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Some effects of sodium pentachlorophenol on activated sludge and minnows.

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Department:

SOME EFFECTS OF SODIUM PENTACHLOROPHENOL ON ACTIVATED SLUDGE AND MINNOWS

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A

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ABSTRACT

The primary purpose of this investigation was to determine the effect of sodium pentachlorophenol on activated sludge. An additional objective was to determine the toxicity of sodium pentachlorophenol to minnows.

All activated sludge units were grown on a glucose and mineral nutrient waste. In addition, a unit acclimated to 100 and 250 mg/l of sodium pentachlorophenol was also studied. The effect of a shock loading of sodium pentachlorophenol was found to depend on the concentration of the loading and also on the group of micro-organisms which were predominating. One activated sludge system was seriously impared at a shock loading of 10 mg/l; yet, another system was not seriously impared until the concentration of the shock loading was 30 mg/l. The acclimated unit significantly reduced the organic content of the glucose waste within six hours; however, the concentration of biological solids was much lower and the oxygen utilization rate was much higher in this system than in the systems receiving the glucose waste only. It was also found that the acclimated unit remained a completely dispersed system. In all cases sodium pentachlorophenol was found to increase the production of cellular protein and to inhibit the production of carbohydrate. Sodium pentachlorophenol was found to be resistant to biological attack.

The studies with minnows established that the 48-hour median

tolerance limit is 1.0 mg/l of sodium pentachlorophenol. It was also established that concentrations as low as 0.4 mg/l are toxic to fish.

ACKNOWLEDGMENT

I would like to express my sincere appreciation to Dr. Don F. Kincannon for his indispensable suggestions and guidance throughout the course of the research and in the preparation of the thesis. I am also deeply grateful to Dr. Kincannon for introducing me to the field of Bioengineering.

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I. INTRODUCTION

The multitude of beneficial uses to which a number of the chlorinated hydrocarbons may be put has resulted in an increasing demand and use of these materials. As a result, increasing concentrations of chlorinated hydrocarbons are finding their way into water disposal systems and eventually to waste treatment plants. Although some of these compounds with carbon to chlorine bonds can be degraded by the activated sludge process, many others are resistant to biologic degradation. It has been shown (1) that the practice of chlorinating the effluents from waste treatment plants results in the considerable production of chlorinated organic compounds instead of their oxidation by an attack on the reducing sites. Since phenol is one of the most common aromatic compounds present in industrial wastes, the chlorination of industrial wastes containing this compound can significantly increase the concentration of chlorinated phenolic compounds in a waste treatment plant effluent.

Many of these chlorinated organic compounds are known to accumulate in the fatty tissue of fishes even though these compounds may be present in only trace quantities. Since low concentrations of these compounds are frequently fatal to aquatic life, it is important that the quantities of these compounds which are present in the streams be closely controlled.

One chlorinated hydrocarbon which has found several applications in industrial processes is pentachlorophenol. This compound has been

used for the control of mildew on textile products and for the control of slime or molds in heat exchange systems. It has also found wide application in the wood preserving industry.

It was the purpose of this investigation to determine the effects of sodium pentachlorophenol on activated sludge and on minnows. In the determinations with activated sludge both acclimated and nonacclimated systems were investigated, and the effects of various pentachlorophenol concentrations on both systems were also studied.

II. LITERATURE REVIEW

The literature contains a considerable amount of information concerning the applications to which sodium pentachlorophenol can be or has been put. Some of its applications, which include its use either alone or in combination with other compounds, are: as an additive to book binding adhesives; as an additive for the prevention of the swelling of wood, cork powder, and organic fiber; as a slime preventing agent in papermaking processes; for the control of snails; for weed control; for impregnating wood; for rotproofing cotton; for the preservation of rubber latex; as a fungicide for textiles, wood products, leather, and asbestos fiber boards; as a herbicide; as a hide preservative; and for providing electrical insulating paper with bactercidial and fungicidial properties. However, the literature contains virtually no information concerning the effects that sodium pentachlorophenol has on the activated sludge process.

An investigation to determine the acute and chronic toxicities of mixtures of sodium pentachlorophenates and sodium salts of chlorophenols with less chlorine was conducted by Roche on several animals. (2) It was found by subcutaneous injection that between 50 and 250 mg/kg of body weight was needed to cause poisoning which was either acute or sub-acute. It was also found that rapid elimination of the product prevents cumulative action and that it did not seem to be a true chronic poison. As a result of this investigation, Roche concluded that

extremely high concentrations would be necessary for sodium pentachlorophenol to be toxic to man.

Motohachi, Izeki, and Moriyoshi (3) have found that the median lethal dose to mice of sodium pentachlorophenol administered orally or subcutaneously was 199 and 63.7 mg/kg of body weight, respectively. An intraveneous injection of 10 mg/kg of body weight was shown to produce a fall of blood pressure and a slight stimulation of respiration in rabbits. Sodium pentachlorophenol also showed a paralytic action on the frog heart.

An investigation was conducted in Russia (4) to determine the allowable concentration of sodium pentachlorophenol in water reservoirs. In this study, sodium pentachlorophenol was administered to guinea pigs (0.25 mg/kg) and rabbits (0.1 mg/kg) for a period of six months, and at the end of this time all indices examined were normal. As a result of these findings, the investigators felt that the maximum allowable concentration of this material in water reservoirs should be determined by its threshold odor concentration (5 mg/l) rather than by a consideration of its toxic effects.

Another important consideration, however, is the effect of sodium pentachlorophenol on aquatic life. Goodnight (5) conducted an investigation to determine the toxicity of sodium pentachlorophenate and pentachlorophenol to fish. He found that these materials are fatal to the more sensitive species of fish in concentrations above 0.2 ppm although

hardier species were able to survive at concentrations of 0.4 or 0.6 ppm. In lethal concentrations, the metabolism of fish was increased; this was evidenced by increased respiratory movements. It was concluded that the toxic effects are apparently due to penetration into the tissues and entrance into the blood stream. Here due to an upset in the metabolic rate, blood pressure was increased and rupture of the smaller capillaries and bleeding resulted. It was also found that even a small increase in the concentration of sodium pentachlorophenate resulted in a large increase in the respiratory rate.

Goodnight (5) also found that the toxicity of sodium pentachlorophenate and pentachlorophenol to fish was increased by lowering the pH of the water and that within reasonable limits the size of the fish, and the temperature and character of the water did not greatly affect the toxicity of these compounds. In another investigation (6) of the factors which affect the 24-hour medium tolerance limit of fish exposed to sodium pentachlorophenate, it was also found that the lower the pH of the test solution the greater was the toxicity. However, contrary to the results of the previous investigation discussed (5) it was found that an increase in temperature resulted in an increase in toxicity. This was attributed to the greater metabolic activity of fish at higher temperatures.

Lyr has presented several articles concerning the effect of pentachlorophenol on fungi (7, 8, 9). He has found that pentachlorophenol

inhibits oxidative phosphorylation in the wood destroying fungi Phellinus igniarius and Collybia velutipes. He has also found that when the concentration of pentachlorophenol was 10^{-6} M, oxygen consumption was increased, but at concentrations of 10^{-4} and 10^{-3} M, oxygen consumption was inhibited or fully suppressed. He, therefore, postulated that pentachlorophenol exerts its action through a mechanism similar to that of 2, 4-dinitrophenol, that is by uncoupling oxidative phosphorylation. He also conducted studies with wood-rotting fungi which were grown as surface cultures in a 3 per cent malt solution at 25°C. After 4 to 21 days the cultures were treated with chlorinated phenols, and the progress of their decomposition was followed by chloride analysis and ultraviolet spectroscopy. He found that all fungi tested which secreted laccase into the culture medium were able to detoxify chlorophenols including pentachlorophenol. He also observed that the stability of halophenols increased with increased chlorination of the benzene ring.

The mitochondria of a cell is the site in which conversion of substrate energy into adenosine triphosphate (ATP) occurs by the process known as oxidative phosphorylation. In the conversion of one mole of glucose to CO_2 and H_2O by the Embden-Meryerhof pathway and the Tricarboxylic Acid Cycle there is a net yield of 38 moles of ATP. Four are formed anaerobically, and 34 are formed by oxidative phosphorylation. Pressman (10) found that pentachlorophenol, an uncoupler of oxidative phosphorylation, has the ability to act on both the DPN-flavin transfer

pathway and the cytochrome b transfer pathway. It has also been shown (11, 12) that the yield of new cells is proportional to the quantity of ATP produced. Therefore, an examination of the effects of pentachlorophenol on mitochondria can provide valuable insight into the effects which may be observed on the cell as a whole when it is exposed to pentachlorophenol.

Studies have been made in which the mitochondria from rat liver were subjected to various concentrations of pentachlorophenol. In a study by Buffa et. al. (13) mitochondria were isolated from the liver of rats injected with 20 mg intraperitoneal doses. Respiratory activity fell 75 per cent in mitochondria removed from the liver and frozen within 15 seconds after injection and fell 100 per cent within 30 seconds. It has also been observed (14) that one minute after intraperitoneal injection of pentachlorophenol, liver mitochondria completely lose respiratory control in that they oxidize pyruvate and L-glutamate significantly faster. In the study by Buffa et. al. (13) the P/O ratio was also observed to follow a parallel drop whereas the adenosine triphosphatase activity of the mitochondria rose 30, 250, and 320 per cent after 15, 30, and 60 seconds, respectively. It was also observed that the P/O ratio fell more rapidly with increasing doses of pentachlorophenol when mitochondria were incubated with pyruvate than when incubated with L-glutamate. The fact that pentachlorophenol inhibits oxidative phosphorylation has been confirmed in other studies (7, 8, 9, 14, 15, 16, 17), and the increased adenosine triphosphatase activity has also been observed (14).

A study was made by Weinback and Garbus (16) to determine the interaction of uncoupling phenols with mitochondria and mitochondrial protein. It was found that in the presence of the minimum concentration of pentachlorophenol required for complete uncoupling of oxidative phosphorylation, the mitochondria accumulated approximately 0.75 μg of pentachlorophenol per mg of mitochondrial protein. It was also found that the mitochondrial protein had an affinity for various uncoupling phenols equal to or greater than intact mitochondria. Pentachlorophenol remained tightly bound to intact mitochondria despite repeated washings with 0.25 M sucrose. However, when bovine serum albumin was included in the washing media, all the pentachlorophenol was removed in a single washing, and the impared mitochondria were readily restored to activity. It was concluded that mitochondrial protein is the binding site for the uncoupling phenols, and that the uptake of pentachlorophenol by intact mitochondria is unrelated to the degree of metabolic activity of the mitochondria.

In another study (17) with the mitochondria from rat liver, it was observed that the addition of pentachlorophenol resulted in swollen and uncoupled mitochondria. However, subsequent addition of serum albumin and adenosine triphosphate restored them to nearly normal. The addition of albumin alone to the normal, swollen, and restored mitochondria increased the capacity for phosphorylation in all, but particularly in the swollen and uncoupled forms. A determination of

the amount of albumin necessary to protect mitochondria from swelling showed that the amount was much less than the amount necessary to protect against uncoupling. It was also observed that morphological changes in mitochondria induced by pentachlorophenol were reversed by albumin and adenosine triphosphate.

A study to determine the effect of variations of bovine serum albumin concentrations on the adsorption of pentachlorophenol has been made by Giglio and Goncalves (18). Interaction between both substances was noted, and determinations at several pH values revealed it was of the anion-anion type. It was also found that there was no coupling of pentachlorophenol with amino acids or with nucleic acids from calf thymus.

It has been shown (19, 20, 21) that the presence of chlorine on a microbial substrate does not necessarily prevent microbial metabolism. However, there appears to be a relationship between molecular structure and resistance to bacterial degradation. The effect of chlorine is evidently modified by several features such as molecular size and the nature, number, and relative position of other substituents, and it appears that the effect of chlorine on metabolism depends entirely on the overall molecular makeup. Okey and Bogan have found (19) that the presence of three or more chlorine atoms on an aromatic ring appears to severely restrict microbial metabolism regardless of the presence of other functional groups; during this study it was also

established that lindane (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) is resistant to metabolic attack.

