# STUDIES ON THE PERFORMANCE OF BIOLOGICAL NITRIFICATION PROCESSES FOR THE REMOVAL OF NITROGENOUS OXYGEN DEMAND FROM WASTEWATERS

By

ENOS LOY STOVER

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1971

Master of Science
Oklahoma State University
Stillwater, Oklahoma
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Thesis Approved:

Don F Kincannon
Thesis Adviser

Dean of the Graduate College

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# CHAPTER I

## INTRODUCTION

All biological growth processes require some form of nitrogen for the synthesis of cellular proteins and nucleic acids. Microorganisms can utilize a range of nitrogen compounds for the synthesis of proteins and nucleic acids under various conditions. In some cases the oxidation state of the nitrogen is changed, while in others it remains the same. Transformations of nitrogen as a source of electron acceptors in anaerobic respiration or the oxidation of nitrogen-containing compounds as a source of energy for synthetic reactions also occur.

All of these transformations occur in oceans, lakes, rivers, streams, estuaries, and sewage treatment processes, where the effects may have extremely important bearing on the condition of the water or the treatment plant performance. Nitrogen exists in wastewaters in the principal forms of ammonia and nitrates. Municipal wastewaters usually have nitrogen contents in the 15-25 mg/l range in untreated and primary settled wastes (1).

It becomes necessary to distinguish between the oxygen uptake due to aerobic organotrophic metabolism of the carbon sources in the wastewater (carbonaceous biochemical oxygen demand) and the oxygen uptake caused by the aerobic autotrophic organisms (2). The aerobic autotrophic organisms concerned here are the nitrifying bacteria, Nitrosomonas and Nitrobacter. These nitrifying bacteria can cause an oxygen uptake which

is sometimes termed the "second-stage uptake" or the nitrogenous biochemical oxygen demand.

Biological nitrification is performed by the two general groups of bacteria mentioned above--Nitrosomonas and Nitrobacter, which oxidize ammonia-nitrogen ($NH_3$-N) to nitrite-nitrogen ($NO_2$-N), and nitrite-nitrogen to nitrate-nitrogen ($NO_3$-N), respectively, as their energy sources. The respective oxidations are carried out as shown in the reactions below:

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

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

Some of the chemical energy released upon this progressive removal of electrons is trapped for biological use. The energy is stored or used in the form of adenosine triphosphate (ATP). This high energy compound can then be used in the reductive or synthetic reactions of the cell. Nitrosomonas and Nitrobacter require an oxygen enriched environment and, in addition, as autotrophs, they require inorganic carbon in the form of carbon dioxide or bicarbonate as their carbon source. The ammonia or nitrite serve as electron donors in the reductive fixation of the inorganic carbon.

Biological nitrification is a vital part of the natural nitrogen cycle. The most common forms of nitrogen found in raw wastewaters are organic nitrogen and ammonia. Complex organic compounds are broken down, by bacteria, into substances that are chemically of simple structure. Organic nitrogen, such as is found in simple amino acids and complex proteins, is converted to dissolved ammonia by the process of

deamination during bacterial metabolism of these organic materials. Ammonia is converted to cellular organic nitrogen by assimilation or amination reactions during bacterial synthesis. When bacteria and other microorganisms die they lyse, producing residual organic nitrogen.

A fraction of the total nitrogen is removed in biological wastewater treatment during carbonaceous removal through bacterial synthesis and correspondent wasting of excess sludge. Any ammonia above that required for bacterial synthesis removed in a biological growth process must be removed by biological nitrification. Nitrification will proceed if nitrifying organisms are present, and proper conditions are provided for the growth and maintenance of these organisms. If the nitrified wastewater is subjected to a proper bacterial population, denitrification will proceed. Denitrification is the process where nitrate and nitrite are subsequently reduced to free nitrogen ($N_2$) gas. The denitrifying bacteria are anaerobic heterotrophic bacteria which require an organic carbon source for synthesis.

Nitrification has been studied intensively by soil scientists for the past century. With the advent of biological wastewater treatment systems, engineers and chemists noticed that nitrification also occurred in some of their treatment plants. Before 1930, wastewater treatment plants were designed with the intended purpose of accomplishing a relatively high degree of nitrification, at least during the warmer summer months. With the development of the biochemical oxygen demand (BOD) test, it became apparent that high degrees of wastewater treatment in terms of BOD removal could be accomplished at marked savings in capital and operating costs by designing to avoid nitrification. Another argument against nitrification was the so-called "rising sludge" problem.

Some plants were plagued with denitrification, resulting in the "rising sludge" problem in the final clarifiers. Under anaerobic conditions in the clarifier, denitrification could occur, releasing nitrogen gas which rises, carrying or bouying sludge to the surface. The main objective in the United States from 1940 until the late 1960s came to be the design of treatment plants to minimize or avoid nitrification.

Even now, nearly fifty years after Streeter and Phelps identified the existence and magnitude of the significant second-stage oxygen demand, design proposals claim as high as "98 percent reduction in 5-day BOD" with inadequate or no consideration given to the second-stage oxygen demand (3). Sanitary engineers generally dismissed the matter of nitrogenous oxygen demand (NOD) of unnitrified effluents on the basis of three premises (4):

a. Nitrification is caused by special organisms, the population of which is minimal in surface waters.

b. The reaction constant for nitrogenous oxidation is small in relation to the constant for carbonaceous matter.

c. Oxidation of ammonia to nitrates simply converts dissolved oxygen to a form in which it is still available to prevent development of anaerobic conditions.

Nitrates and nitrites, as bound oxygen, may replace oxygen as electron acceptor only in special cases. The use of nitrates or nitrites as electron acceptors is called "anaerobic respiration," and can be accomplished by only a few specific microorganisms. The philosophy that unnitrified effluents are not damaging to receiving streams has been undermined by conservationists and biologists who point out that nitrates will not satisfy the oxygen requirement of fish and many other aquatic organisms (5). Oxidizable nitrogenous material in wastewater effluents, when discharged into a stream displaying a high degree

of nitrification, can place a significant demand on the oxygen resources of the stream. Nitrogen in the fixed forms of ammonia and nitrate is considered to be one of the major nutrients supporting blooms of green and non-nitrogen-fixing blue-green algae in surface waters.

Nitrified effluents may be obtained by two general process schemes (biologically). The first method is a single-step process in which both nitrification and carbonaceous removal occur in the same biological reactor. The second method involves a two-step system where the carbonaceous removal and nitrification process are separated. Each reactor can be operated independently of the other in order to obtain optimum growth conditions for the two different types of microorganisms (heterotrophs and autotrophs). The two reactors can be operated at different hydraulic detention times, different sludge retention times, and at different food-to-microorganism ratios. Of course, in both types of systems another stage or reactor would be required to accomplish denitrification.

Ammonia-nitrogen can also be removed from wastewaters by physical-chemical treatment processes. Ion exchange, chlorination-dechlorination, and ammonia stripping processes have all been successfully applied to wastewater treatment. At the present time, it is generally agreed that biological nitrification is, from the technical and economic viewpoints, the most feasible method of removing ammonia from wastewater.

The removal of nitrogenous oxygen demand and, in some cases, complete nitrogen removal from wastewater, is receiving increased attention in water pollution control. Some of the problems involved with nitrogen compounds are the facts that ammonia exerts a considerable

oxygen demand on the receiving water; it is toxic to fish; it exerts an extra chlorine demand and reduces disinfection effectiveness; high concentrations of nitrate can be a health hazard; and both ammonia and nitrate are major nutrients involved in the support of algal blooms and the growth of other aquatic plants.

The reduction of nitrate to nitrogen gas by biological denitrification transforms the nitrogen into a stable form that will not contaminate groundwater or contribute to eutrophication. The three major conditions that are the most difficult to meet in establishing a system for the denitrification of secondary effluent are as follows:

a. oxidation of ammonium ion to nitrate

b. maintenance of an anaerobic system following the oxidation to nitrate

c. provision of an adequate energy source in the anaerobic system for the denitrifying bacteria.

The process of denitrification was beyond the scope of the following research, which was conducted in order to investigate the biological nitrification process in wastewater treatment plants.

As noted above, a mandatory requirement for denitrification is the oxidation of ammonium ion to nitrate. Before a wastewater treatment plant can be designed and operated to accomplish denitrification, it must be designed and operated to accomplish nitrification. If nitrification is not followed by denitrification, nitrite and nitrate nitrogen can serve as nutrients for undesirable algal growth in receiving streams and lakes. However, nitrification eliminates the excessive oxygen demand exerted by ammonia in the receiving streams, nitrification helps eliminate the problems of ammonia toxicity to fish, and helps reduce the chlorine demand of the effluent wastewater.

Therefore, definite advantages are realized if nitrification is achieved in wastewater treatment plants even though a denitrification process is not incorporated into the treatment scheme.

The experimental research reported herein was undertaken in order to obtain operation and design information necessary for the successful functioning of wastewater treatment plants to accomplish nitrification. A synthetic wastewater containing both a carbonaceous and nitrogenous oxygen demand was used as the substrate. Investigations were carried out on both batch reactors and continuous flow systems. The continuous flow studies involved fixed bed reactors as well as fluidized bed reactors. Both one-stage and two-stage systems for the combined and separated carbonaceous and nitrogenous oxygen demand removal were investigated. Parameters measured in both the incoming feed to the reactors and the effluents from the reactors were ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and chemical oxygen demand (COD). Biological solids were also measured in the fluidized bed reactors and in the effluent from those reactors. By measuring the different forms of nitrogen in the influent and effluent, the degree to which nitrification had proceeded in the reactors was determined. Effects upon nitrification due to changing the mean cell residence time, the average time span that a microorganism is retained in the unit before being wasted from the system, in steady state fluidized reactors of both the one-stage and two-stage type were investigated. From these studies, necessary operating conditions and criteria can be established from curves of degree of nitrification versus mean cell residence time.

# CHAPTER II

# LITERATURE REVIEW

## A. Historical Review

Nitrification, like other oxidation processes, was long believed to be due simply to the action of atmospheric oxygen or, in some cases, ozone. Pasteur predicted the biological nature of nitrification and clearly expressed his conviction concerning the essential nature of nitrification in 1862 (6). In 1877, investigations by Storer in America, and Schloesing and Muntz in France, fulfilled Pasteur's prediction (7). Schloesing and Muntz showed that nitrification was not a common property of oxidizing organisms, and that it was favored by a moderate degree of alkalinity and small amounts of organic matter. One common type of microorganism in the form of an ovoid micrococcus was found in all of their nitrifying cultures. Warington began to study nitrification prior to 1878, and continued through 1890 (8). He secured many pure cultures, but never succeeded in isolating the true ammonia oxidizer.

Winogradsky , in 1888, was the first person to isolate an unmistakable nitrifying organism in pure culture. The first American investigators to report the isolation of nitrifying bacteria were Jordan and Richards (8). The main reason previous investigators had failed in their efforts to isolate a nitrifying organism was that they had placed

too much confidence in the ordinary gelatin culture-medium. Winogradsky used a simple nitrifiable solution with the following composition:

| | |
|---|---|
| ammonium sulfate | 1 gm |
| potassium phosphate | 1 gm |
| well water | 1000 cc |
| basic carbonate of magnesia in excess. | |

Much research has been carried out on the techniques and methods for pure culture isolation of nitrifiers since the first isolation by Winogradsky. As a result, many articles have been published on this subject, and a review of this literature is beyond the scope of this investigation.

## B. Types of Organisms

In Bergey's Manual of Determinative Bacteriology (9) are listed five genera of autotrophic bacteria capable of forming nitrite from ammonia. These are Nitrosomonas europaea and monocella, Nitrosococcus, Nitrosospira, Nitrosocystis, and Nitrosogloea, but the last three named are of doubtful validity (10). Imsenecki (11) observed an apparent symbiosis between a heterotrophic bacterium and Nitrosomonas. He suggested such a symbiotic culture to be the reason for the varied nitrifiers classified as separate genera. The possibilities of a symbiotic relationship existing between these bacteria have been discussed by Pandalai (12)(13) and Gunderson (14). Two genera of autotrophic bacteria capable of oxidizing nitrite to nitrate have been well established. These are Nitrobacter agile or winogradskyi and Nitrocystis. Questionable classifications of other genera and species have been described from time to time.

Hofman (15) analyzed the hydrolysates of whole cells of Nitrosomonas for amino acids and sugars. According to his study, Nitrosomonas contain normal amino acid constituents, but their carbohydrate composition is somewhat different from heterotrophs in that no glucose was detected. Loveless and Painter (16) isolated Nitrosomonas cells from activated sludge which were ellipitcal (1.25 μ x 1 μ) and were calculated to weigh about 0.4-0.5 pg ($10^{-12}$g).

## C. Growth Requirements

The growth of nitrifiers is very slow, and the yield of cells per unit of energy source oxidized is low compared to many heterotrophs. Nitrifiers in pure cultures are extremely susceptible to poisons, such as metal ions.

### 1. Nutritional Requirements

Calcium carbonate which is insoluble in water was included in early media to neutralize nitrous acid produced by the oxidation of ammonia-nitrogen. The organisms adhered to the particulate material and could not be readily separated. From these early experiments the erroneous idea developed that suspended particles were necessary. Goldberg and Gainey (17) showed that Nitrobacter did not require the presence of solid particles in the medium for growth, and Engel and Alexander (18) showed that this was also true for Nitrosomonas. Although nitrifiers do not require a suspension on which to grow, they will adhere to one if present.

Nutritional requirements of the autotrophic nitrifiers have not been established in detail. Besides ammonia or nitrite and carbon

dioxide, a minimum concentration of dissolved oxygen is an absolute requirement for growth. Calcium and copper are required by Nitrosomonas, and phosphate, magnesium, and iron are known to be required by both Nitrosomonas and Nitrobacter. Meiklejohn (19) determined the optimum iron concentration to be 6 mg/l for Nitrosomonas europaea and Nitrobacter winogradskyi. The optimum concentration of phosphate for highest growth rate and least lag was determined by Van Droogenbroeck and Laudelout (20) to be as high as 10 mM (310 mg/l as P) for both Nitrosomonas and Nitrobacter. Nicholas, et al. (21) indicated that copper was an essential trace metal involved in an oxidase system of Nitrosomonas. The media used invariably contain potassium and sulfur, but these elements have not been shown to be required. Molybdenum at $10^{-9}$M (0.1 µg/l) gave maximum response of about 11-fold increase in nitrite utilization and cell mass development as shown by Finstein and Delwiche (22). The concentration of sodium salts is another important factor in the growth of Nitrosomonas. Some isolates of Nitrosomonas from activated sludge were shown by Loveless and Painter (16) to be stimulated by the presence of sodium sulfate or sodium chloride.

There is little doubt that some organic compounds augment the growth rate of nitrifiers. Ruban claims that Nitrosomonas can deaminate various purines and oxidize the released ammonia, but this has not yet been confirmed (23). That some organic compounds might be involved in the growth of Nitrosomonas and Nitrobacter has been recently demonstrated by a number of researchers. Ida and Alexander (24) found that many compounds could permeate the cell wall, but no compound tested could replace carbon dioxide. Labeled carbon from sodium pyruvate-2-$C^{14}$ was found widely distributed in cell fractions by Clark and Schmidt (25).

Nitrobacter has been shown to grow with acetate in the absence of nitrite (26), and acetate has even been shown to stimulate the growth of Nitrobacter (27). Clark and Schmidt (28) have also shown that some amino acids give increased production of nitrite and protein by Nitrosomonas, while other amino acids are inhibitory.

2. Temperature

Temperature greatly affects the growth of nitrifiers as in most biological processes. Many researchers have reported relationships for growth rate versus temperature determined from their experimental work (29). Buswell, et al. (30) reported the optimum growth temperature for Nitrosomonas to be in the range of $30^{o}$ to $36^{o}C$. Laudelout and Van Tichelon (31) reported the optimum temperature for Nitrobacter to be at about $42^{o}C$. Wild, Sawyer, and McMahon (32) observed that the rate of nitrification increased through the range of $5^{o}$ to $30^{o}C$ in reasonable agreement with the van't Hoff-Arrhenius law.

3. pH

The pH optima for nitrifying organisms are usually on the alkaline side of neutrality and are not sharply defined. Most studies have been done on the effects of pH value on the oxidation of the substrates by pre-grown cells and not on the growth of the nitrifiers themselves. Hofman and Lees (33) found the optimum pH value to fall between 8.0 and 9.0 on a pre-grown culture of Nitrosomonas. Engel and Alexander (18) observed an even wider pH range (7.0-9.0) giving maximum activity. Another strain of Nitrosomonas isolated from activated sludge by Loveless and Painter (16) had an optimum pH value between 7.2 and 7.8.

Boon and Laudelout (34) found a pronounced optimum at pH 7.8 with 90 percent of maximum activity between pH values 7.8 and 8.6 for Nitrobacter. Wild, Sawyer, and McMahon (32) found pH 8.4 to be optimum for the rate of nitrification.

Errors could be introduced as a result of local "overshooting" of hydrogen-ion concentration when the pH value of the cultures were adjusted (33). Other sources of error might be due to the buffering of the media. If the media used were not buffered, acid produced during the oxidation would lower the pH value, and if buffered, the anion of some of the buffers used might have inhibitory activity. Some researchers believe that part of this discrepancy in pH optima can be attributed to the phenomenon of bacterial acclimation or population selection. They believe that although nitrifying organisms initially are preferential to a pH environment of 7.0 to 8.0, they will acclimate to lower pH environments and re-establish their maximum growth rate. However, Meiklejohn has shown that the pH value of the environment does not necessarily lead to strains which grow best at that pH value (10). She found that organisms isolated from soil of pH 5.0 grew better in the laboratory at pH 7.0 than at pH 5.0. Fred and Graul (35) noted as early as 1916, that acid soils do not possess strains of nitrifying bacteria especially resistant to soil acidity. They found that nitrification increased soil acidity, and that it became necessary to add a basic substance in order to keep the process going.

## D. Energy Considerations

The loss of free energy in the overall reaction carried out by Nitrosomonas as shown below has been reported by various researchers.

$$NH_4^+ + 1\frac{1}{2}O_2 \longrightarrow 2H^+ + H_2O + NO_2^-$$

A free energy loss of about 66 kcal has been reported by Baas-Becking and Parks (36), 76 kcal by Engel (10), and 84 kcal by Nicholas (10). A free energy loss of 17.5 kcal at physiological concentrations of the reactants has been reported by Lees and Nicholas (10) for Nitrobacter. The primary oxidation reaction used by Nitrobacter is shown below:

$$NO_2^- + \frac{1}{2}O_2 \longrightarrow NO_3^-$$

This reaction is almost certainly a one-stage process because of the size of the free energy loss and the generation of reducing power is equivalent to one pair of hydrogen atoms.

Values for an economic coefficient or cell yield coefficient, dry weight of cells produced per weight of nitrogen oxidized, have been reported for both Nitrosomonas and Nitrobacter. Values for Nitrosomonas vary from 0.04 to 0.29 (10)(29). Values for Nitrobacter have been reported from 0.019 to 0.084 (29)(34)(37)(38).

In the process of synthesis, part of the oxygen requirement is obtained from the carbon dioxide used as the carbon source. From the oxidation equations above, the ratio of oxygen used to nitrogen oxidized is calculated to be 3.43 by Nitrosomonas and 1.14 by Nitrobacter. Experimental values of 3.22 and 1.10, respectively, have been reported by Montgomery and Borne (29), and Wezernak and Gannon (39). These lower values consider the oxygen released during the fixation of the carbon dioxide.

## E. Specific Growth Rates and Saturation Constant

Under specified conditions, medium, temperature, pH, et cetera, the maximum specific growth rate, $\mu_{max}$, and saturation constant, $K_s$, are constants for any specific organism from one experiment to another. The maximum growth rate constants reported for Nitrosomonas and Nitrobacter vary over a wide range (10). Values of 0.02 to 2.2 $day^{-1}$ have been reported for Nitrosomonas, while values of 0.03 to 2.5 $day^{-1}$ have been reported for Nitrobacter. This variation could possibly be attributed to limitation of one or more essential nutrients, inhibition by contaminating metal ions, or to strain differences. Saturation constant values for both Nitrosomonas and Nitrobacter vary with temperature. Values in the range of 0.2 to 8.0 mg/l of ammonia-nitrogen for Nitrosomonas and 0.2 to 8.0 mg/l of nitrite-nitrogen for Nitrobacter have been reported. Most saturation constant values reported for both organisms are 2.0 mg/l or less. A value of 1.0 to 2.0 mg/l is commonly used for the saturation constant in theoretical calculations.

## F. Metabolic Intermediate Compounds

Based on the assumption that the energy released is transported by the normal type of high-energy phosphate bond systems and the generation of reducing power is equivalent to three pairs of hydrogen atoms, the conclusion that there should be three stages in the oxidation of ammonia to nitrite, with each stage involving two-electron changes, has been accepted. The first stage has been postulated as the formation of hydroxylamine as shown below:

$$2NH_4^+ + O_2 \longrightarrow 2NH_2OH + 2H^+$$

As early as 1914, Beesley (40) stated that hydroxylated ammonium radical intermediates were probably formed during the oxidation of the ammonium ion. Hofman and Lees (33) found hydroxylamine in trace amounts in cultures of Nitrosomonas, and they also found that hydroxylamine accumulated in these cultures when hydrazine, a selective inhibitor of hydroxylamine oxidation, was added. Engel and Alexander (18) showed that "aged" cells readily lost their ability to oxidize ammonia but not hydroxylamine. A copper-dependent enzyme system suggested by Lees (41), which oxidizes hydroxylamine to nitrite, has been isolated from Nitrosomonas by Nicholas and Jones (42). Rees (43) found that Nitrosomonas europaea derived its energy from the oxidation of ammonia and hydroxylamine, both of which were quantitatively oxidized to nitrite. In spite of this evidence a few researchers are still in doubt as to the role of hydroxylamine in ammonia oxidation (44).

The second oxidation product has been postulated variously as hyponitrite, nitroxyl, dihydroxyammonia, and nitrohydroxylamine. Corbet (45) claimed to have detected both hydroxylamine and hyponitrous acid formed as intermediate compounds during the early stages of nitrification as early as 1934. The steps involved in the oxidation of ammonia to nitrite have been envisioned as shown below:

$$NH_3 \longrightarrow NH_2OH \longrightarrow H_2N_2O_2 \longrightarrow HNO_2$$

Experimental support for this theory as far as hydroxylamine is concerned has been obtained (33)(43).

No intermediates in the oxidation of nitrite to nitrate by Nitrobacter have been observed. Lees and Simpson (37) using a Hartridge reversion spectroscope, indicated that a cytochrome with an absorption

maximum at 551 mμ in the reduced state was intimately concerned with nitrite oxidation. Aleem and Alexander (46) isolated an enzyme system that required both ferrous and ferric iron and that was inhibited by metal-binding agents. Campbell, et al. (47), using labeled carbon dioxide, showed that Nitrocystis oceanus incorporated the carbon dioxide primarily by the reductive pentose phosphate cycle.

## G. Inhibitors of Nitrification

Inhibition can occur by interference with either the general metabolism of the cell or with the primary oxidative reactions. Lees (48) has shown that thiourea, allylthiourea, 8-hydroxyquinoline, salicylaldoxime, and histidine are inhibitory to the primary oxidation reaction of Nitrosomonas. In fact, all chelating agents tested inhibited the formation of nitrite from ammonia. Quastel and Scholefield (49) thought that the inhibition was due to competition with ammonia; however, Lees could detect no competition between ammonia and allylthiourea. The inhibition of nitrite production by the thioureas, which have a peculiarly high affinity for copper, is far more intense than the inhibition by any other of a wide range of chelating agents. This is good evidence in support of the theory that the enzyme concerned in the primary ammonia oxidation by Nitrosomonas is a copper enzyme. Hydrazine also inhibits the oxidation of ammonia--perhaps indirectly--by the accumulation of hydroxylamine. Hydrazine has been found to inhibit selectively the oxidation of hydroxylamine. In the presence of hydrazine, hydroxylamine accumulated and was detected during ammonia oxidation by Hofman and Lees (33).

Lees and Quastel (50) studied the effect of chlorate administration

to nitrifying soils. They found that chlorate ($10^{-4}$-$10^{-5}$M) apparently inhibited the growth of Nitrobacter without affecting its ability to oxidize nitrite. Quastel and Scholefield (49) found at higher concentrations ($10^{-3}$M) chlorate also inhibited nitrite oxidation. Lees and Simpson (37)(51) studied the mechanism of nitrite oxidation in Nitrobacter. They found that chlorate did not inhibit nitrite oxidation directly, but was converted during the course of nitrite oxidation into some compound (possibly chlorite) that did inhibit nitrite oxidation. Cyanate was also found to inhibit nitrite oxidation, but unlike chlorate the inhibition was reversible by washing the cells with water. Nitrite oxidation proceeded at a constant but diminished rate in the presence of cyanate. It appears likely that there was a significant connection between the action of cyanate and of chlorate on the rate of nitrite oxidation on the one hand, and their respective apparent inhibitions of cytochrome reductase on the other. The toxicity of cyanate and chlorate diminished as the concentration of dissolved oxygen decreased.

Various heavy metals as well as some organic compounds will suppress or inhibit the growth of nitrifiers. Loveless and Painter (16) reported concentration values of heavy metals that are inhibitory or toxic to nitrifiers. Other potentially toxic materials include phenolic compounds, phenol, cresol, and halogenated solvents.

## H. Biological Nitrification Processes for Wastewater Treatment

Studies by Downing, Painter, and Knowles (52), Knowles, Downing, and Barrett (53), and Stratton and McCarty (54) have demonstrated the utility of the kinetic approach for describing the phenomenon of

microbial mediated nitrification. Their results indicate that a limiting value of biological solids retention time at 20°C is in the range of 1-2 days, while at 10°C the limiting value is in the range of 4-6 days. Prakasam and Loehr (55) found solids retention time values greater than two days sustained nitrification. However, a minimum sludge age of 3-4 days at approximately 20°C was found necessary for achieving nitrification by Balakrishnan and Eckenfelder (56)(57), Eckenfelder (58), Jenkins (59), Melamed, Saliternik, and Wacks (60), and by Beckman, Avendt, Mulligan, and Kehrberger (61). Essentially complete nitrification should be attained whenever the biological solids retention time based on total system volatile suspended solids is maintained at a value greater than the limiting sludge age value for the nitrifying bacteria at the appropriate pH and temperature, provided that a nitrifying population of microorganisms is present. This statement presumes that the dissolved oxygen concentration will be adequate to sustain nitrifying bacteria, and that toxic substances will not be present in the wastewater. This kinetic approach to biological nitrification should be applicable to both fluidized bed reactor and fixed bed reactor systems.

### 1. Activated Sludge Systems

Barth, Mulbarger, Salotto, and Ettinger (62) conducted field surveys of municipal wastewater treatment plants to determine if deliberate modifications might increase nitrogen removals in these plants. They found that the removal of nitrogen was erratic and not correlated with carbon or solids removal. The digester supernatant was found to be a concentrated point source of nitrogen. This and similar studies

revealed a need for more detailed knowledge of process controls to improve efficiency of nitrogen removal (63). At the present time the basic capability for nitrogen control resides in carefully managed biological systems (64).

In recent years a significant amount of research and development has been devoted to the two-sludge systems for nitrification. Essential features of design are the two-stage concept, which essentially isolates each biological system so that optimum design conditions can be employed to maximize the efficiency of each biological transformation. According to Barth, Brenner, and Lewis (65), Mulbarger (66), Barth (67), and Rimer and Woodward (68), the carbonaceous and nitrogenous oxidations should be separated in order to assure reliable operation.

A two-stage biological system for nitrification is considered necessary in northern climates where wastewater temperatures drop below $65^{o}F$ ($18^{o}C$) (4). In situations where nitrogen removal is required and the nitrification-denitrification route is preferred, many researchers believe that it will be mandatory to accomplish nitrification in a separate biological system. They believe that a large part of the normal BOD will have to be removed before the wastewater enters the nitrification unit. The principal advantage attributed to the two-sludge system as compared to a single combined carbonaceous-nitrification reactor system is that a greater degree of control over the two microbial processes is possible (66).

In activated sludge plants the concentration of nitrifying bacteria at any time will depend not only on the kinetic relationships but also on the rate of loss of nitrifying bacteria from the system

(52). The usual practice at sewage works is to waste sludge at frequent intervals or continuously in order to maintain an approximately constant average concentration of solids in the aeration tanks. The frequency at which sludge has to be removed depends on the rate of growth which, in turn, depends on a number of factors, including strength and nature of the waste, and the detention time of the aeration unit. If the nitrifying organisms are distributed uniformly through the whole sludge mass as in a completely mixed system, and the mass of nitrifying organisms per unit mass of suspended matter is the same in the returned sludge as in the solids lost in the effluent, then the fractional rate of removal of nitrifying organisms from the system at any time will be the same as the fractional rate of removal of sludge solids as a whole.

Therefore, it is not readily apparent why a two-sludge system should be more controllable than a one-sludge system if the concept of mean cell residence time or sludge age is employed to control both nitrifying systems. According to Jenkins and Garrison (69) and Lawrence and McCarty (70), in order to ensure that nitrification occurs at a high efficiency in either one or two-stage systems, the value of the design mean cell residence time selected must be greater than the larger of the values of the limited mean cell residence time required for the nitrifying bacteria. At a given temperature, the limited mean cell residence time, around two to four days, is approximately equal for the ammonia and nitrite oxidizing bacteria (70). Therefore, if the design mean cell residence time is sufficient to sustain ammonia oxidation, nitrite oxidation should also occur. Since the values of $K_s$, a biological "constant" used in the hyperbolic expression relating specific growth rate to substrate concentration, for both conversions

are very small, the process efficiency as measured by nitrate production should be high for steady state values of mean cell residence time reasonably greater than the limited mean cell residence time.

Biological nitrification has been shown to be attainable in a combined carbon oxidation-nitrification activated sludge system by various researchers (61)(71)(72)(73)(74). Johnson and Schroepfer (72) observed aeration basin detention times as low as two to three hours to be adequate with the proper load factor (lb $BOD_5$/day/lb mixed liquor suspended solids). A laboratory scale pilot study using municipal wastewater with one-sludge nitrifying activated sludge systems and two-sludge separate carbonaceous-nitrification systems operated at $20^oC$ and $8^oC$ achieved essentially identical performance (75). Stankewich (29) presented the results of pilot plant nitrification investigations on municipal wastewater using pure oxygen aeration. Essentially complete nitrification was attained in both the second stage of a two-sludge system and in a one-sludge system over the range of mean cell residence times investigated, 4.8 to 40 days.

It seems as though the loading factor or food-to-microorganism ratio in terms of $BOD_5$ or COD and the $BOD_5$ or COD to ammonia-nitrogen ratio have an effect upon the degree of nitrification attainable by the one-sludge system. Johnson and Schroepfer (72) reported that a highly nitrified effluent could be obtained at a load factor of 0.25 to 0.35 lb $BOD_5$/day/lb mixed liquor suspended solids. Beckman, et al. (61) reported the optimum food-to-microorganism ratio to be 0.25 or less for nitrification with the mixed liquor at $50^o$ to $65^oF$. Sawyer (71) operated different activated sludges at $BOD_5$:$NH_3$-N ratios from 8.2:1 to 21.0:1. He noted that the sludges fed at the $BOD_5$:$NH_3$-N = 8.2:1

ratio developed the greatest ability to oxidize nitrogen, while the sludges with $BOD_5$:$NH_3$-N = 16 or more:1 lost most of their nitrogen-oxidizing ability. He could not correlate the variation in nitrifying ability with the $BOD_5$, $NH_3$-N, or organic nitrogen content of the food material.

A high efficiency of nitrification should be achieved if either a one-stage or the second stage of a two-sludge system are operated at a mean cell residence time which is greater than the limiting mean cell residence time for the nitrifying microorganisms. Because the limiting mean cell residence time for the nitrifying bacteria exhibits a high degree of temperature dependence, it becomes necessary to incorporate a sufficient safety factor into the selection of the design mean cell residence time. Jenkins and Garrison (69) recommended that the design mean cell residence time be at least ten days to ensure adequate nitrification. Nitrification can be inhibited by toxic materials, low dissolved oxygen concentrations, and extremes of pH. A conservative design mean cell residence time would tend to minimize deviations in process performance caused by these inhibitions.

## 2. Fixed Bed Reactor Systems

It is generally believed that the trickling filter can bring about nitrification comparable to that of the conventional activated sludge process. Rohlich has shown that nitrification and carbonaceous oxidation can take place simultaneously in trickling filters (76). From studies on biological oxidation of carbohydrate solutions it was found that oxidation of ammonia proceeded best in the lower sections where the concentration of sugar was least (77). The process

variables such as depth of filter, size and type of media, and hydraulic loading can be seen from studies on BOD removal characteristics to greatly influence the degree to which nitrification can be obtained by the trickling filter process. Other factors that influence nitrification are the presence of inhibitors, pH, liquid temperature, and carbonaceous matter in the wastewater.

Many modifications of both the trickling filter and the activated sludge process, mainly modifications classified as either low-rate or high-rate processes, have been advanced within the past few years. Low-rate processes require a lower mass loading rate of organic material per mass of organisms than do high-rate processes. Both low and high-rate trickling filter and activated sludge processes display like characteristics when compared on an equivalent basis (78). Low-rate processes have been reported to: a) give small amounts of sludge, b) remove small amounts of inorganic nutrients, c) produce a nitrified effluent, d) operate at a low food-to-microorganism ratio and a high mean cell residence time, and e) be more stable to shock loading conditions. However, characteristics of high-rate systems are: a) high sludge production, b) a large removal of inorganic nutrients, c) a non-nitrified effluent, d) operation at a low mean cell residence time and a high food-to-microorganism ratio, and e) tendency to be more unstable when shock loaded. Effluent quality and operational characteristics are similar for activated sludge and trickling filter processes if compared on an equitable basis.

In comparing standard and high-rate rock trickling filters, Heukelekian (79) found that a nitrifying population was established in both, but that the nitrate and nitrite production was lower in the

high-rate filter. The high-rate filter had a BOD loading of 2300 lbs/acre-ft/day as compared to only 600 lbs/acre-ft/day for the standard filter. He noted that the nitrogen in the high-rate filter did not change materially with depth, but in the standard filter the nitrogen decreased appreciably.

From studies of high-rate filter performance, Mohlman (80) found that none of the filters loaded at over 2000 lbs/acre-ft/day achieved nitrification. However, the ammonia-nitrogen showed a substantial reduction in the lightly loaded plants, with corresponding high production of nitrates measured in the effluent. He concluded that the nitrifiers were being deprived of the oxygen needed for oxidation, and that the dosing rates of filters must be kept low if nitrification is to proceed.

Grantham, Phelps, Calaway, and Emerson (81) studied the performance of trickling filters using native Florida materials. They found for each filter type studied that nitrification was lower at the higher BOD loading rates. One of their conclusions was that nitrification in a trickling filter follows the monomolecular reaction pattern and depends to some extent on the type of filter medium and loading rates employed. They formulated an equation which defined the rate of oxidation of nitrogen in terms of depth of filter and the concentration of remaining oxidizable nitrogen. Other variables affecting the nitrification capacity of biological processes such as temperature, pH, and the presence of inhibitors were not included in their equation.

Four pairs of filters, one primary 4 ft x 4 ft x 6 ft, in series with a secondary 4 ft x 4 ft x 3 ft, were operated by Sorrels and Zeller (82). Total nitrate production increased with loading to a

maximum loading between 1000 lbs BOD/acre-ft/day and 1480 lbs BOD/acre-ft/day. The secondary filters were definitely the major nitrate producing filters. At a given BOD loading more oxygen was utilized by the primary filters in satisfaction of BOD than in the oxidation of nitrogen. However, in the secondary filter, the oxygen requirement for oxidation of nitrogen exceeded that for the BOD satisfaction.

Balakrishnan and Eckenfelder (77) used a 6-foot deep laboratory scale trickling filter unit to evaluate nitrification at different hydraulic loadings. When the hydraulic loading was increased from 10 MGAD to 30 MGAD in the 6-foot model filter, the percent nitrification decreased from 72 to 52. They, therefore, concluded that hydraulic loading has a significant effect on the degree of nitrification obtainable in a trickling filter process. From predicted performance curves for a temperature range of $15^{o}$ to $30^{o}C$, it was shown that temperature has a great influence on nitrification. Balakrishnan and Eckenfelder developed a relationship for the percent nitrification in trickling filters. From their equation it can be seen that increased nitrification could be achieved by increasing the depth of the filter or by decreasing the hydraulic loading. They also concluded that there appeared to be a correlation between the specific surface of the media and the nitrification rate constant.

From more recent nitrification reports the indication is that it is safe to design for nitrification of 80 to 90 pecent in plastic media biological filters (83). Four studies indicated that short towers were more beneficial than tall towers. Short towers are dictated to provide reasonable ammonia loading rates without excessive high irrigation rates. According to these investigations it should be safe to

design for nitrification of 80 to 90 percent of the ammonia at raw ammonia strengths of up to 20 mg/l, tower irrigation rates of 0.75 to 2.3 gpm/$ft^2$, and ammonia loads of seven to eight pounds of ammonia per 1000 cubic feet of media, temperatures between $40^o$ and $80^oF$, and pH constant within 7.2 to 8.4, and $BOD_5$ in the range of 30 mg/l or less.

Barth (67) reported that a field scale pilot plant at Midland, Michigan, was being used to evaluate the utilization of plastic biological filter media as an attached growth process for second stage nitrification. A research program by the Dow Chemical Company has demonstrated the feasibility of utilizing plastic media biological filters in a stage treatment system to achieve biological nitrification (84). Controlled feed rates to the study pilot plant were taken from the unchlorinated clarified secondary effluent of the Midland Waste Treatment Plant. This effluent contained a low $BOD_5$ (15 to 30 mg/l) with an ammonia concentration in the range of 10 to 20 mg/l ammonia-nitrogen. The system consistently maintained 80 to 90 percent oxidation of the ammonia-nitrogen at flow application rates up to 1.5 gpm/$ft^2$ and variable recycle ratios. There appeared to be a practical limitation of ammonia-nitrogen in the effluent in the range of 1.0 to 1.5 mg/l. Net solids production by the nitrification tower was low. This system has shown consistent and stable performance throughout both summer and winter operation.

Grantham and Seeger (85) noticed that active nitrification appeared to begin between three and nine days after the starting of a trickling filter, depending upon temperature and rock size for "used" rock beds, whereas it took considerably longer (25 days in their investigation) for new rock. Equilibrium, as far as nitrification was concerned,

took much longer time than required for BOD reduction. The degree of nitrification appeared to be more closely related to temperature than the degree of BOD removal. They concluded that the nitrification reaction in the starting of a trickling filter was more dependent upon "seed," temperature, and rock size than was the BOD reduction.

A reactor termed the submerged filter has been developed for the nitrification of secondary effluents. The submerged filter consists of a bed of media through which the wastewater passes in an upward direction. Nitrifying bacteria grow on the surfaces of the stones, and long mean cell residence times are possible. In addition, the upward flow of wastewater allows control of the hydraulic detention time. The characteristics of the submerged filter permit nitrification at very low temperatures or under conditions of variable loading.

Haug and McCarty (86) found that about 90 percent nitrification could be obtained using relatively short detention times of from 30 to 120 minutes with a submerged filter. Preoxygenation of the wastewater and bubble aeration were employed to supply oxygen to the microorganisms. The rate of ammonia oxidation and the degree of bio-film development were observed to be functions of the ammonia-nitrogen concentration. With high ammonia concentrations at the bottom of the filter, oxidation rates and bio-film development were greatest at that point, and decreased along the height of the filter. Mean cell residence times much greater than 10 to 20 days could be computed for this system. The total biological suspended solids concentration in the filter operated for 20 months averaged about 5000 mg/l dry weight.

A field study using laboratory scale submerged rock filters and pure oxygen was conducted by McHarness, Haug, and McCarty (87). After

an initial startup period of 60 days, at a 60-minute detention time, normal steady-state operation resulted in 90 to 96 percent ammonia-nitrogen removal at temperatures ranging from 21° to 27°C. One system employed preoxygenation with pure oxygen at one atmosphere of pressure and required recycle of treated effluent because of limited oxygen solubility, while the other system employed direct bubbling of oxygen into the filter.

Accumulation of high concentrations of organisms in the filter of several thousand mg/l, measured as total suspended solids, permitted biological solids retention times of over 100 days. In general, 90 percent ammonia oxidation of 20 mg/l $NH_3$-N was obtained with a detention time of 30 minutes at 25°C and 60 minutes at 15°C. Detention times of 90 and 120 minutes were required at temperatures of 10°C and 5°C, respectively, in order to achieve the same percent removal.

The submerged filter has advantages of short detention times while maintaining long mean cell residence times. Biological solids separation in a final clarifier and sludge return are not needed. The long solids retention times allow for stable nitrification at varying temperatures, pH, ammonia-nitrogen concentrations, and flow rates. Therefore, the submerged filter is well suited for the treatment of intermittent wastewater discharges if the filter remains aerobic during periods of idleness.

The rotating biological contactor system consisting of closely spaced discs supported by a rod passing perpendicularly through the discs shows great promise as a biological nitrification process. The rotating discs are partially submerged in the wastewater, and the microbial population is alternately passed through the air and the wastewater.

The rotating discs serve three primary purposes (88):

a. to provide media for the support of a fixed microbial growth

b. to contact the growth with the wastewater

c. to aerate the wastewater to provide the dissolved oxygen necessary to maintain aerobic biological activity.

This process has advantages of being noiseless, easy to operate, and requiring a minimum of maintenance and power. Operational characteristics of this process appear similar to both the trickling filter process and the activated sludge process, with important benefits and advantages of each (89)(90).

Antonie (91) reported on the capability of a two-stage rotating disc system to accomplish biological nitrification during the treatment of domestic wastewater. Average wastewater characteristics consisted of 144 mg/l BOD, 323 mg/l COD, 18.2 mg/l $NH_3$-N, and 28.9 mg/l Kjeldahl nitrogen. At a hydraulic loading of approximately 1.0 gpd/$ft^2$, 90-minute residence time, 95 percent $NH_3$-N, 90 percent Kjeldahl nitrogen, and 90 percent BOD removals were achieved. Ammonia-nitrogen effluent concentrations of around 2 mg/l was accomplished without adjustment of normal municipal wastewater pH. The majority of the carbonaceous BOD was satisfied in the first stage of the disc system, allowing nitrifying bacteria to predominate in the second stage and achieve a high degree of ammonia oxidation. European experience has shown that the treatment capacity of this package plant could have been increased by dividing the discs into four rather than two stages (91). This increased treatment capacity would be due to two factors:

a. The development of specific cultures in successive stages would have been enhanced.

b. Both BOD and ammonia oxidation are approximately first order in the disc system; therefore, the improved residence time distribution would have increased the overall BOD and ammonia oxidation rates.

Torpey, Heukelekian, Kaplovsky, and Epstein (92) have reported on the removal of nitrogen and phosphorus by a rotating disc unit with ten sequential stages. The system was operated at a flow of 7.5 to 9 gpm with influent COD and ammonia-nitrogen concentrations of 300 mg/l and 13 mg/l, respectively. The ammonia-nitrogen concentration increased from 13 mg/l to 14.4 mg/l at stage 3, presumably because of the hydrolysis of organic nitrogen in the biological growth. A slight decrease in ammonia-nitrogen concentration was observed in stages 4 and 5. Nitrification started at stage 5 and increased thereafter by about 2 mg/l at each stage throughout the rest of the unit for a total of 10.4 mg/l of combined oxidized nitrogen within 30 minutes of contact time. Effluent COD and ammonia-nitrogen concentrations averaged about 65 mg/l and 5.7 mg/l, respectively.

The amount of nitrification obtainable by the rotating disc process has been shown to be affected by wastewater temperature, BOD loading, and hydraulic loading (93). At a hydraulic loading of 2 gpd/ft$^2$ and wastewater temperature range of 55$^o$ to 71$^o$F, approximately 80 percent of the ammonia-nitrogen was removed, while at the same hydraulic loading and a temperature range of 39$^o$ to 49$^o$F only about 30 to 60 percent ammonia-nitrogen removal was achieved. The percent ammonia-nitrogen removal was also shown to decrease with increasing effluent BOD concentrations. At effluent BOD concentrations of 10 mg/l or less, 90 to 100 percent removal of ammonia-nitrogen was observed.

The rotating disc system appears to be uniquely suited to treating

wastewaters containing a mixture of substrates. Since the system is constructed in a series of stages, the fixed cultures which develop in each successive stage become adapted to treating wastewater as it undergoes a progressively increasing degree of treatment (91). Therefore, if the majority of the carbonaceous BOD is satisfied in the first stages of the disc system, rapid nitrification can take place in the rest of the system with a specialized and established flora of nitrifying microorganisms.

A review of the literature indicates that the removal of nitrogen and nitrogen compounds by biological means is definitely a feasible and practical solution to the nitrogen problems in wastewaters. Both fixed and fluidized bed reactors have been shown to be capable processes for nitrogen removal. Field surveys have found the removal of nitrogen at different plants to be erratic and not correlated with solids or carbon removal. Various authors have attempted to define the nitrification reaction with simple equations. These equations are useful for laboratory investigations where the incoming wastewater and various conditions can be maintained. However, in the field, all of the necessary variables which have a part in the nitrification process are not controllable and cannot be elucidated by simple equations. Since it is not entirely possible to control the wastewater entering a plant, it therefore becomes necessary to control the nitrification process in order to achieve a desired degree of nitrogen removal. Efficient nitrification process controls are not completely worked out. More research is necessary to gain detailed knowledge of process controllability and improve nitrification efficiency.

# CHAPTER III

# MATERIALS AND METHODS

## A. General

A synthetic wastewater was used in all experiments with carbon sources of both glucose and sucrose serving as substrates. Both glucose and sucrose are easily metabolized by most heterogeneous microorganisms and therefore provide an excellent carbon and energy source. Glucose was used in the batch studies and the fluidized bed continuous flow studies. Since the fixed bed reactors required large volumes of synthetic waste, sucrose was utilized because of its high purity and low cost when purchased in technical grade. These carbon sources can be easily monitored by the chemical oxygen demand test. They can be made up in a concentrated form for ease of handling and storage, and the amount applied in the feed to the units can be easily changed by altering the amount of concentrated feed used.

Throughout the investigations the major concern involved the different forms of nitrogen (ammonia, nitrite, and nitrate) measurable in the wastewater, and the removal of the organic carbon was of secondary importance as long as 90 percent or greater removal was obtained. Ammonium sulfate was utilized as the source of nitrogen in the synthetic wastewaters. Like the carbon source, the ammonium sulfate can be made up in a concentrated form, and the carbon to ammonia-nitrogen

ratio can be varied by the amounts of each applied in the feed to the units. Since the carbohydrate carbon source contained no nitrogen, the only source of nitrogen for microbial growth and energy was the ammonium sulfate. Thus, a wastewater with ideal characteristics as far as monitoring the different forms of nitrogen was easily synthesized for the investigative research.

All of the heterogeneous microorganisms employed throughout the investigations were developed from initial sewage seeds from the municipal sewage treatment plant at Stillwater, Oklahoma. The composition of the synthetic wastewaters used as the growth medium in these studies was chosen so that the carbon and energy source (glucose or sucrose) was the limiting growth factor. These heterogeneous populations were employed in both batch studies and continuous flow studies under closely controlled experimental conditions. Both fluidized bed reactors and fixed bed reactors were untilized for the continuous flow investigations.

An initial seed of the autotrophic microorganisms, Nitrosomonas and Nitrobacter, was obtained from the addition of a small amount of cultivated soil to batch units. These batch units contained all of the necessary nutrients including ammonia and excluding organic carbon for the necessary metabolic and energy requirements of the autotrophic microorganisms. These bacteria were also employed in the batch studies and continuous flow studies with both fixed bed reactors and fluidized bed reactors.

## B. Batch Studies

The objective in the first set of batch experiments using the autotrophic nitrifiers and ammonium sulfate as the energy source was to determine the effects, if any, of the organic carbon source (glucose) in the wastewater on the nitrifying microorganisms. Total mixed liquor volume of the batch reactors used was two liters. Oxygen supply to the biological solids and mixing were provided by compressed air through sintered glass diffusers. The compressed air was filtered through cotton and saturated with water by bubbling through tap water.

An acclimated cell suspension taken from the second reactor of the two-stage activated sludge system (described later) was used for these experiments. The ammonium sulfate, inorganic salts, and phosphate buffer were provided as shown in Table I. The pH was monitored throughout the batch experiments with pHydrion papers. Sodium hydroxide was used to keep the batch units adjusted to pH values between 7.5 and 8.0. Temperature in the batch reactors was not controlled; all experiments were run at approximately 20$^{o}$C throughout the period.

The growth medium shown in Table I and a 650 mg/l population of nitrifying organisms were added to each of four batch reactors. Initial samples were withdrawn for measurement of biological solids, chemical oxygen demand, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen. Samples were again withdrawn at 0.5 and one hour for analysis. After the one-hour samples were withdrawn an amount of concentrated glucose solution was added to each of three reactors to give COD values of 143 mg/l, 295 mg/l, and 529 mg/l. The fourth reactor which received no glucose served as the control unit for the

experiment. Samples were again removed for analysis at 1.5 hours, two hours, and hourly thereafter. This same procedure was followed in another batch experiment, in which 455 mg/l, 777 mg/l, and 1139 mg/l of glucose COD were added to three of the reactors. The fourth batch reactor again served as a control (no glucose was added).

TABLE I

COMPOSITION OF FEED FOR 50 mg/l AMMONIA-NITROGEN AS ENERGY SOURCE

| Constituent | Concentration |
|---|---|
| $(NH_4)_2SO_4$ | 250 mg/l |
| $MgSO_4 \cdot 7H_2O$ | 50 mg/l |
| $FeCl_3 \cdot 6H_2O$ | 0.25 mg/l |
| $CaCl_2$ | 3.75 mg/l |
| $MnSO_4 \cdot H_2O$ | 5 mg/l |
| Phosphate buffer, 1.0 M, pH, 7.6 ($KH_2PO_4$ + $K_2HPO_4$) | 5 ml/l |
| Tap water | 50 ml/l |
| Distilled water | to volume |

The objective in the second set of batch experiments was to determine the effects, if any, for the combined addition of both organic carbon source and heterotrophic microorganisms on the autotrophic nitrifying population. The same experimental conditions mentioned in the

previous batch studies were maintained for these investigations. Three batch reactors were employed. The first reactor contained the same growth medium shown in Table I and 480 mg/l of nitrifying organisms. This reactor served as the control unit for the investigations. The second reactor contained the same growth medium plus 450 mg/l glucose COD and 480 mg/l of nitrifiers plus 485 mg/l of heterotrophic microorganisms. The same concentration of ammonium sulfate plus 1000 mg/l of glucose COD and twice the concentration of inorganic salts and phosphate buffer as described in Table I were supplied to the third reactor. It also contained 480 mg/l of nitrifiers and 620 mg/l of heterotrophic organisms. Again, the autotrophic microorganisms were taken from the second reactor of the two-stage activated sludge system (described later), and the heterotrophic microorganisms were taken from the first reactor of the same system. Parameters measured throughout the duration of the experiment were biological solids, COD, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen.

## C. Continuous Flow Experiments

Continuous flow studies involved the utilization of both fluidized bed reactors and fixed bed reactors as previously mentioned. Basic similarities and differences exist between fluidized and fixed bed reactors. Both processes cause stabilization of wastewater by the aerobic conversion of organic matter and certain inorganic matter to cellular material and end products of metabolism. The big difference in the two processes is the method by which the microorganisms are cultivated. In the fluidized bed reactors or activated sludge process, the microorganisms are cultivated in suspension, while in the fixed bed reactors, such as the

biological filter or rotating biological contactor, the microbial growth is adhered to a fixed surface. Another difference is the method by which oxygen is supplied to the systems. Oxygen is supplied by mechanical or diffused air aeration to fluidized reactors, but it is supplied by convection currents to the fixed bed reactors.

## 1. Fluidized Reactor Systems

a. Method of Data Analysis. Since 1950, considerable advancement has been made in the understanding of biological wastewater treatment systems. From the studies of Gaudy and Gaudy, Lawrence and McCarty, Garrett, Busch and Kalinske, McKinney, and Weston and Eckenfelder, the currently accepted mathematical descriptions of the completely mixed activated sludge processes were derived (94). The method utilized for data analysis was that presented by Sherrard, Schroeder, and Lawrence (94) for the mathematical and operational relationships for the completely mixed activated sludge process. By using a variable observed cell yield coefficient, materials balance equations for both microbial growth and oxidation of substrate and energy source can be easily written around the appropriate system boundaries.

Removal efficiency or treatment purification was calculated according to the following expression:

$$E = \frac{100\ (Co-C)}{Co} \qquad (1)$$

where

E = removal efficiency, percent

Co = influent substrate concentration, mg/l

C = effluent substrate concentration, mg/l.

The observed yield coefficient was calculated according to the following relationship:

$$Y_{obs} = \frac{Q_w X + Q_{eff} X_{eff}}{Q(C_o - C)} \quad (2)$$

where

$Y_{obs}$ = observed yield coefficient

$Q$ = influent liquid flowrate, liters/day

$Q_w$ = wasted liquid flowrate, liters/day

$Q_{eff}$ = effluent liquid flowrate, liters/day

$X$ = aeration basin microorganism concentration, mg/l

$X_{eff}$ = effluent liquid microorganism concentration, mg/l

$C_o$ and $C$ have been previously defined.

The magnitude of the variable observed cell yield coefficient for constant environmental conditions and a specific wastewater is dependent upon the mean cell residence time or sludge age which is equal to the reciprocal of the net cell growth rate (95). The mean cell residence time was determined by the following expression:

$$\Theta_c = \frac{VX}{Q_w X + Q_{eff} X_{eff}} \quad (3)$$

where

$\Theta_c$ = mean cell residence time, days

$V$ = volume of aeration basin, liters

The remaining terms are as previously defined.

The observed cell yield coefficient and mean cell residence time based on equations (2) and (3), respectively, are dependent on the aeration basin microorganism concentration, (X). Since reactors with

internal recycle of bacterial cells were utilized, the observed yield coefficient and mean cell residence time were calculated for the aeration chamber plus settling compartment or for the total reactor. An adjustable baffle which could be removed, separated each of these compartments. Therefore, the microorganism concentration, (X), and the volume, (V), based upon the total reactor were appropriately used as shown below:

X = total reactor microorganism concentration, mg/l

V = volume of the total reactor, liters.

Therefore, the mean cell residence time, ($\Theta_c$), can be concluded to be equal to the average time span that a microorganism has been retained in the reactor before being discharged or wasted from the system.

An operational relationship for the calculation of the rate of substrate utilization per unit weight of microorganisms or specific utilization can be obtained. Specific utilization was determined according to the following relationship:

$$U = \frac{1}{Y_{obs}\Theta_c} \tag{4}$$

where

U = specific utilization

$Y_{obs}$ and $\Theta_c$ have been previously defined.

From a materials balance analysis a relationship for the food-to-microorganism ratio can also be developed. Since the specific utilization is essentially equivalent to the food-to-microorganism ratio times the efficiency of treatment, the food-to-microorganism ratio can be represented as shown below:

$$F/M = \frac{U}{E \times 10^{-2}} \quad (5)$$

where

F/M = food-to-microorganism ratio.

U and E have been previously defined.

The food-to-microorganism ratio is defined to be the amount of substrate applied divided by the amount of microorganisms in the reactor per day.

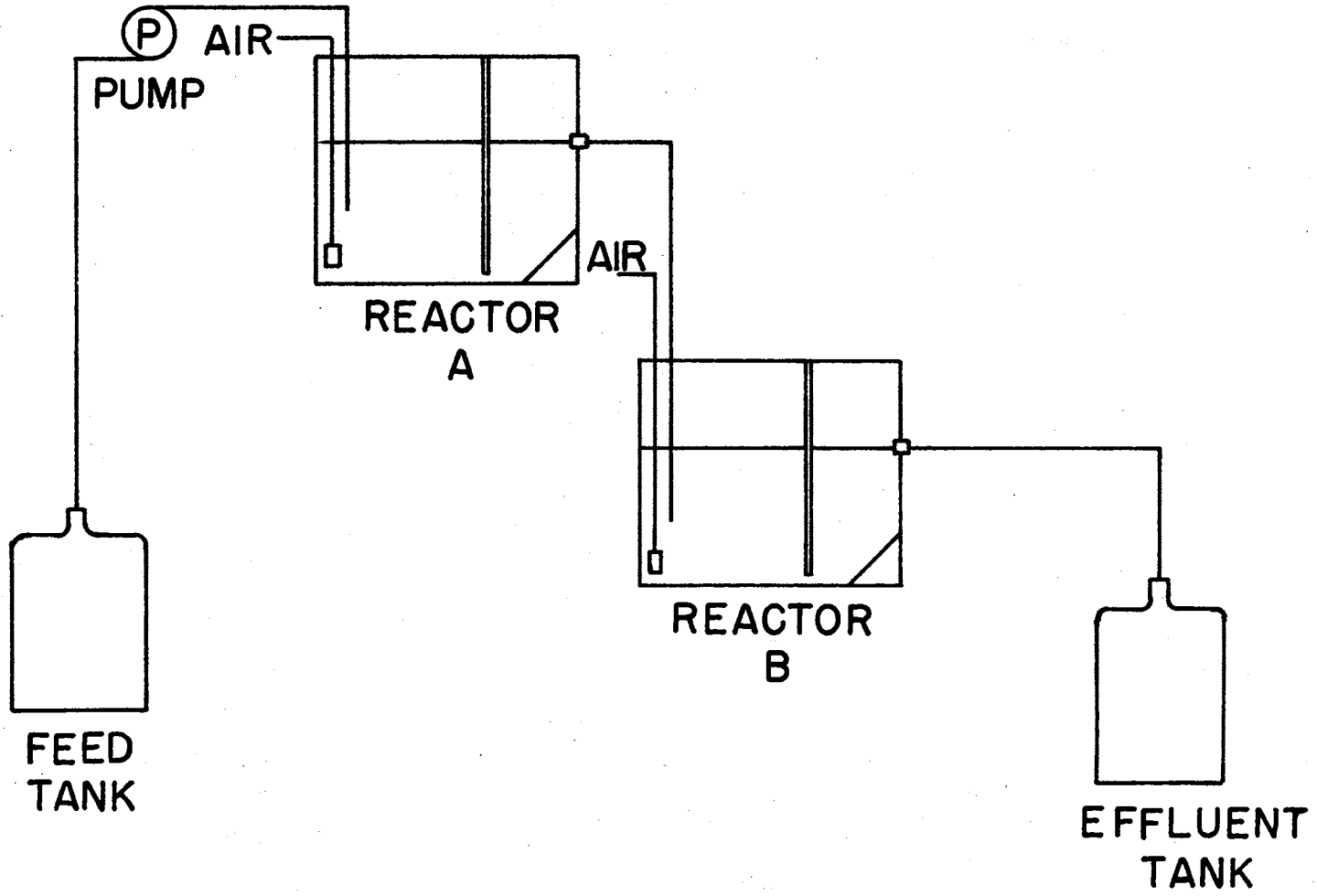
b. Two-Stage Activated Sludge System. The experimental bench-scale pilot plants employed in this investigation are diagrammed in Figure 1. These were plexiglass reactors with internal recycle of biological solids. The aeration and settling chambers were contained in the same reactor and separated by an adjustable plexiglass baffle. Compressed air, which had been filtered and saturated with water, was supplied to each of the units through porous diffusers. The compressed air provided oxygen supply to the biological solids, mixing and "suction" to recycle the settled biological solids from the settling compartment into the aeration chamber. The temperature of the reactors was maintained at room temperature, which was approximately 20°C at all times.

Operation under continuous flow conditions was accomplished by pumping the feed solution to the aeration chamber of the first unit. This first unit was the carbonaceous unit which served to remove the organic constituents from the wastewater. The total volume of the system was 10.2 liters (approximately 6.8 liters aeration capacity and 3.4 liters in the settling chamber). A Milton-Roy dual, positive displacement pump (Mini-pump, Model MM2-b-96R) provided a continuous flow

Figure 1. Diagram of Experimental Two-Stage Activated Sludge System for Separate Carbonaceous Removal and Nitrification

Reactor A = carbonaceous reactor

Reactor B = nitrification reactor


P
AIR
PUMP
AIR
REACTOR
A
REACTOR
B
FEED
TANK
EFFLUENT
TANK

of wastewater to the system at a pumping rate of 10.6 ml/min. This feed rate provided an overall hydraulic detention time of 16 hours (approximately 10.5 hours aeration and 5.5 hours settling). Alternately, each of the feed lines was disinfected by pumping a one-percent solution of Clorox and distilled water. Thus, one of the feed lines was being disinfected while the other was pumping feed solution. This daily alternation of feed lines provided positive control for the retardation of growth in the feed lines. Composition of the synthetic wastewater applied during continuous-flow operations is shown in Table II.

TABLE II

COMPOSITION OF FEED FOR 500 mg/l
GLUCOSE AS SUBSTRATE

| Constituent | Concentration |
|---|---|
| Glucose | 500 mg/l |
| $(NH_4)_2SO_4$ | 250 mg/l |
| $MgSO_4 \cdot 7H_2O$ | 50 mg/l |
| $FeCl_3 \cdot 6H_2O$ | 0.25 mg/l |
| $CaCl_2$ | 3.75 mg/l |
| $MnSO_4 \cdot H_2O$ | 5 mg/l |
| Phosphate buffer, 1.0 M, pH, 7.6 ($KH_2PO_4$ + $K_2HPO_4$) | 5 ml/l |
| Tap water | 50 ml/l |
| Distilled water | to volume |

The effluent flowed by gravity from the settling compartment of the first reactor to the aeration chamber of the second reactor. This second reactor was the nitrification system which biologically converted the ammonia-nitrogen to nitrate-nitrogen. The ammonia-nitrogem remaining in the effluent served as the energy source for the microorganisms contained in the second reactor. The total volume of this system was 9.4 liters (6.2 liters, aeration chamber, and 3.2 liters, settling chamber). The feed rate provided an overall detention time of approximately 14 hours (approximately 9.5 hours aeration and 4.5 hours settling). Effluent from this system flowed by gravity from the settling chamber to a holding tank where it was collected.

Prior to daily sampling, the settling chamber outlet was closed, the feed flow stopped, and the plexiglass baffles separating the aeration and settling chambers were pulled in both systems, allowing the biological solids in both compartments to mix thoroughly. A sample of mixed liquor was then removed from each reactor for biological solids determination. The data represented the sludge concentration in the total system, not in the aeration chamber alone. Also, while the baffles were pulled, a predetermined amount of biological solids was wasted from each system. The baffle was immediately replaced, the settling tank outlet opened, and feeding resumed. Samples were also taken daily from the feed solution for analysis of COD, ammonia-nitrogen, and nitrate-nitrogen. Portions of the effluents from both systems were collected for biological solids analysis, measurement of COD, ammonia-nitrogen, and nitrate-nitrogen. The pH was monitored constantly with pHydrion papers, and occasionally checked with a pH meter. The pH of the nitrifying system was constantly adjusted with sodium hydroxide to maintain

the pH between 7.5 and 8.5.

The mean cell residence time was governed by the amount of wasted solids relative to the solids inventory. The first reactor was operated at a fairly high food-to-microorganism ratio and low sludge retention time. Since nitrifying organisms have low yield coefficients and relatively long generation times as compared to heterotrophic microorganisms, they need longer solids retention times for growth requirements based on the environmental conditions experienced. If the mean cell residence time is kept low enough in the first reactor, the nitrifying population cannot compete in the system and will be washed out. Solids wastage was varied in the second reactor in order to achieve different mean cell residence times. The degree of nitrification achievable by the nitrifying system at various mean cell residence times was monitored. Settling characteristics of the sludge were also checked at various mean cell residence times by measuring zone settling velocities.

