A STUDY OF THE EFFECT OF SPECIFIC GROWTH RATES ON BOTH CHEMICAL AND AUTO-FLOCCULATION OF CELLS GROWN IN A ONCE-THROUGH CHEMOSTAT

By

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1973

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1975

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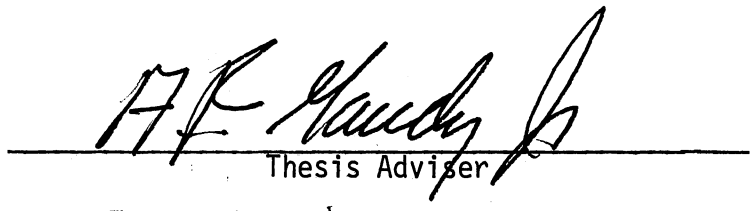

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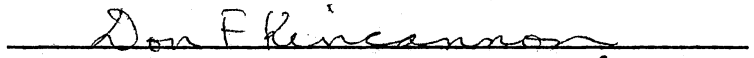
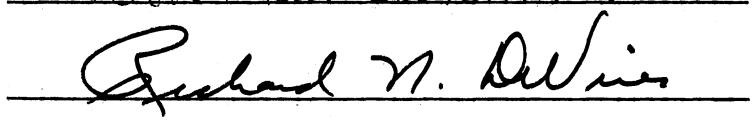




Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to extend my sincere appreciation to Dr. Anthony F. Gaudy, Jr., my principal adviser, for his patience, guidance, and encouragement throughout this study.

I also wish to thank Dr. Joseph Sherrard, Dr. Richard N. DeVries, and Dr. Don F. Kincannon for their encouragement and assistance.

Sue Harmon, John Thomas, and Cindy Anderson deserve special thanks for their encouragement and patience.

I wish to thank my colleagues Norm Bartle, Ray Stall, Donnie Lowe, Steve Reusser, Larry Roach, and all of the students in the department for their assistance and friendship. I also wish to thank Mrs. Grayce Wynd for her excellent typing of the rough draft and final copy of this thesis.

A very special thanks goes to my parents, Dr. and Mrs. Carl S. Kelley, Jr., for their patience and understanding during the past seven years of study.

This investigation was made possible in part through the financial support provided by the Federal Environmental Protection Agency graduate training grant T-9000-78 (formerly WP-238).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

For many years, water courses were used for disposal sites for municipal and industrial wastewater. These receiving streams treated the wastewaters naturally, but this process is a very slow and lengthy one and is a process which is easily overloaded. As the population increased, the streams became more and more polluted, causing fish kills, overgrowth of algae, and hazards to public health. Thus, waste treatment plants are designed to remove these pollutants.

For waste treatment plants to meet present and future requirements, methods of increasing the removal efficiency of the plants must be researched and put into use. Currently, the mixed liquor from such secondary treatment processes as activated sludge, is separated from the purified wastewater by quiescent sedimentation, and this requires that the cells must have previously agglomerated or flocculated. Little is known about bacterial flocculation, and it is one of the purposes of this study to gain an insight into possible relationships between specific growth rate and microbial flocculation. When cells do not autoflocculate sufficiently, then some means of enhancing the natural phenomenon are needed. It is also the purpose of this investigation to gain insight into the effect of coagulants such as ferric and alum salts on bacterial flocculation.

CHAPTER II

LITERATURE REVIEW

Flocculation has been a problem for many, many years; in fact liquid-solid phase separation has been known for 4,000 years. Sanskrit literature, 2000 B.C., suggests that a number of vegetable substances, such as the seed of the *Strychnos potatorium*, were used for coagulants to remove solids from liquids (1). Flocculation can happen naturally, or by the addition of chemicals (polyelectrolytes and metal ions).

A. Auto-flocculation

Flocculation prevails both in the living and non-living world. The study of flocculation of the non-living world has been the concern of colloidal chemists, while the study of flocculation of the living world has been of concern to biochemists. Surprisingly little work has been done with the flocculation process in the treatment of sewage, especially with regard to the flocculation of organisms in the activated sludge process. There has been some research done by Rudolfs and Gehm (2), Heukelekian and Littman (3), Dunbar, Dienert, Cavel, Baly, and Lumb (4) which has contributed useful information, but two men who have contributed some of the basic theories of flocculation are Butterfield and McKinney.

Butterfield in 1935 was able to obtain a pure culture of the

the organism *Zoogloea ramigera* from an activated sludge plant. He proposed that flocculation was caused by the capsule which surrounds the cell (6). The capsule is made of a thick, viscous, sticky, gelatinous material that causes other cells to stick on to it when they collide with a cell (7). Butterfield was able to isolate *Zoogloea ramigera* from the zoogleal mass, or activated sludge floc, by microscopically selecting typical floc particles. He then ran a series of biochemical tests to establish the identity of the organism he isolated. He then ran a series of experiments comparing activated sludge and *Zoogloea ramigera* to remove the pollutants in sewage and to flocculate the mass. The results of these experiments formed the basis of his postulates (6).

Butterfield was not the first to isolate *Zoogloea ramigera*. The first isolation was in 1867 by Itzigsohn. In 1896, Flugge stated that the zoogleal mass in an activated sludge plant was a mass of capsular substance formed by *Zoogloea ramigera*. In 1923, Buswell and Long tried to define the composition of activated sludge and explain the mechanism of purification by activated sludge cells which had flocculated (8). Buswell explained that activated sludge is made up of zoogleal floc and that protozoa on these floc materials were responsible for the purification of wastewater. Two years later, Taylor substantiated Buswell's theory, using a series of experiments (6).

In 1937, Butterfield ran several experiments trying to find other bacteria or organisms capable of producing flocculation, but he found that *Zoogloea ramigera* was the only species which produced floc material in his studies (9). Heukelekian and Littman (10) repeated Butterfield's experiments, and obtained the same results. They also determined that if a large food source is available, there will be large numbers of

swimming bacteria. If there was a shortage of food source, then the bacteria would exist mostly in a flocked state instead of in a free swimming state. It would seem to the author that, since the concentration of substrate or food source determines the cell concentration to some extent, and the amount of substrate present is determined by the specific growth rate or cell age, one might expect that flocculation might be related to cell age or specific growth rate. The most recent addition to the zoogloeal theory was made by Buck and Keefer (11). They isolated an organism or bacteria which they believed to be of the genus *Zoogloea*, but a different species than *Zoogloea ramigera*.

McKinney and Horwood (12) contributed another basic theory of flocculation. In 1952 they were able to do what Butterfield and others tried to do. They isolated several organisms that would produce floc. Their discovery disproved Butterfield's theory that *Zoogloea ramigera* was the only organism or bacteria that would flocculate.

Later (in 1952), McKinney updated his previous theory on flocculation. In his previous works he stated that flocculation is a property of all bacteria. After observing floc particles under a microscope, he reported that floc-producing bacteria grown in soluble substrate were separated only by the capsule while floc-producing bacteria grown in insoluble substrate were separated or composed of a slime layer (13).

McKinney studied the electrical charge on the surface of the bacteria and found that lowering the surface charge below 15 millivolts resulted in auto-agglutination (auto-flocculation) or pure bacteria suspensions, and concluded that the surface charge was the major factor in bacterial flocculation as it existed in activated sludge.

McKinney then ran electrophoresis measurements on 72 different

bacteria to determine the electrical charge on both floc and nonfloc-forming bacteria. The surface charge varied from 4.9 to 20.8 millivolts with the majority running between 6.0 and 12.0 millivolts. A large majority were below the "critical" charge--15 millivolts--for flocculation. It appeared that something else was influencing flocculation besides surface charge (28).

In 1955 and 1957, McKinney made more microscopic examinations of both floc and nonfloc-producing bacteria. He noticed that the nonfloc-producing bacteria were still very motile and active, which indicated that active metabolism of the substrate was still in progress. He noticed that the floc-producing bacteria were lacking motility and activity, which indicated to him that metabolism of substrate had either ceased or slowed down considerably. It should be noted that this might also indicate that the amount of substrate influences flocculation and the amount of substrate available can depend on growth rate or cell age.

McKinney ran experiments using *Alcaligenes faecalis*, and showed that they flocculated within six hours, indicating to him that *Alcaligenes faecalis* was capable of using only a small portion of the substrate. Utilization of this fraction exhausted the food supply, and flocculation occurred. If flocculation were a normal phenomenon which resulted when the food supply was exhausted, then all bacteria would flocculate. McKinney ran this experiment on several other isolated bacteria, and all of the cultures formed floc within seventeen days, and all of the bacteria showed a considerable amount of activity before flocculation. After flocculation, the bacteria showed very little activity, indicating to him that metabolism had ceased. McKinney

concluded that bacterial flocculation depends upon

1) primary surface charge, and

2) energy content of the system and activity of the bacteria (12).

Butterfield and McKinney, along with others, described how and possibly why bacteria flocculate. However, at times, bacteria are nonfloc-producing and the addition of chemicals assists in flocculation. These chemicals include polyelectrolytes and metal ions.

B. Polyelectrolytes

Polyelectrolytes consist of either natural or synthetic polymers. Natural polymers are of several different sources. When microorganisms become "old" or when nitrogen is the limiting nutrient, extracellular polymer excretion increases. The polymers are mainly in the form of bacterial polysaccharides. In addition, many old cells lyse and release polymeric nucleic acids and polypeptides. These polymers serve to flocculate the bacteria cells (7). Again, it might be said that cell "age" might have something to do with flocculation.

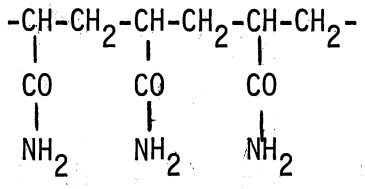
Synthetic polyelectrolytes are long chain, high molecular weight organic polymers that have many active sites along the length of the polymer chain. There are three general classes of polyelectrolytes which can be used. These are

1) anionic

2) cationic, and

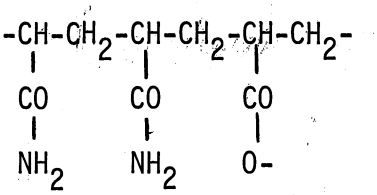
3) nonionic

The three are basically the same except for the charge--anionic being negatively charged, cationic, positively charged, and nonionic being neutrally charged. The basic polyacrylamide structures are



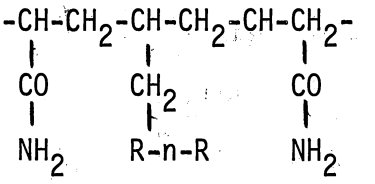
(nonionic polyelectrolyte)

Upon acidification with acrylic acid, the anionic polyelectrolyte is formed



(anionic polyelectrolyte)

Upon addition of quarternary amine, the catonic polyelectrolyte is formed (15)(16).



Although there has been a great amount of work accomplished with the use of polyelectrolytes, there is still much more needed. There are still many unknowns regarding the reasons polyelectrolytes work the way they do.

When flocculation occurs in the presence of polymers, absorption, charge neutralization, and interparticle bridging are possible causitive mechanisms (17). When polyelectrolytes are added to suspended solids, they are absorbed by the organic and the inorganic matter. This absorption is due to the formation of at least one of the following bonds

1) hydrogen bonding

2) electrostatic side-bonding, or

3) double-layer interaction (17)(18)(1)

During this absorption, a great gain or loss of charge or active sites on the polyelectrolyte occurs. When the charge, the Van der Walls' forces, have been reduced on the bacteria, flocculation occurs. When two or more solid particles or bacteria attach to different active sites on the polyelectrolyte, bridging occurs. These bridges yield closely packed flocs of bacteria, and these settle out of the liquid. This bridging effect happens with both natural and synthetic polymers (7).

In 1954, Michaels showed that for a polymer to enhance the flocculation of a suspension of colloids, it had to be absorbed on the colloid surface. Then flocculation could occur by either of two ways

1) the electrokinetic potential of the colloidal surface may be reduced to a level where flocculation occurs, or

2) polymer molecules may absorb one or more colloids and cause bridging between the polymer and the colloids, thus causing flocculation (19)(20).

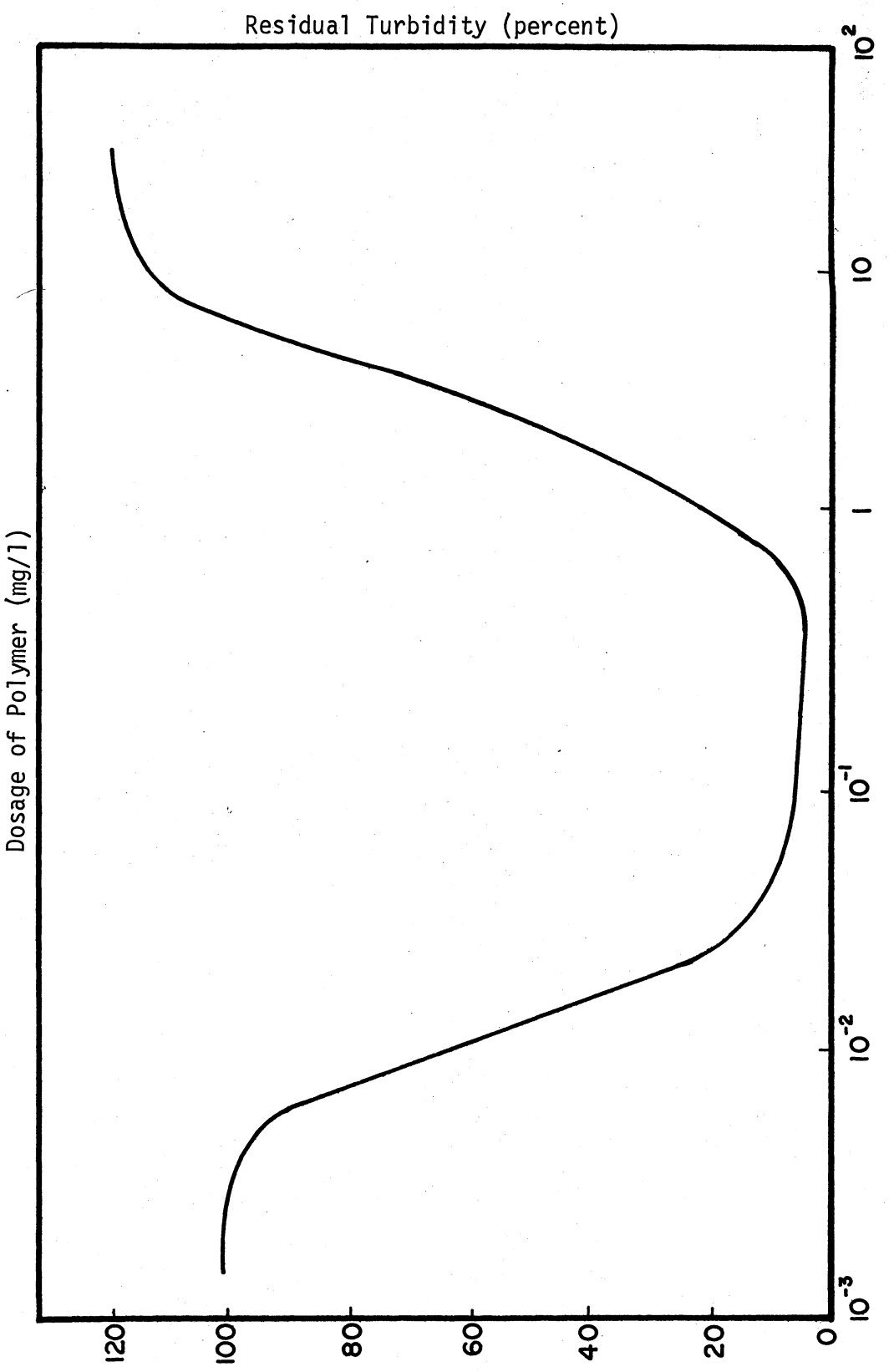
Dosages of polyelectrolytes have a great effect on the efficiency of flocculation. The relationship between cell flocculation and polymer concentration is shown in Figure 1 (21).

Birkner reported that polymer dosages smaller or larger than optimal create incomplete flocculation. He also reported that a change of the polymer dosage resulted in a different size of floc produced. Also, work on optimal dosages of polyelectrolytes has been done at Oklahoma State University by Yu (22), and Woldman (1), in 1962 and 1963, respectively.

Figure 1. Residual Turbidity as a Function of Polymer Dosage (Pulaski, 1968)

Polymer = DEAE dextran (cationic)

Turbidity = kaolinite, 100 mg/l (21)


Residual Turbidity (percent)
120
100
80
60
40
20
0
Dosage of Polymer (mg/l)
10^{-3}
10^{-2}
10^{-1}
1
10
10^{2}

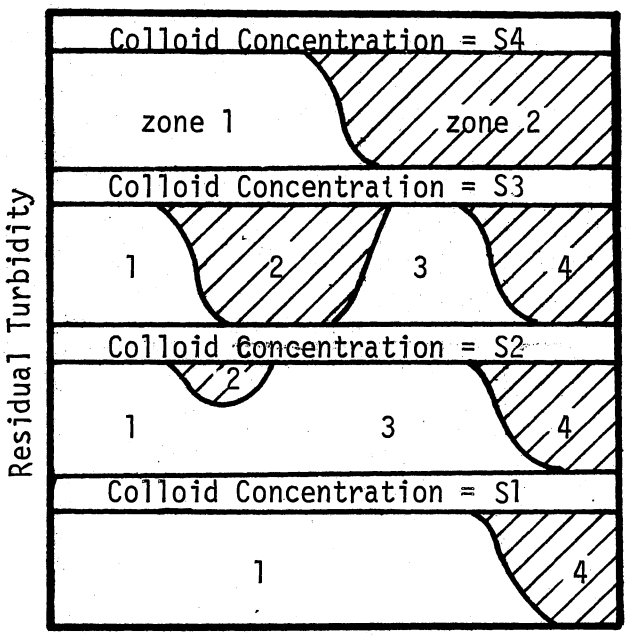
C. Metal Ions

Metal coagulants, such as aluminum sulfate $Al_2(SO_4)_3$ and ferric chloride ($FeCl_3$) have been used for many years in domestic sewage plants as flocculating agents to remove solids from wastewaters. In the flocculation of microorganisms, the efficiency of metal coagulants is dependent on the pH and alkalinity of the wastewater and the relationships between the concentration of bacteria and dosage of metal ions (21)(23).

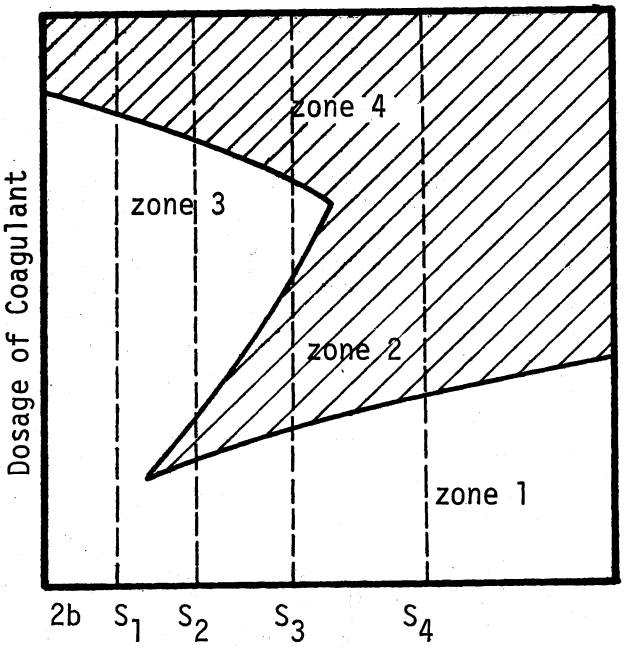
According to Tenney and Stumm, the optimum pH range for effective flocculation is approximately 5 to 6 for alum, and 4.5 and 5.5 for iron (23). Within this pH range, both iron and aluminum hydroxides are formed. Looking at the solubility curves of aluminum and iron, both are quite completely precipitated at pH levels as low as 5, and very little Fe^{+3} and Al^{+3} remain in the water. AT pH 4 or less, the OH^- concentration is insufficient to precipitate Fe^{+3} completely. Al^{+3} is incompletely precipitated at pH less than 5 for the same reason. Such results tend to explain why there must be a residual alkalinity during chemical coagulation.

Concentration of solids in relation to dosage is another important factor in chemical flocculation with Fe^{+3} and Al^{+3}. With reference to Figure 2a, at low dosages, zone I, an insufficient concentration of coagulant has been used to produce flocculation. Increasing the dosage, zone II, permits rapid flocculation and destabilization of bacteria (Figure 2a). A further increase in dosage, zone III, restabilizes the bacteria (Figure 2a). In zone IV, a degree of over-saturation occurs to produce what is termed a "sweep floc (24)(21).

Figure 2. Schematic Representation of Coagulation Observed in Jar Test Using Aluminum (III) or Iron (III) Salts at Constant pH (21)



2a - Dosage of Coagulant



Colloid Concentration, expressed as Concentration at Surface ($\bar{S}$)

Figure 2b is a representation of interrelationships between dosages and bacteria concentration at a constant pH. For systems containing low colloid concentration, sufficient contact opportunity does not exist to produce flocculation. This condition may be found in water treatment plants.

For higher concentration of colloids (S_2 and S_3 in Figure 2b), an increase in dosage of coagulant is required. The destabilization zone (zone II) is observed to widen with an increase of solids concentration (Figure 2a).

For very high colloid concentrations, similar to that found in sludge conditioning in wastewater treatment plants, a high dosage of coagulant is required to cause flocculation of bacteria (Figure 2b).

From the works of Stumm, Tenney, O'Melia, and Morgan, flocculation of bacteria with the aid of aluminum sulfate or ferric chloride depends upon

1) dosage of coagulant

2) pH, and

3) alkalinity.