A study with activated sludge was made by Ingols, Gaffney, and Stevenson (1) to determine the biological activity of halophenols. An activated sludge developed by aerating soil was grown on glucose and peptone in a mineral nutrient medium free of added chloride ions. The sludge was acclimated to the chlorinated phenols by withdrawing the normal food, peptone and glucose, while maintaining a constant level of chlorinated phenol. During its acclimation the sludge changed from its normal yellow-tan color to dark brown. This influence of chlorinated phenols on the predominating species of micro-organisms was also established by the treatment of soil with 50 ppm of sodium pentachlorophenate (21); in this study it was found that a group of micro-organisms could be isolated which was easily stained with methylene blue.

During the investigations with activated sludge (1), the degradability of compounds was determined by ultraviolet adsorption and by determinations of the chloride ion concentrations. An analysis of the degradability tests with sodium pentachlorophenol at a concentration of 100 mg/l indicated that there was no ring degradation after a period of four days and also that no chloride ions were freed at the end of this period.

In a study by Kincannon (22) batch activated sludge units were grown on glucose and mineral nutrients and acclimated for a period of

two weeks to concentrations of sodium pentachlorophenol which varied from 30 to 800 mg/l. Studies on these units were then conducted over six hour periods. The oxygen utilized was observed to be substantially increased at concentrations of sodium pentachlorophenol of 200 mg/l or less; the mixed liquor suspended solids produced were greatly decreased in all cases; and there was a substantial decrease in the rate of removal of organic material in those units acclimated to sodium pentachlorophenol when these values were compared to those obtained from a system grown in a glucose waste only and receiving no sodium pentachlorophenol. It was also observed that no sedimentation occurred in the units which were acclimated to sodium pentachlorophenol.

Methods of chemically treating a waste containing sodium pentachlorophenol have been suggested. Lyr (23) has shown that pentachlorophenol can be destroyed by oxidation with H_2O_2. Hiatt, Hoskins, and Oliver (24) have established that sodium pentachlorophenate can be destroyed by photochemical degradation. Colorimetric analysis of aqueous solutions of sodium pentachlorophenate before and after exposure to light of wave length 290 mμ and to light of wave length 330 mμ indicated that there was a loss of sodium pentachlorophenate from absorption of light in the region 290 to 330 mμ, corresponding to a maximum at approximately 318 mμ. Degradation was found to follow first-order kinetics, and the velocity constant was 3.4×10^{-4}/sec at a light intensity of approximately 0.04 watts per square centimeter between 290 and 330 mμ.

III. MATERIALS AND METHODS

A. Experimental Protocols

1. Batch Activated Sludge Units

The batch activated sludge units which were employed were developed from a sewage seed taken from the primary sedimentation tank of the trickling filter plant located in Rolla, Missouri. These units were developed on a waste containing: glucose, 1000 mg/l; $(NH_4)_2 SO_4$, 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. The units were operated with an aeration volume of 1.5 liters.

After each unit had developed, it was operated in the following manner. Prior to daily feeding, 500 ml of mixed liquor were wasted; the unit was then settled for one hour. Then 500 ml of supernatant were drawn off and wasted. The units were then brought back to the 1.5 liter aeration volume by using distilled water and the waste previously described. All units were operated in this manner for a period of at least 21 days prior to use.

In the studies to determine the effect of long term exposure to sodium pentachlorophenol the pentachlorophenol was added to the batch unit so that its concentration was increased at the rate

of 10 mg/l per day until the desired concentration had been reached. The unit was then operated at this concentration for a period of at least 25 days prior to use. Throughout the entire period the unit was also fed the standard waste. After the response of the system at the desired concentration had been determined, the unit was again fed increasing amounts of sodium pentachlorophenol so that the concentration was increased at the rate of 10 mg/l per day until the next desired concentration was reached. The unit was then operated at this concentration for a period of at least 25 days prior to use.

In the studies to determine the effect of shock loadings, the mixed liquor from a unit receiving the standard waste was divided into two equal parts. Both new units were then brought to volume and one unit was fed the standard waste and sodium pentachlorophenol; the other unit was maintained as a control unit and was fed the standard waste only. At the end of each determination, the control unit was retained and operated as a batch unit until its next use.

2. Bioassay Study

To determine the toxicity of sodium pentachlorophenol, a bioassay study was conducted with minnows being used as the test species. Prior to the test, the minnows were acclimated to the laboratory conditions for a period of approximately one month and

were fed daily during this time. Tap water from the University of Missouri at Rolla water system was used as the dilution water. During the test the fish were kept in five gallon Pyrex bottles with a volume of 18 liters being utilized. A total of six units was used; one unit was maintained as a control, and the other units contained sodium pentachlorophenol at concentrations of 0.4, 0.63, 1.0, 1.6, and 2.5 mg/l. A total of eight fish was used in each unit, and the study was conducted for a period of 96 hours. The other details employed in the test procedure are those specified in Standard Methods (25).

B. Analytical Techniques

1. Substrate Removal

a. Chemical oxygen demand (COD)

One method used to determine waste removal was the COD test. The sample was first passed through a 0.45 μ membrane filter, and the filtrate was used in all determinations. The test was conducted according to the procedure specified in Standard Methods (25).

b. Anthrone

The carbohydrate remaining in solution was measured by the anthrone test as outlined by Gaudy (26) with the exception that 3 ml of sample or aliquot and 9 ml

of anthrone reagent were used instead of those concentrations specified. This was done because previous experience had shown that better results are obtained at these concentrations. Since this test is specific for carbohydrates, it was used to follow the removal of glucose from the system. The difference between the results of the COD and anthrone test can be used to determine whether metabolic intermediates or end products other than carbohydrates are released into the system by the micro-organisms.

2. Respiration

A Warburg respirometer was used to measure respiration. In all cases the temperature was maintained at $20^{o}C$, and the sample volume used was 25 ml. The manometer-flask volumes were determined by using the water method as described by McKinney (27), and the flask constants for direct conversion of manometer readings to oxygen uptake in mg/l were determined by the method outlined in Manometric Techniques (28).

3. Biological Solids

The suspended solids of each unit were determined by the membrane filter technique as outlined in Standard Methods (25). Because of the dispersed nature of the cells in the unit acclimated to sodium pentachlorophenol, the mixed liquor from these units

was first centrifuged for a period of about ten minutes. The supernatant was then easily passed through the filter and collected. Then the "pellet" of solids remaining in each centrifuge tube was removed with a spatula and placed on the filter. The tube and spatula were then washed with distilled water to remove the remaining cells, and the washings were also passed through the filter.

4. Biochemical Composition of Cells

The carbohydrate and protein compositions of the cells were determined by the anthrone and biuret tests, respectively; these tests were conducted according to the procedures outlined by Gaudy (26) with the exception that 3 ml of sample or aliquot and 9 ml of anthrone reagent were used in the anthrone test instead of those concentrations specified by Gaudy.

Since neither of these determinations can be made on whole cells, the assay procedures require that the cell wall be broken down. This was accomplished by boiling the cells in the presence of the anthrone reagent in the test for carbohydrates and by boiling the cells for 15 minutes in 2N KOH before adding the biuret reagent in the test for protein.

5. Sodium Pentachlorophenol Concentration

The rate of change of the concentration of sodium pentachlorophenol in the units with time was determined by the simplified

aminoantipyrine method described in Standard Methods (25). In early investigations, it was found that the color developed for a given sodium pentachlorophenol concentration varied with the length of time that the aminoantipyrine solution had been prepared prior to its use. Therefore, samples of known sodium pentachlorophenol concentration were used with each determination.

IV. RESULTS

A. Batch Activated Sludge Systems

1. Effects of Acclimating an Activated Sludge System to Sodium Pentachlorophenol

The effects of acclimating a system to concentrations of sodium pentachlorophenol of 100 and 250 mg/l are illustrated in Figures 1 and 2. In both cases it can be seen that the glucose was effectively removed from the system in the six hour period, and in both cases the rate of glucose removal followed zero-order kinetics for the first two hours. It can also be seen that in both cases there was a considerable release of metabolic intermediates or end products into the system. At a sodium pentachlorophenol concentration of 100 mg/l, the difference between the COD and anthrone values was 560 mg/l at the end of two hours. In the system acclimated to 250 mg/l, the difference between the COD and anthrone values at the end of two hours was 800 mg/l. The COD of sodium pentachlorophenol is only 0.297 mg of COD per mg of sodium pentachlorophenol; or at a concentration of 100 mg/l, the COD exerted by the sodium pentachlorophenol is only 29.7 mg/l.

It is also of interest to note the large values of oxygen uptake in both systems when these values are considered in relation to the small quantity of biological solids which were present; this is characteristic of the results which would be expected when an uncoupler is present in a system.

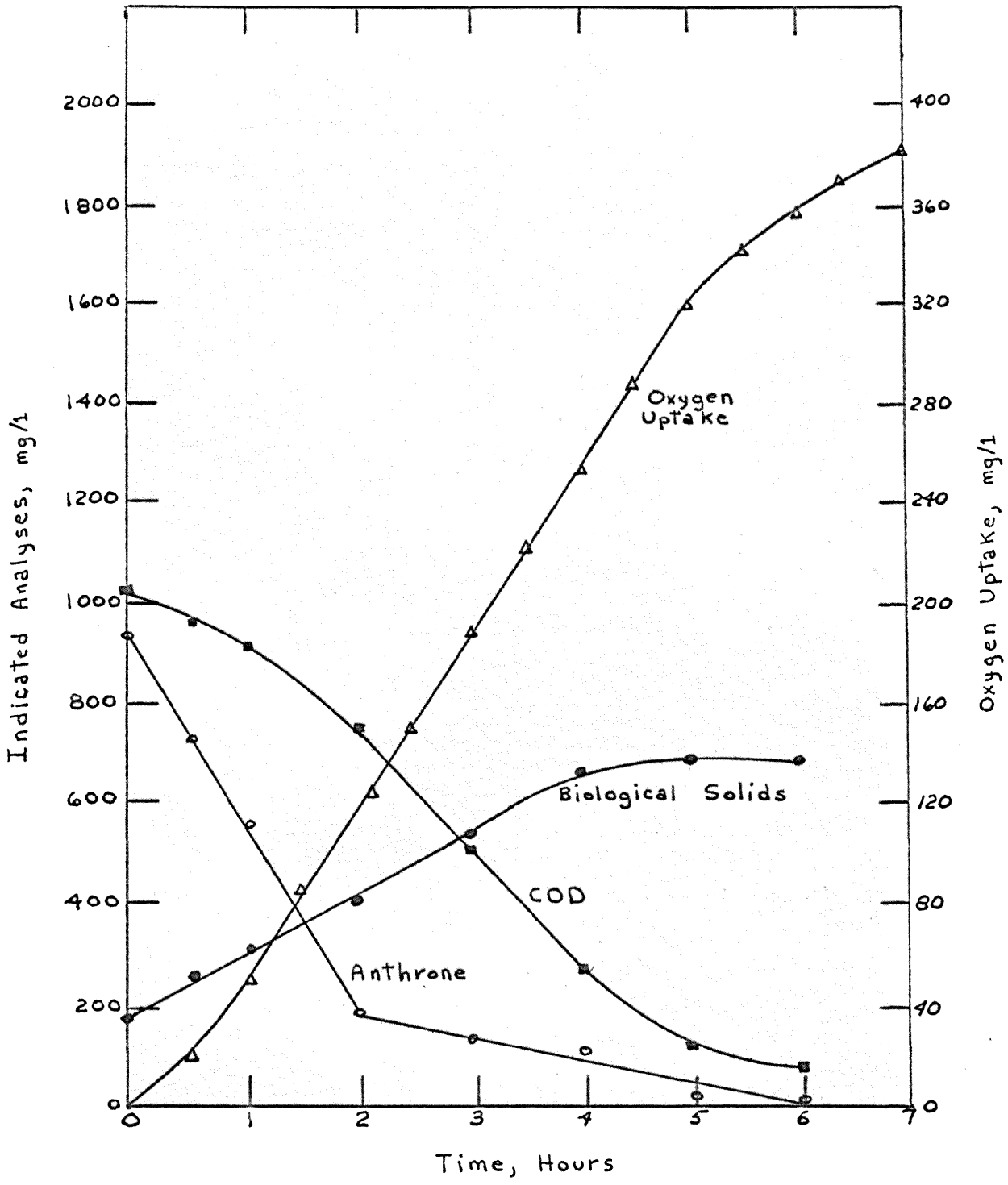

FIGURE 1. Change in System Parameters. Sludge Acclimated to 100 mg/l Sodium Pentachlorophenol.

