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THE EFFECT OF SALINITY ON THE REMOVAL OF SOME ALIPHATIC KETONES

Prepared by
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Tariq A. Mahmoud and William B. Davis

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ABSTRACT

Basic and definitive information pertaining to the effect of salinity on the microbial behavior is essential to determine the fate of organic pollutants discharged into the estuaries, and to determine design and operation parameters of plants subjected to salinity variation.

Twenty-five batch tests were run to determine the long and short term effect of salinity variation on the microbial behavior of cultures established in various salinity levels and tested under different salinity conditions. Aliphatic ketones namely, acetone, 2-butanone and 2-pentanone were used as a carbon source in this study because of their appearance in many industrial wastes. Mixing and aeration was accomplished by using the incubator shaker. Samples were withdrawn periodically and analyzed for the concentration of organisms, concentration of each of the original substrates, total organic carbon, oxygen uptake rate and pH. A hydrogen flame gas-liquid chromatograph, total organic carbon analyzer, Gilson oxygraph were the primary analytical tools used for this study.

Data from batch tests were analyzed and the following conclusions were formulated.

1. The decrease in the substrate removal rates was proportional to magnitude of the salinity shock.
2. The oxygen requirement per unit ketone removed increased

for a culture acclimated to a certain salinity and shocked with lower salinities.

3. In general, it was observed that negative shock magnitudes caused a greater substrate removal retardation than the positive shock magnitudes.
4. Culture acclimated in fresh water suffered a 50% reduction in the substrate removal efficiency when shocked with sea water. While culture acclimated to sea water salinity suffered a 86% reduction in the substrate removal efficiency when shocked with fresh water.
5. The biomass increase per unit ketone removed was higher for cultures established and tested at low salinities.
6. The effect of long term salinity studies clearly indicated that cultures established in fresh water and low salinities have a higher unit rate of removal than the cultures established at greater salinities.
7. The removal of 2-butanone and 2-pentanone occurred first in all test series. The acetone removal started when the concentration of the other two ketones reached low levels (~ 5 mg/l).

Organisms acclimated to ketones in media varying from fresh water to sea water salinity were identified to genera and in two

instances to species. The following conclusions were observed:

1. Two predominant genera, Cephalosporium and Pseudomonas, were common to all salinity levels.
2. Minor species were eliminated with the progressive increase in salinity level.

These two observations lend credence that a reduced removal rate at a higher salinity level was due not to the change in population structure but to the physiological effects of the increased salinity on the uptake and metabolism of the substrate by organisms.

PREFACE

The experimental part of this study was performed in the Environmental Engineering Laboratories at Texas A & M University.

The report was primarily written by the senior author in partial fulfillment of the Doctor of Philosophy requirements under the direction of the junior author who was his major advisor.

Mr. Henry A. Wigley was of great assistance in identifying the microorganisms and in reviewing this report. Mr. Wigley's time and effort is very much appreciated.

The review of this study by Dr. William J. Clark, Dr. Edward A. Hiler, Professor M. D. Jones, and Dr. Tom D. Reynolds is very much appreciated. A sincere appreciation is also extended to Dr. Robert L. Irvine and Dr. William D. Langley for their helpful criticism while conducting this study.

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CHAPTER I

INTRODUCTION

Estuaries provide the environment for biological action on certain pollutants discharged by industries and municipalities located along the thousands of miles of coastline of this planet. Salinity variation is likely to influence the metabolic response of a biomass which may have originated either in treatment systems or natural water bodies. Salinity change is also a major problem in the treatment operation of wastes from some manufacturing process like cheese production, pickle processing, and so forth where the salinity ranges up to the order of 20,000 to 25,000 mg/l. In addition shipboard waste treatment on ocean-going vessels may be significantly influenced by salinity variations. Therefore, basic and definitive information pertaining to the effect of salinity is important to determine the fate of different pollutants discharged into the estuaries, and to determine design and operation parameters of treatment plants subjected to salinity variations.

The concentrations of sodium chloride which will arrest growth of the various types of bacteria have been investigated by microbiologists and biochemists. They have found that the

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salt tolerances vary to a considerable degree from one type of organism to another; however, little experimental material is available explaining in detail why salts in high concentrations exert an inhibitory effect upon the growth of most bacteria. The solution to this question must certainly be preceded by the determination of whether or not salt pervades the cell wall. If so, osmotic inhibitions can be immediately eliminated and the direct effects of salt such as the curtailment of enzymatic activities can be pursued. If not, the salt may damage the cell wall or by osmosis curtail metabolic functions by the removal of cellular water. When dealing with heterogeneous cultures, as encountered in natural or waste treatment systems, this problem is even more complicated. Therefore, this study will be confined to the enumeration of the overall effects of various salinity conditions on the behavioral patterns of microbial populations in waste treatment and natural systems.

Previously, investigations studying the effects of salinity variations on heterogeneous cultures in activated sludge and trickling filter units were faced with numerous technique difficulties. COD, BOD and other techniques were not only very time consuming but their accuracy was affected by the salinity conditions of the sample. An extensive number of tests were possible

in this study because of the availability of sophisticated instruments which not only saved both time and effort, but also, by exhibiting a minimum salt interference, gave more reliable results. Excellent control of tests conditions was possible by using the Environmental Incubator Shaker which provided for identical mixing and aeration, temperature control, and minimum substrate stripping.

In this research, five series of tests were conducted to determine the effects of salinity on the removal of acetone, 2-butanone and 2-pentanone by cultures acclimated to acetone. The objectives of this study were:

1. To study the long term effects of various salinity levels on the microbial behavior.
2. To study the shock effect of selected levels of salinity on culture established in various salinities.
3. To describe the apparent reaction kinetics of each system.
4. To demonstrate the accuracy and precision of the analytical tools such as the Total Carbon Analyzer, Gas Liquid Chromatograph, Gilson Oxygraph and to correlate between their results.
5. To identify predominant species at the various levels of salinity.

CHAPTER II

REVIEW OF LITERATURE

It is well established that bacteria are highly adaptable to salt concentration. Aerobacter aerogenes, for example, can withstand a salinity variation between fresh water and 100,000 mg/l. An extreme example is the presence of some bacteria in the Dead Sea where the salt concentration is about 25%. Fungi are also known to be tolerant to a wide range of salinity variations. Occasionally Phycomycetes are isolated from brines. On the other hand some halophilic organisms cannot tolerate low salinity levels and they lyse when placed in fresh water. A number of marine bacteria species can be transferred to a fresh water medium without problems, while others fail to grow even on a media with 1% NaCl salinity. Marine fungi are unique and can grow in fresh water as well as sea water. For this reason some microbiologists do not believe in the presence of strictly marine fungi.

Almost all of the early work on the effect of ionic concentration was done by microbiologists. Hotchkiss (1) was one of the earliest investigators in this field. She made a comprehensive study of both stimulatory and inhibitory effects of various cations on bacterial growth. When Na^+ is added as NaCl, E. coli growth was

inhibited at 2.0 M level (117,000 mg/l NaCl concentration) and stimulated at up to 0.25 M level (14,500 mg/l NaCl concentration). Levine and Soppeland (2) investigated the effects of various salts on the ability of the bacteria to hydrolyze milk protein and gelatins. The authors used pure cultures isolated from creamery waste. It was found that the minimum NaCl concentration to produce definite inhibition of gelatin hydrolysis was 10,000 mg/l and that the bacterial culture exhibiting the most activity was the most affected by increasing salt concentration. Ingram (3, 4 and 5) found that the endogeneous respiration rate of Bacillus cereus was increased by NaCl concentration up to 0.2 M (11,700 mg/l) but was decreased above that. Winslow and Haywood (6) found that the growth of E. coli was stimulated at NaCl concentration of .005 to 0.25 M. This is in good agreement with Hotchkiss (1) conclusion. Acclimation of E. coli to NaCl was studied by Doudoroff (7). It was found that cells exhibited the greatest degree of adaptability to the salt in the early stationary phase of growth. Cultures harvested before or after this phase exhibit less adaptability.

In an early work by Wood (8) he found that no difference existed between bacterial growth on sea water and fresh water media. This finding was based on a bacterial flora that was largely estuarine. Wood believes that salinity itself does not affect bacterial processes, but merely regulates the strains of bacteria responsible for them.

In the water pollution control area, a fairly recent investigation is of pertinent interest.

As early as 1949, Gotaas (9) observed a decrease in BOD rate constants in dilution exceeding 50% sea water. He also observed that nitrification was inhibited by high chloride concentration.

Stowell (10) cited pilot-plant and full-scale trickling filter performance at San Quentin prison in which the wastewater to sea water ratio was about 1:1. BOD removal up to 90% was obtained when the effluent was recirculated at a ratio of 1:1 to minimize chloride fluctuations.

The effect of sodium chloride concentration on trickling filter slimes was also studied by Lawton and Eggert (11). They observed a reduction in BOD when salt concentrations greater than 20,000 mg/l were applied on slime acclimated to fresh water or when slimes acclimated to 20,000 mg/l salt concentration were subjected to fresh water media.

Carlucci and Pramer (12) reviewed the factors related to reduced survival of bacteria after waste water discharge to the sea. They stated that dilution, adsorption and sedimentation are factors of importance but they do not account fully for the rapid disappearance of bacteria that enters the sea. The authors also concluded that hydrostatic pressure, solar radiation, temperature, salinity, pH, oxidation-reduction potential and nutrient

availability are physio-chemical factors known to influence the activities of microorganisms.

Using a continuous flow reactor, McCarty (13) observed about 90 percent BOD removal at 12 and 24 hour aerator detention at a loading of about 40 lb BOD/1,000 cu. ft. of aerator capacity. Aerator solids were lost during the first day at high salinity when a six hour detention was used.

The effect of various salt concentrations on the extended aeration process was studied by Stewart, Ludwig and Kearns (14). This study was concerned with the treatment and disposal of ship-board wastes. Parallel pilot plants using synthetic wastes made up in fresh water and ocean water were run. Temporary reduction in treatment efficiency was noted when abnormally severe changes in salinity were combined with heavy hydraulic loadings. The length of the recovery period depended upon the duration and the severity of the salinity shock and the loading of the system. Floating sludge problems were encountered without knowing the reason. Also, the sludge concentration in the aerator decreased when ocean water was replaced by fresh water. The coliform count of the effluent was also studied by the authors. They found that the coliform count of fresh water effluent did not differ significantly from that of a system containing 30 percent sea water. The final conclusion of the authors was that the utilization of aerobic biological process for

treating shipboard wastewater under variable salinity conditions was basically feasible.

Mills and Wheatland (15) found no reduction in trickling filter performance when the concentration of NaCl was below 6,600 mg/l. Higher concentrations of NaCl produced effluents higher in COD and ammonia. It required one to three weeks to stabilize performance after a change in NaCl.

In an attempt to reduce pollution in shipping lanes, Ludzack and Noran (16) studied the effect of salinity of the shipboard waste on the activated sludge process and anaerobic digestion. The authors found no detectable changes in the continuous performance of the activated sludge units with chloride concentration of up to 8,000 mg/l, although temporary clarification problems were observed. When operating at a high concentration of salinity, a "shock effect" was observed with sharp changes in chloride concentrations; however, the system adjusted to approximately 80 percent to 90 percent BOD removal after one to three days. This is in very good agreement with the observations of Lawton and Eggert (11). The authors also observed that poor nitrification was characteristic of a change in salt concentration or of sustained operation at high salinities.

Kincannon and Gaudy (17) using laboratory-scale batch activated sludge units investigated an early observation that with high NaCl concentration the accumulated oxygen uptake increased while solid production decreased. Both long term and short term (shock) effects of NaCl were studied. A concentration of 8,000 mg/l had little

or no effect, but at concentration of 20,000 or 30,000 mg/l the efficiency of COD removal and solids production were greatly impaired. In the long term studies there was a severe change in the ratio of respiration to synthesis which was attributed to changes in the metabolic process of the microorganisms.

Sequential substrate removal by activated sludge after a change in salt concentration was studied by Kincannon, Gaudy and Gaudy (18). In this study cells grown using glucose in high salt medium were subjected to a shock loading of salt free medium. The immediate response was the release of cellular components, indicative of lyses. This lysate was metabolized only after an acclimation period following glucose utilization. The authors also concluded that shock loadings to activated sludge plants involving changes in salt concentration affected efficiency and that a longer aeration period is required in order that the system gain acclimation to compounds released in response to shock. The authors also concluded that adaptation to high salt concentrations involves a selection of strains rather than a biochemical adaptation of prevailing species. This is in agreement with Wood's (8) findings.

In another study, Kincannon and Gaudy (19) designed experiments to learn the mechanism of causation for the effects observed in their previous study with particular emphasis on shock loading. Results showed a decrease in substrate removal rate when sludges developed in

fresh water were subjected to slug doses of 30,000 mg/l. No serious stress to the system was noted. Sludges developed in fresh water undergo severe impairment of substrate removal efficiency when slug doses of salt resulted in mixed liquor concentration of 45,000 mg/l. The study also revealed that sludge developed in fresh water was found to be more affected by slug doses of salt than was sludge developed in high salt concentration by rapid change to a fresh water environment. Lyses was observed when cells grown on fresh water environment is transferred to saline water or vice versa. Osmotic shock caused when salt-grown cells were placed in lower salt concentrations, resulted in a more severe disruption of system efficiency when final salt concentration was below 10,000 mg/l. The authors also observed that sludges developed in high salt concentrations were characterized by low carbohydrates and protein content, whereas lipids and RNA were abnormally high. Also sludges acclimated to high salt concentration exhibited a higher ratio of respiration to synthesis than cells grown in fresh water. Changes in settling characteristics were also observed in the higher salt concentration. Sludge developed in 45,000 mg/l concentration generally removes COD at slower rates than did sludge developed in fresh water.

In a very recent study, Kincannon and Gaudy (20) concluded that during the period of increasing the salt concentration to 30,000 mg/l the system could not maintain a high substrate removal efficiency,

but after an acclimation period the system regained its former efficiency. Upon diluting the salt out of the system, a significant rise in cell yield was noted as the salt level passed through the range 8,000 - 10,000 mg/l. It was found that steady operations at a salt level of 8,000 mg/l sustained the cell yield at high levels.

CHAPTER III

INSTRUMENTATION AND MATERIALS

Reactors

The bench scale reactors used for the growth of the cultures were 4-liter resin reaction kettles (Fisher Scientific No. 11-847). Compressed air routed through a cotton filter to remove impurities was supplied to the reactor fluids through a diffusor stone suspended through the central opening of the reactor cover.

Two liter Erlenmeyer flasks were utilized as batch reactors for the batch tests. Aeration and shaking were accomplished through the action of a mechanical shaker, which is described below.

Incubator - Shaker

The incubator shaker used in this study was the Gyrotory Incubator Shaker Model G25, manufactured by the New Brunswick Scientific Company, Inc., New Brunswick, New Jersey. The incubator shaker is a floor mounted, controlled environment incubator incorporating a variable speed shaker equipped with a gyrotory drive mechanism that rotates the shaker platform in a one inch circular orbit. The shaker platform accomodates a wide variety of interchangeable shaker trays, flasks or beakers of various sizes.

Gas Chromatograph

The gas chromatograph used in this study was a Series 1200 Varian Aerograph flame ionization GLC, connected to a Sargent Model SRG recorder and Model 204 disc chart integrator. Pre-purified nitrogen was employed as the carrier gas with pre-purified electrolytic hydrogen and air supplied to the flame ionization detector. Organic components were separated using a 20' x 1/8" coiled stainless steel column packed with 5% Carbowax 20M on 80/100 mesh chromosorb W.

Total Organic Carbon Analyzer

A Beckman Model 915 Total Organic Carbon Analyzer, which utilizes low and high temperature combustion tubes to convert carbonaceous material to carbon dioxide, and a Model 215A Infrared Analyzer for carbon dioxide measurement, was used to determine the amount of the organic carbon in the injected samples. The analyzer is connected to a Beckman Model 1005 ten-inch Laboratory Potentiometric Recorder.

The low temperature combustion tube used phosphoric acid-wetted quartz chips at 150°C to acidify inorganic carbon compounds and to release carbon dioxide.

The high temperature combustion tube used a cobalt oxide impregnated asbestos fiber catalyst at 950°C to oxidize all carbonaceous material.

Filtration Technique

The supernatants of the centrifuge samples removed from the batch reactors were pressure filtered through a 10 ml glass syringe equipped with a membrane filter holder to accommodate a 25 mm filter. Samples withdrawn from suspended solids measurement were vacuum filtered through pre-weighed 47 mm membrane filters. All the membrane filters used had an effective pore size of 0.45 μ .

Gilson Oxygraph

The oxygen uptake rates were determined by using a Model KL Gilson Oxygraph and strip chart recorder. A Clark type electrode assembly was used for its many advantages over the bare platinum electrode. This instrument was obtained from the Gilson Medical Electronics, Middleton, Wisconsin.

Organic Substrate

Acetone, 2-butanone, and 2-pentanone were selected as a carbon source for this study; first because of their frequent presence in industrial wastes, and second because of the extensive experience of the Environmental Engineering personnel at Texas A&M University with the removal patterns of these aliphatic ketones.

Inorganic Salt Media

The following inorganic solutions were prepared and used in distilled water for maintenance of the cultures and for the batch tests.

- Solution 1: Ammonium Sulfate, $(\text{NH}_4)_2 \text{SO}_4$; 117 g/l
- Solution 2: Potassium Dihydrogen Phosphate, KH_2PO_4 ; 10.9 g/l
Potassium Monohydrogen Phosphate, K_2HPO_4 ; 14.0 g/l
- Solution 3: Calcium Chloride, CaCl_2 ; 69.3 g/l
Magnesium Chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 83.5 g/l
Sodium Chloride, NaCl ; 43.6 g/l
- Solution 4: Ferric Chloride, FeCl_3 ; 2.9 g/l

One ml of each solution in one liter of fluid volume provided the following minimum elemental or ionic concentrations.

Sodium, Na^+	17 mg/l
Potassium, K^+	9 mg/l
Calcium, Ca^{++}	25 mg/l
Magnesium, Mg^{++}	10 mg/l
Iron, Fe^{+++}	1 mg/l
Chloride, Cl^-	100 mg/l
Nitrogen, N, in ammonium ion	25 mg/l
Phosphorus, P, in phosphates	5 mg/l
Sulfur, S, in sulfate ion	28 mg/l

The phosphate buffer solution used to stabilize the pH in the cultures growth reactors contains the following:

Potassium Dihydrogen Phosphate, KH_2PO_4 ; 8.5 g/l

Dipotassium Hydrogen Phosphate, K_2HPO_4 ; 21.75 g/l

Disodium Hydrogen Phosphate, Na_2HPO_4 ; 17.5 g/l

Ammonium Chloride; NH_4Cl ; 1.7 g/l

Salinity Source

The various salinity media were prepared using a synthetic sea water bearing the trademark 'Instant Ocean' which is a product of Aquarium Systems of Wickliffe, Ohio. The product is packed in plastic bags containing 27 ounces of synthetic sea water and a vial filled with the trace elements solution. When the contents of one package and the trace elements were dissolved in five gallons of tap water, the average salinity was 35,600 mg/l.

pH Determination

Determinations of pH were made with a Sargent pH meter equipped with an S-30072-15 combination glass-calomel electrode.

CHAPTER IV

EXPERIMENTAL PROCEDURE

Establishment of Heterogeneous Culture and Acclimation to Aliphatic Ketones

Four hundred ml of settled primary sewage was used to inoculate a distilled water medium contained in a 4-liter reactor to provide the initial seed. Metrecal, as a proprietary nutrient containing the necessary growth components for microbes, was added as such to provide a proper C:N:P ratio besides the other trace elements needed for microbial metabolism. The soluble portion of Metrecal was added to the reactor to provide a concentration of 500 mg/l in the three liter aqueous medium, then distilled water was added to the three liter mark.

The mixture was aerated for 24 hours then the air flow was stopped for 15 minutes to allow the sludge to settle. One-half of the supernatant was disposed and the preceding feeding procedure was repeated. This procedure was repeated for fifteen days at which time a considerable amount of sludge had developed and the acclimation to ketones started.

Acclimation to ketones was accomplished by a gradual increase in the ketones with a consequent decrease in the Metrecal. During the acclimation process inorganic salts were added to compensate for the decrease of the inorganic salts added with the Metrecal.

The acclimation process required 20 days at which time the only sources of carbon furnished to the heterogeneous culture was in the form of aliphatic ketones, namely, acetone, 2-butanone and 2-pentanone. Nitrogen, phosphorus and other elements were added in sufficient quantity to sustain the microbial growth.

The pH of the reactor medium was maintained at 7.0 to 7.5 by using a phosphate buffer solution. The buffer solution was added to provide 40 ml of buffer solution per liter of the reactor medium.

The acclimated culture was maintained by a daily feeding of 1000 mg/l acetone. One-third of the mixed liquor was disposed prior to feeding to maintain the microbial viability and concentration, and to control the accumulation of toxic materials. This culture was maintained and labeled as Culture "A".

Acclimation to Salinity

One liter of the aqueous medium in the reactor containing Culture "A" was transferred to another 4-liter reactor. Inorganic salts were then added and the volume of the medium was brought to 3-liters with distilled water and synthetic sea water to create a salinity of 500 mg/l in the 3-liter aqueous medium. Acetone was finally added to provide a substrate concentration of 1000 mg/l. The same procedure was repeated after 24 hours and the salinity level was increased by 500 mg/l. At the end of the tenth day the

salinity level had been elevated to 5000 mg/l. The culture was kept at this level of salinity for an additional two weeks before any batch tests were performed on the culture. This culture was then labeled as Culture "P."

A portion of Culture "B" was acclimated to a salinity level of 10,000 mg/l by using the above procedure. The daily increment in salinity was also 500 mg/l. When the salinity medium had been elevated to the level of 10,000 mg/l the culture was maintained at the same salinity level for an additional two weeks before running any batch tests on the culture. This culture was labeled as Culture "C."

Following the previous procedure Culture "D" was obtained by acclimating portion of Culture "C" to a salinity level of 20,000 mg/l. The daily salinity increment was 350 mg/l. The culture was also maintained at the salinity level of 20,000 mg/l for two weeks before using the culture for any batch test.

Similarly, a portion of Culture "D" was acclimated to a salinity of 35,000 mg/l (normal sea water), using a daily salinity increment of 200 mg/l. At the end of the acclimation period the culture was kept at the 35,000 mg/l salinity for two weeks before running any batch tests on the culture. This culture was labeled Culture "E."

Organisms Identification

After the various cultures (A through E) had been established, species diversity for each culture was determined. Several different types of biological media were employed in the anticipation of the requirements of the various isolates. For the bacteria three types of media were utilized:

1. Difco tryptone glucose extract agar.
2. Tryptone glucose extract agar with 0.4 g/l of yeast extract added.
3. An extract prepared from an autoclaved portion of each culture medium.

Corn meal agar was used for the fungi and liquid 3N Bold's Basal Media described by Bischoff and Bold (21) was employed for the algae.

Samples for the testing of bacteria and fungi were taken from each culture and were agitated for one minute with a Waring Blender. One milliliter of each sample was serially diluted in a sterile buffered solution to yield dilutions of 10^{-2} , 10^{-4} , 10^{-6} and 10^{-8} . Plate dilutions of 2×10^{-3} , 10^{-3} , 2×10^{-5} , 10^{-5} , 2×10^{-7} , and 10^{-7} were obtained by inoculating with 0.1 ml and 0.2 ml samples from each dilution bottle and spreading evenly on the surface with a sterile glass rod. These plates were incubated at room temperature for several days and then individual colonies were aseptically picked and plated several times to insure purity.

An approximate quantitative evaluation was made by counting colonies on the various media and dilutions.

The Pseudomonas Gp IV bacteria were identified by employing accepted microbiological techniques. Significant characteristics included: gram negative rods, oxidase positive reaction, polar flagellation, and no response to Hugh-Leifson glucose oxidation-fermentation medium. Controls were used in all tests.

The Cephalosporium was identified by characteristic conidiophore and conidia morphology. Other organisms were also identified employing accepted techniques.

The algae were tested on a corn meal media by directly adding 1.0 milliliter of each culture and incubating for several days. The corn meal media consisted of corn meal infusion 50 g/l, bacto-destrose 2 g/l, and bacto agar 15 g/l.

Preparation of Culture for Batch Tests

Prior to each test, the contents of the 4-liter reactor containing the particular culture was stirred with a mechanical stirrer for 15 seconds to break down the large flocs of the culture. Then 1500 ml was removed and blended with a waring blender for 15 seconds to disperse the culture uniformly. The dispersed culture was then divided into five equal parts and centrifuged individually at 27,750 x g for ten minutes using a Sorval Super-speed Automatic Refrigerated Centrifuge model RC2-B. The super-

natant was discarded and the five portions of the culture were resuspended in the desired salinity media to correspond to each batch test reactor salinity. The portions of the culture were then transferred to the corresponding batch reactors.

Substrate Removal Test Under Various Salinity Conditions

Five series of experimental tests were conducted in an effort to determine the metabolic response of culture A, B, C, D, and E when subjected to various salinity conditions (Table I). Some of the tests were conducted to determine the effect of long term salinity acclimation of a culture on its metabolic behavior, while the other tests were conducted to determine the effect of the short term change in salinity (shock effect) on the metabolic response of the five different cultures.