Operation of the first system at low mean cell residence times or high growth rates enhance the possibilities for predominance changes in the system, especially for microorganisms with fast growth rates. One environmental condition that has been implied as a cause for proliferation of filamentous organisms is high BOD loading or high food-to-microorganism ratio, which would be the case in a system operated at a low sludge retention time (96). Filamentous microorganisms disrupted the first reactor before the study was complete, and all attempts to re-seed the system failed. Therefore, the decision was made to use the "fixed activated sludge process" described by Randall, Edwards, and King for the remainder of the investigation period (96). This process

designed for filamentous organisms caused no noticeable change in COD and nitrogen content of the effluent wastewater, but did cause less biological solids to be discharged in the effluent from the system.

Conversion of the first reactor to a "fixed activated sludge process" required only the division of the aeration basin into three sections by two screen wire panels approximately 0.5 cm. mesh size suspended at intervals of approximately three inches. The height of the screens above the bottom of the reactor was adjustable. Sintered glass diffusers were placed between the screens and the two end sections of the aeration chamber. The adjustable baffle was replaced by a sharp-crested weir, which separated the aeration chamber from the settling compartment. In this system the filamentous organisms attached themselves to the screen, and there was no recycle of the organisms. The solids which sloughed off the screens consisted of compacted filamentous masses that settled rapidly when washed over the weir into the settling chamber. These solids were wasted from the settling tank daily.

c. One-Stage Activated Sludge Process. The one-stage system is designed to accomplish both carbonaceous removal and nitrification in the same reactor. The bench-scale aeration pilot plants employed in this study were essentially the same as those used in the two-stage system. Compressed air was supplied to these reactors for the same reasons as previously mentioned. The temperature of the reactors was maintained at room temperature--approximately 20$^{o}$C. The pH was monitored constantly with pHydrion papers and maintained at approximately pH 7.5 with sodium hydroxide.

The total volume of the reactors used was three liters

(approximately two liters aeration chamber and one liter settling chamber). A Milton-Roy pump provided continuous flow of wastewater to the system at a pumping rate of 4.2 ml/min. This feed rate provided an overall detention time of 12 hours (approximately eight hours aeration and four hours settling). Composition of the synthetic wastewater applied to the system was essentially the same as that shown in Table II.

Operational procedure of the one-stage system again involved wasting of biological solids from the reactor in order to maintain desired mean cell residence times. The same procedure for solids wasting and collection of samples as described for the two-stage system was again employed. Biological solids, COD, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen were measured at various sludge retention times in order to determine the effect of mean cell residence time upon the degree of nitrification achievable by the system. All systems were well seeded with nitrifying microorganisms before each experiment was initiated.

Systems were operated at different COD to ammonia-nitrogen ratios (COD:$NH_3$-N) to determine the effect of the COD:$NH_3$-N ratio upon the degree of nitrification attainable by the one-stage systems. Composition of the wastewater applied was the same as that shown in Table II with changes in the glucose or ammonium sulfate concentration made to achieve the desired COD:$NH_3$-N ratio. When the glucose was applied at 500 mg/l COD or less, the inorganic salts and phosphate buffer concentrations were added in the same proportions as shown in the table. Inorganic salts and phosphate buffer were increased proportionately as the glucose concentration was increased above 500 mg/l COD. One-stage activated sludge systems were investigated at concentrations of

COD:$NH_3$-N of 100:10, 100:50, 300:30, 500:50, 1000:50, and 1000:100.

2. Fixed Bed Reactors

Differences result between the fluidized and fixed bed reactor processes, due to physical parameters. The removal of compounds in the wastewater is accomplished by incorporation into cellular tissue and oxidation to metabolic end products in both of these processes. Therefore, it would appear that an equivalent basis for comparing the two processes based on data analysis exists as suggested by Kincannon and Sherrard (78). In order to apply the same method for data analysis, as previously mentioned, the weight of microorganisms contained in 1000 cubic feet of media must be obtained. This requires a knowledge of the surface area per cubic foot of media, active film thickness of the microorganisms attached to the media surface, and the dry weight of microorganisms per unit volume. An active film thickness of 70 microns and dry weight of microorganisms per unit volume of 95 $mg/cm^3$ have been reported on a trickling filter by Kornegay and Andrews (97).

a. Biological Filter Process. Bentley (98) has shown that the same method of data analysis previously employed for the activated sludge process can be applied to the biological filter process. The dry weight of biological solids in the system can be determined by using a 70-micron active film thickness, dry weight per unit volume of 95 $mg/cm^3$, and the surface area per cubic foot of media. The plastic media employed for this investigation contained 27 square feet per cubic foot of media. Using these values, 36.5 pounds dry weight of biological solids per 1000 cubic feet of filter media is obtained. Since

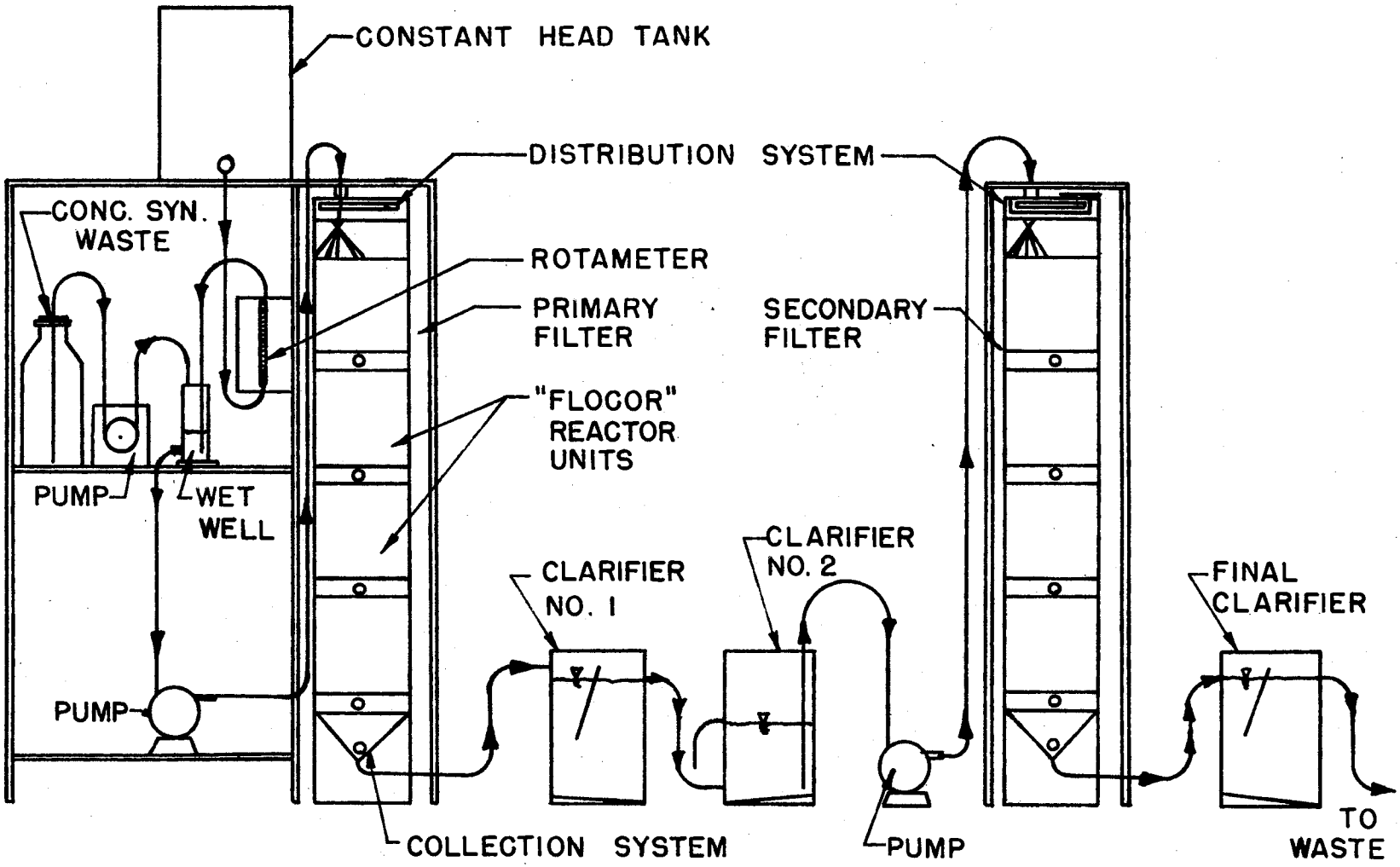
the amount of biological solids in the system and the applied loading is now known, the only other requirement becomes the amount of daily biological solids wastage from the system.

The biological filter was a once-through system with no recycle of biological solids, whereas the activated sludge system did employ solids recycle. A system which employs recycle of biological solids can be controlled or operated by the amount of solids wasted from the system, but a once-through system like the biological filter cannot be operated in such a manner. The controlling factor in the biological filter process then becomes the total organic loading, which regulates the amount of solids wasted from the system. By measuring COD, ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, and biological solids wasted from the system, a comparison of the two processes (activated sludge and biological filter) can be made.

Two identical pilot filter units placed in series operation with two intermediate and a final clarifier were employed in this investigation (Figure 2). Each tower contained four one-cubic foot ( 1 ft. x 1 ft. x 1 ft.) modules of Flocor plastic medium (27 $ft^2/ft^3$) as the contact bed. Void spaces of approximately four inches between each cubic foot module were allowed to permit sampling of the wastewater applied to the system.

All of the investigations were conducted under closely controlled experimental conditions. The only variations applied to the system were the influent organic concentration and hydraulic loading in gallons per day per square foot ($gpd/ft^2$). The hydraulic loadings investigated were 500, 750, and 1000 $gpd/ft^2$. The temperature of the influent wastewater was held at approximately $25^oC$ throughout the duration of study by

Figure 2. Diagram of Experimental Two-Stage Biological Filter System with Intermediate and Final Clarification


CONSTANT HEAD TANK
DISTRIBUTION SYSTEM
CONC. SYN. WASTE
ROTAMETER
PRIMARY FILTER
SECONDARY FILTER
"FLOCOR" REACTOR UNITS
PUMP
WET WELL
CLARIFIER NO. 1
CLARIFIER NO. 2
FINAL CLARIFIER
PUMP
COLLECTION SYSTEM
PUMP
TO WASTE

passing the tap water through a coil of copper tubing immersed in a constant temperature water bath.

The hydraulic flow rate was controlled by a constant head tank which received a continuous flow of tap water that had passed through the water bath. Flow from the constant head tank was regulated by a rotameter. Flow from the rotameter went directly to a wet well, where the tap water and concentrated synthetic waste were mixed. The concentrated solution prepared in either a 20-liter or 40-liter Pyrex bottle was pumped to the wet well by a variable speed Cole-Parmer Master-flex Tubing Pump (Model WZ IR031).

The composition of the synthetic waste using sucrose as carbon source and growth-limiting nutrient is shown in Table III. During feed preparation, 30-60 ml of 16N sulfuric acid was added to assist in dissolving the concentrated waste constituents and to prevent biological growth in the feed bottle and lines. A magnetic stirring bar was used to stir the concentrated feed solution continuously during the feeding period.

The mixed feed was pumped from the wet well to the first filter distribution system by a Teel Rotary-Screw Pump (Model IP610). Oscillating spray nozzles were used to distribute the wastewater across the 1 $ft^2$ horizontal surface area of both filters. Effluent from the first filter was conveyed to the intermediate clarifiers. Waste flow was pumped from the second intermediate clarifier to the second filter by a pump identical to the one described above. Effluent from the second filter flowed into the final clarifier and from there into the sanitary sewer system.

The first filter was originally seeded with microorganisms from

the Stillwater municipal sewage treatment plant. Seeding of the second filter was accomplished by pumping clarified effluent from the first filter. The second filter was also seeded with nitrifying bacteria for nitrification analysis.

TABLE III

COMPOSITION OF FEED FOR 100 mg/l SUCROSE AS SUBSTRATE

| Constituent | Concentration |
|---|---|
| Sucrose | 100 mg/l |
| $(NH_4)_2SO_4$ | 25 mg/l |
| $MgSO_4 \cdot 7H_2O$ | 10 mg/l |
| $K_2HPO_4$ | 6 mg/l |
| $MnSO_4 \cdot H_2O$ | 1 mg/l |
| $CaCl_2$ | 0.75 mg/l |
| $FeCl_3 \cdot 6H_2O$ | 0.05 mg/l |

Experiments consisted of a four-day equilibration period followed by a minimum of three consecutive days of sampling. By obtaining nearly identical values of COD and pH, steady state conditions were ascertained. Samples were collected after each foot of depth. The pH of these samples was determined using a Beckman pH meter. Each sample was filtered, and COD, ammonia-nitrogen, and nitrate-nitrogen analyses

were made of the filtrate. Sloughed biological solids from the filters were removed from the clarifiers on a daily basis.

b. Rotating Disc System. The rotating biological contactor system employed in this investigation was a four-foot long, ten-gallon capacity, miniature unit built by the Autotrol Corporation, specifically for experimental purposes. It consisted of six compartments or stages separated by partitions with holes in the bottom of each partition to allow flow from stage to stage. Five rotating polystyrene discs were contained in each compartment for a total of 30 discs in the entire system. A 1/10 horsepower, 110 volt a.c. electric motor rotated the discs at eleven revolutions per minute. Disc diameters of 23.25 inches gave surface areas of 2.96 square feet for each side of a disc. Total surface area of polystyrene medium available for microbial growth was, therefore, 177.5 square feet. The volume of the rotating discs taken as a cylinder was 11.8 cubic feet, giving a specific surface area of 15 $ft^2/ft^3$.

The waste used in this investigation was essentially the same as that employed in the biological filter investigations (Table III). The carbon source, sucrose, was again the growth-limiting nutrient, and the ammonium sulfate concentration was increased to approximately twice the concentration shown in the table. The wastewater was placed in two holding tanks of approximately 240 liters each. Next, the wastewater was pumped into a holding basin at the head of the rotating disc unit where it was dipped into the first stage by four rotating dippers. In this first stage the actual biological stabilization of the wastewater began. The wastewater flowed through each of the next five stages and

out the effluent line, where it was discharged to a sanitary sewer.

A centrifugal pump with a control valve on the discharge side of the pump was used to pump wastewater from the holding tanks to the first section of the unit. The desired flow rate to the rotating disc system could be maintained by adjustment of the pump discharge control valve. The pump was driven by a 1/3 horsepower, 110 volt a.c. electric motor. A flow rate of 0.5 gpd/$ft^2$ corresponding to a hydraulic detention time of approximately two hours and forty minutes was maintained for these investigations.

A seed of microorganisms was originally added to the unit to aid in the startup of the microbial population. This unit was also seeded with nitrifying bacteria in order to develop a population of the nitrifying autotrophs as well as the heterotrophic microorganisms. Chemical analyses and pH determinations were made on the system to show that equilibrium had been reached before experimentation began.

Seven samples were collected in each sampling period. Influent samples, samples at the end of each stage, and effluent samples were collected. Following collection the pH of the samples was immediately determined, using a Beckman pH meter. The samples were filtered, and COD, ammonia-nitrogen, and nitrate-nitrogen analyses were made on the filtrate. The sampling procedure outlined above was also carried out for quantitative shock load experiments described below.

The quantitative shock load experiments involved increases in the concentration of influent carbon source by approximately two times, three times, and four times, respectively. The inorganic salt concentrations were also increased proportionately. Shock load wastewater was made up in one of the 240-liter holding tanks, while regular

wastewater in the other holding tank was being applied to the rotating disc system. The pump intake line was switched to the holding tank with increased waste concentration for the duration of the shock, and then switched back again for normal operation conditions. Samples were collected for the same analyses mentioned above, before, during, and after the shock load of wastewater was applied to the system.

## D. Analytical Procedures

The analytical methods and techniques employed during this research were the same for both the batch and continuous flow reactors. The tests described herein, except for the ammonia-nitrogen test, were made in accordance with the procedures as listed in "Standard Methods for the Examination of Water and Wastewater" (99). For all of the colorimetric tests, ammonia-nitrogen, nitrite-nitrogen, and nitrate-nitrogen, a Bausch and Lomb "Spectronic 20" was employed. One-half inch diameter by four-inch matched test tubes were used for these colorimetric tests.

### 1. Biological Solids

The weight of biological solids was determined gravimetrically by filtration of the samples through membrane filters (0.45 μm pore size, Millipore Filter Corp., Bedford, Mass.). Filters were placed in aluminum pans and placed in a drying oven for two hours at a temperature setting of 103$^{o}$C. Then the pans were placed in a desiccator for cooling. After cooling, the pans were weighed, and known volumes of samples were filtered with the aid of a vacuum pump. For samples which were difficult to filter, a Sorvall Superspeed Centrifuge type SS-1A, Ivan Sorvall, Inc., was used to reduce the time of filtration. Samples were

centrifuged at a rate of 10,000 rpm for several minutes prior to filtration. The supernatant was carefully filtered first, then the pellet of solids was removed with the aid of a metal spatula and placed on the filter. After filtration, the filters were returned to the pans, placed in the drying oven again for two hours, cooled in the desiccator, and then weighed to determine the biological solids concentration.

2. Ammonia-Nitrogen

The ammonia-nitrogen was determined by a method developed by Niss and described by Ecker and Lockhart (100). Two reagents were required for this analysis. Reagent A contained: 4.7 grams sodium citrate, 1.7 grams citric acid, 9.6 grams phenol, and distilled water to 480 milliliters. Reagent B contained: 6.0 grams boric acid, 8.0 grams sodium hydroxide, 30.0 milliliters of commercial Clorox bleach, and distilled water to 200 milliliters. To 1.0 ml cell-free samples, diluted if necessary to give between 2 and 20 mg/l of ammonia-nitrogen, were added 5.0 ml of reagent A and 2.0 ml of reagent B. These samples were then mixed, heated in a boiling water bath for exactly five minutes, and cooled rapidly to room temperature in ice water. The optical density for these samples was then determined at a wavelength of 615 nanometers against a distilled water-reagent blank. Optical density readings were then compared to a standard curve with known concentrations of ammonia-nitrogen.

3. Nitrite-Nitrogen

Nitrite-nitrogen was determined in accordance with the diazotization method in "Standard Methods" (99). The nitrite concentration was

determined through the formation of a reddish-purple azo dye produced at pH 2.0 to 2.5 by the coupling of diazotized sulfanilic acid with naphthylamine hydrochloride. Cell-free samples were used for analysis by using the filtrate from 0.45 μm pore size membrane filters.

4. Nitrate-Nitrogen

Nitrate-nitrogen was determined by the Brucine Method outlined in "Standard Methods" (99). The reaction between nitrate and brucine produces a yellow color, which can be used for the colorimetric analysis. Again, cell-free membrane filtrate was used for the analysis.

5. Chemical Oxygen Demand

The chemical oxygen demand was determined in accordance with "Standard Methods" (99). Mercuric sulfate and silver sulfate were used for all COD determinations. COD analyses were made on the filtrate of samples filtered through membrane filters.

6. pH

The pH was monitored during experiments with pHydrion papers, and occasionally determined with a pH meter. A Beckman Zeromatic pH meter which was standardized periodically at pH 7.0 and pH 4.0 was employed for the pH determinations.

7. Zone Settling Velocity

Zone settling velocities were determined with the aid of a stopwatch and a 1000-ml graduated cylinder. A graduated cylinder was chosen so that the height of 1000 ml was equal to 1.12 feet. One liter

of mixed liquor suspended solids was placed in the cylinder, shaken well, and allowed to settle. The stopwatch was started at the instant settling of the biological solids began, and readings were taken as the clarified liquid interface reached each 100-ml mark on the cylinder. Next, the interface height in feet was plotted against the time in minutes. The slope of the straight line hindered settling portion of the interface height-versus-time curve gave the zone settling velocity in ft/min, which was converted to ft/hr.

# CHAPTER IV

# RESULTS

Batch units which were to be converted to continuous flow pilot plants to evaluate the performance of both one-stage and two-stage biological systems for nitrification of ammonia in wastewaters were begun on July 20, 1972. Operation and design information obtained from this investigation are presented in the following manner. First, information obtained from batch experiments with the nitrifying microorganisms is presented. Experiments were conducted to determine if the organic carbon source (glucose) inhibited the rate of nitrification achievable by the nitrifying microorganisms. Also included in this phase of the investigation were batch experimental data showing the effects for the combined addition of both organic carbon source and heterotrophic microorganisms on the autotrophic nitrifying population. Second, the operational performance of one-stage and two-stage continuous flow reactors was presented. Included in this phase of the study are data from both fluidized bed reactors (activated sludge) and fixed bed reactors (plastic media biological filter and rotating biological contactor).

## A. Batch Experiments

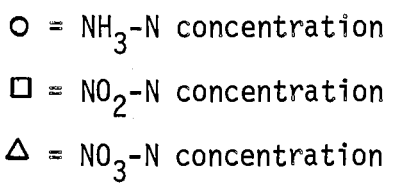
The conversion of ammonia-nitrogen to nitrite-nitrogen to nitrate-nitrogen in a batch reactor with a solids concentration of 700 mg/l

nitrifying microorganisms is shown in Figure 3. A concentration of 40 mg/l ammonia-nitrogen was removed from the system in five hours with almost complete conversion to nitrate-nitrogen. The nitrite-nitrogen concentration started at zero, increased to four mg/l at three hours, and decreased to zero again at five hours. Nitrite-nitrogen did not accumulate in the system, because as the nitrite was being produced from ammonia by Nitrosomonas, the Nitrobacter were oxidizing the nitrite to nitrate to obtain energy.

In the batch experiments where an organic carbon source (glucose) was added to the nitrifying microorganisms to check for inhibition, only COD concentrations of 750 mg/l or greater seemed to have any effect on the rate of nitrification. In all of the experiments where less than 750 mg/l COD was applied, no change in the nitrification rate was observed, and thus, the plots of the different forms of nitrogen versus time were all similar to Figure 3 and are not shown.

Figures 4 and 5 represent three batch studies with 650 mg/l nitrifying microorganisms in which different concentrations of COD were added at 1.5 hours after the experiment was initiated. One unit served as a control and received no COD, while the other two received 777 mg/l and 1139 mg/l COD, respectively. Both of the test reactors with 777 mg/l and 1139 mg/l gave practically identical results, and are therefore represented by one curve on the two figures. It can be seen in Figure 4 that the 50 mg/l of ammonia-nitrogen had been completely removed in seven hours by the control reactor. The two test reactors removed the ammonia-nitrogen at a slower rate and still had a residual of ammonia-nitrogen left after nine hours. Figure 5 shows the rate of nitrate-nitrogen production in the three reactors. The two test

Figure 3. Biological Conversion of Ammonia-Nitrogen to Nitrate-Nitrogen in a Batch Reactor with 700 mg/l Biological Solids



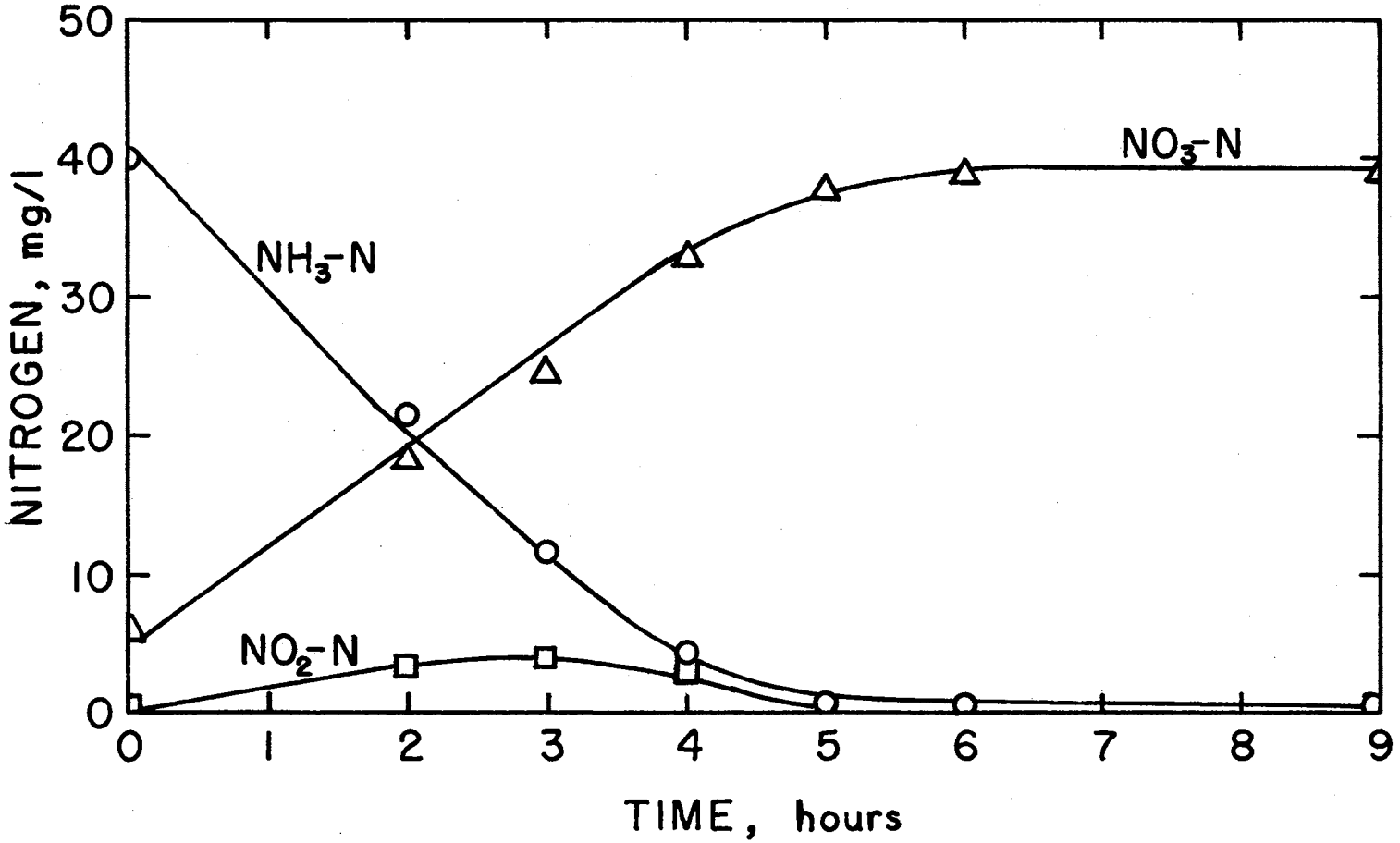

50
40
30
20
10
0
NITROGEN, mg/l
$NH_3$-N
$NO_3$-N
$NO_2$-N
0
1
2
3
4
5
6
7
8
9
TIME, hours

Figure 4. Ammonia-Nitrogen Removal Characteristics in Batch Reactors with 650 mg/l Nitrifying Microorganisms (with and without glucose addition)

O = no COD addition

Δ = 777 mg/l COD addition

Δ = 1139 mg/l COD addition

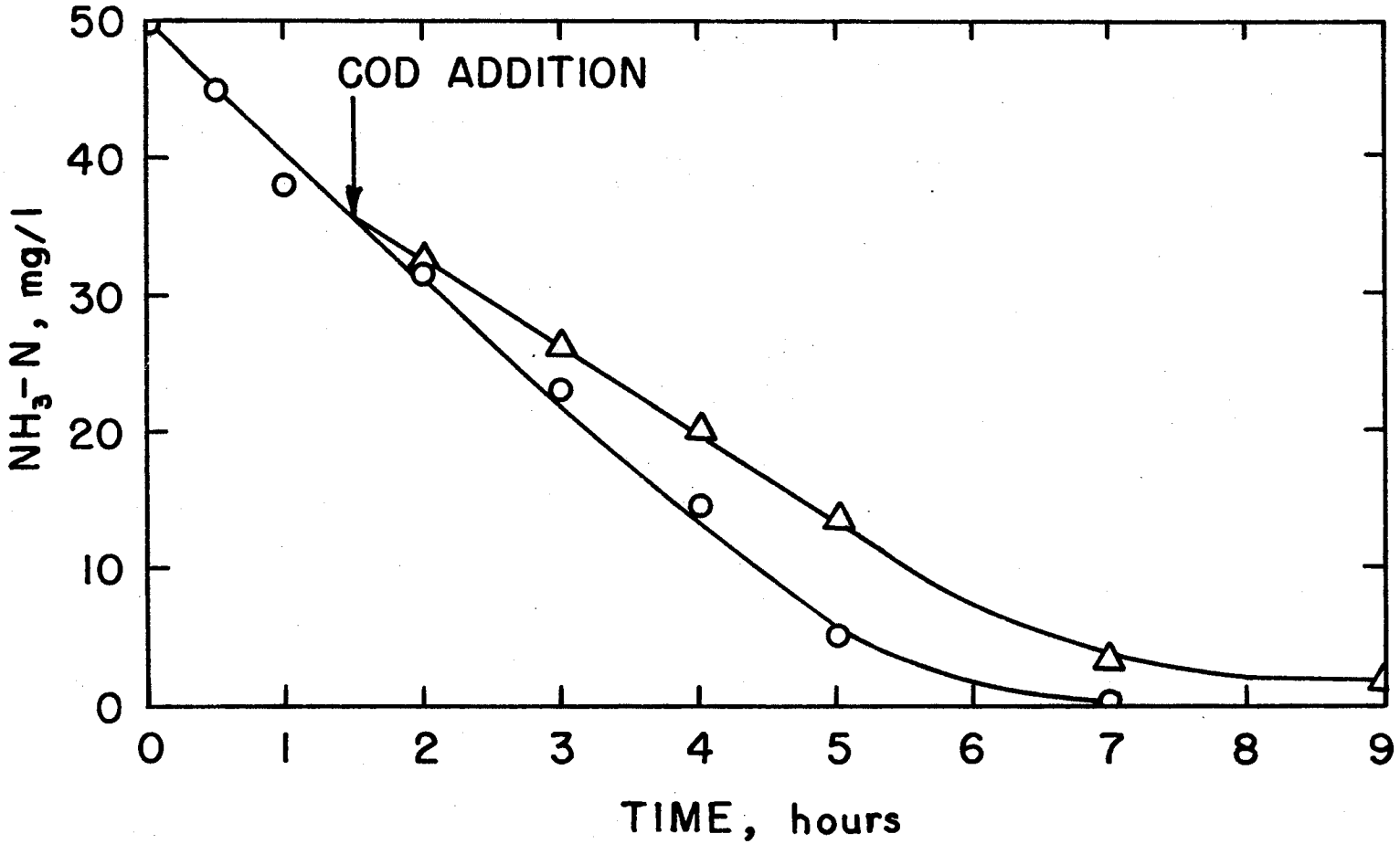
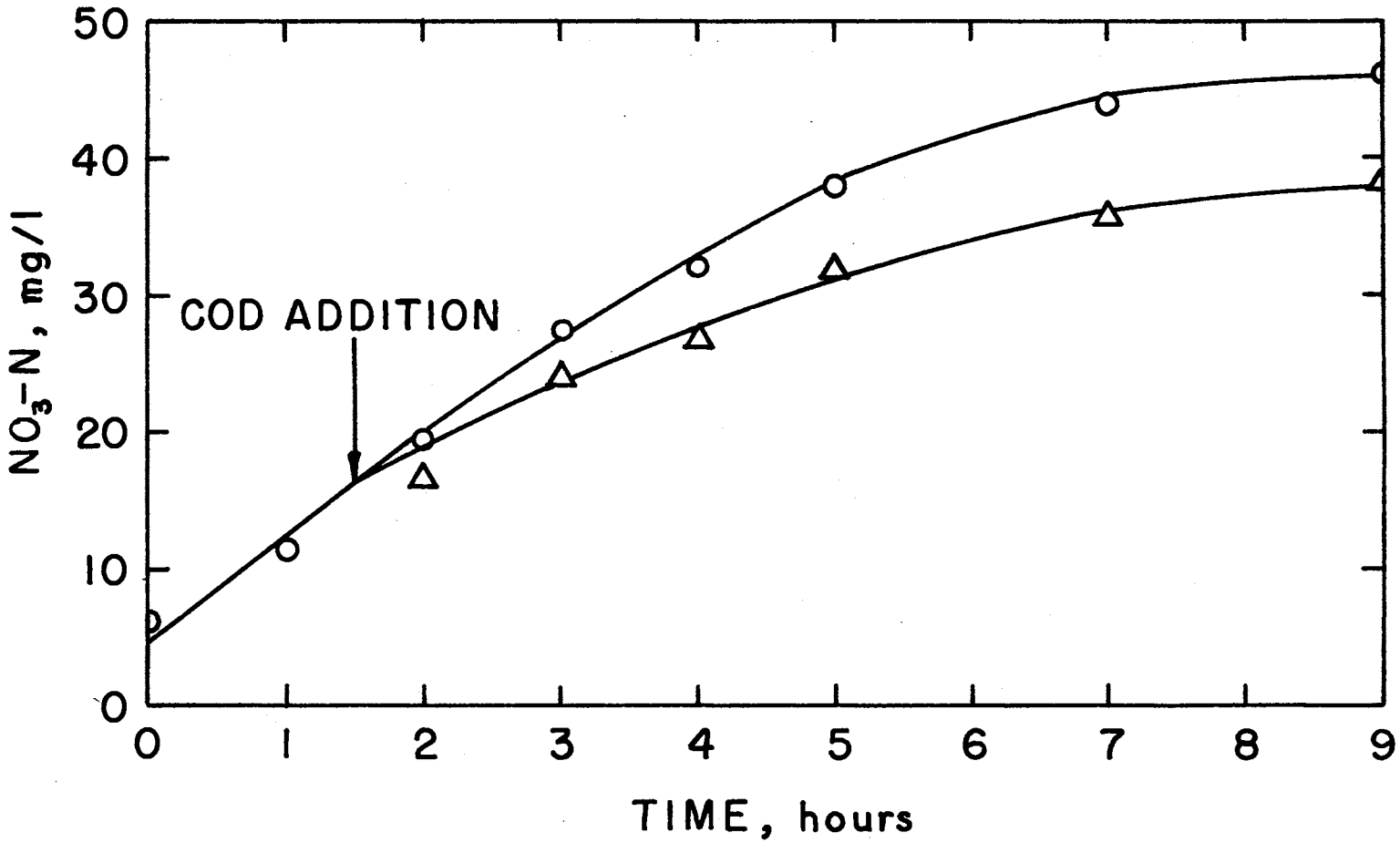

50
40
30
20
10
0
COD ADDITION
$NH_3$-N, mg/l
0
1
2
3
4
5
6
7
8
9
TIME, hours

Figure 5. Nitrate-Nitrogen Production in Batch Reactors with 650 mg/l Nitrifying Microorganisms (with and without glucose addition)

O = no COD addition

Δ = 777 mg/l COD addition

Δ = 1139 mg/l COD addition


50
40
30
20
10
0
$NO_3$-N, mg/l
COD ADDITION
0
1
2
3
4
5
6
7
8
9
TIME, hours

reactors produced nitrate at a slower rate than the reactor with no COD, and therefore produced less nitrate during the nine-hour testing period than the control reactor. After nine hours the control reactor had produced 46 mg/l of nitrate-nitrogen, while the test reactors had produced only 38 mg/l. The nitrogen conversion characteristics of the control reactor and the reactor with 1139 mg/l COD are shown in Table IV.

The combined effects of addition of both organic carbon source and heterotrophic microorganisms on the rate of ammonia-nitrogen removal and nitrate-nitrogen production by the nitrifying population are shown in Figure 6 through Figure 12. At the beginning of these experiments all reactors contained 58 mg/l of ammonia-nitrogen and 480 mg/l of nitrifying microorganisms. Three reactors were again employed--one of which served as the control unit. To one reactor were added 450 mg/l COD and 485 mg/l of heterotrophic microorganisms, while to the other reactor were added 1000 mg/l COD and 620 mg/l of heterotrophic microorganisms.

The ammonia-nitrogen removal characteristics of the three reactors are shown in Figure 6. From two hours through ten hours, all three reactors exhibited zero order kinetics or a straight line relationship of ammonia removal. In Figure 7, the percentage of ammonia-nitrogen that was removed with respect to time is plotted. After ten hours of operation the control reactor had removed only 62 percent of the ammonia-nitrogen, while 85 percent was removed by the reactor with 450 mg/l COD and 485 mg/l heterotrophs. The reactor with 1000 mg/l COD and 620 mg/l heterotrophs had removed 96 percent of its ammonia-nitrogen within the ten-hour testing period.

TABLE IV

BATCH-OPERATED NITRIFYING ACTIVATED SLUDGE WITH AND WITHOUT COD ADDITION, 700 mg/l SOLIDS

| Time (Hours) | Control Unit, (mg/l) | | | Test Unit, (mg/l) | | |
|---|---|---|---|---|---|---|
| | $NH_3$-N | $NO_3$-N | $NO_2$-N | $NH_3$-N | $NO_3$-N | $NO_2$-N |
| 0 | 50.0 | 6.4 | 0.01 | 50.0 | 6.4 | 0.01 |
| 0.5 | 45.0 | 9.0 | - | 45.0 | 9.0 | - |
| 1.0 | 38.0 | 11.5 | 0.19 | 38.0 | 11.5 | 0.19 |
| | | | | 1139 mg/l COD addition | | |
| 1.5 | 33.2 | 12.8 | - | 35.0 | 12.0 | - |
| 2.0 | 32.0 | 19.5 | 0.31 | 33.2 | 16.5 | 0.37 |
| 3.0 | 23.0 | 27.5 | 0.31 | 26.0 | 24.0 | 0.36 |
| 4.0 | 14.8 | 32.0 | 0.26 | 20.0 | 27.0 | 0.31 |
| 5.0 | 5.5 | 38.0 | 0.29 | 13.5 | 32.0 | 0.31 |
| 7.0 | 0.4 | 44.0 | 0.02 | 3.0 | 36.0 | 0.01 |
| 9.0 | 0.4 | 46.0 | 0.01 | 0.9 | 38.0 | 0.01 |

Figure 6. Ammonia-Nitrogen Removal Characteristics in Batch Reactors with 480 mg/l Nitrifying Microorganisms (with and without glucose and heterotrophic microorganism addition)

O = no COD or heterotroph addition

Δ = 450 mg/l COD and 485 mg/l heterotrophic microorganisms

□ = 1000 mg/l COD and 620 mg/l heterotrophic microorganisms

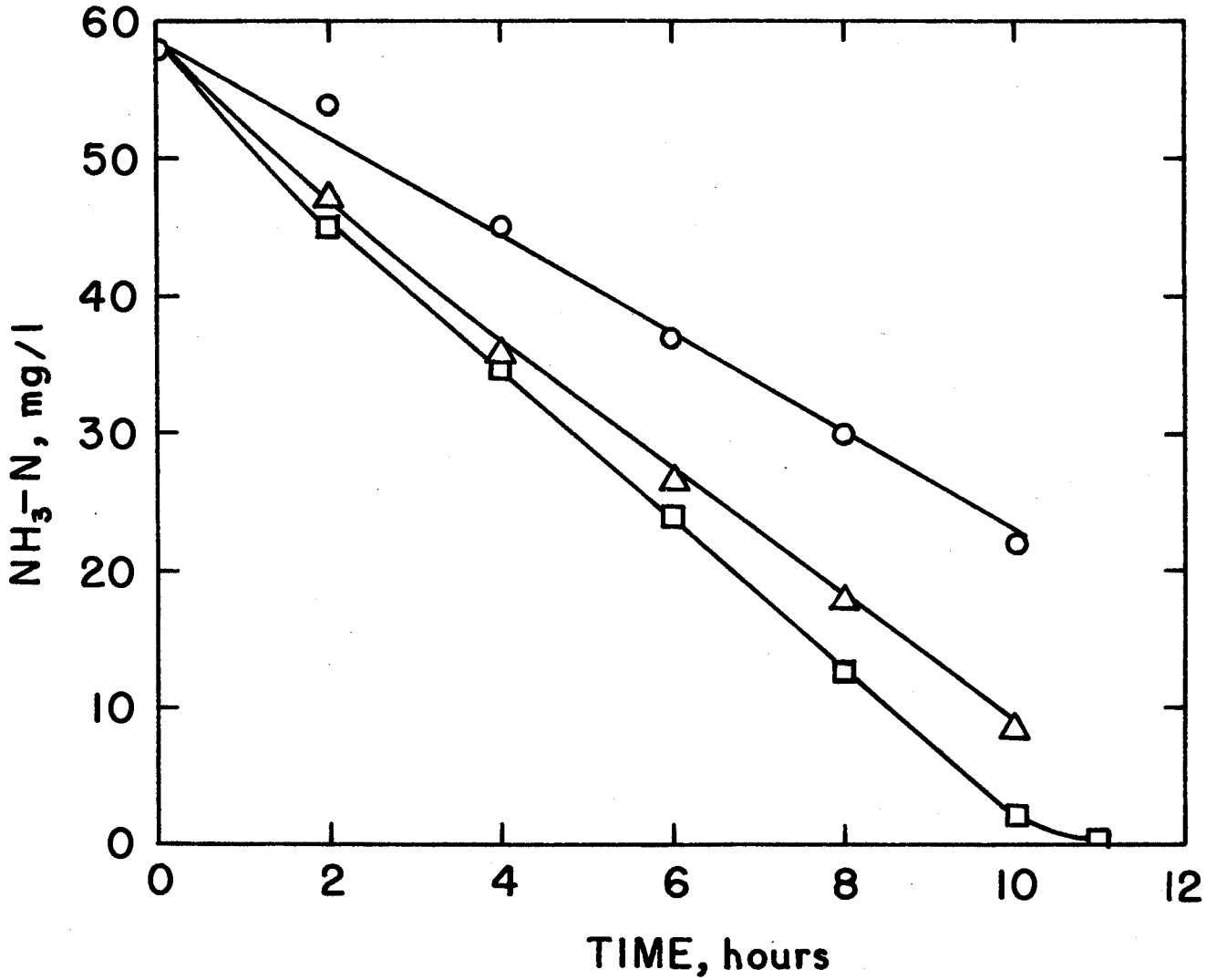
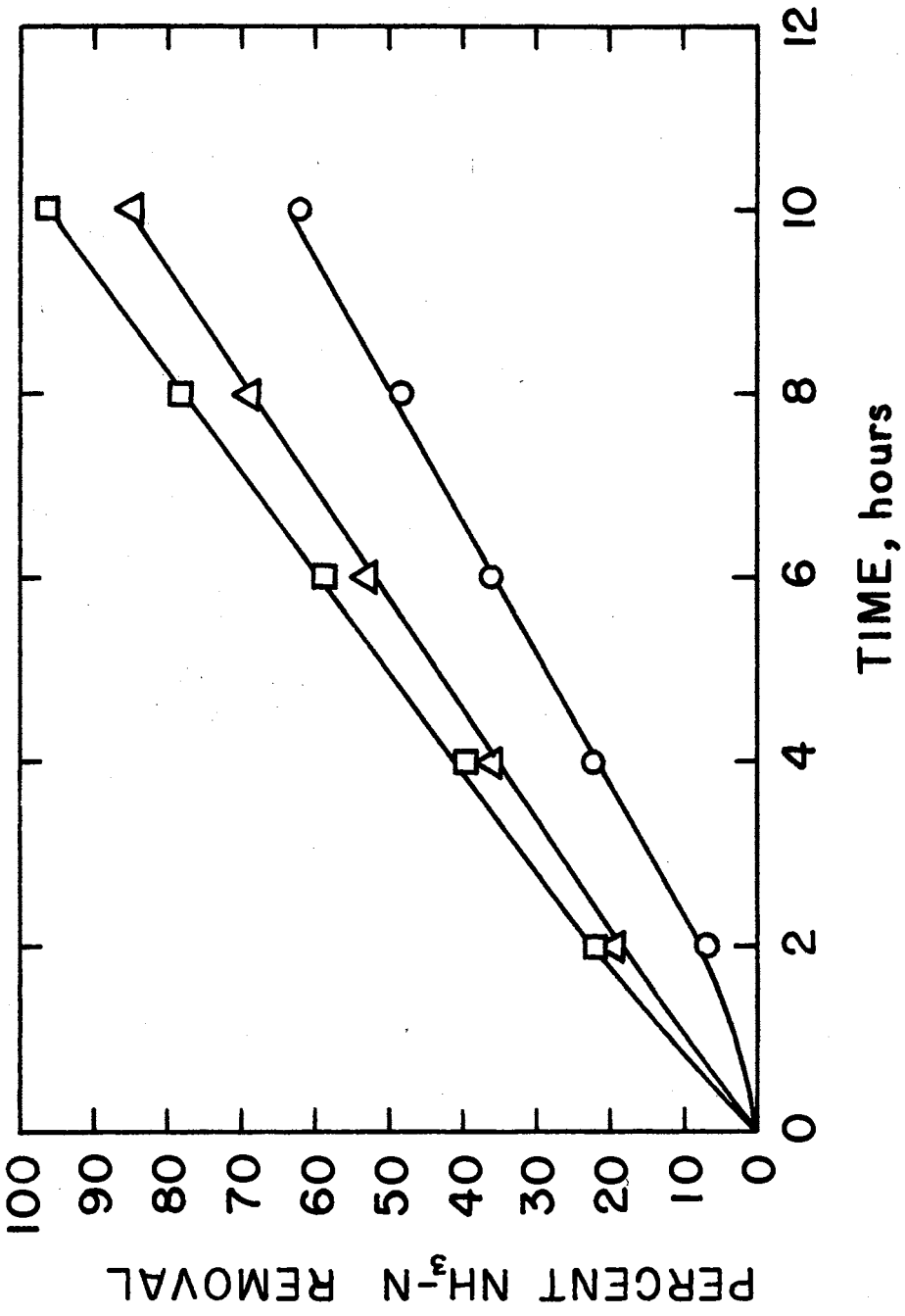

60
50
40
30
20
10
0
0
2
4
6
8
10
12
$NH_3$-N, mg/l
TIME, hours

Figure 7. Percent Ammonia-Nitrogen Removed in Batch Reactors with 480 mg/l Nitrifying Microorganisms (with and without glucose and heterotrophic microorganism addition)

○ = no COD or heterotroph addition

△ = 450 mg/l COD and 485 mg/l heterotrophic microorganisms

□ = 1000 mg/l COD and 620 mg/l heterotrophic microorganisms


PERCENT NH3-N REMOVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
TIME, hours

Nitrate-nitrogen production by the three reactors is shown in Figure 8. The production of nitrate by the two test reactors was essentially the same and is represented by one curve in the figure. In Figure 9, the percent of ammonia-nitrogen removed from the reactors which was converted to nitrate-nitrogen is shown. As can be seen, the control unit converted approximately 91 percent of its ammonia-nitrogen to nitrate-nitrogen, while the percentage of conversion for the two test units was considerably less. The reactor with 450 mg/l COD converted approximately 56 percent of its ammonia-nitrogen to nitrate-nitrogen, while the reactor with 1000 mg/l COD converted only approximately 45 percent of its ammonia-nitrogen to nitrate-nitrogen. The amount of ammonia-nitrogen removed from the reactors which was not converted to nitrate-nitrogen or nitrite-nitrogen was incorporated into the biological cells of the systems. The amount of ammonia-nitrogen removed by incorporation into cellular material is shown in Figure 10. As would be expected, the control unit removed very little ammonia-nitrogen by cell synthesis, since it converted 91 percent of its ammonia-nitrogen to nitrate-nitrogen. The control unit removed only about seven percent of its ammonia-nitrogen by cell synthesis. The 450 mg/l COD and 1000 mg/l COD batch reactors removed about 45 percent and 55 percent of ammonia-nitrogen, respectively, by incorporation into cellular material.

The high percentage of ammonia-nitrogen incorporated into cell matter in the test reactors was due to the large amount of growth of heterotrophic microorganisms as shown in Figure 11 and Figure 12. In eight hours the biological solids concentration increased from 965 mg/l to 1332 mg/l in the reactor with 450 mg/l COD (Figure 11). Over an

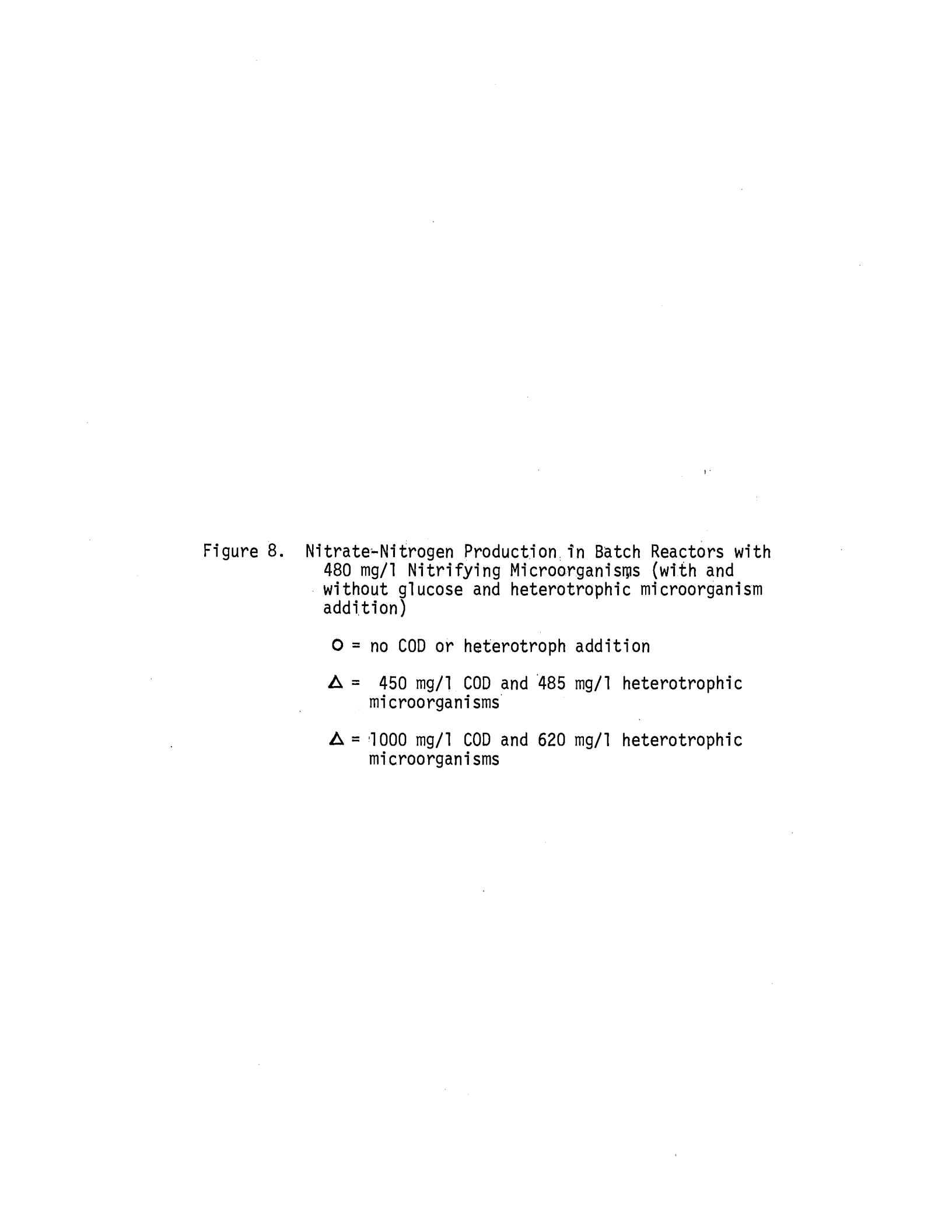


Figure 8. Nitrate-Nitrogen Production in Batch Reactors with 480 mg/l Nitrifying Microorganisms (with and without glucose and heterotrophic microorganism addition)

O = no COD or heterotroph addition

Δ = 450 mg/l COD and 485 mg/l heterotrophic microorganisms

Δ = 1000 mg/l COD and 620 mg/l heterotrophic microorganisms

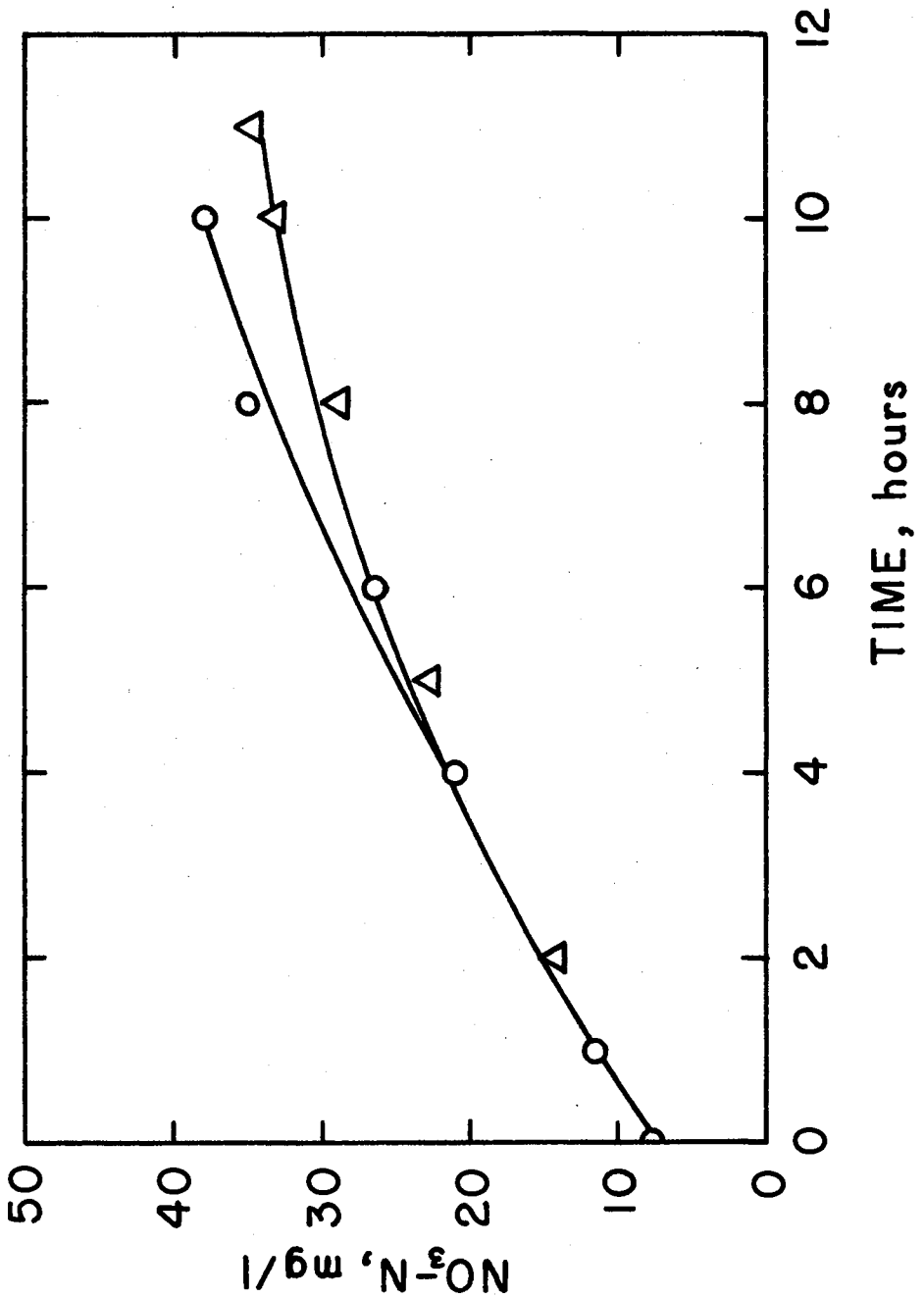

$NO_3^-N$, mg/l
0
10
20
30
40
50
0
2
4
6
8
10
12
TIME, hours

Figure 9. Percent Ammonia-Nitrogen Removed that was Converted to Nitrate-Nitrogen in Batch Reactors with 480 mg/l Nitrifying Microorganisms (with and without glucose and heterotrophic microorganism addition)

○ = no COD or heterotroph addition

△ = 450 mg/l COD and 485 mg/l heterotrophic microorganisms

□ = 1000 mg/l COD and 620 mg/l heterotrophic microorganisms

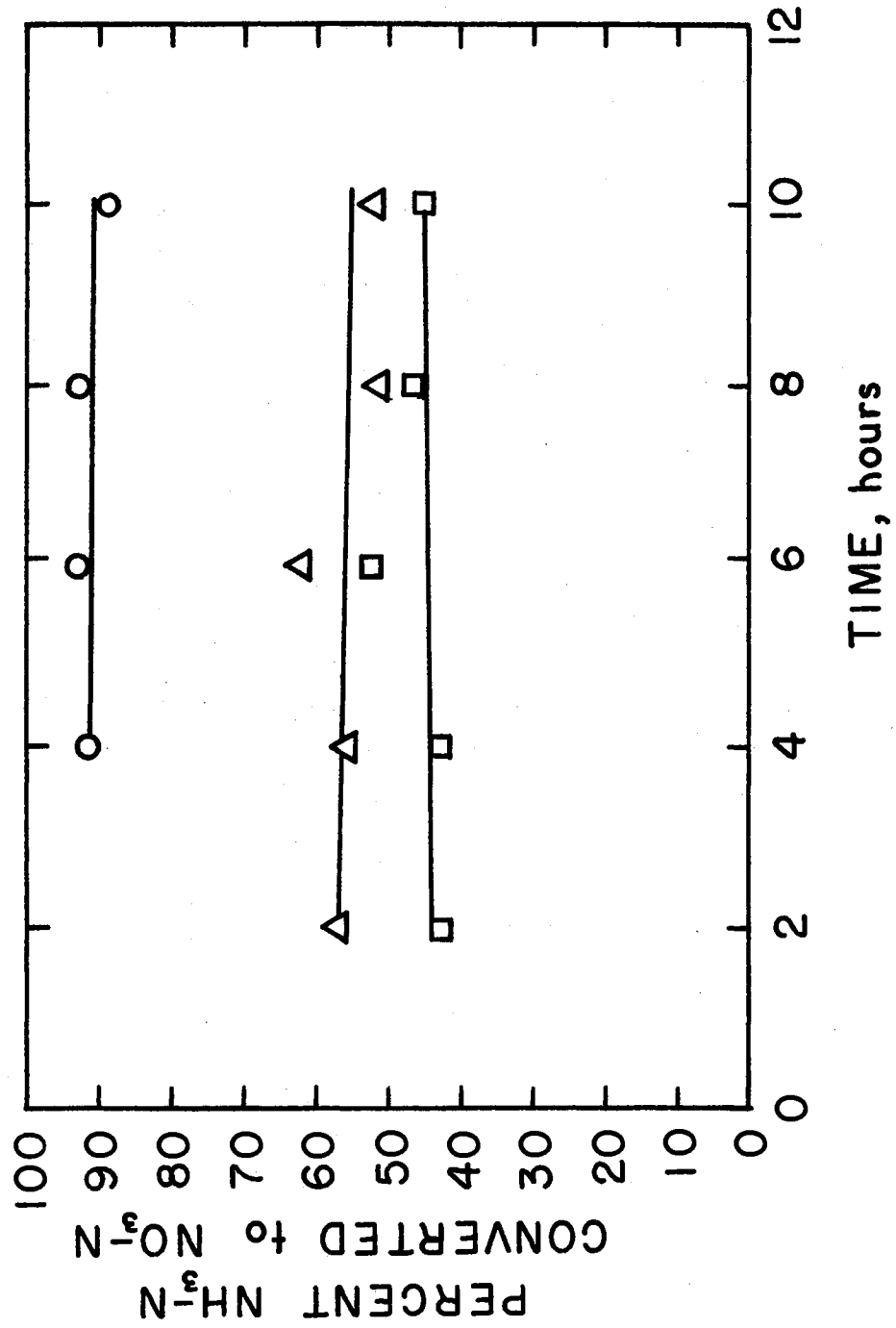

PERCENT $NH_3$-N
CONVERTED to $NO_3$-N
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
TIME, hours

Figure 10. Percent Ammonia-Nitrogen Removed that was Converted to Biological Cell Matter in Batch Reactors with 480 mg/l Nitrifying Microorganisms (with and without glucose and heterotrophic microorganism addition)

○ = no COD or heterotroph addition

△ = 450 mg/l COD and 485 mg/l heterotrophic microorganisms

□ = 1000 mg/l COD and 620 mg/l heterotrophic microorganisms

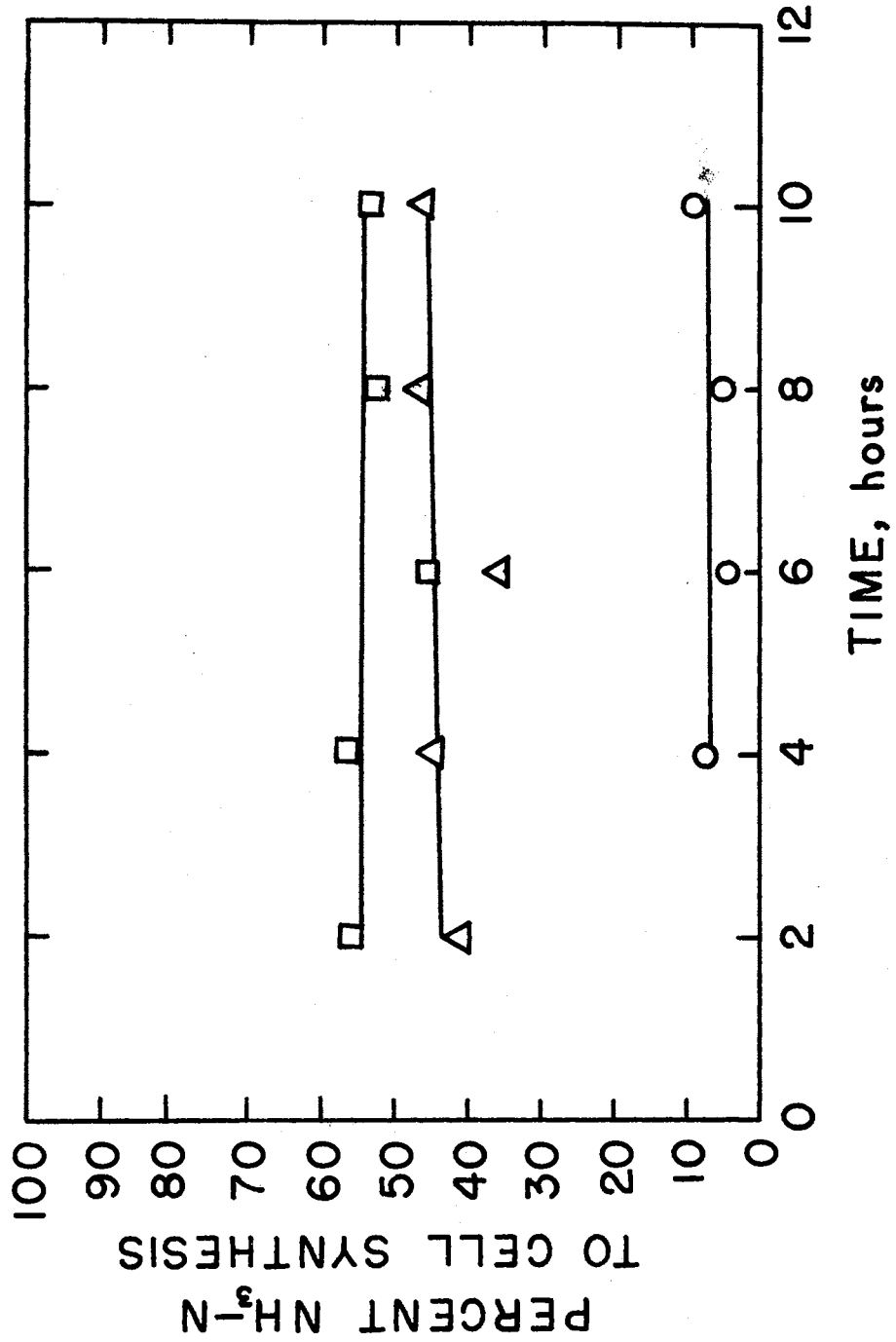
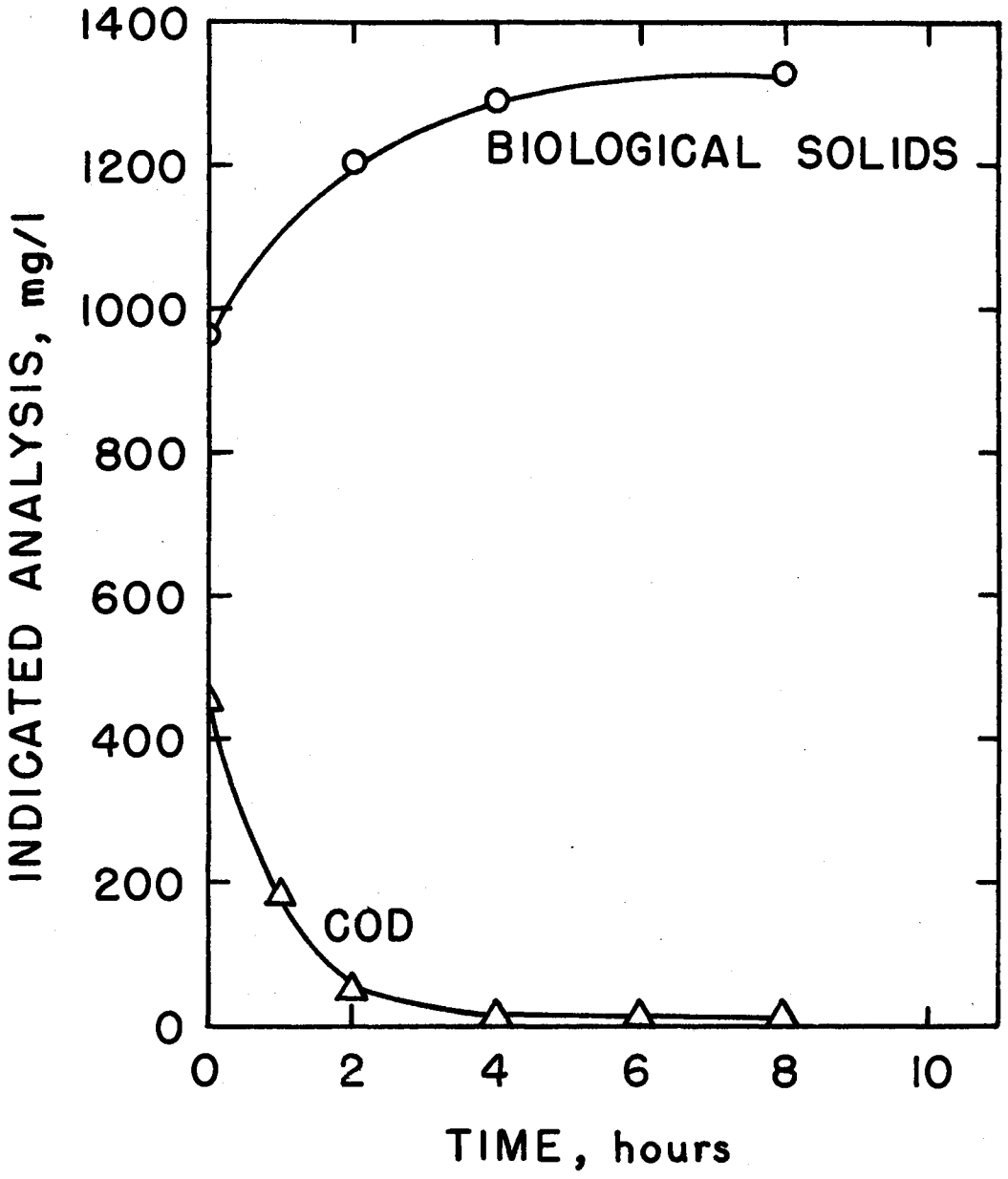

PERCENT NH3–N
TO CELL SYNTHESIS
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
TIME, hours

Figure 11. COD Removal Characteristics and Biological Solids Production in Batch Experiment with 480 mg/l Nitrifying Microorganisms, 485 mg/l Heterotrophic Microorganisms, and 450 mg/l COD


1400
1200
1000
800
600
400
200
0
INDICATED ANALYSIS, mg/l
BIOLOGICAL SOLIDS
COD
0
2
4
6
8
10
TIME, hours

11-hour time period the solids concentration increased from 1100 mg/l to 1600 mg/l in the reactor, with 1000 mg/l COD (Figure 12). The yield in terms of COD for these two reactors were 0.8 and 0.5 mg/l solids per mg/l COD, respectively. The biological solids concentration in the control reactor increased only from 480 mg/l to 498 mg/l. Values of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, COD, and biological solids concentrations for all three reactors during the course of the experiment are shown in Table V.

