STUDIES ON THE RESPONSE OF HETEROGENEOUS POPULATIONS TO VARIOUS TYPES OF SHOCK LOADS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

As communities become larger and industrial development proceeds at an increasing pace, larger quantities of pure water are required. At the same time, this growth in the population and in industrial activities has contributed greatly to the problem of pollution of the aqueous environment, thus tending to limit the useable water supply. The primary concern of industrial management is to produce a maximum profit, and as a consequence, it is not the practice to recover waste materials unless the value of the recovered materials exceeds the cost of recovery. While solutions and suspensions of various materials may be of such concentrations to be considered as strong waste, they are usually too dilute to be amenable to recovery processes, and these effluents are therefore handled as waste materials. While a "waste" material is generally considered as an item to be disposed of as quickly and easily as possible, there is a growing realization that any given industrial plant does not operate entirely independently of another. Polluted water discharged from an upstream plant may affect the cost of operation (production) of downstream plants which use this water. The downstream plant may be a consumer of the upstream plant's product or a supplier of some vital material for the upstream plant. In either case, the economic relationship is disturbed and it is becoming "good business" to exercise

concern over waste material and to act upon this concern by providing treatment of the waste water before discharging it. The concern of industry (as well as municipalities) over the quality of the aqueous environment has not only come about due to self-analysis, but enactment and enforcement of the new federal water pollution control laws and stream standards have played a large role in accelerating concern and action to relieve water pollution. These federal laws are themselves in a sense the result of self-analysis on the part of all people, since they represent the decision of the majority.

The question before industry and municipalities alike with respect to organic matter in waste water is no longer whether to treat the waste but how to treat it. Here economic aspects and reliability of treatment are foremost considerations. It is the author's view that biological treatment is now and will probably remain the most economical means of treatment, and one would not expect any startling developments in means of treatment. However, it is not inconceivable that there will be, through intensive research, new modifications of biological treatment processes and development of ways and means of increasing the rate and efficiency of treatment as well as the reliability of the biological processes.

One of the broad areas in which a great amount of research is needed is the characterization of response of biological systems to changes in environmental (or operational) conditions. Such changes are generally termed "shock loads" (1). They can cause serious disruptions to activated sludge systems. Among the types of shock loads are qualitative organic, quantitative organic, hydraulic, pH, and temperature shocks. Long-term studies on the kinetics and the mechanistic effects

of shock loadings have been undertaken in the Oklahoma State University laboratories; the research included in the present report forms a portion of this overall research effort. In this particular portion of the shock load research it was felt that a variety of shocks should be studied in order to correlate the results with other special studies which had already been conducted or are presently under way in our laboratories pertinent to each type of shock.

In addition, studies are under way on the reliability and efficiency of the so-called "extended aeration" process (no sludge wasting). Studies on the response of this process to quantitative shock loading have already been completed by other researchers in our laboratories, and it was felt that extremely valuable information pertinent to the response to qualitative shocks and to hydraulic shock loadings should be gained while the unit was still operational.

CHAPTER II

LITERATURE REVIEW

Response of Activated Sludge to Shock Loadings

The method of sewage treatment that has become known as the activated sludge process has been widely employed for over half a century. Various researchers have shown the process to be extremely adaptable, and many process modifications have been proposed to meet specific requirements and conditions. During recent years, various modifications of the conventional process have been advanced in attempts to provide a greater degree of purification efficiency. Among these modifications are: tapered aeration (2), step aeration (3), the Kraus process (4), the biosorption process (5), completely mixed activated sludge (6), and the extended aeration or total oxidation process (7). Developments of these modifications have, for the most part, resulted from operational pilot plant and laboratory research studies. Most of the process modifications were devised on the premise that activated sludge processes would operate as plug flow systems and, in general, the design of these plants did not provide for intimate mixing of the waste with the microorganisms in all parts of the aeration tank. During recent years, the geometric design of aeration tanks, as well as the development of new aeration equipment, has undergone sufficient change so that rapid mixing of the incoming waste with the mixed liquor suspended solids is

approached. McKinney (6) has defined "complete mixing" as a basic process in which the incoming wastes are completely mixed with the entire contents of the aeration tank. He felt that the aeration tank can act as a surge tank to level out wide fluctuations in organic loadings, and that the use of the entire mass of the activated sludge to stabilize the organic matter allows better utilization of the air supply. Researchers in the field of basic microbiology have long been interested in the continuous culture of microorganisms, and there has been amassed a considerable amount of theory and experimental data pertaining to continuous culture of microorganisms in systems which can be defined in hydraulic terms as completely mixed. Herbert has reviewed applicable kinetic theory and discussed the various types of reactor systems which can be employed (8).

Gaudy used cells from a completely mixed system to evaluate the effect of qualitative shock loadings under batch conditions, and felt that successful response to qualitative shock loads (changes in the nature of the carbon sources) was dependent on the availability of a readily available nitrogen source and on the physiological condition of the culture (9). He also indicated that acclimation to one substrate may confer acclimation to another substrate. Ramanathan (10), in his study of the kinetic behavior of heterogeneous populations in completely mixed systems observed that the physiological growth parameters (μ_m and k_S) obtained from batch experiments using cells grown in completely mixed reactors were to some extent dependent on the dilution rate. Komolrit (11), working on the effect of qualitative shock loadings in a completely mixed system, indicated that the biochemical response to the shock load was dependent on the dilution rate, the biological solids

concentration, and the sludge activity. He also reported that nitrogen-deficient systems had poor ability to withstand qualitative shock loads.

Krishnan has studied the effect of short term shock loadings under nitrogen-deficient conditions in continuous flow systems (with and without sludge recycle)(12). He indicated that successful response depended upon the dilution rate, availability of an adequate nitrogen source, and sludge recycle. In systems operated without cell feedback, a shock load of nitrogen-deficient waste was characterized by the release of intermediates which were not subsequently utilized. These systems showed a marked decrease in protein content with an accompanying increase in carbohydrate content. Using sludge recirculation, he found that the biochemical composition of the cells did not vary greatly when they were shock loaded under nitrogen-deficient conditions.

Storer (13), working on the kinetic properties of heterogeneous populations in continuous flow units under quantitative shock loading conditions, reported that the transient state caused by increasing the inflowing waste concentration, could not be predicted with accuracy using the Monod equation.

Kincannon (14), in a study on the effect of sodium chloride on activated sludge, reported that completely mixed continuous flow systems operating under steady state conditions could respond satisfactorily to a sodium chloride shock load. He also concluded that the ability of the system to respond successfully to such a shock load was probably dependent upon the predominating species present in the system at the time of the shock, since two independent continuous flow units started from different samples of sewage seed responded differently under the same cultural conditions. He also found that the cell age played an

important role in the response of systems to shock loads of sodium chloride. Young cells were much more severely affected than old cells.

The effect of dilution rate and organic loading on the performance of mixed cultures and activated sludge has been investigated by Cassel, Sulzer, and Lamb (15). They reported that dilution rate can exert a strong selective pressure, thus different species predominate at different dilution rates. This conclusion was based on observation of changes in the color of the mixed liquor, and changes in the number of protozoa found in the mixed liquor. Komolrit studies substrate interaction under shock loading conditions at various dilution rates. He observed that hydraulic loading rate indirectly controlled the manifestation of substrate inhibition by controlling the growth of biological solids in the system (11). George, based on his studies of the biochemical response of continuous flow activated sludge processes to hydraulic shock loads, pH, and temperature shocks, summarized his findings as follows (16):

1. For hydraulic shock loadings in a completely mixed system under constant concentration conditions (incoming waste concentration not changed), an increase in flow rate resulting in a dilution rate greater than 0.25 hr^{-1} caused a deleterious transient response, as indicated by a decrease in biological solids in the effluent. Under constant daily organic loading conditions, a decrease in flow rate resulting in a dilution rate lower than 0.064 hr^{-1} caused a severe transient response. He also indicated that under constant organic loading conditions a change in dilution rate caused a significant change in the steady state yield of cells compared to that observed under constant concentration conditions.

2. In systems initially operating at neutral pH, alkaline shock loads up to pH 7.95 did not cause any severe disruption of purification efficiency. Acid shock loads resulting in reactor pH values of 3.5 or less caused a severe transient response (low biological solids concentration and high effluent COD level).

3. In studies on the effect of temperature shock loads it was found that if the temperature change was not faster than $2^{o}C/hr$, systems operating at detention periods of eight and four hours could recover purification efficiency when shock loaded at temperatures of $47^{o}C$. Large amounts of acetic acid were released as metabolic intermediates and/or endproducts in the system operating at an 8-hour detention time. The acetic acid was subsequently utilized.

Yu (17) studied effects of hydraulic shock loadings in continuous flow activated sludge processes (glycerol was employed as carbon source). The response to a severe increase in dilution rate was a rapid washout of biological solids and high COD concentration in the effluent. The typical response to a severe decrease in dilution rate was somewhat similar to the response to a severe increase in dilution rate. These results were in agreement with George's initial result which was obtained using glucose as carbon source. For shock loadings consisting of decreases in dilution rate, it appeared that the "starvation" conditions imposed on the system caused some cell die-off and lysis. It seems reasonable to postulate that a significant portion of the COD of the effluent consisted of lysis products.

Grady studied carbon-limited and magnesium-limited chemostat systems growing on lysine, and shock-loaded with glucose, fructose, and ribose (18). The results demonstrated that for both carbon and

magnesium-limited reactors, glucose and fructose caused a significant degree of repression of lysine-degrading enzymes.

Another modification of the activated sludge process which can be considered as a completely mixed system is the so-called extended aeration or total oxidation process. The conceptual basis for the process involves two distinct phases of metabolism which occur concurrently in the aerator. In the first phase, a portion of incoming waste is oxidized to provide energy for conversion (synthesis) of the remaining organic material into new cells. In the second phase, "endogenous respiration," the new cells formed are self-oxidized to the final endproduct. This step reduces the quantity of solids to a minimum (ideally, this step eliminates the solids--oxidizes them totally). Such a process could thus minimize the sludge disposal problem, and negate any requirement for anaerobic sludge digestion. A long detention period is generally used to provide time for the endogenous respiration of the excess sludge. Porges, et al. (7) suggested that with extended periods of aeration, the net synthesis of new biological solids could approach zero, and that an activated sludge process could be operated at or near equilibrium solids concentration. However, the results of various investigations on this process have indicated that there is a buildup of biologically inert solids. Forney and Kountz (19) used dry skim milk as a source of organic matter, and indicated that the total oxidation of sludge was not possible. They indicated that approximately 20-25 per cent of the new solids were biologically inert.

As a result of their studies, Washington and Symons (20) felt that inert or inactive volatile solids accumulated at about 10-15 per cent of the ultimate BOD removed. They also reported that the accumulated

inert bio-mass consisted mainly of extracellular polysaccharide. Symons and McKinney also reported that it was not possible to operate an extended aeration batch-fed unit without sludge wasting (21). Extracellular polysaccharides, which they felt were resistant to biodegradation, accumulated in the system during a 35-day experimental period.

Busch and Myrick (22) studied both batch and continuous flow operation of the extended aeration process, and concluded that a portion of the soluble carbon in the waste was channelled into synthesis of inert or inactive microbial cell components which were resistant to biodegradation. Such products accumulated in the system even after 103 days of operation. Ludzack (23) reported that extended aeration treatment resulted in a high escape of solids into the effluent, and that periodic wasting of sludge reduced the suspended solids concentration in the effluent. His results were in agreement with the concept that sludge wasting is required to operate total oxidation systems at an equilibrium level.

Gaudy, et al. (24) in their studies on the operational stability of the extended aeration process concluded that such a process without sludge wasting could operate without continual increase in solids concentration, and they felt that a "total oxidation process" was not inconsistent with sound microbiological concepts pertinent to heterogeneous populations. The results after nearly two years of operation showed that the system was still providing a good substrate removal efficiency, and that the sludge did not accumulate "ad infinitum." There had been periodic cycles of decreasing solids concentration followed by increases in solids concentration. These decreases in solids concentration could not be attributed to solids loss with the effluent,

since all effluent was passed through a Sharples centrifuge, and the solids were returned to the aerator. Thus, when the settleability of the sludge was poor, biological solids were not inadvertently lost with the effluent. Ragthaidee (25) studied an extended aeration activated sludge system under quantitative shock loading conditions (glucose was employed as carbon source). It was found that increasing the feed up to five times (from 500 mg/l glucose to 2500 mg/l glucose) did not affect the biochemical efficiency of the system.

Substrate Interactions in Biological Systems

Investigations in the basic field have shown several mechanisms of metabolic control to be operative in microorganisms. Several investigators studied the effect of glucose on the utilization of other sugars, and reported that glucose caused the cessation of metabolism of the various compounds. The first phenomenon which was observed by Epps and Gale (26), who found that the formation of amino acid deaminases was inhibited by glucose, has been termed the "glucose effect." Monod observed the phenomenon of diphasic growth in medium containing glucose and certain other carbon sources, and postulated that glucose prevented the formation of enzymes essential for degradation of the other carbon sources (27). From these early studies there has been developed a large body of basic information on metabolic controls. The two types of mechanisms which have been generally cited as major controls are "repression" and "feedback inhibition." These mechanisms exert their influence by regulating the flow of metabolites through the metabolic pathways. "Repression" is generally defined as the mechanism which provides control of metabolism of a specific carbon source by decreasing the rate of enzyme formation when such synthesis would result in

overproduction of endproducts as well as temporarily unnecessary enzymes. McFall and Mandelstam (28) have indicated that accumulation of metabolic intermediates could lead to a repression of only those enzymes which would be involved in production of the same intermediates from another carbon source. Therefore they have employed the term "metabolite repression" for this type of control mechanism. The effect is not one which is caused only by glucose. Feedback inhibition (also referred to as "endproduct inhibition") is defined as a control mechanism which inhibits the function or activity of an enzyme already synthesized when the functioning of that enzyme would produce temporarily unnecessary endproducts.

These control mechanisms provide an economical and efficient metabolic regulation for living systems, and they would be expected to apply to a wide variety of microorganisms and might be manifested in natural populations. These effects could have serious ramifications to biological treatment systems under shock loading conditions. They could lead to discontinuities in the kinetics of waste water purification under both qualitative and quantitative shock loading situations. Gaudy provided the first demonstration of the occurrence of sequential substrate removal in heterogeneous (natural) populations (29). He demonstrated diphasic growth for mixed populations of microorganisms; he employed equal amounts of glucose and sorbitol as combined carbon source, and a small inoculum of sorbitol-acclimated cells. He was able to conclude that sorbitol was not utilized until glucose had been metabolized. A break or discontinuous point in the growth curve corresponded to the time of glucose removal. Since a small initial inoculum was employed, this result showed that glucose repressed the synthesis

of sorbitol-degrading enzymes. Later, Gaudy, Komolrit, and Bhatla noted that sequential substrate removal was dependent somewhat upon the age of the cell system (30). Young cell systems exhibited sequential removal of glucose and sorbitol, while concurrent removal of these substrates was observed for old cell systems. The term "young cell" systems was used to describe dispersed cell suspensions grown up into the log phase from small inocula, whereas the term "old cells" was used to identify flocculated systems which settled upon cessation of agitation (typical of activated sludges). Thus it appeared that the complete blockage of removal of one substrate due to the metabolism of another might not be observable in activated sludge systems, wherein starvation conditions (low substrate:cell ratios) existed. It seemed that the maintenance of high biological solids concentration by retention of a high percentage of previously synthesized solids, thus increasing the age of the cell system, might prevent or mask the sequential removal phenomenon. This aspect was deemed of sufficient importance to warrant two separate studies in the bioengineering laboratories. Yu (17) varied average cell age in continuous flow completely mixed activated sludge (once-through systems) by controlling the dilution rate and thereby the average retention time of the cells in the reactor. Glycerol was used as the carbon source, and cells harvested from the system at various dilution rates were employed in batch studies to assess the mode of substrate removal in a glycerol-glucose medium. In his studies there was a tendency toward decreased occurrence of sequential removal for cells harvested from the "chemostat" at lower growth rates (increased age). However, concurrent substrate removal was also observed for cells harvested from faster growing systems. It

was concluded that an increase in the age of the cells did not provide any guarantee that concurrent substrate removal would be exhibited. Heidman (31) operated batch systems in a manner similar to those employed originally by Gaudy, Komolrit, and Bhatla, i.e., wasting one-third of the mixed liquor solids daily. This procedure might be expected to lead to an average age of the cell system of approximately three days. It should be remembered that increasing chronological age (days of operation) does not imply an increasing physiological age. However, this mode of operation did lead to development of the typically flocculated condition of an activated sludge. In a series of eight experiments (conducted over a period of 48 days) in which galactose-acclimated sludge was exposed to glucose-galactose medium, total sequential substrate removal was not observed at any time although in the young cell system (combined unit, Figure 2) there was the most nearly complete manifestation of sequential substrate removal observed in this series of experiments. The second experiment was performed after eight days of operation, and the system was not yet fully flocculated. Comparison of the results of this experiment (combined unit, Figure 3) with those of the previous experiment indicate a greater degree of concurrent removal for the 8-day cell system. The third experiment was performed after fifteen days of operation; the system was in a flocculated condition, and assumedly it was in or approaching a balanced condition with respect to biological solids concentration. Concurrent removal was again observed, and the degree of interference with galactose removal by glucose was approximately the same as for the 8-day experiment. The results on the fifteenth day and on succeeding days were not unlike those of the eighth day, i.e., concurrent removal

was observed, but in all cases the presence of glucose did interfere with galactose removal. Some idea of the relative degree of substrate interference is obtained from Table I in Heidman's thesis. In the last column he has given the ratios of galactose removal rate in combined and in control units for each experiment at various times during each experiment. On the average for young cells (day 1), galactose was removed in the combined unit at approximately two-tenths the rate in the control. For the next experiment (eight days) the ratio was above 0.5, and varied from approximately 0.4 to 0.6 thereafter. Thus, in these studies the rate of galactose removal was retarded twice as much by glucose in the young cell systems as in old cell systems.

Based upon their findings, Gaudy, Komolrit, and Bhatla (30) also suggested that sequential substrate removal could involve not only repression of synthesis of enzymes required to metabolize one of the substrates, but also the blockage of the function or activity of enzymes already synthesized. This suggestion was based on the finding that sequential removal of glucose and sorbitol occurred even when a large inoculum of cells which had been previously acclimated to sorbitol was used as the seeding material. This phenomenon was demonstrated for both heterogeneous populations and a pure culture (a prototrophic strain of Escherichia coli). These workers also reported that the sequential removal of glucose and sorbitol occurred under non-proliferating conditions, and suggested that the mechanism of the blockage which operated in catabolic pathways was similar to the mechanism of feedback inhibition in biosynthetic pathways. Furthermore, Komolrit and Gaudy (32) had conducted studies using a variety of substrate systems under severe shock loading conditions in which one

compound (glucose) was injected into the medium during the period of active removal of another compound to which the cells were acclimated (e.g., sorbitol, dulcitol, mannitol, and glycerol). Upon its addition, glucose metabolism was immediately initiated, and there was a severe blockage of metabolism of the sugar alcohols. These were not again utilized so long as glucose was present above a certain concentration. From these findings, Gaudy's suggestion pertaining to "the existence of a metabolic control mechanism which operates in catabolic pathways in a manner analagous to the mechanism of feedback inhibition which is known to exist in the biosynthetic pathways" seems reasonable. Recently, Zwaig and Lin (33), who used a mutant strain of Escherichia coli which could take up glycerol while actively growing on glucose, have indicated that the blockage of glycerol in their system was, indeed, due to a feedback inhibition mechanism. The first enzyme responsible for the initial degradation of glycerol (glycerol kinase) was blocked and fructose 1,6 diphosphate was found to inhibit the kinase. This work substantiates the conclusions of Gaudy and his co-workers concerning the existence of feedback inhibition in catabolic pathways, and shows that the inhibition can be brought about by metabolic intermediates. Other workers in the pollution control field have verified the findings of Gaudy and his co-workers. Prakasam and Dondero (34) observed sequential substrate removal in a glucose-sorbitol system, using a sorbitol-acclimated sludge. They felt that their results were "in agreement with the original results of Gaudy concerning sequential removal of glucose and sorbitol by sorbitol-acclimated cells." Stumm-Zollinger (35) was much concerned with the heterogeneity of the microbial populations in her attempts to confirm the relevance of Gaudy's findings. Her concern

in this regard has been criticized by Grady (18), and it seems important to point out here that any substrate system, regardless of the initial heterogeneity of the population, enriches for the organisms best suited for that particular system. One growth cycle can change the predominance of various species. It is also important to re-emphasize the fact that pre-acclimation to the substrate being examined for metabolic interference by the presence of another substrate offers the most severe test conditions. Both Stumm-Zollinger and Prakasam and Dondero agreed with Gaudy and his co-workers that increased ageing of the systems (highly flocculated cells), as opposed to young dispersed cells grown up from small inocula, tended to decrease manifestation of sequential substrate removal. This should not, however, be taken as an implication that the control mechanisms are not operative in the flocculated systems unless completely concurrent substrate removal is demonstrated. This aspect has recently been pointed out by Grady (18). Also, in view of Yu's findings which pertain to the age of the cell system as assessed by growth rate in continuous cultures, and Heidman's findings which pertain more to the flocculated nature of the cell system rather than the physiological age, it seems important not to discount substrate interactions and the control mechanisms they set in motion when considering the behavior of activated sludge systems under shock loading conditions. To the contrary, all of the work to date tends to emphasize their importance in heterogeneous or natural microbial populations which are subject to periodic changes in species predominance. The findings of Komolrit and Gaudy (36), and Grady and Gaudy (37), who assessed the effects of metabolic control mechanisms in continuous flow systems under shock loading conditions, offer ample evidence of the

significance and the operation of metabolic control mechanisms in heterogeneous populations. Other research publications pertaining to sequential substrate removal which demonstrate the significance of this consideration are listed in the bibliography (38)(39)(40)(41)(42).

It is apparent from the foregoing review, that most of the research which has been discussed pertaining to delineation of patterns of response of heterogeneous populations to various shock loadings or changes in the environment has been accomplished by Gaudy and his coworkers in the Oklahoma State University bioengineering laboratories. Shock loads to biological treatment processes, particularly to the activated sludge process, have been of concern in the pollution control field for some time but in large measure the knowledge (or lack of it) pertaining to the response has been synthesized from field studies and operational experiences in the field. The long term research effort in the Oklahoma State University bioengineering laboratories represents a major undertaking in which the various shock loads have been applied to chemically-defined systems in known, controlled ways, in a systematic manner. Although much work has been accomplished, the variety of environmental changes which can be examined is large, and much more work seems warranted. Also, when dealing with heterogeneous populations, a very large amount of experimental data is needed in order to discern various trends and effects, since the ecosystem is indeed a complicated one, and such a system is subject to an ever-changing predominance of microbial species. As recently shown by Thabaraj and Gaudy (43), quantitative shock loadings which do not seriously impair the oxygen resources or change the ratio of inorganic and organic nutrients in the system, can cause metabolic responses which precipitate severe changes in

species predominance. This work tends to emphasize the complicated nature of the problem and the need for much more work in order to compile a body of data upon which sound understanding of response (and perhaps prediction and control) may be developed. The work reported in this thesis pertains to a variety of shock loading conditions and, in many cases, the work ties in with and extends past work accomplished in the bioengineering laboratories. In some cases the experiments which were performed provide new avenues of research investigation which lead into work currently under way in these laboratories. This is the case with regard to hydraulic shock loads in multicomponent synthetic waste systems, and the shock loading studies to an extended aeration system. All of the studies reported in this thesis were undertaken in order to gain greater understanding and to enhance useful application of heterogeneous microbial populations in the purification of polluted waters.

Work was undertaken along eight main avenues of approach, and the experimental protocol, results, and discussion sections of this report are subdivided as follows:

1. Response to slug shock loadings.
2. Sequential quantitative shock loadings.
3. Hydraulic shock loading in a multicomponent (three carbon source) substrate system.
4. Hydraulic shock loading in a two-component carbon source system.
5. Quantitative shock loading in a two-component carbon source system.
6. Effect of successive increases in organic loadings.
7. Effect of shock loading to systems operated at high biological

solids concentration.

8. Qualitative and hydraulic shock loadings to extended aeration systems.

CHAPTER III

MATERIALS AND METHODS

A. Description of Apparatus and Standard Synthetic Wastes

1. Experimental Apparatus

The laboratory scale continuous flow activated sludge unit employed in the studies of sections 1 to 6 is shown in Figure 1. The volume of the aeration vessel was 2.5 liters, but the effective volume of mixed liquor under aeration was approximately 2.4 liters, due to the displacement of the mixed liquor by the diffused air. Temperature of the mixed liquor was maintained at $23\pm2^{o}C$. Airflow rate was maintained at 2000 cc/min/l. The inflow of the synthetic waste was regulated by a Milton-Roy Mini-flow pump (Model 4-C-48-R), which was previously calibrated to give the desired flow rate. Suction and delivery lines were made up of tygon tubing with glass junctions. The effluent from the reactor was collected in a flask, and periodically wasted.

Figure 2A is a flow diagram of the continuous flow activated sludge system which was operated for studies conducted at high biological solids concentration, i.e., in the range of 2000 to 2500 mg/l (section 7). The total volume of the system was 9.5 liters (6 liters, aeration chamber, and 3.5 liters, settling chamber). The slide-in baffle divided the reactor into two compartments. Airflow rate was maintained at 2000 cc/min/l; this aeration rate provided a DO (dissolved oxygen)

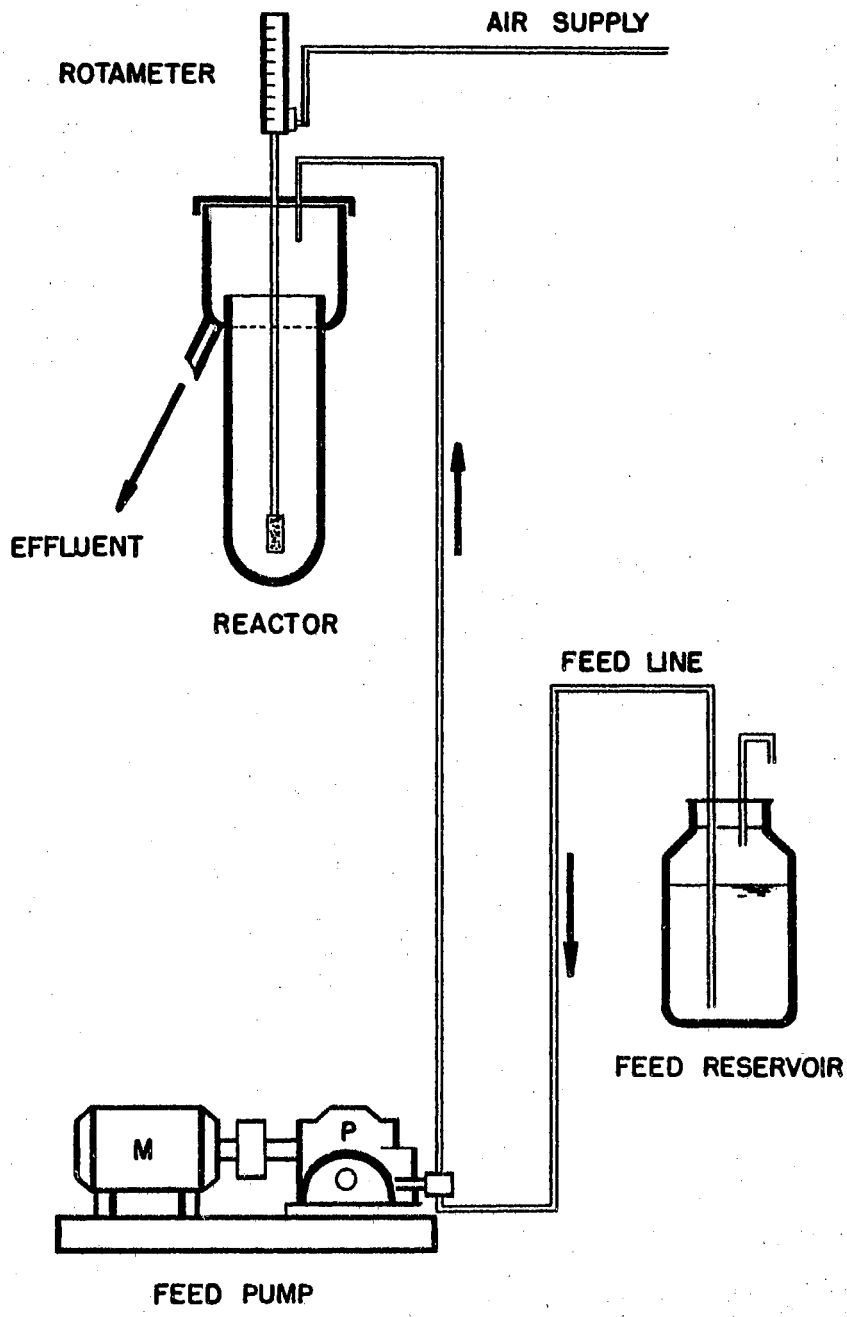

Figure 1 - Schematic representation of the experimental unit.

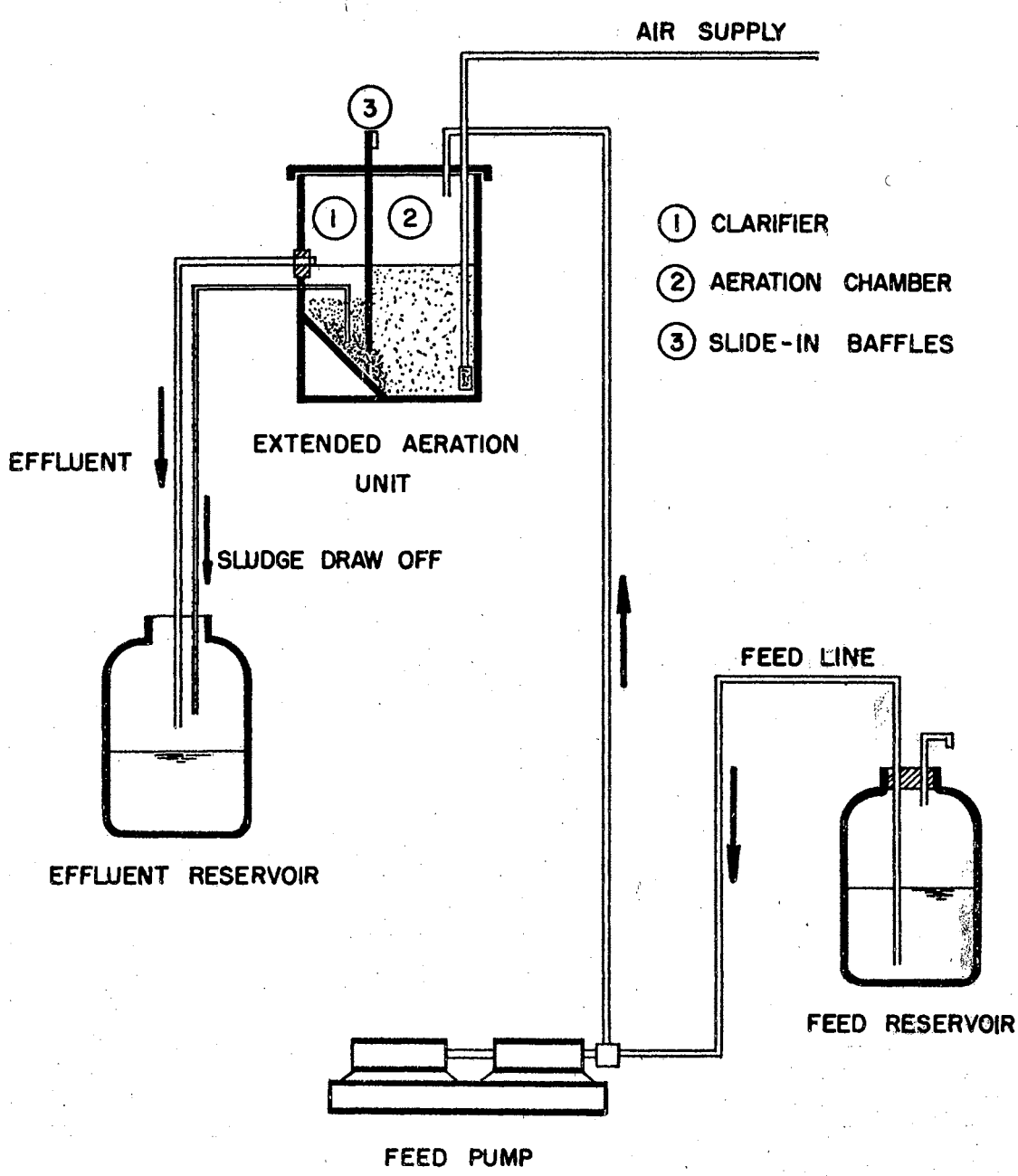

Figure 2A - Schematic flow diagram of continuous flow unit operated at high biological solids concentration.

concentration in the aerator which was always above 6 mg/l and DO concentration in the settling chamber which averaged slightly above 3 mg/l. Compressed air diffused through the mixed liquor provided not only mixing and oxygen supply to the biological solids, but also provided suction to recycle settled sludge from the settling chamber. Temperature of the mixed liquor in the aeration chamber was $23\pm2^{o}C$.

Figure 2B shows a flow diagram for the extended aeration continuous flow activated sludge system. The aeration and settling chamber unit was similar to the one shown in Figure 2A. The mode of operation of the system was such that all mixed liquor was passed from the holding tank through the Sharples centrifuge so that all biological solids were returned to the aerator (section 8). The total volume of the unit was 9.4 liters. The sliding baffle was used to separate the reactor (5.7 liters) and settling chamber (3.7 liters). This corresponds to a mean residence time of seventeen hours in the aeration chamber and seven hours in the settling chamber. Airflow rate and temperature were maintained at the same values as previously mentioned. The extended aeration system employed for these studies was the same as that employed in other studies in the Oklahoma State University bioengineering laboratories.

2. Composition of the Synthetic Wastes for Organic Carbon-limited Systems

The synthetic waste used in the present studies consisted of specific organic carbon sources and essential inorganic salts dissolved in distilled water. The inorganic salts were added to provide a nutritionally balanced synthetic waste. The inorganic salts used per 1000 mg/l of carbon source were: $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$,

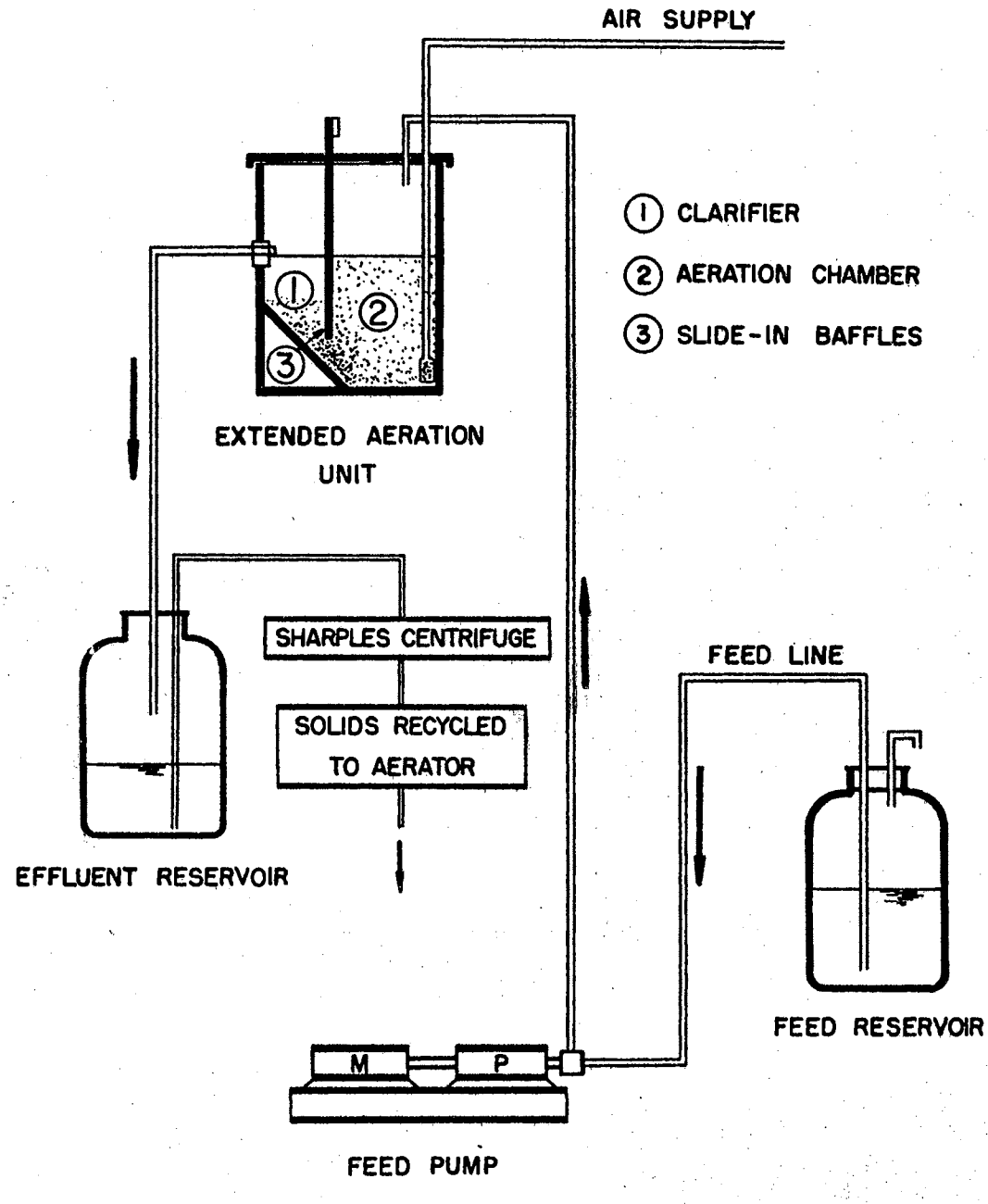

Figure 2B - Schematic flow diagram of continuous flow extended aeration system.

100 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; $CaCl_2$, 7.5 mg/l; tap water, 100 ml/l; 1.0 M potassium phosphate buffer at pH 7.0, 10 ml/l. Whenever the carbon source concentration used was more than 1000 mg/l, the organic salts and buffer were proportionally increased in order to ensure that the inorganic salts and buffer were in excess at all times and that the growth-limiting factor was the organic carbon source. The reason for maintaining the high ratio of COD:N (10:1) was to ensure that the nitrogen source could not become the limiting nutrient.

B. Experimental Protocol - Sections 1 through 6

Before initiating continuous flow operation, two liters of the standard waste containing the desired substrate at 1000 mg/l were inoculated with sewage seed from the primary clarifier of the Stillwater municipal sewage treatment plant, and the aerator (Figure 1) was operated under batch conditions for about twenty-four hours. Then the pump was set at the desired flow rate and the unit was fed continuously for at least four days prior to any particular shock loading experiment. Normally, during this period of "equilibration," samples of the effluent and the reactor liquor were periodically assessed for optical density and biological solids concentration to ensure that complete mixing and a "steady" condition had been attained. Most of the shock loading experiments were conducted only when the system had reached "steady state" except that the shock was applied each twenty-four hours in the study (section 6) on the effect of successive increases in organic loadings.

a) Slug Shock Loadings

Slug doses were administered by injecting a desired substrate solution into the reactor. Prior to the injection of substrate, a portion of the mixed liquor equivalent to the volume of substrate to be added was removed (section 1).

b) Gradual Shock Loadings

Gradual shock loadings (both quantitative and qualitative) were administered by changing the influent feed to a new standard waste of different waste characteristics. The changes in waste characteristics which were used in the present studies were increases in the concentration of the organic carbon sources (sections 2 and 5), or combinations of the previous and new organic carbon sources in various proportions and concentrations (section 6).

During some shock loading experiments, the cells were harvested from the reactor for various batch studies to determine the relationship between the growth rate constant, μ, and substrate concentration. The growth parameters μ_m and k_s were determined by batch experiments. The biological growth was assessed using the membrane filter technique. Substrate concentrations of 50, 100, 200, 300, 500, 800, and 1000 mg/l were used. The growth medium included substrate, inorganic salts, and buffer solution in the same proportions in which they were used in the feed solution for the continuous flow reactor.

c) Hydraulic Shock Loadings

Hydraulic shock loadings were conducted under conditions which may be termed "constant organic concentration" conditions (15). In this type of experiment the substrate concentration in the feed was

maintained constant at all times, and the flow rate (dilution rate) was varied (e.g., 1/24, 1/18, 1/12, 1/8, 1/6, and 1/3 hr^{-1}).

For the batch studies using cells harvested from the continuous flow unit during the hydraulic shock loading experiments in the three-component system (section 3), the experimental batch system consisted of three control reactors, each containing one of the specific carbon sources being studied, and one reactor containing all three carbon sources. The concentration of carbon source in the control system was 400 mg/l, whereas the combined system received 400 mg/l of each carbon source. The inorganic constituents of the medium were the same as those used previously. One hundred ml of seeding population (acclimated seed from the "chemostat") was added; total volume of the mixed liquor in each reactor was 1.5 liters. The batch reactors were aerated vigorously by compressed air admitted to the reactor through porous diffusor stones. Biological solids concentration was determined by the membrane filter technique. Twenty-five ml samples were taken periodically from the reactor, placed in a Servall centrifuge type SS-1, and spun at 12,000 rpm for a period of twenty minutes. The solids were filtered through a Millipore filter (HA, 0.45 μ), and the collected filtrates were used for measurement of total COD; the remaining volume was placed in a freezer for later analysis for specific substrates. Filters were dried for two hours at 103°C and cooled in a desiccator for one hour prior to obtaining the tare weight. Before final weighing, the same drying and cooling procedures were followed. For estimation of intracellular matter by biochemical methods, the wet solids on the filter were scraped off and resuspended in a known volume of distilled water, then preserved in a freezer prior to analysis.

Other batch experiments using cells harvested from the continuous flow unit during studies on hydraulic shock loading in a two-component system (section 4) were performed. In these studies the experimental systems consisted of the glucose control containing 500 mg/l glucose, the glycerol control containing 500 mg/l glycerol, and the combined system containing 500 mg/l of each substrate. The inorganic constituents of the medium were the same as those given previously. The sampling procedures and analytical measurements were the same as those mentioned previously.

Oxygen uptake data were obtained for some experiments using the Warburg respirometer, which was run concurrently with the batch reactors. Before starting aeration in the batch reactors, 40 ml samples of mixed liquor from each reactor were placed in Warburg flasks which contained 1.5 ml of 20 per cent KOH in the center well. The system was maintained at $25^{o}\pm0.5^{o}C$, and an oscillation rate of 100 strokes/min. A ten-minute equilibration period was allowed before the monometers were closed.

Section 7

In studies on the effect of shock loadings at high biological solids concentrations, the system was initiated by aerating a batch of 9.5 liters of the standard waste, inoculated with sewage seed from the primary clarifier of the Stillwater treatment plant, in the unit (Figure 2A) with the baffle wall removed for a period of two days. The baffle was then inserted and the unit was operated under continuous flow conditions at the desired dilution rate for a prolonged period of time in order to obtain a good settling sludge in the clarifier and to

build up the biological solids concentration in the aeration chamber to the range 2000-2500 mg/l. After having operated under these conditions for three weeks, the shock loading experiments (both quantitative and qualitative shock loading) were initiated.

Section 8

In these studies on the effects of qualitative and hydraulic shock loadings to extended aeration systems, the sludge which was previously developed by Ragthaidee (25) was employed. His studies using this laboratory pilot plant were terminated in July, 1968. The author took over the normal operation of the plant and subsequently used this system for qualitative and hydraulic shock load experimentation.

For convenience and clarity of presentation in considering the experimental results obtained under various conditions, the changes in concentration, or composition, of the waste influents and the changes in dilution rate which were employed as shock loadings and the various shock loading conditions will be described in detail in the presentation of each experimental result.

C. Analytical Techniques

1. Biological Solids Determination

The biological solids concentration was determined by the membrane filter technique as outlined in "Standard Methods for the Examination of Water and Waste Water" (44).

2. Chemical Oxygen Demand (COD)

The total COD was measured in accordance with the procedure given in "Standard Methods" (44).

3. Anthrone Test

The carbohydrate content of the filtrate or of the solids suspension was measured by the anthrone method as recommended by Gaudy (45). In this method the concentrated sulfuric acid catalyzes the hydrolysis of carbohydrate and dehydration to furfural or hydroxymethyl furfural, which later condenses with anthrone to give a colored product (46).

4. Periodate Test

Sorbitol and glycerol in the filtrate were determined by the periodate oxidation method of Neish (47) using the recommended modifications of Gaudy, et al. (48).

5. Glucostat Test

In some cases, the glucose concentrations were measured by the enzymatic Glucostat test. The test was performed in accordance with the manufacturer's specification (Method I-a) (49).

6. Galactostat Test

Galactose concentrations of the filtrates were estimated using the Galactostat test which is an enzymatic determination for galactose. The procedure used was in accordance with that given by Worthington Biochemical Corporation (50).

7. Biuret Test

The protein content of the homogenized solids suspension was estimated by the Biuret procedure as outlined by Gaudy (45).

8. Dissolved Oxygen Determination

Dissolved oxygen concentrations in the experimental reactors were estimated by the electronic technique using a galvanic cell oxygen

analyzer (Precision Scientific Co.) which has a sensitivity of ±0.1 mg/l in the temperature range of 5^{o} to 35^{o} C.