For the purpose of testing the microbial behavior of culture "A" when subjected to various salinity levels, the culture was prepared by the previous procedure and was placed in five 2-liter Erlenmeyer flasks. The contents of the first flask was brought to 800 ml using tap water, while synthetic sea water was used to bring the volume of the medium in the second flask to 800 ml. The contents of the other three flasks were brought to 800 ml using a mixture of tap water and synthetic sea water resulting in a media salinity of 5,000, 10,000, and 20,000 mg/l respectively. One and one half (1 1/2) ml each of inorganic solution #1 and #2

TABLE I. - Illustration of Removal Tests

Test Series "A"	Biomass Established in Fresh Water (Culture "A")				
	Tested in Fresh Water (Control)	Shocked with 5000 mg/l	Shocked with 10000 mg/l	Shocked with 20000 mg/l	Shocked with 35000 mg/l
Test Series "B"	Biomass Established in 5000 mg/l Salinity Media (Culture "B")				
	Shocked with Fresh Water	Tested in 5000 mg/l (Control)	Shocked with 10000 mg/l	Shocked with 20000 mg/l	Shocked with 35000 mg/l
Test Series "C"	Biomass Established in 10000 mg/l Salinity Media (Culture "C")				
	Shocked with Fresh Water	Shocked with 5000 mg/l	Tested in 10000 mg/l (Control)	Shocked with 20000 mg/l	Shocked with 35000 mg/l
Test Series "D"	Biomass Established in 20000 mg/l Salinity Media (Culture "D")				
	Shocked with Fresh Water	Shocked with 5000 mg/l	Shocked with 10000 mg/l	Tested in 20000 mg/l (Control)	Shocked with 35000 mg/l
Test Series "E"	Biomass Established in 35000 mg/l Salinity Media (Culture "E")				
	Shocked with Fresh Water	Shocked with 5000 mg/l	Shocked with 10000 mg/l	Shocked with 20000 mg/l	Tested in 35000 mg/l (Control)

were added to each reactor. The flask contents were mixed thoroughly and 100 ml samples were withdrawn by a volumetric pipette from each flask, frozen and stored at 5°C for later suspended solids measurements. The last thing added to each flask was two ml of the ketone stock solution.

The flasks were put in the incubator-shaker for one minute to accomplish thorough mixing of the contents. After the one minute mixing the zero time sample was taken from each flask. Using a volumetric pipette, a 50 ml sample was withdrawn, cooled and stored in a refrigerator for suspended solids measurement. Another 50 ml sample was withdrawn; a portion was placed in a small beaker for immediate oxygen uptake and pH measurements, while the remainder was centrifuged at 27,750 x g for 15 minutes. Using a Vim 10 ml Gabriel Vial Aspirating Syringe equipped with a membrane filter holder to accommodate a 25 mm filter, a portion of the supernatant of the centrifuged sample was filtered and the filtrate retained in a 4 ml vial. Microbial growth in filtered samples was inhibited by adding 20 μ l of 1.5 percent mercuric chloride solution.

After taking the zero time samples, mixing and aeration were resumed. As the biological reaction continued, samples were taken at designated time intervals until such time as the oxygen uptake rate of the cultures dropped sharply indicating the consumption of the primary substrate and intermediates.

All removal tests were carefully temperature controlled at 30°C by the incubator shaker. Proper mixing and aeration were obtained by operating the shaker at 220 rpm.

Biomass Increase

Each sample stored for suspended solids measurement was centrifuged at 27,750 x g for 10 minutes and pressure filtered through pre-weighed 47 mm membrane filters of 0.45 micron effective pore size. Each filter was washed repeatedly with tap water to wash down the inorganic salts outside the cells. The filters and culture were then oven dried to constant weight at 105°C and re-weighed. The difference between the latter weight and the filter weight represented the dry weight of the culture. The suspended solids were then calculated in terms of milligrams per liter, mg/l.

Oxygen Uptake Rate

Oxygen uptake rate was measured by placing 2 ml of the sample removed from the batch reactor at frequent sampling intervals in the sample vessel of the Gilson Oxygraph described earlier. The resultant linear plot of dissolved oxygen (mg/l) versus time (seconds) allowed the calculation of the dissolved oxygen uptake rate in mg/l/hr.

The Gilson Oxygraph was calibrated prior to each batch test by running a triplicate Winkler determination on a tap water

reservoir held at a constant temperature (28.5°C) and aerated at a constant rate. The dissolved oxygen content of the standardization water was found to be 7.7 mg/l.

In an earlier stage of this research the reaction of the Clark electrode toward different ionic concentrations were determined. Samples containing various salinity levels were brought to saturation at constant temperature and their dissolved oxygen contents were measured by the Winkler technique. The saturation points varied from 7.7 mg/l for fresh water to 7.2 mg/l for sea water. The samples were then transferred to the sample vessel of the oxygraph and the electrode reaction was recorded. The initial response of the instrument was almost the same for the different D.O. levels, however, when the oxygen was exhausted by adding a culture to the vessel the base line dropped to the same level regardless of the ionic concentration of the media.

Air Stripping of Ketones

Along with each test an additional reactor was constructed to determine air stripping of the organic components used as the organic substrate. The stripping reactor contained the same amount of substrate and nutrients as the other batch reactors, however, the culture was not added to this reactor. Samples were withdrawn periodically from this reactor and tested chromatographically to determine the concentration of the organic compon-

ents at the various reaction times.

Substrate Components Determination

The analyses of the organic components in all the aqueous solutions were performed by gas-liquid chromatography. The instrument used was described in Chapter III.

Calibration of the GLC was performed prior to each test series. Triplicate determinations were made of a standard acetone, 2-butanone and 2-pentanone solution. Because of the linear response of the flame ionization detector, a single calibration of each compound was considered adequate.

All gas chromatographic analyses were performed on 10 microliter aliquots of the filtrate injected directly into the injection port of the gas chromatograph with a 10 microliter Hamilton syringe. All analyses were made at an electrometer sensitivity setting of flame range 1 and an attenuation of 128. The peak separation of the three ketones used in the test was good at oven temperature of 100°C and a carrier gas flow of 63 cc/min.

One or more gas chromatographic analyses were performed on each 4 ml filtrate taken during an experimental test. Predetermined retention times were used to qualitatively identify individual ketones. Quantitative determination was performed by calculation of the chart integrator counts for each peak. The average of the integrator counts was used when more than one injection

was used, and the individual ketone concentration was calculated by direct proportion to an analysis of a standard solution prepared to contain a known concentration of each ketone.

Organic Carbon Measurement

The total organic carbon in the filtrate of samples withdrawn at various reaction periods is a good indication of the amount of the substrate remaining in the aqueous medium. Previously, COD was a more common parameter utilized to indicate the amount of the substrate present in an aqueous medium. In this study the TOC was measured rather than the COD because of simplicity of TOC technique and because of the chlorides interference in the COD tests.

A Beckman Total Organic Carbon Analyzer was used for the TOC measurements. The instrument was described earlier in Chapter III. Total carbon of the filtrate was determined by injecting 10, 15 or 20 microliters of the sample in the injection port of the total carbon channel of the instrument. The inorganic contents of the sample was determined by direct injection of 20 microliters of the filtrate in the injection port of the inorganic channel. The difference between the total carbon data and the inorganic carbon data represents the total organic carbon (TOC). The instrument was calibrated prior to each test series using standard solutions of potassium hydrogen phthalate organic carbon prepared using

carbon dioxide free distilled water to produce a carbon concentration of 250, 200, 150, 100, 75, 50, and 25 mg/l. The inorganic channel was calibrated by using standard solutions of sodium-carbonate and sodium bicarbonate.

For both standard solutions and test samples, consecutive triplicate recorder peaks with a percentage deviation of less than one percent were considered acceptable.

CHAPTER V

RESULTS AND DISCUSSION

Before presenting the results of this study it is important to define some of the terms used or to be used later on in this manuscript. The term "Control" is used to define the batch reactor in each test series that contains a medium of the same salinity to which the culture being tested has been acclimated. The terms "Shock Effect" or "Short Term Effect" are synonymous and can be defined as the effect of the introduction of a salinity medium on the behavior of a culture originally acclimated (adapted) to a different salinity medium. "Shock Magnitude" is defined as the difference in concentration between the batch test medium and the adaptation medium of any particular culture. Substrate concentration, biomass concentration, oxygen uptake rate and total carbon concentration of any particular batch reactor (system) are referred to as "System Parameters." The change of the system parameters with respect to the test reaction time is referred to as the "Microbial Behavior."

"Long Term Effect" is a term used throughout this manuscript to indicate the effect of salinity adaptation on the culture behavior. This was accomplished by comparing the behavior of the various control systems. The "Unit Rate Removal" was the basis for the behavior comparison of the various control systems. It

is expressed in milligrams of substrate carbon removed per milligrams of biomass present per hour.

The ketone concentration is often represented as ketone carbon or substrate carbon rather than the summation of the concentration of the three ketones used as substrate in the batch tests.

Results of each test series will be presented and discussed individually. System parameters versus reaction time were plotted for each individual reactor. Also the substrate carbon for all reactors within a particular test series was plotted on a single figure for the purpose of easy comparison of the removal rates of the various systems within the particular test series. It is obvious that comparison of the removal rates should be done on the basis of the unit rate removal rather than the substrate carbon and the TOC. However, because of the identical initial biomass in all reactors within a particular series the use of these parameters was justified. Another factor that will sustain this justification is the fact that all batch tests of any particular test series were run simultaneously and under identical temperature, mixing and aeration conditions.

Comparison of the removal rates within the test series was also made by plotting the TOC (total organic carbon) versus the reaction time for the individual reactors. It was observed that the TOC removal pattern followed the pattern of the substrate carbon with the exception of the presence of some residual TOC

which was not encountered in the substrate carbon plot. The presence of the residual TOC made the comparison of the removal rate on the basis of the substrate carbon more practical than the TOC.

Test Series "A"

As was mentioned earlier the culture used in this test series was culture "A" which was originally established in a fresh water medium.

The systems parameters for the five batch reactors are presented in figures 1, 2, 3, 4, and 5. By comparing these figures, it can be seen that the biomass concentration increase was inversely proportional to the shock magnitude. In other words, the culture tested at a salinity medium similar to or close to the culture acclimation (adaptation) salinity showed a higher increase in biomass concentration than the culture subjected to a salinity shock of greater magnitude. The oxygen uptake rates behaved in a similar manner to the biomass concentration increase.

The substrate carbon rates of removal for the five systems in this series are shown in figure 6. This figure clearly indicates that the systems shocked with 5,000 mg/l and 10,000 mg/l salinity removed the substrate almost as fast as the control system, while the system shocked with a salinity of 20,000 mg/l required an additional hour to remove the substrate. A shock of 35,000 mg/l

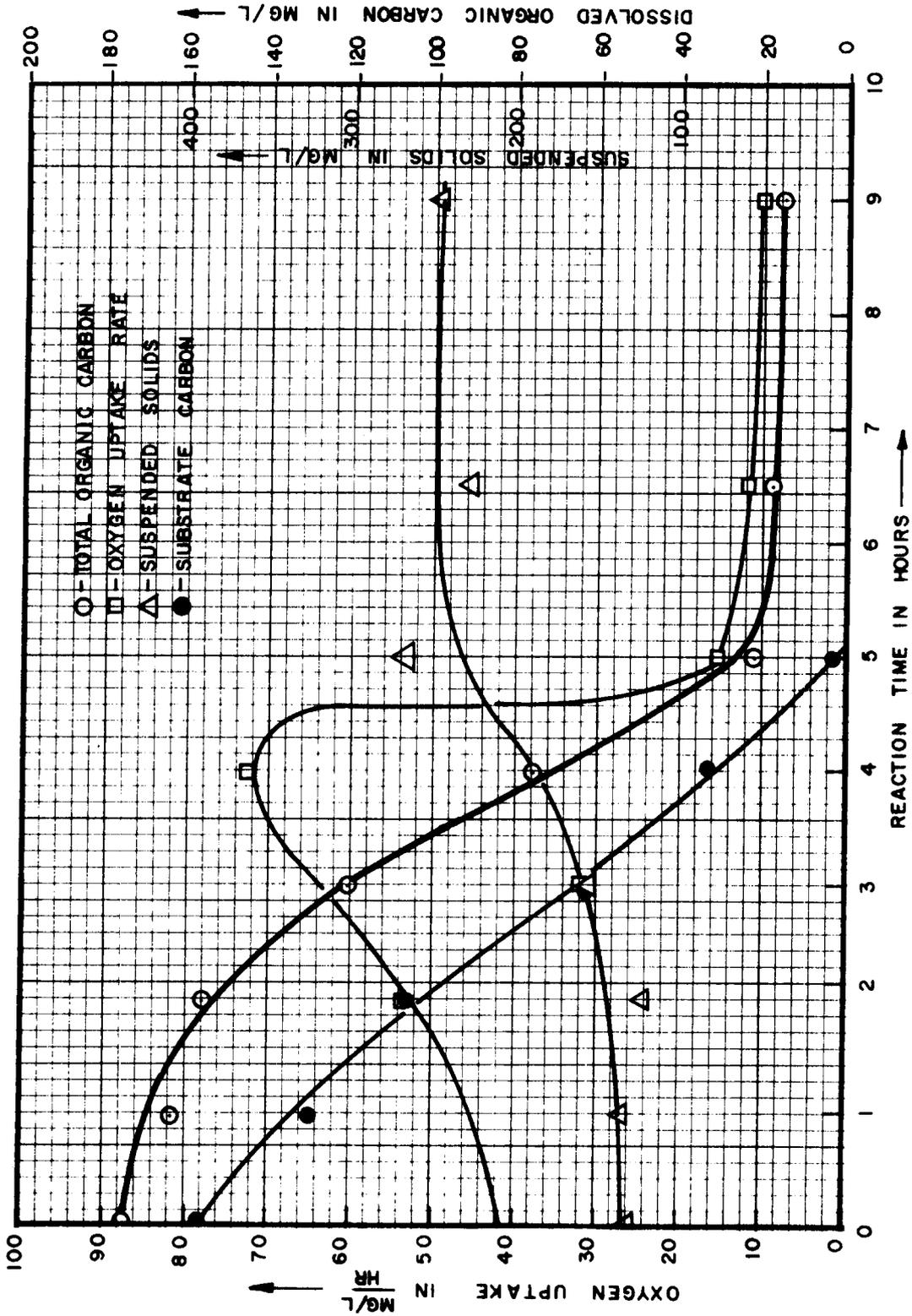


FIGURE 1.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED AND TESTED IN FRESH WATER.

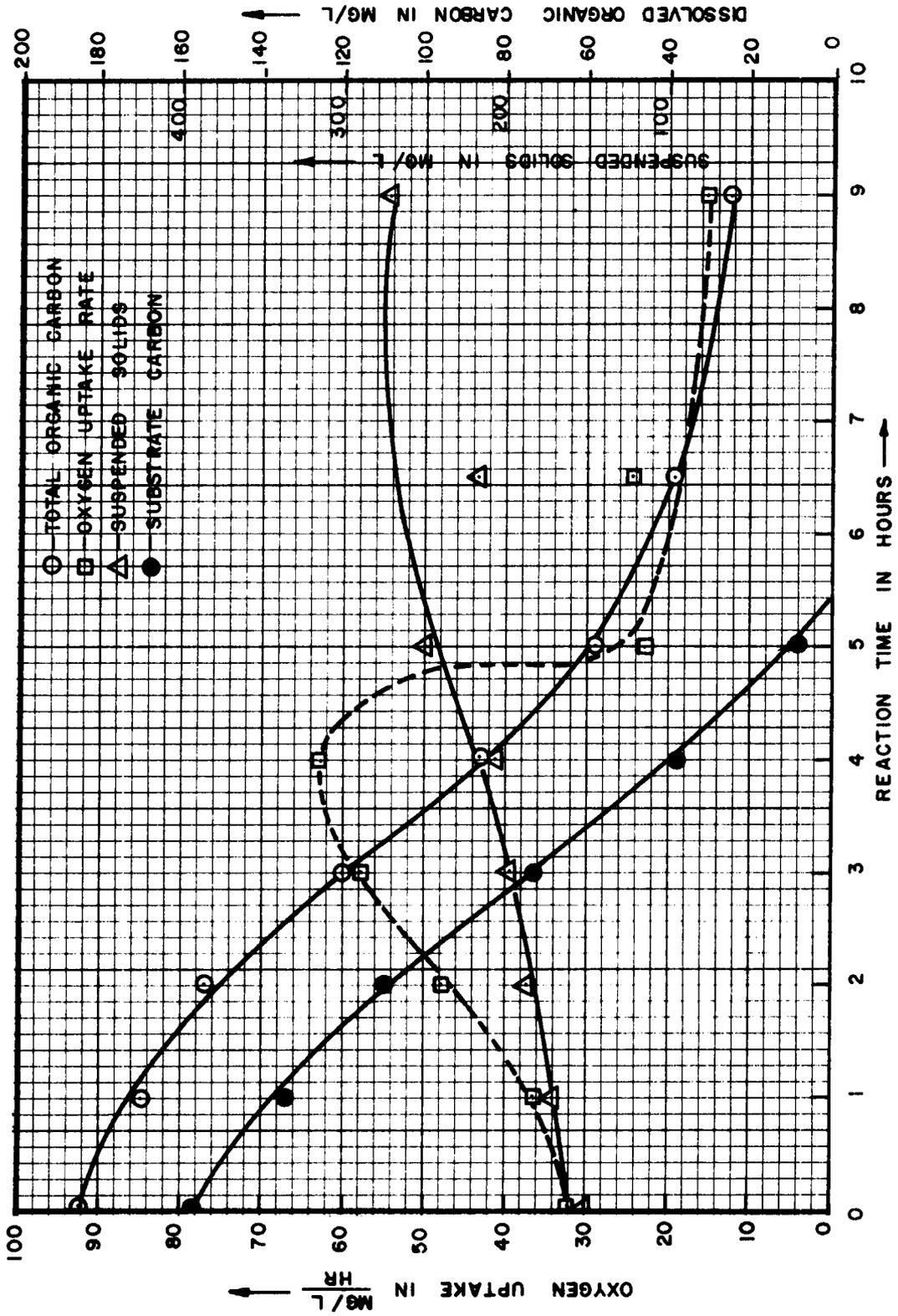


FIGURE 2.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN FRESH WATER AND SHOCKED WITH A SALINITY OF 5,000 MG/L.

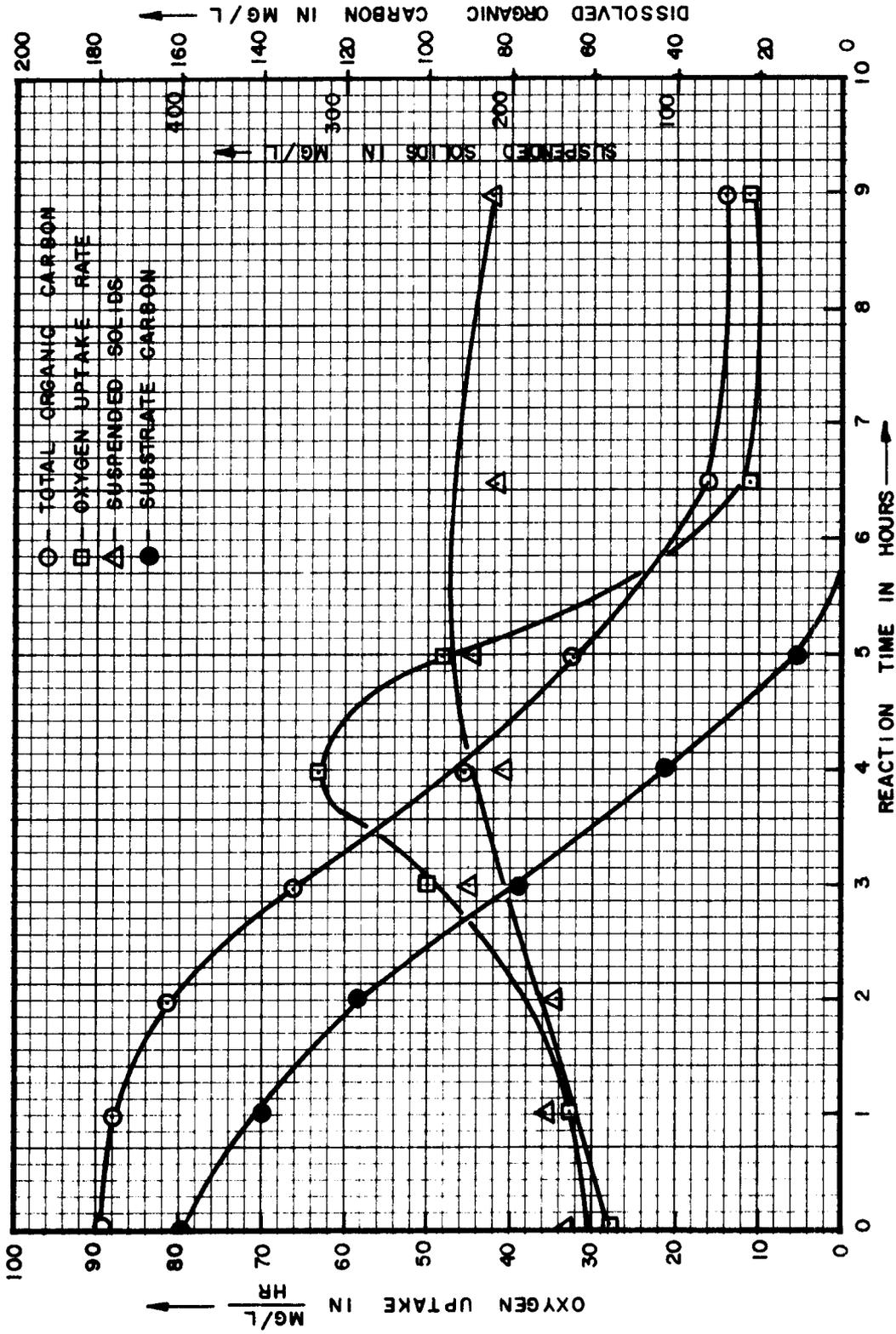


FIGURE 3 - SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN FRESH WATER AND SHOCKED WITH A SALINITY OF 10,000 MG/L.

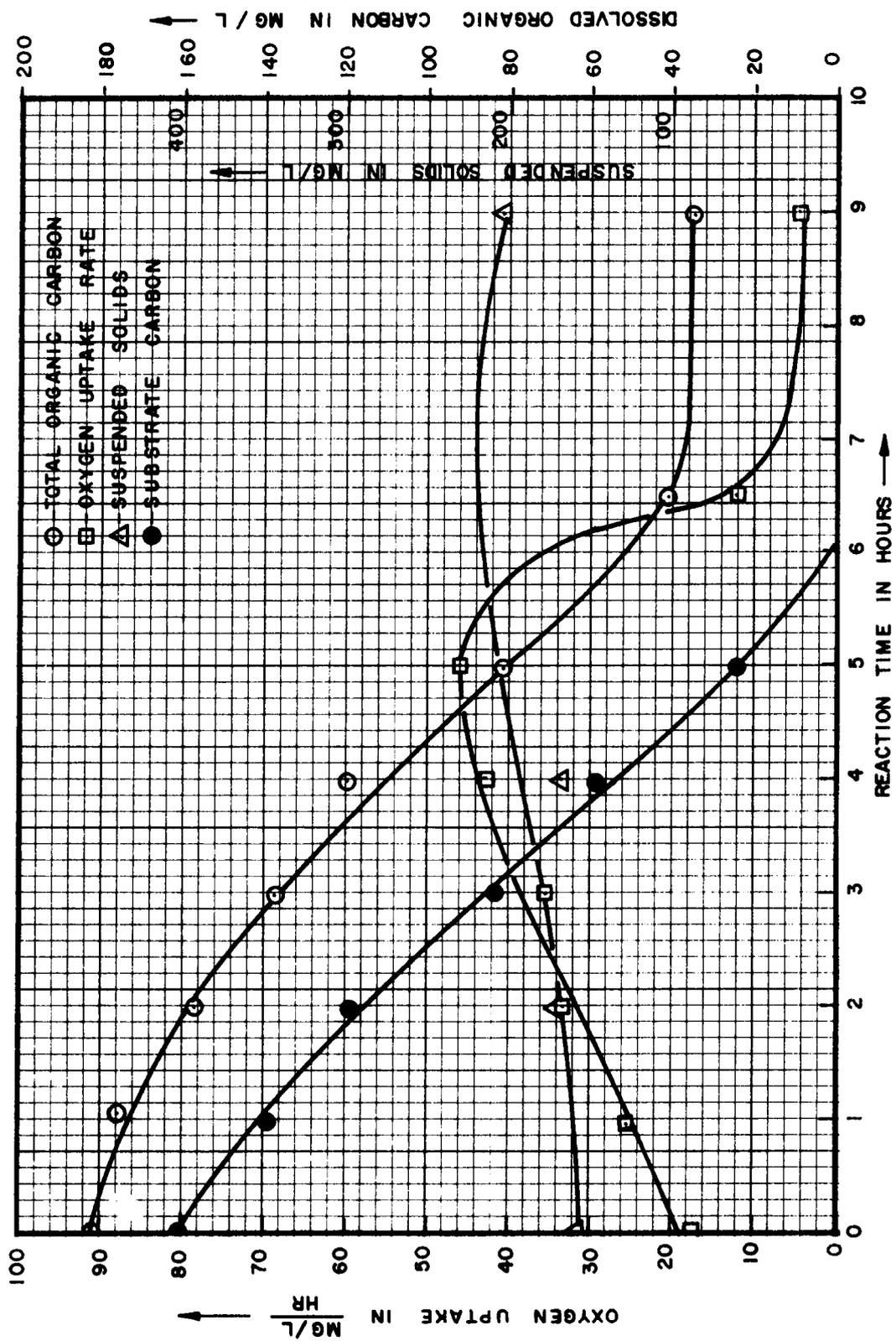


FIGURE 4.— SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN FRESH WATER AND SHOCKED WITH A SALINITY OF 20,000 MG/L.