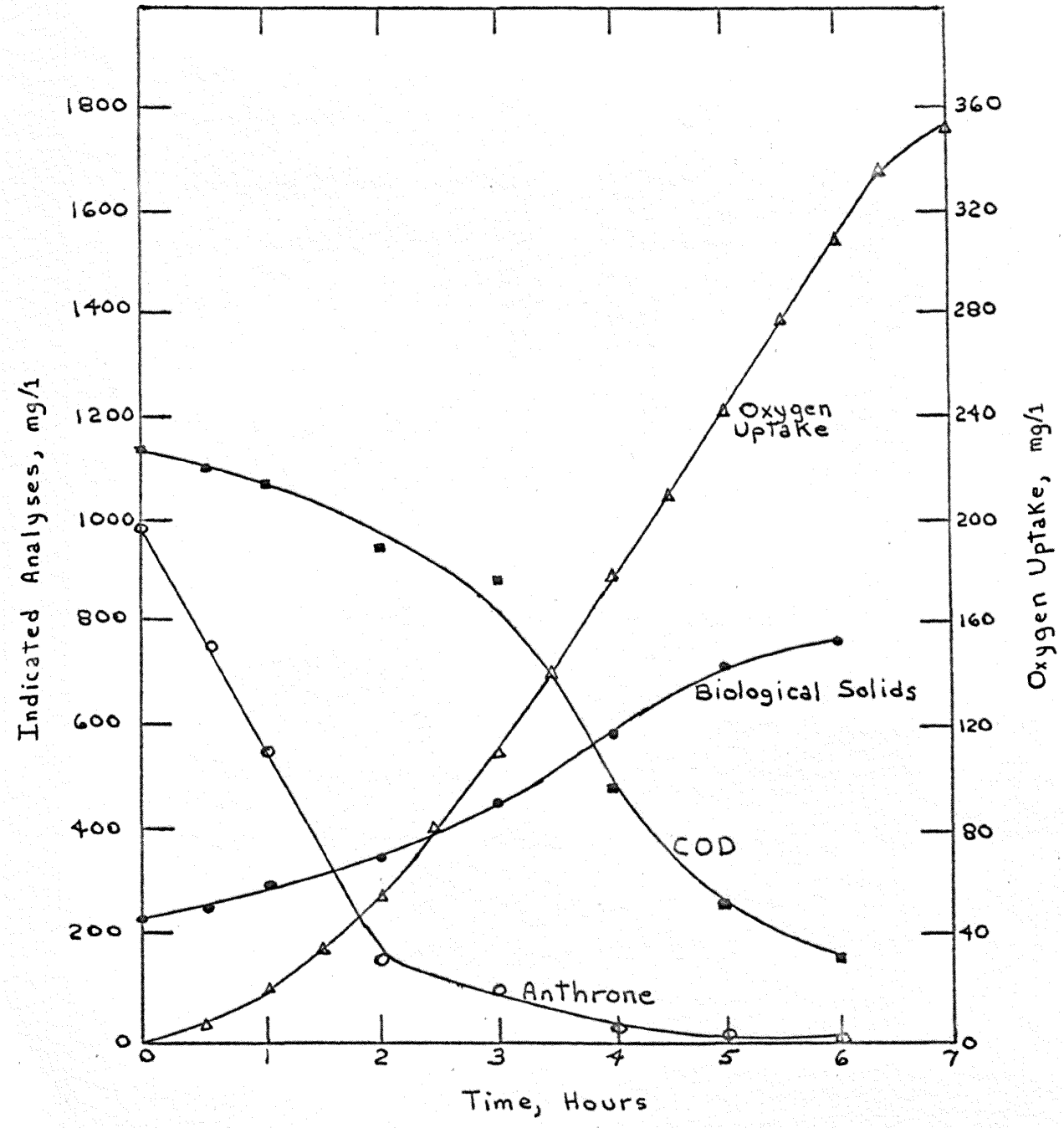

FIGURE 2. Change in System Parameters. Sludge Acclimated to 250 mg/l Sodium Pentachlorophenol.

Determinations of the concentration of sodium pentachlorophenol were made on samples taken from each concentration studied at times 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 hours. In addition, a determination was also made at the end of 22 hours on a sample from the system acclimated to 100 mg/l and at the end of 35 hours on a sample from the system acclimated to a concentration of 250 mg/l. The results of these determinations showed that the sodium pentachlorophenol concentration remained constant throughout the entire period. This indicates that this compound was not subject to biological degradation.

Twenty-five ml of the mixed liquor from the unit acclimated to 100 mg/l of sodium pentachlorophenol were passed through a membrane filter; the cells were washed with 0.03 M phosphate buffer and were then resuspended in the standard waste and brought to a total volume of 25 ml with distilled water. The oxygen utilized by this system was then determined and compared with that utilized by the sodium pentachlorophenol system; the results are shown in Figure 3. It can be seen that there was little effect on the system acclimated to 100 mg/l when it was shock loaded with the standard waste only.

2. Effects of Shock Loading on Activated Sludge System with Sodium Pentachlorophenol

The changes in system parameter for a sludge grown on the

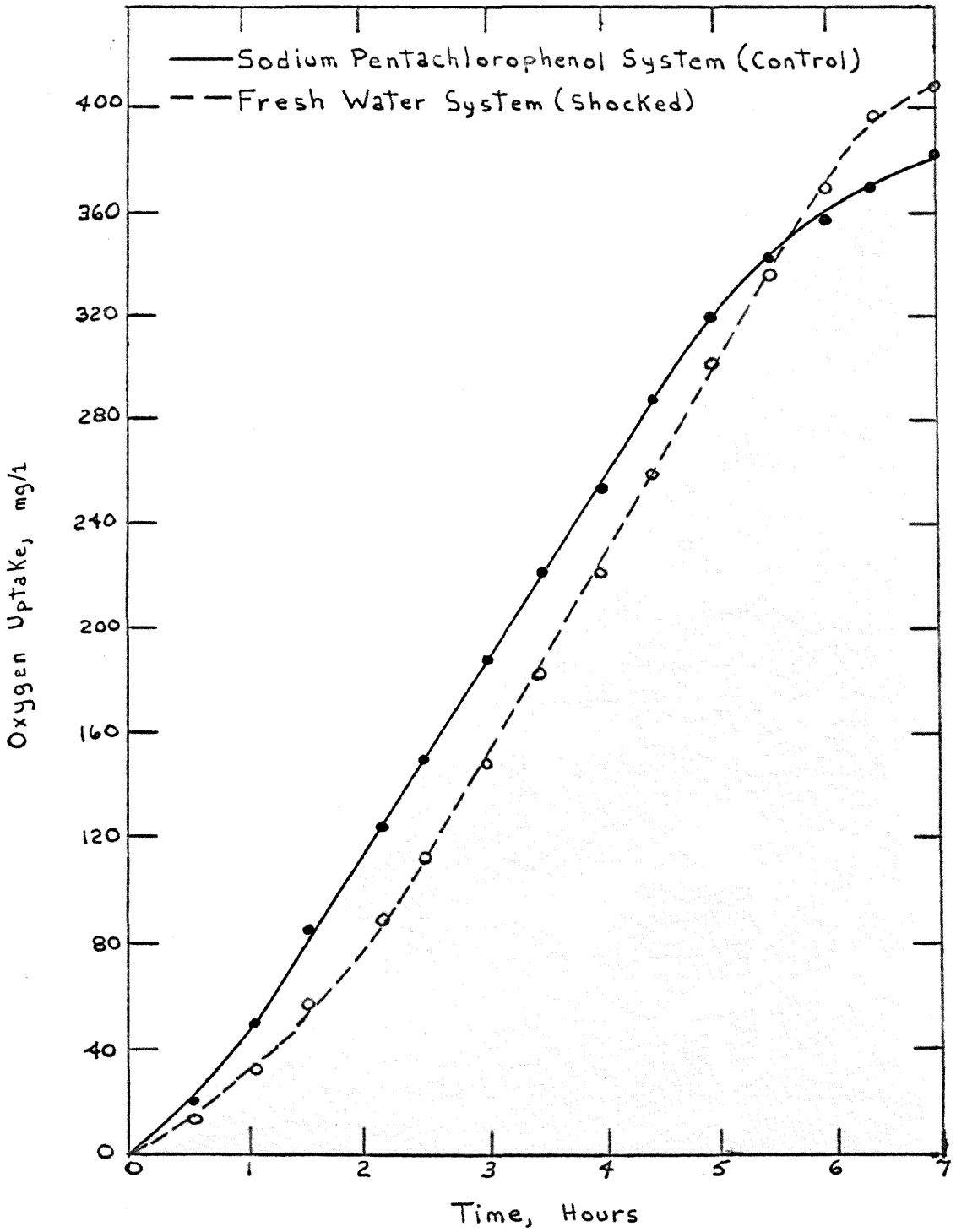

FIGURE 3. Change in Oxygen Uptake . Sludge Acclimated to 100 mg/l Sodium Pentachlorophenol Subjected to Shock Load of Standard Waste.

standard waste and then shock loaded with sodium pentachlorophenol are compared with their respective control systems in Figures 4-8. The control systems were the systems which received the standard waste only with no exposure to sodium pentachlorophenol, and they are represented in Figures 4-8 by the notation, "Fresh Water System (Control)." The data in Figures 4 and 5 were determined from a system in which the cells had a brown appearance; it settled poorly and had the characteristics of a bulking sludge system. The data in Figure 6 were obtained from a system which had a yellow-tan appearance as did the systems represented in Figures 7 and 8. However, although the unit shown in Figure 6 had been operated for a period of 21 days, it remained a completely dispersed system. In contrast to this, the units represented in Figures 7 and 8 were flocculent systems.

The units shown in Figures 4 and 5 were shock loaded with 5 and 10 mg/l of sodium pentachlorophenol, respectively. It can be seen that at these concentrations there was a decrease in the production of biological solids and a decrease in the rate of COD removal when these values are compared with those obtained from the control systems. It can also be seen that these effects were much more pronounced in the system shock loaded with 10 mg/l. In both cases metabolic intermediates or end products were released into the system. The difference between the COD

