INHIBITION OF NITRIFICATION IN BIOLOGICAL SYSTEMS

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To my wife, Rashel, and our children, Siamac, Sepehr, and Siavash, for the joy they bring into my life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

c - Sludge recycle concentration factor, equal to the ratio between the recycle solids concentration, X_R, and the biological solids concentration in the reactor, X

D - Dilution rate. Ratio of the rate of flow, F, and the volume of liquor in the aeration tank, V. It is equal to the reciprocal of the mean hydraulic residence time, $\bar{t}$, in a completely mixed reactor, hr^{-1}

k_d - Maintenance energy coefficient, day^{-1}

K_s - A biological "constant" used in the hyperbolic expression relating specific growth rate to substrate concentration. It is known as the saturation constant. It is numerically equal to the substrate concentration at which specific growth rate is equal to half the maximum specific growth rate for the system, mg/l

S - Substrate concentration, measured as COD, mg/l

S_i - Concentration of substrate in the inflowing feed in continuous flow operation, measured as COD, mg/l

S_o - Initial substrate concentration used in batch growth studies measured as COD, mg/l

S_e - Concentration of substrate in the effluent filtrate measured as COD, mg/l

$\bar{S}_e$ - Steady state concentration of substrate in the effluent filtrate

measured as COD, mg/l

S_t - Concentration of COD in the clarifier effluent, supernatant including non-settled biological solids, mg/l

$\bar{S}_t$ - Steady state concentration of COD in the clarifier effluent, supernatant including non-settled biological solids, mg/l

$\bar{t}$ - Mean hydraulic detention time, hrs

U - Specific substrate utilization rate, day^{-1}

V - Volume of liquor in aerator #1, liters

X - Biological solids concentration, mg/l

$\bar{X}$ - Steady state biological solids concentration in the reactor, mg/l

X_e - Biological solids concentration in the clarifier effluent, mg/l

$\bar{X}_e$ - Steady state biological solids concentration in the clarifier effluent, mg/l

X_R - Biological solids concentration in the recycle flow to the reactor, mg/l

$\bar{X}_R$ - Steady state biological solids concentration in the recycle flow to the reactor, mg/l

X_W - Excess biological solids (sludge wasted), mg/day

$\bar{X}_W$ - Steady state excess biological solids (sludge wasted) mg/day

Y_{t_B} - True mean cell yield obtained during growth at specific growth rate at or near μ_{max} (batch system)

Y_o - Observed mean cell yield obtained during growth at specific growth rate (continuous system with cell recycle)

Y_t - True cell yield

μ_n - Net specific growth rate in continuous system with cell feedback, hr^{-1}

μ_{max} - Maximum specific growth rate for a system in exponential growth, hr^{-1}

α - Recycle flow ratio

Θ_c - Sludge retention time, days

CHAPTER I

INTRODUCTION

The aesthetic, economic, and health considerations involved in the uncontrolled discharge of nitrogen into water have been well documented. Now we are faced with the decision

ment and control, or watch the environment and man's quality of life become further degraded by pollution.

Ammonium ion is the primary form of nitrogen in domestic wastewater treatment plant effluents, and it is commonly found in concentrations of 15 to 25 mg/l. This is a product of deamination of proteins and other nitrogen-containing organics.

Ammonia is toxic to fish in concentrations greater than 2.5 ppm, and can exert a nitrogenous oxygen demand (NOD) upon receiving water. Nitrogenous oxygen demand in the equivalent of 25 mg/l NH_4^+-N is about 114 mg/l, which is much higher than the standard ultimate BOD of conventional treatment plants. This oxygen demand will lower the dissolved oxygen content of the receiving water course, thus endangering aquatic life and overall stream quality. Ammonia also increases the chlorine demand required for disinfection of wastewater effluents. Considerable effort has been made to develop methods for the removal of nitrogen from municipal and/or industrial wastes. Ammonia nitrogen can be removed from wastewater physically, chemically, biologically, or by a combination of any of the above methods. However, it is generally

accepted that biological nitrification from a technical and economic viewpoint is the most feasible method of removing ammonia from wastewater and also, since biological systems for wastewater treatment are in widespread use today, it would be very convenient to be able to establish nitrification within them as a first step in nitrogen control. Although all biological treatment methods, i.e., trickling filter, activated sludge, and rotating biological contactors can support nitrification, activated sludge has received more attention because with the activated sludge process the carbonaceous BOD removal efficiencies tend to be better, and much closer control is possible although it requires more professional operational skills. Thus, the activated sludge process has come under intensive study in an attempt to optimize its use in nitrification.

It is generally believed that biological nitrification is performed by the two bacterial genera, Nitrosomonas and Nitrobacter, which oxidate ammonia to nitrite and nitrite to nitrate, respectively, in a sequential manner as follows:

$$2\ NH_4^+ + 3\ O_2 \xrightarrow{\text{Nitrosomonas}} 2\ NO_2^- + 4\ H^+ + 2\ H_2O \qquad (1)$$

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

$$NH_4^+ + 2\ O_2 \xrightarrow[\text{Nitrobacter}]{\text{Nitrosomonas}} NO_3^- + 2\ H^+ + H_2O \qquad (3)$$

Reaction III is the overall reaction for oxidation of ammonia to nitrate. The chemical energy released upon the completion of this exothermic reaction is estimated by various investigators to be between 72 and 105 K cal per mole of ammonia oxidized (1)(2). This energy is

used for synthesis and metabolic use by the nitrifying bacteria which use inorganic carbon in the form of carbon dioxide (CO_2) or bicarbonate as their carbon source, and ammonia or nitrite as their energy source which also serve as electron donors in the reductive fixation of the inorganic carbon.

On a biochemical level, the nitrification process is more complex than simply the sequential oxidation of ammonia to nitrite by Nitrosomonas and subsequent oxidation of nitrite to nitrate by Nitrobacter. Various reaction intermediates and enzymes are involved (1). More important than understanding of these pathways is knowledge of the response of nitrifying organisms to environmental conditions and a deeper insight into engineering control of the nitrification process.

As a fuller understanding of differences between heterotrophic and autotrophic metabolism and growth characteristics has emerged, steady state and dynamic mathematical models have been developed for nitrifying the activated sludge process (3)(4). However, little is known of the response of a nitrifying activated sludge model to severe changes in the external environment, i.e., shock loading. Complete understanding of the kinetics of nitrification and its response to dynamic inputs is essential before effective control of nitrification will be possible in present and future waste treatment plants. One of the most urgent questions concerning the operation of a nitrification system is the effect of organic material and/or hetrotrophic bacteria on the growth of nitrifying bacteria. It has been widely claimed in the literature that the autotrophic nitrifiers cannot compete successfully in activated sludge systems when carbonaceous BOD levels are high (5)(6). However, no supporting evidence has been

presented regarding either the nature of the competition or the organic compounds involved. The origin of the belief that heterotrophic bacteria and/or organic compounds exert an adverse effect on the nitrifiers is also unclear. It is probably related to the fact that nitrifying bacteria are particularly susceptible to inhibitors of their metabolic process.

The significance of the effects of possible inhibitors in the nitrifying bacteria can be emphasized in the following manner. Nitrifying bacteria do not respond very well to adverse conditions of pH, temperature, and dissolved oxygen, so that a nitrification system can easily be disrupted by improper control of any of these factors. Furthermore, the recovery of a nitrification system is very slow, due to the slow rate of growth of the nitrifying organisms. These problems become almost impossible to overcome if nitrifying organisms are inhibited by organic mater and/or heterotrophic bacteria for extended periods in a nitrifying activated sludge system. No matter what the nature of the inhibitory effect, if the growth rate of the organisms is reduced for an extended period below the minimum rate necessary to maintain the organisms' population in the system, failure of the operation is imminent.

A laboratory scale continuous flow pilot plant activated sludge reactor with total internal cell recycle containing both heterotrophic and nitrifying microorganisms and a synthetic waste containing both carbonaceous and nitrogenous oxygen demand was used in this study. Several different steps and impulse changes were administered to the system running at a steady state and with 100 percent nitrification (whenever possible). Characterization and control of nitrifying

organism responses in this system to environmental changes certainly would be a significant accomplishment, since domestic wastewater treatment plants operate under time-varying loads (7)(8)(9). After characterizing transient response to the particular step or impulse change, the system was run until it attained a new steady state. The major aim of this study was to characterize the nature of the transient response and identify specific inhibitory effects on the biological nitrification process in going from initial to final steady state in step changes. However, another important phase of the study involved subjecting the system to various impulse shock loads quantitatively and observing and investigating any inhibition upon the process of nitrification because of these environmental changes. Also, the aims of this part of the study were to determine if steadiness in effluent substrate concentration as well as reasonable levels of nitrification could be maintained under shock loading conditions and in duration of impulse shock loads, to gain scientific insight into the causative mechanisms for the response, to establish a criterion for design of nitrification plants, activated sludge, or otherwise, and to establish boundary limits for particular shock loads.

CHAPTER II

LITERATURE REVIEW

In 1862, Pasteur predicted the biological nature of nitrification, and clearly expressed his conviction concerning the essential nature of nitrification (10).

As late as 1865, Liebig (10) in addition to other researchers, upheld the view that nitrification was a chemical process.

The important work of Schloesing and Muntz (11) first demonstrated that living organisms were involved directly in the ammonia oxidation reaction. They showed that sewage trickling through a sand column provided nitrate and removed ammonia, and that the reaction was halted completely by introducing chloroform vapor into the column or by heating the column to greater than $100^{o}C$.

In 1878, Warington (12) discovered that nitrate was not produced from ammonium chloride solution unless soil was used as an inoculum. He found that the recovery of ammonia-nitrogen as nitrate was approximately 94 percent. Also, he was the first researcher to demonstrate that the oxidation actually occurred in two stages, and that each stage was accomplished by different organisms. However, Winogradsky was the first person to isolate an unmistakable nitrifying organism in pure culture in 1890. The main reason that previous researchers had failed to isolate a nitrifying organism is that they had placed too much emphasis on the ordinary gelatin culture medium. Winogradsky

used a simple water solution containing ammonium sulfate, potassium phosphate, one gram per liter of each, and basic carbonate of magnesia in excess in well water. He used this solution as a growth medium, and isolated the nitrifying organisms successfully.

It is generally accepted that the biological oxidation of inorganic nitrogen is largely, if not entirely, carried out in an aquatic environment by autotrophic bacteria belonging to the family of Nitrobacteraceae. The Nitrobacteae are strictly autotrophic; that is, they make all of their cellular carbon compounds (protein, lipids, and carbohydrates) from carbon dioxide. They are also aerobic and non-spore forming. Beyond this, however, their characteristics vary. Some are gram-positive; others are gram-negative. The principal genera, Nitrosomonas and Nitrobacter, are the major well defined, active nitrifiers.

In 1890, Winogradsky isolated the first ammonia oxidizing organism, Nitromonas. He later in 1892 withdrew this name and substituted "Nitrosomonas" in its place. Two species were designated, namely, europaea and javanesis. Their chief differences were in the size of the cell, length of flagellum, and their origin. N. europaea was isolated from European soils, and N. javanesis from soils of Java. Winogradsky also isolated a large coccus from the soils of South America, which oxidized ammonia. He called this organism Nitrococcus, but made no species designation. In 1918, Buchanan suggested that this form be called "Nitrococcus americanus." In addition to the above species, Boomeke (14) in 1951 recognized Nitrosomonas oligocarbonogenes, distinguished by its somewhat larger size, greater sensitivity to high temperature and sodium chloride, and lower CO_2 assimilation

per unit of ammonia nitrogen oxidized.

Nitrosomonas

Nitrosomonas is a very small, oval rod often found in pairs, and occasionally in larger aggregates. In young culture it often appears almost spherical; the size is about 0.6 to 0.9 micron. It moves by means of one to two long, polar flagellum which appear to be three to five times as long as the cell body. Nitrosomonas is gram-negative, and forms no spores. It is strictly aerobic, and is very sensitive to acidic conditions.

Nitrobacter

Nitrobacter is a generic name given by Winogradsky to the organisms which form nitrates from nitrites (1). He did not designate any species. He described a species, and it was proposed by Buchanan in 1918 that this organism be called "Nitrobacter winogradskyi," and be taken as the type species.

Nitrobacter is a rod, and is usually found unassociated, but occasionally in pairs or larger aggregates. The size is about 0.5 by 0.8 - 0.9 micron (12). Some are nonmotile, some move rapidly by means of one long thin polar flagellum. The flagellum is often seven to ten times the length of the cell body. Nitrobacter is gram-negative and non-spore forming. They occur in soils, rivers, and streams, and are worldwide in distribution. Under laboratory conditions they grow well only in the complete absence of organic matter (12).

The ability to oxidize inorganic nitrogen is not restricted to

autotrophic organisms (13)(14)(15). However, in no case have the heterotrophic organisms been shown to be able to produce either nitrite or nitrate from ammonium at rates comparable to those of the autotrophic nitrifying bacteria.

Since the early work of Winogradsky, progress has been made toward answering some important questions about these unique organisms. However, due to the difficulties encountered in growing sufficient quantities of nitrifiers in pure culture, it has been only within the last twenty-five years that detailed biochemical studies have been possible. This problem has been solved largely by the development of continuous suspended growth culture techniques ty Engel and Alexander (16), and Goldberg and Gainey (17).

Biochemistry of Nitrification

Nitrosomonas can oxidize ammonia to nitrite, but cannot complete the oxidation of nitrite to nitrate. On the other hand, Nitrobacter is limited to the oxidation of nitrite to nitrate; however, Nitrobacter is not capable of oxidizing ammonia to nitrite. Therefore, to complete the process of nitrification, both groups of organisms must be present. Since complete nitrification is a sequential reaction involving both ammonia oxidizers and nitrite oxidizers, a treatment process for nitrification must be designed to provide an environment suitable for growth of both groups of nitrifying organisms. On a biochemical level, the nitrification process is more complex than simply the sequential oxidation of ammonia to nitrite by Nitrosomonas and the subsequent oxidation of nitrite to nitrate by Nitrobacter. Various reaction intermediates and enzymes are involved.

The stoichiometric reaction for oxidation of ammonium ion to nitrite by Nitrosomonas is

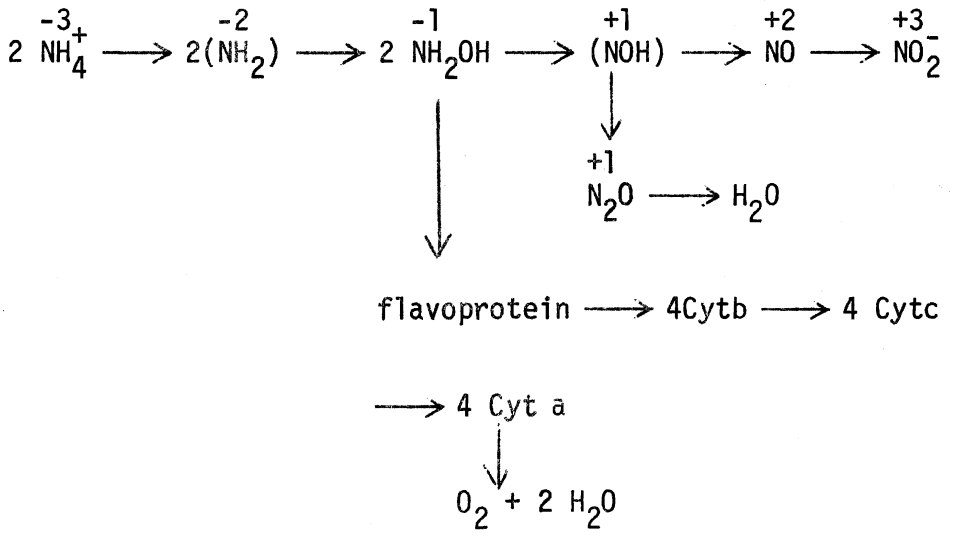
$$2\ NH_4^+ + 3\ O_2 \xrightarrow{\text{Nitrosomonas}} 2\ NO_2^- + 4\ H^+ + 2\ H_2O \qquad (1)$$

The free energy produced by completion of this reaction at physiological concentrations of the reactants has been determined by various investigators: Haug and McCarty, 58 K cal/mole (2), Bass-Becking, 66 K cal/mole (18), Engle, 76 K cal/mole (19), and Nicholas, 84 K cal/mole (20). The generation of reducing power in this reaction is equivalent to three pairs of electrons, since nitrogen is reduced from the oxidation state of -3 in ammonia nitrogen to oxidation state of +4 nitrate nitrogen. This suggests that the reaction takes place in at least three steps since all known chemical reaction mechanisms transfer one pair of electrons at a time. The first postulated step is

$$NH_4^+ + OH^- \longrightarrow NH_4OH \qquad (2)$$

and it involves a very small loss of free energy of approximately 700 K cal per mole and it is thought not to be coupled to energy transfering steps (1). However, Anderson (21) states that this is an endothermic process requiring 47 K cal per mole.

The energy yielding pathway of Nitrosomonas has been the subject of much controversy for some time. The following is what seems to be the consensus of current literature (21)(22)(23)(24)(25)(26)(27):



The first step in the process of oxidation of NH_4^+ is believed to be catalyzed by a copper activated enzyme which has not been identified or isolated at this time. The existence of the enzymatically bound intermediate (NH_2) has not been proven but is widely hypothesized in the literature (26).

Hydroxylamine is a very important naturally occurring stable intermediary product. Through oxidation of hydroxylamine to NO_2^- and its associated electron transport, ATP is generated for use in the cell. The components of the electron transport chain have been generalized as cytochromes b, c, and a, as they resemble the cytochrome system spectrophotometrically, but precise determinations have not been made (22). The link between hydroxylamine and nitric oxide has not been identified conclusively, but the consensus of opinion indicates that it is an enzymatically bound nitroxyl (NOH) compound (26). The final step of oxidation to NO_2^- is rather straightforward, with two cytochromatic redox cycles catalyzing the process.

The efficiency of energy utilization in Nitrosomonas is very low.

This has been attributed to three factors in their metabolism. The autotrophic mode of life requires incorporation of CO_2 into the cell. In Nitrosomonas, enzymes characteristic of the Calvin cycle have been identified. Calvin cycle uses a large amount of energy per atom of carbon assimilated (120 K cal/mole of carbon), thus reducing cell efficiency (28)(29). Another factor is the generation of reducing power in the cell. The normal mechanism with NADH or NADPH is thermodynamically improbable due to the high energy of the end products. A reverse electron transfer mechanism from cytochrome to NADH is used and requires the input of ATP (26)(30). The third factor is necessary for maintaining a nitrite concentration control mechanism whereby nitrite can be reduced enzymatically to N_2O, keeping the concentration of NO_2^- below inhibitory levels in the cell (31)(32). The net result of these factors is that growth yields for Nitrosomonas are very low.

The second stage in nitrification involves the utilization of the nitrite by Nitrobacter. Nitrite is oxidized to nitrate by these organisms, and chemical energy is derived from this oxidation. The relatively simple 2 e^- oxidation of nitrite by Nitrobacter has been well established by biochemists in the last decade, unlike the 6 e^- oxidation of ammonium ion to nitrite by Nitrosomonas (26). Although evidence of the presence of cytochrome a has been found (33), the primary oxidation occurs with iron and cyt c. This cytochrome has a characteristic wavelength of 551 millimicrons and is represented by (cyt 551) in the following schematic:

$$Fe^{+3}(\text{cyt } 551) + NO_2^- \longrightarrow Fe^{+2}(\text{cyt } 551) + NO_2^- \longrightarrow Fe^{+3}(\text{cyt } 551) + NO_3^-$$

Oxidation of nitrite to nitrate yields 17 Kcal/mole of NO_2^- oxidized (34)(35). It represents only the basic primary oxidative, energy yielding reaction. Very high concentrations of cytochrome have been detected in this organism. This is to overcome the fact that the redox potention of cyt 551 is lower than that of nitrite, making the reaction thermodynamically unfavorable (33)(36). Consequently, the ATP levels in the cell are very low, making the cell yield very small--about 0.35 mg/m mole of substrate (36). Factors causing this low yield are the same as those for Nitrosomonas. The overall metabolic processes of Nitrobacter are very similar to those of Nitrosomonas. The generation of reducing power is equivalent to one pair of electrons. No intermediates have been isolated in this enzymic oxidation.

Aleem, Hock, and Varner (37) have shown that reaction

$$NO_2^- + \tfrac{1}{2}\, O_2 \xrightarrow{\text{Nitrosomonas}} NO_3^- \qquad (3)$$

can be separated into two coupled reactions as follows:

$$NO_2^- + H_2O + A \longrightarrow NO_3^- + AH_2 \qquad (4)$$

$$AH_2 + \tfrac{1}{2}\, O_2 \longrightarrow A + H_2O \qquad (5)$$

By tagging the oxygen atom in water, they determined that the oxygen which is incorporated into NO_2^- is derived from water and not from molecular oxygen, as had been assumed. The enzymatic intermediate, AH_2, is regenerated by oxidation with molecular oxygen, which thereby accounts for the oxygen uptake of the reaction.

Heterotrophic Nitrification

In recent years, considerable research has been conducted into the metabolic processes of autotrophic organisms. Since the discovery of nitrifying bacteria in the late 1800s, they have been considered obligate autotrophs, obtaining energy solely from the oxidation of an inorganic substrate (chemolithotroph) and utilizing only carbon dioxide or dissolved carbonate as carbon source. This viewpoint has changed slowly during the last ten years, however, and now many autotrophic organisms have been shown to actually be facultative organisms, capable of growing on organic substrate in the absence of their normal inorganic substrate.

One of the earliest reports of the utilization of organic material by a nitrifying organism was by Clark and Schmidt in 1966 (38). They found that Nitrosomonas europaea could assimilate and utilize pyruvate released as a metabolic intermediate by a yellow-pigmented heterotroph grown in the same culture. Furthermore, the two organisms complemented each other's growth, with the heterotroph utilizing free amino acids released by the autotroph. Continued investigation showed that the Nitrosomonas species was unable to grow in the absence of carbon dioxide, but pyruvate reduced the observed lag phase in batch growth. Pyruvate utilization in this case implies incorporation into metabolic intermediates and no direct oxidation. The following year, Clark and Schmidt (39)(40) found that amino acids were also taken in and utilized by Nitrosomonas europaea. At that time it was also found that several amino acids were inhibitory to this species although, surprisingly, those that were most inhibitory were

taken in to the greatest extent. Although these findings did not alter the viewpoint that Nitrosomonas europaea receives its energy by autotrophic means (chemolithotrophically) they did demonstrate that pre-formed "building blocks" could be taken in and incorporated by the cell into protoplasm.

During this time, Delwiche (41) and Ida (42) were working with Nitrobacter species, and found a number of organic compounds that could be taken in and utilized by them. Acetate was quite readily utilized but no growth or oxidation of nitrite occurred in the absence of carbon dioxide. This led those workers to conclude that Nitrobacter species could not phosphorylate or oxidize the organic substrate to any great extent, and thus true heterotrophic growth was impossible.

The first reported heterotrophic growth was in 1969, when Pope, et al. (43) reported the growth of Nitrobacter agilis heterotrophically on acetate. The ultrastructure of these organisms was compared with those grown autotrophically. Electron micrographs showed a considerable difference in cell composition, although the shape and size were quite the same. The organisms grown in acetate contained a large percentage of electron transport material, presumed to be some sort of polymeric reserve material. More detailed analysis of the cells showed that those grown on acetate were literally stuffed with a reserve material, poly-β-hydroxybutyrate (PHB). As expected, the rate of growth with the organic substrate was very, very slow. Of great interest, however, was the fact that all of the enzymes of the TCA cycle were identified and found to be operative. Presumably the growth rate was limited by some growth factor which the organism could not produce efficiently heterotrophically. Furthermore, it was

found that the growth rate can be greatly increased by the addition of biotin. However, the increase in growth rate was also observed by organisms grown autotrophically upon addition of biotin. The important point was that Nitrobacter agilis could indeed oxidize and grow on the organic substrate. During later experiments by the same workers, it was found that poly-β-hydroxybutyrate began to accumulate quite rapidly in batch experiments where nitrite was present as an energy source (43). As the nitrite became depleted, oxidation of storage material began, and growth continued. However, the rate of growth was very slow, and the energy produced was thought to be used largely for maintenance requirements. It should be noted that this situation occurs only when conditions unfavorable for autotrophic growth prevail.

Further work with both genera of nitrifying bacteria was conducted by Wallace, et al. (26). They demonstrated that several organic compounds were utilized by these organisms and that a large percentage of labelled carbon was found in cell protein. Acetate, pyruvate, α ketoglutarate, succinate, glutamate, and aspartic were all incorporated into cell protoplasm in the absence of the normal nitrogen substrate. It was also discovered that some sort of energy storage materials were available and that they were most likely PHB and a polyphosphate polymer material.

The most significant work with respect to the present investigation was done in 1972 by Pan and Umbreit (44). They worked with mixed cultures of heterotrophic and nitrifying bacteria. Their original hypothesis was that organic material released by nitrifying bacteria which somehow limits their growth would be utilized by the heterotrophic organisms, thereby stimulating growth. Using plate counts, they found

that most of the heterotrophs tested had no effect on the growth of the nitrifiers; however, Candida albicans had a significant stimulatory effect. This response seemed to be highly specific. Mutual interactions of this sort seemed to be rather important in naturally selected populations. Researchers have often observed that when a pure culture becomes contaminated, it is much more hardy than before. Additionally, these investigators also reported that Nitrosomonas could be grown on glucose if a continuous dialysis of the medium was performed (47). They assumed that the organism produces metabolic products which limit its growth, and they suggested this must be removed continuously.

Oxygen Utilization

Oxygen is utilized in the oxidation of both ammonia and nitrite. The stoichiometric quantities required are 3.43 mg oxygen/mg ammonium nitrogen for conversion to nitrite, and 1.14 mg oxygen/mg nitrite nitrogen to be converted to nitrate according to the following reactions:

$$NH_4^+ + \frac{3}{2} O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2\ H^+ + H_2O \qquad (6)$$

$$NO_2^- + \frac{1}{2} O_2 \xrightarrow{\text{Nitrobacter}} NO_3^- \qquad (7)$$

The stoichiometric quantity of oxygen required to convert 1 mg of ammonia nitrogen to nitrate nitrogen is 4.57 mg of oxygen. The quantities of oxygen required can also be expressed in terms of atomic ratio of oxygen to nitrogen (O/N) as 3.0 and 1.0 for reactions VI and VII, respectively.

Bonazzi (45) verified within the limits of manometric accuracy

that the stoichiometric quantity of oxygen was used in these reactions. However, since the work of Bonazzi, several investigators have questioned the amount of oxygen required, and suggested that less than the stoichiometric amount is actually used. The first report concerning this discrepancy was by Buswell and Pagano (46). They claimed that since oxygen is released from carbon dioxide in the synthesis of cell material, less than the stoichiometric quantity of oxygen is required for the oxidation processes. In support of their concept of oxygen released and subsequent utilization, they reported an O/N ratio of 2.75 from the results of an 8-day batch test. It is significant to note that this value was determined three days after the ammonia had been 100 percent oxidized, and it was the only value obtained which was substantially less than 3.0. In addition, the average value for the O/N ratio in their study was 3.11, which is greater than the theoretical value of 3.0.

In 1966, Montgomery and Borne (47) attempted to quantify the oxygen production from CO_2 reduction by assuming theoretical cellular composition of $C_5H_7NO_2$ for the nitrifiers according to the following:

$$5\ CO_2 + NH_3 + 3\ H_2O \longrightarrow C_5H_7NO_2 + 5\ O_2 \qquad (8)$$

From published data they assert that mg oxygen/mg nitrogen oxidized is 3.23 and 1.12 instead of 3.43 and 1.14 for oxidation of ammonia and nitrite nitrogen, respectively. They claim that the discrepancy between the data and the stoichiometric amounts of oxygen required can be accounted for by applying equation VIII.

Wezernak and Gannon (48) used BOD incubation bottles and obtained

corresponding average values of 3.22 and 1.11 mg oxygen/mg nitrogen oxidized, although the standard deviation for each set of data was 0.06 and 0.01, respectively. Jeffrey and Morgan (49) have found that oxygen uptake values in BOD tests for nitrification were within 2.5 percent of the theoretical values.

Reaction VIII represents a biochemical reaction that has no theoretical basis in view of the present knowledge of metabolism for nitrifying bacteria. An understanding of the biochemistry of the Calvin cycle would illustrate that oxygen cannot be released from carbon dioxide, since the oxygen is integrally tied up in the various carbohydrate compounds in the Calvin cycle. Using the empirical formula $C_5H_7NO_2$ to represent cell material is misleading in that it is only an empirical approximation and not a precise measure of cellular material. For a pure culture of nitrifying bacteria, this approximation may easily lead to misconceptions such as those shown in reaction VIII. Considering the degree of accuracy of manometric measurements, most of the results presented (45)(46)(48)(49) only lend confirmation to the concept that a stoichiometric quantity of oxygen is used by the nitrifying bacteria in the oxidation of ammonia to nitrate taking into account the relatively small amount of nitrogen used for synthesis of cellular materials.

Growth Requirements

Macronutrients

Nitrifying bacteria have been shown to grow in a completely inorganic medium, although it is possible that they are not growing at

their maximum growth rate (μ_{max}) due to the unavailability of some unknown micronutrient. The macronutrients CO_2 + O_2 and NH_4^+ for Nitrosomonas and NO_2^- for Nitrobacter are required for growth.

Oxygen Requirements

The requirement for oxygen has been discussed previously, and the stoichiometric requirement of oxygen has been demonstrated by several investigators (24)(34)(35)(45)(46). Oxygen is a limiting substrate at low concentrations, and appears to follow the typical Monod relationship.

The limiting concentration of oxygen which permits nitrification has been reported as 0.5 mg/l (50)(51), although Downing and Scragg (52) reported the limiting value as being less than 0.3 mg/l for a laboratory scale activated sludge system. Wuhrmann (53) noted that in three identically operated high rate activated sludge pilot plants having 1, 4, and 7 mg/l of dissolved oxygen, nitrification was only about ten percent complete at 1 ppm, while it was ninety percent complete at the two higher oxygen concentrations. Downing, et al. (3) concluded that if the concentration of dissolved oxygen exceeded 1 mg/l it is unlikely to limit the rate of nitrification.

In regard to nitrification at higher dissolved oxygen concentrations, Okun (54) reported that nitrification was not affected by dissolved oxygen concentrations up to 33 mg/l. Haugh and McCarty (2) determined that 60 mg/l of dissolved oxygen did not affect the growth rate of the nitrifying bacteria.

Carbon Source

The carbon source for nitrifiers is carbon dioxide, which is available from the atmosphere, respiratory activity of heterotrophic bacteria, and from carbonate alkalinity.

Atmosphere. In equilibrium between water and air, a liter of water contains only about 0.7 mg free carbon dioxide. Because the transfer of carbon dioxide from the atmosphere to the water is such a slow process, the major carbon reserves are the carbonate alkalinity system of water and the heterotrophic bacterial respiratory activity.

Alkalinity. The pK value for the relationship between the ammonium ion and free ammonia is about 6.2.

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$

$$K = \frac{[NH_3][H^+]}{[NH_4^+]}$$

$$pK = - \log K = 6.2$$

The hydrogen ion released by this reaction can react with bicarbonate ion, if available in the water to release additional carbon dioxide (55), according to the following equation:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O \qquad (9)$$

Respiratory Activity

Upon oxidation of organic matter by heterotrophs, carbon dioxide

is released.

$$\text{organic matter} + \text{heterotrophic microorganisms} + O_2 \longrightarrow$$

$$CO_2 + H_2O + \text{new bacteria} \qquad (10)$$

According to Barritt (56), respiratory activity can supply the necessary carbon dioxide for nitrification in acid soils and trickling filters.

It is widely believed that since carbon dioxide is available from the atmosphere, bicarbonate alkalinity, and respiratory activity. it will never be limiting in natural waters. King (55) however, indicated that algal photosynthesis in many systems may be limited by the availability of carbon dioxide. Hence, the nitrification process may also be limited by the lack of available carbon dioxide. This view, however, is generally not accepted.

Other Macronutrients

Other macronutrients required for growth of nitrifying bacteria are ammonia and nitrite for Nitrosomonas and Nitrobacter, respectively. Maximum respiration rate has been reported for Nitrosomonas and Nitrobacter to occur at approximately 100 mg NH_4^+ - N/l and 210 mg NO_2^- - N/l, respectively (1). However, Engel and Alexander (16) reported no inhibition for Nitrosomonas in pure culture at 640 mg/l NH_4^+ - N.

Micronutrients

The requirement for micronutrients by the nitrifying bacteria for growth is similar to that of the heterotrophic bacteria. Some of the

micronutrients appear to be essential, while others are only stimulatory. Since many of these nutrients are required in only trace quantities, it is possible to unknowingly supply them in culture media either as impurities from other compounds or from small quantities present even in ordinary distilled water. Additionally, at higher concentrations, many of these elements appear to be toxic or inhibitory. In general, the micro nutritional requirements for the nitrifying bacteria have yet to be established.

Nitrification Inhibition

Inhibition of the nitrification process can occur by interference either with the general metabolism of the nitrifying cells or with the primary oxidation reactions. Inhibitors of the general metabolism of the cell would not necessarily show up in short-term experiments, whereas those of the primary oxidation reactions would.

Inhibition of primary oxidation reactions may be caused by competitive effects or metal chelating compounds. The general metabolism of cells may be inhibited by uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol, but it is not always easily distinguished which type of inhibition is occurring. When considering the electron transport system, the flavins are inhibited by compounds such as quinacrine, rivanol, and quinine. Cytochromones are adversely affected by cyanide, chlorate, and methyl urethane (1).

Among the most powerful inhibitory substrates are those containing both sulfur and nitrogen attached to the same carbon atom (51). Metal chelating agents such as thiocyanide, thiourea (58) and especially allythiourea also have inhibitory effects, as also do urethanes.

Buswell, et al. (59) reported that the growth rate of Nitrosomonas was reduced by about 25 percent in the presence of 1 mg/l peptone and by 60 percent when 10 mg/l was present. Tomlinson, et al. (60) listed the inhibitory effect of various organic compounds on the oxidation of ammonia by activated sludge. Their experiments showed that long-term effects of inhibitors often differ from their immediate effects. They also found that activated sludge has the ability to adapt to the inhibitor. Quastel and Scholefield (58) found that nitrifying organisms are capable of adapting themselves to the toxic action of guanidine $H\text{-}N{=}C\begin{smallmatrix}\diagup NH_2\\ \diagdown NH_2\end{smallmatrix}$. Organic compounds generally, e.g., glucose (62)(66), glycerol, acetate, butyrate are not toxic, but granulose is toxic to growth of nitrifiers (63). Wild, et al. (56) reported that instantaneous increases or decreases in BOD concentration from 50 to 5 mg/l or 50 to 100 mg/l did not affect the rate of nitrification. It is believed that BOD levels of 40 to 50 mg/l can be tolerated and in fact is necessary in the feed to the nitrification unit (65).

Nitrosomonas Inhibitors. Clark and Schmidt (39) found L-histidine, L-threonine, L-valine, L-methionine, and L-arginine to be inhibitory to nitrifiers. These amino acids were taken up by the cells and utilized in producing cell protoplasm. However, there were no explanations given for inhibitory effects. Low concentrations of organics, especially amines, have been found to be inhibitory as well as the amino acids glycine and alanine. However, no specific inhibitory concentration for these compounds was reported (63). Other amines reported to inhibit ammonia oxidation are trimethylamine, tetramethylamine, chloride, and ethanolamine. The concentrations reported for

50 percent inhibition are approximately 0.005M, 0.01M, and 0.2M, respectively (60)(66). Alcohols, also, have been shown to be inhibitory at high concentrations (60)(66). Methanol, ethanol, and propanol are 100 percent inhibitory at concentrations of 0.005M, 0.09M, and 0.33M, respectively.

The respiratory chain in Nitrosomonas can be inhibited by compounds such as citrate, selenate, arsenate, and pyrophosphate. These compounds inhibit the activity of cytochrome , particularly (25). Light has been reported in the literature (28)(66) to be inhibitory to the nitrification process. This is apparently associated with a pigmented cytochrome.

Nitrobacter Inhibitors. Chlorate ion, CO_3^- was the first reported inhibitor of Nitrobacter. It has been shown to interfere with the action of nitrate reductase and cytochrome 551 in the respiratory chain (28)(35). All halogens have been reported by Lees, et al. (35) to inhibit the oxidation of nitrite when present at high concentrations. Cyanate, arsenate, nitrate, and nitrourea inhibit the enzymatic activity of the cells. These compounds have an ionic structure very similar to the ionic structure of nitrite, and therefore it has been hypothesized that they may compete with the substrate for the active site of the enzyme (28)(35). It was reported by Gould, et al. (67) that the only way to build up the cell mass of Nitrobacter was to continually excrete nitrate from the system. It was also reported that the organism's substrate (NO_2^-) inhibited its growth. This was later found to be caused by the undissociated nitrous acid present at lower pH (68). Classical inhibitors of respiratory chains such as quinacrine, antimycin A, azide,

hydroxylamine, 2,4-dinitrophenol, and a, a' dipyridyl have been reported to be nitrification inhibitors with quinacrine the most significant of the group (26). Table I is a table of compounds found to be inhibitory to nitrification of the activated sludge process in concentrations of less than 20 mg/l (60).

Table II lists ammonia oxidation inhibitors in pure culture.

Table III lists nitrite oxidation inhibitors in pure culutre.

Table IV lists inhibitors of ammonia and nitrite oxidation in pure culture.

Tomlinson, et al. (60) studied the effects of inhibitors in both batch and continuous flow systems and reported that in continuous flow systems, unlike the batch systems, the nitrifying bacteria were found to be able to continue normal growth even in the presence of strong inhibitors such as thiourea, cyanide, dithio-oxamide, and copper. The authors speculated that the inhibitory organic compounds were decomposed by the heterotrophic organisms, consequently reducing the inhibitor concentrations to non-inhibitory levels. However, it is hardly likely that response to copper could have occurred in this way, but no alternate mechanism was suggested for copper inhibition of nitrification. For the most part, inhibition of primary oxidation reactions in the nitrification process and the inhibitor of general metabolism of the cells and cyclymatic inhibition of one form or other and enzyme inhibition play a very important role in the overall mechanism of nitrification inhibition. Consequently, a discussion of enzyme inhibition is pertinent to this literature survey and will be discussed in the following section.