Flocculation occurs by interaction of linear polymers resulting from hydrolysis of aluminum sulfate or ferric chloride with dispersed cells. The ions at the end of these short-chained polymers attach to ionic groups on the bacteria (25)(26).

The purposes of the present study are to

1) compare auto-flocculation with chemically-aided flocculation, using $FeCl_3$ and $Al_2(SO_4)_3$ separately,

2) compare auto-flocculation of cells grown at different specific growth rates, and

3) compare various dosages of metal ions, ferric chloride, and aluminum sulfate on flocculation.

Another aspect of the study, which was not originally proposed, is a brief investigation into the shock loading capability of slow-growing cells. Results of this phase of the work are not included in Chapter IV but are given in Appendix C.

CHAPTER III

MATERIALS AND METHODS

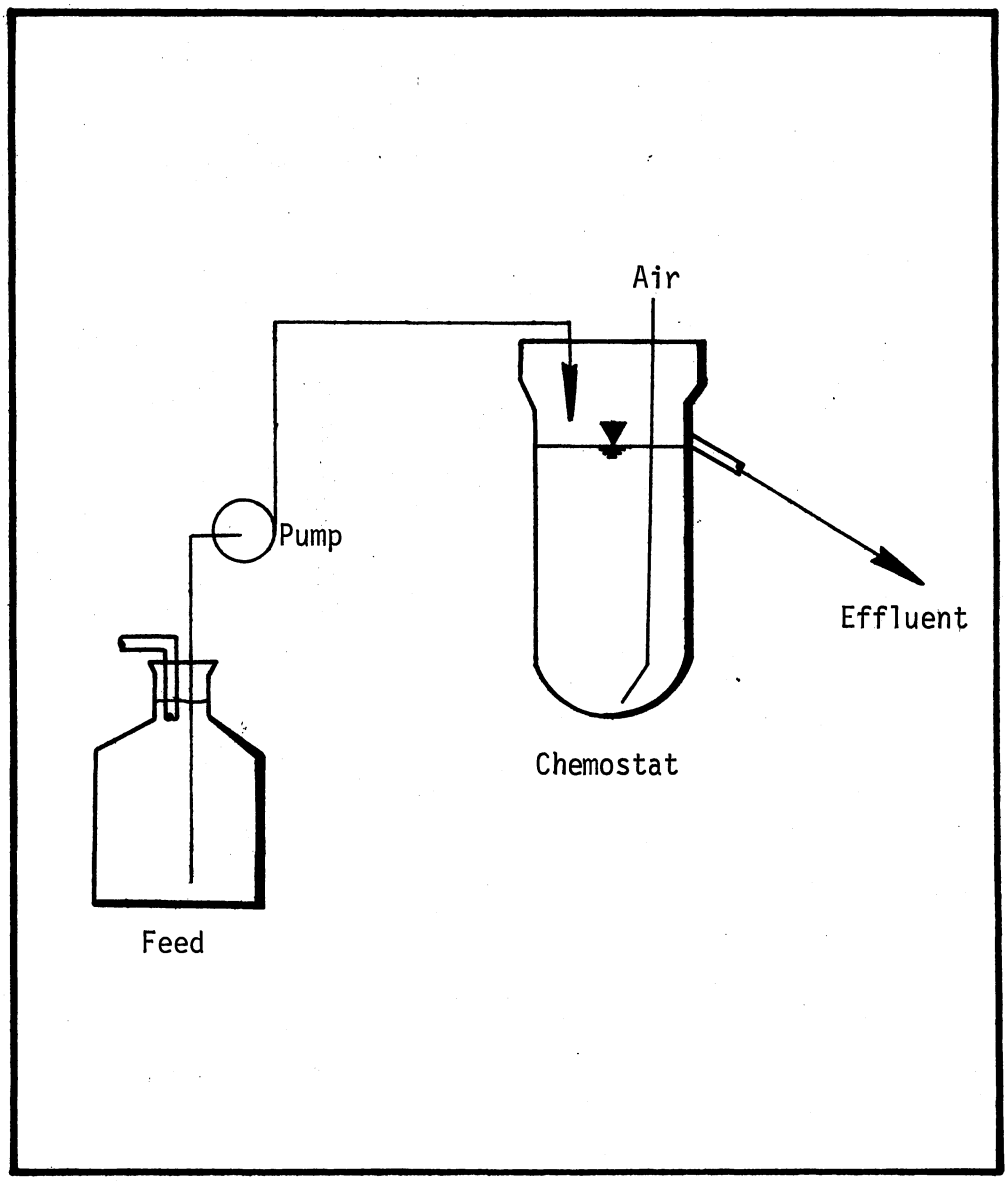
To study the influence of the addition of alum or ferric chloride as a clarification or flocculation aid on high concentrations and dilute concentrations of effluent from a once-through reactor unit and the study of the effect of different specific growth rates on flocculation, a bench scale unit was operated under closely controlled conditions. A description of laboratory apparatus, feed solution, alum and ferric chloride stock solutions, initial startup and daily procedural schedule, analytical procedures, and methods of analyzing the data are given below.

A. Laboratory Apparatus

A diagram of the laboratory setup used in this investigation is shown in Figure 3. A 2.5-liter glass once-through "chemostat" served as the reactor. The aeration volume (volume under air) was 2.1 liter. The reactor was four inches in diameter and fourteen inches in height. The feed rate was changed from experiment to experiment, to provide detention times of 8.0, 24.0, 48.0, or 72.0 $hours^{-1}$ in the reactor.

Air was supplied through two porous diffuser stones at an air flow rate of three liters per minute, which was adequate to provide good mixing and also supply enough oxygen for metabolic requirements of the microorganisms. The air stones were washed thoroughly every three to

Figure 3. Once-through Chemostat Used in Study


Air
Pump
Effluent
Chemostat
Feed

five days and then replaced after each detention time. The air flow was measured using a Gelman airflow meter. To assure that the biological system was safe from oil, foreign liquid, and matter from the compressed air, a filter consisting of cotton fibers was placed in the line ahead of the reactor. A dual, positive displacement pump (Milton Roy, Avondale, Pa.) was used to deliver a continuous flow of synthetic waste to the once-through biological treatment unit. Plastic tubing was used for both the suction side and discharge side of the pump. Alternately, each of the feed lines was disinfected by pumping through it a solution of potassium permanganate. Thus, one set of lines was disinfected while the other set was being used to feed the wastewater to the unit. These quarter-inch diameter lines were replaced several times during the course of this study. The rate of flow was checked daily, using a graduated cylinder and stopwatch.

Complete mixing was checked by several methods. Biological solids samples were taken inside the reactor and from the effluent. Also, chemical oxygen demand was run on the filtrate from the inside of the tank and from the effluent. Optical density was checked on effluent and reactor mixed liquor.

B. Feed Solution

Listed in Table I is the chemical composition of the synthetic wastewater used in this study. The wastewater was designed to have a nominal chemical oxygen demand (COD) of 1000 mg/l (milligrams per liter). Other required nutrients were provided in the concentrations shown in Table I. A one-molar (1M) phosphate buffer system controlled the pH of the system. the feed pH was maintained at approximately 7.2.

Stock solutions of dextrose, salts which contain $MgSO_4$, $FeCl_3$, $MnSO_4$, buffer and ammonia sulfate, were made up using distilled water. Enough distilled water was added to make up two liters (l) of stock solution. New stock solution was prepared once every three weeks in order to guard against contamination.

TABLE I

COMPOSITION OF SYNTHETIC WASTEWATER
(1000 mg/l glucose)

Constituents	Amount
Glucose	1000 mg/l
Ammonia sulfate	500 mg/l
Magnesium sulfate	100 mg/l
Ferric chloride	0.5 mg/l
Manganous sulfate	100 mg/l
Calcium chloride	7.5 mg/l
1 M phosphate buffer solution	10 mg/l
Tap water	100 ml/l

The feed was made up in a 20-liter Pyrex bottle, 10 liters at a time. Three or four liters of distilled water were placed in the bottle, then the correct amounts (measured in a graduated cylinder) of each stock solution were mixed thoroughly with the distilled water in

the glass bottle. Feed solution was prepared daily to keep the feed solution from becoming contaminated.

C. Alum and Ferric Chloride Stock Solution

Stock solutions of alum and ferric chloride were made up for this study. For alum stock, five grams of alum were mixed in a 2-liter volumetric flask (half-filled) and stirred until dissolved. Then the flask was filled to two liters. Thus, the stock contained 2.5 mg/ml of the flocculating chemical. The ferric chloride stock solution was made up in the same way.

D. Initial Startup

The original seed of microorganisms came from the effluent of the primary settling basin at the municipal wastewater treatment plant in Stillwater, Oklahoma. New seed was obtained for each continuous flow run. The reactor was run for one to two days as a batch unit to let the microorganisms acclimate to the synthetic feed.

Following this acclimation period, the reactor was operated on a continuous flow basis. At this time, daily sampling for various analyses was initiated.

E. Daily Parameters

The parameters which were monitored daily are listed in Table II. A 100-ml sample of feed was taken each day. From this, a 10-ml sample was taken for the chemical oxygen demand (COD) analysis; the rest was used to determine the pH.

A 25-ml sample was taken from the effluent and filtered through

0.45 μ membrane filter for the determination of effluent biological solids. A 10-ml sample of the filtrate was taken for the chemical oxygen demand analysis.

TABLE II

PARAMETERS MONITORED ON A DAILY BASIS

1. Feed	
	a) mixing feed
	b) chemical oxygen demand
	c) pH
2. Effluent	
	a) biological solids
	b) chemical oxygen demand, filtered
	c) pH
3. Reactor	
	a) biological solids
	b) chemical oxygen demand, filtered
	c) pH
	d) temperature

F. Analytical Procedures

Feed COD determination was made in accordance with Standard Methods for the Examination of Water and Wastewater (27). Effluent and reactor COD determinations were made in accordance with dilute COD method in Standard Methods (27).

Biological solids concentration was performed by filtering a known volume through a membrane filter (0.45 μ pore size). The filter pad was placed in an aluminum dish and dried at 103°C for two hours. After cooling to room temperature in a desiccator, the tare was weighted for initial weight. After a filter was dried and weighed, a measured volume of the sample was filtered. The filter was then dried for two hours at 103°C, cooled to room temperature in a desiccator, and weighed to determine the final weight.

The pH of the feed and effluent were checked regularly, using a Beckman Expandomatic SS-2 pH meter. The meter was standardized to a pH of 4, 7, and 10, using standard buffer solutions.

Temperature was checked periodically, using a thermometer having a range of -20° to 110°C. The optical density was calculated from readings of percent transmittance using a Bausch and Lomb Spectronic 20 at a wavelength of 535 mm.

The open jar tests were run for each detention time or growth rate after the biological reactor was at a steady state. The jar tests were run on a standard Phipps and Bird jar test apparatus. The jar test apparatus consists of six stirrers and a paddle attached at the end of each stirrer. Each stirrer can be lifted so that a container can be placed on or removed from the apparatus. The stirrers were controlled by a variable speed motor. The range of speed was 0-120 rpm. The containers used for the jar test were 600-ml Pyrex beakers (graduated).

A volume of 250 ml of effluent was placed in each of the six containers. Two blanks were used, one for every sixth run, and the other five jars were used for the settling test. A volume of the effluent in the container was taken out and replaced by an equal amount of alum

solution to give the desired concentration. For example: One ml taken out and replaced with one ml of stock chemical solution gave a concentration of 10 mg/l. Concentrations used were 10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, 60 mg/l, 80 mg/l, 100 mg/l, 120 mg/l, 140 mg/l, and two blanks were used except for 48-hour and 72-hour detention times. For these two detention times, a blank was left out and 240 mg/l was added. This was done to see the effect of high concentrations of alum. A sample of the effluent was taken before the jar test to find initial optical density after the addition of alum, a period of one minute was used for the flash mix and then fifteen minutes was used for flocculation time (5). Samples of 10 ml were taken from the first half-inch of supernatant in each container and placed in sample tubes so optical density could be determined. This was done periodically for every fifteen minutes until the optical density was constant--usually 30-45 minutes. The same procedure was followed for the study in which ferric chloride was employed as coagulant.

Jar tests were also made on a dilute suspension of cells. The same procedure was followed, except the effluent solution was diluted to a concentration of one part effluent to ten parts distilled water.

G. Methods of Data Analysis

Treatment purification, or COD removal efficiency, was determined as follows:

$$E = \frac{S_i - S}{S_i} \times 100$$

where

E = COD removal efficiency, percent

S_i = influent substrate concentration, mg/l

S = effluent substrate concentration, mg/l

Cell yield was determined as follows

$$Y = \frac{X}{S_i - S}$$

Y = yield

X = biological mass, mg/l

Dilution ratio, or specific growth rate, μ, was determined as follows

$$D = \mu = \frac{F}{V}$$

F = flow of effluent, ml/min

V = volume, ml

Efficiency of settling was determined as follows

$$EOD = \frac{OD_i - OD_f}{OD_i} (100)$$

where

EOD = percent decrease in optical density

OD_i = initial optical density

OD_f = final optical density

CHAPTER IV

RESULTS

The laboratory reactor was operated for a period of approximately four months. During these four months, the detention time was varied from eight hours to 72 hours, and jar tests were executed at each detention time when "steady-state" was reached. "Steady-state" conditions were assumed when constant values for reactor solids, reactor COD, effluent COD, and effluent solids were obtained. Tables showing the daily values from which steady-state concentrations of the various parameters were calculated for each detention time are given in Appendix A.

A. Studies at D = 0.125 hr^{-1}

The reactor was run for two days under batch conditions to allow the cells to acclimate to the synthetic wastewater. The reactor was then put into continuous flow and run for fourteen days. The steady-state data are plotted in Figure 4.

The COD removal efficiency was 89.5 percent. The biomass concentration ranged from 412 mg/l to 496 mg/l with an average of 452 mg/l. The average effluent COD was 108 mg/l; the calculated yield, Y, was 0.49. The pH remained constant in the effluent at 6.8; the feed pH was 7.2.

After the reactor ran at steady-state for a few days, jar tests

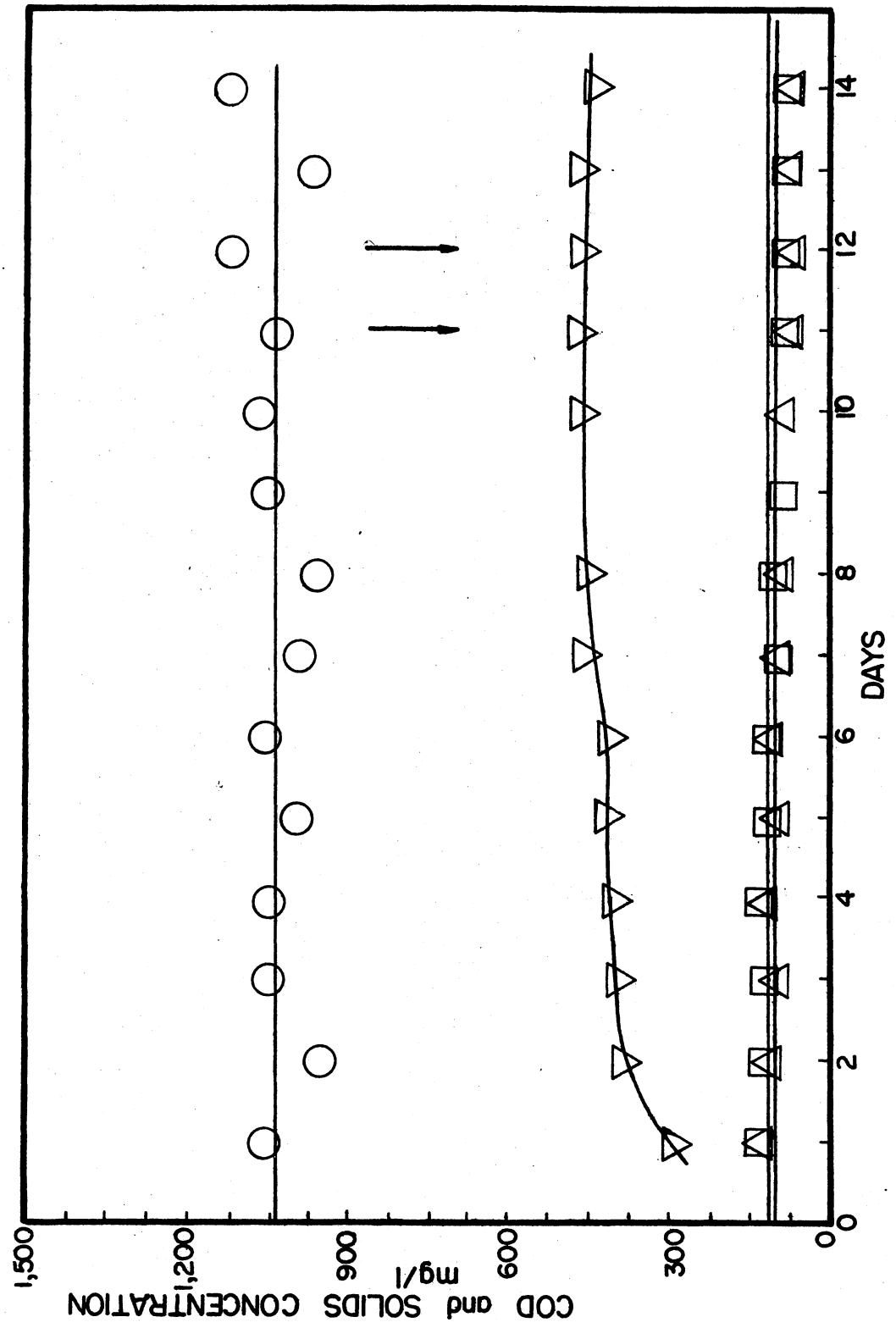
Figure 4. Operational Data for Steady-State at D = 0.125 hr^{-1}
(arrows signify when jar tests were run)

○ = feed (COD)

□ = reactor COD

△ = effluent COD

▽ = biological solids


COD and SOLIDS CONCENTRATION
mg/l
1,500
1,200
900
600
300
0
0
2
4
6
8
10
12
14
DAYS

were run on the effluent (see arrows on Figure 4).

Both alum and ferric chloride were used as coagulants, and tests were run on effluent and a dilute solution of effluent. The results are presented in Figures 5 and 6, 7 and 8.

The results for undiluted effluent (high cell concentration) after addition of ferric chloride and alum as flocculating chemicals are shown in Figures 5 and 6, respectively. As shown in Figure 5, ferric chloride exhibited little effect on the settleability of microorganisms; the same can be said of alum, as seen in Figure 6.

These results indicate that alum was more effective in enhancing settling than was ferric chloride. The best settleability efficiency for ferric chloride was 12.5 percent at 140 mg/l, while for alum it was 28 percent at 100 mg/l.

The results of the addition of ferric chloride or alum as coagulent on dilute concentrations of effluent are shown in Figures 7 and 8, respectively. Both figures show that the addition of ferric chloride or alum effected the settling of bacteria. The settleability efficiency for ferric chloride was 92 percent at a dosage of 100 mg/l, while the efficiency for alum was 97 percent at a dosage of 100 mg/l.

B. Studies at D = 0.042 hr^{-1}

The reactor was run for two days to let the cells acclimate to the synthetic wastewater. The reactor was then put into continuous flow operation and run for eleven days. The steady-state data are plotted in Figure 9.

