A STUDY OF NITRIFICATION KINETICS

by

Hraj Armen Khararjian

Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Sanitary Engineering

APPROVED:

Ernest M. Jennelle, Chairman

Paul H. King

N. Thomas Stephens

February, 1973

Blacksburg, Virginia

ACKNOWLEDGEMENTS

The author wishes to thank Dr. R. D. Walker for his great assistance in clearing the barriers in applying to the Graduate School.

The author also wishes to thank Dr. E. M. Jennelle for his guidance, constructive criticism and friendship during the preceding year.

The author appreciates the cooperation and assistance of Dr. P. H. King and Dr. N. T. Stephens.

To , for his invaluable aid in the laboratory, and for her typing the thesis, thanks are also extended.

And finally, thanks to my parents who have encouraged me to continue my studies and supported me in time of need.

TABLE OF CONTENTS

	Page
LIST OF FIGURES	iv
LIST OF TABLES	v
INTRODUCTION	1
LITERATURE REVIEW	4
MATERIALS AND METHODS	19
RESULTS	25
DISCUSSION	45
SUMMARY AND CONCLUSIONS	56
REFERENCES CITED	57
VITA	59

LIST OF FIGURES

Figure		Page
1.	Chemistry of nine valence states of nitrogen	5
2.	Activated sludge unit	10
3.	Continuous aeration unit	20
4.	Oxygen uptake variation with temperature	26
5.	Oxygen uptake variation with temperature	27
6.	Variation of oxidized nitrogen and oxygen uptake vs time	30
7.	Variation of oxidized nitrogen and oxygen uptake vs time	31
8.	Variation of oxygen uptake with pH under quiescent conditions	32
9.	Oxygen uptake variation with pH under mixing conditions	33
10.	Oxygen uptake variation with pH 6 and 10 under quiescent conditions	34
11.	Oxygen uptake variation with pH 6 and 10 under quiescent conditions	35
12.	Oxygen uptake variation with pH 6 and 10 under mixing conditions	36
13.	Oxygen uptake variation with pH 6 and 10 under mixing conditions	37
14.	Variation of suspended solids and mixed liquor suspended solids with time for the autotrophic unit	41
15.	Variation of suspended solids and mixed liquor suspended solids with time for the autotrophic unit	42
16.	Variation of growth rate constant with temperature	47
17.	Variation of sludge age with temperature	52
18.	Variation of MLSS with organic load at constant sludge ages for 6 hours retention time	54

LIST OF TABLES

Table		Page
I.	Values For Maximum Growth Rate Constant (k) and Saturation Constant (K)	12
II.	Oxygen Uptake For Selected Initial Ammonium Concentration at 20°C and pH 8.3	28
III.	Growth Rate Constants For pH 6, 7, 8, 9 and 10 Without and With Mixing at 20°C	39
IV.	Initial and Final Nitrate Plus Nitrite Nitrogen	40
V.	Averaged Value of MLSS, NH_4-H, NO_3-N COD in The Heterotrophic Unit	43

INTRODUCTION

Most water pollution research reported in recent years regarding nitrogen has dealt with the role of the element in limiting primary production in estuaries and impoundments. However, it is a well established axiom that organic and ammonia nitrogen represents an oxygen liability, and in many instances these forms may account for a significant portion of the total oxygen demand (TOD) of surface waters. For this and other reasons there has been an increased tendency during the last few years for regulatory agencies to impose restrictions on the nitrogenous oxygen demand (NOD) of wastewater effluents. These restrictions require that the wastewater treatment processes be designed and operated in a manner that will assure well nitrified effluents.

The genera of bacteria that are involved in the conversion of ammonia nitrogen (NH_3-N) to nitrate nitrogen (NO_3^--N) have been known for many years and their activity in soil has been of considerable interest to the agronomist in regard to soil fertility. However, few data are available regarding growth parameters for nitrifying bacteria in aquatic systems. Nitrification in wastewater treatment systems has been based almost entirely on empirical methods. This approach was adequate in former years when nitrification was considered as being a phenomena which might occasionally be achieved in wastewater treatment. However today nitrogen transformation is being required in many instances as an integral part of wastewater treatment. This transformation may involve nitrification, or

nitrogen removal through biological, chemical and/or physical processes.

Some environmental engineers feel that biological nitrification is not a reliable process. Work by Mulbarger (1) has indicated that nitrification functions best as a tertiary process following conventional secondary biological treatment for removal of carbonaceous material. Because of the temperature dependence of the nitrifying bacteria, nitrification is required in some instances only during the summer months.

Much of the current confusion regarding nitrification results from a lack of knowledge of the growth parameters related to the biological process. The time has arrived in the evolution of wastewater treatment theory whereby it is necessary that treatment plants be designed and operated on the basis of sound biological relationships. This will require a better understanding of the growth kinetics and yield associated with nitrification.

Growth relationships for heterotrophic bacterial populations were established by Monod (2) in 1949. McKinney (3) et al. have done much to relate the operation of the activated sludge process to established biological principles. Lawrence and McCarty (4) have proposed an unified approach for use in the design and operation of the activated sludge process. When currently available technology is transferred to the design and operating level substantial improvement will be achieved in the removal of carbonaceous material. Similar data are needed on the growth parameters related to the autotrophic nitrifying bacteria. Procedures are needed upon which

sound design and operational practices can be established for NOD control.

The purpose of this investigation was to study the growth relationships in a biological system employing ammonia nitrogen as a substrate. Three bench scale systems were employed in the study; one autotrophic system with only ammonia as substrate, and two mixed heterotrophic-autotrophic systems employing both glucose and ammonia in selected ratios as substrate. The autotrophic system was used as a source of seed for specific experiments.

LITERATURE REVIEW

Nitrogen is probably the most interesting of the chemical elements. This is due to the fact that nitrogen can assume several valance states, and because compounds containing nitrogen play such an important role in both inorganic and organic substances.

Nitrogen, having five valance electrons, forms bonds that are covalent in character. The N-N bond is one of the strongest chemical bonds. This characteristic is responsible for the inertness of nitrogen gas. A relatively large amount of energy is required to cause molecular nitrogen to react with other elements. The resulting compounds have less strongly bound nitrogen. Once elemental nitrogen is fixed (the incorporation of molecular nitrogen into organic compounds) it is readily oxidized and reduced biologically. Nitrogen can exist in nine valance states as shown in Figure (1). In nature the +3, -3, +5, and 0 valance state are most common.

The atmosphere is almost 80 percent nitrogen and serves as a universal reservoir of nitrogen. Through electrical discharge associated with thunder storms, nitrogen combines with oxygen to form nitrate nitrogen (NO_3-N). Rainwater carries the nitrate nitrogen to soil where it serves to nourish plant life resulting in the production of plant protein and other cellular components. Herbivors consume some of the plants and convert plant protein to animal protein. Through respiration and degradation of animal and plant material, ammonia is released. The oxidation of ammonia is termed nitrification. The oxidation reactions are energy yielding and

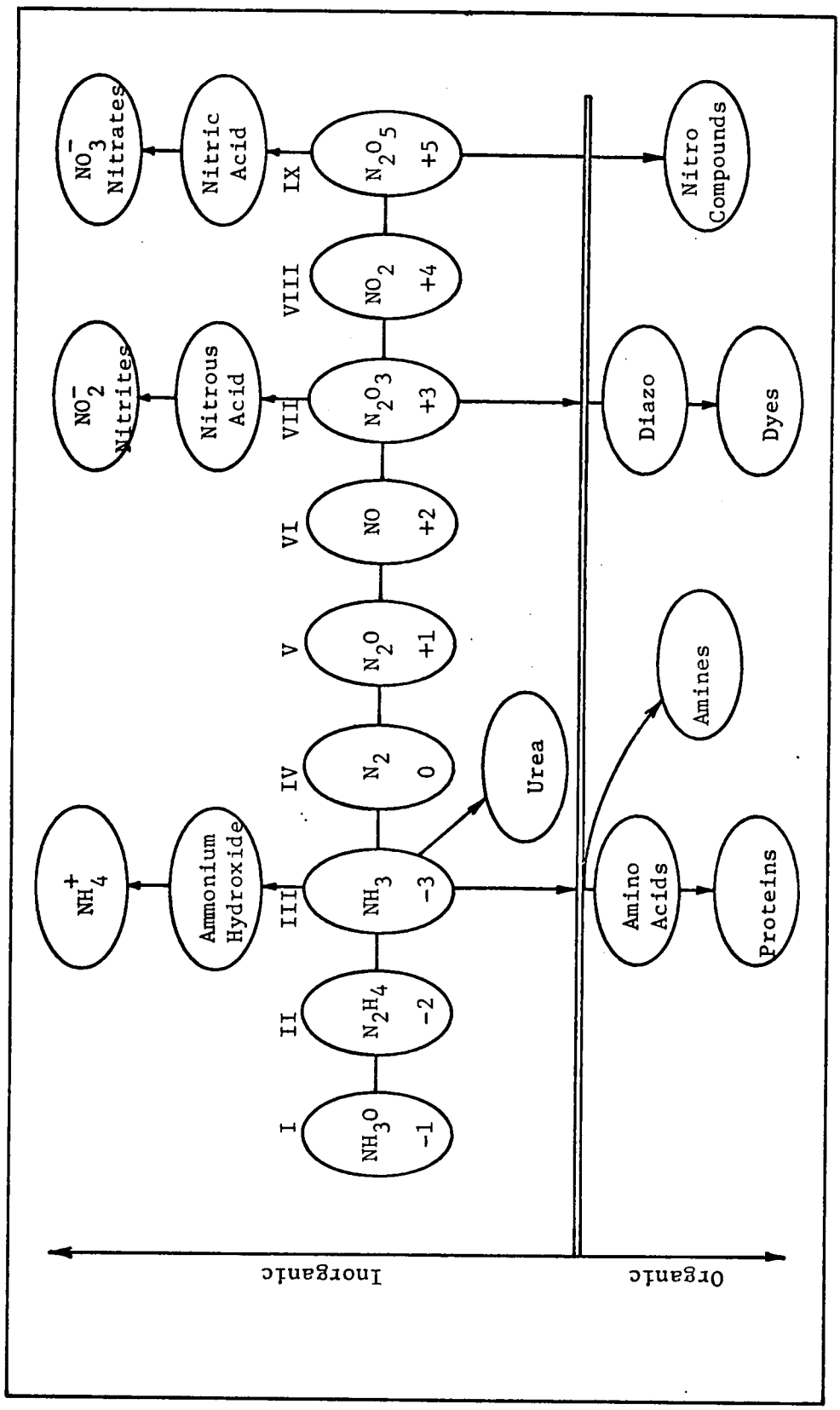

Figure 1. Chemistry of nine valence states of nitrogen.

serve as the energy source for nitrifying bacteria.

Nitrification Process

The autotrophic process of oxidizing ammonia nitrogen to nitrate nitrogen is accomplished in nature mainly by two genera of autotrophic bacteria. Nitrosomonas sp. converts ammonia nitrogen (NH_3-N) to nitrite nitrogen (NO_2^--N). The biological reaction may be shown by the following stoichiometric relationship

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

The intermediates of the biological oxidation pathway have not yet been clearly established. It has been proposed that the reaction proceeds from ammonia to nitrite with hydroxylamine (NH_2OH) and hyponitrite as steps in the pathway: a transformation involving the stepwise formation of -1, +1, +3 oxidation states of nitrogen. Engel and Alexander (5) have concluded that the rapid oxidation of hydroxylamine (there is an absence of a lag period in the oxidation of NH_2OH by ammonium grown cultures) and the stoichiometric conversion to nitrite, is part of the biological pathway in the nitrification process. The oxidation of one gram of nitrogen from ammonia to nitrite releases about 4700 calories of free energy. This energy is used by the bacteria in the process of assimilation of carbon from CO_2 into cellular constituents. The reduction of one gram of carbon from CO_2 to the oxidation level found in cellular constituents requires approximately 10,000 calories of free energy. Thus the cell yield associated with nitrification is not great.

The oxidation of nitrite nitrogen (NO_2^--N) to nitrate nitrogen (NO_3^--N) by Nitrobacter sp. may be shown as

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Lees and Simpson (6) in their investigation of the mechanism of oxidation of nitrite to nitrate have suggested the following steps as the intermediate reactions:

1. $Fe^{+3}_{cyt\ 551} + NO_2^- \rightarrow Fe^{+2}_{cyt\ 551} + NO_2$

2. $Fe^{+2}_{cyt\ 551} + NO_2 + 1/2\ O_2 \rightarrow Fe^{+3}_{cyt\ 551} + NO_3^-$

3. $Fe^{+2}_{cyt\ 551} + NO_2 + ClO_3^- \rightarrow Fe^{+3}_{cyt\ 551} + NO_3^- + ClO_2^-$

4. $Fe^{+3}_{cyt\ 551} + ClO_2^- \rightarrow$ inactivated cytochrome 551

Nitrites act as an electron donor where electrons flow along the cytochrome respiratory chain to oxygen. This is coupled with oxidative phosphorylation in the electron transport, which yields free energy that is used for cell functions.

Kinetics of Nitrification

Downing et al. (7) from a study of the kinetics of nitrification in the activated sludge process proposed that general kinetic equations of the type developed by Monod (2) could be used to define the growth of nitrifying bacteria. Thus the growth rate of Nitrosomonas sp. in the oxidation of ammonia to nitrite can be given in a form similar to the Monod equation as follows:

$$dX_m/dt = \frac{k_m X_m S_1}{K_m + S_1} \qquad \text{----} \qquad (1)$$

where

dX_m/dt = the rate of change in concentration of *Nitrosomonas sp.*

k_m = Maximum growth rate constant as defined by Monod, day^{-1}

K_m = Concentration of substrate at which the growth rate constant is half the maximum value, mg./l

S_1 = Substrate concentration (NH_3-N) in solution at time t, mg./l

The rate of change in ammonia concentration in relation to the change in cell mass is given by:

$$\frac{dS_1}{dt} = \frac{-dX_m}{dt} \Big/ Y_m \qquad \text{----- (2)}$$

where Y_m is the mg of cell yield per mg of ammonia nitrogen oxidized to nitrite.

Nitrification is a consecutive reaction where the product of the first reaction ($NH_3 \rightarrow NO_2^-$) becomes the reactant for the following reaction ($NO_2^- \rightarrow NO_3^-$). In mixed cultures of *Nitrosomonas sp.* and *Nitrobacter sp.* the nitrite required by *Nitrobacter sp.* is supplied by the action of *Nitrosomonas sp.* If the sequence of reactions is allowed to proceed, as ammonia decays, nitrite is formed and quickly oxidized to nitrates. *Nitrosomonas sp.* controls the overall reaction rate in oxidizing ammonia to nitrite. The equation for the rate of growth of *Nitrobacter sp.* can be given as follows (7):

$$dX_b/dt = k_b X_b S_2/(K_b + S_2) \quad \text{-----} \quad (3)$$

$$\text{where } S_2 = S_{2,0} + f_m(X_m - X_{m,0})/Y_m - (X_b - X_{b,0})/Y_b \quad \text{---} \quad (4)$$

in which b, 2, 0 indicate Nitrobacter sp., nitrite nitrogen and initial value respectively. f_m is the ratio of the mass of nitrite formed per unit of ammonia removed. This ratio is theoretically unity and should approach this value in actual conditions.

