PROBLEM NO.  I3)
0272   1034 FORMAT(/16H0PARAMETER ERROR  )
0273   1039 FORMAT(/71H0INDIVIDUAL CONFIDENCE LIMITS FOR EACH PARAMETER (ON LI
0274       1NEAR HYPOTHESIS)    )
0275   1040 FORMAT(/9H0LAMBDA =E10.3, 4X,33HSUM OF SQUARES AFTER REGRESSION = ,
0276       1E15.7)
0277   1041 FORMAT(14H DETERMINANT =  E12.4, 6X, 25H ANGLE IN SCALED COORD. =
0278       1  F5.2, 8HDEGREES  )
0279   1043 FORMAT(23H0TEST POINT SUM OF SQUARES =    E12.4)
0280   2001 FORMAT(/5E12.4)
```

```
0281   2006 FORMAT(5E12.4)
0282        END
0283        SUBROUTINE MATIN(A, NVAR, B, NB, DET)
0284        DIMENSION A(NVAR, 1), B(NVAR, 1)
0285         PIVOTM = A(1,1)
0286         DET = 1.0
0287        DO 550 ICOL = 1, NVAR
0288         PIVOT = A(ICOL, ICOL)
0289        PIVOTM = AMIN1(PIVOT, PIVOTM)
0290         DET = PIVOT * DET
0291  C
0292  C     DIVIDE PIVOT ROW BY PIVOT ELEMENT
0293  C
0294         A(ICOL, ICOL) = 1.0
0295
0296        PIVOT = AMAX1(PIVOT, 1.E-20)
0297         PIVOT = A(ICOL, ICOL)/PIVOT
0298        DO 350 L=1,NVAR
0299   350   A(ICOL, L) = A(ICOL, L)*PIVOT
0300        IF(NB .EQ. 0) GO TO 371
0301        DO 370 L=1,NB
0302   370   B(ICOL, L) = B(ICOL, L)*PIVOT
0303  C
0304  C     REDUCE NON-PIVOT ROWS
0305  C
0306   371  DO 550 L1=1,NVAR
0307         IF(L1 .EQ. ICOL) GO TO 550
0308         T = A(L1, ICOL)
0309         A(L1, ICOL) = 0.
0310        DO 450 L=1,NVAR
0311   450   A(L1, L) = A(L1, L) - A(ICOL, L)*T
0312        IF(NB .EQ. 0) GO TO 550
0313        DO 500 L=1,NB
0314   500   B(L1, L) = B(L1, L)-B(ICOL,L)*T
0315    550 CONTINUE
0316        RETURN
0317        END
0318         SUBROUTINE GASSO(ITYPE, NQ, A, B, C,LL)
0319        DIMENSION A(NQ),B(NQ),C(NQ,NQ)
0320         NP = NQ
0321         NR = NP/10
0322         LOW = 1
0323         LUP = 10
0324    10  IF( NR )15,20,30
0325    15  RETURN
0326    20  LUP=NP
0327        IF(LOW .GT. LUP) RETURN
0328    30  WRITE(LL,500) (J,J=LOW,LUP)
0329        GO TO (40,60,80),ITYPE
0330    40  WRITE(LL,600)(A(J),J=LOW,LUP)
0331        GO TO 100
```

```
0332   60   WRITE(LL,600) (B(J),J=LOW,LUP)
0333        GO TO 40
0334   80   DO 90 I=LOW,LUP
0335   90   WRITE(LL,720)I,(C(J,I),J=LOW,I)
0336        LOW2=LUP+1
0337        IF(LOW2 .GT. NP) GO TO 100
0338        DO 95 I=LOW2,NP
0339   95   WRITE(LL,720)I,(C(J,I),J=LOW,LUP)
0340   100   LOW = LOW + 10
0341         LUP = LUP + 10
0342         NR = NR - 1
0343         GO TO 10
0344   500  FORMAT(/I7,9I7)
0345   600  FORMAT(5E12.4)
0346   720  FORMAT(1H0,I3,1X,F7.4,9F12.4)
0347        END
```

D2. NITOX - Simulation of Mixed Culture Nitrification

PAGE 0001 FTN. 4:28 PM FRI., 1 MAY, 1981

```
0001 FTN4,L
0002       BLOCK DATA NAME
0003       IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0004       DOUBLE PRECISION KM,KO,KS
0005       COMMON /B1/ NOBS,OBS(8,201),DELT,H
0006       COMMON /B2/ SD(4,10),XD(3,10)
0007       COMMON /B3/ KOUNT,S1,S2,PTD,KNT,C1,C2,Q1,QE
0008       COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),
0009      *X(3,15),SRT,DEGC,TS,TH,TR,TD
0010       DATA V(1),V(12),V(13)/27.D0,2.2D0,6.0D0/
0011       DATA KM /5.6D0,6.6D0,12.0D0/
0012       DATA Q(1),Q(13),Q(14)/64.0D0,80.0D0,0.0D0/
0013       DATA KD/.03D0,.05D0,.05D0/
0014       DATA KS/22.D0,.5D0,.5D0/
0015       DATA G /0.45D0,.050D0,.050D0/
0016       DATA SRT,DEGC/10.0D0,15.D0/
0017       END
```

FTN4 COMPILER: HP92060-16092 REV. 2001 (791101)

** NO WARNINGS ** NO ERRORS **