## B. Continuous Flow Investigations

Performance data from both fluidized bed reactors and fixed bed reactors are presented in the following discussion. Both two-stage and one-stage activated sludge systems for nitrification were investigated. Plastic media biological filters and a rotating biological contactor were also studied with respect to nitrification.

### 1. Operational Performance of a Two-Stage Activated Sludge System

The influence of the mean cell residence time on the concentrations of ammonia-nitrogen and nitrate-nitrogen found in the effluent of the second sludge reactor, nitrification reactor, of the two-stage activated sludge process is shown in Figures 13 and 14, respectively. The concentration of ammonia-nitrogen in the feed to the system was approximately 68 mg/l, while in the effluent of the first reactor which served as the feed to the second reactor, the concentration of ammonia-nitrogen was about 49 mg/l throughout the investigation (Figure 13). The concentration of ammonia-nitrogen in the effluent from the second reactor over

Figure 12. COD Removal Characteristics and Biological Solids Production in Batch Experiment with 480 mg/l Nitrifying Microorganisms, 620 mg/l Heterotrophic Microorganisms, and 1000 mg/l COD

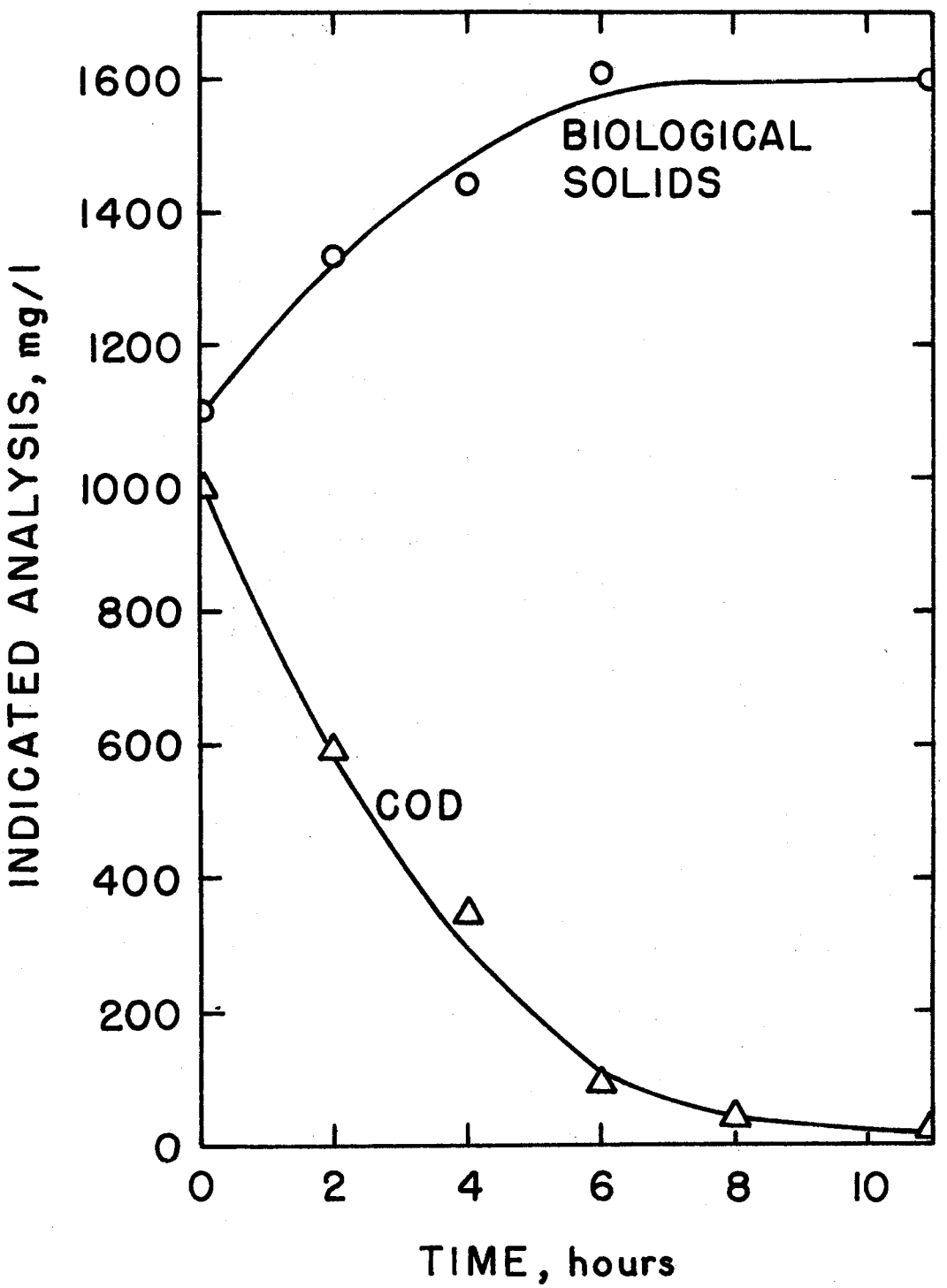

1600
1400
1200
1000
800
600
400
200
0
INDICATED ANALYSIS, mg/l
BIOLOGICAL SOLIDS
COD
0
2
4
6
8
10
TIME, hours

TABLE V

BATCH-OPERATED NITRIFYING ACTIVATED SLUDGE WITH AND WITHOUT COD AND MIXED POPULATION HETEROTROPHIC MICROORGANISM ADDITION, 480 mg/l NITRIFYING MICROORGANISMS PER UNIT

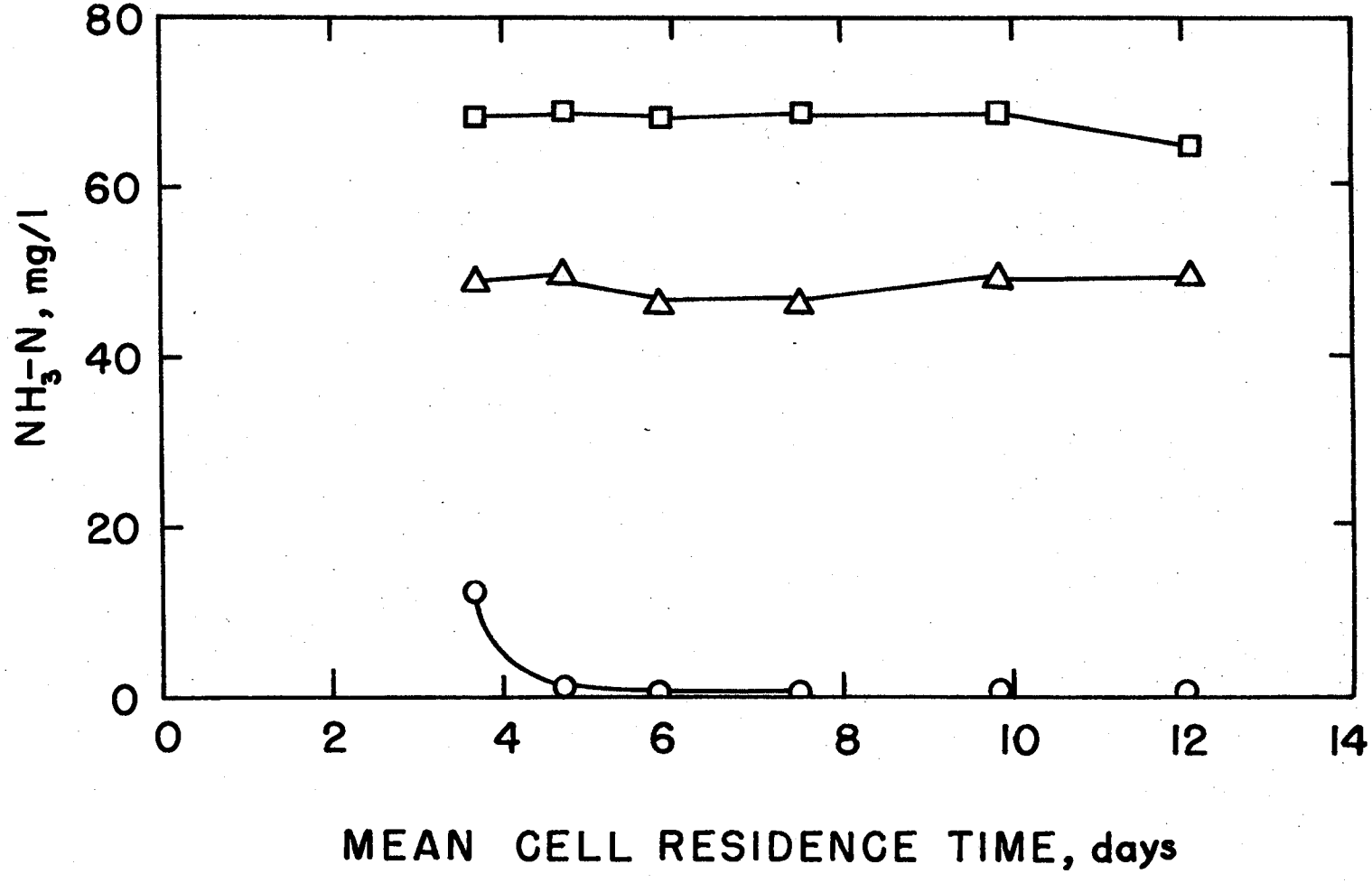
| Time (Hours) | Control Unit, (mg/l) | | | | | Test Unit #1, (mg/l) | | | | | Test Unit #2, (mg/l) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | Solids | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | Solids | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | Solids |
| 0 | 58.0 | 8.0 | 0.1 | 7.9 | 480 | 58.0 | 8.0 | 0.1 | 450.0 | 965 | 58.0 | 8.0 | 0.1 | 1000.0 | 1100 |
| 1 | 57.5 | 11.6 | - | 7.9 | - | 52.0 | 10.6 | - | 181.0 | - | 50.0 | 10.4 | - | - | - |
| 2 | 54.0 | 16.5 | 0.2 | 7.9 | - | 47.0 | 14.3 | 0.3 | 51.2 | 1205 | 45.0 | 13.5 | 0.2 | 590.0 | 1336 |
| 3 | 50.0 | 20.0 | - | 7.9 | - | 41.0 | 17.5 | - | 39.4 | - | 39.0 | 16.5 | - | 465.0 | - |
| 4 | 45.0 | 21.0 | 0.1 | 7.9 | 481 | 36.0 | 20.0 | 0.2 | 19.7 | 1292 | 35.0 | 18.0 | 0.3 | 346.0 | 1443 |
| 5 | 41.0 | 24.5 | - | 7.9 | - | 32.0 | 23.0 | - | - | - | 30.0 | 23.0 | - | - | - |
| 6 | 37.0 | 27.5 | 0.5 | 7.9 | 485 | 27.2 | 27.5 | 0.3 | 19.7 | - | 24.0 | 26.0 | 0.3 | 90.5 | 1608 |
| 8 | 30.0 | 35.0 | 0.5 | 7.9 | 498 | 18.0 | 29.0 | 0.3 | 19.7 | 1332 | 12.8 | 29.0 | 0.3 | 47.3 | - |
| 10 | 22.0 | 38.0 | 0.4 | 7.9 | - | 8.6 | 33.5 | 0.5 | - | - | 2.4 | 33.5 | 0.3 | - | - |
| 11 | - | - | - | - | - | - | - | - | - | - | 0.8 | 35.0 | 0 | 25.7 | 1600 |

Figure 13. Ammonia-Nitrogen Removal Characteristics of the Two-Stage Activated Sludge System (carbonaceous reactor operated at a constant $\Theta_c$ and nitrification reactor operated over a range of $\Theta_c$s)

□ = $NH_3$-N concentration in feed to the system

△ = $NH_3$-N concentration in effluent of the first reactor and influent to the second reactor

○ = $NH_3$-N concentration in effluent of the second reactor


80
60
40
20
0
$NH_3$-N, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

the range of mean cell residence times investigated, 3.7 days to 12.1 days, is also shown in Figure 13. This value decreased from 12 mg/l ammonia-nitrogen at 3.7 days to zero with increasing mean cell residence times.

The variation of nitrate-nitrogen concentration in the effluent of the nitrification reactor with changing mean cell residence times is shown in Figure 14. Nitrate-nitrogen concentration increased from 22.6 mg/l at 3.7 days with increasing mean cell residence times and leveled off at about six days at an effluent concentration of around 35 mg/l. The percent of ammonia-nitrogen removed from the reactor that was converted to nitrate-nitrogen along with the percent of ammonia-nitrogen removed is shown in Figure 15. Both of these parameters increased with increasing mean cell residence times until around six days had been reached, at which time these values leveled off. The percent of ammonia-nitrogen removed that was converted to nitrate-nitrogen increased slower with increasing mean cell residence times than did the percent of ammonia-nitrogen removed from the reactor.

As noted in Figures 13, 14, and 15, the parameters measured all reached values of zero at a mean cell residence time of three days. This value of three days was taken from Figure 16, which shows the relationship of the reactor microorganism concentration with respect to mean cell residence times. Reactor microorganism concentration decreased with lower mean cell residence times. As shown on the graph, the solids concentration became zero at approximately three days and complete washout of the nitrifying microorganisms occurred at sludge ages less than three days. At a mean cell residence time of three days or less under the experimental conditions employed here, no biological

Figure 14. Nitrate-Nitrogen Concentration in the Effluent of the Nitrification Reactor of the Two-Stage Activated Sludge System Versus Mean Cell Residence Time

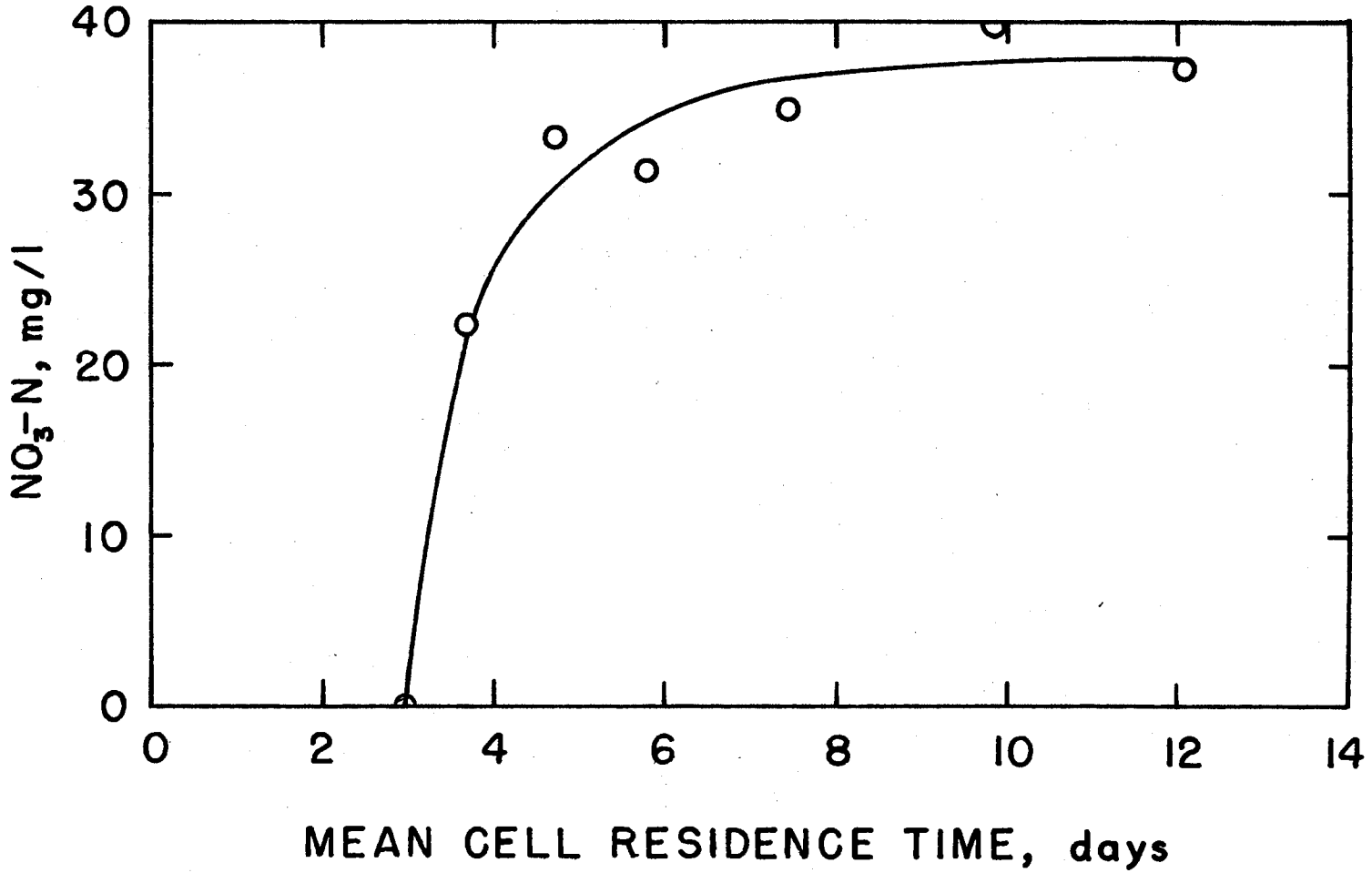

40
30
20
10
0
$NO_3$-N, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 15. Nitrification Efficiency of the Nitrification Reactor of the Two-Stage System Versus Mean Cell Residence Time

O = percent $NH_3$-N removed

$\Delta$ = percent $NH_3$-N removed that was converted to $NO_3$-N

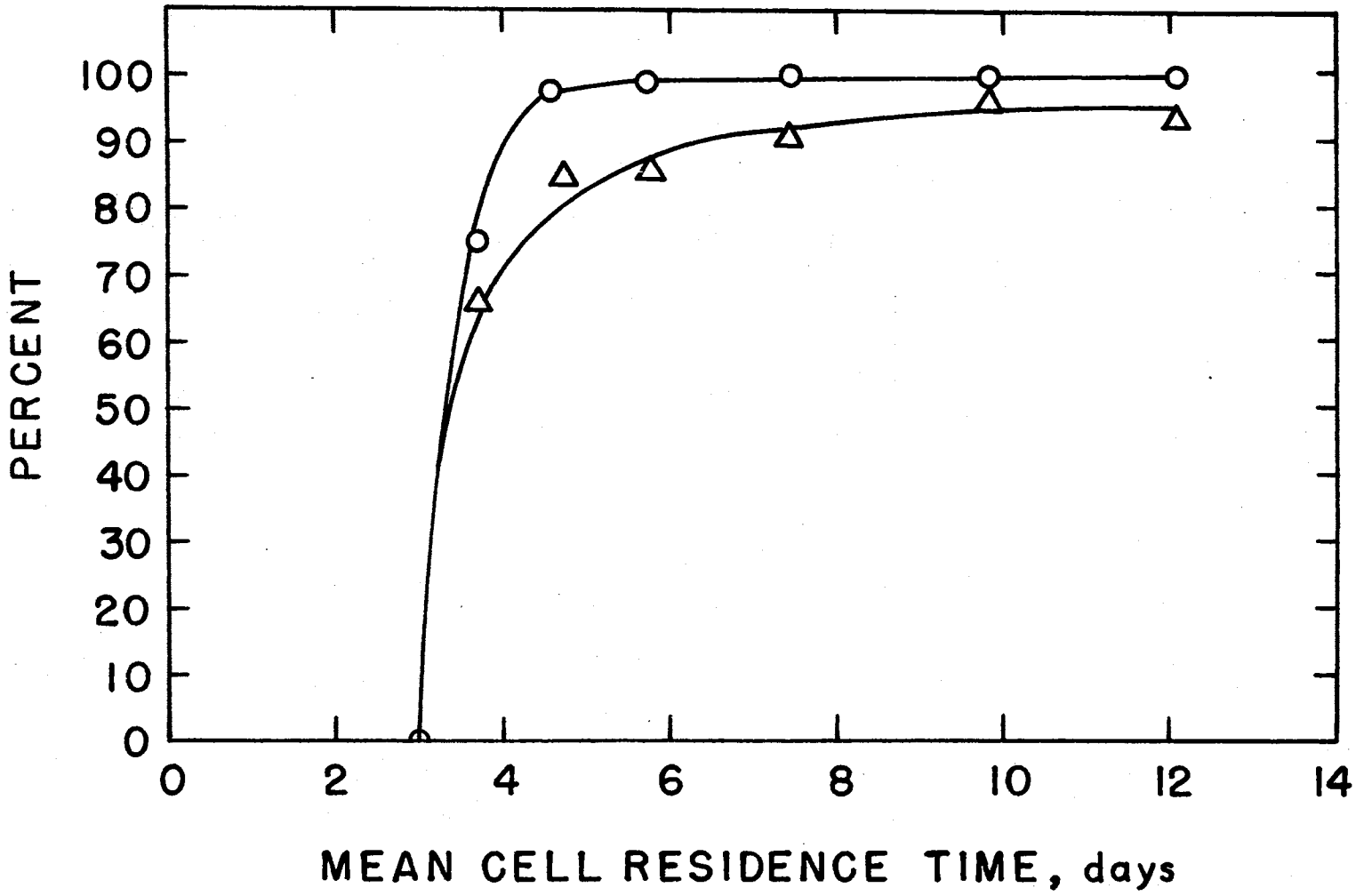
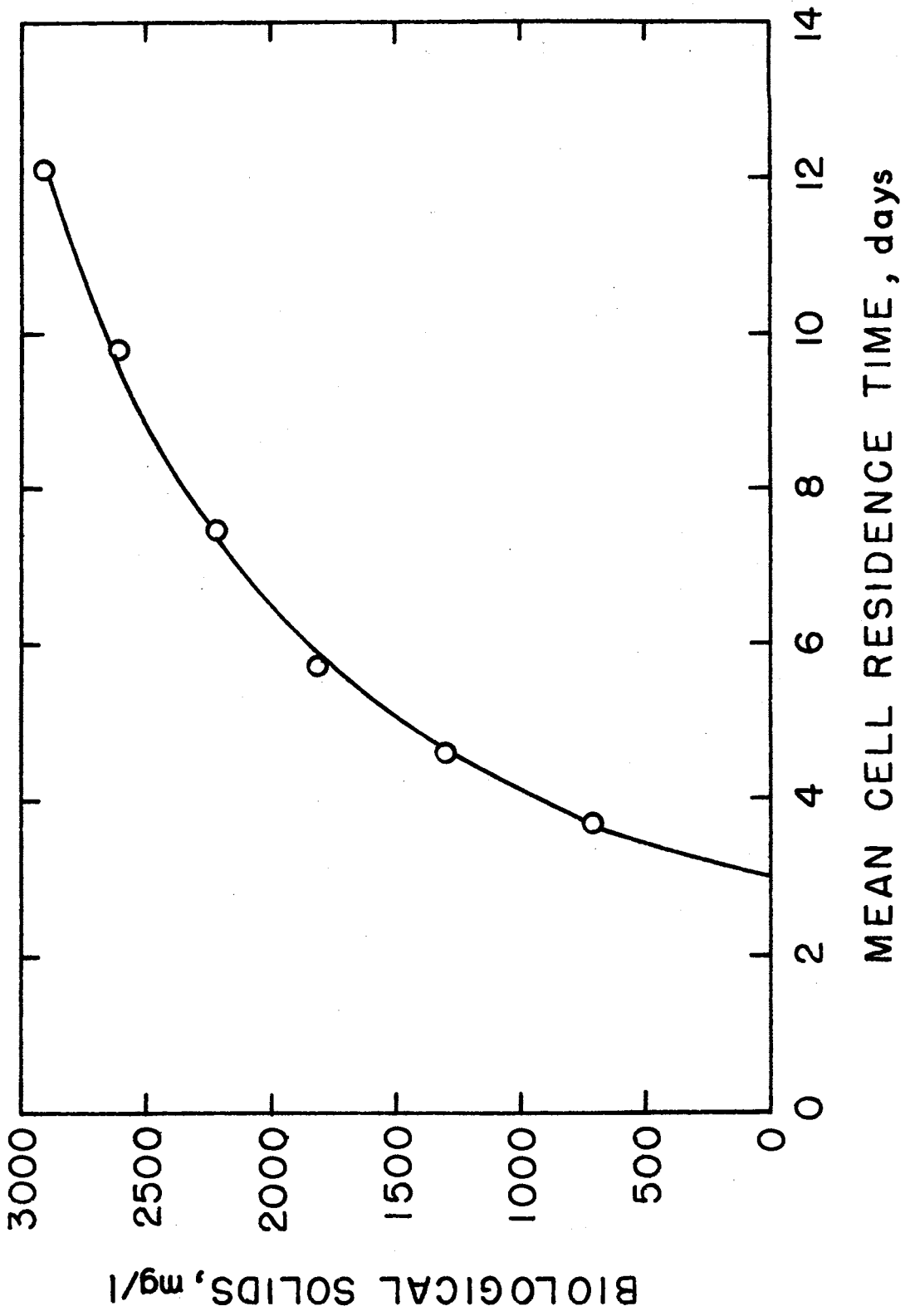

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 16. Nitrification Reactor Microorganism Concentration Versus Mean Cell Residence Time


BIOLOGICAL SOLIDS, mg/l
3000
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

nitrification could be achieved. This is in excellent agreement with other researchers showing the onset of nitrification occurring at approximately a three-day mean cell residence time under normal temperatures (60)(61)(69)(101).

The observed yield coefficient increased as the mean cell residence time decreased, as shown in Figure 17. Two different substrate values were employed to calculate the observed yield coefficient. First, the ammonia concentration in the influent was used, then the ammonia plus the concentration of nitrite produced from the ammonia was used to calculate the observed yield. In both cases the observed yield increased at lower mean cell residence times and flattened out at higher sludge ages. Values of observed yield varied from 4.1 mg of microorganisms produced per mg of ammonia utilized at a sludge age of 3.7 days to 2.8 mg of microorganisms per mg of ammonia at 12.1 days. In terms of ammonia plus nitrite the observed yield varied from 0.52 to 0.33 over the same range of mean cell residence times.

Specific utilization, again based on both ammonia concentration and ammonia plus nitrite concentration, is shown in Figure 18. The specific utilization decreased as the mean cell residence time increased. This decrease was due to the increase in reactor microorganism concentration and relative constancy of the rate of substrate utilization.

The relationship between the food-to-microorganism ratio and mean cell residence time is shown in Figure 19. With increasing mean cell residence time the food-to-microorganism ratio based on both ammonia concentration and ammonia plus nitrite concentration decreased. This can be explained by noting that in Figure 16 the reactor microorganism

Figure 17. Nitrification Reactor Observed Yield Versus Mean Cell Residence Time

O = $Y_{obs}$ in terms of $NH_3$

Δ = $Y_{obs}$ in terms of $NH_3 + NO_2$

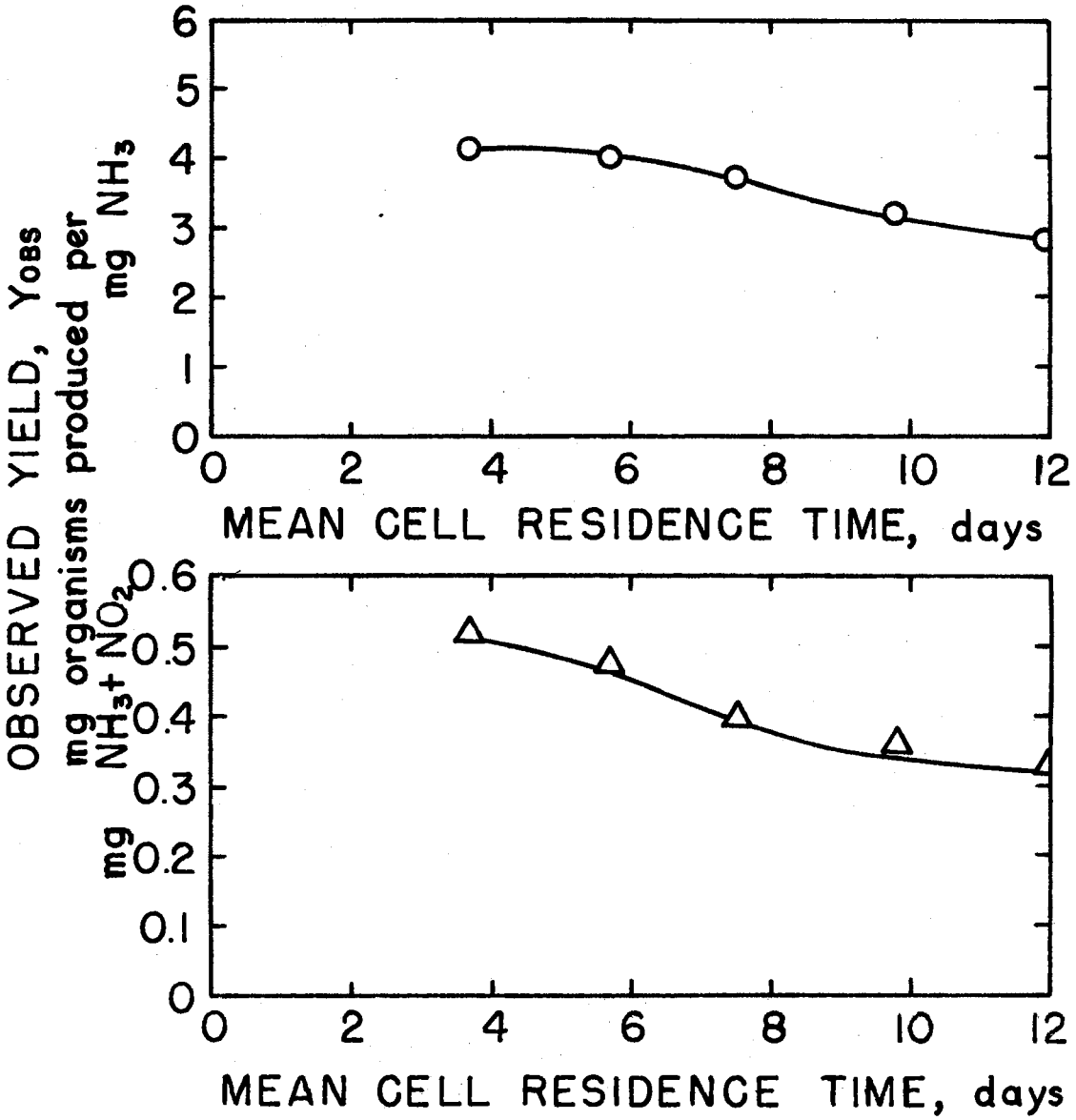

OBSERVED YIELD, Yobs
mg organisms produced per mg NH3
0
1
2
3
4
5
6
0
2
4
6
8
10
12
MEAN CELL RESIDENCE TIME, days
mg NH3+NO2
0
0.1
0.2
0.3
0.4
0.5
0.6
0
2
4
6
8
10
12
MEAN CELL RESIDENCE TIME, days

Figure 18. Nitrification Reactor Specific Utilization Versus Mean Cell Residence Time

O = U in terms of $NH_3$

Δ = U in terms of $NH_3 + NO_2$

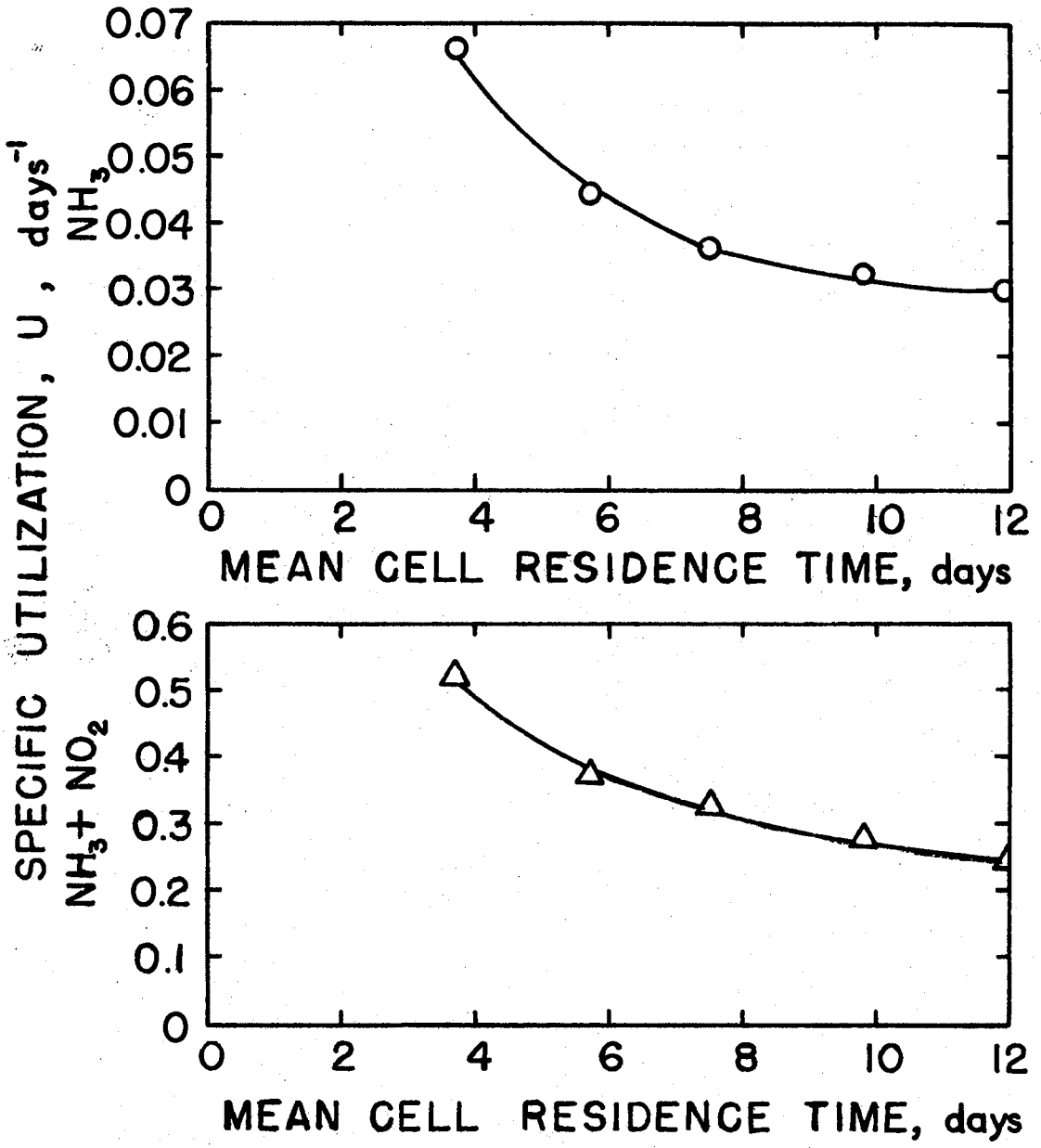
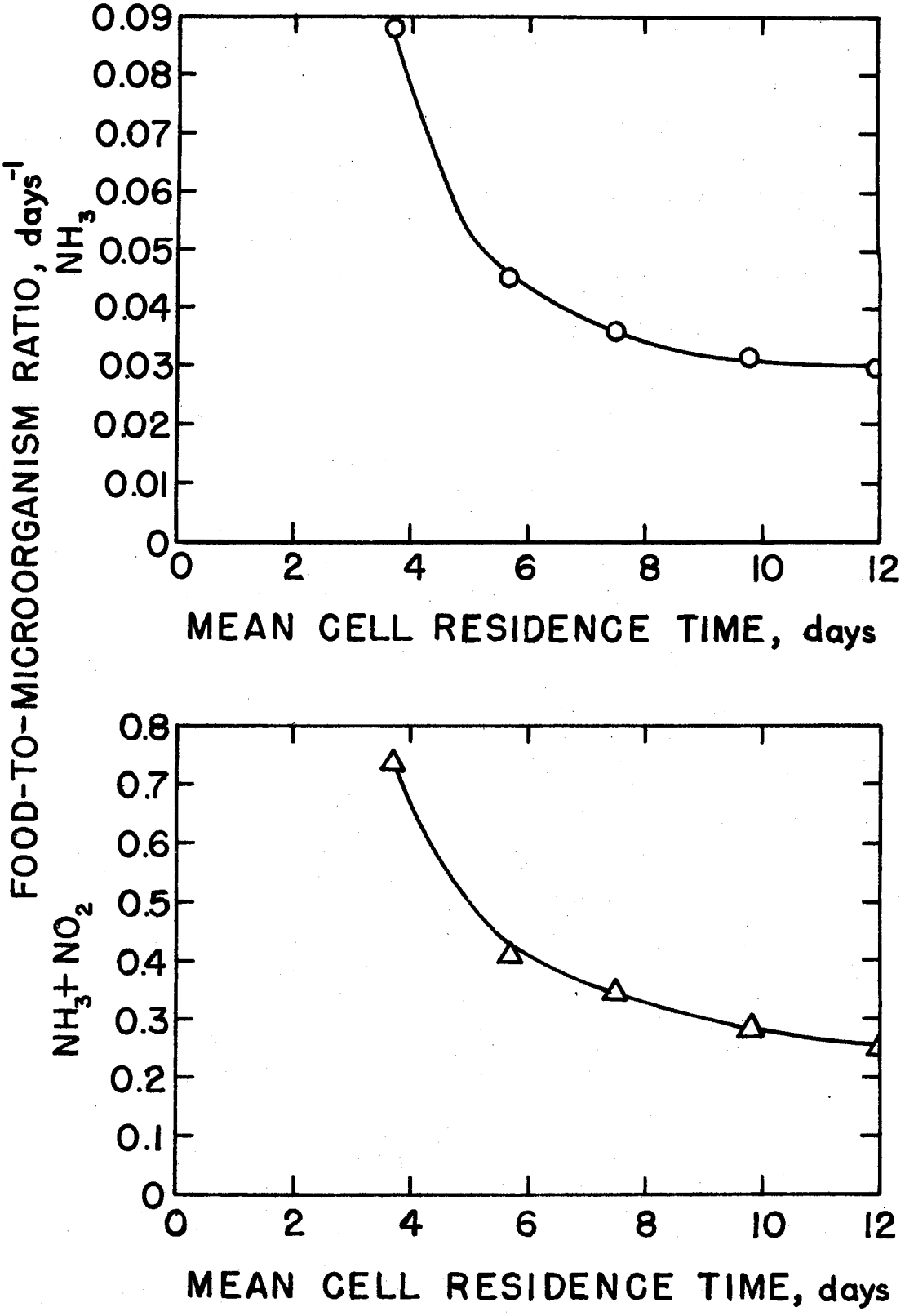

SPECIFIC UTILIZATION, U, days⁻¹
NH3
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
12
MEAN CELL RESIDENCE TIME, days
NH3+ NO2
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
MEAN CELL RESIDENCE TIME, days

Figure 19. Nitrification Reactor Food to Microorganism Ratio Versus Mean Cell Residence Time

O = F:M in terms of $NH_3$

Δ = F:M in terms of $NH_3 + NO_2$


FOOD-TO-MICROORGANISM RATIO, days⁻¹
NH3
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
12
MEAN CELL RESIDENCE TIME, days
NH3+NO2
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
MEAN CELL RESIDENCE TIME, days

concentration increased with increasing sludge age, while the amount of substrate remained relatively constant (Figure 13).

The COD removal efficiency of the carbonaceous unit remained almost constant during this study, as shown in Figure 20. COD removal efficiency measured on membrane filtrate exceeded 90 percent at all times. Filtrate COD in the effluent varied from about 10 mg/l to 40 mg/l. A very small amount of COD, ranging from about 5 mg/l to 20 mg/l, was removed in the nitrification unit.

Zone settling velocities of the sludge mass in the nitrification unit increased with increasing mean cell residence times, as shown in Figure 21. The values for zone settling velocities varied from 5 ft/hr at 3.7 days to about 9.8 ft/hr at 10 days and greater. All of the zone settling velocities plotted in Figure 21 were determined when the pH of the unit was maintained at approximately pH 8.0. A summary of the operational characteristics of the nitrification unit is presented in Table VI.

The effect of varying pH values on the zone settling velocities of a 2800 mg/l concentration of nitrifying microorganisms is shown in Figure 22. When sodium hydroxide was not used to adjust the pH, the nitrifying microorganisms lowered the pH by the production of nitric acid and as a result, decreased the zone settling velocity of the sludge mass. As can be seen from Figure 22, maximum settling rate was achieved at approximately pH 8.5, and the settling rate fell rapidly as the pH dropped.

Figure 20. COD Removal Characteristics of the Two-Stage Activated Sludge System (carbonaceous reactor operated at a constant $\Theta_c$ and nitrification reactor operated over a range of $\Theta_c$s)

□ = COD concentration in feed to the system

△ = COD concentration in effluent of the first reactor and influent to the second reactor

○ = COD concentration in effluent of the second reactor

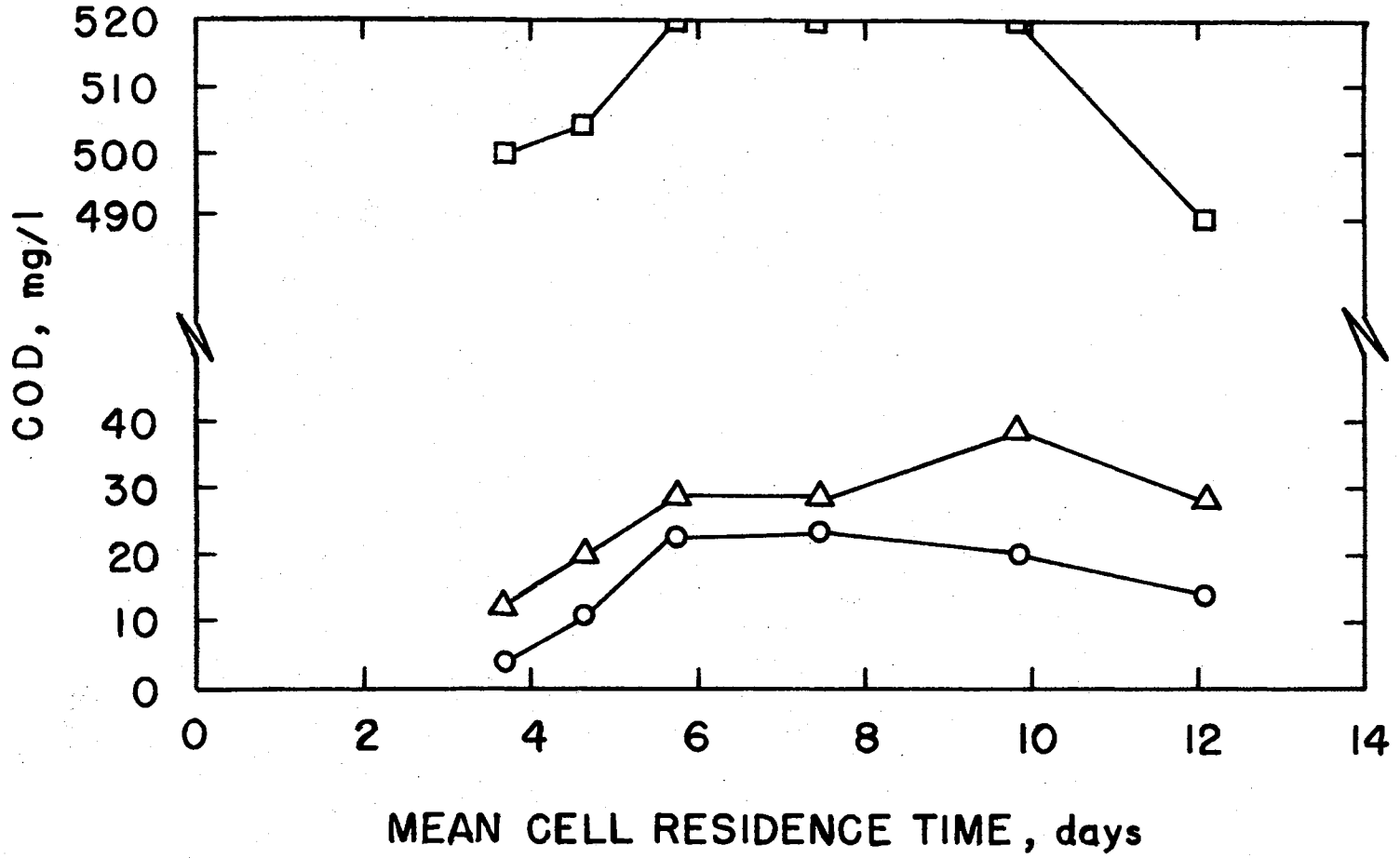

520
510
500
490
40
30
20
10
0
COD, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 21. Nitrification Reactor Microorganism Zone Settling Velocity Versus Mean Cell Residence Time

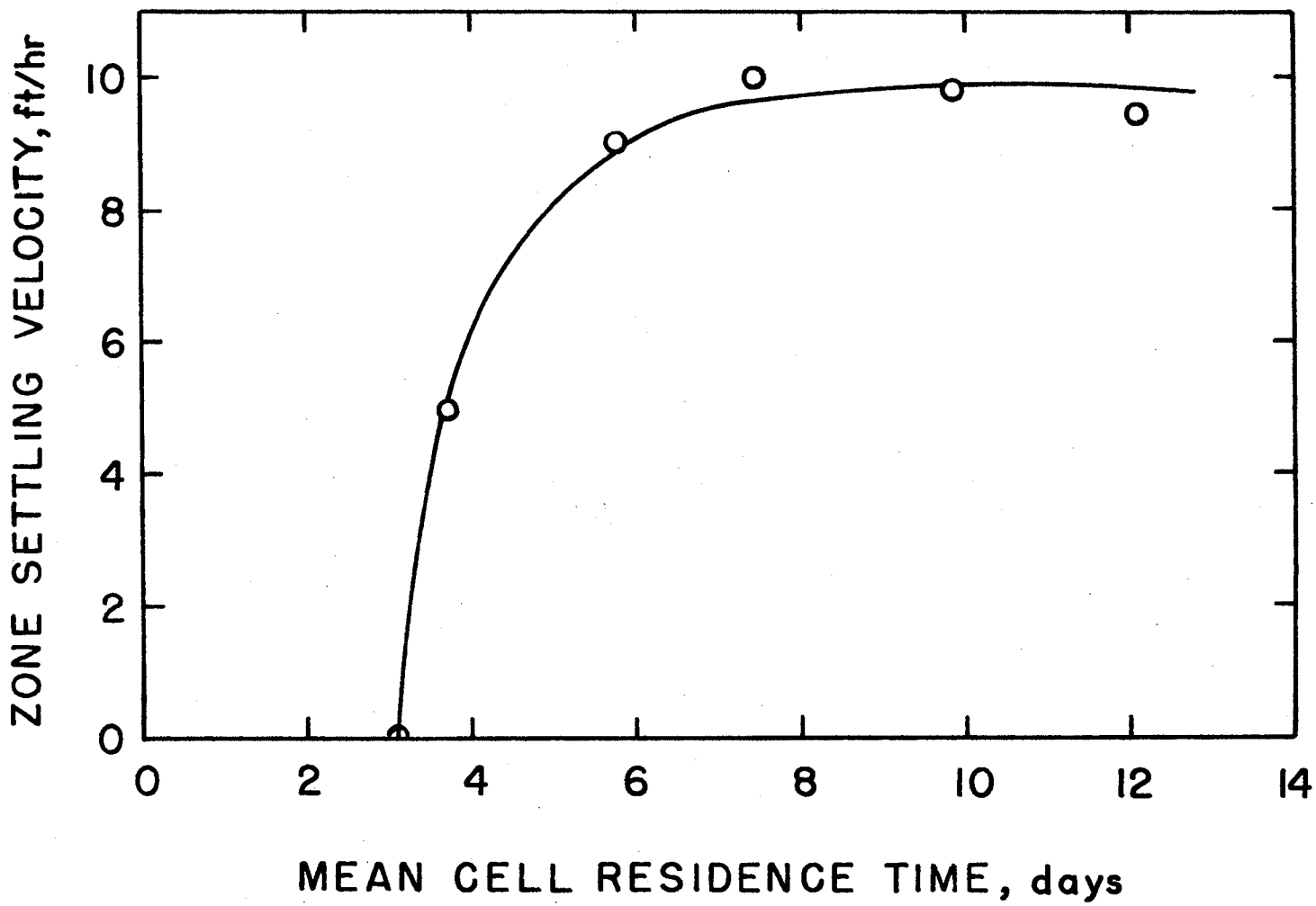
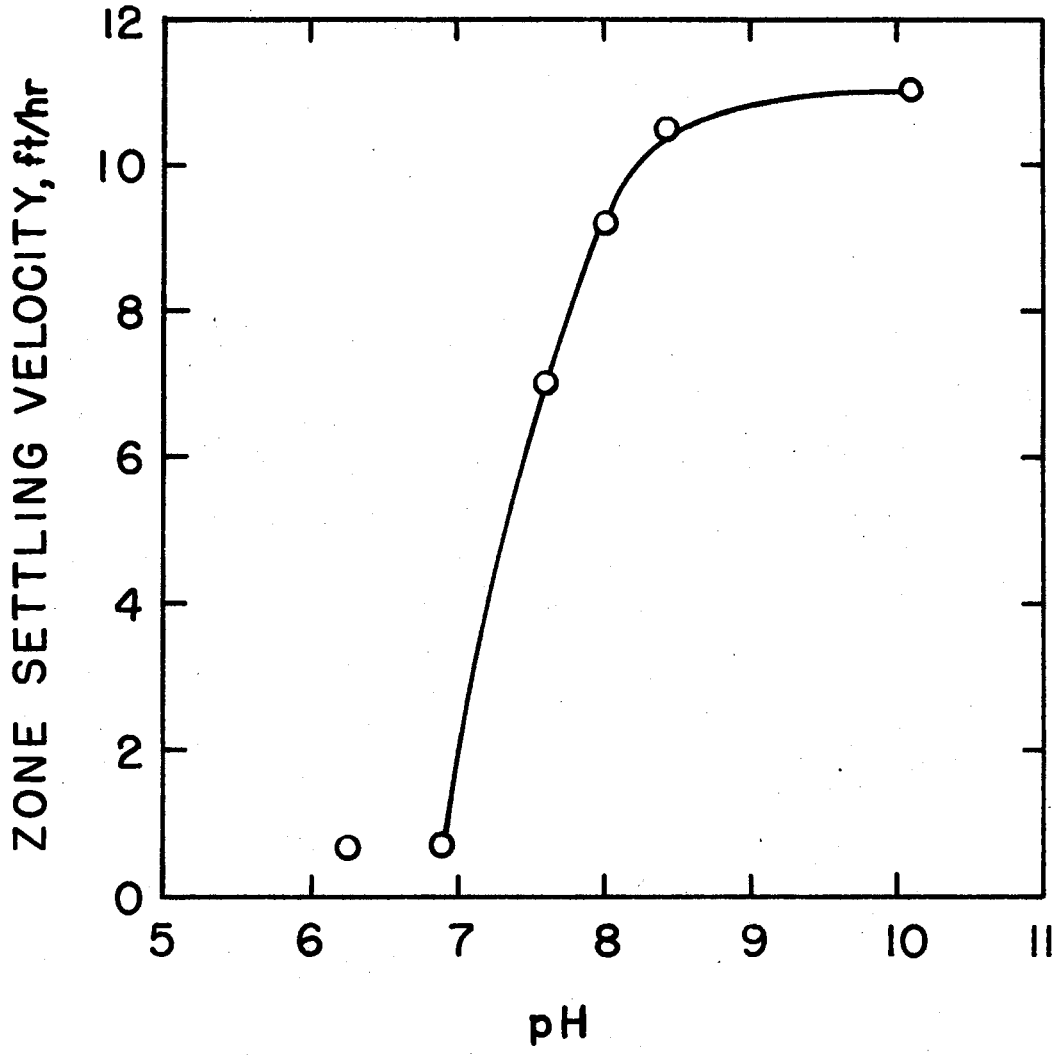

ZONE SETTLING VELOCITY, ft/hr
0
2
4
6
8
10
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

TABLE VI

SUMMARY OF OPERATIONAL CHARACTERISTICS OF THE SECOND STAGE OF THE TWO-STAGE ACTIVATED SLUDGE SYSTEM ($\Theta$ = 14.0 hours)

| $\Theta_c$ (Days) | Biological Solids (mg/l) | Influent, (mg/l) | | | Effluent, (mg/l) | | | $Y_{obs}$ | | U | | F/M | | Zone Settling Velocity (ft/hr) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $NH_3$-N | $NO_3$-N | COD | $NH_3$-N | $NO_3$-N | COD | $NH_3$ | $NH_3$ + $NO_2$ | $NH_3$ | $NH_3$ + $NO_2$ | $NH_3$ | $NH_3$ + $NO_2$ | |
| 3.7 | 700 | 49.0 | 0 | 12.0 | 12.0 | 22.6 | 4.0 | 4.1 | 0.52 | 0.066 | 0.52 | 0.088 | 0.74 | 5.0 |
| 4.5 | 1300 | 49.5 | 0 | 20.0 | 1.2 | 33.8 | 11.0 | - | 0.51 | - | 0.43 | - | 0.47 | - |
| 5.7 | 1820 | 46.0 | 1.1 | 29.0 | 1.0 | 31.4 | 23.0 | 4.0 | 0.48 | 0.044 | 0.37 | 0.045 | 0.41 | 9.0 |
| 7.5 | 2238 | 46.0 | 1.3 | 28.6 | 0.1 | 35.0 | 23.8 | 3.7 | 0.40 | 0.036 | 0.33 | 0.036 | 0.35 | 10.0 |
| 9.8 | 2683 | 49.3 | 2.7 | 39.0 | 0.2 | 42.4 | 20.0 | 3.2 | 0.36 | 0.032 | 0.28 | 0.032 | 0.29 | 9.8 |
| 12.1 | 2889 | 49.1 | 0.6 | 28.5 | 0.1 | 37.5 | 14.3 | 2.8 | 0.33 | 0.030 | 0.25 | 0.030 | 0.26 | 9.4 |

Figure 22. Response of Nitrifying Microorganism Zone Settling Velocity at 2800 mg/l Concentration to Changes in pH


ZONE SETTLING VELOCITY, ft/hr
0
2
4
6
8
10
12
5
6
7
8
9
10
11
pH

## 2. Operational Performance of One-Stage Activated Sludge Systems

Performance data for one-stage activated sludge systems operated to achieve both carbonaceous removal and nitrification in the same reactor is presented in this section. Five different investigations were carried out--some at the same COD:$NH_3$-N ratios and others at different COD:$NH_3$-N ratios. Each system was operated by controlling the daily amount of biological solids wasted in order to maintain a desired mean cell residence time. The mean cell residence time was varied from 1.5 days to 13.5 days, and the degree of nitrification achievable by the systems was monitored.

a. Studies on One-Stage Systems Operated at the Same COD:$NH_3$-N Ratio. Three activated sludge systems were operated at COD:$NH_3$-N ratios of approximately 10:1. These reactors were operated at concentrations of 100 mg/l COD to 10 mg/l $NH_3$-N, 500 mg/l COD to 50 mg/l $NH_3$-N, and 1000 mg/l COD to 100 mg/l $NH_3$-N.