CHAPTER IV

RESULTS

1. Response to Slug Shock Loadings

The system responses due to "immediate" sorbitol shock loadings are shown in Figures 3, 4, 5, 6, and 7. In these figures, as in all figures in this thesis showing response in continuous flow systems, the data to the left of the zero line indicate the prevailing steady state conditions prior to the initiation of the shock, and the data to the right of the line show the post-shock conditions. Negative values of time are used for pre-shock, and positive values for post shock conditions. The shock load was imposed on the system by slug dosing, i.e., by injecting a concentrated solution of sorbitol into the completely mixed activated sludge reactor while continuously feeding 1000 mg/l glucose at a dilution rate of 1/8 hr^{-1}. In the experiment shown in Figure 3, the system had been operating on 1000 mg/l glucose for about four days prior to the shock. Concentrated sorbitol solution was injected into the reactor in sufficient quantity to bring the inital sorbitol concentration to 500 mg/l, while the influent feed was maintained constant at 1000 mg/l glucose. It can be seen that sorbitol was removed within six hours, and there did not appear to be any buildup of metabolic intermediates and/or endproducts. This time period also corresponds to a period of increase in the biological solids concentration. The rise in solids and the

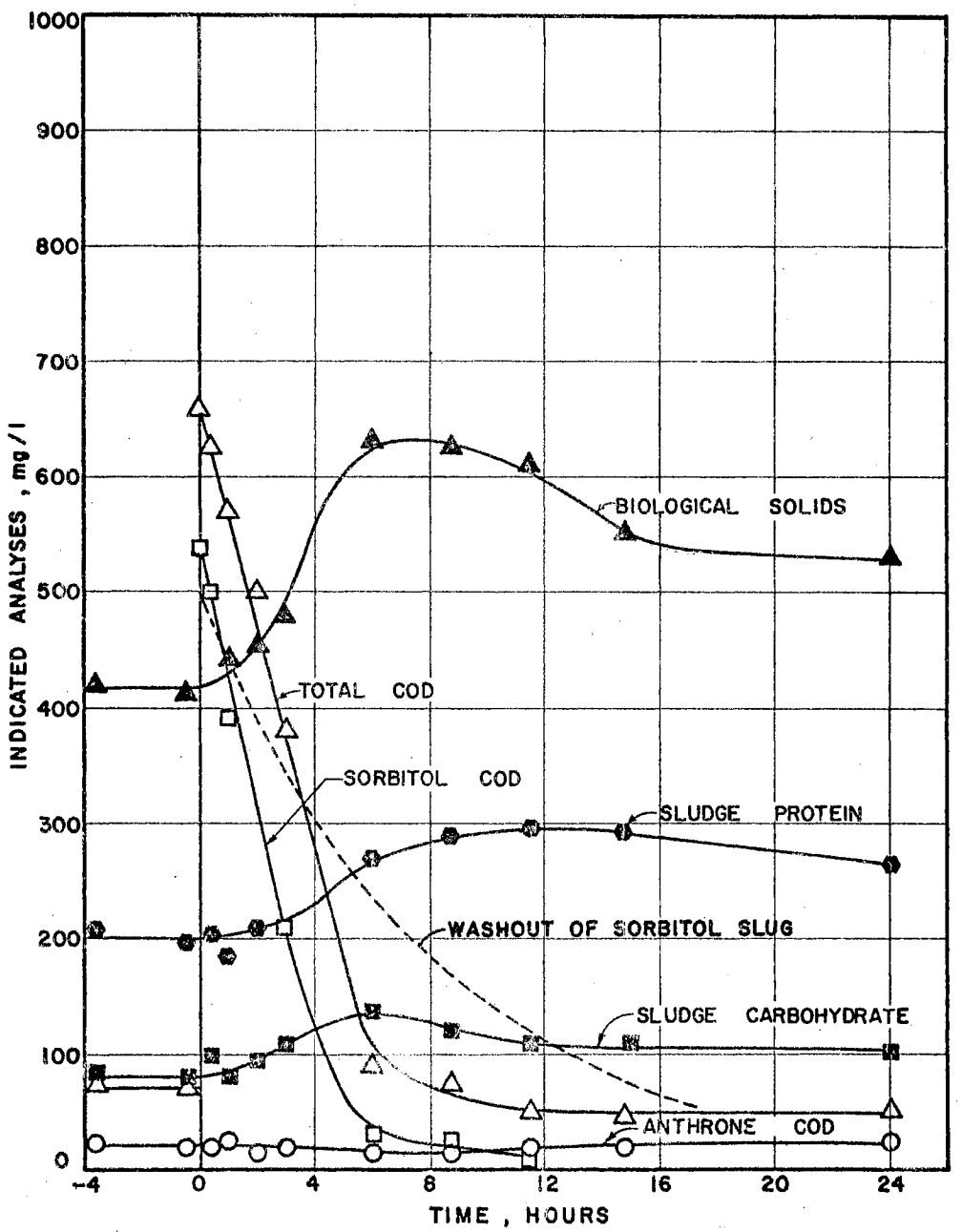

Figure 3 - Biological response to a slug dose of 500 mg/l sorbitol in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l glucose.

accompanying increase in protein and carbohydrate content of the sludge provides indication that the added sorbitol was readily metabolized by the glucose-acclimated sludge. When sorbitol was exhausted, the biological solids concentration dropped to a new "steady state" value of 530 mg/l, which was somewhat higher than the previous steady state value. The dotted curves show the sorbitol concentration which would have been observed if the shock compound had not been taken up by the sludge.

The results shown in Figure 4 were obtained when the influent waste was maintained at 1000 mg/l glucose and enough sorbitol was added to bring its initial concentration in the reactor to 1000 mg/l. It can be seen that most of the sorbitol was removed within four hours. This time period also corresponds to a period of rapid decrease in total COD concentration. After this time the total COD concentration dropped slowly to the same level as before the shock loading (at approximately sixteen hours). The solids level fluctuated widely as a result of this shock. The application of the shock load caused some changes in microbial predominance in the system, as evidenced by changes in color of the mixed liquor. There was very little change in ability of the sludge to metabolize glucose, as indicated by the anthrone curve. The sorbitol COD and total COD removal curves were not parallel after four hours. There was a considerable amount of metabolic intermediates and/or end-products in the medium in comparison with the result shown in Figure 3. The fluctuation in biological solids concentration was accompanied by fluctuation in the protein content of the sludge without much change in carbohydrate concentration.

When an immediate sorbitol shock loading of 1500 mg/l was applied, the results shown in Figure 5 were obtained. There was a wide

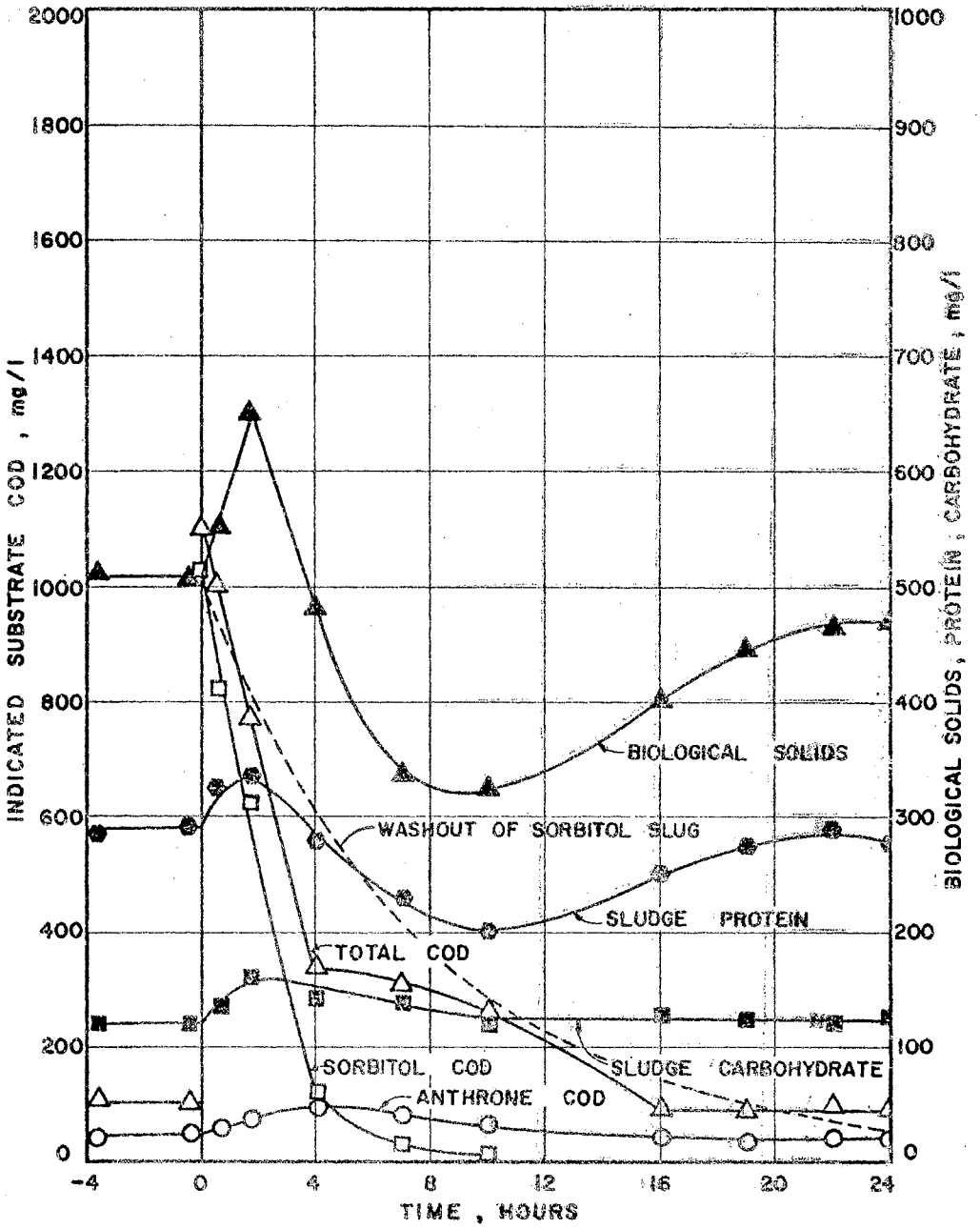

Figure 4 - Biological response to a slug dose of 1000 mg/l sorbitol in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l glucose.

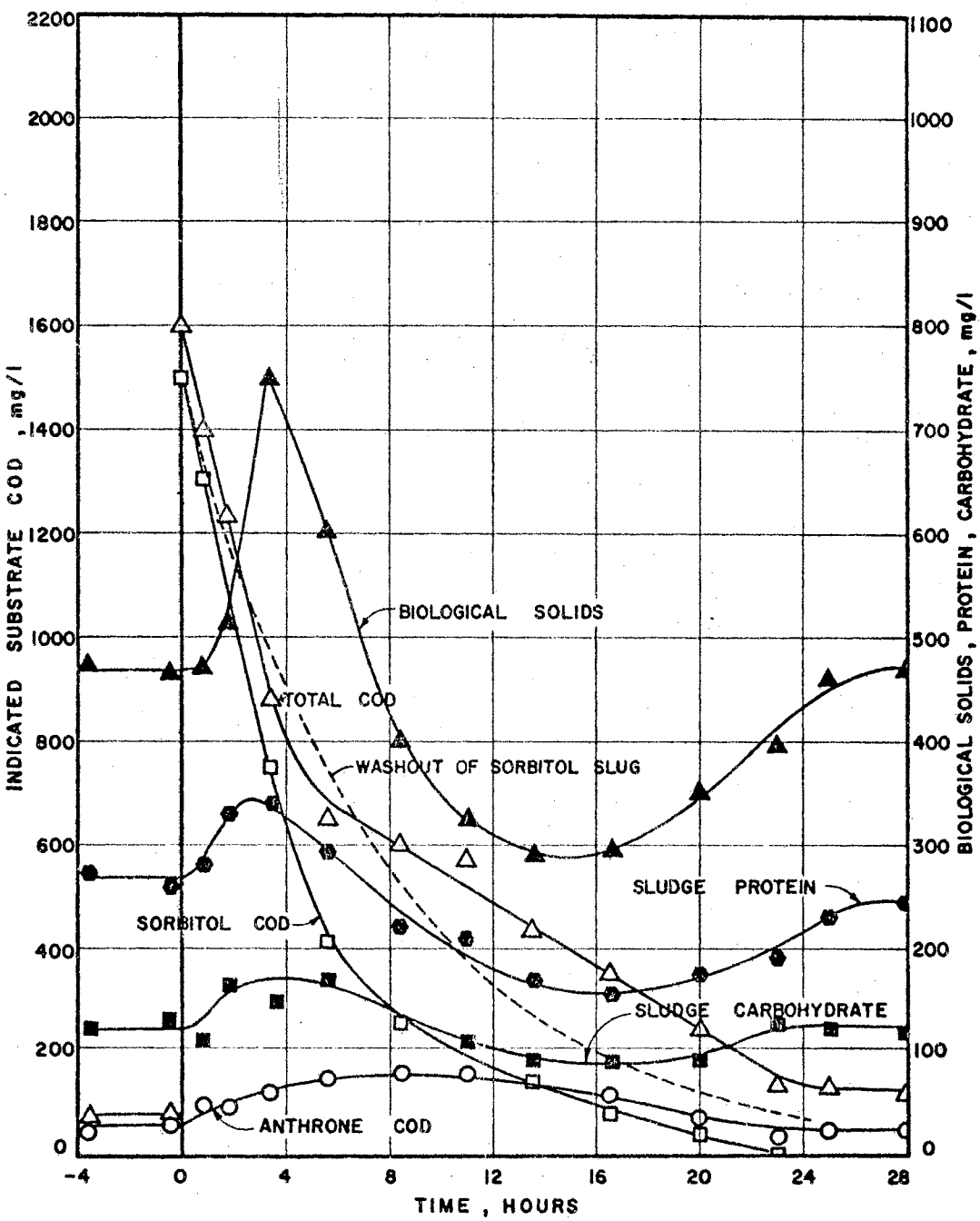

Figure 5 - Biological response to a slug dose of 1500 mg/l sorbitol in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l glucose.

fluctuation in the solids level as a result of the shock loading. It can also be seen that there was a considerable release of intermediates as evidenced by the difference between total COD and sorbitol COD. During this shock loading there were gross changes in microbial predominance, as evidenced by changes in the color of the mixed liquor. This response was similar to that for the previous experiment. It is also apparent that this shock caused a significant increase in glucose (anthrone COD) concentration in the effluent. It is seen that the total COD curve lies above the theoretical washout curve for the sorbitol slug dose attesting to the severity of the metabolic disruption caused by the shock.

The response to a slug shock loading of 2000 mg/l sorbitol is shown in Figure 6. It can be seen that total COD concentration was decreased to a fairly steady level within fourteen hours. There was essentially no change in glucose level in the effluent. The total COD curve lies below the sorbitol washout curve. The response to this shock may be adjudged more successful than the response to the lower level shocks (Figures 4 and 5). In this experiment there was no evidence for gross changes in microbial predominance as compared to the experiments shown in Figures 4 and 5.

Figure 7 shows the shock load response when the slug dose of sorbitol was increased to 2500 mg/l. As with the 2000 mg/l shock, the response to the higher shock level may be adjudged to be a successful one. Sorbitol was metabolized by the glucose-acclimated sludge more rapidly than its removal would have occurred by washout alone. The metabolism of the sorbitol had no apparent deleterious effect on glucose removal as evidenced by the steadiness of the anthrone COD curve.

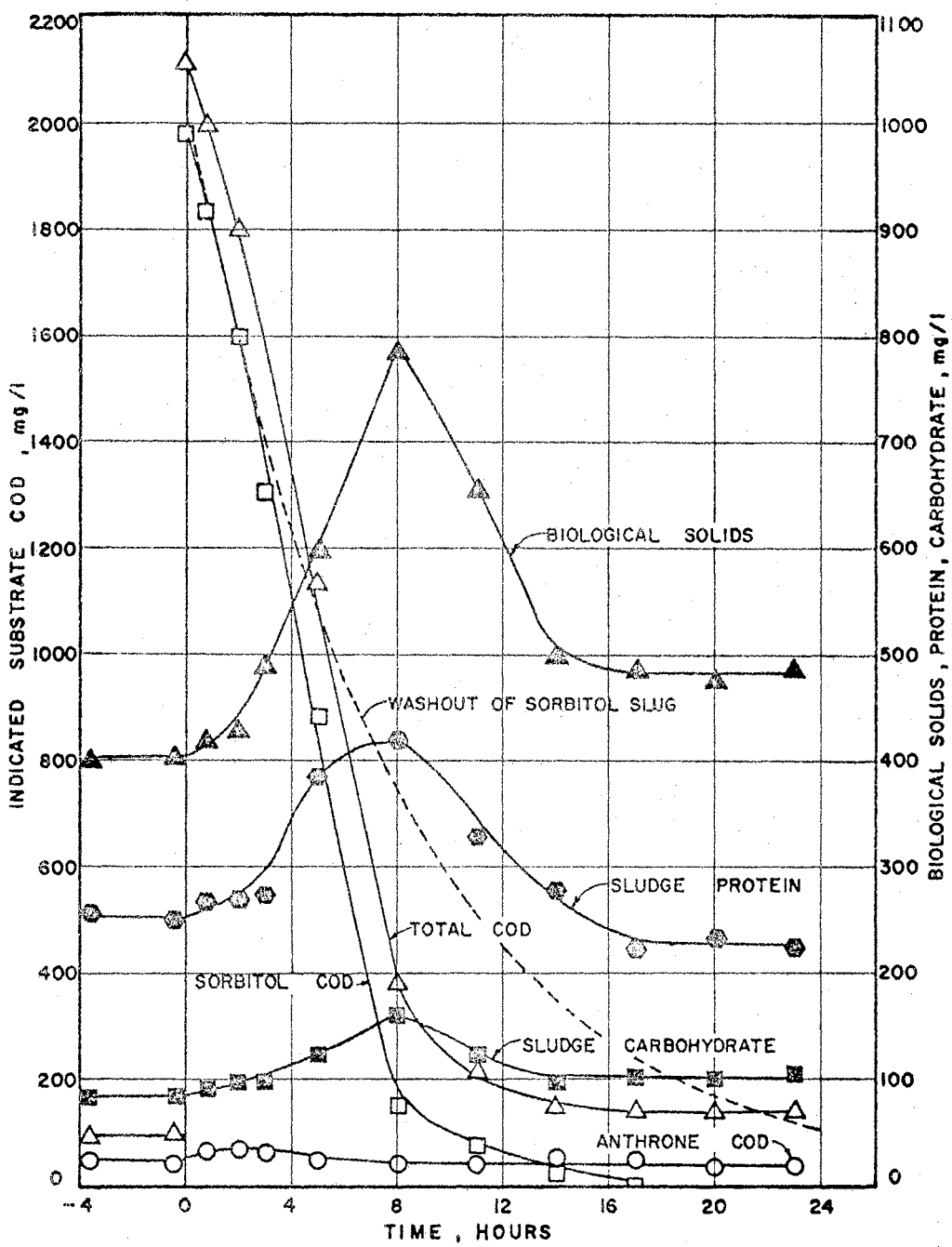

Figure 6 - Biological response to a slug dose of 2000 mg/l sorbitol in a "steady state" continuous flow unit operating at an 8-hour detention time with continuous feeding at 1000 mg/l glucose.

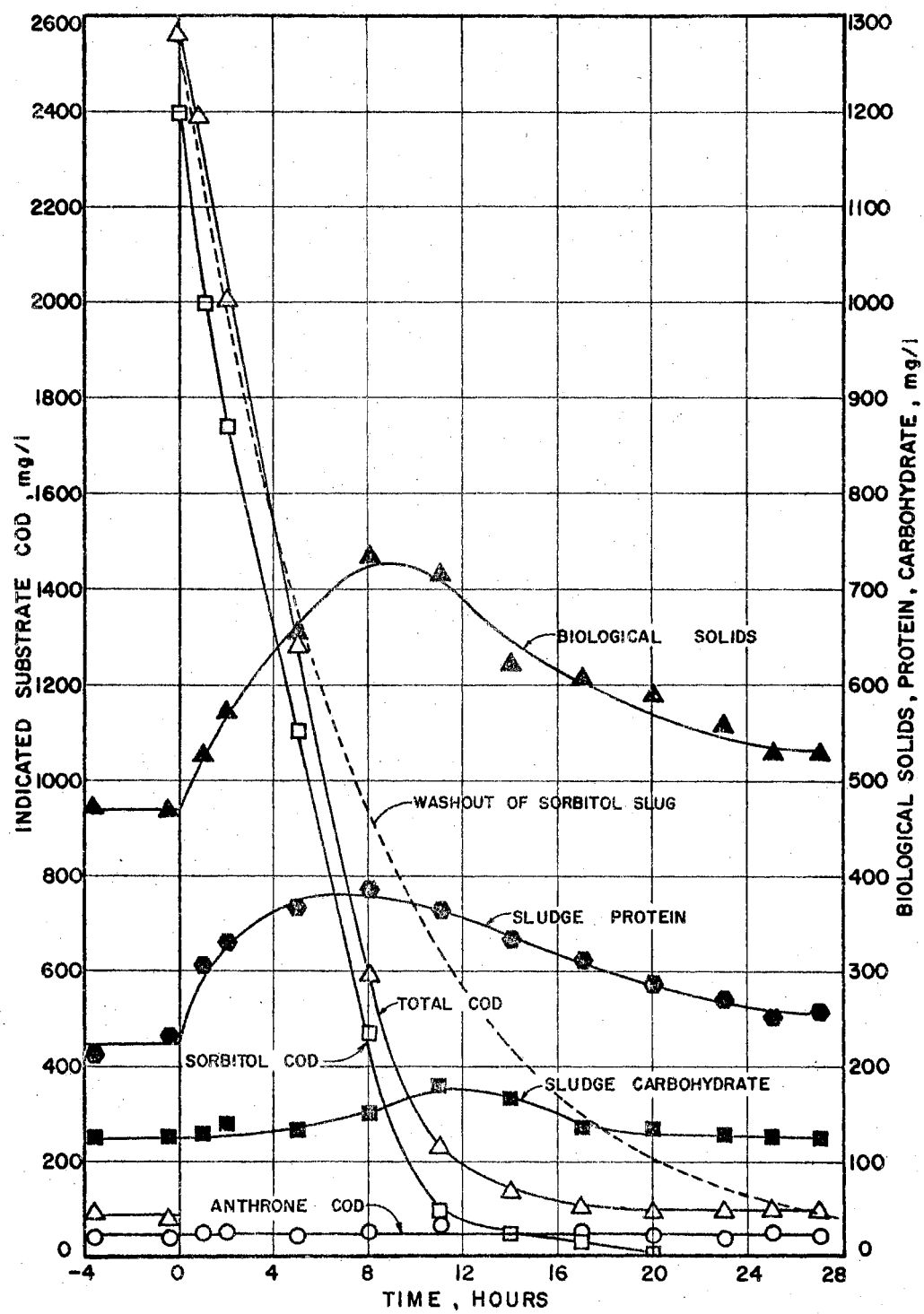

Figure 7 - Biological response to a slug dose of 2500 mg/l sorbitol in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l glucose.

A similar series of experiments was conducted in which the reverse loading conditions were studied. Systems were operated at steady state ($D = 1/8\ hr^{-1}$) with sorbitol at 1000 mg/l as feed substrate, and slug doses of glucose were applied. The responses to these shock loadings are shown in Figures 8, 9, 10, 11, and 12. Figure 8 shows the response when glucose was rapidly injected into the continuous flow unit in sufficient amount to bring its concentration to 500 mg/l, while the influent feed concentration was maintained at 1000 mg/l sorbitol. It can be seen that the total COD concentration in the effluent was returned to a steady level after eight hours. Glucose was readily removed by the sorbitol-acclimated sludge. There was a small but significant increase in sorbitol COD level during the first two hours, providing some evidence that glucose was metabolized at the expense of sorbitol.

When the influent feed was maintained at 1000 mg/l sorbitol and glucose was injected into the reactor to bring its concentration to 1000 mg/l (Figure 9), it can be seen that the rate of increase in biological solids in the system lagged during the first few hours; however, glucose was rapidly removed. There was a larger increase in the sorbitol COD level than was observed at the previous shock concentration; two hours after the shock the sorbitol COD concentration began to decline. By the tenth hour, the previous steady state level was attained. Again it can be seen that an increase in solids concentration was accompanied by an increase in protein synthesis and carbohydrate synthesis, indicating that the additional carbon source was used primarily for growth of the cells, not oxidatively assimilated as storage products.

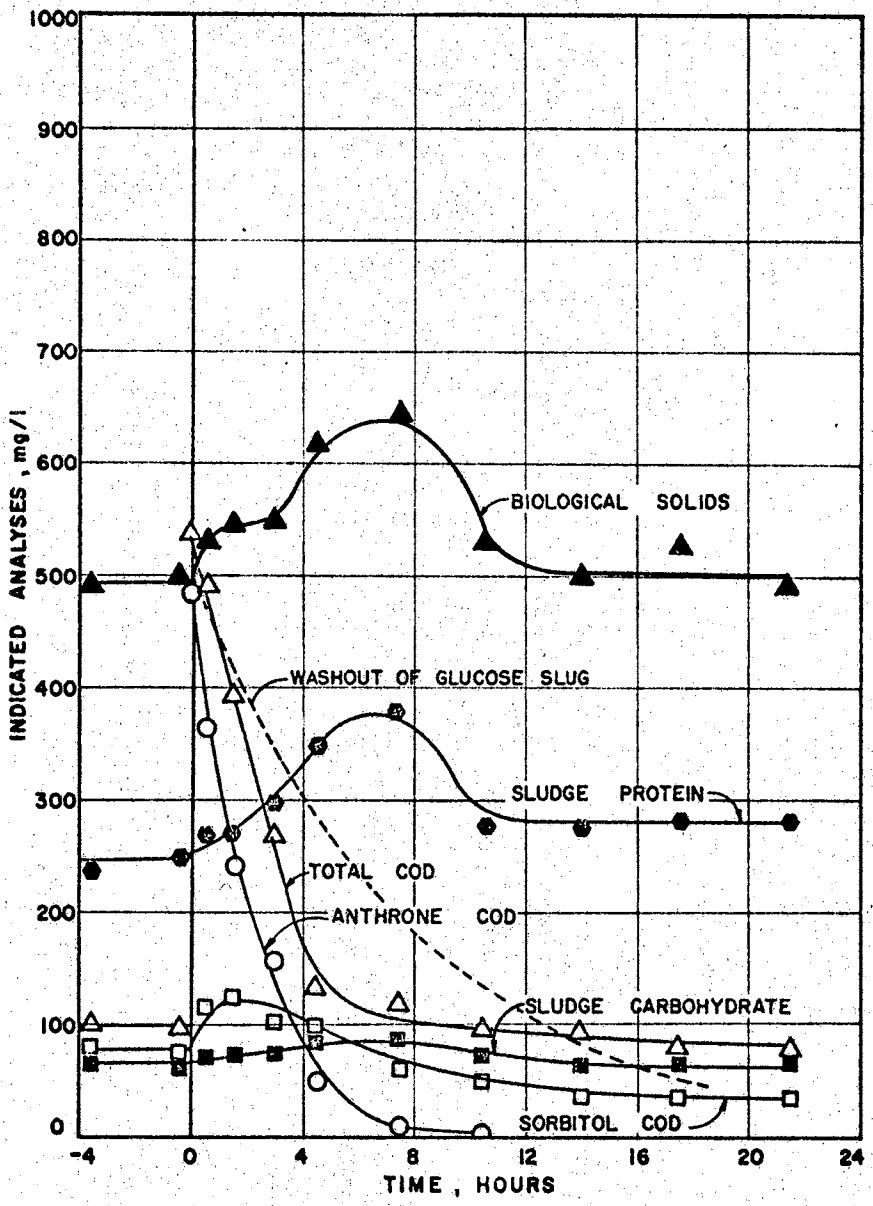

Figure 8 - Biological response to a slug dose of 500 mg/l glucose in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l sorbitol.

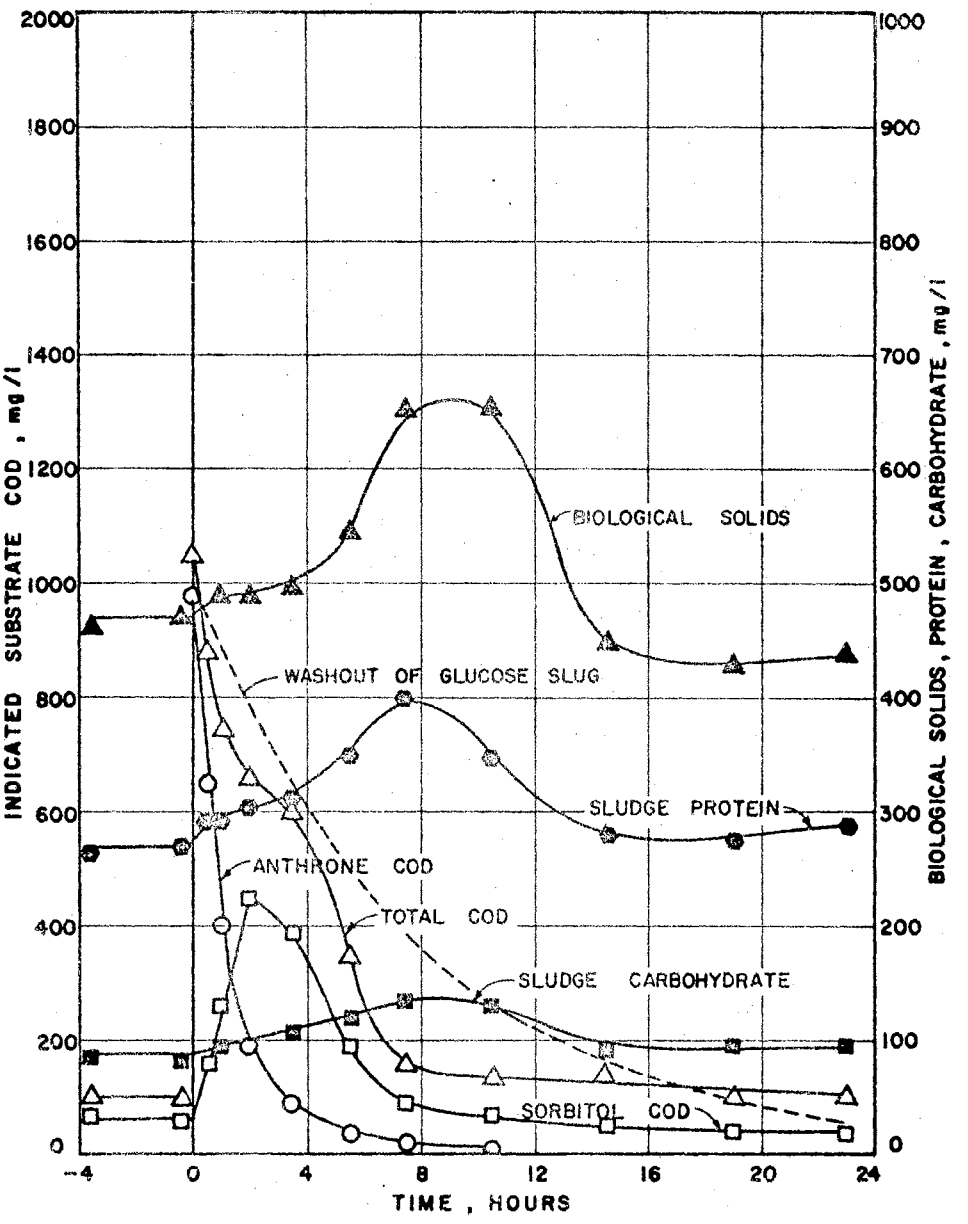

Figure 9 - Biological response to a slug dose of 1000 mg/l glucose in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l sorbitol.

The response to a slug shock loading of 1500 mg/l glucose is shown in Figure 10. It can be seen that the glucose slug dose was removed by the sorbitol-acclimated sludge within six hours. It is apparent that the disruption of sorbitol removal was greater than for the previous shock level (Figure 9). After about fifteen hours, however, the system recovered and both the total COD and sorbitol COD in the effluent were again reduced to the low level which existed before the shock loading was applied. As the severity of the shock was increased (compare Figures 8, 9, and 10), the diphasic nature of the biological solids production and COD removal became more pronounced. The results indicate that the glucose was preferentially metabolized and the extent to which sorbitol was utilized and its rate of utilization was dependent on the amount of glucose present in the system. Again it can be seen that protein synthesis was considerably greater than carbohydrate production.

Figure 11 shows the response when glucose was rapidly added to the system at a concentration of 2000 mg/l. Most of the glucose was removed within eight hours. There was very little increase in the solids concentration during this time, and the sorbitol COD concentration rose within three hours to approximately the same level as it had for the previous shock.

Figure 12 shows the shock load response to a slug dose of 2500 mg/l glucose. It can be seen that the total COD attained a steady state value (approximately 150 mg/l) nineteen hours after the shock. There was a rapid increase in the solids concentration during the first two hours; thereafter the solids concentration levelled off to 610 mg/l at the end of twelve hours; then there was a fairly rapid rise (to 690

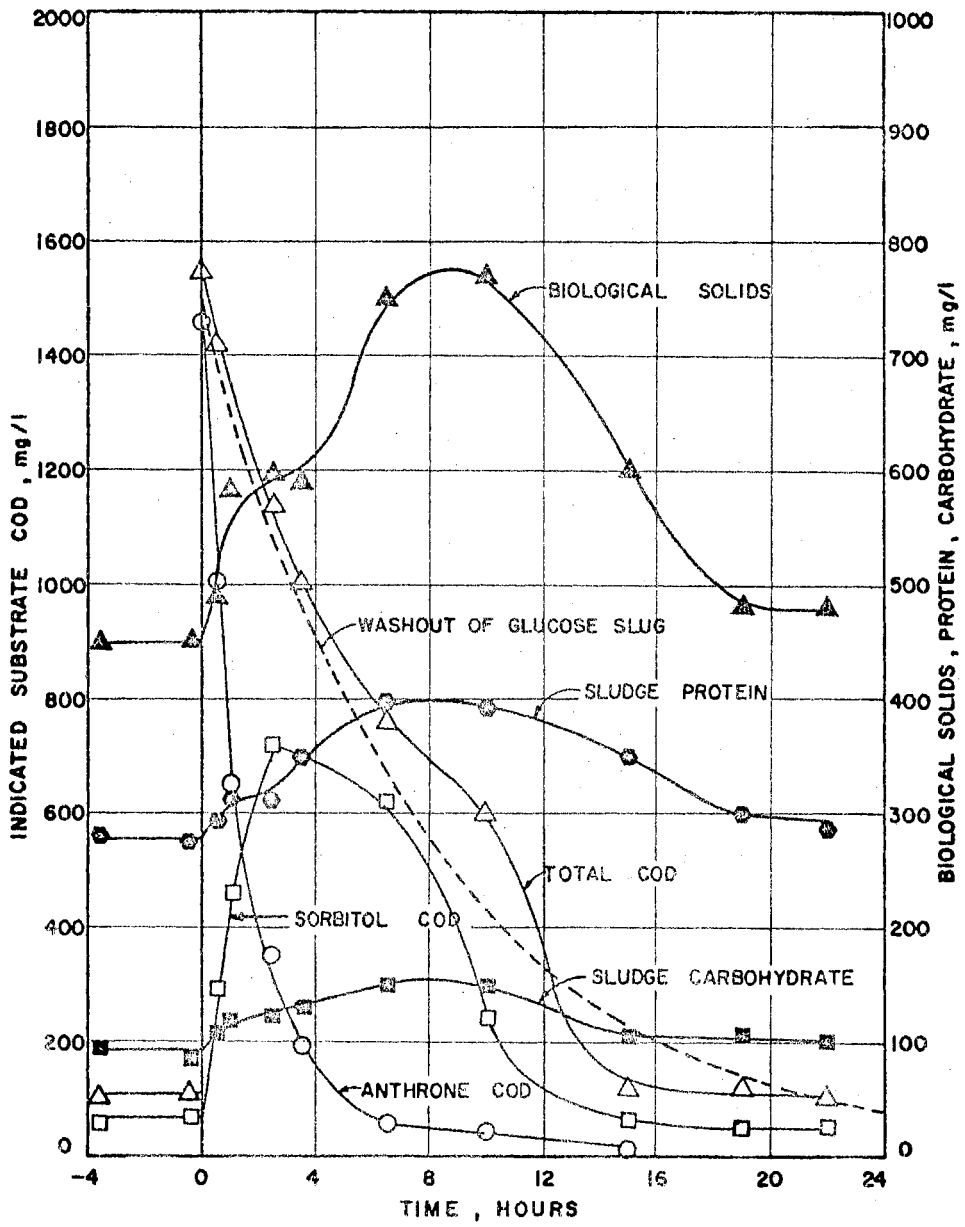

Figure 10 - Biological response to a slug dose of 1500 mg/l glucose at a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l sorbitol.

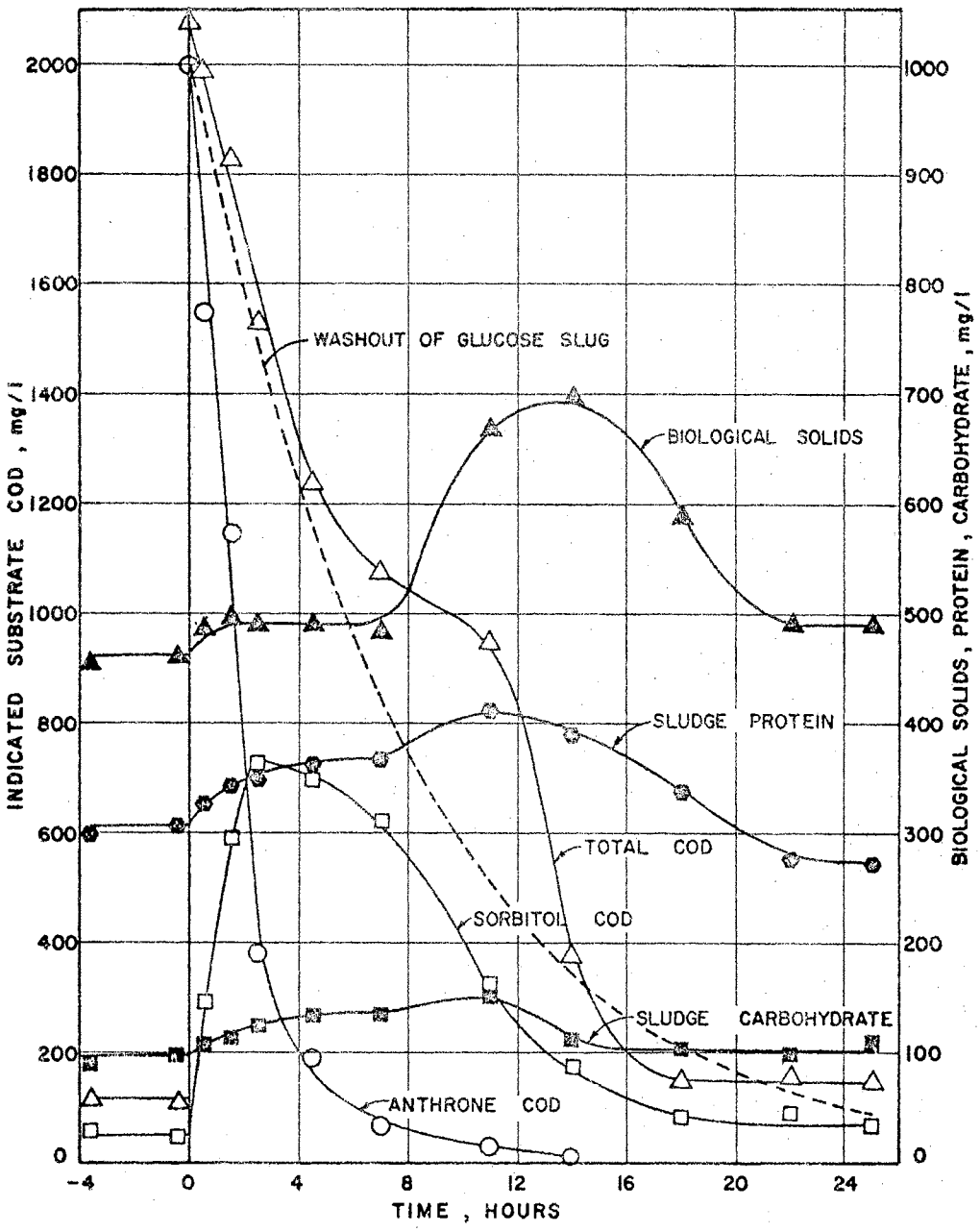

Figure 11 - Biological response to a slug dose of 2000 mg/l glucose in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l sorbitol.

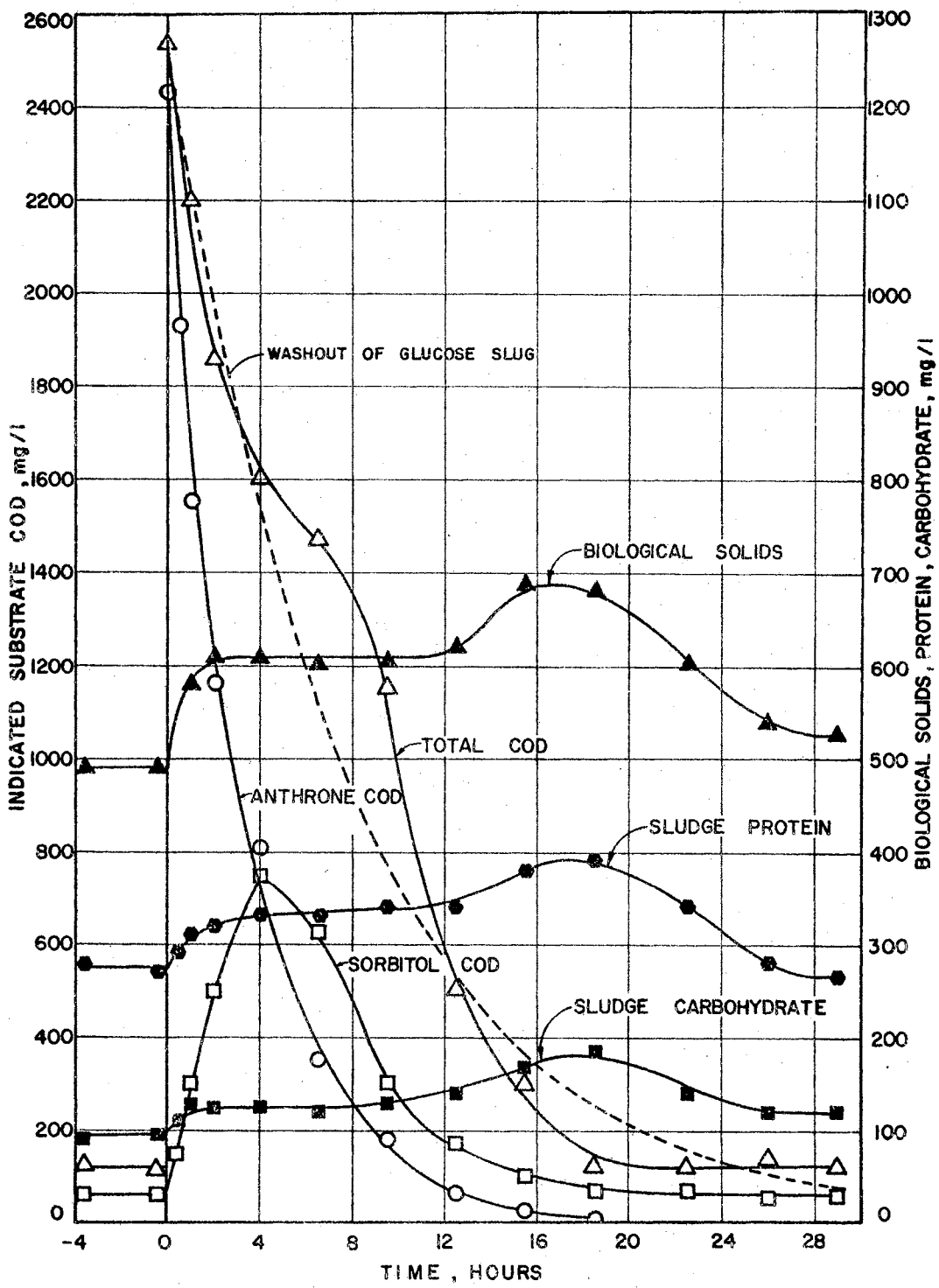

Figure 12 - Biological response to a slug dose of 2500 mg/l glucose in a "steady state" continuous activated sludge unit operating at an 8-hour detention time with continuous feeding of 1000 mg/l sorbitol.

mg/l) during a second phase of COD removal. Again it can be seen that glucose was preferentially metabolized by sorbitol-acclimated sludge, and the extent to which sorbitol COD accumulated in the medium was approximately the same as it was at the 1500 to 2000 mg/l shock levels (compare Figures 10, 11, and 12).

2. Sequential Quantitative Shock Loadings

a) Response to Sequential Stepwise Increases in Sorbitol

The effect of sequential stepwise increases in sorbitol feed concentration, i.e., a series of changes in feed concentration from 1000 mg/l to 1500 mg/l, from 1500 mg/l to 2000 mg/l, from 2000 mg/l to 3000 mg/l, and from 300 mg/l to 4000 mg/l, was investigated. Throughout this series of experiments the reactor was operated at a detention time of eight hours.

Figure 13 shows the response when the influent waste concentration was increased from 1000 mg/l to 1500 mg/l sorbitol. It can be seen that there was no change in the performance of the system as indicated by the total COD and sorbitol COD removal curves. The residual total COD was approximately 90 mg/l, and the sorbitol COD was 50 mg/l throughout the transition period and after attainment of the new steady state condition.

The feed concentration was then increased to 2000 mg/l, and the response is shown in Figure 14. The data plotted at -eighteen hours are the same as those shown at 110 hours in the previous figure. It can be seen that there was a slight increase in the total COD and sorbitol COD as the system approached the new steady state; however, this transition did not result in disruption of COD removal or leakage

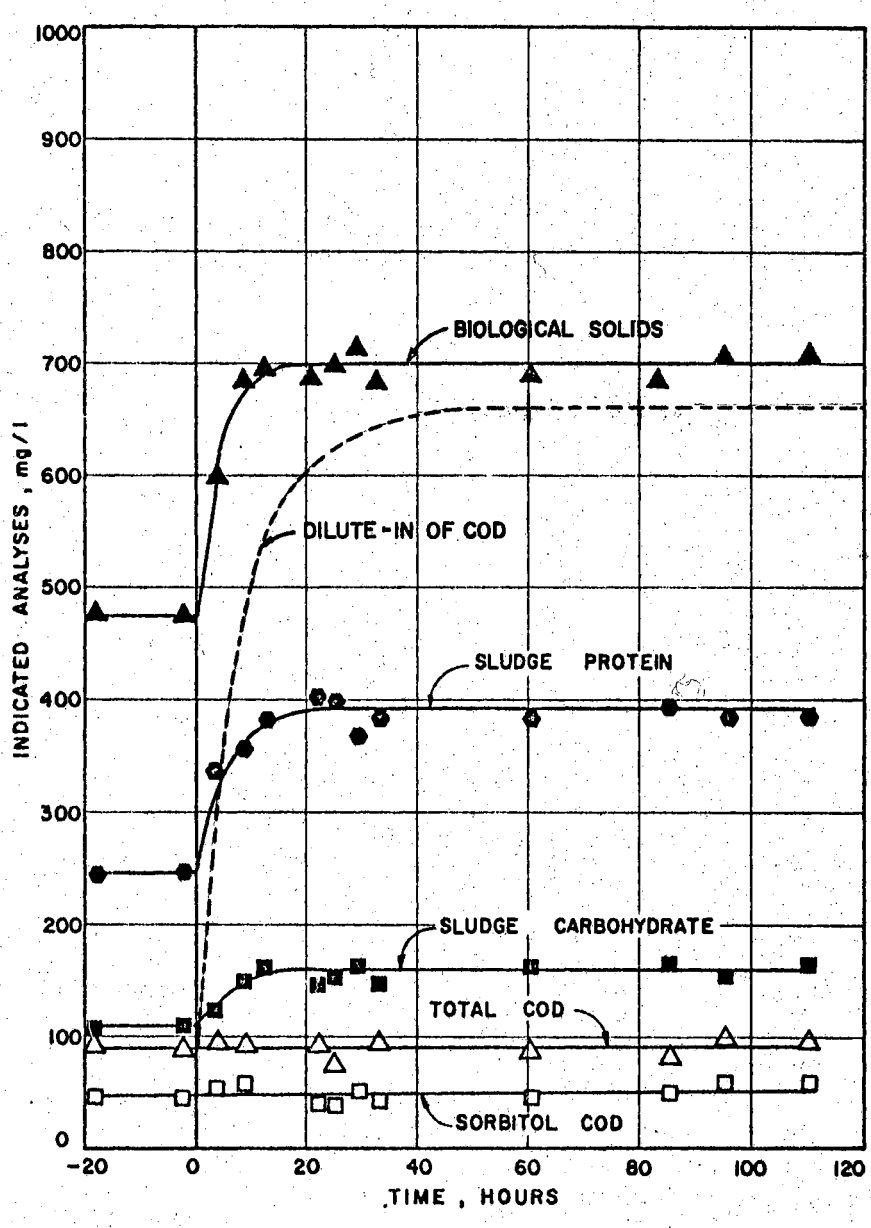

Figure 13 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 1000 mg/l to 1500 mg/l sorbitol.

