STUDY OF THE EFFECTS OF SODIUM CHLORIDE ON ACTIVATED SLUDGE

By

DONNY FRANK KINCANNON

Bachelor of Science
Oklahoma State University
Stillwater, Oklahoma
1959

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1960

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Thesis Approved:

William F. Gaudy Jr.
Thesis Adviser

Elizabeth T. Gaudy

Roger E. Kopfler

Lynnette B. Graves

Dean of the Graduate School

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CHAPTER I

INTRODUCTION

An average of 4,300 billion gallons of rain falls each day on the United States. Most of this is dissipated by evaporation, by run-off into the oceans, and by water uses of plants and animals. It has been estimated that, at present, 315 billion gallons a day are available for man's daily use, with demand amounting to a little more than 320 billion gallons. As our water resources are developed, supplies will increase to approximately 500 billion gallons by 1980 and the demand will increase to 600 billion gallons.

Water is the nation's most used raw material today in industrial plants. The steel industry alone uses 13 billion gallons a day. It takes 770 gallons of water to refine a barrel of petroleum, 320,000 gallons to produce a ton of aluminum, 600,000 gallons to make a ton of synthetic rubber, and 510,000 gallons are needed to fabricate 1,000 yards of woolen cloth.

These figures give only an overall picture of the water problem. While the nation as a whole may have sufficient water, some areas, especially the Southwest, suffer water shortages. A farmer's crops may die of drought, and a city may be forced to ration its water in time of emergency.

Meanwhile, people elsewhere may be fighting floods. To further compound the problem, in the Southwest many natural surface waters are highly saline. At the present, these waters have not been developed for industrial and muncipal uses, but as the water supply becomes more critical such water may be used in increasing amounts for certain industrial needs. If, in turn, these waters become contaminated with organic materials, the biological treatment which may be necessary could be affected by the presence of salt.

Many other factors have contributed to the recent interest in the treatment of organic wastes in saline waters. Wastes of high salt concentration arise from the manufacture of cheese and from pickle processing. Waters containing high concentrations of salt wasted from softening plants may at times be found in streams containing organic pollutants. Also, shipboard wastes contain high concentrations of salt. Thus, increased amounts of saline waters may be found in the waste water stream, and waste treatment plants will be called upon to treat wastes with higher salt concentrations. In the design and operation of such plants it would be of great benefit to have more basic and specific information pertaining to the effects of salinity.

The purpose of this study was to determine the effects of salinity on the activated sludge process of waste treatment and the range of salt concentration which can be successfully handled by the activated sludge process. The analysis of data from this study should provide valuable insights into

the mechanisms and causations of the observed effects from both a basic and applied viewpoint. To provide a better understanding of these effects, studies were conducted in both batch and continuous flow activated sludge systems.

CHAPTER II

LITERATURE REVIEW

The effects of salts on biological viability and respiration were of major importance to the field of microbiology some forty years ago. This interest diminished after a few years but of late has been revived. F. G. Gustafson (1) was one of the early investigators in this field. Using a 0.05 percent glucose medium, he made a comprehensive study of the effects of antagonistic salts on the respiration of the fungus Aspergillus niger. He found that the respiration rate was increased by NaCl concentrations of 0.25 M to 0.5 M and by $CaCl_2$ concentrations of 0.5 M. It was also observed that a 2 M NaCl concentration and a 1.25 M $CaCl_2$ concentration decreased the respiration and he attributed this to an osmotic effect of these salts.

Brooks (2) also observed a stimulation in the rate of respiration in Bacillus subtilis. Sodium chloride stimulated respiration at a 0.15 M concentration while KCl and $CaCl_2$ stimulated respiration at concentrations of 0.2 M and 0.05 M respectively. Higher concentrations caused a decrease in the rate of respiration, these concentrations being 0.5 M for NaCl, 1.0 M for KCl, and 0.25 M for $CaCl_2$.

Holm and Sherman (3), using a one percent peptone

solution as the basic medium, made a study of the effects of various anions in combination with sodium and the effects of various cations in combination with chloride on the growth of Bacterium coli. A 0.2 M concentration was used and they observed that the tube containing NaCl showed growth in the shortest period of time. The culture in one percent peptone solution with no additive was slower in growing than the 0.2 M NaCl solution. In a later study, Sherman and Holm (4) showed that the limiting H-ion zone of growth may be modified by the addition of NaCl to the medium. Fabian and Winslow (5) also studied the effects of various anions in combination with sodium upon the viability of Escherichia coli. They found that NaOH, NaCl, $NaHCO_3$, Na_2CO_3, Na_2SO_4, Na_2HPO_4, NaH_2PO_4, and Na_3PO_4 exhibited the same general qualitative effect. All stimulated the growth of bacteria in low concentrations and inhibited it in higher concentrations.

In 1928, Speakman, Gee, and Luck (6) studied the effect of NaCl on the growth and metabolism of yeast Saccharomyces cerevisiae which was being used in a local brewery. They found that wort containing NaCl up to a concentration of ten percent can be fermented by S. cerevisiae. The yield of the yeast was reduced as the salt concentration increased and there was no evidence of growth stimulation. The lag-phase of the fermentation period was also progressively lengthened by increasing concentrations of NaCl. It is also interesting to note that in their work as the concentration of NaCl was increased up to two percent, the ratio CO_2:sugar utilized

showed a gradual rise. In higher concentrations the ratio gradually fell to a value considerably below the control figure. They suggested an accumulation of intermediates to explain the increased ratio.

The effect of various salts on the ability of bacteria to hydrolyze milk proteins and gelatin was investigated by Levine and Soppeland (7). The organisms studied were pure cultures isolated from creamery wastes. They found that the bacterial cultures exhibiting the most activity were most affected by increasing salt concentrations. The minimum concentration of sodium chloride producing definite inhibition of gelatin hydrolysis was 10,000 mg/l. This was found with the most active organism. In all cases, concentrations of 15,000 to 25,000 mg/l sodium chloride inhibited gelatin hydrolysis.

Winslow and Haywood (8) also made a comprehensive study of both stimulatory and inhibitory effects of various salts on bacterial growth. They found that the growth of E. coli was stimulated in sodium chloride concentrations of 0.005 to 0.25 M. In studying the effects of certain cations in combination with chloride, they found that if the specific potency of sodium is taken as one then the specific potency of potassium is equal to 1, magnesium is equal to 9, and calcium is equal to 12.

Winslow, Walker, and Sutermeister (9) also showed that sodium chloride has a net stimulating effect in low concentrations. They also observed that the E. coli strain showed

throughout the experiment both smooth and rough colonies. When first inoculated, the ratio of S to R types was from 0.4 to 1.1. At the end of twenty-four hours, the ratio of S to R types was from 0.5 to 2.0. They suggested that salts have some effect on the bacterial cell which retards growth during the lag phase of growth and stimulates growth during the logarithmic phase.

West, Gililland, and Vaughn (10) studied the characteristics of coliform bacteria from olives. Using gas formation from glucose metabolism as the criterion of activity, they found that the Escherichia strains were most susceptible to the inhibitory effect of salt and the Aerobacter cloacae cultures were the most resistant.

Several investigators have been interested in the adaptation of bacteria to sodium chloride. Larsen(11) reported that in 1930 Baars showed that the marine bacterium Desulfovibrio aestuarii could be adapted to fresh water by successive culturing in media with less salt until it became identical with its nonmarine counterpart Desulfovibrio desulfuricans. In like manner, he showed that the salt-sensitive D. desulfuricans could be transformed to the salt-dependent D. aestuarii. These transformations were carried out over a sodium chloride range of 0 to 3 percent. Larsen (11) also reports that Rittenberg could not reproduce Baars' work, but Littlewood and Postgate (12) studied the adaptation of seven different strains of Desulphovibrio desulphuricans and showed that the different strains had different abilities to adapt to various levels of

sodium chloride. They could not show any salt-tolerant variants above sodium chloride concentrations of three to four percent.

Hof (13) made a comprehensive study of the growth of bacteria naturally occurring in a salt-free environment in media with a high concentration of salt. She observed that bacteria belonging to the colon group could live in a maximum concentration of sodium chloride of six to twelve percent. Salt also had a very retardative influence on the decomposition of cellulose when garden soil was used as the inoculum. She concluded that generally the salt concentration tolerated in enrichment cultures is higher than that tolerated by the corresponding pure cultures. Hof also isolated obligate halophilic bacteria from an enrichment culture of garden soil and from canal water. She concluded that soil and fresh water bacteria could acquire a halophilic character and suggested that halophilic bacteria from salt waters may be adaptive forms of common bacteria. Ingram (14) did not agree with Hof's work. He pointed out that she did not consider the strong possibility that the organisms recovered at different salt concentrations might be completely and permanently different. Ingram also raised the point that Hof based her conclusion on the assumption that obligate halophiles do not exist in ordinary soil or canal water. Ingram raised the question of whether or not it is just as reasonable to suggest that halophiles do live under these circumstances. If Ingram is correct then it could be reasoned that the effects of salts on biological waste treatment is the selection of halophiles

or if Hof is correct then it could be reasoned that the effects of salts are due to the adaptation of common bacteria to halophilic forms. At the present there is no further evidence in the literature that either of these two theories is correct.

Doudoroff (15) studied the adaptation of E. coli to sodium chloride. He found that cells exhibited the greatest degree of adaptability to salt in the early stationary phase of growth. Very young cultures (log growth phase) and older cultures harvested some time after reaching the stationary phase exhibited a lower degree of adaptability. He also showed that the processes involved are reversible in that acclimatized bacteria readily lost increased ability to reproduce in saline media upon return to a salt-free environment. Thus the adaptation process was not a transformation to an obligate halophilic form. Doudoroff suggested that the adaptation mechanism consists of an individual acclimation, which is independent of reproduction and a selection by the environment of those cells endowed with a wider range of tolerance to normally unfavorable conditions.

The highest concentration of sodium chloride permitting growth before adaptation and after adaptation was studied by Severens and Tanner (16). They found that Salmonella pullorum could tolerate a three percent sodium chloride concentration before adaptation and after 69 days of adaptation, the culture could tolerate an eight percent sodium chloride concentration. Similar results were obtained using

cultures of Eberthella typhosa and Salmonella schotmulleri.

Ingram (17, 18, 19) has made a comprehensive investigation into the effects of salts on the respiration of Bacillus cereus. It was found that the rate of respiration was increased in sodium chloride concentrations less than 0.2 M. Concentrations greater than 0.2 M sodium chloride decreased the rate of respiration. The inhibition of respiration is expressed by Ingram (18) as:

$$\log r = P - Qc$$

where r = rate of respiration; c = salt concentration; and P and Q are constants. Ingram (19) also showed that the concentration of salt in the cell is much less than in the medium, this being as much as one-half or less. He also stated that a bacterial respiratory enzyme may be more sensitive to salts in the living cell than it is when isolated.

A comparison of the effects of salt concentrations on the respiration of a halotolerant bacterium and E. coli was made by Yamada and Shilo (20). They found the E. coli exhibited a maximum rate of respiration at a sodium chloride concentration of one percent, whereas the halotolerant bacterium Bacillus pumilus had a maximum rate of respiration at a sodium chloride concentration of seven to eight percent. They also stated that the main factor which determined the position of the maximum rate of respiration was probably the osmotic pressure.

In 1962, Yoshida (21) reported that sewage microorganisms were adversely affected by 6600 mg/l sodium chloride and killed by 8200 mg/l sodium chloride. This is much lower than any other investigator has ever reported. This is interesting in that Naylor (22) found that at no concentration of sodium chloride is the growth of Aerobacter aerogenes greater than growth obtained in the absence of sodium chloride. It is interesting to note that he also observed maximum growth of Proteus vulgaris at a sodium chloride concentration of 5000 mg/l. Crisley (23) also observed a stimulation of growth for Proteus vulgaris at a sodium chloride concentration of 5000 mg/l. It would appear from these studies that Yoshida's results could be attributed to the isolation of microorganisms that had a low sodium chloride tolerance and are not characteristic of all microorganisms present in sewage. This is also another indication that different types of bacteria display different salt tolerances.

In the pollution control field, Lawton and Eggert (24) have studied the effects of salt on trickling filter slimes. They observed negative reduction in BOD when salt concentrations greater than 20,000 mg/l were applied or when slimes which had become acclimated to salt concentrations greater than 20,000 mg/l were subjected to salt-free wastes. When the slime was shocked with salt concentrations up to 20,000 mg/l it recovered in about one day, but when shocked with 50,000 mg/l sodium chloride it took about five days for recovery to a uniform reduction which was approximately ten

to fifteen percent below the reduction obtained with salt-free wastes. When acclimated slimes were subjected to salt-free wastes, all tubes recovered in one day. It is interesting to compare the negative reduction in BOD for slimes shocked with 50,000 mg/l sodium chloride and slimes acclimated to 50,000 mg/l sodium chloride shocked with a salt-free waste. When shocked with 50,000 mg/l sodium chloride, the effluent BOD of the tube was 68 percent greater than the BOD in the influent. When the slime acclimated to 50,000 mg/l sodium chloride was shocked with a salt-free waste the effluent BOD of the tube was 165 percent greater than the BOD of the influent.

Stewart, Ludwig, and Kearns (25) have studied the effects of salts on the extended aeration process and the applicability of this process for the treatment of shipboard wastes. Temporary reductions in treatment efficiency were noted when abnormally severe changes in salinity were combined with heavy hydraulic and organic loadings. Floating sludge problems were encountered but no definite conclusion as to the cause could be drawn. It was observed that the air diffusing equipment (0.125 inch orifices) became plugged when aerating the synthetic waste containing a high percentage of ocean water. It was also found that the sludge concentration in the aerator decreased when salt water (ocean water) was replaced by fresh water. During the early stages of this run when fresh water was being replaced by ocean water, they found that the total weight of organisms increased but the number of species of protozoa decreased. The reverse took place on returning to

the fresh water waste. As the salinity in the reaction vessel decreased, the number of species of protozoa increased but the total organism weight decreased.

Mills and Wheatland (26) studied the effects of saline sewage on the performance of percolating filters. They found that a continuous addition of 6,600 mg/l sodium chloride caused little, if any, upset in the performance of the filters. Intermittent application of 6,600 or 20,000 mg/l salt, however, appeared to cause a deterioration in effluent quality lasting for a period of about two weeks. They also found that the first addition of salt caused a sudden fall in the amount of oxidized nitrogen in the effluent and an increase in the residual concentration of ammonia, but this effect lasted only one to three weeks.

Ludzack and Noran (27) have investigated effects of salt concentration up to 20,000 mg/l on the activated sludge process. No detectible changes in the performance of activated sludge were observed below sodium chloride concentrations of 5,000 to 8,000 mg/l. High sodium chloride operation was characterized by poor flocculation, higher effluent solids, and a decrease in oxygen demand removal efficiency. Nitrification was also reduced by high sodium chloride operation. It was also observed that a sodium chloride concentration of 20,000 mg/l caused an increase in respiration rate.

The literature records one pilot-plant and full scale plant treating saline wastes. This operation was reported by Stowell (28) in 1954. Salt water is pumped from the bay

and used as service water at San Quentin prison. It was estimated that the chloride concentration would vary from 6,000 to 8,000 mg/l after mixing with fresh water wastes. A high-rate trickling filter pilot plant was constructed and studied. It was found that a slime growth developed within one week and BOD removal was as much as 90 percent in some runs. From the data obtained from the pilot plant study, a full scale trickling filter plant was constructed and operation has borne out the pilot plant studies.

Thus far, this literature review has shown that most pure cultures of bacteria are affected by salts and it has also shown that different types of bacteria or even different strains of the same type exhibit different salt tolerances. The remainder of the review will be devoted to the various theories of causation that have been presented.

Mitchell and Moyle (29) reported that in 1903 Alfred Fischer observed that many Gram-negative organisms could be plasmolysed in salt solutions or in sucrose, but were not plasmolysed by the same concentration of glycerol, urea, chloral hydrate, or other substances of low molecular weight or high lipid solubility. From this Fischer concluded that Gram-negative organisms contained a plasma membrance permeable to glycerol etc., but impermeable to sucrose or electrolyte solutions. Mitchell and Moyle then proposed the hypothesis that the permeability of bacteria to many of their nutrients and waste products is a specific one, dependent upon the presence of the enzymes which transport the nutrients and end-

products of metabolism across the plasma membrane. In 1929 Hill and White (30), studied the possibility of separating certain Gram-positive cocci from Gram-negative bacilli by using a sodium chloride medium. They studied some fifty cultures on media containing sodium chloride concentrations of one to 25 percent. They found that sodium chloride concentrations of two to 20 percent exert marked inhibitory action on the growth of bacilli of the typhoid, paratyphoid, dysentery, and colon groups, and on species of Proteus, Pseudomonas, and Bacillus anthracis. The Gram-positive cocci studied tolerated the high salt concentrations. They also found that when mixtures of cocci and bacilli in different proportions are cultured on appropriate salt agars, the cocci invariably outgrow the bacilli and may sometimes be recovered in pure culture.

Peterson (32) studied the influence of salt upon the bacterial flora and the decomposition of fish. With sodium chloride concentrations of zero to eight percent the flora were heterogeneous, containing both rods and cocci. Between sodium chloride concentrations of 12 to 15 percent the rods were killed, while the cocci were plentiful in a sodium chloride concentration of 18 percent.

Brown and Turner (33) were able to show some correlation between salt tolerances of some Gram-negative bacteria and envelope instability and cation dependence. They also demonstrated a wide variation in salt tolerance by several different strains of Gram-negative bacteria. Two strains of

Spirillum had a maximum tolerance of one percent sodium chloride, whereas a Proteus strain had a maximum tolerance of eight percent.

Henneman (34) studied osmotic influences on respiration of bacterial cells. She observed that Gram-negative organisms show increased turbidity, plasmolysis, and decreased respiration when suspended in solutions of osmotically active substances. Concentrations of 0.1 to 0.8 M sodium chloride, potassium chloride, lithium chloride, and glucose were used in the study. Gram-positive organisms showed no turbidity, plasmolysis, or respiratory responses to sodium chloride or potassium chloride unless washed with water and incubated with phosphate buffers. Thereafter, sodium chloride and potassium chloride inhibited respiration temporarily. Henneman then proposed that since respiration inhibition occurs as plasmolysis and increased turbidity are produced, it may result from modifications in spatial arrangement of enzymes within the cell membrane. Alterations such as this could affect the ability of substrates to penetrate the cell membrane and be metabolized. As supporting evidence she cited the observation that sodium chloride and potassium chloride do not inhibit cell-free preparations of hexokinase, G-6-P dehydrogenase, or cytochrome oxidase, but they do inhibit G-6-P dehydrogenase activity in intact cells.

In 1946 Porter (35) theorized on the mechanism of salt action. He put forth three theories: "(a) ions operate upon the cell by combining with it; (b) they operate without com-

bination but at a distance through radio or other activity, and (c) ions unite with components of protoplasm essential to metabolism...such unions affect the cell because the new compounds cannot operate in the normal protoplasmic economy, or because they operate more actively or less actively or in some other abnormal fashion". Porter did not try to prove any of these theories. Nunheimer and Fabian (31) felt that the effect of a salt is a function of the action of both ions as well as the osmotic pressure involved. Fabian and Winslow (5) also believed in the function of both ions and osmotic pressure.

Ingram (36) suggested that the action of salts on bacterial respiration could partly be explained in terms of "salting out". He pointed out that the material upon which the action is exerted is probably one of the proteins concerned in respiration, perhaps a dehydrogenating enzyme. Ingram also conceded that the main objection to this theory lies in the range of concentrations over which the action is produced. With normal organisms, the salt concentrations are much lower than those causing "salting out". He also points out that there is very little evidence that in normal organisms the dehydrogenating enzymes are less sensitive to salts than the intact cells. A difference in the salt tolerance of enzymes in the intact cell and enzymes in cell extracts may be the source of discrepancy. Ingram could not offer any reason for this, but pointed out that the property must be absent from the enzymes of halophilic organisms, and whatever it is, its absence must be the foundation of the halophilic character.

Scott and his coworkers (37, 38, 39, 40) felt that all organisms require a minimum amount of water and that salts deprive the bacteria of water otherwise available to them. Scott (37) showed that the range of water activities, a_w(a_w = the water vapour pressure of the solution expressed as a fraction of the vapour pressure of pure water at the same temperature) permitting growth of food-poisoning strains of Staphylococcus aureus was virtually independent of the nature of the solutes used in the study. These solutes included sugars and non-toxic salts such as sodium chloride, potassium chloride, and sodium sulfate. Christian and Scott (38) confirmed the findings that the growth of Staphylococcus aureus was independent of the solute. They also observed that for Salmonellae the range of water activities in which growth occurs is much less than for S. aureus. In 1955 Christian (39) proposed that the influence of concentrated solutions of normally non-toxic substances is due to their effects on the availability of water. Marshall and Scott (40) found that for a strain of Vibrio metchnikovi the rate of growth in liquid media and the number of colonies forming on solid media were both greatly increased by reducing the water activity from 0.999 to 0.995.

There have also been several investigators that have studied the effects of salts and osmotic pressure on specific enzymes or metabolic pathways. Clark and MacLeod (41) studied ion antagonism in glycolysis using a cell-free extract of Lactobacillus arabinosus. It was found that inhibition of

glycolysis by Na^+ was reflected primarily in an increase in the lag phase preceding the initiation of glycolysis and that, once established, glycolysis proceeded at a rate which was almost unaffected by the presence of the inhibitory ions.

The effects of osmotic pressure on the respiration of Bacillus subtilis spheroplasts was investigated by Smith (42). She observed an increase in respiration rate as the spheroplasts swelled between sucrose concentrations of 0.8 and 0.4 M, but was unable to explain this response. The same increase was observed in the presence of endogenous substrates or with added substrates or DPNH. On the basis of what is known about other respiratory chain systems, Smith reasoned that the increased respiration rate could result from the uncoupling of oxidative phosphorylation. However, she was not able to establish that this is the explanation of the effect with B. subtilis spheroplasts, since no uncoupler of phosphorylation was found. The author agrees with Smith when she states, "The coupling of respiration to phosphorylation in many kinds of bacteria must be quite labile, since it has been difficult to obtain cell-free extracts of bacteria showing P/O ratios as large as those obtained with mammalian mitochondria."

Baxter and Gibbons (43) investigated the effects of salts on the glycerol dehydrogenases of an extreme halophile, Pseudomonas salinaria, a moderate halophile, Vibrio costicolus; and a non-halophile, E. coli. The glycerol dehydrogenase from E. coli was stimulated by a 0.25 M concentration of either sodium or potassium chloride, but was progressively inhibited

by higher concentrations of both salts, sodium being rather less stimulatory and more inhibitory than potassium. The enzymes from the extreme halophile were found to be much more resistant to salts than the analogous enzymes from a non-halophilic organism or a moderate halophile. Not much difference was observed in non- and moderate halophiles.

The effects of salt on cytochrome oxidase activity in extracts from a halotolerant Micrococcus were reported by Yamada and Asano (44). The activity of this enzyme was more than doubled by sodium chloride concentrations of 1.0 to 2.0 M, whereas, the cytochrome oxidase from a heart muscle preparation showed a decrease in activity in sodium chloride concentrations above 0.1 M. This finding may provide some insight into increased respiration observed with some microorganisms.

When attempting to assess the effects of salts on bacteria it seems that Larsen(11) has asked a very pertinent question, "Does salt penetrate into the cells?" He then philosophizes, "If not, one might envision either a direct action of sodium chloride upon the structure or function of the cell surface, or osmotic damage to the cell. In the latter case, the diffusion of water out of the cell into the saline environment might interfere with metabolic function, or cause damage to the wall. On the other hand, if sodium chloride penetrates freely into the protoplasm, there will be no apparent osmotic problem. In this case, sodium chloride may interfere directly with metabolism, i. e., by affecting the catalytic function of the individual enzymes." This seems to be a very important

question to be answered. Several investigators have attempted to answer this question but have arrived at conflicting conclusions.

Roberts, Abelson, Cowie, Bolton, and Britten (45) have stated that E. coli is freely permeable to anions such as phosphate and sulfate. Littlewood and Postgate (46), using the "thick suspension" technique of Mitchell and Moyle (29), reported that sodium chloride was not diluted with intracellular water below three percent sodium chloride. At four and six percent sodium chloride, water exchange did occur and induced considerable dehydration of the cells without true penetration or loss of viability.

Christian and Waltho(47) studied thirty-two different strains of nonhalophilic bacteria and reported that the potassium contents varied about five-fold and were positively correlated with salt tolerance. They concluded that salt tolerance in non-halophiles is related to their ability to accumulate potassium within the cells. Lubin and Kessel (48) investigated potassium transport in E. coli using K^{42} measurements and flame photometry with similar results. Suspensions of wild-type and potassium transport negative mutants were incubated at 37°C. in media containing $K^{42}Cl$. After incubation, cells were collected on millipore filters, washed briefly with iced high-sodium mineral medium and the radioactivity associated with the cells determined. It was found that the period of rapid uptake was complete within twenty minutes.

Armstrong and Rothstein (49) found that extracellular pH markedly influences the ability of yeast cells to discriminate between K^+ and Na^+. Between pH 6 and 8, the hydrogen ion has no effect. Below pH 4, the hydrogen ion competitively inhibits the transport of each cation.

Miller and Avi-Dor (50, 51) have been able to show the intracellular accumulation of potassium and its effect on respiration. Their results indicate that the rate of glutamate oxidation in E. coli is determined by the intracellular concentration of K^+ rather than by the flux of this ion. They also showed that the increase in intracellular K^+ started to level off at a medium concentration of 3 mM KCl and increased very little with increasing concentrations of potassium chloride in the medium.

A comprehensive study of salt transport across cell membranes has been made by Hendricks (52). He has shown that hydrolysis of ATP is important for transport of sodium chloride, or an exchange of Na^+ or K^+, across plant, animal and artifical membranes. Ions of Na^+, K^+, and Mg^{++} promote the activity of ATPases in membranes of this type. In this same area Blond and Whittan (53) have studied the effects of Na and K on oxidative phosphorylation in relation to respiratory control by a cell-membrane ATPase. Kidney mitochondria were used in the study. They found that the P/O ratio was increased as the result of a stimulatory effect of potassium ions.

Lehninger (54), in studying water uptake and extrusion by mitochondria in relation to oxidative phosphorylation,

found that the protoplast membrane is relatively impermeable to potassium chloride, sodium chloride, and phosphate salts, whereas Na^+ and K^+ enter damaged or swollen mitochondria relatively rapidly. The question of salt transport across a bacterial cell membrane is still not completely resolved.

Many theories regarding the effects of salts on the viability of bacteria have been presented by investigators, but the validity of these theories has not been established. It is felt that the theories presented here are the more pertinent ones that deserve further consideration.

CHAPTER III

THEORETICAL CONCEPTS

A. Batch Activated Sludge Systems

Long, rectangular aeration tanks which are normally used in conventional activated sludge plants approach the "plug" flow concept which may be defined as follows: particles of feed leave the reactor in the same order in which they entered, and do not intermix or interact with each other (55). In most cases batch unit studies are directly applicable to "plug" flow conditions. Laboratory batch reaction data are often expressed in the form:

$$-\frac{dc}{dt} = kC^b \quad \text{.....(I)}$$

in which $\frac{dc}{dt}$ is the change in concentration of the inital substrate with time, k is the kinetic reaction rate constant, and b is the reaction order which may have any value, but is usually in the range between 0.0 and 3.0 and frequently has a value close to 1.0.

The removal of substrate from a batch activated sludge unit is normally considered to be a first-order reaction, but this is an area open to much discussion. In the work to be reported here, the kinetics of substrate removal in batch

units will not be a major area of concern; therefore, only this brief description of kinetics will be presented.

B. Steady State Kinetics

The continuous flow activated sludge unit employed in this study was operated as a completely mixed steady state system. This type of system may be defined as one in which all particles of the feed intermix immediately with all other particles in the reactor. The contents of the reactor are uniform and identical with those of the effluent. Since the contents of the reactor are uniform, the reaction must proceed at only one rate. In biological systems using external control, this rate is controlled by regulating the flow rate and the concentration of limiting nutrients or growth factors. In this study the rate was controlled by the flow rate and the concentration of organic carbon source. All other nutrients were supplied in excess.

The rate of growth for a continuous flow system can be expressed as (56):

$$\frac{dx}{dt} = \mu x - Dx = (\mu - D)\, x \quad \ldots\ldots\ldots (II)$$

In which $\frac{dx}{dt}$ is the rate of change of bacterial population in the reaction vessel.

x is the concentration of organisms

μ is the specific growth rate

D is the dilution rate which is inversely proportional to the mean detention time (T)

Under steady state conditions which were employed in this study the reaction rate by definition must be constant; therefore, the bacterial concentration must be constant and $\frac{dx}{dt}$ is zero. If we set

$$\mu x - Dx = 0$$

then

$$\mu = D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (III)$$

and the growth rate in the reaction vessel is equal to the dilution rate (D). Also since the dilution rate (D) is equal to feed inflow rate (Q) divided by the reaction vessel volume (V), which is constant for any unit, it can be seen that under steady state conditions the growth rate (μ) is mainly controlled by the feed inflow rate (Q). This holds true as long as (μ) does not exceed the maximum growth rate (μm).

The specific growth rate (μ) is also dependent on the substrate concentration in the reaction vessel. According to Herbert and his associates (56), Monod has proposed the following relationship,

$$\mu = \mu_m \left(\frac{S}{K_S + S}\right) \quad \ldots\ldots\ldots\ldots\ldots (IV)$$

where:

S is the substrate concentration in the reaction vessel

μm is the maximum growth rate

K_S is a constant numerically equal to substrate concentration at which the specific growth rate (μ) is equal to one-half of the maximum growth rate (μm).

In a steady state system the substrate concentration remaining in the reaction vessel will be constant and will also be controlled by the dilution rate. By combining equation (III) with equation (IV) the concentration of substrate (S) in a system operated under completely mixed steady state conditions may be obtained as:

$$S = K_s \frac{D}{\mu_m - D} \quad \text{.....(V)}$$

The corresponding concentration for the bacterial population may be expressed as:

$$X = Y (S_r - S) \quad \text{.....(VI)}$$

where:

S_r is the substrate concentration in the inflow feed

S is the substrate concentration in the reaction vessel (which is the same in the effluent)

Y is the yield constant which may be expressed as the ratio of the increase in bacterial weight to the weight of substrate consumed.

This is a very simplified discussion of steady state kinetics, but will suffice for the considerations to be presented in this study. A more complete discussion of steady state kinetics may be obtained elsewhere (56). For the studies to be reported here only one dilution rate (0.125 hr^{-1}) and one inflow substrate concentration (1000 mg/l glucose) was used. The only change to the system was the concentration of

sodium chloride in the inflow feed.

C. The Kinetics of Increase and Decrease of Sodium Chloride Concentration Due to Shock Loading in Steady State Systems.

In this study two shock loading conditions were studied. The gradual shock loading of a fresh water unit with a sodium chloride-containing waste and the gradual shock loading of a unit acclimated to sodium chloride with a fresh water waste. A gradual shock loading was accomplished by changing the inflow feed of the system from a fresh water waste to a sodium chloride waste or by changing from the sodium chloride waste to a fresh water waste. Both conditions were brought about without changing the inflow rate. The kinetics for the increase in sodium chloride concentration can be expressed as (57):

$$\frac{dc}{dt} = C_o D - CD$$

where:

C_o is the concentration of sodium chloride in the inflow

C is the concentration of sodium chloride in the outflow at any time after switching to a sodium chloride feed.

D is the dilution rate, which is inversely proportional to the detention time.

Upon integration of the equation the following expression is obtained:

$$C = C_o (1-e^{-DT})$$

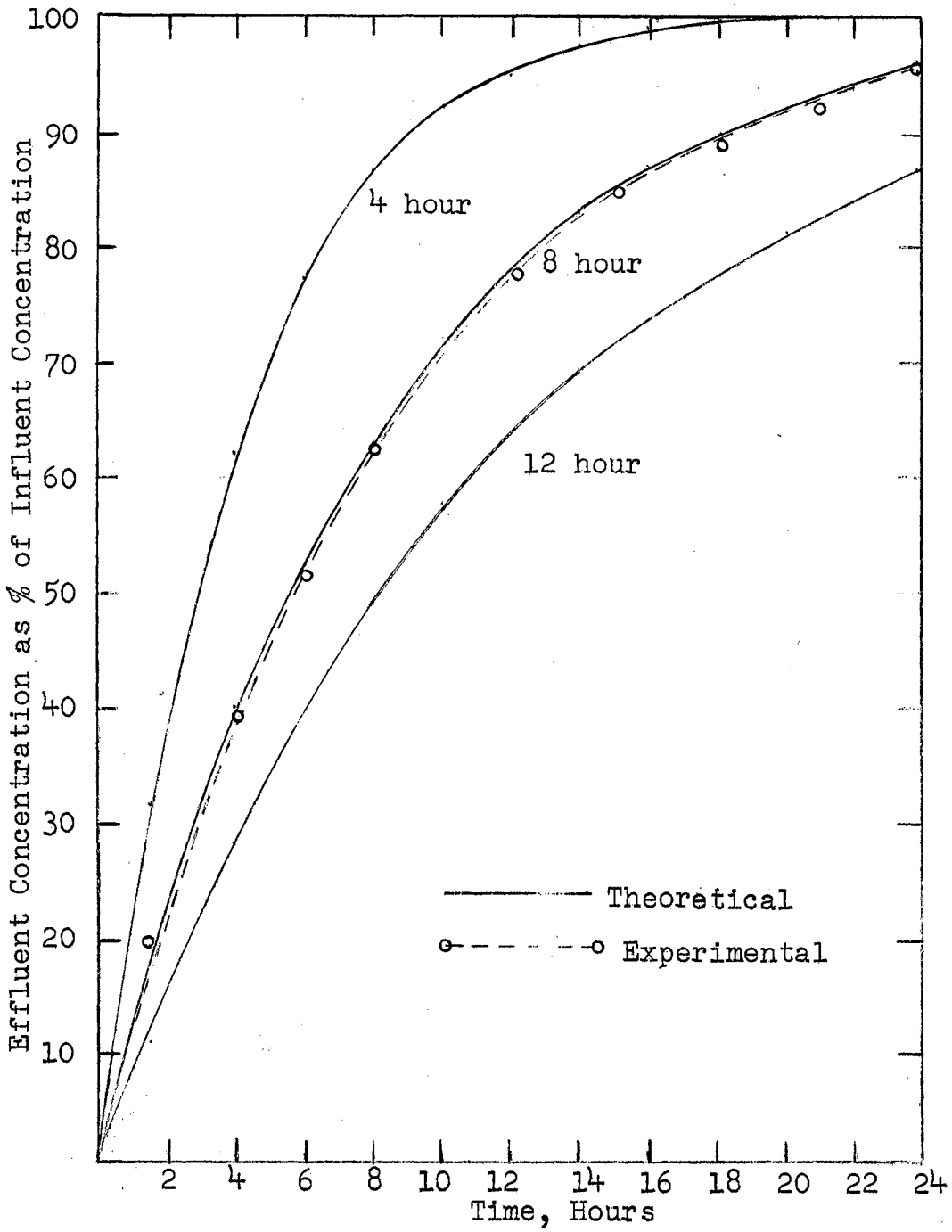

FIGURE 1 INCREASE IN THE SODIUM CHLORIDE CONCENTRATION OF THE SYSTEM DUE TO GRADUAL SHOCK LOADING.

The validity of this expression was checked by measuring the change in chlorides with time in the reaction vessel. The experimental results and the theoretical values are compared in Figure 1. From these results it can be seen that the expression is valid.

The kinetics for the decrease in sodium chloride concentration can also be expressed as:

$$\frac{dc}{dt} = C_oD - CD$$

In the case of a decrease in sodium chloride concentration. C_o is equal to zero and the expression becomes:

$$\frac{dc}{dt} = - CD$$

upon integration this expression yields:

$$\ln C = - DT + k$$

At $T = 0$, $k = \ln C_R$, where C_R is the sodium chloride concentration in the reaction vessel before shocking with the fresh water waste. The expression becomes:

$$\ln C = - DT + \ln C_R$$

From this the final expression of

$$C = C_R e^{-Dt}$$

may be obtained.

The validity of this expression was again checked by measuring the change in chlorides with time in the reaction

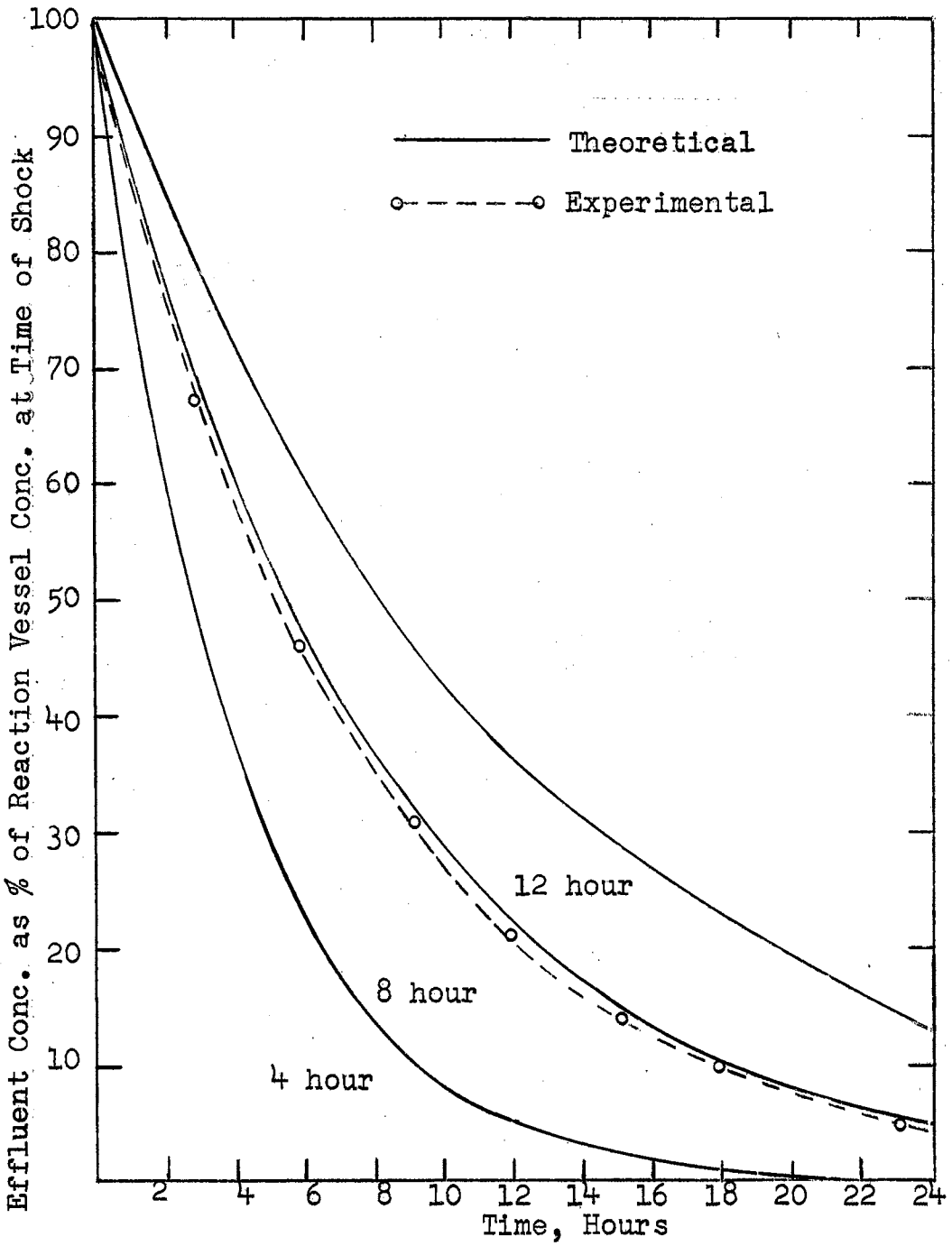

FIGURE 2 DECREASE IN THE SODIUM CHLORIDE CONCENTRATION OF THE SYSTEM DUE TO A GRADUAL SHOCK LOADING OF FRESH WATER.

vessel. The experimental results and the theoretical values are compared in Figure 2. From these results it can be seen that the expression is valid.

CHAPTER IV

MATERIALS AND METHODS

A. Experimental Protocols

1. Batch Activated Sludge Systems

a. Old cells: Batch activated sludge units (1.5 liter aeration volume) were developed from an initial sewage seed taken from the effluent of the primary clarifier of the Stillwater, Oklahoma municipal sewage treatment plant and developed on a synthetic waste containing: glucose, 1000 mg/l; $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $CaCl_2$, 7.5 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; 1 M potassium phosphate buffer (pH 7.0), 10 ml/l; and tap water, 100 ml/l.

Each unit was allowed to come into solids balance and was then operated in accordance with the following procedure. Prior to daily feeding, 500 ml of mixed liquor were wasted and the remaining liquor was settled for one hour. After wasting 500 ml of supernatant, the units were brought to volume with synthetic waste constituents and distilled water to yield the final concentrations given above. The unit was operated in this manner for at least 21 days before being considered as an "old cell" system.

In studies to determine the effects of slug doses of salt,

the mixed liquor from a batch unit was divided into two equal parts. Both new units were then fed the standard waste, brought to volume and one was shock-loaded with the salt concentration under study. The "fresh water" unit was retained as a control. In studies to determine the effects of long term exposure to sodium chloride, an activated sludge which had been operating as a batch unit for 21 days was dosed with the initial concentration of sodium chloride under study. The system was allowed to acclimate for two weeks before determining the response of the system. The unit was then dosed with the next concentration of sodium chloride under study and allowed to acclimate for two weeks. This procedure was followed until all concentrations of sodium chloride under consideration had been studied. A fresh water unit was maintained as a control. In studies to determine the effects of a shock loading of fresh water on sodium chloride-acclimated sludges, activated sludges were acclimated to sodium chloride by gradually dosing a fresh water sludge with increasing amounts of salt. Each unit was operated at the desired salt concentration for at least 30 days prior to studying the effect of dosing with a fresh water synthetic waste. The cells were shocked with fresh water by harvesting the cells from the salt unit by centrifugation and filtration through a millipore filter. The cells were then suspended in a fresh water synthetic waste. The salt unit was retained as a control. In either type of study, samples were collected at regular intervals during a six-hour period immediately after feeding the units.

Respiration, substrate removal and change in biological solids were determined.

b. Young cells: This type of sludge was also started from a sewage seed from the municipal sewage treatment plant and developed on the same synthetic waste as the old cells. In studies to determine the effects of slug doses of salt, the units were operated according to the following procedure. The seeded synthetic waste was aerated for 24 hours. After 24 hours, 50 ml of the mixed liquor were transferred to one liter of freshly prepared synthetic waste. This procedure was followed for three days. On the fourth day the unit was divided into two equal parts. Both new units were then fed the standard waste and one was shock loaded with the salt concentration under study. The fresh water unit was retained as a control. In studies to determine the effects of a shock loading of fresh water on sodium chloride acclimated sludges, activated sludges were allowed to develop in the same manner as young fresh water sludges except that the concentration of salt under study was added to the synthetic waste feed each day. The units were allowed to operate in this manner for seven days. On the eighth day the cells from one-half of the unit were harvested by centrifugation and filtration through a millipore filter. The cells were then suspended in a fresh water synthetic waste. The salt unit was retained as a control. The same sampling procedure was followed and the same analyses were made as those conducted for experiments with the old cells.

