COMPLETION REPORT

RESPONSE OF COMPLETELY MIXED ACTIVATED SLUDGE SYSTEMS TO CHANGES IN THE ENVIRONMENT

A-043 WRRI-OKLAHOMA, OWRRI-USDI
July 1, 1972 - June 30, 1975

PREFACE

In accord with the joint goal of the Oklahoma Water Resources Research Institute, the Center for Water Research in Engineering, and the Bioenvironmental division of the School of Civil Engineering of Oklahoma State University, this research project was designed with the aim of gaining better understanding of factors controlling water quality and reuse. The report deals with description of microbial kinetics when continuous steady growth is subjected to perturbance caused by a change in the external environment.

The accompanying document represents a detailed report on the completion of the project, and embodies pertinent information suggested in OWRT reporting guidelines. The report consists of a summary and conclusion followed by sections entitled: introduction, methodology, results and discussion. Detailed information may be found in the six papers which form the appendices.

It is emphasized that the work of this project did not involve obtaining the experimental data reported in the major papers referenced in this report. The experiments were conducted some years before undertaking the current analytical project. The individuals who share authorship with the principal investigator on four of the papers in the appendices (T. K. George and P. Krishnan) were research assistants

to the PI. They were active a number of years ago in the experimental phases of the work and in making some preliminary analysis of the data.

A. F. Gaudy, Jr., Principal Investigator
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ABSTRACT

This project was undertaken to analyze experimental results previously obtained by the principal investigator dealing with the response of continuously cultured heterogeneous microbial populations, e.g., activated sludge, to step changes in feed flow rate, feed concentration, pH and temperature. The response was characterized by determining pre-shock, transient stage and final "steady state" conditions of effluent substrate and cell concentration and biochemical composition of the biomass. Both once-through and cell recycle systems were studied and effect of specific growth rate and mean hydraulic retention time on response were assessed. In general, an increased retention time, cell recycle and biomass concentration lessened the amount of substrate leakage during the transient stage, whereas, a decreased specific growth rate alleviated disruption of plant efficiency. The report contains an appendix consisting of six papers on these types of shock loading.

KEY WORDS

activated sludge - shock loading - temperature shock - pH shock - hydraulic shock - quantitative shock - effluent quality - step change.

SUMMARY AND CONCLUSIONS

This report includes analyses of experimental results pertaining to the response of mixed microbial populations (activated sludge) to environmental perturbances (shock loads) consisting of step changes in inflow rate (hydraulic shock loads) pH, temperature and concentration of carbon source in the feed stock (quantitative shock loads).

All experiments were accomplished in completely mixed continuous flow reactors. In each case the "steady state" condition in the pre-shock state was defined, the shock administered and the transient response observed until the system approached the new "steady state". The response was characterized with respect to biomass concentration and biochemical composition (protein, carbohydrate, nucleic acid) and effluent quality (total organic carbon, i.e., chemical oxygen demand, COD and specific analysis for carbohydrate, the anthrone test).

Common factors affecting response for all the shock loads were: mean hydraulic retention time, $\bar{t}$, specific growth rate, μ, cell recycle and biomass concentration as well as the rate and mode of administration of the shock load. All of the above factors are interrelated and response to environmental stress cannot be predicted solely on the basis of any one of them. In general, in increased $\bar{t}$, cell recycle and biomass concentration attenuated the amount of substrate leakage during the transient stage, whereas, a decreased μ alleviated disruption of plant efficiency. The results are analyzed in regard to biochemical and ecological response as well as kinetic definition of response patterns. Major articles on these types of shock are included in the appendices to the report.

Regarding engineering guidelines for limits of ability of activated sludge plants to handle shock loading without serious biochemical disruption, it is concluded that with plants operating with mean hydraulic retention times usually employed, i.e., 5-8 hours, an activated sludge process can be expected to successfully withstand a three-fold increase in incoming substrate concentration of organic material to which it is acclimated. The process can be expected to withstand a two-fold hydraulic shock load. Based upon the results herein reported, it is estimated that a system can be protected against significant substrate leakage as well as pH induced predominance of filamentous organisms by control of pH to ± 1 unit from neutrality. Systems operating at reasonably moderate temperatures, e.g., ± 25^{o} C can more readily accommodate increase than decrease in temperature.

INTRODUCTION

The response of microbial populations to change in the environment is a broad basic and applied interest. In the environmental pollution control field heterogeneous or mixed populations are employed and their response to changes in the environment are naturally somewhat more difficult to characterize because of the inherent instability caused by the diversity of species in the population. It is well recognized that such systems will vary in overall biochemical and physical characteristics even under constant environmental conditions. When various types of environmental shocks are administered the situation becomes more complicated and prediction of mechanistic and kinetic response offers a rather exciting and important investigative challenge which has occupied a considerable portion of the principle investigator's research effort down through the years. Some of the problems and the investigational approaches have been recently discussed by the author at an NSF seminar session. The seminar presentation was prepared during the conduct of the present research investigation and formed a portion of the present effort to draw portions of the principal investigator's overall research program into a body of fundamental concepts applicable to the design and operational control of biological purification processes. It is therefore appended to this report and the reader is referred thereto for a more detailed presentation of the overall aims and goals and line of attack which the principal investigator has taken in studying and comparing the behavior of heterogeneous populations under both steady and unsteady conditions.

The current project had one overriding objective. It was considered essential to place before the scrutiny of the investigative and practicing field in the environmental pollution control area, as well as those in the applied microbiology and fermentation areas, various experimental results of the principal investigator and his student co-workers on a variety of environmental shocks. Down through the years the experimental program has been extremely productive of useful data. Although a highly active publication schedule has always been maintained it was quite impossible to maintain the publication schedule at the more rapid pace of the experimental program and it would have been totally unwise and not in keeping with sound investigative principles to have called a moratorium on the experimentation simply in order to keep up with a publication schedule. Thus over the years the principal investigator has amassed a tremendous body of data relative to environmental shock which has yet to be published. It was felt by the author that a separate research project would be ideal for analysis and preparation of some of these materials for publication and the current project was thus submitted. The vital need for such a project was emphasized because of the accelerated move on the part of the regulatory agencies for establishing maximum instantaneous effluent concentrations. Whereas before, average monthly data were thought to be acceptable, the measure of treatment plant efficiency in the future will include not only average data but monthly, weekly or daily maximum concentrations. Thus it can be seen that the reliable and steady delivery of high quality effluent from secondary biological treatment plants for sewage and industrial wastes will become increasingly important. Since in field installations the inputs to these plants are not highly controlled the internal response of the plant as these inputs vary becomes a vital matter for investigation and control. It was hoped

at the beginning of this project that the principal investigator would be able to "catch up" on the majority of unpublished experiments regarding environmental stress or shock loadings. While a great deal of data was analyzed during the conduct of the project and while a considerable amount of these data has been presented for the scrutiny of the field (see appendices), it was not possible to finish complete analysis and reporting of all of the massive results which the PI has. The present report covers analysis of significant investigations in regard to four extremely important types of environmental change or shock load. These are hydraulic shock loads, shock loads consisting of changes in pH, changes in temperature and shock loads consisting of a change in the concentration of incoming carbon source, i.e., the so-called quantitative shock load. The author has been very active in studies of another type of shock load, one consisting of a change in the type of carbon source administered to the reactor system, i.e., the so-called qualitative shock load. These aspects are not covered in the current report.

It should be emphasized that the current project did not involve the conduct of any experimental work. All of the experiments to be described and discussed in this report were conducted several years prior to the initiation of the current project. The materials and methods section which follows provides a brief on the methodology and experimental equipment employed. Detailed discussion of the methodology is provided in the papers appended to this report and in references listed in the literature cited.

METHODOLOGY

The general experimental procedures, analyses performed and laboratory pilot plant reactors were the same as those employed for many other studies reported from the author's laboratory. In all of the work herein to be presented and in many of the studies which have been previously reported a purposeful effort has been made to conduct the entire investigational program as one large series of related experiments. Thus it has always been possible for the PI to correlate and interrelate various investigations in his overall research program. That is to say, insofar as is possible each succeeding experiment has always been related to and helped interpret and explain the results of some previous experiment. Thus the data have had accumulative as well as individual value. One of the ways in which this has been accomplished is through the use of a synthetic waste of known composition, reactors which were hydraulically defined as completely mixed stirred reactors, multiple analyses for various parameters, etc. The type of laboratory growth reactors employed is shown in Figures 1 and 2. When cell recycle to the aeration tank was not practiced (operation as a once-through process) the experimental setup was as shown in Figure 1. However for studies in which cell recycle was employed an additional aeration tank (return sludge aerator, see Figure 2) was employed. The underflow from the clarifier was periodically channeled to the return sludge aerator from which it was pumped to the aeration tank. The hydraulic flow rate, recycle ratio, temperature, pH and feed concentration were subjected to rather precise experimental control and these were varied in known and controlled ways in administering the various environmental changes. Various activated sludges, heterogeneous microbial

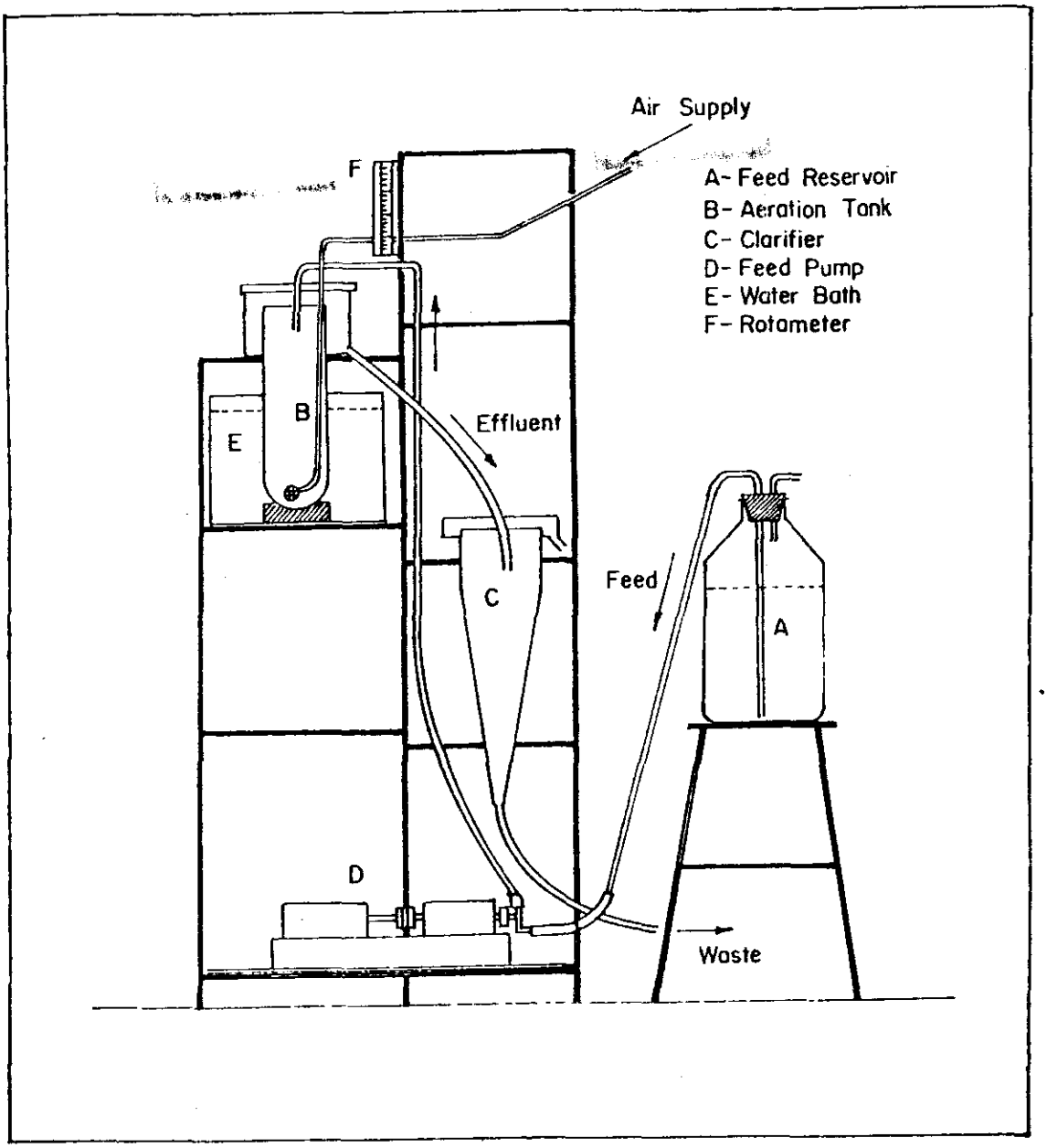

Figure 1. Side view of completely mixed continuous flow reactor employed in laboratory studies of "once-through" systems. A, feed reservoir; B, aeration tank; C, clarifier, D, feed pump; E, water bath; F, rotameter.

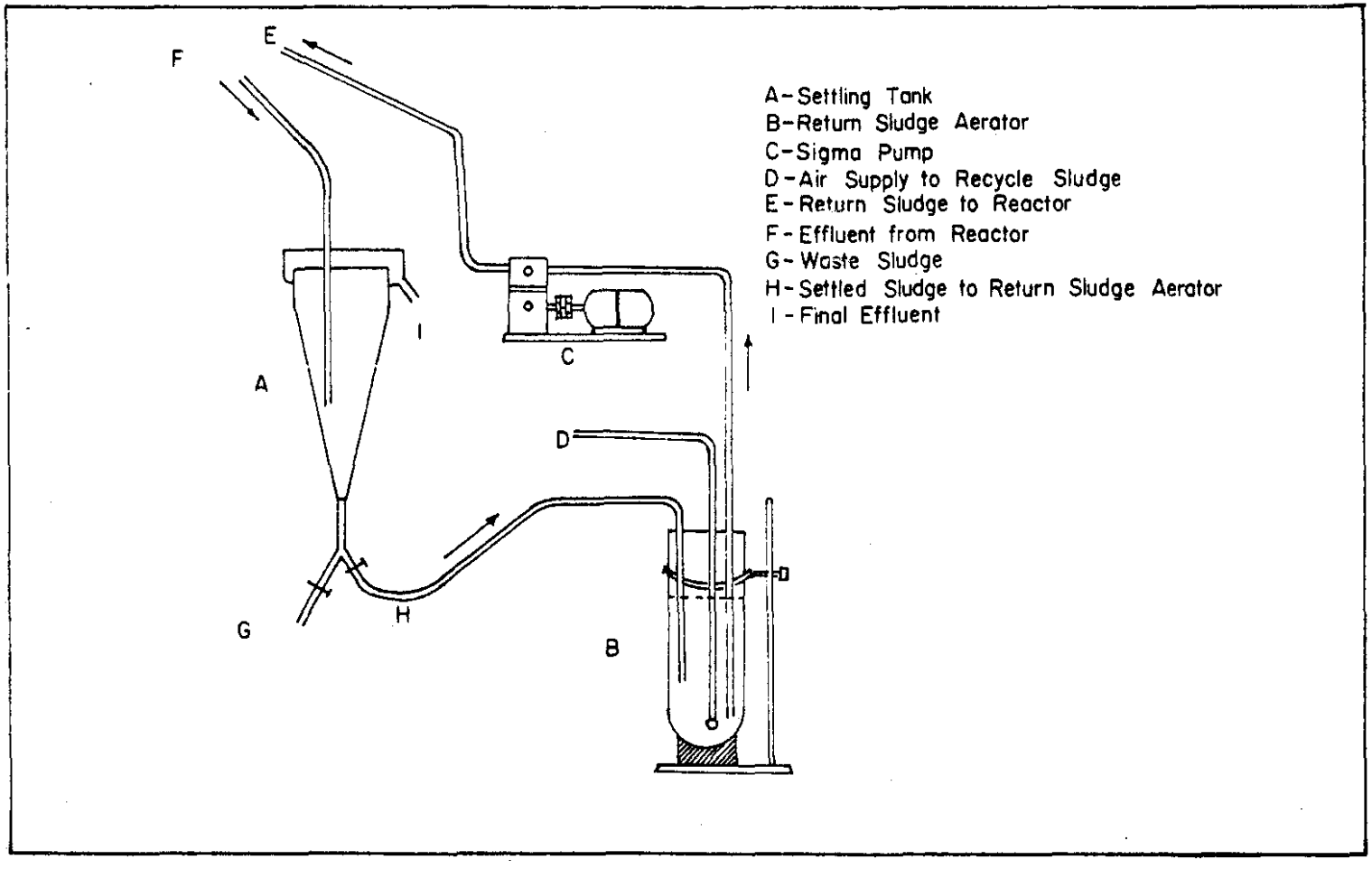

Figure 2. Side view of sludge recycle system added to pilot plant shown in Figure 1 for studies employing cell feedback to reactor.

populations, were developed from initial sewage seeds obtained from the municipal treatment plant in Stillwater, Oklahoma. The system performance was measured by determining the concentration of biological solids in the reactor and in the effluent and in the cell recycle line. Biological solids were always measured by the membrane filter technique. Biochemical composition of the cells were measured in many experiments; cell composition was assayed by running the protein and carbohydrate content of the cells and in some cases RNA, DNA and lipid content. The effluent quality was measured by assessing its chemical oxygen demand (COD) and by analyses for specific components in the effluent (e.g. anthrone and glucostat analyses were employed when the substrate was glucose, etc.). Also in some cases liquid gas chromatography was used to analyze for various metabolic intermediates and/or end products which were determined to be in the effluent on the basis of the difference between the concentration of COD and concentration of specific substrate. Various auxiliary experiments were conducted using cells harvested from the reactor; however these will not be discussed in the current report except where they are particularly critical to analysis of the response of these continuous flow growth reactors.

RESULTS AND DISCUSSION

Hydraulic Shock

One of the types of shock loading which commonly occurs in the field is that type of environmental perturbance which consists of a change in the rate at which the wastewater flows into the reactor. This is commonly called a hydraulic shock loading and since the volume of the reactor is fixed the unit flow rate (commonly called the dilution rate, D), which is the ratio of the incoming flow, F, to the volume of the reactor, V, is subjected to change. It can be shown that the dilution rate, D, is related to and exerts control over the specific biological growth rate, μ. Thus it can be seen that a hydraulic shock precipitates a biological response. Naturally a successful response is one which permits the system to accommodate to the change without excessive deterioration of effluent quality during the period of adaptation or acclimation necessitated by the imposition of the environmental perturbance. Hydraulic shock can be defined simply as a change in the dilution rate with no change whatsoever in the concentration of substrate, S_i. On the other hand hydraulic shock is often accompanied by a considerable change in S_i. Both situations are certainly worthy of experimental investigation. It is also important to emphasize that the immediate past growth history of the biomass can be expected to have some effect on the response to a change in the dilution rate. Thus in the experimental studies to be presented herein it was essential to choose some base line dilution rate. In the studies reported here a dilution rate of 0.125 hrs^{-1} was chosen since this is a dilution rate commonly employed in the field. In the studies shown in the following figures the synthetic waste consisted of a minimal salts medium with

glucose as substrate; temperature was maintained at 25° C and pH was maintained at 7.0.

The next few figures will show some of the responses when dilution rate was changed with no change whatsoever in substrate concentration. Figure 3 shows the response when the dilution rate was decreased fourfold. One would not expect a forced slowdown in growth rate to precipitate a really deleterious transient response with respect to biological solids and substrate removal. It is clear, however, from the figure, that the system did undergo some disturbance. When the dilution rate was halved (see Fig. 4) again there was a slight perturbance, but one certainly would conclude from these results that a decrease in dilution rate while substrate concentration in the feed remains constant, can be accommodated fairly well by the system. The type of hydraulic shock which causes more concern is one consisting of an increase in dilution rate. In Figure 5 it is seen that a doubling in dilution rate caused only a slight transient increase in soluble organic material in the effluent. For a more severe shock, from a D of 0.125 to 0.313 hrs^{-1} (see Fig. 6), the initial pattern of response was the same as in the previous figure. However, the initial dropoff in biological solids concentration and initial rise in effluent COD (see T-COD) was more severe and recovery of initial conditions in X and S was not complete. The lower biological solids concentration and increased effluent COD suggests that at the new dilution rate, the system was beginning to follow its normal dilute-out curve. This surmise is substantiated by noting the initial and final steady state conditions with respect to S and X when an even greater hydraulic shock was applied, i.e., from 0.125 to 0.375 hrs^{-1} (see Fig. 7). The same pattern of response with

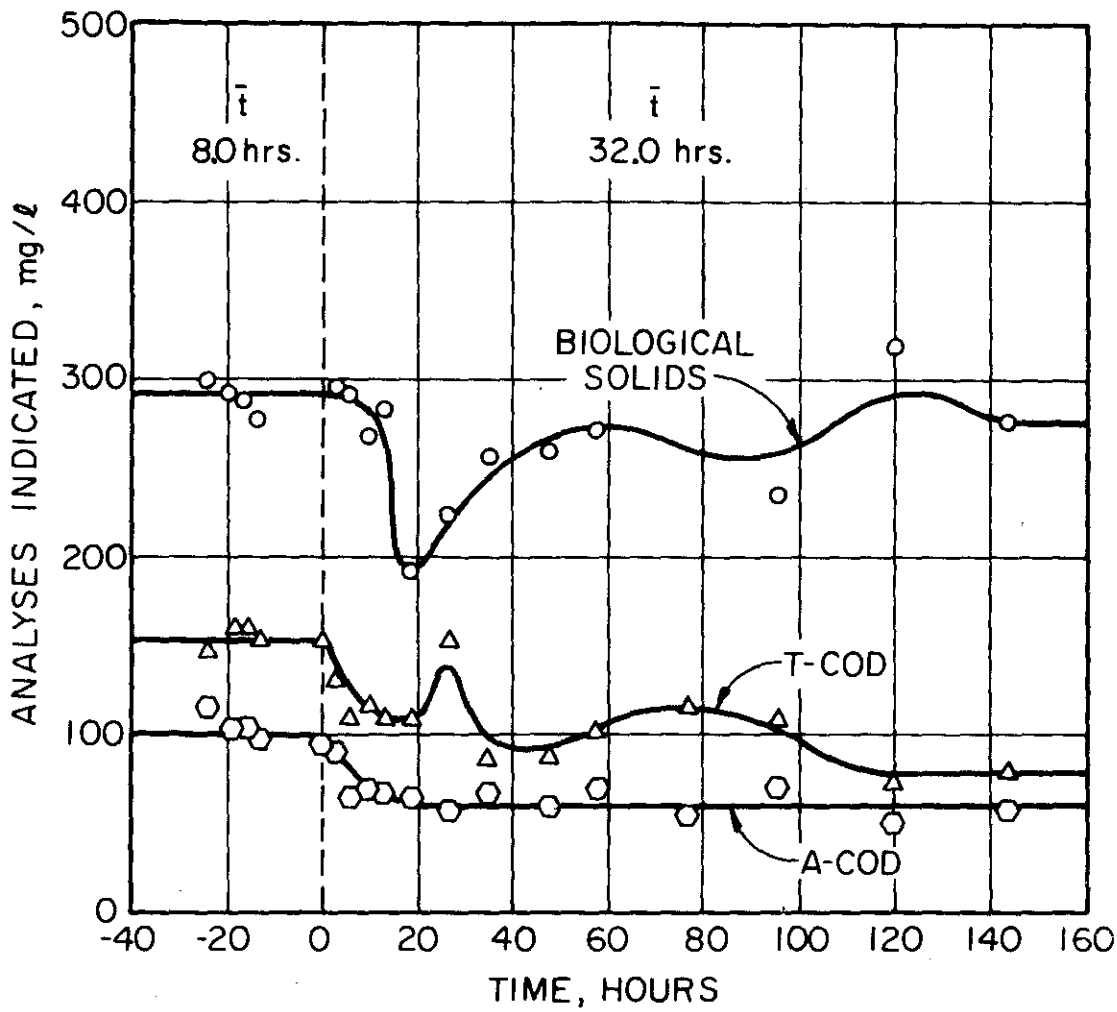

Figure 3. Response to decrease in dilution rate from 0.125 hrs^{-1} to 0.031 hrs^{-1}; S_i = 1000 mg/l glucose; T-COD is total chemical oxygen demand; A-COD is total carbohydrate (anthrone-reactive material) calculated as COD.

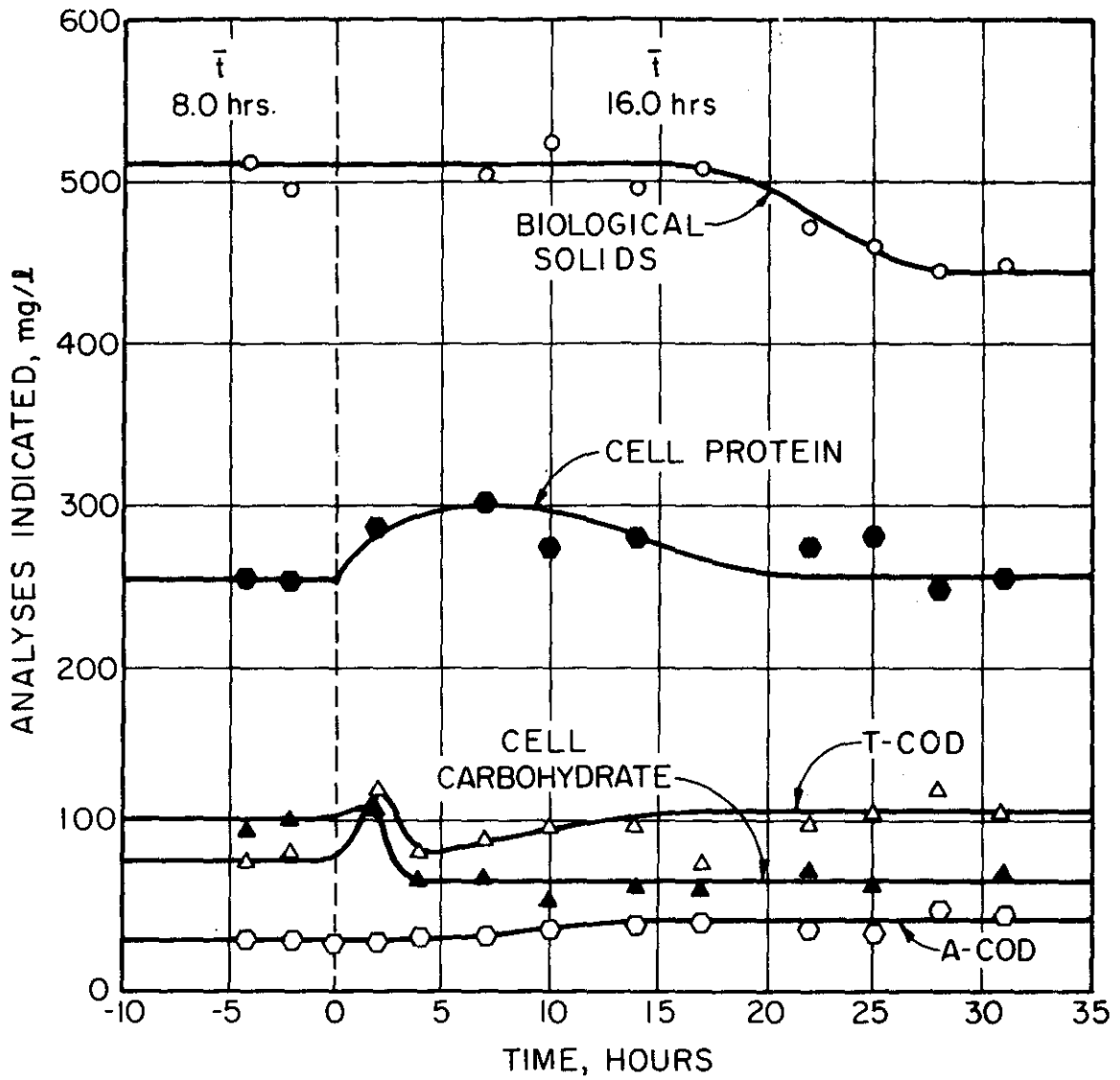

Figure 4. Response to decrease in dilution rate from 0.125 hrs^{-1} to 0.062 hrs^{-1}; S_i = 1000 mg/l glucose.

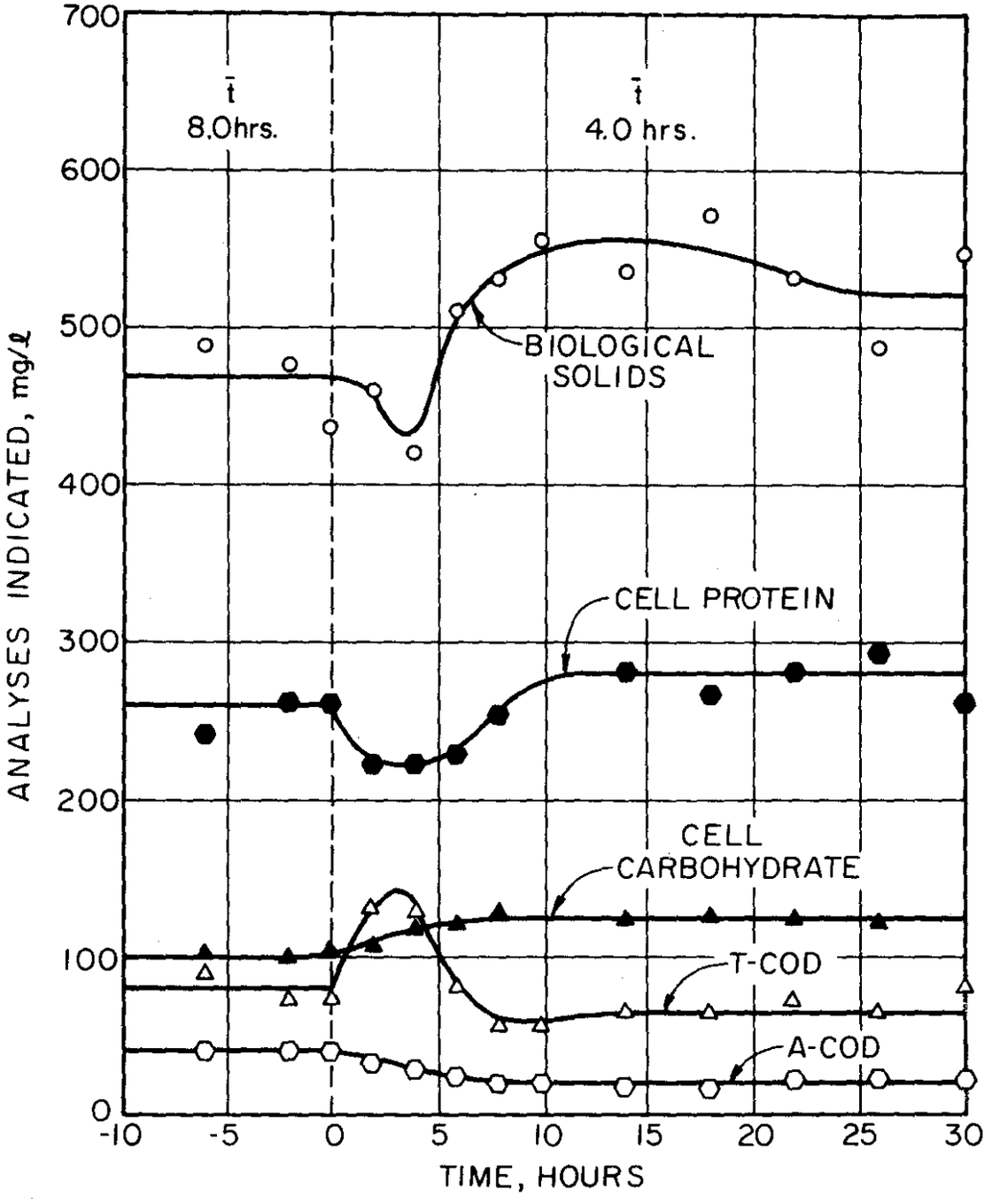

Figure 5. Response to increase in dilution rate from 0.125 hrs^{-1} to 0.25 hrs^{-1}; S_i = 1000 mg/l glucose.

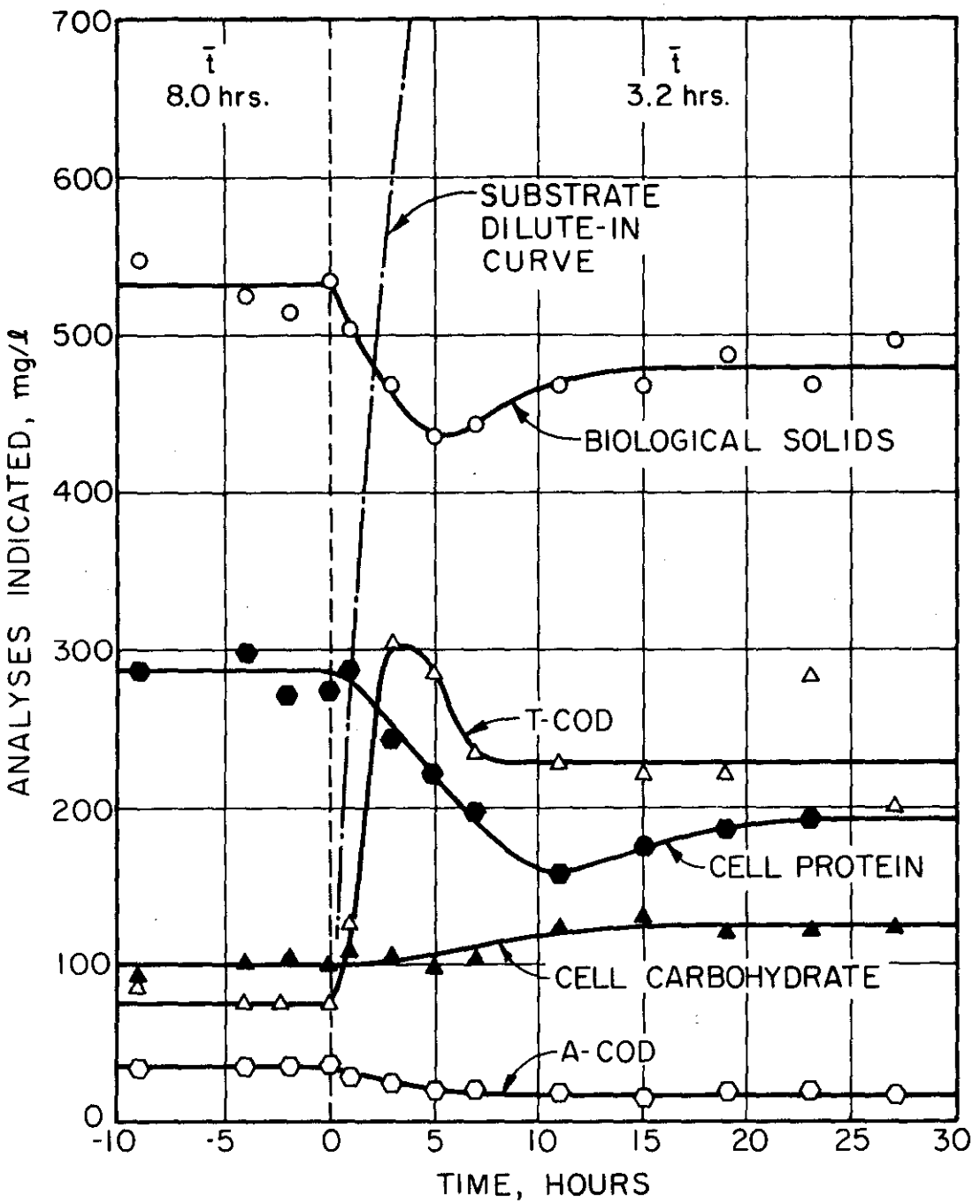

Figure 6. Response to increase in dilution rate from 0.125 hrs^{-1} to 0.313 hrs^{-1}; S_i = 1000 mg/l glucose.

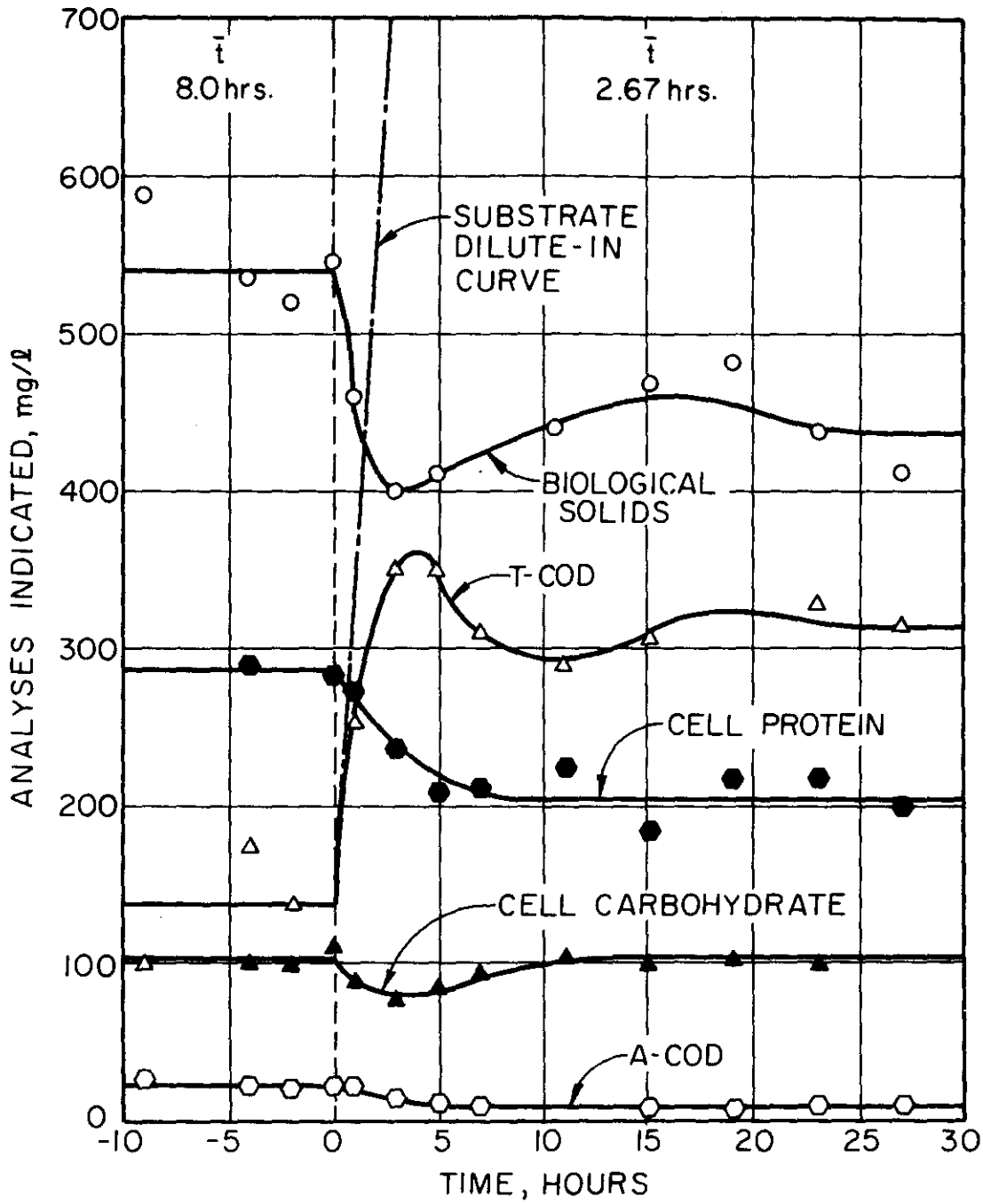

Figure 7. Response to increase in dilution rate from 0.125 hrs^{-1} to 0.375 hrs^{-1}; S_i = 1000 mg/l glucose.

respect to initial and final steady state conditions is seen in Fig. 8, which shows the response when the most severe hydraulic shock was placed upon the system; that is, when dilution rate was changed from 0.125 to 0.437 hrs^{-1}. However, in this case the transient response was somewhat different than it was for the previous two figures. In this figure it is seen that there is a smooth transient as the system approaches the new steady state, whereas in the two previous figures the effluent substrate concentration passed through a maximum and the biological solids concentration passed through a minimum before approaching a new steady state level.

In the hydraulic shock load results thus far shown, the hourly rate of feeding increased when D was increased, and decreased proportionally when D was decreased, since feed substrate concentration remained constant. On the other hand, one can apply hydraulic shock and maintain a constant hourly mass rate of feeding substrate. This requires that when dilution rate is increased, substrate concentration must be decreased proportionally, and when dilution rate is decreased, there must be a concomitant increase in substrate concentration. In these cases, the system receives a quantitative shock along with the hydraulically imposed change in μ. Under such conditions of hydraulic shock it has been found in the principal investigator's laboratory that a decrease in dilution rate is far more deleterious than an increase. That is, the situation is the reverse of that found with the hydraulic shock loading previously described. Figure 9 shows the response when the dilution rate was changed from 0.125 to 0.31 hrs^{-1}. The hourly organic loading was maintained constant, and to do so meant increasing S_i from a 1000 to 4000 mg/l. There was a significant transient in effluent substrate concentration during which the

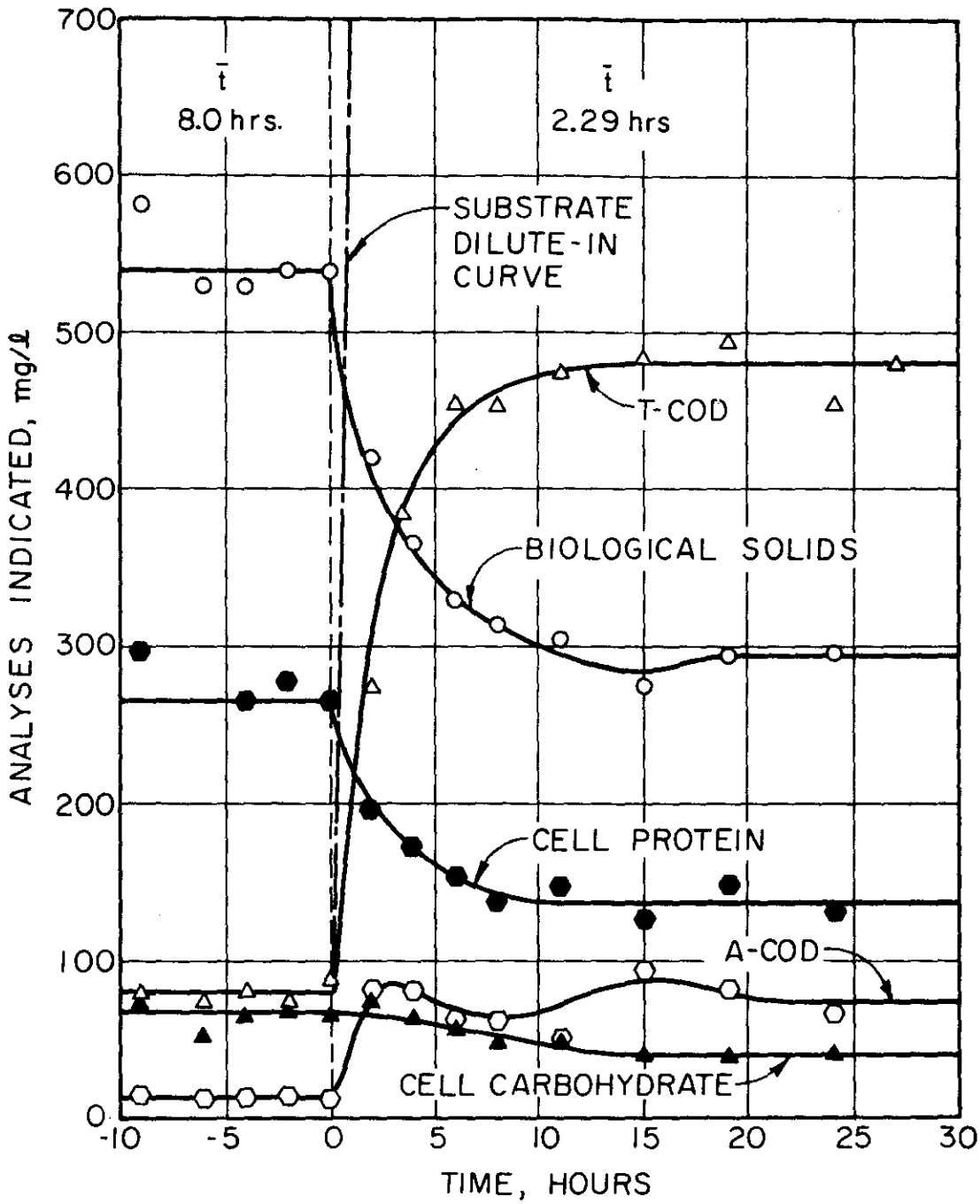

Figure 8. Response to increase in dilution rate from 0.125 hrs^{-1} to 0.437 hrs^{-1}; S_i = 1000 mg/l glucose.

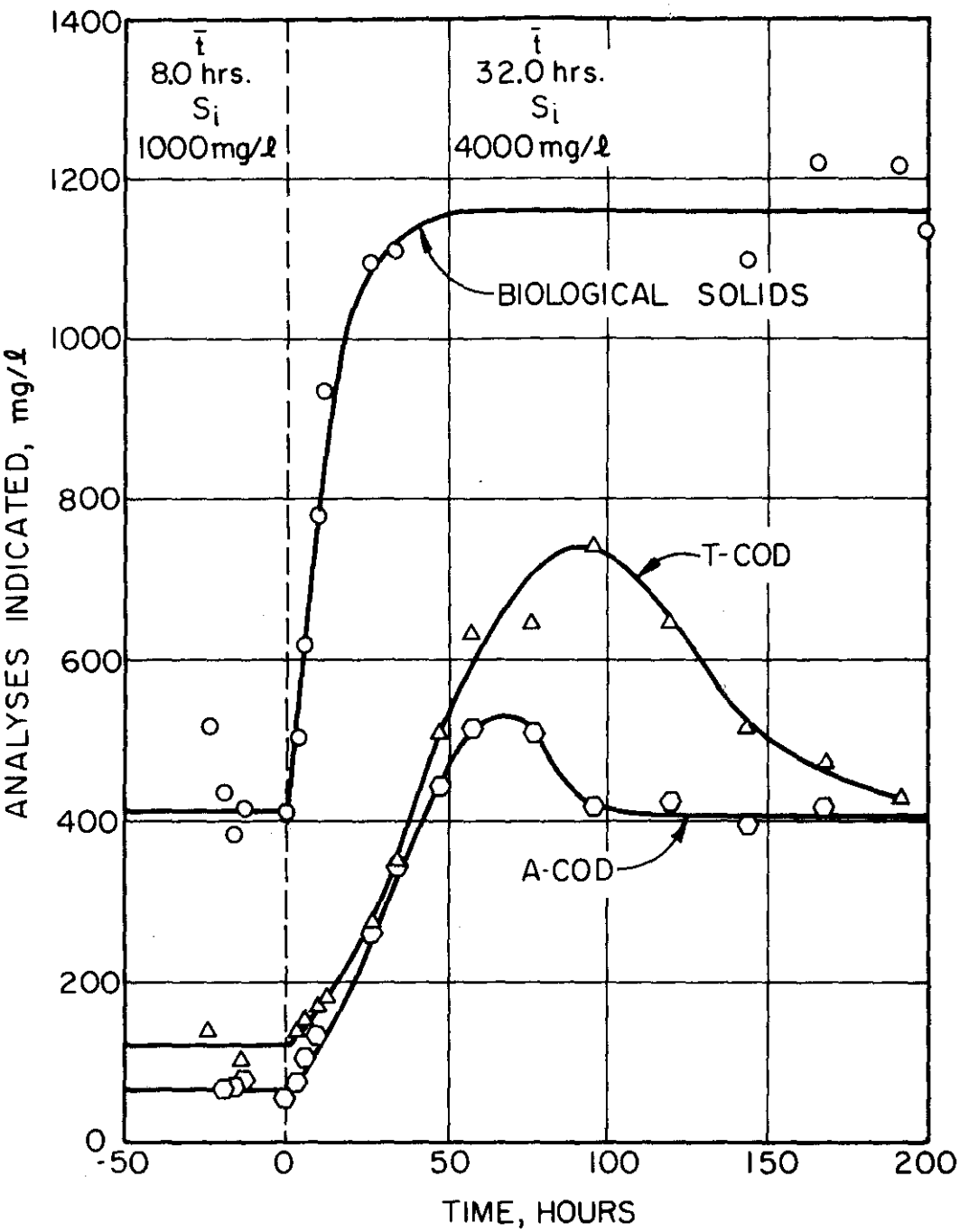

Figure 9. Response to decrease in dilution rate from 0.125 hrs^{-1}, S_i = 1000 mg/l glucose to 0.031 hrs^{-1}, S_i = 4000 mg/l glucose.

efficiency of substrate removal or of purification decreased; however, the system recovered and the COD removal efficiency returned to the preshock level of ± 90%. The response to a less severe decrease in dilution rate (with concomitant increase in S_i) is shown in Fig. 10. In this case there was a smooth transition to the new steady state, and the substrate removal efficiency remained essentially constant.

pH Shock

While there has been much information published regarding effect of pH on microorganisms, there is a relative dearth of experimental information regarding the transition between steady state growth at one pH and steady state growth at another pH. In fact, there are very few systematic studies regarding continuous culture of microorganisms at various steady state pH levels. Thus it was important to gain some insight into types of response and the limit of adaptability for various increases and decreases in pH. Here it was essential to choose some reasonable base level pH, and the one chosen was 6.4 to 6.7. For each of the experiments shown in the next series of figures the initial steady state was assessed, the pH of the feed changed, and the resulting transient behavior of the system examined until the new or final steady state was approached. The major objective was to characterize the response and to obtain some guidelines as to an allowable range in pH in the waste stream. In these studies both once-through and cell recycle systems were employed. The temperature, rate of aeration, etc., were the same as for the hydraulic shock load studies. Source of the population again was municipal sewage; the organisms were acclimated to the synthetic waste which again consisted of a minimal medium in which

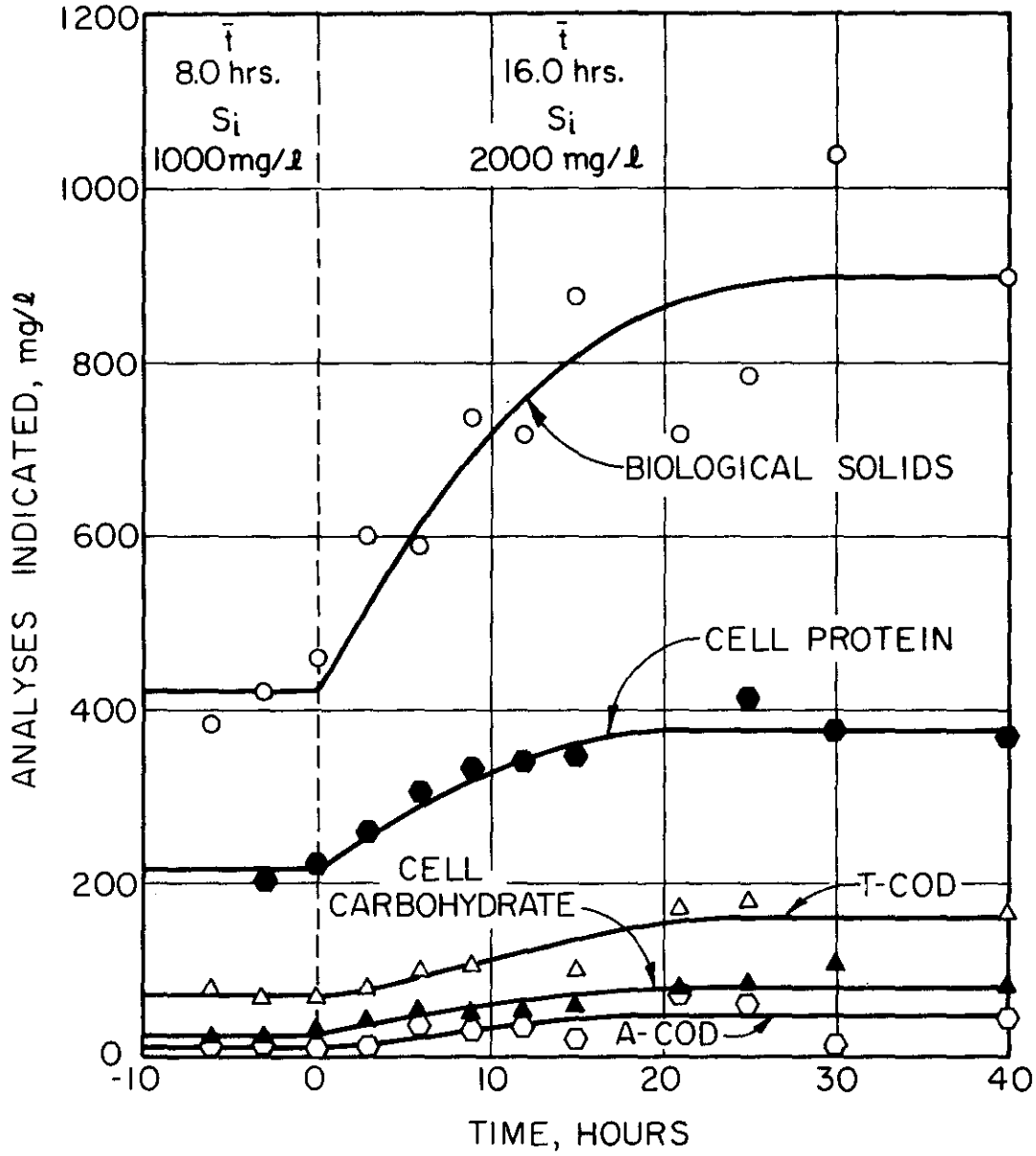

Figure 10. Response to decrease in dilution rate from 0.125 hrs^{-1}, S_i = 1000 mg/l glucose to 0.062 hrs^{-1}, S_i = 2000 mg/l glucose.

glucose was the carbon source. Figure 11 shows the response when a once-through chemostat system was subjected to a change from pH 6.7 previous to the shock (see data to the left of the broken line at time 0) to pH 6.2. It can be seen that there was a relatively short-lived but definite transient in X and S before returning to a steady value. A slightly more severe shock from pH 6.7 to 5.8 to another system again caused a transient response in X but very little disturbance in effluent quality (see Fig. 12). It is interesting to note that in this figure and in the previous one effluent quality was slightly better in the final steady state which resulted at the new pH. In any event, such a mild decrease in pH, while definitely causing a transient disturbance, would have to be adjudged well within the limits of biochemical adaptability of a heterogeneous population. In fact, even in Figure 13, which shows the response to a decrease in pH from 6.4 to 3.5, after a rather severe transient leakage of substrate and dilute-out of cells, there was a fairly rapid recovery. Also plotted in this figure are changes in protein, carbohydrate and DNA content of the biomass. It is seen that there was a considerable drop off in protein and DNA content and a rise in carbohydrate content in response to the lower pH. Also it should be noted that microscopic observations indicated that there was a drastic change in predominance between initial and final steady states. As one might expect, the predominating organisms in the final steady state were filamentous fungi.

When the pH was changed from 6.6 to 3.2 the results were as shown in Figure 14. This shock, which was only slightly more severe than that shown in the previous figure, resulted in a decidedly more severe transient disturbance of both substrate removal efficiency and biological solids concentration. It is seen that the cells diluted nearly completely out

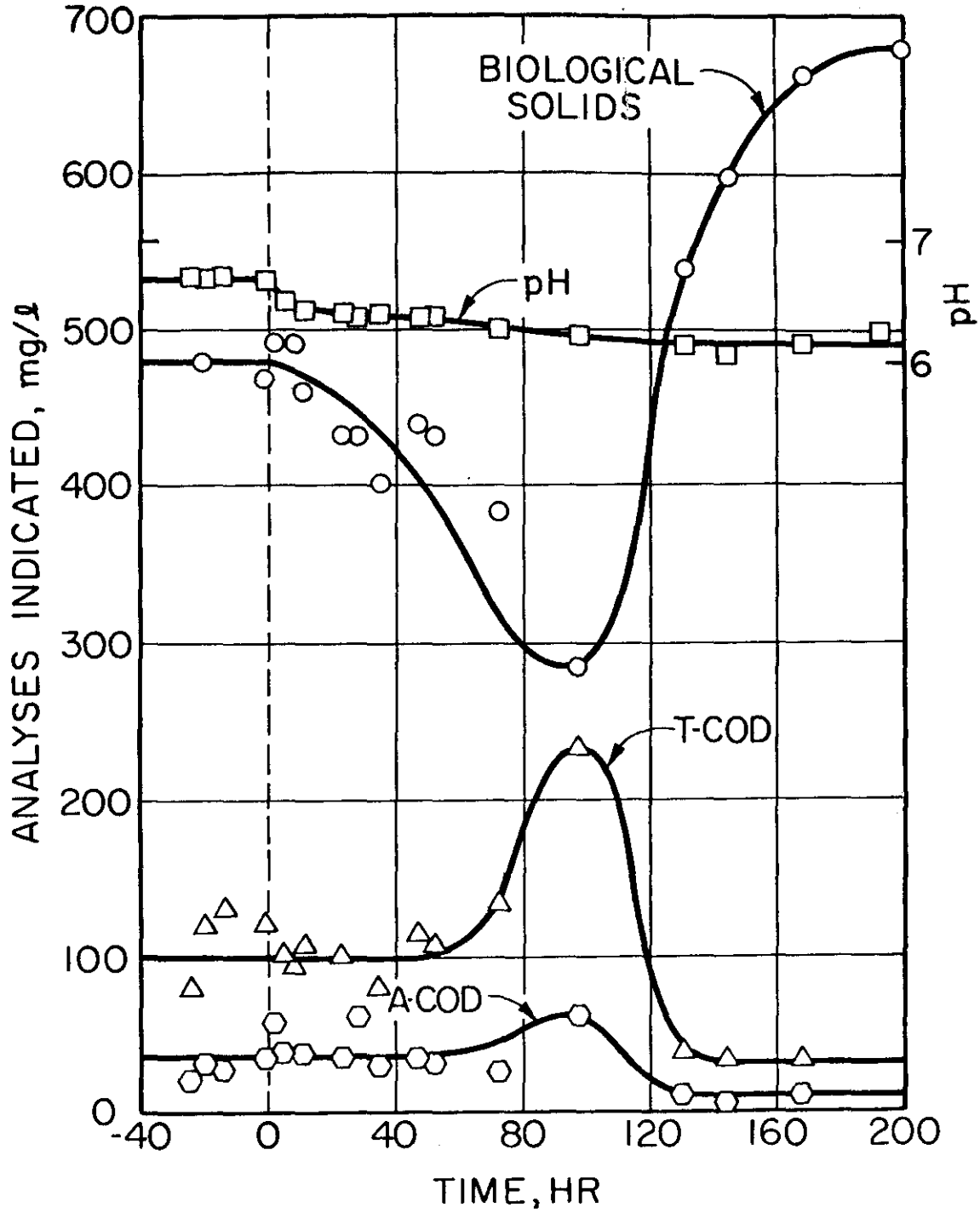

Figure 11. Response to a decrease in pH from 6.7 to 6.2 (no cell recycle).

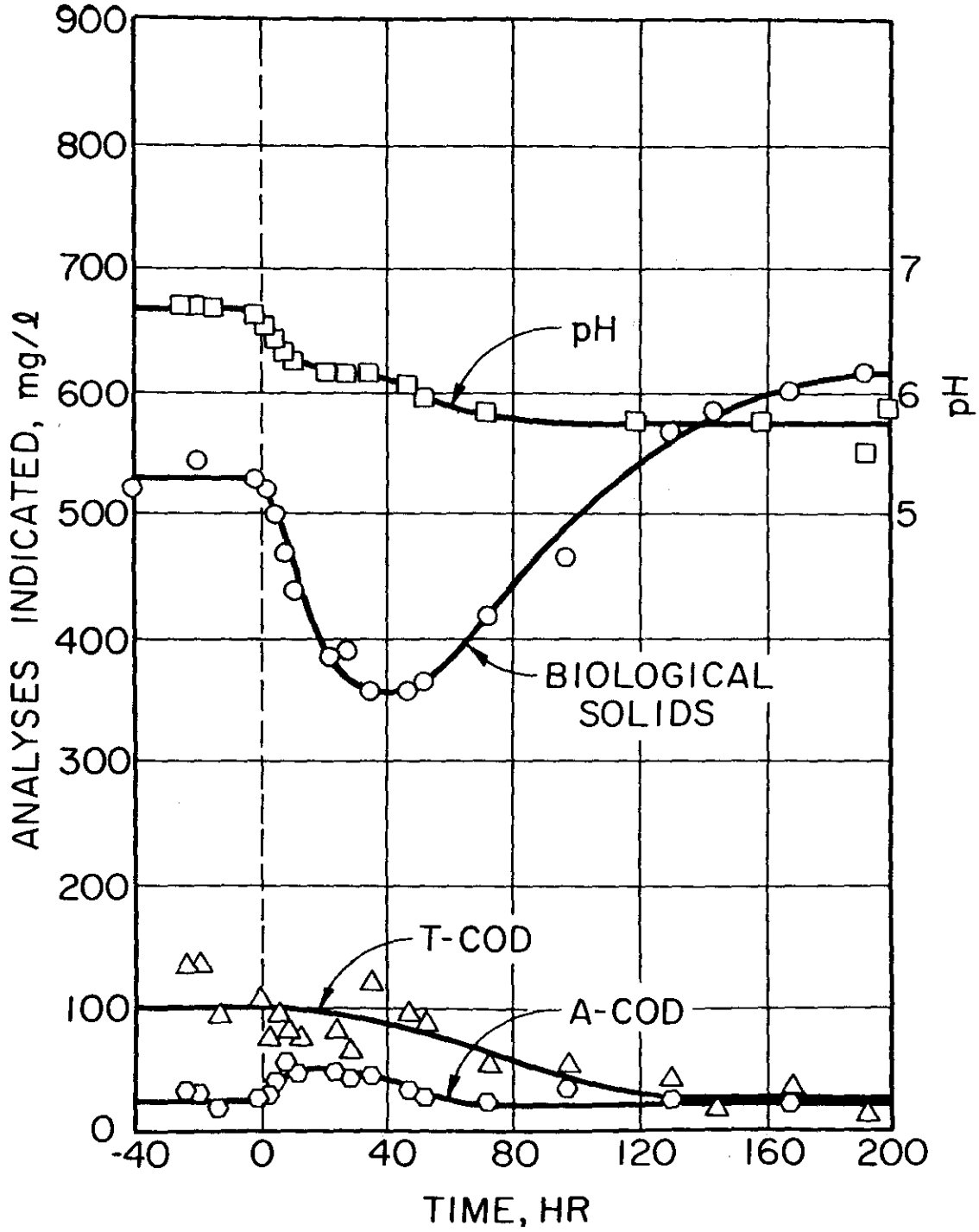

Figure 12. Response to a decrease in pH from 6.7 to 5.8 (no cell recycle).

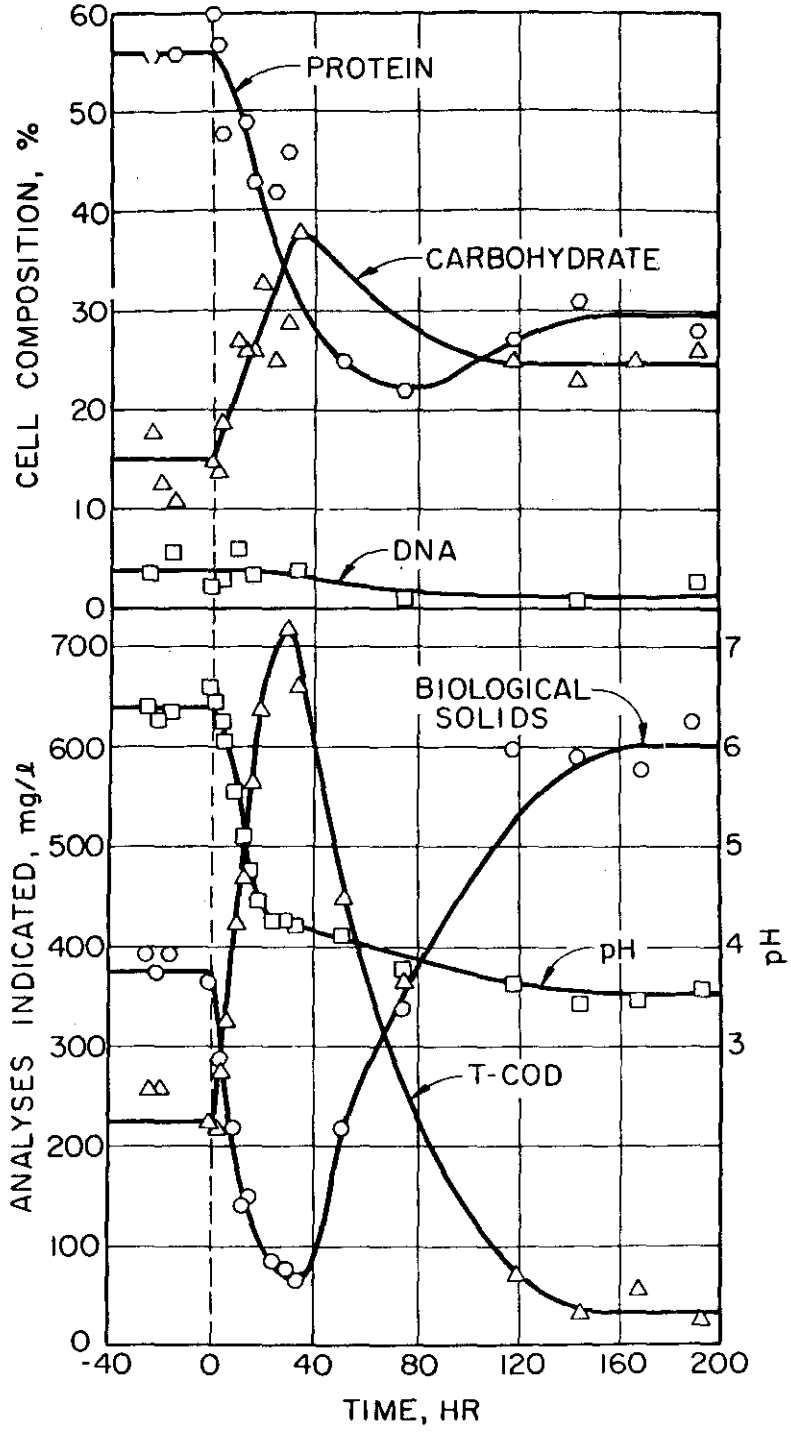

Figure 13. Response to a decrease in pH from 6.4 to 3.5 (no cell recycle).

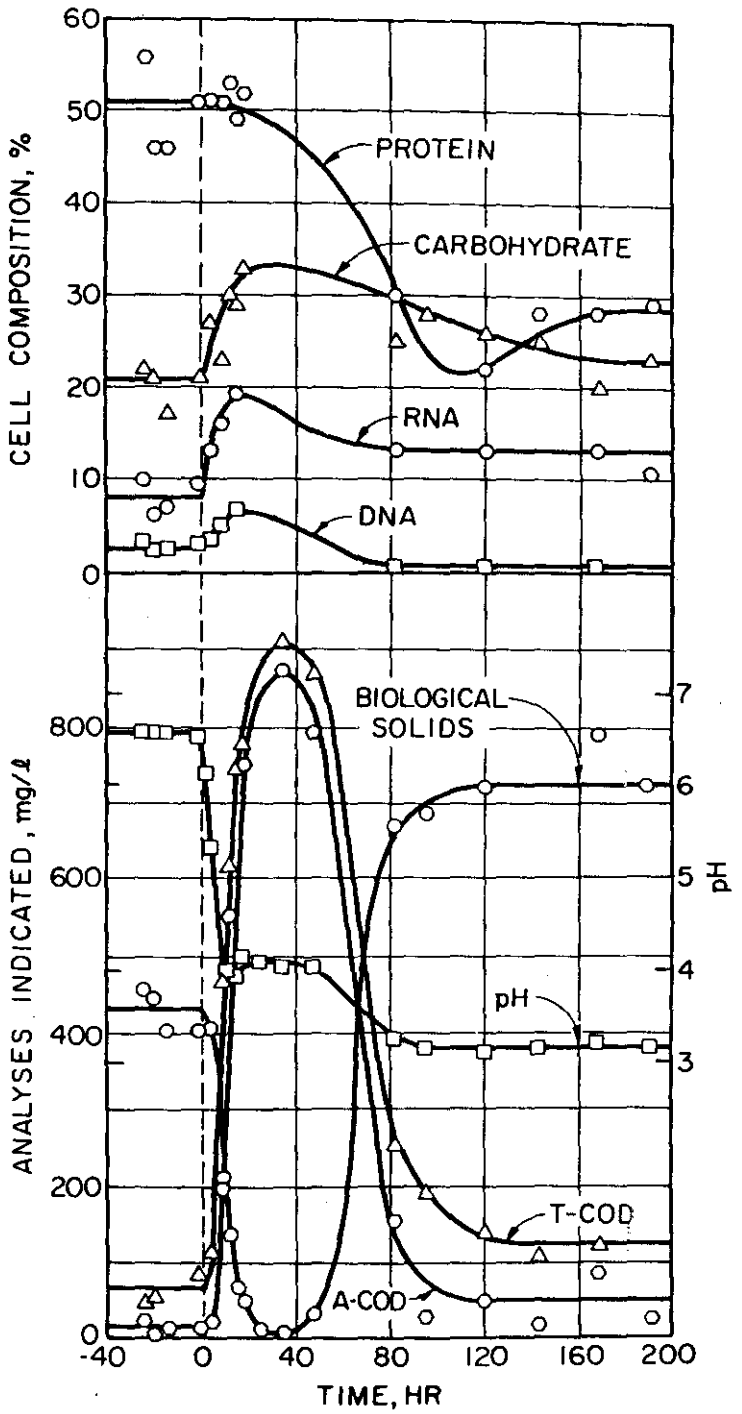

Figure 14. Response to a decrease in pH from 6.6 to 3.2 (no cell recycle).

of the system before a new population began to establish itself. As before, this new population consisted essentially of filamentous fungi. A similar response was registered to a slightly more severe decrease in pH from 6.4 to 3.0 (see Fig. 15). Again, these experiments were run in a once-through chemostat. The general pattern of response was similar to that of the previous figures.

When a slightly more severe shock from pH 6.6 to 2.7 was applied in a once-through chemostat, the system had reached the point of no recovery, as seen in Figure 16. The experiment was terminated after 200 hours, since even though there were very small amounts of biological solids which persisted in the chemostat the system showed no tendency toward recovery. It seems possible that with prolonged aeration the very specific population which is required for existence at such a low pH might have developed, provided, of course, such cells existed in the population prior to the shock. The results of microscopic observations of each system as each experiment progressed left little doubt that the main response mechanism was an ecological shift from individual microorganisms to the filamentous fungi. In general, for experiments wherein cells were recycled, the recycle operation had a tendency to attenuate the transient leakage of substrate. Figure 17 shows one experiment in which total cell recycle of all settleable solids was practiced at a recycle hydraulic flow amounting to 1/3 of the substrate inflow rate. The system was subjected to a very severe shock, i.e., a change in pH from 6.7 to 3.2; there was a rather drastic dilute out of cells but little or no disturbance of substrate removal efficiency. Filamentous forms increased but they were not the predominant forms after the shock.