TABLE I

COMPOUNDS INHIBITING NITRIFICATION IN ACTIVATED SLUDGE AT CONCENTRATIONS OF LESS THAN 20 mg/l MOLARITY

Compound	Formula	Concentration necessary to inhibit oxidation of ammonia by 75%	
		mg/l	Molarity
Thiourea	$(NH_2)_2CS$	0.076	10^{-6}
Thioacetamide	$CH_2.CS.NH_2$	0.53	10^{-6}
Thiosemicarbazide	$NY(NH_2)CS.NH_2$	0.18	$2x10^{-6}$
Sodium cyanide	NaCN	0.65	$2.4x10^{-5}$
Methylisothiocyanate	$CH_3.NCS$	0.8	$1.1x10^{-5}$
Sodium methyl dithiocarbamate	$CH_3.NH.CS.S\ Na$	0.9	$7x10^{-6}$
Dithio-oxamide	$NH_2.CS.CS.NH_2$	1.1	$9.2x10^{-6}$
Allyl isothiocyanate	$CH_2:CH.CH_2.NCS$	1.9	$1.9x10^{-5}$
Mercaptobenzothiazole	$C_6H_4.SC(SH):N$	3	$1.8x10^{-5}$
Phenol	$C_6H_5.OH$	5.6	$6x10^{-5}$
Methyl thiuronium sulphate	$[NH_2.C(:NH).S.CH_3]_2H_2SO_4$	6.5	$2.3x10^{-5}$
Skatole	$C_6H_4NHCH:CCH_3$	7	$5.cx10^{-5}$
Aniline	$C_6H_6.NH_2$	7.7	$8.3x10^{-5}$
m-cresol	$CH_3.C_6H_4.OH$	11.4	$1.1x10^{-4}$
o-cresol	$CH_3C_6H_4.OH$	12.8	$1.2x10^{-4}$
Sodium dimethyl dicarbonate	$(CH_3)_2.N.CS.SNa$	13.6	$9.5x10^{-5}$
Tetramethyl thiuram monosulphide	$(CH_3)_2.N.CS.S.CS.N(CH_3)_2$	16	$7.5x10^{-5}$
Guanidine carbonate	$[(NH_2)_2.C:NH]\ H_2CO_2$	16.5	$9.2x10^{-4}$
p-cresol	$CH_3.C_6H_4.OH$	16.5	$1.5x10^{-4}$
Chloroform	$CHCl_3$	18	$1.5x10^{-4}$
Dimethyl *p*-nitroso-aniline	$(CH_3)_2N.C_6H_4.NO$	19	$1.3x10^{-5}$
Dimethyl ammonium dimethyldithiocarbamate	$(CH_3)_2.N.CS.S.NH_2(CH_3)_2$	19.3	$1.2x10^{-4}$
Allyl alcohol	$CH_2:CH.CH_2OH$	19.5	$3.4x10^{-4}$

TABLE II

KNOWN INHIBITORS OF AMMONIA OXIDATION IN PURE CULTURE

Compound	Molarity	Reference
Acetone	0.14	66
Allythiourea	$1x10^{-6}$	66
Aminoethanol	0.2	66
3-aminotriazole	$1x10^{-3}$	66
L-arginine	$1x10^{-5}$	40
N-butanol	0.11	66
Carbon monoxide	0.05	66
2-chloro-6-trichloromethyl pyridine	$5x10^{-5}$	66
Citrate	$2.5x10^{-3}$	25
Dichlorophenolindiphenol	$1x10^{-3}$	55
Dicyclorohexyl carbodiionide	$5x10^{-5}$	66
Diethyldithiocarbamate		66
Diphenylthiocarbazone	$3x10^{-5}$	66
Dipyridyl	$1x10^{-4}$	66
Ethanol	0.09	66
Ethyl acetate	0.20	66
Ethyl	$1x10^{-4}$	66
L-histidine	$3x10^{-6}$	40
Hydrazide	$2x10^{-3}$	66
L-lysine	$2.7x10^{-5}$	40
Methanol	$5x10^{-3}$	66
L-methionine	$6x10^{-5}$	40
Methylamine	0.01	66
Methylene blue	$1x10^{-4}$	66
Methyl urethane	0.04	28
Peptone	$5x10^{-5}$	57
O-phenanthraline	$5x10^{-5}$	66
Phenazine methosulfate	$5x10^{-5}$	66
N-propanol	.33	66
Pyrophosphate	.03	25
8-quinolinol	$1x10^{-5}$	56
Salicyl aldoxime		28
Tetrachlorosalisylanilide	$1x10^{-5}$	66
Tetramethylammonium chloride	.02	90
Thiosemicarbazide	$1x10^{-5}$	66
L-threonine	$3x10^{-5}$	40
Trimethylamine	0.01	90
L-valine	$1.5x10^{-5}$	40

TABLE III

INHIBITORS OF NITRITE OXIDATION

Compound	Molarity	Reference
Antimycin	$2x10^{-4}$	33
	$5x10^{-3}$	1
Azide	$1x10^{-5}$	1
Chlorate	0.01	35
Nitrourea	$1x10^{-5}$	35
Quinacrine	$1x10^{-5}$	1

TABLE IV

INHIBITORS OF AMMONIA AND NITRITE OXIDATION

Compound	Molarity	Reference
Amino guanidine	$1x10^{-3}$	66
Ammonia	$5x10^{-3}$	1
Arsenate	$5x10^{-3}$	25
Cyanate	$2.5x10^{-3}$	35, 1
2,4 Dinitrophenol	$2x10^{-4}$	66
Nitrate	.04	1
Nitrite	.035	1
Potassium cyanide	$5x10^{-6}$	66, 35
Selenate	$5x10^{-6}$	25

Enzyme Inhibitors. Enzyme inhibitors can be classified as either reversible or irreversible. In reversible inhibition there exists an equilibrium between enzyme and inhibitor, while irreversible inhibition is characterized by a progressive increase with time of the inhibitory effect. Remedial measures for reversible inhibition effects could be simply removing the inhibitor from the system. However, irreversible inhibition effects are permanent and will ultimately reach complete inhibition even with the inhibitor present at very low concentrations. The vast majority of inhibitory effects fall into the reversible category (1).

The presence of inhibitors in an enzyme catalyzed reaction may decrease the reaction rate by interfering with the formation or breakdown of the enzyme-substrate complex. There are several ways in which the inhibitor can influence the kinetics of the reaction. The three most simple and most common types of inhibitors are competitive, noncompetitive, and uncompetitive.

Competitive Inhibition. If the inhibitor molecule I competes with the substrate molecule for the active site where substrate binds to form the dead end complex such as EI according to the following

$$E + I \rightleftharpoons EI$$

thereby essentially removing some of the enzyme molecules from the reaction with substrate (S), this type of inhibitor is called a competitive inhibitor. It can be shown mathematically that the Lineweaver-Burk double reciprocal type equation will have a form such as the following for competitive inhibition:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \left(1 + \frac{I}{K_i}\right) \frac{1}{S} + \frac{1}{V_{max}} \qquad (11)$$

In this type of inhibition, V_{max} remains the same as the uninhibited reaction, but K_m will vary with varying inhibitor concentration, implying that the enzyme affinity for the substrate is changing in response to the inhibitor's action.

Non-competitive Inhibition. This type of inhibition results from an inhibitor which can combine with either the free enzyme or with the enzyme substrate complex. This type of inhibitor generally combines with the enzyme somewhere other than at the active site; consequently, the degree of inhibition is independent of substrate concentration.

$$E + I \longrightarrow EI$$

$$ES + I \longrightarrow ESI$$

but ESI does not dissociate to EI + P. It can be shown mathematically that the Line-weaver-Burk type equation for this type of inhibition will be in the following form:

$$\frac{I}{V} = \left(1 + \frac{I}{K_i}\right) \frac{K_m}{V_{max}} \cdot \frac{1}{S} + \left(1 + \frac{I}{K_i}\right) \frac{1}{V_{max}} \qquad (12)$$

In this type of inhibition, V_{max} for reaction with and without inhibitor will be affected. However, K_m remains unaffected.

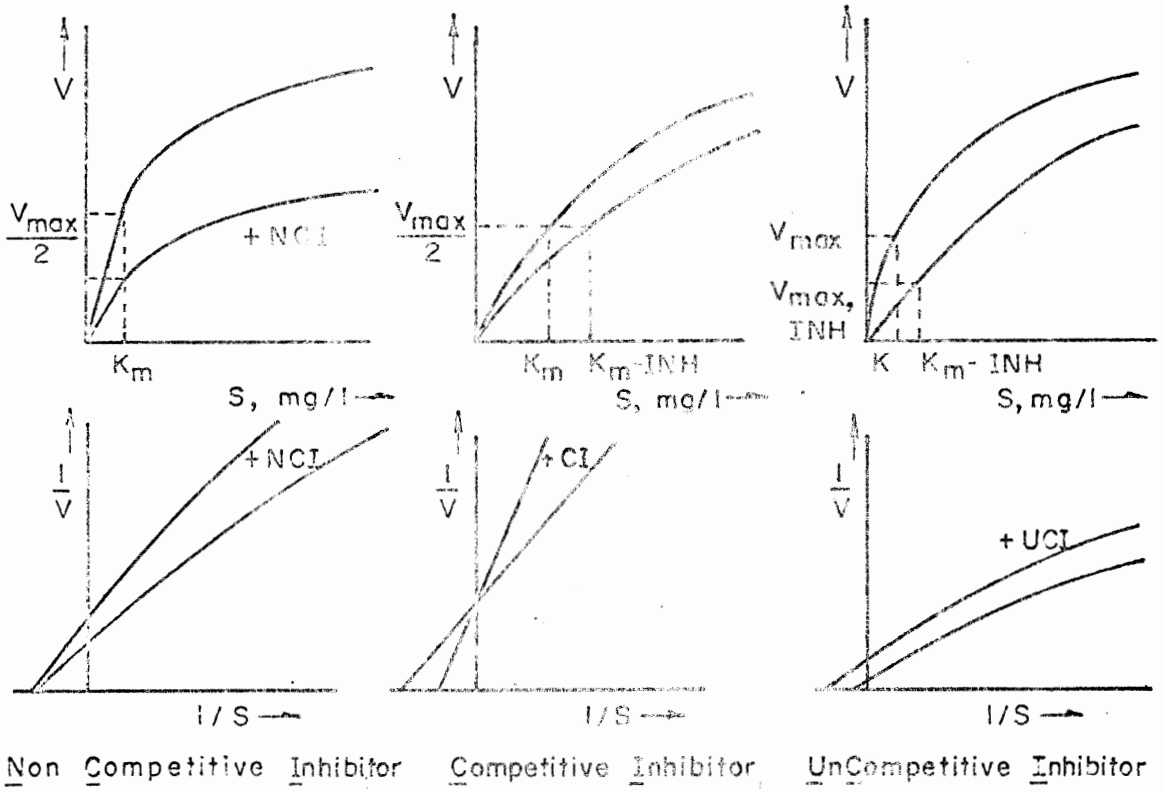
Uncompetitive Inhibition. In this type of inhibition, the inhibitor complexes with the enzyme substrate complex to form a dead end complex.

$$E - S + I \rightleftharpoons ESI$$

It can be shown mathematically that the Lineweaver-Burk type equation for uncompetitive inhibition will be in the following form:

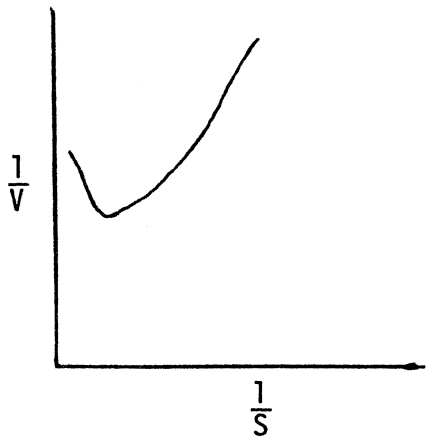
$$\frac{I}{V} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \left(1 + \frac{I}{K_i}\right) \tag{13}$$

Slope of the family of lines representing $\frac{K_m}{V_{max}}$ are the same, but both K_m and V_{max} are affected by an uncompetitive inhibitor. However, the ratio of $\frac{K_m}{V_{max}}$ is not affected.



Non Competitive Inhibitor Competitive Inhibitor UnCompetitive Inhibitor

Substrate Inhibition. There are other types of enzymatic reaction inhibition which do not follow the course of the above classical inhibitions. The data will not plot satisfactorily by any of the methods described above. Such an example may be that of substrate inhibition at high substrate concentrations giving a nonlinear Lineweaver-Burk plot, such as



This type of enzymatic reaction inhibition does not exhibit classical Michaelis-Menten saturation kinetics (hyperbolic). Instead, the plots of velocity versus substrate concentration for these enzymes are sigmoidal. A sigmoidal response generally indicates multiple and cooperative binding sites for substrates (possibly different subunits on the enzyme). The binding of substrate prewumably results in a conformational change in the enzyme. So affinity of the vacant site(s) for the substrate will be affected.

Process Variables. From studies reported, it is apparent that

process variables such as substrate concentration, oxygen, pH, temperature, inhibitors, and kinetic constants of biological nitrification process critically influence the degree of nitrification obtainable in a biological treatment system; therefore, a review of these variables is pertinent to our study, and in the following sections these process variables will be reviewed.

Kinetics of Biological Nitrification

The rate of growth of a bacterial culture is a function of the concentration of some limiting nutrient or substrate. The most widely used mathematical approach for describing the relationship between growth rate and limiting nutrient concentration was reported by Monod (80) in 1942, and is formulated as

$$\mu = \mu_{max} \frac{S}{K_s + S} \tag{14}$$

Monod originally developed this equation to fit the declining phase of bacterial growth for batch cultures. It has been subsequently found to adequately represent batch and continuous cultures (85)(106)(107). His equation is of the same form as that used by Langmuir to describe absorption phenomena and the Michaelis-Menten equation for description of enzyme reaction kinetics. At low substrate concentrations, the rate of growth is directly proportional to the substrate concentration (1st order), since S becomes small when compared to K_s. At high substrate concentrations, the growth rate approaches a maximum value and is independent of substrate concentration (zero order). At higher values of substrate concentrations, the effect of substrate

inhibition can become important.

The growth characteristics of the nitrifying bacteria have been studied by several investigators. The preponderance of evidence indicates that Monod's expression describes the kinetics of nitrification reasonably well. Downing, et al. (3) and Lijkelma (4) have used this continuous growth rate function in their steady state model of nitrification for the activated sludge process. The Monod expression is also used by Poduska and Andrews (88) in their dynamic model of nitrification for the activated sludge process.

The extension of the equation from its use in describing batch cultures to the continuous flow reactor was developed by Monod (89). This model has been further developed and applied to heterogeneous populations by Gaudy, et al. (85). The extension of Monod's growth function from batch to continuous culture was accomplished by utilizing the Monod growth rate function in the appropriate materials balance equations for substrate and organism concentrations in a chemostat with or without recycle as follows:

rate of change in biological solids = rate of increase due to growth − rate of decrease due to outflow

$$V\frac{dx}{d_t} =$$

$$\frac{dx}{d_t} = (\quad - K_d)X - DX$$

rate of change in substrate = rate of increase due to inflow − rate of decrease due to outflow − rate of decrease due to consumption in reactor

$$V\frac{ds}{d_t} = FS_i - FS - V\frac{(\mu - K_d)X}{Y}$$

$$\frac{ds}{d_t} = D(S_i - S) - \frac{(\mu - K_d)X}{Y}$$

where

S_i = influent substrate concentration

$D = \frac{}{V}$ = dilution rate

Y = yield

K_d = decay coefficient

S = reactor substrate concentration

The coefficients μ_{max}, K_s, K_d, and Y are biological constants for particular organisms and particular substrate at particular environmental conditions, and these constants will vary depending on the particular bacterial system being considered, the substrate utilized for growth and the environmental conditions at which growth is taking place.

Kinetic Order of Nitrification Reaction

Zero-Order Reaction

When the reaction rate is independent of the substrate concentration, the rate equation can be described mathematically by the following equation:

$$V = h$$

This results from a high substrate concentration which leads to

a maximum growth rate, indicating no diffusional limitation exists.

From studies by Nelson and cited by Painter (1) it is reported that the rate of ammonia oxidation by Nitrosomonas is linear with time and independent of the NH_3-N concentration. Lees, et al., (90) and Quastel (91) also reported that the rate of nitrification in soil was independent of NH_3-N concentration. Barritt (11) showed that the nitrification phenomenon in soil is the same as in the trickling filter. Aleem, et al. (92) used cell extracts from Nitrobacter in 12 mg/l NO_2-N solution and found that the nitrification rate was zero order from 12 mg/l initial NO_2-N concentration through complete oxidation of the nitrite. Engel, et al. (23) used cell-free extracts of Nitrosomonas europaea and showed that reaction was zero order.

Nicholas, et al. (25) used cell-free extracts and whole cells of Nitrosomonas europaea in separate systems to oxidize hydroxylamine, an intermediate in the oxidation process of ammonia, and found that the reaction was zero order. Anderson (22) used 28 mg/l initial NH_3-N and hydroxylamine in separate systems and reported both systems followed zero order kinetics. Other investigators also have suggested zero order kinetics for nitrification processes up to 60 mg/l of NH_3-N (56) (88)(93)(94)(95)(96)(97). All of the above studies indicate that nitrification rate is zero order.

First-Order Reaction

When the rate of a reaction is proportional to substrate concentration, ∴.

$$V = KS$$

The reaction rate is considered to be first order. Several researchers have used unimolecular or first order rate equation to describe the second or nitrification stage of BOD (99). The second stage, however, may not be due to nitrification but may result from the action of protozoa (100)(101). Other investigators have also used the first order rate equation to evaluate the nitrification process (2)(10).

Autocatalytic Growth Equation

$$V = -\frac{dS}{d_t} = K \cdot S\ (A - S)$$

A = upper limit of substrate concentration

A second order reaction of logistic type has been applied to various biochemical processes, and was used by Wezernak and Gannon (78) to evaluate the nitrification rate in the Clinton River. They reported that the equation provided a more satisfactory comparison than the Monod model. There seems to be no reason to use the autocatalytic reaction equation to predict the nitrification rate in the conventional treatment processes, since the detention time of wastewater in the conventional treatment facilities is usually only a few hours, but the doubling time of organisms is relatively long. Consequently, the autocatalytic reaction equation for nitrification processes is invalid.

Effect of Environmental Conditions on the Nitrification Process

Effect of pH

The pH of the culture medium has been shown to affect the rate of

nitrification of both Nitrosomonas and Nitrobacter in both pure and mixed cultures. Generally, the rate of nitrification exhibits a plateau over a limited pH range and decreases steadily on both sides of the plateau. The pH for optimal rate of nitrification has been reported by many investigators and these are summarized in Table V:

TABLE V

OPTIMUM pH FOR OPTIMAL RATE OF NITRIFICATION

Organism	Culture	Opt. pH Range	Investigator
Nitrosomonas	pure	8.3-9.3	Meyerhof, by Painter (1)
Nitrosomonas	pure	7.5-8.0	Loveless, et al. (69)
Nitrosomonas	pure	8.3-8.6	Lees (29)
Nitrosomonas	pure	7.2-9.0	Engel, et al. (16)
Nitrosomonas	pure	8.0-8.5	Buswell, et al. (59)
Nitrobacter	pure	7.2-8.2	Lees, et al. (35)
Nitrobacter	pure	7.3-8.4	Boon, et al. (68)
mixed	activated sludge	8.0-8.6	Wild, et al. (56)
mixed	activated sludge	8.4-8.5	Rimer, et al. (6)
mixed	activated sludge	7.0-9.4	Hall, by Painter (1)
mixed	activated sludge	8.3-9.3	Meyerhoff, by Painter (1)
mixed	activated sludge	7.4-7.9	Srinath, et al. (97)

The decreased rates of nitrification reported outside of the optimal pH range vary considerably. It has been reported that the activity of nitrifiers decreases by 50 percent for pH values lower than 6.2 and higher than 9.8 (16)(35). Nitrification at pH values as low as 4.5 to 5.0 have been reported in soils by Frederick (70) and extended aeration activated sludge plants by McCarty, et al. (71).

Although the optimal range of pH generally falls between 7 and 9, the differences in the reported optimal pH ranges are also attributable to the pH at which each culture was acclimated. The ability of the nitrifying organisms to acclimate to a new pH is an important factor in deciding the optimal range. Haug, et al. (2) have reported that for a submerged filter, complete acclimation to a pH of 6.0 was obtained within ten days of operation from pH of 7 to 8.5. Acclimation of nitrifying organisms in activated sludge to pH 5.8-6.0 has been reported by Stankewich (72) with a high consistent degree of nitrification.

The nitrification process is a hydrogen ion producing process; consequently, the pH of the reaction medium will be decreased if the medium is not well buffered. Since pH values less than 6.0 affect the rate of nitrification, the alkalinity of the process is an important consideration. For example, in a waste containing 200 mg/l alkalinity as $CaCO_3$ at an initial pH between 7.0-8.5, approximately 20 mg/l ammonium nitrogen can be oxidized without reducing the pH to less than 6.0 (2). The effect of pH changes in biological systems is complex, and usually involves a multiplicity of factors. In a nitrifying system it is difficult to segregate the effects of pH and those caused by a change in unionized HNO_2 or NO_2^- concentrations.

Effect of Temperature on Biological Nitrification

Temperature is another very important factor in nitrifier performance, and several investigators have indicated that nitrification is greatly affected by temperature (2)(3)(5)(34)(51)(64)(67)(73)(75).

Table VI summarizes the optimum temperatures for the nitrification process reported by various investigators.

TABLE VI

EFFECT OF TEMPERATURE ON BIOLOGICAL NITRIFICATION

Investigator	System Studied	Range in Temp. Studied	Opt. Temp. for Process Reported
Balakrishnan, et al. (5)(73)	trickling filter	25-35^{o}	32^{o}
Buswell, et al. (59)	Nitrosomonas isolated from trickling filter	10-37	32
Hopwood and Downing (74) and others (34)(57)	activated sludge	5-30	30
Downing, et al. (3)	activated sludge	7-25	25
Haug and McCarty (2)	submerged filter	5-25	25
Knowles, et al. (75)	activated sludge	10-25	25
Loveless and Painter (69)	Nitrosomonas isolated from activated sludge	10-38	30
Wild, et al. (64)	activated sludge	5-30	30

There are several empirical equations given in the literature in which authors try to correlate rate with temperature. Wild, et al. (64) claim that the rate of nitrification increases through the range of 5-30^{o}C in reasonable agreement with the Van't Hoff-Arrhenius Law.

For Nitrobacter, Laudelout (34) found that the optimum temperature is about 43°C. A preponderance of evidence suggests a temperature range of 25-32°C to be the optimum temperature for the nitrification process. The growth rate decreased rapidly at higher temperatures for both bacteria. Exposure to 53-55°C for ten minutes inactivates Nitrosomonas, and equal exposure to 56-58°C inactivates Nitrobacter (12). At the lower range of operating temperatures, however, nitrification has been maintained, although at lower rates, in pilot scale activated sludge plants at temperatures as low as 5°C (1). Nitrification has been reported by Buswell, et al. (46) to be optimized at 32°C and a minimum number of nitrifiers per ml required to remove 6 mg/l of dissolved oxygen are shown in Table VII:

TABLE VII

EFFECT OF TEMPERATURE ON NITRIFICATION

Medium Temperature	Minimum Population to Remove 6 mg/l Within five days in 1 ml
10	240,000
15	37,000
20	5,000
25	225
30	25

Kinetic Constants of Nitrifying Organisms

For the nitrifying bacteria, the concept of yield is somewhat different, since the yield is defined as the mass of organisms produced per unit mass of inorganic nitrogen oxidized. The nitrifying bacteria do not use ammonia or nitrite for growth in the same sense as heterotrophic bacteria use a substrate for growth since these inorganic nitrogen compounds serve primarily as an energy source and to a much lesser extent, for synthesis. Carbonaceous substrates serve as both an energy and a carbon source for heterotrophs, so that yield is based on the proportion of the substrate which is converted to new cell mass. Since nitrifiers use inorganic nitrogen as their energy source and CO_2 as their carbon source for growth, the yield does not have the same meaning as is more akin to the yield based on ATP employed in measurements with anaerobes.

Yield Constant

According to Lees and Hoffman (29), yield for Nitrosomonas falls as the concentration of nitrite and cellular carbon increases. Later, this view was contested by several investigators (16)(22)(69). These investigators demonstrated that yield constants were more or less independent of concentration of nitrite.

Yield values expressed as weight of dry cells produced per weight of nitrogen oxidized for Nitrosomonas vary from 0.06 reported by Bass-Becking and Parks in 1927 (18) and Skinner and Walker in 1961 (76) to 0.13 reported by Nelson in 1931 [cited by Painter (1)]. Values

reported by Stratton and McCarty (79) are based on thermodynamic calculations and represent the maximum theoretically obtained yields. Tables VIII and IX summarize values of reported nitrification yields.

TABLE VIII

YIELD FOR NITROSOMONAS

Investigator	Yield = $\frac{\text{mg cells}}{\text{mg N oxidized}}$	Reference
Winogradsky	0.059	see 18
Downing, Painter, and Knowles	0.05	3
Skinner and Walker	0.06	76
Loveless and Painter	0.03-0.10	69
Nelson	0.13*	see 1
Meyerhoff	0.06	77
Lees and Hoffman	0.068	29
Knowles, Downing, and Barret	0.05	79
Stratton and McCarty	0.29	79
Downing, Tomlinson, and Truesdale	0.05	57
Wezernak and Gannon	0.098	78

*Based on the assumption that cells contained 50 percent carbon

TABLE IX

YIELD FOR NITROBACTER

Investigator	Yield $\frac{\text{mg cells}}{\text{mg N oxidized}}$	Reference
Lees and Simpson	.02*	35
Boon and Laudelot	.02*	68
Gould and Lees	0.04-0.07	67
Meyerhoff	.06	77
Bass, Becking, and Parks	0.079	18
Knowles, Downing, and Barret	.02	75
Stratton and McCarty	.084	79
Nelson	0.103	1
Mixed Culture		
Stover	3.1**	62

*After reviewing the literature, the preponderance of evidence suggests a value of 0.05 for Nitrosomonas, and 0.02 for Nitrobacter.

**Based on the assumption that all biological solids in the second stage nitrification reactor are nitrifying organisms.

Saturation Constant, K_s

The saturation constant for nitrifying bacteria, K_s, is quite small in comparison to values commonly found for heterotrophic microorganisms. Typically, K_s values are determined by the Lineweaver-Burk technique by utilizing batch experiments at different substrate concentrations. The value of K_s is determined graphically. This method is not readily applicable for nitrifying bacteria because the sensitivity of the readily available analytical techniques for measuring ammonium ion decreases rapidly at the concentrations most needed for determining the saturation constant.

The saturation constant represents the growth limiting substrate concentration at which the growth rate of organisms is one-half the maximum growth rate. The saturation constant, K_s, like the maximum growth rate, is a function of biological characteristics of the organisms as well as the environment to which they are exposed. One of the most important environmental factors is the temperature of the reactor. Knowles, et al. (75) determined an empirical equation to describe saturation constant variation with temperature for Nitrosomonas:

$$\log K_{s_N} = 0.051(T) - 9.158 \qquad \text{XV}$$

For Nitrobacter, they suggested the following equation:

$$\log K_{s_B} = 0.063(T) - 1.149 \qquad \text{XVI}$$

For a temperature range of 8-30°C, the saturation constant K_s for Nitrosomonas and Nitrobacter is expressed as mg NH_4^+ and mg NO_2^- per liter,

respectively. The following tables include the saturation constants for nitrifying bacteria reported in the literature.

TABLE X

SATURATION CONSTANT FOR NITROSOMONAS

Investigator	Temp.	K_s mg/l	Culture	Reference
Downing, et al.	21	0.18*	activated sludge	3
Melamed, et al.	25	0.37*	activated sludge	81
Loveless, et al.	20	1.0	activated sludge	69
Lawrence, et al. Stratton, et al.	15,20,25	2.8,3.6,3.4	synthetic river water	83, 79
Haug, et al.	15,20	0.5-1.0	Warburg analysis	2
Painter	25	3.5	laboratory culture	1
Williamson, et al.	20	.5	laboratory culture	82

*Value determined by dropping mercury electrode technique.

TABLE XI

SATURATION CONSTANT FOR NITROBACTER

Investigator	Temp.	K_s mg/l	Culture	Ref.
Laudelot, et al.	12,18,32	1.4,2.1,9.4	laborature culture	34
Melamed, et al.	25	0.25	activated sludge	81
Lawrence, et al. Stratton, et al.	15,20,25	0.7,1.1,0.7	synthetic river water	83, 79
Painter Lees, et al.	30	6	laboratory culture	1, 35
Painter Gould, et al.	28	6	laboratory culture	1, 67
Painter Laudelot, et al.	32	8.4	laboratory culture	1, 34
Painter	25	5	laboratory culture	1
Williamson, et al.	20	.07	laboratory culture	82
Mixed culture	20	0.18	activated sludge	84

The low values of K_s for nitrifying bacteria indicate that when concentration of ammonium or nitrite is greater than about 5 mg/l, the growth rate will be at a maximum and zero order with respect to substrate concentration. These lower values of K_s are in contrast to the K_s value for heterotrophic bacteria in activated sludge systems which are commonly between 50 and 125 mg/l for heterogeneous populations, as suggested by Gaudy, et al. (85).

μ_{max}, Maximum Specific Growth Rate

μ_{max}, the maximum specific growth rate, represents the mass of microorganisms produced per unit mass of organisms per unit of time when growing at their maximum rate or highest substrate concentration without inhibiting the growth rate. The maximum growth rate is related to K, maximum substrate utilization rate, by the following equation (86):

$$\mu_{max} = y.K \qquad \text{XVII}$$

Values for growth rate of microorganisms are often reported in the literature in terms of doubling time, which is related to the growth rate by the following relation (85)(86):

$$\mu = \frac{\ell n_2}{t_d} \qquad \text{XVIII}$$

where t_d = doubling time or generation time. It should be noted that doubling time or generation time is the time required to double cell numbers, and consequently the time required to double cell mass. These two terms are equivalent only when cell growth is balanced. This

equation is valid during the log growth phase or the exponential growth phase. The maximum growth rate for nitrifying bacteria has been reported by several investigators; values are summarized in Tables XII and XIII.

TABLE XII

MAXIMUM SPECIFIC GROWTH RATE OF NITROSOMONAS

Investigator	Temp.	pH	μ_{max} Days^{-1}	Environment	Ref.
Buswell, et al.	10,15 20,30	-	0.25,0.50 0.94,1.74	activated sludge	59
Engel, et al.	30	8.0	1.5	laboratory culture	16
Gibbs, et al.	30	8.0	1.03	soil	12
Skinner, et al.	28,32	7,7.4	2.1	activated sludge	76
Loveless, et al.	25	7.6,8	.88,1.38	activated sludge	69
Hall	10,18	-	.37,.56	activated sludge	see 1
Lees and Hoffman	30	8.0	.46	laboratory culture	29
Gujer, et al.	12,21	-	0.4, 0.85	activated sludge	see 82
Wuhrmann, et al.	16	-	.57	activated sludge	see 82
Melamed, et al.	25	-	0.17	activated sludge	81
Balakrishnan	23	-	0.37	activated sludge	87
Loehr, et al.	20	-	.7	activated sludge	see 82
Stratton, et al. Lawrence, et al.	15,20,25	-0.21,0.48,0.55		synthetic river water	79,83
Poduska, et al.	23	-	1.08	activated sludge	88
Lawrence, et al.	20	-	0.23,0.5	activated sludge	8

TABLE XIII

MAXIMUM SPECIFIC GROWTH RATE OF NITROBACTER

Investigator	Temp.	pH	μ_{max} Days^{-1}	Environment	Ref.
Boon, et al.	32	-	1.39	activated sludge	68
Stratton, et al.) Lawrence, et al.)	15,21	-	0.28,0.34	(synthetic ((river water	79,83
Poruska, et al.	23	-	1.44	activated sludge	88
Downing, et al.	6,23	7.0	0.03,0.14	activated sludge	3

Knowles, et al. (75) determined the effect of temperature on maximum specific growth rate, μ_{max}, and suggested that the following empirical equation will predict the effect of μ_{max} in the range of 8-30^{o}C reasonably well. Where temperature is expressed in ^{o}C, and μ_{max} in days^{-1},

$$\log \mu_{max}\ N = 0.0413(T) - 0.944$$

$$\log \mu_{max}\ B = 0.0255(T) - 0492 \qquad (19)$$

N = Nitrosomonas

B = Nitrobacter

Using yields of 0.05 and 0.02 for Nitrosomonas and Nitrobacter,

respectively, and doubling times of 11 and 15 days, the maximum substrate utilization can be calculated as 9.25 g NH_4^+ - N g cells/hour for Nitrosomonas, and 2.31 g NO_2^- - N g cells/hour for Nitrobacter.

Loveless and Painter (69) have reported values for maximum specific growth rate of Nitrosomonas which ranged from 0.28 $days^{-1}$ for several conditions. They could not explain the large variation in their results; however, they stated that good replication was obtained during simultaneous determinations. Jenkins (51) demonstrated that the maximum growth rate constant is generally lower in activated sludge as compared to pure conditions.

Nitrification and the Activated Sludge Process

Establishment of nitrification in the activated sludge process depends on maintaining an adequate population of nitrifying bacteria in the aeration tank. To maintain a nitrifying population, a sludge wasting program which retains the slower growing nitrifying bacteria must be utilized. In this regard, sludge age or mean cell residence time, which is equal to the reciprocal of the net growth rate and is defined by equation (20), is the critical factor.

$$\frac{1}{\mu_N} = \Theta_c = \frac{VX}{Q_W X_W + Q_e X_e} \tag{20}$$

where

Θ_c = mean cell residence time, days

V = volume of aeration basin, liters

Q_W = flow of wasted sludge

X_W = concentration of wasted sludge

Q_e = flow of the effluent

X_e = concentration of biological solids in the effluent

The organic carbon concentration in the influent, S_i, controls the growth rate of heterotrophic bacteria and higher organic loadings up to some maximum established by organisms. Growth rate will produce increased sludge quantitites which must be wasted from the system in order to maintain a steady state. An increased wasting rate decreases the Θ_c, thus resulting in a decrease in the fraction of nitrifiers in the mixed liquor. Ultimately, this will lead to cessation of nitrification at higher loading rates. The critical sludge age for nitrification depends on two primary factors: first, the growth rate of the nitrifying bacteria, which is dependent on nitrogen substrate concentration, temperature, pH, dissolved oxygen concentration, and the presence of inhibitory compounds or conditions; second, the net solids production rate of the process, which is dependent on the growth rate and yield coefficient of heterotrophic bacteria.

Until the recent application of mathematical models, based on kinetic principles, some of the basic concepts regarding nitrification in the activated sludge process were not clearly understood. Downing, et al. (3) were among the first investigators to develop a kinetic model in which they presented a theoretical basis for nitrification in the activated sludge process. They concluded that consistent nitrification would be expected in activated sludge plants only if the growth rate of the nitrifying bacteria was greater than the rate of loss through the effluent and sludge wasting streams. Based on this concept and using experimentally determined values for growth rate coefficients, the degree of nitrification in activated sludge plants

was predicted reasonably well by Downing, et al. (3).

Johnson and Schroepfer (108) operated laboratory scale nitrifying activated sludge reactors at 20°C and found that nitrification was obtained at sludge ages greater than three days and was not a function of the hydraulic retention time of the system. This sludge age corresponded to an F/M ratio of 0.3 lb BOD/lb MLVSS/day. Balakrishnan and Eckenfelder (87) concluded that an F/M ratio of 0.3 to 0.4 should not be exceeded if nitrification is to be maintained.

Kincannon and Stover (109) more recently have reported that a sludge age of three days is too low for nitrification to take place, and in fact that at three days the nitrifiers will be washed out of the system in the two-stage activated sludge nitrification process; that a sludge age greater than three days is necessary if nitrification is to be maintained in the activated sludge process. They reported that to achieve complete nitrification, the required mean cell residence time is six days for the nitrification unit of the two-stage process and ten days for the one-stage process. Consequently, to ensure complete nitrification, the design mean cell residence time should be greater than ten days for the one-stage activated sludge plant. This study slso showed that sludge production in the one-stage activated sludge process is almost one-half of that in the two-stage activated sludge.

Shock Loading or Dynamic Forcing

Any sudden change in the physical or chemical environment in a biological system can be defined as shock to the system or shock load (85). Major types of shocks are:

1) quantitative

2) qualitative

3) hydraulic

4) pH

5) temperature

6) toxic

Our major concern in this study deals with quantitative shock loads. This type of shock load involves generally an increase or decrease in the concentration of substrate, S_i, and occurs in all treatment plants. The quantitative shock load can be subdivided into two general classifications: a) step quantitative shock load, and b) impulse quantitative shock load.

Quantitative Shock Loads

Step Shock Loads

In this type of shock load, the S_i is increased or decreased without changing the hydraulic retention time and with no change in flow rate. For nitrifying activated sludge, the meaning of a quantitative shock load is different, since either change in concentration of carbon source or concentration of ammonia source, or changes in both concentrations can be considered quantitative shocks to the system and these shocks can have different effects on the system as a whole. The results are certainly more complicated than in a non-nitrifying activated sludge unit.

Impulse Shock Loads

An impulse quantitative shock load is more severe than a step shock

because a slug dose of concentrated feed is forced on the system. This type of shock, although more severe in nature, is usually shorter in duration. Slug doses to sewers are commonly experienced; when the run to the treatment plant is short, the activated sludge tank may receive a relatively unattenuated slug. The use of a completely mixed reactor is helpful in this regard because of instantaneous dilution of the slug and also dilute-out patterns usually follow in the continuous flow system.

The dilute-out phenomenon can be expressed mathematically by the following equation:

$$C_t = C_o e^{-Dt} \tag{21}$$

where

C_t = reactor concentration at any time, t

C_o = reactor concentration rate after slug dose was administered

D = dilution rate $\frac{1}{day}$

t = time (days)

The quantitative step shock load in case of an increase in substrate concentration will follow a dilute-in curve which can be expressed mathematically by equation 22:

$$C_t = C_o\left(1 - e^{-Dt}\right)$$

where

C_o in this case is the concentration of effluent.