```
0018        PROGRAM NITOX ( ,300)
0019
0020
0021  C*************************************************************************
0022  C       THIS PROGRAM SOLVES A SET OF SIMULTANEOUS DIFFERENTIAL
0023  C       EQUATIONS DESCRIBING THE COMBINED CARBON REMOVAL -
0024  C       NITRIFICATION PROCESS IN A WELL MIXED REACTOR
0025  C       FOLLOWED BY A CLARIFIER WITH TOTAL UNDERFLOW RECYCLE
0026  C       WASTING RATE IS ADJUSTED CONTINUOUSLY TO MAINTAIN
0027  C       DESIRED SRT
0028  C
0029  C
0030  C       Q          FLOWS OF VARIOUS PROCESS STREAMS   L/DAY
0031  C       S(I,J)     SOLUBLE SUBSTRATE CONC'S. "I" REFERS TO THE
0032  C                  SUBSTRATE TYPE, "J" REFERS TO THE PROCESS STREAM
0033  C                  MG/L
0034  C       X(I,J)     MICROORGANISM CONC'S. "I REFERS TO THE SUBSTRATE
0035  C                  TYPE, "J" REFERS TO THE PROCESS STREAM   MG/L
0036  C       N          NUMBER OF EQUATIONS IN THE MODEL
0037  C       Y1         INITIAL VALUES OF THE DEPENDENT VARIABLES
0038  C       MU         MAXIMUM SPECIFIC SUBSTRATE REMOVAL RATES  DAYS-1
0039  C       G          YIELD COEFFICIENTS   MG/MG
0040  C       KS         HALF VELOCITY COEFFICIENTS   MG/L
0041  C       KD         DECAY COEFFICIENTS   DAYS-1
0042  C       V          PROCESS COMPONENT VOLUMES   LITERS
0043  C
0044  C*************************************************************************
0045
0046
0047        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0048        DOUBLE PRECISION KM,KD,KS
0049        COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),X(3,15)
0050       *,SRT,DEGC,TS,JM,TR,TD
0051        COMMON /B2/ SD(4,10),XD(3,10)
0052        COMMON /B3/ KOUNT,S1,S2,PTD,KNT,C1,C2,Q1,QE
0053        COMMON /B1/ NOBS, OBS(8,201), DELT,H
0054        DIMENSION Y1(7),PRMT1(5),DERY1(7),AUX1(16,7)
0055        DIMENSION IDCB(144),IBUF(16),ODTA(8),NAM(3),IT(5),RINIT(7)
0056        EQUIVALENCE (IBUF,ODTA)
0057        EXTERNAL CSTR1,OUTP1
0058  C
0059  C     ...SPEC # OF EQN'S AND INFO FOR DATA STORAGE ON DISC...
0060        DATA N/7/
0061        DATA NAM /2HDA,2HTA,2HEH/
0062        IOPN = 3B
0063        ISEC = 2HER
0064  C
0065  C
```

PAGE 0003 NITOX 4:28 PM FRI., 1 MAY , 1981

```
0066  C
0067  C      ...GO THRU SIMULATION LOOP ONCE FOR EACH SET OF INPUT
0068  C      PARAMETERS...
0069  C
0070  C      DO 200 M=1,2
0071  C      GO TO (1,2) M
0072  C
0073  C      ...INPUT LIST NUMBER 1 ...
0074     1   CONTINUE
0075         DATA Y1 /2.0D0,1.9D0,0.5D0,17.2D0,3430.D0,16.2D0,15.5D0/
0076         S(1,1) = 354.D0
0077         S(2,1) = 20.D0
0078         S(3,1) = 0.D0
0079         S(4,1) = 0.D0
0080         TD = 0.05D0
0081         KOUNT = 0
0082         PRNT1(2) = 5.0D0
0083         KNT = 1
0084         GO TO 8
0085  C
0086  C      ... INPUT LIST NUMBER 2 ...
0087     2   CONTINUE
0088         GO TO 8
0089  C
0090  C      ... INPUT LIST NUMBER 3 ...
0091     3   CONTINUE
0092         GO TO 8
0093  C
0094  C
0095  C      ...SET SYSTEM COMPONENT VOLUMES...
0096     8   DO 9 I=2,11
0097     9   V(I) = 0.
0098  C      ... INITIALIZE PROCESS STREAM CONC'S & FLOWS...
0099         DO 10 I=1,4
0100         S(I,2) = Y1(I)
0101    10   S(I,15) = Y1(I)
0102         DO 15 I=5,7
0103         X(I-4,2) = Y1(I)
0104    15   X(I-4,15) = Y1(I)*Q(13)/Q(1)
0105         Q(15) = Q(13) - Q(14)
0106
0107  C      ...INITIALIZE STORAGE ARRAYS FOR SUBROUTINE SETTLE...
0108         DO 30 K=1,10
0109         DO 20 J=1,4
0110    20   SD(J,K) = Y1(J)
0111         DO 25 J=5,7
0112    25   XD(J-4,K) = Y1(J)
0113    30   CONTINUE
```

PAGE 0004 NITOX 4:28 PM FRI., 1 MAY , 1981

```
0114  C
0115  C     ... SET PARAMETER VALUES FOR INTEGRATION...
0116        NOBS = 1
0117        TS = 0.0D0
0118        TM = 0.0D1
0119        TR = 0.0D0
0120        T = 0.D0
0121        PRMT1(1) = 0.0D0
0122        PRMT1(3) = 0.001D1
0123        PRMT1(4) = 1.0D0
0124        OBS(1.NOBS) = T
0125    35  DO 40 I=1.N
0126        OBS(I+1.NOBS) = Y1(I)
0127    40  DERY1(I) = 1.D0/N
0128  C
0129  C     ...READ SIMULATION STARTING TIME...
0130        CALL EXEC(11.IT.IY)
0131        IT4 = IT(4)
0132        IT3 = IT(3)
0133  C
0134  C     ... CALL HAMMING'S METHOD FOR INTEGRATION ...
0135        CALL HPCG (PRMT1,Y1,DERY1.N,IHLF,CSTR1.OUTP1.AU(1.T)
0136  C
0137  C     ...READ REAL TIME...
0138        CALL EXEC (11.IT.IY)
0139  C
0140  C
0141  C     IF(M .NE. 1) GO TO 50
0142  C     DO 42 I=1.7
0143  C 42  RINIT(I) = Y1(I)
0144    50  CONTINUE
0145  C
0146  C     ...WRITE RESULTS AND INPUT TO DISC FILE DATAEH...
0147  C
0148  C     ...OPEN DISC FILE. EXCLUSIVELY. READ FIRST RECORD..
0149  C
0150        CALL OPEN (IDCB.IERR.MAH.IOPN.ISEC,24)
0151        IF ( IERR .GE. 0) GO TO 200
0152        WRITE (6.615) IERR
0153        STOP1
0154   200  CALL READF (IDCB.IERR.IBUF.16,LEN,1)
0155        IF (IERR .GE. 0) GO TO 210
0156        WRITE (6.616) IERR
0157        CALL CLOSE (IDCB)
0158        STOP2
0159  C
0160  C     ...IF RECORD COUNTER HAS REACHED THE END OF FILE RESET TO 1...
0161  C
```

