A STUDY OF NITRATE VERSUS OXYGEN RESPIRATION IN THE ACTIVATED SLUDGE PROCESS

by

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(ABSTRACT)

Utilization of the activated sludge process is widespread although many of the mechanisms involved are still relatively misunderstood. Incorporation of nitrate respiration (denitrification) into the activated sludge process can have many advantages, but little is known about microbial growth and substrate removal when nitrate respiration is employed.

The primary objective of this study was to evaluate and compare microbial growth and biokinetic coefficients in an aerobic and an anoxic (anaerobic with nitrate as the terminal electron acceptor) activated sludge process. Two bench-scale continuous flow reactors were operated over a range of mean cell residence times with organic carbon as the limiting nutrient. Alkalinity changes were monitored and compared with theory. Engineering applications of the results were discussed.

The maximum microbial yield and endogenous decay coefficient were lower, and the maximum substrate utilization rate was higher for nitrate versus oxygen respiration. Alkalinity production during denitrification was very near the theoretical stoichiometric value of 3.57 mg as $CaCO_3$ per mg NO_3^--N denitrified.

It was concluded that single-sludge systems incorporating organics removal, nitrification, and denitrification can potentially achieve a high degree of nitrogen and organic carbon removal at lower cost than a similar size system incorporating organics removal and nitrification only. Aeration energy savings and reduced sludge production obtained by the utilization of nitrate respiration in single-sludge systems should result in significant cost savings.

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I. INTRODUCTION

The activated sludge process, developed in England in 1914 (1), has a long and successful history in the treatment of domestic and industrial wastewaters. From 1914 to the present, many modifications to the original process have been proposed. In principle, however, there are two major steps involved in successful treatment performance. The first is substrate utilization and the second is separation of microbial solids from the liquid. As shown in Figure 1, a schematic diagram of the activated sludge process, wastewater flows into an aeration basin where a heterogenous culture of microorganisms utilize organics for growth and energy requirements. Oxygen serves as the terminal electron acceptor in the metabolic reactions. Substrate is utilized and new bacteria (sludge) are produced. Solids-liquid separation is achieved through secondary clarification. Some sludge may be returned to the aeration basin while some is wasted from the system.

All domestic and many industrial wastewaters contain nitrogen. Removal of nitrogen from wastewaters is becoming increasingly important because of the many adverse effects nitrogenous materials have on the environment. The ammonia form of nitrogen in small quantities is toxic to fish. Nitrification of ammonia-nitrogen in receiving waters can deplete dissolved oxygen to dangerously low levels. Also, nitrogen is known to be associated with undesirable algae and plant growth in receiving waters.

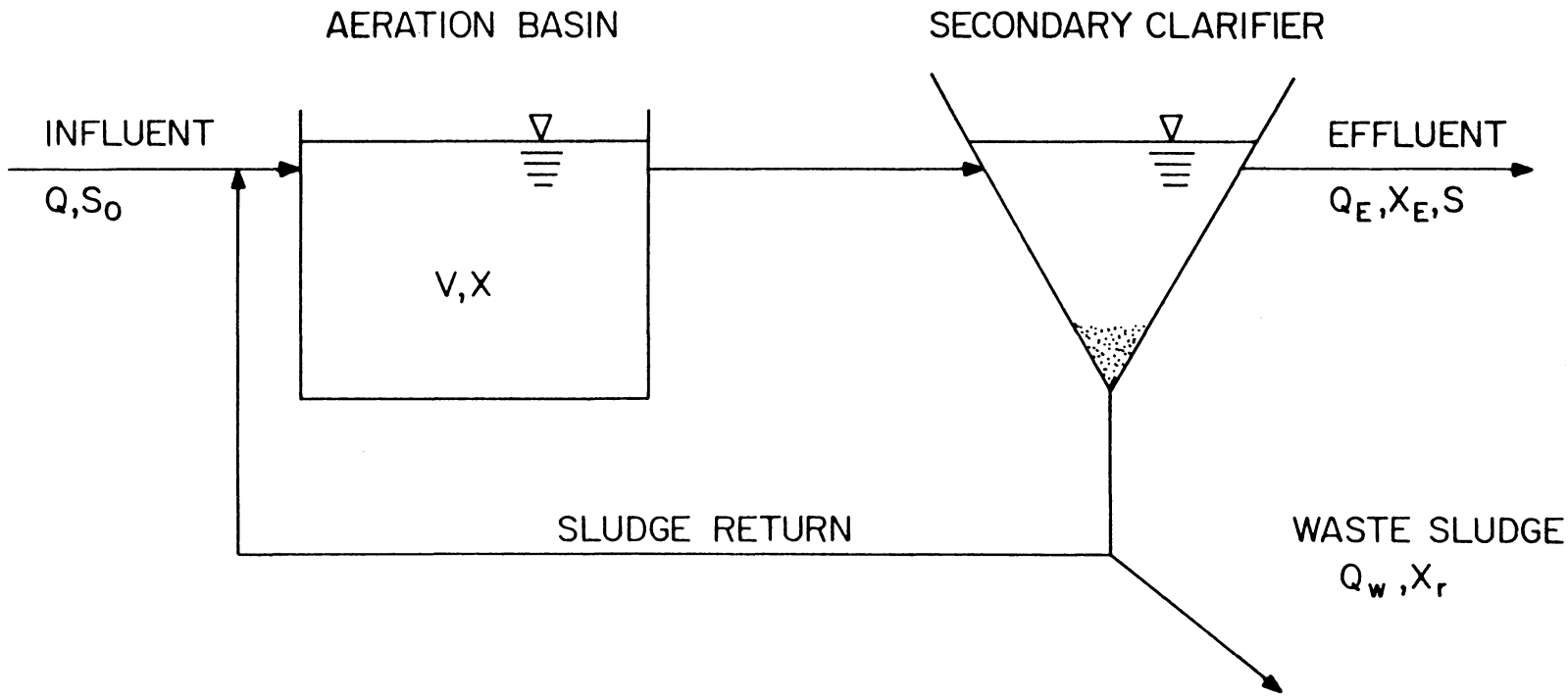

FIGURE 1. FLOW DIAGRAM FOR A COMPLETELY MIXED, SLUDGE RECYCLE ACTIVATED SLUDGE WASTEWATER TREATMENT PROCESS.

Nitrogen removal from wastewaters can be achieved by modifying the activated sludge process to include biological nitrification and denitrification (nitrate respiration). Many different flow schemes have been proposed for nitrogen removal. Some designs include a separate reactor with a separate sludge for biological denitrification (conversion of nitrate to nitrogen gas), and have much higher capital and operating costs than conventional activated sludge because of increased numbers of reactors, increased sludge production, and the use of external carbon sources such as methanol which are required in many cases. A more economical alternative is to remove organic carbon and nitrogen in a single-sludge system.

Single-sludge systems incorporating organics removal, nitrification, and denitrification are complex because a balance between competing microbial processes must be achieved. Oxygen is required for aerobic carbon oxidation and nitrification, whereas an oxygen-free environment is required for denitrification to occur. This is achieved in a single basin by intermittent aeration, or by providing alternating aerated and non-aerated zones within the basin.

A general model to aid in design of single-sludge wastewater treatment systems has been developed by a task group formed by the International Association of Water Pollution Research and Control (IAWPRC), using many of the same concepts of existing activated sludge models (2). The complex nature of single-sludge systems makes it difficult to adequately define the processes involved and their respective process rates, particularly the rate describing microbial

growth of heterotrophs during anoxic conditions. No known literature exists showing direct comparisons of the microbial growth and biokinetic coefficients observed under aerobic and anoxic (anaerobic with nitrate as the terminal electron acceptor) conditions. As a result, the concept for this research project was developed.

Two bench-scale activated sludge reactors were operated under the same conditions except for the terminal electron acceptor employed. One was operated aerobically, utilizing oxygen as the terminal electron acceptor. The other was kept devoid of oxygen, so that only nitrate introduced through the feed solution could be utilized as the terminal electron acceptor. The objectives of this study were to:

(1) Evaluate and compare microbial growth and biokinetic parameters for aerobic and anoxic conditions

(2) Observe alkalinity changes that occur in the two systems and compare the results with theoretical predictions

(3) Assess the implications of the results on the design of activated sludge processes utilizing nitrate respiration.

II. LITERATURE REVIEW

The activated sludge wastewater treatment process is widely used for treatment of domestic and industrial wastewaters. Removal of organic carbon is the primary objective of treatment. Incorporation of nitrification and denitrification into the activated sludge process is necessary to achieve biological nitrogen removal. In this section, a review of organic carbon removal, nitrification, and denitrification in suspended growth biological treatment systems is presented, followed by a review of biokinetic theory as applied to the activated sludge process.

SUSPENDED GROWTH BIOLOGICAL TREATMENT SYSTEMS

A review of some of the important aspects of suspended growth biological treatment systems is presented in this section. For ease of presentation the following three topics will be considered separately: organics removal, nitrification, and denitrification.

Organics Removal

Removal of organic compounds from wastewater is achieved in the activated sludge process by bacteria utilizing organic carbon for growth and energy requirements. Some carbon is incorporated into cell material, and the remainder is oxidized to carbon dioxide. Oxygen supplied via aeration is used as the terminal electron acceptor in the oxidation of organic carbon.

To achieve a required level of treatment, it is necessary to operate the process at a specified mean cell residence time (net

growth rate). This is accomplished by wasting sludge from the secondary clarifier or from the aeration basin. At steady state conditions, the amount of biomass wasted from the system is equal to the amount produced in the system. Organics removal in the activated sludge process is inhibited by extremes of temperature and pH, and presence of toxic materials (3). In an aerobic system, low dissolved oxygen concentrations also inhibit removal of organic carbon compounds.

Activated sludge contains diverse groups of bacteria. In general, they are gram-negative and include members of the genera Pseudomonas, Zoogloea, Achromobacter, Flavobacterium, Nocardia, Bdellovibrio, and Mycobacterium (4). Filamentous Sphaerotilus, Beggiatoa, Thiothrix, Lecicothrix, and Geotrichum can be present in varying numbers (4). Protozoa and rotifers are also present. While it is the bacteria that degrade the waste, protozoa and rotifers act as effluent polishers, consuming dispersed bacteria and biological floc particles (5). The microorganism population in activated sludge is a very complex mixture, and the existing population in any given system is most likely a result of the wastewater composition and particular operating conditions.

All microorganisms require carbon, nitrogen, sulfur, and phosphorus. These elements, combined with oxygen and hydrogen, comprise 96% of the dry weight of a microbial cell (6). Sodium, calcium, iron, manganese, magnesium, chlorine and potassium are required in smaller amounts (6). Trace elements such as molybdenum,

cobalt, copper, boron and zinc are also required in very small amounts, and they occur in sufficient amounts as impurities in other components of a synthetic medium to supply the cells' nutritional requirements (6).

Nitrification

Nitrification, the oxidation of ammonium to nitrate, is necessary to prevent discharges of high levels of ammonium which can: 1) be toxic to fish, and 2) deplete the dissolved oxygen in receiving waters. It is also a first step in biological processes designed for total nitrogen removal. Nitrogen undergoes many transformations in the environment and in wastewater because of biological activity.

Figure 2 is a simplified diagram which shows the relationships between the main biological processes involving nitrogen. The nitrogen cycle is very complex, and a good understanding of the biological processes involved is necessary to understand how nitrification and denitrification occur in the activated sludge process.

Approximately 60% of the total nitrogen in domestic sewage is in the form of ammonium, and 40% is organic nitrogen, which is quickly deaminated or hydrolyzed to ammonium in activated sludge (8). In the presence of nitrifying bacteria and oxygen, nitrate (NO_3^-) is formed according to the following reactions (9):

$$NH_4^+ + 3/2\ O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2H^+ + H_2O \qquad [1]$$

$$NO_2^- + 1/2\ O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \qquad [2]$$

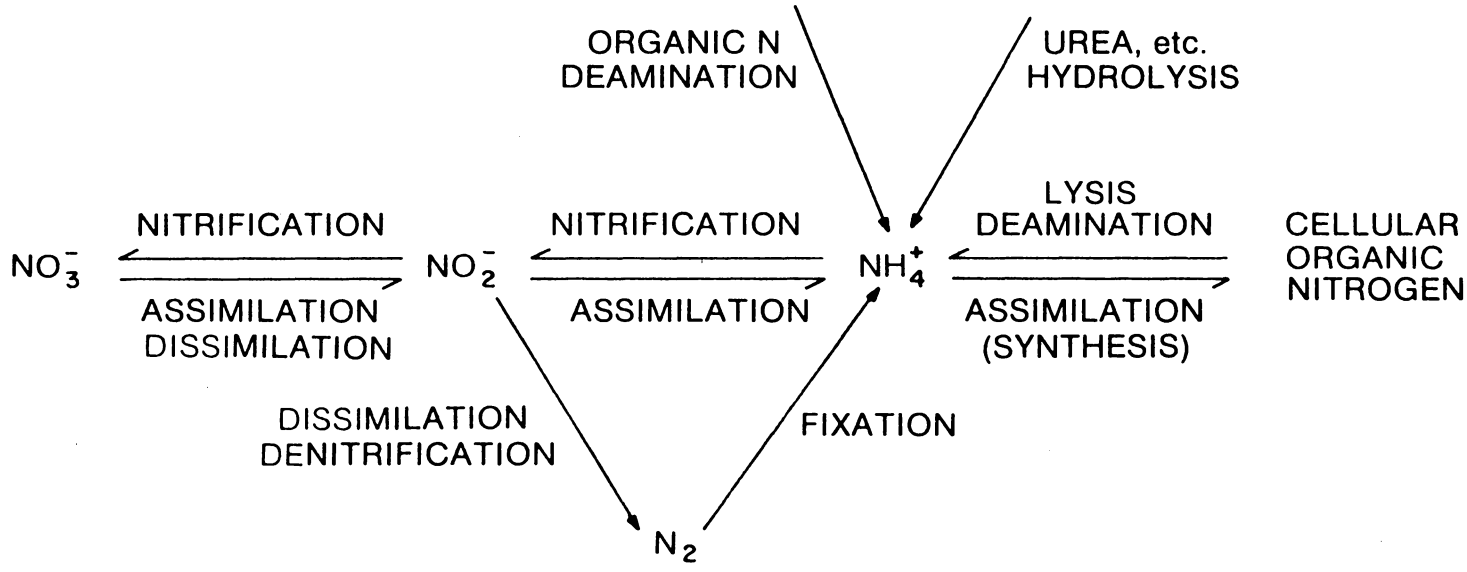

FIGURE 2. BIOLOGICAL PROCESSES INVOLVING INORGANIC NITROGEN. AFTER PAINTER, REFERENCE(7).

Total Reaction

$$NH_4^+ + 2O_2 \xrightarrow{\text{Nitrifiers}} NO_3^- + 2H^+ + H_2O. \qquad [3]$$

Nitrosomonas and Nitrobacter are autotrophic classes of bacteria. Inorganic carbon (carbon dioxide) is used for cell synthesis, and no organic carbon is removed as a result of nitrification.

Theoretically, 4.57 mg of oxygen are required to oxidize one mg of NH_4^+- N, and 7.14 mg of alkalinity as $CaCO_3$ are consumed for each mg of NH_4^+-N oxidized to NO_3^--N. Actual akalinity destruction is almost always less than the theoretically predicted amount (8, 10). This is attributed to the deamination of organic nitrogen to ammonium, which occurs simultaneously with nitrification, and is a net producer of alkalinity, partially offsetting the alkalinity loss. Sherrard et al. (8) have proposed and verified experimentally that alkalinity change in an aerobic treatment system follows the relationship:

$$\Delta \text{ Alk} = 3.57\ [(\Delta \text{ filtrate organic-N}) - (\text{synthesized-N})] - 7.14\ (\Delta\ NO_3^-\text{-N}). \qquad [4]$$

Oxygen must be supplied in sufficient quantities for carbon oxidation and nitrification, otherwise nitrification may be inhibited (10). Nitrification is also inhibited by extremes of temperatures and pH, high organic loadings, low dissolved oxygen concentrations, low alkalinity levels, and presence of toxic materials (10-12). Extra care must be taken to provide suitable environmental conditions when a high degree of nitrification is needed.

Denitrification

Denitrification is the metabolic process by which nitrate is reduced to nitrogen gas. This process is very similar to organic carbon oxidation in aerobic activated sludge except that nitrate (NO_3^-), rather than oxygen (O_2), acts as the terminal electron acceptor, or oxidizing agent, in the oxidation of organic carbon. This process is also referred to as nitrate respiration. Utilization of nitrate as the terminal electron acceptor will occur only when oxygen is not present.