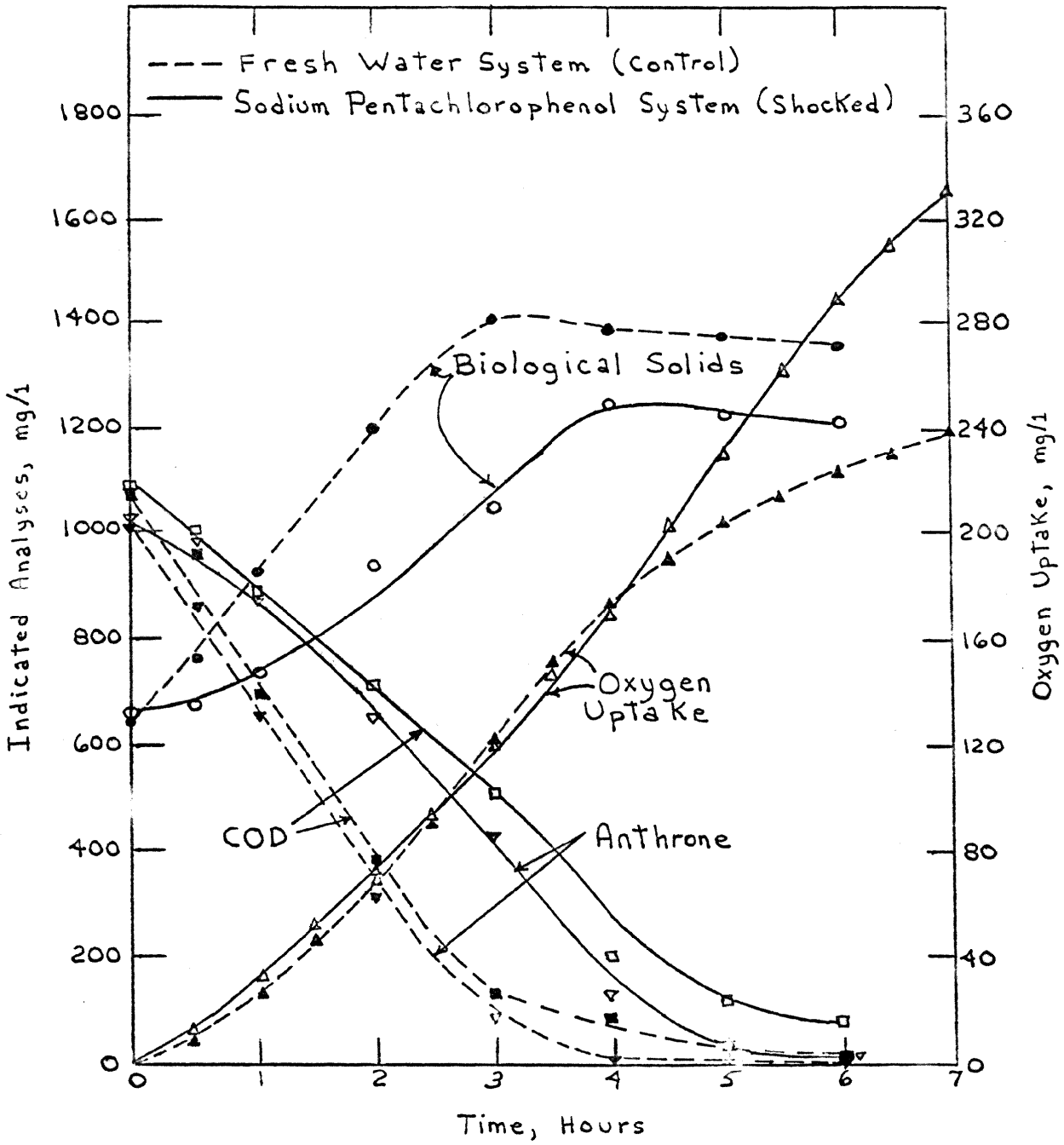

FIGURE 4. Change in System Parameters. Sludge Subjected to Shock Load of 5 mg/l Sodium Pentachlorophenol.

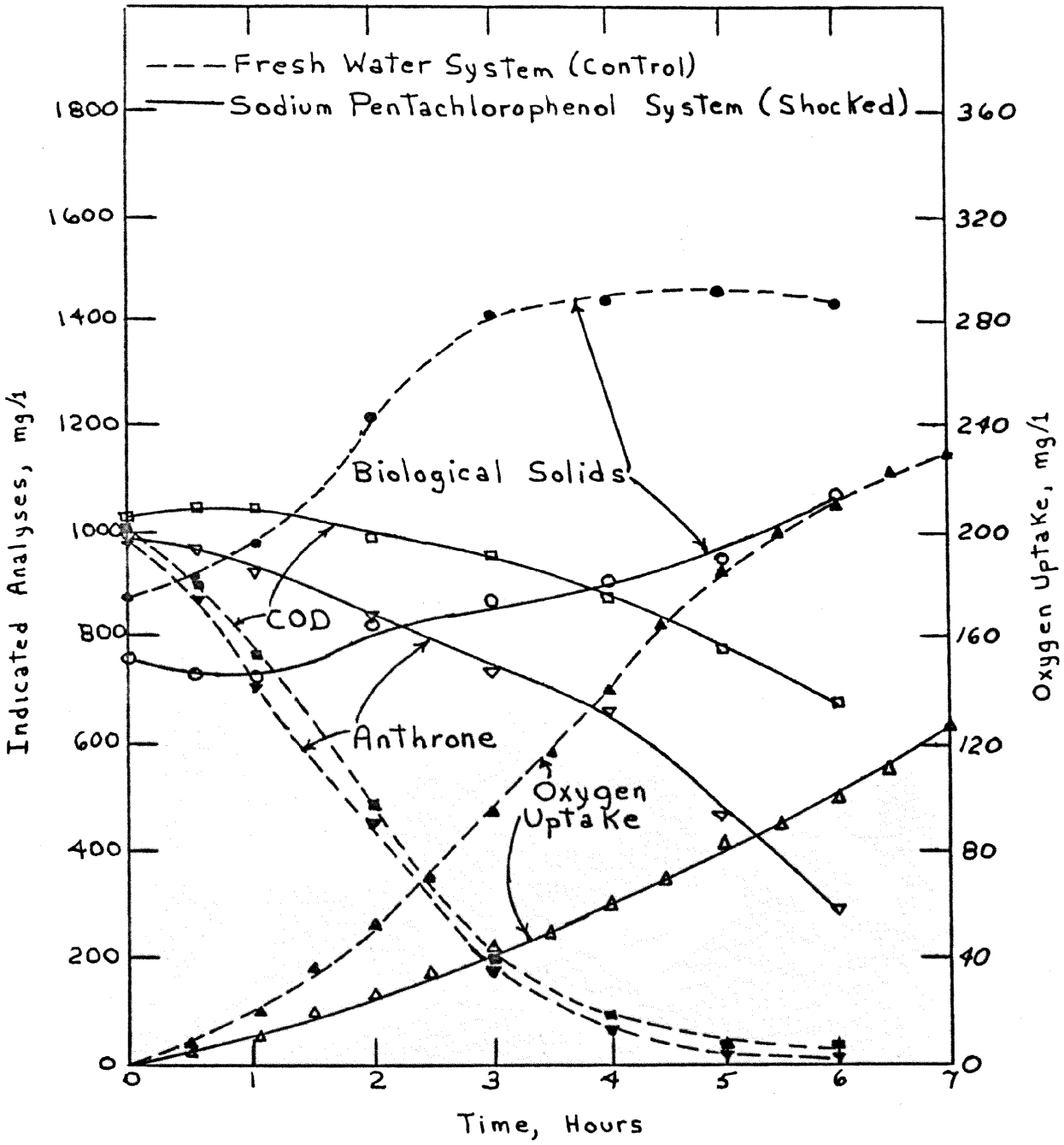

FIGURE 5. Change in System Parameters. Sludge Subjected to Shock Load of 10 mg/l Sodium Pentachlorophenol.

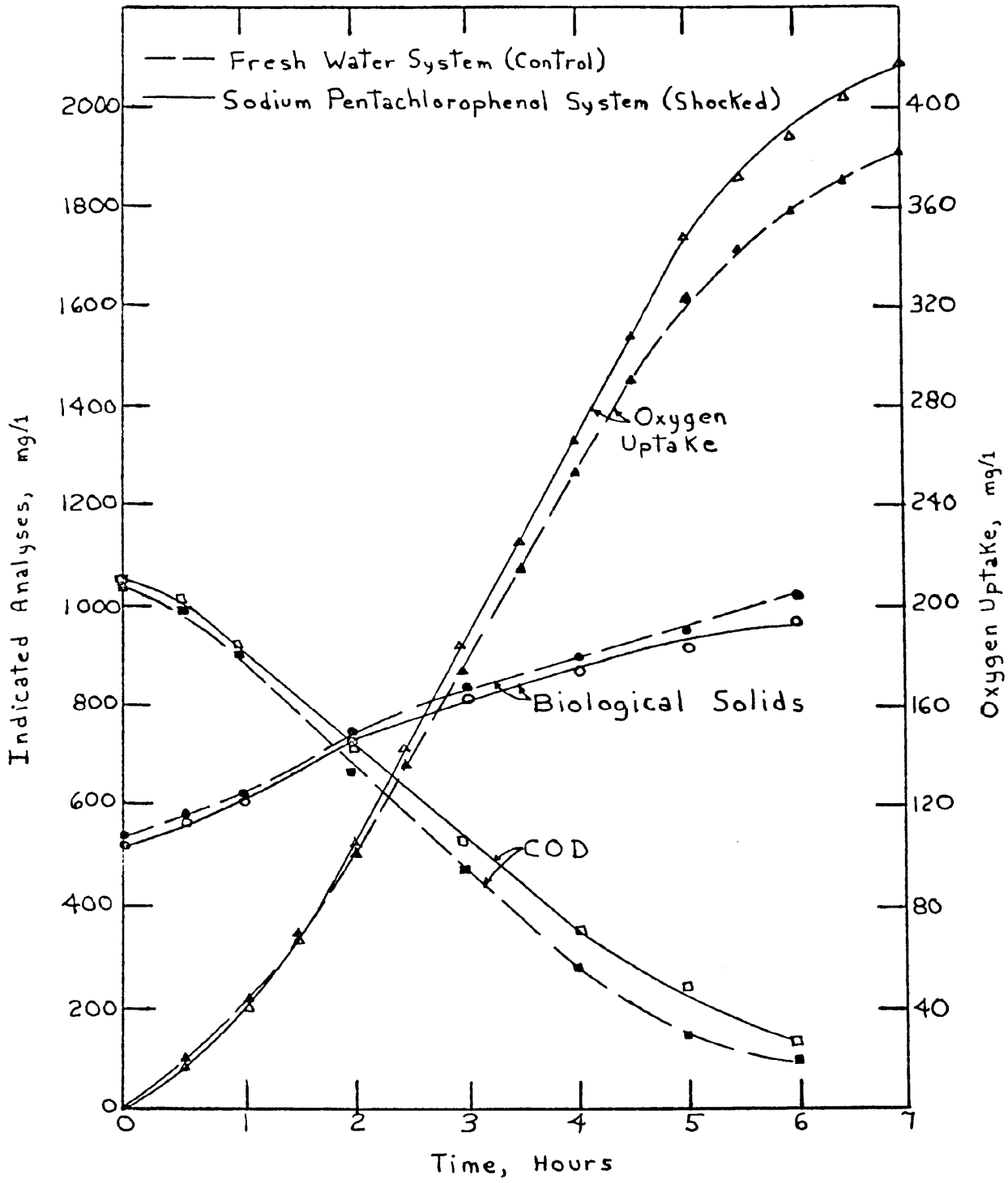

FIGURE 6. Change in System Parameters. Sludge Subjected to Shock Load of 10 mg/l Sodium Pentachlorophenol.