```
0162    210 IF (IBUF(1) .EQ. 601) GO TO 220
0163        IREC = IBUF(1)
0164        GO TO 230
0165    220 IREC = 1
0166    230 CONTINUE
0167  C
0168  C     ...WRITE NOBS AND INPUT TO DISC FILE...
0169        IBUF(1) =NOBS
0170        IBUF(2) = IHLF
0171        IBUF(3) = IT4
0172        IBUF(4)   IT3
0173        IBUF(5) = IT(4)
0174        IBUF(6) = IT(3)
0175        IREC = IREC +1
0176        CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0177        IF(IERR .GE. 0) GO TO 232
0178    231 WRITE(6,617)IERR,IREC
0179        CALL CLOSE(IDCB)
0180        STOP
0181    232 ODTA(1) - SNGL(V(1))
0182        ODTA(2) = SNGL(Q(1))
0183        ODTA(3) = SNGL(Q(15))
0184        ODTA(4) = SNGL(SRT)
0185        ODTA(5) = SNGL(DEGC)
0186        ODTA(6) = SNGL(T)
0187        IREC = IREC+1
0188        CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0189        IF(IERR .LT. 0) GO TO 231
0190        DO 233 I=1,3
0191    233 ODTA(I) = SNGL(KM(I))
0192        IREC = IREC+1
0193        CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0194        IF(IERR  LT. 0) GO TO 231
0195        DO 234 I=1,3
0196    234 ODTA(I) = SNGL(G(I))
0197        IREC = IREC+1
0198        CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0199        IF(IERR .LT. 0) GO TO 231
0200        DO 235 I=1,3
0201    235 ODTA(I) = SNGL(KD(I))
0202        IREC = IREC+1
0203        CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0204        IF(IERR .LT. 0) GO TO 231
0205        DO 236 I=1,3
0206    236 ODTA(I) = SNGL(KS(I))
0207        IREC = IREC+1
0208        CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0209        IF(IERR .LT. 0) GO TO 231
```

```
0210  C
0211  C      ...WRITE ARRAY OBS TO DATAEH...
0212  C
0213         K=0
0214         DO 270 J=1,NOBS
0215         DO 260 I-1,8
0216         K = K+1
0217         ODTA(K) = OBS(I,J)
0218         IF (K .EQ. 8) GO TO 240
0219         GO TO 260
0220     240 IREC = IREC + 1
0221         CALL WRITF (IDCB,IERR,IBUF,16,IREC)
0222         IF(IERR .GE. 0 ) GO TO 250
0223         CALL CLOSE (IDCB)
0224         STOP3
0225     250 K = 0
0226     260 CONTINUE
0227     270 CONTINUE
0228         IREC = IREC + 1
0229         ODTA(1) = SNGL(DELT)
0230         ODTA(2) = SNGL(H)
0231         CALL WRITF(IDCB,IERR,IBUF,16,IREC)
0232         IF(IERR .LT. 0) GO TO 231
0233  C
0234  C      ...ZERO ARRAY IBUF AND WRITE TO RECORD #  THE LAST RECORD
0235  C      NUMBER WRITTEN TO ...
0236  C
0237         DO 280 I=2,16
0238     280 IBUF(I) = 0
0239         IBUF(1) = IREC
0240         CALL WRITF (IDCB,IERR,IBUF 16,1)
0241         IF(IERR .LT. 0) WRITE(6,621)IERR
0242         CALL CLOSE (IDCB)
0243  C
0244  C      ...FORMAT FOR INPUT AND OUTPUT...
0245  C
0246     300 CONTINUE
0247     500 FORMAT(20A4)
0248     501 FORMAT(I5)
0249     502 FORMAT(2F10.2)
0250     503 FORMAT(2F10.3,I5)
0251     504 FORMAT(8F10.1)
0252     505 FORMAT(3F10.4,I5)
0253     506 FORMAT(3F10.3)
0254     507 FORMAT(8F10.1)
0255     600 FORMAT(1H1,20X,20A4///)
0256     601 FORMAT(//25X,'NUMBER OF EQUATIONS = ',I5//)
0257     602 FORMAT(25X,'MODEL PARAMETERS'/30X,'CSTR VOLUME  ',F4.1,1X,'LITERS'
```

```
PAGE 0007  NITOX  4:28 PM  FRI.,  1  MAY , 1981

0258          1)
0259      604 FORMAT(/30X,"SOLIDS RETENTION TIME ",F6.1/30X,"INITIAL CLARIFIER S
0260          1EPARATION COEFFICIENT ",F6.2)
0261      605 FORMAT(/// 5X,"TIME",10X,"COD",8X,"TKN",8X,"NO2-N",8X,"NO3-N",5X,
0262          5 "HETEROTROPHS",5X,"NITROSOMONAS",5X,"NITROBACTER"//)
0263      606 FORMAT(23X,F6.4,19X,F7.3,16X,F10.3/)
0264      607 FORMAT(//25X,"SIMULATION PARAMETERS",/30X,"LOWER BOUND OF INTEGRAT
0265          1ION INTERVAL = ",F10.4/30X,"UPPER BOUND OF INTEGRATION INTERVAL =
0266          2",F10.4/30X,"STEP SIZE = ",F10.4)
0267      608 FORMAT(/30X,"SUBSTRATE REMOVAL RATES, K*(1), K*(2), K*(3) "/35X,
0268          2 3F10.3)
0269      609 FORMAT(/30X,"YIELD COEFFICIENTS, G(1), G(2), G(3) "/35X,
0270          3 3F10.3)
0271      610 FORMAT(/30X,"DECAY COEFFICIENTS, KD(1), KD(2), KD(3) " /35X,
0272          4 3F10.3)
0273      611 FORMAT( 3X,F6.2,9X,F5.1,3(7X,F5.1),9X,F6.0,2(12X,F6.1))
0274      612 FORMAT(/30X,"INITIAL INFLUENT FLOW RATE",F6.1/30X,"INITIAL RETURN
0275          6 FLOW RATE",F6.1)
0276      613 FORMAT(/30X,"HALF VELOCITY COEFFICIENTS, KS1), KS(2), KS(3) " /35X
0277          1, 3F10.3)
0278      614 FORMAT(//5X,"ERROR NUMBER (IHLF) = ",I3)
0279      615 FORMAT(1X,"DISC FILE ERROR OPENING DATAEH FILE =",I7)
0280      616 FORMAT(1X,"DISC ACCESS ERROR READING DATAEH =",I7)
0281      617 FORMAT(1X,"DISC ACCESS ERROR =",I7,"CANNOT WRITE TO DISC
0282          1 RECORD # ",I7)
0283      618 FORMAT(1X,"SIMULATION STARTING TIME = ",I2,":",I2,":")
0284      619 FORMAT(1X,"NUMBER OF STORED OBSERVATIONS =",I5)
0285      620 FORMAT(1X,"SIMULATION FINISHED AT ",I2,":",I2,":")
0286      621 FORMAT(1X,"DISC ACCESS ERROR =",I5," CANNOT WRITE NUMBER OF LAST
0287          1RECORD WRITTEN")
0288      622 FORMAT(1X,"UPPER LIMIT OF INTEGRATED INTERVAL WAS ",F6.2)
0289           STOP
0290           END

FTN4 COMPILER: HP92060-16092 REV. 2001 (791101)