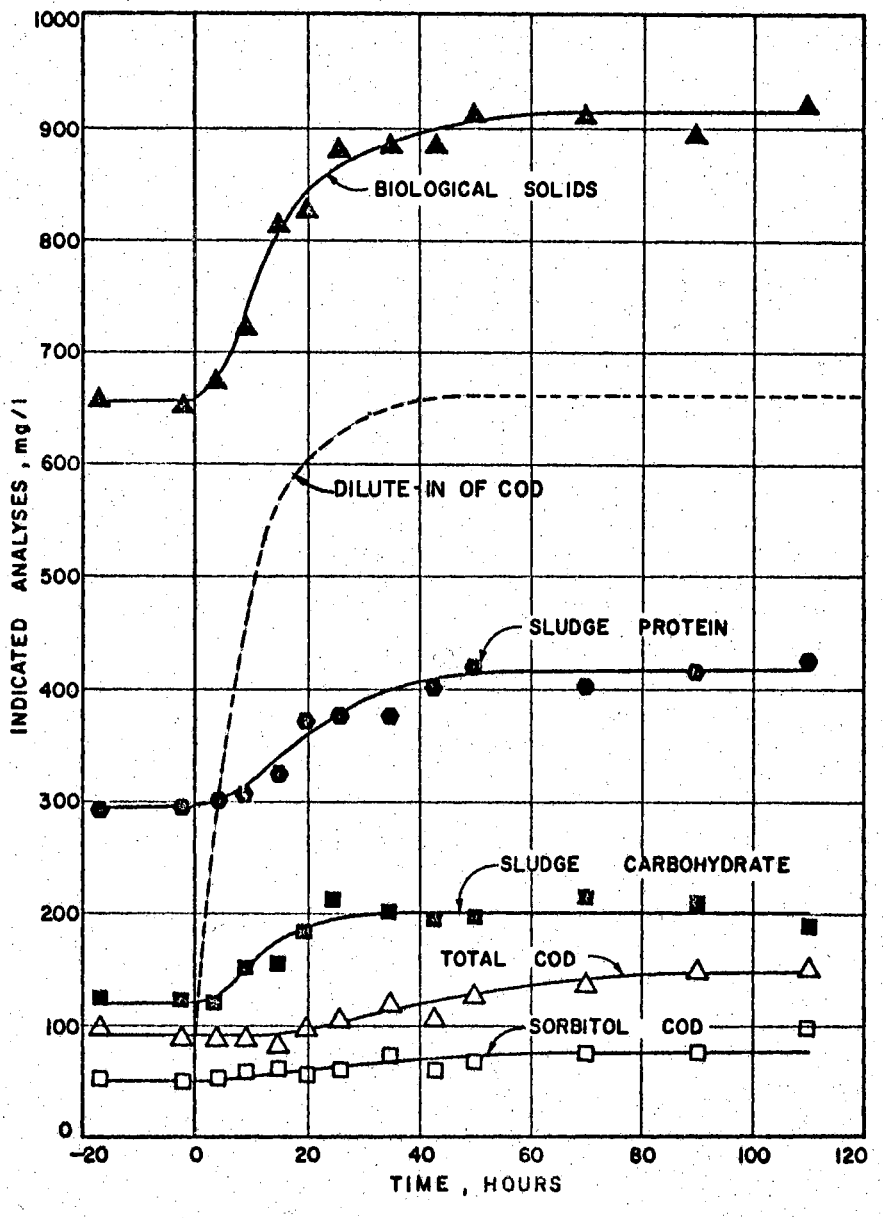

Figure 14 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 1500 mg/l to 2000 mg/l sorbitol.

of COD to an extent greater than that which existed at the new steady state level. Solids concentration increased rapidly enough to prevent a severe transient leakage of COD. The results indicate that the system was capable of handling the applied step increase in feed concentration.

The feed concentration was then increased to 3000 mg/l sorbitol, and the system responded as shown in Figure 15. This fifty per cent increase in feed concentration did not cause a disruptive transient leakage of COD. As with the previous shock, the increase in biological solids concentration occurred with sufficient speed to provide a smooth transition in effluent COD from old to new steady state level. About fifty hours were required before the total COD of the effluent reached a new steady state value.

Figure 16 shows the biochemical response when the feed concentration was changed from 3000 mg/l to 4000 mg/l sorbitol. There was only a slight increase in the total COD during the transient state rise in biological solids concentration. Twenty-four hours were required before the total COD of the effluent reached a new steady state value of 220 mg/l. This value was higher than the effluent COD concentration (180 mg/l) observed at the 3000 mg/l sorbitol feed level. There was also a slight increase in the sorbitol COD over the previous steady state value. It can also be seen that there was a peak or "overshoot" in the biological solids (growth) response. This was not due to synthesis and oxidation of non-nitrogenous "storage" products, since there was a parallel response in the sludge protein curve. For all experiments in this series of shock loadings, it is apparent that the response of the biological solids to the increase in substrate concentration was very

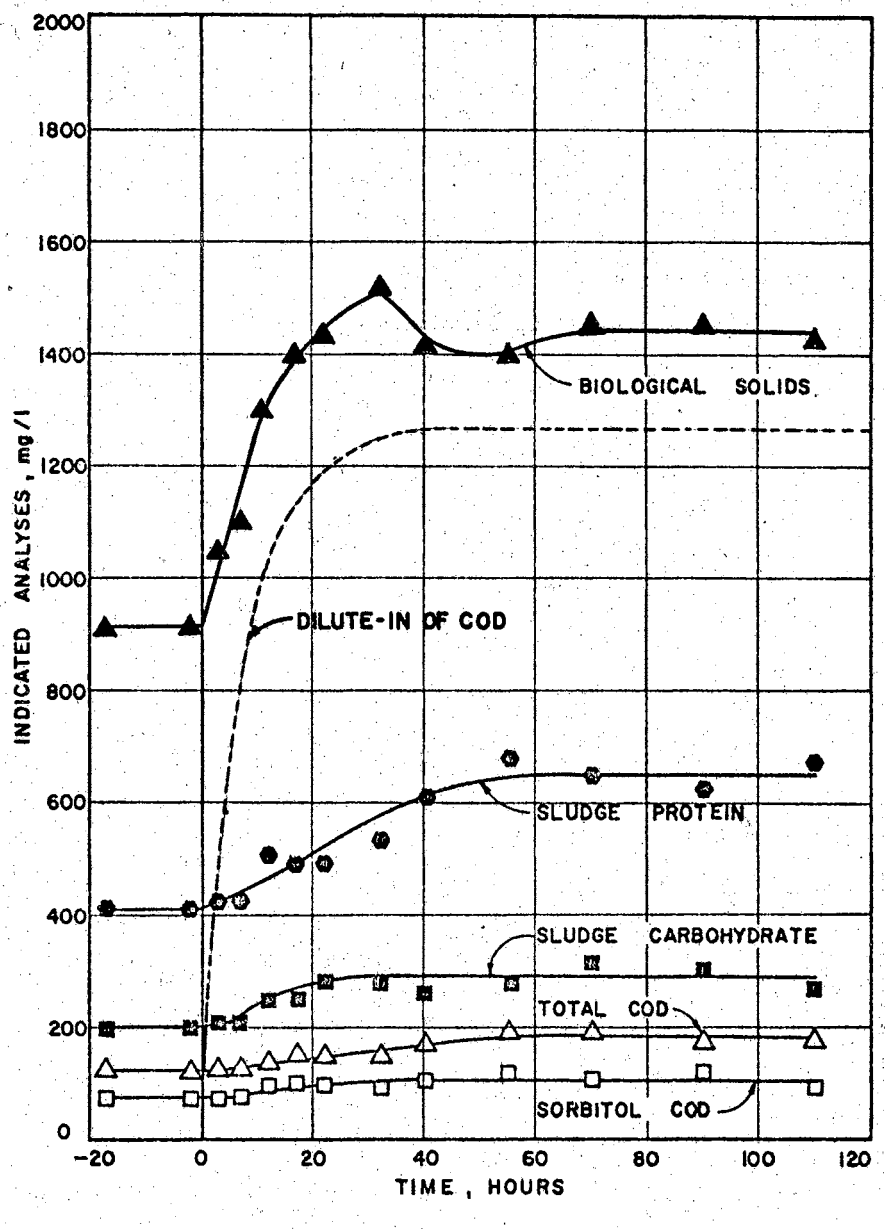

Figure 15 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 2000 mg/l to 3000 mg/l sorbitol.

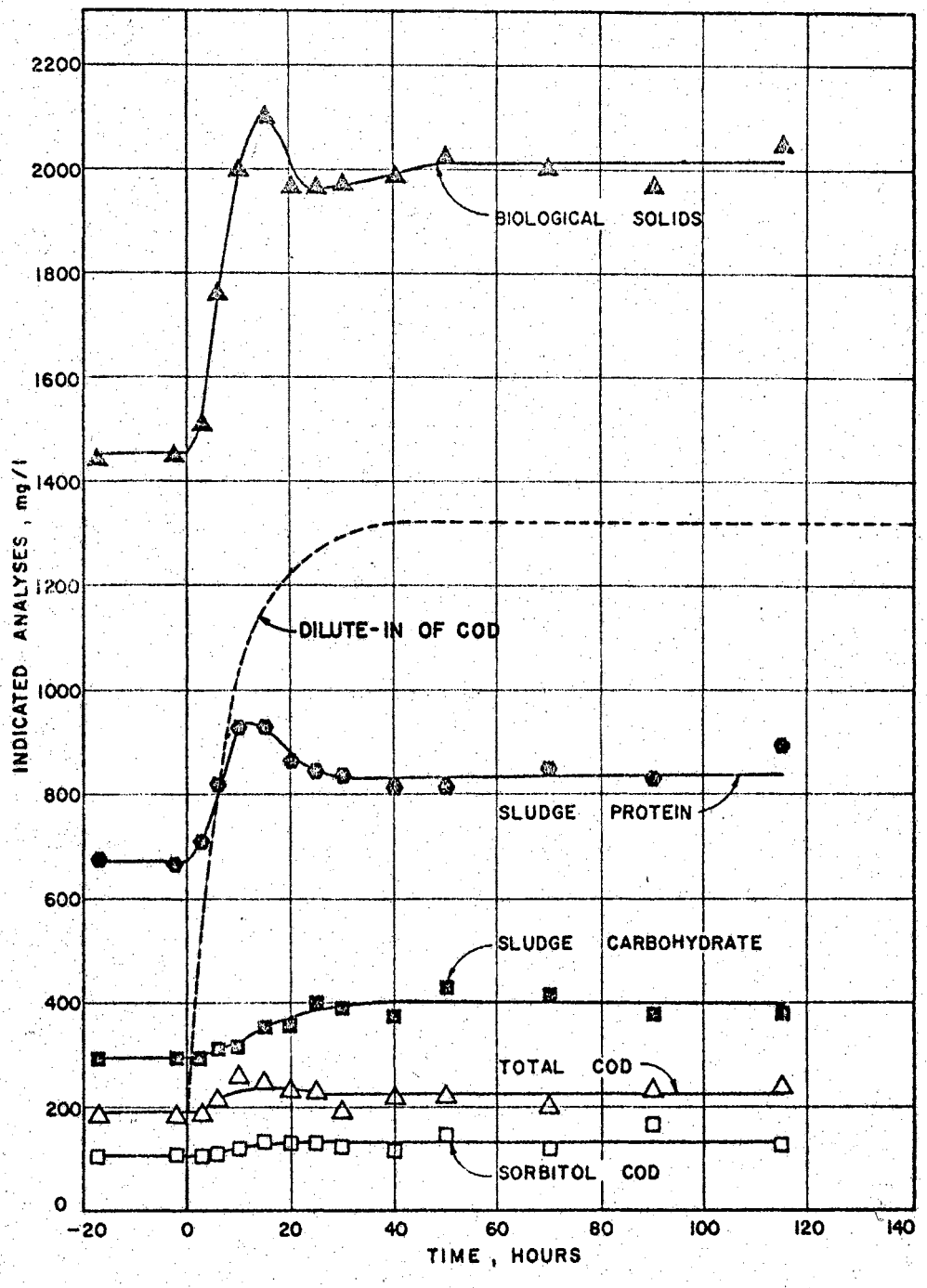

Figure 16 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 3000 mg/l to 4000 mg/l sorbitol.

rapid, and the system was not adversely affected.

b) Response to Sequential Stepwise Increases in Glucose

A series of experiments on sequential quantitative shock loadings similar to those employing sorbitol was conducted with glucose as the carbon source. The effect of sequential stepwise increases in influent glucose concentration from 500 mg/l to 1000 mg/l, 1000 mg/l to 2000 mg/l, and 2000 mg/l to 4000 mg/l glucose, was studied. In addition, during these shock loading experiments, cells were harvested from the reactor effluent during the transient state and steady state, and used in various batch growth studies to determine the relationship between the logarithmic growth rate constant, μ, and substrate concentration, S.

In Figure 17 is shown the response when the influent glucose concentration was changed from 500 to 1000 mg/l. There was an increase of approximately 30 mg/l of the total COD over the previous steady state value; however, after eighteen hours, the total COD was reduced to a value close to the previous steady state value. There was also a slight increase in glucose (anthrone) COD over the previous steady state value. Solids concentration increased to approximately 475 mg/l, which is considerably more than twice the previous steady state solids concentration of 200 mg/l, thus indicating an increase in sludge yield. The increase in solids concentration was accompanied by an increase in protein content and carbohydrate content of the solids. It is seen that the system was capable of handling a shock load of 1000 mg/l glucose without serious disruption of substrate removal efficiency. The arrows on the figure (one prior to changing the feed concentration and one during the time of the transient response) indicate the times when the cells were taken for batch growth rate studies to determine the

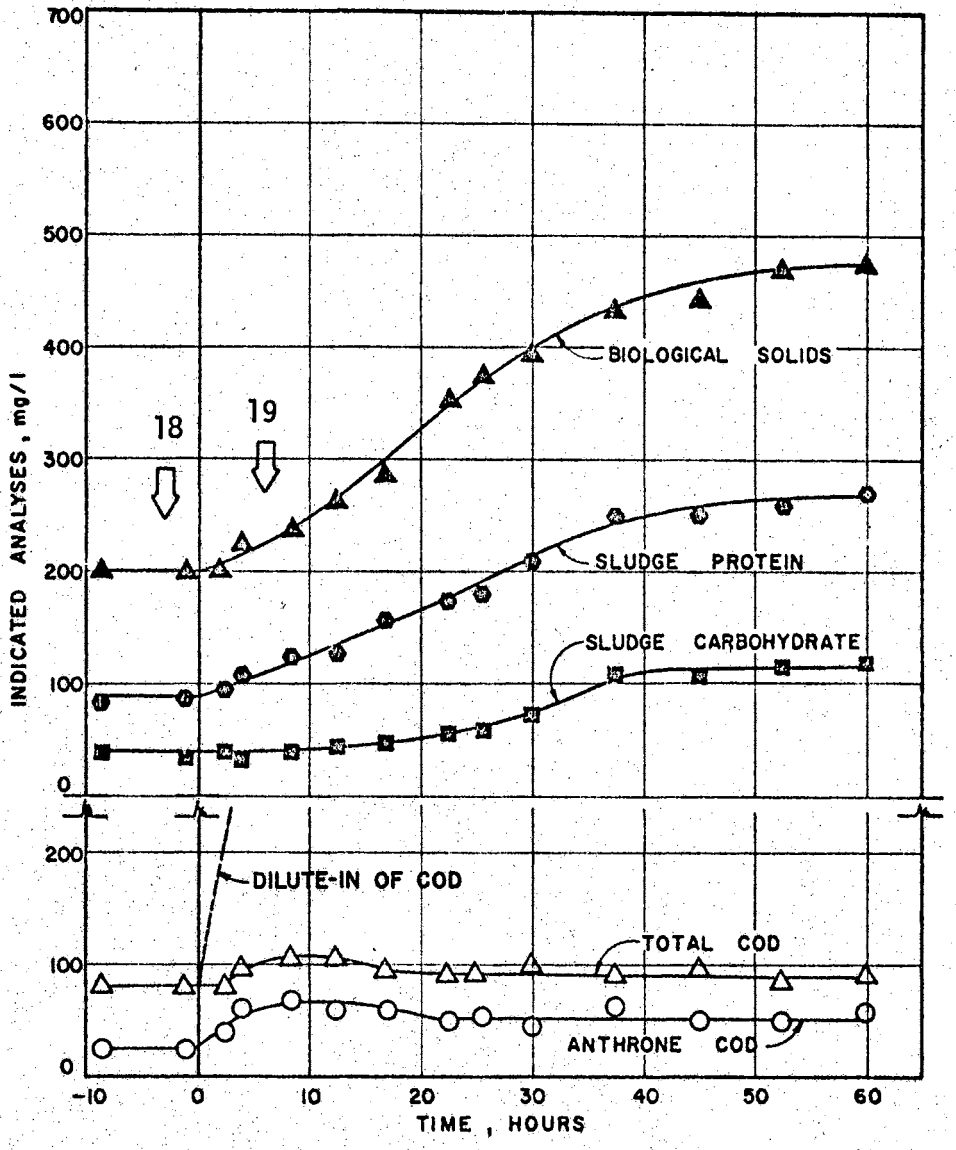

Figure 17 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 500 mg/l to 1000 mg/l glucose.

relationship between μ and S. The number above each arrow indicates the figure number in which the results of the growth rate studies are shown.

Figure 18 shows the relationship between the concentration of growth-limiting factor (i.e., S, glucose) and specific growth rate (μ) for cells harvested during operation at the steady state feeding level of 500 mg/l glucose. In the upper portion of the figure, μ is plotted versus S, and in the lower portion the reciprocal values, $1/\mu$ versus $1/S$, are plotted (i.e., a Lineweaver-Burke plot). It can be seen that the plot of μ versus S approximates a hyperbolic curve in accordance with the Monod relationship, $\mu = \frac{\mu_m \cdot S}{k_S + S}$. Values of μ_m and k_S were calculated using both the upper and lower graphs. It is seen that the reciprocal plot gave somewhat higher values of μ_m and k_S. Also, when these values were inserted into the Monod equation (see curves labeled "Monod theoretical"), they provided a closer fit to the observed data than did the values obtained from a plot of μ versus S. The μ_m and k_S values were found to be 0.290 per hour and 133 mg/l, respectively.

Figure 19 shows the results when cells were taken for growth rate studies during the transient following the change in inflowing glucose concentration from 500 mg/l to 1000 mg/l glucose. The μ_m and k_S values obtained from the Lineweaver-Burke plot were 0.315 per hour and 165 mg/l, respectively. These values were somewhat higher than the values shown in Figure 18.

After a period of operation at the 1000 mg/l feeding level, the inflow glucose concentration was increased to 2000 mg/l glucose. The results are shown in Figure 20. There was a gradual increase in the residual total COD to a fairly constant value of 200 mg/l. The increase in total COD was accompanied by an increase in anthrone COD. Comparison

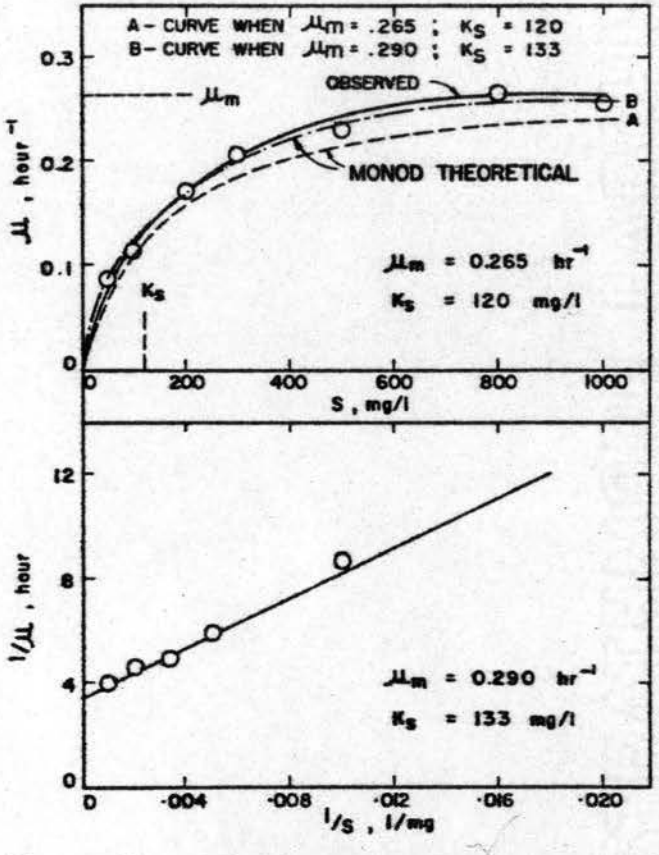

Figure 18 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" with 500 mg/l glucose feed).

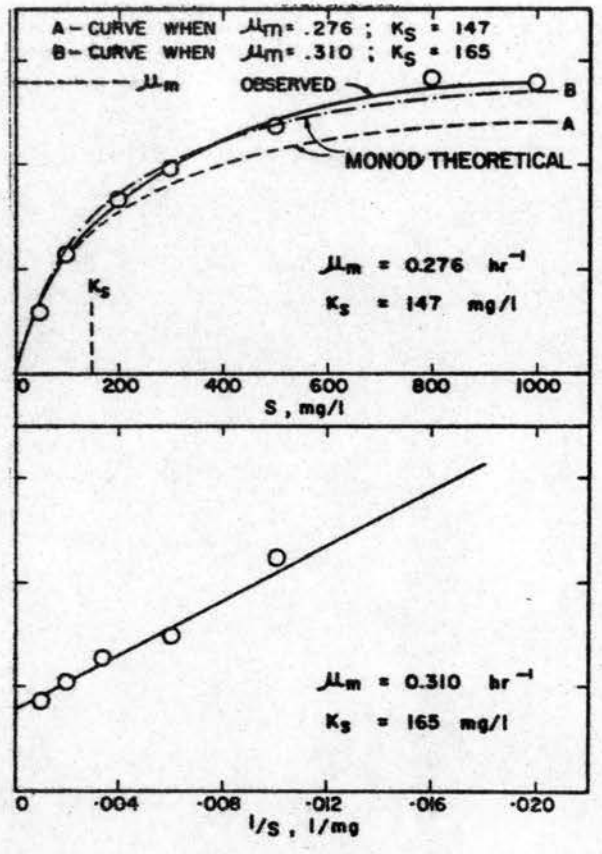

Figure 19 - Relationship between specific growth rate and substrate concentration (cells harvested at 6 hours after changing the influent feed from 500 mg/l to 1000 mg/l glucose).

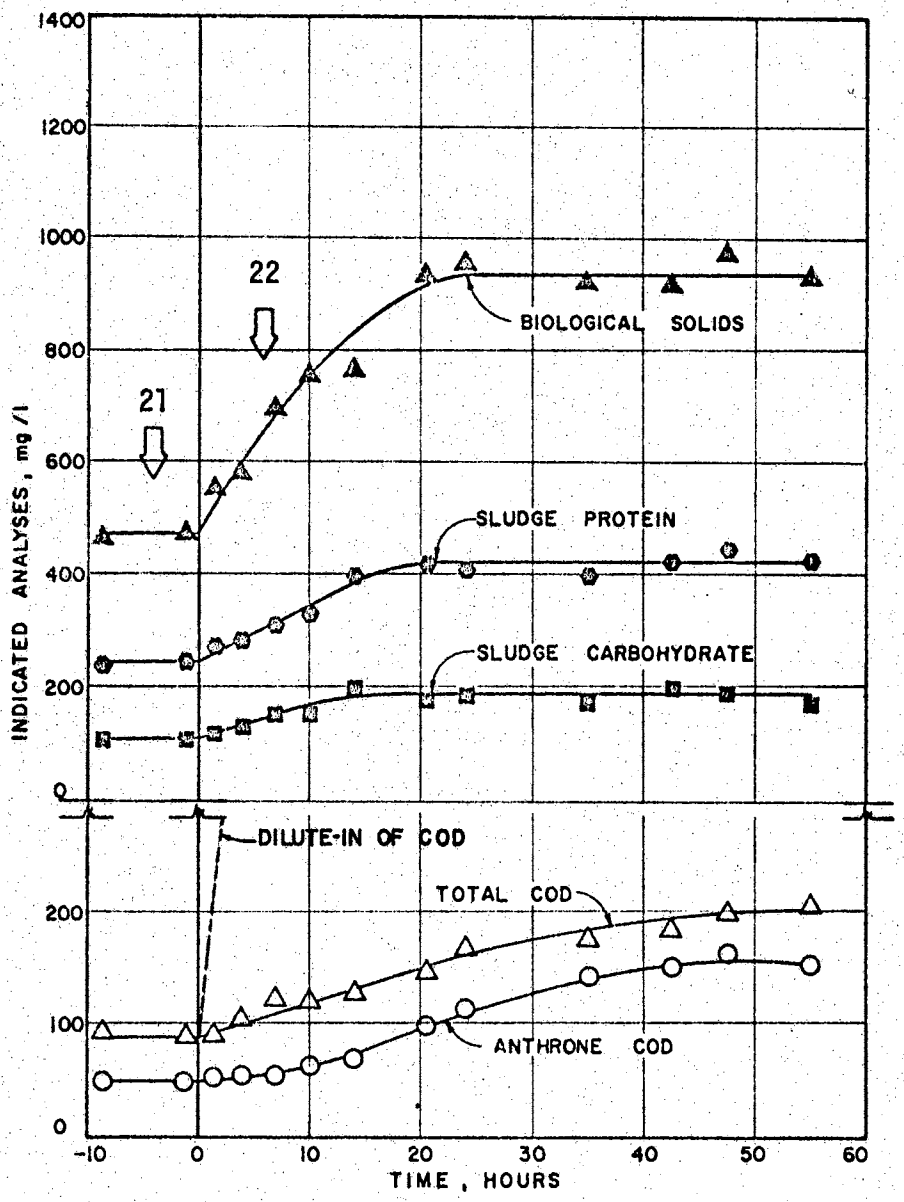

Figure 20 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 1000 mg/l to 2000 mg/l glucose.

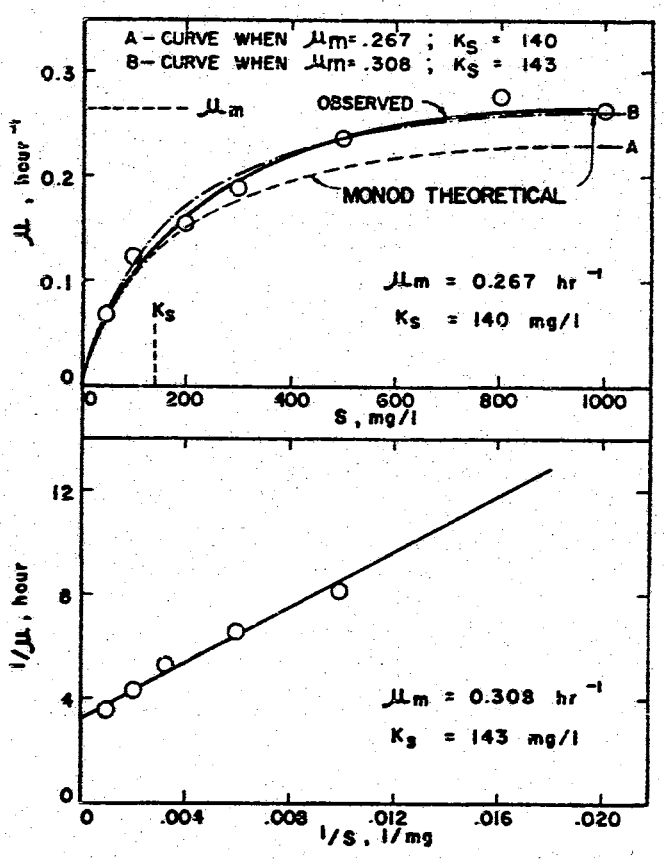

Figure 21 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" with 1000 mg/l glucose feed).

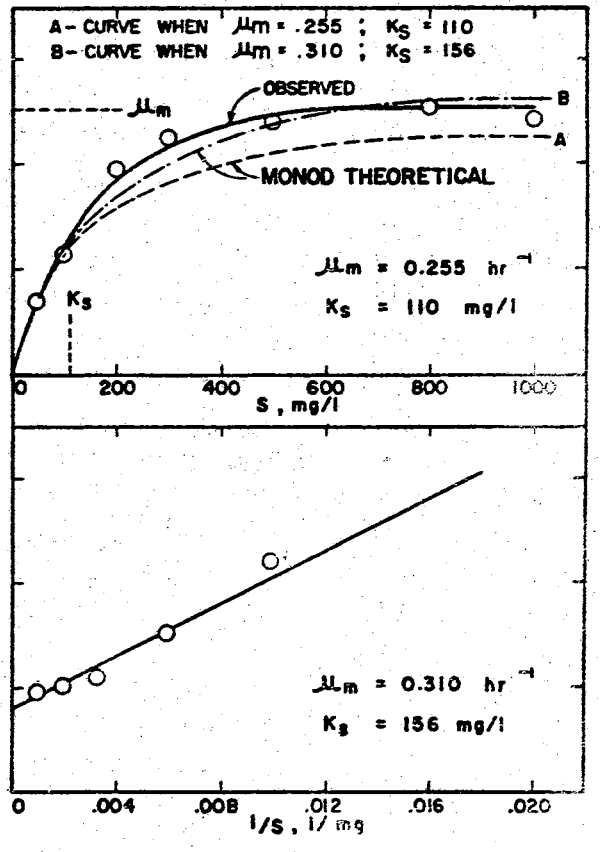

Figure 22 - Relationship between specific growth rate and substrate concentration (cells harvested at 6 hours after changing the influent feed from 1000 mg/l to 2000 mg/l glucose).

of Figures 17 and 20 indicates that the change from 1000 mg/l to 2000 mg/l glucose affected the system more adversely than did the change from 500 to 1000 mg/l glucose. The transient state response in biological solids at the higher feed level (Figure 20) occurred more rapidly than for the previous experiment (Figure 17). However, the solids concentration levelled off and appeared to approach a steady state nearly thirty hours sopner than did the COD concentration.

Figures 21 and 22 show the relationships between substrate concentration and growth rate for the cells harvested from the unit before and after changing the feed from 1000 mg/l to 2000 mg/l glucose (see arrows on Figure 20). These data are in general accord with the Monod relationship, and the values of μ and k_S are in rather close agreement with those shown in Figures 18 and 19.

After a period of steady operation at the 2000 mg/l feeding level, influent glucose concentration was changed to 4000 mg/l glucose. It is seen in Figure 23 that the biological solids level increased rapidly to a maximum of 1990 mg/l within eighteen hours, subsequently dropped to a value of 1340 mg/l, and finally returned to a new steady state value of approximately 2050 mg/l. The total COD of the effluent increased to a value of approximately 560 mg/l during the transient state, and gradually decreased to a new constant value of 250 mg/l in eighty hours. This new steady state COD value was not much higher than the previous steady state value. This shock load caused a rather severe transient disruption of substrate removal efficiency, and the COD in the effluent corresponded to leakage of carbohydrate; there was an extraordinary drop and subsequent recovery in biological solids concentration. This secondary response, i.e., dilute-out of biological solids after the

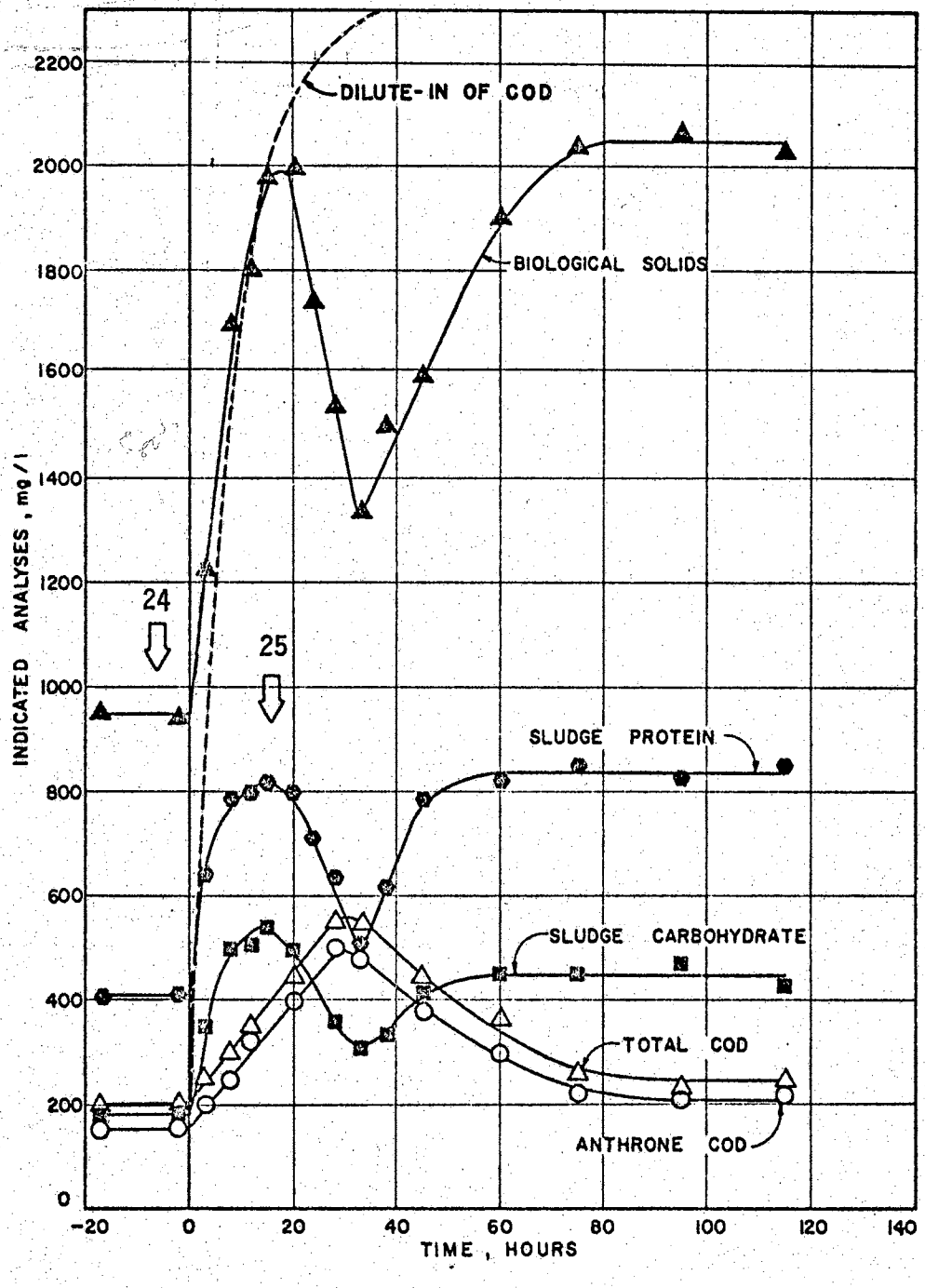

Figure 23 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 2000 mg/l to 4000 mg/l glucose.

early rise, was accompanied by a change in color of the mixed liquor, indicative of a change in predominant microbial species.

Figures 24 and 25 show the relationship between substrate concentration and growth rate using the cells taken from the unit before and soon after changing the glucose concentration from 2000 mg/l to 4000 mg/l. The results are, in general, similar to those previously obtained during steady and transient conditions at the other feeding levels. The value of μ_m shown in Figure 25 is somewhat lower than the values previously obtained during this series of experiments.

Figure 26 shows the biochemical response of the system when the influent glucose concentration was changed from 4000 mg/l to the original feeding level of 500 mg/l glucose. This change resulted in a smooth, non-disruptive transient in biological solids concentration as the cells diluted out in accordance with the decrease in available carbon source. Comparison of the sludge and substrate parameters in this figure after return to the 500 mg/l feeding level with the steady state of the system before changing the feed (Figure 17) show that the series of sequential shocks did not materially change steady state values.

Values of μ_m and k_s before and after return of the feed to the 500 mg/l level are shown in Figures 27 and 28, respectively (also see arrows on Figure 26). The values obtained are in the general range of the previous values.

The values of μ_m and k_s are summarized in Table I. The values of μ_m and k_s for cells harvested during either steady or transient conditions do not differ to any great extent.

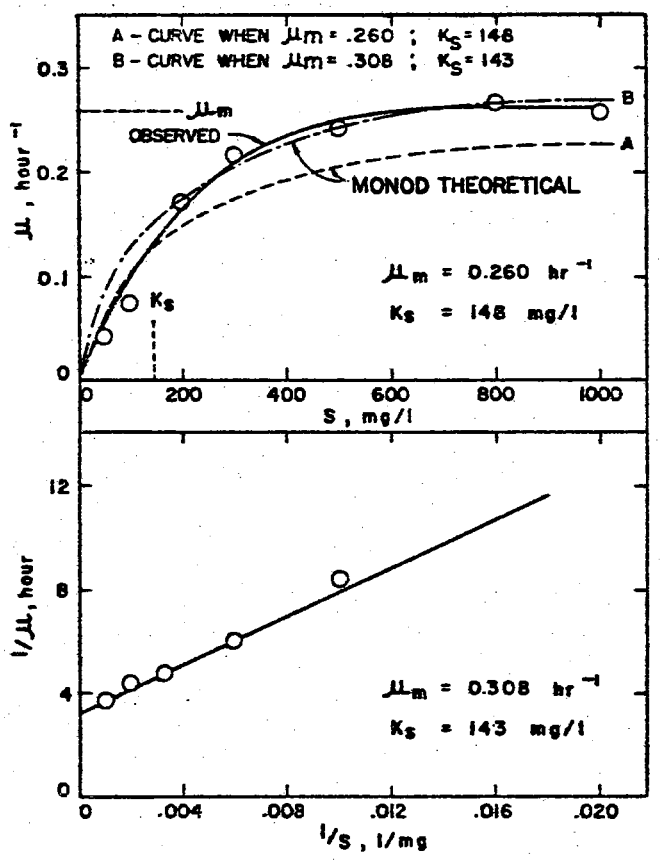

Figure 24 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" with 2000 mg/l glucose feed).

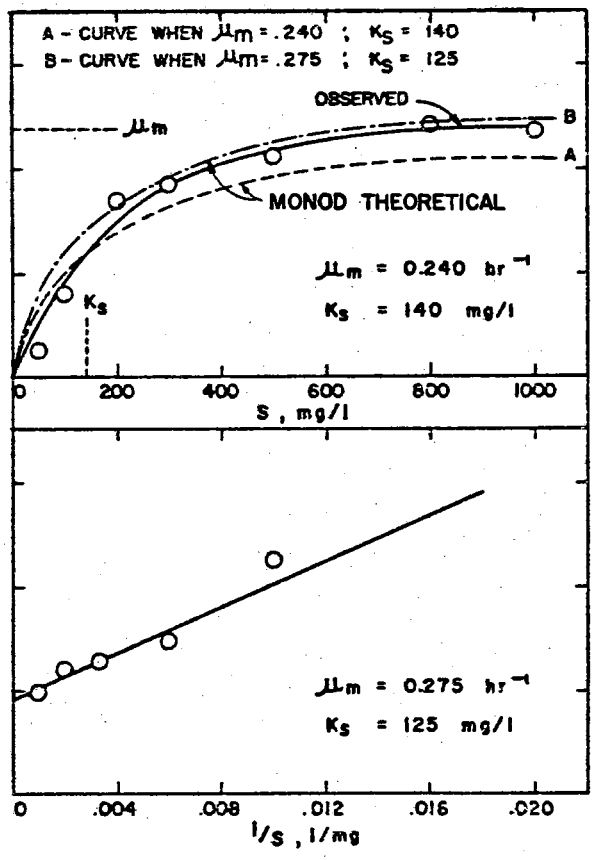

Figure 25 - Relationship between specific growth rate and substrate concentration (cells harvested at 16 hours after changing the influent feed from 2000 mg/l to 4000 mg/l glucose feed).

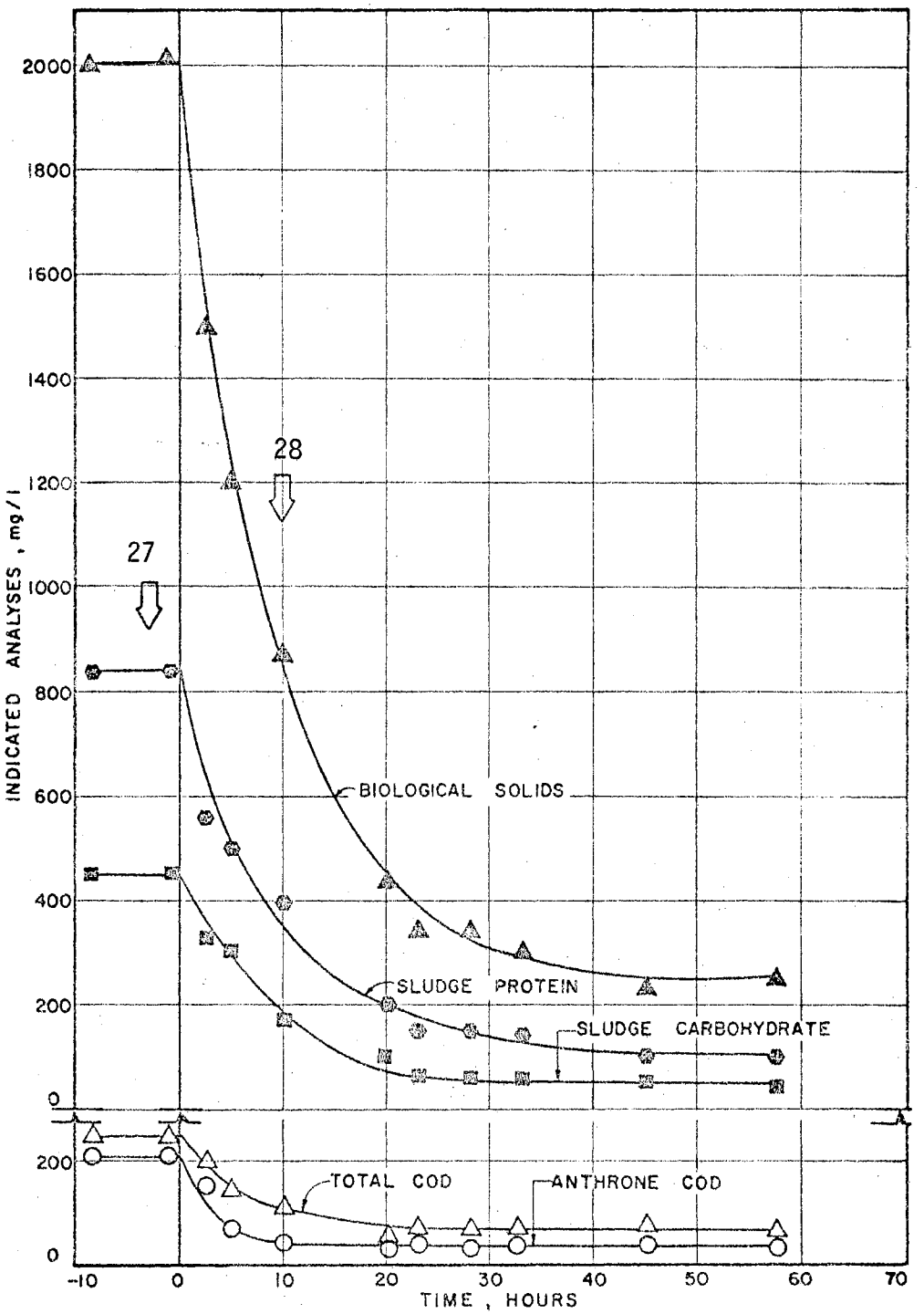

Figure 26 - Biological response in a "steady state" continuous flow activated sludge unit operating at an 8-hour detention time after changing the influent feed from 4000 mg/l to 500 mg/l glucose.

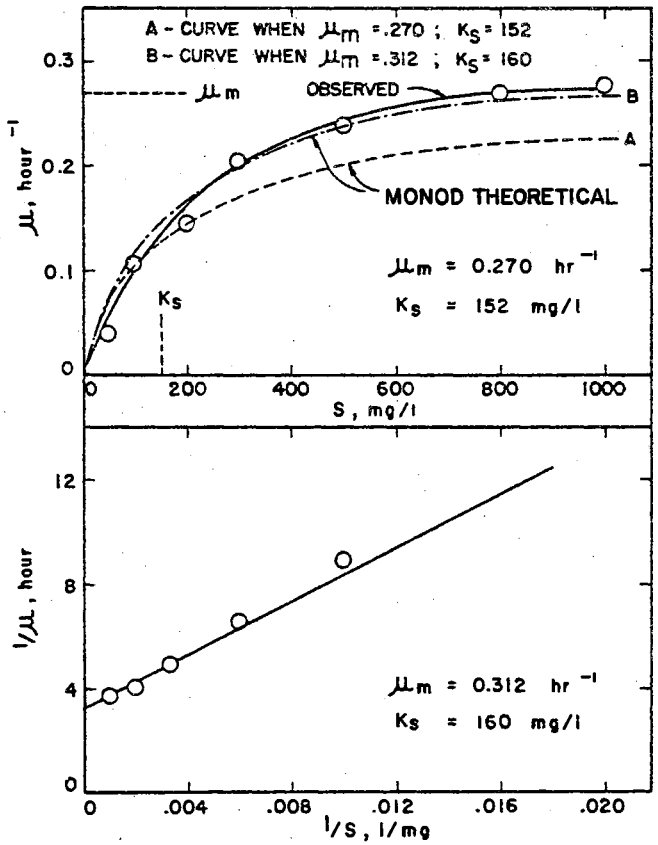

Figure 27 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" with 4000 mg/l glucose feed).

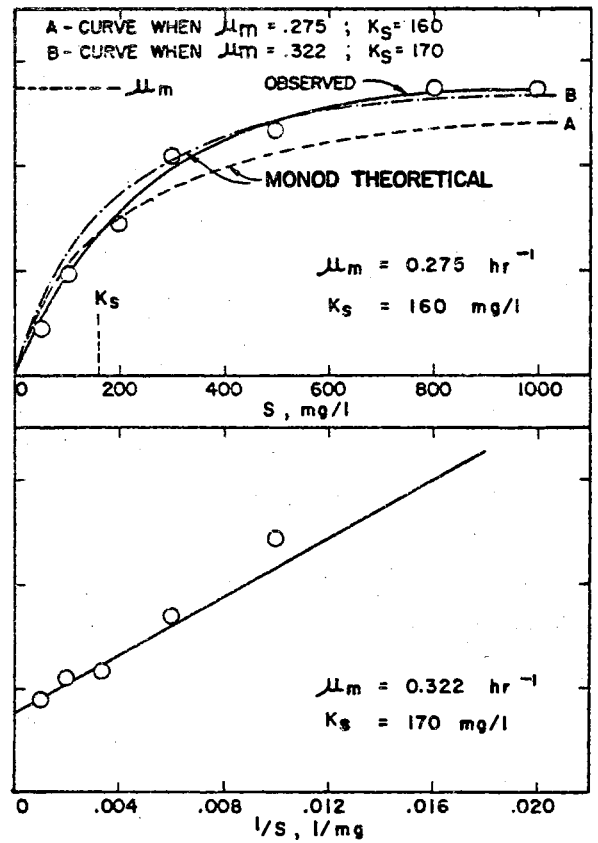

Figure 28 - Relationship between specific growth rate and substrate concentration (cells harvested at 10 hours after changing the influent feed from 4000 mg/l to 500 mg/l glucose).

TABLE I

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS USING CELLS ACCLIMATED TO GLUCOSE
(Dilution rate = 1/8 hr^{-1})

Glucose feed concentration mg/l	Transient State					Steady State						
	μ_{m_1} hr^{-1}	k_{S_1} mg/l	μ_{m_2} hr^{-1}	k_{S_2} mg/l	μ_m hr^{-1}	k_S mg/l	μ_{m_1} hr^{-1}	k_{S_1} mg/l	μ_{m_2} hr^{-1}	k_{S_2} mg/l	μ_m hr^{-1}	k_S mg/l
500	-	-	-	-	-	-	.265	120	.290	133	.278	126
1000	.276	147	.315	165	.295	156	.267	140	.308	143	.288	141
2000	.255	110	.310	156	.284	139	.260	148	.308	143	.284	145
4000	.240	140	.275	125	.258	132	.270	152	.312	160	.291	156
500	.275	160	.322	170	.298	165	-	-	-	-	-	-

μ_{m_1} and k_{S_1} are calculated from the graph μ versus S

μ_{m_2} and k_{S_2} are calculated from the graph $1/\mu$ versus $1/S$

μ_m and k_S are the mean values of μ_{m_1} and μ_{m_2}, and k_{S_1} and k_{S_2}

3. Hydraulic Shock Loading in a Multicomponent (Three Carbon Source) Substrate System

In this series of experiments the responses of a completely mixed activated sludge system to stepwise increases in dilution rate when the feed consisted of glucose, galactose, and glycerol, were studied. The aim of these studies was to determine if the dilution rate, D (or growth rate μ) had an effect on "sequential substrate removal or on the possible order of substrate leakage in the effluent.