Denitrification is believed by many researchers to follow the following pathway (13):

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2. \qquad [5]$$

Many other pathways with different combinations of metabolic end products are possible (14). However, for the pH ranges and environmental conditions of biological wastewater treatment, conversion of NO_3^- to N_2 can be considered a single-step process (7, 15).

Many of the bacteria capable of denitrification are found naturally in activated sludge. Bacteria capable of denitrification include members of the genera Pseudomonas, Achromobacter, Bacillus, Chromobacter, Micrococcus, and Spirillum (7). These are heterotrophic organisms, which can metabolize organics using either nitrate or oxygen as a terminal electron acceptor (11).

Denitrifying bacteria can also convert nitrate to ammonium, which can then be used for assimilation (7). However, assimilation requires expenditure of energy by the organisms, and will not occur as long as

sufficient ammonium is present for growth (11, 16).

Electron transfer pathways (which represent energy produced) are similar for nitrate and oxygen respiration. At a point near the end of the electron transfer pathway, the nitrate pathway branches off (14). Calculations of free energy yields for organics oxidation with nitrate and oxygen indicate that the energy yield with oxygen is slightly greater (17, 18). The ratio of the energy yield of nitrate respiration to oxygen respiration ranges from 83% - 95%, depending on the organic substrate and balanced biochemical equation (17, 18). Since the energy yield is slightly greater for oxygen respiration, oxygen will be used whenever it is present (7, 17) and growth rates will be slightly greater (14).

When oxygen is absent, denitrifying bacteria produce the enzyme nitrate reductase which is necessary to complete the electron transport process for nitrate respiration (19). The similarity between the two processes accounts for the fact that facultative bacteria in a single-sludge system can switch easily from oxygen respiration to nitrate respiration and vice versa (11). It should be noted that while all denitrifiers can utilize oxygen as an electron acceptor, not all aerobes can utilize nitrate (6). Finding an easy method to determine the fraction of denitrifiers present in a sludge with an aeration step is an active area of research (20, 21).

Theoretically, on an electron transport basis, 1 mg NO_3^--N is equivalent to 2.86 mg O_2 (11). Therefore, 100(2.86/4.57) or 62.5% of the nitrogenous oxygen demand could conceivably be recovered by

utilizing nitrate respiration. This can be calculated from the following balanced equation:

$$2\ H^+ + 2\ NO_3^- \longrightarrow N_2 + 2.5\ O_2 + H_2O. \qquad [6]$$

Denitrification is a net producer of alkalinity, theoretically producing 3.57 mg of alkalinity as $CaCO_3$ per mg of NO_3^--N denitrified. Alkalinity production reported in the literature is usually on the order of 2.9 to 3.0 mg of alkalinity as $CaCO_3$ per mg of NO_3^--N removed (10, 11, 22). However, this is for cases where NO_3^--N is also used as the nitrogen source for cellular synthesis. Alkalinity production based on a nitrate removed basis may be lower than the theoretically predicted amount of 3.57 mg because assimilation of nitrate into cellular material was not taken into account. One field study reported alkalinity production ranging from 2.6 to 3.9 mg of alkalinity as $CaCO_3$ produced per mg of NO_3^--N denitrified (23). The production of alkalinity is important because it partially offsets destruction of alkalinity from nitrification in single-sludge systems.

The optimum pH for denitrification is about 7-8 (11, 24). Denitrification will be inhibited by extremes of pH, as well as extremes of temperature, low organic loadings, presence of dissolved oxygen, and presence of toxic materials (10, 11, 24, 25).

BIOKINETIC THEORY FOR SUSPENDED GROWTH BIOLOGICAL TREATMENT SYSTEMS

A review of biokinetic theory for suspended growth biological treatment systems is presented in the following section. Again, for

ease of presentation, the review is divided into three sections: organics removal, nitrification, and denitrification.

Organics Removal

Mathematical models used to describe conventional activated sludge wastewater treatment systems can be found in numerous articles and textbooks (3, 5, 9, 12, 26). Heterotrophic carbon oxidation can be modeled once the proper biokinetic coefficents are determined. The following assumptions and limitations apply to formulation of these models for continuous flow suspended growth biological treatment systems: 1) existence of steady state conditions, 2) growth kinetics of enrichment cultures of mixed populations are similar to those of pure cultures, 3) influent wastewater characteristics and flow are constant, 4) negligible concentrations of microorganisms are present in the influent, 5) neither buildup of biomass nor waste stabilization occurs in the secondary clarifier, and 6) effluent substrate concentration is equal to the reactor substrate concentration.

From materials balance equations it is apparent that one operational control parameter is the mean cell residence time. Mean cell residence time (θ_c) is the average length of time the mixed liquor volatile suspended solids (MLVSS) remain in the reactor. Mean cell residence time can be calculated from the equation:

$$\theta_c = \frac{VX}{Q_w X + Q_e X_e} \qquad [7]$$

where θ_c = mean cell residence time, time,

V = aeration basin volume, volume,

X = aeration basin biomass concentration, mass/volume,

Q_w = biomass wastage liquid flow rate, volume/time,

Q_e = effluent flow rate, volume/time, and

X_e = effluent biomass concentration, mass/volume.

MLVSS provides a good approximation of X for a soluble waste (12). However, when treating a waste such as domestic sewage, this approximation can overestimate the active biomass by a factor between 5 and 10 because of buildup of nonactive volatile solids and endogenous biomass (27).

Effluent substrate concentration of the limiting substrate can be predicted by the relationship:

$$S = \frac{K_s \ (1 + k_d \ \theta_c)}{\theta_c \ (Y_{max} \ k - k_d) - 1} \qquad [8]$$

where S = effluent substrate concentration, mass/volume,

K_s = half saturation constant, substrate concentration at one-half the maximum growth rate, mass/volume,

k_d = biomass decay coefficient, $time^{-1}$,

Y_{max} = maximum biomass yield coefficient, mass/mass, and

k = maximum substrate utilization rate per unit weight of biomass, $time^{-1}$.

The observed yield represents the actual biomass produced per unit of substrate utilized and can be predicted by:

$$Y_{obs} = \frac{Y_{max}}{1 + k_d \ \theta_c} \qquad [9]$$

where Y_{obs} = observed yield coefficient, mass/mass.

The biomass concentration in the aeration basin can be determined by:

$$X = \frac{Y_{max} (S_o - S) \theta_c}{(1 + k_d \theta_c) \ominus} \quad [10]$$

where S_o = influent substrate concentration, mass/volume, and

θ = hydraulic detention time, time.

The reciprocal of the mean cell residence time, termed the specific growth rate, can be calculated with the expression:

$$1/\theta_c = \mu \quad [11]$$

where μ = specific growth rate, $time^{-1}$.

The specific substrate utilization rate is a measure of the unit of substrate removed per unit of biomass per unit time and can be calculated as follows:

$$q = \frac{S_o - S}{\theta X} \quad [12]$$

where q = specific substrate utilization rate, $time^{-1}$.

Rearrangement and substitution of Equations [11] and [12] into Equation [10] yields the following:

$$1/\theta_c = Y_{max} q - k_d. \quad [13]$$

A plot of $1/\theta_c$ vs. q should give a straight line with a slope of Y_{max} and a y-intercept of $-k_d$.

The maximum specific growth rate can be related to the specific growth rate by the expression proposed by Monod (28):

$$\mu = \mu_m \frac{S}{K_s + S} \quad [14]$$

where μ_m = maximum specific growth rate, $time^{-1}$.

The maximum growth rate can also be calculated from the equation (3, 7):

$$\mu m = Y_{max}\, k. \qquad [15]$$

Since μm is directly proportional to k, then k must be related to the specific substrate utilization rate, q, by the Monod expression (5, 28):

$$q = k \frac{S}{K_s + S} \cdot \qquad [16]$$

By taking the inverse of Equation [16], the following expression results:

$$1/q = (K_s/k)(1/S) + (1/k). \qquad [17]$$

Therefore, the values of K_s and k can be determined from a plot of 1/q vs. 1/S. The straight line which best fits the data should have a slope of K_s/k and a y-intercept of 1/k.

Examples of typical biokinetic coefficients for the activated sludge process are given in Table I. However, values for the coefficients are not restricted to the ranges shown and will vary for each system because of wastewater characteristics and specific operating conditions.

In determining the coefficients Y_{max}, k_d, K_s, and k, biological reactors should be operated over a range of effluent substrate concentrations, i.e. θ_c's, with inorganic nutrients and oxygen in excess so that organics as measured by total biodegradable oxygen demand, T_bOD, are the limiting substrate. Data should be taken at steady state conditions, and mean values for all measured parameters should be calculated.

TABLE I

TYPICAL KINETIC COEFFICIENTS FOR AEROBIC ACTIVATED SLUDGE.
AFTER METCALF AND EDDY, REFERENCE (5).

Coefficient	Basis	Value[a] Range	Typical
Y_{max}	mg VSS/mg BOD_5[b]	0.4 - 0.8	0.6
	mg VSS/mg COD	0.25 - 0.4	0.4
k_d	$days^{-1}$	0.04 - 0.075	0.06
k	$days^{-1}$	2 - 10	5.0
K_s	mg/L BOD_5	25 - 100	60
	mg/L COD	15 - 70	40

a: Values reported are for 20°C

b: VSS = volatile suspended solids

Measurements of ultimate biochemical oxygen demand, BOD_u, have been found to consistently underestimate, while chemical oxygen demand, COD, consistently overestimates T_bOD. T_bOD can be determined from the relationship (3, 29):

$$T_bOD = COD - R \quad [18]$$

where T_bOD = total biodegradable oxygen demand, mass/volume,

COD = chemical oxygen demand, mass/volume, and

R = residual (nonbiodegradable) COD, mass/volume.

Residual COD includes substances from the waste as well as non-biodegradable end products of cell lysis and metabolic by-products (29). Residual COD can be variable depending on operating parameters such as biomass concentration, but should be fairly constant over the typical range of operating conditions for activated sludge (29). Determination of residual COD can be accomplished by plotting q vs. effluent COD, with R being the value of the intercept on the effluent COD axis (3). The value for S used in Equations [14] and [16] should be in terms of T_bOD.

The rate of bacterial growth is defined by the following relationships (5):

$$r_g = \mu X \quad [19]$$

where r_g = rate of bacterial growth, mass/volume, time.

Combining equations [14] and [19] results in the expression:

$$r_g = \mu_m \frac{S}{K_s + S} X. \quad [20]$$

The effect of dissolved oxygen on this rate can be represented by a saturation function and the resulting expression is (2):

$$r_g = \mu_m \frac{S}{K_s+S} \frac{S_o}{K_o+S_o} X \qquad [21]$$

where S_o= dissolved oxygen concentration, mass/volume, and

K_o= half saturation constant for oxygen, mass/volume.

The oxygen term serves as a switching function to model the termination of aerobic growth at low dissolved oxygen concentrations. Therefore the value of K_o is very small (2). When T_bOD alone is limiting, equation [20] is adequate.

Nitrification

Many excellent reviews have been written on biokinetic theory of nitrification (3, 7, 9, 11, 12). Nitrification can be modeled with the same method as used for organics removal, because the growth of nitrifiers also follows a Monod relationship, with NH_4^+-N or NO_2^--N being the limiting substrate (11).

Values of K_s for Nitrosomonas and Nitrobacter are approximately 1 mg/l N in full scale activated sludge plants (30). Experimental values listed for k ranged from 0.9 to 30 and 3.9 to 100 $days^{-1}$ for Nitrosomonas and Nitrobacter, respectively (12). Growth yield coefficients are reported as 0.05 - 0.29 and 0.02 - 0.084 mg biomass produced per mg N removed (12). A typical value for k_d is 0.05 $days^{-1}$ for both species (12). Maximum growth rates for nitrifiers are very low, so a high mean cell residence time is required to maintain nitrifier growth and ensure complete nitrification (12).

Denitrification

Microbial growth during denitrification is dependent on carbon and nitrate concentration and can be described as follows (11):

$$\mu_{dn} = \mu_{mdn} \frac{N}{K_N + N} \frac{S}{K_s + S} \quad [22]$$

where μ_{dn} = actual growth rate of denitrifiers, time $^{-1}$,

μ_{mdn} = maximum growth rate of denitrifiers, time $^{-1}$,

N = nitrate concentration, mass/volume,

K_N = half saturation constant for nitrate, mass/volume,

S = organic substrate concentration, mass/volume, and

K_s = half saturation constant for organic substrate, mass/volume.

Kinetic parameters are determined by the same procedure as the one used for organics removal and nitrification (12, 25). Chemostat studies have been shown to be applicable to systems with recycle (31).

Most of the denitrification literature deals with nitrate limited conditions using methanol as the carbon source. Only one reference reports kinetic parameters for a carbon limited study and methanol was used as the carbon source (25). At 20°C, a Y_{max} value of 0.183 mg VSS per mg COD, a k_d value of 0.04 days^{-1}, a K_s value of 9.1 mg/L as T_bOD, and a k value of 10.3 days^{-1} were reported (25).

The value of K_N is very small, usually about 0.1 mg/l N (10, 33, 34). This means that denitrification is relatively unaffected for nitrate concentrations greater than 1 mg/L, and can be considered zero-order with respect to nitrate for design purposes.

Until recently, most denitrification systems have been designed in a post-denitrification mode with methanol as the organic carbon source. Denitrification with methanol is used because of economics, low growth yields, and the fact that methanol does not add additional nitrogen to the system. Much research has been performed with methanol and other non-nitrogen containing compounds as carbon sources, with nitrate being limiting (32 - 34).

Many different flow schemes have been proposed to incorporate nitrogen removal into the activated sludge process (35 - 46). Each configuration has its own relative merits and defects. Two-sludge and three-sludge systems perform well and are relatively easy to design, but are very expensive to construct and operate. In these systems, denitrification is the final step and occurs in a separate reactor. This configuration is sometimes referred to as post-denitrification.

Experiments in the post-denitrification mode usually supply methanol in excess, with nitrate being limiting (24, 33, 34). Therefore, most reported data are in terms of specific nitrate removal rate, q_{dn}, which is also called the denitrification rate. A short aeration period following denitrification is usually required to prevent rising sludge in the final clarifier and to remove any excess methanol, further adding to the expense of this method (34, 35).

In a pre-denitrification system, wastewater organic carbon is utilized as the carbon source for denitrification. Only one reactor is required, but a high rate of mixed liquor recycle is required to supply nitrates to the anoxic section of the reactor.

SINGLE-SLUDGE SYSTEMS

In most cases, 70% - 90% removal of nitrogen can be achieved in a single-sludge system with alternating aerobic or anoxic (nitrate respirating) conditions with no mixed liquor recycle required (23, 36-42, 46). These systems utilize the wastewater organics as the carbon source, eliminating the need for methanol addition. Recent studies show that by utilizing nitrate respiration, high removals of nitrogen and organic carbon can be achieved in a relatively small single reactor (23, 46). Also, operating costs are reportedly lower for single-sludge systems utilizing nitrate respiration than for a similar size aerobic activated sludge system with nitrification, due to aeration energy savings and reduced sludge production (46). In many cases, only minor modifications are necessary to convert an activated sludge plant with nitrification to one utilizing nitrate respiration (23, 46).

The ratio of mass of chemical oxygen demand (COD) removed per mass of NO_3^--N removed varies from waste to waste (11). For methanol, this ratio is reported to be 3.7 (34). This ratio is for the case where NO_3^--N is also the nitrogen source for cell material. For cases where NH_4^+-N is available for cell synthesis, the ratio of COD removed per NO_3^--N removed would be greater.

A mathematical model has been proposed for single-sludge nitrification/denitrification systems (2, 47). In this model, the microbial growth rate for anoxic conditions is described as follows:

$$r_{g2} = \mu_m \frac{S}{K_s + S} \frac{N}{K_N + N} \eta X \qquad [23]$$

where r_{g2} = rate of bacterial growth under anoxic conditions, mass/volume·time,

μ_m = maximum specific growth rate under aerobic conditions, $time^{-1}$,

S = organic substrate concentration,, mass/volume,

K_s = half saturation constant for organic substrate under aerobic conditions, mass/volume,

N = nitrate concentration, mass/volume,

K_N = half saturation constant for nitrate, mass/volume,

η = correction factor for denitrification (<1), and

X = biomass concentration, mass/volume.

The maximum growth rate for aerobic conditions is used in Equation [23] although the maximum growth rate for anoxic conditions can be experimentally determined. This expression implies that the effect of biodegradable substrate, including the value of K_s, is identicial for aerobic and anoxic conditions. A nitrate term is used in the same way an oxygen term is used in Equation [21], i.e., to model the termination of anoxic growth at low nitrate concentrations.