**  NO WARNINGS **  NO ERRORS **   PROGRAM = 02411      COMMON = 00000
```

PAGE 0008 FTN. 4:28 PM FRI., 1 MAY , 1981

```
0291         SUBROUTINE CSTR1 (T,Y1,DERY1)
0292  C
0293  C      THIS SUBROUTINE CONTAINS THE RIGHT HAND SIDES OF THE SET OF
0294  C      SIMULTANEOUS EQUATIONS
0295  C
0296         IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0297         DOUBLE PRECISION KM,KD,KS
0298         COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),X(3,15)
0299        *,SRT,DEGC,TS,TM,TR,TD
0300         DIMENSION Y1(7), DERY1(7)
0301         DIMENSION C(10),R(10),D(10)
0302  C
0303  C      ... REDEFINE AERATION TANK EXIT CONCS ...
0304         DO 15 I=1,4
0305     15  S(I,2) = Y1(I)
0306         DO 20 I=1,3
0307     20  X(I,2) = Y1(I+4)
0308      30 CONTINUE
0309  C
0310  C      ...DEFINE CONVECTIVE , REACTION AND DECAY TERMS FOR THE MODEL
0311         Q(2) = Q(1) + Q(15)
0312         DO 40 I =1,2
0313         C(I) = (Q(1)*S(I,1) + Q(15)*S(I,15) - Q(2)*S(I,2))/V(1)
0314      40 CONTINUE
0315         DO 50 I=3,4
0316         C(I) = (Q(15)*S(I,15) - Q(2)*S(I,2))/V(1)
0317      50 CONTINUE
0318         DO 60 I=5,7
0319         C(I) = (Q(15)*X(I-4,15) - Q(2)*X(I-4,2))/V(1)
0320      60 CONTINUE
0321         DO 70 I=1,3
0322         R(I) = (KM(I)*S(I,2)*X(I,2))/(KS(I)+S(I,2))
0323         D(I) = KD(I)*X(I,2)
0324     70  CONTINUE
0325  C
0326  C      ...DYNAMIC MODEL HAS THE FOLLOWING FORM...
0327         DERY1(1)= C(1) - R(1)
0328         DERY1(2)= C(2) - 0.09D0*G(1)*R(1) - R(2)
0329         DERY1(3)= C(3) + R(2) - R(3)
0330         DERY1(4)= C(4) + R(3)
0331         DERY1(5)= C(5) + G(1)*R(1) - D(1)
0332         DERY1(6)= C(6) + G(2)*R(2) - D(2)
0333         DERY1(7)= C(7) + G(3) *R(3) - D(3)
0334         RETURN
0335         END
```

```
PAGE 0010  FTN.   4:28 PM  FRI.,  1  MAY , 1981

0336        SUBROUTINE OUTP1 (T,Y1,DERY1,IHLF,N,PRMT1)
0337
0338        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0339        DOUBLE PRECISION KM,KD,KS
0340        COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),X(3,15)
0341       *,SRT,DEGC,TS,TM,TR,TD
0342        COMMON /B1/ NOBS,OBS(8,201),DELT,H
0343        DIMENSION Y1(7),PRMT1(5),DERY1(7)
0344  C
0345  C
0346  C     ...NEGATIVE RESULT PREVENT...
0347        DO 10 I=1,N
0348     10 IF(Y1(I) .LT. 0.) Y1(I)=0.01D0
0349  C
0350  C     ...PRINT MODEL OUTPUT VALUES...
0351        IF (T-TS .GE. .050D0) CALL SCRIB (T,Y1,N)
0352  C
0353  C     ...SIMULATE CLARIFIER OPERATION...
0354        IF (T-TM .GE. .1D0) CALL SETTL (Y1)
0355  C
0356  C     ...CALC CURRENT NITRIFIER KM'S...
0357        IF (T-TR .GE. .01D0) CALL RATES (Y1,T)
0358  C
0359  C     ...CHANGE INFLUENT OR OPERATING CONDITIONS...
0360        IF (T .GE. TD) CALL DYNAM(Y1,T)
0361  C
0362        RETURN
0363        END


FTN4 COMPILER: HP92060-16092 REV. 2001 (791101)

**  NO WARNINGS **  NO ERRORS **   PROGRAM = 00130      COMMON = 00000
```

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```
0364        SUBROUTINE SCRIB  (T,Y1,N)
0365
0366  C     ...THIS SUBROUTINE PRINTS OUTPUT VALUES
0367  C        AND PREPARES AN ARRAY (OBS) OF THESE
0368  C       VALUES FOR SUBSEQUENT PLOTTING
0369
0370
0371        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0372        DOUBLE PRECISION KM,KD,KS
0373        COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),X(3,15)
0374       *,SRT,DEGC,TS,TM,TR,TD
0375        COMMON /B1/  NOBS,OBS(8,201),DELT,H
0376        DIMENSION Y1(7)
0377        NOBS = NOBS +1
0378        OBS(1,NOBS) = SNGL(T)
0379        DO 20 I=2,8
0380     20 OBS(I,NOBS) = SNGL(Y1(I-1))
0381        TS = TS + 0.050D0
0382        RETURN
0383        END
```

FTN4 COMPILER: HP92060-16092 REV. 2001 (791101)