Presentation of the experimental data is as follows: A figure is shown depicting the behavior of the continuous flow activated sludge system at the initial dilution rate, during the transient state, and in the final steady state at the new dilution rate. On each figure, arrows indicate the times when the cells were taken from the reactor for batch experiments to determine the order of substrate removal. The numbers shown above the arrows refer to the figure number in which the batch results are presented (control and combined systems--low initial solids concentration).

a) Effect of Changing Dilution Rate from 1/24 to 1/18 hr^{-1}

The continuous flow unit was seeded with a microbial population obtained from the primary clarifier effluent of the municipal sewage treatment plant at Stillwater, Oklahoma. The system was fed a synthetic waste consisting of 300 mg/l glucose, 300 mg/l glycerol, and 300 mg/l galactose. The unit was operated under steady state conditions for four days and a steady state solids concentration of approximately 320 mg/l was attained. The system was then subjected to an increase in flow rate. In Figure 29 are shown the results when the flow rate was increased, causing a change in dilution rate from 0.042 5o 0.055 hr^{-1}

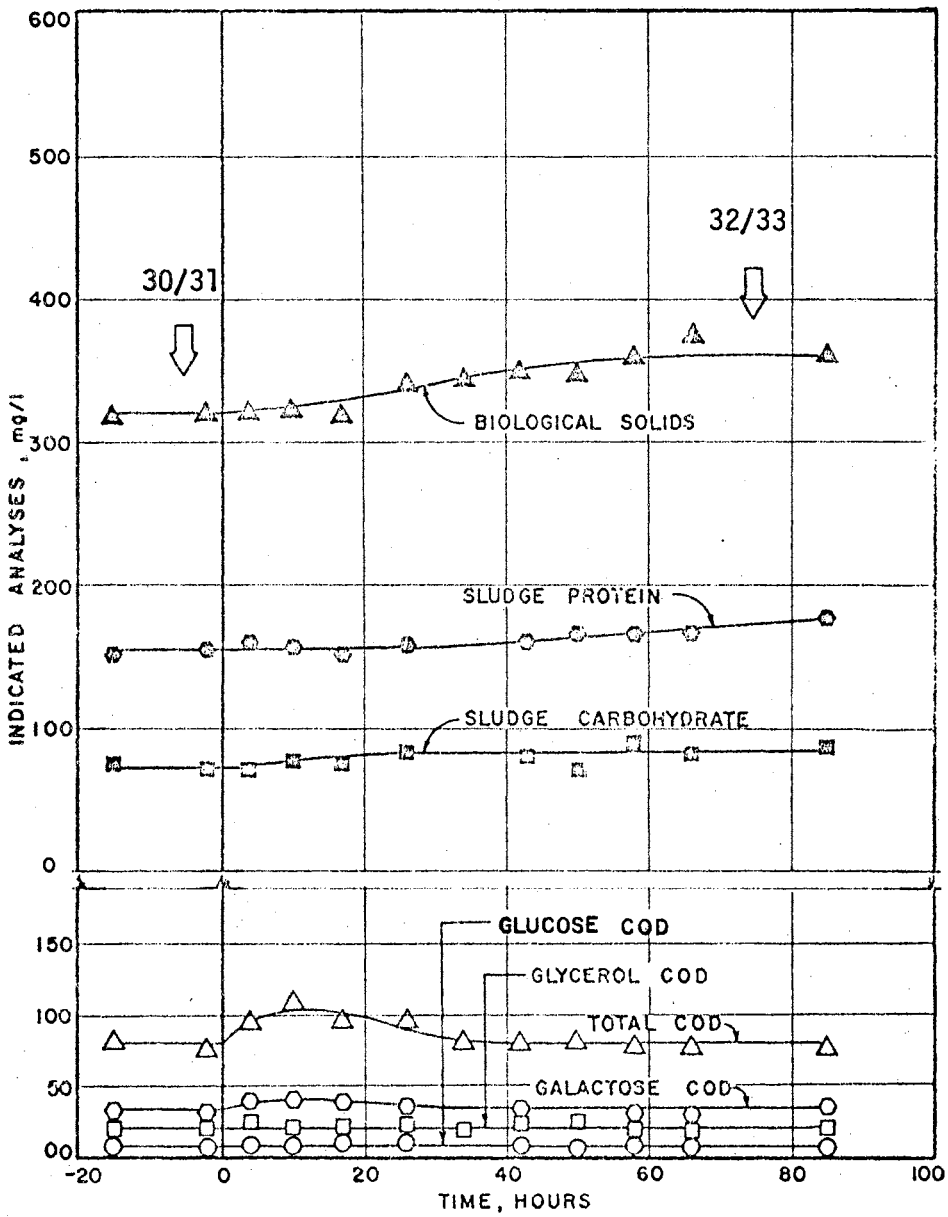

Figure 29 - Response of a continuous flow reactor to a change in dilution rate from 1/24 to 1/18 hr^{-1} (constant feed concentration, 300 mg/l glucose + 300 mg/l glycerol + 300 mg/l galactose).

(detention time from 24 hours to 18 hours). There was a slight increase in biological solids concentration. It is seen that during the transient state there was very little increase in the total COD concentration, and it subsequently returned to the previous steady state value of 80 mg/l at thirty hours. There was no change in glucose COD or glycerol COD in the effluent. There was a very slight increase in galactose COD during the transient state, indicating that the increase in the total COD was attributable to galactose COD. There was a slight increase in biological solids concentration to a new steady state value of 360 mg/l. The increase in biological solids concentration was accompanied by an increase in both protein and carbohydrate content of the solids.

During operation in the steady state at the dilution rate of 1/24 hr^{-1}, cells were harvested from the effluent of the continuous flow activated sludge unit and used in batch studies conducted at low initial biological solids concentration under growth conditions. The results are shown in Figures 30 and 31. It is seen from the control system that glucose was removed at a faster rate than were the other compounds, and a considerable amount of metabolic products remained in the glucose control after the glucose had been removed. The dotted line labeled "intermediates" was obtained by subtracting the specific COD from the total COD for each graph. The substrate removal patterns which were exhibited when the carbon sources were used jointly (Figure 31) indicate that glucose, glycerol, and galactose were removed concurrently. Galactose removal was noticeably slower in the mixed system than it was in the galactose control.

During the operation at the new dilution rate (1/18 hr^{-1}), cells

Figure 30 - Metabolic response under growing conditions in glucose, glycerol, and galactose controls (cells harvested at a dilution rate of 1/24 hr^{-1}).

Figure 31 - Metabolic response under growing conditions in glucose-glycerol-galactose combined system (cells harvested at a dilution rate of 1/24 hr^{-1}).

Figure 32 - Metabolic response under growing conditions in glucose, glycerol, and galactose controls (cells harvested at a dilution rate of 1/18 hr^{-1}).

Figure 33 - Metabolic response under growing conditions in glucose-glycerol-galactose combined system (cells harvested at a dilution rate of 1/18 hr^{-1}).

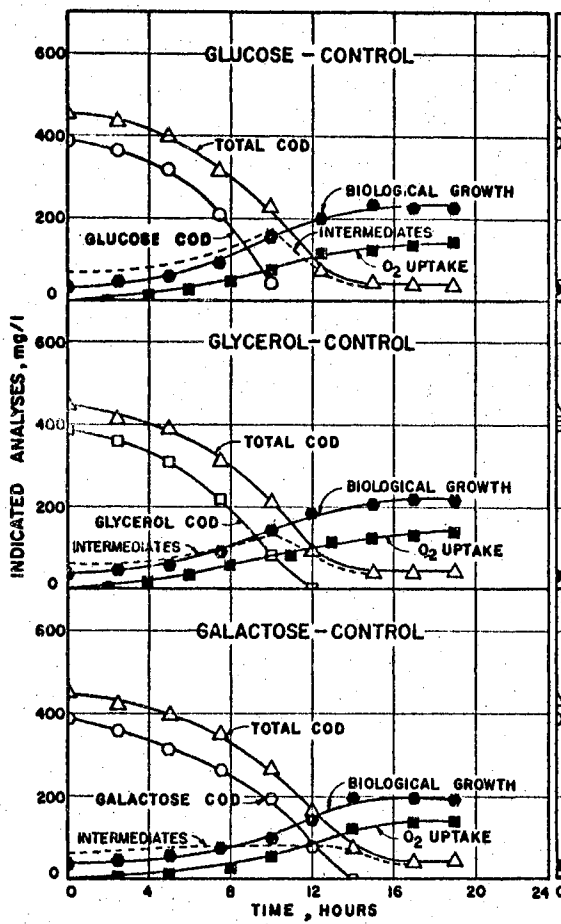

Figure 30

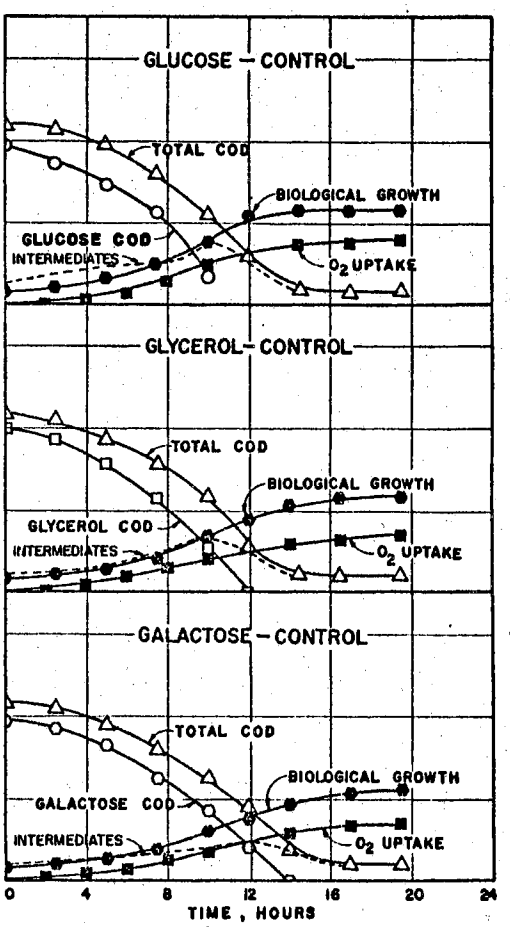

Figure 32

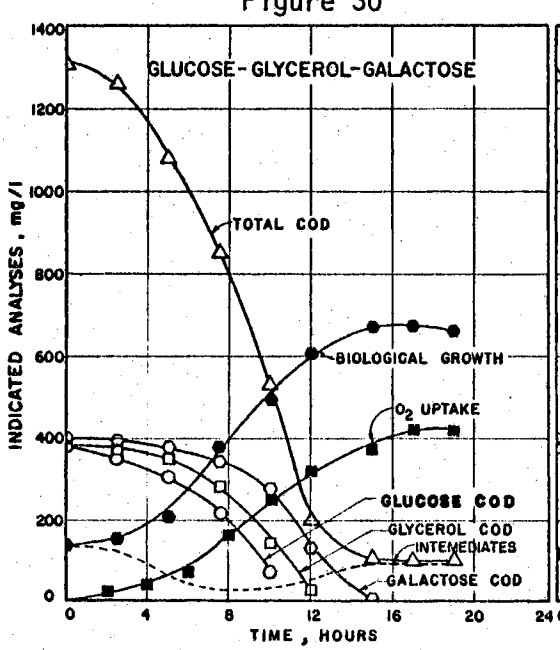

Figure 31

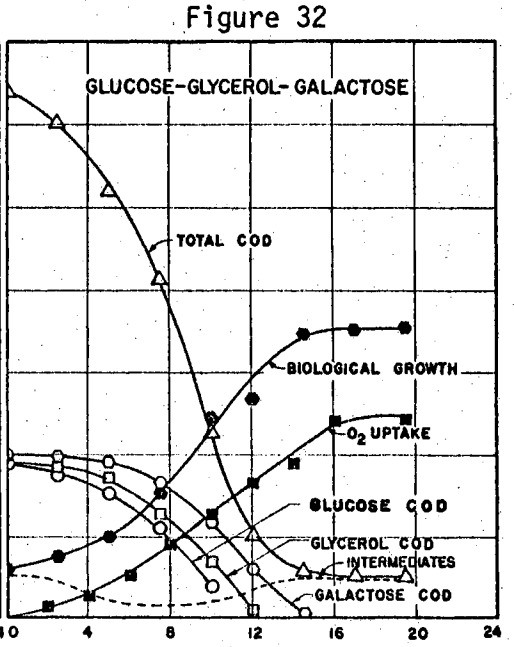

Figure 33

were taken from the effluent of the continuous flow unit and used for batch experiments under growth conditions. The results for the control system (Figure 32) were in general accord with those for the previous experiment (Figure 30). In the combined system (Figure 33), glucose, glycerol, and galactose were removed concurrently, and it is seen that glucose was metabolized faster rate than were the others.

b) Effect of Changing Dilution Rate from 1/18 to 1/12 hr^{-1}

After a period of operation at a dilution rate of 1/18 hr^{-1}, the flow rate was adjusted to yield a dilution rate (or growth rate) of 1/12 hr^{-1}. The behavior of the continuous flow system during this period of operation is shown in Figure 34. There was a slight decrease followed by an increase in the biological solids level as a result of this shock. There was some increase in the total cell protein which accompanied the increase in biological solids concentration; little change was noted with respect to the total cell carbohydrate. It is seen that there was a slight increase in the total COD, to a level of 100 mg/l during the transient state response, and then the concentration slowly decreased to a new steady state value of approximately 70 mg/l, which was slightly lower than the previous steady state value. Again it is seen that there was no change in the glucose COD or glycerol COD in the effluent, but there was a very small increase in galactose COD concentration during the transient state. It is seen from the results that the substrate removal obtained with a dilution rate of 1/12 hr^{-1} compares very well with that of the previous experiment shown in Figure 29.

Eighty hours after applying the change in dilution rate, cells were harvested from the effluent of the continuous flow unit, and used

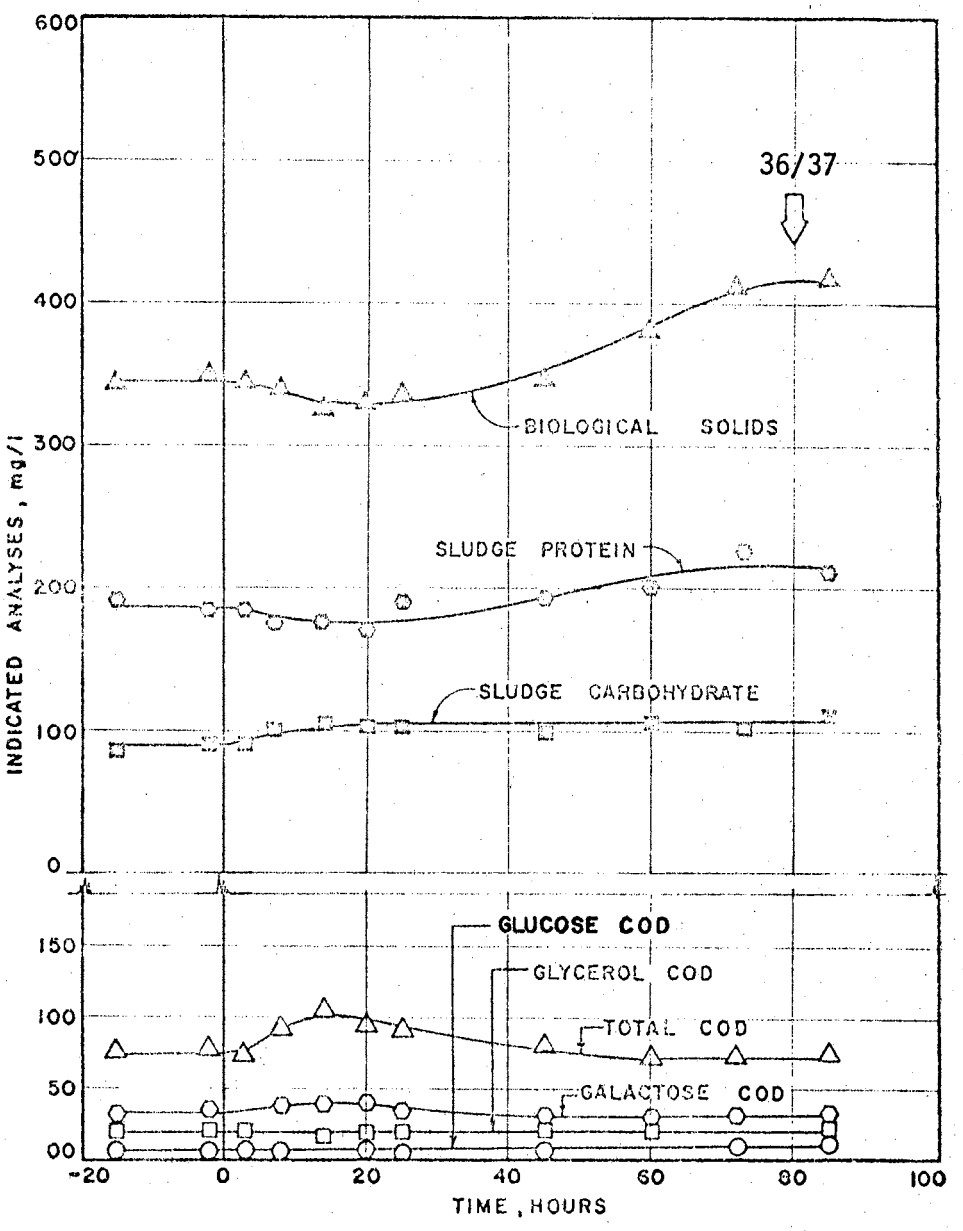

Figure 34 - Response of continuous flow reactor to a change in dilution rate from 1/18 to 1/12 hr^{-1} (constant feed concentration, 300 mg/l glucose + 300 mg/l glycerol + 300 mg/l galactose).

for batch studies under growth conditions in a system employing a low initial inoculum of cells. The results for the control systems (Figure 36) indicate that glucose was metabolized at a faster rate than were the others. In the combined system (Figure 37), the removal of glycerol and galactose was retarded but not totally blocked by the presence of glucose.

c) Effect of Changing Dilution Rate from 1/12 to 1/8 hr^{-1}

In Figure 35 is shown the response when the system was subjected to a change in dilution rate from 0.083 to 0.125 hr^{-1} (detention time from twelve hours to eight hours). The response was similar to that shown in Figure 34. It is interesting to note that in the new steady state the biological solids concentration was higher than at the previous dilution rate. If one compares Figures 29, 34, and 35, it is seen that each increase in growth rate caused the cell yield to increase somewhat. In Figure 35 it is seen that the efficiency of the system was not disrupted as a result of increasing the dilution rate from 1/12 to 1/8 hr^{-1}.

Seventy hours after the change in dilution rate from 1/12 to 1/8 hr^{-1}, cells were harvested from the continuous flow reactor effluent and used for batch experiments at a low initial inoculum under growth conditions; the results are shown in Figures 38 and 39. It is seen in the control systems (Figure 38) that glucose, glycerol, and galactose were utilized at approximately the same relative rates as in the previous batch experiment. There was some production of metabolic intermediates and/or endproducts. There is a clear indication that galactose and glycerol removals were retarded but not totally blocked by the presence of glucose when the compounds were used as a combined source (Figure 39). By the time about half of the glucose was removed from

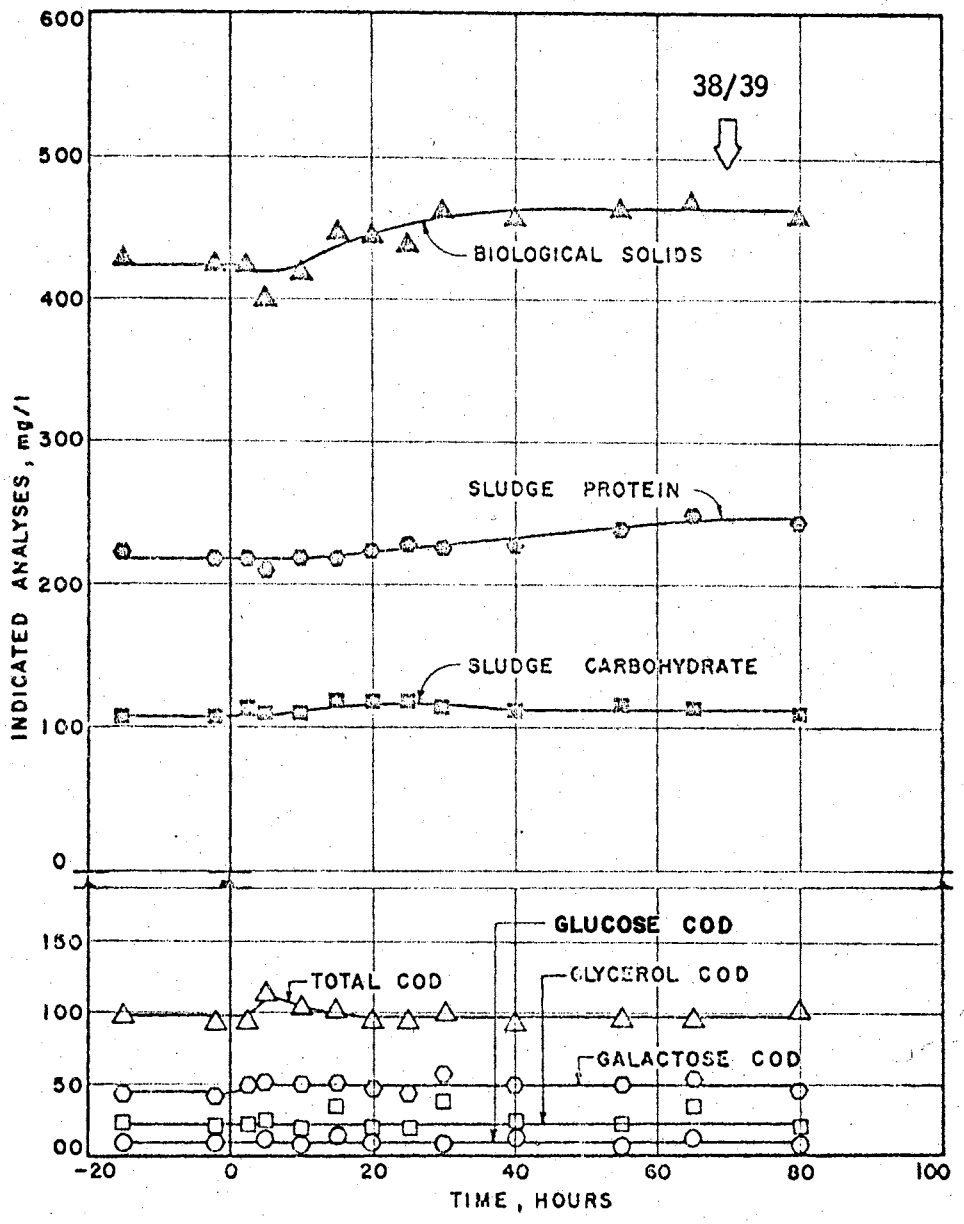

Figure 35 - Response of continuous flow reactor to a change in dilution rate from 1/12 to 1/8 hr^{-1} (constant feed concentration, 300 mg/l glucose + 300 mg/l glycerol + 300 mg/l galactose).

Figure 36 - Metabolic response under growing conditions in glucose, glycerol, and galactose controls (cells harvested at a dilution rate of 1/12 hr^{-1}).

Figure 37 - Metabolic response under growing conditions in glucose-glycerol-galactose combined system (cells harvested at a dilution rate of 1/12 hr^{-1}).

Figure 38 - Metabolic response under growing conditions in glucose, glycerol, and galactose controls (cells harvested at a dilution rate of 1/8 hr^{-1}).

Figure 39 - Metabolic response under growing conditions in glucose-glycerol-galactose combined system (cells harvested at a dilution rate of 1/8 hr^{-1}).

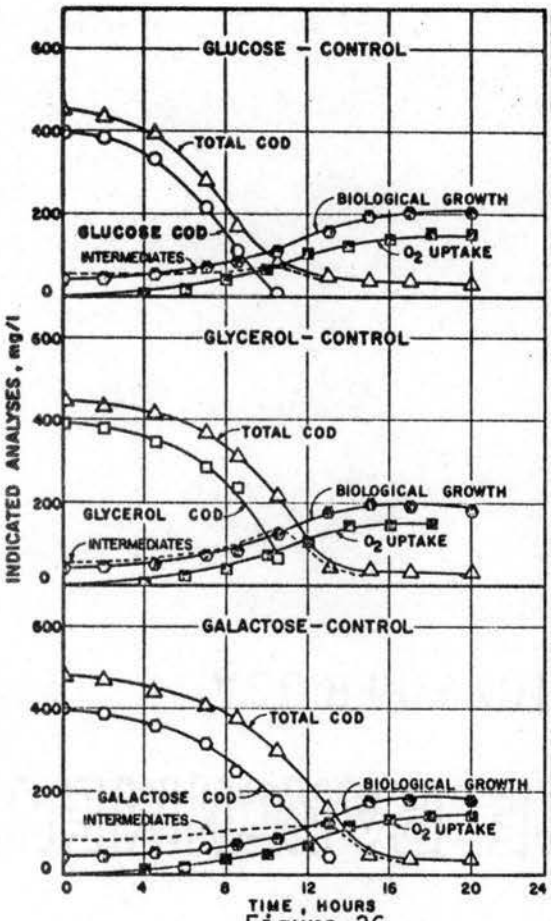

Figure 36

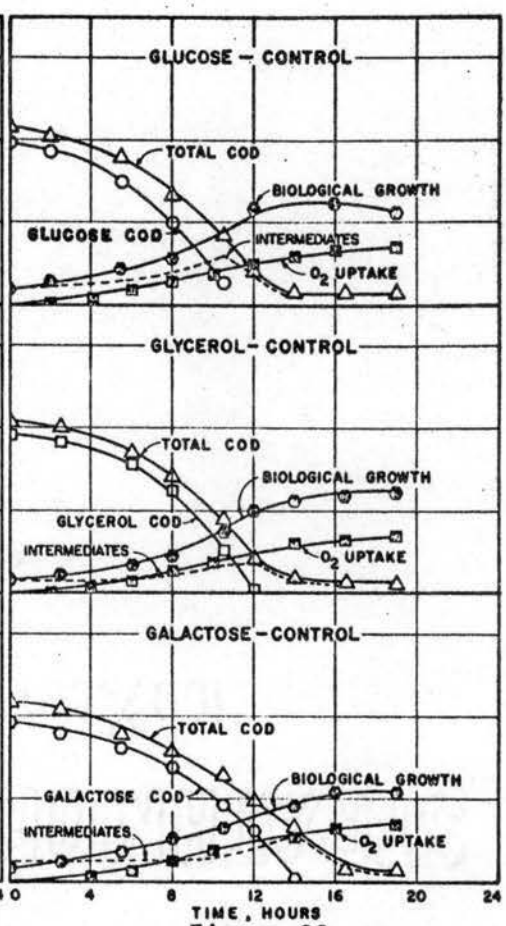

Figure 38

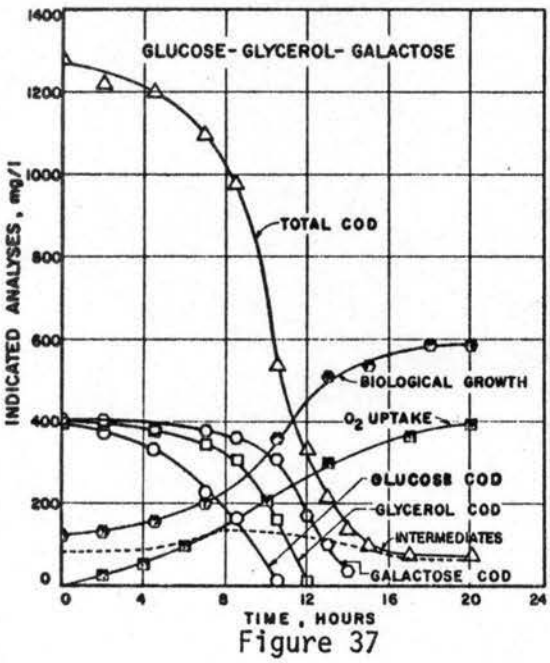

Figure 37

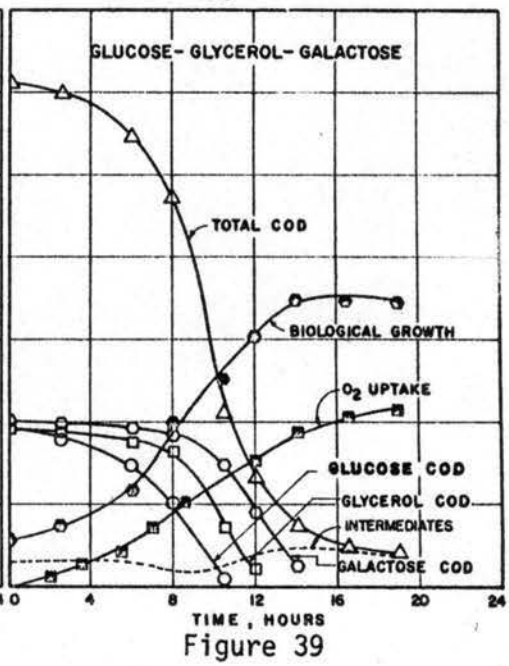

Figure 39

the system, only 40 mg/l glycerol had been removed and 30 mg/l galactose had been eliminated. When all of the glucose had been used, approximately thirty-five per cent of the glycerol and about seventy-five per cent of the galactose remained.

d) Effect of Changing Dilution Rate from 1/8 to 1/6 hr^{-1}

The biochemical response when the system was subjected to a change in dilution rate from 0.125 to 0.166 hr^{-1} (detention time from eight hours to six hours) is shown in Figure 40. As for the previous shock, it is seen that there was no severe transient disruption of COD removal. Solids concentration dropped from approximately 475 mg/l to 420 mg/l, and then rose to 450 mg/l after the shock. It is seen that during the transient conditions immediately after the applied shock, the total COD increased to approximately 125 mg/l, and later returned to the same level as before the shock. There was no change in glucose COD level throughout the experimental period. Galactose COD increased slightly during the transient state. The rising trend in biological solids concentration previously noted at successive increases in dilution rate was not repeated for the change from 1/8 to 1/6 hr^{-1}.

During operation at the new steady state (dilution rate of 1/6 hr^{-1}), cells were harvested from the reactor effluent and used in batch studies. The results for the control systems (Figure 42) indicate that glucose was metabolized at a little faster rate than were the others; glycerol was removed faster than galactose. The behavior of the combined system is shown in Figure 43. Glucose was eliminated in the combined system in approximately the same time as in the control system. Approximately thirty per cent of the glycerol and about seventy-five per cent of the galactose remained in the system at the

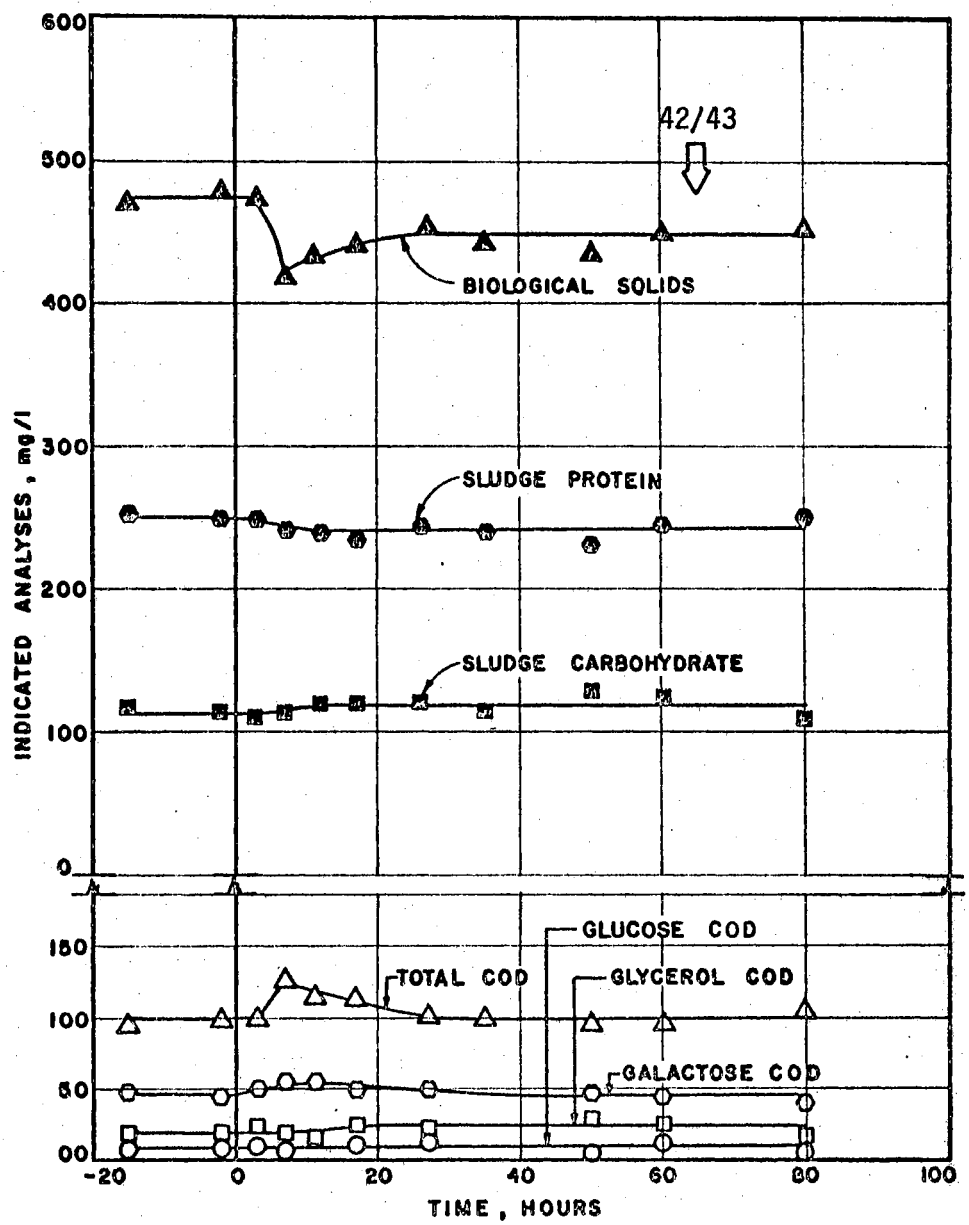

Figure 40 - Response of continuous flow reactor to a change in dilution rate from 1/8 to 1/6 hr^{-1} (constant feed concentration, 300 mg/l glucose + 300 mg/l glycerol + 300 mg/l galactose).

time the glucose concentration reached a very low level.

e) Effect of Changing Dilution Rate from 1/6 to 1/3 hr^{-1}

In Figure 41 are shown the results for a rather severe hydraulic shock loading, i.e., a 100 per cent increase in flow rate, causing a change in dilution rate from 0.166 hr^{-1} to 0.333 hr^{-1} (detention time from six to three hours). The hydraulic shock loading was not assimilated by the system without a very severe transient disruption of substrate removal efficiency. Within thirteen hours after shifting the dilution rate (about four detention times), the biological solids level dropped from 445 mg/l to 130 mg/l, while the total COD increased from 90 mg/l to 490 mg/l. Galactose leakage at this time amounted to 230 mg/l; glycerol leakage amounted to 100 mg/l, and only 50 mg/l glucose passed out into the effluent. The system recovered in forty hours; the biological solids concentration reached a new steady state level of 380 mg/l, which was somewhat lower than the previous steady state concentration. Total COD in the effluent at the new steady state was about 30 mg/l higher than the previous steady state value for the dilution rate of 1/6 hr^{-1}. The COD dilute-in curve (dotted line) indicates the COD concentration which would have been observed had this severe shock caused a complete cessation of metabolism. It is seen that during the first few hours the observed values lie close to this curve.

Shortly after the system had attained its new steady level (see arrow at hour 74, Figure 41), cells were taken from the reactor for batch experiments. The results are shown in Figures 44 and 45. Growth and substrate removal patterns in the control systems (Figure 44) compare very well with those of the previous control systems (Figure 42).

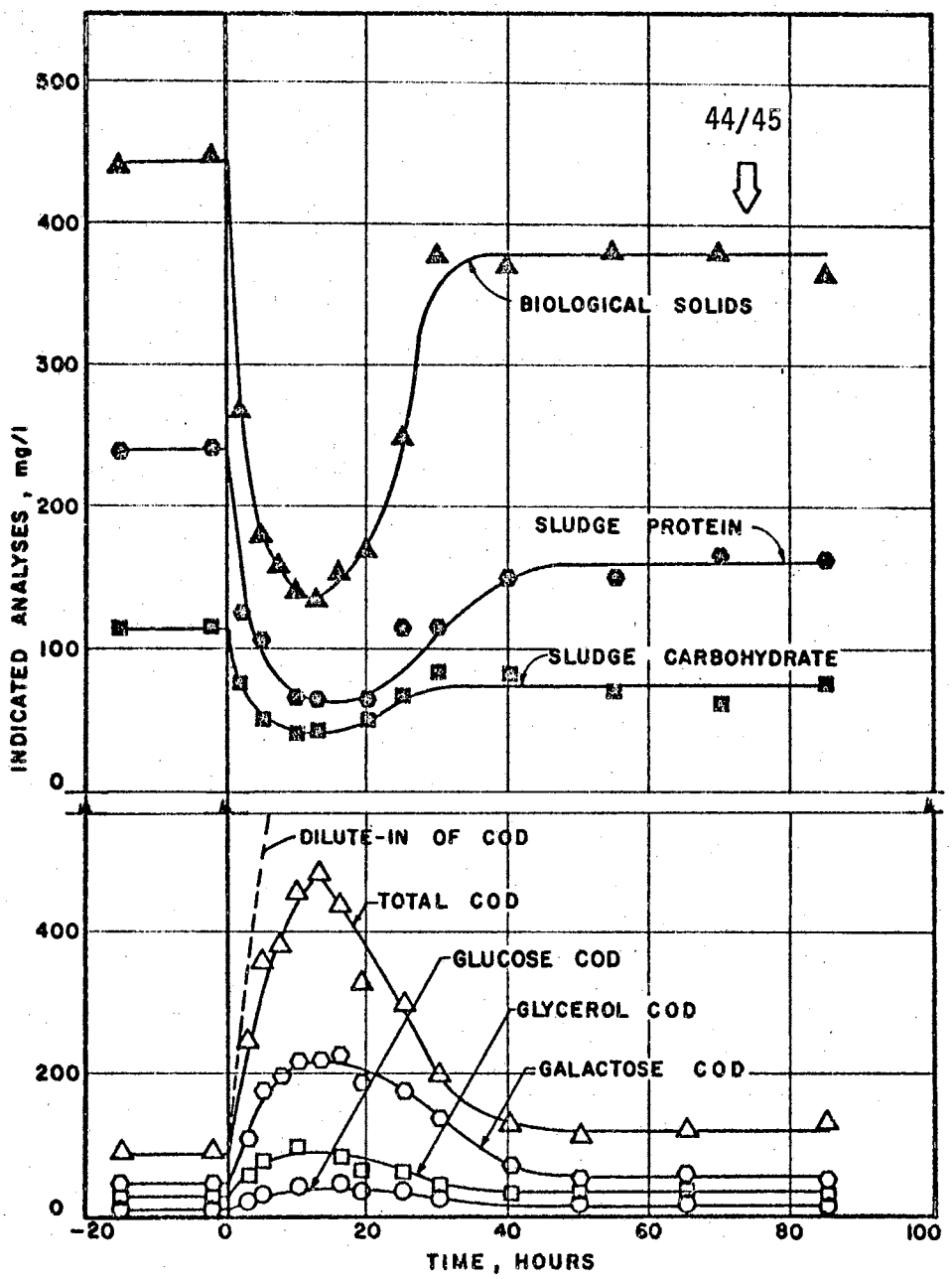

Figure 41 - Response of continuous flow reactor to a change in dilution rate from 1/6 to 1/3 hr^{-1} (constant feed concentration, 300 mg/l glucose + 300 mg/l glycerol + 300 mg/l galactose).

Figure 42 - Metabolic response under growing conditions in glucose, glycerol, and galactose controls (cells harvested at a dilution rate of 1/6 hr^{-1}).

Figure 43 - Metabolic response under growing conditions in glucose-glycerol-galactose combined system (cells harvested at a dilution rate of 1/6 hr^{-1}).

Figure 44 - Metabolic response under growing conditions in glucose, glycerol, and galactose controls (cells harvested at a dilution rate of 1/3 hr^{-1}).

Figure 45 - Metabolic response under growing conditions in glucose-glycerol-galactose combined system (cells harvested at a dilution rate of 1/3 hr^{-1}).

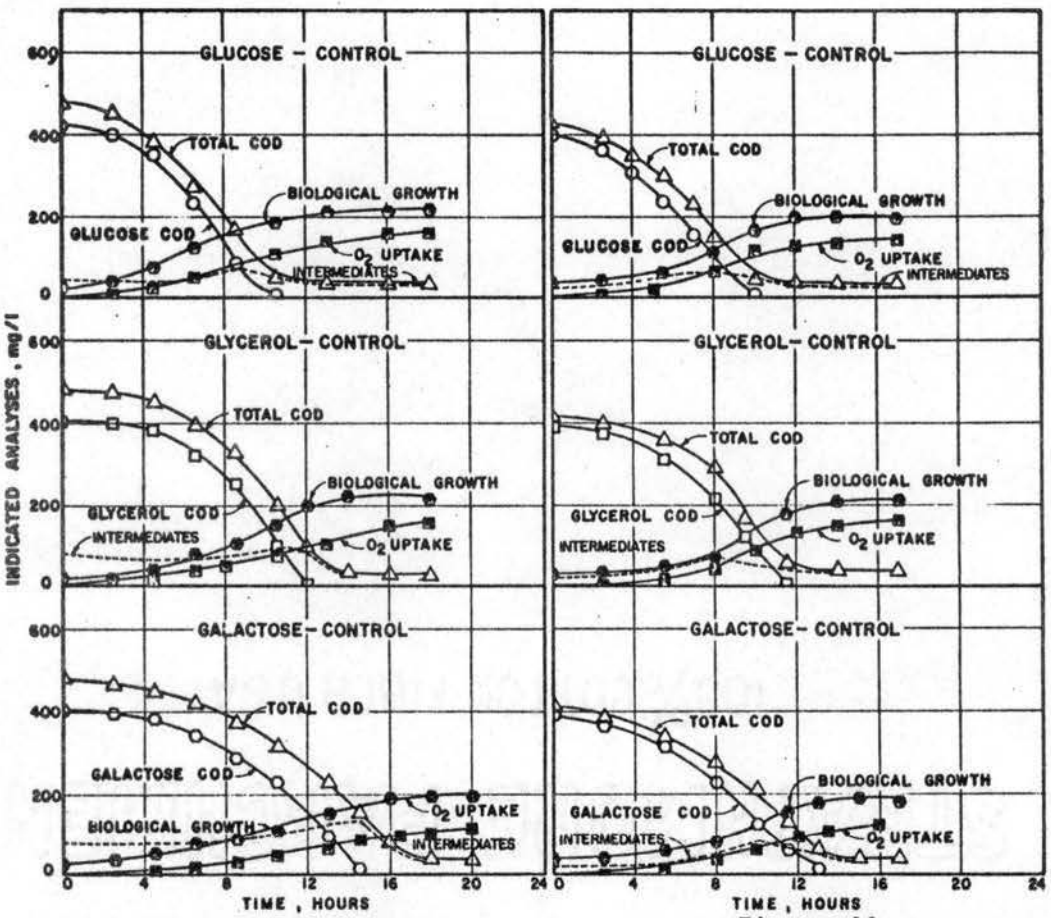

Figure 42

Figure 44

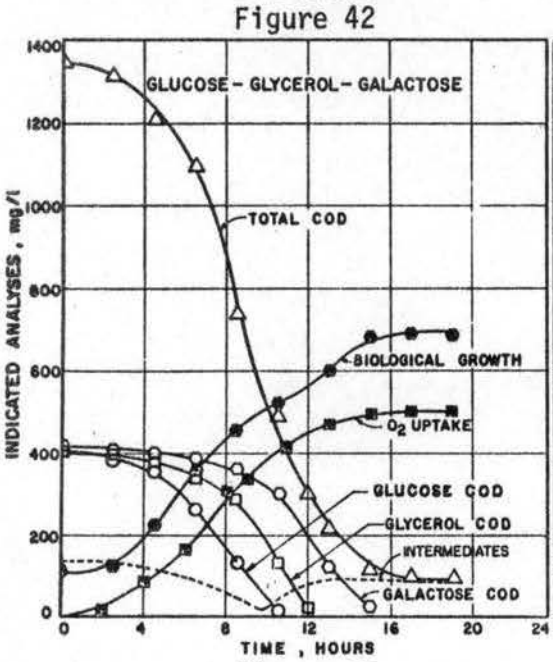

Figure 43

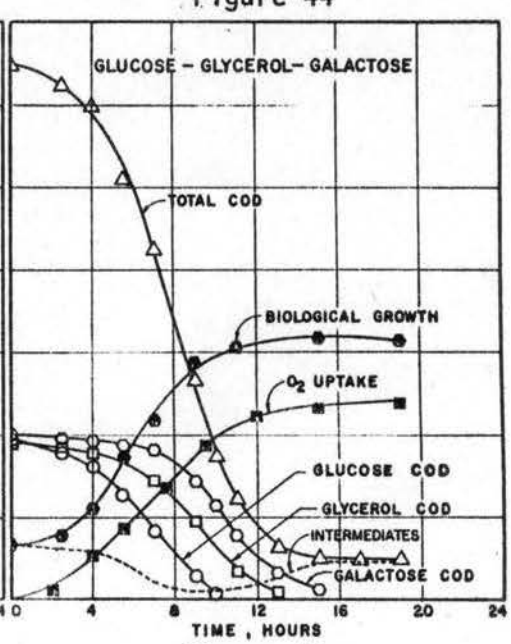

Figure 45

When the compounds were used as a combined carbon source, the removal may be judged to be concurrent; however, glucose did retard the use of glycerol and galactose. When all of the glucose had been metabolized, approximately thirty per cent of the glycerol and about sixty per cent of the galactose remained in the system.

4. Hydraulic Shock Loadings in a Two-Component Carbon Source System

A second series of experiments on hydraulic shock loads was conducted wherein the inflowing feed contained 500 mg/l glucose plus 500 mg/l glycerol, and detention time was sequentially decreased from twenty-four hours to three hours. This study was designed to amplify the work of Komolrit, who used a glucose-sorbitol system, and the work of Krishnan, on a glucose-glycerol system, neither of whom assessed transients produced during hydraulic shocks for these substrates.

The chemostat was operated at detention times of 24, 18, 12, 8, 6, and 3 hours, respectively. During operation in the steady state at each dilution rate, cells were harvested from the reactor effluent for batch experiments under growth conditions in systems employing a low inoculum of cells, in order to determine the typical growth and substrate removal patterns when glucose and glycerol comprised the combined carbon source. A glucose control and a glycerol control were also run. The mode of presentation of the experimental results is similar to that of the previous experiments (section 3).

a) Response to Change in Dilution Rate from 1/24 to 1/18 hr^{-1}

A completely mixed continuous flow activated sludge system was started, employing an initial seed obtained from the primary clarifier effluent of the municipal sewage treatment plant at Stillwater,

Oklahoma. The unit was operated at a 24-hour detention time for five days; then the system was subjected to hydraulic shock loading. Figure 46 shows the biochemical response when the system was subjected to a change in dilution rate from 1/24 hr^{-1} to 1/18 hr^{-1}. There was a slight increase in the biological solids concentration. The cell protein and cellular carbohydrate slowly increased to a level slightly higher than the previous steady state concentrations obtained with a 24-hour detention time. During the transient state there was a very small increase in the total COD to a new steady state value of approximately 60 mg/l.

During operation in the steady state at the initial dilution rate of 1/24 hr^{-1}, cells were harvested from the reactor for the batch experiments (see arrow on Figure 46). The results for the control system (Figure 47) indicate that cells could metabolize glucose and glycerol with approximately the same degree of proficiency. A considerable accumulation of metabolic products (approximately 200 mg/l COD) remained in the glucose control after the glucose had been removed. In the combined system (Figure 48) there is a clear indication that glucose and glycerol were removed concurrently; however, glucose did appear to retard the utilization of glycerol to a small extent.