It appears that Equation [23] is conceptually inaccurate to an extent because it does not represent the actual processes involved and their effect on r_{g2}. Equation [23] uses a correction factor for denitrification, η, to account for a lower maximum specific growth rate under anoxic conditions and to account for the fact that only a

fraction of the biomass in a single-sludge system can utilize nitrate as a terminal electron acceptor. A more conceptually accurate equation would distinguish between the effects of partial ability and partial activity by using the maximum specific growth rate under anoxic conditions while accounting separately for the fact that only a fraction of the biomass can utilize nitrate as a terminal electron acceptor.

SUMMARY

Mathematical models for organics removal, nitrification, and denitrification in activated sludge suspended growth biological treatment systems have been presented and discussed.

Alkalinity production during denitrification is usually reported based on total nitrate removed in systems which also assimilate nitrate into cell material. Alkalinity production reported on this basis may underestimate alkalinity production where sufficient ammonium is present for cell synthesis, which could be the case in a properly operated single-sludge system.

Single-sludge nitrification/denitrification systems appear to be the most economical systems for biological nitrogen removal. Recent studies suggest that aeration costs and sludge production are lower for a single-sludge system than for a similar size conventional activated sludge plant with nitrification, and may perform with greater COD removal efficiency.

Little research has been performed on microbial growth during denitrification with organic carbon as the limiting substrate. More

research is needed in this area to understand how microbial growth and substrate removal occur under nitrate respirating conditions in a single-sludge nitrification/denitrification wastewater treatment plant.

III. EXPERIMENTAL APPROACH AND LABORATORY PROCEDURES

In this chapter the overall experimental approach, including the methods of sampling and analysis and the materials used during this investigation, are described. The two major subsections in this chapter are: Experimental Approach, in which the research objectives are stated; and Laboratory Procedures, in which the methods and materials used in operating the two bench-scale biological reactors are presented.

EXPERIMENTAL APPROACH

The primary objective of this study was to determine biokinetic and growth parameters for "aerobic" activated sludge and for a similar activated sludge system utilizing nitrate respiration ("anoxic" activated sludge). The data collected included MLVSS, alkalinity, pH, COD, NH_4^+-N, and NO_3^--N and NO_2^--N. A listing of all parameters measured daily is presented in Table II. By comparing the two systems side-by-side, information about the relationships governing the two systems should be obtained. The results of this study should provide a better understanding of the processes of substrate removal and anoxic growth involved in a single-sludge wastewater treatment system carrying out organic substrate removal, nitrification, and denitrification.

Another objective of the study was to observe alkalinity changes in the aerobic and the anoxic systems and compare with theoretical predictions.

TABLE II

PARAMETERS MONITORED DAILY

I. Influent Feed

A. Chemical Oxygen Demand
B. Alkalinity
C. Total Kjeldahl Nitrogen
D. pH
E. Dissolved Oxygen Concentration
F. NO_2^--N Concentration *
G. NO_3^--N Concentration *

II. Biological Reactors

A. Mixed Liquor Suspended Solids
B. Mixed Liquor Volatile Suspended Solids
C. pH
D. Dissolved Oxygen Concentration
E. Temperature

III. Filtered Effluent

A. Chemical Oxygen Demand
B. Alkalinity
C. Total Kjeldahl Nitrogen
D. pH
E. NO_2^--N Concentration
F. NO_3^--N Concentration

IV. Unfiltered Effluent

A. Total Suspended Solids
B. Volatile Suspended Solids

* Anoxic Reactor Only

To accomplish the above objectives, two bench-scale continuous flow activated sludge reactors with internal cell recycle were operated over a range of mean cell residence times from 1.5 days to approximately 15 days. The first reactor, termed the aerobic reactor, was operated similar to a conventional activated sludge unit, with air continuously bubbled into the mixed liquor so that oxygen would be utilized as the terminal electron acceptor in the biochemical reactions. The second reactor, termed the anoxic reactor, was designed to exclude all oxygen, so that only nitrate (NO_3^-) introduced into the reactor through the feed would be utilized as the electron acceptor. The feed solution for the anoxic reactor was purged of oxygen by bubbling nitrogen gas into the feed tank under high pressure for 15 minutes each time the feed was prepared. The feed tank and reactor were sealed from exposure to the atmosphere, and nitrogen gas was bubbled under low pressure into the feed and the reactor continuously to maintain a positive nitrogen pressure at all times throughout the experiments. The effluent collection tube was submerged in the effluent collection tank to avoid introduction of air into the reactor from the atmosphere. Dissolved oxygen was monitored to ensure that oxygen was excluded. Schematic diagrams of the aerobic and anoxic systems utilized are provided in Figures 3 and 4, respectively.

LABORATORY PROCEDURES

The materials used in operating the two bench-scale activated sludge reactors are described in this section, followed by a

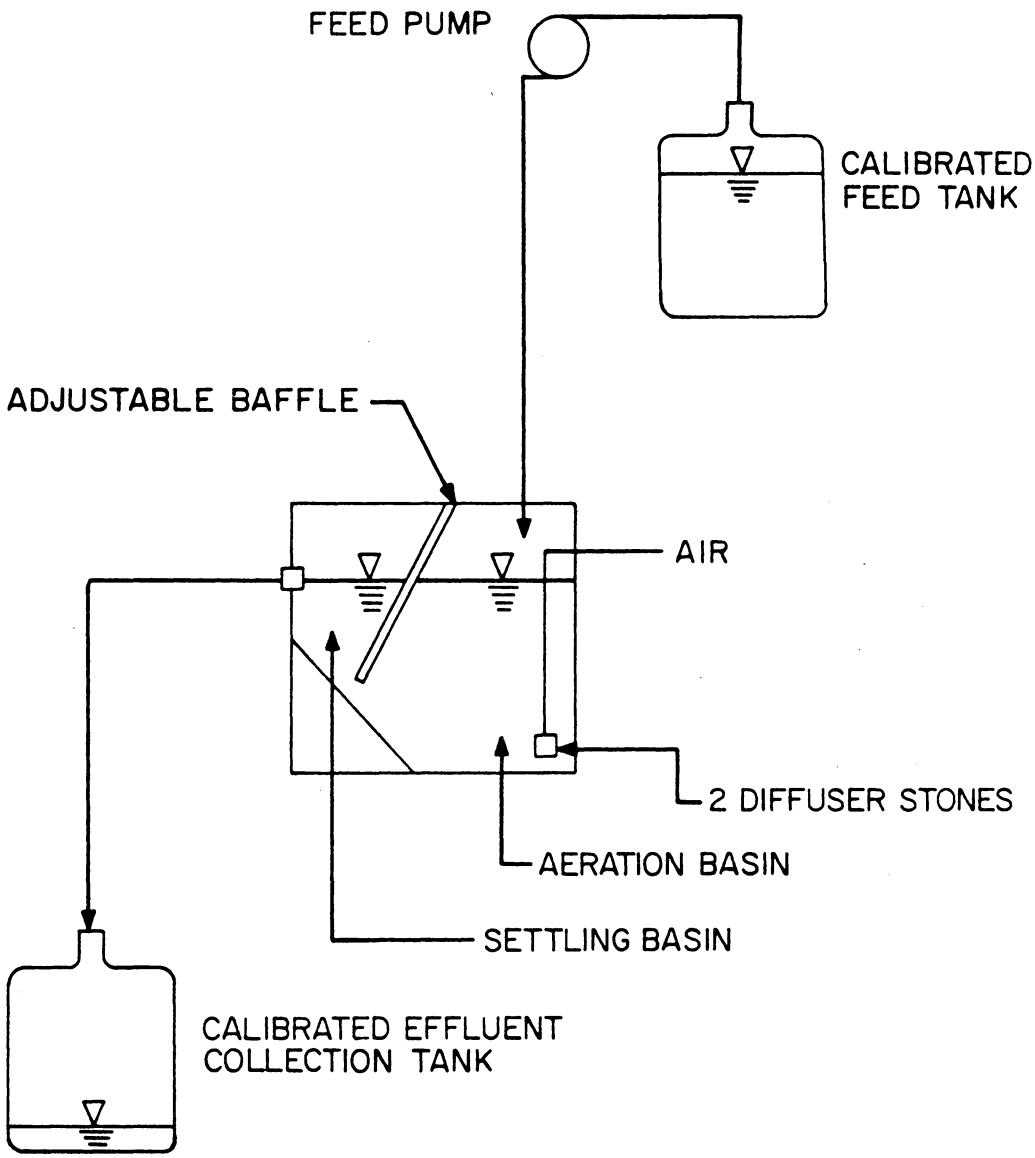

FIGURE 3. SCHEMATIC DIAGRAM OF THE BENCH SCALE AEROBIC ACTIVATED SLUDGE UNIT.

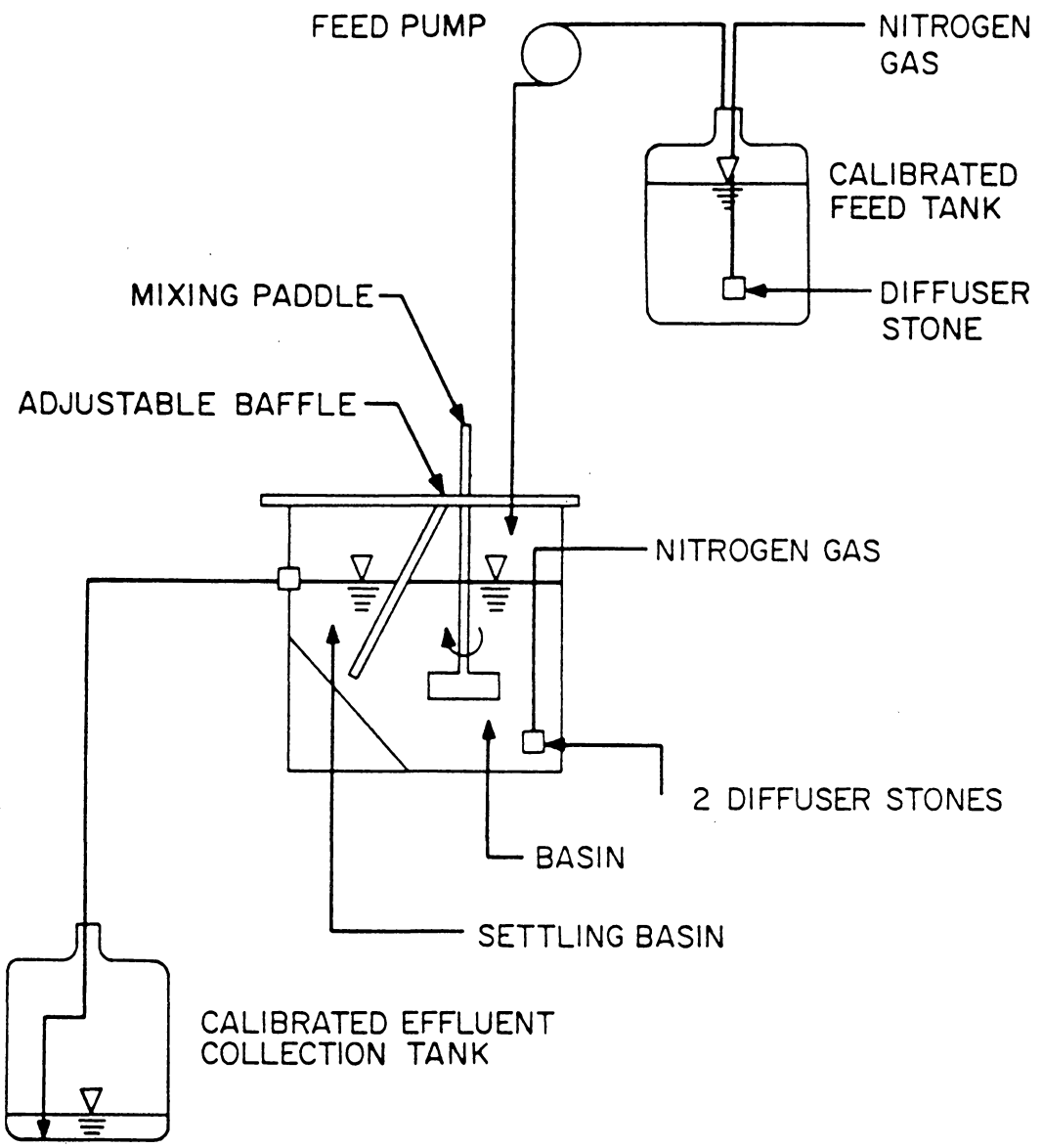

FIGURE 4. SCHEMATIC DIAGRAM OF THE BENCH SCALE ANOXIC ACTIVATED SLUDGE UNIT.

discussion of the experimental procedure and analytical procedures used during the course of this study.

Laboratory Apparatus

The laboratory reactors were constructed of 3/8 inch Plexiglass plastic. Each had a total volume of 8.5 liters, with a 6.3 liter mixed liquor volume, and a 2.2 liter clarifier volume. Mixed liquor chambers and clarifiers were separated by an adjustable baffle. Synthetic wastewater was pumped by a Cole-Parmer (Chicago, IL) Model 7568 peristaltic pump with two Masterflex pump heads through 1/4 inch Tygon tubing from 20 liter calibrated Nalgene carboys to the reactor. A volumetric flow rate of approximately 15 liters per day resulted in a hydraulic detention time of approximately 10 hours (0.42 days) in the mixed liquor basins. Effluent traveled through 1/2 inch Tygon tubing and was collected in 20 liter calibrated Nalgene carboys. Influent and effluent carboys were cleaned daily with a detergent and bleach solution and rinsed thoroughly. Influent and effluent tubing was cleaned, disinfected with bleach, and rinsed with hot water every few days as needed.

Sides of the aerobic reactor were scraped daily in an attempt to remove biological slime. Since the anoxic reactor was enclosed, it was difficult to clean the inside walls, particularly without exposing the mixed liquor to oxygen. However, the sides were cleaned three times throughout the experiment after data collection periods, and then reclosed and operated under anoxic conditions before data were taken again. Biological growth on the sides of the anoxic reactor was

not visibly apparent and therefore was assumed not to be a problem. Dissolved oxygen in the mixed liquor of the aerobic reactor was monitored and never fell below 4.7 mg/l. Vigorous aeration was required to ensure complete mixing and adequate sludge return under the baffle.

Nitrogen gas was supplied to the anoxic feed carboy and anoxic reactor from a compressed nitrogen gas tank. Complete mixing and sludge return was achieved in the anoxic reactor by the use of a mixing paddle powered by a small electric motor. Dissolved oxygen levels less than 0.5 mg/l in the feed and less than 0.1 mg/l in the reactor were maintained so the effects of oxygen respiration in the anoxic reactor could be ignored.

The reactors were located in a constant temperature ($20^{\circ}C \pm 1^{\circ}C$) room. However, approximately one month after the first data were taken, the air-conditioning system failed. It soon became apparent that it would take more than a month for the system to be replaced. Therefore, it was decided to continue the experiment without temperature control. The temperature inside the reactors was monitored and recorded, resulting in a temperature range of $24^{\circ}C \pm 4^{\circ}C$ in the aerobic reactor and $26^{\circ}C \pm 4^{\circ}C$ in the anoxic reactor.

Synthetic Feed

Listed in Table III are the chemicals used in the preparation of the synthetic feeds used in these experiments. Stock solutions were made and used in the daily preparation of the feed solutions. Both stock and feed solutions were prepared using tap water. All of the

TABLE III

COMPOSITION OF INFLUENT TO REACTORS

Compound	Stock Concentration (g)/2 liter	Quantity Used (ml)/18 liter	Final Concentration in 18 liters (mg/l)
Bacto-peptone (Nutrient Broth)	106.00	100	294.0
$MgSO_4.7H_2O$	91.3	20	50.0
$MnSO_4 \cdot H_2O$	9.0	20	5.00
$FeSO_4 \cdot 7H_2O$	4.0	20	2.22
KCl	12.6	20	7.00
$(NH_4)_2SO_4$	84.8	20	47.11
K_2HPO_4	353.6	20	196.44
$NaHCO_3$	100.0	200	555.56
$CaCl_2$	6.75	20	3.75
$Ca(NO_3)_2 \cdot n\ H_2O$	-	*10	555.56

*Grams of $C_a(NO_3)_2 \cdot n\ H_2O$ added to Reactor 1 feed only.

stock solutions were prepared as needed and kept in separate glass jars in the laboratory, except for the bacto-peptone stock solution, which was prepared every five days and kept refrigerated at 4°C to minimize biological growth and degradation.

The only difference in the feeds to the two reactors was that nitrate in the form of $Ca(NO_3)_2 \cdot nH_2O$ was added to the feed of the anoxic reactor. Similar feed solutions, as well as operating techniques, were used so that a direct comparison of the two systems could be made.