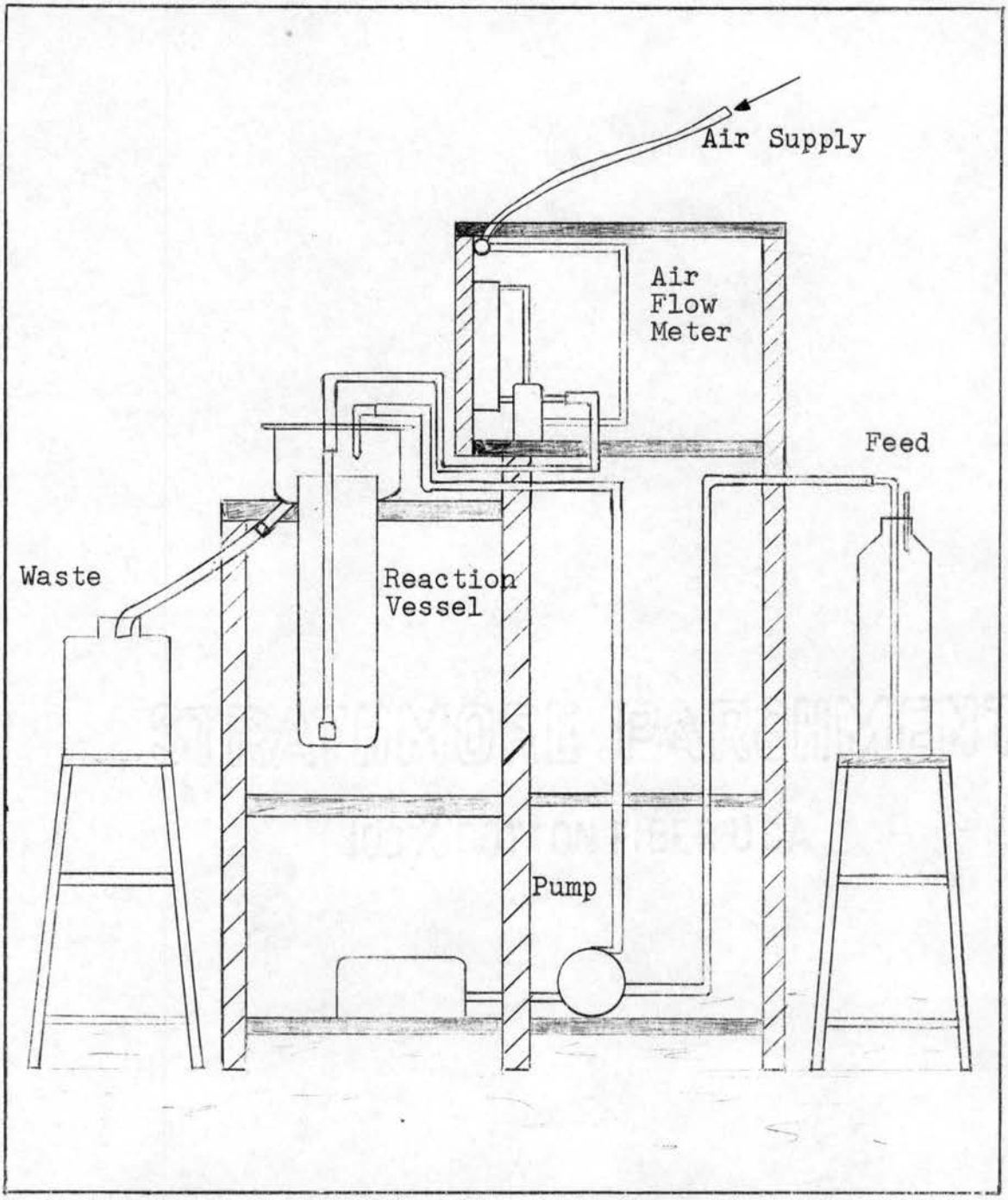

FIGURE 3 SCHEMATIC DRAWING OF THE BENCH-SCALE CONTINUOUS FLOW COMPLETELY MIXED ACTIVATED SLUDGE SYSTEM.

2. Continuous Flow Activated Sludge System

a. Description of apparatus, standard wastes, and operation: A sketch of the bench scale activated sludge unit employed in this study is shown in Figure 3. The aeration volume of the reaction vessel was 2.5 liters with an effective volume of 2.4 liters when the sludge was under aeration. Air was supplied to the system at a rate of approximately 4000 ml/min. The inflow feed was stored in a 10-liter reservior bottle and the flow rate was regulated by a liquid flow meter pump. The outlet of the feed line was made of bent glass tubing and was placed so that there was a two to three inch air gap between it and the mixed liquor of the aeration tank. The system was operated in a laboratory which was maintained at 23°C. ± 3°.

The synthetic waste contained the same constituents and concentrations as that used in the batch activated sludge studies. The wastes contained: glucose, 1000 mg/l; $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $CaCl_2$, 7.5 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; 1 M potassium phosphate buffer (pH 7.0), 10 ml/l; and tap water, 100 ml/l.

After inoculating the system with sewage seed, it was operated as a batch unit for 24 hours. After this period it was operated as a continuous flow unit at a fixed hydraulic rate of 5 ml/min. This provided a detention time of eight hours in the reaction vessel. The unit was thus operated for at least three days to ensure steady state conditions. After this period of equilibration samples were collected daily or

twice daily for two or three days and were analyzed for residual substrate and biological solids. This was done to determine the steady state conditions before applying the shock load with sodium chloride.

The shock loading of sodium chloride was introduced by instantaneously shifting the influent feed from a fresh water waste to a saline waste. Samples were collected every three hours until the saline waste was "diluted into" the reaction vessel, i.e., until the sodium chloride concentration in the reaction vessel was the same as that in the inflow feed. Thereafter, three to four samples were collected daily. The units were allowed to operate at the sodium chloride concentration under study for two to three weeks and were then shock-loaded with fresh water. The fresh water shock was introduced by instantaneously shifting the influent feed from a saline waste to a fresh water waste. Samples were again collected every three hours until the sodium chloride had been completely diluted out of the reaction vessel. Thereafter, three or four samples were collected daily until the unit had again reached a steady state condition.

b. Batch unit studies: In order to study the biochemical response of the activated sludge in the continuous flow system, cells were collected from the effluent of the system and analyzed in batch unit studies. Five hundred ml of the effluent from the continuous flow system were collected before the system was shock-loaded with sodium chloride and also after the system had recovered from the fresh water

shock loading. The 500 ml portion was divided into two equal parts. Both new units were then fed the standard waste and one was shock-loaded with the salt concentration under study. The fresh water unit was retained as a control. In other studies, 250 ml of the effluent of the continuous flow unit were collected during periods when the continuous flow unit was receiving the saline waste. The batch unit was fed the standard waste and the sodium chloride concentration was maintained at the same level as that in the continuous flow unit. In all cases samples were collected at regular intervals during a six-hour period immediately after feeding the units. Respiration, substrate removal and change in biological solids were determined.

3. Related Studies on Effects of Sodium Chloride and The Causation of These Effects.

a. Biochemical composition of the activated sludge: The biochemical composition of the activated sludge was determined on samples from batch unit studies. The carbohydrate and protein contents of the sludge were determined over the six-hour sampling period. Further biochemical characterization of sludge grown in high salt concentrations (45,000 mg/l NaCl) and in fresh water was obtained by analyzing samples collected 24 hours after feeding for protein, carbohydrate, lipid, and ribonucleic acid. Gas chromatography (F & M model 180-C-H-N Analyser) was employed to measure the carbon, nitrogen, and hydrogen content of the sludge.

b. Respiration studies: Old and young cells which had been grown according to procedures previously outlined under "Batch Activated Sludge Systems" were harvested by centrifugation and filtration through a millipore filter and resuspended in a buffer solution or sodium chloride solution. Old and young cells grown in a fresh water medium and in a 30,000 mg/l sodium chloride medium were used in this study. The respiration rates of these cells at various concentrations of sodium chloride and potassium chloride were determined using the Warburg protocol given in Table 1. Biological solids were determined on an initial sample and on the contents of the Warburg flasks at the end of the run.

In another study cells from an "old cell" sodium chloride unit and from an "old cell" fresh water unit were inoculated into a fresh water synthetic waste and placed on the shaker for 24 hours. Each day 1 ml of each unit was again transferred to a freshly prepared fresh water synthetic waste and again placed on the shaker for 24 hours. This procedure was followed for seven transfers. Respiration studies using the Warburg protocol of Table 1 were conducted on cells harvested from these units.

In still another phase of the study a sewage seed was allowed to grow on a shaker apparatus for 48 hours. The standard synthetic waste with a sodium chloride concentration of 30,000 mg/l was used. At the end of 48 hours the cells were harvested and a Warburg respiration study was made. Also 1 ml of the cell suspension was transferred to a freshly

TABLE 1

WARBURG PROTOCOL

Constituent	Concentration	Volume
Carbon source (glucose)	2000 mg/l	10 ml/flask
Buffer Salts Solution		
$(NH_4)_2SO_4$	500 mg/l	
$MgSO_4 \cdot 7H_2O$	400 mg/l	
$FeSO_4 \cdot 6H_2O$	8 mg/l	
$CaCl_2$	7.5 mg/l	
$MnSO_4 \cdot H_2O$	8 mg/l	15 ml/flask
1 M phosphate buffer (pH 7.0)	120 ml/l	
Tap water	80 ml/l	
Distilled water	To 1 liter	
Washed Sludge Suspended in 30,000 mg/l sodium chloride or buffer solution	Determined for each experiment	5 ml/flask
NaCl or KCl	Concentration determined for each flask	10 ml/flask
Total volume reaction mixture		40 ml/flask

Note: For measurements of respiration under non-proliferating conditions the $(NH_4)_2SO_4$ was not included in the buffer salts solution.

prepared synthetic waste containing 30,000 mg/l sodium chloride. Thereafter, each day after 24 hours of growth 1 ml of the cell suspension was again transferred to a freshly prepared saline waste. This procedure was followed for seven transfers. After the seventh transfer the cells were harvested after 16 hours of growth and a Warburg respiration study was conducted.

c. Lysis studies: Studies were made to determine the degree of cell lysis accompanying changes in salt concentration. "Old cells" grown in 45,000 mg/l sodium chloride were harvested by centrifugation 24 hours after feeding and were resuspended in 45,000 mg/l sodium chloride. The optical density was measured at 540 mμ. The cells were again centrifuged and filtered and the COD of the filtrate was determined. The cells were then resuspended in various concentrations of sodium chloride (45,000, 30,000, 20,000, 10,000, 5,000, and zero mg/l) and stirred for ten minutes. The optical density was then measured at 540 mμ. The cells were centrifuged and filtered and the COD of the filtrate was determined. Lysis was measured as decrease in optical density and increase in filtrate COD.

In another study cells grown at various salt concentrations (45,000, 30,000, 20,000, 10,000, and 5,000 mg/l) were harvested shortly after the log-phase of growth by centrifugation. All cells were resuspended in the sodium chloride concentration in which they were grown. The optical density was determined at 540 mμ. The cells were centrifuged and filtered and the COD of the filtrate determined. The cells were then resuspended

in distilled water and stirred for ten minutes. The optical density was then determined at 540 mμ. The cells were centrifuged and filtered and the COD of the filtrate was determined. The degree of lysis was again measured as the decrease in optical density and increase in filtrate COD.

d. Gradient study: The gradient study was made to determine the response of cells acclimated to 45,000 mg/l sodium chloride when they were transferred to a fresh water environment under non-shock-loading conditions. To accomplish such transfer, the salt-grown cells were harvested by centrifugation and then resuspended in a sodium chloride concentration of 45,000 mg/l. The salt concentration was gradually reduced to 2,500 mg/l using a linear gradient mixing device. The cells were then centrifuged and resuspended in a fresh water synthetic waste medium. Under these conditions there was no evidence of lysis. Using the transferred cells, a batch unit study was made to assess their substrate removal efficiency, rate of respiration, and change in biological solids.

e. Diphasic growth studies: Cells acclimated to 45,000 mg/l sodium chloride were harvested by centrifugation and resuspended in a fresh water synthetic waste medium containing: glucose, 400 mg/l; $(NH_4)_2SO_4$, 200 mg/l; $MgSO_4 \cdot 7H_2O$, 50 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $CaCl_2$, 7.5 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; 1 M potassium phosphate buffer (pH 7.0), 10 ml/l; and tap water, 100 ml/l. The system was aerated and samples collected to determine rates of substrate removal and growth

of the organisms. Growth was followed by measuring the optical density. Respiration was followed by using the Warburg respirometer. Another study was conducted to assess the effect of the protein inhibitor chloramphenicol (Parke, Davis & Co.) on the diphasic growth pattern. Cells acclimated to 45,000 mg/l sodium chloride were harvested as before and resuspended in the fresh water synthetic waste medium. The system was divided into two batch units. The units were aerated and after 30 minutes of aeration a chloramphenicol stock solution was added to one unit to obtain a concentration of 100 mg/l. The other unit was followed as a control. The units were sampled at regular intervals. Respiration was followed using the Warburg respirometer. Growth was followed by optical density measurements. Twenty-five ml samples were taken and filtered through a 0.45 μ millipore filter. Substrate removal was assessed using samples of the filtrate. For cell protein determinations, the solids from the 25 ml samples were resuspended in 10 ml of distilled water and this concentrated suspension was used for protein analysis.

B. Analytical Techniques

1. Substrate Removal

a. Chemical oxygen demand (COD): One of the disadvantages of the COD test outlined in Standard Methods (58) is that, under the conditions of the test, dichromate oxidizes chloride quantitatively to chlorine:

$$Cr_2O_7^{=} + 12Cl^- + 14H^+ \longrightarrow 2Cr^{+3} + 6Cl_2 + 7H_2O$$

The chlorine is lost in the gaseous phase. Since 1.0 mg/l Cl is equivalent to 0.23 mg/l O, a correction (mg/l Cl X 0.23) may be made. This correction cannot be applied when silver sulfate is used immediately as a catalyst. When applying this correction chlorides must be determined on a separate sample. This procedure was tried during preliminary investigations and proved very unsatisfactory. Sodium chloride concentrations of 20,000 to 30,000 mg/l provide a COD of 4600 to 6900 mg/l due to chlorides. The COD of the synthetic waste was only 1060 mg/l and after one hour of reaction time this was generally decreased to approximately 300 mg/l. Thus it can be seen that a one percent error in determining the chloride concentration can cause a 15 to 20 percent error in the COD determination.

Cameron and Moore (59) attempted to remove chlorides as AgCl by precipitation and filtration prior to COD analysis. They obtained low COD values with colloidal matter because of co-precipitation. Cripps and Jenkins (60) have developed a method in which they use $HgSO_4$ to form the undissociated $HgCl_2$ which is only slightly oxidized under standard COD conditions. The major disadvantage of this procedure is that the COD flask must be cooled in an ice bath and the sulfuic acid must be added so that the sample does not warm up. When making 30 to 40 COD analysis this procedure could become very laborious. It also requires a chloride correction.

In preliminary work the author tried ion-exchange resins but such a large quantity of resin was required that the method was not feasible. The method finally adopted was precipitation of the chlorides with silver sulfate. The method was tested for low COD values and it was found that with soluble organic material such as glucose very accurate COD values were obtained. Chloride determinations using the Mohr method as outlined in Standard Methods (58) were made on a series of samples and the results showed that all chlorides had been removed. The following procedure was used:

A 25 ml sample was filtered using the membrane filter technique. Silver sulfate was added to the filtrate and mixed. The point at which all chlorides had been precipitated could be observed by a change in color of the solution from a milky white to yellow. The solution was then centrifuged and the supernatant used for the COD test which was conducted according to the procedure outlined in Standard Methods (58).

b. Anthrone (total carbohydrate): Carbohydrate remaining in solution was measured by the anthrone test using the procedures outlined by Gaudy(61). Since the anthrone test is specific for carbohydrates it can be used to follow the removal of glucose from the system. The difference between the results of the COD test and the anthrone test were taken as an indication of the release of metabolic intermediates or end-products by the microorganisms.

2. Respiration

Respiration was measured on a Warburg respirometer. The system was maintained at 25°C. using a shaker rate of 92 oscillations/min. The detailed techniques and calculations are outlined in Manometric Techniques (62) and Standard Methods (58).

3. Biological Solids

a. Membrane filter technique: Total and volatile sludge synthesis was measured by the membrane filter technique as outlined in Standard Methods (58). In some studies the filtered material was transferred from the membrane to tared crucibles for measurement of the total and volatile solids. A 50 ml sample was used in these determinations.

b. Optical density: Biological solids were also determined by measuring the optical density at 540 mμ. A calibration curve of biological suspended solids vs. optical density which was determined by Rao (63) was used.

4. Biochemical Composition of Cells

a. Carbohydrate and protein: The carbohydrate and protein contents of the sludge were measured by using the anthrone and biuret test respectively; the procedures for these tests have been described in detail by Gaudy(61).

b. Lipids: Extraction of lipids from a filtered 25 ml sample of sludge was carried out using 60 ml of a 3:1 ethanol:ether (Skelly F., B.P. 30-60) mixture(64). Lipids were extracted for six hours on a shaker apparatus at 25°C. After extraction the flask contents were filtered through a 0.45μ millipore filter. The filtrate was poured into a COD

flask and the ethanol:ether mixture was evaporated off at 70°C. The flasks were then flushed with a gentle air stream. Blanks and standards were prepared by similarly evaporating 60 ml of the ethanol:ether mixture alone and 60 ml of the mixture containing known amounts of stearic acid. After evaporation the standard procedure for COD analysis was followed. Lipids were reported as equivalent stearic acid.

c. Ribonucleic acid: Cells acclimated to 45,000 mg/l sodium chloride were harvested from a batch-operated unit 24 hours after feeding. The cells were washed twice using a solution containing 45,000 mg/l sodium chloride. Cellular pools and other soluble material were extracted with five percent trichloroacetic acid at 25°C. for 15 minutes. After centrifugation, the residue was extracted with a 2:1 chloroform:methanol mixture at 25°C. for 30 minutes. Nucleic acids were then removed from the residue with five percent trichloroacetic acid at 100°C. for 30 minutes (45). RNA was determined as ribose by the orcinol method described by Morse and Carter (65). An aliquot of the hot TCA soluble material was made to 2.0 ml with distilled water. Five ml of a solution containing 0.1 g of anhydrous ferric chloride in 500 ml of concentrated hydrochloric acid were added. This was followed by 0.3 ml of a solution containing 2.0 g orcinol in 20 ml of 95 percent ethanol. After mixing, the tubes were heated at 100°C. for 20 minutes and then adjusted to 7.3 ml with distilled water. Optical density was measured at 660 mμ. Standards were run using a solution containing 400 μg yeast

RNA (Calbiochem) dissolved in 1.0 ml of five percent TCA.

d. Carbon, nitrogen, and hydrogen: Gas chromatography was employed to measure the carbon, nitrogen and hydrogen content of the sludge. A F&M model 180 C-H-N analyzer was used.

CHAPTER V

RESULTS

A. Batch Activated Sludge Systems

1. Effects of Shock Loading Old Fresh Water Sludge With Sodium Chloride

The changes in system parameters for an old fresh water sludge shock-loaded with sodium chloride are compared with the control system in Figures 4-10. Figures 4, 5, and 6 show that a shock load of 5,000, 8,000, or 10,000 mg/l sodium chloride had little or no effect on the system. The substrate removal (which was complete within two hours of aeration time), the oxygen uptake, and the solids production were approximately the same for the shocked system and the control system.

A sodium chloride concentration of 12,000 mg/l (Figure 7) caused the first variations in system parameters. These variations are slight but it can be seen that the shocked system required approximately one hour longer for effective removal of the substrate. The solids production and oxygen uptake were approximately 20 percent greater in the shocked system. At a sodium chloride concentration of 15,000 mg/l (Figure 8), the shocked system again required approximately one hour longer than the control to remove the substrate.

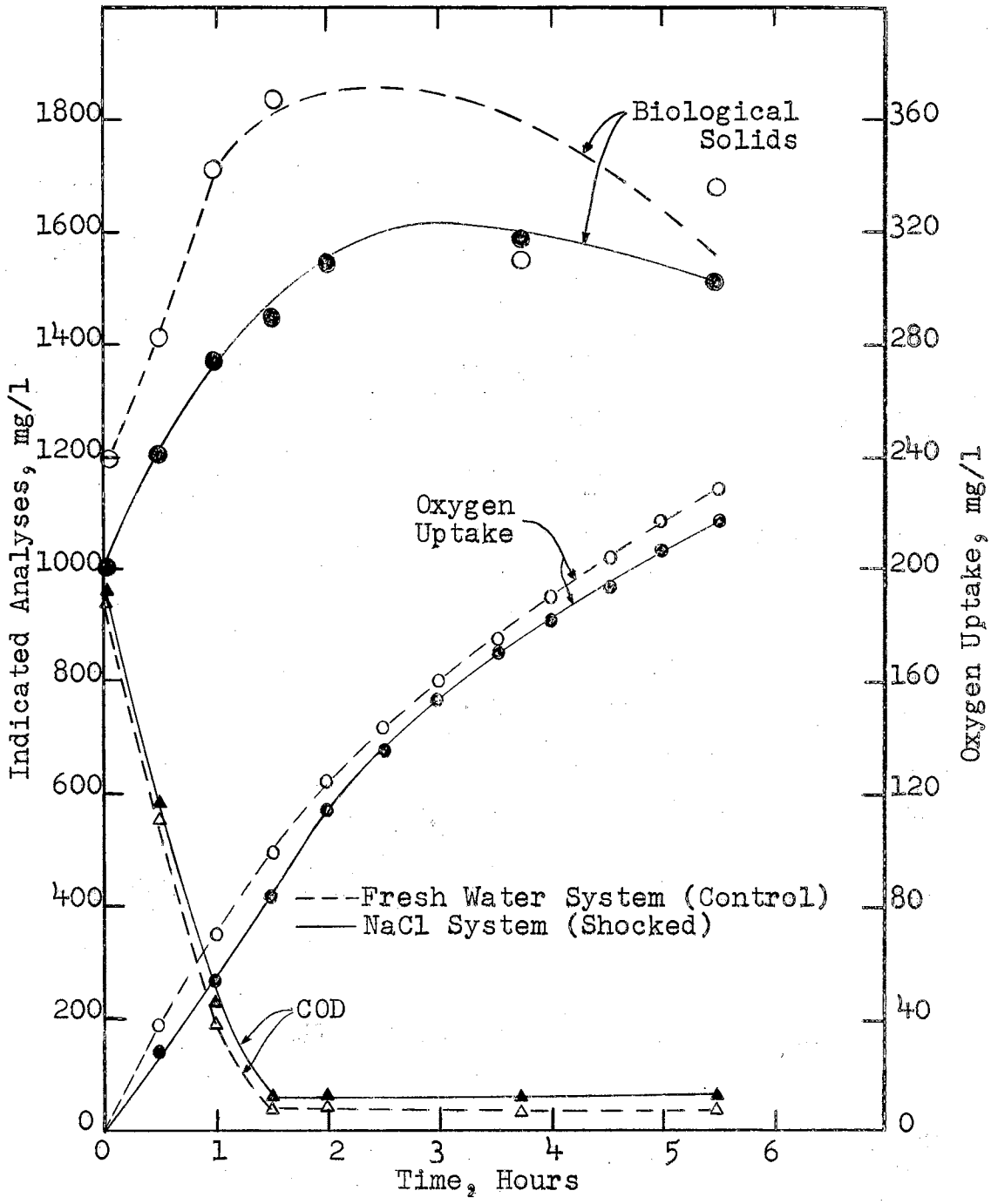

FIGURE 4 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 5,000 mg/l NaCl.

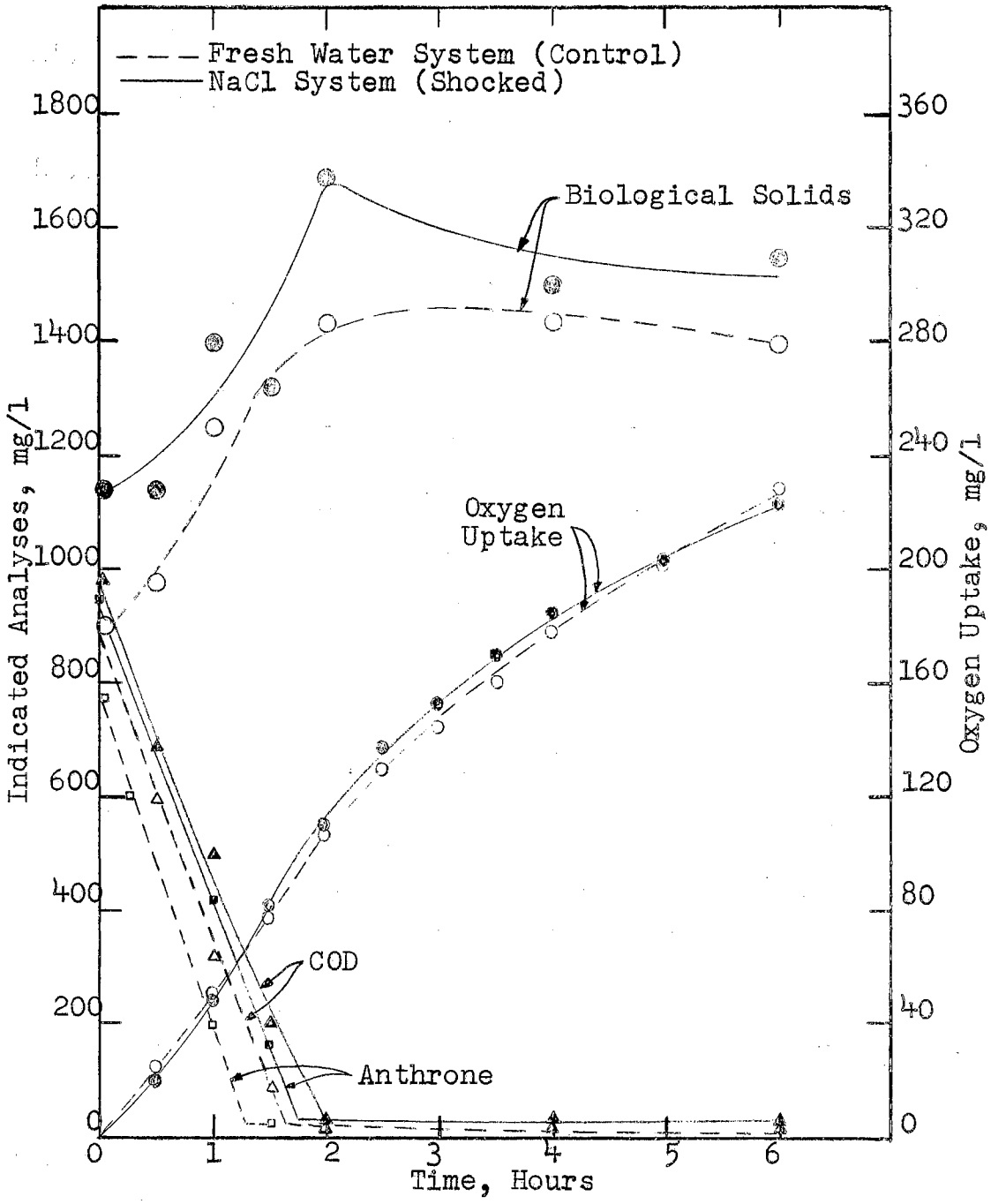

FIGURE 5 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 8,000 mg/l NaCl.

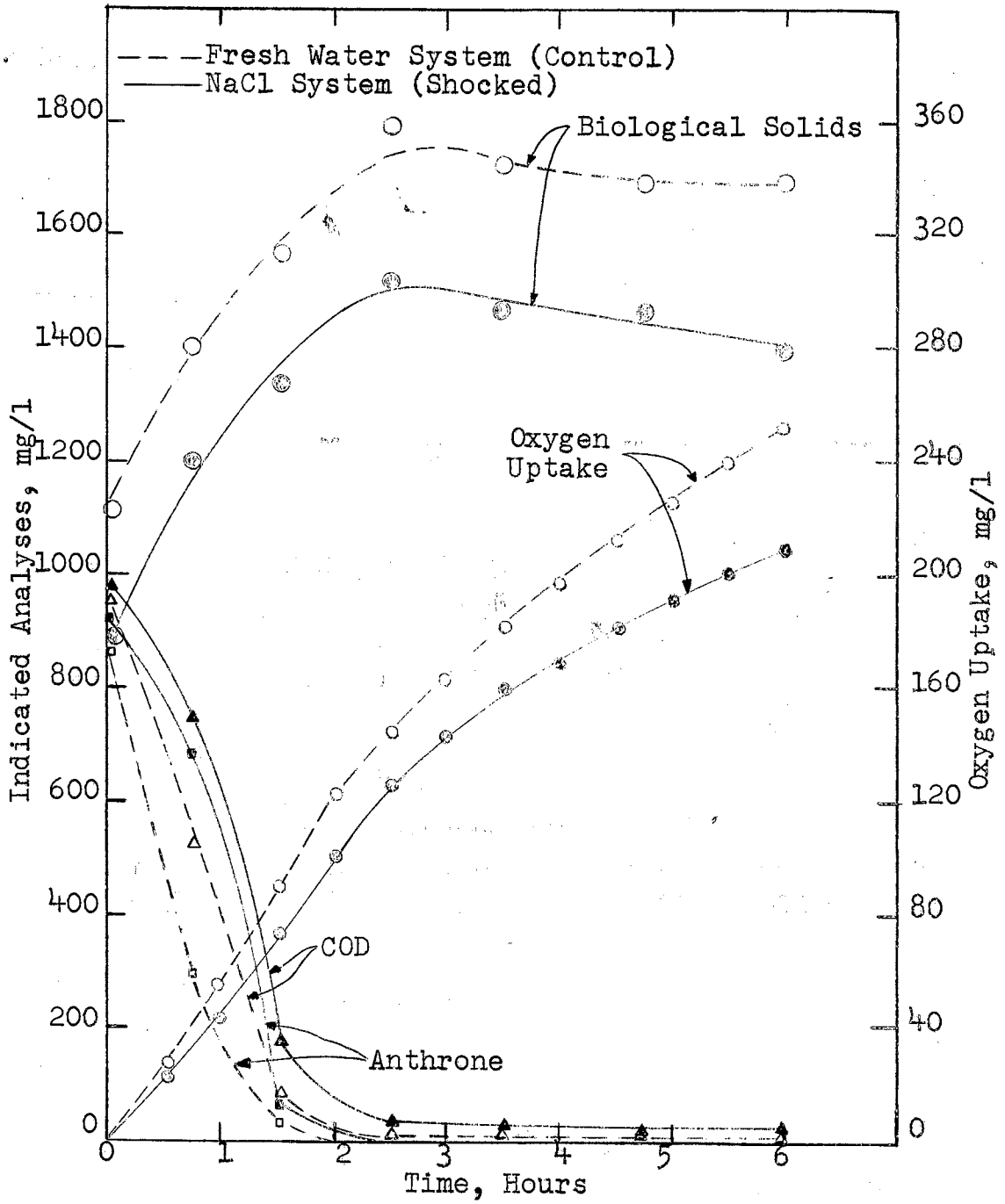

FIGURE 6 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 10,000 mg/l NaCl.

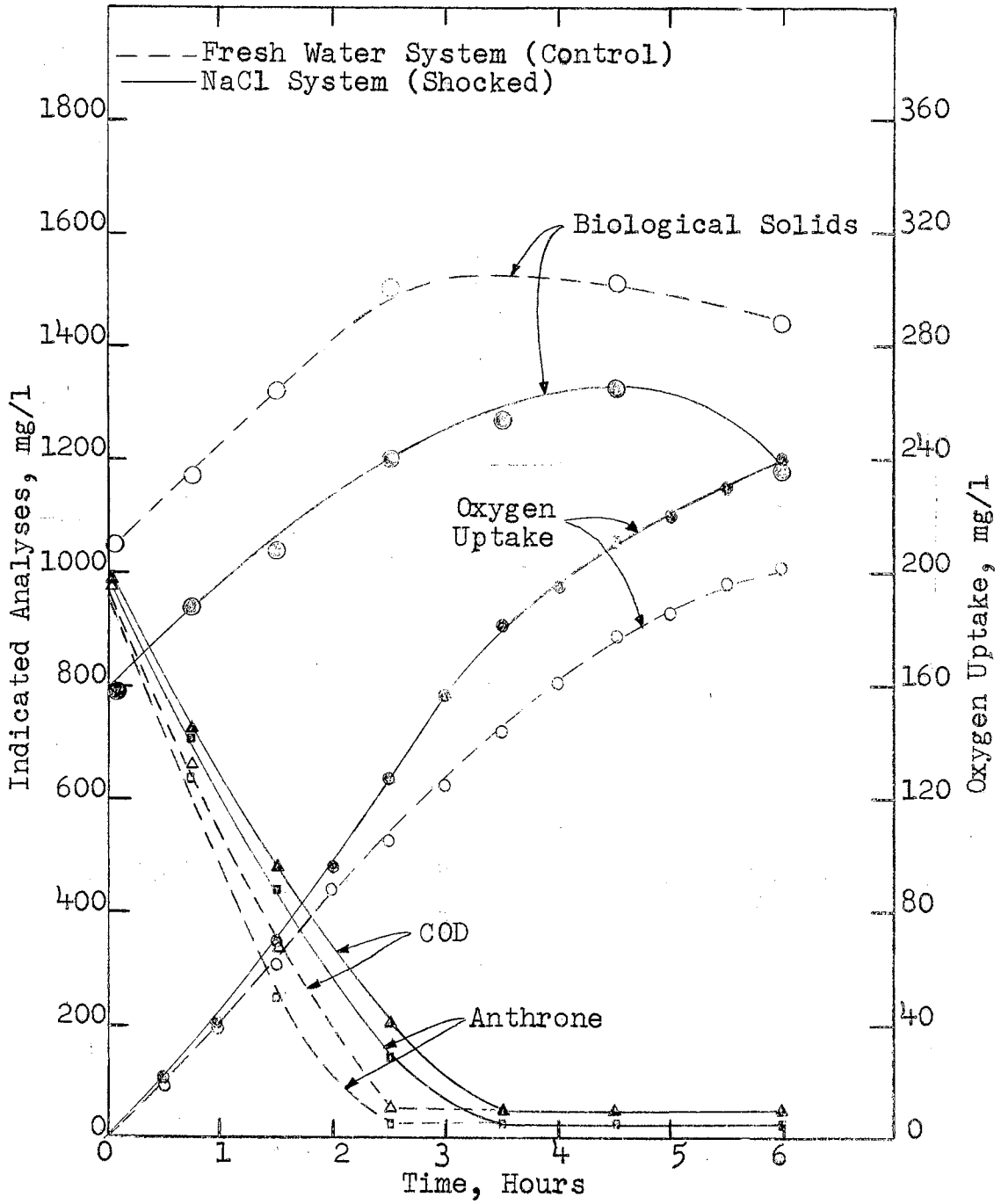

FIGURE 7 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 12,000 mg/l NaCl.

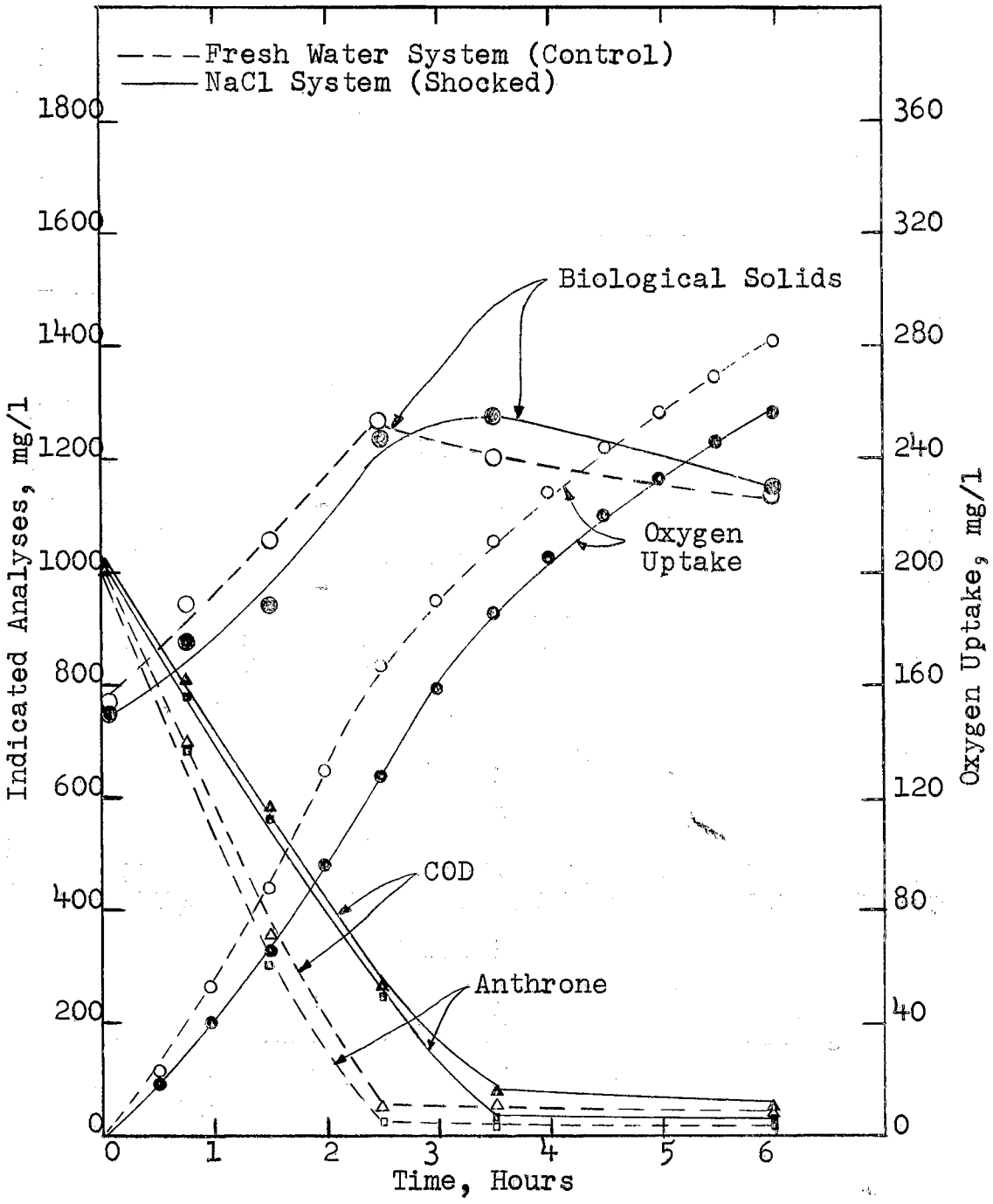

FIGURE 8 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 15,000 mg/l NaCl.

The solids production was approximately ten percent greater in the shocked system than in the control. The effects of a shock load of 30,000 mg/l sodium chloride are shown in Figure 9. The shocked system required approximately two and one-half hours longer to remove the substrate than the control system. It is interesting to note that the difference in solids production was reversed from sodium chloride concentrations of 12,000 and 15,000 mg/l. The solids production was approximately 12 percent greater in the control system than in the shocked system.

In Figure 10 it is seen that a concentration of 45,000 mg/l affected the biochemical response of the system much more severely than the other concentrations. The higher salt concentration apparently caused a considerable degree of lysis and release of cellular constituents. In this study the initial COD due to glucose was 1025 mg/l, whereas the initial COD measured immediately after adding the salt was 1600 mg/l. It is also seen that the released materials were removed from the system prior to utilization of the glucose, since at the end of two and one-half hours only 80 mg/l of the original carbon source (glucose) had been utilized, while the released COD had been reduced by approximately 600 mg/l. The deleterious effect of the salt dosage is best assessed by noting that in the control system glucose removal was essentially complete in two and one-half hours, whereas in the shocked system only 39 percent was removed in six hours. The solids production was approximately 170 percent greater and the

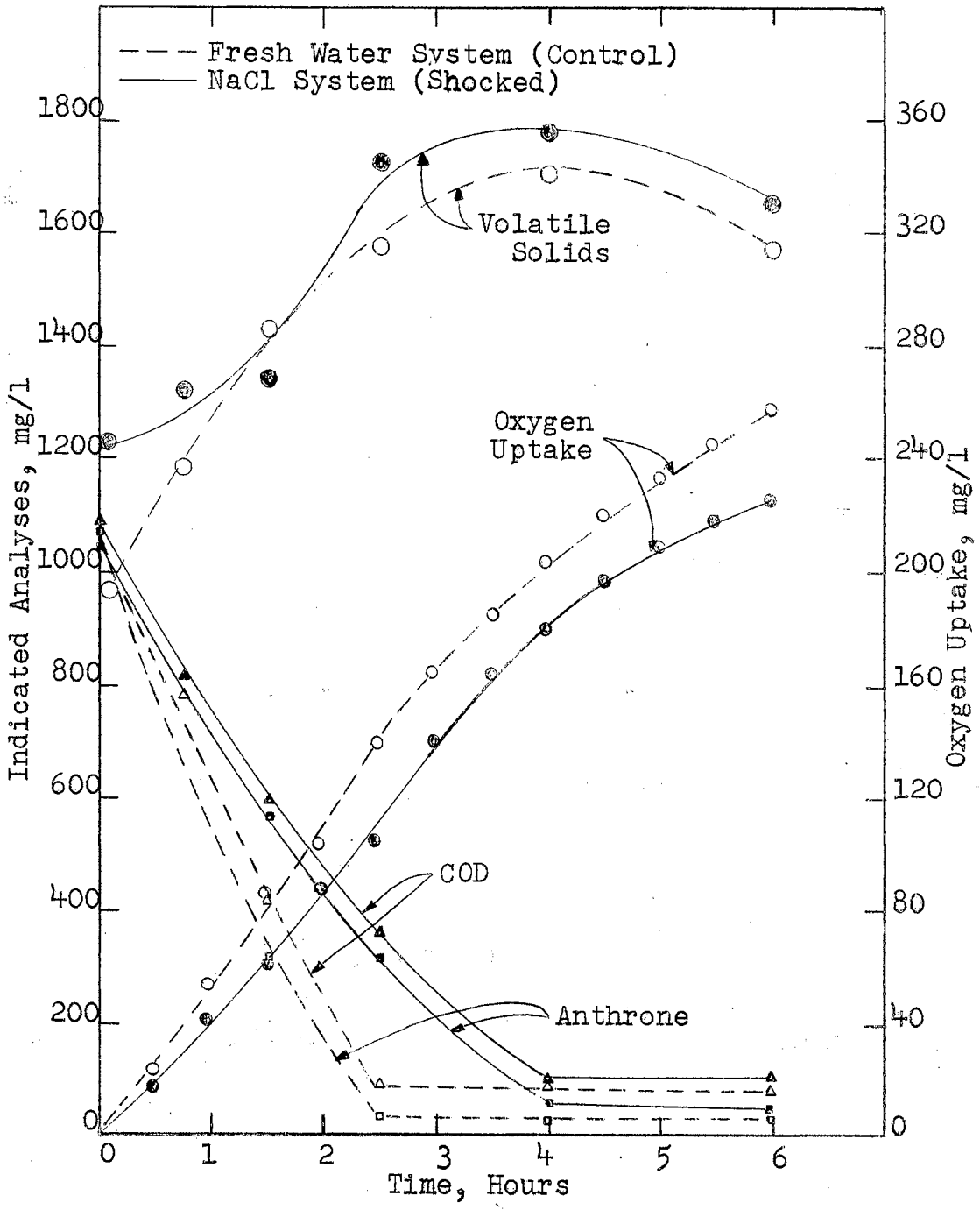

FIGURE 9 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 30,000 mg/l NaCl.

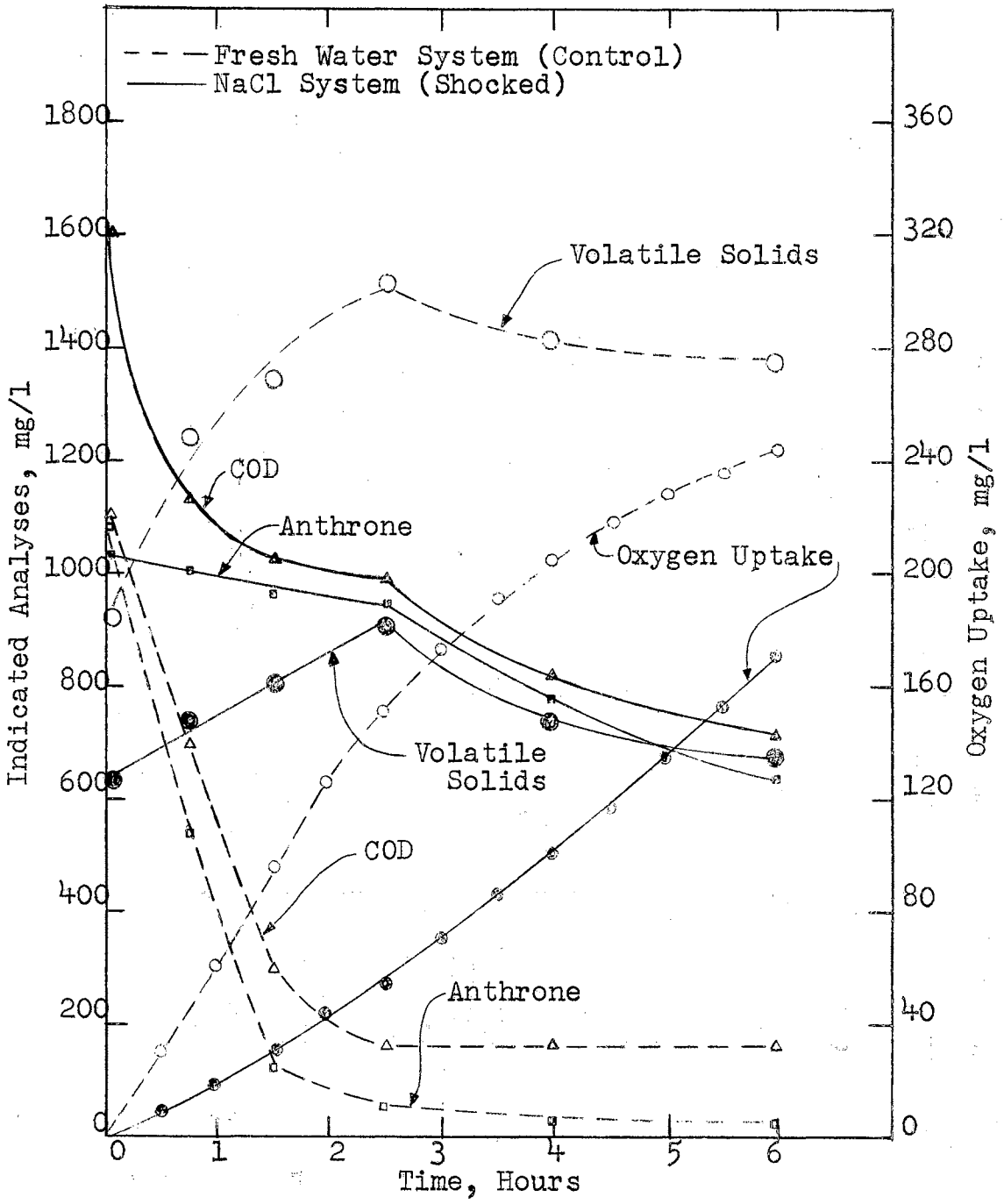

FIGURE 10 CHANGE IN SYSTEM PARAMETERS. OLD FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 45,000 mg/l NaCl.