CHAPTER III

MATERIALS AND METHODS

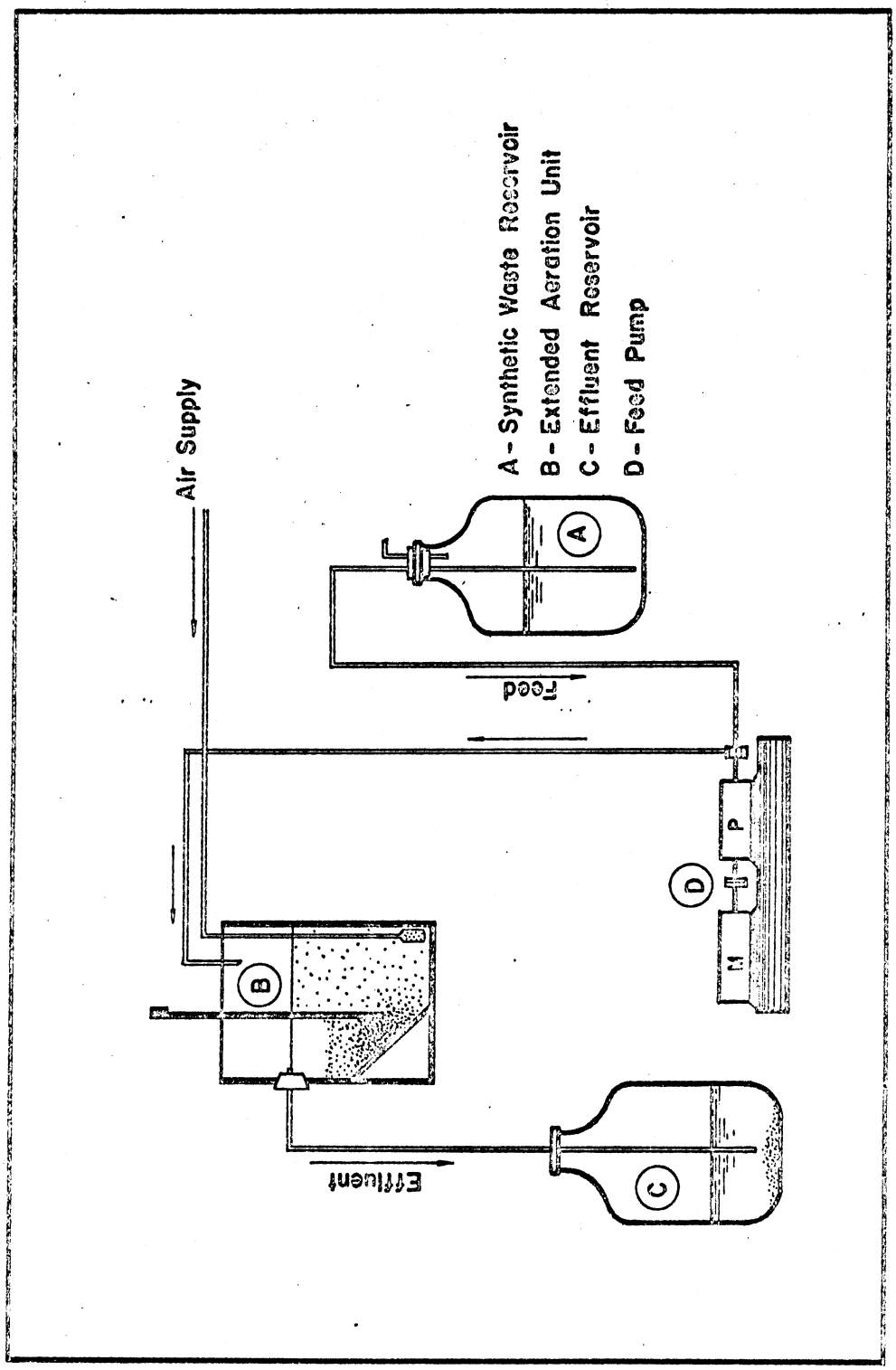
Experimental Approach

In order to understand the kinetics of nitrification and its responses to step and impulse inputs, and to gain insight into the mechanism of nitrification inhibition, a laboratory scale experimental pilot plant reactor was used. A synthetic waste of completely soluble substrate was fed to a continuous flow pilot plant activated sludge reactor at a constant flow rate of 11.4 ml/min. All experiments were conducted under closely controlled conditions of feed rate, pH, and growth rate. The only variation applied to the system was the influent feed concentration, and also in a series of experiments, the growth rate was changed. The chemical oxygen removed was used to measure organic substrate concentration of incoming feed and amount of organic matter in the outgoing effluent, while NH_4^+-N and NO_3^--N and occasionally NO_2^--N were measured to determine the nitrogen concentration. Batch studies were conducted to determine different biological heterogeneous populations and kinetic constants.

Experimental Apparatus

A plexiglass pilot plant activated sludge unit with total internal cell recycle, as shown in Figure 1, was used for continuous flow

Figure 1. Schematic Flow Diagram of a Laboratory-scale Continuous Flow Nitrifying Activated Sludge System With Total Internal Recycle


A - Synthetic Waste Reservoir
B - Extended Aeration Unit
C - Effluent Reservoir
D - Feed Pump
Air Supply
Feed
Effluent
A
B
C
D
P
M

experiments. The aeration and settling compartments were contained in the same reactor and separated by an adjustable plexiglass baffle. The baffle was placed just above the tank bottom, leaving a small gap so that the aeration tank mixed liquor could pass to the settling tank, and sludge could recycle to the aeration tank. The total volume of the system was 10.8 liters (approximately 7.2 liters) of aeration capacity, and 3.6 liters in the settling tank. Compressed air, which had been filtered and saturated with water, was supplied to the aeration compartment through sintered glass diffusers to provide oxygen supply to the biological solids and for mixing the aeration tank solids, and to provide "suction" to recycle the settled biological solids from the settling chamber into the aeration tank. The temperature of the reactor was monitored throughout the study, and was maintained at $20 \pm 2^{o}C$. The pH of the system was monitored very carefully and was adjusted to 7.2 as needed by adding a few drops of ion potassium hydroxide solution.

Operation under continuous flow conditions was accomplished by pumping the feed solution to the aeration tank. A Milton-Roy dual, positive displacement pump (Mini-pump, Model MM2-b-96R) provided a continuous flow to the system, pumping at the rate of 11.4 ml/min. This provided a detention time of 10.5 hours in the aeration tank, and 5.2 hours in the settling tank or total hydraulic detention time of 15.7 hours.

The effluent flowed by gravity from the settling compartment to the holding tank, where it was collected.

The heterogeneous population of bacteria used for the laboratory pilot plant was developed by using an initial inoculum from the primary

clarifier effluent of the Stillwater municipal wastewater treatment plant. These cells were cultivated in batch reactors, using a synthetic waste. After growing a sufficient quantity of cells, continuous flow operation was started.

A completely soluble synthetic waste containing glucose as the carbon source and ammonium sulfate as the nitrogen source was used in these experiments. Glucose is easily metabolized by most heterogeneous populations of microorganisms; consequently, it provides an excellent carbon and energy source. Glucose can be easily monitored by the chemical oxygen demand test, and it can be made up in a concentrated form for ease of handling and storage. The desired concentration of feed could be prepared by using the concentrated solution as a stock solution. A solution contains 200 g of glucose per liter was made and was used as stock solution for preparation of feed. This concentrated glucose solution was prepared as often as every week. 2.5 ml of this solution diluted to a liter will contain 500 mg/l liter of glucose. The ammonium sulfate can also be made up in a concentrated form for ease of handling and storage. A concentrated solution of ammonium sulfate containing 100 g of ammonium sulfate per liter was prepared as often as once a week. This solution served as stock solution for the nitrogen source. 2.5 mg/l per liter of this solution will contain approximately 53 mg/l of NH_3-N. Ammonium sulfate was the sole nitrogen source in the synthetic wastewater. Since all microorganisms require some form of phosphorous for their metabolism and the culture medium should have some buffering capacity, a concentrated (1M) phosphate buffer solution was prepared by dissolving 124.5 g of K_2HPO_2 and 38.75 g of KH_2PO_4 per liter of solution. The pH of the resulting concentrated phosphate

buffer was 7.5. Five ml of 1M phosphate buffer was used per liter of feed solution to provide buffering capacity and a phosphorous source. Most microorganisms require limited amounts of different elements and some trace elements for growth and synthesis to provide necessary salts and trace minerals. A concentrated salt solution containing 0.05 g ferric chloride, 0.75 g/l of calcium chloride, 1.0 g of manganous sulfate, and 10.0 g per liter of magnesium sulfate was prepared. Five ml of this solution was used per liter of feed solution containing 500 mg/l of COD, and increased or decreased proportionately with feed COD variations. The salt solution was prepared as often as every two weeks. Unknown trace minerals were supplied through tap water as ten percent of the feed solution was tap water. These concentrated solutions were prepared for ease of handling and storage, and were kept at a temperature of 1^{o}C in closed sterile containers to avoid contamination and growth. To minimize microbial growth in the feed lines and the feed container, the following procedures were regularly used whenever the feed solution was changed (every 24 hours). The feed containers were cleaned with chromic acid cleaning solution and rinsed several times with tap water, and finally rinsed with distilled water. The feed lines and pumps were disinfected by pumping a five-percent solution of Clorox in water. At all times, one of the feed lines was being disinfected while the other was pumping feed solution to the system. The feed lines were alternated every day to retard microbial growth in the feed lines and pump terminals. Before changing the feed line, the disinfected line was washed several times (at least three times) with tap water and once with the feed solution by gravity flow. This alteration of feed lines provided a positive control for the retardation of growth in the lines.

The initial inoculum for the autotrophic microorganisms, Nitrosomonas and Nitrobacter, were obtained from the addition of a small quantity of cultivated soil to batch units. These batch units contained all of the necessary nutrients for autotrophic metabolism of nitrifiers plus 30 to 40 mg of organic carbon. After cultivation of the nitrifying bacteria in batch, they were transferred to continuous flow reactors several times during a four-month period until full nitrification was developed. This heterogeneous population containing both heterotrophic and nitrifying bacteria was employed for both batch and continuous flow studies under closely controlled experimental conditions.

Sampling Under Steady State Conditions

Prior to sampling, the feed flow was stopped. The effluent outlet was closed, and the plexiglass baffles separating the aeration and settling compartments were pulled out, allowing the biological solids to mix. A sample of mixed liquor (50 ml) was removed from the reactor system for biological solids determination. At the same time, a predetermined amount of mixed liquor (depending on the desired growth rate and previous days' effluent suspended solids) was wasted from the system. The baffle was immediately replaced, and after the sludge blanket in the settling chamber was lowered about half an inch, usually in less than five minutes, the effluent outlet was opened and feed pumps were started. Samples were also taken from the feed solution. A composite sample was taken from the effluent holding tank for effluent suspended solids determinations. Samples during shock loads were from the aeration and settling tanks.

The growth rate or mean cell residence time was governed by the total amount of biological solids withdrawn from the system through daily wasting plus quantity lost in the effluent. Since nitrifying organisms have high generation times and low yield coefficients compared to heterotrophic microorganisms, they require higher Q_c (mean cell residence time) or lower growth rate to be maintained in the system. The mean cell residence times of three days or lower will result in complete washout of nitrifyers from the system (62). Because the nitrifying population in the system cannot compete, the wastage rate will be higher than the growth rate of the nitrifying organisms (62).

Continuous Flow Experiments

After operation was started, the system was operated until steady state was maintained, and nitrification was started after the system was seeded several times by nitrifying organisms from the batch reactors. After complete nitrification was accomplished in the reactor system and it was maintained for at least seven to ten days, shock loading procedures were initiated. Several different shock loads were administered to the system. Two types of dynamic forcings were employed in this study. These were influent concentration steps or quantitative shock loads and concentration impulses or slug dose shock loads.

Influent Concentration Steps on Quantitative Shock Loads

Step changes in influent substrate concentration were accomplished by changing the glucose concentration in the feed and increasing all of the other constitutents in the feed solution proportionately.

Compositions of the feeds used for different shock loads are given in Tables XV, XVI, XVII, and XVIII. This type of shock loading was administered without changing the flow rate. In a different shock load, a NH_3-nitrogen concentration was kept the same as the previous steady state condition, and only the glucose concentration was increased by 100 percent without changing the flow rate. Composition of the effluent solution for this shock load is given in Table XIX. In a similar shock load, glucose concentration in the influent solution was kept the same as the previous steady state, and ammonia nitrogen concentration was increased 100 percent. Composition of the influent solution for this shock load is given in Table XX. This type of dynamic forcing was administered to the system at two different mean cell residence times.

TABLE XIV

COMPOSITION OF GROWTH MEDIUM PER 500 mg/l GLUCOSE AND 50 mg/l NH_3-N

Constituents	Amount
Glucose	500 mg/l
Ammonium sulfate $(NH_4)_2SO_4$	250 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	50 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.25 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	5.0 mg/l
Calcium chloride, $CaCl_2$	3.75 mg/l
1 M phosphate buffer solution, pH 7.0	5 ml/l
Tap water	100 ml/l

TABLE XV

COMPOSITION OF GROWTH MEDIUM PER 1000 mg/l GLUCOSE AND 100 mg/l NH_3-N

Constituents	Amount
Glucose	1000 mg/l
Ammonium sulfate $(NH_4)_2SO_4$	500 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	100 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.5 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	10.0 mg/l
Calcium chloride, $CaCl_2$	7.5 mg/l
1 M phosphate buffer solution, pH 7.0	100 ml/l
Tap water	100 ml/l

TABLE XVI

COMPOSITION OF GROWTH MEDIUM PER 1500 mg/l GLUCOSE AND 150 mg/l NH_3-N

Constituents	Amount
Glucose	1500 mg/l
Ammonium sulfate $(NH_4)_2SO_4$	750 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	150 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.75 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	15.0 mg/l
Calcium chloride, $CaCl_2$	10.25 mg/l
1 M phosphate buffer solution, pH 7.0	15 ml/l
Tap water	100 ml/l

TABLE XVII

COMPOSITION OF GROWTH MEDIUM PER 2000 mg/l GLUCOSE AND 200 mg/l NH_3-N

Constituents	Amount
Glucose	2000 mg/l
Ammonium sulfate $(NH_4)_2SO_4$	1000 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	200 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	1.0 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	20.0 mg/l
Calcium chloride, $CaCl_2$	15.0 mg/l
1 M phosphate buffer solution, pH 7.0	20.0 ml/l
Tap water	100 ml/l

TABLE XVIII

COMPOSITION OF GROWTH MEDIUM PER 500 mg/l GLUCOSE AND 100 mg/l NH_3-N

Constituents	Amount
Glucose	500 mg/l
Ammonium sulfate $(NH_4)_2SO_4$	500 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	50 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.25 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	5.0 mg/l
Calcium chloride, $CaCl_2$	3.75 mg/l
1 M phosphate buffer solution, pH 7.0	5 ml/l
Tap water	50 ml/l

TABLE XIX

COMPOSITION OF GROWTH MEDIUM PER 1000 mg/l GLUCOSE AND 50 mg/l NH_3-N

Constituents	Amount
Glucose	1000 mg/l
Ammonium sulfate $(NH_4)_2SO_4$	250 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	100 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	0.5 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	10.0 mg/l
Calcium chloride, $CaCl_2$	7.5 mg/l
1 M phosphate buffer solution, pH 7.0	10 ml/l
Tap water	100 ml/l

Reverse Shock Loads

After the system achieved a new steady state under new loading conditions, the influent concentration was changed to the previous steady state concentration, and responses of the system were studied under this type of shock.

Impulse Shock Loads

Impulse loading of different amounts of ammonia-nitrogen, glucose, and combined glucose and ammonia- nitrogen concentration were administered to the reactor system operating under steady state conditions by adding an accurately measured quantity of corresponding

concentrated feed solution directly to the reactor. In this type of dynamic forcing, the feed flow rate to the reactor remained unchanged after administration of the impulse shock. The steady state influent containing 500 mg/l glucose and 50 mg/l ammonia nitrogen was fed to the reactor system during the dynamic impulse shock. This type of dynamic test is the most severe forcing for the organisms since they experience a step change in the concentration of the substrate(s) added all at once, but this type of shock is shorter in duration and it will follow a dilute-out pattern.

Batch Growth Studies

During each steady state continuous flow run, cells from the reactor system were employed as initial inoculum for batch growth experiments to determine kinetic constants μ_{max}, K_s, Y_t and b_d, using methodologies described by Gaudy, et al. (85)(106)(107). The medium used for batch growth experiments was the same as that employed in the continuous flow experiments. The cells were grown in 250 ml Erlenmeyer flasks with glucose concentrations ranging from 100 to 1000 mg/l as the limiting nutrient. Initial inoculum concentration was the same in all flasks, with an initial optical density of approximately 0.036 (percent transmission = 92 percent). The total volume of medium fluid per flask in these batch experiments was 40 ml. These flasks were placed on an oscillating shaker (Eberbach), which was adjusted to 100-110 oscillations/min. The growth curves were obtained by measuring optical density at frequent intervals at 560 nm using a Bausch and Lomb Spectronic 20. The initial and final substrate concentrations and suspended solids were measured using COD and membrane filtration

methods, respectively, according to Standard Methods (103), which allowed determination of the batch cell yield, Y_{t_B}. The μ_{max} and K_s were determined by plotting the data obtained from batch growth experiments. The plotting and calculation technique is discussed in the Results section.

Analytical Methods

The analytical procedures employed during this investigation for both batch and continuous flow reactors, except for ammonia nitrogen and volatile organic acid determinations, which were performed according to procedures described in Standard Methods (103). A Bausch and Lomb Spectronic 20 colorimeter with one-half inch diameter by four-inch matched curvettes was utilized for all colorimetric determinations.

Biological Solids

Biological solids were determined gravimetrically by the membrane filter method. Samples were filtered through 0.45 μm pore size membrane filters (Millipore Filter Corp., Bedford, Mass.). Filters were placed in lightweight (about 0.3 g) aluminum pans and placed in a drying oven at 103°C for two hours. The pans containing the filters were then transferred to a desiccator for cooling (about two hours). After cooling, the pans were weighed using a Mettler Gram-atic Balance (Mettler Instrument Corp., Hightstown, N. J.). Known volumes of sampling (usually 50 ml) were filtered with the aid of a vacuum pump. For samples which were hard to filter, a Sorvall Superspeed Centrifuge, Type SS-IA (Ivan Sorvall, Inc., Bristol, Conn.) was used to reduce

filtration time. Samples were centrifuged at a rate of 10,000 rpm for five minutes. The supernatant was filtered, then the pellet of solids was removed from the centrifuge tube and placed on the membrane filter. After filtration was completed, the filters were placed on the corresponding pans and placed in a 103^{o}C oven for a period of two hours. The samples were then removed and cooled in the desiccator, and weighed again. The biological solids were determined from the difference between initial and final weighing.

Ammonia Nitrogen

The ammonia nitrogen for feed and the effluent was determined by a method developed by Niss and described by Ecker and Lockhart (104). The range of this method is between two to 20 mg/l of ammonia nitrogen. Two reagents are required--reagent A containing 4.7 grams of sodium citrate, 1.7 grams of citric acid, 9.6 grams of phenol, in 480 ml of distilled water, and reagent B containing 6.0 grams of boric acid, 8.0 grams of sodium hydroxide, 30 ml of commercial Clorox bleach in 200 ml of distilled water. Samples must be diluted to have the ammonia nitrogen concentration within workable range of the method. 1.0 ml of cell-free sample is required for determination. To the 1.0 ml samples, 5.0 ml of reagent A and 2.0 ml of reagent B are added. Samples are then mixed and heated in a water bath in boiling water for a period of exactly five minutes. Then the samples are cooled rapidly in ice water. The optical density of the blue-colored solution is then measured at 615 nanometers against a blank. The concentration of ammonia nitrogen is determined from a standard curve prepared from known quantities of ammonia nitrogen.

Nitrite Nitrogen

Nitrite nitrogen was determined by the Diazotization Method described in Standard Methods (103), coupling diazotized sulfalinic acid with naphthylamine hydrochloride at pH 2.0 to 2.5. Formation of a reddish purple azo dye is used for determination of nitrite.

Nitrate Nitrogen

Nitrate nitrogen was determined by the Brucine Method outlined in Standard Methods (103). A yellow color is produced, which is used for the colorimetric determination of nitrate. Nitrate-nitrite was also measured by a nitrate specific low electron by means of a 701A Model Orion Research pH/MV Meter. Close agreements were obtained from both methods.

Chemical Oxygen Demand

The chemical oxygen demand (COD) of both influent and filtered effluent were determined in accordance with Standard Methods (103). Mercuric sulfate was used to eliminate chloride intereference, and silver sulfate was used to catalyze oxidation of long-chain fatty acids for all samples.

pH

The pH of the reactor was monitored as often as every hour, especially during the transient state, utilizing an Orion pH/MV Meter which was standardized daily at pH 7.0 and pH 4.0.

Volatile Fatty Acids

An attempt was made to analyze volatile acid by gas chromatography. A Model 810 Research Chematograph (Hewlett Packard Company, Avondale, Pa.) was used for analysis. A six-foot metal column packed with chromsorb 101 was used. This column is capable of detecting all volatile organic acids. The oven temperature was maintained at 200°C. The injection port and detector temperature were held at 272°C and 287°C, respectively. Helium was used as the carrier gas. Helium was supplied at 60 psi and controlled at the #4 position on the rotameter, yielding a flow rate of 140 ml/min. Hydrogen and compressed air were supplied at 30 psi and 33 psi, respectively. Acids are eluted on the basis of the number of carbon atoms, i.e., the higher the number of carbon atoms, the greater is the retention time. The gas chematograph analyzer was turned on the night before the analysis in order that the injection port and detector temperature could attain a desired steady temperature. The carrier gas flow was also turned on at low flow rate to prevent accumulation of unwanted materials on the column. The following morning, the oven was turned on and the gas flows were initiated. Flow of hydrogen gas triggered the lighting of the hydrogen flame, and the recorder was turned on. After the instrument reached equilibrium (in about two hours) indicated by the absence of appreciable drifting in the base, live samples were injected. In all cases, standard or sample site was 5 µl. Samples were carefully injected, using a Hamilton microliter syringe #701 (capacity 10 µl). Range and attenuation were adjusted in order to accommodate the peaks for compounds present in high concentration, and to obtain a well-defined measurable peak for

the compounds present in low concentrations, and to minimize noise in the instrument. It may be pointed out that retention times are independent of attenuation, but are inversely proportional to the carrier gas flow.

Since it was not possible to perform G.C. analyses immediately after obtaining each sample (such a procedure would require continued use of the apparatus over an extended period of time which is economically not feasible and also is physically impossible to do the G.C. analyses during shock loads), samples were frozen immediately after collection for a later analysis. This procedure is acceptable, since a very small percentage of volatile acids are lost during storage (105).

Additional Analyses

In addition to the above mentioned analyses, regular microscopic examinations of mixed liquor from the reactor system were performed to follow changes in predominance and morphological forms of microbes, and the dissolved oxygen of the mixed liquor was measured periodically, using a Weston and Stack Model No. 300 DO meter. The DO was never less than 3 mg/l in the aeration tank.

CHAPTER IV

RESULTS

The experimental results will be presented in four major sections dealing with (a) dynamic step forcing at 10-day Θ_c, (b) dynamic step forcing at 5-day Θ_c, (c) dynamic impulse forcing at 10-day Θ_c, and (d) batch experiments. After each step forcing was administered to the continuous flow unit operating at steady state conditions, the continuous flow was operated under new conditions until a new steady state was established. In all cases the system was operated in the new steady state for several days. The new steady state was terminated by the initiation of another shock. In all cases the experimental run was terminated by operation under steady state conditions. Under each steady state continuous flow run, cells from the aeration tank were employed as initial inoculum for batch experiments to determine μ_{max}, K_s, and Y_{t_B}. In general, the results of each experiment are presented in one figure except for the combined impulse dynamic forcings of glucose and ammonia, in which case each is presented in two separate figures.

In general, each figure will present such parameters as (1) the influent characteristics, which usually include concentrations of feed, COD, and ammonia nitrogen, (2) biological solids concentrations in mg/l, (3) effluent characteristics, which include effluent COD, ammonia and nitrate nitrogen concentrations, and effluent suspended solids

concentration. For all figures, the initial steady state is followed by a transient state followed by the new steady state.

The units along the abcissa are in days. At times, an expanded scale is employed in order to provide enough space for plotting the data which were obtained more frequently during the transient state.

Dynamic Step Forcings - Quantitative Step Shock Loads Operating at 10-day Θ_c

To evaluate the overall system response of a nitrifying activated sludge system operating at steady state with nearly 100 percent nitrification to quantitative shock loads, dynamic step fordings of glucose concentration and ammonia concentration were studied. Responses to two-fold, three-fold, and four-fold increases and decreases in combined glucose and ammonia concentration, two-fold increases in glucose, keeping ammonia constant, and two-fold increases in ammonia, keeping glucose concentration constant, were examined in order to identify any inhibition pattern to the process of one-stage biological nitrification. Results of step quantitative shock loads are summarized in Table XI.

Combined Glucose Ammonia Concentration Step Forcings

1. Two-fold combined glucose and ammonia concentration increase.

The pilot plant was operated in the steady state with nearly 100 percent nitrification. Operating the continuous flow unit at steady state with 100 percent nitrification was a very slow process, since it took nearly four months to develop a combined steady state and 100

percent nitrification in the reactor system. This was accomplished by seeding the continuous flow system with nitrifying microorganisms from the batch reactor periodically, slowly building up the nitrifying population in the heterogeneous system. At initial steady state, the influent COD concentration was 500 mg/l and influent NH_3-N concentration was 50 mg/l. The average steady state values are given in Table XX ; the performance characteristics for this shock load are plotted in Figure 2. The influent COD ranged from 480 to 520 mg/l with an average value of 500 mg/l. The ammonia nitrogen of the influent ranged from 48 to 52 mg/l, with an average value of 50 mg/l. The effluent quality in the initial steady state as measured by filtrate COD, S_e, and effluent ammonia nitrogen and nitrate nitrogen, was exceelent. The mean S_e was 20 mg/l, providing 96 percent removal of the organic substrate; the range of S_e was 14 to 24 mg/l. However, the anthrone test indicated no carbohydrate in the effluent . The mean nitrate nitrogen concentration in the effluent filtrate was 40 mg/l, and the mean ammonia concentration in the effluent was nearly zero, providing 100 percent nitrification of the ammonia nitrogen available after synthesis. The mean biological solids concentration in the effluent, X_e, in this steady state was 25 mg/l, ranging from 15 to 30 mg/l. In general, the system provided very satisfactory treatment and delivered effluent of high quality. The biological solids varied between 3620 and 3788, with a mean value of 3688 mg/l.

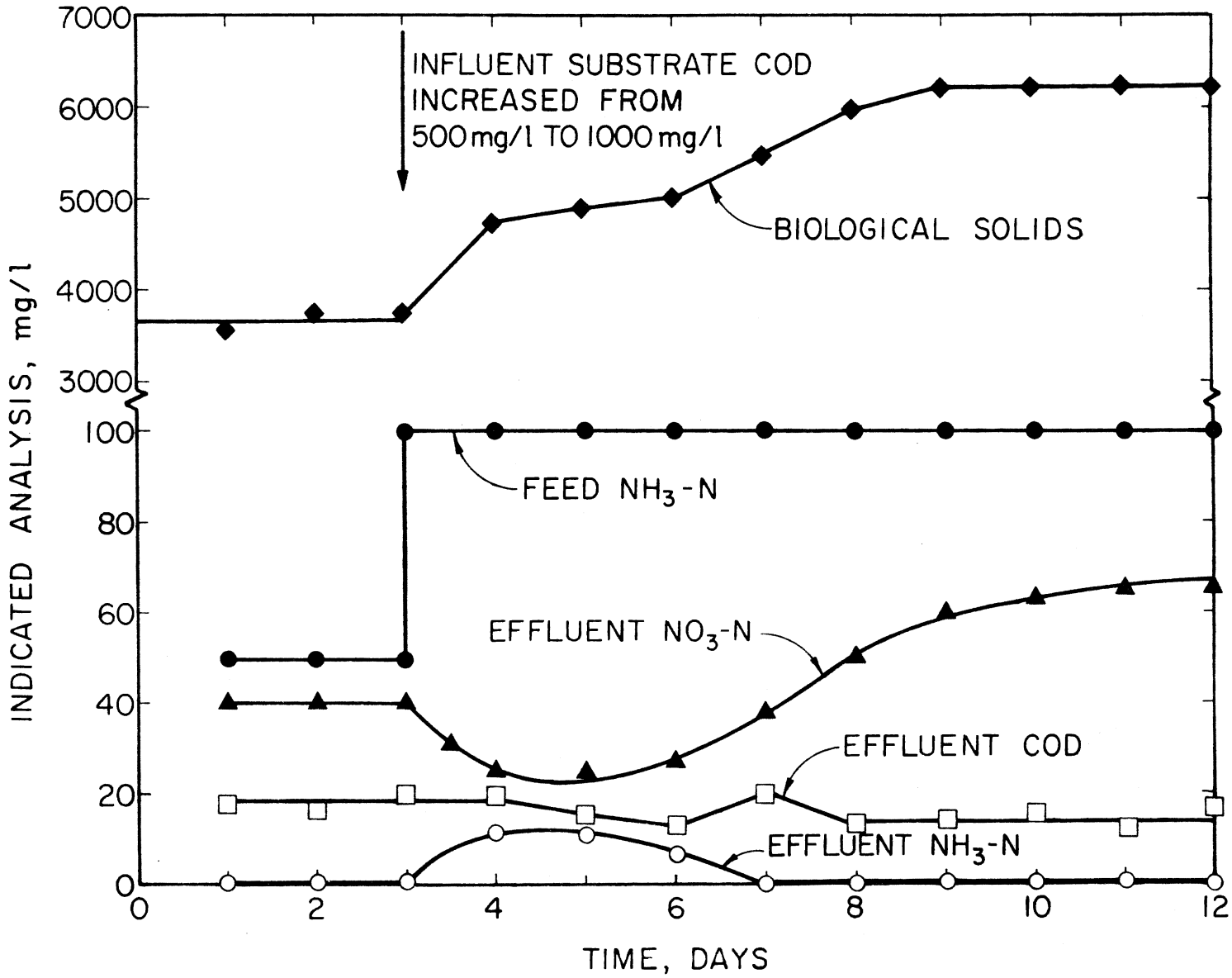
At least ten days of steady data were recorded before starting the shock; however, only three days of steady state are shown in Figure 2. The increase in influent substrate concentration is indicated by a vertical arrow. The ammonia nitrogen in the effluent increased to a

TABLE XX

SYSTEM OPERATIONAL PARAMETERS AT DIFFERENT STEADY STATES

Line	Figure	Θ_c	Feed		Effluent					Y_{Obs}	X	% Nitrification
			COD	NH_3-N	COD	% Eff.	X_e	NO_3-N	NH_3-N			
1	3	10	500	50	20	96.0	25	40	0	0.32	3688	100
2	2	10	1000	100	16	98.4	14	61	0	0.27	6245	100
3	6	10	500	50	14	97.2	16	38	0	0.26	2984	100
			500	51	20	96	19	40	0	0.27	3119	100
4	7	10	1500	150	21	98.6	26	71	55	0.26	8943	56
5	9	10	500	50	20	96.0	18	38	0	0.28	3225	100
6	10	10	2000	200	25	98.8	70	35	75	0.28	2824	32
7	12	10	500	50	20	96	20	40	0	0.28	3216	100
8	13	10	1000	50	30	97	25	20	0.2	0.26	6100	99
9	15	10	500	50	18	96.4	17	39	0	0.27	3200	100
10	16	10	500	100	16	96.8	28	72	14	0.29	3334	84
11	16	10	500	50	50	95	25	40	0	0.29	3286	100
12	19	5	500	50	21	95.8	21	30	10	0.30	1675	75
13	19	5	1000	100	23	97.7	23	8	50	0.29	3176	14

Figure 2. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at Constant Mean Cell Residence Time of 10 Days, Quantitatively Shock Loaded by a Combined Increase in Glucose Concentration from 500 to 1000 mg/l and Ammonia Nitrogen Concentration From 50 mg/l to 100 mg/l

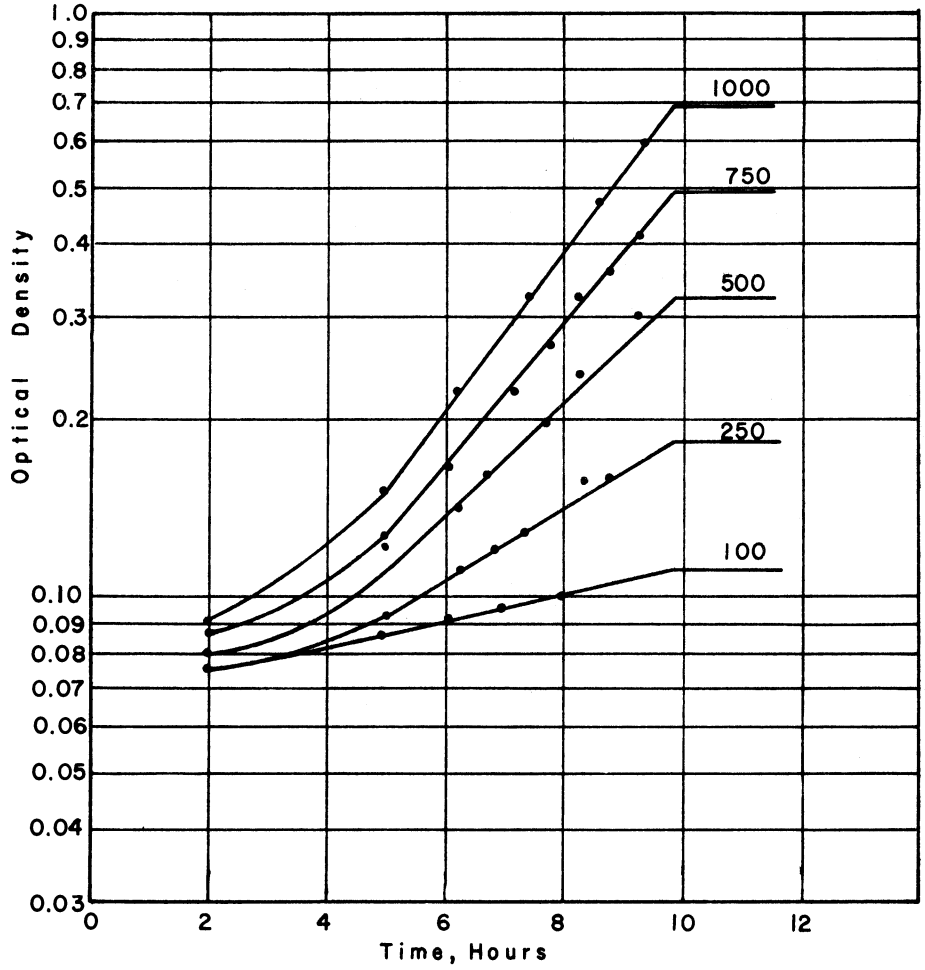

INFLUENT SUBSTRATE COD
INCREASED FROM
500 mg/l TO 1000 mg/l
BIOLOGICAL SOLIDS
FEED NH3-N
EFFLUENT NO3-N
EFFLUENT COD
EFFLUENT NH3-N
INDICATED ANALYSIS, mg/l
TIME, DAYS
7000
6000
5000
4000
3000
100
80
60
40
20
0
0
2
4
6
8
10
12

maximum of nearly 12 mg/l in 24 hours; consequently, the nitrate nitrogen decreased to a minimum of 24 mg/l in two days, a decrease of 40 percent from the steady state nitrate nitrogen concentration. The biological solids concentration in the reactor increased gradually to a value of approximately 6300 mg/l in six days and a new steady state was established with regard to biological solids concentration, X, and effluent characteristics. Influent glucose concentration was kept close to nominal glucose concentration, 1000 mg/l. Influent ammonia nitrogen concentration was kept close to 100 mg/l, ranging from 98 to 106 mg/l. The COD removal ability of the system was not hinered, and there was no leakage of organic substrate during transient state, with the mean S_e, 14 mg/l, ranging from 8 to 20 mg/l. In fact, effluent quality with respect to COD removal was excellent during the transient state. The system recovered from the decrease in nitrification and ammonia leakage just before the start of the new steady state. The effluent suspended solids in the transient perior ranged from 6 to 22 mg/l, with a mean value of 14 mg/l. There was a slight change in the color of the biomass during the transient period from dark brown to a little lighter color, but it changed back to its original color after the transient period. The nitrate-nitrogen concentration increased gradually from a low value of 24 mg/l during the transient period to a mean value of 61 mg/l at the new steady state, ranging from 60 to 65 mg/l. The ammonia nitrogen concentration at the new steady state was also nearly zero. This step dynamic forcing in the combined glucose and ammonia concentration did not deteriorate the effluent characteristics, except for increased nitrate-nitrogen concentration. The nitrate production was hindered, and ammonia nitrogen was leaked out temporarily during the

transient state, but the system recovered within four days from the ammonia leakage and full nitrification was attained within six days, at which time a steady state with respect to biological solids was also established. It should be mentioned that nitrate nitrogen production at the new steady state was about 153 percent of the amount of nitrate-nitrogen produced at the previous steady state. The biological solids in the new steady state ranged from 6144 to 6302, with a mean value of 6245 mg/l. The mean S_e and mean X_e remained quite low at the new steady state, with mean values of 16 and 14 mg/l for S_e and X_e, respectively. Sludge settling characteristics remained reasonably good for a nitrifying activated sludge.

Both during the initial steady state with influent COD of 500 mg/l and influent ammonia nitrogen concentration of 50 mg/l and during the new steady state with influent COD of 1000 mg/l and influent ammonia nitrogen concentration of 100 mg/l, cells from the aeration tank were employed as initial inoculum for separate batch experiments to determine μ_{max}, K_s, and Y_{t_B}. Growth curves obtained from the batch experiments conducted during these continuous flow runs are shown in Figures 3 and 4. The plots shown at the top of these figures are semi-logarithmic plots of the optical density versus time during the growth period. In most of the growth experiments, more frequent optical density readings than those shown in the figures were recorded. The initial substrate concentration, S_o, of the flask is shown in the curves. It can be clearly seen from the figures that exponential growth existed at S_o as low as 100 mg/l glucose. In some of the batch growth experiments long lags were observed, which for the sake of clarity are not shown in the figures.