The influence of the mean cell residence time on the concentration of ammonia-nitrogen and nitrate-nitrogen measured in the effluent of these systems operated at 100 mg/l COD and 10 mg/l $NH_3$-N, 500 mg/l COD and 50 mg/l $NH_3$-N, 1000 mg/l COD and 100 mg/l $NH_3$-N is shown in Figures 23, 24, and 25, respectively. In all three cases over the range of sludge ages investigated the concentration of ammonia-nitrogen in the effluent decreased with increasing sludge age, while the nitrate-nitrogen concentration increased with increasing sludge age. As shown in Figures 26, 27, and 28, the percent of ammonia-nitrogen removed from these systems and the percent of ammonia-nitrogen removed that was

Figure 23. Nitrification Performance Data for One-Stage Activated Sludge System Operated at 100 mg/l COD and 10 mg/l $NH_3$-N

□ = influent $NH_3$-N concentration

○ = effluent $NH_3$-N concentration

△ = effluent $NO_3$-N concentration

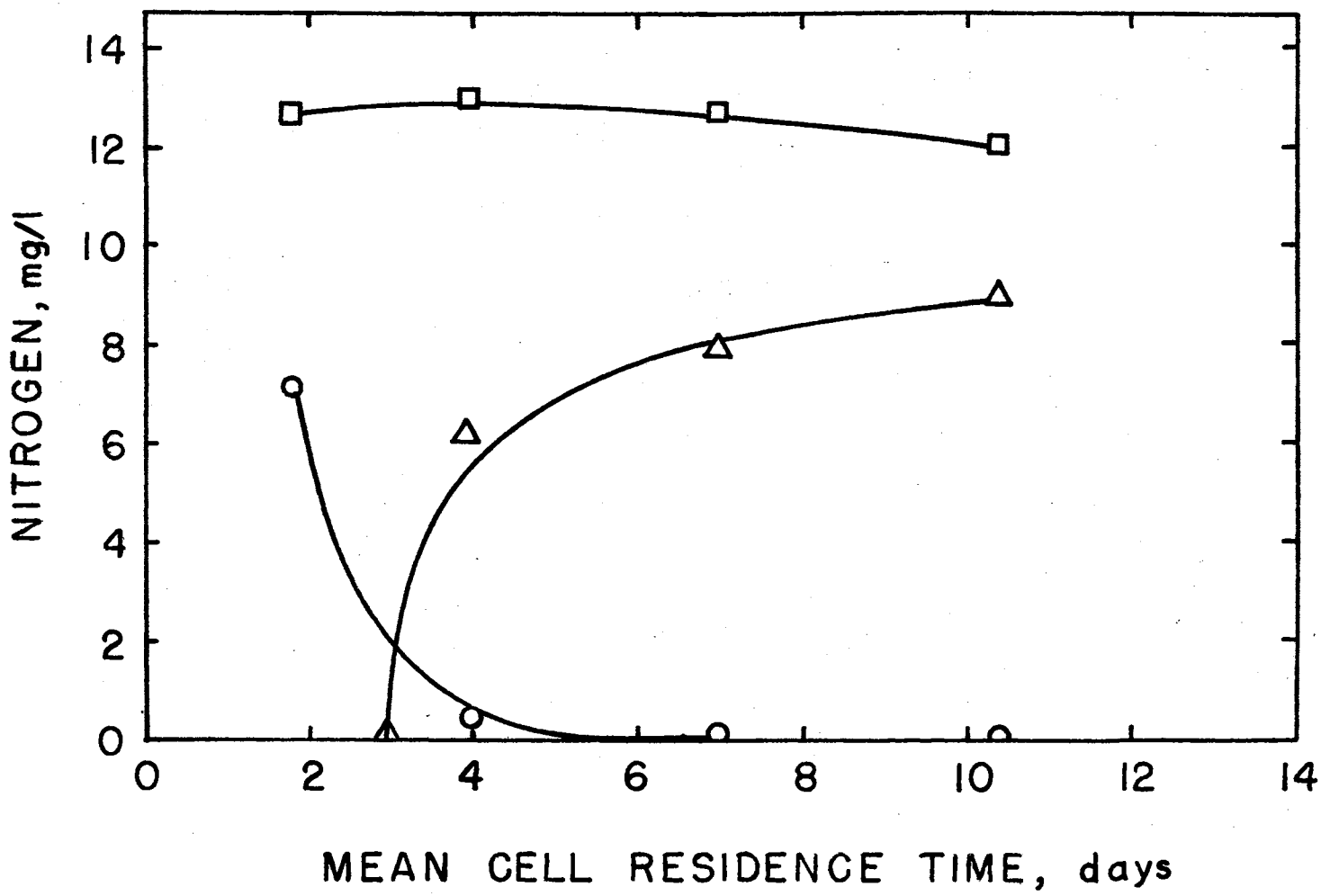

NITROGEN, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 24. Nitrification Performance Data for One-Stage Activated Sludge System Operated at 500 mg/l COD and 50 mg/l $NH_3$-N

□ = influent $NH_3$-N concentration

○ = effluent $NH_3$-N concentration

△ = effluent $NO_3$-N concentration

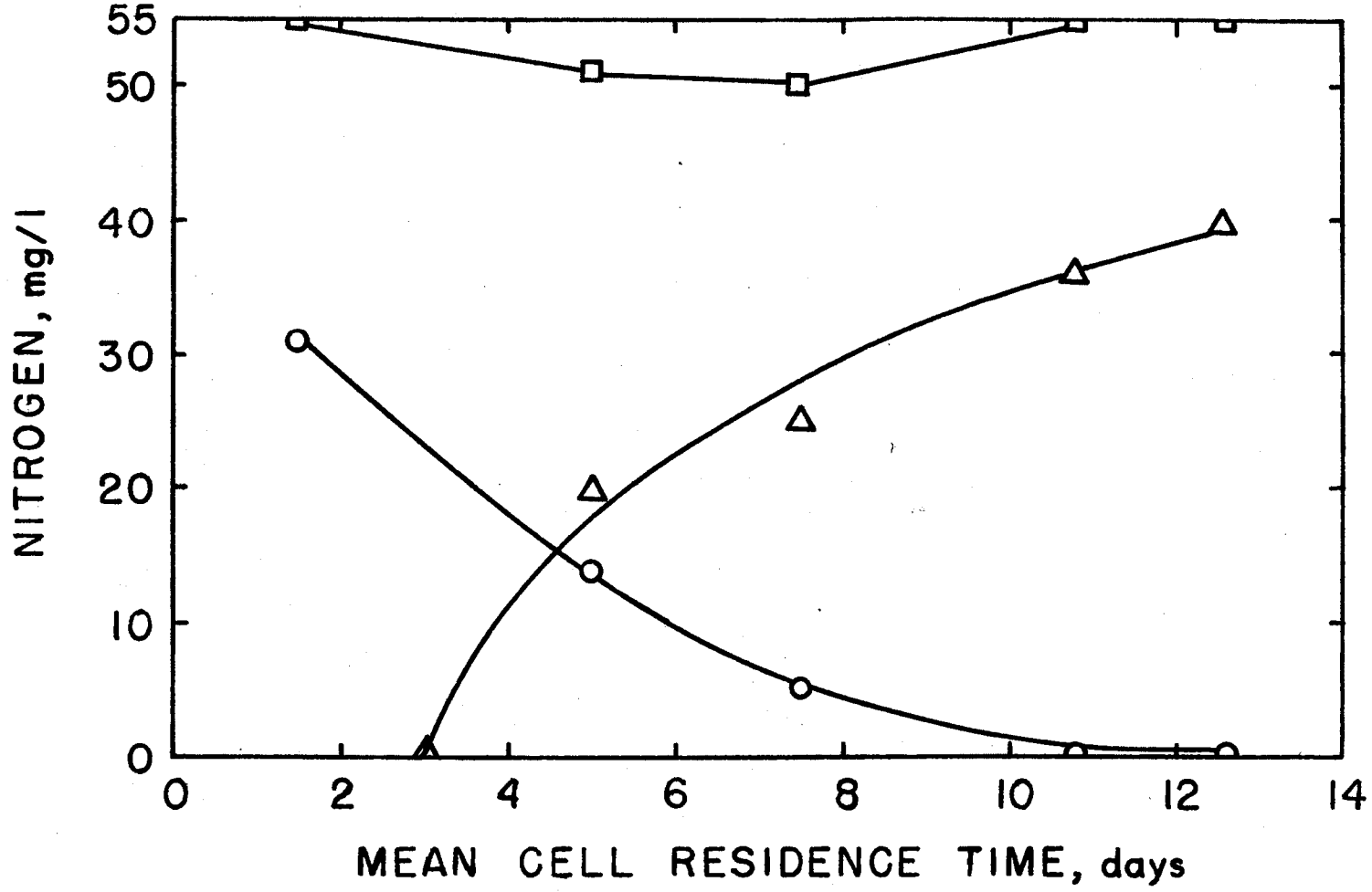

55
50
40
30
20
10
0
NITROGEN, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 25. Nitrification Performance Data for One-Stage Activated Sludge System Operated at 1000 mg/l COD and 100 mg/l $NH_3$-N

□ = influent $NH_3$-N concentration

○ = effluent $NH_3$-N concentration

△ = effluent $NO_3$-N concentration

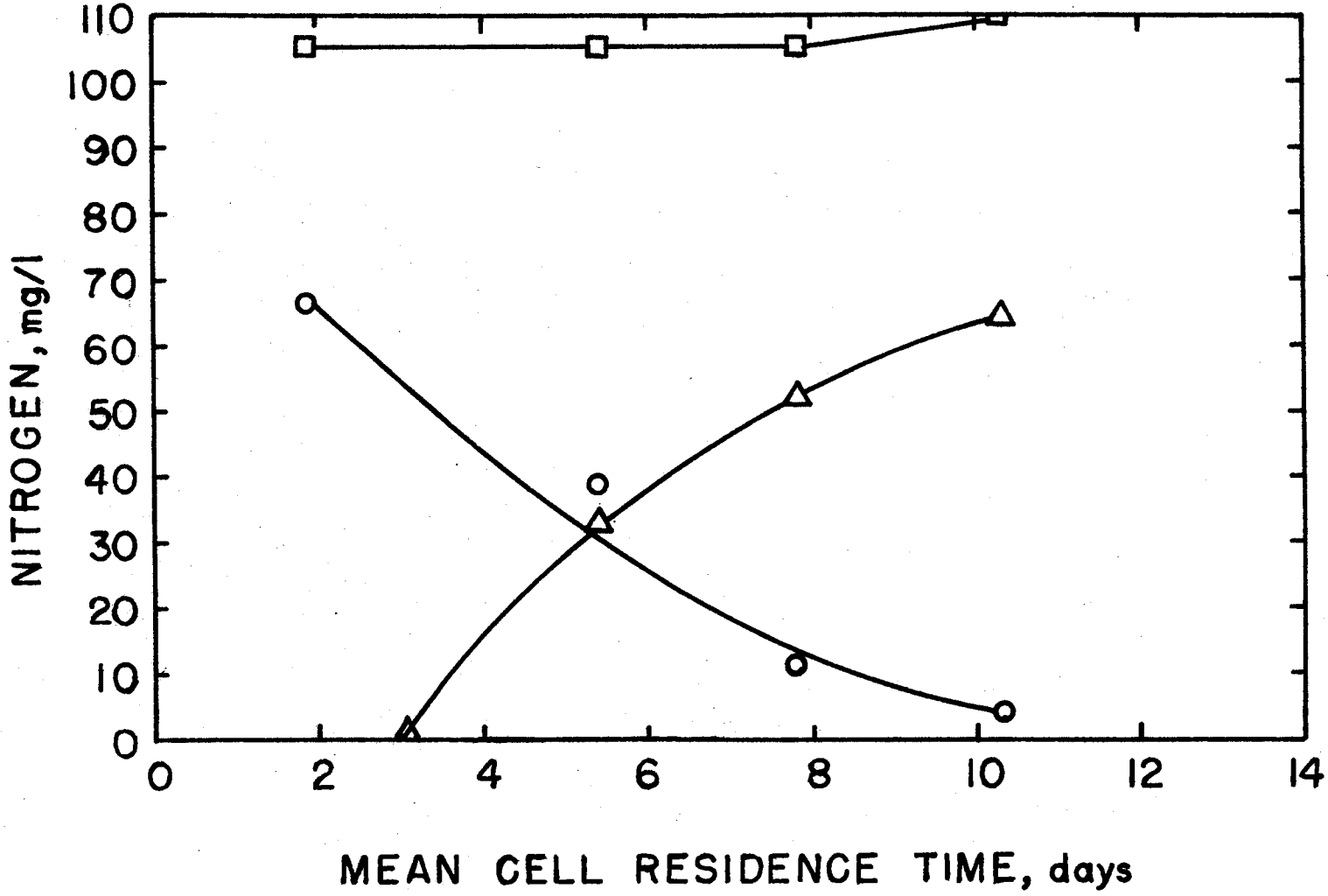

110
100
90
80
70
60
50
40
30
20
10
0
NITROGEN, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 26. Nitrification Efficiency of One-Stage Activated Sludge System Operated at 100 mg/l COD and 10 mg/l $NH_3$-N Versus Mean Cell Residence Time

O = percent $NH_3$-N removed

Δ = percent $NH_3$-N removed that was converted to $NO_3$-N

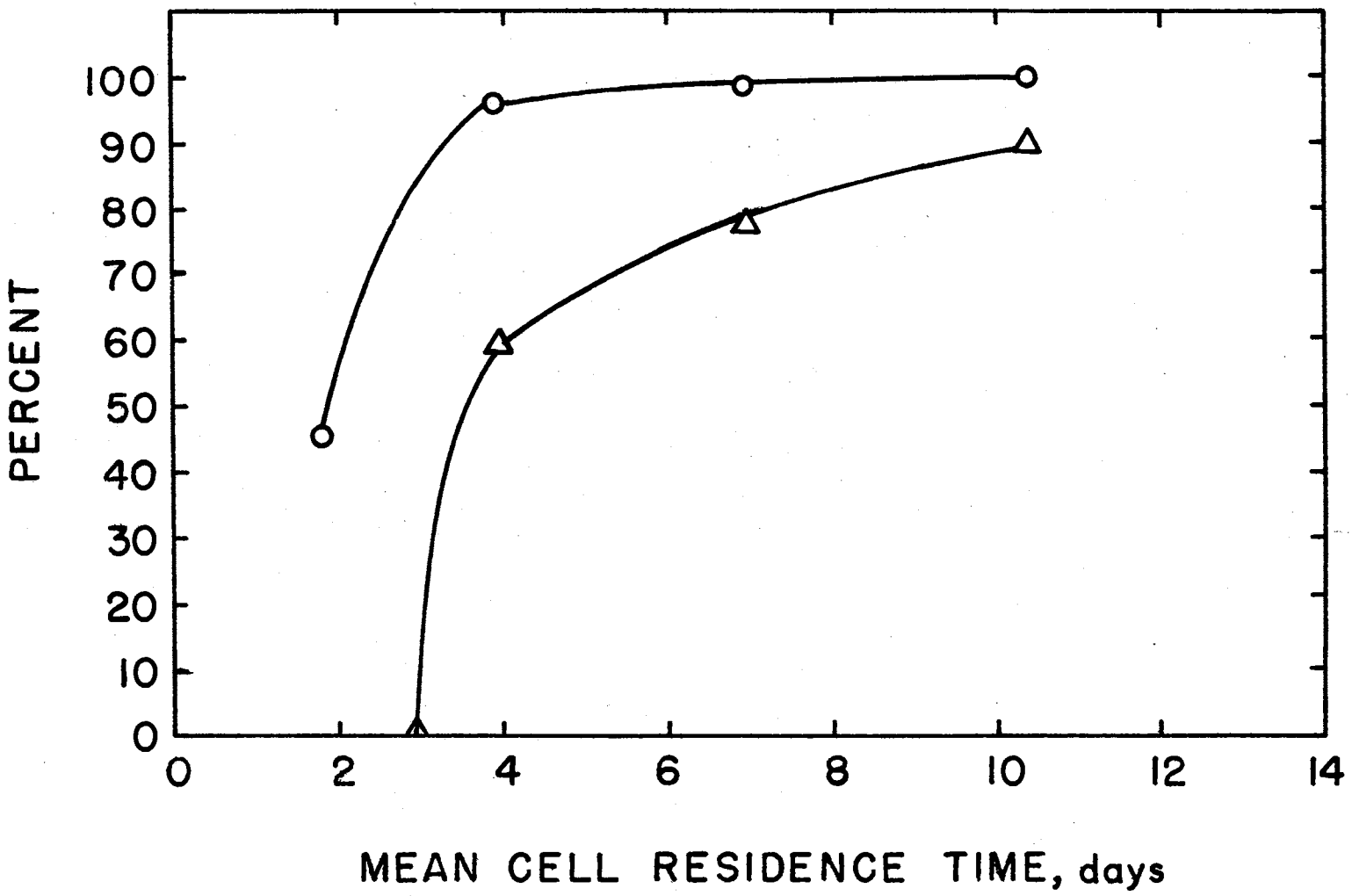

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 27. Nitrification Efficiency of One-Stage Activated Sludge System Operated at 500 mg/l COD and 50 mg/l $NH_3$-N Versus Mean Cell Residence Time

O = percent $NH_3$-N removed

Δ = percent $NH_3$-N removed that was converted to $NO_3$-N

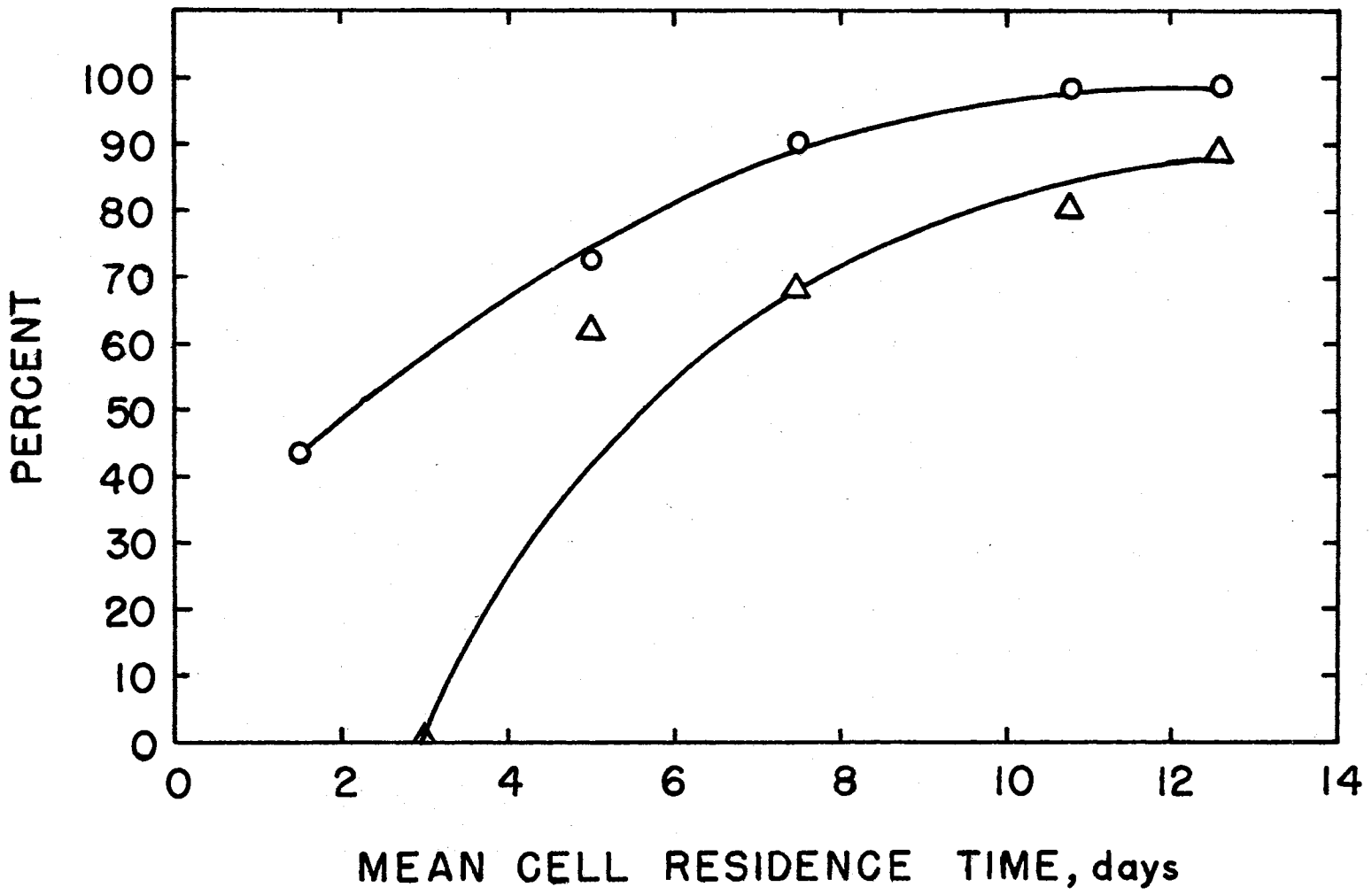
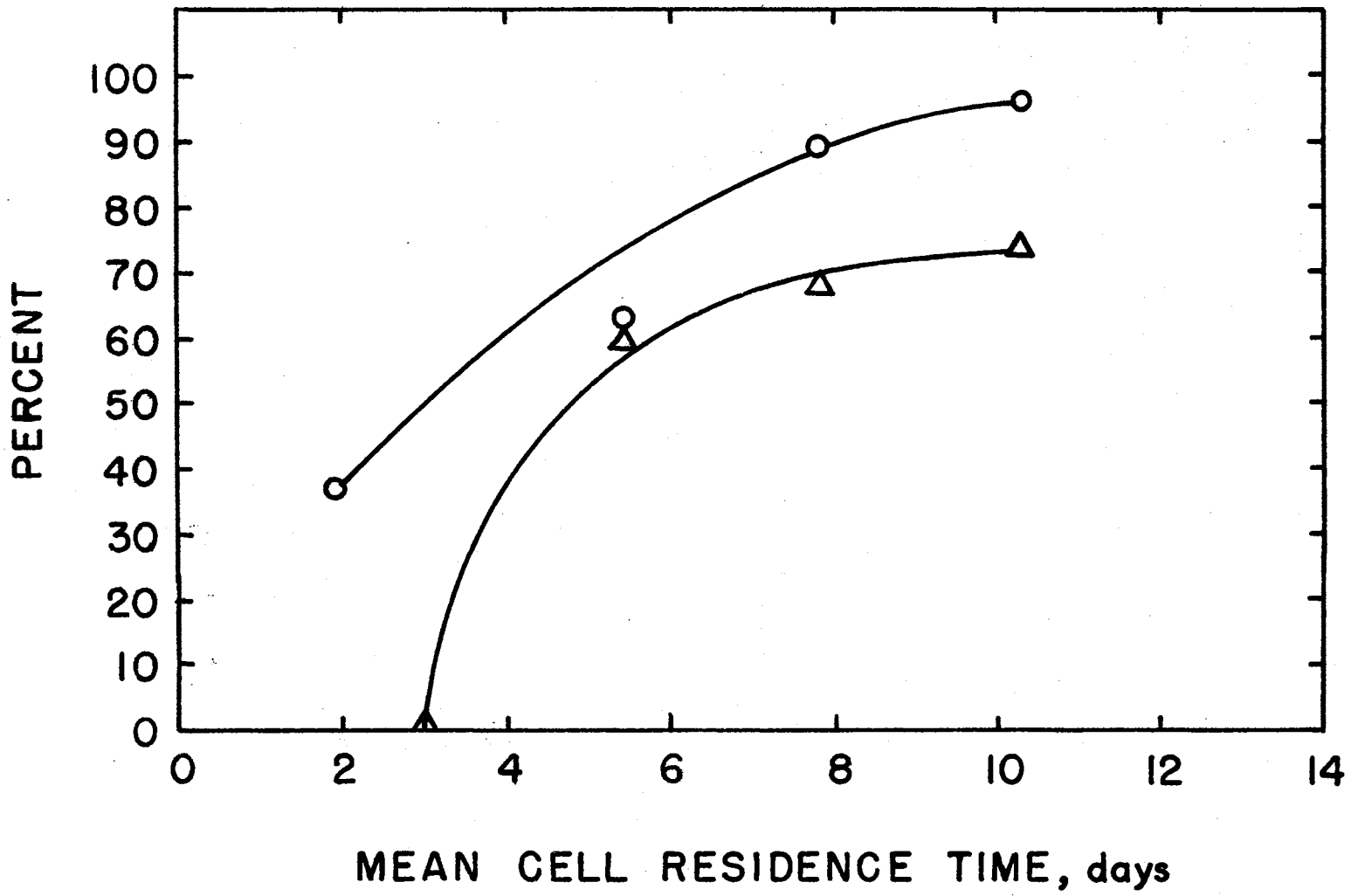

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 28. Nitrification Efficiency of One-Stage Activated Sludge System Operated at 1000 mg/l COD and 100 mg/l $NH_3$-N Versus Mean Cell Residence Time

O = percent $NH_3$-N removed

Δ = percent $NH_3$-N removed that was converted to $NO_3$-N


PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

converted to nitrate-nitrogen both increased with increasing mean cell residence time, the percent of ammonia-nitrogen removed always being greater than the ammonia-nitrogen converted to nitrate-nitrogen. The percent ammonia-nitrogen removed from these systems that was converted to nitrate-nitrogen decreased as the influent COD and $NH_3$-N concentrations increased. At a mean cell residence time of 10 days the percent conversions for the reactors operated at 100 mg/l COD and 10 mg/l $NH_3$-N, 500 mg/l COD and 50 mg/l $NH_3$-N, and 1000 mg/l COD and 100 mg/l $NH_3$-N were about 89 percent, 81 percent, and 73 percent, respectively. The nitrate-nitrogen concentration decreased to zero at a mean cell residence time of approximately three days, but ammonia-nitrogen was still removed at sludge ages below three days due to incorporation of the nitrogen into heterotrophic microorganism cellular matter by synthesis reactions.

The COD removal efficiency remained above 90 percent over the range of sludge ages investigated, as shown in Figures 29, 30, and 31. A summary of the COD removal characteristics of these systems along with the degree of nitrification achieved at the various mean cell residence times studied is shown in Tables VII, VIII, and IX.

A comparison of the ammonia-nitrogen removal characteristics of these three systems is shown in Figure 32. At a mean cell residence time of 10 days or greater the percent of ammonia-nitrogen removed leveled off at about 98 to 99 percent for all three systems. Below 10 days the removal for the two reactors with influent CODs of 500 mg/l and 1000 mg/l dropped off more rapidly than for the reactor with 100 mg/l COD. The percent of ammonia-nitrogen removed that was converted to nitrate-nitrogen increased faster with increasing sludge age for the

Figure 29. COD Removal Characteristics of One-Stage Activated Sludge System Operated at 100 mg/l COD and 10 mg/l $NH_3$-N

□ = influent COD concentration

○ = effluent COD concentration

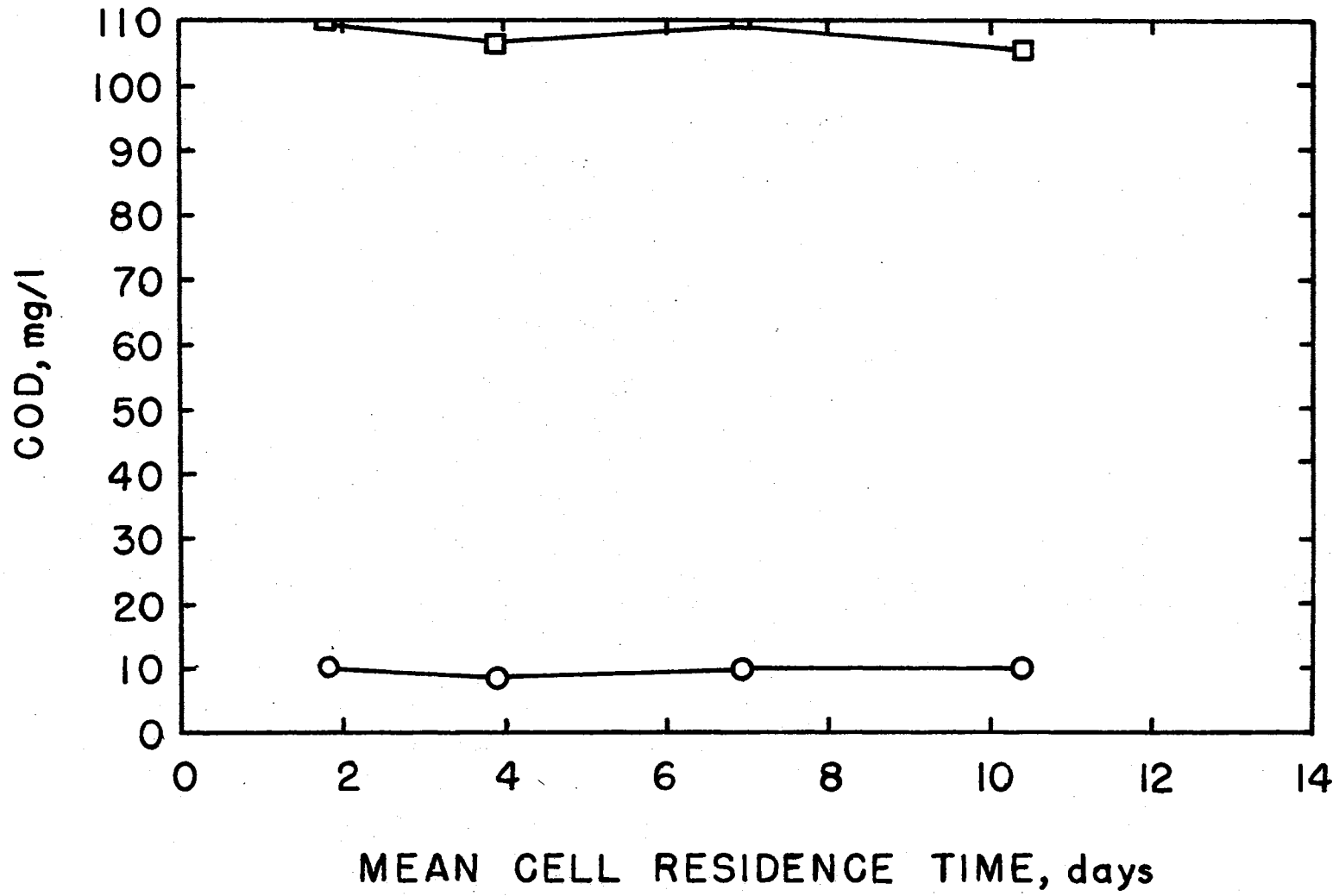

110
100
90
80
70
60
50
40
30
20
10
0
COD, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 30. COD Removal Characteristics of One-Stage Activated Sludge System Operated at 500 mg/l COD and 50 mg/l $NH_3$-N

□ = influent COD concentration

○ = effluent COD concentration

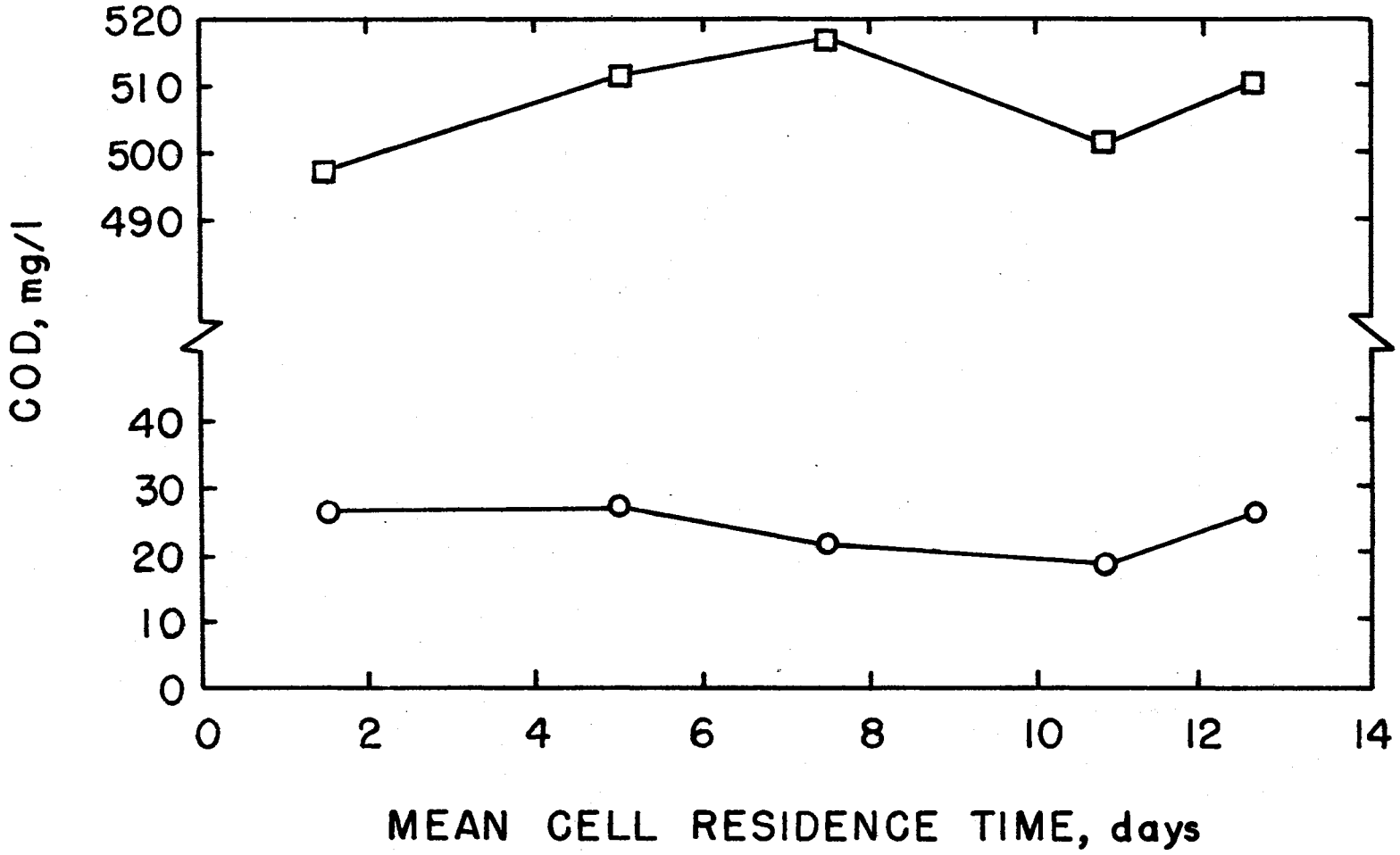

520
510
500
490
40
30
20
10
0
COD, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 31. COD Removal Characteristics of One-Stage Activated Sludge System Operated at 1000 mg/l COD and 100 mg/l $NH_3$-N

□ = influent COD concentration

O = effluent COD concentration

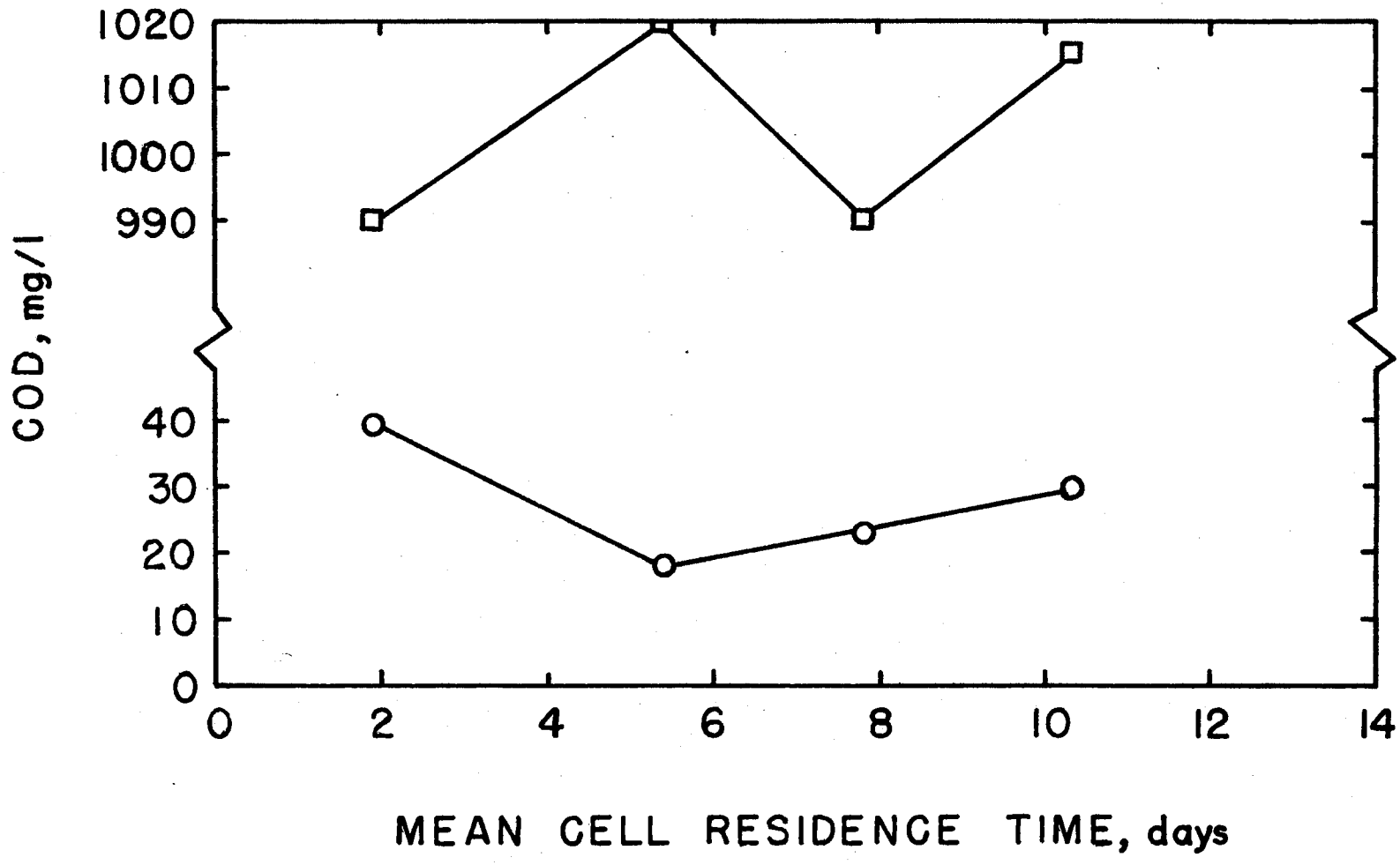

1020
1010
1000
990
40
30
20
10
0
COD, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

TABLE VII

SUMMARY OF OPERATIONAL CHARACTERISTICS OF NITRIFYING ONE-STAGE ACTIVATED SLUDGE SYSTEM FOR COD:$NH_3$-N = 100:10
($\Theta$ = 1.20 hours)

| $\Theta_c$ (Days) | Biological Solids (mg/l) | Influent, (mg/l) | | | | Effluent, (mg/l) | | | | $Y_{obs}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$ | COD |
| 1.8 | 184 | 12.8 | 0 | 0 | 110 | 7.0 | 0 | 0 | 10.0 | 7.8 | 0.51 |
| 3.9 | 354 | 13.0 | 0 | 0 | 106 | 0.5 | 6.2 | 2.8 | 8.0 | 3.7 | 0.47 |
| 6.9 | 550 | 12.8 | 0 | 0 | 110 | 0.2 | 7.9 | 1.7 | 9.8 | 3.3 | 0.39 |
| 10.4 | 757 | 12.0 | 0 | 0 | 105 | 0 | 9.0 | 0.7 | 10.0 | 3.0 | 0.37 |

TABLE VIII

SUMMARY OF OPERATIONAL CHARACTERISTICS OF NITRIFYING ONE-STAGE ACTIVATED SLUDGE SYSTEM FOR COD:$NH_3$-N = 500:50
($\Theta$ = 12.0 hours)

| $\Theta_c$ (Days) | Biological Solids (mg/l) | Influent, (mg/l) | | | | Effluent, (mg/l) | | | | $Y_{obs}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$ | COD |
| 1.5 | 500 | 55.0 | 0 | 0 | 497 | 31.0 | 0 | 0 | 26 | 7.0 | 0.50 |
| 4.9 | 2173 | 51.0 | 0 | 0 | 511 | 14.0 | 20.8 | 0.4 | 27 | 5.8 | 0.45 |
| 7.4 | 2851 | 50.0 | 0 | 0 | 517 | 5.0 | 25.0 | 0.3 | 21 | 4.1 | 0.37 |
| 10.8 | 3780 | 55.0 | 0 | 0 | 501 | 0.5 | 36.0 | 0.6 | 18 | 3.1 | 0.36 |
| 12.6 | 3910 | 55.0 | 0 | 0 | 510 | 0.6 | 40.0 | 0.2 | 26 | 2.8 | 0.32 |

TABLE IX

SUMMARY OF OPERATIONAL CHARACTERISTICS OF NITRIFYING ONE-STAGE ACTIVATED SLUDGE SYSTEM FOR COD:$NH_3$-N = 1000:100
($\Theta$ = 12.0 hours)

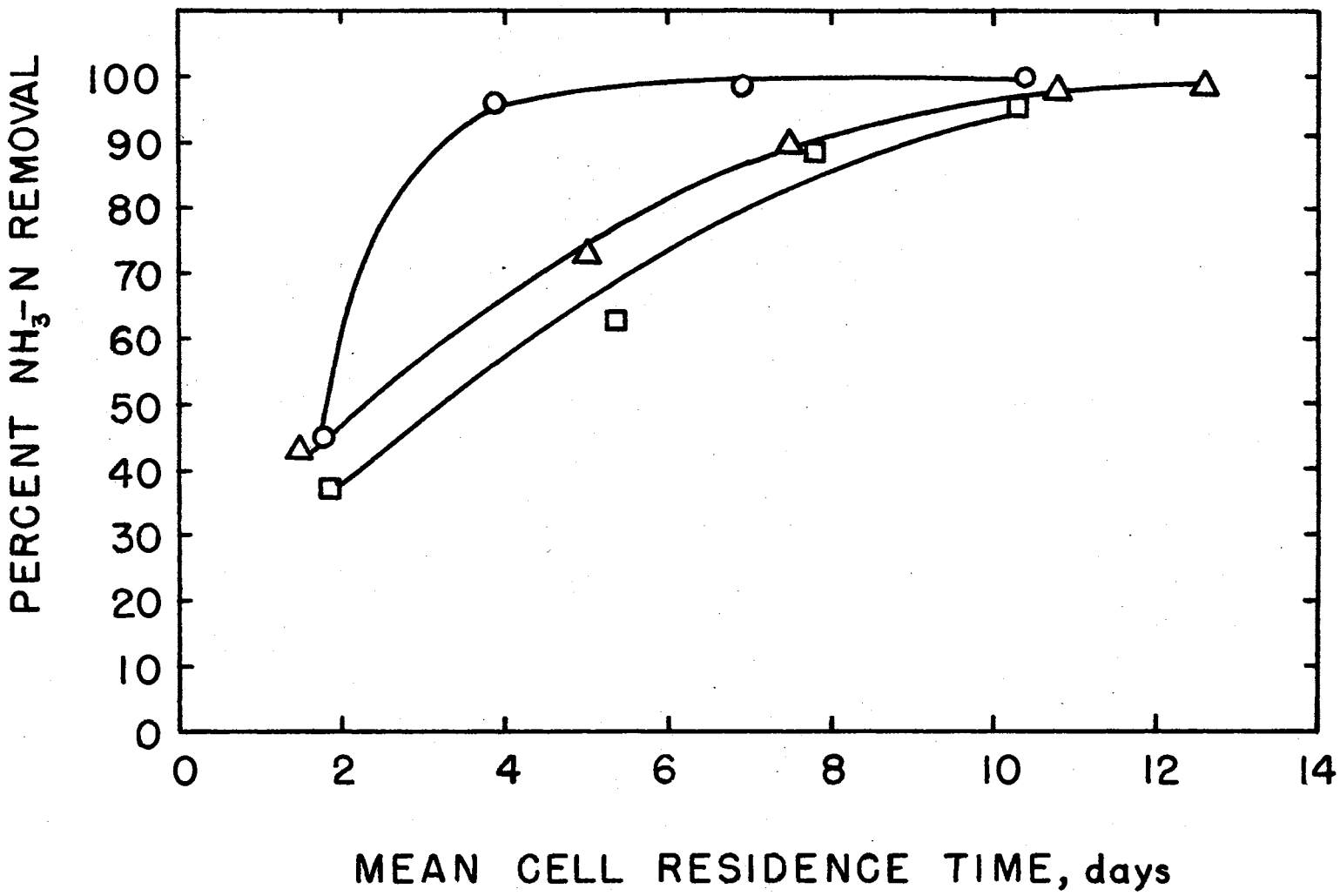
| $\Theta_c$ (Days) | Biological Solids (mg/l) | Influent, (mg/l) | | | | Effluent, (mg/l) | | | | $Y_{obs}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$ | COD |
| 1.9 | 1280 | 105 | 0 | 0 | 990 | 66 | 0 | 0 | 39 | 8.6 | 0.47 |
| 5.4 | 4052 | 105 | 0 | 0 | 1020 | 39 | 33 | 1.8 | 18 | 5.7 | 0.40 |
| 7.8 | 5527 | 105 | 0 | 0 | 990 | 11 | 52 | 0.8 | 23 | 3.7 | 0.37 |
| 10.3 | 6953 | 110 | 0 | 0 | 1015 | 4 | 64 | 0.4 | 30 | 3.2 | 0.35 |

Figure 32. Comparison of Ammonia-Nitrogen Removal Characteristics of One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)


PERCENT $NH_3$-N REMOVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

reactor with influent COD concentration of 100 mg/l than for the reactors with 500 mg/l and 1000 mg/l COD concentrations, as shown in Figure 33. As can be seen in Figure 34, the percent of ammonia-nitrogen removed by cellular synthesis reactions followed the same trend of removal in all three systems. The percent of ammonia-nitrogen channeled into cell synthesis decreased with increasing mean cell residence time, and the largest difference between any two adjacent curves on the graph is only about seven percent.

A plot of reactor biological solids concentration as a function of mean cell residence time is shown in Figure 35. The reactor microorganism concentration increased with higher mean cell residence times. As expected from continuous flow kinetics the reactor microorganism concentrations were larger with the higher substrate concentrations at the same mean cell residence time. At a sludge age of 10 days and influent COD to $NH_3$-N concentrations of 100 mg/l to 10 mg/l, 500 mg/l to 50 mg/l, and 1000 mg/l to 100 mg/l, the reactor solids concentrations were about 800 mg/l, 3700 mg/l, and 6900 mg/l, respectively. The observed yield coefficient computed in terms of both COD and ammonia decreased with increasing mean cell residence time, as shown in Figures 36 and 37, respectively. Computed in terms of COD the observed yield plotted as one curve for all three systems, and in terms of ammonia the yield curves did not deviate appreciably from each other.

In Figures 38, 39, and 40 is shown a comparison of the performance characteristics of four different systems operated at an approximate mean cell residence time of 10 days and COD:$NH_3$-N = 10:1. A reactor operated at 300 mg/l COD and 30 mg/l $NH_3$-N along with the three previously mentioned systems are included in this comparison. The influent

Figure 33. Comparison of Ammonia-Nitrogen Conversion to Nitrate-Nitrogen by One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)

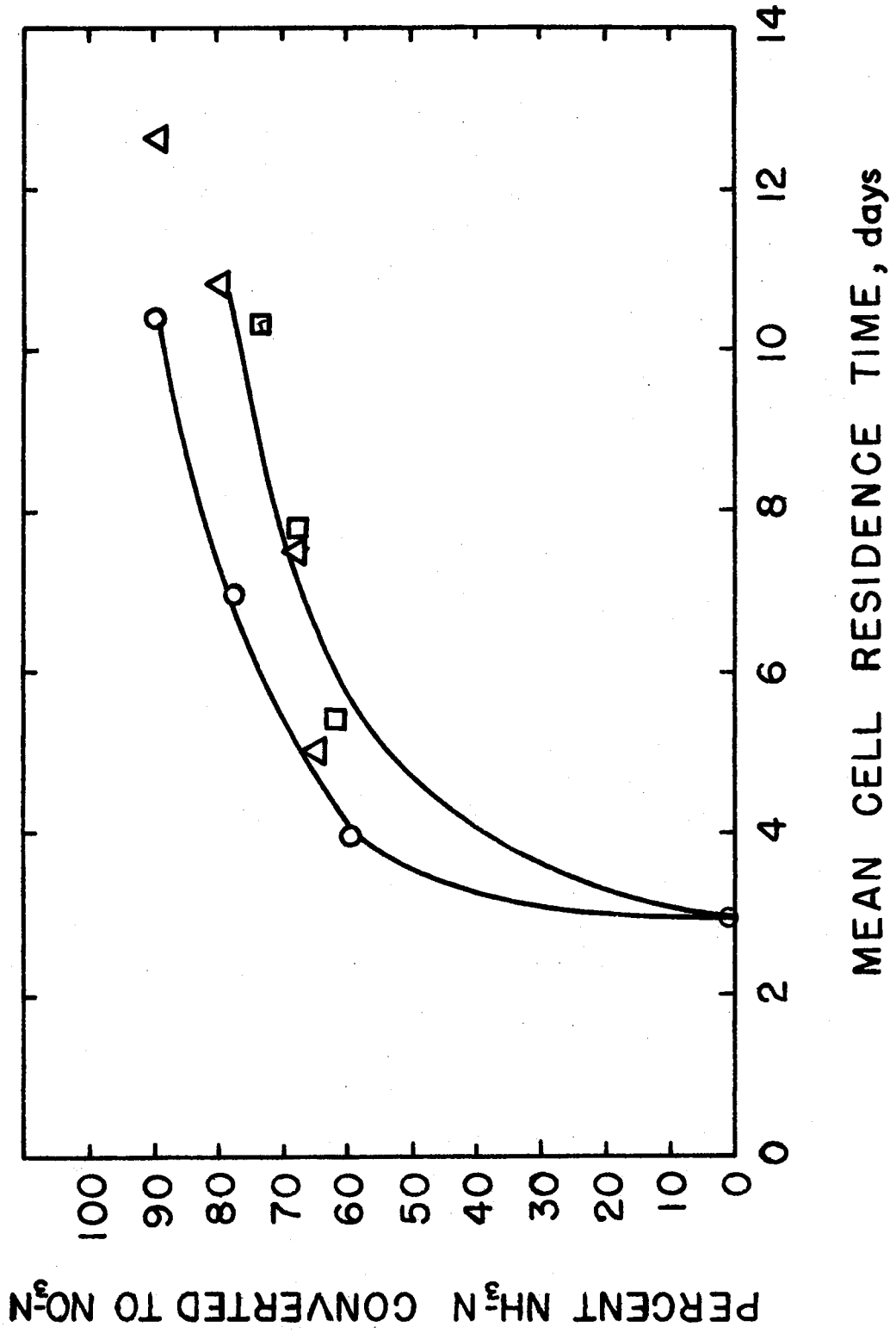

PERCENT NH3-N CONVERTED TO NO3-N
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 34. Comparison of Ammonia-Nitrogen Conversion to Biological Cell Matter by One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)

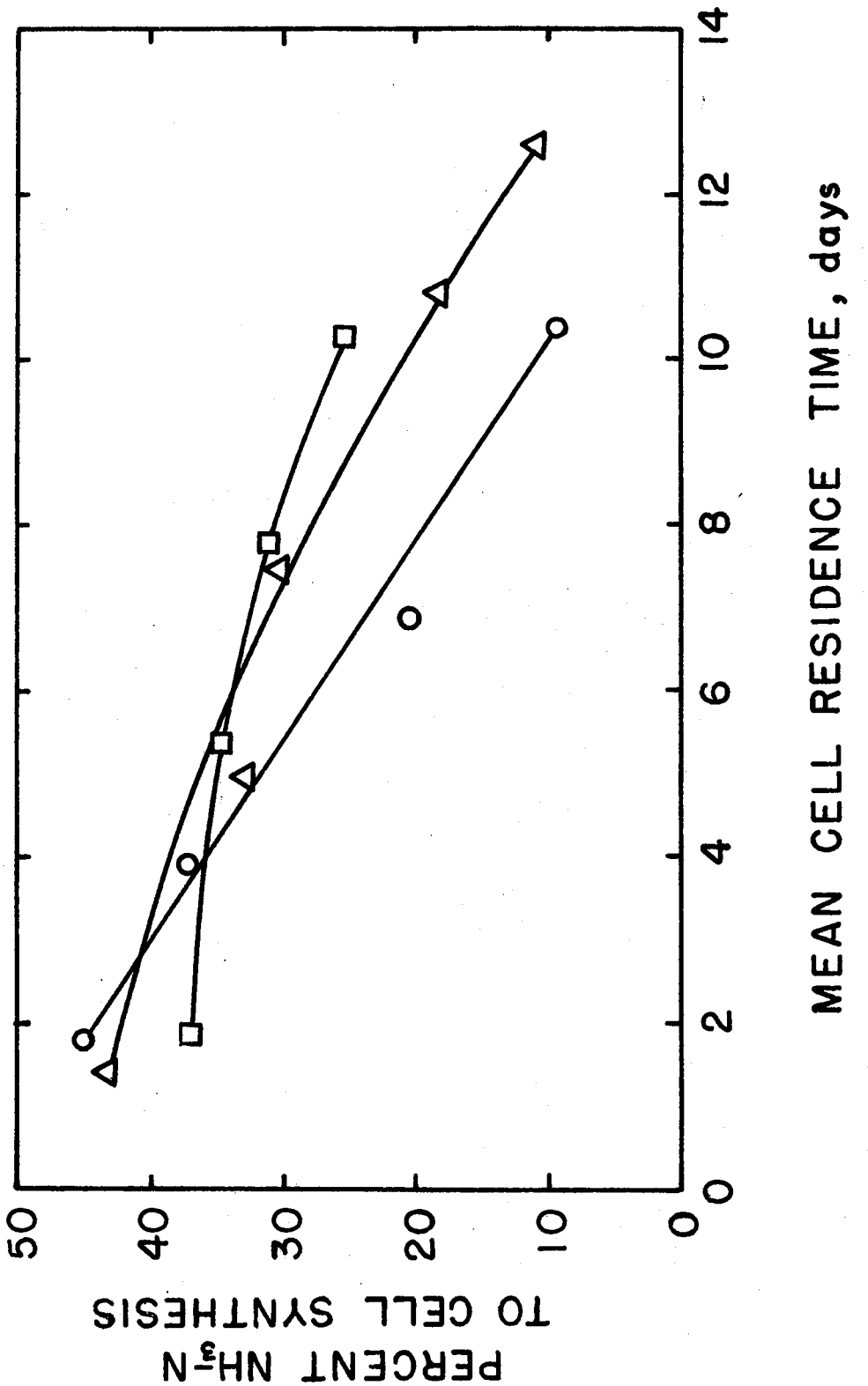

PERCENT NH3-N
TO CELL SYNTHESIS
0
10
20
30
40
50
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 35. Reactor Microorganism Concentration Versus Mean Cell Residence Time for One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)

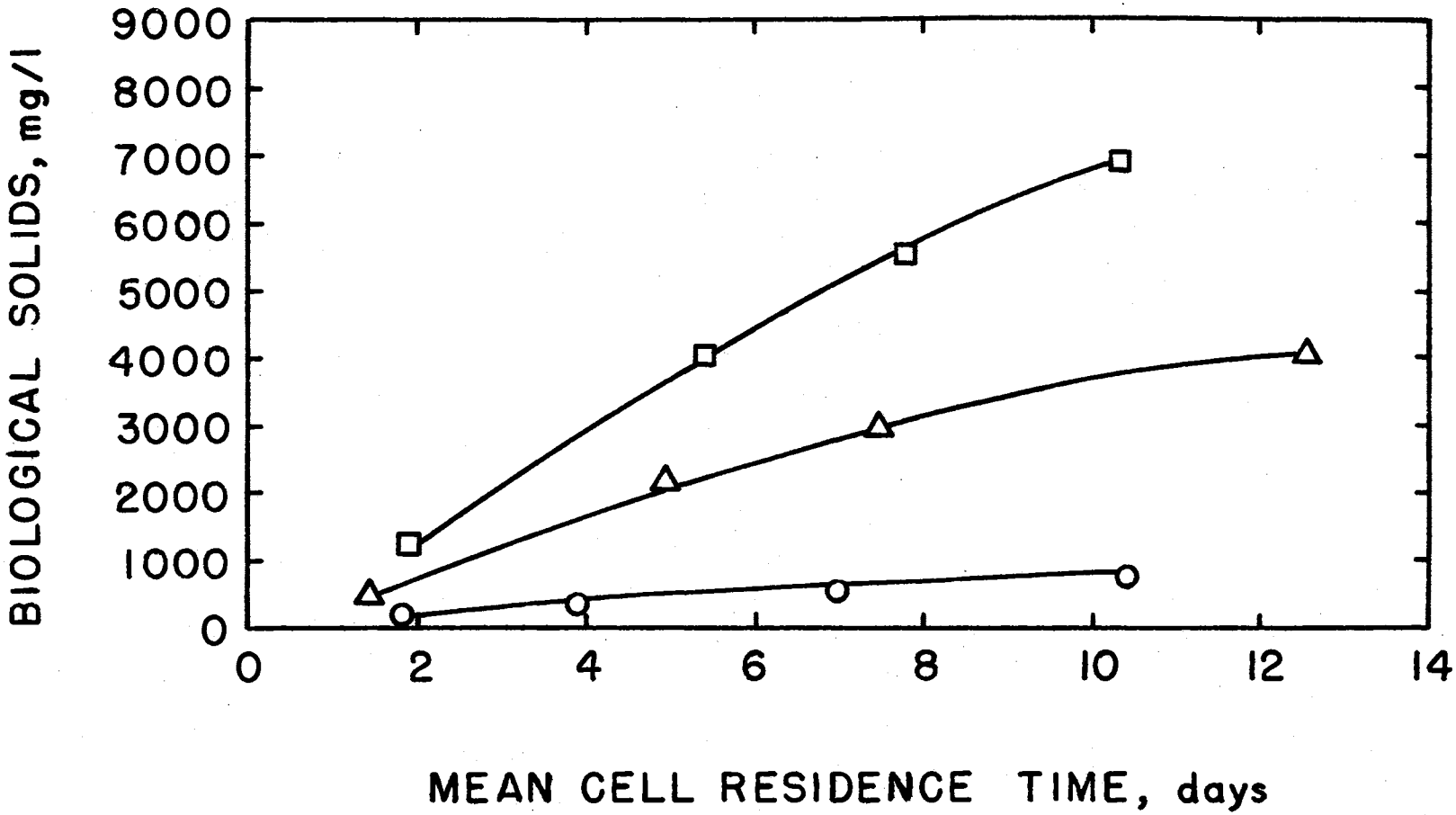
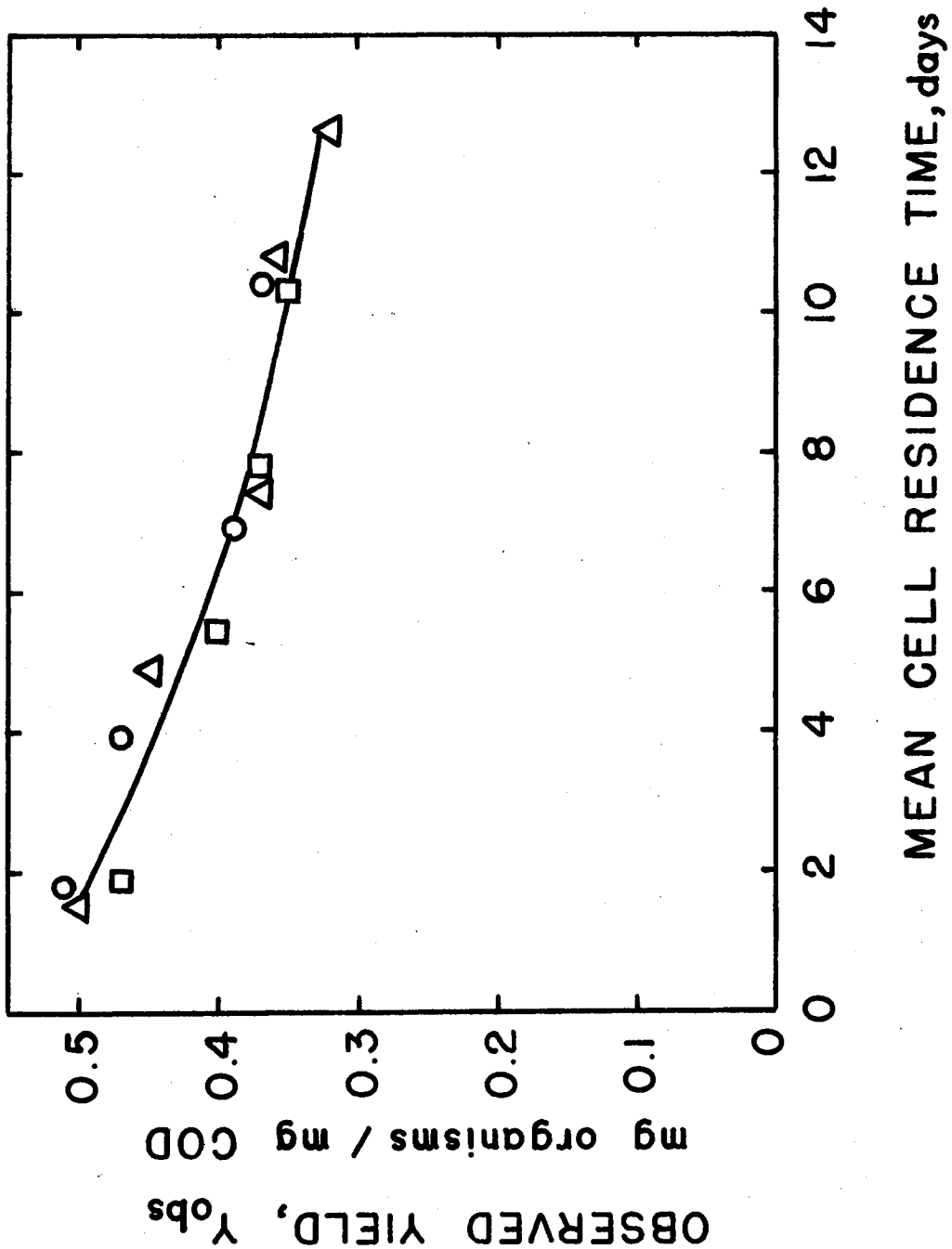

BIOLOGICAL SOLIDS, mg/l
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 36. Observed Yield Computed in Terms of COD Versus Mean Cell Residence Time for One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)


OBSERVED YIELD, $Y_{obs}$
mg organisms / mg COD
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

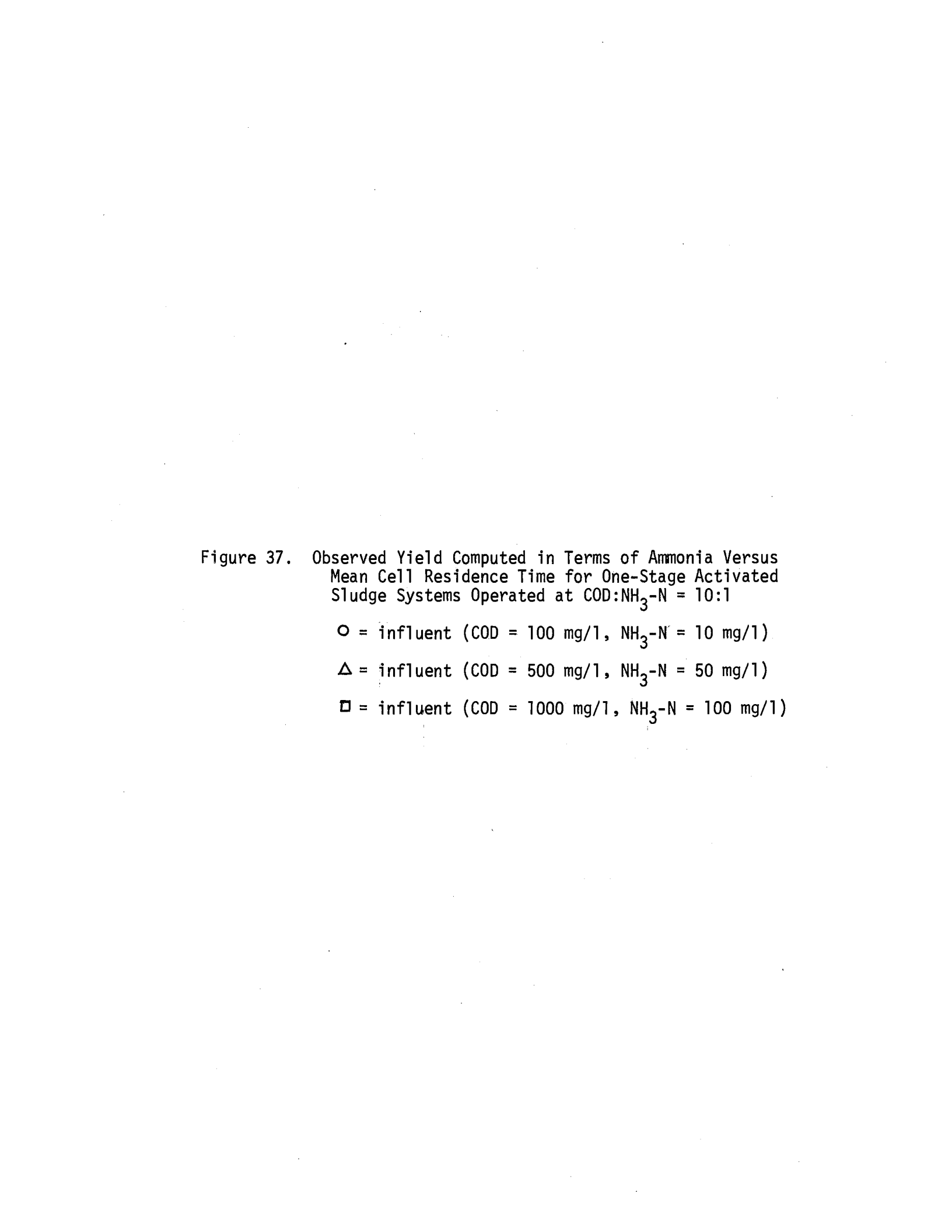


Figure 37. Observed Yield Computed in Terms of Ammonia Versus Mean Cell Residence Time for One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)