The rate of change in nitrite concentration is given by:

$$dS_2/dt = f_m/Y_m(dX_m/dt) - 1/Y_b(dX_b/dt) \quad \text{---} \quad (5)$$

In activated sludge plants as given in Figure (2) nitrification is a function of cell mass as shown by the above kinetic models. In the operation of wastewater treatment plants, the cell mass present depends not only on growth but also on the rate of cell loss through sludge wastage and loss in the effluent. Downing et al. (7) have shown that in order to achieve a significant amount of nitrification in the activated sludge plant at steady state, the following condition must be satisfied

$$(X_m - X_{mo})/X_{mo} \geq \Delta C/C \quad \text{------} \quad (6)$$

where C is the concentration of mixed liquor suspended solids (MLSS) at the inlet end of the aeration unit and ΔC is the increase in MLSS during aeration. The change in mass of Nitrosomonas sp. is also given by:

$$X_m - X_{mo} = Y_m \Delta S_1 \quad \text{------} \quad (7)$$

and a mass balance of ammonia in an activated sludge plant as

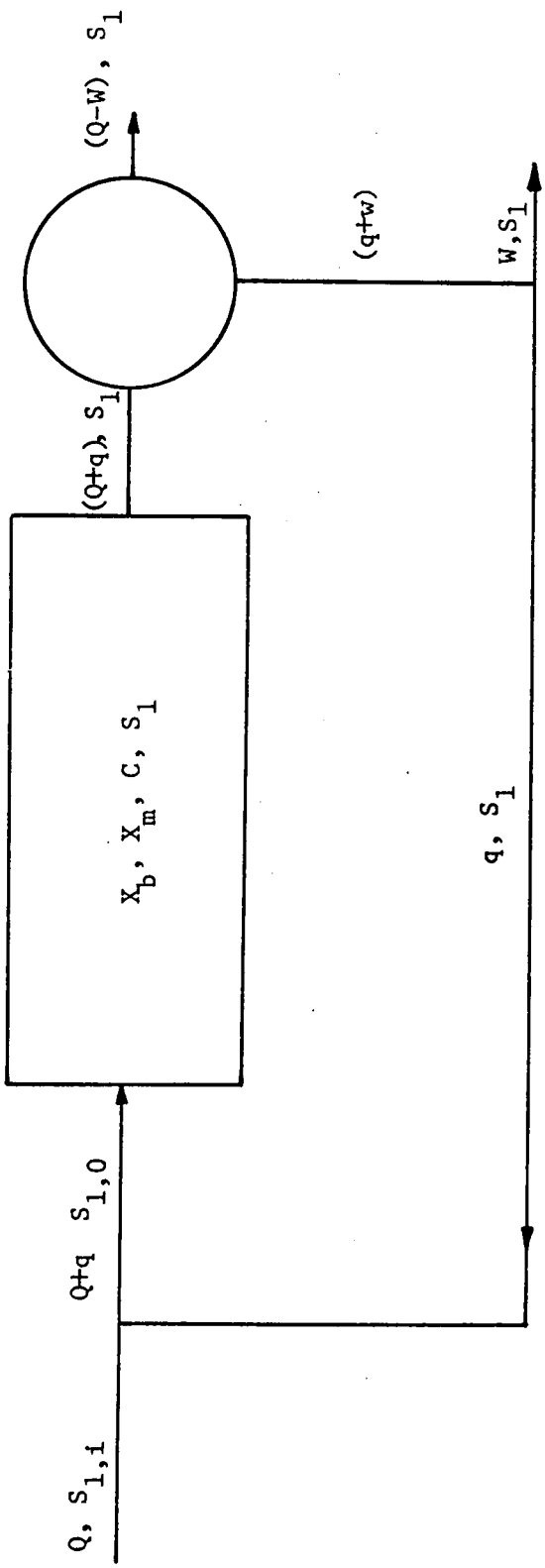

Figure 2. Activated sludge unit.

shown in Figure (2) is given by

$$(1 + p)\ S_{1,0} = S_{1,i} + pS_1 \quad \text{----- (8)}$$

where $S_{1,i}$ is the concentration of ammonia in the sewage and p is the rate of flow of returned sludge divided by rate of flow of settled sewage. Downing et al. (7) showed that by combining equations (6), (7), and (8) the concentration of ammonia in the effluent of activated sludge unit in terms of ΔC is given by:

$$k_m t = \text{Ln}\left(1+\frac{\Delta C}{C}\right) + \frac{K_m \Delta C(1+p)}{S_{1,i}\ (\Delta C+C)+S_1(p\Delta C-C)}\ \frac{\text{Ln}(L+\Delta C/C)\ (S_{1,i}+pS_1)}{(1+p)\ S_1} \quad \text{-- (9)}$$

To solve this equation, it is best to assume a value for S_1 and then determine time (t) by successive approximation.

In this study nitrification rates were monitored by measuring oxygen utilization. This was done because of the difficulty in obtaining instantaneous measurements of the various forms of nitrogen as nitrification progressed. Others have shown that oxygen uptake measurements provide a sensitive mean of obtaining data for aerobic biological activity (18).

Little data are available in the literature relative to the reaction kinetics of the nitrification process. Most of the data reported have been for pure culture experiments. Table (1) shows values of the growth rate constant (k_m) and saturation constant (K_m) as reported by different authors.

There are different opinions expressed in the literature on the effect of ammonium concentration on growth kinetics. On the basis of assuming that biological growth rate for nitrification is

TABLE I

VALUES FOR MAXIMUM GROWTH RATE CONSTANT (k) AND SATURATION CONSTANT (K)

Medium	Temperature	pH	k, Specific Growth Rate Constant day^{-1} Nitrosomonas sp.	K, Saturation Constant mg/l as N Nitrosomonas sp.	Source
Soil	30	--	0.37	---	Duisbag and Puch (16)
Solution	30	--	1.5	---	Engel and Alexander (5)
	29	--	2.2	---	Skinner and Walker (10)
Activated Sludge and Domestic Sludge	20	7.5 - 8.0	0.33	1.0	Downing et al. (7)
			Nitrobacter sp.	Nitrobacter sp.	
Activated Sludge and Domestic Sewage		7.5 - 8.0	0.14	2.1	Downing et al. (7)
Solution		--	1.39	---	Boon and

defined by Monod kinetics (2) as proposed by Downing et al. (7), the growth rate constant will be defined by the initial concentration of ammonia. Buswell et al. (8) working with low concentrations of ammonia, 1-10 mg/l ammonia nitrogen, found that the growth rate constant of Nitrosomonas sp. increased as ammonia concentration increased from 1 mg/l NH_3-N. The growth rate reached a maximum at 5 mg/l NH_3-N then decreased to the value observed for 1 mg/l at 10 mg/l NH_3-N. They reported the same phenomenon for different temperatures. Wild et al. (9) reported that the growth rate was not affected by the initial concentration of ammonia nitrogen, but that concentrations above 60 mg/l NH_3-N was inhibitory.

Yield is defined as the weight of cells formed per weight of nitrogen oxidized. It appears that cell yield is not constant but varies with growth conditions. It is well established in sewage treatment that the low cell yield of the nitrifying organisms relative to that of the heterotrophic fraction of the cell mass can lead to washout of the former if proper operation procedures are not followed. Skimmer and Walker (10) using a synthetic medium consisting of ammonia and phosphate buffer reported a yield of 0.06 mg. of cell produced per mg of ammonia nitrogen oxidized for Nitrosomonas sp. Downing et al. (7) working with activated sludge reported a yield value of 0.05 mg. of cell produced per mg of ammonia nitrogen oxidized. Lees and Simpson (6), Boon and Laudelout (11) using a synthetic medium reported a yield value of 0.02 mg of cell produced per mg of nitrite nitrogen oxidized for Nitrobacter sp.

Downing et al. (7) also reported the same value for activated sludge.

Factors Affecting the Growth of Nitrifying Bacteria

A lot of difficulties have been encountered in wastewater treatment plants employing the nitrification process. Although the growth kinetics for Nitrosomonas sp. and Nitrobacter sp. have been reported. The studies were based on pure culture and have not been adopted to plant operation. In 1963 Downing et al. (7) studied the nitrification process in an activated sludge plant in England, but because the lack of a suitable operating criteria the process is not considered reliable at this time.

Some Factors Affecting the Nitrification Process

1. Temperature. It has been observed at many wastewater treatment plants that nitrification may be achieved during the summer months but not during the remainder of the year. Downing et al. (7) reported that optimum temperature for nitrification was 30°C. They also reported that the growth rate doubled for every 10°C increase in temperature (in accordance with the Van't Hoff Rule) in the range of 6 to 28°C. Wild et al. (9) also reported the same optimum temperature and noted that up to five times the detention time may be needed to accomplish complete nitrification in the colder season to that needed during the summer months. They also concluded that the rate of nitrification increased through the range of 5 to 30°C in reasonable agreement with the Van't Hoff – Arhenius law. They proposed increasing the MLSS to overcome the temperature effects. Beckman

et al. (12) reported a much lower optimum temperature for nitrification. In their study at a combined carbon oxidation-nitrification activated sludge plant, they found an optimum nitrification temperature of 18.3°C and also reported 95 percent ammonia nitrogen removal at 20°C. Ammonia removal decreased to an average of 50 percent at a temperature of 7°C. This is not in agreement with other studies (7) (9) which indicated a relative nitrification rate of 100 percent at 30°C and only about 20 percent at 7°C.

2. pH. Maximum nitrification rates have been reported to occur in slightly alkaline mediums, Hofman and Lees (13) found that Nitrosomonas sp. activity was optimum at pH 8-9, while about 50 percent of the maximum activity was obtained at pH 7. Angel and Alexander (5) obtained a somewhat wider plateau of between pH 7 and 9. Boon and Laudelout (11) reported an optimum pH of 7.8 for Nitrobacter sp. and 90 percent of the maximum activity at both pH 7 and 8.6. The above studies were made using pure culture. Wild et al. (9) reported an optimum pH of 8.4 and that 90 percent of the maximum rate occurred in the range of pH 7.8 to 8.9, while 50 percent in the outside range of pH 7 and 9.8. Beckman et al. (12) reported no significant influence on the rate of ammonia removal in the range of pH 7-8. One of the characteristics of the nitrification process is that it depresses the pH. This is due to the production of hydrogen ions as shown in the following equation:

Nitrosomonas sp.

$$55\ NH_4^+ + 5CO_2 + 76O_2 \rightarrow C_5H_7O_2N + 54NO_2^- + 52H_2O + 109H^+$$

Nitrobacter sp.

$$400NO_2^- + 5CO_2 + NH_4^+ + 19SO_2 + 2H_2O \rightarrow C_5H_3O_2N + 400NO_3^- + H^+$$

If free CO_2 is not available, the nitrifying bacteria have the capability of using bicarbonate ions as a carbon source (14). The stoichiometric equation for this reaction can be written as:

$$2NH_3 + 4O_2 + Ca(HCO_3)_2 \rightarrow Ca(NO_3)_2\ 2CO_2 + 4H_2O$$

From this stoichiometric equation each gram of NO_3-N produced will destroy 3.6 grams of alkalinity as $CaCO_3$. This will lead to the destruction of the buffering capacity of the medium and hence depresses the pH.

3. Biochemical Oxygen Demand (BOD). Available data show that nitrification is maximum in plants treating wastewater having a low BOD:nitrogen ratio. Downing et al. (7) concluded that it is not the carbon:Nitrogen (i.e., BOD:nitrogen) ratio, but rather the increase in heterotrophic sludge production with increases in the BOD:nitrogen ratio of the sewage that is important. As the ratio is increased there will be more wasting of sludge due to greater sludge production. This will lead to washing out the nitrifiers and hence a decrease in nitrification. Wild et al. (9) reported that an instantaneous increase or decrease in BOD concentration from 50 to 110 mg/1 or 50 to 5 mg/1 did not effect the nitrification rate. But it would be expected that a change in the average BOD concentration of the feed would effect the percentage of nitrifiers in the MLVSS and as a result would effect the time necessary to achieve complete nitrification. Johnson and

Schroepfer (15) studied the effect of applied BOD load on the nitrification process. They reported that a load factor of 0.25 to 0.35 mg. of BOD applied per day per mg. of MLSS or less gave a highly nitrified effluent. They concluded that at load factors greater than these limits, little or no nitrification was to be expected. Beckman et al. (12) in a study of a combined carbon oxidation-nitrification process, reported 90-95 percent nitrification with an influent wastewater having a BOD of 250 mg./l They concluded that to achieve nitrification with a mixed liquor temperature of 10-18.3°C, a food to microorganism ratio (F:M) of 0.25 or less was optimum. (F:M ratio is defined as the weight ratio of BOD added to the system per day to the weight of suspended solids in the activated sludge process).

4. Dissolved oxygen (DO). As shown in the following stoichiometric equation for nitrification,

$$2NH_3 + 4O_2 \rightarrow 2HNO_3 + 2H_2O$$

the oxidation of one gram of ammonia nitrogen requires 4.57 grams of O_2. To obtain appreciable nitrification in the activated sludge process the DO concentration maintained in the mixed liquor appears to be a very important parameter. It is generally accepted that the rate of nitrification increases with increasing concentration of DO up to a certain level, above which the rate becomes independent of DO. Downing et al. (7) have reported that complete nitrification was achieved at 0.5 ppm DO while only 50 percent was achieved at 0.3 ppm. Beckman et al. (12) reported that plant-scale studies showed nitrification will be complete at DO levels of 1.6 ppm. They also

concluded that the maintenance of a DO concentration of 2.0 ppm in the aeration tank effluent prevents denitrification from occurring in the final settling tanks, provided that an excessive sludge blanket was not permitted to form.

5. Aeration Period and Biological Retention Time. In order to insure that nitrification occurs at a high efficiency. Downing et al. (7) have reported that there is a minimum period of aeration required to achieve nitrification. This was reported to be roughly equal to the fractional increase in the concentration of activated sludge solids during aeration divided by the growth rate constant of Nitrosomonas sp. in the mixed liquor. They also reported that the minimum period of aeration will also be roughly proportional to the 5 day BOD of the sewage applied and will decrease roughly in inverse proportion to the concentration of sludge in the aeration units. This conclusion has also been reported by Johnson and Schroepfer (15). From this it is obvious that there is a minimum sludge age (θ_c^m) that is required to achieve nitrification. If this minimum sludge age is not maintained in the aeration unit the nitrifiers are washed out of the system and consequently nitrification decreases or ceases.