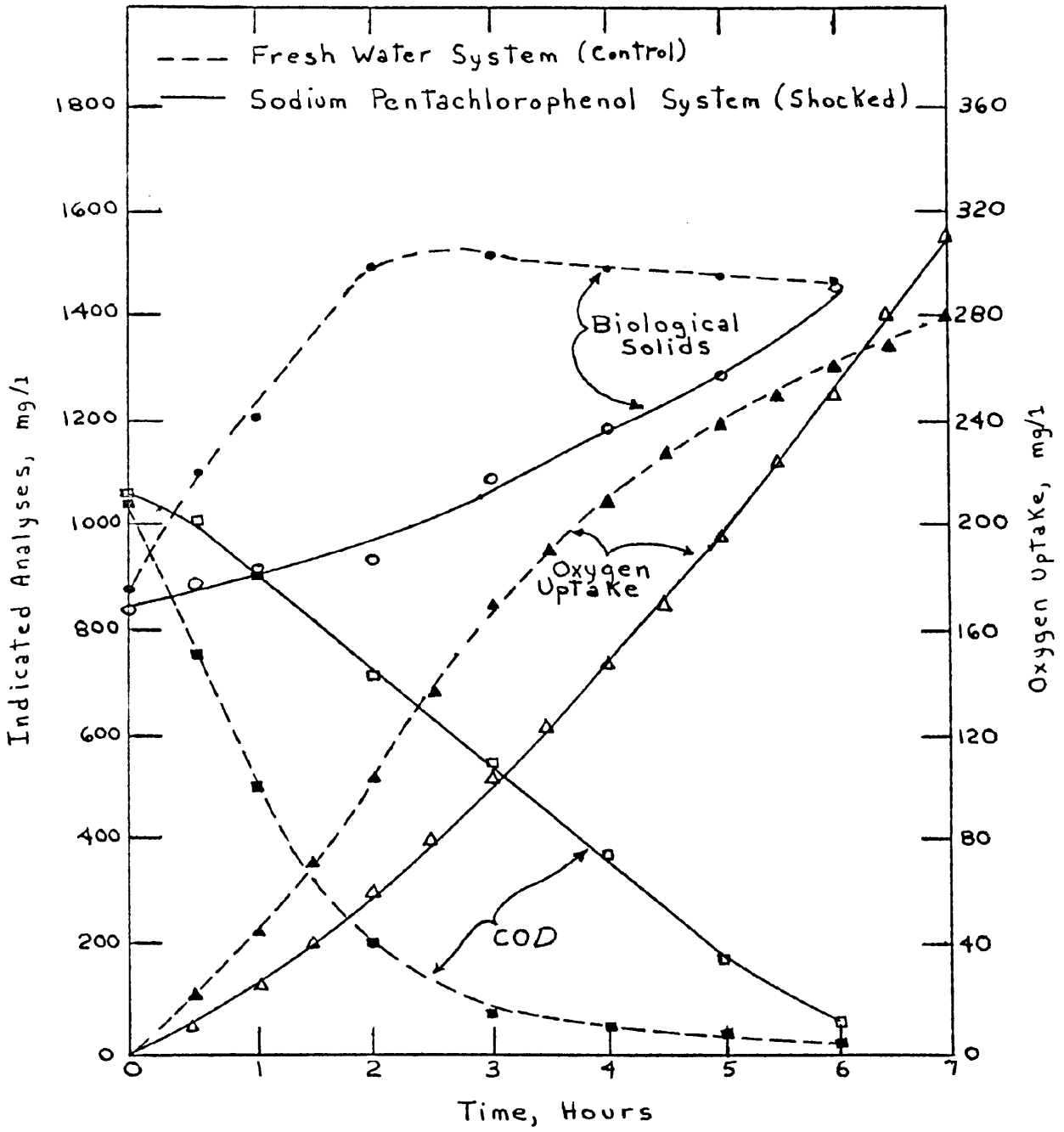

FIGURE 7. Change in System Parameters. Sludge Subjected to Shock Load of 15 mg/l Sodium Pentachlorophenol.

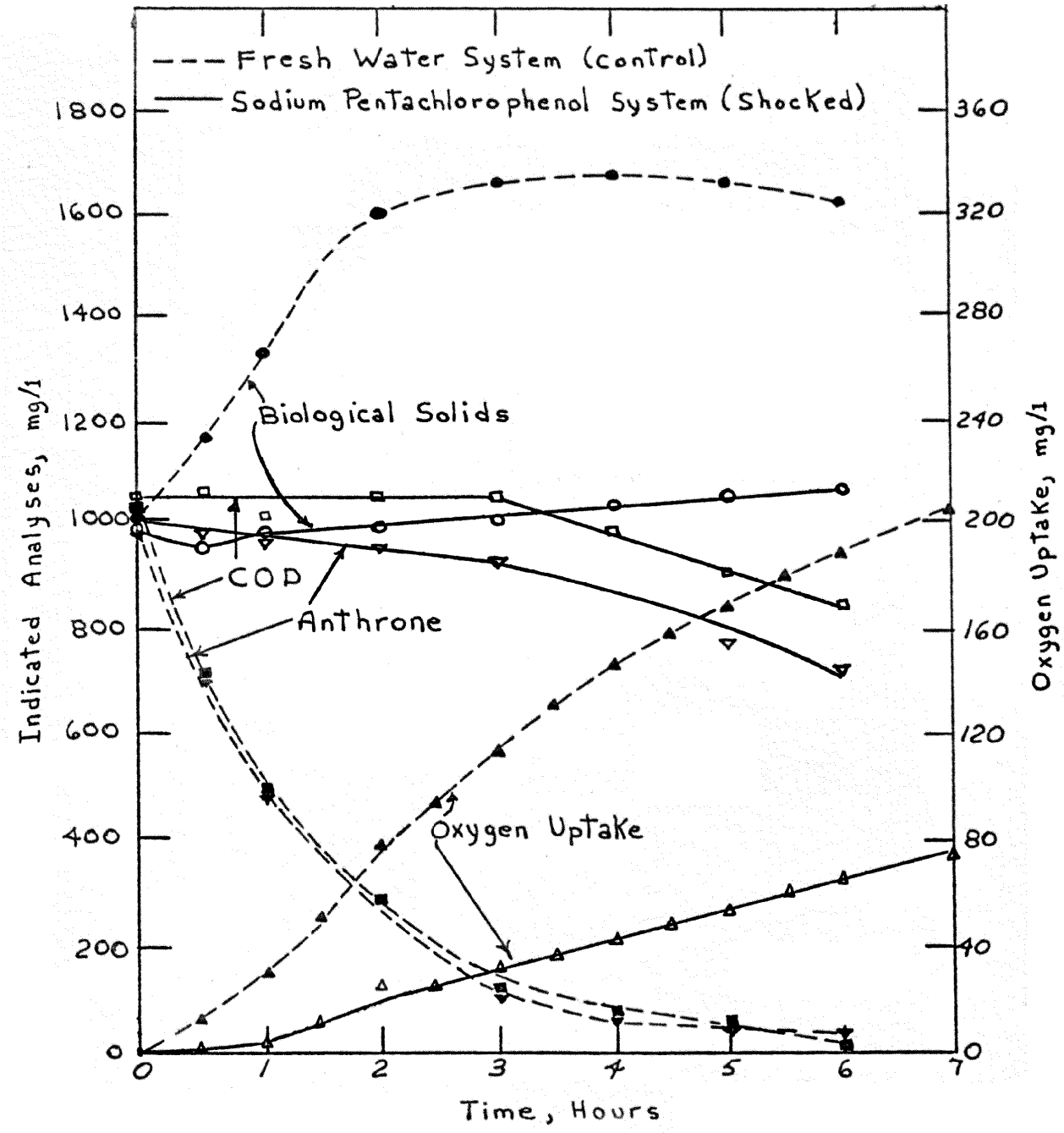

FIGURE 8. Change in System Parameters. Sludge Subjected to Shock Load of 30 mg/l Sodium Pentachlorophenol.

and anthrone values was 71.3 and 388 mg/l at the end of six hours for the systems shock loaded with 5 and 10 mg/l, respectively.

The unit shown in Figure 6 was also shock loaded with 10 mg/l of sodium pentachlorophenol. There was a slight decrease in the production of biological solids, a small decrease in the rate of COD removal, and a stimulation of the rate of oxygen utilization in the system which received the sodium pentachlorophenol as compared to that system receiving the standard waste only. The large quantities of oxygen utilized in both the control and the shocked unit are typical of the results that would be obtained from a young cell system. It is extremely interesting to note the difference in the response of the systems shown in Figures 5 and 6; in one case a shock loading of 10 mg/l drastically affected the behavior of the system while in the other case the response of the shocked system was only slightly different from that of the control unit.

The results of shock loading the flocculent, yellow-tan system with concentrations of 15 and 30 mg/l of sodium pentachlorophenol are shown in Figures 7 and 8, respectively. Although the oxygen utilized by the unit shocked with 10 mg/l was 68 mg/l less than that utilized by the control unit at the end of three and one-half hours it was 32 mg/l greater than the control unit at the end of seven hours. In contrast, the oxygen utilized

by the system receiving 30 mg/l of sodium pentachlorophenol was 128 mg/l less than that utilized by the control system at the end of seven hours. At the end of six hours the biological solids, oxygen uptake, and COD of the unit shocked with 15 mg/l were nearly the same as the control unit. However, the unit which was shocked with 30 mg/l had a COD which was 829 mg/l greater than the control unit and biological solids which were 564 mg/l less than the control unit at the end of six hours. There were also a small amount of metabolic intermediates or end products released into the system shock loaded with 30 mg/l.

Determinations of the concentrations of sodium pentachlorophenol were made on samples taken from the units shock loaded with 10 (Figure 5), 15, and 30 mg/l at times 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 hours. The results of these determinations showed that the pentachlorophenol concentration remained constant throughout the entire period. This indicates that the sodium pentachlorophenol was not degraded by the activated sludge units.

B. Studies of the Effects of Sodium Pentachlorophenol on the Carbohydrate and Protein Composition of the Cells

The effect of a shock loading of sodium pentachlorophenol on cell composition is shown in Figures 9-11. The data in Figure 10 were determined from the cell system represented in Figure 5; this was the

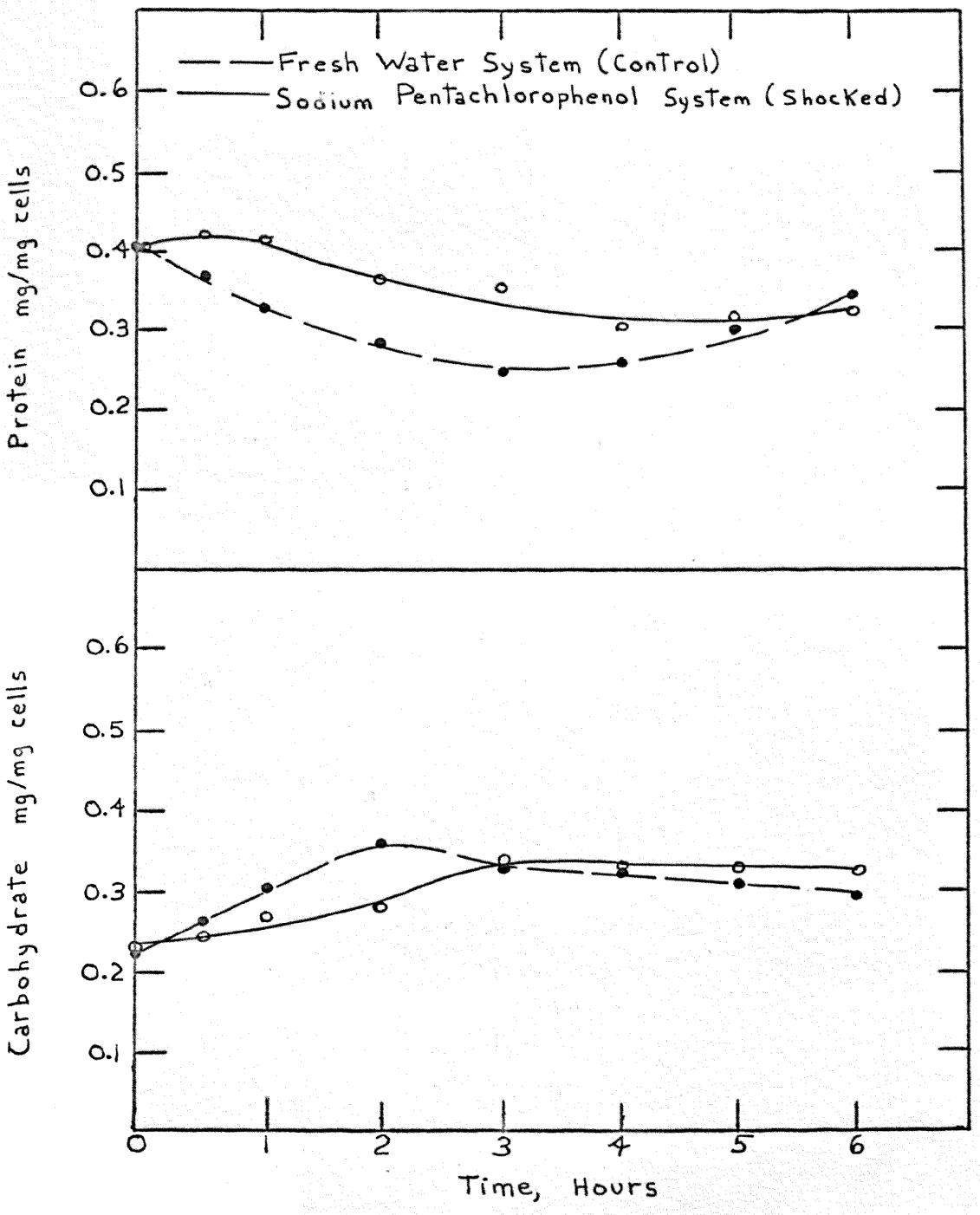

FIGURE 9. Change in Cell Carbohydrate and Protein. Sludge Subjected to Shock Load of 5 mg/l Sodium Pentachlorophenol.