Figure 3. Batch Growth Curves at Various Initial Substrate Concentrations and Relationship Between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Constant Mean Cell Residence Time of 10 days at Glucose Concentration of a 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l. The μ_{max} and K_s Values Obtained From the Plot of $1/\mu_o$ versus $1/S_o$ are 0.69 hr^{-1} and 1041 mg/l, Respectively


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
1000
750
500
250
100
0
2
4
6
8
10
12
Time, Hours

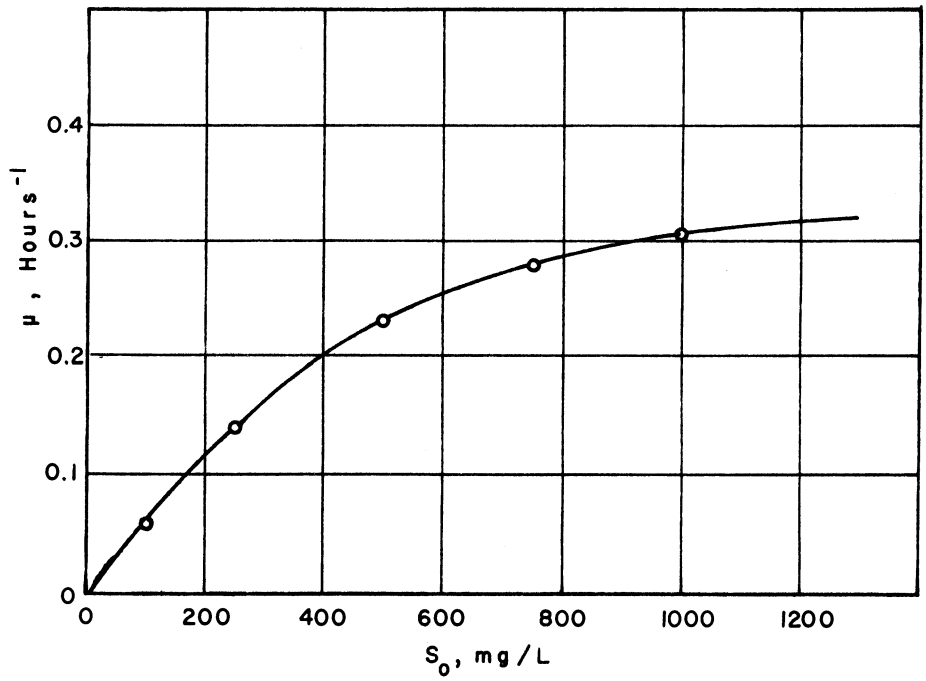
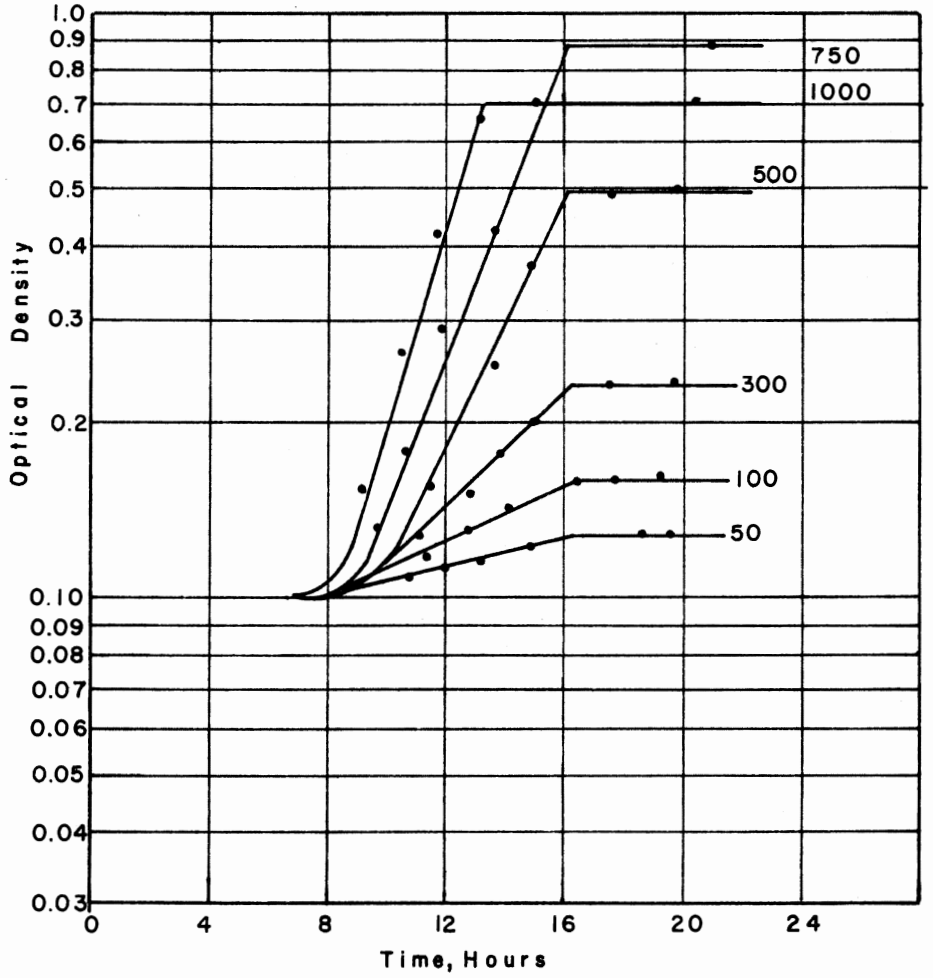
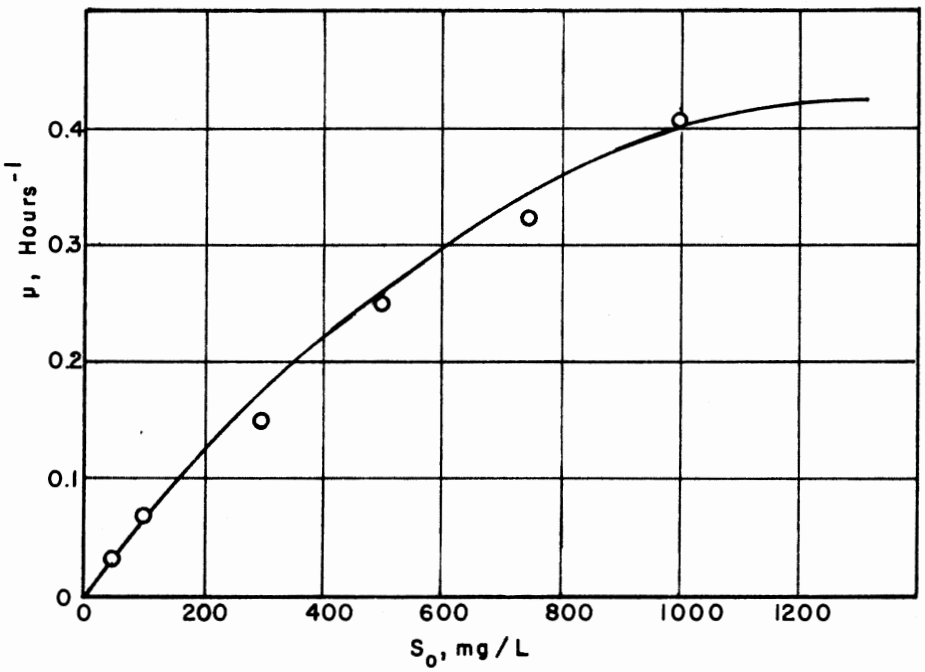

μ, Hours⁻¹
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
S0, mg/L

Figure 4. Batch Growth Curves at Various Initial Substrate Concentrations and Relationship Between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at a Constant Mean Cell Residence Time of 10 Days at a Glucose Concentration of 1000 mg/l and Ammonia Nitrogen Concentration of 100 mg/l. The μ_{max} and K_s Values Obtained From the Pilot of $1/\mu_o$ versus $1/S_o$ are 1.28 hr^{-1} and 2054 mg/l, Respectively


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
750
1000
500
300
100
50
0
4
8
12
16
20
24
Time, Hours


μ, Hours⁻¹
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
S0, mg/L

The lower portion of the figures shows the exponential plot of values of μ at various S_o values, and μ_{max} and K_s were obtained from the double reciprocal plot of $\frac{1}{S_o}$ versus $\frac{1}{\mu}$ as shown in Figure 5, employing the Monod equation:

$$\mu = \frac{\text{max } S_o}{K_s + S_o}$$

and its double reciprocal form

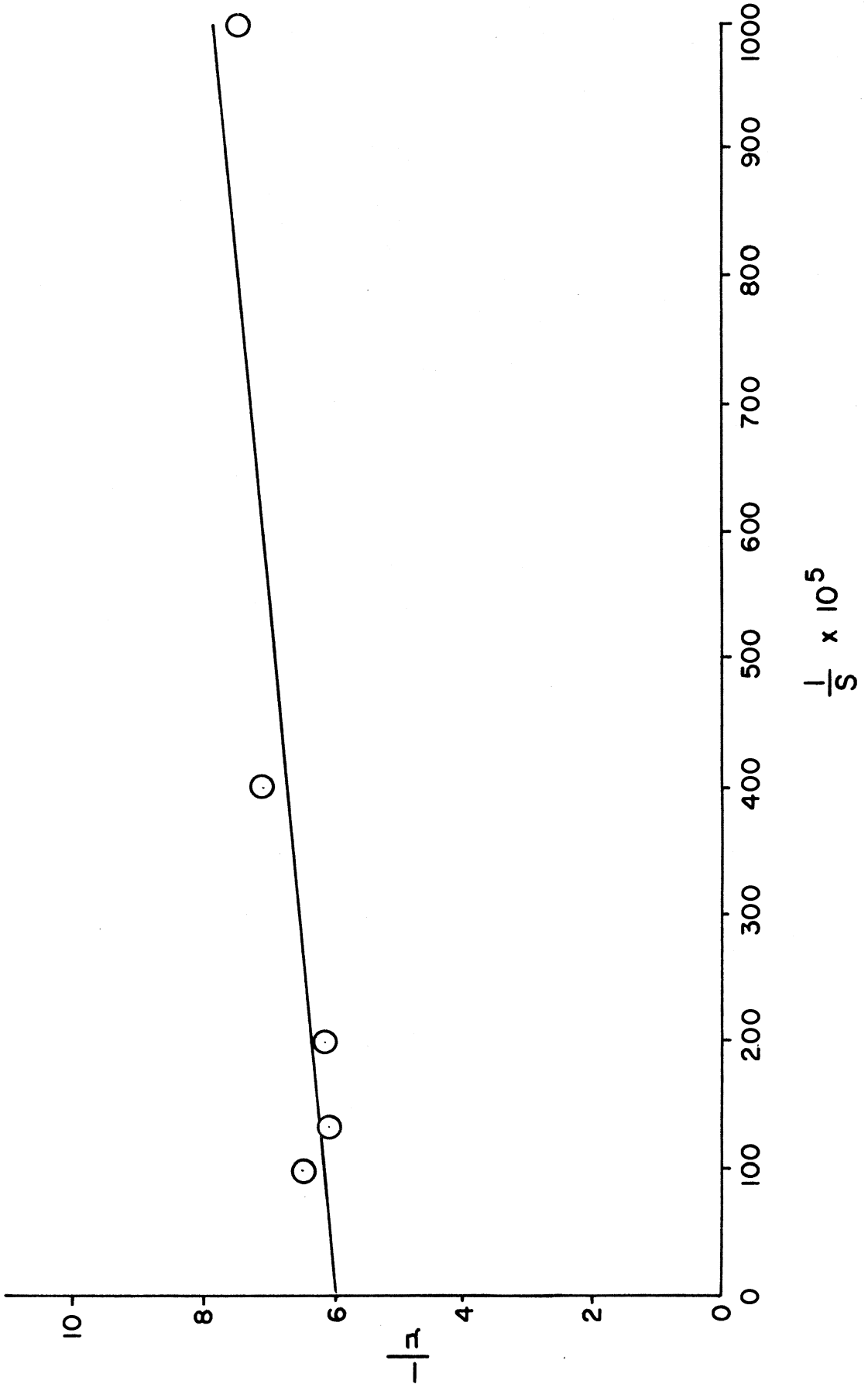
$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}} \frac{1}{S_o} + \frac{1}{\mu_{max}}$$

It can be clearly seen that the slope of $\frac{1}{\mu}$ versus $\frac{1}{S_o}$ is $\frac{K_s}{\mu_{max}}$ and the intercept of the same plot will give $\frac{1}{\mu_{max}}$. The maximum specific growth rates, μ_{max}, observed in these experiments were 0.69 and 1.28 hr^{-1} for cells harvested at steady state from the continuous flow reactor operating at 10-day Θ_c with an influent COD of 500 mg/l and influent ammonia nitrogen concentration of 50, and from the reactor operating at 10-day Θ_c with an influent COD of 1000 mg/l and influent ammonia nitrogen of 100 mg/l, respectively. The values for K_s for the cells harvested from the above reactors under the above conditions are 1041 and 2054 mg/l, respectively. Values of Y_{t_B} obtained from these experiments were 0.53 and 0.52, respectively.

Two-fold Combined Glucose and Ammonia Concentration Decrease

After maintaining the system at the new steady state for ten days, the influent glucose and ammonia concentrations were returned to 500

Figure 5. Reciprocal plot of μ versus S_o to Determine μ_{max} and K_s From Batch Growth Curves


0
2
4
6
8
10
$\frac{1}{\mu}$
0
100
200
300
400
500
600
700
800
900
1000
$\frac{1}{S} \times 10^5$

and 50 mg/l glucose and ammonia nitrogen, respectively. The results of this change are shown in Figure 6. This step decrease in combined influent glucose and ammonia nitrogen concentration did not change the effluent characteristics with the exception of concentration of nitrate-nitrogen in the effluent which decreased steadily following a dilute-out pattern. However, biological solids in the reactor decreased more slowly although steadily. The biological solids decreased from a mean value of 6216 mg/l to a mean steady state value of 2984 mg/l. There was almost no ammonia leakage in the effluent. The mean value for nitrate nitrogen at the new steady state was 38 mg/l. The mean S_e for the transient state and the new steady state was 14 mg/l, ranging from 10 to 20 mg/l effluent. Suspended solids in the effluent in both transient state and the new steady state remained quite low with a mean value of 16 mg/l, ranging from 12 to 20 mg/l. The steady state with respect to biological solids was established in 16 days after the step decrease in influent characteristics. No deleterious effect upon the biological nitrification process either during transient state or in the new steady state as a result of the two-fold decrease in the combined glucose and ammonia concentration at 10-day Θ_c was observed.

Three-fold Combined Glucose and Ammonia Nitrogen Concentration Increase

A steady state with mean S_i of 500 mg/l of glucose and influent mean ammonia nitrogen concentration of 50 mg/l was maintained for almost two weeks, as in the previous run. The average data obtained during this run are shown in Table XX. The performance characteristics before, during, and after the step dynamic forcing are shown in Figure 7.

Figure 6. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at a Glucose Concentration of 1000 mg/l and Ammonia Nitrogen Concentration of 100 mg/l, Operating at Constant Mean Cell Residence Time of 10 Days, Quantitatively Step Shock Loaded by a Combined Decrease in Glucose Concentration from 1000 to 500 and Ammonia Nitrogen Concentration From 100 to 50 mg/l , Respectively

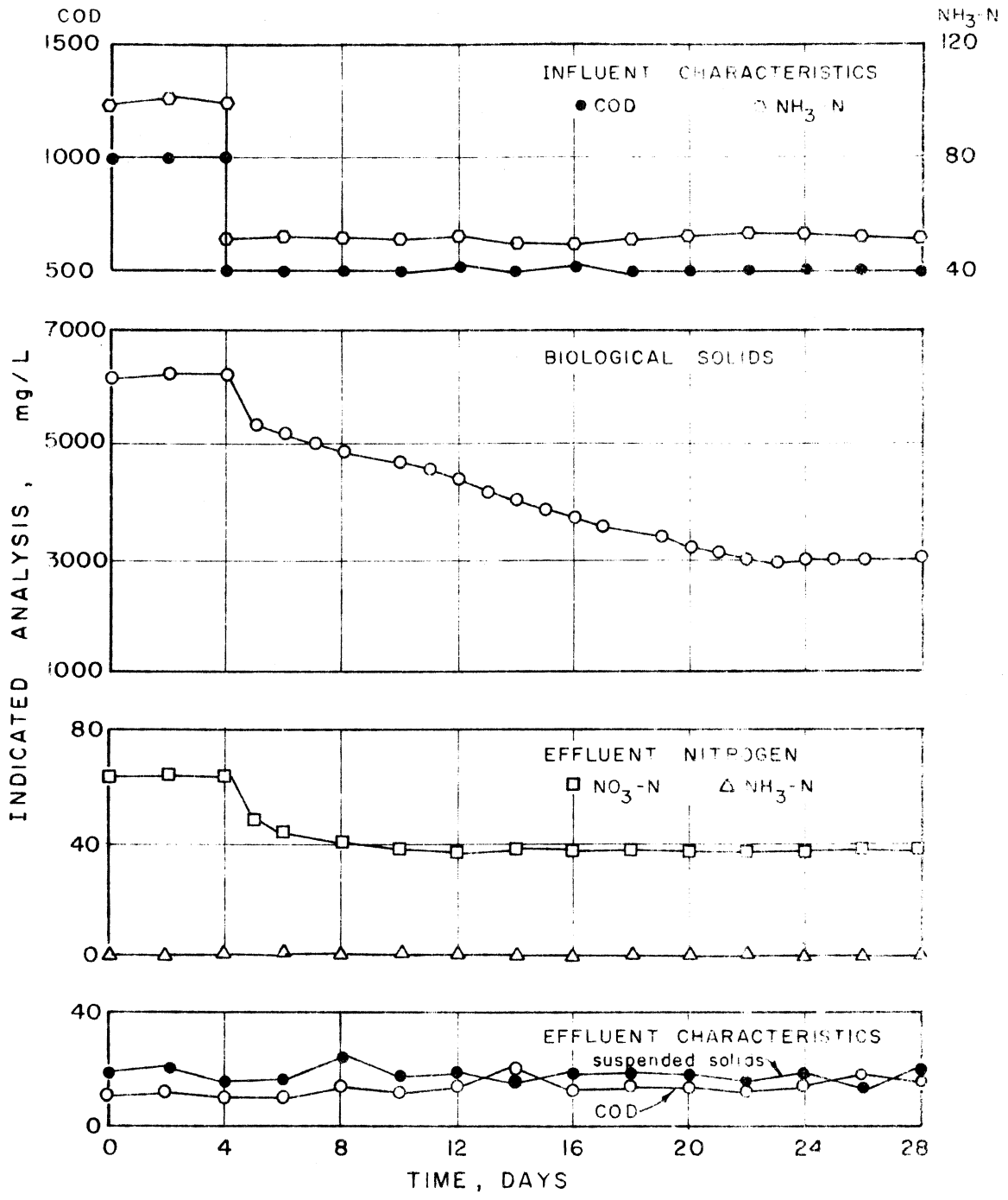
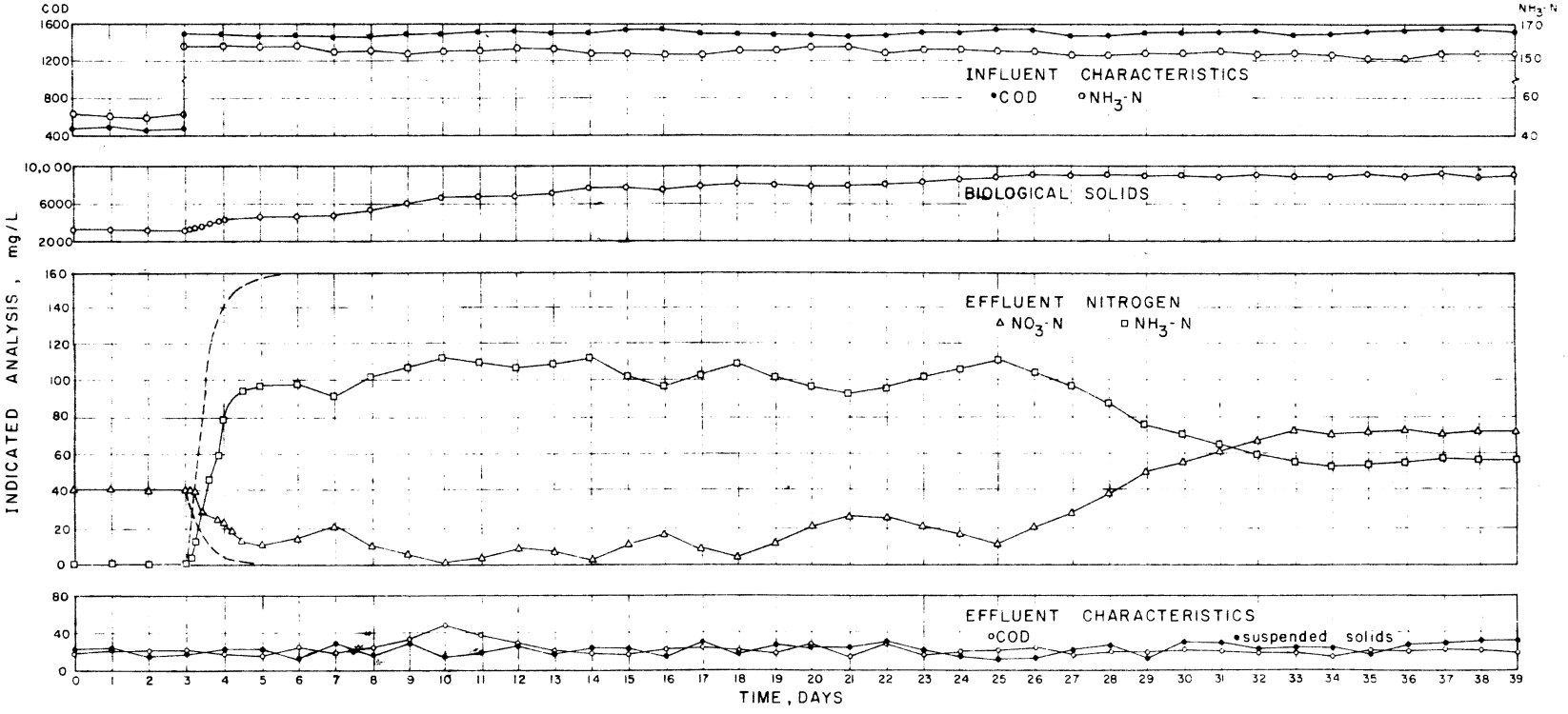

COD
NH3-N
1500
1000
500
120
80
40
INFLUENT CHARACTERISTICS
● COD
△ NH3 N
7000
5000
3000
1000
BIOLOGICAL SOLIDS
INDICATED ANALYSIS, mg/L
80
40
0
EFFLUENT NITROGEN
□ NO3-N
△ NH3-N
40
0
EFFLUENT CHARACTERISTICS
suspended solids
COD
0
4
8
12
16
20
24
28
TIME, DAYS

Figure 7. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at a Constant Mean Cell Residence Time of 10 Days Quantitatively Step Shock Loaded by a Combined Increase in Glucose and Ammonia Nitrogen Concentration From 500 to 1500 mg/l, and from 50 to 150 mg/l, Respectively


COD
NH3-N
INFLUENT CHARACTERISTICS
•COD ○NH3-N
BIOLOGICAL SOLIDS
EFFLUENT NITROGEN
△NO3-N □NH3-N
EFFLUENT CHARACTERISTICS
○COD •suspended solids
INDICATED ANALYSIS, mg/L
TIME, DAYS

The COD of the influent in the initial steady state before the three-fold step shock ranged from 482 to 500 mg/l with a mean influent COD of 500 mg/l. The effluent quality as measured by filtrate COD, S_e, and effluent ammonia nitrogen and nitrate nitrogen was excellent. The S_e range was 16 to 20 mg/l, with a mean value of 20 mg/l, providing 96 percent removal of the organic substrate; however, the anthrone test indicated no carbohydrate in the effluent filtrate. The nitrate-nitrogen in the effluent filtrate ranged from 39 to 41 mg/l, with a mean effluent nitrate nitrogen concentration of 20 mg/l. The ammonia nitrogen in the effluent was very close to zero, providing 100 percent nitrification of ammonia nitrogen available after synthesis. The mean biological solids concentration in the reactor was 3119 mg/l, ranging from 3096 to 3149 mg/l. The mean biological solids in the effluent from the activated sludge system was 19 mg/l, with a range of 16 to 22 mg/l. In general, the system at steady state with influent COD of 500 mg/l and ammonia nitrogen of 50 mg/l, provided very satisfactory treatment and delivered effluent of high quality with respect to COD and NH_3-N removal.

At least ten days of steady data were recorded before administering the step dynamic forcing. However, only three days are shown in Figure 7. After administering the step dynamic forcing (shock load) of 1500 mg/l of glucose and 150 mg/l of ammonia nitrogen to the system, ammonia leakage from the reactor started; within two hours, 2 mg/l of ammonia nitrogen was detected in the system effluent. After 24 hours of operation, 78 mg/l of ammonia nitrogen was detected in the effluent. The ammonia nitrogen in the effluent reached a maximum of 100 mg/l in seven days at the same time the nitrate nitrogen in the effluent

decreased to a value of 28 mg/l in ten hours and decreased to 10 mg/l at two days. The nitrate-nitrogen in the effluent increased to a value of 20 mg/l at four days, and started to decrease after the fourth day to a minimum of 1 mg/l at the seventh day after administration of the shock. It is interesting to note that in the first two days there was a sharp increase in biological solids from approximately 3100 mg/l to 4200 mg/l, after which it leveled for the next two days, corresponding to the increase in nitrate nitrogen concentration. After the fourth day, again there was a sharp steady increase in biological solids in the reactor corresponding to a decrease in nitrate nitrogen concentration from 20 mg/l on the fourth day to the low value of 1 mg/l on the seventh day after the dynamic step forcing. After the seventh day, there was a leveling off in biological solids concentration for the next two days (Figure 7), at which time nitrate nitrogen increased to a value of 10 mg/l on the ninth day. From the ninth to the eleventh day, biological solids again increased and correspondingly, nitrate nitrogen in the effluent decreased to 1 mg/l and ammonia nitrogen increased approximately by the same value as the nitrate decrease. The cycle was repeated approximately by the same value as the nitrate decrease. The cycle was repeated in the next two days. Biological solids leveled, nitrate nitrogen increased 10 to 15 mg/l and correspondingly, ammonia nitrogen decreased by the same amount as the nitrate nitrogen produced. The cycle was repeated again from day 13 to day 15. Biological solids increased, nitrate nitrogen decreased to 4 mg/l, and ammonia nitrogen decreased to 4 mg/l; ammonia nitrogen increased by approximately the same amount that nitrate nitrogen was decreased. From day 16 to day 19, no increase in biological solids was observed. Simultaneously, the

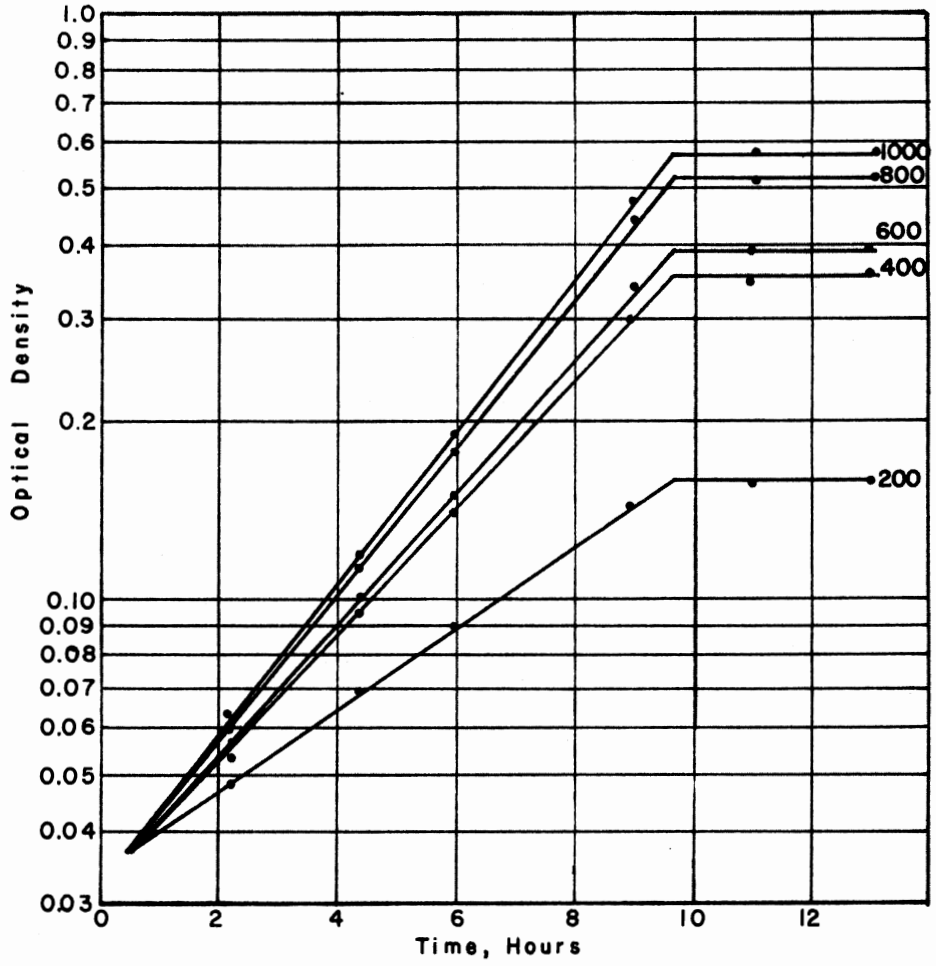
nitrate nitrogen in the reactor increased and ammonia nitrogen was decreased correspondingly. From day 19 after administration of the shock load to day 23, biological solids increased steadily, and nitrate nitrogen decreased steadily to a low value of 10 mg/l from a high value of 30 mg/l at day 19. Ammonia nitrogen increased from a low value of 90 mg/l on day 19 to a high value of 109 mg/l at day 23. After the 23rd day, a steady state with respect to biological solids was established by the system, and nitrate nitrogen increased steadily from 10 mg/l on day 23 to a steady state value of 71 mg/l on day 31. Simultaneously, ammonia nitrogen in the reactor decreased from 109 mg/l at day 23 to a steady state value of 55 mg/l on day 31 after administration of the step quantitative shock load. Steady state with respect to nitrification and biological solids at the new loading (1500 mg/l of glucose and 150 mg/l ammonia nitrogen) was established after a period of approximately one month. The effluent quality with respect to the carbonaceous removal efficiency was excellent. The effluent COD during the transient period ranged from 14 to 48 mg/l with a mean effluent COD value of 21 mg/l. The suspended solids in the effluent from the system ranged from 10 to 30 mg/l, with a mean effluent suspended solids of 21 mg/l. It is interesting to note that during the transient period, increase in reactor biological solids is coupled with a decrease in the nitrate nitrogen level and a simultaneous increase in the reactor ammonia nitrogen.

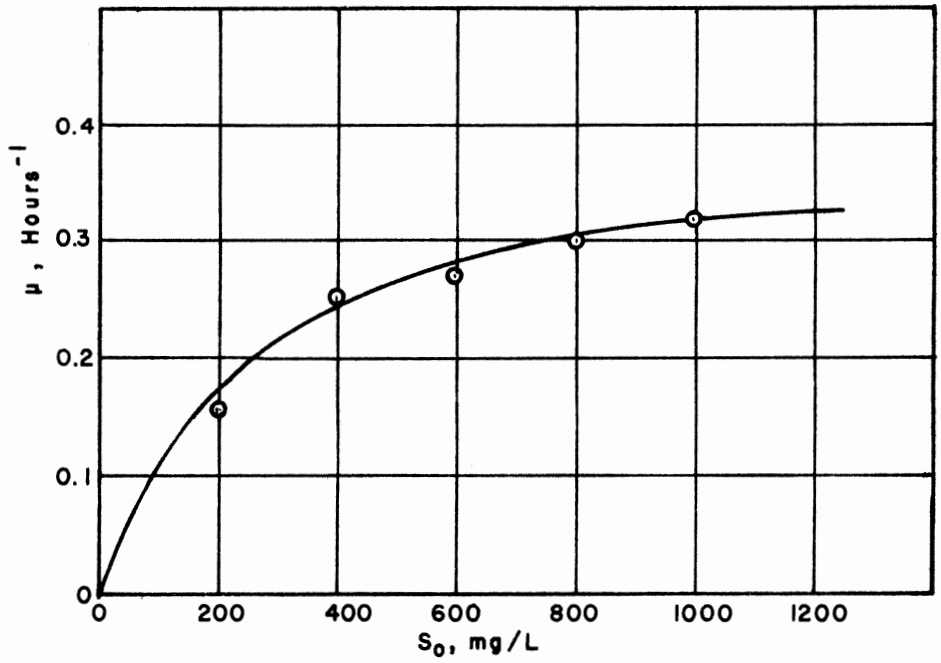
At the new steady state, the efficiency of the system with respect to effluent COD and effluent suspended solids remained quite high. The effluent COD ranged from 14 to 22 mg/l at the new steady state (1500 mg/l glucose and 150 mg/l NH_3-N) with a mean effluent COD of 19 mg/l.

The mean effluent suspended solids at the new steady state was 26 mg/l, with a range of 22 to 30 mg/l. The reactor biological solids concentration at the new steady state ranged from 8786 to 9222, with a mean reactor biological solids concentration of 8943 mg/l. Nitrate nitrogen at the new steady state ranged from 70 to 72 mg/l, with a mean value of 71 mg/l. The effluent ammonia nitrogen concentration at the new steady state ranged from 53 to 57 mg/l, with a mean value of 55 mg/l. It is interesting to note that the system at the new steady state did not produce any more nitrate than it did, although there was plenty of ammonia nitrogen available to be converted to nitrate nitrogen, while in the previous step increase from 500 mg/l glucose and 50 mg/l ammonia nitrogen to 1000 mg/l glucose and 100 mg/l ammonia nitrogen, all available ammonia nitrogen was converted to nitrate nitrogen (see Figure 2). The settling characteristics of the sludge were hindered at the new steady state, a wire mesh with a cloth filter was used to keep the solids from being carried out into the effluent. The settling hindrance was corrected successfully by this procedure.

During the new steady state, cells harvested from the aeration tank were employed as initial inoculum for batch experiments to determine μ_{max}, K_s, Y_{t_B}, as described previously. The maximum specific growth rate obtained for cells harvested at the steady state from the continuous flow system operating at 10-day Θ_c with influent COD of 1500 mg/l and influent NH_3-N concentration of 150 mg/l was 0.43 hr^{-1}. The K_s value of this steady state was 341 mg/l. The value of Y_{t_B} obtained from this experiment was 0.46. This batch study is illustrated graphically in Figure 8.

Figure 8. Batch Growth Curves at Various Initial Substrate Concentrations and Relationship Between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Constant Mean Cell Residence Time of 10 Days at Glucose Concentration of 1500 mg/l and Ammonia Nitrogen Concentration of 150 mg/l. The μ_{max} and K_s Values Obtained From the Double Reciprocal Plot of $1/\mu_o$ versus $1/S_o$ are 0.43 hr^{-1} and 341 mg/l, Respectively


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0
2
4
6
8
10
12
Time, Hours
1000
800
600
400
200

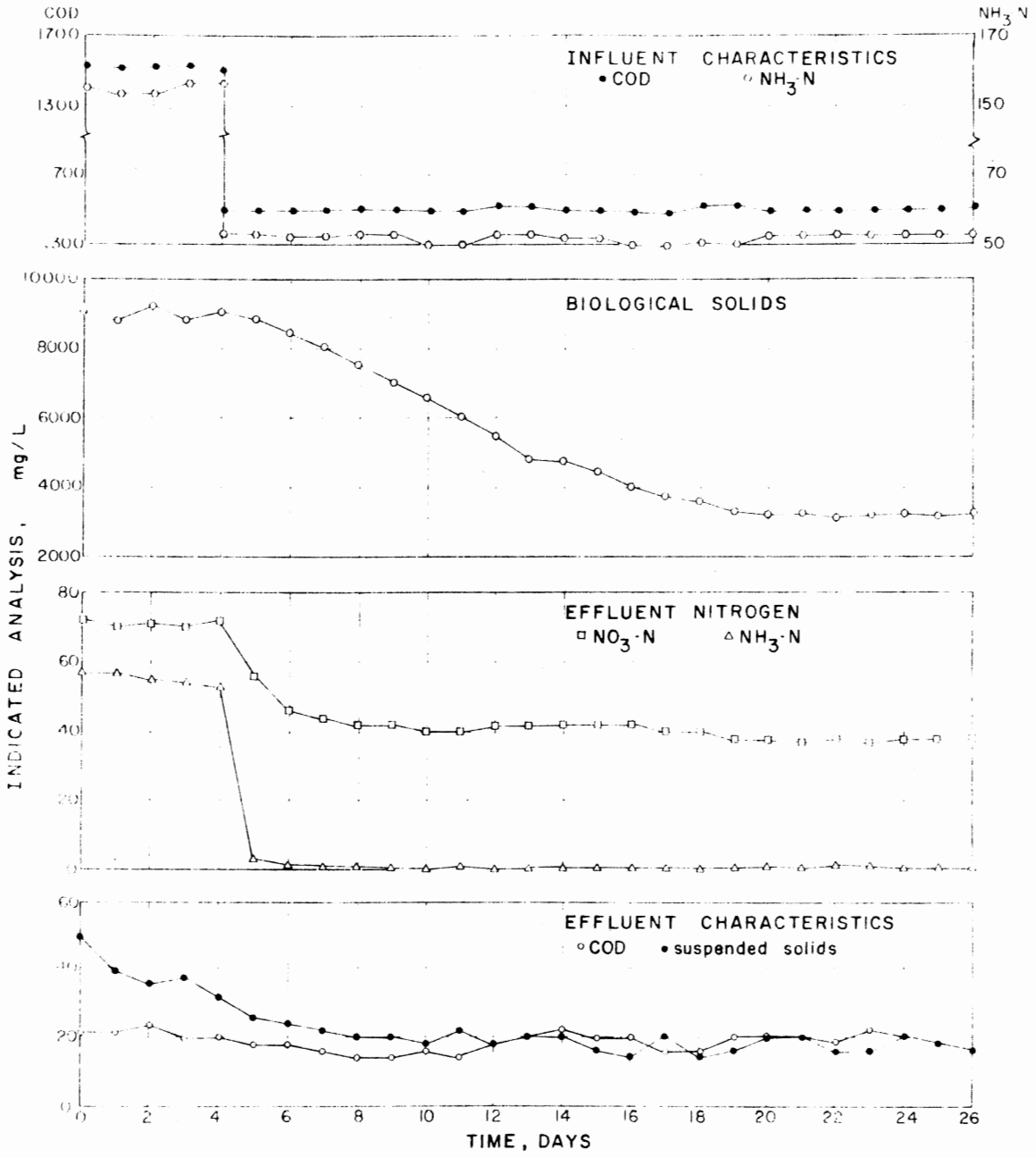

μ, Hours⁻¹
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
S0, mg/L

Three-fold Combined Glucose and Ammonia Concentration Decrease

After maintaining the system at the new steady state (1000 mg/l glucose and 150 mg/l ammonia nitrogen) for approximately two weeks, both glucose and ammonia nitrogen concentrations were decreased to 500 mg/l and 50 mg/l, respectively. The results are shown in Figure 9. There was no significant change in effluent COD or suspended solids. The significant changes involved biological solids concentration in the reactor and nitrate nitrogen and ammonia nitrogen concentration in the system effluent. Biological solids decreased from a mean value of 8943 mg/l to a mean value of 3284 mg/l in fifteen days, after which a steady state with respect to biological solids was established. Effluent ammonia concentration decreased from 53 mg/l to 3 mg/l in 24 hours, and decreased to a value of 1 mg/l in 48 hours. In 72 hours, the effluent contained practically no ammonia. Ammonia nitrogen in the effluent remained practically nil in the transition period. Nitrate nitrogen in the effluent decreased rapidly during the first 24 hours from 72 mg/l to 56 mg/l, and kept decreasing although at a lower rate to a value of 44 mg/l in 48 hours. In six days, nitrate nitrogen in the effluent decreased to 40 mg/l, and remained between 40 to 42 mg/l during the transient period.