The COD removal efficiency was 92.3 percent, and the reactor biomass concentration ranged from 406 mg/l to 480 mg/l with an average of

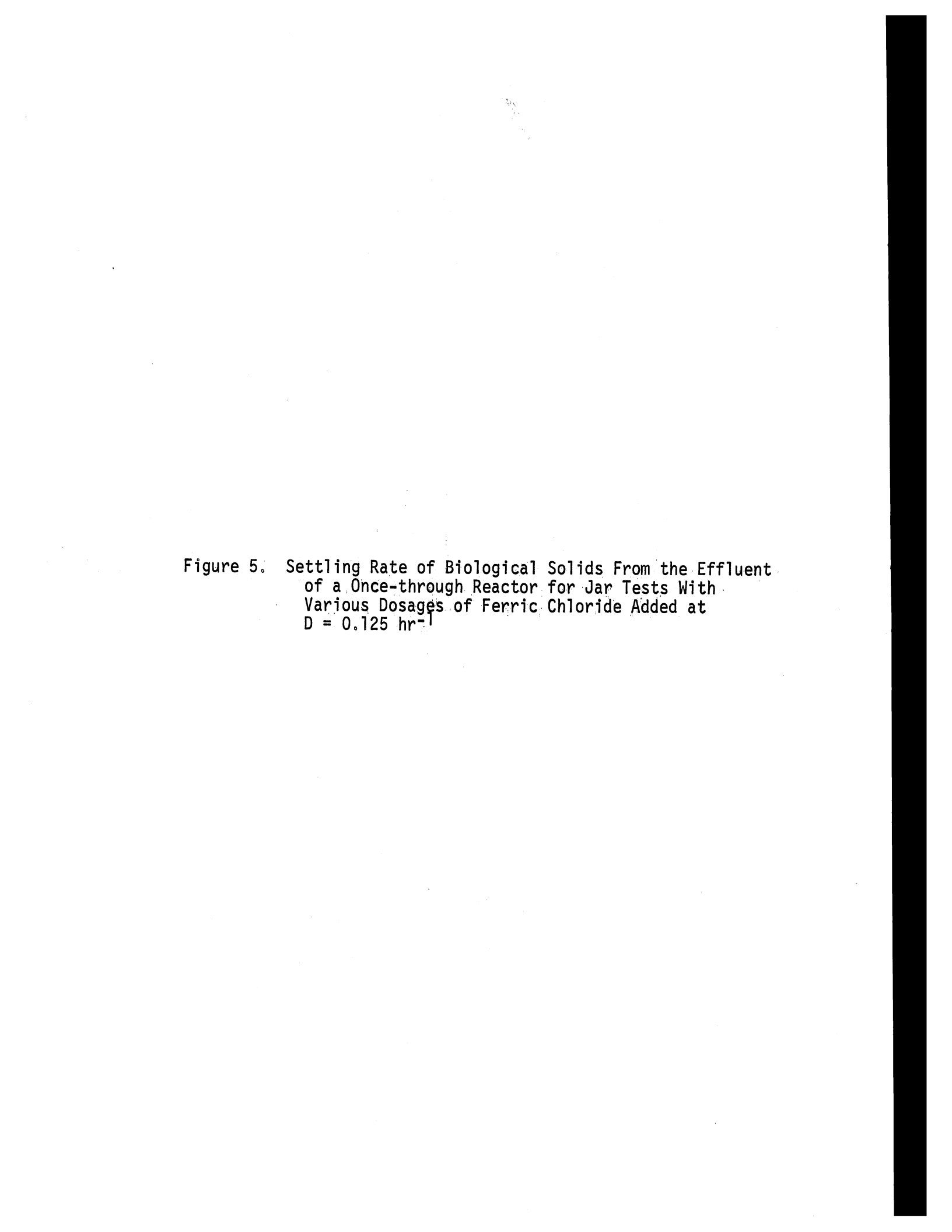


Figure 5. Settling Rate of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at $D = 0.125\ hr^{-1}$

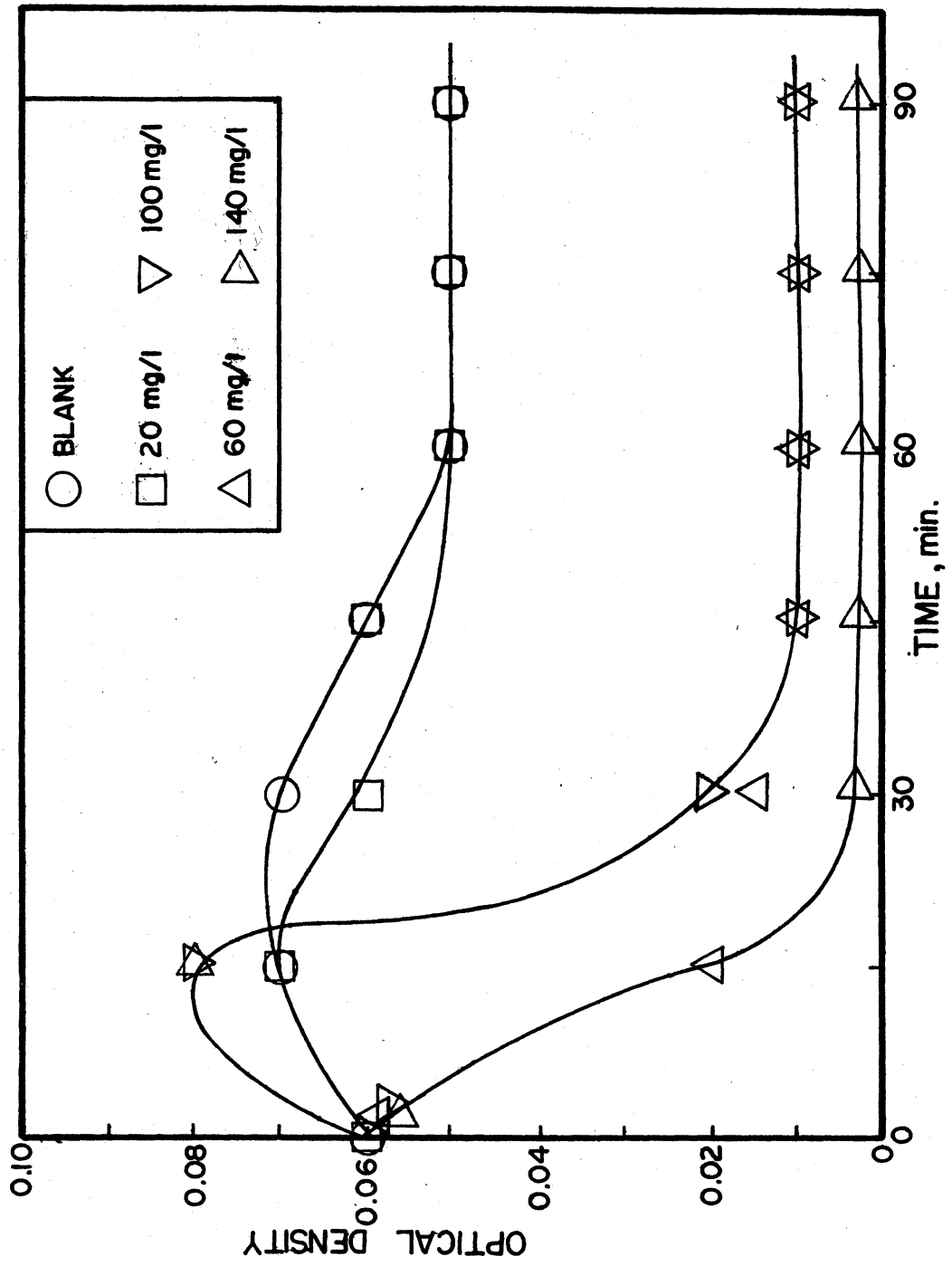

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0.0
0
30
60
90
TIME, min.

Figure 6. Settling Rates of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at $D = 0.125\ hr^{-1}$

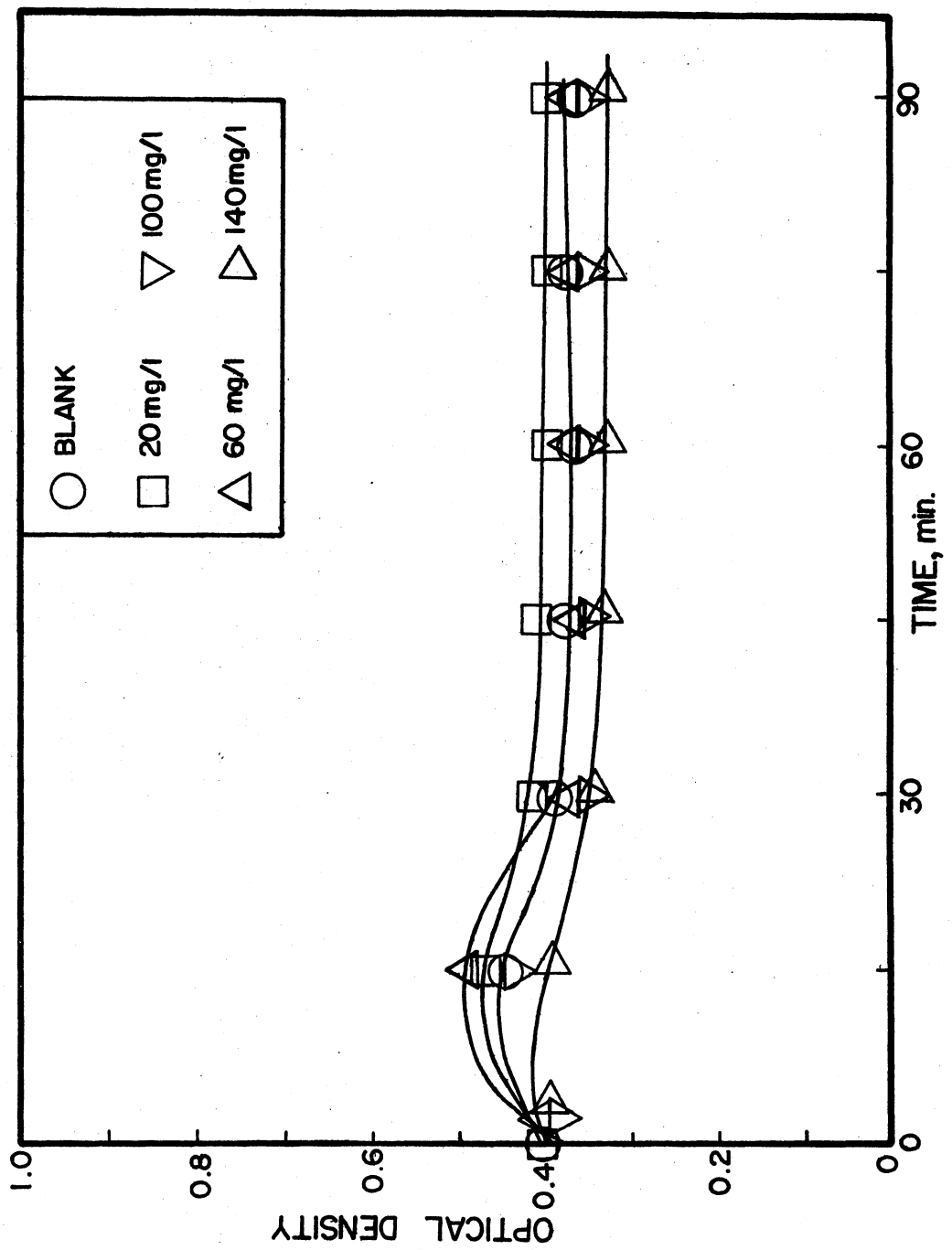

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME, min.

Figure 7. Settling Rates of a Dilute Solution of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at D = 0.125 hr^{-1}

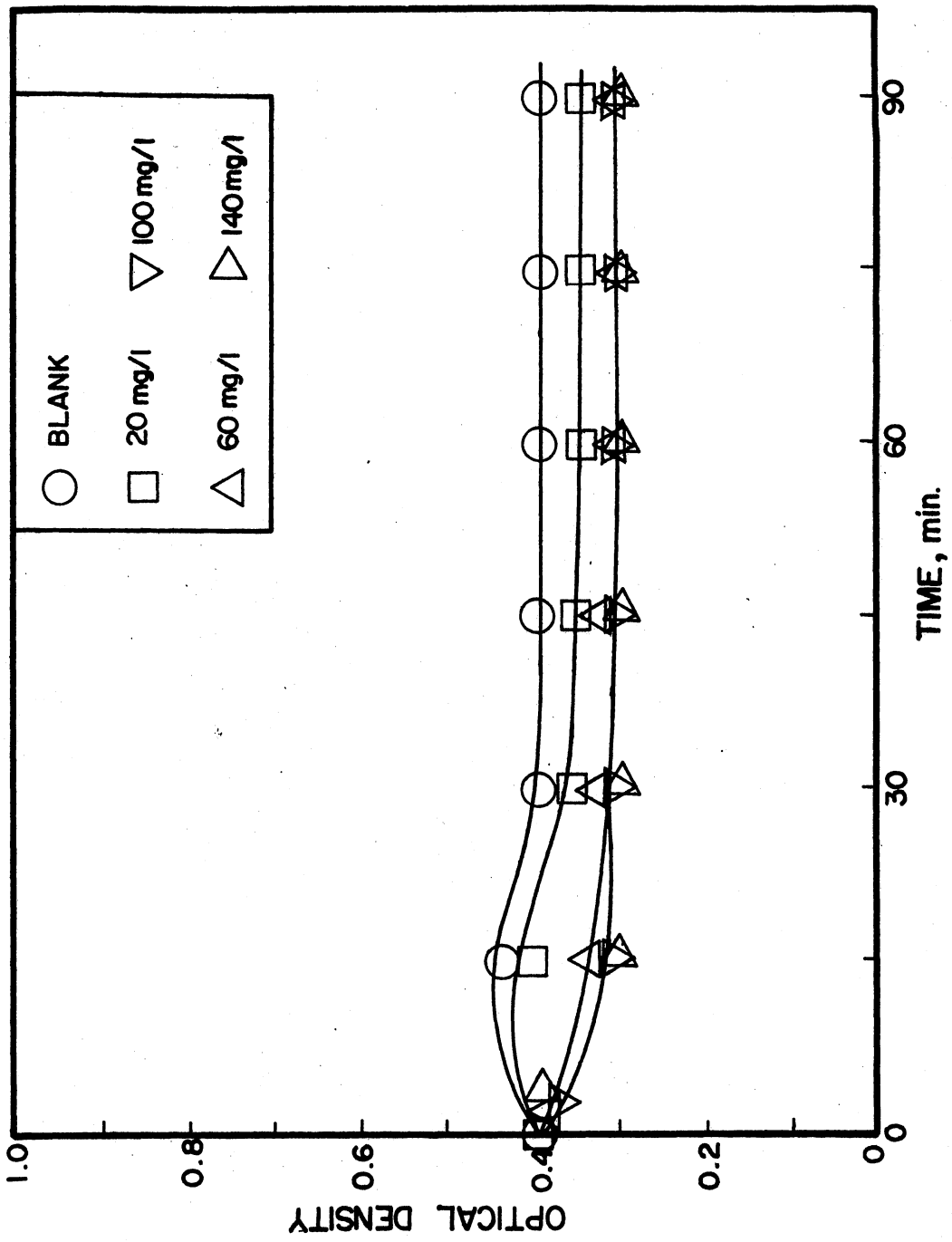

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME, min.

Figure 8. Settling Rates of a Dilute Solution of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at D = 0.125 hr^{-1}

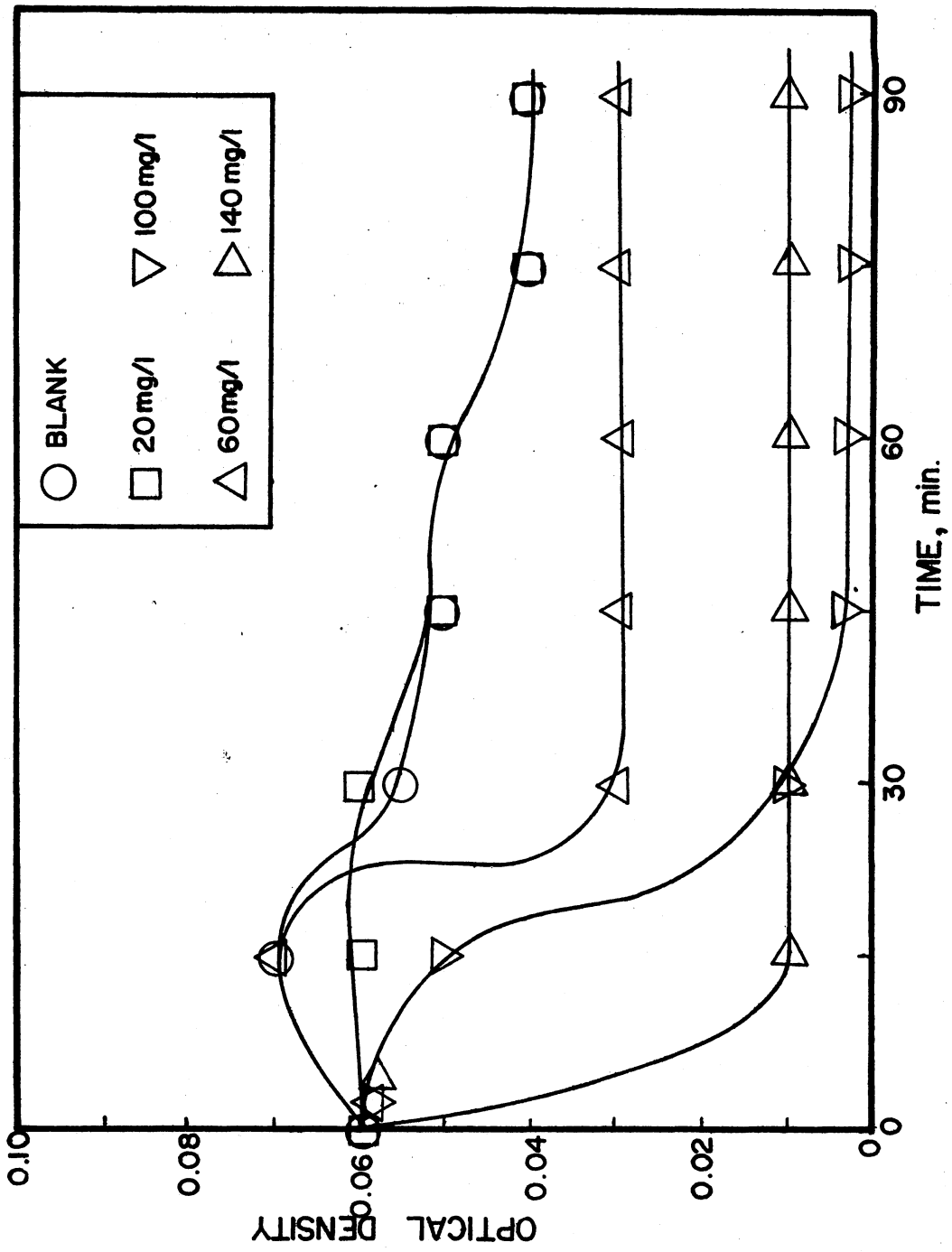

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME, min.

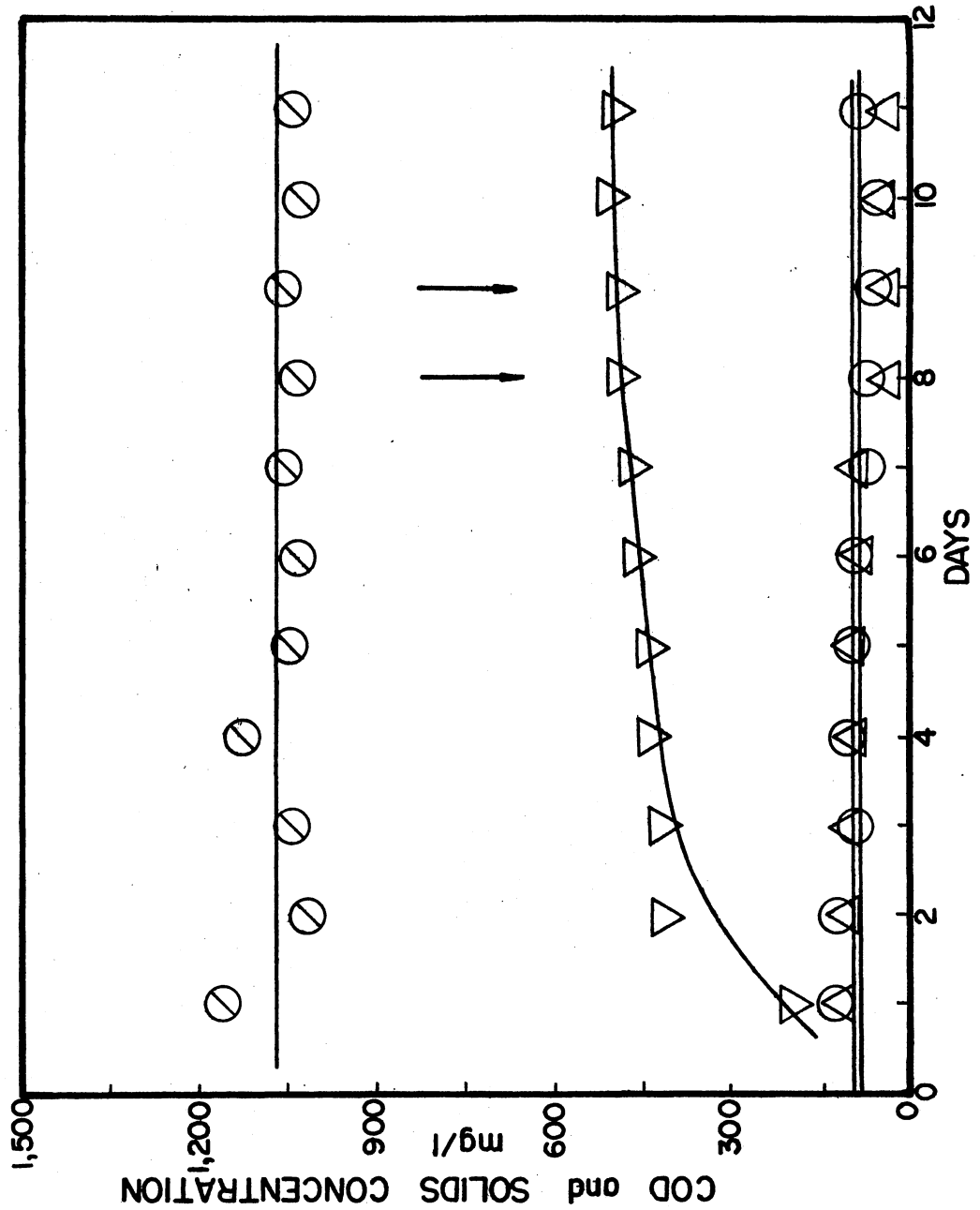
Figure 9. Operational Data for Steady-State at $\theta = 0.042\ hr^{-1}$ (arrows indicate when jar tests were run)


= feed COD


= reactor COD


= effluent COD


= biological solids of reactor


COD and SOLIDS CONCENTRATION
mg/l
1,500
1,200
900
600
300
0
0
2
4
6
8
10
12
DAYS

422 mg/l. The average effluent COD was 81 mg/l; the cell yield, Y, was 0.43. The effluent pH remained constant at 6.8, and the feed pH was 7.2, as before.

After the reactor ran at steady-state for a few days, jar tests were run on the effluent. Results are tabulated in Appendix B and shown graphically in Figures 10, 11, 12, and 13.

Results for addition of ferric chloride and alum to the chemostat effluent are given in Figures 10 and 11, respectively. These figures show that the addition of ferric chloride or alum had little effect on settling of the cells.

The results of the addition of ferric chloride or alum to a dilute solution of effluent are shown in Figures 12 and 13. Both figures indicate that the addition of ferric chloride or alum enhanced settling of the cells. The settleability efficiency for both ferric chloride and alum was 98 percent.