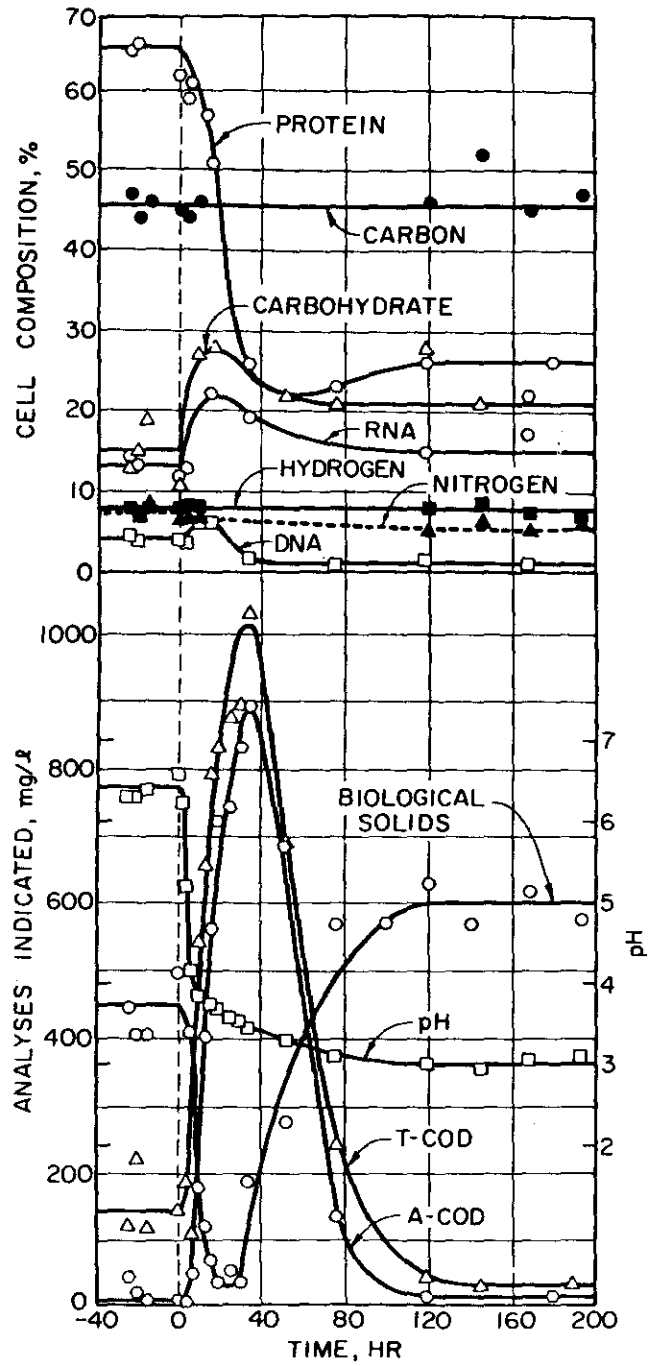

Figure 15. Response to a decrease in pH from 6.4 to 3.0 (no cell recycle).

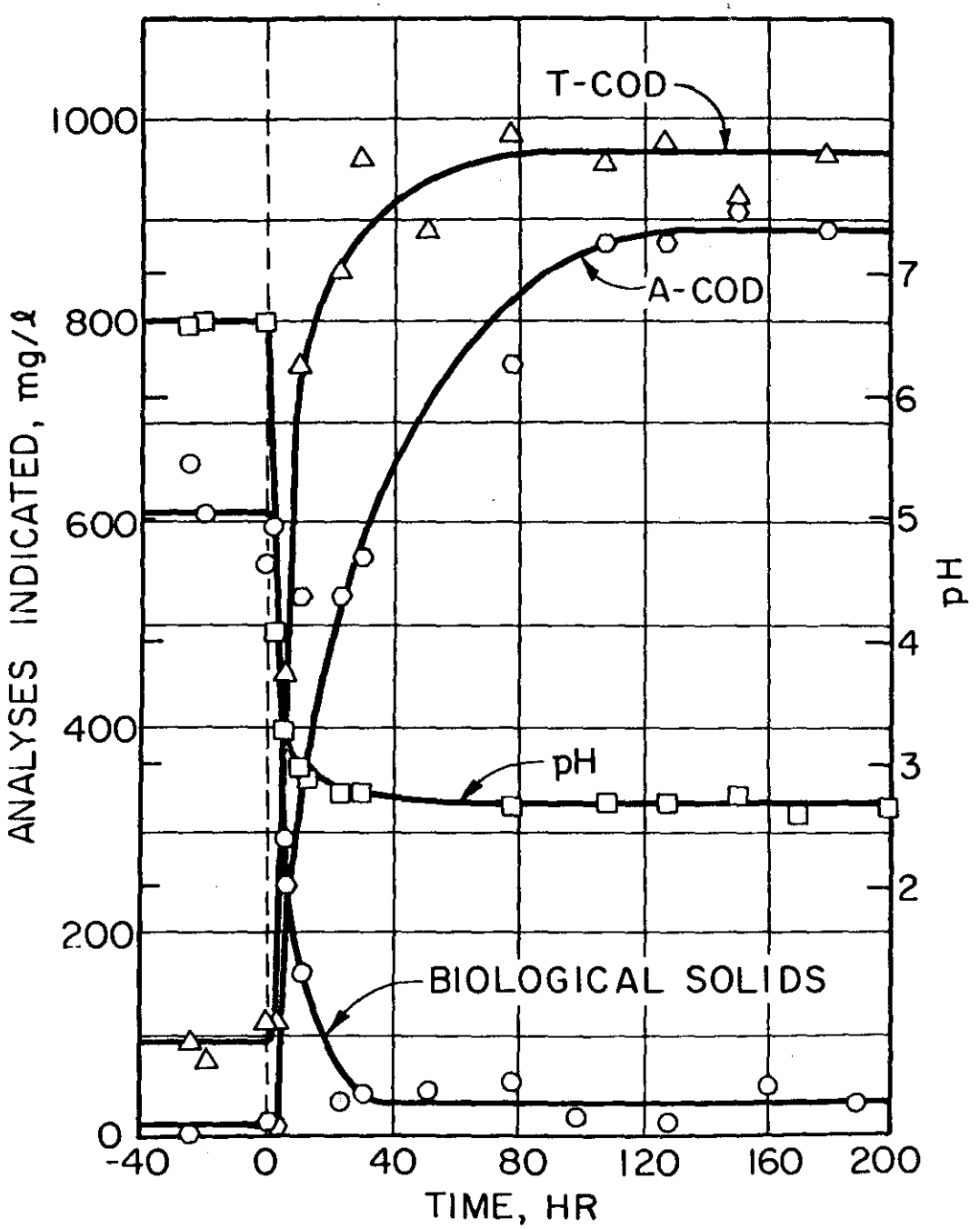

Figure 16. Response to a decrease in pH from 6.6 to 2.7 (no cell recycle).

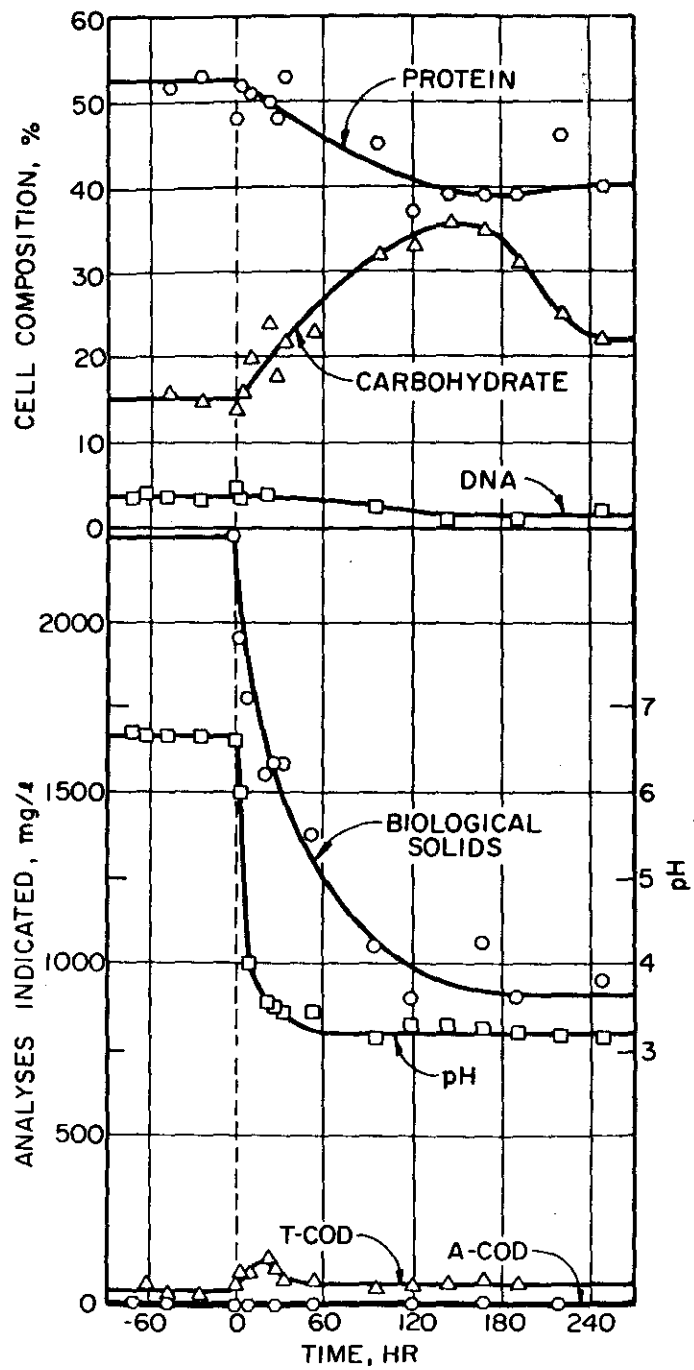

Figure 17. Response to a decrease in pH from 6.7 to 3.2 (total recycle of all settleable cells at 33% of feed flow).

Temperature Shock

Studies on the response to changes in temperature were conducted in once-through chemostats using synthetic waste of the same composition used for the previous studies. As before all populations were developed from an inoculum of municipal sewage. The base line temperature selected for the pre-shocked condition was 25° C and the reactors were run at dilution rates of 0.125 and 0.25 hrs^{-1}. From the base line temperature, systems were subjected to decreases to temperatures as low as 8° C and increases as high as 57.5°C. In the next series of figures showing these results it will be possible to compare the effect of dilution rate or specific growth rate on the ability of these hetrogeneous populations to accommodate the various changes in temperature which were imposed. In general, it will be shown that the systems responded more favorably to increases than to decreases in temperature, and that regardless of the direction of change, there was less leakage of carbon source in the effluent as well as less dilute out of cells during the transient phase in the systems growing at the lower dilution rate. When the temperature was changed over a 12 hour period from 25° to 8° C, neither the system growing at the specific rate of 0.125 or the system at 0.25 hrs^{-1} responded successfully. The experiment was carried out for 200 hours after the shock, but there was no indication of impending recovery after this reduction of temperature to the psychrophilic range. However, when a less severe decrease in temperature (i.e., from 25° to 17.5°C) was studied, there was a dilute out followed by a considerable recovery of substrate removal efficiency. The results of such studies at the two specific growth rates employed are shown in Figure 18. The data presented on the

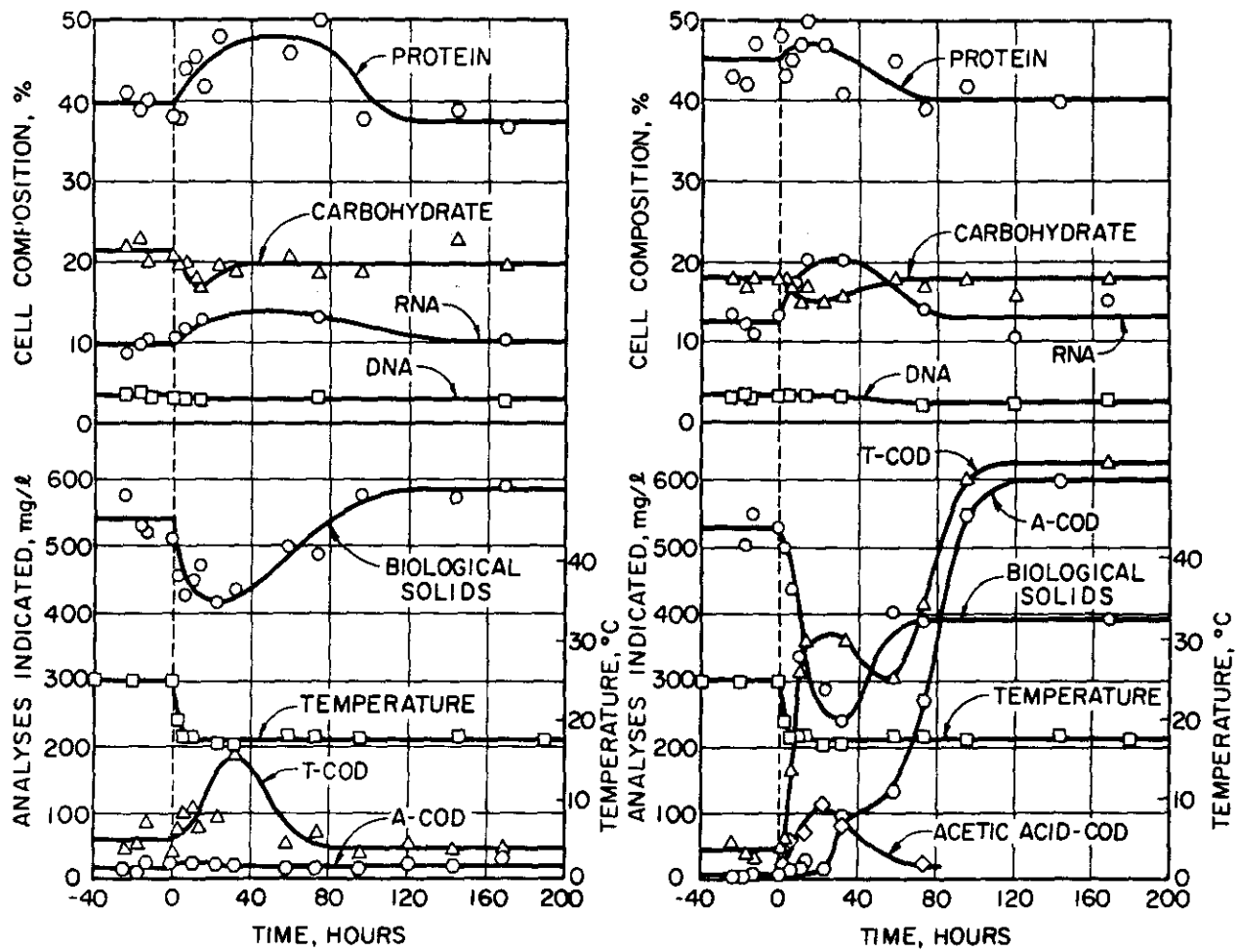

Figure 18. Response of a heterogeneous microbial population growing in a chemostat at 25° C to a decrease in temperature to 17.5° C. Left, dilution rate = $0.125\ hrs^{-1}$; Right, dilution rate = $0.25\ hrs^{-1}$.

left-hand side of the figure show the response of the system run at $D = 0.125\ hrs^{-1}$, and the data on the right show the response for $D = 0.25\ hrs^{-1}$. In each set of data, the values plotted to the left of the lines at time zero represent conditions in the preshock state. It is seen that for the slower growing system (left) there was considerably less disturbance during the transient phase, and there was essentially total recovery in the new steady state at 17.5° C. However, for the faster growing system, (right) there was a more severe and long-lived disturbance and, although the system showed partial recovery there was a considerable (apparently permanent) leakage of carbon source. The feed consisted of 1000 mg/l glucose and it can be seen that approximately 60% of the organic matter contributed by the glucose leaked from the system in the final "steady state". In both systems, it is interesting to note that the transient leakage of soluble organic matter consisted primarily of compounds other than the initial substrate. This is shown by the curves labeled T-COD and A-COD; the former represents total soluble organic matter whereas the latter represents soluble organic matter reactive to the anthrone test. At the higher growth rate, acetic acid was identified as one of the metabolic intermediates and/or end products released. However GLC analysis of samples from the slower growing system revealed an absence of low molecular weight fatty acid. Changes in various parameters of biochemical composition of the sludge are shown in the top portions of the figures.

When a mild increasing temperature change from 25 to 36° C was applied to the system, as shown in Figure 19, there were some fluctuations and an initial decreasing trend in X, but these did not result in any

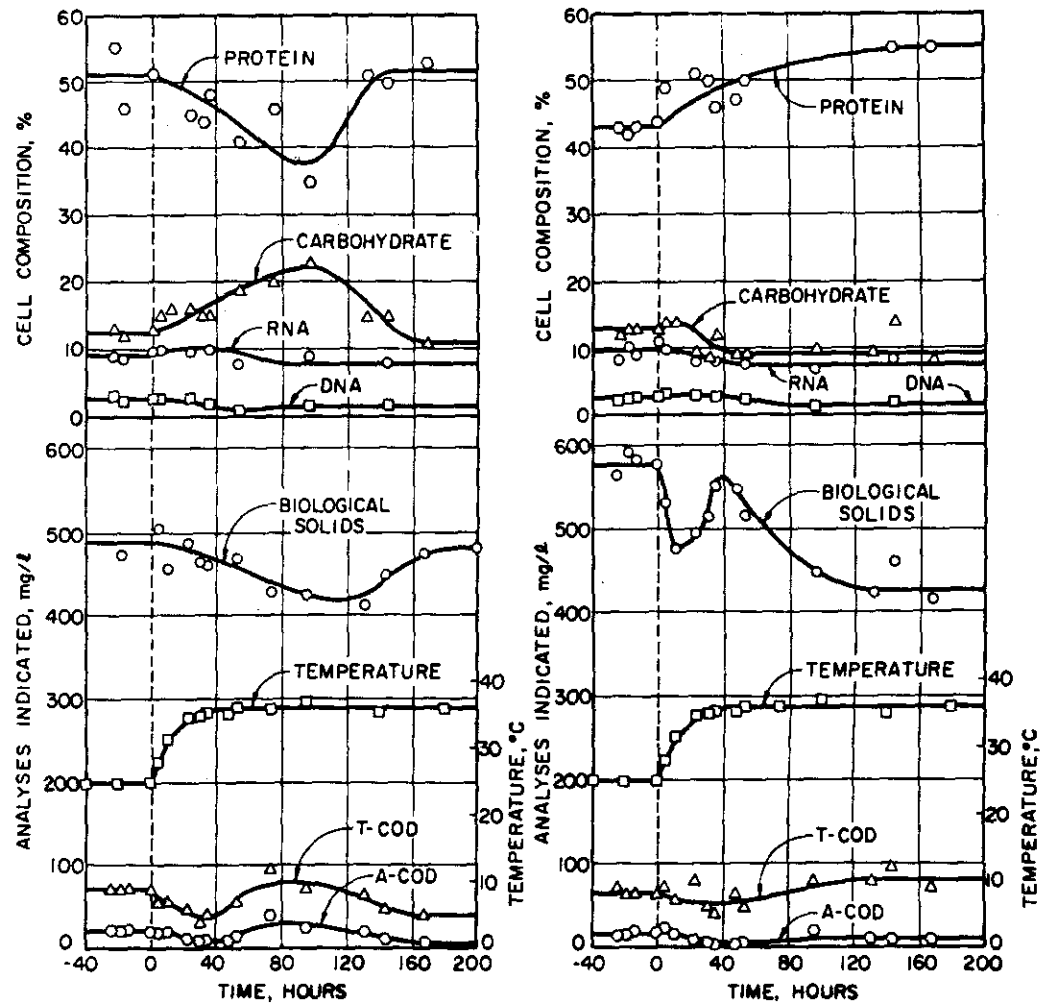

Figure 19. Response of a heterogeneous microbial population growing in a chemostat at 25° C to an increase in temperature to 36° C. Left, dilution rate = 0.125 hrs^{-1}; Right, dilution rate = 0.25 hrs^{-1}.

deterioration of purification efficiency. The most noticeable effect of this shock at either growth rate was a decrease in cell yield and increase in protein concentration in the faster growing system. When the temperature was increased from 25 to 47^{o} C over a 26 hour period, a severe transient leakage of substrate was observed at both growth rates as seen in Figure 20. Here also there was a rather profound effect of growth rate on the severity of dilute-out in X and leakage in S during the transient period. The disturbance in the slower growing system was of less severity and was shorter lived than in the more rapidly growing population. In the slower growing system, dissimilation of the original carbon source proceeded uninterruptedly during the transient. Again at the higher temperature there was a decrease in cell yield. Both systems recovered with regard to substrate leakage in the effluent.

A change in temperature from 25 to 57.5^{o} C led to an apparent inability to recover treatment efficiency (see Figure 21). Even though neither system recovered to the pre-shock level of efficiency, it is amply apparent that the slower growing system did not undergo as severe disruption, and regained a higher degree of substrate removal efficiency than did the faster growing population.

Quantitative Shock Loads

The type of quantitative shock loads in these particular studies consisted of a step increase in inflowing substrate concentration, S_i. This work consisted of a systematic study of response to quantitative shock loadings for the purpose of gaining insight into delineation of the effect of various operational parameters on the ability of the

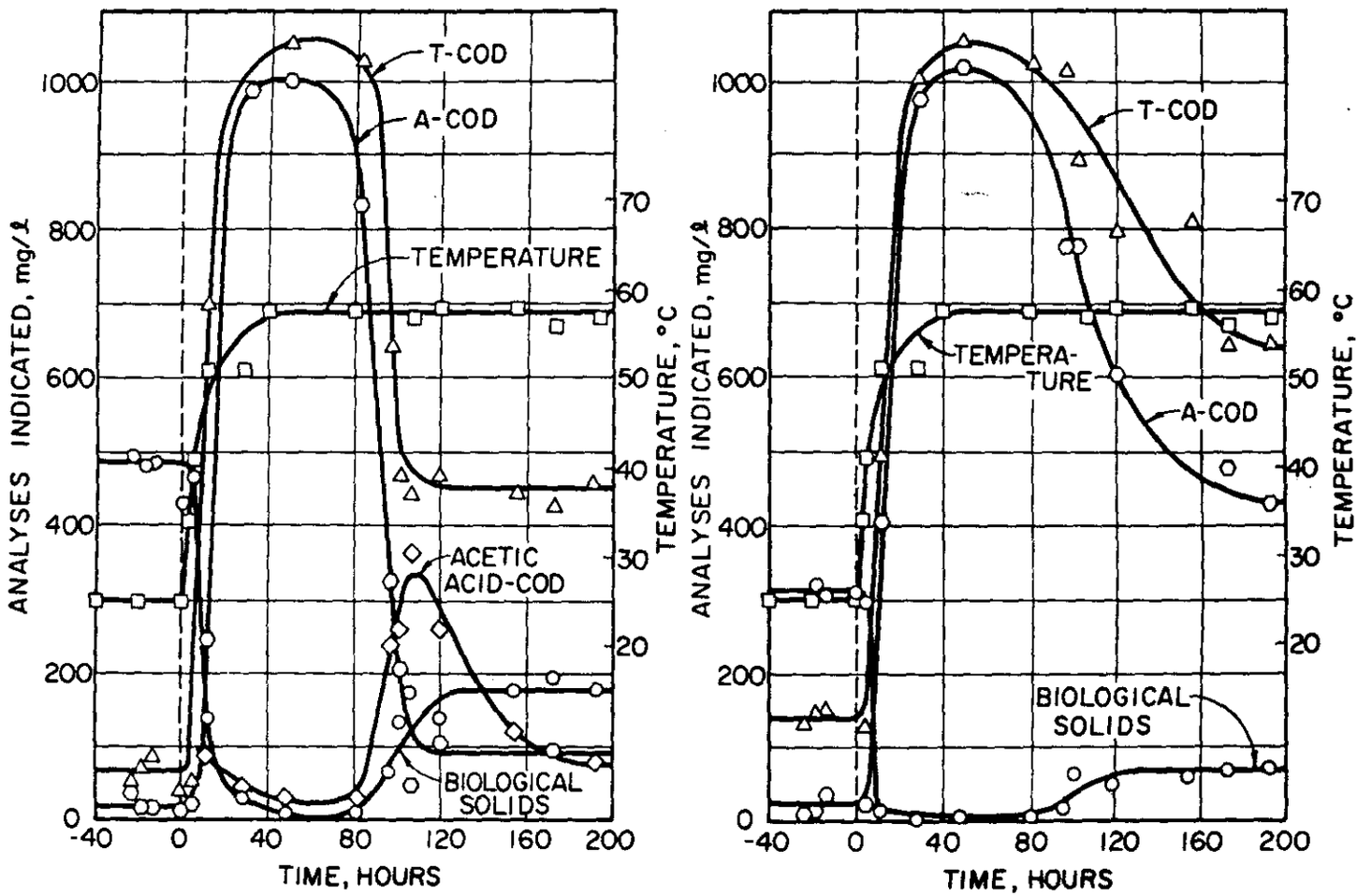

Figure 21. Response of a heterogeneous microbial population growing in a chemostat at 25° C to an increase in temperature to 57.5° C. Left, dilution rate = 0.125 hrs^{-1}; Right, dilution rate = 0.25 hrs^{-1}.

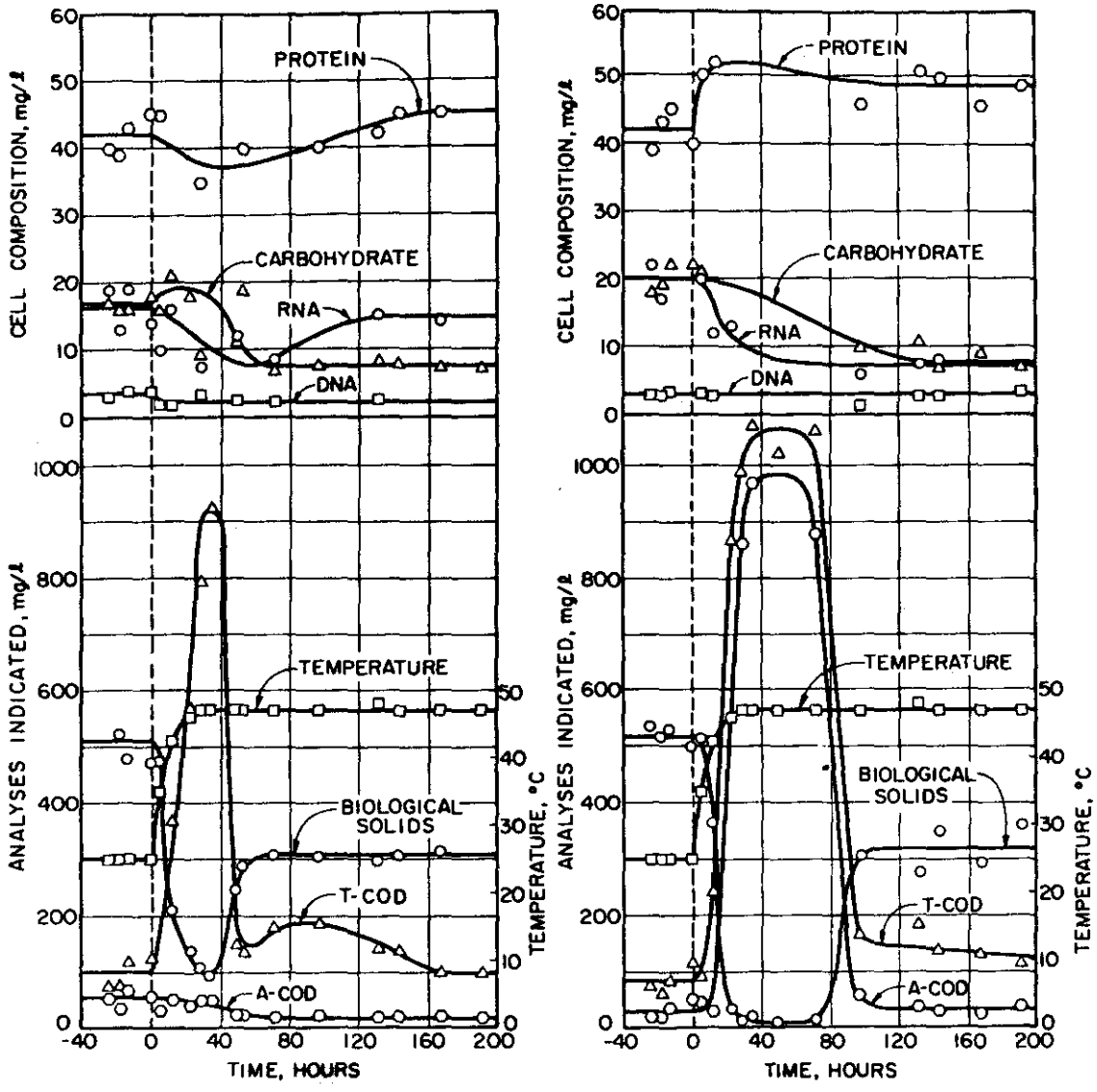

Figure 20. Response of a heterogeneous microbial population growing in a chemostat at 25° C to an increase in temperature to 47° C. Left, dilution rate = 0.125 hrs^{-1}; Right, dilution rate = 0.25 hrs^{-1}.

system to accommodate such shock. Another objective was to arrive at some practical guidelines regarding shock levels which activated sludge systems can handle without significant disruption of treatment efficiency. The experimental approach employed was to compare the shock load behavior of once-through and cell recycle systems at identical hydraulic reactor detention times, $\bar{t}$. Three sets of detention times were selected, 4, 8, and 12 hours. In a once-through system $\bar{t} = \frac{1}{D} = \frac{1}{\mu}$, where D is the dilution rate and is defined as the ratio of inflowing rate of feed flow, F, and the aeration tank volume, V, (i.e., $\frac{F}{V}$); μ is the specific growth rate which, in the steady state condition, is an exponential growth rate constant which can also be defined as $\frac{dX}{dt}\left(\frac{1}{X}\right)$, where the differential is the rate of change in X. When cell recycle is practiced, the mean hydraulic retention time in the reactor is reduced because of the recycle flow. The recycle flow is usually expressed as a fraction, α, of the waste flow and is thus equal to αF. In terms of overall dilution rate, the reactor dilution rate D_1 is given by the following equation:

$$D_1 = D(1 + \alpha).$$

Thus to maintain the same $\bar{t}$ for the reactor it was necessary to reduce the system dilution rate D. This was accomplished by reducing the rate of inflowing waste feed, F. In these studies the recycle flow α was maintained at 0.33.

Figures 22, 23, 24, 25 and 26 are composites of some of the figures given in appendix VI. In general the results show that for the higher $\bar{t}$'s there was an improvement in response with regard to amount of substrate leakage during the transient phase at a given shock concentration. This trend is readily apparent in the once-through systems, e.g., see

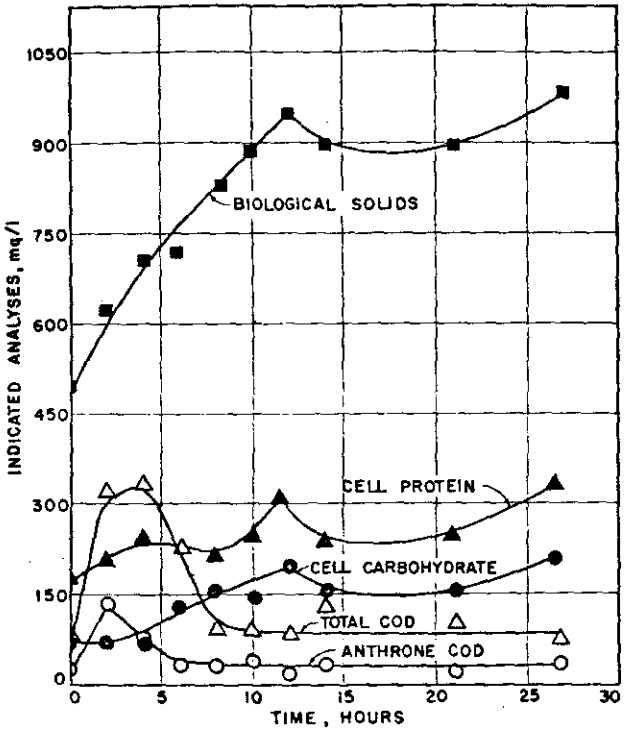

Fig. 1. $\bar{t} = 4$

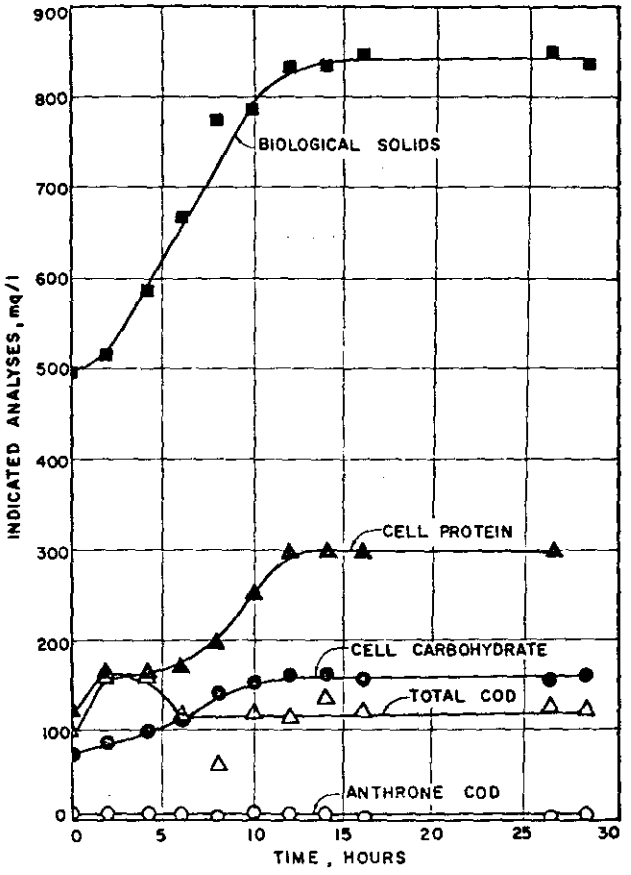

Fig. 4. $\bar{t} = 8$

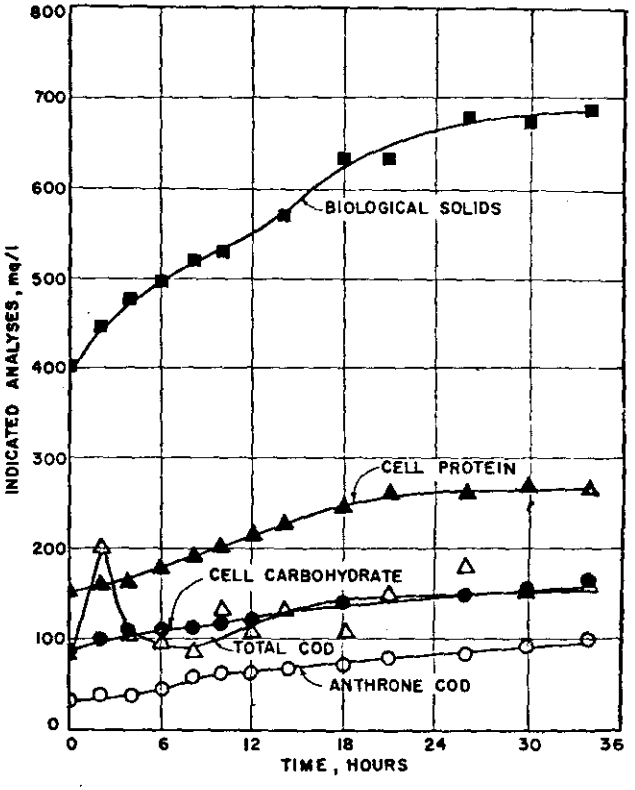

Fig. 7. $\bar{t} = 12$

FIGURE 22. EFFECT OF $\bar{t}$ AT 1000 → 2000 SHOCK LEVEL
ONCE THROUGH SYSTEMS FIGS. 1 → 4 → 7
(numbers refer to figures in Appendix VI)

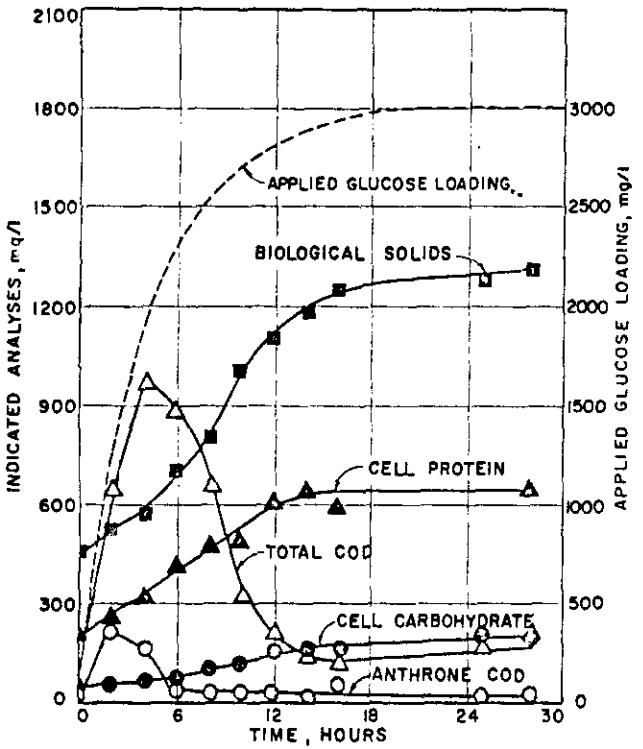

Fig. 2. $\bar{t} = 4$

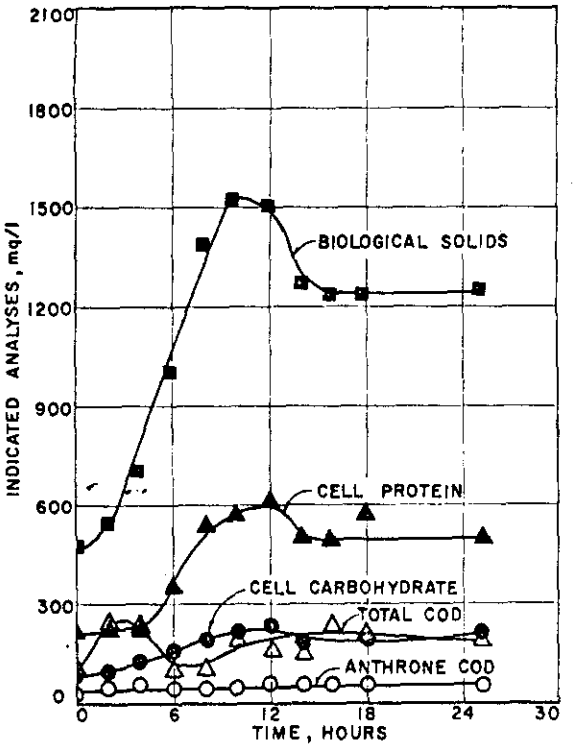

Fig. 5. $\bar{t} = 8$

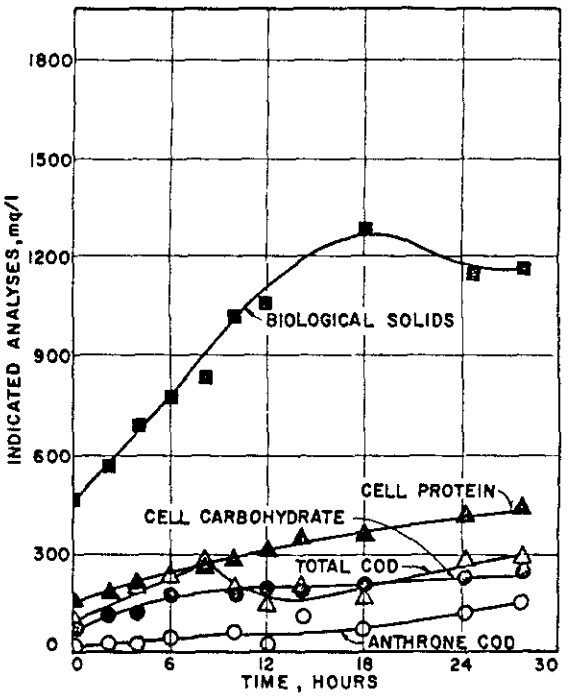

Fig. 8. $\bar{t} = 12$

FIGURE 23. EFFECT OF $\bar{t}$ AT 1000 → 3000 SHOCK LEVEL ONCE THROUGH SYSTEMS FIGS. 2 → 5 → 8
(numbers refer to figures in Appendix VI)

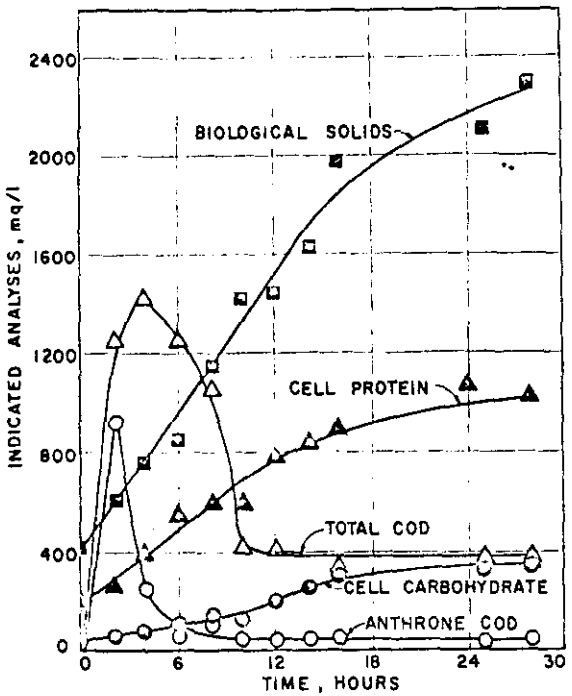

Fig. 3. $\bar{t} = 4$

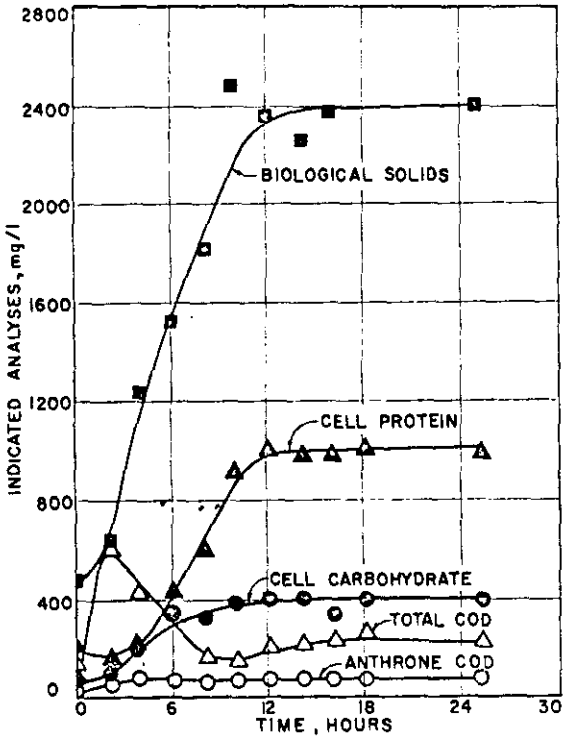

Fig. 6. $\bar{t} = 8$

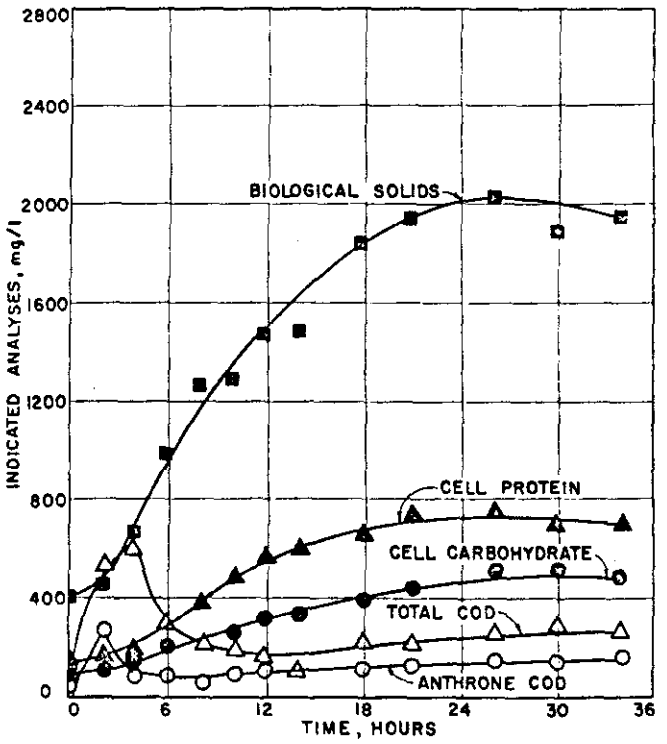

Fig. 9. $\bar{t} = 12$

FIGURE 24. EFFECT OF $\bar{t}$ AT 1000 → 5000 SHOCK LEVEL
ONCE THROUGH SYSTEMS FIGS. 3 → 6 → 9
(numbers refer to figures in Appendix VI)

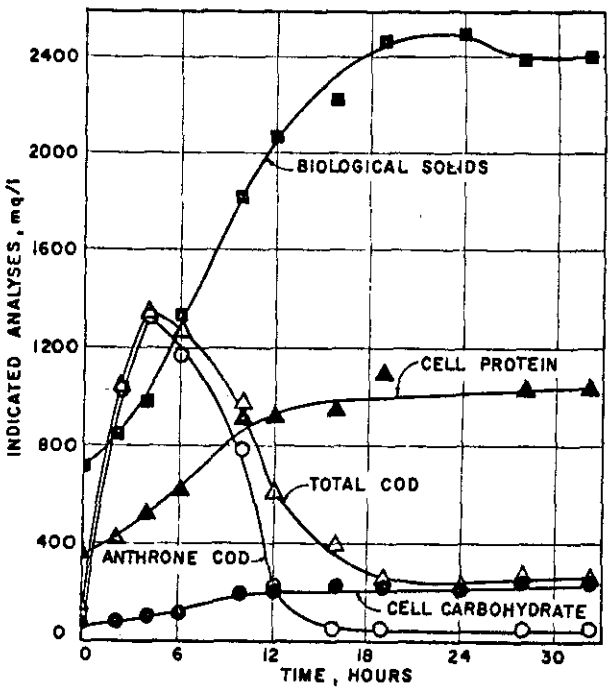

Fig. 3. $\bar{t} = 4$

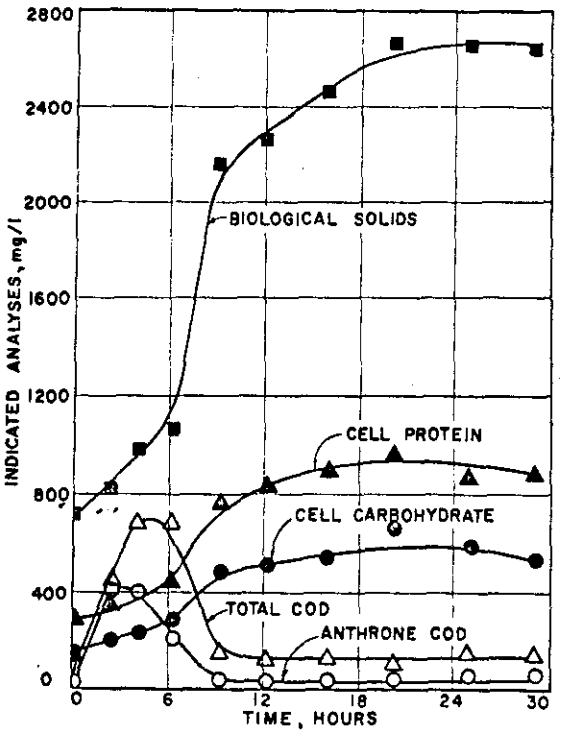

Fig. 6. $\bar{t} = 8$

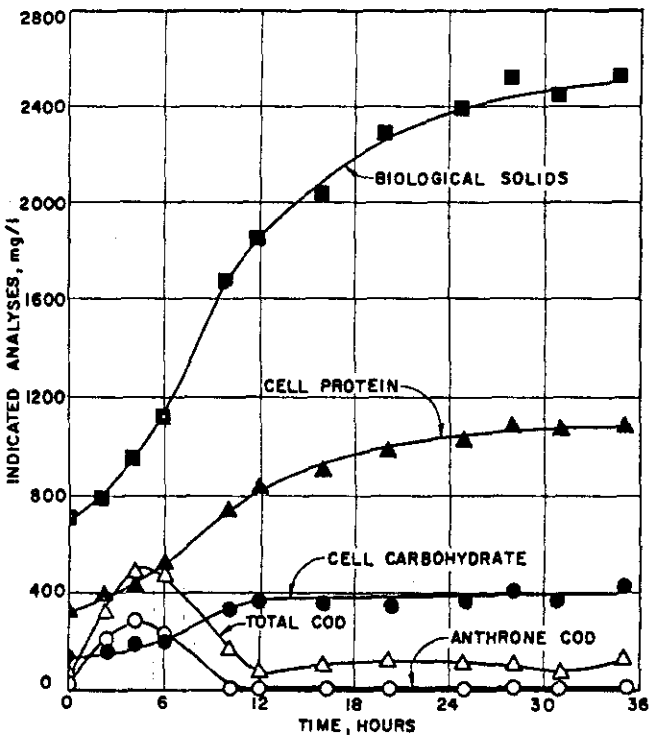

Fig. 9. $\bar{t} = 12$

FIGURE 25. EFFECT OF $\bar{t}$ AT 1000 → 5000 SHOCK LEVEL SLUDGE RECYCLE SYSTEM FIGS. 3 → 6 → 9
(numbers refer to figures in Appendix VI)

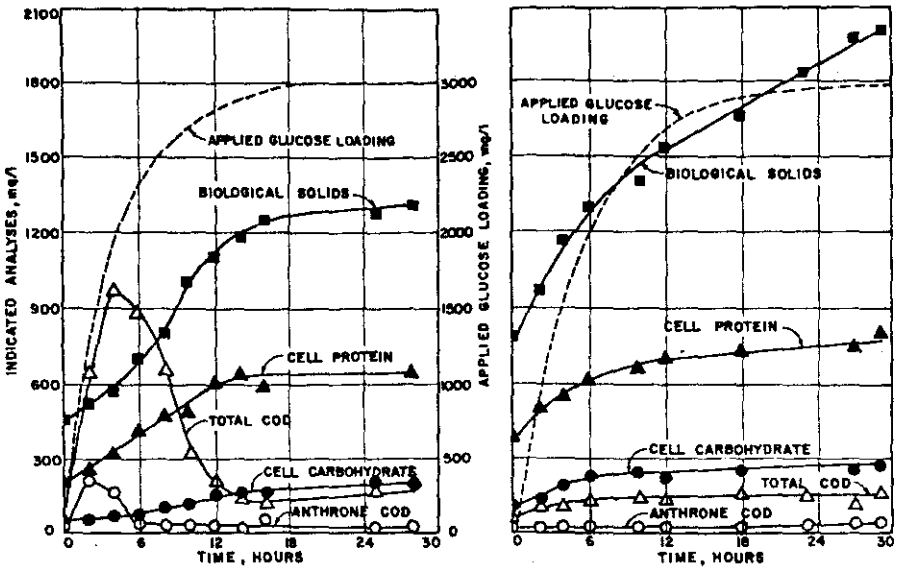

Fig. 2

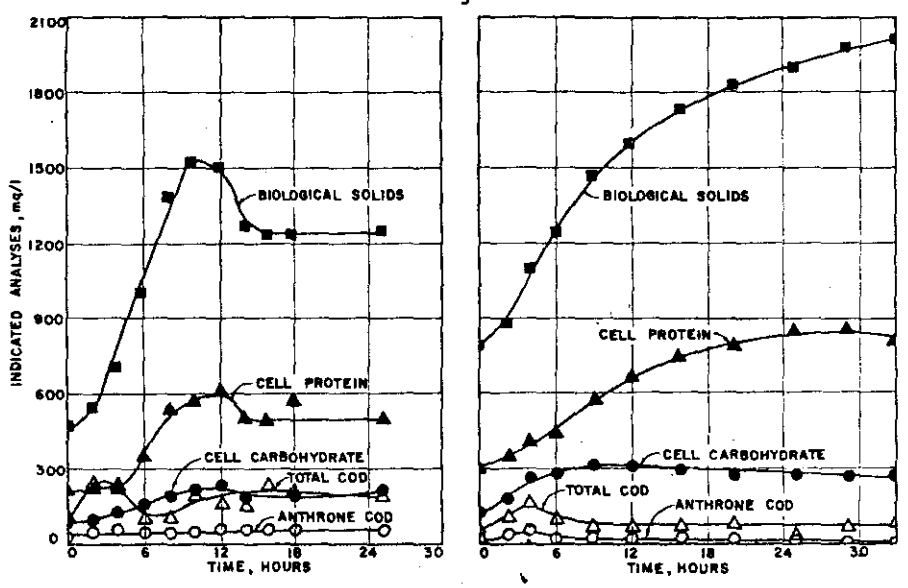

Fig. 5

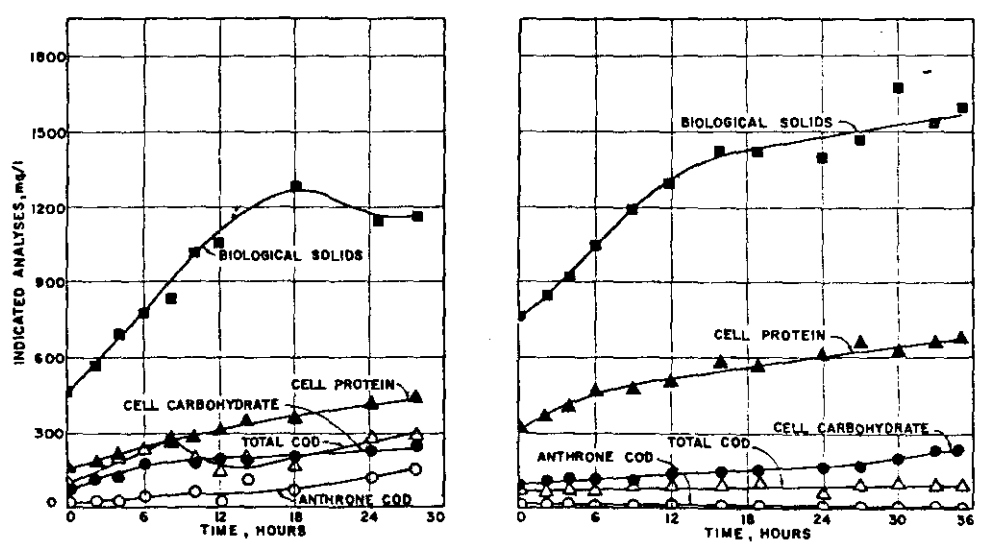

Fig. 8

FIGURE 26. BENEFICIAL EFFECT OF CELL RECYCLE 1000 → 3000 SHOCK LEVEL
FIGURES 2(TOP), 5 & 8; LEFT: ONCE THROUGH, RIGHT: RECYCLE.
(numbers refer to figures in Appendix VI)

APPENDICES

I. Gaudy, A. F. Jr., "Dynamics of Mixed Microbial Populations Under Both Stable and Changing External Environments." Presented at the Second US-Japan Seminar on Dynamics of Microbial Populations, Minneapolis, Minnesota, May 5-10 (1974).

II. George, T. K., and Gaudy, A. F. Jr., "Response of Completely Mixed Systems to Hydraulic Shock Loads." Journal Environmental Engr. Div., ASCE, 99, No. EE5, 593-606 (1973).

III. George, T. K., and Gaudy, A. F. Jr., "Response of Completely Mixed Systems to pH Shock." Biotechnology and Bioengineering, XV, 5, 933-949 (1973).

IV. Gaudy, A. F. Jr., "The Transient Response to pH and Temperature Shock Loading of Fermentation Systems" Presented at the 168th National Meeting American Chemical Society, Atlantic City, New Jersey, September 8-13 (1974), Biotechnology and Bioengineering (In press 1975).

V. George, T. K., and Gaudy, A. F. Jr., "Transient Response of Completely Mixed Systems to Changes in Temperature." Appl. Microbiol., 26, 5, 796-803 (1973).

VI. Krishnan, P., and A. F. Gaudy, Jr., "Response of Activated Sludge to Quantitative Shock Loading Under a Variety of Operational Conditions," Presented at 30th Annual Purdue Industrial Waste Conference, Lafayette, Indiana, May 6-8, 1975.

APPENDIX I

Gaudy, A. F. Jr., "Dynamics of Mixed Microbial Populations Under Both Stable and Changing External Environments." Presented at the Second US-Japan Seminar on Dynamics of Microbial Populations, Minneapolis, Minnesota, May 5-10 (1974).

DYNAMICS OF MIXED MICROBIAL POPULATIONS UNDER BOTH STABLE AND CHANGING EXTERNAL ENVIRONMENTS

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INTRODUCTION

It is a pleasure to discuss some of the work my students and I have undertaken to gain understanding of the biological responses to both long-term and short-term changes in environmental conditions. A large portion of the work we have undertaken deals with "natural" or heterogeneous microbial populations because one of our main interests is the biological treatment of waste substrates wherein mixed populations prevail. The biological treament process which has occupied much of our investigational interest is the so-called activated sludge process, especially as operated in reactors wherein complete mixing exists. One of the overriding purposes for studying environmental response of such systems is to gain an idea of the biological or biochemical factors, as well as physical factors, which determine levels of unused organic substrate carbon (S_e) in the system, and the level of cells (i.e., biomass), X, at the corresponding substrate level. Such information is put to use in predictive formulations for design and operation of the bioengineered process, wherein lies one of our major reasons for engaging in the research.

There are a number of ways one can approach the problem of providing sufficiently accurate descriptions of behavior of activated sludge processes to allow prediction of levels of biomass, X, and effluent substrate, S_e. The approach, or guiding philosophy, we have used is one which recognizes the complexity and uncertainty contributed by

Prepared for the second US-Japan Seminar on the Dynamics of Microbial Populations - May 5-10, 1974, Minneapolis, Minnesota.

heterogeneity in the population, but maintains that such systems are not so dissimilar from single species growth that the theory of continuous culture cannot be used as a starting point. The general theoretical concepts of continuous culture as set forth over two decades ago by Monod (1) and by Novick and Szilard (2), and later elaborated upon by Herbert, Elsworth, and Telling (3), are well known and need not be discussed here. The theory recognizes that there may be small changes in S and X, but these are steadied because of the relationship between the specific growth rate, μ, and the substrate concentration, S. One such relationship which has enjoyed wide usage is the one put forth by Monod and is shown in Equation 1:

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S} \qquad (1)$$

If one runs a chemostat at a particular dilution rate under rather controlled constant chemical and physical conditions in the laboratory, it can be shown that there ensues a rather steady level of X and S. On the other hand, if one disturbs the system by imposing a rather large step increase in, for example, influent substrate concentration, S_i, so that the system does not return to the previous steady state but assumes a new one, the transition stage in X and S is not accurately predicted using the Monod relationship between specific growth rate and substrate concentration. If one administered such a shock and measured at time t an increase in S_e due to the increased S_i, the corresponding value for μ should be computable by Equation 1 and the corresponding value of X could then be computed from a time dependent form of the continuous culture equations. We have performed such experiments and have found that the value of μ predicted using equations incorporating the Monod expression is lower than the observed μ in the system when the sampling time, t, occurs

during the period when S_e is increasing, and the reverse situation exists at a later stage in the transition, i.e., when S_e is decreasing and approaching the new steady state value (4). Such results indicate that μ is not as responsive to S as is stated by Equation 1. Such a finding is also consistent with the concept of growth rate hysteresis as put forth by Perret (5). One way this lag in response can be illustrated is as follows: Figure 1 shows the response in X (top) and in S (bottom) when cells growing in a once-through chemostat at a dilution rate of 0.244 hr^{-1} with S_i at 450 mg/l glucose, measured as chemical oxygen demand (COD), were subjected to a shock loading consisting of a change in S_i to 1450 mg/l glucose COD. The change in S_i was made at time 0, and the data to the left of zero show the initial steady state condition of X and S. Such a shock can be termed a "quantitative" shock, indicating that the sole change was in quantity (concentration) of feed substrate (6). In addition to measuring the filtrate COD concentrations, the substrate was also measured using the anthrone test (filtrate carbohydrate COD). It can be seen in the bottom portion of the figure that there was considerably less leakage of carbohydrate than total organic matter (COD). This aspect of the results is a rather important consideration often encountered in working with heterogeneous populations and sometimes with pure cultures, as well. It can be seen that if one measured S_i by the anthrone test, the apparent leakage would be less severe than that measured by the test for total organic matter (COD) in the filtrate. With regard to environmental pollution control, however, it matters little that the carbon source leaked from the system has been metabolically changed from the original one. What does matter is that the shock caused a significant amount of carbon source to exit the system. It is this applied concern which makes it mandatory for the

applied scientist to employ a general rather than a specific test for substrate.

It can be seen that the system underwent a rather smooth transition between initial and final steady states. In keeping with the theory of continuous culture, the cells responded to the higher concentration of substrate by an increase in specific growth rate, and in approximately eight hours (two mean hydraulic retention times) attained a new steady state. Thus, in a qualitative sense, for this macro-change in S_i, the system followed a course consistent with the course of events predicted by continuous culture theory for micro-changes in S. One may determine if the change in μ is quantitatively in accord with the Monod relationship by determining the slope of the biological solids curve at various times during the transient, and comparing these μ values with those predicted by the Monod equation (Equation 1). One may determine the values for maximum specific growth rate, μ_{max}, and saturation constant, K_s, from batch growth data, and substitution of these "constants" in Equation 1 along with observed substrate values obtained from the filtrate COD data in the bottom portion of Figure 1 permits calculation of corresponding values for μ. These two sets of values for μ at the various concentrations of S observed during the 8-hour transient period are shown in Figure 2. It is seen that during the period while S was increasing to the maximum leakage level (430 mg/l), the observed values of μ lie below those predicted by the Monod relationship whereas in general the situation is reversed as the substrate concentration returns to the steady state level. The hysteresis envelope described by the data is, to be sure, not the smooth one predicted by Perret, but the data are consistent with the concept of growth rate hysteresis. Returning to Figure 1, the dotted lines show the course of change in X and S using

time-dependent forms of the continuous culture equations, and experimentally determined values for μ_{max}, K_s, and cell yield, Y, for the system. It is seen that the model equations provide only a rough approximation in the transient stage.

From work such as this, we have concluded that the general theory of continuous culture employing a Monod-type of relationship between μ and the growth-limiting constituent in the system will probably not suffice for predicting the transient response when the system is shocked.

Stabilizing Dynamic Behavior due to Internal Instability

In view of the above conclusion, one may wonder if the theory of continuous culture is usable for relatively stable conditions of operation. We have done rather extensive work on the growth of heterogeneous populations under fairly steady conditions in once-through chemostats, and have found that even though there are at times gross changes in species predominance, there does develop a relatively steady condition in S and X. There is somewhat more variation in X than in S, because of changes in predominance and concomitant changes in cell yield, Y (7)(8). Figure 3 is an example of the type of data one obtains in a once-through chemostat for heterogeneous populations. It can be seen here that there is considerably more steadiness in S than there is in X. It can also be seen that from such data one can obtain a fairly reliable estimate of average or mean "steady state" values. We have been able to use the continuous culture equations of Herbert (9) to predict levels of X and S at various dilution rates employing experimentally determined values of μ_{max}, K_s, and cell yield, Y. Most of these studies have been conducted at temperatures in the range of 20-25^{o}C using relatively easily-metabolized carbohydrates as carbon source in mineral salts medium. The

microbial populations originate from municipal sewage. The values for μ_{max}, K_s, and Y were generally in the range of 0.4 to 0.6 hr^{-1}, 50 to 150 mg/l, and 0.4 to 0.6 mg/mg, respectively (7)(8)(10)(11)(12). Thus, if one accepts the sufficiency of only an approach to steady state, or a pseudo steady state, the theory of continuous culture can be said to apply to heterogeneous populations when the operational conditions are held fairly steady.

Most activated sludge plants are not operated as once-through chemostats, but incorporate rather massive cell feedback. Equations incorporating cell feedback into continuous culture theory have been employed for some time, and those given by Herbert have been widely used (9). With cell feedback there are two new operating factors which may be distinguished: α, the hydraulic feedback ratio, expressed as the ratio of the rate of inflow feed to rate of flow of feedback slurry, and the feedback concentration factor, c (as employed by Herbert), which is the ratio of cell concentration in the recycle, X_R, and the reactor, X (i.e., $c = X_R/X$). The factors α and c are treated as selectable system constants. Using heterogeneous populations of sewage origin, we have made rather extensive laboratory-scale pilot plant investigations in which we have attempted to run a chemostat with cell feedback holding α and c as system constants (8). Figure 4 shows one such run for which α was 0.25 and c (i.e., X_R/X) was maintained at 1.5. It is rather difficult to operate a system with constant c for heterogeneous populations wherein there is variation in X, because when X changes, X_R must also be changed to keep c as a constant. It can be seen in the figure that c was maintained fairly close to the selected value of 1.5. It can also be seen that the reactor did not attain a steady state in X. Regarding substrate S, there was more fluctuation than was observed in the once-

through system, but compared to the variation for X in this figure, S can be said to have remained fairly steady. It should be noted that the scale for substrate is expanded eight-to-one as compared to the top graphs. In general, it can be adjudged that S remained fairly steady over the 2-month period of operation shown. As unsteady as the condition of X and S may be, one can still take an average of two or three months' data at each dilution rate. Using such data as representative of the "steady state" condition, it was possible to predict the dilute-out patterns using values of the biological constants obtained during the various experimental runs, and to show that the predicted and observed data agreed fairly well.

While it may then be possible to conclude that the general theory of continuous culture for cell recycle systems can be used to predict a general trend in X and S with dilution rate for heterogeneous microbial populations, the mode of operation surely cannot be said to enhance attainment of a very "steady" state. Furthermore, operation with constant c is a monumental operational chore since the operator holding to such a model must change the concentration, X_R, as X changes in order that his mode of operation complies with the stated condition of the model. More important to our current consideration than the physical difficulty of using such a model is that its use in an inherently dynamic system such as one employing heterogeneous populations actually fosters variation in X. For example, consider a situation wherein there has been a slight ecological shift leading to a change in cell yield, Y, causing an increase in X from its former steady value. After noting the increase in X, one must increase X_R in order to maintain c constant. The increase in X_R, instead of decreasing the concentration X to its former value, only serves to displace X further upward, thus causing

greater unsteadiness and further militating against the successful utilization of the model. We were forced to the conclusion that the continuous culture theory with inclusion of cell recycle, as put forth by Herbert, while perhaps satisfactory for pure culture systems, did not suffice for the more ecologically dynamic mixed microbial population.

Notwithstanding the problems of applicability of continuous culture theory, it was desirable to determine if some modification could be devised which might steady the system yet maintain conceptual communion with the original theory of continuous culture. In the interest of providing steadiness in X, it seemed that rather than employ a constant recycle concentration factor, $c = XR/X$, it would be better to make X_R the selectable system constant. Such an assumption in the derivation of the model equations sets a rather severe and somewhat unrealistic constraint at the extreme boundary condition of high dilution rate since the equations do not allow the system to compute to the total washout of cells, i.e., X can never be equal to zero, but can only approach a lower level equal to $X_R\left(\frac{\alpha}{1+\alpha}\right)$. Be that as it may, from a practical standpoint one would not wish to operate a biological waste treatment system anywhere close to the extremely high dilution rates required for washout, and we decided to write a materials balance holding X_R rather than c as a constant (13). The derived equations are shown in Table I and are compared with those of Herbert for cell recycle systems. Ours are somewhat more cumbersome because the derivation leads to the quadratic form, but they are not unduly complicated and are easily computerized. In Figure 5 the dilute-out behavior in S and X as dilution rate is increased is graphically compared with that for Herbert's equations. It can be seen that the assumption of constant X_R adds considerable stability in regard to the "staying power" of the system as dilution rate

increases. Figure 6 shows a flow diagram we envisioned for the process. The essential difference between this diagram and the usual ones including cell feedback is the inclusion of a second aeration tank which serves as a biomass makeup and/or dosing tank. Here the recycle cells are made up to the pre-selected operating level, X_R. The recycled biomass may be thus treated as a chemical (biochemical) dosage to reactor number 1. It was felt that this modification was one which did maintain conceptual communion with continuous culture theory as well as offer some means of engineering control to help stabilize the inherent internal dynamic nature of the system, and we designed laboratory pilot plant studies to determine if this surmise were true. One should recognize that some field modifications of the activated sludge process employ two aeration tanks similarly arranged (e.g., contact stabilization); however, the function of the tanks is not that herein proposed.

Recently, we have run pilot plant studies employing this model, and an example of the type of data obtained is shown in Figure 7 (14). The dilution rate during this 30-day period of operation was 0.125 hr^{-1}. Comparison of these results with those of Figure 4 demonstrates that this mode of operation tends to assist in bringing the system closer to the steady state assumption made in the derivation of the continuous culture equations.

Presently, we are continuing to study the "steady state" behavior under this mode of operation (15). And we are including various auxiliary studies to characterize the biomass. These necessarily include separate batch experiments to determine μ_{max}, K_s, cell yield, etc. Also, because of the practice of cell recycle, it must be remembered that the specific growth rate, μ, is much lower than for a once-through system operating at the same dilution rate. With an X_R value of 10,000 mg/l

and with α and S_i values in ranges usually encountered in the field, specific growth rates of approximately 0.1 day^{-1} may be encountered. At such a low steady state specific growth rate, the cells in the system may be subjected to significant amounts of autodigestion during the residence time in the system; thus, the amounts of excess sludge produced each day (or the observed cell yield) may be lower than that predicted using the so-called "true" cell yield. It can thus be readily appreciated that our current studies on our activated sludge model offer an opportunity to examine the concept of cell maintenance. Thus far we have observed kinetic effects consistent with the cell maintenance concept, but our data have caused us to have some doubt regarding the conceptual validity of the mechanism responsible for the lower cell outputs at lower specific growth rates (16)(17). These studies are continuing because they are of basic significance regarding fundamental growth kinetics in continuous and batch culture; also from a practical standpoint we are interested to know if we should include in our steady state model equations a term for autodigestion. Inclusion of such a term may or may not prove to be a desirable adjustment to the model. In any event, its inclusion would add one new biological "constant" to the model.