The mixture of chemical components was chosen as outlined in Table III to provide all nutrients required for biological growth. Trace nutrients such as copper, molybdenum, and zinc were assumed to be present due to impurities present in the chemicals. The amount of nitrate added to the anoxic feed was chosen so that carbon would be limiting rather than nitrate, based on an assumed chemical oxygen demand (COD) of approximately 5 mg/l required per nitrate-nitrogen (NO_3^--N) denitrified. The theoretical chemical compositions of the feeds are also shown in Table III.

The theoretical COD of the feed solution was 300 milligrams per liter (mg/l) which was provided by the bacto-peptone nutrient broth. The bacto-peptone also supplied most of the total Kjeldahl nitrogen (TKN). Additional nitrogen in the form of ammonium sulfate, $(NH_4)_2SO_4$, was added to maintain a COD:TKN ratio of approximately 5:1. Sodium bicarbonate ($NaHCO_3$) was added to buffer the solution since an increase in hydrogen ion concentration was expected to occur in the

aerobic reactor due to alkalinity destruction occurring during nitrification.

The synthetic feed solutions were prepared daily. A few liters of tap water were added to the clean influent carboys, after which the appropriate amounts of stock solutions and chemicals were added. The carboys were filled to 18 liters and throughly mixed. Any feed solution remaining the following day was discarded, and new feed solution was prepared.

Acclimation and Start-Up

Activated sludge for seeding the reactors was obtained from a bench-scale activated sludge unit being operated in the environmental engineering laboratory at Virginia Polytechnical Institute and State University. This reactor had been seeded with activated sludge from the Lower Stroubles Creek Treatment Plant of the Blacksburg-VPI Sanitation Authority, but had since been acclimated to the same feed solution used in this study.

Heterogeneous microbial cultures were grown aerobically on a batch basis for approximately three weeks, utilizing concentrated feed solutions containing bacto-peptone and nutrients. Once the microbial population reached a concentration of approximately 2500 mg/l, the reactors were set up as shown in Figures 3 and 4, and continuous flow operation was initiated. The desired mean cell residence time (θ_c) was accomplished by wasting a portion of the mixed liquor each day.

Wasting was performed in the anoxic reactor by siphoning the desired amount of mixed liquor with a hand-held siphon pump through an

opening, that was otherwise plugged, in the sealed Plexiglass cover. Wasting from the aerobic reactor was performed in a similar manner.

Mean cell residence time (θ_c) served as the primary control parameter. Relatively constant valves for θ_c were maintained by wasting a volume slightly less than a reciprocal of θ_c times the mixed liquor basin volume, taking into account the cells lost in the effluent. Wasting was performed at the end of each 24 hour operating period, allowing the reactors to stabilize before sampling the next day.

Steady state conditions were assumed to exist when all of the operational parameters remained constant over a period of time. Once an approximate steady state was observed, the reactors were operated for at least two times the θ_c value before data were taken. The parameters in Table III were then monitored daily for a period of 6 days, and the data averaged.

A θ_c of 1.5 days was attained by removing the adjustable baffles and operating the units as chemostats with an 8.5 liter volume. A chemostat is a completely mixed continuous flow biological reactor with no solids recycle, where effluent parameters (pH, solids, etc.) are the same as the mixed liquor parameters, and the hydraulic residence time is equal to the mean cell residence time. This change from normal operational procedures was deemed necessary because sludge settling problems were expected at a θ_c of 1.5 days. A volumetric flow rate of approximately 5.7 liters per day was used. All other operating procedures were the same as described previously.

Collection and Analysis of Samples

Once steady state conditions were attained, a rigorous schedule of sampling and analysis was followed. A 500 milliliter (ml) sample of effluent was collected from the effluent tubing into a flask. A mixed liquor sample ranging in size between 10 to 60 mls was taken. These samples were filtered and total suspended solids and volatile suspended solids analyses were performed. Next, the influent and effluent carboys were emptied, cleaned, and rinsed. The tubing was cleaned as needed. Feed solutions were prepared and samples were taken. The pH and dissolved oxygen of the feed, mixed liquor, and effluent were then measured. Alkalinity was determined on the feed solution and filtered effluent. COD and TKN tests were often performed on the same day the samples were taken. When this was not possible, feed solution and filtered effluent samples were acidified to a pH of 2 with concentrated sulfuric acid and refrigerated at 4° C until COD and TKN tests could be performed, which was always within a week.

Approximately 20 mls of feed solution and filtered effluent were frozen until nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) concentrations could be determined. Since an ion chromatograph was used for these determinations, freezing was chosen as the best method of storing these samples. Acidification with sulfuric acid adds a large number of sulfate ions to the sample, which could interfere with NO_2^--N and/or NO_3^--N determinations.

Analytical Procedures

The analytical techniques used in this study are as follows:

Chemical Oxygen Demand (COD). The COD of the unfiltered synthetic feed solutions and filtered effluents was determined by the dichromate reflux method as outlined in Standard Methods for the Examination of Water and Wastewater (48).

Total Kjeldahl Nitrogen (TKN). TKN analyses were performed on unfiltered synthetic feed and filtered effluent samples using the semi-micro-Kjeldahl method as outlined in Standard Methods (48).

Nitrite Nitrogen (NO_2^--N) and Nitrate Nitrogen (NO_3^--N). NO_2^--N and NO_3^--N were determined on filtered samples using a Dionex 2010i Ion Chromatograph (Dionex Corporation, Palo Alto, CA) equipped with an anion separator column (Dionex P/N 30985), an anion fiber suppressor, and a conductivity detector. The output range was set at 10 μS full scale and the signal was recorded with a Spectra-Physics 4270 integrator. Standards with concentrations of 5 mg/L NO_2^--N and 10 mg/L NO_3^--N were used, and samples were diluted to approximately these same concentrations or less. All testing was performed in accordance with Standard Methods (48).

Alkalinity. The alkalinity of unfiltered feed and filtered effluent samples was determined in accordance with Standard Methods (48). A Fisher Accumet Model 610A pH meter was used to determine the correct end-point pH.

pH. The Fisher Accumet Model 610A pH meter was utilized for pH determinations according to the glass electrode method described in Standard Methods (48). The meter was standardized prior to each use with commercially prepared buffer solutions.

Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS). MLSS concentration was determined as total nonfiltrable residue dried at 103 - 105 C as described in Standard Methods (48). MLVSS concentration was determined as total volatile residue measured after heating to 550 C as described in Standard Methods (48). The total suspended solids (TSS) and volatile suspended solids (VSS) of the effluent were determined in the same manner as MLSS and MLVSS, respectively. Volumes of 10 to 60 mls were used for MLSS/MLVSS determinations, while volumes of 60 to 400 mls were used for TSS/VSS determinations, depending on the filterability of the samples. Since VSS is a better measure of the biomass than TSS, MLVSS and VSS were considered to be the biomass concentrations for purposes of this study. Samples were filtered through 0.45 Whatman Glass Microfibre filters (Whatman Laboratory Products Inc., Clinton, NJ).

Dissolved Oxygen. Dissolved oxygen (DO) concentrations were measured with a YSI Model 54 A Oxygen Meter (Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio). The instrument was calibrated prior to use by reading against air in accordance with the manufacturer's instructions and Standard Methods (48).

Temperature. Temperature of the reactors was monitored using a mercury filled thermometer and in accordance with Standard Methods (48).

IV. RESULTS AND DISCUSSION

To accomplish the objectives of this research, the two continuous flow, completely mixed activated sludge reactors were operated for approximately five months. The first, or aerobic reactor, was aerated at all times and utilized oxygen as the terminal electron acceptor. The second, or anoxic reactor, was devoid of oxygen and utilized nitrate introduced through the feed as the terminal electron acceptor.

Oxygen and nitrate were supplied in excess to the aerobic and the anoxic reactors, respectively, to insure that biodegradable organics would be the limiting substrate. The reactors were operated and data were collected as described in the Experimental Methods and Laboratory Procedures. Raw data collected are presented in tabular form in the Appendix.

The remainder of this chapter is devoted to a presentation of the results of this study, including determination of growth and kinetic parameters for both the aerobic and the anoxic reactors, and a discussion of the results.

SUMMARIES OF STEADY STATE DATA

A summary of the steady state data obtained for the aerobic and the anoxic reactors is presented in Tables IV and V, respectively. Steady state data were assumed to be the daily averages of the raw data presented in the Appendix.

Some interesting observations can be made from the summaries of the steady state data. Table IV shows that in the aerobic reactor, substrate removal followed a normal pattern, with effluent COD values

TABLE IV

SUMMARY OF STEADY STATE DATA FOR AEROBIC REACTOR

Parameter	Value for Given θ_c, days				
	1.5	3.0	6.0	10.0	15.2
COD					
Feed (mg/l)	298	310	304	313	304
Effluent (mg/l)	37	34	28	27	25
TKN concentration					
Feed (mg/L)	57	64	54	56	68
Effluent (mg/l)	0	10	1	0	0
NO_2^--N concentration					
Effluent (mg/l)	3	28	6	9	0
NO_3^--N concentration					
Effluent (mg/l)	47	16	41	38	61
Biological solids					
Mixed liquor (mg/L)	115	709	1115	1606	2237
Effluent (mg/l)		7	24	13	15
pH					
Feed	8.2	8.1	8.1	8.1	8.0
Mixed liquor	8.1	8.0	7.8	7.8	7.4
Effluent		7.9	7.8	7.8	7.3
Alkalinity as $CaCO_3$					
Feed (mg/l)	416	441	419	433	427
Effluent (mg/l)	175	271	226	246	86

TABLE V

SUMMARY OF STEADY STATE DATA FOR ANOXIC REACTOR

Parameter	Value for Given θ_c, days				
	1.5	3.0	6.1	9.6	15.1
COD					
Feed (mg/l)	302	310	312	305	304
Effluent (mg/l)	38	41	26	27	24
TKN concentration					
Feed (mg/L)	62	64	60	68	54
Effluent (mg/l)	49	56	49	57	45
NO_2^--N concentration					
Feed (mg/l)	1	1	1	5	4
Effluent (mg/l)	2	2	0	3	1
NO_3^--N concentration					
Feed (mg/l)	71	73	77	69	68
Effluent (mg/l)	18	12	11	15	12
Biological solids					
Mixed liquor (mg/L)	70	403	678	1185	2327
Effluent (mg/l)		14	9	19	21
pH					
Feed	7.8	7.6	7.5	7.5	7.7
Mixed liquor	8.2	8.1	7.9	8.0	8.5
Effluent		8.1	7.9	8.0	8.4
Alkalinity as $CaCO_3$					
Feed (mg/l)	425	444	473	435	429
Effluent (mg/l)	644	669	715	616	660

becoming smaller as θ_c is increased. A close examination of Table IV reveals that the nitrification data were not as expected. Nitrite accumulated at a θ_c of 3.0 days. This indicates that Nitrobacter may have been inhibited in oxidizing nitrite to nitrate. Also, a higher degree of nitrification occurred at θ_c = 1.5 days, which was operated as a chemostat, than at θ_c = 3.0 days, which is contrary to widely accepted nitrification theory. Possible explanations for this could be that high numbers of nitrifiers were attached to the walls inside the chemostat reactor, shifts in temperature, or experimental or procedural error. The difference in hydraulic detention time should not have had an effect on nitrification.

Other data for the aerobic reactor followed the expected pattern. For example, pH decreases of 0.2 to 0.7 pH units were observed to follow corresponding decreases in alkalinity, i.e., the greater the change in alkalinity, the greater the pH change.

Table V shows a summary of the steady state data for the anoxic reactor. Substrate removal followed a slightly erratic pattern but was comparable to that in the aerobic reactor. Nitrite did not accumulate in the anoxic reactor, reinforcing the assertion that denitrification can be considered a single step reaction (nitrate to nitrogen gas) for design purposes (7). Biological solids concentrations were less in the anoxic reactor than in the aerobic reactor for a given θ_c except for mean cell residence times of approximately 15 days, where the solids concentrations for the two

reactors are nearly equal. Increases in pH of 0.4 to 0.8 pH units were observed to follow corresponding increases in alkalinity.

Average mg of COD removed per mg of nitrate-nitrogen denitrified ranged from 4.4 to 5.1 with an average of 4.9 mg COD/mg NO_3^--N, which agrees well with values reported in the literature. Experimental results also agree strongly with theory, as shown by the following example.

The ratio of change in oxygen to the COD removed in a biological treatment system can be described by the following equation (49):

$$\Delta O_2 / \Delta COD = 1 - f_{cv} Y \qquad [24]$$

where ΔO_2 = change in oxygen, mass,

ΔCOD = change in COD, mass,

f_{cv} = mg COD/mg VSS = 1.42, mass/mass, and

Y = yield coefficient, mass/mass.

The term f_{cv} is used to designate the theoretical COD of a unit of biomass, which can be assumed to be equal to 1.42 (49). Using Y_{max} = 0.272 as determined from Figure 6 for the anoxic reactor and solving Equation [24], the ΔO_2: ΔCOD ratio is found to be 0.614. Assuming 1 mg NO_3^--N is equivalent to 2.86 mg O_2, $\Delta O_2 / \Delta COD = 0.614$ is equivalent to a COD removed per nitrate-nitrogen denitrified ratio of 4.7 mg COD/mg NO_3^--N, which agrees well with the measured value of 4.9.

EVALUATION OF GROWTH AND KINETIC COEFFICIENTS

In evaluating growth and kinetic coefficients, it was necessary to treat the data as if it were taken at the same temperature, even

though actual temperatures were slightly different for different data points due to a breakdown in the air-conditioning system. The average temperatures for the aerobic and the anoxic reactors were 24°C and 26°C, respectively. The anoxic reactor was approximately 2° C warmer at any given time than the aerobic reactor because the covered anoxic reactor captured heat generated from the exothermic denitrification reaction.

A plot of the specific growth rate versus the specific substrate utilization rate for the aerobic reactor is shown in Figure 5. The straight line of best fit was determined by a linear regression analysis. Based on Equation [13], Y_{max} was determined to be equal to 0.503 mg VSS produced/mg COD removed, and k_d was equal to 0.111 $days^{-1}$.

A similar plot for the anoxic reactor is shown in Figure 6. Y_{max} was evaluated to be 0.272 mg VSS/mg COD, and k_d was 0.057 $days^{-1}$. Straight lines as determined by linear regression fit the data points well. Biokinetic values determined from Figures 5 and 6 agree well with values reported in the literature.