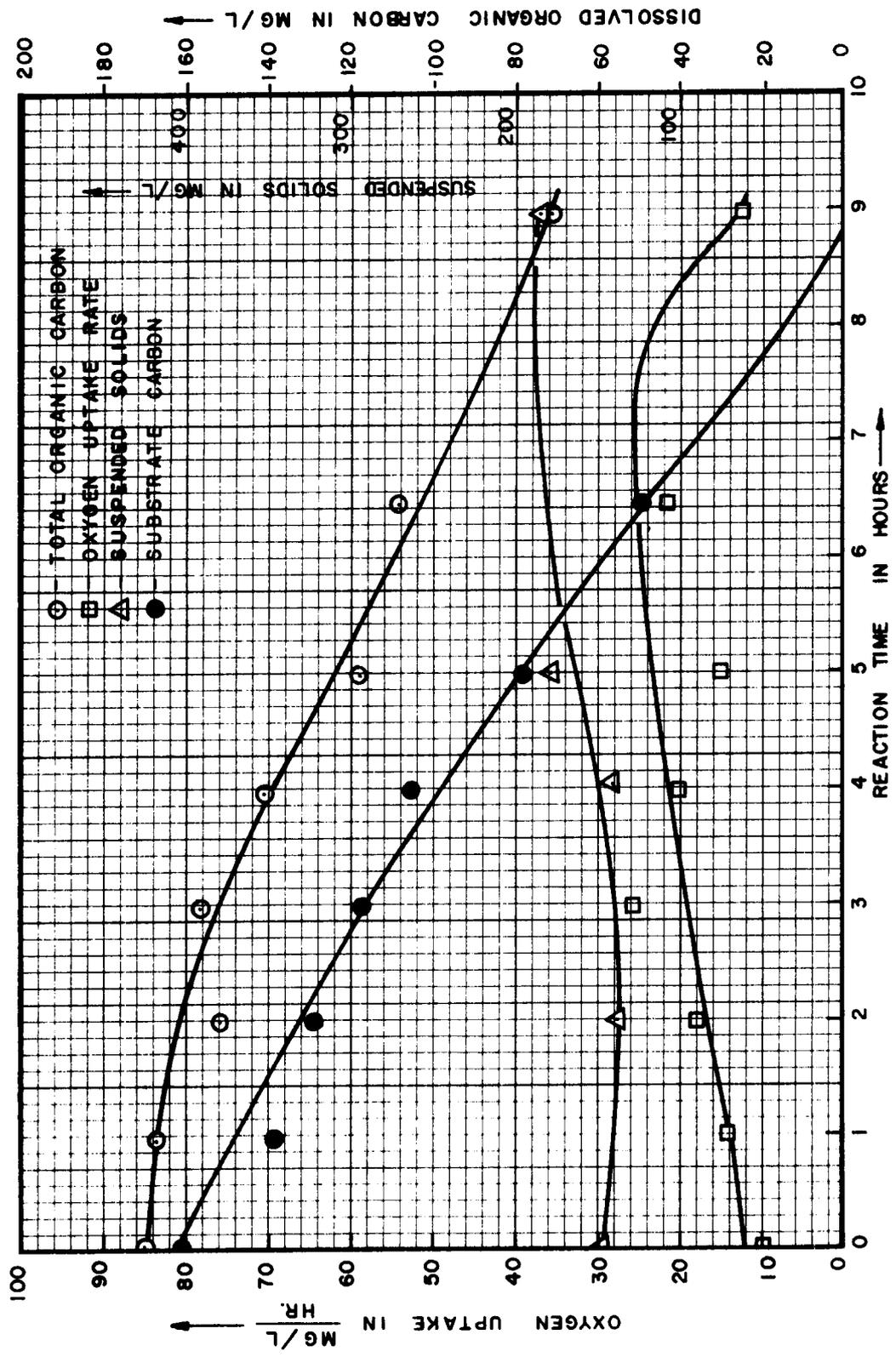


FIGURE 5.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN FRESH WATER AND SHOCKED WITH A SALINITY OF 35,000 MG/L.

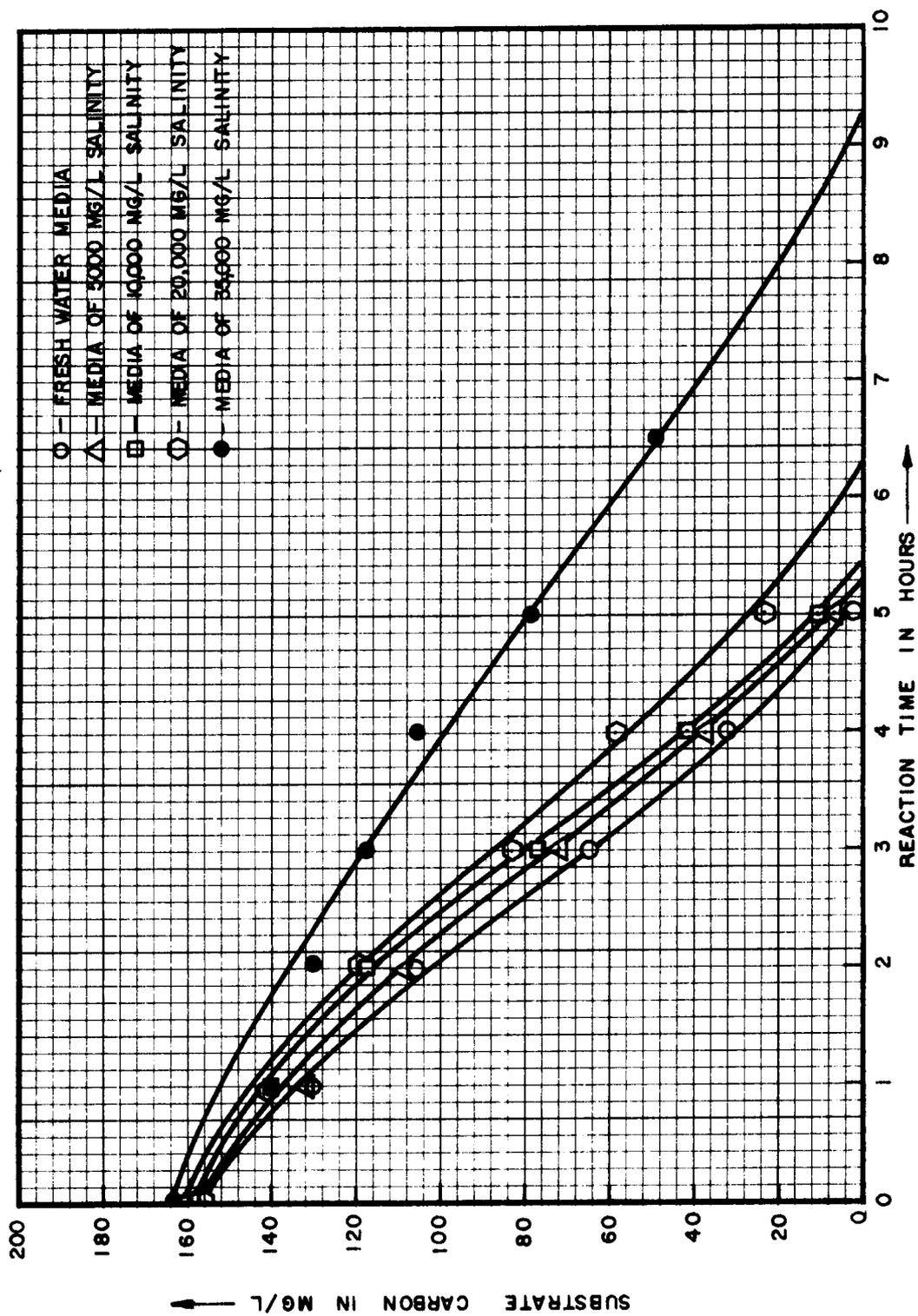


FIGURE 6.— SUBSTRATE CARBON CHANGE WHEN A CULTURE ESTABLISHED IN FRESH WATER IS SHOCKED WITH VARIOUS SALINITY MEDIA.

salinity decreased the removal rate drastically.

The total organic carbon (TOC) concentrations at the various sampling periods for the five systems in this series are shown in figure 7. The removal pattern of the biodegradable portion of the TOC followed the pattern of the substrate carbon described earlier. The residual TOC was higher for systems shocked with higher salinity. This residual TOC was proportional to the magnitude of salinity shock. The high level of residual TOC could be a result of either an undetected intermediate or a cell lysate.

Test Series "B"

As was defined earlier, culture "B" is a culture originally acclimated to aliphatic ketones and adapted to a salinity level of 5,000 mg/l. In this test series the behavior of culture "B" was determined under various salinity levels by using five batch reactors.

The system parameters for the five batch reactors are presented in figures 8, 9, 10, 11, and 12. From these figures it can be concluded that a shock magnitude of \pm 5,000 mg/l or + 15,000 mg/l did not affect the ultimate oxygen uptake rate considerably, while a shock magnitude of 30,000 mg/l decreased the ultimate oxygen uptake rate considerably. The biomass increase, in general, appeared to decline with the increase in the positive shock magnitude.

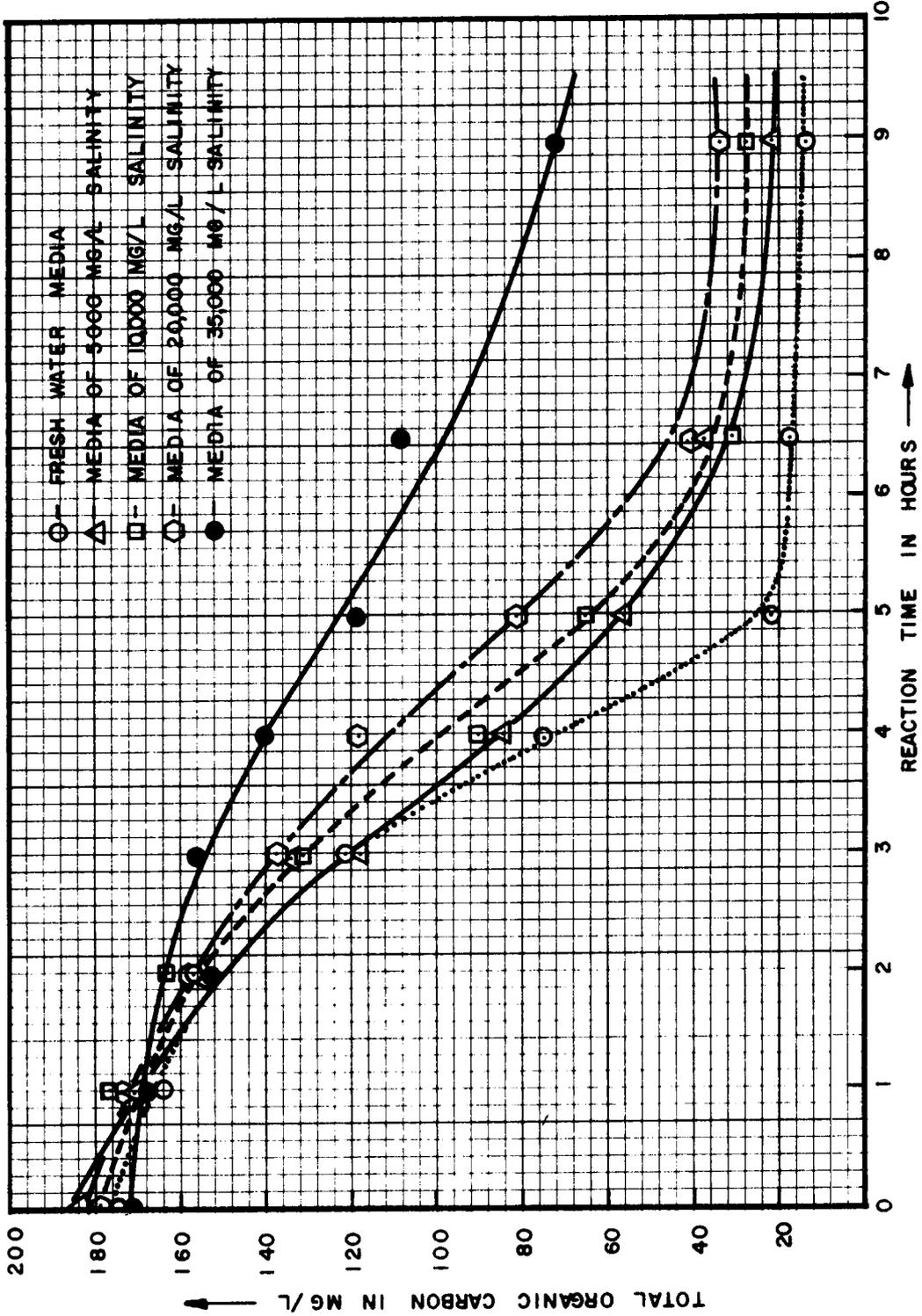


FIGURE 7. - TOTAL ORGANIC CARBON CHANGE WHEN A CULTURE ESTABLISHED IN A FRESH WATER MEDIA IS SHOCKED WITH VARIOUS SALINITY MEDIA.

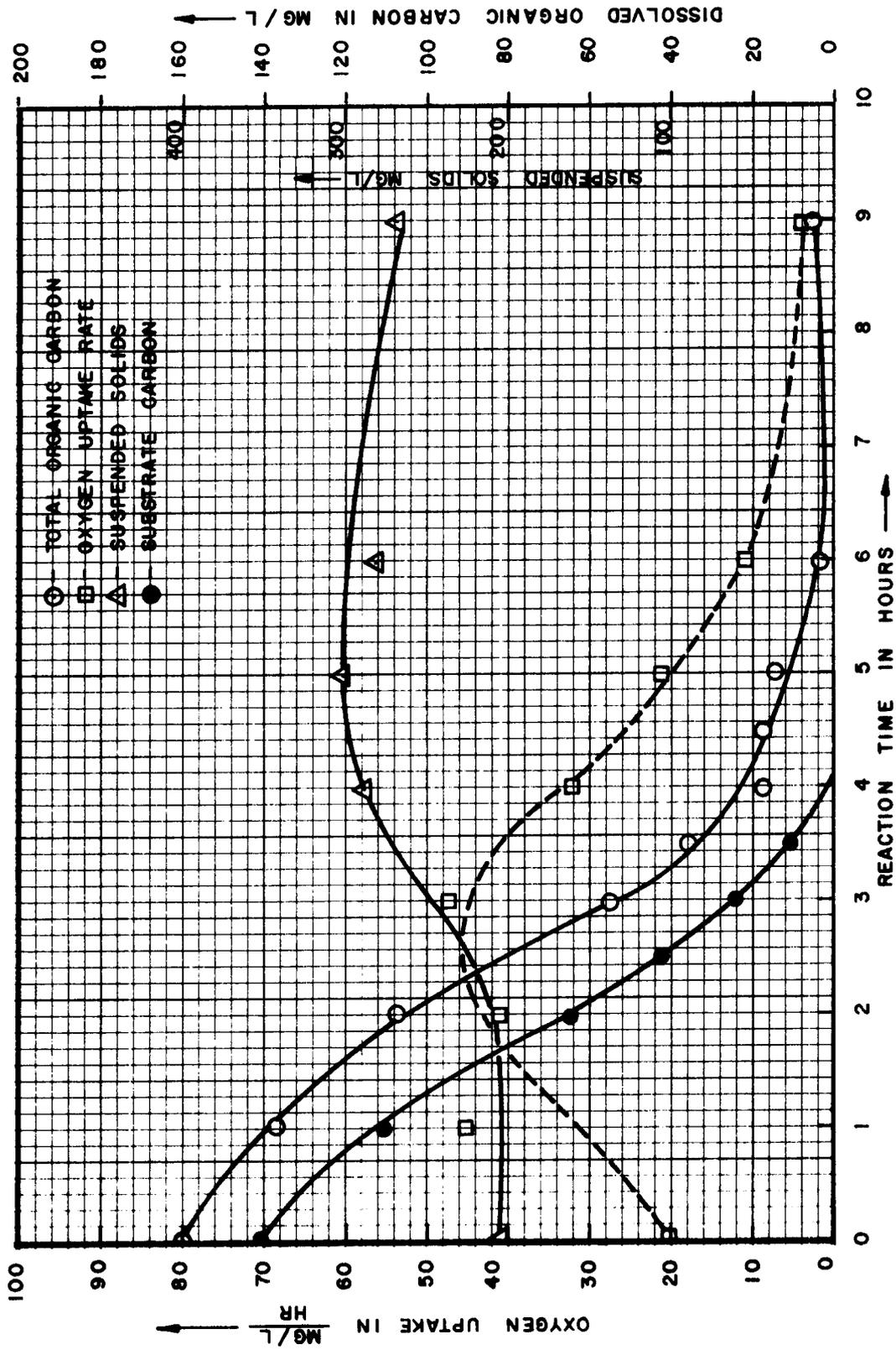


FIGURE 8. - SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 5000 MG/L AND SHOCKED WITH FRESH WATER.

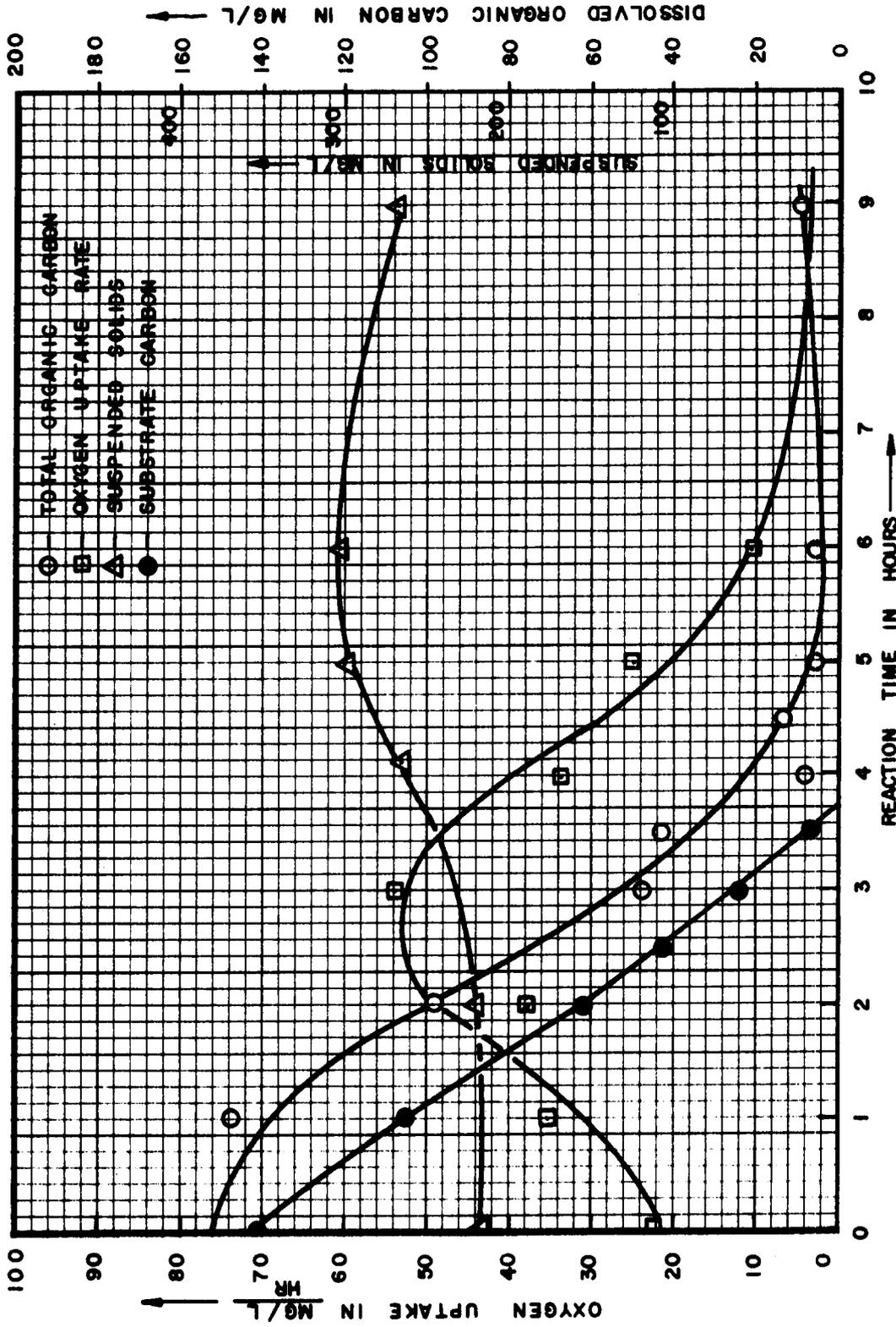


FIGURE 9.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED AND TESTED IN A SALINITY OF 5000 MG/L.

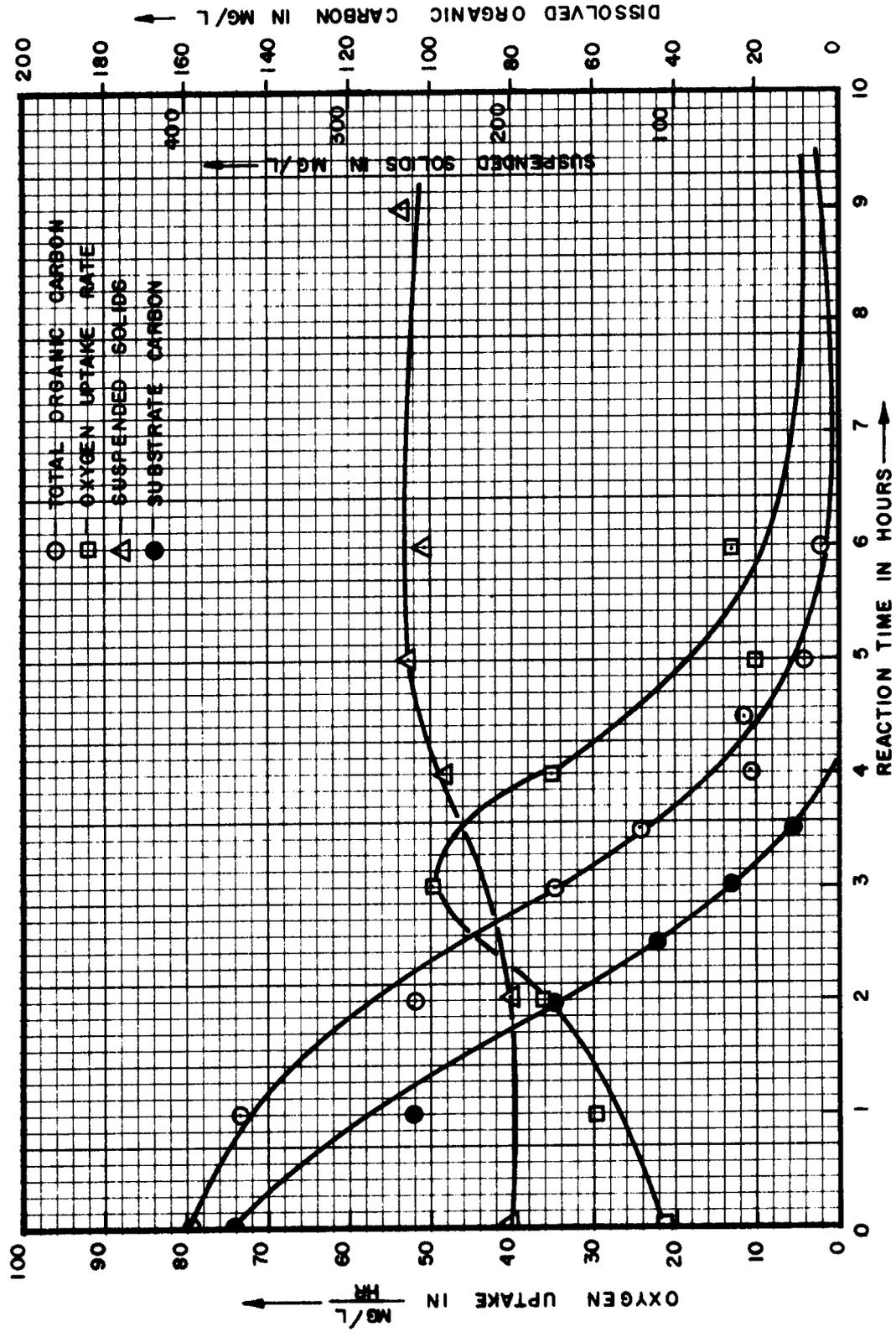


FIGURE 10.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 5000 MG/L AND SHOCKED WITH A SALINITY OF 10,000 MG/L .

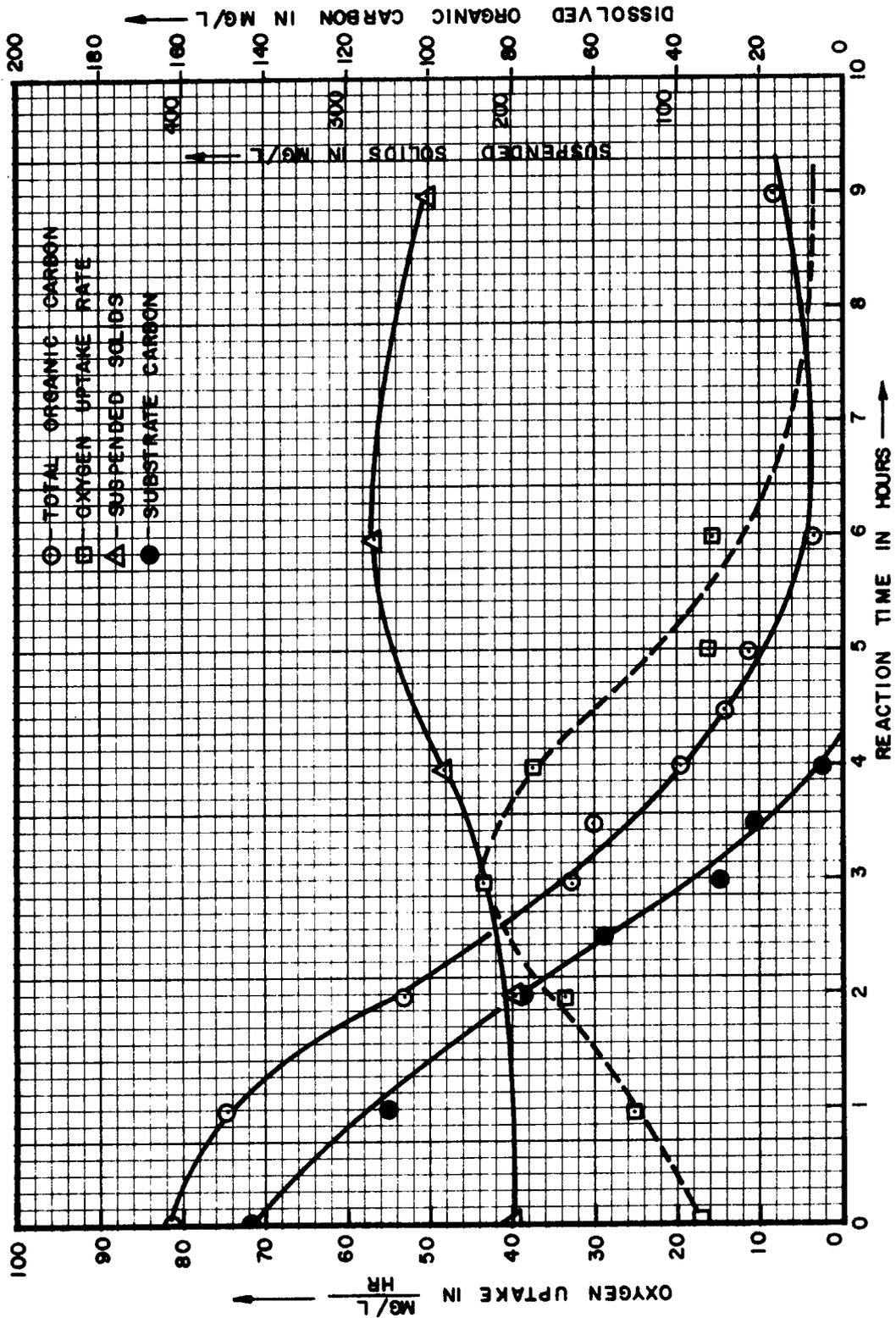


FIGURE 11. - SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 5000 MG/L AND SHOCKED WITH A SALINITY OF 20000 MG/L.