Dynamic Behavior Due to External Stress (Shock Loadings)

To this point, we have discussed some ways and means (constant X_R) by which we have been able to bring some modicum of steadiness to an inherently dynamic (heterogeneous) microbial system when we operate the system under rather steady conditions of S_i, temperature, pH, flow rate, etc. We are planning to perform experiments on this system wherein we administer perturbations such as the type shown in Figure 2 for once-

through systems. In fact, at this writing we have already accomplished some preliminary shock loading experiments involving a three-fold increase in S_i, and the system does seem to exhibit a high degree of stability in S to such shocks. However, the great amount of our shock load work was not designed to test the adaptability of this particular system or model. Our interest in shock loading has been pursued over a long period of time; in fact, it pre-dates by many years the development of the model I have just described. Some of our shock loading studies have been conducted in batch systems when such an experimental design seemed best for the particular matter being investigated. Also, although we have conducted many continuous culture system shock loading studies during which cells were recycled, most of the shock load studies were conducted using once-through completely mixed culture reactors. Perhaps the reason for employing once-through systems which is most readily appreciated by fellow experimentors is the difficulty of operating systems with cell recycle. Not only does cell recycle complicate the analysis of the response, but the physical difficulties of keeping the plant operating in accord with the model (with either constant c or X_R) during the transient are many. However, such problems can be overcome, and the main reasons for our interest in "once-through" system responses are more fundamentally significant than those of expediting the experiment. From a practical engineering standpoint, cell recycle attenuates the severity of substrate leakage largely because of the high concentration of biomass, whereas the once-through system is less resistant to change. In addition to the lower biomass concentration, such systems operate with relatively young cell ages, i.e., higher specific growth rates. As yet we do not really know all the reasons why these two factors (high biomass concentration and slower specific growth

rate) attenuate the degree of substrate leakage during shock. However, most of our data indicate that such is the case. Thus, if one sets limits of tolerance based upon the once-through response, he may expect that they will be on the conservative side and provide some engineering safety factor against substrate leakage. From a basic standpoint of trying to understand mechanistic principles governing both biochemical and ecological response and of attempting to describe the transient mathematically, the once-through system is the obvious starting place. When one contemplates the complex biochemical and ecological mechanisms which may be set in motion when various external perturbances are imposed upon the system, there is some reason to doubt that transient models for adequate description of the response of heterogeneous populations will become available in the near future. However, it should be apparent that useful descriptive models, whether conceptually sound or not, cannot be forthcoming without the obtaining of experimental results against which to test the response predicted by any of the possible models. Our approach has thus been to examine responses to various shocks in once-through systems with a definite aim of establishing practical (presently conservative) limits of adaptability to external changes and of gaining useful data with which to characterize the response patterns. While we cannot at this time present a model with which to predict the transient response, we have amassed and are continuing to amass a large body of experimental data under known and controlled conditions, and I shall devote the remainder of the report to presenting some of these results. I hope I may be forgiven if I avoid making sweeping general conclusions. We have amassed so much data that not all of it has been fully analyzed in conceptual detail as yet. General trends and patterns are emerging, and brief mention of these will be provided.

Response to Quantitative Shock Loadings

With regard to the quantitative shock load, it might be recalled that Figure 1 showed the general trend in response to an increase in S_i. That was a response we would tend to call a biochemical or immediate response to the change; that is, it represents a response similar to one which might also occur with a pure culture. In a gross sense, it is an en-masse response of the biomass behaving like an "average" culture or species. For such studies, pure cultures might serve as useful tools for for investigation, and the mode of study essentially parallels the work undertaken by microbial kineticists. However, Figure 8 shows a response that probably would not have occurred had not the population been a heterogeneous or mixed population (18). There are many parameters shown in this figure, but I would like to call your particular attention to the bottom set of graphs depicting the condition of the effluent for this once-through chemostat operation (dilution rate = 0.125 hr^{-1}). The system had attained a relatively steady state condition with S_i at 1000 mg/l (glucose). The feed concentration was then changed to 2000 mg/l and the response was a rise in biological solids concentration with no transient leakage of substrate. The immediate biochemical response was a success; the system accommodated a doubling of S_i without any leakage of carbon source or disruption of the steady state in S. However, more than 30 hours after administering the shock there was a rather severe disruption. We can account for this upset as an ecological response induced by the shock load in S_i. The severe transient leakage occurred during a period when there was a drastic change in species predominance in the system. The shift in predominating species was marked by a change in color of the mixed liquor, and microscopic examination revealed a drastic change in morphological characteristics of the cells comprising the biomass. The

shift could be attributed only to the change in S_i. A more severe shock which caused a transient substrate leakage during the immediate response revealed that the carbon source in the effluent COD consisted largely of compounds other than the feed substrate. These results are shown in Figure 9. It can also be seen that there was again a secondary or ecological response. The elaboration of metabolic intermediate products by the existing population during the early or metabolic en-masse response may be a factor which contributes to the shift in predominance which comprises the secondary response. In these cases, the secondary responses are much more disruptive than the en-masse metabolic response. One might say that results such as these emphasize the difference between pure, or unnatural, cultures and mixed, or natural, populations. If nothing else, they emphasize the complexity of the mixed system and the difficulty one might have in modeling transient response. There certainly would seem to be a need for caution in applying the necessarily simplistic mathematical model approach to describing or predicting the kinetic course of the transient in S and X due to quantitative shock loadings. Thus, our decision to characterize the response by various shocks through rather careful experimentation rather than spend time, perhaps futilely, seeking to model the response may be appreciated.

Hydraulic Shock Loads

Another type of shock which occurs quite frequently is one which involves a change in flow rate, F. The accompanying change in D and thus in μ ($\mu \sim D$) is one which, like the response to a quantitative shock, requires either an increase or decrease in specific growth rate. Hydraulic shocks can come about simply as a change in D with no change at all in S_i or, as often happens, they may be accompanied by a

considerable change in S_i. For example, oftentimes an increase in flow rate after a storm is accompanied by a decrease in S_i, due to dilution of the waste water. Both situations are worthy of investigation. Also, the immediate past growth history of the cells can be expected to have some effect on the response to the change in D. For example, cells grown at D of 0.1 hr^{-1} before the change might be expected to respond differently than a population grown at a D of 1.0 hr^{-1} when D is changed to 2.0 hr^{-1}. In the studies I shall briefly describe (19), we chose a base line dilution rate of 0.125 hr^{-1} because this dilution rate usually places the system on a flat, stable portion of the dilute-out curves in X and S, and this D is not an uncommon one for field reactors in the pollution control field. Synthetic waste consisted of minimal salts medium with glucose as substrate; temperature was 25C and pH was maintained at 7.0.

The next few slides will show some of the responses when dilution rate was changed with no change whatsoever in substrate concentration. Figure 10 shows the response when the dilution rate was decreased fourfold. One would not expect a forcible slowdown in growth rate to precipitate a really deleterious transient response with respect to biological solids and substrate removal. It is clear, however, from the figure, that the system did undergo some disturbance. When the dilution rate was halved (see Figure 11), again there was a slight perturbance, but one certainly would conclude from these results that a decrease in dilution rate while substrate concentration in the feed remains constant, can be accommodated fairly well by the system. The type of hydraulic shock which causes more concern is one consisting of an increase in dilution rate. In Figure 12 it is seen that a doubling in dilution rate caused only a slight transient increase in soluble organic material

in the effluent. As can be seen, the soluble COD (see T-COD) rose from approximately 80 mg/l to a maximum of 130 mg/l within the first three hours, but returned to its former steady state level within the first six hours after changing dilution rate. It is also seen that the organic carbon in the effluent was not the original substrate. The curve labeled "A-COD" represents the amount of anthrone reactive material in the soluble portion of the effluent, and it is seen that the transient rise in COD consisted of materials other than carbohydrate. For a more severe shock from a D of 0.125 to 0.313 hr^{-1} (see Figure 13), the initial pattern of response was the same as in the previous figure. However, the initial dropoff in biological solids concentration and initial rise in effluent COD (see T-COD) was more severe and the recovery of initial conditions in X and S was not complete. The lower biological solids concentration and increased effluent COD suggest that at the new dilution rate, the system was beginning to follow its normal dilute-out curve. This surmise is substantiated by noting the initial and final steady state conditions with respect to S and X when an even greater hydraulic shock was applied, i.e., from 0.125 to 0.375 hr^{-1} (see Figure 14). The same pattern of response with respect to initial and final steady state conditions is seen in Figure 15, which shows the response when the most severe hydraulic shock was placed upon the system; that is, when dilution rate was changed from 0.125 to 0.437 hr^{-1}. However, in this case, the transient response was somewhat different than it was for the previous two figures. In this figure, we see a smooth transient as the system approaches the new steady state, whereas in the two previous figures, the effluent substrate concentration passed through a maximum and the biological solids concentration passed through a minimum before approaching the new steady state level.

In all of the hydraulic shock load results thus far shown, the hourly rate of feeding increased when D was increased, and decreased proportionally when D was decreased, since feed substrate concentration remained constant. On the other hand, one can apply hydraulic shock and maintain a constant hourly rate of feeding substrate. This requires that when dilution rate is increased, substrate concentration must be decreased proportionally, and when dilution rate is decreased, there must be a concomitant increase in substrate concentration. In these cases, the system receives a quantitative shock along with the hydraulically imposed change in μ. We have made studies under this condition of hydraulic shock, and we find that in this case, a decrease in dilution rate is far more deleterious than an increase. That is, the situation is the reverse of that found with the hydraulic shock loadings previously described. Figure 16 shows the response when the dilution rate was changed from 0.125 to 0.031 hr^{-1}. The hourly organic loading was maintained constant, and to do so meant increasing S_i from 1000 to 4000 mg/l. There was a peaked transient in effluent substrate concentration during which the efficiency of substrate removal or of purification decreased; however, the system recovered and the COD removal efficiency returned to the pre-shock level of $\pm$ 90 percent. The response to a less severe decrease in dilution rate (with concomitant increase in S_i) is shown in Figure 17. In this case there was a smooth transition to the new steady state, and the substrate removal efficiency remained essentially constant.

Response to pH Shock

The heterogeneous populations in biological treatment systems are, at times, subjected to a variation in the pH of the inflowing medium.

There has, of course, been much information published regarding the effect of pH on microorganisms; however, there is a relative dearth of experimental information regarding the transition between steady state growth at one pH, and steady state growth at another pH. Indeed, there are very few systematic studies regarding continuous culture of microorganisms at various steady pH levels. Thus, it seemed important to us to gain some insight into responses one might expect to observe and the limit of adaptability for various increases and decreases in pH. Here it was essential that we choose some reasonable base level pH, and the one chosen was 6.4 to 6.7. For each of the experiments shown in the next series of figures, the initial steady state was assessed, the pH of the feed changed, and the resulting transient behavior of the system examined until attainment of the new or final steady state was approached (20). Our major objective was to characterize the response and to obtain some guidelines as to an allowable range of change in pH in the waste stream. In these studies we did, in addition to operation of once-through chemostats, operate some systems with cell recycle. The temperature, rate of aeration, etc., were the same as for the hydraulic shock load studies. The source of the population again was municipal sewage; the organisms were acclimated to the synthetic waste which again consisted of a minimal medium in which glucose was the carbon source. Mild alkaline shocks, for example a change from pH 6.6 to 8.0, did not result in any serious disruption in X and S. Since we were using a phosphate buffer system to bring about the changes in pH, it was difficult to study response to shocks of a higher pH. In any event, it is usually shocks on the acid side which are to be expected in waste streams. Figure 18 shows the response when a once-through chemostat system was subjected to a change from pH 6.7 previous to the shock (see

data to the left of the broken line at time 0) to pH 6.2. Here we see a relatively short-lived but definite transient in X and S before S returns to a steady value. A slightly more severe shock from pH 6.7 to 5.8 to another system again caused a transient response in X, but very little disturbance in effluent quality (see Figure 19). It is interesting to note that in this figure and in the previous one, effluent quality was slightly better in the final steady state which resulted at the new pH. In any event, such a mild decrease in pH, while definitely causing a transient disturbance, would have to be adjudged well within the limits of biochemical adaptability of a heterogeneous population. In fact, even in Figure 20, which shows the response to a decrease in pH from 6.4 to 3.5 after a rather severe transient leakage of substrate and dilute-out of cells, there was a fairly rapid recovery. In this figure we have also plotted the changes in protein, carbohydrate, and DNA content of the biomass. It is seen that there was a considerable dropoff in protein and DNA content and a rise in carbohydrate content in response to the lower pH. Also, it should be noted that microscopic observations indicated that there was a drastic change in predominance between initial and final steady states. As one might expect, the predominating organisms in the final steady state were filamentous fungi.

When a once-through chemostat was subjected to a drop in pH from 6.6 to 3.2 in another experiment, the results were as shown in Figure 21. This shock, which was only slightly more severe than that shown in the previous figure, resulted in a decidedly more severe transient disruption of both substrate removal efficiency and biological concentration. It is seen that the cells diluted nearly completely out of the system before a new population began to establish itself. As before, this new population consisted essentially of filamentous fungi. A similar

response was registered to a slightly more severe decrease in pH from 6.4 to 3.0 (see Figure 22). Again, these experiments were run in a once-through chemostat. The general pattern of response was similar to that of the previous figure; it would seem that there may be some possibility for mathematical description of these peaked transient responses.

When a slightly more severe shock from pH 6.6 to 2.7 was applied in a once-through chemostat, the system had reached the point of no recovery, as is seen in Figure 23. The experiment was terminated after 200 hours, since even though there were very small amounts of biological solids which persisted in the chemostat, the system showed no tendency toward recovery. It seems possible that with prolonged aeration, the very specific population which is required for existence at such a low pH might have developed, provided, of course, such cells existed in the population prior to the shock. The results of microscopic observations of each system as each experiment progressed leaves little doubt that the main response mechanism was an ecological shift from individual microorganisms to the filamentous fungi.

In general, for experiments wherein cells were recycled, the recycle operation had a tendency to attenuate the transient leakage of substrate. Figure 24 shows one experiment in which we practiced total recycle of all settleable cells at a recycle hydraulic flow amounting to one-third of the substrate inflow rate. When such a system was subjected to a very severe shock, i.e., a change in pH from 6.7 to 3.2, there was a rather drastic dilute-out of cells but little or no disruption of substrate removal efficiency. Filamentous forms increased, but they were not the predominant forms after the shock.

Response to Temperature Shock

We have also examined the effects of changes in temperature on the transition between pre- and post-steady state conditions (21). These studies were conducted in once-through chemostats using synthetic waste of the same composition used for the previous studies. As before, all populations were developed from an inoculum of municipal sewage. The base line temperature selected for the pre-shock condition was $25^{o}C$, and the reactors were run at dilution rates of 0.125 and 0.25 hr^{-1}. From the base line temperature, systems were subjected to decreases to temperatures as low as $8^{o}C$ and increases to as high as $57.5^{o}C$. In the next series of figures showing these results, we will be able to compare the effect of dilution rate or specific growth rate on the ability of these heterogeneous populations to accommodate the various changes in temperature which were imposed. In general, it will be shown that the systems responded more favorably to increases than to decreases in temperature, and that regardless of the direction of temperature change, there was less leakage of carbon source in the effluent as well as less dilute-out of cells during the transient phase in the systems growing at the lower dilution rate. When the temperature was changed over a 12-hour period from 25^{o} to $8^{o}C$, neither the system growing at the specific rate of 0.125 or the system at 0.25 hr^{-1} responded successfully. The experiment was carried out for 200 hours after the shock, but there was no indication of impending recovery after this reduction of temperature to the psychrophilic range. However, when a less severe decrease in temperature (i.e., from 25^{o} to $17.5^{o}C$) was studied, there was a period of dilute-out followed by a considerable recovery of substrate removal efficiency. The results of such studies at the two specific growth

rates employed are shown in Figure 25. The data presented on the left-hand side of the figure show the response for the system run at D = 0.125 hr^{-1}, and the data on the right show the response for D = 0.25 hr^{-1}. In each set of data, the values plotted to the left of the dotted line at time zero represent conditions in the pre-shock state. We can see that for the slower growing system there was considerably less disturbance during the transient phase, and there was essentially total recovery in the new steady state at 17.5°C. However, for the faster growing system, there was a more severe and long lived disturbance and, although the system showed partial recovery, there was a considerable (apparently permanent) leakage of carbon source. The feed consisted of 1000 mg/l glucose, and it can be seen that approximately 60 percent of the organic matter contributed by the glucose leaked from the system in the final "steady state." In both systems, it is interesting to note that the transient leakage of soluble organic matter consisted primarily of compounds other than the initial substrate. This is shown by the curves labeled T-COD and A-COD; the former represents total soluble organic matter whereas the latter represents soluble organic matter reactive to the anthrone test. At the higher growth rate, acetic acid was identified as one of the metabolic intermediates and/or end products released. However, GLC analysis of samples from the slower growing system revealed an absence of low molecular weight fatty acid. Changes in various parameters of biochemical composition of the sludge are shown in the top portions of the figures.

When a mild increasing temperature change from 25 to 36°C was applied to the system, as shown in Figure 26, there were some fluctuations and an initial decreasing trend in X, but these did not result in any deterioration of purification efficiency. The most noticeable

effect of this shock at either growth rate was a decrease in cell yield and increase in protein concentration in the faster growing system. When the temperature was increased from 25 to 47°C over a 26-hour period, a severe transient leakage of substrate was observed at both growth rates as seen in Figure 27. Here also we see a rather profound effect of growth rate on the severity of dilute-out in X and leakage in S during the transient. The disturbance in the slower growing system was of less severity and was shorter lived than in the more rapidly growing population. In the slower growing system, dissimilation of the original carbon source proceeded uninterrupted during the transient. Again, at the higher temperature there was a decrease in cell yield. Both systems recovered with regard to substrate leakage in the effluent.

A more severe temperature shock (see Figure 28) which placed the system in the thermophilic range, i.e., a shock from 25 to 57.5°C, led to severe transient disruption and an apparent inability to recover treatment efficiency, at least within the 200-hour period after changing the temperature. Even though neither system recovered to the pre-shock state, it is amply apparent that the slower growing system did not undergo as severe disruption, and regained a higher degree of substrate removal efficiency than did the faster growing population.

There was, in these experiments, a pattern of cell dilute-out and substrate leakage followed by recovery which might eventually be subjected to a reasonable mathematical or predictive analysis for the transient state; however, at this time it seems rather difficult to establish the adequacy of a physiological or mechanistic basis for the response. One might expect an increase in specific growth rate for an increase in temperature and mathematical relationships might be thus established. In all probability, there may be some sort of hysteresis

effect or kinetic lag involved, similar to that shown for quantitative shock loadings. Also, the ecological response would have to be considered, and this is a complicating factor which will require much more experimental research than mathematical model-making. For those shocks involving an increase in temperature there was, during the period of cell dilute-out and recovery, some evidence for changes in species predominance as indicated by changes in morphology within the biomass. For the high temperature shock, the thick, short rods which predominated in the biomass prior to the shock were, in the recovery phase, replaced by thin, elongated cells.

Response to Qualitative Shock Loadings

There is yet one more type of shock which has been of particular interest in our laboratories for many years. This type of shock involves a change in the chemical composition, that is, the qualitative analysis of the incoming feed stock. When the nature of the compounds in a waste stream changes, e.g., from predominantly carbohydrate to mixed carbohydrate and proteinaceous waste materials, one might expect there would be a period of acclimation and/or adaptation before the system could accommodate to the new waste stream. Acclimation might require enzyme induction and some constituents in the wastewater might repress such induction; that is to say, the sequential or diauxic growth on two or more substrates as shown by Monod for pure cultures, might also be manifested in heterogeneous populations. The experiment shown in the next figure is one which I performed many years ago to gain some initial insight into the possible occurrence of sequential growth when the population consisted of a heterogeneous biomass (see Figure 29)(22). The

figure shows a growth experiment in which a mixed population which had been originally obtained from sewage and acclimated over a prolonged period of time to sorbitol was subjected to growth on a mixture of glucose and sorbitol. The optical density curve suggests that sequential growth occurred and the curves for glucose removal and removal of chemical oxygen demand (total soluble organic matter) leave little doubt that glucose was removed first, followed by removal of sorbitol. Thus, there was sequential growth and glucose repressed the synthesis of enzymes required to metabolize sorbitol, even though the small initial inoculum was acclimated to sorbitol. This experiment was the first of many in a study of this type of shock load which is indeed still continuing in our laboratory, and it has from time to time involved the energies, talents, and laboratory facilities of Professor Elizabeth Gaudy and some of her graduate students in microbiology.

Of particular significance in considerations of biological waste water treatment, it should be emphasized that the blockage of removal of one compound by the presence of another in the waste stream is not uniquely dependent upon repression of enzyme synthesis (23). Figure 30 shows the response of Escherichia coli under non-proliferating conditions (nitrogen source was withheld from the system). The cells were acclimated to sorbitol prior to the experiment, and therefore contained a complement of pre-synthesized enzyme, and one can see in this experiment that a rather high initial inoculum was employed. Thus, substrate removal was not dependent upon growth of the cells nor was metabolism of sorbitol dependent upon synthesis of new enzyme. Regardless, it can be seen that glucose was removed and sorbitol was not removed during the incubation period. Results such as these in pure culture studies as well as studies with heterogeneous populations indicated to us that

there was another blockage mechanism in addition to repression to enzyme synthesis, i.e., there was a mechanism which involved blockage at the functional rather than genetic level. We reasoned that such a mechanism would probably involve a feedback control somewhat similar to the feedback controls which were known to exist in biosynthetic pathways. From results such as these, one might reason that in a biological treatment system in which the microbial population is extemely high, the introduction of certain compounds as shock loads to the system could in the most severe case immediately block the metabolism of the waste components to which the sludge had been previously acclimated, and in less severe cases, seriously retard the rate of their removal. Figure 31 shows that such blockage certainly can occur (24). Here we see a case wherein a system was actively removing sorbitol to which it had been previously, thoroughly, acclimated, and upon injection of glucose to the system, sorbitol removal was blocked until the injected glucose had been metabolized. This figure, as with the previous two, represents the results of batch experiments. It is also interesting to examine the response of a continuous culture system to such shock loads. Figure 32 shows the response in a once-through chemostat operating at a dilution rate of 0.25 hr^{-1} ($\bar{t}$ = 4 hours) when the inflowing feed was changed from 1000 mg/l sorbitol (to which this heterogeneous population had been thoroughly acclimated) to 1000 mg/l sorbitol + 1000 mg/l glucose (25). It is seen that neither sorbitol nor glucose leaked in the effluent; however, a considerable amount of metabolic intermediates and/or end products did leave the system in the transient phase between the initial and final steady states. Figure 33 shows the response for a similar system when the inflowing feed was changed from 1500 mg/l sorbitol to 1500 mg/l sorbitol + 1500 mg/l glucose. In this case, at the

higher organic loading there was more severe leakage of organic substrate in the effluent, and there was, within the first four hours after changing the feed composition, a small leakage of sorbitol. Figure 34 shows the response to a somewhat more severe shock. In this case, the system was metabolizing 500 mg/l of glycerol prior to the shock, and after the shock the feed consisted of 500 mg/l glycerol + 1500 mg/l glucose. In this case, it can be seen that the addition of glucose caused glycerol to appear in the effluent for a considerable period of time after the shock. Figure 35 shows the response to a shock load situation in which a once-through chemostat operating with the detention time of six hours with a heterogeneous biomass growing on 1000 mg/l of lysine was subjected to a change in feed consisting of 1000 mg/l lysine COD + 1000 mg/l glucose COD (26). It is seen in the bottom portion of the figure that the shock resulted in a transient response in which the carbon source leakage in the system peaked at 900 mg/l. During the transient, the system showed both leakage of lysine and glucose. The top portion of the figure represents the enzymatic capability of the cells toward lysine (ec/p) as well as the specific substrate removal rate with respect to lysine (rr/p). The ec/p values represent an approximate (whole cell) assay for lysine degrading enzyme. Further explanation and the significance of these rather important parameters can be found in references (26)(27) and (28). When the same shock load was applied to a system which had been growing in pre-shock steady state at a dilution rate of 0.083 hr^{-1}, i.e., a detention time of 12 hours, the response was as shown in Figure 36. Comparison of the response in this figure with that shown in the previous figure indicates that the system which had been growing at a slower rate prior to the shock accommodated it more successfully, i.e., there was less

leakage of substrate. One might say in analysis of the data that the lower dilution rate or specific growth rate also dictated a slower application of the shock substrate, thus leading to the lesser disturbance. It is significant to note, however, that there was also greater repression of specific ability to metabolize lysine (see ec/p values) in the system which was operating at the higher dilution rate, i.e., the faster growth rate.

For all of the shock loads thus far presented, it is emphasized that the carbon source was the growth-limiting nutrient. When constituents in the medium other than the carbon source are the limiting factors for growth, either prior to the shock or after the shock, the biochemical mechanism of response may undergo considerable alteration. For example, we have found in some studies in which nitrogen or magnesium, rather than the carbon source, limited growth; that the response to qualitative shock loading is more deleterious when the system is growing at a slower growth rate than at a higher one. That is, the degree of severity of the response with respect to substrate leakage is reversed from the situation wherein carbon source is the growth-limiting nutrient. We have attempted to explain this difference on the basis of production of metabolite inhibitor and the factors which control the pool of metabolites which may contain the metabolic components responsible for the blockage. We have some evidence that the controlling metabolite(s) may block at or close to the substrate entry level, i.e., they may prevent the substrate from entering the cell.

Our studies on response to all of the types of shock loadings as well as those on growth and physiology of mixed microbial populations in general, are continuing at both the fundamental and applied levels. At the more basic level, we are interested in the physiological and

ecological occurrences during metabolism under relatively constant and under changing environmental conditions. Chemical and ecological understanding of mechanism of response is, in our view, more important than mathematical description since mechanistic understanding is a necessary prerequisite to making kinetic formulations and models of real scientific utility and predictive significance. Mathematical modeling of kinetics without sufficient, mechanistic, conceptual input is rather like putting the cart before the horse or, if you will, assuming a problem has been solved because it has been described. It seems that often we engineers and scientists tend to accord basic significance to a mathematical description of a process rather than to the underlying concept which the mathematical formulation is describing. On the other hand, it must be recognized that there are times when predictive formulas are vitally needed to accomplish some aims and that they may have to be empirically developed because the urgency of their application to a need may not permit the luxury of time for scientific determination of mechanism. In such cases, empirical formulations lacking perhaps in mechanistic depth are highly justificable so long as their successful use does not generate an intellectual and investigational lassitude regarding mechanistic causation or encourage replacement of conceptual theory with conceptual dogma.

The foregoing comments on investigational philosophy or attitude are surely not included as a lecture or "sermon," but are stated simply to explain our approach to the study of the dynamics of microbial populations. We are attempting to gain a basic mechanistic understanding (biochemical and ecological) and are also seeking to gain practical guidelines for mathematic description of processes and limits of accommodation to environmental change.

We believe our model for operation of heterogeneous biomass systems with cell feedback has proven and will continue to prove to be useful in steadying the inherent or internal dynamics of the system which is a result of the heterogeneity of the population regardless of the steadiness or unsteadiness of the external environment. Under conditions of severe change in external environment (i.e., shock loading conditions), some guidelines are emerging with respect to ability of continuous culture systems to accommodate to change. The results of the once-through chemostat studies shown here permit us to set tentative guidelines which are conservative for systems with cell feedback. With respect to quantitative and hydraulic shock loads, systems operating at dilution rates commonly employed in field processes for wastewater treatment can be expected to accommodate without serious disruption a change in D or in S_i of 100 percent. In general, all of the results presented tend to suggest that the growth history prior to the shock may play a significant role in determining the nature of the response. In general, the system growing at the slower growth rate prior to the shock responded more successfully (for example, see the results from the temperature shock). It also seems reasonable that the more biomass in the reactor, the less will be the leakage of substrate during the shock. Cell recycle systems should thus be particularly advantageous, since they both lower the specific growth rate and increase $\bar{X}$ compared to once-through systems. For our recycle model, the following expression is applicable:

$$\mu = D(1 + \alpha - \alpha X_R/\bar{X})$$

Thus, it can be seen that in addition to the hydraulic control imparted by D and α, the recycle solids concentration, X_R, plays a significant role in determining μ as well as providing a high concentration of

biomass to resist change. Also, S_i can affect μ since it affects $\bar{X}$. Also, we cannot overlook a factor which may have an effect on response, i.e., the cell or biomass age, θ_c. We have observed, for example, less deleterious blockage of one substrate by introduction of another for populations of greater cell or biomass age. Since $\mu = 1/\theta_c$, the role of the specific growth rate in determining the response to change may be of considerable significance. Also, from an ecological point of view, slower growth rate enhances the opportunity for greater diversity and co-existence of Eubacteriales and higher forms of microorganisms, and thus stability in the ecosystem. Thus, we can see possible relations between some of the controllable variables which may enhance ultimate control over the dynamics of biological response to change. And while we see no end to our work, we remain happy in its pursuit.

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17. Response to decrease in dilution rate from 0.125 hr^{-1}, S_i = 1000 mg/l glucose to 0.062 hr^{-1}, S_i = 2000 mg/l glucose.

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21. Response to a decrease in pH from 6.6 to 3.2 (no cell recycle).

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25. Response of a heterogeneous microbial population growing in a chemostat at 25°C to a decrease in temperature to 17.5°C. Left, dilution rate = 0.125 per hr; right, dilution rate = 0.25 per hr;

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29. Substrate removal and growth in a multicomponent system, glucose, 300 mg/l plus sorbitol, 300 mg/l sludge acclimated to sorbitol (batch reactor).

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31. Response of sorbitol-acclimated sludge to shock loading with glucose with the use of young cells under growing conditions; shock was applied after sorbitol metabolism was initiated (batch reactor).

32. Shock load response in a steady-state continuous-flow activated sludge unit after change of influent waste composition from 1000 mg/l sorbitol to 1000 mg/l sorbitol + 1000 mg/l glucose.

33. Shock load response in a steady-state continuous flow activated sludge unit after change of influent waste composition from 1500 mg/l sorbitol to 1500 mg/l sorbitol + 1500 mg/l glucose.

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35. Effect of glucose shock load on a carbon-limited reactor growing on L-lysine at a 6-hr detention time ($D = 0.167\ hr^{-1}$). Time plot of response in the reactor when the influent was changed by the addition of 1000 mg/l glucose as COD. Zero time is the time at which the feed was changed; negative time is that prior to administration of the shock. Symbols: ⊙, biological solids concentration; ●, protein concentration; ⊡, total COD; ◧, lysine COD; ◒, glucose COD; ■, metabolic intermediate COD; ◬, lysine removal rate per unit of protein (rr/p); ▲, lysine enzymatic capability per unit of protein (ec/p).

36. Effect of glucose shock load on a carbon-limited reactor growing on L-lysine at 12-hr detention time ($D = 0.083\ hr^{-1}$). Symbols: ⊙, biological solids concentration; ●, protein concentration; ⊡, total COD; ◧, lysine COD; ◒, glucose COD; ■, metabolic intermediate COD; ◬, lysine removal rate per unit of protein (rr/p); ▲ lysine enzymatic capability per unit of protein (ec/p).

TABLE I

COMPARISON OF STEADY STATE EQUATIONS ACCORDING TO MODELS OF HERBERT AND OF RAMANATHAN AND GAUDY

HERBERT c CONSTANT $\left(c = \frac{X_R}{\bar{X}}\right)$	RAMANATHAN AND GAUDY X_R CONSTANT
$\bar{X} = \frac{Y}{1+\alpha-\alpha c}(Si - \bar{S}_e)$	$\bar{X} = \frac{Y[Si-(1+\alpha)\bar{S}_e]+\alpha X_R}{1+\alpha}$
$\bar{S} = \frac{Ks\, D(1+\alpha-\alpha c)}{\mu_{max} - D(1+\alpha-\alpha c)}$	$\bar{S} = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$ $a = \mu_{max} - (1+\alpha)D$ $b = D\left[Si-(1+\alpha)K_s\right] - \frac{\mu_{max}}{1+\alpha}\left[Si + \frac{\alpha X_R}{Y}\right]$ $c = K_s D\, S_i$
$\mu = D(1+\alpha-\alpha c)$	$\mu = D\left(1+\alpha-\alpha\frac{X_R}{\bar{X}}\right)$

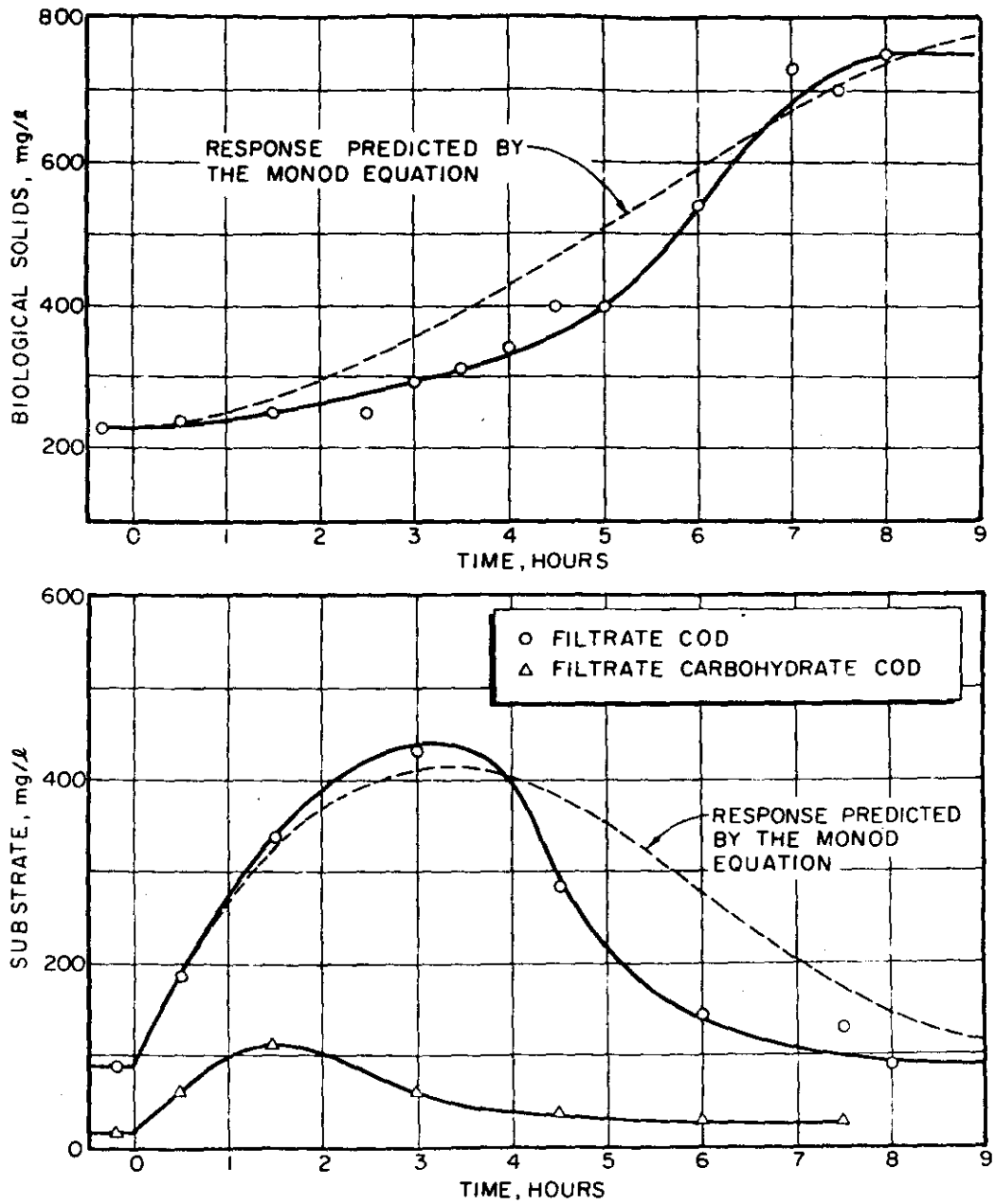
800
600
400
200
BIOLOGICAL SOLIDS, mg/ℓ
RESPONSE PREDICTED BY THE MONOD EQUATION
0 1 2 3 4 5 6 7 8 9
TIME, HOURS
600
400
200
0
SUBSTRATE, mg/ℓ
○ FILTRATE COD
△ FILTRATE CARBOHYDRATE COD
RESPONSE PREDICTED BY THE MONOD EQUATION
0 1 2 3 4 5 6 7 8 9
TIME, HOURS

Figure 1

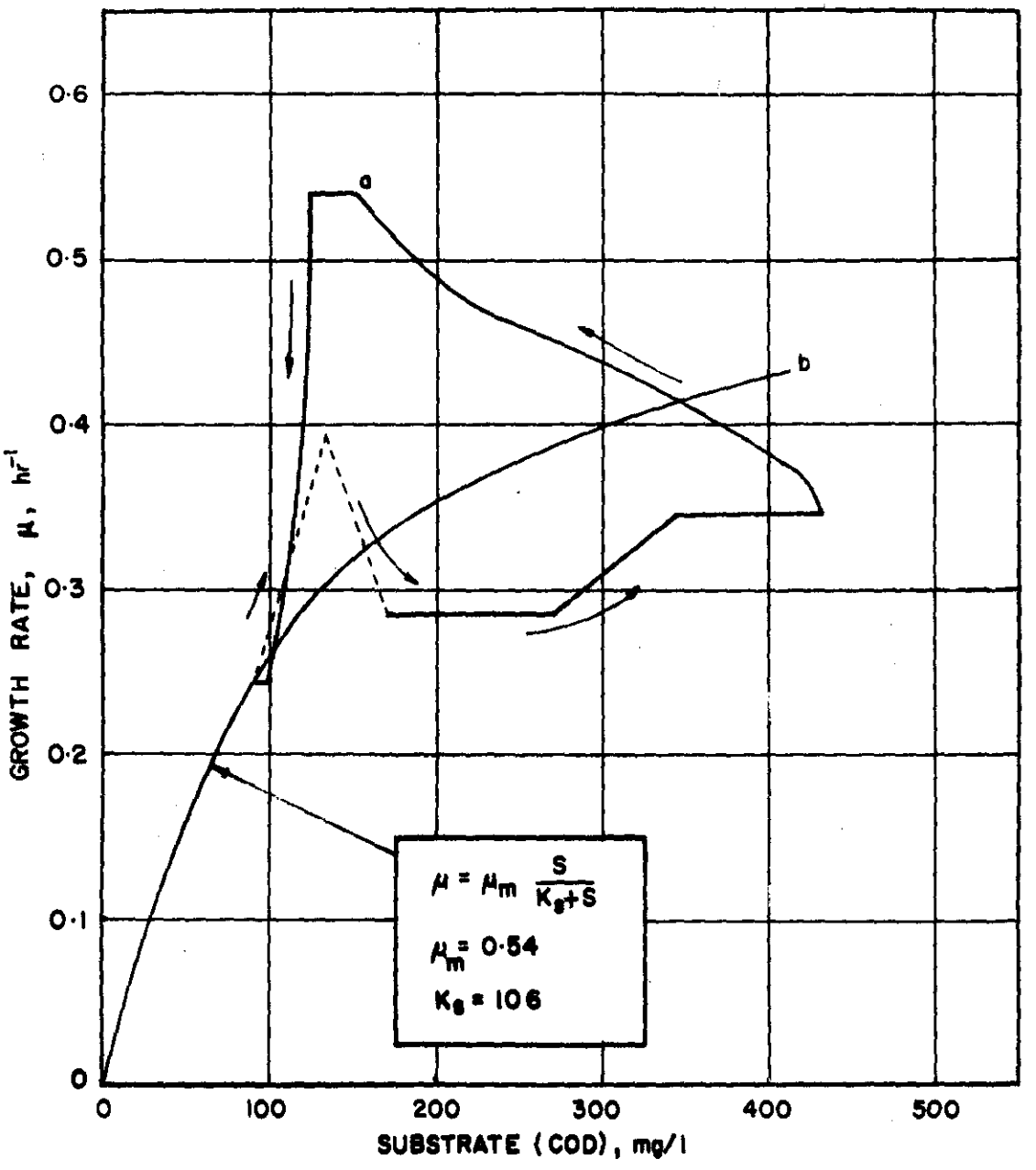
0·6
0·5
0·4
0·3
0·2
0·1
0
GROWTH RATE, μ, hr⁻¹
a
b
$\mu = \mu_m \frac{S}{K_s+S}$
μ_m = 0·54
K_s = 106
0
100
200
300
400
500
SUBSTRATE (COD), mg/l

Figure 2

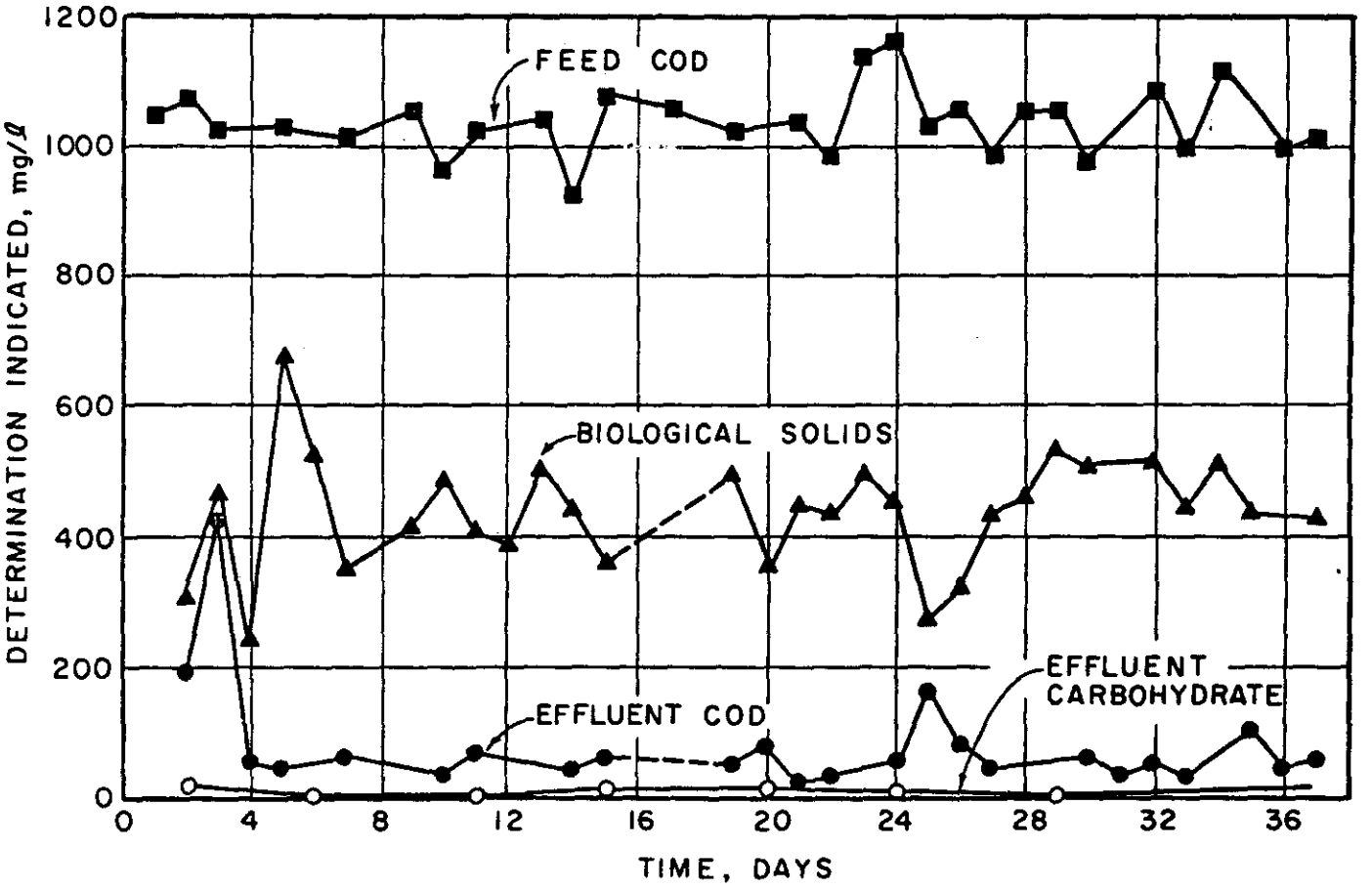
1200
1000
800
600
400
200
0
DETERMINATION INDICATED, mg/ℓ
FEED COD
BIOLOGICAL SOLIDS
EFFLUENT COD
EFFLUENT CARBOHYDRATE
0
4
8
12
16
20
24
28
32
36
TIME, DAYS

Figure 3

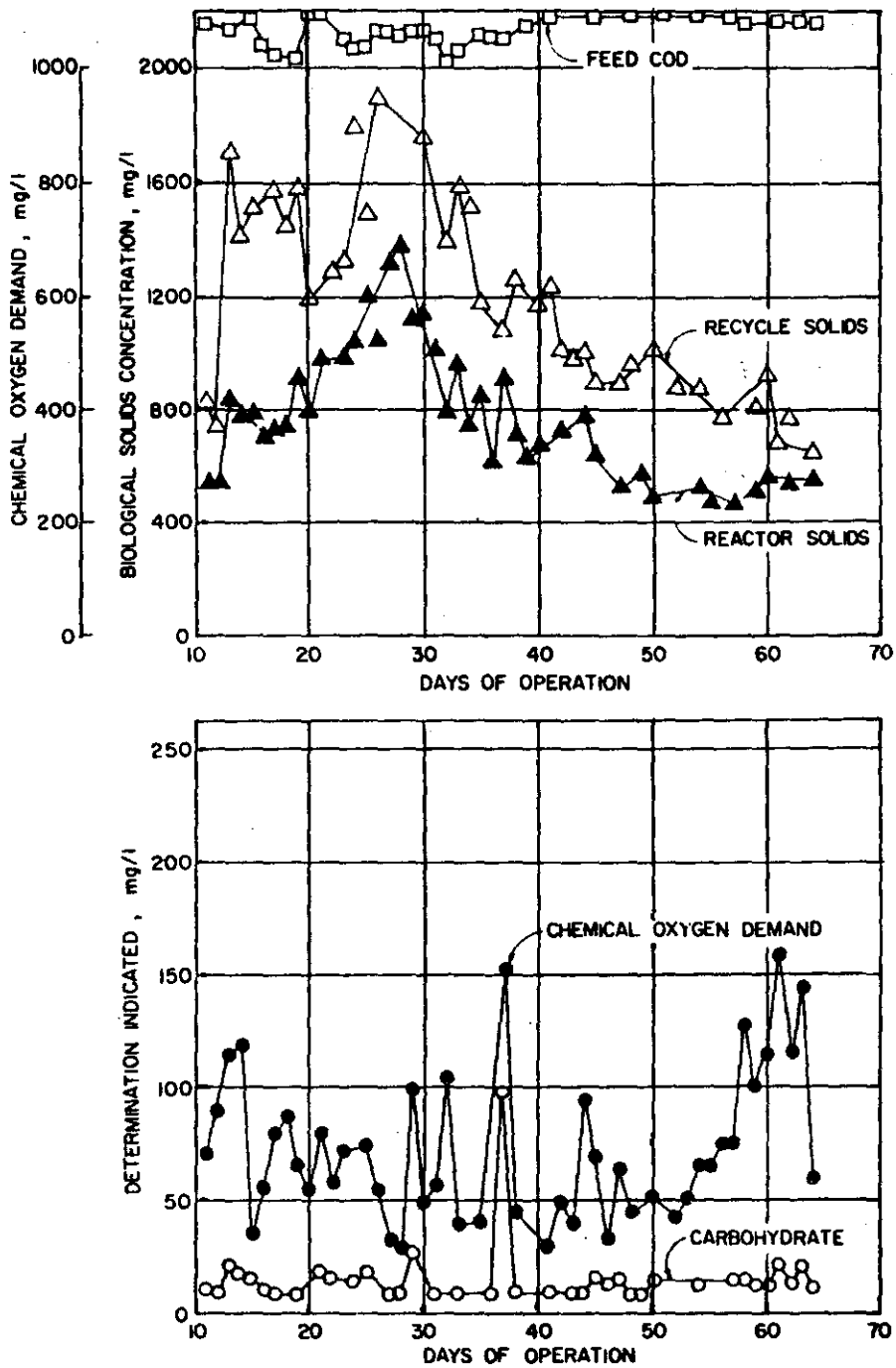
CHEMICAL OXYGEN DEMAND, mg/l
BIOLOGICAL SOLIDS CONCENTRATION, mg/l
FEED COD
RECYCLE SOLIDS
REACTOR SOLIDS
DAYS OF OPERATION
DETERMINATION INDICATED, mg/l
CHEMICAL OXYGEN DEMAND
CARBOHYDRATE
DAYS OF OPERATION

Figure 4

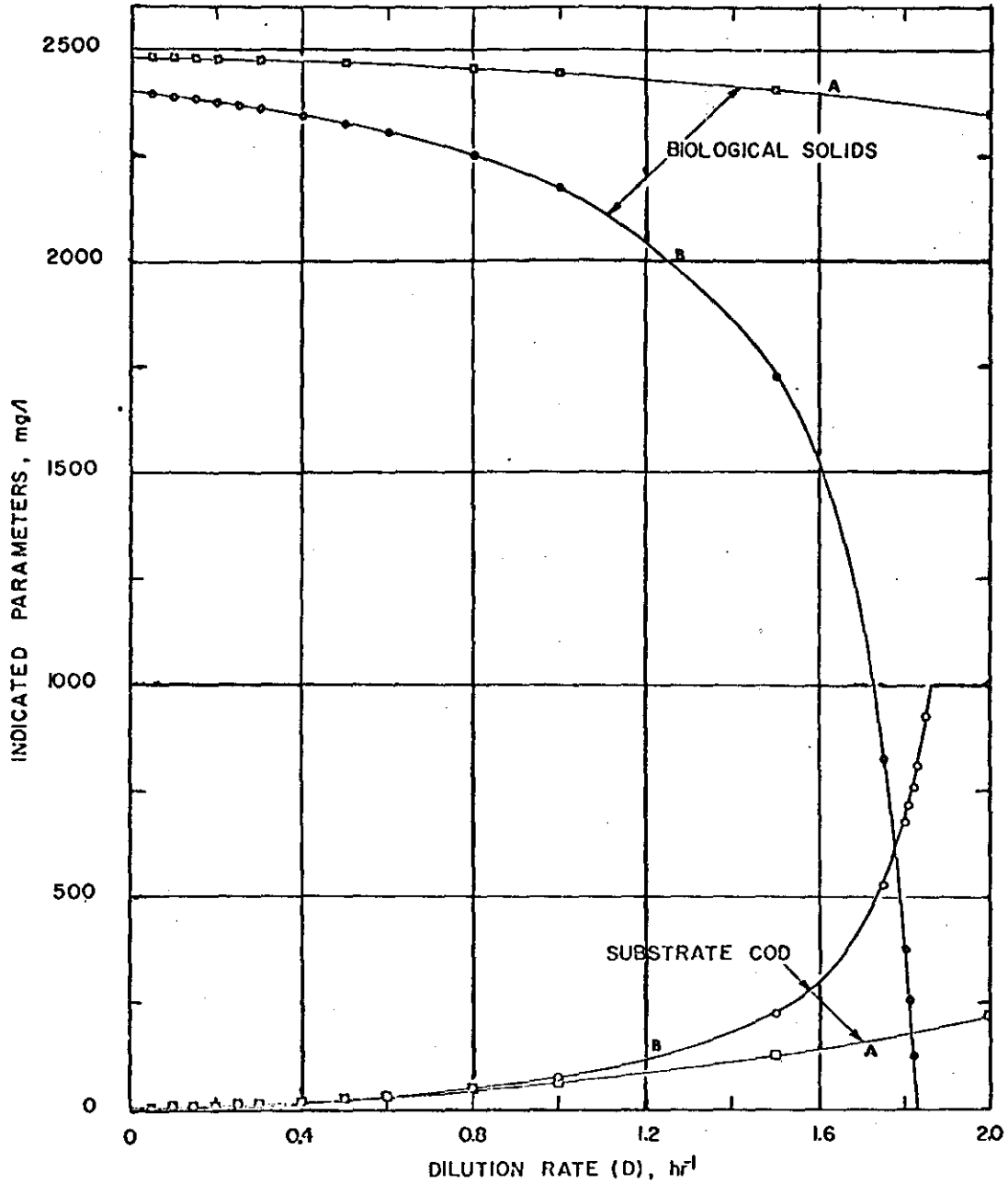
2500
2000
1500
1000
500
0
INDICATED PARAMETERS, mg/l
BIOLOGICAL SOLIDS
A
B
SUBSTRATE COD
A
B
0
0.4
0.8
1.2
1.6
2.0
DILUTION RATE (D), hr⁻¹

Figure 5

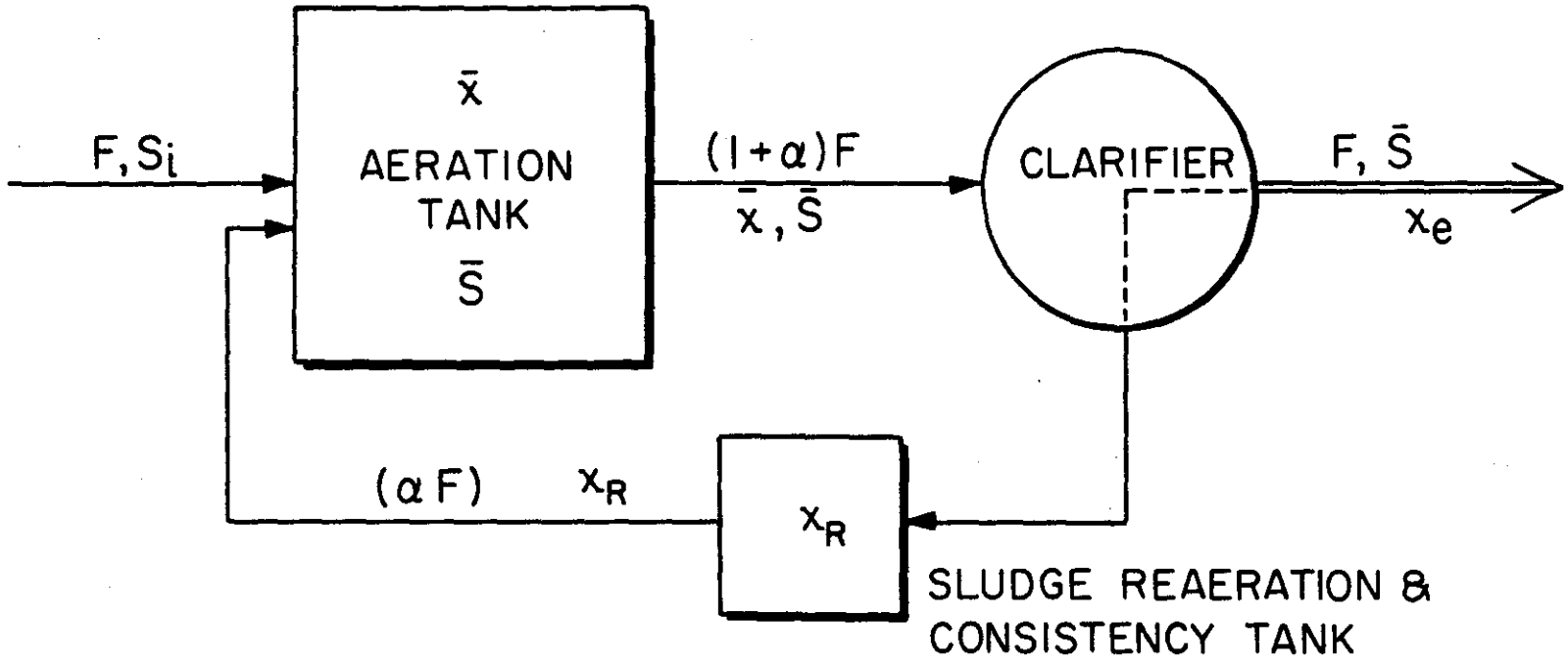
F, Si
$\bar{x}$
AERATION TANK
$\bar{S}$
(1+α)F
$\bar{x}$, $\bar{S}$
CLARIFIER
F, $\bar{S}$
x_e
(α F)
x_R
x_R
SLUDGE REAERATION & CONSISTENCY TANK

Figure 6

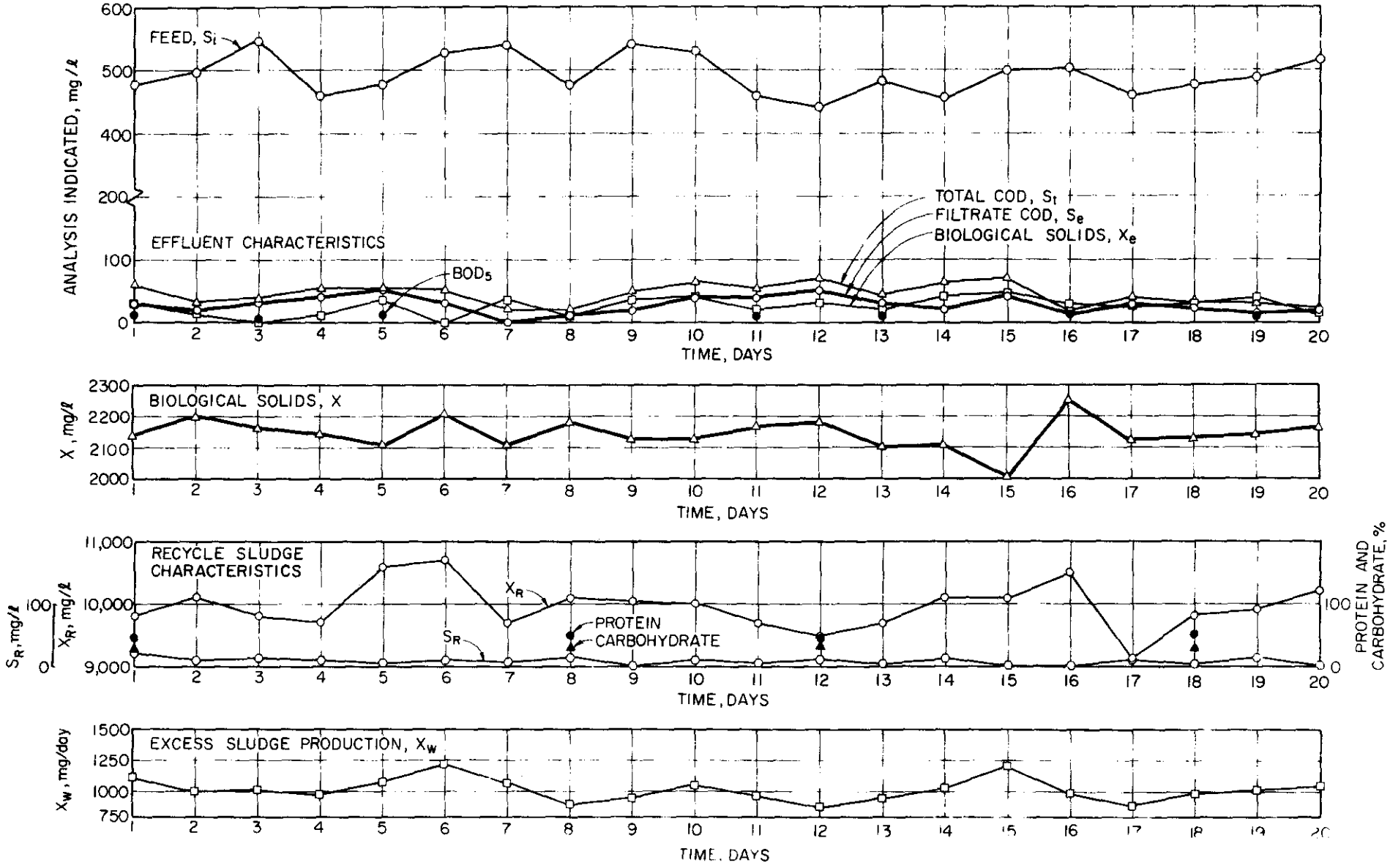

Figure 3 OPERATIONAL CHARACTERISTICS FOR AN ACTIVATED SLUDGE PROCESS WITH CONSTANT X_R AT AN S_i OF 500 mg/ℓ

Figure 7

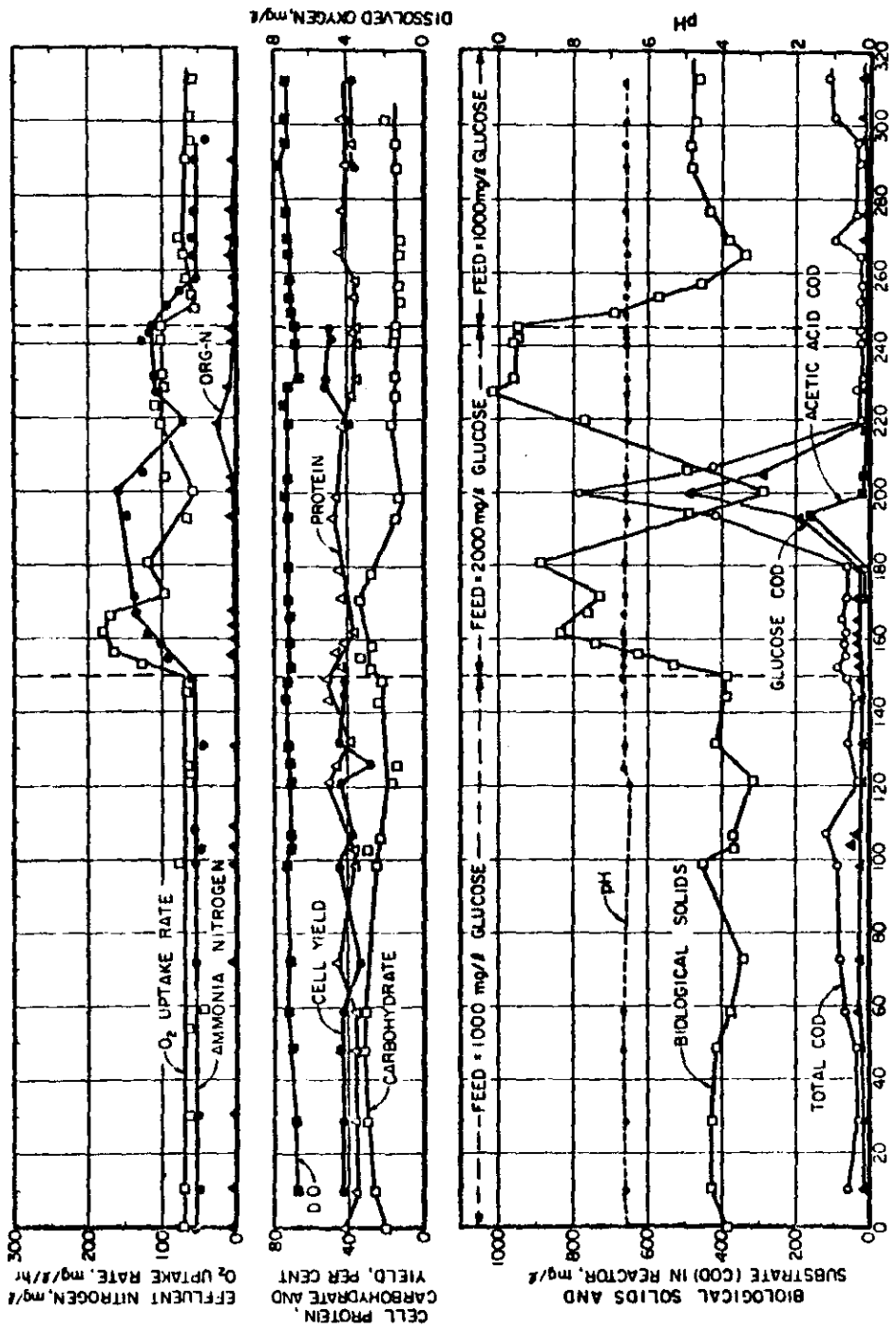
EFFLUENT NITROGEN, mg/ℓ
O2 UPTAKE RATE, mg/ℓ/hr
O2 UPTAKE RATE
AMMONIA NITROGEN
ORG-N
CELL PROTEIN, CARBOHYDRATE AND YIELD, PER CENT
CELL YIELD
PROTEIN
CARBOHYDRATE
DO
DISSOLVED OXYGEN, mg/ℓ
BIOLOGICAL SOLIDS AND SUBSTRATE (COD) IN REACTOR, mg/ℓ
FEED = 1000 mg/ℓ GLUCOSE
FEED = 2000 mg/ℓ GLUCOSE
FEED = 1000 mg/ℓ GLUCOSE
pH
BIOLOGICAL SOLIDS
TOTAL COD
GLUCOSE COD
ACETIC ACID COD
TIME, hrs

Figure 8

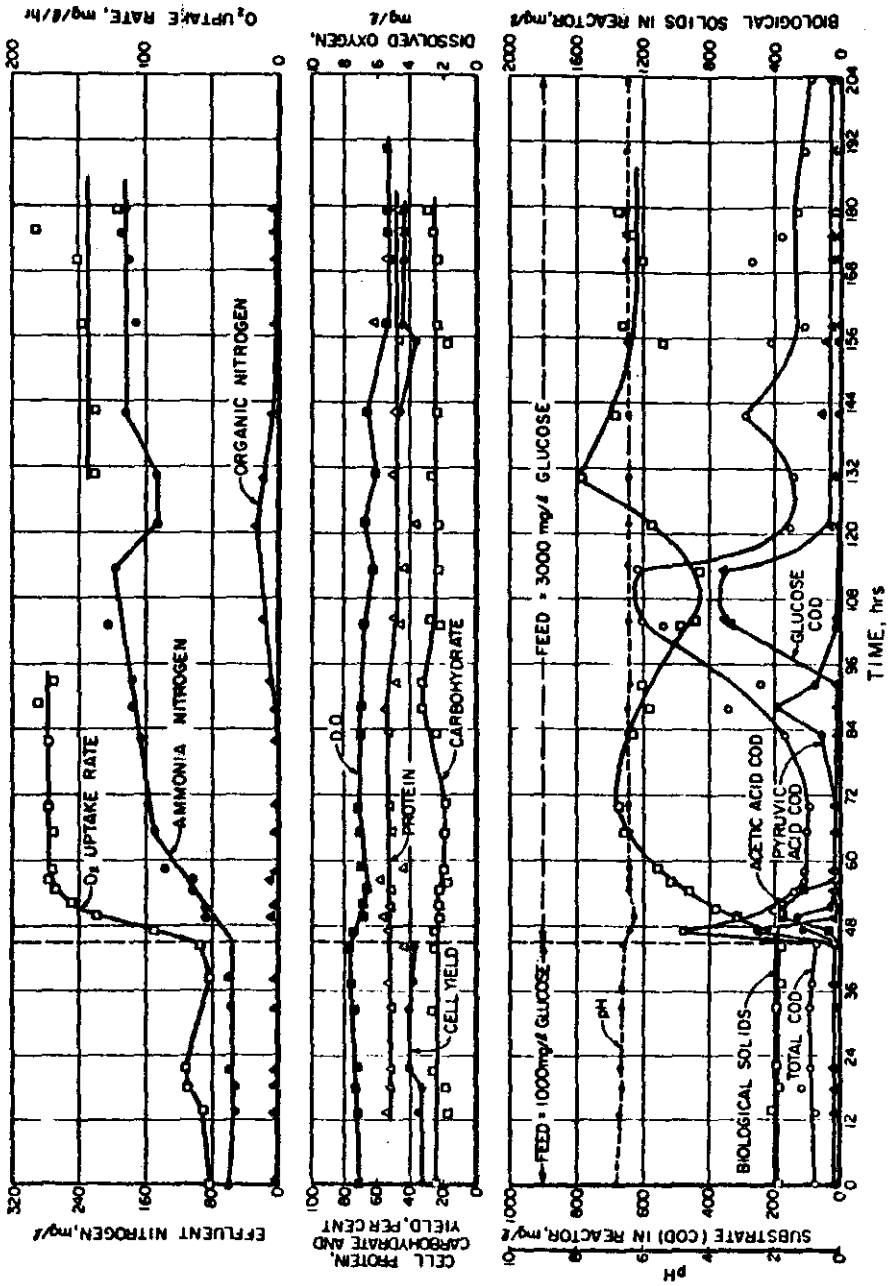
BIOLOGICAL SOLIDS IN REACTOR, mg/ℓ
DISSOLVED OXYGEN, mg/ℓ
O₂ UPTAKE RATE, mg/ℓ/hr
SUBSTRATE (COD) IN REACTOR, mg/ℓ
CELL PROTEIN, CARBOHYDRATE AND YIELD, PER CENT
EFFLUENT NITROGEN, mg/ℓ
pH
FEED = 1000 mg/ℓ GLUCOSE
FEED = 3000 mg/ℓ GLUCOSE
BIOLOGICAL SOLIDS
TOTAL COD
ACETIC ACID COD
PYRUVIC ACID COD
GLUCOSE COD
D.O.
PROTEIN
CARBOHYDRATE
CELL YIELD
O₂ UPTAKE RATE
AMMONIA NITROGEN
ORGANIC NITROGEN
TIME, hrs