The maximum yield coefficient for the aerobic reactor was almost double that of the anoxic reactor. Observed yield for the aerobic reactor was greater than that for the anoxic reactor until they coincide at a mean cell residence time of approximately 15 days. Observed yields coincided because endogenous decay in the aerobic reactor occurred at a much faster rate than in the anoxic reactor, as exhibited by the values of k_d. Endogenous decay of microorganisms

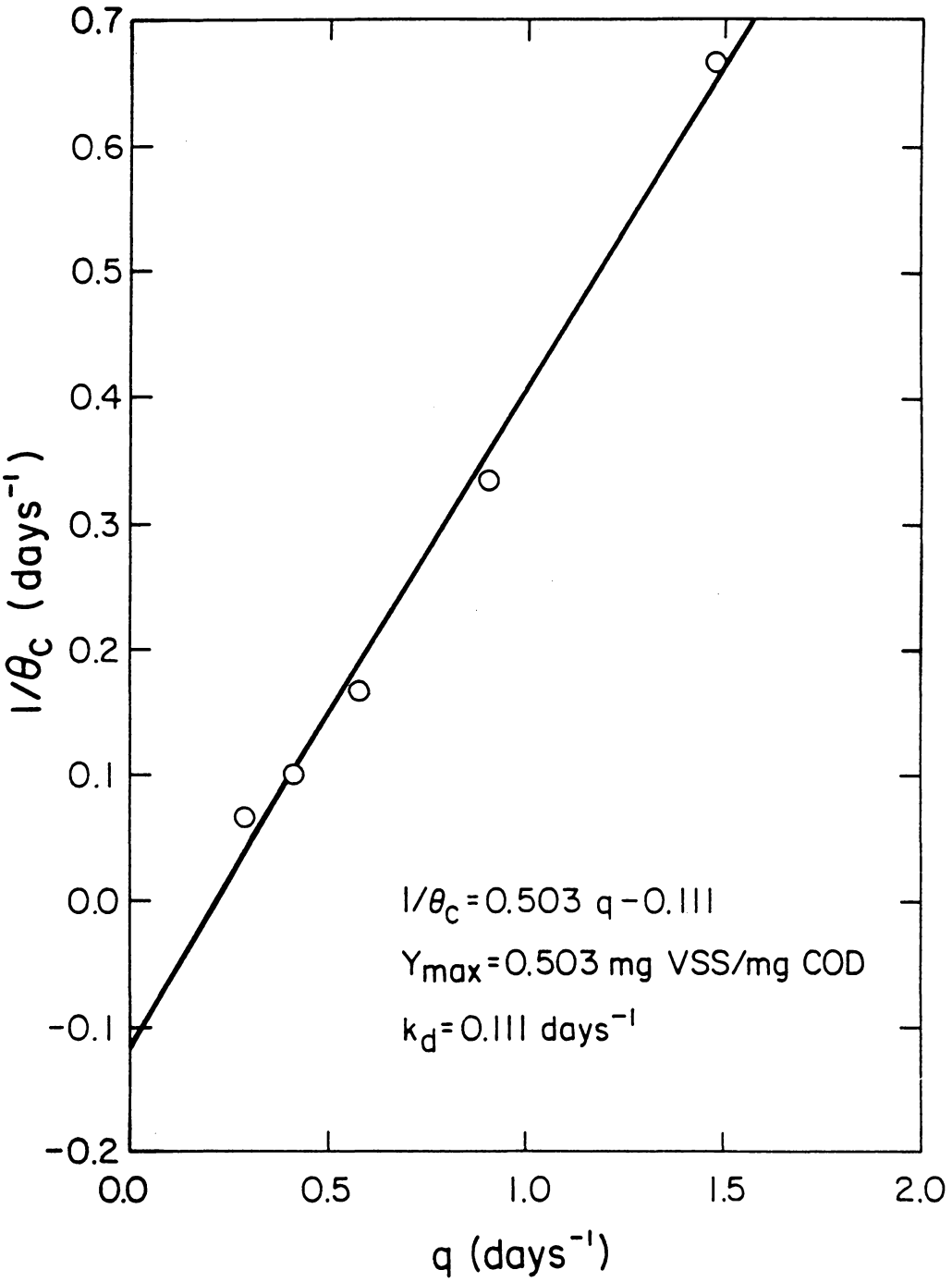

FIGURE 5. SPECIFIC GROWTH RATE VERSUS SPECIFIC SUBSTRATE UTILIZATION RATE FOR THE AEROBIC REACTOR.

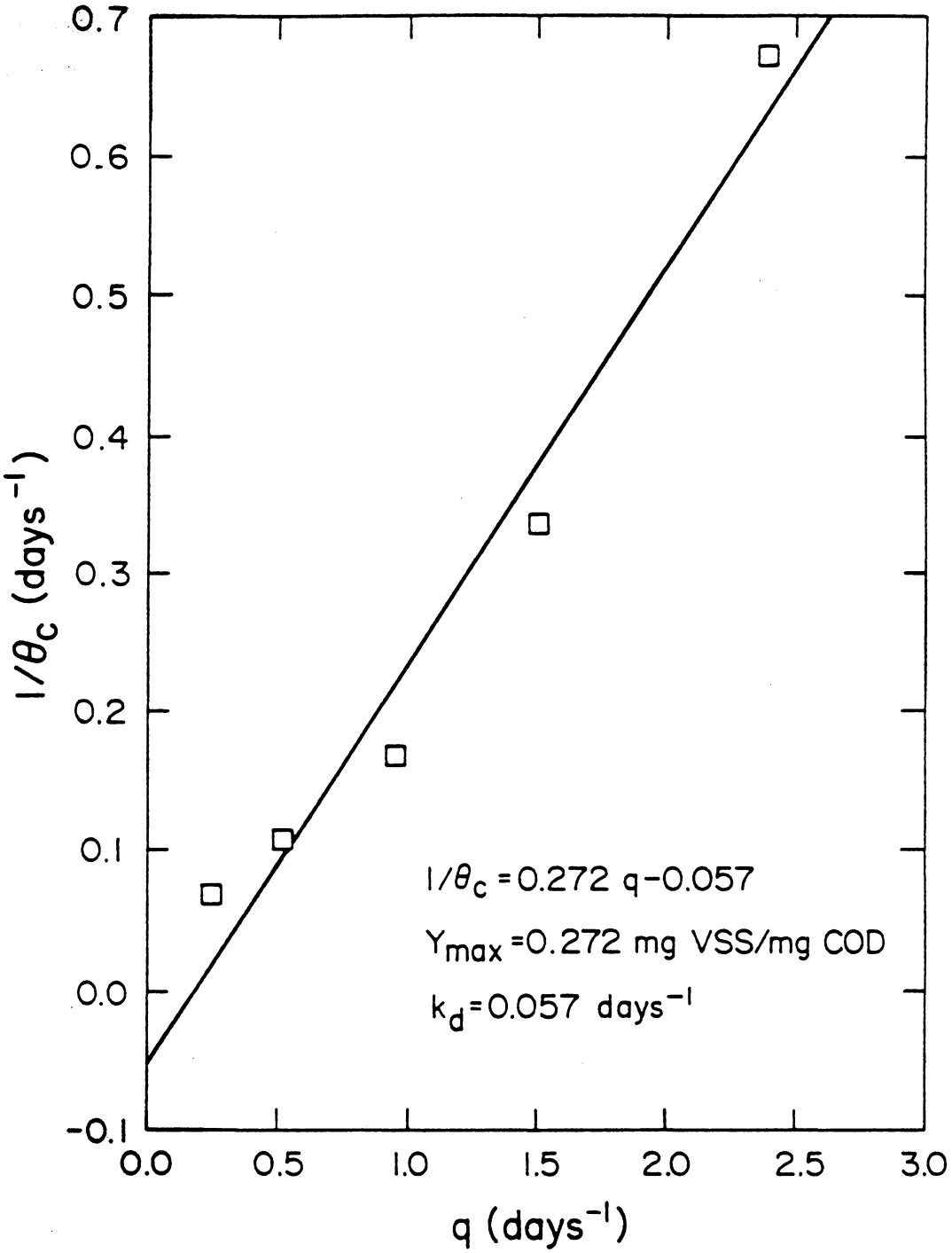

FIGURE 6. SPECIFIC GROWTH RATE VERSUS SPECIFIC SUBSTRATE UTILIZATION RATE FOR THE ANOXIC REACTOR.

occurred slower when nitrate was used as the terminal electron acceptor than when oxygen was used as the terminal electron acceptor.

To determine the kinetic coefficents K_s and k, an evaluation of the residual COD, R, for each reactor was necessary. By plotting the specific substrate utilization rate versus the effluent COD, as shown in Figure 7, residual COD for both the anoxic and the aerobic reactors was estimated to be 22 mg/L. R was subtracted from the effluent COD, S_e, to determine the values of S for Figures 8 and 9.

It is reasonable that the residual CODs were equal for the anoxic and the aerobic reactors. In Figure 7, the point with the smallest value of q is given the most weight in determining R, and the biological solids concentrations in the two reactors were nearly equal for these two points.

An evaluation of the kinetic coefficients K_s and k for the aerobic reactor was performed by plotting 1/q versus 1/S as shown in Figure 8. Linear regression was used to determine the line of best fit. According to Equation [17], K_s was determined as 67 mg/L and k was found to be 6.7 $days^{-1}$. Figure 9 shows a similar plot for the anoxic reactor. K_s for the anoxic reactor was 76 mg/L and k was 11.8 $days^{-1}$. The K_s value of 76 mg/L for the anoxic reactor is comparable to the value for K_s of 67 mgt/L for the aerobic reactor, demonstrating that the effect of biodegradable substrate concentration on microbial growth is about the same whether nitrate or oxygen is utilized as the terminal electron acceptor. Values determined for the kinetic coefficients K_s and k agree well with values reported in the

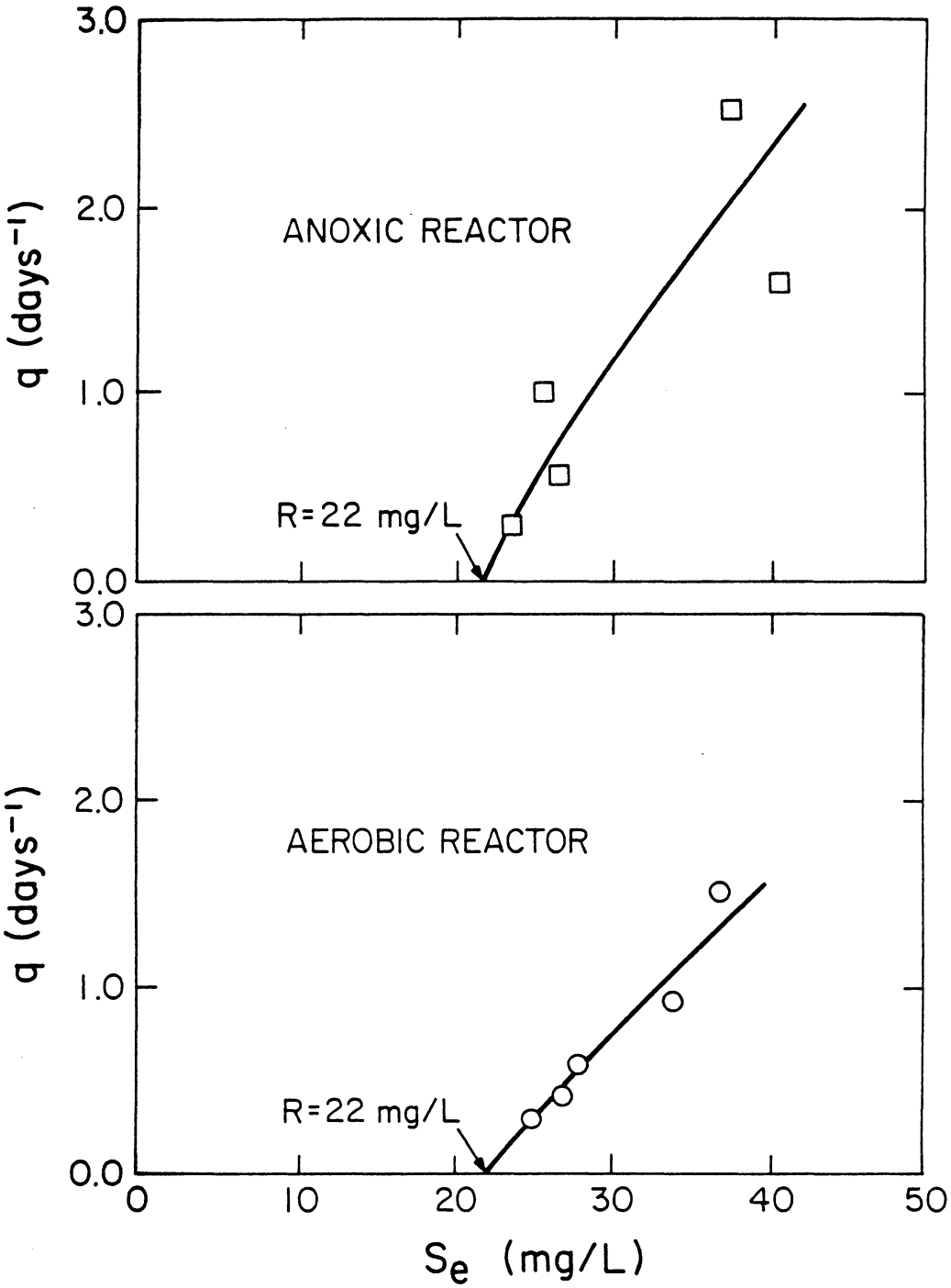

FIGURE 7. EVALUATION OF RESIDUAL COD FOR THE ANOXIC AND AEROBIC REACTORS FROM PLOTS OF SPECIFIC SUBSTRATE UTILIZATION RATE VS. EFFLUENT COD.

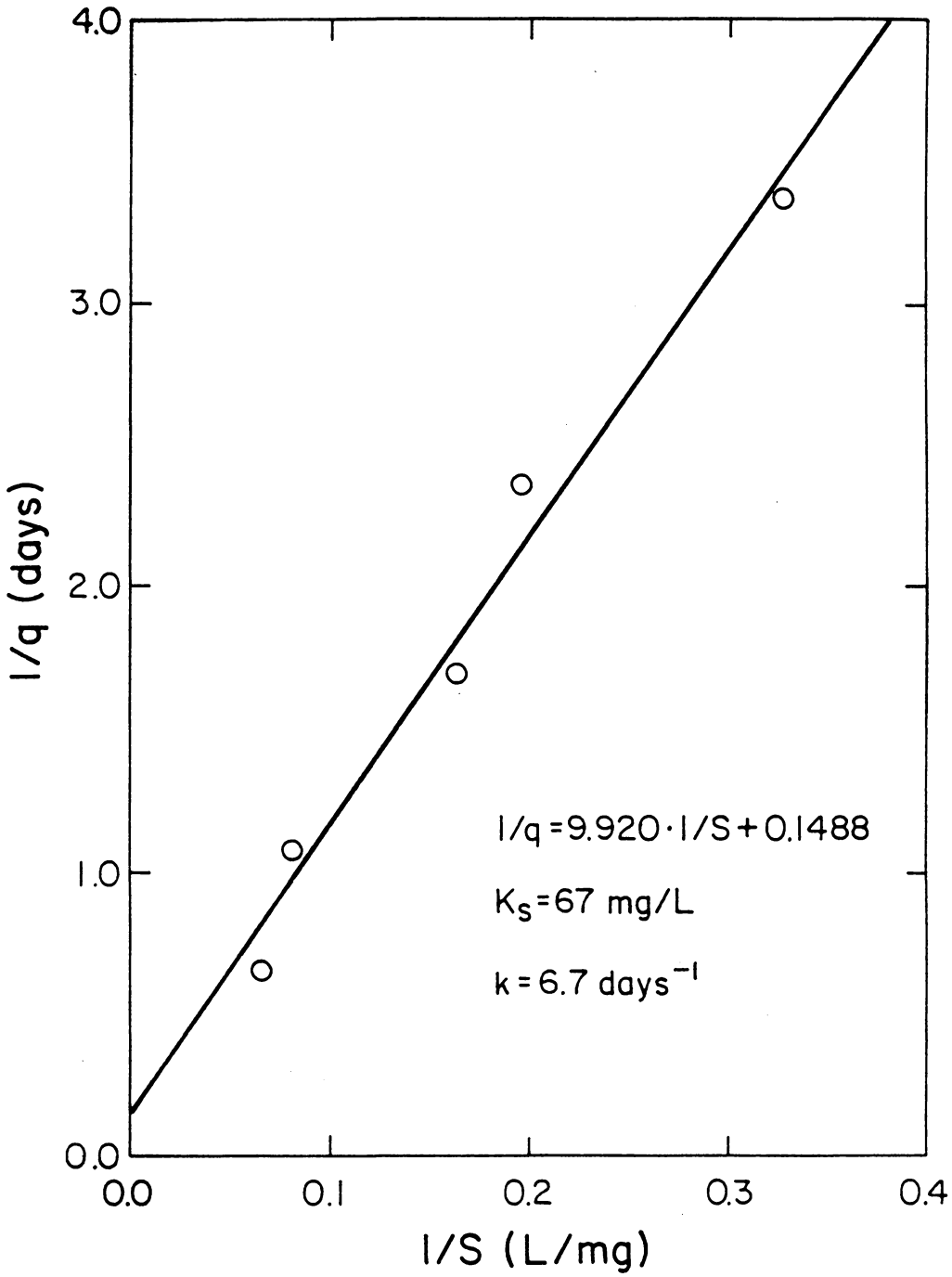

FIGURE 8. EVALUATION OF KINETIC COEFFICIENTS FOR THE AEROBIC REACTOR.