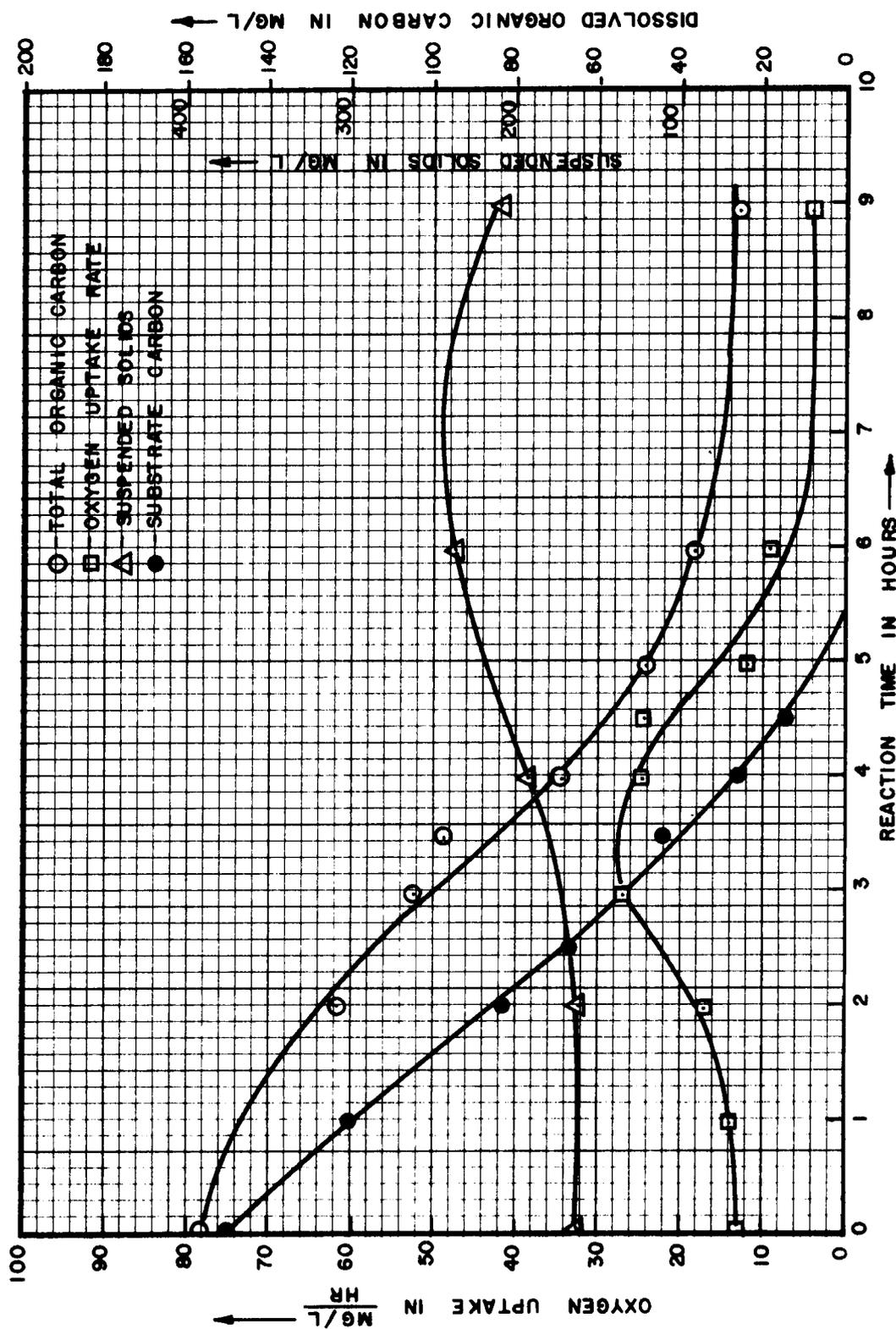


FIGURE 12.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 5000 MG/L AND SHOCKED WITH A SALINITY OF 35,000 MG/L.

The changes in substrate carbon concentration when culture "B" was shocked with the various salinity media are shown in figure 13. As can be detected from this figure, a shock magnitude in the order of $\pm 5,000$ mg/l did not affect the substrate carbon removal rate, while a shock magnitude of + 15,000 mg/l slowed down the reaction rate considerably. Again a shock magnitude of + 30,000 mg/l drastically suppressed the activity of the system.

The total organic carbon (TOC) concentration changes for the five systems are presented in figure 14. It is obvious that the removable TOC followed the pattern of the substrate carbon removal. The higher residual TOC phenomenon which was observed in test series "A" for higher salinity shocks was also present in this test series.

Test Series "C"

Culture "C" defined in the previous chapter as the culture originally acclimated to aliphatic ketones and adapted to a salinity of 10,000 mg/l. In this test series portions of culture "C" were tested for behavior under various salinity conditions.

The results of the various behavior components were plotted versus time in figures 15, 16, 17, 18, and 19. Comparison of these figures indicated that the biomass concentration increase was not affected by a salinity shock of magnitude in the order of $\pm 10,000$ mg/l, while a salinity shock of 35,000 mg/l (shock magnitude of

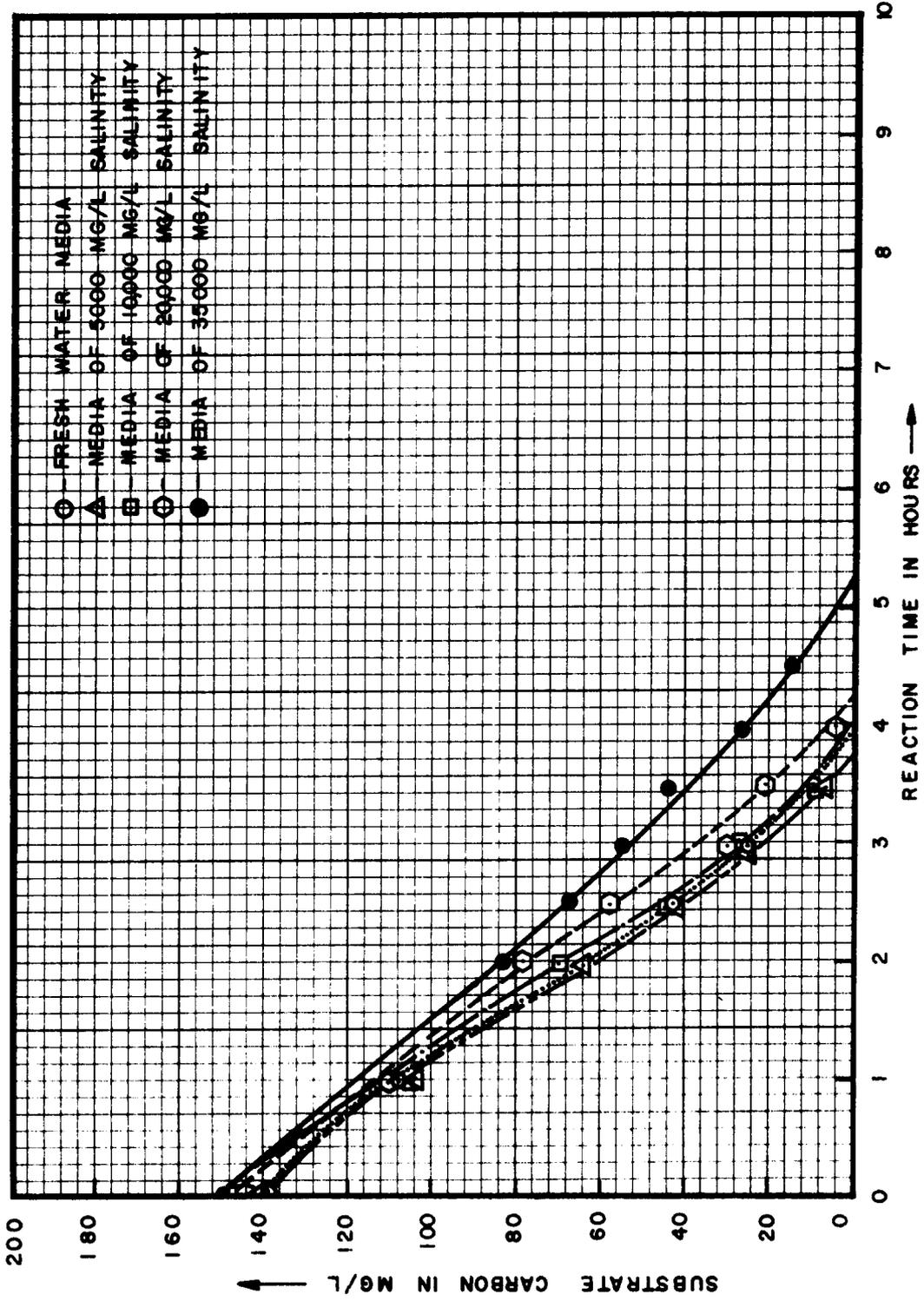


FIGURE 13.— SUBSTRATE CARBON CHANGE WHEN A CULTURE ACCLIMATED TO A SALINITY OF 5000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

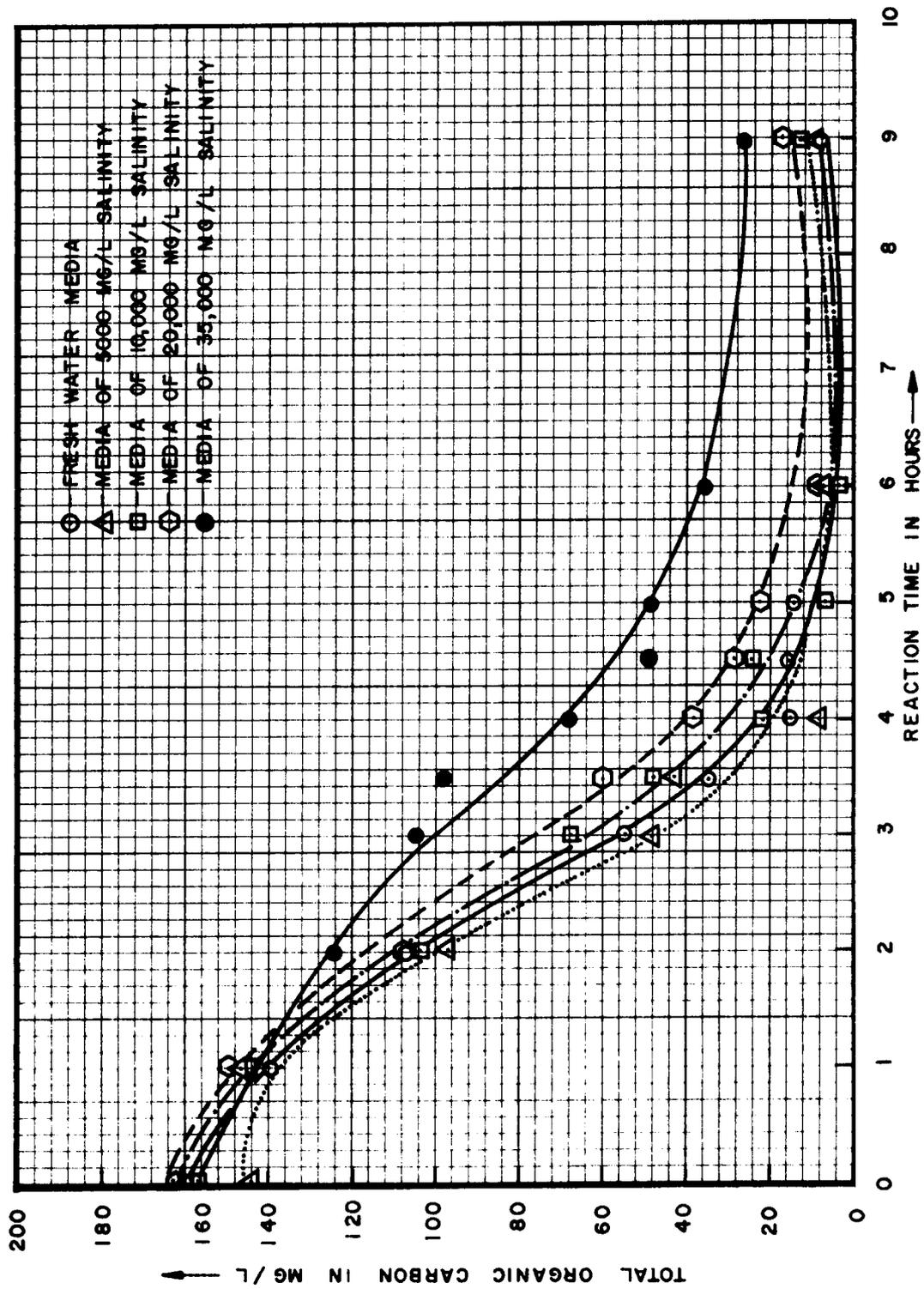


FIGURE 14.- TOTAL ORGANIC CARBON CHANGE WHEN A CULTURE ACCLIMATED TO A SALINITY OF 5000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

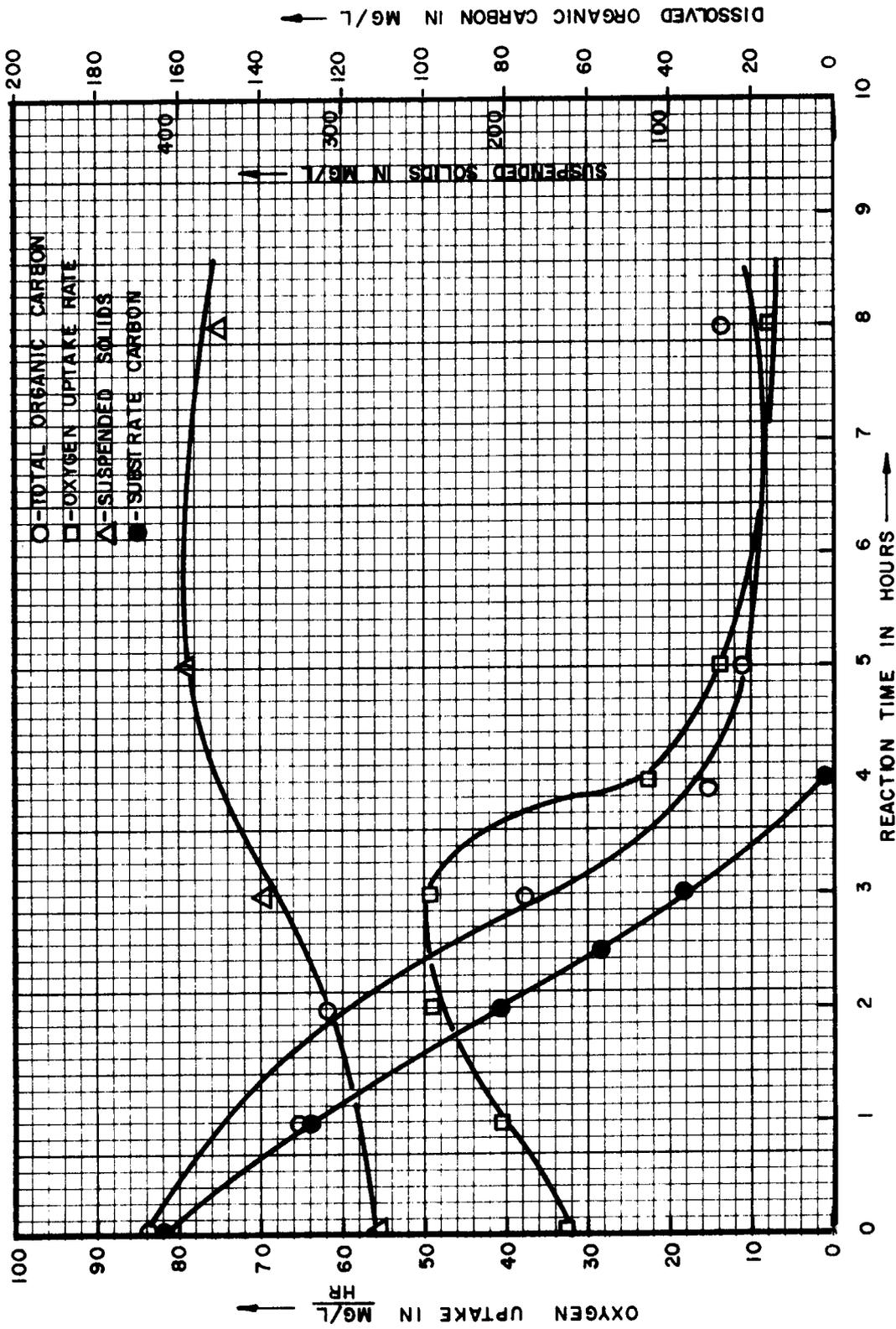


FIGURE 15.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 10,000 MG/L AND SHOCKED WITH FRESH WATER.

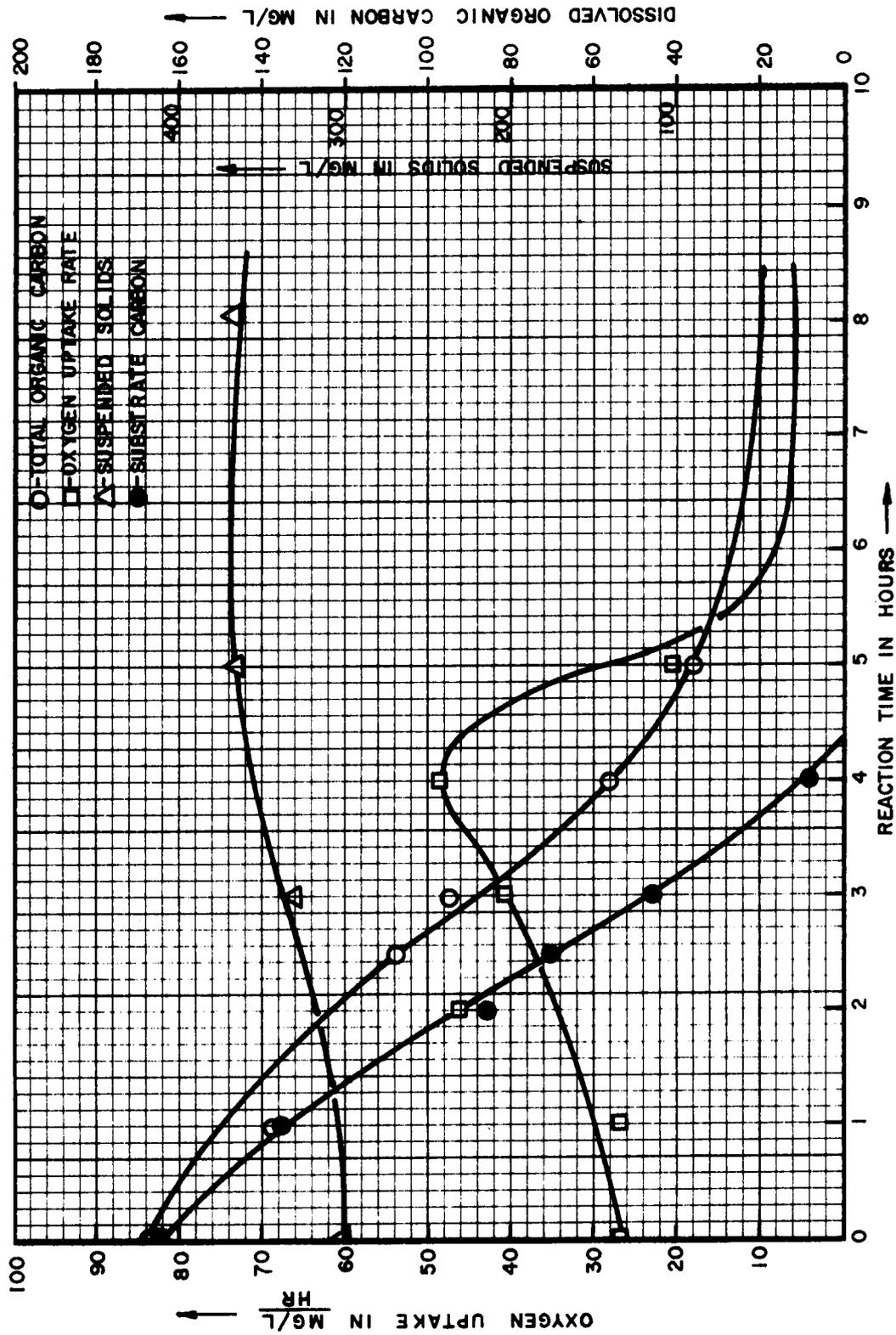


FIGURE 16.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 10,000 MG / L AND SHOCKED WITH A SALINITY OF 5000 MG / L .

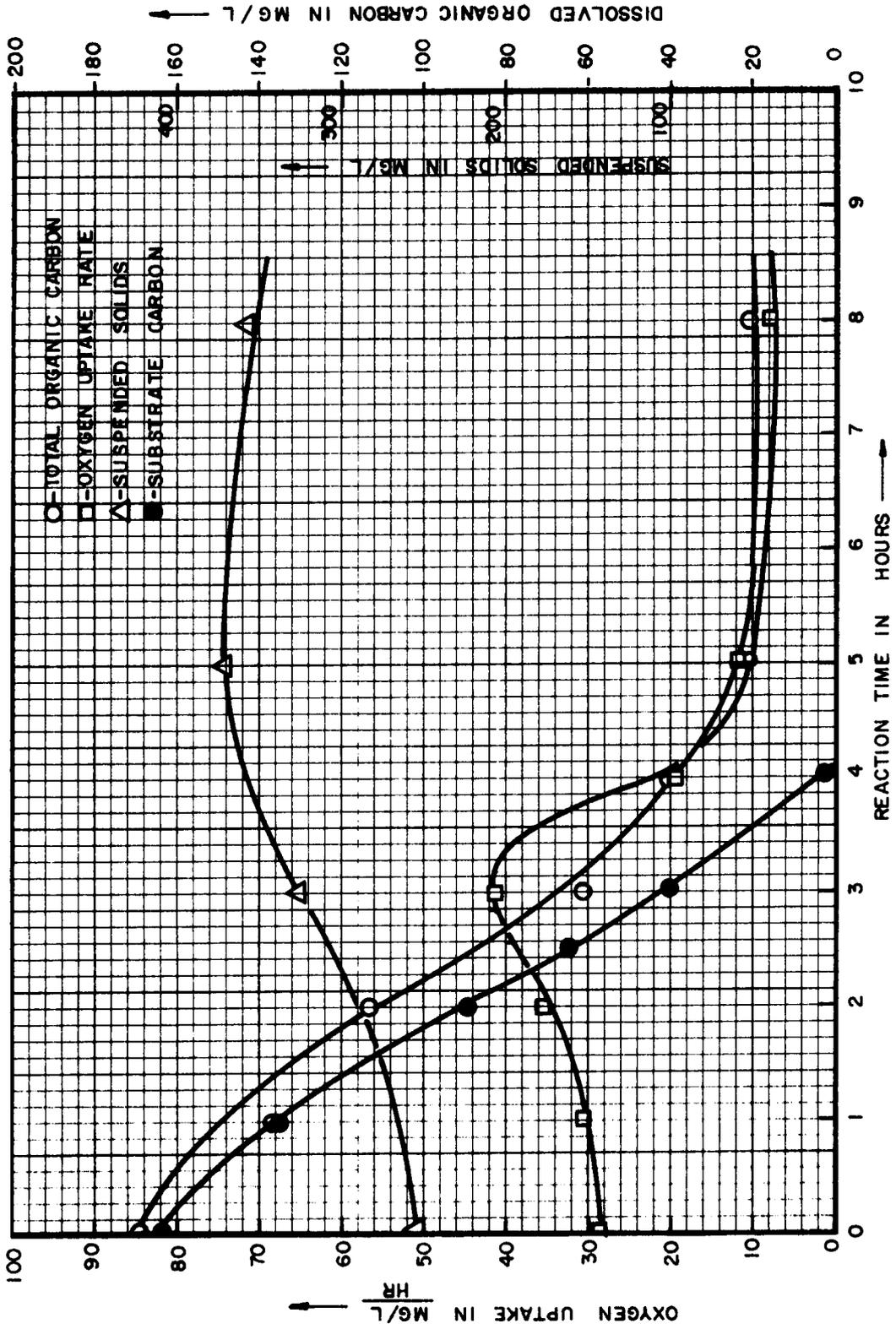


FIGURE 17.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED AND TESTED IN A SALINITY OF 10,000 MG/L.