While there has been considerable interest in nitrification in wastewater treatment during the past few years, there is very little definitive data available regarding the design and operation of treatment facilities in a manner that will assure nitrification. For this reason federal and state regulatory agencies do not in general require that nitrification be accomplished in wastewater treatment.

MATERIALS AND METHODS

A major part of this study involved the establishment and maintenance of a nitrifying bacterial population. This was accomplished with an 11 liter chemostat having a design as shown in Figure 3. A variable speed Sigmamotor finger pump was used to give a feed rate of 5 milliliters per minute, giving a retention time of 12 hours. The chemostat was covered to exclude light and thus prevent the growth of algae. Trickling filter effluent was used as seed at start-up and a synthetic medium containing the following constituents (19) was used as feed throughout the study:

Compounds	conc. mg/l
Ammonium Sulfate	2,000
Dihydrogen Potassium Phosphate	250
Dipotassium Hydrogen Phosphate	750
Ferrous Sulfate	10
Manganese Sulfate	10
Magnesium Sulfate	30
Calcium Chloride	20

The above solution contains 424.2 mg/l NH_3-N. All nitrogen concentrations herein are expressed as nitrogen. Because domestic sewage generally approximates 25 mg/l NH_3-N(15), the feed was diluted to be within this value. A dilution of 1:16 was used to give an NH_3-N concentration of 26.5 mg/l in the feed solution. The feed solution was prepared daily. The main carbon source was CO_2 and bicarbonate. A stock solution containing 64 gr/l of sodium bicarbonate was prepared. Five ml. of this stock bicarbonate solution was added to every liter of the synthetic feed prepared. Anhydrous sodium carbonate was also added at a concentration of 50 mg/l to raise the

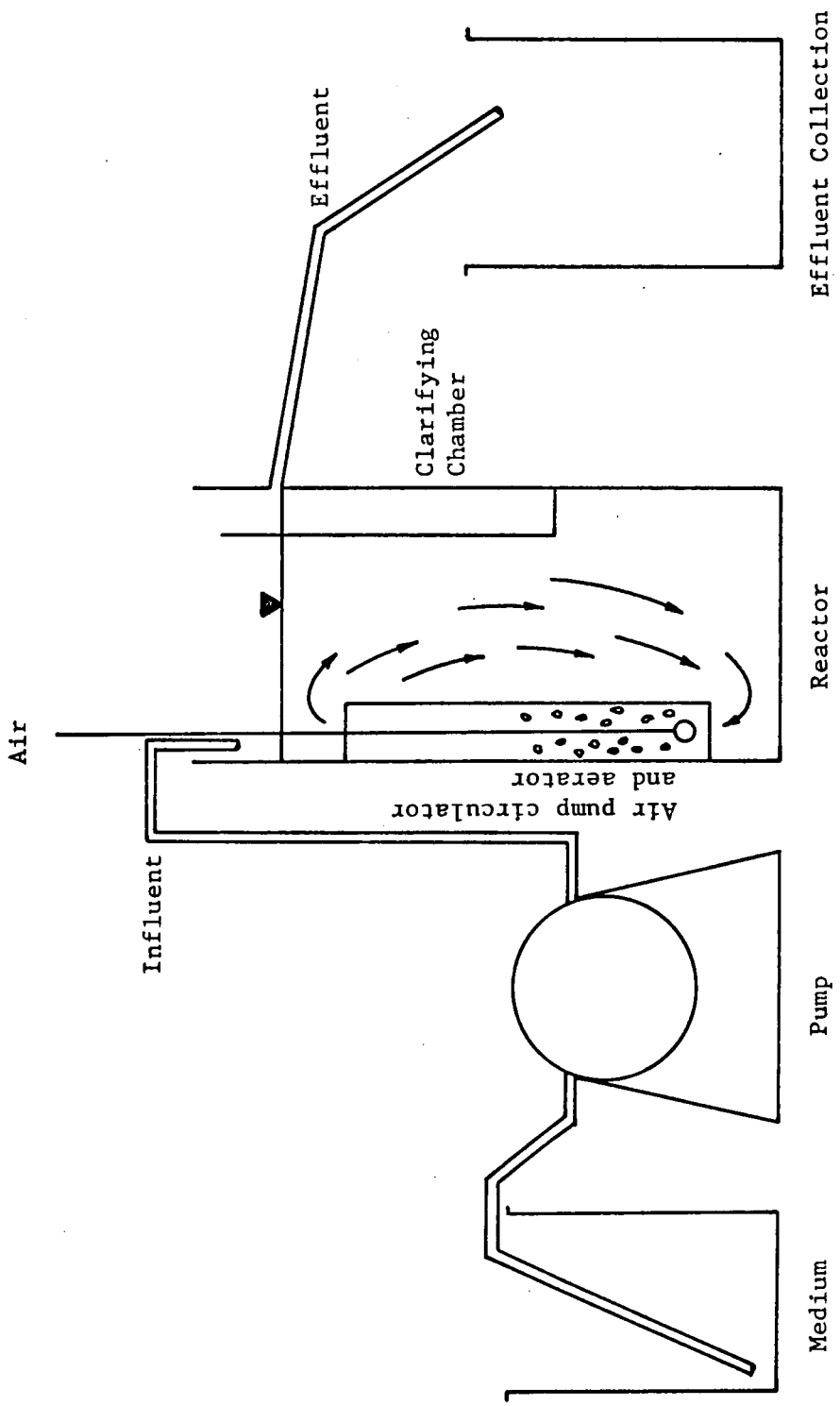

Figure 3. Continuous Aeration Unit.

pH of the feed solution. This gave a pH of 9.0 in the feed solution and maintained a pH of between 8.0 and 8.5 in the reactor medium.

Development of the Nitrifying Culture. Trickling filter effluent was first aerated in a batch reactor containing the synthetic medium. Every day for a week the solids were settled and the supernatant withdrawn prior to adding fresh medium. After a week the MLSS was transferred to the continuous flow system shown in Figure 3. The system was operated for a month with a flow rate of 2.5 ml/min during which time the nitrate content of the influent and effluent was measured to assure the development of a nitrifying biomass. The flow rate of 2.5 ml/min gave a detention period of 3 days. After the first month the flow rate was increased to 5 ml/min to give detention time of 12 hours. Nitrate content of both the influent and effluent were monitored during the assimilation period.

Growth Rate Determination and the Effect of Ammonia Concentration on the Growth Rate Constant. Several procedures can be used to measure growth rate. These include: (a) gravimetric monitoring of change in cell mass; (b) microscopic numeration of cell concentration; (c) monitoring of change in the concentration of reactant or product; (d) the monitoring of some other physical or chemical parameter related to biological activity. An evaluation of the methods available for measuring nitrification resulted in the selection of oxygen uptake as the most suitable method of monitoring the progression of the nitrification process. It was thought that this method provided the most sensitive and otherwise suitable procedure for use.

A stock solution of 10 gr/l of ammonium sulfate was prepared; this contained 2.121 g/l as NH_3-N. Appropriate amounts of this stock solution were added to dilution water to give 10, 20, 30, 40 and 50 mg/l NH_3-N. The dilution water used was prepared as described in Standard Methods (17). This medium contained identical minerals to the medium used for feeding the culture except manganese was not present and ammonium chloride was not added to the dilution water. The dilutions were made in liter flasks, to which was added five milliliters of sodium bicarbonate from stock solution of 64 gr/l as a carbon source, and five milliliters of mixed liquor from the chemostat as inoculum. The dilute solutions with the inoculum were poured into acid cleaned BOD bottles. An extra BOD bottle was used as a blank which contained everything that the ammonia diluted solution had except the ammonium sulfate. The pH was adjusted to 8.3 using anhydrous sodium carbonate. The BOD bottles were stored in dark shelves at 20°C in a constant temperature room. Initial and 24 hour readings of the DO were made using a YSI model 54 oxygen meter. Initial and final nitrate concentration were measured using the cadmium reduction method as described in Standard Methods (17).

The Effect of pH on Growth Rate. Two sets of experiments were conducted to determine the effect of pH on growth rate. One set was mixed by using quiescent conditions. A medium containing 10 mg/l NH_3-N was prepared as described above. pH adjustments were made using 0.1N H_2SO_4 and anhydrous sodium carbonate to yield a pH 6, 7, 8, 9 and

10 in the respective dilutions. The DO readings were made with a YSI oxygen meter. The initial and final nitrate nitrogen concentrations were determined.

Effect of Temperature on the Growth Rate. Experiments were conducted at 7°C, 20°C and 30°C to determine the effect of temperature on growth rate. An initial ammonia concentration of 10 mg/l NH_3-N was used along with the other inorganic salts (19). The DO readings were made with a YSI oxygen meter. Nitrate and nitrite nitrogen concentrations were determined by analyzing the content of one bottle each time DO determinations were made.

Determination of Cell Yield. While the chemostat contained a sedimentation chamber, solids build up was not sufficient to require solids wastage from the unit. Mixed liquor suspended solids (MLSS) was monitored and when steady-state conditions were achieved, the suspended solids content of the effluent was determined as a measure of the cell yield. The effluent was collected on a daily basis and mixed prior to withdrawing samples for suspended solids determination. 250 ml samples were filtered using fiberglass filter pads.

Effect of Organics on Nitrification Process. Activated sludge from an activated sludge plant located at a rest stop on Route I 81 east of Radford, Virginia was used as a nitrifying sludge source. Two continuous flow units having a design as shown in Figure 3 were prepared and fed with 50 and 100 mg/l COD augmented with the previously described synthetic media (19). The units were fed at a rate of 11 ml/min giving a detention time of 16.7 hours. Stock solu-

tions of dextrose and glutamic acid, each containing 10 gr/l were prepared for use as an organic carbon and amino acid source. The 100 mg/l COD media contained five milliliters of each of the two solutions. Five milliliters of bicarbonate from the 64 mg/l stock solution was added to the feed solution which was prepared daily. Sufficient amounts of the inorganic synthetic medium (19) were added to give the desired ammonia nitrogen concentration. The mixture was then diluted to a liter. For 50 mg/l COD, 2.5 ml of each dextrose and glutamic acid was added. The two units were operated for 1.5 weeks at an ammonia concentration in the feed of 16.5 mg/l NH_3-N until equilibrium was established before starting data collection. Data collection was continued for 12 days, then the NH_3-N was increased to 27.7 mg/l. The increase of NH_3-N was continued on a weekly basis until trace amounts were monitored in the effluent of the units at a feed concentration of 49.2 mg/l NH_3-N at which the units were shut down.

Influent and effluent ammonia nitrogen concentration was measured using the distillation procedure as described in Standard Methods (17) with boric acid being used as the indicating solution. Nitrate nitrogen content of the influent and effluent was also determined. MLSS were measured in accordance with the procedure described in Standard Methods (17).

Microscopic studies were conducted on the sludge from both the autotrophic and heterotrophic systems to observe the visual characteristics of the floculant mass. A binocular microscope with phase contrast optics was used.

RESULTS

Effect of Ammonia Concentration on Growth Rate. The daily oxygen uptake for different NH_3-N concentrations are shown in Table II. The data indicate that there was no significant difference in the oxygen uptake rate for the different ammonia concentrations employed. Taking the daily oxygen uptake for the 10 mg/1 NH_3-N concentration at 20°C as representative values for the other NH_3-N concentrations, two plots were prepared. A linear plot is shown in Figure 4 and a semi-log plot of the data is shown in Figure 5. These plots indicate that the growth followed zero order kinetics. From Figure 5 taking second, third, and fourth days oxygen uptake values as representative for the consecutive oxidation of ammonia nitrogen to nitrate nitrogen (the first day oxygen uptake was assumed unrepresentative for the consecutive reactions because the initial nitrite concentration was zero, the fifth day oxygen uptake was assumed unrepresentative because Downing et al. (7), and others (12) (15) have concluded that DO tensions below 0.5 mg/1 inhibits nitrification) the growth rate constant was calculated to be 0.643 day^{-1} at 20°C and pH 8.3. The initial and final nitrate plus nitrite nitrogen was 0.26 and 3.25 mg/1 as N respectively (these measurements were also made for the set of bottles containing 10 mg/1 NH_3-N).

Effect of Temperature on Nitrification. On measuring the oxygen uptake for 30°C on a daily basis, the DO dropped to 1.2 mg/1 after two days of incubation for the first experiment. The experiment

TABLE II

OXYGEN UPTAKE FOR SELECTED INITIAL AMMONIUM CONCENTRATION AT 20°C and pH 8.3

Concentration ppm as NH_4-N	Oxygen Uptake ppm Time - Days				
	1	2	3	4	5
10	0.5	1.8	4.3	6.9	7.8
20	0.5	1.8	4.2	6.9	7.8
30	0.5	1.7	4.3	7.3	7.8
40	0.5	1.7	4.2	7.0	7.8
50	0.5	1.7	4.2	6.9	7.8

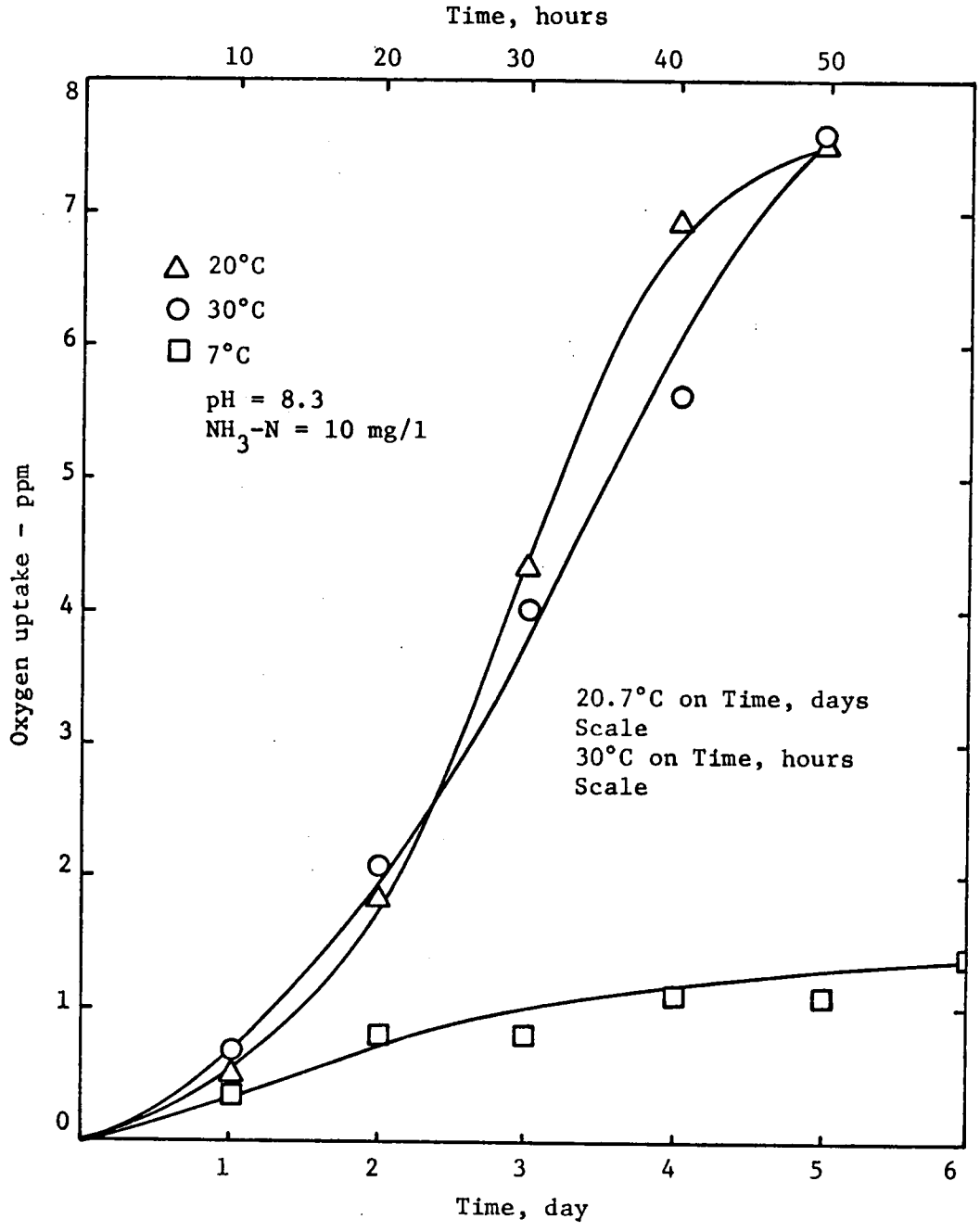

Figure 4. Oxygen uptake variation with temperature.