A new steady state was established in about 15 days, but in this new steady state, biological solids ranged between 3155 to 3284 mg/l, with a mean value of 3225 mg/l. The effluent ammonia nitrogen concentration in the new steady state was negligible, as it was very close to zero--less than 0.1 mg/l. The nitrate nitrogen in the effluent at the

Figure 9. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at a Glucose Concentration of 1500 mg/l and Ammonia Nitrogen Concentration of 150 mg/l, Operating at a Constant Mean Cell Residence Time of 10 Days, Quantitatively Shock Loaded by a Combined Decrease in Glucose Concentration From 1500 to 500 mg/l, and Ammonia Nitrogen Concentration of 150 mg/l to 50 mg/l, Respectively

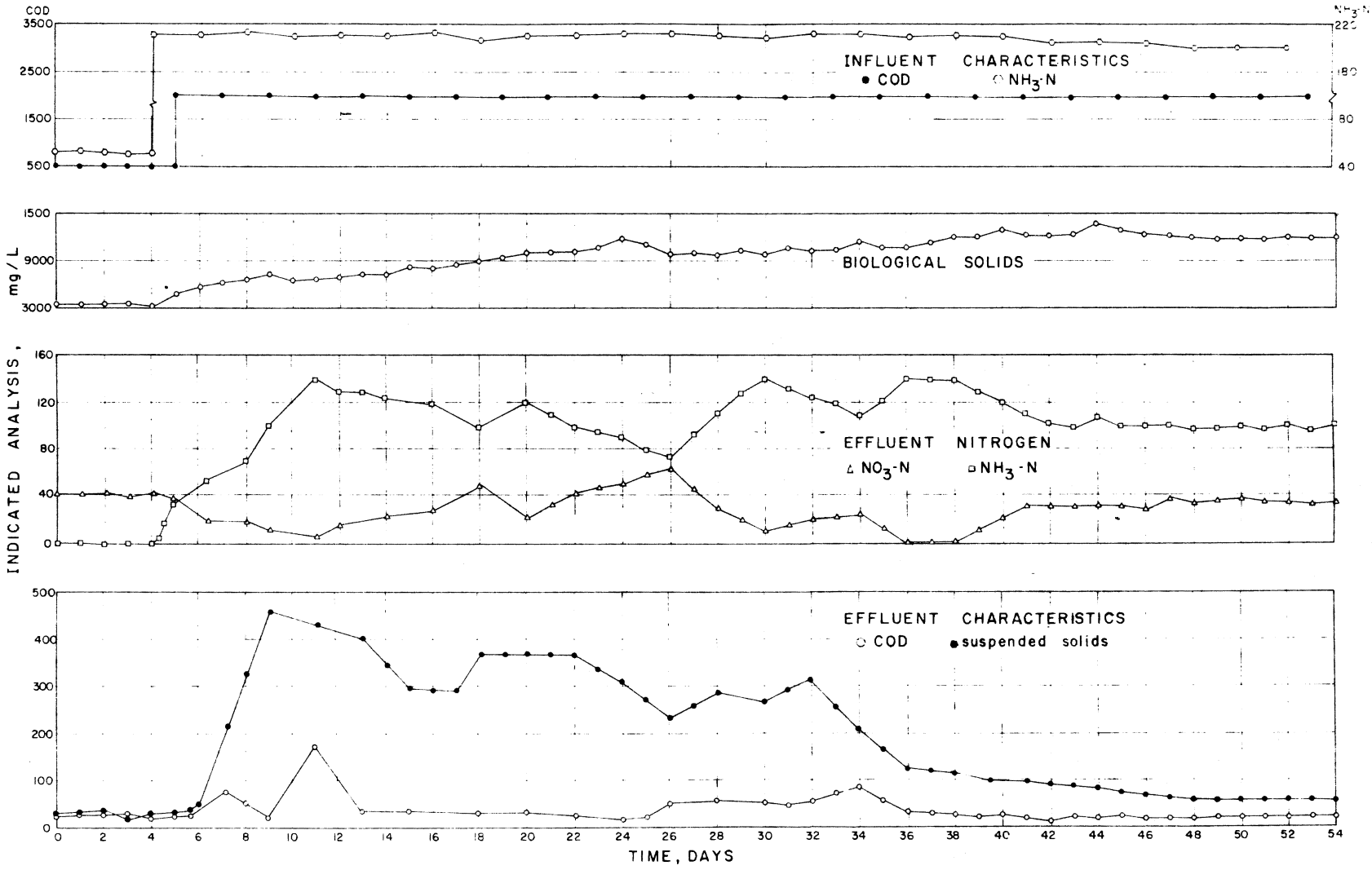

COD
NH3 N
INFLUENT CHARACTERISTICS
• COD ○ NH3-N
BIOLOGICAL SOLIDS
EFFLUENT NITROGEN
□ NO3-N △ NH3-N
EFFLUENT CHARACTERISTICS
○ COD • suspended solids
INDICATED ANALYSIS, mg/L
TIME, DAYS

new steady state ranged between 37 and 38 mg/l, with a mean value of approximately 38 mg/l. The COD and suspended solids in the effluent at the new steady state had a mean value of 20 and 18 mg/l, respectively. The effluent COD range was between 16 and 20 mg/l at the new steady state, and suspended solids range was also between 16 and 20 mg/l. The settling characteristics improved, and there was no need to use the filter to avoid escape of biological solids in the effluent. This procedure was discontinued, and the system was operated without the filter. It is interesting to note that at this new steady state with a loading of 500 mg/l glucose and 50 mg/l ammonia nitrogen, all available ammonia nitrogen was converted to nitrate nitrogen, which was also the case at previous steady states with the same loading. However, the biological solids in these steady states were slightly different, indicating a different steady state with the same nitrification efficiencies.

Four-fold Combined Glucose Ammonia Concentration Increase

The new steady state at influent concentration of 500 mg/l glucose and 50 mg/l ammonia nitrogen was maintained for almost two weeks before the new shock load was applied. The average data obtained during this run are shown in Table XX. Almost immediately after administering the four-fold dynamic step forcing the nitrate production was suppressed. Ammonia nitrogen leaked out into the effluent, and biological solids started to increase steadily and more or less in the same manner as the previous shocks. The performance characteristics before, during, and after the shock are shown in Figure 10. Effluent characteristics were monitored in terms of effluent COD, S_e, and suspended solids

Figure 10. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at a Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at Constant Mean Cell Residence Time of 10 Days, Quantitatively Shock Loaded by a Combined Increase in Glucose Concentration From 500 to 2000 mg/l and Ammonia Nitrogen Concentration from 50 to 200 mg/l, Respectively


COD
3500
2500
1500
500
INFLUENT CHARACTERISTICS
● COD ○ NH_3-N
220
180
80
40
1500
9000
3000
mg/L
BIOLOGICAL SOLIDS
INDICATED ANALYSIS,
160
120
80
40
0
EFFLUENT NITROGEN
△ NO_3-N □ NH_3-N
500
400
300
200
100
0
EFFLUENT CHARACTERISTICS
○ COD ● suspended solids
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54
TIME, DAYS

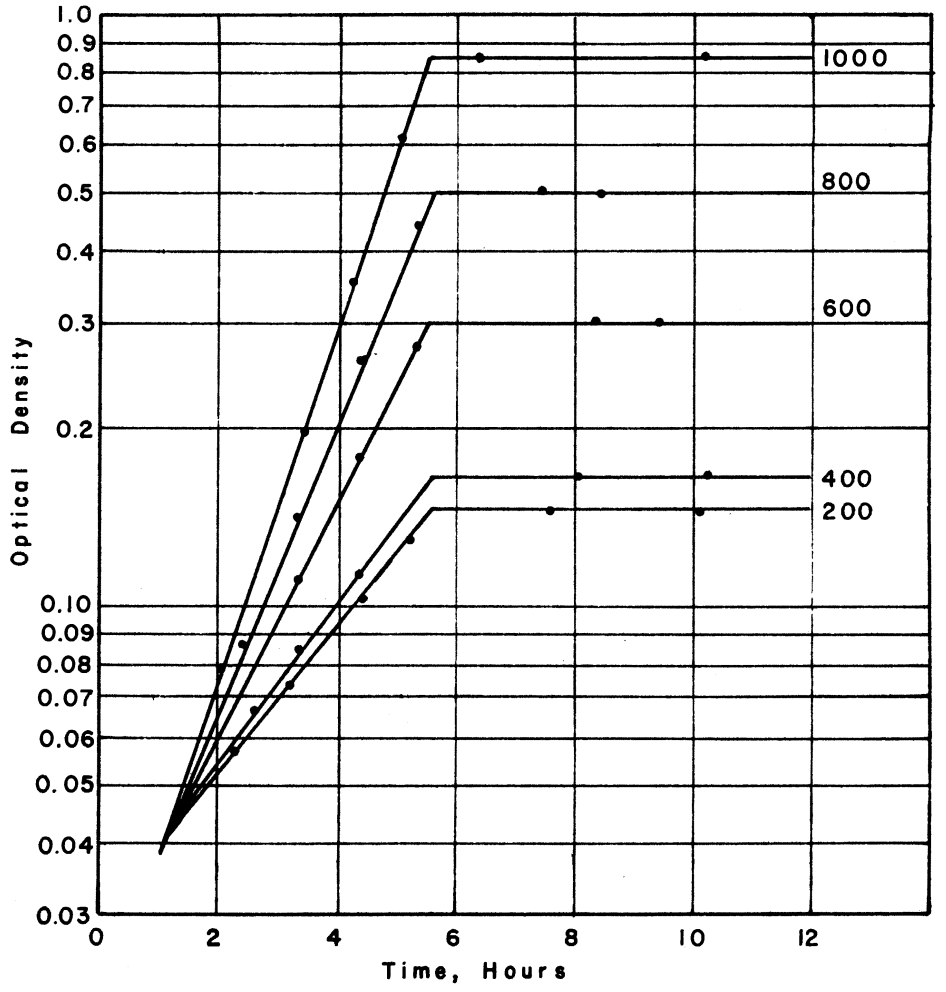
concentration, X_e. Also, spot checks were made with the anthrone test on the effluent, S_e, to determine the concentration of carbohydrate in the effluent; however, no carbohydrate was detected. The effluent was also used to determine volatile organic acids by gas chromatography. No volatile organic acid was detected in the effluent during the initial steady state, transient period, or the new steady state. Similar to the response of the system to previous combined quantitative step shock loads, nitrate production was initiated as soon as a leveling off of the biological solids occurred, but nitrification was depressed when the biological solids in the reactor increased rapidly. This pattern of inhibition of nitrification occurred in cycles corresponding to increases in biological concentration in the effluent as in previous shocks (see Figure 10).

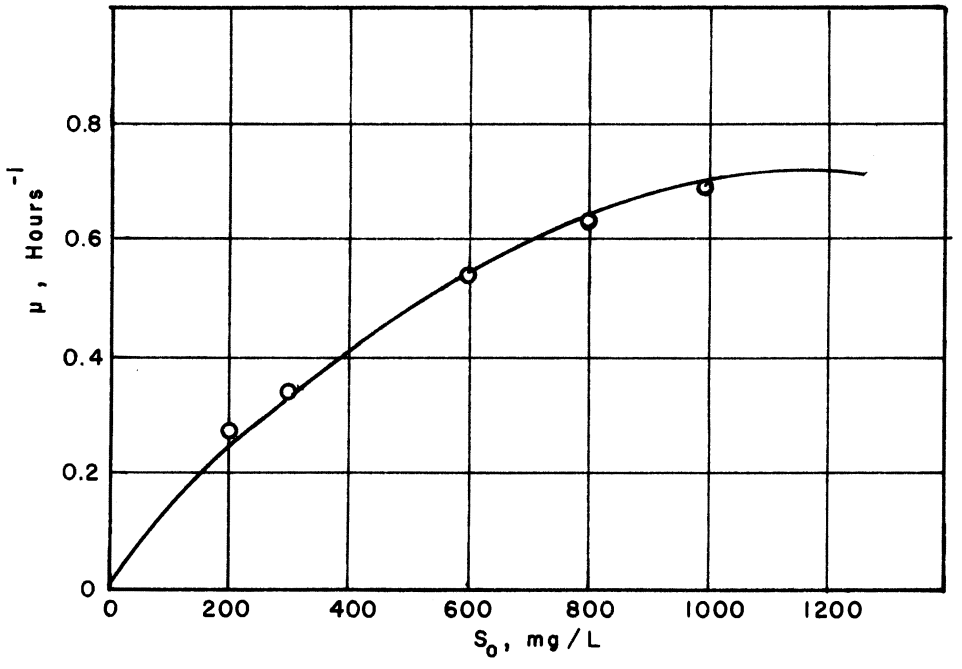
In the transient period, the system effluent characteristics with respect to biological solids concentration and COD removal efficiency changed significantly. S_e increased to 75 mg/l in 55 hours temporarily and then leveled off; the suspended solids in the effluent increased to 215 mg/l and reached a maximum of 465 mg/l on day 4, while S_e remained low. On day 6, the S_e increased sharply to 170 mg/l, while the effluent suspended solids level was 345 mg/l. COD removal characteristics recovered rapidly; however, the effluent suspended solids in the effluent remained relatively high for 30 days, after which in order to keep the constant Θ_c in the unit, the wasting procedure was stopped and when the system recovered, the volume of wasted sludge was reduced to account for the biological solids lost in the system effluent. At times it was necessary to recycle the settled effluent solids in order to keep a constant Θ_c.

Thirty-six days after the four-fold combined glucose ammonia nitrogen step, quantitative shock load was initiated, the system reached a new steady state. The system characteristics at the new steady state are summarized in Table XX. The nitrate nitrogen did not recover to the original level. After 40 days of operation of the step dynamic forcing, the mean steady state nitrate-nitrogen concentration was only 35 mg/l while ammonia nitrogen in the effluent was between 75 and 80 mg/l. The biochemical removal efficiency at the new steady state with respect to COD removal was high (98.8 percent). The mean effluent COD was 25 mg/l; the effluent biological solids, X_e, even at the new steady state remained relatively high, with a mean value of 70 mg/l. The average biological solids in the new steady state was 12,824 mg/l. The settling characteristics were relatively good, considering the high concentration of biomass in the reactor. The filter used in the previous experiment was also used for this experiment; however, the suspended solids in the effluent remained relatively high. The color of the biomass changed from dark brown in the initial steady state to a light yellow to cream color during the transient period, to a light golden brown color during the new steady state. It did not return to its original dark brown color at the new steady state.

Cells harvested from the aeration tank at the new steady state were employed as initial inoculum for batch experiments to determine biological kinetic constant for the biomass at the new steady state as described previously. The growth curves are shown in Figure 11. The maximum specific growth rate, μ_{max}, observed in these experiments was 1.01 hr^{-1} for cells harvested at steady state from the continuous flow reactor operating at 10-day Θ_c with an influent COD concentration of

Figure 11. Batch Growth Curves at Various Initial Substrate Concentrations and Relationship between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Constant Mean Cell Residence Time of 10 Days at Glucose Concentration of 2000 mg/l and Ammonia Nitrogen Concentration of 200 mg/l. The μ_{max} and K_s Values Obtained From the Plot of μ_o versus $1/S_o$ are 1.01 hr^{-1} and 596 mg/l


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
Optical Density
1000
800
600
400
200
0
2
4
6
8
10
12
Time, Hours

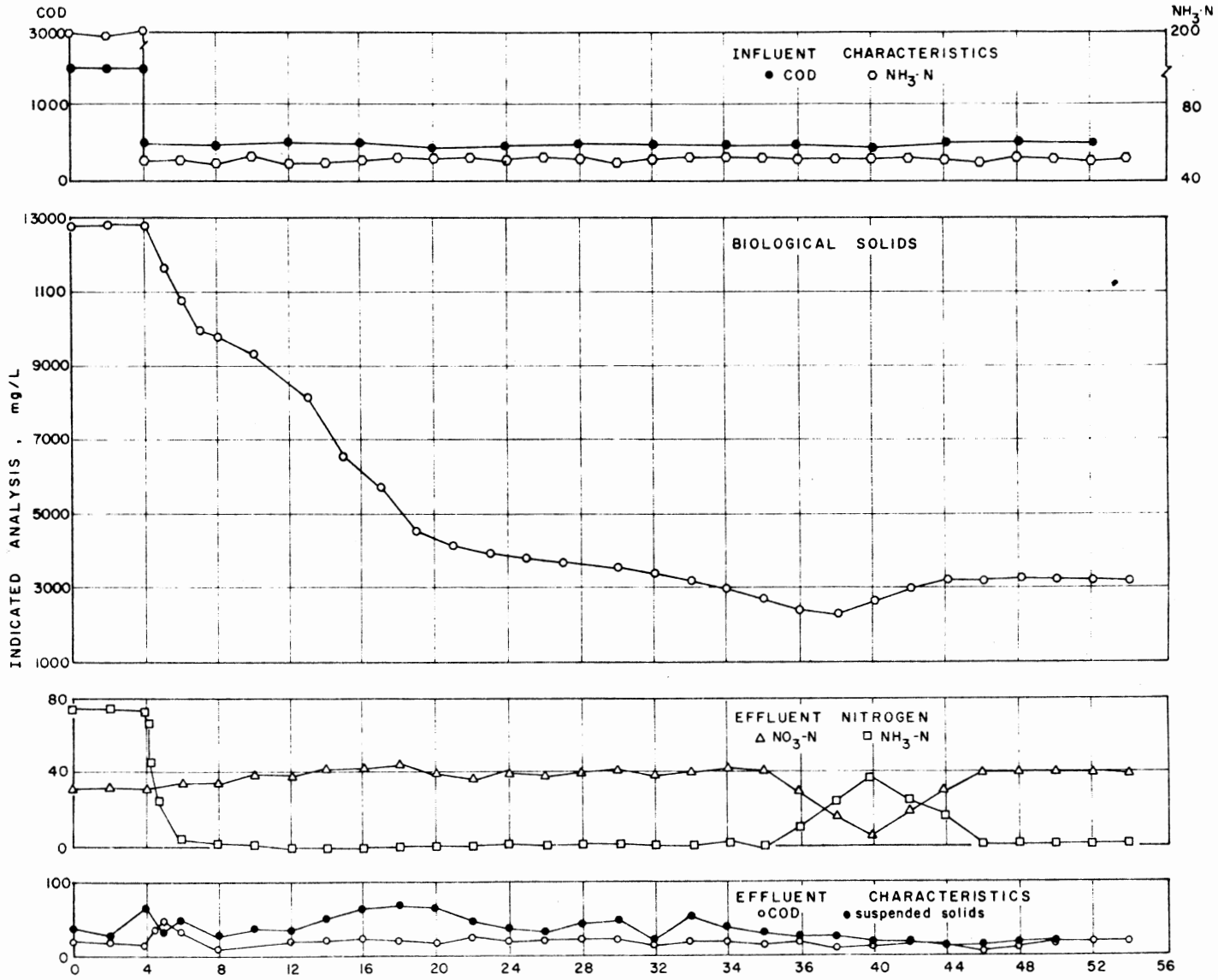

0.8
0.6
0.4
0.2
0
μ, Hours⁻¹
0
200
400
600
800
1000
1200
S0, mg/L

2000 mg/l and ammonia nitrogen concentration of 200 mg/l. The K_s value for these cells harvested from the above reactor under the same conditions was 596 mg/l. The value of Y_{t_B} obtained was 0.51.

Four-fold Combined Glucose Ammonia Concentration Decrease

After eight to ten days of steady state operation at a glucose concentration of 2000 mg/l and ammonia nitrogen concentration of 200 mg/l, the influent concentration was changed to 500 mg/l glucose and 50 mg/l ammonia nitrogen. There was no significant change in biochemical efficiency with respect to COD removal. The system characteristics before, during, and at the new steady state are sshown in Figure 12. The biomass in the reactor decreased steadily with a more rapid rate initially to a value of about 2300 mg/l from an initial value of 12,800 mg/l in about 38 days. It is interesting to note that the biomass concentration decreased to a concentration much less than the concentration of biomass at the previous steady state with the same concentrations of constituents at the previous steady state. However, the system recovered in six days with respect to COD removal efficiency and established a new steady state in 44 days after the four-fold decrease in combined glucose and ammonia nitrogen concentration. At the new steady state, the biomass concentration was about the same as those in the previous steady state condition, with influent of the same composition. The mean biological solids concentration at the new steady state condition was 3216 mg/l, with a range of 3150 to 3228 mg/l. The system characteristics at the new steady state are summarized in Table XX. The effluent nitrate nitrogen increased slightly during the transient period

Figure 12. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 2000 mg/l and Ammonia Nitrogen Concentration of 200 mg/l, Operating at a Constant Mean Cell Residence Time of 10 Days, Quantitatively Step Shock Loaded by a Combined Decrease in Glucose Concentration From 2000 to 500, and Ammonia Nitrogen Concentration From 200 to 50 mg/l


COD
NH3-N
INFLUENT CHARACTERISTICS
● COD ○ NH3-N
BIOLOGICAL SOLIDS
INDICATED ANALYSIS, mg/L
EFFLUENT NITROGEN
△ NO3-N □ NH3-N
EFFLUENT CHARACTERISTICS
oCOD ● suspended solids

from 35 mg/l to a maximum of 46 mg/l in 12 days, and leveled off to a value of around 40 mg/l during most of the transient period. Thirty-four days after the decreased shock, nitrate nitrogen in the effluent decreased to a minimum of 6 mg/l, but recovered to a mean steady state value of 40 mg/l in 46 days after the shock load. It is interesting to note that the minimum in nitrate concentration occurred when the biological solids was increasing from an earlier decrease, and it is also pertinent to point out that effluent ammonia nitrogen reached a maximum when nitrate nitrogen concentration fell to a minimum. The ammonia nitrogen in the effluent decreased rapidly after administration of the four-fold combined glucose and ammonia reverse shock load. The effluent ammonia nitrogen concentration was lowered to 6 mg/l in two days after the shock; it was lowered to 4 mg/l in four days, and there was no ammonia nitrogen in the effluent after the eight days following the shock. The new steady state effluent ammonia nitrogen concentration was zero, indicating 100 percent nitrification of all available ammonia after synthesis. The mean suspended solids in the effluent in the new steady state was 20 mg/l. The settling characteristics of the system improved from the previous steady state. The color of the biomass changed to darker brown during the transient period and was a dark brown similar to the one at the previous steady state with the same influent and operating conditions.

Glucose Concentration Step Shock Loads Keeping Ammonia Concentration Constant

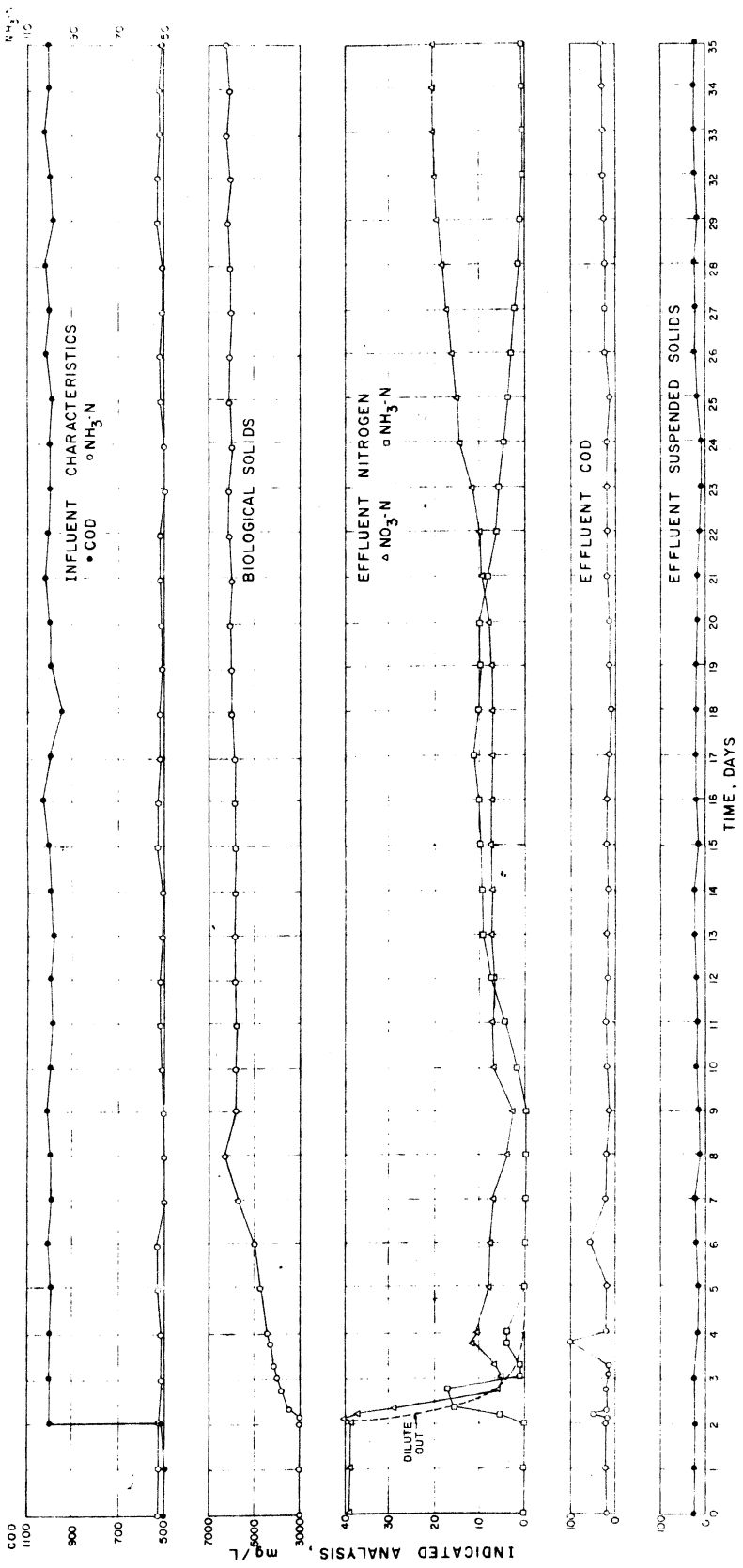
Two-fold Glucose Concentration Increase. Before administering this shock, the system was operated at steady state with influent

glucose concentration of 500 mg/l and influent ammonia nitrogen concentration of 50 mg/l for approximately two weeks with complete nitrification of all available ammonia. The mean steady state values for different parameters at this initial steady state are summarized in Table XX. The system characteristics at initial steady state during the transient period and the new steady state are shown in Figure 13. Only three days of new steady state are shown in Figure 13. Upon administration of the two-fold increase in influent glucose concentration, the biological solids concentration in the reactor started to increase fairly rapidly. In two days the biomass concentration increased to about 4000 mg/l from an initial value of approximately 3100 mg/l. The biomass concentration reached a value of 6300 on day 6, and leveled off to a steady state value of between 5900 and 6000 after day 7. The nitrate nitrogen concentration in the reactor increased by 1.5 mg/l within the first two hours, but then started to decrease sharply. Within eight hours it decreased to a value of 295 mg/l and decreased to 7 mg/l in 18 hours. The dashed line in Figure 13 represents the theretical dilute-out curve calculated from the system dilution rate utilizing equation (21),

$$C_t = C_o e^{-Dt}$$

The nitrate nitrogen in the reactor reached a low concentration of 5.5 mg/l in 26 hours, after which it increased to 12 mg/l 42 hours after the shock load. A gradual decrease in the reactor's nitrate nitrogen concentration was observed after this temporary increase. The nitrate nitrogen concentration in the system was lowered to 3.5 mg/l on day 7.

Figure 13. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Concentration of 50 mg/l Operating at a Constant Mean Cell Residence Time of 10 Days Quantitatively Step Shock Loaded by an Increase in Glucose Concentration From 500 to 1000 mg/l While Keeping Ammonia Nitrogen Concentration at an Initial Level of 50 mg/l


INFLUENT CHARACTERISTICS
• COD ○ NH_3-N
BIOLOGICAL SOLIDS
EFFLUENT NITROGEN
△ NO_3-N □ NH_3-N
DILUTE OUT
EFFLUENT COD
EFFLUENT SUSPENDED SOLIDS
INDICATED ANALYSIS, mg/L
TIME, DAYS

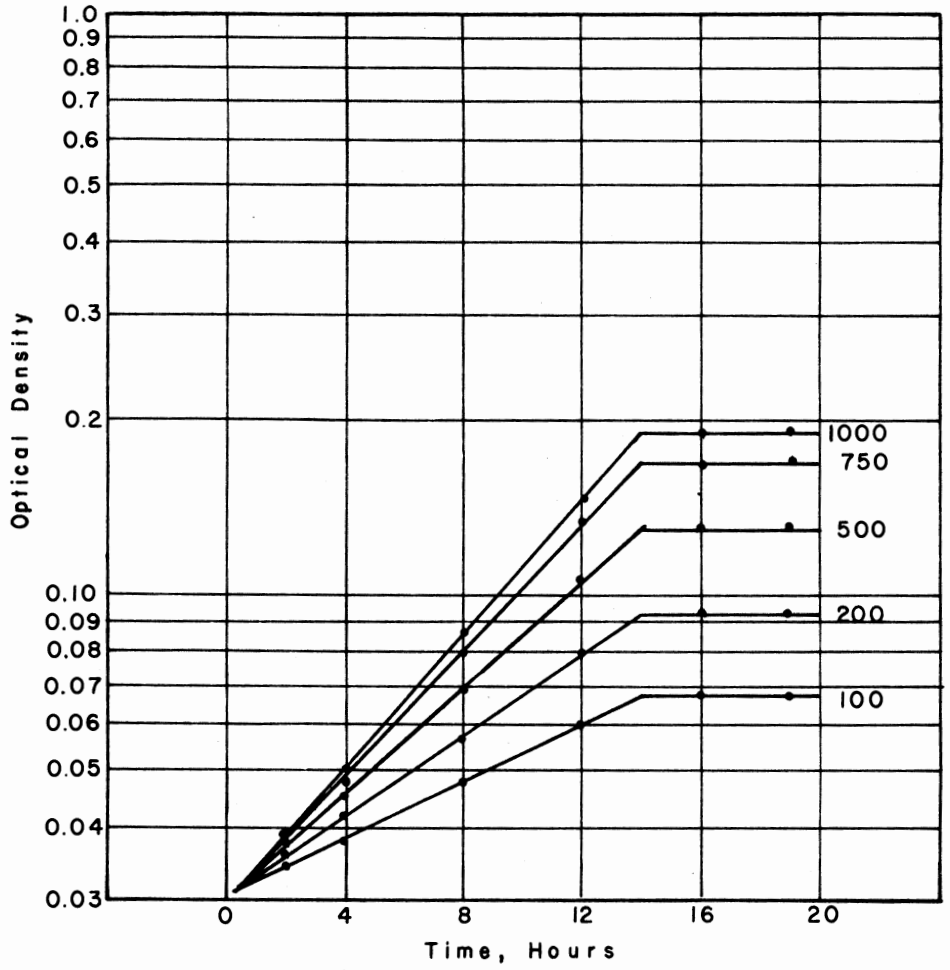
After the eighth day, a pseudo steady state with respect to nitrate nitrogen concentration was observed, where it remained steady between 7 and 8 mg/l. After day 17, a gradual increase in nitrate nitrogen in the reactor was observed until it reached a new steady state on day 28 with the mean nitrate nitrogen concentration of 20 mg/l. Ammonia nitrogen concentration at the initial steady state was approximately zero after administration of the shock load. Ammonia nitrogen in the reactor increased to 5.5 mg/l in four hours and increased to a maximum of 17.5 mg/l in eighteen hours, after which time ammonia nitrogen in the reactor decreased sharply to 1.5 mg/l in 26 hours. In three days, the ammonia nitrogen concentration decreased to less than 0.3 mg/l. The system efficiency with respect to COD removal ability was almost unaffected except for a mild increase in effluent COD from a mean value of 20 mg/l at the initial steady state to 50 mg/l in four hours after the step increase in glucose concentration and another temporary increase in effluent COD to 98 mg/l 42 hours after the shock. The average effluent COD during the transient period and at the new steady state was about 30 mg/l. The effluent suspended solids concentration remained unaffected by this step increase. The system characteristics at the new steady state with influent mean glucose concentration at 1000 mg/l and mean ammonia concentration of 50 mg/l are summarized in Table XX.

The color turned to light dream during the transition period from an initial dark brown color, and turned to a golden brown at the new steady state. Cells harvested from the aeration compartment of the new steady state were employed as initial inoculum for batch experiments to determine the biological kinetic constant for the heterogeneous

population at the new steady state, as described previously. The growth curves are shown in Figure 14. The maximum specific growth rate, μ_{max}, for the heterogeneous population determined in these experiments was 0.28 hr^{-1} for cells harvested at steady state from the continuous flow reactor. Operating at 10-day Θ_c with influent COD of 1000 mg/l and ammonia nitrogen concentration of 50 mg/l, the K_s value for the cells harvested from the above reactor under the same conditions was 149 mg/l. The yield was determined to be 0.67. No carbohydrate was detected in the effluent except for the temporary periods where S_e was increased. No organic volatile acid was detected at the initial steady state, during the transient period or at the final steady state.