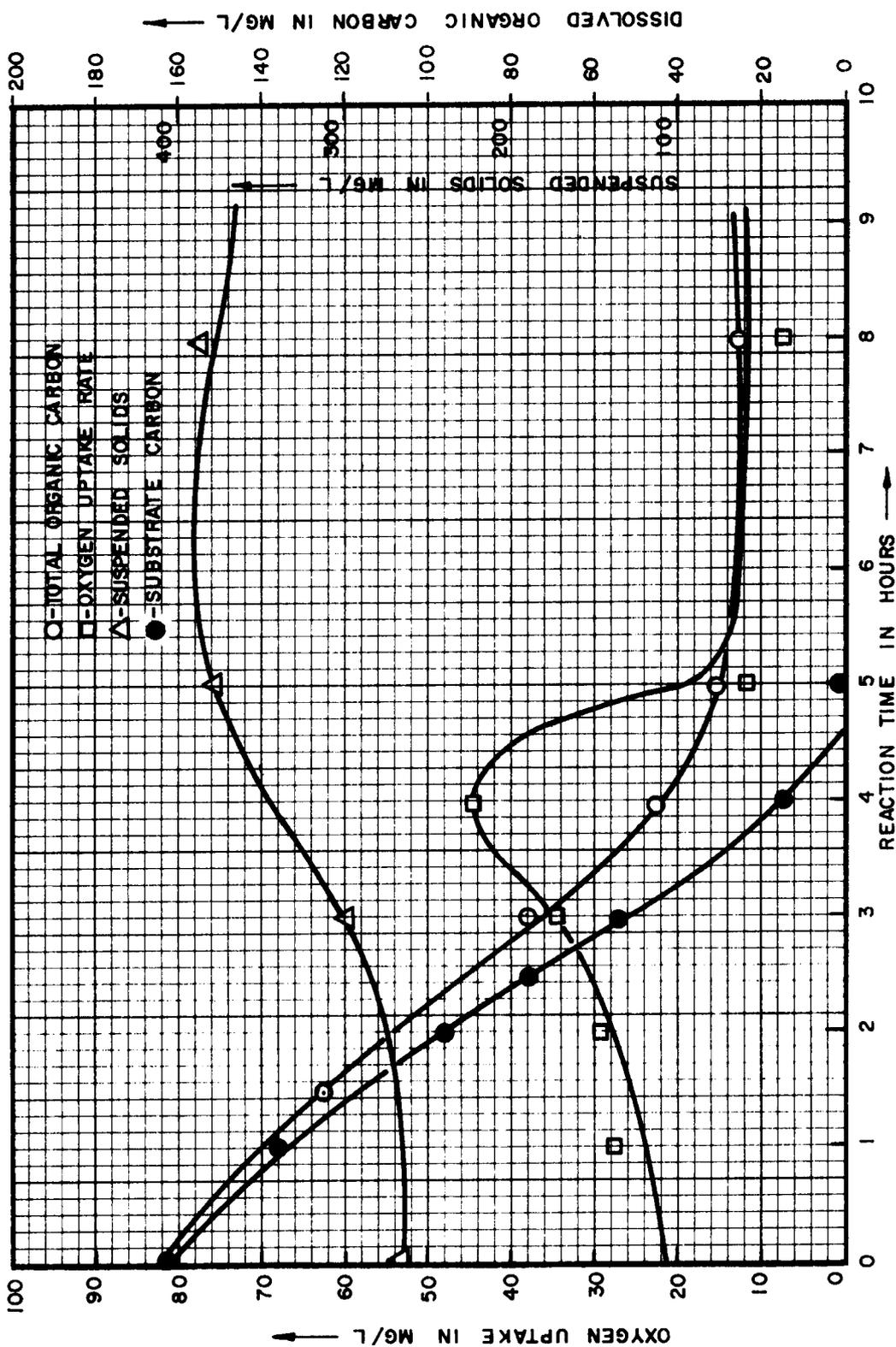


FIGURE 18.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 10,000 MG/L AND SHOCKED WITH A SALINITY OF 20,000 MG/L.

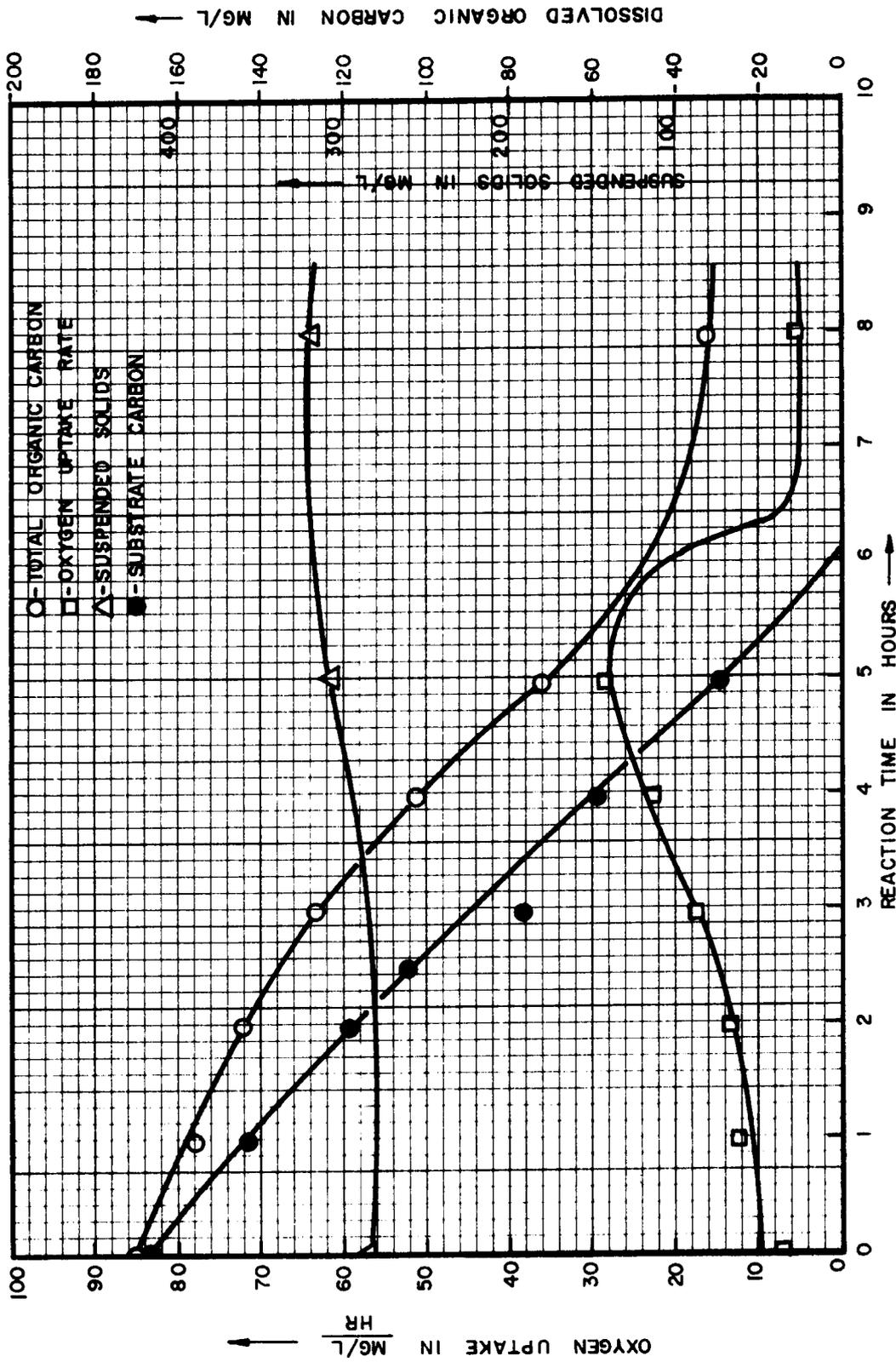


FIGURE 19.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 10,000 MG/L AND SHOCKED WITH A SALINITY OF 35,000 MG/L .

+ 25,000 mg/l) reduced the increase in the biomass concentration for this particular system.

The ultimate oxygen uptake rate of a system shocked with a salinity of 20,000 mg/l did not differ from the ultimate oxygen uptake rate of the control system (10,000 mg/l salinity medium), while a salinity shock of 35,000 mg/l did suppress the ultimate oxygen uptake rate considerably. On the other hand the negative salinity magnitudes (- 5,000 and - 10,000) resulted in a significant increase in the ultimate oxygen uptake rate and oxygen uptake per unit carbon removed.

The substrate carbon concentrations versus the reaction time for the five systems within this test series are plotted in figure 20. This figure clearly indicates that a shock magnitude in the order of $\pm 10,000$ mg/l did not significantly affect the substrate carbon removal, while a shock magnitude of + 25,000 mg/l (salinity shock of 35,000 mg/l) resulted in a considerable slow down in the rate of removal of the substrate carbon.

Figure 21 represents the total organic carbon (TOC) concentration versus time for the five batch reactors within this test series. It is clear that the removable part of the TOC behaved similarly to the substrate carbon removal described above. Also indicated in figure 21 is that the residual TOC did not vary with the shock magnitude except for the system shocked with 35,000 mg/l salinity where a higher residual TOC is indicated. The fact that

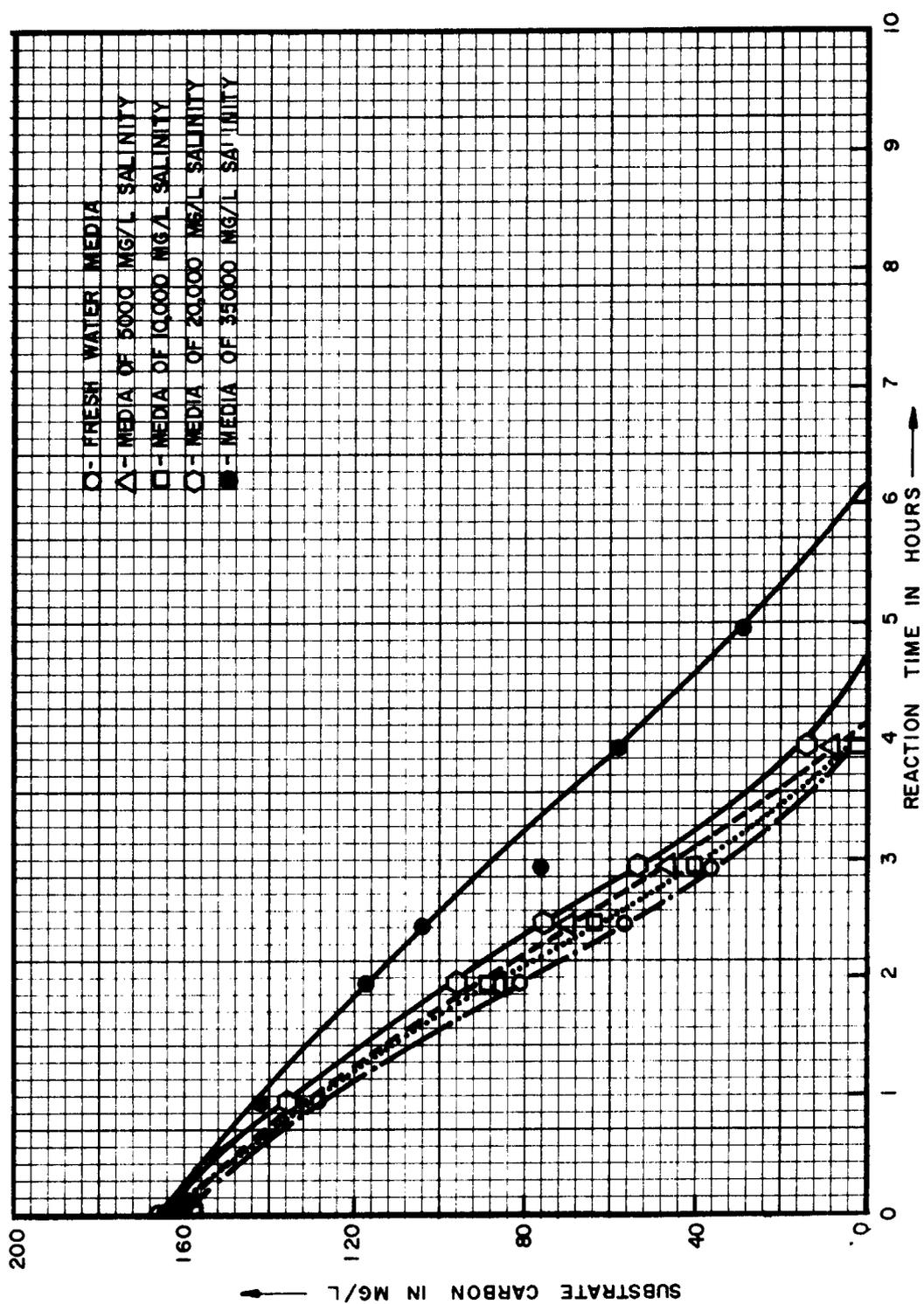


FIGURE 20.-SUBSTRATE CARBON CHANGE WHEN A CULTURE ACCLIMATED TO A SALINITY OF 10,000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA .

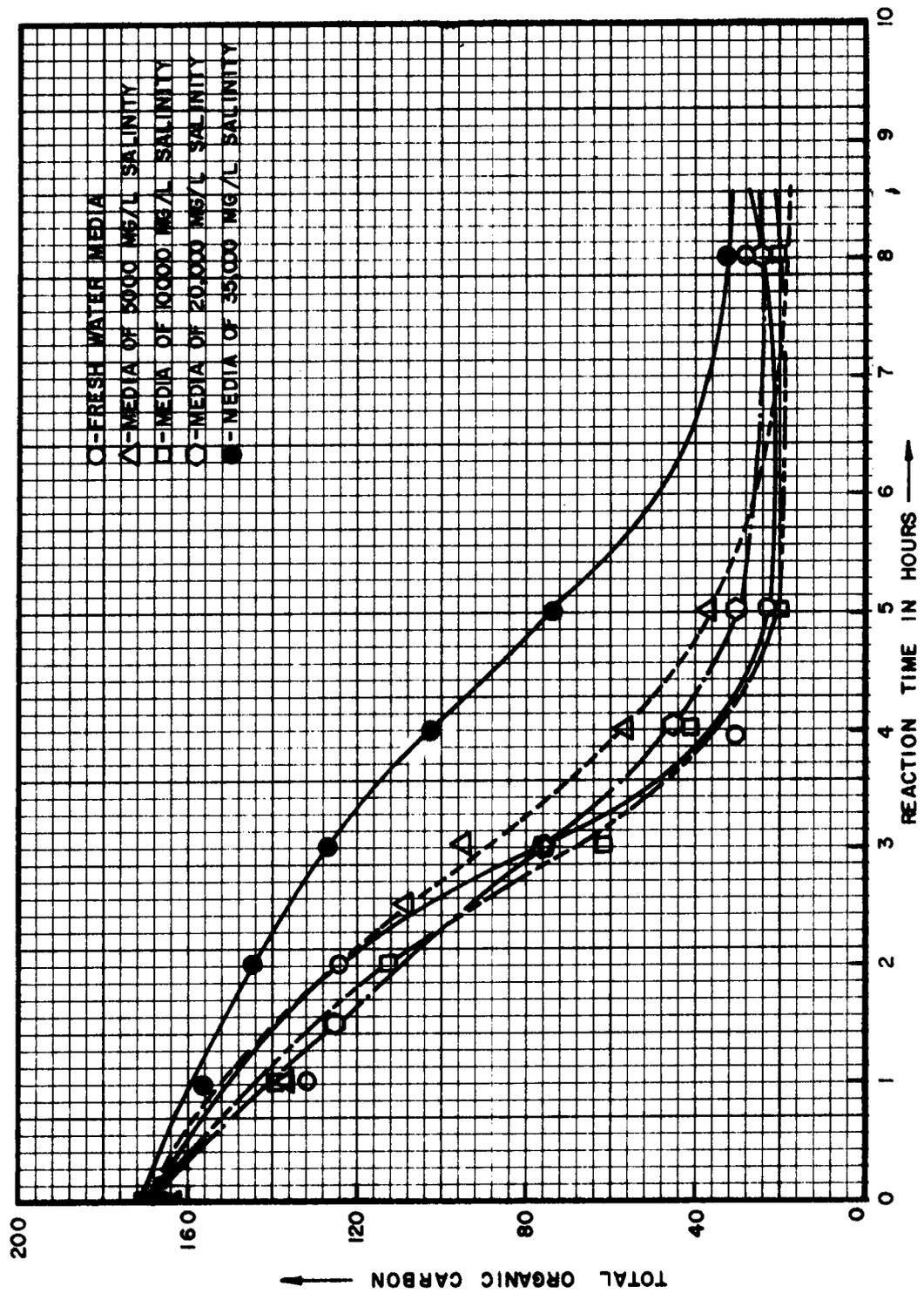


FIGURE 21.- TOTAL ORGANIC CARBON CHANGE WHEN A CULTURE ACCLIMATED TO A SALINITY OF 10,000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

the residual TOC in the systems of this series is lower than that of previous series, could be due to the fact that culture "C" did not lyse as much as culture "B" under shock conditions.

Test Series "D"

Culture "D" was defined earlier as a culture originally acclimated to aliphatic ketones and adapted to a salinity of 20,000 mg/l. In this test series equal portions of culture "D" were subjected to various salinity shocks and the behavior of the various systems was compared with the control system.

Parameters for the various systems are plotted in figures 22, 23, 24, 25, and 26. It is obvious from these figures that the ultimate oxygen rates for the systems subjected to a shock magnitude of - 10,000 mg/l, - 15,000 mg/l, and + 15,000 mg/l did not vary considerably. However, systems shocked with - 10,000 mg/l and - 15,000 mg/l demonstrated a higher oxygen uptake per unit substrate removed than the control systems, while the system shocked with fresh water (shock magnitude of - 20,000 mg/l) demonstrated a very low oxygen uptake rate due to a drastic decrease in the organic removal rate.

The biomass concentration increase was not affected significantly by salinity shocks having magnitude ranges of - 15,000 mg/l to + 15,000 mg/l. Systems shocked with fresh water (shock magnitude of - 20,000 mg/l) suffered a slight loss in the biomass concentration

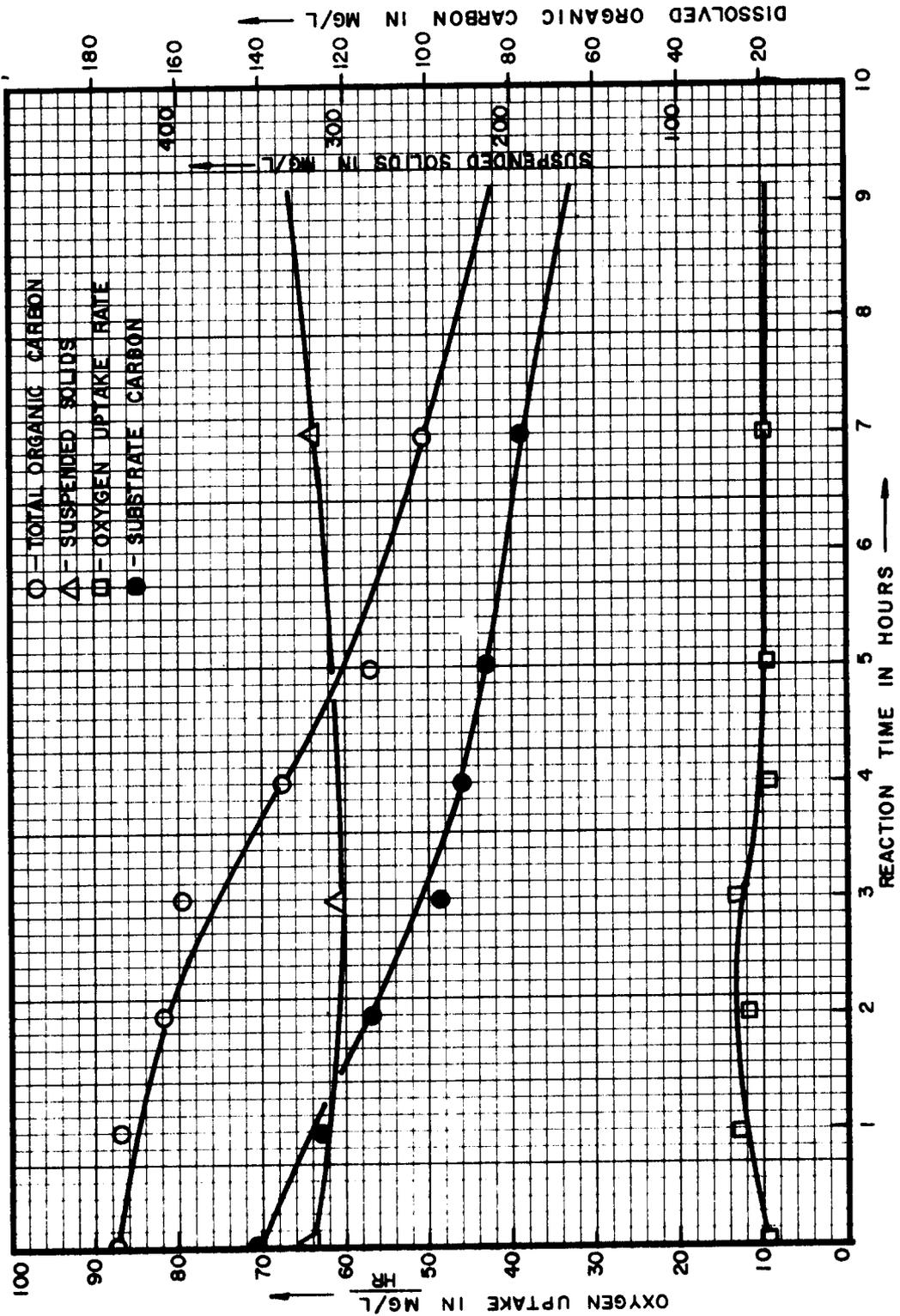


FIGURE 22.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 20,000 MG/L AND SHOCKED WITH FRESH WATER.

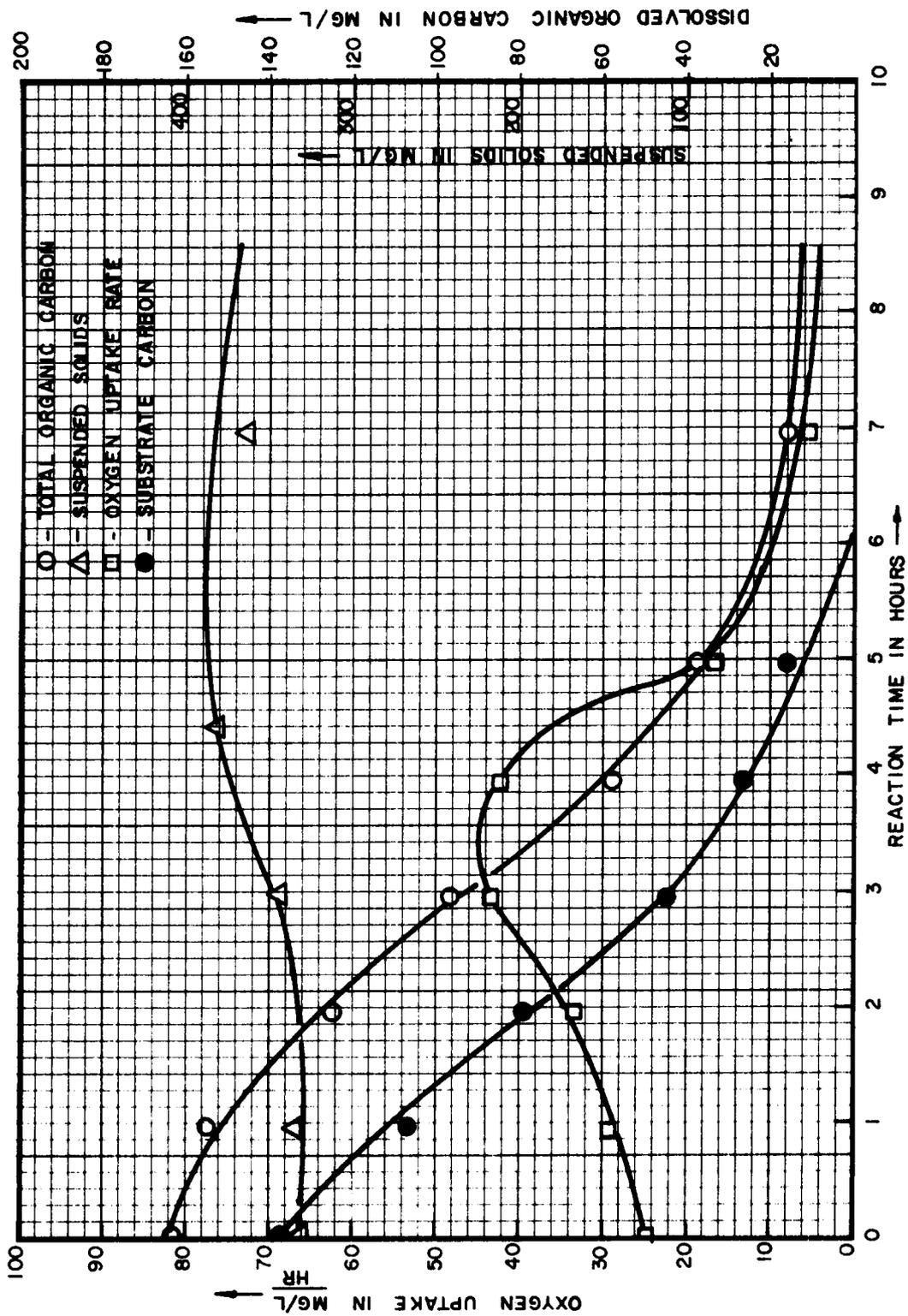


FIGURE 23.— SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 20,000 MG/L AND SHOCKED WITH A SALINITY OF 5000 MG/L.