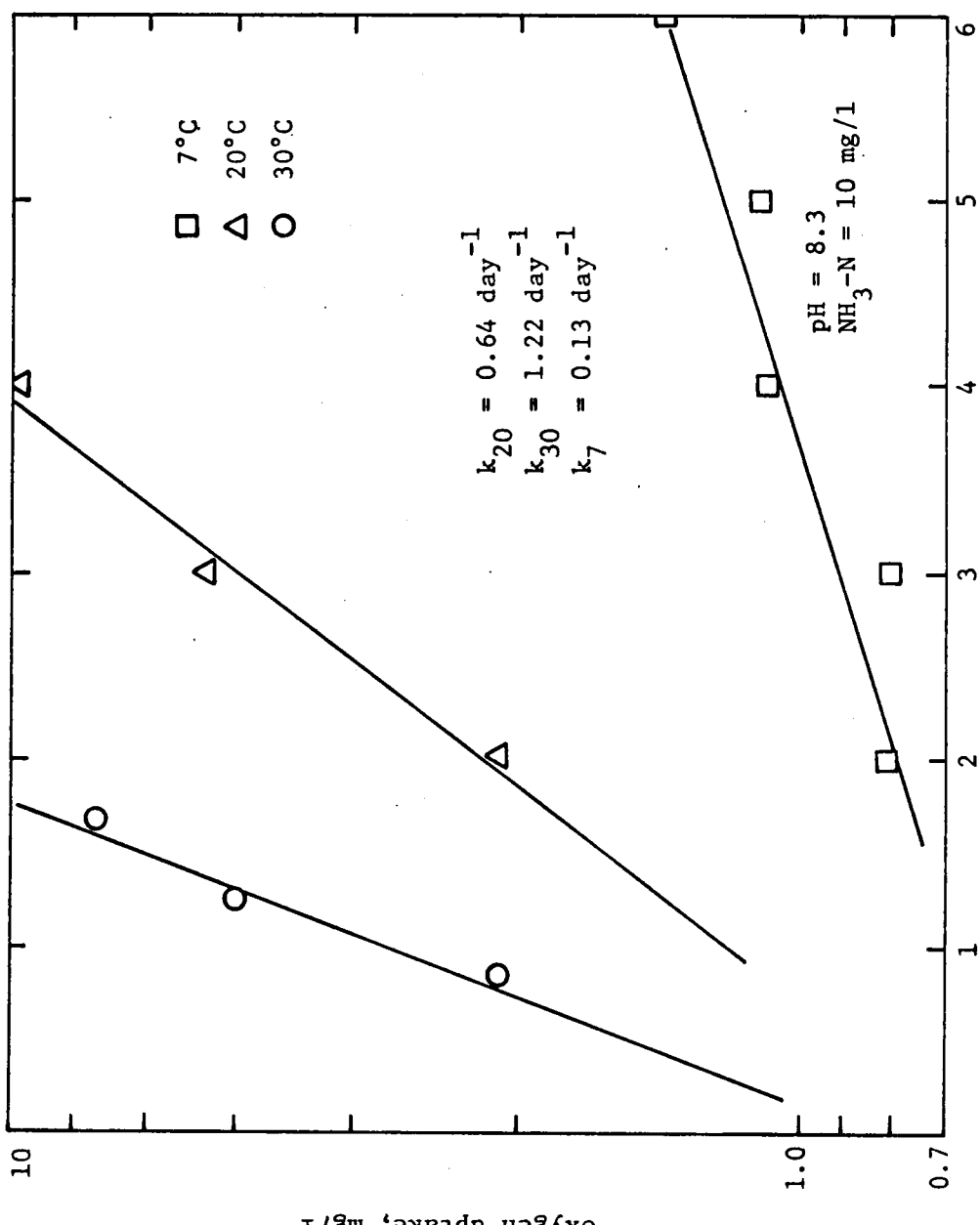

Figure 5. Oxygen uptake variation with temperature.

was repeated and DO readings were taken at 10 hour intervals. The linear and semi-log plots of the oxygen uptake are shown in Figures 4 and 5. The growth rate constant at 30°C and pH 8.3 was calculated to be 1.225 day^{-1}.

The oxygen uptake data for 7°C are also shown in Figures 4 and 5 and the growth rate constant was 0.13 day^{-1} at 7°C and pH 8.3.

This study was also concerned with comparing the values of growth rate constant determined from oxygen uptake data with other methods. The second procedure employed was measuring the change in oxidized nitrogen concentration (nitrite plus nitrate nitrogen) with time. Semi-log plots of the change in oxidized nitrogen and oxygen uptake for temperatures 20°C and 30°C are shown in Figures 6 and 7 respectively. The growth rate constant determined from oxidized nitrogen and oxygen uptake at 20°C were 0.54 day^{-1} and 0.66 day^{-1}; for 30°C they were 1.16 day^{-1} and 1.2 day^{-1}.

Effect of pH and Mixing on Growth Rate. Besides studying the effect of pH on the growth rate, the effect of mixing on the oxygen uptake was also studied. Oxygen uptake data obtained under quiscent conditions at pH 7, 8, and 9 are shown in Figure 8. Oxygen uptake data obtained under mixing conditions are shown in Figure 9. Linear and semi-log plots of oxygen uptake for pH 6 and 10 are shown in Figure 10 and 11 under quiscent conditions, and Figures 12 and 13 show the data for the experiments conducted under mixing conditions. The plots for pH 6 and 10 indicate that the oxygen uptake rate kinetics approaches zero order outside the pH range 6 and 10.

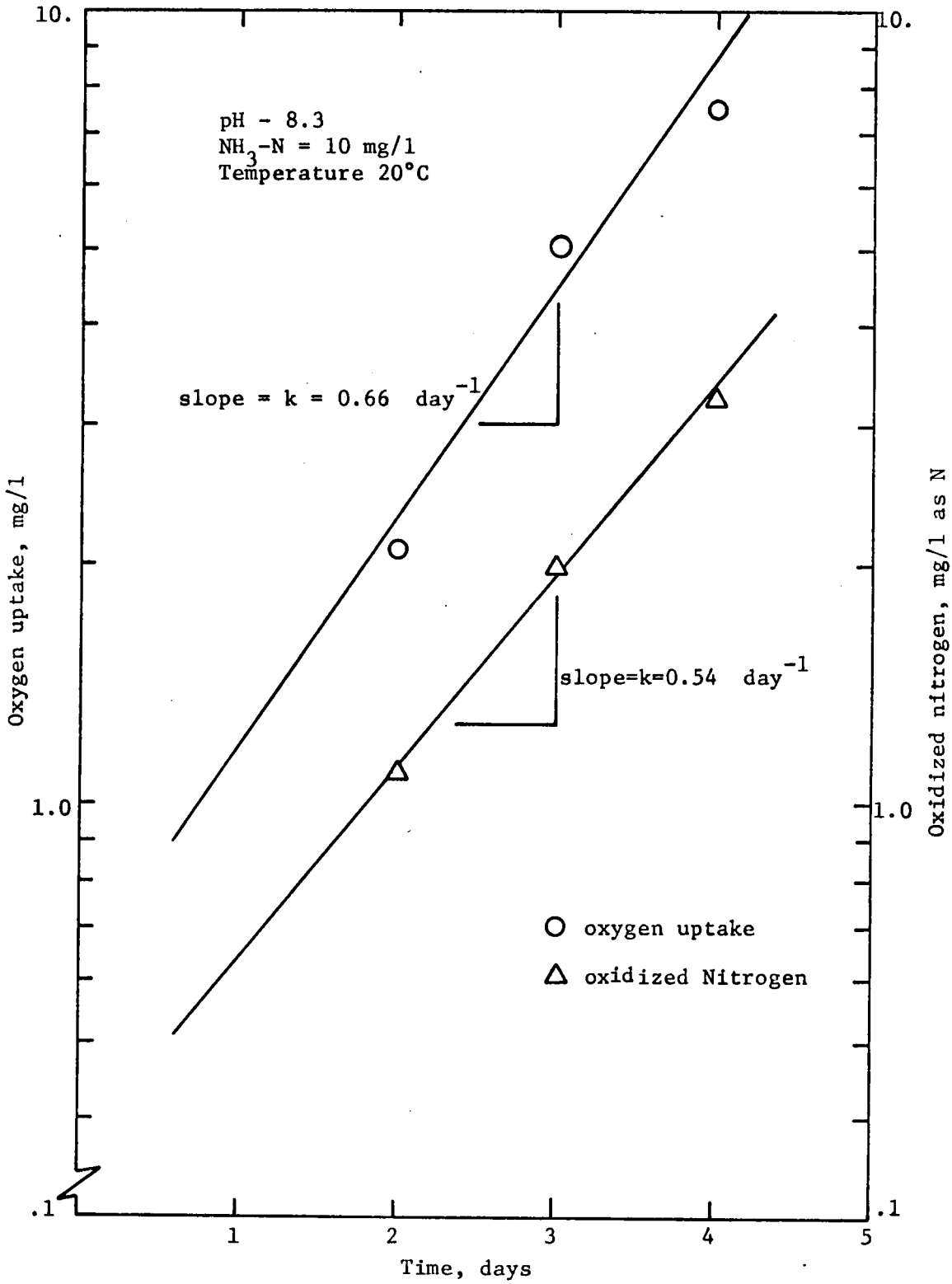

Figure 6. Variation of oxidized nitrogen and oxygen uptake vs time.

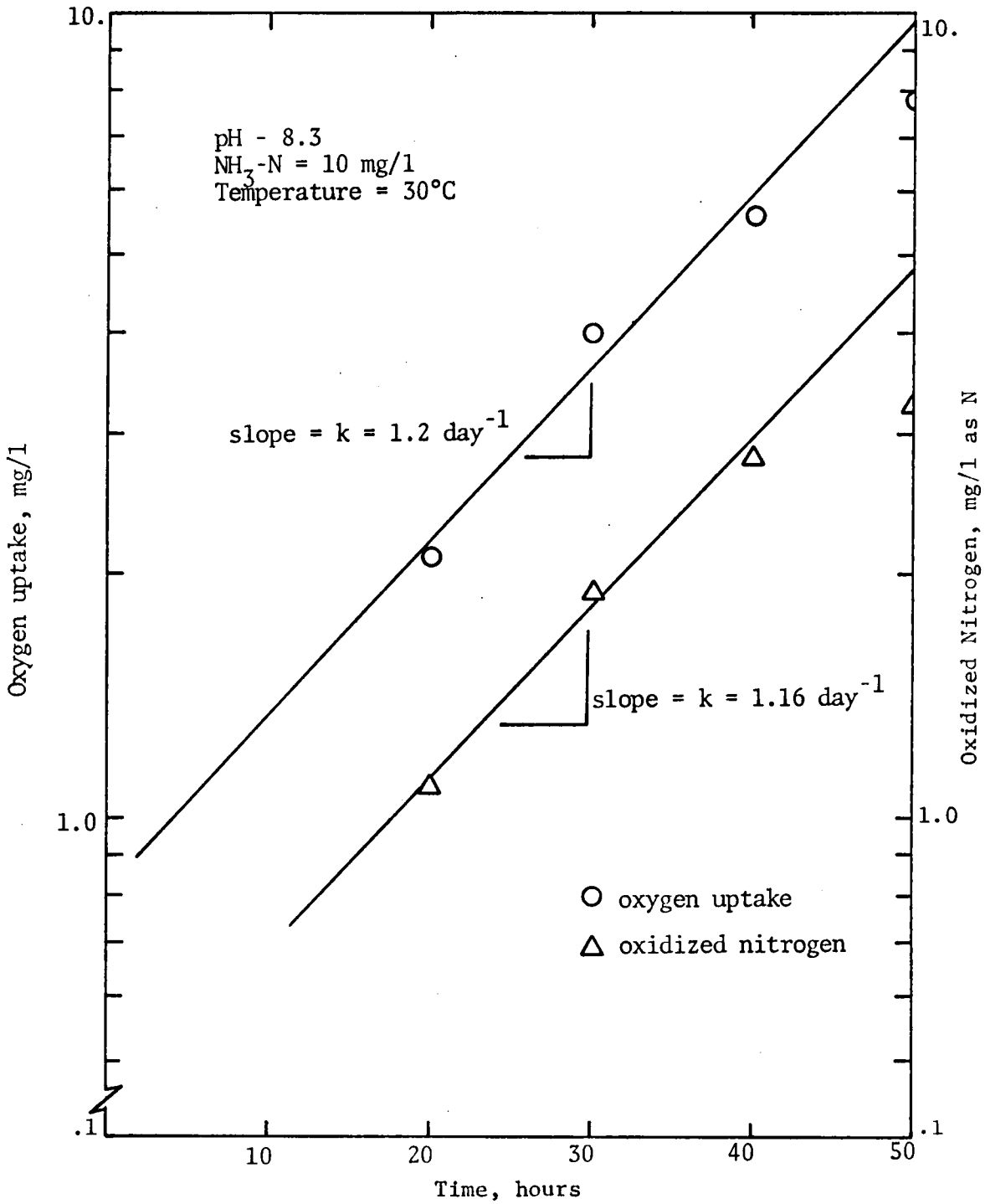

Figure 7. Variation of oxidized nitrogen and oxygen uptake vs time.