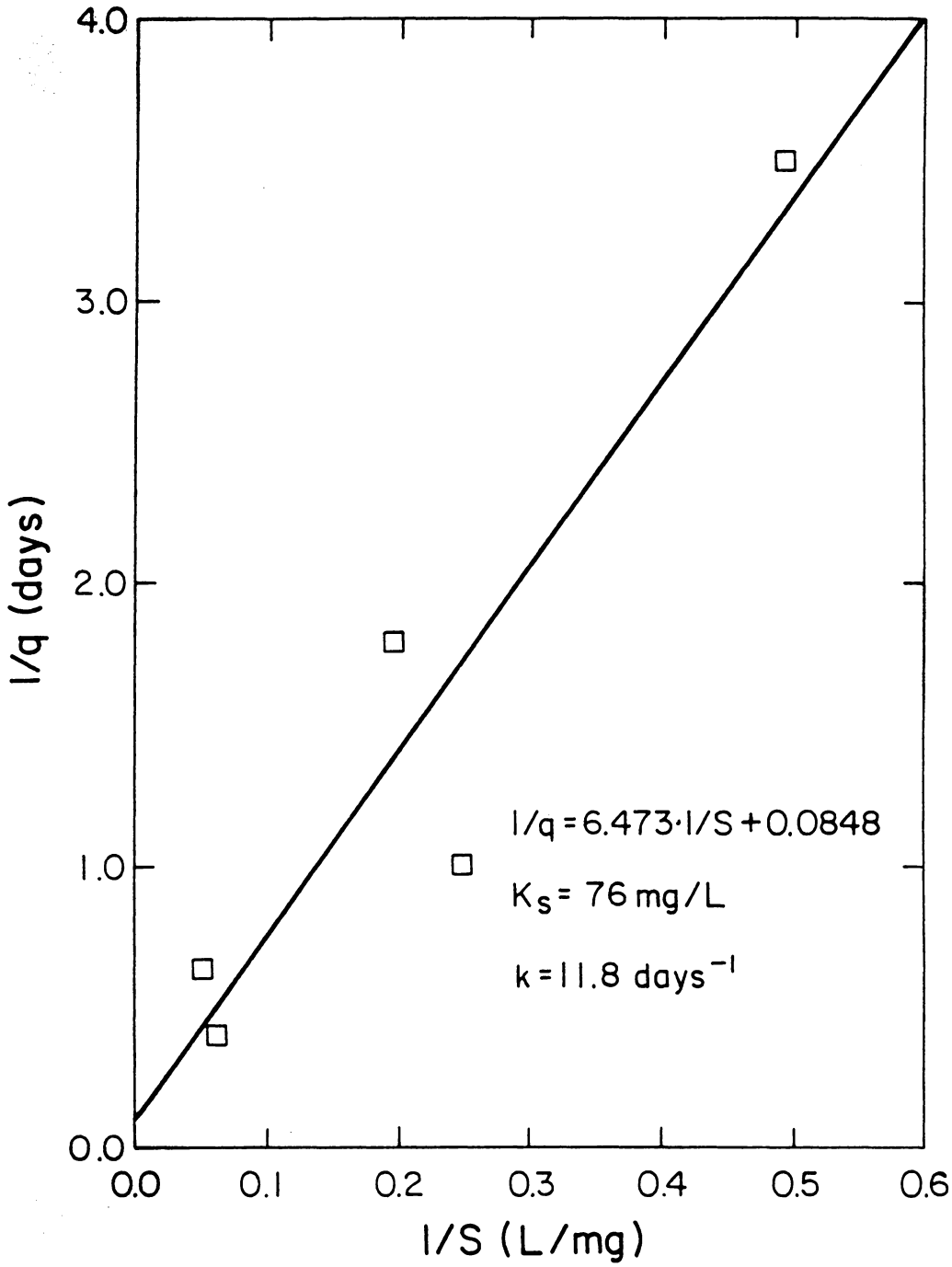

FIGURE 9. EVALUATION OF KINETIC COEFFICIENTS FOR THE ANOXIC REACTOR.

literature. The data points follow a straight line very well. In Figure 9, it can be seen that the data points for the anoxic reactor are more erratic than those in Figure 8. However, the straight line of best fit as determined by linear regression showed a high degree of correlation as exhibited by a correlation coefficient of 0.94.

Maximum substrate utilization rate, k, was significantly greater for the anoxic reactor than for the aerobic reactor. Biodegradable organic substrate is utilized more quickly per unit of biomass when nitrate serves as the terminal electron acceptor than when oxygen is used.

Values for the maximum specific growth rate, μ_m, were calculated from Equation [15] to be 3.4 $days^{-1}$ for the aerobic reactor and 3.2 $days^{-1}$ for the anoxic reactor. These values are what would be expected based on calculations of free energy yields of reactions similar to those occurring in the two systems. Energy yields for the oxidation of glucose with oxygen and nitrate as terminal electron acceptors can be described as follows (14):

	ΔG_7^o *
$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$	-686
$5\ C_6H_{12}O_6 + 24\ H^+ + 24\ NO_3^- \longrightarrow 12\ N_2 + 30\ CO_2 + 42\ H_2O$	-649

*Standard free energy adjusted to pH 7, in kcal/mole.

The ratio of standard free energies of nitrate respiration of glucose to oxygen respiration of glucose is 0.946, while the ratio of maximum growth rates found in this study is 0.942.

To see how well the experimental data fit the model, the specific substrate utilization rate versus the biodegradable substrate concentration, S, was plotted. The curve describing the model as generated from Equation [16] was also plotted. A plot of q versus S for the aerobic reactor is shown in Figure 10. The values for k and K_s as determined from Figure 8 were used in Equation [16] to generate the theoretical curve. Figure 11 shows q versus S for the anoxic reactor. Values for k and K_s as determined in Figure 9 were used to generate the curve in this figure.

Figures 10 and 11 show that the theoretical curves of specific substrate utilization rate versus biodegradable substrate concentration adequately describe the data. Note that the scales are different for the two figures. A closer examination of the Figures 10 and 11 reveals that the curve in Figure 11 describing the anoxic reactor is much steeper than the curve describing the aerobic reactor in Figure 10. The maximum substrate utilization rate must be higher for the anoxic reactor if the curves are to adequately fit the measured data points.

SUBSTRATE REMOVAL

Figure 12 shows the relationship between the specific substrate utilization rate and mean cell residence time for both the anoxic and the aerobic reactors. Specific substrate utilization rate is greater in the anoxic reactor for mean cell residence times up to 15 days, suggesting that the design mean cell residence time that is

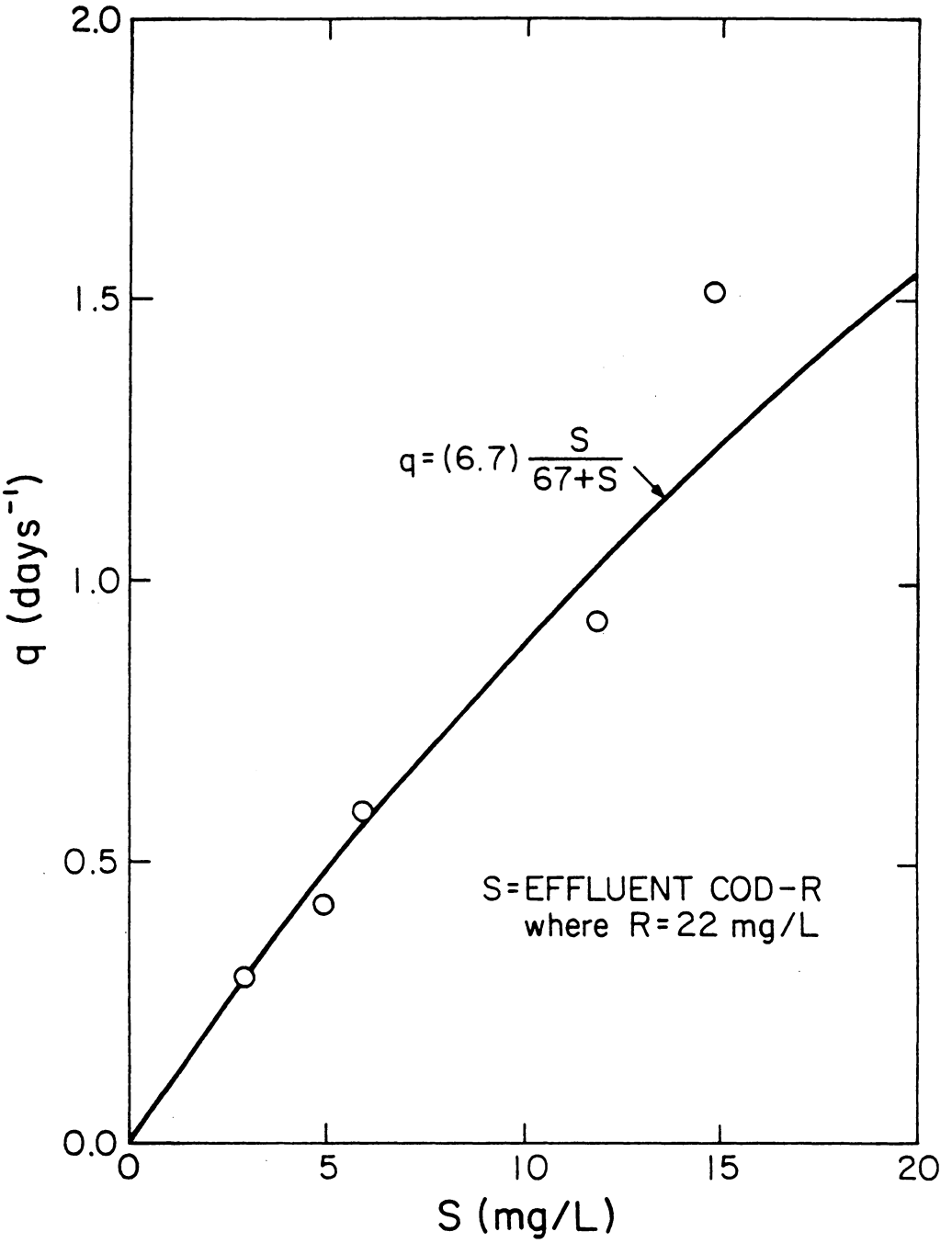

FIGURE 10. SPECIFIC SUBSTRATE UTILIZATION RATE VERSUS BIODEGRADABLE SUBSTRATE CONCENTRATION FOR THE AEROBIC REACTOR.

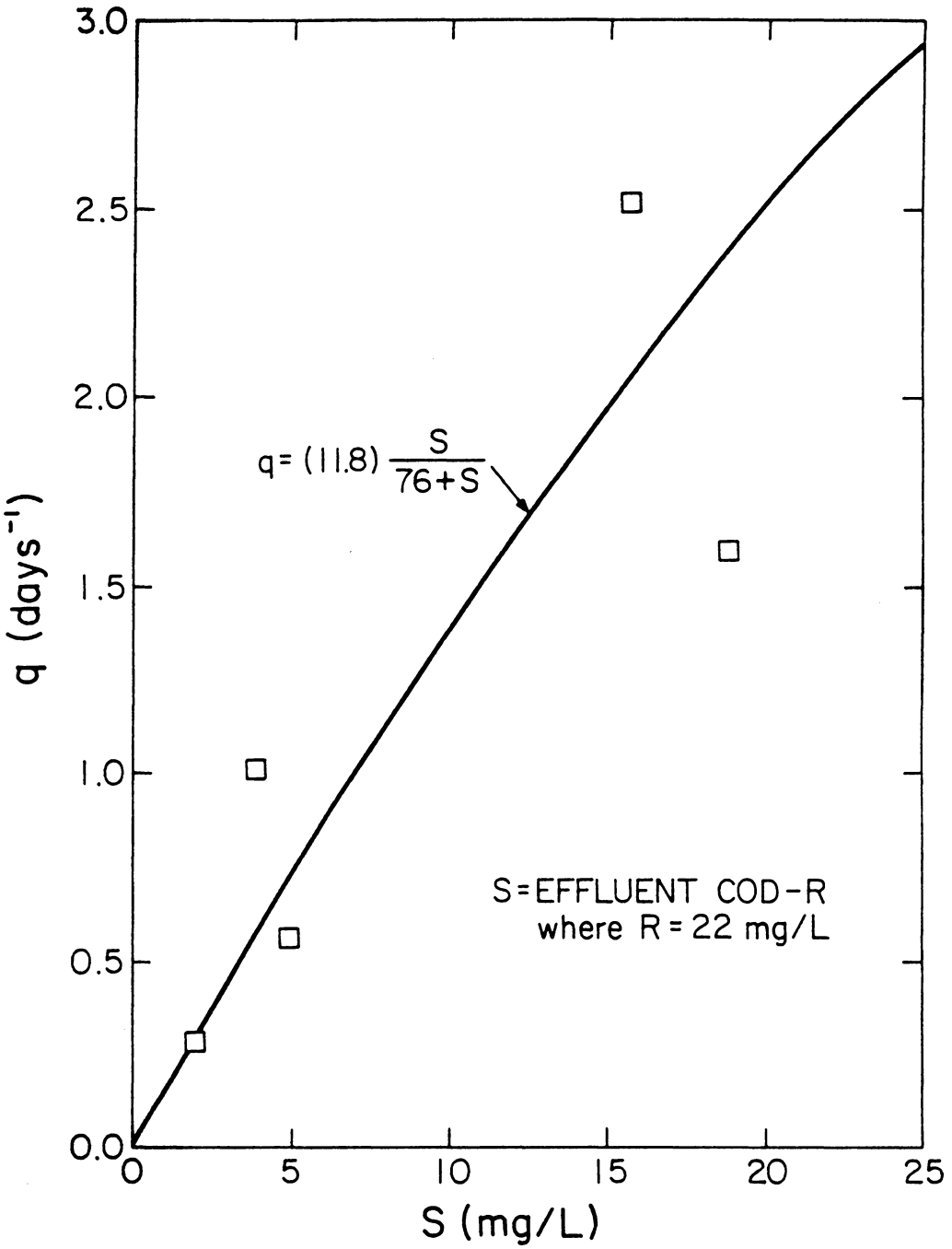

FIGURE 11. SPECIFIC SUBSTRATE UTILIZATION RATE VERSUS BIODEGRADABLE SUBSTRATE CONCENTRATION FOR THE ANOXIC REACTOR.

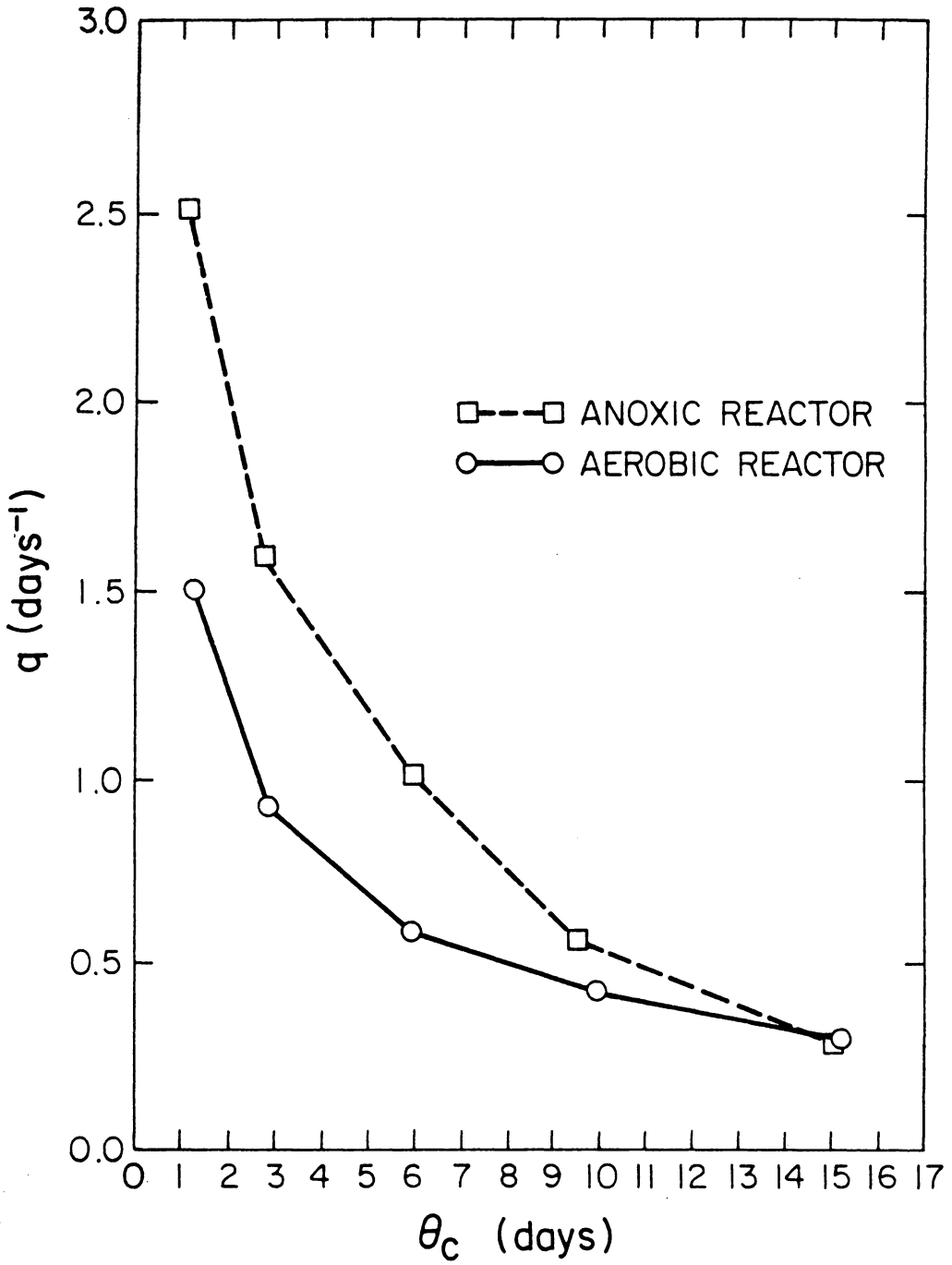

FIGURE 12. SPECIFIC SUBSTRATE UTILIZATION RATE VS. MEAN CELL RESIDENCE TIME FOR THE ANOXIC AND AEROBIC REACTORS.

most efficient for substrate removal in a single-sludge system is less than 15 days. However, this result is not directly applicable to a single-sludge system, because the observed yields of denitrifiers in single-sludge systems would be less than observed yields in the anoxic reactor of this study because denitrifiers would show a higher degree of decay when they are periodically exposed to air (10).