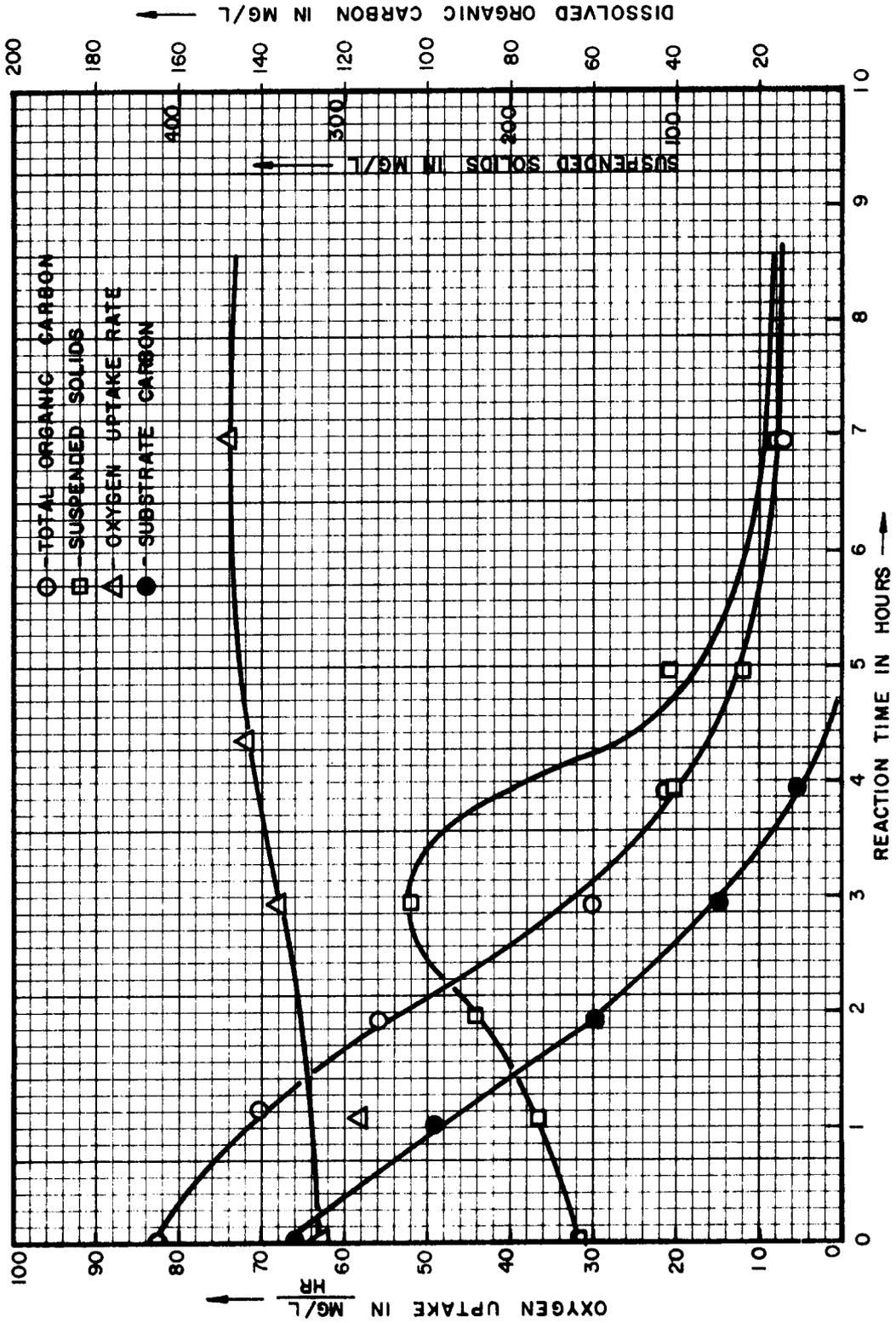


FIGURE 24.-SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 20,000 MG/L AND SHOCKED WITH A SALINITY OF 10,000 MG/L.

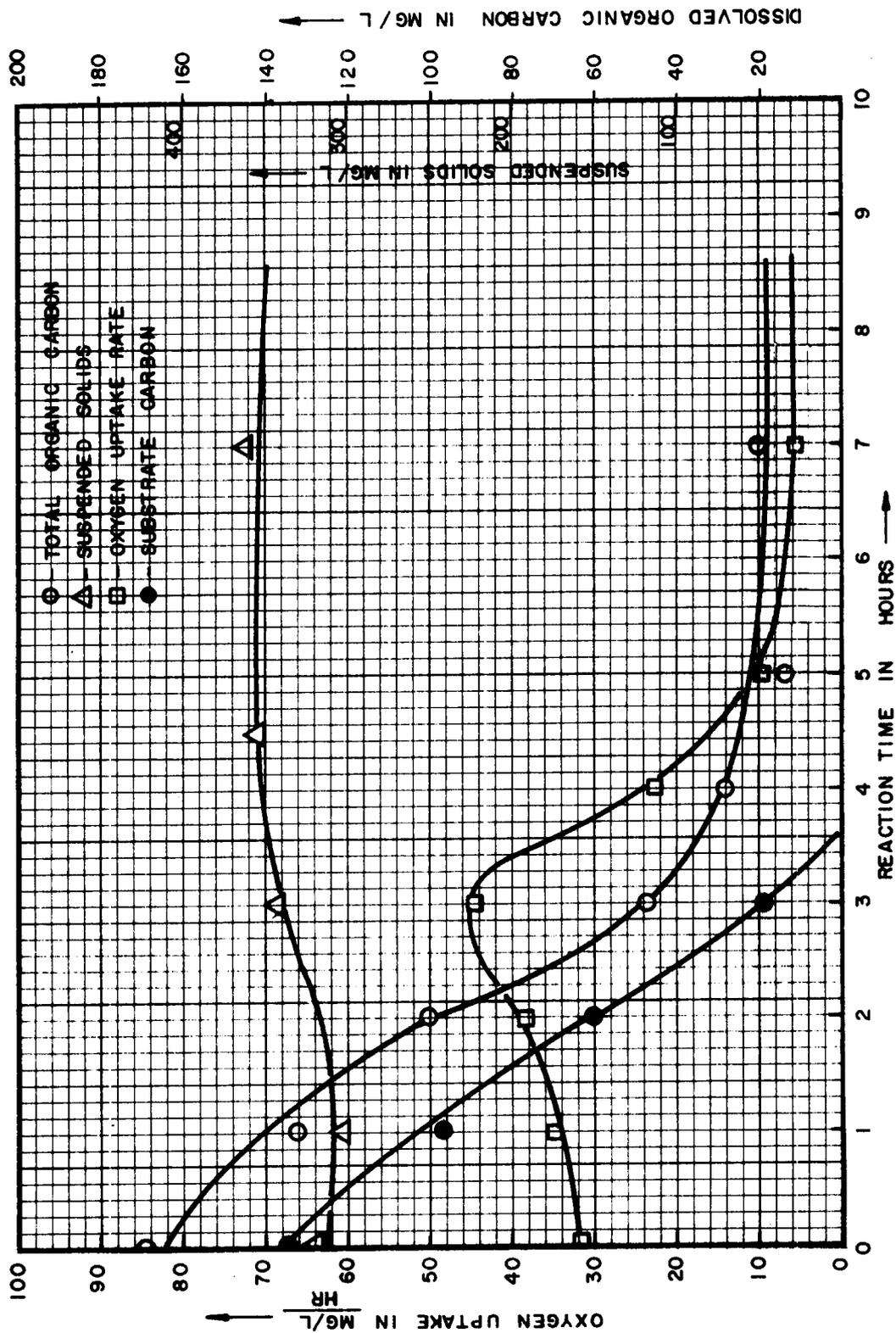


FIGURE 25.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED AND TESTED IN A SALINITY OF 20,000 MG/L.

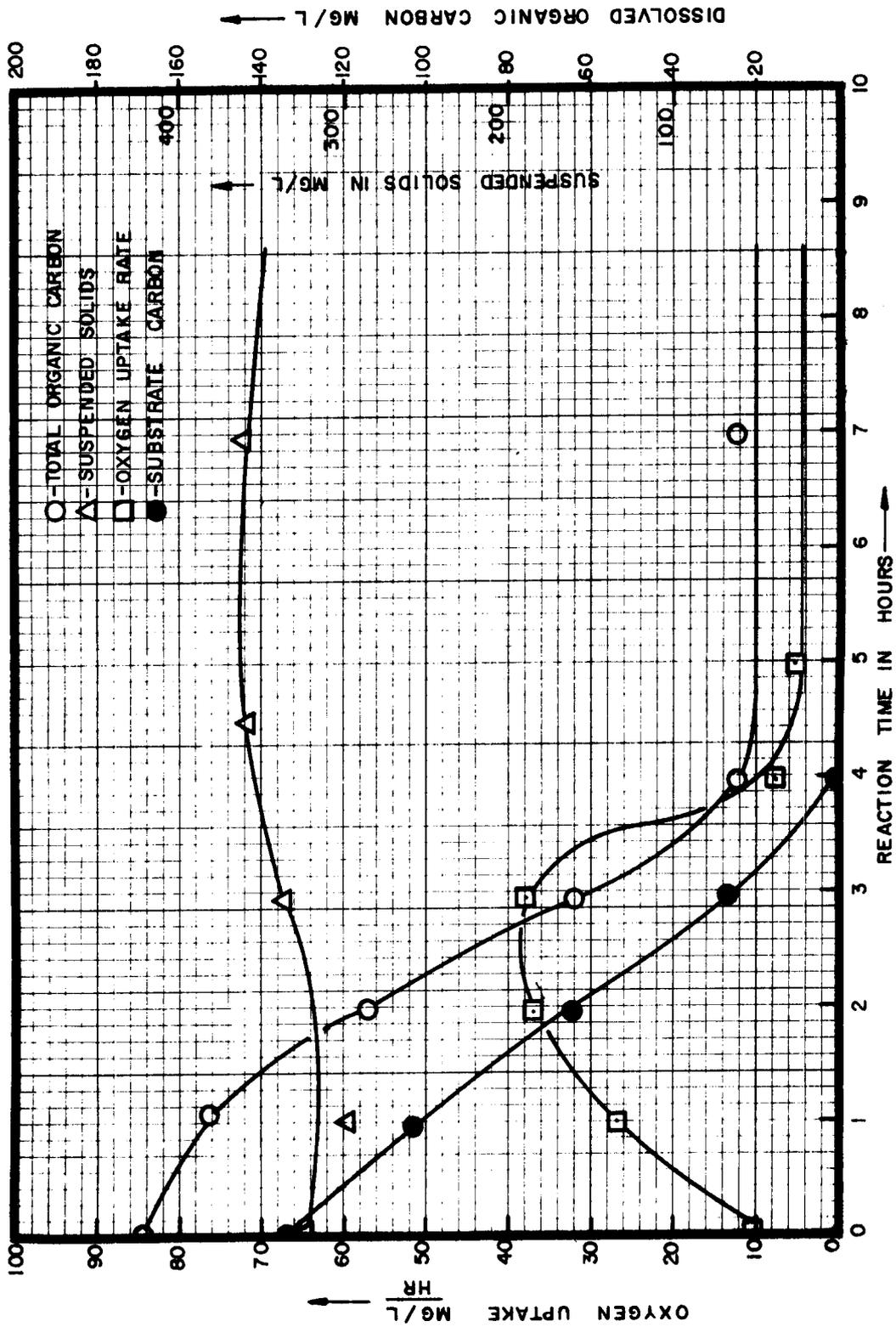


FIGURE 26.-SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 20,000 MG/L AND SHOCKED WITH A SALINITY OF 35,000 MG/L.

during the first three hours of reaction; however, the system recovered its original biomass concentration but never did gain any substantial increase.

The substrate carbon concentration versus time for the five systems are simultaneously plotted in figure 27. The comparison of the five graphs of this figure indicates that a shock magnitude ranging from - 10,000 to + 15,000 mg/l did not significantly affect the substrate removal rates of the shocked systems, while a shock magnitude of - 15,000 mg/l did slow down the removal rate considerably. On the other hand, the substrate carbon removal was suppressed drastically when the system was shocked with fresh water (shock magnitude of - 20,000 mg/l). This system demonstrated a low removal rate during the first three hours of the reaction. The removal rate slowed down as the reaction time progressed but the system failed to remove the substrate at the end of the ten hours allowed for the reaction.

Total organic carbon concentration (TOC) versus time is plotted in figure 28. The removal of the biodegradable portion of the TOC followed the substrate carbon removal pattern. Residual TOC was generally low and it did not vary from system to system. This phenomenon could be due to minimal cell lysing of culture "D". No conclusion could be made on the level of final residual TOC for the system shocked with fresh water because of the incomplete reaction. Also the difference between the TOC and the

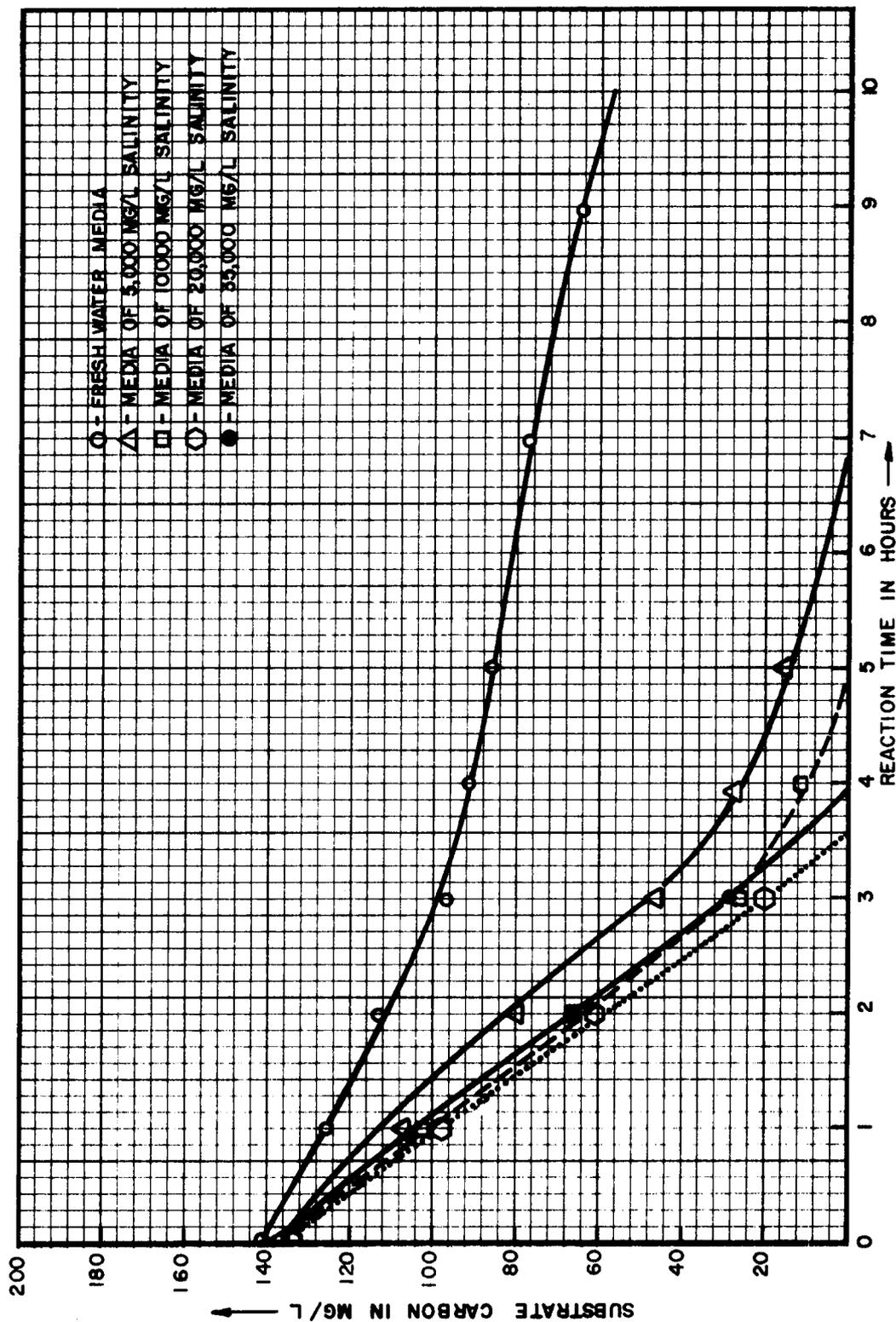


FIGURE 27.-SUBSTRATE CARBON CHANGE WHEN CULTURE ACCLIMATED TO A SALINITY OF 20,000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

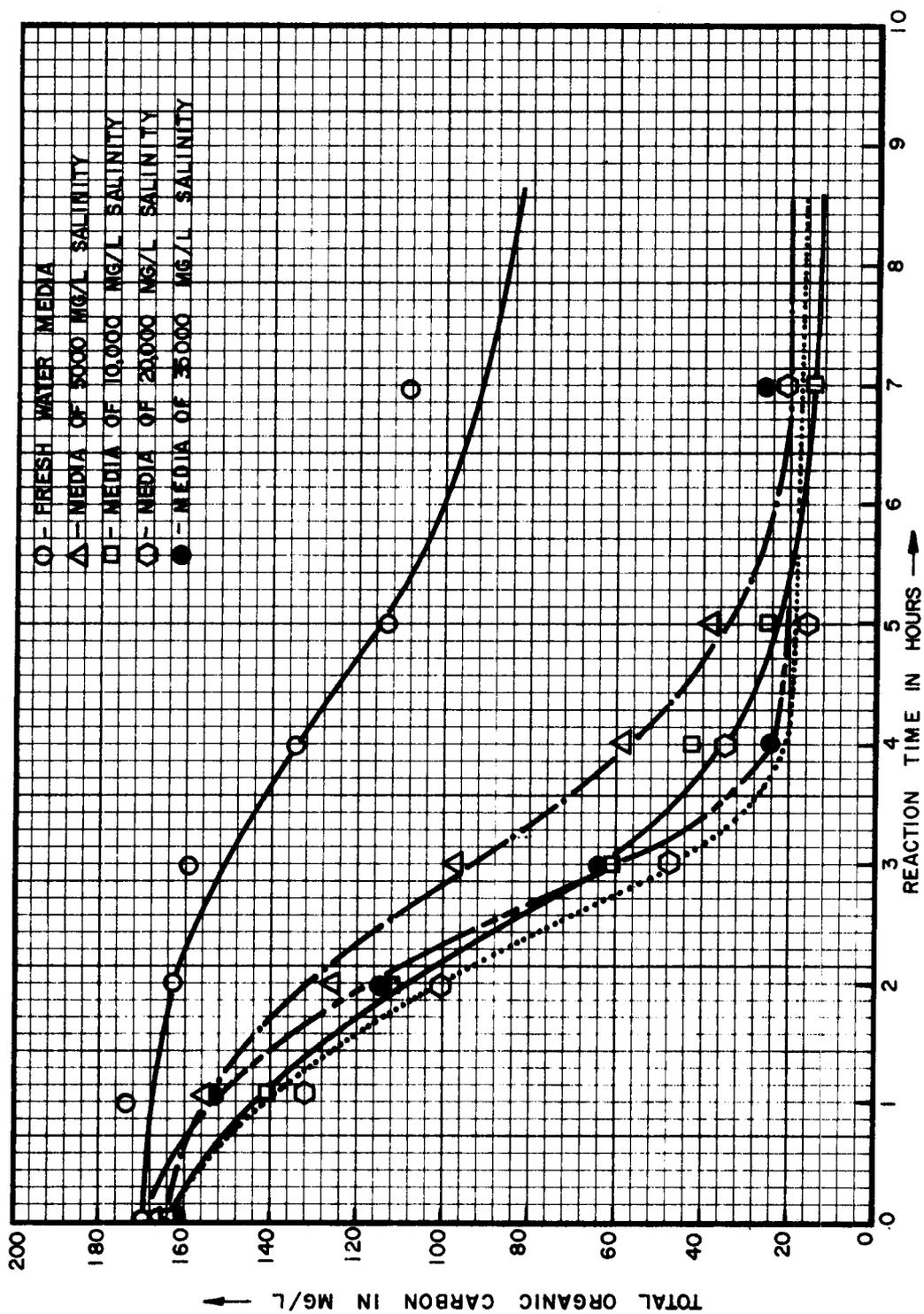


FIGURE 28.- TOTAL ORGANIC CARBON CHANGE WHEN A CULTURE ACCLIMATED TO A SALINITY OF 20,000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

substrate carbon is a rough indication of the low residual TOC in this system.

Test Series "E"

Culture "E" was defined earlier as a culture acclimated to aliphatic ketones and to a salinity of 35,000 mg/l (Normal Sea Water Salinity). The metabolic behavior of this culture under various salinity shock conditions was tested by running five batch tests simultaneously.

The system parameters for the five batch reactors are presented in figures 29, 30, 31, 32, and 33. From these figures it can be concluded that systems subjected to a shock magnitude of - 10,000 mg/l and - 5,000 mg/l demonstrated a higher oxygen uptake per unit substrate removed than the control system, while the system shocked with fresh water demanded a very low level of oxygen uptake. Systems subjected to a shock magnitude of - 20,000 mg/l and - 10,000 mg/l demonstrated a higher ultimate oxygen uptake rate. The biomass concentration in the system shocked with fresh water suffered a substantial loss, while the biomass concentration of the other systems displayed an increase that was inversely proportional to the shock magnitude.

The substrate carbon concentration changes when culture 'E' was shocked with various salinity media are shown in Figure 34. This figure indicates that a salinity shock of 20,000 mg/l (shock magnitude of -15,000 mg/l) did not affect the substrate removal

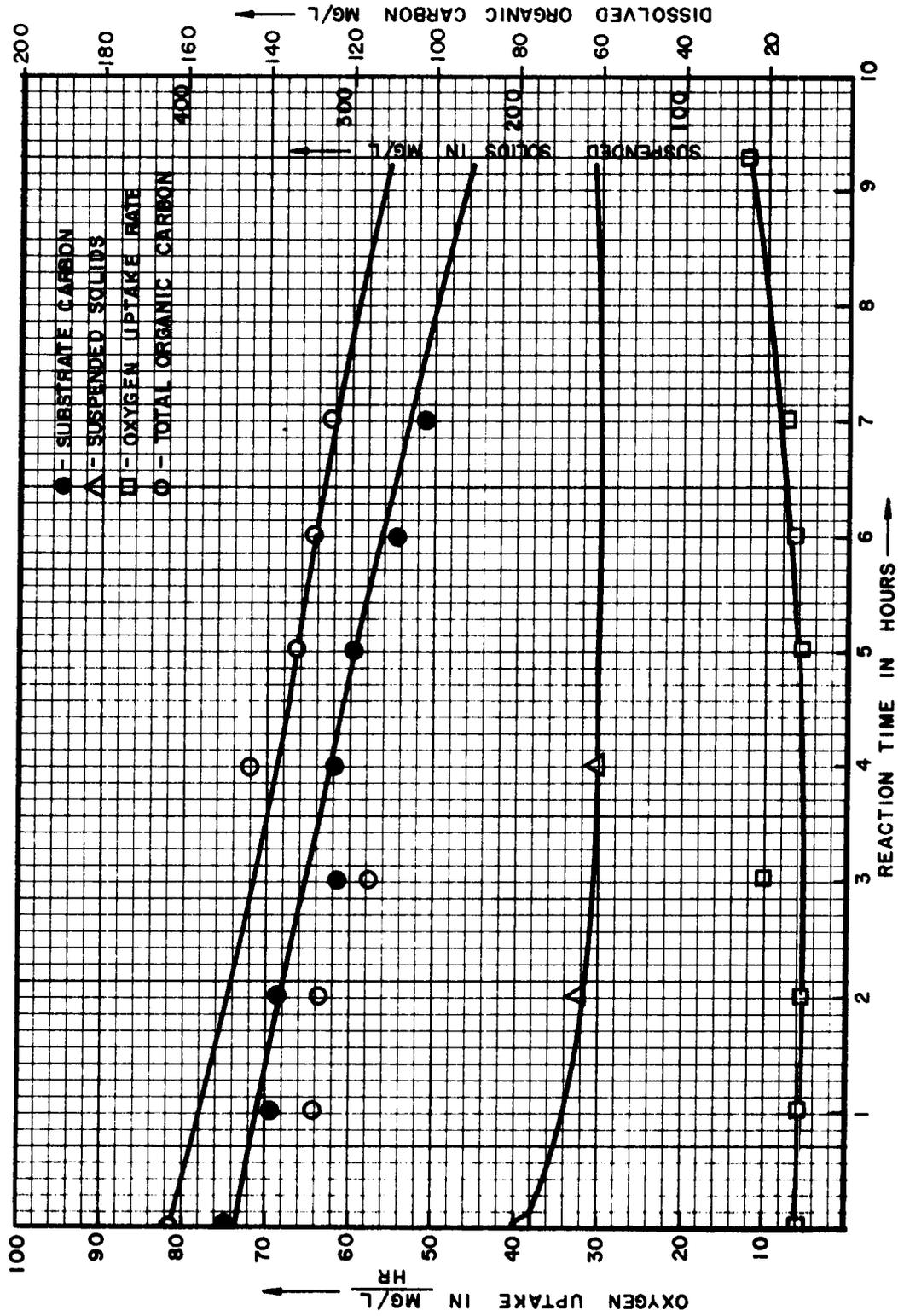


FIGURE 29.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 35,000 MG/L AND SHOCKED WITH FRESH WATER.