C. Studies at D = 0.021 hr^{-1}

The reactor was run for three days to let the cells acclimate to the synthetic wastewater. After they were acclimated, the reactor was put into continuous flow operation for fifteen days. The "steady-state" data are plotted in Figure 14. There was some solids flocculation prior to day 6; however, in calculating average steady-state conditions, all data shown in Figure 12 were employed.

COD removal efficiency was 90.9 percent. The reactor solids ranged from 250 mg/l to 412 mg/l, with an average of 341 mg/l. The average effluent COD was 99.6 mg/l, and the cell yield, Y, was 0.36 mg/l. The effluent pH ranged from 6.8 to 7.0, and the pH of the feed

Figure 10. Settling Rates of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at D = 0.042 hr^{-1}

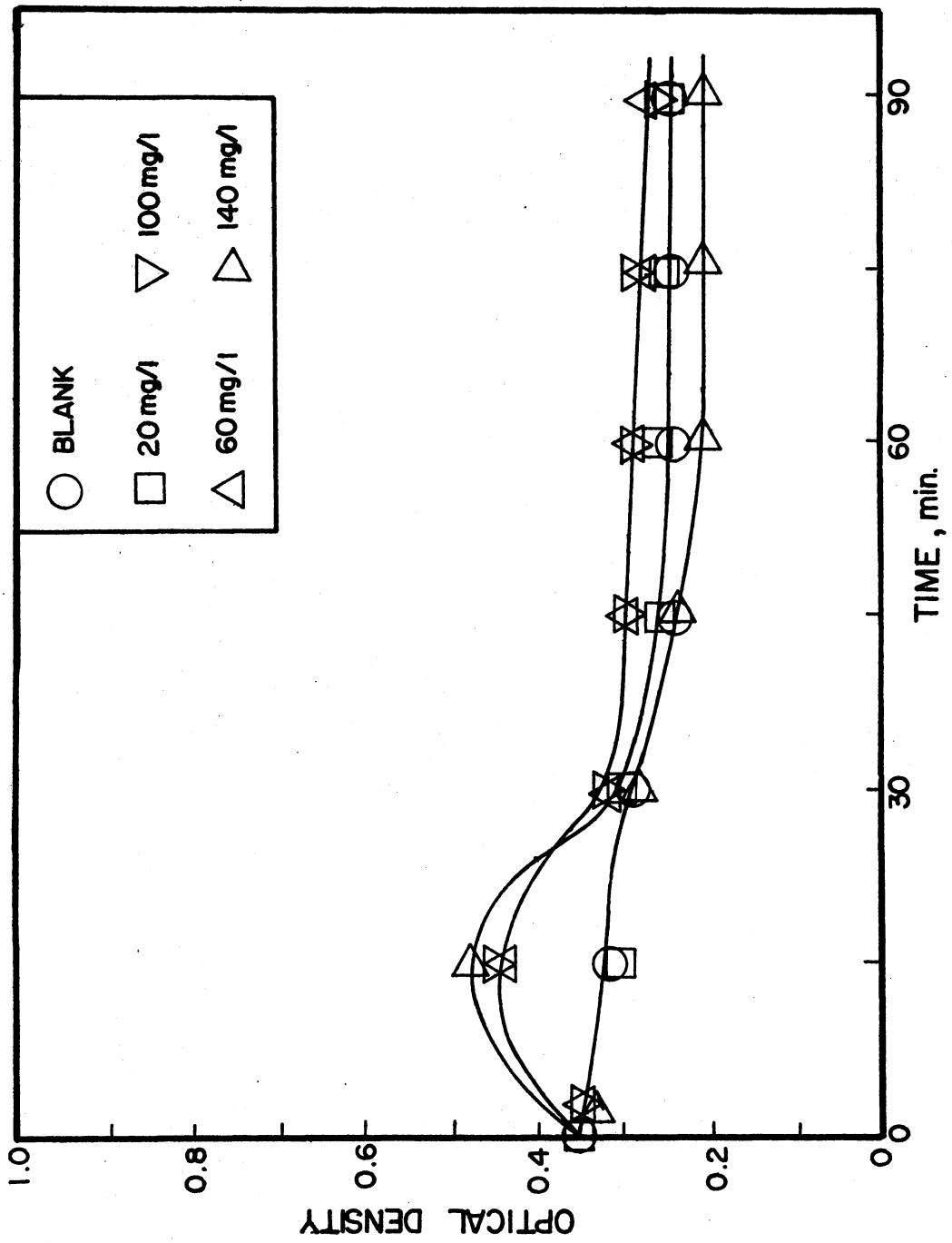
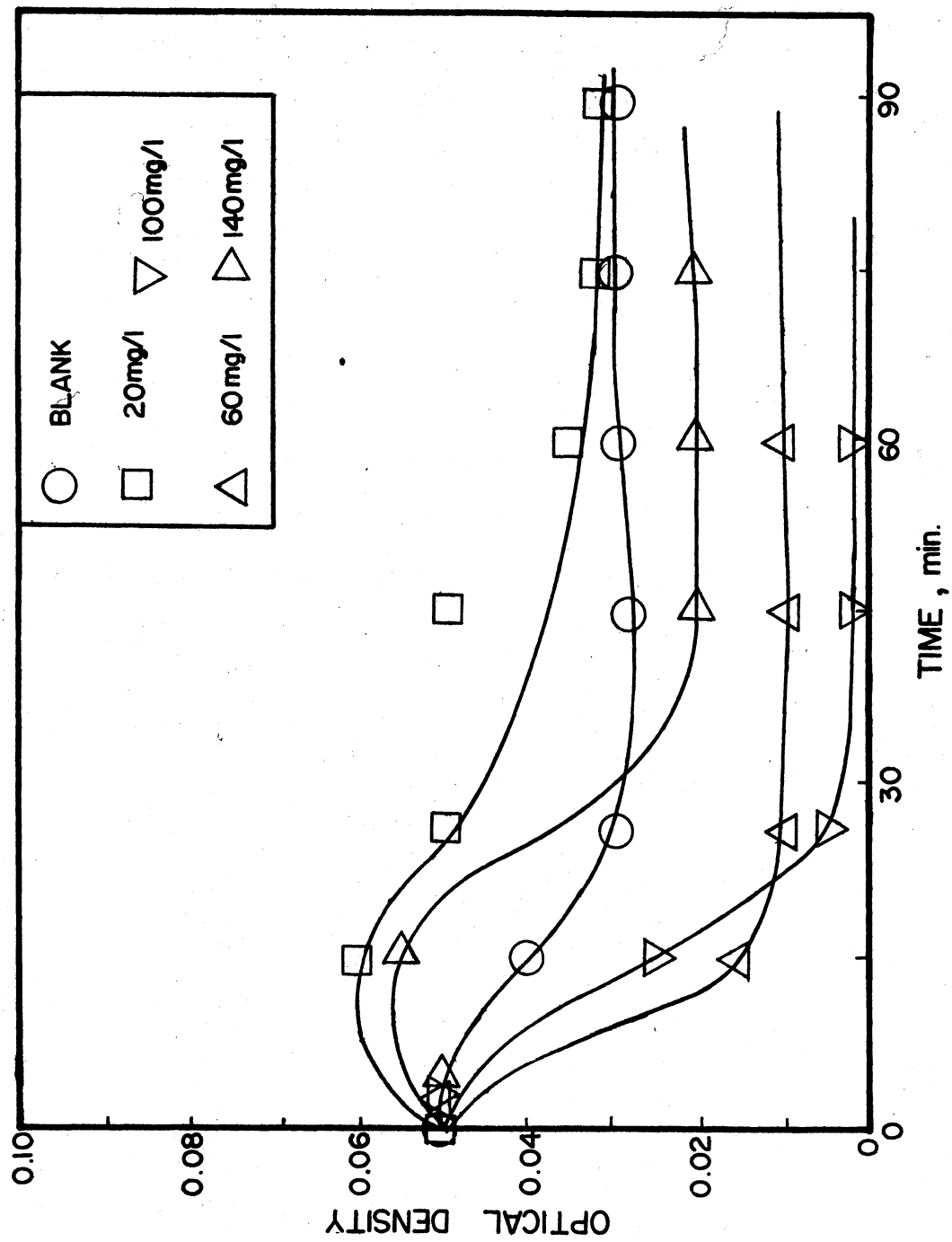

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME , min.

Figure 11. Settling Rates of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at $D = 0.042\ hr^{-1}$


BLANK
20mg/l
60mg/l
100mg/l
140mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME , min.

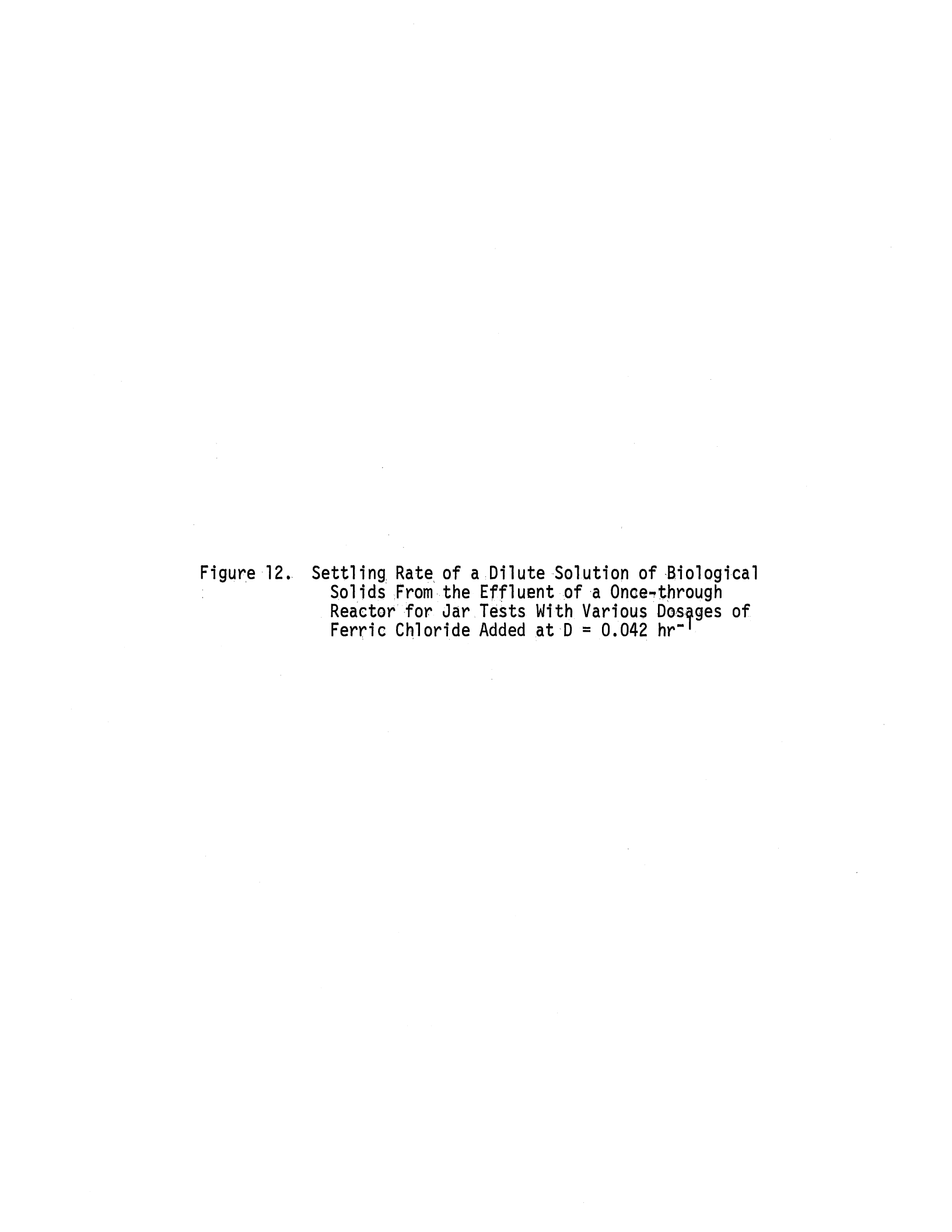


Figure 12. Settling Rate of a Dilute Solution of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at D = 0.042 hr^{-1}

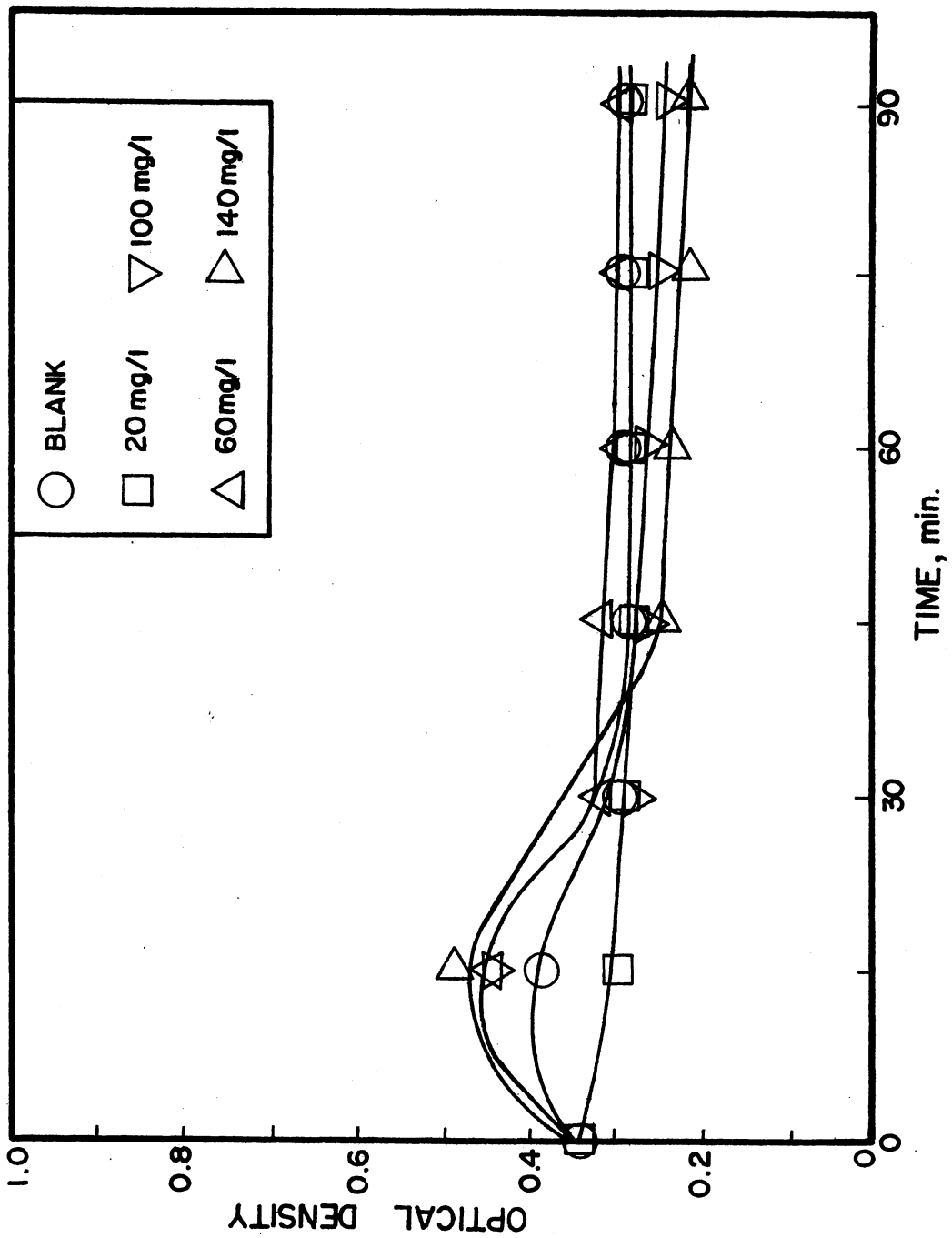

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
30
60
90
TIME, min.

Figure 13. Settling Rates of a Dilute Solution of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests with Various Dosages of Alum Added at D = 0.042 hr^{-1}

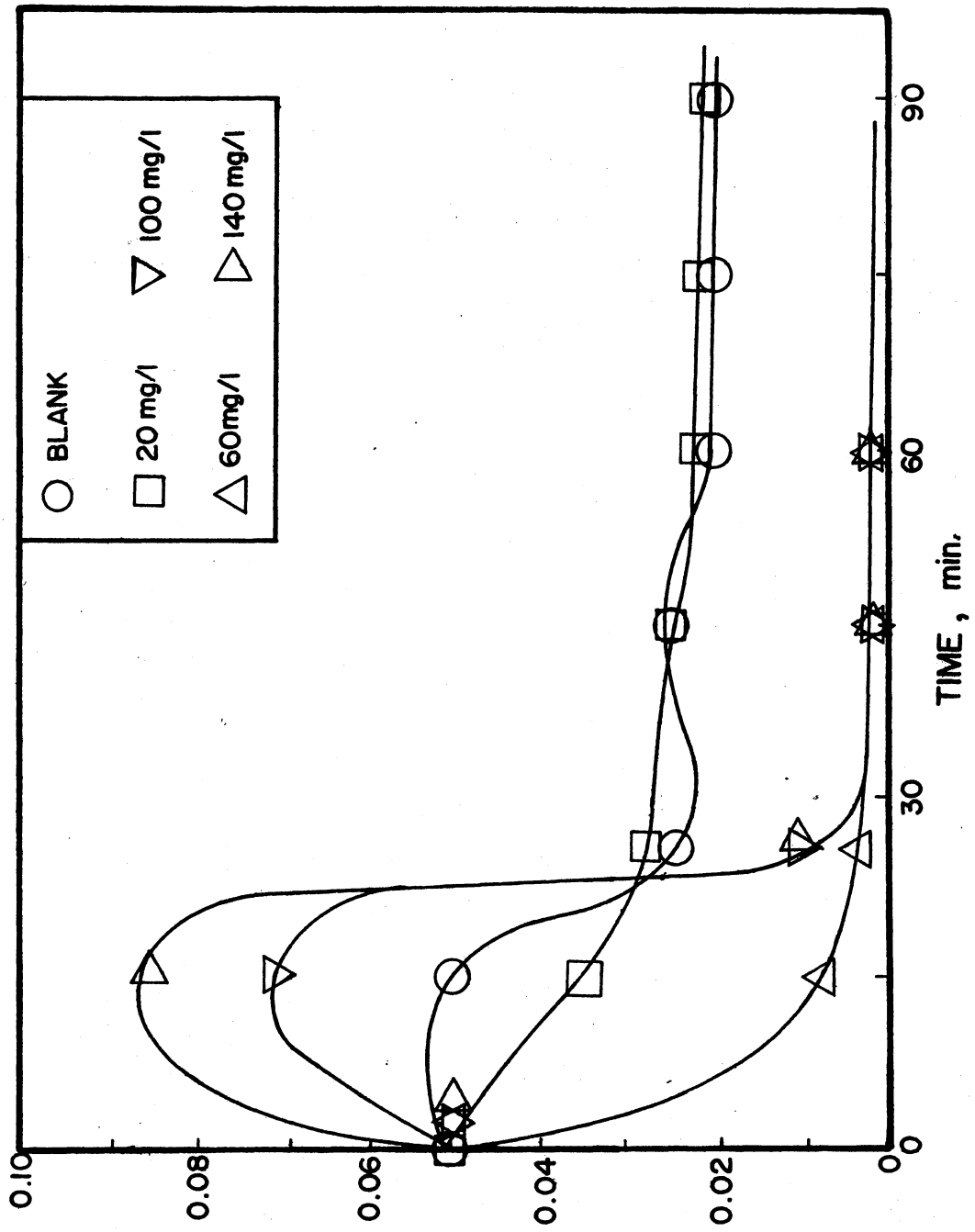
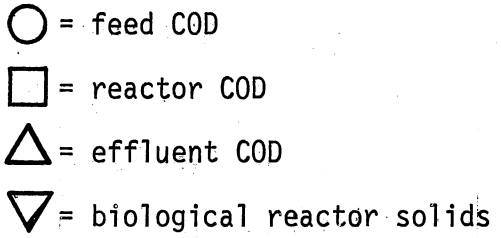
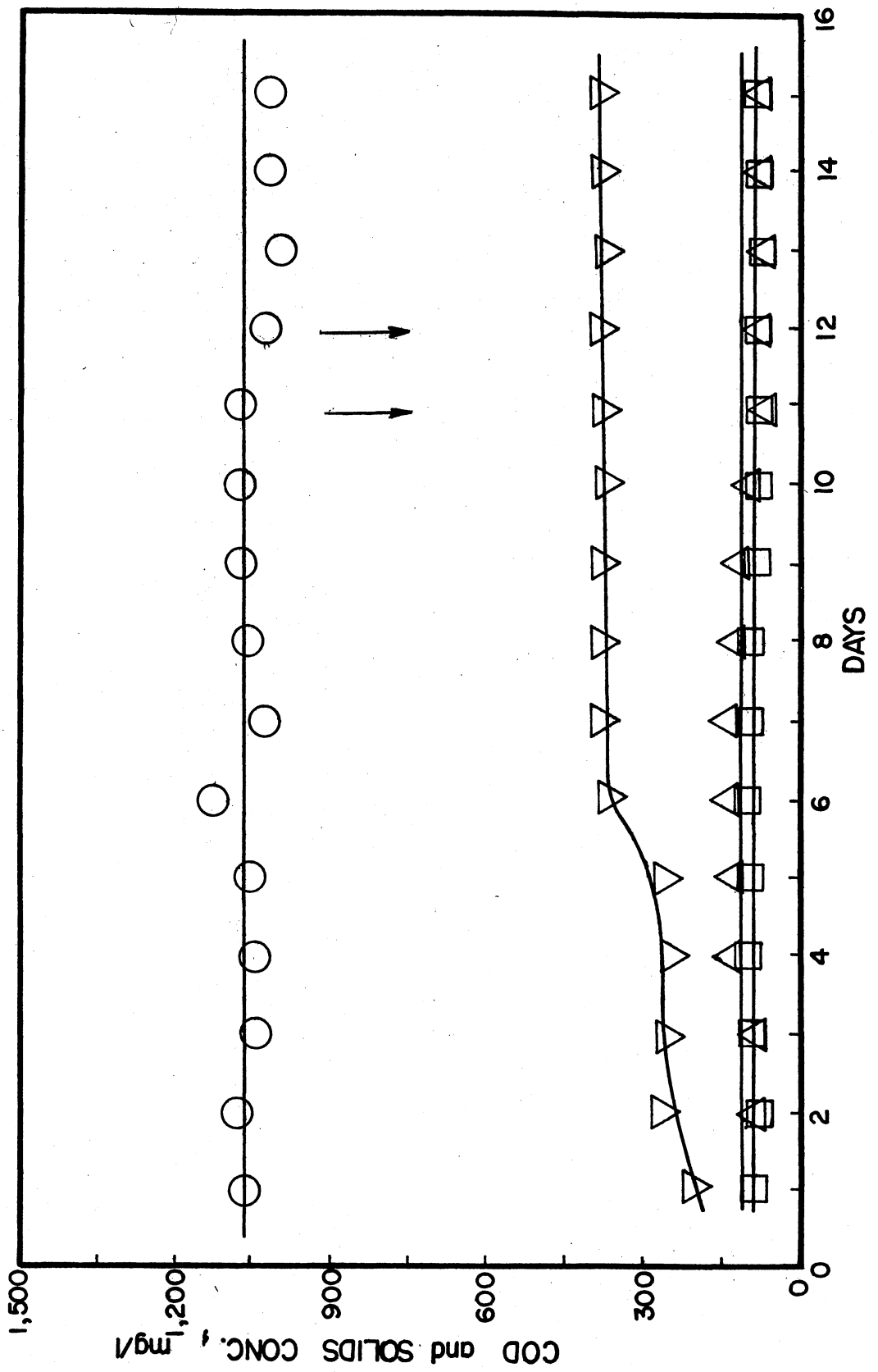

BLANK
20 mg/l
60mg/l
100 mg/l
140 mg/l
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME, min.