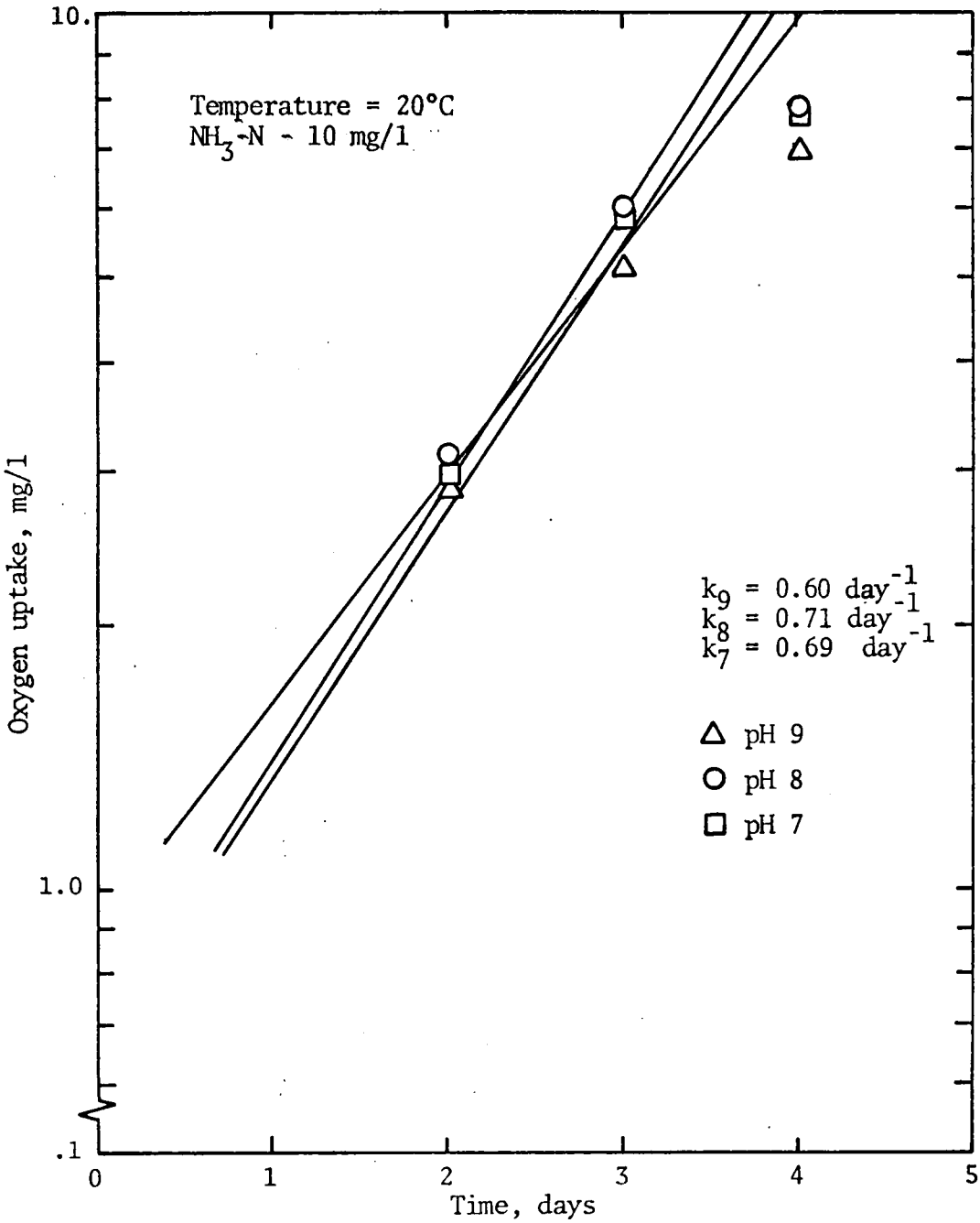

Figure 8. Variation of oxygen uptake with pH under quiescent conditions.

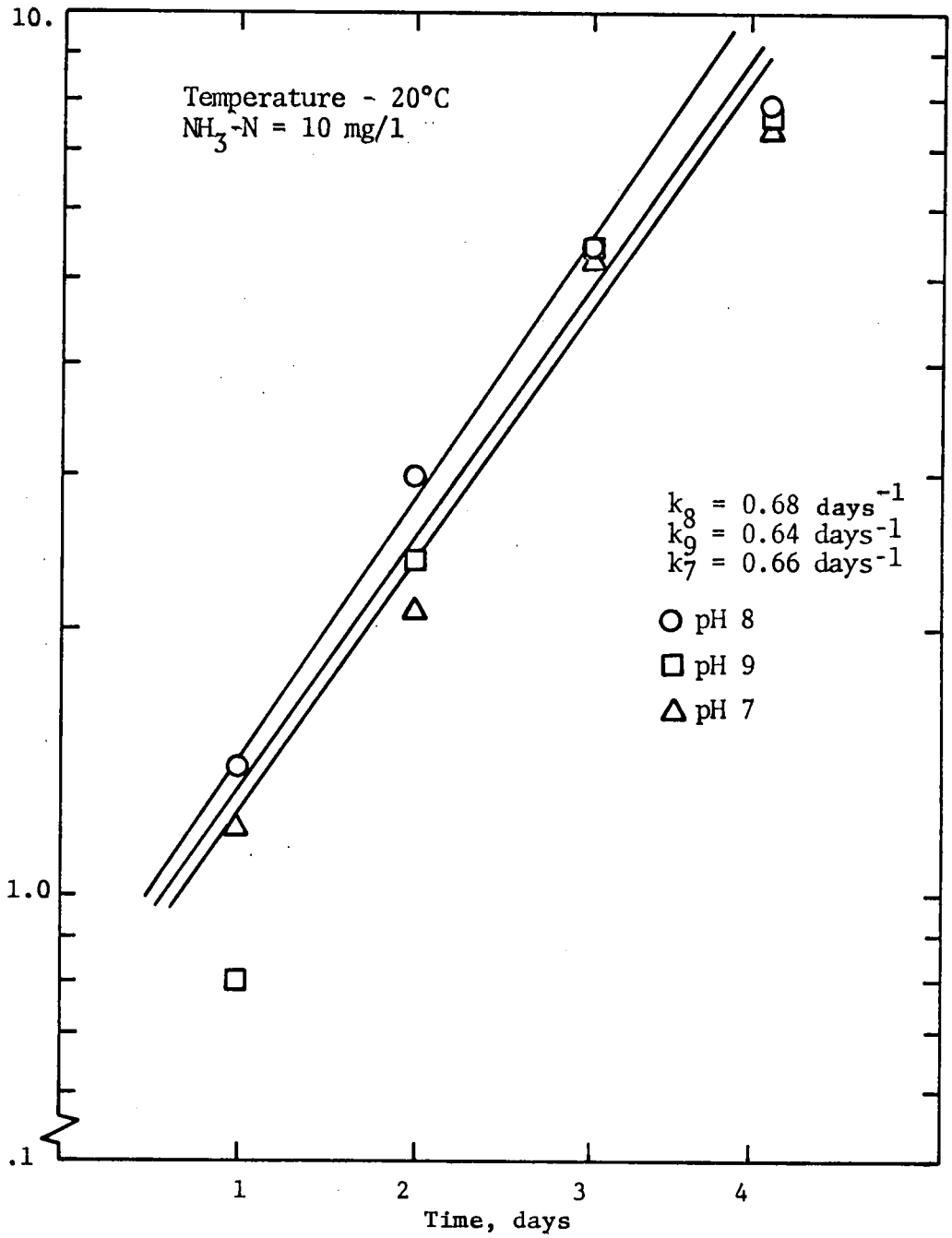

Figure 9. Oxygen uptake variation with pH under mixing conditions.

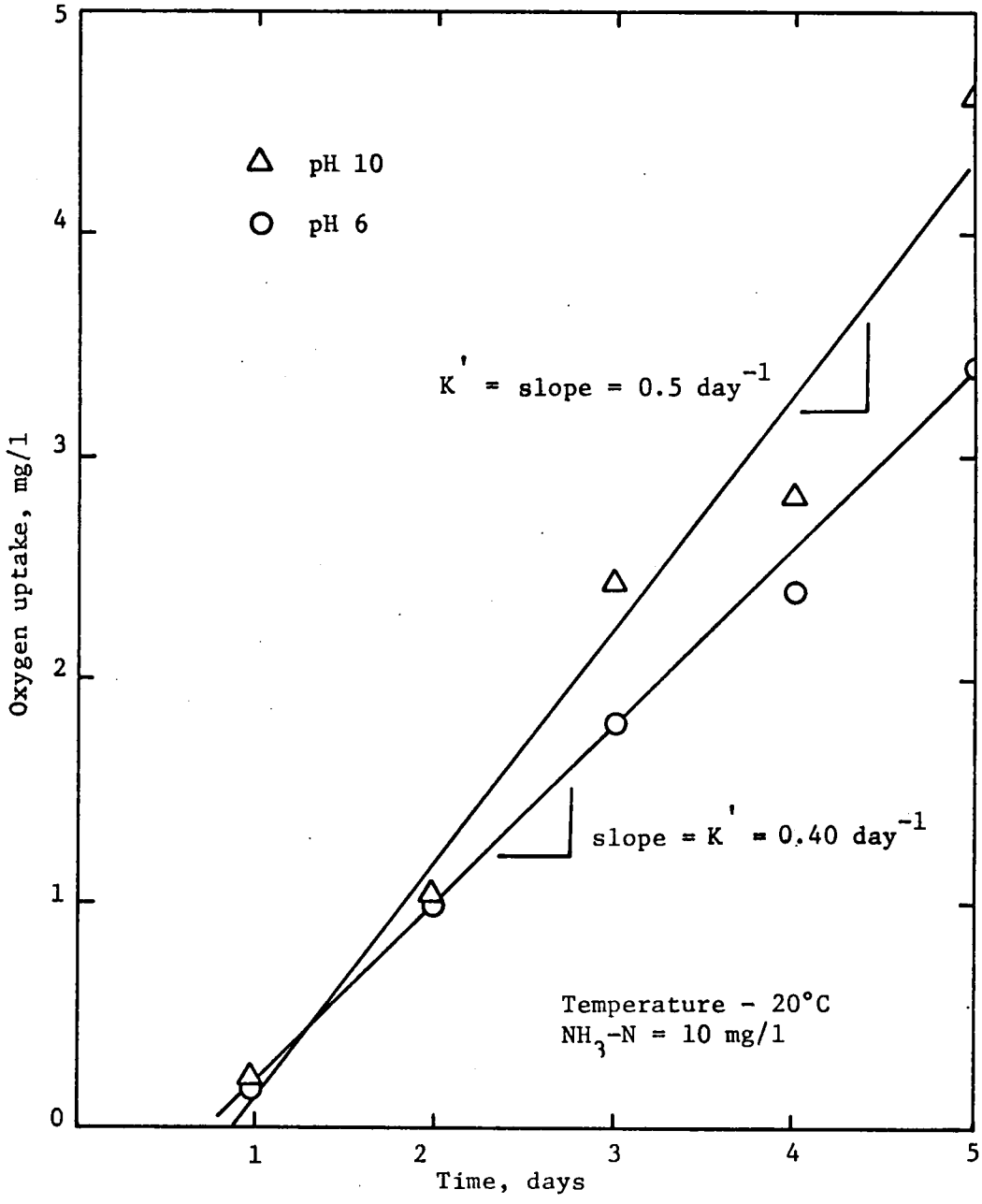

Figure 10. Oxygen uptake variation with pH 6 and 10 under quiscent conditions.

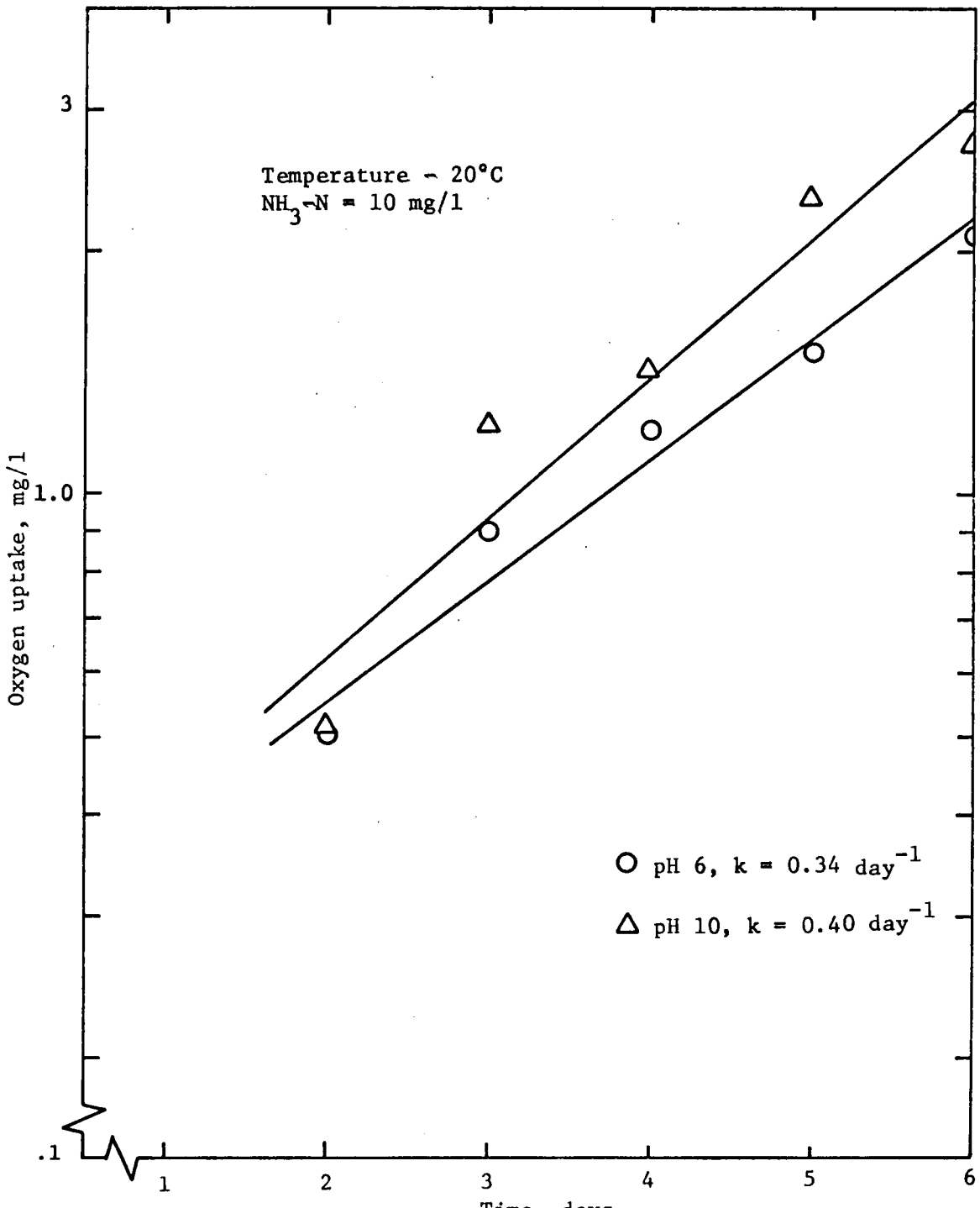

Figure 11. Oxygen uptake variation with pH 6 and 10 under quiscent conditions.