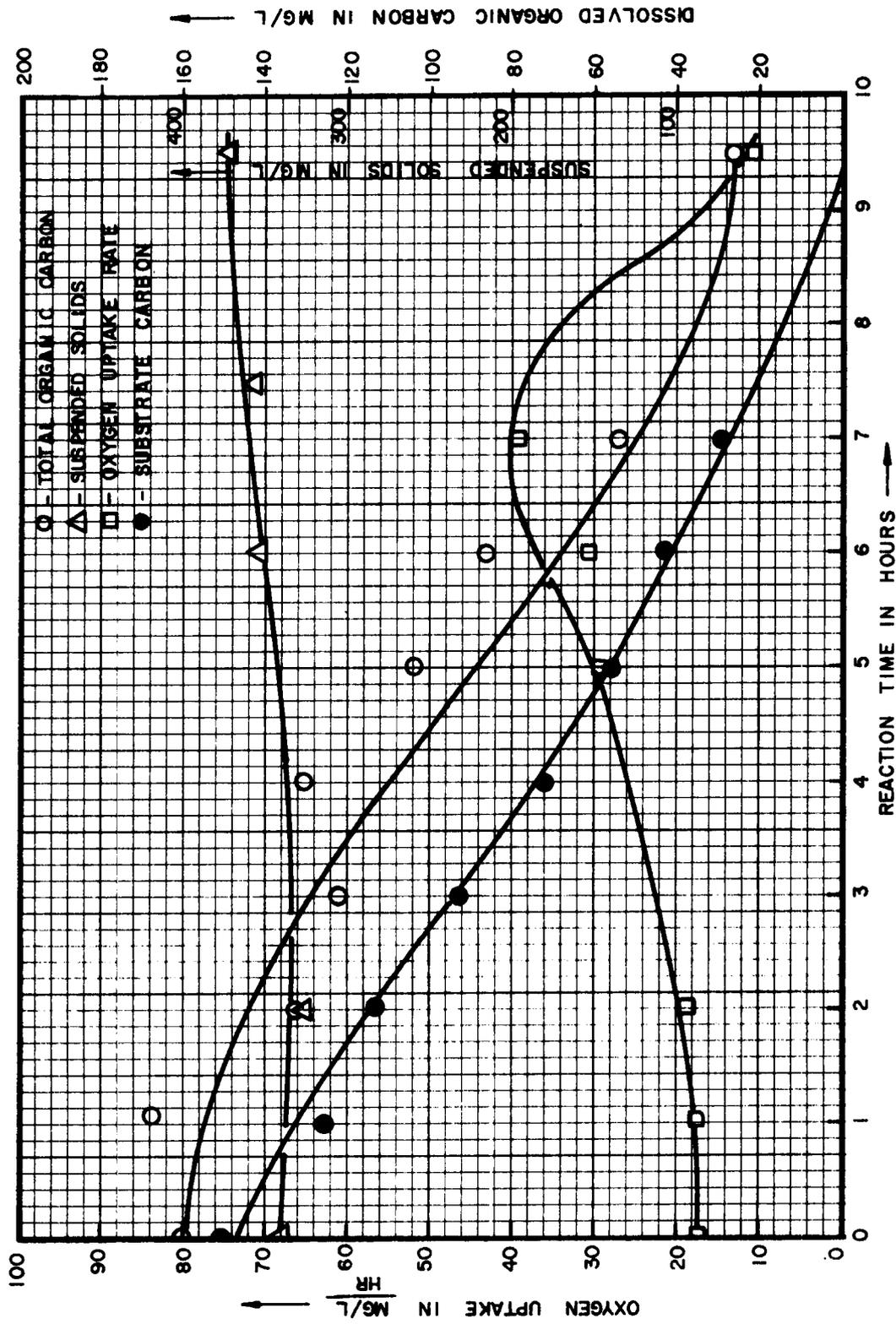


FIGURE 30.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 35,000 MG/L AND SHOCKED WITH A SALINITY OF 5,000 MG/L.

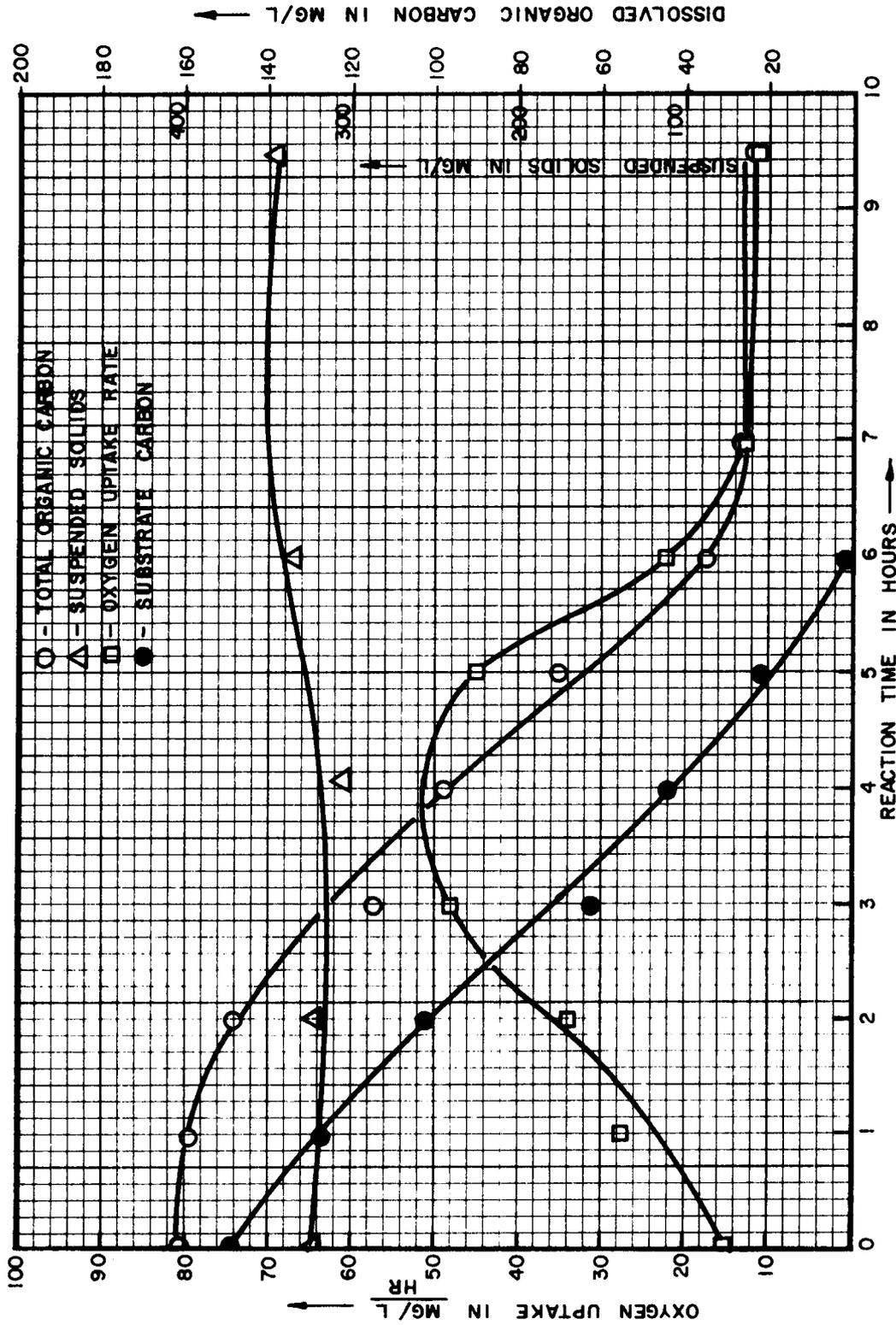


FIGURE 31.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 35,000 MG/L AND SHOCK WITH A SALINITY OF 10,000 MG/L.

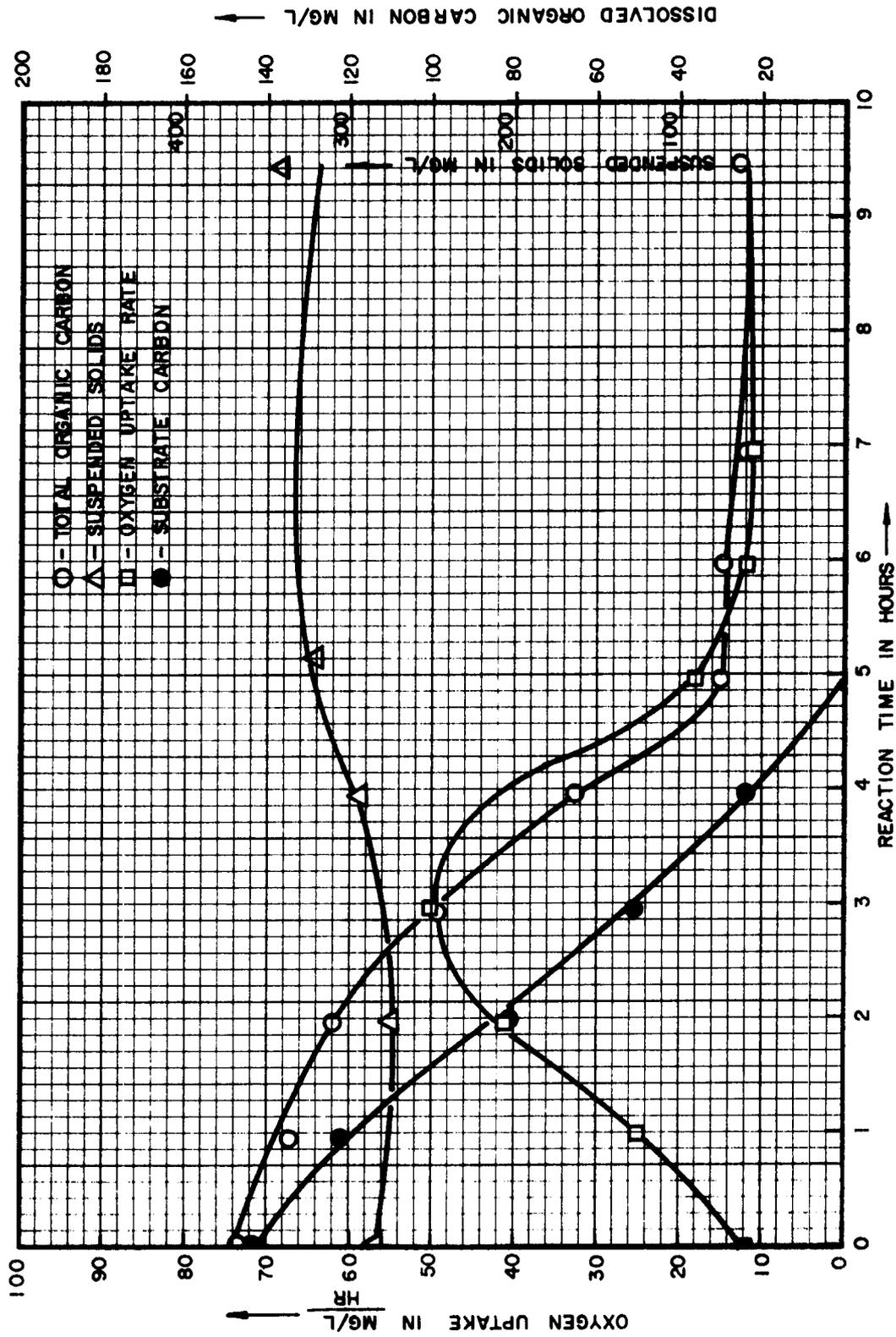


FIGURE 32. - SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED IN A SALINITY OF 35,000 MG/L AND SHOCKED WITH A SALINITY OF 20,000 MG/L.

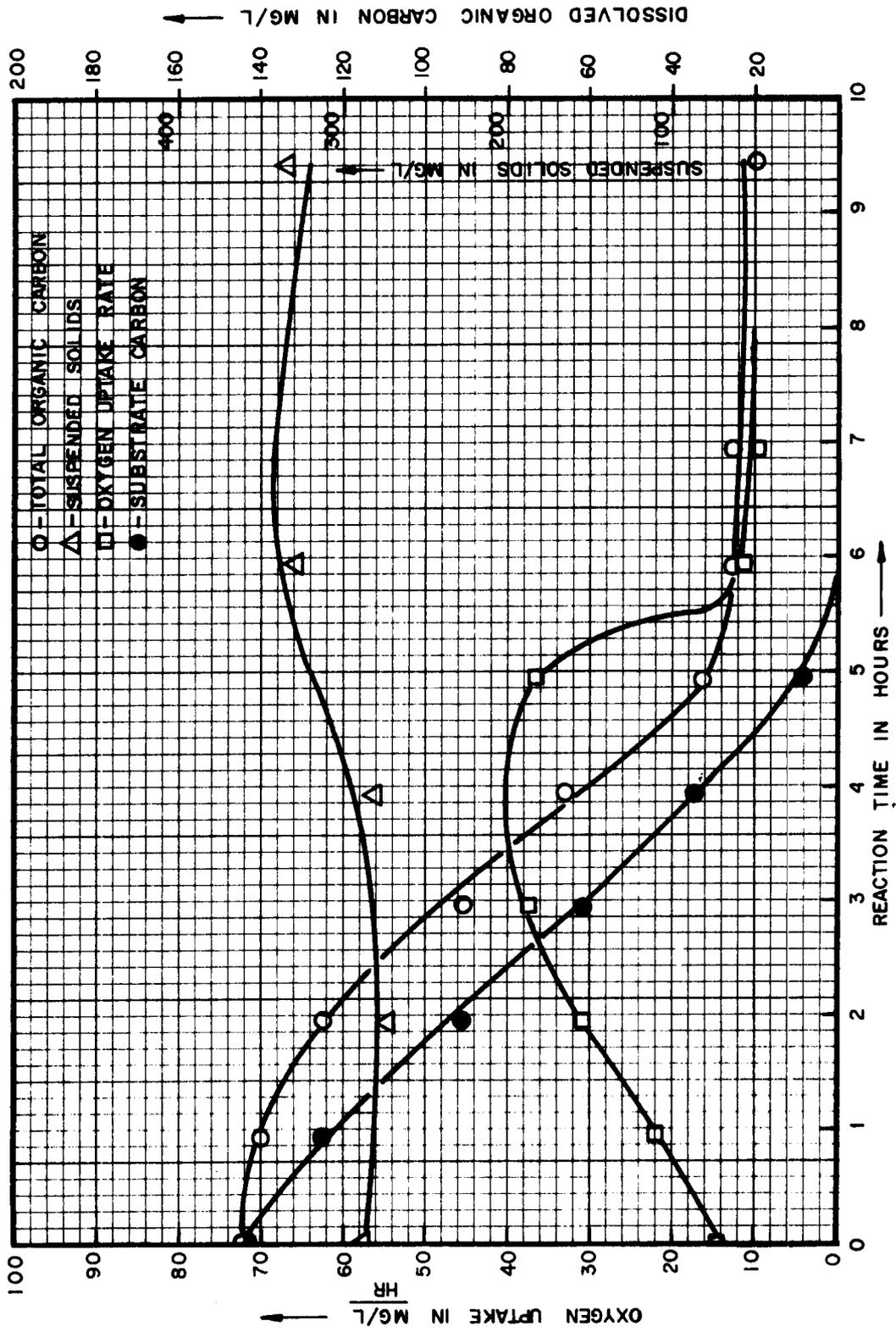


FIGURE 33.- SYSTEM PARAMETERS FOR A CULTURE ESTABLISHED AND TESTED IN A SALINITY OF 35,000 MG/L .

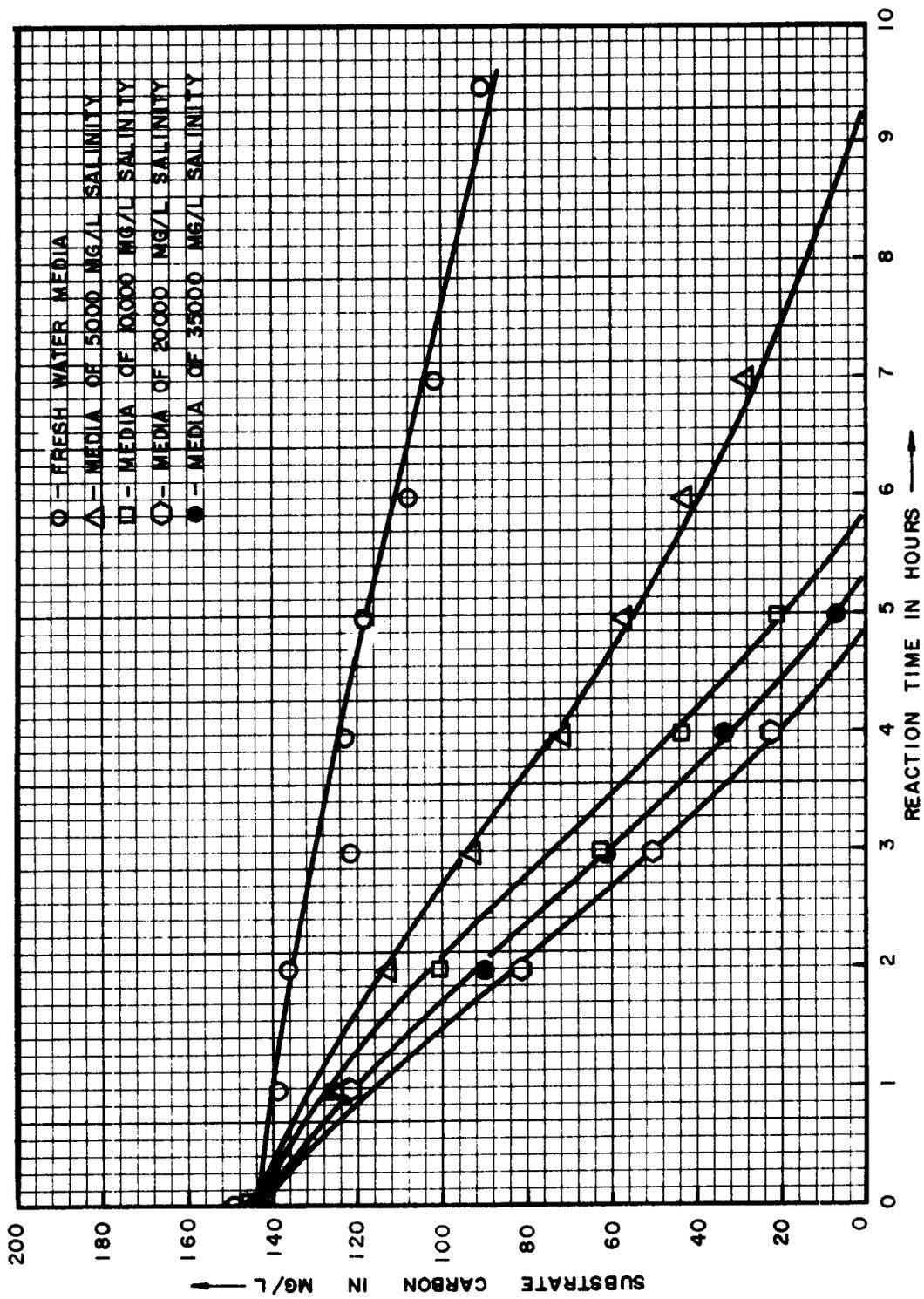


FIGURE 34. - SUBSTRATE CARBON CHANGE WHEN CULTURE ACCLIMATED TO A SALINITY OF 35,000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

rate, while a salinity shock of 10,000 mg/l (shock magnitude of -25,000 mg/l) reduced the substrate removal rate slightly. A salinity shock of 5,000 mg/l (shock magnitude of -30,000 mg/l) greatly reduced the substrate removal rate of the system. The system shocked with fresh water (shock magnitude of -35,000 mg/l) suffered the most drastic reduction of the substrate removal rate.

The total organic carbon (TOC) concentration changes for the five reactors are presented in Figure 35. This figure indicates that the biodegradable portion of TOC followed the pattern of removal of the substrate carbon. The residual TOC was generally low and it did not vary from system to system.

Long Term Effect of Salinity

The long term effect of salinity on the microbial behavior was determined by the comparison of the five control systems was based on substrate carbon unit rate removal of each control system. Comparison of the substrate carbon concentration changes for the five control systems was not possible because of variations in the initial biomass concentration, and because of the fact that each control batch test was run at a different time. The unit removal rate was calculated by dividing the rate of removal of substrate carbon concentration by the corresponding instantaneous biomass concentration.

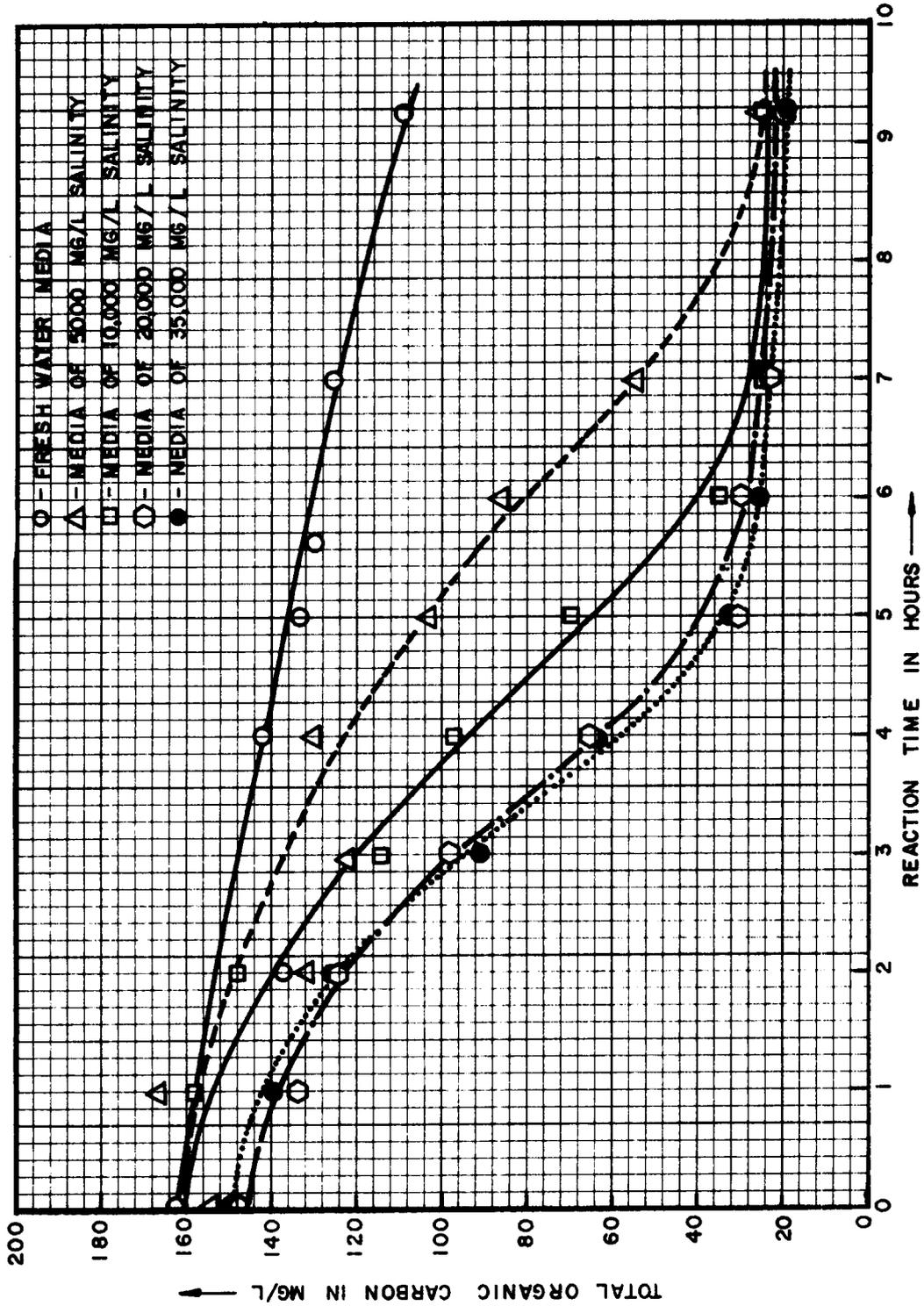


FIGURE 35. - TOTAL ORGANIC CARBON CHANGE WHEN CULTURE ACCLIMATED TO A SALINITY OF 35,000 MG/L IS SHOCKED WITH VARIOUS SALINITY MEDIA.

The unit rate of removal of the five control systems is plotted versus instantaneous substrate carbon in Figure 36. This figure shows that the unit rate of removal was reduced as the salinity level increased. This indicates that an increase in salinity levels will decrease the overall metabolic activity of an acclimated heterogeneous culture. The reason for this decline in the overall activity of the culture will be discussed later in the light of the species diversity of the five cultures tested.

The term unit reaction rate can be expressed in milligrams of substrate carbon per milligrams of biomass per hour or:

$$- \frac{1}{C_b} \cdot \frac{\Delta C_s}{\Delta t}$$

where:

C_b is the biomass concentration in mg/l

C_s is the substrate concentration in milligrams
of substrate carbon per liter

t is the reaction time in hours

In every batch system, the substrate removal is due to either a biological activity or physical stripping or both activities simultaneously. Therefore, the rate of removal of the substrate will be a function of the rate of the two activities.