The batch growth results when cells were harvested seventy-five hours after the change in dilution rate are shown in Figures 49 and 50. It is seen that in the control systems, glucose was removed more rapidly than was glycerol, and there was some accumulation of metabolic intermediates and/or endproducts in the glucose system. Comparison of the previous glucose control (Figure 47) with the control system shown in Figure 49 indicates that glucose was eliminated at a slightly faster rate in the system shown in Figure 49, production of intermediates was

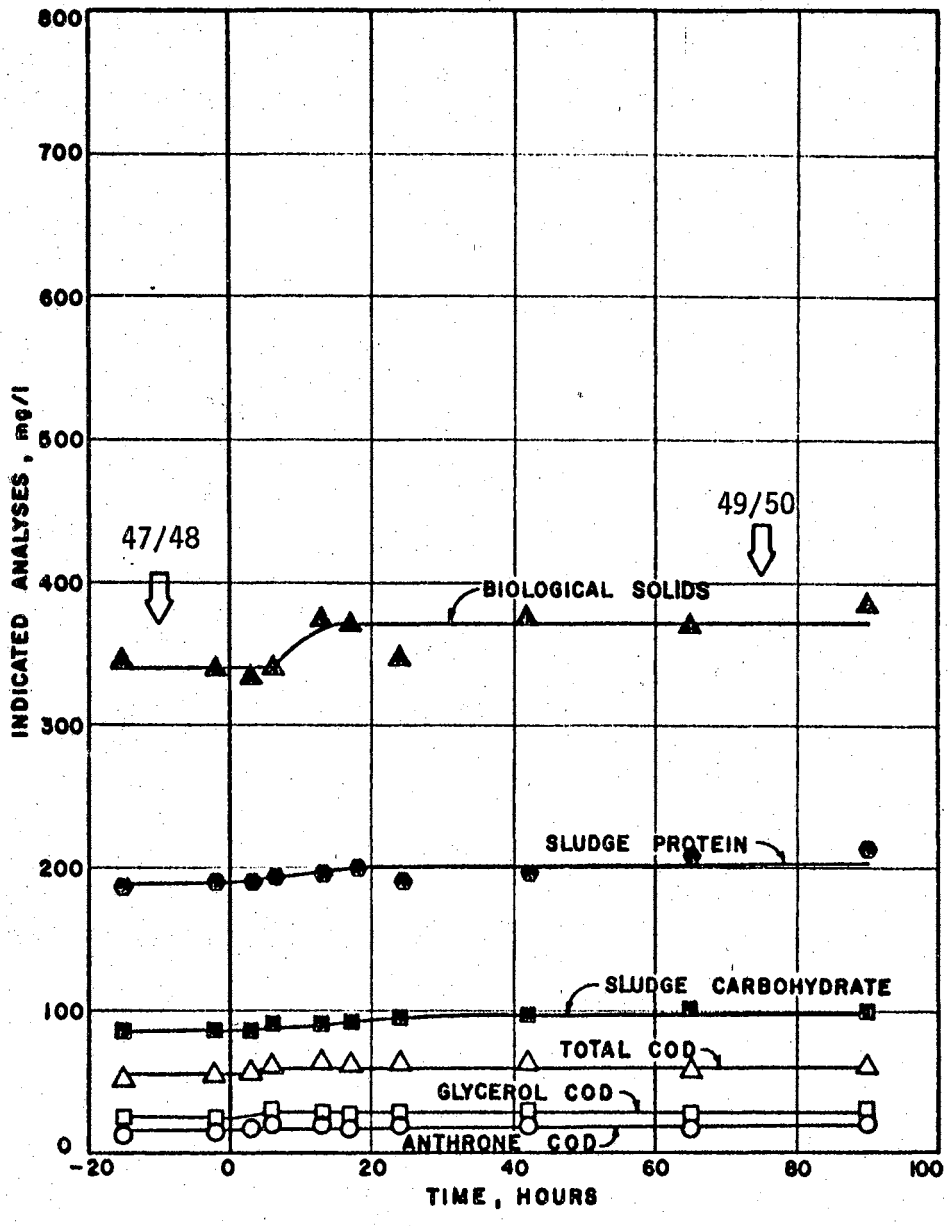

Figure 46 - Response of continuous flow reactor to a change in dilution rate from 1/24 to 1/18 hr^{-1} (constant feed concentration of 500 mg/l glucose + 500 mg/l glycerol).

Figure 47 - Metabolic response under growing conditions in glucose and glycerol controls (cells harvested at a dilution rate of 1/24 hr^{-1}).

Figure 48 - Metabolic response under growing conditions in glucose-glycerol combined system (cells harvested at a dilution rate of 1/24 hr^{-1}).

Figure 49 - Metabolic response under growing conditions in glucose and glycerol controls (cells harvested at a dilution rate of 1/18 hr^{-1}).

Figure 50 - Metabolic response under growing conditions in glucose-glycerol combined system (cells harvested at a dilution rate of 1/18 hr^{-1}).

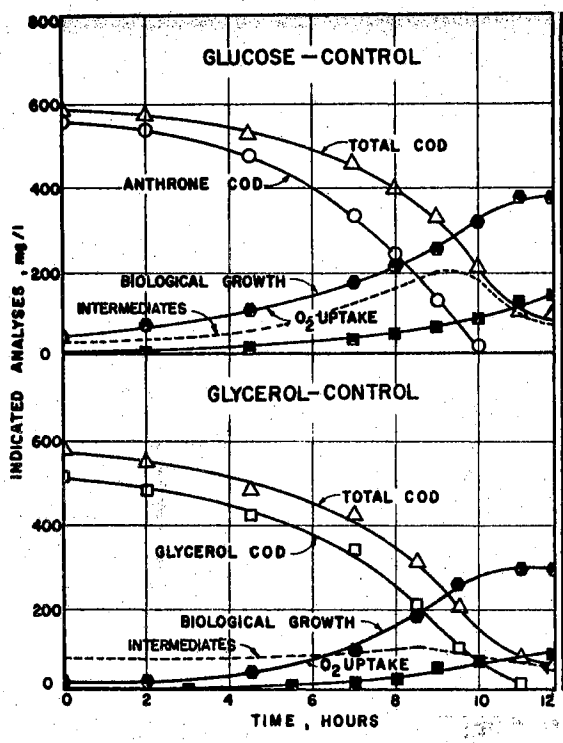

Figure 47

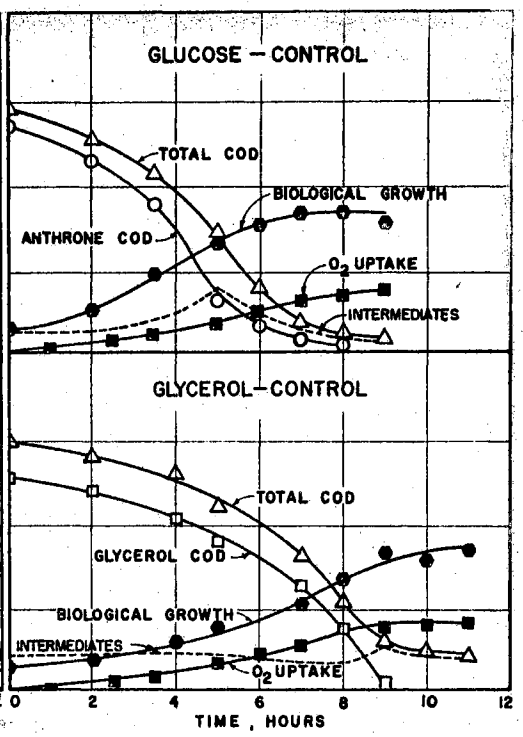

Figure 49

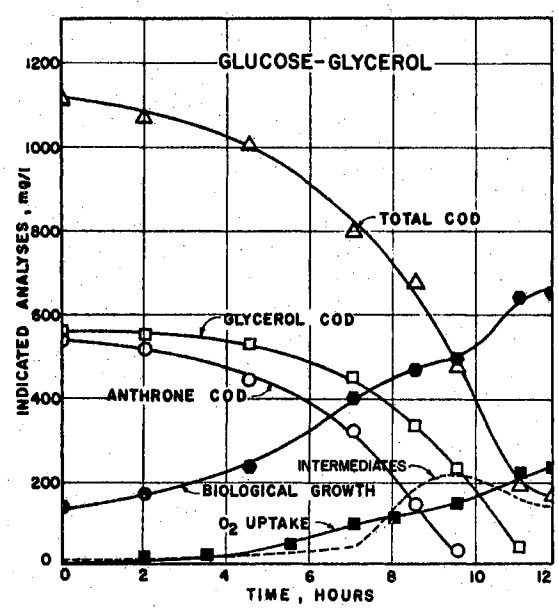

Figure 48

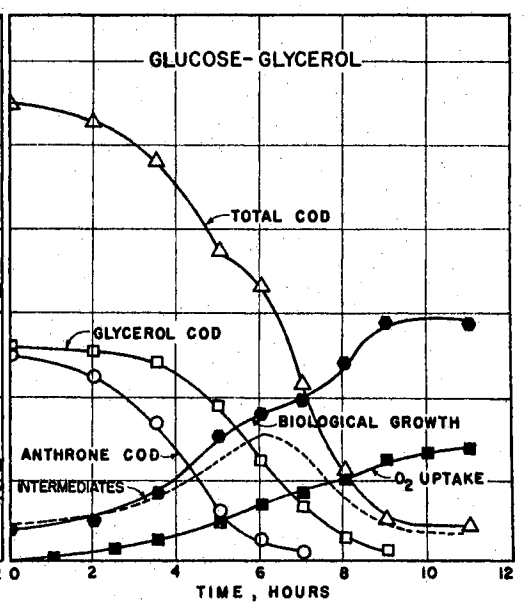

Figure 50

less, and the total COD removal was considerably improved. In the combined system (Figure 50), glucose appeared to retard glycerol removal during the first four hours. When fifty per cent of the glucose was eliminated from the system, only 70 mg/l of the glycerol had been eliminated. There was a large accumulation of metabolic products in the medium during the first six hours; these were later removed at a rather rapid rate after the exhaustion of glucose.

b) Response to Change in Dilution Rate from 1/18 to 1/12 hr^{-1}

Figure 51 shows the response of the system when the dilution rate was changed from 1/18 to 1/12 hr^{-1}. During the transient state after the shock, there was an increase in the biological solids concentration to a maximum of 450 mg/l in sixteen hours, and there was a subsequent reduction to a new steady state value of 420 mg/l, which was higher than the previous steady state solids concentration. There was an increase in sludge yield coefficient. The cell protein increased to a level slightly higher than the previous steady state concentration. The cellular carbohydrate increased initially, but later was reduced. During the transient state there was very little disruption of substrate removal efficiency. The response of the system to this hydraulic shock loading was similar to that obtained for the experiment shown in Figure 46.

Eighty hours after the change in dilution rate from 1/18 to 1/12 hr^{-1}, cells were harvested and used for a batch experiment under proliferating conditions; the results are shown in Figures 53 and 54. In the control system, glucose was metabolized more rapidly than was glycerol. When the glucose and glycerol were used as combined carbon source, the removal of glycerol was noticeably retarded but not

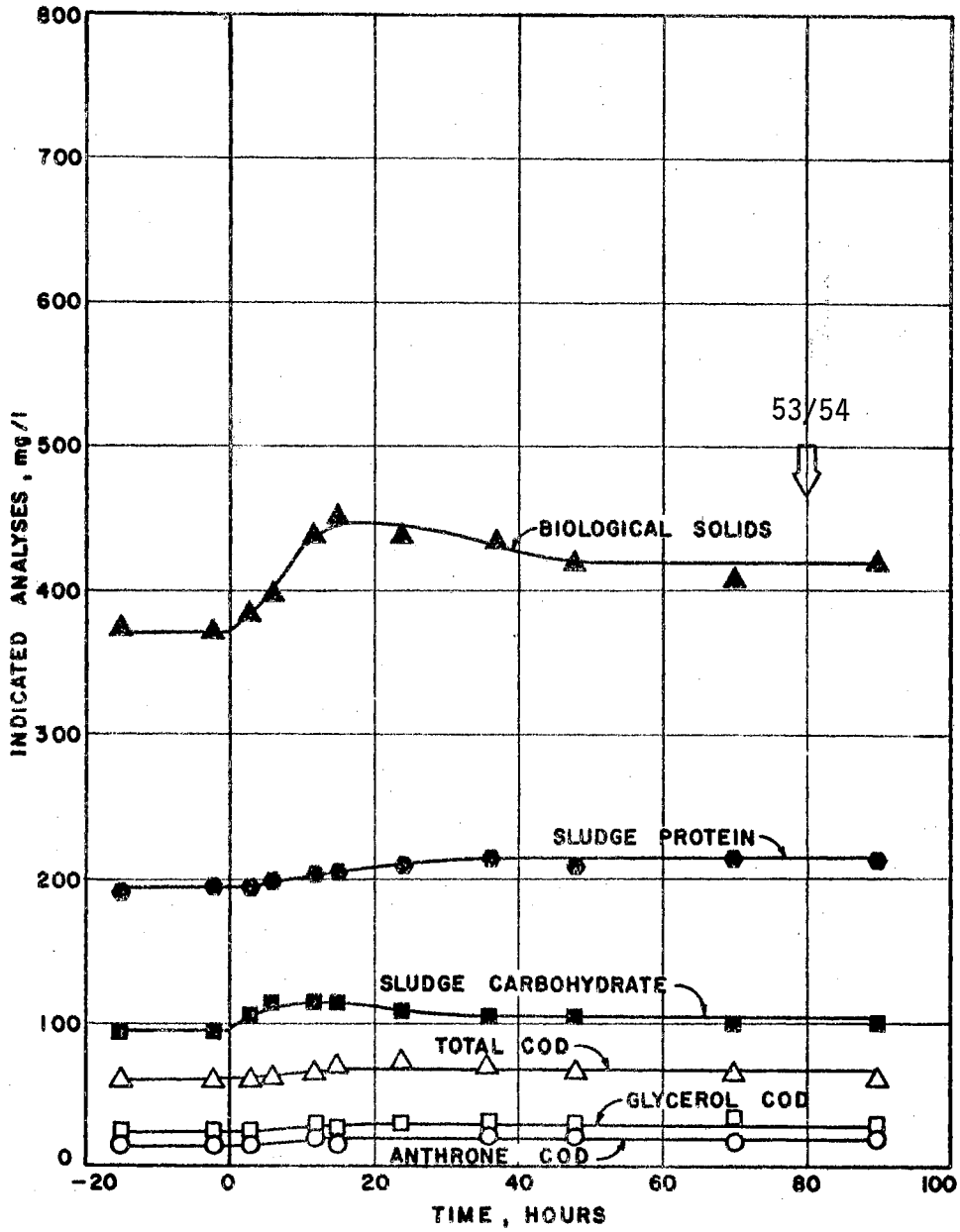

Figure 51 - Response of continuous flow reactor to a change in dilution rate from 1/18 to 1/12 hr^{-1} (constant feed concentration of 500 mg/l glucose + 500 mg/l glycerol).

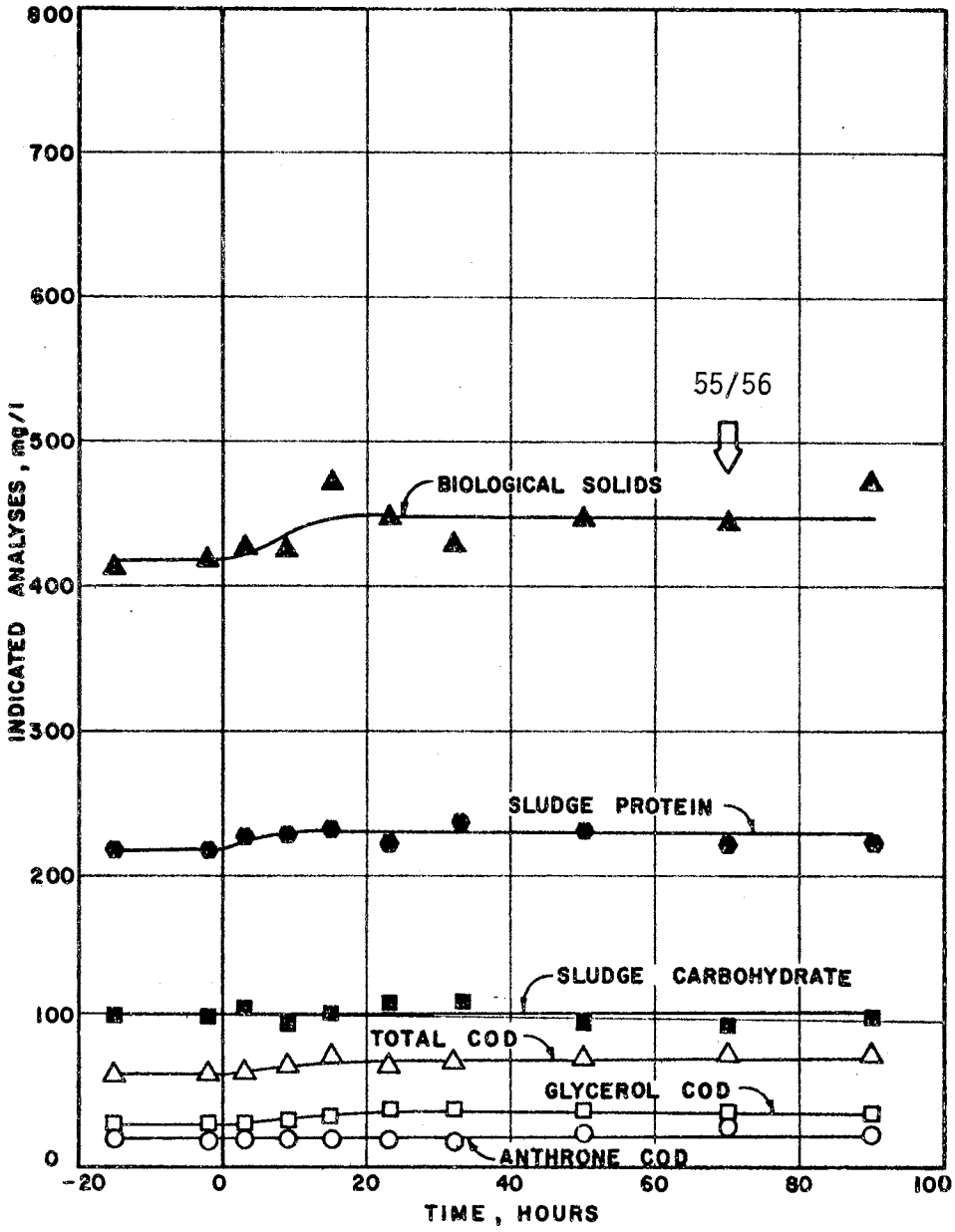

Figure 52 - Response of continuous flow reactor to a change in dilution rate from 1/12 to 1/8 hr^{-1} (constant feed concentration of 500 mg/l glucose + 500 mg/l glycerol).

Figure 53 - Metabolic response under growing conditions in glucose and glycerol controls (cells harvested at a dilution rate of 1/12 hr^{-1}).

Figure 54 - Metabolic response under growing conditions in glucose-glycerol combined system (cells harvested at a dilution rate of 1/12 hr^{-1}).

Figure 55 - Metabolic response under growing conditions in glucose and glycerol controls (cells harvested at a dilution rate of 1/8 hr^{-1}).

Figure 56 - Metabolic response under growing conditions in glucose-glycerol combined system (cells harvested at a dilution rate of 1/8 hr^{-1}).

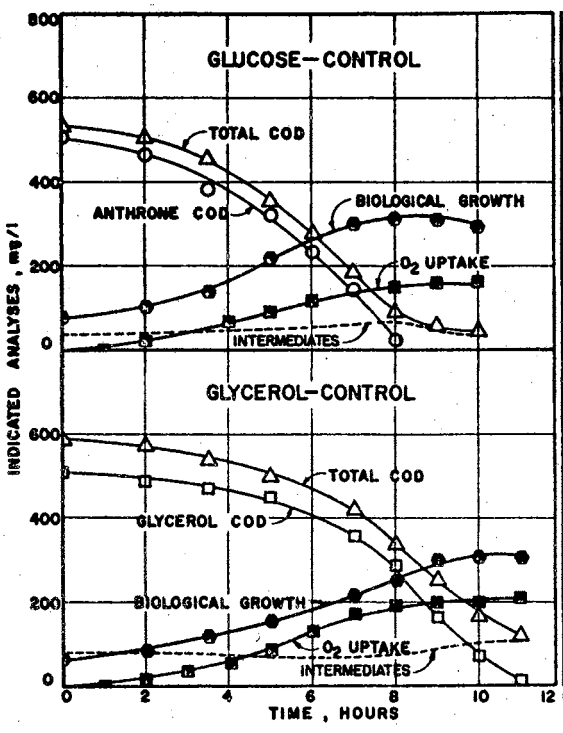

Figure 53

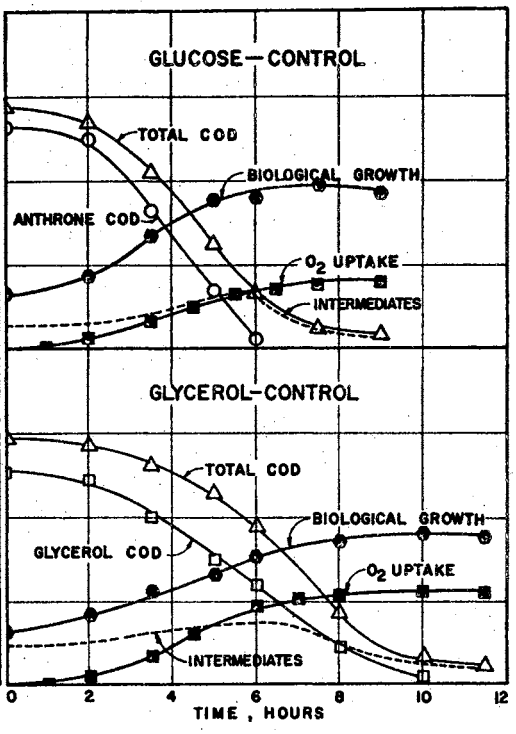

Figure 55

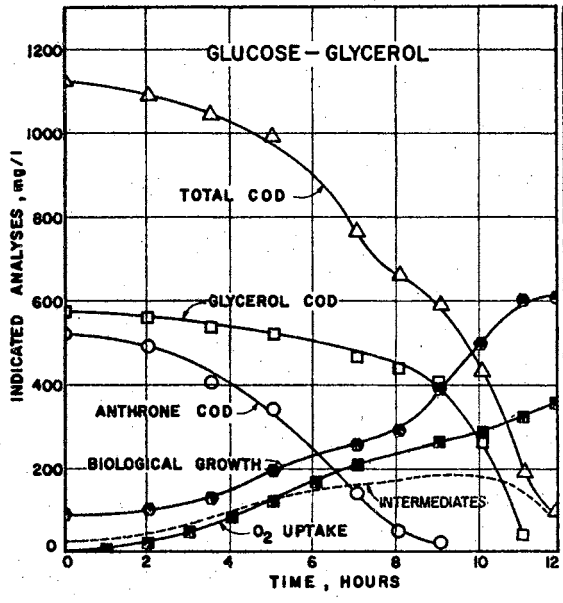

Figure 54

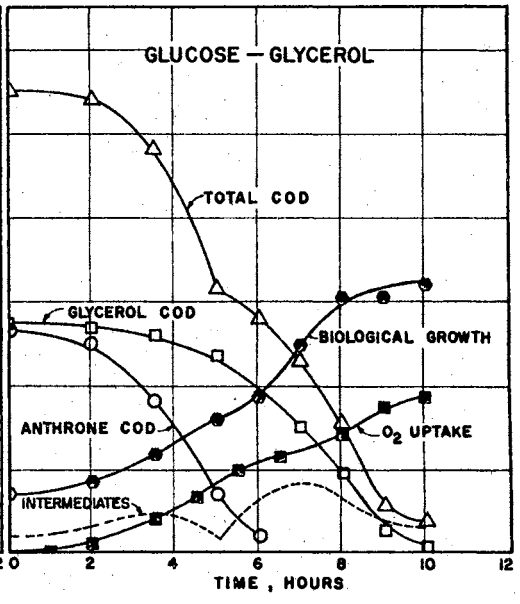

Figure 56

totally blocked by the presence of glucose. When glucose had reached a very low level (approximately 50 mg/l), only 100 mg/l of the glycerol had been removed.

c) Response to Change in Dilution Rate from 1/12 to 1/8 hr^{-1}

Figure 52 shows the biochemical response of the system when the detention time was changed from twelve to eight hours. As with the previous hydraulic shocks, the system responded satisfactorily. Biological solids concentration increased from approximately 420 mg/l to a new steady state solids level of 450 mg/l within sixteen hours, indicating a slight increase in the cell yield coefficient. The performance of this system was in general similar to that at the previous dilution rates (Figures 46 and 51).

After operation at the new dilution rate of 1/8 hr^{-1} for seventy hours (see arrow at Figure 52), cells were taken for batch experiments. The results are shown in Figures 55 and 56. From the control systems it is seen that glucose was utilized at a faster rate than was glycerol. When glucose and glycerol were used as joint carbon source (Figure 56), glycerol removal was retarded in the presence of glucose and the total COD curve was distinctly diphasic in character. The first phase was associated primarily with glucose removal, whereas the second phase corresponded to removal of glycerol.

d) Response to Change in Dilution Rate from 1/8 to 1/6 hr^{-1}

Figure 57 shows the response when the dilution rate was changed from 1/8 hr^{-1} to 1/6 hr^{-1}. During the transient state, the biological solids level rose from a value of approximately 450 mg/l to a transient peak value of approximately 520 mg/l at sixteen hours after the shock,

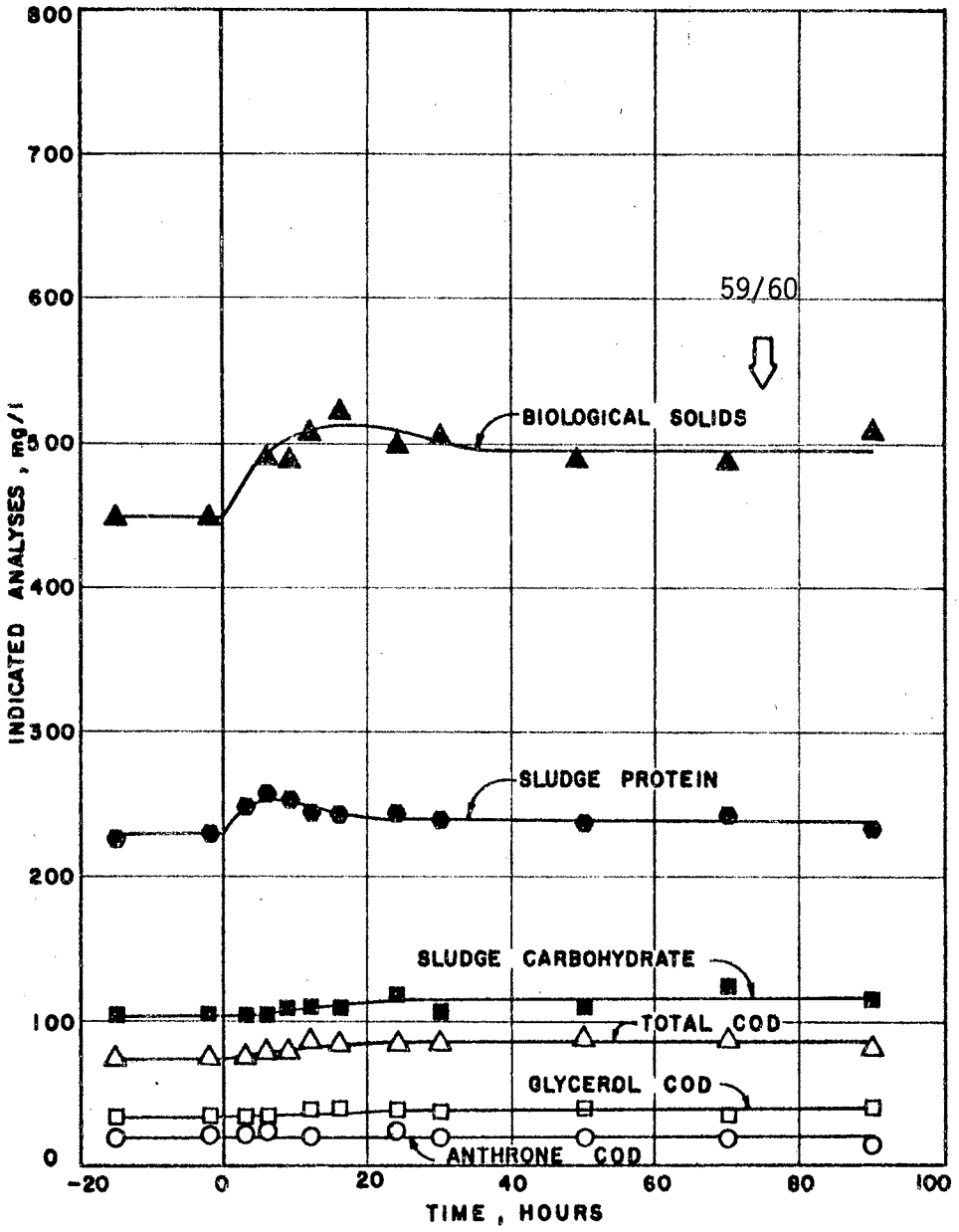

Figure 57 - Response of continuous flow reactor to a change in dilution rate from 1/8 to 1/6 hr^{-1} (constant feed concentration of 500 mg/l glucose + 500 mg/l glycerol).

and thereafter decreased to a new steady state value of 500 mg/l at thirty hours. Initially, the total cell protein curve paralleled the biological solids curve, but after six hours it began to decrease to a new constant value slightly higher than the previous steady state concentration. There was a slow increase in the cellular carbohydrate. The total COD rose slightly from a steady state value of nearly 75 mg/l to a new constant level of 85 mg/l twenty hours after the application of the shock. There was no significant change in anthrone COD or glycerol COD in the effluent. It was observed again that an increase in flow rate tended to increase the cell yield coefficient of the system.

The results of the experimentation in batch systems employing cells harvested seventy-five hours after changing the dilution rate to 1/6 hr^{-1} are shown in Figures 59 and 60. The results for the control systems (Figure 59) indicate that glucose was removed more rapidly than was glycerol. When glucose and glycerol were used as combined substrate (Figure 60), each was metabolized somewhat more slowly than when fed as sole carbon sources (compare Figures 59 and 60).

e) Response to Change in Dilution Rate from 1/6 hr^{-1} to 1/3 hr^{-1}

Figure 58 shows the response when the dilution rate was increased from 1/6 hr^{-1} to 1/3 hr^{-1}. The efficiency of the system was disrupted severely. The biological solids decreased from approximately 500 mg/l to nearly 380 mg/l, and then rose to a lower new steady state level of 460 mg/l. During the period of cell washout, the total COD rose to nearly 370 mg/l, but rapidly returned to a lower value in response to the recovery in solids concentration. A considerable portion of the effluent COD was attributable to glycerol. Anthrone COD increased from

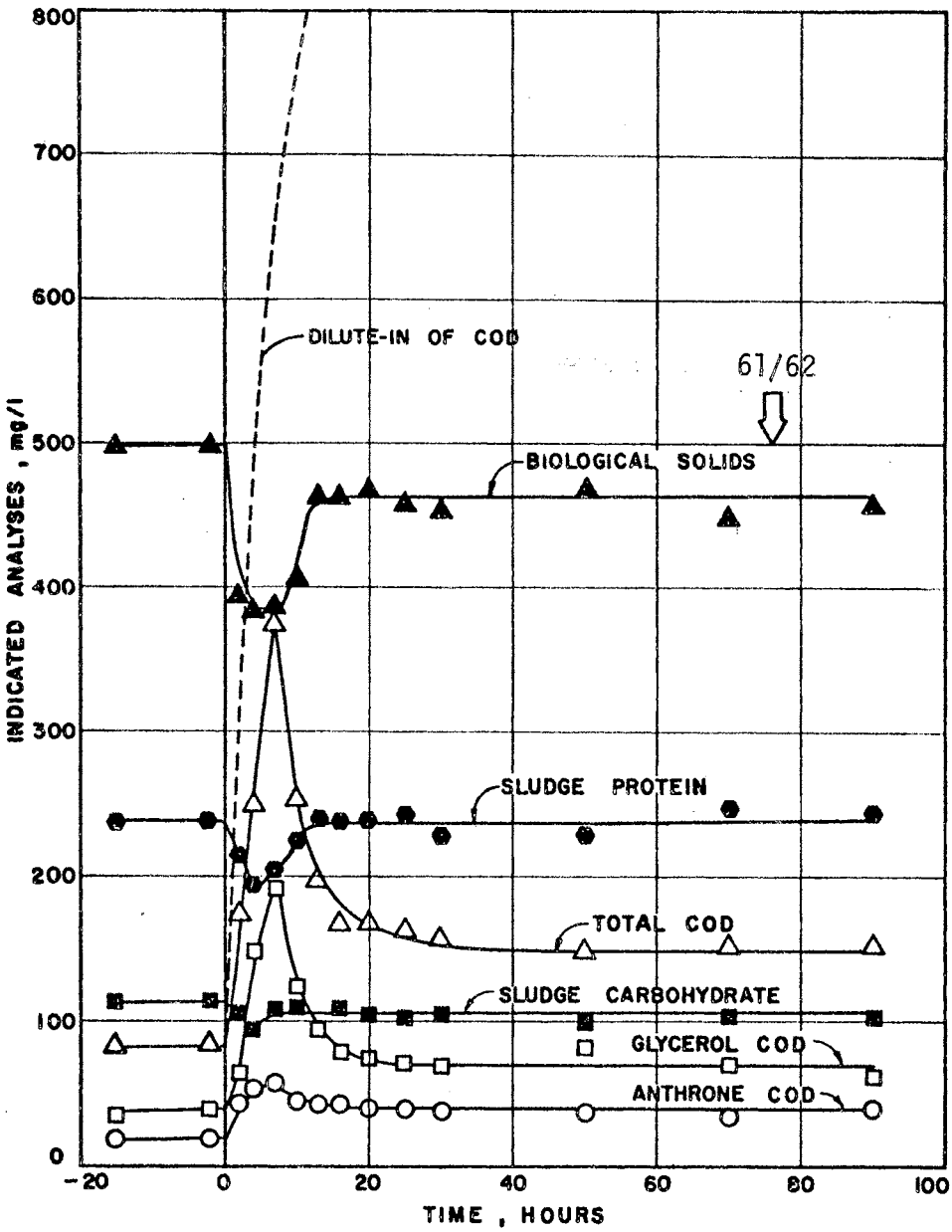

Figure 58 - Response of continuous flow reactor to a change in dilution rate from 1/6 to 1/3 hr^{-1} (constant feed concentration of 500 mg/l glucose + 500 mg/l glycerol).

Figure 59 - Metabolic response under growing conditions in glucose and glycerol controls (cells harvested at a dilution rate of 1/6 hr^{-1}).

Figure 60 - Metabolic response under growing conditions in glucose-glycerol combined system (cells harvested at a dilution rate of 1/6 hr-1).

Figure 61 - Metabolic response under growing conditions in glucose and glycerol controls (cells harvested at a dilution rate of 1/3 hr^{-1}).

Figure 62 - Metabolic response under growing conditions in glucose-glycerol combined system (cells harvested at a dilution rate of 1/3 hr^{-1}).

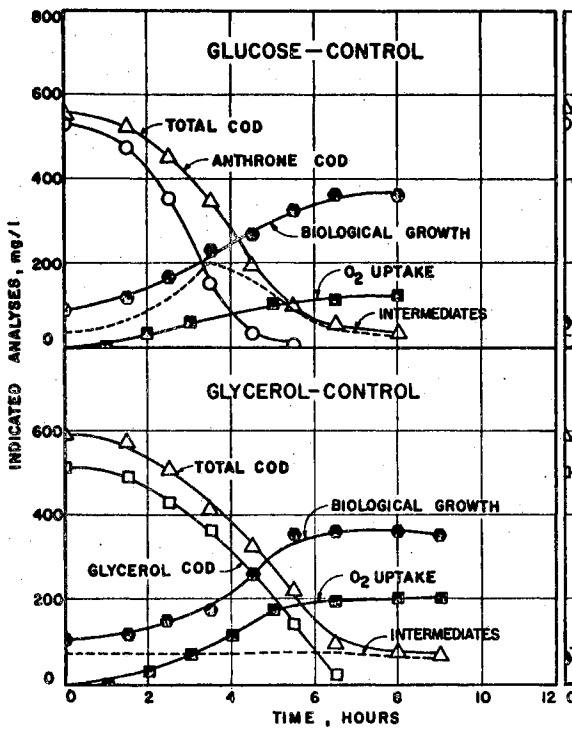

Figure 59

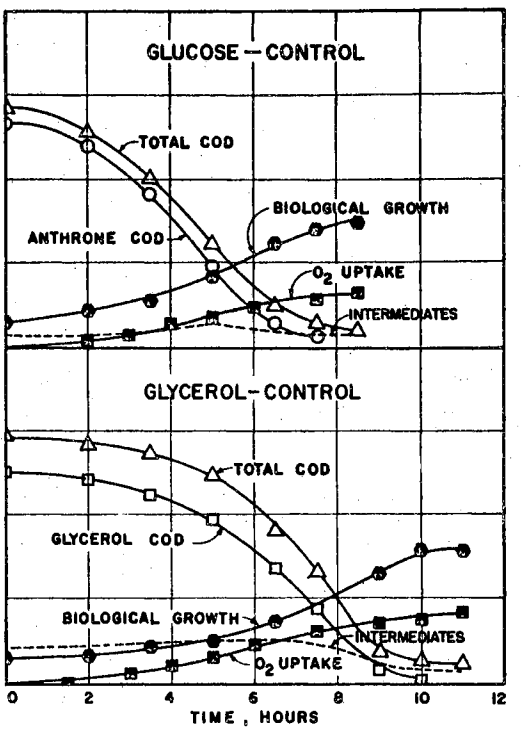

Figure 61

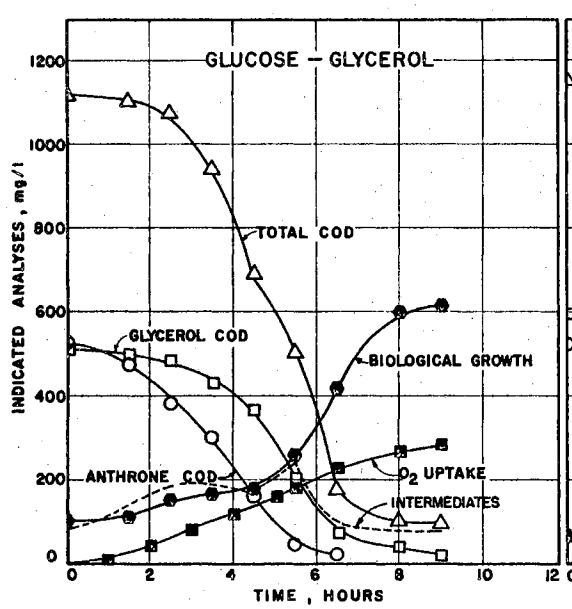

Figure 60

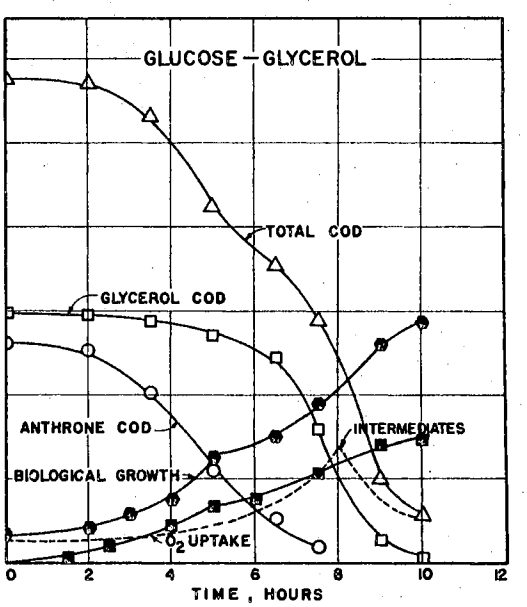

Figure 62

a value of approximately 20 mg/l to a transient peak value of nearly 55 mg/l, and thereafter returned to a new steady state value of approximately 40 mg/l in thirteen hours. Comparison of the effluent total COD curve with the dilute-in curve during the early portion of transient response indicates that the immediate response was a drastic pause in growth (and substrate removal) before the system could adjust to the new growth rate.

Results of batch studies using cells harvested for experimentation at the new dilution rate (see arrow, Figure 58) are shown in Figures 61 and 62. In the control systems (Figure 61), glucose was metabolized at a faster rate than was glycerol. When the compounds were used as a joint carbon source, the glycerol utilization was significantly retarded by the presence of glucose; after the exhaustion of glucose, glycerol metabolism proceeded quite rapidly.

5. Quantitative Shock Loadings in a Two-Component Carbon Source System

In many experiments previously accomplished in the bioengineering laboratories, the effect of quantitative, qualitative, and combined shock loads has been assessed. However, the effect of a quantitative shock loading in a system which had been previously operating on a multicomponent carbon source had not been investigated. It was felt that such studies could make a useful input to the body of environmental response data which has been obtained.

In these studies the dilution rate was maintained at 1/8 hr^{-1} while increasing the feed concentration from 500 mg/l to 4000 mg/l (equal portions of glucose and glycerol) in a series of shocks in which the organic load was doubled each time. The performance of the system was assessed for each steady state condition and each transient

response to the quantitative shocks. During operation at each steady state, cells were harvested from the effluent for batch studies to determine μ_m and k_S on the glucose-glycerol substrate. The results are presented in a manner similar to those of the previous sections (3 and 4).

The system was started using an initial seed obtained from the primary clarifier effluent of the municipal sewage treatment plant at Stillwater, Oklahoma. At the start of continuous flow operation, the synthetic waste consisted of 250 mg/l glucose plus 250 mg/l glycerol. The system was operated in the steady state for three days, then the feed was changed to 500 mg/l glucose plus 500 mg/l glycerol. The response is shown in Figure 63. During the transient state, the total COD increased to a maximum of 155 mg/l, and was gradually reduced to a new steady state value of 100 mg/l after thirty-six hours. There was essentially no change in the anthrone COD in the effluent during the transient state; however, glycerol COD reached a maximum of 80 mg/l, then was reduced to a new steady state value of 45 mg/l after thirty-six hours. The biological solids concentration increased from approximately 230 mg/l to a maximum of 570 mg/l, and then decreased to a fairly constant value (490 mg/l) after seventy hours. There was a concomitant increase in protein and carbohydrate.

During operation at the initial steady state feeding level (see arrow at minus ten hours, Figure 63), cells were taken from the effluent reactor for batch experiments to study the relationship between growth rate (μ) and substrate concentration on equal portions of glucose and glycerol as joint carbon source. The batch experiments were run in a manner similar to those of the previous experiment (section 2). In

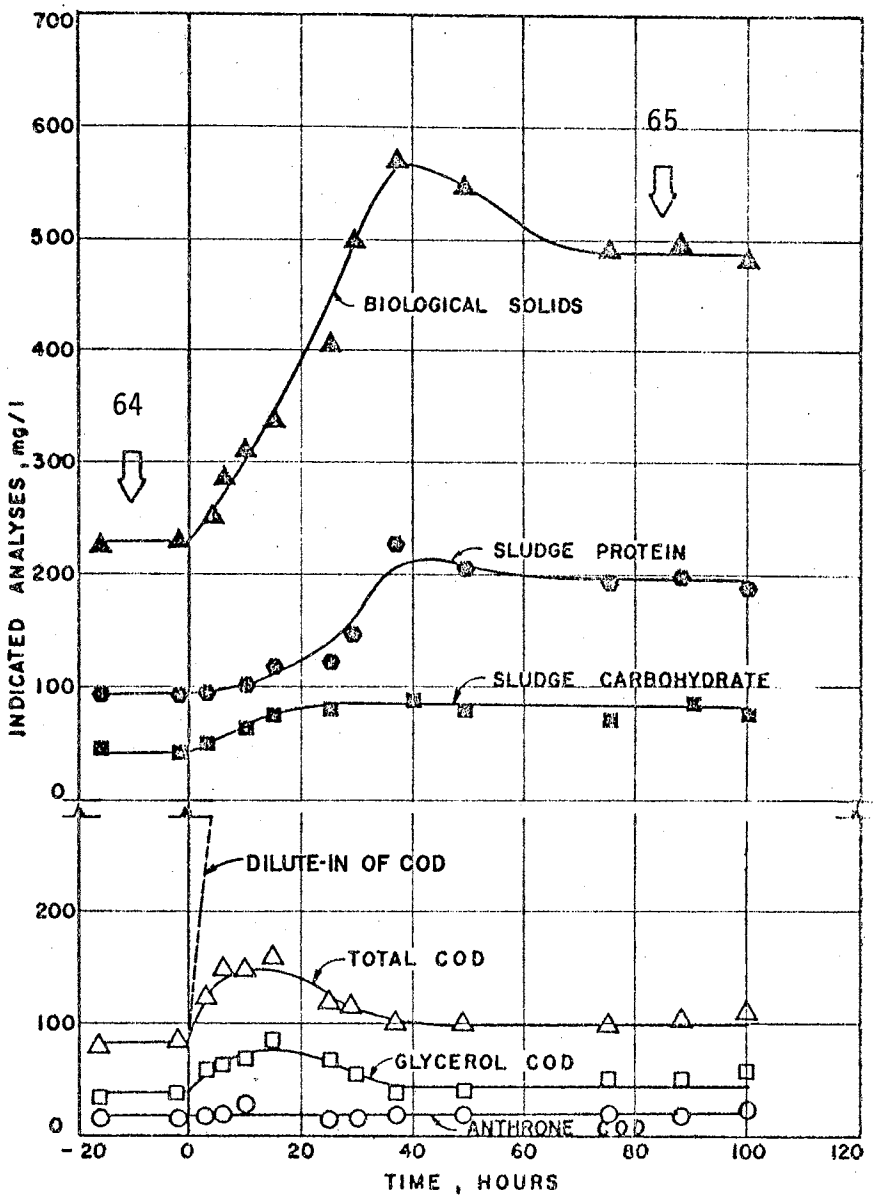

Figure 63 - Response of a system shock loaded from 250 mg/l glucose + 250 mg/l glycerol to 500 mg/l glucose + 500 mg/l glycerol (dilution rate 1/8 hr^{-1}).

Figure 64, curve A was plotted from the μ_m and k_S values obtained from a plot of μ versus S (Monod), and curve B was plotted from values obtained from a plot of $1/\mu$ versus $1/S$ (Lineweaver and Burke plot). The μ_m values obtained with the combined glucose and glycerol as carbon source compare very well with those obtained on glucose as sole carbon source (see Figure 18).

Shortly after the continuous flow unit had attained its new steady state level (see arrow at hour 85, Figure 63), cells were again harvested for batch experiments to investigate the relationship between growth rate and substrate concentration. The value of μ_m obtained with glucose and glycerol as joint carbon source was found to be 0.294 hr^{-1} (reciprocal plot, Figure 65). This value is slightly lower than the μ_m value obtained with glucose alone (Figure 21).

The system was operated for a few days at the new feeding level, then the influent was changed from 500 mg/l glucose plus 500 mg/l glycerol to 1000 mg/l glucose plus 1000 mg/l glycerol. The response is shown in Figure 66. There was an increase in the total COD concentration from approximately 100 mg/l to a fairly constant value of 235 mg/l after thirty-five hours. The glycerol COD concentration reached a new steady state level of 140 mg/l after thirty hours; however, there was no change in glucose (anthrone) concentration in the effluent as a result of this shock. It is seen that the general response of the system with respect to solids and COD was similar to that shown in Figure 20 for a comparable shock situation when glucose was the sole carbon source. A major point of interest concerning the response shown in Figure 66 is that the increased glucose in the feed appeared to cause a steady level of leakage in glycerol (periodate) COD.