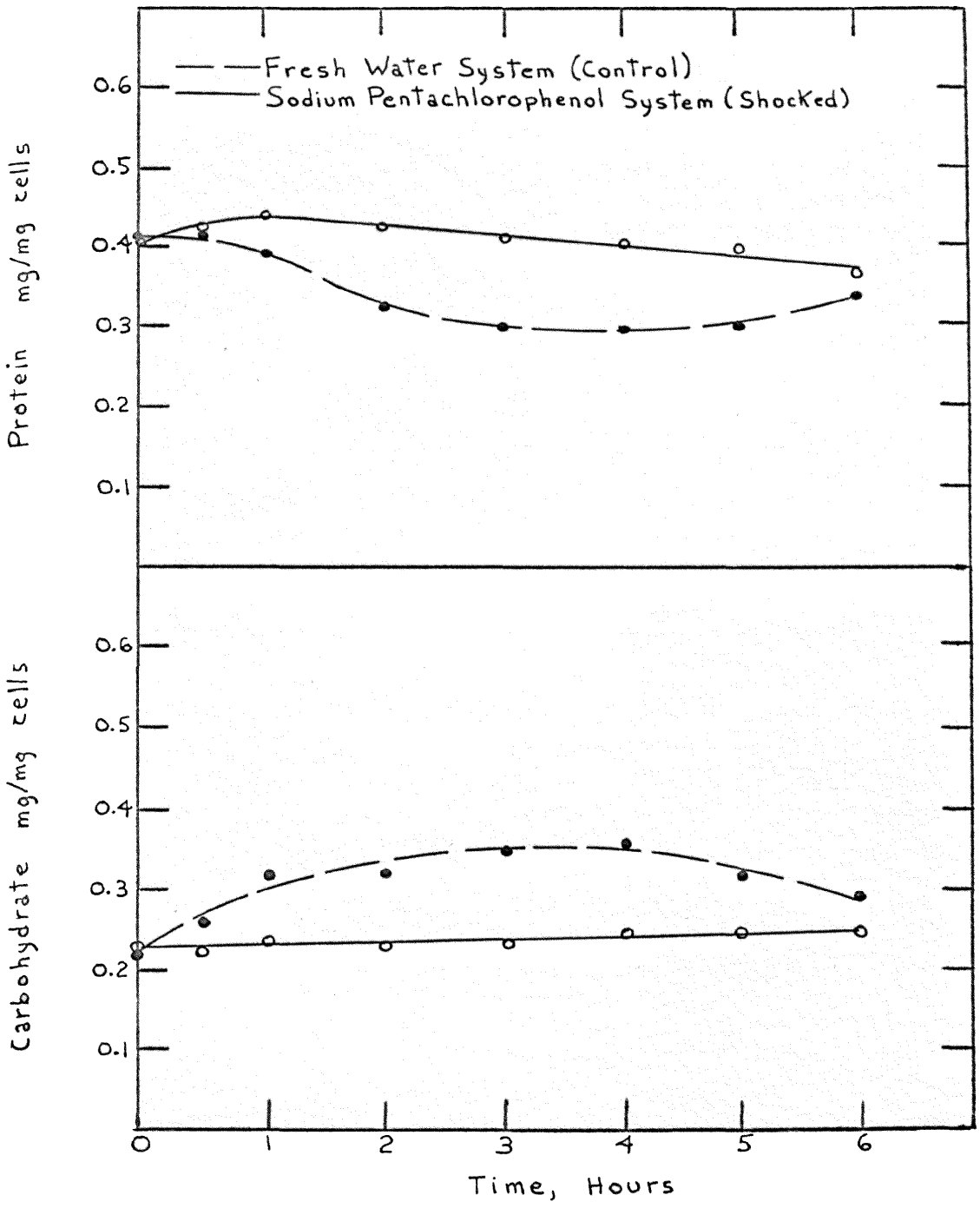

FIGURE 10. Change in Cell Carbohydrate and Protein. Sludge Subjected to Shock Load of 10 mg/l Sodium Pentachlorophenol.

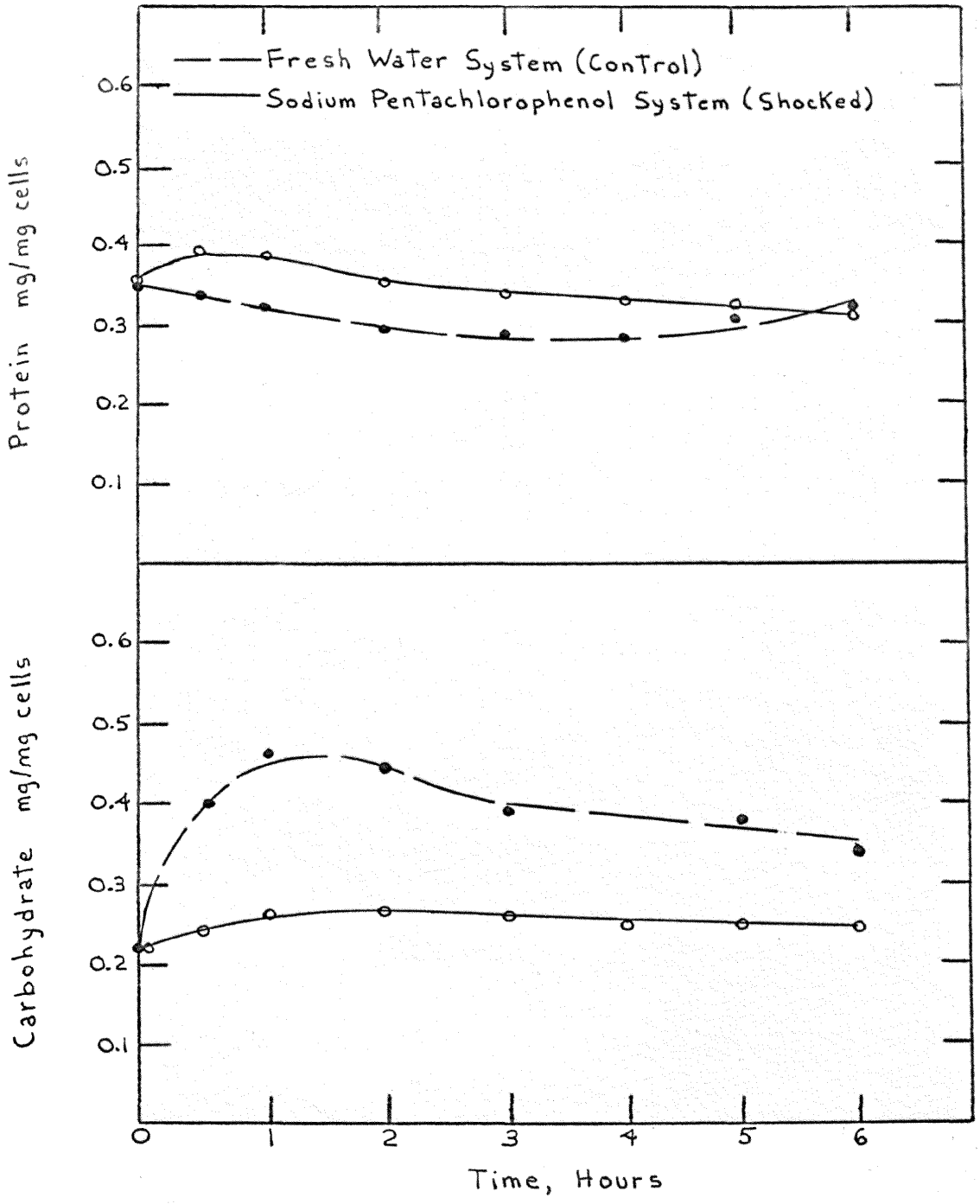

FIGURE 11. Change in Cell Carbohydrate and Protein. Sludge Subjected to Shock Load of 30 mg/l Sodium Pentachlorophenol.

unit with the brown appearance. In all cases the production of cellular carbohydrate in the systems which were shock loaded lagged behind its production in the control system. A shock loading of 5 mg/l resulted in nearly equal cell carbohydrate composition for the shocked and control units at the end of three hours, but at shock loadings of 10 and 30 mg/l the carbohydrate composition of the cells in the shocked system was considerably less than in the control system throughout the six hour period. In contrast to this behavior, the protein composition of the cells in those units which were shock loaded always increased slightly immediately after being shock loaded and then decreased at a much slower rate than the protein composition of those cells in the control unit.

The effect on the cell carbohydrate and protein content of acclimating a system to 250 mg/l of sodium pentachlorophenol is shown in Figure 12. It can be seen that the production of protein was stimulated even more in an acclimated system than in a system which was shock loaded. Also the initial carbohydrate content of the cells acclimated to 250 mg/l was 40 per cent less than in those systems which were grown on the standard waste only.

C. Bioassay Study

The results of the bioassay study are given in Table I. It can be seen that the 48-hour median tolerance limit is 1.0 mg/l. Concentrations of sodium pentachlorophenol of 1.6 and 2.5 mg/l proved to be

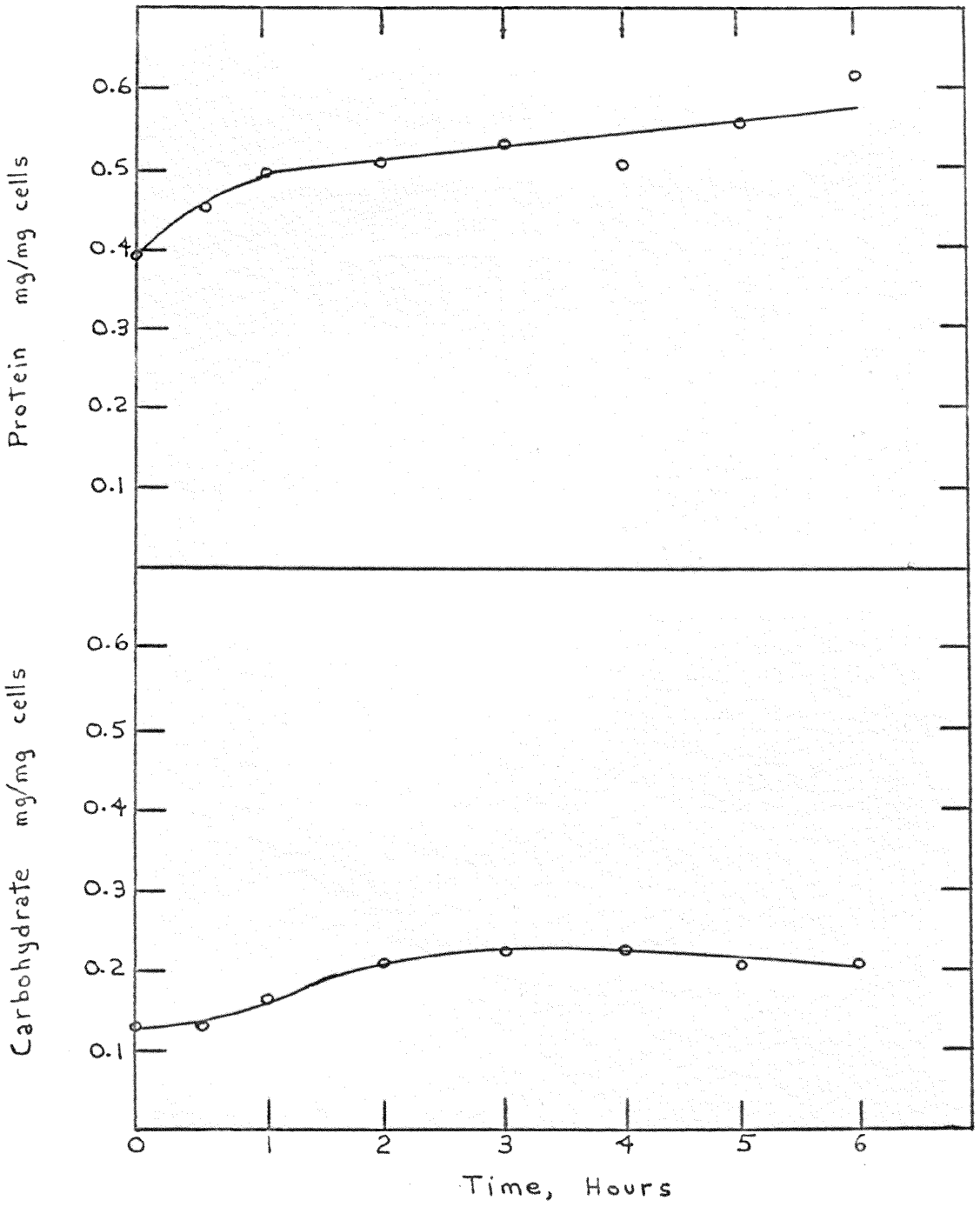

FIGURE 12. Change in Cell Carbohydrate and Protein. Sludge Acclimated to 250 mg/l Sodium Pentachlorophenol.