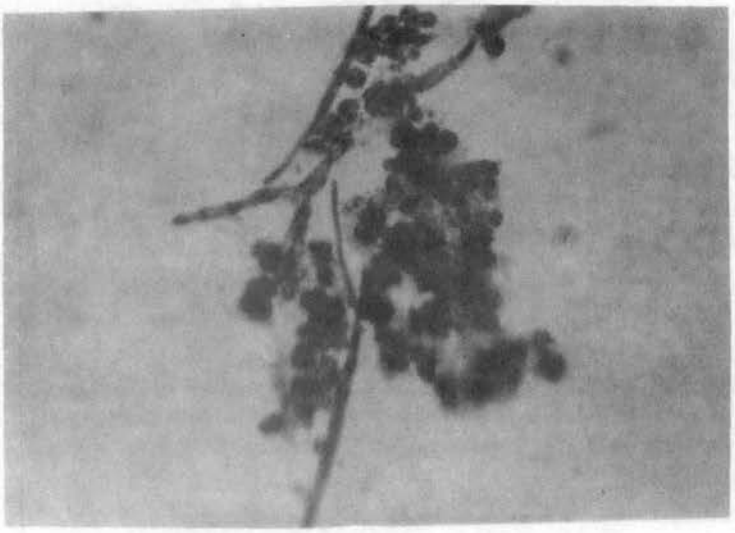

FIGURE 11 PHOTOMICROGRAPH OF SLUDGE FROM A CONTROL UNIT (x 2620)

FIGURE 12 PHOTOMICROGRAPH OF OLD FRESH WATER SLUDGE 5 HOURS AFTER BEING SHOCK LOADED WITH 10,000 mg/l NaCl. (x 2620)

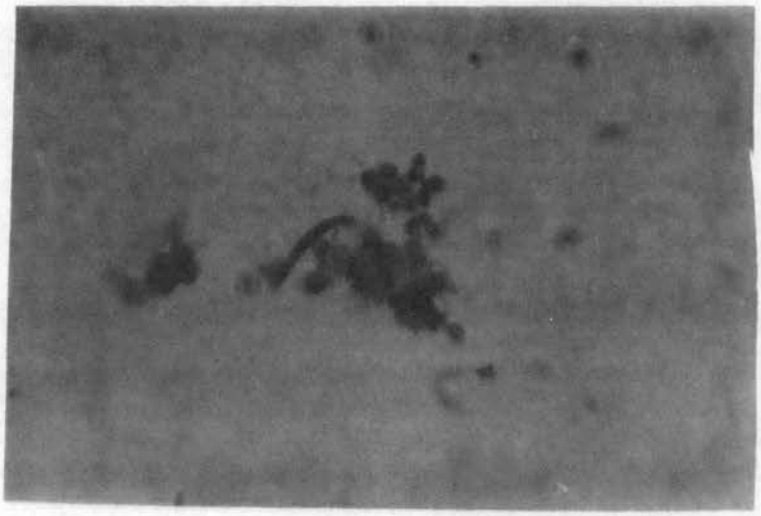

FIGURE 13 PHOTOMICROGRAPH OF OLD FRESH WATER SLUDGE 5 HOURS AFTER BEING SHOCK LOADED WITH 15,000 mg/l NaCl. (x 2620)

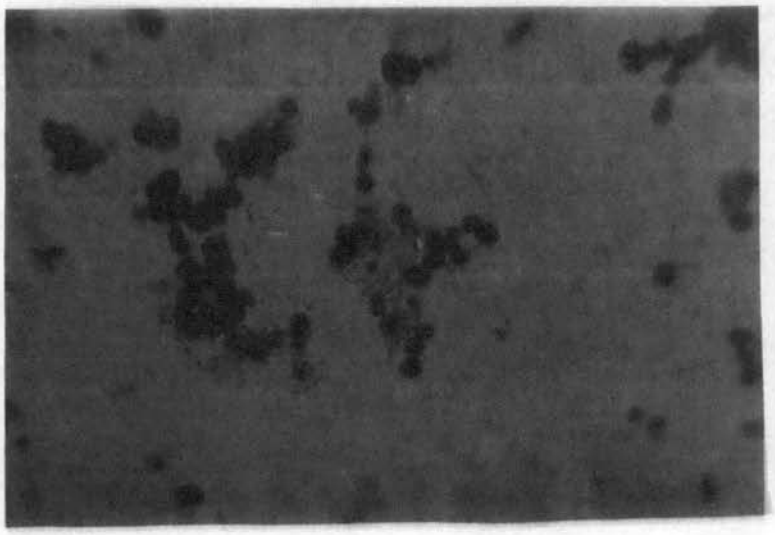

FIGURE 14 PHOTOMICROGRAPH OF OLD FRESH WATER SLUDGE 5 HOURS AFTER BEING SHOCK LOADED WITH 30,000 mg/l NaCl. (x 2620)

oxygen uptake 40 percent greater in the control than in the shocked system.

Microscopic examinations were made on the sludge from the units and the photomicrograph (Figure 11) of the control unit shows a typical activated sludge composed of large floc particles together with bacteria, protozoa, and fungi. Photomicrographs were also taken of the shock-loaded systems approximately five hours after being shocked with the sodium chloride. Figure 12 shows that a sodium chloride concentration of 10,000 mg/l had no affect on the appearance of the sludge. The sludge was still composed of large floc particles together with bacteria, protozoa, and fungi. The unit shocked with 8,000 mg/l sodium chloride also had the same appearance. Sodium chloride concentrations of 12,000, 15,000, and 20,000 mg/l caused the sludge to be a little more dispersed and this is shown by Figure 13. At a sodium chloride concentration of 30,000 mg/l the sludge became even more dispersed and fewer predators were observed (Figure 14). Several tetrad forms of large cocci were also observed.

2. Effects of Long Term Exposure to Sodium Chloride

The changes in system parameters under long term exposure to various concentrations of sodium chloride are compared with the control system in Figures 15 - 23. Figures 15, 16, 17, 18, and 19 show that a long term exposure to sodium chloride concentrations of 1500, 3000, 7500, 12,000, or 15,000 mg/l had little or no effect on the system. The substrate was

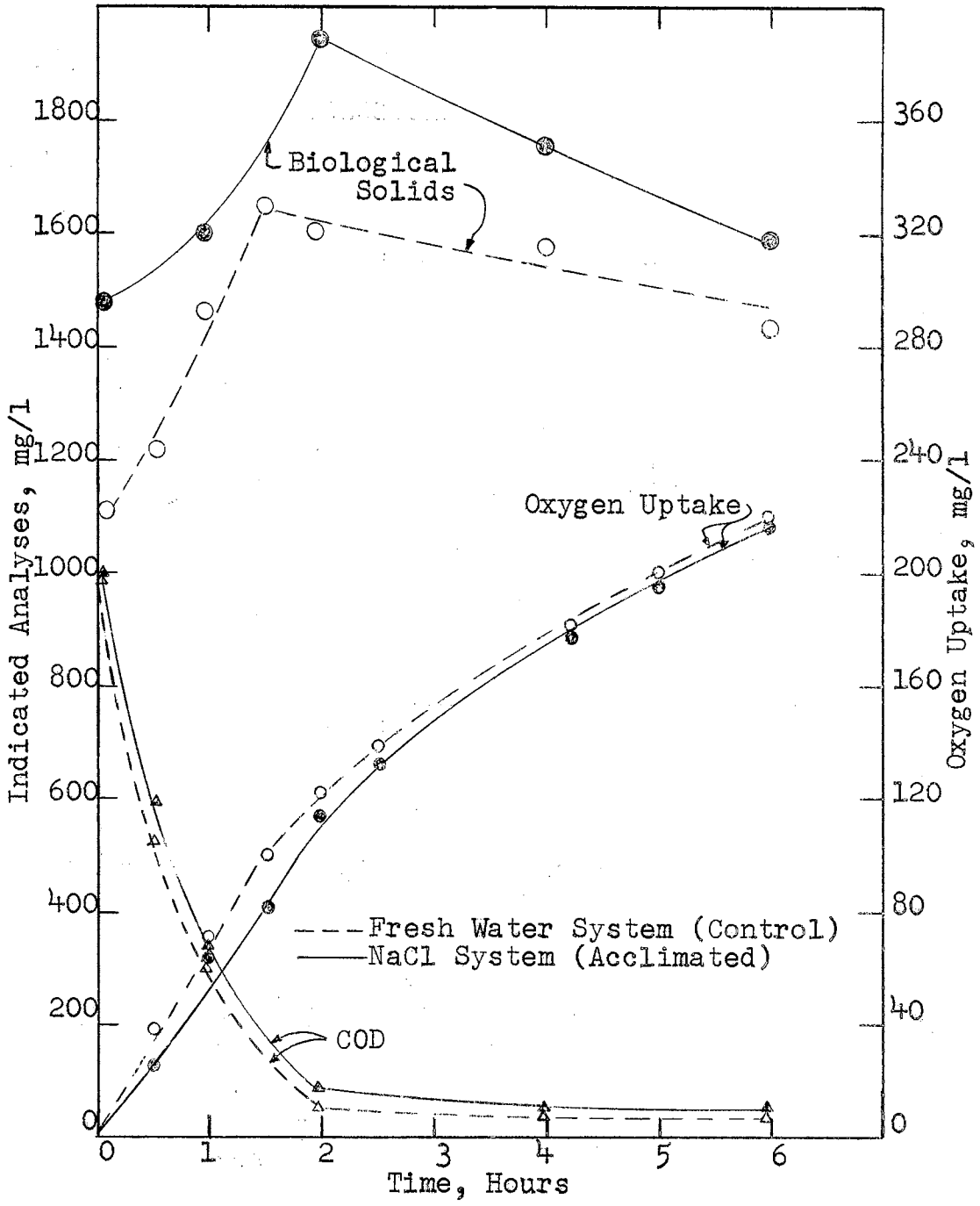

FIGURE 15 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 1500 mg/l NaCl.

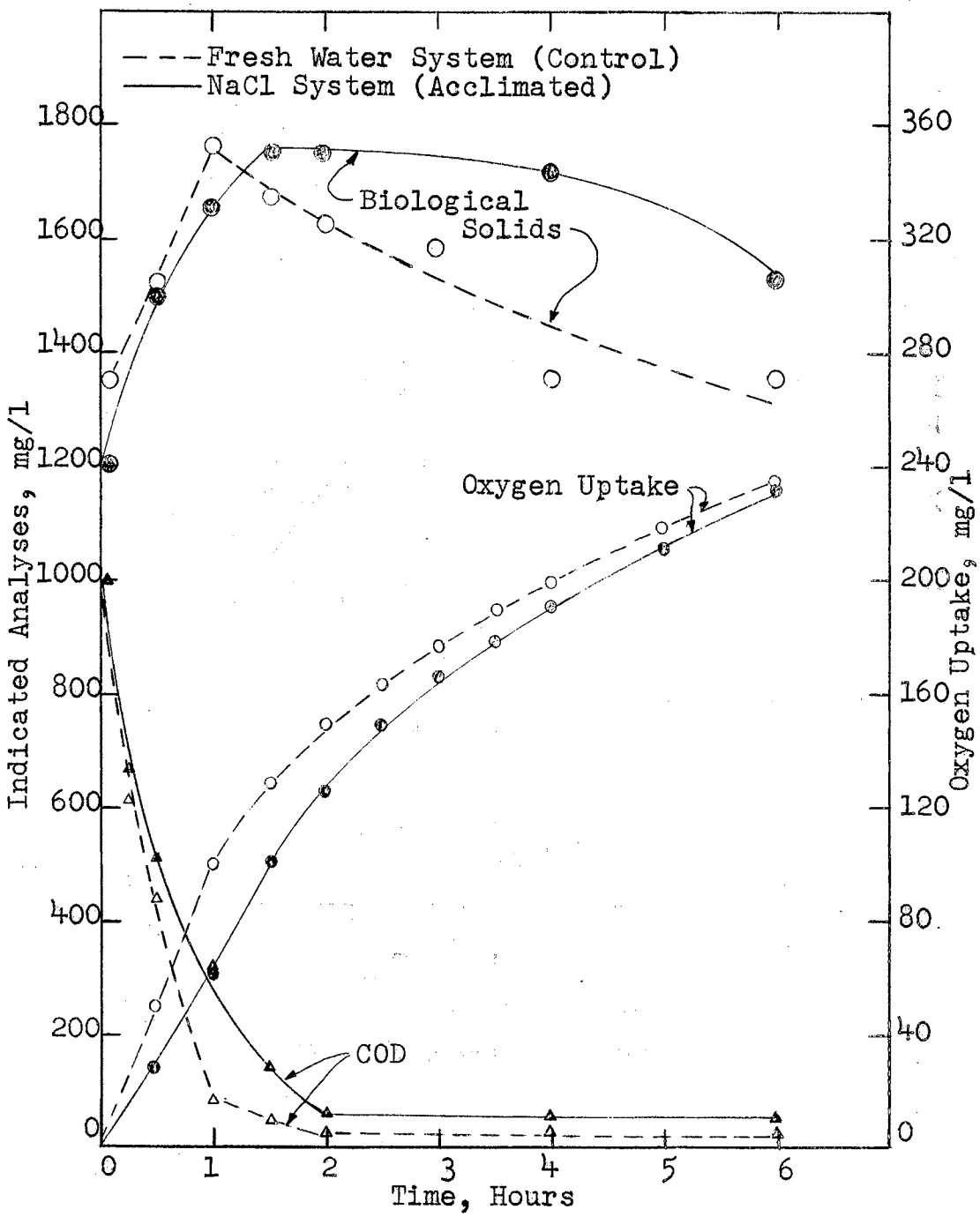

FIGURE 16 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 3,000 mg/l NaCl.

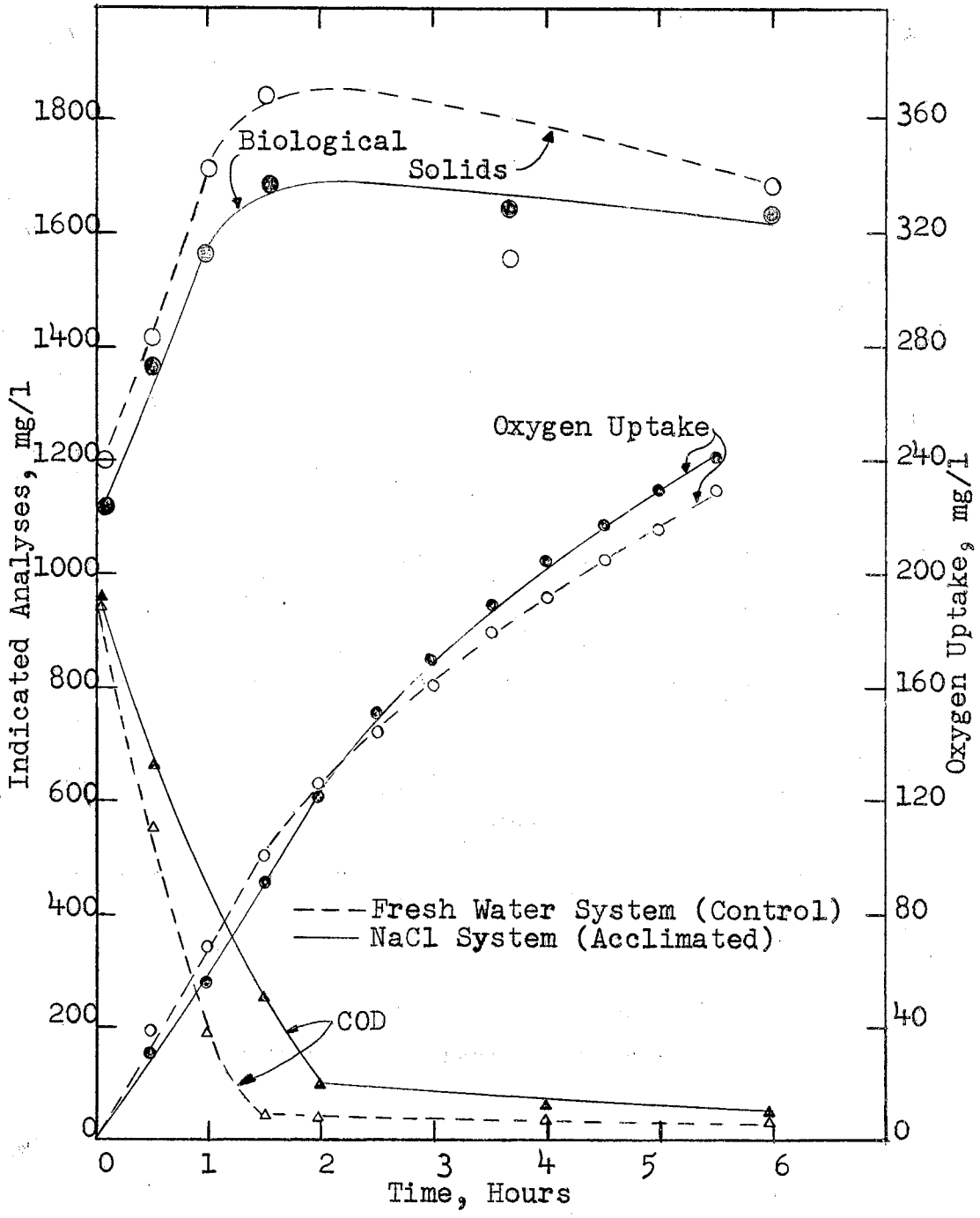

FIGURE 17 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 7,500 mg/l NaCl.

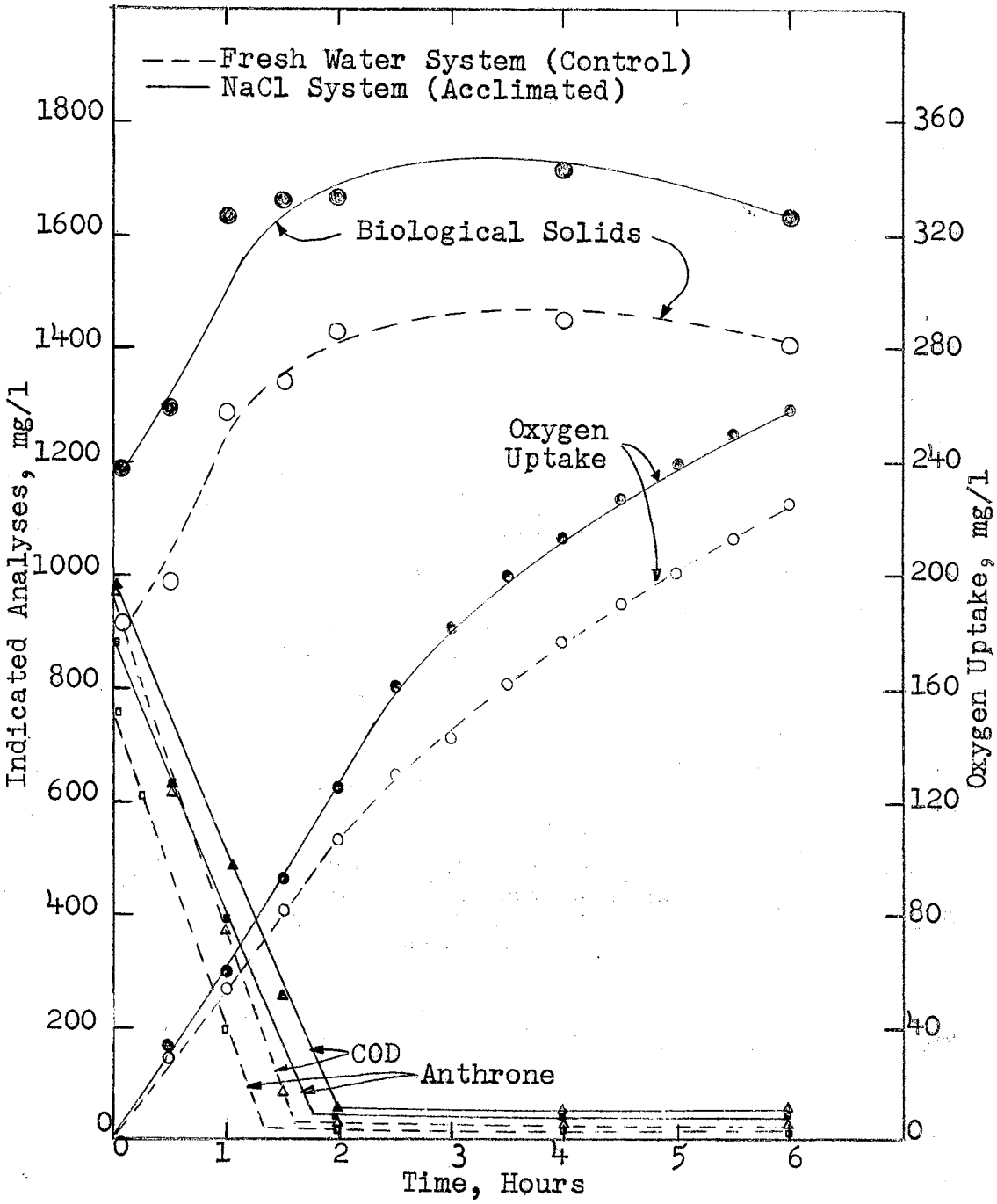

FIGURE 18 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 12,000 mg/l NaCl.

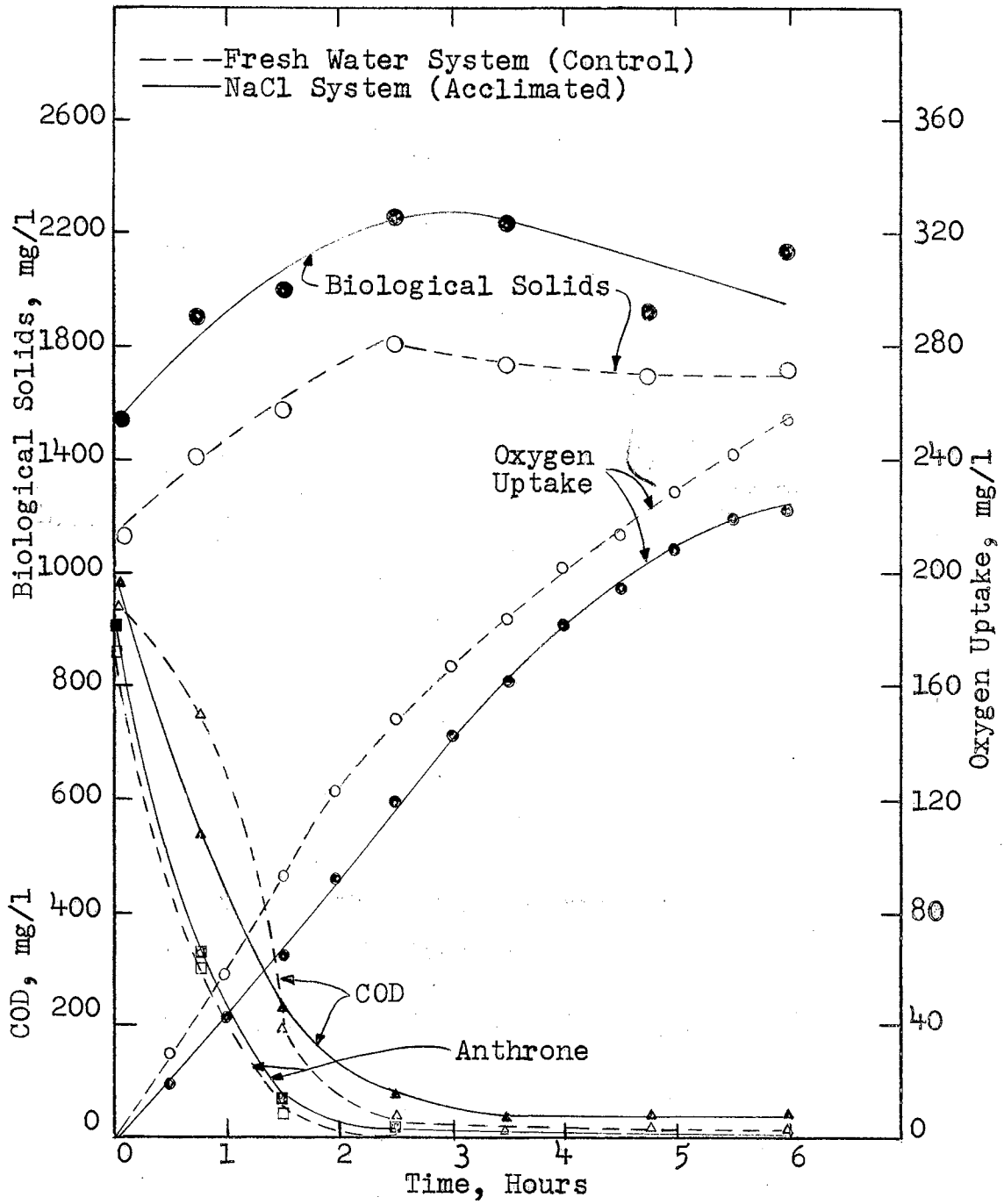

FIGURE 19 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 15,000 mg/l NaCl.

effectively removed within two hours by both the control and experimental units in each experiment. The solids production and the oxygen uptake were approximately the same for both units, and the point of maximum solids production corresponds to the point of maximum COD removal.

The first distinct variations in system performance were observed at a sodium chloride concentration of 18,000 mg/l (Figure 20). The substrate was effectively removed by both units in two and one-half hours, but the solids production in the sodium chloride unit was 38 percent greater than in the control and the oxygen uptake in the sodium chloride unit was 63 percent greater than in the control. Thus a definite stimulation by the sodium chloride was observed. A sodium chloride concentration of 22,500 mg/l (Figure 21) still showed some delayed stimulation, but this stimulation was not nearly as much as 18,000 mg/l sodium chloride. The production of solids and the oxygen uptake in the sodium chloride unit was approximately ten percent greater than in the control system. The sodium chloride unit also required one hour longer than the control unit to remove the substrate.

The effects of long term exposure to 30,000 mg/l sodium chloride are shown in Figure 22. The solids production in the control unit was 33 percent greater than in the sodium chloride unit, but the oxygen uptake was the same in both units. An inhibitory effect on solids production and no effect or a slight stimulatory effect on oxygen uptake by the sodium chloride is indicated. It is also interesting to note

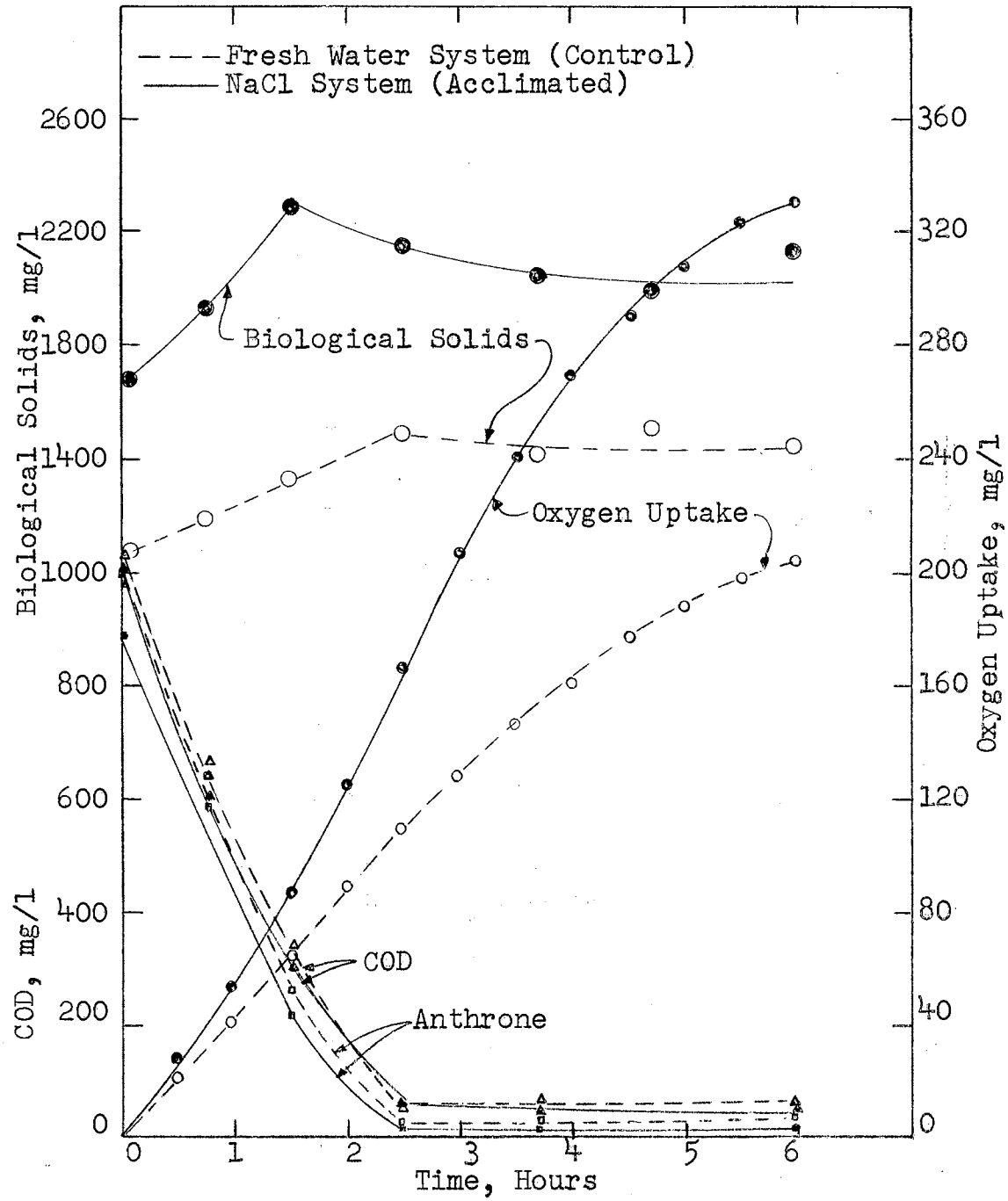

FIGURE 20 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 18,000 mg/l NaCl.

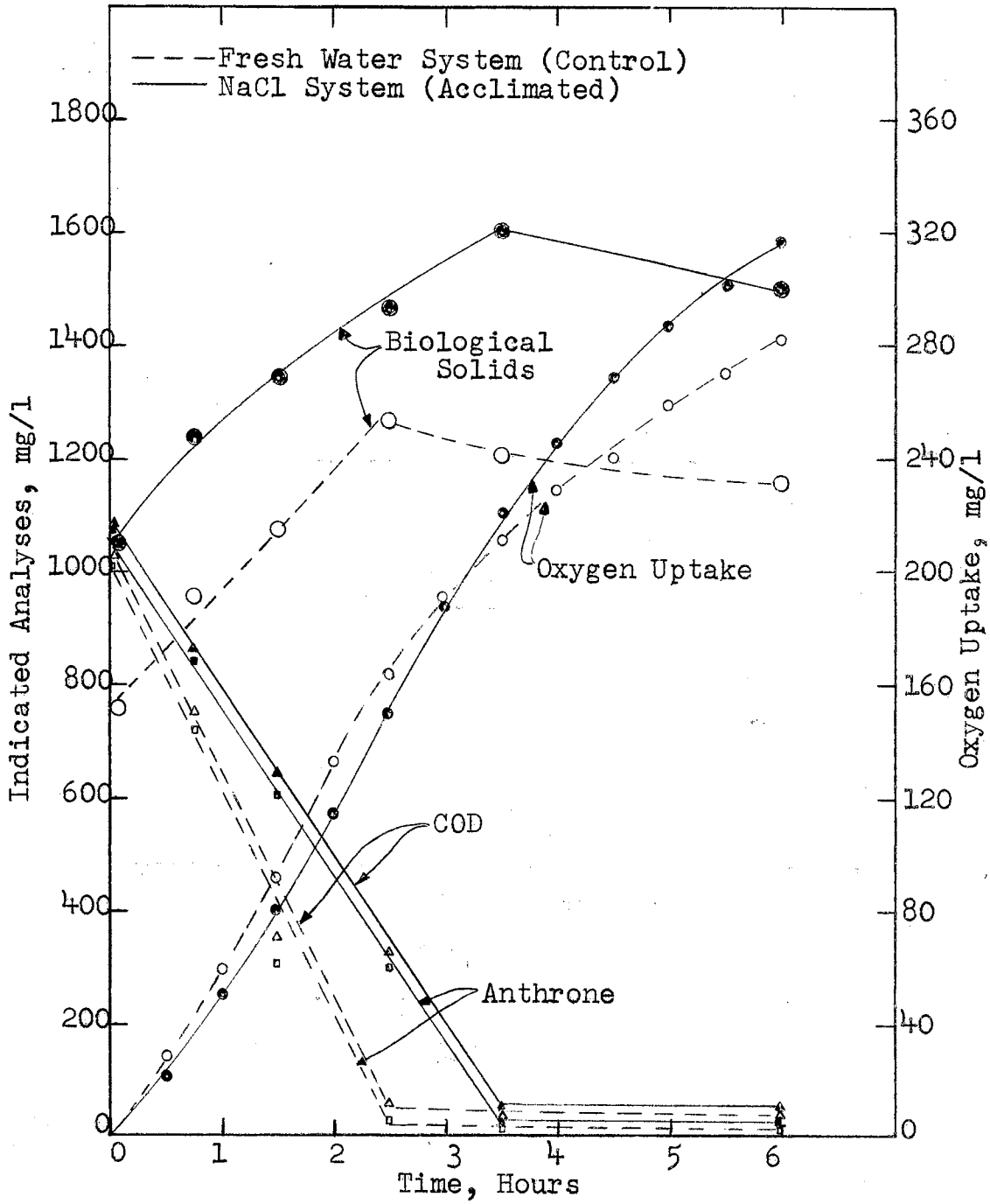

FIGURE 21 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 22,500 mg/l NaCl.

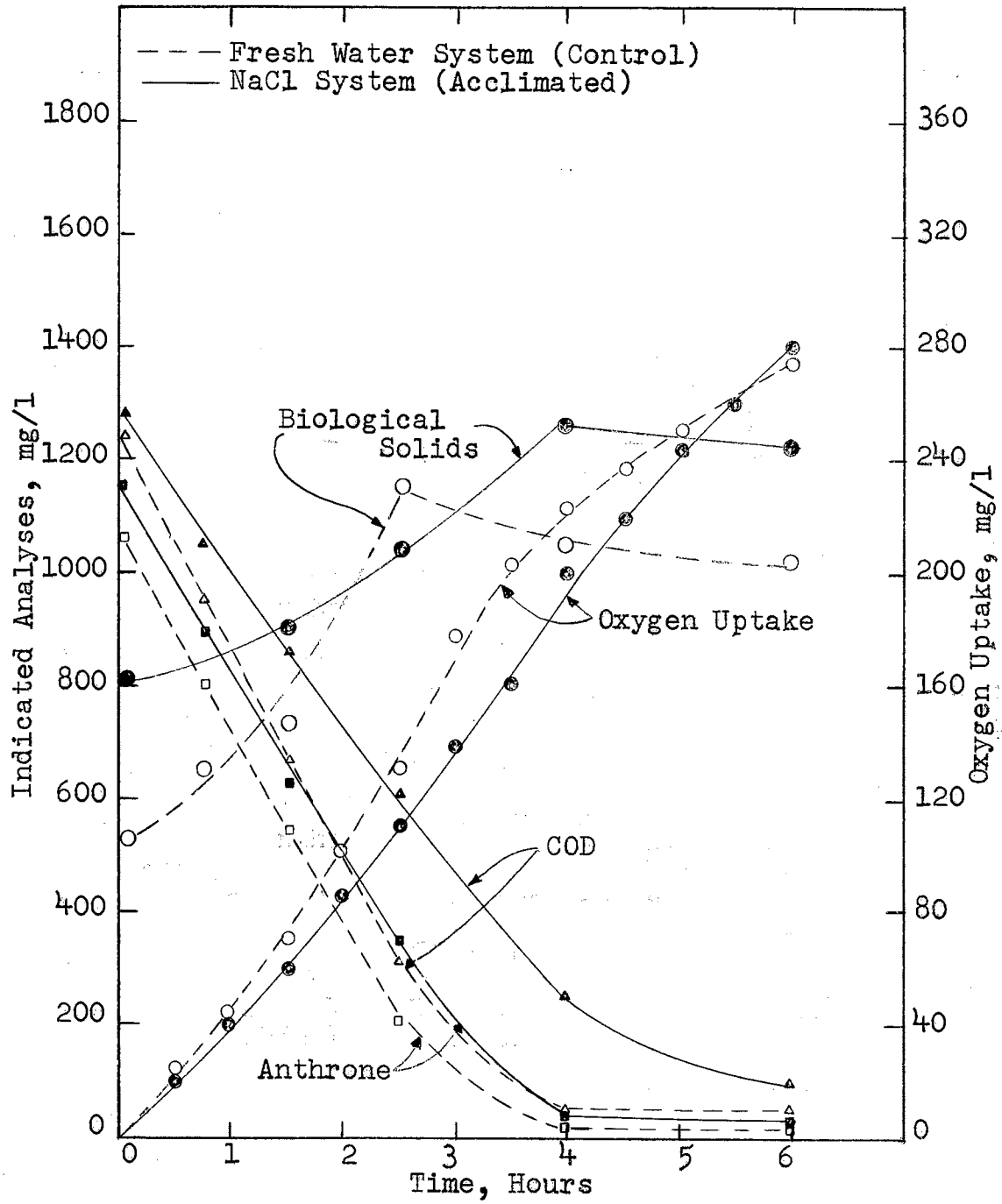

FIGURE 22 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 30,000 mg/l NaCl.

that the glucose was removed in the same length of time by both units, but 250 mg/l of COD still remained in the sodium chloride unit at the point of maximum glucose removal, whereas only 50 mg/l of COD still remained in the control unit. These results indicates a greater release of metabolic intermediates or end products during glucose metabolism in the presence of NaCl.

Figure 23 shows very clearly the effects that high concentrations of sodium chloride (45,000 mg/l) can have on an activated sludge system. It is seen that the efficiency of COD removal was greatly impaired. At the end of six hours of aeration 280 mg/l of COD still remained in the sodium chloride unit even though the glucose had been effectively removed in four hours of aeration. Both the COD and glucose were removed in two and one-half hours in the control unit. From these data it would appear that considerable amounts of metabolic intermediates or end products were again released during glucose metabolism. Also, synthesis of new sludge was greatly impaired in the sodium chloride unit. The increase in solids in the control unit was 130 percent greater than in the sodium chloride unit. There was very little difference in the total oxygen uptake, even though the sodium chloride unit exhibited an initial lag in oxygen uptake.

Microscopic examinations were also made on these systems and may be compared with the typical activated sludge system shown in Figure 11. Figure 24 shows a photomicrograph of a sludge under long term exposure to 12,000 mg/l sodium chloride. It can be seen that 12,000 mg/l sodium chloride had no effect

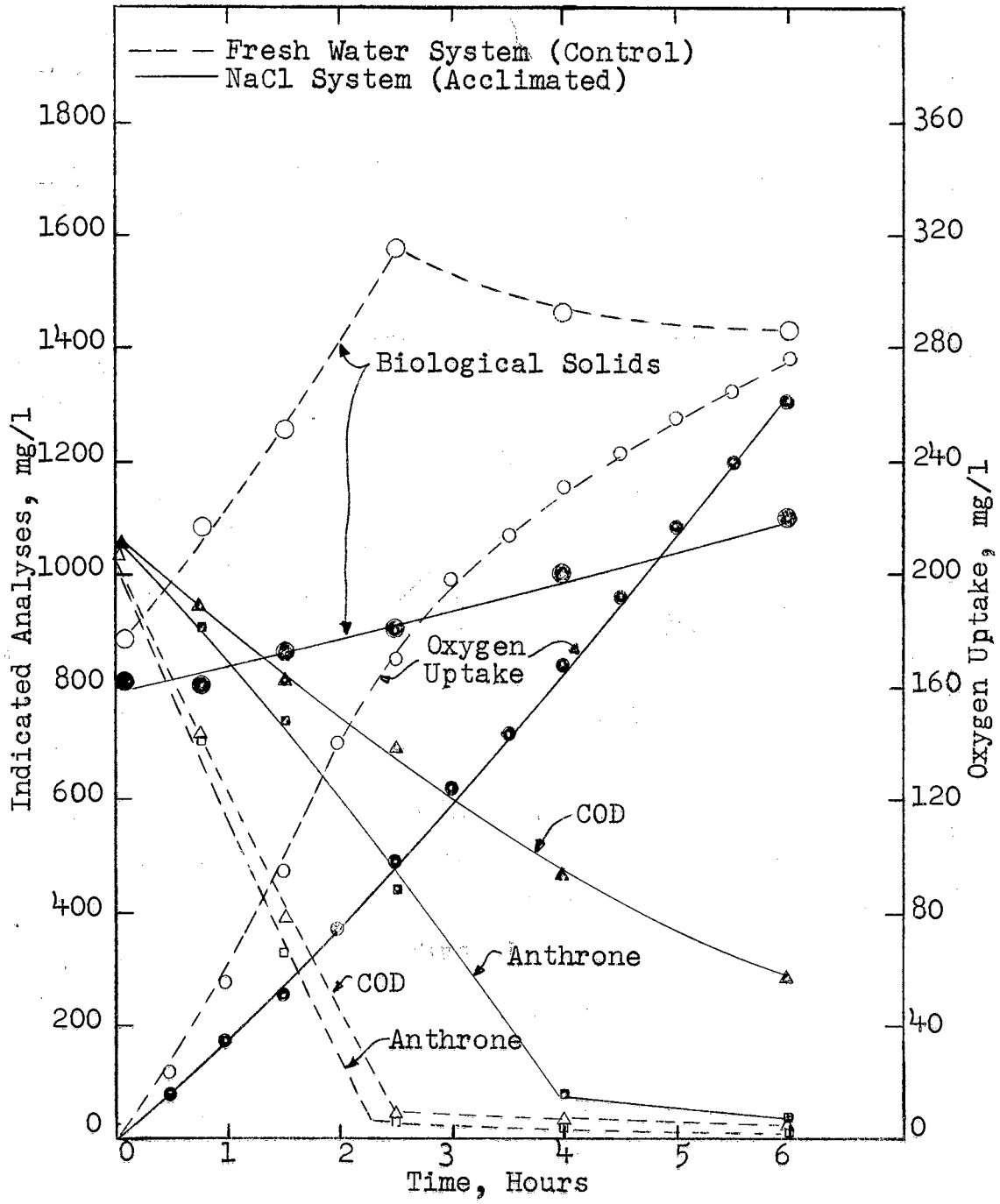

FIGURE 23 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 45,000 mg/l NaCl.