Figure 9

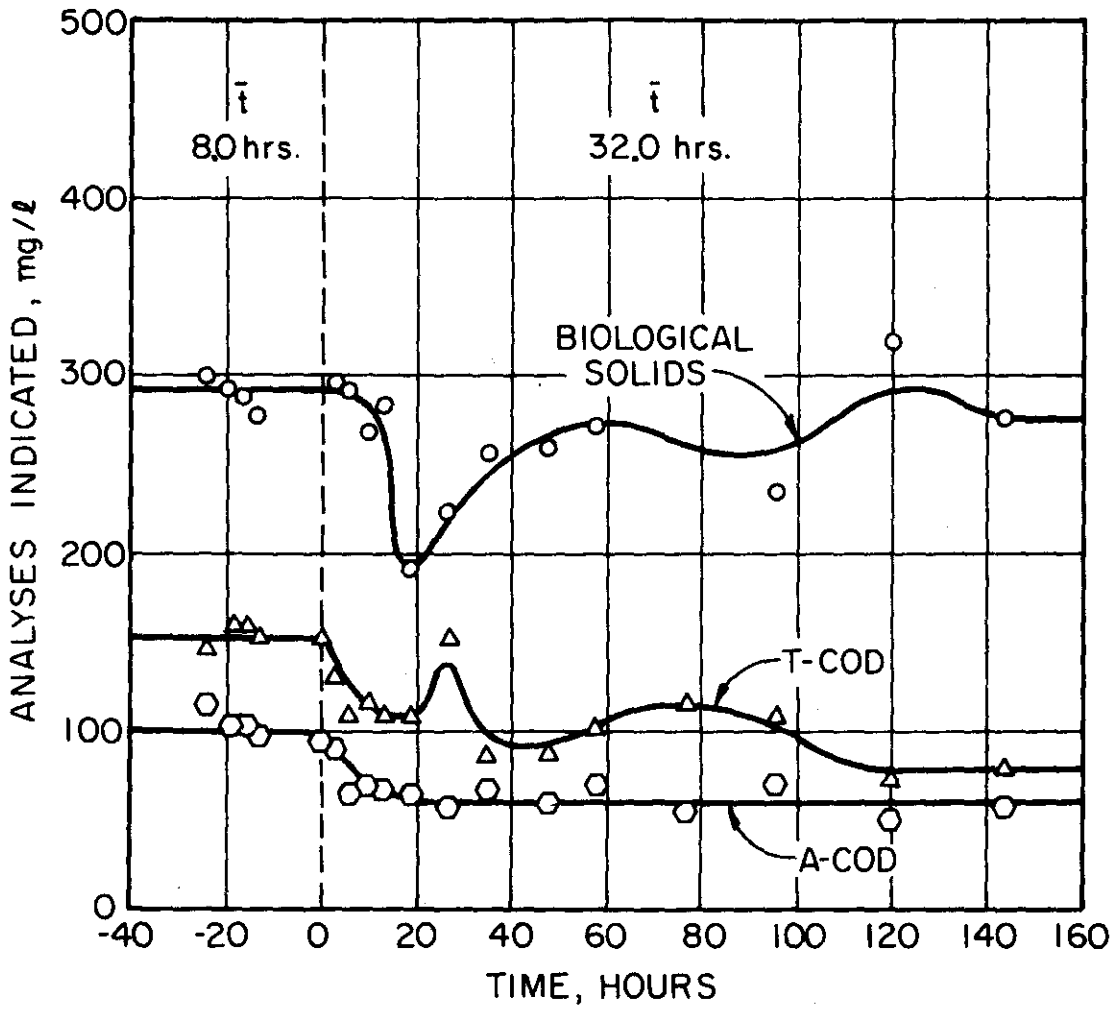
500
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
t̄
8.0 hrs.
t̄
32.0 hrs.
BIOLOGICAL SOLIDS
T-COD
A-COD
-40
-20
0
20
40
60
80
100
120
140
160
TIME, HOURS

Figure 10

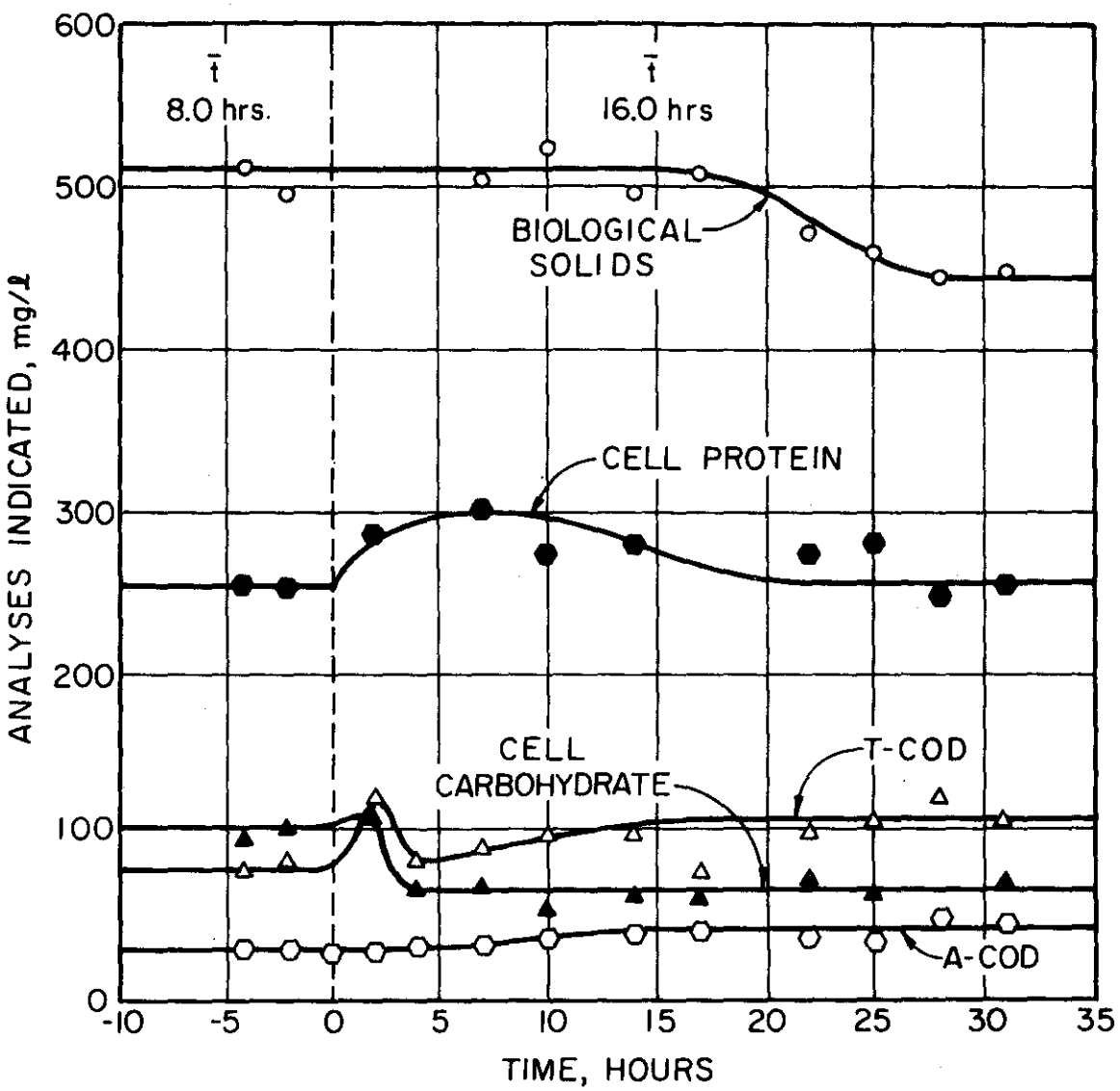
600
500
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
$\bar{t}$
8.0 hrs.
$\bar{t}$
16.0 hrs
BIOLOGICAL SOLIDS
CELL PROTEIN
CELL CARBOHYDRATE
T-COD
A-COD
-10
-5
0
5
10
15
20
25
30
35
TIME, HOURS

Figure 11

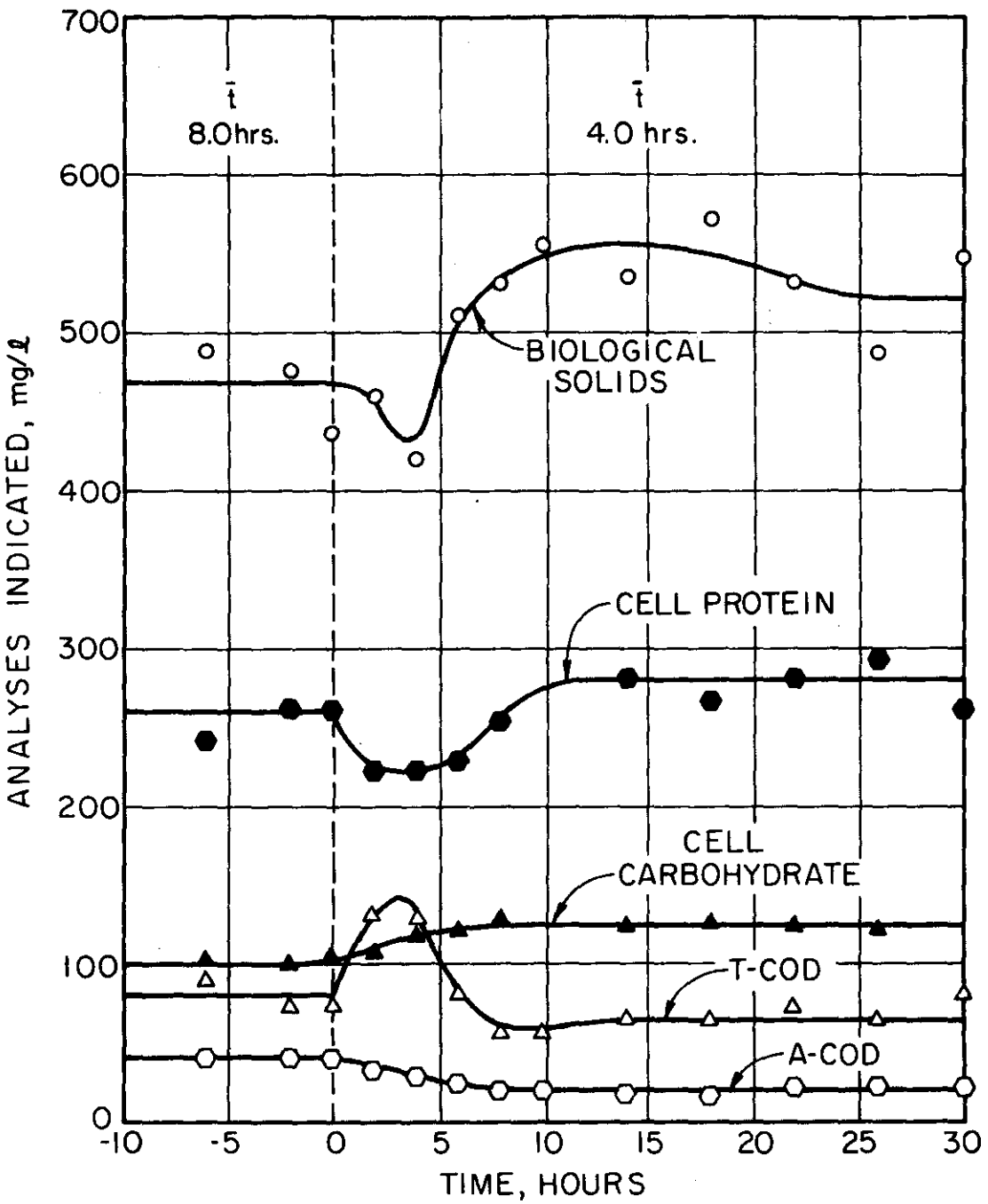
700
600
500
400
300
200
100
0
-10
-5
0
5
10
15
20
25
30
t̄
8.0hrs.
t̄
4.0 hrs.
BIOLOGICAL SOLIDS
CELL PROTEIN
CELL CARBOHYDRATE
T-COD
A-COD
ANALYSES INDICATED, mg/ℓ
TIME, HOURS

Figure 12

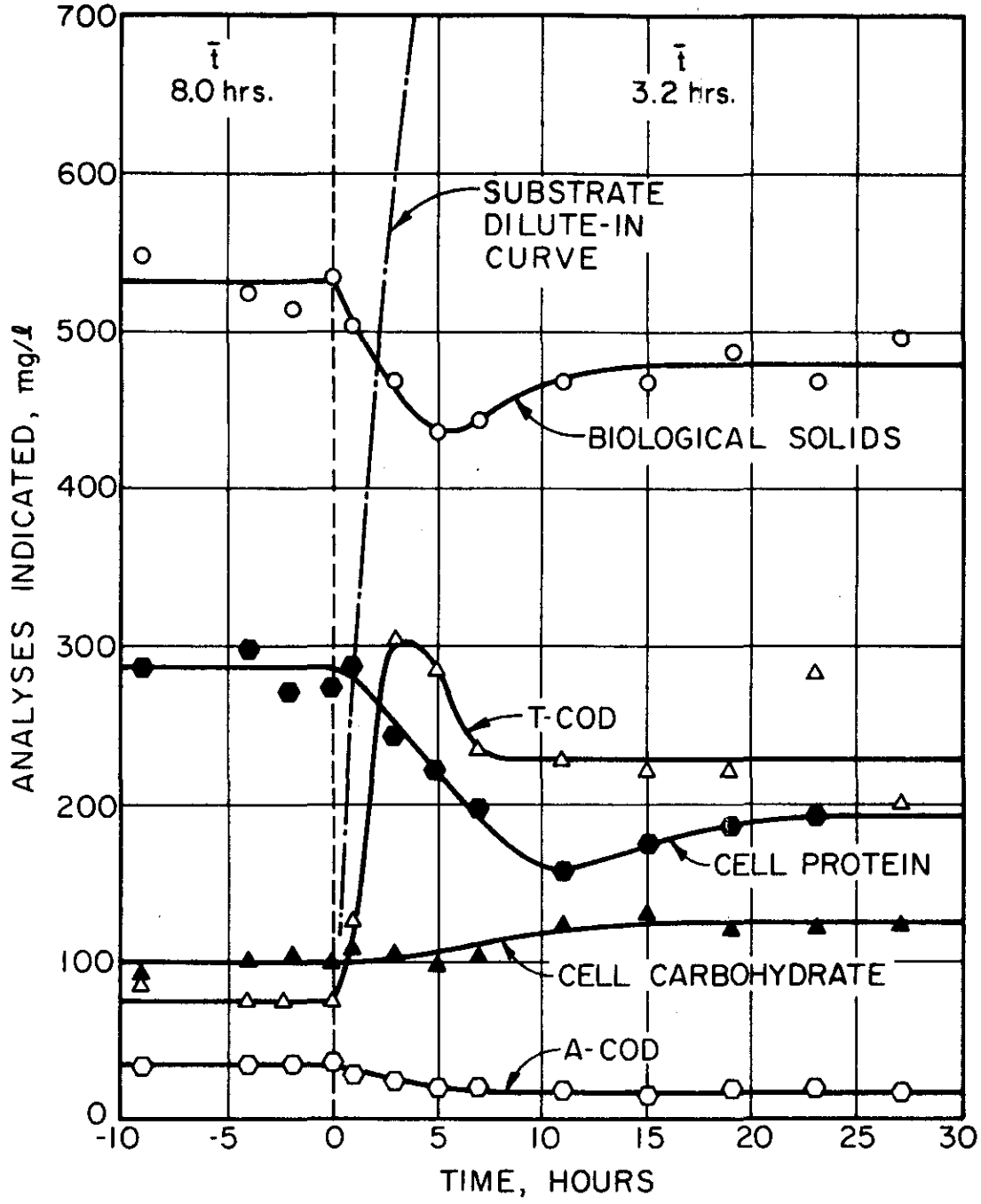
700
600
500
400
300
200
100
0
-10
-5
0
5
10
15
20
25
30
$\bar{t}$
8.0 hrs.
$\bar{t}$
3.2 hrs.
SUBSTRATE
DILUTE-IN
CURVE
BIOLOGICAL SOLIDS
T-COD
CELL PROTEIN
CELL CARBOHYDRATE
A-COD
ANALYSES INDICATED, mg/ℓ
TIME, HOURS

Figure 13

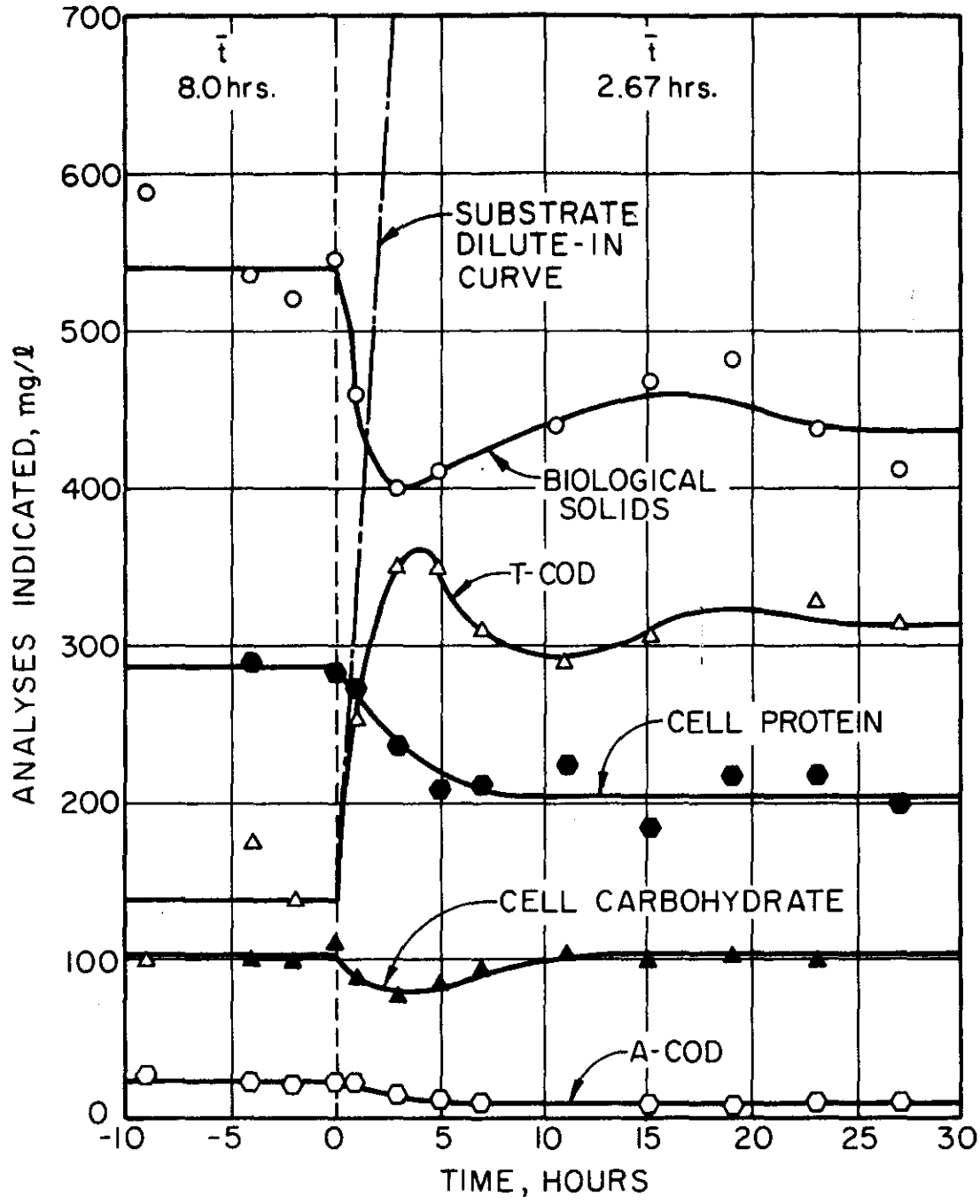
700
600
500
400
300
200
100
0
$\bar{t}$
8.0 hrs.
$\bar{t}$
2.67 hrs.
SUBSTRATE
DILUTE-IN
CURVE
BIOLOGICAL
SOLIDS
T-COD
CELL PROTEIN
CELL CARBOHYDRATE
A-COD
ANALYSES INDICATED, mg/ℓ
-10
-5
0
5
10
15
20
25
30
TIME, HOURS

Figure 14

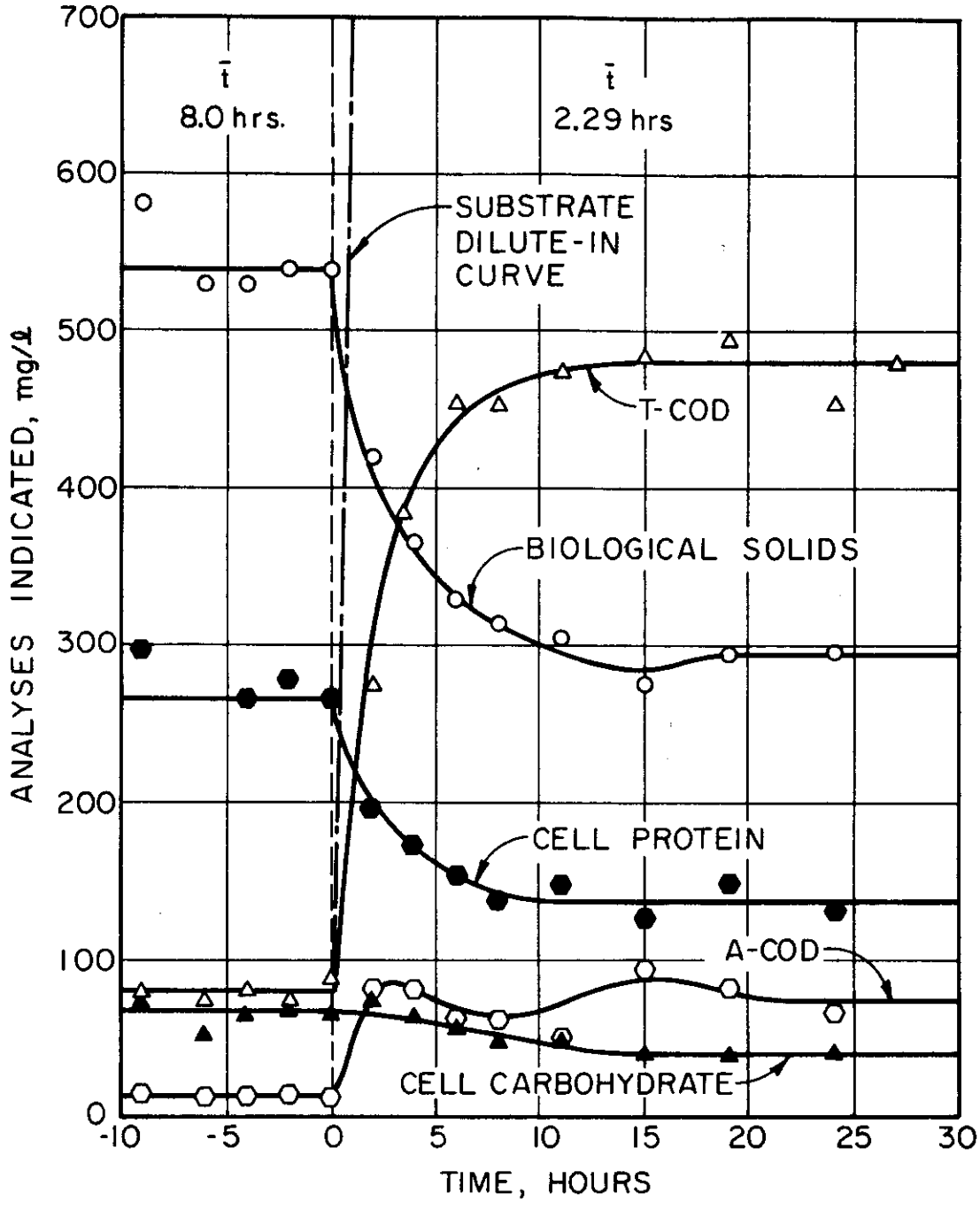
700
600
500
400
300
200
100
0
-10
-5
0
5
10
15
20
25
30
$\bar{t}$
8.0 hrs.
$\bar{t}$
2.29 hrs
SUBSTRATE
DILUTE-IN
CURVE
T-COD
BIOLOGICAL SOLIDS
CELL PROTEIN
A-COD
CELL CARBOHYDRATE
ANALYSES INDICATED, mg/ℓ
TIME, HOURS

Figure 15

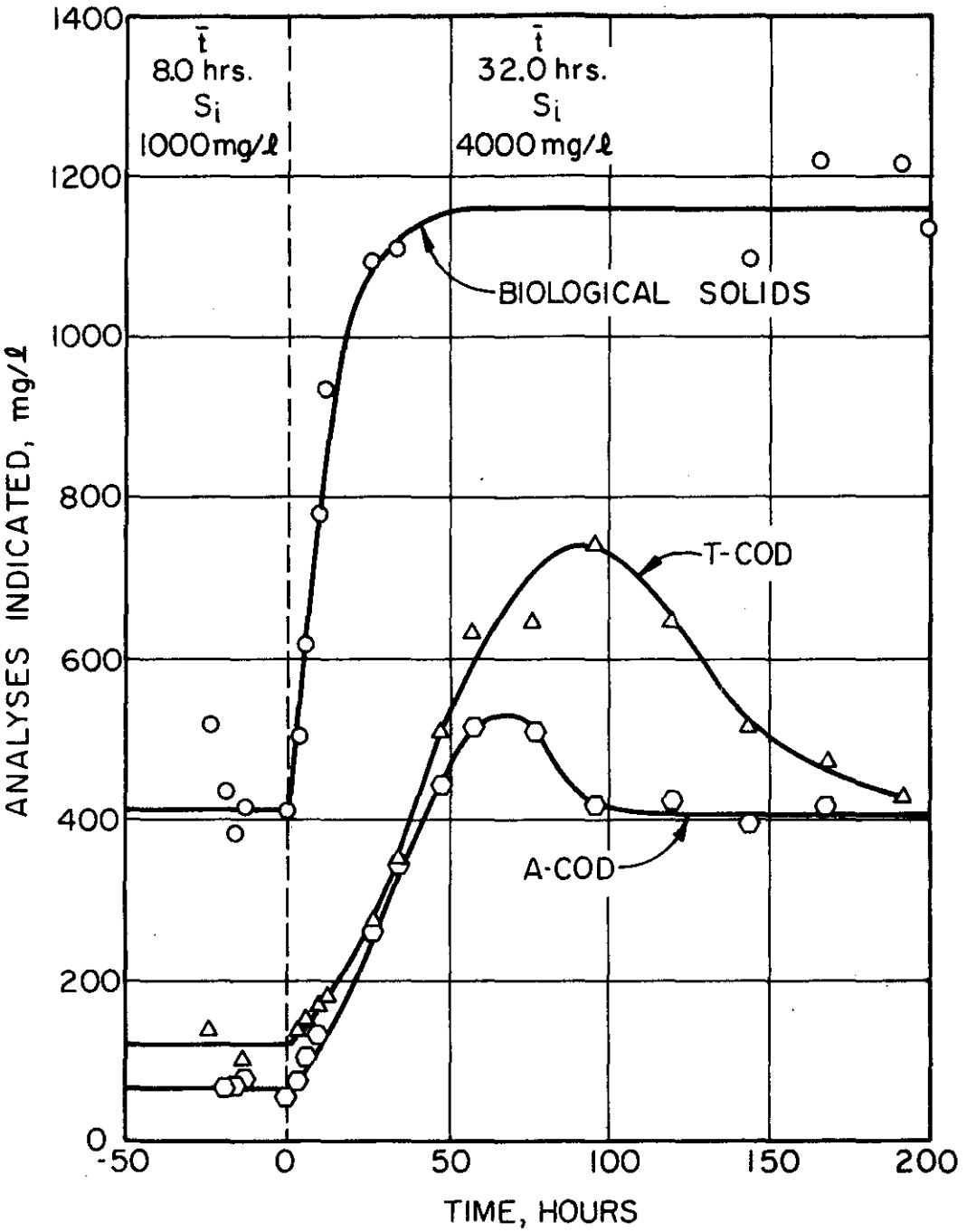
1400
1200
1000
800
600
400
200
0
-50
0
50
100
150
200
ANALYSES INDICATED, mg/ℓ
TIME, HOURS
t̄
8.0 hrs.
Si
1000 mg/ℓ
t̄
32.0 hrs.
Si
4000 mg/ℓ
BIOLOGICAL SOLIDS
T-COD
A-COD

Figure 16

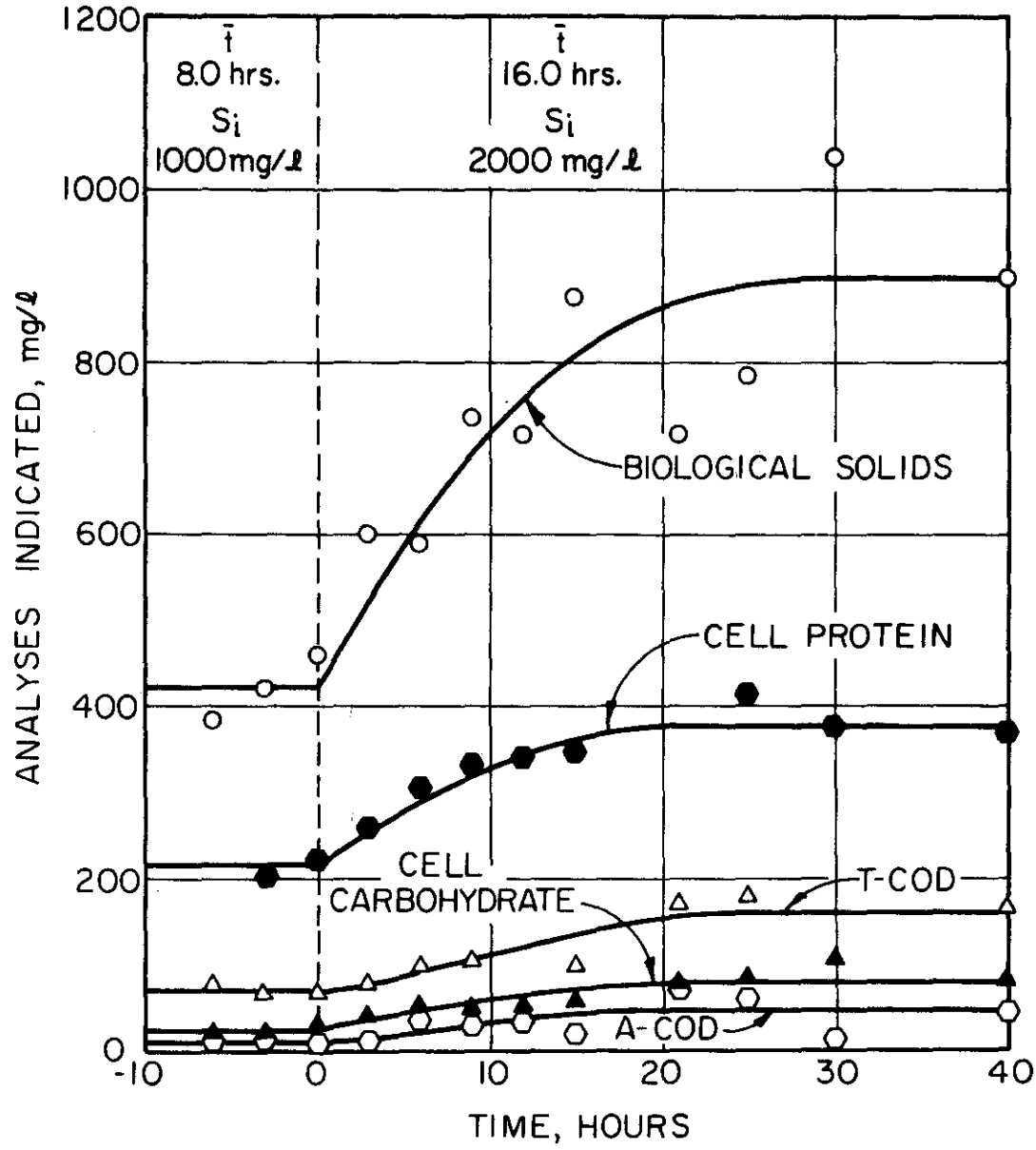
1200
1000
800
600
400
200
0
-10
0
10
20
30
40
ANALYSES INDICATED, mg/ℓ
TIME, HOURS
t̄
8.0 hrs.
Si
1000mg/ℓ
t̄
16.0 hrs.
Si
2000 mg/ℓ
BIOLOGICAL SOLIDS
CELL PROTEIN
CELL
CARBOHYDRATE
T-COD
A-COD

Figure 17

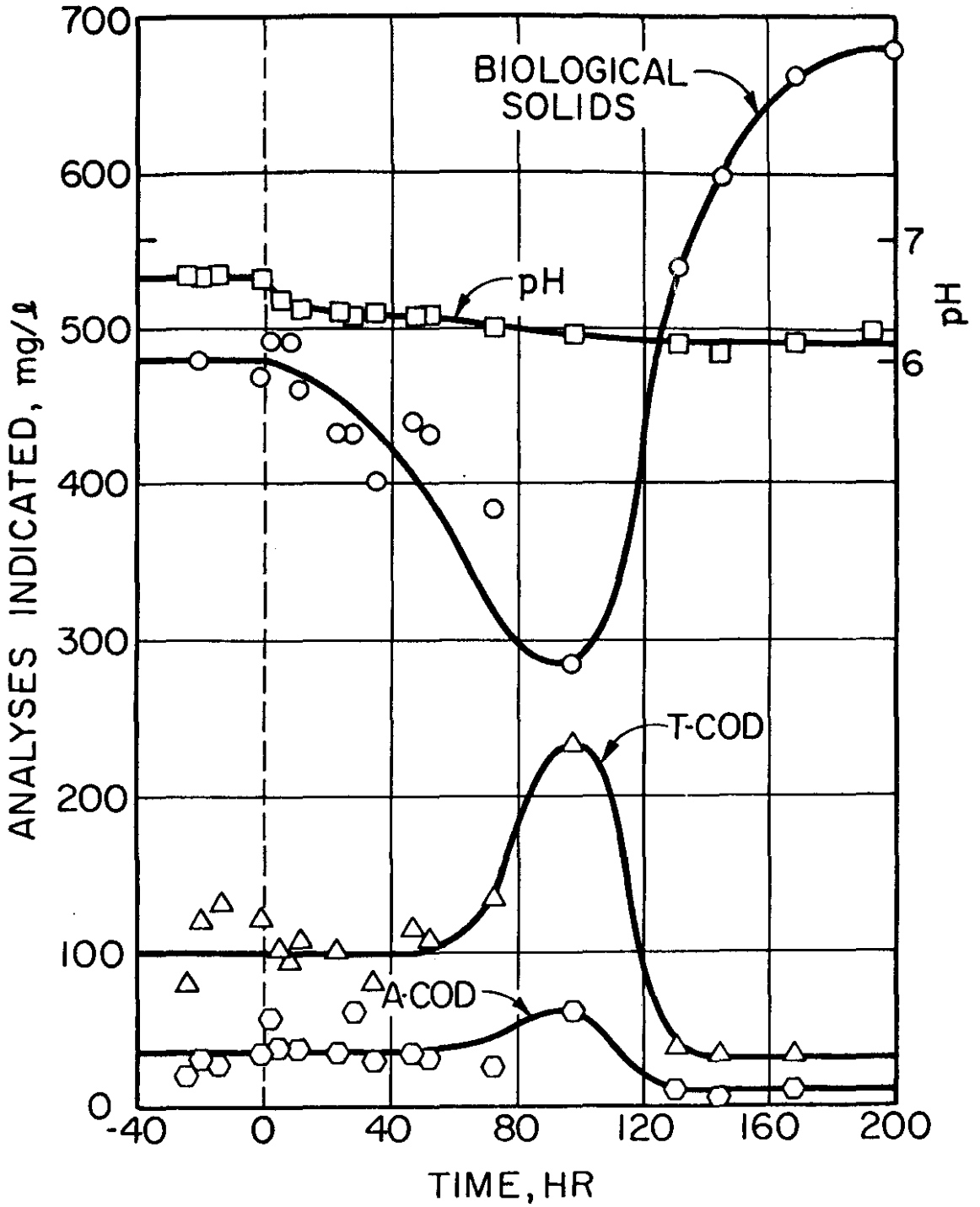
700
600
500
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
BIOLOGICAL SOLIDS
pH
T-COD
A-COD
7
6
pH
-40
0
40
80
120
160
200
TIME, HR

Figure 18

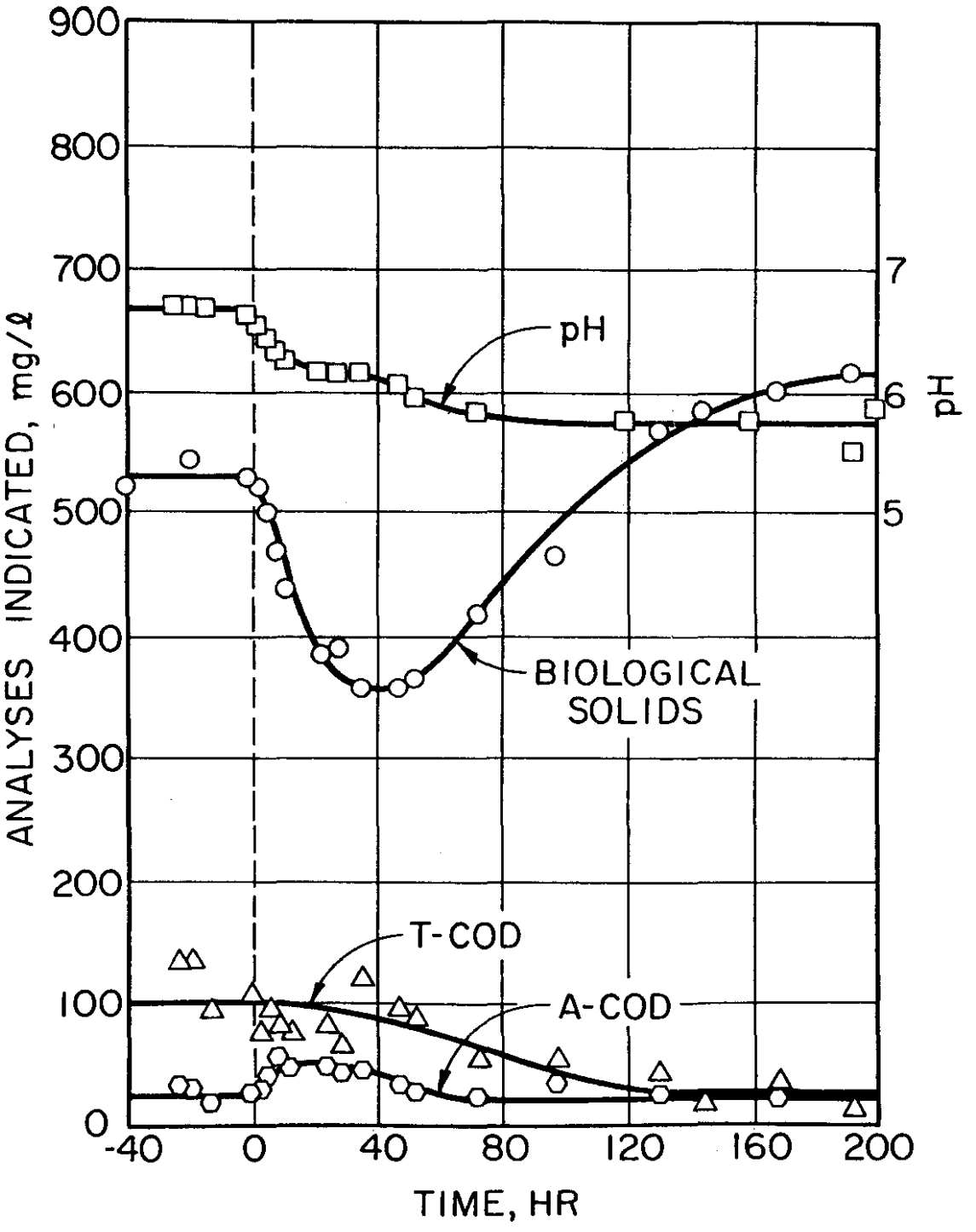
900
800
700
600
500
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
7
6
5
pH
pH
BIOLOGICAL SOLIDS
T-COD
A-COD
-40
0
40
80
120
160
200
TIME, HR

Figure 19

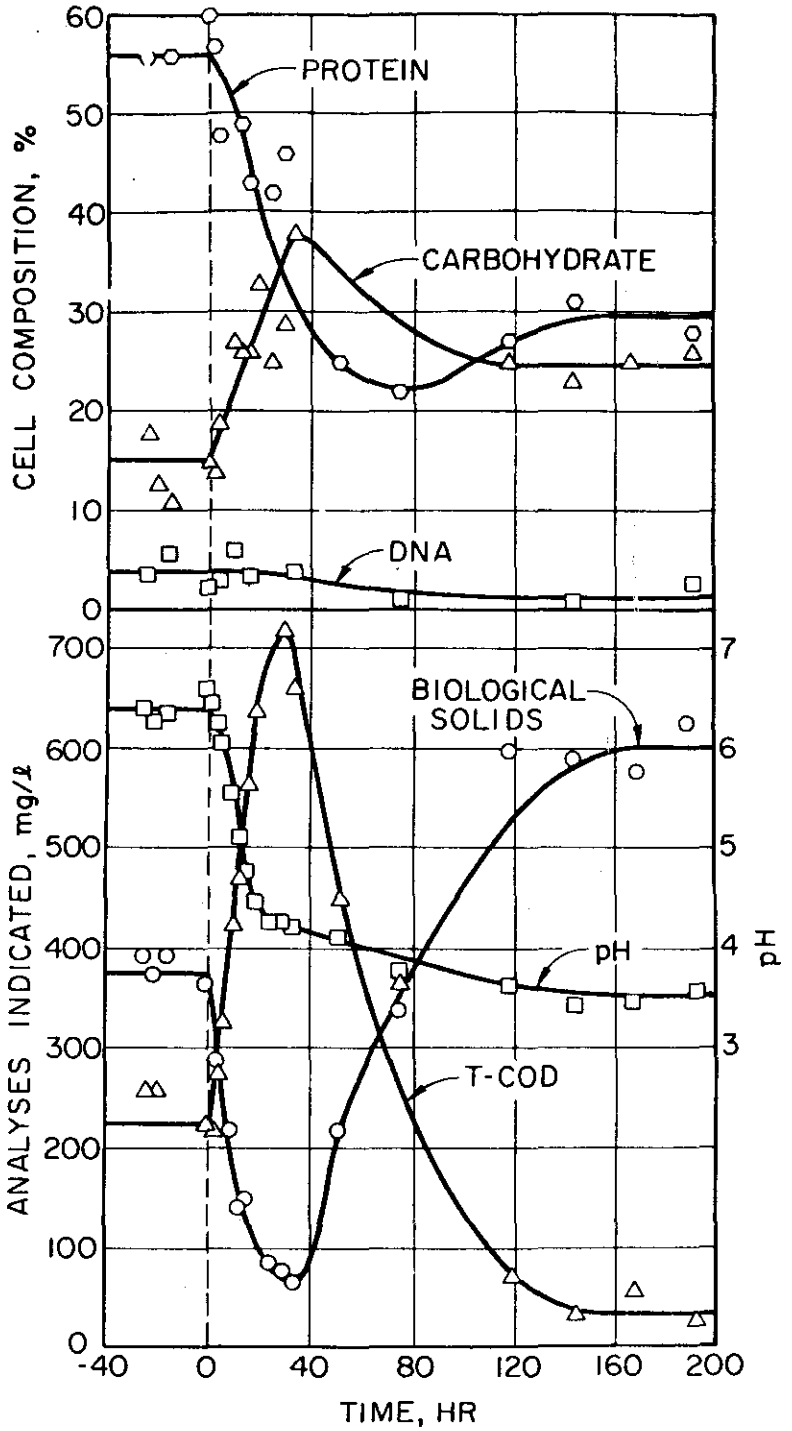
PROTEIN
CARBOHYDRATE
DNA
BIOLOGICAL SOLIDS
pH
T-COD
CELL COMPOSITION, %
ANALYSES INDICATED, mg/ℓ
pH
TIME, HR
60
50
40
30
20
10
0
700
600
500
400
300
200
100
0
7
6
5
4
3
-40
0
40
80
120
160
200

Figure 20

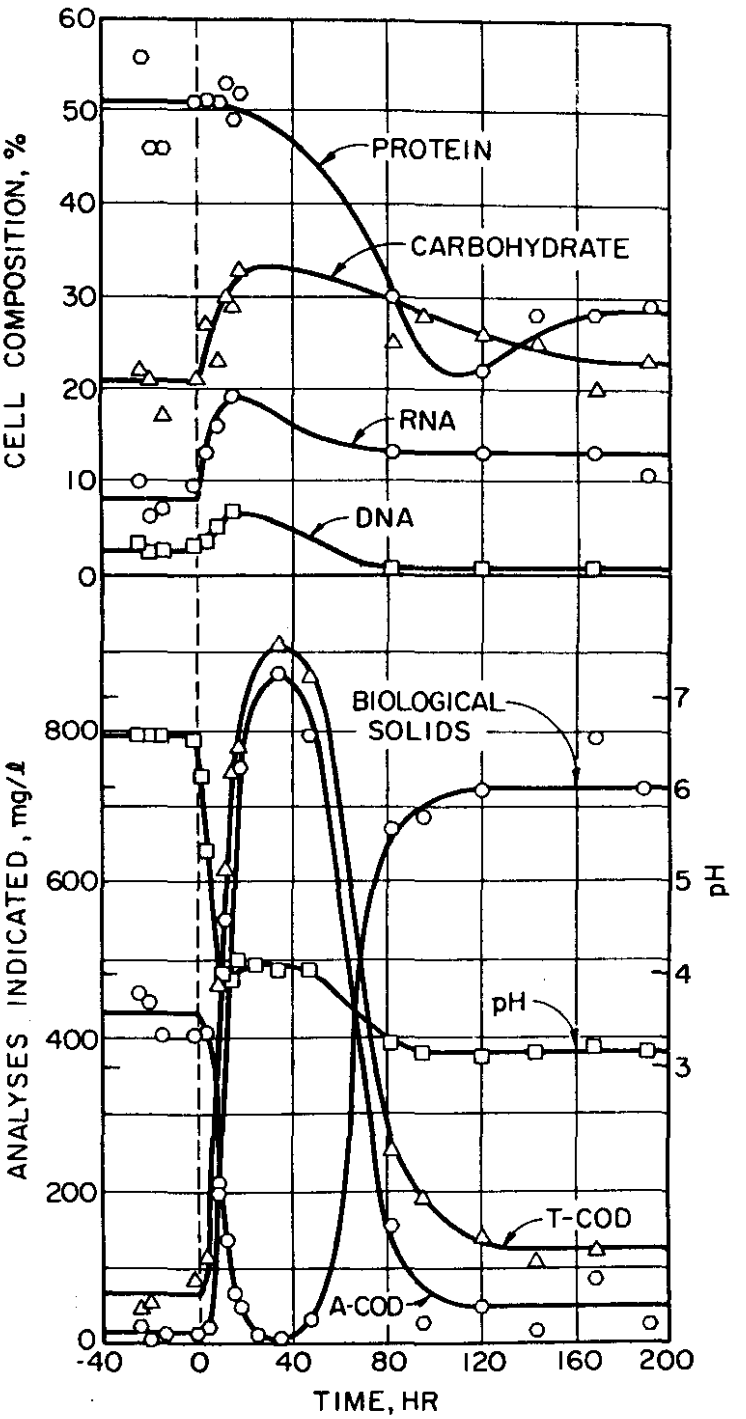
CELL COMPOSITION, %
ANALYSES INDICATED, mg/ℓ
pH
TIME, HR
PROTEIN
CARBOHYDRATE
RNA
DNA
BIOLOGICAL SOLIDS
pH
T-COD
A-COD
60
50
40
30
20
10
0
800
600
400
200
0
7
6
5
4
3
-40
0
40
80
120
160
200

Figure 21

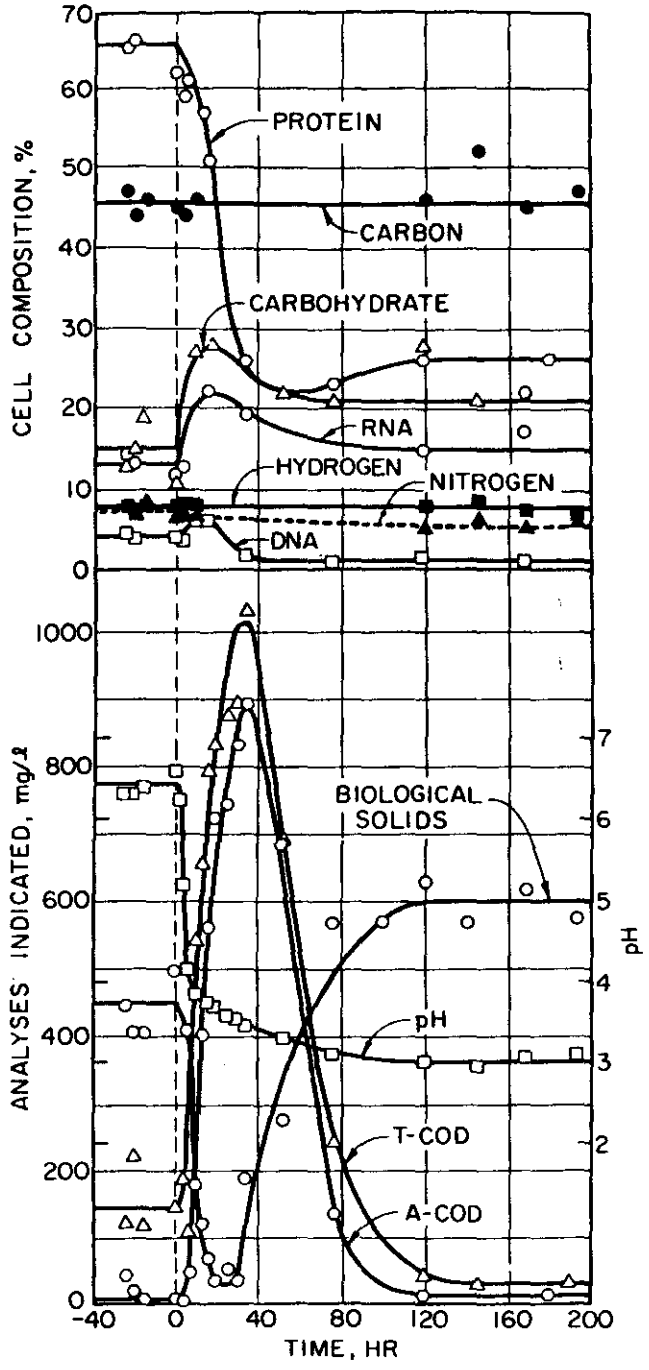
CELL COMPOSITION, %
ANALYSES INDICATED, mg/ℓ
pH
TIME, HR
PROTEIN
CARBON
CARBOHYDRATE
RNA
HYDROGEN
NITROGEN
DNA
BIOLOGICAL SOLIDS
pH
T-COD
A-COD
70
60
50
40
30
20
10
0
1000
800
600
400
200
0
7
6
5
4
3
2
-40
0
40
80
120
160
200

Figure 22

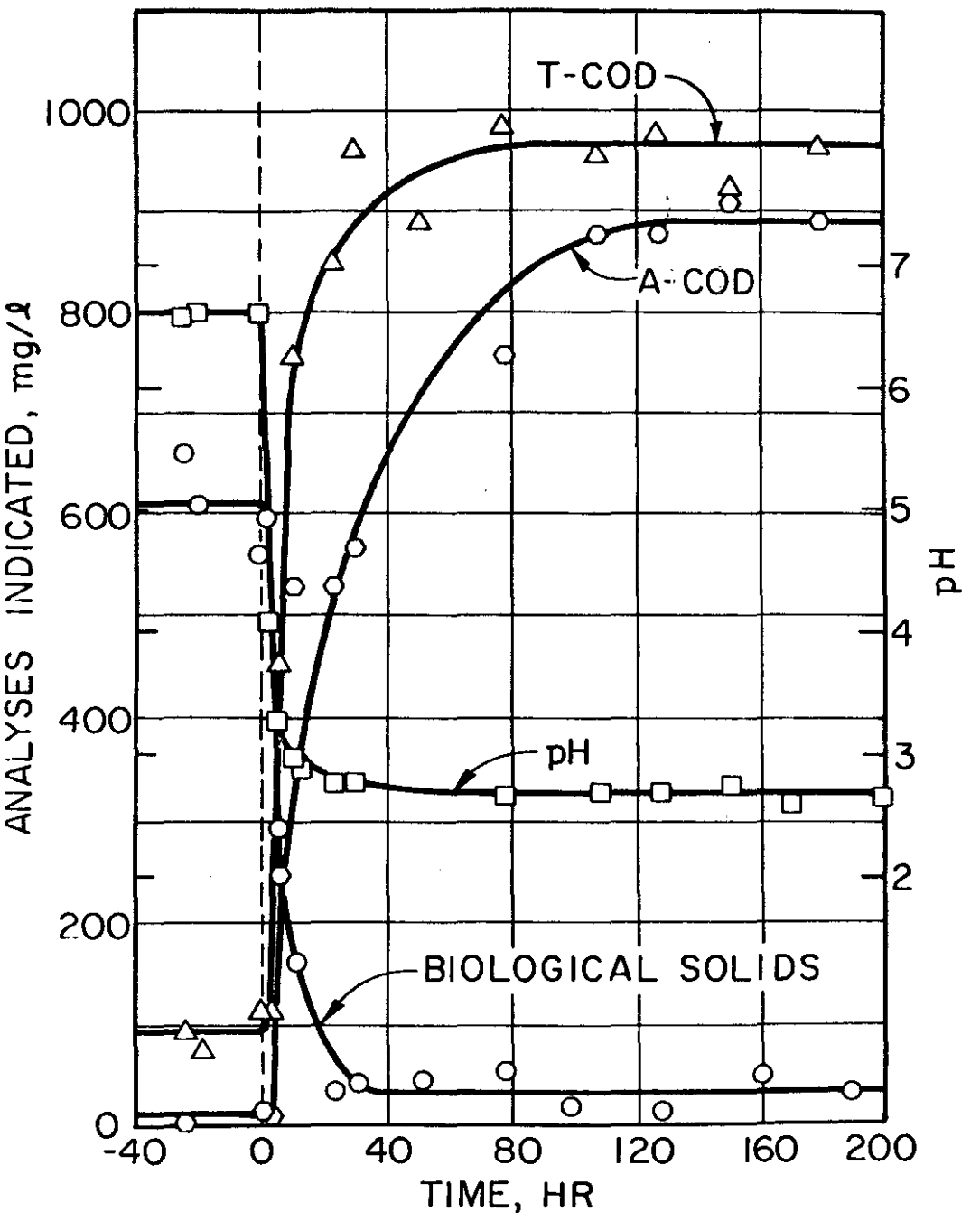
T-COD
A-COD
pH
BIOLOGICAL SOLIDS
ANALYSES INDICATED, mg/ℓ
pH
TIME, HR
0
200
400
600
800
1000
2
3
4
5
6
7
-40
0
40
80
120
160
200

Figure 23

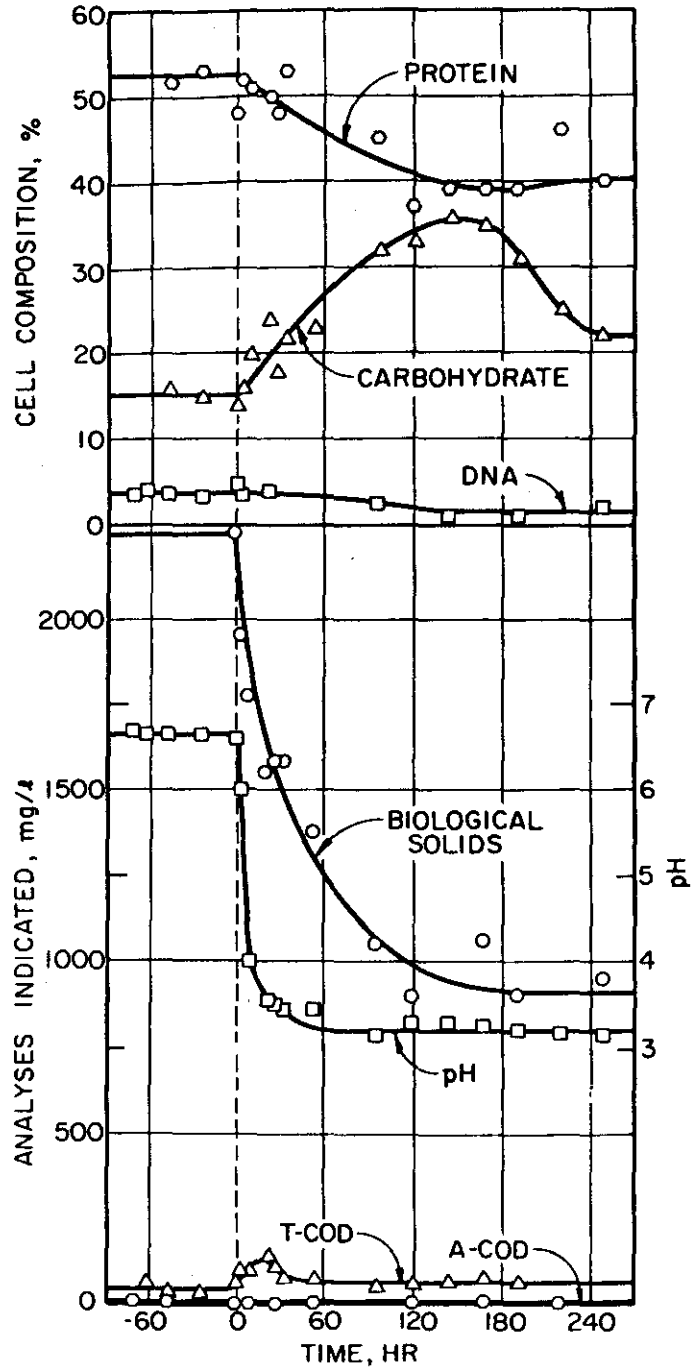
CELL COMPOSITION, %
60
50
40
30
20
10
0
PROTEIN
CARBOHYDRATE
DNA
ANALYSES INDICATED, mg/ℓ
2000
1500
1000
500
0
BIOLOGICAL SOLIDS
pH
7
6
5
4
3
T-COD
A-COD
-60
0
60
120
180
240
TIME, HR

Figure 24

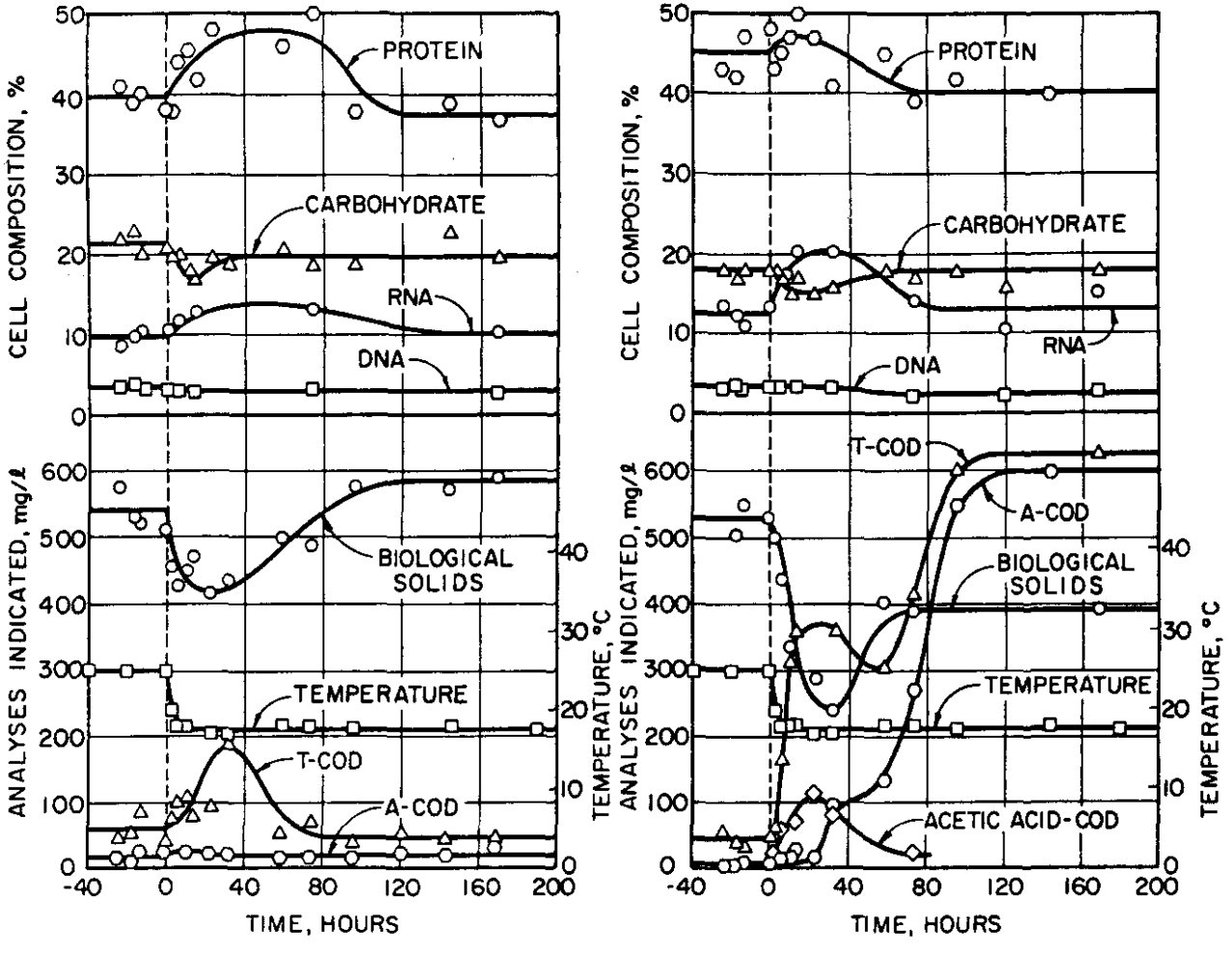
PROTEIN
CARBOHYDRATE
RNA
DNA
BIOLOGICAL SOLIDS
TEMPERATURE
T-COD
A-COD
ACETIC ACID-COD
CELL COMPOSITION, %
ANALYSES INDICATED, mg/ℓ
TEMPERATURE, °C
TIME, HOURS

Figure 25

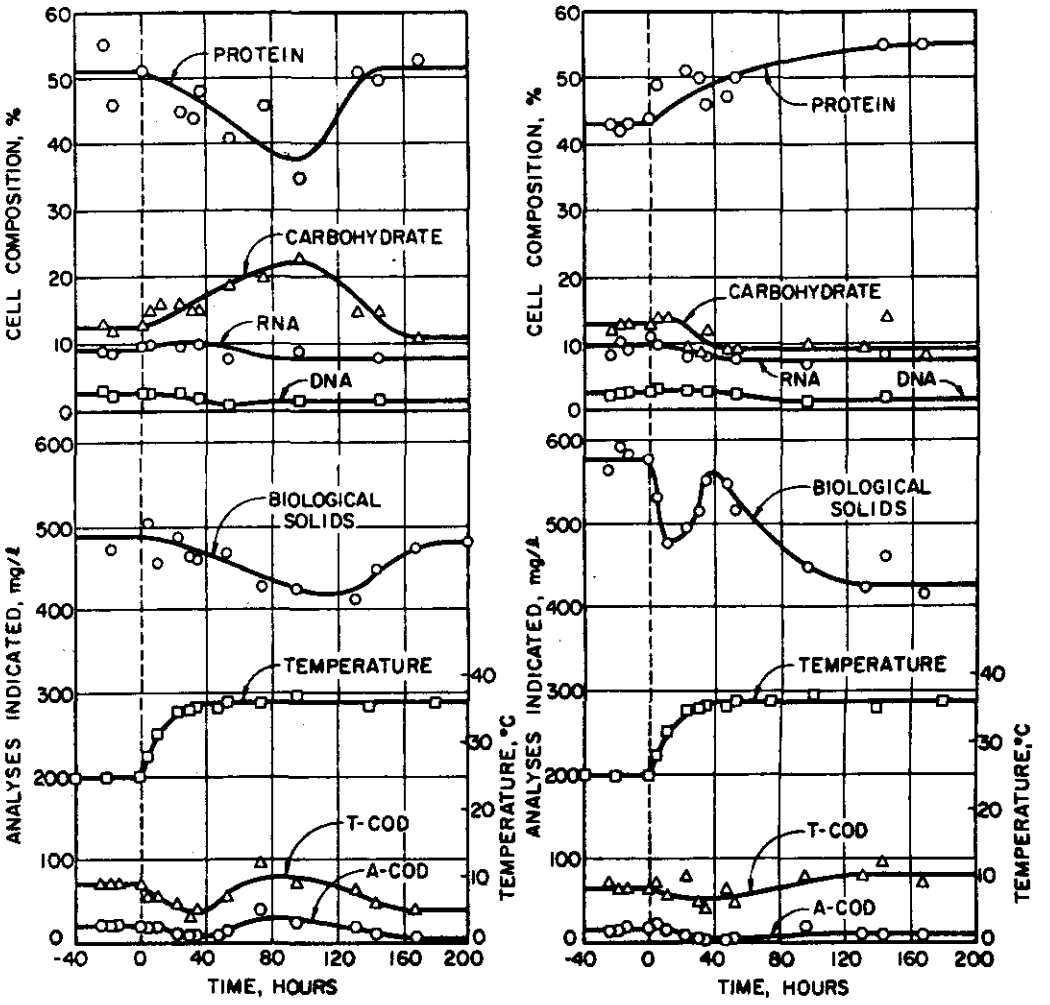
CELL COMPOSITION, %
PROTEIN
CARBOHYDRATE
RNA
DNA
ANALYSES INDICATED, mg/ℓ
BIOLOGICAL SOLIDS
TEMPERATURE
T-COD
A-COD
TEMPERATURE, °C
TIME, HOURS

Figure 26

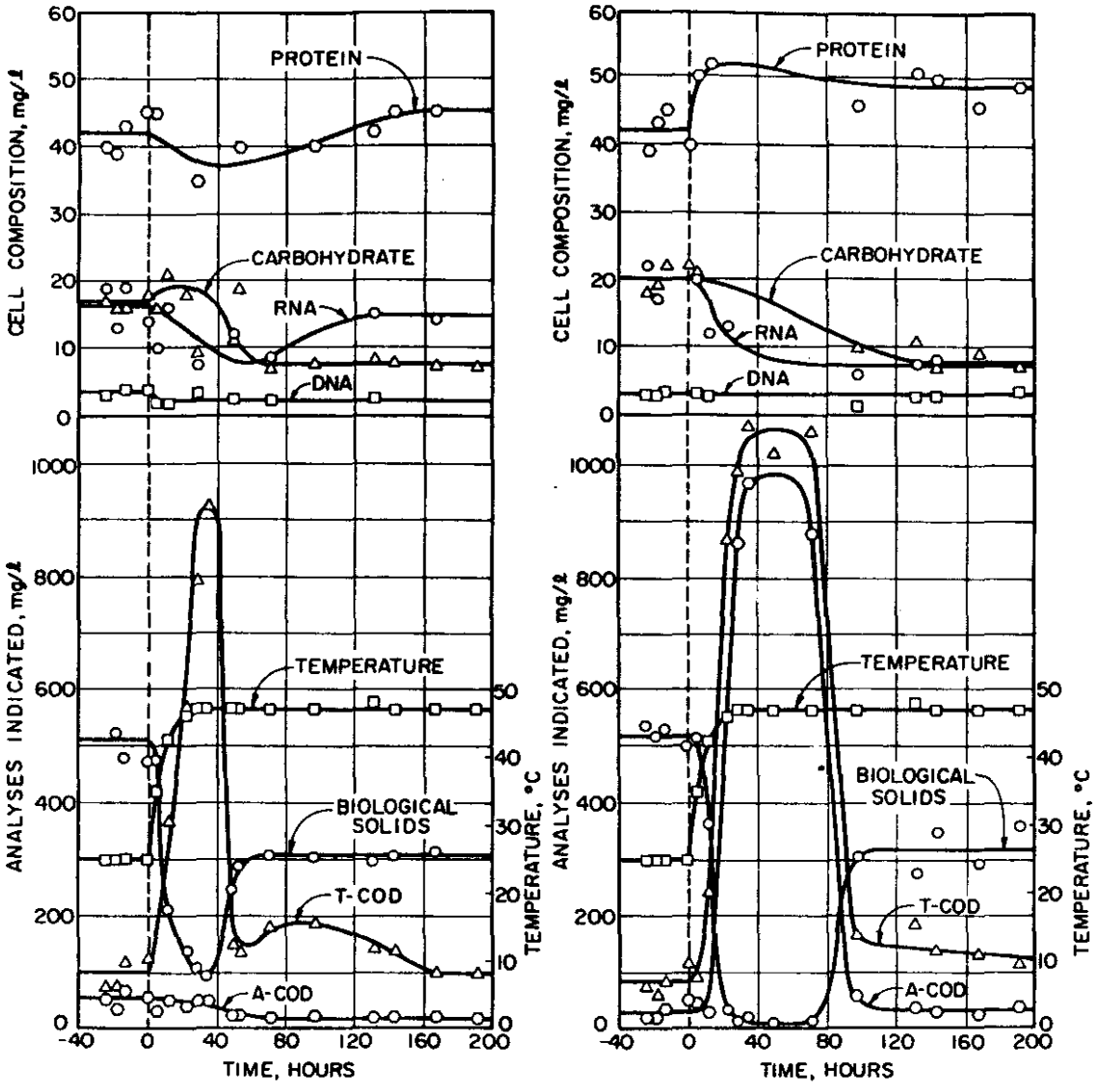
PROTEIN
CARBOHYDRATE
RNA
DNA
TEMPERATURE
BIOLOGICAL SOLIDS
T-COD
A-COD
CELL COMPOSITION, mg/ℓ
ANALYSES INDICATED, mg/ℓ
TEMPERATURE, °C
TIME, HOURS

Figure 27

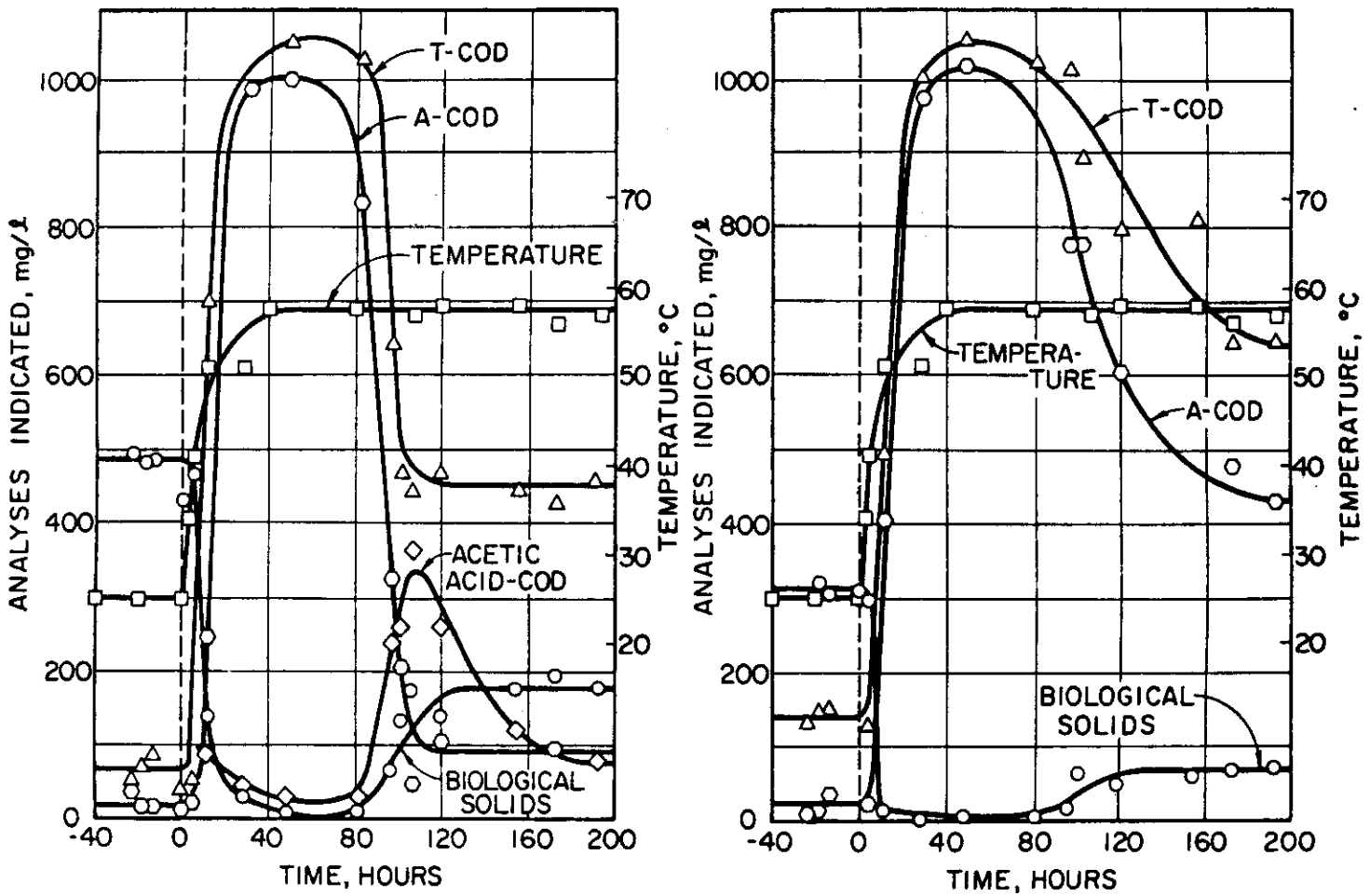
T-COD
A-COD
TEMPERATURE
ACETIC ACID-COD
BIOLOGICAL SOLIDS
ANALYSES INDICATED, mg/ℓ
TEMPERATURE, °C
TIME, HOURS
0
200
400
600
800
1000
20
30
40
50
60
70
-40
0
40
80
120
160
200
T-COD
TEMPERA-TURE
A-COD
BIOLOGICAL SOLIDS
ANALYSES INDICATED, mg/ℓ
TEMPERATURE, °C
TIME, HOURS