** NO WARNINGS ** NO ERRORS ** PROGRAM = 00069 COMMON = 00000

PAGE 0012 FTN. 4:28 PM FRI., 1 MAY , 1981

```
0384        SUBROUTINE SETTL (Y1)
0385        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0386        DOUBLE PRECISION KH,KD,KS
0387        COMMON /B4/ Q(15),S(4,15),KH(3),G(3),KD(3),V(15),KS(3),X(3,15)
0388       *,SRT,DEGC,TS,TM,TR,TD
0389        COMMON /B2/ SD(4,10),XD(3,10)
0390        DIMENSION Y1(7),F(3)
0391        DIMENSION   SOL(3)
0392
0393  C     ...THIS SUBROUTINE PERFORMS THREE SETS OF CALCULATIONS
0394  C        WHICH APPROXIMATE THE CLARIFIER FUNCTION
0395  C        (1) REACTOR EFFLUENT CONCS ARE STORED IN A TIME DELAY
0396  C            ARRAY TO ACCOUNT FOR TIME LAG IN CLARIFIER
0397  C        (2) APPROPRIATE VALUES ARE DRAWN FROM THIS ARRAY TO
0398  C            USE AS TIME-DELAYED CONCS IN CLARIFIER OVERFLOW
0399  C            AND UNDERFLOW
0400  C        (3) THE RETURN SLUDGE STREAM (Q(15)) IS REDUCED BY A
0401  C            FACTOR WHICH APPROXIMATES THE DESIRED WASTING RATE
0402  C            TO MAINTAIN A PARTICULAR SRT
0403
0404
0405  C     ...BEFORE OLD VALUES ARE REPLACED, PERFORM PRELIMINARY
0406  C        CALCULATIONS FOR WASTING
0407  C     ...(1) CALC SOLIDS LOSS IN CLARIFIER OVERFLOW OVER THE
0408  C            LAST ING STEPS IN MILLIGRAMS...
0409        Q(11) = Q(2)
0410        Q(12) = Q(11) - Q(13)
0411  C
0412  C     ...SPECIFY TOTAL EFFLUENT SOLIDS CONC...
0413  C
0414  C     X12 = 30.D0
0415  C
0416  C     ...CALC FRACTION OF NS,NB,HETER...
0417        DO 10 I=1,3
0418        F(I) = Y1(I+4)/(Y1(5)+Y1(6)+Y1(7))
0419  C
0420  C     ...CALC INDIVIDUAL EFFLUENT SOLIDS CONCS...
0421     10 X(I,12) = X12*F(I)
0422        TOL = Q(12)*X12*0.1D0
0423
0424  C     ...(2) CALC AVE SYSTEM SOLIDS SINCE LAST CALCULATION
0425  C            ASSUMING ALL SOLIDS RESIDE IN REACTOR
0426        DO 20 I=1,3
0427     20 SOL(I) = V(1)*(XD(I,1)+Y1(I+4))/2.
0428        SYSOL = SOL(1) + SOL(2) + SOL(3)
0429
0430  C     ...(3) CALCULATE WASTE SOLIDS MASS OVER NEXT
0431  C            TIME STEP IN MILLIGRAMS...
```

```
0432        WSOL = ((SYSOL/SRT)*.1D0) - TOL
0433
0434  C     ...NOW UPDATE DELAY ARRAY HOLDING PAST CONC VALUES
0435  C        OF AERATION TANK EFFLUENT
0436        DO 50 K=1,9
0437        I = 11-K
0438        DO 30 J=1,4
0439    30  SD(J,I) = SD(J,I-1)
0440        DO 40 J=1,3
0441    40  XD(J,I) = XD(J,I-1)
0442    50  CONTINUE
0443        DO 60 J=1,4
0444    60  SD(J,1) = Y1(J)
0445        DO 70 J=5,7
0446    70  XD(J-4,1) = Y1(J)
0447
0448
0449  C     ...CALCULATE TIME DELAYS FOR OVERFLOW AND UNDERFLOW
0450        TLAG12 = V(12)/(Q(12)*.1D0)
0451        TLAG13 = V(13)/(Q(13)*.1D0)
0452        IF ( TLAG13 .LT. 1.0D0 ) TLAG13 = 1.0D0
0453        IF ( TLAG12 .LT. 1.D0) TLAG12 =1.0D0
0454        L12 = IDINT(TLAG12)
0455        L13 = IDINT(TLAG13)
0456
0457  C     ...WITHDRAW TIME DELAYED VALUES FROM DELAY ARRAY AND CALC
0458  C     SOLIDS CONCS IN EXIT STREAMS BY MASS BALANCE...
0459        DO 85 J=1,4
0460        S(J,12) = SD(J,1+L12)
0461        S(J,13) = SD(J,1+L13)
0462    85  S(J,15) = S(J,13)
0463        DO 95 J=1,3
0464    95  X(J,15) = (Q(11)*XD(J,1+L13) - Q(12)*X(I,12))/Q(13)
0465
0466  C     ...CALC WASTE STREAM FLOW RATE, Q(14)...
0467        Q(14) = WSOL/((X(1,15)+X(2,15)+X(3,15))*.1D0)
0468        Q(15) = Q(13) - Q(14)
0469
0470        TM = TM + .1D0
0471
0472        RETURN
0473        END
```

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```
0474        SUBROUTINE RATES (Y1,T)
0475        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0476        DOUBLE PRECISION KM,KD,KS
0477        DOUBLE PRECISION KM1,KM2,KM3
0478        COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),X(3,15)
0479       *,SRT,DEGC,TS,TM,TR,TD
0480        COMMON /B3/ KOUNT,S1,S2,PTD,KNT,C1,C2,Q1,QE
0481        DIMENSION Y1(7)
0482  C
0483  C****************************************************************************
0484  C
0485  C      THIS SUBROUTINE UPDATES NITRIFIER KM'S AS FUNCTIONS OF
0486  C      SRT (DAYS) AND TKN AND COD LOADINGS EXPRESSED AS
0487  C      GRAMS OF SUBSTRATE REMOVED PER LITER OF REACTOR
0488  C      VOLUME PER DAY
0489  C      FOR DYNAMICALLY CHANGING INFLUENT CONDITIONS (KOUNT ) 0),
0490  C      SUBSTRATE LOADINGS ARE CALCULATED AS THE DIFFERENCE
0491  C      BETWEEN THE THEORETICAL CONC FOR A CONSERVATIVE
0492  C      MATERIAL AND THE ACTUAL SIMULATED AERATOR CONC
0493  C
0494  C****************************************************************************
0495  C
0496  C
0497        IF (KOUNT .LT. 1) GO TO 50
0498  C
0499  C     ...CALC SUBSTRATE CONC (C1,C2) FOR A CONSERVATIVE MATERIAL...
0500        TAU = V(1)/Q(1)
0501        C1 = S(1,1)*(1.D0-DEXP(-(T-PTD)/TAU))+S1*DEXP(-(T-PTD)/TAU)
0502        C2 = S(2,1)*(1.D0-DEXP(-(T-PTD)/TAU))+S2*DEXP(-(T-PTD)/TAU)
0503        QE = Q(1)*(1.D0-DEXP(-(T-PTD)/TAU))+Q1*DEXP(-(T-PTD)/TAU)
0504        GO TO 100
0505  C
0506   50   C1 = S(1,1)
0507        C2 = S(2,1)
0508        QE = Q(1)
0509  C
0510  C      (1) CALC CURRENT REACTOR SUBSTRATE LOADINGS
0511   100  COD = ((C1-Y1(1))*QE)/(V(1)*1000.D0)
0512        TKN = ((C2-Y1(2))*QE)/(V(1)*1000.D0)
0513  C     IF (T .GE. .48D0) WRITE (6,603) COD,TKN
0514  C
0515  C      (2) CALC NS AND NB KM'S
0516        KM1 = 5.6D0
0517        KM2 = (.28-.55*TKN+.003*SRT*COD)*24.D0
0518        KM3 = (.84-.42*COD)*24.D0
0519  C
0520  C      (3) ADJUST ALL KM'S FOR TEMPERATURE
0521        DEGK = DEGC + 273.D0
```

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```
0522        KM(1) = KM1*DEXP((21.325D0/DEGK)*(DEGK-295.D0))
0523        KM(2) = KM2*DEXP((25.420D0/DEGK)*(DEGK-295.D0))
0524        KM(3) = KM3*DEXP((28.968D0/DEGK)*(DEGK-295.D0))
0525  C
0526        TR = TR +.01D0
0527  C
0528        RETURN
0529        END
```