FIGURE 24 PHOTOMICROGRAPH OF A SLUDGE UNDER LONG TERM EXPOSURE TO 12,000 mg/l NaCl. (x 2620)

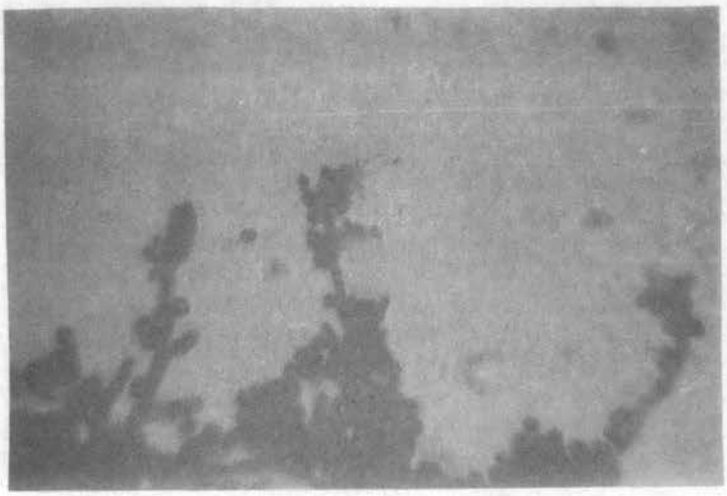

FIGURE 25 PHOTOMICROGRAPH OF A SLUDGE UNDER LONG TERM EXPOSURE TO 18,000 mg/l NaCl. (x 2620)

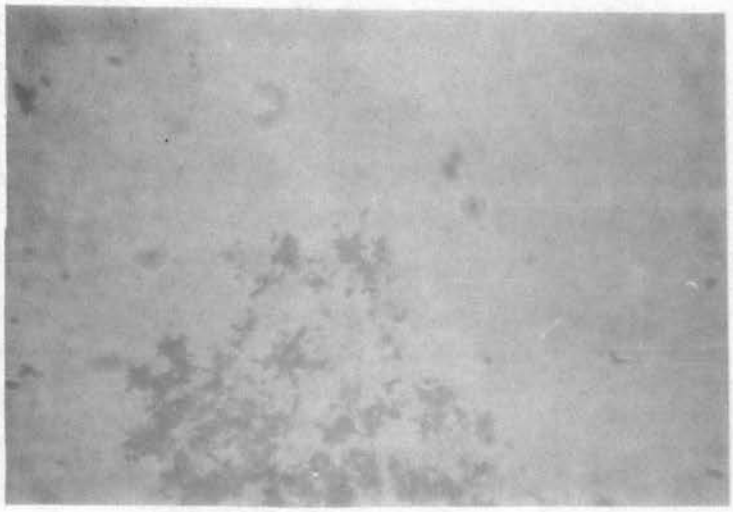

FIGURE 26 PHOTOMICROGRAPH OF A SLUDGE UNDER LONG TERM EXPOSURE TO 30,000 mg/l NaCl. (x 2620)

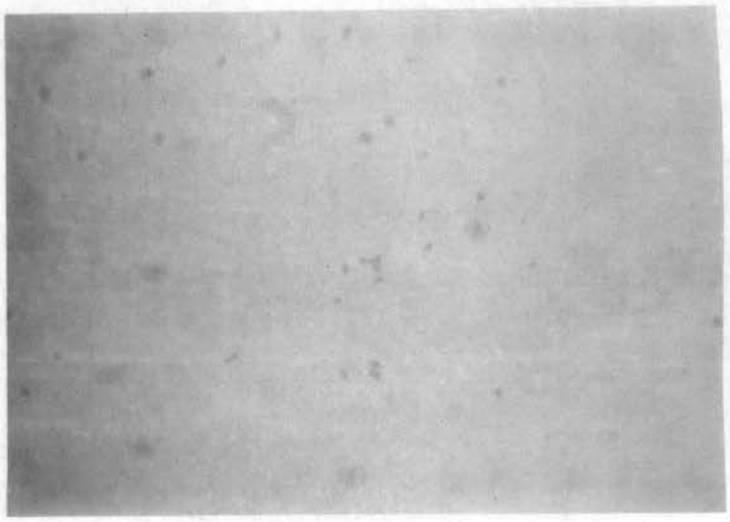

FIGURE 27 PHOTOMICROGRAPH OF A SLUDGE UNDER LONG TERM EXPOSURE TO 45,000 mg/l NaCl. (x 2620)

on the appearance of the sludge. The sludge consisted of large floc particles together with bacteria, protozoa, and fungi. Sodium chloride concentrations of 15,000, 18,000, and 22,500 mg/l produced a sludge that was a little more dispersed but still had floc particles and predators. Figure 25 shows a photomicrograph of a sludge under long term exposure to 18,000 mg/l sodium chloride. This sludge is characteristic for all three concentrations.

Figure 26 shows the changes in sludge characteristics when grown in 30,000 mg/l sodium chloride. The sludge was dispersed, with very few large floc particles in evidence. A few predators were present but were not nearly as numerous as before. The predominating organisms are small cocci. Figure 27 shows a photomicrograph of a sludge after long term exposure to 45,000 mg/l sodium chloride. The sludge was rather dispersed, very few predators were found, and the predominating bacteria were small cocci. It is interesting to note that the bacteria were much smaller than those observed in the control or the shocked systems.

3. Effects of Shock Loading an Old Sludge Acclimated To Sodium Chloride With Fresh Water

The responses of glucose-acclimated sludges, grown in synthetic wastes containing 10,000, 30,000, and 45,000 mg/l sodium chloride, when shocked with fresh water medium are shown in Figures 28, 29, and 30. In Figure 28 it is seen that an old sludge grown in 10,000 mg/l sodium chloride was

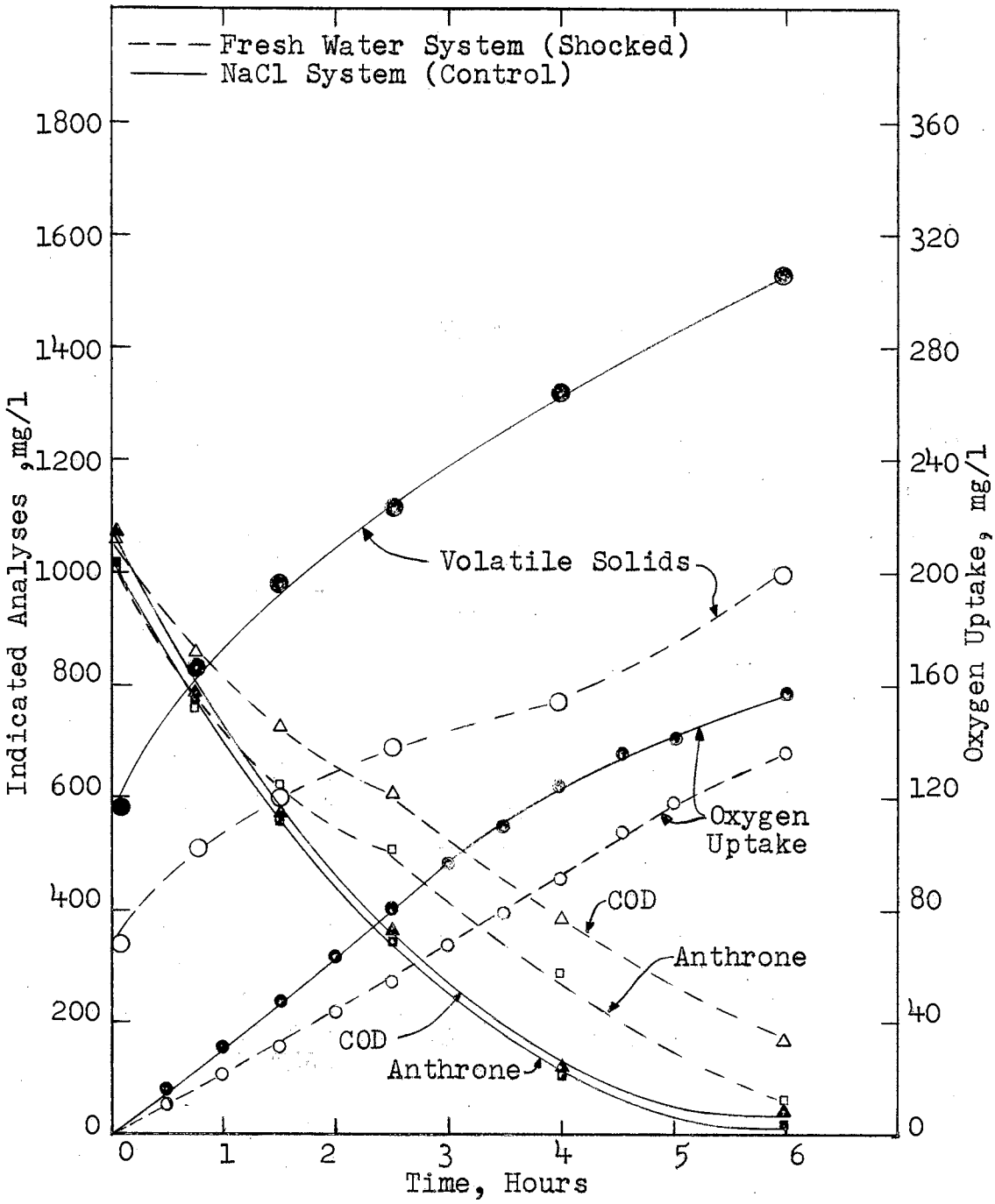

FIGURE 28 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 10,000 mg/l NaCl SHOCKED WITH FRESH WATER.

affected very little when shocked with fresh water. The COD removal in the fresh water-shocked system was retarded very little and the oxygen uptake was approximately the same in both units, as was the increase in solids concentration. The total solids production was a little greater in the sodium chloride unit which might indicate a stimulation in growth by the salt. Comparison of the initial glucose and COD values immediately after dosing with fresh water indicates that there was no release of cellular components due to the fresh water shock.

In Figure 29 it is seen that an old sludge grown in 30,000 mg/l sodium chloride could effectively remove the substrate when salt concentration remained at this level, whereas the same sludge system devoid of sodium chloride underwent a considerable loss of efficiency. Comparison of the initial glucose and COD values immediately after dosing with fresh water indicates that there was an immediate release of cellular components. The glucose concentration was 1015 mg/l, whereas the COD concentration was 1200 mg/l. Comparison of the COD and glucose removal curves indicates a continual release of cellular components or metabolic intermediates for the first two and one-half hours of aeration. This was shown by a gradual decrease in COD while there was a rapid decrease in glucose. This was further borne out by the solids curve. The solids concentration decreased slightly during the first two hours of aeration. The loss in efficiency of the fresh water shocked system is shown by the fact that

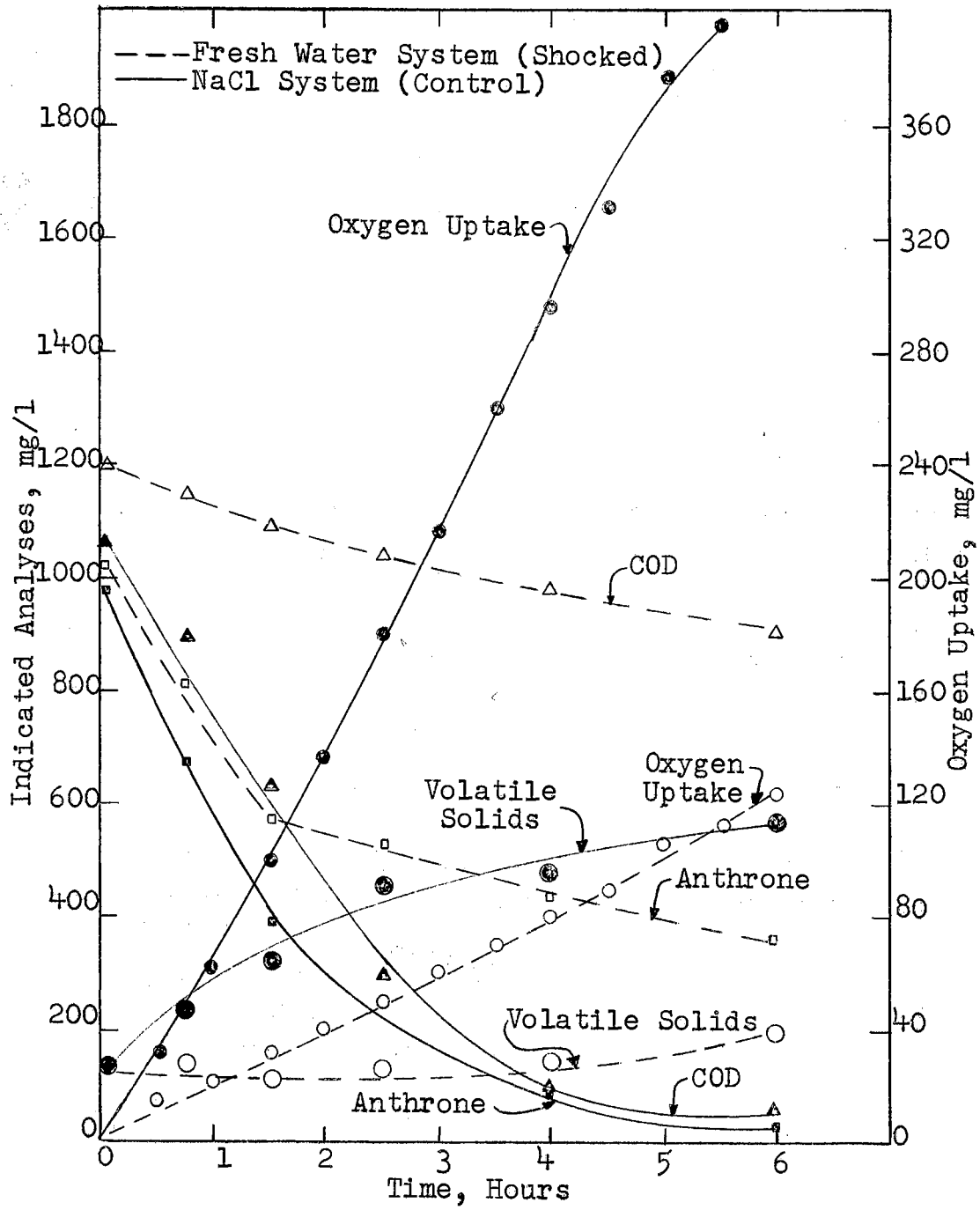

FIGURE 29 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 30,000 mg/l NaCl SHOCKED WITH FRESH WATER.

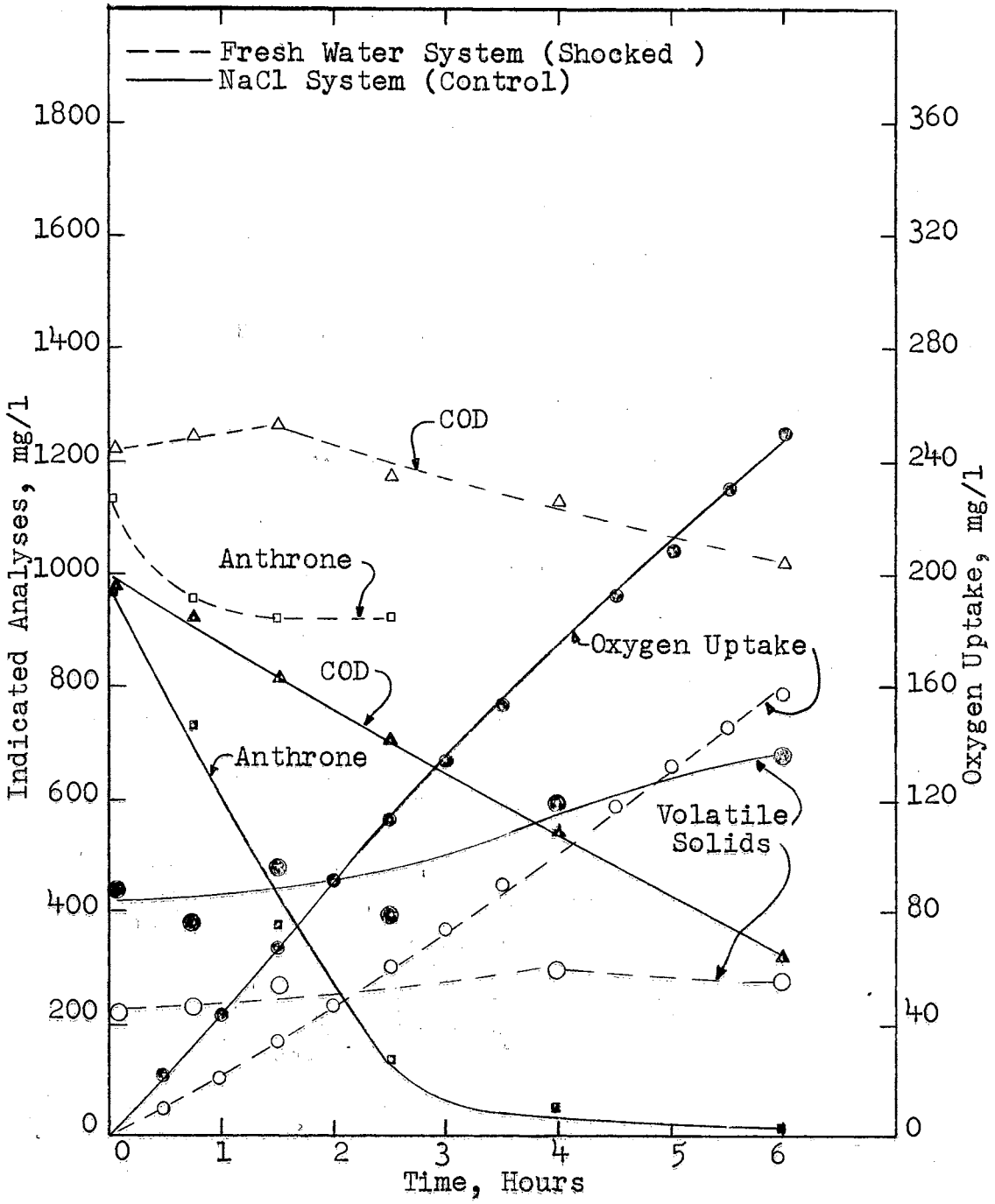

FIGURE 30 CHANGE IN SYSTEM PARAMETERS. OLD SLUDGE ACCLIMATED TO 45,000 mg/l NaCl SHOCKED WITH FRESH WATER.

only 200 mg/l of COD was removed in six hours of aeration. Also the solids production in the salt unit was 620 percent greater and the oxygen uptake 250 percent greater than in the fresh water-shocked system.

In Figure 30 it is seen that an old sludge grown in 45,000 mg/l sodium chloride could remove glucose in a reasonable aeration period when the salt concentration was maintained at the same level. However, the removal of total COD was greatly retarded as compared with the results shown in Figure 29 for sludge grown on 30,000 mg/l salt. From these data it would appear that considerable amounts of metabolic intermediates or end products were released during glucose metabolism. When a portion of the same sludge was placed in the growth medium made up in fresh water, an initial increase in COD of approximately 200 mg/l resulted, indicating an immediate release of cellular constituents. There was a slow rise in COD during the first one and one-half hours of aeration. It is seen that very little glucose was removed, and it is also evident on the basis of oxygen uptake and biological solids production that the salt-grown sludge could not respond successfully to the rapid decrease in salt concentration.

4. Effects of Shock Loading Young Fresh Water Sludge With Sodium Chloride

The changes in system parameters for a young fresh water sludge shock-loaded with sodium chloride are compared with the control system im Figures 31 and 32. Figure 31 shows that a

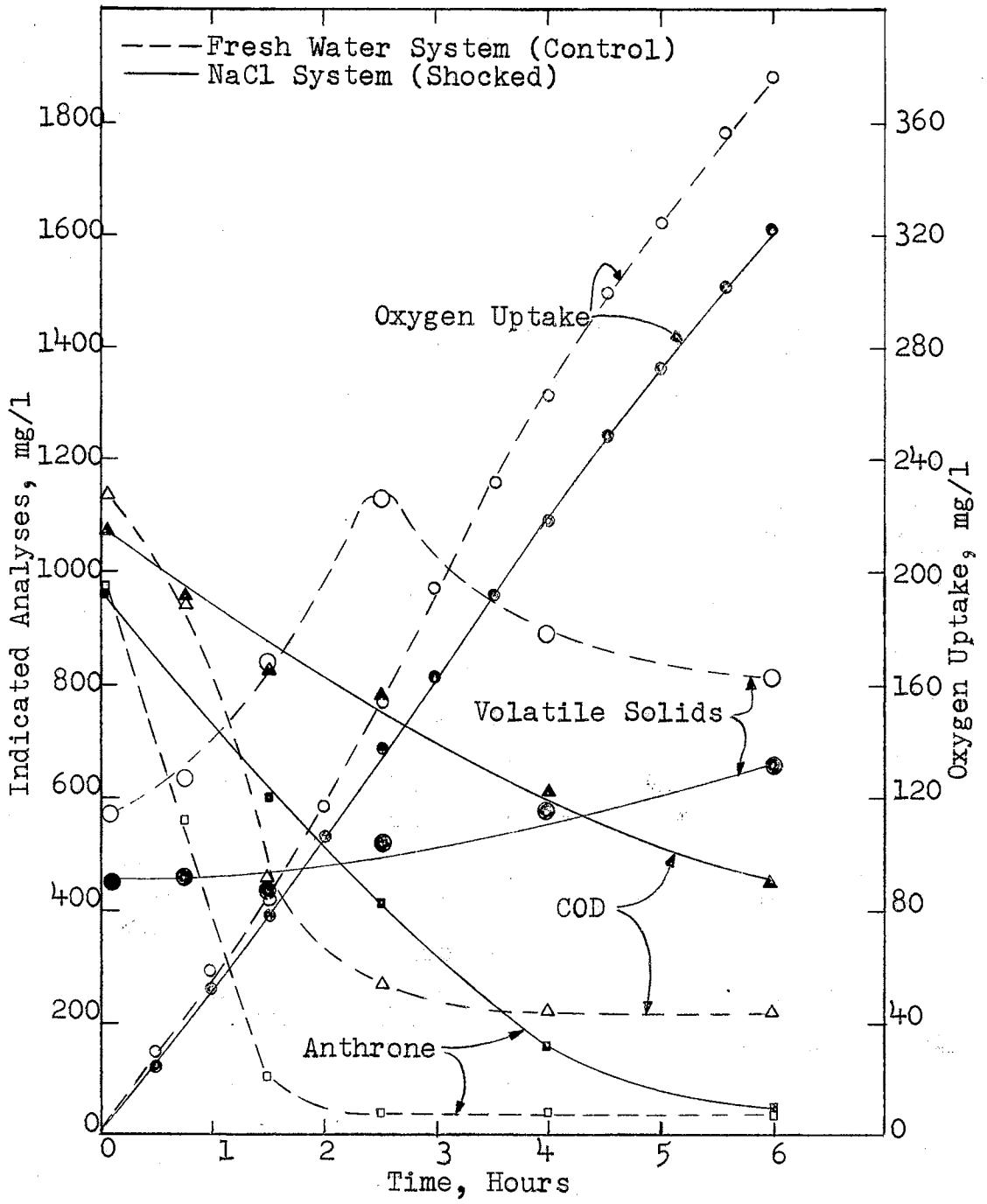

FIGURE 31 CHANGE IN SYSTEM PARAMETERS. YOUNG FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 30,000 mg/l NaCl.

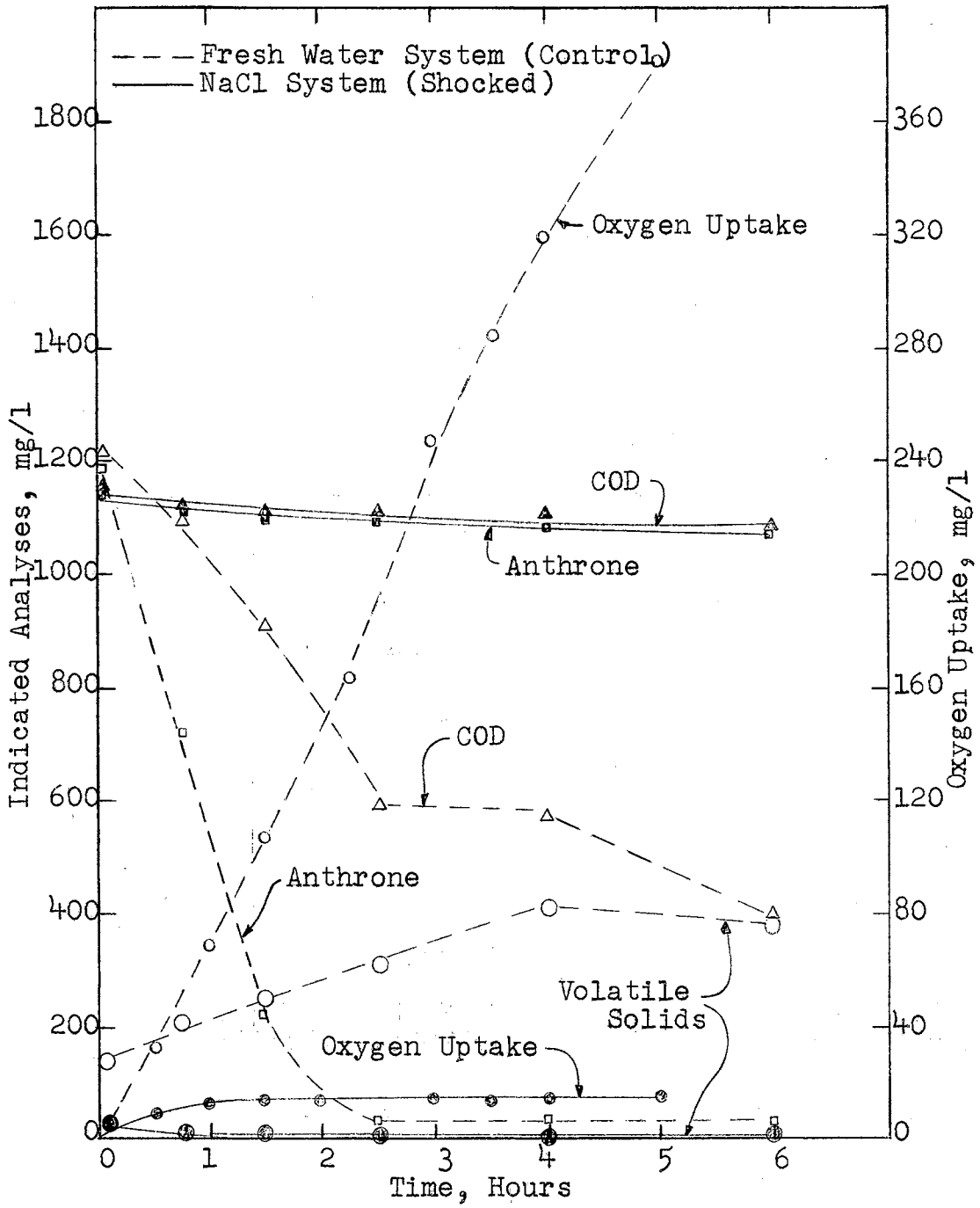

FIGURE 32 CHANGE IN SYSTEM PARAMETERS. YOUNG FRESH WATER SLUDGE SUBJECTED TO SHOCK LOAD OF 45,000 mg/l NaCl.

shock loading of 30,000 mg/l sodium chloride causes a considerable loss in efficiency. The substrate was effectively removed in two and one-half hours in the control system, whereas 450 mg/l of COD still remained in the shocked system after six hours of aeration. The glucose was completely removed in six hours in the shocked system thus indicating the release of metabolic intermediates or end products during glucose metabolism. The increase in solids in the control was 160 percent greater than in the shocked system, whereas the oxygen uptake was only 12 percent greater indicating a considerable shift in the ratio of respiration to synthesis.

In Figure 32 it can be seen that total destruction of system efficiency resulted from a shock loading of 45,000 mg/l sodium chloride. The biological solids decreased to zero and there was no oxygen uptake or substrate removal observed after the first hour of aeration. It is interesting to note that metabolic intermediates or end products were being released in the control system during glucose metabolism and that the sludge required an apparent acclimation period before they were removed.

5. Effects of Shock Loading A Young Sludge Acclimated To Sodium Chloride With Fresh Water

The responses of young glucose-acclimated sludges, grown in synthetic wastes containing 10,000, 30,000, and 45,000 mg/l sodium chloride, when shocked with fresh water medium are shown in Figures 33, 34, and 35. In Figure 33 it is seen that a

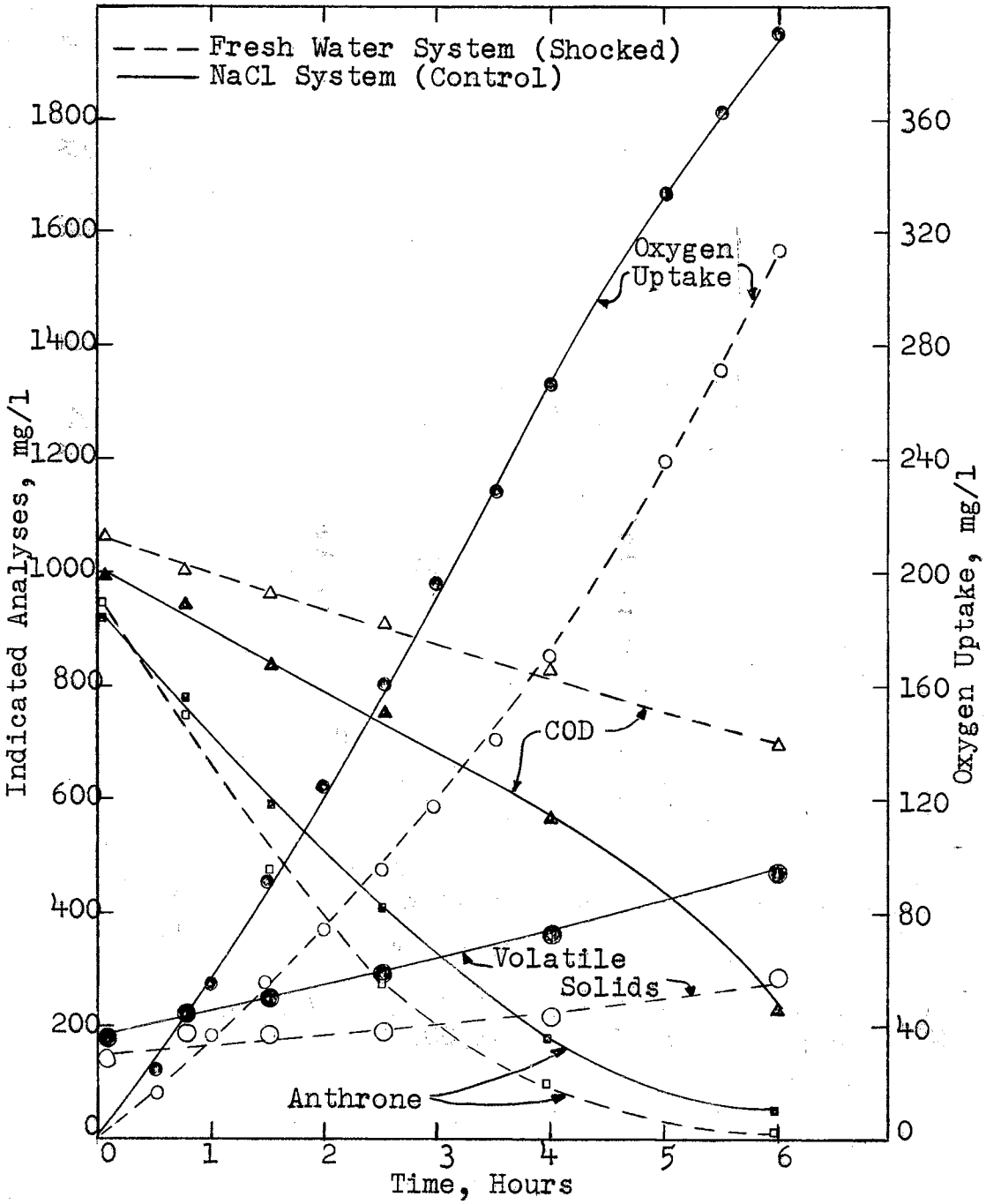

FIGURE 33 CHANGE IN SYSTEM PARAMETERS. YOUNG SLUDGE ACCLIMATED TO 10,000 mg/l NaCl SHOCKED WITH FRESH WATER.

young sludge grown in 10,000 mg/l sodium chloride was affected very little when shocked with fresh water. The substrate removal, solids production, and oxygen uptake were fairly similar for both systems. The oxygen uptake of the shocked system did show a lag as compared to the control. Both systems released a considerable amount of metabolic intermediates or end products during glucose metabolism.

In Figure 34 it is seen that a young sludge grown in 30,000 mg/l sodium chloride could remove glucose in a reasonable aeration period when the sodium chloride concentration was maintained at that level. Considerable amounts of metabolic intermediates or end products were released. When a portion of the same sludge was shocked with a fresh water medium, an initial increase of COD of approximately 100 mg/l resulted, indicating an immediate release of cellular components. However, the system recovered rapidly and effectively removed the COD in six hours of aeration. The increase in solids was approximately 100 percent greater in the shocked system and the oxygen uptake approximately 20 percent greater than in the acclimated system. It is interesting to compare these results with those obtained when an old sludge grown in 30,000 mg/l sodium chloride was shocked with fresh water. It was seen in Figure 29 that when the old sludge was dosed with fresh water the system underwent a considerable loss of efficiency, whereas the young sludge recovered from the initial shock and had an overall efficiency better than the control.

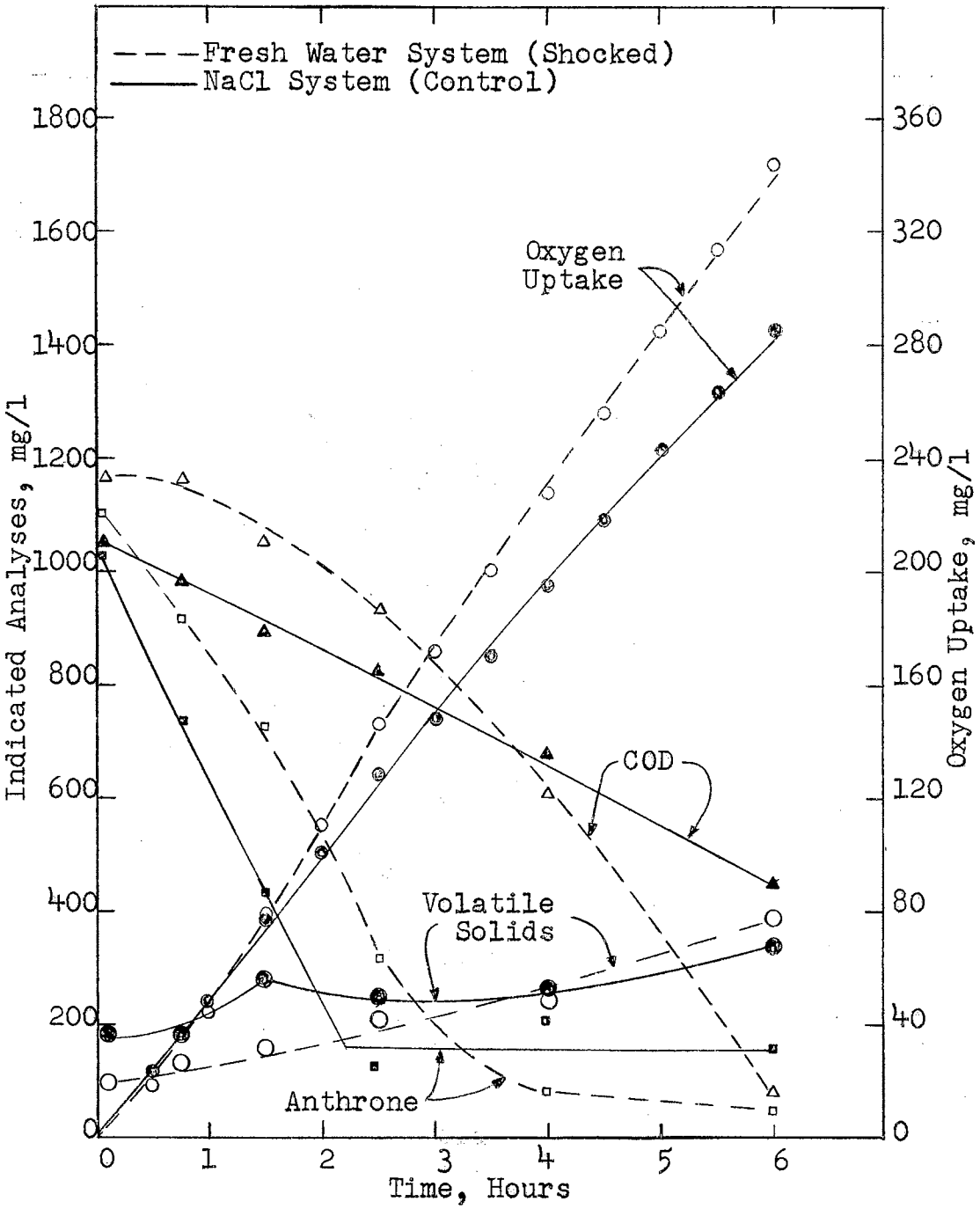

FIGURE 34 CHANGE IN SYSTEM PARAMETERS. YOUNG SLUDGE ACCLIMATED TO 30,000 mg/l NaCl SHOCKED WITH FRESH WATER.

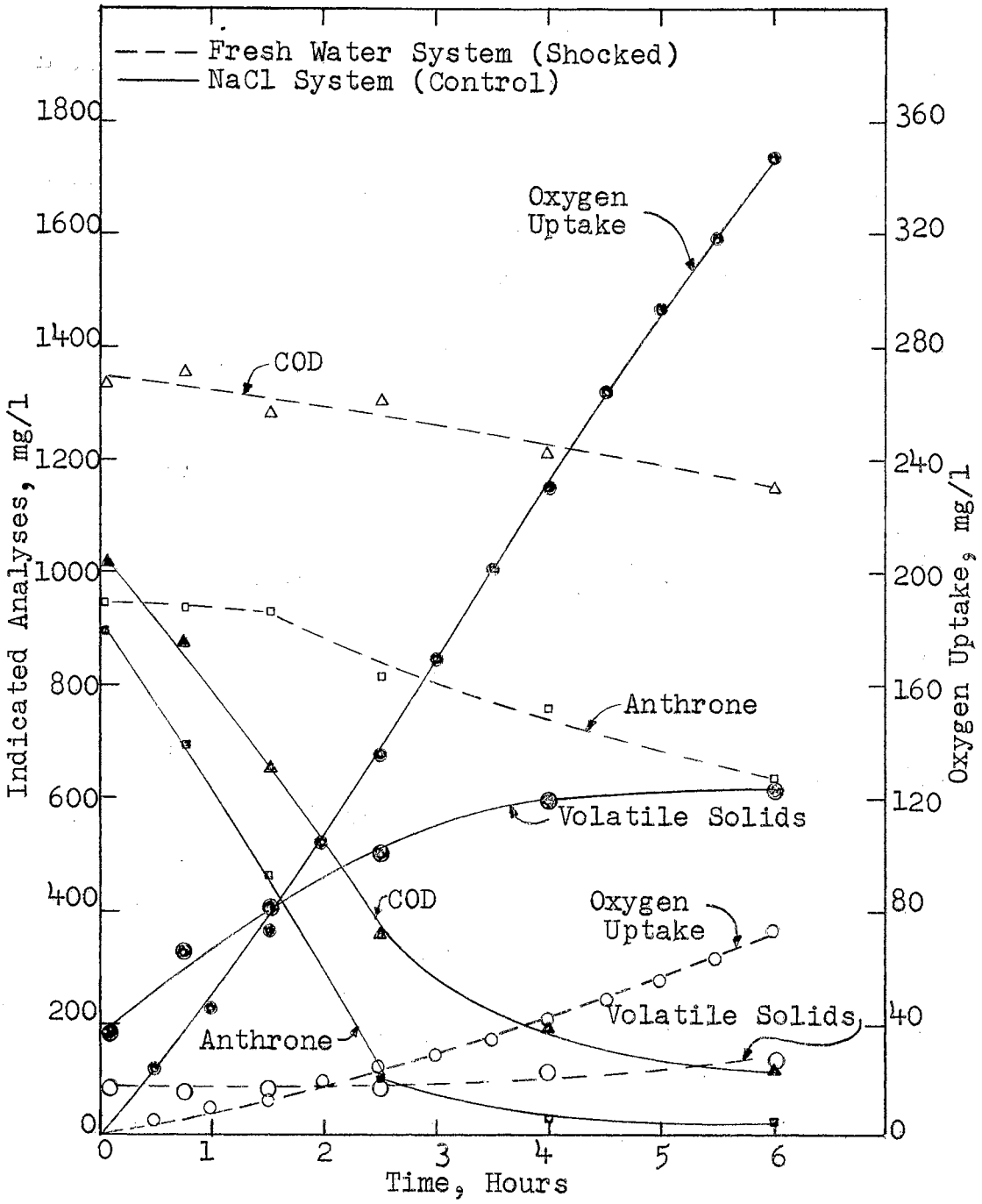

FIGURE 35 CHANGE IN SYSTEM PARAMETERS. YOUNG SLUDGE ACCLIMATED TO 45,000 mg/l NaCl SHOCKED WITH FRESH WATER.

In Figure 35 it is seen that a young sludge grown in 45,000 mg/l sodium chloride could effectively remove the glucose when the salt concentration was maintained at that level. However, when a portion of the same sludge was shocked with a fresh water medium, an initial increase of COD of approximately 400 mg/l resulted, again indicating an immediate release of cellular components, and almost complete destruction of system efficiency occurred. Only 180 mg/l of COD was removed in six hours of aeration with an accumulated oxygen uptake of only 70 mg/l and an increase in solids of only 50 mg/l.

B. Continuous Flow Activated Sludge System

1. Response of System To Shock Loading of 30,000 mg/l Sodium Chloride

a. Response to gradual shock loading: Figure 36 shows the general response of a continuous flow activated sludge system to a gradual shock loading of 30,000 mg/l sodium chloride. In the steady-state condition before the shock loading the volatile solids were maintained at approximately 320 mg/l with a residual COD of 120 mg/l and a glucose residual of 15 mg/l. It is seen that the residual COD is approximately 100 mg/l greater than the residual glucose. Such residual COD cannot be attributed solely to the interference of the inorganic salts in the waste medium. According to Komolrit (57) the COD due to the inorganic salt interference for this medium will never exceed 30 to 50 mg/l. Therefore, these results tend to indicate that metabolic

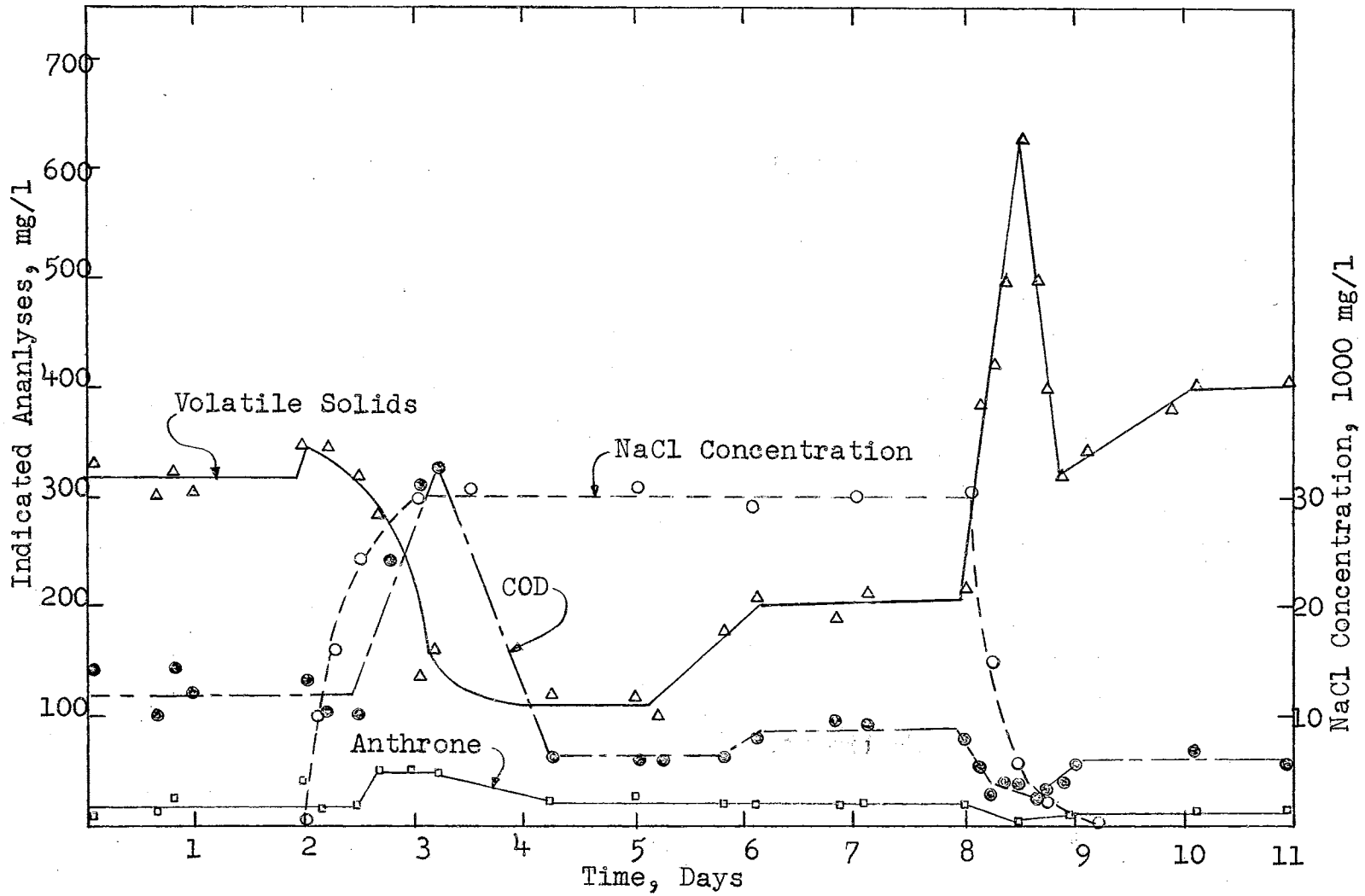

FIGURE 36 RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 8 HOUR DETENTION AND SHOCK LOADING OF 30,000 mg/l NaCl.

intermediates or end products are being released during glucose metabolism. The release of these materials was also observed in the earlier batch studies. It is interesting to note that the volatile biological solids were increased by approximately 50 mg/l at the lower concentrations of sodium chloride (8,000 to 10,000 mg/l) and as the salt concentration increased beyond this level the volatile solids decreased to a low of 110 mg/l. During this decrease in solids there was a sharp increase in residual COD and glucose, but as soon as the solids began to level off, the residual COD and glucose dropped sharply. The COD decreased to a value less than before the salt shock, whereas the glucose decreased to a value slightly greater than before the shock. Between three and four days after the salt shock the system response changed without any change in the sodium chloride concentration. The volatile solids increased by approximately 100 mg/l and the residual COD increased by 25 mg/l. There was no change in the residual glucose.