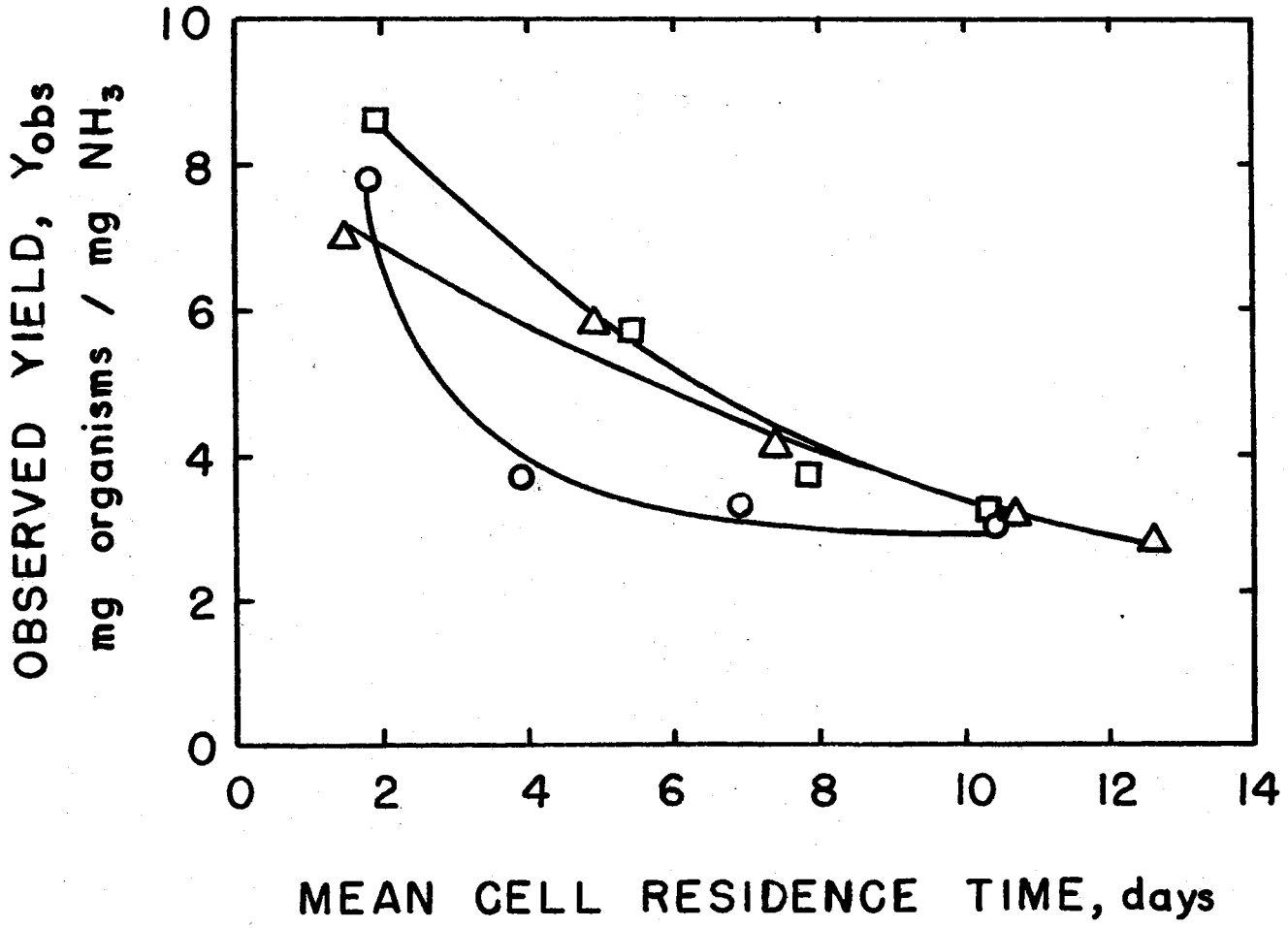

OBSERVED YIELD, $Y_{obs}$
mg organisms / mg $NH_3$
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 38. Ammonia-Nitrogen Removal Characteristics for One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1 and at a 10-day Mean Cell Residence Time

□ = percent $NH_3$-N removed

○ = percent $NH_3$-N removed that was converted to $NO_3$-N

△ = percent $NH_3$-N removed that was converted to biological cell matter

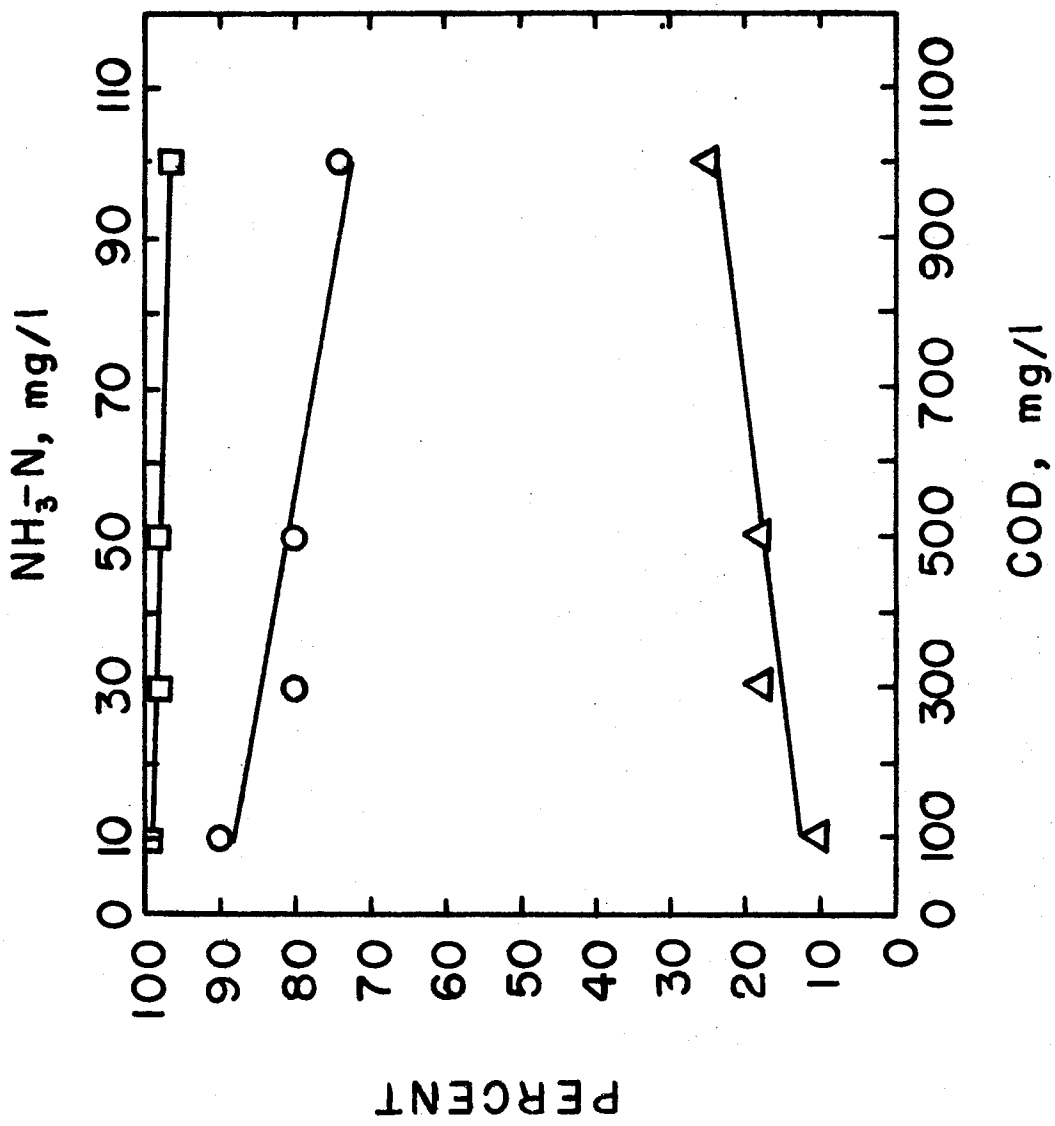

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
10
30
50
70
90
110
$NH_3$-N, mg/l
0
100
300
500
700
900
1100
COD, mg/l

Figure 39. Reactor Microorganism Concentration for One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1 and at a 10-day Mean Cell Residence Time

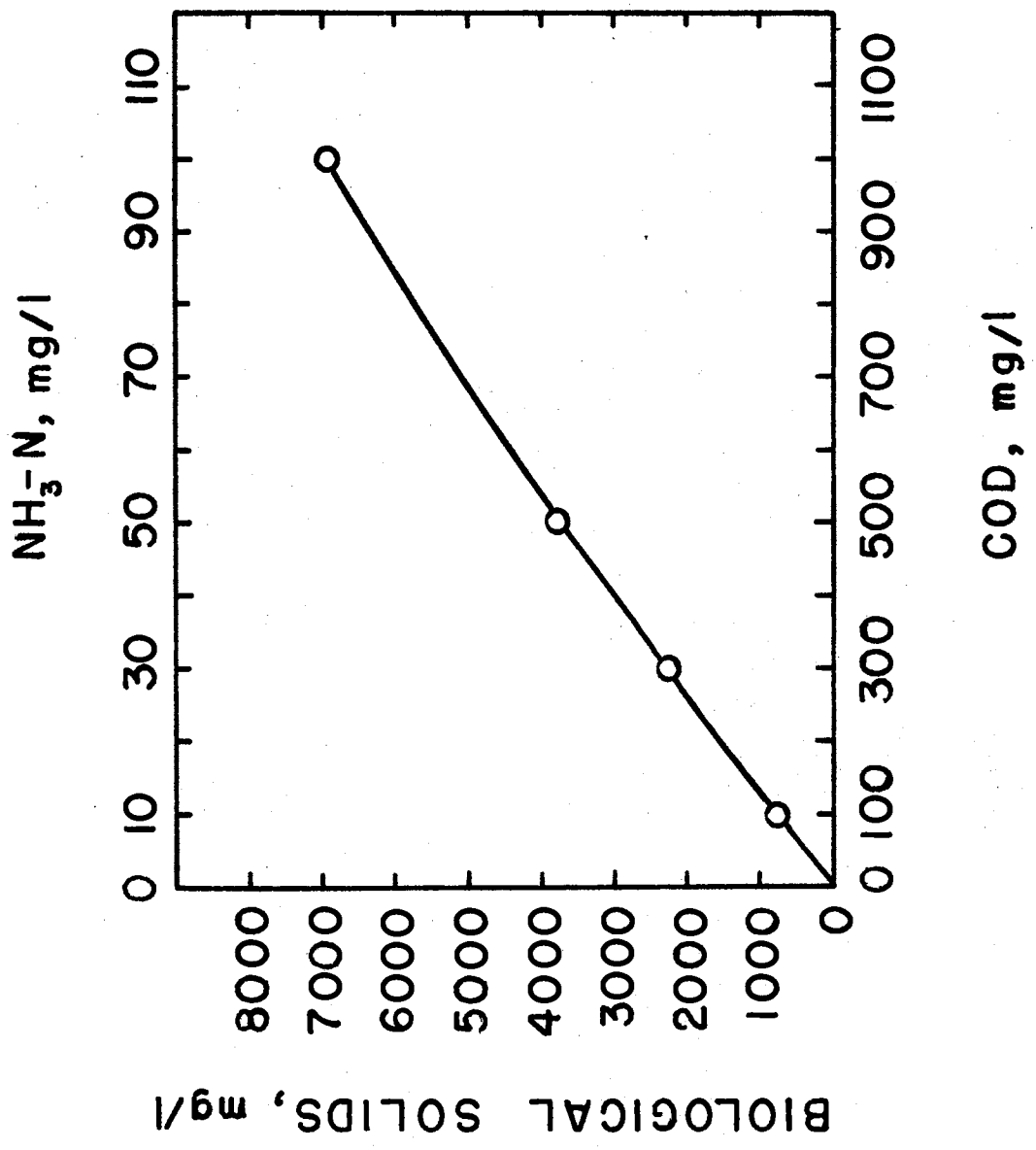
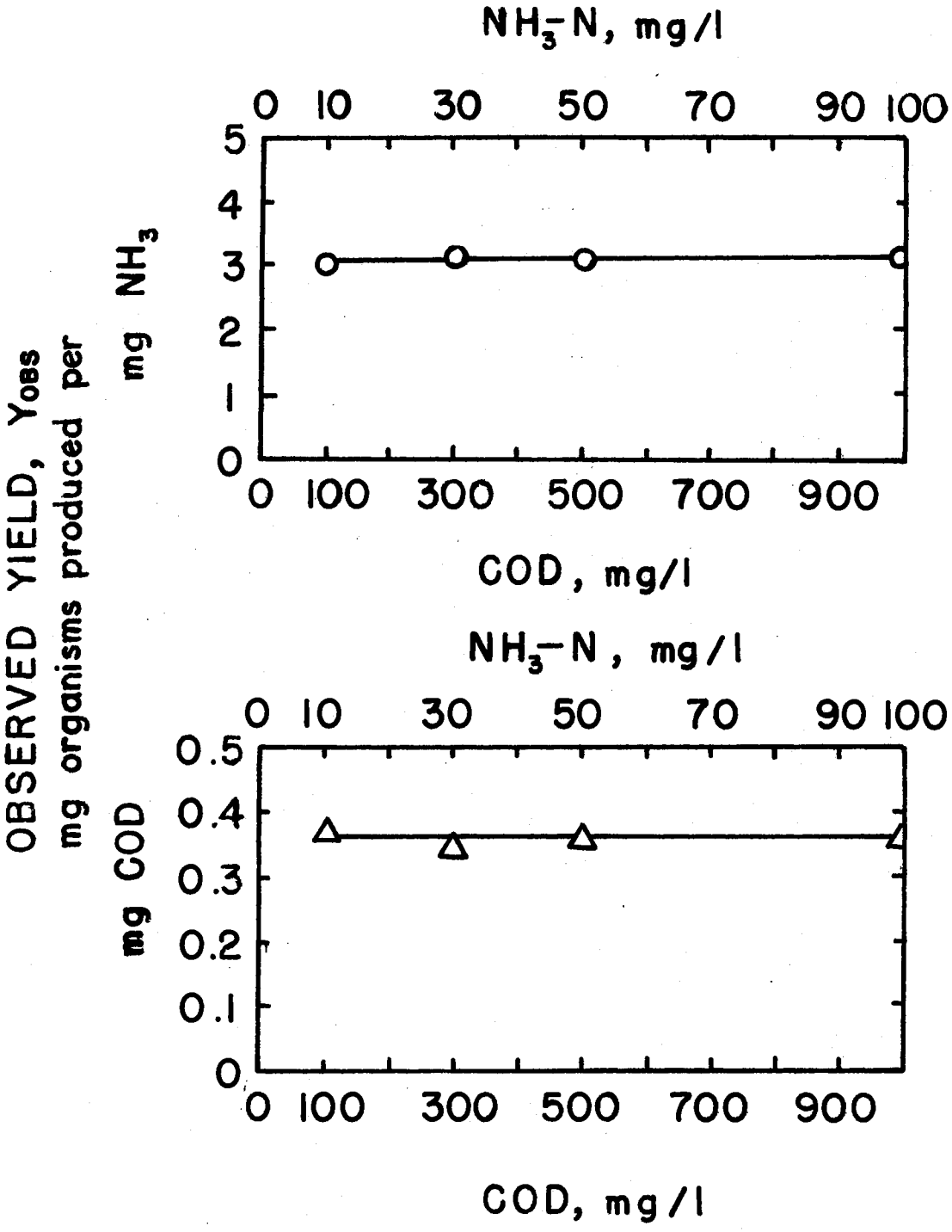

BIOLOGICAL SOLIDS, mg/l
0
1000
2000
3000
4000
5000
6000
7000
8000
0 100 300 500 700 900 1100
COD, mg/l
0 10 30 50 70 90 110
NH3-N, mg/l

Figure 40. Observed Yield for One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1 and at a 10-day Mean Cell Residence Time

O = $Y_{obs}$ in terms of $NH_3$

Δ = $Y_{obs}$ in terms of COD


OBSERVED YIELD, $Y_{OBS}$
mg organisms produced per
mg $NH_3$
$NH_3$-N, mg/l
0 10 30 50 70 90 100
5
4
3
2
1
0
0 100 300 500 700 900
COD, mg/l
$NH_3$-N, mg/l
0 10 30 50 70 90 100
mg COD
0.5
0.4
0.3
0.2
0.1
0
0 100 300 500 700 900
COD, mg/l

COD concentrations range from 100 mg/l to 1000 mg/l, and the influent ammonia-nitrogen concentrations range from 10 mg/l to 100 mg/l.

As can be seen in Figure 38, the percent ammonia-nitrogen removal remained constant at about 98 to 99 percent for all four systems at a sludge age of 10 days. Percent ammonia-nitrogen converted to nitrate-nitrogen decreased approximately 15 percent between influent concentrations of 100 mg/l and 1000 mg/l COD--but the percent of ammonia-nitrogen to cell synthesis increased approximately 15 percent between these same influent COD concentrations.

Biological solids concentration increased with increasing influent COD concentrations, as shown in Figure 39. The reactor microorganism concentration increased from 757 mg/l at 100 mg/l COD and 10 mg/l ammonia-nitrogen to 6953 mg/l at 1000 mg/l COD and 100 mg/l ammonia-nitrogen. In all four systems the observed yield coefficient in terms of both COD and ammonia was constant at the 10-day sludge age (Figure 40). The observed yield remained at a constant 3.1 based on ammonia and 0.36 based on COD.

b. Studies on One-Stage Systems Operated at Different COD:$NH_3$-N Ratios. Three different activated sludge systems were operated at COD:$NH_3$-N ratios of approximately 2:1, 10:1, and 20:1. These reactors were operated at influent concentrations of 100 mg/l COD to 50 mg/l $NH_3$-N, 500 mg/l COD to 50 mg/l $NH_3$-N, and 1000 mg/l COD to 50 mg/l $NH_3$-N. Results from the reactor operated at 500 mg/l COD and 50 mg/l $NH_3$-N have already been presented in the preceding section.

The concentration of ammonia-nitrogen in the effluents from all three systems decreased with increasing mean cell residence time, while

nitrate-nitrogen increased, as shown in Figures 41, 24, and 42. Only 15.7 mg/l nitrate-nitrogen was produced at a 13.5-day sludge age from the reactor with influent concentrations of 1000 mg/l COD and 50 mg/l ammonia-nitrogen. Ammonia-nitrogen removal and conversion to nitrate-nitrogen both increased with increasing mean cell residence time, as shown in Figures 43, 27, and 44. In all cases the amount of nitrate-nitrogen produced was less than the ammonia-nitrogen removed due to ammonia-nitrogen removal by synthesis and a small amount of nitrite-nitrogen leaving the system.

COD purification efficiency measured on membrane filtrate remained above 90 percent over the range of mean cell residence times investigated, except for one value from the 100 mg/l COD and 50 mg/l $NH_3$-N reactor, as can be seen in Figures 45, 30, and 46. At a mean cell residence time of 6.5 days this system achieved only about 80 percent COD removal, but at lower sludge ages the removal increased again to around 90 percent. This would not be an uncommon occurrence for such a system loaded at a low COD loading due to the residual COD usually observed and the accuracy of the COD test at such low values. The COD removal characteristics along with nitrogen concentrations obtained at the various sludge ages are shown in Tables X, VIII, and XI.

In Figure 47 is shown a comparison of the ammonia-nitrogen removal characteristics of these three reactor systems. At a sludge age of approximately 10 days the removal stabilized at about 98 to 99 percent in all three systems. Below 10 days, ammonia-nitrogen removal dropped off much more rapidly in the system operated at 500 mg/l COD and 50 mg/l $NH_3$-N than in the other two systems. The removal in the two systems operated at 100 mg/l and 1000 mg/l COD remained high until about a

Figure 41. Nitrification Performance Data for One-Stage Activated Sludge System Operated at 100 mg/l COD and 50 mg/l $NH_3$-N

□ = influent $NH_3$-N concentration

○ = effluent $NH_3$-N concentration

△ = effluent $NO_3$-N concentration

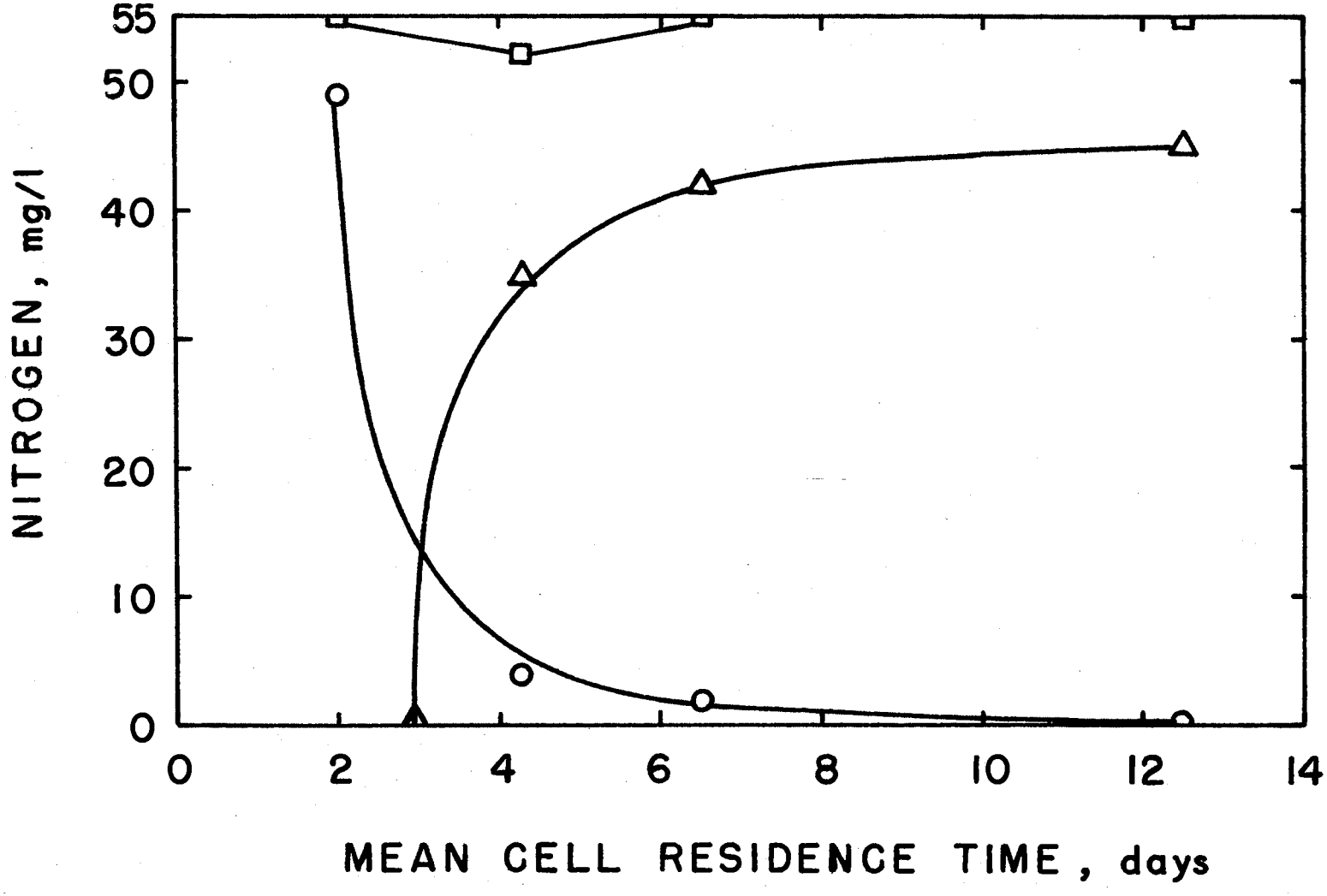

55
50
40
30
20
10
0
NITROGEN, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 42. Nitrification Performance Data for One-Stage Activated Sludge System Operated at 1000 mg/l COD and 50 mg/l $NH_3$-N

□ = influent $NH_3$-N concentration

○ = effluent $NH_3$-N concentration

△ = effluent $NO_3$-N concentration

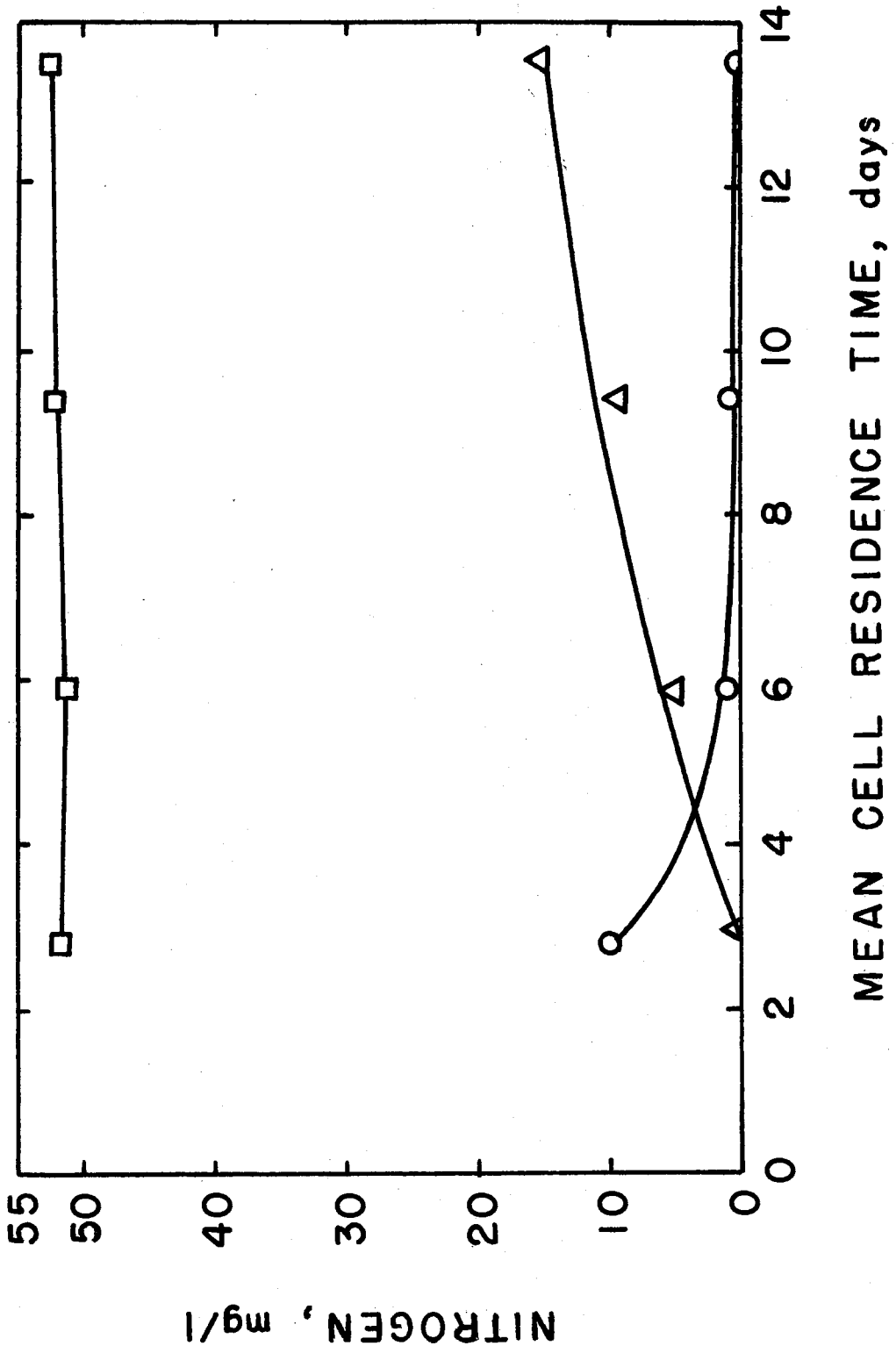

NITROGEN, mg/l
55
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 43. Nitrification Efficiency of One-Stage Activated Sludge System Operated at 100 mg/l COD and 50 mg/l $NH_3$-N Versus Mean Cell Residence Time

O = percent $NH_3$-N removed

Δ = percent $NH_3$-N removed that was converted to $NO_3$-N

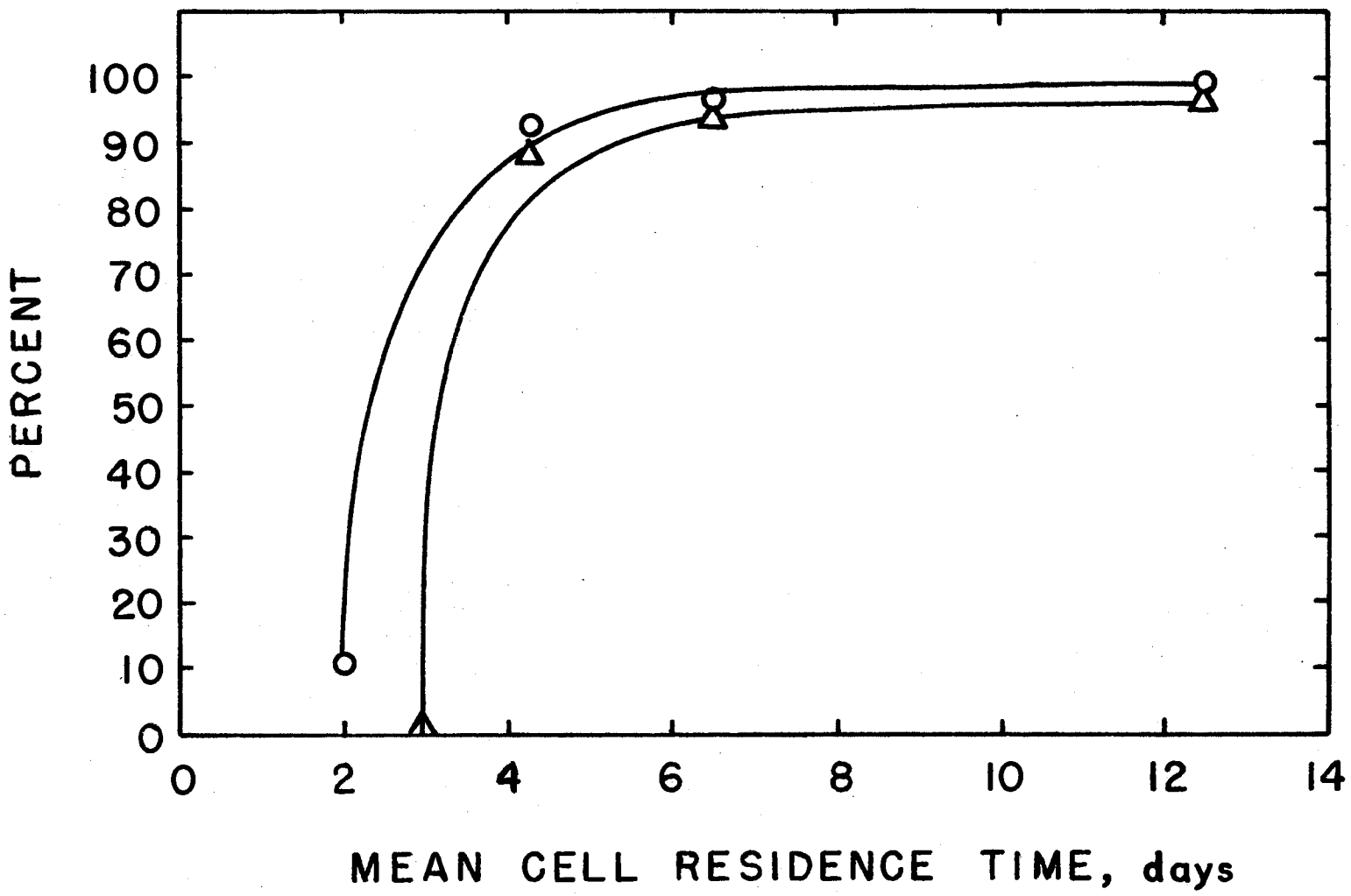

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 44. Nitrification Efficiency of One-Stage Activated Sludge System Operated at 1000 mg/l COD and 50 mg/l $NH_3$-N Versus Mean Cell Residence Time

O = percent $NH_3$-N removed

Δ = percent $NH_3$-N removed that was converted to $NO_3$-N

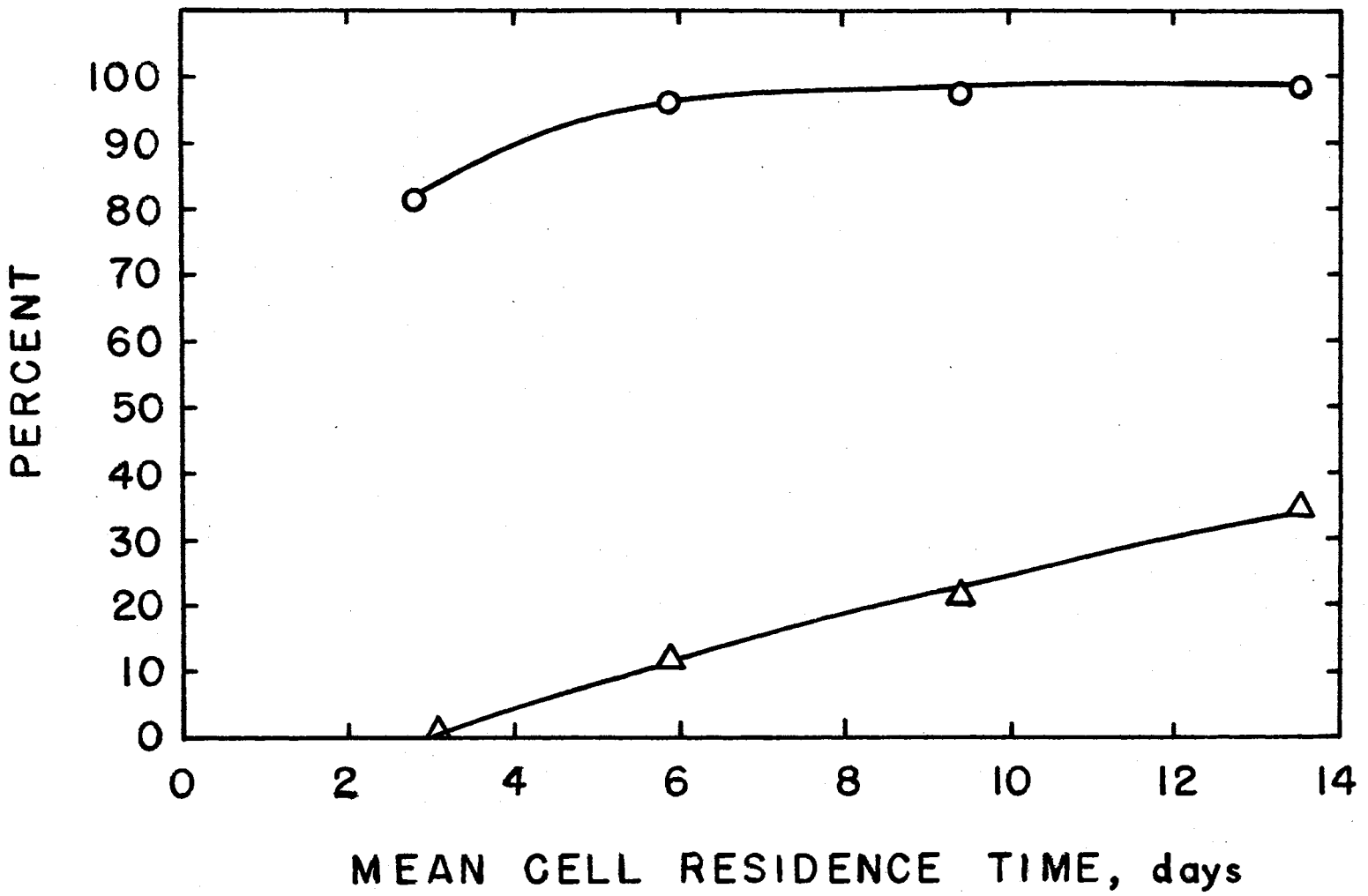

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 45. COD Removal Characteristics of One-Stage Activated Sludge System Operated at 100 mg/l COD and 50 mg/l $NH_3$-N

□ = influent COD concentration

○ = effluent COD concentration

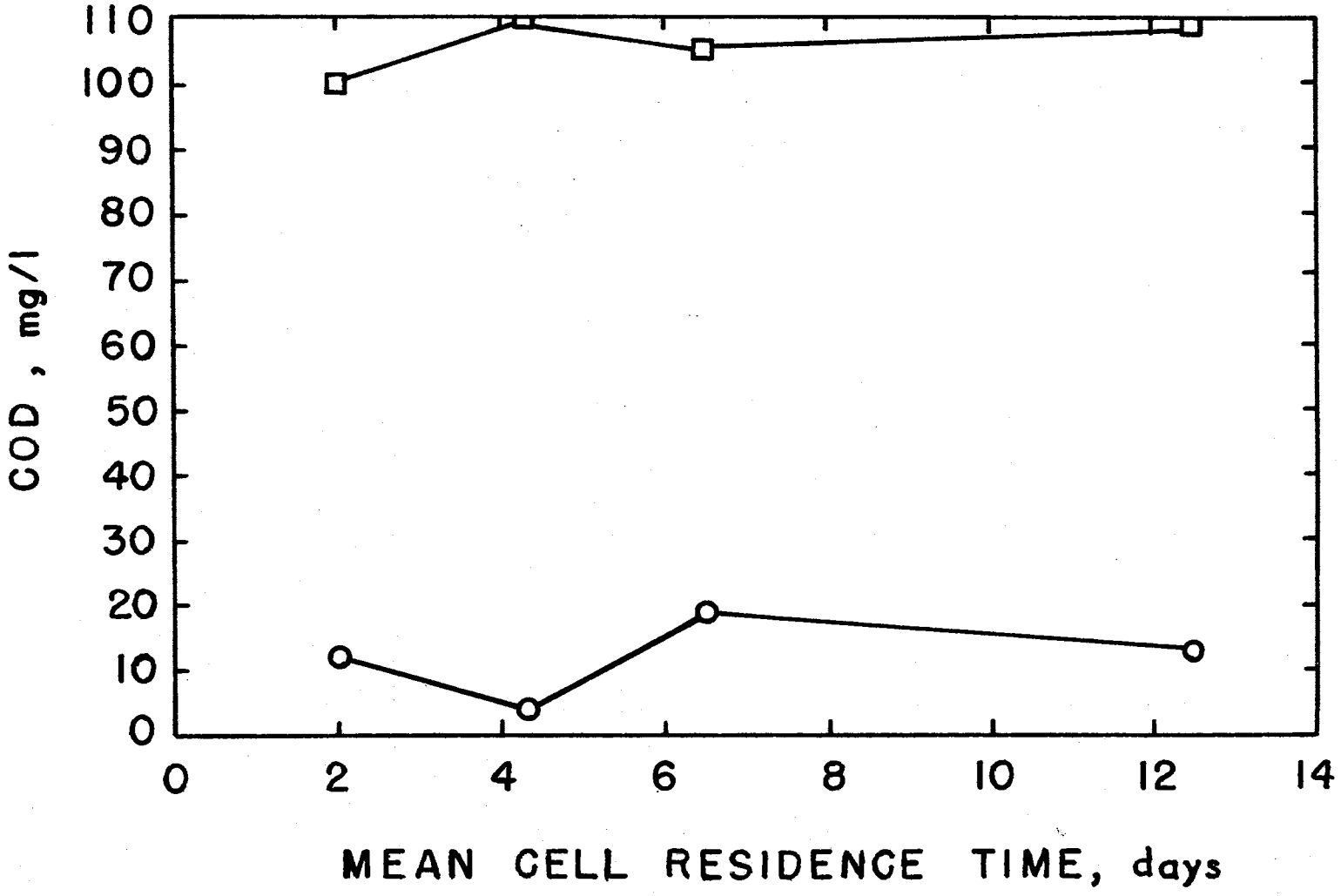

110
100
90
80
70
60
50
40
30
20
10
0
COD , mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 46. COD Removal Characteristics of One-Stage Activated Sludge System Operated at 1000 mg/l COD and 50 mg/l $NH_3$-N

□ = influent COD concentration

○ = effluent COD concentration

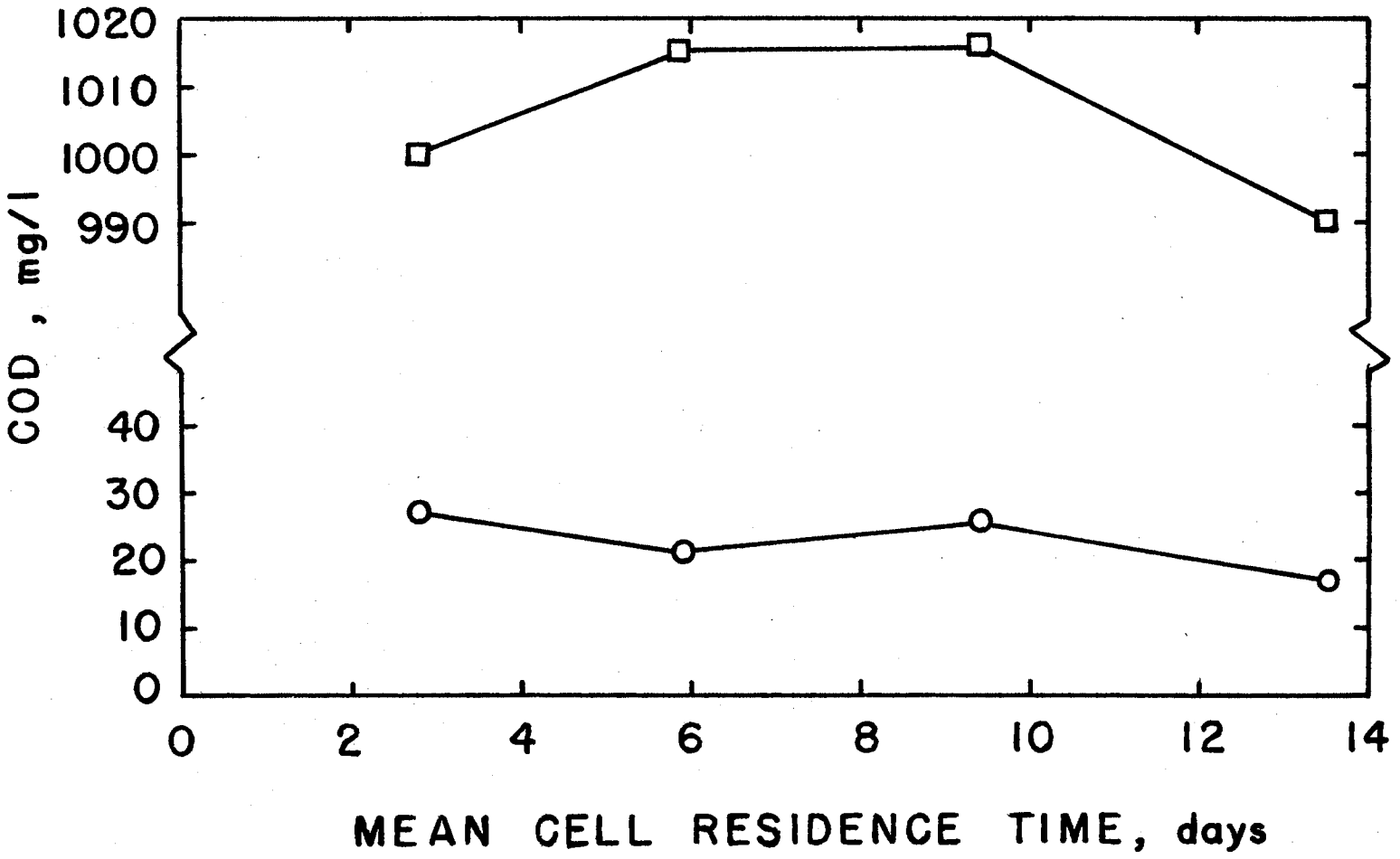

1020
1010
1000
990
40
30
20
10
0
COD, mg/l
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

TABLE X

SUMMARY OF OPERATIONAL CHARACTERISTICS OF NITRIFYING ONE-STAGE ACTIVATED SLUDGE SYSTEM FOR COD:$NH_3$-N = 100:50 ($\Theta$ = 12.0 hours)

| $\Theta_c$ (Days) | Biological Solids (mg/l) | Influent, (mg/l) | | | | Effluent, (mg/l) | | | | $Y_{obs}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$ | COD |
| 2.0 | 182 | 55.0 | 0 | 0 | 100 | 49.0 | 0 | 0 | 12 | 7.5 | 0.52 |
| 4.3 | 800 | 52.0 | 0 | 0 | 111 | 4.0 | 35.0 | 0.5 | 4 | 2.0 | 0.87 |
| 6.5 | 1575 | 56.0 | 0 | 0 | 105 | 2.0 | 42.0 | 0.4 | 19 | 2.2 | 1.10 |
| 12.5 | 2584 | 55.5 | 0 | 0 | 109 | 0.4 | 45.0 | 0.1 | 13 | 1.8 | 1.00 |

TABLE XI

SUMMARY OF OPERATIONAL CHARACTERISTICS OF NITRIFYING ONE-STAGE ACTIVATED SLUDGE SYSTEM FOR COD:$NH_3$-N = 1000:50
($\Theta$ = 12.0 hours)

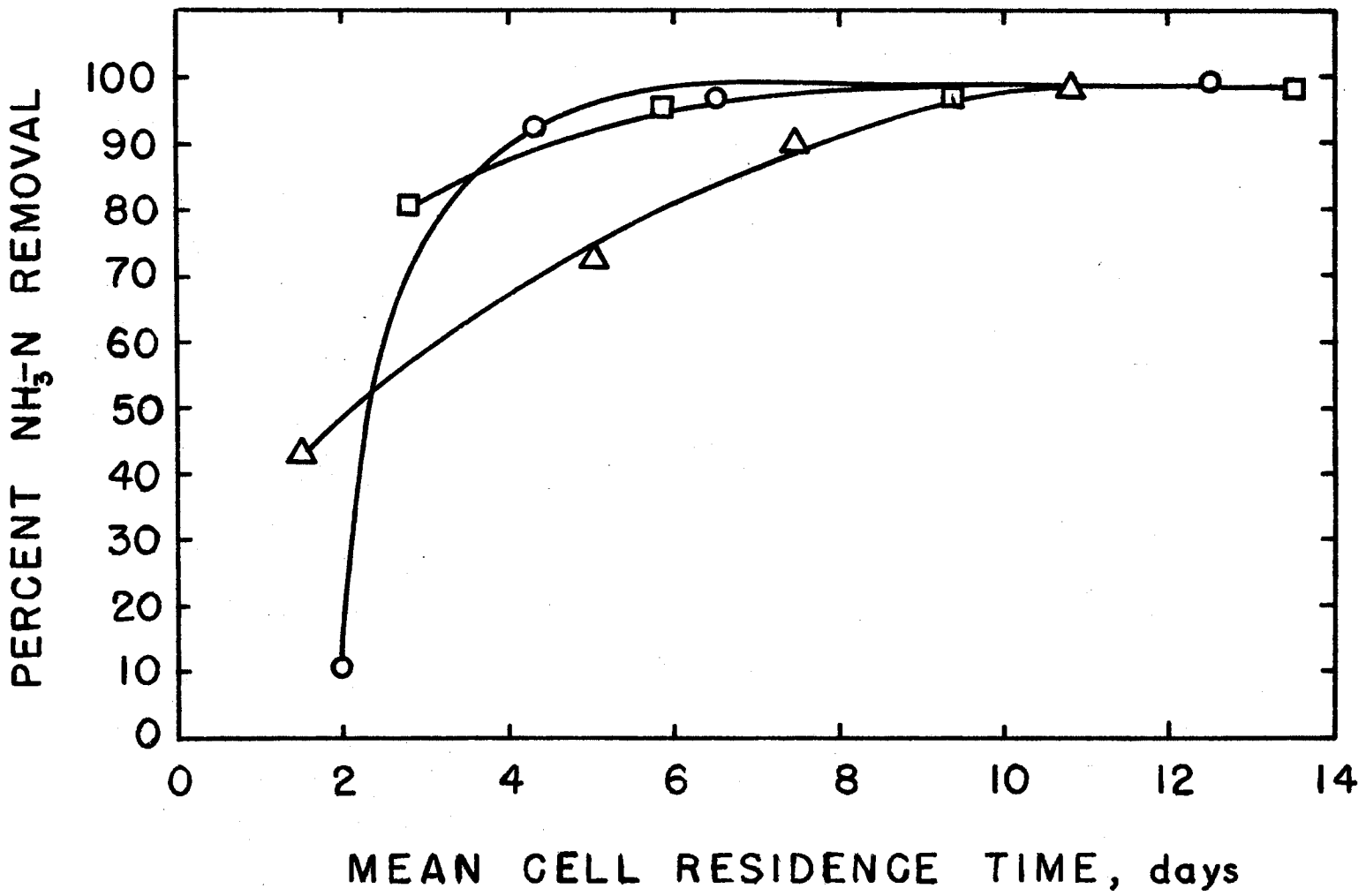
| $\Theta_c$ (Days) | Biological Solids (mg/l) | Influent, (mg/l) | | | | Effluent, (mg/l) | | | | $Y_{obs}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$-N | $NO_3$-N | $NO_2$-N | COD | $NH_3$ | COD |
| 2.8 | 1895 | 53.0 | 0 | 0 | 1000 | 10.0 | 0 | 0 | 27 | 7.8 | 0.44 |
| 5.9 | 4720 | 52.5 | 0 | 0 | 1015 | 2.0 | 5.0 | 0.46 | 21 | 7.8 | 0.40 |
| 9.4 | 6180 | 54.0 | 0 | 0 | 1016 | 1.4 | 9.2 | 0.20 | 26 | 6.1 | 0.33 |
| 13.5 | 7240 | 55.0 | 0 | 0 | 990 | 0.9 | 15.7 | 0.10 | 17 | 4.9 | 0.27 |

Figure 47. Comparison of Ammonia-Nitrogen Removal Characteristics of One-Stage Activated Sludge Systems Operated at Different COD:$NH_3$-N Ratios

○ = influent (COD = 100 mg/l, $NH_3$-N = 50 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 50 mg/l)


PERCENT $NH_3$-N REMOVAL
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

four-to-five day sludge age had been reached.

Ammonia-nitrogen removed converted to nitrate-nitrogen decreased significantly with increasing COD:$NH_3$-N ratio (Figure 48). At a sludge age of 10 days and COD:$NH_3$-N = 2:1, approximately 95 percent conversion was achieved, while at the same sludge age and COD:$NH_3$-N = 10:1 and COD:$NH_3$-N = 20:1, only about 80 percent and 25 percent conversion to nitrate, respectively, was accomplished. This reduction in nitrification can be explained by Figure 49, which shows the ammount of ammonia-nitrogen removed by conversion to cellular nitrogen to increase with increasing COD:$NH_3$-N ratio. Approximately only five percent of the ammonia-nitrogen was converted to cellular nitrogen at a 10-day sludge age by the reactor operated at 100 mg/l COD and 50 mg/l ammonia-nitrogen. The reactors with influent concentrations of 500 mg/l COD to 50 mg/l $NH_3$-N and 1000 mg/l COD to 50 mg/l $NH_3$-N converted about 20 percent and 75 percent of the ammonia-nitrogen to cellular nitrogen at the 10-day sludge age.

In Figure 50 is shown a comparison of the reactor biological solids concentration as a function of sludge age for these three systems. The solids concentration increased with higher mean cell residence times and appeared to start leveling off at about 12 days. Both Figure 50 for different COD:$NH_3$-N ratios and Figure 35 for the same COD:$NH_3$-N ratio show similar characteristics of sludge increase and leveling off with increased sludge age.

The observed yield coefficient determined in terms of COD and ammonia is shown in Figures 51 and 52, respectively. For the reactor with COD:$NH_3$-N = 20:1 or 1000 mg/l COD and 50 mg/l $NH_3$-N, the observed yield

Figure 48. Comparison of Ammonia-Nitrogen Conversion to Nitrate-Nitrogen by One-Stage Activated Sludge Systems Operated at Different COD:$NH_3$-N Ratios

○ = influent (COD = 100 mg/l, $NH_3$-N = 50 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 50 mg/l)

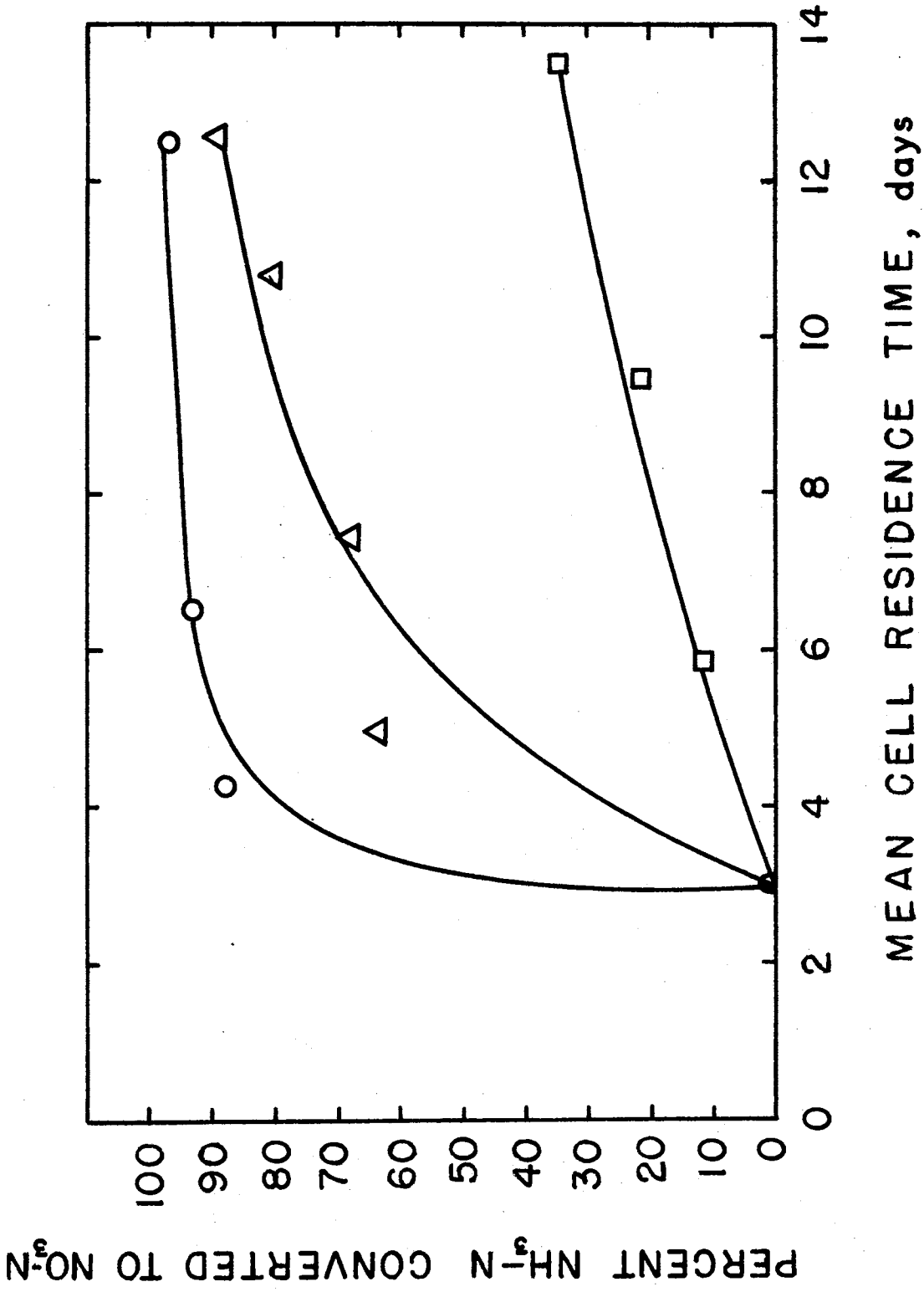

PERCENT NH3-N CONVERTED TO NO3-N
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 49. Comparison of Ammonia-Nitrogen Conversion to Biological Cell Matter by One-Stage Activated Sludge Systems Operated at Different COD:$NH_3$-N Ratios

○ = influent (COD = 100 mg/l, $NH_3$-N = 50 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 50 mg/l)

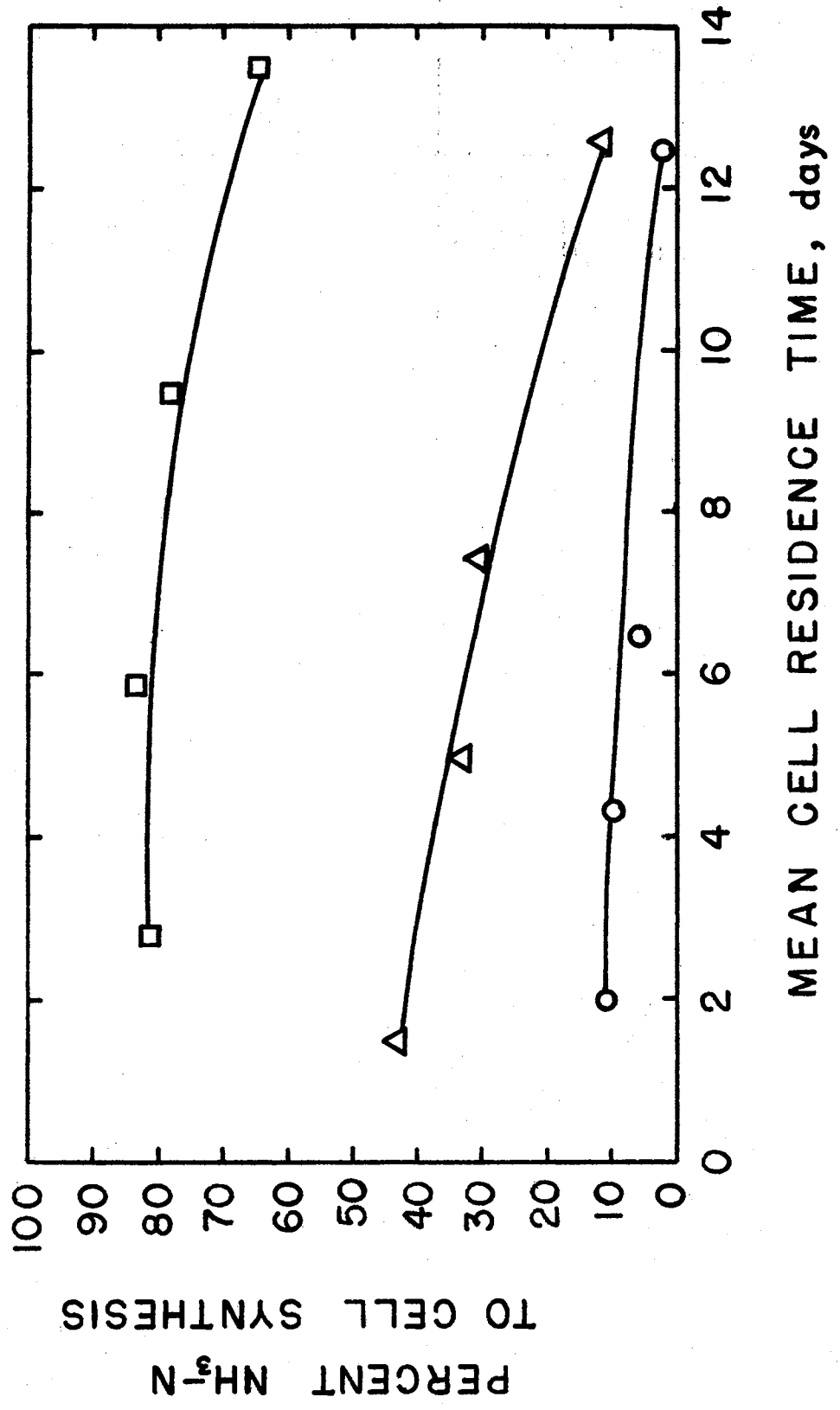

PERCENT $NH_3$-N TO CELL SYNTHESIS
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 50. Reactor Microorganism Concentration Versus Mean Cell Residence Time for One-Stage Activated Sludge Systems Operated at Different COD:$NH_3$-N Ratios

O = influent (COD = 100 mg/l, $NH_3$-N = 50 mg/l)

Δ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 50 mg/l)

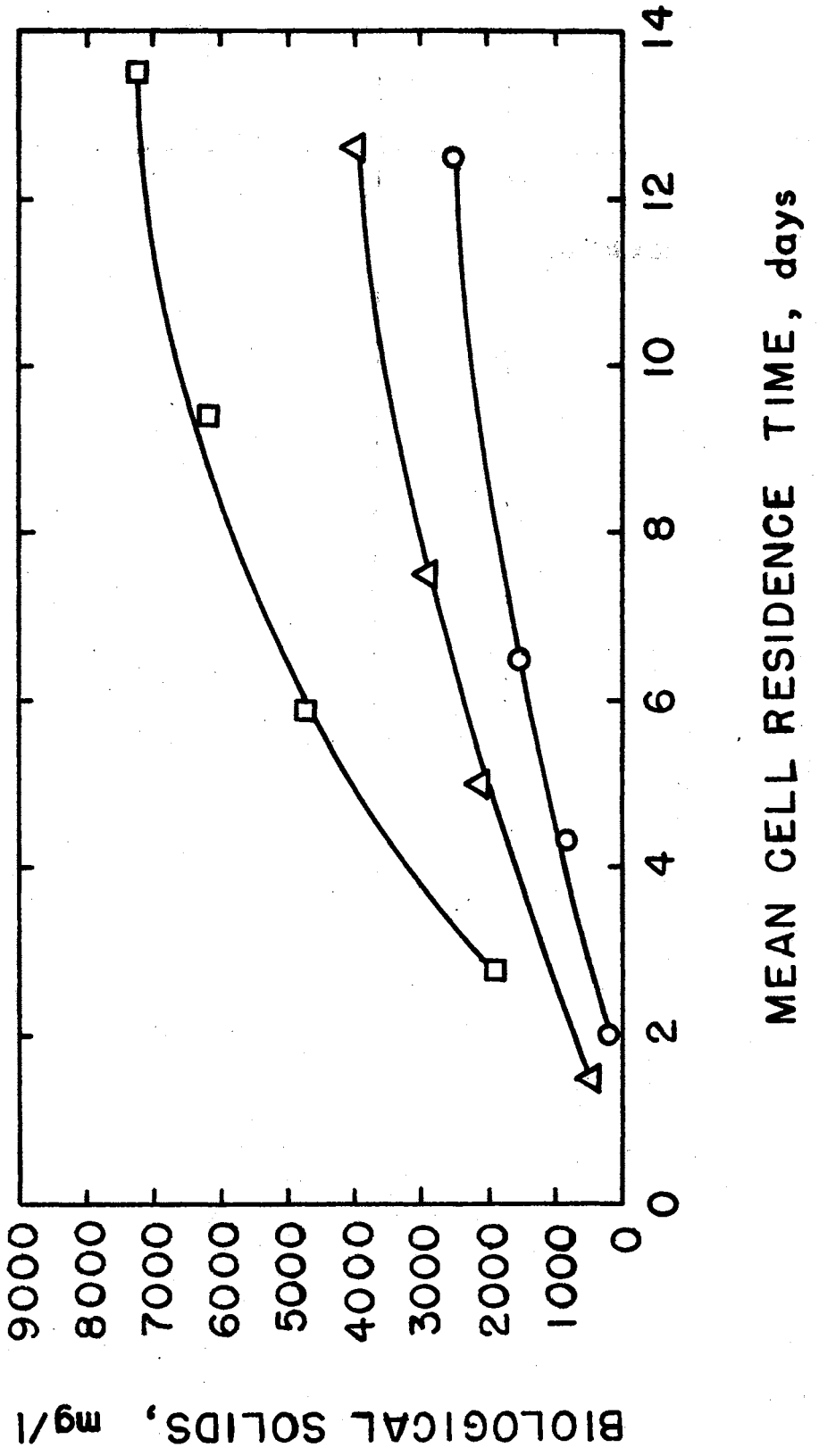

BIOLOGICAL SOLIDS, mg/l
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 51. Observed Yield Computed in Terms of COD Versus Mean Cell Residence Time for One-Stage Activated Sludge Systems Operated at Different COD:$NH_3$-N Ratios

○ = influent (COD = 100 mg/l, $NH_3$-N = 50 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 50 mg/l)

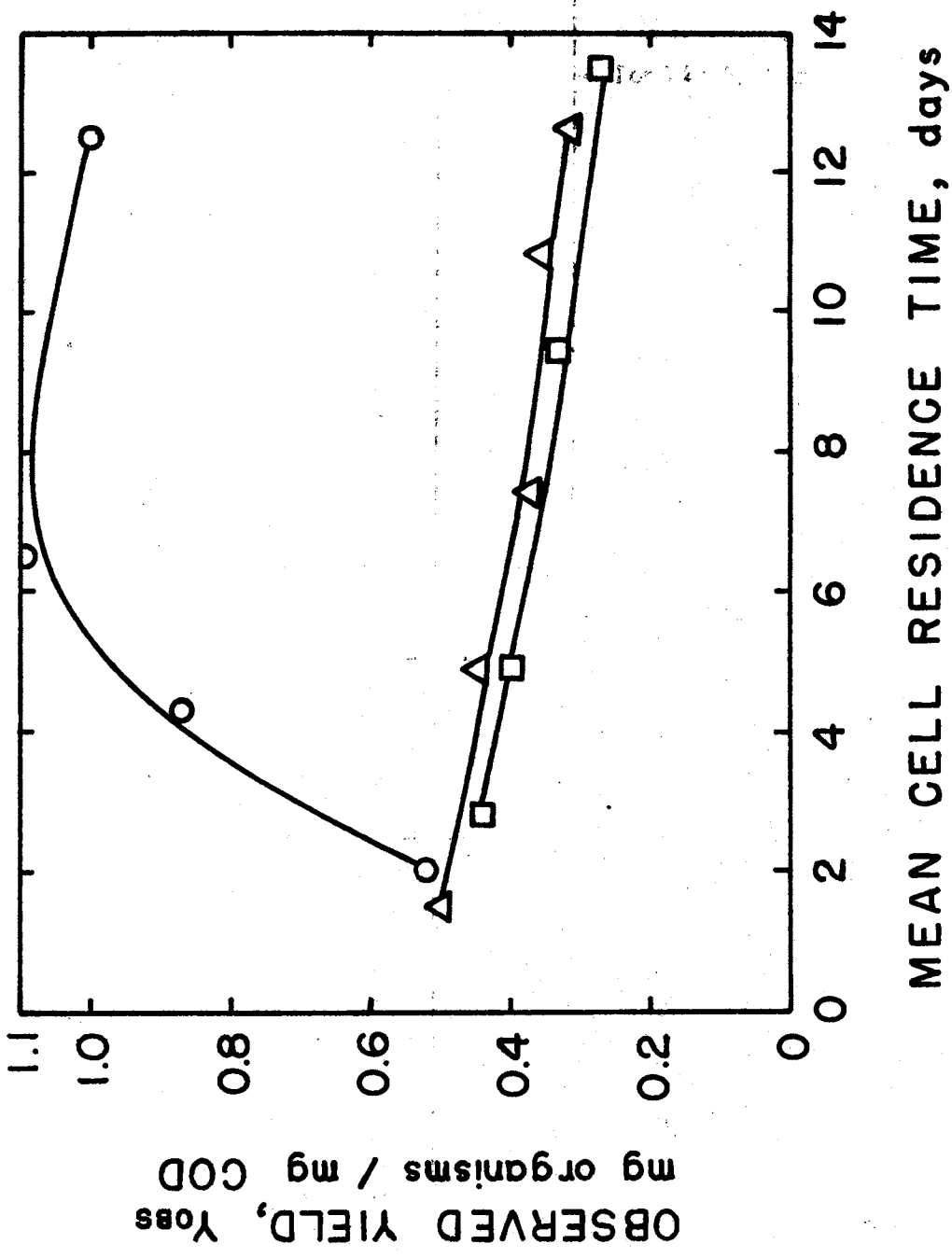
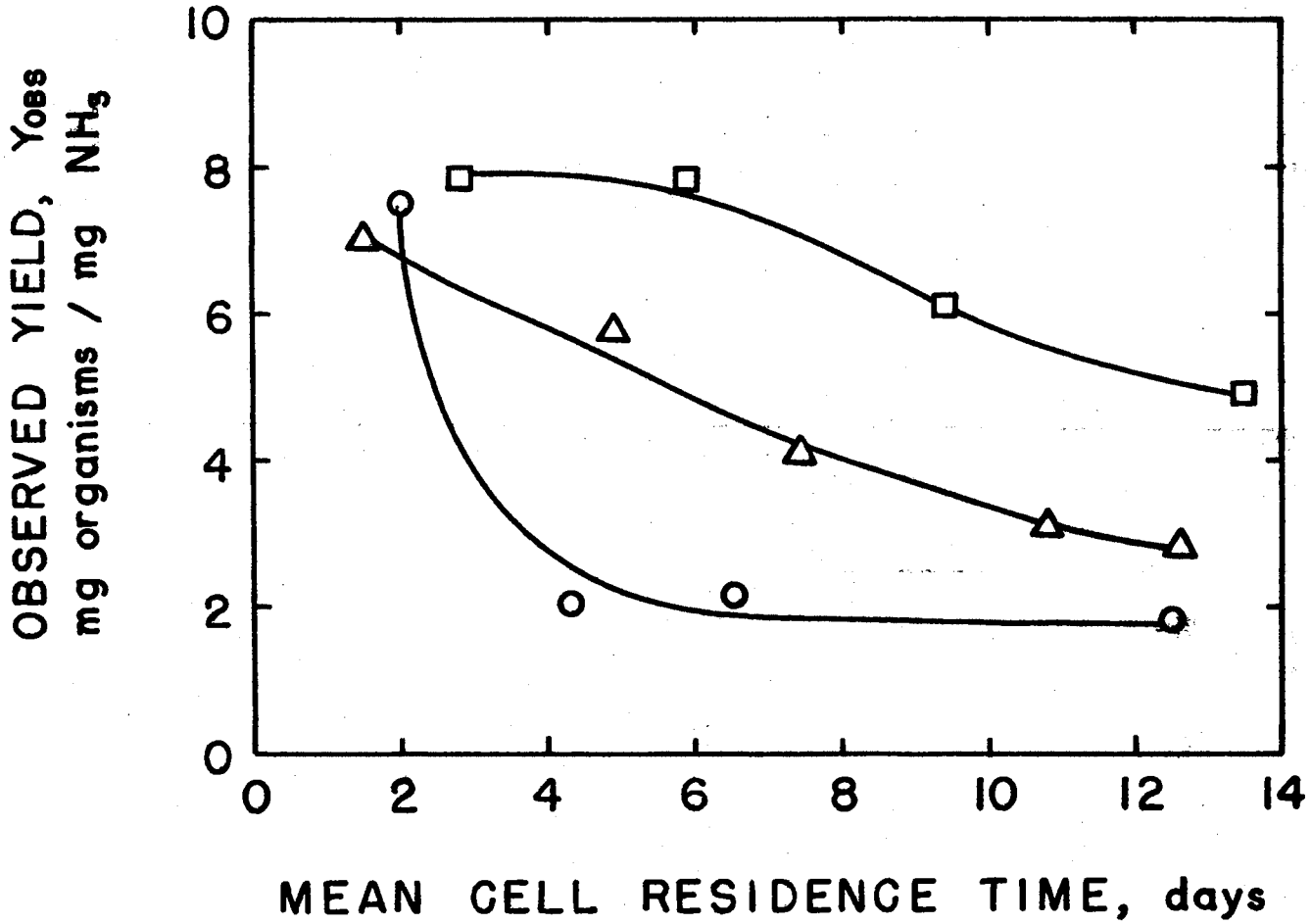

OBSERVED YIELD, $Y_{obs}$
mg organisms / mg COD
1.1
1.0
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

Figure 52. Observed Yield Computed in Terms of Ammonia Versus Mean Cell Residence Time for One-Stage Activated Sludge Systems Operated at Different COD:$NH_3$-N Ratios

○ = influent (COD = 100 mg/l, $NH_3$-N = 50 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 50 mg/l)


OBSERVED YIELD, $Y_{obs}$
mg organisms / mg $NH_3$
10
8
6
4
2
0
0
2
4
6
8
10
12
14
MEAN CELL RESIDENCE TIME, days

curve in terms of COD proved to be nearly equal to the yield curve for COD:$NH_3$-N = 10:1, as shown in Figures 36 and 51. The yield curve for COD:$NH_3$-N = 20:1 was approximately parallel to the yield curve for COD:$NH_3$-N = 10:1, and slightly less in value--the greatest difference being only about 0.05 mg of microorganisms per mg of COD. For the reactor operated at 100 mg/l COD and 50 mg/l $NH_3$-N (COD:$NH_3$-N = 2:1), the yield increased from about 0.5 mg organisms per mg COD at two days to a value of 1.1 mg organisms per mg COD at a little over six days mean cell residence time. Then the yield started to decrease with increasing sludge age, and at 12.5 days the yield had decreased to 1.0 mg organisms per mg COD. The yields greater than 1.0 were due to a significant amount of growth from the high concentration of ammonia-nitrogen in the influent to the system. Since only 10 percent or less of the ammonia-nitrogen (5 mg/l or less) was channeled into cell synthesis, 90 percent or more (45 mg/l or more) was available as an energy source for the autotrophic nitrifying organisms.