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** NO WARNINGS ** NO ERRORS ** PROGRAM = 00531 COMMON = 00000

```
0530        SUBROUTINE DYNAM (Y1,T)
0531        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0532        DOUBLE PRECISION KM,KD,KS
0533        COMMON /B4/ Q(15),S(4,15),KM(3),G(3),KD(3),V(15),KS(3),X(3,15)
0534       *,SRT,DEGC,TS,TM,TR,TD
0535        COMMON /B3/ KOUNT,S1,S2,PTD,KNT,C1,C2,Q1,QE
0536        DIMENSION Y1(7)
0537  C
0538  C
0539  C*********************************************************************
0540  C
0541  C      THIS SUBROUTINE CHANGES INFLUENT OR OPERATING CONDITIONS
0542  C      AT SPECIFIED VALUES OF T TO SIMULATE DYNAMIC OPERATION
0543  C      AFTER EACH SUBROUTINE CALL, THE TIME PERIOD BEFORE THE
0544  C      NEXT UPDATE IS SET
0545  C
0546  C*********************************************************************
0547  C
0548  C
0549        KOUNT = 1
0550  C
0551        IF(T .LE. 4.375D0) GO TO 2
0552        IF(T .GT. 4.375D0) KNA = 16
0553        S(2,1) = 15.3D0
0554        GO TO 100
0555    2   IF(T .LE. 4.125D0) GO TO 3
0556        IF(T .GT. 4.125D0) KNA = 15
0557        S(2,1) = 22.5D0
0558        GO TO 100
0559    3   IF(T .LE. 3.875D0) GO TO 4
0560        IF(T .GT. 3.875D0) KNA = 14
0561        S(2,1) = 9.0D0
0562        GO TO 100
0563    4   IF(T .LE. 3.5D0) GO TO 5
0564        IF(T .GT. 3.5D0) KNA = 13
0565        S(2,1) = 15.3D0
0566        GO TO 100
0567    5   IF(T .LE. 3.125D0) GO TO 6
0568        IF(T .GT. 3.125D0) KNA = 12
0569        S(2,1) = 21.3D0
0570        GO TO 100
0571    6   IF(T .LE. 2.875D0) GO TO 7
0572        IF(T .GT. 2.875D0) KNA = 11
0573        S(2,1) = 9.5D0
0574        GO TO 100
0575    7   IF(T .LE. 2.375D0) GO TO 8
0576        IF(T .GT. 2.375D0) KNA = 10
0577        S(2,1) = 15.1D0
```

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```
0578          GO TO 100
0579     8    IF(T .LE. 2.125D0) GO TO 9
0580          IF(T .GT. 2.125D0) KNA = 9
0581          S(2,1) = 19.5
0582          GO TO 100
0583     9    IF(T .LE. 1.875D0) GO TO 10
0584          IF(T .GT. 1.875D0) KNA - 8
0585          S(2,1) = 10.9D0
0586          GO TO 100
0587    10    IF(T .LE. 1.375D0) GO TO 11
0588          IF(T .GT. 1.375D0) KNA = 7
0589          S(2,1) = 15.3D0
0590          DEGC = .10D0
0591          GO TO 100
0592    11    IF(T .LE. 1.29D0) GO TO 12
0593          IF(T .GT. 1.29D0) KNA - 6
0594          DEGC = .7D0
0595          GO TO 100
0596    12    IF(T .LE. 1.125D0) GO TO 13
0597          IF(T .GT. 1.125D0) KNA = 5
0598          S(2,1) = 20.5D0
0599          GO TO 100
0600    13    IF(T .LE. 1.0D0) GO TO 14
0601          IF(T .GT. 1.0D0) KNA = 4
0602          DEGC = 10.0 D0
0603          GO TO 100
0604    14    IF(T .LE. 0.875D0) GO TO 15
0605          IF( T .GT. 0.875D0) KNA = 3
0606          S(2,1) = 10.0D0
0607          GO TO 100
0608    15    IF(T .LE. 0.5D0) GO TO 16
0609          IF(T .GT. 0.5D0) KNA = 2
0610          S(2,1) = 14.3D0
0611          GO TO 100
0612    16    S(2,1) = 19.6D0
0613          DEGC = 15.0D0
0614          KNA = 1
0615          GO TO 100
0616  C
0617   100    IF(KNA .NE. KNT) GO TO 200
0618          S1=C1
0619          S2=C2
0620          Q1=QE
0621          PTD=T
0622          KNT=KNT+1
0623   200    CONTINUE
0624          RETURN
0625          END
```

```
0626  C
0627  C
0628        SUBROUTINE HPCG(PRMT,Y,DERY,NDIM,IHLF,FCT,OUTP,AUX,X)
0629  C
0630  C
0631        IMPLICIT DOUBLE PRECISION (A-H,P-Z)
0632        COMMON /B1/ NOBS,OBS(8,201),DELT,H
0633        DIMENSION PRMT(1),Y(1),DERY(1),AUX(16,1)
0634        N=1
0635        IHLF=0
0636        X=PRMT(1)
0637        H=PRMT(3)
0638        PRMT(5)=0.
0639        DO 1 I=1,NDIM
0640        AUX(16,I)=0.
0641        AUX(15,I)=DERY(I)
0642      1 AUX(1,I)=Y(I)
0643        IF(H*(PRMT(2)-X))3,2,4
0644  C
0645  C     ERROR RETURNS
0646      2 IHLF=12
0647        GOTO 4
0648      3 IHLF=13
0649  C
0650  C     COMPUTATION OF DERY FOR STARTING VALUES
0651      4 CALL FCT(X,Y,DERY)
0652  C
0653  C     RECORDING OF STARTING VALUES
0654        CALL OUTP(X,Y,DERY,IHLF,NDIM,PRMT)
0655        IF(PRMT(5))6,5,6
0656      5 IF(IHLF)7,7,6
0657      6 RETURN
0658      7 DO 8 I=1,NDIM
0659      8 AUX(8,I)=DERY(I)
0660  C
0661  C     COMPUTATION OF AUX(2,I)
0662        ISW=1
0663        GOTO 100
0664  C
0665      9 X=X+H
0666        DO 10 I=1,NDIM
0667     10 AUX(2,I)=Y(I)
0668  C
0669  C     INCREMENT H IS TESTED BY MEANS OF BISECTION
0670     11 IHLF=IHLF+1
0671        X=X-H
0672        DO 12 I=1,NDIM
0673     12 AUX(4,I)=AUX(2,I)
```