Six days after being shocked with sodium chloride the influent waste was changed from the salt waste to a fresh water waste. As the sodium chloride concentration decreased, the volatile solids increased very sharply to a value approximately twice that of steady-state conditions before the salt shock. A peak in solids production is indicated at a sodium chloride concentration of approximately 7,500 mg/l. Below this salt concentration the solids dropped sharply and finally leveled off at 405 mg/l. This solids level was 85 mg/l greater than the steady-state level before the salt shock.

During the sharp increase in solids the residual COD and glucose were decreased and both leveled off at values less than the steady-state levels before the salt shock.

b. Response to immediate shock loading - batch unit studies: In order to analyze the biochemical response of the sludge in the continuous flow unit to an immediate shock load, sludge was harvested from the continuous flow unit and studied in batch units. Figure 37 shows the biochemical response to an immediate shock loading of 30,000 mg/l sodium chloride of a sludge harvested before the continuous flow unit was subjected to a sodium chloride shock loading. The initial COD and glucose concentration of the control were higher than the shocked system because the sodium chloride was added to the shocked system in a volume of water and the control unit was not adjusted to this new volume. Thus, the volume of the control unit was less than the shocked system and the same volume of glucose from a stock solution was added to both units. In all later studies the volume of the control unit was adjusted with distilled water to that of the shocked system. It can be seen that a shock load of 30,000 mg/l sodium chloride had a very deleterious effect on the efficiency of the system. The fresh water system removed 67 percent of the COD in six hours aeration time, whereas the shocked system removed only 11 percent of the COD in six hours. The oxygen uptake in the shocked system was only one-half that in the control and the solids production in the shocked system was only one-fifth that of the control.

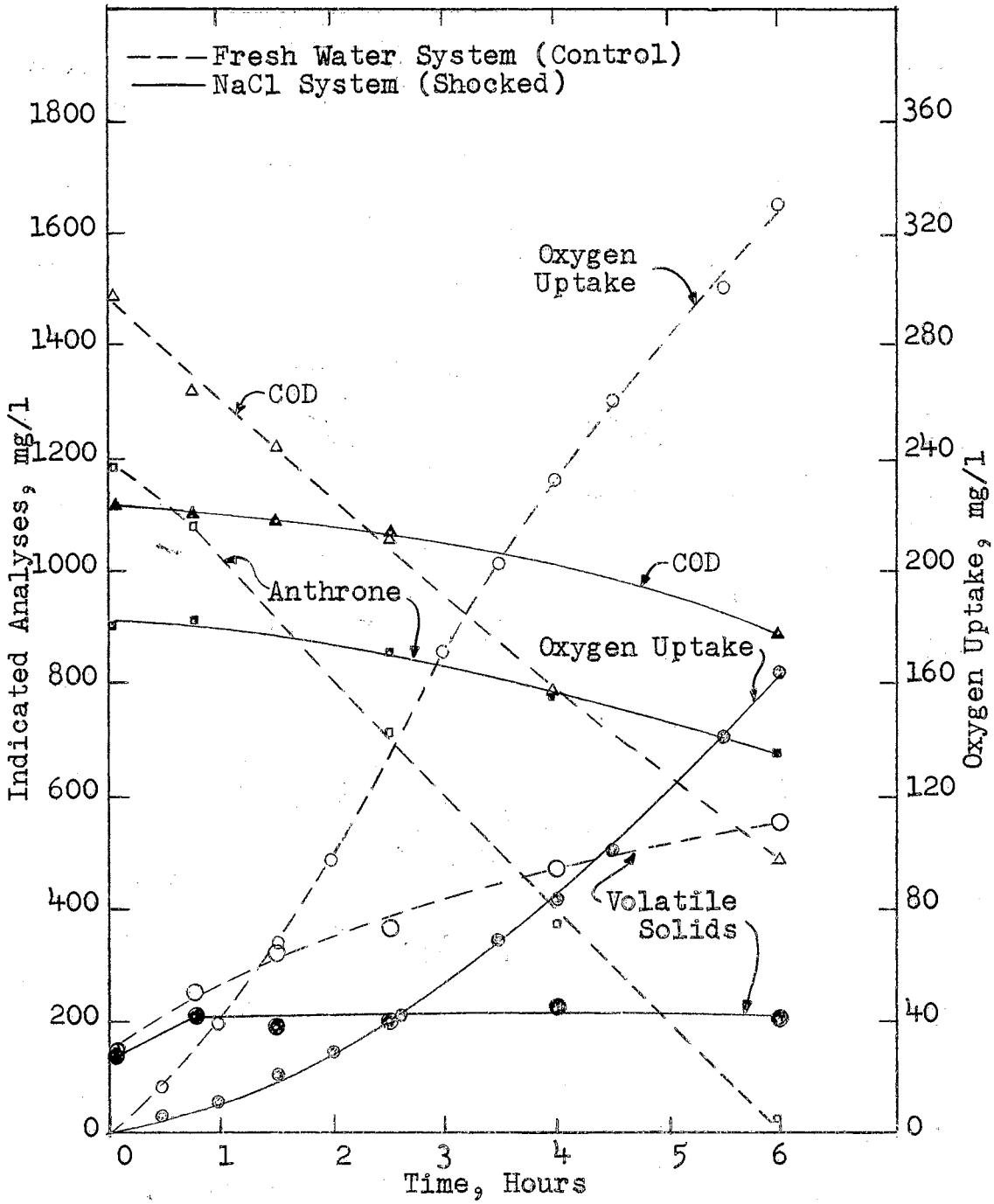

FIGURE 37 RESPONSE TO IMMEDIATE SHOCK LOADING OF 30,000 mg/l NaCl. SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT BEFORE BEING SUBJECTED TO NaCl SHOCK LOADING.

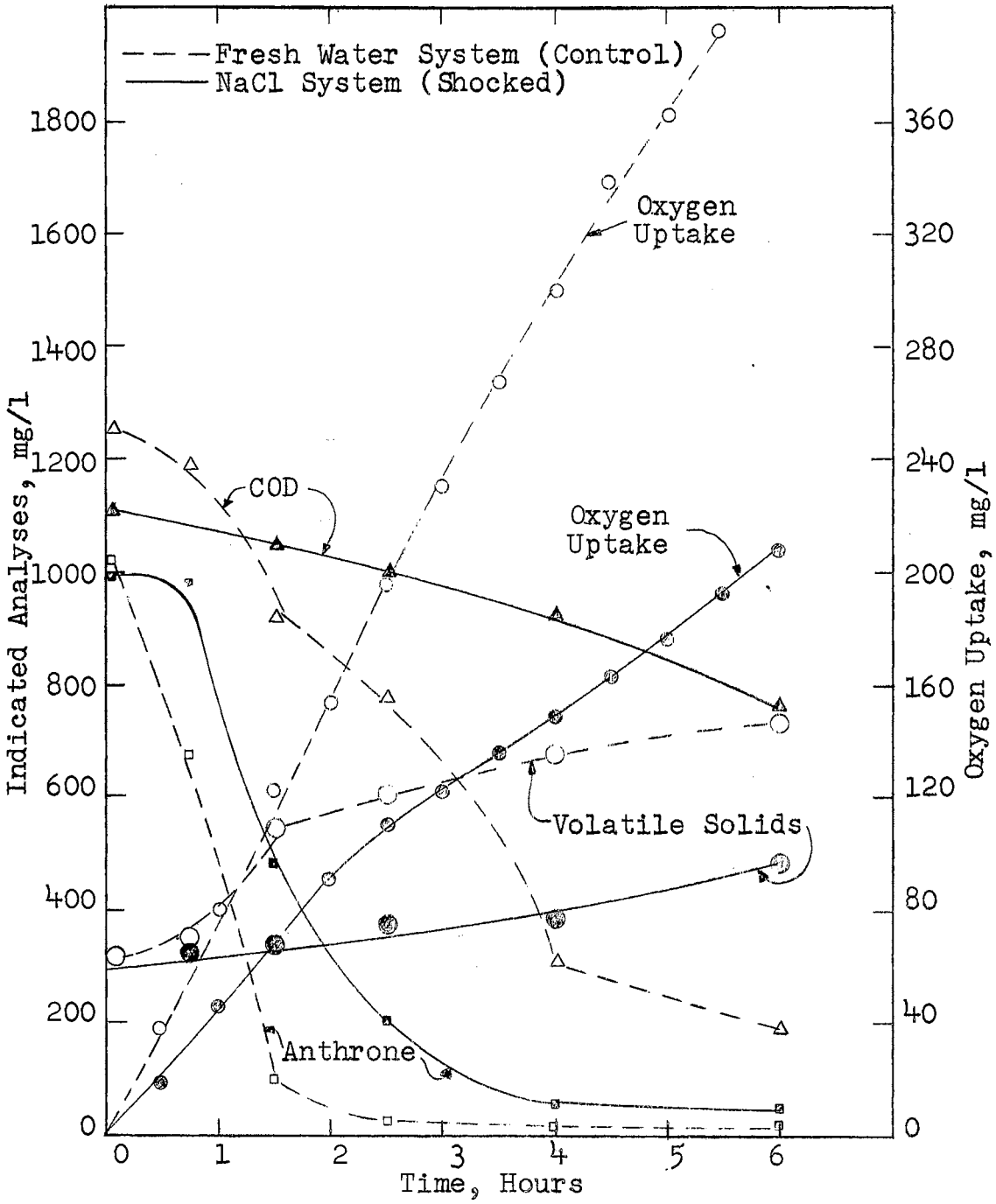

FIGURE 38 RESPONSE TO IMMEDIATE SHOCK LOADING OF 30,000 mg/l NaCl. SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT AFTER 30,000 mg/l NaCl HAD BEEN DILUTED OUT OF SYSTEM, ELEVENTH DAY OF OPERATION.

Figure 38 shows the response to an immediate shock loading of 30,000 mg/l sodium chloride of a sludge that was harvested after the sodium chloride waste had been diluted out of the continuous flow unit and the unit had again reached steady-state conditions. It is seen that the 30,000 mg/l sodium chloride shock loading again had a very deleterious effect on the efficiency of the system. The control system removed 86 percent of the COD in six hours aeration, whereas the shocked system removed only 31 percent of the COD in six hours. The glucose was effectively removed from the shocked system in four hours but very little was used for growth or respiration; most was released as metabolic intermediates or end products.

c. Biochemical response of acclimated sludge - batch unit studies: The biochemical response of a sludge grown in a continuous flow unit and acclimated to 30,000 mg/l sodium chloride is shown in Figure 39. The glucose was effectively removed in three hours and 90 percent of the COD was removed in six hours of aeration. It is seen that metabolic intermediates or end products were released during glucose metabolism and these were metabolized after the glucose removal was completed. A slight retardation in the rate of oxygen uptake at the point of complete glucose removal suggests a short acclimation period before the intermediates or end products were metabolized.

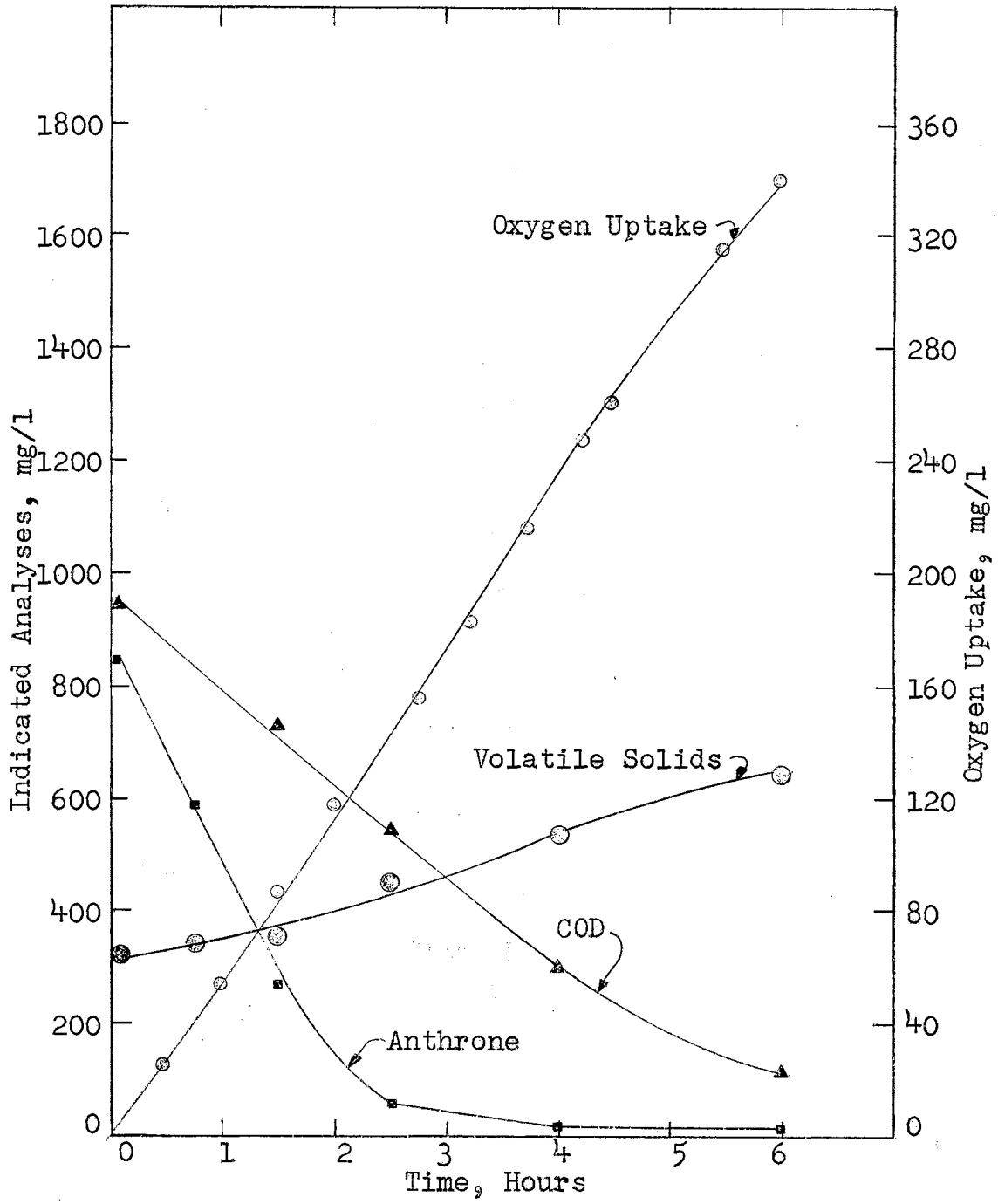

FIGURE 39 BIOCHEMICAL RESPONSE OF SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT ACCLIMATED TO 30,000 mg/l NaCl AT SEVENTH DAY OF OPERATION.

2. Response of System To Shock Loading of 45,000 mg/l Sodium Chloride

a. Response to gradual shock loading: Figure 40 shows the general response of a continuous flow activated sludge system to a gradual shock loading of 45,000 mg/l sodium chloride. In the steady-state condition before the shock loading, the volatile solids were maintained at approximately 315 mg/l. This agrees very well with the system that was shocked with 30,000 mg/l. A residual COD of 75 mg/l and a glucose residual of 10 mg/l was characteristic of the steady-state. The glucose residual compares very well with the previous system, whereas the residual COD is somewhat lower. This could indicate that the sludge in this system released less metabolic intermediates or end products than the system shocked with 30,000 mg/l sodium chloride, thus, making it a more efficient sludge. Both units were started independently from sewage seed. The volatile solids again increased at the lower concentrations of sodium chloride and decreased at the higher concentrations. At approximately four days after the addition of sodium chloride the solids concentration leveled off at a value of 190 mg/l. The solids remained at this level for approximately two days and then began increasing. After this the volatile solids cycled from a high point to a low point and continued to do so until the waste was changed from a salt waste to a fresh water waste. The residual COD showed the same type of pattern as the volatile solids except that the residual COD was at a high point at the time the volatile solids were at a low point.

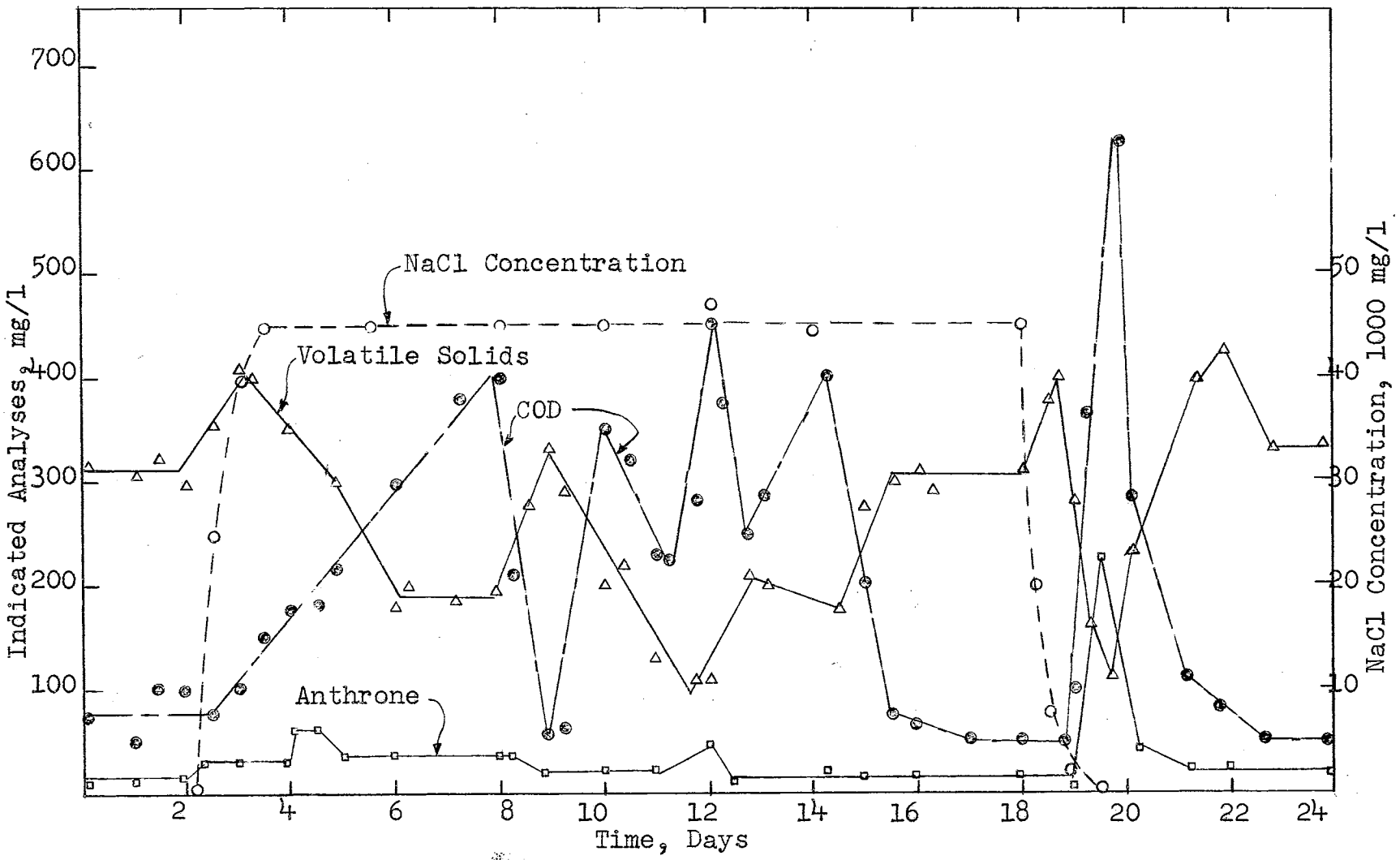

FIGURE 40 RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 8 HOUR DETENTION AND SHOCK LOADING OF 45,000 mg/l NaCl.

The residual glucose varied during the run but never more than 20 to 30 mg/l, whereas the residual COD varied by as much as 350 mg/l. It appears that the difference in volatile solids was due to the ability of the sludge to metabolize the metabolic intermediates or end products released into the medium during glucose metabolism.

After 16 days of operation with a salt waste, the influent was changed from the salt waste to a fresh water waste. As the sodium chloride concentration decreased the volatile solids increased until a sodium chloride concentration of approximately 8,000 mg/l was reached. At this concentration the volatile solids decreased very rapidly. During the increase in solids the residual COD and glucose remained approximately the same, but as the solids decreased the residual COD and glucose increased very sharply until the residual COD reached a maximum of 625 mg/l and the residual glucose reached a maximum of 225 mg/l. Approximately two days after changing the influent waste to a fresh water waste the system began adjusting to the shock and reached a steady-state condition very similar to that before the sodium chloride shock loading.

Photomicrographs were taken of sludges from the continuous flow unit. Figure 41 shows a photomicrograph of the sludge before the unit was shocked with sodium chloride. It is seen that the sludge consisted of dispersed rods and cocci, some diplo forms, a few protozoa and practically no floc particles. Figure 42 shows a photomicrograph of the sludge after its acclimation to 45,000 mg/l sodium chloride. It is seen that

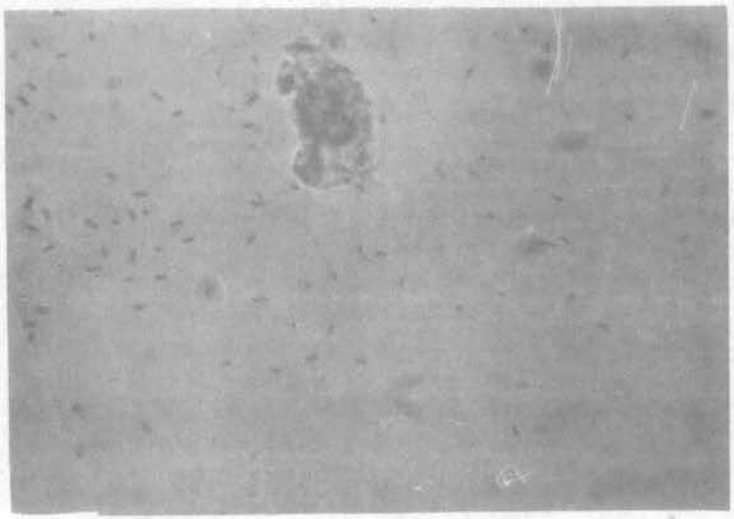

FIGURE 41 PHOTOMICROGRAPH OF A SLUDGE FROM CONTINUOUS FLOW UNIT BEFORE NaCl SHOCK. (x 2620)

FIGURE 42 PHOTOMICROGRAPH OF SLUDGE FROM CONTINUOUS FLOW UNIT 14 DAYS AFTER BEING SHOCKED WITH 45,000 mg/l Na Cl. (x 2620)

FIGURE 43 PHOTOMICROGRAPH OF SLUDGE FROM CONTINUOUS FLOW UNIT 24 HOURS AFTER BEING RETURNED TO FRESH WATER WASTE (x 2620)

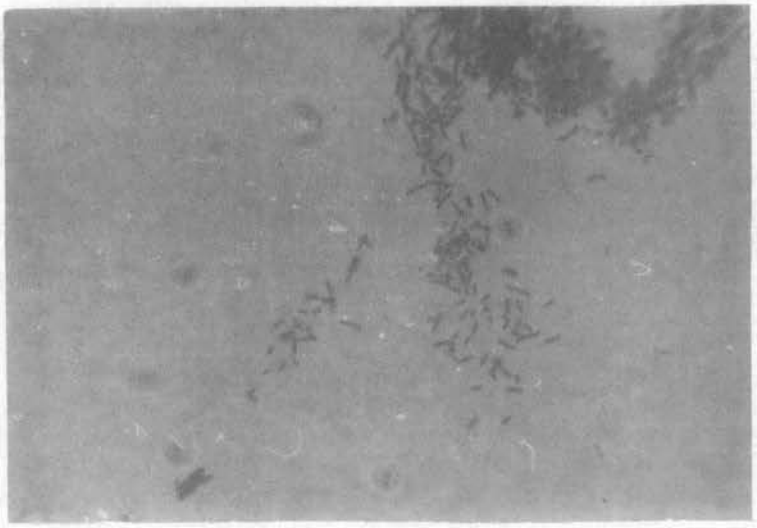

FIGURE 44 PHOTOMICROGRAPH OF SLUDGE FROM CONTINUOUS FLOW UNIT OPERATING IN STEADY-STATE AFTER BEING RETURNED TO A FRESH WATER WASTE (x 2620)

the sludge consists almost entirely of very small single cell and diplo cocci. There were very few predators present. Figure 43 shows the sludge 24 hours after the unit had been returned to a fresh water waste. The system was exhibiting its greatest degree of lysis. The residual COD was at its high point and the solids were at a low point. It is seen that the sludge consisted almost entirely of chains of cocci and short rods. Figure 44 shows the sludge after again obtaining steady-state conditions. The sludge consisted of diplo cocci and chains of cocci and rods. The chains are generally shorter than those observed in Figure 43. It is evident that the morphology of the system changed after being shocked with 45,000 mg/l sodium chloride.

b. Response to immediate shock loading - batch unit studies: The effects of an immediate shock loading of 45,000 mg/l sodium chloride on a sludge harvested from a continuous flow system operating under steady-state conditions before being shocked with sodium chloride are shown in Figure 45. It is seen that the shock loading did have a deleterious effect on the efficiency of the sludge. The increase in volatile solids in the shocked system was only one-fifth that in the control and the total oxygen uptake for the six hour test period was less than one-half that in the control. The shocked system did effectively remove the glucose in the six hours but only 15 percent of the COD was removed. It should also be noted that in the control unit the glucose was effectively removed in two and one-half hours, whereas only 32 percent of

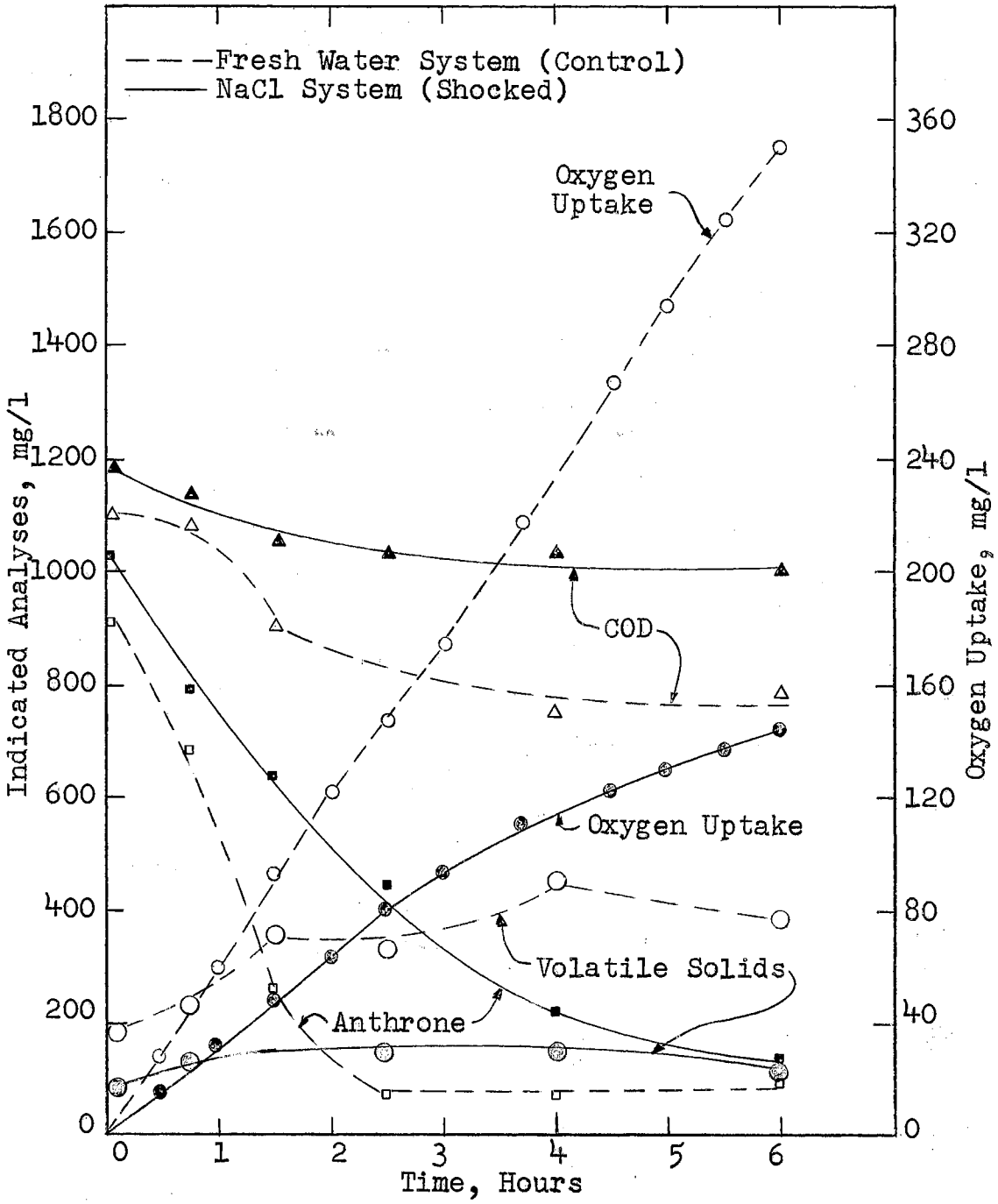

FIGURE 45 RESPONSE TO IMMEDIATE SHOCK LOADING OF 45,000 mg/l NaCl. SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT BEFORE BEING SUBJECTED TO NaCl SHOCK LOADING.

the COD was removed in six hours. Thus, in both units a considerable amount of metabolic intermediates or end products were released during the glucose metabolism.

At this point it was felt that it would be advantageous to make a study of shock loading a sludge from this same unit with 30,000 mg/l sodium chloride. This was done in order to evaluate the differences in effects of two different concentrations of sodium chloride on sludges harvested from the same continuous flow unit. Sludge was harvested for the 30,000 mg/l sodium chloride study two days after the 45,000 mg/l sodium chloride study (Figure 45) was conducted. The results of the 30,000 mg/l study are shown in Figure 46. The oxygen uptake for the shocked system was approximately one-half of that for the control and the increase in solids for the shocked system was about one-sixth that of the control. The glucose was effectively removed from the shocked system in six hours and 36 percent of the COD was removed. The system which was shocked with 30,000 mg/l sodium chloride was affected in a manner similar to the system that was shocked with 45,000 mg/l sodium chloride, but when one examines the control systems for the two tests a completely different response is observed for each control. In Figure 46 it is seen that the control system had effectively removed the glucose in one and one-half hours and after a short acclimation period the remaining COD was removed at a fairly rapid rate. This acclimation period produced a diphasic growth curve and a diphasic oxygen uptake curve. In the control system of Figure 45, it is seen that an acclimation

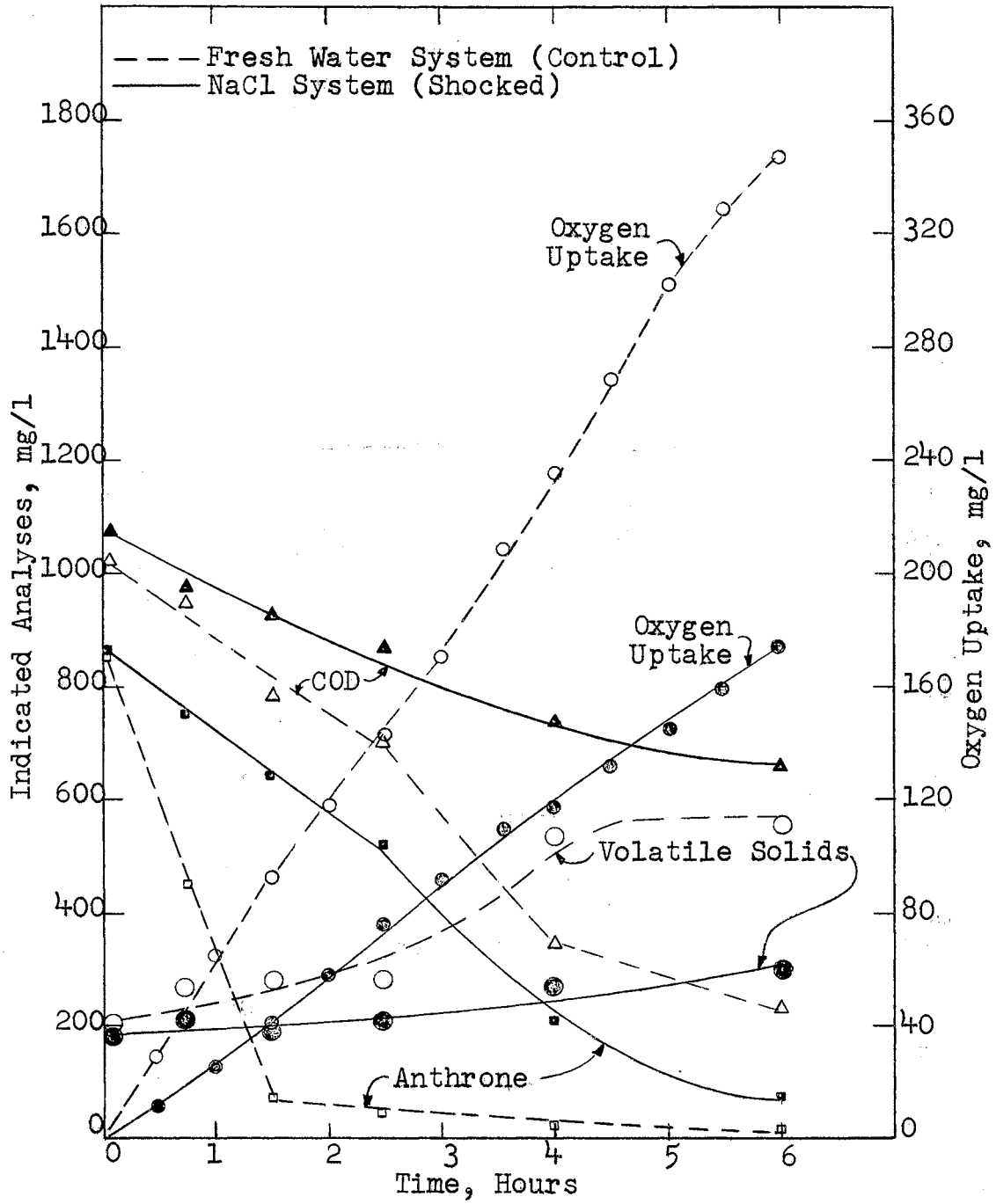

FIGURE 46 RESPONSE TO IMMEDIATE SHOCK LOADING OF 30,000 mg/l NaCl. SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT BEFORE BEING SUBJECTED TO NaCl SHOCK LOADING.

to the remaining COD did not occur during the six hour test period and the volatile solids of the system decreased. This indicates that endogenous respiration was taking place. It is possible that if the test had been conducted for a longer period of time, an acclimation to the metabolic intermediates or end products would have been observed and the remaining COD would have been removed. The main consideration to be delineated here is that the biochemical responses of the two control units were different even though they were harvested from the same continuous flow unit only two days apart.

Figure 47 shows the response to an immediate shock loading of 45,000 mg/l sodium chloride to a sludge that was harvested after the sodium chloride waste had been diluted out of the continuous flow unit and the unit had again reached steady-state conditions. It is seen that the sodium chloride had a very serious effect on the efficiency of the system. The volatile solids in the shocked system increased by 50 mg/l the first hour and thereafter decreased, even though 980 mg/l of glucose remained in the system. Forty-four percent of the glucose was removed in six hours but only seven percent of the COD was removed. It is also interesting to note that the efficiency of the control system was greatly retarded when compared to the other control systems. Only 14 percent of the COD was removed in six hours and yet 85 percent of the glucose was removed in three hours. This indicates that the sludge which survived the fresh water shock loading was not as efficient as the sludge developed before the sodium chloride

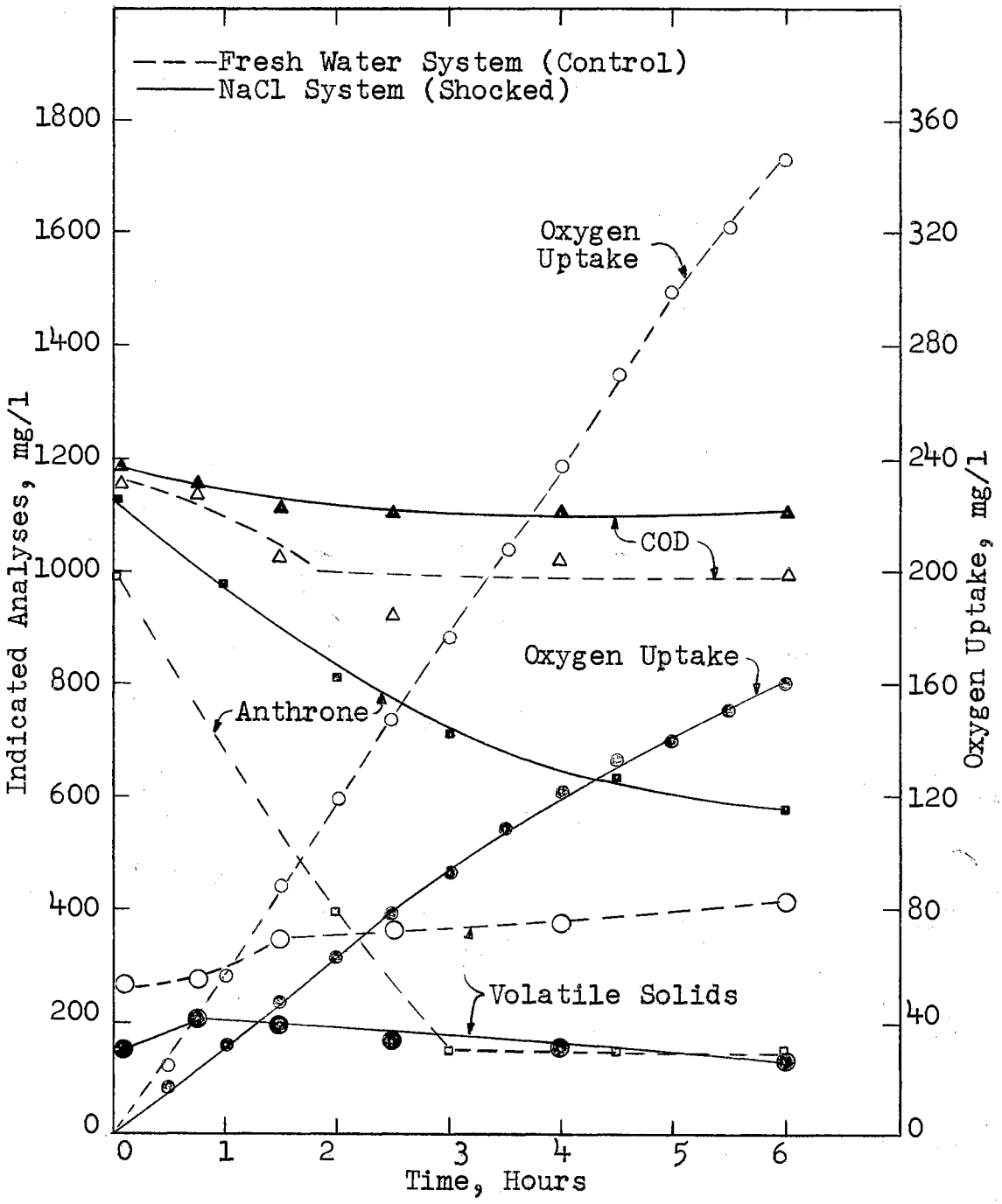

FIGURE 47 RESPONSE OF IMMEDIATE SHOCK LOADING OF 45,000 mg/l NaCl. SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT AFTER 45,000 mg/l NaCl HAD BEEN DILUTED OUT OF SYSTEM.

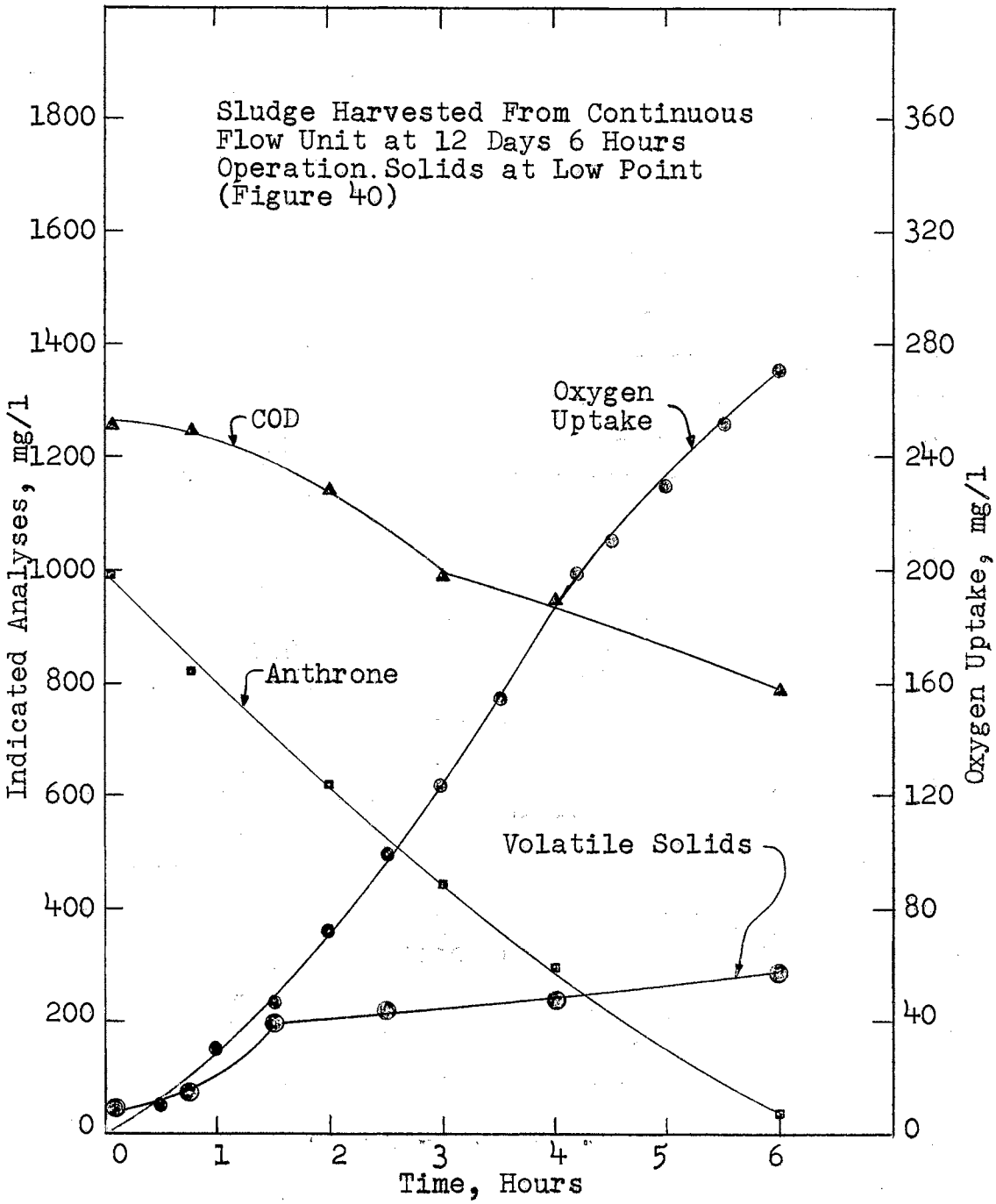

FIGURE 48 BIOCHEMICAL RESPONSE OF SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT ACCLIMATED TO 45,000 mg/l NaCl.