TABLE I

Bioassay Study

Time		Concentration 0.40 mg/l		Concentration 0.63 mg/l		Concentration 1.0 mg/l		Concentration 1.6 mg/l		Concentration 2.5 mg/l		Control Unit	
Hrs.	Min.	No. Dead	Length,* Weight	No. Dead	Length, Weight	No. Dead	Length, Weight	No. Dead	Length, Weight	No. Dead	Length, Weight	No. Dead	Length, Weight
3	20							1	6.6, 1.8	3	6.7, 1.9		
											6.5, 1.8		
											6.0, 1.5		
4	10									1	6.7, 2.0		
4	40							1	6.0, 1.6	1	6.7, 2.0		
5	20									1	5.9, 1.6		
5	50							1	6.3, 1.6	1	6.5, 1.9		
7	00							1	6.3, 1.6	1	6.5, 1.9		
9	10							3	6.0, 1.5				
									5.9, 1.5				
									6.7, 1.9				
10	10							1	6.7, 1.9				
11	00							1	6.4, 1.8				
15	15					1	6.7, 1.9						
16	20					1	6.0, 1.6						
24	00												
35	30	1	6.0, 1.5										
40	30			1	6.0, 1.6	1	6.5, 1.9						
48	00					1	6.7, 2.0						
91	45					1	6.5, 1.8						
96	00												

*All lengths are expressed in cm and all weights are expressed in grams.

much more toxic and resulted in all fish being killed in eleven and seven hours, respectively. At concentrations of 0.4 and 0.63 mg/l the toxic effects were much less pronounced since only one fish was killed at each of these concentrations.

There was evidence that capillary rupture resulted in death because blood was present around the gills of many of the fish which died. At a concentration of 2.5 mg/l there was a noticeable increase in the respiration rate; the gills of the fish exposed to this concentration moved much more rapidly than did those of the fish in the control unit. Also at this concentration the fish underwent a noticeable loss of equilibrium before death. They darted from one side of the container to the other, and at times they were swimming upside-down. In contrast to this behavior, the fish which died at concentrations of 0.4 and 0.63 mg/l were noticeably impared for a period of nineteen and eleven hours before their death, respectively. However, in this case the fish lay motionless at the bottom of the container moving their gills slowly for the periods indicated prior to their death.

At the end of the 96-hour test period all surviving fish appeared to be in good condition. This was also the case for those fish in the control unit.

V. DISCUSSION

A. Batch Operated Systems

1. Unit Acclimated to Sodium Pentachlorophenol

During the daily operation of the unit acclimated to sodium pentachlorophenol, some interesting and important observations were made which warrant discussion. The color of the acclimated unit was light green throughout the entire period of study in contrast to the yellow-tan color or the brown color of the units receiving the standard waste only. The finding that there is a change in the predominating species of micro-organisms present when an activated sludge system is exposed to sodium pentachlorophenol is in agreement with the observations made by Ingols (1). It was also found that no sedimentation occurred. The cells remained completely dispersed even after standing quiescently for a period of one hour. Kincannon (22) has also found that units acclimated to various concentrations of sodium pentachlorophenol will remain completely dispersed systems.

Another important observation was that the biological solids present 24 hours after the unit acclimated to sodium pentachlorophenol was fed were only present in concentrations of about 200 mg/l. Yet, the biological solids present in the flocculating unit receiving the standard waste only were present in significantly higher concentrations; even after this unit was divided into two

equal parts and brought back to the 1.5 liter aeration volume with distilled water and the standard waste, the biological solids averaged about 800 mg/l.

The combination of low biological solids and no sedimentation would cause almost insurmountable problems at an activated sludge waste treatment plant which received sodium pentachlorophenol in addition to other wastes. The presence of this uncoupling agent would greatly decrease the amount of biological solids produced. Also, the fact that the system would remain completely dispersed would make final sedimentation of no value; there would be no clear effluent to discharge, and the sludge could not be concentrated. Therefore, there would be little return sludge, and this would magnify the problem caused by the low production of biological solids.

Another interesting observation was that the oxygen utilization was extremely high in the unit acclimated to sodium pentachlorophenol when this was considered in relation to the low quantity of biological solids present in the system. To illustrate this, the variation in the rate of oxygen utilization with time was determined for the unit at concentrations of sodium pentachlorophenol of 100 and 250 mg/l and also for the control unit shown in Figure 4 since this unit is typical of the oxygen utilized by all of the flocculent control units. 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 biological solids at that point. The results are shown in Figure 13.

The large initial difference in the values obtained for the unit acclimated to sodium pentachlorophenol is due to the fact that the unit acclimated to 250 mg/l exhibited a lag phase in the rate of oxygen utilization whereas the unit acclimated to 100 mg/l did not. The important thing to consider, however, is the fact that the oxygen utilization rate was significantly higher in the unit acclimated to sodium pentachlorophenol than it was in the flocculent unit which received the standard waste only. This finding is in agreement with the fact that sodium pentachlorophenol functions as an uncoupler of oxidative phosphorylation.

It is also significant that a system can acclimate and function well at concentrations of sodium pentachlorophenol which are extremely detrimental to a unit shock loaded with this material. On the other hand, the oxygen uptake curves presented in Figure 3 indicate that the system acclimated to a concentration of sodium pentachlorophenol of 100 mg/l was only slightly affected by a shock loading of the standard waste.

The COD remaining at the end of six hours was 89.2 and

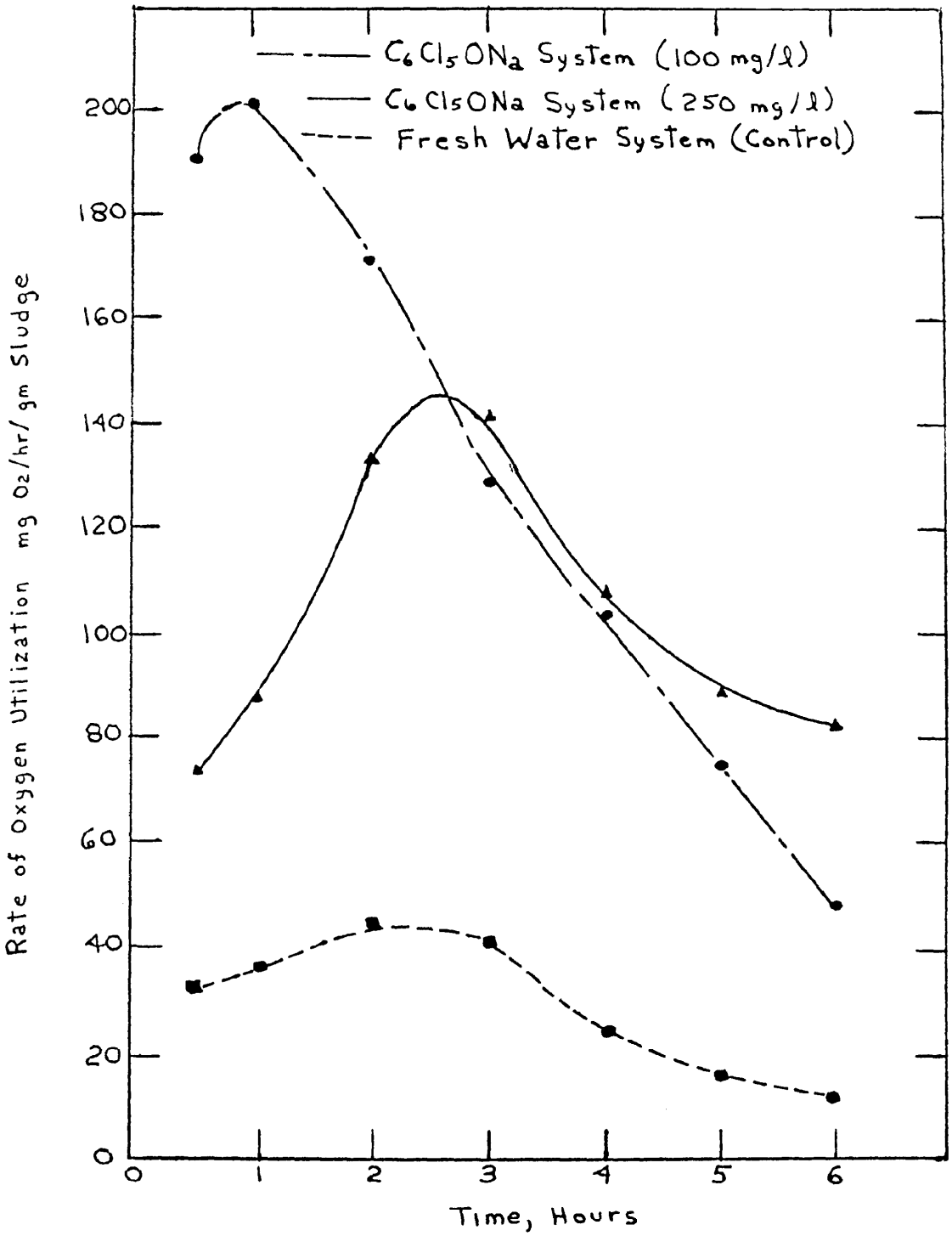

FIGURE 13. Variation in Rate of Oxygen Utilization with Time of Aeration.

166 mg/l for the unit acclimated to 100 and 250 mg/l of sodium pentachlorophenol, respectively. In both cases the glucose was essentially removed from the unit at the end of the six hour period, and the remaining COD was caused by metabolic intermediates or end products and the sodium pentachlorophenol in the system. However, the COD of the effluent at the end of six hours was 680 mg/l for the unit shown in Figure 5 which was shock loaded with 10 mg/l and 848 mg/l for the unit which was shock loaded with 30 mg/l, and in both of these cases there was still a significant quantity of glucose remaining.

It can be seen that the glucose was removed significantly faster than the COD in the unit acclimated to sodium pentachlorophenol. In both cases the rate of glucose removal followed zero-order kinetics for the first two hours, and in both cases the glucose was essentially removed within five hours. It can also be seen that there was a considerable release of metabolic intermediates or end products. At the end of two hours the difference between the COD and anthrone values was 560 mg/l in the system acclimated to 100 mg/l of sodium pentachlorophenol and 800 mg/l in the system acclimated to 250 mg/l. However, a substantial portion of these metabolic intermediates or end products were removed from the system once the glucose concentration had fallen to about 200 mg/l.

The determinations of sodium pentachlorophenol concen-

trations made during both studies on the acclimated units showed that the concentrations did not vary during the investigations, and this indicates that sodium pentachlorophenol is not subject to biological degradation. This is in agreement with the recent investigation conducted by Ingols (1).

Acclimating a system to sodium pentachlorophenol resulted in significant changes in the carbohydrate and protein composition of the cells when this composition is compared with the carbohydrate and protein contents of the cells in the unit receiving the standard waste only. The carbohydrate and protein content of the cells acclimated to a concentration of 250 mg/l of sodium pentachlorophenol is compared with the carbohydrate and protein content of the cells in the control units which are shown in Figures 5 and 8. The results are shown in Figure 14. It can be seen that the production of protein was stimulated and the production of carbodydrate was decreased when the system was acclimated to a concentration of 250 mg/l.