Figure 28

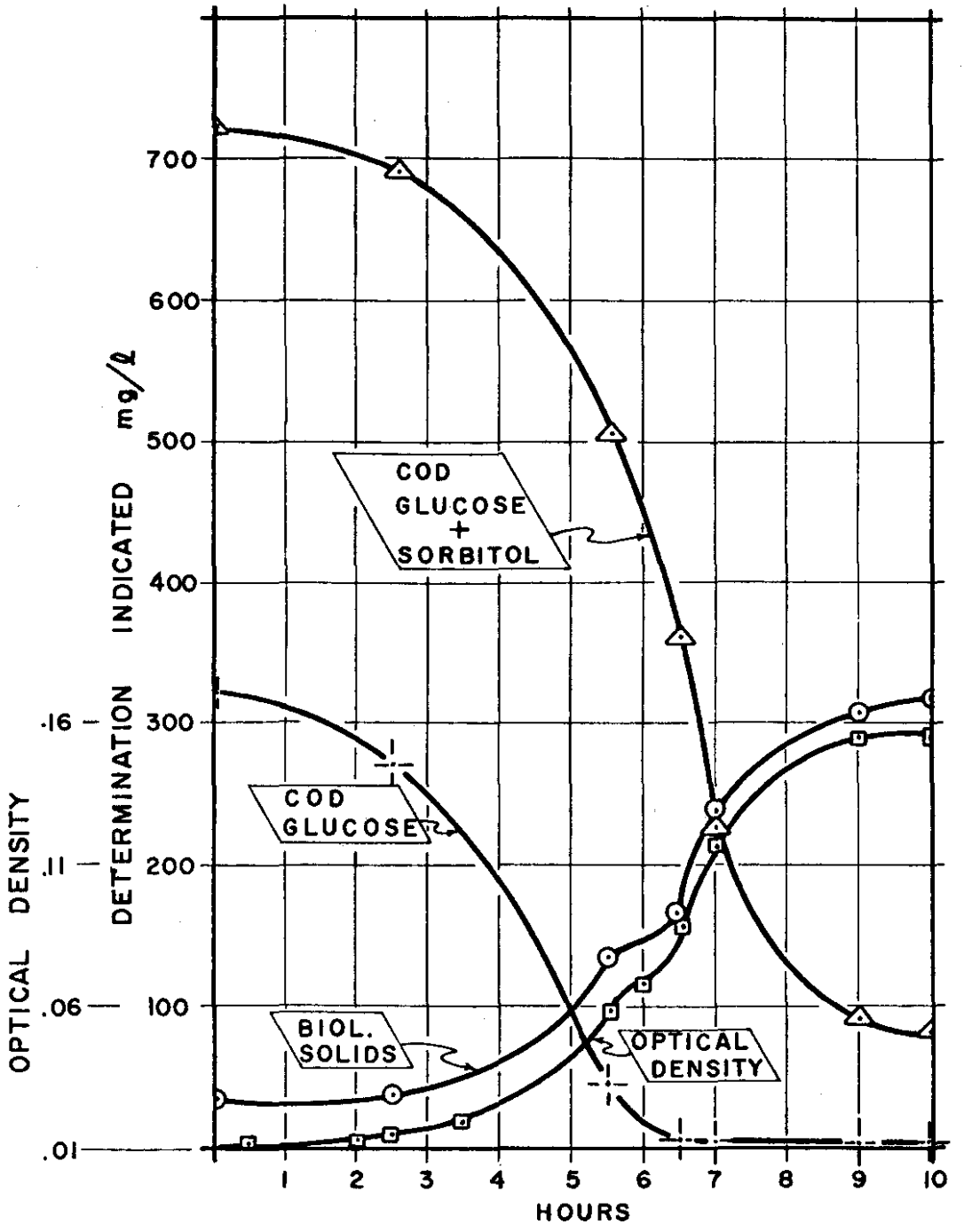
OPTICAL DENSITY
DETERMINATION INDICATED mg/l
.16
.11
.06
.01
700
600
500
400
300
200
100
COD GLUCOSE + SORBITOL
COD GLUCOSE
BIOL. SOLIDS
OPTICAL DENSITY
1
2
3
4
5
6
7
8
9
10
HOURS

Figure 29

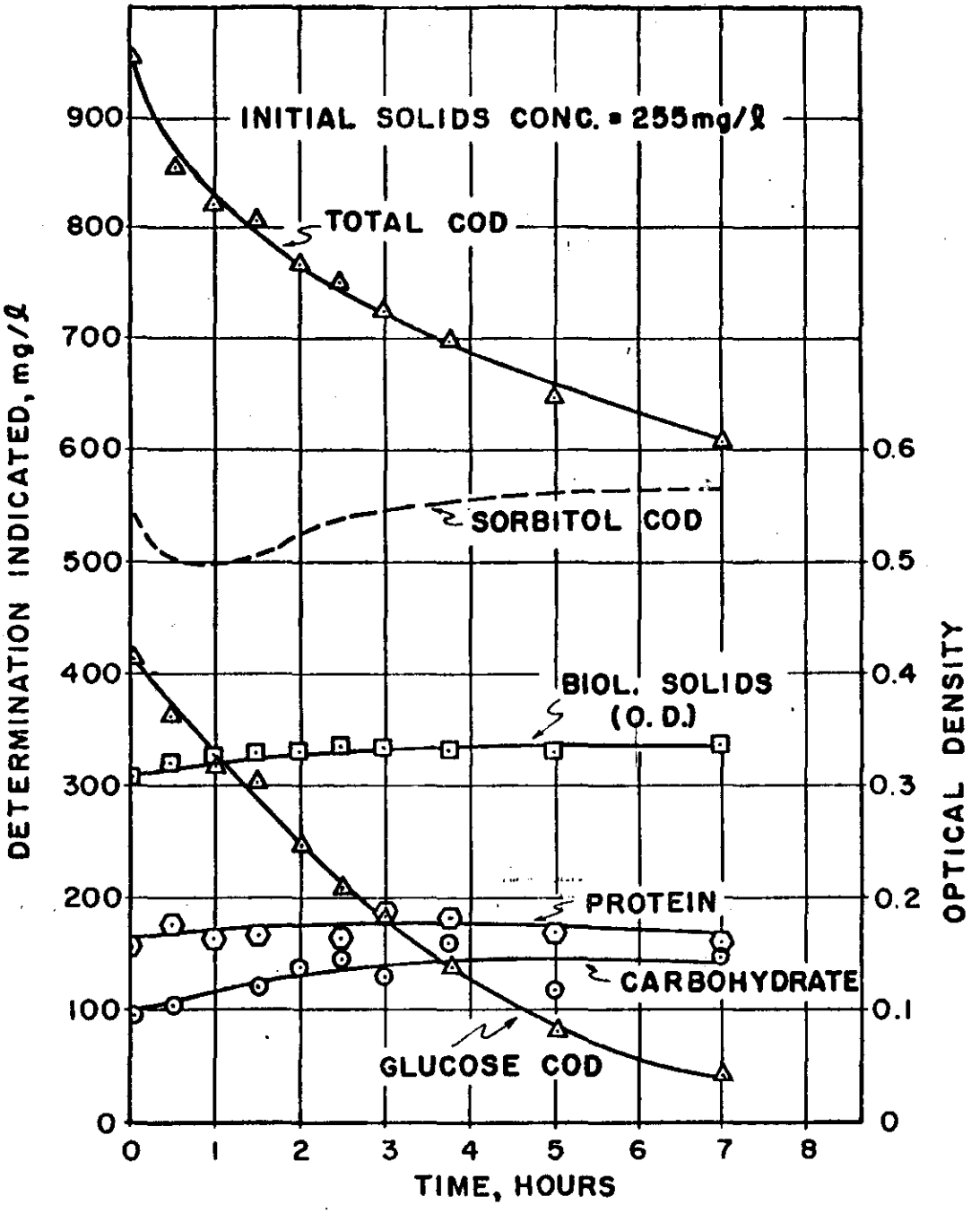
INITIAL SOLIDS CONC. = 255mg/ℓ
TOTAL COD
SORBITOL COD
BIOL. SOLIDS (O.D.)
PROTEIN
CARBOHYDRATE
GLUCOSE COD
DETERMINATION INDICATED, mg/ℓ
OPTICAL DENSITY
TIME, HOURS
0
100
200
300
400
500
600
700
800
900
0.1
0.2
0.3
0.4
0.5
0.6
1
2
3
4
5
6
7
8

Figure 30

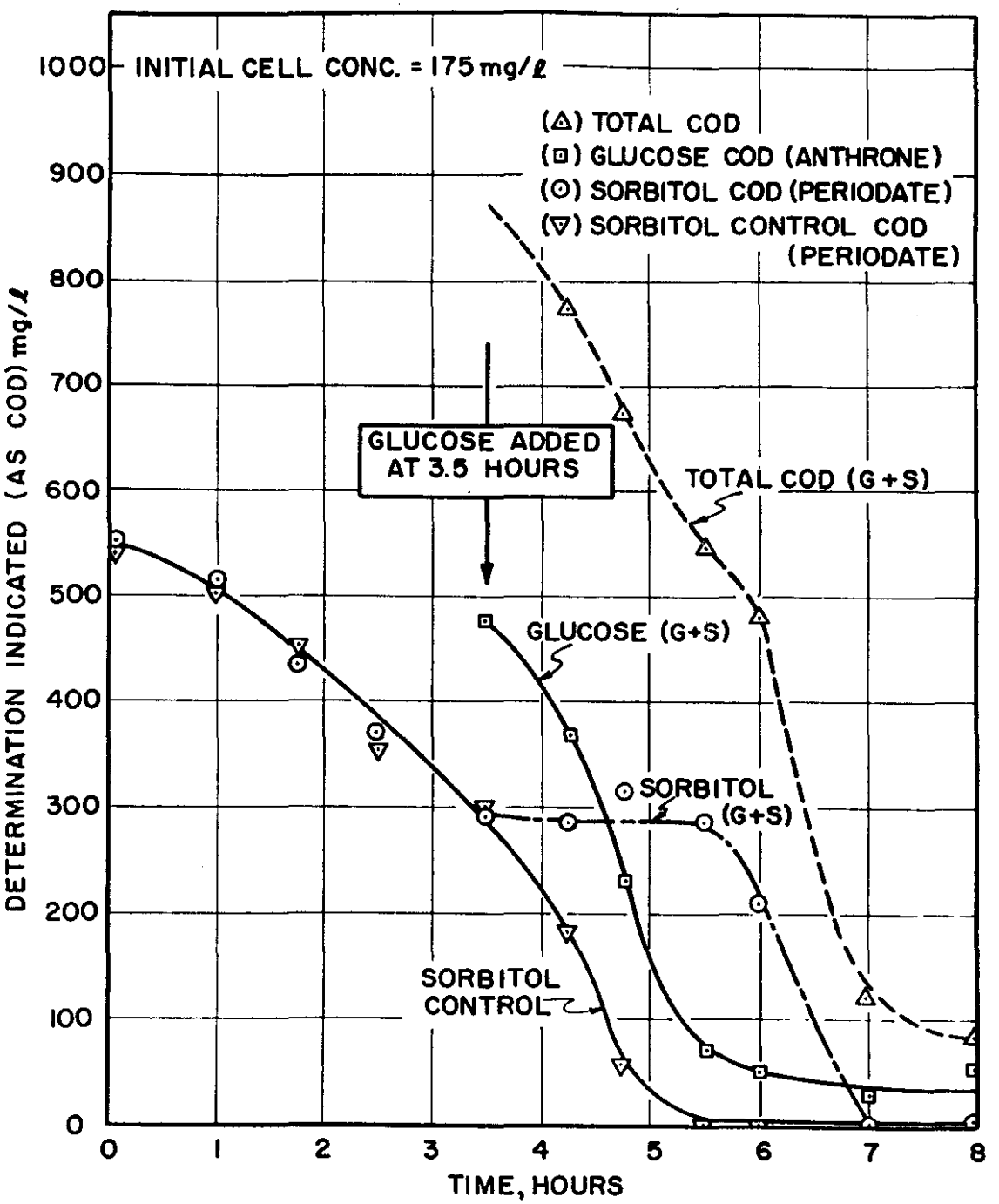
INITIAL CELL CONC. = 175 mg/ℓ
(△) TOTAL COD
(⊡) GLUCOSE COD (ANTHRONE)
(⊙) SORBITOL COD (PERIODATE)
(▽) SORBITOL CONTROL COD (PERIODATE)
GLUCOSE ADDED AT 3.5 HOURS
TOTAL COD (G+S)
GLUCOSE (G+S)
SORBITOL (G+S)
SORBITOL CONTROL
DETERMINATION INDICATED (AS COD) mg/ℓ
TIME, HOURS
0
100
200
300
400
500
600
700
800
900
1000
0
1
2
3
4
5
6
7
8

Figure 31

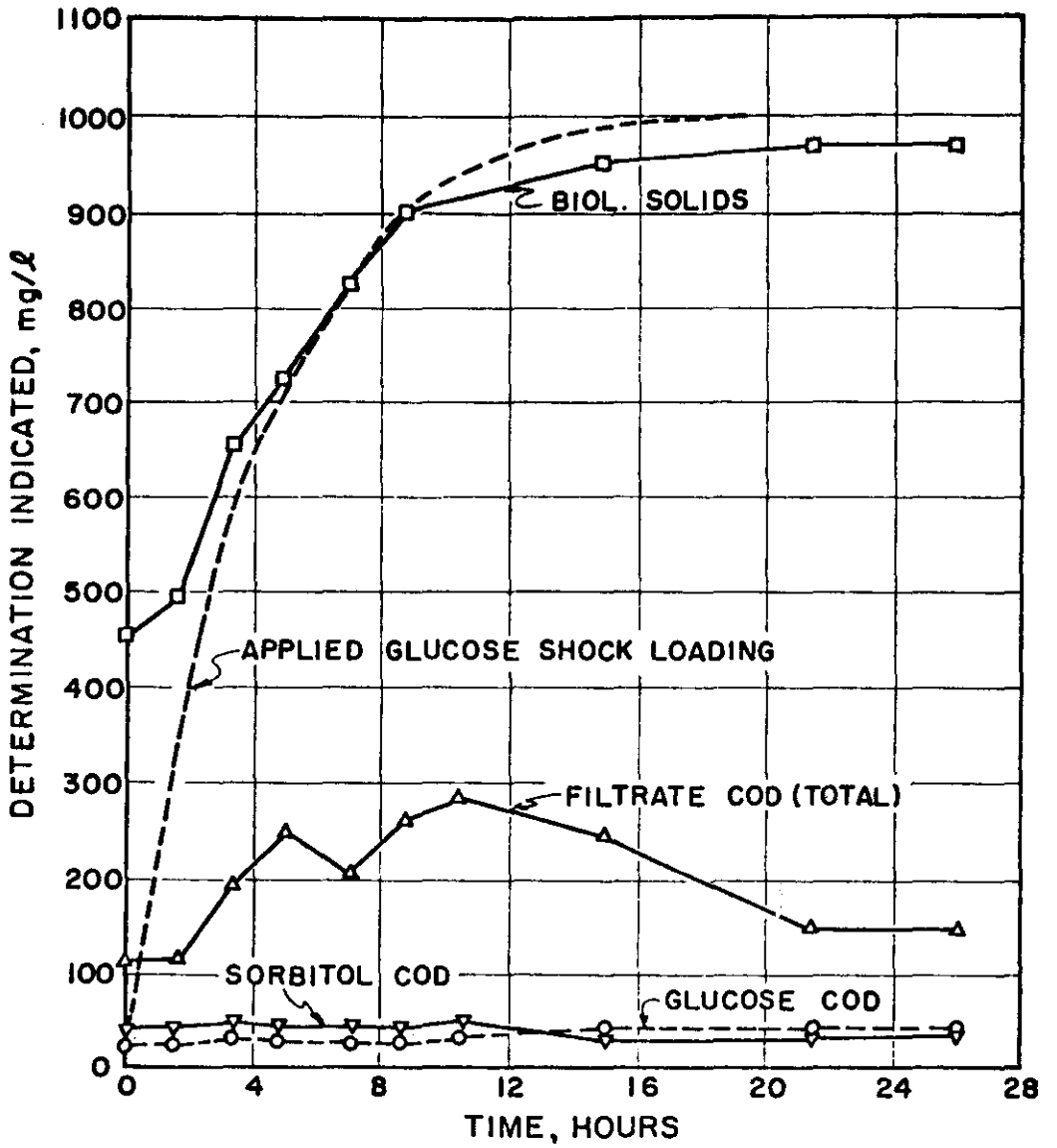
1100
1000
900
800
700
600
500
400
300
200
100
0
DETERMINATION INDICATED, mg/ℓ
BIOL. SOLIDS
APPLIED GLUCOSE SHOCK LOADING
FILTRATE COD (TOTAL)
SORBITOL COD
GLUCOSE COD
0
4
8
12
16
20
24
28
TIME, HOURS

Figure 32

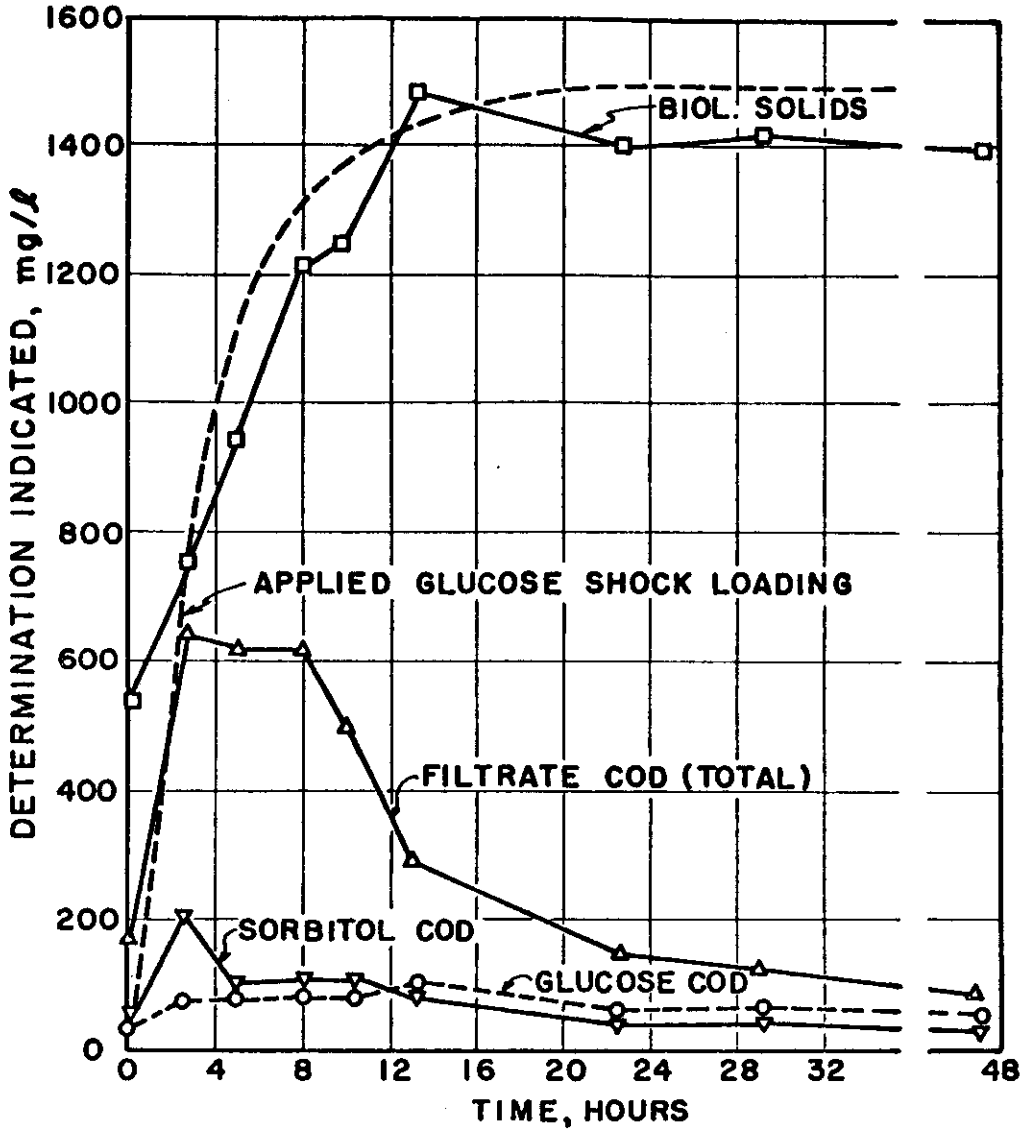
1600
1400
1200
1000
800
600
400
200
0
DETERMINATION INDICATED, mg/ℓ
BIOL. SOLIDS
APPLIED GLUCOSE SHOCK LOADING
FILTRATE COD (TOTAL)
SORBITOL COD
GLUCOSE COD
0
4
8
12
16
20
24
28
32
48
TIME, HOURS

Figure 33

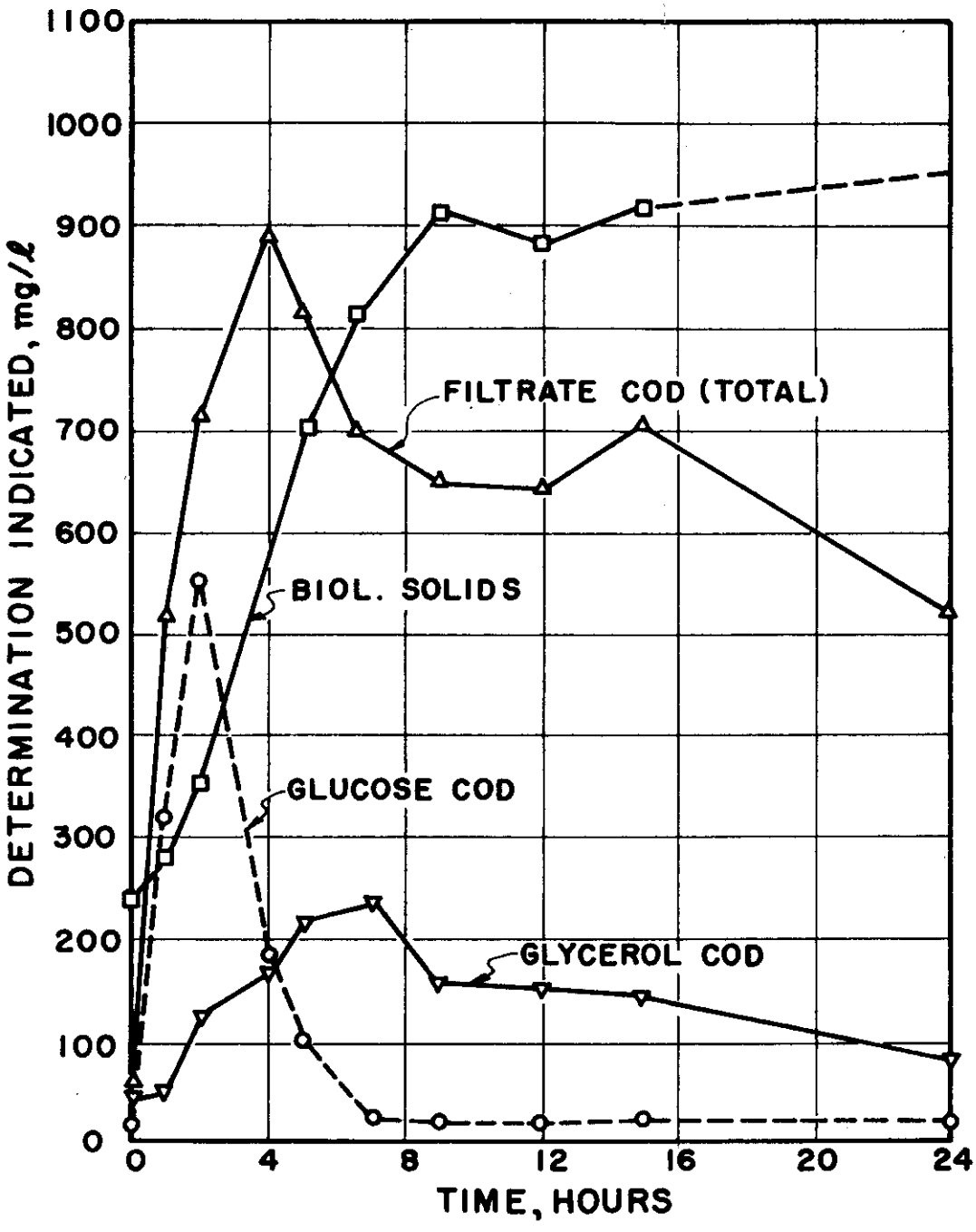
1100
1000
900
800
700
600
500
400
300
200
100
0
DETERMINATION INDICATED, mg/ℓ
FILTRATE COD (TOTAL)
BIOL. SOLIDS
GLUCOSE COD
GLYCEROL COD
0
4
8
12
16
20
24
TIME, HOURS

Figure 34

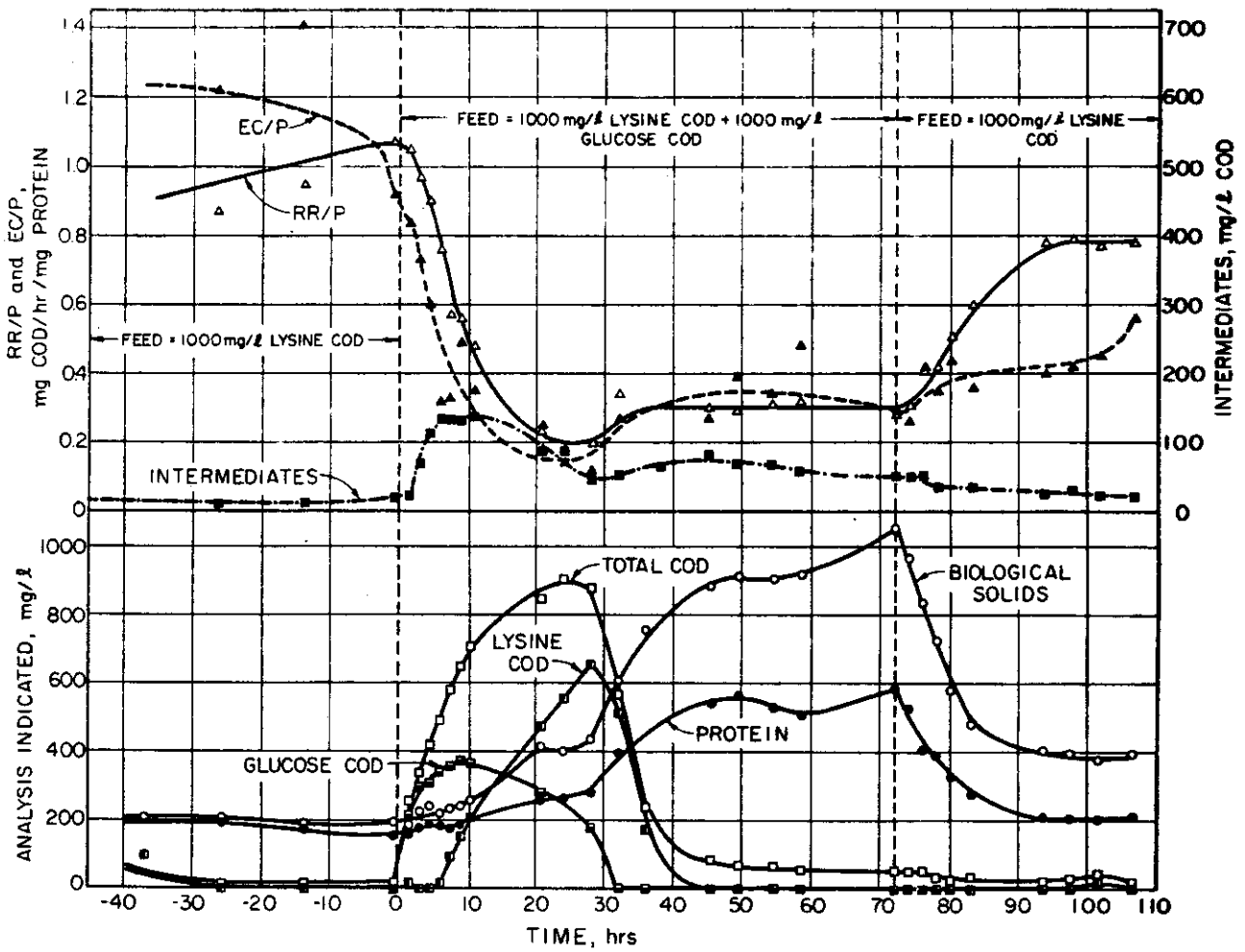
RR/P and EC/P,
mg COD/hr/mg PROTEIN
INTERMEDIATES, mg/ℓ COD
ANALYSIS INDICATED, mg/ℓ
TIME, hrs
EC/P
RR/P
FEED = 1000 mg/ℓ LYSINE COD + 1000 mg/ℓ GLUCOSE COD
FEED = 1000mg/ℓ LYSINE COD
FEED = 1000mg/ℓ LYSINE COD
INTERMEDIATES
TOTAL COD
BIOLOGICAL SOLIDS
LYSINE COD
PROTEIN
GLUCOSE COD

Figure 35

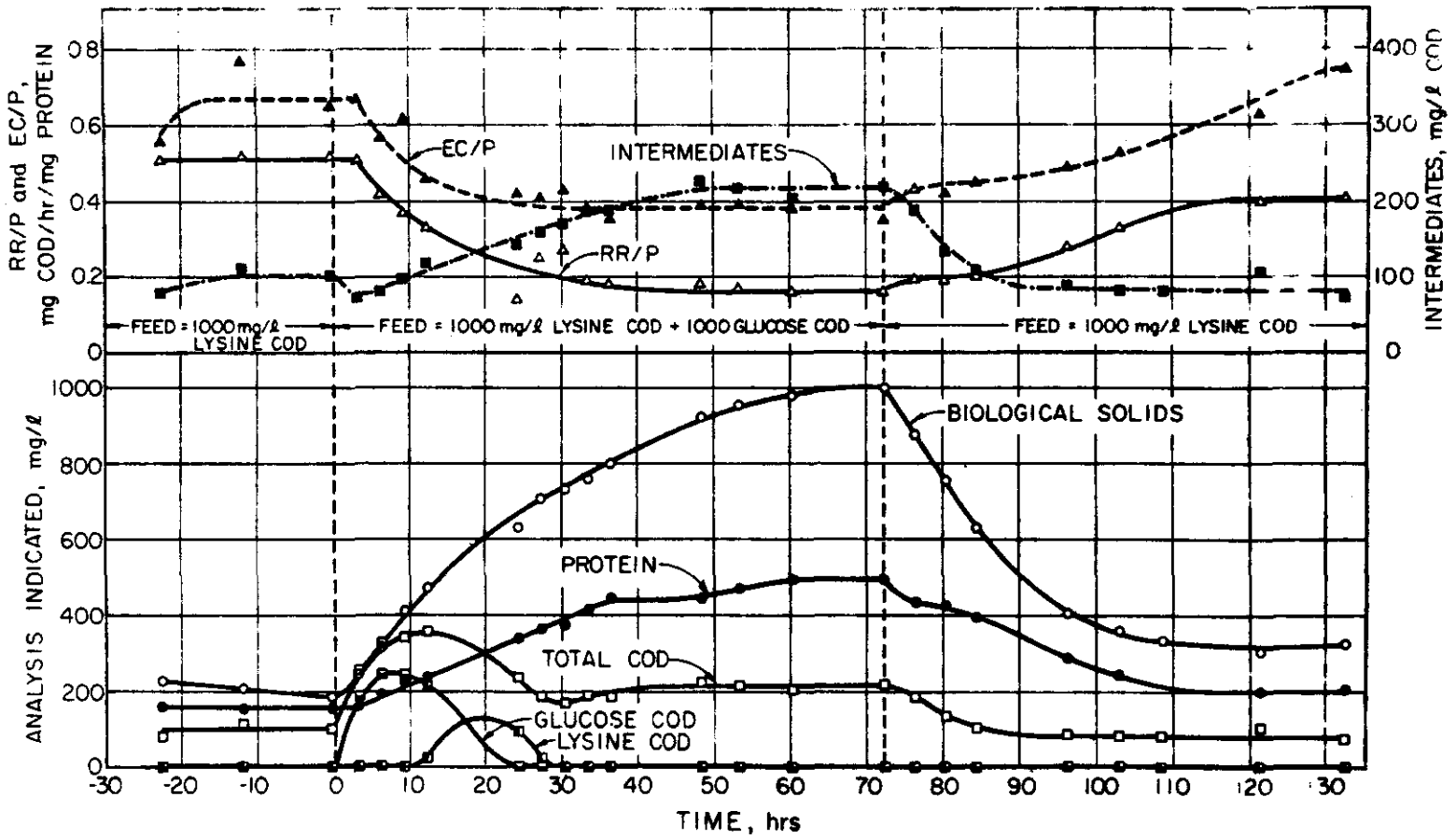
RR/P and EC/P,
mg COD/hr/mg PROTEIN
INTERMEDIATES, mg/ℓ COD
EC/P
INTERMEDIATES
RR/P
FEED = 1000 mg/ℓ LYSINE COD
FEED = 1000 mg/ℓ LYSINE COD + 1000 GLUCOSE COD
FEED = 1000 mg/ℓ LYSINE COD
ANALYSIS INDICATED, mg/ℓ
BIOLOGICAL SOLIDS
PROTEIN
TOTAL COD
GLUCOSE COD
LYSINE COD
TIME, hrs

Figure 36

APPENDIX II

George, T. K., and Gaudy, A. F. Jr., "Response of Completely Mixed Systems to Hydraulic Shock Loads." Journal Environmental Engr. Div., ASCE, 99, No. EE5, 593-606 (1973).

RESPONSE OF COMPLETELY MIXED SYSTEMS TO HYDRAULIC SHOCK LOADS

By
T. K. George[1] and A. F. Gaudy, Jr.[2], F. ASCE

ABSTRACT

Responses of completely mixed, once-through continuous culture systems of heterogeneous microbial populations of sewage origin were systematically examined under two conditions of hydraulic shock loading. All hydraulic shocks were applied as step changes in dilution rate (either increases or decreases) from a base level of D = 0.125 hr^{-1}. Eight experiments were conducted with constant concentration of inflowing feed carbon source, and eight under conditions of constant daily organic loading rate. Biochemical response was assessed primarily by measurements of biological solids concentration and effluent concentration of carbon source. It was found that under conditions of constant feed concentration, increases in flow rate were most detrimental and an increase of up to 100 percent could be accommodated without serious disruption of biochemical efficiency. Under constant organic loading conditions, decreases in flow rate were more detrimental and a decrease of 100 percent could be accommodated without serious transient disruption.

Based upon these results, it is tentatively recommended that activated sludge processes be protected with surge basins to

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[1]T. K. George, Professor of Civil Engineering, Engineering College, Trichur, Kerala, India
[2]A. F. Gaudy, Jr., Edward R. Stapley Professor of Civil Engineering Chairman Bioenvironmental Engineering Program, Oklahoma State University, Stillwater, Oklahoma, 74074.

accommodate changes in flow rate in excess of 100 percent of the average design flow.

INTRODUCTION

Development of kinetic models for design and operation of activated sludge processes is facilitated by assuming steady state conditions. However, it is well known that perturbations, or shock loadings, which tend to disrupt the steady state, can occur. Research on various types of shock loadings has been a prominent investigative interest in the authors' laboratories for many years, and various reports on experiments dealing with the quantitative and qualitative types of shock loading have been reported. In the present report, results of some experimentations on hydraulic shock loading are presented.

Grady (6) has recently reviewed much of the literature pertinent to shock loadings and development of possible predictive formulations for depicting the transient behavior with respect to biological solids and effluent substrate concentrations, and this need not be reviewed here. In general, there are relatively few reports in the pollution control area dealing with the systematic study of hydraulic shock loading, i.e., studies in which the dilution rate, D (or hydraulic inflow rate), was systematically changed over a wide range of values.

Some data on transient behavior after a change in dilution rate for pure culture systems have been reported (1)(5)(8)(9). Such work has shown that the specific growth rate, μ, does not adjust instantaneously. While studies with pure cultures are useful in helping to provide data for possible "models" depicting transients, they may not be translatable to the more complex systems of natural or heterogeneous

microbial populations. Models for such populations may eventually be evolved, but such evolution will depend upon availability of large amounts of data. Also, there is a more immediate need for practical guidelines regarding ranges of severity of perturbances which can be tolerated by such systems. The work herein presented was undertaken largely with the latter objective in mind.

Experimental Plan

The types of hydraulic shock considered were broadly classified into two general types; in one, herein called "constant feed concentration," the change in D (increase or decrease) was imposed with no change in the inflowing substrate concentration; whereas in the second, the change in D was accompanied by a compensating change in feed substrate concentration so that the unit (daily) organic loading remained constant--herein termed "constant daily organic loading" conditions. Since most conditions of hydraulic shock lie between these, it was of interest to examine both. A change in D may be a simple step change with the new flow rate holding for a considerable time so that the system approaches a new steady state, or the change may occur in cyclic pulses of predictable or unpredictable amplitude and periodicity. In the present study, the former mode was selected.

While cell or sludge recycle is an inherent feature of most activated sludge processes, once-through systems were selected for study. Reasons for such selection lie essentially in the fact that once-through systems are more sensitively "poised" and more responsive in a biochemical and ecological sense than are cell feedback systems. Thus, such systems can be expected to provide a more conservative estimate of possible limits of change in dilution rate which an activated sludge system might accommodate successfully, i.e., accommodate without

serious disruption of biochemical efficiency of substrate removal.

In addition to the above factors, the dilution rate in the initial steady state (i.e., past growth history) can affect the response. It seemed important to set some base line dilution rate for the initial steady state, and in the present study all hydraulic shocks were imposed from an initial D = 0.125 hr^{-1}, i.e., detention time $\bar{t}$ = 8 hours. This specific growth rate (μ = D in the systems studied) is slow enough so that the system is not operating near the critical or washout D and effluent substrate is low; also, it is a dilution rate not uncommonly employed in the field.

MATERIALS AND METHODS

The continuous flow completely mixed reactors employed in these studies were 2.5-liter Pyrex glass aeration vessels. The experimental apparatus (reactors, pumps, water bath, etc.) was the same type used previously by Gaudy, et al. and has been described elsewhere (4). The reactors were adjudged to be completely mixed in accordance with technique previously described and employed by Komolrit and Gaudy (7). Compressed air was supplied through carborundum diffusers at an airflow rate of 5 l/min. All experiments were run at a reactor temperature of 25 $\pm$ 1°C.

Glucose was employed as carbon source; the composition of the medium per liter of feed was: glucose, 1000 mg; $(NH_4)_2SO_4$, 500 mg; $MgSO_4 \cdot 7H_2O$, 100 mg; $MnSO_4 \cdot H_2O$, 10 mg; $CaCl_2 \cdot 2H_2O$, 7.5 mg; $FeCl_3 \cdot 6H_2O$, 0.5 mg; 1 M phosphate buffer (pH = 7.0), 10 ml; tap water, 100 ml, and distilled water to volume.

To initiate each experiment, a sewage seed obtained from the effluent of the primary clarifier of the municipal sewage treatment

plant at Stillwater, Oklahoma, was aerated 24 hours with two liters of the synthetic waste. This provided ample time for development of a dense microbial population. The reactor was then run on a continuous flow basis for a period of two to four days during which time a "steady state" condition with respect to biological solids concentration and substrate concentration developed, and the pre-shock condition of the system was established. A hydraulic shock was then applied and the transient behavior assessed until a new steady state was approached. After each experimental run, the apparatus was drained and cleaned, and a heterogeneous population was again developed from a fresh sewage seed for the next experiment in the series.

Samples of the mixed liquor (and/or effluent from the reactor) were filtered through tared membrane filters (pore size 0.45 μm) for determination of biological solids concentration. Protein and carbohydrate content of the biological solids were measured by biuret and anthrone analyses, respectively (3). In some cases, the composition of the bio-mass was further delineated by analyses for RNA (orcinol test)(10), and DNA (diphenylamine test)(2). In a few experiments, the carbon, hydrogen, and nitrogen contents of the sludge were determined (C-H-N Analyzer, Hewlett-Packard Company, Avondale, Pa.). The membrane filtrate was analyzed for chemical oxygen demand (COD)(13), as well as total carbohydrate concentration (anthrone test)(3). Throughout these studies, frequent checks were made on pH, temperature, and DO (electrometrically) in the reactor. The phosphate buffer in the medium maintained pH at 6.9 $\pm$ 0.1 throughout each experiment, and the airflow rate employed was sufficient to keep the DO concentration at 5.0 mg/l or above.

RESULTS

Hydraulic Shock Under Conditions of "Constant Feed Concentration"

Decrease in Dilution Rate

Three experiments in which the dilution rate was decreased were performed. Figure 1 shows the biochemical response when D was decreased to 25 percent of the prior steady state value, i.e., from 0.125 hr^{-1} to 0.031 hr^{-1}, or from a detention time of 8 hours to 32 hours. Data points to the left of the dashed vertical line (time zero) were obtained at D = 0.125 hr^{-1}; data to the right were obtained after making the change in D. There was some fluctuation in biological solids concentration and in effluent quality as measured by either the total COD concentration in the filtrate (T-COD), or by the anthrone determination expressed as chemical oxygen demand (A-COD). Only carbohydrates are measured by the anthrone test; therefore the difference between the "total COD" and the "anthrone COD" represents soluble organic matter of a non-carbohydrate nature produced by the microorganisms and is hereafter referred to as metabolic intermediates and/or endproducts. It is seen that the disturbance caused by this shock was slight as regards effluent quality (COD); the response was a successful one. The change in D (or specific growth rate, μ) was rather a drastic one (a four-fold decrease), and it did cause some cells to "dilute out" during the first 20 hours. The slight dilute-out was in all probability precipitated by a shift in microbial predominance which was observed to accompany the change in dilution rate. Changes in predominance were assessed by noting changes in the apparent color of the mixed liquor and by microscopic examination in wet mount. It is also significant to note that the sludge yield, Y, in the initial

"steady state" was rather low--slightly less than 30 percent (solids level $\pm$ 300 mg/l for feed concentration of 1060 mg/l COD). RNA and DNA analyses were performed, but are not plotted; there was no significant change in nucleic acid composition in response to the shock.

Figure 2 shows results when the dilution rate was decreased from 0.125 to 0.0625 hr^{-1}. This shock resulted in a mild perturbation in COD removal efficiency. As adjudged by the rather small and short-lived disturbance of effluent quality (filtrate COD concentration), the experiment provided evidence that halving the rate of inflow did not significantly disrupt the system.

Another experiment was run in which the dilution rate was decreased by 25 percent (0.125 to 0.094 hr^{-1} or $\bar{t}$ = 8.00 to 10.7 hr). This change in dilution rate caused only minimal fluctuation in system parameters and sludge composition.

Increases in Dilution Rate

A fifty percent increase in flow rate, from D = 0.125 to 0.188 hr^{-1}, did not cause any significant disturbance in effluent substrate concentration, as seen in Figure 3. There was a slight decrease in protein content and a concomitant increase in carbohydrate content of the sludge.

When the flow rate was doubled, there was a distinct transient rise in effluent COD concentration (see Figure 4). This transient leakage of substrate did not consist of the original carbon source, but of metabolic intermediates and/or endproducts of a non-carbohydrate nature released by the bio-mass in response to the increased specific growth rate forced by the change in dilution rate. The increase in effluent COD was rather short lived; recovery was achieved in eight hours or less, and the response may be adjudged successful, i.e., this

the curve. Substituting these values in the equation, one can solve simultaneously for μ_{max} and K_s. For example, simultaneous solution of the equation using values of S at D = 0.3 and 0.4, yield μ_{max} and K_s values of 0.53 hr^{-1} and 124 mg/l, respectively; using values of S at D = 0.25 and 0.35, values of 0.5 hr^{-1} and 106 mg/l, respectively, are obtained. These values are within the general range of values found in other studies employing heterogeneous populations reported from this laboratory (4)(11)(12).

Hydraulic Shock Under Conditions of "Constant Daily Organic Loading"

In this series of experiments, the organic loading (mg COD fed per unit of time) was maintained constant in the pre- and post-shock periods. Thus a change from D = 0.125 hr^{-1} ($\bar{t}$ = 8 hrs) to D = 0.031 hr^{-1} ($\bar{t}$ = 32 hrs) was accompanied by a change in feed concentration from 1000 to 4000 mg/l.

Decrease in Dilution Rate

Figure 9 shows the response when D was changed from 0.125 hr^{-1} to 0.031 hr^{-1}. In response to the higher feed concentration, the biological solids concentration rose rather sharply, but not rapidly enough to allow removal of the increased feed substrate. There was a peaked transient rise in effluent substrate concentration and significant loss of substrate removal efficiency during the transient phase which continued for a considerable period of time. By the time the experiment was terminated, the substrate removal efficiency had returned to its pre-shock level ($\pm$ 90 percent); consequently the substrate level in the effluent was four times greater than in the pre-shock condition. In any event, it is evident that this system could not accommodate the shock without significant transient disruption

even though the mass of substrate fed per unit time remained constant. It is important to note here that the response was not due to lack of DO at the higher substrate concentration. DO values below 5 were never observed. The values of protein, carbohydrate, RNA, DNA, and C-H-N content of the sludge are not plotted. In general, the content of these constituents of the bio-mass remained within commonly expected ranges.

A more favorable response was obtained when the dilution rate was changed from 0.125 to 0.062 hr^{-1}. It is seen in Figure 10 that in this case, a peaked transient leakage of substrate (overshoot) did not ensue. Instead, there was a rather smooth, gradual transition as this system approached the new "steady" state. The values of biological solids concentrations vary widely, and from these data alone it would be impossible to conclude that the system was approaching the new steady state. However, the values for protein and carbohydrate content of the bio-mass together with the effluent analyses for COD and anthrone-reactive material provide rather strong indication that the system had attained a new steady state in which the substrate removal efficiency (not effluent quality) remained essentially the same as in the pre-shock conditions, and that the transition was a smooth non-disruptive one. A milder shock, D = 0.125 to 0.094 hr^{-1}, also resulted in a smooth transition.

Increase in Dilution Rate

As under conditions of constant concentration, an increase in dilution rate forces the system to grow at a higher specific growth rate ($\mu = D$). However, since μ is also affected by substrate concentration (an increase in S engenders an increase in μ), the lowering of substrate concentration by decreasing the feed may then foster a

affected by the four-fold decrease in specific growth rate imposed by the new hydraulic regime. When the flow rate was increased, the system exhibited a noticeable transient increase in effluent substrate concentration when D was doubled. A small peaked transient of short duration ensued, and S soon returned to its former steady state level (see Figure 4). At higher increases in D, the peaked transient leakage of substrate with concomitant decrease and recovery in X was also developed (see Figures 5 and 6). However, the new steady state at the high D values fell along the natural dilute-out curve for these populations, as was shown in Figure 8. At still higher values of D, the transition curve for substrate did not exhibit a peak, i.e., there was a smooth rise to the new "steady" substrate concentration; there was no "overshoot" and recovery (see Figure 7).

Thus, in these studies there is some evidence for damped oscillatory response (Figures 5 and 6), and at higher magnitudes of change in D the oscillating nature of the response is almost completely damped out. Mor and Fiechter observed a transition from a damped oscillatory transient response to one of a smooth transition depending largely upon the past history of the culture, i.e., the magnitude of dilution rate, D, prior to initiating the step increase in D (9). In the present study, the initial steady state dilution rate was held constant and a similar effect was noted. However, it is extremely difficult to make a comparative analysis of studies accomplished with heterogeneous populations and those employing pure cultures (Mor and Fiechter employed S. cerevisiae). Also, in the present study, effluent COD in the new steady state for experiments in which D was increased by more than 100 percent (see Figures 5, 6, and 7) was due primarily to metabolic intermediates and/or end products. The distinct differences

between dissimilation of the original carbon source and metabolic incorporation of the carbon contained in the substrate comprise another complicating factor which is more prevalent in heterogeneous population studies than in studies employing pure cultures, and is a factor not usually given consideration in "model making." Nonetheless, it is the heterogeneous population system which is of importance in biological treatment processes, and it is the total removal of carbon, not of specific substrate, which is important. Much work is needed using both pure and heterogeneous populations with various past histories (e.g., initial steady states) of the systems and magnitudes of change, etc., in attempts to gain an understanding of response. Such understanding is a prime requisite for construction of adequate models of practical utility. For the present, it seems best to use the results in attempts to gain insight into practical guidelines.

From a practical point of view, the results of these shock load studies provide evidence that hydraulic shocks (with S_i constant) constituting an increase in D (decrease in retention time) are more deleterious than are decreases in D, and that for a design $\bar{t}$ of $\pm$ 8 hours, an increase in D of 100 percent can be successfully accommodated, i.e., it will lead to little or no disruption in metabolic efficiency of the system.

When hydraulic shocks of like magnitude are imposed upon the system under conditions of constant daily organic loading, a decrease in dilution rate comprises the more severe perturbance since the system must also accommodate the concomitant rise in S_i (quantitative shock). For example, when D was decreased to one-fourth its former value at a constant concentration of S_i (see Figure 1), there was minimal disturbance, whereas the same hydraulic change under constant organic

loading conditions caused rather severe transient disturbance (peaked COD response) before the system approached a new steady state condition, as was seen in Figure 9. Shock loadings consisting of increases in dilution rate with concomitant decreases in S_i led to rather orderly wash-out of biological solids to the new steady level with small fluctuations in effluent COD. From the standpoint of providing guidelines for accommodation to shock, the data indicate that when the hydraulic shock comes on to the system under conditions of relatively constant organic loading, D can be halved (with S_i doubled) without causing serious disruption of COD removal efficiency. Also, it seems reasonable to say that D can be doubled (with S_i halved) without serious disruption.

Although biological response to a rather wide range of hydraulic shock loadings has been presented in this report, the situations examined by no means bracket the extremes of hydraulic shock loads and various modes of their application to biological systems. Also, the response measured was the biological response of the bio-mass and, in some instances of hydraulic shock, the bio-physical effects, e.g., sludge settleability, may be of major concern. However, in cases wherein removal of the organic substrate depends upon successful metabolism of the organic matter (and in most cases it does), metabolic considerations must take precedence over the bio-physical response. No amount of settleability can metabolize the waste substrate. Also, there are engineering expedients to enhance separation of the mixed liquor, whereas there is less control over the biological response. Therefore, some tentative engineering conclusions based upon the results of these studies seem warranted.

CONCLUSIONS

Based upon these data, it seems reasonable to conclude that for completely mixed activated sludge reactors designed for operation with a mean hydraulic residence time of approximately eight hours, the system can be expected to accommodate, without serious disruption of biochemical efficiency of substrate removal, hydraulic shocks consisting of step increases in flow rate up to 100 percent with no change in concentration of incoming substrate (constant concentration conditions). Decreases in flow rate greater than 100 percent can be accommodated. Under conditions of constant daily organic loading, such a system can be expected to accommodate a decrease in flow rate of 100 percent (with 100 percent increase in inflowing substrate concentration). Increase in flow rate of 100 percent can also be accommodated.

It seems reasonable to recommend that in the interest of providing more steady and reliable performance with regard to substrate removal efficiency, activated sludge systems be afforded protection (i.e., by insertion of an equalization or surge basin) against a change in flow rate greater than 100 percent.

ACKNOWLEDGMENT

The experimental phases of this work were conducted under a research grant WP-00075 from the Federal Environmental Protection Agency. Portions of the analysis of the data and manuscript preparation were supported by a research grant A-043 from the Oklahoma Water Resources Research Institute USDI.

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APPENDIX II

The following symbols are used in this paper:

D	Dilution rate. Ratio of the rate of inflow, F, and the volume of liquor in the aeration tank, V. It is equal to the reciprocal of the mean residence time in a completely mixed reactor.
K_s	A biological "constant" used in the hyperbolic expression relating specific growth rate to substrate concentration. It is known as the saturation constant. It is numerically equal to the substrate concentration at which the specific growth rate is equal to ½ the maximum specific growth rate for the system.
S	Substrate concentration.
$\bar{S}$	Steady state concentration of substrate in a completely mixed continuous flow reactor.
S_i	Concentration of substrate in the inflowing feed in continuous flow operation.
$\bar{t}$	Mean hydraulic residence time in a completely mixed continuous flow reactor, V/F.
X	Biological solids concentration, weight per volume.
$\bar{X}$	Steady state biological solids concentration in a completely mixed continuous flow reactor.
μ	Specific growth rate in an exponential phase of growth.
μ_{max}	The maximum specific growth rate for a system in exponential growth.

TITLES FOR FIGURES

Figure

1. Response to a Decrease in Dilution Rate From 0.125 hr^{-1} to 0.031 hr^{-1}; S_i = 1000 mg/l Glucose. T-COD is Total Chemical Oxygen Demand. A-COD is total carbohydrate (anthrone-reactive Material) Calculated as COD.

2. Response to a Decrease in Dilution Rate From 0.125 hr^{-1} to 0.062 hr^{-1}; S_i = 1000 mg/l Glucose.

3. Response to an Increase in Dilution Rate From 0.125 hr^{-1} to 0.188 hr^{-1}; S_i = 1000 mg/l Glucose.

4. Response to an Increase in Dilution Rate From 0.125 hr^{-1} to 0.25 hr^{-1}; S_i = 1000 mg/l Glucose.

5. Response to an Increase in Dilution Rate From 0.125 hr^{-1} to 0.313 hr^{-1}; S_i = 1000 mg/l Glucose.

6. Response to an Increase in Dilution Rate From 0.125 hr^{-1} to 0.375 hr^{-1}; S_i = 1000 mg/l Glucose.

7. Response to an Increase in Dilution Rate From 0.125 hr^{-1} to 0.437 hr^{-1}; S_i = 1000 mg/l Glucose.

8. Steady State Concentrations $\bar{X}$ and $\bar{S}$ at Various Dilution Rates.

9. Response to a Decrease in Dilution Rate From 0.125 hr^{-1}, S_i = 1000 mg/l Glucose to 0.031 hr^{-1}, S_i = 4000 mg/l Glucose.

10. Response to a Decrease in Dilution Rate From 0.125 hr^{-1}, S_i = 1000 mg/l Glucose to 0.062 hr^{-1}, S_i = 2000 mg/l Glucose.

11. Response to an Increase in Dilution Rate From 0.125 hr^{-1}, S_i = 1000 mg/l Glucose to 0.188 hr^{-1}, S_i = 667 mg/l Glucose.

12. Response to an Increase in Dilution Rate From 0.125 hr^{-1} S_i = 1000 mg/l Glucose to 0.25 hr^{-1}, S_i = 500 mg/l Glucose.

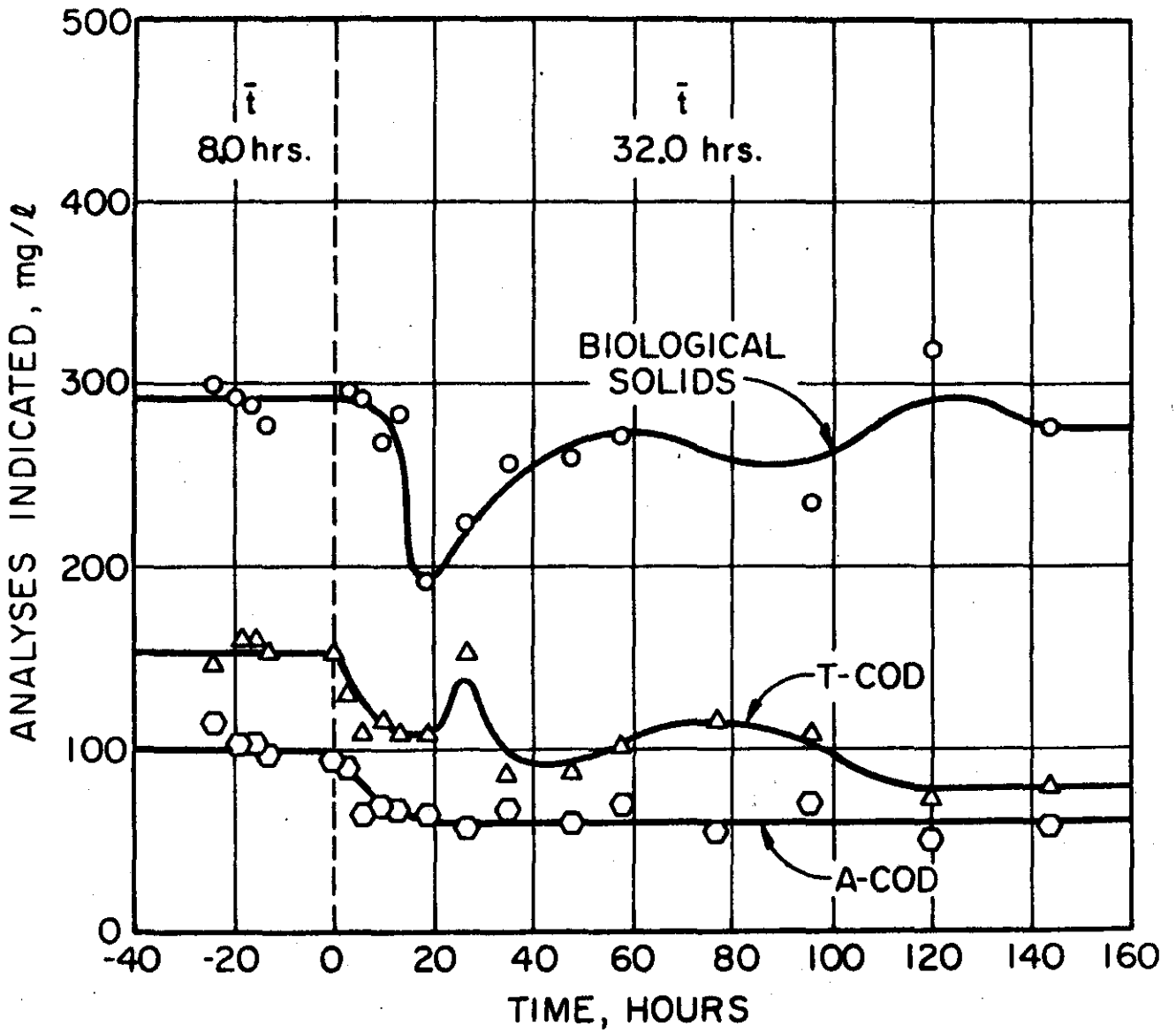
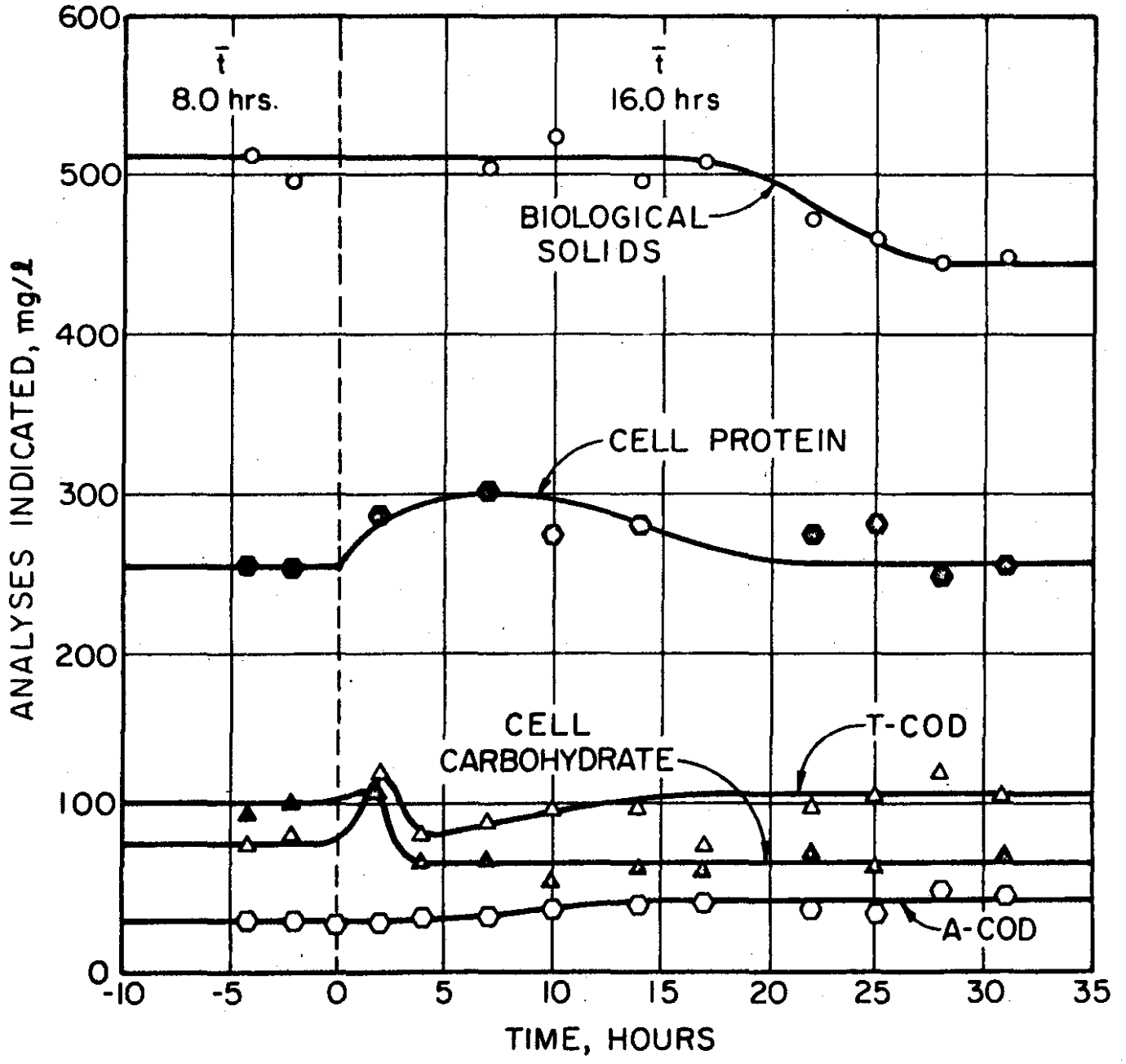

500
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
t̄
8.0 hrs.
t̄
32.0 hrs.
BIOLOGICAL SOLIDS
T-COD
A-COD
-40
-20
0
20
40
60
80
100
120
140
160
TIME, HOURS

Figure 1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400
300
200
100
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$\bar{t}$
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$\bar{t}$
16.0 hrs
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CELL PROTEIN
CELL CARBOHYDRATE
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A-COD
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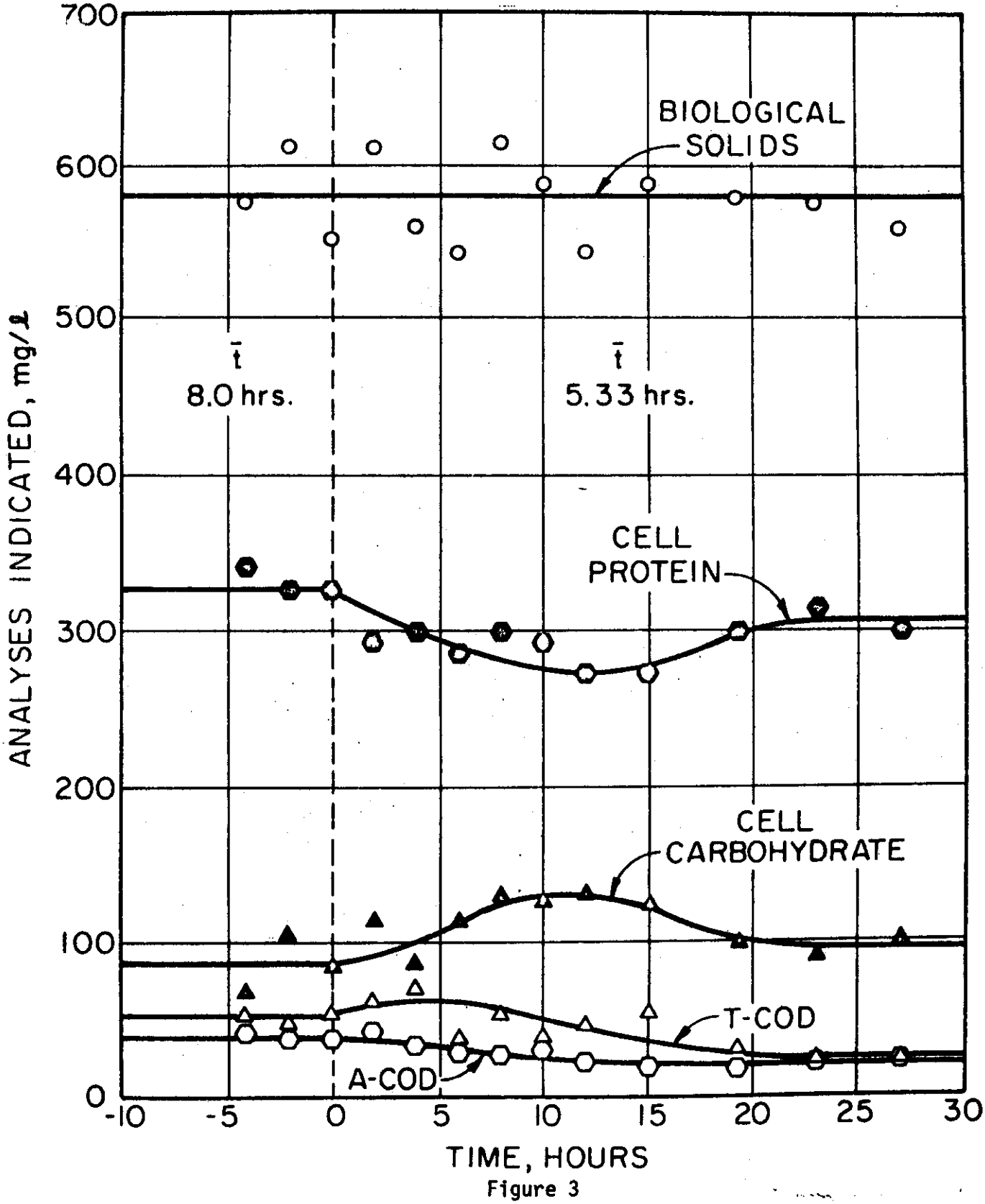
-5
0
5
10
15
20
25
30
35
TIME, HOURS

Figure 2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600
500
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
BIOLOGICAL SOLIDS
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$\bar{t}$ 5.33 hrs.
CELL PROTEIN
CELL CARBOHYDRATE
T-COD
A-COD
-10
-5
0
5
10
15
20
25
30
TIME, HOURS

Figure 3

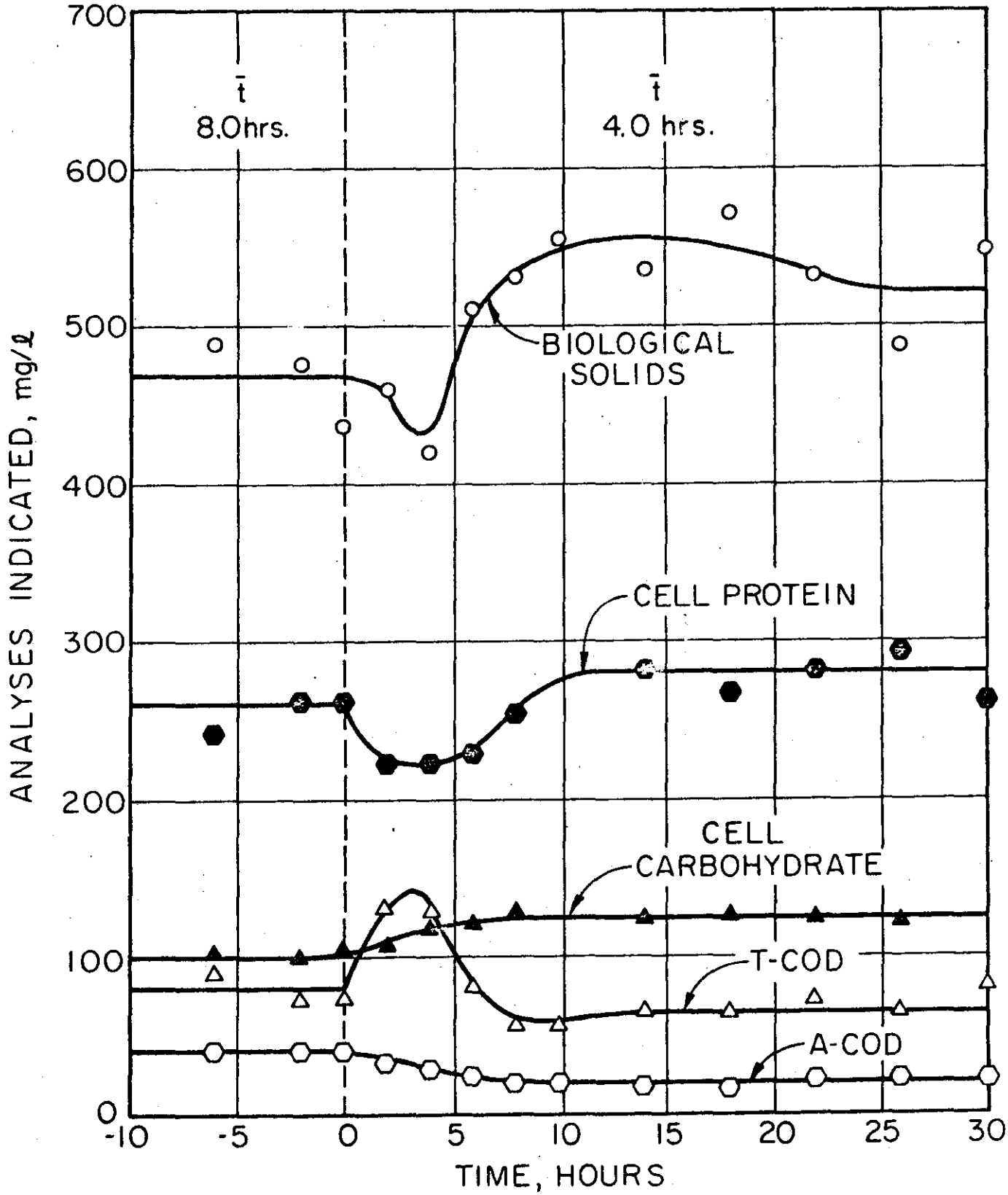

700
600
500
400
300
200
100
0
-10
-5
0
5
10
15
20
25
30
$\bar{t}$
8.0hrs.
$\bar{t}$
4.0 hrs.
BIOLOGICAL SOLIDS
CELL PROTEIN
CELL CARBOHYDRATE
T-COD
A-COD
ANALYSES INDICATED, mg/ℓ
TIME, HOURS