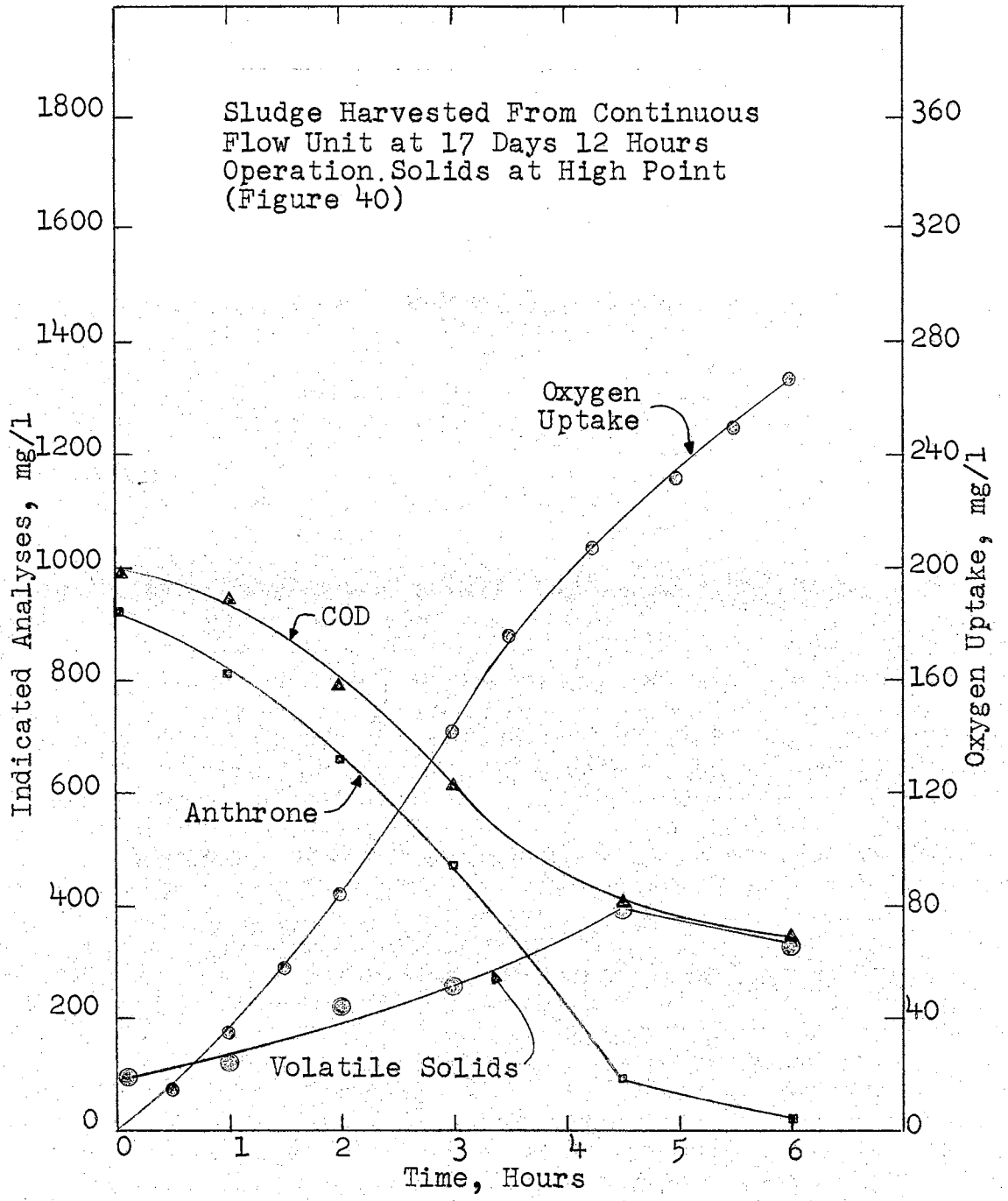

FIGURE 49 BIOCHEMICAL RESPONSE OF SLUDGE HARVESTED FROM CONTINUOUS FLOW UNIT ACCLIMATED TO 45,000 mg/l NaCl.

shock loading.

c. Biochemical response of acclimated sludge - batch unit studies: Figure 48 shows the response of a sludge harvested from the continuous flow unit after acclimation to 45,000 mg/l sodium chloride. The solids were at a low level when this sludge was harvested. The glucose was removed in six hours, but only 38 percent of the COD was removed in six hours. Figure 49 shows the response of a sludge from the same unit but harvested when the solids were at a high level. The solids concentration was reduced in the batch study to correspond to the solids concentration in Figure 48. It is seen that this sludge was more efficient than the sludge harvested at the solids low point. Sixty-six percent of the COD was removed in six hours, whereas the other sludge only removed 38 percent in six hours. It is also interesting to compare these two sludges with the sludge acclimated to 30,000 mg/l sodium chloride (Figure 39). It is seen that the sludge acclimated to 30,000 mg/l sodium chloride was much more efficient than either sludge acclimated to 45,000 mg/l.

3. Response of System To Gradual Shock Loading of 8,000 mg/l Sodium Chloride.

In the gradual shock loading studies with 30,000 and 45,000 mg/l sodium chloride, it was observed that the volatile solids concentration in the continuous flow unit increased at low sodium chloride concentrations. A sodium chloride concentration of approximately 8,000 mg/l appeared to be the most

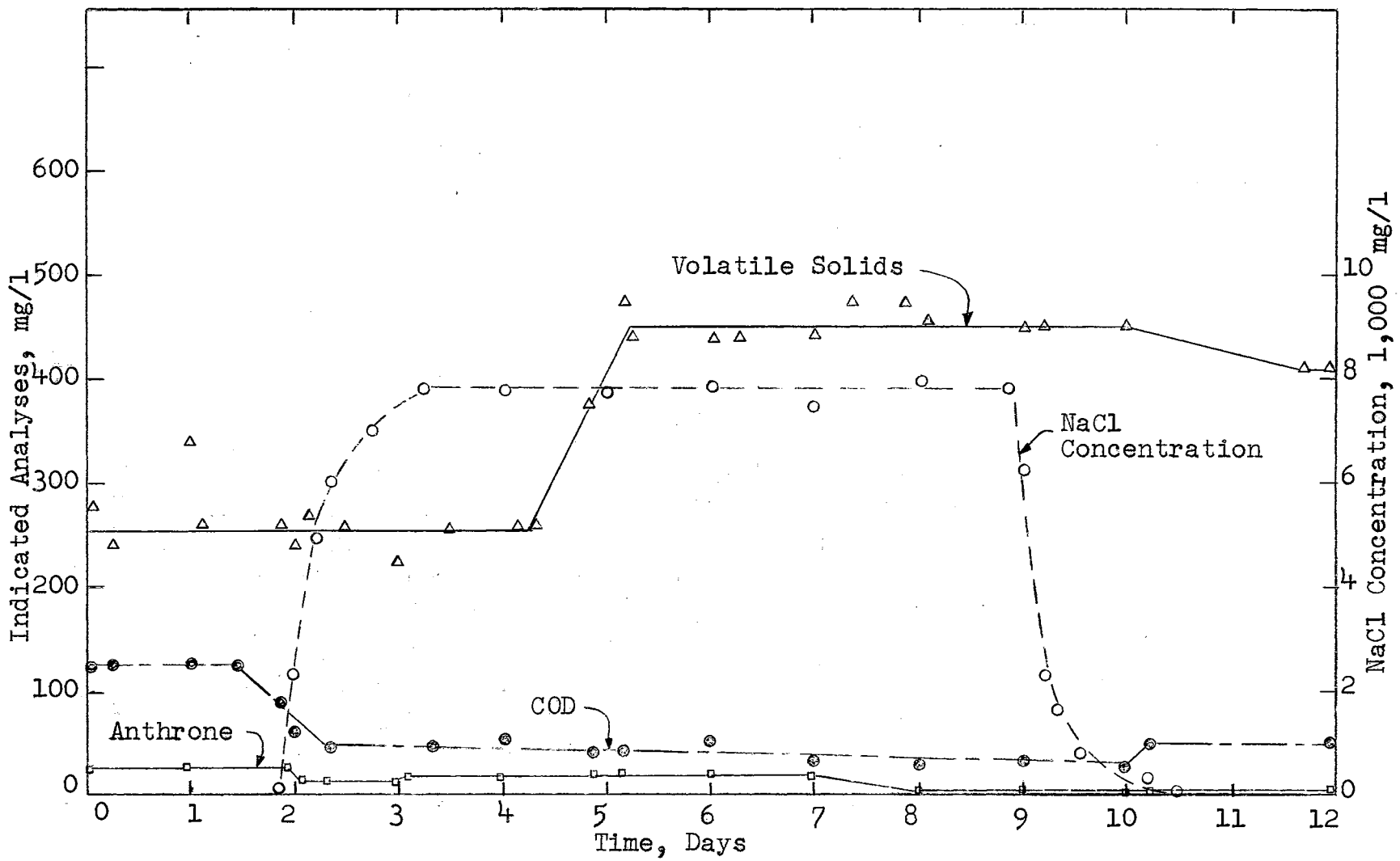

FIGURE 50 RESPONSE OF THE STEADY STATE CONTINUOUS FLOW ACTIVATED SLUDGE UNIT AT 8 HOUR DETENTION TIME AND SHOCK LOADING OF 8,000 mg/l NaCl (After T.K. George).

stimulating. Therefore, it was felt that a continuous flow unit should be operated at a sodium chloride concentration of 8,000 mg/l for several days to see whether an increase in biological solids could be maintained due to sodium chloride. The results of this experiment are presented in Figure 50. It is seen that the solids concentration for steady-state conditions before adding the salt waste was approximately 260 mg/l. The solids concentration remained at this value for four days after being shocked with 8,000 mg/l sodium chloride, but on the fourth day the solids began to increase to a concentration of 450 mg/l. They remained at this level until the influent waste was again changed to a fresh water waste. The solids concentration then decreased but after three days had not decreased to a concentration as low as before the salt shock. Thus, a sustained increase in biological solids was observed at a sodium chloride concentration of 8,000 mg/l.

C. Related Studies on Effects of Sodium Chloride and The Causation of These Effects

1. Biochemical Changes Due to Sodium Chloride Concentrations

Changes in carbohydrate and protein content of the sludge during the aeration period for batch activated sludges acclimated to various sodium chloride concentrations are compared to the composition of sludge growing in fresh water medium in Figure 51. Sodium chloride concentrations up to 12,000 mg/l did not seriously affect the protein and carbohydrate content, but at the 30,000 mg/l and 45,000 mg/l sodium chloride levels

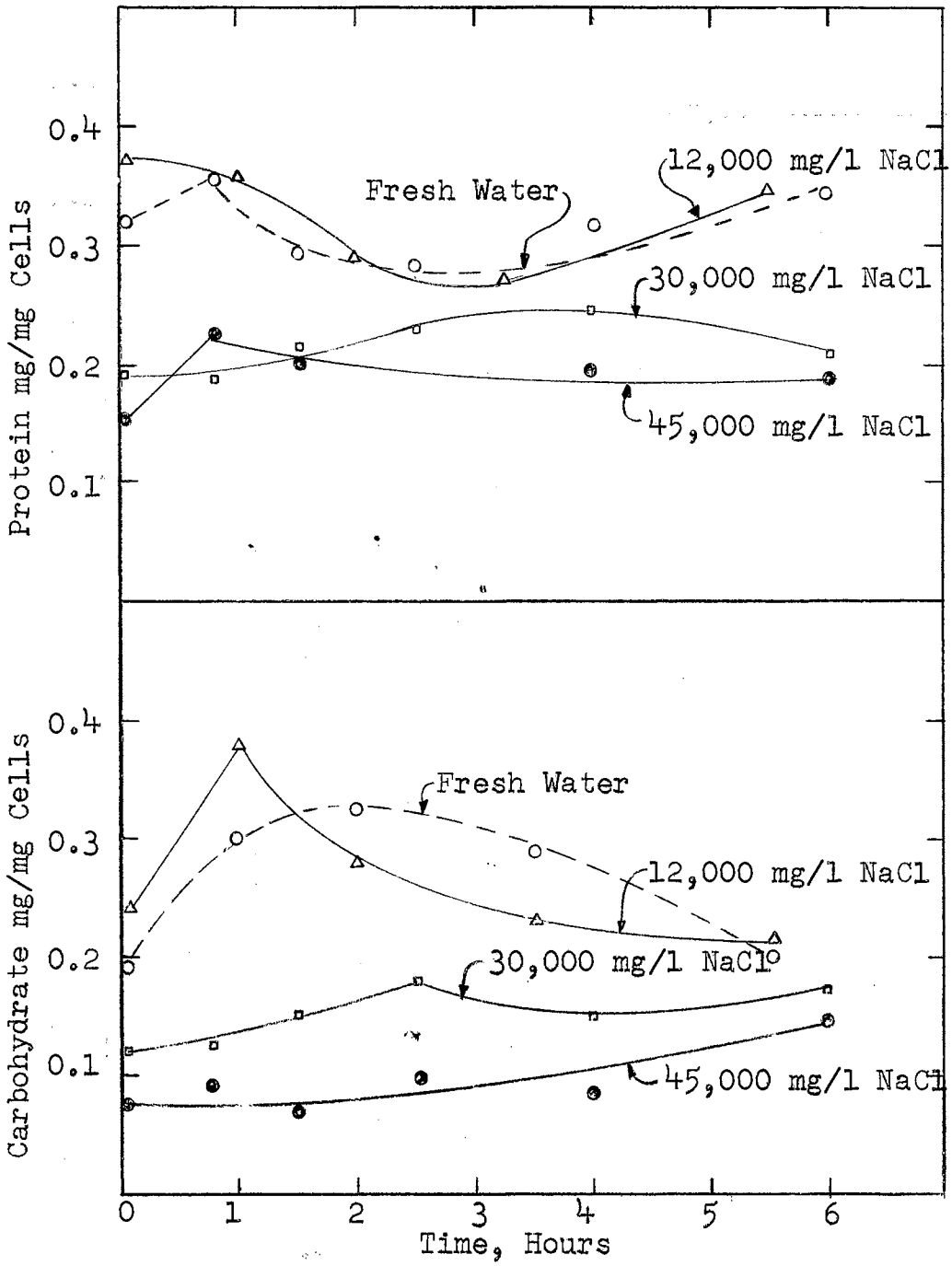

FIGURE 51 EFFECT OF NaCl ON CARBOHYDRATE AND PROTEIN CONTENT OF SLUDGE ACCLIMATED TO VARIOUS SALT CONCENTRATIONS.

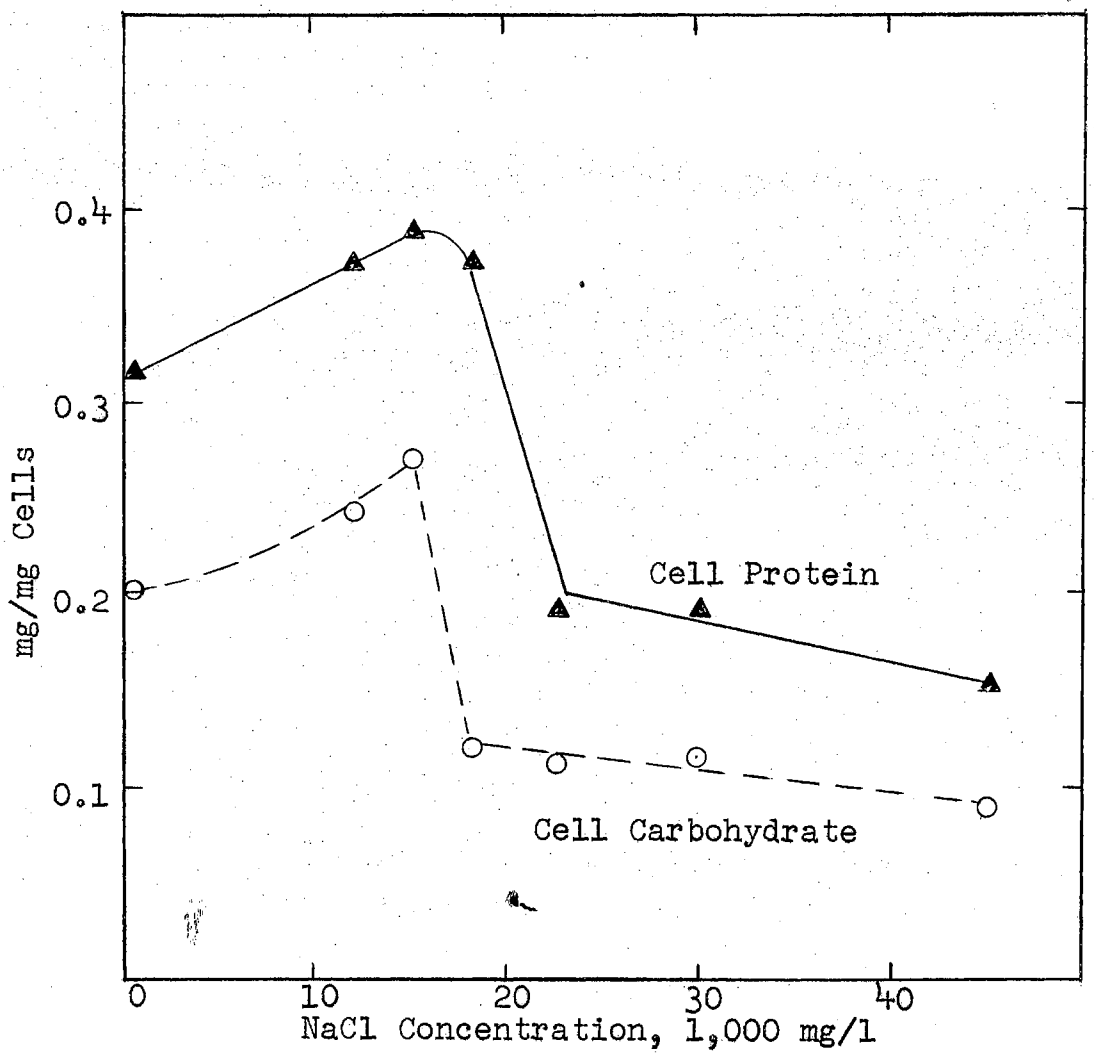

FIGURE 52 CELL PROTEIN AND CARBOHYDRATE 24 HOURS AFTER FEEDING FOR CELLS ACCLIMATED TO VARIOUS CONCENTRATIONS OF NaCl.

the carbohydrate and protein contents were reduced by approximately 50 percent. This is further borne out by results shown in Figure 52. The carbohydrate and protein content of the sludge 24 hours after feeding was increased slightly up to a salt concentration of approximately 15,000 mg/l. Above this level both the protein and carbohydrate content of the sludge dropped sharply to a level approximately 50 percent less than a sludge grown in fresh water.

A more complete biochemical analysis of sludge harvested from a unit acclimated to 45,000 mg/l sodium chloride is compared with sludge from a fresh water unit in Table II. The normal ranges for bacterial cells (66) are also given for comparative purposes. The values for the fresh water sludge compare well with the average values, whereas, the biochemical

TABLE II

COMPOSITION OF CELLS
(gm/100 gm volatile solids)

Unit	Carbohydrate	Protein	Lipids*	RNA	Carbon	Hydrogen	Nitrogen
NaCl	11.8	15.7	33.2	19.2	50.7	7.8	11.5
Fresh Water	22.2	47.0	18.7	7.7	53.1	8.3	10.7
Average Values (66)	15-20	50	10-15	10	50	10	8-15

* Lipids are expressed as stearic acid

composition of sludge grown in 45,000 mg/l is considerably different. The carbohydrate content of this sludge was approximately half and the protein content one-third that of the fresh water sludge. Conversely, the lipid content was approximately double and the RNA content two and one-half times that of the fresh water sludge. Even though the biochemical analyses of the fresh water and the sodium chloride sludge differ, Table II shows that the carbon, hydrogen and nitrogen content are the same for both sludges. Both sludges compare well with accepted values for elemental composition.

2. Studies On The Effects of Salts on Respiration

a. Effects of NaCl and KCl on old and young cells: The effects of sodium chloride and potassium chloride on old and young cells are shown in Figures 53, 54, 55, and 56. For comparative purposes the oxygen uptake for each study as a percentage of the control is given in Table III. In general as the salt concentration increased the accumulated oxygen uptake decreased, and potassium chloride adversely affected the oxygen uptake less than sodium chloride. The salts had less effect on acclimated cells than on non-acclimated cells and old cells were affected less than the young cells. In studies on the effects of salts on old cells acclimated to 30,000 mg/l sodium chloride (Figure 54), it was found that a salt concentration of 15,000 mg/l very slightly increased the oxygen uptake.

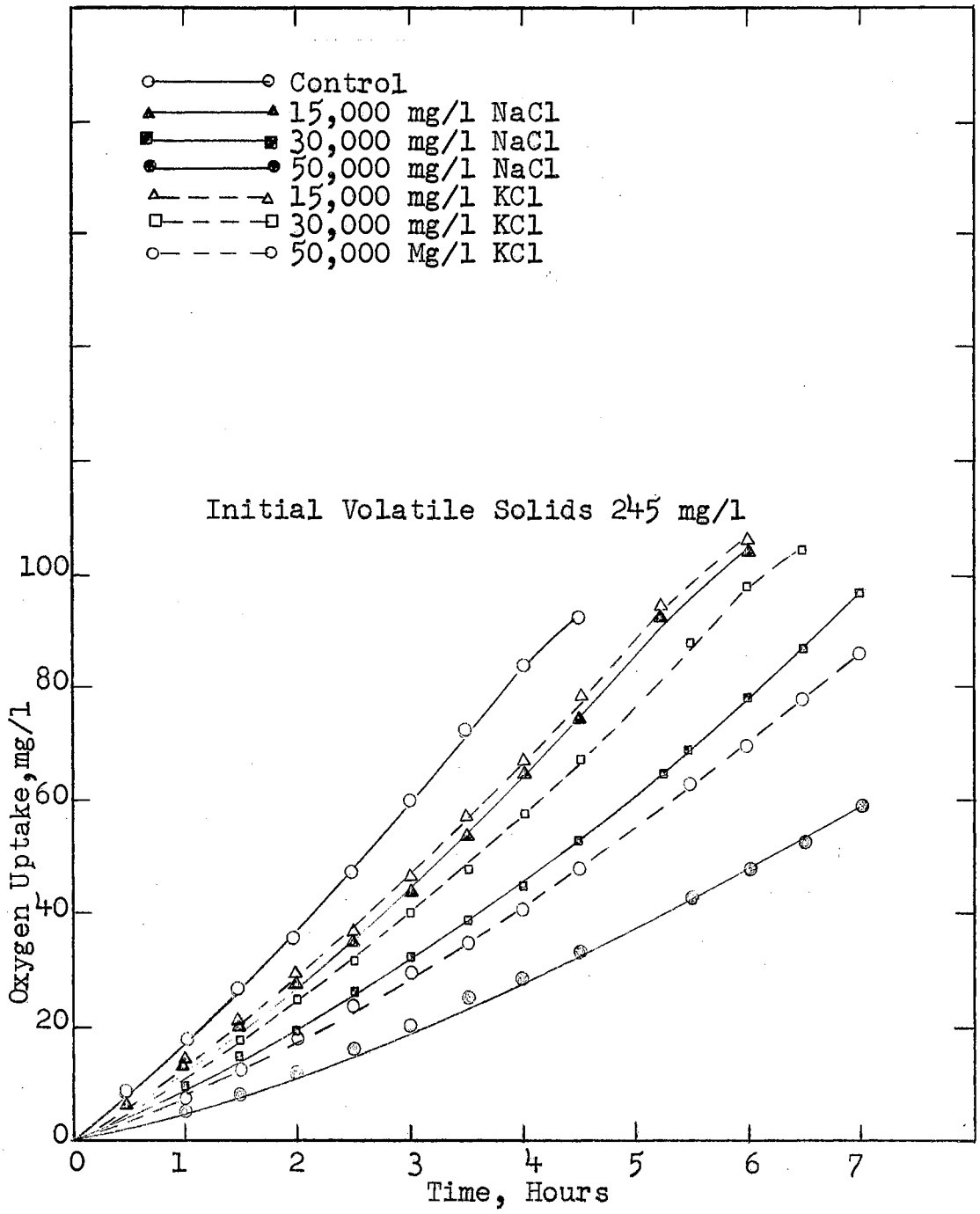

FIGURE 53 EFFECTS OF NaCl AND KCl ON OLD CELLS NOT ACCLIMATED TO NaCl OR KCl.

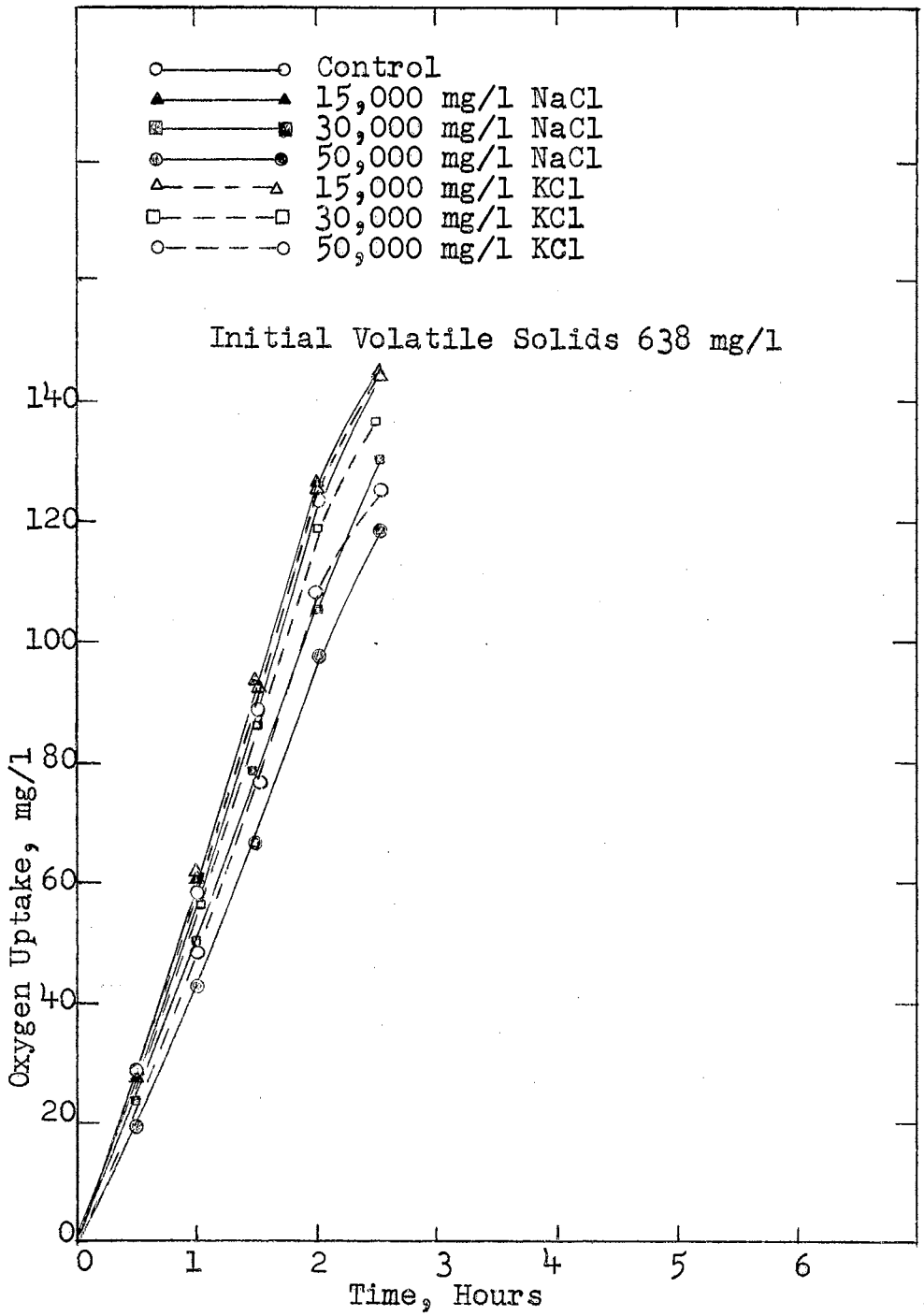

FIGURE 54 EFFECTS OF NaCl AND KCl ON OLD CELLS ACCLIMATED TO 30,000 mg/l NaCl.

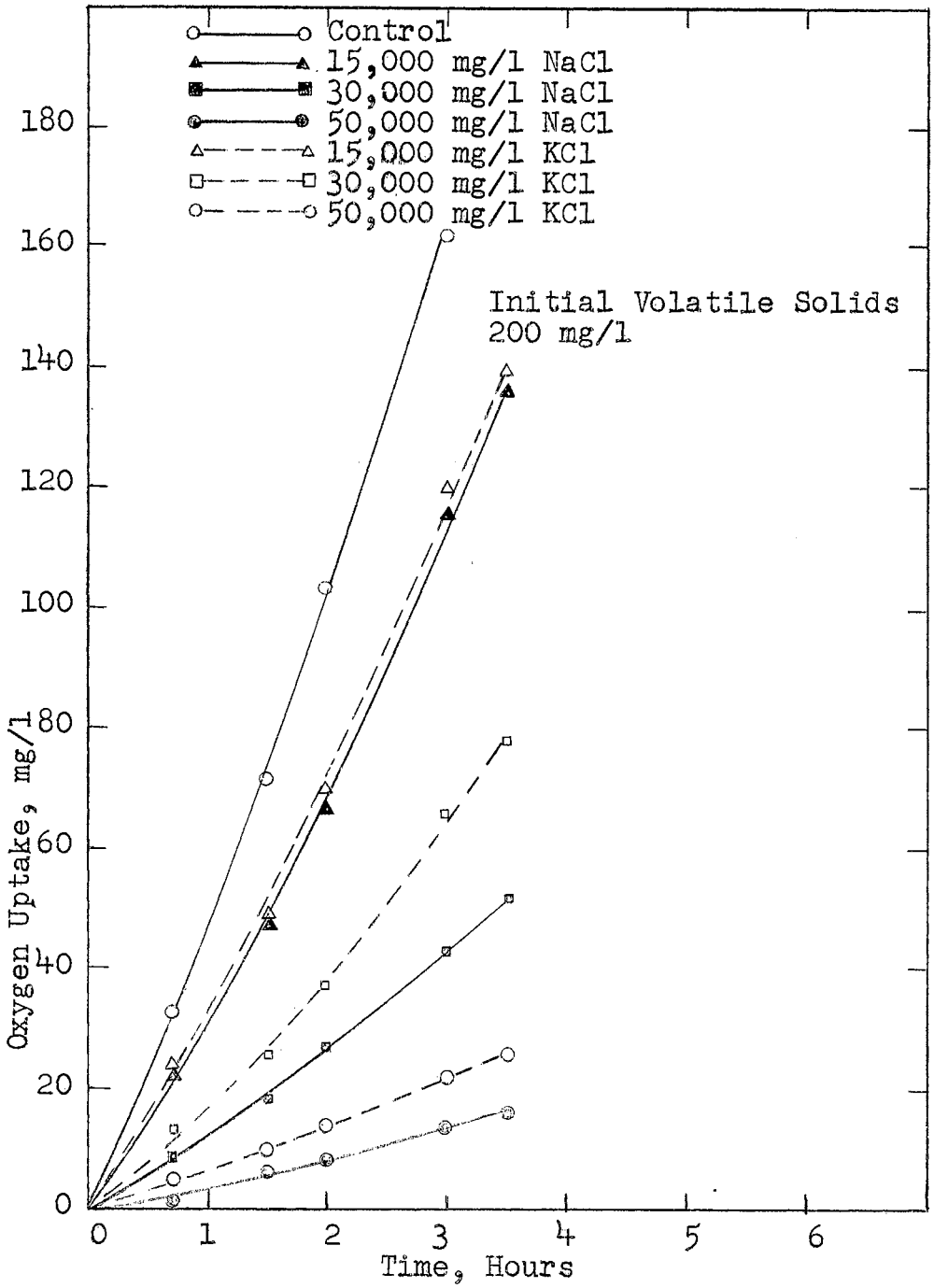

FIGURE 55 EFFECTS OF NaCl AND KCl ON YOUNG CELLS NOT ACCLIMATED TO NaCl OR KCl.

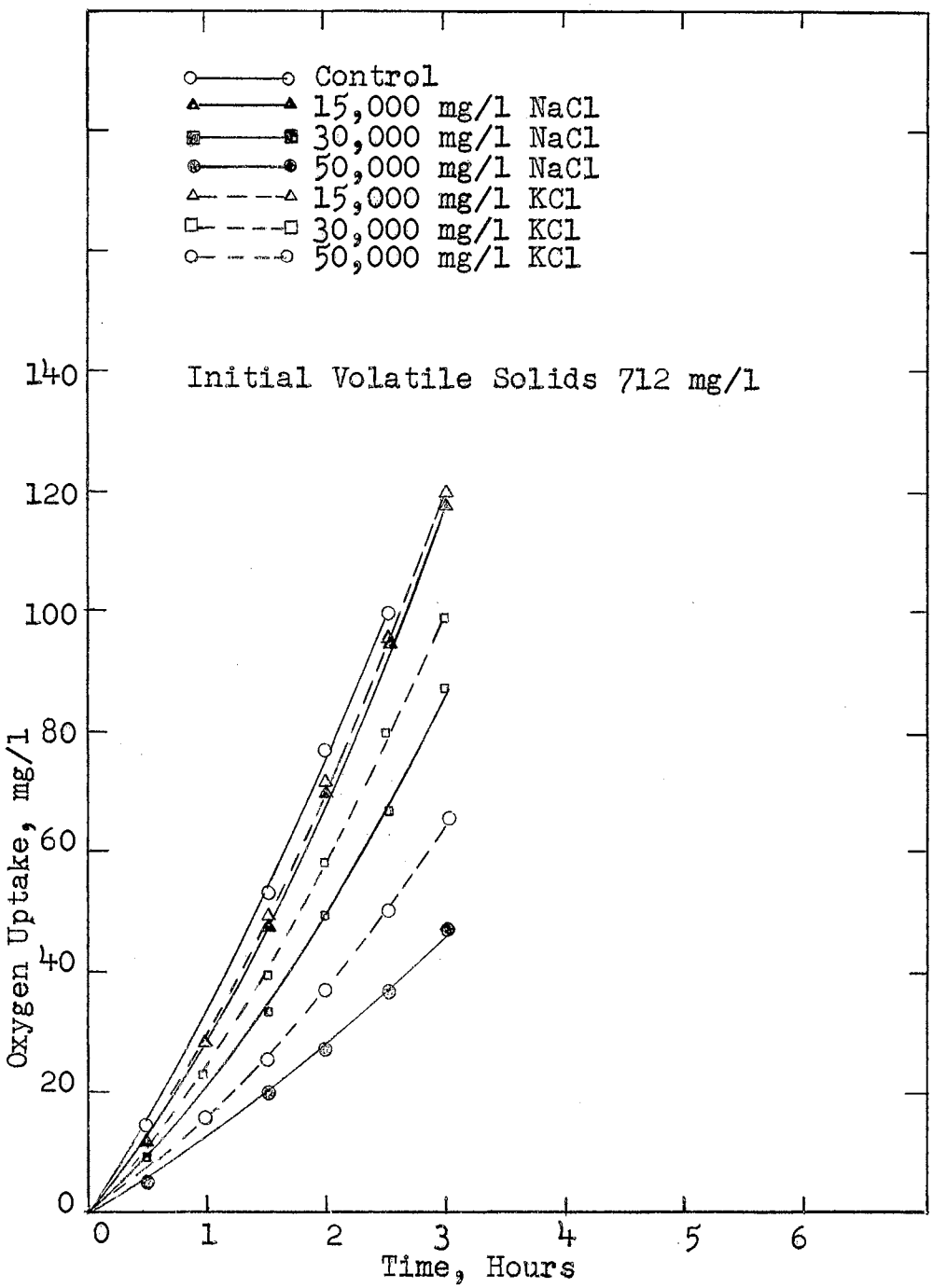

FIGURE 56 EFFECTS OF NaCl and KCl ON YOUNG CELLS ACCLIMATED TO 30,000 mg/l NaCl.

TABLE III

COMPARATIVE OXYGEN UPTAKE

Salt Concentration mg/l	Oxygen Uptake as Percentage of Control			
	Old Cells Not Acclimated	Old Cells Acclimated 30,000 mg/l NaCl	Young Cells Not Acclimated	Young Cells Acclimated 30,000 mg/l NaCl
NaCl				
15,000	77	102	71	94
30,000	53.5	89.5	26.5	66
50,000	34.5	78	8	36
KCl				
15,000	79.5	102	73.5	95
30,000	68	96.5	40	80
50,000	49	88	13	50
Combinations				
15,000 NaCl 50,000 KCl	36 (38)*	73 (90)	11.5 (9.2)	32 (47)
30,000 NaCl 30,000 KCl	34.5 (36)	75.5 (86)	12 (16)	29 (53)
50,000 NaCl 15,000 KCl	26 (27)	62.5 (81)	9.5 (5.9)	23 (34)

* Figures in parenthesis indicate the expected percentage oxygen uptake calculated from the effect of each salt taken separately. i.e., for 15,000 NaCl + 50,000 KCl, 0.77 x 0.49 = 0.38.

It is also of interest to note in Table III that when the systems were fed combinations of sodium chloride and potassium chloride the effect of the two salts on cells not acclimated was a summation of the effects of the two salt concentrations taken separately. However, for cells acclimated to 30,000 mg/l sodium chloride the effect was much more severe than the expected effect when considering the combined effect of each salt concentration taken separately.

b. Comparison of the effects of NaCl on old cells and cells transferred several times in NaCl-free medium: Figure 57 compares the effect of sodium chloride on cells from an old cell sodium chloride unit at 30,000 mg/l and an old cell fresh water unit after both had been transferred five times in fresh water medium and allowed to grow up for 24 hours after each transfer. It is seen that there was essentially no difference in the oxygen uptake of the two systems. A sodium chloride concentration of 15,000 mg/l slightly increased the oxygen uptake, whereas greater concentrations depressed the oxygen uptake.

A more comprehensive study was then made to compare the oxygen uptake before and after transfers. Figure 58 shows the effects of sodium chloride on old cells acclimated to 30,000 mg/l sodium chloride before being transferred and Figure 59 shows the effects of sodium chloride on old cells from a fresh water unit before being transferred. It can be seen that there was a considerable degree of difference in the oxygen uptake characteristics of the two systems. The

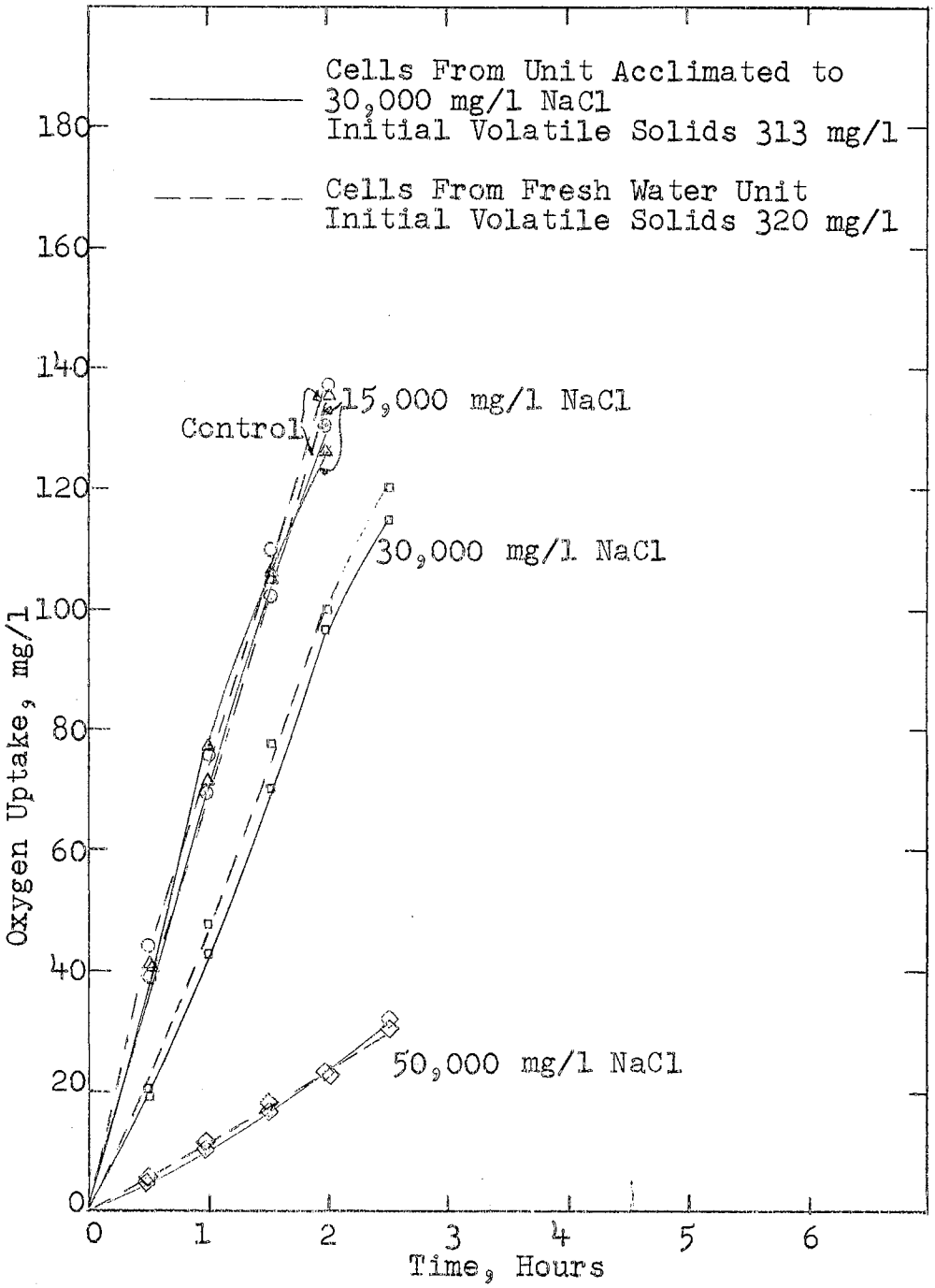

FIGURE 57 EFFECTS OF NaCl ON OLD CELLS TRANSFERRED FIVE TIMES IN FRESH WATER MEDIUM

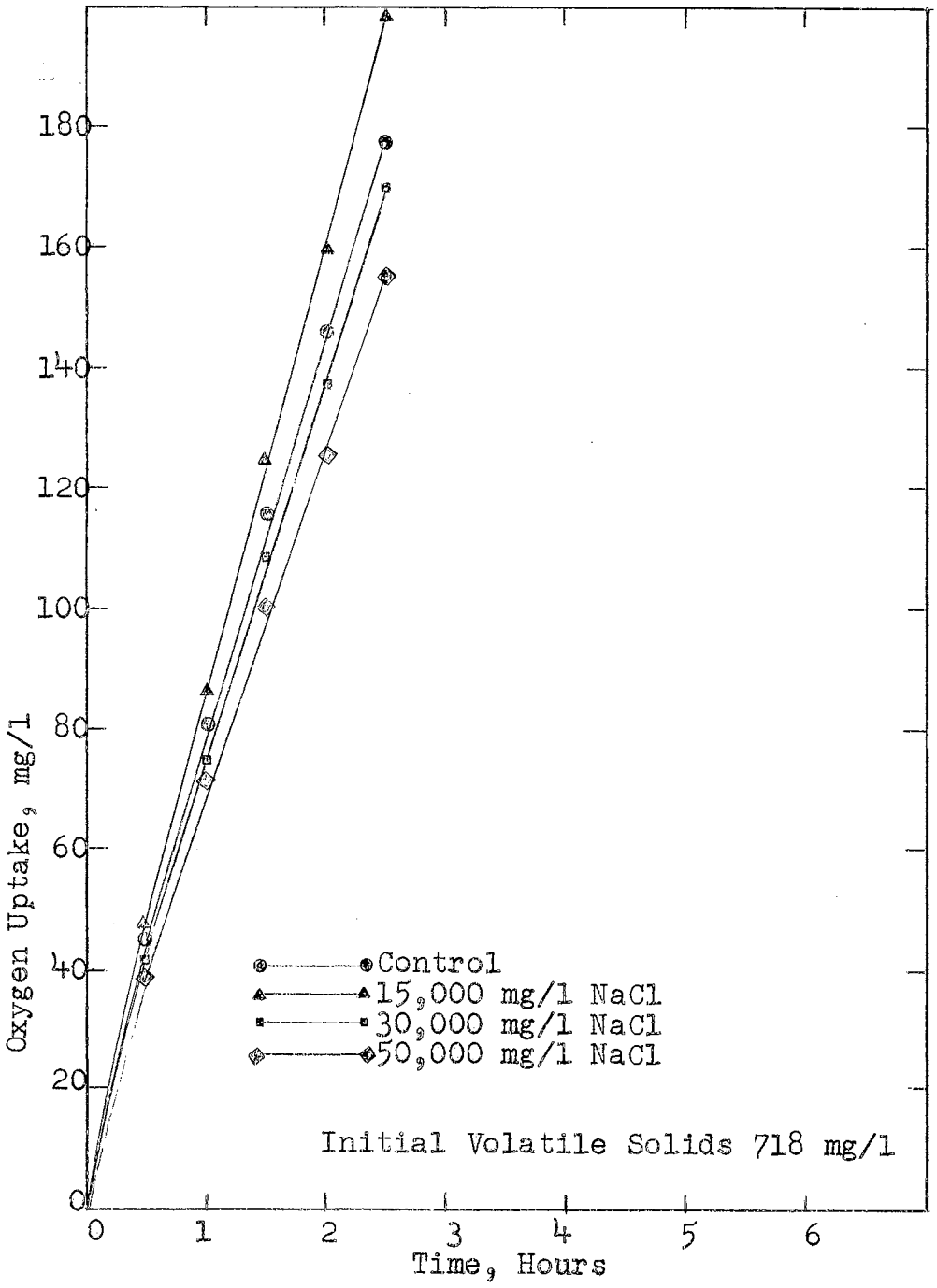

FIGURE 58 EFFECTS OF NaCl ON OLD CELLS ACCLIMATED TO 30,000 mg/l NaCl BEFORE TRANSFERS. NON-PROLIFERATING CONDITIONS.