COD removal efficiencies were plotted as a function of mean cell residence time in Figure 13 to determine if either the anoxic or the aerobic reactor exhibited greater COD removal capabilities. It appears that COD removal efficiencies were essentially the same for both reactors. The anoxic reactor performed equally as well as the aerobic reactor in terms of COD removal efficiency, even though the maximum growth yield coefficients were very different. The amount of growth depends on the terminal electron acceptor used.

ALKALINITY CHANGES

The alkalinity changes due to nitrification and denitrification are plotted in Figure 14 as a function of mean cell residence time. A solid horizontal line was drawn to show the average changes in alkalinity per NO_x-N reduced in the anoxic reactor and per NO_x-N produced in the aerobic reactor.

Alkalinity destruction due to nitrification ranged from 3.9 to 5.6 with an average of 4.5 mg of alkalinity as $CaCO_3$ destroyed per mg of NO_x-N produced, less than the theoretically predicted value of 7.14. This observation, however, was expected. The feed contained large quantities of organic nitrogen, which when deaminated to

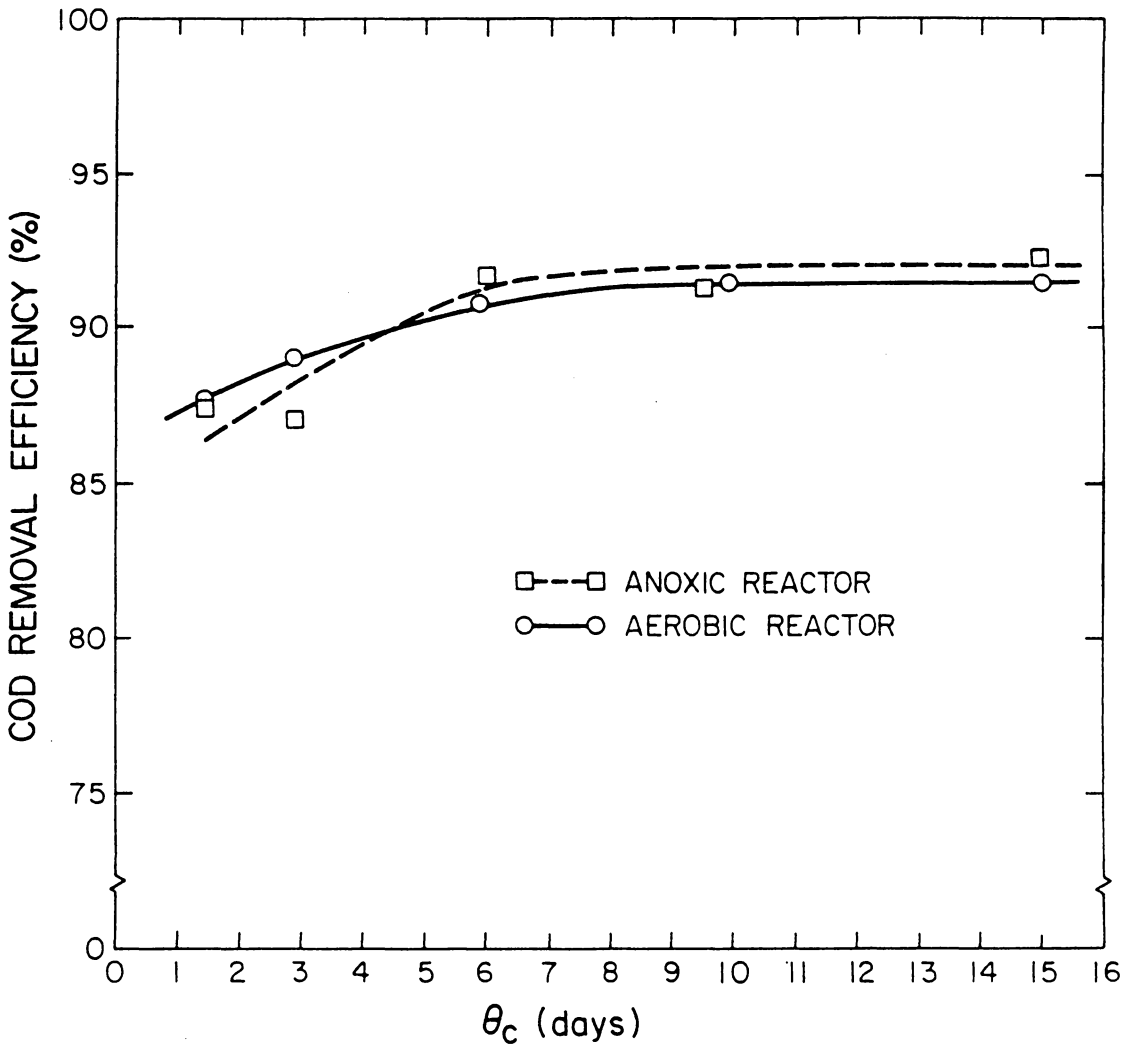

FIGURE 13. COD REMOVAL EFFICIENCIES FOR THE ANOXIC AND AEROBIC REACTORS VERSUS MEAN CELL RESIDENCE TIME.

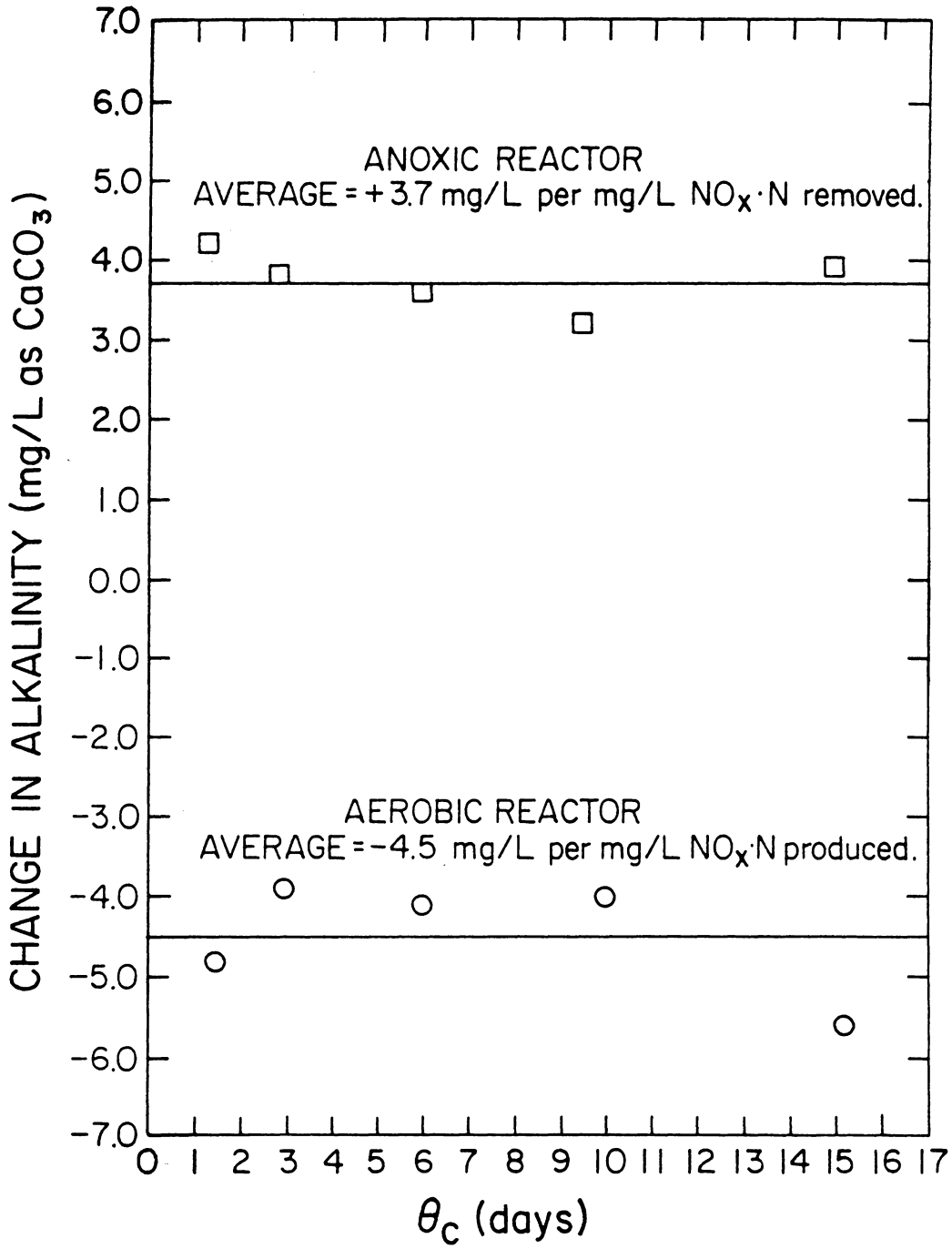

FIGURE 14. ALKALINITY RELATIONSHIPS IN THE ANOXIC AND AEROBIC REACTORS AT DIFFERENT MEAN CELL RESIDENCE TIMES.

ammonium produces alkalinity, partially offsetting alkalinity destruction due to nitrification. The measured value of 4.5 mg of alkalinity as $CaCO_3$ destroyed per mg of NO_x-N produced agrees well with values found by Sherrard et.al. (8) for wastewaters with similar organic nitrogen concentrations. Domestic wastewater contains a smaller percentage of organic nitrogen than the synthetic waste used in this study. Therefore, alkalinity destruction would be closer to the theoretical value of 7.14 for domestic wastewater.

In Figure 14, the data point showing alkalinity change in the aerobic reactor at a mean cell residence time of 15.2 days shows a much greater change than the other points. Influent TKN for this data point is higher than for the other points, even though the organic nitrogen supplied by the bacto-peptone remained the same. Therefore, the ratio of ammonium N to organic N was greater for this data point, and alkalinity change would be expected to be closer to the theoretical 7.14 mg/L as $CaCO_3$.

Alkalinity production due to denitrification in the anoxic reactor ranged from 3.2 to 4.2 with an average of 3.7 mg of alkalinity produced per mg of NO_x-N reduced, which agrees very well with the theoretical value of 3.57. For cases where sufficient ammonium is available for cell synthesis and reduction of NO_x-N is due strictly to denitrification, the theoretical alkalinity production of 3.57 mg as $CaCO_3$ per NO_x-N removed provides a good estimate for engineering purposes.

A summary of important results determined in this investigation is presented in Table VI. A discussion of the engineering applications of these results is presented in the following chapter.

SINGLE-SLUDGE SYSTEMS

Care should be taken in applying these results to the design of single-sludge nitrification/denitrification systems treating domestic or industrial wastewaters. Some of the growth and kinetic parameters determined from separate anoxic and aerobic systems are not directly applicable to a much more complex single-sludge system. However, some generalizations can be made from the results of this study.

The equation proposed by Grady et al. (2) for microbial growth under anoxic conditions for single-sludge systems as described by Equation [23] appears to be conceptually inaccurate. It does not represent the actual processes involved and their effect on the rate of bacterial growth and it does not distinguish between the effects of particl activity and partial ability of the biomass in a single-sludge system. An equation such as the following may be more accurate:

$$r_{g2} = \mu_m \frac{S}{K_s + S} \frac{N}{K_N + N} f_{dn} X \qquad [25]$$

where r_{g2} = rate of bacterial growth under anoxic conditions, mass/volume·time,

μ_m = maximum growth rate under anoxic conditions, time $^{-1}$,

S = organic substrate concentration, mass/volume,

TABLE VI

SUMMARY OF LABORATORY RESULTS

Coefficient	Basis	Aerobic Reactor (Avg. T = 24° C)	Anoxic Reactor (Avg. T = 26° C)
Y_{max}	mg VSS/mg COD	0.503	0.272
k_d	$days^{-1}$	0.111	0.057
k	$days^{-1}$	6.7	11.8
K_s	mg/L T_bOD	67	76
μ_m	$days^{-1}$	3.4	3.2
Alkalinity change per mg/L $NO_x \cdot N$ produced or removed	mg/L as $CaCO_3$	-4.5	+3.7

K_s = half saturation constant for organic substrate under anoxic conditions, mass/volume,

N = nitrate concentration, mass/volume,

K_N = half saturation constant for nitrate, mass/volume,

f_{dn} = fraction of denitrifiers, and

X = biomass concentration.

The values of K_s determined in this study were approximately the same for nitrate and oxygen respiration, and so K_s for aerobic conditions could be used in Equation [25] as it was in Equation [23]. Equation [23] incorporates the effect of the lower maximum growth rate under anoxic conditions and the effect of only a fraction of the biomass being able to utilize nitrate as electron acceptor into one correction coefficient, η. Maximum growth rate, μ_m, under anoxic conditions can be experimentally determined, and it should be fairly constant for a given wastewater and set of conditions. Therefore, Equation [25] should be used to define the rate of growth under anoxic conditions in a single-sludge system because it more accurately represents the actual processes involved in anoxic bacterial growth and distinguishes between the effects of partial activity and partial ability of the biomass in a single-sludge system.

V. ENGINEERING APPLICATIONS

In this chapter, a discussion of engineering applications of the results of this study is presented. The long and successful history of wastewater treatment with the fully aerobic activated sludge process often makes it the treatment of choice whenever secondary treatment is required. The results of this study illustrate that aeration may not be required if sufficient amounts of nitrate are available for use as the terminal electron acceptor, as is the case for many industrial wastes. There are also many advantages to utilizing nitrate respiration where nitrates are produced as a result of nitrification. Design engineers need to be aware of and consider any alternatives that will reduce capital and/or operating costs. The results of this study indicate that the utilization of nitrate respiration may be warranted in the design and/or operation of many domestic and industrial activated sludge wastewater treatment plants.

ADDITION OF NITRATES INSTEAD OF AERATION

Perhaps the most dramatic engineering application of the results of this investigation is that aeration is not required in the activated sludge process to remove organic carbon as long as electron acceptors other than oxygen are available. Addition of sufficient amounts of nitrate compounds to wastewaters can eliminate the need for oxygen. The long and successful history of aerobic treatment makes consideration of the alternative of nitrate addition unlikely. The addition of alternative electron acceptors may become more

favorable when it is desired to reduce or eliminate aeration because of rising energy costs.

Addition of nitrates would probably not be suitable for domestic wastewater because most of the ammonium and organic nitrogen in the wastewater would pass through the treatment system and be released in the effluent. However, nitrogen deficient wastewaters such as those from many food processing establishments and paper and pulp mills could be treated in this manner with very little nitrogen in the effluent. Energy would be required for mixing only and the energy savings could more than offset the cost of chemical addition. Lower sludge production would be obtained than with aerobic treatment while maintaining the same degree of organic carbon removal, further reducing costs.

There are some disadvantages to adding nitrates as an alternative to aeration. Addition of calcium nitrate would cause precipitation of calcium carbonate. Addition of sodium nitrate or potassium nitrate would add dissolved solids. Sludge settling problems may occur in the secondary clarifier because of nitrogen gas bubbles from denitrification causing rising sludge. A short period of aeration prior to clarification may be desirable to remove any excess organic material, to increase the rate of endogenous decay, and to prevent denitrification in the secondary clarifier.

Clearly, more research in the form of bench-scale or pilot plant studies is needed to optimize this method of wastewater treatment. The energy savings resulting from mixing instead of aeration will vary

for different wastewaters, but should be significant in most cases. As energy costs continue to rise, alternatives to high energy aeration will become more important.

INDUSTRIAL WASTEWATERS CONTAINING NITRATES

Nitrate respiration should be considered for activated sludge processes treating wastewaters containing nitrates for many reasons. If sufficient quantities of nitrate are available to serve as the terminal electron acceptor, aeration may not be required. Observed sludge yields from such a system should be lower than if all substrate were removed aerobically, except possibly at high operating mean cell residence times, where observed yields for nitrate and oxygen respiration may coincide. Any reduction in aeration requirements and sludge production can result in cost savings. Nitrogen removal is also achieved, and the cost savings should make this an attractive alternative regardless of whether nitrogen removal is required.