Two-fold Glucose Concentration Decrease Keeping Ammonia Nitrogen Concentration Constant. After the system was operated for 10 to 15 days at the new steady state, the influent glucose concentration was decreased to the initial concentration of 500 mg/l, keeping ammonia nitrogen concentration at 50 mg/l. The system characteristics before the system was shock loaded are summarized in Table XX. After initiation of the two-fold decrease step shock in glucose concentration (Figure 15), the biological solids concentration in the reactor started to decrease at a relatively constant rate. The system established a new steady state with respect to the biomass concentration at about 10 days. Concentration of nitrate nitrogen in the reactor started to increase sharply after administration of the two-fold decrease in glucose concentration step shock from a mean nitrate nitrogen value of 20 mg/l to 24 mg/l in 12 hours to 32 mg/l in two days, and increased to 40 mg/l in eleven days. The mean value for concentration of nitrate

Figure 14. Batch Growth Curves at Various Substrate Concentrations and Relationship Between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Steady State with a Constant Mean Cell Residence Time of 10 Days at Glucose Concentration of 1000 mg/l and Ammonia Nitrogen Concentration of 50 mg/l. The μ_{max} and K_s Values Obtained from Plot of $1/\mu_o$ versus K_s are 0.28 hr^{-1} and 149 mg/l


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
1000
750
500
200
100
0
4
8
12
16
20
Time, Hours

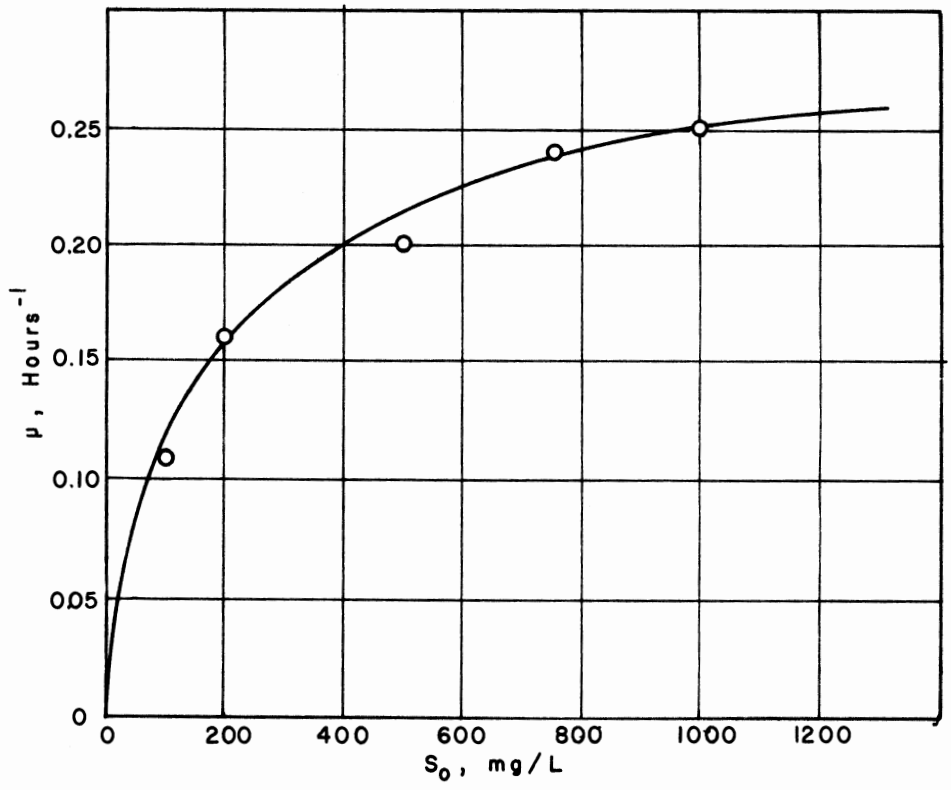
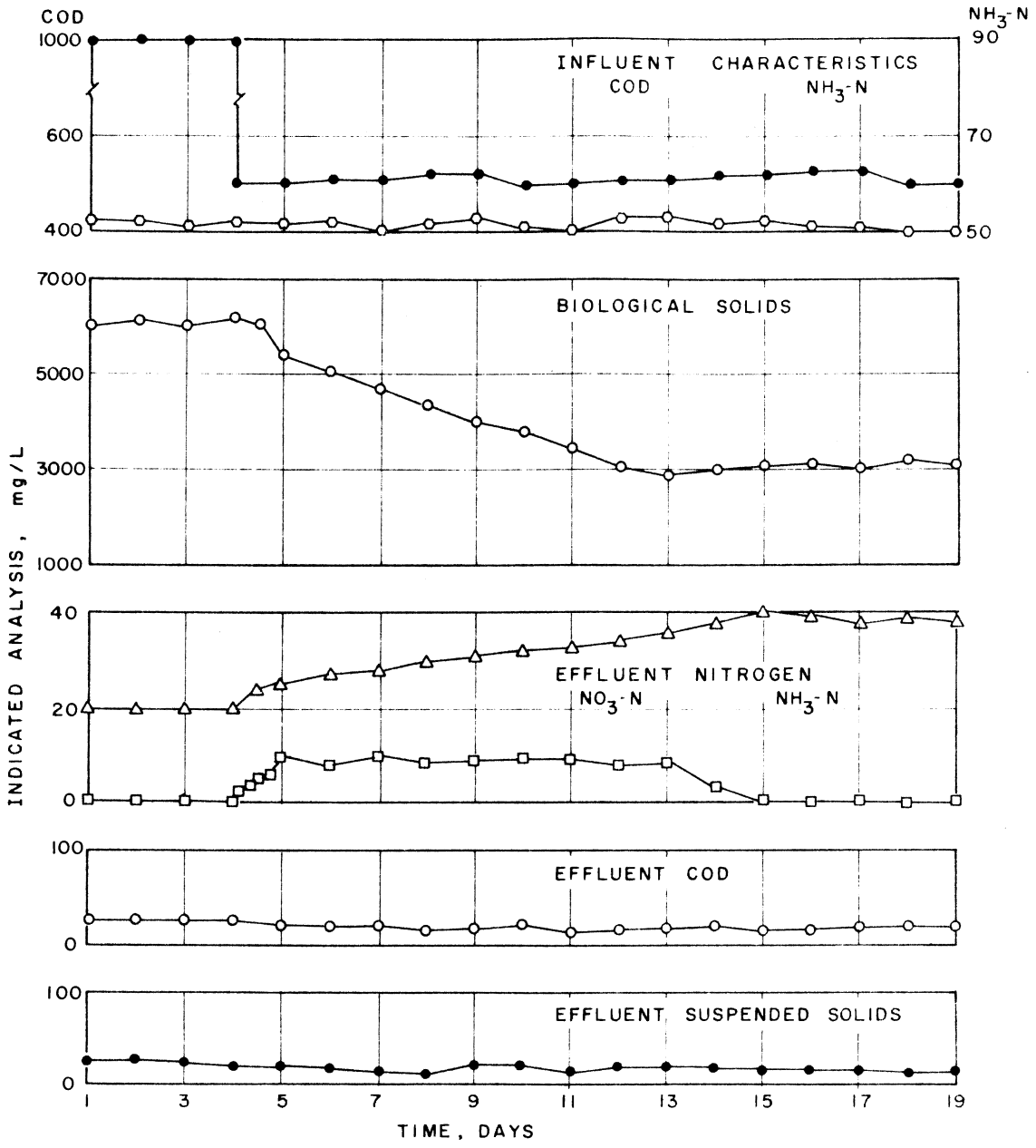

μ, Hours⁻¹
0.25
0.20
0.15
0.10
0.05
0
0
200
400
600
800
1000
1200
S0, mg/L

Figure 15. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 1000 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at a Constant Mean Cell Residence Time of 10 Days Quantitatively Step Shock Loaded by a Decrease in Glucose Concentration From 1000 mg/l to 500 m/gl While Keeping Ammonia Nitrogen Concentration at an Initial Value of 50 mg/l


COD
NH3-N
INFLUENT CHARACTERISTICS
COD
NH3-N
BIOLOGICAL SOLIDS
EFFLUENT NITROGEN
NO3-N
NH3-N
EFFLUENT COD
EFFLUENT SUSPENDED SOLIDS
INDICATED ANALYSIS, mg/L
TIME, DAYS

nitrogen at the new steady state was 39 mg/l. Ammonia nitrogen in the effluent increased upon application of the step two-fold glucose concentration decrease. In six hours after initial of the step two-fold decrease, ammonia concentration increased from approximately zero to 3 mg/l in 24 hours; the effluent contained ammonia nitrogen at a concentration of 10 mg/l. Ammonia nitrogen concentration remained relatively steady between 9 and 10 mg/l between day 1 and day 9. After the ninth day, ammonia nitrogen concentration in the effluent decreased steadily to approximately zero at the new steady state. The COD removal efficiency of the system was unaffected by this shock load, with a mean effluent COD of 18 mg/l ranging between 15 and 25 mg/l. Suspended solids concentration in the effluent was also unaffected by this shock. The mean effluent suspended solids concentration for the new steady state was 17 mg/l, ranging between 15 and 22 mg/l. The color of the biomass was changed to a darker brown color at the new steady state. The system characteristics at the new steady state are summarized in Table XX, where the mean values for different parameters are listed. Settling characteristics of the system remained reasonably good for a nitrifying activated sludge system. Various system parameters are represented graphically in Figure 15. No volatile acids were detected in the system effluent at the initial steady state, during the transition period, or at the final steady state.

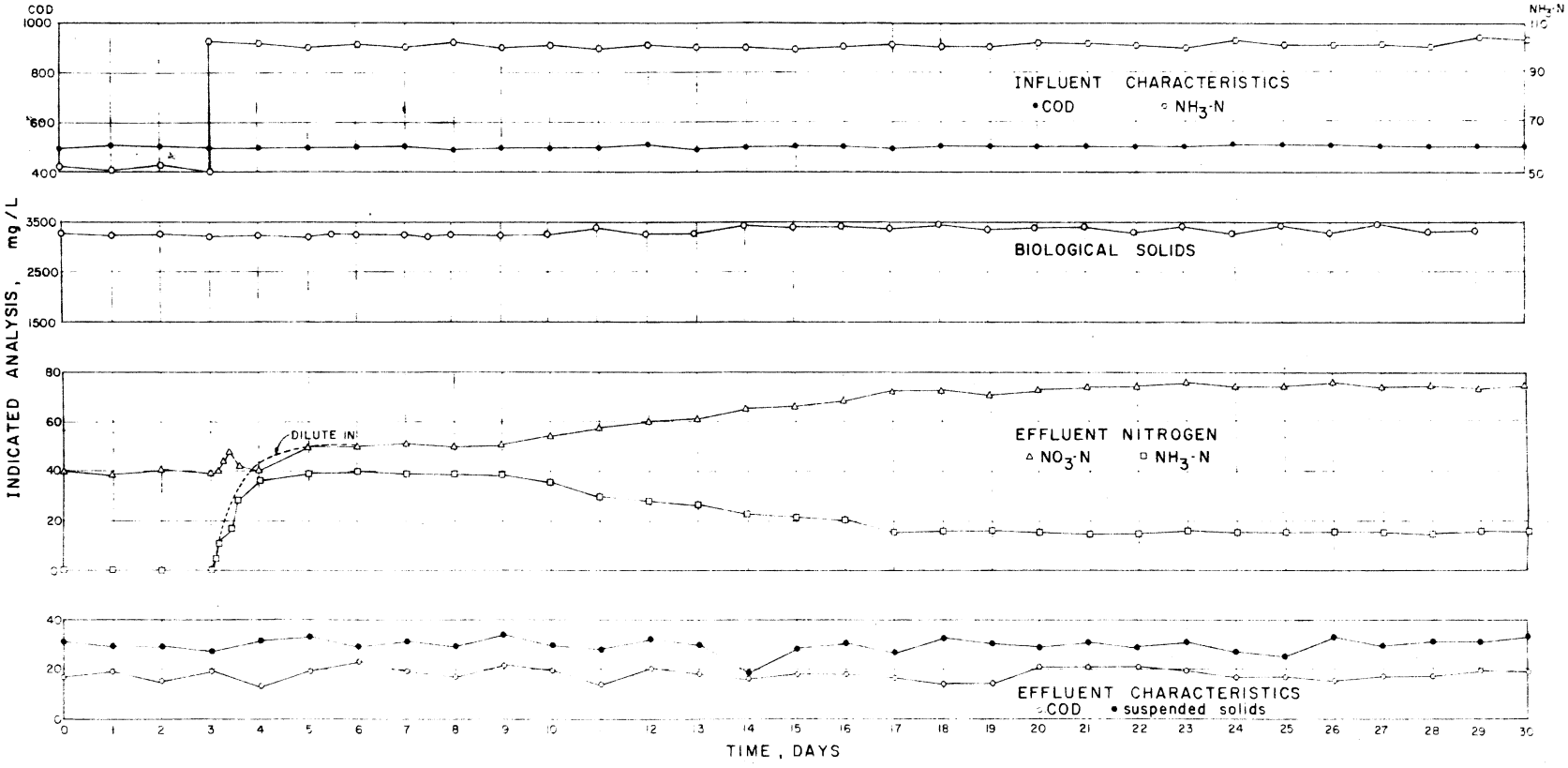
Ammonia Concentration Step Shock Loads
Glucose Concentration Constant

Two-fold Ammonia Concentration Increase. The system was operated at steady state at influent glucose concentration of 500 mg/l and

ammonia nitrogen concentration of 50 mg/l for approximately two weeks operating at 10-day mean cell residence time. The performance characteristics of the system in the initial steady state are given in Table XX. Several system parameters during the initial steady state transition period and the final steady state are illustrated in Figure 16. The two-fold step ammonia nitrogen increase did not affect the biological solids concentration in the reactor to any significant extent. The mean biological solids concentration at the new steady state was 3206 mg/l, ranging from 3190 to 3322 mg/l.

Nitrate nitrogen in the effluent increased to 48 mg/l in the first 10 hours, then decreased to its initial level in 24 hours. Forty-eight hours after administration of the shock load, nitrate nitrogen in the effluent increased to 50 mg/l at which level it stayed steady during the next four days, after which it started to increase gradually to a final mean steady state level of 72 mg/l with a range of 71 to 75 mg/l. Ammonia nitrogen leaked out into the effluent upon application of the step shock load. The reactor contained 10 mg/l ammonia nitrogen six hours after the shock, and 17 mg/l in 12 hours. In 16 hours the ammonia nitrogen concentration in the reactor increased to 29 mg/l. Ammonia nitrogen in the reactor reached a maximum of 40 mg/l in three days after the shock, where it remained relatively constant for the next five days. After the ninth day, ammonia nitrogen in the reactor started to decrease gradually to 8 mg/l on day 17, at which time the system had established a new steady state with respect to nitrification efficiency. The system performance characteristics for the new steady state with influent glucose concentration of 500 mg/l and mean influent ammonia nitrogen concentration of 100 mg/l are

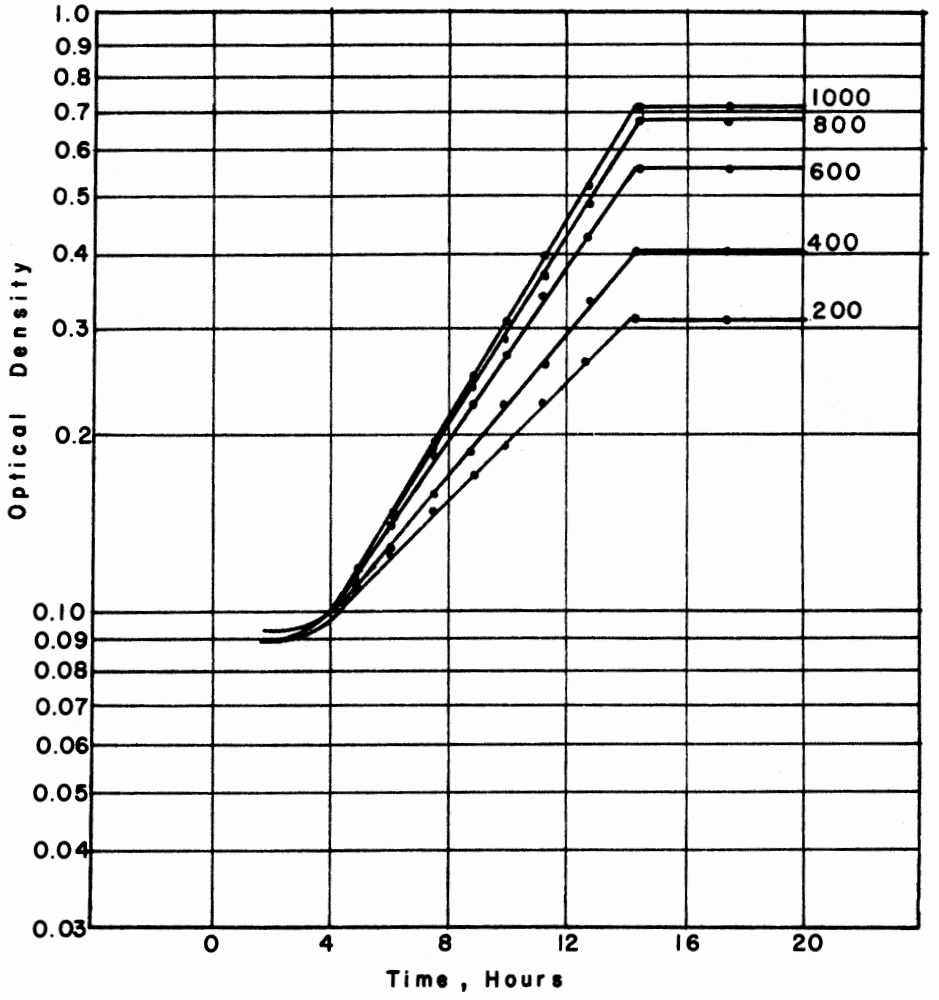
Figure 16. Operational Charactistics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Concentration of 50 mg/l Operating at a Constant Mean Cell Residence Time of 10 Days Quantitatively Step Shock Loaded by an Increase in NH_3-N Concentration From 50 to 100 mg/l While Keeping Glucose Concentration at an Initial Level of 50 mg/l

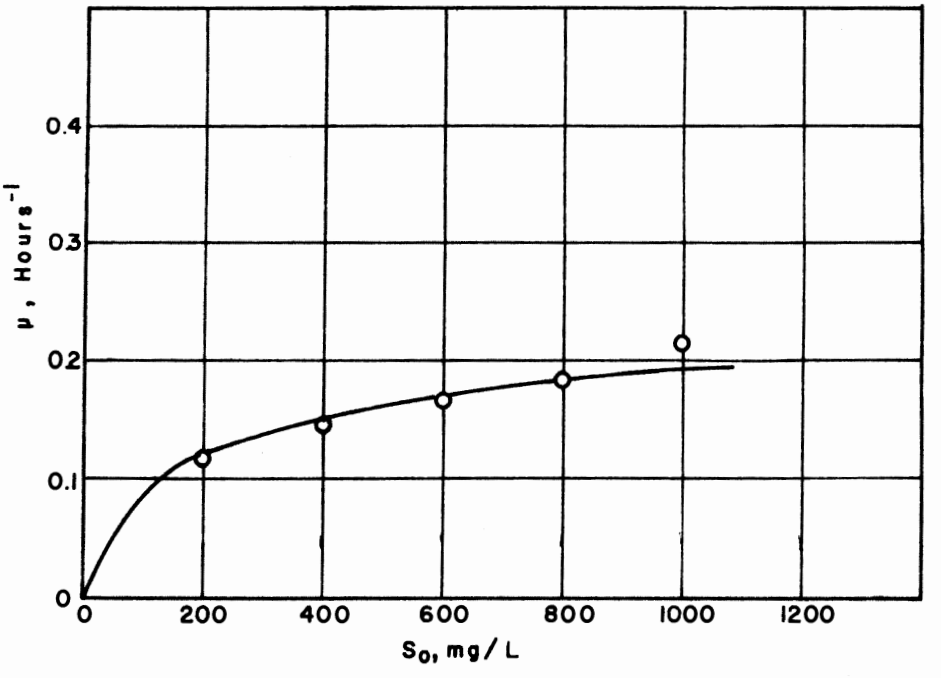

COD
1000
800
600
400
NH3-N
110
90
70
50
INFLUENT CHARACTERISTICS
•COD ○ NH3-N
INDICATED ANALYSIS, mg/L
3500
2500
1500
BIOLOGICAL SOLIDS
80
60
40
20
0
DILUTE IN
EFFLUENT NITROGEN
△ NO3-N □ NH3-N
40
20
0
EFFLUENT CHARACTERISTICS
○COD • suspended solids
0 1 2 3 4 5 6 7 8 9 10 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
TIME, DAYS

summarized in Table XX. The effluent quality with respect to effluent COD, S_e, and effluent suspended solids, X_e, were excellent at the initial steady state, during the transient period, and at the new steady state with mean COD of 16 mg/l at the new steady state, ranging from 14 to 21 mg/l. The mean effluent suspended solids value for the new steady state was 28 mg/l, ranging from 23 to 35 mg/l. No volatile organic acid was detected by gas chromatography in the effluent in the initial steady state, during the transient period, or at the new steady state. The biomass color turned to even darker brown at the new steady state.

Cells harvested from the aeration chamber at the new steady state were employed as initial inoculum for batch experiments to determine the biological kinetic constants for the biomass at the new steady state. The growth curves are shown in Figure 17. The maximum specific growth rate, μ_{max}, observed in these experiments was 0.23 hr^{-1} for cells harvested at steady state from the continuous flow reactor operating at 10 day Θ_c with influent COD of 500 mg/l and ammonia nitrogen concentration at 100 mg/l. The K_s value for these cells harvested from the above reactor under the same conditions was 216 mg/l. The value of Y_{t_B} obtained was 0.50. After steady state was maintained for two weeks, the system influent was changed back to the original 500 mg/l glucose and 50 mg/l ammonia. The biological solids in the reactor did not change. The nitrate nitrogen in the effluent was reduced to a mean value of 40 mg/l after 30 hours, and no ammonia nitrogen was detected in the effluent even 6 hours after the new shock. Like biological solids, the effluent COD and suspended solids were not affected by this shock. The performance characteristics for the new steady state are

Figure 17. Batch Growth Curves at Various Substrate Concentrations and Relationship between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Steady State With a Constant Mean Cell Residence Time of 10 Days at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 100 mg/l. The μ_{max} and K_s Values Obtained From Plot of $1/\mu_o$ versus $1/S_o$ are 0.23 hr^{-1} and 216 mg/l


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
1000
800
600
400
200
0
4
8
12
16
20
Time , Hours


μ , Hours⁻¹
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
S₀, mg/L

listed in Table XX. No volatile organic acids were detected during the transient period or the steady state.

Quantitative Shock Loads Operating at 5-day Θ_c

After steady state was established at 10-day Θ_c, the mean cell residence time for the continuous flow system was lowered to five days in order to study the effect of the mean cell residence time upon the inhibition pattern observed in previous experiments. This was accomplished by doubling the amount of sludge wasted from the system. As a result of doubling the amount of wasted sludge, the system lost its stability and nitrification was gradually decreased unitl it was completely stopped. The system lost its nitrifying ability, and efforts to restore nitrification were not successful. After approximately three months of operation and seeding, nitrification at 5-day Θ_c was accomplished, but the system was much less stable, and nitrification was never completely accomplished. The highest amount of nitrate produced was about 30 mg/l. The system performance characteristics with mean influent glucose concentration of 500 mg/l and mean ammonia nitrogen concentration of 50 mg/l are summarized in Table XX. Biological solids in the reactor were lowered to a new value of 1876 mg/l. Operating the system at a 5-day mean cell residence time, the mean nitrate nitrogen in the effluent was 30 mg/l, and mean ammonia nitrogen in the effluent operating with mean cell residence time of five days was 10 mg/l. Effluent COD and suspended solids concentrations were 21 mg/l each.

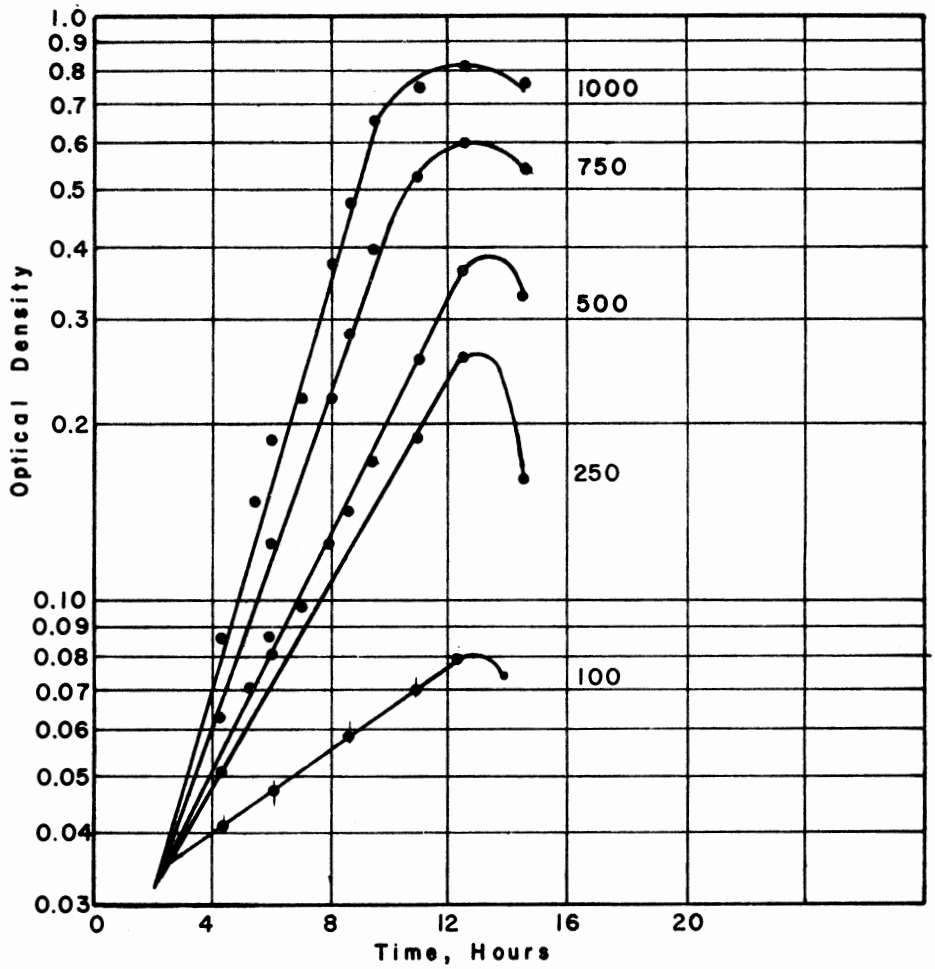
Step Quantitative Shock Loads at 5-day Θ_c

Two-fold Combined Glucose and Ammonia Step Increase Operating at 5-day Θ_c

The continuous flow unit was operated at 5-day Θ_c for approximately two weeks at steady state before application of the step qncrease in combined glucose and ammonia influent concentrations. Cells harvested at this steady state were employed in batch experiments to determine the biological kinetic constant for the heterogeneous population, as described previously. The maximum specific growth rate, μ_{max}, was determined to be 0.65 hr^{-1}. The growth plots for this batch experiment are shown in Figure 18. K_s was determined to be 636 mg/l and Y_{t_B} was 0.55.

The mean biological solids concentration at this steady state was 1836 mg/l, ranging from 1714 to 1936 mg/l. Mean effluent nitrate nitrogen concentration was 30 mg/l, ranging from 27 to 32 mg/l. The mean effluent ammonia nitrogen concentration was 10 mg/l, ranging from 8 to 13 mg/l. The mean effluent COD was 21 mg/l, ranging between 18 and 30 mg/l. The suspended solids concentration at initial steady state was 21 mg/l, ranging from 15 to 25 mg/l. Upon administration of the two-fold combined glucose-ammonia increase shock load, biological solids increased to 2000 in about 31 hours, and to a maximum of 4023 in 18 days after the shock. After day 18, the biomass concentration decreased and established a new steady state with biomass concentration of 3176 mg/l. The system operational characteristics in the initial steady state, during the transient period, and in the new steady state are shown in Figure 19. Nitrate-nitrogen concentration decreased from 30

Figure 18. Batch Growth Curves at Various Substrate Concentrations and Relationship Between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Steady State With a Constant Mean Cell Residence Time of 5 days at Glucose Concentration of 500 mg/l and Ammonia Nitrogen concentration of 50 mg/l. The μ_{max} and K_s Values Obtained from Plot of $1/\mu_o$ and $1/S_o$ are 0.61 hr^{-1} and 635.6 mg/l


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
1000
750
500
250
100
0
4
8
12
16
20
Time, Hours

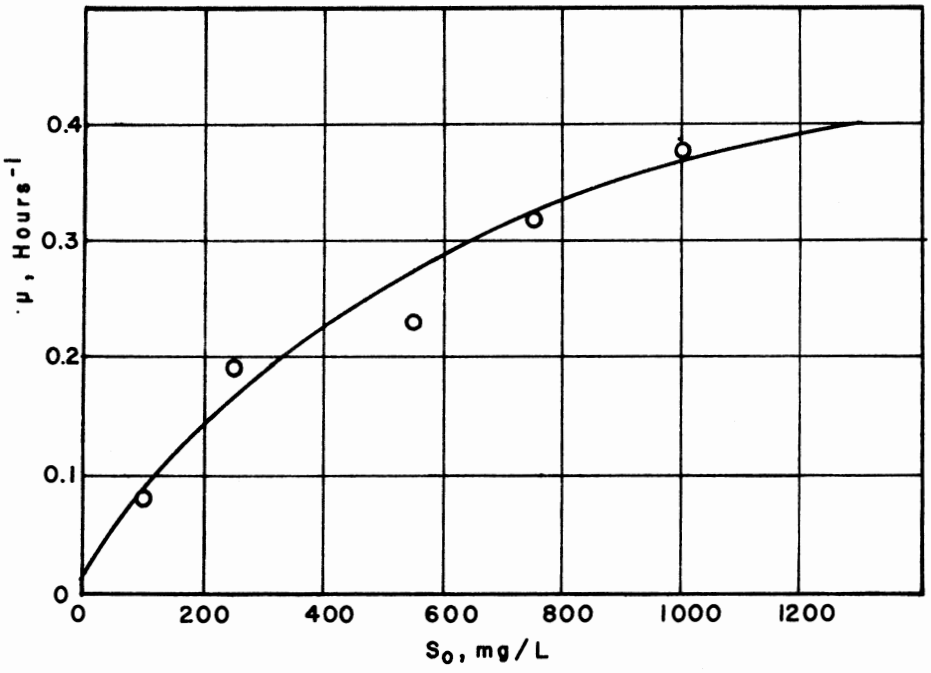
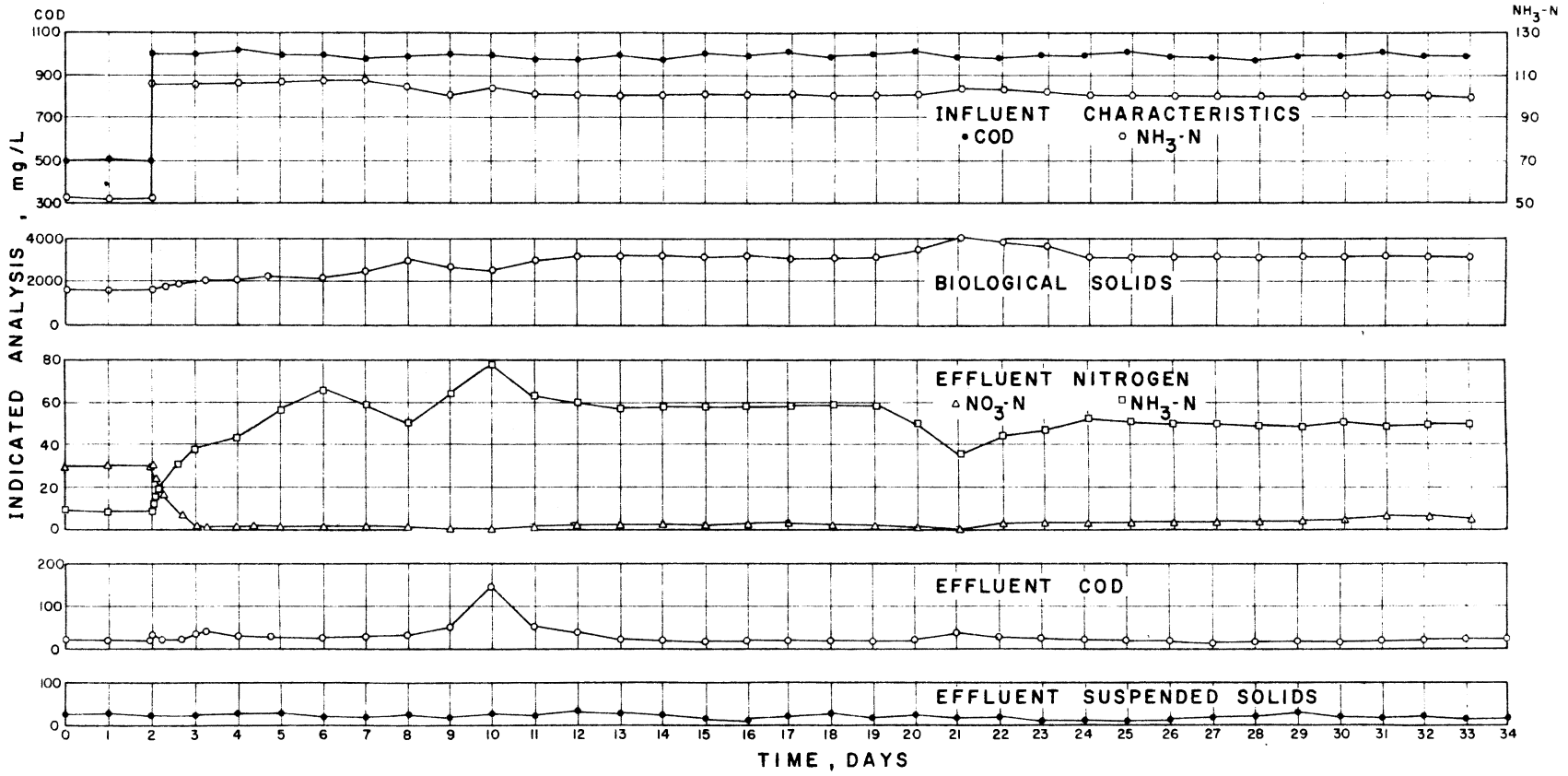

μ, Hours⁻¹
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
1200
S0, mg/L

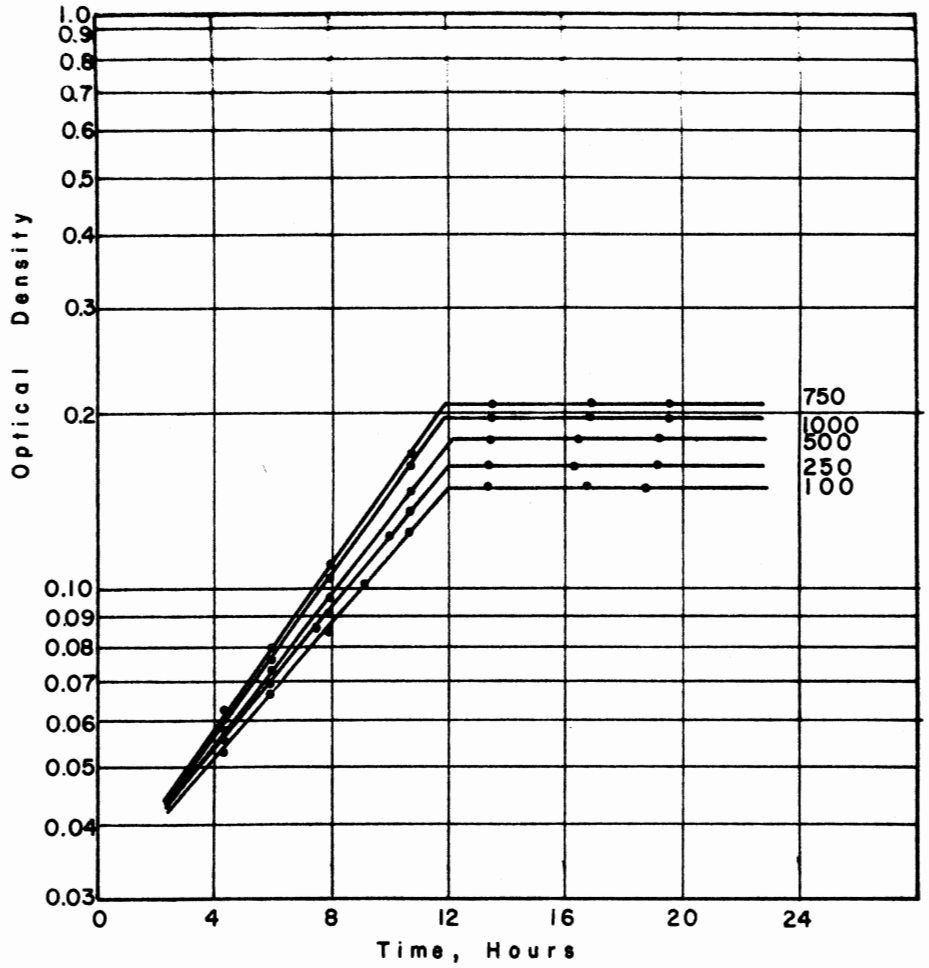
Figure 19. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Concentration of 50 mg/l Operating at a Constant Mean Cell Residence Time of 5 Days Quantitatively Step Shock Loaded by an Increase in Combined Glucose Concentration from 500 to 1000 mg/l and Ammonia Nitrogen Concentration from 50 to 100 mg/l


COD
NH3-N
INFLUENT CHARACTERISTICS
• COD
○ NH3-N
BIOLOGICAL SOLIDS
EFFLUENT NITROGEN
△ NO3-N
□ NH3-N
EFFLUENT COD
EFFLUENT SUSPENDED SOLIDS
INDICATED ANALYSIS, mg/L
TIME, DAYS

mg/l to 18 mg/l in eight hours, to 10 mg/l in 18 hours, and to about 1 mg/l in 24 hours, where it stayed steady between zero and 1 mg/l until 19 days after the shock load. After day 19, nitrate nitrogen increased to steady state concentration of about 8 mg/l. The effluent ammonia nitrogen concentration increased almost immediately after the shock load was applied to the system. After six hours, the effluent ammonia nitrogen increased from 10 to 20 mg/l. In 24 hours, the effluent ammonia nitrogen concentration had increased to 38 mg/l and it gradually increased to a maximum of 79 mg/l seven days after the shock load was applied. Then it decreased gradually to a new steady state value of 50 mg/l. The shock load caused a cessation of the nitrification process. The system COD removal efficiency was almost unaffected for seven days. At day 7, the effluent COD was increased to 149 mg/l temporarily, after which it decreased to a new steady state concentration of 23 mg/l. Effluent suspended solids concentration was unaffected by this step shock load, and the new steady state effluent mean suspended solids concentration was only 23 mg/l. No anthrone or volatile organic acid was detected in the effluent during the initial or final steady state. There was about 100 mg/l carbohydrate at day 10, when effluent COD was 149 mg/l in the transient state. However, no volatile organic acid was detected in the system.

Color of the biomass became much lighter after the shock load. Cells harvested from the reactor during this steady state were employed for batch growth experiments to determine the biological kinetic constants for the heterogeneous population at the steady state. The batch growth curves for these experiments are shown in Figure 20. Maximum specific growth rate, μ_{max}, for cells harvested from the system operating

Figure 20. Batch Growth Curves at Various Substrate Concentrations and Relationship Between μ and S_o for Cells Harvested From the Nitrifying Activated Sludge Pilot Plant Operating at Steady State With a Constant Mean Cell Residence Time of 5 Days at Glucose Concentration of 1000 mg/l and Ammonia Nitrogen Concentration of 100 mg/l. The μ_{max} and K_s Values Obtained From Plot of $1/\mu_o$ versus $1/S_o$ are 0.16 hr^{-1} and 23 mg/l


Optical Density
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0
4
8
12
16
20
24
Time, Hours
750
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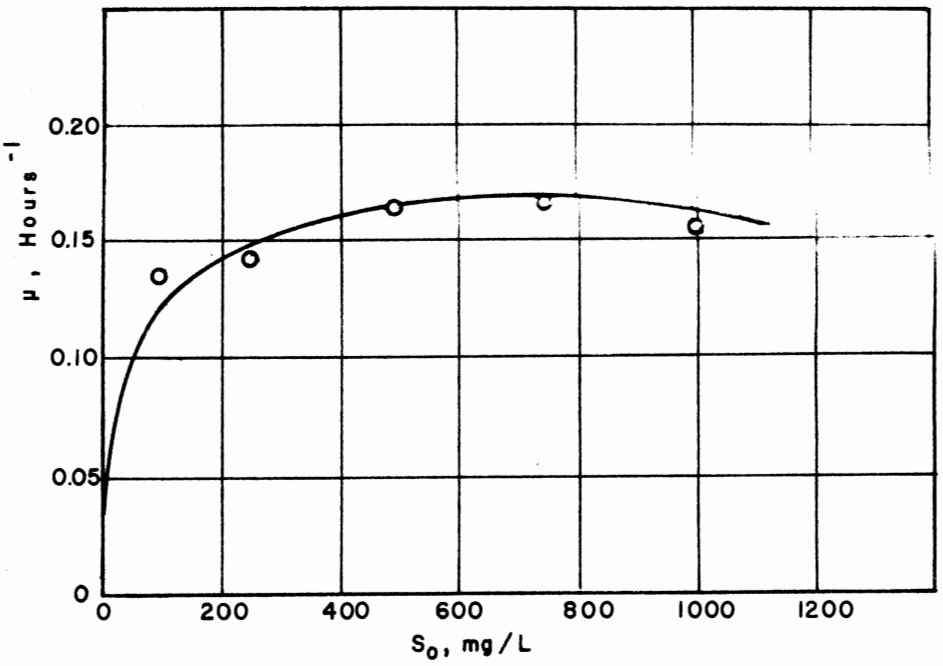
500
250
100


μ, Hours⁻¹
0.20
0.15
0.10
0.05
0
0
200
400
600
800
1000
1200
S0, mg/L

at 5-day mean cell residence time at the new steady state with influent mean glucose concentration of 1000 mg/l and influent mean ammonia nitrogen concentration of 100 mg/l was 0.16 hr^{-1}. K_s of these cells was 23 mg/l; Y_{t_B} was 0.51.