Figure 4

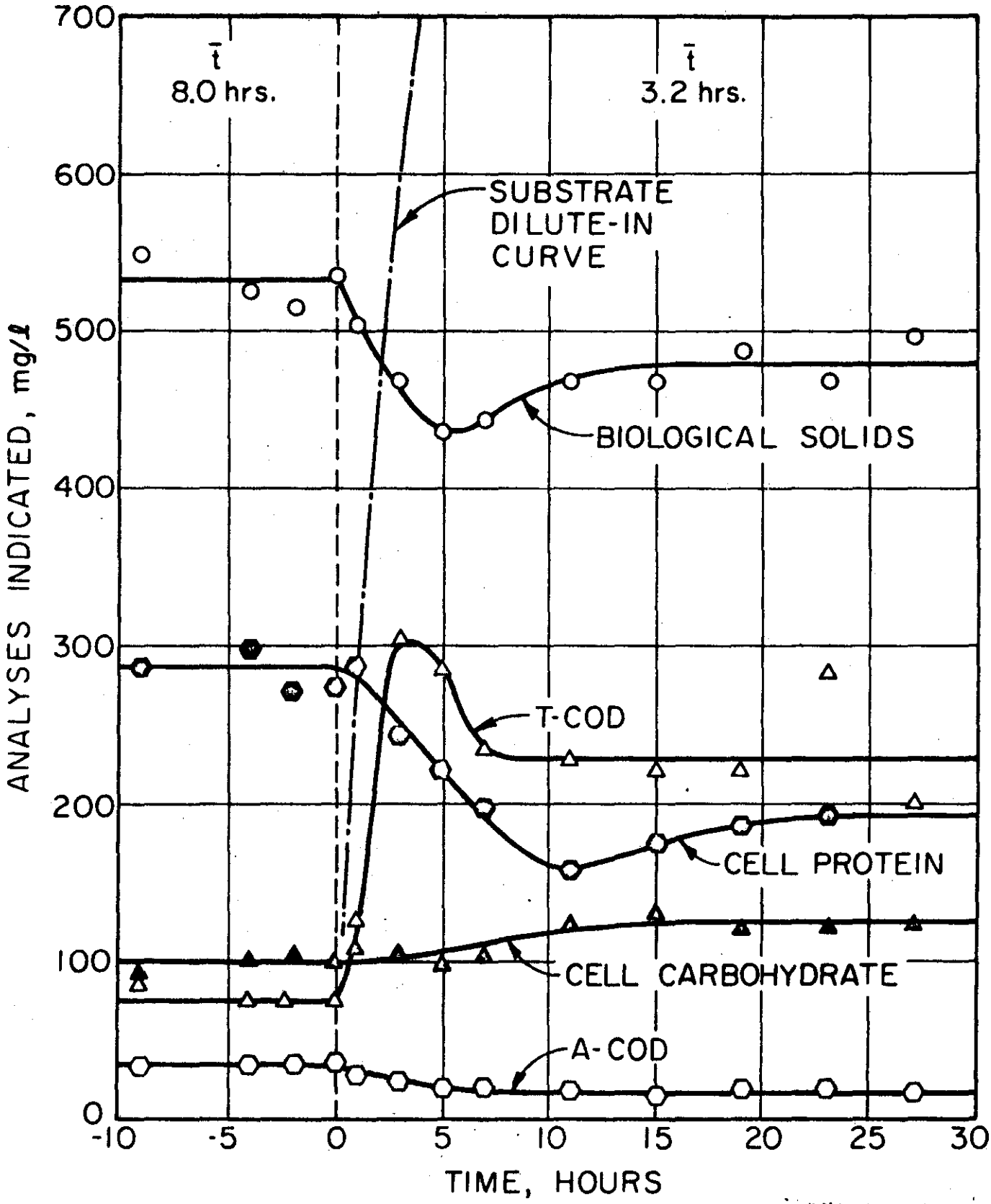
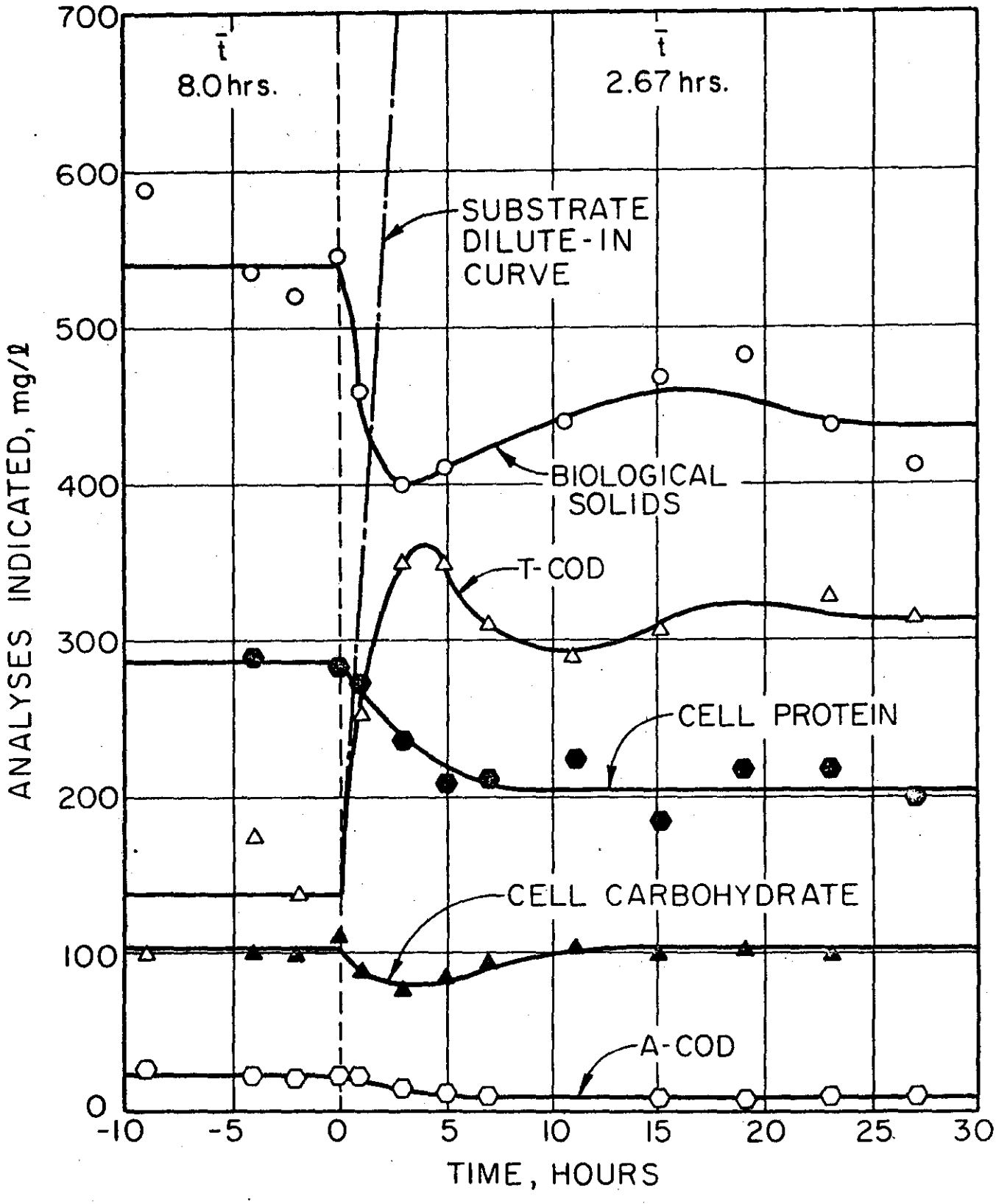

700
600
500
400
300
200
100
0
-10
-5
0
5
10
15
20
25
30
$\bar{t}$
8.0 hrs.
$\bar{t}$
3.2 hrs.
SUBSTRATE
DILUTE-IN
CURVE
BIOLOGICAL SOLIDS
T-COD
CELL PROTEIN
CELL CARBOHYDRATE
A-COD
ANALYSES INDICATED, mg/ℓ
TIME, HOURS

Figure 5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600
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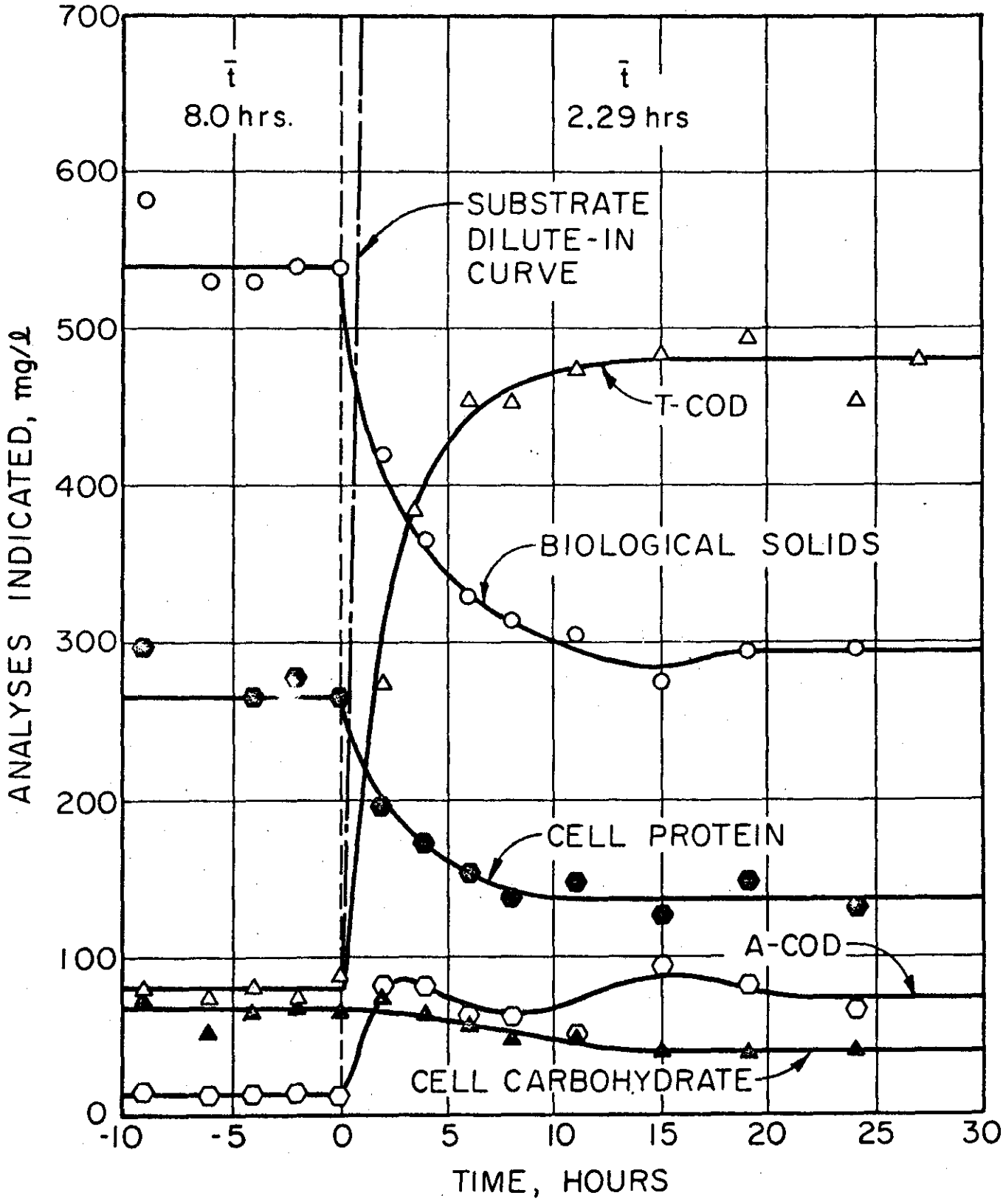
400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
$\bar{t}$
8.0 hrs.
$\bar{t}$
2.67 hrs.
SUBSTRATE
DILUTE-IN
CURVE
BIOLOGICAL
SOLIDS
T-COD
CELL PROTEIN
CELL CARBOHYDRATE
A-COD
-10
-5
0
5
10
15
20
25
30
TIME, HOURS

Figure 6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400
300
200
100
0
ANALYSES INDICATED, mg/ℓ
$\bar{t}$
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$\bar{t}$
2.29 hrs
SUBSTRATE
DILUTE-IN
CURVE
T-COD
BIOLOGICAL SOLIDS
CELL PROTEIN
A-COD
CELL CARBOHYDRATE
-10
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0
5
10
15
20
25
30
TIME, HOURS

Figure 7

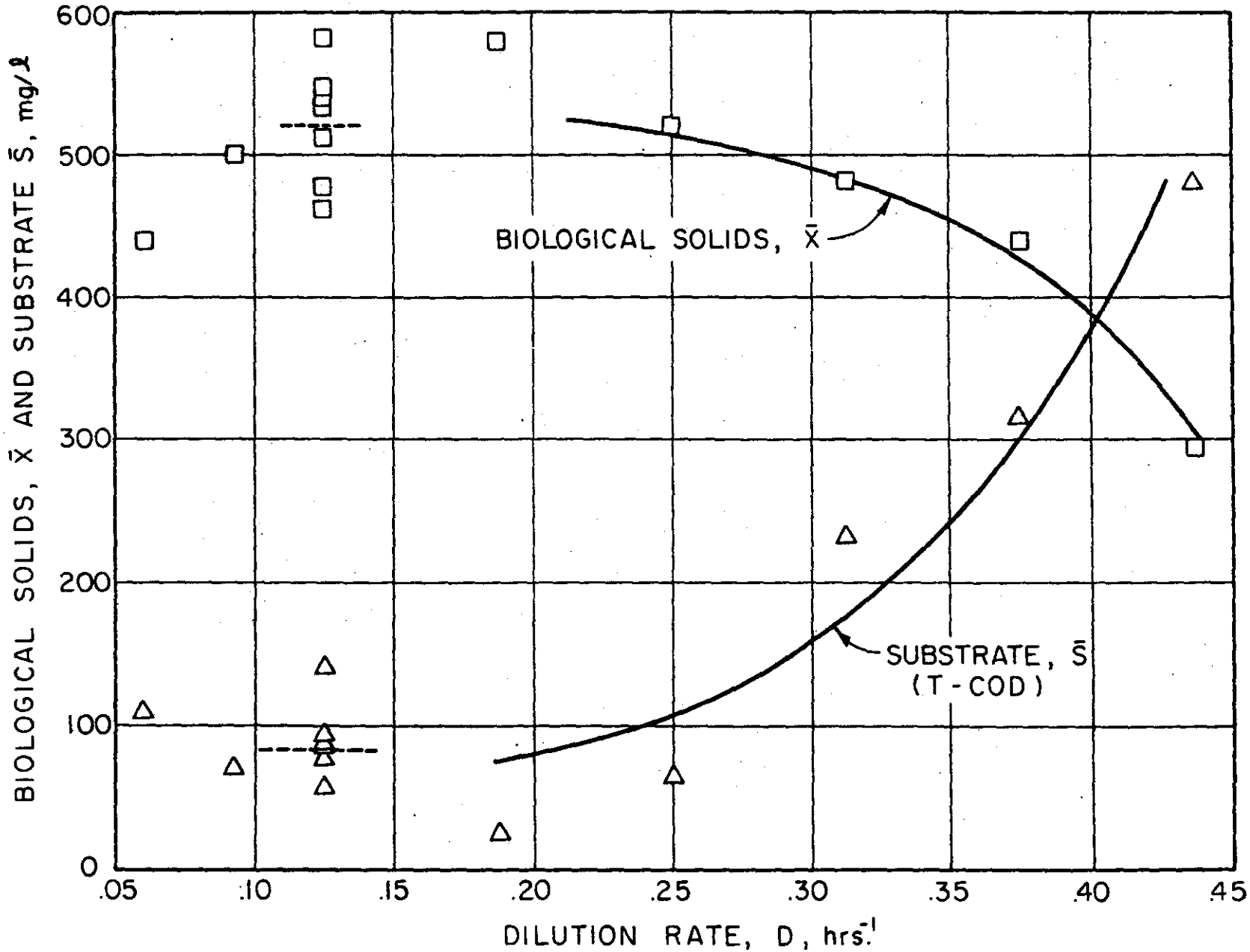
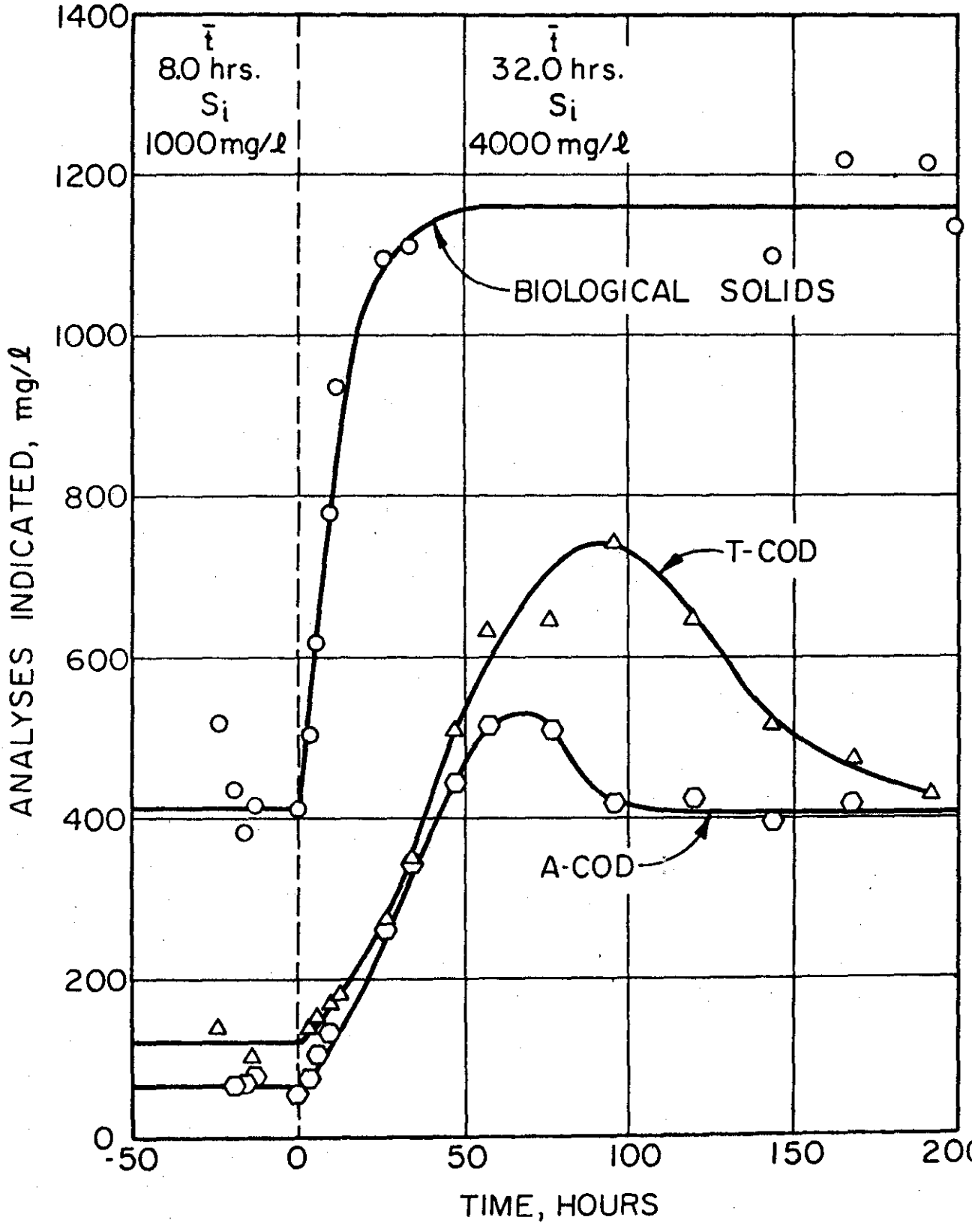

BIOLOGICAL SOLIDS, $\bar{X}$ AND SUBSTRATE $\bar{S}$, mg/ℓ
600
500
400
300
200
100
0
.05
.10
.15
.20
.25
.30
.35
.40
.45
DILUTION RATE, D, hrs.$^{-1}$
BIOLOGICAL SOLIDS, $\bar{X}$
SUBSTRATE, $\bar{S}$
(T-COD)

Figure 8


1400
1200
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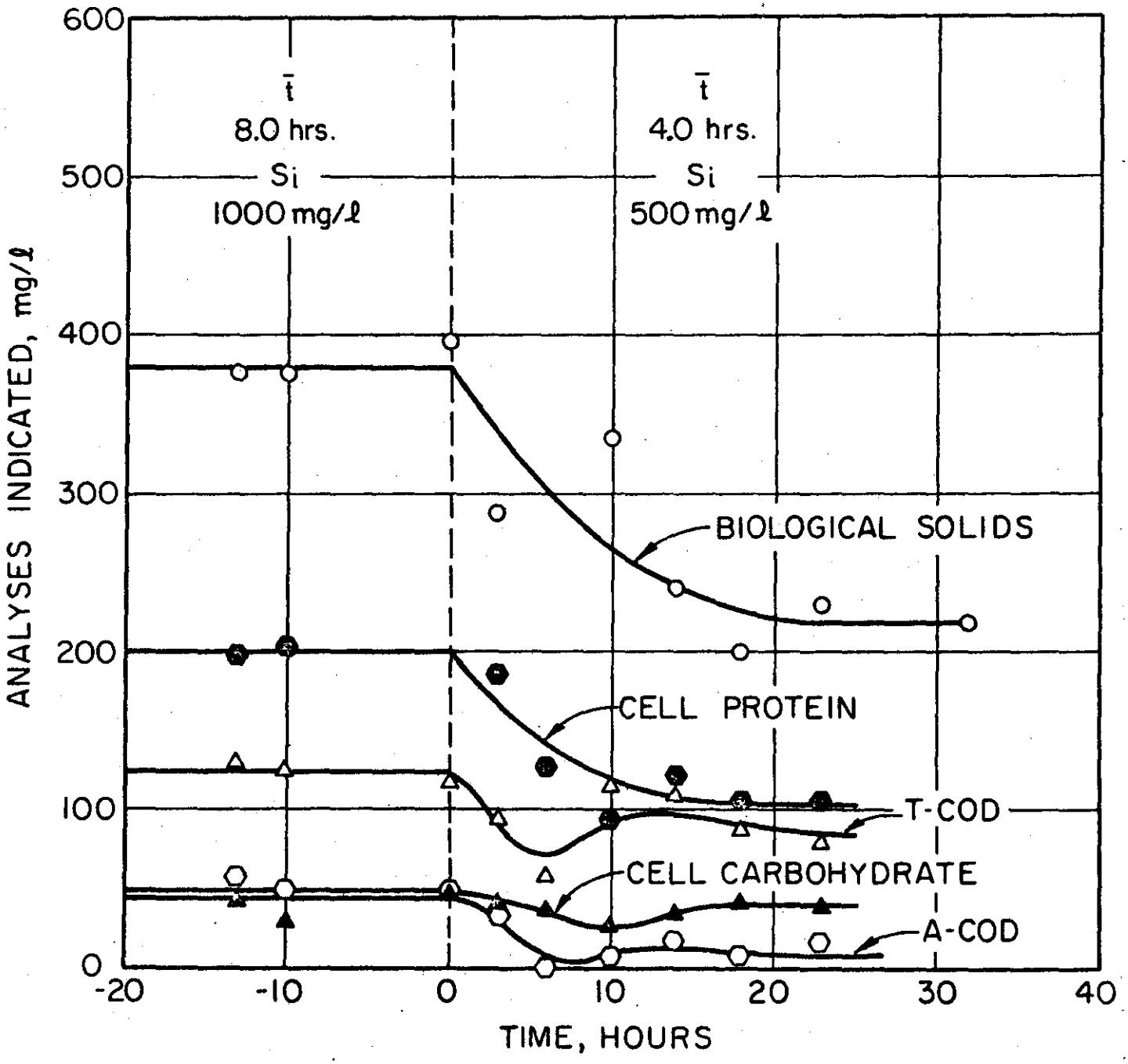
800
600
400
200
0
-50
0
50
100
150
200
ANALYSES INDICATED, mg/ℓ
TIME, HOURS
t̄
8.0 hrs.
Si
1000 mg/ℓ
t̄
32.0 hrs.
Si
4000 mg/ℓ
BIOLOGICAL SOLIDS
T-COD
A-COD

Figure 9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500
400
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200
100
0
-20
-10
0
10
20
30
40
ANALYSES INDICATED, mg/l
TIME, HOURS
t̄
8.0 hrs.
Si
1000 mg/l
t̄
4.0 hrs.
Si
500 mg/l
BIOLOGICAL SOLIDS
CELL PROTEIN
T-COD
CELL CARBOHYDRATE
A-COD

Figure 12

APPENDIX III

George, T. K., and Gaudy, A. F. Jr., "Response of Completely Mixed Systems to pH Shock." Biotechnology and Bioengineering, XV, 5, 933-949 (1973).

RESPONSE OF COMPLETELY MIXED SYSTEMS TO pH SHOCK

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Stillwater, Oklahoma 74074

SUMMARY

The response of aerobically growing heterogeneous microbial populations of sewage origin to step increases and decreases in pH were studied in both once-through and cell recycle systems. The pH range studied was 2.7 to 8.0. All studies were conducted at a dilution rate of 0.125 hr^{-1}, and all shocks were administered from a base or pre-shock pH level of 6.4 to 6.7. In each experiment, the pre-shock or initial "steady state" was assessed, the pH of the feed changed, and the resulting transient behavior of the system examined until attainment of the new or final "steady state" was approached. The major objectives of the work were to characterize the nature of the response with respect to bio-mass and effluent substrate concentrations, types of microbial populations present and chemical composition of the bio-mass, and to obtain guidelines as to allowable change in pH in waste streams. It was found in once-through systems that substrate removal efficiency recovered from pH levels as low as 3.0 after rather long periods of transient leakage of substrate. Cell recycle attenuated the severity of substrate leakage. In all cases of severe acid shock, the microbial population changed from predominantly bacterial-protozoan to one consisting predominantly of filamentous fungi. Changes in chemical composition of the sludge (protein and carbohydrate content) were

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consistent with the population changes. Based upon the results, it can be conservatively estimated that changes in pH of no more than one unit from the neutral pre-shock range can be tolerated without possible disruption of biochemical efficiency of substrate removal.

INTRODUCTION

The effect of pH on growth and microbial products has been of wide interest in areas of basic microbiology and in commercial fermentations. The system pH is also of critical concern in the biological treatment of waste waters, since neutralization of large volumes of waste water to maintain optimum pH for growth and purification efficiency can be very costly. Thus, much work has been done to develop microbial systems which can provide acceptable treatment efficiency of some industrial wastes in acid or alkaline ranges, e.g., Heukelekian and Gellman (1). Recently, Randall, Edwards, and King have reported treatment efficiencies of approximately 85% (based on removal of COD) at pH values as low as 2.6 (2). There may be some doubt as to how well systems acclimated to acid or alkaline extremes of pH can adjust to shock loadings, since the pH is bound to exert a selective influence on the species present in a biological treatment process, e.g., activated sludge. The lowered diversity of species in the system can be expected to restrict the range of adaptive response to the variety of environmental changes to which treatment processes are subjected, such as changes in concentration and kind of organic substrate(s), flow rates, temperature, pH, etc.

While there have been many investigations regarding operation at various levels of pH, there have been fewer investigations to

characterize system response with respect to changes in pH. Recently, Clark and Speece (3) have presented data on the transient response (effect on methane production) of continuous flow anaerobic digestion systems to changes in pH. From a "steady state" level of 8, the system could recover completely from decreases in pH to as low as pH 5 provided the duration of the depressed pH was less than 12 hours. Such results demonstrate the importance of rapid corrective action in such finely poised ecosystems as exist in anaerobic digestion processes. In the area of basic microbial kinetics, studies on the response of pure cultures to various changes in pH (step, pulsating, etc.) have been helpful in devising models to describe the response (4).

In the present experimentation, the aim was to study systematically the effects of step changes in pH on continuously cultured heterogeneous microbial populations in completely mixed aerobic systems. From the standpoint of possible "model making" from the data, the use of heterogeneous populations interjects a complicating factor since it can engender an ecological as well as biochemical response. However, as related to biological treatment of waste waters, it is the heterogeneous population which is of major importance. The aim of the study was to provide general characterization of the response with regard to cell and substrate concentration and composition of the cells. While such information can prove useful in helping provide data for mathematical modeling of the response, the main purpose of the present study was to help establish guidelines for allowable pH changes which a biological treatment plant might accommodate without serious disruption of treatment efficiency.

It is realized that the biochemical response to a shock loading

is in all probability related to and affected by the pre-shock conditions of growth. With regard to changes in pH, the past growth history with respect to dilution rate, D, and pH in the initial steady state seem of prominent significance. In the present study, D was maintained constant at 0.125 hr^{-1}, and the initial pH levels were maintained at slightly below neutral (pH 6.4 to 6.7).

MATERIALS AND METHODS

The reactors employed in these studies were custom-built 2.5-liter Pyrex vessels. The experimental apparatus (reactors, pumps, water bath, etc.) were the same type as those employed previously in our research, and are described in more detail elsewhere (5)(6)(7). Compressed air was supplied through carborundum diffusers at an airflow rate of 5 l/min. Temperature was maintained at 25 $\pm$ 0.5°C. The reactors were adjudged to be completely mixed, in accordance with techniques described and employed previously by Komolrit and Gaudy (8).

The synthetic waste employed consisted of the following: glucose, 1000 mg/l; $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; $CaCl_2 \cdot 2H_2O$, 7.5 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; tap water, 100 ml/l; 1 M phosphate buffer solution, 10 ml/l. The phosphate buffer solution was made up using suitable ratios of H_3PO_4, KH_2PO_4, K_2HPO_4, and/or K_3PO_4 to yield the various pH values used during the study.

Throughout the investigation, the COD:N ratio was maintained at 10:1, and COD:P ratio was maintained at 3.4:1. All pH changes were effected by changing ratios of acid and basic phosphates; thus, the range of change in pH in these studies was governed by the pH values which could be maintained by the buffer system. Extremely high pH

values were avoided because they led to precipitation of salts in the synthetic waste.

Each experiment was initiated by inoculating the synthetic waste with a 20-ml sample of sewage obtained from the effluent of the primary clarifier of the municipal sewage treatment plant at Stillwater, Oklahoma. The system was run under batch conditions for 24-48 hours, after which the feed solution was continuously pumped in at $D = 0.125\ hr^{-1}$. In "once-through" systems, continuous flow operation was continued at the base line pH value (6.4-6.7) for a period of two to five days, during which the initial "steady state" condition was assessed. For experiments in which cell recycle was employed, a period of two to three weeks in the pre-shock condition was allowed for attainment and characterization of the initial steady state condition. Shock loadings were applied by changing (increasing or decreasing) the pH of the influent feed. The transient response to the step change was assessed, as was the condition at the new steady state.

Biological solids concentration was measured by the membrane filter technique (0.45 μm pore size); protein and carbohydrate of the biological solids were measured by the biuret and anthrone techniques, respectively (9). In some cases, the cells were also analyzed for RNA, using the orcinol test (10), and for DNA, using the diphenylamine test (11). During one experiment, the carbon-hydrogen-nitrogen content of the sludge was also measured (C-H-N analyzer, Hewlett-Packard Co., Avondale, Pa.). The membrane filtrate was analyzed for total chemical oxygen demand (T-COD)(12), and for total carbohydrate concentration (anthrone test) calculated as COD (A-COD)(9). Frequent

checks were made on pH (Beckman Zeromatic pH meter), temperature, and dissolved oxygen (Precision Scientific DO analyzer) in the reactor.

EXPERIMENTAL RESULTS

The biochemical response to a step change in pH from 6.6 to 8.0 is shown in Figure 1. Data plotted to the left of the dashed line through zero on the time axis represent the "steady state" condition at the initial pH level. The change in pH caused only slight fluctuation in the substrate removal measured by either the COD or anthrone test; there was some fluctuation in biological solids concentration. Protein content of the sludge decreased somewhat, and there was an accompanying rise in carbohydrate content.

Figure 2 shows the results when the same change in pH was applied to a system in which sludge was recycled. The recycle flow was 33.3 percent of the feed flow, and the biological solids concentration in the recycle was 3500 mg/l $\pm$ 10 percent. In this case, the resulting pH in the reactor was 7.65. The fact that the pH did not rise quite as much as it did in the previous experiment may be attributable to the high biological solids concentration. There was essentially no change in the effluent substrate concentration. The rise in biological solids concentration was accompanied by a rise in protein, carbohydrate, and DNA in the sludge. Thus, the percent composition remained nearly the same as before the shock.

When a somewhat milder alkaline shock (from pH 6.7 to 7.3) was applied to a once-through system, there was essentially no change in the biological parameters.

When the pH was decreased from the base level of 6.7 to 6.2 in

a once-through system, there was a discernible transient disruption of the substrate removal ability of the system (see Figure 3). The biological solids concentration gradually decreased from 480 to 290 mg/l within approximately 100 hours after changing the feed pH. The effluent substrate concentration was not affected until after the 50th hour. Thereafter, the effluent COD rose to approximately 230 mg/l with the maximum COD leakage occurring at the time of lowest solids concentration in the system. COD removal in the final steady state was considerably better than before the shock and there was an apparent increase in cell yield (note the final solids concentration of 680 mg/l). The drop in pH was a gradual one, requiring approximately 120 hours to reach 6.15.

In Figure 4 it is seen that a slightly more severe shock in a once-through system, from pH 6.7 to 5.8, precipitated a more rapid dilute-out of cells, but in contrast with the previous result, no excess leakage of COD ensued. Microscopic examination of the sludge revealed no gross change in species predominance in either experiment. It is also interesting to note that in both experiments the biological solids concentration was higher in the final state (or as the system approached steady state). Analyses for sludge composition were not obtained during the experiments shown in Figures 3 and 4.

When the system was stressed by a decrease of three pH units (6.4 to 3.5), a rather drastic response was obtained, as seen in Figure 5. Within 40 hours, the biological solids concentration dropped from 375 to 65 mg/l. During this time, the pH dropped rather sharply to slightly above pH 4, and then there was a gradual decline to pH 3.5 during the period of solids recovery. The effluent COD curve was

essentially the mirror image of the biological solids concentration curve. Approximately five days after the change in pH was initiated, the system recovered. In the new steady state, the biological solids concentration was higher and the effluent COD concentration lower than at the more neutral pH level of the initial steady state. There were drastic changes in protein and carbohydrate content. Cell protein dropped from 56 percent to a low of 22 percent during the transient, then rose to a new steady state level of only 29 percent. The carbohydrate content rose from 15 to 38 percent, and then decreased to 25 percent in the final steady state. There was also a reduction in the DNA content of the bio-mass. The sludge composition in the final steady state is more representative of fungi than of bacteria. Microscopic examinations made during the experiment indicated that in the initial steady state, the bio-mass consisted predominantly of clumped bacteria and protozoa, whereas after making the change in pH, filamentous forms predominated.

A similarly severe pH shock (6.5 to 3.3) was applied to a system in which cells were recycled. For this experiment, the average concentration of recycle sludge was 1000 mg/l, and the hydraulic recycle ratio was 33.3 percent. The biological solids concentration in the reactor in the pre-shock condition was 625 mg/l. As seen in Figure 6, the shock caused a rapid dilute-out of cells and substrate. This system recovered somewhat more rapidly than the one shown in Figure 5, and the maximum substrate concentration in the effluent was lower than in the case with no cell feedback. The drop in protein and increase in carbohydrate content of the sludge was similar to that observed in the previous experiment. However, unlike the previous case, the

protein content of the sludge remained at approximately 50 percent for nearly 40 hours, during which time most of the substrate leakage and solids dilute-out occurred. These data suggest that due to the recycle of cells, the ecological or adaptive response (shift to filamentous fungal forms) was delayed by the recycle of non-filamentous species. Microscopic examination of the sludge indicated that the previously noted shift from clumped bacteria in the initial steady state to filamentous forms in the final state again occurred.

A similar pH shock was applied to a system in which a higher recycle sludge concentration was employed. Nearly all of the settleable sludge was returned from the clarifier to the aeration tank at a hydraulic recycle ratio of 33 percent of the feed flow rate. This led to a pre-shock level of 2250 mg/l biological solids. In this case, when the pH was changed from 6.7 to 3.2, there was only minor leakage of substrate. As seen in Figure 7, the effluent COD rose only to 150 mg/l and within 40 hours returned to the pre-shock level. However, the drastic effect on sludge settleability is indicated by the drop in biological solids concentration (2250 to 900 mg/l). In this experiment, there was an increase in filamentous forms after the shock, but they did not predominate. The sludge consisted predominantly of loosely clumped bacteria in a lattice or web of filamentous forms. The drop in protein and increase in carbohydrate content were again evidenced, although the change in sludge composition was not as severe as in the two previous experiments. Thus, the cell composition data correlated rather well with microscopic observations of gross change in predominant species.

It was seen in Figure 5 that the once-through system could

recuperate from a rather severe shock consisting of a change in pH from 6.4 to 3.5. It was of interest to examine response patterns to even more severe changes, and to determine at what pH these systems might for all practical purposes lose their recuperative power. Figures 8, 9, and 10 show responses to increasingly severe depressions of pH. Decreasing the pH to 3.2 caused leakage of a higher concentration of COD (900 mg/l) than did a drop to 3.5 (Figure 5, 700 mg/l). It is seen in Figure 8 that the leakage of COD was in all probability leakage of original substrate (in any event, it was carbohydrate material). Changes in sludge composition and predominant forms were essentially the same as for the previous experiments.

In Figure 9 it is seen that the same general pattern of recuperative response was repeated when the pH was lowered to 3.0. It is also interesting to note that in all of the once-through systems which received acid shocks, the biological solids concentration in the recovery state was higher than in the pre-shock condition.

In this experiment, samples were also processed through the C-H-N analyzer to assess relative changes in elemental composition in response to the shock. There was very little change except for the expected drop in nitrogen content.

Finally, as seen in Figure 10, within 200 hours the system gave no indication of recovery from a shock loading at a final pH of 2.7. The fact that some substrate was being removed and some growth persisted in the mixed liquor might portend eventual recovery.

DISCUSSION

The present studies were conducted to gain an insight into the

transient responses of mixed (heterogeneous) microbial populations to changes in pH. The once-through systems studied might be expected to provide a conservative gauge of the magnitude of allowable change in pH which an activated sludge can accommodate without serious disruption of biochemical efficiency. The most severe alkaline shock (pH 6.6 to 8.0 with no sludge recycle) caused little or no disturbance to the system. The nature of the buffer system (and mineral medium) precluded studies at higher pH values; it also determined the ranges of acid shocks which could be applied.

From the present results, the magnitude of allowable decrease in pH cannot be closely defined. It is possible to conclude that a drop of approximately one pH unit can be accommodated with little disruption, although even changes of this magnitude or less can cause sufficient perturbance to be of concern (e.g., see Figures 3 and 4, once-through systems). It is also apparent (see Figure 9) that systems can be expected to recover biochemical efficiency (after rather prolonged periods of transient leakage of substrate) after changes of pH from neutral to approximately pH 3.0.

The phosphate buffer system did not readily facilitate the study of response to the range of pH 3.5 to 4.5, since the buffering capacity in this range is poor; however, for the acid shocks examined, a definite trend or pattern of response evolved. The more severe the acid shock applied, the greater was the transient in effluent COD and the greater was the dilute-out of cells before recovery was initiated. There did not appear to be any direct correlation between severity of shock and time to recover the former state of substrate removal efficiency.

The general graphic characterization of response was fairly well defined and may be of possible use in mathematical modeling studies, but these data might best be employed in this regard for comparison with results obtained from systematic studies using more defined populations (e.g., one microbial and one fungal species) and designed specifically to obtain kinetic data for modeling growth response to an exchange of favorable environments for each species.

There is little doubt that the primary response mechanism was an ecological one involving a decided shift in predominating species from bacterial and protozoan communities to filamentous fungi. However, there was some indication (discussed below) of partial "en masse" acclimation of the dominant population of the initial "steady" state condition. The characteristic shift in predominating species was noted in frequent microscopic examinations, and the change in biochemical composition of the sludge, notably the lowering of protein content, is consistent with values for fungi (13). Such a change in predominating species is expected, since low pH values are known to be deleterious to growth of protozoa and to favor predominance of fungi and yeasts. The present study attests to the relatively short time period required for the fungi to become predominant. However, it seems clear from comparison of results shown in Figures 5, 6, and 7, that the predominating bacterial-protozoan populations in the pre-shock state were not altogether ineffectual in responding to the change in pH (i.e., there may have been some "en masse" acclimation of the dominant population). In each case (Figures 5, 6, and 7), the decrease in pH was of the same order of magnitude (slightly more severe proceeding from Figure 5 through 7), and the primary difference

was the existence of progressively higher biological solids concentration in the pre-shock state (due to cell recycle) which resulted in progressively less leakage of substrate in the transient response. From a practical standpoint, it may certainly be said that maintenance of high biological solids concentration (by cell recycle) exerts a beneficial effect on the biochemical response since it attenuates the transient loss of substrate. The same can be said in the case of hydraulic shock (7) and shocks involving increases in feed substrate concentrations (14).

The adverse effect of low pH on sludge settleability was evident in Figure 7. While this represents a serious practical problem, it is ultimately not as serious as loss of biochemical efficiency. The former may be subjectable to remedial and/or preventive engineering expedients (e.g., mechanical or chemical enhancement of mixed liquor separation), whereas the biochemical efficiency is a property of the biological material and less subject to engineering enhancement under adverse environmental conditions.

While there are some species of bacteria which can flourish at the low pH ranges examined in this study, the successful response was due to the versatility of the fungi. All experimental runs were started with single inocula of fresh sewage (pH 6.6 to 7.2), and contained trace quantities of filamentous organisms. Fungi were present, but not dominant, during the pre-shock state at neutral pH levels. The fact that filamentous fungi can proliferate over a wide range of pH also indicates that if the shock pH had been relieved, i.e., pH returned to the neutral pre-shock range, the reversal of predominating species to naturally flocculating bacterial-protozoan populations

would be expected to be slow. Thus, remedial addition of chemical flocculants might have to be applied for an extended period of time after the shock was relieved. Remedial mechanical enhancement of separation of filamentous organisms might be implemented by adding, in series with the clarifier, a second aeration tank equipped with vertical flow-through screens such as the apparatus employed first by Kato and Sekikawa (15) in their studies at high pH values and by Randall, et al. (2), in their studies under acid conditions. The former researchers suggested a reversed flow sheet for difficultly-treated organic wastes. However, with regard to protecting against malfunction due to acid shock loadings, it would seem more advantageous to place the entirely fluidized process (activated sludge) ahead of the screened tank, i.e., "fixed activated sludge process" originated by Kato and Sekikawa. Thus, the screens could be employed for entrapment of filamentous organisms escaping the activated sludge clarifier. The matted sludge from the screened aeration tank could then be separated in a clarifier, and these solids wasted, thus helping to purge the system of filamentous forms after the shock was relieved.

The need for such remedial expedients and equipment in cases where periodic acid shocks are anticipated might be obviated by providing for monitoring of the pH and for pH adjustment ahead of the activated sludge chamber. Such an operation is subject to a considerable amount of automation and, depending upon the specific situation, e.g., buffering capacity of the waste, etc., the cost of this preventive measure might be considerably lower than the cost of the remedial measures described above. In accordance with the present

experimentation, it can be conservatively estimated that the system should be protected against significant substrate leakage, as well as pH-induced predominance of filamentous organisms, by control of pH to $\overset{+}{-}$ one unit from neutrality.

ACKNOWLEDGMENT

The experimental phases of this work were conducted under a research grant WP-00075 from the Federal Environmental Protection Agency. Portions of the analysis of the data and manuscript preparation were supported by a research grant A-043 from the Oklahoma Water Resources Research Institute USDI.

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LIST OF FIGURES

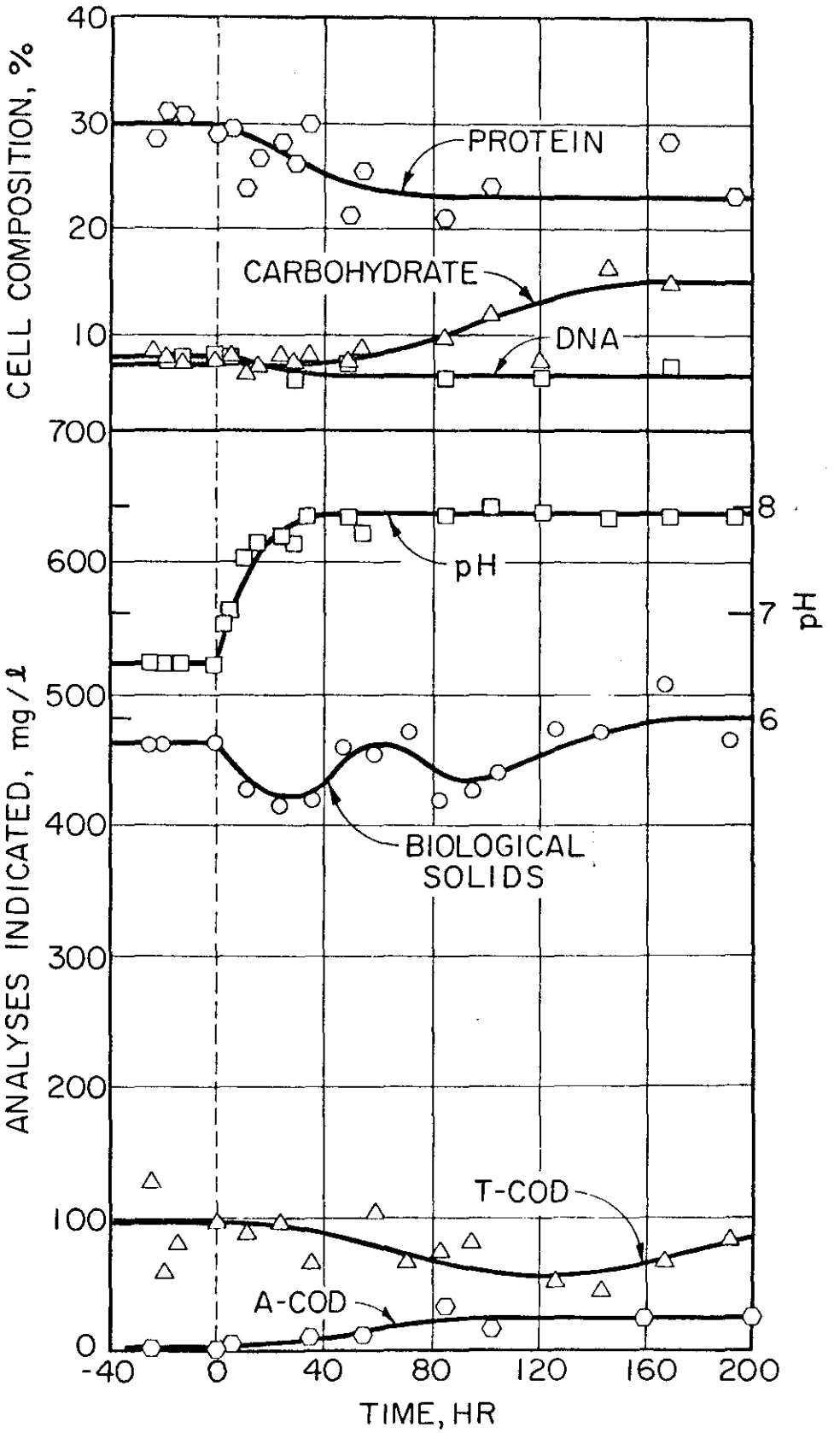

Figure 1. Response to an increase in pH from 6.6 to 8.0 (no cell recycle). T-COD is total COD of filtered effluent, and A-COD is total anthrone-reactive material (carbohydrate) in filtered effluent calculated as COD.

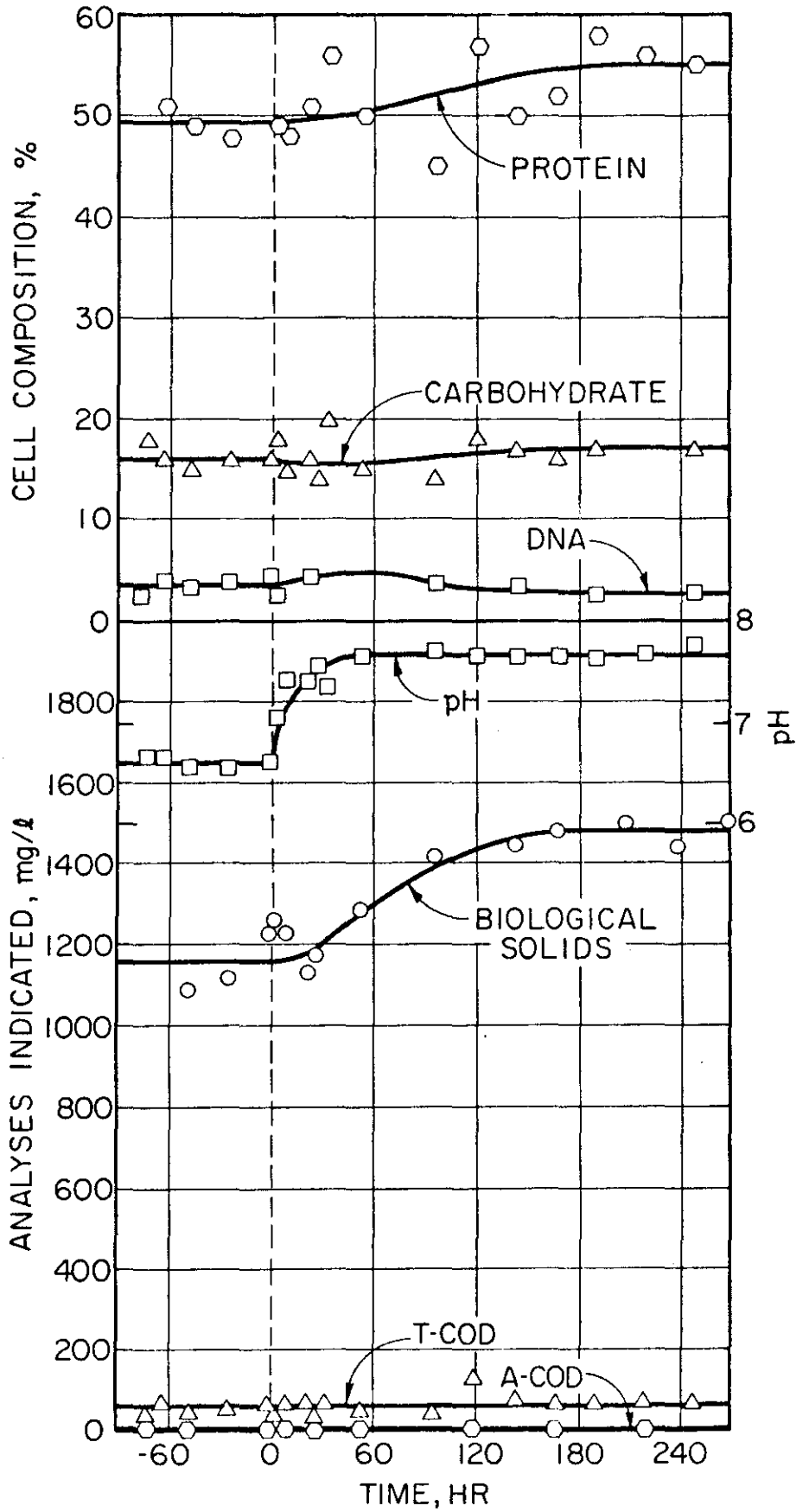

Figure 2. Response to an increase in pH from 6.6 to 7.7 (3500 ± 350 mg/l cells recycled at 33.3 percent of feed flow).

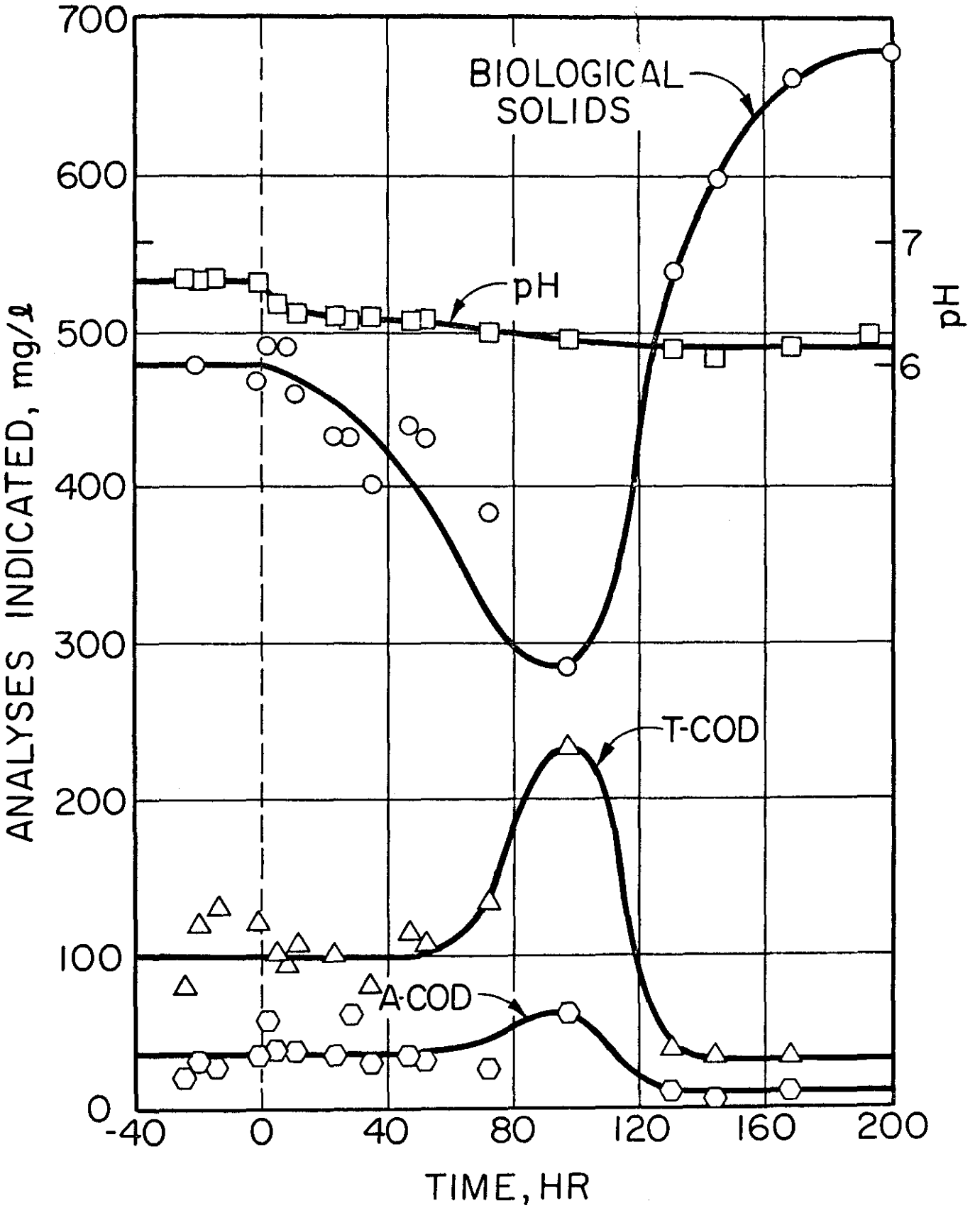

Figure 3. Response to a decrease in pH from 6.7 to 6.2 (no cell (recycle).

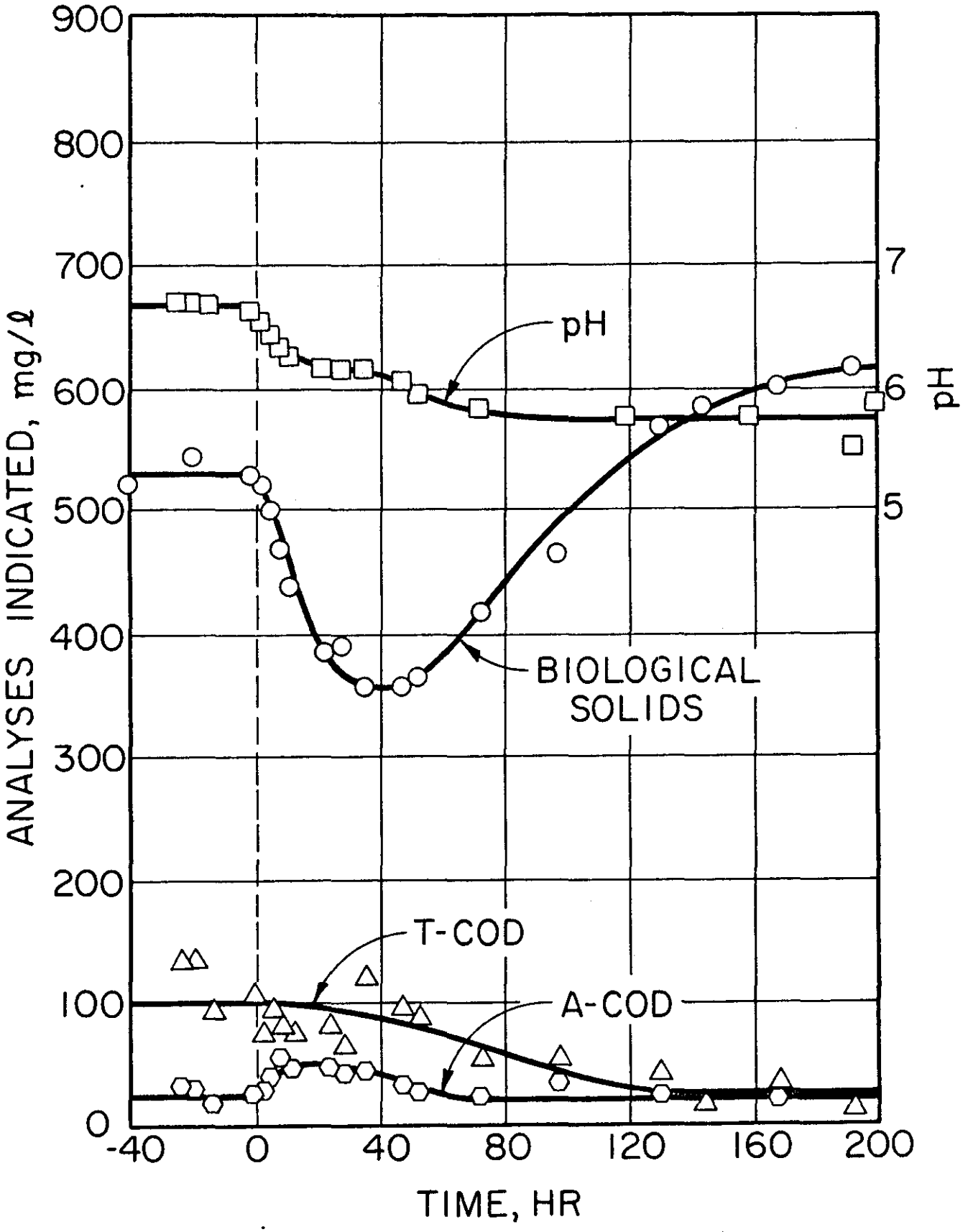

Figure 4. Response to a decrease in pH from 6.7 to 5.8 (no cell recycle).

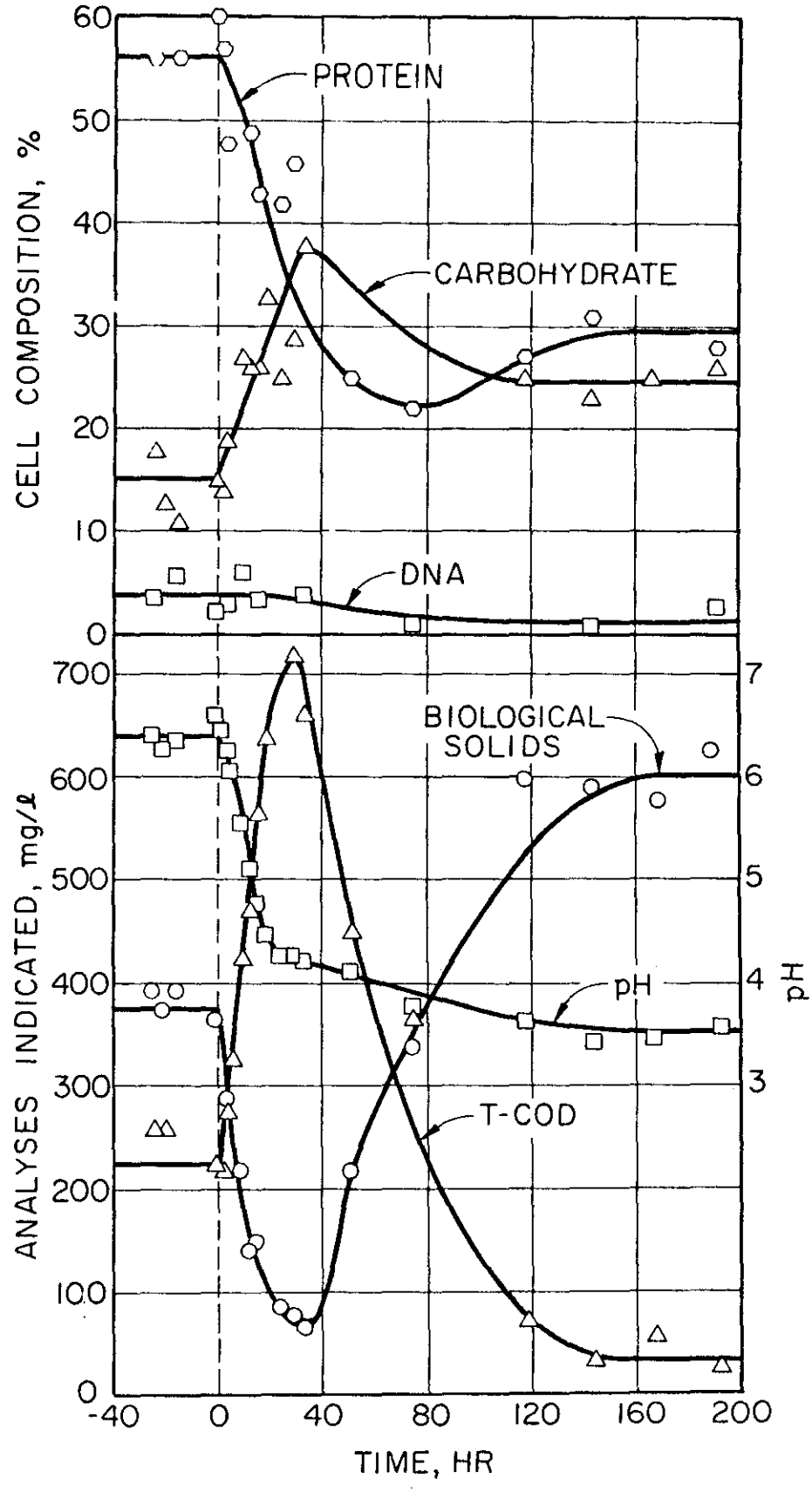

Figure 5. Response to a decrease in pH from 6.4 to 3.5 (no cell recycle).

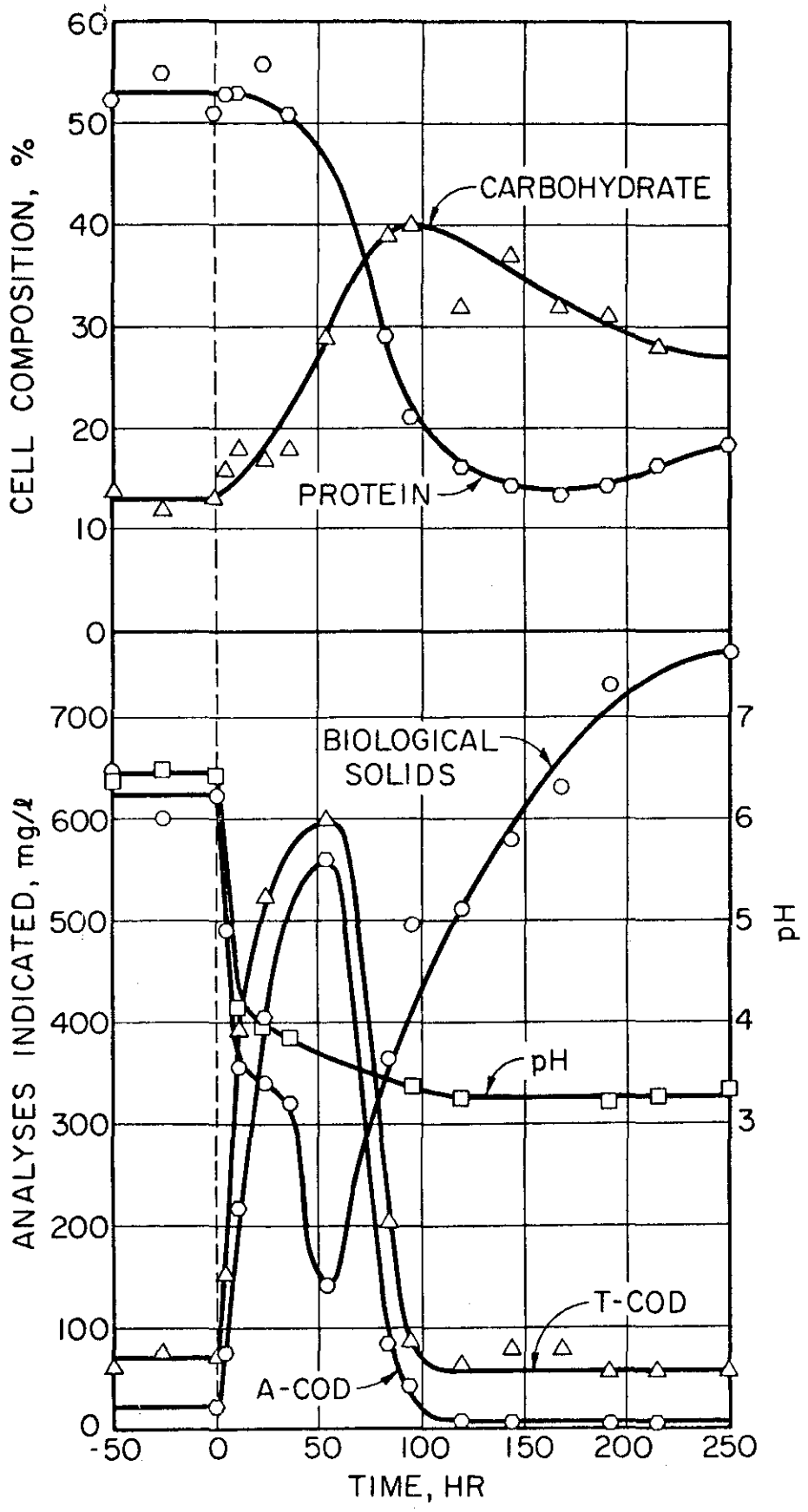

Figure 6. Response to a decrease in pH from 6.5 to 3.3 (± 1000 mg/l cells recycled at 33.3 percent of feed flow).

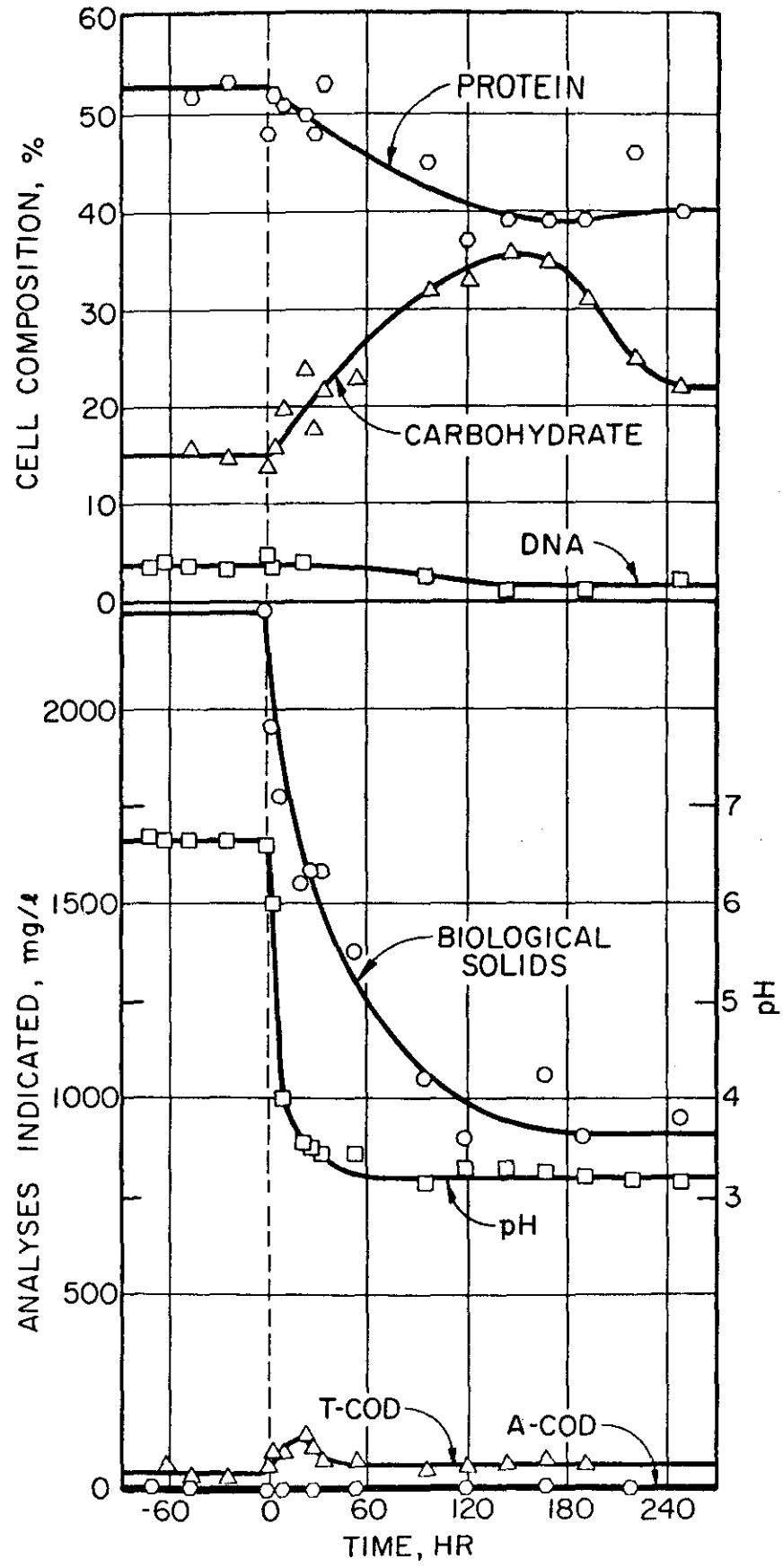

Figure 7. Response to a decrease in pH from 6.7 to 3.2 (total recycle of all settleable cells at 33.3 percent of feed flow).