```
0674        H=.5*H
0675        N=1
0676        ISW=2
0677        GOTO 100
0678  C
0679     13 X=X+H
0680        CALL FCT(X,Y,DERY)
0681        N=2
0682        DO 14 I=1,NDIM
0683        AUX(2,I)=Y(I)
0684     14 AUX(9,I)=DERY(I)
0685        ISW=3
0686        GOTO 100
0687  C
0688  C     COMPUTATION OF TEST VALUE DELT
0689     15 DELT=0.
0690        DO 16 I=1,NDIM
0691     16 DELT=DELT+AUX(15,I)*DABS(Y(I)-AUX(4,I))
0692        DELT=.06666667*DELT
0693        IF(DELT-PRMT(4))19,19,17
0694     17 IF(IHLF-10)11,18,18
0695  C
0696  C     NO SATISFACTORY ACCURACY AFTER 10 BISECTIONS. ERROR MESSAGE.
0697     18 IHLF=11
0698        X=X+H
0699        GOTO 4
0700  C
0701  C     THERE IS SATISFACTORY ACCURACY AFTER LESS THAN 11 BISECTIONS.
0702     19 X=X+H
0703        CALL FCT(X,Y,DERY)
0704        DO 20 I=1,NDIM
0705        AUX(3,I)=Y(I)
0706     20 AUX(10,I)=DERY(I)
0707        N=3
0708        ISW=4
0709        GOTO 100
0710  C
0711     21 N=1
0712        X=X+H
0713        CALL FCT(X,Y,DERY)
0714        X=PRMT(1)
0715        DO 22 I=1,NDIM
0716        AUX(11,I)=DERY(I)
0717    220Y(I)=AUX(1,I)+H*(.375*AUX(8,I)+.7916667*AUX(9,I)
0718       1-.2083333*AUX(10,I)+.04166667*DERY(I))
0719     23 X=X+H
0720        N=N+1
0721        CALL FCT(X,Y,DERY)
```

```
0722         CALL OUTP(X,Y,DERY,IHLF,NDIM,PRMT)
0723         IF(PRMT(5))6,24,6
0724      24 IF(N-4)25,200,200
0725      25 DO 26 I=1,NDIM
0726         AUX(N,I)=Y(I)
0727      26 AUX(N+7,I)=DERY(I)
0728         IF(N-3)27,29,200
0729   C
0730      27 DO 28 I=1,NDIM
0731         DELT=AUX(9,I)+AUX(9,I)
0732         DELT=DELT+DELT
0733      28 Y(I)=AUX(1,I)+.3333333*H*(AUX(8,I)+DELT+AUX(10,I))
0734         GOTO 23
0735   C
0736      29 DO 30 I=1,NDIM
0737         DELT=AUX(9,I)+AUX(10,I)
0738         DELT=DELT+DELT+DELT
0739      30 Y(I)=AUX(1,I)+.375*H*(AUX(8,I)+DELT+AUX(11,I))
0740         GOTO 23
0741   C
0742   C
0743   C     ******************************************************************* HPCG
0744   C     THE FOLLOWING PART OF SUBROUTINE HPCG COMPUTES BY MEANS OF
0745   C     RUNGE-KUTTA METHOD STARTING VALUES FOR THE NOT SELF-STARTING
0746   C     PREDICTOR-CORRECTOR METHOD.
0747     100 DO 101 I=1,NDIM
0748         Z=H*AUX(N+7,I)
0749         AUX(5,I)=Z
0750     101 Y(I)=AUX(N,I)+.4*Z
0751   C     Z IS AN AUXILIARY STORAGE LOCATION
0752   C
0753         Z=X+.4*H
0754         CALL FCT(Z,Y,DERY)
0755         DO 102 I=1,NDIM
0756         Z=H*DERY(I)
0757         AUX(6,I)=Z
0758     102 Y(I)=AUX(N,I)+.2969776*AUX(5,I)+.1587596*Z
0759   C
0760         Z=X+.4557372*H
0761         CALL FCT(Z,Y,DERY)
0762         DO 103 I=1,NDIM
0763         Z=H*DERY(I)
0764         AUX(7,I)=Z
0765     103 Y(I)=AUX(N,I)+.2181004*AUX(5,I)-3.050965*AUX(6,I)+3.832865*Z
0766   C
0767         Z=X+H
0768         CALL FCT(Z,Y,DERY)
0769         DO 104 I=1,NDIM
```

```
0770    1040Y(I)=AUX(N I)+.1747603*AUX(5.I)-.5514807*AUX(6,I)
0771       1+1.205536*AUX(7.I)+.1711848*H*DERY(I)
0772        GOTO(9.13.15,21),ISW
0773  C     ****************************************************************** HPCG
0774  C
0775  C
0776  C     POSSIBLE BREAK-POINT FOR LINKAGE
0777  C
0778  C
0779  C     STARTING VALUES ARE COMPUTED.
0780  C     NOW START HAMMINGS MODIFIED PREDICTOR-CORRECTOR METHOD
0781    200 ISTEP=3
0782    201 IF(N-8)204.202.204
0783  C
0784  C     N=8 CAUSES THE ROWS OF AUX TO CHANGE THEIR STORAGE LOCATIONS
0785    202 DO 203 N=2 7
0786        DO 203 I=1.NDIM
0787        AUX(N-1 I)=AUX(N,I)
0788    203 AUX(N+6.I)=AUX(N+7 I)
0789        N=7
0790  C
0791  C     N LESS THAN 8 CAUSES N+1 TO GET N
0792    204 N=N+1
0793  C
0794  C     COMPUTATION OF NEXT VECTOR Y
0795        DO 205 I=1.NDIM
0796        AUX(N-1.I)=Y(I)
0797    205 AUX(N+6.I)=DERY(I)
0798        X=X+H
0799    206 ISTEP=ISTEP+1
0800        DO 207 I=1.NDIM
0801       0DELT=AUX(N-4.I)+1.333333*H*(AUX(N+6.I)+AUX(N+6.I)-AUX(N+5.I)+
0802       1AUX(N+4.I)+AUX(N+4.I))
0803        Y(I)=DELT-.9256198*AUX(16.I)
0804    207 AUX(16.I)=DELT
0805  C     PREDICTOR IS NOW GENERATED IN ROW 16 OF AUX. MODIFIED PREDICTOR
0806  C     IS GENERATED IN Y. DELT MEANS AN AUXILIARY STORAGE.
0807  C
0808        CALL FCT(X.Y.DERY)
0809  C     DERIVATIVE OF MODIFIED PREDICTOR IS GENERATED IN DERY
0810  C
0811        DO 208 I=1.NDIM
0812       0DELT=.125*(9.*AUX(N-1.I)-AUX(N-3.I)+3.*H*(DERY(I)+AUX(N+6 I)+
0813       1AUX(N+6.I)-AUX(N+5.I)))
0814        AUX(16.I)=AUX(16.I)-DELT
0815    208 Y(I)=DELT+.07438017*AUX(16.I)
0816  C
0817  C     TEST WHETHER H MUST BE HALVED OR DOUBLED
```