After operation for ninety-five hours at the new steady state

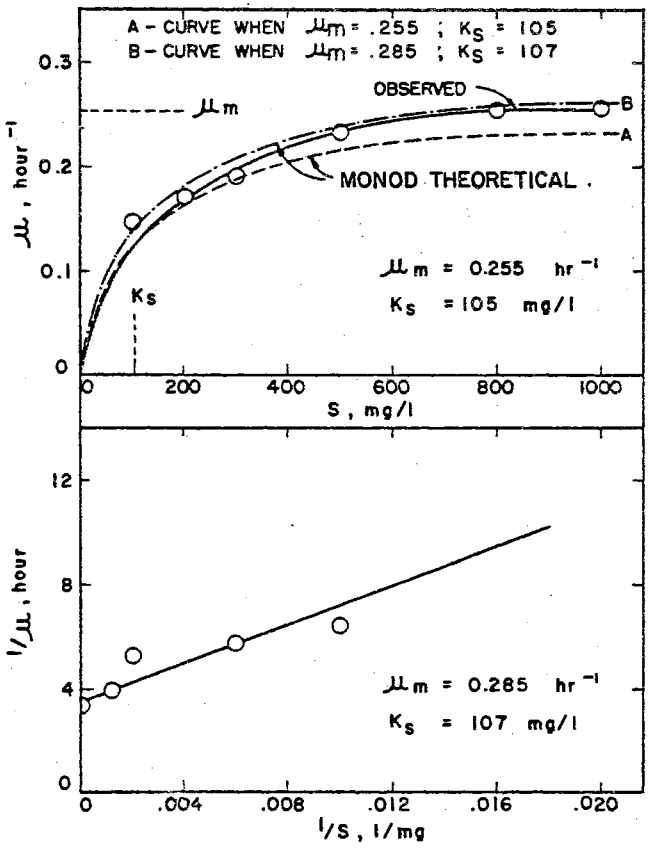

Figure 64 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" operated with 250 mg/l each of glucose and glycerol).

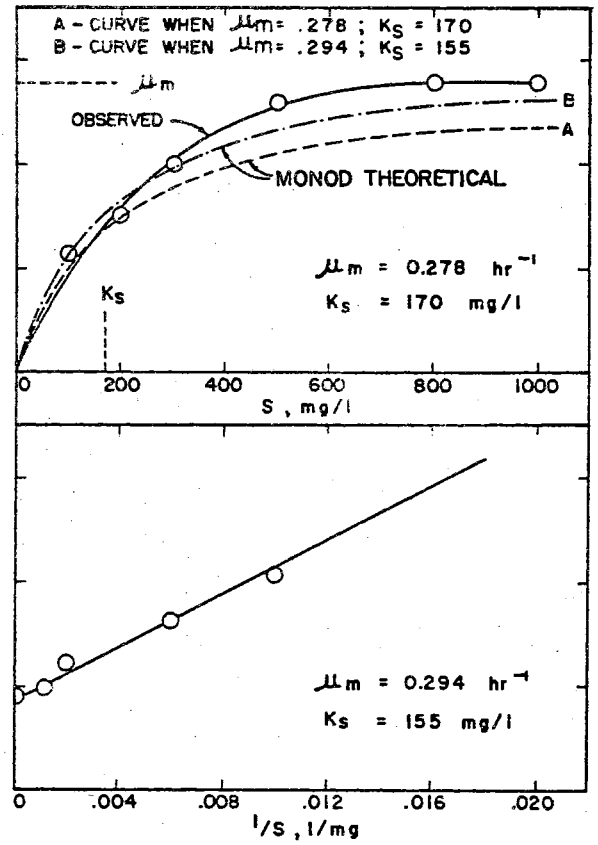

Figure 65 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" operated with 500 mg/l each of glucose and glycerol).

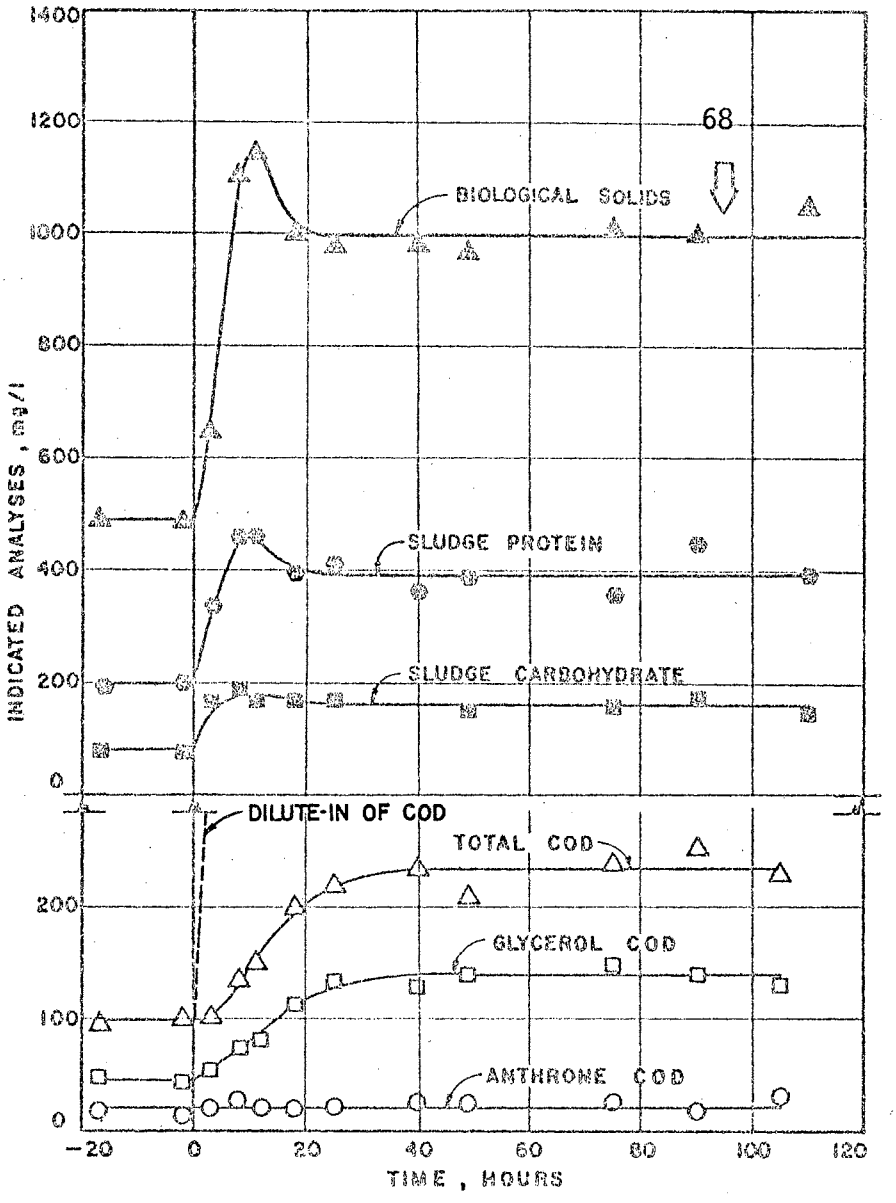

Figure 66 - Response of a system shock loaded from 500 mg/l glucose + 500 mg/l glycerol to 1000 mg/l glucose + 1000 mg/l glycerol (dilution rate 1/8 hr^{-1}).

feeding level, cells were harvested from the effluent to assess the growth rate under batch conditions (see arrow, Figure 66). The results of the growth study are shown in Figure 68. The μ_m value obtained with glucose plus glycerol as the combined carbon source was found to be 0.270 hr^{-1}, which is slightly lower than that of the previous experiment with glucose medium as shown in Figure 22.

Figure 67 shows the response when the influent was increased from 1000 mg/l glucose plus 1000 mg/l glycerol to 2000 mg/l glucose plus 2000 mg/l glycerol. There was a significant leakage of substrate during the transient state. A maximum of 1000 mg/l total COD escaped into the influent in six hours. Within fourteen hours, the concentration was reduced to the pre-shock level. Thereafter the total COD increased slowly and a new steady state value of 300 mg/l was attained. The final total COD was somewhat higher than the previous steady state value. At the peak in effluent COD it is seen that approximately half of the COD could be attributed to metabolic intermediates, and that considerably more glycerol COD (periodate) than glucose COD (anthrone) escaped.

The early phase of the transient response is particularly interesting. It can be estimated from the theoretical COD dilute-in curve (dotted curve) for the shock that the effluent COD would have been approximately 1500 mg/l at the time the maximum effluent COD was recorded if none of the additional 1000 mg/l glucose plus 1000 mg/l glycerol had been removed. The transient COD peaked at 1000 mg/l, indicating that approximately 500 mg/l of the shock COD was removed by the cells. At this point in the transient response the biological solids concentration had increased by approximately 500 mg/l, indicating that essentially all of the substrate had been channelled into sludge

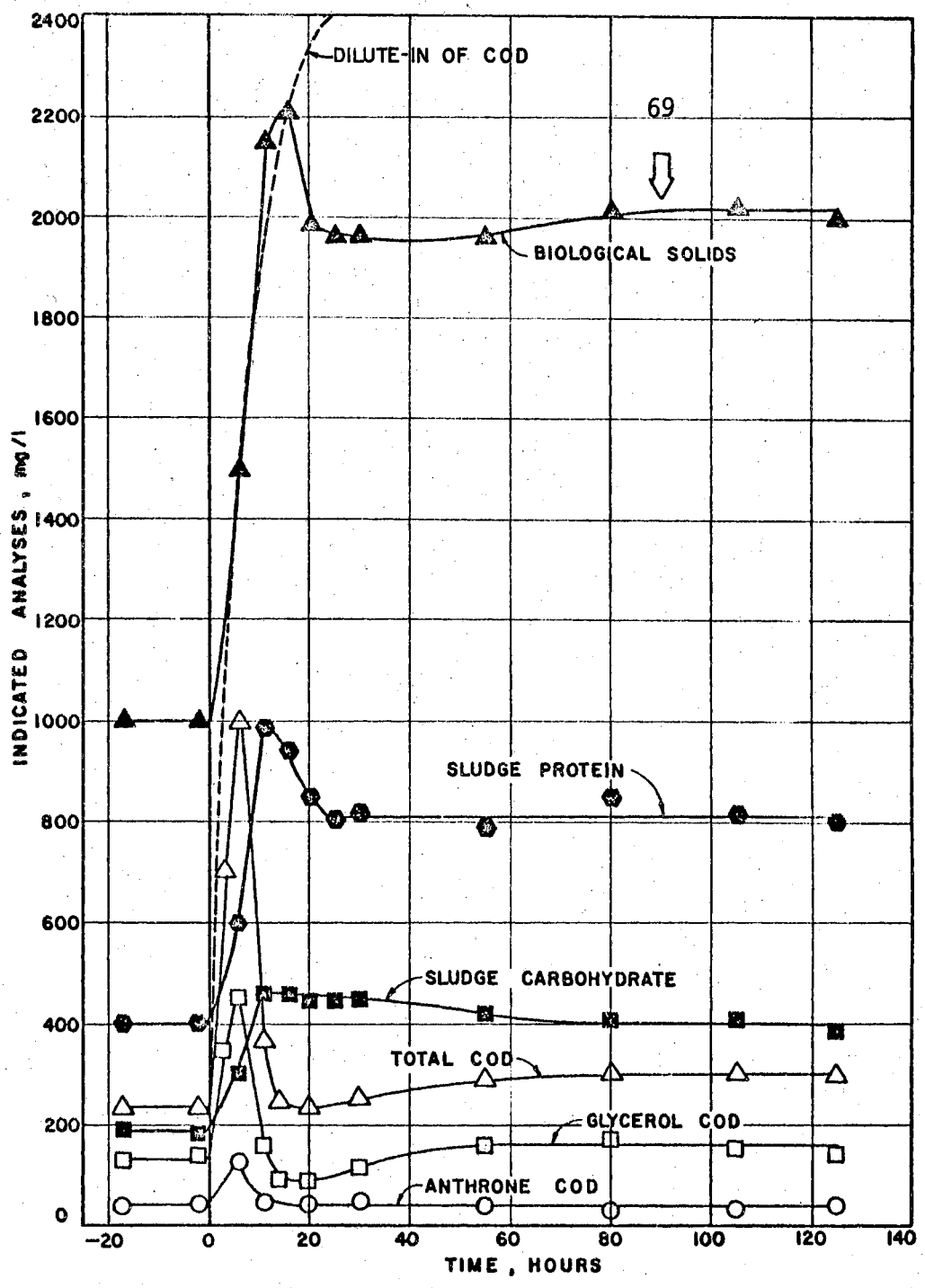

Figure 67 - Response of a system shock loaded from 1000 mg/l glucose + 1000 mg/l glycerol to 2000 mg/l glucose + 2000 mg/l glycerol (dilution rate 1/8 hr^{-1}).

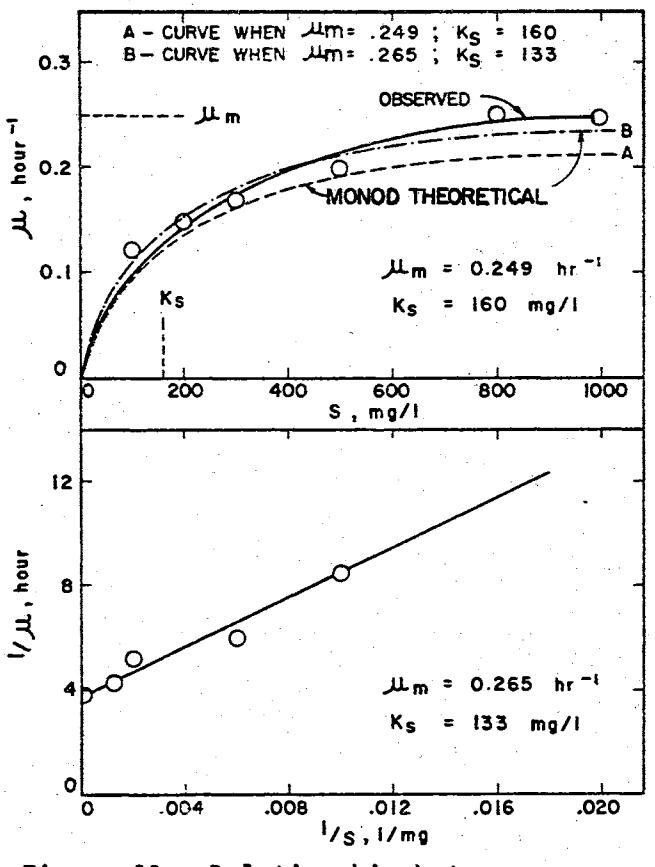

Figure 68 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" operated with 1000 mg/l each of glucose and glycerol).

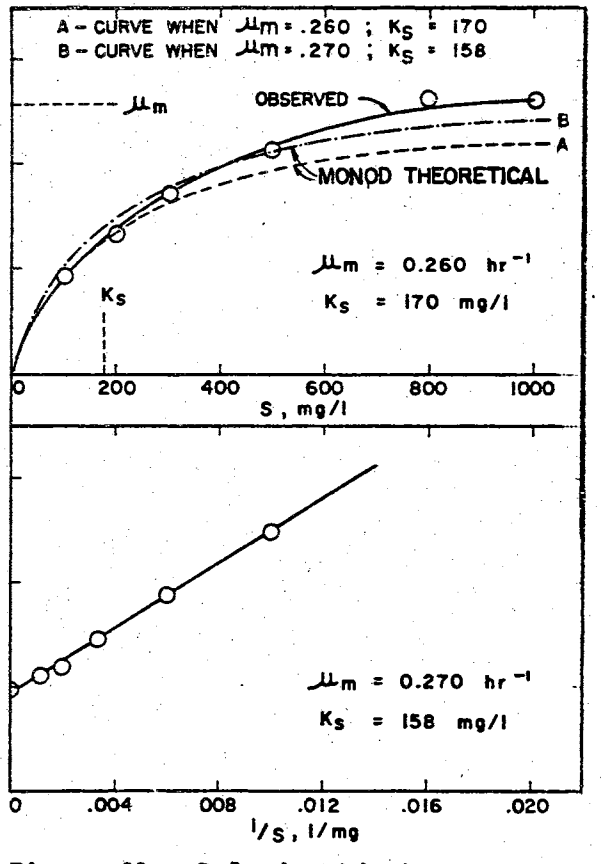

Figure 69 - Relationship between specific growth rate and substrate concentration (cells harvested from the "steady state" operated with 2000 mg/l each of glucose and glycerol).

synthesis. While a sludge yield of 100 per cent does not seem realistic, the results do indicate that there was a rather large increase in sludge yield during the early part of the transient stage. By the time the solids concentration attained a peak value, approximately 2200 mg/l at seventeen hours, the effluent COD had returned to approximately the level which existed before the shock, and the sludge yield was approximately the same as it had been before the shock.

Ninety hours after changing the influent feed concentration from 1000 mg/l glucose plus 1000 mg/l glycerol to 2000 mg/l glucose plus 2000 mg/l glycerol, cells were harvested for batch studies under growth conditions (see arrow, Figure 67). The results which are shown in Figure 69 indicate a μ_m value of 0.270 hr^{-1}. This value is slightly lower than the μ_m value obtained with glucose as single carbon source (Figure 25).

Values of μ_m and k_s are summarized in Table II. It is seen that the value of μ_m while not constant, varied over a rather small range. Comparison of the results shown in Tables I and II indicates that for the cells taken from the reactor under steady state conditions, the μ_m values obtained with glucose medium as single carbon source were slightly higher than those obtained with glucose plus glycerol as joint carbon sources.

6. Effect of Successive Increases in Organic Loadings

This portion of the study was designed to determine the extent of interference between waste components in the continuous flow activated sludge process with successive increases in organic loadings. In the previous studies the systems were shocked and a new steady state was attained, and after assessing the new steady condition, another shock

TABLE II

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS USING CELLS ACCLIMATED TO GLUCOSE AND GLYCEROL AS COMBINED CARBON SOURCE

($D = 1/8\ hr^{-1}$)

Glucose plus glycerol (equal portions) feed concentration mg/l	Growth parameter for cells taken from the steady state continuous flow unit					
	μ_{m_1}	k_{S_1}	μ_{m_2}	k_{S_2}	μ_m	k_S
	hr^{-1}	mg/l	hr^{-1}	mg/l	hr^{-1}	mg/l
500	.255	105	.285	107	.272	106
1000	.278	170	.294	155	.286	162
2000	.249	160	.265	133	.257	146
4000	.260	175	.270	158	.265	166

μ_{m_1} and k_{S_1} are calculated from graph of μ versus S

μ_{m_2} and k_{S_2} are calculated from graph of $1/\mu$ versus $1/S$

μ_m and k_s are the mean values of μ_{m_1} and μ_{m_2}, and k_{S_1} and k_{S_2}

was applied. In the present study the feed was changed each twenty-four hours regardless of the condition of the system.

In this study, glucose was fed at 1000 mg/l, and after establishing steady state conditions, the feed was changed from 1000 mg/l glucose to 1000 mg/l glucose plus 1000 mg/l glycerol. Thereafter, the glucose concentration was maintained at 1000 mg/l and the glycerol concentration was increased each twenty-four hours in increments of 1000 mg/l until the 3000 mg/l level was reached.

Two separate sets of experiments were conducted: one at D = 1/12 hr^{-1}, and another at D = 1/8 hr^{-1}. Figure 70 shows the response when the feed was changed from 1000 mg/l glucose to 1000 mg/l glucose plus 1000 mg/l glycerol at a dilution rate of 1/12 hr^{-1}. There was a rapid increase in the level of total COD in the filtrate. A maximum level of 340 mg/l was attained shortly after administering the shock loading; a new "steady state" level of 250 mg/l was obtained within eighteen hours. There was only a slight transient increase in anthrone COD. Glycerol COD rose to approximately 185 mg/l and then decreased to approximately 150 mg/l. The biological solids concentration did not increase significantly during the period of maximum leakage of COD. Acclimation to glycerol took place within five hours.

Figure 71 shows the response when the feed was changed to 1000 mg/l glucose plus 2000 mg/l glycerol. The first plotting point in Figure 71 coincides with the last plotting point in Figure 70. A relatively high increase in the total filtrate COD can be noted; it rose to a new steady state level of 440 mg/l, which is nearly twice the previous effluent COD concentration. There was no change in anthrone COD. The increase in total filtrate COD in general paralleled the

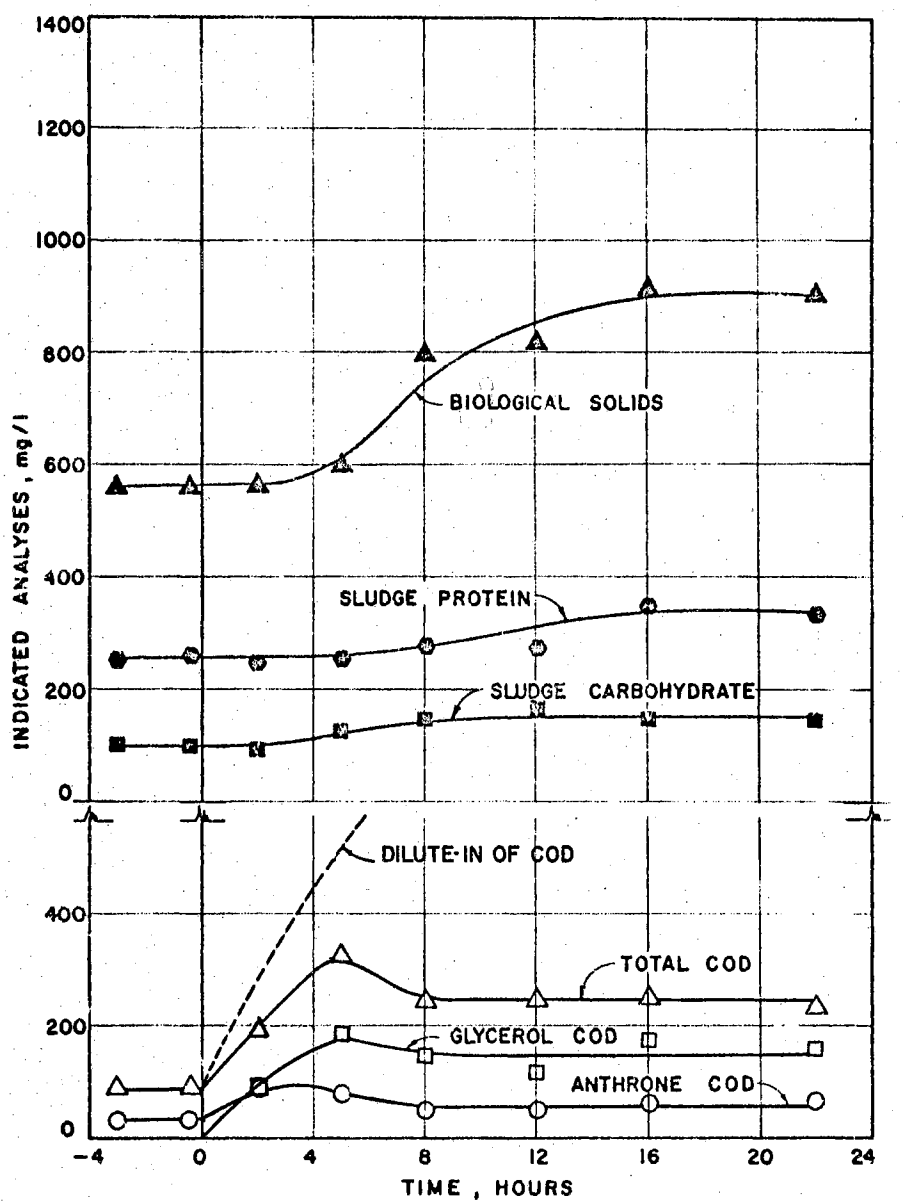

Figure 70 - Response of a system shock loaded from 1000 mg/l glucose to 1000 mg/l glucose + 1000 mg/l glycerol (dilution rate 1/12 hr^{-1}).

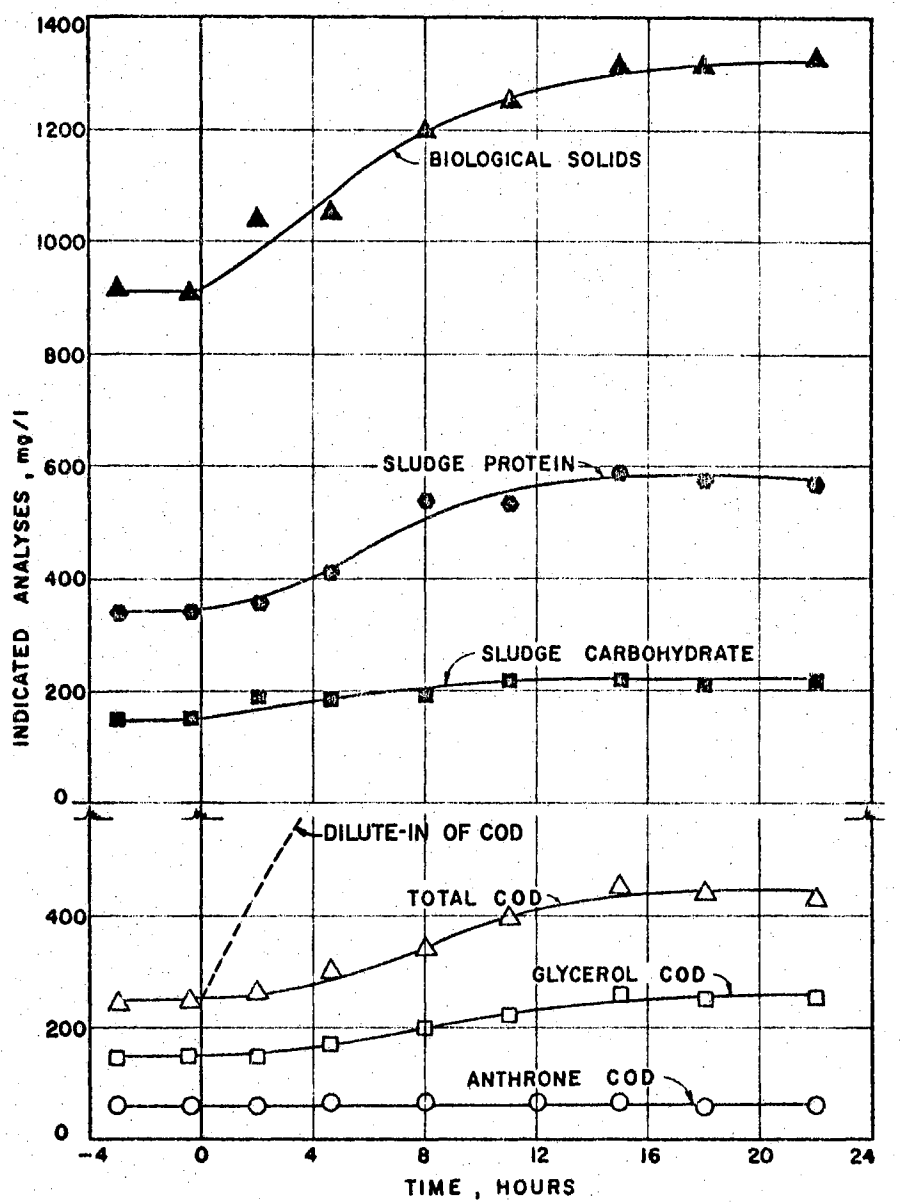

Figure 71 - Response of a system shock loaded from 1000 mg/l glucose + 1000 mg/l glycerol to 1000 mg/l glucose + 2000 mg/l glycerol (dilution rate 1/12 hr^{-1}).

glycerol COD curve. A significant increase in biological solids was observed, with no apparent lag or acclimation period. It is also observed that the increase in biological solids concentration was accompanied by proportionately greater protein than carbohydrate synthesis.

Figure 72 shows the response when, after twenty-four hours of operation, the feed was changed by increasing the inflow waste strength to 1000 mg/l glucose plus 3000 mg/l glycerol. There was an increase in total filtrate COD to a level of 580 mg/l shortly after changing the influent feed. The concentration thereafter decreased and attained a new steady state value of 500 mg/l in ten hours. There was no significant effect on the efficiency of removal of glycerol; however, the glucose COD (anthrone) was rapidly increased to a new steady state level of 185 mg/l within two hours. There was a steady increase in the biological solids concentration to approximately 2040 mg/l within fifteen hours after the shock was applied. Again it can be seen that the increase in the biological solids was accompanied by an increase in both protein and carbohydrate content of the solids.

A similar set of experiments was performed for which the system was operated at a dilution rate of 1/8 hr^{-1} rather than 1/12 hr^{-1}. Figure 73 shows the response when the feed was changed from 1000 mg/l glucose to 1000 mg/l glucose plus 1000 mg/l glycerol. It is seen that even though the inflowing waste concentration was increased two-fold, there was very little effect on the COD removal efficiency as indicated by the level of total COD. It is interesting to compare these results with the results obtained in Figure 70 (dilution rate of 1/12 hr^{-1}) in which the system was shock-loaded with the same level of substrate. At

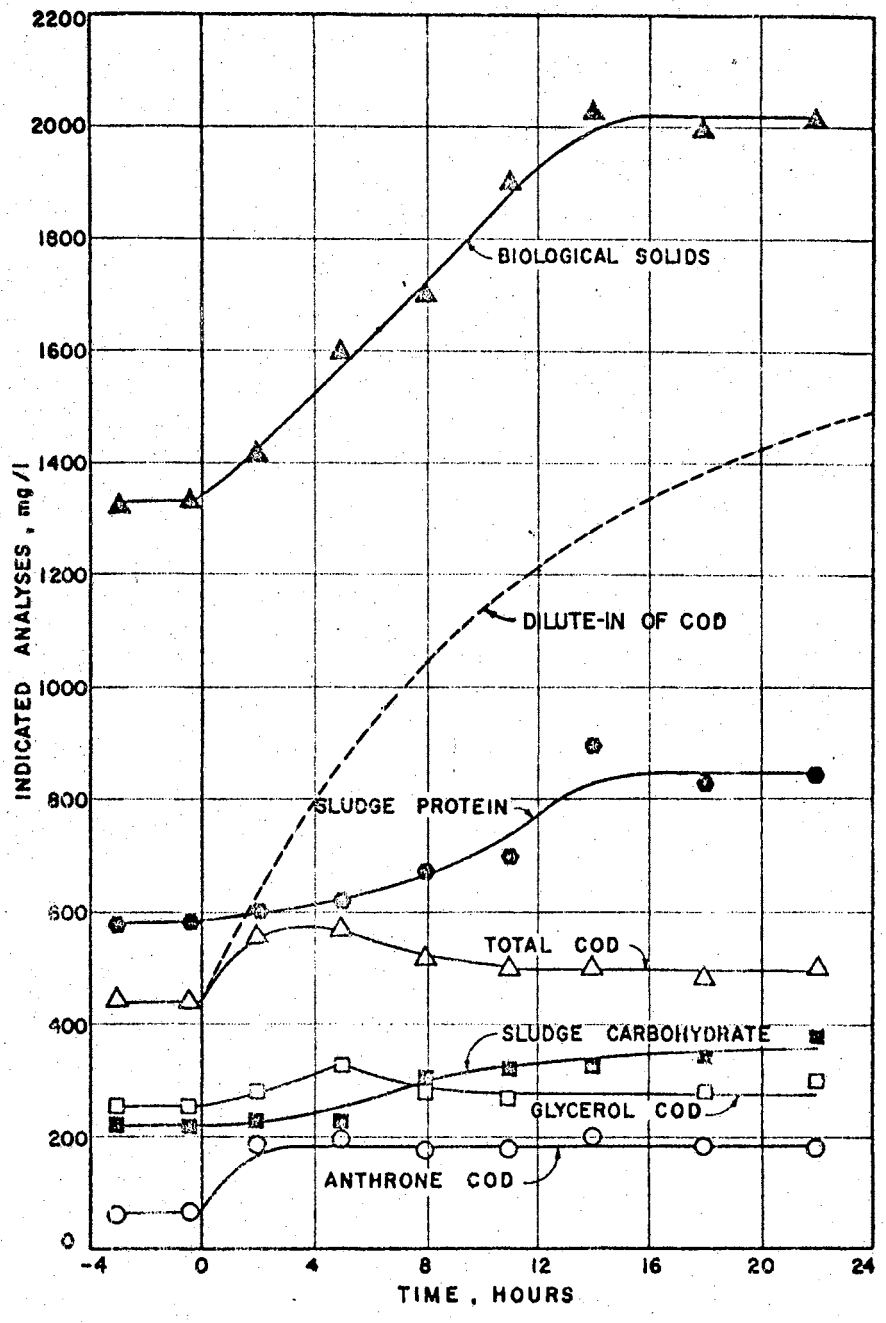

Figure 72 - Response of a system shock loaded from 1000 mg/l glucose + 2000 mg/l glycerol to 1000 mg/l glucose + 3000 mg/l glycerol (dilution rate 1/12 hr^{-1}).

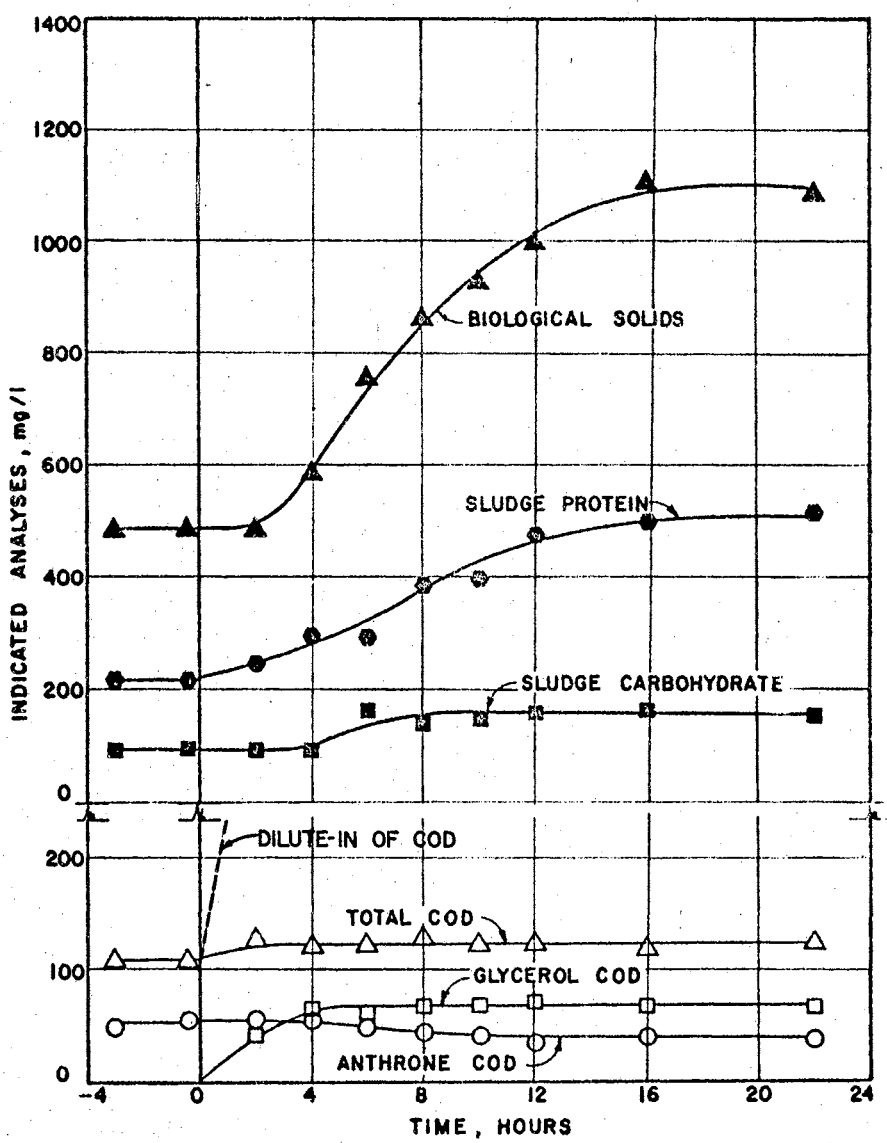

Figure 73 - Response of a system shock loaded from 1000 mg/l glucose to 1000 mg/l glucose + 1000 mg/l glycerol (dilution rate 1/8 hr^{-1}).

the higher growth rate (Figyre 73) there was less leakage of COD even though the rate of COD "dilute-in" was higher. The biological solids increased to an apparent steady state level of approximately 1100 mg/l after the shock.

Figure 74 shows the results when the influent waste concentration was changed to 1000 mg/l glucose plus 2000 mg/l glycerol. There was essentially no effect on the removal efficiency as indicated by the total COD, anthrone, and glycerol COD of the effluent. There was a smooth transient increase in the biological solids concentration to a maximum level at approximately twenty-two hours. As adjudged by the shape of the solids curve, the system was approaching a new steady state at this time. The increase in biological solids concentration was also accompanied by a concomitant increase in both cell protein and carbohydrate.

The results shown in Figure 75 were obtained when a change in influent feed composition to 1000 mg/l glucose plus 3000 mg/l glycerol was made. Again it can be seen that there was apparently no significant effect on removal efficiency as measured by the levels of glucose COD and glycerol COD. There was a slight increase in the level of the total filtrate COD to the transient peak of 140 mg/l. The total COD concentration then returned to a level slightly higher than the previous steady state value. Comparing the responses at similar feed levels for the two dilution rates (i.e., Figures 75 versus 72, 74 versus 71, and 73 versus 70), it is seen that a more favorable response with respect to system efficiency was obtained at the lower detention time.

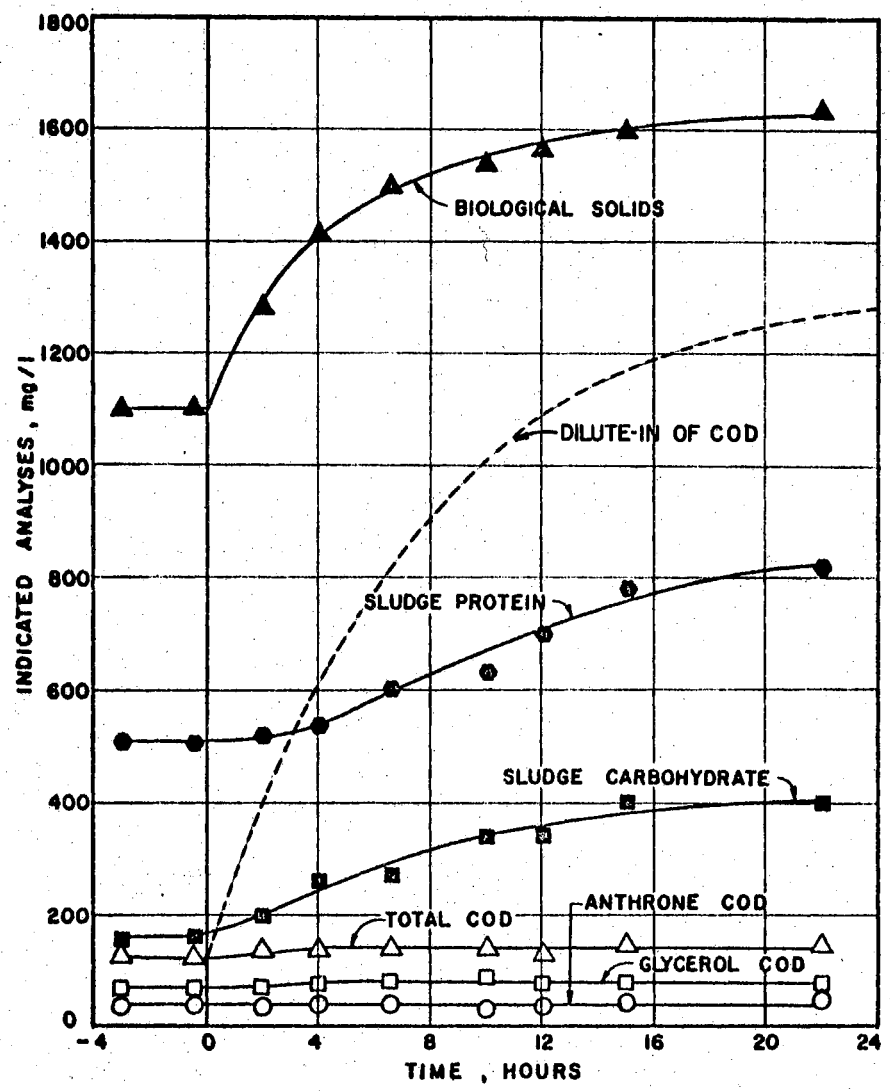

Figure 74 - Response of a system shock loaded from 1000 mg/l glucose + 1000 mg/l glycerol to 1000 mg/l glucose + 2000 mg/l glycerol (dilution rate 1/8 hr^{-1}).

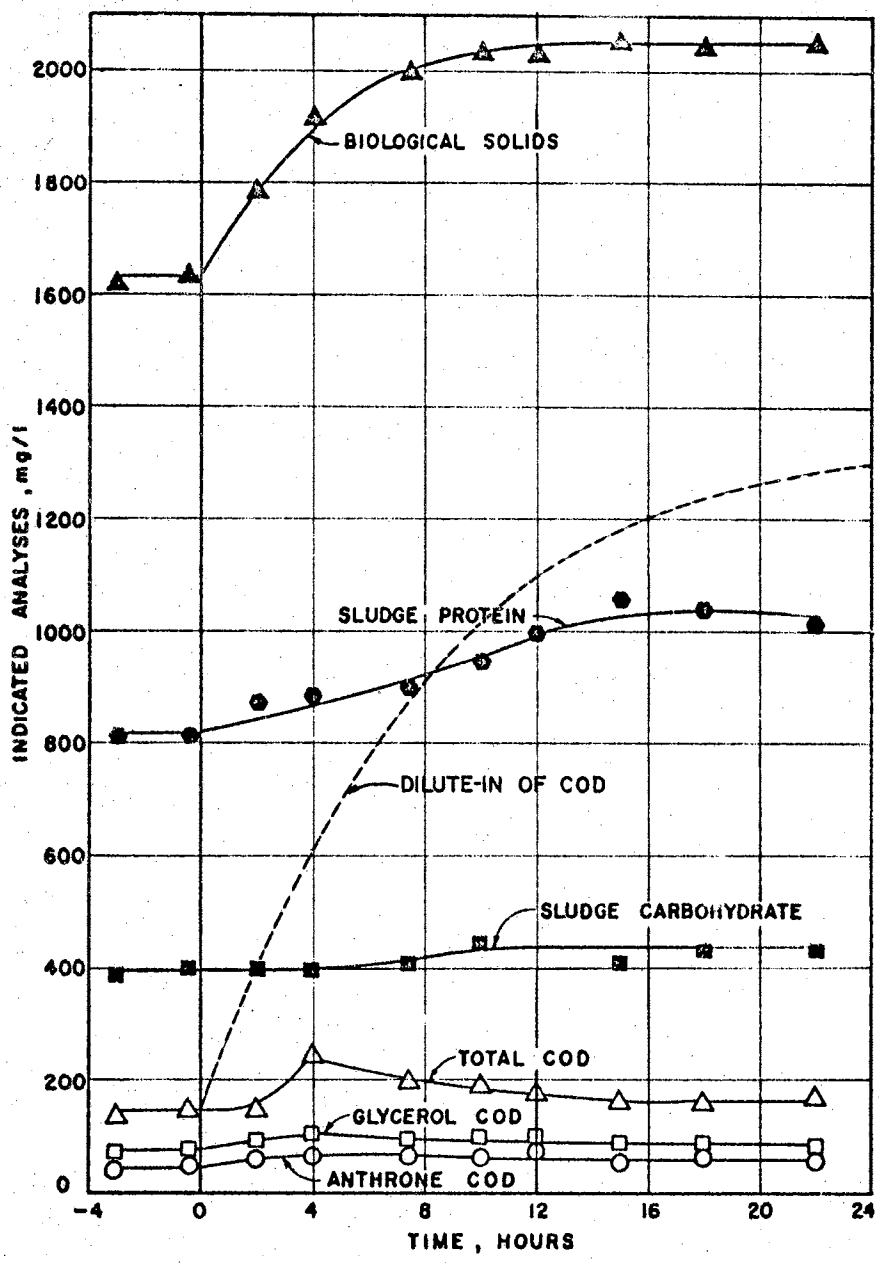

Figure 75 - Response of a system shock loaded from 1000 mg/l glucose + 2000 mg/l glycerol to 1000 mg/l glucose + 3000 mg/l glycerol (dilution rate 1/8 hr^{-1}).

7. Effect of Shock Loading to Systems Operated at High Biological Solids Concentration

Much of the previous work on shock loadings to completely mixed continuous flow systems had been conducted in systems of the "once-through" type; i.e., sludge recycling was not practiced and the biological solids in the system at the time of administering the shock load was determined simply by the cell (sludge) yield for the particular substrate and by the dilution rate of the system. It was important to obtain more data on the effect of biological solids concentration in determining the response to various shock loadings. Many continuous activated sludge systems are operated with an aeration solids level of between 2000 and 2500 mg/l, and experiments were designed in which this concentration was maintained in the aerator by recycling sludge from the settling chamber (see experimental unit in Figure 2A). Two types of shock load were studied. The conditions of the study were as follows:

1) Quantitative Shock Loading: The dilution rate was maintained at 1/24 hr^{-1}. Increases in inflowing feed concentration were administered to determine the resultant transient response for shock load levels of 1000 to 1500 and 1000 to 3000 mg/l glycerol.

2) Qualitative Shock Loading: Employing a dilution rate of 1/12 hr^{-1}, the continuous flow activated sludge unit was operated at an inflowing glycerol concentration of 1000 mg/l while varying inflowing glucose concentrations (1000, 2000, 3000, 4000, and 5000 mg/l). The response of the system in both the transient state and steady state was assessed.

Quantitative Shock Loadings

The continuous flow unit was started using an initial seed taken

from the primary clarifier effluent of the municipal sewage treatment plant at Stillwater, Oklahoma. The system was fed a synthetic medium containing 1000 mg/l glycerol, and was operated under steady state conditions for three weeks. During this time a good settling sludge was developed, and the biological solids in the aeration chamber was built up to and maintained in the range of 2000 to 2500 mg/l. During the operation period, solids concentration in the aerator was controlled by wasting a portion of the settled sludge from the clarifier. Such operation required a great deal of investigational effort, and it was necessary to waste solids at least three times daily. This method of controlling steady state solids level in the aeration tank was different than that employed by either Ramanathan or Krishnan, who maintained a separate aerated sludge tank from which sludge was recycled to the aerator.

Figure 76 shows the response when the influent waste concentration was increased from 1000 mg/l to 1500 mg/l glycerol. There was no change in the concentration of total COD (filtrate). The level of approximately 40 mg/l prior to the shock remained steady during the shock. Glycerol COD, as measured by the periodate test, was not detected at any time. In order to assess the effect on sludge settleability, the biological solids concentration of the settling tank effluent was measured. The shock loading did not seriously affect sludge settleability.