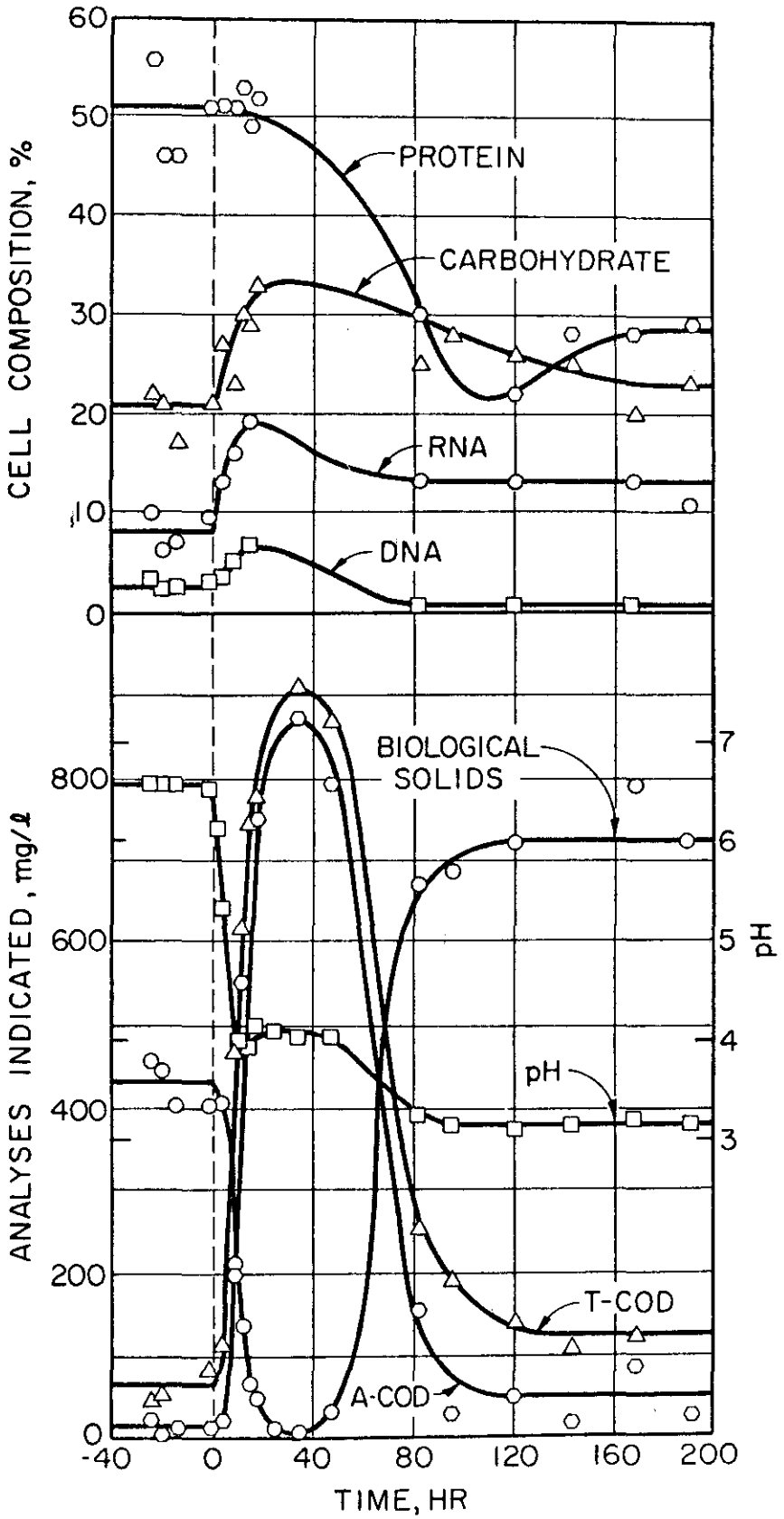

Figure 8. Response to a decrease in pH from 6.6 to 3.2 (no cell recycle).

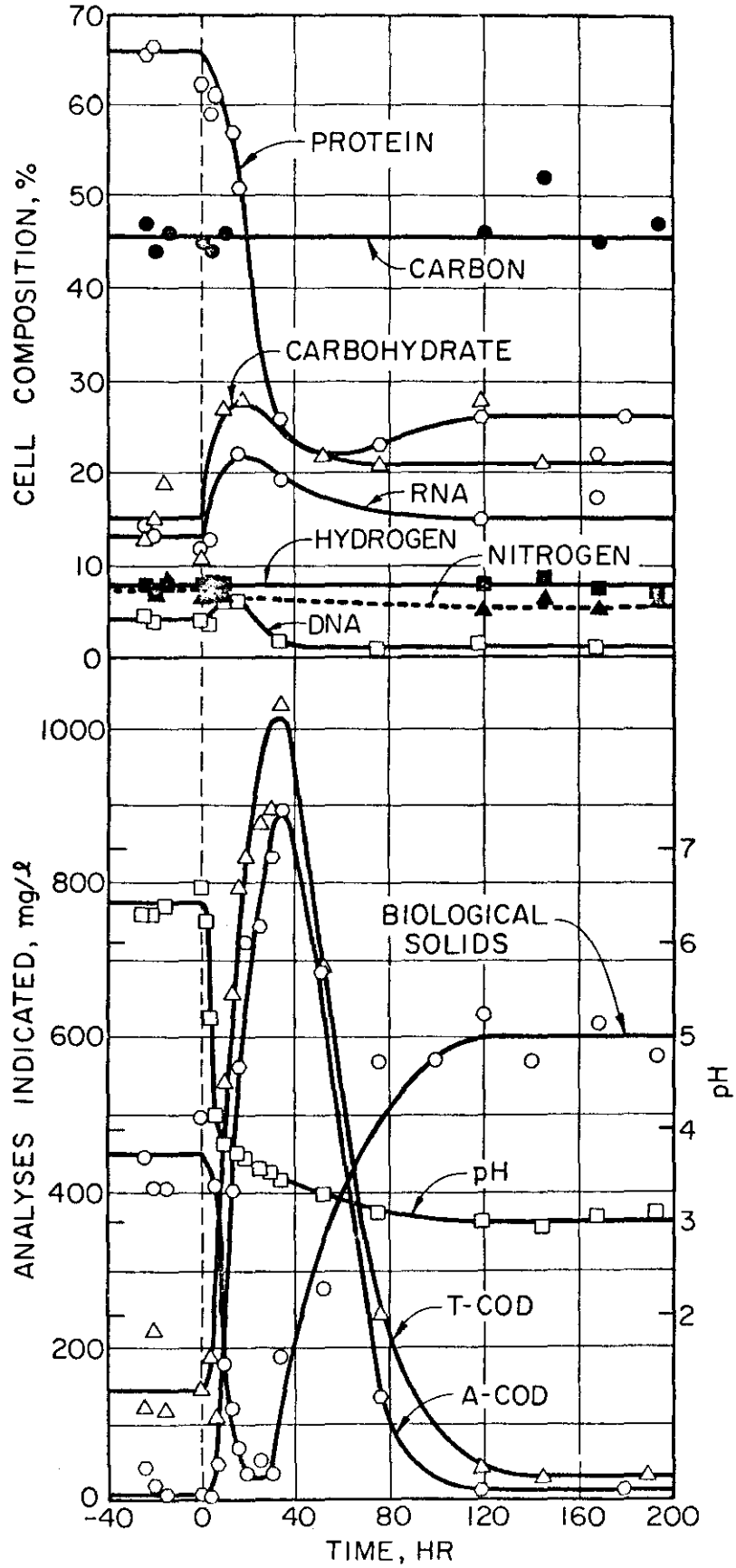

Figure 9. Response to a decrease in pH from 6.5 to 3.0 (no cell recycle).

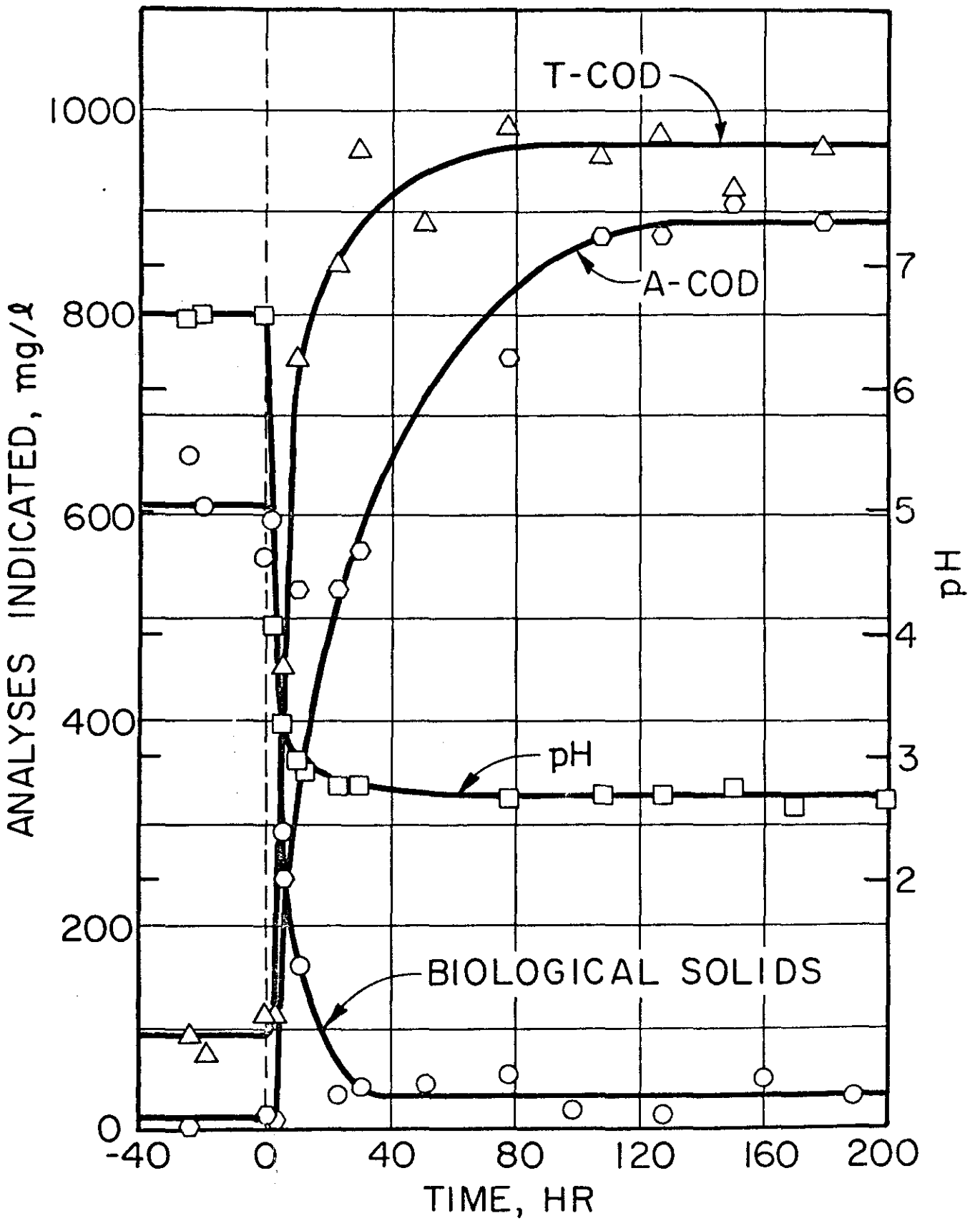

Figure 10. Response to a decrease in pH from 6.6 to 2.7 (no cell recycle).

APPENDIX IV

Gaudy, A. F. Jr., "The Transient Response to pH and Temperature Shock Loading of Fermentation Systems" Presented at the 168th National Meeting American Chemical Society, Atlantic City, New Jersey, September 8-13 (1974), Biotechnology and Bioengineering, (In press 1975).

THE TRANSIENT RESPONSE TO pH AND TEMPERATURE SHOCK LOADING OF FERMENTATION SYSTEMS

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The control of pH and temperature in industrial fermentation processes is an extremely important factor in seeking optimization of process controls, as demonstrated, for example, by the recent study of Rai and Constantinides on the kinetics of the gluconic acid fermentation[1]. The product is of sufficient worth that such controls can be profitable. Usually, less attention is paid to determination of the allowable range of temperature and pH variation, which could occur without seriously hampering the quality and amount of product. On the other hand, when employing biological processes for treatment of wastes, establishment of precise operational optima and close control of these parameters are not practiced, and there is greater cause for concern over the response of the system to a changing environmental condition with respect to pH and temperature as well as tolerable limits of variation for these control variables. These differences in emphasis may be attributed in the main to differences in the sale price of the product and its required quality or purity. In addition, the "natural"

Prepared for presentation at the Symposium on Fermentation Kinetics and Modeling, Division of Microbial Chemistry and Technology, 168th National Meeting, American Chemical Society, Atlantic City, New Jersey, September 8-13, 1974.

or "heterogeneous" microbial population in the waste treatment system imparts a unique potential adaptability to changes in chemical and physical environment, and this "enrichment culture" principle can permit a wider band of operational flexibility with respect to pH and temperature, as well as other environmental variables.

Notwithstanding some of the understandably different attitudinal approaches in practical application, there is a definite similarity and unity of purpose in obtaining fundamental kinetic description of transient response and understanding of the biological mechanisms of which the kinetic response is a manifestation. This unanimity of scientific purpose was demonstrated quite well during the recent U.S.-Japan Seminar on Dynamics of Microbial Population wherein the author[2] and other participants discussed some of their work in this area.

In our laboratories, we have been studying the response of heterogeneous microbial populations to changes in various environmental conditions (shock loadings) and recently we have published some of our studies on response to step changes in pH and temperature[3][4]. Since these system control variables are of considerable importance to fermentation processes generally, this session offers a good opportunity to review and correlate these findings.

Like many other research laboratories, we are interested in mathematical modeling of processes and have presented much data regarding so-called steady state models under essentially constant operational conditions. Because of the difficulties of modeling heterogeneous populations, we have in general avoided, or at any rate put off, such attempts regarding modeling of the transient upon disruption of the steady state. In a large portion of our studies, we have sought to

obtain experimental results for state variables under varying but interrelated conditions for control variables, the aim being primarily to gain a generalized graphical description of the course of response from an initial to a final steady state when the system is subjected to step changes in the control variable. The hope is that in time, from such experimental results, we or others may be able to devise mathematical description. However, from a basic standpoint, we are more interested in the mechanisms of response, and hold that the ultimate mathematical model of such responses must be a mathematical description of the mechanism, not simply a description of the experimental data, even though we certainly recognize the usefulness of such empirical predictive descriptions from an engineering point of view. From an immediate practical standpoint, we wish to employ the results to provide guidelines regarding allowable limits of shock which can be accommodated within tolerable limits (time and concentration) of disruption of effluent quality.

MATERIALS AND METHODS

Experimental results herein presented were obtained in laboratory "chemostats" (2.5 liters aeration volume) which were shown to be completely mixed with respect to substrate and cells. Thus, in the steady state the specific growth rate, μ, was equal to the dilution rate, D, when the system was operated without cell recycle, which was the condition of operation for most of the experiments. During the pH shock studies some experiments were run with cell recycle. In all experiments minimal medium containing essential mineral salts, phosphate buffer and ammonia ion as nitrogen source was employed. In all studies

glucose was the source of carbon and it was the growth-limiting nutrient. All experiments were begun with initial inocula of municipal sewage which served as the source of heterogeneous microbial populations.

In the studies in which pH was changed all systems were run at $D = 0.125\ hr^{-1}$ and all shocks (acid or alkaline) were applied from a base level pH near neutrality. This base level was chosen as one which is close to normal pH conditions in field installations. Also it allows wide diversity in species of the microbial population. After establishing the average values for the steady parameters in the initial steady state at the base level pH, the pH in the feed solution was changed. The feed pH was controlled by varying the molar ratio of phosphate buffer salts. Changes in various state parameters (substrate concentration, cell concentration, cellular carbohydrate, protein, RNA, DNA, etc.) were measured during the transient following the step change. Individual experiments were terminated when the system approached a final steady state.

The temperature shock studies were conducted at two steady state growth rates, $\mu = D = 0.125\ hr^{-1}$ and $0.25\ hr^{-1}$. From a base level temperature of 25 C, step changes, up or down, were applied by changing the temperature in an externally circulating water bath in which both reactors (one at each μ) were immersed. It was observed that the rates of temperature change in both reactors were essentially the same. More detailed descriptions of the materials and experimental protocols can be found in the literature[3][4].

pH SHOCK

The results of the pH shock studies offer some insight into the effect of past growth history, with respect to specific growth rate and biological solids concentration, on the response to change in pH.

Because of the buffer system employed only a few alkaline shocks were applied. These were rather mild increases in pH and the system handled them with little or no leakage of substrate. Mild acid shocks, i.e., from pH 6.7 to 6.2 and pH 6.7 to 5.8 produced transient periods of cell dilute-out and some leakage of carbon source. Recovery was fairly rapid and there was no microscopic evidence for gross change in microbial species comprising the bio-mass[3].

When increasingly more severe decreases in pH were applied, a definite pattern of response evolved. Values for the state variables are shown to the left of time zero in Figure 1. Upon changing the pH of the feed from 6.4 to 3.5, there was a rather rapid diluting out of the bio-mass and a concomitant increase in carbon source in the effluent. The carbon source concentration is labeled T-COD, i.e., total COD, indicating the results of the chemical oxygen demand test, or total oxidizable organic material. Thus for these results it is not known if the deterioration in effluent quality was due to leakage of the original substrate or of partially metabolized intermediates. The shock was accompanied by a rather drastic change in the biochemical composition of the bio-mass. The transient phase was characterized by rising carbohydrate and decreasing protein content, a slight overshoot and in the final steady state, a much depressed protein content and an increased carbohydrate content. These changes in biochemical composition indicate a change in species predominance from bacterial to fungal.

Microscopic examinations made throughout the study showed that the biomass was comprised predominantly of clumped bacteria and protozoa in the initial steady state and of filamentous forms after the change in pH.

The attenuating effect of cell recycle on the severity of disturbance in the transient phase is shown in some degree in Figure 2. In this case the shock was slightly more severe (from pH 6.5 to 3.3) and cells were recycled. Even though the recycled cell concentration was rather low, approximately 1000 mg/l, the extent of the substrate leakage was less, 600 mg/l COD versus 720 mg/l in Figure 1 and this system recovered much more rapidly. Both COD and anthrone analyses were run during this experiment and it can be seen that disruption in "purification" efficiency was due to leakage of carbohydrate. Thus if there were metabolites other than the original carbon source (glucose) they were probably ones early in the substrate degrading scheme. We have on many occasions run glucostat analyses along with anthrone determinations and have found significant differences on some occasions but most generally when there is leakage of metabolites they do not react with anthrone. In the majority of cases they are low molecular weight acids, e.g., acetic acid. In the case of acid shocks, however, the leaked materials may not be immediate metabolic products of partial breakdown of the original substrate but could be storage and possibly structural components leaked due to disruption of membranes and cell walls by the environmental stress. The point to be delineated is that such phenomena are not usually considered in making mathematical models but they are nonetheless real biological complications which should serve to warn against oversimplifying any predictive model for the transient phase.

The attenuation in the severity of disruption of system efficiency during the transient phase when cell recycle is practiced may involve a number of factors. First, cell recycle lowers the specific growth rate in the steady state. In the once through system $\mu = D$ whereas in the cell recycle system μ is given by equation (1).

$$\mu = D(1 + \alpha - \alpha \frac{X_R}{X}) \qquad (1)$$

In this equation α is the hydraulic feedback ratio, in the present case 0.33; X_R is the concentration of cells in the recycle flow, in this case ± 1000 mg/l. Since the steady state cell concentration, X, prior to the shock was approximately 620 mg/l and D was 0.125 hr^{-1}, the specific growth rate, μ, was 0.1 hr^{-1}, i.e. 20% lower for the system shown in Figure 2 than for the one shown in Figure 1. Cell recycle also increases the cell concentration, X, in the reactor and the presence of a greater bio-mass concentration might be expected to reinforce the system against leakage of carbon source. Also, there can be an ecological consequence of cell recycle which probably plays a significant role but may in one sense be more deleterious than helpful since it tends to retard an adaptive response, i.e. resists a change in species predominance. In Figure 2 there is some evidence that the predominance shift was delayed. The protein content did not decrease significantly for 40 hours after administering the shock whereas in Figure 1 the protein content of the bio-mass began dropping much earlier.

Figure 3 shows the response when total cell recycle of all cells settling in the system clarifier was practiced. The hydraulic recycle ratio was again 0.33. The recycle cell concentration, X_R, was not determined but it can be seen that the reactor cell concentration, X,

was rather high, i.e., 2250 mg/l, because of the total recycle of cells. It is seen that there was very little leakage of substrate due to this rather severe shock in pH, 6.7 to 3.2. The major effect was on settleability of the bio-mass. The biological solids concentration dropped from 2250 to 900 mg/l. There was an increase in filamentous forms but they did not predominate.

From these three figures one can conclude that heterogeneous microbial populations exhibit considerable ability to recuperate from rather severe changes in pH. It was of interest to determine the extent to which pH could be lowered without loss of recuperative power in a reasonable time period. Shocks were applied which changed pH from 6.6 to 3.2, 6.4 to 3.0 and 6.6 to 2.7. In the first two cases, the patterns of response were essentially the same as that shown in Figure 1 for less severe shock but the extent of substrate leakage and cell dilute-out was greater and the time to recover was lengthened. Finally when the pH was changed to 2.7, this system showed little or no sign of recovery within the 200 hr period of observation after applying the shock, or 25 mean hydraulic retention times.

TEMPERATURE SHOCK

The apparent effect of the steady state specific growth rate on the severity of response is shown more clearly by the results of the temperature shock studies. In this series of experiments cell recycle was not practiced but dual once-through systems operated at dilution rates $D = \mu = 0.125$ and $0.25\ hr^{-1}$ were simultaneously subjected to identical temperature shock. Each system was operating at 25 C prior to the change in temperature. Other than dilution rate all conditions

of operation were identical in each reactor. Since heterogeneous populations were studied it cannot be said with certainty that the distribution of species in each reactor was the same but there were at any rate no grossly apparent differences in the populations.

A decrease in temperature from 25 to 8 C caused a drastic disruption of substrate removal efficiency and a severe (nearly total) dilute-out of cells with no sign of impending recovery 200 hrs after administering the shock.

The results of a milder cold shock from which both systems recovered is shown in Figure 4. As in previous figures, data to the left of time zero shows the initial steady state condition. The faster growing system is shown in the right-hand graph. The drop in temperature from 25 to 17.5 C occurred at the same rate in both systems and was completed in 6 hr (0.75 and 1.5 mean hydraulic retention times). It is apparent that the slower growing system (left-hand graph) responded much more successfully. In this system there was a small rise in COD (T-COD) and no disturbance at all in concentration of anthrone-reactive material (see A-COD) due to the change in temperature. However the system growing at D = 0.25 hrs^{-1} could not successfully accommodate the shock. For the first 20 hours after the shock there was little change in concentration of anthrone-reactive material but there was a rapid rise in the COD in the effluent. Some of the dissimilation products registered as acetic acid (Polypac 2 column, Model 810 Hewlett-Packard Co.). Forty hours after administering the shock the system appeared to be poised to recover but from 40 to 100 hrs both the T-COD and A-COD rose to 600 mg/l. During the time the COD was rising, the biological solids underwent an unexpected recovery attaining a level of 390 mg/l. This corresponded to a cell yield of 90% as compared

to one of 50% prior to the shock. Cell yield of 90% is not unheard of but it certainly does appear to be abnormal. The result was not due to incomplete mixing of reactor contents since the cell concentration in the effluent and the reactor were the same. In both systems during the transient phase there was a rise in RNA and protein content and a decrease in carbohydrate content of the cells.

A mild increase in temperature from 25 to 36 C was accomplished in both systems in 40 hr (5 X $\bar{t}$ at D = 0.125 hr^{-1} and 10X $\bar{t}$ at D = 0.25 hr^{-1}) and produced only slight and short-lived purturbance in effluent quality in either system. However, when a more drastic increase in temperature was applied (25 to 47 C), there was a quite severe disruption in effluent quality as seen in Figure 5. The change in temperature was completed in 26 hr in both systems. The results leave little doubt regarding the tendency for steady state systems growing at slower specific growth rates to respond more successfully to environmental change. It is interesting to note that in the slower growing system substrate dissimilation proceeded uninterruptedly (compare T-COD and A-COD) whereas at the higher dilution rate the COD in the effluent was essentially all due to anthrone-reactive material.

The rather amazing resilience of these heterogeneous populations is demonstrated in Figure 6 which shows the response when the temperature was raised to the thermophilic range (25 to 57.5 C). At the lower growth rate there was, after a severe transient disruption, a partial recovery in which the original substrate was subject to nearly the same degree of dissimilation as in the preshock state. There was however permanent (at least for 200 hr) leakage of nearly 50% of the organic feed stock (see T-COD), presumably as partially metabolized intermediates, in an

apparent final steady state condition. Again it is quite apparent that the faster growing system was less successful in accommodating the shock.

DISCUSSION

In other studies on shock loadings to continuous culture systems we have noted that cells growing at lower specific growth rates accommodate shock loadings better, i.e., leak less substrate, than do systems growing at faster specific growth rates. For example, this general trend has been found in studies for which the shock consisted of changes in concentration of the incoming carbon source, i.e. quantitative shock loads, (Krishnan and Gaudy, unpublished data) and in studies wherein the shock consisted of changes in the types of carbon source in multi-component feeds (qualitative shock loads) e.g. carbohydrate-amino acid systems (5) and carbohydrate-alcohol systems (Komolrit and Gaudy, unpublished data). With regard to the latter type of shock we were able to form a reasonable hypothesis to explain the effect. The hypothesis involves the concept of metabolite inhibition and the effect of rapid growth rate on the production and accumulation of metabolic pools which may contain the compound(s) responsible for the feedback inhibition[5]. The formation of mechanistic hypotheses for the apparent effect of immediate past growth rate when the shock consists of a change in concentration of carbon source, or change in pH or temperature as in the present instance, seems considerably more difficult. About all that can be said with any degree of certainty is that the effect does exist and it does seem to be of general occurrence and applicable to a variety of environmental changes. For some shock load studies one might argue that since the change in the control variable is usually made by

adjusting the incoming feed composition, the simple fact that the environmental change is being administered hydraulically more slowly when the system is growing more slowly may provide an explanation for the effect. Also one might argue that if cell recycle is practiced the growth rate becomes an apparent function of hydraulic recycle ratio, cell concentration in the recycle and cell concentration in the reactor (which is in turn affected by substrate concentration in the feed) as given by equation (1) and these factors rather than μ itself may be controlling the response. As previously mentioned the cell concentration in the reactor at the time of the shock seems to be one of the factors which could play a pivotal role in determining the response. The results shown in Figure 3 surely attest to this possibility. Even though much more research is needed in order to determine the separate effects of various conditions making up the immediate past history of the cells, it is possible to conclude, because of the results of the temperature shock studies, that there is a separate effect of μ. In Figures 4 and 5 cell concentration in each system prior to the shock was essentially the same. In either set of results the only difference was the hydraulic rate of feeding, i.e. μ. Furthermore the hydraulic rate of feeding in this particular case was not a factor in controlling the rate of administering the change in temperature. The temperature change occurred at essentially the same rate in both the fast and slower growing systems. Even though heterogeneous populations were used and allowing for the fact that there is no way we can say with surety that the populations in each reactor were the same, there is clear evidence that the system with the lower dilution rate, i.e. slower specific growth rate, responded to the shocks, in each case, with less transient disturbance in effluent

quality and less dilute-out of cells. Thus it seems that the longer mean residence time ($\bar{t} = \frac{1}{D} = \frac{1}{\mu}$) and accompanying greater contact time in the reactor allows more time for the cells to adjust to the new conditions. Such a simple initial line of thinking does not really explain anything of the mechanism of response (which is probably dependent upon the type of shock) but may be of some help in formulation of individual response hypotheses since this hydraulic factor when translated to biochemical reaction time seems to be one of the factors which will be common to any of the various possible biological response mechanisms. Also in our temperature shock studies we found that in general these heterogeneous systems which were studied responded better to increases than to decreases in temperature. Since within limits an increase in temperature serves to increase reaction velocity (or reduce the required reaction time) the result is consistent with a (tentative) general impression that any operational procedure which serves to increase retention time or provide for more effective reaction time can serve to enhance ability to accommodate environmental change.

Obviously from an engineering standpoint cell recycle can accomplish greater mean cell residence time without large increases in reactor volume. Also from the standpoint of "ecological" engineering of the process, providing for a longer mean residence time (slower growth rate) by increasing the cell recycle serves also to increase the cell concentration in the reactor which may in itself exert an independent attenuating effect on leakage of carbon source during shock loading. The increase in cell concentration serves to lower the organic loading per unit mass of cells, thus increasing the degree of starvation conditions in the reactor and increasing the amount of predation, lysis and satellite

growth which in turn increases the diversity of species, thereby expanding the ecological scope of potentially successful response.

Heterogeneity of the population, to say the least, complicates any effort at predictive description of response because one must deal with the shifts in species which may be caused by the change (the ecological response) in addition to the "en masse" biochemical response of the cells predominating at the time of the environmental change. In other studies on increases in substrate concentration, we have obtained some evidence that these possible responses can occur sequentially rather than, or as well as, concurrently, with the ecological response being significantly delayed and in such cases we have observed much more deleterious conditions with regard to substrate leakage.[6] On the other hand in these studies on pH shock it was amply apparent that a successful response to rather drastic changes in pH was almost entirely brought about due to the ecological response. In closing, it may be discerned that these results provide some guidance regarding degree or magnitude of change which may be tolerated without serious upset of purification efficiency. However, the many complexities involved with heterogeneous populations engender as much reluctance to make sweeping general conclusions as they do desire for obtaining greater and greater amounts of experimental data under controlled conditions.

ACKNOWLEDGMENTS

The experimental phases of this work were conducted under research grant WP00075 for the Federal Environmental Protection Agency. This manuscript was prepared under partial support of research grant A-043 from the Oklahoma Water Resources Research Institute, U.S. Department of the Interior.

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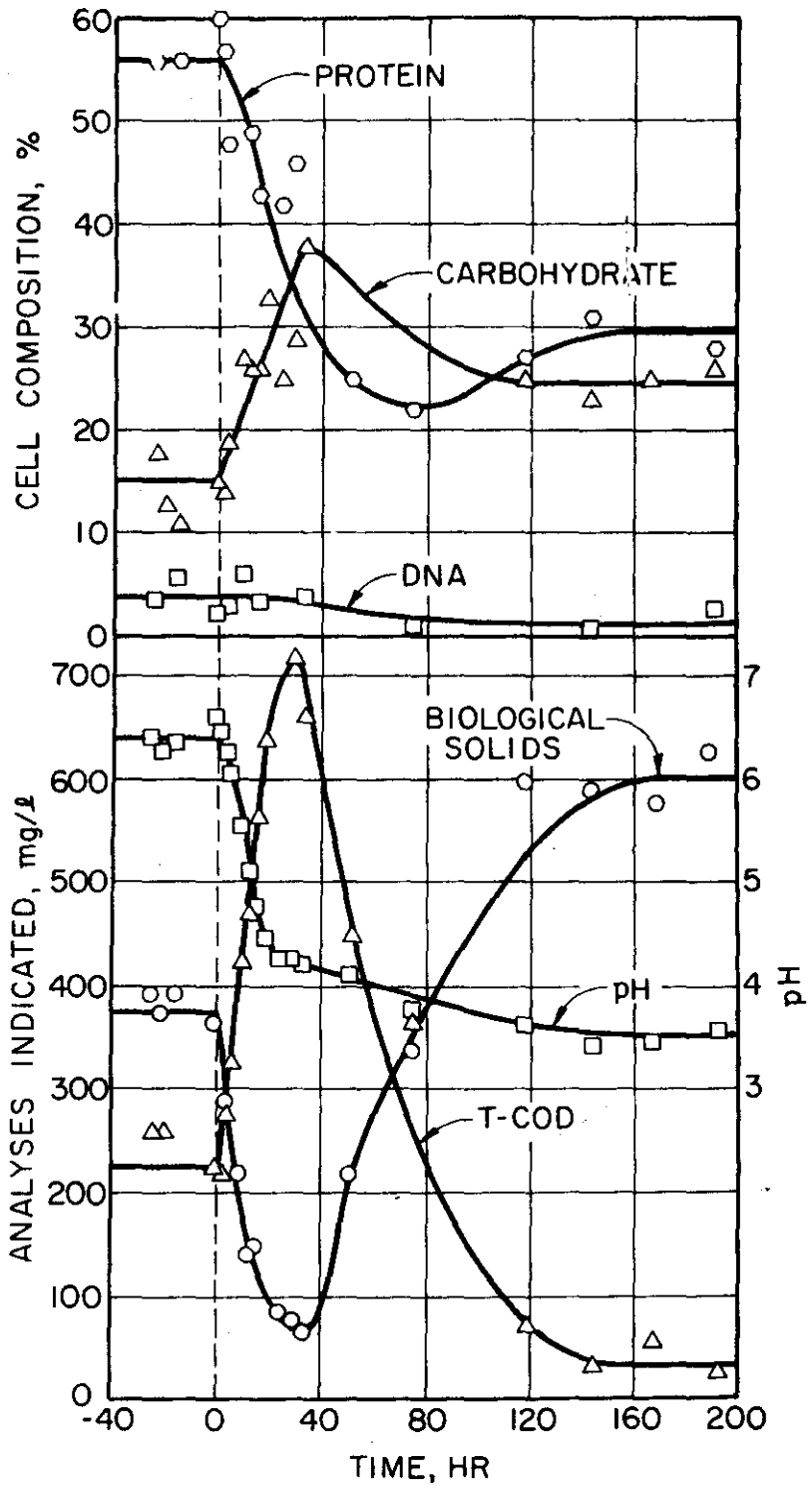

Figure 1. Response of a Heterogeneous Microbial Population Growing in a Once-through Chemostat to a Decrease in pH from 6.4 to 3.5

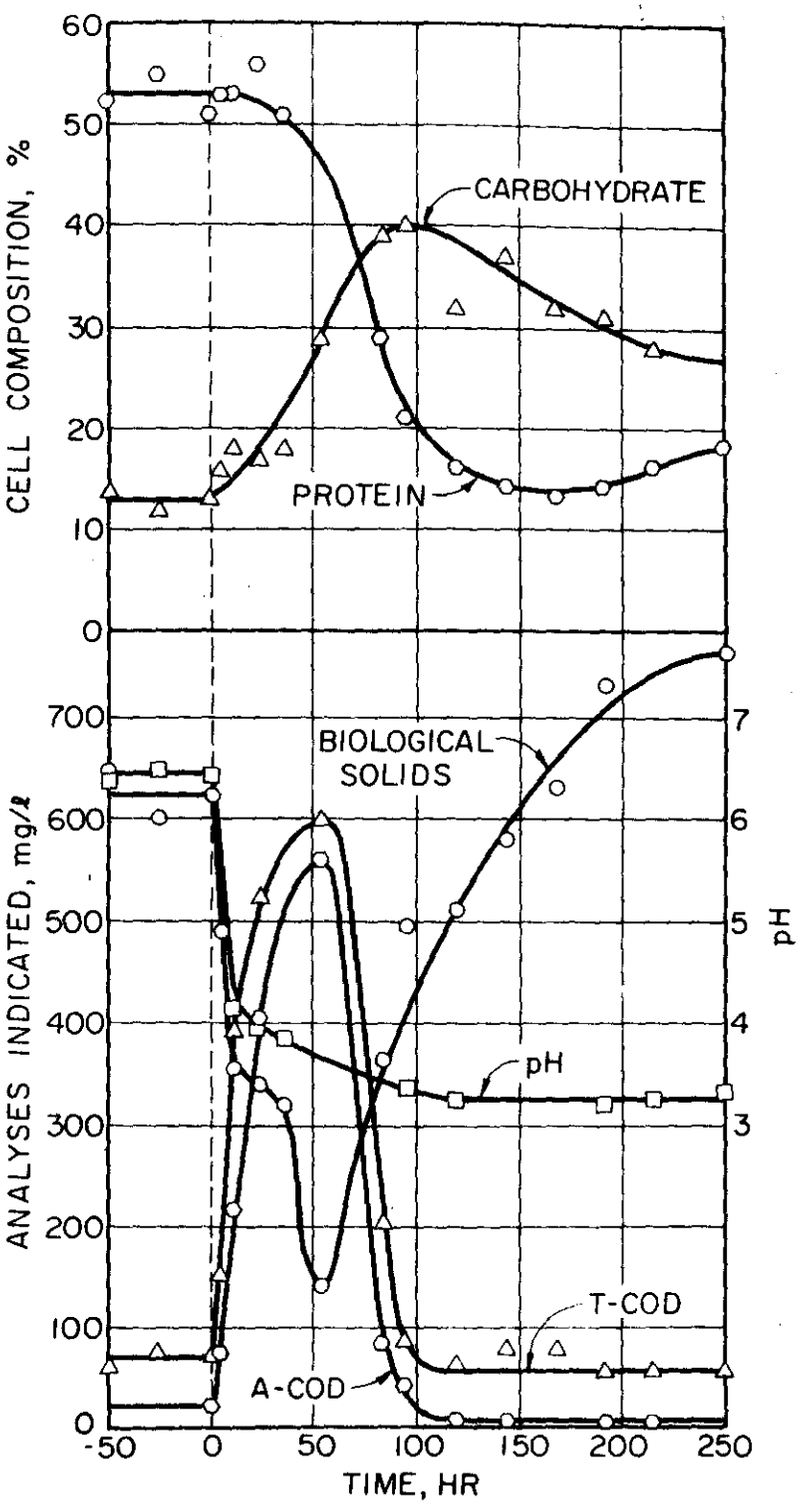

Figure 2. Response of a Heterogeneous Microbial Population Growing in a Chemostat to a Decrease in pH from 6.5 to 3.3 (± 1000 mg/l Cells Recycled at 33.3 Percent of Feed Flow)

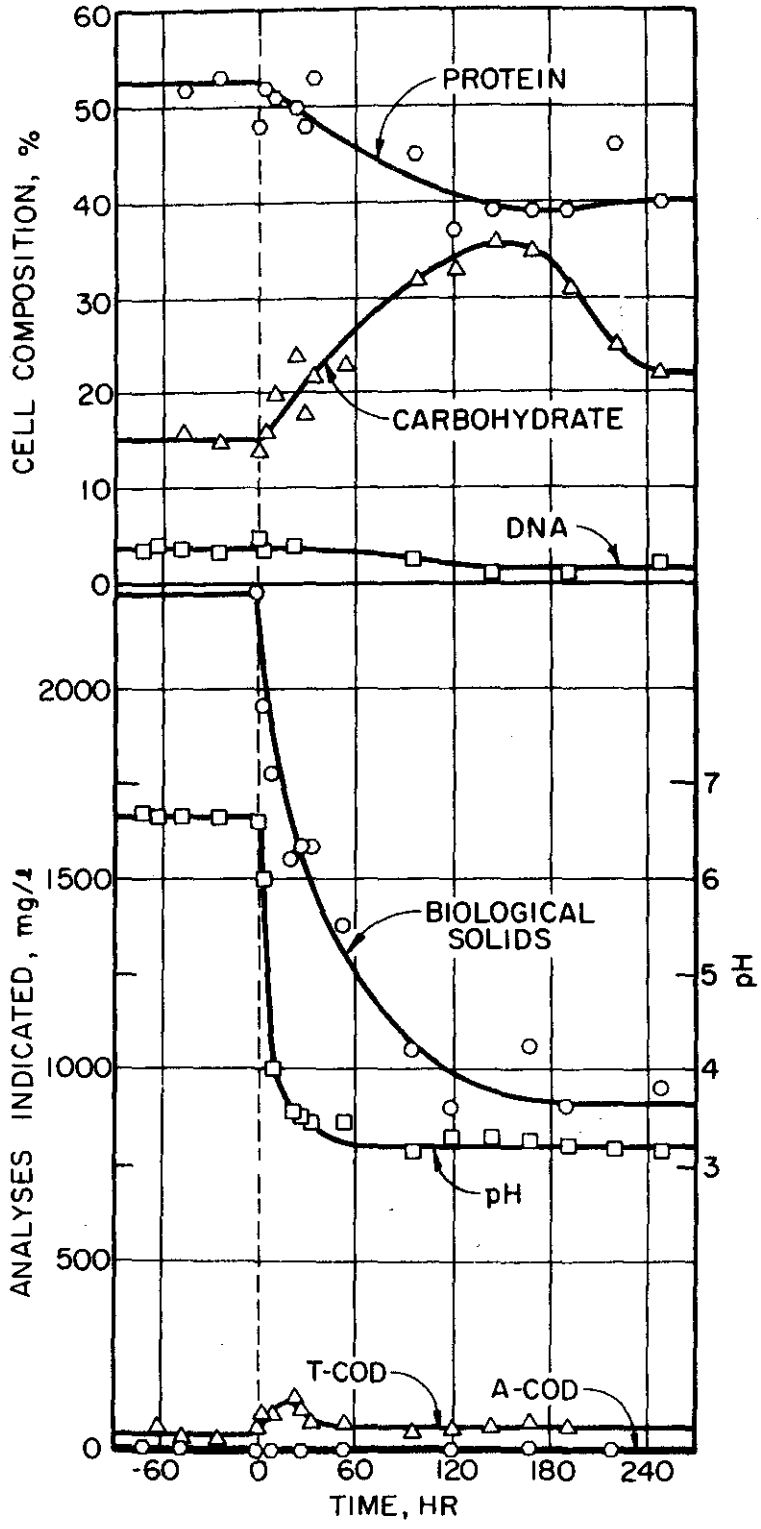

Figure 3. Response of a Heterogeneous Microbial Population Growing in a Chemostat to a Decrease in pH from 6.7 to 3.2 (total recycle of all settleable cells at 33.3 percent of feed flow)

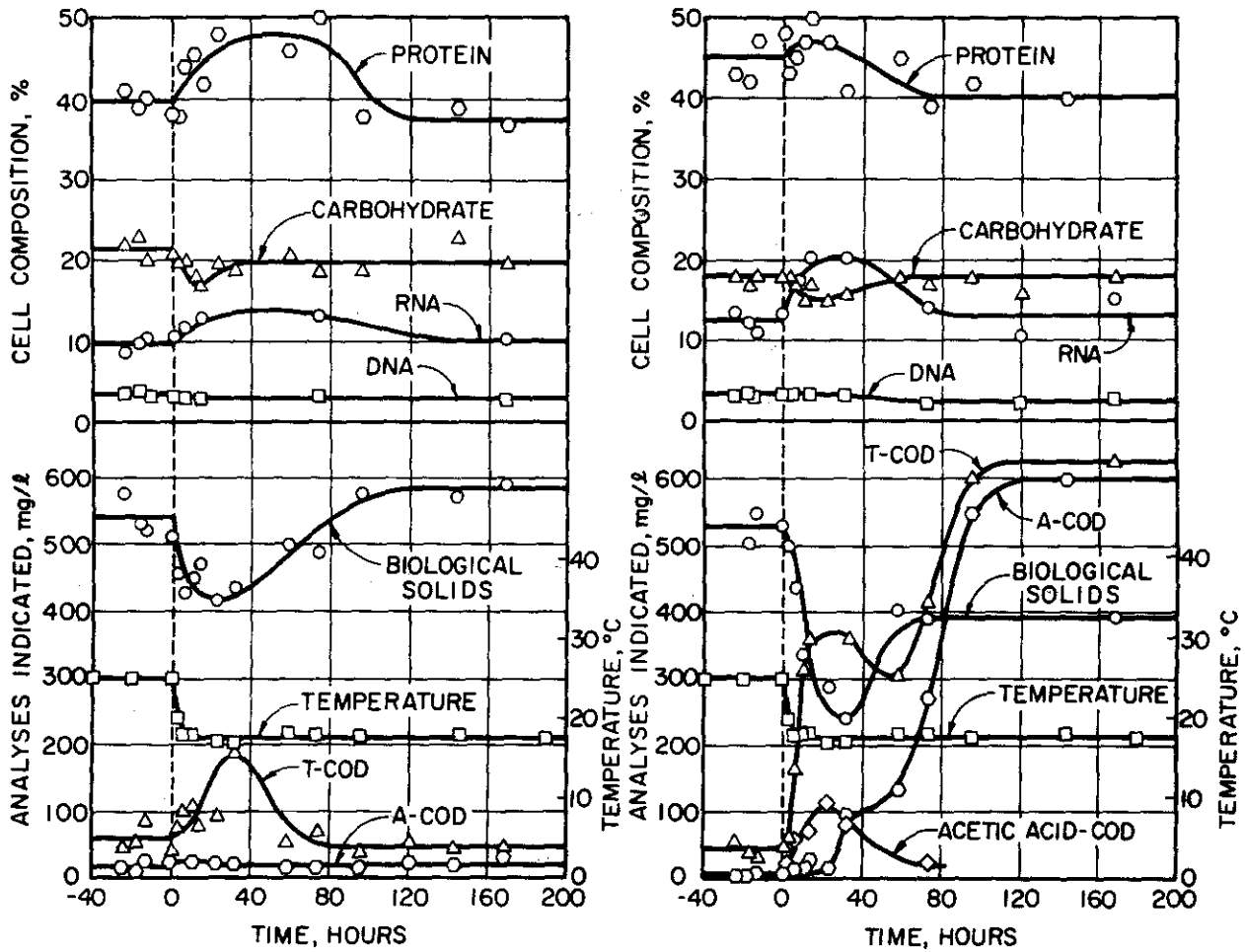

Figure 4. Response of a Heterogeneous Microbial Population Growing in a Once-through Chemostat at 25 C to a Decrease in Temperature to 17.5 C. Left, Dilution Rate = 0.125 hr^{-1}; Right, Dilution Rate = 0.25 hr^{-1}.

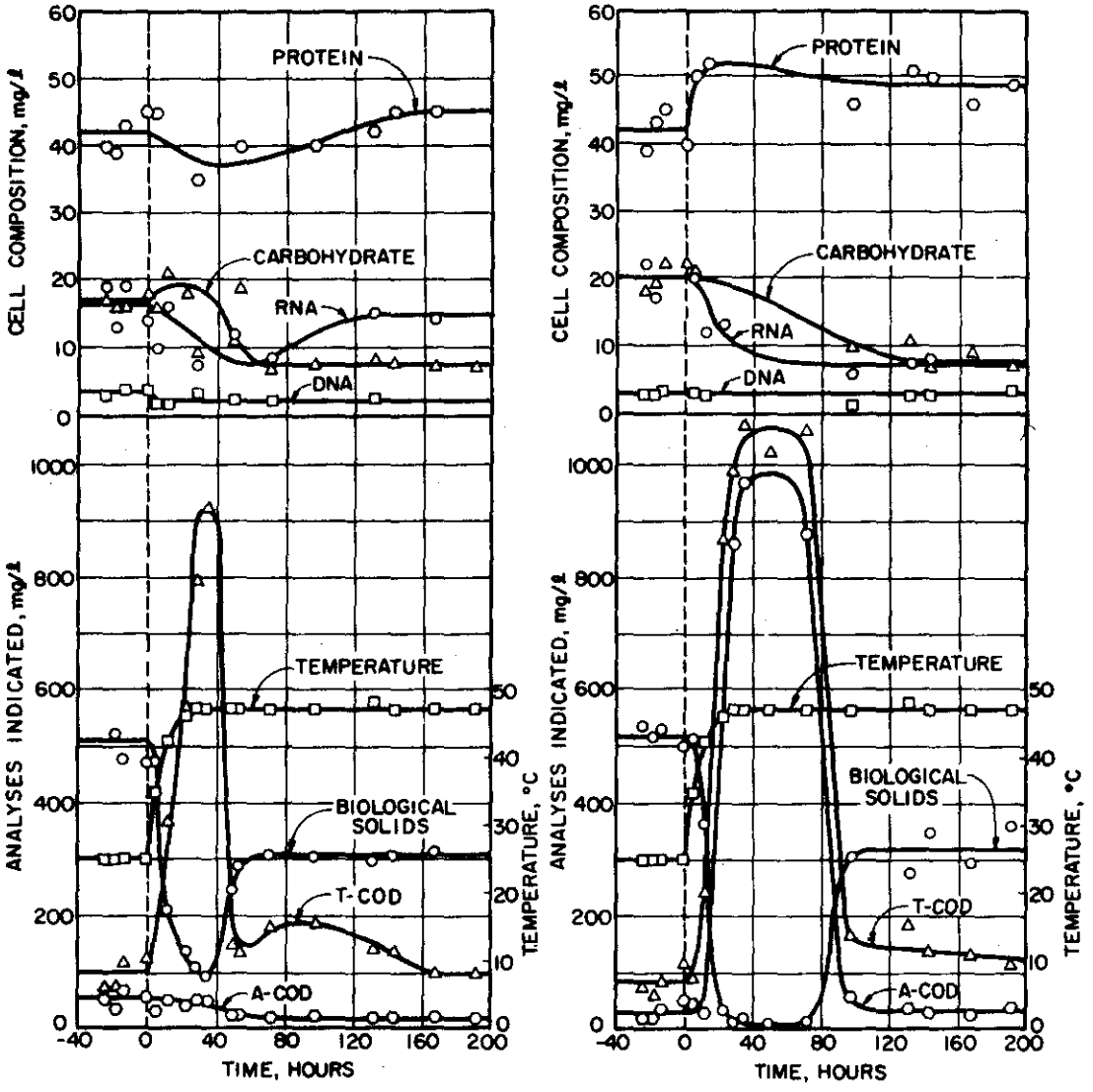

Figure 5. Response of a Heterogeneous Microbial Population Growing in a Once-through Chemostat at 25 C to an Increase in Temperature to 47 C. Left, Dilution Rate = 0.125 hr^{-1}; Right, Dilution Rate = 0.25 hr^{-1}.

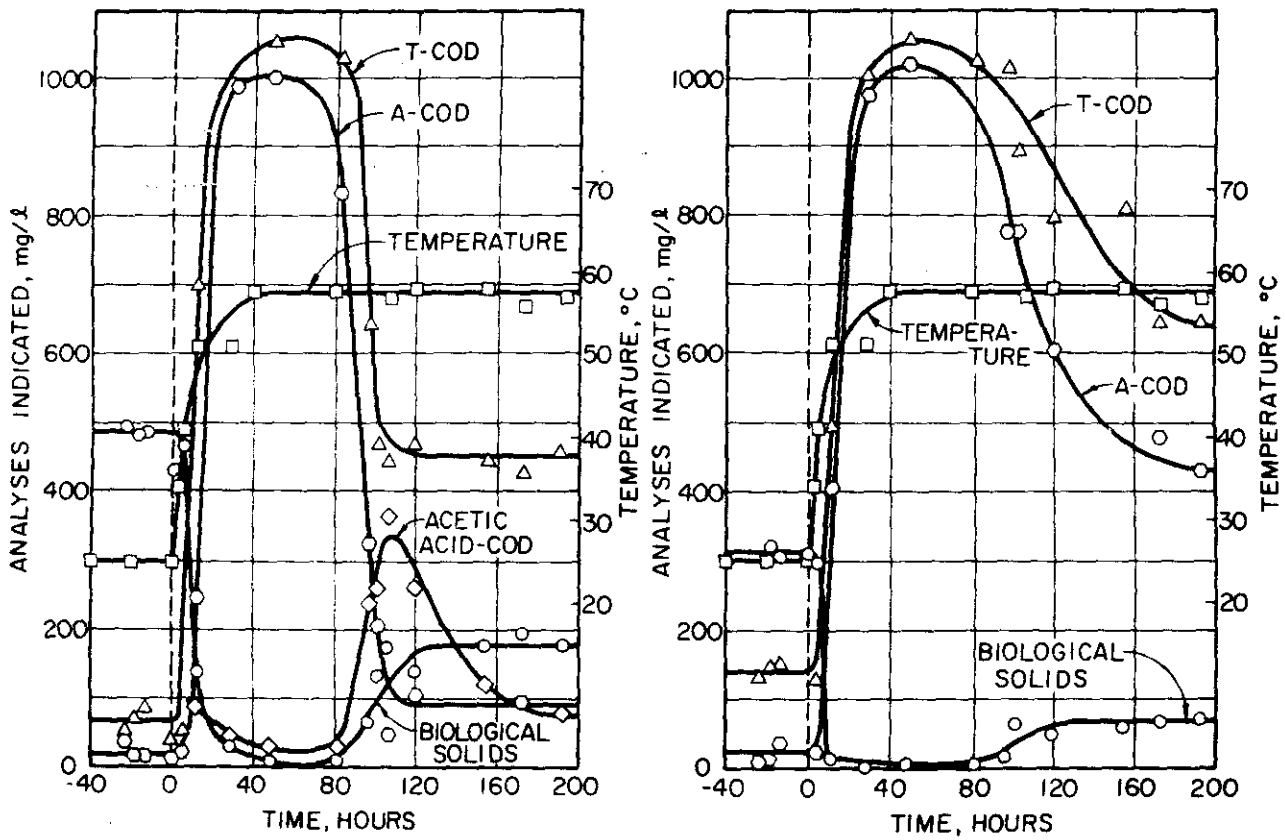

Figure 6. Response of a Heterogeneous Microbial Population Growing in a Once-through Chemostat at 25 C to an Increase in Temperature to 57.5 C. Left, Dilution Rate = 0.125 hr^{-1}; Right, Dilution Rate = 0.25 hr^{-1}.

APPENDIX V

George, T. K., and Gaudy, A. F. Jr., "Transient Response of Completely Mixed Systems to Changes in Temperature." Appl. Microbiol., 26, 5, 796-803 (1973).

APPLIED MICROBIOLOGY, Nov. 1973, p. 796-803
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Vol. 26, No. 5
Printed in U.S.A.

Transient Response of Continuously Cultured Heterogeneous Populations to Changes in Temperature

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Received for publication 19 June 1973

Completely mixed, once-through continuous culture systems of heterogeneous microbial populations of sewage origin were systematically examined for response to changes in reactor temperature. Systems were operated at two dilution rates of 0.125 and 0.25 per h. "Steady state" conditions of the systems were assessed with the reactors operating at 25 C. From this base line, temperature was decreased to as low as 8 C and increased to as high as 57.5 C. Response was assessed in the ensuing transient phase as the system approached a new "steady state." The response was measured by changes in amount and type of carbon source in the reactor effluent as determined by the chemical oxygen demand test, the anthrone test, and gas chromatography. Biological solids concentration and cell composition (protein, carbohydrate, ribonucleic acid and deoxyribonucleic acid) were also determined. These systems responded more favorably to increases than to decreases in temperature. Regardless of the direction of change, the system with the lowest dilution rate (D = 0.125 per h) responded more successfully; i.e., there was less leakage of carbon source in the effluent and less dilute-out of cells during the transient phase.

Although there has been much investigational interest regarding the effects of temperature on the growth and composition of chemostatically grown cells under "steady state" conditions, there is scant experimental data in the literature regarding the transitional response to step increases or decreases in temperature, or both. Such aspects are of general interest in the area of continuous culture of microorganisms and of considerable applied interest regarding the understanding and control of microbial processes such as biological treatment, e.g., by activated sludge, of organic-laden wastewaters wherein heterogeneous populations rather than pure or specific mixtures of species are employed.

Much of the work on effects of temperature on microbial growth and physiology through 1966 has been reviewed by Farrel and Rose (5). There has been recent interest in the caloric values of cells grown at various temperatures, and it would appear that the calories per gram of cells remain essentially unchanged regardless of growth temperature (12, 14). There is continuing controversy regarding the effect of growth temperature on chemical composition (deoxyribonucleic acid [DNA], ribonucleic acid [RNA], protein, etc.) (2, 6, 10). The differences in data appear to arise from variations in experimental or environmental conditions of growth.

Little or no quantitative data describing the transient response to changes in temperature are available. With regard to heterogeneous populations, Dougherty and McNary (4) performed some pilot plant studies on activated sludge by using orange juice as substrate and noted changes in predominant species and some deterioration in effluent quality after gradual increases in temperature over the range of 21 to 36 C. Brezonick and Patterson (1) noted an increase in adenosine 5'-triphosphate content of activated sludge over the temperature range of 9 to 37 C, with a marked decrease at 45 C.

The results presented herein form a part of a long-term and continuing investigation of the effect of various environmental perturbances to chemostatically growing mixed microbial populations, i.e., shock loadings to activated sludge processes. Of particular interest in these studies was response to both increases and decreases in

temperature from a common base-line temperature, and we were interested in assessing the effect, if any, which initial growth rate or dilution rate might have on the nature and extent of the transient phase between initial and final steady states. These results are presented to provide possible guidelines for limits of tolerance of activated sludge processes to temperature shock and because the experimental picturization of changes in system parameters in the transient phase may have some usefulness in the general area of microbial kinetics and physiology, even though the response involves transient ecological as well as physiological reaction to the environmental stress.

MATERIALS AND METHODS

The continuous-flow growth reactors employed were once-through chemostats (2.5-liter Pyrex glass) and have been previously described in detail (8). Prior to receiving a temperature shock, the units were maintained at 25 C ± 0.5 C. Temperature was controlled by a Precision Lo/Temptrol (Precision Scientific) which was connected to the water bath in which the reactors were placed. Aeration through carborundum diffusers was maintained at 5,000 ml/min. The growth medium contained: glucose, 1,000 mg/liter; $(NH_4)_2SO_4$, 500 mg/liter; $MgSO_4 \cdot 7H_2O$, 100 mg/liter; $MnSO_4 \cdot H_2O$, 10 mg/liter; $CaCl_2 \cdot 2H_2O$, 7.5 mg/liter; $FeCl_3 \cdot 6H_2O$, 0.5 mg/liter; tap water, 100 ml/liter; 1 M phosphate buffer solution (pH, 7.0), 10 ml/liter, and distilled water to volume.

Each experiment was initiated by inoculating the synthetic waste with a sample of sewage obtained from the effluent of the primary clarifier of the municipal sewage treatment plant at Stillwater, Okla. The reactors were run 2 to 4 days under continuous-flow conditions at the "steady state" to establish the base-line condition prior to the change in temperature. Each step change in temperature was applied by adjusting the temperature of the water flowing into the water bath. Thus, the "shock" was not immediate. In all cases, the temperature was closely monitored so that the rate of temperature change was known. Experiments were conducted at two dilution rates: D = 0.125 per h (8-h mean hydraulic retention, t) and 0.25 per h (t = 4 h).

Frequent samples were taken to assess the response of the biomass. The concentration of biological solids was determined by the membrane filter technique (15) by using 0.45-μm pore size filters. The filtrate was analyzed for chemical oxygen demand (COD) (15) and carbohydrates by the anthrone test (7). The filtrate was also analyzed by gas-liquid chromatography for volatile (acetic) acids by using a Polypac-2 column (model 810 Hewlett-Packard Co., Avondale, Pa.). Protein and carbohydrate contents of the biological solids were determined, respectively, by the biuret and anthrone analyses (7). RNA and DNA contents of the biological solids were determined, respectively, by the orcinol (13) and the diphenylamine (3) reactions, by using a trichloroacetic acid extract of the cells. Frequent checks on the pH of the reaction liquor were made, and the reactors were also checked frequently for complete mixing (11).

RESULTS

Five long-term continuous-flow experiments were conducted in which like changes in temperature, increases and decreases from the base temperature at 25 C, were administered to chemostat systems operating at dilution rates of 0.125 and 0.25 per h. The temperature shock range was from 8 to 57.5 C.

In Fig. 1, as in all succeeding figures, data to the left of the dashed vertical line (negative time scale) depict the preshock "steady state" condition. The temperature change was initiated at time zero, and the responses are shown to the right (positive values on the time scale). In all cases, the graph on the left depicts response at D = 0.125 per h; response at D = 0.25 per h is shown on the right. The curve identified as "substrate dilute-in curve" in Fig. 1 represents the calculated value of the reactor (or effluent) COD in the absence of metabolism, i.e., if metabolism had stopped at the time of changing the temperature. The curve labeled "T-COD" depicts the total chemical oxygen demand of the filtrate. The curve labeled "A-COD" is the carbohydrate concentration in the filtrate calculated to its COD value as hexose sugar (e.g., COD glucose = mg of glucose per liter × 192/180). The difference between T-COD and A-COD may be taken as a measure of noncarbohydrate metabolic intermediates or end products produced by the organisms, or both. The curve labeled "acetic acid COD" results from gas-liquid chromatographic analysis. In the experiment shown in Fig. 1, the only chromatographic peak detected corresponded to acetic acid, and the amount was very small (± 30 mg/liter) in the system operating at D = 0.25 per h; none was detected at the lower dilution rate. Prior to the change in temperature, both systems provided excellent substrate removal efficiency. The cell yield was somewhat higher for the higher dilution rate. The change from 25 to 8 C was effected in 12 h. It is apparent that neither system responded successfully as the temperature was decreased to the psychrophilic range; there was no indication of impending recovery after 200 h of operation at the post-shock temperature. It appears that the lower dilution rate permitted a greater degree of dissimilation of substrate (compare A-COD curves) and slightly greater utilization of the

organic carbon (compare T-COD curves). There was some indication of "overshoot" with regard to A-COD in the system with lower D, whereas there was a smoother transition at the higher dilution rate.

When a less severe decrease in temperature, from 25 to 17.5 C, was applied (see Fig. 2), the response of the system growing more slowly in the preshock state was much more successful than that of the more rapidly growing one. The change in temperature was effected in 6 h, i.e., 0.75 and 1.5 mean hydraulic retention times, respectively, for the two systems. There was essentially no leakage of anthrone-reactive material in the system of lower D, and there was only a short-lived transient rise in T-COD which corresponded to a transient decrease in biological solids concentration. During the tran-

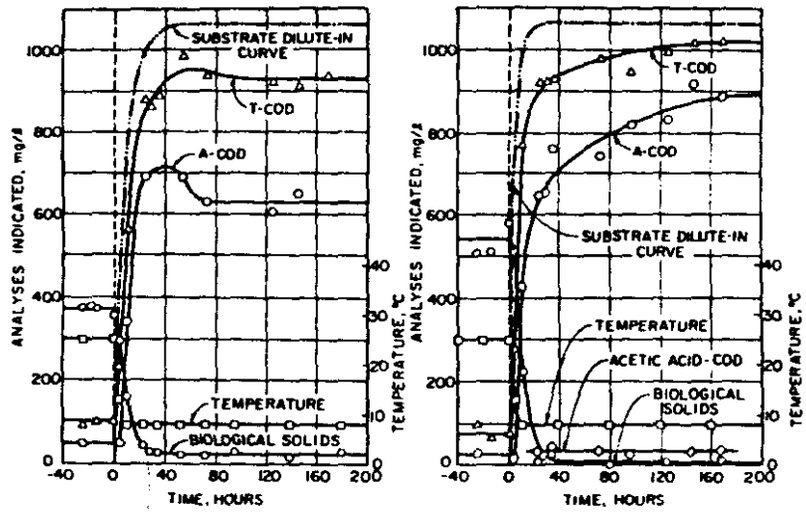

FIG. 1. *Response of a heterogeneous microbial population growing in a chemostat at 25 C to a decrease in temperature to 8 C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.*

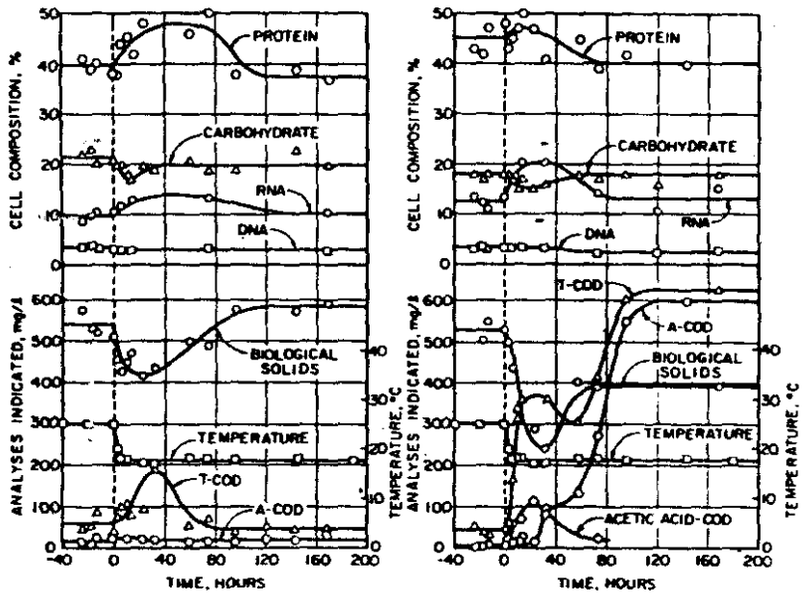

FIG. 2. *Response of a heterogeneous microbial population growing in a chemostat at 25 C to a decrease in temperature to 17.5 C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.*

sient stage, there was an increase in protein and RNA content of the biomass and a slight decrease in carbohydrate content. Although this system successfully accommodated the decrease in temperature, the one at D = 0.25 per h could not accommodate the change. During the first 20 h, the effluent COD rose sharply but there was essentially no leakage of carbohydrate. During this period, acetic acid concentration rose to 114 mg/liter. The concentration of biological solids decreased from 510 to 240 mg/liter. The system appeared to be on the verge of recovery approximately 40 h after changing the temperature, but both T-COD and A-COD rose to nearly 600 mg/liter 100 h after initiating the shock. There was an unexpected recovery of the concentration of biological solids to nearly 390 mg/liter concomitant with the rise in effluent COD, leading to a cell yield of approximately 90% as compared to one of approximately 50% in the preshock condition. The high cell concentration was not due to incomplete mixing, i.e., retention of cells in the reactor, since the concentrations of cells in the reactor and the effluent were the same. As in the case of the system with lower D there was, during the transient stage, an increase in RNA and protein content and a decrease in carbohydrate content of the biomass.

The mildest increasing temperature shock studied was one from 25 to 36 C. The change was effected over a period of approximately 40 h ($5 \times \bar{t}$ at D = 0.125 per h and $10 \times \bar{t}$ at D = 0.25 per h). Successful responses occurred at both dilution rates (Fig. 3). At the lower dilution rate, there was only slight fluctuation in effluent quality and a slow, but completely reversible, decrease in the concentration of biological solids. There was a decrease in protein content and a concomitant increase in carbohydrate content, but these parameters, along with the concentrations of effluent COD (S) and biological solids (X), returned to the preshock level. At the higher dilution rate, there was initially a rapid loss of biological solids but the biomass concentration recovered rapidly, followed by a slow decrease to the new "steady state" level. The fluctuations and decreasing trend in X did not result in any deterioration in purification efficiency. The most noticeable effects were the decrease in cell yield and increase in protein content of the biomass.

When the systems were subjected to a more severe increase in temperature, from 25 to 47 C over a period of 26 h, a severe transient leakage of substrate ensued at both dilution rates. The effect of lower dilution rate in attenuating the severity of dilute-out in X and leakage in S in the transient phase is amply demonstrated in these experiments. It is also seen that, for the system with lower D, dissimilation of the substrate proceeded without interruption, whereas in the system of higher D, the leakage of anthrone-reactive material paralleled the T-COD concentration. Both systems recovered the preshock level of treatment efficiency, and the higher operating temperature led to a lower cell yield (see Fig. 4).

An increase in temperature to the thermophilic range, i.e., from 25 to 57.5 C, led not only to severe transient disruption of the system but to inability to recover treatment efficiency within 200 h after changing the temperature. Again the system with lower D evidenced the more successful response. Analyses for sludge composition were not performed, since the cell concentration was extremely low in the transient phase. It is important to note that the deleterious response could not be attributable to deficiency of dissolved oxygen. The lowest dissolved oxygen concentration recorded was 3 mg/liter, a value much in excess of oxygen concentration usually found to limit metabolism of microorganisms (see Fig. 5).

DISCUSSION

In these studies, the temperature shocks, either increases or decreases, were applied at equal rates of change to each of two comparable systems which were growing at specific growth rates of 0.25 and 0.125 per h in the initial steady state, and it is amply apparent that, regardless of the direction of temperature change, the system with lower D exhibited a greater degree of accommodation to the shock. The same effect has been observed in other shock-loaded systems for which the carbon source was the growth-limiting nutrient. For example, systems with lower D have been observed to leak less substrate during transient response to qualitative shock, i.e., changes in the type of compounds comprising the carbon source in multicomponent substrate systems, e.g., carbohydrate-amino acid systems (9) and carbohydrate-alcohol systems (unpublished data, Komolrit and Gaudy). We have also observed in other studies (Krishnan and Gaudy, unpublished data) that systems with lower D respond more favorably to quantitative shock, i.e., changes in the concentration of the inflowing carbon source.