```
0818        DELT=0.
0819        DO 209 I=1,NDIM
0820    209 DELT=DELT+AUX(15,I)*DABS(AUX(16,I))
0821        IF(DELT-PRMT(4))210,222,222
0822  C
0823  C     H MUST NOT BE HALVED. THAT MEANS Y(I) ARE GOOD.
0824    210 CALL FCT(X,Y,DERY)
0825        CALL OUTP(X,Y,DERY,IHLF,NDIM,PRMT)
0826        IF(PRMT(5))212,211,212
0827    211 IF(IHLF-11)213,212,212
0828    212 RETURN
0829    213 IF(H*(X-PRMT(2)))214,212,212
0830    214 IF(DABS(X-PRMT(2))-.1*DABS(H))212,215,215
0831    215 IF(DELT-.02*PRMT(4))216,216,201
0832  C
0833  C
0834  C     H COULD BE DOUBLED IF ALL NECESSARY PRELEEDING VALUES ARE
0835  C     AVAILABLE
0836    216 IF(IHLF)201,201,217
0837    217 IF(N-7)201,218,218
0838    218 IF(ISTEP-4)201,219,219
0839    219 IMOD=ISTEP/2
0840        IF(ISTEP-IMOD-IMOD)201,220,201
0841    220 H=H+H
0842        IHLF=IHLF-1
0843        ISTEP=0
0844        DO 221 I=1,NDIM
0845        AUX(N-1,I)=AUX(N-2,I)
0846        AUX(N-2,I)=AUX(N-4,I)
0847        AUX(N-3,I)=AUX(N-6,I)
0848        AUX(N+6,I)=AUX(N+5,I)
0849        AUX(N+5,I)=AUX(N+3,I)
0850        AUX(N+4,I)=AUX(N+1,I)
0851        DELT=AUX(N+6,I)+AUX(N+5,I)
0852        DELT=DELT+DELT+DELT
0853    2210AUX(16,I)=8.962963*(Y(I)-AUX(N-3,I))-3.361111*H*(DERY(I)+DELT
0854       1+AUX(N+4,I))
0855        GOTO 201
0856  C
0857  C
0858  C     H MUST BE HALVED
0859    222 IHLF=IHLF+1
0860        IF(IHLF-10)223,223,210
0861    223 H=.5*H
0862        ISTEP=0
0863        DO 224 I=1,NDIM
0864       0Y(I)=.00390625*(80.*AUX(N-1,I)+135.*AUX(N-2,I)+40.*AUX(N-3,I)+
0865       1AUX(N-4,I))-.1171875*(AUX(N+6,I)-6.*AUX(N+5,I)-AUX(N+4,I))*H
```

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```
0866      0AUX(N-4,I)=.00390625*(12.*AUX(N-1,I)+135.*AUX(N-2,I)+
0867      1108.*AUX(N-3,I)+AUX(N-4,I))-.0234375*(AUX(N+6,I)+18.*AUX(N+5,I)-
0868      29.*AUX(N+4,I))*H
0869       AUX(N-3,I)=AUX(N-2,I)
0870   224 AUX(N+4,I)=AUX(N+5,I)
0871       X=X-H
0872       DELT=X-(H+H)
0873       CALL FCT(DELT,Y,DERY)
0874       DO 225 I=1,NDIM
0875       AUX(N-2,I)=Y(I)
0876       AUX(N+5,I)=DERY(I)
0877   225 Y(I)=AUX(N-4,I)
0878       DELT=DELT-(H+H)
0879       CALL FCT(DELT,Y,DERY)
0880       DO 226 I=1,NDIM
0881       DELT=AUX(N+5,I)+AUX(N+4,I)
0882       DELT=DELT+DELT+DELT
0883      0AUX(16,I)=8.962963*(AUX(N-1,I)-Y(I))-3.361111*H*(AUX(N+6,I)+DELT
0884      1+DERY(I))
0885   226 AUX(N+3,I)=DERY(I)
0886       GOTO 206
0887       END
```

FTN4 COMPILER: HP92060-16092 REV. 2001 (791101)

** NO WARNINGS ** NO ERRORS ** PROGRAM = 03012 COMMON = 00000

APPENDIX E

List of Abbreviations

A	-Arrhenius frequency factor ($time^{-1}$)
ANOVA	-Analysis of variance
ATP	-Adenosine triphosphate
BOD_5	-Five day biochemical oxygen demand ($mg \cdot L^{-1}$)
C:N	-Carbon to nitrogen ratio
COD	-Chemical oxygen demand ($mg \cdot L^{-1}$)
E	-Arrhenius activation energy ($cal \cdot g\text{-}mole^{-1}$)
F	-F statistic
FA	-Fluorescent antibody
HRT	-Hydraulic residence time (time)
k	-Specific substrate removal rate ($time^{-1}$)
k_D	-Endogenous decay coefficient ($time^{-1}$)
k'_{max}	-Maximum specific substrate removal rate ($time^{-1}$)
K	-Reaction rate coefficient ($time^{-1}$)
K_s	-Half saturation coefficient for substrate removal ($mg \cdot L^{-1}$)
MLSS	-Mixed liquor suspended solids ($mg \cdot L^{-1}$)
MLVSS	-Mixed liquor volatile suspended solids ($mg \cdot L^{-1}$)
MPN	-Most probable number

MSLOF	-Mean square due to lack of fit
MSPE	-Mean squares due to pure error
NH_4-N	-Ammonium nitrogen
NO_2-N	-Nitrite nitrogen
NO_3-N	-Nitrate nitrogen
NO_x-N	-Oxidized inorganic nitrogen
$\hat{q}$	-Specific nitrification rate ($time^{-1}$)
r_i	-Rate of change of nitrogen concentration ($mg \cdot L^{-1} \cdot time^{-1}$)
R	-Universal gas constant ($cal \cdot g\text{-}mole^{-1} \cdot {}^{o}K^{-1}$)
S	-Substrate concentration ($mg \cdot L^{-1}$)
SRT	-Solids retention time (time)
SRT_c	-Critical solids retention time (time)
T	-Temperature (degrees)
TIC	-Total inorganic carbon
TKN	-Total Kjeldahl nitrogen
TOC	-Total organic carbon
TSS	-Total suspended solids
VSS	-Volatile suspended solids
X	-Microorganism concentration ($mg \cdot L^{-1}$)
X_a	-Added nitrifier concentration ($mg \cdot L^{-1}$)
Y	-Biomass yield coefficient ($mg \cdot mg^{-1}$)
μ	-Specific microorganism growth rate ($time^{-1}$)
μ_{max}	-Maximum specific microorganism growth rate ($time^{-1}$)
τ	-Time parameter (time)