Figure 14. Operational Data for Steady-State = 0.021 hr^{-1} (arrows indicate where jar test study was run)




COD and SOLIDS CONC., mg/l
1,500
1,200
900
600
300
0
0
2
4
6
8
10
12
14
16
DAYS

was 7.2.

After the reactor was maintained at steady-state for a few days, jar tests were run as for the cells harvested at the previous dilution rates and the data are tabulated in Appendix B.

The results of studies on the addition of ferric chloride and alum to reactor effluent are found in Figures 15 and 16, respectively. The addition of ferric chloride or alum enhanced, slightly, the rate of settling compared to the blank sample. However, it is interesting to note that at this specific growth rate, the cells showed a tendency to flocculate and settle without addition of coagulant. The settling efficiency of the blank was 81 percent, whereas addition of 140 mg/l ferric chloride (see Figure 15) increased the efficiency to 89 percent. For addition of alum, the efficiency was also 89 percent.

The results of adding ferric chloride and alum to dilute effluent are presented in Figures 17 and 18, respectively. Both ferric chloride and alum were effective in enhancing the settling of the cells. Ferric chloride caused a greater degree of flocculation and settling than did alum. The settling efficiency of the blank was 57 percent for alum jar tests, and 67 percent for the ferric chloride jar tests. Addition of 100 mg/l of alum yielded an efficiency of 71 percent, whereas from 100 m/gl ferric chloride the efficiency was 97 percent.

D. Studies at D = 0.014 hr^{-1}

Both reactors were started up and run for a few days as batch reactors to let the cells become acclimated to the synthetic wastewater. After a few days, both reactors were placed into continuous flow operation for 21 days. The steady-state data for both reactors A and B are

Figure 15. Settling Ratio of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride at D = 0.021 hr^{-1}

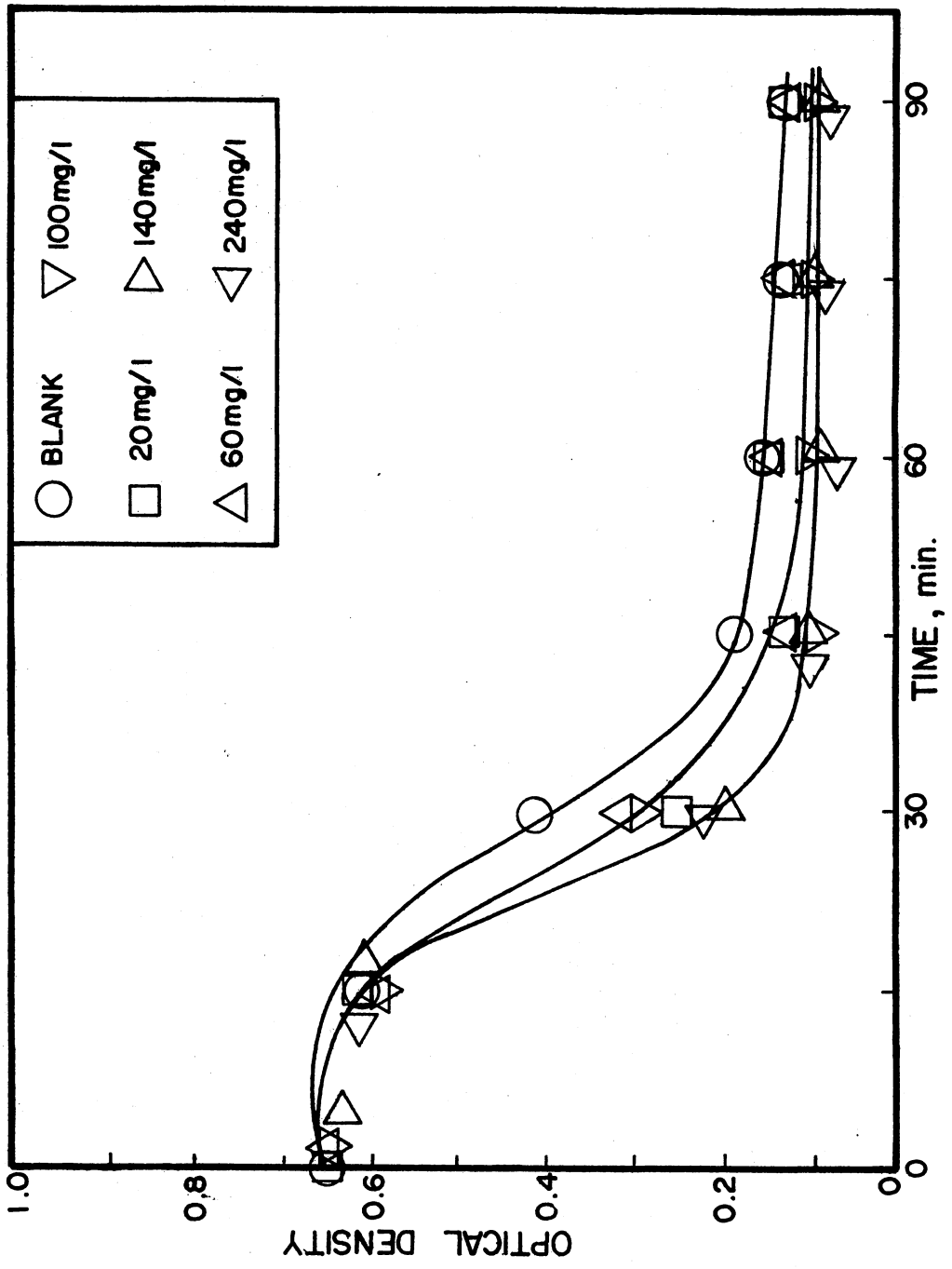

BLANK
100mg/l
20mg/l
140mg/l
60mg/l
240mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME, min.

Figure 16. Settling Rates of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at $D = 0.021\ hr^{-1}$

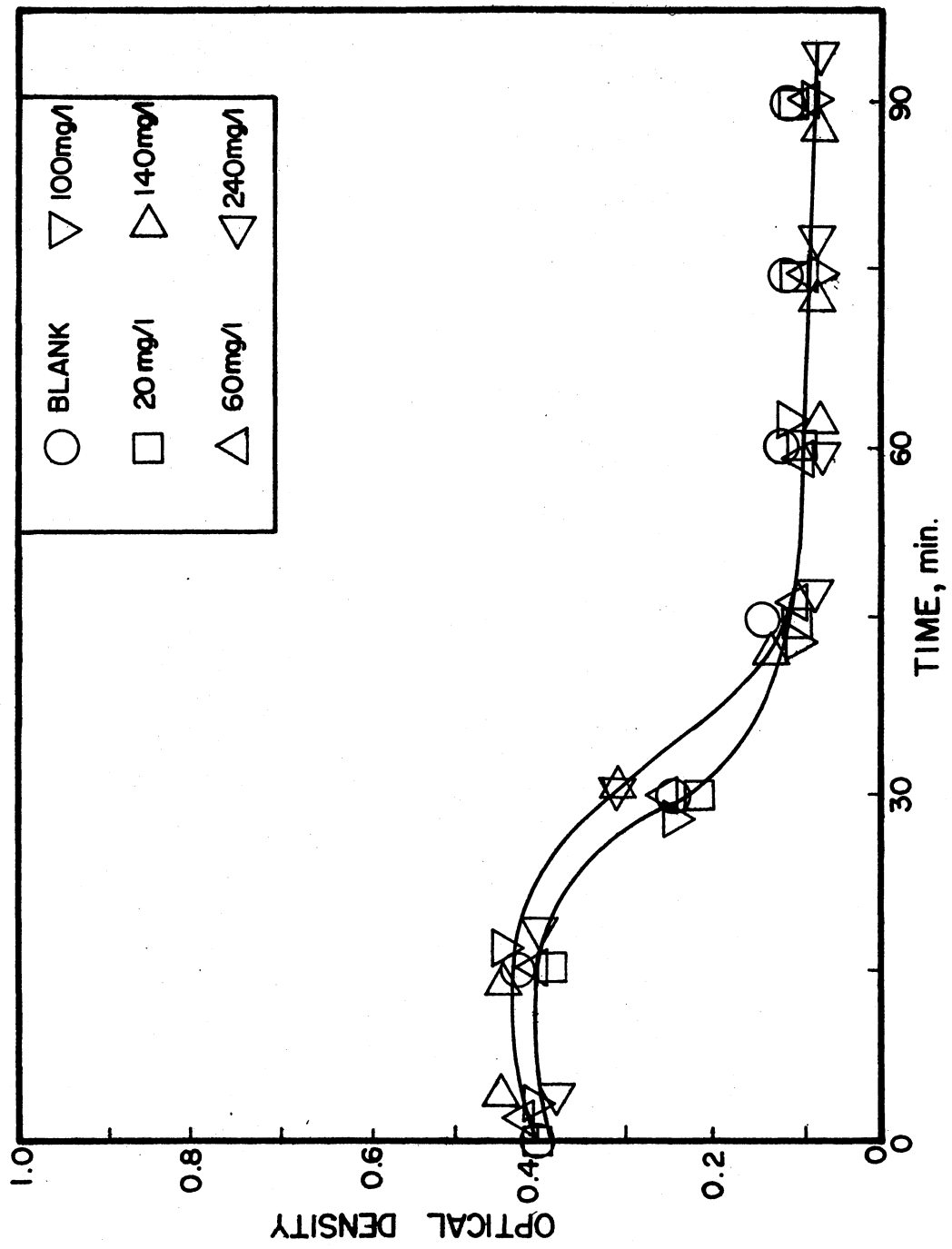

BLANK
20 mg/l
60mg/l
100mg/l
140mg/l
240mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME, min.

Figure 17. Settling Rates of Biological Solids From a Dilute Solution of Effluent From a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at $D = 0.021\ hr^{-1}$

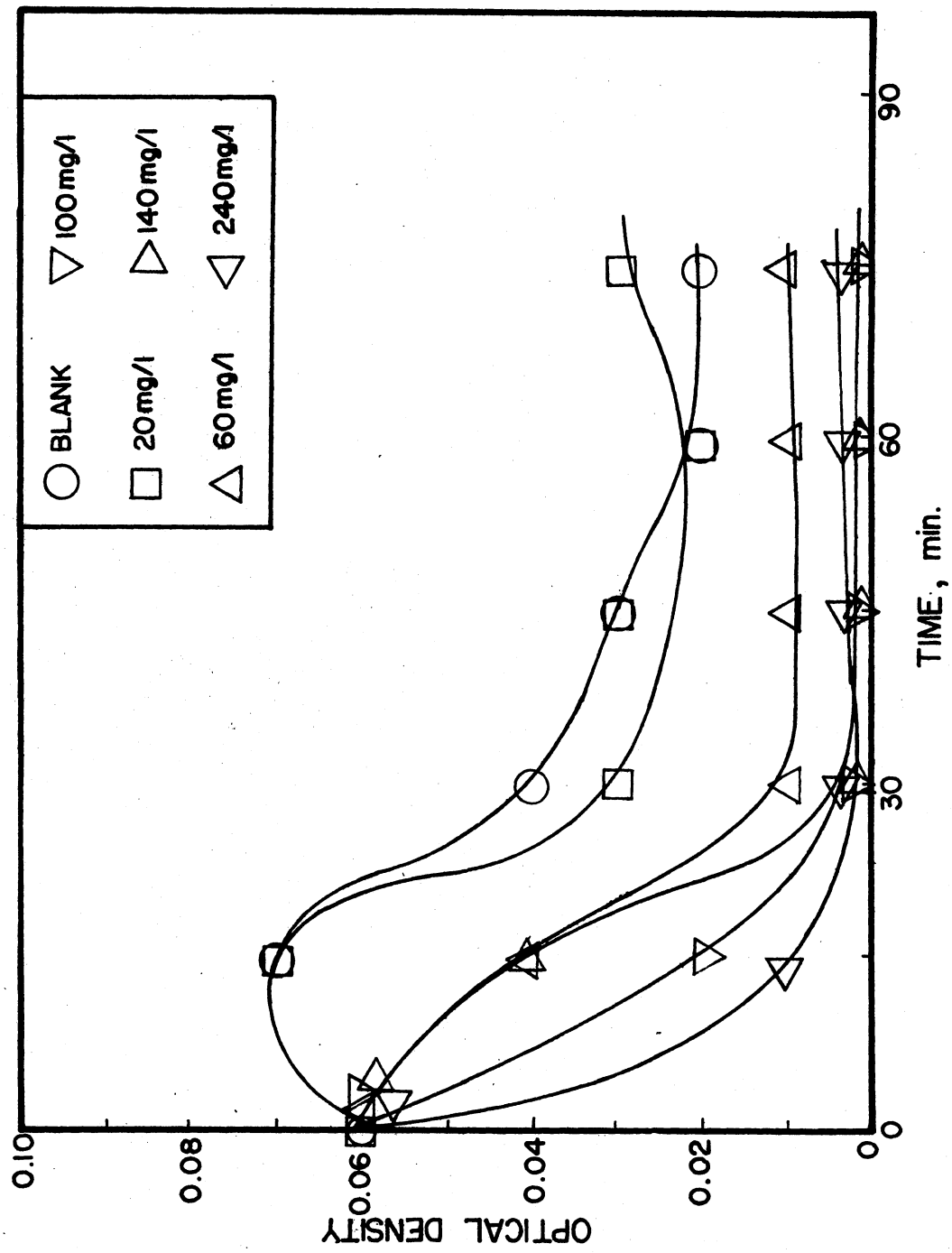
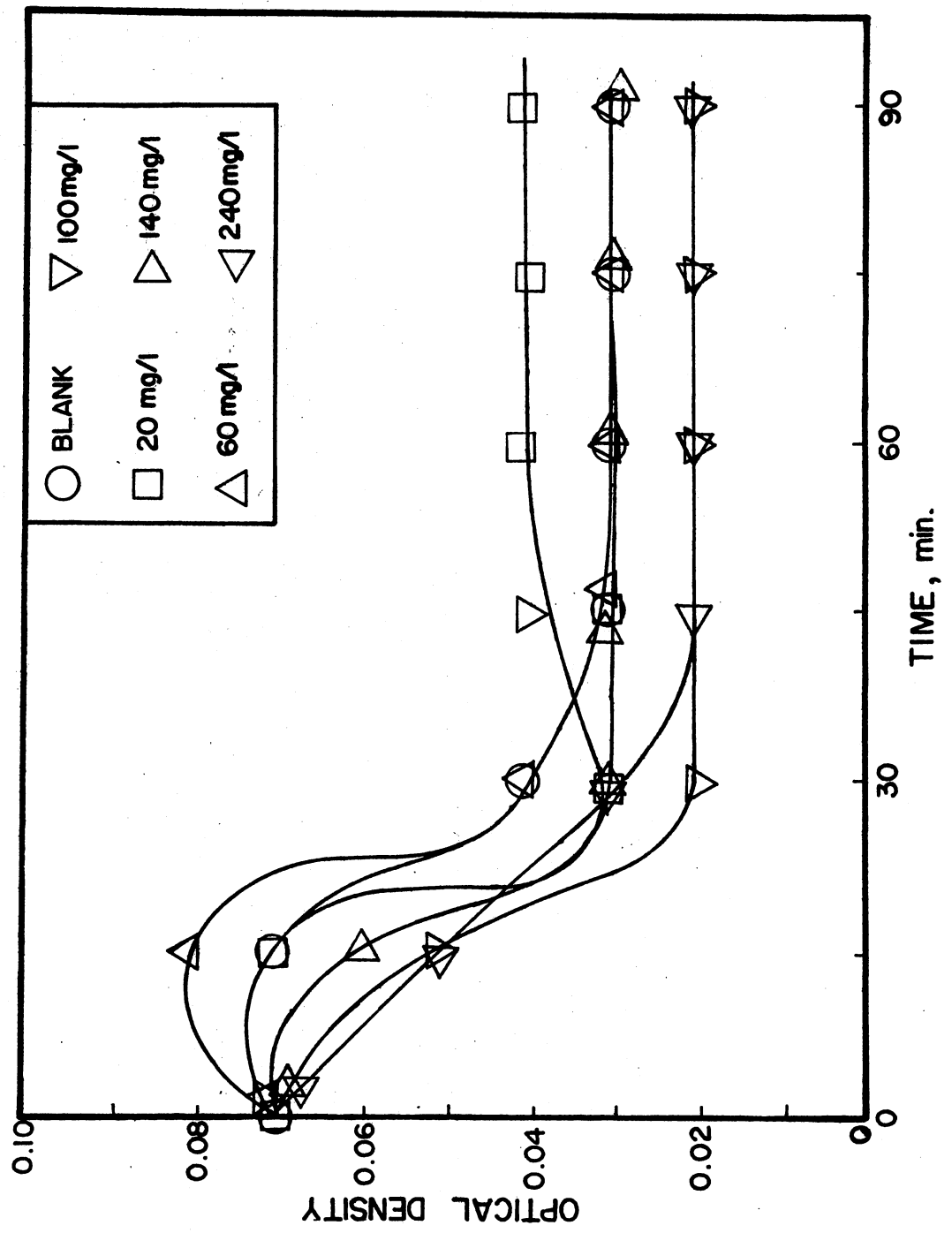

BLANK
100mg/l
20mg/l
140mg/l
60mg/l
240mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME, min.

Figure 18. Settling Rates of Biological Solids From a Dilute Solution of Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at D = 0.041 hr^{-1}


BLANK
20 mg/l
60 mg/l
100mg/l
140mg/l
240mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME, min.

presented in Figures 19 and 20.

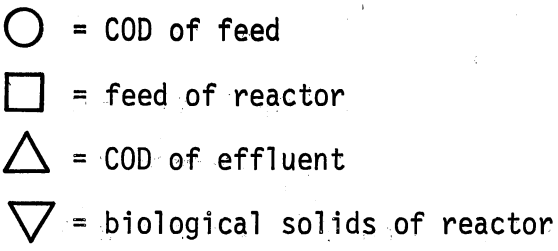
The COD removal efficiency for reactor A was 92.5 percent. The biological solids in the reactor A ranged from 450 mg/l to 620 mg/l, with an average of 526 mg/l. The average effluent COD was 71.2 mg/l, and the cell yield, Y, was 0.53 mg/l. The pH remained constant at 6.8, and the feed pH was 7.2.

The COD removal efficiency of reactor B was 93.3 percent. The biological solids ranged from 470 mg/l to 600 mg/l with an average of 533 mg/l. The average effluent COD was 76.2 mg/l, and the cell yield, Y, was 0.54 mg/l. The pH of the effluent and the feed remained constant at 6.8 and 7.2, respectively.

After the two reactors were maintained at steady-state for a few days, jar tests were run on the effluent of the reactors. In order to obtain enough reaction fluid for the jar tests, the effluent from both reactors was combined. The steady-state data indicated that both reactors were maintaining approximately the same COD removal and biological solids. Thus, the assumption was made that they were in fact two portions of the same cell system and could be validly mixed. Raw data for the jar test may be found in Appendix B.