Similar trends for various types of system perturbances by no means imply similar mechanisms of metabolic or ecological response, but

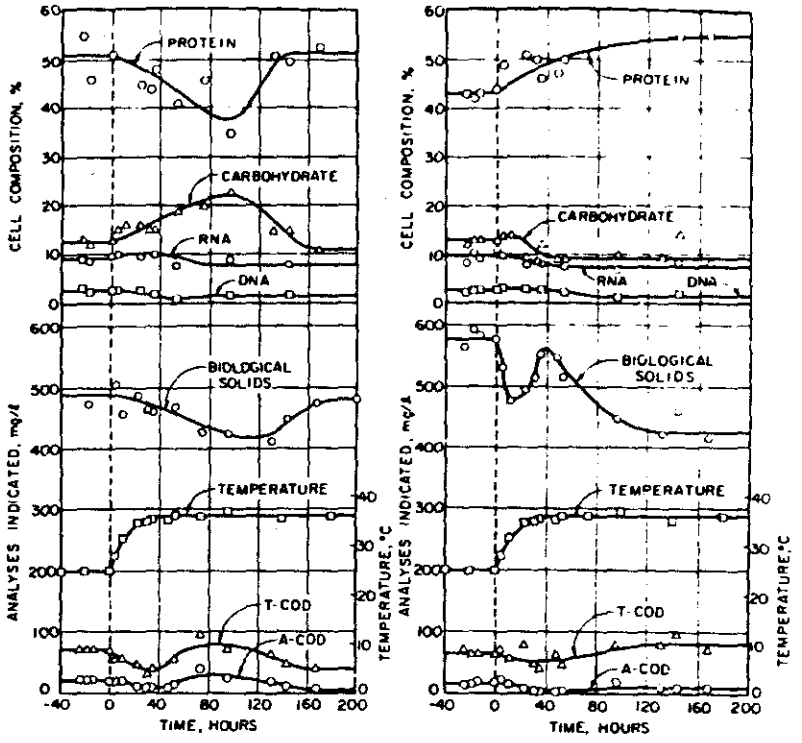

FIG. 3. *Response of a heterogeneous microbial population growing in a chemostat at 25 C to an increase in temperature to 36 C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.*

the general trend does tend to explain the rather good ability of activated sludge systems to successfully withstand, without serious metabolic malfunction, the various environmental stresses which are imposed upon them. The specific growth rate, μ, for such systems is naturally rather low because of cell feedback, i.e., $\mu = D(1 + \alpha - \alpha X_R/X)$, wherein α is the hydraulic feedback ratio, X_R is the cell concentration in the feedback, and X is the concentration of cells in the aeration tank. In addition, cell feedback provides a much greater concentration of biomass in the reactor than could exist without feedback, and we have observed in some experiments that a higher cell concentration also attenuates the transient leakage of substrate. Both results of operation with cell feedback (lower μ and higher X) can combine to provide apparent greater protection against various types of environmental stress. Indeed, there may be some doubt as to which factor most affects the resistance to environmental shock. For this reason, studies in once-through systems are apropos to activated sludge process research because they provide the investigator with a tool with which to separate the effects. The present study on temperature shock, as well as other shock load study results mentioned above, would seem to leave little doubt that the specific growth rate at which the cells were growing in the preshock steady state has a separate and a rather significant influence on the response. It is also quite possible that the response is more greatly influenced by the hydraulics of the system than by the preshock physiological condition of the cells as influenced by μ or X at the time of applying the stress. A longer mean hydraulic retention time (i.e., 1/D = t) may, simply by retaining cells in the reactor longer, provide more time for adjusting to the new conditions.

In studies cited above on changes in type and concentration of substrate, the imposed change was usually administered at a rate governed by the hydraulic feed rate, D. Thus, the change in concentration or type of substrate, or both, would be administered more slowly for lower values of D in accordance with the calculatable dilute-in curves. However, for the present study, the rate of temperature change in each system was the same, since the temperature shock was not administered via a change in the inflowing medium, and the difference in response can be attributed solely to the different dilution rates (with allowance for possible differences in the populations in the reactors prior

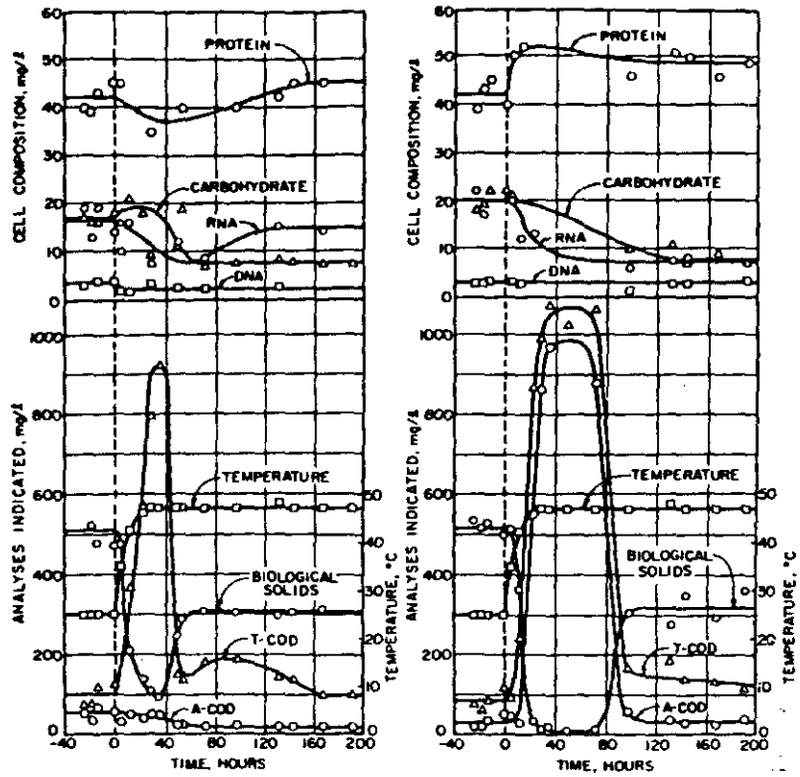

FIG. 4. *Response of a heterogeneous microbial population growing in a chemostat at 25 C to an increase in temperature to 47 C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.*

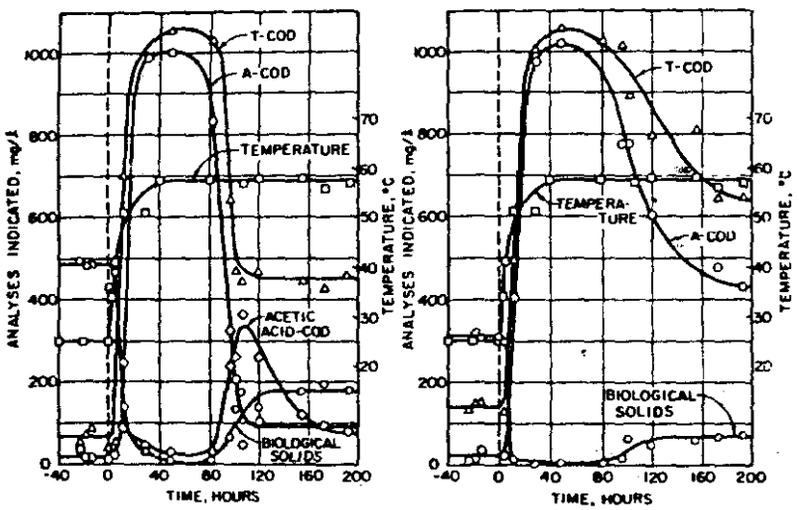

FIG. 5. *Response of a heterogeneous microbial population growing in a chemostat at 25 C to an increase in temperature at 57.5 C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.*

to the shock). It would appear that the lower dilution rate provides a greater intra- or intercellular response time which permits the more favorable metabolic response.

With regard to the composition of the biomass, the lowering of temperature (see Fig. 2) caused an early decrease in carbohydrate and an increase in RNA and protein contents during

the transient stage. The cell composition in the final "steady state" was approximately the same as in the preshock state, and there was a slight rise in cell yield. The increase in protein and RNA accompanied by a decrease in carbohydrate is indicative of rapid growth. Since the lower temperature would be expected to decrease the growth rate of the population predominating prior to the shock, the change in biomass composition may be indicative of an adaptive response in which cells which could grow rapidly at the cooler temperature were being selected, while cells incapacitated at the lower temperature were diluting out of the system. The higher carbon source concentration in the reaction liquor during the transient stage would also tend to increase the specific growth rate of the cells which were able to grow. It is noted, however, that if such a change in predominance took place it was not reflected in a noticeable change in the morphological appearance of the biomass as adjudged by frequent microscope examination during the experiment.

Dilution rate apparently affected the change in protein content in response to an increase in temperature (Fig. 3, 4). In both cases, the population which was growing more slowly in the preshock condition responded with a decrease in protein content, whereas in the more rapidly growing system protein content increased during the transient phase. There was, in general, a decreased cell yield at the elevated temperature. During the period of cell dilute-out and recovery, there was some evidence for changes in species predominance for all three temperature increases as indicated by changes in morphology; however, an ecological (or, in any event, a morphological) shift was particularly evident at the 47 and 57.5 C temperatures. Prior to the shock, short, thick rods predominated in the biomass. These began to dilute out as the concentration of biological solids decreased, and they were replaced in the recovery phase by thin, elongated cells.

Although there was, in these experiments, a general pattern of cell dilute-out and substrate leakage followed by recovery, and although the severity and duration of dilute-out were greater with greater changes in temperature from the base of 25 C, it is somewhat difficult to determine if these responses can be modeled mathematically. The ultimate utility of a model for the transient stage depends upon the adequacy of its physiological (mechanistic) basis. Although there have been attempts, by using a systems approach, to devise such predictive models for single species systems without demonstration of a mechanistic basis for them, the utility of such approaches for heterogeneous population systems would seem rather minimal. For natural populations, the problem is much more complicated, and successful modeling will, in any event, depend upon the availability of experimental results obtained in controlled experiments which provide a record of the response as measured by a number of significant parameters. The results herein presented are intended to help satisfy this need. Also, they provide some guidelines regarding the magnitude of change which a natural system can accommodate, and it may be tentatively concluded that systems operating at reasonably moderate temperatures, e.g., ± 25 C, can more readily accommodate increases than decreases in temperature. This may be due in part to the fact that the most general effect of a nonlethal rise in temperature is an increase in growth rate, either of the existing predominants or of cells selected by the higher temperature.

ACKNOWLEDGMENTS

The experimental phases of this work were conducted under research grant WP-00075 from the Federal Environmental Protection Agency. Portions of the analysis of the data and manuscript preparation were supported by a research grant A-043 from the Oklahoma Water Resources Research Institute, U.S. Department of the Interior.

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APPENDIX VI

Krishnan, P., and A. F. Gaudy, Jr., "Response of Activated Sludge to Quantitative Shock Loading Under a Variety of Operational Conditions," Presented at 30th Annual Purdue Industrial Waste Conference, Lafayette, Indiana, May 6-8, (1975).

RESPONSE OF ACTIVATED SLUDGE TO QUANTITATIVE SHOCK LOADING UNDER A VARIETY OF OPERATIONAL CONDITIONS

by

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The response of biological treatment processes, e.g., activated sludge, to shock loadings has been of long standing investigational interest in the authors' laboratories. Understanding and characterization of response to environmental change present very difficult and complex problems because of the many types of shock and the manner in which they can be administered as well as the biological heterogeneity of the biomass eliciting the response. Work in the authors' laboratories has encompassed quantitative shock (i.e., changes in concentration of feed, S_i) as well as qualitative shock (i.e., changes in the nature of the substrate). The most recent work on shock loadings published from this laboratory has concerned hydraulic shock[1], "temperature shock"[2] and "pH shock"[3]. The present report deals with quantitative shock loading in both once-through and cell recycle systems with the shock administered as a step increase in feed concentration, S_i. In the last report from this laboratory dealing with the quantitative shock, Thabaraj and Gaudy showed that in addition to the shock-induced transient and subsequent attainment of a new steady state in response to an increase in S_i there may also be a secondary response of an ecological nature which can be more disruptive of treatment efficiency than the immediate biomass

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Paper presented at the 30th Annual Purdue Industrial Waste Conference, Lafayette, Indiana, May 6, 7, 8, 1975.

response[4]. The present interest centers on the biomass response, and a systematic study was designed which provided for parallel comparison of once-through and cell recycle systems, holding mean hydraulic retention time, $\bar{t}$, the same in both, and varying levels of shock substrate and growth rate (μ) or mean cell residence time (θ_c). All of these factors as well as biomass concentration, X, mass loading rate and others may affect response patterns. In the steady state prior to the shock, all of these factors contribute to the uniqueness of the near-term past growth history of the biomass and this determines, along with the mode of administering the shock, the response patterns which will be manifested. Grady[5] and Schaezler et al[6] have reviewed the work of many researchers and the general impression among many is that specific growth rate, μ, or its reciprocal, sludge age, θ_c, as well as mean hydraulic retention time, $\bar{t}$, prior to the shock play a significant role in determining the response. Schaezler et al[6] did not show the corroborating experimental data but they state that in general their results demonstrated that slower growing cultures responded to increases in S_i better than more rapidly growing systems. Unfortunately they chose to use only the Glucostat analysis as a measure of substrate and the utility of their information to the environmental pollution control science suffers because they have no measure of the response in regard to the amount of the organic matter exiting in the effluent. It has been shown by Storer and Gaudy[7] as well as Thabaraj and Gaudy[4] that in response to an increase in S_i heterogeneous populations can dissimilate the original organic carbon source much faster than they assimilate it, thus elaborating various organic metabolic products into the medium. Therefore, it seems unwise

to employ such an analysis as Glucostat as the sole measure of organic carbon source in work relating directly to environmental pollution control. Also caution must be exercised in attributing better response simply to the lower growth rate because in a once-through system lower growth rates are brought about by decreasing the dilution rate D, i.e., by increasing $\bar{t}$. Thus the same step increase in influent substrate concentration is applied at different rates to systems operating at different values of D and the resulting difference in mass loading rate may influence the response pattern. However it is significant to note that in the recent report on thermal shock loading by George and Gaudy[2] in which the step increase in temperature was applied at the same rate independently of dilution rate, D, or specific growth rate, μ, systems growing at lower values of μ responded more favorably, i.e., showed less leakage of carbon source during the transient phase.

The present systematic study of response to quantitative shock loading was undertaken to gain insight toward delineation of the roles of the various parameters cited above and to arrive at some practical guidelines regarding shock levels which activated sludge systems can handle without significant disruption of treatment efficiency.

MATERIALS AND METHODS

Apparatus

In these studies continuous flow completely mixed activated sludge pilot plants were run in the laboratory as both once-through and cell recycle systems. The reactors were the same as those described in previous reports[8][9][10][3]. When sludge recycling was not employed, the compressed air flow rate was 4000 ml/minute and the effective volume of

the aeration tank was 2.4 liters. The air flow was increased to 5000 ml/minute when sludge recycle was employed to ensure complete mixing of mixed liquor solids and the effective volume of the aeration vessel was 2.2 liters. Temperature was maintained at 25 C ± 0.5 by a thermostatically controlled water bath. Synthetic waste water was fed through a pump (Milton Roy Co., Avondale, Pa.). A dual pump system was employed and one pump and set of feed lines was cleansed and disinfected while the other delivered synthetic waste to the reactor. Return sludge was fed to the reactor through a peristaltic pump (Sigma Motor Co., Middleport, N.Y.).

Synthetic Waste

In all experiments the synthetic waste consisted of a readily available carbon source, glucose. The concentration of mineral salts employed per 1000 mg/l of carbon source was as follows: $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4.7H_2O$ 100 mg/l; $FeCl_3.6H_2O$, 0.5 mg/l; $MnSO_4.H_2O$, 10 mg/l; $CaCl_2$, 7.5 mg/l; 1.0 M phosphate buffer pH 7.0, 10 ml/l; tap water, 100 ml/l and distilled water to volume. All shocks were applied to systems growing initially in the "steady state" on the above medium at an inflowing substrate concentration of 1000 mg/l. When quantitative shocks consisting of increases in inflowing substrate concentration, S_i, to 2000, 3000 and 5000 mg/l were applied, the salts concentrations were proportionally increased.

Analytical Parameters

Biological solids concentrations were measured by the membrane filter technique (pore size, 0.45μ). Protein and carbohydrate contents of the biomass were measured by the biuret and anthrone analyses[11], respectively. Effluent quality was measured by COD[12] and anthrone analyses on the membrane filtrate. Dissolved oxygen was monitored electrometrically.

Operational Plan and Experimental Protocol

An aim of the present study was to compare the shock load behavior of once-through and cell recycle systems at identical hydraulic reactor detention times, $\bar{t}$. Three sets of detention times were selected, 4, 8 and 12 hours. In a once-through system $\bar{t} = \frac{1}{D} = \frac{1}{\mu}$, where D is the dilution rate and is defined as the ratio of the inflowing rate of feed flow, F, and the aeration tank volume, V, (i.e., F/V); μ is the specific growth rate which, in the steady state condition, is an exponential (logarithmic) growth rate constant which can also be defined as $dX/dt\ (\frac{1}{X})$ where the differential is the rate of change in X. When cell recycle is practiced, the mean hydraulic retention time in the reactor is reduced (dilution rate increased) because of the recycle flow. The recycle flow is usually expressed as a fraction, α, of the waste flow and is thus equal to αF. In terms of dilution rate, the reactor dilution rate D_1 is given by equation 1

$$D_1 = D(1 + \alpha) \qquad (1)$$

Thus to maintain the same $\bar{t}$ for the reactor (or D_1) it is necessary to reduce the system dilution rate, D, i.e., F/V. This was accomplished by reducing the rate of inflowing waste feed, F. In the present studies the recycle flow ratio, α, was maintained at 0.33. These hydraulic relationships for the current study, calculated from equation 1, are shown in Table 1. The reduction in D to maintain the same reactor hydraulic retention time has a consequence of lowering the mass rate of organic feed loading and has ramifications regarding analysis of shock loading results which will be discussed in a later portion of this report.

In these experiments efforts were made to operate the recycle systems in accordance with Herbert's model embodying the theory of continuous

culture[13]. In this model the recycle cell concentration factor c is the ratio of concentration of recycle sludge, X_R, and the mixed liquor suspended solids concentration X. A value for c of approximately 2.5 was selected in these studies since this would provide an X approximately twice that in the once-through system. It was felt that this value of c, although lower than that which might be reasonably obtained in an actual activated sludge plant, could be obtained in these studies without serious experimental problems and would provide sufficient difference in X to gain insight into effects of recycle solids on the shock load response. The recycle solids concentration varied somewhat for each experiment but was in general 2000 mg/l ± 10%. All systems were operated with an incoming substrate concentration, S_i, of 1000 mg/l glucose prior to the shock. Shocks consisting of step increases in glucose concentration to 2000, 3000 and 5000 mg/l were administered.

Each continuous flow unit was started from a sewage seed obtained from the municipal treatment plant at Stillwater, Oklahoma. Each system was operated at the steady state with respect to X and S for at least 3 days prior to administering a shock. The once-through systems attained a relatively steady condition in one or two days. When sludge recirculation was practiced, normally 3 weeks were required before flocculated cells which would settle in the clarifier were developed. Prior to any experimentation, the reactors were checked for complete mixing in accordance with procedures previously described by Komolrit and Gaudy[8]. Also throughout the experimentation complete mixing with respect to suspended solids in the reactor was checked periodically. The units were judged to be completely mixed at all times during the study.

RESULTS

In Figure 1, it is seen that when a once-through system growing in the steady state at $\bar{t}$ = 4 hours with an inflowing substrate concentration, S_i, of 1000 mg/l glucose was shocked by an increase in S_i to 2000 mg/l, there was a disruption of substrate removal efficiency lasting approximately two mean hydraulic retention times. The initial steady state condition is shown at zero on the time axis. The COD of the mixed liquor filtrate rose from 70 to 340 mg/l whereas the COD due to carbohydrate in the filtrate (anthrone COD) rose only to 140 mg/l, indicating microbial elaboration of metabolic products. The system appeared to have completed the initial response within 12 hours as indicated by the biomass response (suspended solids, protein and carbohydrate).

When an increase in S_i to 3000 mg/l was applied, the response was as shown in Figure 2. The results for the once-through system are shown on the left and those for the same shock for the system employing cell recycle ($c = 2.5$, $\alpha = 0.33$) are shown on the right. The dotted line curves show the dilute-in patterns of S_i during the shock. These curves show the value of substrate concentration in the unit if substrate utilization had stopped at the time the shock was applied. They are plotted on this figure to show the difference in rate of load application between the once-through and cell recycle systems brought about by the fact that the mean hydraulic retention time in both was held constant at 4 hours. It is seen that the necessary reduction in inflow rate of feed causes a slightly slower shock loading rate which undoubtedly helps account for the more successful response to the increase in S_i. It should also be emphasized that the steady state mass loading per unit of time (i.e. lbs glucose COD/1000 cu ft/day) is higher

for the once-through system. The pattern of response of the once-through system was similar to that for the 2000 mg/l shock except that the transient leakage of organic carbon (total COD and anthrone COD) was more severe and the time to recover was longer. It is readily apparent that the decreased loading rate coupled with the higher biomass concentration in the reactor prior to the shock enabled the cell recycle system to accommodate the three-fold increase in S_i without any reduction in substrate removal efficiency.

When S_i was increased to 5000 mg/l there was severe leakage of substrate in both types of systems as seen in Figure 3. The major difference in response was that while the peak COD leakage was approximately the same for both systems a much greater amount of non-carbohydrate COD was produced in the once-through reactor. Also the residual COD after the transient stage in effluent substrate was considerably higher in this system.

The general pattern of response at this hydraulic retention time (4 hours) as substrate concentration was increased from 2000 to 3000 to 5000 mg/l, in the case of the once-through system was one of progressively greater substrate leakage in the transient phase with the major portion of the COD in the effluent being attributable to metabolic products of the original carbon source rather than leakage of that carbon source itself. Sludge recirculation prevented leakage of COD at the 3000 mg/l feed level but not at the 5000 mg/l level.

When the mean hydraulic retention time was increased to 8 hours and a step change from 1000 to 2000 mg/l feed was applied, the response was considerably less deleterious than at $\overline{t}$ = 4 hours. There was very little leakage of COD during the transient stage (see Figure 4).

A 200 percent increase in S_i can be expected to cause only slight perturbance in effluent quality in the transient phase as seen in Figure 5.

A step change from 1000 to 3000 mg/l carbon source was readily accommodated by both the once-through and the recycle system. In this figure, as in Figures 2 and 3, ($\overline{t}$ = 4 hours) the effluent COD in the final steady state was somewhat lower in the cell recycle system.

At $\overline{t}$ = 8 hours, subjecting the systems to a five-fold step increase from 1000 to 5000 mg/l produced only a short-lived transient leakage of substrate (see Figure 6). At this high shock level cell recycle did not exert an attenuating effect on the amount or duration of leakage of carbon source in the transient stage.

Increasing $\overline{t}$ to 12 hours had little effect on the leakage of COD in the effluent at the 2000 mg/l shock level. There was only a slight transient increase in effluent COD, as seen in Figure 7. The COD peaked at a slightly higher concentration than at the 8 hour residence time (compare with Figure 4); however, in the main, the amount of COD in the effluent during the transient phase was approximately the same. The lack of improvement in level of effluent COD in the transient stage at the 12 hour as compared with the 8 hour residence time suggests that there would be little to be gained in protection from shock at the level herein employed by the 50 percent increase in $\overline{t}$. In fact, the slower growth response in the biomass parameters (biological solids, protein and carbohydrate) could prove to be a detriment in cases of multiple or sequential shocks coming on the system before completion of a transient response.

The response to an increase in feed from 1000 to 3000 mg/l is shown in Figure 8. In the once-through system there was only a slight transient increase in COD but there was considerable fluctuation in effluent quality;

whereas in the cell recycle system the response was characterized by a steadiness and absence of any transient rise in effluent COD.

The 5000 mg/l shock level at this $\bar{t}$ produced only a slight transient disturbance in effluent quality in both systems as shown in Figure 9. Proportionately less non-carbohydrate COD was produced in the cell recycle system, in keeping with the trend of responses to the previous shocks at this level at $\bar{t}$'s of 4 and 8 hours (compare with Figures 3 and 6). As in all previous cases the effluent COD in the final steady state was lower in the system in which cell recycle was practiced.

DISCUSSION

The results show rather clearly that as $\bar{t}$ is increased there is, in the main, improvement in the response with regard to amount of substrate leakage during the transient phase at a given shock concentration of substrate. This trend is most readily seen in the once-through systems (e.g., compare Figures 1, 4 and 7; 2, 5 and 8; and 3, 6 and 9) but is also seen in the cell recycle systems at the high shock level (Figures 3, 6 and 9). Also an examination of both the once-through and cell recycle systems at each $\bar{t}$ for increasing shock concentrations reveals that the higher the $\bar{t}$ the less severe was the increase in substrate leakage as the shock loading was increased. The results also provide an indication that cell recycle, even at the low recycle sludge concentration factor employed in these studies, had some beneficial effect with regard to transient substrate leakage at the 3000 mg/l, i.e., the 3-fold, shock level (see Figures 2, 5 and 8). It appeared, however, that the 5000 mg/l shock level exceeded the ability of this particular cell recycle program to attenuate substrate leakage over that observed

in the once-through system. In addition to its effect on substrate leakage during the transient, cell recycle appeared to smooth out fluctuations in the effluent substrate curve during the transition phase and in all cases provided lower effluent substrate concentrations after re-establishment of a steady state in response to the step change in S_i.

In analyzing the results, one of the most apparent facts regarding an increase in $\bar{t}$ is that it indicates a slower growth rate (in a once-through system: $\bar{t} = \frac{1}{D} = \frac{1}{\mu}$). Thus it might be said that cells with a "slow growth" history prior to the shock can adjust more readily to the change. Cell recycle also slows the specific growth rate. In such systems μ is not equal to the dilution rate, D, but is related to it in accordance with the following equation[10][13]:

$$\mu = D\,(1 + \alpha - \alpha \cdot c) \qquad (2)$$

The term in parentheses includes the hydraulic recycle ratio, α, and the sludge concentration factor, c, which is equal to the ratio of the concentration of sludge in the recycle and in the aeration tank (X_R/X). Since this term is always less than 1, μ is always lower in a cell recycle system than in a once-through system with the same system dilution rate. Thus if slow growth rate prior to the shock is an asset, cell recycle systems can be said to possess such an advantage in this regard especially when one considers the very low specific growth rates (high cell ages) normally employed in the field.

Using the average experimental value of X_R for each system and the value of X in the steady state prior to applying a shock loading, the approximate values of μ were computed in accordance with equation 2 and are given in Table 2. Had the sludge concentration factor, c, been held more precisely constant at 2.5 and had the cell yield not been subject to

some changes during the preshock "steady state", the μ values for the recycle systems at any given $\bar{t}$ would be expected to be the same. Using the nominal value of $c = 2.5$, the values of μ at $\bar{t}$ of 4, 8 and 12 hours are 0.095, 0.048 and 0.031/hr^{-1} respectively. These values are seen to compare rather well with actual values for the systems prior to the shocks at the 3000 mg/l level. Values of c calculated from the "steady state" data were 2.43, 2.54 and 2.52, respectively. However during the runs prior to shocks at the 5000 mg/l level, the values of c calculated from the data were 3.14, 2.73 and 2.92 at $\bar{t}$'s of 4, 8 and 12 hours, respectively. Thus the actual estimated values of μ were lower than those called for in the experimental design. It is recalled that during these experiments the aim was to employ Herbert's operational strategy, i.e., to hold c as a system constant. It is now known that this mode of operation is not a particularly good one for maintenance of the steady state with heterogeneous populations and revised kinetic model equations and data in support of holding X_R rather than c as a design and operational constant have been presented[14][15][16]. In any event, it is clearly seen that μ in the once-through system was significantly higher than in the comparable cell recycle system. In general the specific growth rate with cell recycle was approximately 40 percent of the once-through value, i.e., close to the designed value, prior to the 3000 mg/l shocks but varied from approximately 20 to 30 percent that of the once-through value prior to the 5000 mg/l shocks. However, it is interesting to note that at the 5000 mg/l shock level comparison of once-through and cell recycle results revealed that cell recycle did not have a significant attenuating effect on the transient leakage of substrate. However, increasing $\bar{t}$ in either the once-through or the cell recycle systems (which also decreases μ) did

exhibit an attenuating effect. The beneficial effect of cell recycle was most in evidence at the 3000 mg/l shock level (200 percent increase). In addition to the decreased specific growth rate, the recycle system operates with higher biological solids concentrations which in itself can be expected to provide a biological buffer against transient leakage of substrate during a step increase in S_i. Furthermore as shown previously (see feed dilute-in curves, Figure 2) holding $\bar{t}$ the same in both types of systems required a decrease in feed flow rate (in accordance with equation 1); thus the rate at which the increase in feed concentration was administered to the system was less for the cell recycle system. It can be seen that cell recycle can be expected to help the system accommodate the shock in a number of ways. Factors bringing about lower growth rate through recycle and the accompanying higher biological solids concentration in the aeration tank also depress the mass organic loading rate. The organic loading in pounds glucose/1000 cu feet/day employed in these studies is given in Table 3. The loadings listed at S_i = 1000 mg/l represent those in each system prior to the shock. Loadings for the recycle system at the 2000 mg/l post shock level are given even though recycle systems were not shocked at this low shock level (since they responded successfully at the higher shock level). It is immediately apparent that these loadings are rather high and in accordance with the hydraulic relationship of equation 1 (also Table 1) the recycle system received a unit loading 75 percent that of the once-through system. Comparing the daily loading rate values in the table with the transient responses in Figures 1 through 9 it is noted that the most successful response from the standpoint of prevention of substrate leakage was that exhibited by the recycle system at $\bar{t}$ = 4 hours when S_i was changed from 1000 to 3000 mg/l (Figure 2). In this case the loading rate was increased

from the preshock level of 282 to 846 lbs glucose/1000 cu ft/day. However for an increase from 94 to only 470 lbs/1000 ft^3/day ($\bar{t}$ = 12 hr, shocked to S_i = 5000; Figure 9 cell recycle system) there was a significant transient increase in effluent substrate concentration. The same can be said at $\bar{t}$ = 8 (Figure 6) for the system originally loaded at 141 and shocked to 705 lbs/1000 ft^3/day. Thus a faster growing, more highly loaded recycle system could accept a 3-fold shock at a higher mass loading rate more readily than could a slower growing system shocked to a 5-fold increase at a lower mass loading rate even though the rate of application or diluting in of the substrate was slower at the higher $\bar{t}$'s (i.e., lower specific growth rates). The cell concentration prior to the shock in all three cases cited above was approximately the same so that this factor did not enter into the response. Thus when viewed from a mass loading standpoint, lower values of μ (or higher Θ_c) may lessen the total shock mass loading capability of the system.

It should be emphasized that the intensity of shock or percent increase in loading may be of more critical concern than the total increase in mass loading. Comparison of the behavior of the recycle systems of Figures 2 and 6 is particularly interesting in this regard. The increase in mass loading was the same in both cases: 846-282 = 564, Figure 2; 705-141 = 564, Figure 6. However, the system of Figure 2 was subjected to a 3-fold increase, whereas that of Figure 6 was subjected to a 5-fold increase. The data in Figure 2 show excellent effluent quality throughout the transient response whereas Figure 6 shows significant transient leakage.

Although there is reason to believe that there is a decrease in unit mass loading capacity at lower μ's (higher Θ_c's) it is also reasonable to

expect that within limits this lower unit capability can be counteracted by higher biological solids concentration in the aeration tank. In most systems in the field the biological solids concentration, X, is very high in relation to S_i and other factors such as the reserve storage capacity of the sludge, e.g., oxidative assimilation for carbon storage compounds, may effectively take up the carbon source depending on the nature of the substrates in the waste stream. Such uptake of substrate did not appear to play a role at the biochemical solids concentrations employed in the present studies. An examination of the figures shows that there was no disproportionate synthesis of nonproteinaceous material, e.g., carbohydrate, during the early portion of a successful response. Therefore, the biochemical analyses seem to be in accordance with a growth, or reproductive response, model.

In seeking to uncover some of the mechanisms and factors governing response to increases in S_i, it would seem that neither mass loading rate alone nor intensity of change alone can provide satisfying fundamental biochemical clues or even useable guidelines regarding limits of shock which might be accommodated by activated sludge systems. The fact remains, however, that at all three $\bar{t}$'s and at the rather high growth rates (0.099 to 0.031 hr^{-1}) herein employed, the cell recycle system accommodated a three-fold step increase in S_i and in total mass loading without an increase in effluent substrate concentration during the transient stage. As $\bar{t}$ was increased, i.e., μ decreased (Figures 2, 5 and 8) there was no indication that the response was deteriorating. Although the results presented are based on filtrate COD, it is important to emphasize that in the cell recycle systems sludge settleability was excellent at all times. Only after the 5000 mg/l shock was there any evidence of deterioration in settleability; in those

systems the high shock level resulted in an increase in filamentous organisms and imparted a bulking tendency to the sludge. In general based upon the biochemical responses and observations on flocculation and settling characteristics it seems there is some safety in extending the findings to the field condition, wherein exist lower growth rates and higher biological solids concentration, and it is concluded that for cases of quantitative shock consisting of a step change in S_i, activated sludge systems can be expected to accommodate, with little or no leakage of substrate in the transient phase, a 200 percent increase in S_i.

ACKNOWLEDGEMENTS

The experimental phases of this work were conducted in 1964-5 with partial support from a research grant WP-00075 USPHS. Analysis of the data and manuscript preparation were conducted under a research project A-043-Okla. OWRRI-USDI.

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TABLE 1

RELATIONSHIP BETWEEN MEAN HYDRAULIC RETENTION TIME, $\bar{t}$, REACTOR DILUTION RATE, D_I, AND DILUTION RATE, D.

ONCE THROUGH			CELL RECYCLE		
$\bar{t}$	D_I	D	$\bar{t}$	D_I	D
hrs.	$hrs.^{-1}$	$hrs.^{-1}$	hrs.	$hrs.^{-1}$	$hrs.^{-1}$
4	0.25	0.25	4	0.25	0.188
8	0.125	0.125	8	0.125	0.094
12	0.083	0.083	12	0.083	0.062

TABLE 2

PRE-SHOCK SPECIFIC GROWTH RATE, μ, OF EACH SYSTEM AT THREE MEAN HYDRAULIC RETENTION TIMES.

SHOCK S_i (mg/ℓ)	$\bar{t}$ = 4 HOURS		$\bar{t}$ = 8 HOURS		$\bar{t}$ = 12 HOURS	
	WITH RECYCLE	NO RECYCLE	WITH RECYCLE	NO RECYCLE	WITH RECYCLE	NO RECYCLE
2000	-	0.25	-	0.125	-	0.083
3000	0.099	0.25	0.046	0.125	0.031	0.083
5000	0.053	0.25	0.040	0.125	0.020	0.083

TABLE 3

MASS ORGANIC LOADING RATE IN POUNDS COD PER 1000 CUBIC FEET PER DAY AT THREE MEAN HYDRAULIC RETENTION TIMES.

S_i (mg/ℓ)	$\bar{t}$ = 4 HOURS		$\bar{t}$ = 8 HOURS		$\bar{t}$ = 12 HOURS	
	WITH RECYCLE	NO RECYCLE	WITH RECYCLE	NO RECYCLE	WITH RECYCLE	NO RECYCLE
1000	282	375	141	188	94	125
2000	564	750	282	375	188	250
3000	846	1125	423	563	282	375
5000	1410	1875	705	938	470	625

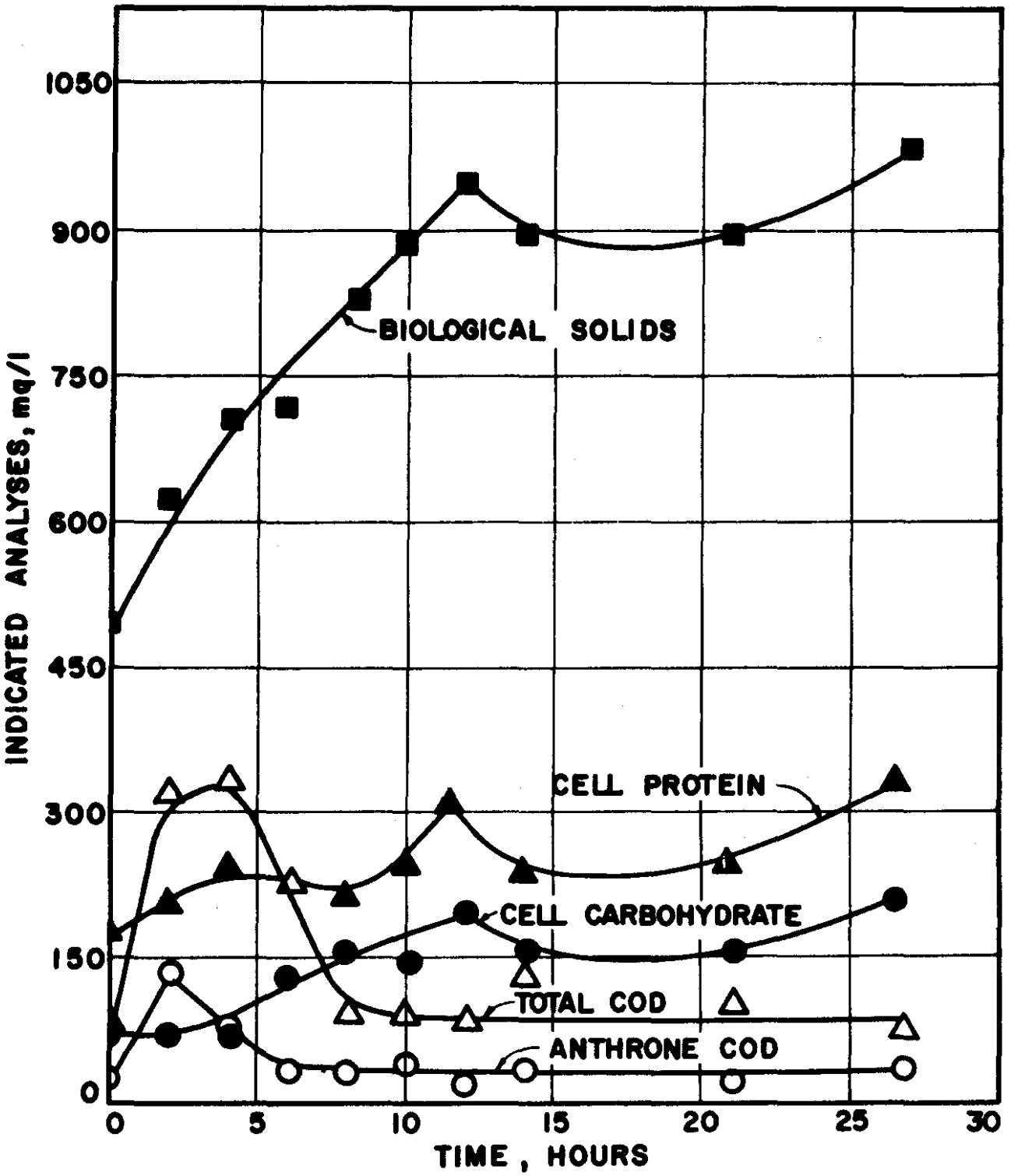

FIGURE 1. RESPONSE TO SHOCK LOAD; S_i 1000→ 2000 mg/l AT $\bar{t}$ = 4 HOURS; ONCE THROUGH SYSTEM.

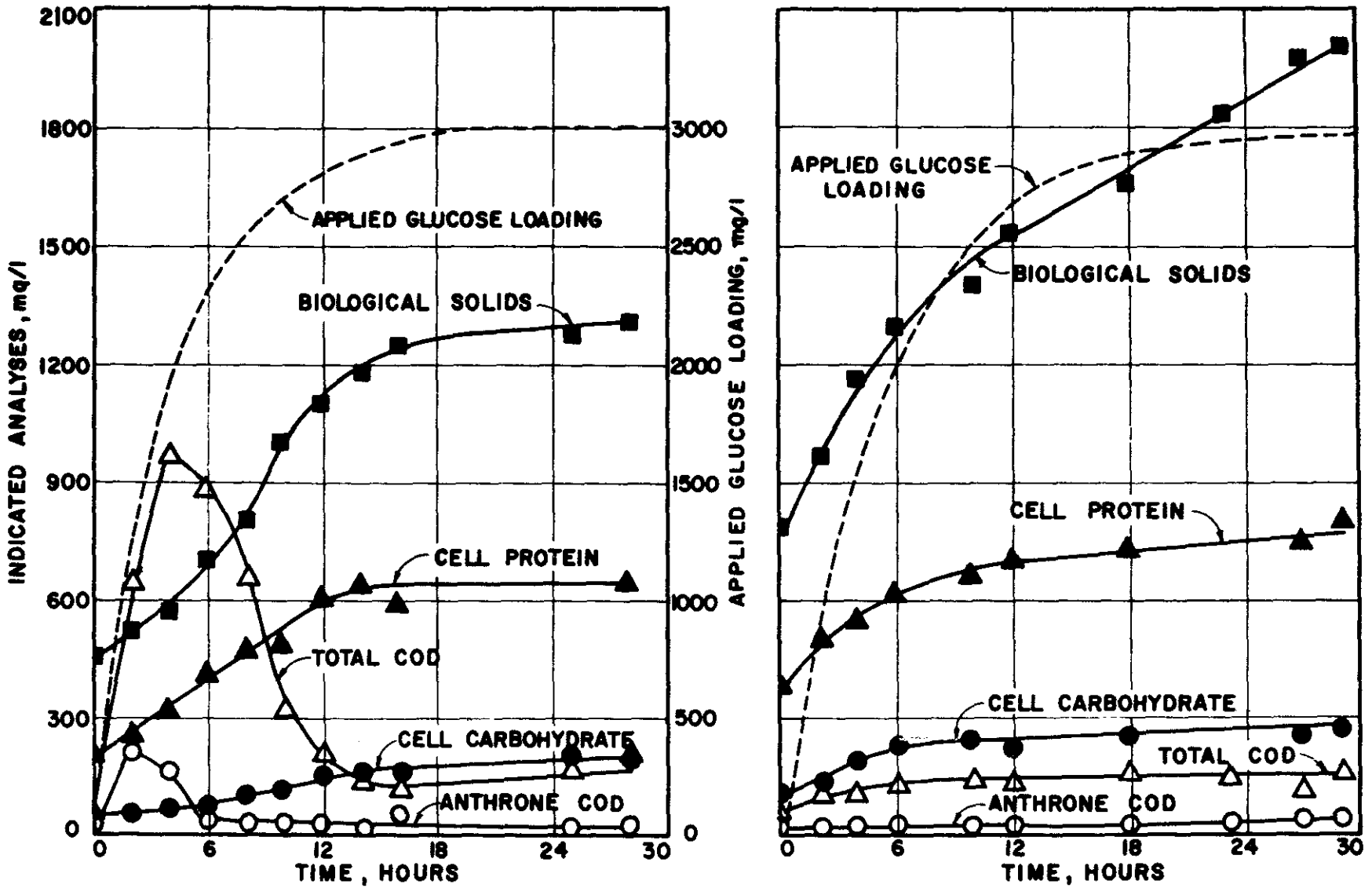

FIGURE 2. RESPONSE TO SHOCK LOAD; S_i 1000→3000 mg/l AT $\bar{t}$ = 4 HOURS; LEFT: ONCE-THROUGH, RIGHT: CELL RECYCLE

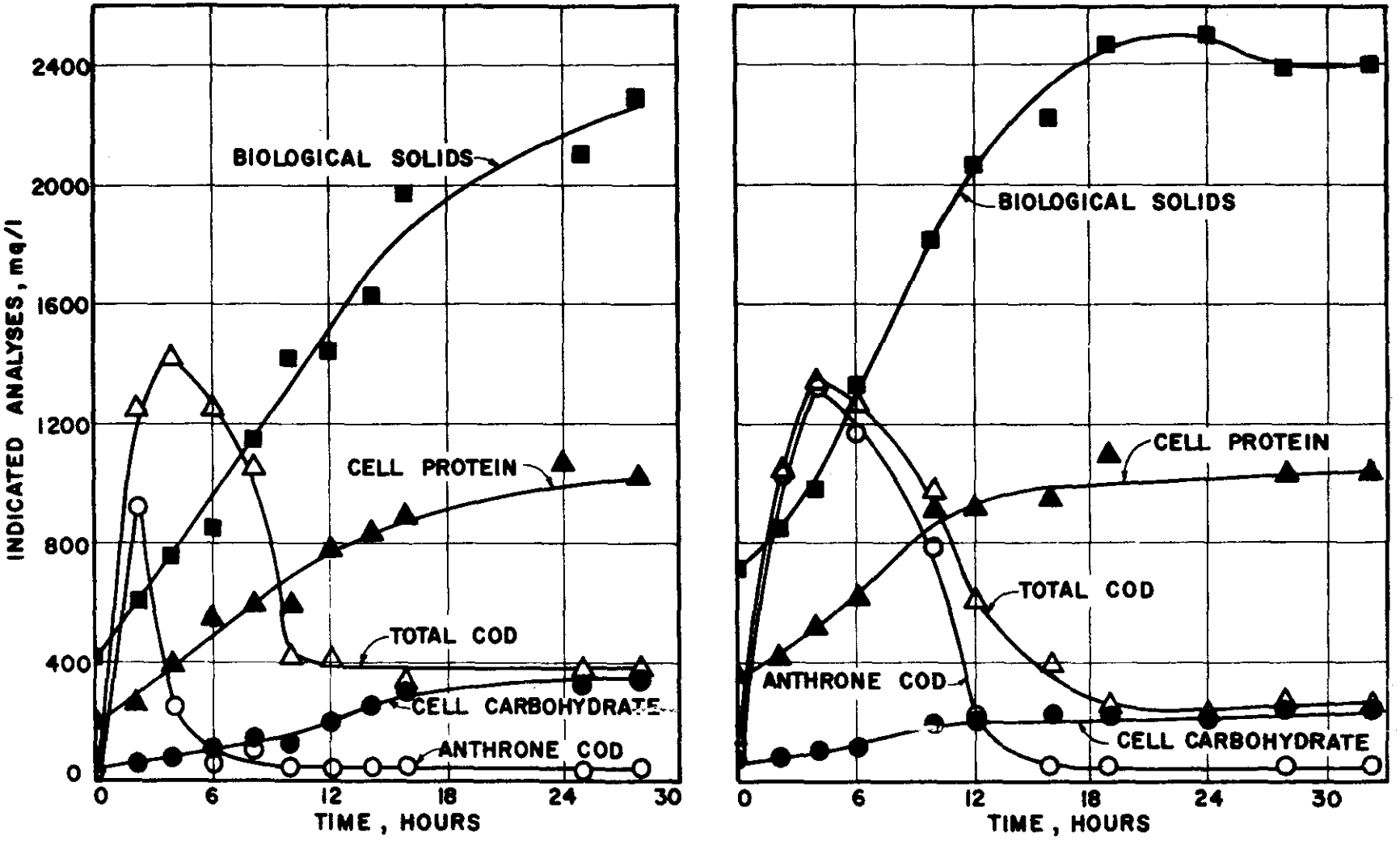

FIGURE 3. RESPONSE TO SHOCK LOAD; S_i 1000→5000 mg/l AT $\bar{t}$ = 4 HOURS; LEFT: ONCE THROUGH, RIGHT: CELL RECYCLE

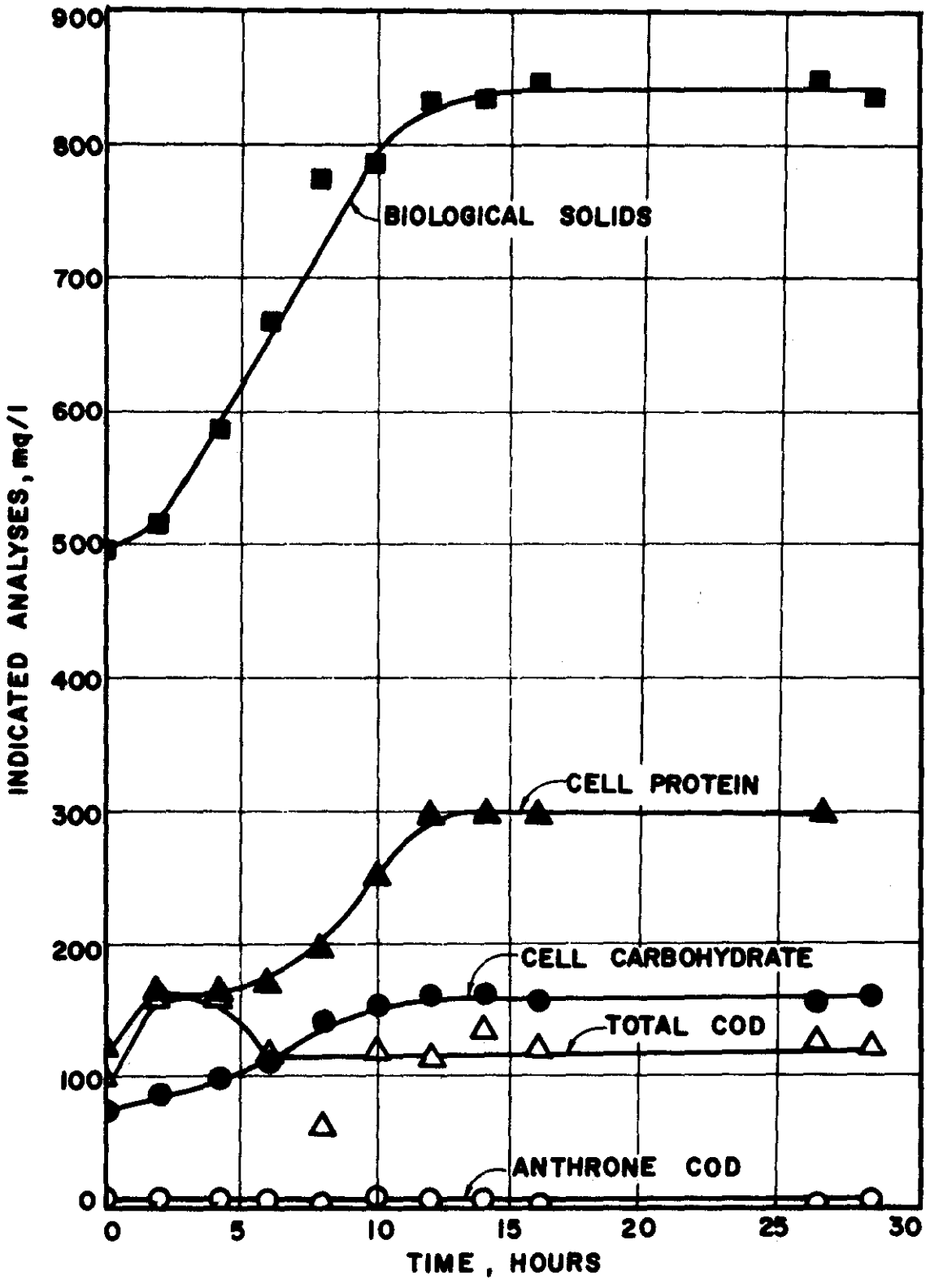

FIGURE 4. RESPONSE TO SHOCK LOAD; S_i 1000→2000 mg/l AT $\bar{t}$ = 8 HOURS; ONCE THROUGH SYSTEM

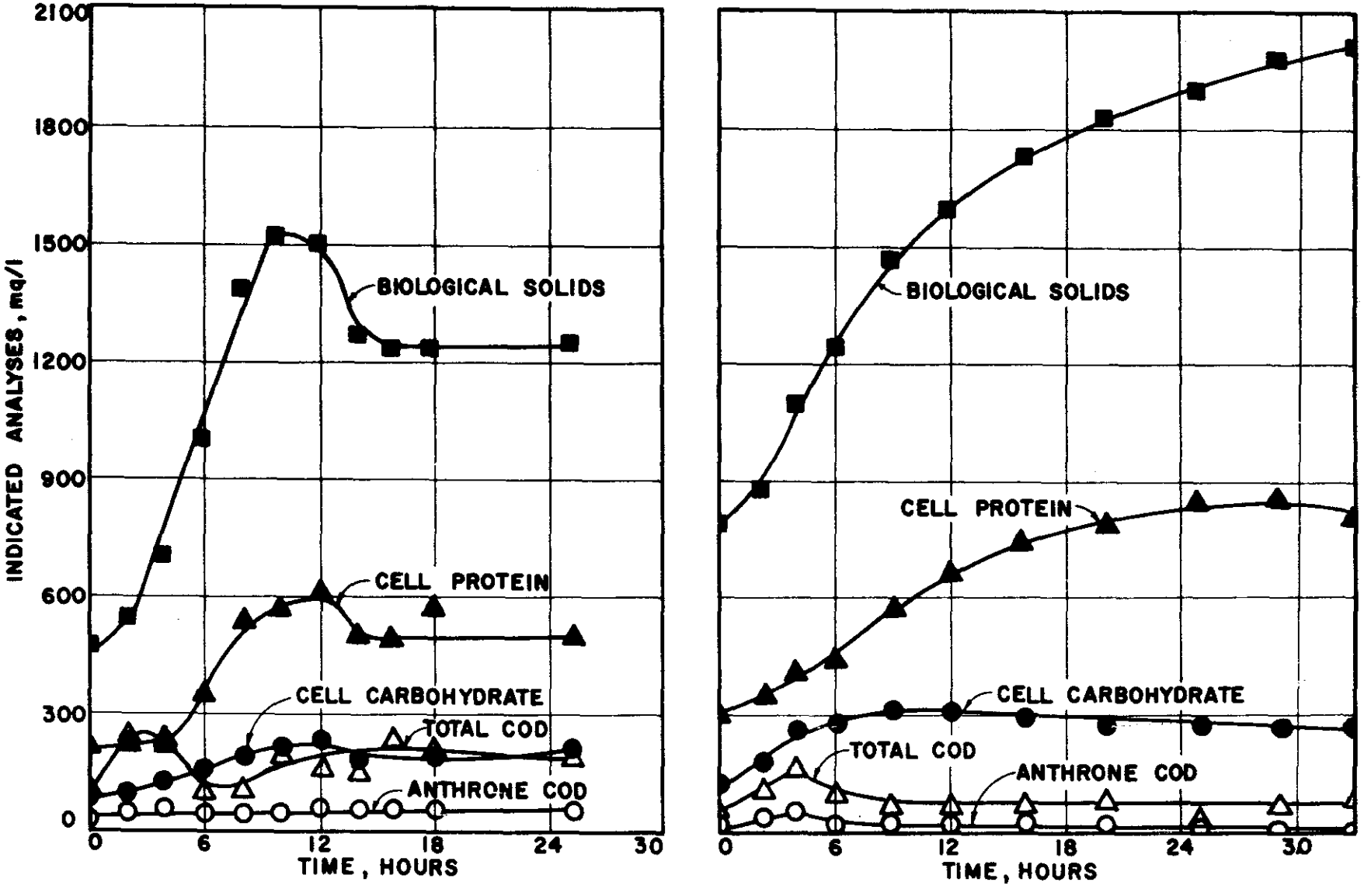

FIGURE 5. RESPONSE TO SHOCK LOAD; S_i 1000 → 3000 mg/l AT $\bar{t}$ = 8 HOURS; LEFT: ONCE THROUGH, RIGHT: CELL RECYCLE

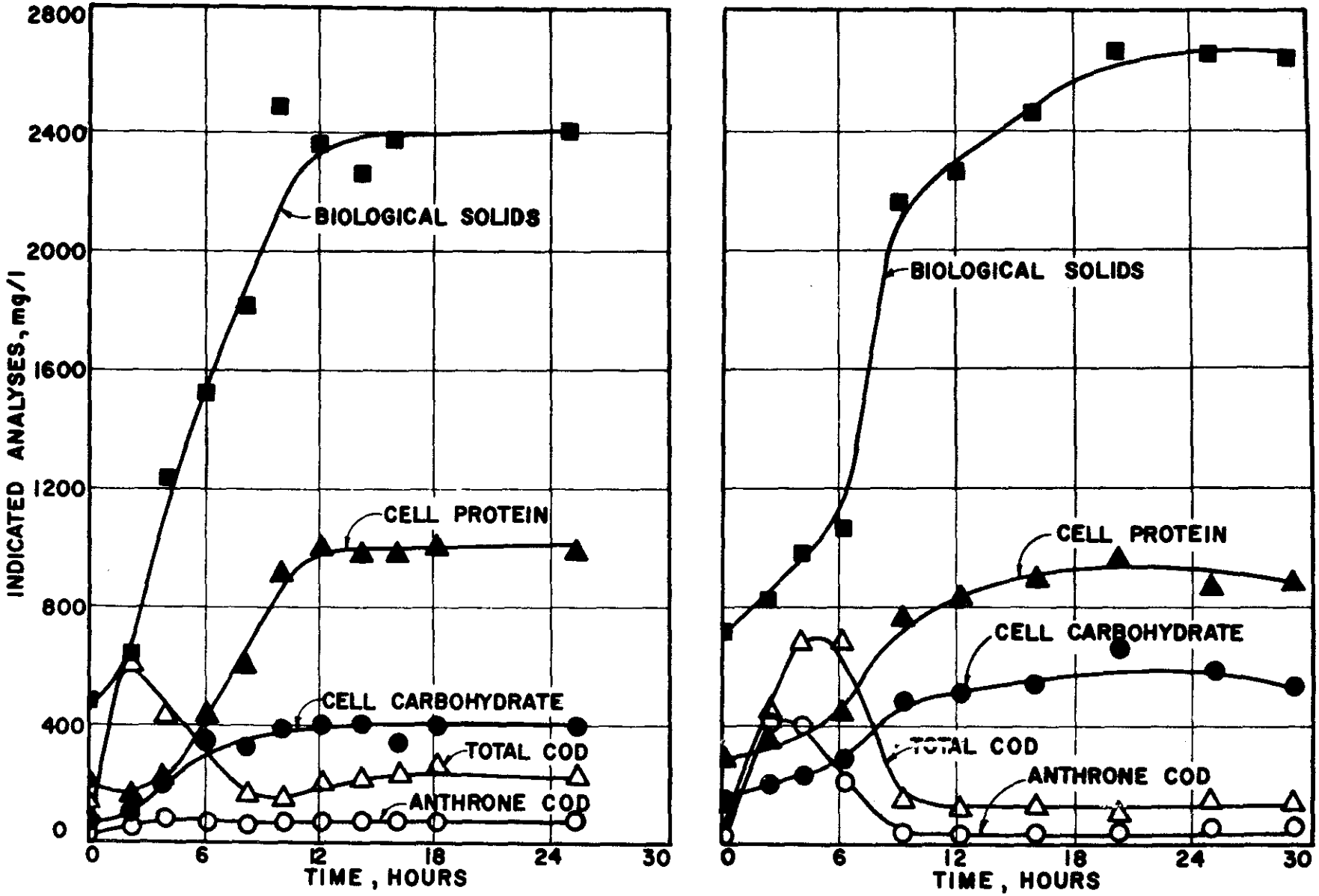

FIGURE 6. RESPONSE TO SHOCK LOAD; S_i 1000→5000 mg/l AT $\bar{t}$ = 8 HOURS;
LEFT: ONCE THROUGH, RIGHT: CELL RECYCLE

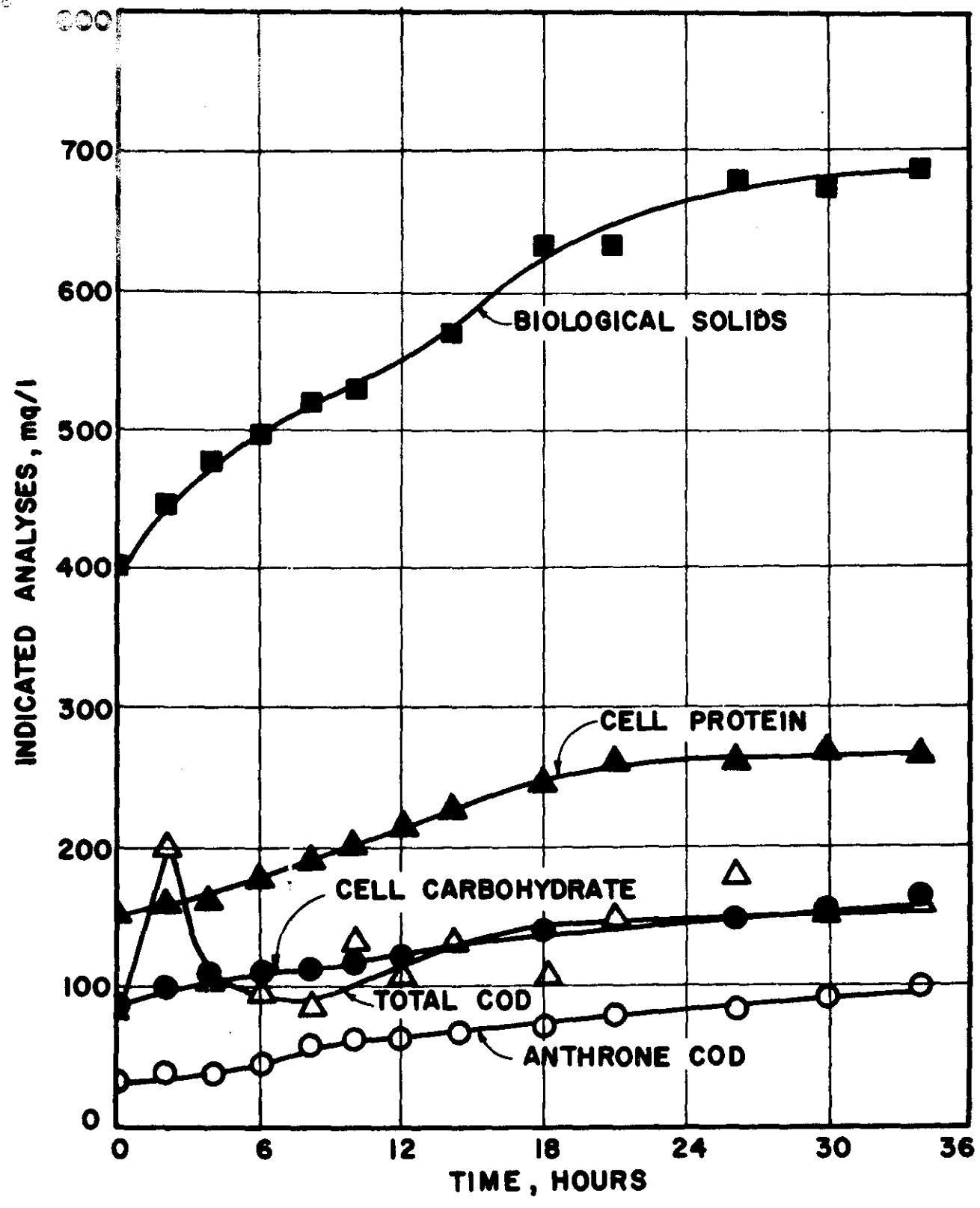

FIGURE 7. RESPONSE TO SHOCK LOAD; S_i 1000→2000 mg/l AT $\bar{t}$ = 12 HOURS; ONCE THROUGH SYSTEM

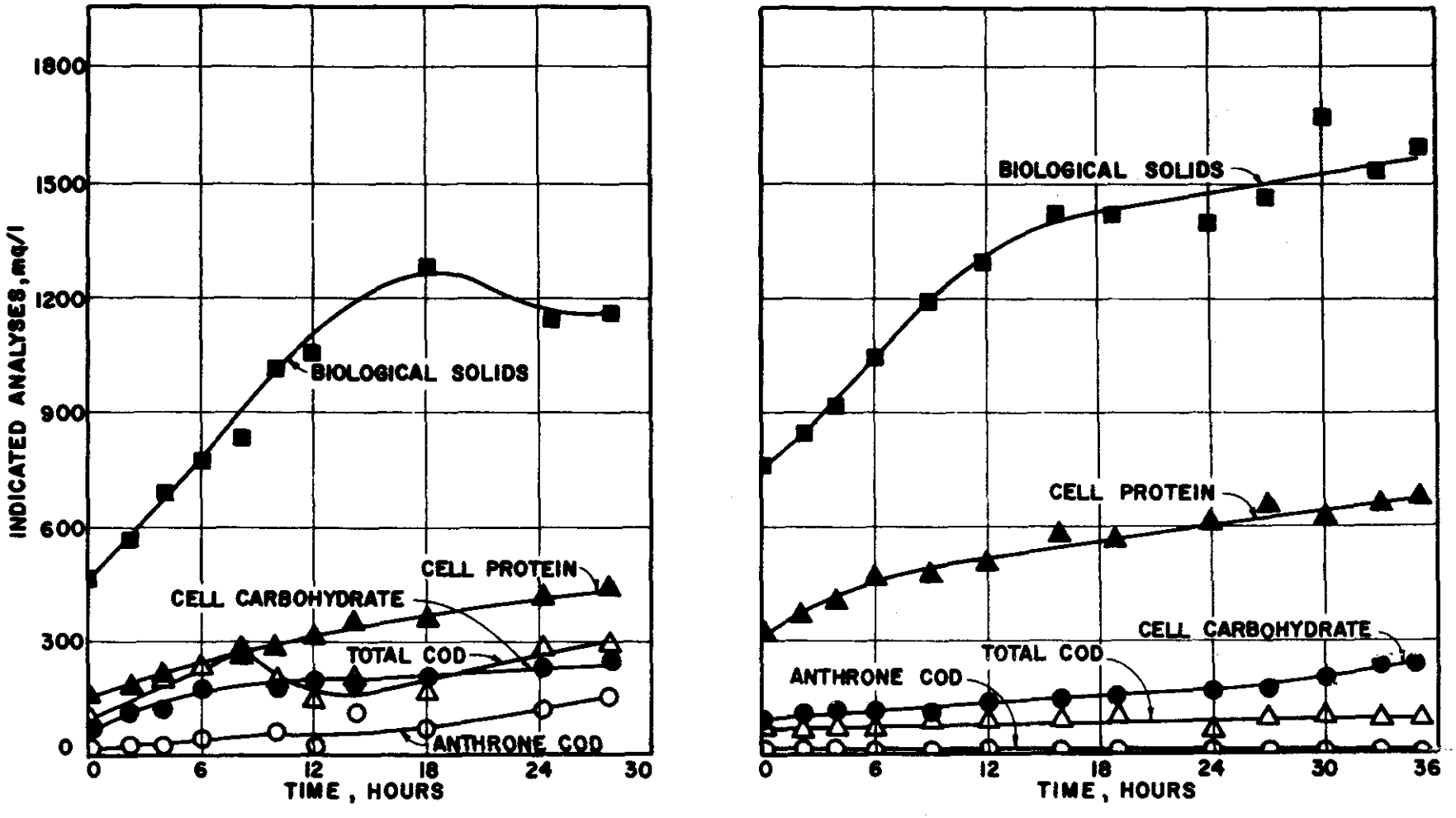

FIGURE 8. RESPONSE TO SHOCK LOAD; S_i 1000→3000 mg/l AT $\bar{t}$ = 12 HOURS;
LEFT: ONCE THROUGH, RIGHT: CELL RECYCLE

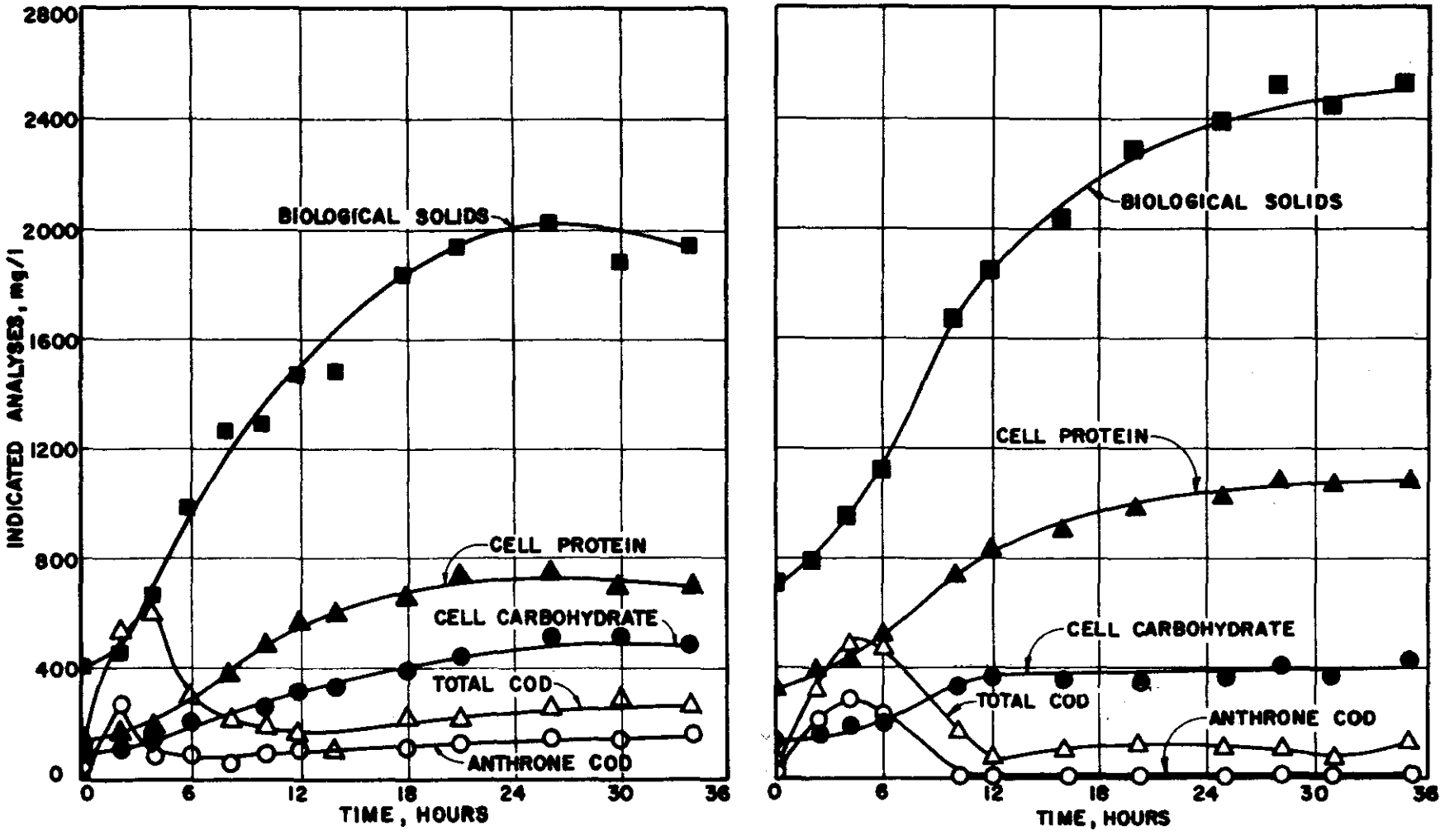

FIGURE 9. RESPONSE TO SHOCK LOAD; S_i 1000→5000 mg/l AT $\bar{t}$ = 12 HOURS;
LEFT: ONCE THROUGH, RIGHT: CELL RECYCLE