In all three systems the observed yield in terms of ammonia decreased with increasing mean cell residence time, as shown in Figure 52. Over the range of sludge ages investigated the observed yields from each of the three systems deviated significantly from one another. The yields from the system with COD:$NH_3$-N = 20:1 were much higher than the yields from the system with COD:$NH_3$-N = 10:1 which were substantially higher than the yields from the system with COD:$NH_3$-N = 2:1. These very high and widespread yield values in terms of ammonia were due to the variance in the influent COD concentrations, while the ammonia-nitrogen concentration (50 mg/l) remained the same in all systems. At the higher COD concentrations (500 mg/l and 1000 mg/l) a

very high proportion of the total growth in the systems was carbonaceous growth.

3. Performance of Combined Two-Stage Biological Filter at Various Organic Loadings

This biological trickling filter system was operated at approximately COD:$NH_3$-N = 20:1 and at various influent COD concentrations and hydraulic flow rates. Flow rates of 500, 750, and 1000 gpd/$ft^2$ were employed for this investigation. The total organic and ammonia-nitrogen loadings for each experimental run were calculated by multiplying the average influent COD or $NH_3$-N concentration by the flow rate and then converting by use of the proper coefficients into units of lbs COD/day/1000 $ft^3$ or lbs $NH_3$-N/day/1000 $ft^3$. The results presented are for the combined filter volumes.

a. Nitrogen Removal by Biological Synthesis Reactions. Shown in Figures 53, 54, and 55 are the relationships of $NH_3$-N and COD remaining with depth in both the primary and secondary filters for various organic carbon and $NH_3$-N concentrations and flow rates, without any nitrification taking place. Effluent nitrate-nitrogen and nitrite-nitrogen concentrations were consistently zero even though the system had been seeded with nitrifying microorganisms. According to a plot of applied COD (lbs/day/1000 $ft^3$) versus mean cell residence time obtained by Bentley (98) from an investigation of this same reactor system, all of the results shown here were procured at mean cell residence times of less than two days. Therefore, the ammonia-nitrogen removed from the system was incorporated into bacterial cell matter.

Figure 53. Relationship of Ammonia-Nitrogen and COD remaining with Depth of the Biological Filter Operated at a Constant Flow Rate of 500 gpd/$ft^2$ and Varying $NH_3$-N and COD Concentrations ($NH_3$-N removal due to cell synthesis only)

O = influent (COD = 256 mg/l, $NH_3$-N = 15 mg/l)

Δ = influent (COD = 554 mg/l, $NH_3$-N = 34.8 mg/l)

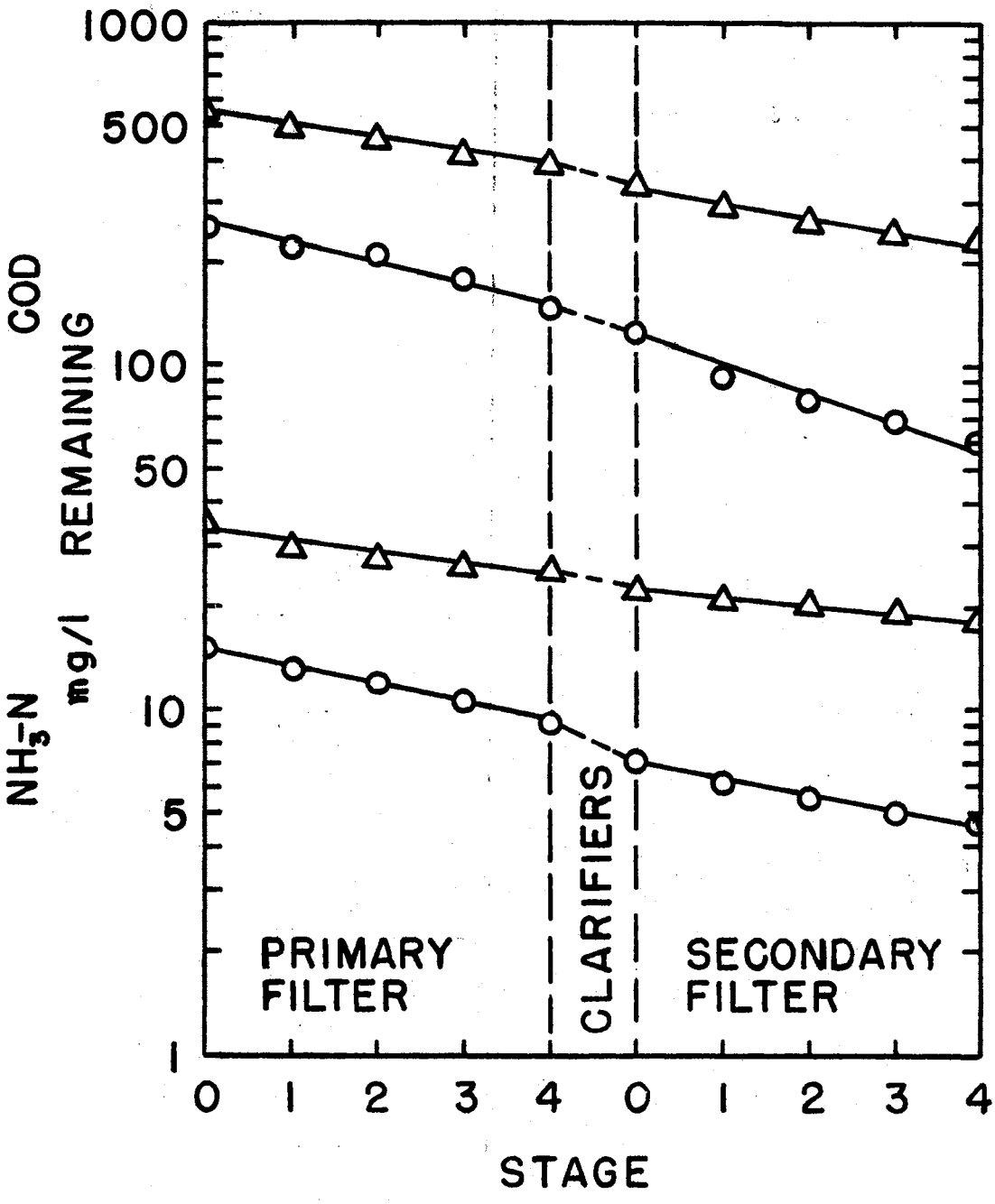

1000
500
100
50
10
5
1
COD
NH3-N mg/l REMAINING
PRIMARY FILTER
CLARIFIERS
SECONDARY FILTER
0 1 2 3 4 0 1 2 3 4
STAGE

Figure 54. Relationship of Ammonia-Nitrogen and COD Remaining with Depth of the Biological Filter Operated at a Constant Flow Rate of 750 gpd/ft$^2$ and Varying $NH_3$-N and COD Concentrations ($NH_3$-N Removal due to cell synthesis only)

o = influent (COD = 290 mg/l, $NH_3$-N = 17.5 mg/l)

Δ = influent (COD = 202 mg/l, $NH_3$-N = 11.4 mg/l)

□ = influent (COD = 149 mg/l, $NH_3$-N = 8.7 mg/l)

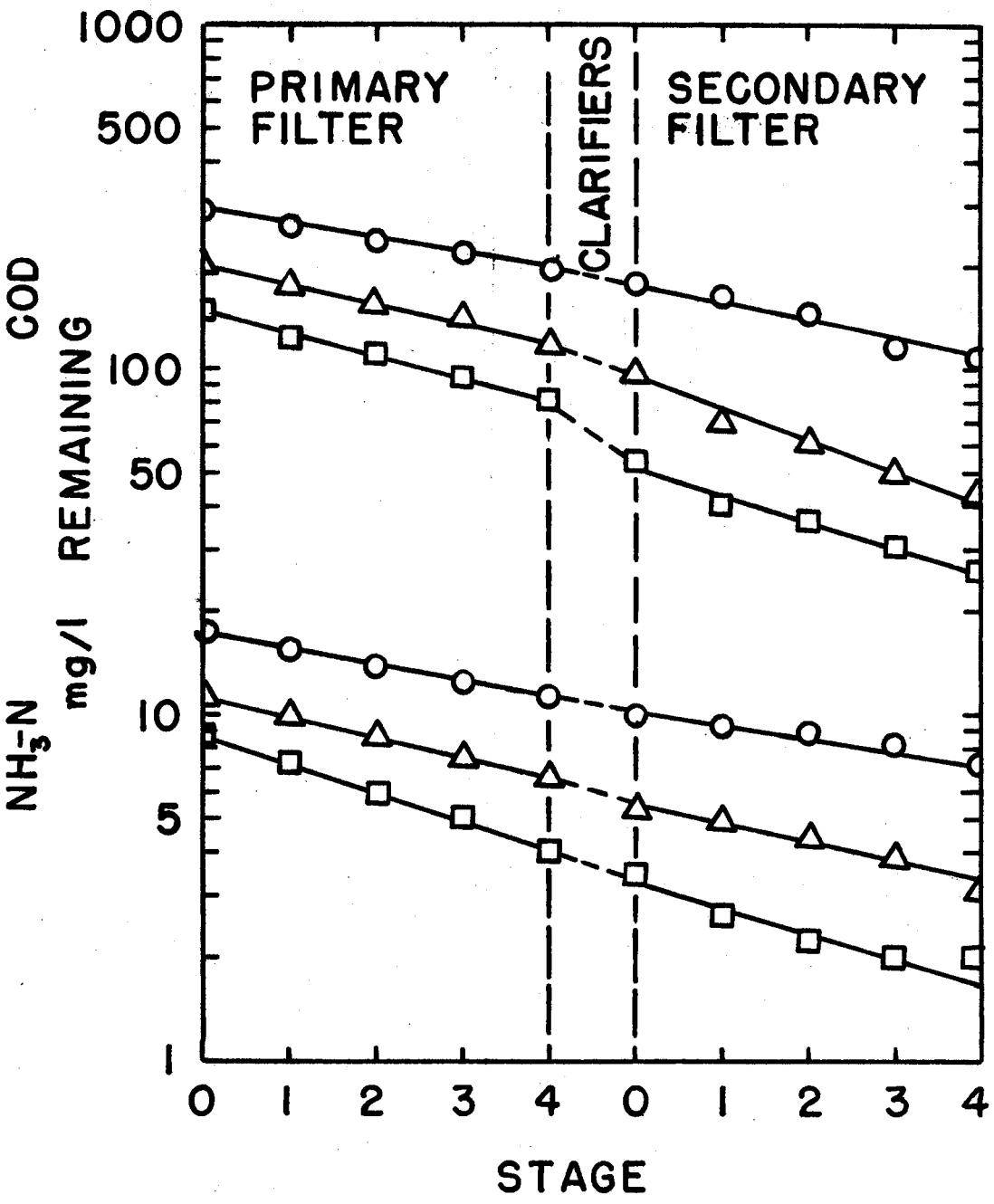
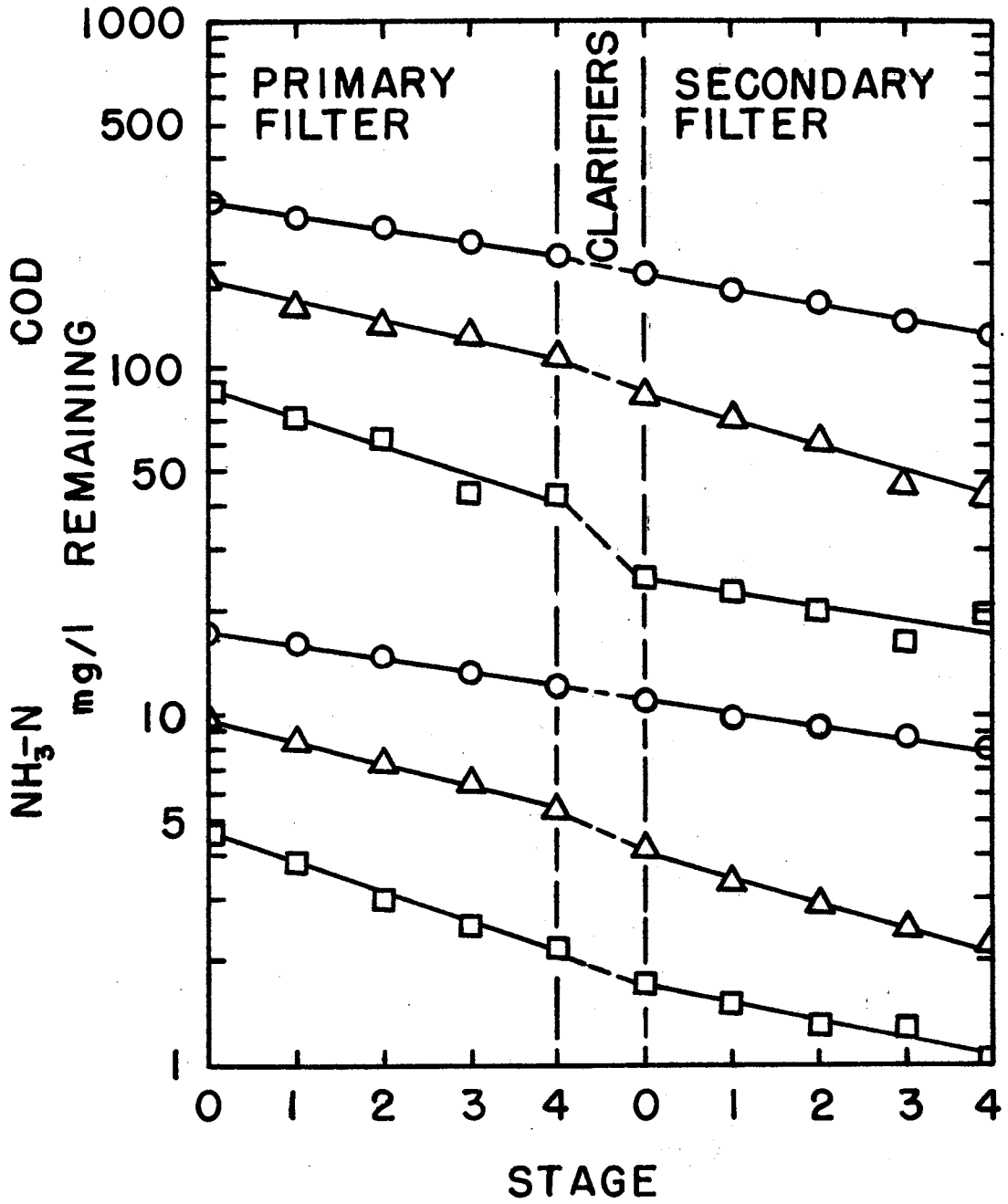

1000
500
100
50
10
5
1
PRIMARY FILTER
CLARIFIERS
SECONDARY FILTER
COD
$NH_3$-N mg/l REMAINING
0 1 2 3 4 0 1 2 3 4
STAGE

Figure 55. Relationship of Ammonia-Nitrogen and COD Remaining with Depth of the Biological Filter Operated at a Constant Flow Rate of 1000 gpd/ft$^2$ and Varying $NH_3$-N and COD Concentrations ($NH_3$-N removal due to cell synthesis only)

○ = influent (COD = 300 mg/l, $NH_3$-N = 17.6 mg/l)

△ = influent (COD = 177 mg/l, $NH_3$-N = 9.9 mg/l)

□ = influent (COD = 84 mg/l, $NH_3$-N = 4.7 mg/l)


1000
500
100
50
10
5
1
COD
$NH_3$-N
mg/l REMAINING
PRIMARY FILTER
CLARIFIERS
SECONDARY FILTER
0 1 2 3 4 0 1 2 3 4
STAGE

It can be seen in these semi-logarithmic plots that both COD and $NH_3$-N remaining with depth are straight lines. This indicates that the removal of both COD and $NH_3$-N is first order with respect to filter depth. By calculating the slopes of the lines plotted in Figures 53, 54, and 55, substrate removal rates, (K), were obtained. Removal rates in terms of both COD and $NH_3$-N were calculated from these figures for the primary filter, and plotted as shown in Figure 56. These values along with influent and effluent concentrations are shown in Table XII. The first order decreasing rate of removal for both COD and $NH_3$-N with respect to applied COD loading and corresponding applied $NH_3$-N loading plotted as identical curves in Figure 56.

The relationship of percent $NH_3$-N removed with $NH_3$-N applied (lbs/day/1000 $ft^3$) over a wide range of loadings is shown in Figure 57. An ammonia-nitrogen removal efficiency of 80 percent was achieved at a loading of six lbs $NH_3$-N/day/1000 $ft^3$ by simple conversion of the substrate nitrogen into cellular nitrogen. As the ammonia-nitrogen loading was increased, the removal efficiency decreased and appeared to be leveling out at about 30 percent removal. The COD removal efficiency versus COD loading in lbs/day/1000 $ft^3$ at a COD:$NH_3$-N = 20:1 ratio for this same system, as studied by Marcangeli (102), received only from zero to 11 percent more COD removal than ammonia-nitrogen removal.

b. Biological Filter and Activated Sludge Nitrogen Removal by Synthetic Processes. In Figure 58 is shown the percent of $NH_3$-N removal with respect to $NH_3$-N to microorganism ratio for the biological filter process. The $NH_3$-N to microorganism ratio was calculated by using a 70 μ active film thickness, as described in the preceding

TABLE XII

SUMMARY OF PERFORMANCE CHARACTERISTICS FOR THE COMBINED TWO-STAGE BIOLOGICAL FILTER SYSTEM AT VARIOUS FLOW RATES AND INFLUENT CONCENTRATIONS

| Flow Rate ($gpd/ft^2$) | Influent | | | | Effluent | | Substrate Removal Rate $K_1$ | |
|---|---|---|---|---|---|---|---|---|
| | $NH_3$-N (mg/l) | $NH_3$-N Loading (lbs/day/ 1000 $ft^3$) | COD (mg/l) | COD Loading (lbs/day/ 1000 $ft^3$) | $NH_3$-N (mg/l) | COD (mg/l) | $NH_3$-N | COD |
| 500 | 15.0 | 7.8 | 256 | 133 | 4.9 | 60 | -0.125 | -0.133 |
| 500 | 34.8 | 18.2 | 554 | 289 | 18.0 | 236 | -0.088 | -0.094 |
| 750 | 8.7 | 6.8 | 149 | 117 | 2.0 | 27 | -0.170 | -0.156 |
| 750 | 11.4 | 8.9 | 202 | 158 | 3.0 | 45 | -0.129 | -0.134 |
| 750 | 17.5 | 13.7 | 290 | 227 | 7.0 | 109 | -0.103 | -0.099 |
| 1000 | 4.7 | 4.9 | 84 | 88 | 1.0 | 20 | -0.189 | -0.185 |
| 1000 | 9.9 | 10.3 | 177 | 185 | 2.2 | 44 | -0.146 | -0.133 |
| 1000 | 17.6 | 18.4 | 300 | 313 | 7.9 | 125 | -0.089 | -0.086 |

Figure 56. Substrate Removal Rate for the Biological Filter Versus Applied Loading (lbs/day/1000 $ft^3$) at Various Flow Rates and Concentrations ($NH_3$-N removal due to cell synthesis only)

O = K in terms of COD

Δ = K in terms of $NH_3$-N

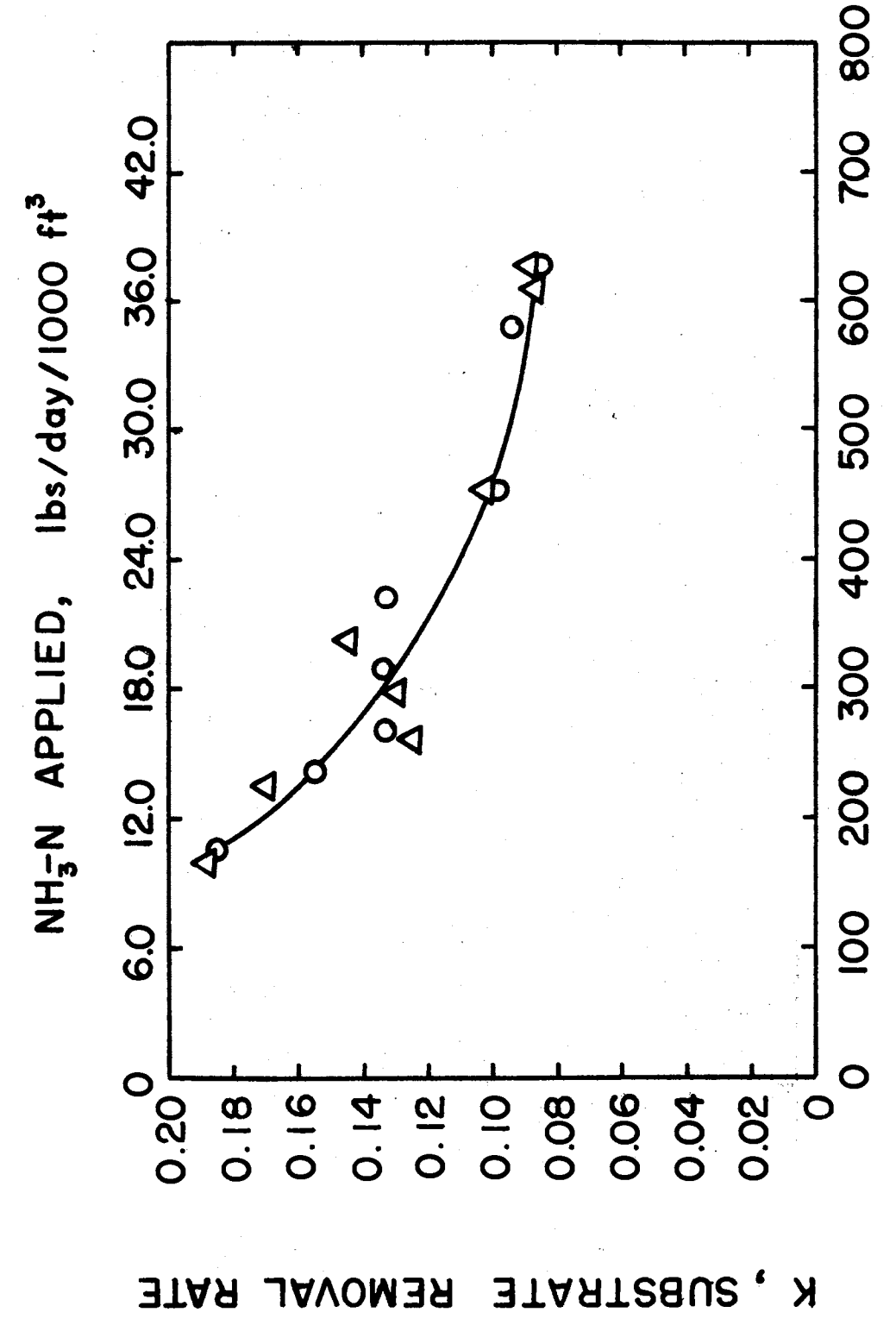

K , SUBSTRATE REMOVAL RATE
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
0
6.0
12.0
18.0
24.0
30.0
36.0
42.0
NH3-N APPLIED, lbs/day/1000 ft3
0
100
200
300
400
500
600
700
800
COD APPLIED, lbs/day/1000 ft3

Figure 57. Relationship of Percent Ammonia-Nitrogen Removed by Cell Synthesis with $NH_3$-N Applied (lbs/day/1000 $ft^3$) to the Biological Filter at Various Flow Rates and Concentrations

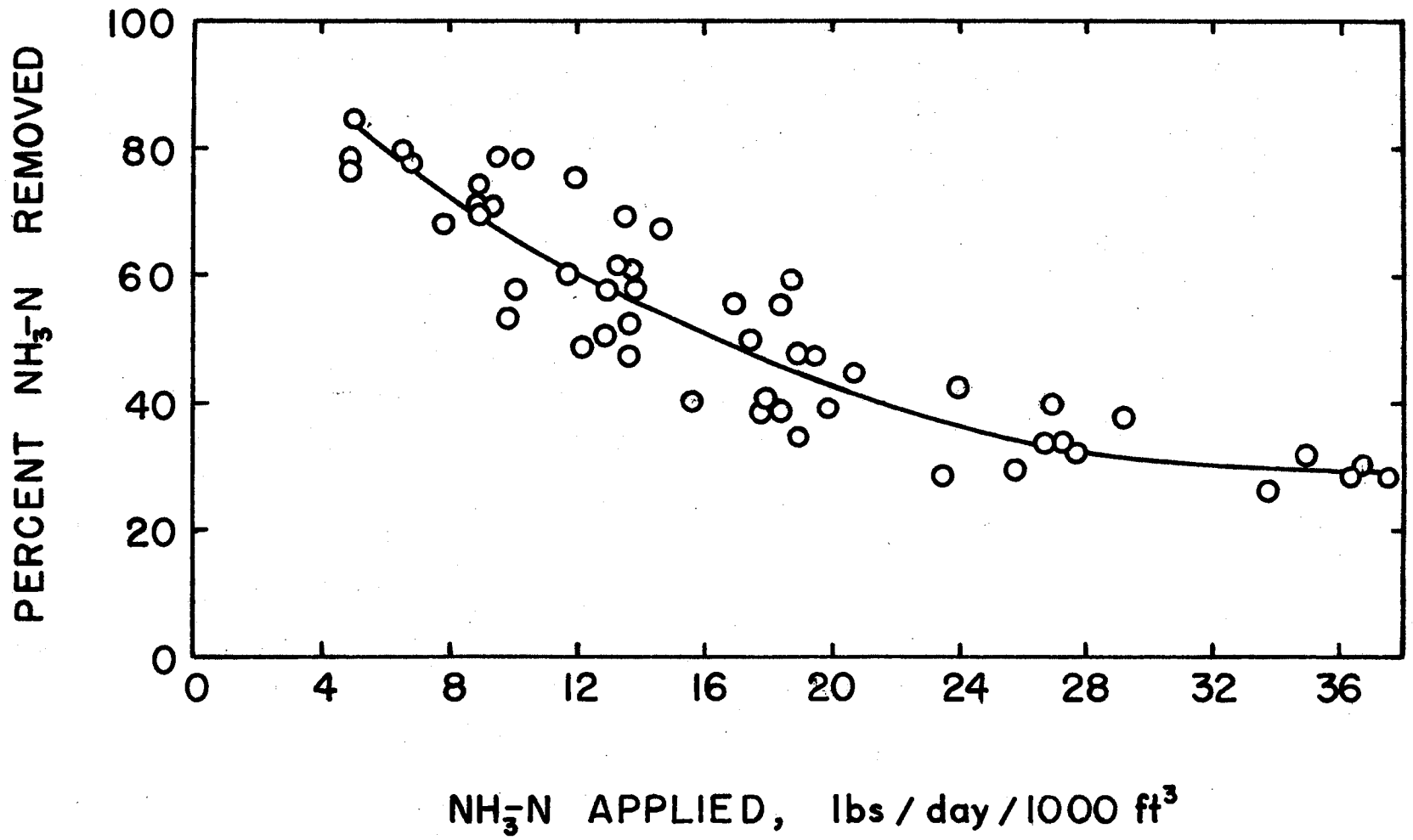
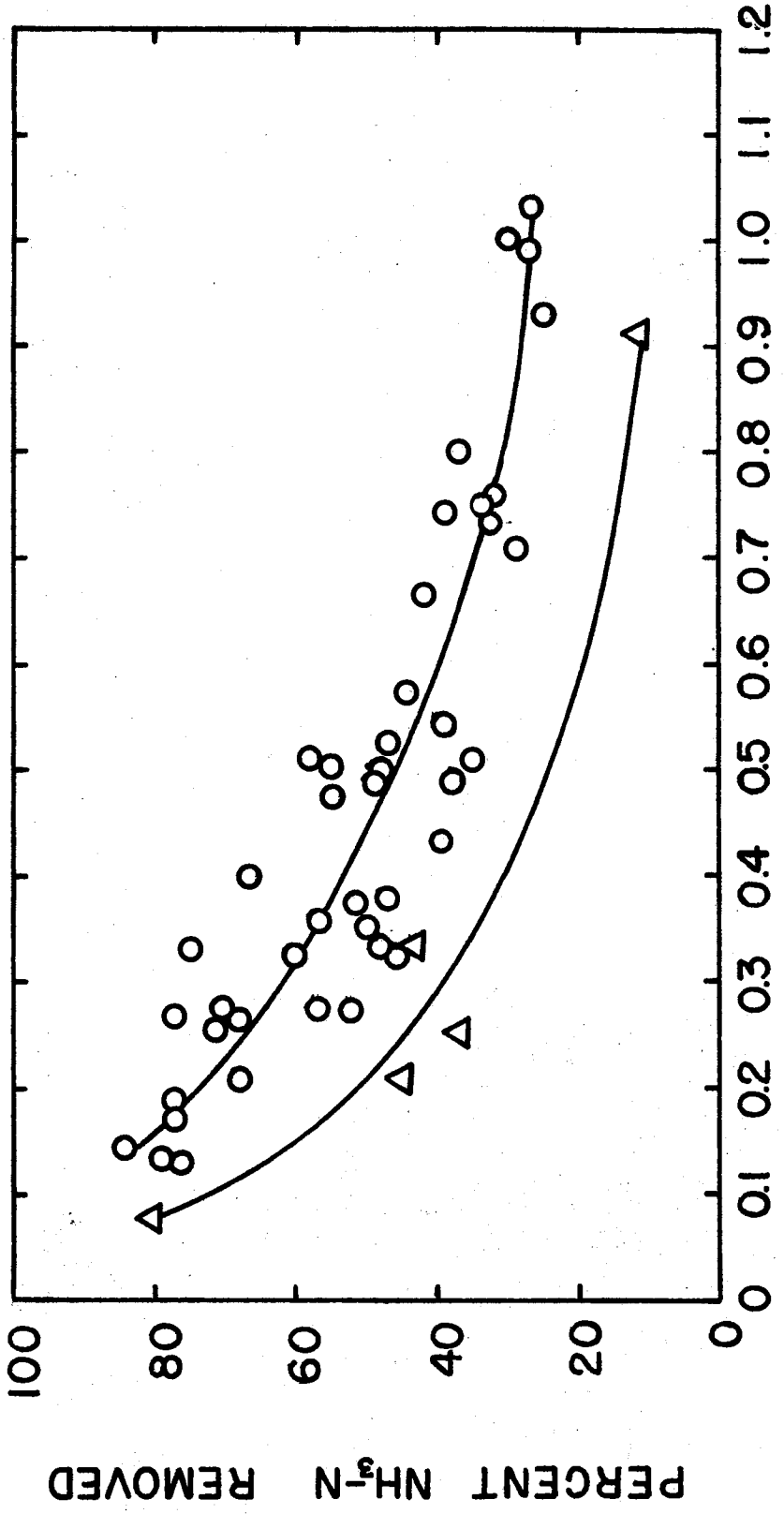

PERCENT $NH_3$-N REMOVED
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
$NH_3$-N APPLIED, lbs / day / 1000 $ft^3$

Figure 58. Percent Ammonia-Nitrogen Removal by Cell Synthesis Versus Ammonia-Nitrogen to Microorganism Ratio for Laboratory Activated Sludge and Biological Filter Systems

O = biological filter process

Δ = one-stage activated sludge systems


PERCENT NH3-N REMOVED
0
20
40
60
80
100
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
NH3-N TO MICROORGANISM RATIO, days-1

chapter. At an $NH_3$-N to microorganism ratio of about 0.15 $days^{-1}$ the biological filter removed 80 percent of the ammonia-nitrogen. The ammonia-nitrogen removal decreased with increasing $NH_3$-N to microorganism ratio. According to Bentley (98), the mean cell residence time varied only from about 0.7 to 1.8 days over the range of investigations shown in Figure 58.

Also shown in Figure 58 is the percent of $NH_3$-N removal with respect to $NH_3$-N to microorganism ratio for the one-stage activated sludge process without nitrification. The activated sludge system removed 80 percent of the ammonia-nitrogen at an $NH_3$-N to microorganism ratio of about 0.09 $days^{-1}$ as compared to 0.15 $days^{-1}$ for the biological filter. As with the biological filter, ammonia-nitrogen removal decreased with increasing $NH_3$-N to microorganism ratio; the two curves remained approximately parallel to each other. Mean cell residence times investigated for the activated sludge system, ranging from 1.5 to 2.8 days, were higher than those from the biological filter system.

c. Ammonia-Nitrogen Removal by Nitrification. Since nitrification was not achieved by the biological filter at the higher organic loadings and low sludge ages, it was deemed necessary to lower the total organic loading or increase the mean cell residence time in order to accomplish nitrification. The influent COD concentration was lowered to approximately 53 mg/l at a hydraulic loading of 1000 $gpd/ft^2$ for a total organic loading of 55 lbs COD/day/1000 $ft^3$. The mean cell residence time corresponding to this organic loading was approximately five days. The COD concentration was reduced to approximately 23 mg/l by the time the fifth foot had been reached, and remained at this same concentration throughout the rest of the reactor.

At this lower organic loading, nitrification began in the fifth foot of the system and continued throughout the rest of the system, as shown in Figures 59 and 60, for two different experimental investigations. Ammonia-nitrogen in the effluent was completely reduced to zero for the first time in any experiments observed. As shown in Figure 59, the ammonia-nitrogen was completely removed even before the eighth foot had been reached. Nitrate-nitrogen increased from 0.005 mg/l at the beginning of the fifth foot to approximately 0.3 mg/l in the effluent from the eighth foot of the system. Between the first and the fourth foot of the system no nitrification occurred, as can be seen by the nitrate-nitrogen concentrations; therefore, no data were collected between these points.

Since all but the residual COD had been removed before the fifth foot, the secondary filter did not remove any COD and therefore functioned similarly to the second sludge system of the two-stage activated sludge system. As such, it should be possible to compare the second sludge system and the secondary filter in terms of nitrification. At this loading the $NH_3$-N to microorganism ratio was 0.046 $days^{-1}$ in the secondary filter. In the second sludge system of the two-stage activated sludge system at a mean cell residence time of five days the $NH_3$-N to microorganism ratio was 0.055 $days^{-1}$, as shown in Figure 19. The ammonia-nitrogen removals achieved by the biological filter and the activated sludge systems were 100 percent and 98 percent, respectively. Approximately 80 percent of the ammonia-nitrogen removed was oxidized to nitrate-nitrogen in the activated sludge process, while only about 40 percent was oxidized in the biological filter. This suggests that possibly a larger amount of ammonia-nitrogen was being removed by

Figure 59. Nitrification Performance Data for the Biological Filter Operated at a Hydraulic Loading of 1000 $gpd/ft^2$ and a Total Organic Loading of 55 lbs $COD/day/1000\ ft^3$ (influent $NH_3$-N = 1.8 mg/l, influent COD = 53 mg/l, effluent COD = 23 mg/l)

O = $NH_3$-N concentration

Δ = $NO_3$-N concentration

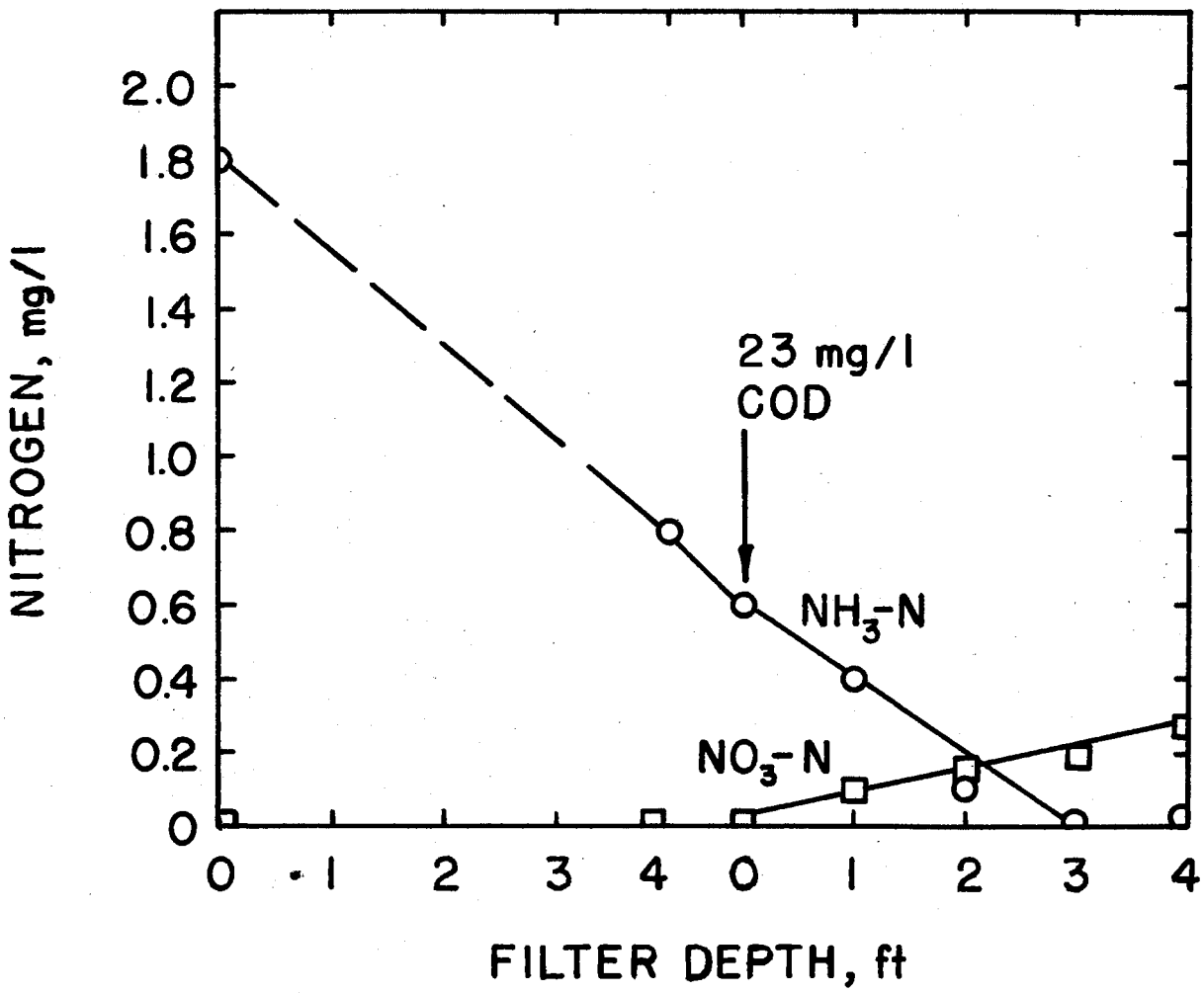
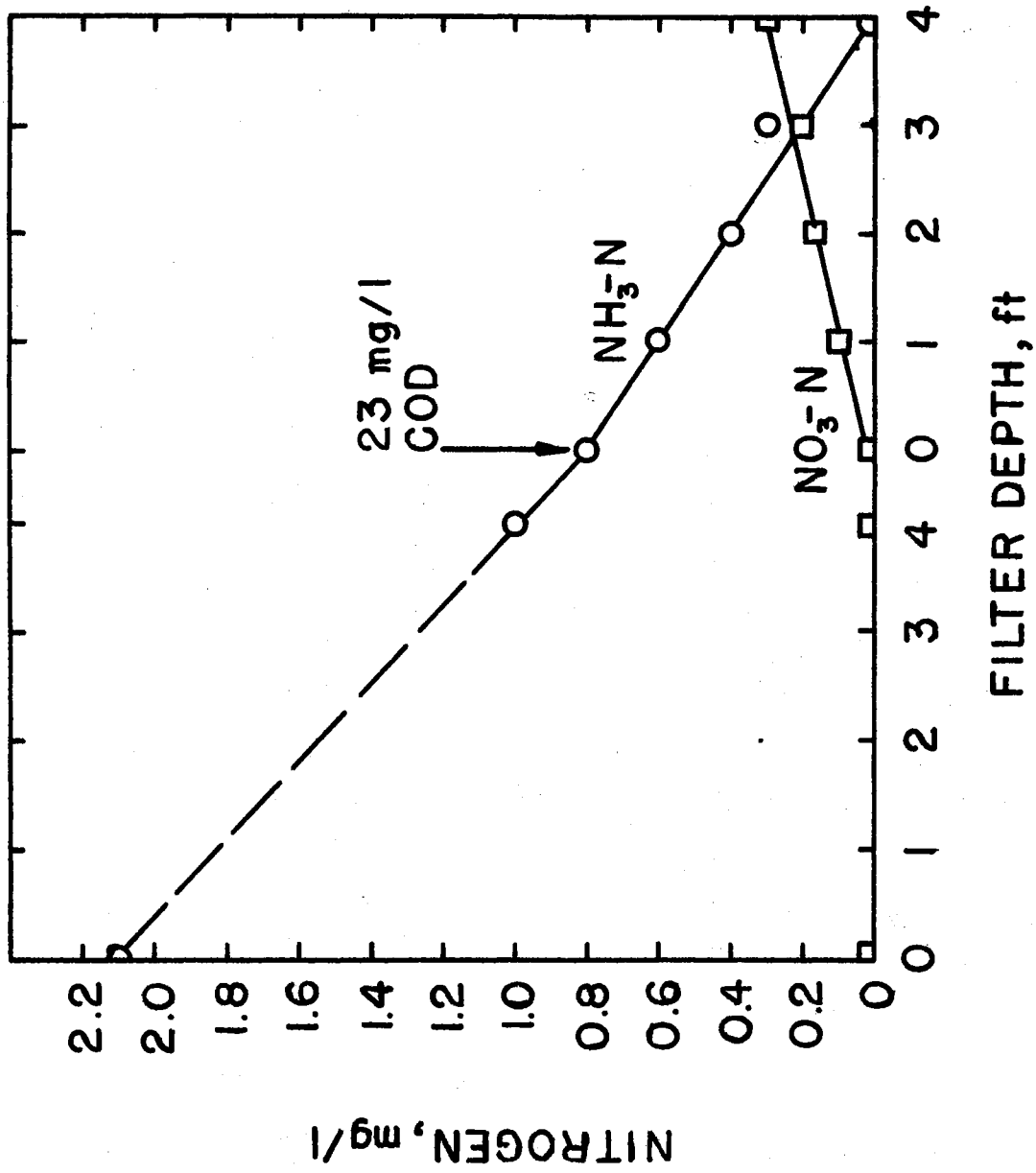

NITROGEN, mg/l
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
23 mg/l
COD
NH3-N
NO3-N
0 · 1 2 3 4 0 1 2 3 4
FILTER DEPTH, ft

Figure 60. Nitrification Performance Data for the Biological Filter Operated at a Hydraulic Loading of 1000 gpd/$ft^2$ and a Total Organic Loading of 55 lbs COD/day/1000 $ft^3$ (influent $NH_3$-N = 2.1 mg/l, influent COD = 53 mg/l, effluent COD = 23 mg/l)

O = $NH_3$-N concentration

Δ = $NO_3$-N concentration


NITROGEN, mg/l
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
FILTER DEPTH, ft
0
1
2
3
4
0
1
2
3
4
23 mg/l
COD
$NH_3$-N
$NO_3$-N

synthesis reactions in the biological filter, or that more nitrogen was discharged as nitrite-nitrogen.

## 4. Nitrification Performance of a Rotating Biological Contactor

### a. Removal Capabilities of the Rotating Biological Contactor.

Ammonia-nitrogen removal characteristics of the rotating disc unit utilizing synthetic wastewater at a hydraulic loading of 0.5 gpd/ft$^2$ and influent COD concentration of 250 mg/l and $NH_3$-N concentration of 27.6 mg/l are shown in Figure 61. In the first stage 82 percent of the $NH_3$-N was removed, and all of the $NH_3$-N had been removed by the end of the fifth stage, as shown in Figure 62. COD removal in the first stage was 81 percent, increasing to 88 percent in the second stage, and remaining at 88 percent throughout the rest of the reactor. Ammonia-nitrogen and COD removal characteristics are shown in Table XIII and Figure 62.

Nitrification, as shown by nitrate-nitrogen production in Figure 61, started in the first stage of the system. In the first stage 5.3 mg/l nitrate-nitrogen was produced, and the concentration increased to 12.2 mg/l in the effluent from the system. Ammonia-nitrogen converted to nitrate-nitrogen by the unit was 44.2 percent. The pH decreased from 7.2 in the influent to pH 7.0 in the effluent.

The removal rates, "$K_1$" for $NH_3$-N and COD and "$K_2$" for $NH_3$-N, achieved by the unit at this same loading is also shown in Table XIII. In Figure 63 is shown the percent $NH_3$-N and COD remaining at each stage of the unit for the same experiment depicted in Figures 61 and 62. It can be seen that a first order decreasing rate of removal

Figure 61. Nitrification Performance Data for the Rotating Disc Process Operated at a Hydraulic Loading of 0.5 $gpd/ft^2$ and Influent COD Concentration of 250 mg/l and $NH_3$-N Concentration of 27.6 mg/l

O = $NH_3$-N concentration

□ = $NO_3$-N concentration

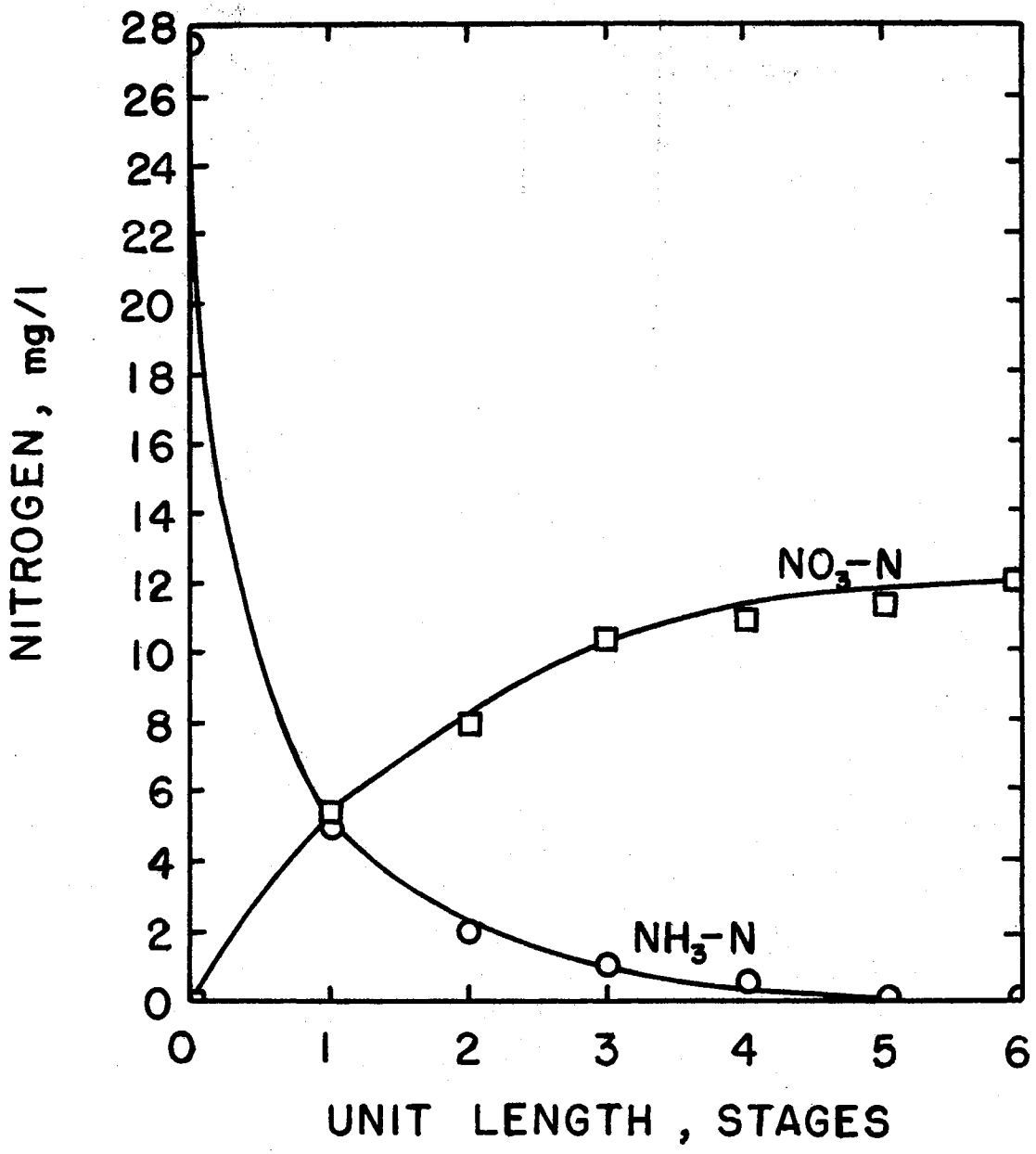

NITROGEN, mg/l
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
UNIT LENGTH, STAGES
NO3-N
NH3-N

Figure 62. COD Removal and Nitrification Efficiency of the Rotating Disc Process Operated at 0.5 gpd/$ft^2$ and Influent COD and $NH_3$-N Concentrations of 250 mg/l and 27.6 mg/l, respectively

○ = percent $NH_3$-N removed

△ = percent COD removed

□ = percent $NH_3$-N removed that was converted to $NO_3$-N

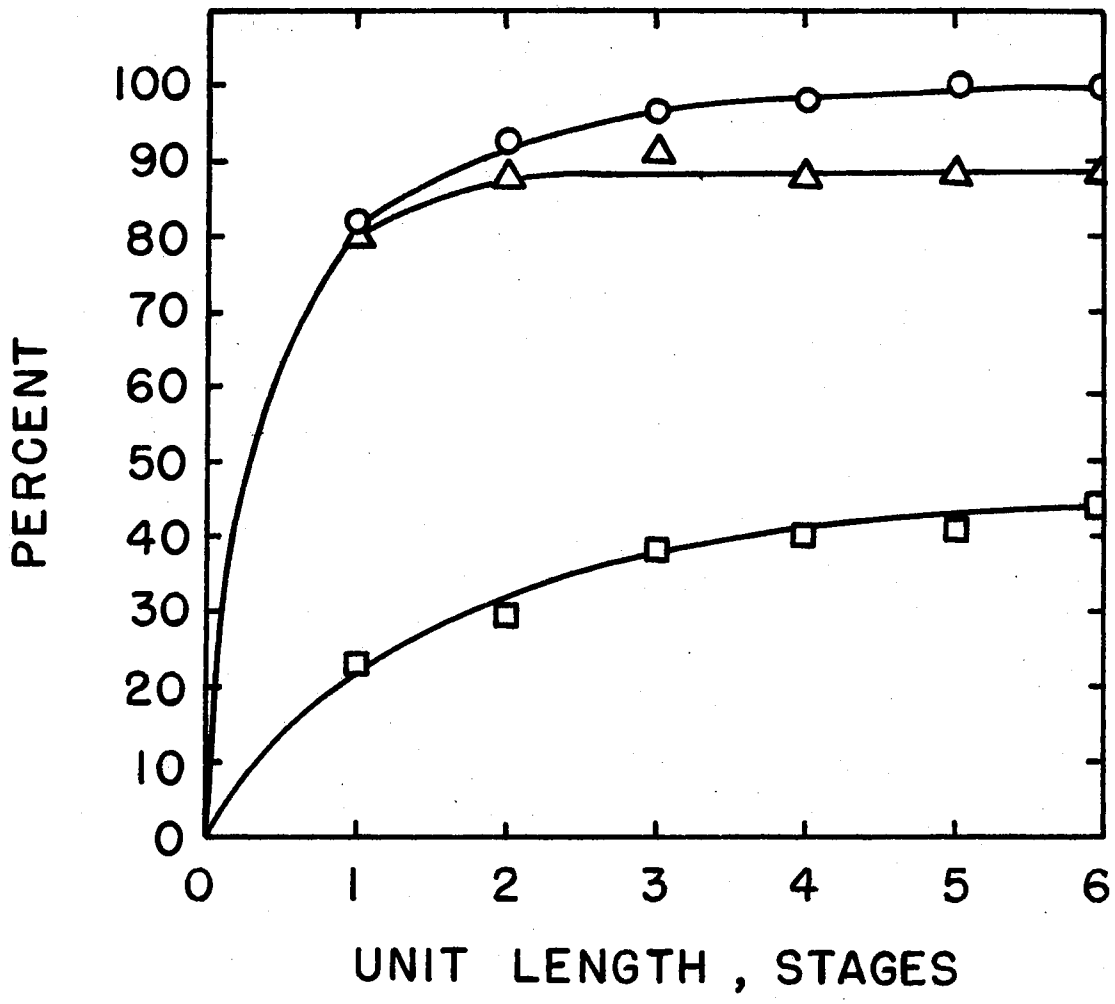

PERCENT
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
UNIT LENGTH , STAGES

TABLE XIII

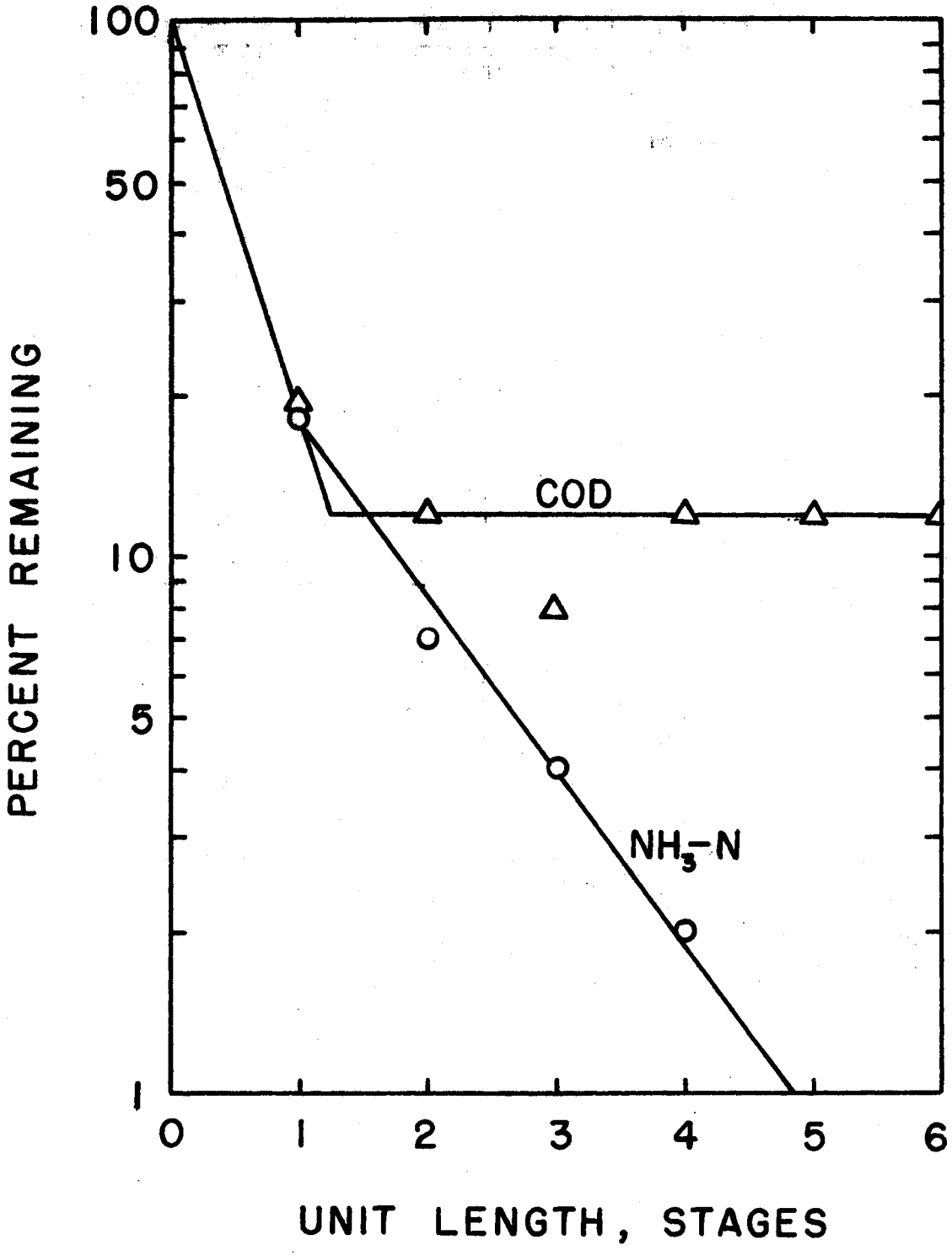
SUMMARY OF PERFORMANCE CHARACTERISTICS FOR THE ROTATING BIOLOGICAL CONTACTOR OPERATED AT 0.5 gpd/ft$^2$

| Stage | $NH_3$-N (mg/l) | Percent $NH_3$-N Removed | $NO_3$-N (mg/l) | Percent Conversion | COD (mg/l) | Percent COD Removed | pH | Substrate Removal Rate (K) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $NH_3$-N | COD |
| Influent | 27.6 | | 0 | | 250 | | 7.2 | | |
| 1 | 5.0 | 82 | 5.3 | 23.4 | 46 | 81 | 7.1 | $K_1$ = -1.708 | $K_1$ = -1.693 |
| 2 | 2.0 | 93 | 7.9 | 29.6 | 31 | 88 | 7.0 | | |
| 3 | 1.0 | 96 | 10.3 | 38.7 | 19 | 92 | 7.0 | $K_2$ = -0.768 | |
| 4 | 0.5 | 98 | 10.8 | 40.0 | 31 | 88 | 7.0 | | |
| 5 | 0 | 100 | 11.3 | 41.0 | 31 | 88 | 7.0 | | |
| (Eff) 6 | 0 | 100 | 12.2 | 44.2 | 31 | 88 | 7.0 | | |

Figure 63. Relationship of Percent Ammonia-Nitrogen and COD Remaining per Unit Length in Stages of the Rotating Disc System Operated at 0.5 gpd/$ft^2$ and Influent COD and $NH_3$-N Concentrations of 250 mg/l and 27.6 mg/l, respectively

O = percent $NH_3$-N remaining

Δ = percent COD remaining


100
50
10
5
1
PERCENT REMAINING
COD
NH3-N
0
1
2
3
4
5
6
UNIT LENGTH, STAGES

occurred for the unit with the synthetic wastewater. It is important to note the distinct slope change following the first stage for both $NH_3$-N and COD. All but the residual COD had been removed by the first stage. The removal rates for both $NH_3$-N and COD in the first stage were -1.708 $stage^{-1}$ and -1.693 $stage^{-1}$, respectively. The removal rate for $NH_3$-N in the rest of the unit was -0.768 $stage^{-1}$. The ammonia-nitrogen removal rate in the first stage of the system was more than double the removal rate in the rest of the system where ammonia-nitrogen was removed by nitrification only. COD removal characteristics for the rotating disc system at this loading were in close agreement with results obtained by Garrett (90) on the same system with similar wastewater.

b. Shock Load Results for Nitrogen Removal. Performance characteristics of the unit before, during, and after quantitative shock loads of wastewater were applied to the unit are shown in Tables XIV and XV. Results are shown for two of the shock load experiments in which the influent COD and ammonia-nitrogen concentrations were increased by approximately two and four times, respectively. The values shown in these two tables are average values taken from the various sampling periods.