Concentration Slug Dose Shock Loads

Impulse tests of combined glucose and ammonia nitrogen, and ammonia nitrogen were studied by adding an accurately measured quantity of concentrated feed solution directly to the reactor. This type of dynamic test or shock load is a most severe forcing for the organisms, since they experience a step change in the concentration of the material added. A step change is much less severe dynamic forcing for the organisms. It should be noted that a slug dose or an impulse is a theoretical function which can be only approximated in practice by a pulse of short duration.

Three ammonium nitrogen concentration impulse forcings and two combined glucose and ammonia concentration impulses were applied in these studies. Influent glucose concentration to the unit at steady state was 500 mg/l; mean ammonia nitrogen in the system's influent was 50 mg/l. Mean biological solids concentration during the steady state before application of the first ammonia impulse was 3250. The continuous flow unit was operated at 10-day mean cell residence time for at least five days before application of each impulse forcing. The following concentration impulses were applied to the system operating at steady state at 10-day Θ_c. Results of different concentration impulse shock loads are summarized in Table XXI.

TABLE XXI

PERFORMANCE CHARACTERISTICS OF THE SYSTEM BEFORE THE CONCENTRATION IMPULSES AT 10-DAY Θ_c

Line	Figure	Average Biolog. Solids (mg/l)	Reactor NO_3-N (mg/l)	Reactor NH_3-N (mg/l)	Reactor COD (mg/l)	Effluent Suspended Solids (mg/l)	Impulse Concentration pH	Impulse Concentration Glucose	Impulse Concentration NH_3-N (mg/l)
1	21	3250	40	0	12	21	7.2	0	86
2	22	3224	40	0	20	19	7.2	0	148
3	23	3266	40	0	17	23	7.2	0	255
4	24	2325	39	0	19	21	7.2	55	610
5	25	3362	39	0	18	19	7.2	304	3500

Ammonia Impulses

85 mg/l NH_3-N Concentration Impulse. To the continuous flow system operating at 10-day mean cell residence time with complete nitrification, a slug dose of concentrated ammonium sulfate solution was added so that, after mixing, the reactor concentration was 86 mg/l NH_3-N. The system performance characteristics before this 86 mg/l slug dose of ammonium are listed in Table XXI. The mean biological solids concentration before the concentration impulse was 3250 mg/l. The system was producing 40 mg/l of nitrate nitrogen with no effluent ammonia nitrogen. Effluent COD, S_e, was 12 mg/l, and suspended solids concentration in the effluent was 21 mg/l. As a result of administration of the ammonia concentration impulse, nitrate nitrogen increased steadily to

a maximum of 63 mg/l in 12 hours from the initial steady state concentration of 40 mg/l, after which nitrate nitrogen in the reactor decreased to the original 40 mg/l in 24 hours. This shock had no significant effect on COD removal efficiency or biological solids concentration, or effluent suspended solids concentration, as is shown in Figure 21. Ammonia concentration in the reactor decreased steadily faster than the theoretical dilute-out curve, which is shown by the dashed line in Figure 21. This concentration impulse did not inhibit the nitrification process or the biochemical efficiency of the heteotrophic microorganisms.

150 mg/l Ammonia Nitrogen Concentration Impulse

The system was maintained at steady state with complete nitrification for at least five days before application of ammonia concentration impulse. The system performance characteristics just before the impulse was applied are summarized in Table XXI. The mean biological solids concentration of 3224 mg/l, reactor mean nitrate and ammonia concentration of 40 and 0 mg/l, respectively, and reactor COD and suspended solids of 21 and 19, respectively. Application of the shock did not affect the biological solids concentration or COD, nor the effluent suspended solids concentration of the system. However, nitrate nitrogen was increased from an original steady state concentration of 40 mg/l to a maximum of 72 mg/l in 16 hours, and leveled off to the original steady state concentration in 36 hours. Ammonia nitrogen in the reactor decreased faster than the theoretical dilute-out curve, as shown by the dashed line in Figure 22, where responses of the system to the 150 mg/l ammonia nitrogen concentration impulse are

Figure 21. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at a Constant Mean Cell Residence Time of 10 Days Quantitatively Impulse Shock Loaded by Ammonia Nitrogen Concentration of 85 mg/l in Addition to Continuous Flow Influent

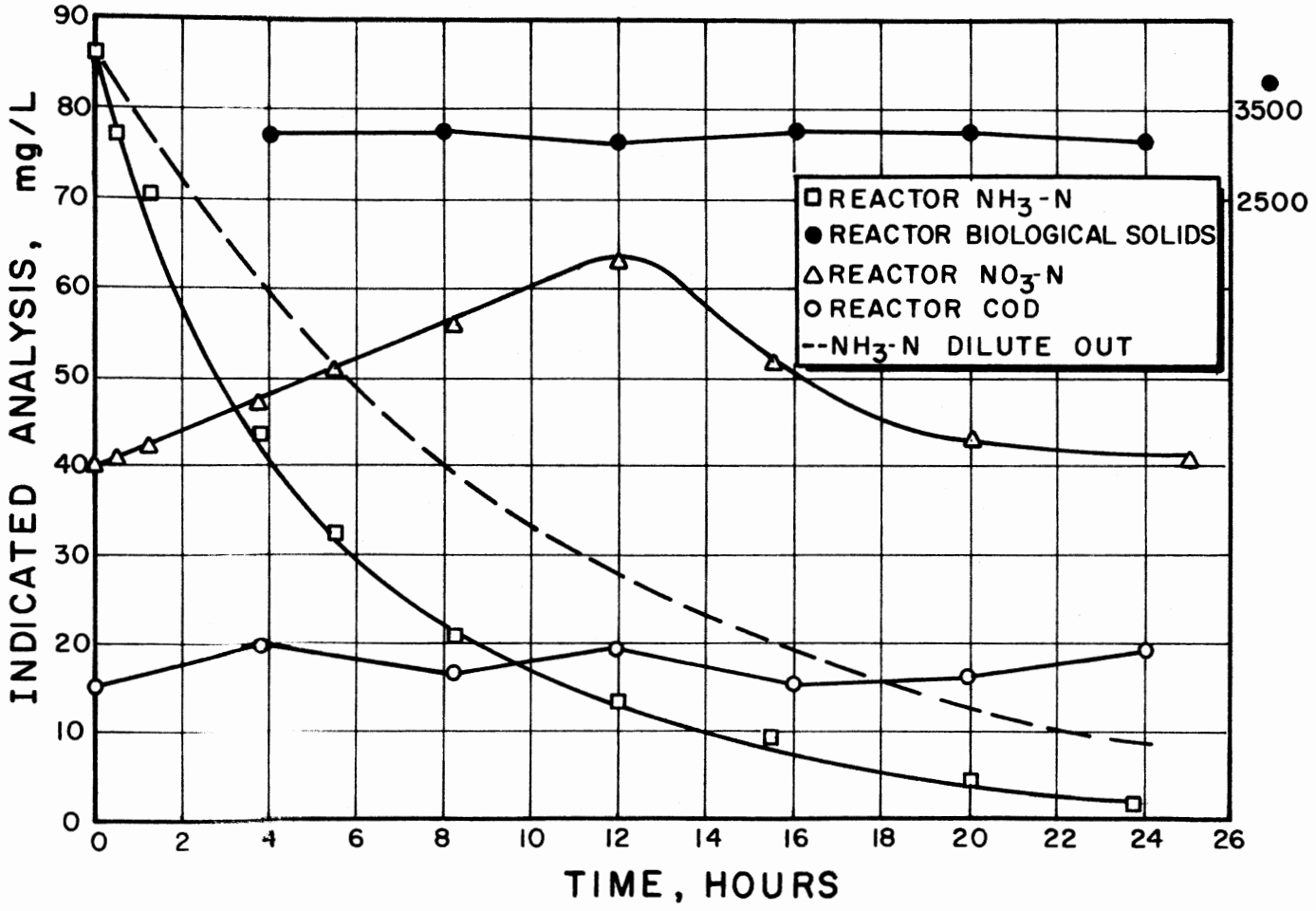
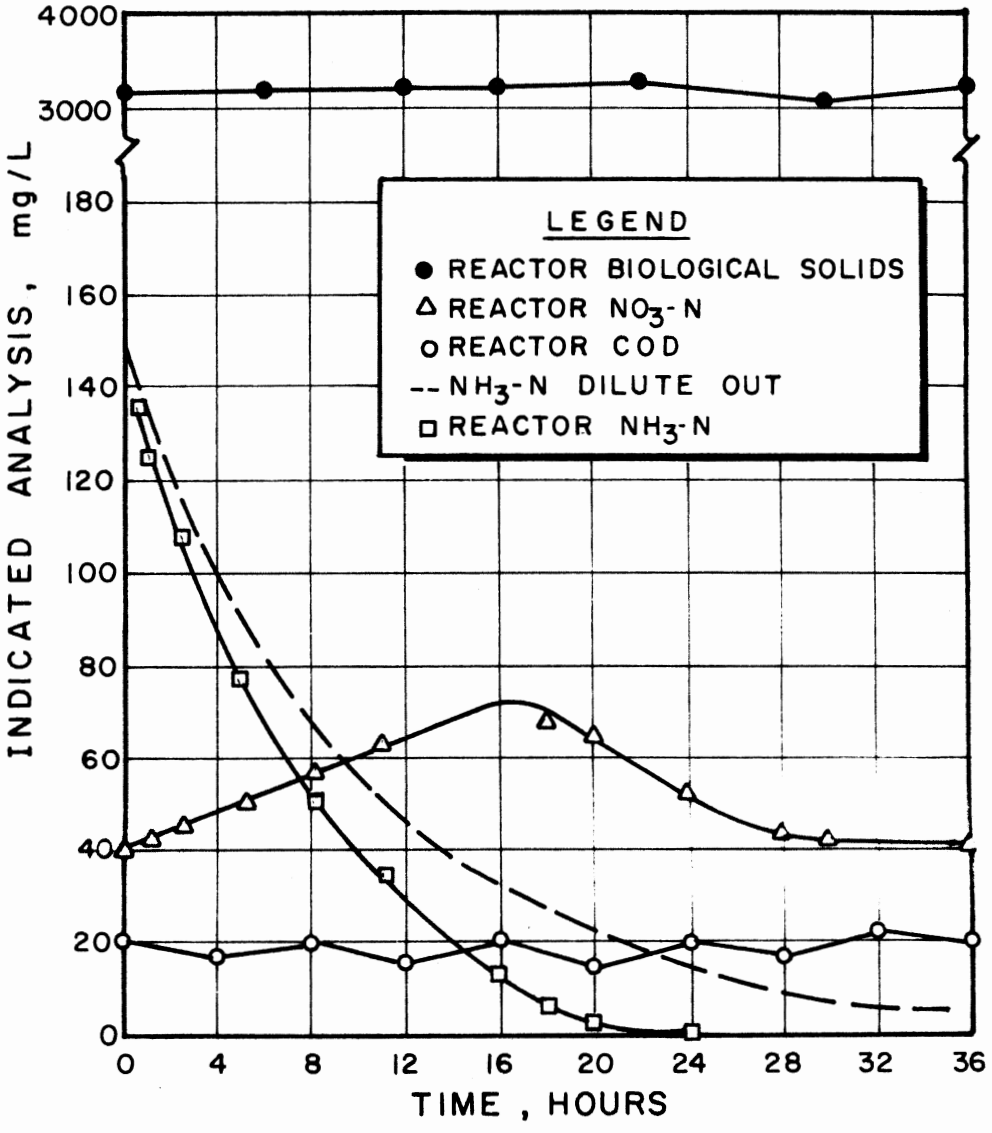

INDICATED ANALYSIS, mg/L
TIME, HOURS
REACTOR NH3-N
REACTOR BIOLOGICAL SOLIDS
REACTOR NO3-N
REACTOR COD
--NH3-N DILUTE OUT
3500
2500
0
10
20
30
40
50
60
70
80
90
0
2
4
6
8
10
12
14
16
18
20
22
24
26

Figure 22. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at Constant Mean Cell Residence Time of 10 Days Quantitatively Impulse Shock Loaded by Ammonia Nitrogen Concentration of 150 mg/l in Addition to Continuous Flow Influent


4000
3000
180
160
140
120
100
80
60
40
20
0
INDICATED ANALYSIS, mg/L
LEGEND
REACTOR BIOLOGICAL SOLIDS
REACTOR NO3-N
REACTOR COD
--NH3-N DILUTE OUT
REACTOR NH3-N
0
4
8
12
16
20
24
28
32
36
TIME, HOURS

illustrated. The continuous flow influent to the reactor was the same as the previous ammonia impulse forcing, 500 mg/l glucose and 50 mg/l ammonia nitrogen.

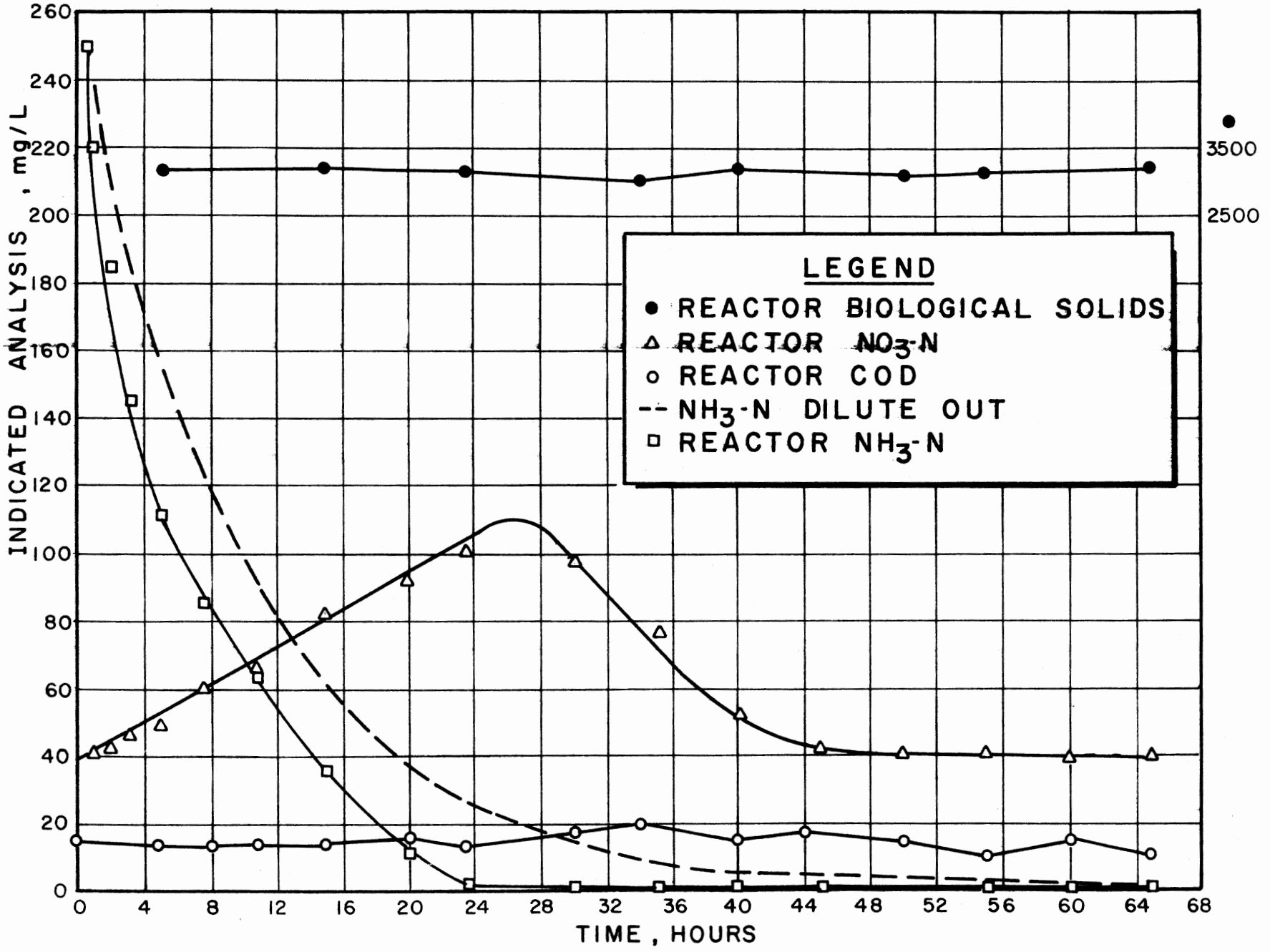
250 mg/l Ammonia Nitrogen Concentration Impulse

Again prior to application of this shock load, the sysem ran under steady state conditions operating at 10-day mean cell residence time with influent glucose concentration of 500 mg/l and ammonia nitrogen of 50 mg/l for at least five days. System performance characteristics prior to this shock load are summarized in Table XXI. Mean biological solids concentration in the reactor was 3266 mg/l, with NO_3-N and NH_3-N concentrations of 40 and zero mg/l, respectively. Application of the 255 mg/l NH_3-N concentration impulse did not affect biological solids concentration, COD, or suspended solids concentration in the effluent. However, nitrate nitrogen in the reactor was increased to a maximum of 110 mg/l in 26 hours, after which it started to decrease to its original steady state nitrate nitrogen concentration of 40 mg/l in 50 hours after the slug dose shock load. Ammonia nitrogen in the reactor decreased faster than the theoretical dilute-out curve shown by the dashed line in Figure 23, where the responses of the continuous flow system to the 250 mg/l ammonia nitrogen impulse forcing are shown graphically.

Combined Glucose and Ammonia Concentration Impulses

600 mg/l Glucose and 55 mg/l NH_3-N Concentration Impulse. Prior to shock loading the system, the combined concentration impulse system

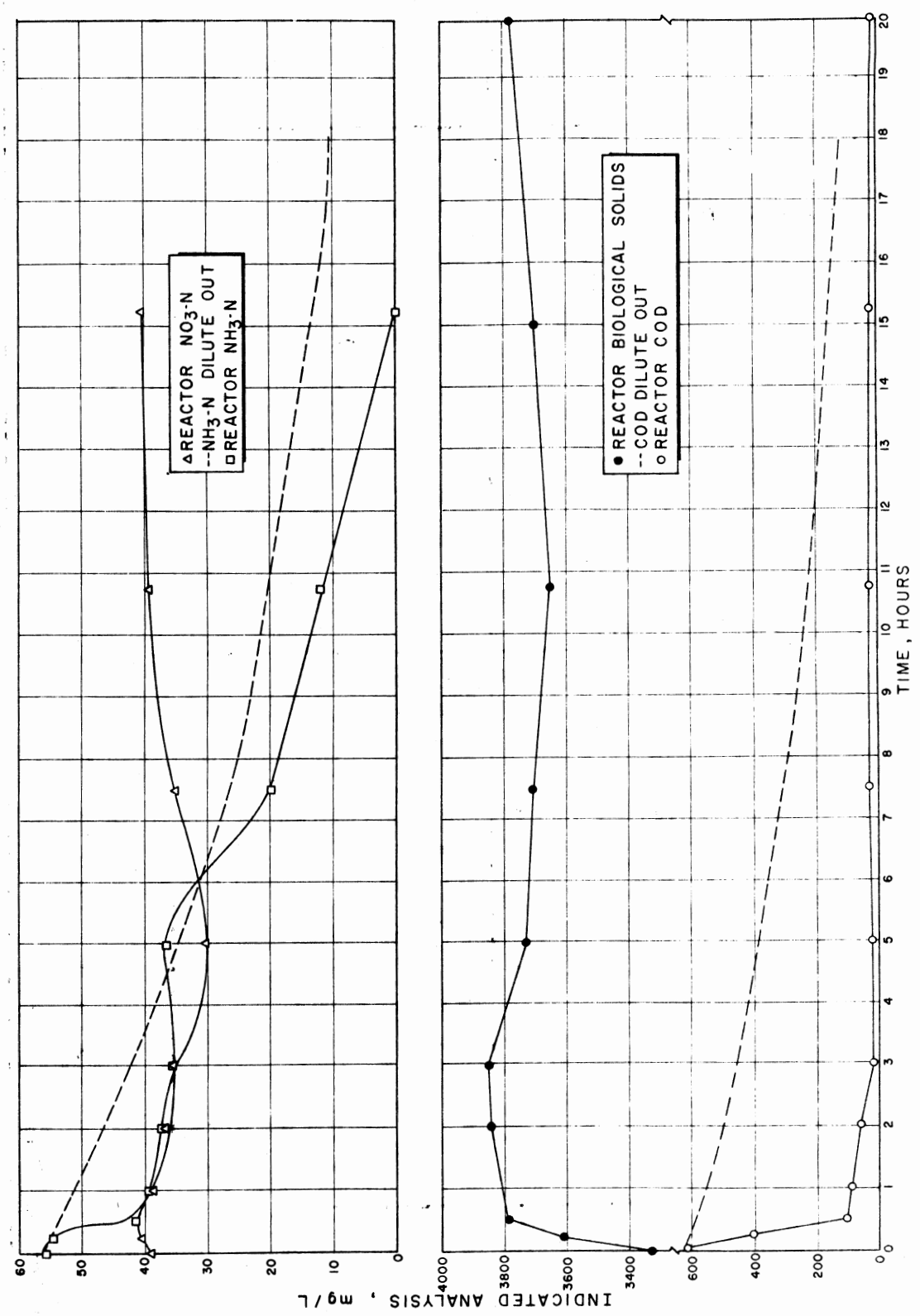
Figure 23. Operational Characteristics of a Continuous Flow Nitrification Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 250 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at Constant Mean Cell Residence Time of 10 Days Quantitatively Impulse Shock Loaded by Ammonia Nitrogen Concentration of 250 mg/l in Addition to Continuous Flow Influent


INDICATED ANALYSIS , mg/L
TIME , HOURS
LEGEND
REACTOR BIOLOGICAL SOLIDS
REACTOR NO3-N
REACTOR COD
NH3-N DILUTE OUT
REACTOR NH3-N
3500
2500
0
20
40
60
80
100
120
140
160
180
200
220
240
260
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68

was maintained at least for five days at steady state with complete nitrification. System performance characteristics prior to the shock are summarized in Table XXI. The responses of the system to the combined glucose and ammonia concentration impulse forcing is shown in Figure 23. The mean biological solids concentration prior to shock was 3325 mg/l. Other parameters, effluent nitrate nitrogen, ammonia nitrogen, COD, and suspended solids were 40, zero, 19, and 21 mg/l, respectively.

Upon administration of the concentration impulse of combined glucose and ammonia, biological solids in the reactor started to increase to 3780 mg/l in 30 minutes to a maximum of 3845 mg/l in three hours, after which biological solids started to decrease gradually to a final concentration of approximately 3700 mg/l. Nitrate nitrogen in the reactor increased to 415 mg/l in 30 minutes, and decreased to a minimum concentration of 30 mg/l in five hours. After 15 hours, it leveled off to its original steady state level. Ammonia nitrogen in the reactor decreased sharply for the first 30 minutes after administration of the combined concentration impulse shock load, after which time the reactor ammonia nitrogen leveled off to a steady 36 mg/l in five hours. After five hours, the reactor ammonia nitrogen was decreased sharply to zero effluent ammonia nitrogen in 15 hours after the shock. Effluent COD decreased much faster than the theoretical COD dilute-out represented by the dashed line in Figure 24. Most of the COD was removed within 30 minutes. It is clearly shown that nitrification was repressed by this impulse shock load. Here again it is obvious that this repression of nitrification process is occurring simultaneously with the rapid biological growth of heterotrophic microorganisms due to increased

Figure 24. Operational Characteristics of a Continuous Flow Nitrification Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at Constant Mean Cell Residence Time of 10 Days Quantitatively Impulse Shock Loaded by Glucose Concentration of 610 mg/l and Ammonia Nitrogen Concentration of 55 mg/l in Addition to Continuous Clow Influent

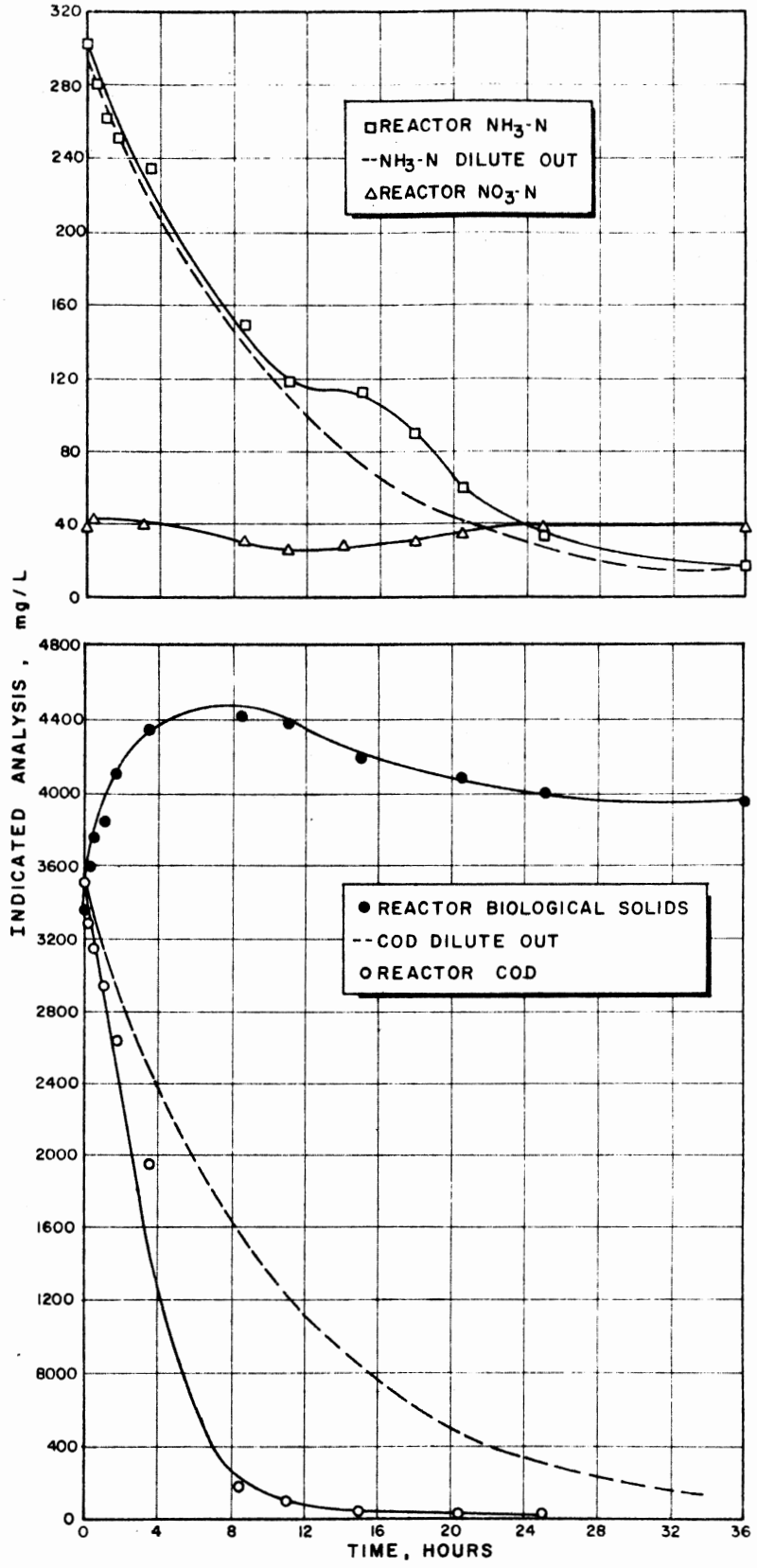

△REACTOR NO_3-N
--NH_3-N DILUTE OUT
□REACTOR NH_3-N
● REACTOR BIOLOGICAL SOLIDS
--COD DILUTE OUT
○ REACTOR COD
INDICATED ANALYSIS, mg/L
TIME, HOURS

carbon source in the system.

Combined 3500 mg/l Glucose and 300 mg/l Ammonia Nitrogen Concentration Impulse

After operating the system at steady state for several days in the continuous flow system operating at 10-day Θ_c with influent ammonia nitrogen concentration of 50 mg/l and effluent glucose concentration of 500 mg/l with complete nitrification, the combined concentration impulse shock load was applied to the system. The biological solids started to increase rapidly. The system performance characteristics before administration of the shock load are summarized in Table XXI. Figure 25 is a graphical representation of the continuous flow system responses to this combined concentration impulse. Biological solids increased sharply from an initial steady state value of 3362 to 4100 mg/l in less than two hours, and to a maximum of 4430 mg/l in 8½ hours after the shock load. The system stabilized with respect to biological solids in 36 hours with biomass concentrations of 3960 mg/l. Practically all COD was removed in 8½ hours. Nitrate nitrogen was decreased from the initial steady state value of 39 mg/l after a very small increase to 26 mg/l in 8½ hours. Nitrate nitrogen increased to the original level in 24 hours. Ammonia removal followed the theoretical dilute-out curve indicated by the dashed line in Figure 25 very closely and less rapidly than the dilute-out of about eight hours after the shock load. Reactor COD decreased at a faster rate than that predicted by the theoretical dilute-out curve. It is clearly demonstrated that the nitrification process was repressed by this combined concentration impulse shock load.

Figure 25. Operational Characteristics of a Continuous Flow Nitrifying Activated Sludge System With Total Internal Cell Recycle at Glucose Concentration of 500 mg/l and Ammonia Nitrogen Concentration of 50 mg/l Operating at Constant Mean Cell Residence Time of 10 days Quantitatively Impulse Shock Loaded by Ammonia Nitrogen Concentration of 300 mg/l in Addition to Continuous Flow Influent


□REACTOR NH_3-N
--NH_3-N DILUTE OUT
△REACTOR NO_3-N
● REACTOR BIOLOGICAL SOLIDS
--COD DILUTE OUT
○ REACTOR COD
INDICATED ANALYSIS, mg/L
TIME, HOURS
0
40
80
120
160
200
240
280
320
400
1200
1600
2000
2400
2800
3200
3600
4000
4400
4800
8000
4
8
12
16
20
24
28
32
36

Batch Experiments

The effect of several acetate ion concentrations (25, 75, 150 mg/l) on nitrifiers was studied in batch reactors containing only the nitrifying organisms. Figures 26, 27, and 28 graphically represent the effects of different acetate concentrations inthe batch reactor containing an initial concentration of between 50 and 60 mg/l of ammonia nitrogen. In all experiments, acetate enhanced the ammonia removal rate. In all cases, initial nitrate production rates were lower; however, higher quantities of nitrate were detected in batch reactors containing the acetate.

Figure 26. Effect of 25 mg/l of Acetate Ion on Biological Nitrification in Batch Systems

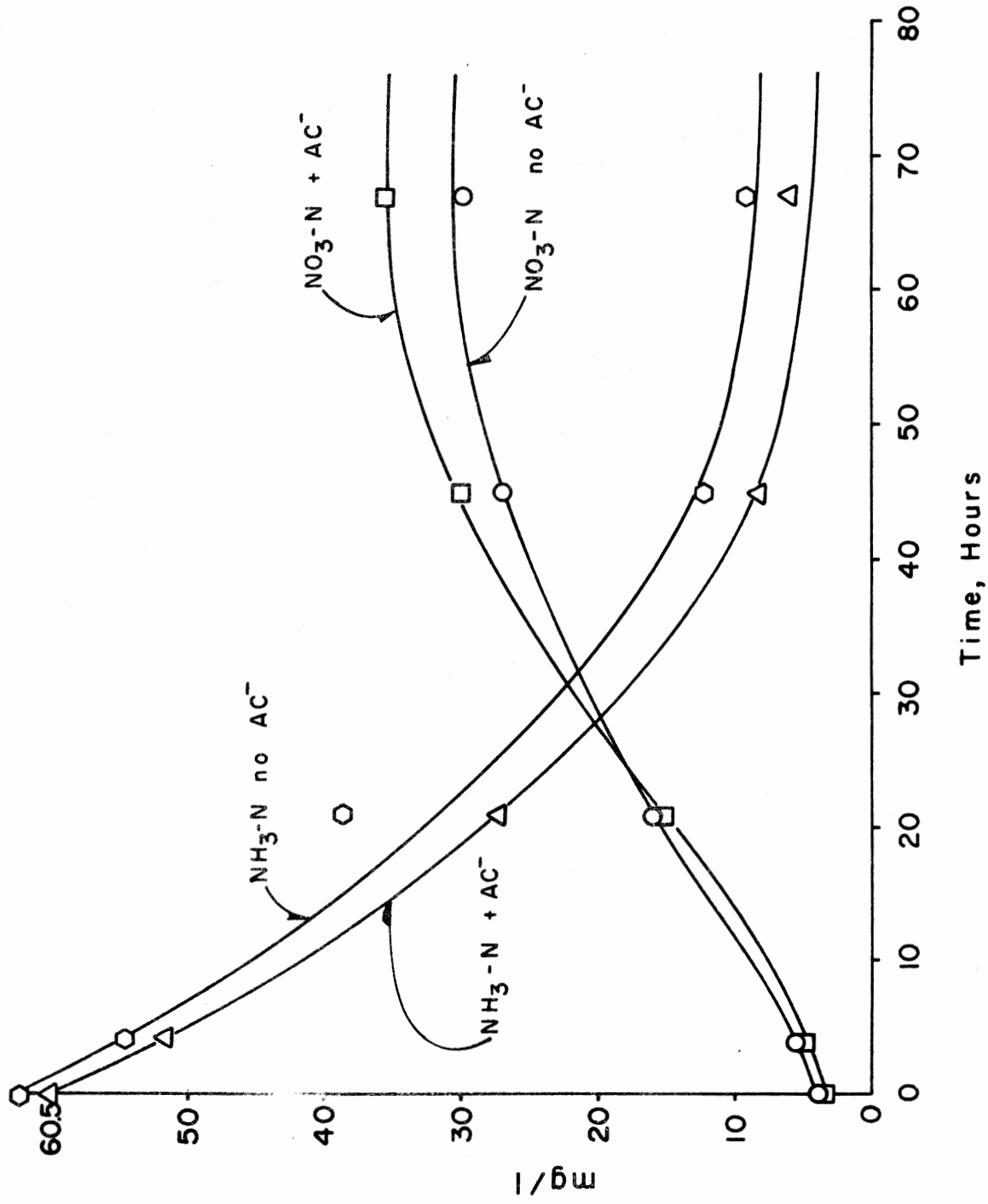

mg/l
60.5
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
Time, Hours
NO_3-N + AC^-
NO_3-N no AC^-
NH_3-N no AC^-
NH_3-N + AC^-

Figure 27. Effect of 75 mg/l of Acetate Ion on Biological Nitrification in Batch Systems