2. Units Shock Loaded with Sodium Pentachlorophenol

An examination of the results obtained from those systems which were shock loaded with sodium pentachlorophenol indicates that the response of any particular system was a function of both the predominating species of micro-organisms present and the concentration of the shock load. It will be recalled that shock loadings

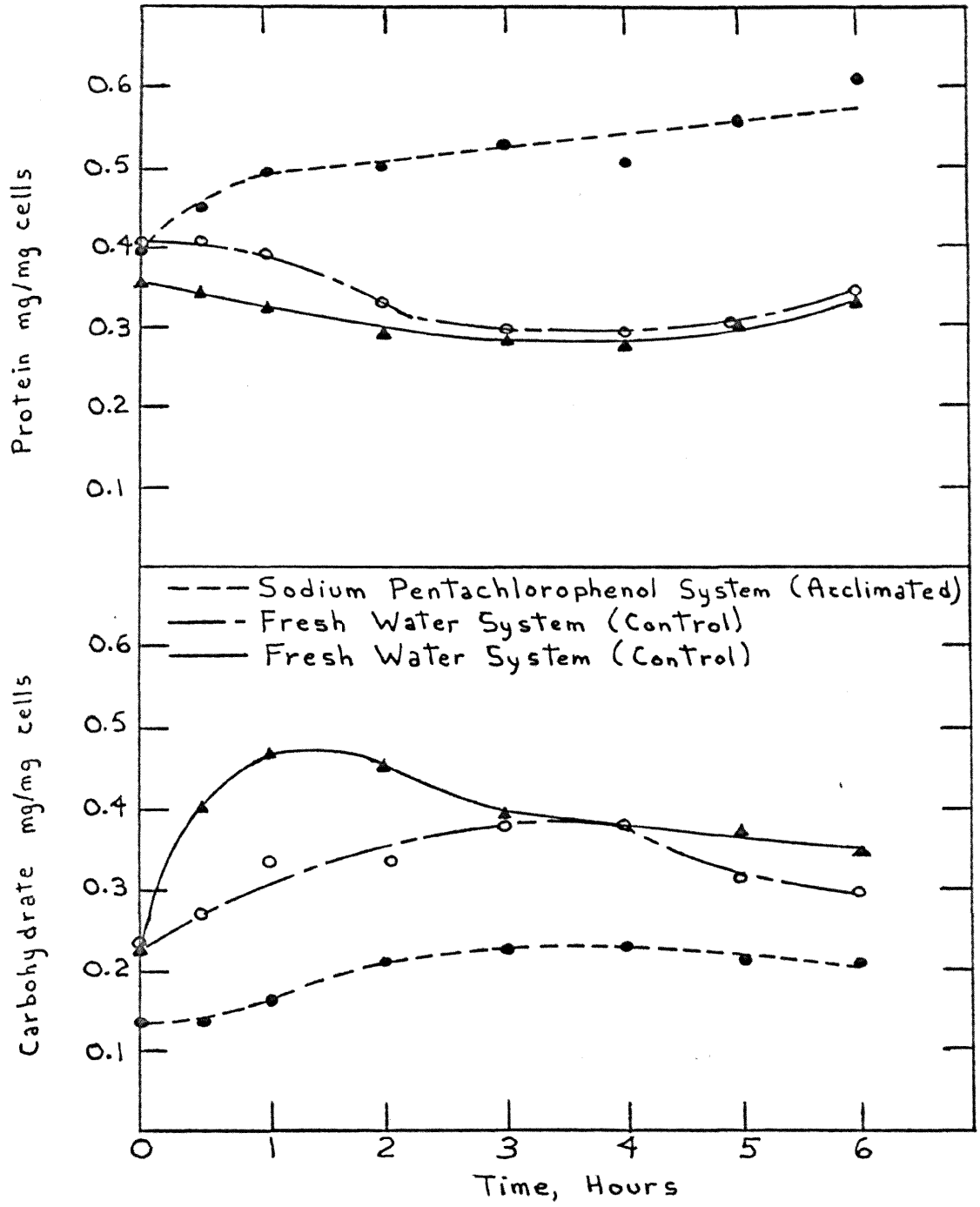

FIGURE 14. Change in Cell Carbohydrate and Protein. Comparison of System Acclimated to 250 mg/l Sodium Pentachlorophenol with Systems Receiving Standard Waste Only

of 5 and 10 mg/l were made on a system with a brown appearance, and that shock loadings of 10, 15, and 30 mg/l were made on a system with a yellow-tan appearance. At the time of the shock loading of 10 mg/l on the system with the yellow-tan appearance, this system had been operated for 21 days, but it still remained a dispersed system. All other studies on the effects of shock loadings of sodium pentachlorophenol were made on flocculent systems.

A comparison of the units which were shock loaded with 15 and 30 mg/l with the units of brown appearance which were shock loaded with 5 and 10 mg/l of sodium pentachlorophenol reveals some striking similarities in behavior for units shock loaded with different concentrations. The unit shock loaded with 30 mg/l had an initial decrease in biological solids, a final COD of 848 mg/l, and a much smaller rate of oxygen utilization than the control unit. The unit shock loaded with 10 mg/l (Figure 5) also had an initial decrease in biological solids, a much smaller rate of oxygen utilization than the control unit, and a final COD of 680 mg/l. The units which were shock loaded with 5 and 15 mg/l also possess characteristics which are somewhat similar, although the effect of the shock loading of 15 mg/l was greater on the yellow-tan system than the shock loading of 5 mg/l was on the other system.

The data in Figures 5 and 6 illustrate the difference in

the response of the two predominating systems when they were both shock loaded with 10 mg/l of sodium pentachlorophenol. Although the system with the yellow-tan appearance possessed the characteristics of a young cell system while the system with the brown appearance did not, it is still evident that the response of a system to a given concentration of sodium pentachlorophenol is affected by the predominating species of micro-organisms present. In one system the effect of the shock loading was very slight; yet the system with the brown appearance was drastically affected and responded quite similarly to that system shock loaded with 30 mg/l.

In all cases the COD remaining at the end of six hours in those systems which were exposed to sodium pentachlorophenol was greater than in the control systems; this difference ranged from only 58.2 mg/l for the system shocked with 5 mg/l to 829 mg/l for the system shock loaded with 30 mg/l. Also in those systems in which anthrone determinations were made (Figures 4, 5, and 8) it was found that some metabolic intermediates or end products were present. However, it was only in the system which was exposed to 10 mg/l (Figure 5) that there was a considerable difference in the COD and anthrone values; this difference was 388 mg/l at the end of six hours.

It was also observed that the lower concentrations of sodium

pentachlorophenol stimulated the rate of oxygen utilization whereas the higher concentrations resulted in a greatly decreased rate of oxygen utilization. The effect of any given concentration on the oxygen utilization rate was also determined by the predominating species of micro-organisms present which in turn determined the response of the system as a whole. This effect on the oxygen utilization rate would be extremely important in any activated sludge treatment plant which employed tapered aeration because the oxygen utilized by a system exposed to low concentrations of sodium pentachlorophenol would increase rather than decrease along the length of the aeration tank.

The determinations of the sodium pentachlorophenol remaining which were made at various times on the units receiving shock loads of 10 (Figure 5), 15, and 30 mg/l showed that the sodium pentachlorophenol was not subject to biological degradation. Therefore, physical or chemical methods must be employed to degrade this waste.

It can be seen (Figures 9-11) that shock loadings of sodium pentachlorophenol resulted in a change in the carbohydrate and protein contents of the cells which was different from the change observed in those cells in the units receiving the standard waste only. The change in protein composition is especially apparent in Figures 10 and 11. In both cases the units which were shock

loaded underwent an initial decrease in the concentration of biological solids, and yet there was an increase in the protein composition of the cells; in contrast to this behavior the protein composition of the cells in the control unit decreased and at the same time there was an increase in the concentration of biological solids. It can also be seen that the carbohydrate composition of the cells receiving 10 and 30 mg/l of sodium pentachlorophenol remained less than the carbohydrate composition of the cells in the control unit throughout the six hour period, but that the carbohydrate compositions of the cells in the unit receiving a shock load of 5 mg/l and the cells in the control unit were almost identical at the end of three hours.

B. Bioassay Study

The results of the bioassay study have shown that the 48-hour median tolerance limit of sodium pentachlorophenol to the minnows tested was 1.0 mg/l. It was also established that concentrations as low as 0.4 mg/l were detrimental to fish life. Goodnight (5) has established that the lethal concentration varied from 0.2 ppm to greater than 0.6 ppm depending upon the species of fish which were tested. The important thing to consider, however, is that sodium pentachlorophenol is toxic to fish in low concentrations. Therefore, since it has been established that sodium pentachlorophenol is not subject to biological degradation by the

activated sludge treatment process, any attempt to determine the permissible concentration of this material in wastewater must take into consideration the aquatic life which is present in the receiving stream in addition to considering the effect upon the treatment plant itself.

VI. SUMMARY AND CONCLUSIONS

As a result of the information obtained during the course of this investigation, the following may be concluded:

A. An activated sludge system acclimated to concentrations of sodium pentachlorophenol of 100 mg/l or greater will remain a completely dispersed system. The quantity of biological solids produced will be substantially decreased, and the oxygen utilization rate will be substantially increased when these values are compared with those obtained from a system grown under similar conditions with the exception that no sodium pentachlorophenol is present.

B. The production of protein is stimulated and the production of carbohydrates is decreased when a system is exposed to sodium pentachlorophenol. This is true whether the system is acclimated or shock loaded.

C. The effect that a shock loading of sodium pentachlorophenol will have upon a system is determined not only by its concentration but also by the group of micro-organisms which are predominating.

D. As determined by a comparison of oxygen uptake curves, a system acclimated to 100 mg/l of sodium pentachlorophenol can withstand a shock loading of the standard waste without serious effects.

E. A system can acclimate to concentrations of sodium pentachlorophenol which are at least ten times greater than the concentration

necessary to impair a system which is shock loaded and still function to significantly reduce the organic content of a waste. The COD of the unit acclimated to sodium pentachlorophenol at concentrations of 100 and 250 mg/l was 89.2 and 166 mg/l, respectively at the end of six hours. In contrast, shock loadings of 10 (Figure 5) and 30 mg/l produced effluents with COD of 680 and 848 mg/l, respectively at the end of six hours.

F. There is no evidence to indicate that sodium pentachlorophenol is subject to biological degradation either when activated sludge units are acclimated to or shock loaded with this material.

G. Activated sludge units acclimated to sodium pentachlorophenol release significant quantities of metabolic intermediates or end products during the metabolism of a glucose waste. The quantity of metabolic intermediates or end products released into a system which is shock loaded with sodium pentachlorophenol depends upon the magnitude of the shock loading and the micro-organisms which are predominating; increasing concentrations of sodium pentachlorophenol were found to increase the quantity of metabolic intermediates or end products released for any given system.

H. Sodium pentachlorophenol is toxic to minnows at concentrations of 0.4 mg/l. The 48-hour median tolerance limit is 1.0 mg/l.

VII. RECOMMENDATIONS FOR FUTURE WORK

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

A. It would be of benefit to know the highest concentration of sodium pentachlorophenol that a system can acclimate to without becoming a completely dispersed system.

B. Isolation and identification of the predominating species of micro-organisms present in a system acclimated to sodium pentachlorophenol may prove of value.

C. Identification of the metabolic intermediates or end products which are released when a system is exposed to sodium pentachlorophenol may provide valuable information concerning the metabolic control mechanisms which are affected.

D. It may be desirable to determine if a trickling filter could be used to treat wastes which contain sodium pentachlorophenol. It is possible that final sedimentation would not be affected, and thus the effluent would be free of high concentrations of micro-organisms.

E. Chemical or physical methods for removing sodium pentachlorophenol from wastewater should be investigated. It is felt that activated carbon may prove to be extremely beneficial in removing this waste.

F. It would be interesting from a basic standpoint to determine if the addition of bovine albumin to a waste containing sodium pentachlorophenol would enable an activated sludge system to better withstand shock loadings.

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VITA

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