Anoxic (anaerobic with nitrate as the terminal electron acceptor) reactors can be covered to prevent any oxygen from entering the system. The only energy required is for mixing to keep the biomass suspended in the reactor, which should be much less than the energy that would be required for aeration. Anoxic activated sludge treatment is particularly suited to nitrate-rich industrial wastewaters such as those from poultry and uranium processing establishments.

SINGLE-SLUDGE SYSTEMS

Articles in the literature report that many existing activated sludge plants designed to achieve nitrification can be easily modified to single-sludge nitrification/denitrification systems (23, 46). Beer (23) suggested that a single-sludge system utilizing nitrate respiration may have a greater capacity for organics removal compared with fully aerobic activated sludge systems. Ip et al. (46) implied that sludge production was lower and organics and suspended solids removal were superior in a plant modified to include nitrate respiration. In their study, a total nitrogen removal of approximately 90% was achieved.

The results obtained from this study support the contentions that substrate removal can be greater and cell yields lower in an activated sludge process utilizing nitrate respiration. Hence, utilization of nitrate respiration in the activated sludge process can result in nitrogen removal in addition to an effluent of equal or superior quality in terms of COD removal. Aeration requirements and sludge production should also be reduced for the process while obtaining the same degree of treatment. Reduced aeration energy requirements and reduced sludge handling and disposal would result in significant cost savings.

ALKALINITY CHANGES

Incorporating nitrate respiration into the activated sludge process can have a beneficial effect on alkalinity. Nitrification consumes alkalinity, and nitrification is inhibited when alkalinity is

deficient. Significant decreases in pH can result in low alkalinity wastewaters. Many activated sludge facilities with nitrification may need to add alkalinity to prohibit inhibition of nitrification and large decreases in pH.

Results of this study showed that denitrification produces alkalinity very close to the theoretical stoichiometric value of 3.57 mg as $CaCO_3$ per mg NO_x-N denitrified. Lower values for alkalinity production reported in the literature (2.9 to 3.0 mg/mg) are for cases where nitrate is also used for cellular nitrogen and calculations were made on the basis of total nitrate-nitrogen removed (10). Nitrate respiration produces alkalinity in the system, partially offsetting the consumption of alkalinity by nitrification. Addition of alkalinity may be avoided in systems where it might otherwise be required, resulting in cost savings.

VI. SUMMARY AND CONCLUSIONS

Two bench-scale activated sludge units were operated over a range of mean cell residence times with organic carbon as the limiting nutrient. One reactor was aerobic and the other anoxic (anaerobic with nitrate as the terminal electron acceptor). Growth and kinetic coefficients for the two units were evaluated and compared, and a comparison between them was presented in Table VI. Alkalinity changes were monitored and compared with theory. Engineering applications of the results were also discussed.

From the results of this investigation, the following conclusions were formed:

1. The maximum microbial yield and endogenous decay coefficients were lower, and the maximum substrate utilization rate was higher for nitrate respiration versus oxygen respiration.
2. The values of the half-saturation constants, K_s, were approximately the same for the nitrate and oxygen respiration systems.
3. Alkalinity production during denitrification was very near the theoretical stoichiometric value of 3.57 mg as $CaCO_3$/mg NO_3^--N denitrified when sufficient quantities of ammonium where present for cell synthesis.
4. The equation proposed by Grady et al. (2) to describe microbial growth under anoxic conditions appears to be conceptually inaccurate. The following equation may be more accurate because it represents the actual processes involved

and their effect on the rate of bacterial growth while distinguishing between the effects of partial activity and partial ability of the biomass under anoxic conditions in a single-sludge system:

$$r_{g2} = \mu_m \frac{S}{K_s + S} \frac{N}{K_N + N} f_{dn} X$$

where r_{g2} = rate of bacterial growth under anoxic conditions, mass/volume·time,

μ_m = maximum growth rate under anoxic conditions, time^{-1},

S = organic substrate concentration, mass/volume,

K_s = half saturation constant for organic substrate under anoxic conditions, mass/volume,

N = nitrate concentration, mass/volume,

K_N = half saturation constant for nitrate, mass/volume,

f_{dn} = fraction of denitrifiers, and

X = biomass concentration.

5. Utilization of nitrate respiration in the activated sludge process should be considered in the design of many wastewater treatment plants, particularly those that treat wastewaters containing nitrates or where nitrates are formed as a result of nitrification.

6. Single-sludge systems incorporating organics removal, nitrification, and denitrification can potentially achieve a high degree of nitrogen and organics removal at a lower cost than a similar sized system incorporating organics removal and nitrification only. Aeration energy savings and reduced sludge production obtained by the utilization of nitrate respiration in single-sludge systems should result in significant cost savings.

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APPENDIX

TABLE A-I

Raw Data for θ_c = 1.5 Days in Aerobic Reactor (T = 20°C)

Date	COD		Biological Solids	Flow Rate	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	(L/day)	(days)
9/30	292	29	113	5.0	1.7
10/1	308	59	119	6.0	1.4
10/2	302	56	115	6.0	1.4
10/3	302	28	123	6.0	1.4
10/4	281	28	116	6.0	1.4
10/5	301	24	110	5.0	1.7
AVG.	298	37	115	5.7	1.5

TABLE A-I (Continued)

Date	TKN		NO_2^--N	NO_3^--N	Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./Eff.
9/30	61	0	1	59	428	162	8.1/7.9
10/1	48	0	3	49	428	178	7.9/7.9
10/2	54	0	3	44	395	181	8.1/8.0
10/3	64	0	4	42	432	181	8.3/8.3
10/4	57	0	3	44	399	176	8.4/8.3
10/5	59	0	4	46	413	173	8.4/8.3
AVG.	57	0	3	47	416	175	8.2/8.1

TABLE A-II

Raw Data for θ_c - 3.0 Days in Aerobic Reactor (T = 25° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
9/6	312	28	780	6	15	2000	3.0
9/7	308	24	868	4	15	2040	3.0
9/8	304	40	716	5	15	2010	3.0
9/9	300	28	708	8	15	1955	3.0
9/10	312	40	588	10	15	1845	3.0
9/11	324	44	592	10	15	1850	3.0
AVG.	310	34	709	7	15	1950	3.0

TABLE A-II (Continued)

Date	TKN		NO_2^--N	NO_3^--N	Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./ML/Eff.
9/6	64	2	27	19	413	228	8.0/7.9/7.8
9/7	65	7	24	18	404	252	8.1/7.9/7.8
9/8	65	13	28	19	428	254	8.0/7.9/7.9
9/9	64	16	40	19	432	285	8.2/8.0/8.0
9/10	64	14	22	8	532	304	8.1/8.1/8.0
9/11	64	9	25	11	437	304	8.1/8.1/8.0
AVG.	64	10	28	16	441	271	8.1/8.0/7.9

TABLE A-III

Raw Data for θ_c - 6.0 Days in Aerobic Reactor (T = 20° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
7/27	303	31	1110	31	15	640	6.0
7/28	307	31	1100	29	15	665	6.0
7/29	311	28	1130	19	15	830	6.0
7/30	299	24	1110	23	15	750	6.0
7/31	307	35	1150	26	14	740	6.0
8/1	299	20	1090	17	15	825	6.0
AVG.	304	28	1115	24	15	742	6.0

TABLE A-III (Continued)

Date	TKN		NO_2^--N	NO_3^--N	Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./ML/Eff.
7/27	52	1	4	35	405	222	8.1/7.8/7.9
7/28	51	0	4	41	418	230	8.0/7.7/7.8
7/29	52	1	3	41	415	220	8.1/7.8/7.9
7/30	55	2	7	42	425	225	8.2/7.6/7.8
7/31	58	0	7	42	448	253	8.0/7.9/7.9
8/1	54	0	8	43	400	205	7.9/7.8/7.7
AVG.	54	1	6	41	419	226	8.1/7.8/7.8

TABLE A-IV

Raw Data for θ_c - 10.0 Days in Aerobic Reactor (T = 28° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
8/24	306	30	1595	17	15	470	10.0
8/25	302	22	1635	10	15	540	10.0
8/26	310	26	1660	13	15	515	10.0
8/27	319	26	1590	11	15	525	10.0
8/28	319	26	1630	9	15	545	10.0
8/29	323	30	1525	15	15	480	10.0
AVG.	313	27	1606	13	15	513	10.0

TABLE A-IV (Continued)

Date	TKN		NO_2^--N	NO_3^--N	Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./ML/Eff.
8/24	55	0	10	34	438	255	8.1/7.8/7.8
8/25	53	0	7	39	440	258	8.1/7.8/7.8
8/26	58	0	11	35	418	250	8.0/7.8/7.8
8/27	58	0	8	37	425	240	8.2/7.7/7.7
8/28	54	0	9	39	440	240	8.2/7.7/7.7
8/29	55	0	9	41	435	235	8.1/7.9/7.8
AVG.	56	0	9	38	433	246	8.1/7.8/7.8

TABLE A-V

Raw Data for θ_c - 15.2 Days in Aerobic Reactor (T = 20° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
7/7	274	20	2110	10	15	345	15.2
7/8	284	27	2180	19	15	285	15.2
7/9	321	28	2100	11	14	340	15.3
7/10	320	30	2230	15	16	305	15.3
7/11	307	27	2380	20	15	290	15.2
7/12	320	20	2420	12	14	350	15.2
AVG.	304	25	2237	15	15	319	15.2

TABLE A-V (Continued)

Date	TKN		NO_2^--N	NO_3^--N	Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./ML/Eff.
7/7	76	0	0	64	425	98	8.0/7.4/7.4
7/8	67	0	0	52	430	92	8.1/7.4/7.2
7/9	63	1	0	62	384	88	8.0/7.3/7.2
7/10	72	1	0	70	472	84	8.1/7.4/7.4
7/11	69	0	0	64	438	82	8.0/7.3/7.2
7/12	63	0	0	55	412	72	8.0/7.3/7.2
AVG.	68	0	0	61	427	86	8.0/7.4/7.3

TABLE A-VI

Raw Data for θ_c = 1.5 Days in Anoxic Reactor (T = 22°C)

Date	COD		Biological Solids	Flow Rate	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	(L/day)	(days)
9/30	304	35	66	5.0	1.7
10/1	304	37	78	6.0	1.4
10/2	290	44	69	6.0	1.4
10/3	327	44	75	6.0	1.4
10/4	297	28	74	6.0	1.4
10/5	289	37	58	5.0	1.7
AVG.	302	38	70	5.7	1.5

TABLE A-VI (Continued)

Date	TKN Inf. (mg/L)	TKN Eff. (mg/L)	NO_2^--N Inf. (mg/L)	NO_2^--N Eff. (mg/L)	NO_3^--N Inf. (mg/L)	NO_3^--N Eff. (mg/L)	Alkalinity Inf. (mg/L as $CaCO_3$)	Alkalinity Eff. (mg/L as $CaCO_3$)	pH Inf./Eff.
9/30	63	54	0	2	69	10	461	646	7.6/8.1
10/1	64	54	8	2	72	22	447	618	7.7/8.2
10/2	61	37	0	2	84	19	416	638	7.7/8.2
10/3	56	40	0	1	70	22	428	622	7.9/8.3
10/4	63	54	0	1	70	19	397	715	7.8/8.1
10/5	64	56	0	1	63	14	401	622	7.9/8.3
AVG.	62	49	1	2	71	18	425	644	7.8/8.2

TABLE A-VII

Raw Data for θ_c - 5.0 Days in Anoxic Reactor (T = 27° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
9/20	310	50	403	19	15	1595	3.0
9/21	318	41	417	19	14	1535	3.0
9/22	331	29	420	13	15	1680	3.0
9/23	302	53	402	15	16	1565	3.0
9/24	308	45	403	7	15	1870	3.0
9/25	292	28	373	10	15	1750	3.0
AVG.	310	41	403	14	15	1666	3.0

TABLE A-VII (Continued)

Date	TKN (mg/L)		NO_2^--N (mg/L)		NO_3^--N (mg/L)		Alkalinity (mg/L as $CaCO_3$)		pH
	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf./ML/Eff.
9/20	64	52	0	4	66	8	432	637	7.6/8.3/8.3
9/21	67	57	0	1	84	15	479	677	7.6/8.0/8.0
9/22	67	51	8	1	59	18	445	663	7.5/7.9/7.9
9/23	70	61	0	7	67	2	437	689	7.6/8.3/8.3
9/24	59	56	0	0	85	13	437	675	7.7/8.2/8.2
9/25	56	57	0	1	79	14	432	670	7.4/8.1/8.1
AVG.	64	56	1	2	73	12	444	669	7.6/8.1/8.1

TABLE A-VIII

Raw Data for θ_c - 6.1 Days in Anoxic Reactor (T = 30° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
8/13	321	27	652	5	15	935	6.0
8/14	310	23	720	9	15	865	6.0
8/15	263	27	676	8	15	870	6.1
8/16	298	31	680	7	15	895	6.1
8/17	341	23	656	13	15	750	6.1
8/18	341	23	683	14	15	745	6.1
AVG.	312	26	678	9	15	843	6.1

TABLE A-VIII (Continued)

Date	TKN		NO_2^--N		NO_3^--N		Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./ML/Eff.
8/13	62	50	2	0	82	16	490	775	7.4/7.8/7.9
8/14	59	50	0	0	82	14	428	670	7.5/7.9/7.9
8/15	55	48	3	0	62	9	600	795	7.6/7.9/8.0
8/16	58	53	0	0	78	5	438	715	7.4/7.8/7.8
8/17	64	49	0	0	80	10	435	648	7.4/7.8/7.8
8/18	59	46	0	0	79	10	448	685	7.5/7.9/7.9
AVG.	60	49	1	0	77	11	473	715	7.5/7.9/7.9

TABLE A-IX

Raw Data for θ_c - 9.6 Days in Anoxic Reactor (T = 27° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
9/5	304	24	1240	16	15	530	8.8
9/6	308	40	1160	19	15	420	9.6
9/7	300	24	1156	22	15	420	9.0
9/8	304	28	1168	14	15	450	10.1
9/9	308	24	1188	24	15	330	10.1
9/10	308	20	1200	18	15	405	10.1
AVG.	305	27	1185	19	15	426	9.6

TABLE A-IX (Continued)

Date	TKN		NO_2^--N		NO_3^--N		Alkalinity		pH
	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L as $CaCO_3$)	Eff. (mg/L as $CaCO_3$)	Inf./ML/Eff.
9-5	70	67	0	1	71	14	451	637	7.6/8.0/8.0
9/6	72	46	0	2	64	14	425	598	7.4/8.3/8.3
9/7	70	59	18	4	74	20	411	620	7.5/7.7/7.7
9/8	67	57	0	0	57	19	444	610	7.5/7.7/7.7
9/9	63	47	8	8	79	11	401	618	7.6/8.1/8.1
9/10	63	63	6	0	67	11	480	610	7.6/8.2/8.2
AVG.	68	57	5	3	69	15	435	616	7.5/8.0/8.0

TABLE A-X

Raw Data for θ_c - 15.1 Days in Anoxic Reactor (T = 22° C)

Date	COD		Biological Solids		Flow Rate	Waste Sludge	θ_c
	Inf. (mg/L)	Eff. (mg/L)	Mixed Liquor (mg/L)	Eff. (mg/L)	(L/day)	(ml/day)	(days)
7/20	324	25	2190	25	15	250	15.1
7/21	274	19	2470	24	15	275	15.1
7/22	318	31	2450	21	15	290	15.1
7/23	321	22	2250	17	15	305	15.1
7/24	306	30	2250	18	15	300	15.1
7/25	279	18	2350	18	15	305	15.1
AVG.	304	24	2327	21	15	288	15.1

TABLE A-X (Continued)

Date	TKN (mg/L)		NO_2^--N (mg/L)		NO_3^--N (mg/L)		Alkalinity (mg/L as $CaCO_3$)		pH
	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf./ML/Eff.
7-20	58	50	1	1	73	24	395	670	7.7/8.6/8.5
7/21	62	48	11	0	57	13	510	660	7.8/8.5/8.4
7/22	52	38	5	0	57	2	427	667	7.9/8.5/8.4
7/23	47	40	0	2	78	13	427	640	7.8/8.5/8.5
7/24	54	48	2	0	77	9	385	665	7.6/8.4/8.3
7/25	48	45	6	0	64	12	430	655	7.5/8.2/8.1
AVG.	54	45	4	1	68	12	429	660	7.7/8.5/8.4

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