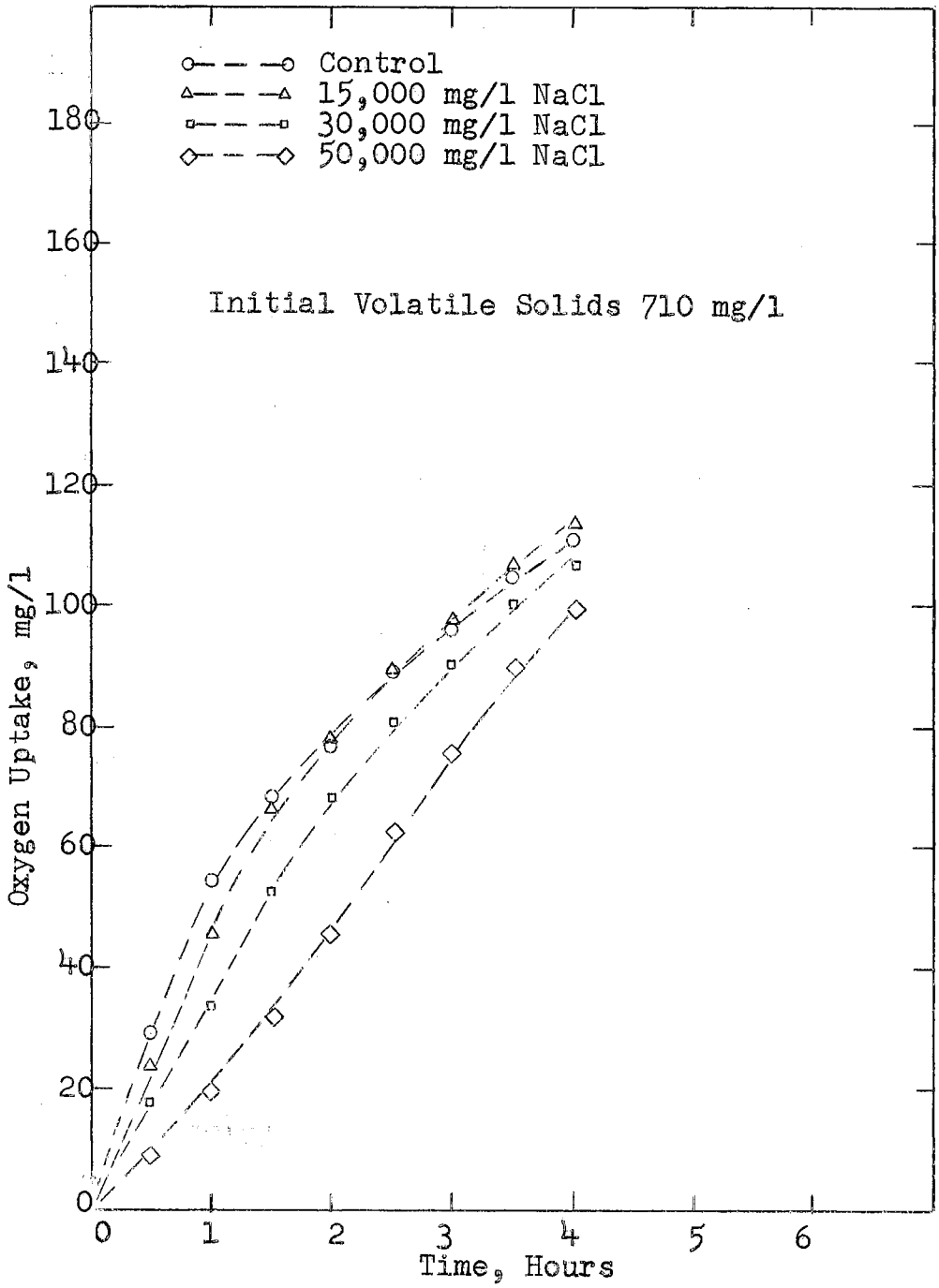

FIGURE 59 EFFECTS OF NaCl ON OLD CELLS FROM FRESH WATER UNIT BEFORE TRANSFERS. NON-PROLIFERATING CONDITIONS.

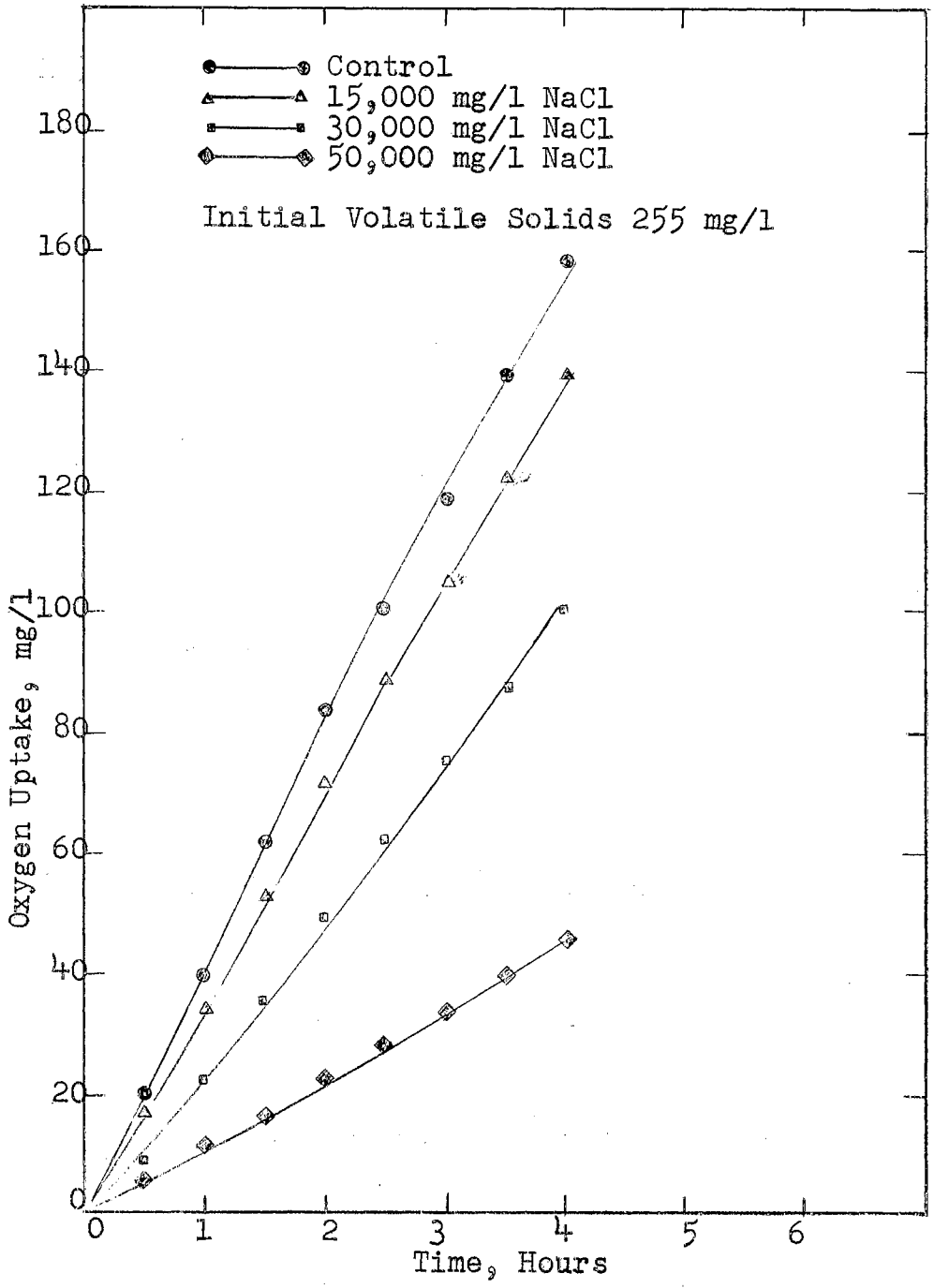

FIGURE 60 EFFECTS OF NaCl ON OLD CELLS ACCLIMATED TO 30,000 mg/l NaCl AFTER BEING TRANSFERRED SEVEN TIMES IN FRESH WATER MEDIUM. NON-PROLIFERATING CONDITIONS.

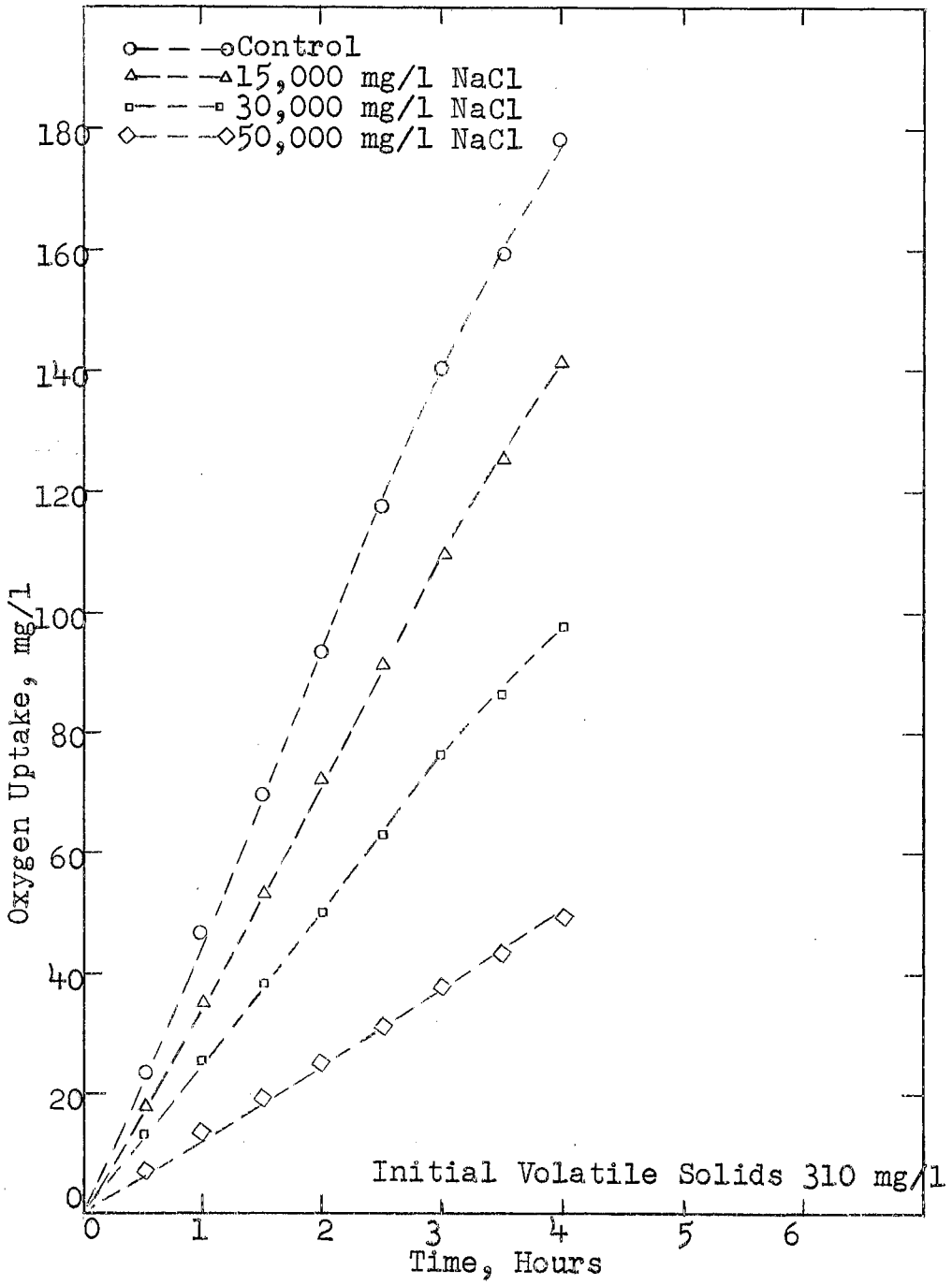

FIGURE 61 EFFECTS OF NaCl ON OLD CELLS FROM FRESH WATER UNIT AFTER BEING TRANSFERRED SEVEN TIMES IN FRESH WATER MEDIUM. NON-PROLIFERATING CONDITIONS.

rate of oxygen uptake was much less for the fresh water cells. In the acclimated cell studies a sodium chloride concentration of 15,000 mg/l stimulated the oxygen uptake and concentrations of 30,000 mg/l and 50,000 mg/l yielded a slight depression of oxygen uptake. Figure 60 and 61 show the same systems after being transferred seven times in fresh water medium. From these figures it can be seen that there is essentially no difference in the effects of sodium chloride on the two systems. The rate of oxygen uptake in the old cell sodium chloride unit is still a little greater than in the old cell fresh water unit. All three concentrations of sodium chloride depressed the rate of oxygen uptake. The effect of the transferring is further borne out by the Q_{O_2} of the systems. These are given in Table IV. It is noted that in general the Q_{O_2} values were

TABLE IV

Q_{C_2} ($mgO_2/hr/mg$ sludge) FOR CELLS
BEFORE AND AFTER TRANSFER OPERATION

NaCl Con. mg/l	Before Transfers				After Transfers			
	NaCl Unit		Fresh Water		NaCl Unit		Fresh Water	
	Q_{O_2}	Percent of Control	Q_{O_2}	Percent of Control	Q_{O_2}	Percent of Control	Q_{O_2}	Percent of Control
Control	99		84		155		143	
15,000	111	112	63	75	136	87	114	73
30,000	92	93	49	58	98	63	78	55
50,000	84	85	28	33	44	28	39	27

higher after the transfers than before indicating that the age of the cells have an affect on the rate of oxygen uptake. After the seven transfers the cells would be classified as young cells, whereas before the transfers they were classified as old cells. It is also of interest to note that even though the Q_{O_2} values were higher after the transfers, the Q_{O_2} values for the sodium chloride unit when represented as a percent of the control are a great deal less after the transfers than before. However, the Q_{O_2} values represented as a percent of the control are approximately the same for the fresh water unit.

c. Effect of degree of acclimation to NaCl on respiration: The effects of sodium chloride on cells grown up for 48 hours from a sewage seed in a medium containing 30,000 mg/l sodium chloride are shown in Figure 62. Also, the effects of sodium chloride on cells from this same unit after being allowed to grow for 18 hours after being transferred seven times in a 30,000 mg/l sodium chloride medium are shown in Figure 63. It is seen that the sodium chloride had a much more deleterious effect on the oxygen uptake after the transfers than before. It is important here to remember that the cells were harvested after 48 hours growth in the first case and after only 18 hours in the latter case. The cells grew very slowly in 30,000 mg/l sodium chloride and approximately 44 to 46 hours were required to reach the end of the log growth phase. Thus, the cells harvested at 48 hours were harvested after the log growth phase, but the cells harvested at 18 hours were harvested during the log growth phase. When cells are

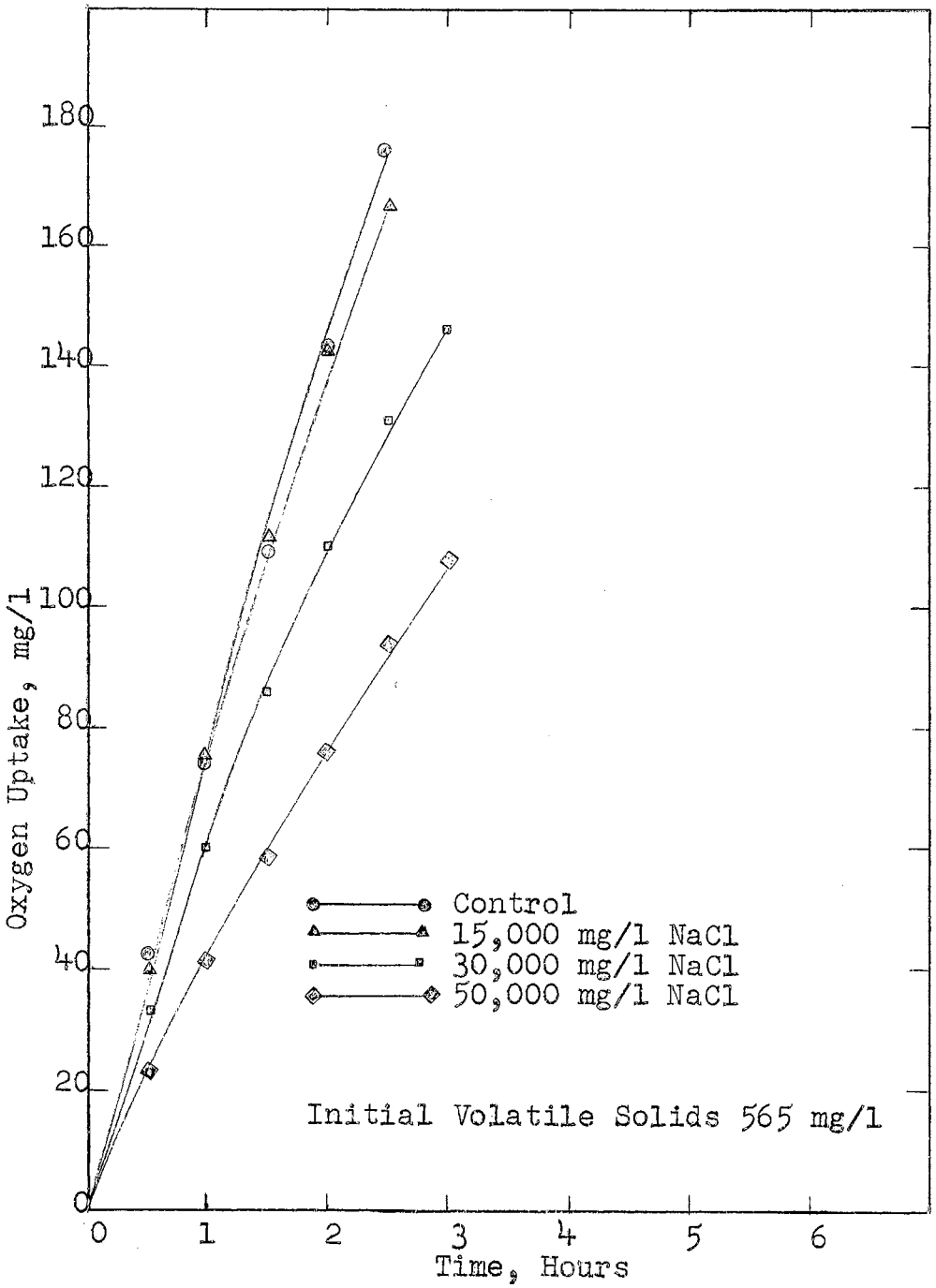

FIGURE 62 EFFECT OF NaCl ON CELLS HARVESTED AFTER 48 HOURS GROWTH IN 30,000 mg/l NaCl. NON-PROLIFERATING CONDITIONS

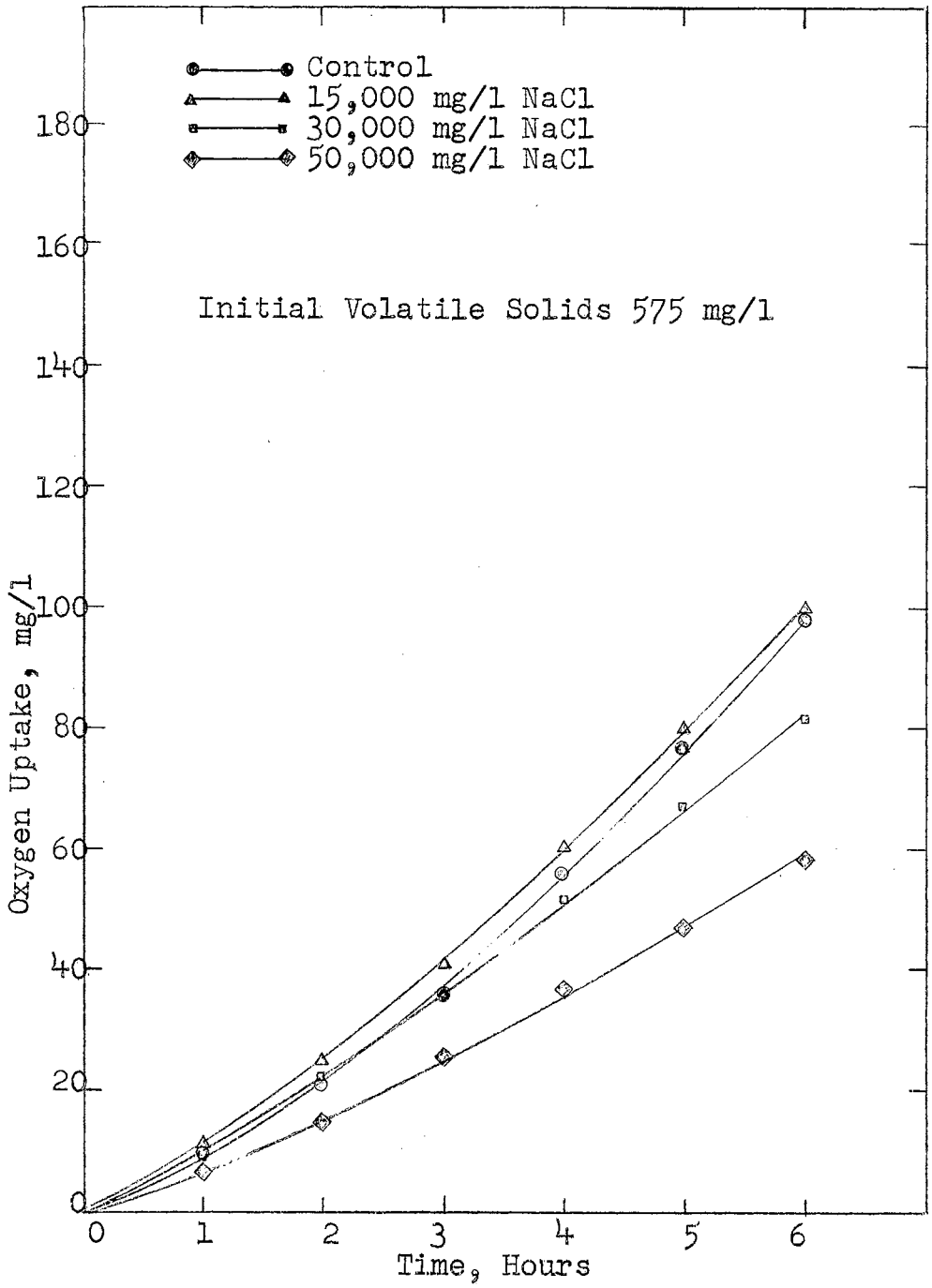

FIGURE 63 EFFECT OF NaCl ON CELLS HARVESTED AFTER 18 HOURS GROWTH AFTER BEING TRANSFERRED SEVEN TIMES IN 30,000 mg/l NaCl MEDIUM NON-PROLIFERATING CONDITIONS.

grown in high salt concentrations, it appears that the growth phase in which they are harvested has a great deal to do with their rate of oxygen utilization, even more so than the degree of acclimation.

3. Lysis Studies

a. Change in degree of lysis when cells grown in 45,000 mg/l sodium chloride are placed in various concentrations of sodium chloride: The effects of placing cells grown in 45,000 mg/l sodium chloride in lower salt concentrations and in fresh water are shown in Figure 64-A. The release of COD and the decrease in biological solids were measured as indicators of the degree of cell lysis. The maximum concentration of released COD and the maximum decrease in solids occurred when the cells were placed in medium which contained no added sodium chloride. At the cell concentration employed (1150 mg/l), the maximum amount of COD released was 705 mg/l. It is seen that 10,000 mg/l was the critical salt level. Above and below this value the degree of lysis remains proportional to the change in salt concentration, but lysis was not severe when the cells were placed in salt concentrations above 10,000 mg/l.

b. Change in degree of lysis when cells grown in various concentrations of sodium chloride are placed in fresh water: Figure 64-B shows changes in COD and biological solids when young cells grown in various concentrations of sodium chloride were placed in fresh water medium. At the cell concentration used in the study (555 mg/l), the maximum COD

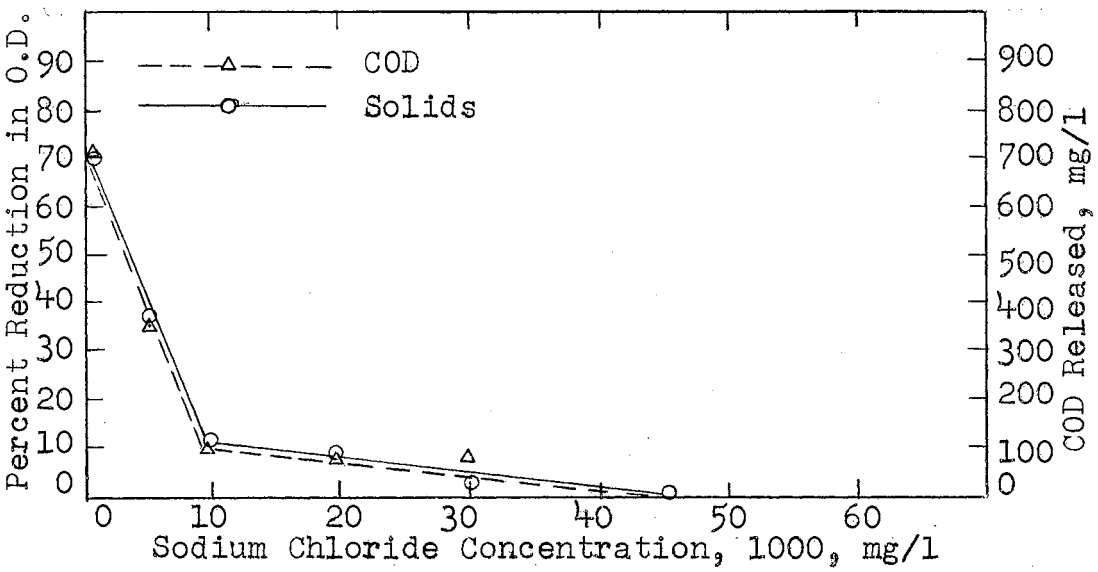

FIGURE 64-A CHANGE IN COD AND SOLIDS WHEN CELLS GROWN IN 45,000 mg/l NaCl ARE PLACED IN VARIOUS CONCENTRATIONS OF NaCl.

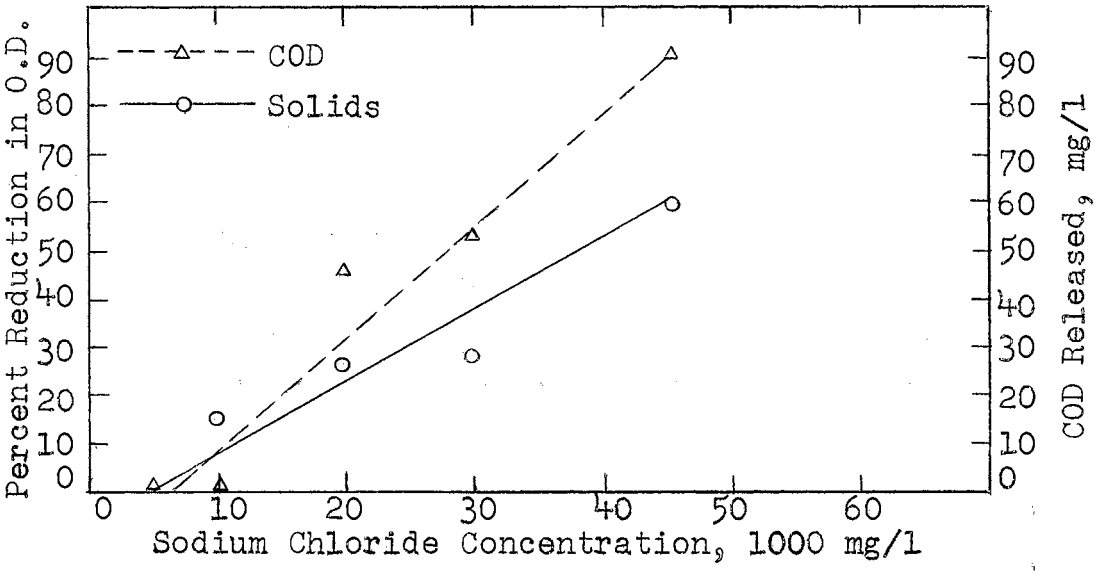

FIGURE 64-B CHANGE IN COD AND SOLIDS WHEN CELLS GROWN IN VARIOUS CONCENTRATIONS OF NaCl ARE PLACED IN FRESH WATER.

released was 90 mg/l. This was for cells grown in 45,000 mg/l sodium chloride. It is seen that, in general, there was a linear relationship between the change in salt concentration and the degree of lysis. It is interesting to note that the maximum concentration of COD released in these studies was not solely a function of cell concentration. For example, under the experimental conditions employed for the study shown in Figure 64-A, 705 mg/l of COD were released when 1150 mg/l of sludge developed at 45,000 mg/l sodium chloride were placed in fresh water. However, when 555 mg/l of young cells harvested shortly after the log phase of growth in 45,000 mg/l sodium chloride were placed in fresh water (Figure 64-B) only 90 mg/l of COD were released. These results suggest that the physiological condition of the cells exerts some control over the degree of lysis.

4. Change in System Parameters When Sludge is Acclimated to 45,000 mg/l Sodium Chloride and Then Transferred Through A Linear Gradient to Fresh Water

It was of interest to determine the response of cells acclimated to 45,000 mg/l sodium chloride when they were transferred to a fresh water environment under non- shock-loading conditions. To accomplish such transfer, the salt-grown cells were harvested and the salt concentration was gradually reduced to fresh water using a linear gradient mixing device. Under these conditions there was no evidence of lysis. Using the transferred cells, a study was made to assess their substrate removal efficiency; the results of this study are shown in

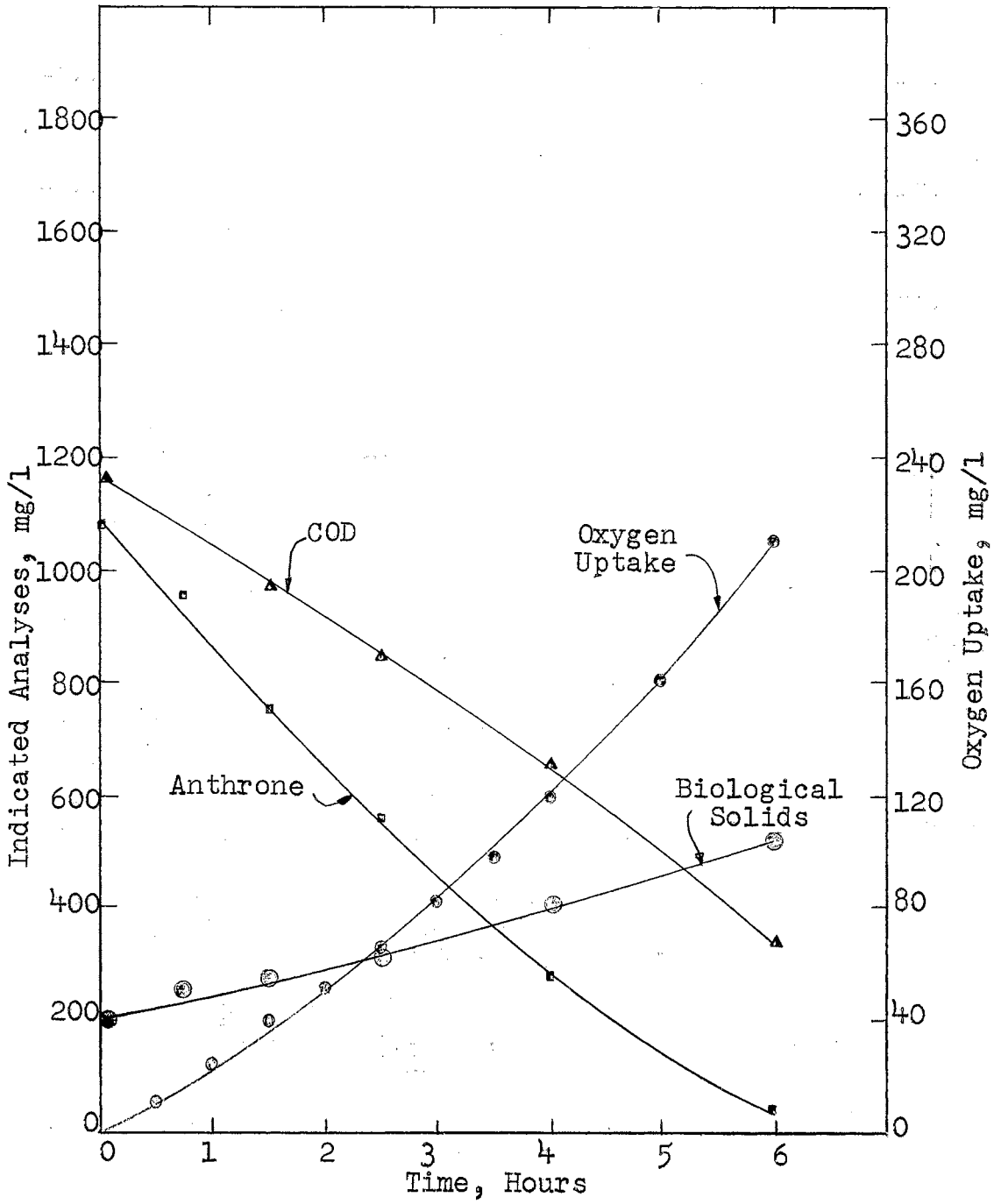

FIGURE 65 CHANGE IN SYSTEM PARAMETERS. SLUDGE ACCLIMATED TO 45,000 mg/l NaCl AND TRANSFERRED THROUGH A LINEAR GRADIENT TO FRESH WATER.

Figure 65. The biochemical response as measured by COD and glucose removal, oxygen uptake, and sludge production is in general comparable to the response of acclimated cells grown in medium containing 45,000 mg/l sodium chloride (Figure 23). Thus, it appears that the sludge has approximately the same response in fresh water as it has in a salt medium.

5. Studies on Diphasic Growth

a. Changes in system parameters during long term aeration studies: Figure 66 shows the variations in biochemical parameters of a sludge acclimated to 45,000 mg/l sodium chloride and then shock-loaded with a fresh water medium. It is seen that the fresh water shock caused a considerable amount of lysis in that the initial glucose concentration was only 360 mg/l, whereas the initial COD concentration was 1070 mg/l. It is interesting to note that after the removal of the glucose there was a break in both the solids curve and the oxygen uptake curve. After all glucose had been removed there remained 820 mg/l of COD. The COD concentration remained at this level for some time before it was removed by the system. Also, as the system started to remove the remaining COD the solids and oxygen uptake began to increase again. An apparent acclimation period was required after glucose removal before the remaining COD could be metabolized by the sludge.

b. Effect of chloramphenicol on system parameters: Chloramphenicol, an inhibitor of protein synthesis, was employed to study the possibility that new enzyme(s) must be synthesized before the material released due to cell lysis could be meta-

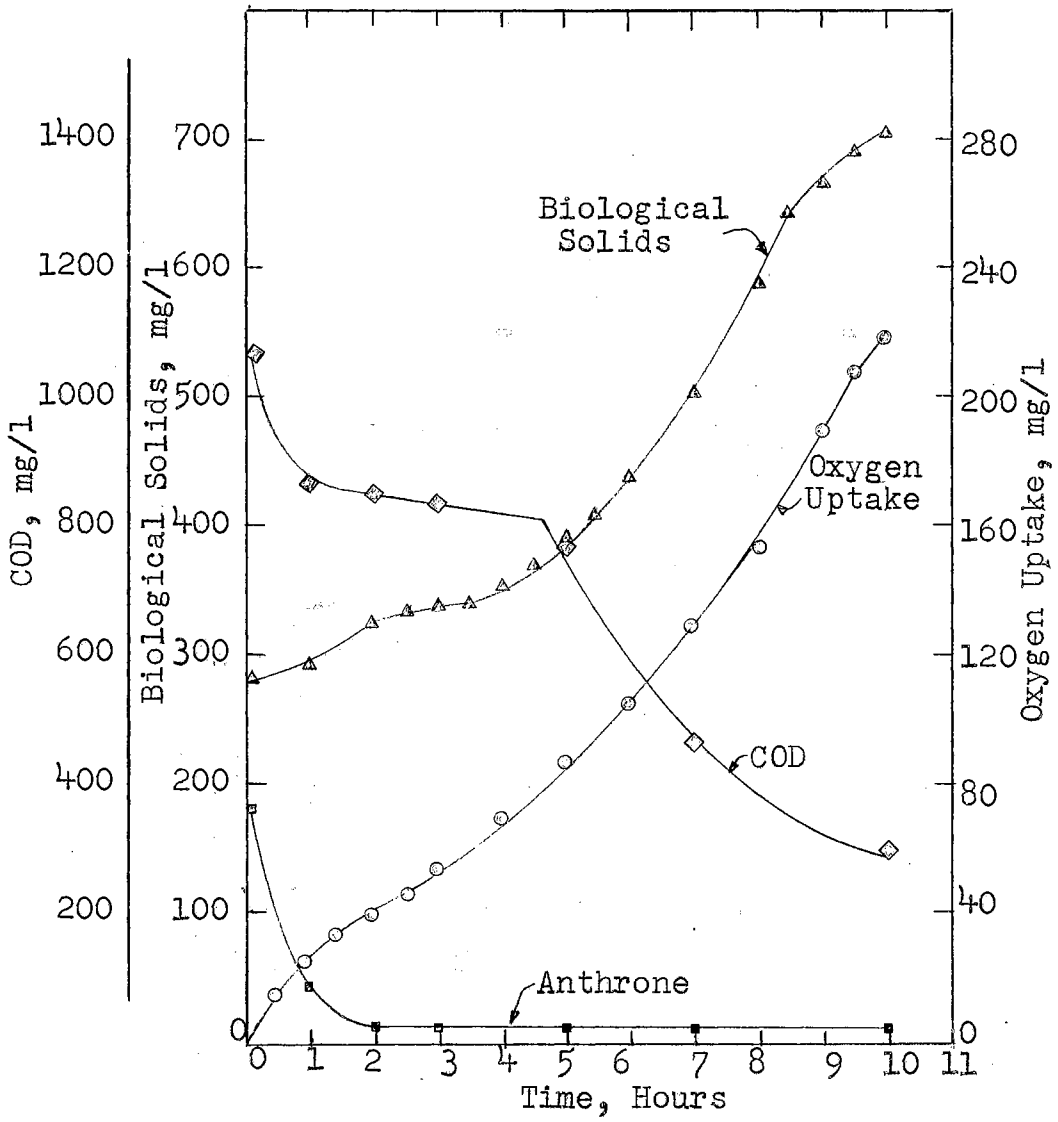

FIGURE 66 LONG TERM AERATION STUDY USING CELLS ACCLIMATED TO 45,000 mg/l NaCl AND SHOCKED WITH FRESH WATER.

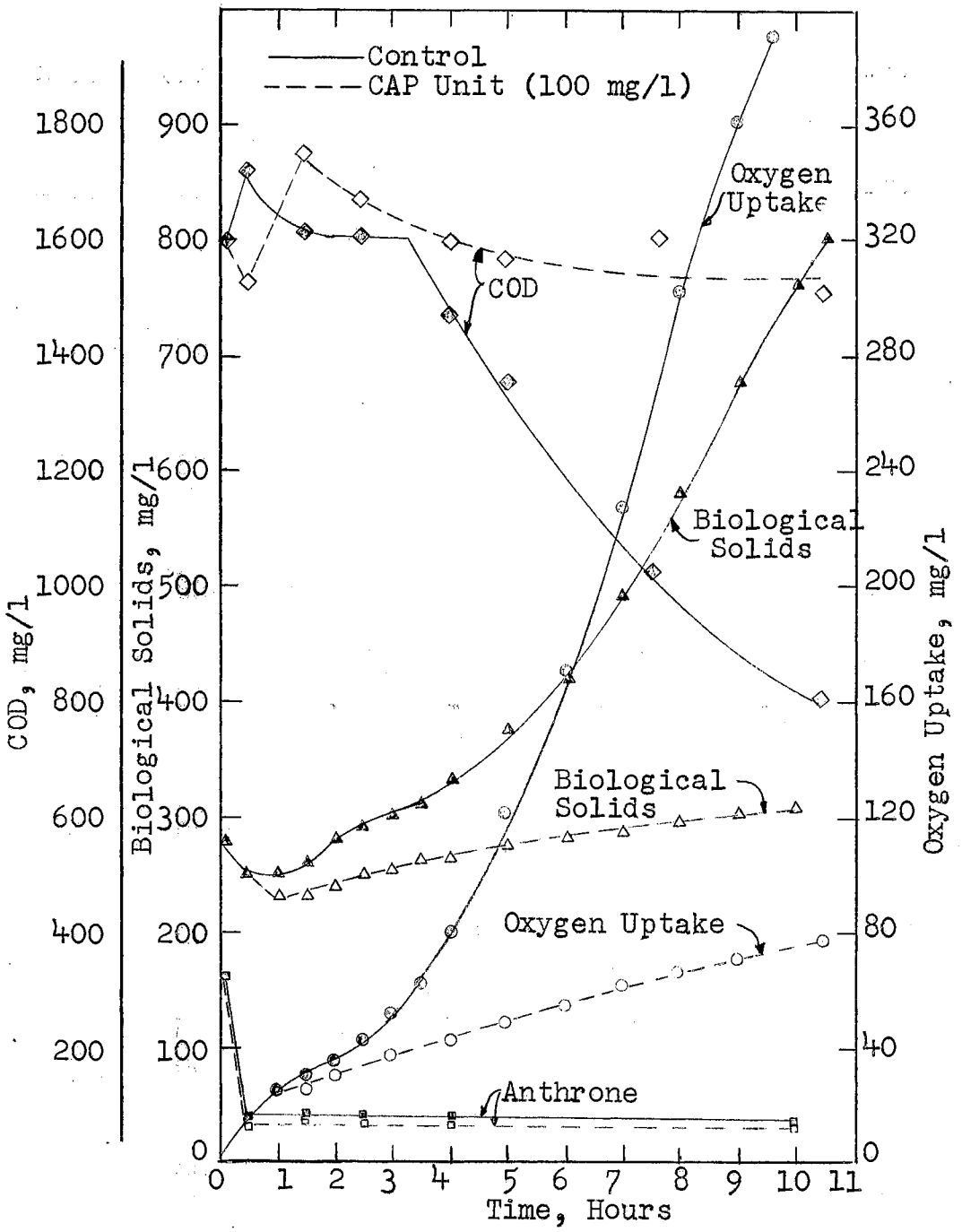

FIGURE 67 EFFECT OF CHLORAMPHENICOL ON SYSTEM PARAMETERS.