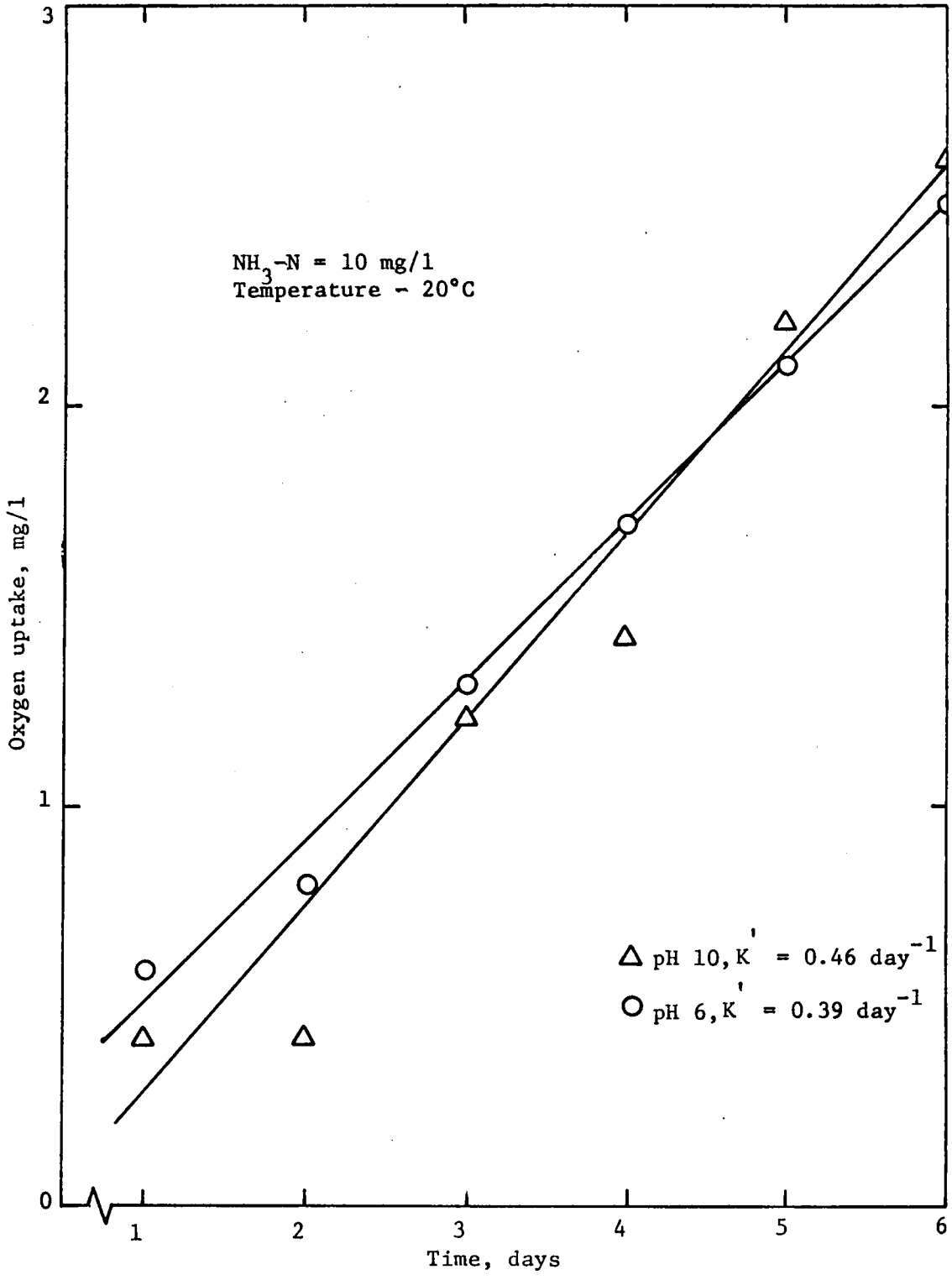

Figure 12. Oxygen uptake variation with pH 6 and 10 under mixing conditions.

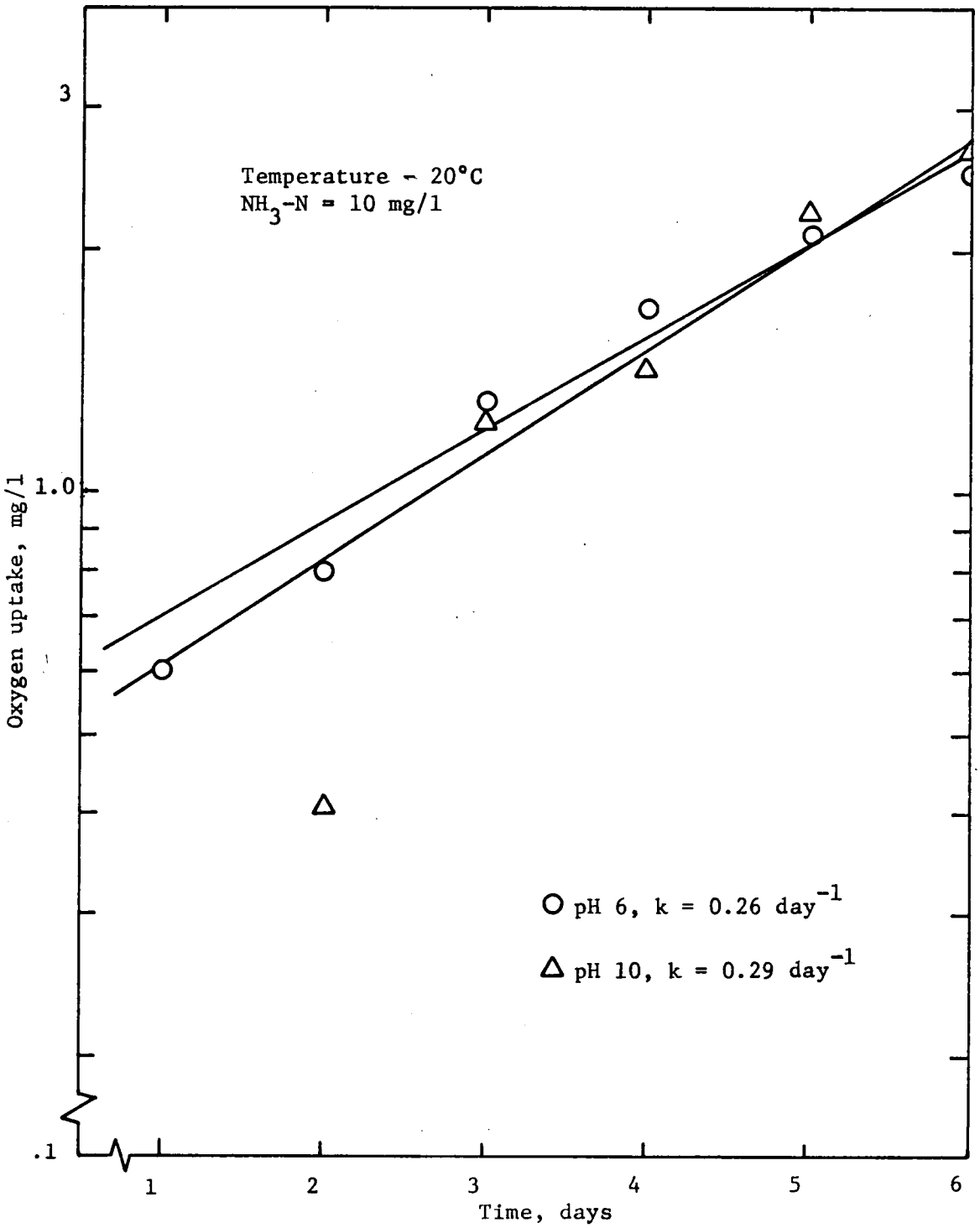

Figure 13. Oxygen uptake variation with pH 6 and 10 under mixing conditions.

Table III summarizes the growth rate constants for pH 6, 7, 8, 9 and 10 under both quiscent and mixing conditions. Table IV gives the initial and final nitrate plus nitrite nitrogen concentrations for experiments conducted under quiscent conditions at pH 6, 7, 8, 9 and 10.

Cell Yield. The suspended solids data for the chemostat liquor and effluent are presented in Figure 14. The average suspended solids of the effluent was 6.474 mg/l for an NH_3-N concentration in the chemostat feed of 26.5 mg/l. The ammonia concentration of the effluent was zero. The yield was determined by dividing the suspended solids concentration of the effluent by the oxidized nitrogen (it is equal to the ammonia concentration of the chemostat feed). This gave cell yield of 0.244 mg of cell produced per mg of ammonia nitrogen oxidized.

Effect of Organics on the Nitrification Process. The MLSS concentration for the heterotrophic reactors is shown in Figure 15. During the operation of the units it was observed that the effluent of the unit receiving the higher COD in the feed contained lower suspended solids than the effluent of the unit with lower COD. Suspended solids content of the effluent was measured on a daily basis during the last two weeks of the operation of the units. The results are shown in Figure 15. Table V gives the averaged values of influent and effluent COD and nitrogen data for the influent and effluent ammonia and oxidized nitrogen concentrations.

The results obtained from microscopic studies showed that the sludges of the three continuous flow reactors were different. In

TABLE III

GROWTH RATE CONSTANTS FOR pH 6, 7, 8, 9 and 10

WITHOUT AND WITH MIXING AT 20°C

pH	Growth Rate Constant, k, day^{-1} Without Mixing	With Mixing
6	0.34	0.26
7	0.69	0.64
8	0.71	0.68
9	0.60	0.66
10	0.40	0.29

TABLE IV

INITIAL AND FINAL NITRATE PLUS NITRITE NITROGEN WITH INITIAL NH_3-N of 10 mg/l

pH	$NO_3 + NO_2$ -N Initial	Final
6	0.26	0.81
7	0.26	2.3
8	0.26	2.4
9	0.26	2.5
10	0.26	0.85

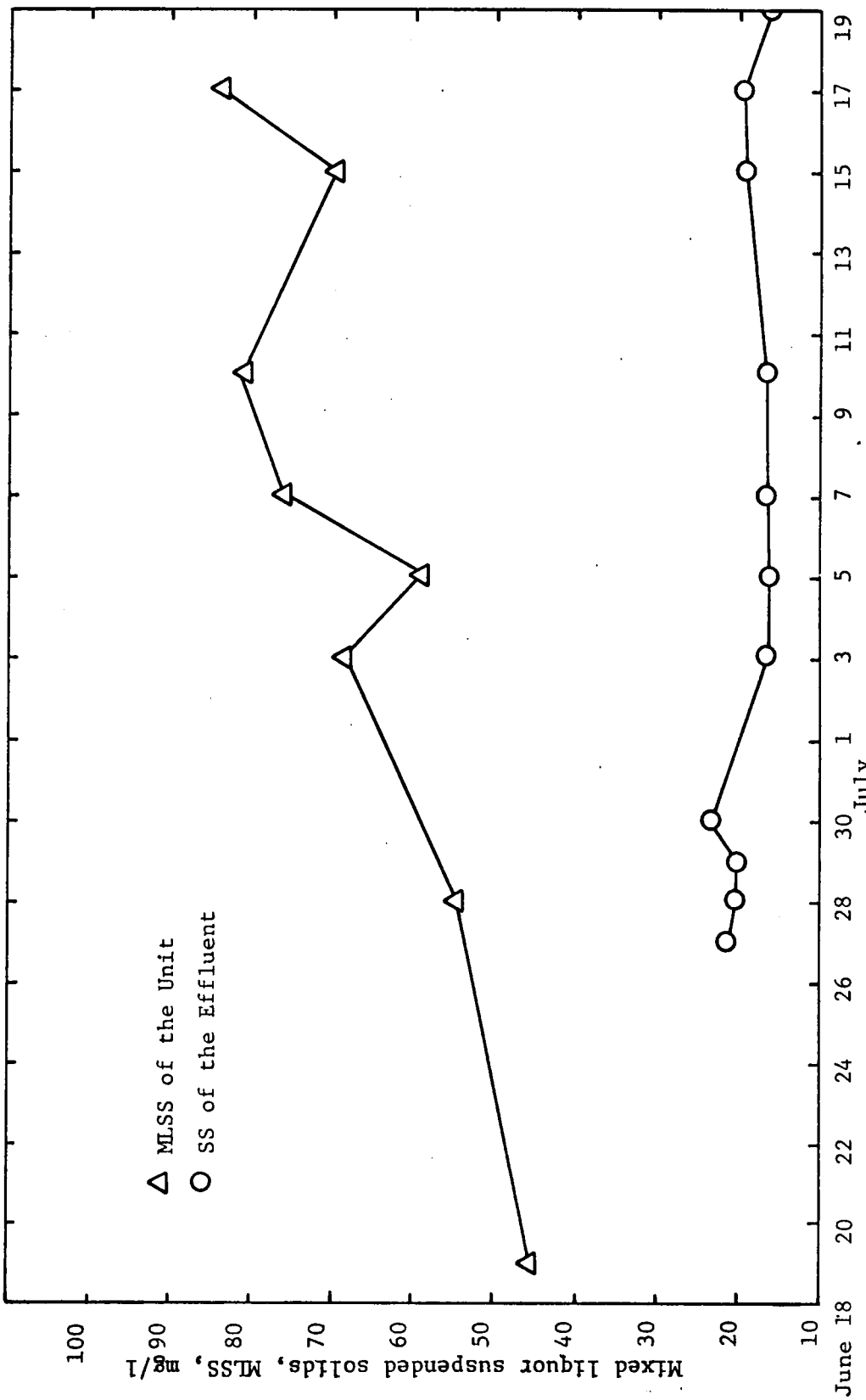

Figure 14. Variation of Suspended Solids and Mixed Liquor Suspended Solids with time for the autotrophic unit.