Throughout the 48-hour period during which the feed level was held at 1500 mg/l glycerol, biological solids were not withdrawn from the settling tank. The reactor, i.e., aeration and settling chambers (see Figure 2A) is so constructed that sludge recycle occurs hydraulically; the sludge is internally recycled. Partly because of the increased feed

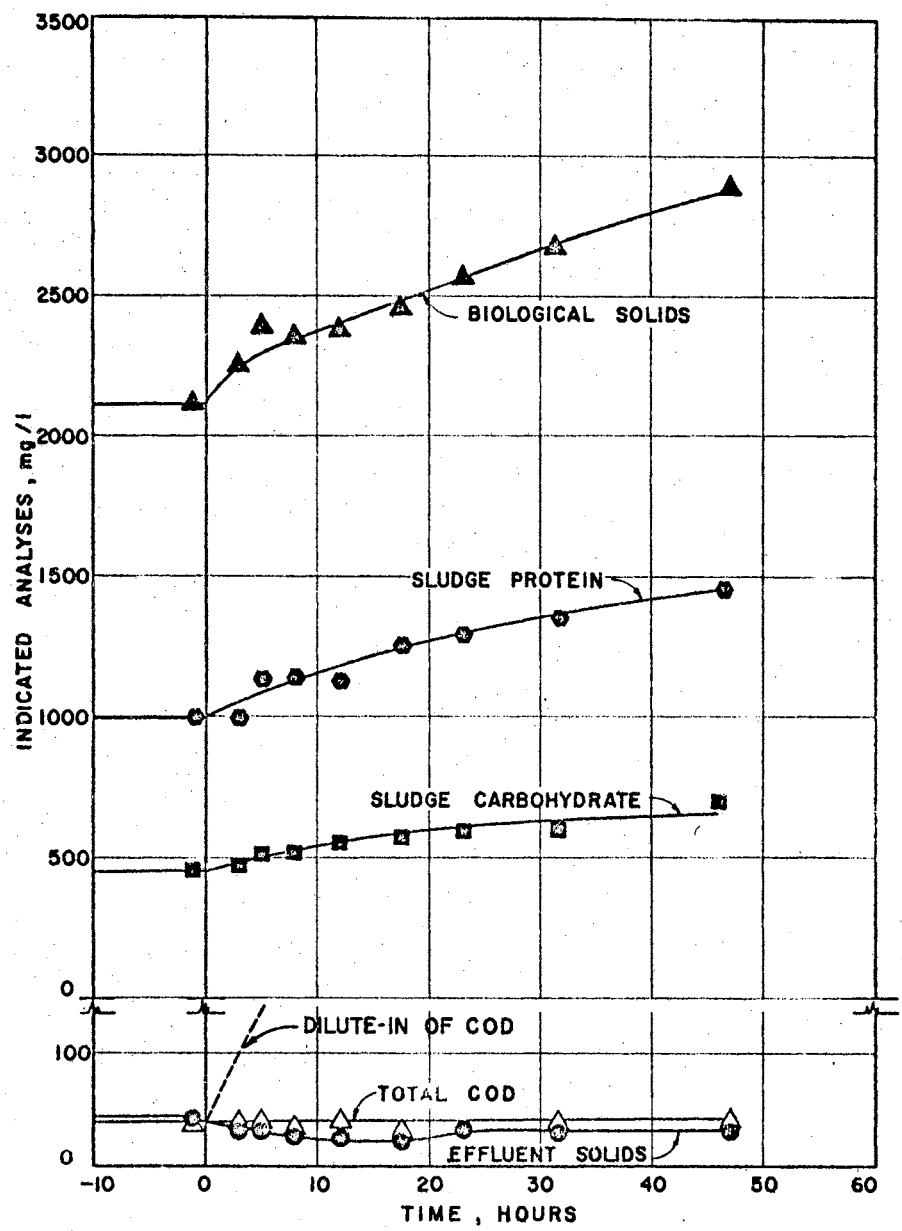

Figure 76 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 1500 mg/l glycerol (detention time in aeration chamber = 24 hours).

and partly because of the natural or uncontrolled sludge recycle, the biological solids concentration rose steadily. It would have been interesting to resume the control procedure for the aeration solids level to assess steady state operation at the new feeding level. However, the major interest was in assessing the shock response with respect to effluent characteristics. Since it was obvious that the system had successfully responded to the shock, the substrate concentration was returned, after forty-eight hours, to the 1000 mg/l feeding level and the aeration solids concentration, by controlled wasting of sludge, was returned to the 2000 mg/l level. After two days of operation at this original feeding and biological solids level, the feed concentration was changed from 1000 to 3000 mg/l glycerol.

Figure 77 shows the response to this more severe shock load. Even though the inflowing feed was increased three-fold, there was apparently no significant effect on the metabolic efficiency of the system, as indicated by the total COD curve. The filtrate COD was slightly increased, from 40 mg/l to a new steady value of 60 mg/l, within eighteen hours. Again no glycerol COD was found in the effluent. There was a slight increase in the supernatant solids level (from 30 to 55 mg/l).

Qualitative Shock Loadings

After returning the feeding level to 1000 mg/l glycerol, the flow rate was adjusted to yield a dilution rate of 1/12 hr^{-1}, and the solids level was again controlled to the 2000-2500 mg/l level. The system was operated under this new steady condition for one week, then a series of qualitative shock loads was administered. At all times glycerol (1000 mg/l) was fed to the aerator; however, glucose was also added to the

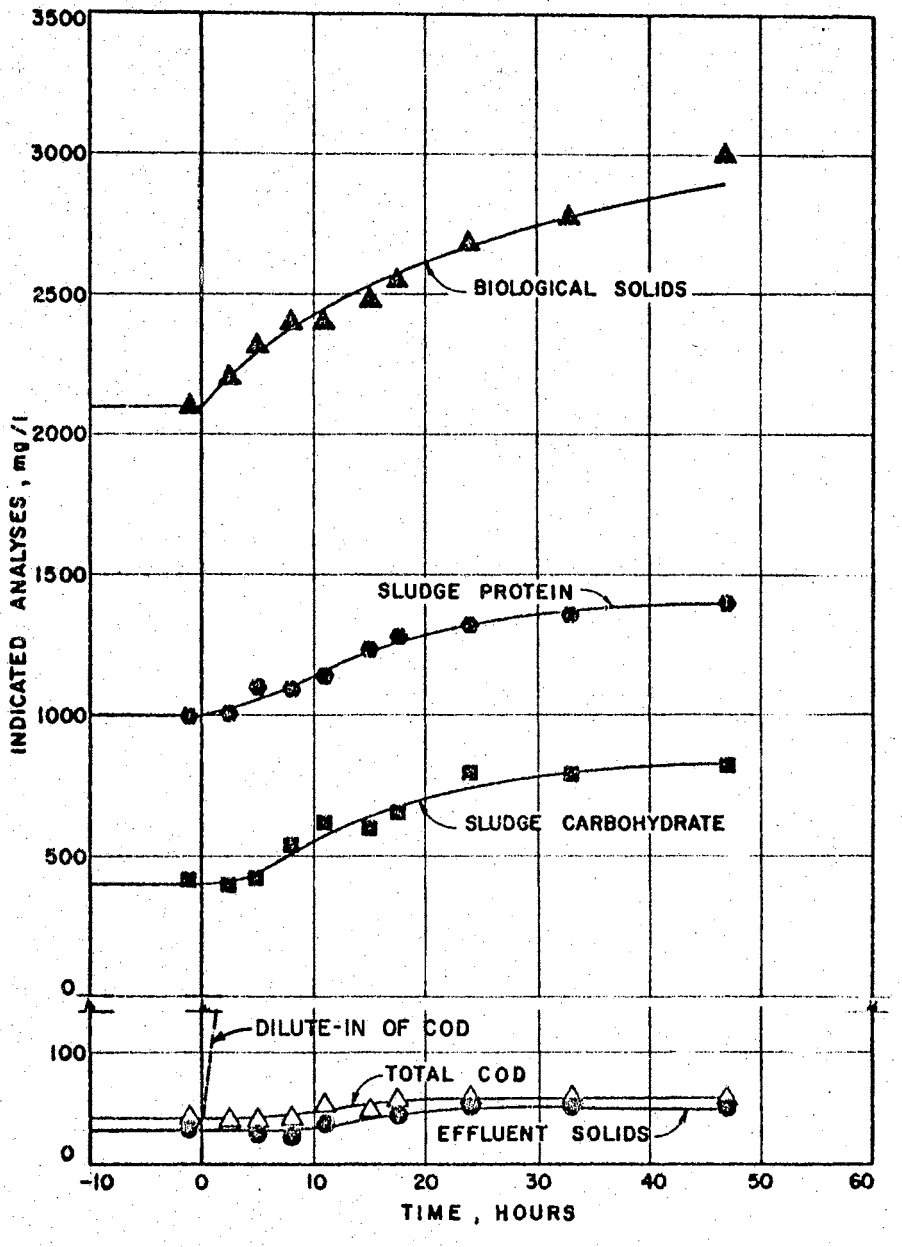

Figure 77 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 3000 mg/l glycerol (detention time in aeration chamber = 24 hours).

feed in varying concentrations, thus comprising the qualitative shock. With respect to total COD in the feed, this type of change also represents a quantitative shock loading.

Figure 78 shows the response when the feed composition was changed from 1000 mg/l glycerol to 1000 mg/l glycerol plus 1000 mg/l glucose. There was no change in the total filtrate COD. Analysis for glycerol was performed, but none could be detected in the effluent. A small amount of glucose COD (anthrone) appeared in the effluent (approximately 10 mg/l). There was no significant change in the supernatant solids level. Biological solids in the aeration chamber accumulated to a level of approximately 3550 mg/l in forty-seven hours. A significant increase in both the protein and carbohydrate content of the solids occurred. The feed was returned to the original 1000 mg/l glycerol level after forty-eight hours; the solids concentration was adjusted to the 2000-2500 mg/l level. The system was operated under steady condition for two days prior to applying the next shock.

Figure 79 shows the results when the influent feed was changed from 1000 mg/l glycerol to 1000 mg/l glycerol plus 2000 mg/l glucose. Again there was no change in total COD (filtrate). The value of total filtrate COD was approximately 60 mg/l throughout the experimental period. Glucose COD (anthrone) was found to be 10 mg/l, and no glycerol COD appeared in the effluent (as determined by periodate analyses). The biological solids concentration rose continually during the 48-hour period; there was also a concomitant rise in protein and carbohydrate. The biological solids concentration in the clarifier supernatant increased from 60 mg/l to a fairly constant level of only 90 mg/l, indicating that the shock load did not seriously impair the settling efficiency.

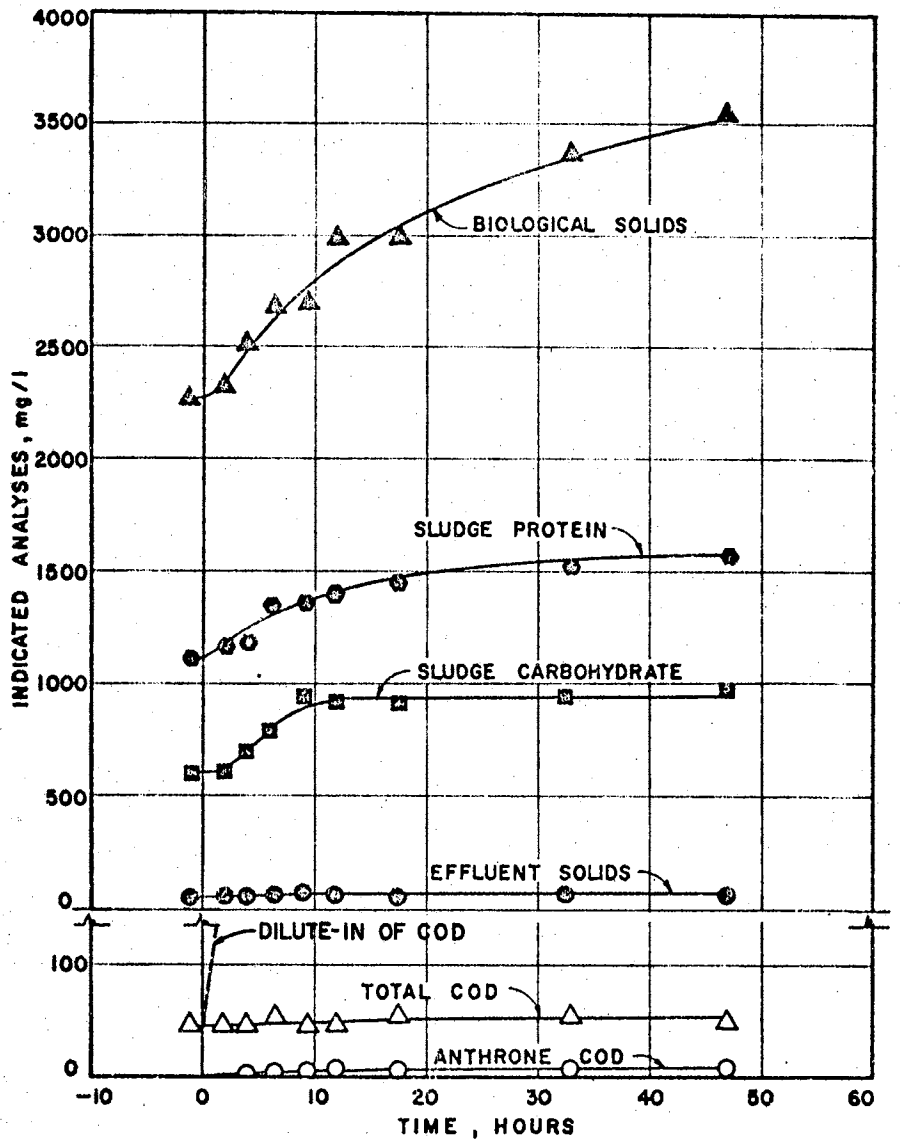

Figure 78 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 1000 mg/l glycerol + 1000 mg/l glucose (detention time in aeration chamber = 12 hours).

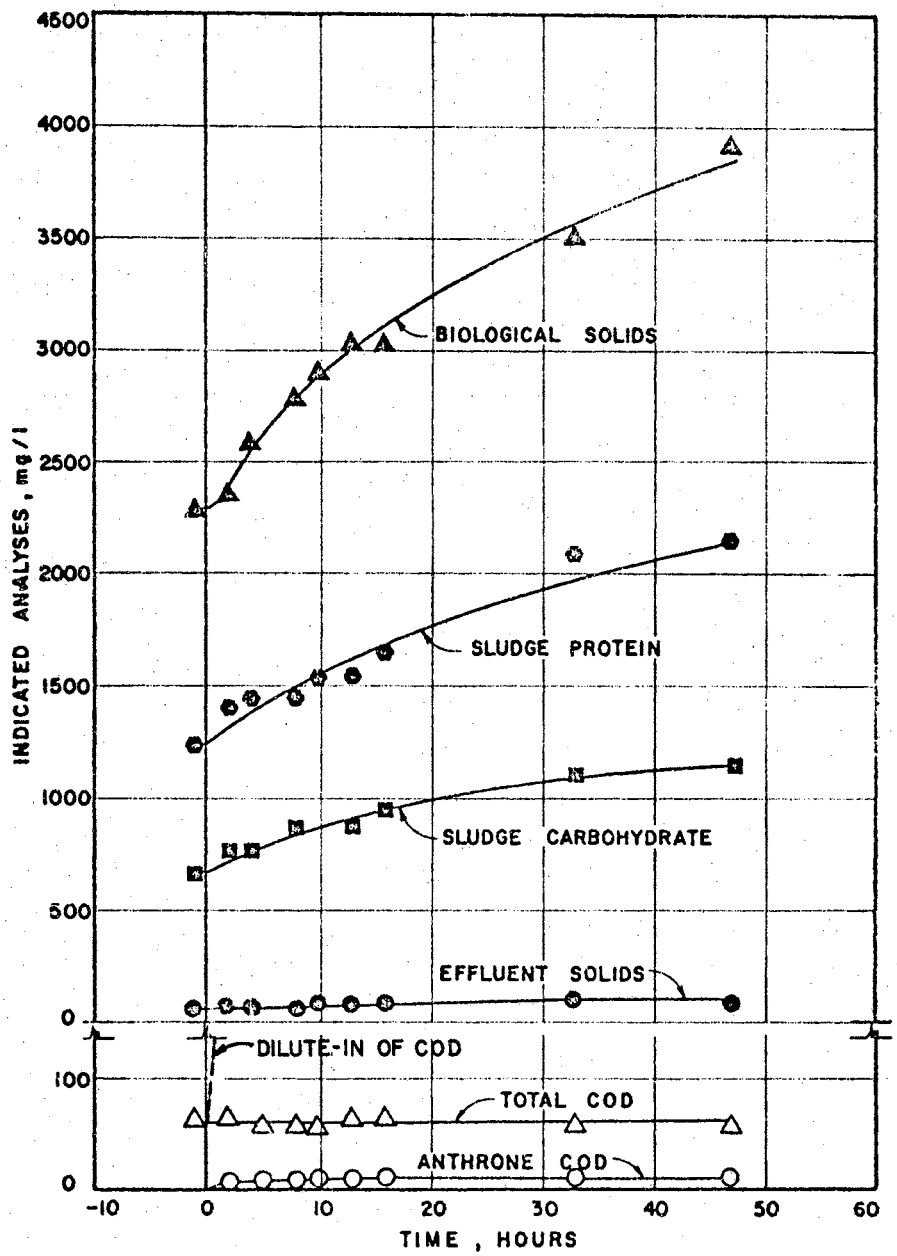

Figure 79 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 1000 mg/l glycerol + 2000 mg/l glucose (detention time in aeration chamber = 12 hours).

The response when the influent feed concentration was increased from 1000 mg/l glycerol to 1000 mg/l glycerol plus 3000 mg/l glucose is shown in Figure 80. There was only a slight increase in the total filtrate COD, although the influent feed was increased to four times its previous concentration. The total filtrate COD reached a fairly constant value of 95 mg/l within sixteen hours. There was a slight increase in glucose COD (anthrone) to approximately 15 mg/l. Again no glycerol COD was found in the effluent. Even at this high level of glucose (3000 mg/l), the metabolism of glycerol was not affected. The suspended solids concentration in the effluent rose from 60 mg/l to approximately 75 mg/l, indicating that the shock did not seriously affect the settling characteristics of the sludge.

The results when the influent feed was changed from 1000 mg/l glycerol to 1000 mg/l glycerol plus 4000 mg/l glucose are shown in Figure 81. There was a substantial increase in the total filtrate COD, and an increase in the glycerol COD and glucose COD were noted. A significant amount of metabolic products accumulated and was subsequently eliminated. The total COD increased from 80 mg/l to the transient peak value of 470 mg/l in thirteen hours, and thereafter returned to a level slightly higher than the previous value. During the period of disruption and recovery of substrate removal there was a little change in the suspended solids concentration in the clarifier effluent, indicating that the effect of the shock on sludge flocculating and settling characteristics was negligible.

Figure 82 shows the results observed when glycerol-acclimated sluge at 1000 mg/l glycerol influent concentration was shock-loaded with 1000 mg/l glycerol plus 5000 mg/l glucose. The severity of disruption

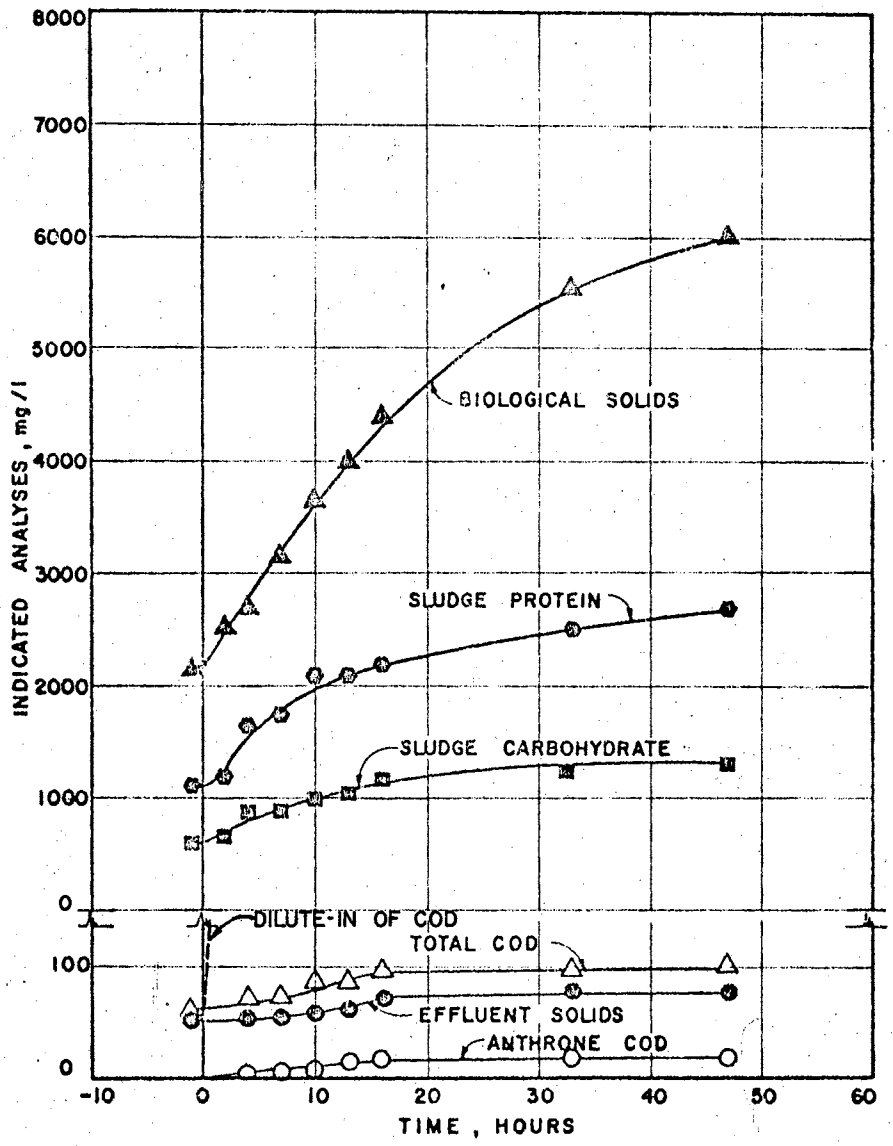

Figure 80 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 1000 mg/l glycerol + 3000 mg/l glucose (detention time in aeration chamber = 12 hours).

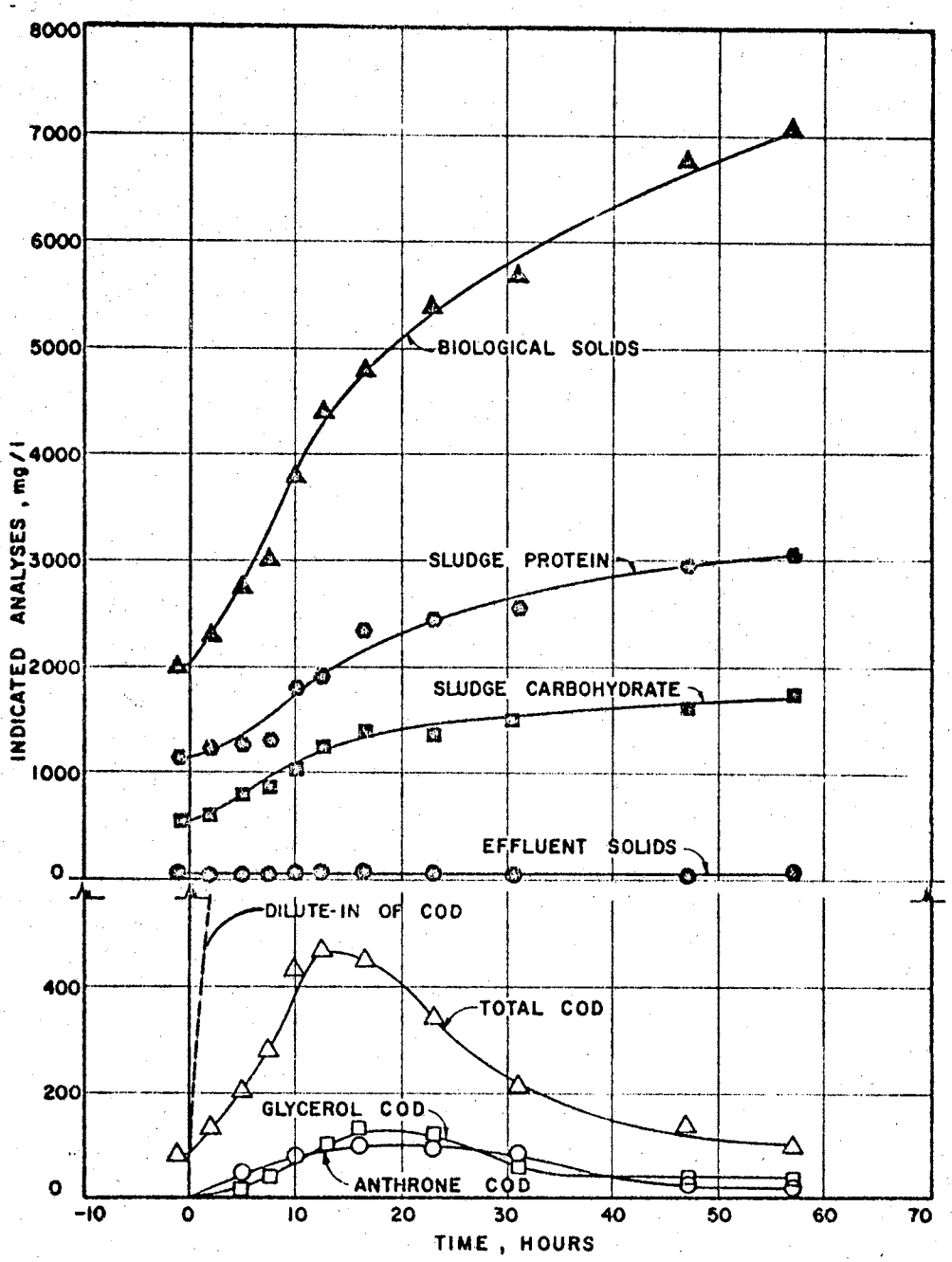

Figure 81 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 1000 mg/l glycerol + 4000 mg/l glucose (detention time in aeration chamber = 12 hours).

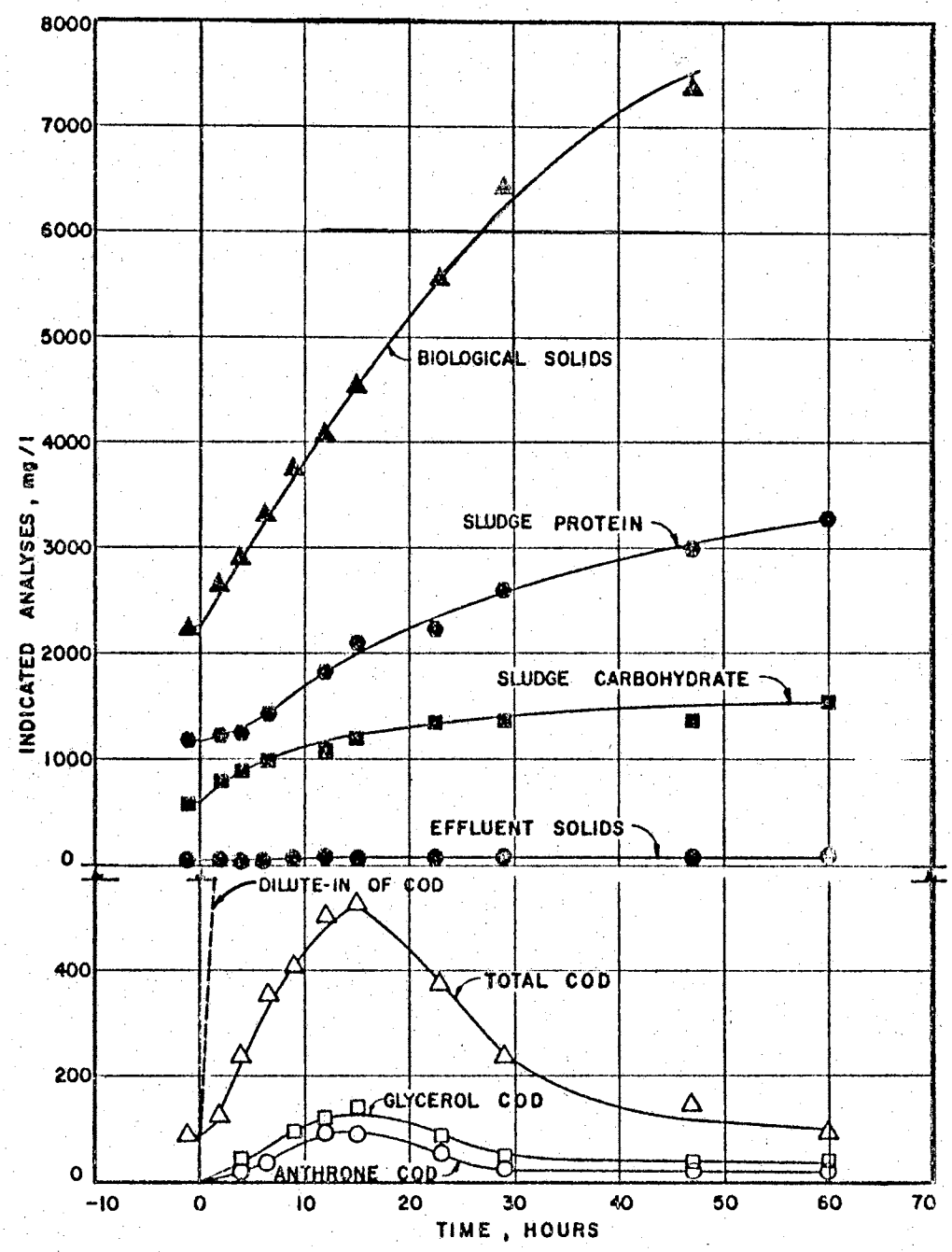

Figure 82 - Response of a system operated at high solids concentration to a change in feed from 1000 mg/l glycerol to 1000 mg/l glycerol + 5000 mg/l glucose (detention time in aeration chamber = 12 hours).

of the system was somewhat greater than for the previous shock, but again recovery was rather rapid. Within fifteen hours after shifting the waste influent concentration, the total COD rose from 90 mg/l to the transient peak of 540 mg/l, and then returned to a normal level in fifty-five hours. As with the previous shock, there was a considerable increase in the glycerol COD and glucose COD during the transient state, and a greater amount of metabolic products was released. It is significant to note that sludge retention in the settling chamber was not impaired by the shock.

8. Qualitative Shock Loadings and Hydraulic Shock Loadings to Extended Aeration Systems

Rather extensive research efforts have been under way in the Oklahoma State University bioengineering laboratories on the extended aeration (total oxidation) process. In view of recent findings concerning the possible usefulness and expanded scope of applicability of the process, further studies on its response to various shock loadings were important. The present study was designed to examine the response of an extended aeration system to qualitative shock loadings and to hydraulic shock loadings. Ragthaidee (51) had studied the response to quantitative shock loadings and found that shock loadings up to five-fold increase in feed COD did not seriously impair the biochemical efficiency of an extended aeration system. Other work in this laboratory pertains to the long-term operational behavior of the extended aeration activated sludge under non-shock loading conditions (24).

Before presenting the experimental results for the present shock load studies, it is appropriate to provide a brief history of the development of the extended aeration activated sludge used in these

experiments. The system was developed by seeding a synthetic waste, containing glucose, with effluent taken from the primary clarifier of the municipal sewage treatment plant at Stillwater, Oklahoma. After a few days of "batch" growth to allow acclimation, the extended aeration unit (total volume = 9.4 liters) was operated (on March 31, 1967) under continuous flow feeding with 1000 mg/l glucose as the substrate. The overall detention time in the aeration tank and settling chamber was maintained at twenty-four hours. All biological solids were returned to the aeration chamber except a small portion (15 ml) taken daily, or nearly so, for analysis. On October 12, 1967 (196 days after starting the unit), approximately half of the system was transferred to a second unit of the same type, and both systems were diluted to 9.4 liters with tap water. Since the biological solids concentration was now approximately halved, the feed concentration was proportionally reduced to 500 mg/l glucose. One unit was retained to continue studying the long-term behavior of the extended aeration system (24); the other was used for the studies on the response to quantitative shock loadings.

Since there were no data available in the literature or from studies in our own laboratory on the ability of the extended aeration process to accommodate qualitative shock loadings and little was known about their response to hydraulic shock loadings, it was considered essential to continue response studies using the system which had been employed for the quantitative shock load investigations. Before subjecting this system to further shock loadings, it was desirable to operate for a while under steady feeding conditions at an inflowing feed concentration of 500 mg/l glucose.

After Ragthaidee had completed his work on quantitative shock

loads, the writer assumed the responsibility for operation of the unit. The system was returned to the steady feeding condition (500 mg/l glucose) for 90 days. It is emphasized that during this period, as for all succeeding and all preceding periods of operation under steady or shock conditions, no biological solids were purposely or inadvertently wasted. Although few analyses were carried out during the 90-day period, all regular operational procedures (e.g., centrifugation of effluent and return of all solids to the aerator) were rigorously carried out. Near the ending of this period, samples were taken to determine the status of the system before applying the first qualitative shock loading. From this time forward, performance data for the system during the qualitative shock loading studies are shown in Figure 83 and succeeding figures. Hour "zero" on Figure 83 occurred on October 4, 1968. At this time the biological sludge was 554 days old, i.e., the system had been run with total solids retention (except as previously noted for sampling) for 554 days. After determining the "steady" behavior, the feed was changed to 250 mg/l glucose plus 250 mg/l glycerol, and the system performance was assessed. After the new steady state was attained, the feed was returned to 500 mg/l glucose, and a new steady state was reached. The feed then was changed to 500 mg/l glucose plus 500 mg/l glycerol, and the transient and new steady states were again assessed. Then the feed was returned to the original 500 mg/l glucose. Similar shocks up to 3000 mg/l of each substrate were applied.

Reactor characteristics as well as effluent characteristics are given in the figures. The curve labeled "total COD" is the COD of the mixed liquor filtrate, whereas the curves labeled "anthrone COD" and "glycerol COD" were plotted using anthrone and periodate results

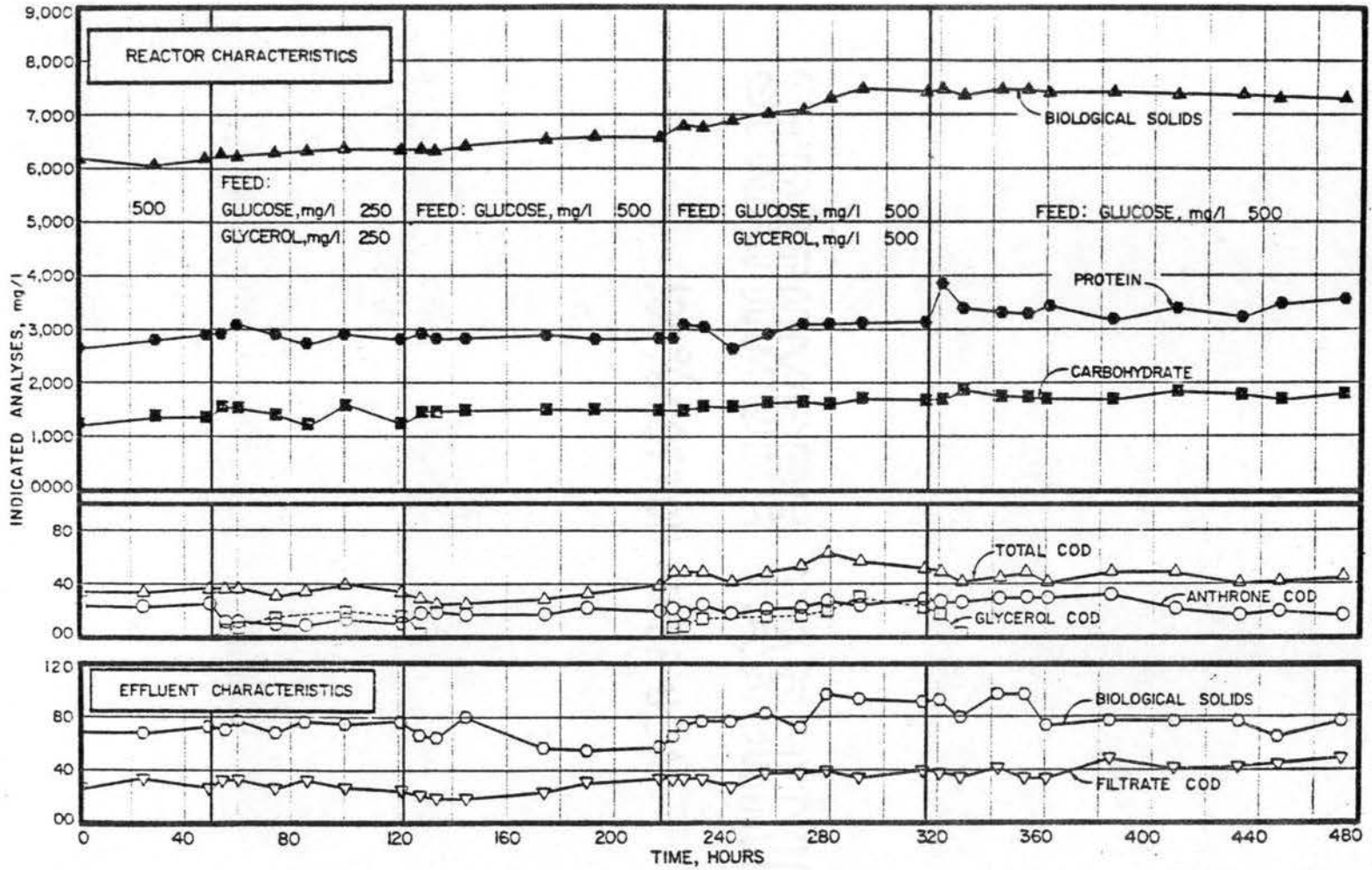

Figure 83 - The biochemical response of an extended aeration system to qualitative shock loadings (normal feed = 500 mg/l glucose); a series of changes in the inflowing feed concentration from 500 mg/l glucose to 500 mg/l glucose + 500 mg/l glycerol.

calculated to their respective COD values. Effluent characteristics refer to the condition of the effluent from the settling chamber. This condition was assessed by determining biological solids concentration and filtrate COD. The COD of the "whole" effluent was not measured, but can be estimated by summing the filtrate COD and the COD equivalent (52) of the biological solids.

It is important to note that the practice of removing the interior wall to allow mixing of solids in the settling and aeration compartment was followed, as in previous studies under non-shock loading conditions (24). However, in the present study it was desired to assess transient effects on clarification efficiency as well as biochemical efficiency; therefore, during assessment of the transient state, the baffle wall could not be removed just prior to sampling, since this would interfere with assessment of settling tank effluent characteristics with respect to biological solids concentration. Thus, the biological solids concentration recorded as a "reactor" characteristic refers to the solids concentration in the aeration tank, not the biological solids in the total system, i.e., reactor plus settling tank. During this series of experiments the removable baffle was in place at least six hours prior to sampling.

From hours 0-50, samples were taken to assess the operation at the steady feed level of 500 mg/l glucose. Although at this time the system had been aged approximately 1.5 yr and had been previously subjected to a series of quantitative shock loads, the purification efficiency was approximately 95 per cent. The mean biological solids concentration was approximately 6200 mg/l. The filtrate COD of the effluent fluctuated between 25 and 35 mg/l. The solids concentration

of the effluent was approximately 70 mg/l.

After fifty hours, the influent composition was changed from 500 mg/l glucose to 250 mg/l glucose plus 250 mg/l glycerol. The biological solids concentration in the aeration chamber increased from 6200 mg/l to a fairly constant value of 6300 m g/l. There was some fluctuation in the cellular protein and carbohydrate. The COD concentration and solids concentration of the effluent remained fairly constant throughout this experimental period (hours 50 to 122). In the aeration chamber, glucose COD (anthrone) was reduced to a level of approximately 10 mg/l (there was a lower concentration of glucose in the feed), and glycerol COD (periodate) appeared in the filtrate (approximately 20 mg/l).

At 122 hours, the influent feed was returned to the original feed, 500 mg/l glucose. There was no disruption in system efficiency due to this change. During this period (hours 122 to 218), the biological solids concentration rose gradually to approximately 6600 mg/l, and there was no significant change in the cellular protein and carbohydrate.

At 218 hours, the system was shock-loaded by changing the influent feed concentration from 500 mg/l glucose to 500 mg/l glucose plus 500 mg/l glycerol . The biological solids rose steadily to approximately 7400 mg/l. During this period the effluent solids concentration increased to approximately 90 mg/l, and there was also a slight increase in the filtrate COD of the effluent. The purification efficiency was approximately ninety-six per cent. In the aeration chamber, there was no significant change in glucose COD level. The glycerol COD rose to a level of 30 mg/l during this period (hours 218 to 317).

At 317 hours, the feed was returned to the original concentration

of 500 mg/l glucose. During the period of operation at this feeding level (hours 317 to 480), the biological solids concentration decreased slightly. The solids concentration in the plant effluent decreased from 90 mg/l to approximately 75 mg/l. The COD removal efficiency remained at approximately 95 per cent. The system was operated at this feed level until hour 540.

Figures 84 and 85 show the performance of the extended aeration system during the ensuing series of changes in feed composition. At hour 540, the influent feed composition was changed from 500 mg/l glucose to 1000 mg/l glucose plus 1000 mg/l glycerol. The biological solids concentration rose steadily to approximately 9900 mg/l during the period of operation at this level, hours 540 to 636 (see Figure 85). The effluent solids concentration increased from 75 mg/l to a maximum of approximately 210 mg/l during this period, indicating that the flocculating and settling characteristics of the sludge were decidedly retarded due to this shock. There was no significant change in effluent filtrate COD. The biolchemical COD removal efficiency was about ninety-seven per cent. In the aerator, the total COD (filtrate) rose from 50 mg/l to 70 mg/l; there was no significant change in the glucose COD level, which rose to approximately 25 mg/l during this period. The increase in total COD in the aerator was attributable mainly to glycerol COD. The filtrate COD of the settling tank effluent never exceeded 60 mg/l, even under the high level shock load applied during this period. The COD value of the clarifier effluent filtrate was generally 10 to 20 mg/l lower than the total filtrate COD in the aeration chamber.

From hours 636 to 708, the influent feed was returned to its

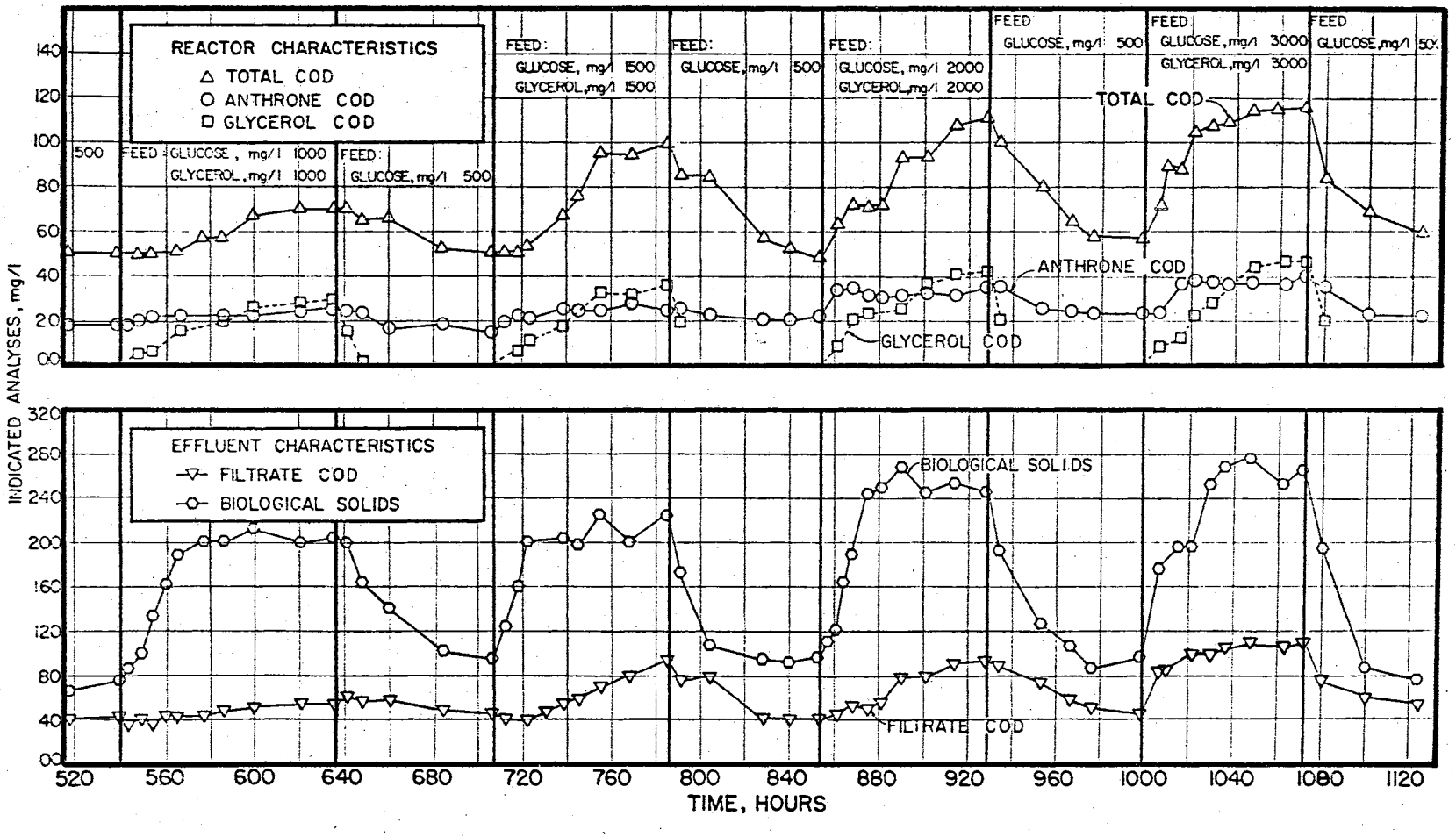

Figure 84 - The biochemical response of an extended aeration system to qualitative shock loadings (normal feed = 500 mg/l glucose); a series of changes in the inflowing feed concentration from 500 mg/l glucose to 3000 mg/l glucose + 3000 mg/l glycerol.

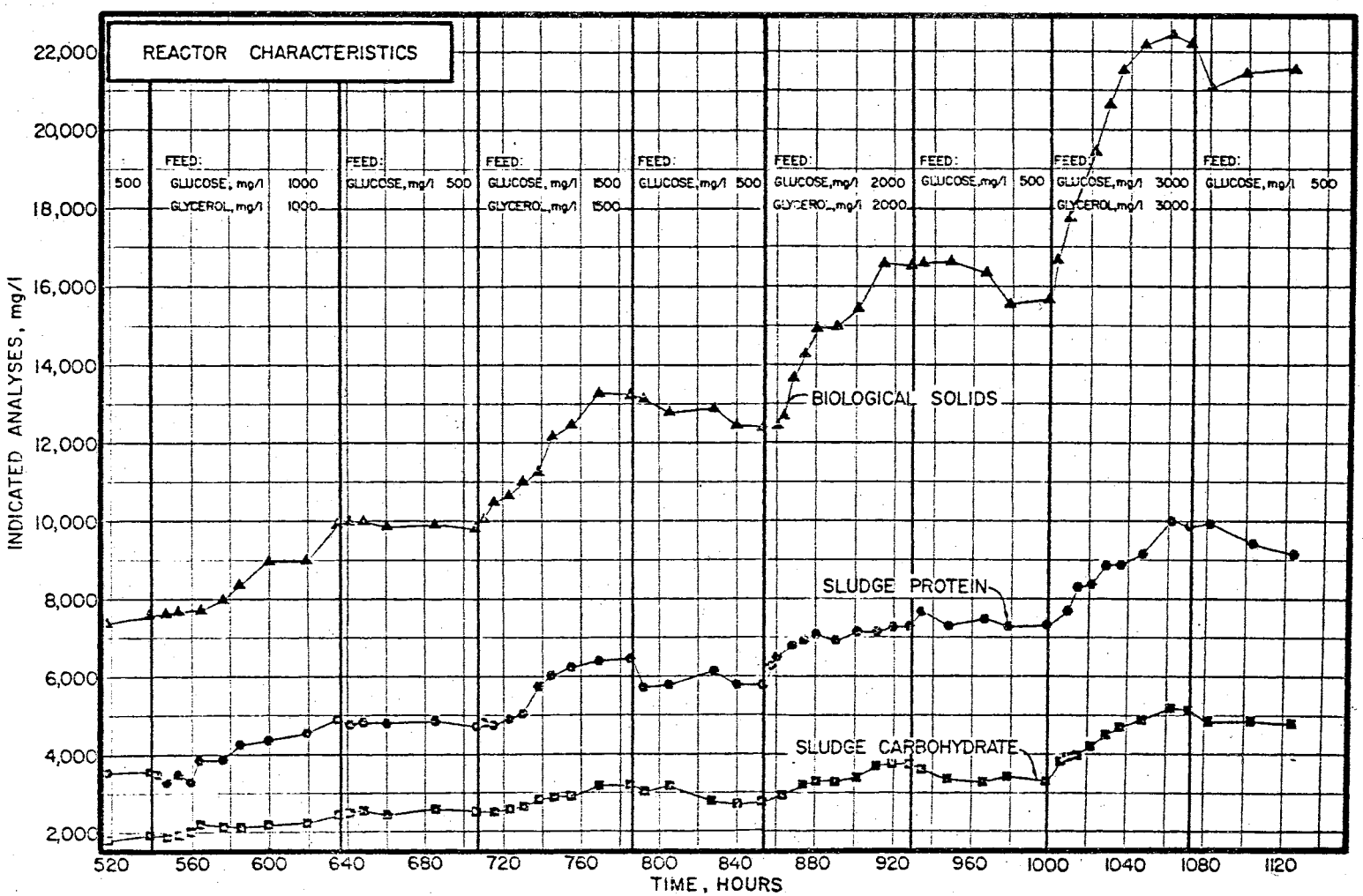

Figure 85 - Biological solids growth in an extended aeration system during a series of changes in the influent feed concentration from 500 mg/l glucose to 3000 mg/l glucose + 3000 mg/l glycerol.

original composition, 500 mg/l glucose. The biological solids concentration remained at a fairly constant level of 9900 mg/l throughout this period. There was no noticeable change in protein and carbohydrate content of the sludge. The effluent solids concentration dropped to approximately 95 mg/l, and there was also a very small decrease in the effluent filtrate COD concentration.