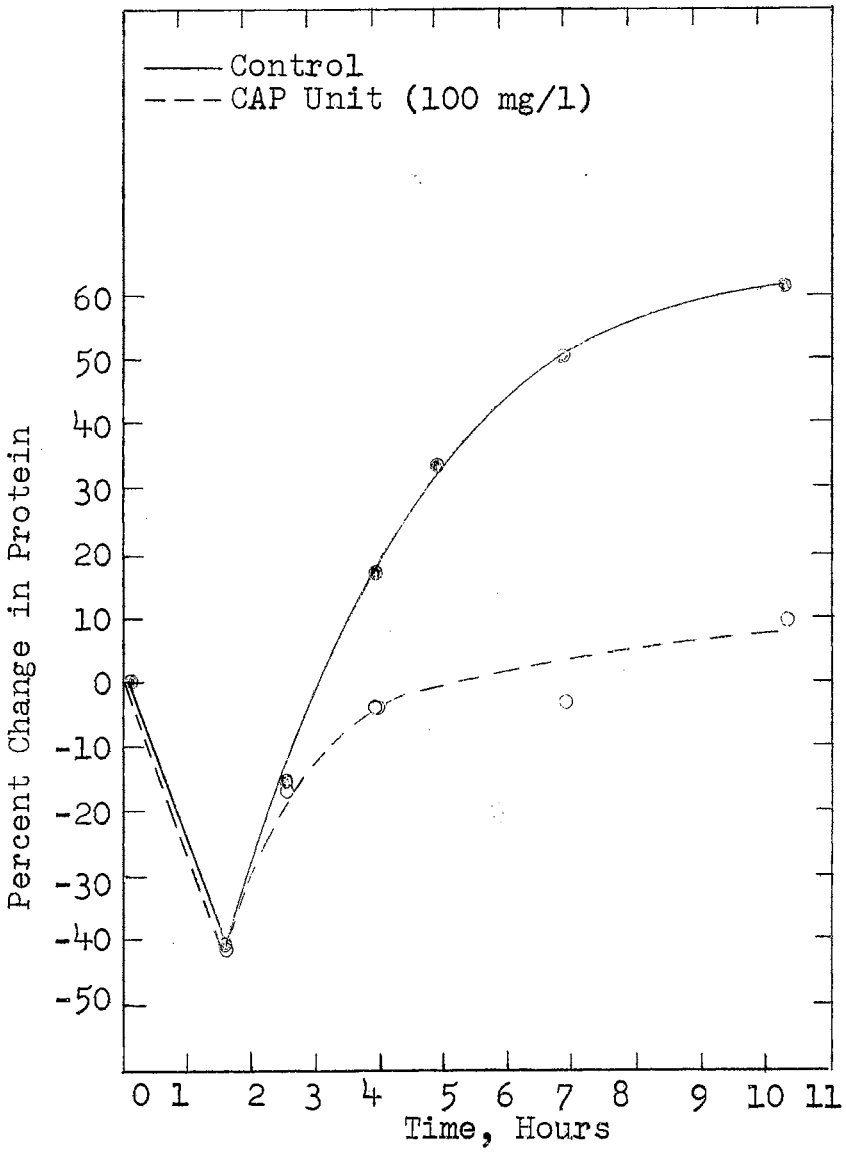

FIGURE 68 EFFECT OF CHLORAMPHENICOL ON PROTEIN SYNTHESIS.

bolized. It was intended to block protein synthesis in the system before the enzyme(s) could be synthesized.

Chloramphenicol was added to the system 30 minutes after the substrate (glucose) was added to give a final concentration of 100 mg/l. Figure 67 compares the effects of chloramphenicol with a control system. It is seen that the oxygen uptake, solids production, and COD removal were blocked by the chloramphenicol after the glucose was removed, whereas the control system after a short acclimation period effectively removed the remaining COD with a high increase in solids and a high rate of oxygen uptake.

Figure 68 shows the effects of chloramphenicol on protein synthesis. It is seen that protein for both sludges decreased during the initial lysis period and both increased after the lysis was completed. The chloramphenicol unit eventually increased only to a level equal to that before lysis, whereas the control increased to a level 60 percent greater than the initial value. A definite inhibition of protein synthesis is observed. It can also be seen that the chloramphenicol was not effective as a protein inhibitor until after two and one-half hours of aeration. It may be that during lysis the cells are unable to take up the chloramphenicol, thus imparting a delayed action. However, the required enzyme system was not induced until after three and one-quarter hours of aeration. This is indicated by the COD removal curve for the control system (Figure 67). Therefore, the delayed action of the chloramphenicol was of no consequence.

CHAPTER VI

DISCUSSION

A. Batch Operated Systems

1. Biochemical Responses

From the results of this study it can be seen that high concentrations of sodium chloride cause a decrease in the rate of substrate removal. This is true for both shock loads and long term exposures. A fresh water sludge shocked with sodium chloride concentrations up to and including 30,000 mg/l could remove all COD in a reasonable period of time. A shock load of 45,000 mg/l sodium chloride caused a much more deleterious effect on the sludge. Severe impairment of system efficiency ensued when an "old" fresh water sludge was shocked with 45,000 mg/l sodium chloride (Figure 32).

It is interesting to note that fresh water sludges could acclimate to sodium chloride concentrations up to and including 15,000 mg/l with no apparent change in the biochemical response of the sludge. At sodium chloride concentrations of 18,000 mg/l and 22,500 mg/l the substrate removal rate was decreased somewhat, but a definite stimulation of oxygen uptake and solids production was observed. Sludges developed in higher salt concentrations (45,000 mg/l NaCl) in general

removed COD at a slower rate than did sludge developed in fresh water and there was a greater tendency for the "high salt" sludge to release metabolic intermediates or end products during glucose metabolism than was found for sludge developed in fresh water.

It can also be seen from this study that sludges developed in waters with low salt content can withstand shock-loadings of high sodium chloride concentration somewhat better than sludges developed in high salts medium can withstand a rapid decrease in salt concentration. The results shown in Figure 9 indicated approximately 30 percent decrease in substrate removal efficiency when a fresh water sludge was dosed with 30,000 mg/l of sodium chloride; however, when an activated sludge acclimated to 30,000 mg/l sodium chloride was dosed with fresh water medium (Figure 29), substrate removal efficiency was reduced by approximately 75 percent. At the 45,000 mg/l salt level severe impairment of system efficiency ensued when fresh water sludge was shocked with salt (Figure 10), and nearly total destruction of efficiency was observed when sludge acclimated to 45,000 mg/l salt was subjected to fresh water medium (Figure 30).

An interesting result of the study which is not shown in the figures is the change which occurred in the settling characteristics of the sludge. In two separate studies, sludges developed at either 30,000 mg/l or 45,000 mg/l sodium chloride were dispersed systems practically devoid of any flocculating tendency. However, this does not appear to be a

general characteristic of sludges developed in high salt concentration since in one previous study the system did flocculate at sodium chloride concentrations of 30,000 mg/l and 45,000 mg/l. In all three cases the biochemical response of the sludge was the same.

2. Effects of Sludge Age

From the results of this study it can be seen that the responses of the old sludge to high concentrations of sodium chloride are quite different from those of young sludges. Under shock load conditions the substrate removal efficiency of the young sludge (Figures 31 and 32) was much more severely impaired than that of the old sludge (Figures 9 and 10). In fact, at a concentration of 45,000 mg/l salt the efficiency of the young sludge was completely destroyed. It is true that under long term exposure conditions there was very little difference in the response of old and young sludges (Figures 23 and 35), indicating that the young sludge could acclimate to high salts as satisfactorily as the old sludge but could not withstand the initial shock as well as the old sludge (Figures 10 and 32).

In studies to determine the effects of salts on the rate of respiration, it was found that young sludges had a higher Q_{O_2} than the old sludges (Table IV), indicating a greater activity for the young sludges. Levine and Soppeland (7) found in their studies that the bacterial cultures exhibiting the most activity were most affected by increasing salt concentrations. Komolrit (57) reported that glucose was not

readily degradable by either sorbitol- or mannitol-acclimated old sludges but was degradable by a similarly acclimated young sludge. Komolrit felt that glucose permease, required by the cells for glucose uptake, or an initial enzyme step required to bring glucose into the Embden-Meyerhoff-Parnas pathway was absent in the old cell sludge and had to be induced. His work thus showed that under organic shock loads also, young and old sludges respond differently.

A possible reason why old and young sludges respond differently to a salt shock may lie in the amount of dispersion exhibited by each sludge. The young sludges are completely dispersed systems, whereas the old sludges form floc particles and have a low degree of dispersion. If the sodium chloride does not penetrate the cell then the action of the salt must be upon the structure or function of the cell surface, or through osmotic damage to the cell. If the salt does penetrate the cell then the action would be an interference with metabolism. In either case, the old cell existing in floc particles would have less exposed surface area than the dispersed young sludge. Therefore, the old sludge would have some protection from the action of the salt, whereas the young sludge would be fully exposed. It is interesting to note that one effect of the shock loading of 30,000 mg/l was the dispersion of the floc particles. This is shown by comparing the photomicrographs in Figures 11 and 14.

The explanation just presented does not offer any suggestion as to why a more active organism is affected more

severely by sodium chloride than a less active organism when both are dispersed. An explanation of this could be that the permeability of organisms varies with their activity. The more active the organism the more permeable the cell membrane. Thus, the more active the organism the greater the permeability of the salt into the cell which may then interfere with some metabolic process. The problem can not be fully resolved until the question "Does salt penetrate into the cells and to what degree?" is answered.

The role of sludge age in the response of the bio mass to high sodium chloride concentrations is further emphasized when the biochemical responses of sludges from the continuous flow units are considered. It was observed in these studies that the deleterious effect of the salt fell between that observed for a young sludge and an old sludge. In the continuous flow studies a detention time of eight hours was employed. This produced a sludge with an age that must be classified somewhere between a young and old sludge. The effect of sludge age is especially important when considering the types of waste treatment being employed. Most conventional activated sludge plants in operation today employ long, rectangular aeration tanks in which "plug" flow tends to occur. However, completely mixed aeration tanks are becoming more popular as a means of treatment and will undoubtedly become even more popular in the future. In both types of activated sludge systems some degree of engineering control of sludge age can be obtained by varying the detention time and the volume and

concentration of recycled sludge. Thus, the effect of the sodium chloride on the system will vary in accordance with operational procedures which promulgate the development of older or younger sludges. Therefore from these studies it seems apparent that the sludge age that is to be maintained in the aeration tank must be considered when employing an activated sludge system to treat wastes containing high concentrations of sodium chloride.

3. Effects on Respiration

From the results of the studies on respiration it can be seen that in general as the salt concentration increased the accumulated oxygen uptake decreased. In cases where an old sludge had been acclimated to sodium chloride, a salt concentration of 15,000 mg/l stimulated the oxygen uptake. This was true for both sodium chloride and potassium chloride. There was very little difference between the effects of sodium chloride and potassium chloride. Sodium chloride did depress the oxygen uptake slightly more than potassium chloride. It was also observed that the acclimation to sodium chloride was not permanent. After an acclimated sludge had been transferred seven times in fresh water medium, the sludge had completely lost its increased ability to withstand a sodium chloride shock (Figures 58 and 60). It is also interesting to note that the growth phase at which the sludge was harvested had a pronounced effect on its ability to adjust to the salt shock. The activity of cells harvested during the log growth phase (Figure 63) was much more severely retarded than that of

cells harvested shortly after the log growth phase (Figure 62). This finding agrees very well with Doudoroff's (15) work with E. coli.

It seemed apparent from batch unit studies that a change in the ratio of respiration to synthesis occurs at high concentrations of sodium chloride. In order to analyze this effect more thoroughly, the percentage of substrate respired with increasing aeration time is shown in Figure 69, and the ratio of respiration to synthesis for various sodium chloride concentrations is shown in Figure 70. These parameters were determined using the data from the batch unit studies (Figures 4 to 23).

The percentage of substrate respired was determined by first converting the accumulated oxygen uptake at various time periods to the amount of substrate respired using the stoichiometric relation: mg/l oxygen x $\frac{180}{192}$ = mg/l substrate respired. This was then divided by the concentration of substrate removed during the various time periods. Figure 69 shows that in all cases the percent substrate respired increased with time. The rate of increase under conditions of long term exposure at 30,000 mg/l and 45,000 mg/l sodium chloride was slightly less than the other cases. It is interesting to note that after one hour of aeration 21 percent of the substrate removed was channelled into respiration in the 45,000 mg/l unit while in the control only approximately nine percent was channelled into respiration. After six hours the portion respired was 32 percent for the high salt system and approxi-

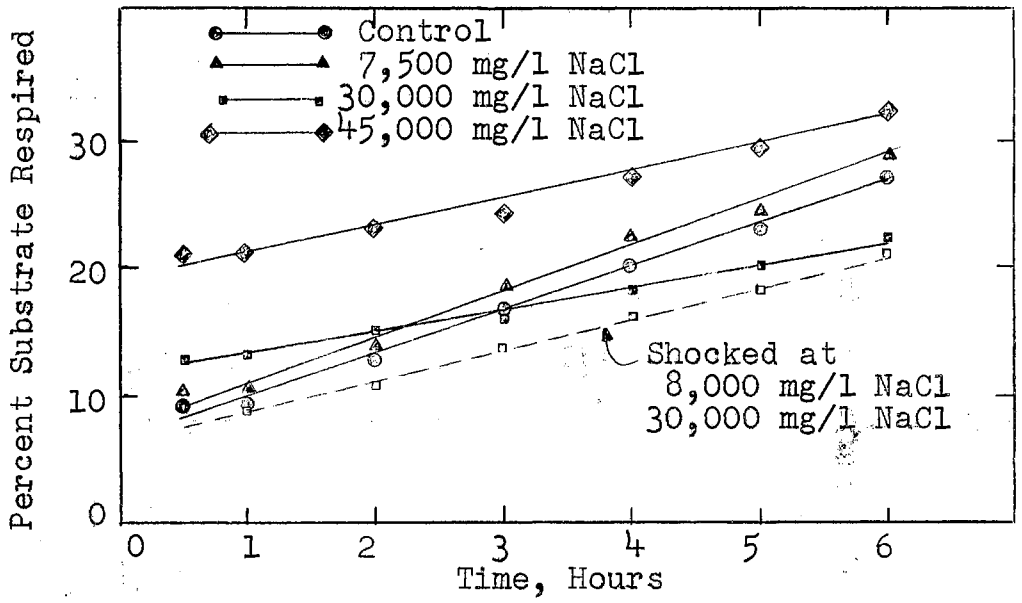

FIGURE 69 VARIATION OF PERCENT SUBSTRATE RESPIRED WITH TIME.

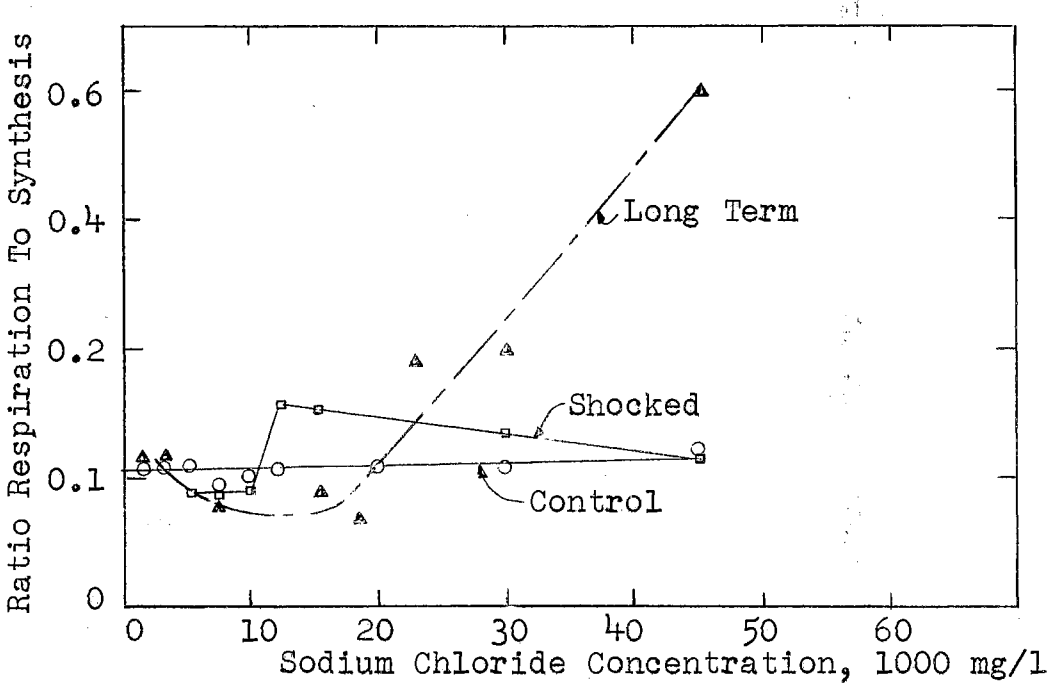

FIGURE 70 VARIATION OF THE RATIO OF RESPIRATION TO SYNTHESIS WITH CONCENTRATION OF SODIUM CHLORIDE.

mately 26 percent for the control. A long term exposure to 7,500 mg/l sodium chloride or shock conditions had very little effect on the percent substrate respired.

Figure 70 clearly shows a change in the ratio of respiration to synthesis at high concentrations of sodium chloride. The ratio of respiration to synthesis was determined at the point of maximum solids production. The amount of substrate respired was determined as previously described, and then divided by the increase in biological solids. For the control systems a constant ratio of 0.22 was maintained throughout the investigation. For shock exposure, the ratio of respiration to synthesis for a sodium chloride concentration of 5,000 mg/l was 0.19 and the ratio remained at this value up to the 10,000 mg/l salt level. At a concentration of 12,000 mg/l the ratio increased to 0.26 and then decreased linearly to a value of 0.22 at 45,000 mg/l sodium chloride. In the long term exposure study the ratio of respiration to synthesis was decreased at sodium chloride concentrations of 5,000 mg/l to 18,000 mg/l. At higher concentrations the ratio was sharply increased to a value of 0.80 at a sodium chloride concentration of 45,000 mg/l. These results show that sodium chloride causes a change in the biological population that results in greater use of the substrate for growth at lower salt concentrations and a greater use of the substrate for respiration than for sludge synthesis at higher salt concentrations. These changes that occur in the biological population will be discussed in greater detail later.

The significance of the change in the ratio of respiration to synthesis to plant operation is that as the ratio is increased more of the substrate is channelled into respiration, and less into synthesis. This means that less sludge will be produced and in turn a problem of maintaining the proper solids concentration in the aeration tank may be encountered.

In analyzing the accumulated oxygen uptake curves obtained from both the long term exposure and shock load studies, it was observed that at high sodium chloride concentrations the maximum rate of oxygen uptake occurred later than in the control. To verify this observation, the variation in the rate of oxygen utilization with time of aeration was determined and is shown in Figure 71. The rate of oxygen utilization was obtained by determining the slope of a line drawn tangent to a point on the accumulated oxygen uptake curve, calculating the oxygen used for one hour at this rate and dividing by the total weight of solids at that point.

From Figure 71 it can be seen that there is essentially no difference in the rate of oxygen utilization in the control, long term unit, or shock load unit at a sodium chloride concentration of 8,000 mg/l. Also, at 30,000 mg/l sodium chloride there is no difference in the time pattern of the rate of oxygen utilization between the control and shock load unit, but there is a shift in the location of the point of maximum oxygen utilization for the long term unit. Here the maximum rate of oxygen utilization has very definitely been moved further along the time scale. At 45,000 mg/l sodium chloride

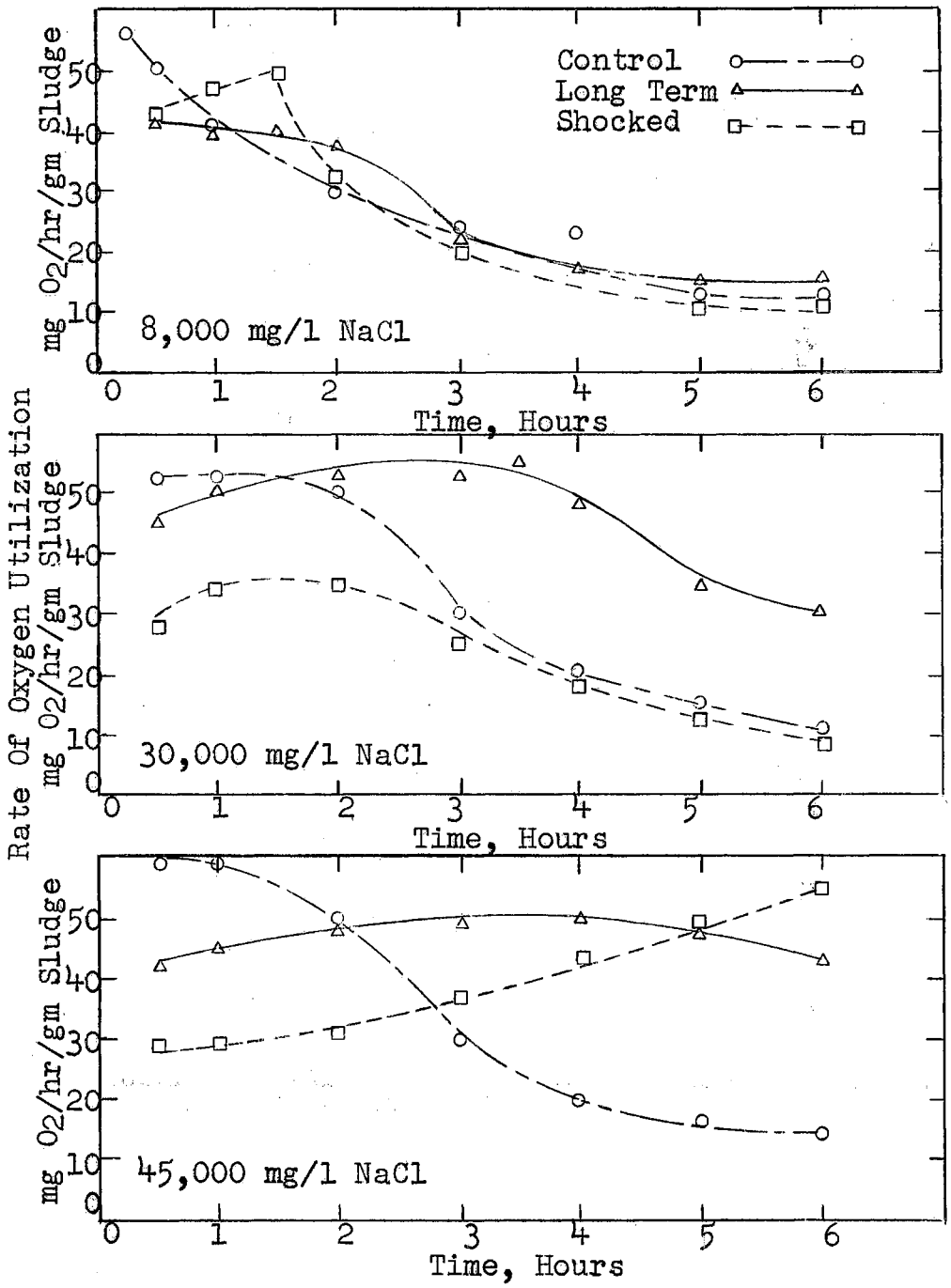

FIGURE 71 VARIATION IN RATE OF OXYGEN UTILIZATION WITH TIME OF AERATION

there is a shift in the point of maximum oxygen utilization for both long term exposure and shock load conditions. It should be noted that the oxygen utilization at six hours, which would be equivalent to the effluent end of the aeration tank in plug flow systems, is still fairly high, almost three times greater than the control for the long term studies and three and one-half times greater for shock load studies. In all cases the control exhibited a high initial oxygen uptake rate which in no more than two hours began to decrease rapidly to the final endogenous level.

The shift in the point of maximum oxygen utilization could be a very important consideration in aeration tank design. In the waste treatment field purification of a waste is thought of in terms of BOD removal or COD removal. In using these terms the primary interest is in the total oxygen required to stabilize a particular waste, but it is also important to determine the distribution of the oxygen demand in the aeration tank in order to design the aeration system. Oxygen must be supplied at a rate equal to or greater than the rate of utilization or the treatment efficiency will be impaired. In the long, rectangular aeration tanks used in conventional activated sludge processes, the oxygen uptake rate varies with time of aeration as the sludge passes through various growth phases. If the ratio of BOD or COD to biological solids is low, the initial rate of oxygen utilization rapidly decreases to a low, relatively constant, rate which is considered to be the endogenous level. If the BOD or COD to solids ratio is

high, the initial rate of oxygen utilization tends to be maintained at a high level for some time before it falls off rapidly to the endogenous level. This distribution of the rate of oxygen utilization has been verified in the field and has been reported by several investigators (67, 68, 69). The "tapered aeration process", which has become a widely adopted method for arranging diffuser tubes in conventional activated sludge systems, is based upon this principle.

This analysis has shown that the location of the point of maximum rate of oxygen utilization is moved further down the aeration tank when a system is subjected to high concentrations of sodium chloride. These results indicate that if an existing treatment plant employing tapered aeration were called upon to treat a high sodium chloride waste, the efficiency of the treatment process could be greatly reduced. Tapered aeration designed for normal wastes would not supply oxygen at the optimum rate and location required for efficient operation.

B. Continuous Flow Systems

It can be seen from the results of this study that continuous flow, steady-state systems can in general withstand a gradual shock-loading of high concentrations of sodium chloride. A gradual shock load of 30,000 mg/l sodium chloride (Figure 36) caused an initial decrease in system efficiency but the system recovered within two days to yield a COD removal slightly better than before the shock. It is also interesting to note that the sludge did not acquire a permanent acclimation to

sodium chloride. As soon as the continuous flow unit was again fed a fresh water waste, the acclimation to sodium chloride was lost. This is shown in Figure 38. The efficiency of a sludge harvested after the continuous flow unit had been fed a fresh water waste was very seriously retarded when the sludge was shocked with 30,000 mg/l sodium chloride.

A gradual shock loading of 45,000 mg/l sodium chloride (Figure 40) caused an initial decrease in system efficiency and approximately six days were required for recovery. It is interesting to note that this recovery was not permanent; instead the system oscilated between low solids-high effluent COD and high solids-low effluent COD conditions. The biochemical responses of these sludges at these different conditions varied noticableably. The sludge corresponding to high solids-low effluent COD (Figure 49) was much more efficient than the sludge corresponding to low solids-high effluent COD (Figure 48) conditions. When the system was returned to a fresh water waste a much more deleterious effect was observed than when the sludge was shocked with sodium chloride. This helps to confirm the findings during the batch unit studies that sludges developed in fresh water are less drastically affected by a slug dose of salt than is a sludge developed in high salt concentration by a rapid change to a fresh water environment.

One interesting aspect of the continous flow studies was the confirmation of the observation in batch studies that low sodium chloride concentrations stimulate the production of

biological solids during glucose metabolism. Figure 50 shows that the biological solids in the continuous flow unit were increased by 80 percent when subjected to a sodium chloride concentration of 8,000 mg/l. This increase in solids was maintained throughout the period that the unit was subjected to the salt waste. This provides evidence that the stimulation was not temporary but would be maintained as long as the unit was subjected to the salt waste.

One aspect of this study that is not evident from the results presented is that the response of a continuous flow system to high concentrations of sodium chloride depends upon the nature of the biological population at the time of the shock. Another continuous flow unit was operated to obtain photomicrographs of the sludge. The biological solids before the shock loading were at approximately the same level as the other continuous flow units. The unit was gradually shock loaded with 45,000 mg/l sodium chloride. Forty-eight hours after initiation of the shock loading, the efficiency of the system was completely destroyed. The other unit was not affected nearly as severely as this. This indicates that the biological populations of the two systems were different and had different tolerances to sodium chloride. The fact that the predominating species in a continuous flow unit will change spontaneously is further borne out by the biochemical response of the batch unit control systems. Figures 37, 38, 45, 46, and 47 show the biochemical responses of sludges harvested from a continuous flow unit receiving a fresh water waste.

The biochemical responses of the control units for each of these studies are different as indicated by the figures, thus pointing out that a change in predominating species had occurred. Novich and Szilard (71, 72) observed spontaneous mutations in the chemostat when tryptophan, ammonium chloride, phosphate, or lactate was used as the controlling growth factor. This led to the observation that if a bacterial strain is grown over a long period of time in the chemostat a mutant might arise which grows faster under the prevailing conditions than the parent strain. If this happens, practically the entire bacterial population in the chemostat will change from the parent strain to the new strain. In the continuous flow studies discussed here a heterogeneous population (sewage seed) was employed which provides an excellent opportunity for a change in predominating species. In light of these facts, it is very difficult to predict the effect of high salts concentration on a continuous flow system unless the character of the biological population can be controlled. Indeed, this is an area for future work.

C. Special Considerations

A significant response to rapid changes in salt concentration was the immediate release of cellular constituents as evidenced by an increase in soluble COD and a decrease in biological solids. These findings may explain the negative BOD reduction reported by Lawton and Eggerts(24), and the reduction in weight of organisms reported by Stewart, Ludwig,

and Kearns (25), which ensued following a change from ocean water to a fresh water waste. In studies specifically designed to assess the degree of lysis in response to rapid changes in salt concentration (Figures 64-A and 64-B) it was found that cells grown in 45,000 mg/l salt underwent some degree of lysis when placed in lower concentrations of sodium chloride, but when they were placed in concentrations below 10,000 mg/l there was a drastic increase in the degree of lysis (Figure 64-A). When cells grown in various levels of salt concentration were placed in salt-free medium, the degree of lysis was approximately linear with salt concentration in the original growth medium. From the data obtained it appears that cells grown in salt concentrations between 5,000 and 10,000 mg/l do not undergo a considerable amount of lysis when placed in fresh water. It is interesting to compare the degree of lysis for equal changes in salt concentrations under both test conditions. For example, in Figure 64-A it was seen that when old cells grown in 45,000 mg/l salt were placed in 10,000 mg/l sodium chloride, representing a decrease of 35,000 mg/l, there was approximately a 12 percent decrease in optical density, whereas when young cells grown in 30,000 mg/l sodium chloride were placed in fresh water there was a decrease in optical density of nearly 30 percent (Figure 64-B). Thus it would appear that a relative change from a high to a low concentration of salt is not as significant in regard to cell lysis as is a smaller decrease involving a change to fresh water. The difference in degree of lysis in the two cases mentioned above

is made even more significant by the fact that young cells showed less tendency to lyse when both types of cells were treated in the same way, i.e., when salt concentration was reduced from 45,000 mg/l to zero. Therefore,it seems apparent that three factors are involved in determining the degree of lysis, i.e., age of cells, magnitude of change in salt concentration and final salt concentration into which the cells are placed. Other factors being equal, the change into salt-free medium appears to exert the most pronounced effect.

Another significant aspect is the diphasic growth that occurred following lysis. In Figure 66 it was seen that a plateau in oxygen uptake and cell growth was generated between the time when all glucose had been removed and the time when the remaining COD was metabolized. The first stage was due to the metabolism of the glucose substrate and the second stage was due to the metabolism of the cellular components released during lysis. The second stage cannot be attributed to protozoa because microscopic examinations were made and no protozoa were observed in the system. The occurrence of this diphasic growth suggests that a new enzyme or enzyme system is induced during the plateau period between the two phases. This is further borne out by the chloramphenicol studies reported in Figure 67. The blocking of protein synthesis by chloramphenicol before the total removal of glucose completely prevented the occurrence of the second-stage growth. Bhatla (70) reported the occurrence of diphasic oxygen uptake for a pure culture. This diphasic oxygen uptake was due to the

secondary metabolism of volatile acids released as intemediates during glucose metabolism. He suggested that the diphasic growth of the pure culture was due to one of the following:

(1) The volatile acid-utilizing system is an induced enzyme system and the formation of the required enzymes is repressed by glucose.

(2) A transport enzyme (a permease) must be synthesized before the volatile acids can be brought into the cell and it is repressed in the presence of glucose.

It appears that one of these explanations would also explain the diphasic growth observed with lysed cells, except that instead of volatile acids cellular constituents are involved.

However, it must be pointed out that when a fresh water sludge was shock loaded with 45,000 mg/l sodium chloride (Figure 10) an osmotic lysis occurred and in which the lysed cellular material was removed before the glucose. In this case, the medium contained a high salt concentration whereas other systems in which diauxie was observed were fresh water systems. A possible answer to why the cells used the lysed material before the glucose could be that the presence of the salt inhibited glucose metabolism. The role of the salt in this occurrence has not been investigated. A second possible explanation is that the material released in the two situations may be entirely different in composition. A shock of high salt would tend to damage the cell membrane rather than cause a complete burst of the cell, whereas the reverse would be true for a fresh water shock. Thus, probably in the salt

shock the material released would be some readily metabolized compounds from the cellular pools. Furthermore as shown in Table II the biochemical composition of cells grown in fresh water and cells grown in high concentrations of sodium chloride are drastically different and material released might therefore be quite different, thus leading to an entirely different response.

The changes in protein and carbohydrate content of the cells (Figure 51) offer some insight into the effect of sodium chloride on the composition of the sludge. It was seen that at the 30,000 and 45,000 mg/l salt levels the carbohydrate and protein content of the cells was approximately half that of cells grown at either 12,000 mg/l salt or in fresh water. The analysis shown in Table II indicates that the decrease in carbohydrate and protein content was made up in part by an increase in lipid and RNA of the salt-grown cells. It is interesting to note that such drastic differences in biochemical composition were not reflected in the elemental composition (C-H-N). This finding itself seems very significant since on the one hand it indicates that the elemental composition, and the empirical formula which can be derived therefrom, offers no clue as to the biochemical composition, and on the other hand it indicates that the elemental composition of cells is a fairly constant value regardless of the biochemical composition. The difference in the biochemical composition of the sludge seems so great as to indicate that the differences in biochemical behavior between sludges acclimated to high salt

concentration and those grown in fresh water may not principally be due to the effect of sodium chloride on a particular sludge, but to the selection of species which can proliferate in high salt concentration. This tentative conclusion is supported by the results of the linear gradient study in which sludge acclimated to 45,000 mg/l sodium chloride was placed in fresh water under conditions which prevented the osmotic shock leading to cell lysis (Figure 65). Comparison of the results shown in Figure 23 and Figure 65 indicate that the substrate removal, oxygen uptake, and sludge growth patterns are similar for sludge acclimated to 45,000 mg/l salt, regardless of whether the cells are growing in a high salt concentration or salt-free environment. Thus the response is adjudged characteristic of the sludge, not of the presence of sodium chloride.

The photomicrographs add further support to the theory of selection of species. In the long term batch activated sludge studies it was observed that the sludge from a fresh water unit consisted of large floc particles together with bacteria, protozoa, and fungi (Figure 11), whereas a sludge from a unit under long term exposure to 45,000 mg/l sodium chloride consisted of very dispersed organisms, mainly single cell cocci (Figure 26). There were very few predators present in the sludge. Thus there was a very definite change in the morphological characteristics of the sludge. The sludge in the continuous flow unit before being shocked with sodium chloride consisted of dispersed single cell cocci and rods, some chains, and protozoa (Figure 41). After acclimation to 45,000 mg/l

sodium chloride the proportion of cocci to rods increased and the cells were generally smaller (Figure 42). When the system reached steady-state conditions after being returned to a fresh water waste the sludge consisted of short chains of cocci and rods and diplococci (Figure 44). Again there was a very definite change in the morphological characteristics of the sludge. It was observed in Figure 9 that a shock load of 30,000 mg/l sodium chloride had very little effect on the response of the system. An examination of the photomicrograph of a sludge shocked with 30,000 mg/l sodium chloride (Figure 14) reveals that there was no morphological change in the sludge. The effect of the salt was to disperse the sludge but there was no apparent change in the bacteria.

Therefore, based upon the difference in biochemical composition of fresh water sludge and high salt sludge, the linear gradient study, and the change in morphology of the sludge as shown by the photomicrographs it is concluded that the effect of sodium chloride on an activated sludge, which consists of a heterogenous population, is to foster the selection of species that can proliferate in high sodium chloride concentrations. Many investigators in the microbiological field have observed that different species have different tolerances to salts; therefore it does not seem unreasonable that in a heterogenous population only those species that can tolerate the salt concentration under study will survive. In making this conclusion the author does not mean to imply that sodium chloride does not have a bio-

chemical effect on microorganisms, but only that the effects observed on a heterogenous population seem to be due primarily to the selection of species which possess the characteristics observed in biochemical studies.

The author feels very strongly that sodium chloride does have a biochemical effect on individual microbial cells. Indeed such biochemical effect may provide the selective pressure on a mixed population which causes changes in predominance. It is suggested that the biochemical effect could be due to one or both of the following:

(1) The uncoupling of oxidative phosphorylation. On the basis of what is known about other respiratory chain systems, it could be reasoned that uncoupling does take place. In fact, Smith (42) has suggested that uncoupling may be an effect of salt but has pointed out the difficulty of studying this problem. The coupling of respiration to phosphorylation in many bacteria seems to be quite labile in that it has been difficult to obtain active cell-free extracts and especially difficult to obtain cell-free extracts of bacteria showing P/O ratios as large as those obtained with mammalian mitochondria. When considering heterogenous populations the problem is magnified in that if an active cell-free extract is obtained it probably will not be representative of the heterogenous populations.

(2) One of the control mechanisms involved in protein synthesis is affected by sodium chloride. In the biochemical analysis of a sludge acclimated to 45,000 mg/l sodium chloride,

it was observed that the protein content of the sludge was approximately one-third and the RNA two and one-half times average accepted values. It has also been observed in these studies that the production of biological solids in the presence of high salt concentrations is greatly reduced. Ingram (36) suggested that the action of salt on bacterial cells is the "salting out" of key enzymes. If his theory is correct then it is possible that this key enzyme is one involved in protein synthesis. It is also possible that the action of the salt on the enyzme is something other than salting out. One of the objections to this theory is that it does not explain the low carbohydrate and high lipid content of the sludge. The resolution of this theory requires a full study of the control mechanisms involved in protein synthesis.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The study has uncovered many unexpected effects of sodium chloride. All of these effects could not be completely investigated within the scope of the present study and will be left for future work. However, the following conclusions can be made from the study reported herein.

1. High concentrations of sodium chloride cause a decrease in the rate of substrate removal. This is true for both long term exposure and shock loading conditions. There is a noticeable decrease in substrate removal rate when sludges developed in fresh water are subjected to slug doses of 30,000 mg/l sodium chloride; however, this does not appear to cause serious distress to the system. Old sludges developed in fresh water undergo severe impairment of substrate removal efficiency when subjected to slug doses of salt resulting in mixed liquor concentrations of 45,000 mg/l and the efficiency of a young sludge is completely destroyed at 45,000 mg/l.

2. Sludge developed in fresh water is less drastically affected by a slug dose of salt than is a sludge developed in high salt concentration by a rapid change to a fresh water environment.

3. The physiological condition of the cells, which is operationally defined as cell age, plays an important role in the response of the cells to shock loads of sodium chloride. "Young cells" are much more severely affected than "old cells".

4. The completely mixed continuous flow activated sludge system operating under steady-state conditions could in general respond satisfactorily to a sodium chloride shock load. The ability of the system to respond satisfactorily to a shock load is probably dependent upon the predominating species present in the system at the time of the shock, since two completely independent continuous flow units started from different samples of sewage seed responded differently under the same cultural conditions.

5. Low sodium chloride concentrations stimulate the production of biological solids. This was observed in both batch and continuous flow studies.

6. The ratio of respiration to synthesis can undergo a severe change when the sludge is subjected to long term exposure to high concentrations of sodium chloride. Shock loading causes a much less severe change.

7. The point of maximum rate of oxygen utilization is delayed further along the aeration tank in a plug flow system. This dislocation of oxygen uptake rate is of such significance that it must be considered when designing the aeration system.

8. When sludges which are acclimated to a high concentration of salt are placed in a fresh water environment, the immediate response involves a release of cellular components

indicative of lysis. The osmotic shock causes a more severe disruption of system efficiency when the final salt concentration is below 10,000 mg/l. When sludges grown in salt concentrations between 5,000 and 10,000 mg/l are placed in fresh water, the degree of lysis appears to be negligible.

9. When cell lysis occurred, the response of the system was characterized by diphasic growth, the first stage except in one experiment being due to glucose metabolism and the second stage being due to the metabolism of the released cellular constituents. A new enzyme system(s) had to be synthesized before the released components could be metabolized.

10. Sludges grown in high salt concentrations were characterized by low carbohydrate and protein content, and abnormally high lipid and RNA content.

11. A release of metabolic intermediates or end products was noted during glucose metabolism for sludges growing in high salt concentrations. This appeared to be more prevalent in the young sludge population.

12. When sludges acclimated to high salt concentrations are transferred to fresh water under non-shock-loading conditions, the biochemical response to exogenous substrate under growth conditions is similar to the response obtained for the same sludge in the presence of high salt concentration. Photomicrographs revealed that a morphological change occurred in the biological population when subjected to high salt concentrations. These results are taken as in indication that the acclimation to high salts involves primarily a selection of

species rather than a biochemical acclimation of prevailing species.

CHAPTER VIII

SUGGESTIONS FOR FUTURE WORK

In view of the present study it is felt that the following investigations would be of value.

1. The study should be extended to include other salts such as calcium chloride, magnesium chloride, and titanium or aluminum chloride. This would provide the effect of divalent and trivalent ions on activated sludge. Further work should also be conducted using salts other than chlorides. It would be well to study carbonates, bicarbonates, and sulfates. This would provide a much broader analysis of the effect of inorganic wastes on the activated sludge method of sewage treatment.

2. It would also be well to study these different inorganic compounds in combinations with each other. In most wastes several inorganic compounds will be present and it would be of benefit to know the antagonistic or synergistic interaction between these various salts.

3. A more detailed study of the selection of species by salts should be conducted. This should involve the actual isolation and identification of organisms. It would also be desirable to determine if species can be selected which can withstand repeated large changes in salt concentration.

4. Before the true effect of salts on the bacterial cell can be determined, it must be established whether or not the salts penetrate the bacterial cell and to what degree. To accomplish this, it is felt that radioactive tracer techniques should be established that would leave no question regarding cell penetration.

5. Studies should be conducted to establish whether or not salt causes an uncoupling of oxidative phosphorylation. This could be accomplished very easily if techniques could be developed for obtaining an active bacterial extract.

6. The possibility of salt affecting any of the control mechanisms in protein synthesis should be studied.

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VITA

Donny Frank Kincannon

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDY OF THE EFFECTS OF SODIUM CHLORIDE ON ACTIVATED SLUDGE

Major Field: Engineering

Biographical:

Personal Data: Born January 17, 1933 in Olustee, Oklahoma.

Education: Attended grade school in Amarillo, Texas, and high school in Altus, Oklahoma; completed requirements for the Bachelor of Science degree from Oklahoma State University, in January 1959; completed requirements for the Master of Science degree from Oklahoma State University in May 1960; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in May 1966.

Professional Experience: Served in the U. S. Army from April 1953 to January 1956. Student Civil Engineer, Corps of Engineers. Hydraulic Engineer, Bureau of Reclamation. Sanitary Engineer, Texas State Department of Health. Instructor, Department of Civil Engineering, Arlington State College, Arlington, Texas.

Membership in Honorary and Professional Societies: Sigma Xi, Chi Epsilon, American Society of Civil Engineers, American Society for Engineering Education, Water Pollution Control Federation.

Publications: Author and co-author of the following publications:

1. "Certification of Sewage Works Operators", Manual for Sewage Plant Operators, edited by W. S. Mahlie, Texas Water and Sewage Works Association, Austin, Texas, 1964.

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