The results of studies on effluent are shown in Figures 21 and 22, respectively. At this low specific growth rate, the blank systems settled very well. Thus, the effect of ferric chloride and alum was very small. Addition of 60 mg/l ferric chloride caused more rapid settling, but within a 30-minute period the blank exhibited approximately the same degree of clarification (see Figure 19). The settling efficiency of the blank for the ferric chloride addition was 83 percent, while for the blank in the alum test it was 82 percent. The efficiency of settling

Figure 19. Operational Data of Steady-State of Reactor A at $D = 0.014\ hr^{-1}$ (arrows indicate where jar tests were studied)



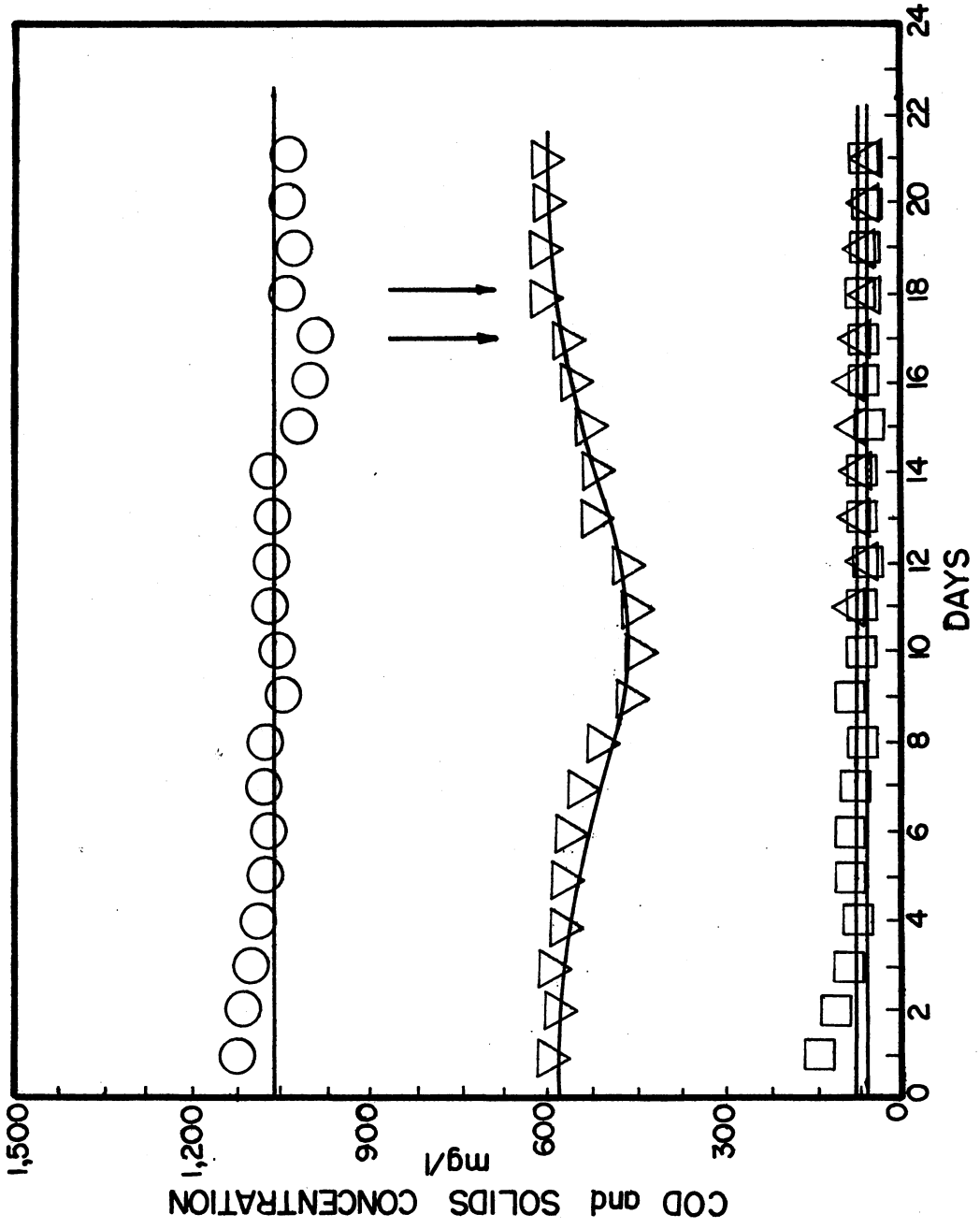
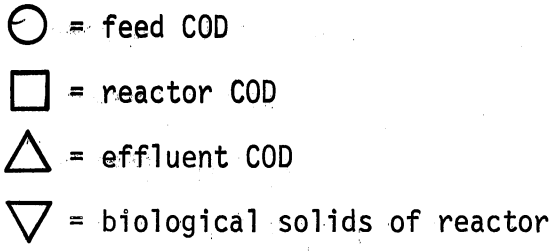

COD and SOLIDS CONCENTRATION
mg/l
1,500
1,200
900
600
300
0
0
2
4
6
8
10
12
14
16
18
20
22
24
DAYS

Figure 20. Operational Data of Steady-State for Reactor B at D = 0.014 hr^{-1} (arrows indicate where jar test studies were made)



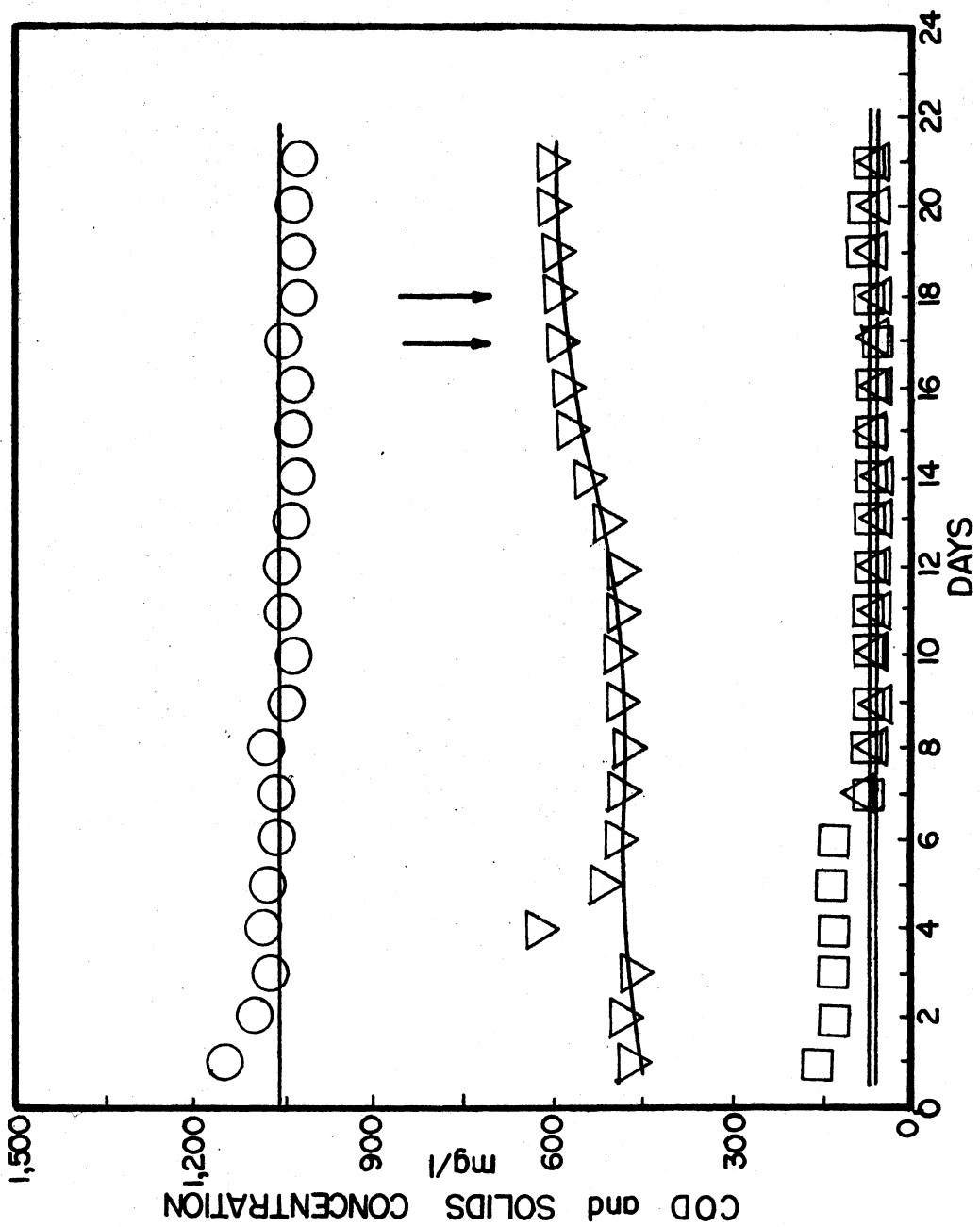

COD and SOLIDS CONCENTRATION
mg/l
1,500
1,200
900
600
300
0
0
2
4
6
8
10
12
14
16
18
20
22
24
DAYS

Figure 21. Settling Rates of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at 0.014 hr^{-1}

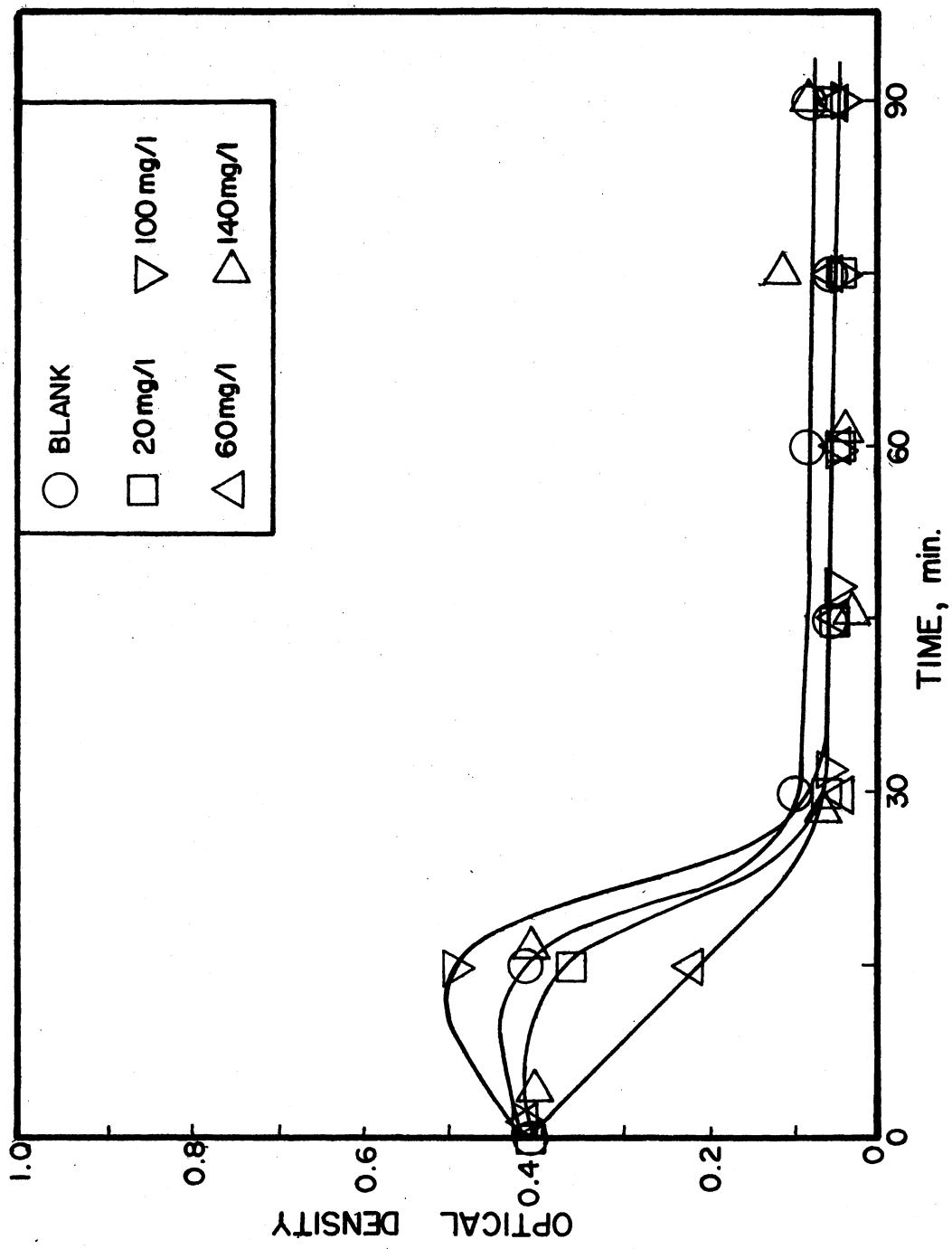
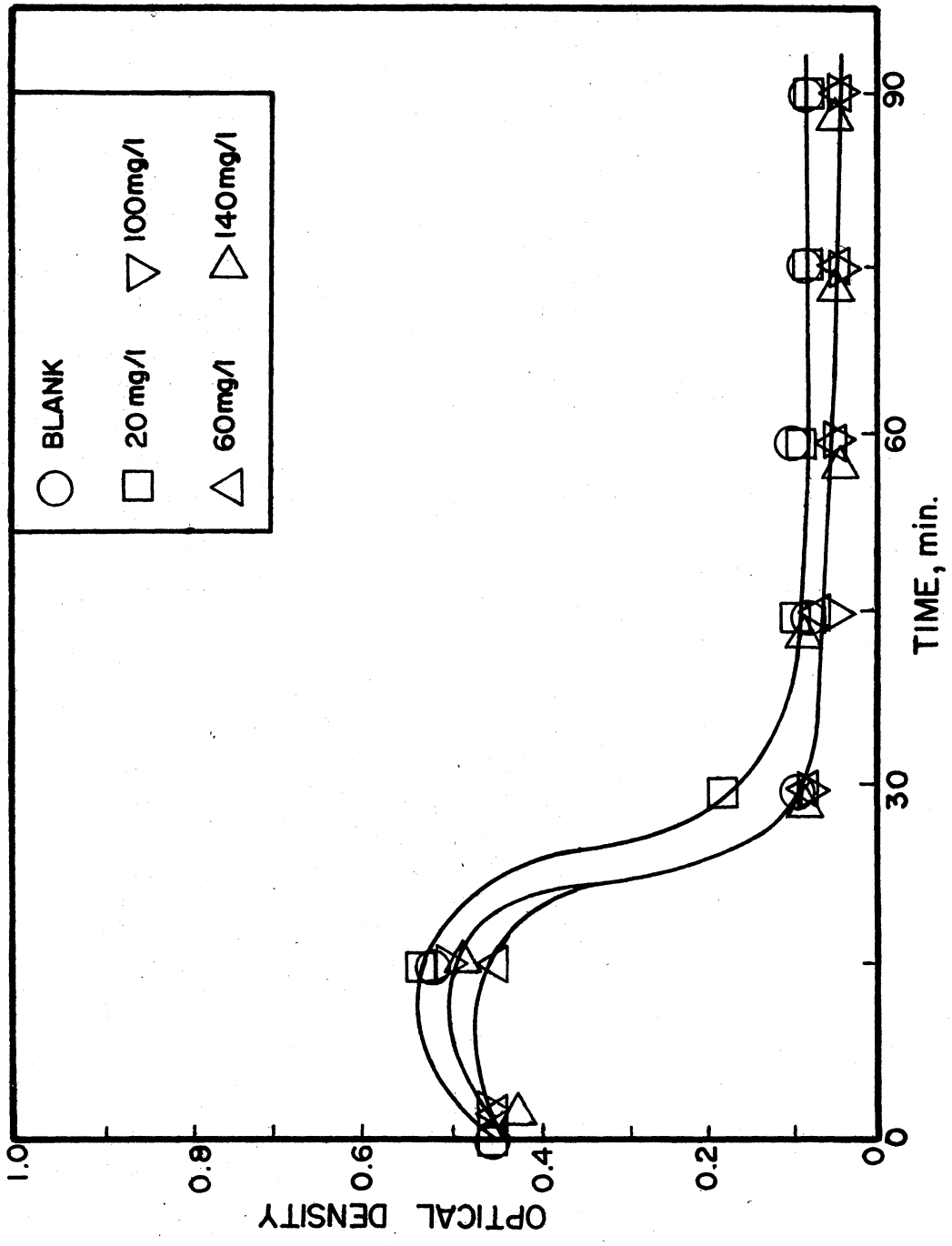

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME, min.

Figure 22. Settling Rates of Biological Solids From the Effluent of a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at D = 0.014 hr^{-1}


BLANK
20 mg/l
60mg/l
100mg/l
140mg/l
OPTICAL DENSITY
1.0
0.8
0.6
0.4
0.2
0
0
30
60
90
TIME, min.

for addition of ferric chloride was 93 percent, whereas for alum it was 91 percent.

The results for the addition of ferric chloride and alum to diluted effluent are found in Figures 23 and 24, respectively. The effect of either ferric chloride or alum was noticeable compared to the settling characteristics of the blanks. The efficiency for the blank in the ferric chloride jar test was 67 percent, while the efficiency for the blank in the alum jar test was 83 percent. The efficiency of settling with the addition of 60 and 100 mg/l of ferric chloride was 93 percent.

Since two reactors were now running in the steady-state at a detention of 72 hours, it seemed a good opportunity, before shutting down the operation, to perform some shock loading experiments. This was especially significant because of a long-standing interest in shock loads in the author's laboratory and because some of the other fellow student researchers were at present conducting shock loading experiments. Thus, the detention time was decreased to four hours in one reactor and two hours in the other reactor, and the response to these severe hydraulic shocks was measured. Since this line of experimentation was very different from the main subject of the present report, these results are not presented in this chapter but can be found in Appendix C. Briefly, both shocks led to nearly complete dilute-out of the cells. Had there been any significant amount of cells left after the shock, the author intended to perform jar tests to determine if the cells which had settled readily at $D = 0.014\ hr^{-1}$ would again exhibit the non-settling tendencies exhibited in the present study on the higher growth rate.

Figure 23. Settling Rates of Biological Solids From a Dilute Solution of Effluent From a Once-through Reactor for Jar Tests With Various Dosages of Ferric Chloride Added at $D = 0.014\ hr^{-1}$

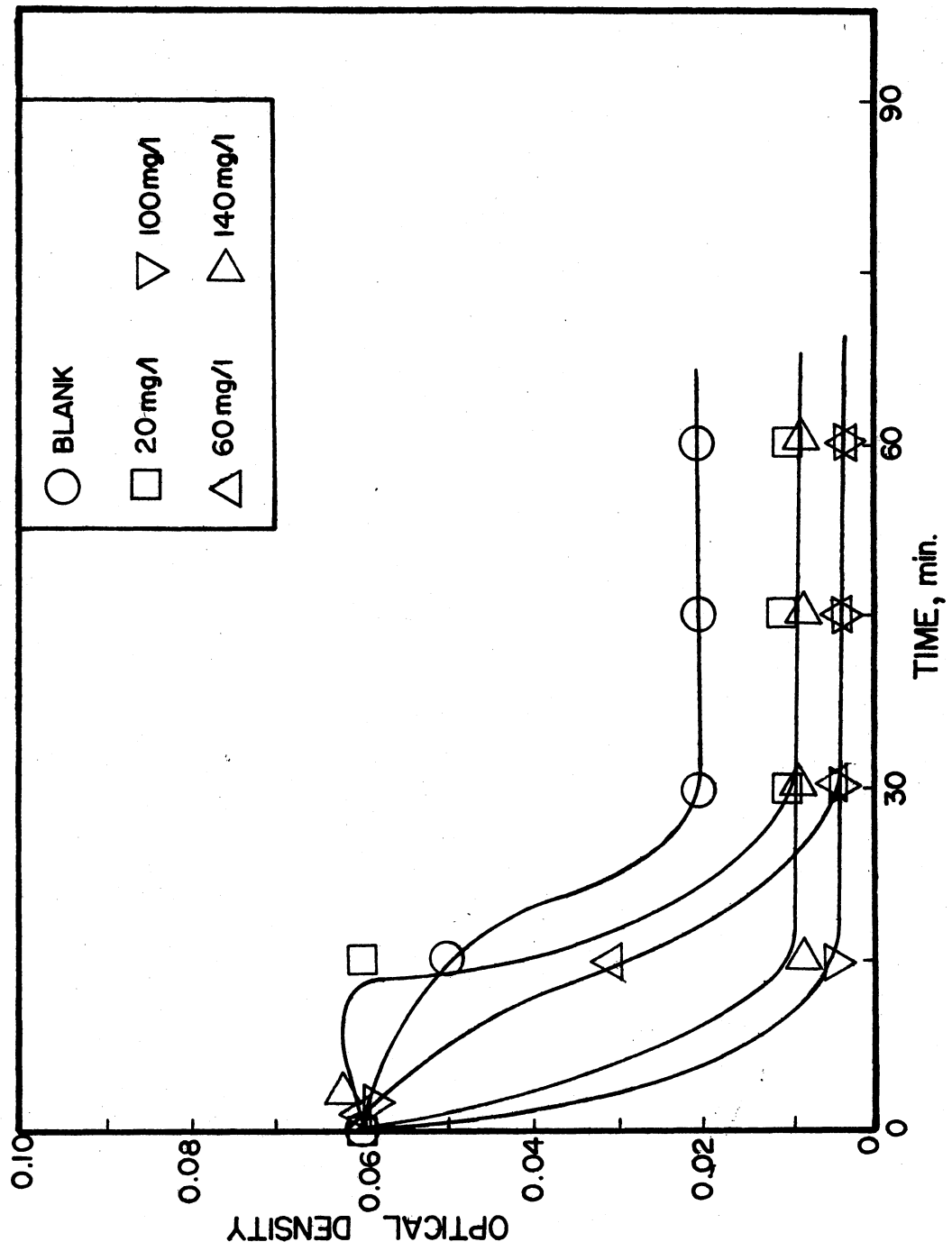
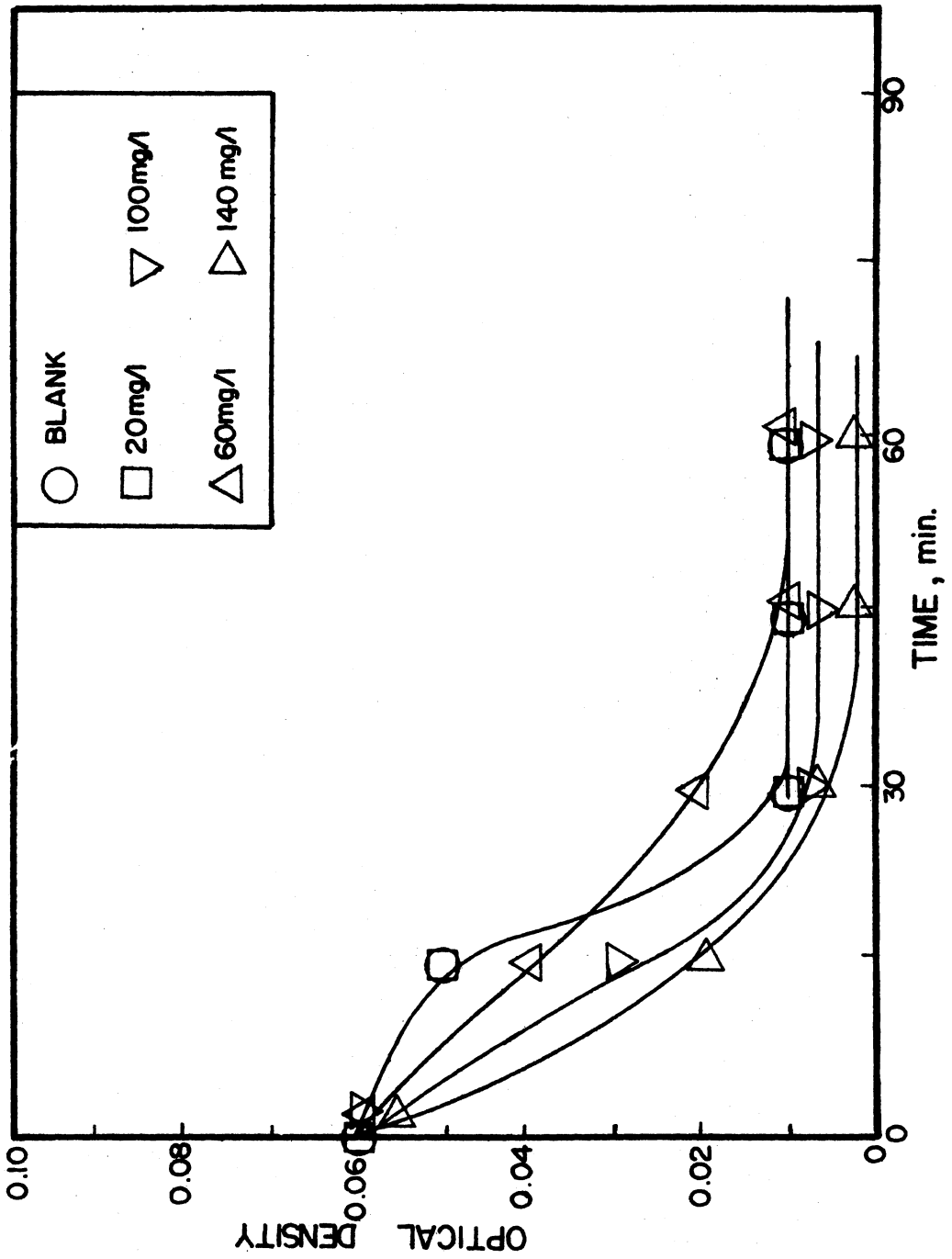