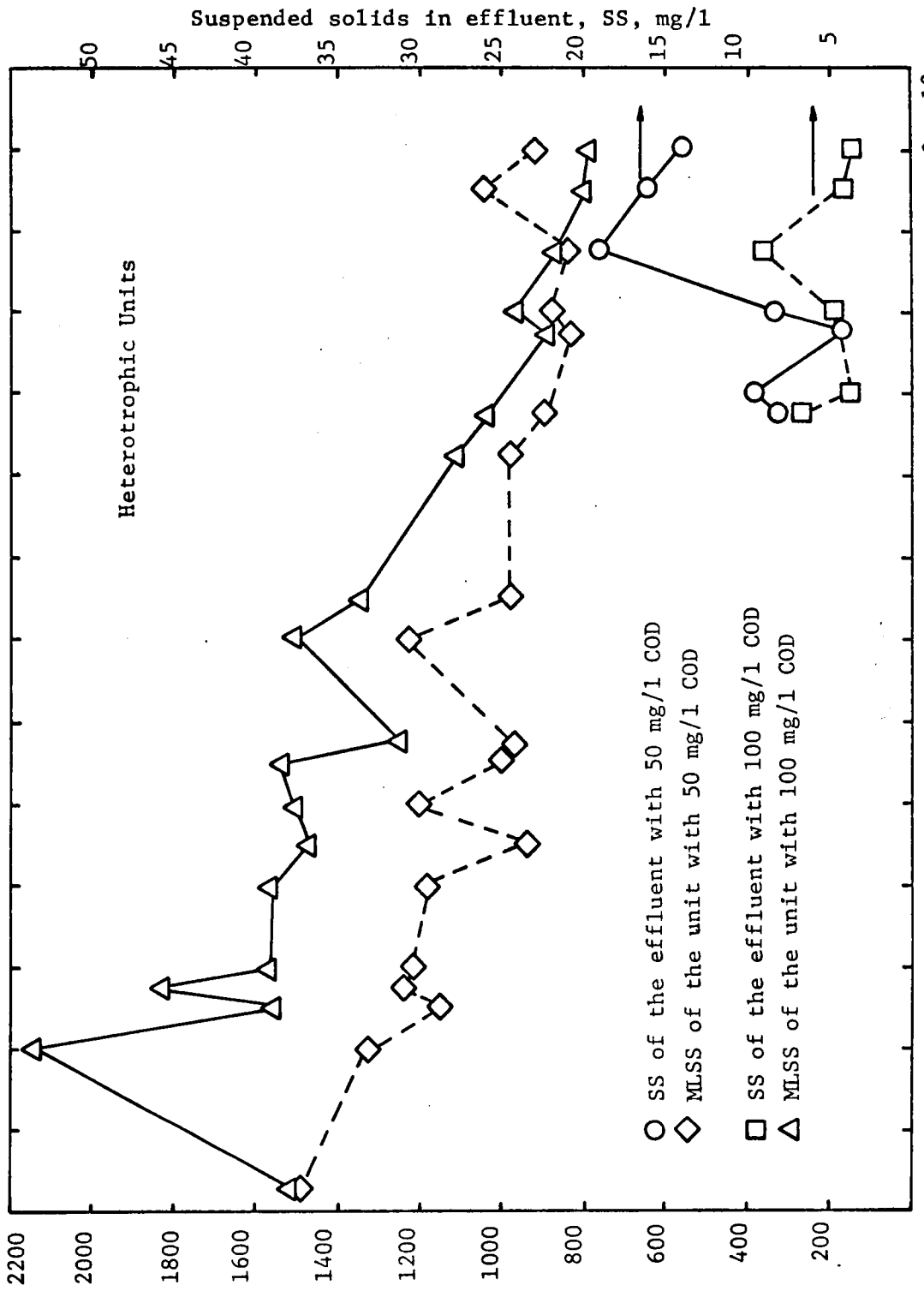

Figure 15. Variation of suspended solids and mixed liquor suspended solids with time.

TABLE V

AVERAGED VALUE OF MLSS, NH_4-H, NO_3-N

COD IN THE HETEROTROPHIC UNIT

COD mg/l		MLSS mg/l		NH_4-N mg/l		NO_3-N mg/l	
Inf.	Eff.	Unit	Eff.	Inf.	Eff.	Inf.	Eff.
103.6	25.96	1570.7	--	16.5	0.	0.85	15.72
				27.7	0.	0.85	18.27
				49.2	0.224	--	--
51.8	21.6	1136.4	--	15.85	0	0.85	14.77
				27.7	0	0.85	17.3
				49.0	0.2	--	--
103.6	--	8.85	4.5	--	--	--	--
57.8	--	901.7	11.6	--	--	--	--

the heterotrophic unit receiving higher COD concentration in the feed, the dominant bacterial mass was filamentous organisms while for the unit receiving the feed solution having a COD of 50 mg/l. filamentous organisms were observed but in much lower concentrations. The mixed liquor from the latter reactor contained a higher concentration of free swimming organisms. No filamentous growth was observed in the autotrophic unit and the organisms consisted of small clusters. There were fewer free swimming organisms present in the mixed liquor of the autotrophic unit than in heterotrophic units.

DISCUSSION OF RESULTS

The growth rate observed in this study indicates that nitrification kinetics are zero order and not substrate concentration dependent as reported by Downing et al. (7) and Buswell et al. (8). The data presented in Table II show that the nitrification rate was not a function of ammonia nitrogen concentration. Wild et al. (9) also reported that the nitrification rate was independent of ammonia nitrogen concentration and that the nitrifiers work at maximum efficiency at all times.

Oxidation rate calculated from oxidized nitrogen data were similar to those calculated from the oxygen uptake data. The difference in the data presented in Figure 6 and 7 is thought to be due to the less sensitive wet chemistry involved in the oxidized nitrogen determination. Thus, it appears that oxygen uptake measurements can be adopted as a reliable procedure for determining the growth parameters of the autotrophic nitrification process. In fact this method was found to be simpler and more accurate with the sensitive dissolved oxygen meters that are available than the wet chemistry procedures; change in cell mass; or microscopic numeration of cells.

It was found, as shown by the data in Figures 8 and 9, and Table III, that there was no significant difference observed in the rate of nitrification within the pH range of 7 to 9. Although the maximum nitrification rate was observed at pH 8, the rate at pH 7 and 9 was not significantly different. This trend was also reported

by Beckman et al. (12) who reported that within the range pH 7 to 9, pH had no significant influence on the rate of ammonia removal. The results obtained at pH 6 and 10, as shown in Figures 10, 11 or 12 and 13, indicate the oxygen uptake kinetics approaches zero order outside the pH range 7 to 9. Monod (2) refers to this type of growth as linear growth, and reports that it is caused by the activity of one enzyme or a group of enzymes being limited to a constant rate. Mixing did not cause a significant change in the activity rate as shown by the data in Table III.

It was observed that temperature had a significant influence on the rate of oxygen uptake as shown by the data reported in Figure 5. The rate of nitrification increased as temperature was increased. These results show good correlation with results reported by others. A plot of the growth rate constants at 7°C, 20°C, and 30°C as shown in Figure 16 gave the following linear relationship.

$$k_T = 0.046T - 0.22 \quad \text{------} \quad (10)$$

where k_T is the growth rate constant at temperature T, in days^{-1} and T is the temperature in degrees Centigrade. The relationship can also be written in the following form:

$$k_T = k_{20} + 0.046(T - 20) \quad \text{------} \quad (11)$$

where k_{20} is 0.70, the growth rate constant at 20°C. The equation is applicable for the temperature range of 5-30°C and pH 7 to 9. This relationship is reasonably in agreement with the Van't Hoff's -

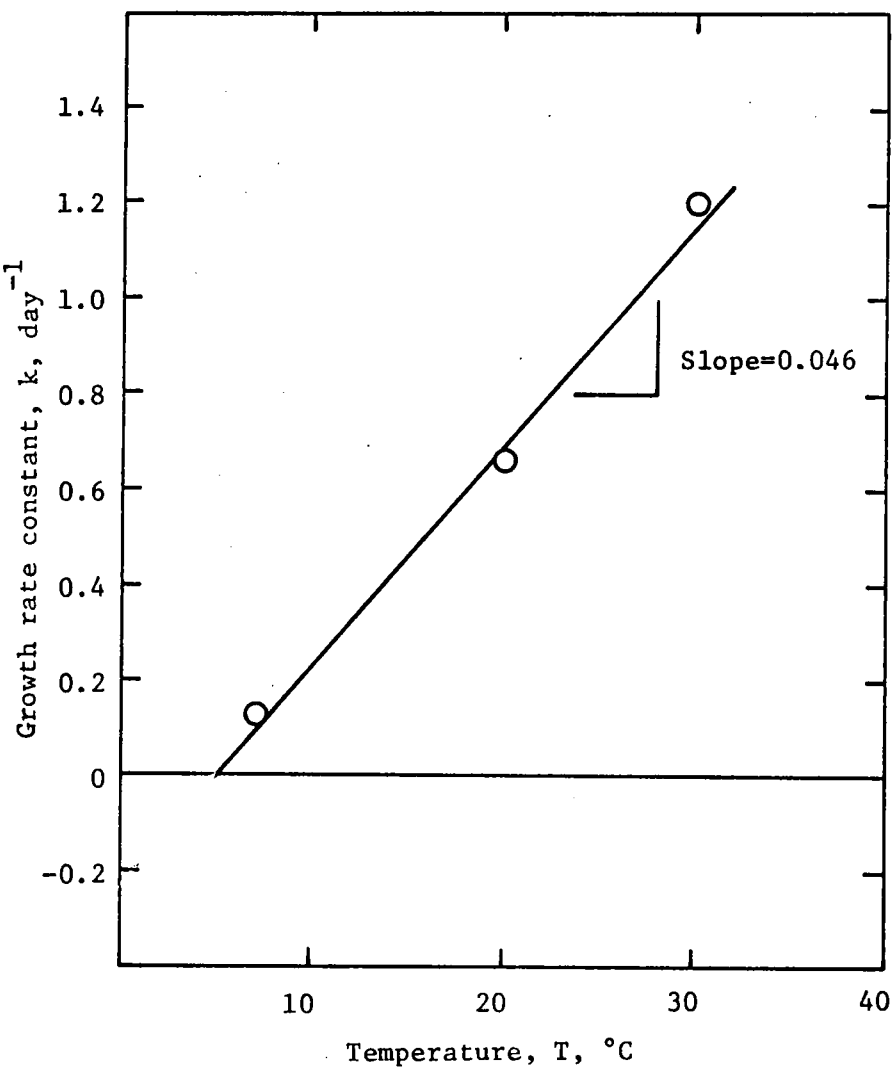

Figure 16. Variation of growth rate constant with temperature.

Arhenius law which states that the rate of a reaction will double with every 10°C increase in temperature.

The cell yield obtained in this study was somewhat higher than the values given for a pure culture (7). This might have been caused by the lysing of dead autotrophic cells thus providing a limited substrate for heterotrophic activity in the chemostat. During the chemostat operation, it was observed that the solids did not stay in suspension but rather attached to the inside periphery of the unit. The reason for this phenomena is not known but it is mentioned in the literature that it is necessary for the nitrifying organisms to adhere to solid surface in order to achieve their maximum activity (7). The sides of the chemostat were scraped daily to suspend the growth.

The results obtained also show that low DO levels are inhibitory to nitrification. In some experiments nitrification was inhibited at DO tensions of 0.5 mg/l on others it appeared to be inhibited at 0.3 mg/l DO. This trend agrees with data reported by Downing et al. (7). They found that DO levels as high as 1 mg/l were inhibitory.

It was observed as shown by the data in Table V that at normal NH_3-N levels, the BOD:nitrogen ratio did not have any effect on the nitrification rate. A trace of ammonia nitrogen appeared in the effluent of the heterotrophic units at a feed concentration of 49 mg/l of NH_3-N as N. This is not thought to indicate that high concentrations of ammonia inhibits nitrification but may indicate that the

ammonia level exceeded the oxidation capacity of nitrifiers in the biomass.

A reasonable level of BOD in the feed appears to enhance treatment efficiency while not interfering with the nitrification process. As shown by the data presented in Figure 15, the reactor receiving the feed containing the greater organic content produced the better quality effluent. Beckman et al. (12) as well as Schroepfer (15) have reported that nitrification is attainable in the activated sludge process having a BOD of 250 mg/1.

As the ammonia concentration in the feed of the heterotrophic units was increased above 15 mg/1 NH_3-N problems with floating sludge were observed in the clarifier which led to a sudden drop of the solids in the mixed liquor as shown by the data in Figure 15. After checking the nitrogen balance of the effluent and influent (theoretically the influent ammonia nitrogen as N should be equal to the effluent nitrate plus nitrite nitrogen as N) a loss of nitrogen was observed when the influent NH_3-N was increased from 15.85 mg/1 to 27.7 mg/1 as shown by the data in Table V. One explanation of this loss is that denitrification was occurring in the solids settling chamber. It appears that higher nitrate levels in the sludge will promote denitrification which interfers with sedimentation.

Application of Nitrification Kinetics to the Activated Sludge Plant

Problems in achieving nitrification, especially during

colder weather, have been encountered by many operators of activated sludge plants. Adequate data are not currently available for designing activated sludge processes to achieve nitrification on a continuing basis. While Downing et al. (7) have developed the kinetics for the process, the model is not readily adaptable to process design. As indicated by Downing et al. (7), it is necessary to maintain the nitrifiers in the unit in order to achieve nitrification. In other words one should maintain a suitable sludge age which is defined as:

$$\theta_c = X_T/(\Delta X/\Delta t)_T \qquad \text{------} \qquad (12)$$

where θ_c is the sludge age in days, X_T is the total active nitrifying microbial mass within the treatment system in mg/l, and $(\Delta X/\Delta t)_T$ is the total quantity of active nitrifying mass produced daily in mg/l. Since this study showed that the nitrifiers are independent of the initial NH_3-N concentration, it follows that:

$$(\Delta S_1/\Delta t)/Xt = k \qquad \text{-------} \qquad (13)$$

For the pH range of 7 to 9, and a temperature range from 5 to 30°C, the linear temperature relationship found in this study, equation 10, can be substituted in equation 13 to give:

$$X_T = (\Delta S/\Delta T)/(0.046T - 0.22) \qquad \text{-------} \qquad (14)$$

From definition of cell yield:

$$(\Delta X/\Delta t)_T = Y\ \Delta S/\Delta t \qquad \text{-----} \qquad (15)$$

Substituting equations 14 and 15 in equation 12 we get

$$\theta_c = 1/(0.046T - 0.22)Y \qquad \text{----} \qquad (16)$$

Thus in order to achieve nitrification during all seasons of the year, it is necessary to maintain a sludge age relative to the operating temperature. Substituting the yield Y of 0.244 mg. of cell produced per mg. of ammonia nitrogen oxidized into equation 16, gives

$$\theta_c = 4.1/(0.046T - 0.22) \quad \text{----} \quad (17)$$

A plot of this equation is shown in Figure 18 which indicates that the required sludge age to achieve nitrification at 10°C is almost five times that of 30°C.