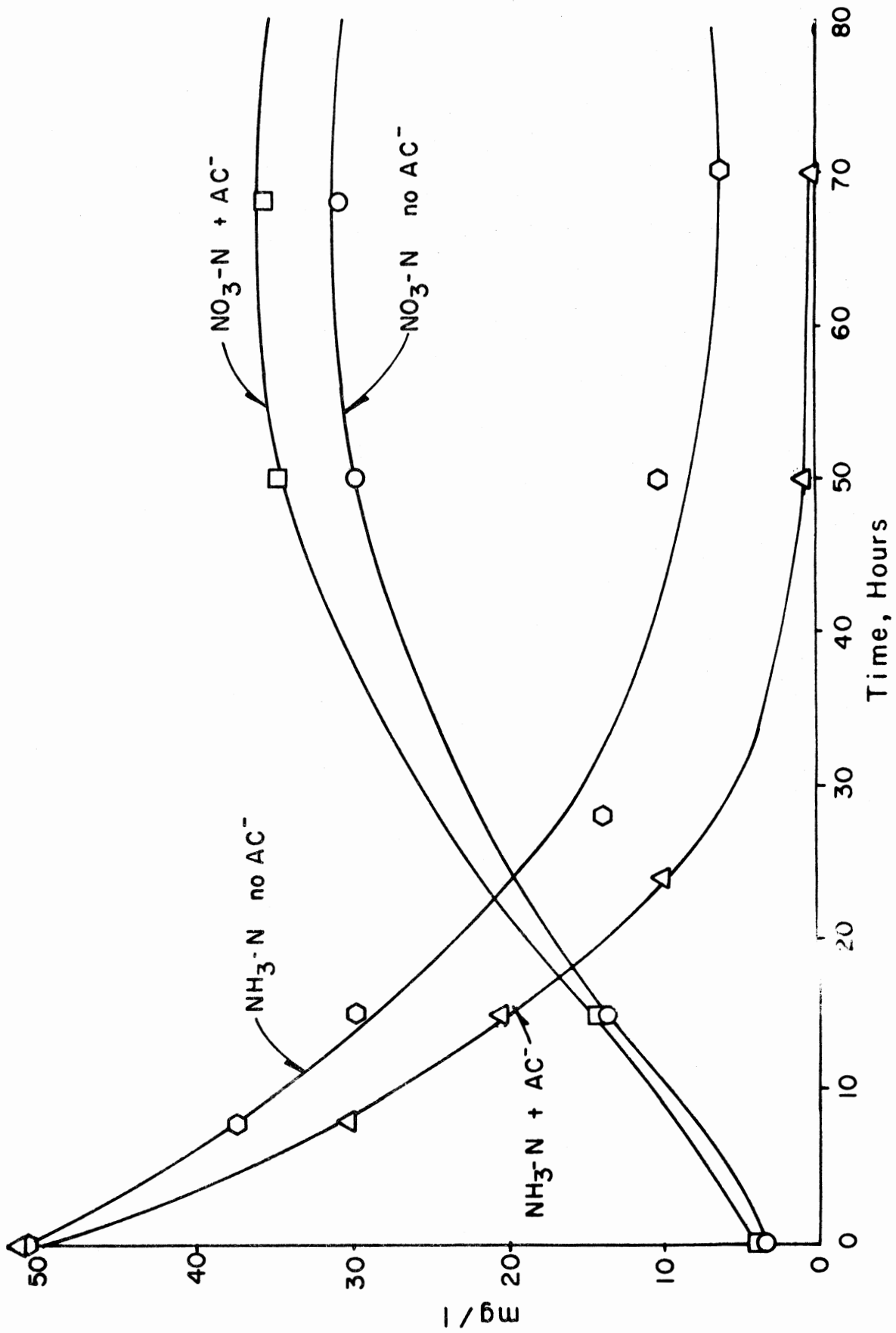
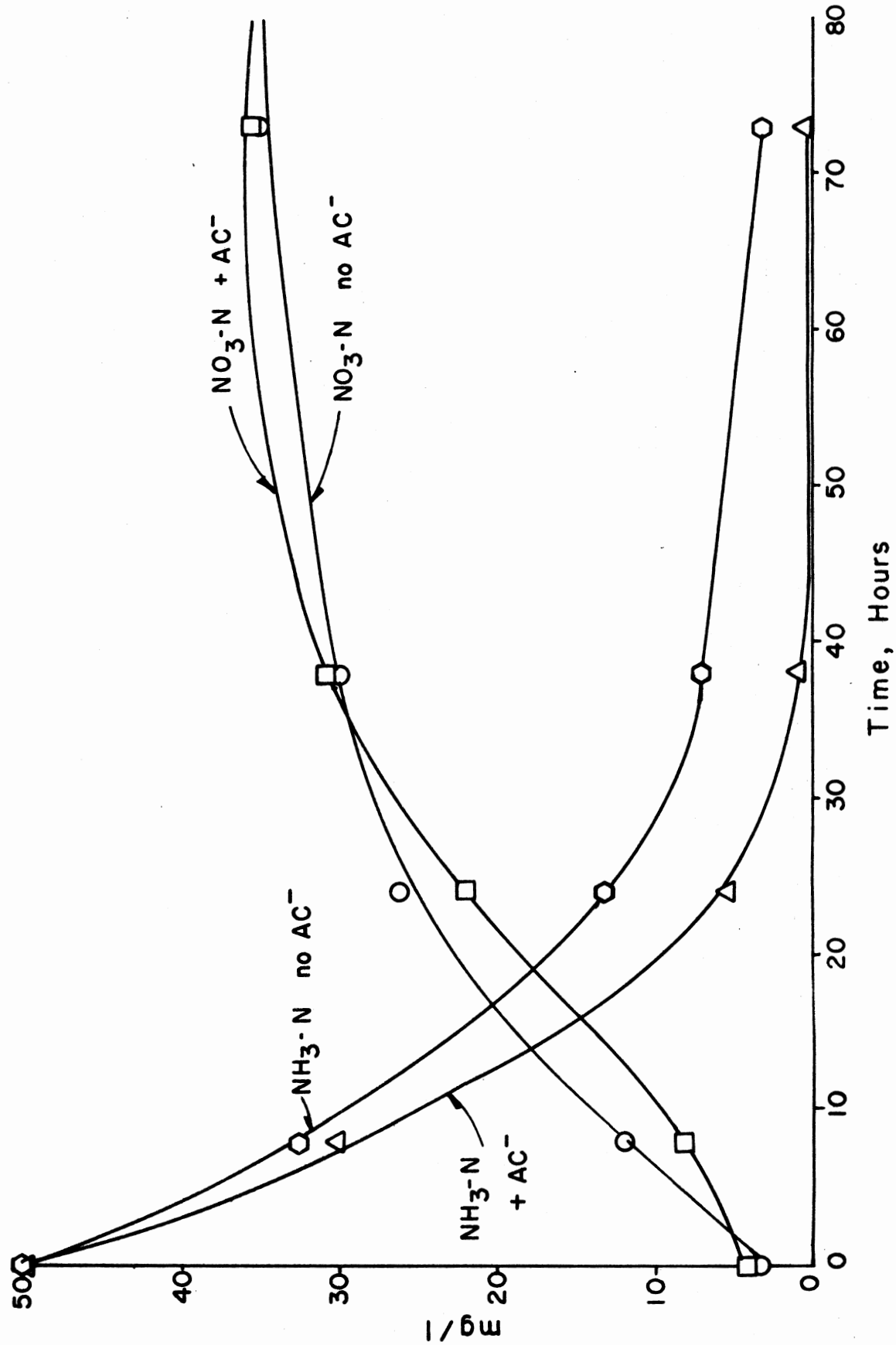

NO_3-N + AC^-
NO_3-N no AC^-
NH_3-N no AC^-
NH_3-N + AC^-
mg/l
Time, Hours
0
10
20
30
40
50
60
70
80

Figure 28. Effect of 150 mg/l of Acetate Ion on Biological Nitrification in Batch Systems


NO_3-N + AC^-
NO_3-N no AC^-
NH_3-N no AC^-
NH_3-N + AC^-
mg/l
Time, Hours
0
10
20
30
40
50
60
70
80

CHAPTER V

DISCUSSION

Inhibition of biological nitrification in a one-stage nitrifying activated sludge process can occur by interference either with the general metabolism of the nitrifying microorganisms or with the primary oxidation reaction. Inhibition of the general metabolism of the nitrifying microorganisms would not necessarily be detected in short-term experiments, while inhibitors of the primary oxidation reactions would. Consequently, both long-term and short-term experiments were conducted to investigate inhibition of the nitrification process. It is widely believed that organic matter and/or heterotrophic microorganisms in some way inhibit the nitrifying microorganisms. The specific nature of this inhibition has never been defined, but many environmental engineers and investigators in the field apparently accept the existemce of some inhibition pattern by organic compounds and or heterotrophic microorganisms as evidenced by the way in which nitrification is discussed in the literature. A majority of investigators suggest low organic loadings to the system in order for the nitrification process to occur in the system. At low organic loadings, a relatively inactive heterotrophic population will predominate, which will stimulate nitrification. Often it is assumed that high BOD concentration and the associated heterotrophic activity will limit the activity of the nitrifying microorganisms. On a metabolic level, one of several

possibilities comes into perspective; namely, a competition concept, inhibition, or lack of some essential nutrient. Assuming that inorganic salts are present in excess, the two constituents which the two types of bacteria actually compete for are (1) dissolved oxygen, and (2) ammonia. If ammonia is present in excess of the nutritional requirements of the heterotrophic microorganisms, then if a sufficient supply of dissolved oxygen is present, competition will not occur. Inhibition could be due to either the original organic substrate present or some metabolic by product released by the heterotrophic microorganisms. An additional effect may be removal of some essential nutrient by the heterotrophic bacteria which will limit activity of nitrifying bacteria which can probably be categorized as competition. In this study, experiments were designed so the ammonia nitrogen or dissoved oxygen concentration would not be limiting to growth activity of nitrify microorganisms.

It has been shown that glucose or organic matter does not inhibit the nitrification process in the system with a glucose concentration of about 800 mg/l (61)(62). Subsequently, a series of experiments were designed to study the effect of different aspects of heterotrophic metabolism upon the biological nitrification process in a one-stage nitrifying activated sludge system. In the first series of experiments, the long-term effect of heterotrophic metabolism upon the nitrification process was studied. In the first experiment, a continuous flow activated sludge system operating at a 10-day mean cell residence time with complete nitrification of all available inorganic nitrogen was subjected to a step quantitative shock load of combined organic carbon source and nitrogen source of two-fold magnitude. As a result of an

increase in carbon source, the system must adjust by increasing the biological solids in the reactor since the reactor microorganism concentration, X, should increase with the increasing influent substrate concentration according to the following equation:

$$X = \frac{\Theta_c}{\Theta} \cdot \frac{Y\left(S_o - S\right)}{1 + K_d\Theta_c} \tag{22}$$

where

S_o = influent substrate concentration, mg/l

S = effluent substrate concentration, mg/l

Θ = reactor hydraulic detention time = $\frac{V}{F}$

Y = yield constant

K_d = maintenance energy or decay coefficient, day^{-1}

Since in a recycle system the effluent substrate concentration will remain relatively constant for all practical purposes and also the growth in the system was kept constant by keeping a constant mean cell residence time by controlling the quantity of wasted sludge, and since all other variables in the above expression are constant except the influent substrate concentration, the reactor biological solids concentration, X, must increase with increasing influent substrate concentration, S. In order to predict the reactor biological solids concentration, one must obtain the yield constant and also the maintenance energy or decay coefficient. It can be shown that the growth rate is a function of the yield coefficient, Y, specific substrate utilization rate, U, and decay coefficient, K_d, according to the following equation:

$$U_n = \frac{1}{\Theta_c} = Y_t U - K_d \tag{23}$$

A plot of $\frac{1}{\Theta_c}$, or observed net growth rate, versus specific substrate concentration utilization rate will yield both Y and K_d: Y is the slope of the line and K_d is the $\frac{1}{\Theta_c}$ axis intercept. These constants can be used in equation (22) to predict the biological solids concentration at a particular Θ_c and a particular influent substrate concentration. Constants Y_t and K_d can also be determined by plotting a reciprocal of the observed yield, $\frac{1}{Y_o}$, versus a reciprocal of the observed net specific growth rate, $\frac{1}{\mu_{o_n}}$. Since

$$\frac{1}{Y_o} = \frac{1}{Y_t} + \frac{K_d}{Y_t} \cdot \frac{1}{\mu_n} \tag{24}$$

Equation (23) was used by Schultz, et al. (112), and equation (24) by Merr, et al. (113). Y_o and Y_t are observed and true yield value, U, is the specific substrate utilization rate, day^{-1}. $U_n = \frac{1}{\Theta_c}$ was kept constant during these studies at desired values of S and 10 days by controlling the quantity of wasted sludge.

$$U_n = \frac{1}{\Theta_c} = \frac{X_w}{VX} \tag{25}$$

where

$$X_w = F_w X_w + X_e \left(F - F_w\right) \tag{26}$$

X_w, F_w, F, and X_e are average values for each parameter during the steady state. Observed yield, Y_o, was calculated by the following equation:

$$Y_o = \frac{X_w}{F(S_i - S_e)} \tag{27}$$

Figures 29 and 30 show the maintenance plots as per equations (23) and (24). Since the system operated only at two different constant specific growth rates and special effort was made to keep Θ_c, mean cell residence time, as nearly as possible to the predicted experimental values of 5 and 10 days, subsequently only two points are shown on the graphs but these points are average values for more than ten steady state conditions. The "true yield" value from these plots is determined to be 0.35, and K_d to be 0.028 day^{-1}. The yield of 0.35 calculated from the continuous flow system is very much different than the yield determined from batch experiments. The mean value for the true yield determined from batch studies is 0.53, with a range of 0.46 to 0.67. Table XXII summarizes the values of different biological kinetic constants obtained from batch data. The first column is a summary of mean cell residence time at which the continuous flow unit was operated at steady state before the cells were harvested for the batch study. The second column summarizes the glucose and ammonia nitrogen ratio used for influent for the continuous flow unit. The third, fourth, and fifth columns in Table XXII summarize the values of maximum specific growth rate, $hours^{-1}$, saturation constant, K_s, and yield constant, Y_{t_B}, respectively. It is interesting to point out that values of the saturation constant, K_s, for various steady states vary within a very large range--from a low value of 23 mg/l for cells harvested from the system operating condition summarized on line one of Table XXII, at 5-day Θ_c, with influent glucose concentration of 1000 mg/l and ammonia nitrogen

Figure 29. Plot of Maintenance Energy to Determine "True Yield," Y_t, and Maintenance Coefficient, K_d, Utilizing Equation (23)

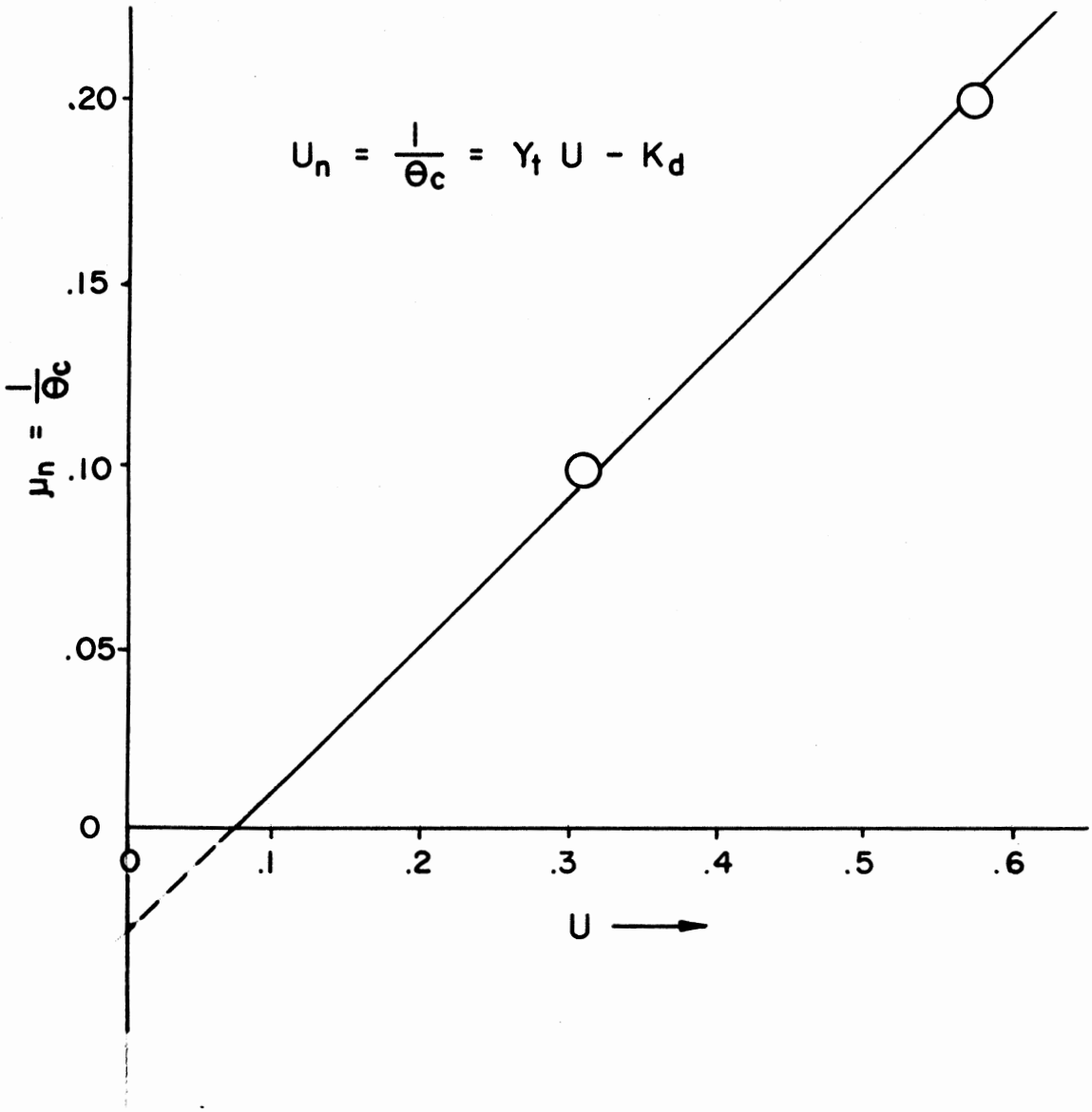
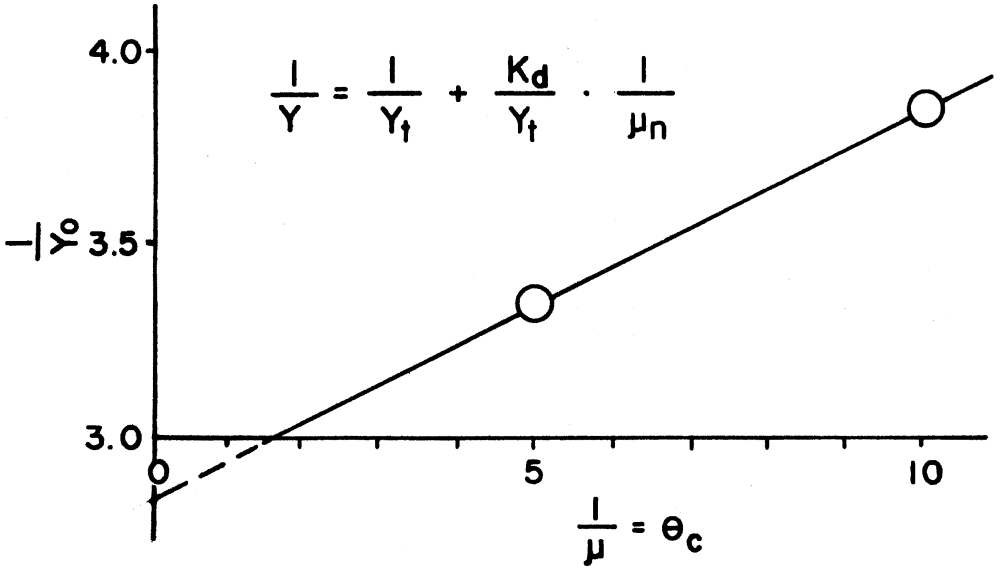

$U_n = \frac{1}{\theta_c} = Y_t\, U - K_d$
$\mu_n = \frac{1}{\theta_c}$
.20
.15
.10
.05
0
0
.1
.2
.3
.4
.5
.6
U

Figure 30. Plot of Maintenance Energy to Determine "True Yield," Y_t, and Maintenance Coefficient, K_d, Utilizing Equation (24)


4.0
3.5
3.0
0
5
10
$\frac{1}{Y} = \frac{1}{Y_t} + \frac{K_d}{Y_t} \cdot \frac{1}{\mu_n}$
$-\frac{1}{Y}$
$\frac{1}{\mu} = \theta_c$

concentration of 100 mg/l. At this particular operating condition, a very small quantity of nitrate was being produced in the continuous flow unit, indicating a small population of nitrifiers in the system with a maximum nitrate-nitrogen production of 14 percent of total available ammonia nitrogen after synthesis.

TABLE XXII

SUMMARY OF BATCH EXPERIMENT RESULTS

Line	Θ_c (day)	$\frac{\text{Glucose}}{NH_3}$	μ_{max} H_2-1	K_s (mg/l)	Y_{t_B}
1	5	$\frac{1000}{100}$	0.16	23.4	0.51
2	5	$\frac{500}{50}$	0.61	635.6	0.55
3	10	$\frac{500}{100}$	0.23	216.2	0.50
4	10	$\frac{500}{50}$	0.69	1040.6	0.53
5	10	$\frac{1000}{100}$	9.28	2053.5	.5
6	10	$\frac{1500}{150}$	0.43	340.6	0.46
7	10	$\frac{2000}{200}$	1.01	595.9	0.51
8	10	$\frac{1000}{50}$	0.28	149.2	0.67
Avg.	-	-	0.59	632	.53

The largest saturation constant, K_s value determined from the

batch experiment was 2054 mg/l for operating conditions summarized in line 5 of Table XXII with a mean cell residence time of 10 days, influent glucose concentration of 1000 mg/l, and influent ammonia nitrogen of 100 mg/l operating at steady state with complete nitrification of all available ammonia nitrogen after synthesis, although K_s values are not within the reported values for organisms of sewage origin as suggested by Gaudy, et al. (85), which is reported to be within 125 to 250. However, these values are well within the range of values reported in the literature. Lower values of K_s have been reported by Monod (8) and some low values within our low range have been reported by Gaudy, et al. (85). High values for K_s have been reported by Schaefer (114). The saturation constant, K_s, governs the shape or sharpness of the curvature of the plot of specific growth rate, μ, versus initial substrate concentration, S_o, for batch growth experiments. The higher the value of K_s, the flatter the curve and the more slowly μ becomes asymptotic to the maximum specific growth value as substrate concentration is increased in the batch growth experiments. Conversely, the lower the value of K_s, the sharper the curve, the faster becomes asymptotic to μ_{max} as substrate concentration is increased in the batch experiments. In this case, μ is very sensitive to small changes in substrate concentration at lower range (85)(86). The average value for K_s for all eight separate batch experiments employing cells harvested from the continuous flow nitrifying activated sludge system at various operating conditions was 632 mg/l. Although no apparent specific relationship exists between nitrification and the values of K_s determined for cells harvested at steady state at various operational conditions in a nitrifying activated sludge continuous flow system, it

is the author's opinion that the preponderance of experimental data indicates that nitrification will affect biological kinetic constants of heterogeneous microorganisms determined by batch experimentation using cells harvested from continuous flow nitrifying activated sludge systems.

Sufficient data were not collected to determine K_s and μ_{max} from continuous flow data, but the limited data suggest a low value of about 50 mg/l for K_s determined from the continuous flow data. More data are necessary to determine the effect of nitrifiers on biological kinetic constants of the system. Maximum specific growth rates for the above batch experiments varied from 0.16 to .28 hr^{-1} for cells harvested from the nitrifying activated sludge system at conditions summarized in lines 1 and 6 of Table XXII, respectively. The continuous flow system was operating with influent equivalent glucose concentration of 1000 mg/l for both systems, and equivalent influent ammonia concentration of 100 mg/l for both systems operating at a constant mean cell residence time of 5 and 10 days, respectively. The mean maximum growth rate constant for all eight separate batch experiments was 0.53 hr^{-1}. Continuous flow data available are not sufficient to predict maximum specific growth rate for the system; however, the available data suggest a maximum specific growth rate of 0.15 hr^{-1}.

Continuous Flow Studies on Inhibition

Quantitative shock loads of combined glucose and ammonia and glucose alone, both step and impulse, affected the nitrification process. Figure 2 indicates as a result of the increase in influent glucose concentration from 500 mg/l to 1000 mg/l, the biological solids

increased rapidly and as a result of this rapid increase, the nitrification process was inhibited although temporarily, but indeed the rapid heterotrophic growth brought about by increased substrate concentration did affect the nitrification rate, in the one-state nitrifying activated sludge system. Nitrate production was reduced significantly, indicating inhibition of nitrifiers. As soon as the system established a steady state with respect to biological solids concentration, the inhibition phenomenon was removed and full nitrification was established within the next few days. It is apparent that the system possessed the capability of accepting a shock load concentration of twice the previous influent concentration so far as system response to COD removal is concerned. However, the system's nitrification capability was suppressed and inhibition temporarily--probably by some metabolic intermediate which is apparently produced to only a significant extent during the rapid growth phase of the shock loading. From the experimental results it is obvious that if this is the case, the concentration of inhibitory compound is very low, since the system effluent COD did not increase significantly during the transient period, as one would expect the inhibitory metabolic intermediate to be an organic compound. Consequently, if this were the case, it would have exerted a chemical oxygen demand which would have been detected in the effluent COD. Since no significant change in the effluent COD was observed, one could conclude that the inhibitory compound concentration was less than a few mg/l. Another possibility would be that the inhibitory products are used up by some of the heterotrophic bacteria, but not before they exert their inhibitory effect upon nitrifiers. This was also observed by Krishnan (115) where he reported that in

systems with recycle, when they were quantitatively shock loaded, intermediates were not observed, where systems without recycle exhibited the release of metabolic intermediates and/or end products. He further reported that the release of metabolic intermediates was a function of the glucose concentration in the influent and the detention time of the system. The higher the glucose concentration and the shorter the detention time, the greater the production of intermediates for the system without cell recycle. Intermediates were probably formed in both systems with and without recycle. However, in the recycle system, intermediates were metabolized by some microorganisms; consequently, they were never detected.

Figures 6 through 9 represent shock loads of 3- and 4-fold combined glucose and ammonia to the system. Again, successful biochemical responses were observed with respect to COD removal. However, a similar pattern of nitrification inhibition was observed during the transient period for both of these shocks. Rapid heterotrophic growth similar to the previous shock load caused a temporary suppression of the nitrification process--although this temporary suppression was much longer in duration than the previous shock load--nevertheless, the suppression was temporary until the system attained a new steady state with respect to biological solids concentration.

The responses to the gradual (step) shock loading can be predicted and modeled based upon the principles of continuous culture operation of a completely mixed continuous flow system with recycle. In such systems, the rate of growth of microorganisms population is determined and is a function of the dilution rate. In the steady state, biological solids concentration is a function of substrate concentration and

the cell yield for the particular substrate and biological species present. When the system is operated at a growth rate which is below maximum specific growth rate, the observed net growth rate in a once-through system is

$$\mu_N = D \tag{28}$$

and for a recycle system

$$\mu_N = (1 + \alpha - \alpha c)D \tag{29}$$

In these experiments, the growth rate was much below maximum specific growth rate and was controlled by the amount of wasted sludge. The system was operated under steady state condition for several days before steady state was disrupted by increasing the supply of influent substrate (glucose and ammonia) or just the glucose. The system responded quite readily by a rapid increase in growth rate. Under increased loading conditions, the system may act as a batch-operated system with respect to substrate removal and increase in biological solids concentration rate, but after the transient period, the biological solids in the system leveled off and once again glucose became the limiting nutrient. Steady state was established, and growth rate was controlled by equation (29).

In the transient state, the growth response to the gradual step shock load is controlled primarily by the rate of increase of incoming substrate concentration in the reactor. If the rate of increase of substrate concentration minus the amount of substrate oxidized is greater than or equal to the rate of biological solids production, the

system will respond successfully to the shock with respect to COD removal. However, if the rate of increase in substrate concentration exceeds the rate of biological solids production taking into account the amount of substrate oxidized, then the substrate will be lost in the effluent until the biological solids concentration is increased to a level at which the substrate removal rate would equate to the rate of substrate inflow.

Step Decrease Quantitative Shock Loads

Neither the nitrification process nor the COD removal efficiency of the system was affected significantly by 2-, 3-, or 4-fold combined glucose and ammonia nitrogen concentration step decrease quantitative shock loads (see Figures 6, 9, and 12). Biological solids decreased gradually until a new steady state with respect to biological solids was attained, since a fixed relatively small volume of reactor mixed liquor was wasted daily (to keep constant mean cell residence time). This was the reason that a new steady state with respect to biological solids concentration was established after a relatively extended transition period. Inhibition based on the rapid biological growth was observed in the 4-fold combined step decrease quantitative shock load where the biological solids concentration decreased from a steady state value of approximately 13,000 mg/l gradually in a period of about one month to a low level of approximately 2400 mg/l. After this period, biological solids concentration started to increase rapidly, causing inhibition of the nitrification process (see Figure 12). Once again it is clearly observed that rapid growth of heterotrophic microorganisms is concurrent with inhibition of the nitrification process since we did

not observe any inhibition during the decreasing phase of biological solids even though the biological solids level decreased below the original level at the previous steady state. However, as soon as rapid biological growth was initiated, the nitrification process was inhibited.

Two-fold Ammonia Nitrogen Step Quantitative Increase Shock Load

The two-fold ammonia nitrogen step quantitative increase shock load did not disturb the system's COD purification efficienty (Figure 16). However, ammonia nitrogen did leak out of the system because of slow growth of the nitrifying organism. The system reached a steady state with respect to nitrification; however, some ammonia was always left in the effluent. The two-fold quantitative step shock load with ammonia nitrogen did not cause any deleterious effect upon the nitrification process, although ammonia nitrogen was leaked out in the effluent because of increased ammonia nitrogen in the influent, and the system response was very slow to accommodate the additional ammonia nitrogen in the influent because of the slow growth rate of nitrifying organisms. However, even approximately one month after administration of the shock load, although the system seemed to be in steady state, about 18 mg/l of ammonia nitrogen was left in the effluent. This could have been due to slow growth of the nitrifiers or lack of sufficient supply of CO_2 in the system, since CO_2 production was maintained at the original level by not altering the influent glucose concentration. However, almost doubling the nitrate production subsequently required increasing the quantity of CO_2. Another possible explanation of this

experiment (see Figure 16) would be that the rate of incoming ammonia nitrogen is greater than the maximum specific growth rate of nitrifying organisms.

Two-fold Quantitative Step Shock Load With Glucose

In this quantitative step shock load, ammonia nitrogen concentration was kept constant while influent glucose concentration was doubled. Doubling the glucose concentration caused suppression of the nitrification process (see Figure 13). This inhibition pattern because of simultaneous rapid heterotrophic biological growth, was very similar to the inhibition caused in the first experiment (Figure 2) where both influent glucose and ammonia nitrogen concentration were doubled. However, in this experiment, ammonia nitrogen concentration in the transition period was zero for an extended period of time (approximately five days). This is, of course, due to increased ammonia nitrogen requirement for heterotrophic growth. However, after steady state with respect to biological solids was reached, ammonia nitrogen leaked out in the effluent for a rather long period. This observation may be explained in the following manner: Since it has already been shown that rapid heterotrophic growth inhibits nitrifiers (Figures 2, 7, 10), and since increased glucose concentration causes rapid growth, the nitrifiers are inhibited. Since during this period influent ammonia nitrogen concentration is not sufficient to supply both heterotrophic and nitrifying microorganisms, all ammonia nitrogen is used by heterotrops during the initial stages of the transient period. However, once a new steady state with respect to biological solids is established,

there will be some ammonia nitrogen available for utilization by the nitrifying organisms. However, since nitrifiers were inhibited severely, both by heterotrophic growth and by the unavailability of ammonia nitrogen for a period of approximately five days, the nitrifying population was reduced to such an extent that it caused leakage of ammonia nitrogen in the system. It required another three weeks for the system to reach its original steady state concentration of nitrifying organisms even after steady state with respect to biological solids concentration was established. This step increase in influent glucose concentration did not deteriorate the quality of the influent with respect to COD removal capability.

Combined Quantitative Impulse Shock Loads of Glucose and Ammonia

The response of the system with respect to nitrification to this type of shock load was similar to step shock loads of a similar nature. A slug dose of glucose caused rapid biological growth (Figures 24 and 25) and, subsequently, inhibition of the nitrification process. However, since the glucose concentration was diluted out in a relatively short period, the effect of inhibition is not as distinct as the step quantitative shock loads. However, the inhibition is definitely apparent since ammonia nitrogen concentration in both cases is higher than those predicted by theoretical dilute-out. This observation can be explained in the following manner: Since the nitrification process is inhibited by the rapid heterotrophic growth, the ammonia nitrogen in the system influent is leaked out since it is not being nitrified and the ammonia nitrogen leakage together with ammonia nitrogen left

in the reactor from the impulse dosage will cause the total ammonia nitrogen in the system to be greater than the concentration predicted by the theoretical dilute-out curve. This increase in the system's ammonia nitrogen concentration is coincided by a small temporary decrease in the system's nitrate nitrogen concentration--again an occurrence of the inhibition phenomenon. The system response with respect to COD removal capacity was good. This result is in agreement with those predicted by Krishnan (114) from his shock loading studies at lower biological solids concentrations and lower solids recycle ratios and, consequently, lower mean cell residence time.

Quantitative Impulse Shock Loads
by Ammonia Nitrogen

This type of shock load did not interfere with the system biochemical efficiency with respect to COD removal ability. Nitrate nitrogen in the system increased almost immediately after administration of ammonia nitrogen concentration impulse in all cases, with the system nitrate nitrogen concentration following a straight line relationship with respect to time with very similar slopes in all three shock loads (Figures 21, 22, 23). There was no lag phase observed with respect to nitrate nitrogen production. The result of ammonia impulse forcings shows that no inhibition of nitrification occurred for ammonia nitrogen concentrations up to approximately 260 mg/l. Engel and Alexander (16) reported that ammonia nitrogen concentrations as high as 640 mg/l did affect the ammonia oxidizing ability of Nitrosomonas. The results of the ammonia impulse shock loads (Figures 21, 22, 23) clearly indicate no lag in the growth rate for the ammonia oxidizers. This has also

been confirmed by other investigators (38)(36)(52)(67)(82). Also, since substrate storage can cause lags, it is interesting to note that presently there are no significant nitrogen storage products known for bacterial systems. The utilization of inorganic nitrogen by the nitrifying bacteria was then assumed to represent a concomitant increase in the growth rate and that no lag existed. This result also supports further evidence that nitrification is zero order.

Engineering Significance

Since treatment plants are operated under time-varying loading conditions, a steady state with biological solids is unlikely. Consequently, such a system cannot be expected to produce a highly nitrified effluent reliably, since periodic quantitative shock loads of impulse and step are being applied to the system continuously. Although these shocks may be of short duration, in light of information provided within, the nitrification process will be suppressed by this shock load. One may argue that the inhibition of nitrification caused by these shock loads is temporary and the system will recover after the shock; however, by the time the system recovers from one shock, chances are that it is being subjected to or already has been subjected to another shock load of one type or another. This further suppresses the treatment process. A possible remedial measure for this type of inhibition would be an equalization basin ahead of the activated sludge aeration unit to buffer the effect of these types of shock load by providing an effluent of more or less uniform quality with consisten composition to the activated sludge system.

An alternative measure would be a two-stage activated sludge

system which has its own problems (109), higher quantities of sludge, more clarification requirements. For a two-stage activated sludge system, the final clarifying capacity is almost twice that of a single stage activated sludge process since each stage of the two-stage process must have its own integral clarifier and sludge return system. The higher equipment (aeration and clarification) cost and higher sludge disposal cost in case of operation of a carbonaceous unit at lower mean cell residence time make the two-stage nitrification activated sludge system economically less feasible and consequently less appealing. Other alternatives would be fixed bed reactor systems--namely, biological towers and rotating biological contactors. Both biological towers and multi-stage rotating biological contactors can provide high degrees of nitrification even with high concentrations of ammonia nitrogen concentration (111). The required equalization basin for a single stage nitrification activated sludge system is not required for biological towers or rotating biological contactors since in case of biological towers, the first few feet of the tower, and in case of rotating biological contactors, the first few stages (1, or 1,2) will act as a buffer zone for reducing the impact of organic surges to the system. Thus, rapid growth rates and increases of heterotrophic microorganisms will be initiated before it reaches the nitrification organisms which will predominate in the lower section of the biological towers or downstream stages of rotating biological contactors (usually after the first stage). This buffer zone may also act as a filter for a wastewater containing materials which may be toxic to the nitrifying population, such as known organic inhibitors which may be biodegraded in initial stages, or heavy metals which may

be adsorbed by the sludge in the initial stages of both biological towers and rotating biological contactors. Consequently, it is this author's opinion that in light of the new findings of this study, the fixed bed reactor would provide more reliable nitrification than the fluidized bed reactors. However, when this type of operation is not practical, a two-stage nitrifying activated sludge system should be considered. One-stage activated sludge systems may be used to achieve nitrification as a last resort, provided an equalization basin is designed ahead of the activated sludge unit.

Inhibition and Rapid Heterotrophic Growth

It is apparent in all experiments that every time the system is inhibited, the inhibition pattern is accompanied by rapid heterotrophic growth. In all glucose or combined glucose and ammonia shock loads, nitrification was inhibited to varying degrees, the severity of the response depended upon the severity and duration of the shock due to the increase in organic carbon source loading which in turn caused rapid heterotrophic growth. In no case in these experiments was nitrification inhibited because of a decrease in organic carbon source or increase in concentration of ammonia titrogen source. The reason is obviously that a decrease in organic carbon source or an increase in ammonia nitrogen does not cause rapid heterotrophic biological growth. Although the exact mechanism of this inhibition was not determined, it is clearly shown that nitrification is inhibited during the rapid growth of heterotrophic microorganisms. This inhibition is temporary in nature and is removed once the rapid heterotrophic biological growth is curtailed and steady state conditions with respect to biological

solids concentration is reached. This finding is not in agreement with that of Hockenbury, et al. (115) which indicates that inhibition is due to organic matter.

CHAPTER VI

CONCLUSIONS

1. Rapid heterotrophic growth in one-stage nitrifying activated sluge systems will inhibit the nitrification process. However, inhibition is temporary and will last only so long as rapid heterotrophic growth is taking place. Inhibition is relieved when rapid heterotrophic growth ceases.

2. Unsteady conditions will inhibit nitrification, but nitrification is restored as soon as a steady state with respect to biological solids concentration is established.

3. Quantitative step and impulse shock loads of organic carbon source will inhibit nitrification. The degree of inhibition is dependent upon the concentration and duration of the shock load. However, in all cases, inhibition is temporary and nitrification is restored when the system recovers from the consequences of the shock load. Again, inhibition is occurring simultaneously with rapid heterotrophic growth.

4. Quantitative step and impulse shock loads of ammonia nitrogen source will not affect biological nitrification, since no rapid growth of heterotrophic microorganisms results from ammonia nitrogen alone.

5. This study supports the zero-order reaction rate equation for the biological nitrification process.

6. Biological kinetic constants determined from batch studies employing cells from the continuous flow nitrifying activated sludge

system are different than those reported for cells from continuous flow non-nitrifying systems. Consequently, this would lead one to believe that biological nitrification will affect the biological kinetic constants, μ_{max}, K_s, Y_{t_B}, and K_d.

7. The depression of the nitrification rate when the organic loading is high is not caused entirely by the deficit of dissolved oxygen as suggested by some investigators, since the oxygen level during the transient state did not drop below 3 mg/l at any time, but nitrification was inhibited.

CHAPTER VII

SUGGESTIONS FOR FUTURE STUDY

Based on the findings of this study, the following suggested experiments are pertinent for future investigation involving the nitrification process.

1. Study the effect of step and impulse quantitative shock loads for biological towers.

2. Study the effect of step and impulse quantitative shock loads similar to the one in the study for rotating biological contactors.

3. After completion of the first two studies, make a comparison of the different systems to find the most feasible process economically and operationally.

4. Determine the effect of qualitative shock loads to the nitrifying system and the different systems, and compare.

5. Study the effect of quantitative shock loads similar to those in this study in two-stage activated sludge systems.

6. Develop micro-analytical techniques to identify and quantify the cause of inhibition of nitrification during the rapid growth of heterotrophic bacteria.

7. Determine and compare predominant types of microorganisms at different stages in biological towers.

8. Investigate various strategies for improving the average level of nitrification in treatment plants. These might include the use of

equalization basins and timing of the addition of digester supernatant to the nitrifying system.

9. Study effects of nitrification on different biological kinetic constants.

10. Study effects of other types of shock loads upon the nitrification process utilizing different systems, and compare.

11. Study the biological nitrification quantitative shock loads in a system with automatic pH control.

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