BLANK
20 mg/l
60 mg/l
100 mg/l
140 mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME, min.

Figure 24. Settling Rates of Biological Solids From a Dilute Solution of Effluent From a Once-through Reactor for Jar Tests With Various Dosages of Alum Added at D = 0.014 hr^{-1}


BLANK
20mg/l
60mg/l
100mg/l
140 mg/l
OPTICAL DENSITY
0.10
0.08
0.06
0.04
0.02
0
0
30
60
90
TIME, min.

CHAPTER V

DISCUSSION

In this investigation, auto-flocculation was compared to flocculation aided with chemicals, using various dosages of ferric chloride and alum for cells grown at four specific growth rates. Studies were made using cells in the effluents (high concentrations) and using 1/10 dilutions of the effluent (low concentrations).

A. Jar Tests on Effluent Cells (high concentration)

The addition of ferric chloride or alum to high concentrations of cells from the effluent of a once-through reactor was not effective in that the dosed systems exhibited the same lack of flocculation and settling as did the blank systems. Some concentrations of metal ions hindered settling and actually caused dispersion of the cells. For example, when the specific growth rate was $D = 0.042\ hr^{-1}$, Figures 10 and 11 showed that the addition of 60 mg/l of ferric chloride or alum interfered with the settling of the cells. In Figure 10, the addition of 100 mg/l of ferric chloride caused an apparent dispersion of the cells. In these experiments, the addition of 20 mg/l of either ferric chloride or alum had no effect on settling. However, the addition of 100 mg/l and 140 mg/l of ferric chloride or alum caused some formation of floc particles. Only at the 140 mg/l dosage were the floc particles

dense enough to settle to an extent which showed improvement in clarity compared to the results at zero dosage. The settling efficiency of the blank for the ferric chloride study (Figure 10) was 29 percent, while with a dosage of 140 mg/l of ferric chloride, the settling efficiency was 38 percent. The settling efficiency of the blank for the alum study (Figure 11) was 21 percent, while with the addition of 140 mg/l of alum, the settling efficiency was 38 percent.

The results of the studies at other growth rates yielded essentially the same results, i.e., little benefit from the addition of the chemical coagulants. However, it is important to note that settling efficiency of the blanks varied according to the specific growth rate. This aspect is discussed separately below. The addition of certain dosages of metal ions caused dispersion of the cells and other dosages caused some formation of floc particles, but the ions never affected the settling or flocculation of the cells to any significant extent. These results are shown in Table III with the settling efficiency of the blank and the dosage which created the optimal settling efficiency with ferric chloride and alum.

Michaels in 1954 showed that for a polymer to affect flocculation and settling, the polymer had to be absorbed on the surface of the colloid. When this occurred, flocculation could then take place by either reduction of electrokinetic potential of the colloid surface, or bridging of colloid particles (19)(20). In the present study, polymers were not employed but the same principles of charge reduction and agglomeration by bridging (colloid-metal-colloid) might be applicable, and since a rather high concentration of colloids, i.e., bacteria, was present, there may have been enough metal ions to reduce the Van der Waal

forces and other surface charges contributin to the electrokinetic potential. Also, the salts which were added to the feed for nutrients may have caused interference with the reduction of the charges on the bacterial surface. Bridging may not have occurred because of a lack of such sites on the cell, or it may have been that the surface charge was so large that the cells repelled each other.

TABLE III

COMPARISON OF EFFECT OF CHEMICAL DOSAGES ON CLARIFICATION OF CHEMOSTAT EFFLUENT

	Blank		Chemical			
Growth Rate (hr^{-1})	Ferric Chloride (percent)	Alum (percent)	Dosage (mg/l)	Ferric Chloride (percent)	Dosage (mg/l)	Alum (percent)
0.125	8	10	80, 120	13	140	28
0.042	29	71	140	38	140	38
0.021	81	70	240	83	240	83
0.014	87	84	120, 240	93	50, 100 120, 240	87

According to Stumm and O'Melia, there are three factors which influence destabilization of bacteria by Fe^{+3} and Al^{+3}; these are dosages of coagulant, pH, and colloid concentration (26). The pH of the effluent used for the jar tests were 6.8 and 6.9. These values are higher than the optimal pH of ferric chloride--4.5 to 5.5--and for

alum, 5 to 6 (23). When adding ferric chloride or alum to water, the pH will normally drop according to the dosage or concentration of the alum or ferric chloride. For example, when 150 mg/l of alum (15 ml) were added to 235 ml of tap water, the pH dropped from 6.2 to 5.8. However, the system under study was highly buffered, and the pH could be expected to remain at 5.8-6.9. (pH was not checked after addition of Fe^{+3} or Al^{+3} salts.)

B. Jar Tests on Dilute Effluent Cells (low concentration)

The addition of ferric chloride or alum to a dilute solution of effluent from a once-through reactor was found to greatly enhance settling of bacteria. These coagulants were effective only at concentrations of greater than 50 mg/l. Concentrations less than 50 mg/l of coagulant caused a dispersion of cells.

When making up the dilute solution of effluent, distilled water was added to the effluent of the reactor. This caused a dilution of the salts, lowering the electrolyte concentration. This, coupled with the lower bacteria concentration, apparently created conditions favorable to chemical enhancement of flocculation and settling of the diluted cells. The settling efficiency of the blanks and dosages which caused the greatest flocculation and settling are shown in Table IV.

Since the colloid concentration was low, there should be no intermediate zone between zones of flocculation (zone II in Fibure 2b). It seems that flocculation and settling occurred in the "sweep floc" zone, zone IV in Figure 2a (21)(24).

TABLE IV

COMPARISON OF EFFECT OF CHEMICAL DOSAGES ON CLARIFICATION OF DILUTE CHEMOSTAT EFFLUENT

	Blank		Chemical			
Growth Rate (hr^{-1})	Ferric Chloride (percent)	Alum (percent)	Dosage (mg/l)	Ferric Chloride (percent)	Dosage (mg/l)	Alum (percent)
0.125	10	25	140	96	100	90
0.041	10	49	80, 100 120, 140	98	80, 100	98
0.021	66	57	80, 100 120, 140	98	50, 100 120, 240	71
0.014	66	83	60, 80 100, 120	93	140	93

C. Auto-flocculation at Different Specific Growth Rates

In Table V it is shown that the settling tendencies of the cells are increased as the specific growth rate decreases, or as the cell "age" increases. The settling efficiency for D = 0.125 hr^{-1} was 28 percent, while the settling efficiency for D = 0.014 hr^{-1} was 83 percent.

Butterfield proposed that flocculation was caused by the capsule of the cell, while Heukelekian and Littman obtained the same results as did Butterfield, but they also proposed that bacteria flocculate when there is a shortage of substrate available (9)(10). McKinney noticed that the cells flocculated when they were lacking motility and activity.

All of the theories of flocculation indicate that the amount of

substrate could in some way play a major role in determining whether flocculation occurs. On the other hand, the amount of substrate in solution is also determined by the growth rate or the cell age of the system.

TABLE V

COMPARISON OF EFFECT OF SPECIFIC GROWTH RATE ON AUTO-FLOCCULATION OF CHEMOSTAT EFFLUENT

Growth Rate (hr^{-1})	Blank for Ferric Chloride Jar Test (percent)	Blank for Alum Jar Test (percent)
0.125	8	13
0.041	79	38
0.021	81	87
0.014	87	97

It is known from observations made in the author's laboratory during growth studies over a long period of years that cell suspensions go from dispersed to flocculent phases as cell age increases, i.e., growth rate decreases (29).

Liao (30) in his batch studies with various pure cultures of floc-forming bacteria, concluded that species possessing high μ_{max} values flocked somewhat better than those with a lower maximum growth rate capability. Since his studies were in batch systems, the high μ_{max}

might be interpreted as a capability for removing more substrate in a given amount of time and thus these cells flocked better because of lowered substrate concentration. On the other hand, if the cells grew faster, they would be proportionately older at a given harvesting time. It is, of course, difficult to correlate findings in continuous flow and batch studies, but there does not appear to be any inconsistency between the present findings in continuous systems and similar observations by Gaudy (29) and the findings in batch pure culture work by Liao.

Also, the correlation between low growth rate and settling efficiency of the cells can be observed in current studies in these laboratories. Studies currently under way by Mr. N. Bartle as well as Mr. Randy Bradley, show an apparent correlation between μ and settling velocity. Settling velocity may possibly be associated with tightness of floc. In addition, studies have been made by Mr. Saleh showing that at low growth rates, excellent clarity of effluent is maintained even during shock load conditions wherein substrate concentration in the reactor (and effluent) are significantly raised above that during steady operation. Thus, it would appear that low growth rate rather than absence of substrate or low substrate concentrations as per the finding of Littman and Heukelekian (10) plays a determining role in effectuating flocculation (31)(32).

Also, another possibility to explain the apparent effect of cell age is that as the cells become older, a significant portion of the population undergoes lysis. The macromolecules of the cells may function as natural polymeric polyelectrolytes, thus enhancing cell flocculation. In fact, Pavoni, Tenney, and Echelberger (33) have

suggested that exocellular bacterial polymers such as polysaccharide protein, RNA and DNA, enhance bacterial flocculation. They concluded that this mechanism occurred during the endogenous phase. While this interpretation may be debated, it does seem that release of such polymers may be expected during very slow growth under the "starvation" conditions which exist in activated sludge processes. The conditions for flocculation are not well defined and there is much need for continued study of this complicated process. The fact that addition of bacterial polymer (e.g., polysaccharides) will not unequivocally cause flocculation was demonstrated in the author's laboratory in an experiment in which five preparations of bacterial polysaccharide harvested from pure cultures of sewage origin were added to dispersed suspensions of young heterogeneous populations (34). The polysaccharides employed were those used as substrate by Obayashi and Gaudy (35). They showed that microbial extracellular polysaccharide could be readily used by an acclimated heterogeneous population. Thus, if such polymers aided flocculation, they could in time also serve as a source of substrate.

CHAPTER VI

CONCLUSIONS

The operation of a once-through chemostat using the effluent for jar tests with the addition of either ferric chloride or alum has led to the following conclusions:

1. Some dosages of ferric chloride and alum caused a dispersion of cells and interfered with settling.

2. Flocculation of bacteria from the effluent was not enhanced by addition of ferric chloride or alum.

3. Flocculation of a dilute solution of effluent was enhanced by the addition of ferric chloride or alum.

4. Specific growth rate appears to be a major factor in flocculation and settling, with lower specific growth rate yielding more flocculant cell suspensions.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

Based on the findings of this study, the following suggestions are presented for future studies on the factors affecting flocculation:

1. Study the effect of ferric chloride or alum on flocculation of both effluent and dilute effluent, with pH adjustment.

2. Study the differences of flocculation characteristics of various pure cultures of bacteria with different morphological characteristics, e.g., capsulated versus non-capsulated bacteria, motile versus non-motile bacteria.

3. Study the effect of specific growth rate on flocculation over a wide range of growth rates.

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APPENDIX A

DATA FOR STEADY-STATE AT DIFFERENT GROWTH RATES

TABLE VI

DATA FOR STEADY-STATE
($D = 0.125\ hr^{-1}$)

Date (1974)	Day	Feed COD	Tank COD	Effluent COD	Tank Solids	Effluent Solids	pH
8-26	1	1056	124	122	280	272	6.8
8-27	2	944	122	118	412	392	
8-28	3	1048	112	106	416	400	
8-30	5	1008	114	106	432	416	
8-21	6	1056	114	112	440	424	
9- 1	7	1008	108	104	480	460	6.8
9- 2	8	944	112	107	408	452	
9- 3	9	1088	110	-	464	-	
9- 4	10	1056	-	106	-	486	6.8
9- 5	11	1040	108	104	468	460	
9- 6	12	1080	106	102	464	456	
9- 7	13	944	102	100	496	456	6.8
9- 8	14	1056	102	101	456	440	

$$Y = \frac{452}{1024 - 113}$$

$$Y = .49$$

TABLE VII

DATA FOR STEADY-STATE
($D = 0.042\ hr^{-1}$)

Date (1974)	Day	Feed COD	Tank COD	Effluent COD	Tank Solids	Effluent Solids	pH
9-10	1	1152	117	113	180	166	
9-11	2	992	113	109	420	406	6.8
9-12	3	1032	98	105	420	413	
9-13	4	1100	105	103	440	426	
9-14	5	1048	97	94	446	426	6.8
9-15	6	1032	81	74	460	440	
9-16	7	1056	78	74	466	440	
9-17	8	1032	64	50	486	460	6.8
9-18	9	1056	61	54	486	473	
9-19	10	1032	77	57	493	480	
9-20	11	1040	64	57	486	480	6.7
	Average:	1062	86	81	434	422	

$$Y = \frac{422}{1052 - 81}$$

$$Y = 0.43$$

TABLE VIII

DATA FOR STEADY-STATE
($D = 0.021\ hr^{-1}$)

Date (1974)	Day	Feed COD	Tank COD	Effluent COD	Tank Solids	Effluent Solids	pH
10- 6	1	1048	-	-	28	-	
10- 7	2	1064	80	-	204	-	
10- 8	3	1096	77	96	256	259	6.8
10- 9	4	1048	86	80	264	212	6.8
10-10	5	1048	106	138	252	224	6.9
10-11	6	1064	90	138	264	240	7.0
10-12	7	1128	110	135	375	367	6.9
10-13	8	1020	109	138	392	360	6.9
10-14	9	1070	106	124	376	340	
10-15	10	1096	88	106	412	348	6.9
10-16	11	1096	83	89	372	352	
10-17	12	1096	80	65	386	352	7.0
10-18	13	1032	80	74	404	372	
10-19	14	986	84	70	376	364	6.8
10-20	15	1015	86	72	384	356	
10-21	16	1025	83	80	388	348	6.9
	Average:	1058	89.8	99.6	341	321	

$$Y = \frac{321}{1058 - 99.6}$$

$$Y = 0.36$$

TABLE IX

DATA FOR STEADY-STATE (Tank #1)
($D = 0.014\ hr^{-1}$)

Date (1974)	Day	Feed COD	Tank COD	Effluent COD	Tank Solids	Effluent Solids	pH
11-22	1	1136	147	-	596	-	6.8
11-23	2	1128	104	-	592	-	
11-24	3	1104	87	-	590	-	
11-25	4	1096	80	-	576	-	6.8
11-26	5	1072	91	-	570	-	
11-27	6	1072	108	-	568	-	
11-28	7	1080	105	-	550	-	
11-29	8	1080	84	-	500	-	6.9
11-30	9	1040	108	-	480	-	
12- 1	10	1064	85	-	450	-	
12- 2	11	1080	72	88	460	400	6.9
12-3	12	1072	71	67	458	440	
12- 4	13	1072	67	75	540	470	6.7
12- 5	14	1080	68	76	540	480	
12- 6	15	1008	60	79	560	620	6.7
12- 7	16	1000	64	71	583	530	
12- 8	17	992	67	69	591	580	
12- 9	18	1048	64	66	620	584	6.8
12-10	19	1032	60	64	599	582	
12-11	20	1040	64	65	604	590	6.8
12-12	21	1048	63	64	616	594	
	Average:	1060.4	78.6	71.2	552	533	

$$Y = \frac{533}{1060.4 - 71.2} \qquad Y = 0.54$$

TABLE X

DATA FOR STEADY-STATE (Tank #2)
($D = 0.014\ hr^{-1}$)

Date (1974)	Day	Feed COD	Tank COD	Effluent (COD)	Tank Solids	Effluent Solids	pH
11-22	1	1160	160	-	496	-	6.8
11-23	2	1104	93	-	504	-	
11-24	3	1088	91	-	480	-	6.8
11-25	4	1110	107	-	628	-	
11-26	5	1104	-	-	528	-	
11-27	6	1080	96	-	500	-	6.8
11-28	7	1072	67	71	480		
11-29	8	1110	63	63	476	-	6.8
11-30	9	1056	64	59	483	-	
12- 1	10	1000	64	68	480	484	6.8
12- 2	11	1080	71	70	475	460	
12- 3	12	1072	61	71	470	465	
12- 4	13	1040	68	63	513	490	6.9
12- 5	14	1016	68	63	540	500	
12- 6	15	992	64	71	580	527	6.8
12- 7	16	1000	63	68	588	570	
12- 8	17	1048	64	68	595	582	6.9
12- 9	18	1008	64	64	599	588	
12-10	19	1032	60	67	594	575	6.8
12-11	20	1040	75	67	592	580	
12-12	21	1008	60	79	593	585	
	Average:	1058	70.4	67.4	533	533	

$$Y = \frac{533}{1058 - 67.4} \qquad Y = 0.54$$

APPENDIX B

RESULTS OF JAR TESTS AT DIFFERENT GROWTH RATES

TABLE XI

RAW DATA FOR JAR TEST WITH FERRIC CHLORIDE ADDED
D = 0.125 hr^{-1}

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.4	.4	.4	.4	.4	.4	.44	.44	.44	.44	.44	.44
15	.44	.44	.46	.41	.42	.41	.51	.49	.43	.44	.39	.40
30	.38	.39	.40	.39	.39	.39	.48	.38	.37	.36	.36	.35
45	.38	.39	.40	.39	.39	.39	.44	.37	.37	.36	.35	.34
60	.37	.38	.39	.38	.39	.38	.38	.39	.38	.36	.36	.35
75	.37	.38	.40	.39	.39	.38	.38	.38	.38	.36	.36	.35
90	.36	.37	.40	.39	.39	.39	.38	.38	.38	.36	.36	.35

TABLE XII

RAW DATA FOR JAR TEST WITH ALUM ADDED
$D = 0.125\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.39	.39	.39	.39	.39	.39	.39	.39	.39	.39	.39	.39
15	.43	.42	.40	.38	.38	.37	.40	.33	.31	.30	.33	.3
30	.38	.37	.36	.37	.35	.34	.36	.32	.31	.30	.31	.3
45	.38	.36	.36	.36	.35	.34	.36	.31	.30	.30	.3	.3
60	.37	.36	.35	.36	.33	.34	.36	.30	.29	.30	.3	.3
75	.38	.35	.34	.34	.35	.33	.35	.30	.29	.29	.29	
90	.37	.35	.34	.36	.35	.34	.34	.30	.29	.29	.29	

TABLE XIII

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH FERRIC CHLORIDE ADDED
D = 0.125 hr^{-1}