Once the sludge age required to achieve nitrification has been established, it could be applied to the equation developed by Lawrence and MacCarty (4) for a completely mixed activated sludge unit with solids recycle to obtain the MLSS required in order to achieve nitrification. The equation is given by:

$$X_H t = [Y_H \theta_c (F_0 - F_1)]/(1 + b\theta_c) \quad \text{-----} \quad (18)$$

where

X_H = microbial mass concentration, mg/l

t = V/Q = Hydraulic retention time, days

V = reactor volume, gallons

Q = flow rate of liquid through the reactor, gpd

$F_0 - F_1$ = Organic load removed, mg/l*

b = microorganism decay coefficient, day^{-1}

Y_H = Yield for Heterotrophic microbial mass.

Equation 18 may be written as:

---

*Biomass yield from nitrification becomes insignificant at normal levels of BOD and kjeldahl nitrogen found in domestic sewage. Thus, sludge age has been based on BOD loadings.

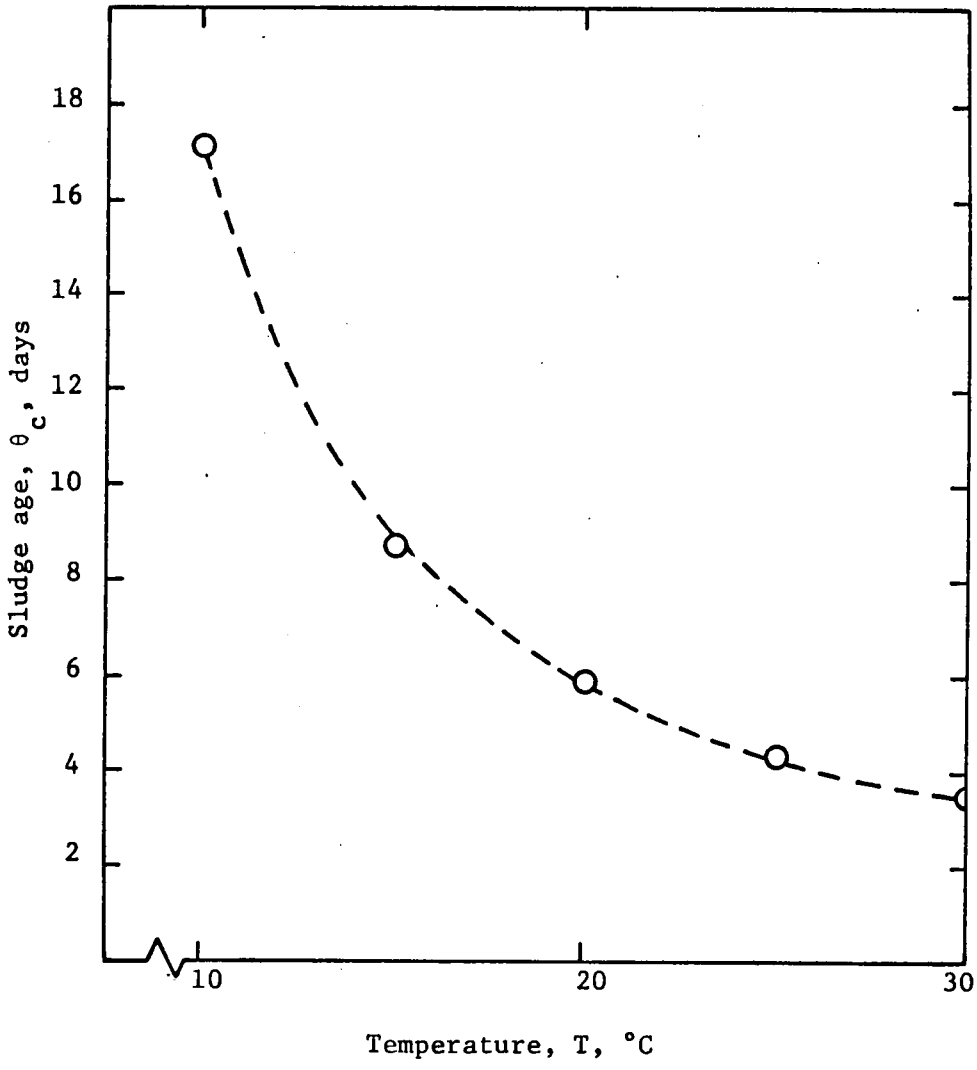

Figure 17. Variation of sludge age with temperature.

$$X_H V = [Y\theta_c Q(F_0 - F_1)]/(1 + b\theta_c) \quad \text{------} \quad (19)$$

In the design of an activated sludge system the engineer can select the critical θ_c from Figure 18, determine the X_H which can be handled in the solids separation system and calculate the reactor volume to provide the required retention time.

Selecting Y to be equal to 0.6, b as 0.05 and t as 6 hours, a family of curves can be constructed for determining the MLSS level required to maintain a desired sludge age as the strength of the waste varies. These curves are shown in Figure 18.

At 20°C the sludge age required to achieve nitrification as shown in Figure 18 is 5.85 days. Assuming that the average domestic sewage contains 250 mg/1 BOD, the MLSS level required to achieve nitrification is about 2700 mg/1 (from Figure 19) thus it is evident why nitrification is often accomplished during the summer season where the sewage temperature is greater than 20°C since most activated sludge plant operators maintain a MLSS concentration of more than 2000 mg/1. On the other hand if the temperature of the sewage being treated drops to 10°C a sludge age of 17.1 days would be required and in turn a MLSS level of about 5600 mg/1 in the aeration unit must be maintained. Thus for a fixed volume reactor, the solids concentration varies inversely with the temperature of the medium and for strong wastewater, nitrification may be difficult to achieve during the winter months. Maintaining a MLSS of 5600 mg/1 would cause two major problems.

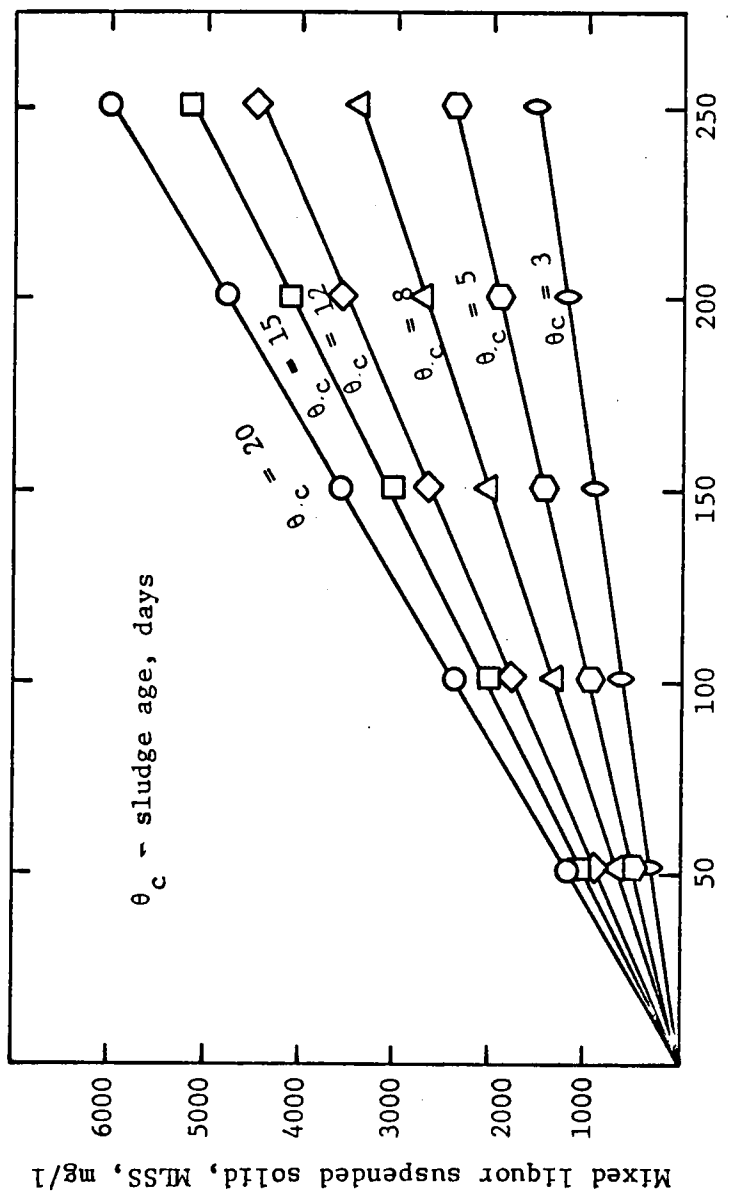

Figure 18. Variation of MLSS with Organic Load at Constant Sludge ages for 6 Hours Retention time.

1. Maintaining an adequate oxygen level in the aeration tank.
2. Problems in the solids separation process due to the increase in the solids flux, thus allowing solids to be lost in the effluent.

To overcome these operational difficulties the MLSS could be reduced to normal levels by reducing the influent BOD to the secondary treatment process. The required sludge age at 10°C is 17.1 days, and for BOD of 100 mg/1 the MLSS level to be maintained is 2300 mg/1 which is a normal level of activated sludge unit. The reduction in BOD can be accomplished by employing chemical coagulation as a part of the primary treatment during the winter months.

Thus the operator can control the treatment process so as to achieve nitrification throughout the year. By adding metal salts as a part of the primary process, the operator can remove phosphorus and accomplish nitrification without resorting to the so-called tertiary processes.

It was mentioned in the discussion that problems with floating sludge was observed in the heterotrophic units as the nitrate concentration was increased. Thus good settling and rapid solids return is needed to avoid anaerobic condition in the clarifiers and hence preventing denitrification from occurring.

SUMMARY AND CONCLUSIONS

1. The growth rate of nitrifying organisms was found to be independent of ammonia concentration in the range of 10 to 50 mg/l NH_3-N.

2. The nitrification rate was dependent on temperature. For the temperature range of 5° to 30°C the rate was defined by: $k_T = 0.046T - 0.22$.

3. Within a pH range of 7 to 9, pH had no significant influence on the nitrification rate. Outside of this pH range, the nitrification rate decreased.

4. For complete nitrification on a continuous yearly basis, relative sludge ages for the operation temperatures must be maintained, as given by the following equation: $\theta_c = \frac{4.1}{0.046T - 0.22}$

5. The presence of organic material did not have any effect on the nitrification rate. In fact, heterotrophic growth was found desirable because of overall floc characteristics and related improvement in effluent quality.

REFERENCES CITED

1. Mulbarger, M. C., "Nitrification and Denitrification in Activated Sludge System," Journal Water Pollution Control Federation, 43, 2099-2070 (1971).

2. Monod, J., "The Growth of Bacterial Cultures," Annual Review of Microbiology, 3, 371-394 (1949).

3. McKinny, R. E., "Mathematics of Complete-Mixing Activated Sludge," Journal of Sanitary Engineering Division, ASCE, 88, No. SA3, Proc. paper 3133, 87-113 (1962).

4. Lawrence, A. W., and McCarty, P. L., "Unified Basis for Biological Treatment Design and Operation," Journal of the Sanitary Engineering Division, ASCE, 96, No. SA3, paper 7365, 757-778 (1970).

5. Engel, M. S., and Alexander, M., "Growth and Autotrophic Metabolism of Nitrosomonas Europea," Journal of Bacteriology, 76, 217-222 (1949).

6. Lees, H., and Simpson, J. R., "The Biochemistry of the Nitrifying Organism," Biochemical Journal, 65, 297-305 (1957).

7. Downing, A. L., Painter, H. A., and Knowles, G., "Nitrification in the Activated Sludge Process," Journal Institute of Sewage Purification, Part 2, 130-153 (1964).

8. Buswell, A. M., Shiota, T., Lawrence, N., and Meter, I. V., "Laboratory Studies on the Kinetics of the Growth of Nitrosomonas with Relation to the Nitrification Phase of the BOD Test," Applied Microbiology, 2, 25-25 (1954).

9. Wild, H. E., Jr., Sawyer, C. N., and McMahon, T. C., "Factors Affecting Nitrification Kinetics," Journal Water Pollution Control Federation, 43(a), 1845-1854 (1971).

10. Skimmer, F. A., and Walker, N., "Growth of Nitrosomonas Europea in Batch and Continuous Culture," Archeiv Fur Microbiology, 38, 339-349 (1961).

11. Boon, B., and Laudelaut, H., "Kinetics of Nitrite Oxidation by Nitrobacter Winogradskyi," Biochemical Journal, 85, 440-447 (1962).

12. Beckman, W. J., Avendt, R. J., Mulligan, T. J., and Kehrberger, G. J., "Combined Carbon Oxidation-Nitrification," Journal Water Pollution Control Federation, 44(10), 1916-1931 (1972).

13. Hofman, T., and Lees, H., "The Biochemistry of Nitrifying Organism:4. The Respiration and Intermediary Metabolism of Nitrosomonas," Biochemical Journal, 54, 579 (1953).

14. "Bergey's Manual of Determinative Bacteriology," 7th Edition, The Williams and Wilkins Company, Baltimore, Maryland, 1957.

15. Johnson, W. K., Schroepfer G. J., "Nitrogen Removal by Nitrification and Denitrification," Journal Water Pollution Control Federation, 36(8), 1015 (1964).

16. Dursbag, P. C., and Buehrer, T. F., "Effect of Ammonia and Its Oxidation Products on Rate of Nitrification and Plant Growth," Soil Science, 78, 37 (1954).

17. "Standard Methods for the Examination of Water and Wastewater," 13th Edition, American Public Health Association, New York, N. Y. (1971).

18. Jennelle, E. M., and Gaudy, A. F., Jr., "Studies on the Kinetics and Mechanism of BOD Exertion in Dilute Systems," Biotechnology and Bioengineering, 12, 519 (1970).

19. "Microbiology," Michael J. Pelczar, Jr. and Roger D. Reid, McGraw Hill, New York, N. Y., 1958.

The vita has been removed from the scanned document

A STUDY OF NITRIFICATION KINETICS

by

Hraj A. Kharajian

(ABSTRACT)

One autotrophic and two heterotrophic chemostats were operated during the study period. The autotrophic unit was operated using an inorganic synthetic ammonium medium to provide a culture of nitrifying organism and to study cell yield. The two heterotrophic units were operated with a feed containing 50 and 100 mg/1 COD respectively along with the inorganic ammonium medium to determine the effects of heterotrophic activity on the nitrification process. The parameters monitored included mixed liquor suspended solids, suspended solids concentration in the effluent and influent; effluent ammonia and nitrate nitrogen concentration.

The culture from the autotrophic unit was used as inoculum of nitrifying organisms for the other phases of the study. These phases of the study included determination of the effect of ammonium concentration, temperature and pH on nitrification. Temperature was found to be the main controlling factor for nitrification. An equation for the sludge age required at operating temperature was developed and a graph was prepared to determine the MLSS required, for a given influent BOD and the determined sludge age, that will assure nitrification on yearly basis.

Changes in the pH within the range of 7 to 9 were found to have no significant influence on the nitrification rate. The nitrification rate decreased at pH outside this range.

It was found that the presence of heterotrophic activity had no effect on the nitrification. In fact the presence of heterotrophic biomass was found desirable because of the improvement in the effluent quality.