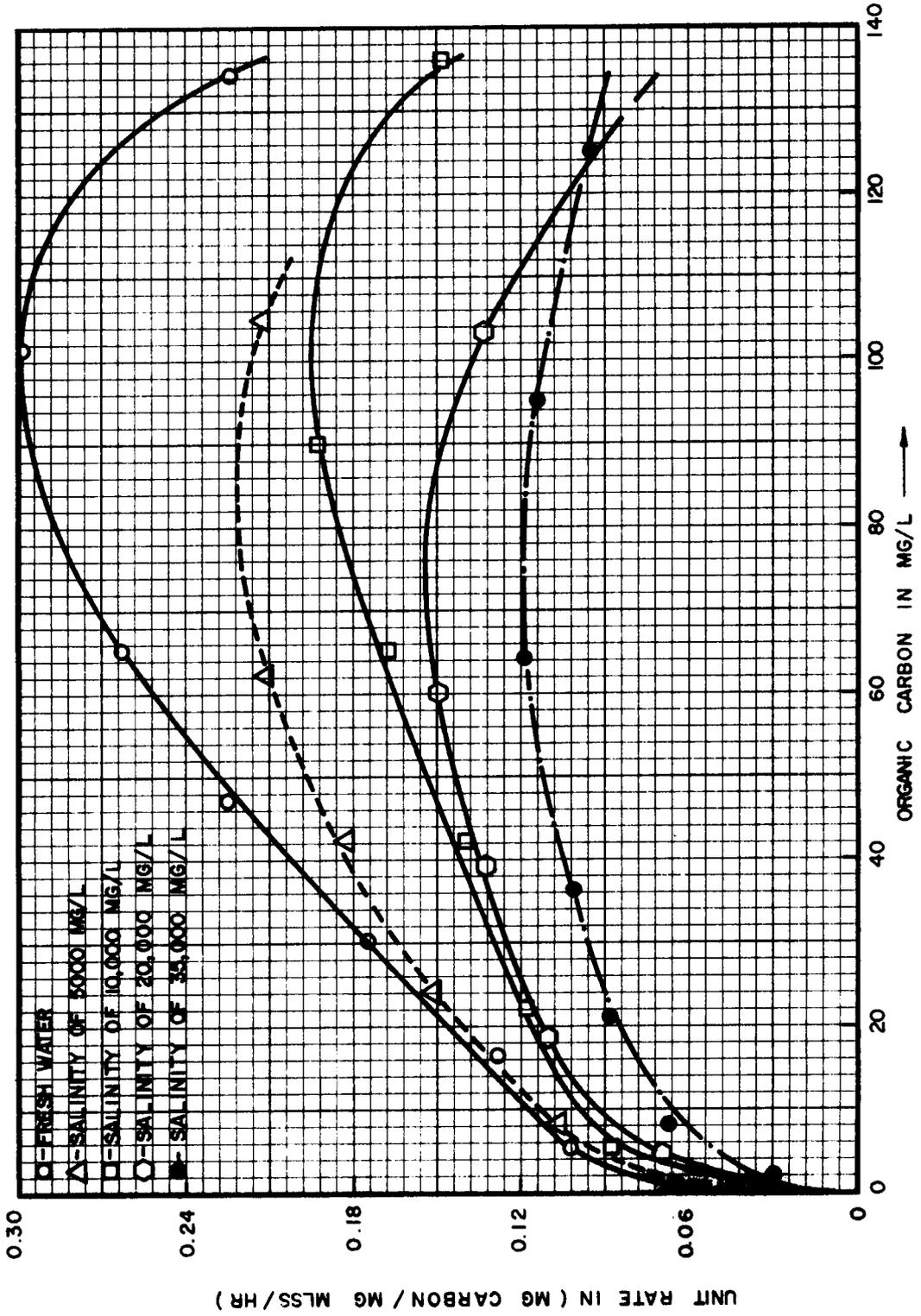


FIGURE 36.-UNIT RATE vs. SUBSTRATE CARBON FOR CULTURES ACCLIMATED AND TESTED IN VARIOUS SALINITIES.

i.e.

$$r_r = f(r_p, r_b)$$

where:

r_r is the overall rate of removal

r_p is the physical rate of removal

and r_b is the biological rate of removal,

$$r_b = K C_s C_b \dots \dots \dots (1)$$

but

$$r_p = r (C_s, T, S, C_b, A \dots \dots \text{etc.})$$

where:

T is the temperature

S is the solubility of the substrate

A is a factor to describe mixing and aeration

Both active and inactive systems were run under identical conditions of mixing, aeration and temperature. C_b was zero for the inactive system and it varied with time for the active system. Conventionally the effect of C_b on r_p has been assumed to be negligible,

therefore,

$$(r_p)_{\text{active}} = (r_p)_{\text{inactive}}$$

The use of a solid free reactor to determine the physical removal of organics was based on this assumption.

In an active system r_r , is the overall rate of removal of the substrate,

$$r_r = r_p + r_b$$

In this study $r_p = 0$ because no stripping was detected. This will simplify the previous equation to:

$$r_r = r_b = r = - \frac{d C_s}{dt} = - \frac{\Delta C_s}{\Delta t}$$

or in other words, the substrate removal is solely due to the microbial activity.

Substitute in Equation 1,

$$- \frac{\Delta C_s}{\Delta t} = K C_s C_b$$

dividing by C_b ,

$$- \frac{1}{C_b} \frac{\Delta C_s}{\Delta t} = K C_s$$

K is determined by plotting

$$- \frac{1}{C_b} \cdot \frac{\Delta C_s}{\Delta t} \text{ versus } C_s$$

and is shown in Figure 36 for each of the test series.

The Effect of Salt Concentrations on the Instrumentation Response

Van Hall and Stenger (22) studied the effects of various salts on the response of the TOC and reported a definite interference by some ions on inorganic carbon measurements however, the effect of NaCl was not reported in this study.

To determine the effect of NaCl on the TOC response, a side test was run by preparing several standards having various salinity levels. The TOC responded identically to standards of similar carbon concentrations regardless of the salinity level of the standards.

Salt accumulation inside the GLC column could create back pressure problems, thus delaying the instruments response. To eliminate this problem a glass tube was installed at the GLC injection port to allow salt precipitation inside the tube. Since samples injected in the gas liquid chromatograph carried through the column as a vapor, it is expected that the presence of inorganic salts in the liquid phase of the samples should exert no influence on the GLC response. This assumption was tested by comparing standards containing different salinity levels and no variation in the GLC response was detected.

One of the major problems in the biomass concentration determination was the variation in salt concentration of the various samples. A thorough washing of filtered samples with tap water

was emphasized during the biomass concentration determination. No increase in TOC of the wash water was detected which implies that excessive washing did not reduce the organic contents of the cell mass.

A side test was performed to determine the effect of salt concentration on the response of the Oxygraph. The dissolved oxygen concentrations of various sea and fresh water samples were measured by the Oxygraph and the Winkler technique simultaneously. The response of the oxygraph to fresh and sea water samples containing dissolved oxygen between zero and six milligrams per liter was almost identical. The response of the oxygraph varied slightly for dissolved oxygen higher than six milligrams per liter. This variation was not important to our study since the dissolved oxygen in the samples tested during the batch tests was always below six milligrams per liter.

Sequence of Removal of Organic Components

In an earlier phase of this study, acetone was used as the sole organic source in some of the batch tests and instantaneous uptake was observed. The system behaved similarly when 2-pentanone and 2-butanone were tested individually. In all test series, 2-butanone and 2-pentanone were removed prior to acetone. Acetone removal started only after the other two ketones concentrations reached low levels. A typical sequence of removal behavior is shown in Figure

37. This phenomenon of interdependent or competitive removal was previously described by Langley (23).

Substrate Stripping

A solids free reactor was used with some of the test series to determine the portion of ketones which was physically stripped from the systems as a result of the mechanical mixing.

The amount of the substrate stripped from the active system during the substrate uptake would be difficult to evaluate precisely due to the difference between active and inert systems, such as the indeterminable effects of biological solids on the retention of solute at the air-fluid interface.

Fortunately, no substrate stripping was detected during the first ten hours of the batch test and all the substrate removal in the active systems was due to the biomass activity. This observation simplified the description of the kinetics of the systems and the calculations of the unit rates.

Sociological Adaptation

Organisms acclimated to acetone in media varying from fresh water to sea water salinity were identified to genera and in two instances to species an approximate quantitative evaluation

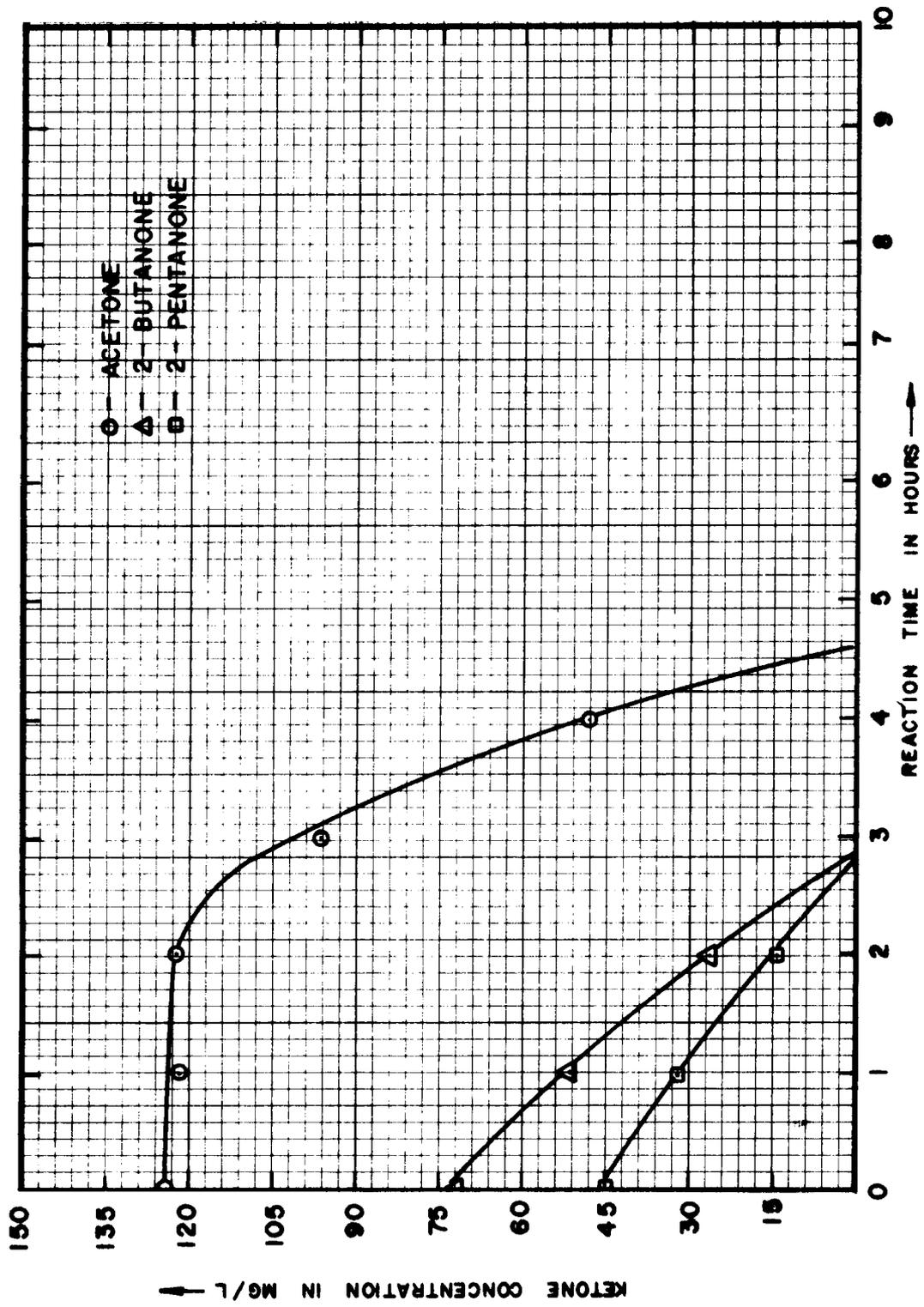


FIGURE 37.- TYPICAL REMOVAL PATTERN OF ACETONE , 2-BUTANONE, AND 2- PENTANONE

was obtained by counting the colonies on each plate and dividing by the appropriate dilution factor. Total numbers determined from different dilutions were averaged to obtain an approximation. As shown in Table II this technique was sufficient since the predominant organisms were obvious. In summary the predominant organisms were:

1. Culture 'A' - (Established in fresh water media)
 - a) Pseudomonas spp.
 - b) Cephalosporium spp.
 - c) Aeromonas formicans
 - d) Baccillus cereus
 - e) Saccharomyceteae
 - f) Aeromonas spp.
 - g) Chlorella spp.
2. Culture 'B' - (Adapted to 10,000 mg/l salinity)
Same social structure as Culture 'A'.
3. Culture 'C' - (Adapted to 10,000 mg/l salinity)
Same social structure as Culture 'A' and 'B'
4. Culture 'D' - (Adapted to 20,000 mg/l salinity)
 - a) Cephalosporium spp.
 - b) Pseudomonas spp.
 - c) Chlorella spp.

TABLE II. - Predominate Organisms in Cells Per Milliliter

ORGANISM	CULTURE				
	A	B	C	D	E
Pseudomonas Gp IV	6×10^5	8×10^5	4×10^6	22×10^6	13×10^6
Pseudomonas Gp IV	5×10^4	5×10^4	13×10^3	-	-
Bacillus cereus	6×10^3	9×10^3	23×10^3	Nil	-
Aeromonas sp.	4×10^3	6×10^3	3×10^3	-	-
Aeromonas formicans	$<10^3$	$<10^3$	Nil	-	-
Cephalosporium	*	*	*	*	*
Saccharomycetaceae	500×10^3	450×10^3	12×10^3	-	-
Chlorella	*	*	*	*	*

* Present, but not countable by this method.

- Not Present.

5. Culture 'E' - (Adapted to 35,000 mg/l salinity)

- a) Cephalosporium spp.
- b) Pseudomonas spp.

This transition from a fresh water system to a sea water system was accompanied by a change in the microbial population; however, two predominant genera, Cephalosporium and Pseudomonas, were common to all salinity levels. Thus, this change was characterized by the retention of the most predominant organisms together with the suppression of the lesser contributing organisms. The elimination of the minor species could be attributed to the sensitivity of these species to a medium of high ionic strength, but it is more likely that the elimination was due to the retardation of the metabolic activity and consequently the reproduction of the species. As shown in Table III the total organic carbon removed per unit biomass produced was increased as the salinity was increased. Or in other words, the cell yeild was decreased as the salinity level was increased. The effect of a reduced reproduction rate on the dominancy of a species is very obvious, especially in a system where a portion of the culture is disposed periodically.

These observations are quite significant in that they lend credence to the notion that the reduced removal rates of substrate at higher salinities was due not to the change in population but to the physiological effects of the increased salinity

TABLE III. - The Effect of Salinity
Acclimation on the Substrate - Biomass Relations.

Culture	Salinity Level	* $\frac{\Delta\text{TOC}}{\Delta C_b}$				** $\frac{\Delta C_b}{\Delta\text{TOC}}$
		run 1	run 2	run 3	av.	
A	0,000	1.33	1.43	1.23	1.33	0.75
B	5,000	1.63	-	1.28	1.45	0.68
C	10,000	1.40	1.36	1.33	1.36	0.73
D	20,000	2.27	3.70	1.13	2.37	0.42
E	35,000	2.85	2.17	-	2.51	0.39

* Substrate removed (As TOC) per unit biomass produced.

** Cells yield coefficient.

on the uptake and metabolism of the substrate by the organisms.

A unicellular type alga, chlorella spp. was present in culture A, B, C and D. It was present in the sampling plastic tube of the reactor containing Culture 'E'. This is an indication that this alga can tolerate 35,000 mg/l salinity but did not survive in the reactor itself because of the light limitation.

General Discussion

There are conflicting viewpoints concerning the functional and structural inhibitory effects of salt concentrations on the metabolic activity of microorganisms. In this study, no attempt was made to analyze and explain the mechanisms of the microbial behavior under various salinity conditions; however, one phenomenon that was studied was cell lysing. With few exceptions, salinity shocks did not affect the magnitude of the TOC level of systems shocked with various salinity levels; however, this is not a firm indication that cell lysing did not occur. Nucleic acids (DNA, RNA), amino acid pools, and other vital cell constituents may lyse without being detected through the TOC measurements because of the limitation of TOC analysis accuracy. Another argument for the absence of cell lysing (using TOC technique) is that the major portion of the culture mass was composed of fungal hyphae which is known for salinity shock resistance because of the thickness of the cell walls. Another possibility that may

have occurred in the systems is the immediate utilization of cell lysate, but the absence of an oxygen uptake response in the concerned systems appears to eliminate this possibility.

The high residual TOC observed in some of the systems could be either unidentified residual intermediates or cell lysate. The former explanation is more likely because of the absence of such high residual TOC for systems shocked with lower salinities where lysing due to osmotic effects is more probable.

The major concern of this study was to determine the behavior of the various systems to provide a more knowledgeable approach to process design. The familiarity with the systems parameters will also give a better understanding of what is occurring in the systems in a natural system subjected to salinity conditions similar to those studied.

The progressive slow down in the unit rate removal for cultures established at higher salinity levels was apparent. This phenomenon has been explained by others to be the result of a population shift due to the increase in the salinity level. In this study a population change was also observed but consisted of the elimination of the minor species only, rather than the establishment of new species. The conclusion that the shift in population is the main reason for the removal rate slow down is not a safe conclusion. Since the predominant groups existed at all salinity levels, the change in the metabolic behavior

of these groups could provide a satisfactory explanation. Further study of the metabolic response of the individual predominant species is essential before any concrete conclusion can be made.

The decrease in the substrate removal rate was proportional to the magnitude of the salinity shock; however, another important factor was the salinity level under which the culture was shocked. For example, a culture was more sensitive to a shock with a fresh water medium than one of higher salinity. In addition, different test series responded differently to the same shock magnitudes.

Industrial effluents discharged into natural systems are sometimes required to meet certain criteria. A 20 ppm BOD₅ is a classical criterion. Such criterion is usually specified without reference to the nature of the relation between the salt concentration of the effluent and the receiving body of water. In fact the BOD₅ that will be exerted on the natural system will not only depend on the organic loading, but also on the nature of the salinity shock. This factor should be considered when setting criteria.

The numerous problems associated with biological treatment of wastes under variable salt concentrations make process and unit design extremely challenging. The fact that the oxygen requirement is a function of both organic loading and salinity shock conditions will add to the complexity of aerator design.

In addition, the effect of salinity on the settling characteristics which were observed in this study may result in adding new considerations to the design of final clarifiers. Configuration and size may differ from the conventional design.

Because these studies were carried out exclusively in batch systems, the effect of variable salinities on the organic and hydraulic loading could not be determined. This information must be obtained from cultures developed in continuous flow reactors.

CHAPTER VI

CONCLUSIONS

From the above mentioned results the following was concluded:

1. A salinity shock of magnitude in the order of $\pm 10,000$ did not affect the metabolic response of heterogeneous cultures considerably.
2. Culture acclimated to fresh water and shocked with sea water showed a 50% reduction in the substrate removal efficiency.
3. Culture acclimated to sea water and shocked with fresh water showed a 86% reduction in substrate removal efficiency.
4. Culture acclimated to 5,000, 10,000, and 20,000 mg/l salinity and shocked with various salinity levels suffered a reduction which varied with both acclimation conditions and shock magnitudes.
5. In general, using similar shock magnitudes, it was observed that the negative shock magnitudes caused a greater reduction in the substrate removal than did the positive shock magnitude.
6. In general, cultures acclimated to high salinity levels demonstrated a higher oxygen uptake per unit substrate removed and sometimes a higher ultimate oxygen uptake rate than the corresponding control system.

7. Generally, the biomass increase of cultures established in fresh water or in low salinities were higher than the biomass increase of cultures established in high salinity levels.
8. The transition from a fresh water system to a sea water system was accompanied by a change in the microbial population. This change characterized by the elimination of the minor species and the retention of the two pre-dominant genera, Cephalosporium and Pseudomonas.

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APPENDIX
DATA TABULATION

TEST SERIES 'A'

TABLE IV. - Oxygen Uptake Rate (mg/l/hr.)

S** \ T*	0	1	2	3	4	5	6.5	9
0000	34	49	53	62	73	16	12	10
5,000	32	36	48	57	63	22	24	15
10,000	28	33	39	50	63	48	11	11
20,000	17	25	33	36	43	46	12	—
35,000	10	15	18	26	21	16	22	13

TABLE V. - Biomass Concentration (mg/l)

S** \ T*	0	1	2	3	4	5	6.5	9
00000	132	134	123	155	182	270	230	245
5,000	150	170	185	197	203	250	215	270
10,000	168	177	172	222	205	220	210	210
20,000	157	—	170	—	168	—	—	203
35,000	148	—	140	—	143	177	—	185

* Time in hours

** Salinity in mg/l

TABLE VI. - Total Organic Carbon Concentration (mg/l)

S**\T*	0	1	2	3	4	5	6.5	9
0000	176	164	157	122	74	22	18	16
5,000	184	170	154	119	85	57	38	24
10,000	178	176	163	132	90	65	32	28
20,000	182	178	157	137	120	81	41	36
35,000	171	167	153	157	141	119	109	72

TABLE VII. - Substrate Carbon Concentration (mg/l)

S**\T*	0	1	2	3	4	5	6.5
0000	158	130	106	64	32	2	0
5,000	157	133	108	72	37	7	0
10,000	159	140	117	77	42	10	0
20,000	162	139	119	83	58	23	0
35,000	183	140	130	118	106	78	49

* Time in hours

** Salinity in mg/l

TEST SERIES 'B'

TABLE VIII. - Oxygen Uptake Rate (mg/l/hr)

S**\T*	0	1	2	3	4	5	6
0000	21	45	41	48	32	21	11
5,000	23	35	38	54	34	25	11
10,000	22	30	36	50	35	11	13
20,000	17	25	34	44	37	16	16
35,000	13	14	18	27	25	12	10

TABLE IX. - Biomass Concentration (mg/l)

S**\T*	0	2	4	5	6	9
0000	205	206	288	304	280	268
5,000	218	220	266	298	304	265
10,000	200	202	244	266	256	268
20,000	200	200	242	---	284	252
35,000	165	164	192	---	238	208

* Time in hours

** Salinity in mg/l

TABLE X. - Total Organic Carbon Concentration (mg/l/hr)

S**\T*	0	1	2	3	3.5	4	4.5	5	6	9
0000	160	136	106	55	36	17	17	14	3	6
5,000	146	149	97	48	43	9	14	7	6	9
10,000	159	147	104	69	48	21	24	9	4	--
20,000	163	150	107	65	60	38	28	22	7	16
35,000	158	121	124	106	98	69	49	49	38	26

TABLE XI. - Substrate Carbon Concentration (mg/l)

S**\T*	0	1	2	2.5	3	3.5	4	4.5
0000	140	109	64	42	24	10	0	0
5,000	141	105	62	41	24	7	0	0
10,000	148	104	69	44	26	10	0	0
20,000	144	110	78	57	29	21	4	0
35,000	150	---	83	67	55	45	26	15

* Time in hours

** Salinity in mg/l

TEST SERIES 'C'

TABLE XII. - Oxygen Uptake Rate (mg/l/hr)

S**\T*	0	1	2	3	4	5	8
0,000	32	40	49	49	22	14	8
5,000	27	27	46	40	49	21	-
10,000	29	31	35	41	19	12	8
20,000	17	28	30	35	44	12	7
35,000	7	12	13	18	22	28	5

TABLE XIII. - Biomass Concentration (mg/l)

S**\T*	0	3	5	8
0,000	279	345	395	376
5,000	300	330	368	362
10,000	256	326	374	360
20,000	268	298	380	387
35,000	287	---	307	319

* Time in hours

** Salinity in mg/l

TABLE XIV. - Total Organic Carbon Concentration

S** \ T*	0	1	1.5	2	2.5	3	4	5	8
0000	168	130	---	124	---	75	30	22	27
5,000	168	138	---	---	108	95	56	35	--
10,000	171	138	---	113	---	61	40	21	20
20,000	163	---	125	---	58	76	45	30	25
35,000	169	155	---	144	---	126	102	72	32

TABLE XV. - Substrate Carbon Concentration

S** \ T*	0	1	2	2.5	3	4	5	8
0000	164	128	81	57	36	2	0	0
5,000	165	136	86	71	46	8	0	0
10,000	166	136	89	64	40	2	0	0
20,000	163	137	96	76	54	16	1	0
35,000	167	143	118	104	76	59	29	0

* Time in hours

** Salinity in mg/l

TEST SERIES 'D'

TABLE XVI. - Oxygen Uptake Rate (ma/l/hr)

S** \ T*	1	2	3	4	5	7
0000	13	11	13	9	9	9
5,000	29	33	44	43	17	6
10,000	37	44	52	20	21	9
20,000	35	38	44	22	10	6
35,000	27	37	38	8	6	-

TABLE XVII. - Biomass Concentration (mg/l)

S** \ T*	0	1	3	4.5	7
0000	322	---	304	---	318
5,000	330	336	344	384	364
10,000	316	292	340	260	370
20,000	320	302	342	356	364
35,000	326	300	338	358	362

* Time in hours

** Salinity in mg/l

TABLE XVIII. - Total Organic Carbon Concentration (mg/l)

S**\T*	0	1	2	3	4	5	7
0000	160	174	163	159	134	113	100
5,000	164	155	125	97	58	38	16
10,000	167	141	112	60	42	24	14
20,000	169	132	100	47	28	15	20
35,000	169	153	114	64	24	---	25

TABLE XIX. - Substrate Carbon Concentration (mg/l)

S**\T*	0	1	2	3	4	5	7
0000	141	125	113	96	91	86	76
5,000	137	107	79	45	27	16	0
10,000	136	99	59	30	10	0	0
20,000	135	97	60	18	0	0	0
35,000	135	103	64	28	0	0	0

* Time in hours

** Salinity in mg/l

TEST SERIES 'E'

TABLE XX. - Oxygen Uptake Rate (mg/l/hr)

S**T*	1	2	3	5	6	7	9.25	9.5
0000	5	5	10	6	7	8	12	--
5,000	17	18	29	30	31	39	--	11
10,000	27	34	48	45	22	18	--	11
20,000	25	41	50	18	12	11	--	--
35,000	22	31	37	36	12	10	--	--

TABLE XXI. - Biomass Concentration (mg/l)

S**T*	0	2	4	5.33	6	7	9.25	9.5
0000	193	160	150	---	---	---	155	---
5,000	345	326	---	---	354	356	---	370
10,000	323	320	304	---	332	---	---	342
20,000	318	274	292	320	---	---	---	344
35,000	304	272	280	---	328	---	---	335

* Time in hours

** Salinity in mg/l

TABLE XXII. - Total Organic Carbon Concentration (mg/l)

S**T*	0	1	2	3	4	5	6	7	9.25	9.5
0000	163	128	127	115	144	133	129	125	110	--
5,000	155	167	132	122	130	103	86	54	---	25
10,000	155	158	148	144	97	69	34	25	---	24
20,000	148	134	124	98	65	30	29	22	---	25
35,000	145	140	125	90	65	32	25	24	---	19

TABLE XXIII. - Substrate Carbon Concentration (mg/l)

S**T*	0	1	2	3	4	5	7	9.25
0000	151	139	137	122	123	120	108	92
5,000	150	125	113	93	72	57	44	0
10,000	149	126	101	61	44	21	0	0
20,000	142	122	82	50	24	0	0	0
35,000	145	125	91	61	34	7	0	0

* Time in hours

** Salinity in mg/l