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06
15	.07	.07	.07	.075	.085	.082	.05	.019	.04	.08	.08	.08
30	.07	.065	.06	.062	.058	.06	.05	.015	.03	.02	.004	.002
45	.065	.065	.06	.06	.055	.055	.048	.01	.02	.01	.004	.02
60	.005	.058	.048	.05	.049	.04	.048	.01	.01	.01	.003	.02
75	.05	.052	.049	.05	.043	.04	.047	.01	.01	.005	.003	.02
90	.05	.05	.049	.05	.042	.04	.047	.01	.01	.005		

TABLE XIV

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH ALUM ADDED
$D = 0.125\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06
15	.07	.07	.061	.07	.07	.058	.065	.07	.051	.05	.05	.06
30	.055	.055	.06	.058	.06	.048	.055	.033	.005	.013	.008	.01
45	.50	.051	.049	.05	.05	.040	.051	.028	.005	.002	.005	.01
60	.049	.048	.047	.05	.05	.035	.05	.03	.005	.002	.01	.01
75	.043	.042	.041	.048	.05	.038	.05	.03	.0048	.002	.01	.01
90	.043	.042	.041	.049	.05	.038	.05	.03	.005	.002	.01	.01

TABLE XV

RAW DATA FOR JAR TEST WITH FERRIC CHLORIDE ADDED
$D = 0.042\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.34	.34	.34	.34	.34	.34	.34	.34	.34	.34	.34	.34
15	.32	.30	.30	.32	.32	.32	.32	.44	.45	.45	.46	.47
30	.28	.26	.26	.26	.27	.28	.25	.31	.32	.32	.30	.28
45	.24	.25	.26	.25	.26	.26	.25	.30	.30	.28	.27	.24
60	.24	.25	.25	.26	.26	.26	.24	.29	.28	.28	.25	.21
75	.24	.24	.25	.25	.26	.26	.23	.27	.28	.25	.25	.21
90	.24	.24	.25	.25	.26	.26	.24	.27	.28	.25	.25	.21

TABLE XVI

RAW DATA FOR JAR TEST WITH ALUM ADDED
$D = 0.042\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.34	.34	.34	.34	.34	.34	.34	.34	.34	.34	.34	.34
15	.37	.36	.31	.36	.35	.38	.44	.44	.46	.44	.46	.48
30	.28	.28	.28	.28	.28	.29	.29	.31	.29	.27	.28	
45	.28	.28	.27	.27	.27	.28	.29	.32	.29	.26	.27	.24
60	.27	.28	.26	.27	.27	.28	.29	.29	.29	.25	.27	.23
75	.28	.27	.27	.27	.27	.28	.29	.28	.29	.25	.26	.22
90	.27	.27	.26	.26	.26	.27	.28	.28	.29	.25	.26	.22

TABLE XVII

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH FERRIC CHLORIDE ADDED
$D = 0.042\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05
15	.05	.03	.035	.05	.04	.035	.045	.008	.002	.07	.08	.085
25	.025	.025	.028	.025	.023	.025	.03	.005	.01	.01	.01	.01
35	.025	.02	.025	.023	.023	.028	.025	.004				
45	.025	.02	.025	.03	.023	.028	.025	.01				
60	.02	.02	.022	.023	.03	.03	.024					
75	.02	.023	.025	.021	.028	.03	.02					

TABLE XVIII

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH ALUM ADDED
$D = 0.042\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	Blank	60mg	80mg	100mg	120mg	140mg
0	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05
15	.04	.045	.06	.06	.06	.06	.04	.015	.025	.045	.05	.055
25	.03	.03	.05	.038	.045	.045	.03	.01	.005	.001	.025	
35	.03	.03	.048	.035	.04	.04	.028	.01	.001	.001	.02	.02
45	.028	.03	.048	.035	.04	.039	.03	.01	.001	.001	.02	.02
60	.03	.028	.035	.055	.04	.038	.03	.01	.001	.001	.02	.02
75	.03	.03	.032	.04	.04	.035						

TABLE XIX

RAW DATA FOR JAR TEST WITH FERRIC CHLORIDE ADDED
$D = 0.021\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.64	.64	.64	.64	.64	.64	.64	.64	.64	.64	.64	.64
15	.6	.6	.61	.59	.62	.62	.59	.59	.57	.64	.6	.62
30	.41	.26	.24	.34	.29	.33	.31	.18	.28	.22	.19	.22
45	.17	.18	.13	.13	.13	.12	.11	.1	.09	.16	.1	.1
60	.15	.14	.14	.13	.14	.13	.1	.1	.1	.28	.09	.08
75	.12	.12	.11	.11	.12	.12	.1	.1	.09	.1	.1	.09
90	.12	.12	.12	.11	.11	.11	.1	.1	.09	.1	.1	.09

TABLE XX

RAW DATA FOR JAR TEST WITH ALUM ADDED
$D = 0.021\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4	.4
15	.43	.42	.38	.4	.4	.4	.4	.4	.42	.4	.43	.4
30	.24	.2	.21	.19	.13	.21	.25	.27	.23	.29	.31	.3
45	.14	.11	.11	.11	.12	.11	.10	.09	.11	.10	.10	.09
60	.12	.11	.11	.10	.11	.10	.10	.09	.10	.09	.09	.08
75	.13	.11	.11	.11	.11	.10	.1	.09	.09	.09	.09	.07
90	.12	.11	.11	.11	.11	.1	.1	.09	.09	.09	.09	.07

TABLE XXI

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH FERRIC CHLORIDE ADDED
$D = 0.021\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06	.06
15	.07	.07	.07	.09	.08	.08	.04	.01	.02	.03	.04	.12
30	.04	.03	.03	.04	.05	.04	.01	0	0	.01	0	.1
45	.03	.02	.03	.03	.03	.03	.01	0	0	0	0	.1
60	.02	.02	.02	.03	.03	.03	.01	0	0	0	0	.09
75	.02	.02	.03	.03	.03	.03						

TABLE XXII

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH ALUM ADDED
$D = 0.021\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
15	.07	.07	.07	.07	.07	.07	.08	.04	.05	.07	.06	.05
30	.04	.04	.03	.04	.04	.03	.04	.02	.02	.02	.03	.03
45	.03	.03	.03	.04	.04	.03	.03	.02	.04	.02	.03	.02
60	.03	.03	.04	.03	.04	.03	.03	.02	.02	.02	.03	.02
75	.03	.04	.04	.04	.04	.03	.03	.02	.02	.02	.03	.02
90	.03	.04	.04	.04	.04	.03	.03	.02	.02	.02	.03	.02

TABLE XXIII

RAW DATA FOR JAR TEST WITH FERRIC CHLORIDE ADDED
$D = 0.014\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.39											
15	.42	.39	.36	.34	.38	.35	.23	.21	.48	.31	.39	.23
30	.09	.07	.06	.06	.07	.09	.05	.06	.06	.05	.06	.06
45	.06	.07	.05	.06	.06	.04	.05	.05	.06	.04	.04	.05
60	.07	.06	.05	.05	.07	.05	.05	.05	.05	.04	.04	.03
75	.06	.06	.05	.05	.06	.05	.05	.05	.05	.04	.12	.03
90	.07	.07	.05	.05	.06	.04	.05	.05	.04	.04	.07	.03

TABLE XXIV

RAW DATA FOR JAR TEST WITH ALUM ADDED
$D = 0.014\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.44											
15	.52	.51	.53	.55	.53	.5	.44	.42	.50	.57	.48	.46
30	.10	.12	.17	.13	.15	.25	.09	.09	.08	.13	.09	.14
45	.07	.08	.078	.078	.07	.07	.07	.05	.05	.04	.05	.03
60	.09	.07	.07	.07	.07	.07	.05	.05	.05	.05	.05	.04
75	.08	.07	.07	.07	.07	.07	.05			.04		.04
90	.07	.07	.07	.07	.068	.06						

TABLE XXV

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH FERRIC CHLORIDE ADDED
$D = 0.014\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.06											
15	.05	.06	.05	.05	.06	.08	.03	.004	.004	.008	.02	.16
30	.02	.01	.01	.03	.02	.04	.004	.004		.008	.008	.14
45	.02	.01	.01	.02	.02	.03	.004	.008		.004	.008	.14
60	.02			.02								
75												
90												

TABLE XXVI

RAW DATA FOR JAR TEST ON DILUTE EFFLUENT WITH ALUM ADDED
$D = 0.014\ hr^{-1}$

CONC / TIME	Blank	10mg	20mg	30mg	40mg	50mg	60mg	80mg	100mg	120mg	140mg	240mg
0	.06											
15	.05	.04	.05	.05	.04	.05	.04	.04	.03	.02	.02	.03
30	.01	.01	.01	.01	.0	.01	.02	.008	.008	.01	.008	.01
45	.01	.01	.01	.01	.01	.01	.01	.008	.008	.01	.004	.008
60	.01	.01	.01	.01	.01	.01	.01					
75												
90												

APPENDIX C

RESULTS OF SHOCK LOAD STUDIES WITH D = 0.014 hr^{-1} TO 0.25 hr^{-1} AND D = 0.014 hr^{-1} TO 0.5 hr^{-1}

Shock Loads

Shock loads to biological waste treatment plants can be defined as any sudden change in the environmental or operational condition under which the microorganisms responsible for purification exist in the system. Since shock loads have caused problems at treatment plants for many years, much research has been undertaken on shock loads.

Shock loads can be classified into several types (36):

Quantitative Shock Loads

This type of shock load involves either an increase or decrease in the concentration of organic carbon source in the influent, while the flow, F, remains constant. This type of shock load occurs in the treatment plant every day.

Qualitative Shock Loads

This type of shock load involves a change in the composition of the carbon source in the influent, e.g., glucose to acetic acid. This type of shock load can take place whenever an industry changes processes of manufacturing or changes to a different product line, etc.

Toxic Shock Loads

This type of shock load i nvolves the addition of chemical compounds to the influent which could slow down or stop the metabolism of the cell. These chemical compounds could be heavy metals, organic compounds, e.g., phenol, and inorganic compounds, e.g., cyanide. This type of shock load can arise from industrial activity.

pH Shock Loads

This type of shock load occurs when the pH of the reaction liquor changes. This may result from industrial plants causing either acid or basic conditions in the wastewater. A sudden change in pH can be very harmful from a biochemical point of view.

Temperature or Thermal Shock Loads

This type of shock load occurs when there is a change in the temperature of the reaction liquor.

A successful response to the various shock loads will be dependent on several factors (37):

1) severity of the shock
2) rapidity of the shock
3) detention time for the treatment system
4) physiological characteristics of the sludge
5) biomass concentration in the system
6) dissolved oxygen concentration in the aerator
7) number of different species present in the system and the versatility of those species predominating at the time of the shock.

Hydraulic Shock Loads

Change in D from 0.014 hr^{-1} to 0.25 hr^{-1}: The reactor was operting at steady-state before the hydraulic shock load was applied (Figure 25). The flow rate, F, was increased so that D = 0.25 hr^{-1}; the plan was to take samples until the reactor had diluted out or accepted the shock load and assumed a new steady-state level.

The results of the shock load are shown in Figure 25. The biological solids did not dilute out as fast as the calculated dilution of the biological solids using equation (1), (38).

$C = C_o e^{-t/t_d}$

C = concentration at any time

C_o = initial concentration of reactor

t = time

$t_d = V/Q$

The COD of the reactor also did not dilute in as fast as the calculated values using equation (2)

$C = C_o(1-e^{-t/t_d})$

C = concentration at any time

C_o = concentration of influent

t = time

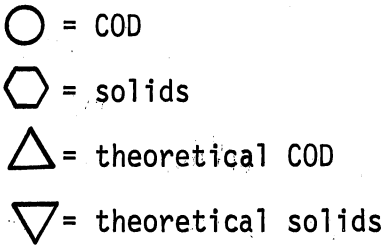
$t_d = V/Q$

It is apparent that growth at the very low specific growth rate prior to the shock prevented the cells from making successful response, although it is seen by comparing the theoretical dilute-out and observed curves for S and X that at the new dilution rate, some of the cells in the system responded but apparently did not possess the high $\mu_{max.}$ needed to exist in the reactor at the new dilution rate.

Change in D from 0.014 hr^{-1} to 0.5 hr^{-1}: The reactor was operating at steady-state before the shock load was applied (Figure 26). The flow rate, F, was increased so that it became 0.5 hr^{-1}, and samples were taken as before.

The results of the shock load are presented in Figure 26. The

Figure 25. Response of a System Shock Loaded From Dilution Rate of 0.014 hr^{-1} to 0.25 hr^{-1}



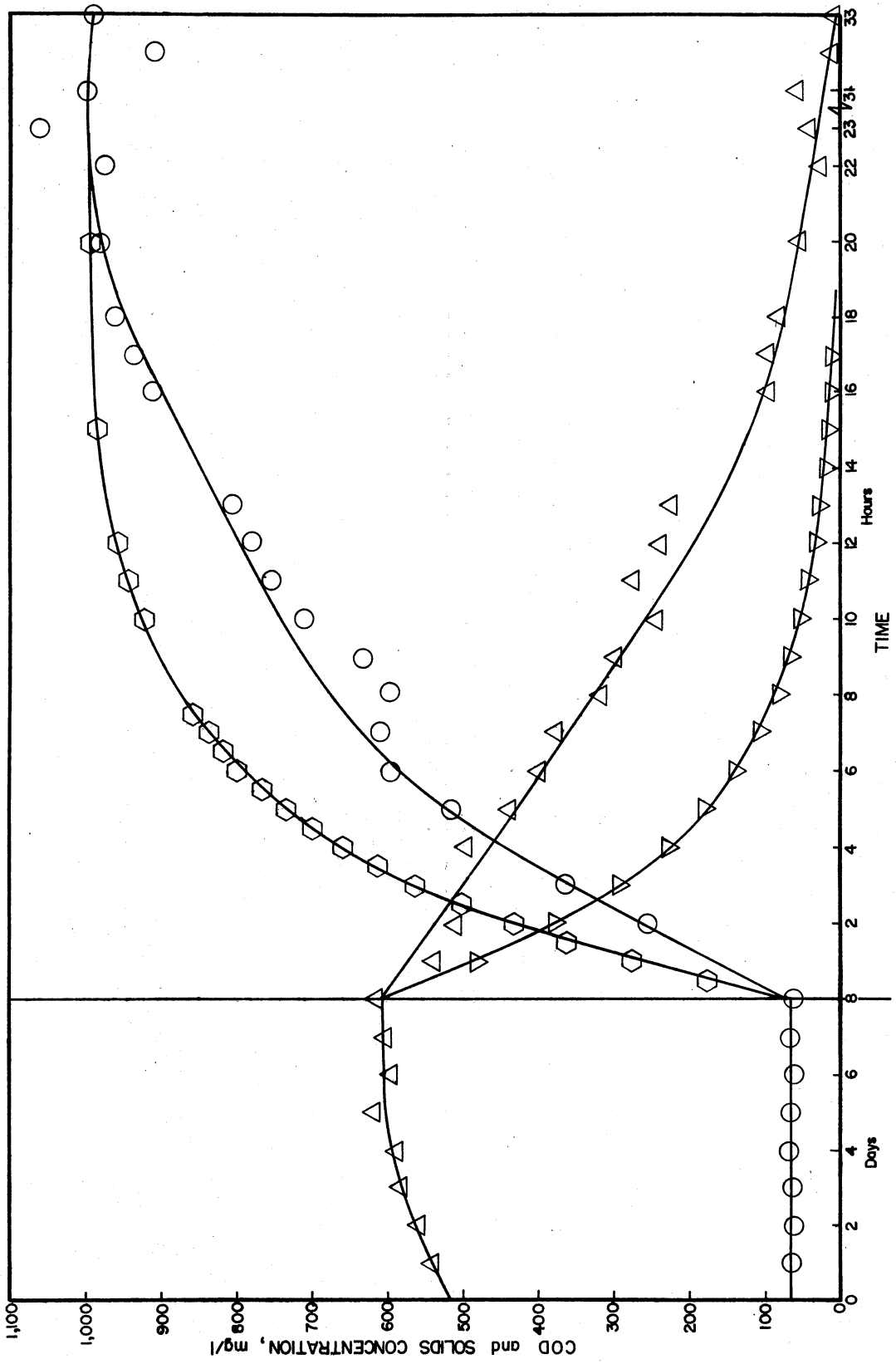
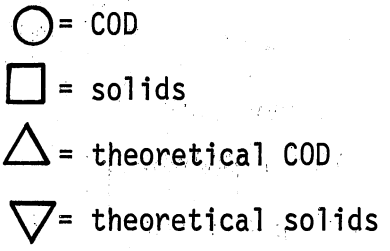
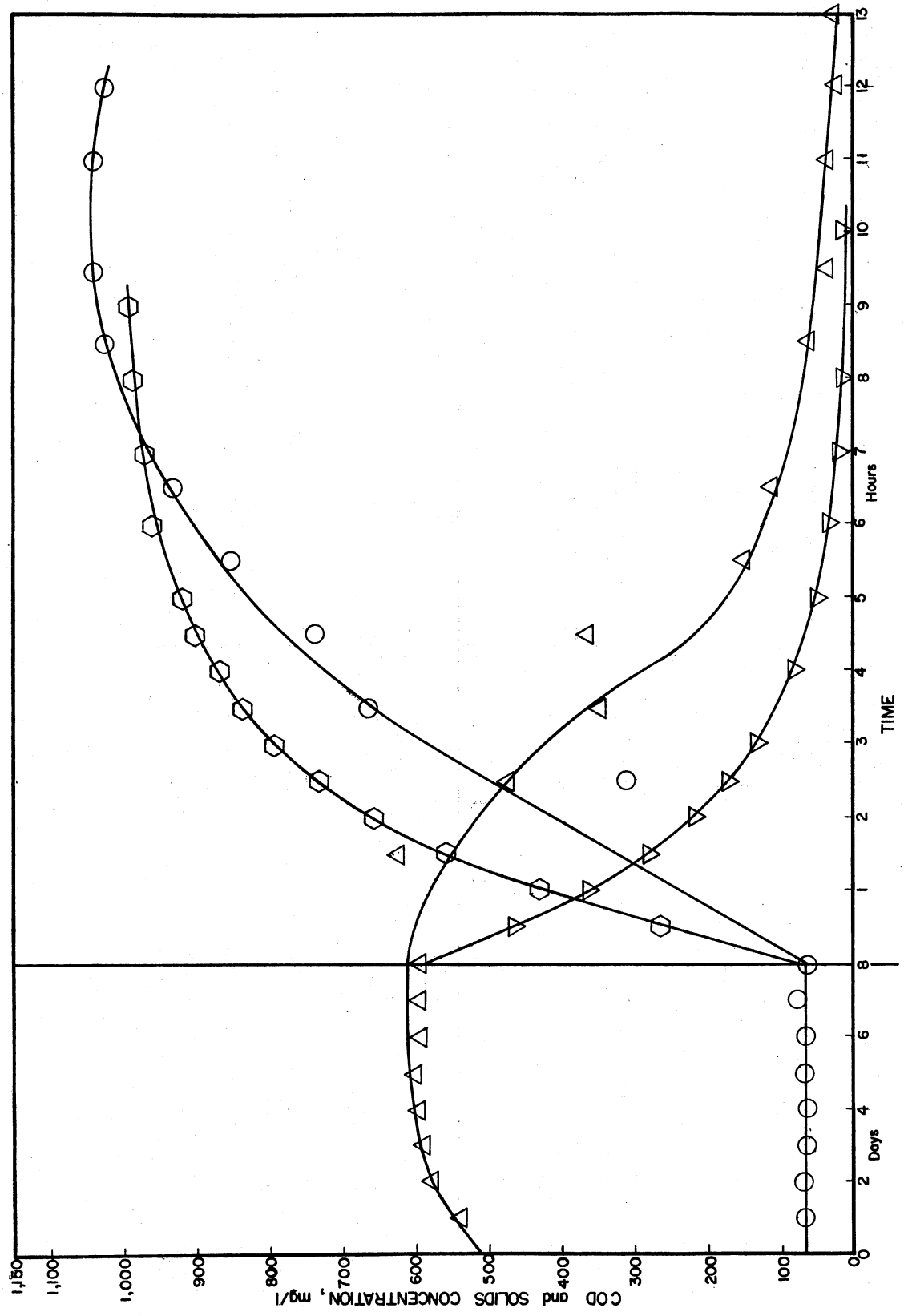

COD and SOLIDS CONCENTRATION, mg/l
1,100
1,000
900
800
700
600
500
400
300
200
100
0
2
4
6
Days
0
2
4
6
8
10
12
14
16
18
20
22
23
31
33
Hours
TIME

Figure 26. Response of a System Shock Loaded From Dilution Rate of 0.014 hr^{-1} to 0.5 hr^{-1}




COD and SOLIDS CONCENTRATION, mg/l
1,150
1,100
1,000
900
800
700
600
500
400
300
200
100
0
2
4
6
8
Days
1
2
3
4
5
6
7
8
9
10
11
12
13
Hours
TIME

biological solids did not dilute out as fast as the theoretical values calculated using equation (1). The COD did not dilute in as fast as the calculated values using equation (2). The observed curves and the curves calculated for no growth lie closer than those for the previous shock, as would be expected. It is, however, interesting to note that even at this severe shock load condition, a partial growth response was evidenced. It is also interesting to note that total cell dilute-out did not quite occur, i.e., there was a very small concentration of cells in the unit when the experiment was terminated. It is possible that after a more prolonged period of aeration, the few cells remaining could have adapted to the new growth rate.

VITA

Carl Sellers Kelley, III

Candidate for the Degree of

Master of Science

Thesis: A STUDY OF THE EFFECT OF SPECIFIC GROWTH RATES ON BOTH CHEMICAL AND AUTO-FLOCCULATION OF CELLS GROWN IN A ONCE-THROUGH CHEMOSTAT

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