At hour 708, the feed was changed to 1500 mg/l glucose plus 1500 mg/l glycerol. The biological solids concentration increased steadily to approximately 13,500 mg/l during the period of feeding at this level (hours 708 to 786). The increase in biological solids concentration was accompanied by an increase in the cell protein and carbohydrate. During this period the effluent solids concentration increased to a maximum level of 210 mg/l, indicating that the settleability of the sludge was disrupted severely. The effluent filtrate COD rose steadily to approximately 95 mg/l. The COD removal efficiency was approximately ninety-six per cent. In the aeration chamber, the total COD rose to approximately 100 mg/l; there was only a slight increase in glucose COD, and again it is evident that the increase in total COD was due mainly to leakage of glycerol COD.

From hours 786 to 853, the inflowing feed was returned to the original composition, 500 mg/l glucose. During this period the supernatant solids concentration was reduced to approximately 95 mg/l (Figure 84). The aeration solids concentration (Figure 85) dropped to approximately 12,500 mg/l, and it is evident that a portion of the sludge was autodigested, since no solids were wasted and the effluent COD decreased. The filtrate COD of the effluent dropped to approximately 40 mg/l. TheCOD removal efficiency remained at approximately

ninety-six per cent.

Between hours 853 and 929, the system was fed 2000 mg/l glucose plus 2000 mg/l glycerol. The biological solids concentration increased rapidly to approximately 16,600 mg/l. There was a concomitant increase in protein and carbohydrate concentration in the solids. As with the two previous shocks, the solids concentration of the effluent increased (in this case to approximately 265 mg/l), indicating that the sludge settleability was severely disrupted due to this severe shock loading. The clarifier effluent filtrate COD rose steadily to approximately 90 mg/l. The COD removal efficiency remained high throughout this period. The glucose COD in the aeration tank increased slightly to approximately 35 mg/l. The glycerol COD increased to approximately 43 mg/l during this period, and again the rise in total COD was attributable mainly to leakage of glycerol COD.

From hours 929 to 1000, the feed was maintained at the original level, 500 mg/l glucose. The solids concentration in the aeration chamber decreased to approximately 15,600 mg/l, with no significant decrease in protein and carbohydrate content of the solids. Again there is evidence for autodigestion of excess solids. The solids concentration and COD concentration of the clarifier supernatant recovered to "normal" values forty-eight hours after shifting the influent feed concentration.

Between hours 1000 and 1072, the system was operated at a feed of 3000 mg/l glucose plus 3000 mg/l glycerol. At this severe shock loading level it is again evident that flocculation and settleability of the sludge was severely retarded. The solids concentration in the supernatant rose steadily to approximately 275 mg/l. The filtrate COD

in the supernatant increased from 45 mg/l to approximately 115 mg/l. The biochemical response was excellent, and the COD removal efficiency remained at approximately 95 per cent. The biological solids concentration in the aerator rose to approximately 22,800 mg/l during this experimental period, and there was a concomitant increase in both protein and carbohydrate content of the solids. As for previous shocks, the rise in total COD in the reactor was due mainly to glycerol COD.

It is evident that flocculation and settling of the sludge was hampered when the system was subjected to this severe shock load. It is also seen that the sludge flocculation and settleability recovered very well after shifting the influent feed concentration to its normal level of 500 mg/l glucose (at hour 1072). It is emphasized that the COD removal efficiency of the system was not adversely affected during this series of shock loads which encompassed an increase in substrate from 500 up to 6000 mg/l as well as qualitative changes in substrate composition. The loss of solids in the effluent appeared to be accompanied by some shifts in predominating species. The color of the mixed liquor in the aerator and the sludge in the settling tank was a rather dark brown, whereas the apparent color of the settling tank overflow, imparted by the cells suspended in it, was yellow-green. From visual observation of the system, the loss of solids appeared to be caused not by deflocculation of cells, but by production of excess non-flocculated cells.

The system was operated for 158 hours at the original loading level (500 mg/l glucose and overall detention time of twenty-four hours), then at hour 1231, the flow rate was changed in initiation of a series of hydraulic shock loadings. During these shock loading studies, the feed

concentration was maintained at 500 mg/l glucose.

At hour 1231, the dilution rate was increased from 1/24 to 1/2 hr^{-1}; the response is shown in Figure 86. The biological solids concentration decreased from 22,000 mg/l to approximately 21,500 mg/l. The solids concentration and COD concentration in the supernatant decreased slightly during this period. It is emphasized that the solids were not lost or wasted. The drop in solids concentration in the aeration chamber was representative of either an increase in the autodigestion rate, or of increased accumulation of solids in the settling chamber. During the period of decreasing solids concentration, the pH dropped from ±6.5 to 5.7, and a small amount of froth appeared on the liquid surface in the clarifier. The COD removal efficiency was approximately ninety per cent.

From hours 1280 to 1400, the dilution rate was returned to its original level, 1/24 hr^{-1}. Initially, the biological solids increased slightly to approximately 21,800 mg/l. The effluent filtrate COD was in the range of 45 to 60 mg/l. The supernatant solids rose slightly to approximately 55 mg/l. There was no froth on the surface of the clarifier compartment. During this period of operation the pH was adjusted in gradual steps by addition of NaOH solution. The pH was increased from 5.7 to 6.8-7.0 over a period of eighty hours. Throughout the remainder of the experiment it remained in the normal operating range of 6.8 to 6.3.

At hour 1400, the system was subjected to a severe hydraulic shock load; the dilution rate was changed from 1/24 to 1/6 hr^{-1}. There was a rather sharp decrease in biological solids concentration in the aerator. The solids concentration in the settling tank effluent rose to

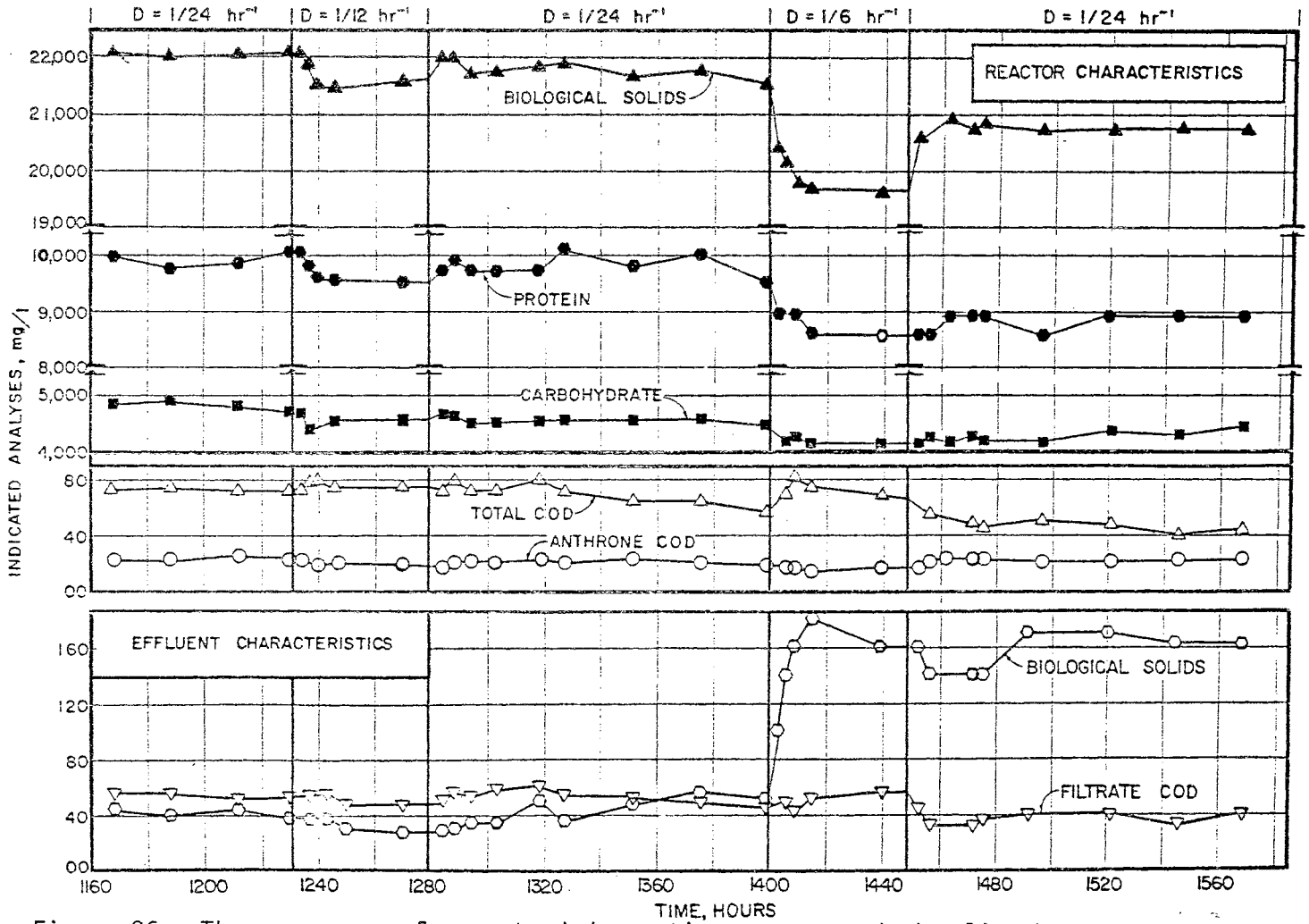

Figure 86 - The responses of an extended aeration system to hydraulic shock loadings (constant feed concentration of 500 mg/l glucose), a series of changes in dilution rate from 1/24 to 1/6 hr^{-1}.

approximately 180 mg/l. From these results it must be concluded that the decrease in biological solids concentration in the aerator was due in part to some wash out of the solids from the settling chamber. There was no significant change in the filtrate COD of the effluent, indicating an entirely successful biochemical response. The COD removal efficiency remained at approximately ninety per cent. The pH did not decrease during this period, but remained at approximately 6.5.

From hours 1449 to 1592, the system was operated at the original dilution rate, 1/24 hr^{-1} (see Figures 86 and 87). The biological solids concentration rose to approximately 20,700 mg/l, but the biological solids concentration in the supernatant was not reduced; it fluctuated in the range of 140 to 170 mg/l. The COD removal efficiency was approximately ninety-two per cent; the pH remained in the normal operating range, i.e., 6.8 to 6.3.

As adjudged by the appearance of the settling tank effluent, the loss of biological solids in the effluent which occurred in response to the previous shock (D, 1/24 to D, 1/6 hr^{-1}) did not appear to be accompanied by a change in microbial predominance. The sludge in the aeration tank and in the settling tank retained the dark caramel color as before, and the solids in the clarifier effluent appeared to be fragments of the same material. The apparent color of the clarifier effluent imparted by the suspended solids was essentially the same as that of the mixed liquor, and the effluent filtered more rapidly than the samples taken during solids leakage in response to the qualitative-quantiative shock loads previously administered.

Since the clarity of the effluent did not recover in the period of 143 hours (hour 1449 to 1592) after returning to the original dilution

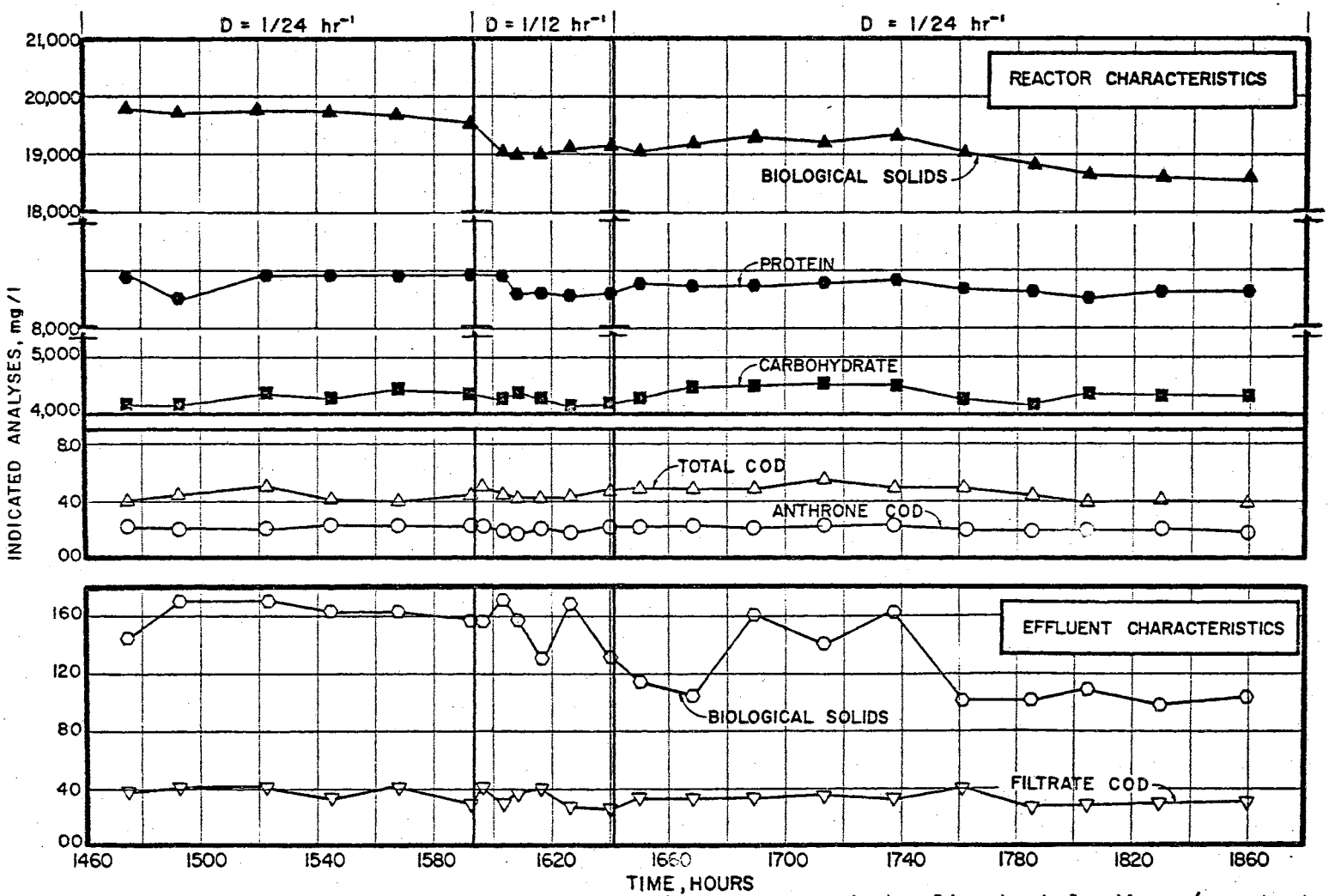

Figure 87 - The responses of an extended aeration system to hydraulic shock loadings (constant feed concentration of 500 mg/l glucose); a series of changes in dilution rate from 1/24 to 1/12 hr^{-1}.

rate of 1/ 24 hr^{-1}, it was decided to apply another hydraulic shock to determine whether sludge retention would deteriorate further. The dilution rate was changed from 1/24 to 1/12 hr^{-1} (see Figure 87, hours 1592 to 1632). The biological solids concentration in the aerator decreased somewhat, and the supernatant solids fluctuated in the range of 130 to 170 mg/l during this period.

From hours 1632 to 1860, the dilution rate was changed to the normal dilution rate, 1/24 hr^{-1}. The biological solids in the aerator gradually decreased to approximately 18,600 mg/l. Again it is emphasized that the decrease in solids concentration in the mixed liquor was not due to the wasting of sludge. During the period of gradual decrease in solids concentration, a slight amount of froth formed on the surface of the secondary clarifier. The COD removal efficiency (biochemical) remained at approximately ninety-four per cent. The suspended solids concentration in the settling tank effluent decreased, but did not attain the low level which existed prior to administering the severe hydraulic shock (D, 1/24 to D, 1/6 hr^{-1}).

CHAPTER V

DISCUSSION

The biological responses to a rather wide range of environmental changes and conditions have been investigated. Some of the experiments were similar to those conducted by past workers in the bioengineering laboratories, and the present work was accomplished to verify and extend these past studies. On the other hand, some of the studies represent newly-designed experimental efforts. Since the amount of experimental work was so extensive, an attempt is made in the present section to summarize the findings reported in the various subsections of the results and to bring the major trends of the responses into focus.

1. Response to "Slug" Shock Loading

In this series of experiments, two comparative situations were studied. First, a continuous flow system was slug-loaded with increasing concentrations of sorbitol, while the original carbon source, glucose, was fed continuously at 1000 mg/l (Figures 3, 4, 5, 6, and 7). Then a similar system was continuously fed sorbitol, and slug doses of glucose were administered (Figures 8, 9, 10, 11, and 12). In the first case, one might have expected that a considerable acclimation period would have been needed to initiate sorbitol metabolism and that the presence of glucose might have prevented or seriously retarded this

acclimation process. Such did not appear to be the case since, in general, sorbitol was removed more rapidly than would have occurred solely by dilute-out of the slug dose, and there was a fairly rapid increase in the biological solids concentration. In two cases (Figures 4 and 5) there was evidence that a change in predominating species took place in response to the shock and there was a rather severe secondary transient. The initial rise in biological solids was followed by a rather sharp decline, and there was a considerable amount of COD in the effluent. The occurrence of a secondary transient accompanied by (or caused by) a change in microbial predominance has also been observed by Thabaraj and Gaudy (43) in their work on the effect of oxygen tension on the ability of a completely mixed activated sludge to accommodate quantitative shock loadings. The occurrence of the secondary transient was not dependent upon oxygen tension, since they observed it at both high and low dissolved oxygen concentrations in the reactor. In the present studies the DO concentration was rather high (6.0 mg/l). The change in predominance, therefore, cannot be ascribed to an effect of DO. Also, it was not associated with an extensive shock, i.e., it did not occur at the two highest shock levels (Figures 6 and 7). Occurrence of this type of response appears to be dependent upon the nature of the predominating species prior to the shock.

When the situation was reversed, and glucose was administered as the slug dose (Figures 8, 9, 10, 11, and 12), the response was a more severe disruption of the system. As the glucose "slug" was increased, greater concentrations of "sorbitol COD" appeared in the medium (see Figures 8, 9, and 10), attesting to the fact that the concentration of glucose plays a significant role in controlling the manifestations of

enzyme repression and/or inhibition of sorbitol-utilizing enzyme(s). This result is in accord with the results of Komolrit (11). However, beyond a certain concentration of glucose (or ratio of glucose to sorbitol), sorbitol leakage appears to remain constant. In the present study, the 1500, 2000, and 2500 mg/l glucose doses resulted in essentially the same maximum concentrations of "sorbitol COD." It is emphasized that sorbitol was assessed by the periodate test, and both Heidman and Su have observed accumulations of periodate-reactive metabolic products during metabolism of glucose. Thus, it may be possible that some of the periodate-reactive material recorded as sorbitol in these studies may actually have been products of glucose dissimilation. However, if this were the case, one would expect that the concentration of periodate-reactive material would be related to the amount of glucose added. Therefore, the "sorbitol COD" would have continued to increase at the high glucose doses. Such was not the case. The maximum sorbitol COD recorded was approximately 700 mg/l (at 1500, 2000, and 2500 mg/l glucose doses). If the periodate-reactive material were produced as a transient byproduct of glucose dissimilation, its concentration at the 2500 mg/l glucose shock might have exceeded 1000 mg/l.

If any generalization can be drawn at this time, it would appear from these studies that a system can withstand a slug dose of a compound for which some acclimation or production of inducible enzyme(s) is expected, more successfully than it can withstand a slug dose of a compound known to exert a repressive (or inhibitive) effect upon the substrate to which the system is already acclimated.

2. Sequential Quantitative Shock Loadings

a) Response to Sequential Stepwise Increases in Sorbitol Concentration

The responses of a system operated at an 8-hour detention time with sorbitol as carbon source to sequential stepwise increases in sorbitol feed concentration (range of concentration from 1000 to 4000 mg/l) were shown in Figures 13, 14, 15, and 16. This series of shock loads did not cause serious malfunction of the system with respect to total effluent COD concentration. The total effluent COD varied with substrate concentration in the feed, i.e., the higher the sorbitol concentration in the feed, the greater was the amount of total COD which appeared in the effluent. However, it is apparent that for all experiments herein reported, the COD removal efficiency was approximately ninety-five per cent. In all experiments under these gradual shock loading conditions it is apparent that the response to the shock load involved a rather smooth transient increase in the biological solids concentration until it reached a new equilibrium, and the percent yield of solids was in the range from 46.5 to 52.3. The results observed in this study support the finding of Krishnan (12). In his studies on the effect of gradual shock loadings of systems with and without sludge recirculation, using glucose as carbon source, he found that among factors which control the response to gradual shock loadings are detention time and solids concentration in the aerator. In his studies, higher solids concentration (approximately 800 mg/l in his case) gave protection against gradual shock loadings up to a certain increase in glucose concentration beyond which the solids did not afford any protection, e.g., up to 3000 mg/l glucose at 4-hour detention time. The results of the present study indicate that if increases in organic

loading (shock loads) can be applied gradually in a sequential manner, allowing attainment of new steady state conditions between incremental increases in loading, the biochemical potential for accommodating quantitative shock loads may be increased.

b) Response to Sequential Stepwise Increases in Glucose Concentration

The responses to sequential stepwise increases in glucose feed concentration were shown in Figures 17, 20, and 23. When the system was subjected to a doubling of the previous influent concentration, the system (see Figures 17 and 20) responded successfully (from 500 mg/l to 1000 mg/l, and from 1000 mg/l to 2000 mg/l glucose) without a significant rise in the effluent COD. At these shock loading levels, the COD removal efficiencies of the system were found to be approximately 90.5 to 91.5 per cent. However, it is very clear (Figure 23) that there was a severe disruption of COD removal efficiency in the transient state for the system in which the feed was increased from 2000 to 4000 mg/l glucose.

The response started in a successful manner, i.e., there was a rapid rise in biological solids concentration. The rise was faster than that in Figure 20, and much faster than that shown in Figure 17. One would expect the initiation of a successful response to be a more rapid rise in solids at this energy level, since there was more total substrate and biological solids present. Even though the initial response could be adjudged successful, and it may be concluded that the pattern and rate of response was consistent with results which might be expected based upon the previous two shocks, there followed a secondary response which involved a severe dilute-out of cells. This

response was accompanied by a change in the color of the mixed liquor, indicative of a change in predominating microbial species. This effect has also been observed by Thabaraj and Gaudy (53). Such occurrences are complicating factors in any attempt to categorize or predict limits of successful response to shock loadings. The results do, however, offer a clear indication that two distinct responses, i.e., metabolic and ecological, can be involved. The results to date indicate that they occur sequentially, the first by the predominant species indigenous to the system prior to the shock, and the second by the increase in the population of cells which were formerly not predominant in the system.

It is recalled that during these shock load studies, samples of the cells were taken for growth rate studies during steady and during transient conditions (Figures 18, 19, 21, 22, 24, and 25). The μ_m and k_s values were summarized in Table I. It is interesting to note that μ_m values during transient or steady state were approximately the same except the value obtained using cells taken during the transient in response to the 4000 mg/l glucose shock. In this case, the μ_m value was somewhat lower than the others. The cells used in the growth rate study were harvested at the time of peak solids concentration in the primary (first) response. At this time there had been a slight change in the color of the mixed liquor (it became more apparent as solids concentration decreased). Thus the change in color can be correlated to the decrease in μ_m. While the concomitant occurrence of these events does not offer much basis for theorizing as to the cause for the secondary response, they do provide corroborative indications that it did involve a change in predominating species.

3. Hydraulic Shock Loadings in a Multicomponent (Three Carbon Source) Substrate System

In this section and in section 4 (two-component carbon source system) which follows, the results of initial (exploratory) studies on the response of cells growing on multicomponent source systems to changes in growth rate (hydraulic shocks) are discussed. Currently, a more exhaustive study employing a wide variety of multicomponent carbon source systems is being conducted in the Oklahoma State University bio-engineering laboratories by J. J. Su. The results of the studies herein reported seemed to warrant the more exhaustive studies being made by Su. The results of Mateles, et al. (54), George (16), and Yu (17) indicate that hydraulic shocks can cause rather severe transient disruption of system efficiency. In the present study, using three- and two-component carbon sources, the hydraulic shock may be termed, in accordance with the nomenclature adopted by George, "constant concentration" shocks. The only change made in administering these shocks was hydraulic flow rate; the concentration of incoming feed remained constant, thus, when D was increased, the amount of organic matter entering the reactor per unit of time was increased, i.e., the daily organic loading was increased.

For the studies reported in the Results, section 3, the steady state dilution rate before imposing the shock load was 1/24 hr^{-1}, and the inflow concentration of 900 mg/l substrate (300 mg/l glucose plus 300 mg/l glycerol plus 300 mg/l galactose) was maintained. It is important to note that the system successfully responded to increases in flow rate up to fifty per cent (Figures 29, 34, 35, and 40) with very little rise in the effluent COD during the transient state. In response

to the increased inflow rate, the biological solids concentrations decreased slightly during the transient state. However, there was no appreciable change in solids concentration as it attained a new steady state, and the effluent COD level was approximately the same as it had been in the previous steady state conditions. For the more severe hydraulic shock loading (Figure 41), the system was overloaded by one hundred per cent; i.e., detention time was decreased from six to three hours. There was a severe disruption of COD removal efficiency and a rapid wash out of biological solids during the transient state. The system recovered forty hours after applying the shock.

It is interesting to note that at all times under steady and transient conditions the order of substrate leakage from higher to lower concentration was galactose > glycerol (periodate) > glucose. At all times, except during the severe transient (Figure 41), very small concentrations were found. During this severe transient, a proportionately high concentration of galactose was observed. The results of the continuous flow studies correlate very well with those of the accompanying batch studies (Figures 30, 31, 32, 33, 36, 37, 38, 39, 42, 43, 44, and 45) using cells harvested at the various dilution rates. In the control systems of each set of experiments,the rate of removal followed the same order, and in accompanying combined systems, glucose was always removed first, followed by glycerol and galactose. A comparison of the effects of each substrate on the removal of the others can be made in a general way by comparing the combined system with its accompanying control for each set of experiments. It is realized in all cases when making comparison of amounts of substrate removed in the controls with amounts removed in the combined system at a particular time, that the

biological solids concentration in the combined system was always higher because of the greater amount of total carbon source present. Since biological solids concentration can exert an effect on the rate of removal, a direct comparison of the controls and combined system as a measure of effect of one substrate on the removal of another provides a rather conservative assessment. For example, in Figure 31 (cells harvested at D = 1/24 hr^{-1}) it is seen that all three substrates were removed concurrently; the order of removal rates was glucose > glycerol > galactose. Comparison of Figure 31 with Figure 30 (control systems) shows that the glucose removal curves in the combined and control unit were essentially identical. Making the same type of comparison for the other two substrates, it is seen that during the early phase, i.e., the first five hours, glycerol and galactose removals were somewhat less in the combined system than in the respective control systems. Thus the results suggest a slight retardation or blockage of metabolism of these substrates in the combined system. This effect was somewhat more in evidence for cells harvested at D = 1/18 hr^{-1} (compare Figures 32 and 33). As the dilution rate was increased, the interference with removal became more pronounced (compare Figures 36 and 37, 38 and 39, 42 and 43, 44 and 45). It is rather interesting to note that the past growth history of the cells affected their response in batch. Greatly increased growth rate (or decreased cell age) provided greater manifestation of substrate interference by either repression or inhibition. The results suggest, as do others obtained in past studies in the bioengineering laboratories (17)(30)(31) that there is a tendency for greater manifestation of substrate blockage in batch systems, i.e., a shift toward a more sequential mode of substrate removal, as the age of the cells

decreases. This effect is also shown in continuous culture during transition to higher growth rate, i.e., during severe hydraulic shocks. In the present study it was also evidenced in the order of residual substrate levels during steady state operation.

4. Hydraulic Shock Loadings in a Two-Component Carbon Source System

These experiments were similar to those of Section 3, except for the nature of the substrate, and the responses to the hydraulic shock loadings were in general accord with the pattern observed in the previous experimentation (see Figures 46, 51, 52, 57, and 58). At the severe shock (D 1/6 hr^{-1} to D 1/3 hr^{-1}, Figure 58), the pattern of solids washout and COD leakage was the same as that exhibited in the previous experiment at the same shock level (see Figure 41). A large portion of the COD leakage was attributable to metabolic intermediates and/or endproducts. Also, as in the previous case, much of the more slowly metabolized compound, in this case glycerol (periodate), appeared in the effluent in much greater concentrations than did the more rapidly metabolized compound glucose.

Also in general agreement with the trends observed in the batch studies of section 3, the batch studies in the present series of experiments using cells harvested at the various dilution rates indicate a tendency toward a more sequential mode of substrate removal as dilution rate (growth rate) increased. This effect can be readily seen by comparison of Figures 47 and 48 (D = 1/24) and Figures 61 and 62 (D = 1/3 hr^{-1}). It is emphasized that this trend provides simply a rough guide or rule of thumb indicating that excessive leakage of one compound due to a blockage of removal by another would be more likely to occur in systems operating at a high dilution rate (low detention time) than in

systems operating at high detention times. There is no guarantee that lower dilution rates will provide total protection from the blockage effect. For example, the cells employed in Figures 53 and 54 (D = 1/12 hr^{-1}) had a history of slower growth (greater age) than the cells used in Figures 61 and 62 (D = 1/3 hr^{-1}) yet the tendency toward sequential removal was in evidence. Yu found in his studies over a wide range of dilution rates that the occurrence of a more sequential mode of removal was more prevalent when cells previously growing at a fast growth rate (dilution rate) were used than when slower growing cells were used. But he also observed some cases where nearly sequential removal was observed using slowly growing cells. Employing growth rate as an assessment of cell age, the results of the present studies (sections 3 and 4) are in general accord with those of Yu. Even by ageing cells in various ways in batch experiments, as did Tsay (55), and Heidman (31), there was no way to provide absolute protection against the retarding effect of one substrate on the removal of another. Concerning Heidman's results, it can be said that in a very young system of batch-grown cells there was exhibited a greater degree of substrate blockage than for older systems, but a considerable degree of retardation of blockage was observed no matter how long the system was aged (see pages 15 and 16, Chapter II, this manuscript).

5. Quantitative Shock Loadings in Two-Component Systems

In this series of experiments the two-component system employed in the hydraulic shock studies, i.e., glucose and glycerol, was used to study response to quantitative shock loadings consisting of equal parts of each carbon source. The system was run at D = 1/8 hr^{-1} and was always permitted to come into a new steady state prior to administering

each new shock loading. Shocks from 500 to 1000 (Figure 63), 1000 to 2000 (Figure 66), and 2000 to 4000 mg/l COD (Figure 67) were applied. Thus the loading was doubled during each shock.

During the first doubling of substrate (Figure 63), the "typical" rise and subsidence in effluent COD was observed, and glycerol (periodate) leaked in the effluent. The effluent characteristics in the new steady state were not unlike those before the shock, except for a slightly higher total COD and glycerol COD. The effluent response required approximately forty hours. The biological solids concentration had peaked at this time, but the solids response continued for an additional forty hours during which time there was a gradual decline to a new steady state level. When the organic loading was again doubled (Figure 66) the response in biological solids was much more rapid, due, presumably, to the greater quantity of substrate and the higher initial solids concentration. There was no "typical" rise and subsidence in effluent COD concentration. There was, instead, a significant and permanent increase in effluent COD. The anthrone level remained the same, and most of the increased COD was attributable to glycerol (periodate) COD. In regard to correlation of the solids and COD responses, the situation was the reverse of that observed for the previous shock. Comparing the new steady states after each shock, it can be seen that the lesser loading (although the relative magnitude of the shocks was the same) yielded the more favorable response. In Figure 63 it can be seen that the overall efficiency of COD removal was increased in response to the shock, whereas in Figure 66 the overall efficiency with respect to COD removal was slightly depressed after the shock. This pattern was not continued when the loading was again doubled (Figure

67). Instead, the pattern was similar to that observed in Figure 63, although the transient disruption was much more severe. It is seen in Figure 67 that upon return to the steady state, the overall system efficiency with respect to effluent quality was improved over what it had been prior to the shock. It is felt that this system responded as rapidly as can probably be expected of any comparable system operated without artificial controls, e.g., return of biological solids either prior to or during the shock loading. When one compares the dilute-in curve for the shock with the effluent COD and biological solids curves, it is seen that nearly all of the substrate which was removed is accounted for as biological solids. This result was mentioned previously, and it does not seem that a one hundred per cent yield is plausible in view of the fact that one cannot attribute the rapid rise in biological solids to adsorption of the substrate (note the rise in protein content of the sludge). The results do, however, seem to indicate that the cells were incorporating substrate (i.e., synthesizing cellular material) at or close to the maximum rate of which they were capable, and at a greater efficiency than during the steady state condition. Storer and Gaudy have also noted an apparent increase in cell yield during the early stages of transient response to quantitative shock loading (56). However, the reasons for this apparent increase remain somewhat obscure.

The values of μ_m and k_S obtained in the separate growth rate studies at each steady state were approximately the same, regardless of the substrate level in which the cells were growing prior to use in the growth rate studies. It is interesting to note that there was no evidence for diauxic growth in the curves from which the growth rates

were obtained.

6. Effect of Successive Increases in Organic Loadings

The aim of these studies was two-fold. First, it was desired to assess the behavior of a system under successive increases in organic loading without regard to attainment of a new steady state before each increase in loading. Secondly, it was desirable to compare the responses for two dilution rates (1/12 and 1/8 hr^{-1}). In both sets of experiments a two-component substrate consisting of glucose and glycerol was employed; however, in both series of shocks the feed consisted solely of glucose prior to administering the first shock. When the feed was changed from 1000 mg/l glucose to 1000 mg/l glucose plus 1000 mg/l glycerol at the 12-hour detention time (Figure 70), there was a decided lag in the response. The lag was attributed to the need for an acclimation period before the cells could metabolize glycerol. During this period (4 to 5 hours), the biological solids remained essentially at the previous steady state level, and the glycerol (periodate) curve was essentially parallel to the glycerol dilute-in curve. The lag in response was not observed during successive increases in loading (Figures 71 and 72) which consisted of increases in glycerol concentration.

When the same type of experiments was run at the 8-hour detention time, the lag in response to the first change (Figure 73) was much less severe than it had been at the slower growth rate, and the disturbance to the system during the transient was barely noticeable. At the next shock level (Figure 74) there was essentially no disturbance in COD removal efficiency. Even at the highest level (Figure 74), the disturbance was minimal. It is apparent from the results that

additional detention time did not benefit these systems regarding ability to accommodate these shock loadings. On the contrary, the faster growing (younger) cells acclimated more speedily to glycerol and were more efficient in its utilization in the presence of glucose. Some caution should be exercised regarding generalization from the results of only two sets of experiments, especially when dealing with natural populations. It does, however, seem reasonable to expect that faster growing cells might initiate an adaptive response faster, especially in view of the fact that the only change in feed was addition of a compound to which the cells had to acclimate, i.e., there was no change in the glucose concentration. It is interesting to note that at either dilution rate, the presence of glucose did not prevent acclimation to and continued removal of glycerol. Such may not have been the case if lower detention periods had been used. Work now being conducted in the bioengineering laboratories by J. J. Su should shed more light on the effect of dilution rate on permanent leakage of one substrate in the presence of others in the feed.

It is interesting to note that in all cases in these experiments the concentration of glucose (anthrone) in the effluent (reactor) was fairly low. Indeed, its presence may have helped initiate acclimation to glycerol. From their studies on yeast, McQuillan and Halvorson (57) have found that whereas glucose at high concentrations caused repression of enzyme synthesis, at low concentrations it stimulated induction of enzyme synthesis. It would certainly seem logical to conclude that a shock loading with a so-called "B" compound (acclimation required) to a system being fed an "A" compound (acclimation rapid or none required) can lead to a less severe response than the reverse shock situation

under comparable operational conditions (dilution rate, total organic loading, temperature, etc.).

7. Effect of Shock Loading to Systems at High Biological Solids Concentration

The steadying effect provided by maintenance of a biological solids concentration in the reactor which is higher than that supported naturally by the system (i.e., operation with solids recycle) was demonstrated by the results presented in Figures 76 and 77). The detention time was purposely made rather long (twenty-four hours), since the effect of shock loading on sludge settleability as well as biochemical efficiency of the system was assessed. When these studies were designed, there was some expectation that sludge flocculation and settling would deteriorate under shock loading conditions, and there was some concern that due to de-flocculation it would be difficult to maintain the desired solids level. The results indicate that even after tripling the organic loading (1000 to 3000 mg/l glycerol, Figure 77), such was not the case. The results obtained in this brief study of the quantitative shock load both substantiate and extend the conclusions of Krishnan concerning the beneficial effects of solids recycle with regard to ability of the system to accommodate quantitative shock loads.

The succeeding studies (Figures 78 through 82) in which the detention time was twelve hours and a glycerol-acclimated system was shocked by addition to the glycerol feed of increasing amounts of glucose, offer a more penetrating view into the beneficial effects of cell recycle or maintenance of artificially high aeration solids levels in the reactor. It is emphasized that in this series of experiments

the system received a quantitative as well as a qualitative shock, and the shock substrate, glucose, was one which would be expected to interfere with metabolism of the original carbon source (glycerol). There was essentially no interruption of efficiency up to a shock level of four hundred per cent increase in organic loading (see Figures 78, 79, and 80). The quality of the effluent with respect to soluble COD as well as suspended solids remained excellent. Glycerol (periodate) analyses were run, but there was essentially no periodate-reactive material present. The feeding of 3000 mg/l glucose (shock) with 1000 mg/l glycerol did not lead to any leakage of glycerol COD, and the accompanying sludge synthesis did not lead to serious leakage of unflocculated cells. A transient leakage of soluble COD did occur when the loading was increased from 1000 mg/l glycerol to 1000 mg/l glycerol plus 4000 mg/l glucose (see Figure 81). Over fifty per cent of the accumulated COD was attributable to metabolic intermediates and/or endproducts. Under this severe shock condition, glycerol (periodate) COD did appear in the effluent, but there was nearly as much glucose (anthrone) COD present. Glucose was not metabolized at the expense of glycerol. Again, there was essentially no effect on the suspended solids level in the settling chamber effluent.

When the system was subjected to an even more severe shock (5000 mg/l glucose, see Figure 82), the transient leakage of COD was only slightly greater than that at the previous shock level. Again there was no increase in suspended solids in the effluent even though the biological solids in the aeration chamber rose to over 7000 mg/l in response to the shock. It is emphasized that, in this series of experiments, the shock organic loading was relieved after assessing each

shock. It is not known whether good quality effluent, with respect to suspended solids could have been sustained over a long period of time at these high loadings. It does seem apparent, however, that rapid deterioration of flocculating and settling characteristics is not necessarily a consequence of shock loadings. In these studies, at least, the efficiency of the system was governed by the metabolic response. The maintenance of a high solids concentration prior to the shock, coupled with the fact that the system was capable of retaining the solids synthesized in response to the shock, were jointly responsible for the successful metabolic response. It is emphasized that during assessment of the response to the shocks, solids were not wasted from the settling tank. The ability of this type of upflow clarifier to retain excess solids and return underflow to the aeration tank (see Figure 2A) in all probability played a significant role in the overall success of the response. Based upon visual observations during these experiments, the sludge blanket through which the effluent had to pass is believed to have functioned as a rather successful filter of unflocculated cells during the 50 to 60 hour period of each shock.

8. Qualitative Shock Loadings and Hydraulic Shock Loadings to Extended Aeration Systems

The results shown in Figures 83, 84, and 85 indicate that an extended aeration system could accommodate a considerable range of shock loading (qualitative and quantitative), provided no solids were wasted, i.e., the solids level was allowed to increase as the level of shock was increased. It cannot be said that the response to the change from 500 mg/l glucose to 3000 mg/l glucose plus 3000 mg/l glycerol would

have been as successful as it was had the shock been placed upon the system at the beginning of the series of experiments when the aeration solids concentration was 6000 mg/l instead of 15,500 mg/l. The beneficial effects of higher aeration solids concentration which were observed in other results reported in this thesis (section 7) would be expected to be operative under conditions of total cell recycle (extended aeration) as well as for systems in which cells were purposely wasted. It is, however, significant that the metabolic response with respect to effluent characteristics (e.g., filtrate COD, anthrone, periodate) was excellent, and there was no excessive leakage of glycerol. The system responded rather well in all respects through the one hundred per cent increase in loading (500 mg/l glucose plus 500 mg/l glycerol). However, when the loading was increased from 500 mg/l glucose to 1000 mg/l glucose plus 1000 mg/l glycerol, there was a noticeable rise in total filtrate COD and a very significant rise in biological solids level in the clarifier effluent, and at a high shock loading the effect was even more pronounced. It is interesting to note that the level of suspended solids in the effluent remained high while the feed level was high, but dropped off each time the feed was reduced to the original 500 mg/l glucose level. This effect was evidenced even though there was always a net increase in the biolgical solids level in the reactor as the loading was increased. Thus the leakage of biological solids did not appear to be due to high biological solids concentration, but to the nature of the loading.

Comparison of biological solids in the effluent (Figure 84) and in the reactor (Figure 85) indicates that upon return to the 500 mg/l glucose feeding level between each increase in feed there was a slight

decrease (although never as much as the previous increase) in the solids concentration in the reactor. Since no solids were lost or wasted, the decrease in solids provides some evidence for net autodigestion during the period of normal feeding.

After approximately 158 hours of operation at the 500 mg/l glucose level, the overall detention time of the system was changed from 24 to 12 hours, and the response to this hydraulic shock load (Figure 86) was a successful one in every respect. Upon administering a reverse shock load (return to 24-hour detention time) there was no change in effluent or reactor characteristics. When an extremely severe hydraulic shock (24 to 6-hour detention time) was applied, biological solids concentration in the clarifier effluent rose significantly and did not return to the previous level upon release of the hydraulic shock, i.e., return to the 24-hour detention time. When the severe shock was applied, the solids in the effluent appeared to be fragments of the floc, i.e., not unflocculated microorganisms which came into predominance due to the faster growth rate. After release of the shock, the solids level in the effluent decreased but did not, even after prolonged operation at the normal flow rate, return to the level which existed before the severe shock was applied. Thus, release of solids during the high flow rate could not be attributed solely to hydraulic tearing of the floc. Apparently, some permanent or long-term damage to mixed liquor separation had been effected by the hydraulic shock.

CHAPTER VI

CONCLUSIONS

1. During shock loadings, a secondary response involving a change in predominating microbial species can develop. This response, which was observed during slug and gradual shock loadings in the present work, followed the primary (metabolic) response which did not appear to be accompanied by a change in predominance.

2. Slug dosing with glucose to a system operating on sorbitol yielded a more severe disruption of metabolism than slug dosing with sorbitol to a system operating on glucose.

3. The general kinetic characteristics, μ_m and k_s, for cells harvested at various steady states following sequential stepwise increases in organic loading, were similar.

4. Increasing severity of hydraulic shock loading in two- and three-component carbon source media lead to leakage of the carbon sources in the same order as that observed in batch systems.

5. Increasing severity of quantitative shock loading in a two-component medium (glucose and glycerol in equal portions leads to significant leakage of glycerol (periodate) COD. In experiments employing the same substrates but holding glucose feed concentration constant while increasing glycerol concentration, there was a less severe leakage of glycerol, indicating that the severity of blockage

was associated with the ratio of glucose to glycerol in the feed. Extrapolating this finding to the more general case, it seems valid to conclude that increasing concentrations of the substrate from which the metabolite repressor may be produced (i.e., so-called "A" compounds) can cause more severe disruption in multicomponent media than can equivalent increases in the compound subject to repression (the so-called "B" compounds). This general conclusion seems justified in view of the fact that the concentration of repressor which can be present depends in a large measure upon the concentration of the "A" compound.

6. The results shown in section 7 clearly indicate the beneficial effects of maintaining a high biological solids concentration (by sludge recycle) during shock loadings. With aeration solids at approximately 2000 mg/l and a detention time of twelve hours, the system could withstand with essentially no disruption of efficiency a combined quantitative-qualitative shock of a most severe nature, i.e., a fourfold increase in loading in which all of the increased loading consisted of a compound (glucose) which would be expected to block metabolism of the original compound (glycerol). The success of the system was in all probability due to solids retention in the aerator brought about by the internal recycle feature of the reactor-settling chamber construction of the system. While separate aeration tank and settling tank designs would appear to be more controllable under steady operation because a definite recycle flow and recycle solids concentration can be maintained, such an arrangement may be disadvantageous for accommodation of shock loadings. The type of reactor used in the present study permits biological solids to build up rapidly in the aeration chamber, where they are most needed during the shock.

7, The extended aeration activated sludge employed in these studies was one which had been aged a considerable time prior to assessing its shock loading response. Therefore, the results and the conclusions based upon them can be said to apply to a rather authentic extended aeration sludge. The process was relatively free of metabolic disruption due to combined quantitative and qualitative shocks and hydraulic shocks which were applied. It would seem that part of the reason such systems can accommodate severe shock loadings is due to the high biological solids concentration. The results of the present study, as well as those of Ragthaidee and of Yang, indicate that retention of solids (i.e., no wasting of solids) does not cause severe deterioration of the metabolic activity of the sludge. Thus the benefits of high aeration solids and the protection against shock loads which they provide, are imparted essentially unimpaired to extended aeration sludges. The results of the present study indicate that the hydraulic shock load gives the most cause for concern and that the nature of the deleterious response is escape of solids in the clarifier effluent, not a biochemical inability to remove soluble carbon source. Since biological solids can vary in extended aeration systems, and since response to shock loads is known to be related to biological solids concentration, more general conclusions than those which have been drawn should not be made.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

1. Some of the work which could be fruitfully extended based upon the present results is already under way in the bioengineering laboratories. For example, an intensive study by J. J. Su on the effect of changing dilution rate (both transient and steady conditions) in multi-component carbon source systems is nearing completion.

2. Concerning the shock load response of the extended aeration process, it would be of interest to study the response to shocks under nitrogen-deficient conditions. Such shocks could lead to rapid accumulation of cellular carbohydrate (possibly some of it extracellular in nature) which could affect subsequent autodigestive cycles. Except for temperature and pH shocks, this is essentially the only type of shock not yet investigated in this laboratory with regard to the extended aeration process.

3. In view of the fact that changes in microbial predominance were noted at times throughout these studies as well as in nearly all studies involving heterogeneous populations, continuation of a program of experimentation designed to characterize microbial interaction in known mixtures of species should in the long run provide valuable insight into the overall significance and role of predominance changes.

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