In Figures 64 and 65 are shown the ammonia-nitrogen concentrations in the influent, wastewater leaving each stage, and in the effluent for the two shock load experiments. Ammonia-nitrogen concentration in the influent before the first shock load experiment was 25 mg/l, and the concentration in the effluent was zero, as shown in Figure 64. During the shock, the influent concentration was increased to 55.5 mg/l causing

TABLE XIV

SUMMARY OF RESULTS FOR QUANTITATIVE SHOCK LOAD NUMBER ONE AT TWICE THE LOADING

| Stage | $NH_3$-N, (mg/l) | | | $NO_3$-N, (mg/l) | | | COD, (mg/l) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | During | After | Before | During | After | Before | During | After |
| Influent | 25.0 | 55.5 | 25.0 | 0 | 0 | 0 | 234 | 440 | 224 |
| 1 | 2.0 | 25.1 | 2.6 | 3.5 | 1.0 | 1.1 | 105 | 59 | 43 |
| 2 | 0.2 | 18.1 | 1.4 | 8.8 | 3.8 | 3.8 | 56 | 59 | 29 |
| 3 | 0 | 14.3 | 0.2 | 9.6 | 5.9 | 5.9 | 26 | 41 | 29 |
| 4 | 0 | 12.0 | 0.2 | 10.2 | 8.2 | 8.6 | 13 | 39 | 29 |
| 5 | 0 | 10.3 | 0 | 11.3 | 10.2 | 11.3 | 26 | 35 | 29 |
| (Eff) 6 | 0 | 9.0 | 0 | 11.7 | 11.3 | 14.4 | 18 | 27 | 25 |

TABLE XV

SUMMARY OF RESULTS FOR QUANTITATIVE SHOCK LOAD NUMBER TWO
AT FOUR TIMES THE LOADING

| Stage | $NH_3$-N, (mg/l) | | | $NO_3$-N, (mg/l) | | | COD, (mg/l) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | During | After | Before | During | After | Before | During | After |
| Influent | 27.6 | 119.0 | 28.6 | 0 | 0 | 0 | 250 | 835 | 235 |
| 1 | 5.0 | 96.0 | 4.0 | 5.3 | 1.6 | 2.3 | 46 | 468 | 50 |
| 2 | 2.0 | 83.5 | 2.8 | 7.9 | 2.3 | 6.8 | 31 | 319 | 46 |
| 3 | 1.0 | 78.0 | 1.6 | 10.3 | 3.6 | 9.0 | 19 | 275 | 42 |
| 4 | 0.5 | 73.5 | 1.0 | 10.8 | 5.2 | 10.4 | 31 | 236 | 42 |
| 5 | 0 | 66.0 | 0.5 | 11.3 | 7.0 | 11.7 | 31 | 171 | 42 |
| (Eff) 6 | 0 | 55.0 | 0 | 12.2 | 8.6 | 12.4 | 31 | 104 | 27 |

Figure 64. Ammonia-Nitrogen Removal Characteristics of the Rotating Disc Process Before, During, and After a Two-Fold Quantitative Increase in Substrate Concentrations

○ = influent to system

□ = effluent stage 1

⬡ = effluent stage 2

△ = effluent stage 3

● = effluent stage 4

■ = effluent stage 5

⬢ = effluent stage 6 (system effluent)

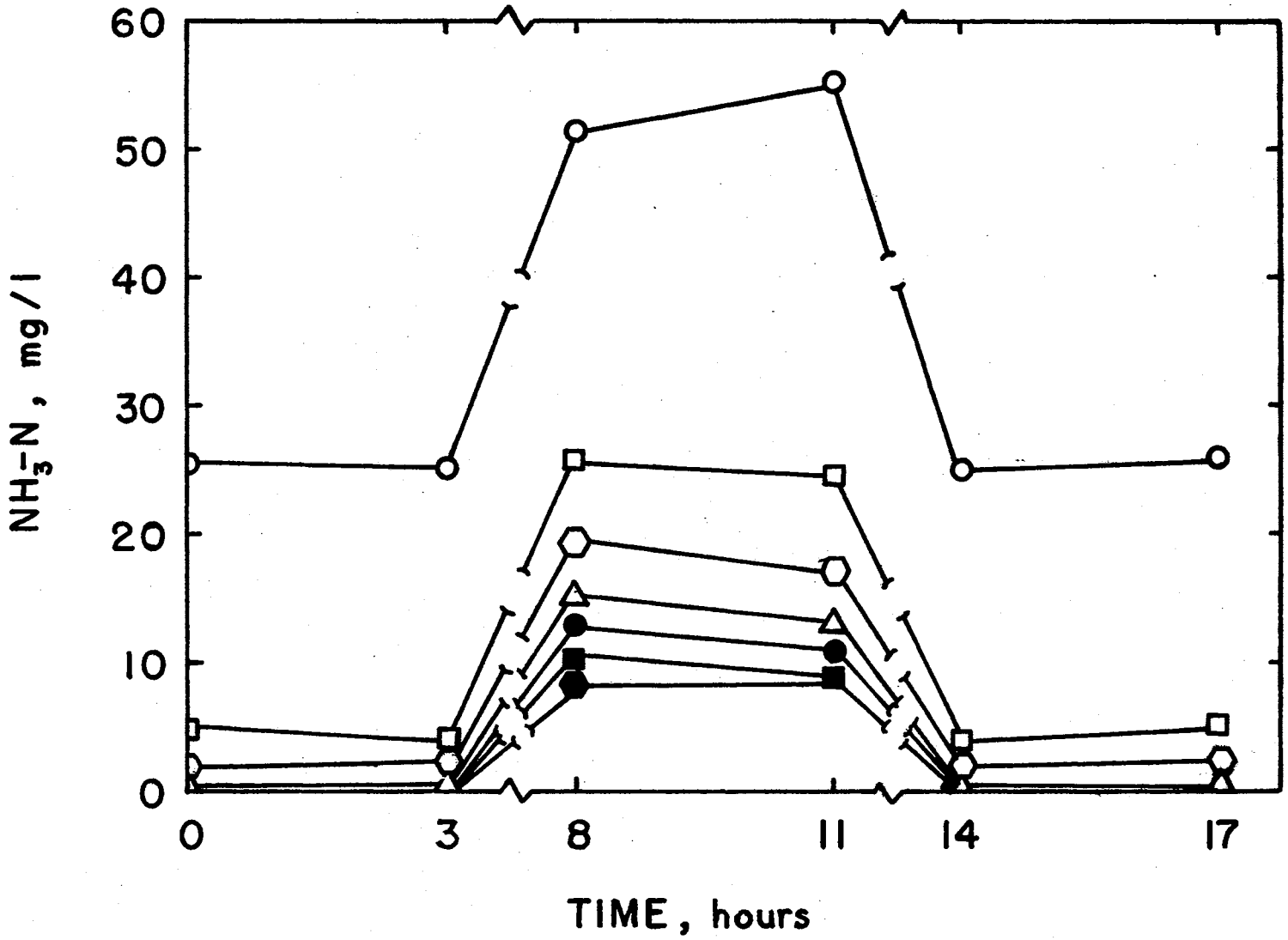

60
50
40
30
20
10
0
$NH_3$-N, mg/l
0
3
8
11
14
17
TIME, hours

Figure 65. Ammonia-Nitrogen Removal Characteristics of the Rotating Disc Process Before, During, and After a Four-Fold Quantitative Increase in Substrate Concentrations

○ = influent to system

□ = effluent stage 1

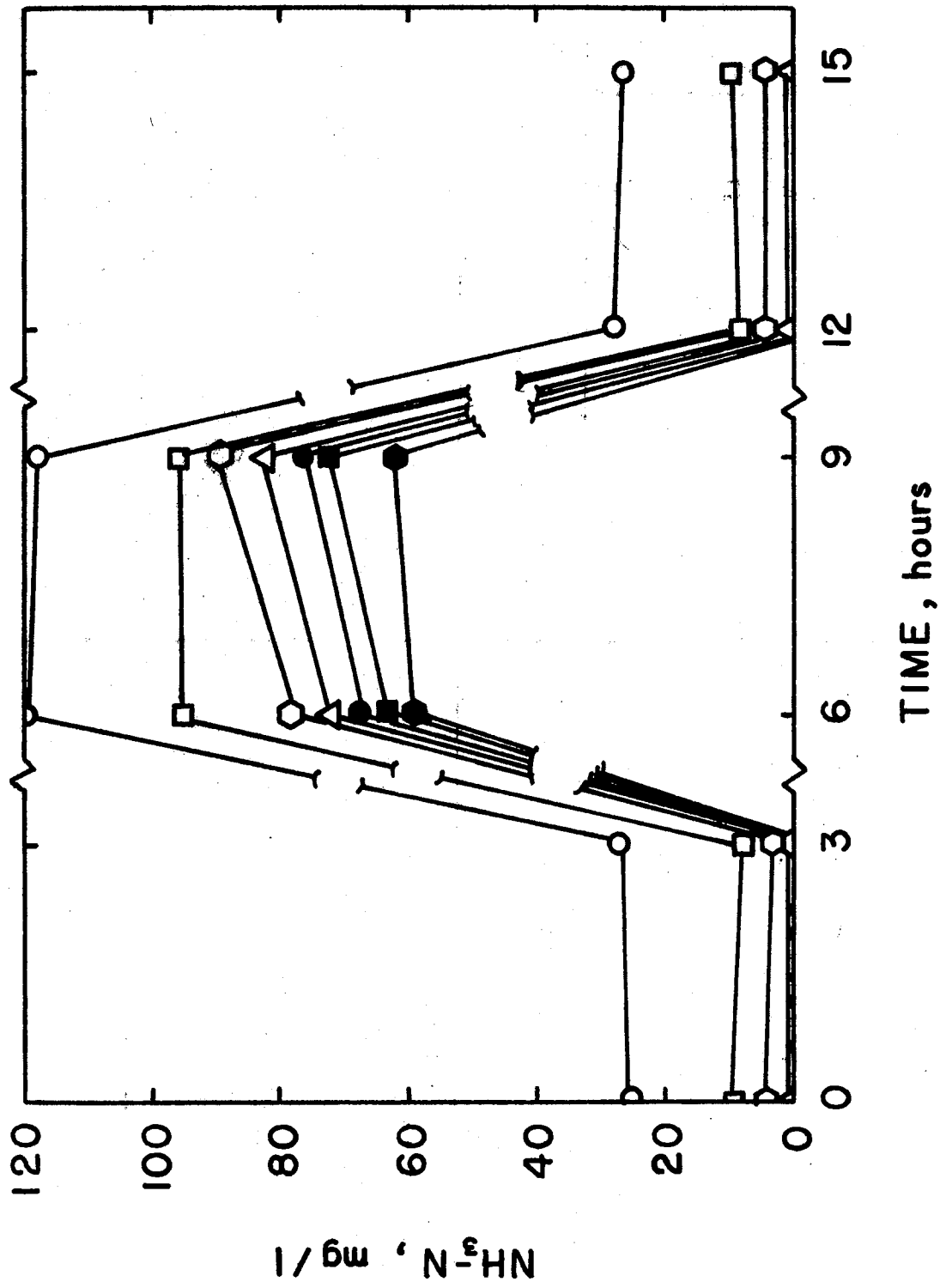
⬡ = effluent stage 2

△ = effluent stage 3

● = effluent stage 4

■ = effluent stage 5

⬢ = effluent stage 6 (system effluent)


120
100
80
60
40
20
0
$NH_3$-N , mg/l
0
3
6
9
12
15
TIME, hours

the effluent concentration to increase to 9 mg/l. In the second shock load experiment the influent ammonia-nitrogen concentration was increased from 27.6 to 119 mg/l causing the effluent concentration to increase from zero to 55 mg/l, as shown in Figure 65. Values of COD concentrations corresponding to these two experiments were influent increases from about 240 mg/l to 440 mg/l and 835 mg/l, while effluent increases were from about 25 mg/l to 27 mg/l and 104 mg/l, respectively.

Approximately 26 mg/l ammonia-nitrogen was removed before the shock loads were applied, while 46.5 mg/l and 64 mg/l were removed during the first and second shock loads, respectively. The percent of ammonia-nitrogen remaining per unit length in stages for the two shock loads and before is shown in Figure 66. As can be seen, the percent of ammonia-nitrogen remaining increased with increasing influent COD and $NH_3$-N concentrations of the shock loads applied.

The increased amounts of ammonia-nitrogen removed during the shock loads were not removed by biological nitrification but, instead, were removed by incorporation of the nitrogen into biological cell material, as can be explained by Figures 67 and 68. In Figure 67 is shown the concentration of nitrate-nitrogen produced by each stage of the system during and before the two shock load experiments. Nitrate-nitrogen not only failed to increase, but it decreased with increasing COD and $NH_3$-N concentrations during the shocks to the system. The concentration of nitrate-nitrogen in the effluent decreased from approximately 12 mg/l before the shocks to 11.3 mg/l and 8.6 mg/l during the two shock load experiments. However, excellent COD removal was maintained by the system during the period of increased COD, as shown in Figure 68. Approximately 90 percent COD removal was achieved by the rotating disc system

Figure 66. Percent Ammonia-Nitrogen Remaining per Unit Length in Stages Before and During the Quantitative Shock Loads to the Rotating Disc Process

○ = percent $NH_3$-N remaining before shock loads

□ = percent $NH_3$-N remaining during the two-fold substrate increases

△ = percent $NH_3$-N remaining during the four-fold substrate increases

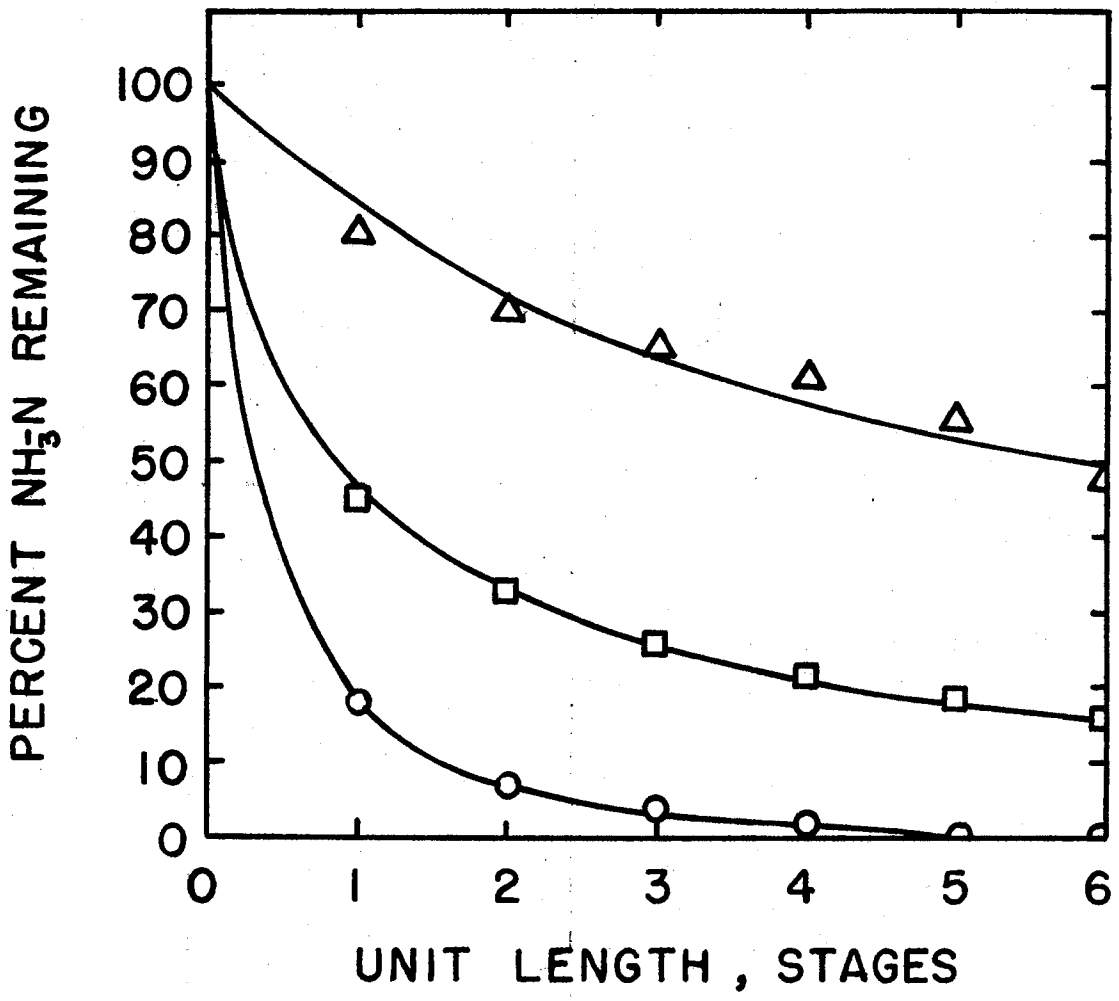

PERCENT $NH_3$-N REMAINING
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
UNIT LENGTH, STAGES

Figure 67. Nitrate-Nitrogen Production per Unit Length in Stages Before and During the Quantitative Shock Loads to the Rotating Disc System

○ = $NO_3$-N production before shock loads

□ = $NO_3$-N production during the two-fold substrate increases

△ = $NO_3$-N production during the four-fold substrate increases

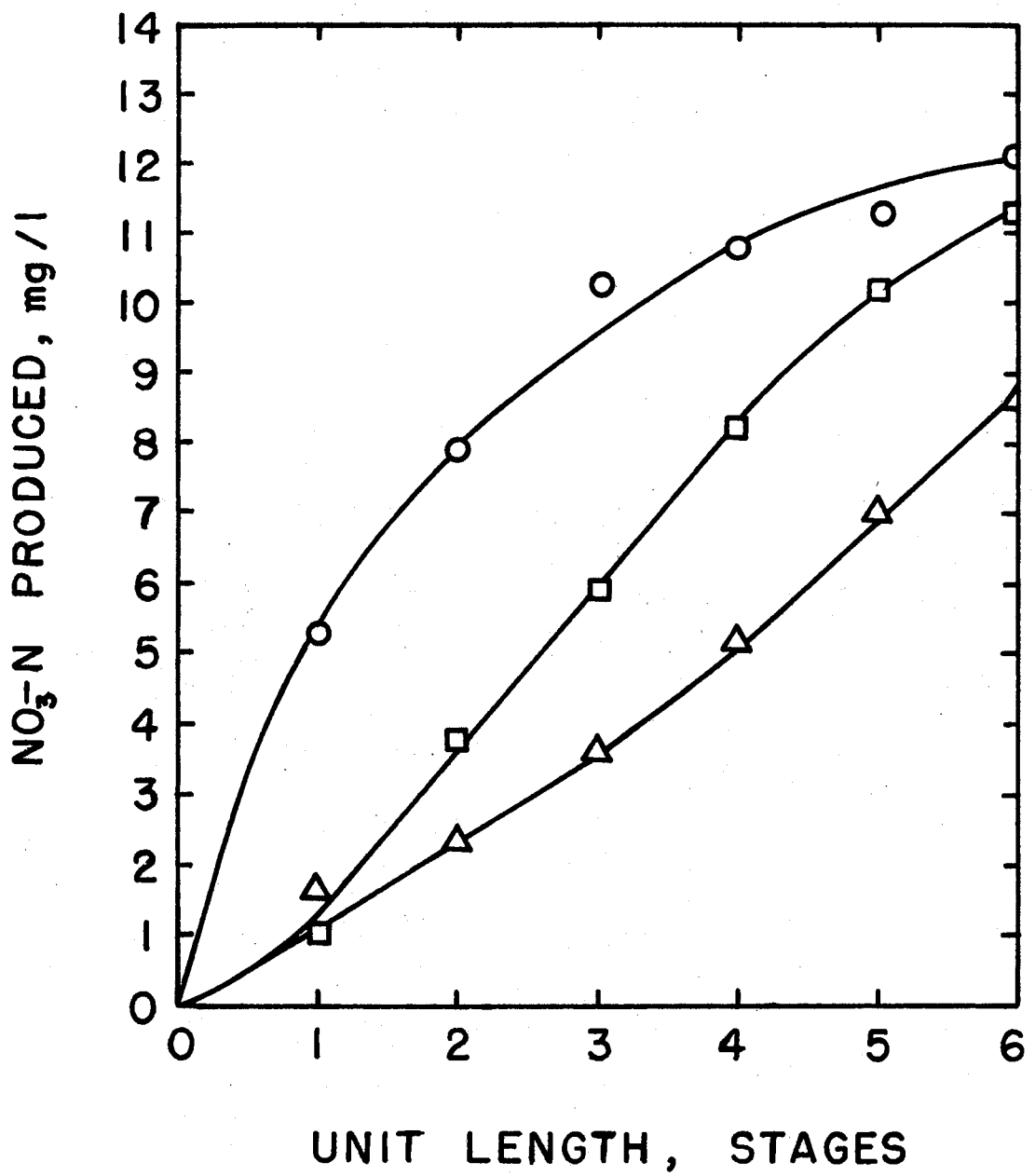
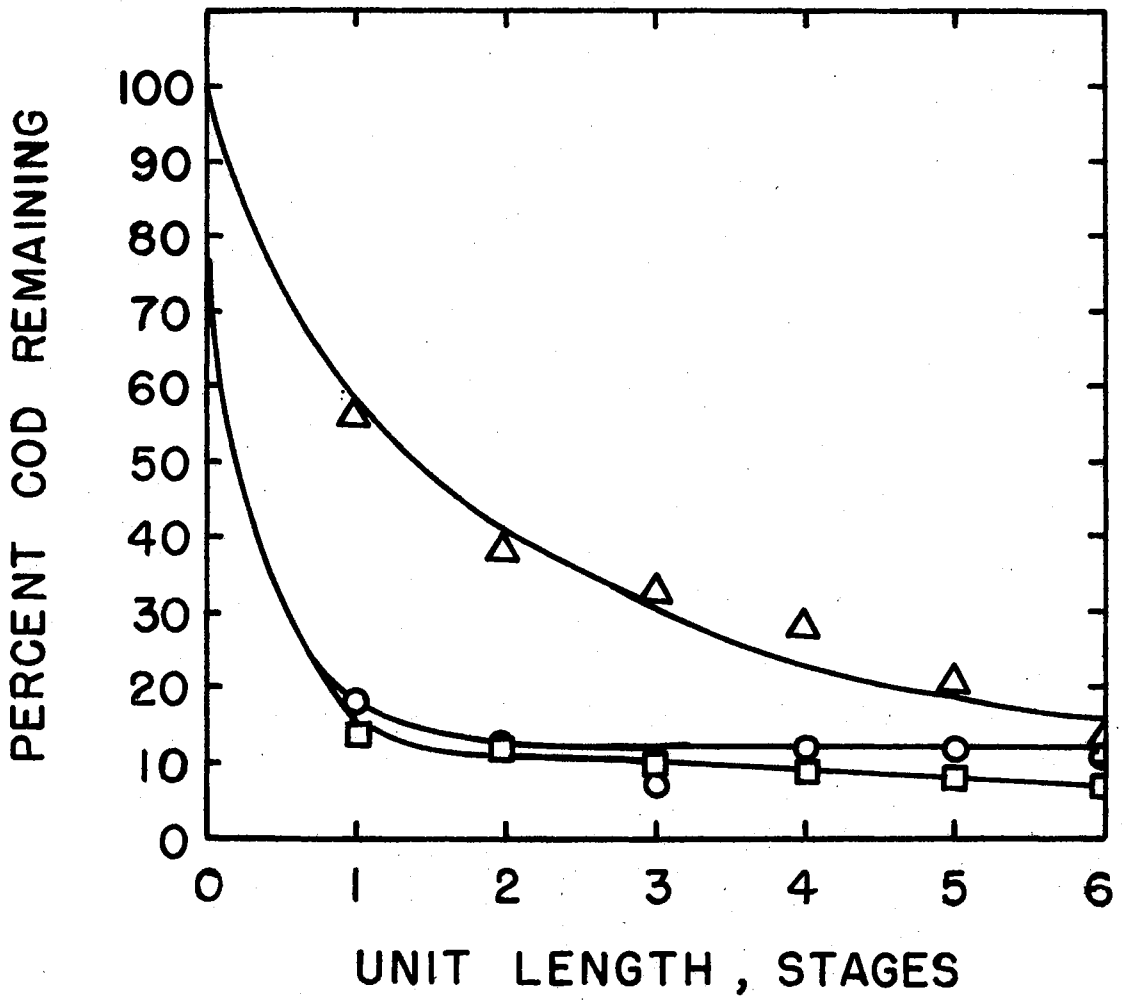

14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
6
NO3-N PRODUCED, mg/l
UNIT LENGTH, STAGES

Figure 68. Percent COD Remaining per Unit Length in Stages Before and During the Quantitative Shock Loads to the Rotating Disc Process

○ = percent COD remaining before shock loads

□ = percent COD remaining during the two-fold substrate increases

△ = percent COD remaining during the four-fold substrate increases


PERCENT COD REMAINING
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
UNIT LENGTH, STAGES

before and during both shock load experiments. The same COD removal during times of increasing COD occurred because of an increase in growth of the heterotrophic microorganisms since the reactor was a once-through system. This increase in growth requires more nitrogen, as shown in Figures 6, 7, 10, 11, and 12 with batch studies, and therefore more ammonia-nitrogen was removed by synthesis of new cell material.

# CHAPTER V

## DISCUSSION

The nutritional requirements of the autotrophic nitrifying bacteria, Nitrosomonas and Nitrobacter, have not been established in detail. A minimum concentration of dissolved oxygen along with ammonia or nitrite and carbon dioxide are mandatory requirements for growth. Temperature and pH also greatly affect the growth of the nitrifying microorganisms. Many compounds have been found to cause inhibition of nitrification by interference with the general metabolism of the cell or with the primary oxidative reactions. Downing, Tomlinson, and Truesdale (103) found that many of the substances they examined could reduce the rate of nitrification of an unadapted activated sludge grown from purely domestic sewage. However, only a relatively few appeared to be inhibitory at concentrations of the order in which they would ever be likely to be present in sewage.

From batch experiments it was determined that concentrations of the synthetic wastewaters used throughout this investigation would not be inhibitory to the nitrification process. In all experiments where organic substrate concentrations of less than 750 mg/l COD were applied to batch reactors containing nitrifying microorganisms, no change in the nitrification rates were observed. However, when organic substrate concentrations of 777 mg/l and 1139 mg/l were applied to the same type systems, inhibition of the rates of nitrification were observed, as

seen in Figures 4 and 5. Both ammonia-nitrogen removal and nitrate-nitrogen production decreased at these organic loadings. In completely mixed activated sludge units, substrate concentrations of this magnitude would seldom be observed, and the conclusion can therefore be made that the organic substrate by itself would not inhibit the nitrification process in such systems.

Batch investigations were also conducted to determine the characteristics of ammonia-nitrogen removal and nitrate-nitrogen production with both organic carbon source and heterotrophic microorganism addition to the nitrifying bacteria. As can be seen in Figures 6, 7, 8, and 9, these additions had a profound effect on the nitrification capabilities of the nitrifying bacteria. The ammonia-nitrogen removal was most specifically affected because of the amount that was incorporated into heterotrophic biological cell material. Much of the ammonia-nitrogen was removed simply by biological synthesis due to the tremendous growth of the heterotrophic bacteria. Therefore, faster ammonia-nitrogen removal rates were observed in these reactors.

The nitrate-nitrogen production in the reactors with organic carbon and heterotrophs was slightly less than that produced in the strictly nitrifying reactors. This could be partly explained by the inhibition effect of this high concentration of organic carbon source, as previously explained. One possibility might be the production of metabolic byproducts by the heterotrophic microorganisms which were inhibitory to the nitrification process. Another reasonable explanation would be competition between the heterotrophs and the autotrophs for the ammonia-nitrogen present in the wastewater. A significant proportion of the ammonia-nitrogen is incorporated into biological cell

material, and thus is not available as an energy source for the nitrifying bacteria. The dissolved oxygen concentration was maintained high enough that oxygen was not a limiting factor.

### A. Nitrification Characteristics of the Two-Stage Activated Sludge System

The utilization of the synthetic wastewater provided a carbon and nitrogen source that did not vary qualitatively from day to day. The influent temperature and reactor temperature remained fairly constant at approximately 20°C. The hydraulic flow rate was carefully controlled and remained the same throughout the duration of the study. By wasting a specific amount of biological solids from the reactor, the mean cell residence time was carefully controlled. Therefore, a controlled experimental environment was maintained for this system throughout the investigation period.

The influence of the mean cell residence time on the degree of nitrification obtainable by the second sludge system of the two-stage activated sludge system was established under the previously mentioned conditions. Ammonia-nitrogen concentration in the influent to the nitrification reactor remained approximately constant, while that in the effluent varied over the range of sludge ages from three days to about eight days. Essentially complete nitrification was obtained by the system at mean cell residence times of six days or greater.

Under the experimental conditions employed, complete washout of the nitrifying bacteria resulted at a sludge age of around three days or less. Since the reciprocal of the mean cell residence time is equal to the specific growth rate, the maximum specific growth rate, $\mu_{max}$, for

this system would be 0.33 $day^{-1}$ or 0.014 $hour^{-1}$. This value is in complete agreement with results obtained by other researchers (56)(57)(58)(59)(60)(61). At a mean cell residence time of three days or less, no nitrification of the ammonia-nitrogen could be expected. The concentration of ammonia-nitrogen in the influent and in the effluent would be approximately the same. The nitrate-nitrogen concentration in the effluent increased from zero at a three-day mean cell residence time to around the maximum value obtained by the unit at a six-day mean cell residence time. Nitrate-nitrogen production leveled off at the six-day sludge age and did not get appreciably greater as the sludge age was increased. Stankewich (29) obtained complete nitrification with the second step nitrification system at sludge retention times of seven to nine days.

As would be predicted by the mathematical model (94), the reactor biological solids concentration increased with increasing mean cell residence time over the range investigated. The solids concentrations seem a little high for an influent ammonia-nitrogen concentration of about 50 mg/l. However, the energy source for these autotrophic nitrifiers was both ammonia plus the nitrite produced from the ammonia. The carbonaceous growth from the influent COD to this system was not significant since the highest influent COD was around 40 mg/l, and the most COD ever removed by the system was about 20 mg/l. Similar solids concentrations were also reported by Stankewich (29) for the nitrification reactor of a two-stage system operated at very low influent BOD concentrations. The biological solids concentration seemed to start leveling off at the higher sludge ages, but the highest sludge age investigated was 12.1 days (Figure 16). Higher values of sludge age

would need to be investigated before a definite statement could be made. The observed yield coefficient decreased as the mean cell residence time increased (Figure 17) and also seemed to start leveling off at the higher sludge ages.

The reactor microorganism concentration, X, should increase with increasing mean cell residence time, $\Theta_c$, as shown in the equation below:

$$X = \frac{\Theta_c}{\Theta} \frac{Y(S_o - S)}{1 + K_d\Theta_c} \tag{6}$$

where

$S_o$ = influent substrate concentration, mg/l

$S$ = effluent substrate concentration, mg/l

$\Theta$ = reactor hydraulic retention time, V/Q

$Y$ = a yield constant, mass of microorganisms per mass of substrate utilized

$K_d$ = maintenance energy coefficient, $day^{-1}$.

In a system where the influent substrate concentration remains constant, the effluent substrate concentration will remain approximately constant or decrease with increasing mean cell residence time. Thus, since all of the other variables are constant except the mean cell residence time, the reactor microorganism concentration must increase with increasing sludge age.

In order to predict the reactor microorganism concentration, it becomes necessary to know the yield constant and the maintenance energy coefficient. The mean cell residence time can be shown to be a function of specific utilization, U, yield constant, and maintenance energy coefficient with a plot of specific growth rate, $1/\Theta_c$, versus specific utilization rate. The data can be linearized according to an equation

of the form:

$$\frac{1}{\Theta_c} = YU - K_d \qquad (7)$$

where Y is the slope of the line and $K_d$ is the vertical axis ($1/\Theta_c$ axis) intercept. These constants can then be used in equation (6) where the biological solids concentration can now be determined for the desired mean cell residence time value.

When the nitrification reactor data were linearized, the following equations were obtained:

$$\frac{1}{\Theta_c} = 4.80U - 0.05 \qquad \text{(Figure 69)}$$

and

$$\frac{1}{\Theta_c} = 0.65U - 0.07 \qquad \text{(Figure 70)}$$

In Figure 69, the specific utilization rate was plotted in terms of ammonia, while in Figure 70, the specific utilization rate was plotted in terms of ammonia plus nitrite. The solution of these two equations obtained in Figure 69 and Figure 70 provide values of Y = 4.80, $K_d$ = 0.05 $day^{-1}$ and Y = 0.65, $K_d$ = 0.07, respectively. Now these constants along with the hydraulic detention time employed and the influent and effluent substrate concentrations (Figure 13) can be used with the desired mean cell residence time value to determine the reactor biological solids concentration at this specific value of sludge age. Employing these constants and mean cell residence times of four days and ten days, reactor biological solids concentrations of 1150 mg/l and 2630 mg/l, respectively, were obtained. These calculated values are in excellent agreement with experimentally observed values (Figure 16).

Figure 69. Specific Growth Rate Versus Specific Utilization Rate (U in terms of ammonia) for the Nitrification Reactor of the Two-Stage Activated Sludge System

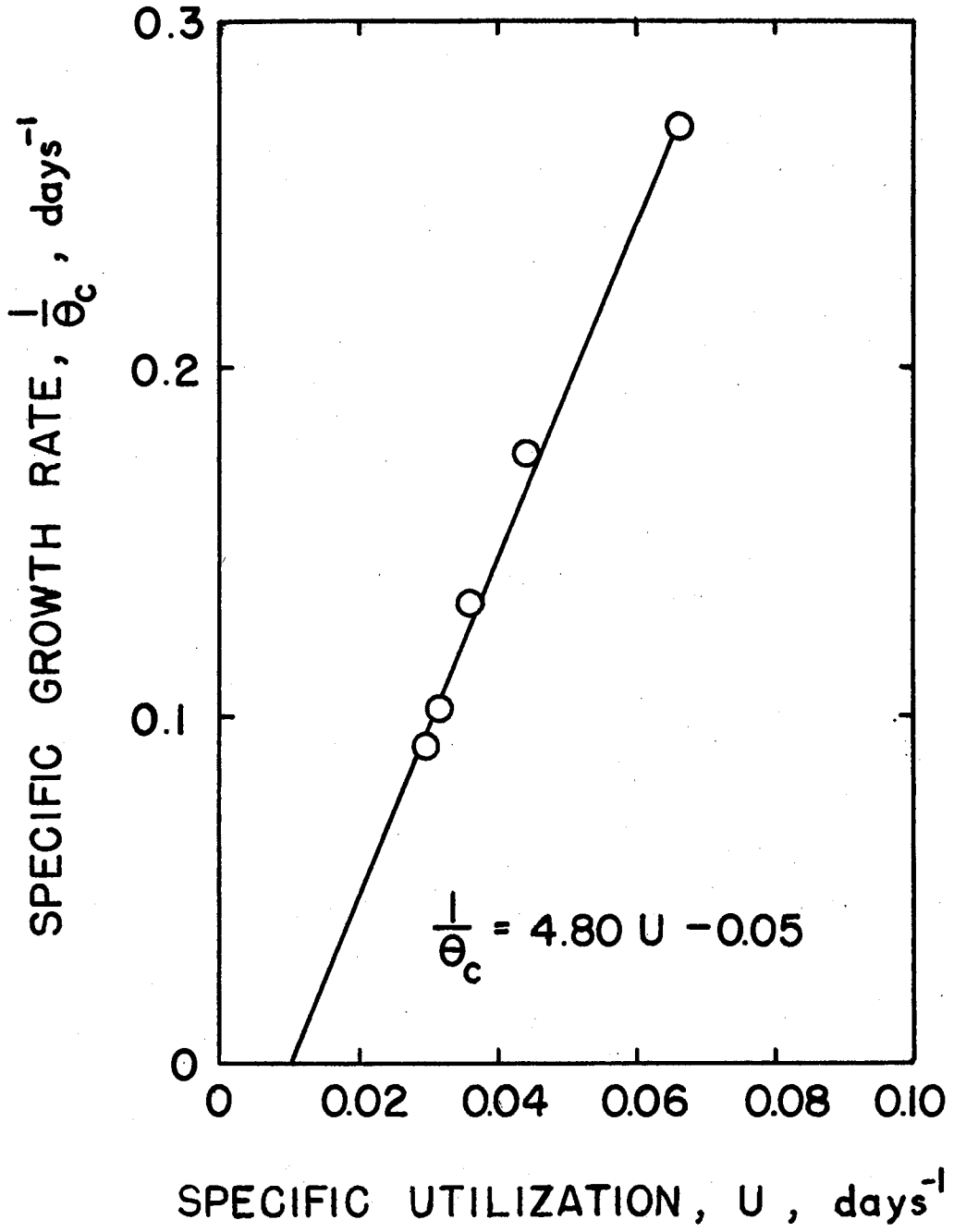
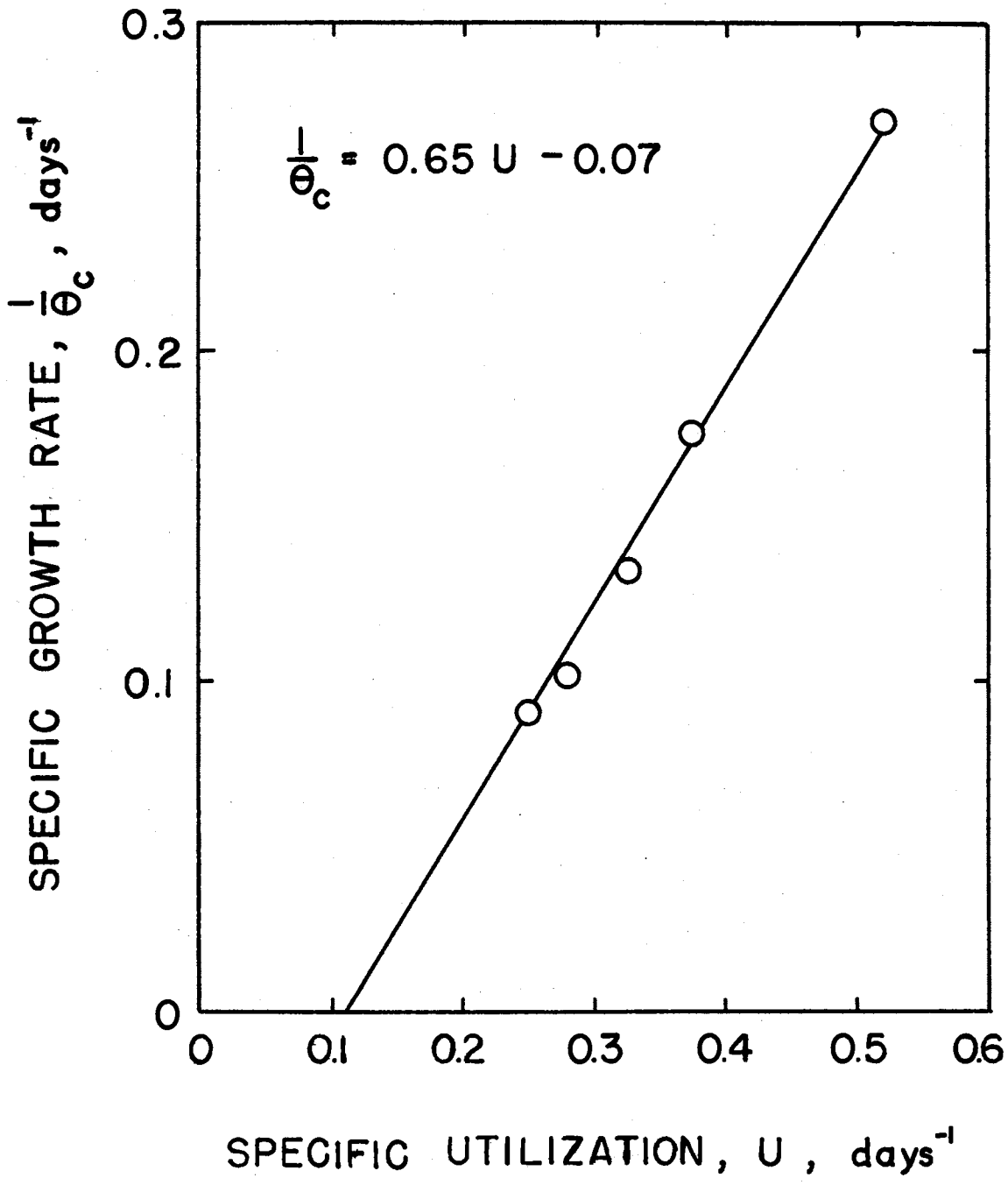

0.3
0.2
0.1
0
0
0.02
0.04
0.06
0.08
0.10
SPECIFIC GROWTH RATE, $\frac{1}{\theta_c}$, days$^{-1}$
$\frac{1}{\theta_c}$ = 4.80 U −0.05
SPECIFIC UTILIZATION, U, days$^{-1}$

Figure 70. Specific Growth Rate Versus Specific Utilization Rate (U in terms of ammonia plus nitrite) for the Nitrification Reactor of the Two-Stage Activated Sludge System


0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
$\frac{1}{\theta_c} = 0.65\ U - 0.07$
SPECIFIC GROWTH RATE, $\frac{1}{\theta_c}$, days$^{-1}$
SPECIFIC UTILIZATION, U, days$^{-1}$

In the reviewed literature the yield coefficients for the nitrifying bacteria are reported separately for Nitrosomonas and Nitrobacter grown in pure cultures. As such, the energy sources used for computing these yield values are ammonia and nitrite, respectively. However, in a nitrifying activated sludge system it is not possible to compute separate yield coefficients for these two organisms. A total combined yield coefficient for Nitrosomonas and Nitrobacter must be calculated. Either ammonia or ammonia plus nitrite can be used to compute the observed yield. As can be seen in Figure 17, it makes a significant difference by which method the observed yield coefficient is calculated. The true yield must be computed in terms of ammonia plus nitrite; however, this presents problems since it requires the knowledge of how much nitrite was produced from the ammonia. Therefore, the simplest and easiest method would be to compute the observed yield in terms of ammonia only.

Specific utilization and food-to-microorganism ratio both decrease with increasing mean cell residence times, as shown in Figures 18 and 19. Again, these values cannot be calculated for Nitrosomonas and Nitrobacter treated separately, but they must be computed for the combined total system. A large difference in values obtained is also noted by using ammonia plus nitrite or by using ammonia alone. These values can be computed either way, but again the computations involving only ammonia are simpler and easier to carry out.

The zone settling velocity of the sludge mass increased with increasing mean cell residence times (Figure 21). Settling characteristics of the sludge at mean cell residence times below six days were very poor. Nearly maximum zone settling velocity had been reached at

the six-day sludge age. At values of sludge age greater than six days the settling velocities remained approximately constant. Sludge settling velocities were also affected by the pH of the system, as shown in Figure 22. Maximum zone settling velocities were achieved at approximately pH 8.0. At pH values above 8.0 the settling velocity was nearly constant, while at pH values below 8.0 the settling velocity of the sludge mass decreased very rapidly with decreasing pH values. Therefore, optimum pH of the nitrifying bacteria, pH value around 8.0, also produces optimum settling characteristics in the sludge mass. In treatment plants employing recirculation of biological solids, the concentration of solids that can be recycled is dependent upon the settling characteristics of the sludge mass. Settling characteristics of the sludge mass also become extremely important in treatment plants where clarification is the only method of solids removal before discharge.

The nitrification reactor also removed a small fraction of the COD that was discharged by the carbonaceous reactor. At least 90 percent of the wastewater COD was consistently removed by the carbonaceous reactor, which was operated at a low mean cell residence time in order to limit nitrification. Never more than 40 mg/l COD was discharged from this reactor. The nitrification reactor removed anywhere from 5 mg/l to about 20 mg/l of the COD that was discharged by the first reactor.

Stall and Sherrard (101) wrote balanced stoichiometric equations to represent biochemical reactions taking place in both one and two-step activated sludge systems with the use of a mathematical model. To write these balanced equations it was necessary to know the chemical composition of the wastewater and to calculate the observed yield coefficient for organic removal and nitrification. The organics were

represented as $C_6H_{12}O_6$, nitrogen was present as $NH_3$-N, and the other nutrients were present in sufficient quantities to not limit growth. They used observed yield coefficients of 0.44 and 0.29 for carbon removal at sludge age values of two and ten days, respectively. A value of 0.13 was used for the observed yield due to nitrification at a sludge age of 10 days. A cell formula of $C_5H_7O_2N$ was used to represent cellular protoplasm for both carbon and nitrogen removal organisms.

The carbonaceous reactor of the two-step system was operated at a sludge age of two days in order to control nitrification, while the nitrification reactor was operated at 10 days. The balanced stoichiometric equation for the first reactor is shown below:

$$20C_6H_{12}O_6 + 20.6NH_3 + 45O_2 \longrightarrow 15C_5H_7O_2N + 5.6NH_3 + 45CO_2 + 90H_2O$$

The ratio of biological cells produced per organics utilized is the observed yield or $15C_5H_7O_2N/20C_6H_{12}O_6$ = 0.44 mg volatile suspended solids per mg COD. Ammonia-nitrogen not incorporated into cellular material in the first reactor is either oxidized to nitrate-nitrogen or incorporated into the nitrifying microorganisms in the second reactor. Stall and Sherrard used the ratio of cells produced per $NH_3$-N utilized to be the observed yield; 0.13 mg volatile suspended solids per mg $NH_3$-N. Using this observed yield value they obtained the balanced stoichiometric equation shown below:

$$5.6NH_3 + 0.462CO_2 + 7.8918O_2 \longrightarrow 0.0924C_5H_7O_2N + 5.5076NO_3^- + 16.1532H^+$$

The equations shown above for the two reactor system were obtained by using an influent wastewater COD:$NH_3$-N ratio of 400:30.

There exists a significant difference in the observed yields used

to balance these equations for the first reactor and the second reactor. In the carbonaceous unit the organic carbon source, glucose, serves both as an energy source and carbon source for the heterotrophs, and, therefore, the yield value obtained is a true yield value. However, in the nitrification reactor the observed yield is calculated in terms of ammonia-nitrogen which only serves as an energy source for the nitrifiers. The carbon source used by these autotrophs is carbon dioxide. The yield of nitrifying bacteria measured in terms of carbon dioxide would be a very complicated and involved calculation to be pursued in a system such as used here. The observed yield is thus calculated in terms of the energy source. In the nitrification reactor equation the only energy source considered is the ammonia. Since Nitrosomonas and Nitrobacter are both contained in this reactor and therefore contribute to the observed yield obtained, nitrite as well as ammonia must be considered as part of the energy source. Ammonia serves as energy source for Nitrosomonas, and nitrite serves as energy source for Nitrobacter.

Haug and McCarty proposed the following equations for the synthesis and metabolism of the nitrifying organisms based on their calculated yield coefficients (29).

Nitrosomonas:

$$29NH_4^+ + 37\ O_2 + 5\ CO_2 \longrightarrow C_5H_7O_2N + 28\ NO_2^- + 57\ H^+ + 26\ H_2O$$

Nitrobacter:

$$96\ NO_2^- + 43\ O_2 + 5\ CO_2 + NH_4^+ + 2H_2O \longrightarrow C_5H_7O_2N + 96 NO_3^- + H^+$$

These equations yield an overall oxygen requirement of 4.04 pounds of oxygen per pound of ammonia completely oxidized. This ratio varies with the age of the culture as does the yield coefficient, but

according to these researchers it should always be between 4.0 and 4.6 pounds of oxygen per pound of ammonia completely oxidized.

If these two equations for Nitrosomonas and Nitrobacter are combined, the following equation is obtained:

$$30NH_4^+ + 68NO_2^- + 80\ O_2 + 10CO_2 \longrightarrow 2C_5H_7O_2N + 96NO_3^- + 58H^+ + 24H_2O$$

A smiliar equation is obtained with the data reported in the previous chapter at a mean cell residence time of 10 days as shown below:

$$30NH_3 + 68NO_2^- + 51.5\ O_2 + 25CO_2 \longrightarrow 5C_5H_7O_2N + 93NO_3^- + 55H^+$$

This equation yields an overall oxygen requirement of 3.2 pounds of oxygen per pound of ammonia completely oxidized. The difference in the two equations and the oxygen requirements is due to the different values of yield coefficient that were used to balance the equations.

Nitrosomonas seem more frail than most other bacteria when grown in pure culture (104). Various workers have experienced difficulty in trying to maintain their cultures in a viable state for long periods. The majority of the strains isolated in the past have been lost. Gunderson (104) lost all of his cultures after 12 to 25 months under a variety of conditions, and Meiklejohn (105) lost all of hers after about 18 months storage, again under varying conditions. The nitrifying activated sludge system cultured during this investigation was also lost at one time during the study period. The microorganisms seemed to change to a fine grain sandy-type material. The reactor had to be "re-seeded" with nitrifiers and started all over again.

Factors controlling the aging and death of bacteria under otherwise non-toxic conditions are little understood. The frequently

observed death of Nitrosomonas in liquid media is usually not caused by exhaustion of substrate or oxygen. It has been suspected that the accumulation of nitrite might have killed the bacteria, but Nitrosomonas can tolerate large quantities of nitrite although it is toxic to many organisms. Nitrite would not accumulate in a nitrifying activated sludge system because it would be immediately oxidized to nitrate by Nitrobacter. A possible explanation for these experiences is that they might be brought about by inadequate control of trace elements. More consistent results could possibly be achieved with a medium more carefully balanced with trace elements and buffered against toxic effects by the inclusion of adequate chelation capacity. Another possible explanation might be that lethal mutations occur in the nitrifying bacteria or that a phage interferes.

Another problem encountered in the operation of the two-stage activated sludge system involved the operation of the carbonaceous reactor at a low mean cell residence time. Operation at a low mean cell residence time necessarily creates a high food-to-microorganism ratio in the reactor. High BOD loading or high food-to-microorganism ratio along with low nitrogen concentration, low oxygen tension, and drastic change in pH are some environmental conditions that have been implied as causes of bulking sludge (96). Bulking is the major cause of operational difficulty with the activated sludge process and, in most cases, bulking occurs when environmental conditions favor the growth of filamentous microorganisms. The proliferation of filamentous microorganisms in the carbonaceous reactor presented problems at times during the investigation period.

## B. Ammonia-Nitrogen Removal Characteristics of the One-Stage Activated Sludge System

A synthetic wastewater providing the same composition of carbon and nitrogen source as previously described for the two-stage system was also used for the one-stage system. Reactors were operated at different concentrations of COD and ammonia-nitrogen. The hydraulic flow rates were carefully controlled, and the reactors were operated by wasting of biological solids to establish desired mean cell residence times, while temperature of the reactors remained fairly constant at about 20°C. A controlled experimental environment was maintained for these one-stage reactor systems.

### 1. Nitrification at the Same COD:$NH_3$-N Ratio

Data from three activated sludge reactors operated at different mean cell residence times and at concentrations of 100 mg/l COD to 10 mg/l $NH_3$-N, 500 mg/l COD to 50 mg/l $NH_3$-N, and 1000 mg/l COD to 100 mg/l $NH_3$-N were presented in the preceding chapter. Ammonia-nitrogen and nitrate-nitrogen in the effluents from all three systems varied over the range of mean cell residence times from three days to about ten days. Essentially complete nitrification was obtained by these three systems at a sludge retention time of 10 days or greater. The degree of nitrification decreased with decreasing sludge age below 10 days, as shown in Figures 32 and 33. Nitrification decreased to zero at a sludge age of three days, but ammonia-nitrogen was still removed at lower sludge ages. This limit on nitrification was again due to the washout of the nitrifying bacteria from the system. The removal of ammonia-nitrogen

was strictly due to the incorporation of the ammonia into heterotrophic cell matter at sludge ages below three days.

The decrease in ammonia-nitrogen removal and nitrification below 10 days in all three reactors operated at the same COD:$NH_3$-N ratio did not proceed at the same rate. Percent ammonia-nitrogen removal and percent nitrification in the units operated at 500 mg/l and 1000 mg/l COD dropped off faster than in the unit operated at 100 mg/l COD. The units operated at 500 mg/l and 1000 mg/l COD fell off at approximately the same rate. At a sludge age of around two days the percent ammonia-nitrogen removed in the 100 mg/l COD reactor was about the same as that removed by the other two reactors, although between 10 days and two days the percent removal in the 100 mg/l COD reactor dropped off much slower.

The percent ammonia-nitrogen removal at two days was the same in all three systems simply because no nitrifiers were present, and the removal was due to cell sythesis only. The percent ammonia-nitrogen removed that was converted to cellular material was approximately the same in the 500 mg/l and 1000 mg/l COD reactor, and this was about the same or greater at most sludge ages than that for the 100 mg/l COD system (Figure 34). Therefore, the decrease in ammonia-nitrogen removal from 10 days to two days in these two systems was due simply to an insufficient population of nitrifying microorganisms.

One explanation for an inadequate population of nitrifiers would be a competition-type relationship between the heterotrophic and autotrophic microorganisms. The heterotrophic microorganisms are capable of faster growth rates and are not as sensitive to environmental changes as are the nitrifiers, and would, therefore, be capable of a crowding out type of effect upon the nitrifiers. Production of compounds toxic to

the nitrifying microorganisms by the heterotrophic microorganisms must also be considered as a possible explanation for the depressed rate of nitrification. A similar effect was shown in the batch studies previously described where a depression of the nitrification rate was observed upon the addition of carbon source and heterotrophic organisms to a nitrifying population.

Performance data presented by Saidi (106) and Murthy (107) for the extended aeration activated sludge process are in agreement with the results discussed above. When the organic loadings to the systems were increased the reactors biological solids concentrations began increasing immediately and the dominant form of effluent nitrogen changed from nitrate-nitrogen to ammonia-nitrogen. After Saidi changed the influent substrate concentration from 500 mg/l COD to 1000 mg/l COD, the conversion of ammonia-nitrogen to nitrate-nitrogen in the system practically ceased for a period of 10 to 12 days. The biological solids concentration was constantly increasing during this 10 to 12 day period. Murthy's data showed the same results when he gradually increased the substrate concentration from 1000 mg/l COD to 1500 mg/l COD over an eight-day period. During this period the reactor microorganism concentration increased, while the ammonia-nitrogen concentration in the effluent increased significantly, and, therefore, the nitrate-nitrogen concentration in the effluent decreased. This depression of nitrification extended over approximately a 15-day period. At the end of this 15-day period the reactor solids concentration had leveled off. An extended aeration activated sludge pilot plant operated by Yang (108) also showed periods of varying solids concentration and varying degrees of nitrification.

The concentration of nitrifying bacteria depends on the kinetic relationships involved and on the rate of loss of nitrifying bacteria in the effluent and in the surplus sludge wasted from the system. If nitrifying microorganisms are distributed uniformly through the sludge mass, as they would be in a completely mixed system, and the mass of nitrifying organisms per unit mass of suspended matter is the same in the solids lost and in the returned sludge, then the fractional rate of removal of the nitrifying organisms from the system at any time is the same as the fractional rate of removal of the sludge solids as a whole. Therefore, the fraction of the total sludge mass that was nitrifying microorganisms could not be maintained at a high enough level to accomplish complete nitrification in these systems below a sludge age of 10 days.

A lower fraction of nitrifying organisms could accomplish complete nitrification in the 100 mg/l COD and 10 mg/l $NH_3$-N system and not accomplish complete nitrification in the other two systems because of the tremendous difference in ammonia-nitrogen concentrations applied to the systems. One reactor received 10 mg/l ammonia-nitrogen while the other two units received 50 mg/l and 100 mg/l. At a sludge age of six days, approximately 30 percent of the influent ammonia-nitrogen was converted to cell materials (Figure 34). This left only 7 mg/l of ammonia-nitrogen to be oxidized in the 100 mg/l COD reactor, while 35 mg/l and 70 mg/l ammonia-nitrogen were present to be oxidized in the 500 mg/l COD and 1000 mg/l COD reactors, respectively. This is a significant difference in amounts of ammonia-nitrogen available to be oxidized to nitrate-nitrogen.

Although the ammonia-nitrogen removal and nitrification decreased

with decreasing sludge age below 10 days, the COD removal efficiency remained approximately constant. The COD removal efficiency remained above 90 percent over the range of sludge ages investigated in all three reactor systems.

Biological solids concentrations increased with increasing mean cell residence times, as shown in Figure 35. The increase in the solids concentration was lowest at 100 mg/l COD and highest at 1000 mg/l COD due to the vast difference in amount of organic carbon source supplied to the reactors. As would be expected, the solids concentration at 500 mg/l COD was approximately halfway between the solids concentrations at 100 mg/l and 1000 mg/l COD.

The reactor microorganism concentration should increase with increasing mean cell residence time, as shown in equation (6). The yield constant, Y, and the maintenance energy coefficient, $K_d$, must be determined in order to predict the reactor microorganism concentration at various sludge ages. The mean cell residence time can be related to constant yield by a plot of specific growth rate versus specific utilization rate. An equation of the form

$$\frac{1}{\Theta_c} = YU - K_d \qquad (7)$$

is obtained. The following equation

$$\frac{1}{\Theta_c} = 0.55U - 0.07 \qquad \text{(Figure 71)}$$

describes the linear relationship of the data from the reactors operated at COD:$NH_3$-N = 10:1. The solution of this equation yields the values of Y = 0.55 and $K_d$ = 0.07 $day^{-1}$.

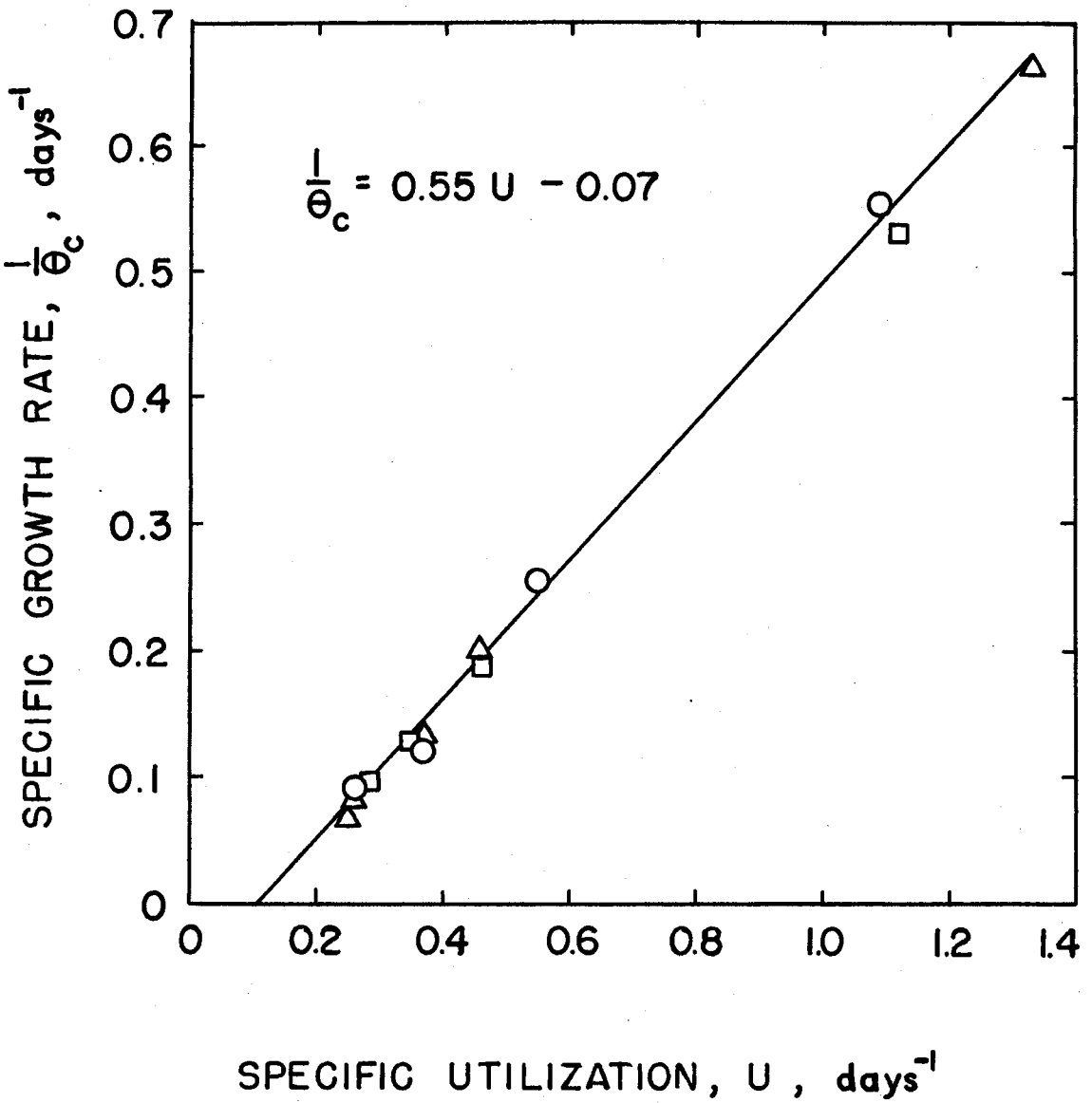
These constants along with the reactor hydraulic detention time

Figure 71. Specific Growth Rate Versus Specific Utilization Rate (U in terms of COD) for the One-Stage Activated Sludge Systems Operated at COD:$NH_3$-N = 10:1

○ = influent (COD = 100 mg/l, $NH_3$-N = 10 mg/l)

△ = influent (COD = 500 mg/l, $NH_3$-N = 50 mg/l)

□ = influent (COD = 1000 mg/l, $NH_3$-N = 100 mg/l)


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
$\frac{1}{\theta_c} = 0.55\ U - 0.07$
SPECIFIC GROWTH RATE, $\frac{1}{\theta_c}$, days$^{-1}$
SPECIFIC UTILIZATION, U, days$^{-1}$

employed and the influent and effluent COD concentrations (Figures 29, 30, and 31) can be used to determine the reactor biological solids concentration at the desired mean cell residence time. Employing these values and mean cell residence times of four days and 10 days, reactor microorganism concentrations were obtained as shown below:

$COD:NH_3-N = 100:10$

| | |
|---|---|
| 4 days | X = 344 mg/l |
| 10 days | X = 650 mg/l |

$COD:NH_3-N = 500:50$

| | |
|---|---|
| 4 days | X = 1650 mg/l |
| 10 days | X = 3110 mg/l |

$COD:NH_3-N = 1000:100$

| | |
|---|---|
| 4 days | X = 3340 mg/l |
| 10 days | X = 6300 mg/l |

These calculated solids concentration values are in close agreement with the experimentally observed values (Figure 35).

Observed yield coefficients computed in terms of both COD and ammonia decreased with increasing mean cell residence times over the range investigated (Figures 36 and 37). The observed yield coefficient, in terms of COD, plotted as a single curve for all three reactors. This is because the same $COD:NH_3-N$ ratio was applied to all three systems, and the COD concentration was the driving force in determining the solids concentration. The COD concentration was ten times higher than the ammonia-nitrogen concentration. In terms of ammonia the observed yield curves for these three systems plot close to one another, especially the 50 mg/l and 100 mg/l $NH_3-N$ curves. Again, the reason for this occurrence is that all three systems were operated at the same $COD:NH_3-N$ ratio of 10:1. Since the $COD:NH_3-N$ ratio is constant, the yields in

terms of $NH_3$-N should roughly be a constant factor times the yields in terms of COD, especially for the reactors operated at the higher COD loadings. At the higher COD loadings the COD is a much stronger factor in determining the fraction of organisms that are heterotrophs in the total system, than the ammonia-nitrogen is in determing the fraction that are autotrophs especially at low sludge ages.

Results from four systems operated at COD:$NH_3$-N ratios of 100:10, 300:30, 500:50, and 1000:100 and at an approximate sludge age of 10 days to compare nitrification performance are shown in Figures 38, 39, and 40. Essentially complete nitrification had been achieved by all four systems at this sludge age. Approximately 80 percent of the ammonia-nitrogen was oxidized to nitrate-nitrogen, and the other 20 percent was incorporated into biological cell matter. At this COD:$NH_3$-N ratio of 10:1 with varying COD and $NH_3$-N concentrations, nearly identical nitrification results were obtained at a sludge age of 10 days. However, below a 10-day solids retention time the results were not consistent. The degree of nitrification attained by the processes fell off faster with the higher loading rates than with the lower loading rates. Therefore, in order to achieve a high degree of nitrification in systems operated at a COD:$NH_3$-N = 10:1 ratio, a mean cell residence time of 10 days or greater should be used for design and operation.

The biological solids concentration in these reactors at 10 days increased with increasing influent COD concentrations, as seen in Figure 39. Observed yield in terms of both COD and ammonia was constant for all four systems. In terms of COD the observed yield was 0.36, and in terms of ammonia it was 3.1 at the 10-day mean cell residence time.

## 2. Nitrification at Different COD:$NH_3$-N Ratios

The results from three one-stage activated sludge systems operated at COD:$NH_3$-N ratios of 100:50, 500:50, and 1000:50 were presented in the preceding chapter. Again the ammonia-nitrogen and nitrate-nitrogen concentrations in the effluents from these three systems varied over the range of sludge ages from three days to about 10 days. At mean cell residence times of 10 days or greater, essentially complete nitrification was obtained by all three reactors. Nitrification decreased with decreasing sludge age below 10 days until the limiting value of three days was reached where nitrification stopped completely.

Approximately 95 percent ammonia-nitrogen was removed at six days by the reactors operated at 100 mg/l COD to 50 mg/l $NH_3$-N and 1000 mg/l COD to 50 mg/l $NH_3$-N, while only 82 percent was removed by the reactor operated at 500 mg/l COD to 50 mg/l $NH_3$-N (Figure 47). The 95 percent removal obtained by the two reactors at six days was due to two entirely different causes. At the six-day solids retention time the reactor operated at 100 mg/l COD and 50 mg/l $NH_3$-N converted 92 percent of the ammonia-nitrogen removed to nitrate-nitrogen, while the reactor operated at 1000 mg/l COD and 50 mg/l $NH_3$-N converted only 14 percent of its ammonia-nitrogen to nitrate-nitrogen. The reason that only 14 percent ammonia-nitrogen was oxidized to nitrate-nitrogen was because most of the ammonia-nitrogen was required for cell synthesis (Figure 49). At six days this system required 81 percent of the ammonia-nitrogen for cellular synthesis.

The extent of nitrification achievable by a system is dependent on the COD:$NH_3$-N ratio. In the presence of an excess of carbonaceous

material available for bacterial growth requirements, soluble ammonia-nitrogen is utilized in the formation of insoluble organic nitrogenous compounds in the sludge. Ammonia-nitrogen removal by synthesis reactions is the dominating or controlling process over the ammonia-nitrogen oxidation processes. This competition or crowding effect that the heterotrophic microorganisms exhibit over the autotrophic nitrifying microorganisms can be seen in Figures 47, 48, and 49. On the other hand, any ammonia-nitrogen in excess of that required to remove the available carbonaceous material may be oxidized to nitrate-nitrogen. This dependence upon COD:$NH_3$-N ratio is well established in Figures 48 and 49. As the COD:$NH_3$-N ratio increases, a greater percentage of the ammonia-nitrogen is required for bacterial synthesis, and, therefore, less ammonia-nitrogen is available for oxidation. The nitrifiers cannot compete with the heterotrophs for the ammonia-nitrogen, and for this reason only excess ammonia-nitrogen above that required for synthesis of new cells can be oxidized to nitrate-nitrogen. The 95 percent ammonia-nitrogen removal at six days in these reactors was achieved under two entirely different situations. In one reactor most of the ammonia-nitrogen was oxidized to nitrate-nitrogen, while in the other most of the ammonia-nitrogen was incorporated into cell matter.

These results are in agreement with results reported by Corbet and Wooldridge (109) in 1940. They aerated mixtures of sewage and activated sludge and found that some nitrified and some did not. The absence of nitrification was not due to the absence of the appropriate microorganisms or to the presence of excessive amounts of ammonia-nitrogen. They found that the extent of nitrification was dependent upon the carbon to nitrogen ratio of the mixtures. Sawyer (71) also aerated different

mixtures of sewage and activated sludges and found that the oxidizing ability of the mixtures varied with the BOD:$NH_3$-N ratio. The systems were operated at BOD:$NH_3$-N ratios of 8.2:1 to 21.0:1. He found that the sludges fed at the 8.2:1 ratio developed the greatest ability to oxidize nitrogen, while the sludges fed with 16:1 or greater ratios lost most of their nitrogen oxidizing ability. He could not, however, correlate the variation in nitrifying ability with the BOD, $NH_3$-N, or the organic nitrogen content of the food materials.

The ammonia removal and ammonia converted to nitrate curves for the one-stage system operated at 100 mg/l COD and 50 mg/l $NH_3$-N were almost identical to the curves obtained for the nitrification reactor of the two-stage system (Figures 15 and 43). This was because of the very low influent COD concentrations applied to each system. The influent to the second reactor of the two-stage system contained from 15 mg/l to 40 mg/l COD and 50 mg/l $NH_3$-N. Therefore, the only difference in the influent to the two systems was from 60 mg/l to 85 mg/l COD. The biological solids concentrations in the two reactor systems over the range of sludge ages investigated were nearly identical (Figures 16 and 50). Just as in the two-stage system, this one-stage system achieved essentially complete nitrification at a sludge age of six days. One-stage activated sludge systems at low COD or BOD loadings can accomplish nitrification to the same degree as two-stage systems even at low values of mean cell residence times--provided that no toxic compounds are present to inhibit nitrification.

Observed yield curves in terms of COD for the reactors operated at 500 mg/l and 1000 mg/l COD decreased with increasing mean cell residence times and were parallel to each other with only 0.04 mg organisms per

mg COD difference. This is explained by the high COD loadings of these reactors. The total amount of growth from the organic carbon is higher than the growth from $NH_3$-N since these reactors contained 10 and 20 times more carbon source than $NH_3$-N source. Therefore, the organic carbon source rather than the nitrogen source was the dominating factor controlling sludge production. In the system operated at the low COD loading of 100 mg/l the ammonia-nitrogen was the dominating factor, as can be seen in Figure 51, by the increase in observed yield coefficient from a sludge age of two days to six days. The maximum yield coefficient, which was greater than one, and the earliest point of complete nitrification both occurred at a sludge age of six days. At two days the observed yield was due strictly to the growth of carbonaceous organisms, since the nitrifiers could not grow in the system under the environmental conditions employed. As the mean cell residence time increased the nitrifying fraction of the total population increased until complete nitrification was attained at the six-day sludge age. The observed yield coefficient increased as the population of nitrifying organisms increased in the reactor. When maximum nitrification was achieved at six days the observed yield coefficient then started a decreasing trend, as it should.

In terms of ammonia the three observed yield curves were distinctly separate curves decreasing with increasing mean cell residence time, as shown in Figure 52. The influent ammonia-nitrogen concentration was 50 mg/l in all three reactors, while the COD concentrations were 100 mg/l, 500 mg/l, and 1000 mg/l. Since the ammonia-nitrogen was constant, the greatest yields were produced by the largest concentration of organic carbon source, 1000 mg/l COD. The next greatest yields were produced by

500 mg/l COD, and the lowest yields were produced at 100 mg/l COD where the ammonia-nitrogen concentration was a more significant factor in determining the yields. From Figure 52 can be seen the significance of the yields from the organic carbon source as compared to the yields from ammonia-nitrogen at very low COD loadings. The observed yields in terms of ammonia at a constant ammonia-nitrogen concentration increase greatly with increasing COD concentrations.

Problems encountered in the operation of the one-stage activated sludge systems consisted of the occurrence of both bulking and rising sludge. Both bulking and rising of sludge in activated sludge plants have been problems of major concern for many years. Activated sludge systems can be, and are, frequently disrupted by the proliferation of filamentous forms to such a degree that bulking sludge occurs and causes partial or complete failure. The rising sludge problem involves the rising or buoying up of a sludge blanket by bubbles of gas which have been found to be nitrogen gas (110)(111). Some microorganisms possess the enzymes necessary to reduce nitrate to nitrite to nitrogen gas (denitrification). Under anaerobic conditions beneath the sludge blanket in a clarifier these organisms can produce nitrogen gas which buoys the sludge blanket to the surface.

As previously mentioned, one of the environmental conditions that has been stated as a cause of bulking sludge is drastic changes in pH. Even though a phosphate buffer at pH 7.6 was used in the wastewater, the pH of the reactors could not be maintained by this buffer. The reactor pH was constantly decreased by the production of nitric acid by the nitrifiers. Sodium hydroxide was used to adjust the reactor pH, but still the pH value of the reactors fluctuated between about 6.0 and 8.0.

The pH remained constant without adjustment only at the low sludge ages when nitrification was not present in the systems. All of the reactors were plagued with filamentous growth and bulking sludge at one time or another during the investigation period.

The rising of activated sludge has been shown to be correlated with the presence of nitrites and/or nitrates. In all of the one-stage nitrifying systems, rising sludge was observed occasionally in the settling compartments. The rising sludge was not a major problem, however, because the reactors employed internal recycle of the biological solids. Most of the time the settling sludge was well aerated and did not remain in the settling chamber for very long, and thus a sludge blanket did not develop and produce anaerobic conditions. Rising sludge could partially or totally disrupt treatment plants where clarifiers with long detention times allowed anaerobic conditions to be maintained and sludge blankets to be formed for long periods of time.

## C. Comparison of One-Stage Versus Two-Stage Nitrifying Systems

Many researchers believe that in order to accomplish nitrification a separate biological system is a mandatory requirement. They believe the principal advantage is that a greater degree of control over the two microbial processes is possible. The results of this investigation demonstrate that when the concept of mean cell residence time is employed to control both the one-sludge and the two-sludge nitrifying systems that the one-sludge system can also be controlled to accomplish nitrification.

During exponential growth, both the rate of accumulation of

biological solids and the total amount of solids synthesized are relatable to the concentration of substrate in definable fashion, which can be quantitatively estimated by three biological systems constants, maximum specific growth rate, saturation constant, and sludge or cell yield (112). These biological kinetic constants determined in batch systems are related to physical and hydraulic characteristics of continuous flow systems. This kinetic approach can be applied to nitrifying systems, since these biological constants can be determined for the nitrifying bacteria. However, numerous problems are encountered with this kinetic approach. Values for these biological constants as reported in the literature are extremely low, and thus they are difficult to determine. Also, in a mixed culture system it is generally not possible to determine what fraction of the system biomass is metabolizing a given substrate. However, a simplifying approach can be taken to describe continuous flow biological nitrification treatment processes through the utilization of sludge age or mean cell residence time. For a specific wastewater and constant environmental conditions the variable observed cell yield coefficient is dependent on the net cell growth rate, $\mu$, or its reciprocal, the mean cell residence time, $\Theta_c$.

$$\mu = \frac{1}{\Theta_c} \quad \text{or} \quad \Theta_c = \frac{1}{\mu}$$

Some researchers have reported that nitrification can be attained in one-stage activated sludge systems if the proper load factor or lb $BOD_5$ applied per day per lb mixed liquor suspended solids is maintained in the system (61)(72). The COD or BOD to $NH_3$-N ratio of the influent wastewater does have an effect on the degree of nitrification achievable by one-stage systems especially at low mean cell residence times.

One-stage systems operated at COD:$NH_3$-N ratios of 20:1, 10:1, and 2:1 achieved complete nitrification at sludge ages of 10 days or greater. One reactor operated at influent COD and $NH_3$-N concentrations of 1000 mg/l and 100 mg/l, respectively, achieved essentially complete nitrification at a mean cell residence time of around 10 days. Another reactor operated at influent COD and $NH_3$-N concentrations of 100 mg/l and 50 mg/l, respectively, attained complete nitrification at a mean cell residence time as low as six days. This reactor produced nitrification curves nearly identical to those produced by the second sludge of a two-sludge system with an influent $NH_3$-N concentration of 50 mg/l. However, at sludge ages below 10 days there was much discrepancy in the degree of nitrification attainable by these systems at different COD:$NH_3$-N ratios.

One-stage as well as two-stage activated sludge systems can be operated and controlled to accomplish complete nitrification. The operational difference between the two systems is simply the value of the limiting mean cell residence time. For the second sludge system of the two-stage system the limiting mean cell residence time to achieve complete nitrification is six days, while for the one-stage system the limiting value is from six days to 10 days, depending on the influent COD:$NH_3$-N ratio. A limiting mean cell residence time of 10 days should therefore be applied to one-stage activated sludge systems. In order to ensure that nitrification occurs at a high efficiency in either system, a value of the design mean cell residence time selected must be greater than the limiting mean cell residence time for the nitrifying microorganisms.

In one-stage activated sludge systems operated at high influent COD

and low ammonia-nitrogen concentrations, the kinetic approach involving food-to-microorganism ratios, specific utilization rates, and observed yields in terms of COD gives good consistent results. Likewise, in one-stage activated sludge systems operated at high ammonia-nitrogen and low COD concentrations, this kinetic approach yields good results in terms of ammonia-nitrogen. However, between these two types of loadings where significant amounts of biological growth occur from both the COD and ammonia-nitrogen source, this kinetic method yields inconsistent, unreliable results in terms of both COD and ammonia-nitrogen.

Both one-stage and two-stage activated sludge systems have advantages associated with a mean cell residence time approach when considering phenomena such as nitrification. In enrichment cultures containing only nitrifying bacteria, nitrifying reactor of two-sludge system, it is possible to measure accurately the substrate, e.g., ammonia or ammonia plus nitrite, and to determine values of specific utilization and food-to-microorganism ratio. However, a system based on specific utilization or food-to-microorganism ratio is handicapped in mixed culture operations, since it is generally not possible to determine what fraction of the system biomass is metabolizing a given substrate. Depending on the COD or BOD to $NH_3$-N ratio, the major fraction of the biomass could be involved in either carbonaceous removal or nitrogenous oxidations. As shown in the preceding chapter, it is possible, at a constant value of mean cell residence time and constant ammonia-nitrogen concentration, for two one-stage systems to report widely varying values of observed yields and thus specific utilization or food-to-microorganism ratios for ammonia-nitrogen oxidation if the two systems are operated at different influent carbonaceous waste concentrations. Similar problems

are encountered in attempting to relate nitrification performance to food-to-microorganism ratio for carbonaceous removal. Specific utilization, food-to-microorganism ratios, and observed yields in terms of either COD or BOD or ammonia-nitrogen can vary tremendously for two reactors operated at different COD or BOD to ammonia-nitrogen ratios at the same mean cell residence time. Such problems have led to confusion and erroneous conclusions in attempting to develop food-to-microorganism ratio type criteria for design and control of nitrification. The use of the mean cell residence time approach focuses on the central issue, i.e., growth rate of the nitrifying bacteria, and avoids unnecessary confusion.

The single stage carbonaceous removal and nitrification process must be operated at a low food-to-microorganism ratio in order to achieve the required minimum sludge retention time necessary, based on the environmental conditions experienced. Solids production per unit of COD or BOD removed will be low, since the operating food to biomass ratio is low. Only one clarifier is required in this process scheme, excluding a primary clarifier. Nitrification may not occur in a single stage system if toxic compounds are present.

Each stage of the two-stage carbonaceous removal and nitrification process has its own integral clarifier and sludge return system. Therefore, the final clarifier capacity required for the two-stage nitrification process would be about twice that required for the single stage process. The first stage can be operated at low sludge retention times and fairly high food-to-microorganism ratios, while the second stage can be operated at high sludge retention times and low food-to-microorganism ratios. More sludge will be produced by the two-stage system, due to the difference in operating conditions, i.e., values of

mean cell residence times utilized. Waste sludge production can be calculated by using the following equation:

$$P_x = \frac{YQ(S_o - S)}{1 + K_d\Theta_c} = \frac{VX}{\Theta_c} \quad (8)$$

where

$P_x$ = waste sludge production per unit time

Q = influent waste flow rate

V = aeration basin volume

Y, $K_d$, $\Theta_c$, X, $S_o$, and S have been previously defined. If a wastewater flow of 1 MGD containing 500 mg/l of soluble, biodegradable COD and 50 mg/l of ammonia-nitrogen flowed into a one-stage system operated at a 10-day sludge age or a two-stage system with the first reactor operated at a two-day sludge age and the second reactor operated at a 10-day sludge age and hydraulic detention times as described in the preceding chapter for the experimental units, the following amounts of waste sludge would be produced daily:

| | |
|---|---|
| One-stage system | 1150 lbs |
| Two-stage system | 1870 lbs |

As a result of more sludge production, larger sludge disposal facilities will be required for two-stage systems than for one-stage systems.

The total oxygenation tank volume of a two-stage system may be approximately equal to that of a one-stage nitrification system (29)(73). Nitrification may be possible in a two-stage system when it is not possible in a one-stage system if materials toxic to nitrifiers are present in the wastewater. Toxic organics that are biodegradable can be oxidized in the first stage, and heavy metals may precipitate or be absorbed with the sludge, enabling them to be removed with the waste sludge from

the first stage. The first stage may also act as a buffer zone by reducing the impact of organic surges to the nitrification stage enabling better process control.

The COD or BOD to $NH_3$-N ratio of the wastewater to be treated is an extremely important factor to be considered in the design of biological growth processes for ammonia-nitrogen removal. If the COD or BOD to $NH_3$-N ratio is high enough, it becomes possible to convert all of the $NH_3$-N into cellular material in a one reactor system (Figure 49). By proper operating conditions the amount of nutrients such as nitrogen and phosphorus that is incorporated into biological cell matter can be controlled to an extent. The observed yield by increasing with decreasing mean cell residence times allows for higher removal of nutrients at lower sludge ages than at higher sludge ages. At low sludge ages it may be possible to convert most of the ammonia-nitrogen into cell matter, or at longer sludge ages, nitrification may be accomplished by the same reactor. Proper analysis of the wastewater to be treated may reveal that the design of a two-stage nitrification process would be totally unwarranted.

Problems encountered in both the one-stage and two-stage nitrification processes include pH reduction by the production of nitric acid. If insufficient alkalinity is present in the wastewater, the one-stage system and the nitrification reactor of the two-stage system will necessarily require pH adjustment or the addition of a buffer. Rising sludge may be a problem in the one-stage system, whereas it would not occur in a two-sludge system. The organisms present in the second reactor do not possess the necessary enzymes to produce nitrogen gas from nitrate or nitrite, and the first reactor is operated at a low mean cell residence

time in order to suppress nitrification. Another problem could be bulking sludge in the first reactor of the two-stage system and in the one-stage system due to the proliferation of filamentous organisms. Environmental conditions possibly associated with these systems, such as drastic changes in pH and high food-to-microorganism ratios have been implied as causes of bulking sludges.

## D. Nitrogen Removal With Biological Filters

A controlled experimental environment was maintained for the investigations involving the biological filter process. Hydraulic flow rates and substrate concentrations were carefully controlled throughout each experiment. The influent temperature of the wastewater was maintained at approximately $25^{o}C$.

It has been shown that nitrification and carbonaceous oxidation can both take place in trickling filters. Process variables such as depth of filter, size and type of media, and hydraulic loading have been shown to influence the degree to which nitrification can be obtained by the trickling filter process. Of course, other factors such as pH, liquid temperature, presence of inhibitors, and carbonaceous matter in the wastewater influence nitrification as they would in any biological nitrification process.

Cook and Kincannon (113) found that the COD removal efficiency was dependent on the amount of total COD (lbs/day/1000 $ft^3$) rather than its concentration or flow rate. They suggested the use of the total COD loadings as a basis for comparison. This has been verified by experimental data from other investigations for varying COD concentrations and flow rates. It was found during this investigation that the same

procedure could also be applied to ammonia-nitrogen removal by incorporation into cellular materials (Figure 57).

Percent ammonia-nitrogen removed versus ammonia-nitrogen applied in lbs/day/1000 $ft^3$ at flow rates of 500 gpd/$ft^2$, 750 gpd/$ft^2$, and 1000 gpd/$ft^2$ and various COD and $NH_3$-N concentrations with COD:$NH_3$-N = 20:1, plotted as a single curve. The curve obtained was identical in appearance to curves of percent COD removal versus COD applied in lbs/day/1000 $ft^3$ obtained by other researchers using the same type of media. No nitrification was achieved by this system over the range of loadings shown here, even though the filter had been well seeded with nitrifiers, because of the high COD loadings and low mean cell residence times experienced. This aspect of the biological filter will be discussed later. Since a biological cell is approximately 50 percent carbon and 15 percent nitrogen by dry weight every time 100 mg of carbon is converted to cell material, 30 mg of nitrogen is also converted to cell material. Therefore, the COD and ammonia-nitrogen removal characteristics of a system at a constant COD:$NH_3$-N ratio should exhibit identical properties.

This aspect of ammonia-nitrogen removal as compared to COD removal is best shown by the substrate removal rate curve. The substrate removal rates in terms of both COD and ammonia-nitrogen were first order decreasing rates of removal, as can be seen in Figures 53, 54, and 55. The relationship between substrate removal rate and applied loading in terms of both COD and $NH_3$-N, plotted as a single curve (Figure 56). As the total applied COD and $NH_3$-N loading increased the substrate removal rate decreased over the range of loadings investigated. Similar substrate removal rate curves in terms of COD have been reported by

various researchers.

Fixed bed reactor loadings are normally given in terms of pounds COD or BOD per day per 1000 cubic feet of media, gallons per acre per day, and gallons per acre-foot per day, while the actual surface area of media available for biological growth and the quantity of microorganisms present are neglected. Therefore, a better means of describing the loading applied to a fixed bed reactor would be the use of a food-to-microorganism ratio. This approach was suggested by Kincannon and Sherrard (78) and shown by Bentley (98) to be a better means of describing fixed bed system loadings than conventionally used terminology. Bentley suggested a design procedure resulting from the results of his investigation as outlined below:

1. The desired amount of organics to be removed should be expressed as a percentage of the applied organic loading. Applied organic loading in terms of 1000 cu ft of filter medium can be used to determine the volume of filter medium that is required.

2. Organic loading can be converted to a corresponding food-to-microorganism ratio.

3. Food-to-microorganism ratio can be related to mean cell residence time. The design mean cell residence time can thereby be determined.

4. Design mean cell residence time can be used to predict the total amount of solids wasted from the system each day.

5. Quality of the system effluent can be predicted in regard to COD and biological solids which are discharged per day.

This type of approach will enable system performance of different fixed bed reactors to be compared on a more equitable basis. This type of design approach can be applied to other types of media and to different substrates. For the ammonia-nitrogen removal data previously presented, this technique was used. The result was the curve of

ammonia-nitrogen removal versus ammonia-nitrogen to microorganism ratio, as seen in Figure 58. Percent ammonia-nitrogen removed decreased with increasing ammonia-nitrogen to microorganism ratio in a similar pattern as percent ammonia-nitrogen removed versus the applied ammonia-nitrogen loading. At an applied loading of 36 lbs $NH_3$-N/day/1000 $ft^3$ and an $NH_3$-N to microorganism ratio of 1.0 $day^{-1}$, the ammonia-nitrogen removal seemed to level off at about 30 percent.

It was determined from Bentley's results that the reason the filter would not nitrify was because the organic loadings were too high and thus the mean cell residence times were too low. Since the biological filter does not employ recirculation of biological solids, it is a once-through system, the controlling factor becomes the total loading applied to the system instead of the amount of biological solids wasted from the system. Therefore, in order to increase the mean cell residence time high enough to achieve nitrification, it was necessary to decrease the total organic loading applied to the system. The mean cell residence time was increased to around five days at which time complete nitrification of the wastewater was attained (Figures 59 and 60). Nitrification started in the first foot of the second tower or the fifth foot of the whole system and proceeded throughout the rest of the system.

All but the residual COD had been removed by the time nitrification began in the fifth foot of the system. Here again is an indication of the crowding out effect of the heterotrophic carbonaceous organisms over the nitrifying organisms, previously discussed. Nitrification began only after all of the COD had been removed, and therefore the competition between the nitrifying and carbonaceous organisms

had ceased.

## E. Comparison of Activated Sludge and Biological Filter Performance

The removal of organic and nitrogen compounds from wastewaters occur as a result of microbial metabolic activities by both the biological filter and activated sludge process. Organic and nitrogen compounds are incorporated into cellular materials and oxidized to metabolic end products. A summary of the similarities and differences of these to biological processes is shown in Table XVI (78).

Kincannon and Sherrard (78) have suggested that mean cell residence time values and food-to-microorganism ratios could be used in the comparison of activated sludge and biological filter processes. An investigation by Bentley (98) has supported their conclusions and found that additional parameters were also applicable in terms of organic carbon source. He found that the following relationships could be expected to hold true for both types of processes. High values of food-to-microorganism ratio correspond to low sludge age values, and low values of food-to-microorganism ratio correspond to high sludge age values. Biological solids production is dependent on mean cell residence time. Solids production is high at low sludge ages, and solids production is low at high values of sludge age. Effluent quality improves with increasing mean cell residence times. Observed yield coefficients and specific utilizations are dependent on the mean cell residence times of operation. The observed yield coefficient and specific utilization decrease with increasing sludge age.

The investigation reported herein supports Kincannon and Sherrard's

TABLE XVI

BASIC SIMILARITIES AND DIFFERENCES BETWEEN THE TRICKLING FILTER AND ACTIVATED SLUDGE PROCESS

| Similarities | Differences |
|---|---|
| 1. Wastewater stabilized by conversion of organic matter to cellular material and end products of metabolism | 1. Sludge recycled to activated sludge process while clarified effluent recycled to trickling filters |
| 2. Stabilization of wastewater organics is an aerobic reaction | 2. Microbial growth in suspension in activated sludge process and adhered to surface in trickling filter |
| 3. Level of treatment that can be achieved is similar | 3. Oxygen supplied by mechanical or diffused air aeration to activated sludge process, but supplied by convection currents in trickling filter |
| | 4. Operational problems |

conclusions in terms of ammonia-nitrogen removal. The concepts of food-to-microorganism ratio and mean cell residence time are valid means of comparison for activated sludge and biological filter processes for ammonia-nitrogen removal as well as COD removal. When properly described applied loadings are employed the biological filter and activated sludge processes can be compared on an equal basis. The activated sludge and biological filter processes used in this investigation differed due to the recirculation of biological solids. The activated sludge system employed solids recycle and could thus be controlled by the amount of sludge wasted from the system. However, the biological filter system was a once-through system, and the sludge wasted from the system was controlled by the total load applied to the system. These processes, however, should be comparable at the same food-to-microorganism ratio and mean cell residence times.

Ammonia-nitrogen removal by synthetic reactions decreased with increasing $NH_3$-N to microorganism ratio in both the biological filter and activated sludge process (Figure 58). The two ammonia-nitrogen removal curves remained approximately parallel to each other over the range of $NH_3$-N to microorganism ratios investigated. A higher efficiency of removal was consistently maintained by the biological filter system. The differences in the removals achieved by the two systems are probably due to the fact that the two systems were operated at different mean cell residence time values and at different temperatures. Mean cell residence times investigated in the activated sludge system ranged from 1.5 days to 2.8 days, while those in the biological filter system were less. Also, the biological filter was operated at $25^{o}C$, and the activated sludge systems were operated at only $20^{o}C$.

In the nitrification experiments with the biological filter, all of the COD except the residual COD had been removed before the fifth foot of depth had been reached. Nitrification of the ammonia-nitrogen began in the fifth foot and proceeded through the rest of the unit. Thus, from the fifth foot on down the biological filter functioned as a biological nitrification system. The $NH_3$-N to microorganism ratio for this part of the filter was 0.046 $days^{-1}$ and the mean cell residence time was approximately five days. In the nitrification reactor of the two-stage activated sludge system the $NH_3$-N to microorganism ratio was 0.055 $days^{-1}$ at a sludge retention time of five days (Figure 19). Excellent ammonia-nitrogen removals were achieved by both systems; however, the activated sludge system oxidized a larger percentage of its ammonia-nitrogen to nitrate-nitrogen. A larger percentage of nitrogen was incorporated into cellular matter in the biological filter, or a larger percentage was discharged from the system as nitrite-nitrogen.

## F. Nitrification Characteristics of the Rotating Biological Contactor

The hydraulic flow rates and substrate concentrations were carefully controlled during each experiment. A carbon and nitrogen source was provided that did not vary qualitatively from day to day. The influent temperature of the wastewater remained fairly constant at approximately 20°C. In considering these factors, it is concluded that a controlled experimental environment was maintained for this fixed bed reactor process.

Many researchers involved with the rotating disc process become

concerned with either the hydraulic flow rate or the concentration of substrate applied to the system instead of the total applied loading. The COD, BOD, or $NH_3$-N removal efficiency can also be shown to be dependent on the amount of total COD, BOD, or $NH_3$-N (lbs/day/1000 $ft^2$) applied to the system. COD relationships have been established for rotating disc systems treating synthetic wastewater and slaughterhouse wastewater (90)(89).

At a hydraulic loading of 0.5 gpd/$ft^2$ and influent COD and $NH_3$-N concentrations of 250 mg/l and 27.6 mg/l (COD:$NH_3$-N = 10:1), respectively, the rotating disc system attained complete nitrification. An effluent ammonia-nitrogen concentration of zero was consistently maintained by the system (100 percent ammonia-nitrogen removal). The majority of the COD was removed in the first stage of the rotating disc system. Nitrification began in the first stage and proceeded throughout the rest of the system. The first stage removed 82 percent of the ammonia-nitrogen, and all of the ammonia-nitrogen had been removed by the end of the fifth stage (Figure 62).

The same type of removal characteristics were reported by Antonie (91) for a rotating disc system treating domestic wastewater. He found that the majority of the carbonaceous BOD was satisfied in the first stage, allowing the second stage to achieve a high degree of nitrification. The statement has been made that nitrification begins in the rotating disc process when the wastewater BOD concentration approaches 30 mg/l (114). High degrees of nitrification have been achieved by the rotating disc process for the treatment of a high ammonia and low BOD content sludge supernatant (115). At an overall ammonia-nitrogen loading of 25 lbs/1000 $ft^3$/day (740 to 830 mg/l) and a reactor temperature

of 22°C, the removal of ammonia-nitrogen in the sludge supernatant through a four-compartment system was in excess of 99 percent. In spite of the high rate of oxygen transfer, the dissolved oxygen in the reactor (operated at 3 rpm) was maintained at 2 mg/l or greater. The maximum rate of oxygen transfer for ammonia-nitrogen oxidation alone was 620 pounds of oxygen per 1000 $ft^3$ aeration volume per day. Even at this high ammonia-nitrogen loading, most of the ammonia-nitrogen was removed by the first stage or compartment of the rotating disc system just as it was in this investigation at the lower ammonia-nitrogen loading.

First order decreasing rates of substrate removal were exhibited by this system for both COD and $NH_3$-N. Since most of the COD was removed in the first stage, a single substrate removal rate was observed. This was in agreement with results obtained by Garrett (90) using a synthetic wastewater with the same constituents. However, for ammonia-nitrogen a two-phase substrate removal rate was observed. The first phase which was the faster removal rate extended through the first stage of the system. The slower, second phase removal rate, started in the second stage and continued through the fifth stage where all of the ammonia-nitrogen was removed. Two phases of ammonia-nitrogen removal are explained by the fact that in the first stage ammonia-nitrogen is removed by both synthesis of cell materials and nitrification, and, therefore, a fast rate of ammonia-nitrogen removal is observed. Since most of the COD was removed in the first stage, the remaining ammonia-nitrogen was removed at a slower rate in the rest of the system by nitrification only.

Since little nitrification occurred in the first stage, most of

the ammonia-nitrogen was removed by incorporation into cellular materials. The $NH_3$-N and COD removal rates in the first stage of the system were nearly identical (Figure 63). The $NH_3$-N removal rate was -1.708 $stage^{-1}$, and the COD removal rate was -1.693, $stage^{-1}$. The ammonia-nitrogen removal rate should be a little higher than the COD removal rate, due to nitrification of some of the ammonia-nitrogen.

The rotating disc process has been shown by Antonie (116) to be effective in the processing of fluctuating wastewater flows. The purpose of the shock load experiments was to determine the effect, if any, on the ammonia-nitrogen removal capabilities of the system after increased concentrations of COD and $NH_3$-N were applied to it for a period of time. Influent COD and $NH_3$-N concentrations were increased by about two times and four times during the shock loads.

Before the shock loads were applied, approximately 26 mg/l ammonia-nitrogen was removed, but during the first and second shock loads 46.5 mg/l and 64 mg/l of ammonia-nitrogen, respectively, were removed. The percent of ammonia-nitrogen remaining increased with increasing influent COD and $NH_3$-N concentrations of the shock loads applied (Figure 66). The increased amounts of ammonia-nitrogen removed during the shock loads was due to the incorporation of the ammonia-nitrogen into cellular material instead of by oxidation of the ammonia-nitrogen to nitrate-nitrogen. Excellent COD removal was maintained by the system during the period of increased COD, as seen in Figure 68. Approximately 90 percent COD removal was maintained before, during, and after the shock loads of wastewater were applied. The increased quantities of COD removed was due to increased growth of the carbonaceous microorganisms. The increase in growth required more ammonia-nitrogen for synthesis, as

noted in the batch reactor investigations.

Nitrate-nitrogen concentrations not only failed to increase with increasing $NH_3$-N and COD concentrations during the shocks to the system but decreased instead (Figure 67). The nitrate-nitrogen concentration produced in each stage of the system decreased with increasing COD and $NH_3$-N concentrations. More ammonia-nitrogen was available for nitrification, but less oxidation of ammonia-nitrogen to nitrate-nitrogen occurred. Here again is shown the previously described effect of carbon source and carbonaceous microorganisms on the degree of nitrification obtainable by the nitrifying population. As the influent COD concentration to the system was increased, the carbonaceous microorganism growth increased, and as a result depressed the nitrification rate of the system. This is in agreement with results from the batch experiments and with results from continuous flow activated sludge systems operated by Saidi (106) and Murthy (107). Since a sufficient number of nitrifiers were present in these systems to accomplish nitrification before the COD was increased and since the nitrification rate was depressed when the carbonaceous biological solids began increasing, it appears that the carbonaceous microorganisms might produce intermediary metabolic byproducts that are toxic to the nitrifiers while growing at these fast growth rates.

Both fluidized and fixed bed reactors can accomplish high degrees of ammonia removal if designed and operated properly. Definite advantages and disadvantages are associated with the one-stage and two-stage approaches for biological oxidation of ammonia-nitrogen to nitrate-nitrogen. One-stage systems can achieve degrees of nitrification comparable to two-stage systems when operated properly. Variables which

affect the nitrification capacity of any of these biological processes are temperature, pH, presence of inhibitors, total applied loading, and COD or BOD to $NH_3$-N ratios. A specific advantage of fixed bed reactors over fluidized bed reactors in processes such as nitrification, whether one-stage or two-stage, is the development of fixed cultures in successive stages which become adapted to treating wastewater as it undergoes progressively increasing degrees of treatment. For example, if the majority of the COD or BOD is removed in the first stages, rapid nitrification can take place in the rest of the system with a specialized and established flora of nitrifying organisms. The nitrifying population can also be developed in successive stages of Nitrosomonas followed by Nitrobacter.

# CHAPTER VI

# CONCLUSIONS

The results of this investigation support the following conclusions:

1. At a temperature of 20°C nitrification ceases in biological processes at a mean cell residence time of approximately three days or less.

2. The maximum specific growth rate, $\mu_{max}$, of the nitrifying microorganisms at 20° C was found to be 0.33 $day^{-1}$ or 0.014 $hour^{-1}$ since the specific growth rate, $\mu$, is equal to the reciprocal of the mean cell residence time ($\mu = \frac{1}{\Theta_c}$).

3. Complete nitrification can be attained at a six-day or greater mean cell residence time in the nitrification reactor of a two-stage system at 20°C.

4. One-stage combined carbonaceous-nitrification systems can achieve degrees of nitrification comparable to two-stage separated carbonaceous nitrification systems when designed and operated properly.

5. The COD to $NH_3$-N ratio greatly affects the degree to which nitrification can be achieved in one-stage systems. The limiting mean cell residence time is affected by the COD to $NH_3$-N ratio. At least a 10-day or greater mean cell residence time should be applied to one-stage nitrification systems.

6. One-stage nitrification systems when operated at low influent COD and high ammonia-nitrogen concentrations, produce nitrification data

nearly identical to that produced by the nitrification reactor of a two-stage system.

7. Ammonia-nitrogen removal by incorporation into biological cell matter is a more efficient means of removal than the oxidation of ammonia-nitrogen to nitrate-nitrogen. Nitrification and carbonaceous microorganism synthetic processes are competitive for ammonia-nitrogen with the synthetic processes being the dominating factor.

8. Active synthesis of heterotrophic microorganisms at high growth rates inhibit or depress nitrification rates.

9. The concept of food (ammonia-nitrogen) to microorganism ratio provides an excellent method of describing fixed bed reactor loadings, in terms of ammonia-nitrogen removed by incorporation into cellular materials.

10. The oxidation of ammonia-nitrogen to nitrate-nitrogen and the incorporation of ammonia-nitrogen into cellular materials can be equitably compared in the fluidized and fixed bed reactor systems in terms of mean cell residence time and food ($NH_3$-N) to microorganism ratio, respectively.

11. Fixed bed biological systems obtain similar ammonia-nitrogen removal efficiencies as fluidized bed systems when operated at the same food-to-microorganism ratios.

12. When properly operated, biological filters and rotating biological contactors as well as fluidized biological systems are excellent processes for removal of ammonia-nitrogen from wastewaters.

13. Nitrifying microorganisms do not respond to spontaneous increases in substrate concentrations as well as carbonaceous microorganisms.

# CHAPTER VII

## SUGGESTIONS FOR FUTURE STUDY

Based on the findings of this study, the following suggestions are presented for future investigations involving ammonia-nitrogen removal from wastewaters:

1. Determine limiting mean cell residence times for nitrification under different conditions of pH and temperature.

2. Determine effects of qualitative and quantitative shock loads on the limiting mean cell residence time for nitrification.

3. Conduct a more detailed nitrification investigation with the biological filter for comparison with the fluidized reactor systems.

4. Apply the sludge age method to the rotating biological contactor for comparison with the biological filter and the fluidized bed reactor system.

5. Determine predominate type of microorganism with respect to stages in the fixed bed reactor systems.

6. Conduct detailed chemical analyses to determine cause of decreased rate of nitrification with addition of organic carbon source and carbonaceous microorganisms.

7. Investigate ammonia-nitrogen removal by high-rate biological processes for incorporation of the ammonia-nitrogen into biological cell matter for the harvesting of protein.

SELECTED BIBLIOGRAPHY


1. Advanced Waste Treatment and Water Reuse Symposium, Session Two, sponsored by the Environmental Protection Agency, Dallas, Texas (1971).

2. Gaudy, A. F. Jr., "Biochemical Oxygen Demand." In Water Pollution Microbiology. Ed. by Ralph Mitchell, 305-332 (1972).

3. Mt. Pleasant, R. C., and Schlickenrieder, W., "Implications of Nitrogenous BOD in Treatment Plant Design." Journal of the Sanitary Engineering Div. Proceedings of the American Society of Civil Engineers, 97, 709-719 (1971).

4. EPA Technology Transfer Seminar Publication, "Nitrification and Denitrification Facilities." 1-16 (1973).

5. Courchaine, R. J., "Significance of Nitrification in Stream Analysis-Effects on the Oxygen Balance." Journal Water Pollution Control Federation, 40, 835-847 (1968).

6. Jordan, E. O., "Nitrification." General Bacteriology, 504-509 (1910).

7. Barritt, N. W., "The Nitrification Process in Soils and Biological Filters." Annals of Applied Biology, 20, 165-185 (1933).

8. Gibbs, W. M., "The Isolation and Study of Nitrifying Bacteria." Soil Science, 8, 427-481 (1919).

9. Bergey's Manual of Determinative Bacteriology, 7th Ed., Breed, R. S., Murray, E. G. D., and Smith, N. R., The Williams and Wilkins Company, New York (1957).

10. Painter, H. A., "A Review of Literature on Inorganic Nitrogen Metabolism in Microorganisms." Water Research, 4, 410-424 (1970).

11. Imsenecki, A., "Symbiosis Between Myxobacteria and Nitrifying Bacteria." Nature, London, 157, 877 (1946).

12. Pandalai, M., "Symbiotic Aspects of Nitrification." Nature, London, 158, 484-485 (1946).

13. Pandalai, M., "Nitrification in Presence of Organic Matter." Science, 84, 440-441 (1936).

14. Gunderson, K., "Observations on Mixed Cultures of Nitrosomonas and Heterotrophic Soil Bacteria." Plant and Soil, 7, 26-34 (1955).

15. Hofman, T., "The Biochemistry of the Nitrifying Organisms. 3. Composition of Nitrosomonas." Biochemical Journal, 54, 293-295 (1953).

16. Loveless, J. E., and Painter, H. A., "The Influence of Metal Ion Concentrations and pH Value on the Growth of a Nitrosomonas Strain Isolated from Activated Sludge." Journal of General Microbiology, 52, 1-14 (1968).

17. Goldberg, S. S., and Gainey, P. L., "Role of Surface Phenomena in Nitrification." Soil Science, 80, 43-53 (1955).

18. Engel, M. S., and Alexander, M., "Growth and Autotrophic Metabolism of Nitrosomonas europaea." Journal of Bacteriology, 76, 217-222 (1958).

19. Meiklejohn, J., "Iron and the Nitrifying Bacteria." Journal of General Microbiology, 8, 58-65 (1953).

20. Van Droogenbroeck, R., and Laudelout, H., "Phosphate Requirements of the Nitrifying Bacteria." Antonie Van Leeuwenhoek Journal of Microbiology and Serology, 33, 287-296 (1967).

21. Nicholas, J. D., Wilson, P. W., Heinen, W., Palmer, G., and Beinert, H., "Use of Electron Paramagnetic Resonance Spectroscopy in Investigations of Functional Metal Components in Microorganisms." Nature, London, 196, 433-436 (1962).

22. Finstein, M. S., and Delwiche, C. C., "Molybdenum as a Micronutrient for Nitrobacter." Journal of Bacteriology, 89, 123-128 (1965).

23. Lees, H., "Energy Metabolism in Chemolithotropic Bacteria." Annual Review of Microbiology, 14, 83-98 (1960).

24. Ida, S., and Alexander, M., "Permeability of Nitrobacter agilis to Organic Compounds." Journal of Bacteriology, 90, 151-156 (1965).

25. Clark, C., and Schmidt, E. L., "Effect of Mixed Culture on Nitrosomonas europaea Simulated by Uptake and Utilization of Pyruvate." Journal of Bacteriology, 91, 367-373 (1966).

26. Stanier, R. Y., Doudoroff, M., and Adelberg, E. A., "The Nitrifying Bacteria." The Microbial World, 600-605 (1970).

27. Delwiche, C. C., and Finstein, M. S., "Carbon and Energy Sources for the Nitrifying Autotroph Nitrobacter." Journal of Bacteriology, 90, 102-107 (1965).

28. Clark, C., and Schmidt, E. L., "Growth Response of Nitrosomonas europaea to Amino Acids." Journal of Bacteriology, 93, 1302-1308 (1967).

29. Stankewich, M. J. Jr., "Biological Nitrification with the High Purity Oxygenation Process." Paper presented at the 27th Purdue Industrial Waste Conference, Union Carbide Corporation (1972).

30. Buswell, A. M., Shiota, T., Lawrence, N., and Van Meter, I., "Laboratory Studies on the Kinetics of the Growth of Nitrosomonas with Relation to the Nitrification Phase of the BOD Test." Applied Microbiology, 2, 21-25 (1954).

31. Laudelout, H., and Van Tichelon, L., "Kinetics of the Nitrite Oxidation by Nitrobacter winogradskyi." Journal of Bacteriology, 79, 39-42 (1960).

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

33. Hofman, T., and Lees, H., "The Biochemistry of the Nitrifying Organisms. 4. The Respiration and Intermediary Metabolism of Nitrosomonas." Biochemical Journal, 54, 579-583 (1953).

34. Boon, B., and Laudelout, H., "Kinetics of Nitrite Oxidation by Nitrobacter winogradskyi." Biochemical Journal, 85, 440-447 (1962).

35. Fred, E. B., and Graul, E. J., "Some Factors that Influence Nitrate Formation in Acid Soils." Soil Science, 1, 317-338 (1916).

36. Baas-Becking, L. G. M., and Parks, G. S., "Energy Relations in the Metabolism of Autotrophic Bacteria." Physiological Reviews, 7, 85-106 (1927).

37. Lees, H., and Simpson, J. R., "The Biochemistry of the Nitrifying Organisms. 5. Nitrite Oxidation by Nitrobacter." Biochemical Journal, 65, 297-305 (1957).

38. Gould, G. W., and Lees, H., "The Isolation and Culture of the Nitrifying Organisms. Part 1. Nitrobacter." Canadian Journal of Microbiology, 6, 299-307 1960).

39. Wezernak, C. T., and Gannon, J. J., "Oxygen-Nitrogen Relationships in Autotrophic Nitrification." Applied Microbiology, 15, 1211-1215 (1967).

40. Beesley, R. M., "Experiments on the Rate of Nitrification." Journal of the Chemical Society, 105, 1014-1024 (1914).

41. Lees, H., "Effect of Copper-Enzyme Poisons on Soil Nitrification." Nature, London, 158, 97 (1946).

42. Nicholas, D. J. D., and Jones, O. T. G., "Oxidation of Hydroxylamine in Cell-Free Extracts of Nitrosomonas europaea." Nature, London, 185, 512-514 (1960).

43. Rees, M. K., "Studies of the Hydroxylamine Metabolism of Nitrosomonas europaea. I. Purification of Hydroxylamine Oxidase." Biochemistry, 7, 353-366 (1968).

44. Yoshida, T., and Alexander, M., "Hydroxylamine Formation by Nitrosomonas europaea." Canadian Journal of Microbiology, 10, 923-926 (1964).

45. Corbet, A. S., "The Formation of Hyponitrous Acid as an Intermediate Compound in the Biological or Photochemical Oxidation of Ammonia to Nitrous Acid. II. Microbiological Oxidation." Biochemical Journal, 29, 1086-1096 (1935).

46. Aleem, M. I. H., and Alexander, M., "Cell-Free Nitrification by Nitrobacter." Journal of Bacteriology, 76, 510-514 (1958).

47. Campbell, A. E., Hellebust, J. A., and Watson, S. W., "Reductive Pentose Phosphate Cycle in Nitrosocystis oceanus." Journal of Bacteriology, 91, 1178-1185 (1966).

48. Lees, H., "The Biochemistry of the Nitrifying Organisms. 1. The Ammonia-Oxidizing Systems of Nitrosomonas." Biochemical Journal, 52, 134-139 (1952).

49. Quastel, J. H., and Scholefield, P. G., "Biochemistry of Nitrification in Soil." Bacterial Reviews, 15, 1-53 (1951).

50. Lees, H., and Quastel, J. H., "Bacteriostatic Effects of Potassium Chlorate on Soil Nitrification." Nature, London, 155, 276-278 (1945).

51. Lees, H., and Simpson, J. R., "The Use of Cyanate and Chlorate in Studies on the Relation Between the Nitrite Oxidation and the Reduction of a Cytochrome System in Nitrobacter." Biochemical Journal, 59, XVi-XVii (1955).

52. Downing, A. L., Painter, H. A., and Knowles, G., "Nitrification in the Activated-Sludge Process." Institute of Sewage Purification Journal and Proceedings, 130-158 (1964).

53. Knowles, G., Downing, A. L., and Barrett, M. J., "Determination of Kinetic Constants for Nitrifying Bacteria in Mixed Culture, with the Aid of an Electronic Computer." Journal General Microbiology, 38, 263-278 (1965).

54. Stratton, F. E., and McCarty, P. L., "Prediction of Nitrification Effects on the Dissolved Oxygen Balance of Streams." Environmental Science and Technology, 1, 405-410 (1967).

55. Prakasam, T. B. S., and Loehr, R. C., "Microbial Nitrification and Denitrification in Concentrated Wastes." Water Research, 6, 859-869 (1972).

56. Balakrishnan, S., and Eckenfelder, W. W., "Nitrogen Relationships in Biological Treatment Processes. I. Nitrification in the Activated Sludge Process." Water Research, 3, 73-81 (1969).

57. Balakrishnan, S., and Eckenfelder, W. W., "Nitrogen Removal by Modified Activated Sludge Processes." Journal of the Sanitary Engineering Division Proceedings of the American Society of Civil Engineers, 96, 501-512 (1970).

58. Eckenfelder, W. W., "Manual of Treatment Processes." Water Resource Management Series, 1, 9-11 (1969).

59. Jenkins, S. H., "Nitrification." Water Pollution Control, 68, 610-618 (1969).

60. Melamed, A., Saliternik, C., and Wacks, A. M., "BOD Removal and Nitrification of Anaerobic Effluent by Activated Sludge." Paper presented at the 5th International Water Pollution Research Conference (July-August, 1970).

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

62. Barth, E. F., Mulbarger, M., Salotto, B. V., and Ettinger, M. B., "Removal of Nitrogen by Municipal Wastewater Treatment Plants." Journal Water Pollution Control Federation, 38, 1208-1219 (1966).

63. Ludzack, F. J., and Ettinger, M. B., "Controlling Operation to Minimize Activated Sludge Effluent Nitrogen." Journal Water Pollution Control Federation, 34, 920-931 (1962).

64. Barth, E. F., "Perspectives on Wastewater Treatment Processes--Physical-Chemical and Biological." Journal Water Pollution Control Federation, 43, 2189-2194 (1971).

65. Barth, E. F., Brenner, R. C., and Lewis, R. F., "Chemical-Biological Control of Nitrogen and Phosphorus in Wastewater Effluent." Journal Water Pollution Control Federation, 40, 2040-2054 (1968).

66. Mulbarger, M. C., "Nitrification and Denitrification in Activated Sludge Systems." Journal Water Pollution Control Federation, 43, 2059-2070 (1971).

67. Barth, E. F., "Design of Treatment Facilities for the Control of Nitrogenous Materials." Water Research, 6, 481-483 (1972).

68. Rimer, A. E., and Woodward, R. L., "Two-Stage Activated Sludge Pilot-Plant Operations at Fitchburg, Massachusetts." Journal Water Pollution Control Federation, 44, 101-116 (1972).

69. Jenkins, D., and Garrison, W. E., "Control of Activated Sludge by Mean Cell Residence Time." Journal Water Pollution Control Federation, 40, 1905-1919 (1968).

70. Lawrence, A. W., and McCarty, P. L., "Unified Basis for Biological Treatment Design and Operation." Journal of the Sanitary Engineering Division Proceedings of the American Society of Civil Engineers, 96, 757-778 (1970).

71. Sawyer, C. N., "Activated Sludge Oxidations. V. The Influence of Nutrition in Determining Activated Sludge Characteristics." Sewage Works Journal, 12, 3-17 (1940).

72. Johnson, W. K., and Schroepfer, G. J., "Nitrogen Removal by Nitrification and Denitrification." Journal Water Pollution Control Federation, 36, 1015-1036 (1964).

73. Greene, R. A., "Complete Nitrification by Single Stage Activated Sludge." Paper presented at the 46th Annual Conference of the Water Pollution Control Federation (October, 1973).

74. Horstkotte, G. A., Niles, D. G., Parker, D. S., and Caldwell, D. H., "Full-Scale Testing of a Water Reclamation System." Journal Water Pollution Control Federation, 46, 181-197 (1974).

75. Lawrence, A. W., "Modeling and Simulation of Slurry Biological Reactors." Paper presented at the 8th Annual AEEP Workshop (December, 1972).

76. Reeves, T. G., "Nitrogen Removal: A Literature Review." Journal Water Pollution Control Federation, 44, 1895-1908 (1972).

77. Balakrishnan, S., and Eckenfelder, W. W., "Nitrogen Relationships in Biological Treatment Processes. II. Nitrification in Trickling Filters." Water Research, 3, 167-174 (1969).

78. Kincannon, D. F., and Sherrard, J. H., "Trickling Filter Versus Activated Sludge, When to Select Each Process." Paper presented at the 28th Purdue Industrial Waste Conference (May, 1973).

79. Heukelekian, H., "Similarities and Differences Between a Biofilter and a Standard Filter." Sewage Works Journal, 20, 1032-1040 (1948).

80. Mohlman, F. W., "High-Rate Filter Performance." Sewage Works Journal, 20, 618-625 (1948).

81. Grantham, G. R., Phelps, E. B., Calaway, W. T., and Emerson, D. L. Jr., "Progress Report on Trickling Filter Studies." Sewage and Industrial Wastes, 22, 867-874 (1950).

82. Sorrels, J. H., and Zeller, P. J. A., "Heavy Loadings on Trickling Filters." Journal Water Pollution Control Federation, 35, 1184-1197 (1963).

83. Richard, J., Data for Nitrification and Denitrification with Plastic Media Biological Filters (1973).

84. Duddles, G. A., Richardson, S. E., and Barth, E. F., "The Application of Plastic Media Trickling Filters in Biological Nitrification Systems." Paper presented at the 45th Annual Conference of the Water Pollution Control Federation (October, 1972).

85. Grantham, G. R., and Seeger, J. C. Jr., "Progress of Purification During the Starting of a Trickling Filter." Sewage and Industrial Wastes, 23, 1486-1492 (1951).

86. Haug, R. T., and McCarty, P. L., "Nitrification with Submerged Filters." Journal Water Pollution Control Federation, 44, 2086-2102 (1972).

87. McHarness, D. D., Haug, R. T., and McCarty, P. L., "Field Studies of Nitrification With Submerged Filters." Paper presented at the 46th Annual Conference of the Water Pollution Control Federation (October, 1973).

88. Antonie, R., "The Bio-disc Process: New Technology for the Treatment of Biodegradable Industrial Wastewater." Reprint from Chemical Engineering Symposium Series, 67, No. 107 (Water-1970).

89. Stover, E. L., "Response of the Bio-Disc Process to Slaughterhouse Wastewater Treatment." Master's Thesis, Oklahoma State University, Stillwater, Oklahoma (1972).

90. Garrett, D. A. S., "A Study of Removal Characteristics of the Rotating Biological Contactor." Master's Thesis, Oklahoma State University, Stillwater, Oklahoma (1973).

91. Antonie, R., "Application of the Bio-disc Process to Treatment of Domestic Wastewater." Paper presented at the 43rd Annual Conference of the Water Pollution Control Federation (October, 1970).

92. Torpey, W. N., Heukelekian, H., Kaplovsky, A. J., and Epstein, R., "Rotating Disks with Biological Growths Prepare Wastewater for Disposal or Reuse." Journal Water Pollution Control Federation, 43, 2181-2188 (1971).

93. Birks, C. W., Data for Ammonia-Nitrogen Removal with Rotating Biological Contactors (1972).

94. Sherrard, J. H., Schroeder, E. D., and Lawrence, A. W., "Mathematical and Operational Relationships for the Completely Mixed Activated Sludge Process." Paper presented at the 24th Annual Oklahoma Industrial Waste and Advanced Water Conference (April, 1973).

95. Sherrard, J. H., and Schroeder, E. D., "Relationship Between the Observed Cell Yield Coefficient and Mean Cell Residence Time in the Completely Mixed Activated Sludge Process." Water Research, 6, 1039-1049 (1972).

96. Randall, C. W., Edwards, H. R., and King, P. H., "Microbial Process for Acidic Low-Nitrogen Wastes." Journal Water Pollution Control Federation, 44, 401-413 (1972).

97. Kornegay, B. H., and Andrews, J. F., "Kinetics of Fixed-Film Biological Reactors." Journal Water Pollution Control Federation, 40, R460-R469 (1968).

98. Bentley, T. L., "Application and Comparison of Activated Sludge Design and Operational Control Parameters to an Experimental Fixed-Bed Reactor." Master's Thesis, Oklahoma State University, Stillwater, Oklahoma (1974).

99. Standard Methods for the Examination of Water and Wastewater, 13th ed., American Public Health Association, New York (1971).

100. Ecker, R. E., and Lockhart, W. R., "Specific Effect of Limiting Nutrient on Physiological Events During Culture Growth." Journal of Bacteriology, 82, 511-516 (1961).

101. Stall, T. R., and Sherrard, J. H., "One Sludge or Two Sludge?" Water and Wastes Engineering, 11, 4, 41-44 (1974).

102. Marcangeli, G. E., "Study on the Performance of an Experimental Two-Stage Trickling Filter Employing a Plastic Medium." Master's Thesis, Oklahoma State University, Stillwater, Oklahoma (1973).

103. Downing, A. L., Tomlinson, T. G., and Truesdale, G. A., "Effect of Inhibitors on Nitrification in the Activated-Sludge Process." Institute of Sewage Purification Journal and Proceedings, 537-550 (1964).

104. Gunderson, K., "Preservation of Nitrosomonas." Nature, London, 179, 789 (1957).

105. Meiklejohn, J., "The Isolation of Nitrosomonas europaea in Pure Culture." Journal of General Microbiology, 4, 185-191 (1950).

106. Saidi, H., "Studies on the Hydrolytically-Assisted Extended Aeration Process and on Pre-hydrolysis of Sludge in Aerobic Digestion Processes." Master's Thesis, Oklahoma State University, Stillwater, Oklahoma (1974).

107. Murthy, K. S. N., "Operational Performance and Nitrifying Characteristics of a Hydrolytically-Assisted Extended Aeration Process at High Organic Loadings." Master's Thesis, Oklahoma State University, Stillwater, Oklahoma (1974).

108. Yang, P. Y., "Studies on Extended Aeration Activated Sludge and a Modification of the Process Employing Chemical Hydrolysis of Portions of the Return Sludge." PhD Thesis, Oklahoma State University, Stillwater, Oklahoma (1972).

109. Corbet, A. S., Wooldridge, W. R., "The Nitrogen Cycle in Biological Systems. I. Some Conditions Affecting the Distribution of Nitrogenous Compounds During Treatment of Sewage by the Activated Sludge Process." Biochemical Journal, 34, 1015-1025 (1940).

110. Sawyer, C. N., and Bradney, L., "Rising of Activated Sludge in Final Settling Tanks." Sewage Works Journal, 17, 1191-1209 (1945).

111. Wooldridge, W. R., and Corbet, A. S., "The Nitrogen Cycle in Biological Systems. II. Changes in Mixtures of Sewage and Activated Sludge Entailing the Liberation of Nitrogen." Biochemical Journal, 34, 1026-1035 (1940).

112. Gaudy, A. F. Jr., and Gaudy, E. T., "Biological Concepts for Design and Operation of the Activated Sludge Process." Project Report for the Water Quality Office, Environmental Protection Agency, 17090 FQJ (1971).

113. Cook, E. E., and Kincannon, D. F., "An Evaluation of Trickling Filter Performance." Water and Sewage Works, 118, 4, 90-95 (1971).

114. Antonie, R. L., Kluge, D. L., and Mielke, J. H., "Evaluation of a Rotating Disk Wastewater Treatment Plant." Journal Water Pollution Control Federation, 46, 498-511 (1974).

115. Lue-Hing, C., Obayashi, A. W., Zenz, D. R., Washington, B., and Sawyer, B. M., "Nitrification of a High Ammonia Content Sludge Supernatant by Use of Rotating Discs." Paper presented at the 29th Purdue Industrial Waste Conference (May, 1974).

116. Antonie, R., "Response of the Bio-disc Process to Fluctuating Wastewater Flows." Paper presented at the 25th Purdue Industrial Waste Conference (May, 1970).

# VITA

Enos Loy Stover

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES ON THE PERFORMANCE OF BIOLOGICAL NITRIFICATION PROCESSES FOR THE REMOVAL OF NITROGENOUS OXYGEN DEMAND FROM WASTEWATERS

Major Field: Civil Engineering

Biographical:

Personal Data: Born November 19, 1948, in Shawnee, Oklahoma, the son of Mr. and Mrs. Euell Stover.

Education: Graduated from Sulphur High School, Sulphur, Oklahoma, in May, 1967; received the degree of Bachelor of Science in Civil Engineering from the Oklahoma State University, Stillwater, Oklahoma, in July, 1971; received the degree of Master of Science in Bioenvironmental Engineering in July, 1972; completed requirements for the degree of Doctor of Philosophy in Civil Engineering at the Oklahoma State University in July, 1974.

Professional Experience: Undergraduate research assistant, Oklahoma State University, October, 1970, to August, 1971; bioenvironmental engineering trainee, September, 1971, to July, 1974; graduate teaching assistant in bioenvironmental engineering, Oklahoma State University, September, 1973, to May, 1974.

Membership in Professional Societies: Associate Member Oklahoma Society of Professional Engineers and National Society of Professional Engineers; Member, American Society of Civil Engineers; Water Pollution Control Federation.

Membership in Honorary Societies: Chi Epsilon, Phi Kappa Phi.